An Introduction to the Microscopical Study of Diatoms

Robert B. McLaughlin

Edited by John Gustav Delly and Steve Gill

Editorial note:

Many readers will recognize the name of Robert B. "Mac" McLaughlin as the author of a regular Diatom column published in *The Microscope* during the 1980's and 1990's. He also wrote two books in the Microscope Series on Accessories and Contrast Methods. He was a contributor to the diatom literature, including numerous papers printed in the *Quekett Microscopical Club Journal* and, indeed, has had a diatom named for him, *Gomphonema mclaughlinii* (Reichardt). Over 50 years ago, while still in Alaska, and prior to his work on the diatoms of the Kenai Deposit, Mac started to write a diatom textbook for himself acting more as a compiler or organizer of material from, generally, older, scarcer diatom works. He was, in his words, like a fish out of water, and wished to self-educate so as to be able to do meaningful work with the microscope and diatoms. He read and studied extensively, initially for his own amusement and edification, and succeeded to a remarkable degree. Then, one day, thinking that he might help others by letting them know what he had learned were the important things to study, where to find information and publications, and, in short, distill his hard-won knowledge, he started "the book". For illustrations he wanted to use figures from Friedrich Hustedt's publication, *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden*, (ABB.XI T.4, S.I., 1929); the publisher Cramer had told him that the work was long out-of-print, and that there would be no problem about using illustrations from it. Mac's book eventually came to be over 525 typed papers, while still needing some appendices, index, table of contents, revision, and editing.

> "Collecting and preparing diatoms, as well as disclosures about examination and culture methods."

More than 25 years ago, a publisher told Mac that they wanted to prepare his book for distribution, but, incredulously, they made no progress over a ten year period! Mac was, understandably, disheartened by the whole venture, and the book was put away; in addition, he had moved to Santa Fe. The matter of the book was brought up at The Hooke College of Applied Sciences, and it was decided that it would be a valuable contribution to make this knowledge available by some means for those wishing to start the microscopical study of diatoms. As it turned out, Mac was by then an octogenarian, his eyesight was poor, and reading a chore. He had donated his equipment, samples, and references to the California Academy of Sciences to be with the donations from Hanna. Being thus unable to do anything more with the book, he gave permission to the American Editor at Hooke College of Applied Sciences to do with it as he saw fit. After much discussion, it was decided to

> References to Hustedt in the main body of the book should assume that the detail referred to may be in any of his volumes.

abandon search for an editor or anybody else to attempt to complete it, and to publish it online, as it is, even to include Mac's own sketches and handwritten notes. There is precedent for this tack, as in the re-publication of Mann's *The Bullet's Flight*, with Pope's hand-written marginalia, and Lee's *Note-Book of an Amateur Geologist*.

Mac no longer had the Hustedt book he intended to use for illustrations, but readers will find adequate illustrative material in Hustedt's three-part *Die Kieselalgen* or in Schmidt's three-volume *Atlas der Diatomaceen-Kunde*, both of which have been reprinted.

Thus, the Hooke College of Applied Sciences feels that rather than let Mac's 525+ page manuscript on diatoms, descend, unseen, into oblivion, it would be better to transcribe it as best we can and let it stand on its own merits. Whilst it is true that some of the text and conjecture is now dated and superseded, the majority of the content is as useful to both amateur and professional as it was when Mac first wrote it. The manuscript was in a semi-edited state. Where Mac authorized any correction it has been included, simple typographical errors have been corrected, any other editing has been at the discretion of the Editors, including the addition of an extensive bibliography.

The layout was designed for US Royal sized paper with a view to it being printed. The formatting of the document reflects this intention. Space has been left in the margins for user's own notes.

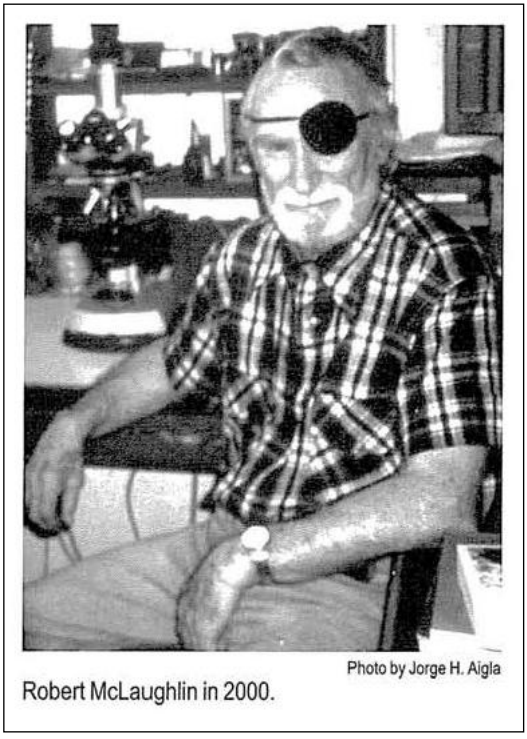
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Many of the chemical procedures described are included in a historical context. Though some might still be used by trained and suitably equipped chemists it is not recommended that others use the procedures or techniques described without first fully understanding the dangers and ramifications of misuse etc. Should you choose to embark on any of the procedures then you do so at your own risk and the Editors will not and cannot be held responsible.

Remembering Robert B. "Mac" McLaughlin
1922 – 2012



Robert McLaughlin in 2000.

During the time of the final editorial process it was learned that Mac had died (on 6th April 2012) having turned 90 years old in January of that year. A brief biographical note follows:

Mac, as he was known to his friends, will be remembered by long-time readers of *The Microscope* as the author and editor of the popular "Diatoms" column, which appeared in each quarterly issue for 10 years in 1985-1995. He also wrote two highly respected books in the Microscope Series: *Accessories for the Light Microscope* (1975) and *Special Methods in Light Microscopy* (1977).

In addition, he authored or co-authored numerous articles on diatoms for specialty journals, including a major contribution (with John L. Stone), "Some Late Pleistocene Diatoms of the Kenai Peninsula, Alaska" (*Nova Hedwigia*, 1986). Mac's contribution to the diatom and microscopy literature was formally recognized when he received the Annual Award of the State Microscopical Society of Illinois. The ultimate recognition of Mac as a diatomist came when he had a diatom named after him: *Gomphonema mclaughlinii* E. Reichardt. He proudly displayed a photomicrograph of this diatom on the letterhead of his stationery. Incidentally he was also mentioned by name in a crime novel by Patricia Cornwell as an expert in diatom identification during a forensic examination of trace evidence. Mac had received his Federal Communications Commission assigned Radio Operator License in 1938, when he was 16 years old. He built his own radio station, of course, and was assigned the call sign W6QIN.

In 1940, Mac joined the United States Navy for six years, and at boot camp in San Diego, California, he qualified for Aviation Radio School. He was subsequently sent to the Naval Air Station at Seattle, Washinton, where he became a radio operator aboard a PBY aircraft. The squadron went to Kodiak, Alaska, in early summer 1941. It was here that he became a high-speed Navy telegrapher. The Navy did not furnish bugs (a kind of telegraph key with springs and adjustable weights that allows for

adjustment of speed and touch) at the time, and Mac supplied his own - a MacKey. Mac's "fist" was beautiful; his tone was pure, and his well-formed characters were characteristically recognizable - it was a pleasure to copy his transmissions.

After Pearl Harbor, Mac and a buddy of his volunteered for a mission to install a one receiver/one transmitter radio station for weather reporting at Chernofski Bay on the other end of Unalaska Island. After a year at Umnak, Mac was transferred to be in charge of the Amchitka Transmitter Station at Kirilof Point. Then it was back to the States, where he became Chief-in-Charge of the Naval Transmitter Station at Pensacola, Florida.

In 1946, Mac went to Tri-State College in Angola, Indiana, and graduated with a Bachelor of Science degree in Electrical Engineering. He then moved to Anchorage, Alaska and the Civil Aeronautics Administration, where he was responsible for both the Intra-Alaska and Trans-Oceanic sites. In 1961, Mac became interested in diatoms, and started his studies on the diatoms found in Alaska's Kenai Peninsula. It was during this time that he started his unpublished 500-page personal manuscript notebook on diatom structure and identification, which is the subject of this book.

His letters, which were always typed on his "mill" (manual typewriter), and his Morse code transmissions were always full of fascinating detail about his diatom studies. A selection of these communications was shown to the Editor of *The Microscope* at the time, and Mac was invited to write a regular column on diatoms. Fortunately, he accepted, and provided 10 years of articles and two books.

Mac's microscopy lab in Santa Fe, which was housed in his radio shack, was equipped with a Leitz CM microscope and a Zeiss Standard microscope, Interestingly, while in Santa Fe, Mac became a Research Associate of the Museum of New Mexico, where, from 1981 to 1991, he specialized in the microscopical examination of folk and fine art. One project he worked on involved the question of the origin of a specific piece of Southwest Indian pottery. Mac sampled the clay used to make the pottery, and found diatoms mixed with the clay. He identified the diatom flora, and then proceeded to examine clays from possible sites. He ended up pinpointing the exact origin of the clay, and thus, the tribe responsible for making that particular pottery piece. Successes like these gave him great satisfaction. As a side interest, Mac enjoyed collecting full plate blocks of postage stamps, and, of course, stamps that depicted microscopes.

When Mac started to have trouble with his eyes, he replaced his Zeiss photomicrographic camera with a video unit, so that he could view his images on a TV monitor. When it became apparent that his failing eye-sight would no longer permit him to do serious diatom work, he donated his reference collection of more than 4,000 diatom reference slides, books, and other equipment to the California Academy of Sciences, so that they could be added to what G Dallas Hanna had already established at the Academy. Mac, however, retained one microscope and about 200 diatom slides and spent his final days recording notes on these 200 slides so that his daughter Susan might have them; she has every intention of using her father's microscope and viewing the specimens while reading his notes.

He will be missed by all those who enjoyed his diatom publications and also by his many friends across the world.

Although "Mac" never saw this version of his manuscript we feel that had he done so and been able to see it to completion he would have added the following:

For my daughter Susan
and
to beginning diatomists everywhere.

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PART I

CHAPTER 1.

1. MORPHOLOGY.

Diatoms are microscopic unicellular algae. Whatever the shape or size of the cell, it consists of the same basic parts: the nucleus, cytoplasm, plasma membrane, and the cell wall.

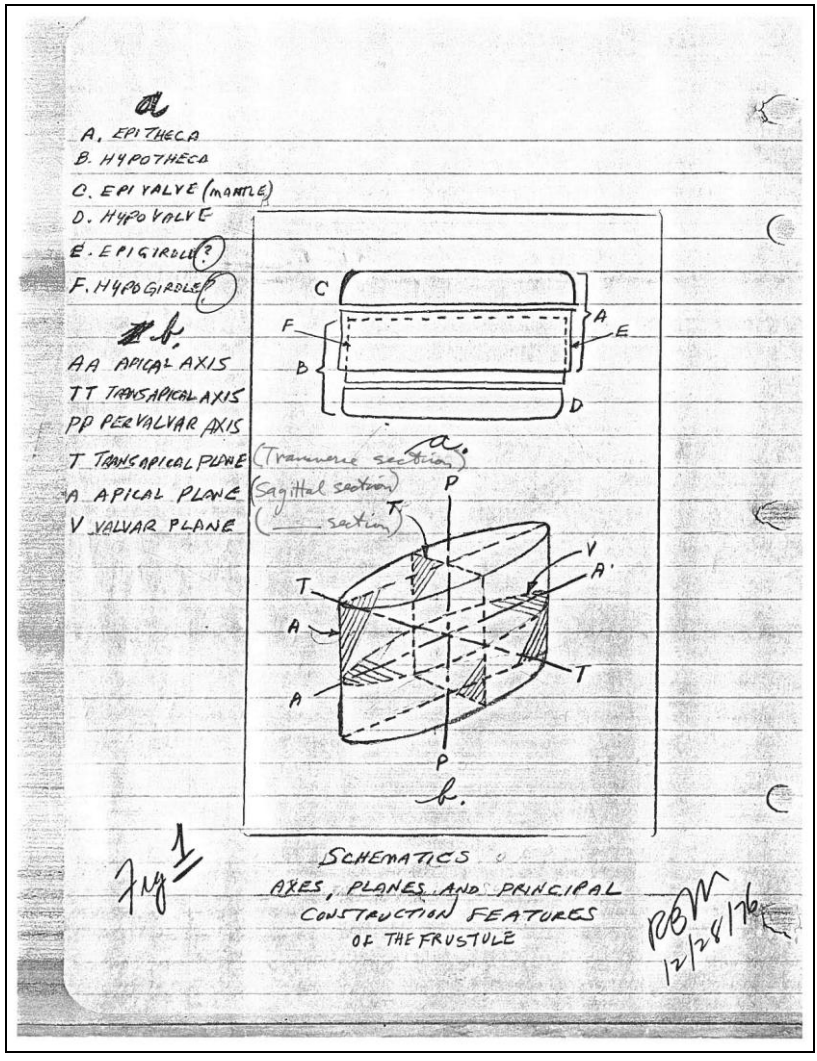
1.1. Structure of the Cell Wall

Emphasis in this Introduction is placed on the structure of the diatom cell wall, as opposed to the living contents, as, in the main, interest in diatom study is directed toward the structure and microstructure of the cell wall. Classification of diatoms and their identification pertinent to many areas of investigation is based on the structure of, and markings on the cell wall as revealed by the light microscope (LM). It is therefore imperative that the student of diatoms possesses a good basic knowledge of that structure and the terminology associated with it.

1.2. General Structure

The cell wall of a diatom is composed of a siliceous material and consists basically of four distinct parts to form a box-like structure; a "top", "bottom", and two slip-fitting, encircling "sides", something on the order of a pill box. The whole siliceous box, or epiderm, of a diatom is called a frustule (see Figure la). The components of the frustule then are the epivalve (upper valve or mantle), hypovalve (lower valve), and the two connecting bands (the hypogirdle and epigirdle). One of the latter is associated with the upper valve and the other with the lower valve, the two sub-assemblies being termed the epitheca and hypotheca respectively. The lower valve, with its connecting hypogirdle, is just slightly smaller than the upper assembly, allowing the upper, epigirdle, band to slip over the lower one. The two connecting bands are collectively regarded as the girdle, although occasionally they are individually referred to as girdles. Some authors also refer to the girdles as the cingula (singular cingulum). Usually a portion of the valve is bent, more or less at a 90 degree angle, and is called the valve mantle. It is on the edge of the valve or, more specifically, the valve mantle that the girdle band is fitted to the valve.

> Cingulum (pl.), Cingula (sing.) *Latin. cingulum - a girdle or belt.*



Friedrich Hustedt: Die Kieselalgen Teil I. (Figs. 1 & 3.)

Figure 1.

The entire frustule may assume many forms ranging from that of a flattened cylinder to complex and even twisted structures. The outline of a diatom is a diagnostic feature in classification and identification, and unless otherwise noted indicates an outline of a valve view of the frustule. There is a terminology associated with the outlines and shapes of diatoms. Figure 2 indicates a representative group of diatom outlines and the terms used in describing them.

1.3. Symmetry

The very wide variety of geometric shapes and features connected with diatom morphology results in numerous patterns. When a motif such as a particular structural feature of the valve or frustule is repeated systematically, the result is a periodic pattern.

Diatom structure by its very nature then, is amenable to being referenced to some system of symmetry. Symmetry in the diatom frustule may be regarded as the correspondence in size, shape, and relative position of its parts that are on opposite sides of a dividing line or median plane, or that are distributed about a center or axis.

The symmetry of diatoms is related to an established set of axes and associated planes. Figure 1b illustrates the principal axes and planes to which diatom symmetry is referred. The pervalvar axis P-P joins the center points of the two valves (upper and lower). The apical axis A-A (sometimes called the sagittal axis) connects the two ends (apices) of a valve such as in the genus *Navicula*. The transapical or transverse axis T-T lies perpendicular to the apical axis on the valve of the diatom. In circular forms the transapical and apical axes are the same length (the diameter) which is then sometimes referred to as the transvalvar axis. The apical and transapical axes determine the valve surfaces or basic symmetry planes. The apical (AA) and pervalvar (PP) axes define the apical plane (A) or the sagittal section. The transapical (TT) and pervalvar (PP) axes define the transapical plane (T) or the transverse section.

The apical (AA) and transapical (TT) axes define the valvar plane (V).

The symmetry of the various genera of diatoms differs in relationship to these three axes. How the symmetry differs is important to systematic description of the genera of diatoms.

Not only is the symmetry of the diatom frustule important to systematic description, but perhaps is even more important to the student of diatoms in forming a three-dimensional conception of various types of frustules that ordinarily are seen in the light microscope as two dimensional objects. Although by examining the frustule in a number of optical sections one can build a three-dimensional mental picture. The visual concept is considerably enhanced by other knowledge of spatial relationships of the various parts. Comparison might be drawn to the value of the knowledge of descriptive geometry and mechanical drawing to macro-objects.

For this latter reason, as well as for the former, considerable space is devoted to basic symmetrical relationships of the diatom frustule in this book.. It is felt that the beginning diatomist, or any other diatomist, not familiar with these concepts, can benefit by familiarization with them. Because of the great variety of configurations of the diatom frustule and the sometimes complex relationship of its various parts, any of a number of basic symmetry systems could be formulated. Friedrich Hustedt (1929) provided an extensive system to describe diatom symmetry many years ago. I have freely translated from the German, and paraphrased and adapted much of his text and illustrations in the following material on diatom symmetry.

The definition of symmetry previously stated requires, in part at least, that there is a correspondance in size of parts on opposite sides of a symmetry plane. Since the

epitheca of a diatom is larger than the hypotheca, then the so-called basic symmetry plane determined by the apical and transapical axis in reality becomes an orientation plane or plane of similarity. In the strictest sense it cannot become a plane of symmetry and the pervalvar axis could never be termed iso-polar. However, because the inherent construction of diatoms is such that the difference in size between the epitheca and hypotheca is very small we can, for practical purposes, regard the axes and planes described as adequate for diatom symmetry purposes. For that reason, and on the basis of practicality, the pervalvar axis can then be either isopolar or heteropolar.

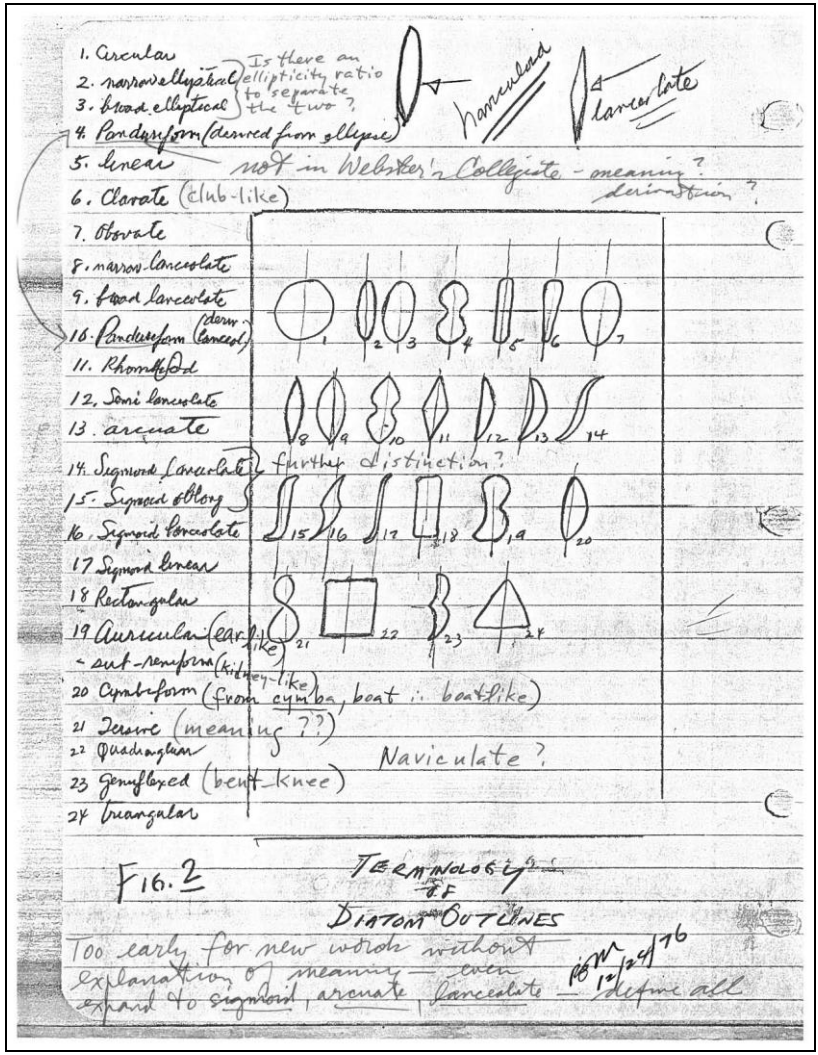


Figure 2.

The designation isopolar or heteropolar, with reference to any of the axes of symmetry is, in part, descriptive of diatom symmetry. For instance, the pervalvar axis of *Navicula peregrina* (Figure 3A) intersects the epitheca and hypotheca of the

frustule at points that are termed poles of the axis. If those points or poles intersect surfaces that are identical in structure the axis is termed isopolar. If they intersect surfaces that are not identical, as in *Achnanthes brevipes* (Figure 3B) for instance, the axis is termed heteropolar. For the two examples the former determine that the pervalvar axis is isopolar and the latter is heteropolar. Similar conclusions may be drawn for the other two axes (apical and transapical) in reference to particular diatoms.

In *Gomphonema olivaceum* (3C) the transapical axis is isopolar, whereas in *Cymbella tumida* (3D) it is heteropolar. In the former, although the outline is curved, the intersecting surfaces are the same. In the latter the intersections of the transapical axis are on dissimilar surfaces, one being curved much more than the other. In *Surirella linearis* (3E) the apical axis is isopolar, but in *Rhopalodia vermicularis* it is heteropolar. From this it is seen that a diatom frustule may be described in a limited way by using a polar designation for each of the three axes of symmetry.

On the basis of the symmetrical relationships of diatom structure there are two major divisions recognized.

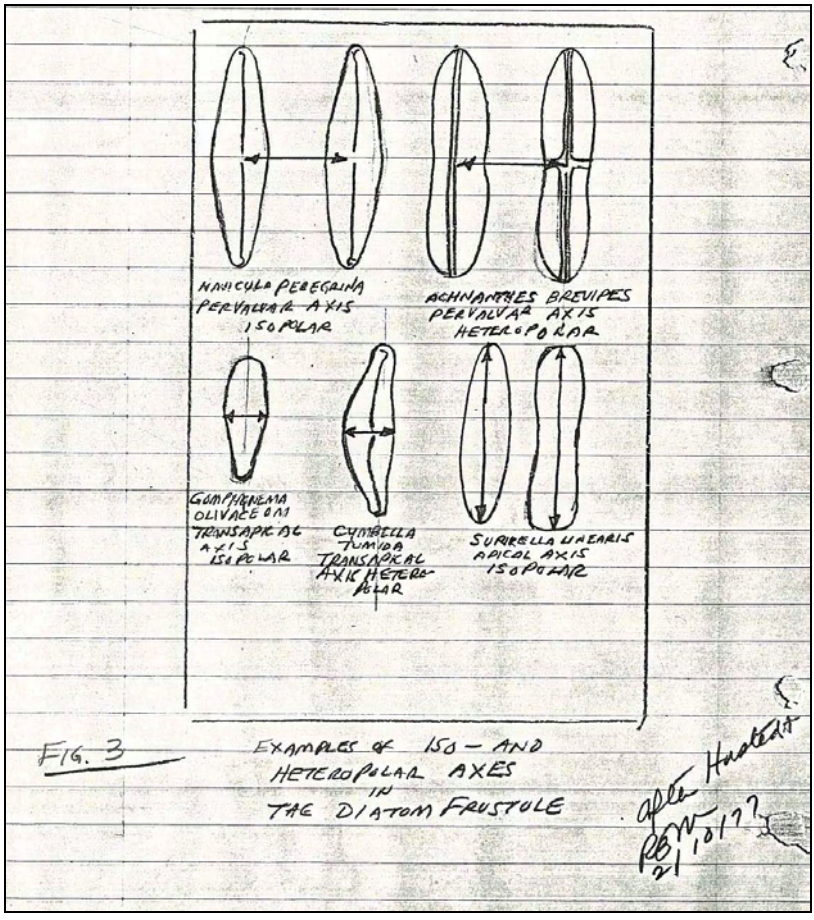
(1) A group in which the pervalvar axis is so isopolar (with the practical assumption mentioned previously). There are at the very least two isopolar or several heteropolar transverse axes possible which are perpendicular to the pervalvar axis. When the transverse axes are of equal length, the form is monoaxial, and when they are of unequal length, they are heteroaxial (such as in bilateral diatoms).

    This group is termed line-symmetrical.

(2) The second group is plane-symmetrical. Outside of the heteropolar or isopolar pervalvar axes, there are only two transverse axes possible, of which at least one is heteropolar.

    It is not possible to express the attributes of all diatom forms in terms of pure symmetry. Some frustule constructions involve twisted, or torsive, forms that complicate the symmetrical relationships. For such cases there are three main types of symmetry concepts that often suffice:

    (a) Mirror Symmetry. One valve is the mirror image of the other.
    (b) Diagonal Symmetry. One valve as compared with the other is rotated about the pervalvar axis 180 degrees.
    (c) Antisymmetry. A combination of (a) and (b) above. The mirror image of one cell-half is rotated 180 degrees.



Friedrich Hustedt: Die Kieselalgen Teil I (Figs. 4, 5, 6. 7, & 8)

Figure 3.

1.3.1. Line-Symmetrical Frustules

i. Monaxial Frustules (Figure 4).

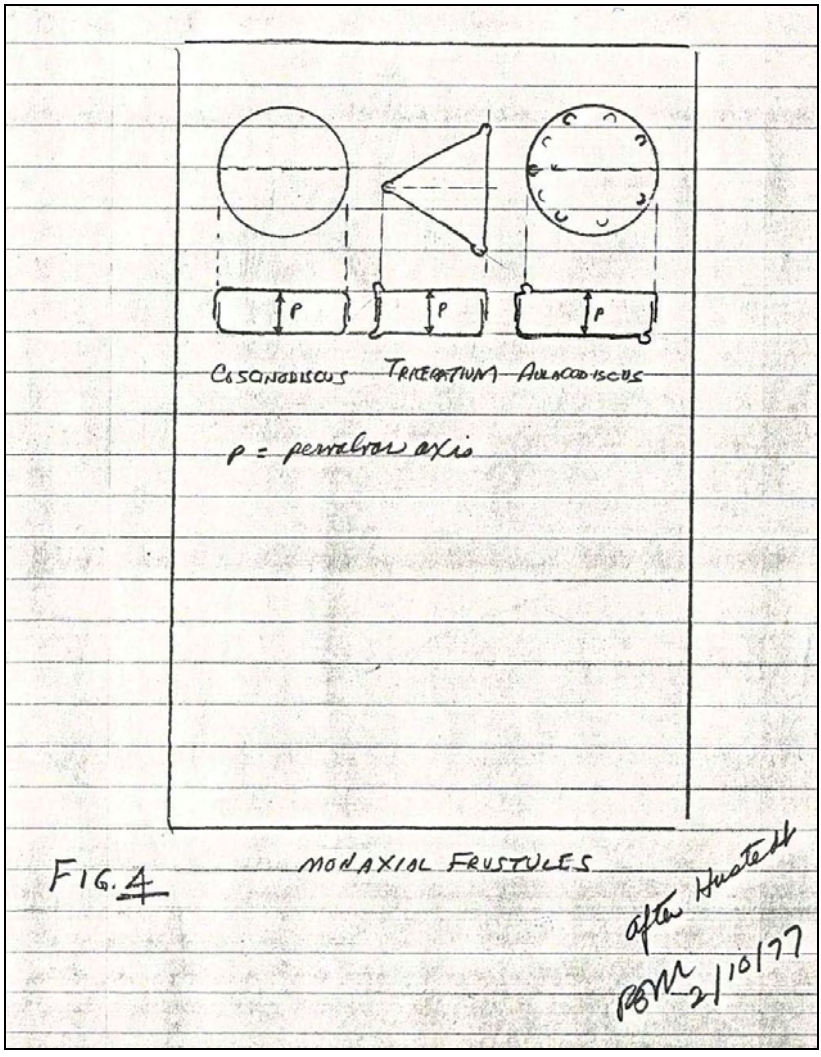
Examples of monaxial frustules are in the genera *Coscinodiscus*, *Triceratium* and *Aulacodiscus*. A short analysis in each case will provide insight into the variations possible.

Coscinodiscus. Pervalvar axis is isopolar, with an infinite number of isopolar transvalvar axes. The cell referred to the valvar plane (Figure 1b) is mirror-similar, and each of the innumerable median planes divides the frustule in two mirror symmetrical halves. All axes are straight.

Triceratium (a three-process form). The pervalvar axis is isopolar. There are three heteropolar transverse axes from the processes to the center of the

opposite side. The frustule is mirror-similar to the valvar plane, and to each of the meridian planes is mirror-symmetrical. All axes are straight.

Aulacodiscus (a form with five processes). The pervalvar axis is isopolar, and there are five isopolar transverse axes from the processes through the center to the opposite side. The frustule is diagonally-similar to the valvar plane and for each of the five median planes is mirror-symmetrical. All axes are straight.

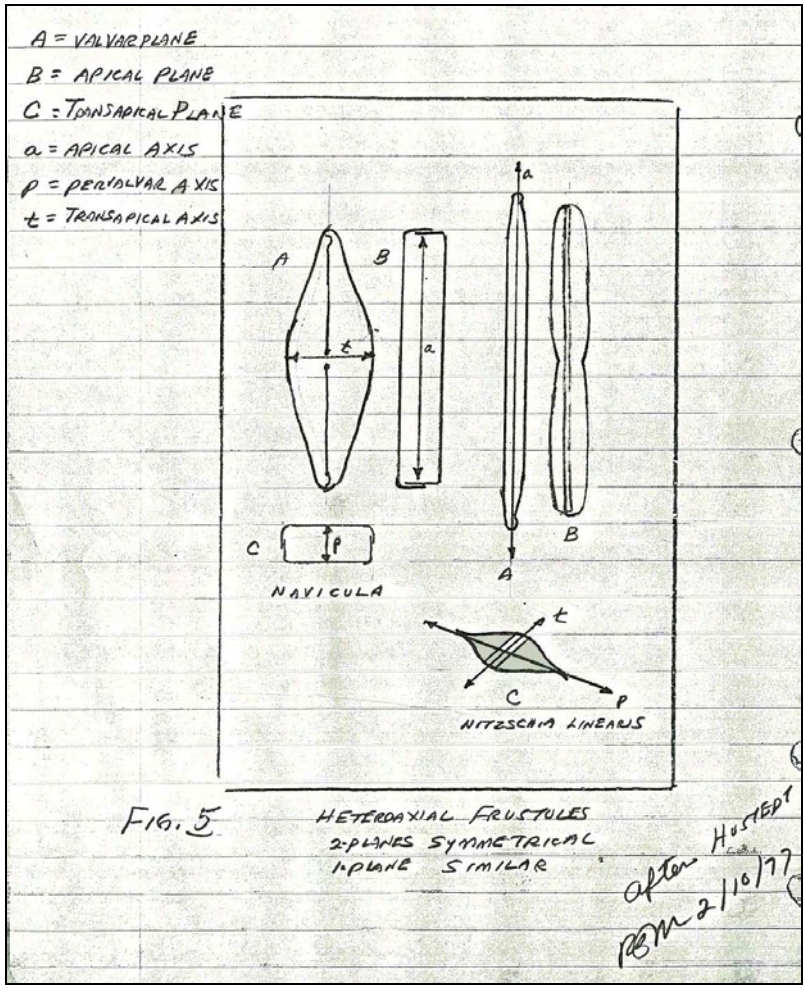


Friedrich Hustedt: Die Kieselalgen Teil I (Figs. 10, 11, & 12)

Figure 4.

ii. Heteroaxial Frustules

Examples of this type-form are divided into two different cases. The first case is that in which the frustule is symmetrical in two planes and similar in one. Two generic types illustrate this (Figure 5).



Friedrich Hustedt: Die Kieselalgen Teil I (Figs. 13 & 14)

Figure 5.

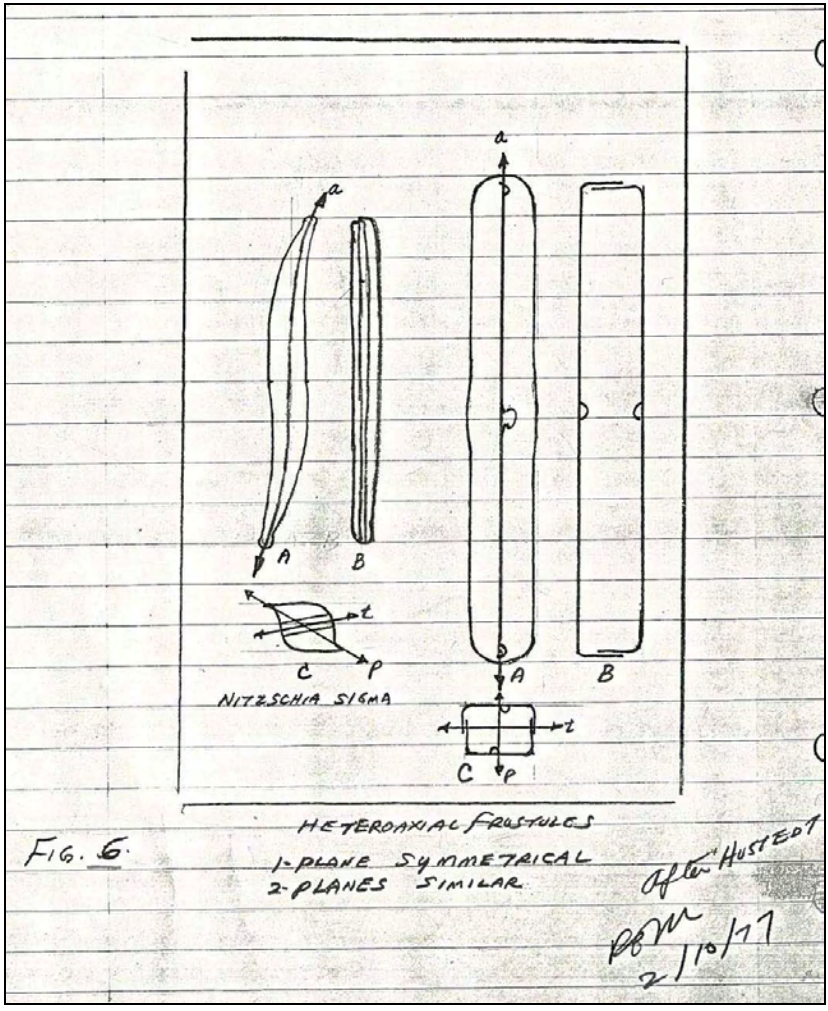
Navicula linearis (form with a straight raphe). Three-straight isopolar axes. The frustule to the valvar plane is mirror-similar, and to the apical and transapical planes is mirror-symmetrical.

The second case is that in which the frustule is symmetrical to one plane and similar in two (Figure 6). Two examples of this are:

Nitzschia sigma. Three isopolar axes, of which the apical axis is not straight but S-shaped. Both other axes are straight. The frustule to the valvar plane is anti-similar, for the epitheca is a similar mirror-image of the hypotheca, but is twisted by 180 degrees. To the apical and transapical plane the frustule is anti-similar or anti-symmetrical, for in both cases one half is always the mirror-image of the other rotated 180 degrees. The reason for this

relationship in this species is the curvature of the apical axis. This causes the keel to curve in a concave direction on one side and a convex direction on the other side.

Pinnularia viridis. Three straight isopolar axes. The frustule is diagonal-similar to the valvar plane and apical plane, because the raphe system on each cell-half is oppositely oriented to the other in relation to the apical axis. The frustule is mirror-symmetrical to the transapical plane.

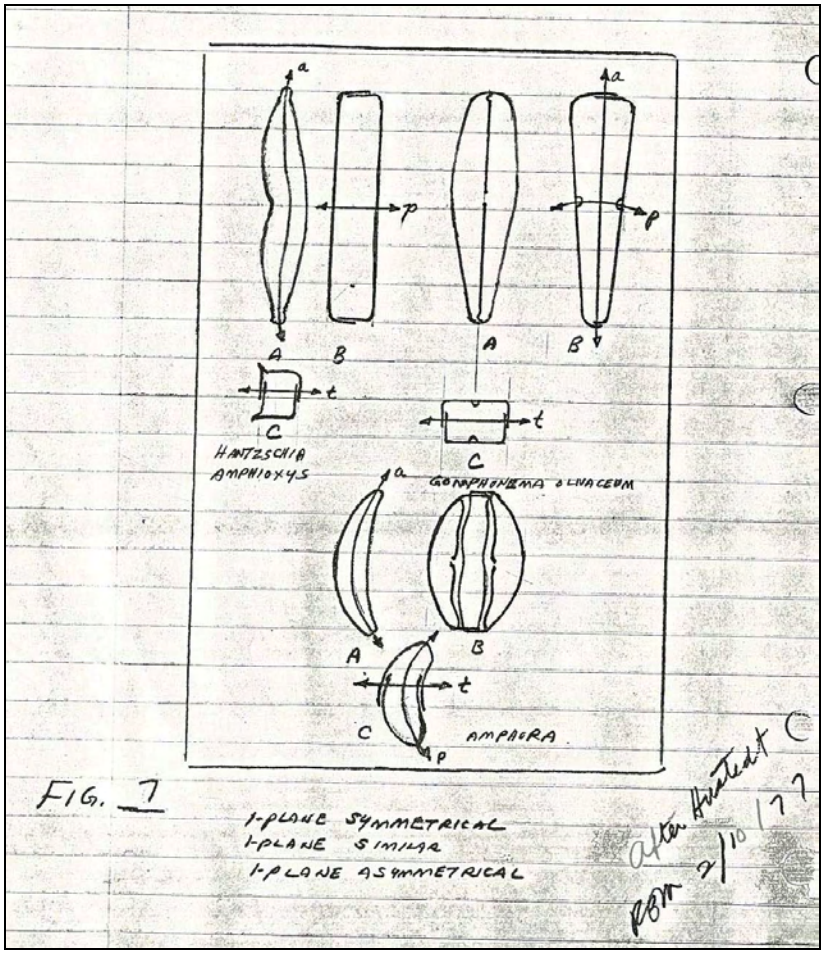


Friedrich Hustedt: Die Kieselalgen Teil I (Figs. 15, & 16)

Figure 6.

1.3.2. Plane-Symmetrical Frustules (Figure 7)

Examples of this group are found in the genera *Hantzschia*, *Gomphonema*, *Amphora*, *Achnanthes* and *Gomphocymbella*. These include several types of symmetrical relationships.



Friedrich Hustedt: Die Kieselalgen Teil I (Figs. 17, 18, & 19)

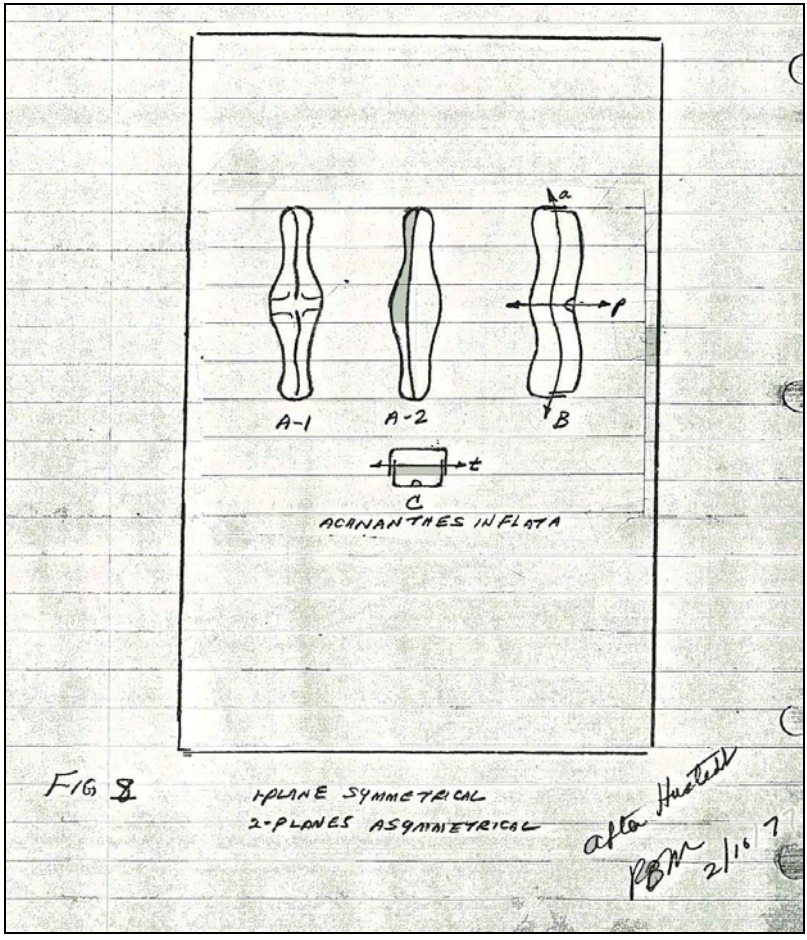
Figure 7.

The first case is that the frustule is to one plane symmetrical, one plane similar, and one plane asymmetrical. Three examples of this case are:

Hantzschia. Three isopolar axes. The pervalvar and transapical axes are straight, and the apical axis is curved. The frustule is mirror-similar to the valvar plane, to the transapical plane mirror-symmetrical, and to the apical plane asymmetrical. The curved apical plane divides the frustule into ventral and dorsal halves.

Gomphonema olivaceum. The pervalvar and transapical axes are isopolar, and the apical axis is heteropolar. The apical and transapical axes are straight, while the pervalvar axis is curved. The frustule is mirror-similar to the valvar plane, to the apical plane mirror-symmetric, and to the transapical plane asymmetric.

Amphora. The pervalvar and apical axes are curved and isopolar. The transapical axis is heteropolar and straight. The frustule is mirror-symmetrical to the valvar plane, to the transapical plane mirror-symmetrical, and to the apical plane asymmetric.



Friedrich Hustedt: Die Kieselalgen Teil I (Fig. 20)

Figure 8.

The second case is when the frustule is symmetrical to one plane and asymmetrical to two. An example of this case is:

Achnanthes inflata (Figure 8). The pervalvar axis (p) is heteropolar, and the apical (a) and transapical (t) axes isopolar. The pervalvar (p) and transapical (t) axes are straight and the apical axis (a) is undulate crooked. The frustule to the apical and valvar planes is asymmetric, as the pseudoraphe of one valve is eccentrically displaced, while the raphe on the other valve (A1) runs with the apical plane.

Pseudoraphe:
Latin. *pseudo - a counterfeit, a sham. Greek. rhaphe - a seam.*
A line of thickened silica, apex to apex, which is not a raphe but acts as a dividing line between the opposing transverse striae.

The third case is when the frustule is similar to one plane and asymmetrical to two.

Gomphocymbella (Figure 9). The pervalvar axis is isopolar, while the apical and transapical axes are heteropolar. The pervalvar and apical axes are curved and the transapical axis straight. The frustule is mirror-similar to the valvar plane (A), and to the apical (B) and transapical (B) planes is asymmetric.

1.4. Girdle Bands

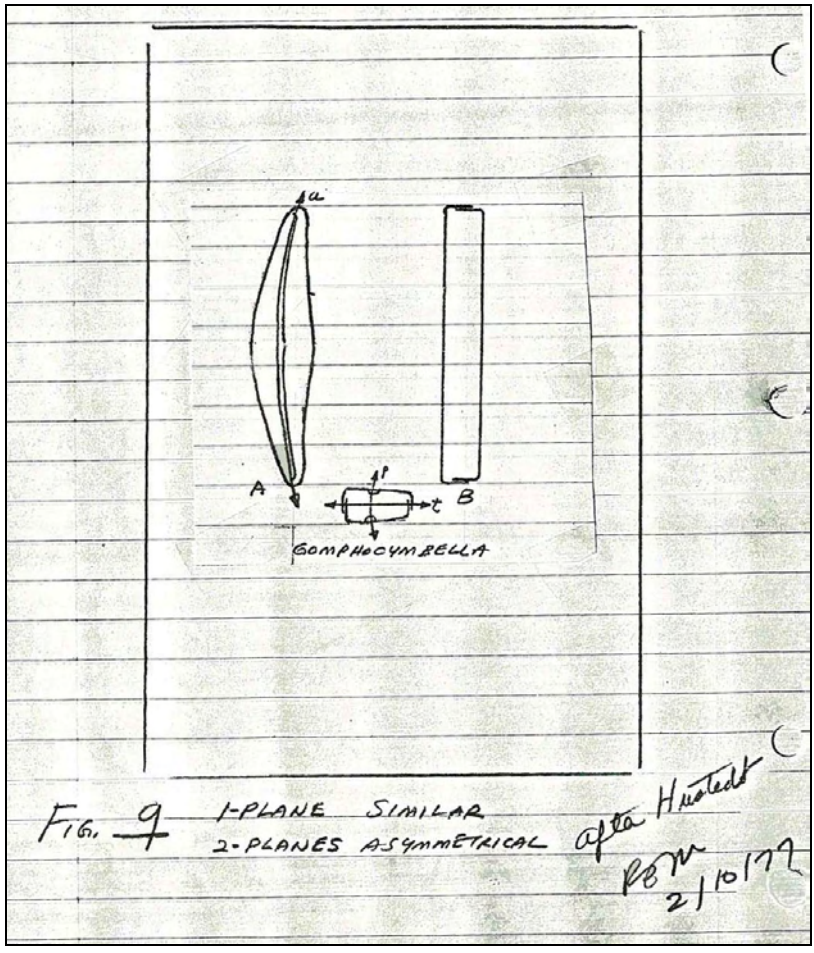
These bands are of silica and variously termed the hoop, girdle, zone, or connective, in reference to their part in uniting the two halves of the frustule and delineating a natural dividing point for it.

Hyaline:
Latin. *hyalus - glass.*
A region of thickened silica. Often bearing no features but not necessarily so.

They are usually closed bands with parallel edges, and hyaline in appearance. However, diatom girdle bands are sometimes sculptured or decorated to a minor degree. In some species of *Pinnularia* for instance, the connecting bands are vertically marked (in the girdle-view) with many fine lines for most of their length. Other markings on the girdle are often similar to those upon the valve, but usually are larger or at least more elongated. Some girdles exhibit finely punctate patterns.

The width of the frustule girdle (both girdle bands) varies considerably. In the genera *Synedra* and *Nitzschia* it is very narrow, and in *Navicula* it is rarely wider than the width of a valve. However, in *Biddulphia* it may become wider than the diameter or width of the valve. In some genera (*Rhizosolenia* etc.) the girdle width is so great as to cause the diatoms to almost never be seen in valve-view. In these latter cases the girdle is usually complex in structure, consisting of a number of bands or plates. A simple girdle, or zone, is considered to be that which is composed of only two girdle bands each of which is associated with its respective valve. Even in this simple girdle structure there is some variation, as in *Hemidiscus cuneiformis* the girdles are wedge-shaped, and in a number of diatoms in the genera *Coscinodiscus* the girdles have a groove or cut (sometimes diagonally) which leaves those ends free and able to flex. When this type of construction is seen, the “cuts” invariably are not coincident from band to band, but offset laterally from one another.

Although not cemented or solidly connected with the valve mantle, the girdle band may be very finely serrated and in some circular forms at least, fit into a stepped-edge or groove in the valve mantle, thus assuming a more or less firm position. When living, the diatom contents with its associated plasma membrane, and the usual exuded gelatinous covering of the frustule serve to assist in providing a strong but somewhat flexible connection between the parts of the frustule. When diatoms are cleaned for study and these binding constituents are thereby removed, the connecting bands may become detached from the valves.



Friedrich Hustedt: Die Kieselalgen Teil I (Fig. 21)

Figure 9.

1.5. Intercalary Bands

When the girdle of a diatom is designated as complex, it indicates additional girdle bands, and markings and structure associated with them. These additional bands are

located between the valve mantle and the girdle band proper, and are called intercalary bands. They may number from one to many. In *Rhabdonema* for instance, there may be 30 or more such bands forming the girdle. In some diatoms intercalary bands are difficult to distinguish with the light microscope, and for that reason may be more common than indicated in the literature.

Intercalary Bands, Intercalary Rings - *In combination. Latin. inter - between. Latin. calare - to proclaim. plus Middle English. band.*
These are bands of silica that sit between the valve and the girdle band or between girdle bands. They may or may not exist and there may be a variable number. Where they fit with the valve they sometimes form a septum.

lntercalary bands appear to be connected to the valve mantle and the girdle by a series of folds, horn-like processes, or knife-like serrated edges. Again, as mentioned previously, the plasma membrane internally, and the outer mucilage covering of the frustule, probably in most cases, keep these complex structures together in the living state. This assumption however does not offer a complete explanation of the way these structures are kept intact, as even when cleaned vigorously, some of the most complex structures hold together. The means by which this part of diatom structure is maintained is very imperfectly known.

Hendey (1964) indicates that diatom cells are capable of considerable growth upon the pervalvar axis by increasing the width of the girdles, taking place presumably by an extension of their edges. Patrick and Reimer (*The Diatoms of the United States* 1966, 1975) state that the number of intercalary bands would seem to indicate the age of certain frustules, as in young cells they may be less numerous than in older ones. The actual mechanism by which such construction is accomplished is not known. Mostly, the intercalary bands are of a fairly constant number regardless of age.

Norman Ingram Hendey (1903-2004)

Dr. Ruth Patrick b. Topeka, Kansas 1907
Charles W. Reimer b. 14th May 1923 d. 29th November 2008

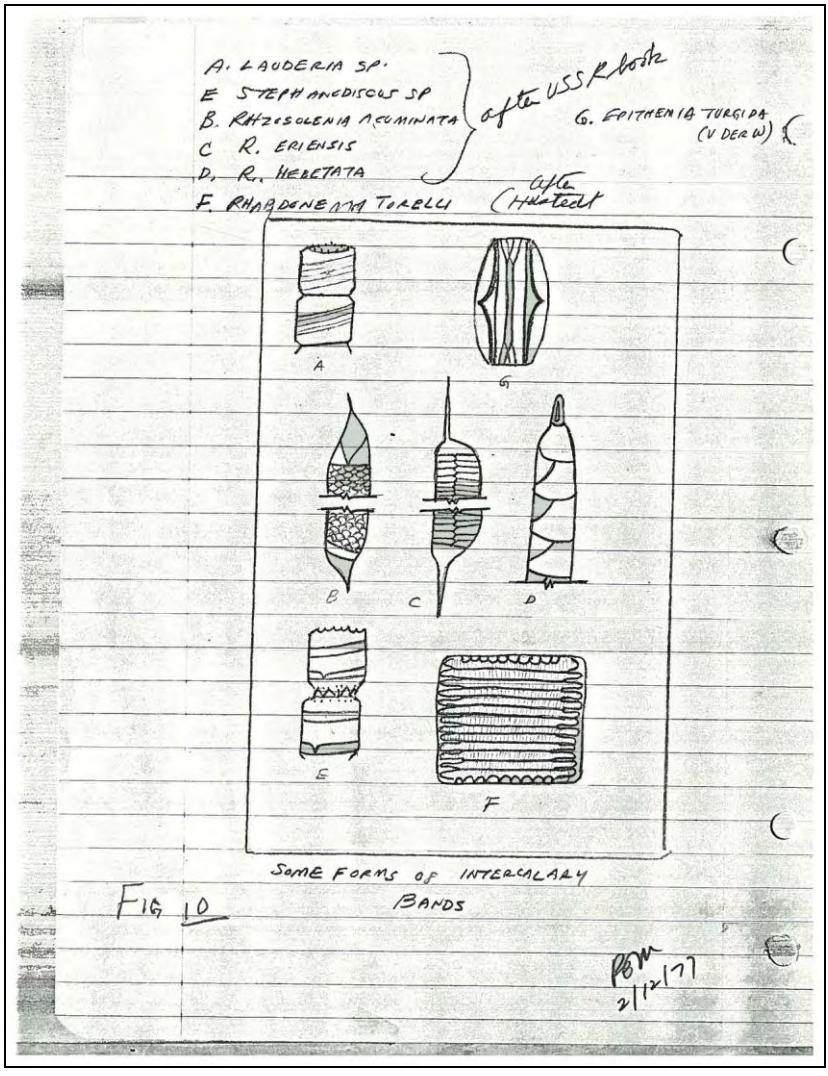


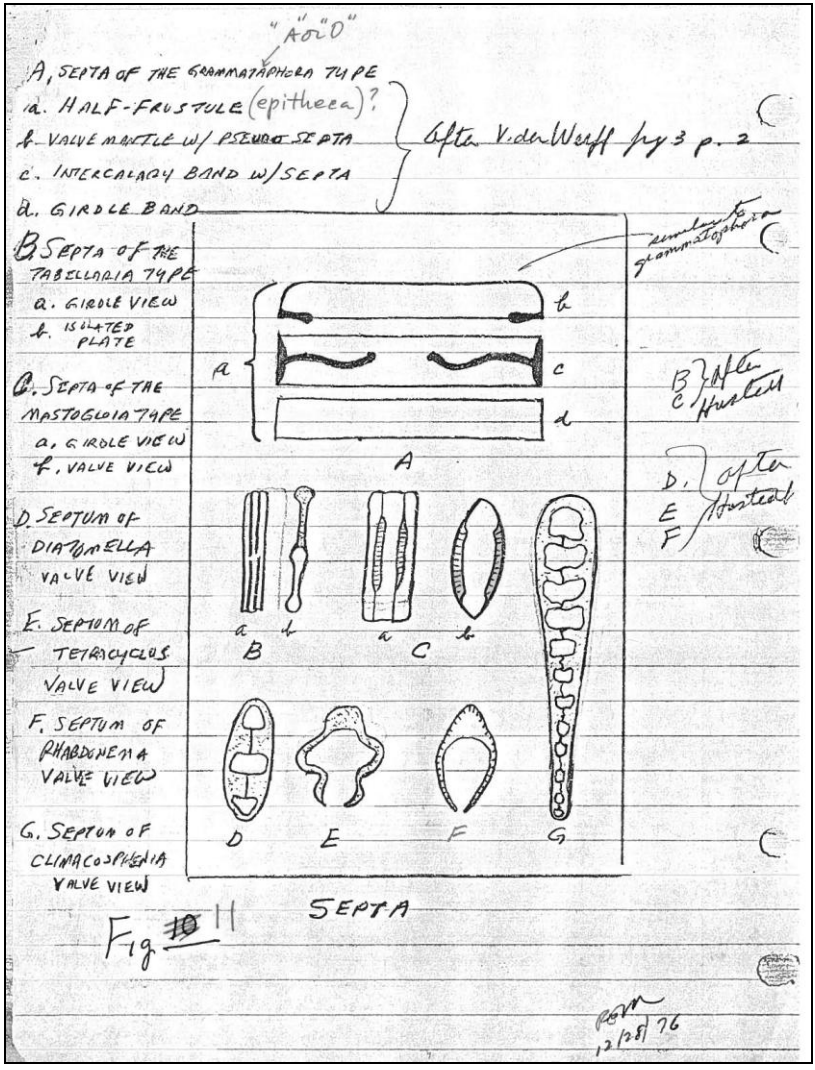
Figure 10.

These bands take on a number of different forms and configurations. Some are hyaline, and identical or nearly so, with the girdle bands. Others are marked, and of quite different form and construction. They may be continuous bands, open-ended bands, and/or make various forms of connection with preceding or succeeding ones. They may spiral around the frustule or be composed of plates or scales of a size relatively large or small compared with the frustule. In the genus *Rhizosolenia* in particular, the latter arrangement is common (Figure 10). Hendy believes the imbricated arrangement of these tile-like plates compensates for the weakly siliceous nature of such cells. He indicates the high production rate of these species makes a high demand on the available silica in sea-water, and that a structure of this type imparts

Imbricate –
Latin. imbrex - a tile.
Overlapping.

stability and elasticity to withstand the stresses and strains of ocean action. At the same time it overcomes the silica deficiency of a cell having so large a surface area and so great a length-to-width ratio.

In some series of intercalary bands the first and last are unbroken, but between them are broken (interrupted) bands with a narrow projecting tongue or wedge in the middle of the bands opposite the break, which fits into or under the interruption in the adjoining band.



Most of these figures: Friedrich Hustedt: Die Kieselalgen Teil I (Fig. 25)

Figure 11.

1.6 Septa (Figure 11)

Intercalary bands often grow inwardly to form a plate-like structure known as a septum. Not all intercalary bands do this, but when they do, the various types of plate structures or septa formed are characteristic, to a degree, of a particular genus of diatom. One or more of these septa may occur in one frustule, sometimes 20, 30, or more in such genera as *Rhabdonema*, for instance.

Septa(e) (pl.), Septum (sing.) -
Latin. septum - an enclosure, a hedge, fence, barrier, or wall.
A piece of silica that projects from a girdle band into the cell thereby dividing the cell into compartments.

The septa may develop from the apices of the valve only, or they may develop from the sides only, or even extend completely across the frustule. They are always perforated in the latter case with large holes (Figure 11), and in most cases, are more open than closed, surface wise. In some cases they develop only in about one half of the valve, and in the *Tabellaria* type extend from alternate apices from band to band (Figure 11B). It will be noted that the intercalary band here is continuous, but the septum extends only about halfway the length of the frustule. In *Tetracyclus* (Figure 11E) the septa developed is more at the ends than at the sides, but the structure is opened at one end. Other forms of septa are illustrated in Figure 11. In Figure 11A the *Grammatophora* type of septa is illustrated in an apical (or sagittal) section. Note here that the valve mantle itself has produced slightly into the interior of the frustule a pseudo-septum at each end. This view indicates a general characteristic thickening of the valve/intercalary band material at the point of extension.

The surface of the septa may be smooth, irregular, undulating and/or perforated. In some cases, thickened siliceous ribs may develop and be attached to the valve itself, forming chambers between the valve and septum. In *Mastogloia* (Figure 11C) these ribs are marginal only, but in *Epithemia* and *Denticula* they extend completely across the valve (Figure 12).

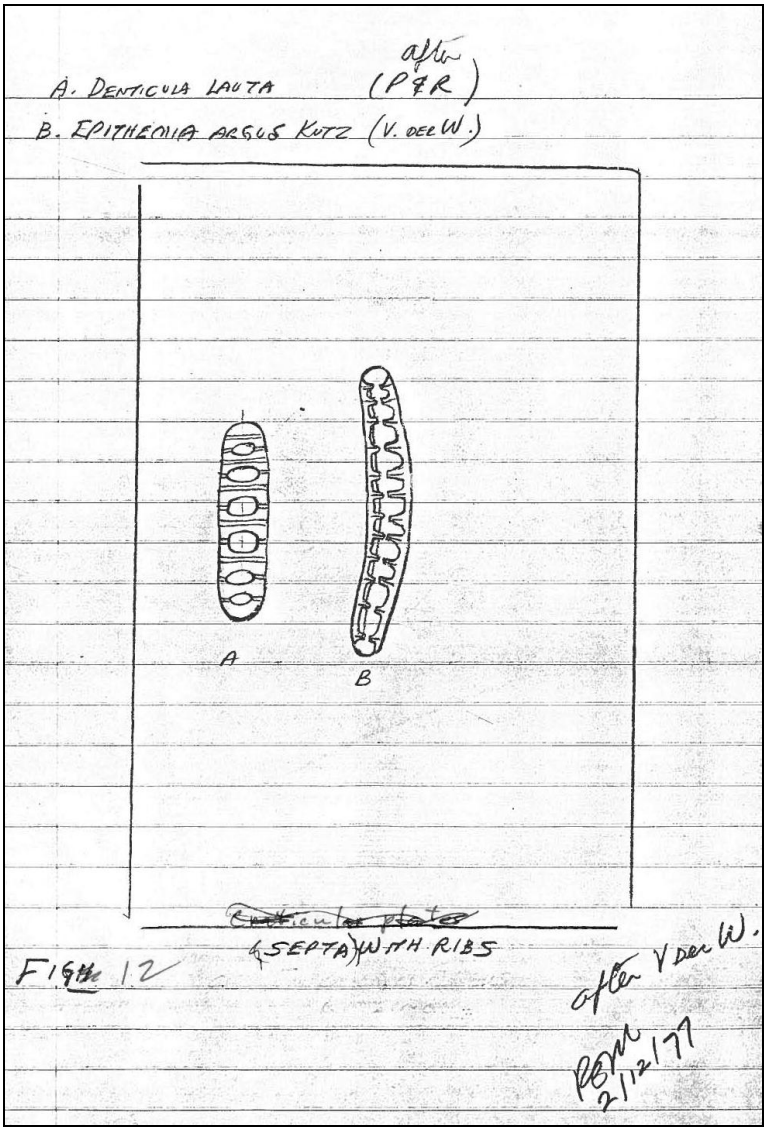
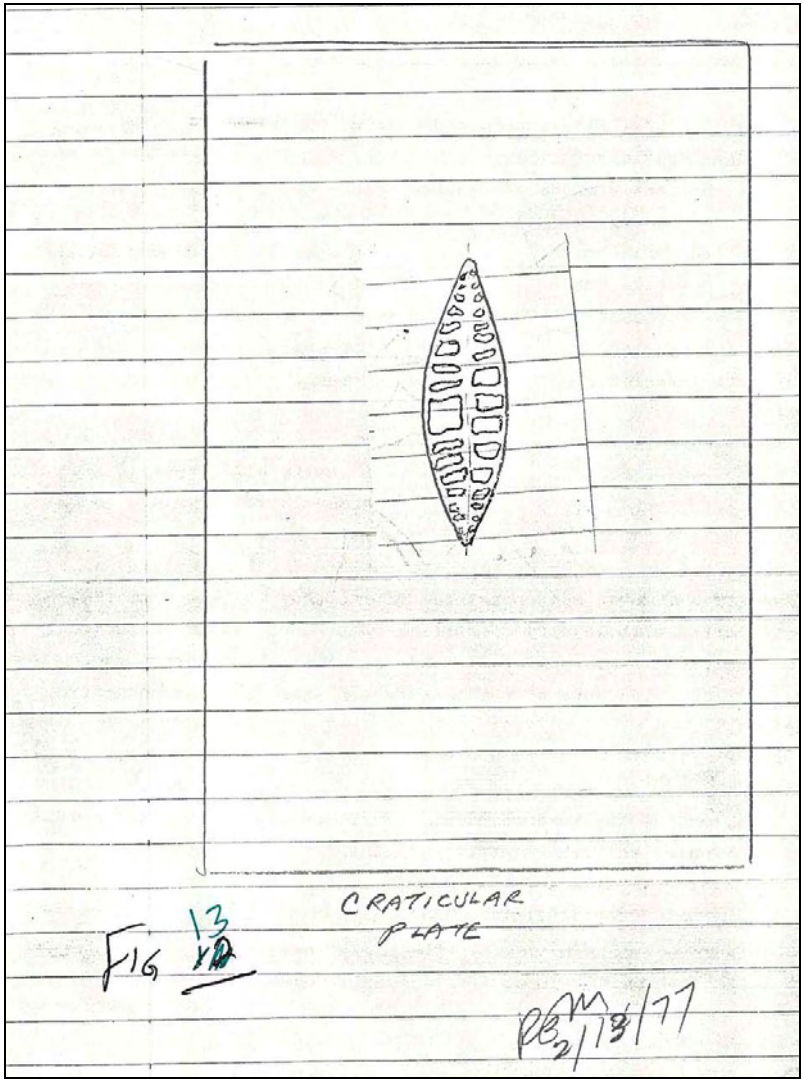

A. DENTICULA LAUTA (after P&R)
B. EPITHEMIA ARGUS KUTZ (V. DER W.)
A
B
4 SEPTA WITH RIBS
after V. Der W.
PGM 21/2/77

Figure 12.



Friedrich Hustedt: Die Kieselalgen Teil I (Fig. 26)

Figure 13.

1.7. Craticular Plates (Figure 13)

Diatoms sometimes contain internal structures termed craticular plates. They have the outline of the valve and consist of a strongly siliceous median rib and less strongly silicified transverse ribs. The diatom which is most commonly associated with the term is probably *Navicula cuspidata*, as such plates are often seen with it. Craticular plates also have been found with *Eunotia* spp. and *Meridion*

Craticular - *Latin:Greek. Krater - a mixing bowl.* Cup-shaped. Also thick silica bars that are normally cup or mug-shaped though not necessarily formed like a cup.

circulare. Sometimes the plate is not flat and parallel to the valve, but curved or bent. These curious structures, at one time (ca. 1868) prompted the establishment of a separate genus, *Craticula*. However subsequent investigations by diatomists discredited that concept. A craticular plate as occurring with *Navicula cuspidata* is illustrated in Figure 13. The function of such plates is not understood and there have been various opinions expressed over many years regarding it. Cleve, for instance, considered these formations in *Navicula* to be abnormalities or monstrosities. Pfitzer considered them to be analogous to a double valve, and some at one time considered the craticular "state" to be a defense against change of salinity in the water. Mann found occurrences of the craticular plates with *Navicula cuspidata* to vary with environment.

Per Theodor Cleve
(1840 - 1905)

Ernst Hugo Heinrich Pfitzer
(1846 - 1906)

Dr. Albert Mann
(1853 - 1935)

1.8. *Liostephania*

Payne (*Liostephania and Its Allies*,1922) believed that diatoms described as belonging to the "genus" *Liostephania* were actually the internal plates of other diatoms, particularly the genus *Asterolampra*. The greatest number of these "diatoms" have been found in the Eocene deposits of Barbados, and have also occurred in the cores of Miocene age taken from the equatorial Pacific. Early researchers considered them variously as craticular plates, inner valves, and in the past they were included under the generic names of *Actinogonium* and *Dictyolampra* as well as *Liostephania*.

Frederick William Payne
(1852 – 1927)

Hanna and Brigger (1970) consider these objects as being formed by ordinary precipitation of silica in cavities where diatoms have been, and also in the interiors of complete frustules. Upon examination and manipulation by mechanical finger, they are found to be solid discs, either polygonal with rounded corners, or circular. They are very limited in geographical distribution, do not occur often, and at present do not fall well into botanical nomenclatural systems, They are still largely fossils of uncertain origin and value.

G Dallas Hanna
(b. 24th April 1887 Carlisle (Arkansas) d. 20th November 1970 California)

Albert Leon Brigger
(1892-1981)

Other casts of diatom interiors, or of complete frustules, have been found and reported on for many years. For instance, pyritized diatoms from Galveston, Texas were reported on in a meeting of the New York Microscopical Society in 1892. The casting material, being what it was, created no illusions as to its origin and method of formation. However, casts of deposited material which is essentially the same as that of the diatom frustule, are not nearly as obvious in their origins. This factor has prolonged and delayed the current, and no doubt proper, interpretation of the origin of *Liostephania*.

1.9. Valve Structure

1.9.1 Size

A micron is usually represented: µm

The dimensions most commonly used in describing diatom size are the length and width of the bilateral diatom valve in valve view, and the diameter of circular diatoms in valve view. The unit of measure is the micrometer (10^{-6}m) or one millionth of a meter. In older texts the term "micron" is used to express the same unit.

The length is a measure of the distance from one apex (pole) to the other, and the width is a measure of the valve at the widest point. Diatoms of complex outline are sometimes dimension designated at particular points. The dimensions do not ordinarily include the lengths of any processes or extensions such as spines, awns and other structures appended to the valve. In much of the literature the length of awns, spines and other processes are not given. When known, they should be. In all diatoms it is of value to know the dimension along the pervalvar axis. Far too few authors provide any information of this nature.

The largest diatoms are found among such genera as *Coscinodiscus*, *Thalassiothrix*, *Rhizosolenia*, and *Isthmia*. Among the largest are *Coscinodiscus rex*, *Thalassiothrix longissima*, and *T. antarctica* which in the largest dimensions are more than 3000 micrometers (3 millimeters). These are very large indeed when a diatom of 500 micrometers diameter is ordinarily considered very large.

Albert Collier
Alice Murphy

The smallest diatoms are usually found among the *Achnanthes*, *Fragilaria*, *Navicula*, and *Melosira*. Many species of these genera are 20 micrometers or less in their largest dimension. Collier and Murphy (1962) have reported observing diatoms as small as 0.75 micrometer in diameter from the Gulf of Mexico. One of the minute diatoms they reported, a new species, *Chaetoceros galvestonensis*, has a frustule of only slightly greater length than 4 micrometers.

Linear, Lineate - *Latin. linearis - pertaining to or consisting of lines.* Long and Narrow with parallel sides.

Cuneiform - *Latin. cuneus - a wedge.* Having a wedge shape.

1.9.2.Shape

Cymbiform - *Latin. cymbalum - the hollow of a vessel.* Margins unequally curved in opposite directions.

The valve view of a diatom is the usual shape described in diatom literature. As most diatoms are seen in valve view, this is an important recognition feature. Most diatoms in the girdle view appear rectangular, and exhibit few differentiating features.

Terminology associated with the shapes generally originates with geometric figures and variations of them, or is based on fancied similarity with objects of special shapes such as the lance-head (lanceolate), the ear (auricular), and the kidney (reniform), etc. Figure 2 illustrates some of the more common shape terms used in the literature.

Classifications, to be discussed later, in some broad cases, are based on general shape such as the pennate and centric divisions. Hendey (1964) has, out of the immense diversity of form, distinguished shape-groups designated as (1) linear, (2) cuneiform, (3) cymbiform, (4) carinoid, (5) discoid, (6) gonoid, and (7) solenoid. He has tabulated certain characteristics that are common to specific shape-groups, not necessarily in accord with strict taxonomic rules, but to assist in perception and discussion of various genera.

Carinoid, Carinate - *Latin. carina - a keel.* Possessing a keel.

Discoid - *Greek. discos* Like a disc.

Gonioid - *Greek. gonia - an angle.* Angled.

Solenoid - *Greek. solen - a pipe.* A cylindrical coil.

Of considerable importance in the identification of diatoms is the contour of the valve surface. This feature is perhaps most important in the centric diatoms where often the valve markings are not as distinctive as in the pennates. Where valve markings are similar and difficult to differentiate, the contour of the valve surface may be determinative of a species.

The contour of the valve surface may be determined by careful microscopical examination in successive focusing planes, or it may be very clear in a girdle view of the diatom valve. In circular diatoms the variations in the valve surface are often in concentrically higher and lower zones with from one to several alternations or undulations. It is not unusual for the hypovalve inflations and depressions to be oppositely phased with those of the epivalve. In some diatoms this is often characteristic of a species. In other cases of circular diatoms the surface may be flat, convex, or concave to a more or less marked degree. Figure 14 illustrates some of the surface variations in circular diatoms from a girdle view. The various shapes shown are representative only and not all-inclusive of the variations that occur.

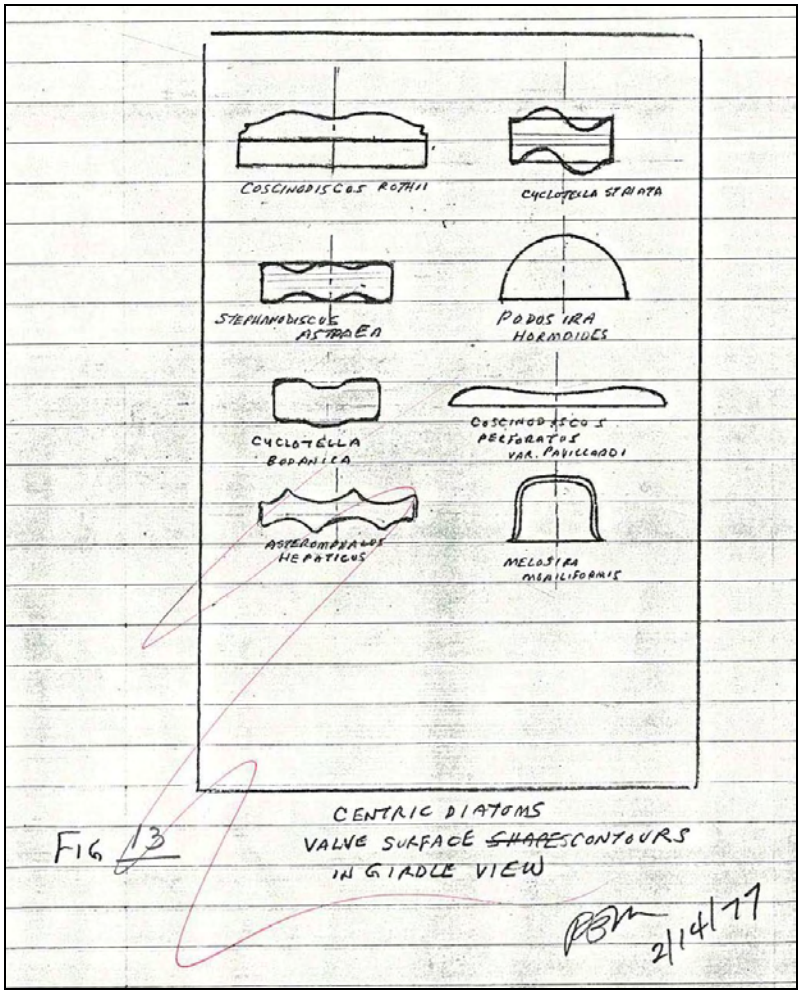


Figure 14.

The pennate diatom valves are more often than not flat, smoothly curved, and less likely to exhibit undulatory surfaces. Outstanding exceptions to this are the genera *Campylodiscus* and *Cymatopleura*, which show strong curvatures and undulations of the valve surfaces.

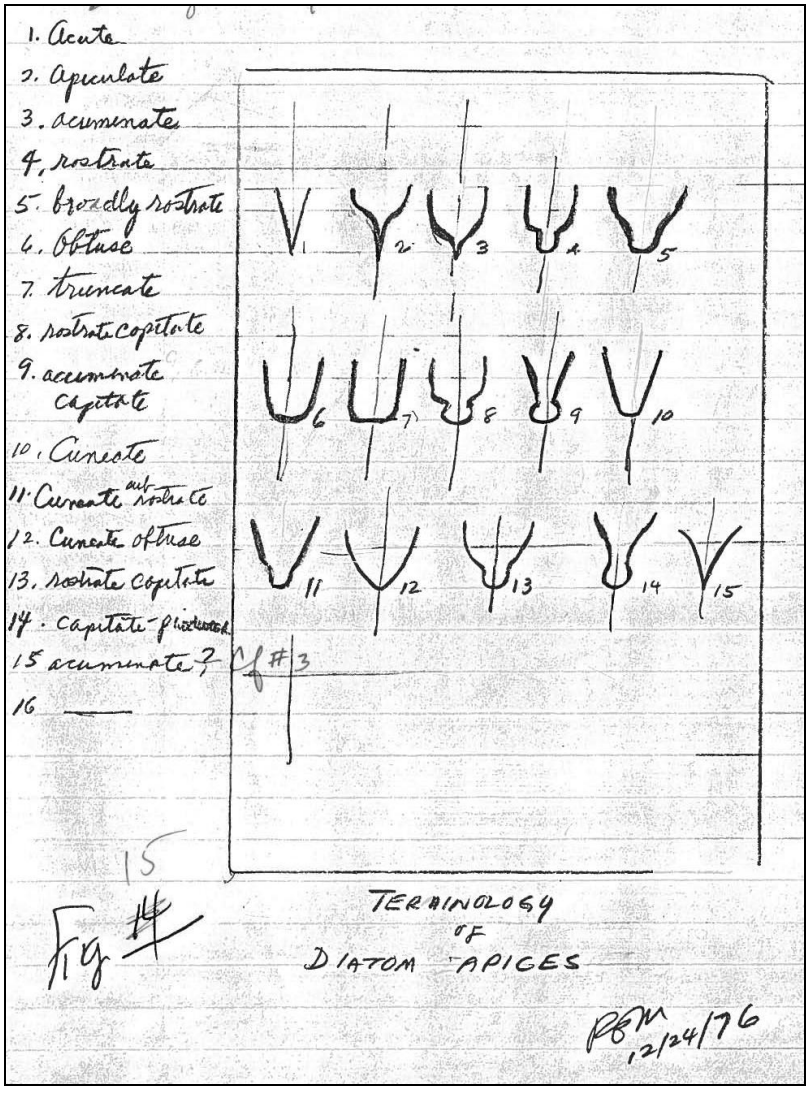


Figure 15.

Another very important aspect of shape or outline, is that of the apices of the bilateral diatoms in valve view. As in the general outline, the shapes of the ends are described with a special, but not standard, terminology. Examples of that terminology, with the shapes of the ends they describe are illustrated in Figure 15. Unfortunately not all authors use exactly the same terms to describe the various shapes of the extremities. However, the illustrations do cover most of the fundamental shapes encountered, and will be of assistance in making identification from the literature in comparison with what is observed with the microscope.

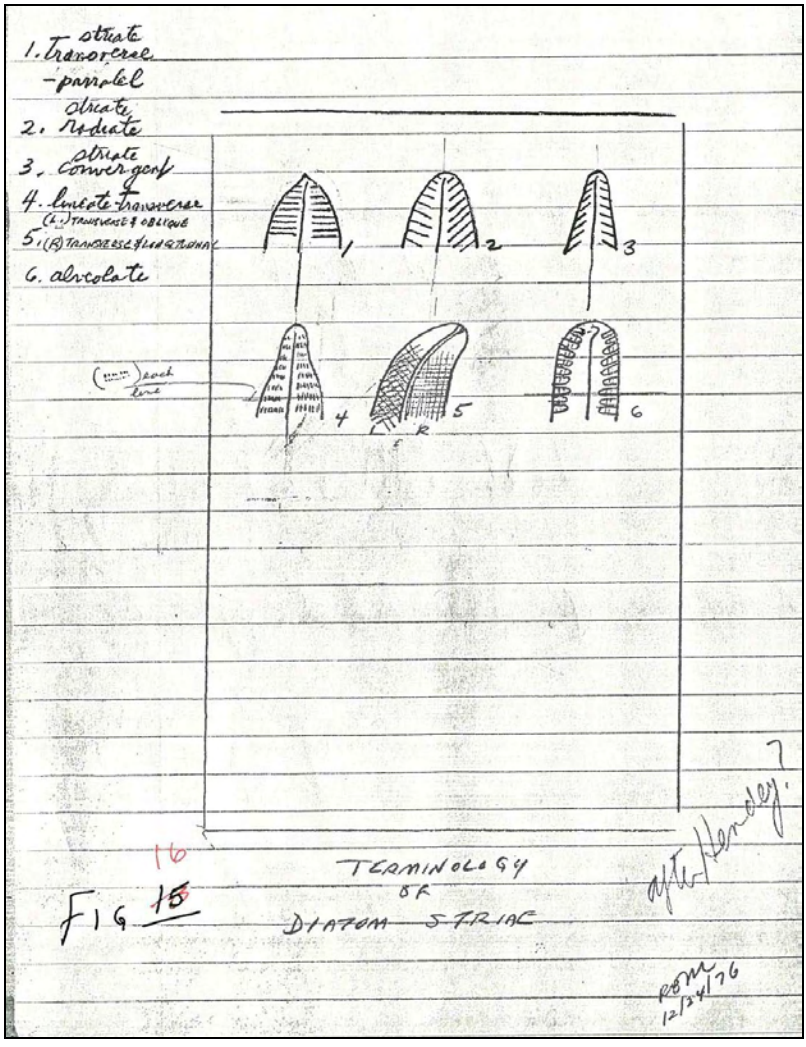
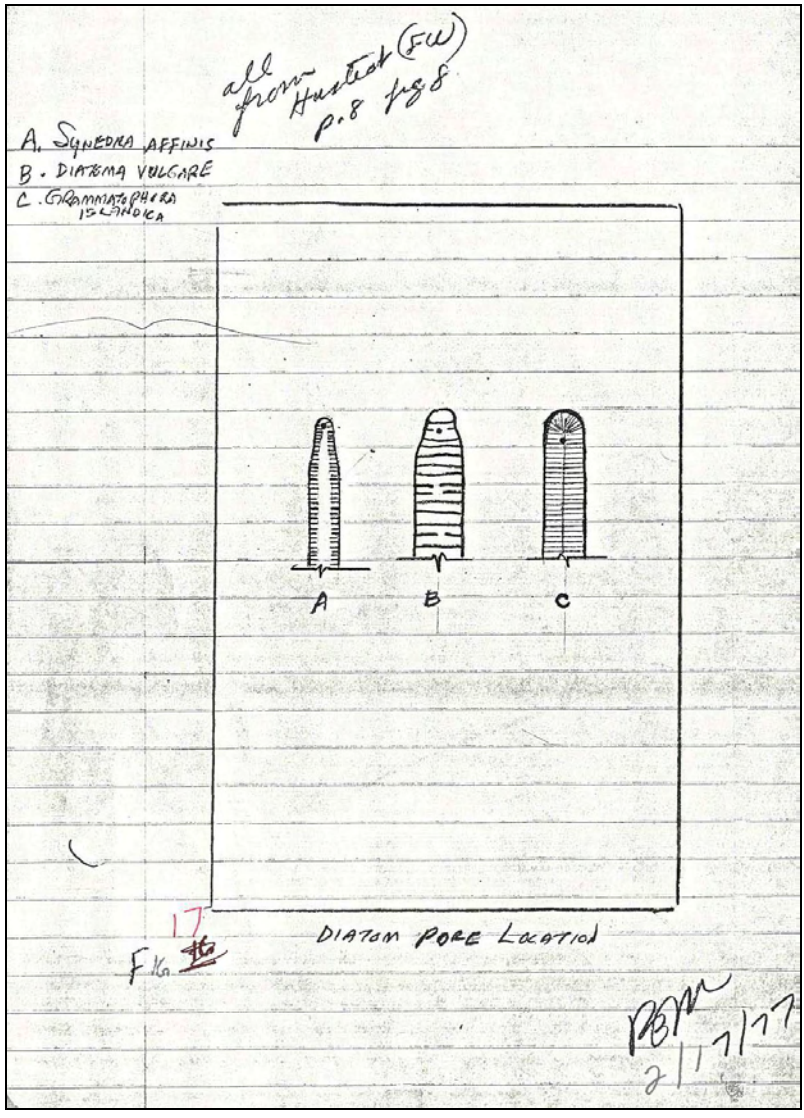

1. striate transverse - parallel
2. striate undulate
3. striate convergent
4. lineate transverse
(L) TRANSVERSE & OBLIQUE
5. (R) TRANSVERSE & LONGITUDINAL
6. alveolate
(.....) areolae
1
2
3
4
5
6
L
R
FIG 16
TERMINOLOGY OF DIATOM STRIAE
12/3/76

Figure 16.



Friedrich Hustedt: Die Kieselalgen Teil I (Fig. 38)

Figure 17.

1.9.3. Striae, Punctae, and Pores. (Figures 16 and 17)

Striae (pl.), Stria (sing.)
Latin. stria - a furrow, groove or channel.
A line of pores, punctae, spots or dots.

With the light microscope, appearances of fine lines in various spacings and orientations on the valve surface (especially in the pennate diatoms) are manifestations of very closely spaced holes or pores. When their size and spacing is such that, at the particular magnification and resolution capability used, they cannot be separated optically, they appear as lines or striae (Figure 16).

When they can be resolved and separated, the pores (holes) appear as small dot-like markings termed puncta. They may be arranged in regular or irregular, straight, curved, transverse, radiating, or convergent lines on the valve surface.

Puncta are actually very small (usually circular) simple openings in the cell wall. Hendey indicates the size limits of such openings are fairly narrow, ranging from 0.5 to 1.0 micrometer in diameter, with most at the lower limit.

Punctae (pl.), Puncta (pl. & sin.), Punctum (sing.) -
Latin punctum; punctus - a prick, small hole or puncture. a point or a dot made in a waxen tablet as the sign of a vote.
Spots or dots on the valve and girdle bands.

In the diatom literature, pores are referred to variously as dots, beads, pearls, depressions, and perforations. Some of these terms were used at a time when the actual nature of the pore was not understood. The term puncta (punctum singular) has survived and is in use in modern description for the pattern arrangements probably because it describes the light microscope appearance better than any other (the term pore is preferably reserved to describe specialized openings in the valve). Characteristically, regardless of other terms used, the electron microscope has established that they are perforations, holes, or pores, rather than thickenings or depressions or other sculpture as the terms “beads” and “pearls” convey.

In some diatoms there are apparent specialized pores (Figure 17) that secrete both a thick and thin mucilage. Patrick and Reimer term these “jelly pores” and Hustedt “Gallertporen”. The pores are strategically located on the valve evidently to perform one of two functions. The pore that secretes a thick gelatinous substance to form a pad for attachment to a substrate or to another diatom is generally located close to one of the apices of the valve (Figure 17). Other pores more distant from the apices usually secrete a thinner material which covers the valve surface. *Grammatophora* as an example, has a specialized pore, near the base of a spine, located at the end of the valve, which secretes a thick substance (Figure 17c).

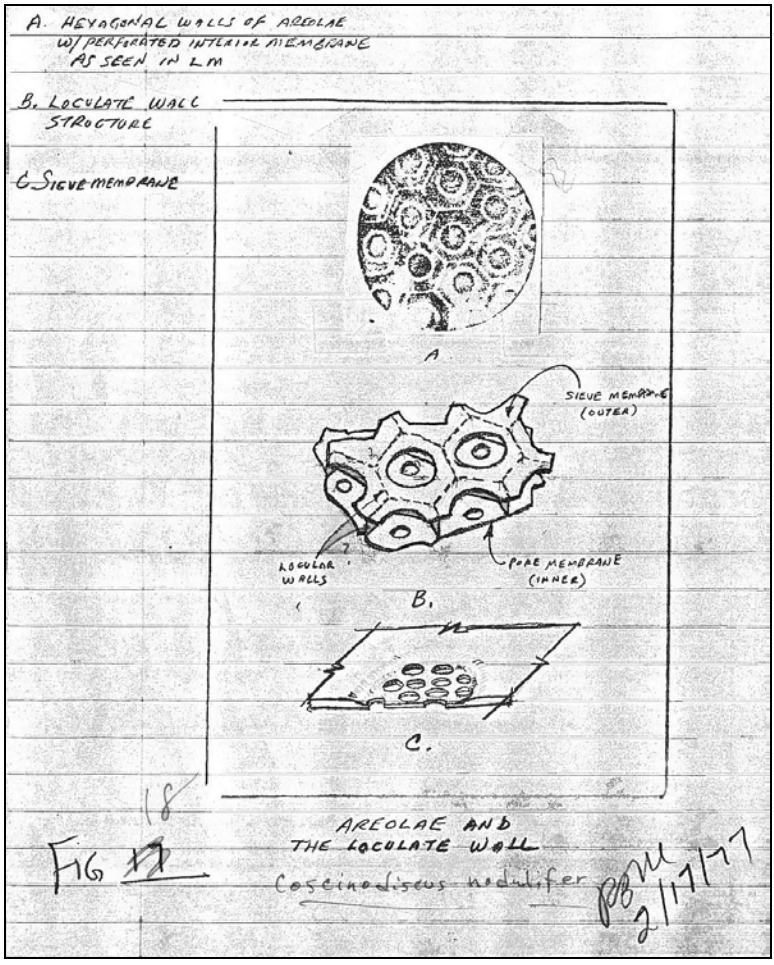


Figure 18.

1.9.4. Areolae (Figure 18)

Aereolae (pl.), Aereola (sing.)
also Areolae (pl.), Areola (sing.), Areoles
-
Latin. areola - a small open space.
The name given to the regular perforations seen in the valve or girdle. Usually hexagonal or polygonal.

These are larger structures than puncta or pores and are usually of a higher degree of complexity. They are in the form of a depressed box or chamber, or a box-like structure within the cell wall of the valve, having perpendicular sides much like the cells of a honeycomb. They may be wholly or partly closed upon either their inner or outer surfaces by membranes, usually perforate or multiperforate. Many of the outer membranes especially, are very delicate and subject to corrosion and destruction, which may account for the fact that they are often not reported in older descriptions of species now known to be so equipped. Either natural corrosion or cleaning processes may completely destroy the extremely delicate structure. Perforations in these outer membranes are often very

fine, their diameters or lesser dimension being in the vicinity of 0.2 to 0.3 micrometer. These finer details of areolae are sometimes difficult to see with the light microscope excepting at high magnification and resolution capability - then with difficulty. The electron microscope has initially revealed, or confirmed, much of this type of structure in recent years.

The areolae in shape may be hexagonal (as in many *Coscinodiscus*), sub-circular (*Isthmia*), elliptical or elongated (*Pinnularia*) or irregular. although areolae is a term used by a number of authors to describe elongated chambers in the *Pinnularia*, Hendey recommends using alveolus to more correctly indicate an elongated channel-like structure. Alveolus is a Latin word meaning “trough” and is far more suitable a term in the case of the *Pinnularia*. The alveolae of *Pinnularia* spp. usually have openings at the ends of the “trough” rather than holes or perforations in the bottom. The openings in the trough ends contribute to the impression of a set of double-lines near the edge of the *Pinnularia* valve.

1.9.5. Canaliculi

These are narrow, tubular channels through the valve wall or extension of the valve wall. They are found only in a few genera, and in particular occur in the alae of *Surirella* spp., connecting the raphe with the interior of the cell. Hendey points out that the elongated areolae or alveolae of *Pinnularia* are sometimes erroneously referred to as canaliculi, a practice which should be abandoned. (Refer to Figure 18).

Canaliculi (pl.), Canaliculus (sing.) - *Latin. canalis - a water pipe.* Small furrow or channel.

Ala(e), Aloe - *Latin. ala - a wing.* A wing. An extension to the valve that forms a flange.

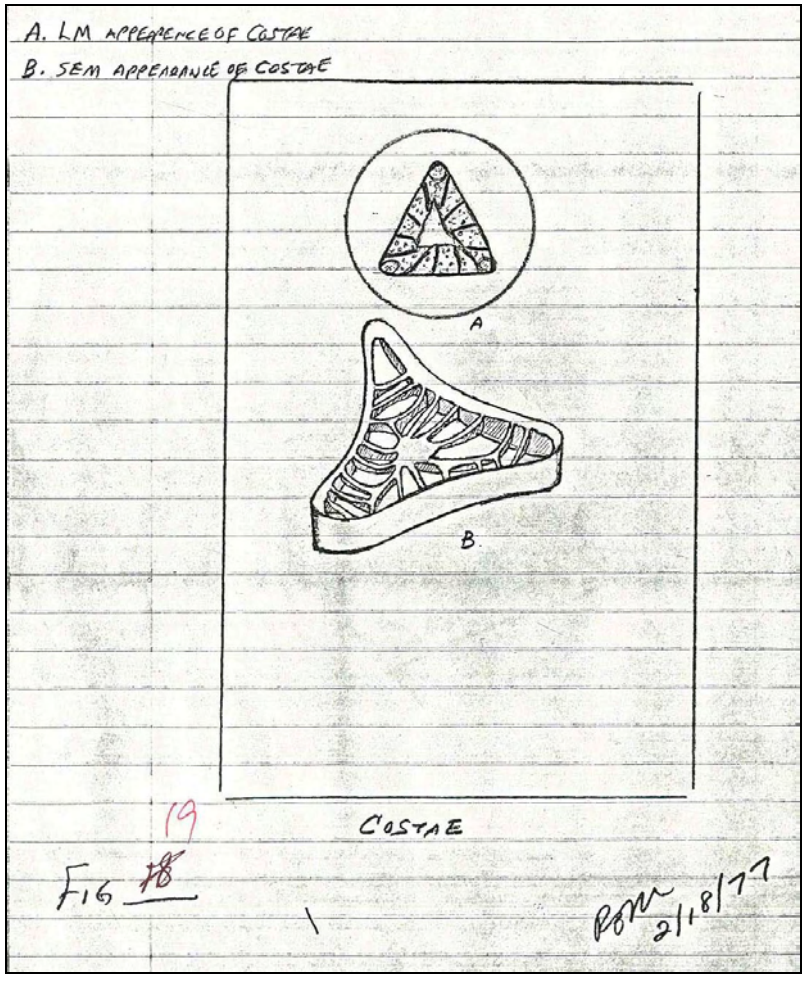


Figure 19.

1.9.6 Costae (Figure 19)

Costae (pl.), Costa (sing.) -
Latin. costa - a rib, a side, a wall.
Siliceous thickenings in the valve. Usually appearing as double lines. Most often appearing towards the margins. Sometimes ribs.

Ribs or solid units of structure formed by heavy deposition of silica, and used to strengthen the valve structure are called costae. This term also has had its abuses, being used to describe areolae, alveolae (*Pinnularia*) and other channel-like appearances. In the early days of diatom study, some structures were imperfectly understood and the term costae used quite indiscriminately. Figure 19 illustrates costae as the term is properly interpreted. The drawing (Figure 19b) has been made from a scanning electron microscope (SEM) micrograph taken of a triangular-shaped diatom, with other details omitted to emphasize the costate structure. The view is from the underside of the valve and shows the costae joined to the valve mantle and extending to a more or less central point.

Other types of diatoms exhibit similar costae, although they may not result in a framework such as in this example; perhaps only being in the form of individual ribs, transverse to the apical axis of a bilateral diatom for instance. Costae often are pierced by openings of various shapes and relative sizes. In the light microscope (LM) ribs or costae usually appear as heavy black lines in brightfield illumination.

1.9.7. Cell Walls

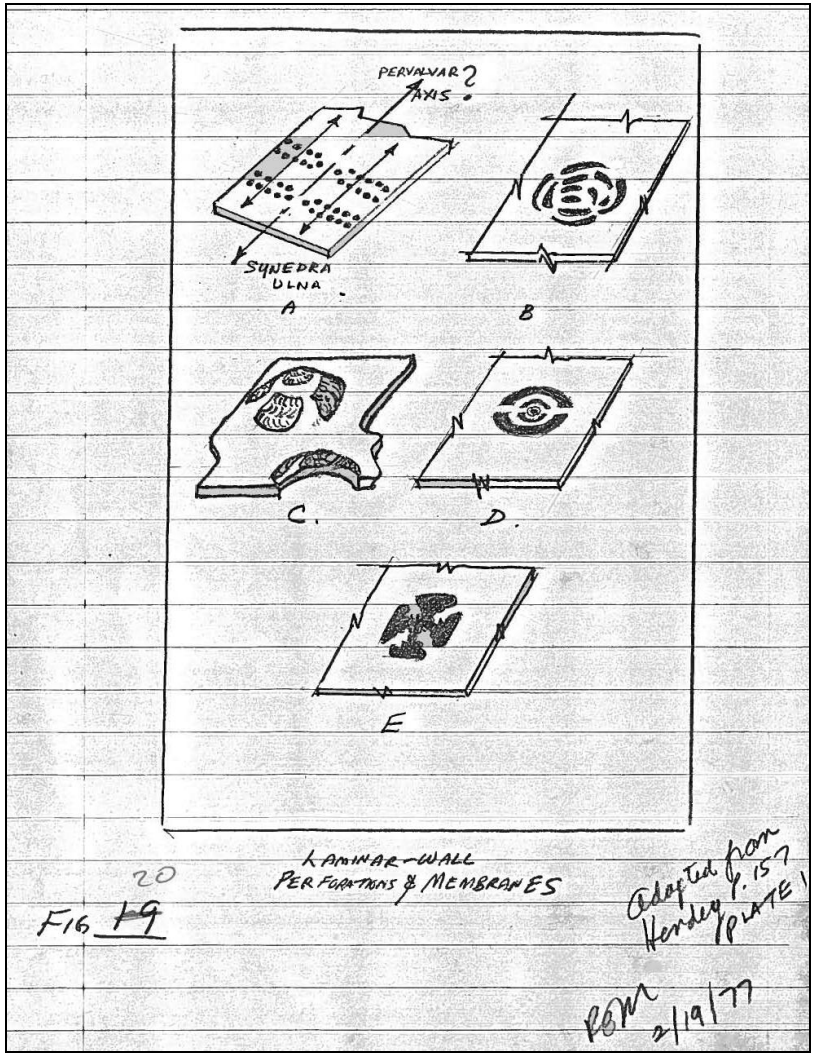


Figure 20.

1.9.7.1. Single-layer Cell Wall (Laminar type) (Figure 20)

From extensive studies with the electron microscope Hendey (1959) has been able to differentiate two different basic types of cell well construction in diatoms.

The cell-wall in the first type is a single-layer of silica through which simple pores or pore canals extend. This cell wall is termed laminar and may be either very thin or relatively thick, the latter sometimes supporting a complex arrangement of pore canals as exist in *Triceratium plano-concavum*. Patrick and Reimer describe this particular feature as three pore canals originating in a wart-like structure on the valve surface of *T. plano-concavum* which join together to form a main canal.

The pore (puncta) openings are modified in a number of ways by various types of silica layers (membranes) extending across them.

According to Hendey (1959) they may be:

(1) Multiperforate in rows as in *Synedra ulna* (Figure 20A).
(2) Reticulate as in *Achnanthes longipes* (Figure 20B).
(3) Incised and compound as in *Cocconeis stauroformis* (Figure 20C).
(4) Of plate-like marginal outgrowths as in *Rhaphoneis amphiceros* (Figure 20D).
(5) Dendriform marginal outgrowths as in *Didymosphenia geminata* (Figure 20E).

In the foregoing listed example species, the valve is a single stratum with or without costal thickenings.

1.9.7.2. Two-layer Cell Wall (Loculate type)

A double layer of silica separated by vertical walls forming a chamber network. In some diatoms these chambers are exceedingly small. For instance, Patrick and Reimer cite *Pleurosigma* and *Gyrosigma* as good examples. The minute chambers are opened to the exterior by one or two slit-like-pores and to the interior by circular ones, the combination in the microscope appearing as a minute sieve. It is the arrangement of these areolae which produces the three directional lines in *Pleurosigma* and the transverse and longitudinal striae in *Gyrosigma* when viewed with the LM. The slits in *P. angulatum* may be only about 0.6 micrometer in length and perhaps less than a tenth of a micrometer in width. The hole in the other silica layer is about 0.4 micrometer in diameter. Individual chambers possibly average about 0.5 micrometer in diameter.

A much larger chambered structure that illustrates the loculate wall very well is found in the genus *Coscinodiscus*. Figure 18 illustrates various aspects of the wall construction for the species *Coscinodiscus nodulifer*. Figure 18C shows the type covering on the cell wall exterior. It is a thin extension of the outer membrane and is termed the sieve membrane. The stratum that is interior is perforated in each locular chamber by a hole of about 0.7 micrometer diameter. The sieve membrane holes are less than half this, being only about 0.3 micrometer in diameter. The areolae upper opening (covered by the sieve membrane) is approximately 2.5 to 3.0 micrometers in diameter. Estimates from SEM micrographs of the thickness of the sieve

membrane are in the vicinity of about 0.1 micrometer; making this a very delicate structure subject to damage by natural corrosion and by the cleaning process. The vertical walls of the areolae in *C. nodulifer* are mostly in the shape of a hexagon, approximately 3.0 micrometers thick and separate the two strata about 2.5 micrometers. Where the corners (rather junctions) of the hexagons are, there is a slightly thickened area on the upper (outside) sieve membrane. In the light microscope these thickenings appear as lumens or points of brighter area at six points around the opening. Because the sieve membrane, when present, is very thin and its perforations are near the resolution limit of the LM, it is not easily seen. Also because of the relative thickness and height of the areolae walls, the appearance in the LM is as in Figure 18A where their outline (hexagons) and the opening in the lower pore membrane, is all the structure evident, at least on casual examination.

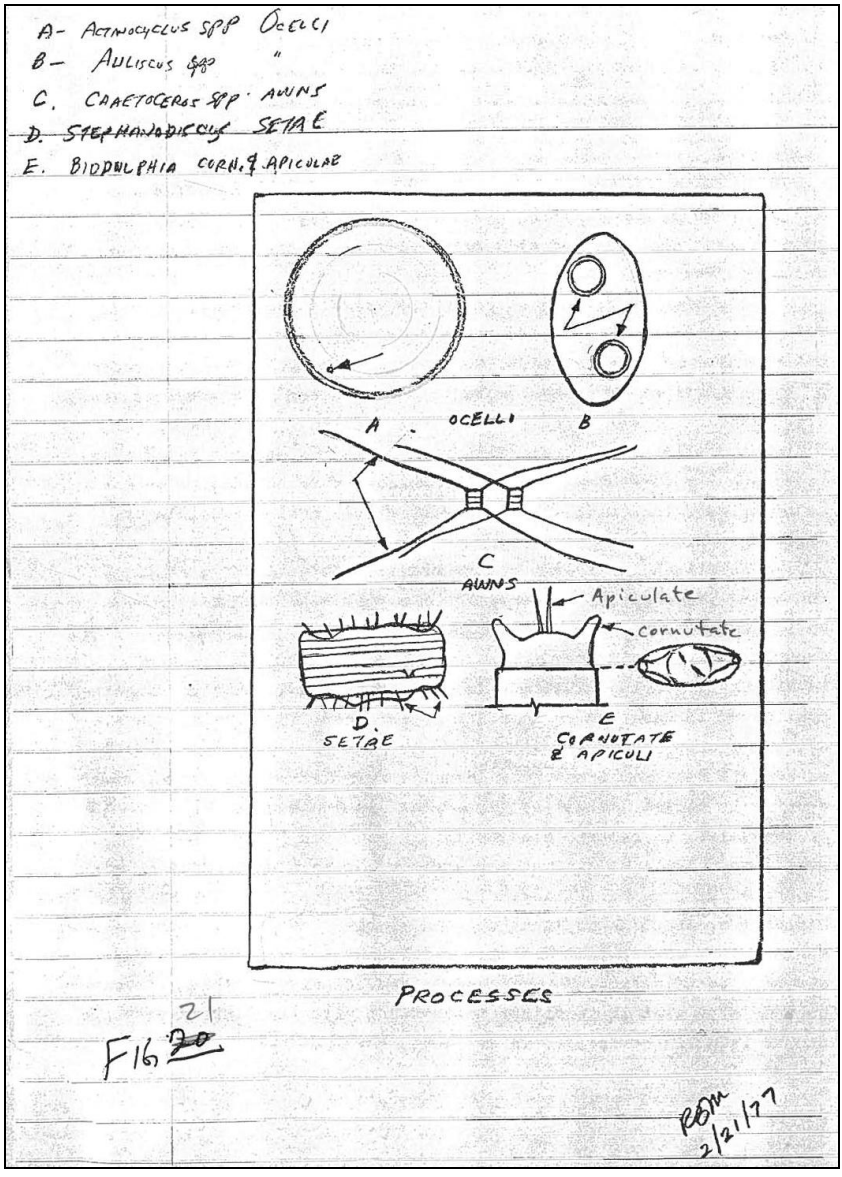


Figure 21.

1.9.8. Processes (Figure 21)

The valve surface of diatoms is often modified by extensions and outgrowths of various sizes, lengths, and shapes. The word process is used to describe them in general. However, many of them are of definite and distinctive forms and can therefore be designated with specific adjectives. There is really no standardized terminology for them, but some of the more common encountered in the literature are:

Cornutate: A short horn-like outgrowth arising from the apices or angles of a valve. The tops or upper surfaces of such processes are often perforated with many small pores. The genera *Biddulphia* and *Triceratium* both offer excellent examples of this type of process. (Figure 21E)

Boss: A process as above, somewhat shorter and stouter, and one which is in the angles of a valve. A specific example are these that are present in *Triceratium arcticum*.

Apiculi: Processes which are smaller than the above, usually very slender, and which may have open ends. Examples are found in *Biddulphia* spp. (Figure 21E), and specifically represented by *Skeletonema costatum*.

Spinnulae: These are very small processes, slender, and closed at the ends.

Awns: Very much elongated, and they may be hollow for much of their length. The term is used extensively in descriptions of the genus *Chaetoceros*. (Figure 21C)

Setae: Similar to the above, awn, but much shorter, more hair- or bristle-like, less robust, more like spines. Usually located at the periphery of centric diatom valves as in *Stephanodiscus* spp. (Figure 21D)

Ocelli: Short hyaline, rather flattened processes, almost button-like in appearance. They range from barely discernable and small, as in *Actinocyclus* spp., to rather large flattened and very obvious "eyes" in *Auliscus*, in which they are variously termed as nodules, pseudoocelli, and pseudonodules. (Figures 21A and 21B)

Mastoid: An adjective which describes a large circular and mounded process. Sometimes used to describe the ocelli of *Auliscus* spp.

Cylindrical, oval, bulbous, etc.: Processes of *Aulacodiscus* spp. are quite varied in shape and generally rise considerably above the valve surface, are hyaline and assume pear shapes, etc. that are normally pierced with circular channels or tubes communicating with the valve interior. There are usually three or more disposed symmetrically about the center, at the valve margin.

There are so many different types and versions of processes, it would be impossible to completely describe all of them. However, their principal function is to maintain contact between contiguous diatom frustules or to a common substrate and so assist in colony formation. They accomplish this in one or both of two methods. One is by adhesion furnished by gelatinous stalks or pads to stones, algae or other foreign bodies, or to adjoining frustules. The other is by mechanical linkages to other frustules whose processes are so designed as to furnish connection of an enmeshed nature or by hooked or curved extremities. This latter method is no doubt aided in most, if not all cases by the exudation of adhesive mucus from the ends and/or along the surfaces of such interconnecting appendages. Their shape, length, type of ends etc., are usually determinative of the type and shape of colony formation.

Location of the processes on the valve is variable. In some forms the processes are not superimposed (one valve and the other), but may alternate in position. This can be seen with the microscope by focusing first on one valve and then the other of a complete frustule. Examples of this condition are found often in species of *Aulacodiscus*, *Actinocyclus*, *Asterolampra*, *Asteromphalus*, and *Eupodiscus*.

In triangular forms and in those in which the processes lie on the major axis of the valve, the processes of the entire frustule are superimposed.

Sometimes two valves of the same diatom can bear a different number of processes. This condition has been observed and reported on by Kitton in *Aulacodiscus kittonii*, *A. africanus*, *A. oregonus*, *A. pulcher*, *A. argus*, *A. rogersii*, and *A. margaritaceus*.

The terms "rimportule" and "labiate process" have been used in recent years to describe certain SEM appearances. They are openings, usually flush, but sometimes raised circular pores, near the margin of centric diatoms, projecting into the interior of the valve, there opening by a slit, sometimes lipped, that is transverse to the radius of the valve. In centric diatoms this feature is found to be more common as SEM investigations are extended to more and more species. Genera as diverse as *Actinoptychus*, *Asterolampra*, *Roperia*, *Actinocyclus*, *Hemidiscus*, and *Coscinodiscus* contain species having these processes. Further work with the SEM in this regard may be a basis for changes in diatom classification. The dimensions and location of this microstructure is such that it is either not evident with the light microscope or has been misinterpreted in the past. For instance, the "stout-spines" or "apiculi" just inside the margin of *Coscinodiscus nodulifer* described by many early authors, are the vertical optical projection of a widened solid interstitial space between areolae, which is the top-cover portion of the rimportule continuation into the interior of the valve.

Figure 22 illustrates the marginal rimportules as they appear in SEM micrographs, both exterior and interior to the valve. The illustrations were drawn from SEM micrographs, omitting other detail to emphasize the labiate processes. There is also a nodule located near-center of the *C. nodulifer* valve. It is perforate and communicates to the interior of the valve, opening beneath the pore membrane via a lipped horn-like opening; this feature is also considered to be a labiate process.

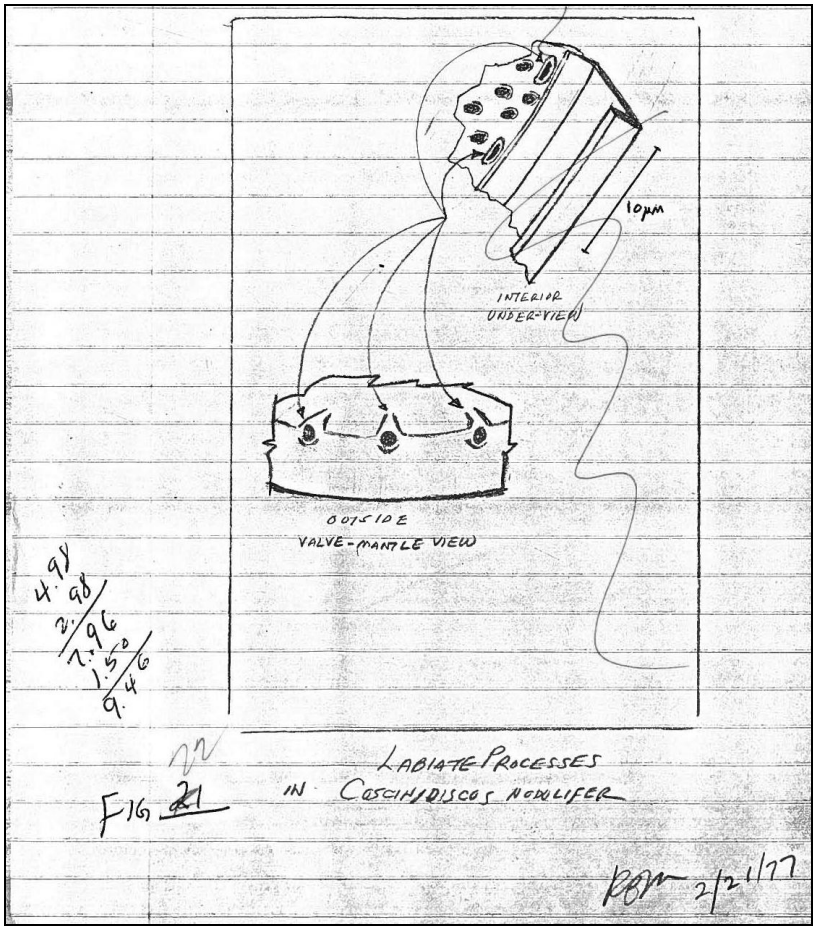


Figure 22.

The functions of many of the processes, as mentioned previously, are fairly evident, but the function or functions of the labiate processes at this time are still very much in question.

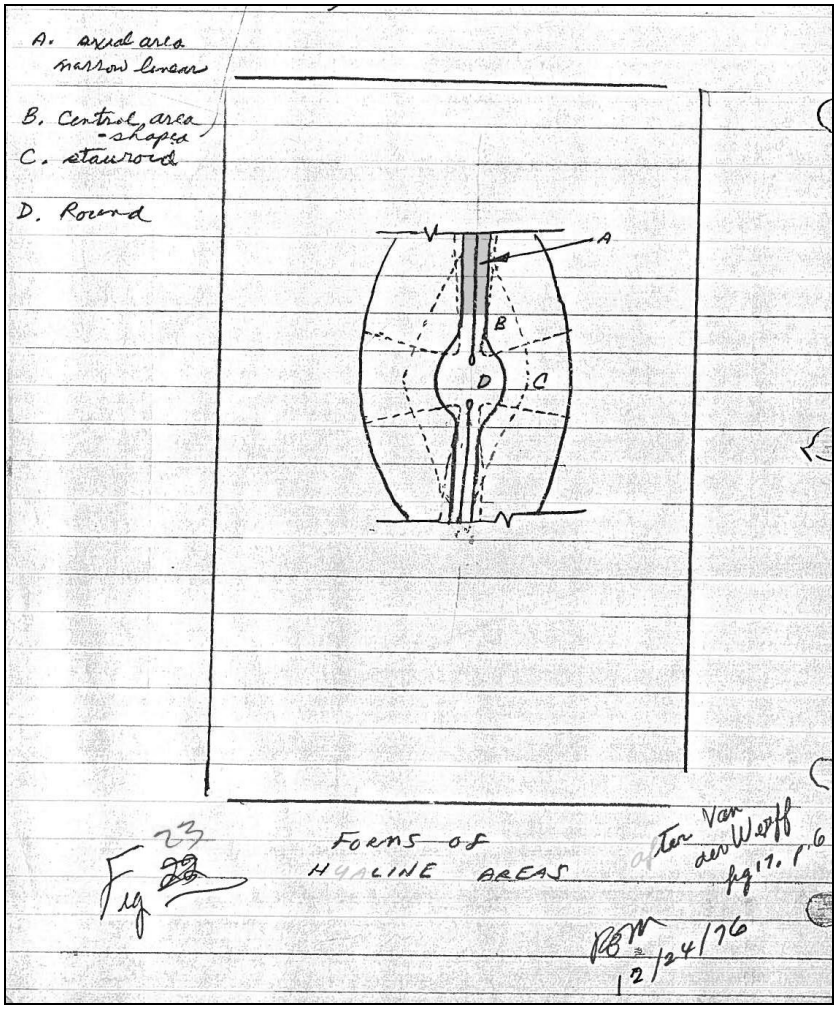


Figure 23.

1.9.9. Hyaline Areas (Figure 23)

The valves of diatoms, in addition to the structure previously covered, also exhibit hyaline areas that are localized and distinctive. In the bilateral diatoms these areas are quite important in identification and classification, and they have been assigned, on a rather non-standard basis, specific descriptive terms. There are two of major importance in diatom description and recognition.

There is usually present in the apical axis, a hyaline space called the axial area (Figure 23A). The shape of this area may be narrow-linear, broad and curved, or variously shaped. It sometimes is not completely hyaline, but may have irregular markings. Especially when a raphe is present, it may have puncta, single isolated, grouped, and in rows, on one or both sides of the raphe. The area may be thickened relative to the rest of the valve, or bordered by thickened areas or ribs situated along

both sides of the apical axis, as in *Frustulia* spp. The shape and general make-up of this area is generally constant for species and intra-specific determinations.

The central area (Figure 23B) is a special part of the axial area lying between the valve spices. It is generally outlined by the variations in lengths of the rows of puncta transverse to the apical axis. The central area may be well defined even in valves without a raphe, but generally is more variable in its outline and shape when a raphe, with its associated central nodule, is present. The central area may be square or rectangular as in *Synedra*, or vary from small circular to many different shapes. The central area may not be entirely hyaline, but sometimes contains isolated or grouped puncta or other types of markings. When it contains isolated puncta as in *Gomphonema* spp. they are sometimes referred to as the stigmae. The central area may be formed from an enlargement of the central nodule in valves with a raphe. In *Stauroneis*, the nodule is thickened and extends throughout the area, even to the valve edges, in the form of a stauros or cross-shape (Figure 23C). In other species, such as *Navicula lyra* the central area may be extended outward from the central nodule laterally and then curve longitudinally for most of the length of the valve forming a characteristic "lyre" shape. Many of the extensions, especially where the axial area is narrow, as in the *Navicula lyra* above, are thickened and thereby provide additional strength to the valve.

In the bilateral diatoms the axial hyaline area may become very narrow and straight. In diatoms in which only one valve has an actual raphe, the other valve often exhibits an axial area that is narrow and somewhat on the order of a raphe in appearance. In these diatoms that hyaline axial area is designated the pseudoraphe. The striae and other valve markings on the pseudoraphe or rapheless valve may be identical with those on the raphe valve in their distribution and relative location, or they may be quite dissimilar. The genera *Achnanthes*, *Cocconeis*, and *Rhoicosphenia* show examples of this type of structure.

In most centric diatoms there are also hyaline areas present. In some cases the hyaline area is well-defined, and because of its shape and projection above the surface of the valve, is classed with the processes. In other cases certain areas form the base from which processes arise, in the shape of a small hyaline "plateau", the edges of which are defined by the arrangement of valve pores. Some hyaline areas in centric diatoms are located near the center or at center, and are in the older literature referred to as an umbilicus. This latter term is the product of light microscope observation. Researches with the electron microscope, especially the scanning electron microscope (SEM), will do much to clarify some of the rather ambiguous and general terms assigned in the past to this type of diatom structure.

1.9.10. The Raphe

The name derives from a Greek word meaning seam. The raphe is not present in all diatoms. It is considered such an important and individualistic feature of diatom structure however that its presence or absence forms the basis, of several important classification schemes. It is a feature of a great number of genera of bilateral diatoms and in the older literature was referred to as the median line. It may be present on one or both valves, and may be observed running down the center, terminating in a

nodule at each end of the valve and interrupted at the central portion by another nodule.

Despite its deceptively simple appearance, in the light microscope, especially on superficial examination, it is a rather complex structure. The variations in its structure and terminations in the polar and central nodules are characteristic and constant enough to make it very valuable in the classification and identification of diatoms.

It is a cleft or fissure in the valve ranging in cross section from a simple slit, normal to the valve surface, to a horizontal "V", or zigzag, proceeding from the outer to inner surface of the valve. It may vary in character in its run down the length of the valve from the central nodule to the apical end. Even a simple slit may alter its angle with respect to the valve surface one or more times in that distance, or it may even change from a simple slit to a horizontal "V" and back again. Figure 24 shows how the raphe might appear in the light microscope and an accompanying cross-sectional view. It will be noted for instance, that in Figure 24C the raphe at section C-C has a zigzag form, but on both sides of this it would have a cross section like that of Figure 24B.

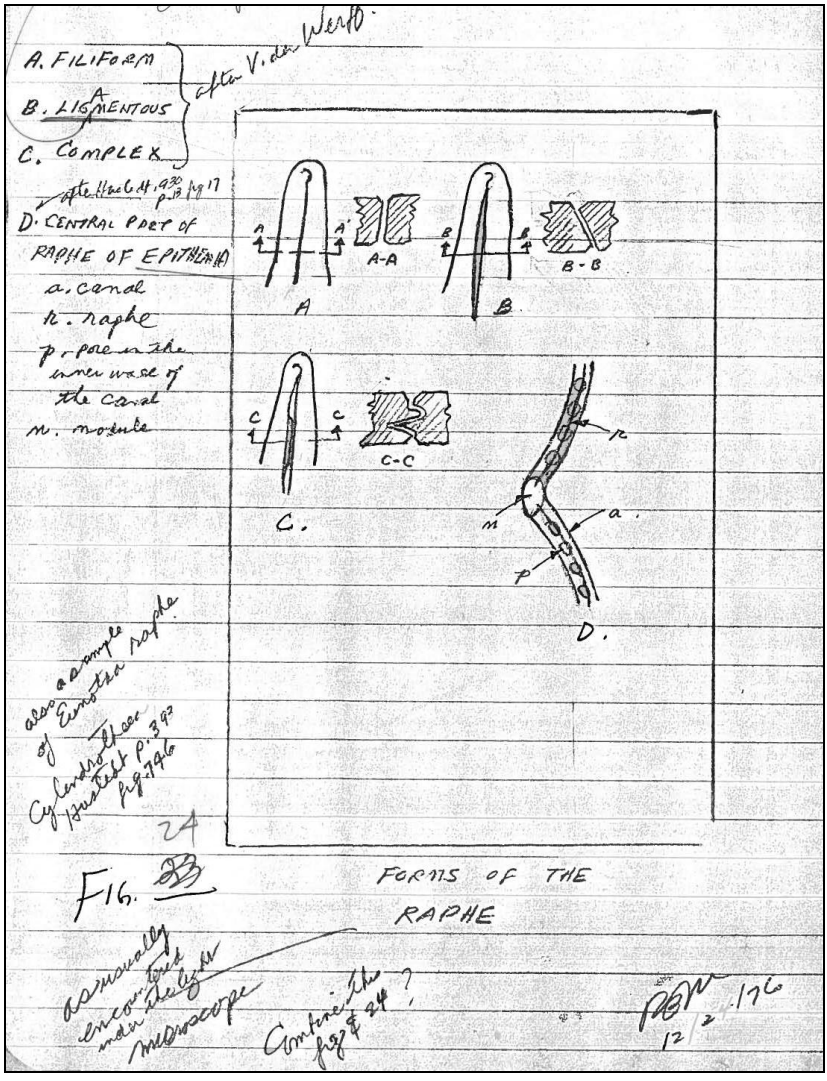


Figure 24.

The raphe from the central nodule to the one polar nodule on the one hand is the mirror image of that from the central nodule to the other pole of the valve. The terminal fissures under that condition are turned or hooked to the same side on a given valve. However, from the epivalve to the hypovalve, the terminal fissures are usually diagonally symmetrical, being turned in opposite directions. The central nodule from one valve to the other is generally diagonally symmetrical as well. There are few exceptions to this arrangement.

If a raphe is a vertical or sloped fissure it generally appears as in Figures 24A and 24B and is considered simple and so designated in diatom descriptions. The vertical fissure appearing as a single line is sometimes termed filiform, distinguishing it from the sloping fissure which is assigned the term ligamentous. If the raphe

consists of a combination of a horizontal “V” and vertical and/or sloping fissures in one view, it is termed complex (Figure 24C).

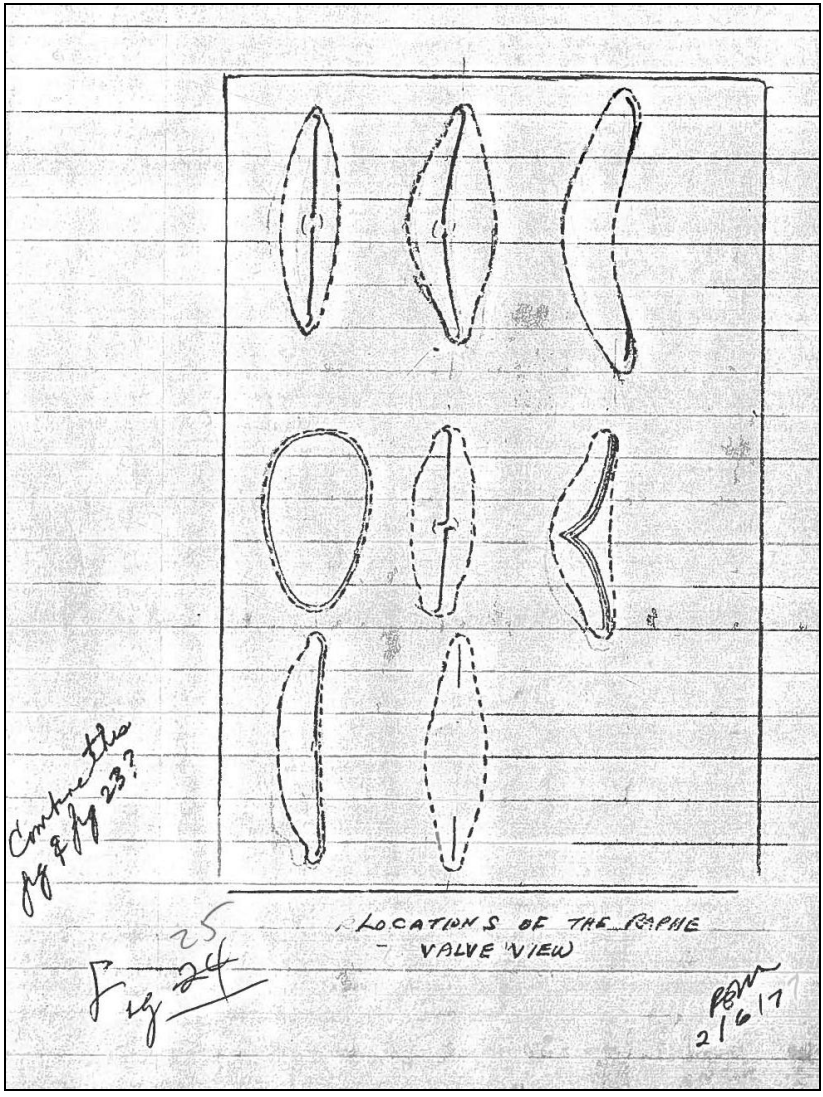


Figure 25.

The raphe is not always straight or located at the center of a valve (Figure 25). In *Cymbella* it is curved and slightly to one side of the apical axis of the valve. In *Eunotia* there are two raphe systems in each valve. They are not joined as in many of the *Navicula*, and are curved. The raphe slit in that case is usually seen in the terminal or polar nodules and extends for a variable but short distance into the valve mantle. The ends of the raphe which might be regarded as primitive terminal fissures, branch into the protoplasm at angles. No true central nodule is present in the genus *Eunotia*.

In some genera the terminal nodules may be elongated and the terminal fissures not well developed. This is the case, for instance, in *Amphipleura* and *Frustulia* where the shapes of the terminal nodules are very characteristic. In *Frustulia* and *Frickea* the raphe lies between two siliceous ribs running on each side of the apical axis. In *Amphipleura* the raphe branches are very short with a long space in the center. In *Amphora* the raphe is quite excentric to the apical axis. The approaches of each branch of the raphe into the central nodule is sometimes very characteristic, as in *Neidium* an abrupt turn in opposite directions is often evident.

The cleft or raphe fissure is not wholly open, being in many cases closed in its middle region; that is, along the bend or in cross-section about the point of the horizontal "V". Thus in reality there may be two cleft-like fissures, one on the inner side and one on the outer side of each valve.

The most common example of a highly developed complex raphe occurs in the genus *Pinnularia*. For that reason, and also because some of the species are very large and the raphe more easily studied, the *Pinnularia* type raphe is often used as an example of raphe structure.

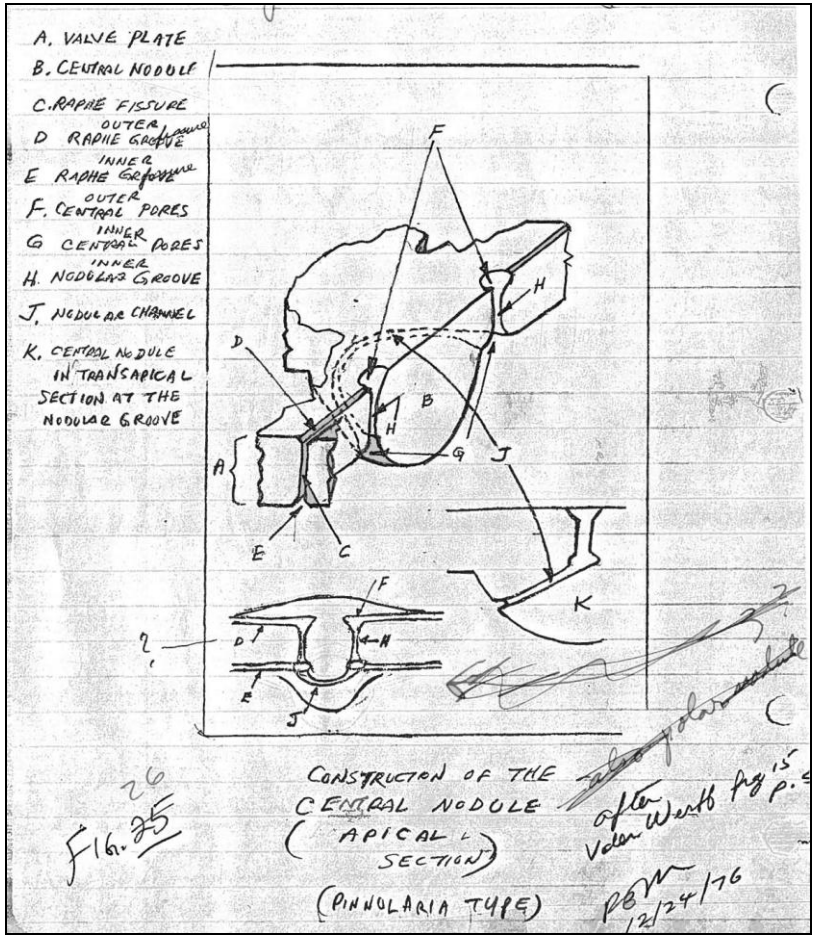


Figure 26.

Figure 26 illustrates an idealized apical section through a *Pinnularia*-type raphe. Various parts and features of the raphe construction in this area are very interesting. The central nodule is a swollen solid outgrowth of the valve plate, not hemispherical in shape, but rather like a peaked inverted dome situated slightly off-center. Each branch of the raphe, joins with the central nodule. The upper cleft or outer fissure of the raphe, opening to the outside of the valve, is joined at the central nodule with the lower cleft, or inner fissure opening to the valve interior, via a central pore. Each of these central pores has an upper portion; the outer central pore, a lower portion; the inner central pore, and a middle portion termed the nodular groove. The inner and outer portions of the pores are rather funnel shaped and the mid-portion somewhat constricted. The lower ends, or inner pore portions of the two central pores are joined via a nodular channel. This connection provides for a continuous flow of protoplasm between the inside and outside of the valve.

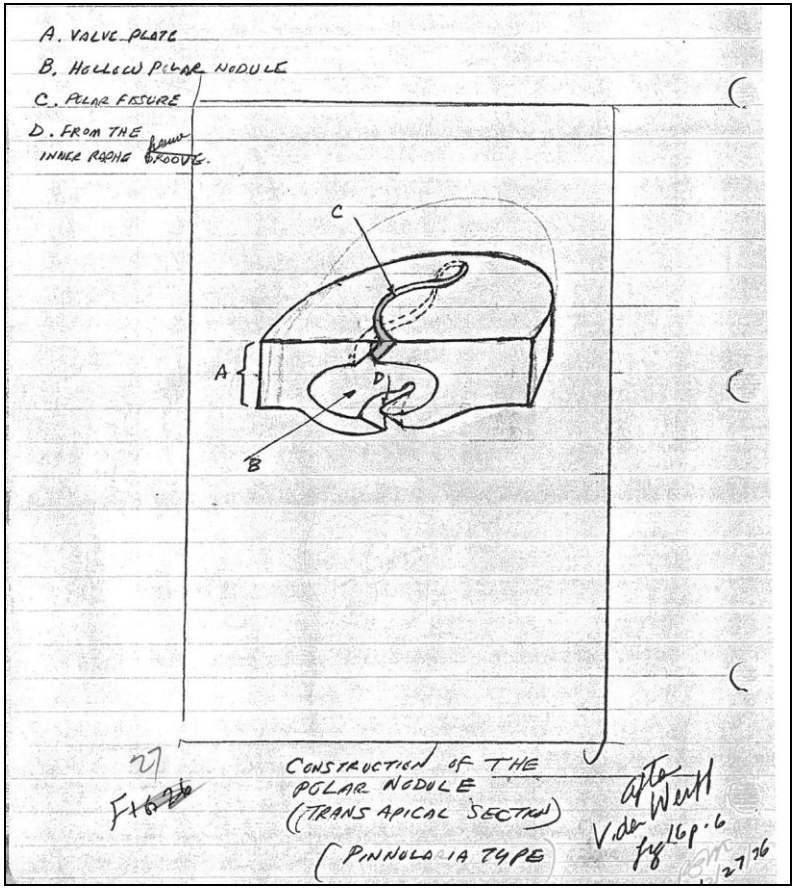


Figure 27.

At the polar ends of the valve the inner and outer fissures of the raphe terminate in an enlarged hollow hyaline body termed the polar, or terminal, nodule. Figure 27 illustrates an idealized transapical section of a *Pinnularia*-type polar nodule The terminating fissure is twisted and curved, often in the shape of a hook. Examination of the drawing shows the fissure first sloping in one direction then in another as it

proceeds on its curving course. The dotted lines are the lower edge of the fissure and when they appear to the left of the solid lines (representing the upper edge) the fissure is slanting upwards and to the right, and when they appear to the right the fissure is then twisted upward and to the left. The inner fissure is shorter and terminates in a more or less funnel-shaped opening which communicates with the valve interior.

Raphe constructions in the various genera have many variations in the placement, shape, cross-section and dimensions of the raphe fissure and in the central and polar nodule terminations; however they are, in essentials at least, of the same general form.

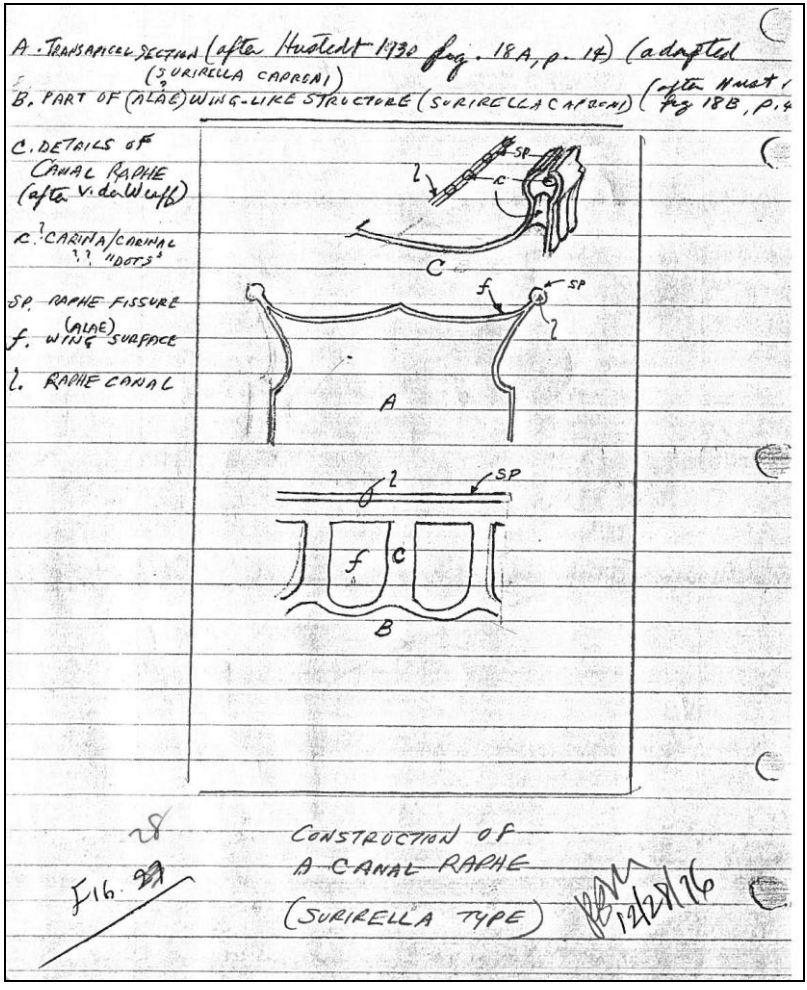


Figure 28.

There is another type of raphe represented in a number of diatom genera. It is the so called canal raphe. It differs essentially in that it is a simple fissure in a tubular channel called the canal. The bottom of the canal is perforated by a series of holes through which the protoplasm comes into contact with the raphe. Figure 28

illustrates the details and placement of the raphe in the genus *Surirella*. In this genus the canal raphe is in the top of the wing-like structure in the margin of the valve. Figure 28A is a transapical section through the valve of *Surirella caproni* (after Hustedt). The relative location of the raphe fissure, the raphe canal, and the wing-like surface or alae are provided in this figure. In Figure 28B there appears a part of the wing-like structure of the same diatom (after Hustedt) in apical section, providing another view of the same relationships. In addition this view shows the holes (C) that open at the bottom of the canal and communicate as extended passages, to the valve interior. In this view they are shown as alternating with portions of the wing surface. In Figure 28C is a perspective view of canal raphe construction (after Van der Werff).

Albert van der Werff (1903-1991)

In *Surirella* the raphe is sometimes interrupted at one or both poles of the valve, but in other cases runs across one pole of the valve. The pole at which the two ends of the raphe terminate is thought of as the terminal nodule. Because of its relatively narrow dimensions and its placement on the outer-upper edge of the wing, this raphe is difficult to see with the light microscope.

In *Epithemia* the cylindrical canal extends through the cell wall in a curved and recurved path across the valve in an apical direction. The fissure opens to the outside and holes or pores, much as in *Surirella* communicate with the interior. There is a central nodule at the apex of the two curved portions of the raphe canal. Figure 24D illustrates details of an *Epithemia* canal raphe, and Figure 25 shows its relative location on the valve.

In *Rhopalodia*, the canal raphe occupies the extreme edge of the valve in girdle view, and is often not distinct in valve view.

In *Nitzschia* the structure is very similar to that of *Epithemia*, excepting it is closely associated with a keel. In this group the raphe fissure itself is either difficult to see, or separate from other structure, with the light microscope. However, the "carinal dots" spoken of in describing diatoms of the genus *Nitzschia* especially, are usually meant to be either the communicating pores of the raphe canal or the solid material spaces between them.

1.9.11. Special Morphological Nomenclature.

In the previous material the morphology and associated terminology applies to a wide range of diatom cell-wall structure. There are a few genera of diatoms with either unique or very specialized structure that deserve additional attention. Although basically the same as other diatoms in a general way, some of their peculiarities of structure are constant and outstanding enough in the microscopical image, as to warrant special nomenclature.

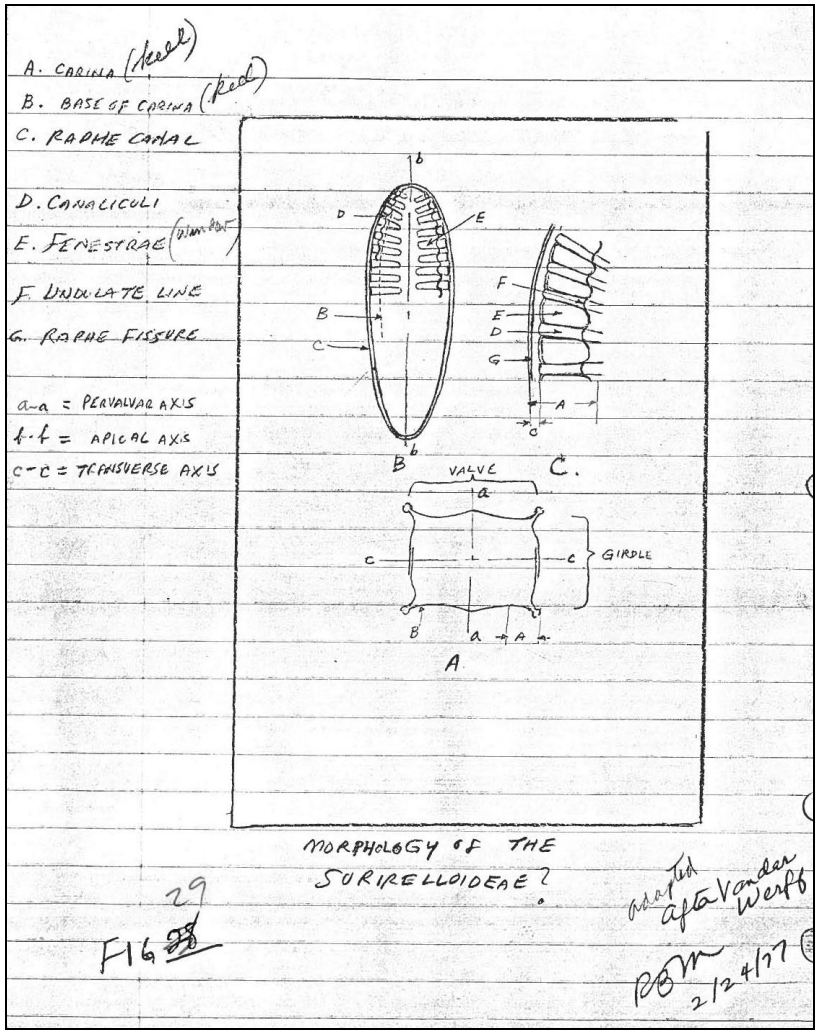


Figure 29.

Figure 29 illustrates the special terms used with *Surirella* and related genera. The unique location of the raphe, the type of raphe and its spatial relationship to the rest of the valve, and its means of communication with the cell contents are the basis for some specific terminology.

> Fenestrated - Window-like or divided into shiny portions.
> Ala(e) - A wing. An extension to the valve that forms a flange.

Each of the valve surfaces develops as a wing-like structure on each side, arising at varying distances from the apical axis. A series of thickened siliceous widened ribs is part of the wing structure. The wings are often referred to as alae and the ribs as fenestrae. Between the fenestrae are canals that open at the bottom of the raphe canal and communicate to the interior of the frustule and the included protoplasm. These canals are properly termed canaliculi.

The surface of the valve from each side of the apical axis, in transapical section, gives the impression of a wing-like structure and serves as a basis for the term alae. However, some authors prefer to regard the frustule, in transapical section, as having four keels, one at each corner. When so regarded they use the word carina to indicate that projection which carries the canal raphe. The canal raphe consists of the raphe fissure, the raphe canal, and the openings at the bottom of the canal which are the "tops" of the canaliculi opening to the interior. A more detailed illustration of this is included as Figure 28C.

The Naviculoid genus *Diploneis* is another diatom form having some special terms associated with its valve structure (Figures 30 and 31). The raphe is enclosed in a thickened and prominent rib which runs along the apical axis and is termed the median costa. Each of the costae run from the central nodule toward the apical ends of the valve, not necessarily reaching it. The central area includes the central nodule, and its breadth and shape is an important diagnostic feature and often specific. The central area is expanded and continues along both sides of the median costae to form an H-shaped hyaline area, the cross member of the "H" formed from the central area and nodule, and the vertical members, running adjacent to the median costae. The vertical members are referred to as the horns. The width of the horns are usually very narrow, but sometimes wide enough to have distinct width and a characteristic shape.

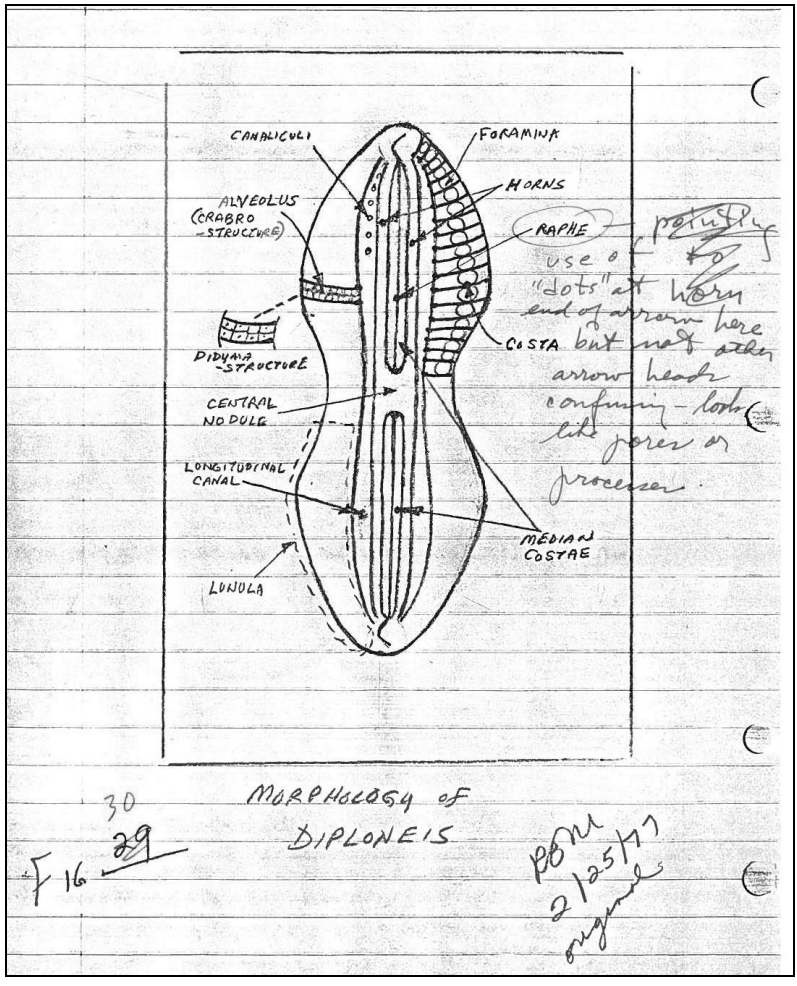

CANALICULI
FORAMINA
HORNS
ALVEOLUS (CRABRO -STRUCTURE)
RAPHE
use of "dots" at horn end of arrow here but not other arrow heads confusing - looks like pores or processes.
COSTA
DIDYMA -STRUCTURE
CENTRAL NODULE
LONGITUDINAL CANAL
MEDIAN COSTAE
LUNULA
MORPHOLOGY OF DIPLONEIS
30
29
16
2/25/77

Figure 30.

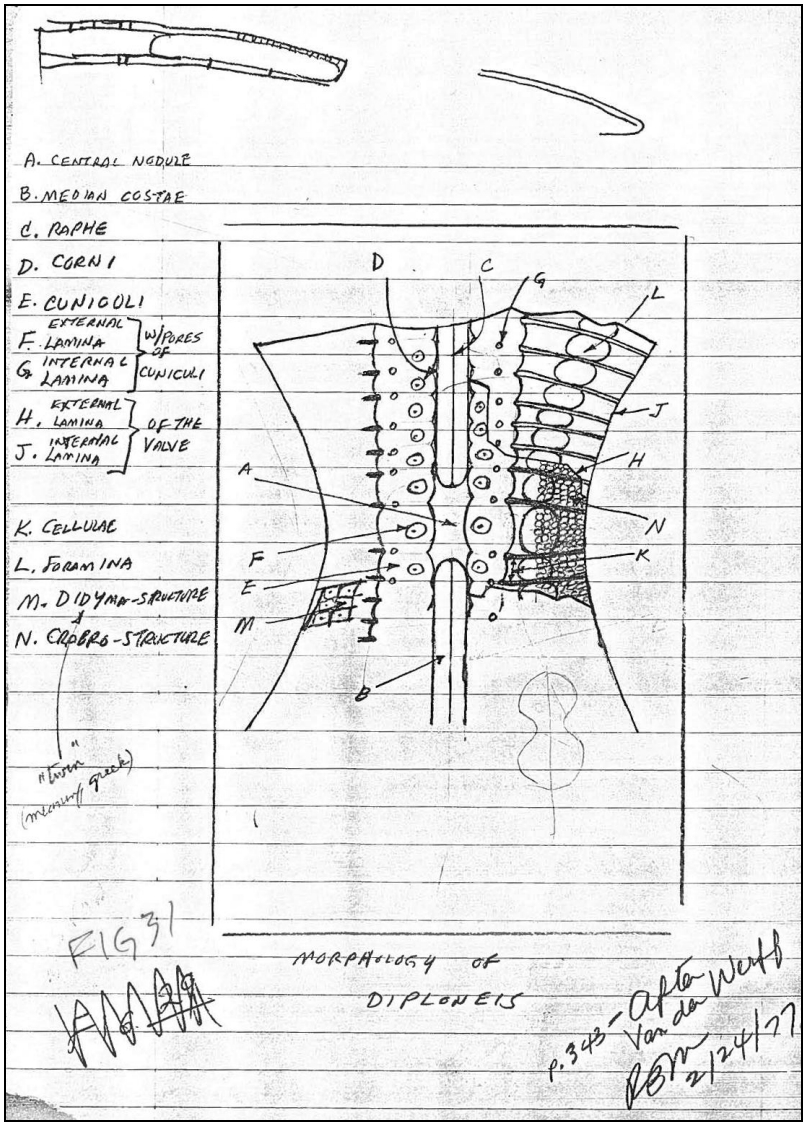


Figure 31.

Beginning at the outer margins of the horns and continuing outward to the edge of the valve, the valve has a double-layered wall with an inner and outer siliceous lamina that is characteristically chambered. The first distinctive portion of the valve is a longitudinal canal which runs adjacent to the "horns" and which often contains pores arranged in various groups or patterns. The width and shape of the longitudinal canal is often characteristic and specific. It is sometimes called the "furrow", and van der Werff refers to it as the cunicula (or under-passage). From the outboard edge of the longitudinal canal to the edge of the valve the structure incorporates thickened siliceous ribs or costae to form transapical chambers between them. The longitudinal canal may, in some species, connect to the rib-chambers via pores, or it may be completely closed off from them by a solid longitudinal wall

partition. The floors of the costae-bounded chambers may open to the cell interior via one or more pores or even rather large openings which van der Werff terms the foramina. The costae-bounded chambers are further subdivided by one of two usual types of cellular arrangements. One is termed a crabro-structure in reference to a hornets nest cell, and the other is a didyma-structure of open cells and pores combined. Sometimes the transapical chambers are divided into smaller chambers by longitudinal ribs running the length of the valve and crossing the costae. The upper, or outer, lamina of the valve is usually very finely punctate in rows between the costae. Often, these puncta are so fine as to be scarcely visible. The portions of the valve structure encompassing the rib or costate structure form a more or less lunate shaped area in each segment on each side of the raphe (that is, above and below the transapical axis) which is sometimes called the lunula.

The costae vary in distribution, relative attitude and number, with different species. They may be arranged transverse parallel, radiate, convergent, or in combinations thereof, in relation to the apical axis, and thereby constitute another determinative factor in identification.

A third diatom form that is assigned some special nomenclature is the genus *Melosira*. The various terms are illustrated in Figure 32A. Figure 32B is a transvalvar section of *Melosira juergensi* to illustrate a very finely punctate condition that sometimes occurs in this genus. The holes or puncta are very fine, and the valve wall relatively thick. The holes are more properly termed porecanals or canaliculi under that condition.

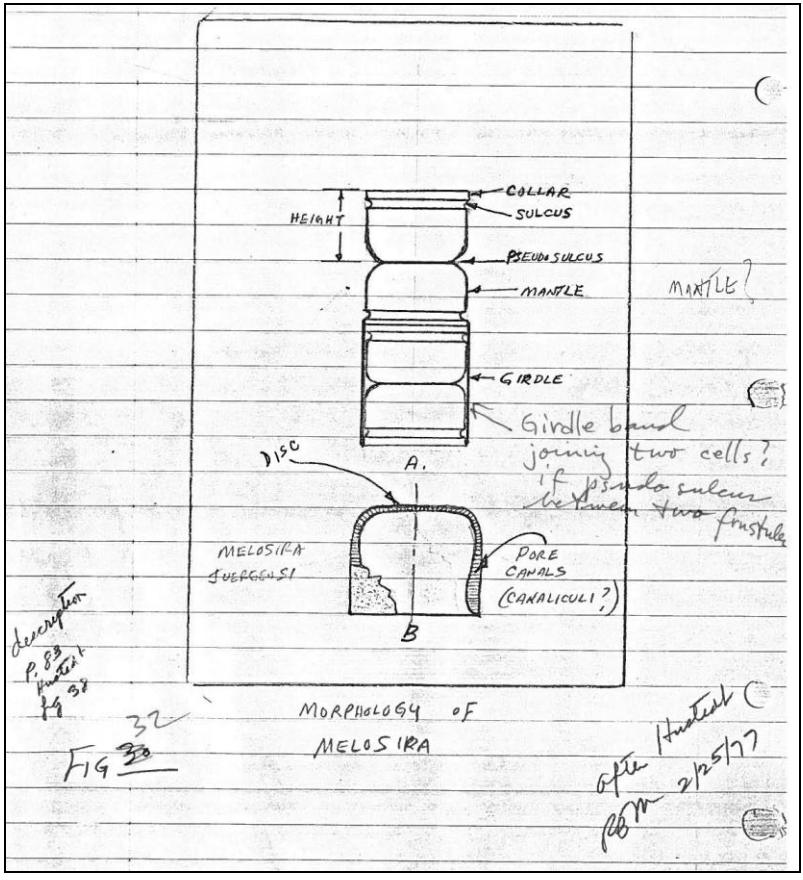


Figure 32.

Because of the shape and size of *Melorisa* frustules in general, and as they often appear in chain-like groups under the microscope, the girdle aspect is often seen. This condition has contributed considerably toward the development of special nomenclature.

The end-surface of the valve is called the disc, and the cylindrical portion the mantle. An inset groove ringed around the mantle is termed the sulcus. The latter projects into the inside of the valve as a ring-shaped septum. Correspondingly the edge where two valves of adjacent frustules are joined is called the pseudosulcus. These are always open "grooves", but because of the overlapping girdleband, may appear to be closed. The short part of the mantle between the sulcus and girdleband is called the neck or collar.

Because the length of the cell is dependent upon the growth of the girdleband, and that growth is variable, the height of this particular genus is taken as the height of a single valve. It is measured in the long axis of the cell from the center of the disc to the beginning of the girdleband.

1.10. Polymorphism

Ronald Stenning Wimpenny.
(b. 10th August 1898 d. 1971)
The Fisheries Laboratory, Lowestoft (Ministry of Agriculture and Fisheries)

Dr. Robert (Johan) Wilhelm Kolbe
(1882 – 1960)

Lloyd H. Burckle

The quality of a given species assuming more than one form or variations on the form, and other evidence of polymorphism is not rare in diatoms. Very great differences in size alone is a polymorphic feature that occurs more often than has been realized in the past. Wimpenny (1936) for instance, found temperature increases were causative in the increased diameter of *Rhizosolenia styliformis* and *R. alata*. Kolbe and Burckle and McLaughlin (1977), have made extensive investigations of size variations in *Coscinodiscus nodulifer* A.S. ranging as high as 18 to 1.

Chains of polymorphic species may contain frustules of very dissimilar contour, and in some instances the-two valves of a frustule, usually alike, may be different from one another in one or more respects. For instance, differing valves in species normally symmetrical are exemplified by *Navicula notabilis* and *Coscinodiscus punctatus*, one valve bearing densely packed granules, the other with granules scattered towards the center. Frustules of *Coscinodiscus superbus* may consist of two convex valves, or two concave, or one convex and one concave; on the concave valves the punctation is open, on the convex it is closely radiated from a small central area. *Triceratium pentacrinus* may have one smooth valve and one valve spiny. An example of dimorphism, trimorphism, or polymorphism may occur in species with processes. The processes may be different in the same frustule (from valve to valve). Differences in size, number and shape of processes in this instance have been recorded. In *Cerataulus laevis* for instance, most valves have very pronounced processes, while in others they are greatly diminished in size.

There is certainly some evidence that polymorphism as observed and reported may be due to either misinterpretation of the biological stage of the observed specimen, or lack of information on previous life history. For instance, it is true that the auxospore cell of a diatom may be very different in length, width, outline, and in certain markings, from the “normal” or vegetative cell. Even separate genera have been established in the past on the basis of observation of auxospores. Another morphological condition that is sometimes difficult to differentiate from polymorphism is the effect ecological conditions have in which the particular diatom has grown. Monstrous and structurally deformed frustules are often encountered in long-time artificial cultures, and also in specimens collected from natural environments. Many of these are rather obvious in their radical departures from normal growth, but some subtleties and preliminary manifestations of these causatives certainly may have accounted for reports of polymorphism, especially on the basis of isolated, single, or individual specimens.

1.11. Abnormal Forms

P. Miquel, Paris
De la culture artificielle des Diatomees 1892

Henri-Ferdinand Van Heurck (b. Antwerp 1838, d. 1909)

In cultivations of diatoms, if certain physical and chemical elements are intentionally exaggerated, and when diatoms grow in these media, they assume deformed, bizarre, and monstrous forms. Dr. Miquel, a pioneer in diatom culture, termed these cultivations teratological. Distortions of structure, absence of normal structure and modifications of symmetry all may be combined to produce nearly unrecognizable forms in the extreme. The various irregularities are generally manifested by indented or deformed outlines, double or multiple centers, and by unsymmetrical varied markings. Van Heurck discusses these variations at length.

Abnormal forms also exist in natural habitats, and are reported from time to time, no doubt the result of similar ecological excesses or deprivations. There is also good reason to believe that fossil forms may be distorted in shape by enormous pressures they may be subjected to in the matrix during many thousands or millions of years.

1.12. Descriptive Terms and Features and Examples

Of the various terms and phrases used to describe the diatom frustule some are rather general, and in a manner of speaking, there are diatom genera and/or species which are good examples of them. The following is included to assist the novice diatomist in becoming familiar in a general way with the best diatom examples of certain types of morphological features.

Table 1.

Morphological Feature	**Genus/Genera/Species**
Arched	*Campylodiscus*.
Bent	*Achnanthes*.
Twisted	*Scoliopleura*.
Radial undulations	*Actinoptychus*
Concentric undulations	*Craspedodiscus; Actinocyclus.*
Transverse undulations	*Cymatopleura.*
Inside struts and buttresses (costae)	*Biddulphia tuomeyi*; *Terpsinoe*; *Anaulus*; *Entogonia*.
Radial costae	*Arachnoidiscus*
Separable diaphragms and septa	*Grammatophora*; *Rhabdonema*.
Fixed transverse septa	*Porpei*; *Diatoma*.
Single small ocellus (near margin of valve)	*Actinocyclus*; *Euodia*; *Roperia*.
Large excentric ocellate process	*Monopsis*.
Large central hyaline circle	*Omphalopsis*; *Porodiscus*.

Central pseudonodule on each valve	*Glyphodesmis*.
Central psuedonodule on one valve only	*Cyclophora*.
Two small ocelli at the ends of a central longitudinal hyaline space	*Fenestrella*.
Small oval ocelli around the margin	*Rattrayella*.
Two or more large ocelli on raised processes	*Auliscus*; *Pseudo-Auliscus*; *Huttonia*; *Eupodiscus*; *Glyphodiscus*; *Craspedodiscus*.
Processes terminating in flat or cushion-shaped hyaline areas sometimes finely punctate	*Biddulphia*; *Cerataulus*.
Valve view crescentric	*Amphora*.
Raphes side by side - girdleview	*Amphora*.
Keels side by side - girdleview	*Hantzschia*.
Raphes one above the other - close to concave side of valve - valve view	*Amphor*a.
Crest running across top of valve	*Goniothecium*; *Odontella*.
Ridge or plate in the form of a web along surface of valve	*Odontotropis*.
A series of humps and crests or ridges on the upper valve	*Cheloniodiscus*.
Plate in the form of a crest or fringe	*Stephanopyxis limbata*.
Plate in the form of a crest or fringe around the valve close to the edge like the brim of a hat	*Xanthiopyxis*.
Heels run side by side in girdle view	*Hantzschia*.
Keels diametrically opposed	*Nitzschia*.
Raphe on a sigmoidal keel	*Amphiprora*.
Valve view sigmoid	*Pleurosigma*; *Gyrosigma*.
Club-shaped (clavate)	*Actinella*; *Clavicula*; *Rhopalodia, Gomphonema*.
Naviculoid, very convex, slightly twisted spirally	*Scoliopleura*; *Alloioneis*.
Margins of valves form wings or alae	*Surirella*.
Prominent hyaline processes symmetrically disposed	*Aulacodiscus*.
Furrows in valve view symmetrically arranged	*Aulacodiscus*.
Triangular valve view	*Triceratium*; *Trinacria*.
Panduriform-valve view	*Diploneis*.
Arcuate valve view	*Cerataulus*.
Wedge-shaped	*Rhopalodia, Gomphonema*.
Cymbiform	*Cymbella*.
Needle-like	*Synedra*.

1.13. The Cell Contents

1.13.1. The Elementary Plant Cell

The cell content (inside the cell-wall) is called the protoplast and is considered to consist of three basic parts. They are the plasma membrane, the cytoplasm (sometimes referred to as the cytosome), and the nucleus.

Although it is not the intent to enter into a lengthy discussion of the biology of the diatom, it does seem appropriate to provide at least a brief outline of cell contents and function. The following paragraphs include a very brief, but modern, concept of a generalized plant cell and its functions. It is intended that they will provide a basis for understanding the specific parts arrangements and protoplast morphology of the diatom. Figure 33 is a generalized drawing of a plant cell which illustrates the parts and functions to be described.

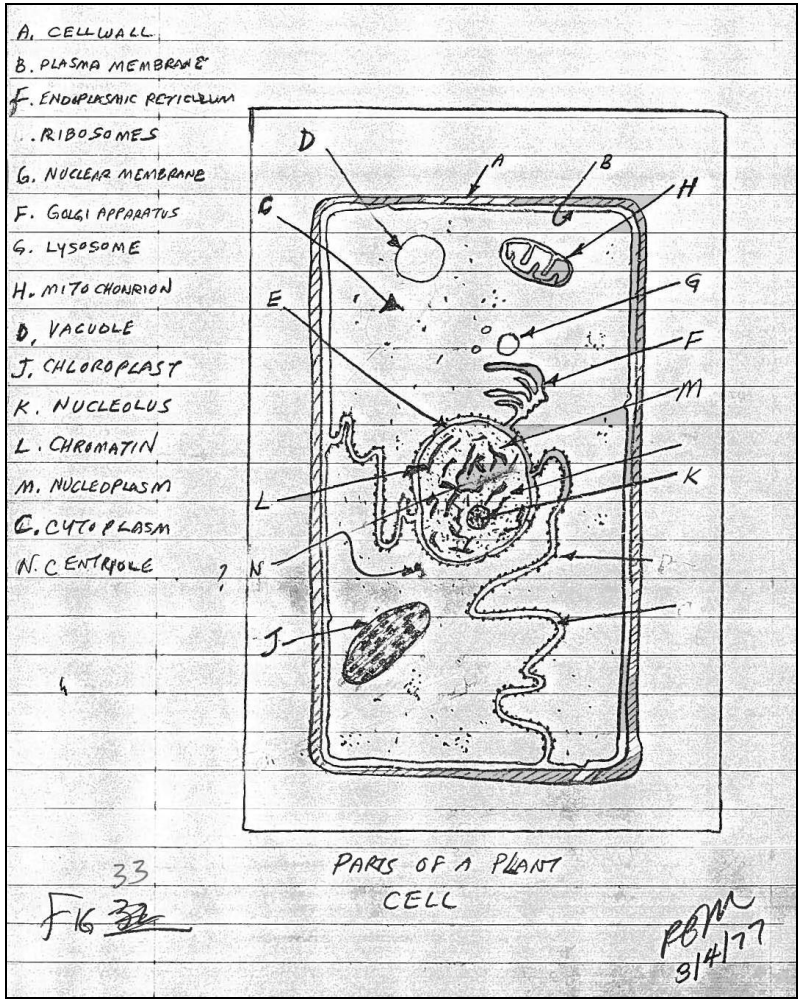


Figure 33.

The plasma membrane controls what enters and leaves the cell, the cytoplasm is where growth and energy release occur, and the nucleus is the site of control for both growth and reproduction.

The plasma membrane is believed to consist of an inner and outer layer of protein molecules and a middle layer of lipid molecules. Small molecules enter and leave the cell through small pores, larger solid particles enter by a process of phagocytosis, and water and dissolved materials enter by a process of pinocytosis. Food, water and wastes may be stored in the cell inside vacuoles.

Phagocytosis - the cellular process of engulfing solid particles.
Pinocytosis – the cellular process of engulfing fluids. Often termed 'cell-drinking'.

Phagocytosis and pinocytosis involves at some points along the plasma membrane a sinking inward and the "pinching off" of membrane-lined vacuoles.

At other points along its surface, the plasma membrane is continuous with a network of membrane-lined canals called the endoplasmic reticulum. These canals criss-cross the cytoplasm enabling various materials to be transported throughout the cell.

The assembly of protein molecules takes place at darkly-staining bodies called ribosomes scattered along the surfaces of the endoplasmic reticulum or lying free in the cytoplasm.

Although growth is most obvious in the cytoplasm it is under control of the nucleus. The nucleus produces Ribonucleic Acid (RNA) molecules that go out into the cytoplasm and control protein production by becoming part of the ribosomes where protein production takes place. Near the nucleus of the cell, elements of the endoplasmic reticulum fold back and forth to form a cup shaped stack of membranes called the Golgi complex. The membranes of the Golgi complex differ from those of the endoplasmic reticulum in that they lack ribosomes. The Golgi complex also seems to help transport the materials it stores by budding off some of these materials into floating membrane-lined water "bubbles" called vacuoles. It is thought that the lysosome, a vacuole-like body full of digestive enzymes, may be formed as a bud from the Golgi complex. Lyso refers to "dissolving power" and "some" means body. So the function of the lysosome is to dissolve food. If it were not for the surrounding membrane, the digestive enzymes inside the lysosome could dissolve the cell itself. The food in a phagocytic vacuole may be digested by enzymes released when the phagocytic vacuole fuses with a lysosome.

The mitochondria also consist of (in part) "sandwiched" membranes of protein and lipid molecules. It is thought that the young mitochondrion may bud off from one of the cells membranes. There is an inner membrane in a mitochondrion which is folded inward to form "shelves" called cristae. The latter are thought to serve as "energy assembly lines" where molecules are broken down to produce energy. Therefore the mitochondrion is considered the "powerhouse" of the cell, as it releases energy for cell activities.

Another structure concerned with energy reactions is the chloroplast. It has a double membrane structure similar to that of the mitochondrion. The chloroplast is concerned with energy accumulating reactions rather than energy releasing

reactions. The chloroplast "traps" light energy for use by green plants. Plastid is a general term that includes all chloroplast-like structures in a cell. White plastids are called leucoplasts, red plastids are called rhodoplasts, etc.

Chlorophyll, the green colored chemical that actually absorbs light energy is located within the chloroplast in stacks of membranes called grana. Just as the inner sandwiched membranes of the mitochondrion are folded to form cristae, so the inner membrane of the chloroplast is folded and stacked to form the grana. The green plant cell produces its large molecules using the energy of light that is absorbed by the chlorophyll located in the grana within its chloroplasts.

The process of releasing energy for cellular activities is called respiration, so the site for most cellular respiration is the mitochondrion. The process of building up or synthesizing large molecules using the energy of light (photo) is called photosynthesis. The site of photosynthesis is the chloroplast.

The electron microscope reveals there "are several smaller structures included in the nucleus. The nucleus is separated from the cytoplasm by a nuclear membrane continuous with the endoplasmic reticulum.

Small pores allow small molecules to pass through the plasma membrane and larger pores allow larger molecules to pass between the nucleus and the cytoplasm through the nuclear membrane. The nucleoplasm is a watery solution in the nucleus containing much dissolved protein. Floating in the nucleoplasm, a darkly-staining structure there is called the nucleolus, and is believed to be composed principally of RNA.

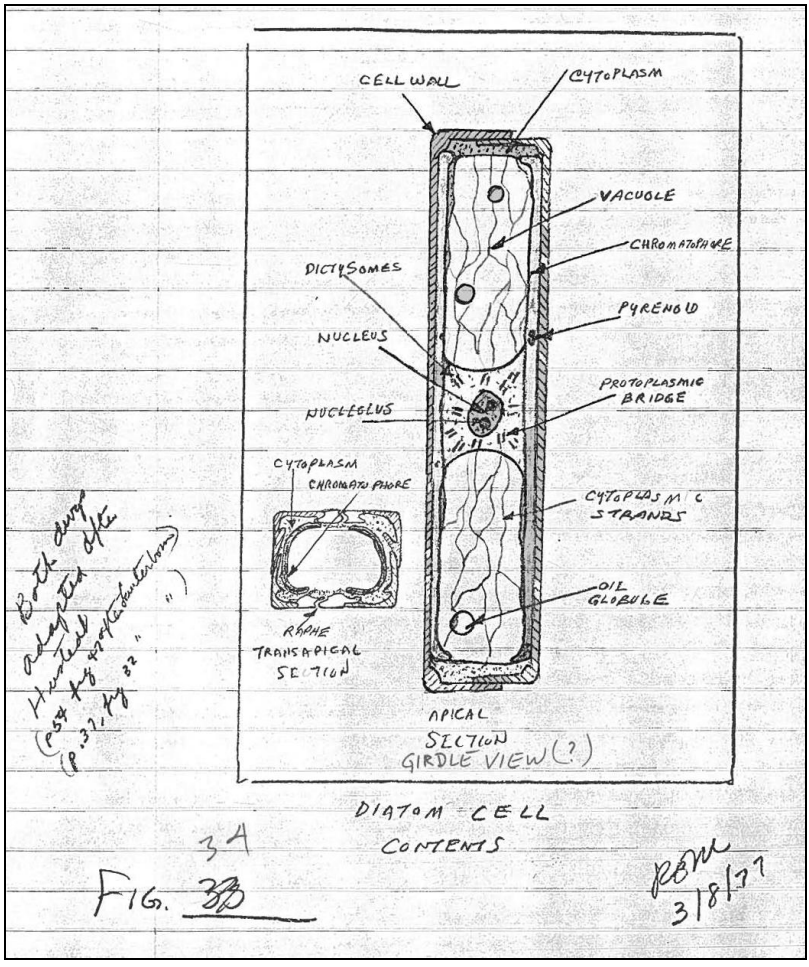
Before the nucleus divides, a tangled network of darkly-staining structures called chromatin can be seen. The chromatin is made up of individual threads called chromosomes. As the nucleus prepares to divide, the long, thin, partly invisible threads in the nucleus coil so that the entire individual chromosomes are visible. These individual "threads", or chromosomes, are specifically arranged Deoxyribonucleic Acid (DNA) molecules and carry the cells' hereditary information. Specifically, the set of DNA molecules arranged in chromosomes actually control growth and reproduction of the cell. The RNA molecules that control protein production at the ribosomes are made by DNA molecules found in the chromosomes of the nucleus. The RNA made by the DNA of the chromosomes seems to be stored in the nucleolus, and then goes to the cytoplasm where it controls protein production.

1.13.2. The Diatom Cell

The cell-wall of the diatom is the frustule, the morphology of which has been treated at some length in preceding sections of this chapter.

The very brief previous discussion of the generalized plant cell was intended as a background for understanding the morphology of the protoplast of the diatom cell. Figure 33 shows some details of a plant cell which are usually not encountered in the study of diatoms with the light microscope, and in an arrangement strictly arbitrary, portraying no particular type of plant cell. The diatom has some definite

morphological characteristics which will be described in the following paragraphs. This material should serve as a guide as to what is seen in living diatom material, and as a basis for understanding certain treatment methods of the cell contents to be described later.



Friedrich Hustedt: Die Kieselalgen Teil I (Fig. 47)

Figure 34.

Referring to Figure 34 the cell contents include a single nucleus with one or more fairly conspicuous nucleoli. The nucleus in bilateral diatoms usually is situated along the pervalvar axis and centered in the frustule. The resting nucleus contains chromatin granules and is variable in size and shape depending upon the species of diatom. The nucleus may be spheroid, ovate, ellipsoidal, or even reniform in shape. In centric diatoms it is usually spherical. A very minute feature of the nucleus is the centriole. It is a small spherical body which in mitosis forms the center of the astral rays. Hustedt states that in *Surirella* it appears as a minute body of about 1.5 to 2.0 micrometers in diameter. Most writers refer to it as the "centrosome". Centrosome in more modern usage is considered to be the dense zone surrounding the centriole in a cell center and is also called the microcentrum. It is not easily seen, but proper staining procedures will bring it out quite adequately for the light microscope.

A large vacuole is present and is usually centrally located. However, in the bilateral diatoms it is normally divided into two portions by the so-called protoplasmic bridge (see Figure 34) located on the pervalvar axis. In centric diatoms wherein the nucleus lies near one of the valves this disposition of vacuoles is not the case. In both centric and pennate diatoms, thin cytoplasmic strands may traverse the vacuole, and illustrations by Hustedt (after Lauterborn) show this.

Robert Lauterborn
(1869 - 1952)

Under comparatively low-magnification, the cytoplasm of diatoms appears simply fine granular, but with the aid of the best objectives, and under favorable conditions, where the layer of cytoplasm is thin, it is often possible to recognize a distinctively reticulate structure. It may be observed, for instance, in the small masses of cytoplasm which occupy the extremities of the frustule in *Pinnularia oblonga*. Lauterborn (1896) reported "double-rods" in the cytoplasm near the nucleus and West (1916) noted them as well, but admitted no known function for them. Drum (1963) has described such occurrences which he terms dictyosomes. They resemble Golgi-complex bodies found in most plant and animal cells. They are visible under favorable conditions in *Pinnularia major* with the light microscope, and are illustrated in Figure 34.

George Stephen West
(son of William West)
(1876 – 1919)

R. W. Drum

Diatoms contain chloroplasts, or perhaps more correctly chromatophores (the term chloroplast usually being reserved for the green plants) which, dependent upon the species of diatom, are variously shaped and disposed in the cytoplasm. In Figure 35 the apical section shows only the edges of the chromatophores, which cover the entire inside sides or girdle areas of the frustule, not quite covering the raphe of either valve. The transapical section shows this, and also that the cytoplasm fills the cavities in the frustule. This latter condition prevails to maintain a close contact with pores and other openings in the valves through which active transport of nutrients and waste must take place.

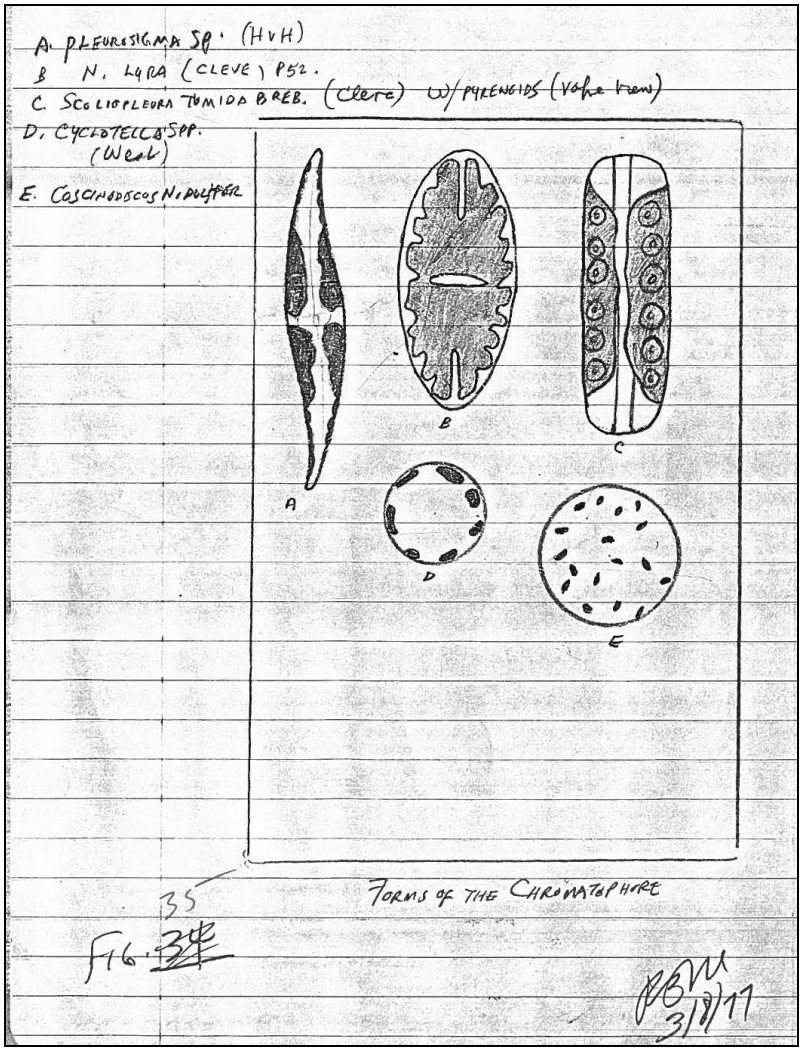


Figure 35.

Chromatophores assume different shapes, appearing in the form of plate-like structures of many forms, as granules, and as disc-shaped or ovate bodies. The larger plate-like forms are encountered often in the bilateral diatoms and the smaller discoid bodies more often in the centric diatoms. The larger forms may number one or two in a frustule, but they may be very numerous and be scattered at random throughout the frustule, even into spines and other hollow processes of the valve.

Although the forms of the chromatophores are variable they are typical in a general way for certain species. In *Melosira* they are in the form of numerous small irregular lobed plates located near the upper cell surface; in *Coscinodiscus* they are often numerous small lobed or roundish discs scattered over the valve surface; in *Cylindrotheca* small roundish granules, and in *Chaetoceros* they may be either numerous small round discs, or occur in many large plates, or even in only one or two large plate-like forms. In the pennate diatoms, the larger forms take on varied

shapes. For instance, *Pleurosigma* often exhibits plate-shaped chromatophores with more or less incised edges, and in *Campylodiscus* there may be only two large plates, lying on the valves, with edges bluntly rounded, often breached in the center, and with a plicated or folded surface.

Hilmar Günther von Schoenfeldt (more correctly Schönfeldt) (1840 – 1920)

Pfitzer (187l) developed a classification of diatoms based on the structure of the chromatophores, and von Schoenfeldt listed centric and pennate diatoms, of freshwater and brackish water origins, with descriptions of their chromatophores. However, it often happens that in the same genus, there are several different chromatophore shapes and/or structures. Also, because of their inconsistency in size, shape, and disposition within the frustule, they are not considered a suitable or distinguishing feature on which to base major classifications.

Constantin Mereschkowsky (1855-1921) Russian botanist

However, within a genus the attention to the shape, size, distribution and type of chromatophore may serve to group and separate species. Hustedt indicates that Mereschkowsky proposed a plan for dividing diatoms according to the construction and/or arrangement of the chromatophores as they collectively form the endochrome. His scheme involved two major divisions such as if the endochrome was formed from one plate or more than one plate. In the poly-plate division he further subdivided by 2 and 4 plates and then into such constituent makeups of the endochrome as small discs, granules, etc.

The chromatophore is laminated (as the chloroplast of green plants) and is surrounded by a membrane. The color of the chromatophore is usually of a golden or brownish yellow color and sometimes, but rarely, of a greenish color, as in *Navicula cuspidata*. It contains chlorophyll but the green color of that constituent is masked by other chemical pigments present.

Pigments present include chlorophyll a, chlorophyll c which absorbs a relatively higher proportion of blue light than red than chlorophyll a, and a complex mixture of yellow pigments. The yellow pigments consist of carotenes and xanthophyll. Recent work has shown that the carotenoids possess a degree of photo-synthetic activity which enables the diatom to take advantage of conditions of habitat where chlorophyll is less efficient. The absorption spectra of the various pigments in diatoms and their interaction in energy transfer is very imperfectly understood. Hendey discusses this matter at some length and in greater detail.

The chromatophores contain oil droplets and pyrenoids. The oil droplets in the chromatophore appear to have the same density as the oil bodies or globules found in the other parts of the protoplast. At least a single discoid pyrenoid is believed to be in each chromatophore, although many diatoms appear to have none at all. The pyrenoid is a highly refractive protein body and serves as the center for the deposition of starch. It is a lens-shaped to barrel-shaped shiny entity, but not always sharply defined in the chromatophores. It may be variable in number and disposition. Hustedt indicates that the pyrenoids which occur in multiple are generally smaller than those appearing singly. He also notes that the location of the

pyrenoid(s) can be differentiated on a general basis from those that are centrally located within the chromatophore, and those that occupy positions near the edges. Mereschkowsky, according to West had recorded instances where pyrenoids partially or entirely emerged from the chromatophores and under the latter situation appeared as free colorless bodies on their inner surfaces.

Aside from the oil droplets found in the chromatophores which may or may not be obvious with the light microscope, there invariably is very obvious globules of oil which appear distributed in the protoplast. The oil appears in one or more globules or bodies of comparatively large size and is easily soluble in ether and blackened by osmic tetroxide. They have been shown to be a food-reserve by keeping diatoms in tap-water for considerable periods in closed containers, in which case the oil is completely used up, according to West.

Mereschkowsky, according to Hustedt , also formulated a systematic scheme based on the appearance of oil-bodies as variable in number and location, or whether the number and location was constant. In the latter constant category he further subdivided those diatoms in which the oil bodies were near the edge or surface of the chromatophores, and those that were lying free mostly in the vicinity of the raphe.

Butschli's red corpuscles are extra-nuclear bodies, spherical in shape, which are not dissolved in alcohol or ether and do not become black with osmic tetroxide. They can be stained with Haematoxyln and Methylene blue in a treatment which will be covered later.

In many diatoms they are distributed throughout the frustule, but in some, such as *Pinnularia major*, *Stauroneis acuta*, and *Gyrosigma attenuatum*, they are located at definite locations. In *Navicula cuspidata* for instance, these bodies are comparatively large, are two in number, and are located on each side of the protoplasmic bridge just under the raphe.

Konstantin Ignatevich Meyer
(1881 – 1965)

Dr. Otto Heinzerling

Because of the work of Meyer and Heinzerling, and because as a rule, volutin (inclusion bodies of nucleic acid and related substances) is found in all diatoms, Hustedt concludes that the Butschli bodies are a firm volutin constituent of the cells. The importance of these bodies function-wise is not certain, but they may serve as a nitrogenous reserve material for the new formation of living substance. He notes that with cell division, the volutin-granules dissolve, but soon after completed division occurs they are again in their old location and condition.

One further morphological feature, of the diatom deserves mention. Although its manifestation is not within the contents of the cell, it is internally generated. That feature is a mucilaginous covering of the diatom frustule. Apparently all diatoms in the living state possess mucus covering to some degree, sometimes rather thick, and sometimes so thin as to not be detectable unless suitably stained. The thickness of the covering may vary, depending upon the condition of the diatom physiologically. The covering may extend to making up various pads and stipes of the material for colony formation and for adherence to various substrates. Special pores, as

mentioned previously, are sometimes involved in such mucus distribution and concentration.

CHAPTER 2.

2. PHYSIOLOGY

The various processes and activities which occur within the living diatom, and the functioning of it within its environment are known only very imperfectly. Although the culturing of diatoms and investigation of their response to the presence or absence of various physical, chemical, and photo-stimuli had been undertaken before 1900, it was not realized until recently that the physiology of diatoms is very diverse, and it would therefore be extremely presumptive to attempt any kind of generalization in that regard. Therefore, in the following paragraphs, only a very brief treatment of major areas of diatom physiology will be presented. The student who would enter into this area of diatom study will need to consult the literature of specialists for his background and assistance.

2.1. Nutrition

Originally diatoms were believed to be entirely autotrophic, or completely self-nourishing and capable of synthesizing food from inorganic compounds, as all chlorophyll containing plants.

However, West mentioned that several saprophytic forms had been described, including the colorless diatoms *Nitzschia putrida* and *N. leucosigma*. He also indicated that Karsten succeeded in producing a saprophytic form of *Nitzschia palea* by cultivating it in favorable nutritive media such as glycerin or grape sugar. The term saprophytic applies more directly to a nourishment obtained from dead organic matter, as is the case with most fungi. A better modern term for this latter means of nourishment is heterotrophic.

George Karsten
(1863 – 1937)

The modern viewpoint, supported by recent researches, is that diatoms are largely autotrophic, but that at least some are heterotrophic, dependent on an outside source of food, as do all animals, saprophytes and parasites.

The nutritive requirements of diatoms may be generally divided into vitamins, organic substances, and minerals. It has not been shown that all diatoms require vitamins or of what kind, and the term “organic substances” is very inclusive and non-specific. Mineral requirements are better understood, but any generalizations, even in this regard, are not advisable without some qualification. The following information is included with the understanding that it is only true from mainly isolated and specific experiments and investigations, and in no way applies generally to any and all diatoms.

Diatoms, in some cases, require vitamins. As the result of investigations on a number of different species it has been found that variously vitamin B12 (Cobalamine) and theomine, were required for normal growth or to maintain a normal condition of the frustule.

The need for organic substances has been long indicated from the importance of soil extract, and extracts from sea water and algae for maintaining proper growth. These requirements had been recognized from early cultivation experiments, but the precise nature of these substances was not determined and still has not been to any degree of exactness.

Mineral requirements of diatoms are similar to those of most plants. They include phosphates, nitrogen (in the form of ammonium or nitrates), sulfates, calcium, magnesium, potassium, iron, manganese, and silicon. The function of and best forms in which these minerals are assimilated by the diatom, is known only superficially. The requirement for a minimum supply of silica for reproduction seems fairly obvious, and it has been found that calcium seems to be one of the most important elements for diatom nutrition, but what its most important function is, is still not resolved. It is thought to be one of the constituents of the buffering system that controls pH, and it may have several other chemical roles in the biology of the diatom cell. Iron has been found to be necessary for rapid growth. Various forms of iron, in colloidal, particulate and in ferric form, have been found to be utilizable by diatoms of varying species and habitat.

2.2. Growth

The interpretation of what is considered growth in diatoms might at first seem to be difficult, as the frustule itself, once formed, and excepting for possible expansion along the pervalvar axis, is fixed in size.

However, the concept of growth is not always bound with a change in size, but in the changes that take place from birth, or origin, to an adult stage wherein procreation may take place. Growth in diatoms then is considered to be that change in physiological condition to the point where the cell is ready to divide or otherwise reproduce. Another aspect of growth is the manner of growth. In the latter are the considerations of how the diatom lives during the growing period. Many live isolated as individuals, or in colonies. Some are attached to various substrates and some are entirely pelagic. These two growth concepts will be briefly treated here, with more emphasis on the latter, or manner of growth, as it has some significance, both in the internal physiology of the cell, and in identification for the diatomist.

The use of the term Pelagic here is in error and should actually be ‘planktonic’.

The growth of diatoms to get to the point of reproduction is of course linked with nutrition and other conditions of the growth environment. Of the latter, temperature and the amount of light available, play major parts.

Diatoms have been shown to flourish, dependent upon the species, over quite a range of temperatures. Some are quite tolerant of a wide range of temperatures and others are very intolerant and sensitive and require a rather carefully regulated narrow range in which to reproduce at all.

Diatoms appear to flourish in temperatures well below the stated 20 degrees.

Temperatures which in general will support growth of diatoms are within the range of 20° to 30°Centigrade. This is a generalization however, and specific diatoms may flourish at temperatures somewhat less or greater. Some diatoms can adapt to temperatures outside of their normal range, some cannot. Temperatures as low as -10°Centigrade have been withstood for 24 hours by *Nitzschia putrida* for instance, without harm.

Optimum temperatures are, for specific diatoms, those temperatures or ranges of temperature which promote the most rapid growth. This is of course connected with the efficiency of photosynthesis and other aspects of environment. The rate of division, or growth, varies widely from species to species and what is considered rapid for one might be slow for another. Some diatoms, according to Patrick and Reimer divide several times in a 24 hour period, and others may remain healthy many weeks without division.

The photosynthesis process depends upon light, but its efficiency varies greatly with the species. The proportions of the various pigments in the cell, the depth at which the diatom lives, and the length of the photosynthetic cycle, manifested in various geographical regions, all affect it. As might be expected, photosynthesis in diatoms increases with light intensity, reaching a maximum value at about 10 kilolux, according to Lewin and Guillard (1963).

Dr. Joyce (M.) Chismore Lewin
b. 1926

Robert Russel Louis Guillard
b. 1921

The mode or manner of growth is variable among diatoms. It is not a consistent characteristic as to genera, and some species vary in assuming one mode of existence or another. However, it is of interest to list some of the more common modes and the genera in which certain species may assume that particular manner of growth.

Table 2.

Mode	**Description**	**Genera/Species**
Stipitate or pendiculate	Attached by a gelatinous stalk to stones, algae, or other foreign bodies. Some species of the following genera assume these modes of growth.	*Achnanthes, Arachnoidiscus, Brebissonia, Climacosphenia, Cocconeis, Cymbella, Entopyla, Gephria, Gomphonema, Licmophora, Podocystis, Synedra*

Chains or filaments	Some of which at the beginning have been anchored by an end or corner to algae or other objects. Some species of the following genera assuming this life-style.	*Achnanthes, Bacillaria, Bacteriastrum, Bacteriosira, Biddulphia, Chaetoceros, Climacosira, Diatoma, Dimerogramma, Entopyla, Eucampia, Eunotia, Fragilaria, Gephryia, Glyphodesmis, Grammatophora, Guinardia, Lauderia, Melosira, Meridion, Plagiogramma, Podosira, Hyalodiscus, Rhabdonema, Rhizolsolenia, Rutilaria, Striatella, Tabellaria, Terpsinoe, Tetracyclus*
Zigzag chains	When a complete face to face junction is interrupted and the frustules form an irregular zigzag chain united at the corners or extremities. These chains may consist of a hundred frustules, or at times only of two or three.	*Biddulphia, Diatoma, Grammatophora, Tabellaria,* and *Terpsinoe*. *Fragilaria, Rhabdonema,* and *Climacosira* may form blocks of frustules and the blocks then form a chain united at the corners of the blocks.
Ribbon-like groups	Forms a ribbon-like group with the individual frustules sliding one upon another longitudinally. forming a longitudinal rod-like filament.	*Bacillaria paradoxa*
Spiral filaments	Diatoms arranged in spiral-like filaments in a closely coiled arrangement do so as a result of their cuneate or wedge-shaped frustules.	Species of the genera *Corinna*, *Eucampia*, and *Meridion* are examples. *Meridion*, a prime example of this manner of growth has been found however, attached "head to tail" alternately; forming straight filaments.
Side-by-side groups	Frustules are attached to one another along the girdle, or zone, in small groups instead of at the valve face.	*Pinnularia* spp. and species of *Navicula* sometimes assume this mode.
Irregularly branched filaments	A condition extant when the attachment of the process is made indiscriminately at any portion of an adjacent frustule.	*Isthmia nervosa*, and *I. enervis* are examples of this arrangement.

Chains – straight	Frustules fastened together by exudation of mucus from spines often show this form.	*Stephanopyxis corona* is an example.
Chains - straight or slightly curved	Union by claws	Species of *Hemiaulus, Trinacria* and similarly equipped genera often appear in this style of grouping.
Cylindrical straight chains	This usually effected by spines or horns arranged parallel to the pervalvar axis as in species of *Rhizosolenia.*	*Rhizosolenia*
Chains by awns	Junction maintained by the union of gelatinous coating of awns.	*Chaetoceros* spp. are good examples.
Chains by single-threads of plasma (more or less silicified)		This style of intra-attachment and manner of growth is exemplified by *Thallassiosira nordenskioldii*.
Chains by a circlet of plasma threads		*Stephanosira karsten*, and *Coscinosira polychorda* show this form
In masses of mucus	A number of species exhibit this way of life	*Mastogloia, Frustulia, Dickieia*, and *Schizonema*
In cylinders of mucus	(straight or branching)	Some species of *Schizonema, Berkeleya, Cocconema, Homoeocladia Cymbella*
Chains by marginal threads		*Coscinodiscus heliozoides*, *Stephanodiscus zachariasi*, *Coscinodiscus sol*, and species of *Gosslierella*.

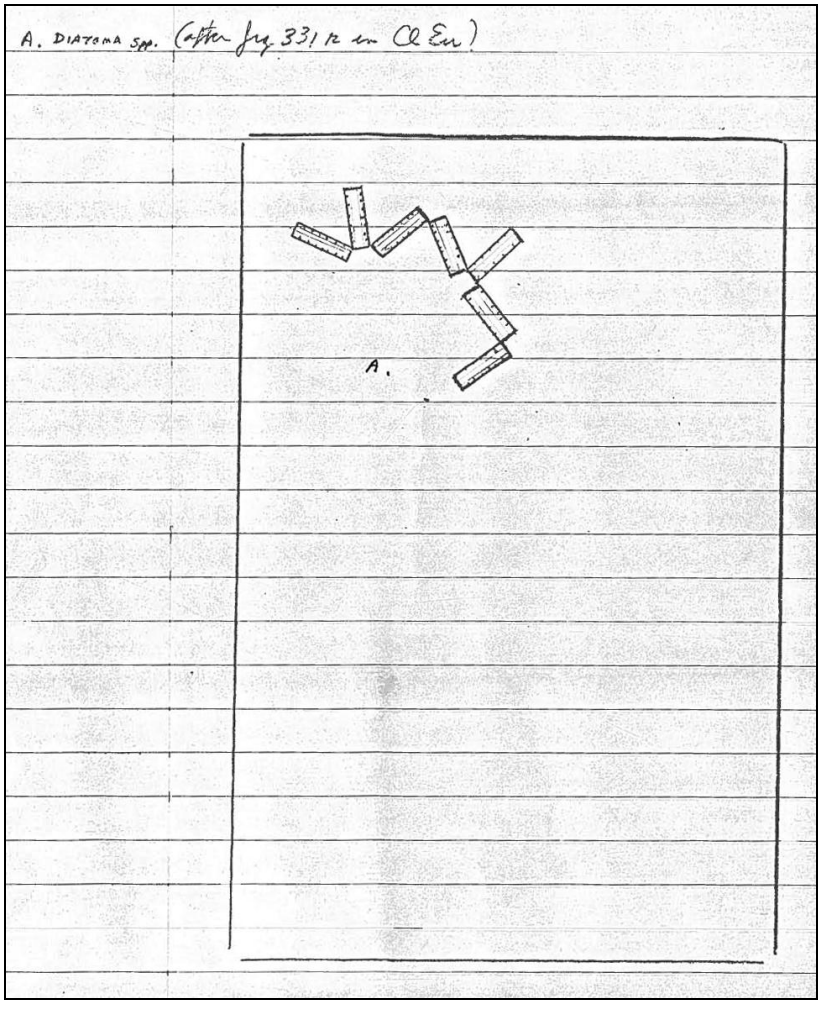


Figure 36

2.3. Movement

Movement in diatoms takes place both internally and externally. Internal movements involve the shifting of the nucleus in some diatoms as auxospore germination takes place, and the change in shape of the chromatophores under differing lighting conditions. External movement has been under study by microscopists for many years. During that time various reasons for, and theories to account for it, have been propounded.

The motion, that is the movement of the frustule without outside motive power, is in a definite direction and manner, and often of differentiating types of movement, dependent upon the species. For many years there has been recognized a connection between the presence of a raphe or a keel, and the motile power of a diatom, as the power of movement is confined to genera possessing a raphe on one or both valves, or possessing a keel (with a canal raphe). It is now the consensus that only diatoms possessing a raphe have the power off such movement.

The problem of examining the raphe for evidence in the question of diatom mobility is difficult because cytoplasm with a relatively low refractive index is surrounded by a frustule with a relatively high one. The raphe, where the inquiry is centered, obscures the protoplasm underneath by refraction. Nonetheless various ingenious schemes have been devised by experimenters over the years to determine, either directly or indirectly, the actual mechanism by which such motion takes place. Some of these methods will be described later in this book. Most recent studies have established streaming of cytoplasm in the raphe as the cause of movement. Sections of cytoplasm when examined in the electron microscope have shown linear bundles of microfibrils similar to the smooth muscle fibrils common in animals. Contraction of these fibrils could cause expansion of material in the raphe and thus cause movement. Upon motion, diatoms characteristically leave a continuous fibrous trail, invisible in the brightfield and darkfield light microscope, upon the substrate. Study of this trail and its constituency have been made by the use of certain stains. It is thought that the trail substance originates in cytoplasmic particles known as crystalloid bodies, and staining reactions show that this material is similar, if not identical, to diatom volutin. It seems that the origin of the propulsive force is in the contraction of the microfibrils, but that the movement is caused by the reaction of the expelled trail on the substratum.

Types of movement exhibited by diatoms are various and dependent, to a degree, on the shape of the raphe and/or mode of living. A well known instance of the mode of living having some influence on the type of motion is that of *Bacillaria paradoxa*. The rod-like diatoms lying one against the other form a ribbon-shaped band or filament. A terminal frustule may suddenly begin to slide along the contiguous frustule, which acts in a similar manner to a third and so on throughout one portion of the filament or packet. Meanwhile, the same action is going on at the other end of the group, but in the opposite direction. The central frustule appears to remain stationary, or nearly so. The lateral extension continues until each frustule is in contact with its neighbor for only a very small portion of its length. Then the filament is again contracted by the frustules sliding back upon each other. This changed direction of movement continues until the frustules are again only slightly in contact. The direction of movement is again reversed and so on. When the filament is elongated to its utmost extent, it is extremely rigid; An increase in temperature increases the rapidity of movement. The foregoing description has been taken from Taylor. Patrick and Reimer indicate the lining up of *B. paradoxa* takes about 80 seconds at 20°C., and the sliding out at the rate of 10 micrometers per second. They also mention that the rate of movement may be altered by a change of osmotic pressure in the medium or by the presence of narcotics.

Taylor also notes that *Nitzschia seriata* is found in chains which are extended like a fully opened series of *Bacillaria paradoxa*. These chains move like single rods, and the cohesion of the frustules is so strong, that if the chain is broken, a small fragment of the valve remains attached to its neighbor.

Other movements seem to be influenced by the shape of the raphe, as in *Navicula* the movement is straight (back and forth), in *Amphora* curved, and in *Nitzschia* curved with two different radii, according to Patrick and Reimer. There also are swaying movements exhibited about one apex of a frustule with certain diatoms.

Straight moving diatoms such as in the genera *Navicula*, often move in a continuous motion in one direction for many times their own length, pushing debris and other materials out of their path, before reversing the direction.

Some diatoms in their motions react to various wavelengths of light which influences their general direction of travel. White light seems to attract them, and on that basis some types of diatoms may be separated from debris by methods to be described later. Patrick and Reimer indicate that other wavelengths of light, blue, red, etc., may produce a negative or repellent response in certain species.

Under normal conditions of living, the diatom cells are only motile for short periods of time. Usually this is just after the beginning and just before the end of the light period. There appears to be daily rhythm of movement in otherwise undisturbed diatoms.

While it may be that rapid rectilinear motion is only found in diatoms possessing a raphe, Hendey points out that there are many forms with a raphe that have no apparent motion, and that Boyer cited *Mastogloia thwaites* as an example of this.

Charles Sumner Boyer (b. 24th September 1856 d. 1928) Philadelphia

Even after more than a century of investigation the movement of diatoms is still very imperfectly understood. Although, as included above, some very revealing and probably correct hypotheses have now been established as to the mechanism of movement, very little is known yet of the types of movements and how they are affected environmentally in specific diatoms.

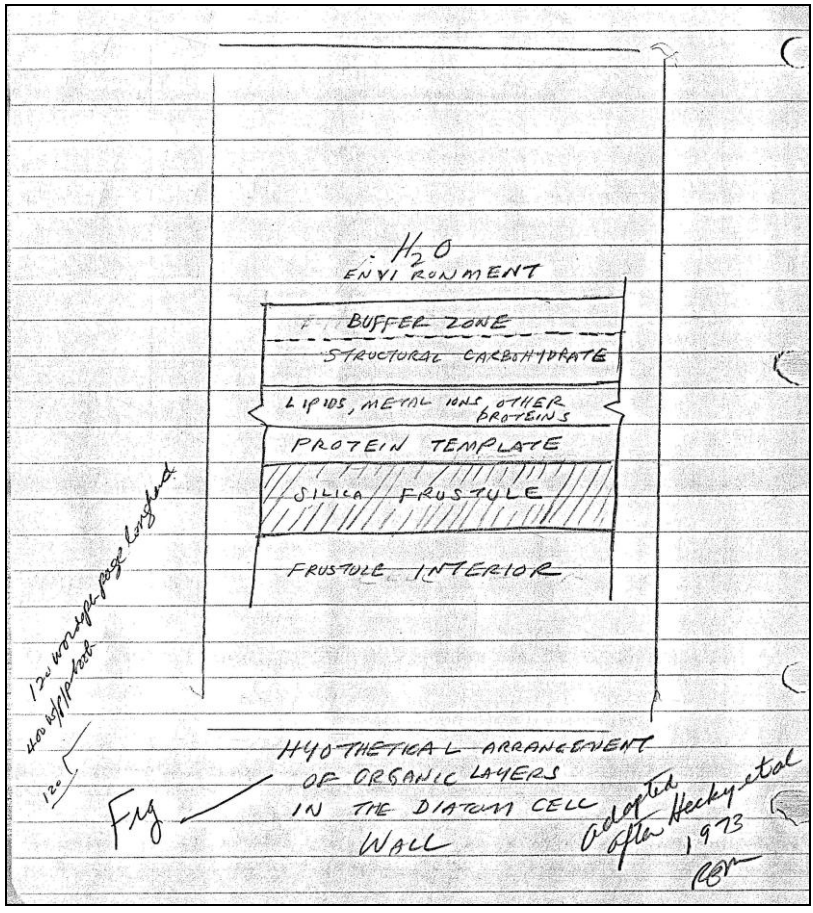


Figure 37.

2.4. Formation of the Cell wall

The cell wall of living diatoms is considered by many to be mainly composed of hydrated amorphous silica with small amounts of the oxides of aluminum and iron.

Prof. Robert E. Hecky

Hecky et al (1973) analyzed the chemical composition of the cell walls of diatoms. They suggested that the silicon depositing mechanism in diatoms is the condensation of silicilic acid, in epitaxial order (i.e. mimics the orientation of the substrate), on a protein template enriched in serine and threonine.

The nature of the template and the polysaccharides in the cell wall may determine the solubility of diatom frustules in various environments. The solubility of diatom frustules could be, if known with any exactitude, a very important factor in micropaleontology, wherein the "corrosion" of diatom frustules in the thanatocoenosis can be time or environmentally related.

An illustration of a hypothetical arrangement of organic layers in the diatom cell wall, based on the findings of Hecky et al is included as Figure 37.

Einsele and Grim (1938) indicate living diatom silica to have a specific gravity of 2.07 and Patrick and Reimer that fossil diatom silica is 2.00 and that evidence suggests the latter to be composed of alpha-quartz.

W. Einsele
J. Grim

The thickness of the diatom frustule is variable, and in tropical plankton types in particular, may be quite thin, and/or lightly silicified. This factor must be taken into account in deciding on the method of cleaning frustules for study.

Alpha-quartz is the most common form of quartz in the earth's crust. Alpha -quartz however, in polarized light, is anisotropic and diatoms are not!

CHAPTER 3.

3. REPRODUCTION

Diatoms reproduce principally by vegetative cell division in which mitosis occurs, and by auxospore formation.

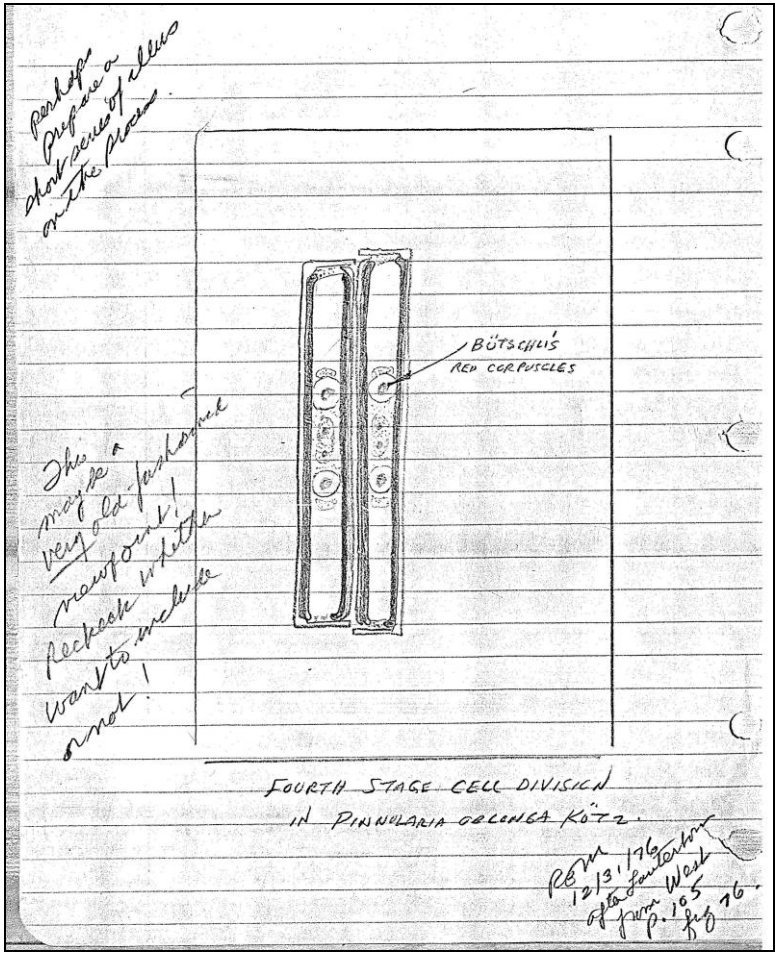


Figure 38

3.1. Vegetative Cell Division

This is the most common method of reproduction in the diatoms and has been observed by many workers. The following is a general description of the process which applies to most diatoms.

First there is a slight increase in volume of the cell by slippage along the girdle. The mitotic spindle lies in the pervalvar axis of the cell and a distinct centrosome is

present near each pole of the spindle in many species of diatoms, having been observed and illustrated particularly in the larger forms, as in *Pinnularia* spp.

There is mitotic division of the cell, usually taking place at night or in the early morning hours, and because of the small size of the chromosomes in many diatoms, is difficult to observe. However, the process in larger species in *Pinnularia* and *Nitzschia* for instance, has been observed and illustrated many times.

Immediately following the mitotic division of the nucleus, or almost simultaneously, there is a division of the protoplast. It begins as an infolding in the plane of the girdle of the peripheral layer of the cytoplasm.

After the cell membranes are formed upon the completion of protoplast division, new siliceous valves, at first very delicate, are formed on each divided membrane.

The two connecting bands of the original girdle become separated, each forming one half the girdle of the daughter cells.

Owing to the formation of a pair of new valves within the girdle of the old ones, it would appear that the newer half of each successive generation becomes reduced in size by twice the thickness of a connecting band. Many earlier investigators believed that there was no way in which the new cell could expand, or that the new valves could increase in size, and that successive diminution followed a mathematical progression to a minimum size where either death resulted or regeneration through auxospore formation took place.

However, some of them observed that the successive diminution in size did not take place, at least not in all cases. They reasoned that since the new valves are only feebly silicified until they have been extruded beyond the confines of the connecting band of the old girdle, that further expansion could take place at that time.

Patrick and Reimer indicate that many modern workers have shown that successive diminution does not always take place as formerly thought, and that indeed cultures lasting over several years, in some cases, have shown no decrease in size. They further indicate that this is not necessarily a species characteristic, as differing clones of the same species showed differing patterns of growth in this respect.

Chromatophore division takes place during mitosis. According to Patrick and Reimer, if many small ones are present they divide equally into the new cells, and their division takes place later. When one chromatophore is present it divides longitudinally, and when two are present they may divide longitudinally or transversely. The pyrenoids increase in number by division.

When the new frustules are forming they do so “like ice on the surface of a pond” according to Taylor and are not “molded” as a metal in a die. If the valves of a frustule are different or dissimilar, the new valves appropriately form, each with its characteristic markings and protuberances. The silicate crust is formed gradually, the growth being from the center outwards. New girdles at the margins of the new valves are formed in due time, sometimes before and sometimes after the parting of the daughter cells. Taylor also says that in *Ditylum* the part first silicified is the central spine, and in *Climacosphenia* the markings grow from the smaller or basal end towards the apex or wide ends. In *Coscinodiscus Janischii* there is an increase in

structure from the center to the margin, the projections in the areolations becoming longer until the single areolations become two, and these further increase in size and number as the circumference is approached. Septa, at least in *Grammatophora*, appear to be formed subsequent to their adjacent valves.

The question arises of course as to how, or rather when, the protuberances such as processes, spines and awns are formed. Taylor indicates that the spines of *Ditylum*, *Corethron* and *Attheya*, and the awns of *Chaetoceros* are developed within the parent frustule in the course of division, and in the last two named genera they straighten out and silicify completely after release from the enclosing zone. In the case of *Corethron* he indicates they spread away from the body of the frustule after liberation.

Taylor's information mostly was obtained from such classical references as Van Heurck's Treatise, W. Smith's Synopsis of the British Diatomaceae and appropriate older journals, and not from direct observation. Details of the reproduction of most diatoms have never been observed, and probably the majority of such observations on record were made more than a half-century ago.

Reverend William Smith
(1808 – 1857)

Although, as mentioned previously, all diatoms do not show a progressive mathematically determined diminution, reduction in size does take place in most diatoms. The reduction in size has an influence on the shape and aspect ratios of the frustule, and should be an important consideration in the classification of forms, varieties, and even species. Geitler (1932) made studies on the cell size and shape of diatoms and found (in the pennates) that the smaller cells generated through successive vegetative divisions are not necessarily proportional in their various dimensions. He found the length of the apical axis decreases at a faster rate than the transapical dimension does and that smaller cells were therefore wider than the larger preceding ones. He found the same to be true of the relationship between the apical and pervalvar axis dimensions, thus making the smaller frustules "thicker", or relatively greater in the girdle dimension, than their predecessors. The form details also were noted to be modified in that the smaller cells were more rounded, and even that an increase in striation density accompanied a size decrease.

Professor Dr. Lothar Geitler
b. 18th May 1899
d. 1st May 1990

This leads to the conclusion that since various dimensions, their ratios, and valve details may be considerably altered during successive vegetative divisions, that designation of species should not be attempted without a full study of as complete a series as possible.

3.2. Auxospores

The rate at which cell division progresses, varies dependent upon many factors, not all completely understood. At some time, however rapidly the division takes place, there is a significant reduction in the size of the daughter cells until they reach a

point where they form auxospores which produce cells of a size near that of the original mother cell.

Investigations by Geitler (1932) have demonstrated that the auxospores are not formed by minimum-size individuals as once believed. Size alone is no longer considered the only factor instituting auxospore formation, but that physiological and ecological conditions are of considerable influence as well.

The point at which auxospore formation takes place must be a critical one. For in effect, to produce a larger individual, the resources of the cell must be at just the right physiological, chemical, and physical point. What combinations of conditions is suitable for initiating this action is not exactly known. Certainly it seems evident that more than one condition is responsible. If the triggering conditions do not correspond with the resources, there will be no auxospore and possibly the vegetative cycle will continue to a minimum size and ultimate death.

It seems evident, that auxospore formation is not a favored activity, but is an act of protection against extinction, Not an act that is favored by a plentiful - or more plentiful than usual, supply of nutrients. Nature is economical. The only reason for the release of energy and resources for auxospore production and the development of a larger individual, needing more energy for its own life cycle, is survival -- or a set of conditions that signify that survival is threatened. The economical natural method of reproduction would, on the face of it, be by the vegetative or cell-division method. There is good reason to believe that the non-sexual (self-division) mode of reproduction is the result of an abundant supply of nourishment, and the sexual method (or asexual) (parthenogenic or agamic) in the formation of auxospores is of a deficiency in this respect. This is more in conformance with the economy of nature, and further, auxospore formation is probably not instituted only by a nutritional deficiency, but by other factors as well that might indicate impending disaster.

Since size alone is evidently not a trigger for auxospore formation as once believed, then rejuvenance to a larger sized individual might take place at any size if the signifying conditions are extant. If we can assume that auxospore production can take place at any size, then it may be a basis to explain extraordinary size ranges found in some diatom species.

Whatever the conditions favorable for auxospore production are, the result is formation of special cells (auxospores) from which emerge cells of maximum size with new siliceous valves eventually bearing all the characteristics of the species. There are several methods of auxospore formation and in some a sexual process is involved where gametes are formed which fuse to produce a zygote, the zygote developing to produce an auxospore. Auxospore formation is complex, and although it has been detailed for some species, for most it is unknown or uncertain. Various authors have, over the years, separated the production of auxospores into a number of categories or classifications, usually numbering 4 or 5.

The classification following is taken from Patrick and Reimer who modified that of Geitler (1932) with the inclusion of the results of more modern research.

3.2.1. Normal Type A. Two mother cells each produce two gametes, which copulate in pairs to produce two auxospores.

3.2.1.1. The gametes are undifferentiated or isogamous; the apical ends of the auxospores are perpendicular to the apical axis of the mother cells. Examples are *Amphora coffeaformis, A. cymbelloides, A. ovalis, A. ovalis* var. *pediculus, A. pusio, A. veneta, Auricula hyalina, Denticula van heurckii, Epithemia sorex, E. turgida, E. zebra, E, zebra* var. *saxonica, Navicula radiosa, Rhopalodia gibba, R. gibba* var. *ventricosa, Surirella ovata.*

3.2.1.2. Each mother cell produces a wandering and resting gamete. The apical axes of the auxospores are parallel to those of the mother cell. *Achnahthes lanceolata, A. minutissima, Amphipleura pellucida, Anomoeneis sculpta, A. serians, Brebissonia boekii, Cymbella affinis, C. caespitosa* var. *pediculus, C. cistula, C. gastroides, C. helvetica, C. lacustris, C. lanceolata, C. parva, C. sumatrensis, C. ventricosa, C. ventricosa* var. I and II, *Frustulia rhomboides* var. *saxonica, Gomphonema constrictum, G. constrictum* var. *capitatum, G. geminatum, G. olivaceum, G. parvalum* var. *micropus, Navicula crucigera, N. cuspidata* var. *ambigua, N. directa, N. pygmaea, N. ramosissima, N. scopulorum, N. subtilis, N. viridula, Nitzschia hybrida, N. longissima, N. sigmoidea, N. subtilis, Pinnularia gibba, P. hemiptera, P. viridis, Rhoicosphenia curvata, Schizonema lacustrea.*

3.2.1.3. One mother cell produces two wandering gametes and one mother cell produces two passive gametes. The apical axis auxospores are parallel to those of the mother cell. *Navicula halophila, Synedra ulna, S. rumpens* var. *fragilaroides.*

3.2.1.4. The gametes behave according to no rule; the auxospore position varies. *Achnanthes brevipes, A. lanceolata, A. longipes, Denticula tenuis, Navicula didyma, N. fonticola, N. hybrida, Nitzschia longissima, Pleurosigma nubecula.*

3.2.2. Normal type B. Spermatozoa and an egg cell are formed. Produces two spermatozoids. One oogonium produces on egg cell. A spermatozoid enters the egg and a zygote is formed. *Melosira varians* is an example.

3.2.2.1. Four spermia are produced from a spermatogonia cell. One oogonium produces one egg cell. A spermium enters the egg and a zygote is formed. *Cyclotella sp., Biddulphia rhombus, Cerataulus smithii, Rhabdonema adriaticum*. In *Biddulphia granulata* two eggs per oogonium are formed.

3.2.3. Reduced Type A. Two mother cells each build one gamete, these fuse to form a single auxospore.

3.2.3.1. The gametes behave isogamously. *Cocconeis pediculus, C. placentula, C. placentula* var. *klinoraphis, C. placentula* var. *tenuistriata, Cymatopleura solea, Eunotia arcus, E. flexuosa, E. formica, E. pectinalis, Navicula cryptocephala* var. *veneta, Rhoicosphenia curvata, Surirella capronii, S. splendida, S. striatula, S. calcarata*.

3.2.3.2. The gametes behave anisogamously. *Navicula seminulum, Cocconeis pediculus, C. placentula, C. placentula* var. *pseudolineata.*

3.2.4. Reduced Type B. One mother cell develops an auxospore through automixis (the fusion of two nuclei within the cell).

3.2.4.1. Two gametes of one mother cell copulate with each other. *Achnanthes subsessilis, Cyclotella meneghiniana, Gomphonema constrictum* var. *capitatum, Gomphonema angustatum*.

3.2.4.2. The sexual nuclei of a mother cell copulate. *Amphora normanii, Chaetoceros borealis, C. densus, Grammatophora marina, Nitzschia palea*.

3.2.5. Reduced Type C. The auxospore formation is apomictic (parthenogenetic).

3.2.5.1. From one mother cell there develops through vegetative division two auxospores. *Cocconeis pediculus, Cymbella* spp., *Libellus constrictus, Rhabdonema arcuatum, Synedra affines, Tabellaria* sp.

3.2.5.2. From one mother cell (the mother cells may pair) there develops one auxospore.

3.2.5.2.1. Parthenogenetically. *Cocconeis pediculus, C. placentula, C. placentula* var. *klinoraphis, C. placentula* var. *euglypta, Cymatopleura elliptica, C. solea, Cymbella ventricosa* var. I, *Meridion circulaire, Navicula grevillei, Surirella gemma*.

3.2.5.2.2. Purely vegatatively. *Melosira* sp.

It will be noted that some species of diatoms produce auxospores through more than one method. For instance, *Cocconeis placentula* var. *klinoraphis* produces auxospores by two mother cells building a gamete which fuse and form a single auxospore or, one mother cell may parthogenetically develop an auxospore. There are other examples of multiple-mode auxospore generation by diatoms in the previous listings.

The foregoing classification of methods of auxospore production is one of many elucidated by various authors over the years, and represents a modern viewpoint based on recent investigations. There will be, no doubt, further revisions of such classifications as knowledge of the complex process is increased.

At the onset of auxospore formation, there is nearly always a considerable mucilaginous secretion which surrounds the cells. Whatever the mode of auxospore formation is, the auxospore cell may be very different in length, width, outline and certain markings from the vegetative cell. The number of striae, in some species investigated remains the same for mother, auxospore, and vegetative cells. However, in others there may be a less dense striae distribution in the auxospore. than in the vegetative cell. It is usually only after several successive vegetative divisions that the normal outline and other aspects of frustule details are recovered.

A brief word-picture of the process in one type of auxospore production is instructive. The frustule first surrounds itself with a thick layer of mucus which it secretes; then the cytoplasm dilates and under its pressure the two valves are separated. The cytoplasm set free in the mucilaginous mass surrounds itself with a membrane. This is the auxospore which rapidly increases in size and develops an outward shape more or less like the original cell. The wall soon becomes silicified and sometimes assumes the markings characteristic of the species at once. In other cases the individual characteristics only develop after several vegetative divisions.

It is not appropriate herein to describe in detail the various processes of auxospore production which have been observed in a number of species. The above is only a generalized description of one type of auxospore formation. References in the Appendix will need to be consulted for more detailed and specific information.

3.3. Resting Spores.

After a period of active vegetative life, in certain diatom genera there develops a type of body technically termed a statospore.

Hustedt indicates that resting spores are found only in the centric, not the pennate forms, and then primarily in pelagic diatoms. The usual structure of the vegetative cell is, for the most part, absent. The spore formation for different genera is also different.

In the freshwater diatom, *Attheya zachariasi* the spore forms through the contraction of the cytoplasm, in the central space enclosed by the girdleband. It then forms, one after the other, both thick-walled frustules, with the separate girdlebands missing. The pervalvar axis is very short. Upon the death of the mother cell, the spore becomes free and sinks to the mud at the bottom where it is usually found. Hustedt

further describes the formation of resting spores in the marine genus *Chaetoceros* as being similar to that of *A. zachariasi*, although the characteristic awns are missing in the spore. Instead, the spore surfaces possesses many short spines or are covered with longer spines, of which some may be drawn out into very bizarre forms.

In almost all cases the resting spores are generated in specific forms and with characteristics that are often indispensable in their determination by species.

The previous description of resting spore formation was that of what is considered a simple process. Some much more complicated processes are found in other species of diatoms such as in the genus *Melosira*.

Hustedt describes the formation of resting spores in the species *M. arctica* as an example of a complicated process.

Before the true origin of resting spores was known, many were described as individual diatom species or genera. Especially in fossil deposits, where the resting spores were found without any trace of the external walls of the mother cells, was this practice carried out.

Ehrenberg for instance, gave many of them generic names, *Goniothecium*, *Dicladia*, *Hercotheca*, etc. Many "species" of Goniothecium, for example, were found to be spores of *Chaetoceros*, *Hemidiscus*, etc.

Hustedt indicates that even today, many fossil genera occupy a rather insecure position classification-wise, because of our incomplete knowledge of resting spore formation and their origins. In some pelagic forms, especially, the frustules are so weakly silicified as to not be preserved at all, wherein the thick-walled spores are practically indestructible. This is reason to believe that perhaps forms now counted as separate genera may eventually prove to be resting-spores.

3.4. Microspores

The term microspore is antiquated, but is in use in so much of the older literature, it is used to head up this section. In current usage in botany it is defined as the smaller of two kinds of spores produced by a heterosporous plant; a spore which develops into a male gametophyte. In reference to diatoms, microspores are small motile cells that are usually referred to as spermatozoid cells (male gametes) in recent literature. Diatom spermatozoid cells have been known with certainty to have a single flagellum since von Stosch's research in 1951, although in some of the older references they are described incorrectly to have two flagella.

Prof. Hans Adolf von Stosch
b. 4th June 1908
d. 8th January 1987

The number of such spores produced varies with the species, but has been reported to be from 8 to 128 (the latter number in a species of *Coscinodiscus*). Most reports indicate they are formed by successive division of the nuclei, accompanied by a cleavage of the chromatophores after each nuclear division. The true nature and purpose of these bodies is not yet completely understood. Although Karsten (1904) described what he considered to be a complete cycle of their activity in the reproduction of *Corethron valdiviae*, little has been done since to further corroborate

his observations, or to provide conclusive proof of their ultimate fate in any species. Their true nature and purpose has yet to be shown, and much careful observation will be required to properly assess their role, if any, in diatom reproduction.

CHAPTER 4.

4. DISTRIBUTION AND ECOLOGY

The amount of literature on the distribution of diatoms environmentally and geographically is very great. Almost from the beginning, floral studies and lists have been made in large numbers relating to where diatoms live and under what conditions they thrive. It is not possible to cover all of this information in even an abbreviated way in this treatment. There are several excellent references which will do much to summarize the enormous volume of information on this aspect of diatom study. Particularly useful are publications by Kolbe (1932), Patrick (1948), and Cholnoky (1968). There are many others no less useful in specific geographic and ecological areas. A list of such references, and a commentary on their main points of usefulness in the study of diatoms, is included in Part III of this book. All that will be attempted in this chapter will be to merely outline the salient points of diatom distribution and ecology.

Béla Jenö Cholnoky (1899-1972)

The conditions essential for the growth of diatoms are moisture and light. Dependent also upon temperature and chemical conditions in their environment, they are found in all parts of the world at all latitudes, from the lower regions of the oceans to high altitude locations in mountains. Two major divisions of their environment may be made into marine and freshwater habitats. Marine genera and species are normally well separated from freshwater diatoms and those of one group are not usually found in the habitat of the other. However, at estuaries, in tidal pools and backwaters there are some diatoms which flourish in those environments that cannot be considered strictly marine or freshwater in nature. There are species which can live in either salt or brackish water, some are common to brackish and fresh water, and a few can live either in the sea or in fresh water. But as a rule there is a sharp distinction between marine and freshwater species both generically and specifically.

Marine diatoms found in the plankton are mostly centric, as for example *Chaetoceros, Biddulphia, Thallasiosira, Coscinodiscus, Ditylum*, and *Rhizosolenia*. Fresh water plankton diatoms are mostly pennate as in *Tabellaria, Asterionella, Synedra, Fragillaria, Nitzschia,* and *Surirella*, with a few centric forms, *Cyclotella, Melosira,* and *Rhizosolenia*. In fact, with few exceptions discoid diatoms are marine, and bacillar or naviculoid forms are common to both salt and freshwater.

4.1. Fresh and Brackish water Diatoms

The science that deals with life in inland waters and all the factors which influence it, is called limnology. To truly understand, in detail, the life of diatoms in fresh and brackish waters all limnological factors would have to be known. All of the processes of other forms of life, chemical and physical conditions extant, and their interactions is necessary for a complete understanding of diatom distribution. The following paragraphs will only present an outline of diatom distribution as to what

types of habitats diatoms occupy, and some of the chemical and physical factors relating to them.

4.1.1. Water Habitats

These habitats are considered as those in which the diatom is immersed in a water environment, as in lakes, ponds, rivers, streams, pools, and reservoirs.

4.1.1.1. Lakes

Lakes are comparatively large bodies of water with considerable depth as opposed to ponds. Because of their size they may support a "plankton", benthic (bottom), and epiphytic flora. The "plankton" diatoms of lakes are not true plankton as occur in marine habitats, but are neritic forms which spend the vegetative part of their existence afloat.

Patrick and Reimer divide the plankton diatoms into small forms or nanoplankton, and larger forms or net plankton, Examples of nanoplankton are *Stephanodiscus hantzschii*, *Cyclotella glomerata*, and *C. comta*. The net plankton, many in colony formations or of comparatively large size, are represented by such genera as *Rhizosolenia*, *Synedra* and *Asterionella*.

Epiphyte: Greek. epi - upon, Greek. phyton - a plant
Attached to a plant, anything from a tree to a piece of algae, for physical support, but does not draw nourishment from it.

Neritic:
Derived Latin. nereis - a sea-nymph.
Relating to the shallow waters along a coastline.

Benthic or bottom forms, are diatoms living on the substrate, in shallow or deep water, most being represented by diatoms with a raphe. Examples are species in the genera *Campylodiscus*, *Navicula*, *Nitzschia*, *Pleurosigma*, and *Surirella*.

Epiphytic forms are those which grow attached to other substrata such as submerged rocks or dead organic material. They may be attached by gelatinous stalks or stipes as are often found in species of *Cymbella* and *Gomphonema*, or more sessile-like as in the genera *Achnanthes* and *Cocconeis*. *Nitzschia* sp. has been reported as living within the tissues (endophytically) of sphagnum leaves, and certain species of *Frustulia* and *Eunotia* are known to often live in the tops of sphagnum plants.

This natural distribution of different species of diatoms in various parts of a lake has been of considerable use to the geologist. By studying the distribution of frustules in fossil deposits the contours of ancient lakes or lagoons may be determined.

4.1.1.2. Other Quiet Water Habitats

Aside from lakes, perhaps the most common quiet water habitats; are ponds, pools, reservoirs, bogs, swamps, and backwaters. All of these habitats support diatom flora of one kind or another, on a temporary or permanent basis, Some diatoms can

survive long periods of desiccation, provided they are not dried too rapidly, and in pools, ditches, and similar water environments that may dry up and subsequently be replenished, the diatoms soon revive and reappear.

In fairly large pools or ponds, especially those with some depth, a distribution of floating, neritic and benthic forms may appear, quite similar to the distributions found in lakes.

In reservoirs diatoms are encountered on the walls and other construction features that are covered by water, some causing problems including corrosion of concrete, filter clogging, and taste and odor in the water supply.

Diatoms living in bogs and swamps are usually represented by quite characteristic flora dependent upon the chemical, physical, and biological makeup of the bogs and swamps.

4.1.1.3. Rivers and Streams

Rivers and streams provide a habitat for diatoms in which the water is moving, at least to some degree. The water movement varies in most rivers and streams from very swiftly moving areas near the center to relatively quiet areas near the banks, including eddying and/or pooling water. The swiftness of the water flow influences various types of diatom appearances. For instance, in fairly swift water planktonic diatoms are inhibited, and slime forms are not able to grow under such conditions. In fast flowing streams the most successful diatoms are those that attach themselves to their substrate via mucus in stalks or masses. *Achnanthes, Cocconeis, Cymbella,* and *Gomphonema* are examples cited by Patrick and Reimer. They further indicate that the swiftness of the current may even effect the shapes of diatoms. As an example, *Desmogonium* in fast flowing waters is long with scarcely capitate ends, while in quiet water it is short with broad capitate ends.

River plankton is usually found where the current is much reduced, along the edges or in the stream bed or in eddies and pools near the banks.

Large rivers have well-developed plankton populations in regions of slower water movement, in the estuary, for example, whereas streams not having slower water regions generally do not support plankton diatoms to the same degree. Patrick and Reimer indicate the main plankton genera found in large rivers are *Asterionella, Cyclotella, Diatoma, Fragilaria, Melosira, Nitzschia, Synedra,* and *Stephanodiscus*, and in the United States particularly *Melosira, Cyclotella, Stephanodiscus, Fragilaria, Tabellaria,* and *Synedra*.

4.1.1.4. Other Moving Water Habitats

Springs (including hot springs) and waterfalls also support diatom populations, sometimes directly on rocks that are submerged in the running or bubbling water, and sometimes on substrates that are only moistened by splashed and sprayed water. The latter condition prevails along rivers and streams too, where rapid flows spray island and/or bank rocks, stones or soil. According to Patrick and Reimer the pool

spring is a much better diatom habitat than the spring which forms a waterfall, and they note that *Odontidium heimale* var. *mesodon* is especially suited to such habitats. Although a number of diatoms flourish in the hot springs of Nevada and California, living in temperatures of from 70 to 80°C., the usual flora of hot springs consists of eurythermal diatoms (ones that withstand a rather wide range of temperatures). In the Dutch East Indies the flora of hot springs has been found to be the same as that in running water of 45°C.

The Dutch East Indies became Indonesia following World War II.

4.1.1.5. Aerial Habitats .

Many diatoms are found living in locations where water does not immerse them continually and may only be supplied as spray, rain and/or snow, condensation, dew, seepages, and other non-constant and intermittent sources. Types of habitats included in this classification are mosses, tree trunks, damp stones and rocks, caves, leaves, and soil. Plants which thrive in air securing their water from rain or dew, such as orchids, are known as aerophytes. Diatoms which can withstand the rigors of widely varying water conditions, as those living in the habitats above, may be considered aerophilous forms.

According to Patrick and Reimer the following diatoms are found in:

a. rocks or moss kept wet by seeping springs;
Pinnularia borealis, Melosira roesena, Navicula fragilarioides, and *N. confervaceae*; also certain species of *Cymbella, Gomphonema, Synedra, Achnanthes,* and *Cystopleura*. (The latter genus name is not recommended for use by VanLandingham). (Most diatoms formerly listed as species of *Cystopleura* are recommended to be included in the genera *Epithemia* and *Rhopalodia*.)

b. very wet moss such as the tops of sphagnum;
Species of the genera *Eunotia* and *Frustulia* are abundant.

c. caves;
Fragilaria construens var. *venter, Melosira dickiei, M. roseana, Navicula Kotschyi, N. perpusilla,* and *Pinnularia borealis*.

d. spray zones (lakes, rivers, etc.);
Achnanthes, Cymbella, Gomphonema, Epithemia, and *Denticula*.

Diatoms are less often encountered in woods soil than in field or garden soil, and are most plentiful in the top centimeter. *Hantzschia amphioxys, Navicula atomus, N. nitrophila, N. mutica, N. contenta* f. *biceps, Pinnularia habifouriana, P. brebissonii,* and *P. borealis* are the more common soil species,

George Krasske
(1889 – 1951)

According to Patrick and Reimer, Krasske (1929) reported *Eunotia fallax* var. *gracillima*, *Melosira Dickiei*, *Navicula contenta*, *N. Krasskei*, and *N. sohrensis* living among protococcus on dry rocks.

In dry moss some very small forms occur. Included are *Navicula contenta* var. *parallela*, and var. *elliptica*, *N. mutica* var. *cohnii* instead of *N. mutica*, and smaller forms of *N. fragilarioides*, *Pinnularia borealis*, and *Melosira roeseana* rather than its long filamentous varieties.

4.1.1.6. Chemical and Physical Factors

Chemicals important in diatom distribution are calcium, iron, silicon, nitrogen, sulfur, copper, and chromium. Some of them inhibit growth of certain species and some of them are essential for any diatom growth. The hydrogen-ion concentration (pH) is one of the most important chemical factors influencing diatom occurrence and distribution. So important in fact, that several workers have been able to group diatoms according to their occurrence in waters having specific pH ranges. Most diatoms have some tolerance over a range of pH, as it varies at least somewhat throughout the day, usually reaching its highest value in the afternoon. The effects of light, temperature, and turbulence are also quite influential as to the distribution and occurrence of diatoms. The possible combinations of interactions of chemical and physical factors influencing diatom growth are enormous in number.

A means of summarizing this very great number of ecological possibilities is through diatom spectra. Diatom spectra are compilations of the physico-chemical tolerances of individual diatom species. These spectra when plotted against species abundance, can reflect the ecology of an assemblage of diatoms.

A listing of these groups illustrates the various physical and chemical factors considered important in the distribution of diatoms.

pH Spectrum (Hustedt 1937 - 39)

1. Acidobiontic forms - found at pH lower than 7 with their optimum of development at a pH below 5.5.
2. Acidophilous forms - found at pH of about 7, but with their optimum below 7.
3. Indifferent forms - appearing at a pH of approximately 7.
4. Alkaliphilous forms - also appearing at a pH of approximately 7, but with the optimum above 7.
5. Alkalibiontic forms - appearing in only alkaline water.

Current Spectrum (Hustedt 1937 - 39)

1. Limnobiontic forms - especially appearing in stagnant water.
2. Limnophilous forms - having their optimum development in stagnant water.
3. Indifferent forms - widespread both in stagnant and running water.

4. Rheophilous forms - having their optimum in running water.

5. Rheobiontic forms – in a special way connected with running water.

Halobin Spectrum (Kolbe 1927)

1. Oligohalobous forms - mainly widespread in fresh water i.e. water containing less than 5% of salt.
    a. Halophobous forms - almost shunning salt, found particularly in water deficient in chloride.
    b. Indifferent forms - freshwater forms proper.
    c. Halophilous forms - mainly widespread in freshwater but thriving reasonably well in slightly brackish water.
2. Mesohalobous forms - brackish water forms - mainly widespread in water containing 5 - 20% of salt.
3. Euhalobous forms or marine forms proper - having their optimum development in water containing 30-40% salt.

Saprobic Spectrum (Collingsworth et al 1967)

R. C. Collingsworth

1. Polysaprobic zone - typical of the zone of decomposition, little or no oxygen.
2. Mesoprobic
    a. Zone A - zone of oxidation, amino acids abundant.
    b. Zone B - zone of terminal oxidation, final recovery zone.
3. Oligosaprobic - clean water zone.
4. Katharobic - pristine water zone.

Habitat Spectrum

1. Plankton forms - generally found in the plankton.
2. Benthic and epilithic forms - found living on the bottom or attached to rocks.
3. Epiphytic forms - attached to other plants but not growing parasitically on them.

In this spectrum in some cases diatoms might be assigned as euplankton (belonging to the true plankton), tychoplankton (spending part of its life on the bottom), and littoral (being found in the littoral zone).

Nutrient Spectrum

1. Eutrophic forms - found in relatively shallow lakes and ponds rich in organic matter and nutrients with oxygen depletion occurring in the hyolimnion during the summer.

2. Oligotrophic forms - found in deep lakes where the waters are continuously oxygenated and the organic matter produced in relation to water volume is low.
3. Dystrophic forms - found in lakes that are low in calcium carbonate, high in humus content, and very poor in nutrients.

4.1.1.7. Geographic Distribution

It can be said that diatoms are cosmopolitan, and that generally wherever there is water they will be found. It is also true that many genera and species of diatoms are more or less cosmopolitan, occurring in many parts of the world. It is not possible to separate geographical and environmental factors in the considerations of diatom distribution. Where diatoms thrive, they do so by reason of the living conditions extant at that particular location. On the other hand, there are certainly locations favorable, or even ideal, in many parts of the world, for some species of diatoms to flourish, yet they are not found there. The worldwide distribution of diatoms is, of course, influenced by the means by which they may be transported from one location to another. Diatoms may be distributed by winds, ocean currents, geological events, birds, animals, and by man himself with his many modes of transportation. So little is known about what diatoms do exist in certain regions of the world that any attempt at describing geographic distribution must be extremely superficial. The following information must be regarded in that light.

Certain genera, species, and groups of forms occur in certain deposits, or countries, and others seem to predominate in certain localities and material. In the same country different regions may contain fairly typical assemblages. This is especially true in countries having a variety of climates and/or terrain features. The United States has humid (forest), subhumid (grassland), semiarid (steppe), and arid (desert) areas which contributes to the differences of diatom flora in the eastern, central, and western parts of the country. On the other hand, there is a similarity between the flora of the eastern U.S. and Western Europe, New Zealand and Great Britain, and between Campeachy Bay and the Phillipines. In South America, for similar reasons, there seems to be a variation in the endemic genera and species according to the region investigated, and there appears to be definite flora differences between northern and southern Africa.

Because of the dearth of information regarding diatom floras in many parts of the world, really definitive comparisons cannot be made. However, the student can gain considerable information on particular areas by consulting the literature e.g. Europe, central Europe in particular has been reported on extensively, including Germany, France, Holland, Switzerland, Austria, Hungary, and Belgium. Great Britain and the countries of northern Europe such as Sweden, and Finland are also represented as well. Patrick and Reimer's work on the United States promises to be a very valuable reference, and there are new and important works appearing on the diatoms of the USSR, and Southern Africa too.

4.2. Marine Diatoms

The habitats of marine diatoms are mainly two; those of the littoral or near shore areas, and the deep sea. The littoral species grow attached to, or are closely associated with bottom-features, and the deep sea diatoms are strictly planktonic (pelagic), spending their existence afloat.

Factors affecting marine diatoms are similar to those of fresh water types; namely physical and chemical. Temperature, water viscosity, water salinity, light, and nutrients. Water viscosity varies with temperature and affects the ability of diatoms to float, an important factor in planktonic life.

It has long been recognized by oceanographers that the different seas and oceans of the world have different characteristics both physical and chemical. Although the waters are contiguous between the Atlantic, Pacific, Arctic, and Antarctic Oceans for example, they all exhibit unique and individual characteristics.

The diatom flora of the various oceans reflects some of these differences. There are distinct Arctic and Antarctic floras, and the Temperate and sub-Tropical floras differ to a large extent.

Tropical forms as a class are large and elaborately ornamented and excel in the variety and beauty those of more northern regions, and there is no preponderance of naviculoid forms, elongated diatoms of rather plain design, which occur in large numbers in colder waters.

In the Antarctic seas, in the region of pack-ice, diatoms are a major constituent of the surface life of the sea. At times the water, and even the ice, is a dull yellow color from the vast number of diatoms contained therein.

The Arctic seas show the same abundance of diatoms, sometimes to the extent that the surface of the sea appears colored green for several acres. The flora of the Arctic is generally quite distinct from that of the Antarctic. For instance, *Triceratium arcticum* f. *balaena*, and *Campylodiscus helianthus* are characteristic in the Arctic. On the other hand the freshwater diatom *Fragilariaopsis antarcticum* found on Antarctic icebergs and ice-floes, is also found in material from the Atlantic deeps, probably because the ice has been transported into those warmer waters to melt.

Though diatoms are found as pelagic forms on or near the surface of the oceans, a greater diversity of forms appears as the littoral zone is approached at coastlines. Diatoms in the littoral zone are called neritic, those living farther out at the lowest depths, benthonic.

The Atlantic and Pacific Oceans have different floras, and in certain areas of those oceans where upwelling occurs, prolific diatom growth is evident. In the equatorial Pacific for instance, in the general vicinity of several degrees north and south of the equator, the equatorial currents and countercurrents promote upwelling and consequent nutritional enrichment of upper waters. There appears to be a connection between this situation and the abundance and size distributions of certain diatoms deposited in the bottom sediments.

Limits of ocean depths at which diatoms flourish is dependent to a great extent upon the depth to which light penetrates the water. In tropical regions, the greatest

abundance of diatoms is at between 100 and 400 meters. Mann says the limit is probably something below 100 fathoms, and Gran found plankton most abundant between 10 and 20 meters below the surface, but rarely below 100 meters.

Haakon Hasberg Gran
(1870 – 1955)

Diatom surges or blooms occur in the seas as well as in freshwater habitats. It has been noticed that in early spring when water is at its coldest, the upper layers of the ocean will suddenly become filled with myriads of diatoms, which after persisting for a few weeks, disappear as suddenly as they come; and again in the autumn a second maximum of diatoms might occur. Sometimes in different months different forms are prevalent, giving distinct characters to the gatherings. For instance, according to Taylor at Hong Kong in January very few diatoms are obtained. In February the *Coscinodiscae* are most plentiful. In March and April numerous species of *Rhizosolenia* and *Chaetoceros* make their appearance, and about the end of April nearly every diatom has disappeared.

CHAPTER 5.

5. CLASSIFICATION

Current systematic classification of diatoms is based on the general and detailed structure of the cell wall as it is revealed by the light microscope. The transmission and scanning electron microscopes (TEM, SEM) must be considered an extension of the light microscope in classification and identification. The use of scanning electron microscope micrographs can aid the diatomist in consistently identifying the same species. Also, continuing research, especially with the SEM, may in time revise a number of concepts regarding diatom cell wall construction and eventually form a basis for re-classification of at least certain genera or species.

The first formal attempt at scientific classification of the diatomaceae was made by Agardh in 1824. From that time to the present there have been many major classification schemes proposed and in use by various authors. In the early years of the development of classification schemes the diatomaceae were generally regarded as having an animal nature although Ralfs in 1845 was inclined to the opposite opinion. The development over the years of the various schemes of classification has been based on external morphology. That this base is not a natural one is recognized, but explainable because of the great preponderance of fossil diatoms with respect to the total number of individuals in addition to the large number of species. Also, most of the fossil species still exist in the living condition, further promulgating the convenience of a taxonomy based on external characteristics.

Carl Adolph Agardh
(1785-1859)

John Ralfs
English Botanist
b. 13th September 1807
at Millbrook, near
Southampton, England
d. 14th July 1890
Penzance, Cornwall,
England

Schemes proposed to base genera and species entirely on ecological and/or growth pattern criteria; are not consistent with the traditional external morphology doctrine of classical diatom taxonomy. A classical definition of a species is that it is an aggregate of individuals which have been proved to have descended from a common ancestor, or are so similar to one another that they may be presumed to have done so. In diatom species determination this is done on the basis of cell wall characteristics alone, disregarding internal cytological criteria. That this situation will eventually have to be rectified is clear. However, it will be very dependent upon extensive and lengthy life history, and other biological investigations, of possibly tens of thousands of species. It will not be a rapid change.

DNA ‘fingerprinting’ is revolutionising taxonomy and the ‘change’ is set to become more rapid than ‘Mac’ could have envisaged. However, forms known only from fossil deposits still have to be ‘shoe-horned’ into the classifications.

Meanwhile, the student of diatoms must be content with classification mainly based on external features of the frustule. Even this systematic taxonomy is in a state of flux, and several major schemes are in common use. It seems as though nearly every

new major contribution to diatom literature includes a new classification scheme or at least a modification of an existing one.

The student can do nothing more than recognize the fact of extreme confusion in this area and deal with it accordingly, on a case by case basis. In making generic and specific determinations he must reconcile himself to dealing with a number of different classification methods and their accompanying synonomy. In making original determinations of new species or genera he is wise to "adopt" one of the current classification schemes to work within.

The earlier classifications of diatoms were based mainly on the shape of the frustule. Other following systems took into account the growth of the cells and arrangement of the endochrome. The latter considerations could not be applied fully to species known only as fossils. Divisions of diatom shapes considered as centric (having a structure that is arrayed with reference to a central point), and pennate (feather-like with structure arranged each side of a median line), are also considered in a number of classification schemes. The absence or presence of a raphe has also been an important differentiation in diatom taxonomy. The movement or non-movement capability of diatoms has been used as a point of differentiating diatom groupings. Habitat, mode of living, size, exterior structure, interior structure; outline of form, and distribution of the endochrome have all been considered as important distinguishing features upon which to base a system of classification. Not all of these can be applied across both living and fossil forms, and some of them are not stable enough to support firm classification formats.

The object of classification is to ultimately sort diatoms into small groups so as to render their recognition and identification easy. One may suspect that taxonomy is a reflection of the order-seeking mind of man rather than a reflection of a natural state. This statement is particularly true in the classification of diatoms because of difficulties in correlating living and fossil forms, and the very great number of species.

In establishing any scheme of classification, the use of constant characteristics is very important, and trifling differences are not. Occurrence of characteristics in a great number of forms is important and a basis for classification. The following paragraphs will briefly touch on the relative importance of various diatom characteristics for taxonomic purposes.

5.1. Mode of Growth

This refers to the habit and would include the attached, unattached, planktonic, etc. types of existences diatoms live. Cleve thought this to be a matter of little import for taxonomic purposes as in many cases the same species of diatoms are found at times to be attached and at others unattached. A variable such as this is very difficult to encompass in a classification.

5.2. Size

Limits of dimensions are generally thought to be fairly definite, the larger forms of each species being twice as large as the smallest. However, there are numerous exceptions to this "rule", and many "species" have been established entirely on the basis of size alone, a very questionable practice. Specific exceptions to the "twice as large as the smallest" view are *Amphora ovalis*, *Achnanthes brevipes*, *Pinnularia viridis*, *Coscinodiscus nodulifer*, and *Nitzschia dissapata*, to name but a few. Too few observations and measurements have contributed to our misconceptions on size ranges. More thorough study of diatom series, investigations of their nutrient requirements for growth, and the complexities of auxospore formation are needed to clarify this characteristic and assess its true taxonomic value.

5.3. Form of the Frustule.

The shape of a diatom frustule is fairly constant and an important characteristic. However, there are some variations which should be allowed for which in times past have been the basis for the establishment of numerous "varieties", and even new "species". Teratological forms of diatoms are perhaps uncommon, but certainly not rare. The effects of both physical and chemical excesses or deficiencies are known to contribute to these monstrously shaped and disfigured frustules. Why the more subtle changes in the shape of the frustule continue to generate large numbers of varieties is not clear. If the form or shape of the frustule is taken as an **individual** identifying characteristic of a diatom rather than **the** identifying characteristic, the proliferation of varieties can be, at least, somewhat curbed.

5.3.1. Zone

The zonal aspect of diatoms has little variation and is an important characteristic that has been too much neglected in classification. The complexities of the zone are important features taxonomically that should be used more often.

Zone:
Greek. *zone - a girdle.* This word used alone refers to the girdles and the sides to which they attach. It is also used to refer to an area surrounding another feature.

5.3.2. Symmetry or Asymmetry.

As a generic distinction this may, when combined with other characteristics be of value.

5.3.3. Central Nodule.

As a specific characteristic it is in most cases very valuable, as it is constant in the same species. It assumes definite and readily identifiable generic forms as *Stauroneis* (extends transversely), *Diploneis* (into horns following the median closely), *Frustulia* (enclosed between siliceous ribs), *Amphipleura* (forks resembling

those of *Diploneis*), and *Navicula lyratae* (horns distinct from median-lyriform with large lunate areas).

5.3.4. Raphe

This is very valuable specifically, but not of sufficient constancy for generic distinction. Many Naviculoid forms of differing genera have similar raphe types.

5.3.5. Terminal Fissures.

The direction the terminal fissures take with respect to the median is a specific characteristic of great value. They usually take the same direction in a given valve in the *Naviculae*.

5.3.6. Axial and Central Areas.

The form, presence or absence of these characteristics is valuable specifically.

5.3.7. Longitudinal Lines.

The presence or absence of longitudinal lines appears to be of great importance generically. *Diploneis*, *Caloneis*, *Amphora*, and *Pinnularia* are examples in which this characteristic plays a minor classification role.

5.3.8. Valve Structure.

Puncta, striae, alveoli, costae, septa, and ribs are of great importance both specifically and generically. Their placement, arrangement, number and direction with respect to reference axes of the frustule and/or valve, are subject to only slight variations.

5.3.9. Cell Contents.

The endochrome including the chromatophores and other bodies usually exhibit position and form that is fairly constant for groups of allied species. However, these characteristics are not known at all for fossil forms, nor for most living forms.

Cleve considered characteristics such as valve structure, presence or absence of longitudinal lines and the nature of the non-striate parts of the valve to be paramount in classification.

As to where to limit the detailing of minutiae and whether or not classifications should account for them is a question that most authorities have various opinions on. Although most agree that there is a necessity to condense or limit the number of species,

Sir Nicholas Yermoloff KCB, KCVO, FLS.
Military Attaché at the Russian Embassy
b. circa 1854 Russia
d. 1924 London

it is difficult to argue against recording and describing the most insignificantly appearing details, as in the words of Yermoloff "It is a process of differentiation, the reverse process of synthetic grouping would be impossible without it". Notwithstanding this latter concept, the tendency is for modern workers to streamline and adjust the taxonomy to a less cumbersome form.

The various classification schemes devised in more recent times are formulated in accordance with the International Rules of Botanical Nomenclature as revised from time to time. These rules are established to aim at a fixity of names, to avoid or reject the use of forms and names which may cause error, ambiguity or confusion, and to avoid all useless creation of names. Scientific names are in Latin, and if taken from another language a Latin termination is given to them, except in a few cases sanctioned by custom. Probably the majority of diatom names are Greek in origin, followed by Latin, and then by other languages.

5.4. Classification

The classification proposed by Hendey (1964) is an example of modern thought. It assigns every individual diatom to a species, every species to a genus, every genus to a family, every family to a suborder, and all suborders to an order, the order to a class, and the class to a division. The system within this concept is as follows:

Since the original work Classification schemes have been revised. The reader is advised to consult later documentation.

Division Chrysophyta
Class Bacillariophyceae
Order Bacillariales
Suborder Coscinodiscineae
Family Coscinodiscaceae
Family Hemidiscaceae
Family Actinodiscaceae
Suborder Aulacodiscineae
Family Eupodiscaceae
Suborder Auliscineae
Family Auliscaceae
Suborder Biddulphineae
Family Biddulphiaceae
Family Anaulaceae
Family Chaetoceraceae
Suborder Rhizoeoleniineae
Family Leptocylindraceae
Family Corethronaceae
Family Rhizosoleniaceae
Suborder Fragilariineae
Family Fragiliariaceae
Suborder Eunotiineae
Family Eunotiaceae
Suborder Achnanthineae
Family Achnanthaceae

Suborder Naviculineae
Family Naviculaceae
Family Auriculaceae
Family Gomphonemaceae
Family Epithemiaceae
Family Bacillaraceae
Suborder Surirellineae
Family Surirellaceae

It will be noted that the family name is the generic name with an ending of -aceae, and the suborder with an ending of -ineae. For instance, the genus *Surirella* is in the family *Surirellaceae* and the suborder *Surirellineae*.

Other classification systems, instead of incorporating Division, Class, Order, Suborder, Family, and Genus, might be constructed as Division, Subdivision, Order, Family, and Genus. In some cases subfamilies are included in the structure. The variety of systems that have been used, and are still in use, reflect the thinking of the particular diatomist/taxonomist as to what the major divisions are. In older taxonomic structures especially, the genera were divided into sections and families into tribes instead of the more modern subfamily. The student will encounter all of the variations possible in working with the diatom literature of the past and present.

As to what system of classification is best is a perplexing question. Some older classification schemes live on, or have been revised by modern workers. The diatomists who have published extensively support and promote their systems by the sheer volume of their work. A classification system is not sacrosanct as it represents only the opinion of the one who made it, and need not be accepted without doubt. Its acceptance will depend upon the validity of the arguments advanced in its support, and the weight attached to it will depend upon the reputation of the originator.

There are a number of classification schemes that divide diatoms into centrate and pennate types. “The divisions of the Centricae and Pennatae are so fundamental, probably representing two lines of descent, that they cannot be disregarded. Also, in view of the fact that all centric diatoms are non-motile, it might be much wiser to restrict the differentiation between motile and immotile forms entirely to the Pennatae. The differentiation would then coincide with the degree of development of the raphe, which is without doubt the most important morphological structure of the pennate diatom”. The preceding quote is part of the argument advanced by Schuett and West upon their modification of Mereschkowsky’s scheme of Mobiles and Immobiles.

F. Schuett

Friedrich Hustedt, probably the most energetic diatom worker of all, and the originator of an immense literature on diatoms, follows a basic Centricae/Pennatae scheme in his works. It seems therefore important to include that which he adopted herein, as the student of diatoms will, without doubt, be using these most important references.

The basic two divisions are the Centricae and Pennatae. They are defined as follows:

5.4.1. Centricae.

Frustule centric, rarely of bilaterally symmetrical construction, with concentric, radial or irregular, never pinnate structure. Raphe or pseudoraphe missing. Valve surface circular, polygonal or elliptical, rarely boat-shaped or irregular. Auxospore formation always asexual, microspores and resting-spores observed in some species.

5.4.1.1.Centricae

i. Discoideae

Frustule disc-like, flat or cylindrical, Transverse section usually circular; as a rule without horns and outgrowths.

ii. Solenoideae

Frustule rod-like cylindrical, many times longer than a diameter. Transverse section usually circular.

iii. Biddulphiodeae

Frustule box-shaped, valve with two or more poles, usually with horns or outgrowths thereon.

iv. Rutilaroideae

Frustule boat-shaped with an irregular or radiating structure.

5.4.2. Pennatae.

Frustule truly bilaterally symmetrical. Constructed with and almost always pinnate, rarely irregular form. Raphe or pseudoraphe present. Valve surface for the most part rod- or boat-shaped. Auxospore formation sexual or reduced, microspores not found with certainty.

The following is freely translated from the German and is taken from the Hustedt "key" format.

5.4.2.2. Pennatae (defined above)

(1). Araphideae

v. Fragilarioideae

Any apical oriented valve fissures missing. Pseudoraphe present.

(2). Raphidiodeae

vi. Eunotioideae

A rudimentary raphe present, running from a weakly developed terminal nodule for a short distance toward the center. Central nodule missing.

Peronieae

The rudimentary raphe on only one valve.

Eunotieae

Both valves with a rudimentary raphe.

(3). Monoraphideae

One valve with a full developed raphe.

vii. Achnanthoideae

(4). Biraphideae

Both valves with a full developed raphe.

viii. Naviculiideae

Raphe in the para-apical axis, without keel, or if keeled, without keel puncta.

ix. Epithemoideae

Raphe not on the para-apical axis, sometimes on an excentrically disposed keel without keel puncta.

x. Nitzschioideae

Both valves with a canal raphe, keel puncta or wing canals present, central pores missing. Each valve with an often excentrically disposed keel as a continuation of the valve surface.

xi. Surirelloideae

Frustule with two side-disposed wings, on which the canal raphe runs.

In the present state of knowledge on the biology of various genera, the many systems of classification advanced thus far are based a great deal upon arbitrary factors. Nonetheless, this does not detract from their usefulness in conveniently dividing various forms into definite and easily recognizable groups. This is one of the principal objects of classification and further improvements of the system(s) in use will undoubtedly result from electron microscope research and continuing biological studies of the diatom.

CHAPTER 6.

6. USES OF DIATOMS

6.1. In Nature

Diatoms are probably the greatest fundamental food supply for the marine world, and of almost the same importance for freshwater life. They are at the beginning of a food chain which ultimately is a very major factor in the welfare of the human race. They are used as food by lower animal forms and crustaceans in the oceans which in turn support fish and other larger marine life. They form the chief support of the pteropods, medusae, copepods and ostracods which live in their company, and are also one of the principal sources of nourishment for molluscs, oysters, and holothurians, whose stomachs always contain quantities of these plants.

Diatoms are probably the most important of all the algae groups, and the most abundant in the total number of individuals produced. They are the most important group in the plankton flora and probably photosynthesize more food than the rest of the plant world combined.

In their life process they liberate oxygen and because of their large numbers contribute considerably to the purification of the waters in which they live. This in turn improves conditions for the purposes of higher life forms. This influence alone is of inestimable value in the quantity and quality of all life forms throughout the world.

6.2. In Science

Scientifically diatoms are interesting in many ways, and are becoming increasingly important in specific areas of biology and geology. The “ubiquitous diatom”, as it has often so appropriately been termed, exhibits some curious, puzzling, or suggestive geographical distributions. Some forms are distributed over most of the world, others strictly endemic to certain areas, or to specific oceans. Just as other floras vary from country to country, so diatoms may help to indicate the place of origin of the material containing them. This is particularly so in fossil deposits many of which have a distinct diatom flora. For instance, material from Oamaru, New Zealand is easily differentiated from that of California deposits. Living diatoms of certain genera or species show remarkable endemism in even the same country, as has been mentioned in a previous chapter.

In oceanography diatoms have been of considerable use in the measurement, direction, extent, and velocity of ocean currents. Their small size enables them to be carried for hundred or thousands of miles. A bed of diatoms some 1200 miles long and 20 miles in breadth consisting of *Coscinodiscus rex*, lying between the islands of Guam and Luzon (125 to 145 degrees longitude East) can only have been formed by long continued and constant currents. The proliferation of diatoms within a few degrees of the equator in the Pacific Ocean is indicative of upwelling, and thus improved nutrient conditions, as a result of currents and countercurrents. Study of

diatoms in deep sea cores in the same vicinity are being made to assist in expanding knowledge of paleoclimatology.

From an examination of the species or genera contained it is generally possible to say whether a deposit was formed in fresh, brackish or salt water.

Diatom presence in general may denote the potability of water, or in some cases, of its poisonous nature to humans. The diatom as an indicator of ecological conditions in lakes and streams is very important. In recent years, great advances have been made in methods to assess water quality, lake, stream, and river pollution, and in the detection and control of industrial wastes affecting our water resources. It may be that the use of diatoms as ecological indicators in general will be their greatest scientific use to man.

In geology, diatoms may indicate the location of primeval lakes or seas, the contours of their beds may be indicated by the presence of pelagic or littoral forms, and the climate at the time, whether temperate, tropical, or arctic may be ascertained.

Some unexpected, normally brackish water, species can sometimes be found in waste water gullies and canals, due mainly to the influx of water contaminated by road salting during the winter.

We cannot ignore the importance of diatoms in another science related area. It is in the development of the microscope itself. Even many diatomists of today are unaware of the important role the very plants they study with the microscope has had in the advance of the instruments optics.

Diatoms were objects of microscopical examination in the very early days of microscopy. Diatomists, that is persons specializing in the scientific study of diatoms, were as rare then as today. However, there were quite considerable numbers of amateur microscopists who were intrigued with the beauty and geometrical symmetry of these plants. Enthusiasts pressed their microscopes to the utmost to determine minute structure and demanded better and better instruments from the makers. The market for microscopes during those early days was, in a large measure, dominated by these enthusiasts, and manufacturers did all they could to improve their instruments in accord with their patrons interests. There can be no doubt that the endeavor to make visible the finer structure of diatoms, ranging in some instances to 100,000 to the inch, gave rise to a rivalry, which has worked marvels in the improvement of microscopes and lenses. To this end certain diatoms were used as delicate tests for the perfection of lenses.

John Dawson Sollitt (5th October 1794 – 1868). Headmaster of Hull Grammar School

Jacob Whitman Bailey (1811 – 1857) American Naturalist

Taylor says it is claimed that their suitability for this purpose was first discovered by J. D. Sollitt, a Hull (England) microscopist, in 1841, when lines were seen on *Pleurosigma hippocampus*. Specimens were sent to various London microscopists, Smith, Ross, Powell and Lealand, to Nachet in Paris, and to Professor Bailey in the United States. In ensuing years microscope and lens makers often indicated diatoms that could be resolved by their objectives. A veritable family of diatoms, in a mix of genera and species, became known as “test Diatoms”. For

instance, *Pleurosigma angulatum*, remarkable for its constancy of markings, was known to every microscopist or user of optical instruments, and has long been, and even today is, an integral part of literature bearing on applied microscopy.

Although in more recent times other means of testing microscope objectives are used, *Pleurosigma angulatum* still appears in the literature from time to time in discussions involving microscope optics. As an example, a quite lengthy article in *The Microscope*, entitled "Light and Electron Microscope Studies of *Pleurosigma angulatum* for Resolution of Detail and Quality of Image", appeared in 1966. In the *Encyclopedia of Microscopy and Microtechnique* by Peter Gray (1975), *Pleurosigma angulatum*, *Amphipleura pellucida*, and *Surirella gemma* are represented in light microscope photomicrographs and TEM micrographs, as illustrations in the discussion on resolution.

The use of diatoms for actual testing of objectives is now outmoded, but they are still excellent objects for the training of microscopists. The adjustment and manipulation of the light microscope to resolve certain detail in specific diatoms must be very precise, and is a test of the microscopists technical ability with his instrument. It is fair to say a good diatomist must be a good microscopist. Anyone who uses a light microscope in any field can benefit by resolution exercises on diatoms.

6.3. In Industry

Probably there are over a thousand specific uses for diatoms in industry. The structure of diatom frustules when in mass-multiple aggregation provides unique properties obtainable in no other way.

The raw material which is generally suited to industrial purposes is called diatomite, or diatomaceous earth. Large deposits of both fossil-fresh and fossil-marine diatoms are mined in various countries of the world. The United States is the leading, if not dominant, producer of diatomite. Within the U.S. the principal producing States, in order of production are California, Nevada, Washington, Arizona, and Oregon. Other countries which produce significant quantities of diatomite are France, Germany, Italy, and Denmark.

Diatomaceous earth may be distinguished from other formations of a similar appearance by its insolubility in acids, its extreme lightness, power of absorbing liquids, and its properties of polishing metals. It is usually whitish, or light in color.

Its principal uses are in filtration, insulation, fillers, and in abrasives. Depending upon the particular diatom constituency, diatomaceous earth is sometimes porous enough that it can absorb an amount of liquid equal to three-quarters its gross volume. Probably its unique properties will, by the very nature of them, not be duplicated by man. It will continue to be desirable for many industrial uses because of its lightness, purity, inertness, heat resistance, and high porosity.

It is not possible in a limited space to list all of its uses. However, in the following paragraphs an attempt will be made to indicate its broad scope of application within several major areas.

6.3.1. Filtration

It has been used in the filtration of water, beer, acids, gelatine, syrups, milk, oils, and penicillin. In filtration procedures in the sugar industry it has been added to sugar solutions as an anti-clogging aid.

6.3.2. Insulation

Diatomaceous earth has been used successfully in the insulation of blast furnaces, boilers, kilns, ovens, and houses. Bricks of diatomaceous earth insulate and improve the efficiency of fireplaces. It also serves as a sound insulator as well as a heat insulator. Diatoms have been used in soundproofing material for room partitions and ceilings and for improving acoustics in general. Additionally it is, or has been used as packing for refrigerators, steam pipes, and insulation of cold storage facilities for preserving meats and fruit.

6.3.3. Fillers

Diatoms are used as fillers in paints, imparting a softness or luster, and to add a quality of hardness to silica paints for surfacing concrete and bricks. It is useful as a bonding and reinforcing material in paints, ceramics and plastics because of its adherent and interlocking qualities. When washed and calcined diatoms have been used in the manufacture of dynamite under the names of Randannite and Kieselguhr. Dynamite in this case is essentially nitro-glycerine absorbed into the interstices of dried diatomaceous earth. It serves to insulate in its minute cavities small particles of nitroglycerine in such a way as to render the liquid practically solid, and at the same time to obviate the danger of free nitroglycerine being exploded by shock. At an early time when dynamite as such, was used as a propellant for projectiles in guns, the diatomaceous earth scored the gun barrels and it was therefore necessary to use a substitute. It has also been used mixed with tar for roofing to prevent running and to render the tar less sticky in the hot sun, and thus improve resistance to cracking or checking.

It is sometimes employed as an absorbent in the manufacture of colors, sealing wax, and rubber erasers. When mixed in small quantities with rubber it has the property of increasing its strength and resiliency. It also has been used as a substitute for Fullers earth in the manufacture of cloth. It has been used as a pigment for paints used by the Indians of the Southwestern United States in decorating pottery. Additional filler uses include mixture of diatoms in mineral pastes, soaps, stucco, white lead, drain pipes, plaques, tiles, porcelain, and artificial stone and bricks.

6.3.4. Abrasives

For the purpose of polishing, diatoms are superior to powders composed of amorphous silica. They have been used in mild abrasive polishes for silver, other metals, wood, shells, and in toothpaste and

Diatoms from Sozodont toothpowder is a commonly found preparation.

toothpowders. The use of diatomaceous earth in many applications goes back to ancient times. Taylor reports that it was known to the Greeks and Romans, and that Stasbo and Vitruvius wrote of building stones which would float in water. Emperor Justinian instructed his architects to use them in the construction of the dome of Hagia Sophia in Constantinople.

From these ancient applications to space-age rockets, diatoms have found increasing use in mans endeavors. They will no doubt, continue to be a valuable industrial product in times to come.

CHAPTER 7.

7. MOUNTANTS

7.1. Introduction

Mountants are used in microscopy for the following reasons:

1. To increase specimen visibility.
2. To provide a greater depth of field.
3. To aid in decreasing glare, through elimination of reflection.
4. To make use of high aperture objectives possible.

Mounting media used in diatom work may be for either permanent or temporary mounts. For whatever type of mount, a mountant is used primarily to provide an improvement in visibility and perception of the subject matter.

A colorless particle (diatom) can only be made visible with ordinary light microscopy if its refractive index differs from that of the surrounding medium. Contrast will be proportional to the difference between the refractive index of the mountant and the diatom. It is for this reason that diatoms are commonly mounted in a medium of higher or lower refractive index than they themselves possess. Mounting diatoms dry; that is, in air, has at least in the past, not been an uncommon practice. Air has an index of 1.0 and being lower than that of diatoms provides considerable visibility. However, depth of field is decreased with dry mounts as compared with regular fluid or resin mounts. For instance, the apparent thickness of a layer of material is less than a similar layer of air in direct proportion to the indices of refraction. The axial magnification of a lens {the microscope objective for instance) is equal to the square of the lateral magnification. If the lateral magnification is 4.0, the aerial image of a hemispherical object of 1 mm. diameter will be a hemi-ellipsoid 4.0 mm. in diameter and 16 mm. high (or long). The higher index of refraction mounting media minimizes this effect and is important in visual examination, and very important photo-micrographically. Since the refractive index of air is considered as 1.0, high aperture objectives will have their aperture reduced/slightly less than 1.0 when they are used to examine a dry mount, with a resultant loss of resolution. Also, diatoms may show some defects in air that have a tendency to disappear in resinous or fluid mounts.

Although a diatom may contain structure within it on a resolvable scale, with the optics in use, it may not be visible microscopically. This lack of visibility is frequently due to poor contrast, in that the diatom possesses the same light transmitting or reflecting qualities as the surrounding or background medium. Diatoms possess markings and evidence structural detail in a wide range of relative coarseness (or fineness) with coarsely marked diatoms (*Pinnularia* spp. etc.) a very high index mountant will cause the microscopical image of those details to be extremely contrasty to opaque. On the other hand, very small diatoms finely marked

(*Achnanthes* spp. etc.) benefit very much on being mounted in a higher refractive index medium.

When diatoms are mounted in strewn preparations and considerable debris or other undesired material accompanies them, a mountant can sometimes be selected, because of its refractive index, that minimizes the visibility of all but the diatoms. This may be of great advantage in making population studies and/or related statistical counts.

Diatomists, as a group, probably have experimented with, and used, more different mountants than any other microscopical workers. The great dependence of diatom classification, and identification, on extremely fine frustular structure has been largely responsible. Some of the mounting media previously used or experimented with, were variable in their results, not stable, or have been replaced by others. The modern diatomist may, in general, use only one preferred mountant for the majority of his work. However, there is, from time to time, a requirement for a mountant having different properties. Therefore this chapter provides information on a variety of mountants that will allow some latitude in selection. Comments on the characteristics of some of these media are also included. Actual mounting technique is provided in the next chapter.

Table 3

Visibility Index

Medium	**R.I.**	**Index of Visibility ([R.I. of medium – R.I. of Diatom Silica] x 100])** *R.I. of Diatom Silica = 1.434*
Air	1.000	43.4
Water	1.334	10.0
Canada Balsam	1.515	8.1
Styrax	1.580	14.6
Hyrax	1.700	26.6
Realgar	2.400	96.6

Table 3a.

Relative Visibility Index

index of visibility (medium a) / index of visibility (medium b)

Medium	**Canada Balsam (a)**	**Water (a)**	**Styrax (a)**	**Hyrax (a)**	**Air (a)**	**Realgar (a)**
Canada Balsam (b)	[1]	1.23	1.80	3.28	5.36	11.93
Water (b)	0.81	[1]	1.46	2.66	4.34	9.66
Styrax (b)	0.55	0.68	[1]	1.82	2.97	6.61
Hyrax (b)	0.30	0.37	0.55	[1]	1.63	3.63
Air (b)	0.19	0.23	0.34	0.61	[1]	2.23
Realgar (b)	0.08	0.10	0.15	0.28	0.45	[1]

7.2. Index of Visibility

The quantitative effectiveness of differing refractive indices between a diatom and a mountant may be expressed by a so-called “visibility” index. This is defined as the difference between the refractive index of the mountant and the diatom; expressed as a whole number.

Referring to Table 3 the index of visibility is shown for diatoms mounted in various media. Note the high visibility provided by air mounting versus even fairly high R.I. mountants such as Styrax and Hyrax. This table indicates for instance that a diatom would be more than three times as visible mounted in Hyrax as in canada balsam. For example: (1.515 - 1.434) is 0.081 (index of visibility is 8.1) and (1.700 - 1.434) is 0.266 (index of visibility 26.6)·and therefore 26.6 divided by 8.1 is 3+ (times the visibility of the one medium over the other – See Table 3a.).

The visibility of diatoms in any other medium whose refractive index is known can be compared in the same manner. However, visibility as such, is dependent upon brightness as well as contrast and upon other factors too. Therefore, this type of comparison figure is a guide only to the effectiveness of changing the R.I. of a mountant. This index figure could seem to indicate that the higher the refractive index, the greater the visibility of the diatom. A very high index of refraction medium gives an object the appearance of being very dense to almost opaque, and for specimens of comparatively gross characteristics is not to be recommended.

The commonly accepted dividing line between mountants of "high" and "low" refractive index is that of Canada Balsam. Its longevity and popularity as a useful mountant has been largely due to its refractive index, which nearly matches that of most glass. Its R.I. does vary however from approximately 1.510 to 1.550. Mountants with refractive indices above and below these limits are considered to be "high" and "low" index of refraction media respectively.

7.3. Depth of Field

If the depth of field of a microscope objective in air is D, then the depth of field in a medium of refractive index n is D x n. When the field depth of an objective is computed to be D, on a specimen mounted in a medium of index n_1, and it is desired to know the field depth in some other medium with an index of n_2, the new depth of field is given by:

$$D_2 = D_1 \frac{n_2}{n_1}$$

The depth of field D1 of a microscope is independent of the magnification and dependent only on the wavelength of the light, the refractive index of the medium, and the numerical aperture of the objective in the relationship,

$$D_1 = \lambda \frac{\sqrt{n^2 - (N.A.)^2}}{(N.A.)^2}$$

Where:

λ = wavelength of the light in micrometers.
n = refractive index of mounting medium.
NA = numerical aperture of objective.

Wavelength of light is normally represented in whole units of nanometres (nm). The nanometre was formerly known as the **millimicron**, since it is 1/1000 of a micron (micrometre), and was often denoted by the symbol **mμ** or (more rarely) **μμ**

For instance, the depth of field of an objective with an N.A. of 0.65 in a mounting medium of refractive index 1.52, using light of a wavelength 0.540 micrometer, is 1.768 micrometers. This indicates that there will be produced, by the objective, a continuous in-focus image above and below the focus plane for a distance of 1.768 divided by 2, or 0.888 micrometer. This image with this depth of field can be reproduced by photomicrography. When using the microscope visually the eye accommodation increases the depth of field by the relationship:

$$\frac{250x10}{M} micrometres$$

If for instance the preceding objective of 0.65 N.A. had a magnifying power of 40 diameters and a 10X ocular were in use, the eye accommodation would be approximately 1.56 micrometers and the visual depth of field then will be 1.768 + 1.56 or 3.328 micrometers. This accounts for the fact that visually examined diatoms always seem to be more completely in focus than their photomicrographic representations. As an example of the improvement in field depth obtained by going from a lower to a higher refractive index medium for mounting diatoms and under the conditions above, consider the following:

For a medium such as Canada Balsam, with an R.I. of 1.52, going to a high index medium such as Hyrax (R.I. 1.70) will increase the strictly optical field from 1.768 micrometer to about 1.980 or more than 0.2 micrometer. The visual depth of field is increased from 3.328 to about 3.770 micrometers, or more than 0.45 micrometer. The improvement in depth of field is nearly 12%, and while not a great amount, when coupled with the improved contrast, combines to provide a strikingly improved visual image and a much sharper appearing photomicrograph.

7.4. Mountant Mixes

The refractive index of any liquid can be raised or lowered by the addition of another liquid with which it is miscible. The resulting refractive index can be calculated with a fair degree of accuracy, according to Shillaber, by;

Charles Patten Shillaber (Author of – Photomicrography in Theory and Practice 1944)

$$N(v_1 + v_2) = n_1v_1+n_2v_2$$

Where:
n is the desired index and,
n_1 is the index of liquid 1.
n_2 is the index of liquid 2.
v_1 is the volume of liquid 1.
v_2 is the volume of liquid 2.

The application of this equation should give results within approximately 0.001. Exact checks however, should be made with a refractometer.

Over the years there have been a number of mountant mixes compounded by diatomists in attempting to obtain high refractive index media. Many of them accomplished that purpose, but were undesirable for other reasons, such as dark color, proclivity to bubbles, difficulty in hardening, poor tenacity or adherence to glass, and so on. However, there are a few which have become quite useful and even preferred by a number of workers.

Many diatomists examine diatoms in temporary mounts in water only and thereby do not realize the full capability of the light microscope for examining objects in other fluid media. There are a number of oils and other fluids which are of a sufficiently high refractive index as to make them attractive for examining diatoms as temporary mounts or in making photomicrographs. Some of these mountants are included in this chapter, especially those which should be on hand at all times.

7.5. Factors Affecting Refractive Index

The refractive index of a substance is affected by both heat and light. For instance, a l degree C. change in temperature can effect a change in the refractive index of an immersion oil of about 0.0004. The refractive index increases as the temperature decreases and vice versa.

As the wavelength of the light varies so does the refractive index of a substance. As the wavelength increases (moving toward the red end of the spectrum) the R.I. decreases, and as the wavelength decreases, as toward the ultraviolet end of the spectrum, the R.I. increases.

Commercial mountants and immersion oils usually have their refractive indices specified at the D-line (589 nanometers) of the spectrum since this is near the peak of the visual brightness curve. Therefore the index n_D has been chosen by optical designers as the basic index for ray tracing and for the specification of focal length. Two other indices, one on either side of n_D are then chosen for purposes of lens achromatization. The most frequently used ones are n_C for the red end of the spectrum and n_F for the blue end. Refractive indices for mountants and immersion oils are most frequently expressed for these different Fraunhofer lines (D, C, and F). Likewise, dispersion, an expression of the range of refractive indices for a given medium, is generally limited between the F and C lines and indicated by $n_F - n_C$.

7.6. Diatom Mountants

All modern commercially available mountants no longer contain Aroclors or PCBs, though Aroclors still seem to be available.

The following mountants are listed to indicate the wide range used in diatom work with their general characteristics. In addition, some mountants are listed and discussed which are no longer in general use, or have been discontinued entirely, but may be encountered in older slide collections. Knowledge of the latter is of general interest to the diatomist, and in some cases may provide a basis for obtaining better visual or photographic images in working with

old collections. **Caution.** Many modern mounting media for diatoms contain Aroclor, and polychlorinated biphenyl (PCB) which, when used over long periods of time, or ingested, or absorbed through the skin, are toxic. Precautions should be taken in using these media to keep them away from the skin and avoid ingestion.

(A number of mountants carry the suffix "rax" which implies something that can be stretched.)

7.6.1. Hyrax R.I. 1.70

A synthetic resin invented by Hanna (1930). It is a condensation product similar in many respects to other "plastics". Hyrax is a clear substance of pale amber color, soluble in benzene, xylene, or toluene, becoming hard when the solvent is evaporated by heat. It is not soluble in alcohol; it is neutral and does not become acid. The refractive index of Hyrax is rather uncertain, but it ranges from 1.66 to as high as 1.70 or 1.75. The index of visibility for diatoms mounted in it is more than 3, assuming the refractive index of the diatom silica to be 1.43.

It is supplied in a quite viscid liquid form and may require thinning prior to use, preferably with xylene. After a period, Hyrax mounts will darken somewhat, but no crystallization products are usually reported.

A similar product currently available in the United States and the UK is Zrax.

Because of its very viscous nature and the low-boiling point of solvents used (xylene, benzene, toluene) with it, Hyrax has a definite proclivity for bubble production. In order to obtain the advantages of its high refractive index (as with other mountants), all trace of solvents must be removed in the final mount. A recommended technique for applying this specific mountant to minimize bubbles is included in the chapter on mounting diatoms. It is used extensively by diatomists.

7.6.2. Canada Balsam R.I. 1.515 - 1.530.

A natural resin from the balsam fir, *Abies balsamea*, a tree native to North America. The refined and paper filtered product (as it should be purchased) is aromatic, clear, light in color, with a refractive index near that of most glass. Despite assurances by commercial producers that it is neutral, it usually is not, being somewhat acidic. This latter quality may limit its use with certain stained objects.

Upon drying, it forms a hard transparent mass with good adhesion properties to glass, and is fairly inert chemically. Almost any organic solvent is suitable, and the alcohols, chloroform and turpentine are sometimes used. The solvent used determines the drying time. For instance, turpentine-balsam stays liquid for a long time, and chloroform-balsam dries very quickly.

Balsam can be purchased in various forms, but that recommended is generally called xylene-balsam. It is made by drying out the natural oils from Canada Balsam and dissolving it in xylene and filtering it through paper.

A method of making a neutral balsam is described by Lee (1928). Balsam may be kept quite neutral enough for most purposes by including a marble chip in the storage bottle.

Arthur Bolles Lee (1849-1927). *The Microtomists' Vade-Mecum.*

The preponderance of diatom mounting does not concern itself with stained specimens, and therefore the acidity problem is of lesser importance to the diatomist. Where acidity is undesirable, there are a number of neutral synthetic resins with the same index as Canada Balsam and with more desirable qualities.

Canada Balsam tends to darken considerably in long exposure to sunlight and therefore slides should be stored in a dark place. The bottle is best not exposed to sunlight either with proper precautions in their preparation and storage, Canada Balsam mounts have withstood long periods of time with little or no change of appearance or other properties, showing no granulation or cracking. Canada Balsam was, with little doubt, the first medium used to make the first permanent diatom mounts, probably about 1832 (not later than 1835). In fact nearly all diatom mounts up to about 1849 were either mounted dry (air) or in balsam after being cleaned.

This medium is still used in certain cases of diatom mounting to be discussed later. However, the diatomist usually has less of a requirement for this medium than for the higher refractive index mountants.

7.6.3. Styrax R.I. 1.60

A natural resin. Sometimes referred to as storax or liquidamber, it is obtained from the tree *Styrax officinalis* which is well distributed over the world. In the United States the tree *Liquidambar styraflua* is a source, and the resin derived from it is referred to as liquidamber.

Liquidambar orientalis is the source described in the British Pharmacopia and the resin product in that case is called gum styrax. This mounting medium was introduced in microscopical technique by the famous diatomist Henri Van Heurck in 1883.

Styrax **is not** generally available commercially in a form ready to use as a diatom mountant. The liquid resin Storax (the raw material) **is** available however. The best process for preparation of the purchased raw material involves a lengthy hardening and bleaching period in sunlight. The material so treated must be exposed to direct sunlight, as attempting the process under glass has proven to be unsatisfactory.

If raw storax can be obtained, the refined product is a good mountant for diatoms. The following process is recommended.

Crude storax is a dirty grayish resin, aromatic in odor, and viscous. It usually contains dirt, flies and other insects, and bits of bark and twigs which must be removed as a first step. The crude material is dissolved in chloroform. Chloroform is used instead of xylene or toluene in this step because in the subsequent boiling step the latter solvents react with the water, while chloroform is rapidly evaporated out in

the process. The chloroform dissolves the crude styrax away from the bark, twigs, etc., and evaporates out before boiling actually takes place.

After adding chloroform to the crude material it is passed through filter paper. It will come through the paper, accompanied by the chloroform, in a golden syrup, with a slight metallic tinge. If the material is purchased already filtered, this chloroform and filter step in the process can be eliminated. The filtered material is placed in a receptacle such as a boiling flask, beaker, or pan to which three times its volume in distilled water is added. The mixture is then boiled for five minutes. A strong spicy odor is produced indicating that some of the essential oils and acids are being removed, which is the purpose of this step. After five minutes of boiling allow the Storax to settle to the bottom and decant the supernatant water. Again add three times the Storax volume of distilled water and repeat the process. This repeated boiling should be done at least three or four times, to assure maximum removal of the oils and other undesired constituents.

The Storax is then spread thinly on glass plates. Small window pane sizes of glass (about 12 inches on a side) are convenient, and a very thin application can be made by rolling a glass rod, after dipping in the processed Storax, across the plates. The Storax will become cloudy in appearance after it is applied in this thin layer.

The glass plates are then placed on a flat surface in bright sunlight. The thin layer of the processed Storax on the plates is then stirred several times daily with a glass rod. In about two days the Storax assumes a clear yellowish color, and in approximately two weeks becomes stiff. During this period it should continue to be stirred several times a day. in another week or two (dependent upon sunlight and temperatures) it will become impossible to stir and when cold can be chipped off the glass with a conchoidal fracture. At this point it should be scraped off the glass sheets and stored in bottles as is. During the sunlight process the Storax should get no hotter than about 90°C. on the glass, or it will rapidly darken. The quicker it hardens, the better. During this process insects are attracted, and there is bound to be an accumulation of them in the Storax. The larger ones are easily picked out, but the smaller ones are ignored and are chipped off and stored with the hardened Storax which product diatomists have termed ‘Styrax’.

For use, a small amount is dissolved in chloroform (xylene, or toluene will suffice) and passed through filter paper twice. In order to filter properly the solvent-styrax is necessarily made very thin. It can be thickened to a desired consistency by supporting the cap loosely on the neck of the mountant bottle so that the solvent may evaporate sufficiently without dust entering the bottle. One reported disadvantage of Styrax is that it hardens very slowly and often does not become completely hardened. To harden it, the addition of the natural gum colophony is sometimes resorted to. However, this lowers the refractive index, and therefore renders the mixture less useful in resolving fine structure in the mounted forms. Other reported disadvantages of Styrax include cloudiness and the production of grainy precipitates. However, proper preparation of the raw material will prevent these difficulties.

The general unavailability of the processes Styrax and the lengthy processing of the raw Storax has limited its current use among diatomists.

7.6.4. Naphrax R.I. 1.70

A synthetic resin invented by Col. Wm. D. Fleming (1943/1954). This high index mountant is made in a similar process to that of Bakelite. The phenol in the process for Bakelite is replaced by napthalene, using glacial acetic acid as a solvent, and sulfuric acid as a catalyst.

Colonel William D. Fleming
3rd President of the Microscopical Society of Southern California (1948)

Dependent upon the care with which it is made, Naphrax has a refractive index in the vicinity of 1.70. It is pale amber in color, and in thin layers as used in diatom mounts, the color is barely noticeable and not objectionable. Naphrax is very intolerant of moisture, and mounts must be thoroughly dry before applying it. If moisture remains when it is applied, there is the possibility that the resulting diatom mount may become cloudy or precipitates may form. Some workers also report a tendency to bubble production that is bothersome in slide preparation. However, with proper precautions, and careful technique, this mountant is excellent for much diatom mounting, and is used extensively. It is available commercially, in England and the United States. Solvents are xylene, toluene, and benzene. Toluene is suggested as the best solvent to use. Solutions in benzene are apt to be annoying from the skin formed on the medium, while making the mount. On the other hand, xylene solutions are apt to take an unduly long time to harden under the coverglass.

Mounts made with this resin do not harden as quickly as those with balsam. In mounting diatoms the hardening time may be shortened considerably by heating the slide mount up to the boiling temperature of the solvent.

7.6.5. Pleurax R.I. 1.75 - 1.77

A synthetic medium invented by Hanna. It is a sulfur-phenol resin. Solvents are 95% ethyl alcohol, isopropyl alcohol, and acetone. It has a lemon-yellow color and has been used in diatom mounting. When mounting diatoms in this medium particular attention must be paid to complete removal of any water. A 95% wash solution of alcohol to dehydrate the diatoms is recommended. It is not satisfactory with a gum fixative.

7.6.6. Caedax R.I. 1.550

A synthetic mountant, and a cyclohexanone. It is neutral to water, white, and does not discolor with age. It is sensitive to excessive heat (changing color) and to water. It has excellent penetrating qualities and is used extensively in micropaleontological work. Solvent is xylene. A preferred substitute for Canada Balsam, as it is absolutely neutral and has little coloring of its own. Caedax does not mix with even small traces of water. Preparations therefore have to be dehydrated carefully. Caedax does not polymerize over a period of years, and therefore may be re-dissolved in xylene, repeatedly. It is manufactured by R. Merck of Darmstadt, Germany, and available through most chemical supply houses.

7.6.7. MM-165 R.I. 1.62 - 1.65 (Editor's Note: Possibly Aroclor based - MeltMount)

A synthetic medium marketed by R. P. Cargille in the U.S. It is fairly viscous and has a tendency to bubble production. Xylene or toluene are solvents. A very slight yellowish coloration is present.

7.6.8. Balsam of Tolu R.I. 1.618 - 1.640

A natural resin similar to Styrax, it comes from the central American tree *Myroxylon balsanum*. It has one of the highest of refractive indices of all natural resins. It is found named as a mountant in some older diatom slides of 50 years or more in age. It is not used at present to the writers knowledge. Solvents are as for most other natural resins.

7.6.9. Gum Thus R.I. unknown

Storax (Styrax) is also a candidate for the frankincense of biblical times.

A natural resin from the spruce fir, genus *Boswellia*, of which there are several species. It is possible that this material is the frankincense of biblical times. It appears frequently named as the mountant in old diatom slide collections. No longer used.

7.6.10. Cassia Oil R.I. 1.6

Because of its high refractive index, this oil has in the past been used as a medium for mounting diatoms. For temporary mounting of diatoms, in photomicrography for instance, this oil is useful. However, because of the difficulties in securing long lasting seals, permanent mounts using it are no longer attempted; as they were in the past.

7.6.11. Realgar R.I. 2.3 - 2.5

An artificially prepared Arsenic disulfide melt. One of the original uses of this very high-index of refraction medium was in mounting diatoms. Its name stems from the naturally occurring mineral, and its primary use is still as a mountant for diatoms. It is ordinarily not used unless the diatom markings are very fine, as the very high contrast produces extremely opaque heavy-appearing images on those with gross characteristics. Therefore, it is more often used in examining the minute and delicately sculptured freshwater diatom forms.

Regretfully, a pure mixture of realgar is difficult to prepare, and therefore opinions as to its permanence as a diatom mountant vary. However, Hustedt indicates that he had realgar-mounted diatom preparations in his slide collection that remained unchanged in more than 40 years.

Hustedt describes a method for preparing realgar of lasting qualities which is repeated here for those who wish to attempt it. **Caution!** Because of the very

poisonous gases generated during this process, it should be carried out under an efficient hood in the laboratory.

Completely pure realgar (arsenic disulfide) is obtained by sublimation. This is then heated in equally pure arsenic bromide until dissolved. The cooled and filtered resulting mass appears greenish-yellow in color, and is of a viscous consistency. To improve its permanence add $^1/_6$ to $^1/_4$ parts of sulfur by volume to the mass, completely dissolving it by repeated warming.

Heating, in a test-tube, 7 parts by weight of arsenic and 25 parts by weight, of sulfur, also produces realgar. However, this medium is a deep reddish color. An amber color may be produced by subliming the arsenic twice and recrystalizing the sulfur from carbon disulfide. Only moderate heat is required to melt realgar.

In the *Journal of the New York Microscopical Society* for 1895, an "artificial" realgar was described as being composed of 1 part sulfur to 1.7 parts of arsenious acid. The realgar then being sublimed to the coverglass, and thence fused to the slide.

Any preparation of the actual mountant itself, or slide preparations using realgar as a mountant, must be carried out with great care and all contact with the mountant or fumes from it must be avoided.

7.6.12. Alpha-Monobromonaphthalene R.I. 1.66

The high refractive index of this material has long attracted the diatomist. Permanent mounts using it, because of the difficulty of making long-lasting seals, are largely a thing of the past. However, for temporary diatom examination, or in the photomicrography of diatoms, this medium has good qualities. It penetrates the minute cavities well, and because of its low viscosity entrains few air bubbles. It can be mixed with oils to lower its refractive index. The R.I. is quite variable and may differ from that given above. It darkens somewhat with age.

7.6.13. Dammar R.I. 1.529

A natural resin, sometimes called gum dammar or agathis, obtained from the trees of the *Dammara* genus. Solvents are the same as for other resins as Canada Balsam, etc. This is very seldom, if at all, used at present in mounting diatoms.

7.6.14. Sandarac R.I. (variable)

A natural resin, from the tree *Callitris quadrivalvis* native to Morocco, also known as juniper resin. This resin has in the past been used in making diatom mounts and appears named from time to time in old slide collections.

Recently, in the observation of the diatom cytoplasm, and to minimize the visibility of the frustule markings, this resin has again found use. It is modified to alter its refractive index. The finest gum juniper is dissolved in the minimum of a mixture of one part castor oil B.P. to twelve parts pure amyl alcohol. The solution is diluted

until by observation, the correct R.I. is found, namely when about equal parts, by weight, of resin and solvents, have been used. When this medium is used, the stained diatoms should be dehydrated in alcohol and transferred to amyl alcohol before mounting.

7.6.15. Aroclor 5442 R.I. 1.66

R. I. Firth used Aroclor 5442 dissolved in Chloroform

A chlorinated diphenyl synthetic resin. There is a series of Aroclors, of which this is only one. Among the dozen different Aroclors there are four of particular interest to the microscopist (numbers 1254, 1260, 5442, and 5460). Aroclor 5442 is used successfully in mounting diatoms, and has been used in a mix with Styrax (to be described later) as a diatom mountant.

Aroclor 1254 is a mixture of polychlorinated biphenyls (PCBs), a class of environmental toxins which cause a wide spectrum of neurotoxic effects. Learning and memory deficits are the profound effects of PCBs which may be related to hippocampal dysfunction.

Aroclor 5442 softens at 46 - 52°C. (115- 126°F), but in use is kept hotter at about 158°C., at which temperature it is very fluid. It is stable to heat and oxidation and is inflammable. Ringing to prevent oxidation (as necessary with most natural resins) is not required. It is permanently thermoplastic and therefore can be heated and reheated as many times as desired without detriment to the final mount. This mountant is soluble in xylene. In recent years the chlorinated polyphenyl compounds have gained a rather bad reputation because of their poisonous nature. However, as with all dangerous, or potentially dangerous compounds, proper precautions in handling them is the key to safety. This medium deserves more attention as a diatom mountant than it has received previously. Other members of the Aroclor family have been used in mounting diatoms. Aroclor 5460 (soluble in toluene or xylene) with a refractive index of 1.670 is valuable for use as a temporary diatom mountant in the field because of its portability. It is a brittle resin supplied in flakes. The flake material is carried in a vial. If the diatoms are previously dried on a coverglass, the slip is placed on a microslide, a few of the flakes are shaken out next to

the slip and a match flame applied beneath the slide. The Aroclor will flow underneath the slip. A temporary high-refractive index mount is then available for examination with a portable microscope. Aroclor 5460 alone does develop cracks and is unsuitable for permanent mounts. However, a mix of equal parts of Aroclor 5460 and coumarone resin in toluene (R.I. 1.64) has been suggested as a permanent diatom mountant. A mix of Styrax and Aroclor described next has been most successfully and more widely adopted by the diatom worker.

7.6.15. Styrax-Aroclor R.I. 1.64

A mix of a natural and synthetic resin invented by Brigger (1960). Complete preparation of this mix is described in the reference. It is a clear to very pale amber

color, hardens as soon as the solvents are evaporated, and does not darken if overheated. The mix is a great improvement over Styrax alone, as it is clearer and is assured of hardening. Aroclor 5460, a brittle resin, supplied in flakes, with a refractive index of 1.67 is used in an equal part, by weight, with Styrax and other chemicals in the process. The process described by Brigger is a comparatively easy one, which anyone with some care can perform, and which is comparatively free from any real dangers to the compounder. Although not used to as great an extent as Hyrax for instance, this mountant has become a favorite of many leading diatomists. The term Styrax A has been used in referring to this mix.

7.6.16. Piperine Mixtures R.I. 1.63

Piperine by itself has a high refraction index (1.68), is practically colorless in thin layers, and is permanent. However, it has the undesirable property of crystallization in a short time, making preparations so affected, unusable. When used for mounting diatoms, therefore, it has normally been employed as a constituent of a mix. According to Hustedt antimony bromide or colophony was chosen by Van Heurck as additives for the prevention of crystallization. In some of the older slide collections, featuring "piperine" as the mountant, it was no doubt, a mix of this sort.

Molten piperine dissolves the tri-iodides of arsenic and antimony and forms solutions that are fluid at slightly over 100°C. and are resin-like and amorphous when cold. However, the difficulty in obtaining sufficiently pure arsenic and antimony iodides either makes the preparation costly, or difficult of accomplishment. Larsen and Berman give a good account of the preparation of mixtures of piperine and iodides resulting in refractive indices within the range of 1.68 to 2.10. Most of the mixtures so obtained are strongly colored, ranging from an amber to dark red color, making their use very limited.

Harry Bermann
Esper S. Larsen

Hustedt describes a piperine-coumarone medium, after Kolbe, that is useful as a diatom mountant, as follows:

Equal parts, by weight, of piperine and coumarone resin are melted together in a porcelain crucible. It is advisable that the piperine be melted first and then the coumarone, with continual stirring, is gradually added. The mass, after becoming completely melted, is further heated until bubbles begin to form. The duration and degree of heat are of decisive importance to the quality of the medium. Afterward the mass is cooled to a viscous consistency, and poured out in individual drops on an iron plate to hasten cooling and hardening into clear solidified globules. If it is found that the molten mix is becoming too viscous during the pouring, it can be rewarmed. A solidified globule is usually sufficient to make two to four slide preparations.

The refractive index of this mixture is (according to Kolbe) of from 1.63 to 1.65, depending on the duration and degree of heat used.

Piperine is a crystalline alkaloid; a nitrogenous compound of plant origin present in pepper. It is an amide of piperidine and piperic acid, its chemical formula being $C_{17}H_{19}NO_3$. It can be purchased from chemical supply houses, but it is expensive, with a minimum purchase of 25 grams.

Coumarone resin is the product of polymerization of a fraction of benzene known commercially as solvent naphtha. It has been well known in industry for more than seventy years and is well documented in the literature. Its use as a mounting medium for microscopic specimens has been experimented with since the late 1920's. Its refractive index is approximately 1.59.

Other piperine mixes have been compounded with varying degrees of success, most of them unsatisfactory, in that they often form extensive crystals in the resinous mass.

7.6.17. Coumarone Resin R.I. 1.59 - 1.60

A synthetic resin (as above) which has been largely neglected by the diatomist in favor of more popular and readily accessible media. Frison (1952) made a study of this resin as a mounting media, and concluded that it was better for mounting diatoms than Styrax.

Karel Edouard Frison
(1888-1973)
Belgian

His medium consists simply of a mix of the resin with xylene. His preparation is as follows:

Three quarters of the volume of a 550 ml. wide-mouthed bottle is filled with unpulverized resin, which is then covered with xylene to a height of l mm. above the resin. Stirring should be done from time to time with a strong stirring rod. The use of a glass rod is not recommended as it breaks too easily. A nickel spatula or a stainless steel rod or knife, is best for the purpose, The material will be in solution in two or three days time without heating. The mixture is left in the bottle which constitutes a stock solution. Impurities and dust, which are present, will slowly settle to the bottom of the bottle. The clear part at the top is used as required. In this way filtration is avoided, which would, in any case, be very slow or almost impossible in practice.

To protect against any possible separation of the hardened resin (in amount) from the glass slide, add about 1% by volume, of castor oil.

Coumarone has a tendency to become yellow under the influence of air and light. Sealing the coverglass, and storage in light-tight containers will minimize it.

Prof. Georges
Deflandre
(1897 – 1973)

Another mix of coumarone resin is that devised by Deflandre (1933). His formula; coumarone resin 20 parts, xylene 80 parts, monobromonaphthaline 1 part, all by volume. Note that this is a coumarone-xylene medium similar to that of Frison, but using monobromonaphthalene instead of castor oil as a plasticizer.

7.6.18. Methylene Iodide R.I. 1.74

A non-resinous mounting medium that can be used to advantage with diatoms. It flows freely, has a medium vapor pressure, and is colorless. Mounts made with this

medium can be sealed with paraffin. However, it is somewhat unstable and may darken over a period of time. It is soluble in all proportions in alcohol or ether, but only slightly soluble in water.

Methylene iodide will dissolve sulfur, the refractive index being raised to 1.78 for a saturated solution. The sulfur is easily put into the iodide in excess by heating some of the liquid with the sulfur in a test-tube.

This medium is not recommended for permanent diatom mounts, but should be advantageous for temporary mounts for photomicrography for instance.

CHAPTER 8.

8. MOUNTING

8.1. Introduction

To facilitate the study of diatoms, they are ordinarily mounted on glass microslides for examination with a light microscope. In some instances they are prepared for examination by the electron microscope. The emphasis in this chapter is on the methods, techniques, and special apparatus used in making permanent preparations for the light microscope. A brief portion is devoted to some elementary considerations involving the electron microscope. The latter instrument is now used in diatom research in two forms; the transmission electron microscope (TEM), and the scanning electron microscope (SEM). Preparation methods for each are very briefly treated as, at present the use of electron microscopy for the study of diatoms is rather restricted, and very highly specialized. The limited availability of such instrument and the specialized expertise required for their use does not warrant lengthy treatment in a book devoted to the elementary study of diatoms.

Electron Microscopes are now commonly used in diatom studies in academic environments.

Two general categories of microslide preparations are used for diatom study, temporary and permanent. Permanent preparations are divided into two major divisions of strew slides and selected slides. Other specialized preparations such as arranged groups, group assemblages, type, and genus slides, also fall into the permanent selected category.

Strewn slides serve in compiling lists of species from samples collected, aid in correlating diatom associations, and in obtaining statistical data for biological and geological purposes.

The selected slide with an individual selected diatom located inside a black circle becomes a museum specimen which can be catalogued and referred to by later workers at will. Other types of selected preparations provide for detailed study and comparisons of diatom morphology, ecological relationships and generic and specific relationships in taxonomical investigations. The further purposes, methods, apparatus, and techniques for studying such preparations are included in Part III of this book.

Many of the techniques described in the following paragraphs for making diatom slides, whether they are strews or selected preparations are old. The making of such preparations with very specialized apparatus and/or with such meticulous care may seem to be a waste of valuable time to the modern worker. However, the methods have been used for many years to produce long-lasting slide preparations suitable for reference and study by generations of workers in this highly specialized field. It seems appropriate that contemporary diatomists should offer the same advantages to their future counterparts.

8.2. Microslides and Coverglasses

Before embarking on detailed procedures for preparing diatoms for study, it is appropriate to consider the main materials of which permanent (or temporary) mounts are made.

Essential to good diatom preparations is the condition and quality of microslides and coverglasses. Microslides (and particularly coverglasses) should be free of blisters, bubbles, striae, pits, and cracks. Flat, parallel, plane surfaces are most important. The glass should be annealed, and the edges of microslides of good quality are ground smooth to prevent chipping and crack development. Microslides of "non-corrosion" designation should only be used, and of a thickness that is as close as possible to l mm. This is the most convenient thickness; being rugged, yet thin enough for almost all optical purposes. Thick microslides should be avoided, as they can cause problems in proper focusing of the field diaphragm with the substage condenser of the microscope. Very thin slides are too easily broken, and they may cause trouble in holding immersion oil between the substage condenser and the bottom of the microslide when the former is critically adjusted for high power microscopical examination. Commercial slides are available with all of the desired characteristics, pre-cleaned, in a usual minimum pack of one-half gross. The slide thickness usually ranges from 0.97 to 1.07 mm., and the length and width dimensions 75 and 25 millimeters respectively.

Microslides for special temporary mounts or culture study purposes are also available and will be described in detail in the study section following.

Coverglasses for diatom mounting are perhaps more exacting in their requirements than those for any other type of work with the light microscope. Because diatoms are more often mounted on the coverglass, and the latter thereby handled considerably more than is usual in other types of work, they must possess superior physical qualities. Chance Brothers glass, manufactured in England, is recommended as the best coverglass material to be obtained. It is stable, non-corroding, and far less brittle than other glass. It is available in the United States through Arthur C. Thomas Company. If it cannot be obtained, then the best quality coverglasses possible should be used.

Round covers are recommended for most diatom mounts, as they are easily and neatly sealed with the aid of a ringing turntable. The most used size is probably 19 mm. in diameter. This size is convenient for strews and certain types of selected slides. Smaller diameters (10, 12 or 16mm), are desirable for individual selected diatom mounts. In some special cases, circular covers will not provide sufficient area. This is particularly true in studies involving large statistical sampling.

Rectangular coverglasses of up to 24 x 60 mm. are readily available for that purpose.

The thickness of coverglasses used is very important in minimizing spherical aberration in the microscopical image. Two thicknesses are sufficient to accommodate any objective of modern manufacture; 0.17 and 0.18 mm. Coverglasses as close to this as possible should be obtained for mounting diatoms on. Which thickness is used, is dependent upon the designed correction for the microscope objective in use. Purchase number $1^1/_2$ coverglasses if possible, as they

range in thickness from about 0.16 to 0.19 mm., and a large proportion of them will be 0.17 and/or 0.18 mm. in thickness. If this "number" cannot be purchased, then the next best choice is number 1 thickness. These are rather thin and range from 0.13 to about 0.16 mm. Some in this selection will be found as thick as 0.17 or 0.18 mm. The last choice is the number 2 which ranges from 0.18 to about 0.25 mm., there being a minority of the thinner slips.

Whatever range of cover thickness is purchased, they should be measured and sized with some kind of thickness gauge. An ordinary machinists micrometer is satisfactory for the purpose. Coverglass gauges, specifically designed for this type of measurement, have been made for many years by major suppliers of microscopical apparatus. However they rather difficult to obtain today.

The tolerance for coverglass thickness varies with the numerical aperture of the objective, ranging from plus or minus 0.3 to plus or minus 0.003 mm. for a high dry objective of N.A. 0.85. The details of what deviation in coverglass thickness means in the final image and the techniques of microscope adjustment to minimize the effect of such deviations is covered more thoroughly in the following section on diatom study. Suffice it to say at this point that coverglass thickness should be as close as possible to either 0.17 or 0.18 mm (dependent upon the objective design).

8.3. Mounting on Coverglass vs. Microslide

Diatoms are mounted (fastened in some manner) either on the underside of the coverglass or on the microslide, according to the preference of the mounter. As to which is preferable, the mounting on the underside of the coverglass is recommended. It is a time-proven method of superior results which has been recommended and used by most of the leading diatomists of past and present. There are those that proclaim a preference for mounting diatoms on the microslide, largely as a matter of ease and convenience, and who decry the coverglass mounting method as unnecessarily more difficult and far too particular for the results obtained. Nothing could be further from the truth. Mounting on the cover is not difficult, and is as easily accomplished as mounting on the microslide, and the method, when properly accomplished, provides for minimum spherical aberration in the final image, no mean accomplishment, and quite worthy of any "extra" care taken.

For certain specific diatoms, or for mounts which are not required to be revealing of the finest detail, then mounting on the microslide may be an acceptable method. However, the recommended method in this book is to mount diatoms on the coverglass, whether strews or selected slides are being prepared, and the techniques described are all directed to that end, unless specifically otherwise noted. In the following section on diatom study, a more detailed justification for mounting on the coverglass is provided.

8.4. Temporary Mounts

This type of mount for diatoms is used rather infrequently and therefore the treatment here is correspondingly brief. However, there are a number of advantages

in this type of preparation for preliminary examination, and for photomicrography, that are often not realized. Temporary preparations for long-term live-specimen study are included in part III of this book.

A procedure for preparing a slide satisfactory for most generic determinations in the field is to place a drop of the diatom-containing material on a microslide. It is then held over a flame until it boils, and then steamed for a few seconds longer. A drop of water or glycerine is applied and topped with a coverglass. Examine immediately with the microscope. A semi-permanent slide following this procedure can be made using glycerine jelly as the mountant.

This type of preparation is not suitable for detailed examination and specific determination of diatoms. In some cases, especially where freshwater forms are to be examined, the debris may interfere with the very small forms. Then perhaps an incinerated preparation, as described previously, will be a better approach.

8.5. Strew Slide Preparation

8.5.1. Preliminary Cleaning

Strew slides will ordinarily be made up using previously cleaned diatoms that have been stored in vials, either in the dry state or in a preserving liquid. In mounting diatoms from storage, it is necessary to remove preservatives, such as alcohol or formalin, before proceeding.

The diatoms, with the preservative, are removed from the storage vial or bottle and transferred to a small flask. Add distilled water to a height of about 3 inches. Agitate the mixture and allow to settle. The supernatant is poured off and more distilled water added. This process is repeated a number of times until the preservative is eliminated. The storage bottle or vial is also rinsed with distilled filtered water. The diatoms are then returned to the storage vial where they are again rinsed by two or three settlings using distilled water. Diatoms stored in hydrogen peroxide may be mounted directly, but washing by repeated settlings is recommended.

The procedure above may seem extremely meticulous, but it will ensure clean diatoms for mounting and eliminate any detritus that may have carried over from the cleaning process, and/or that might have accumulated during long storage, This preliminary washing just prior to mounting from storage, is carried out in quantity when the preparation is to be a series of strews or spreads from which diatoms are to be further selected for other types of mounts. If only a very few diatoms are to be mounted, or a single strew is contemplated this preliminary washing is carried out in a reduced way by shaking the storage bottle and taking a small sample from it with a pipette. This small sample from it is then washed as described in a test-tube or other appropriately small container.

If the diatoms have been stored dry then a sample large enough for the purpose is taken from the storage vial and washed several times by repeated settlings in a testtube or flask, dependent upon the volume of the material, before proceeding. For a single strew slide or a spread slide, one or two samples of an amount to be

contained on the end of a toothpick will probably suffice. In any event, whatever the method of storage, the diatoms are given a preliminary wash and kept at the ready in distilled water for the next step.

It is well to mention here that pipettes used in transferring cleaned diatom material from storage, or for other steps in the mounting procedure, must be clean and free from any contaminant. Pipettes should be cleaned with a feather or pipe cleaner and stored in distilled water to which a small amount of hydrogen peroxide has been added. For work which is very critical as to contaminants or foreign material, it is best to use only one pipette for each type of diatom material being handled and discard it after use. Disposable pipettes are obtainable in many different sizes very economically. Pipettes operated by a rubber bulb are very convenient and easy to use. However, with very little practice pipettes using the mouth and/or finger are easily mastered. A convenient sized pipette for diatom work of the latter variety is of a 1.0 ml. capacity (with $^{1}/_{10}$ ml. divisions) about 27 mm. in length with a body bore of approximately 5 mm. and a tip bore constricted to about 1 or 1.5 mm. A flexible rubber or plastic mouth tube fastened to the upper end of such pipettes makes them a bit more maneuverable in some operations.

If the mix of diatoms in the distilled water waiting to be mounted is too dense, then it should be diluted further in distilled water in a vial or test-tube such that there is a slightly cloudy appearance to the liquid. The ideal condition is obtained when the diatoms are applied if they are in a single layer not too widely dispersed on the glass substrate. If the material is too dense, diatoms will be so thickly distributed as to pile up on one another in the strewn preparation, interfering with observation and identification. If it is too sparse, the diatoms will be so distant from one another as to prevent a ready analysis to be made of the diatoms present.

The procedure for making spread slides and store slides has been detailed in Chapter 5. The remaining information concerns itself with the making of permanent strew preparations.

In addition to the cleaning methods described previously for coverglasses, Hustedt recommends the following. The coverglasses are boiled for about one quarter of an hour with concentrated nitric acid in a boiling flask, washed free of acid in distilled water, dehydrated completely of every trace of water through alcohol treatment, and finally preserved in pure sulfuric ether.

Whatever the cleaning method, the coverglass is picked up from storage with coverglass forceps, or other convenient tweezers, and placed on a warm hotplate or other warmed metal surface. The plate should not be too hot before the diatoms are applied. Alternatively the cover (or several covers, dependent upon their size) can be placed on a microslide before being placed on the warming plate. This latter method provides easier handling of the covers in succeeding steps.

8.5.2. Application to Coverglass

When the cover/s is/are warm, add a drop of distilled water to it just sufficient to spread to the edge. The size of the drop and amount that can be retained on the cover

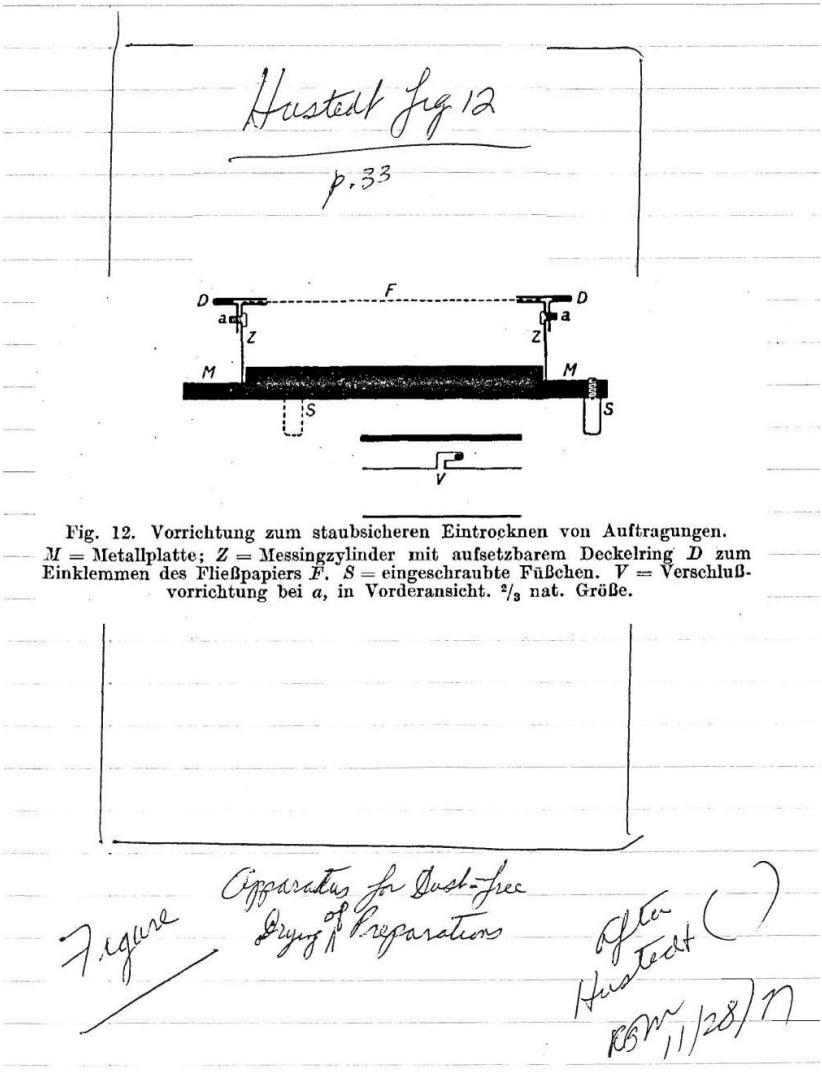
without overflowing must be determined by experience. It is surprising however, how much water can be retained on a single coverglass if it is really clean.

Next a suspension of the diatom material in the proper density (as described before) is dropped into the water on the coverglass from a height of a few centimeters. The amount of material to be dropped is dependent upon the size of the cover and the density of spread desired. Experience again will determine this. The dropped material will settle through the water and ideally spread in an evenly distributed layer on the coverglass. The temperature of the hot plate is raised until bubbles begin to appear in the water drop. If the heat rise is too rapid, or the temperature becomes too great, there will be established eddies and turbulence in the water that destroys the even distribution of diatoms.

8.5.3. Drying

At this point the coverglass may be either removed from the hot plate and the water allowed to evaporate, or remain, with the applied heat driving off all moisture.

If the latter, faster method is adopted, the heat must be very carefully controlled to drive off the water without disturbing the even distribution of the diatom material. If sufficient time is available, the slow evaporation of the water is more desirable.



Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 12)

Figure 39.

In the natural evaporation of the water, which may take several hours, or even an overnight period of time, the covers with their diatom load, must be protected from airborne dust. Hustedt describes a very elegant little drying chamber that suits the purpose admirably. Figure 39 illustrates the salient features. It consists of a circular metal plate M (aluminum, brass, or copper are excellent) about 8 mm. thick and 12 mm. in diameter, with a concentric edge area 1.5 mm. broad and half as thick as the central portion. The central portion is covered with a brass collar about 2 mm. in height of which the upper edge has been bent inward forming a flat ring of about 8 mm. in breadth. On this edge, held in place by a retaining ring of metal, lies a disc of

blotting paper. The fabrication of this cover ring D and its fastening to the brass collar Z is detailed in the figure indicating an open slotted closure V and a positioning pin a on each side of the cover assembly. The entire apparatus is supported by three little feet S of brass threaded into the base.

The size described is suitable for the drying of preparations on coverglasses which can be laid in multiple on the central portion, being separated and positioned with the aid of a needle. If coverglasses are supported on microslides (or if the preparations to be dried are on microslides) then a larger diameter chamber of perhaps double the size will be found more useful.

In practice, the preparations are placed on the central portion of the plate and the cover with the blotting-paper top is put in place. The blotting-paper takes up the evaporating moisture readily, and at the same time is a protection from dust. Even during perhaps week-long periods of selecting individual diatoms from such preparations, the protection against dust is quite adequate with this little chamber.

If the diatom material contains large forms, the drying by the continual application of gentle heat is appropriate. Very fine or small diatoms should be dried at room temperature {possibly for 2 or 3 days). If the very small forms are dried too rapidly they will adhere to the glass and cannot be picked off.

A good substitute for the drying chamber of Hustedt is a porous wooden box, such as a cigar-box, recommended by Burke. The porous wood allows evaporation to take place very evenly and slowly, and such a box will accommodate a large number of coverglasses or microslides. The preparations should be retained on a "carrier" or platform that can easily be lifted in and out of the box.

Dr. Joseph F. Burke
A member of the New York Microscopical Society

For those with more complete laboratory facilities, a vacuum desiccator may be used to dry and evaporate the suspension on the cover. The advantage of drying in a vacuum with a desiccant, such as anhydrous calcium sulfate, is that water particles are removed from the upper surface of the liquid without heat and consequent agitation - thus insuring that the suspension remains in an even uniform layer.

Evaporation without the aid of heat may, of course, be effected without the drying chambers mentioned above. The use of small watch glasses is ideal in protecting individual coverglass preparations against dust and dirt for the rather lengthy time required.

For a strew slide to be most useful the diatomaceous material should be in a uniform single-diatom layer. It is essential that all traces of water be removed from the distributed diatoms as many mountants are extremely sensitive to it. Precautions in drying the preparation at this stage will materially assist in making a superior permanent mount.

If drying by applied heat is preferable from the standpoint of the time saved (and if fairly large diatoms are involved), then the use of a thermostatically controlled electric hot-plate is very useful. However, very good results may be obtained by the method used in the past by Hustedt. He recommends the use of a square thin copper plate of 15 or 20 mm. on a side, 1.5 mm. thick supported by a tripod. Heat is applied

at one corner of the copper plate by an alcohol lamp. For gentle heating of coverglass preparations they are placed at the diagonally opposite corner of the plate, being heated by conduction through the metal plate. Multiple coverglasses are accommodated in this manner, and when a higher rate of heating is desired the location of the alcohol lamp, in relationship to the preparations, is changed.

8.5.4. Choosing the Mountant

Kenneth E. Lohman
b. 1897
U. S. Geological Survey

As mentioned in a previous chapter, the visibility of diatoms improves when mounted in the higher refractive index media. However, very coarsely marked forms such as in certain species of *Pinnularia*, the image of the structure yielded may be too dense and obscure. Also, according to Lohman (1972), where a strew slide might contain considerable quartz, feldspar, mica, etc., as might be especially so in examining sediments, which is difficult to remove without the loss of very small diatom forms, the higher R.I. media can cause "visual interference". In that or similar cases, the use of a mountant with a refractive index near 1.515, such as Canada Balsam or Caedax, is in order. The undesired particles are then rendered nearly invisible and cause less visual interference with the examination of the diatoms. At the other extreme, where very small and delicately marked diatom forms are to be studied, the use of very high refractive index mountants, such as realgar, is indicated.

A preliminary microscopical examination of any diatom material before mounting is always advisable whether fossil or fresh. By this means, important information is obtained as a basis for future treatment, with which a choice of mountant can be guided.

8.5.5. Minimizing Bubbles

In mounting diatoms, whether as selected mounts or in strews, the vexing problem of bubbles is always present to some degree or another. Some mountants are prone, because of their viscosity, boiling point, and/or other characteristics, to bubble production during the mounting procedures. Mountants with such characteristics cannot always be avoided, and it is true that almost any resinous mountant (natural or synthetic) will be troublesome, to some degree, in this respect. Bubble production can be minimized and/or eliminated with good technique and bubbles can be removed by various means.

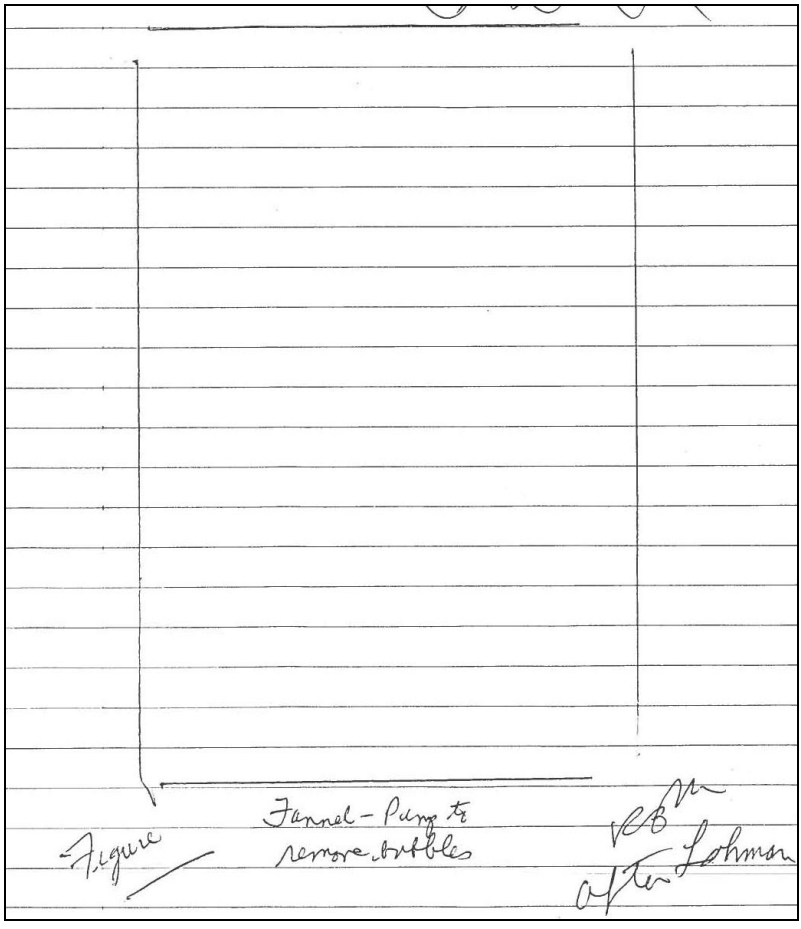


Figure 40.

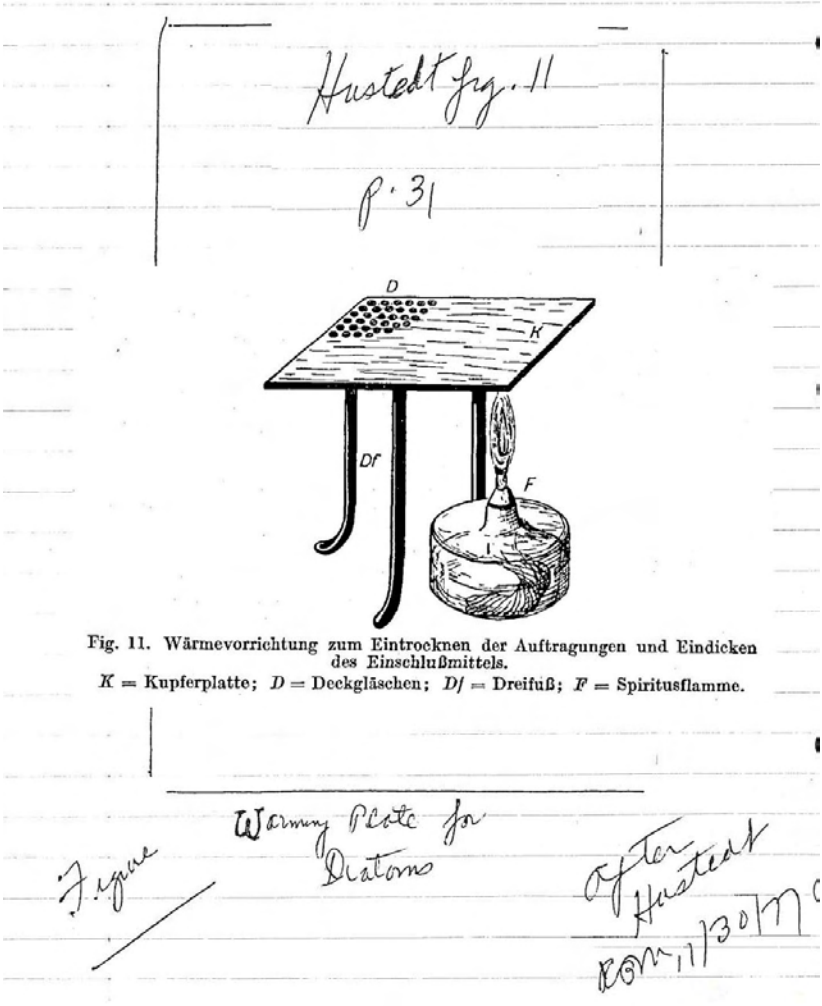
In almost any procedure, using almost any mountant, bubble formation and entrapment can be minimized at the beginning by application of the mountant solvent to the dry strewn diatoms on the coverglass. Xylene for instance, a common solvent for many mountants, is dropped on the strewn diatoms and the so treated preparation examined with the microscope, or a suitable magnifier. When bubbles have ceased to appear, it is apparent that the solvent has penetrated all voids and driven out entrapped air. When the mountant is applied, its viscosity is reduced by the solvent action and thereby entrains less air in entering the voids. When the rare case occurs that this procedure does not remove all entrapped air, the method developed by Lohman, illustrated in Figure 40 will do so. An inverted glass funnel is connected to a Richards pump. The edge of the funnel that is in contact with the hotplate is ground to fit that surface and prevent leakage of air. This light suction is particularly effective with strews containing whole frustules in which entrapped air is difficult to remove.

The use of different mountants requires procedures, sometimes tailored to the characteristics of the one used. It is not feasible here to note all of the methods for all possible mountants used in diatom mounting. Therefore the following procedures

have been selected for some of the more commonly used mounting media. They will serve as guides to the methodology in using others. Although the procedures described are for the preparation of strew slides, the techniques of application and ultimate treatment, insofar as the mountant at least, apply to the preparation of selected slides as well, unless otherwise noted.

8.5.6. Mounting in Canada Balsam or Styrax

Mounting in these two natural media is similar, and most procedures associated with them are applicable to other natural resins.



Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 11)

Figure 41.

With the strewn material on the coverglass completely dry it is placed on a warm metal plate. To assure complete dryness the temperature of the hotplate should be at about 125°C. This temperature is sufficient to drive off any residual moisture but below the boiling point temperature for xylene (140°C.). The strew is left on the plate long enough to completely remove any water. An electric hot plate or a copper plate heated by an alcohol flame as in Figure 41 is adequate. Then, with a pipette, a drop of xylene is applied to the diatoms. Before the heat drives off all of the solvent a drop of the mountant (of a fairly thick consistency) is placed on the diatoms. As the coverglass warms, the mountant will spread to the edge of the coverglass and bubbles will begin to appear. Additional heat hardens the mountant (if an alcohol flame is used, care must be taken that the mountant is not set afire).

If the medium is allowed to harden under room temperature (being protected from dust) it will take anywhere from 4 or 5 days to a week to harden. This lengthy procedure is not recommended. Instead, the temperature of the hotplate is adjusted to hasten the hardening. The degree of hardening of the mountant that is accomplished in this way is learned by experience. Too high a temperature will boil the mountant too rapidly, forming bubbles and ultimately resulting in the mountant becoming darker colored, with the possibility of crack development. Styrax is particularly prone to this result if heated too rapidly at a high temperature. The mountant when cool should be of a hardness that it can just be dented with the point of a needle, and yet not fluid at all. Styrax has another disturbing quality if heated too hot and too rapidly. It will tend to separate from its glass substrate when receiving mechanical shock.

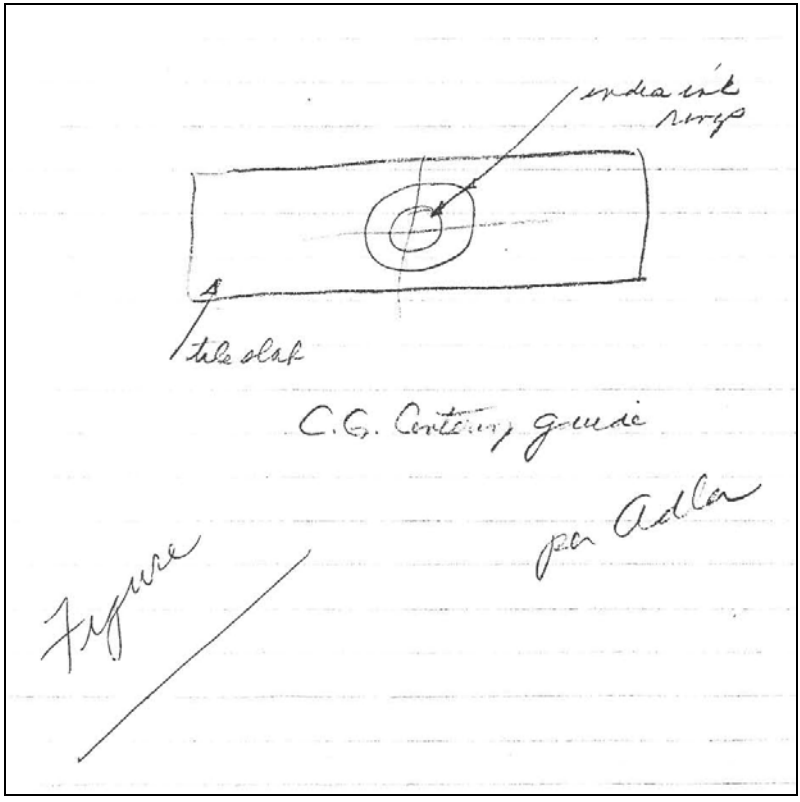


Figure 42.

After the mountant is properly hardened, the coverglass is placed on a specially marked piece of white poster board, or card material. Referring to Figure 42 the outline of a microslide, with several concentric circles at its center is drawn with india ink on the white surface. The concentric circles correspond in diameter to commonly used cover glasses. The prepared coverglass is aligned with an appropriate circle. If the coverglass is carried on a microslide for convenience of handling, the procedure is the same, except the microslide is aligned with the ink outline and the cover centered as before, with the aid of a needle, to an ink circle. A perfectly clean, warmed, microslide is used to pick up the coverglass, using the outlined rectangle to align it such that the coverglass is picked up at the center. This not only provides for a pleasing appearance of the preparation, but is of practical importance in later work. The warming of the slide prior to pick-up assures adherence and minimizes the formation of bubbles between it and the mountant layer.

The micro-slide with the coverglass uppermost, is then placed on a warming plate and heated until the mountant spreads to the edge of the coverglass. Some bubbles will appear and will usually work their way to the edge where they will burst or can be punctured with the aid of a needle. Pressure on the coverglass to force excess mountant laterally to the edge must be done very carefully if at all. Both Canada Balsam and Styrax possess a viscosity such that the vertical pressure is not relieved

through lateral flow at the same rate as it is applied and therefore the transmitted vertical pressure is likely to crush the diatom frustules. Clamping the coverglass by use of a spring clip is sometimes recommended by certain workers to force the excess mountant out and keep the coverglass level and from "floating upward" as the mountant hardens. This should only be done if there is no possibility of crushing large forms, or if some type of protection against it has been taken beforehand. The protection often takes the form of some type of spacer to provide the protection, and is more often used in selected mounts. The various means of accomplishing this are covered later in discussing selected mounts.

The excess mountant will leave without pressure through simple warming, and not more will leave than the largest forms in the strew will permit. The latter holds true only if gentle warming is used. After cooling, the excess mountant may be removed by scraping with a sharp knife or razor blade and cleaning with alcohol, xylene, or benzene. With some practice however, the amount of the media originally applied to the cover can be gauged such that there is little, if any, excess.

A properly made preparation will have the following attributes:

(1) The coverglass and microslide are parallel to one another. An inclined coverglass can be quickly leveled through light pressure with a needle if the preparation is warmed. If a great number of impurities are in the strew such as large sand grains etc., that contribute to difficulties in leveling the coverglass, the preparation must be redone. In some cases, if the original preparation is warmed, the coverglass with the diatoms and bulk of the mountant can be lifted off and transferred to another microslide. The heavy sand grains will mostly remain with a part of the resin on the first microslide.

(2) The mountant forms an almost colorless layer just thick enough to be suitable for the largest diatom forms and no thicker. It extends to the edge of the coverglass in a symmetrical cone-shaped slope. Strong coloration of the resin is usually due to the application of high heat in the hardening step.

(3) After cooling, the coverglass is firmly fixed in place to the extent that immediate examination of the preparation with oil immersion objectives is possible without fear of displacing the cover. Should this not be the case, the hardening of the mountant is not complete. If there has been a mistake in preparation, it sometimes can easily be adjusted. For instance, warming of the preparation allows the cover to be adjusted with a needle. If insufficient mountant has been applied, the preparation is placed on a hotplate and warmed. Then an additional drop of mountant is applied at the edge of the coverglass. It will, by capillary action, enter and fill the void.

A sealing ring is not always absolutely necessary, but is appropriate for neatness. It is a means of providing some protection of the resin from reaction with air and thereby promotes durability. Also a sealing ring acts as a mechanical fastener of the preparation to the microslide.

Styrax particularly, in some cases will separate from the glass if subjected to shock, such as if the slide were dropped. The sealing ring prevents this. Shellac, which can be colored if desired, is a good sealant. This subject will be gone into in more detail in the following treatment of selected mounts.

Shellac is a resin secreted by the female lac bug.
It is used in French polish and is available as flake from many hardware suppliers.

It will be found that Styrax, or mountant with approximately the same refractive index, is sufficient to resolve most diatom structure. In only a few cases will it be found necessary to resort to mountants with a higher refractive index. This is especially true if good objectives are used and careful attention to lighting and adjusting the microscope is attended to. The latter will be discussed at length in the study section following in Part III.

It cannot be emphasized too strongly that high heating temperatures should, in general, be avoided. The step wherein the mountant is gently warmed and hardened before the coverglass and microslide are joined is the best procedure. If the coverglass and microslide are joined when the mountant is very fluid, the generation of bubbles, and their action in the enclosed space results in displacement of diatoms laterally, or causing them to "sink" down into the medium, and at the same time creates a greater danger of the coverglass "pulling" down and eventually crushing the diatoms as it hardens.

With very viscous media, as are most of the higher refractive index mountants, strong heating can easily shatter the diatoms completely, or at the very least drive them out of position laterally (if in a selected mount) or clump them disadvantageously in a strewn mount.

The procedure herein described, is in general, applicable to most natural resins, including Canada Balsam, Styrax, etc. With the possible exceptions mentioned previously Canada Balsam and other mountants of the same index are used only occasionally. If one has not seen diatoms mounted in a medium of the refractive index of Styrax or higher (in some cases) it is not realized just how great the advantage in visibility and resolution of detail can be.

8.5.7. Mounting in Hyrax

Styrax has been described insofar as its preparation and use in mounting primarily because of its antiquity in respect to mounting diatoms. It was one of the first mountants used other than canada balsam, and thousands of old diatom collections contain slides of diatoms mounted in that medium. Several contemporary mounters still use it to advantage, and some of its shortcomings such as a tendency to forming crystals, or cracking or crazing, are obviated by careful preparation of the resin to begin with, and in proper procedures in its use. However, it is, at this time, not generally available, and especially not in a finished form ready to use.

Hyrax is no longer available. However, Prof. Bill Dailey has produced a product with very similar properties – Zrax.

Hyrax however, with the approximately same refractive index, is generally available in both the United States and England. It is obtained in a form

ready to use, and has the advantage over Styrax of quicker hardening and of being able to withstand higher temperatures. Hustedt indicated that it would, if found to be stable, replace Styrax as a diatom mountant. Since that time it has proven to be a very stable and reliable medium, and is probably, along with Naphrax, one of the most commonly used diatom mounting medium.

Due to the low boiling point of the solvents (xylene, toluene, benzene) used, and the extremely viscous nature of the mountant itself, bubbles are a major problem with Hyrax. This is particularly true as every trace of solvent must be expelled if advantage is to be taken of its high refractive index. The following technique will assure the best results.

A drop of xylene is placed on the diatoms on the coverglass, and allowed to penetrate thoroughly in order to remove any bubbles of air from the interior of the diatoms. Meanwhile a cold drop of Hyrax is placed in the center of a cold microslide. The coverglass is then picked up, as before, with the microslide, inverted and placed on a warming plate. (It is assumed that the centering template previously described is used to center the location of the coverglass). The temperature of the warming plate should be such that the hand can just bear to remain there for a few seconds. The preparation is left on the plate for some forty eight hours. The length of time that the microslide should be allowed to remain on the hot plate can be judged by the fact that when properly hardened, any Hyrax which has exuded from under the coverglass remains hard at the temperature of the plate.

The slide is then allowed to cool and the excess mountant cleaned off with a cotton swab moistened in benzene and the slide returned to the hotplate for a further twenty four hours. At the end of this time the slide is cleaned by placing it in methylalcohol and rubbing it with cotton, dried off with a cloth and replaced on the hotplate for as long as desired, perhaps up to a week, After this the coverglass may be ringed if desired.

If insufficient time is available to follow the very careful procedure above, a much faster means can be employed that is nearly as successful. After the usual flooding with xylene, the Hyrax is applied to the slide and the cover picked up as before. The slide is then inverted (with the coverglass uppermost) and the cover pressed down with a needle until the Hyrax reaches the coverglass edge. (Alternatively, the coverglass can be picked up with sharply pointed forceps and inverted and placed on the Hyrax). The properties of Hyrax are such that, unlike the natural resins, it can be pressed down without harming the diatoms. The preparation is then placed on a hotplate and the heat raised to bring the mountant to a state of vigorous boiling to completely eliminate bubbles. The preparation is then placed on a heat-sink of thick brass or aluminum to cool. Hyrax will withstand the high heat necessary to drive off the solvent without subsequent discoloration, but the heat should only be applied for as long as is necessary to accomplish that and no longer. The vigorous boiling to remove the solvent results in a multitude of bubbles and turbulence of the medium which can easily dislodge the diatoms on the coverglass, allowing them to move about both laterally and vertically, spoiling an evenly distributed layer.

8.5.8. Mounting in Naphrax

This mountant is very intolerant of moisture. The diatom material must be thoroughly dry before applying it. The cover, with diatoms, should be dried for at least 15 minutes at 80°C., then rinsed off with three changes of xylene or toluene, after which the Naphrax is applied. The mechanical procedures in handling coverglass and microslide are the same as described previously for other mountants.

After the Naphrax is applied to the cover it should be put back on the hotplate for at least an hour before being joined to the microslide. The preparation is then heated further to drive off the remaining solvent. If overheated, Naphrax will become darker in color.

8.5.9. Mounting in Piperine-Coumarone

The mountant mix as prepared according to previous directions results in small globules or buttons of hardened material. Each globule is cut with a knife into 2 to 4 small pieces. A piece is placed on the center of a microslide and melted over heat. The coverglass with the dried diatoms is heated (best on a metal plate) and laid (diatom side down) on the melted drop of mountant. Then the heating is continued until the mountant becomes fluid and spreads to the edge of the coverglass. In heating this material air bubbles are produced, which are removed by lifting and inclining the coverglass slightly with the aid of a needle. After cooling, the medium immediately becomes hard. The excess at the edge of the cover is then completely removed with the help of alcohol or xylene.

A lacquer ring is not absolutely necessary (according to Hustedt) but is desirable for neatness and as a protection when using immersion objectives for examination.

Clamping or weighting the coverglass down is only permissible with the mountant if it is sure no damage to large frustules will take place, or if additional protection in the form of spacers has been provided.

The finer structure of diatoms appears correspondingly better in this medium than in Styrax for instance, in direct relation to its increased refractive index.

Higher heat than is usual with the mountants discussed previously is necessary with this medium. It is useful in making selected mounts as well.

8.5.10. Mounting in Realgar

A drop of the medium produced as described previously, is placed in the center of a microslide and the coverglass applied with the diatoms down. The preparation is then heated intensively for a considerable time until no gas bubbles appear. **Caution! Danger! Poisonous fumes!** At this point, the mass becomes red, but after cooling assumes a bright yellow color and becomes hard. There may be some bubbles and fissures remaining in the hardened finished product, but they are fixed in place and will not wander about in the preparation. A lacquer ring is very desirable, especially to aid in preventing the coverglass from becoming separated from the microslide through mechanical shock.

8.5.11. Mounting Diatoms Dry

Some diatoms possess weakly silicified cell walls that in a high refractive index medium become nearly invisible. They are sometimes advantageously mounted dry to improve the visibility of detail. In such cases, the coverglass with a dry strew of diatoms is mounted without the embedding medium. The microslide for this purpose must be meticulously clean (A wash with acidified alcohol is a good cleaning fluid in this case.)

In order that the diatoms are not crushed against the microslide, and to fix it in the center of the assembly, a lacquer ring, whose inner diameter is about 2 mm. less than the outside diameter of the coverglass, is employed. The lacquer ring is applied to the microslide using a ringing turntable. The ring must be very thoroughly dried and of a thickness sufficient to protect the largest diatom in the strew. Dependent upon the ringing material employed, drying may take several days or even weeks. This type of mounting is appropriate for incinerated strews as described before. Even chemically cleaned diatoms applied as strews to coverglass may advantageously be "incinerated" to remove all traces of extraneous materials and moisture which contribute to the shortened life of such preparations.

A microslide with a properly applied and dried ring is rubbed clean with a chamois just prior to assembly. The ring should be about l mm. in width. The microslide with the coverglass in position, is then placed on a not-too-hot warming plate to soften the lacquer ring, and the coverglass gently pressed into contact. The warming must be very carefully controlled or the ringing material may run under the coverglass and spoil the preparation. For the same reason avoid pressing down on the coverglass too hard. In the completed preparation there must be no gaps between the coverglass and the ring through which air and/or moisture can pass. A sealing ring is then applied for both mechanical and hermetic reasons.

Dry preparations are not absolutely permanent, and even if properly sealed some damage may become apparent in a few years time due to mold on the microslide. In those cases the coverglass can be carefully loosened and applied to another prepared microslide, thus renewing the preparation for another period. If good quality microslides have not been used, diatoms sensitive to silicic acid may become destroyed through "corrosion" and renewal of the preparation thereby impossible. In the preparation of dry mounts it is imperative, for protection against such damage, that the very best quality microslides and coverglass be used. In any event, a dry preparation is only made when a resin mount is not feasible. While they may be admirably suitable as a short lived preparation, or for photomicrographic purposes, they definitely are not best for durability.

8.6. Selected Slide Preparation

8.6.1. Ringing Turntable

Strew-slide preparation is the simplest form of diatom mounting and requires little in the way of special apparatus with the possible exception of a ringing turntable. In

the preparation of selected diatom slides however, there has been developed a number of interesting and useful devices to aid in the technique of making such preparations. Common to both strew and selected diatom preparation is the ringing turntable. It is used in a number of ways in the manufacture and finishing of such slides and it is appropriate to describe it at this point. Other special apparatus will be described along with techniques requiring, or aided by, their employment.

In its fundamental form a ringing turntable is a simple apparatus. It consists essentially of a rotatable platform upon which a coverglass and/or a microslide may be fastened. Upon rotation of the platform, imparting a circular motion to its load, circular formations of various substances may be applied. India ink locating rings, lacquer or varnish coverglass seals, circular built-up cell-walls for liquid mounts, or supports for dry preparations, and finishing materials such as shellac, varnishes, paints and enamels, for protection and decorative purposes, are among the applications. Figure 43 illustrates such a device. Although electric motor driven turntables have been devised, the hand operated type illustrated is, by far, the most common and is satisfactory for all purposes in the making of diatom preparations. The turntable itself is of sufficient mass that when given a pull by hand it continues to rotate for a considerable period of time. The availability of these little devices commercially has become less and less through the years and they are difficult to obtain today. Some of the versions available in the past had a self-centering device that positioned the microslide in the exact center of the turntable. As can be seen from the illustration there are only three essential parts to the device. A turntable, a means of securing the microslide to it, and some kind of guide or rest for the brush or applicator. For the making of good rings the latter is essential to steady the applicator.

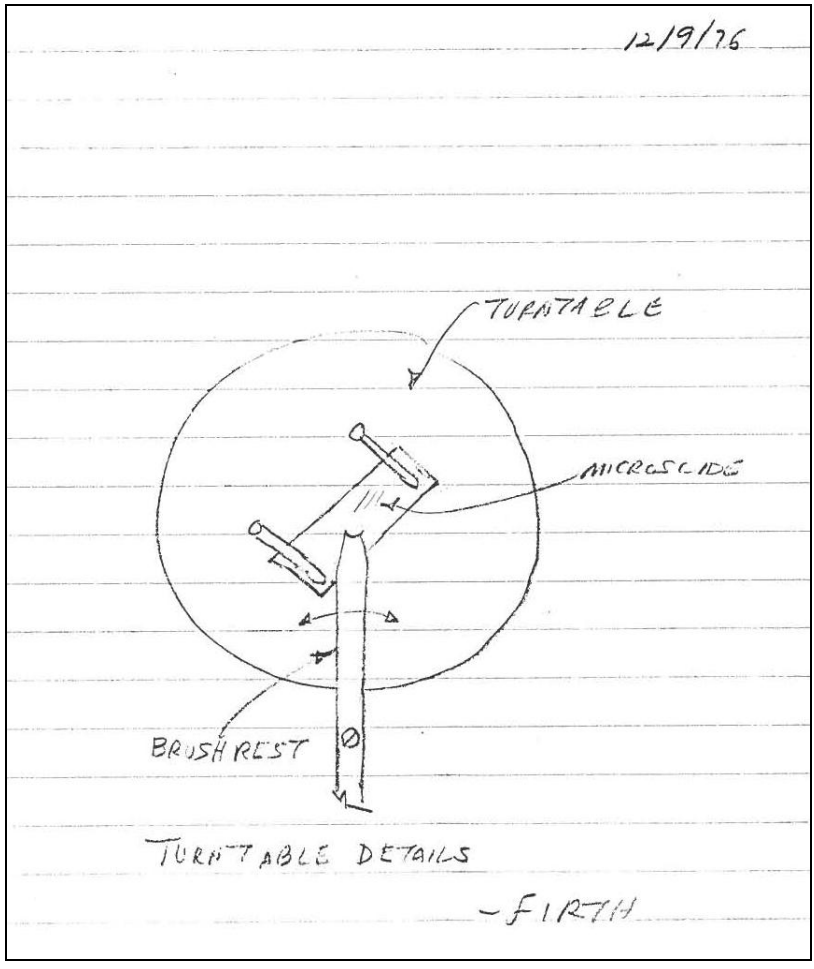


Figure 43.

As an aid to centering the microslide on the turntable two rings may be scribed on the metal surface of 1 inch and $3^{1}/_{8}$ inches diameters (Figure 44). A detachable white paper disc the size of the turntable with the above two guide rings in black ink provides better visibility for the work and is very useful. For instance, a red circle on the paper the size of black rings to be made on the coverglass or microslides indicates the position of the brush and shows up the black ring as the brush touches the glass.

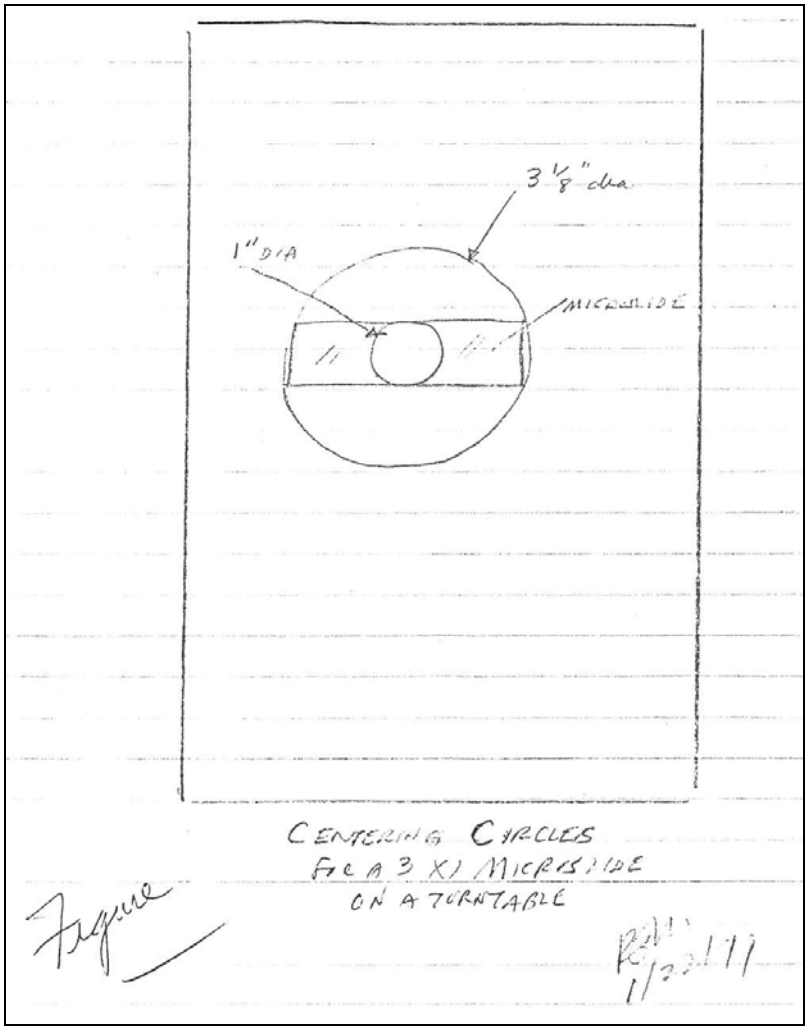

3 1/8" dia.
1" DIA
MICROSLIDE
CENTERING CIRCLES
FOR A 3 X 1 MICROSLIDE
ON A TURNTABLE
Figure

Figure 44.

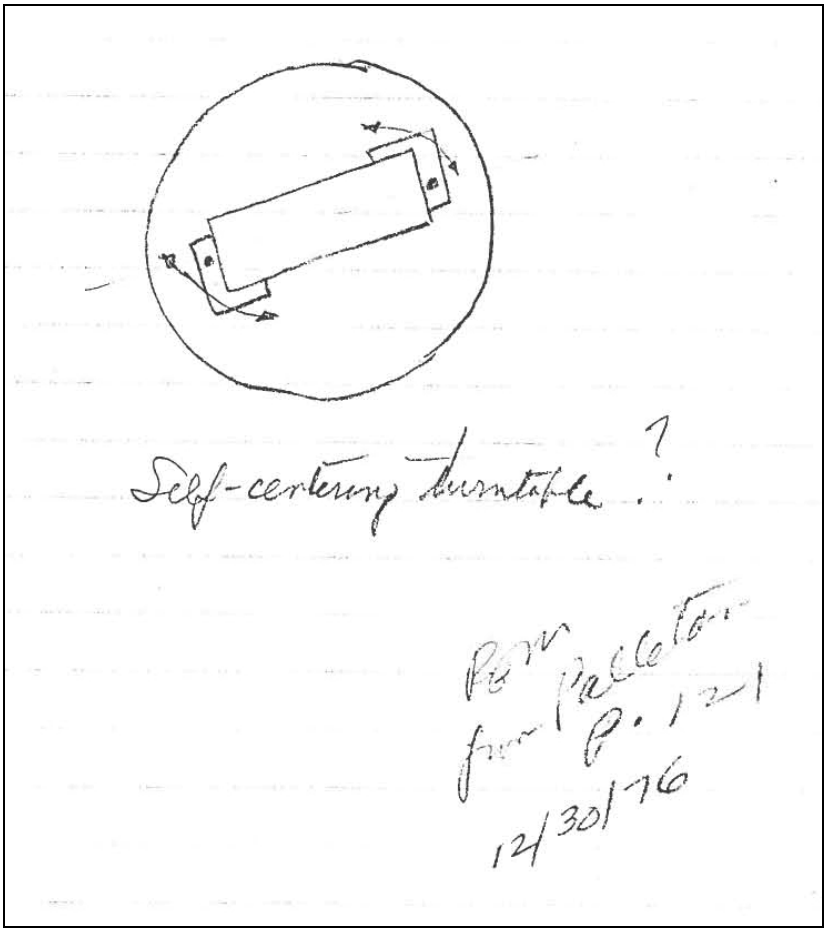


Figure 45.

An important function of the turntable in selected diatom mounting is providing a black locating ring on the coverglass where the diatom(s) are to be mounted. One purpose of the locating ring is to indicate exactly where only one or two (or at most a limited number) of diatoms are. Another is to enable one to find the proper microscope focus which can be a matter of difficulty in the case of delicate very transparent forms, and a matter of danger with a high aperture objective with a very short working distance. Also, the locating ring may serve as a medium (black ink) for a star test in correcting for effective coverglass thickness, either by adjustment of a collar on an objective, or by microscope tubelength adjustment. The proper adjustment can be a matter of resolving the diatom or missing complete resolution entirely. Procedures for making such adjustments are provided in Part III of this book.

The making of very fine ink locating rings is one of the most difficult procedures to master in diatom slide preparation. However, difficulties can be minimized with the proper selection and use of materials, and with practice. The india ink used should

be of the best quality, of uniform thickness and run easily, It should be examined under the microscope for too much granularity with a 10X objective.

The best quality sable brush (00 to 0000 size) is used. It should be kept very clean by rinsing and wiping frequently. The inking brush should be held on the coverglass for 2 or 4 seconds with a fast spin of the turntable, as the relatively long spin improves the edge of the inked circle. The circle applied will ordinarily be of about $^{1}/_{10}$ inch in diameter. Completed rings are examined under the microscope at 100X for uniformity.

In application the brush with its load of ink is held against the rest but not touching the glass. The turntable is then given a pull and the tip of the brush lowered to apply the ink. Experience alone will dictate how much ink to use and how much pressure is applied to the brush tip. Too much ink will result in a large blob being applied, and too dry a brush will not provide a uniform solid black ring.

The coverglass is fastened to a microslide in one of two ways. A small drop of distilled water is applied to the microslide and the coverglass placed on it and the combination put on a hot plate. The heat will evaporate most of the water and pull the coverglass securely down on the slide. After any necessary cooling the combination is put on the turntable. It will be found easy enough to separate the two by pushing the coverglass laterally off the microslide with the point of a needle. Alternatively the coverglass can be fastened to the slide with a drop of glycerin. However, this latter method requires some eventual cleanup.

8.6.2. Preparing the Coverglass

The first step in making a selected diatom slide is preparing the coverglass. The diatom is normally fastened to the coverglass by some sort of adhesive, such that during subsequent operations it does not become dislodged and remains in the orientation in which placed. Although the usual orientation of a diatom is to allow examination of the outer valve surface, that is not the only aspect of value. Lamentably, many professional, as well as amateur preparations, show only one or two valves and usually only one face of the valve. It is rare for slides or drawings to show zonal aspects of diatom structure. It is recommended that both inner and outer aspects of valves be provided in selected mounts and if at all feasible, at least one orientation providing a zonal view. Detached girdles, especially those showing markings, edge details, and/or joint features also should be mounted. All of this may require the parts to be in various positions; flat, on edge, and even perhaps at angles for oblique views. These arrangements necessarily call for some kind of adhesive to fix the location and orientation.

There are two general types of adhesives used in fastening diatoms to a glass substrate. One type is a water-soluble one which can be remoistened and dried repeatedly and the other is one which remains moist for a considerable length of time and then sets permanently. There are variations of both types and examples are as follows:

8.6.2.1. Dextrine Adhesive

This particular fixative is recommended by R. I. Firth, an experienced diatom mounter of long standing. Use brown dextrine (described by British Chemists as British gum).

Robert Isaac Firth
(1902-1982)

(a) Dissolve the powder in distilled water with a little formalin. It is important to get a perfect solution free from microscopic lumps of undissolved gum.
(b) Pass the solution through filter paper into a perfectly clean wide mouthed bottle having a ground glass stopper if available - otherwise a plastic screw-on top. It is important that the bottle be wide mouthed.
(c) Apply gentle heat to the bottle (which should be covered loosely with a paper cap to prevent admission of dust) until so much of the water has been driven off that the solution has the consistency of a golden syrup or motor oil. The heat should be so gentle that it takes a couple of days. If more than gentle heat is applied the solution may become lumpy.
(d) Now filter a little glycerine into the solution sufficient to make it about half the consistency of golden syrup or motor oil. It is important that there is a perfect mix of glycerine and gum, use a glass stirring rod for the purpose.
(e) The fixative when applied remains moist for some hours. Apply the smallest amount and spread as thinly as possible with the tip of the little finger. Examine the film applied at 100X to quality control it for cleanliness.
(f) When mounting the diatom push it about ever so slightly as to assure contact with the gum.
(g) Apply gentle heat for 3 or 4 minutes until the glycerine is driven off and the diatom is fixed permanently in position.

It will be noted that this fixative is permanent once the heat has been applied and the glycerine is driven off. Although it remains moist for hours, it might dry too much in lengthy preparations involving large numbers of mounted diatoms or their parts, or if the work must be interrupted for a time.

Also, the brown dextrine is not readily available, which makes this fixative undesirable from that standpoint. However, if a small amount of the gum can be procured, enough fixative for years is assured.

For single diatom mounts, or for work of relatively short duration this adhesive is of proven utility as the excellence of mounts produced by Mr. Firth over the years has demonstrated.

8.6.2.2. Gum Tragacanth Mucilage

A popular and successful adhesive for fixing diatoms to the coverglass is a water solution of gum tragacanth. The gum is easily obtained and has been used by many diatomists and still is.

Generally a 1% solution, by volume, of the tragacanth in water is used. It should be filtered before use. A variation of this adhesive was proposed by Fuge (1938) and his directions for preparation are repeated here as follows:

Rev. Joseph Dingley Palmer Fuge (1874-1944)

(a) Into a dry one ounce bottle put 30 to 40 drops of alcohol.
(b) From the tip of a penknife add as much powdered tragacanth as represents the bulk of a split pea, and shake well.
(c) Add a half-ounce of water. Shake again and it is ready.

Before application to a cover, it is recommended that this mucilage be filtered once or twice, and examined under 100X with the microscope for cleanliness. It is applied very thinly to the coverglass with the finger tip or with a needle and spread with a finger.

The main advantages of this adhesive is its ready availability, easy preparation and that it can be moistened and dried again and again during the mounting process. Also, many coverglasses may be prepared with this fixative and stored for future use. A successful procedure is to place the diatoms on the dried fixative surface and moisten it with the breath which fixes the diatom in place as it dries. If it needs to be repositioned it can be again moistened with the breath and moved.

The thickness of the mucilage is not critical and is easily adjusted in the making by the use of more or less water. It is essential that it be filtered before use, especially if it has been stored for a period of time. Storage in a dropping or squeeze bottle is recommended.

8.6.2.3. Gelatine Adhesive

A very successful adhesive, used perhaps by more diatomists than any other, is a gelatine - acetic acid – ethyl alcohol mix. Various compounding proportions have been proposed and used successfully. One such mix described by Adler is repeated here.

Herman Adler - (1930-1990).

The required amounts of materials are:

Gelatine 6 grams
Distilled water 50 grams
Glacial acetic acid 50 grams
Ethyl alcohol 8 grams.

Preparation is as follows:

(a) Place the gelatine and water in a 200 ml. flask.
(b) Place the flask in a water-bath and agitate until the gelatine is in solution.
(c) Cool and add acetic acid and alcohol.
(d) Filter, discarding the first few drops.
(e) Store in tightly stoppered bottle.
(f) Filter occasionally, and especially before use.

The fixative can be applied as previously described for the others, or roll a thin glass rod previously dipped in it over the coverglass. It is allowed to dry under cover to exclude dust. It can be used to prepare a stock of coverglasses as with the tragacanth mucilage. The moist breath is used to affix diatoms laid upon it. A more convenient recipe by volume is that of Patrick using 12 parts of glacial acetic acid, 2 parts of gelatine, and 1 part alcohol. The acid is added to the gelatine and heated in a water bath after which the alcohol is added. It should be filtered while still warm.

It should be noted that if the gelatine fixatives are not relatively fresh, they will not hold reliably. Once dried on the cover however, they will remain effective for months. It is best to shake up a new batch of the fixative each time a supply of coverglasses is to be prepared.

In all cases of application of the adhesives it is important that the coverglasses be clean and dry, and that as soon as possible they are put under cover to dry (in the case of the latter two) free of dust.

The india-ink locating ring is applied as previously described, over the dried gelatine-acetic acid (or dried gum tragacanth) mucilage and the coated and ringed covers stored in a Petri dish.

The dextrine fixative of Firth does not lend itself to mounting on the cover with an ink locating ring. If the ink ring is to be dispensed with then this adhesive may be used on the cover. Otherwise application of the fixative in its liquid state to a cover will destroy the ink ring. Therefore, when using this fixative the diatom mounting is usually done on the microslide, and the ink ring is on the cover, negating at least a portion of the latter's utility.

Another aspect of preparing coverglasses for diatom mounts is the provision of supports to protect the selected diatoms from being crushed. Over long periods of time some mountants act to pull the cover down and damage can result to the carefully selected and arranged diatoms and/or their parts. In some older preparations diatom mounters used three large spaced diatoms of the genera *Coscinodiscus*, *Triceratium*, or similar forms to protect the smaller subject diatoms of the slide. Of course, in time, they themselves are subject to crushing, and although a charming and interesting application, other more practical means are recommended.

Spacers of varied designs have been used with success over the years. Materials commonly used are metal and glass, the metal generally being in a thin beaten or foil form.

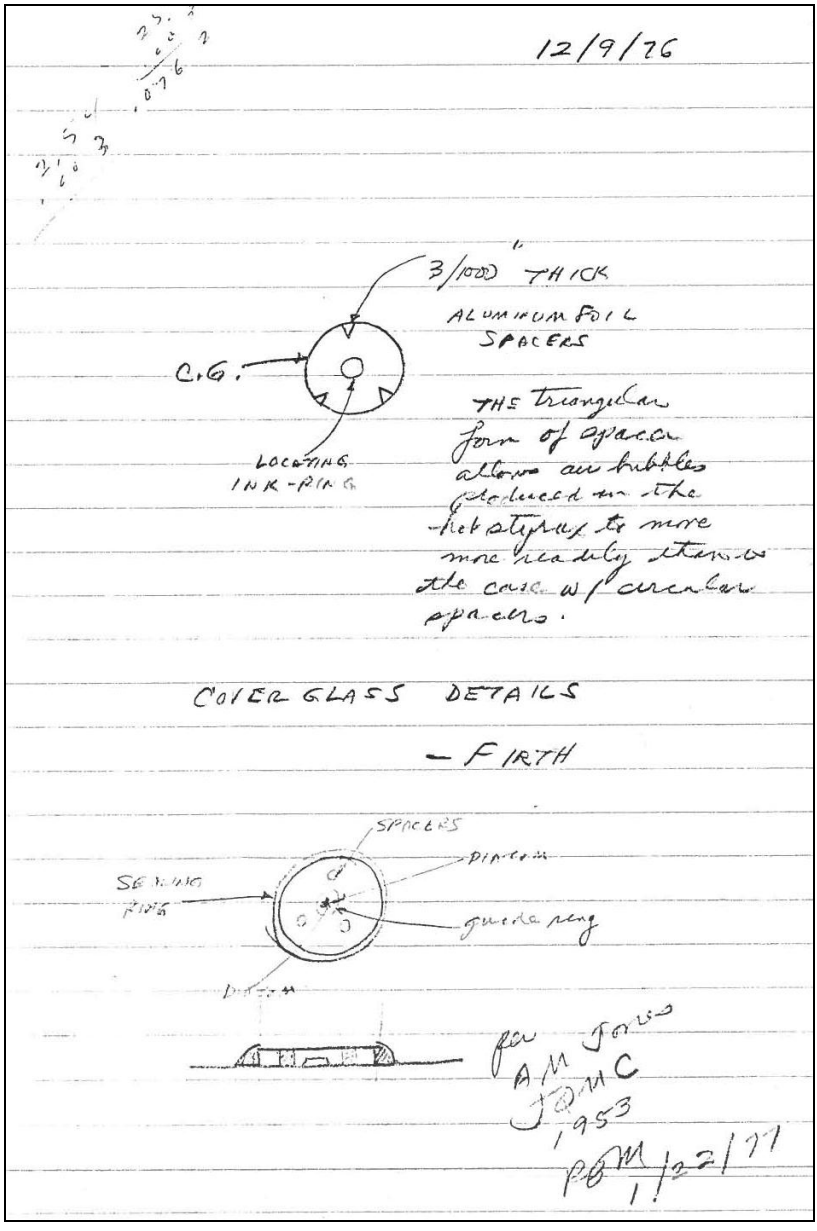


Figure 46.

Simple and effective spacers can be made from coverglass. It is thin, of a very uniform thickness, yet thick enough to protect almost any kind of diatom mount, Coverglass, or fragments, are crushed in a mortar with a pestle to obtain pieces

about a millimeter in their largest dimension, and stored in a small vial. These pieces can be selected with the aid of a moistened brush and applied to the coverglass which has already been prepared with a fixative and ink-ring. Three selected pieces of glass are placed smoothside down on the cover, in an equi-spaced pattern between the ink-ring and the edge. The moisture from the brush is enough to fasten them to the cover. Some more particular mounters prefer cutting a coverglass into tiny triangles with a diamond point, but the random shapes work just as well.

Aluminum foil, about 0.003 inch thickness, cut into triangles of about 1 mm. on a side are sometimes used. When these are fastened at the edge of the coverglass with the points of the triangles facing toward the center it is claimed that bubbles from hot Styrax move out from under the cover more readily than is the case with circular spacers. See Figure 46. To smooth and flatten such spacers rub them between two glass microslides.

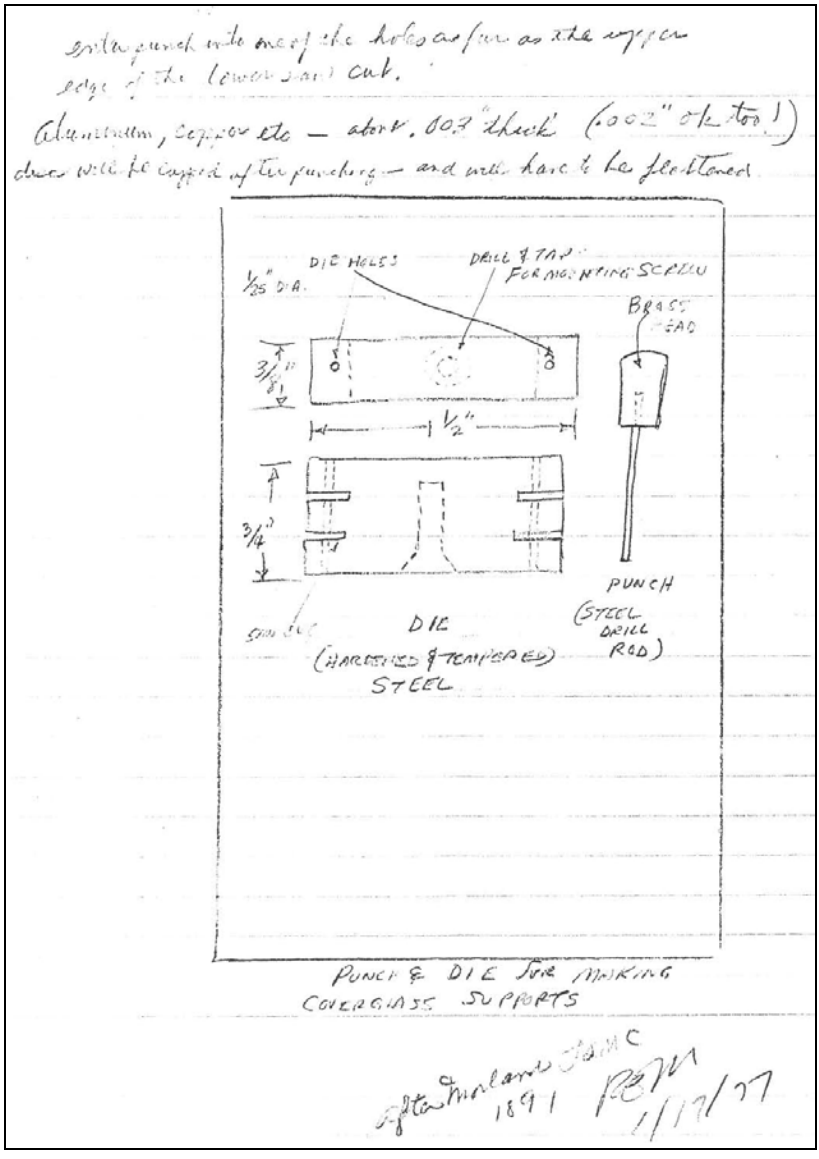

punch into end of the holes as far as the upper edge of the lower slot cut.
Aluminum, copper etc — about .007 thick (.002" or less!)
discs will be cupped in the punching — and will have to be flattened
DIE HOLES
1/25" DIA.
DRILL & TAP FOR MOUNTING SCREW
BRASS HEAD
3/8"
1/2"
3/4"
PUNCH
(STEEL DRILL ROD)
DIE
(HARDENED & TEMPERED) STEEL
PUNCH & DIE FOR MAKING COVERGLASS SUPPORTS
1891
1/17/77

Figure 47.

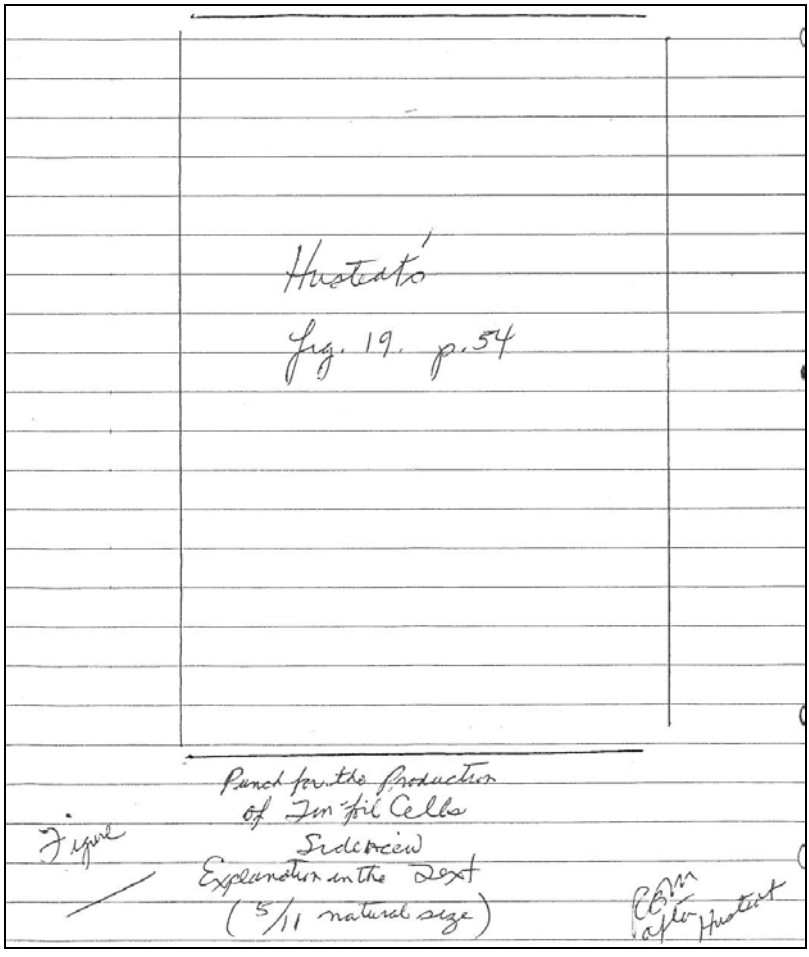
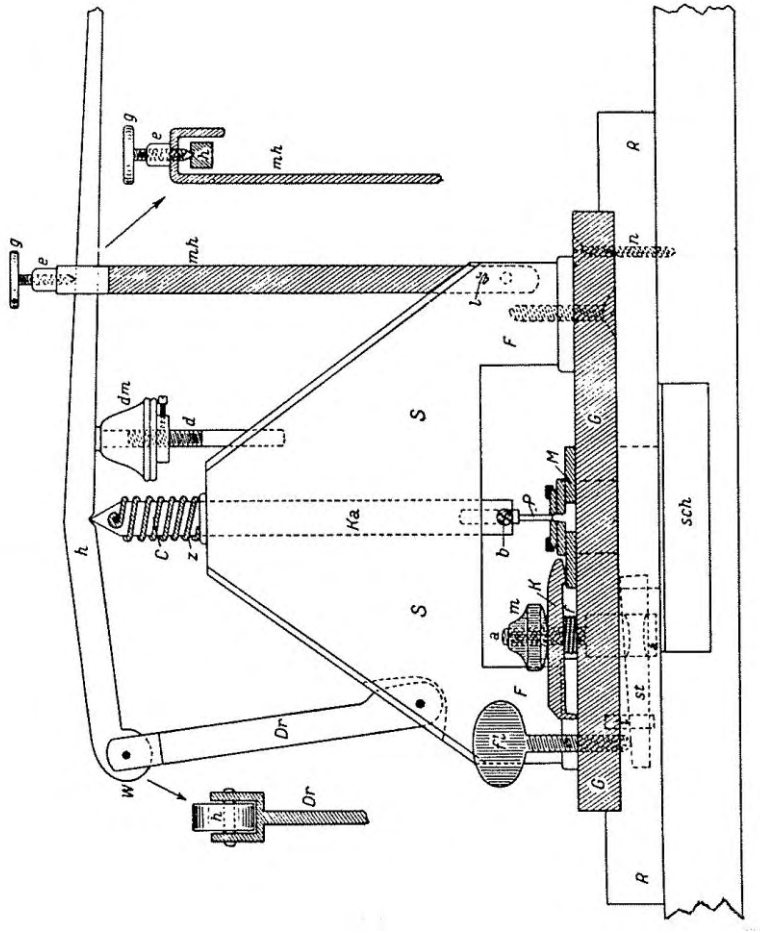


Figure 48 (Notebook).



Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden*. Fig. 19. Pg. 54.
Punch for the Production of Tin-foil Cells (Side View)

Figure 48.

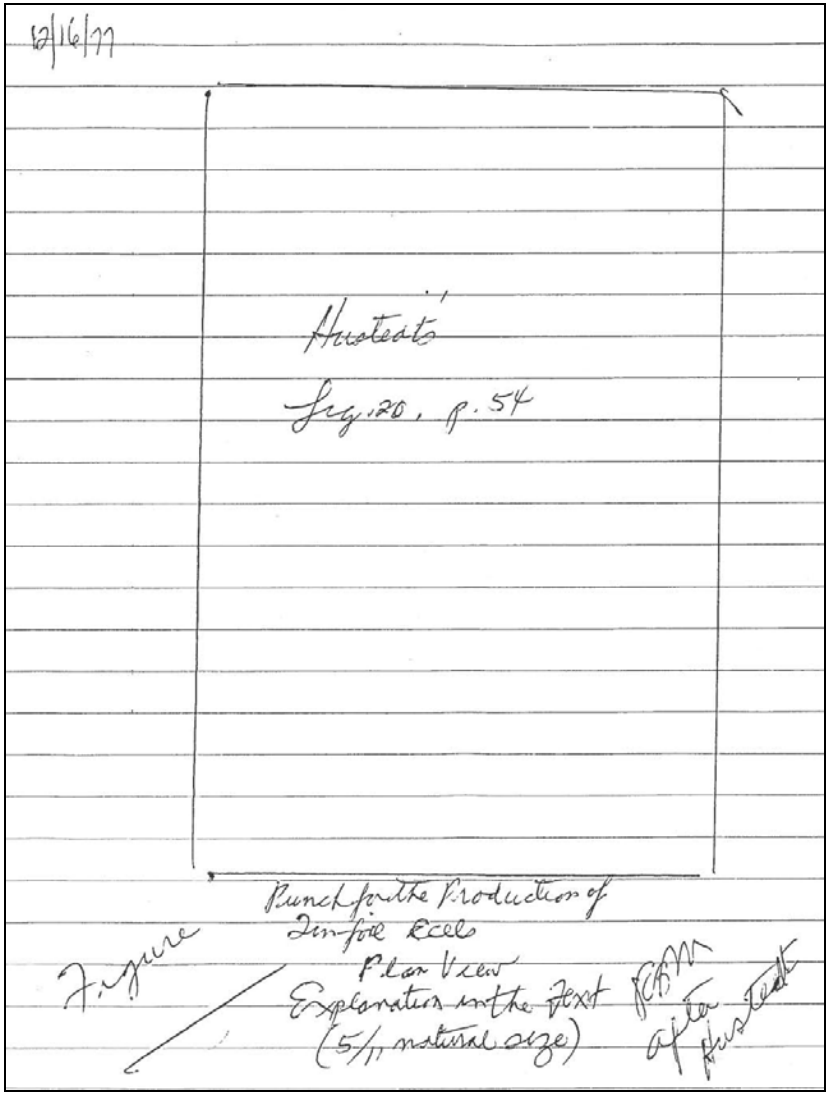
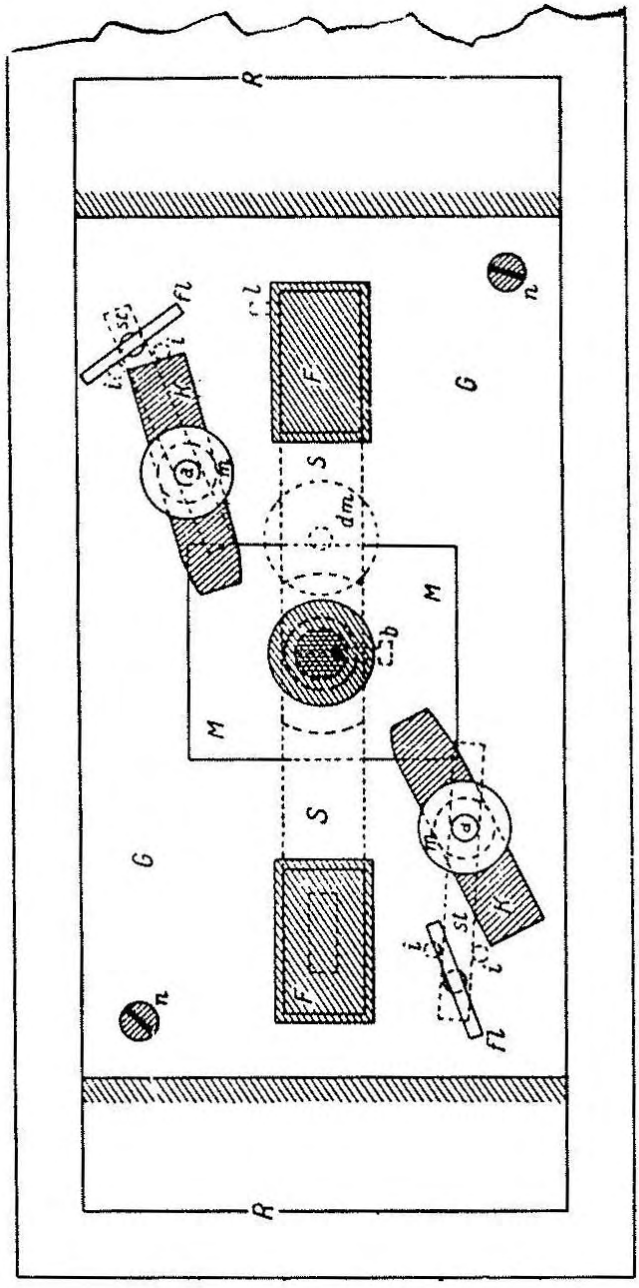


Figure 49 (Notebook).



Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden*. Fig. 20. Pg. 54.
Punch for the Production of Tin-foil Cells (Plan View)

Figure 49.

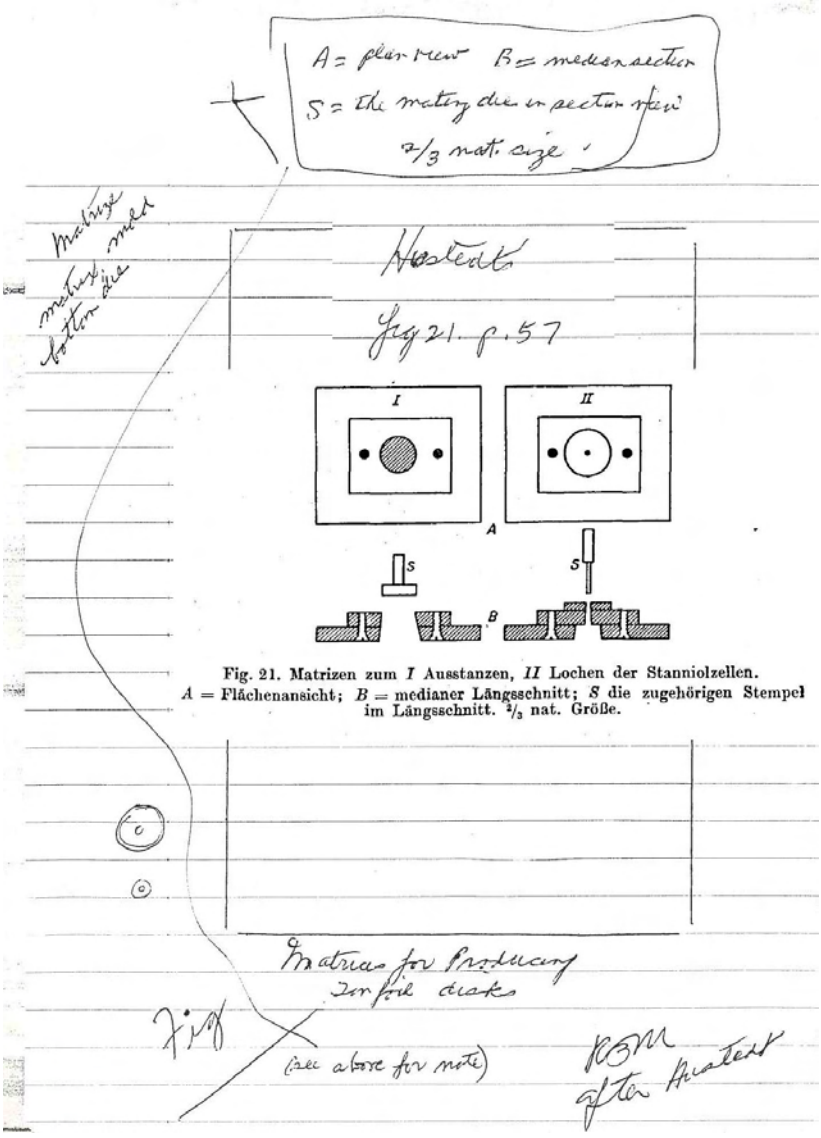


Fig. 21. Matrizen zum *I* Ausstanzen, *II* Lochen der Stanniolzellen. *A* = Flächenansicht; *B* = medianer Längsschnitt; *S* die zugehörigen Stempel im Längsschnitt. 2/3 nat. Größe.

Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 21)

Figure 50.

Henry Morland
(1838 - 1925)

Round spacers of aluminum, copper, etc. of from 0.002 to 0.003 inch in thickness are fabricated by an ingenious little punch and die devised and described by Morland (1891). The details of such an apparatus are included in Figures 47-50. After the material is punched it will be found that the discs are cupped and will have to be flattened before use. The die is made from

hardened and tempered steel and the punch from a steel drill rod. In operation the punch is entered into one of the die-holes as far as the upper edge of the lower saw-cut.

Another type of spacer that was popular in times past, took the form of a quite large diameter flat metal disc with a hole in the center. It was mounted concentrically with a round coverglass, and the diatoms mounted on the coverglass in the space left by the hole, the combination forming a kind of cell that protected and located the diatoms at the same time.

Hustedt discusses various methods of spacing the coverglass from the microslide and includes the use of glass and gelatine rings as well as the means previously described. He also mentions that Eulenstein had constructed an apparatus to fabricate punched metal discs but that details of construction were not known. Hustedt's own design for such an apparatus is repeated here to further protect against loss of such knowledge, and to afford a more general appreciation of these unique devices through the English language.

Theodor Eulentstein
d. 1875
of Stutgardt

With reference to Figures 48 and 50 the following is freely translated from Hustedt's description.

> The base plate G of the punch is a smooth polished steel-plate of $8^1/_2$ x 16 mm., and 11 mm. thick, with a central opening of about 2 mm. in diameter.
>
> On the centerline of the long dimension of the base plate there is a vertical steel plate S, about 15.5 mm. thick, in the form of an isosceles trapezoid with the base corners having cut-out feet F. The base lines of this trapezoid are respectively 13 and 2.7 mm. long, the feet 2.5 mm. wide and 2.1 mm. high, so that there is, under the center of the vertical plate an open space of 8 x 2.1 mm., which serves to accommodate the matrix M. The matrix, that will be described more completely later, is held with the help of two diagonally positioned strong steel clamps K, that through a very firmly tightened double screw arrangement, prevents any slipping or sliding. The clamps are 43 mm. long, 10 mm. wide at the rear end and slightly tapered at the front end. They are made from 4 mm. thick steel, and possess little feet at their ends. The front foot that holds the matrix is flat and broad, and the rear one, that bears on the base plate, narrow and somewhat higher. They have a hole at the center through which passes screw *a* from under the base-plate mating with clamping nut *m*. A brass expansion spring is inserted between the clamp and base plate. The lower part of the screw is made long-cylindrical with a diameter of 11 mm. and projects about 12 mm. beneath the base plate. The cylindrical part of the screw is also drilled out and takes a strong steel wedge shaped pin 5 mm. long. A second screw (wing screw) f1, which is 2.7 mm. from the first, and passes downward through the base plate, bears on the smaller end of this pin.

Final tightening of the clamp is made with its help. Two guide pins *l* prevent sidewise movement of the wedge-shaped pin.

In the perpendicular centerline of the vertical plate there is a channel Ka about l mm. in diameter, through which slides an 11 mm. long precisely fitting steel cylinder *c*. The lower end of this cylinder is drilled out in its center to receive the die P. A heavy set-screw *b* clamps the die in place and limits the upward travel of the cylinder, controlled by the strong spring E, after each punching. On one slanted side of the plate S, the vertical arm Dr pivots in a slotted opening. It terminates at the same height as the steel cylinder and is hinged there at W to a horizontal lever arm *h* . This horizontal arm bears on the upper end of the steel cylinder carrying the die, and to prevent it from sliding off has a groove ground-in to fit the sharpened wedge-shaped top of the cylinder.

Sunk into the other side of the upright plate S is a long vertical standing screw *d* containing a bell-shaped nut *dm*, adjustable in height, which limits the extent of the lever arm movement.

A 2 mm. thick by 8 mm. wide brass strip *mh*, 16 mm. long, which at one end has been bent at right angles twice to form a hook or catch with one side 13 mm., the other of 2 mm., and with a lateral space of 1 mm., that is wide enough to contain the horizontal lever arm, is employed for centration. The long side of this brass strip has some vertical aligned holes at its lower end that can be pushed onto a steel stud *l* that is located laterally on the foot of the upright plate about 11 mm. below the center part of the horizontal lever arm extending beyond the cylinder Ka. This apparatus provides a temporary fixed vertical depth adjustment of the die, allowing both hands to remain free to center the matrix.

The entire apparatus is mounted on a wood frame R and fastened to it by diagonally opposite screws *n*. Beneath the plate G there is a sufficiently deep hollow space and in the lower board the drawer *sch*.

There are two kinds of matrices; one serves to punch out, and the other to put a hole in the tinfoil disk (Figure 49). All matrices consist of two rectangular 5 mm. thick, plane-polished steel plates, the larger of which is 4.5 x 3.8 mm. and the smaller being about 2 x 3 mm. Both are centered with respect to the other and screwed together, so that the larger one forms a base plate. A central hole is bored through both that slightly tapers wider from top to bottom to allow the punched out tinfoil disc to fall as freely as possible. The upper opening of the hole is equal in diameter to the coverglass diameter to be used. As different coverglass sizes are employed there must be a corresponding number of matrices available. In general one with a diameter of 8 to 9 mm. will suffice, as larger forms merely use more material, while small ones are somewhat troublesome in their manipulation, although Hustedt himself had hundreds of such preparations only 5 mm. in diameter.

The matrix for making a hole in the tinfoil disc is different from the one described above merely in that in the center it has a 3 mm. thick disc-shaped top piece whose upper surface is precisely plane, polished, and as smoothly

burnished as possible. It's central hole is for the purpose correspondingly much smaller, and varies between 1 and 3 mm. in diameter being tapered wider in a downward direction.

When type slides are to be prepared with the help of tinfoil cells, the hole is required to be larger to provide a greater surface for the selected diatoms.

A large number of dies are used with the matrices, the upper parts of which are fastened to the sliding cylinder Ka with a set screw. The lower parts possess a mating diameter to the corresponding opening in the matrices. These must be very exactly fitted, sharply edge-ground and polished. Moreover, there is a requirement for a number of small brass rings with two counter-bored holes. The lower opening corresponds with the diameter of the disc-shaped centerpiece hole-forming matrix, and the upper is the size of the coverglass employed. The necessary number of these is determined by the number of matrices and the kinds of coverglasses.

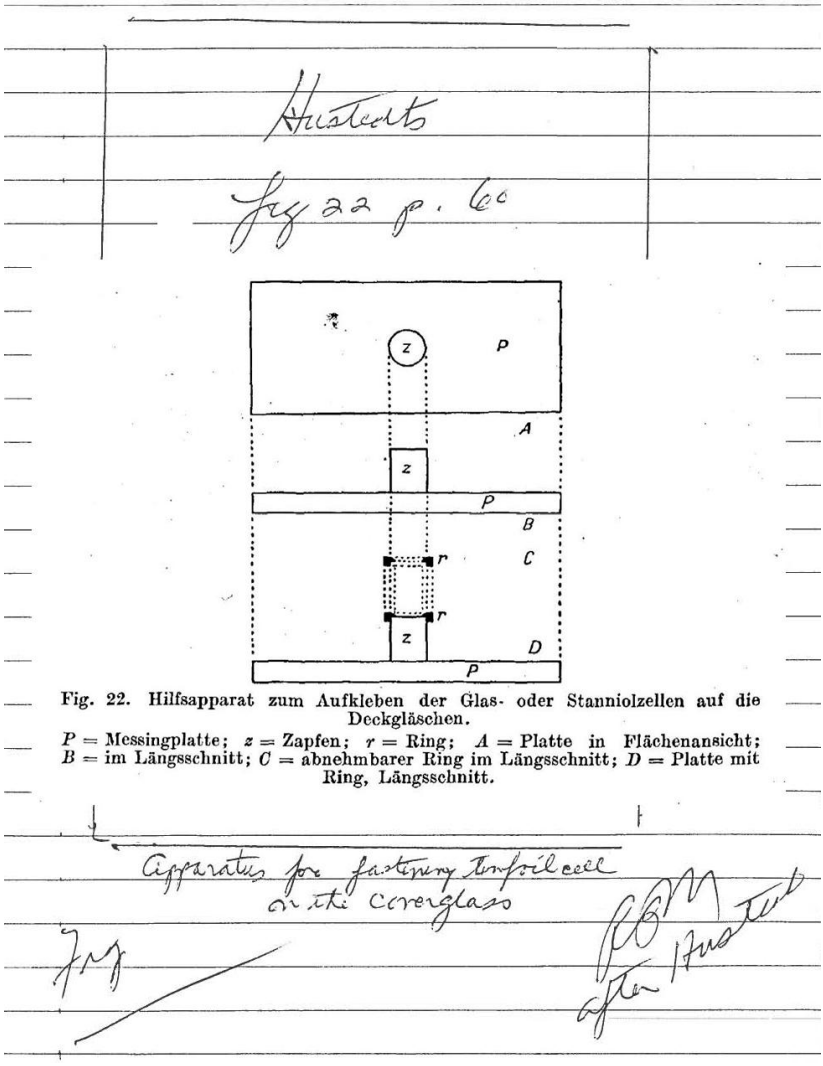
The work with the punch proceeds in the following manner. A die of the proper size for punching a disc is fastened into the sliding cylinder with the setscrew and the mating matrix is slid beneath the clamps on the base plate bringing it into the approximate center position and tightening the clamps sufficiently as to allow only slight movement. The cylinder is lowered with the lever arm until the lower surface of the die is almost touching the upper surface of the matrix and fixed at this lowered position by the use of the adjusting screw *g*, and locking nut *l* on the previously described vertical brass strip *mh*. The matrix is now slid into alignment, centering it's opening precisely with the die, and locking firmly into place by additional tightening of the clamp screws. The vertical brass strip *hm* is then removed and the depth of movement of the cylinder adjusted with the bell-shaped nut and set screw dm that is in the vertical plate. It is raised or lowered until the die only penetrates the opening in the matrix just far enough to cut through the tinfoil. From a stock of tinfoil small plates of about 10 x 15 mm. are cut out and protected, under light pressure, between cardboard sheets. After the adjustments are made the punching can begin. The finished discs fall through the hole in the matrix into the drawer below and in a short time a large quantity of such discs are produced. The hole is somewhat more troublesome, as the little plates must be individually taken up and fitted. The clamping and centering of the hole-die and matrix is accomplished in the same way as described above, but on the disc-shaped center part of the matrix a matching brass ring is placed whose upper opening is the size of a punched-out disc. Touching the tinfoil with the fingers produces minute drops of moisture not easily removed. Therefore the discs are picked up individually with tweezers and laid in the ring. With a downward pressure of the die a larger or smaller circular opening is punched and with the raising of the die the punched disc is lifted up. It must be carefully removed with tweezers, if some other means, such as a stripping device in the form of a brass angle fastened to the upright plate, has not been provided.

With the punching out and perforation of the little plates it is difficult to avoid bending them. In use they must be flat and smooth, and therefore

should be pressed between flat plates with fairly strong pressure. For this purpose a strong piece of plate glass at least 1.5 mm. thick and a steel plate about 1 mm. thick are employed. They both must be flat and their surfaces mate perfectly with one another. A sheet of thick smooth cardboard is laid upon the steel plate and the tinfoil discs with their dull sides down, are placed upon it. They are then covered with the glass plate which in turn is covered with a thick layer of blotting paper. The whole assembly is best pressed for a short time in a copying press, or in lieu of that, with a screw clamp. The pressure must be applied at the exact center, especially if the copy-press is employed, or the glass may break, or the discs will become only partly smoothed and flattened. After several minutes the plates with the tinfoil discs are taken out of the press. They have been clearly impressed into the cardboard, but the other side mostly adheres to the glass and is simultaneously polished. If they cannot be easily removed by tweezers, the task may be aided by heating the glass plate. To give the discs a more pleasing appearance they can be embossed in different ways, by putting between the tinfoil and glass plate a uniform textile fabric, silk gauze, or muslin. A re-pressing without the inserted material is worthwhile, as the little plates then adhere to the coverglass better. The completed discs are kept in suitable small glass tubes in which a cotton plug beneath the cork prevents them from being bent from accidental shaking. The bottom of the tube is leveled by the use of an inserted flat-cut cork disc.

The tinfoil discs are fastened to the coverglass on which diatoms are to be mounted. This requires no further measures, other than putting it upon the fixative layer that is on the glass. For the purpose it is only necessary to use a gelatine solution. As soon as the layer is dry a tinfoil disc is flattened on a clean microslide, a coverglass is picked up with tweezers, the tinfoil breathed upon, and the coverglass laid, fixative side down, on it. The moist breath on the upper surface of the tinfoil softens the gelatine and a light pressure is sufficient to fasten the coverglass and tinfoil disc together. Care must be taken to make sure that the two surfaces are joined completely and evenly, as later the mountant or lacquer may penetrate between the tinfoil and coverglass, at the least spoiling the appearance of the preparation, if not resulting in a complete separation of the coverglass. With some practice it is easy to lay the coverglass centrally so that the edges of both parts are in coincidence. Besides, one can usually construct a device to assure absolutely accurate work. The simplest way is to fasten three small pieces of not too thick glass to a microslide, arranged so that they form a circular enclosing space the size of the tinfoil disc and coverglass respectively. The tinfoil is placed first in this enclosure, and the coverglass follows, necessarily assuring coincidence with the tinfoil disc. Further, the hole-punching matrix can be employed, in that the matching ring for the tinfoil disc and coverglass is so limited in size as to provide exact congruence. Because a steel matrix would suffer from the frequent moistening, a little brass-plate apparatus is employed for the purpose that is constructed in a similar way. Refer to Figure 51. P is the brass-plate base, *z* is a brass peg, *r* the counterbored brass ring previously described, A and B are plan and elevation views respectively,

C is a cross-section of the removable ring *r*, and D shows the ring and support together in an elevation view.



Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 22)

Figure 51.

Glass cells, commercially available, may be employed instead of tin cells, and are utilized and handled in much the same manner as described for the tinfoil discs.

Tinfoil cells can be made without the apparatus described. They can be made with the aid of a simple iron punch. However, there are difficulties in obtaining a properly centered hole and one which is as finely and cleanly punched.

8.6.3. Placing the Diatoms

When microslides, coverglasses, and diatom specimen material are prepared and ready for use, the next step is the actual placing of the diatom(s) on the coverglass. In performing this task it is possible to do so manually with a hand-held bristle described previously, transferring the diatom(s) from the store slide to the coverglass and placing it in the desired position, while observing with the microscope. However, considerable practice and a steady hand is necessary to do this. To provide for more positive and repeatable results and a modicum of confidence in performing such hand manipulations, a number of ingenious devices have been used. Hustedt describes a rather elaborate preparation microscope setup that employs a large dissecting microscope of about 100X magnification. In order to provide complete steadiness, freedom of hand movement, and protection from fatigue, large wooden arm rests are substituted for the hand rests. Möller (circa 1890) used a similar arrangement with heavily padded arm rests. It is doubtful if contemporary diatom mounters use hand-held bristles for the work, except as incidental tools.

Johann Diedrich Möller
(1844 - 1907)

The most common and useful apparatus for manipulating diatoms is the mechanical finger, of which several versions have been previously described. It matters little as to what style is used as long as the operator is familiar and proficient with it. It is used primarily to lift the diatom from a store slide and deposit it on the coverglass and manipulate it into the desired orientation prior to embedment in a mounting medium. The transfer procedures involved are similar to those described in making store slides from spreads. However, an additional factor is introduced, in that it is desired to deposit the diatom in a particular location and orientation, which requires more attention as to how it is picked up and how it is laid down. Also, examination of the valve or frustule (or other part) while on the bristle, is necessary to determine its ultimate suitability for mounting. The diatom is examined under magnification while being rotated on the bristle to ascertain its general physical condition and to detect any undesirable breaks, cracks, or missing appendages, before positioning it on the cover. The magnification used for such purposes is about 100X which affords sufficient working space for manipulations beneath the objective. Very small diatoms are a problem and very difficult to mount on a selected basis, although it can be done after much practice. Sizes down to about 15 to 20 micrometers (largest dimension) are at about the lower limit which can be handled in this manner.

Once the diatom is placed in the desired location and orientation, it is fixed in place by breathing on it with the moist breath (with the water soluble fixatives). The coverglass is then transferred to a warming plate (hotplate etc.), flooded with xylene or other solvent of the mounting medium, which is allowed to evaporate, assuring the elimination of any remaining moisture.

Then, after cooling, another small drop of xylene is applied to the diatom(s) and sufficient mountant added. The coverglass is then picked up with a clean warm microslide, inverted, and placed on a hotplate to drive off the solvent. When using Styrax, Burke recommends that it be applied thinly to the cover which has the diatoms and allowed to stand overnight in a dust free location. On the following day it is placed on a hotplate with a temperature of about 80°C. to drive off the solvent,

and while still hot is inverted on a warm microslide and gently pressed into place. With the combination of different mountants and different mounters, many procedures have been devised, and it is not possible to cover them all in a limited space. Therefore, only a few specific procedures will be described to guide the would-be mounter. Sufficient information is provided to safeguard against common pitfalls, regardless of the specific step by step procedures followed.

Special apparatus to assist in making selected diatoms slides ranges from the very simple to quite complex. Some are merely of convenience value to provide more ease of centering such as the templates of Figures 44 and 45. Others are useful to assist in handling one or more coverglasses (Figure 52), or to speed up the laborious selecting, picking, and transfer operations, especially where a large number of preparations are to be made. Of the latter type Hustedt describes several which are repeated here.

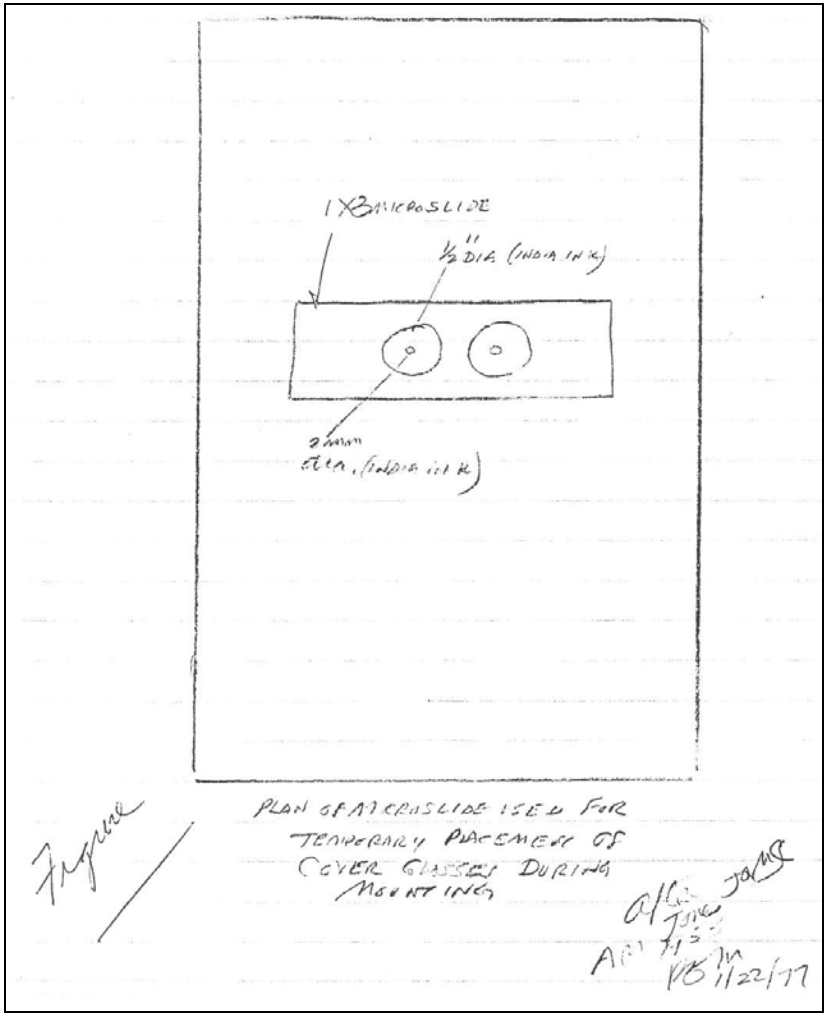
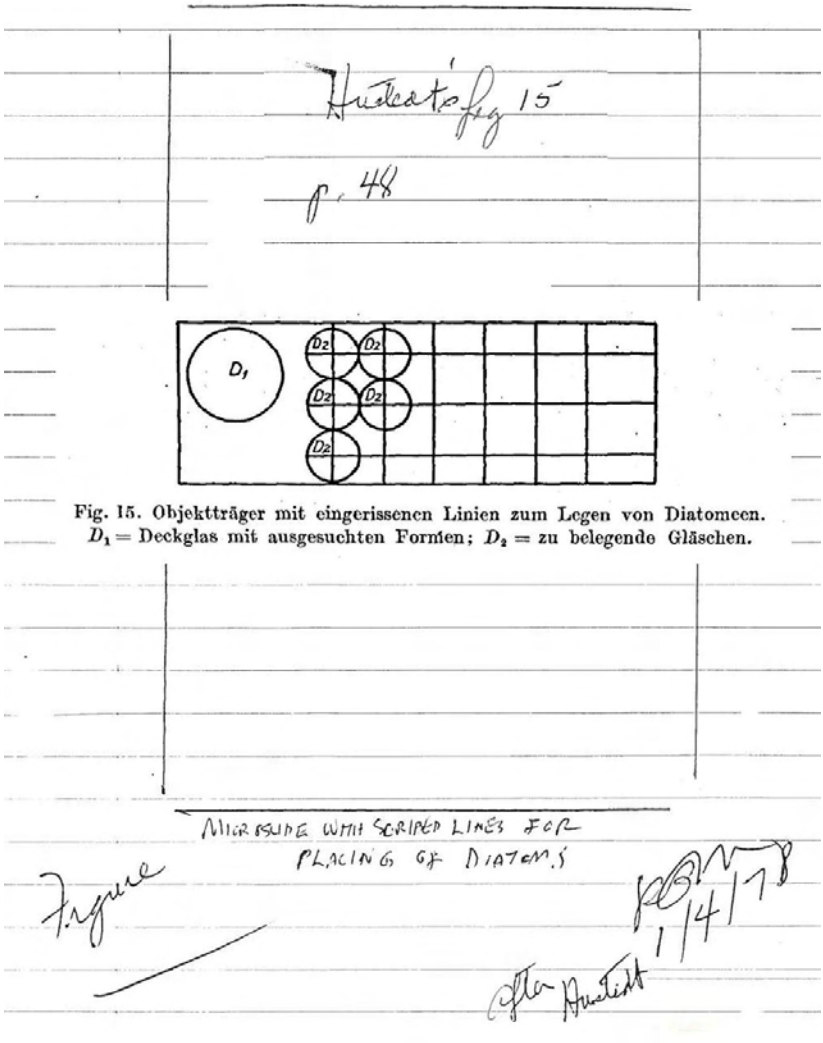


Figure 52.

For instance, instead of using two microslides (the store and object slide) he shows how a large diameter coverglass used as a store source and smaller coverglasses for the selected object diatoms can be employed to speed up the work. Reference is made to Figure 53. A standard size microslide is marked, with the aid of a writing diamond or carbide-tip scriber, in a network of scribed lines such that the small covers D can be placed on their intersections. The large "store" cover D, of perhaps 18 mm. diameter, is placed on an unmarked end of the microslide and transfers of selected diatoms made from there to the appropriate small covers. The assembly is easily carried on the mechanical stage of a microscope. The coverglasses can be temporarily fastened to the microslide with petroleum ether (ligroin). With the preparation of many coverglasses it may not be advisable to utilize a gelatine fixative, because as the breath is used to fix a diatom on one coverglass, it may disturb the location or orientation of one previously laid on another. The moistening process, with the breath, of the fixative layer on which the diatom lies, is observed and controlled through observation with the microscope, as the positioning and orientation may be guided with a handheld bristle up to the moment of final setting of the fixative.



Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 15)

Figure 53.

A fixative of a film of shellac on the coverglass is warmed to soften it. The diatom sinks into the thin layer of shellac and is fixed in position when cooled. The dextrine fixative of Firth can also be used on the cover (under the restrictions mentioned previously), with the diatom(s) being laid in it while it is still soft, being fixed in position upon heating. An objection to the shellac or other similar fixatives is that there is no possibility of later easy corrections to the diatom positioning as there is when using a gelatine one.

An elaborate device for the selection and placing of diatoms was devised by E. Debes and is described by

E. Debes, Leipzig

Hustedt. The device was primarily for use with a "preparation microscope" such as the dissecting microscopes used by Hustedt and Möller. However, it could be adapted to a modern stereomicroscope to provide a very elegant selecting and mounting apparatus. The following description by Hustedt is freely translated from the German.

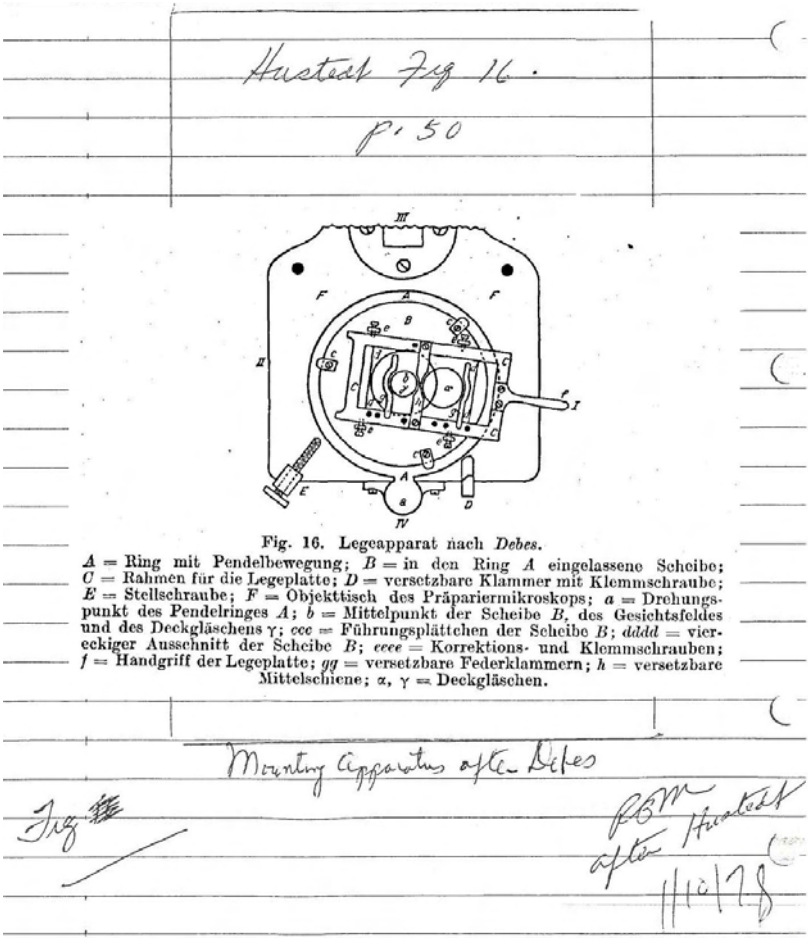
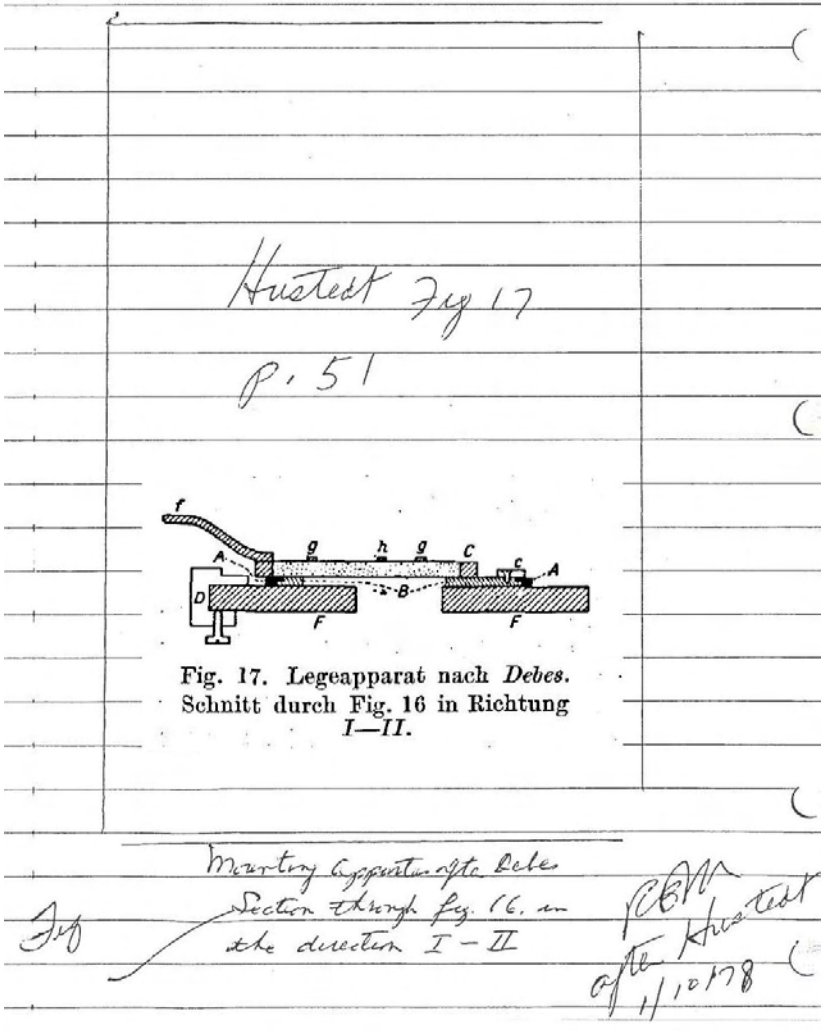


Fig. 16. Legeapparat nach *Debes*.
A = Ring mit Pendelbewegung; *B* = in den Ring *A* eingelassene Scheibe; *C* = Rahmen für die Legeplatte; *D* = versetzbare Klammer mit Klemmschraube; *E* = Stellschraube; *F* = Objekttisch des Präpariermikroskops; *a* = Drehungspunkt des Pendelringes *A*; *b* = Mittelpunkt der Scheibe *B*, des Gesichtsfeldes und des Deckgläschens γ; *ccc* = Führungsplättchen der Scheibe *B*; *dddd* = viereckiger Ausschnitt der Scheibe *B*; *eeee* = Korrektions- und Klemmschrauben; *f* = Handgriff der Legeplatte; *gg* = versetzbare Federklammern; *h* = versetzbare Mittelschiene; α, γ = Deckgläschen.

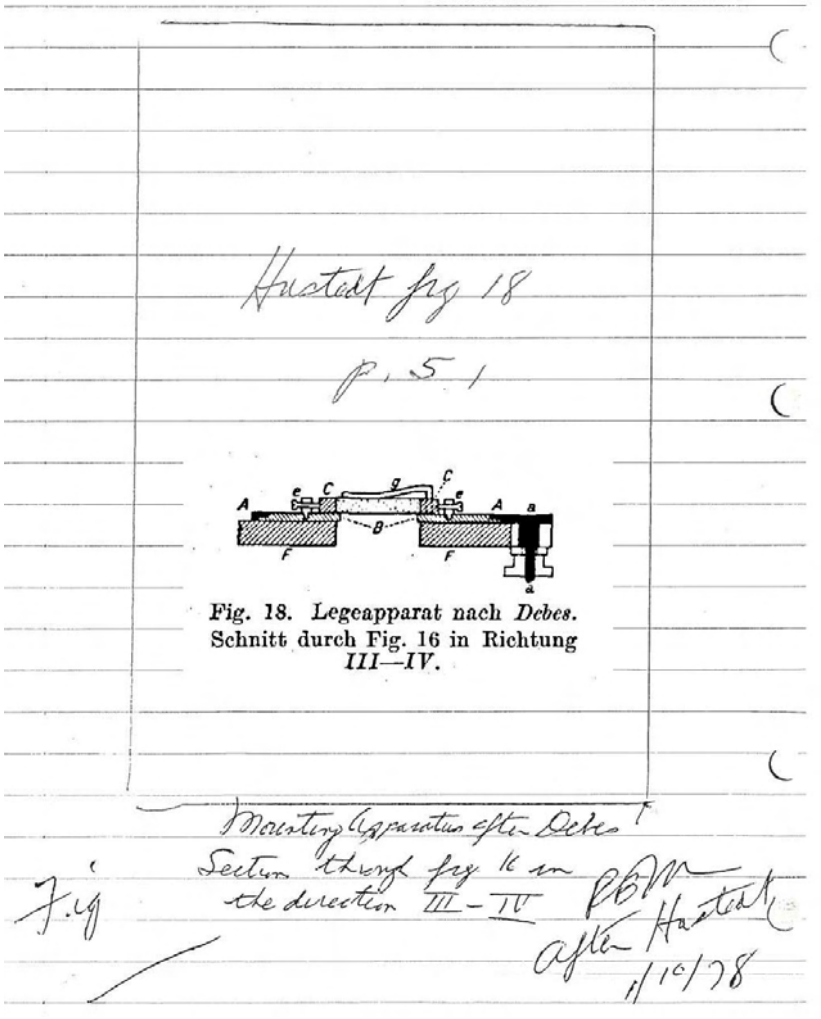
Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 16)

Figure 54.



Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 17)

Figure 55.



Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 18)

Figure 56.

The assembled apparatus consists of two main parts, the mounting-plate and the motion mechanism associated with it. Refer to Figures 54-56. The motion mechanism consists of a gliding ring A, on the stage of the microscope, within which is enclosed, and inset, a disc B. The ring A swings pendulum-like on the axis pin a which is inserted into a pivot that is capable of being tightened or loosened.

The center of the pendulum movement of A passes through the exact center of the field of view. The inset plate B is rotatable about the same center *b* and is provided with three little screw-tightened guide plates *c*. Plate B also possesses an excentrically located large rectangular opening *dddd*. The lateral movement of A is limited in one direction by the clamp D, which is adjustable in position and fixed by

a setscrew, and in the other by the adjusting screw E which is firmly connected to the stage by its nut.

The mounting plate consists of a small rectangular plate-glass plate in a metal frame C that has a little handle *f* on one of the short sides. The upper surface of the glass plate and the frame lie in the same plane. The mounting plate is divided into two parts by a metal strip *h* which is rounded out in the center on both sides, and fastened by fine screws located at the ends, in corresponding holes in the frame. To hold the coverglasses immovable in position, two clamps *g*, each of which is rounded out on one side, lie on the surface of the mounting plate, each being positioned and rotated on the long side of the frame by means of a little pin at one end. To accommodate different sizes of coverglasses, the strip *h* and clamps *g*, are adjustable in position and the frame possesses a corresponding number of holes for the purpose. The frame and mounting plate is adjustable on plate B by the use of four setscrews eeee, bearing on the long sides of the rectangle, whose nuts are fixed in plate B. A groove is cut into each long side of the frame in which the screws grip and which makes possible a sliding adjustment.

To make initial adjustments a coverglass, with its center marked by an ink-dot, is used. It is placed firmly against the right rounded-out side of strip *h*, and fixed in place by the right clamp *g*. To locate the center of the field view the ring A is moved to the right by handle *f*, frame C adjusted by sliding and fixed by setscrews *eeee*, and final centration determined by rotation of plate B. The clamp D is adjusted and fixed at such a location on the microscope stage as to make it possible to always return to the center of the coverglass. The setscrew E is adjusted to bring a predetermined zone of the store-cover into the field of view so that no area is overlooked.

The advantage of this simple apparatus for manipulation is quite evident. With the bristle in practically one position the transfer of a picked diatom from the store cover to the exact center of the target-cover is accomplished with one rapid swinging motion by handle *f*. While it was devised primarily for use with hand held bristles, it should prove to be equally useful with a mechanical finger. It would have a considerable advantage in speed of transfer, from store-position to target-position, over the rather slow motions of a mechanical stage.

8.6.4. Applying the Mountant

After the diatom(s) have been selected, placed, and fixed on the coverglass the mountant is applied. Former remarks apply in regard to prior application of solvent to minimize bubbles, and in the general precautions to be taken with different mountants.

Since in the selected mounts only one, or a few, diatoms are mounted, and the time expended is quite great, it is imperative that they be free of bubbles. In a strewn mount, due to the great number of similar diatoms present, the appearance of one bubble, for instance, is of far less importance than one bubble in a one or two specimen mount. Pre-application of the solvent, use of a thin medium, and judicious use of heat are all excellent steps in limiting bubbles in the completed preparation.

Certain genera or species of diatoms create greater mounting difficulties than others. Among them are *Auliscus, Diploneis, Surirella,* and *Melosira*. *Auliscus* has a deep girdle and when mounted on the girdle edge is more apt to trap air and thereby promote bubble retention. In the chain-forming diatoms, as *Melosira,* there is a much greater chance of trapped air and resulting bubbles. With these diatoms or similarly constructed ones, the preliminary application of the mountant solvent is almost mandatory. The cover (or microslide) with the attached diatom is picked up with tweezers and held at an angle to the horizontal. A small drop of xylene (or other solvent) is applied to a spot just above the diatom and allowed to run down to cover the diatom. Examine it under low power. If air is still trapped it will gradually be seen to disappear. When the air is gone, apply a drop of mountant in a similar manner. The solvent is then driven off by warming and the cover and microslide joined by the usual procedure.

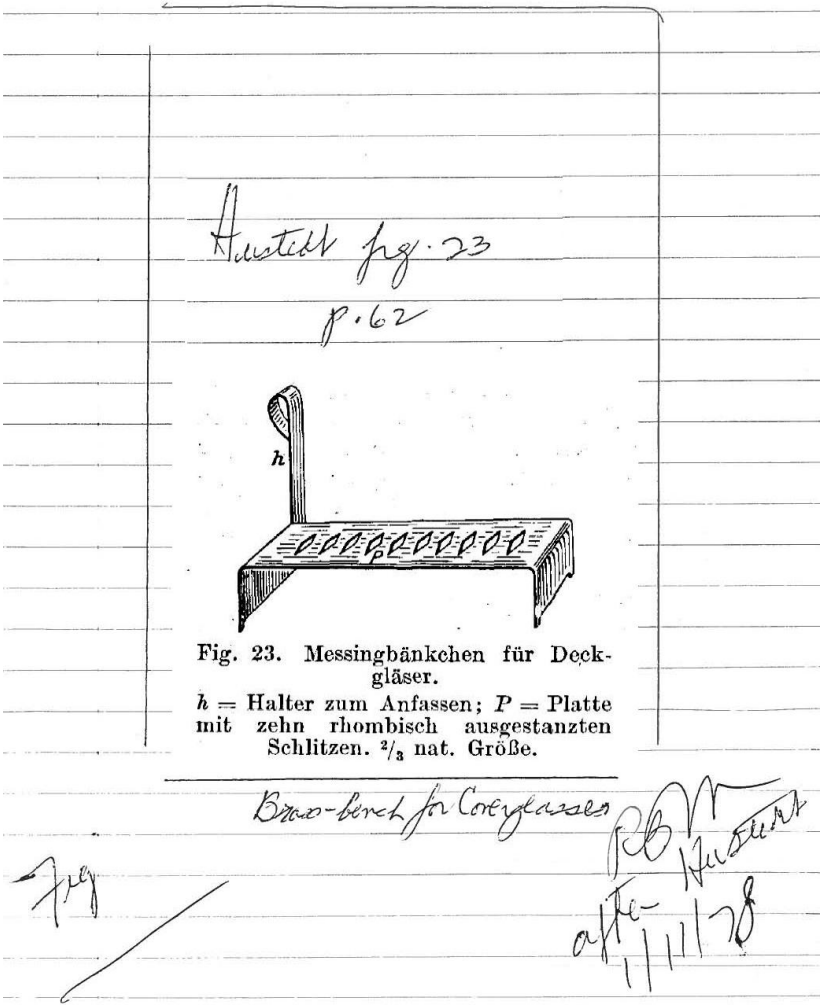
Spacers, when used, often are a point of generation for bubbles. Naphrax, for instance issues hundreds of bubbles from these points and it is sometimes difficult to get completely rid of them. The Styrax-Aroclor mountant mix previously described creates minimal problems of this sort and may be preferred when using spacers.

If the drop of mountant is applied to the microslide, in most cases it is advisable to touch only one edge of the coverglass (with the diatom) to it, the latter being allowed to settle gently. Bubbles in the mountant (unless trapped in diatoms) will usually migrate toward the edge of the coverglass and dissipate. When they are present, do not heat too quickly but let stand until they move toward the edge of the cover.

Bubbles are apt to form under diatoms which are not flat, particularly domed ones, and sometimes refuse to move when heated. Turning the slide face-down over the heat is sometimes successful, allowing the bubble to rise and escape between the edge of the diatom and the glass substrate.

In some stubborn cases the application of high heat (if the mountant can stand it without coloration) while rocking the slide to and fro will cause a bubble to dislodge and escape.

In cases where diatoms are mounted on the microslide and the mountant has been hardened by heat, bubbles are less likely to occur if a heated cover glass is placed on a cold slide which is then placed on the hot plate.

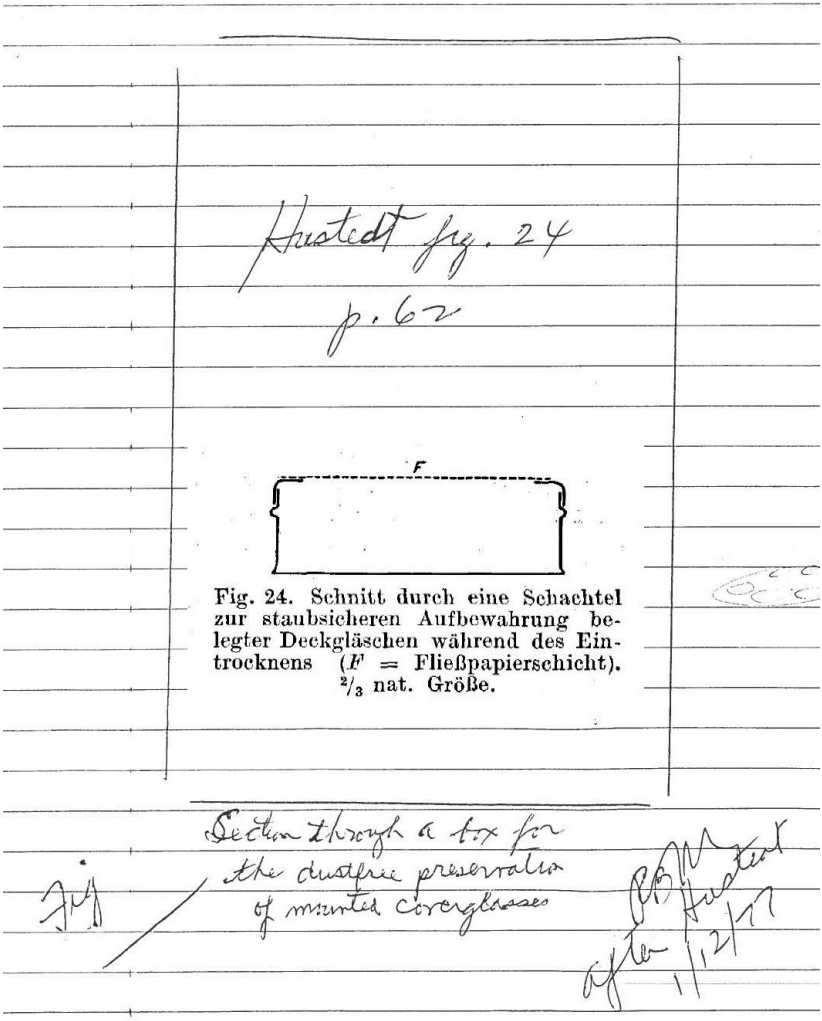


Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 23)

Figure 57.

Hustedt has described a little stand to assist in the treatment of a number of coverglasses at one time. Refer to Figure 57. A small bench is made out of a thin sheet of brass into which have been cut a number of rhombic-shaped slots somewhat shorter than the diameter of coverglasses to be treated. In use, the coverglasses with the mounted diatoms fixed thereon, are placed upright in the slots. The bench is then placed in a covered glass bowl containing sufficient solvent to cover the coverglasses. After a day, the bench is removed from the solvent bath and the coverglasses are laid specimen-side up at the edge of a ground glass plate of about 15 mm. in diameter. They are then slid with a needle across the plate to the opposite edge, the ground glass thus siphoning off the surplus solvent. The covers are then

placed in a separate container, a small drop of mountant applied, and allowed to harden at ordinary room temperature.



Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 24)

Figure 58.

In order that the hardening can take place protected from dust, they are put into a covered container. Most of the lid of a flat sheet-metal box is cut out so that only about 5 or 10 mm. of the edge remains and the opening covered with a sheet of blotting paper. Refer to Figure 58. Further, a support is made by punching a number of holes, with a diameter of about l mm. greater than the covers, in a small piece of cardboard about 1.5 mm. thick. This is then covered with a second piece of cardboard, unpunched, of the same size. Refer to Figure 59. The little support is of a size to fit into the previously described box, and can be laid upon its bottom and

covered completely. The mounted covers are laid in the cutouts, which are purposely colored with black ink to improve the visibility of the mounting medium. To avoid mistakes the cutouts are numbered so that necessary observations can be recorded for the individual mounted covers, in a notebook.

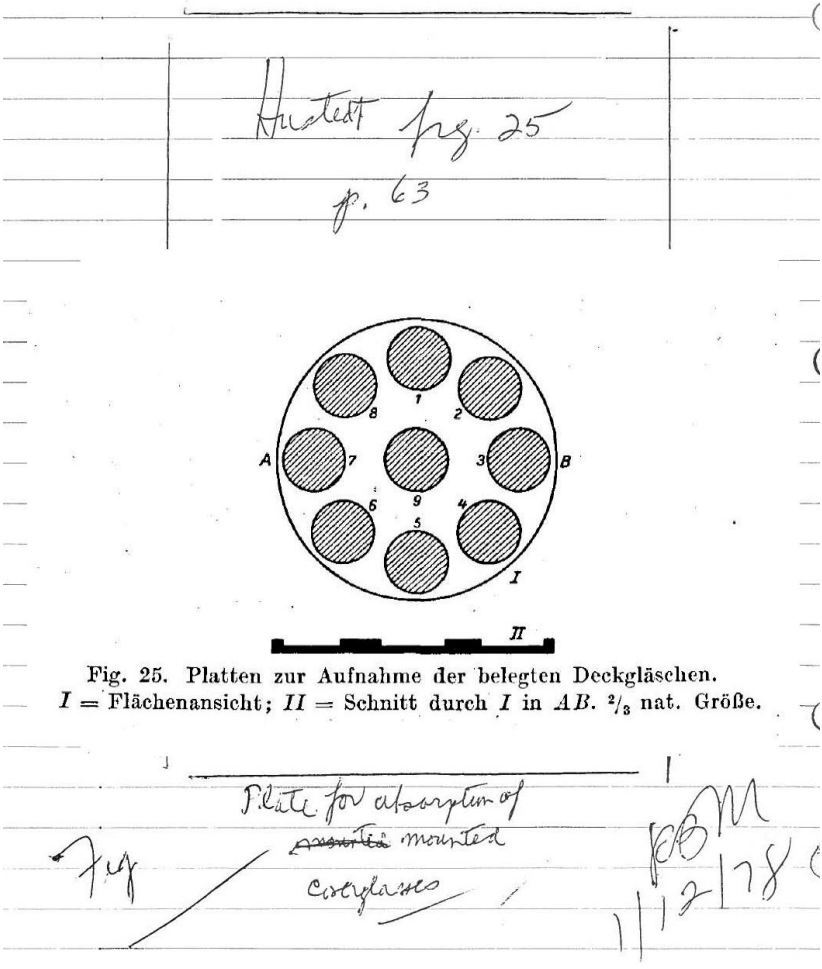


Fig. 25. Platten zur Aufnahme der belegten Deckgläschen.
I = Flächenansicht; *II* = Schnitt durch *I* in *AB*. ⅔ nat. Größe.

Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 25)

Figure 59.

The next day, if Styrax is the mountant being used, the first drop of the Styrax solution has, upon drying, formed a ridged ring at the edge of the glass, the mountant being in a thinner layer at the center. A second small drop of Styrax will eliminate this disadvantage which could easily lead to the formation of bubbles in the preparation. After about four or five days the mountant is thick enough that the coverglass can be placed on a microslide. To avoid unnecessary work, all covers are examined once more with the microscope. Then a small drop of thin mountant

(Styrax solution) is applied to the microslide which is then used to pick up the coverglass. The fresh drop softens the hardened mass somewhat, so that a light pressure is sufficient to bring the cover into the correct position. With this process the formation of bubbles in the preparation is completely eliminated. The microslide could be warmed and then, without the addition of the drop of mountant, will pick up the cover and the combination further warmed to allow the mountant to spread to the edge. It is necessary in each case to exercise caution and avoid too high a heat. The Styrax must become fairly hard before a lacquer finishing ring is applied.

The making of selected mounts appears, after the foregoing description to be more difficult than it really is. With some experience it is possible in a relatively short time to prepare a great number of such preparations.

8.7. Finishing the Slide

Although in many cases it is not mandatory for diatom slides to be sealed and/or ringed it is always a desirable procedure. It provides a more pleasing appearance, and if done properly, contributes to the lasting properties of the preparation. In some cases it is a requirement, and if not done, early degradation and spoiling of valuable specimen material will result.

Two major reasons for sealing or ringing microslide covers are (1) mechanical and (2) chemical. As mentioned previously, some mountants do not adhere to glass well, and may detach with mechanical shock. Also, many mountants are adversely susceptible to long exposure to air, moisture, and/or immersion oils or fluids. The mountant may react to these conditions by oxidizing or by precipitation of minute crystals throughout its mass, making observations of the diatoms with the microscope practically impossible, or cracks may develop, even to the extent of destroying diatom frustules. Long exposure to air, in addition, may cause darkening of the mountant to an objectionable degree.

Sealing the edge of the coverglass, where the mountant is exposed, and in sufficient quantity to overlap the cover edge and microslide surface, is a major protective step in the preparation of diatom slides. Properly mounted and protected diatom preparations can have an almost indefinite life for future study and reference.

All materials used to ring the coverglass will not be good sealants. Some ringing materials are used as finishes to provide a more attractive appearance to the finished product, but contribute little insofar as protection is concerned. Therefore there is a distinction between sealing and ringing, one implying protection, the other sometimes but not necessarily so.

Cements or sealants made of drying oils harden by chemical change and sometimes are troublesome after long periods of time. Shellac sealants, on the other hand, harden simply by deposition from solution and exhibit long-lasting properties of stability, and are therefore, preferable in most cases.

One of the most useful of sealants is Shellac. Adler recommends degummed and de-waxed flake Shellac dissolved to a syrupy consistency in methyl alcohol. It should be fairly thick but even flowing. Burke recommends orange Shellac dissolved in

alcohol to a proper consistency, and Firth uses white Shellac. Any of the Shellacs may be colored by the addition of lamp-black or other coloring materials.

Shellac is applied to the cover with a turntable and small, preferably, camel-hair brush. First only a small thin amount is applied to seal up the edge of the cover and allowed to dry overnight. A second and final coat is then applied. If the applied final ring is not very precise, a chisel-point penknife or scalpel, may be used to push the edge into place. A somewhat greater control over the edge and smoothness of application can be obtained by using a brush which has a flat straight, not a pointed, tip. A number 2 size is most useful.

In England Murrayite is used extensively for sealing museum jars and covers. It is a synthetic cement which is dissolved in benzol to a thin syrupy consistency. It is permanently waterproof, alcohol-proof, and glycerine-proof. Covers are sealed with a brush and turntable as above allowing 10 hours between coats.

A most useful material for sealing and ringing diatom slides is the commonly available gold-size. It is very tenacious in its adhesion to glass and when dry is tough and pliable. It also can be colored and used in the final finishing.

Various slide ringing cements, including asphaltum, are readily obtainable from chemical supply houses. Table 4 lists a number of materials which can be used as coverglass sealing compounds including a few for temporary mounts. Of them all, Shellac or gold size perform as well as any, are convenient, and adjustable in consistency.

Table 4

Slide Coverglass Sealing Compounds	
Permanent	**Temporary**
Shellac	Any heavy oil
Gold size	Glue
Murrayite	Latex
Gum Dammar	Vaseline
Varnishes	Paraffin
Lacquers	Beeswax
Asphaltum	Plasticine
Fingernail Polish	
Sealing Wax and Alcohol	
Celluloid in Acetone	
Clearite	
Aroclors	
Iso-butyl methacrylate in Xylene	
Brown Cement	
Bitumen	
Hobbyist Paints (e.g. Humbrol)	

A good seal should not crack, even with age. Cracking can be avoided, in part, by applying several thin coats, drying between them, rather than with one thick coat.

Adding Castor Oil to bitumen/asphaltum will prevent cracking.

This latter method of application also provides for additional strength to the seal through lamination. During long periods of storage, additional coats of sealant from time to time will assure permanency.

8.8. Special Preparations

8.8.1. Type Slide

Mounting diatoms for special purposes usually entails the employment of techniques of selected diatom mounting in multiple and ordered arrangements. The diatoms are selected from spreads and transferred to the prepared coverglass with a mechanical finger in a systematic manner consistent with the purposes of the preparation. Included in this type of mount are arranged groups, genus, and type slides. Arranged "group" is a general term indicating diatoms arranged in a geometric pattern, sometimes simple, sometimes very complex, and usually for strictly aesthetic purposes. A genus slide is one which contains many species of one genus, as a rule from different localities. The term "type slide" is in common use by diatomists to indicate one which contains a number of species typical of the locality shown on the label.

The major difference in preparing "type" or other arranged slides is the technique and/or devices used to place the diatoms in specific ordered locations on the coverglass. For instance, a very fine line to divide the microscope field of view and/or align specimens can be provided by fastening a spider-web, or hair, across the diaphragm of a Huygenian ocular, or one can be placed between two coverglasses of about $^{3}/_{4}$ inch diameter and laid on the eyepiece diaphragm. The line is always in focus with the field and diatoms can be positioned along it with great precision.

Hustedt describes the preparation of type slides and his instructions are repeated here, being freely translated and adapted from the original German. His concept of a type slide includes those which contain a systematic order of forms from a particular material (locality) or a greater or lesser number representing genera or groups or species within genera, perhaps from a number of different materials and/or locations. This style of preparation lends itself admirably to the characterization of localities and habitats of diatoms and can be a very powerful reference. More of this later in the following section of this book. He refers to the outstanding preparation Universum Diatomaceerum Möllerianum of J. D. Möller (c.1904) which contains 4036 individually placed diatoms in nine panels in a space of 6 by 6.7 mm. It was Möller's intent to systematically mount all known species of diatoms on a single slide. This preparation is undoubtedly the most ambitious and colossal undertaking in the history of diatom mounting.

The establishment of a systematic order on the cover is considerably aided, especially where large numbers of diatom are involved, if the species or genera are first separately transferred from the spread or store-slide to another individual cover or microslide. Then, in the order determined for their mounting, they are used to supply the diatoms laid in the type array.

The coverglass that is to contain the type-material is prepared as for individual selected mounts, complete with fixative and ink ring or cell, as appropriate. The area required inside the ring or cell may be considerably greater than that for a single diatom and its size should therefore be adjusted accordingly. The diatoms should be mounted in a pre-determined scheme that lends itself to ready and systematic reference. Concentric circles or parallel rows are suggested, the latter being preferred. Hustedt recommends marking, with a diamond (or carbide) scriber, a network of lines spaced about 0.1 mm. covering an area of about 6 by 6 mm., on a microslide. This net of 5600 squares serves as a guide to laying the diatoms in order on the coverglass which rests on the network-marked microslide during mounting. Instead of a marked microslide, a coverglass could be so marked as a guide, being placed upon a microslide under the coverglass being mounted. To avoid a constant refocusing of the microscope during the work, a coverglass, of corresponding thickness to the network-marked one, is placed under the store coverglass from which the diatoms to be mounted are selected. If the stored diatoms are on a microslide, some similar corresponding adjustment in thickness will need to be made.

Commercially available network-marked graticules (reticles) can also be employed for this work. They are available in all manner of scales, grids, circles and other patterns. Although their primary purpose is for measuring and counting, there is no reason they cannot be used for mounting such diatom preparations as well. They are small and ordinarily fit within the ocular of the microscope. In the Huygenian ocular the reticle is placed on the diaphragm which lies between the eye-lens and field-lens of the ocular and is then always in focus with the field of view. With Ramsden oculars the focal plane lies just beyond the field lens (in the direction of the objective) and therefore reticles must be placed in the same location. The Ramsden ocular is usually constructed such that the eye lens and field lens can be removed as a unit cell from just above the field lens diaphragm location. To insert the reticle, the lenses are removed from the tube and the reticle placed upon the diaphragm. The Ramsden ocular is superior in many respects to the Huygenian and where good image quality of the ocular micrometer net is desired, it is to be preferred. If a binocular head is being used only one eyepiece need be equipped with the net reticle.

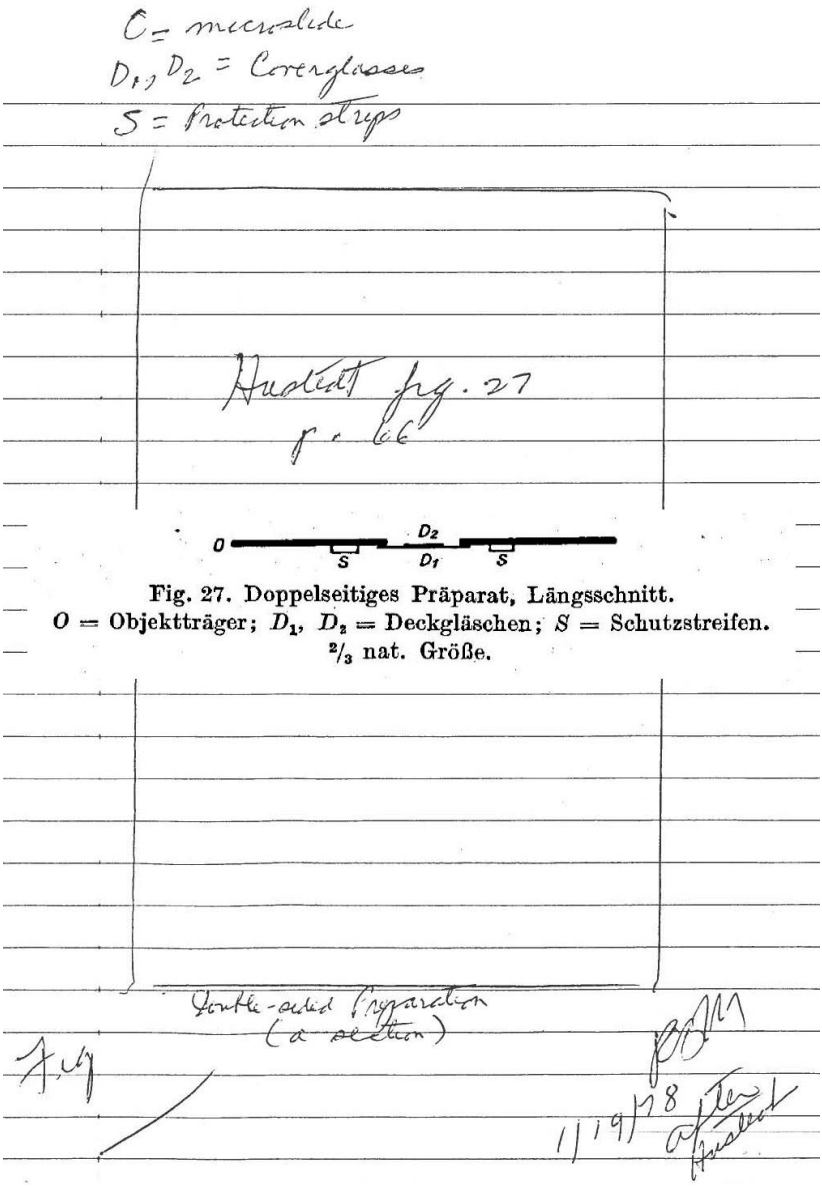
As each individual form is placed on the prepared coverglass it is fastened in place by breathing on it. Through repeated and continuous breathing on each form as it is laid, the layer of fixative is softened such that delicate forms may sink too far into it, or that a previously laid diatom might become shifted in position thus spoiling the entire preparation. Hustedt therefore recommends covering the coverglass with a small glass cover of about a centimeter in height and diameter between each laying operation. However, he is referring to the condition wherein a dissecting microscope is in use and the mounters head is necessarily very close to the work. With the use of a conventional microscope stand (stereo or non-stereo) the worker is far enough removed from the mounting site as to have his breath pose no danger to the laid diatoms. With a sufficiently thin layer of fixative the diatoms should not sink into to it as he has indicated.

The embedment of the type material mounted in this manner is accomplished in the same way as for single selected mounts. The preliminary treatment with the solvent of the mountant, just prior to its application, is recommended.

8.8.2. Double-sided Preparations

It is worthwhile, although perhaps not completely necessary, and especially with dorsoventrally constructed diatoms, to be able to examine both sides. Or, in many cases, it is of advantage to be able to examine the inside, as well as the outside, surface of a valve to become acquainted with cell-wall construction, perforations etc. Examinations of this sort are not entirely satisfactory with dry or loose unprepared material that is encountered in spread or store slides, and the conventional mount does not provide a means for such examination. To take full advantage of the resolution obtainable with a diatom mounted in a suitable contrasting medium, a double-sided preparation is used. This is of considerable advantage when studying species of the genera *Amphora* and *Nitzschia*. The diatoms are embedded in a mountant contained between two coverglasses. Reference is made to Figure 60. The microslide O is a 25 x 75 mm. cardboard rectangle, with a hole of 15 mm. diameter punched out of its center. Coverglasses of 18 mm. diameter are used, or one of 18 mm. diameter and one of lesser diameter is satisfactory. The 18 mm. coverglass D is fastened to the cardboard slide with shellac, thus forming a 15 mm. diameter cell, into which the coverglass D with the mounted diatom is fitted. The large coverglass is protected from pressure by two glass strips S fastened to the slide.

It might be more advantageous to make up the two-coverglass embedment previous to fastening it to the cardboard slide. In that way, any heat required during preparation will not effect the cardboard.



Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 27)

Figure 60.

8.8.3. Labeling

Permanent labels for microscope slides always have posed some problems. Over long periods of time adhesives change and labels can become detached. Even with the level of present-day technology the same problems in making labels of paper

adhere permanently to glass are with us. However, if certain precautions are taken prior to, and during application of labels their permanency, or at least a very long life, can be assured.

The first prerequisite in attaching labels is for the glass surface to be absolutely clean and free of oil, greasy residues, or other dirt. The portions of the slide that will receive the label should be cleaned more carefully than any other, and preferably just prior to labeling.

Gum arabic is a customary slide label adhesive but will not adhere well to any but the cleanest of surfaces. An adhesive with superior qualities can be made as follows: Dissolve 120 grams of gum arabic in a quarter liter of water, and 50 grams of gum tragacanth in a separate, same volume of water. After a few hours the tragacanth solution is shaken until it froths and mixed with the gum arabic solution. This mix is strained through linen. Then add 150 grams of glycerine previously mixed with $2^1/_2$ grams of oil of thyme. (This recipe is taken from Lee – *The Microtomists' Vade-Mecum*).

Labels may be purchased already printed and gummed, and generally are the best in the long-run. In applying these it will be found advantageous if both sides of the label are moistened, which minimizes buckling and curling during drying.

Self-adhesive labels are attractive, but the chlorinated rubber, which is the basis of most of these adhesives, may oxidize over a period of time and the label loosen or curl away from the glass. To prevent this, after the inking is dry the label has a thin layer of clear finger-nail polish applied, effectively sealing the surface and the edges to the glass.

An old, but effective; label, is to apply a thin coat of balsam on the end of the slide, and allow it to dry thoroughly. The inking is done on the balsam layer, and after becoming dry another layer of balsam completes the job.

The ink used for labels should be waterproof, permanent, and preferably black. If at all possible the label should be printed rather than written in script. Anyone who has attempted to decipher the scrawlings, or even very elegant calligraphy of old microscope slides will agree with this. The printing of very small fine letters is accomplished quite easily with appropriately sized crow-quill pen points, or the fine versions of the modern Rapidograph fountain pens (manufactured by Koh-i-noor) used by draftsmen.

Printing of microscope labels in quantity, can be done quite reasonably on a small manually operated printing press. Printing equipment and accessories recommended for printing microscope labels has been quoted at less than 200 U.S. dollars at the time of this writing.

Even the best of labels may become detached in time with the danger of losing identification of the diatom slide contents. This possibility can be protected against by applying the slide number on the underside of the microslide with a writing diamond or carbide-tip marker. The number is referenced in a card or similar catalog which contains all label information plus other data about the slide.

The data to be included on the slide label will vary somewhat, depending upon the content and purpose of the slide. More of this will be discussed in the following section on the study of diatoms. However, in any case, there would be an indication of the mountant used, and the coverglass thickness in mm. if known, as these two items are usually of considerable importance in critical diatom examination. Also, generally it will be found advisable to use a label at each end of the diatom slide so as to provide sufficient space for data. Usually it is important that the label contains data as to what the origin of the diatom is, as to locality and/or habitat, fossil or recent, freshwater or marine, and the name of the preparer and the date of preparation. In strews, note of special or dominant forms might be included. In order to conserve label space a set of abbreviations is often used. For instance RF = recent freshwater; FF = fossil freshwater; FM = fossil marine, etc. These are generally recognized by most diatomists, although there may be some variations in them due to language. For any one particular worker at least, it is preferable that a consistent abbreviation system be adhered to and that they be defined or referenced in a card catalog, for instance.

Abbreviations of mountants, at least of the more common and older types are also generally recognized. Styrax is abbreviated St.; Piperine-coumarone = Pip. cum.; Naphrax = Nx; Hyrax = Hx, etc. However, where there may be any confusion generated they should be spelled out or at least referenced in the slide card file.

8.8.4. Mounting in Special Media

8.8.4.1. Low-Index of Refraction Media

For various reasons it may be desirable to mount diatoms in special mounting media to emphasize frustular details not imaged at their best with the usual mountants. It has been pointed out previously that a high index of refraction media may well obscure details of the more coarsely marked diatoms. For instance the very dark border image, especially with highly arched valves, obscures the finer details of the edge structure. In that case Hustedt recommends using low-index media such as a reasonably volatile oil. Oil of turpentine, olive oil, oil of cloves, cedarwood oil, and similar fluids penetrate the diatom frustule or valve quickly, make the walls transparent, and therefore provide better images of the wall edges and its construction features such as pores, septa, girdles and intercalary bands.

8.8.4.2. High-index of refraction Media

The resinous high index of refraction media used for permanent preparations furnish in all cases the necessary contrast and visibility for the resolution of the finest structural details possible with the light microscope.

However, at times it may be required, in the course of diatom study, to wish to observe diatom frustules with the advantages of the high index of refraction media, but not with the attendant, perhaps, lengthy mounting procedures usually necessary with the resinous media used in permanent preparations.

For the purpose of temporary preparations, or for the condition where further temporary changes in the mounting media might be desirable in the course of study, a number of fluid media are available to serve the purpose.

Hustedt considers the following as suitable: quinoline, colorless, with a refractive index of 1.624; methylene iodide, with a slight reddish color, with a refractive index of 1.74; monobromonaphthaline, colorless, refractive index 1.66; phenylmustard oil, with a yellowish color, index of refraction 1.65; phenylsulfide, of a deep yellow-green color, index of refraction: 1.95; and carbon disulphide, colorless, with an R.I. of 1.628.

All of these materials may be applied without further preparation directly to dry diatoms. They evaporate rather readily as they are all quite volatile. For a longer lasting preparation a temporary enclosing ring may be applied.

Among the more solid media the following are of importance (after Hustedt):

Arsenite of stannous chloride-glycerine

To prepare this, six parts of stannous chloride and two to two and half parts of arsenious acid are weighed. The stannous chloride is boiled for a short time in a test-tube, and during the heating and an equal amount of glycerine is added, heated further and shaken until a clear solution is obtained. To this the arsenious acid is added very slowly, and the whole is heated and shaken until all is in solution. After cooling, the result is a thickly fluid colorless mass that has excellent lasting properties. Hustedt indicates his material made in 1915 lasted 15 years without change!

A drop of this mountant is placed on a diatom-loaded cover, picked up with a microslide and strongly heated, which creates a lively development of bubbles. After cooling, the bubbles disappear for the most part and the medium becomes brownish colored. Its refractive index may perhaps equal that of a piperine mixture. The preparation ordinarily soon becomes turbid, but can be cleared by renewed heating.

Stannous chloride- Glycerine—Gelatine

A glycerine-gelatine with the consistency of honey is first produced by heating clear gelatine with pure glycerine. In 8 grams of this material dissolve 40 grams of pure stannous chloride with the aid of heat. The solution at first appears to be milky or turbid, but with boiling in a test-tube becomes clear and with the color of Canada Balsam. With this latter boiling very strong bubbling occurs, so the test-tube should only be about a quarter full. It is applied to a coverglass as balsam would be. It remains hygroscopic however, and a temporary protection ring is advisable. It is recommended that a wax ring be used over which a ring of lacquer is applied. The refractive index should be about 1.70.

Hustedt also describes the preparation of an antimony-bromide-glycerine-arsenious acid mix, but does not recommend its use.

8.9. Notes and Techniques

The following will provide additional guidance to the diatom mounter.

In filtering mountants, and fixatives, the time is shortened by using a fluted filter paper only, with no glass funnel. Mountants may have to be quite thin for filtering purposes and then allowed to thicken by evaporation, or heating, if appropriate.

Clean the mountant bottle prior to storing mountant in it by the use of the mountant solvent which itself is preferably filtered before use.

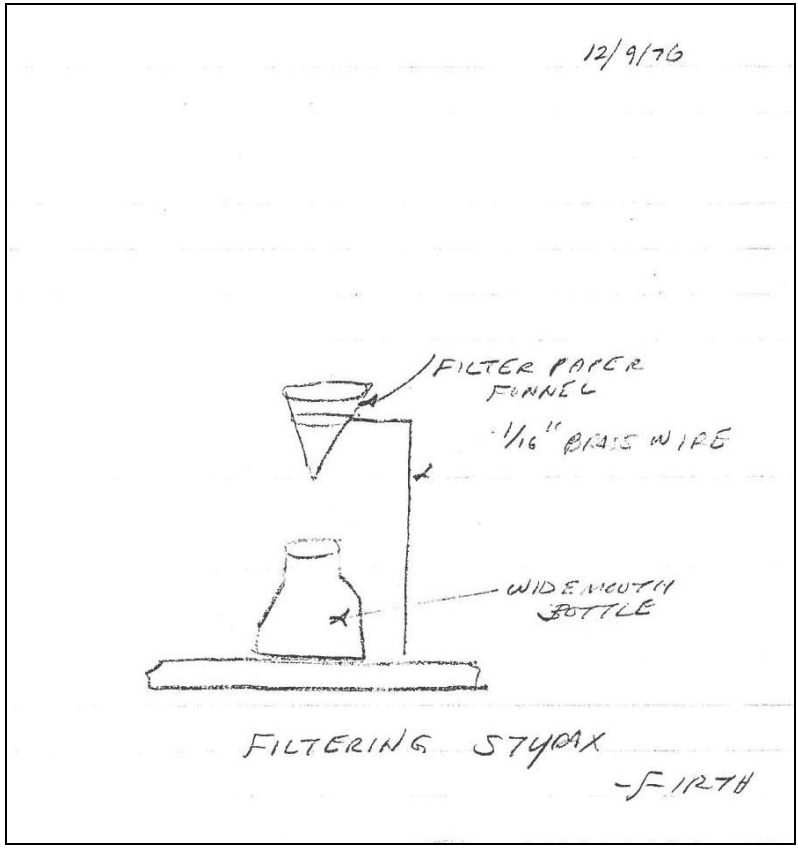


Figure 61.

In labeling, better adherence will be obtained if the partially applied label is inverted and pressed and adjusted against a surface such as blotting paper. This technique provides for some movement in adjusting the label position, and at the same time a good contact of the adhesive and glass surface.

Microslides can be given final cleaning just before mounting proceeds with water and a good rubber eraser. After this the surface is wiped with a linen cloth and examined at 100X under the microscope for cleanliness.

In preparing spread slides, breathe on the slide to clean it and wipe with a linen cloth. Drop the suspended material from a height of 3 or 4 mm. to get a good spread. The slide is then rested at a slope with one end on a wooden block to drain off excess water.

An automobile cigarette lighter can be used as a very small heater in making picked diatom slides. Mounted on a small stand and furnished power with a heavy duty transformer, it can be operated by a foot switch leaving both hands free. It is useful in the final heating of a mount wherein the remaining solvent bubbles off. Col. Wm. D. Fleming has used such a device for years in mounting with Naphrax. Beeswax can be used to secure a bristle on the end of a mechanical finger or hand operated wand. It is also useful in temporarily fastening coverglasses to a microslide for picking and mounting, or other operations.

To avoid untidy and dirty preparations the microslides, diatom specimen materials, and coverglasses, should not be left uncovered at any stage of the work any longer than absolutely necessary.

Two covers on one microslide during the mounting process saves space on a hot plate. If they are close enough together they may be covered with a single watch glass.

Some mounters make a small india ink mark on the edge of the coverglass to facilitate its correct orientation on the microslide.

If a frustule or diatom has retained a bubble which has taken some time to remove, do not ring the cover for a day or two as the bubble sometimes reappears and further heating may be required.

An inverted glass pie-dish is excellent for protecting a large number of microslides and/or coverglasses in stages of preparation, from dust.

8.10. Electron Microscope Mounts

Mounting diatoms for study with an electron microscope is quite different from that for the light microscope, and because the diatomist himself does not ordinarily go further than to provide cleaned material for such preparations, only a brief treatment of technique is provided here. Two major types of electron microscope are now used in the study of diatoms, each having its advantages, and each requiring, by virtue of its operation a different type of specimen mounting.

8.10.1. The Transmission Electron Microscope (TEM)

Referring to Figure 62 the transmission electron microscope (TEM) is compared with an optical light projection microscope. The object diatom O is supported on a diaphragm that is transparent to the electron beam. The magnetic objective and

projection lenses image the diatom on a screen. The intermediate image in each case is at I and the final image at I.

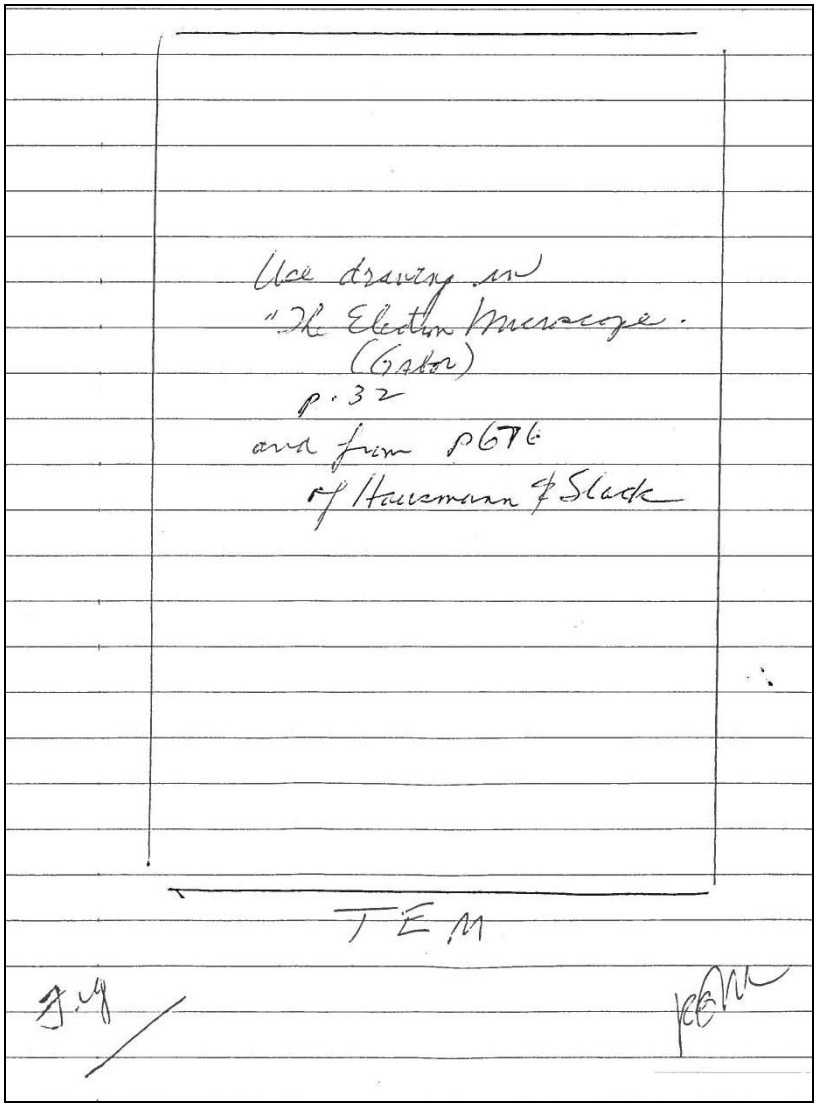


Figure 62.

The mounting of diatoms for examination by this type of electron microscope entails placing them on the support which ultimately is located in the objective compartment of the instrument.

The supporting film must be thin enough to offer little or no opacity and scattering to the electron beam. The films employed are collodion (nitro-cellulose), ethyl-cellulose, or polyvinyl formal resin (Formvar), among others.

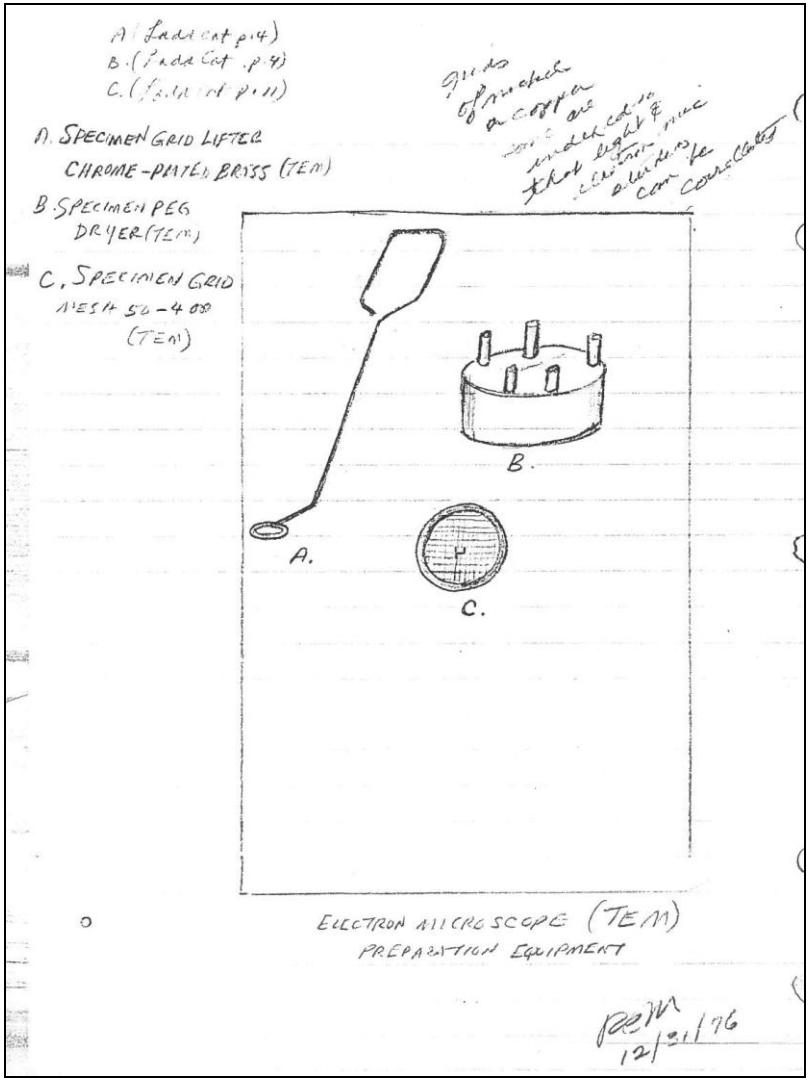


Figure 63.

Hendey describes a typical procedure in mounting diatoms as follows:

> a. Allow a drop of nitro-cellulose (5% in amyl acetate) to fall upon the surface of clean distilled water in a glass dish about 20 mm. in diameter.
>
> b. The drop should spread out radially and evenly (irregular spreading indicates the water surface is not clean, and such films should be gently swept off which acts to clean the surface). Films of this sort are in the vicinity of 100 angstroms in thickness.
>
> c. A metal specimen support (Figure 63c) grid is laid upon the film surface (or as many as can be accommodated and/or required).

d. A special ring tool (Figure 63a), usually made of chrome-plated brass, is plunged beneath the surface and brought up under the grid to lift it slightly above the level of the film.

e. The film is cut around the edge of the grid and from the stem of the lifting tool with a mounted needle and lifted free.

f. The ring is inverted over a cylindrical peg mounted in a special stand (Figure 63b). The film is now on the upper surface of the grid.

g. Any residual water is removed from the filmed grid with a piece of filter or blotting paper.

h. The grids are placed in a desiccator to dry and until required.

i. The diatom material is dropped on the film surface and returned to the desiccator until dry.

j. The preparation on the filmed grid is now ready to be placed in the specimen chamber of the microscope. Sometimes this entails placing the mesh with the preparate over the opening of a specially designed cartridge which, when loaded into the object chamber, provides positioning on a repeatable basis.

Ferdinand Reynold. Schoeman
b. 1943

Robert Eldred Mostert "Archie" Archibald
b. 1940

Schoeman and Archibald indicate that with specimens large in comparison with the grid openings a film support is not always necessary - the diatoms being placed directly on the grid surface. Also, they recommend that the diatom material should be suspended in de-ionized water to which several drops of 50% ethanol has been added to reduce static electrical charges.

There is a great variety of support grids available for use in electron microscopes with grid mesh sizes ranging from 50 to 1000 or more. The hole sizes (interstices) may vary from 450 micrometers to as little as 12 micrometers. The grids are very delicate, being about 0.8 mil. thick. A common diameter for such grids is 3 mm. Some grids are indexed with number-letter systems, through a photo-engraving process, to provide for easy correlation between light and electron microscope studies, and some are equipped with large holes or slots in addition to the regular mesh. Grids with the centers marked in some way to facilitate orientation and location are also available.

The meshes that will be found to be most useable for diatom work are the 200, 300, and 400 mesh grids. The 200 mesh is for the larger forms in *Pinnularia*, *Synedra*, etc.; the 500 is suitable for the majority of specimen material with a varied species composition and specimen size, and the 400 for very small sizes of diatoms.

With very large diatoms, in comparison with the grid openings, a film support is not always necessary - the diatoms being placed directly on the grid surface. Choices of this sort will have to be made on the basis of what is to be shown in the micrograph.

If a whole frustule or valve is to be depicted, or whether only a portion is involved may decide whether this is desirable.

Although not absolutely necessary in TEM examination, it is sometimes advantageous to "shadow" the diatoms with a metallic film, providing greater contrast in the image. Platinum is a common shadowing metal, being applied by a high-vacuum coating device at an oblique angle.

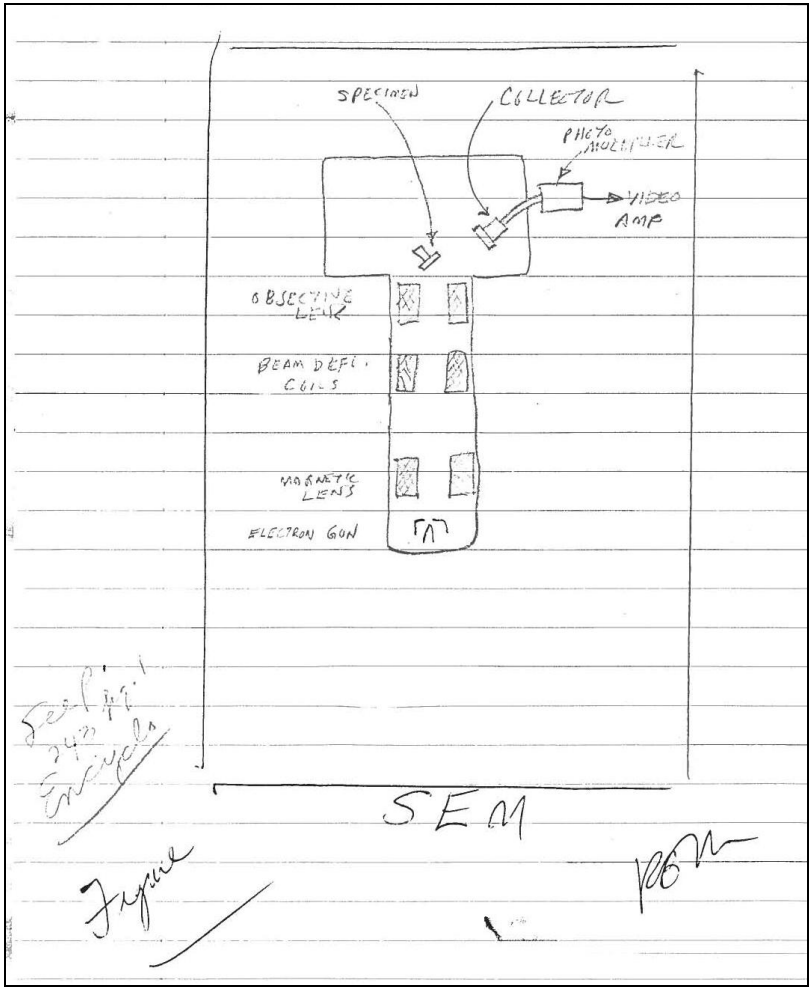


Figure 64.

8.10.2. The Scanning Electron Microscope (SEM)

Reference is made to Figure 64. In this type of electron microscope the diatom surface is scanned by an electron beam. Electrons of both "reflected" and secondary categories are produced from the diatom surface in accordance with its variation, collected, and ultimately by video techniques, a visual image is produced on a kinescope, or display tube. Collection and processing of the secondary electrons has been found to give the most informative image and is the normal mode of operation

of the SEM. This system is essentially a specialized reflection method of electron microscopy and therefore the specimen surface is most important in image formation.

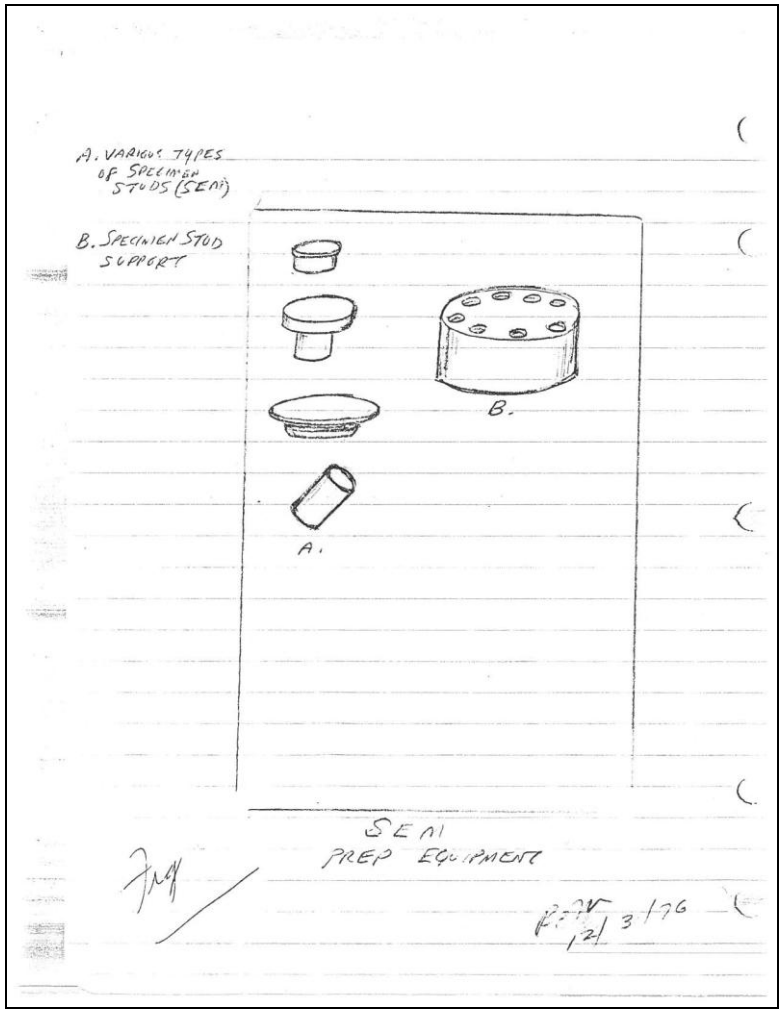


Figure 65.

The diatom material is mounted on a viewing stud (reference Figure 65A), which is ordinarily of aluminum or brass and may take on various forms dependent upon the specimen and/or specific model of microscope in use. Studs are commonly about 2.5 mm. in diameter. The diatoms may be affixed to the top of SEM studs with adhesives or merely dropped on the stud surface and allowed to air-dry in a dust-free location.

The top of the studs can be furnished with mica discs which provides a smooth clean surface and excellent specimen support. Special miniature spatula-needles are available for making dispersions directly on SEM studs, and they are also useful in

applying an adhesive if desired. The studs are conveniently held in a special support while being worked upon or during specimen drying (Figure 65B).

As with preparation of diatoms for TEM examination, Schoeman recommends that cleaned material be suspended in de-ionized water to which several drops of 50% ethanol have been added to reduce static. For best results with most SEMs, the diatoms are coated with gold, providing a brighter image and improving resolution through prevention of charge accumulation on the specimen surface. Schoeman indicates that a lighter metallic coating with less obscuration of fine detail can be obtained by placing the stud (with diatoms) in a 1% osmium tetroxide vapor at room temperature for 16 - 50 hours. Having a high vapor pressure at room temperature, the osmium penetrates the small pores in the diatom valves.

Schoeman outlines the procedure he considers best for preparing diatoms for SEM viewing as follows:

a. Cleaned diatoms are suspended in de-ionized water to which several drops of 50% ethanol have been added.
b. Brass viewing studs (10 mm. in diameter) are coated with a mono-layer of 2% collodion in amyl acetate and allowed to dry at room temperature.
c. Several drops of the diatom suspension are placed on the collodion covered brass stub, and allowed to air-dry.
d. The diatom material on the stub is then coated with gold/paladium for 2 to 5 minutes in a Hummer Technics D.C. Sputter Coater or similar.

In some cases, instead of dropping material on the stud and effectively providing a "strew", it may be desired to examine one particular diatom frustule, valve, or other part at a particular orientation. In that case it should not be difficult to place such material with the aid of a mechanical finger. Working with a stereomicroscope at from 40X to 100X will provide the necessary working distance and make the task comparatively easy of accomplishment. The same can be said of "selected" preparations for the TEM.

End of PART I

PART II

Collection and Preparation Methods

CHAPTER 1.

1. INTRODUCTION

This section deals with the practical aspects of collecting and preparing diatoms for microscopical study. The variety and sophistication of the collecting apparatus and the preparation methods used will depend to a great extent upon the purpose of the study and the resources available. It is intended here to provide a sufficiently broad coverage of these subjects as to enable a beginning student of diatoms to do a creditable job of preparing them for study. Also, sufficient information is provided to allow some choice of method according to resources available, intent of the study, and preferences of the investigator.

Many methods of collection and preparation have been presented by individual diatom workers over the years. In almost all cases those methods are the personal preference of the individual, or an adopted method of another, with modifications of apparatus and/or procedures. Even in the majority of such cases however, it is assumed by the particular author that the reader is fully acquainted with the reasons for the procedures described or the means used. Of course that is not always the case and therefore usually limits the uninitiated to following exactly the process described or not using it at all.

There are almost as many techniques of collecting and preparing diatoms as there are diatomists. Many different techniques accomplish the same result and it would be extremely presumptuous to name a best method or procedure. In preparing this section a great number of methods and techniques, from the distant past to the present, have been studied and distilled into a limited selection of techniques and procedures that will provide good results. Whenever the method and/or procedures are directly attributable to an individual worker, the origin is cited.

In the Appendix a rather complete list of individual papers and articles on these subjects is listed for reference. They range from the early days of diatom study to the present and encompass the spectrum of collection and preparation methods.

In this section a limited number of step-by-step procedures are presented from among which the worker may choose to suit his own situation. In addition, considerable space is devoted to the reasons for the use of specific recommended chemicals and techniques which will provide a basis for anyone to formulate a procedure of his own according to his inclination and resources.

I have drawn heavily upon the works of Friedrich Hustedt. Many of his procedures, techniques, and apparatus are included in these chapters that have only been available previously in the German language.

CHAPTER 2.

2. COLLECTION

2.1. Collecting Apparatus and Materials

A listing of various equipment that will assist in making diatom collections follows with a brief description of the items and their use. Actually, very minimal means are necessary for most collecting work. However, for convenience and for special situations, certain pieces of apparatus are essential, and the diatomist will be guided by his individual requirements. In many limnological investigations it is important to collect various data regarding the water from which diatoms are gathered. Temperature, salinity, pH, turbidity, and other qualities may be of interest. As this information is most appropriately obtained at the time of diatom collection, materials and means for detection and measurement in the field are included.

In the list following, a number of pieces of apparatus use netting in various configurations and for a variety of purposes. In the description of types of collecting nets the term “bolting-cloth” or “bolting silk” is frequently encountered. A brief discussion of this term in connection with nets is in order. Bolting is an old word meaning sieving or “sifting through” a sieve. It is applied to silk or other cloth having a characteristic firm weave. The strands of the warp run straight through the length of the piece, while the threads of the weft are looped around each strand of the warp, locking the whole fabric together, so that it cannot stretch in any direction, thus maintaining a great uniformity of the openings. It is manufactured in different mesh sizes, measured by the number of threads per inch, and the number of fibers in the thread varies with the mesh size. The holes are square and the count, therefore, the same in each direction, the finest being about 200 to the inch, Bolting cloth mesh numbers refer to the meshes per lineal inch. For example, the number 25 silk bolting cloth has 200 meshes per lineal inch or 40,000 apertures to the square inch. Number 0 has 38 meshes to the lineal inch or 1,444 apertures to the square inch.

Standard silk bolting cloth numbers and their corresponding meshes are:

No. 0; 38 mesh
No. 6; 74 mesh
No. 12; 125 mesh
No. 20; 173 mesh
No. 25; 200 mesh

A very strong equivalent to standard bolting cloth is a commercially available nylon monofilament screen cloth made to the same mesh sizes. Number 20 is the finest mesh that can be used in routine collecting. The medium counts are most suitable for pond nets, as the finest grades will not permit the free passage of water. In certain types of diatom collecting it is important to known the maximum size of material a given netting will pass, and that it is of such a consistent construction as to give

repeatable results. Also, for some qualitative sampling, the use of such accurately constructed sieving allows more easy calculation of the rate of water passage through it. For casual collecting, substitutes for bolting cloth are quite satisfactory.

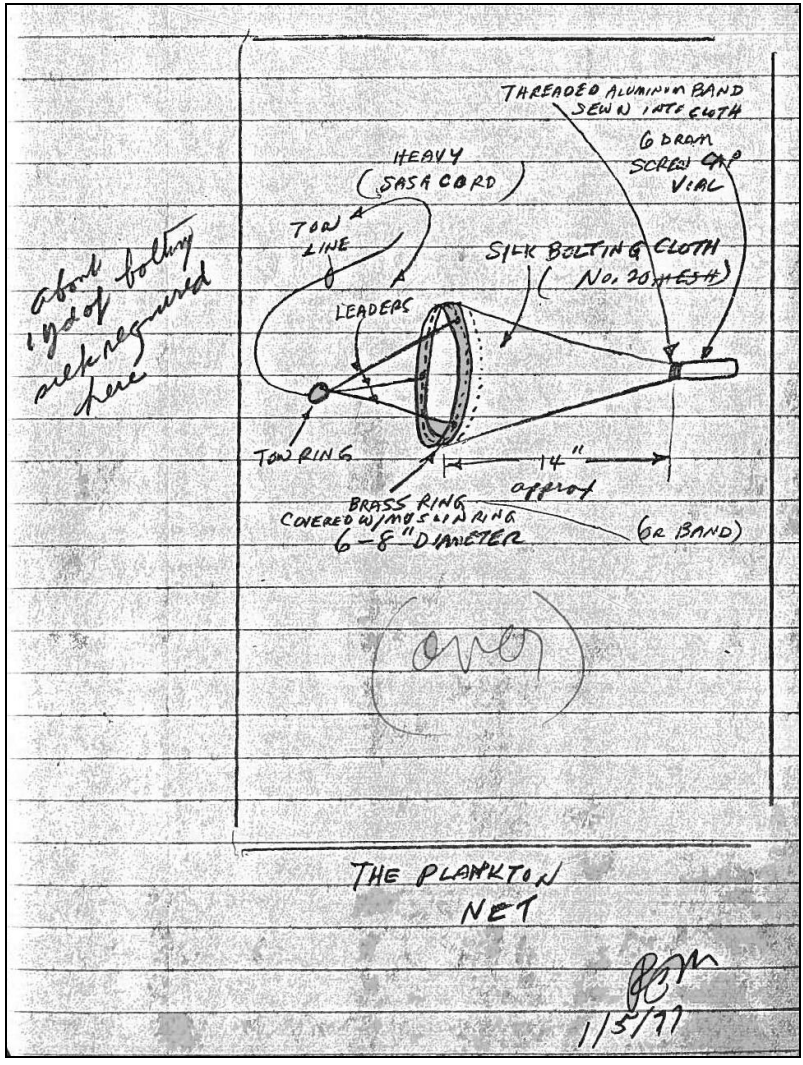


Figure 66.

2.1.1. Plankton Net

For gathering plankton forms this type of net may be towed from a boat, or in small sizes may be mounted on a long handle and swept through the water. It also can be thrown into the water and pulled in by a tow-line. A conical fine-meshed net, it is illustrated in one form and size in Figure 66. The mesh size quite common is number 20 bolting mesh. At the narrow end of the net a small collecting jar or conical vial of about 50 ml. capacity, is attached, About one yard of bolting cloth is required to make a net of the dimensions illustrated. The net should be towed from a boat at slow speeds (not more than 2 knots), usually just below the surface. Approximately

15 or 20 minutes of towing is sufficient to provide ample specimen material. Some commercial versions of plankton nets use a fine-mesh nylon cloth instead of bolting silk, for additional strength.

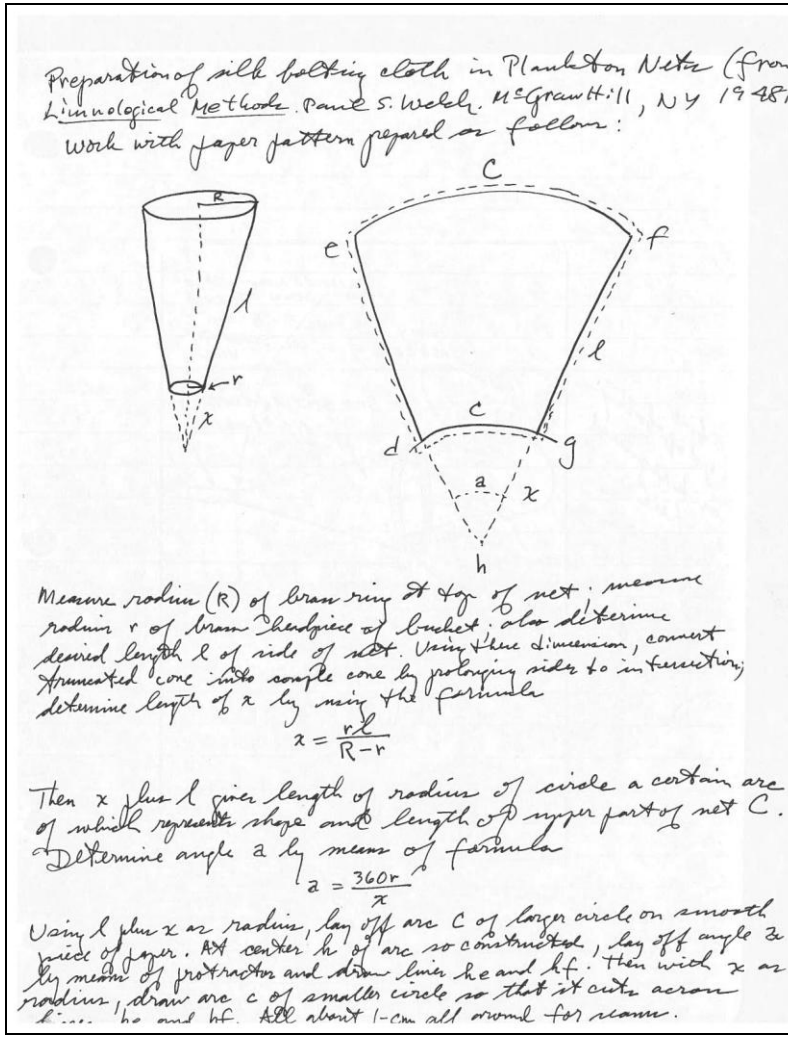


Figure 67.

2.1.2. Bottom Sampling Dredge

A device that is used to bring up a constant volume of sub-strata and the organisms contained in it. Most samplers cover a very small area of about 8 inches by 5 inches. The principal type of mechanism is a pair of clam-shell jaws that are held open by spring action until closed by a release operated from above. When closed the sample remains undisturbed as it is raised to the surface. The parts are normally constructed of brass and bronze. This type of dredge is extremely effective in acquiring material on soft bottoms of sand and/or silt, but is not satisfactory in rock or gravel bottoms.

2.1.3. Secchi Disk

A weighted white plastic or white-painted metal disk 20 centimeters in diameter, used to determine the turbidity or degree of visibility of natural water supplies. The disk is lowered into the water until it disappears from sight. It is raised and lowered several times to get an average depth reading from graduated line. It is also used with the Forel-Ule Scale to determine the color of the water supply.

2.1.4. Forel-Ule Color Scale

François Alphonse Forel and Willi Ule

Provides a means to perform color analysis of water. The Forel-scale (I - X) is primarily for offshore blue to green waters. The Ule-scale (XI — XXII) is for the yellowish to brown inshore waters. Permanent color standards are provided with these comparators, mounted in specially designed plastic comparator blocks with special light filters. For best results the comparator is viewed over a Secchi Disk which has been lowered to a depth of one meter.

2.1.5. Sounding Lead and calibrated Line

A two-pound lead weight attached to a heavy duty braided nylon line that is marked in meters is valuable for determining depths. Twenty meters is a convenient length.

2.1.6. Thermometer

A commercial version of a thermometer suitable for limnology is one which has engraved graduations in a yellow back tubing for easy visibility, with a range of from -5 C. to +45°C. with 0.5°C. divisions. It is furnished with a nickel-plated brass jacket with window openings and perforations which reduce time-lag to a minimum.

2.1.7. Water Sampling Bottle

A weighted bottle (250-300 ml.) that can be obtained in various configurations for taking water samples at different depths from about one to twenty meters.

2.1.8. Water Test Kits

These can be obtained commercially (e.g. HACH; LaMotte) in both salt-water and fresh-water versions. They are used to determine levels of dissolved oxygen, carbon dioxide, pH, nitrates, phosphates, silica, calcium, magnesium, total hardness, ion exchange, ion chlorination, oxidation, taste, odor, and color.

2.1.9. Hydrogen-ion Concentration (pH) Measurement

The measurement of pH can be of almost any degree of accuracy required. For very general and casual measurement, pH-paper indicators are convenient for use in the field. They indicate vivid color differences in acid, neutral, and alkaline water, and by matching colors with a color chart, provide a rough numerical pH value. For accurate measurement, pH meters of great sophistication can be obtained for both field and laboratory use.

Digital display portable aquarium pH testers are readily available, cheaply.

2.1.10. Dip Net

A small short-handled net similar to those used for aquariums is useful to collect materials containing diatoms from water environments, such as those associated with filamentous algae and other water plants. Larger "pond-life" dip nets have handles about three feet in length, and are also very useful. Some are equipped with a detachable glass vial at the tip. In that case the net mesh is usually the same as with "plankton" nets, about 173 to the inch.

A telescopic handle as used by fishermen on their landing nets is a useful addition.

The best diatom locations are always just beyond your reach (or so it always appears) so a pair of waders might also usefully be packed.

2.1.11. Scraper Net

Used for scraping along the bottom and sides of ponds and streams to gather debris to which diatoms may be attached. It has four sides made of galvanized sheet metal and a bottom of fine-mesh brass screening.

2.1.12. Plankton Sieves

Sets of sieves can be obtained commercially that are very convenient for separating the catch in the field. Sets are usually made up of six sieves of approximately 6 inches in diameter and 3 inches in depth with brass wire or stainless steel screens. U.S. Bureau of Standards mesh sizes 10, 18, 35, 60, 120; and 230 are included. The latter two are especially useful for plankton analysis and diatom separation.

2.1.13. Grappling Hook

A very effective means of collecting large algae forms that diatoms maybe epiphytic upon, and other bottom materials which might be a substrate for diatom growth. It may be thrown from the shore or towed slowly behind a boat. One with an overall length of about 8 inches weighing about 2 pounds is satisfactory. A rope or wire of

at least 100 pound strength is recommended. Smaller versions of about half the dimensions above are also very useful. A satisfactory substitute can be easily made of stout copper wire twisted, with a ring at the other end to which is attached a length of strong cord.

2.1.14. Plant Grappling Bar

Ideal for collecting marine or freshwater plants. A bar about 24 inches in length is equipped with 12 strong metal teeth. The 6 inch teeth are slightly curved for maximum collecting capacity. Used with a tow line.

2.1.15. Piling Scraper

Primarily used for removing firmly attached marine specimens from pilings. A curved 16 inch blade is made to accommodate most sizes of pier supports. The blade is attached to a handle of 5 or 6 feet in length. A bag of appropriate size netting is attached to catch the scraped material. All parts are of a non-corrosive metal.

2.1.16. Mud Sucker (Figure 68)

A semi-quantitative device used for taking a surface sample of mud and its contained micro-organisms in deep water. When it touches the bottom a brass weight is allowed to slide down the line and trip the catch. After being tripped, mud is sucked up into the rubber bulb.

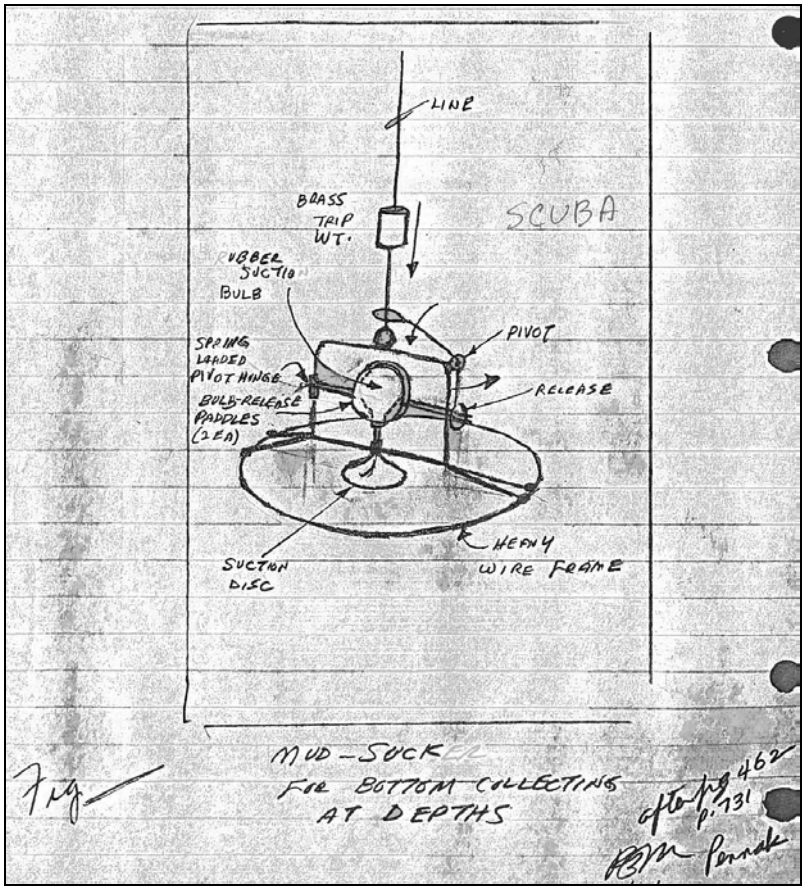


Figure 68.

2.1.17. Spoon (one sharpened edge)

An ordinary spoon with one edge sharpened is convenient for scraping small amounts of diatom growths from rocks and the substrates. Attached to a pole or handle of several feet in length, it can be used in more inaccessible locations.

2.1.18. Strainer

A kitchen wire strainer of 4 inches diameter to separate out the larger pieces and debris from the catch.

2.1.19. Jars and/or Bottles

Preferably wide-mouthed. Quart, pint and half-pint sizes are satisfactory to temporarily retain collections until returned to the laboratory. If, however, you are on a full-day walking trip then carrying large volumes of water around can be extremely tiring. If possible use a portable microscope to check your collection in the field to see whether it is worthy of transport back to the laboratory.

2.1.20. Pipettes

These should be equipped with rubber bulbs for convenience in gathering small quantities of specimen materials. Various sizes are available.

Some British microscopists use a Turkey Baster for collecting larger volumes and mud.

White photographic developing/fixing trays are also extremely useful in the field. Nowadays they are generally plastic and very light. The ceramic ones of yesteryear are particularly heavy.

2.1.21. Plastic Bags

These are used to transport moist or wet plant specimens that may contain epiphytic diatoms, or other wet material for the same reason. Even completely liquid samples may be transported in this manner. Generally more satisfactory than newspaper wrappings.

2.1.22. Aspirator Bottle

A small aspirator bottle is useful for gathering small quantities of liquids from localized collecting points. The narrow nozzle may be easily directed for selective collecting.

2.1.23. Glass Vials

Glass vials 3 inches long by 1 inch diameter are convenient for small samples or for storing concentrated samples collected by other means. Cork seal for transportation. Test-tubes are good substitutes.

2.1.24. Bucket

An ordinary pail or bucket of small size is useful for initial gathering of fairly large amounts of material which is to be further concentrated before transport. White or bright colored ones are to be preferred. Some can be had with a snap-on lid.

2.1.25. Plankton Bucket

A bucket with a sloping bottom and a centrally located drain hole connected to a bottle can be used to concentrate large gatherings down to predominantly diatomaceous material.

2.1.26. Labels

Labels are important to keep records and prevent mixing of collections. Use pencil or waterproof ink.

2.1.27. File Cards

A number of file cards are convenient to keep data on, each being reserved for an individual specimen collection.

2.1.28. Pocket Magnifier

For very rough examination of small appearances a pocket magnifier of 10X/15X, or 20X is convenient. A Coddington or similar wide-field glass is preferable.

2.1.29. Rubber Bands

A handful of these are useful to close the tops of plastic bags, serve as retainers for temporary filtering sieves and for binding labels and data cards.

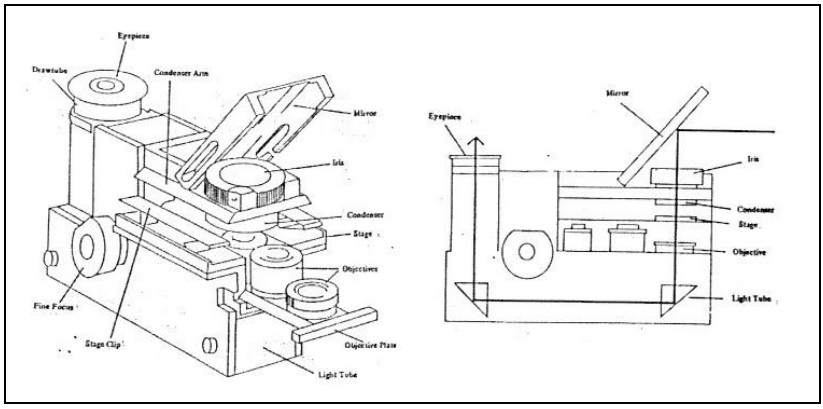
2.1.30. Portable Microscope

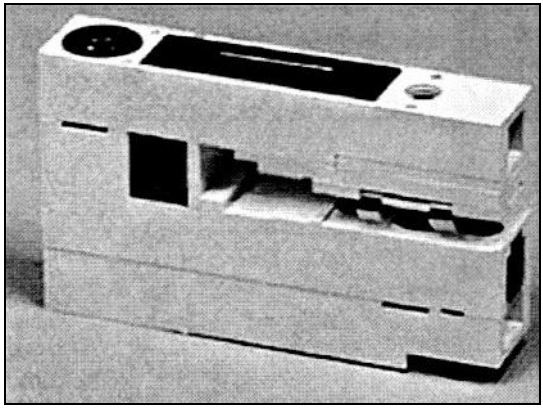
For more detailed examination of field material at lake, stream, or shore. A number of excellent versions are now available commercially.

John Norris McArthur (1901 – 26th April 1996)

The McArthur Microscope devised by Dr. John McArthur of England is a precision instrument, capable of advanced work in the field and small enough to be easily carried in the hand or pocket and often comes with a belt pouch. Another advanced type of instrument is the Model H made by Nikon of Japan which is equipped, or can be equipped with practically any conveniences found in a conventional research stand.

For most field work, much less sophisticated instrumentation is quite satisfactory. Two microscopes that are quite adequate in this respect are readily available. One is the Open University Model of the McArthur portable. It's capabilities are considerably reduced from the advanced model. However, it is very light in weight. and convenient for field use. It is made of lightweight/high-impact plastic and of compact design, being only about 3 inches by 5 inches by l inch in dimensions.





Magnifications are obtained by a 10x Huygenian ocular and a double or triple objective assembly. Objectives are 8X, 2OX, and 40X, providing magnifications of 80X, 200X, and 400X. A double objective model providing only 80X and 200X magnification is also available at reduced cost. A second portable or "pocket" microscope of considerable abilities is one made by Tiyoda of Japan. It is a hand-held type also, completely enclosed in a hinged steel case that is opened for use. The stage is coupled to the focusing adjustment knob on the side post, and the objective remains fixed, whereas in the Open University type the stage remains fixed and the objectives are moved to focus. The mechanical tubelength of the Tiyoda is 160 mm. There is no illuminating apparatus; the microscope is simply directed toward a source of light such as the sky, any room light or even a white wall. Almost any objective in the Tiyoda line of microscopes (and probably any RMS threaded objective) can be used with this little instrument.

The most generally useful combination is a planchromatic objective of 20X, 0.40 N.A. with a 10X ocular.

For work in the field, identification of many genera of diatoms can be made at about 200X. For species determination, in most cases, increased magnification is required up to 400X or more, making the use of such portable instruments for this purpose of doubtful value, excepting those of advanced design.

With the advent of a considerable increase in environmental research, more and more emphasis is being placed on microscopy in the field. The instruments mentioned above will serve the diatomist admirably.

2.1.31. Pocket Knife

An ordinary pocket knife is an indispensable tool. It can be used as a scraper, or in cutting loose bits of material with attached diatoms, as well as for its more common cutting uses.

2.1.32. Scissors

Useful to cut paper strips for temporary labels, to cut plants and/or plant parts up for storage and transport.

2.1.33. Preservative (6-3-1)

6 parts of water, 3 parts 95% alcohol, and 1 part commercial formalin. Added in an amount equal to the volume of the specimen (and its water medium) it serves as an excellent preservative.

2.1.34. Glycerine

If 5 cc. of glycerine per 100 cc. of preservative is added it will protect against complete loss should the preservative evaporate.

2.1.35. Formalin

A 3 to 5% water solution is about the simplest preservative for field use. A good mix is 3 cc. of commercial formalin to 37 cc. of water. Commercial formalin is a 37 to 40% solution of formaldehyde, H_2CO, and is slightly acidic. It may be neutralized by maintaining a slight deposit of magnesium carbonate in the bottom of the reagent bottle.

2.1.36. FAA (Formalin-aceto-alcohol)

A general purpose fixative in which material may be kept for several days without harm. Numerous modifications of this fixative are used. The following is an excellent preservative as well as a killing agent when the material is to be later prepared for staining or cytological work. To 50 cc. of 95‘% alcohol add 50 cc. of glacial acetic acid, 10 cc. of commercial formalin, and 35 cc. of water. Proprionic acid may be substituted for the acetic acid.

2.2. Freshwater Diatoms

2.2.l. Planktonic

Planktonic diatoms (or at least those which spend a large portion of their lives afloat) may be collected primarily from freshwater lakes, but in some cases from large ponds. These diatoms are best collected using a net. Whereas no. 20 bolting cloth is usually satisfactory for the collection of much of the algae and zooplankton, freshwater diatoms are often very small. Nanoplankton nets of 10 to 28 micrometers mesh are best used for collecting these diatoms.

For qualitative sampling a towed net, either from a boat or sweeping by hand, is satisfactory. However, because of the difficulty in determining just how much water passes through the net in a given time, ordinary tow nets are not satisfactory for quantitative work.

For quantitative work there are plankton samplers equipped with a flow-meter to indicate the total mount of water passing through the mouth of the net. They are usually used for gross quantitative zooplankton sampling, but might be satisfactory for diatom work if the net mesh size useful with the flow-meter is not too large to obtain a satisfactory sample of the smaller diatoms. Quantitative sampling requires accurate knowledge regarding the amount of water in relation to the quantity of material. Measured amounts of water may be poured through plankton nets, although the procedure may be extremely slow.

Nets towed behind a boat should be weighted to track properly and make gatherings from specified depths. A coarse screen at the conical front end of such plankton nets keeps out vegetation and debris but allows small organisms to enter. When the net is retrieved it should be held upright so that the organisms are concentrated in the vial. The latter is carefully removed and the contents emptied into a collecting vial or jar. In flowing water collect where brown foam gathers at eddies or where it is trapped by a fallen branch. A brown appearance does not always indicate diatoms, but such indications should be collected and examined microscopically.

2.2.2. Benthic

These diatoms may live either near or on, but not attached to the substrate. The bottom mud, pebbles and rocks or shallow areas often contain these forms. The finest of silts and muds often contain the greatest proportions of diatoms. In shallow ponds sunlight causes diatoms to form a brownish layer on surfaces of the mud. A characteristic coloring of diatoms when they are present in profusion is chocolate to deep-brown, even nearly black, or occasionally a dark greenish brown. Collection is often easily accomplished by scraping off the material from the substrate with a spoon. If the light is strong and the day is advanced it may be in the form of a mat of nearly pure diatoms. Mud gatherings present cleaning difficulties, but many species not otherwise encountered are collected in this way. The use of an aspirator bottle is also very satisfactory where the water is shallow enough to allow the nozzle to operate. When water drains down over rock faces, the dark brown or black color is

indicative of diatom growth. Scrape into a specimen vial or fold into filter paper for transport.

The presence of diatoms will give a brownish tinge to the water in the collecting or aspirator bottle. In strong sunlight there is the appearance of twinkling specks.

2.2.3. Epiphytic and Attached Forms

Many diatoms are epiphytic on leaves and/or stems of water plants and are collected by gathering their host plants from the water by dredges, hooks, and by hand. The plant material should be squeezed over a wide mouthed collecting jar. The plants included will be from the bottoms of lakes at considerable depths to shallow areas, and even mosses and similar plants in relatively dry areas. Certain plants such as Elodea or submerged grasses are often a substrate for diatoms.

Sphagnum mosses, especially older moss which has turned black or brown, in the bottoms of waterways and ditches is usually more prolific of algae than the young bright green moss. If any moss feels gelatinous, it could mean diatoms in profusion. On sunny afternoons diatoms are often trapped by filamentous algae such as spirogyra. When acting as a host for diatoms it lacks its usual green color, becoming brownish, indicating diatoms in abundance. A handful, gently drained, and then squeezed over a wide-mouth collecting bottle will yield fluid containing diatoms. Sometimes if the algae is placed in a large collecting jar with water and then beaten with a glass rod to dislodge the diatoms the yield will be greater. The fluid containing the diatoms is then poured off into another collecting jar.

The algae can also be gathered and transported to the laboratory in plastic bags or in folded newspaper, where the separation treatment may perhaps be carried out more thoroughly. In these cases where the plant is collected, the cleaning process in the laboratory often entails destruction of the plant. Generous gatherings should be made to assure a reserve of material. Utricularia (bladderwort) especially when it occurs in soft water or acid lakes, is a veritable net itself and squeezings from it often contain diatoms.

Many attached forms appear as coatings on rocks, fuzzy films on submerged sticks and other debris, on wet stones near or in waterfalls and dripping cliffs. The rims of dams, submerged glass, shells, crockery, old rotting wood, trunks of trees, and even the backs of snapping turtles, are often the source of excellent diatom specimens.

2.2.4. Soil

A considerable variety of small forms are usually present in soil. The diatoms generally are restricted to about the top centimeter or two of the terrain. The matrix (soil) is collected and preserved, to be later separated from diatoms in a laboratory cleaning process. The number of specimen; is generally small and fairly large amounts of soil should be collected for processing. Several liters of material is usually satisfactory.

2.2.5. Fossil

Fossil deposits of freshwater diatoms are numerous and present throughout the world. The large commercial deposits are evident from their very size alone.

Smaller deposits are recognized by the whitish or grayish material which is extremely light in weight and very absorbent. In some cases this material may underlie swampy or boggy ground and be thoroughly impregnated by water. In the latter case the material has a rather fetid odor, and may be colored or mottled with oxides of iron.

Deposits, even small ones, should be collected carefully and systematically. Collections of broken-off and fallen material at the base of old diatomaceous cliffs are not nearly as desirable as samples collected from known fixed locations. Collecting should be at least from three different levels with notes on the locations below surface level indicated on sample labels. If possible, corings should be taken to provide a continuous sampling, the core later being extracted and divided into intervals according to level. An iron pipe, of a centimeter or two inside diameter, with a sharpened lower end, and as long as can be handled efficiently, is useful for coring in soft diatomaceous earth. The pipe can be driven into the ground or top of the deposit with a sledge hammer or weighty engineers hammer. The top of the pipe is protected by a stout block of wood. If the pipe diameter is selected such that a solid rod will fit inside, the removal of the core is simplified. This particular procedure is only satisfactory if the material is diatomaceous earth of considerable softness. If the material is too soft it will be too compressed when driven out of the core-pipe; If too hard, this simple coring procedure will not work.

Fossil diatoms are sometimes found in hard matrix and collection is similar to that of rock and minerals. The geologists pick, a small two pound hammer, and assorted cold chisels are useful in these cases.

2.3. Marine Diatoms

2.3.1. Planktonic

The most productive area for marine plankton diatoms as a rule is from 15 to 20 miles offshore and 10 to 20 meters below the surface. Excepting for the longer, and perhaps stronger towlines required, collecting is similar to that in lakes. Marine plankton diatoms are larger, in general, than freshwater ones and the plankton nets of no. 20 or no. 25 bolting cloth will yield good catches. The materials for marine collecting must be stronger and of materials minimally affected by salt water. It is to be noted that the season for making marine plankton diatom gathering varies with latitude. Some times of the year at a given latitude the presence of such forms is minimal at best. At other times veritable “blooms” occur as in freshwater habitats.

2.3.2. Benthic

The most prolific habitat of marine and associated forms for the casual collector is the flat sandy beaches which gradually merge into salt marshes. Search sands after long exposure to sunlight as many forms are attracted to the surface by intense light at low tide. Ripples in the sand left by receding tides, especially those which still hold water, are often productive of plentiful specimens. In the bottoms of the ripple troughs look for color or oxygen bubbles. Look for tiny patches one-quarter to one-half inch in diameter on sandy beaches, as they are often very profitable in diatoms. If the sand is gathered with the diatoms and some water, the sand will usually settle rapidly and diatoms can be poured off into a second storage bottle.

Along shores, bays, and in harbors, the pilings exposed to seawater, the bottom of boats and buoys, and beach debris are usually very productive. Scraping with a knife blade, spoon, or piling scraper as appropriate, is the usual gathering method. Diatoms, sometimes in gelatinous tubes, collect in late winter or early spring and may be attached to piling and other marine locations. Further from the beach, in saltwater marshes and meadows, the surface of water in ditches reached by the tide are often covered with bubbles and a brown scum. This should be skimmed off and collected. The moist or wet sides of such ditches can be scraped with a spoon when a brownish coating is evident. Pressing with a spoon held horizontally will allow diatoms and water to fill it. It is rare to find diatoms in sharply shelving, stony, or sandy beaches, or those that are covered by deep water at high tide.

2.3.3. Epiphytic

Submerged grasses in salt-marshes and other forms of plants indigenous to these areas often are a host for diatoms.

Among the algae, red seaweeds should be particularly well examined for closely adhering forms and those attached by stipes. Green seaweeds are not usually as good a source of diatoms, but some types harbor them, and all should be examined. Filamentous forms of diatoms may become entangled with floating algae and can often be separated in great quantity and purity from that source. As with freshwater collecting the algae can be transported back to the laboratory for separating out the diatoms, or the plant material can be beaten in water to effect separation, the water being poured off with the suspended diatoms into a collecting jar.

Another source of marine diatoms not really “epiphytic” or “attached” is from the stomachs of various marine animals, especially those which feed on diatoms directly. The ones which nearly always contain diatoms are the salpae, limpets, oysters, and holothurians. The contents of the stomach are drawn off with a rubber-bulb pipette and placed in collection vials.

2.3.4. Fossil

Fossil marine diatoms are found in the sediments of the ocean depths and are gathered by rather sophisticated means. Because the top sedimentary layer is of recent origin, corings are taken at considerable depths.

A simple form of corer consists of a long hollow metal tube with a weighted upper end. When the tube is dropped overboard, the weight drives the open end into the sediment and the sediment is then returned intact and undisturbed. The tube usually has a plastic liner for easy removal and storage of the core. Fins help guide the tube on a straight path. More modern corers have a smaller release weight which allows the corer to free-fall the last few feet to the bottom; at the same time it takes a small core of the upper few centimeters of the surface. Within the larger corer a piston rides upward as the tube is driven into the sediment. The piston uses water pressure to overcome friction between the corer and the sediment and helps to collect a more complete sample. The piston also prevents disturbance of the sample. Sometimes, a small glass ball is utilized in the corer. As the corer, called a "ball-breaker" makes contact with the bottom, the glass ball breaks and the sound, picked up by the ships underwater sound detector, is used to determine the exact depth at which the core was recovered. Cores of over 60 feet in length have been recovered with the piston corer.

Sediment accumulates on the ocean bottom at various rates, dependent upon water density, temperature, currents, and the nature of the sedimentary material. A rate of a centimeter of accumulation per 1000 years is not uncommon. Therefore long cores can be very revealing of the ancient deposits and truly fossil diatoms recovered in that manner.

The cores are removed, and a string, or other means, is used along its length to maintain its physical and magnetic orientation before being cut up into convenient lengths for storage. Records are kept of the many sections so that diatoms (and other organisms) studied in the future can be correlated in time and with other fossils.

Enormous fossil marine deposits are also found on land. In the United States marine deposits most notable are those in Maryland and California. Oamaru, New Zealand, is another famous locality for fossil marine diatoms . The collecting of land-based marine diatoms is essentially the same as those of freshwater origin. Most important is the recording of complete information regarding the location of specimen material.

2.4. Preservation and Transportation

What procedures are followed in preserving and transporting diatom material from the field to the laboratory is largely dependent upon the ultimate objective of making the collection. If life studies are desired, then of course methods to preserve the diatoms in the living state must be made. If the object involves the study of the cell contents in a non-living state, then immediate fixing procedures may be in order. Studies involving examination and analysis of frustule morphology are understandably less demanding of these preliminary precautions.

2.4.1. The Living State

The diatoms should be gathered and transported in as near the real life situation as possible. For specimens which are planktonic this is easily accomplished. Attached and epiphytic specimens should be transported in large collecting jars with the substrate material or host plant parts insofar as possible. They should be transported with just enough water to cover the material well, leaving ample space for this is especially important if the container is to be stoppered or otherwise covered before arriving in the laboratory. Some gatherings can degenerate and gases of sufficient quantity aggregated to blow corks out and scatter the material. Immediately upon arriving at the laboratory the material should be transferred to wide shallow containers, such as Petri dishes, for good aeration.

2.4.2. Cytological Studies

For the eventual study of cell contents, live diatoms collected in the field can be treated just as they were to be used in life studies. However, it is quite convenient to remove the living material from substrates or host plants as previously described (for attached and epiphytic forms) and kill and fix them before transporting. A simple 3 to 5% neutral formalin solution can be used to preserve the diatoms. FAA (formalin-aceto-alcohol) general purpose fixative is recommended as the best all-around treatment however.

Pre-treatment of this nature provides for the killing, fixing, and preservation of the material in as near a natural state as possible and is especially important when transportation time might be lengthy. Also it precludes the sometimes annoying gathering of living diatoms in sides and corks, creating additional recovery problems at the laboratory.

2.4.3. Frustule and Fossil Studies

If the diatoms are collected alive, and are destined to be used in morphology (frustule) studies wherein the cell contents are completely removed in a cleaning process, it matters little how they are transported from the latter standpoint. However, if the gathering is one from the mud or other substrate wherein considerable matrix matter is involved, it might be advantageous to transport alive. The reason for this is that the diatom's natural proclivity for moving toward a source of white light (in some cases) can be used to separate them from debris and thereby obtain a collection more easily cleaned.

If the future cleaning process is considered not to be adversely affected by excessive mud, silt, or other debris, then killing with one of the preservatives mentioned previously is in order. Transporting and/or storing dead diatoms for future cleaning poses fewer problems in the future.

If the collection is strictly fossil in nature; that is frustule and/or matrix only, transportation poses no problems. However, contamination of one fossil sample to another is a problem both in collecting and transporting, and eventually in the laboratory work of cleaning the diatoms. It is imperative for careful geological or

paleontological investigation that there be no cross contamination. The problem can be eliminated by placing specimens in cloth bags and then in plastic bags.

2.5. Miscellaneous Notes

In collecting diatoms and preparing them for transportation back to the laboratory there are a number of helpful techniques available to make the work easier.

In making collections in rapidly moving water wherein the material is scraped off or otherwise dislodged from the substrate, block the flow of water on the upstream side, if possible, with a board, stone, brick or other impediment, to prevent washing away specimen material as it is freed.

To concentrate the catch use the "two-bottle" method. Place stones, shells, etc., with water in one bottle, shake and pour off into a second bottle, discarding the material in the first. Add more solid (fresh) material to the second bottle, shake and pour back into the first. This procedure is continued, concentrating the material from many pieces of debris, rocks, shell fragments, etc. The method is particularly useful in marine shore-collecting, but is good in freshwater collecting as well.

Collect generous gatherings, as there should always be a reserve of material in case of accidents. A quarter of an ounce in bulk of a clean gathering in a half-ounce bottle is usually sufficient. Pint or quart size jars are more appropriate for non-pure diatom gatherings.

Collecting on sunny days is generally more productive as scum on water at that time is sometimes composed of pure diatoms. Also in pond collecting the diatoms are only an inch or two below the water level and more easily seen on the surface of the mud.

Keep collecting bottles with specimen material away from bright sunlight. Under the influence of bright light diatoms give off bubbles of oxygen which carry them to the surface. With any evaporation of the water they then become very firmly attached to the bottle side, making removal at the laboratory more difficult. If they are not required live at the laboratory, adding formalin preservative to the collection will prevent problems such as this.

If the specimen volume is small and it is required for cytological purposes, an .amount of preservative (FAA) of equal to the specimen and its water medium, should be added in the field. If 5 cc. of glycerine per 100 cc. of fluid is added it will protect against total loss should the preservative evaporate.

If corks are used in collecting vials or bottles they can be kept free of diatoms, so that they can be used again, by using waxed paper to cover the bottom and sides when forced into the bottle.

Storage bottles or vials should not be used for more than one collection. It is better to discard them than run the risk of future contamination, or to perform the time consuming work of chemical cleaning.

Field collection bottles of pint or quart size can be cleaned for re-use. However, they must be thoroughly cleaned with soap and brush, after which a dilute acid rinse, and then a final rinse in de-ionized water is in order.

Pack mosses, seaweeds, large masses of algae, grasses, and other plants in plastic bags secured with rubber bands. Mud gatherings also may be packed in this manner, especially if mixed with other diatom substrates such as rocks and pebbles that might break glass. The more liquid specimens in bulk should be collected and transported in jars of suitable size. The rich scrapings and pure gatherings are kept in glass vials or tubes.

Collecting data is very important and as much as possible should be recorded at the collecting site, cards (3 inches x 5 inches) can be cut into strips and inserted into vials etc. Each collecting container and its stopper or lid should carry a number which can be cross referenced into a notebook. The latter should be of paper that is little affected by water and of a strength to withstand rough field use. Engineers field notebooks are excellent for the purpose.

Records should include as a minimum the date, location, and name of the collector, Other data such as the general nature of the water - fresh, brackish, estuarine, or marine; from sandy or muddy locales, rock faces, splash zones, etc. is very desirable. More specific data on all aspects of the water properties, especially pH in freshwater gatherings is valuable. Time of day and general weather conditions will be of interest in any comparative work at a future time.

A more or less pure concentration of live freshwater diatoms may be obtained in the laboratory from mud collections in the following way. Cover a flat accumulation of the mud gathering with a white cloth and place near a light. Diatoms will migrate from the mud through the mesh of the cloth. Some types of diatoms are not motile enough for this process to work well. However, if sufficient time is allowed for the migration, diatoms in small amounts are obtained in a very clean condition. A variation is to use two pieces of cloth, one slightly smaller and above the other, when diatoms have penetrated to the upper surface, the top cloth can be peeled off, leaving the lower one in place on the mud surface.

CHAPTER 3.

3. EXAMINATION OF CELL CONTENTS

3.1. Introduction

The cell contents of diatoms may be examined in live specimens to a limited degree. The contrast in the light microscope is insufficient however to reveal all but the most obvious internal features. Phase contrast and differential interference contrast microscopy can be used to advantage for certain types of investigations. These latter methods and the equipment necessary for their implementation will be discussed at greater length in a subsequent chapter. At this point, the examination of internal features of the diatom cell will be restricted to those using a conventional light microscope in bright field, or simple modifications of bright field.

There are two major reasons for employing staining techniques. One is to provide light amplitude and/or color contrast between various parts of the cell contents, and the other is to provide a diagnostic indicator (the stain) which has certain affinities for specific cell parts. This latter feature is especially valuable in analyzing functions of different cell parts. The degree to which certain of them stain, whether they stain at all, and under what conditions, is revealing of the nature of the cytological materials and their functions, providing a greater insight and understanding of the particular diatom being examined.

The preparation and treatment of diatoms for this type of examination ranges from the very simple to the quite complex. As the cleaning and preparation of diatoms for morphological studies of the frustule entails destruction of the cell contents, it seems logical to present preparation for cytological studies first.

3.2. The Living cell and Gelatinous Formations

Although one can learn more about internal cell structure through fixing and staining of killed diatoms, there are certain advantages to the examination of living material. In a thorough study of any diatom species, this phase of examination should not be neglected. The original color, form, and condition of the chromatophores shows up best in fresh living material. The various formations of gelatinous substances associated with diatoms are often easily observable in the living cell, and the movement of certain diatoms can only be studied in this state.

In order to protect larger diatoms from being crushed or restricted in movement as the coverglass pulls down, upon gradual evaporation of the specimen fluid, some kind of spacers are recommended, especially during prolonged examination. Glass threads of suitable diameter inserted beneath the coverglass or small pieces of coverglass are excellent for the purpose.

A simple method in studying the movement mechanism of diatoms is to have them immersed in an ink-emulsion. India-ink and other coloring materials are useful in this respect if the particles are fine enough. Also, sepia and carmine are easily

employed for this purpose. In these media the streaming of cytoplasm, and currents induced by that means, are made visible.

The same type of immersion is recommended for examination of gelatinous substance formations. The ink particles do not penetrate the jelly-like substances and the latter then appear as bright halos around or between the individual cells.

3.3. Killing and Fixing

It is not the intent here to provide a course of instruction in microtechnique. Other books on the subject are admirably suited to that purpose and far more authoritative. However, some interesting and important information, especially as it applies to working with diatoms, is certainly in order.

Living diatoms, except for a very few instances, cannot be stained without first killing and fixing them. The purpose in this operation is to render the internal (and external in some cases) cell parts in as lifelike condition as possible, and to make that condition durable enough such that it remains unchanged throughout any other treatment such as staining and mounting. In most cases the two procedures (killing and fixing) are simultaneous.

An important factor in fixing is that the agents used do not expand or contract any parts of the cell contents. Sometimes two fixing agents having opposite effects can be used in a properly proportioned mixture to accomplish this. Chromic and acetic acids are often used for instance, in much botanical work for that reason. Further fixing, or preserving, is quite often then accomplished by alcohol, or by preservatives such as weak formalin.

3.3.1. Fixing the Cell Contents

For this step there have been many reagents and procedures used and recommended by different workers. They are not all equally successful in all cases. It is therefore advisable that not one single method be available, but that there are a number of them to choose from. In cases where the particular material is being treated for the first time it should be divided into a number of portions and different methods of fixing carried out to determine the best one. Also, it may be that several methods are about equally successful, but that chemicals on hand demand a choice of only one. For the fixing of the diatom cell contents without regard to possible future staining operations, the following first seven reagents are recommended by Hustedt.

Fritz Richard Shaudinn
b. 19th September 1871
d. 22nd June 1906
Co-discoverer of the causative agent of syphilis.

Fixing solution, after Shaudinn:

7% Aqueous solution of mercuric chloride, l part.
Absolute alcohol, 2 parts.

Chromic-acetic acid, after Flemming:

Chromic acid solution 70cc.
Glacial acetic acid 5cc.
Distilled water 90cc.

Walther Flemming
b. 21st April 1843
d. 4th August 1905
German biologist.

Chromic-osmic-acetic acid, after Flemming:

1% Chromic acid solution 180cc.
2% Osmic acid solution 25cc.
Glacial acetic acid 12cc.
Distilled water 210cc.

Picric-sulfuric acid:

Picric acid (cold saturated) 100cc.
Sulfuric acid 2cc.
Distilled water up to 300cc.

Fixing solution after Bouin (1827):

Picric acid solution (saturated) 15cc.
Formalin 5cc.
Glacial acetic acid 1 cc.

Pol André Bouin
(1870 – 1962)
French Histologist.

Fixing solution after von Rath:

Otto von Rath

Saturated Picric acid solution 200cc.
1 gram Platinum chloride dissolved in water 10cc.
Glacial acetic acid 2cc.
Osmic acid (2% sol.) 25cc.

Fixing solution after Zenker:

Potassium dichromate 2 grams
Sodium sulfate 1 gram
Mercuric chloride 5 grams
Glacial acetic acid 5 grams
All mixed with 100 cc. of distilled water

Friedrich Albert von Zenker
(1825 – 1898)
German pathologist.

Fixing solution- Allen's modification of Bouin's fluid:

Compound a saturated solution of Picric acid in the filtered water in which the diatoms were found growing 75cc
Formalin 15cc.
Glacial acetic acid 10cc.
Urea crystals 1 gram

In a procedure describing the use of the latter fixing-agent, Subrahmanyan recommends using a large amount of it with the material to be fixed, about 3 to 1, leaving it to work for 5 to 12·hours.

R. Subrahmanyan

A simple means of preparing diatoms for ultimate examination of the internal contents is to treat them as one would filamentous algae forms. They are killed in osmic acid (osmic tetroxide) and further fixed in weak formalin.

Osmic tetroxide is an extremely poisonous and volatile chemical used in a 1% solution in water. It is an excellent and rapid killing and fixing agent, As now purchased; it usually comes contained in a hermetically sealed glass ampoule or container. To prepare the solution the container is crushed in an appropriate amount of water allowing the released osmic acid crystals to dissolve. This compound is easily oxidized by light and dust and therefore should be kept in a blackened dropping bottle. Solutions have a much greater permanence if they contain 1% chloroplatinic acid, or if one gram of the latter is added to every 100 ml. of the osmic acid solution.

If specimens are in a drop of water on a slide, fixation is accomplished by inverting it over the mouth of the osmic acid bottle. A few drops of the solution are sufficient for fixing specimens in 5 or 10 ml. of water. All fixed material should be washed in several changes of water. If the material is colored black or dark brown by the action of the osmic acid, place it in commercial hydrogen peroxide until sufficiently bleached. Osmic acid is, unfortunately, very expensive.

Another fixing agent is made by adding two to 10 parts of glacial acetic acid (99.5% CH_3COOH) to 100 parts of saturated mercuric chloride. A mercuric chloride solution by itself preserves, as a fixing means, the finest structural relationships of the nucleus, and the plasma, with nuclear division.

Alcohol is also used in varying concentrations as a fixative and preservative. By alcohol is meant ethyl alcohol (C_2H_5OH). It is usually supplied at about 95% concentration (190 proof). Absolute alcohol (100%) can be difficult to get hold of and with taxes etc, very expensive. It can be made by the following procedures.

Dehydrate 95% alcohol with anhydrous copper sulfate. Crystals of Copper sulfate (cupric) should at first be heated in an evaporating dish until the water of crystallization is driven off and a white powder remains. Place some of this powder in a bottle and add 95% alcohol. The water in the alcohol immediately combines with the copper sulfate, turning it blue. Add more anhydrous copper sulfate until it no longer turns blue. Then quickly filter the alcohol into a dry bottle and stopper with a tight cork or ground glass stopper.

To make various alcohol dilutions is comparatively simple. An example: to make a 60% solution, add 35 ml. of water to 60ml, of 95% alcohol. Other concentrations are obtained on the same basis.

Formalin is a standard 37% to 40% solution of formaldehyde gas. It is in itself pure, or as a 10% or yet weaker solution, through dilution of the standard solution, a good fixing agent. An excellent mixture, very suited to the fixing and preservation of

freshly collected material, is 30 parts water, 15 parts 95% alcohol, 12 parts 37% formalin, and 1 part glacial acetic acid.

3.4. Stains and Staining

In the process of staining, the dye or coloring agent becomes attached to the structure to be stained, and remains attached through out the various procedures involved in making a complete preparation and final diatom mount. The most common reason for permanent attachment of the dye differentially is not mechanical adhesion, but is that the electrical charge on the dye becomes balanced by the charge on the part or structure dyed. The degree or adhesion of the stain to the structure can be increased by the action of so-called mordants. Sulfates are the most widely used mordants in microtechnique - particularly the double-sulfates known as alums.

Other methods of differential staining are dependent upon the solubility of the dyestuff (oil soluble, water soluble etc.); or by simple difference in permeability between different types of material. Different sized molecules of colored dyes will permeate different portions of the diatom and its interior components with different facility.

Dyes attached by mordants may be removed by ionized solutions, usually acid, or with the use of solutions of the mordant itself. This is, in addition, another basis for differential staining. It is also evident why acid-containing mountants are often detrimental to stained diatom preparations.

Staining may be either direct or indirect. In the direct method the coloring agent is applied to the diatoms which are removed when sufficient dye has been absorbed; In the direct method, the diatoms are soaked in the stain solution, then differentiated. Direct staining is simple and very satisfactory in most diatom work.

Stained diatoms are preferably mounted in resins which are not soluble in water. A great many stains fade rapidly in the commonly used water soluble media. As natural resins (such as Canada Balsam) are usually quite acidic, stained diatoms are better mounted in a neutral synthetic medium, assuring color permanence.

The following listed chemicals and stains are useful in diatom work, at least some of which should be on hand. The modern name by which they are ordinarily ordered, the form they take, and usual minimum ordering quantities are included. The uses to which they are often put are included also. It is to be noted that modern stains are both certified and non-certified. Stains that are not certified should be filtered before use. Those that are certified do not require filtering (this is especially important when the stains are mixed, as with crystal violet, etc.) Many of the more common reagents used in work involving staining diatoms can now be purchased already prepared for use. Where it seems appropriate, directions are also given for preparation of them by the diatomist. Oftentimes this is most desirable because of the small quantities involved, infrequent use, the problems of storage for long periods of time, and the fact that fresh reagents usually provide better results.

1. Analine Red

Stains the gelatinous substance of the stipe of stalked diatoms.

2. Bismark Brown

Generic name is Basic Brown 1. Color index no. 21000. A certified stain (by the Biological Stain-Commission, Inc. - Rochester, N.Y.). Stains the Butschli bodies red-brown, and gelatinous stipes brown.

3. Basic Fuchsin

Generic name is Basic violet 14. Color index no. 42510.
A certified stain. This is a magenta-red basic dye widely used as a nuclear stain. It also may be used to advantage with other stains, e.g., Methylene Blue, Haematoxyln and Eosin.

4. Carbol Fuchsin (A mixture of basic fuchsin and phenol).

Minimum purchase 10 grams. This mixture can also be easily made up as follows:
100 parts of a 5% water solution. of phenol (carbolic acid), and 1 part fuchsin dissolved in 10 parts alcohol are mixed together. Stains the diatom *Asterionella gracillima* Heib. gelatinous envelopes very little, but colors the filaments quite strongly.

5. Osmic Tetroxide (Osmic acid).

Minimum purchase 0.25 gram. In very weak solution it blackens the diatom oil globules intensively. The Butschli bodies are not blackened however.

6. Haematoxylin

Generic name is Natural Black 1. Color index no 75290. A certified stain. A natural coloring matter commonly used as a nuclear stain. A principal constituent of Delafield's Haematoxylin.

Francis Delafield
b. 3rd August 1841
d. 17th July 1915
American Physician

7. Delafield's Haematoxylin

A general-purpose nuclear stain. May be purchased under the name pre-mixed, or can be prepared as follows:
1 gram of haematoxylin crystals in 10 ml. absolute alcohol. Add this drop by drop to 100 ml. of a saturated aqueous solution of aluminum ammonium sulfate. Expose the mixture to air and light for several weeks to "ripen". Then filter, add 25 ml. glycerin and 25 ml. methyl alcohol. This reagent is a very important staining means for the diatom cell, auxospores, stipes, the gelatinous cap of the perizomium, and Butschli bodies.

8. Methylene Blue

Generic name is Basic Blue 9, color index no. 52015, A certified stain. An excellent intravital stain for diatoms. Make up a stock of

0.01 gram methylene blue in 100 ml. of absolute alcohol. Add enough of this solution to water containing diatoms to tinge it a light blue. Also colors the gelatinous stipe and outer covering of the diatom frustule, and shows through coloration that the points of the perizonium, which are found under the top (or cap), are converted from gelatin.

In a solution of 1:1000 it will color certain inclusions or the cell in very characteristic ways. However, if the nucleus itself begins to color, the cell will die. The Butschli bodies will color bluish at the beginning with reddish edges and later to red-violet.

This stain is also used (in part) in staining the diatom "trail".

9. Eosin B

Generic name Acid Red 91. Color index no. 45400. A certified stain. Colors the gelatinous stipe but not the Butschli bodies, and makes the centrosomes visible.

10. Basic Fuchsin

Generic name Basic violet 14. Color index no. 42510. A certified stain. A basic analine dye. An ammoniacal fuchsin solution colors the chromatophores red.

11. Gentian Violet (methylrosanhline chloride)

Minimum purchase 1 oz. Stains diatom cell bodies excepting the nucleus.

12. Methyl Green (methylene green)

Generic name is Basic Green 5. Color index no. 52020. Not a certified stain. After fixing the diatoms with mercuric chloride, they are stained for a short time in methyl green. The lobes of the chromatophores will appear a ruby-orange color with yellowish shadings. Longer exposure to the stain produces a rosy coloration of the chromatophores.

13. Nigrosin

Stains the gelatinous sheath of diatoms after they have lain for some time in a glucose-peptone solution.

14. Picric-Nigrosin solution

To make: Add to 3 saturated water solution of picric acid a water/nigrosin solution until the liquid appears a deep olive green.

Diatom material, after lying in this solution is washed with water or 50% alcohol. Examined in glycerine or mounted in Canada Balsam, after application of clove oil, a beautiful blue tone is apparent. Picric-nigrosin fixes and stains at the same time. It is very well suited to the staining of auxospores.

15. Picric-Sulfuric Acid (Picric sulfate?)

    To make: 100 cc. of a cold saturated solution of picric acid is mixed with 2 cc. of concentrated sulfuric acid, filtered and diluted in 400 cc. of water.

    After being fixed with this solution, diatoms upon being washed in alcohol, assume a yellow coloration. If they are instead treated with Haem-alum, the chromatophores will become yellow-grayish, and if with saffranin they become bright-rose colored.

16. Safranin

    Generic name Basic Red 2. Color index no. 50240. A certified stain. Stains the centrosomes red, although the nuclei are not stained. After fixing with picric-sulfur acid solution (as above) the chromatophores color bright-rose.

17. Haem-alum

    Mayer made this from 1 gram. Hematein (generic name Natural Black 1) dissolved in 50 cc. of 90% warmed alcohol, placed in 1 liter of water, in which 50 grams of alum have been dissolved. This solution he called Haem-alum. After settling, decant and add some thymol as a preservative. It is useable immediately. It stains the gelatinous envelope of the plasma-body a deep blue. With fixing through picric sulfate, the chromatophores, owing to the haem-alum, retain a yellow-grayish coloration.

3.5. Selective Staining of Individual Parts

The investigator is usually interested in studying various parts of the diatom cell on a selective basis. In the following paragraphs each area of cell investigation is presented with one or more recommended preparation methods.

3.5.1. The Nucleus

With most diatoms the cell nucleus can be observed even though unstained. However, the details of its structure, and especially its activity in reproduction, can only be studied after careful fixing and staining.

Nuclear division is found at various times of the day. But mostly in the early morning or night hours. Therefore to study cell division the diatom material must be either collected and fixed at specific times of the day, or gatherings from cultures made at appropriate times for the fixing and staining procedures to follow.

Geitler obtained excellent results through fixing with mercuric chloride-alcohol, Flemmings solution, or Baugartel's picric acid-mercuric chloride-alcohol-haemalum solution, and afterwards staining with iron-alum-haematoxylin (Heidenhain) Delafield's haematoxylin, and with safranin-light green. The staining by safranin-light

Martin Heidenhain
b. 7th December 1864
d. 14th December 1949
German Anatomist

green is especially good for the study of auxospore formation, as revealed in the earlier stages by red-coloration of the gelatinous material. The stained diatoms are then transferred through alcohol steps, oil of cloves, and xylene, and finally mounted in neutral Canada Balsam.

Dr. Konrad Gemeinhardt b. 1883

Gemeinhardt turned to a simple method in his examination of the genus *Synedra*. The material first is allowed to stand for from 12 to 24 hours in Bouin's solution. Shorter treatment than 12 hours in the fixing solution is not satisfactory, while with a stay longer than 24 hours, difficulties will be experienced in rinsing. Under the latter condition appearances of maceration also may occur.

Staining is successful in a 5% solution of Haemalum in warm distilled water. The material, immediately after rinsing, is brought into the staining solution and requires only 15 minutes to stain. Differentiation is not necessary. If the material, after fixing, has lain in alcohol, the staining time will be doubled.

The mounting of such stained material is accomplished in the following way. First a drop of 10% liquefied gelatine solution is put in the center of a microslide. Immediately thereafter a small drop of the diatom material in distilled water is added, and thorough mixing of the two fluids accomplished with the aid of a needle. The mixture is then allowed to solidify evenly. The microslide is then taken through the alcohol steps, oil of cloves, xylene, and finally mounted in Canada Balsam. To avoid shrinkage of the cell contents, it is advisable that as small as possible alcohol steps are taken, and that the length of time in each step is not under five minutes. As an expedient, it is further recommended that before placing the material in pure alcohol, it be passed through a mixture of alcohol and oil of cloves. In this way the clarification of the opaque gelatine layer in pure oil of cloves takes place more quickly.

Unfortunately it happens that in many nuclear investigations the chromatophores are very troublesome. They, through the nuclear stain, become themselves strongly colored, and often through their position completely mask the nucleus.

3.5.2. The Centrosome

Fixation of the centrosomes is accomplished with some advantage by the use of an Osmic acid mixture. They are intensely stained by safranin and to a somewhat lesser degree by haematoxylin.

To stain with safranin the Flemming fixative is used. The fixed material is then allowed to remain in a 2% solution of potassium dichromate for ten minutes, then transferred to a 1% solution of potassium permanganate for five minutes, and finally stained in an alcoholic solution of safranin.

Karsten, after fixing with picric-osmic-acetic acid platinum chloride, obtained good results with eosin as a stain.

3.5.3. Dictyosomes

Otto Heinzerling

These are the "Doppelstabschen" of Hustedt, and according to Heinzerling are best fixed with an osmic acid fixative. A diluted solution of safranin is especially suited to staining these bodies, after remaining several days in the stain they become colored dark red.

3.5.4. The Chromatophores.

The chromatophores are susceptible and sensitive to all fixing means, and react upon careless treatment with physical modifications and distortions. The lobes and processes are especially easily retracted. An examination of them in the living condition is therefore always to be recommended. They are best fixed with Bouin's fluid, or with an osmic acid mixture. The frequently disturbing pigment can through longer treatment with alcohol or formalin, be removed. Analine dyes are most suited to staining the chromatophores, and with the same fixing means, aged haemalum, Delafield's haematoxylin, and acid-fuchsin give good results.

von Schoenfeldt indicates that the use of an ammoniacal fuchsin solution is of some success in staining these bodies. Ammonia is added to a basic concentrated alcoholic solution of fuchsin until it becomes straw yellow. The material is treated in this solution and transferred to Canada Balsam or Styrax without going through any alcohol steps (alcohol would immediately remove the stain). After staining, cleanse in water, dry, and flood with xylene before placing in Canada Balsam-xylene, or Styrax-xylene.

3.5.5. The Pyrenoids

In many cases the pyrenoids may be easily studied in the living state. With some diatoms however, careful staining is required to make them visible. For fixing in this case the osmic acid mixture or Bouin's solution are best. One can then stain with eosin, methyl green-orange, picric acid-nigrosin, or haematoxylin. If the staining is to be accomplished with safranin, the material must remain up to a week in the solution, and yet the results are not always pleasing, as the staining is mostly rather indistinct.

3.5.6. Oil Globules

In the living cell the oil droplets are frequently confused with the volutin globules. A 1% solution of osmic acid quickly colors the oil droplets, with the diatoms, a very dark brown. With Sudan III a red coloration is obtained, while with naphthol-blue, a blue one. The oil bodies are dissolved in alcohol, ether, chloroform, benzene, and xylene. They are not dissolved in *eau de Javelle* (hypochlorite of potash) and are not colored through application of sulfuric acid. According to Heinzerling, after a treatment with a 30% sodium hydroxide solution of about three hours, the original round oil droplets become angular, getting slowly smaller and disappearing after

five hours. Whether they are dissolved is not known for sure, and on account of the indistinct saponification their substance is uncertain.

3.5.7. The Butschli Bodies

Hustedt considers these to be volutin, and in the living cells they can be stained red-violet with a very dilute solution of methylene blue, and red-brown with Bismark brown, For fixation of the volutin bodies the use of a picric acid mixture is satisfactory, and staining thereafter with a dilute haematoxylin. According to Meyer the most distinct staining is through a 1:10 solution of methylene blue, following which differentiation is accomplished by 1% sulfuric acid. By this method the cell contents are decolored while the volutin remains a dark blue. Previous fixing with osmic acid produces the same results. To bring the volutin into solution the living cell is crushed, while observing under the microscope, by a light pressure on the coverglass. The surrounding water, at ordinary temperature, is sufficient to dissolve the volutin. With the employment of concentrated nitrate of saltpeter, or a 10 or more percent sodium carbonate solution the crushing of the cell can be dispensed with as the reagents penetrate the cell and dissolve the volutin.

3.5.8. The Plasma Membrane

Examinations by Liebisch disclosed the existence of a special membrane located immediately inside the silica frustule. According to chemical reactions it consists of a pectin substance.

This inner membrane may be examined by first treating a cell (or cells) with chloral hydrate which ruptures the outer girdleband with its accompanying frustule(s). The cell is then killed by application of dilute hydrochloric acid, and then boiled for a short time (all being done on the microslide). After washing thoroughly, the membrane will stain an intensive blue with methylene blue, and orange-red with safranin. A strong red coloration of the membrane is given, by: the application of ruthenium-red. Haematoxylin and gentian-violet also may be employed in staining this feature.

All staining can be undertaken with complete cells, or one can, by the use of 5% to 40% hydrofluoric acid, dissolve away the siliceous frustule. This latter operation must be carried out in a paraffin dish as the acid attacks glass. After treatment the cell remains are thoroughly washed in distilled water, brought for a time into a ferric sulfate solution, and after repeated washing, into a solution of ferrous cyanide of potassium. Such steps, with the subsequent application of Prussian blue result in a blue staining of the membrane, while the plasma itself remains unstained in its natural color of yellowish green.

3.5.9. Permanent Preparations of Fixed and/or Stained Diatoms

According to Hustedt, the most suitable mountant for stained diatoms is Canada Balsam. It must be ascertained however, that neutral Canada balsam is used, as if it

is even slightly acid, the adherence of some of the staining dyes are often adversely affected.

The specimen material is treated in the same way as previously described; that is through various alcohol steps and from there to xylene and finally being transferred to Canada Balsam. To limit shrinkage of the cell contents the alcohol steps begin with very weak solutions and proceed in very gradual concentrations. In each succeeding step the material is allowed to remain slightly longer than in the previous step. It is essential that the last alcohol step be completely water-free, or with the addition of xylene, the preparation will become spoiled by a turbid, or cloudy appearance.

Now and then a disadvantage of mounting in balsam occurs. The individual cells may, more or less, change their positions under the coverglass, even at times as far as to the edge of the preparation. To prevent this from happening, the cells are fastened to the coverglass with the aid of a thin gelatin solution in the way previously described. The gelatin is allowed to just barely begin to dry without completely drying out. Then, with the diatoms lying in the gelatin on the coverglass in this way, hardening in alcohol is undertaken. The clarification of the gelatin layer is attained, as mentioned previously, by the insertion of the oil of cloves treatment between the alcohol and xylene steps.

In many cases it is desirable to later examine a mounted cell from the girdle view as well as the valve view. Karsten (1896) described the following way this might be accomplished.

The collected material of a catch, or the greater part of it is washed into a flat glass dish, and is treated with additions of a mercuric chloride solution until it becomes clear and antiseptic.

A small quantity of this mercuric chloride treated water is drawn up with a pipette and deposited on a microslide. A cover glass likewise made antiseptic by mercuric chloride is ringed around the edge with glycerine-gelatin and placed over the material on the slide, sealing it airtight. If the gelatin ring is appropriately thick enough, diatom cells in the fluid can be rotated by slight pressure on the cover glass with a needle. The gelatine ring is elastic enough to provide suitable play in movement which is transmitted to the fluid and thence to the diatoms. (The procedure is very similar to “crystal rolling” performed in examining microcrystals under the microscope in various viscous fluids.) After several days duration there will be evaporation of the fluid, gradually reducing the available range of movement. The preparation can be sealed with Canada Balsam to further delay the evaporation, but the mount will not be a satisfactory permanent one.

CHAPTER 4.

4. CLEANING THE FRUSTULES

4.1. Need for Cleaning

4.1.1. Introduction

As the classification and identification of diatoms is dependent upon the morphology of the frustule, they must be rendered as clean as possible for examination under the microscope. In order to resolve some of the minute structure and markings, the cell contents and all other parts, excepting the siliceous frustule, must be removed and/or destroyed. There is always the possibility that during cleaning, various constituents of diatom frustules may become loosened or corroded away. Therefore cleaning methods should be adapted to suit the particular material to be processed. The minimum or least drastic cleaning, consistent with the microscopical examination requirements, should always be used.

There is no best way of cleaning diatoms as the origin of the material may be recent or fossil, freshwater or marine, and contained within many different substrates or matrices. The material may, in some, cases, be of almost pure diatoms, and require little, if any, cleaning for the purpose of microscopical examination. On the other hand, the diatoms may be embedded in solid rock or intimately mixed with mud and other debris to such an extent that very lengthy and extreme methods of cleaning are required. Various methods and combinations of methods must be used, often by trial one after the other. Thus it is appropriate that a number of different cleaning methods be available to the diatom worker.

Of the various chemicals used in cleaning diatoms, each one has a specific purpose for which it is included in the process. If enough is known about the diatom sample material as to its constituency, or the absence of organic matter for instance, some chemicals need not be used and the process thereby shortened.

It is true that the siliceous frustules of diatoms can usually withstand very heroic treatment with concentrated acids. However, there are diatoms which are weakly silicified, especially those of marine origin, in which care must be taken not to damage or destroy the frustules during cleaning. Also, the use of alkalis in the cleaning of diatoms must be very carefully controlled, or severe corrosion or destruction will result. A few specific diatoms are of such delicate construction that they are very difficult to clean and mount permanently and very specialized procedures must be used to do so.

The purpose for which the diatoms are to be cleaned also has a bearing on the methods used. For temporary examination of diatoms in the living state cleaning may not, of course, be necessary. If the diatoms are numerous and very little, if any, organic matter is present; all that is necessary to obtain good to at least satisfactory slides, is by shaking in distilled water and drying on a coverglass.

If the ultimate use of the diatom is for the purpose of becoming part of a herbarium collection and a reference specimen, then it is appropriate that cleaning be of the best method possible. Cleaning an assemblage of diatoms such that ecological relationships among various genera and species is preserved, calls for a different method for instance, than if an individual diatom is to be selected and mounted for morphological studies.

The facilities for cleaning also play an important part in selecting a method. If the cleaning must be indoors with no provision for fume disposal, for instance, the use of hot, boiling, or fuming acids, is to be avoided.

In the following paragraphs various cleaning methods for diatom material are described to cover most, if not all, conditions that will be encountered by the investigator.

4.2. The Stages of Cleaning

The cleaning of diatoms can, in general, be divided into a few distinct stages or steps:

a. The removal, by mechanical means, of mud, debris and other waste.

b. The oxidation of organic matter and dissolving of as much mineral matter as possible with acids and other chemicals.

c. The breaking up and removal and/or freeing of diatoms from encrusting and insoluble residues by means of alkalis and mechanical processes.

4.2.1. Chemicals

Quite a wide variety of chemicals are used in the various methods of diatom cleaning. The following list, with comments relative to purpose, will provide some background and a basis for some choices. All chemicals may be of the cheapest commercial grades. Salts may be either of sodium or potassium according to availability. As a rule, sodium salts are more reasonable in price than potassium salts.

Nitric Acid (concentrated)

A strong oxidizing agent. Used to remove calcium and ether salts which might be precipitated or hardened by subsequent treatment with sulfuric acid, and to oxidize as much organic matter as possible. (Some organic substances, if treated directly with sulfuric acid will char, producing a quantity of carbon). The use of this acid should be avoided when cleaning weakly siliceous plankton specimens.

Hydrochloric Acid (dilute)

Used to rid diatomaceous samples of carbonates and/or iron compounds. Boiling for 50 minutes will usually disintegrate most calcareous material. If the diatom material is in limestones, or when particles of shells or foraminifera are present, this reagent is definitely called for. When carbonates are not present, boiling in hydrochloric acid is unnecessary.

Sulfuric Acid (concentrated)

A strong oxidizing agent, especially when hot and concentrated. Oxidizes organic matter present, including cellulose. When boiling, sulfuric acid liberates white fumes of anhydrous sulfuric acid. The latter being very poisonous, some sort of fume removal must be used. This acid, if concentrated, can be used cold to oxidize organic matter thus obviating the necessity for a fume hood, etc. However, the process is slow and may take several days. In some cases, the cold acid is not active enough to do a complete job.

Sodium or Potassium Chlorate

A bleaching agent. Its oxidizing action on certain colored compounds turns them colorless. This chemical is used in conjunction with sulfuric acid to completely oxidize and/or bleach organic material accompanying the diatoms. In some processes it is placed first (in crystal form) with the mass of material to be treated, and the sulfuric acid then added. Conversely some procedures call for adding the acid first and then crystals of the bleaching agent. Other chemicals employed for the same purpose in cleaning diatoms are sodium dichromate, sodium nitrate, potassium nitrate; potassium chlorate, potassium bichromate, potassium permanganate, and hydrogen peroxide. Which is used is dependent upon the originator of the process and his preferences. Many can be used interchangeably in different processes and not affect the results adversely. Sodium chlorate, potassium chlorate, and potassium nitrate are most often called for in cleaning procedures recommended by older authors. Potassium permanganate and hydrogen peroxide are used in some of the most modern methods.

Oxalic Acid (crystals)

Occasionally useful in solid form for the reduction of manganese oxide when potassium permanganate is used, and in strong solution for the removal of iron sometimes present in fossil material.

Sodium Acetate (super-saturated solution)

Preliminary to cleaning is used to break up fossil material containing diatoms that will not respond to other methods. Sodium thiosulfate works as well, and is more economical to use.

Sodium Carbonate (washing soda)

When material (fossil) will not break up by soaking in distilled water, boiling in this chemical frequently causes it to fall to a powder, This is used sparingly - a piece about the size of a pea for 6 oz. of liquid, or a tablespoon per quart of water. An alkali, it may damage diatom frustules, and as soon as the purpose of breaking up the mass is accomplished, complete washing is in order to eliminate it. The alkali should be as weak as possible, and the time of exposure to it short, only long enough to accomplish the result.

Potassium Permanganate (saturated solution)

An oxidizing agent used in a method to clean fresh diatom gatherings.

Hydrogen Peroxide (37% solution)

An oxidizing agent used in a method to clean fresh diatom gatherings.

Chlorox (Trade Name)

A household disinfectant formed by passing chlorine into dilute sodium hydroxide. The reaction products are sodium chloride, sodium hypochlorite and water. No effort is made to separate the NaCl. It is marketed as a mixture, the sodium hypochlorite and sodium chloride each constitutes about 5% of the mixture, the remainder being water. It can be used to remove small amounts of organic matter from fresh diatom gatherings. Lohman considers it easier to use, much more economical, and practically as effective as hydrogen peroxide for the purpose.

Ammonium Hydroxide (dilute solution)

Mud pellets may be broken up by dilute ammonium hydroxide and vigorous shaking at intervals for 24 hours or longer. Also, diatoms soaked in the dilute solution and shaken once or twice a day for a week will usually be freed from fine debris. Household ammonia is a satisfactory substitute.

Sodium Hexametaphosphate (5% solution)

This chemical and other soultions of soluble phosphates lower the surface tension of water. They are used to disperse a suspension and thus materially

aid in the removal of fine clay fractions. They hydrolyze in water to make basic solutions.

Phenolphthalein

An indicator, can be used to indicate changes from acid to base condition and vice versa. The acid condition is colorless, the base condition color is red. The pH at which the color changes is from 8 – 10.

Methyl Orange

An indicator to check acidity. Its acid color is red and base color yellow. The pH at which the color changes takes place is from 3 - 4.5.

Litmus

Acid color red and base color blue. The pH at which the color changes is from 6 - 8.

Heavy Liquids

Heavy liquids used in separating mineral fractions can be used in diatom work. For instance, mixtures of bromoform and absolute ethyl alcohol have been used.

Citric Acid

Has been used in breaking up clay materials containing diatoms. Used alternately with potassium acetate (boiling).

4.2.1.1. Precautions

Many of the chemicals used in cleaning diatoms, especially the acids and oxidizing agents, are extremely active, poisonous, and/or corrosive. Reactions in steps of some methods are violent, accompanied by much heat and copious fumes.

Therefore, it is essential that proper precautions be taken to protect against accident. All cleaning operations involving such dangerous materials should be carried out either in the open air, or with facilities for fume removal. Fumes released during some reactions are not only dangerous to man, but may be extremely injurious to microscopical equipment. Therefore cleaning, if possible, should be carried out remote from microscopical equipment or fume removal facilities utilized.

Glassware used should be heat resistant borosilicate types. When heating flasks containing acids and other corrosives it is better to use heating jackets, hot plates or

sand baths to distribute applied heat. Too localized heating can cause extreme "bumping" and ejection of highly dangerous fluids.

In the detailed cleaning instructions to follow, comments are included regarding violent reactions and types of apparatus and glassware recommended to be used.

4.2.2. Cleaning through Incineration

The incineration process of cleaning diatoms is doubtless more desirable than through acids and/or other chemicals. Its simplicity, and the obtaining of specimen material ready to mount in a very short time, is attractive in itself. It has the distinct advantage that colonial arrangements of diatoms are often preserved in their natural arrays, and that certain ecological relationships (genera and species) are undisturbed. Also, some diatoms can withstand the incineration treatment, but not a chemical one.

A disadvantage is that this method can only be used when the material is practically sediment-free. Also colonial arrangements of diatoms quite often exhibit one aspect or view of the frustule, to the exclusion of others. In addition, the method does not, as a general rule, clean the frustules of diatoms as well as chemical means, and their transparency therefore is not sufficient in many cases to reveal certain structural details, whether they are surface or interior ones.

The incineration method, then, is generally reserved for the following conditions:

1. Very little material is available, which might, through chemical cleaning methods, become further diminished or lost.
2. When very delicate forms are to be cleaned, especially plankton material that might be destroyed by acids.
3. If it is necessary that the colonial relationships of frustules be maintained.

The process is basically the application of a flame to a coverglass upon which the specimen material lies, ultimately completely destroying the organic material present and leaving, ideally, only diatom frustules.

It is essential that aside from the diatom frustules and organic material, there are no remaining salts accompanying them, or they, after incineration, will definitely affect the clarity of the preparation. Therefore, it is necessary to wash the specimen material in distilled water by repeated decantations prior to the incineration.

The material to be incinerated is mixed with distilled water and a drop applied to a coverglass. The amount of water should be proportioned to the amount of diatoms, such that after shaking in a test-tube or vial, there is a slightly turbid appearance. This will assure a plentiful spread of diatoms in the final mount.

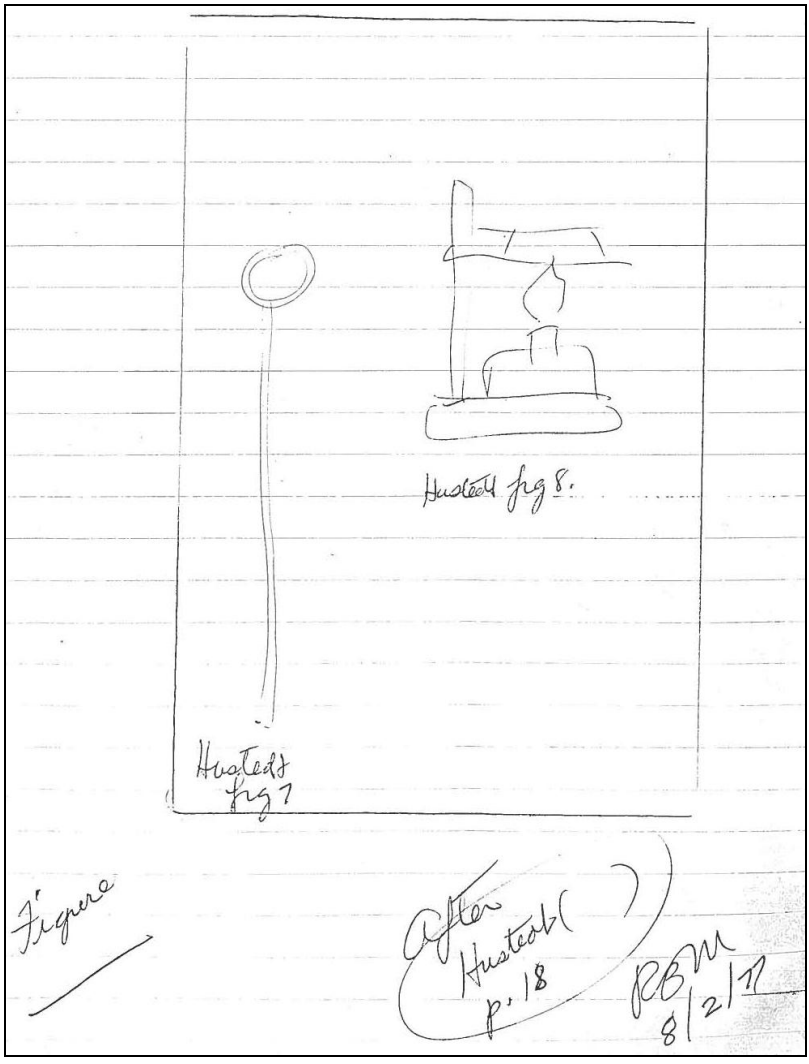
Coverglasses are preferably circular and as thin as possible. The former condition is preferable from a permanent slide standpoint, and the latter condition assures adequate incineration without an excessively high temperature flame source. In this type of mount, wherein possibly the clarity of the diatom frustules is not of the best,

coverglass thickness relating to the best use of microscopic objectives is of lesser importance. The coverglass should be, prior to use, as free from foreign matter as ordinary cleaning can make it.

The drop of fluid containing the diatom material is applied to the coverglass and the latter placed in a dust free place to dry undisturbed. Then the coverglass is placed on a thin platinum or silver sheet and heated to a red-heat. It is advisable not to choose too great a flame, but better to apply the heat for a longer time; burning to a gray ash. After cooling, the incineration is inspected with the microscope, and if necessary, the heating process repeated until the result is considered successful.

To hold the thin metal sheet that supports the coverglass during the incineration, a number of methods can be devised. Hustedt describes two. Reference is made to Figure 69. One of the methods he describes is that attributed to Elger. It consists of an iron plate (a) of about 12 mm. diameter and 12 mm. thick as a base which carries a vertical steel post (b) of 12 mm. diameter and approximately 18 mm. in height. A brass cylinder of about 2 mm. in height (c) is positioned and fastened in place by means of a set screw (d). The brass cylinder is fastened to a brass ring (e) of about an outer and inner diameter of 8 mm. and 6 mm. respectively and about 2 mm. thick. On this ring there are fastened six, 2 to 3 mm. high, brass pegs (f). The pegs are bent inwardly at their upper free ends and the ends are filed flat such that they all lie in a horizontal plane. A 0.5 mm. thick sheet of platinum supported on the pegs serves as a hot plate (g) to hold coverglasses. The heat source used is an alcohol lamp that sits on the iron base plate. The height of the assembly is adjusted such that the hot plate is struck by the full flame of the lamp. The plate can be completely occupied with coverglasses. The incineration takes approximately 20 minutes. Less contaminated material and delicate forms require correspondingly less time.

Dr. Albert Elger
Eutin in Holstein



Friedrich Hustedt: Die Kieselalgen Teil I (Figs. 85 & 86)

Figure 69.

A simple coverglass holder is illustrated in Figure 69. Simply a brass handle about 20 mm. in length fitted on one end with a loop or ring about 2 mm. in diameter, the coverglass is carried on a silver plate in a recessed edge of the ring. The disadvantages of this type of aid are that only one coverglass at any one time may be heated, and repeatable results are difficult to attain holding it by hand. Also, if the position of the carrier is not very carefully controlled, the coverglass or the metal plate may bend or melt. The previously described apparatus is much more satisfactory.

Very satisfactory incineration may be done by the aid of improvised holders available in the laboratory. Common ring stands are easily adapted for the purpose, for instance. High intensity heat can shorten the time for incineration, but care must be taken to avoid overheating as the coverglass being very thin, is easily warped or melted. Small propane torches are available that serve the purpose admirably.

4.3. Chemical Cleaning

There are two major methods of chemically cleaning diatomaceous material. One involves the use of boiling acids, and is the most widespread, and the other uses potassium permanganate, The latter is rather restricted in use but very advantageous under certain conditions. Both the acid and permanganate methods have numerous variations. Later in this section, a detailed procedure will be presented for each.

However, before any chemical cleaning of diatom frustules can take place, there are certain preliminary steps to be taken. These preliminaries primarily are aimed at separating, as much as possible, the diatom frustules from other matter so that chemical cleaning be most effective. These procedures include breaking down fossil material. There are separation methods that are specifically devised for separating diatoms from debris such as sand grains and other chemically insoluble material, and for grading diatoms into specific size ranges that apply after chemical cleaning has taken place. These procedures will be dealt with later. The separation methods considered here are preliminary to chemical treatment.

4.3.1. Separation of Material

4.3.1.1. Fresh Material

By fresh material is meant that which is not fossil, but has been collected from a recent environment. It may consist of diatoms mixed with such undesired items as sticks, stones, plant parts, mud and silt, shells, and sand grains. The material is separated from the larger sized debris, as much as possible by mechanical means. The “two-bottle” technique described earlier is effective, either in the field, or in the laboratory in eliminating much of the larger debris from the collection. Squeezing or beating plant parts such as leaves and stems is also a way in which most diatoms may be separated from that upon which they are epiphytic or on which they are living as a substrate.

In some cases where the diatoms may be epiphytic and it is suspected that they will not be easily freed or that their numbers are limited, plant material is cut up into small pieces and included in the chemical cleaning, ultimately becoming completely destroyed.

After the obvious separation by the aforementioned means, the use of sieves is resorted to. A series of sieves from 20 mesh through 300 to 400 mesh are very useful to the diatomist. At this preliminary stage the large mesh sieves are used to wash the diatoms through into other containers, retaining the larger debris on the

sieve, which is ultimately discarded. A portion of the mix is placed on a 20 mesh sieve for instance, and a stream of water from a wash bottle, or other source, used to thoroughly wash the diatoms off the debris. Several washings such as this may be resorted to, ensuring all of the diatoms being freed from larger pebbles or grains of sand, plant parts, etc. The material passed through will consist of diatoms and small-sized detritus. This sieving procedure may be carried out with increasingly smaller mesh sizes until it is judged that to go further would retain the diatoms on the sieve with the debris. For this preliminary sieving 20 or 30-mesh sieves will pass all but the largest diatoms usually encountered. Of course, as a control, the material retained on the mesh may be examined for very large forms if they are suspected.

The 30 mesh sieve will pass diatoms in excess of 500 micrometers in diameter. If some knowledge as to the maximum size of diatoms in the material is known, smaller sieves can be used. Dependent upon the type of sieve (its construction) the following diatom diameters will be retained on the following mesh sizes.

300 mesh	60-70 micrometers
150 mesh	120-140 micrometers
75 mesh	240-280 micrometers
30 mesh	600-700 micrometers
20 mesh	900-1050 micrometers

It will be noted that mesh sizes are the number per lineal inch, do not take into account the thickness of the mesh material (wire), or how it is constructed, and are for diameters of circular diatoms. Long, acicular forms may pass through or be retained depending upon their orientation with respect to the openings.

A technique, perhaps better than the use of a stream of water, especially applicable when the detritus is of comparatively small sizes, is to use the sieve immersed in water. The sieve, with the material to be separated, is suspended in a larger container of water, such that the water level is above the sieve material and its load, but not above the top of the sieve sides. Gentle to and fro motion will cause the enclosing water to wash the suspended material and diatoms and fine debris to fall through the mesh into the larger container.

However the sieving operation is accomplished, or to whatever degree, the result should be that all of the diatoms with the least foreign material should be retained for further treatment.

After sieving has separated the larger unwanted material out, the diatoms and smaller sand grains may be further separated by settling. The material is shaken up in a quantity of water and placed in a test-tube, beaker, or sedimentation flask, in accordance with the amount of material and/or inclination of the diatomist, and allowed to settle. Only the obvious larger and heavier material is allowed to settle in this step, and the supernatant, consisting of diatoms and very fine debris, decanted for further processing, Too long a settling at this stage may well cause the loss of larger forms. The separation of very fine debris is better delayed until chemical cleaning is completed.

4.3.1.2. Fossil Material

Fossil diatomaceous material may occur in either marine or freshwater deposits and be contained in a soft friable "earthy" to hard, rocky matrix. In general, this material requires preliminary breaking up prior to chemical cleaning. Since the range of hardness of fossil material is great, it may, in some cases, be broken up by gentle pressure of the fingers, or it may be necessary to use a much more mechanical force. In any event the fossil material should be broken up into pieces of approximately a centimeter in the largest dimension. If very hard, this may be done with a hammer and sharp chisel with as light blows as possible, or with cutting pliers or pincers. As a first trial, especially if the material is fairly friable, pieces are placed in a quantity of water, shaken, and allowed to soak for 24 hours. If it does not in this time fall to a powder, harsher treatment is called for.

The cut-up or broken pieces should then be placed in the bottom of an Erlenmeyer flask. Enough to cover the bottom of the flask is sufficient. Small amounts of material are more successfully treated than large quantities. Flasks of 150, 250, or 500 ml. capacity are usually satisfactory. Distilled water, equivalent to several volumes of the solid material, is added and brought to a boil. With much fossil material boiling in water will be sufficient for it to disintegrate into a sludge.

An Erlenmeyer Flask is commonly known as a Conical Flask.

If boiling in water is insufficient then boiling in dilute alkali may be tried. Boil the material in a dilute solution of washing soda (sodium carbonate). No exact strength is required, but it should be very dilute. Start with a tablespoon of sodium carbonate to a quart of water. As the material breaks up and the solution becomes milky, decant this milky solution into a large volume of water from the unbroken pieces and continue boiling with fresh washing soda solution. This will remove the disintegrated material from longer exposure and boiling. As this material settles in the water, the surplus water should be decanted. Wash the accumulated disintegrated material by repeated decanting until free from alkali. Prolonged exposure to alkali, even weak solutions can be damaging to the diatom frustules. Therefore, this operation should be as short as possible, and the material thoroughly washed immediately after breakup is complete.

Some fossil material will not break up with boiling in alkali. In such cases an attempt to break up the material by the use of crystallizing salts is in order.

After washing alkali from the material, allow it to dry thoroughly. Then prepare a super-saturated solution of either sodium acetate or sodium thiosulfate ("hypo"). Prepare by boiling the crystals with about 5% of the quantity of distilled water until a residue of crystals remains. The diatom material is, with the saturated salt solution, heated (if necessary) to about 60°C., providing penetration of the salt into specimen material. On cooling, the salt solution crystallizes and breaks up the lumps. After cooling to room temperature, if the material is placed in a refrigerator overnight the breaking up may be more complete. This process of boiling, cooling, and freezing, may have to be repeated several times. When it is broken up as much as possible, the sodium acetate is washed out with repeated decantations and the material is then returned to boiling in alkali as necessary. Depending upon the amount of material

being treated, the crystallization process may be carried out in a sturdy metal pan with sloping sides, a porcelain evaporating dish, or similar container. The important factor here is that fragile containers may be subject to breakage during the alternate liquid-solid phases.

4.3.2. Acid Treatment

4.3.2.1. Fume-Disposal

Many of the processes produce copious and very corrosive fumes. The simplest method of handling them is to do all such associated processes in the open air, staying well away from the fumes.

However, this is not usually possible for the average diatom worker. Performing such tasks on the hearth of a fireplace, where there is a strong chimney draft, is another way to dispose of such problems, but that is also not usually an available possibility, Laboratory workers may have access to proper fume hoods, which solves the problem admirably. Most diatom workers, even those with access to modern laboratories, do not have ready access to fume hoods, mainly because the size of their operation and time demand is small. Many methods have been described that allow acid treatments, with their accompanying fumes, to be carried out in enclosed spaces with minimum possibility of danger to man and equipment. Some of them will be described in the following paragraphs.

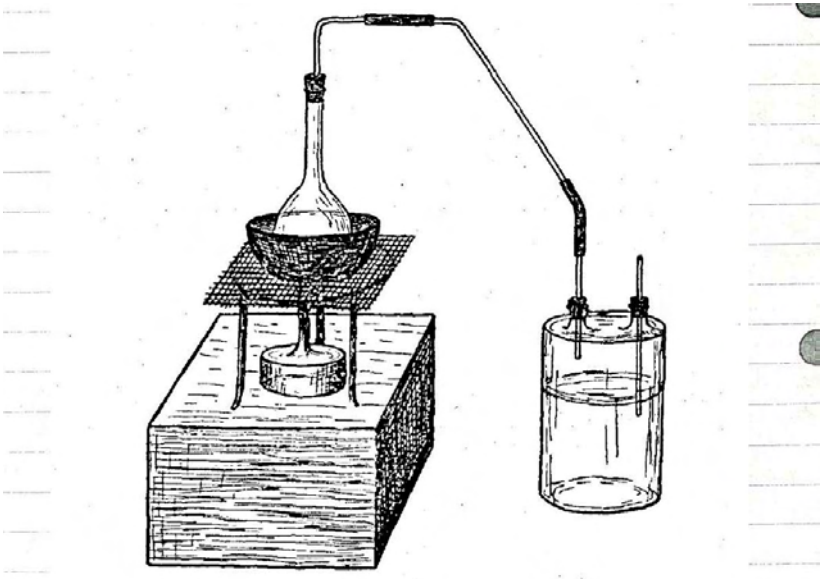


Fig. 9. Vorrichtung zum Kochen von Diatomeenmaterial in Säuren.

Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 9)

Figure 70.

Hustedt describes a system (attributed to Kolbe) whereby the reaction flask is part or an enclosed system. Referring to Figure 70 the flask is heated in a sand bath supported on a tripod; The flask is stopper equipped with glass tubing terminating in a large bottle of water. Note that the glass tubing terminates in air and the fumes expelled from the reaction flask cannot escape the wash-bottle excepting through diffusion into the water. This system is an enclosed passive one, and with prolonged boiling of material in the flask, the pressure in both the containers may equalize and eventually decrease its effectiveness.

A modern positive version of this old system is more effective. Its action is positive in that fumes are removed from the reaction flask by aspiration. Refer to Figure 70. The diatom material with the corrosive liquid (acids, etc.) is placed in a Kjeldahl flask. The latter is a pear-shaped flask with a long neck useful in acid digestions. It is supported by a ring stand or other appropriate support, preferably with a heating

mantle or in a sand bath. The setup as far as the receiving bottle is very similar to that in Figure 71. Note however, that the glass tubing in this case, terminates submerged in the receiving bottle. A second tube is connected through glass tubing and rubber hose connections to a Richards filter pump. The fumes are aspirated into the receiving bottle and dissolved into the contained water by action of the Richards pump and a water tap. The water in the receiving bottle is a sodium carbonate solution with phenolphthalein indicator added. The solution will neutralize acid fumes dissolved and the indicator will show when the soda solution should be renewed, by a color change. The Richards pump is brass and, to protect it against the acid fumes, the receiving bottle with its neutralizer is necessary. All rubber connections exposed to fumes should be as short as possible. No definite strength for the soda solution is required. About a handful in a gallon bottle half full of water will be sufficient.

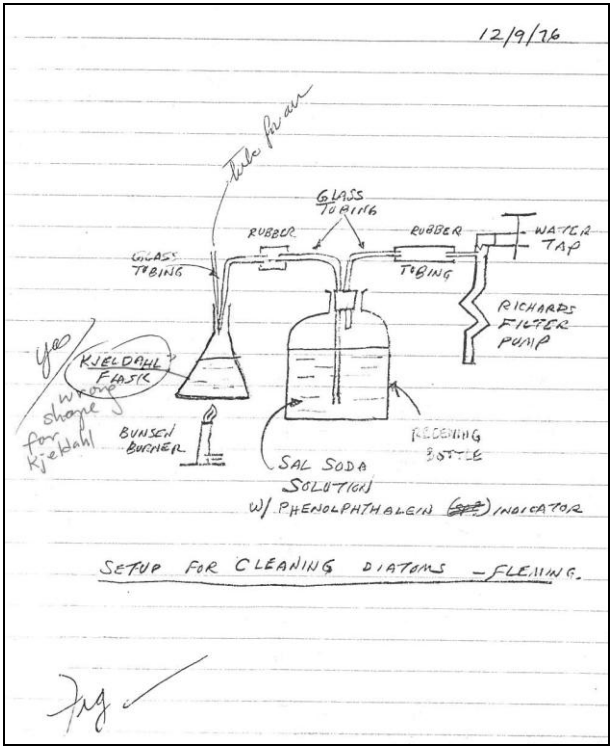
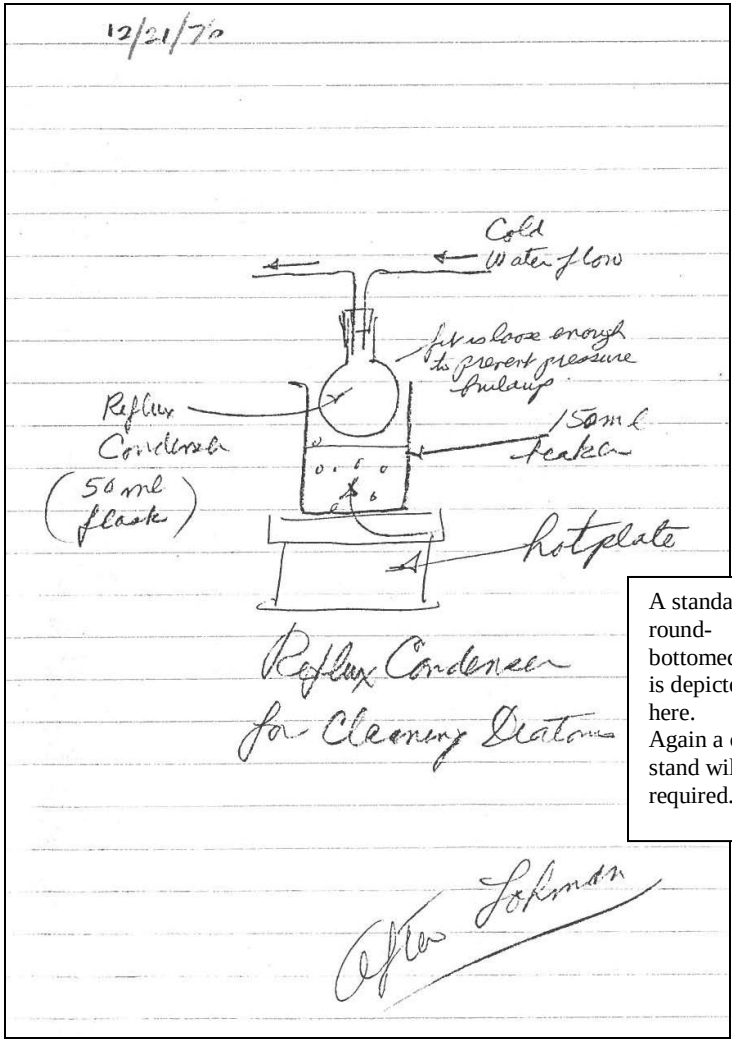


Figure 71.

A Kjeldahl Flask is usually pear-shaped. That depicted in this illustration is commonly known as a Conical Flask. The main advantage of the Kjeldahl Flask in diatom cleaning is that there are no awkward corners for material to get stuck in. The disadvantage is that it doesn't sit on a tripod stand and gauze all that easily. You will need to use a clamp stand.

The apparatus can be simplified by the use of a polyethylene filter pump for use with corrosive vapors. The receiving bottle with its neutralizer could then be eliminated and direct connection with the pump (and water tap) made. However, the Richards pump is more powerful and positive in its action, and generally more useful. Therefore, the receiving bottle is a small price to pay for the added effectiveness.



A standard round-bottomed flask is depicted here. Again a clamp-stand will be required.

Figure 72.

Alternatively you may wish to use a reflux condenser that is made for the purpose. They are cheap and readily available.

The use of a reflux condenser has some advantages. Refer to Figure 72. Cold water is passed through a round bottomed flask with appropriate fittings. The flask is selected to be of a size to just fit close enough within the top of a beaker to prevent pressure buildup. The steam and acid vapors from the boiling material in the beaker condense on the cold surface of the flask instead of escaping into the surrounding air. Lohman recommends a 150 ml. beaker and a 50 ml. borosilicate extraction flask fitted with a 2-hole no. 6 rubber stopper. The flask may be supported with a ring stand and a burette clamp. No fume hood is required, and the acid will not boil away, as it is returned as a condensate to the original solution. As has been mentioned before, small amounts of material are more successfully treated and with this method of fume disposal, that is particularly true.

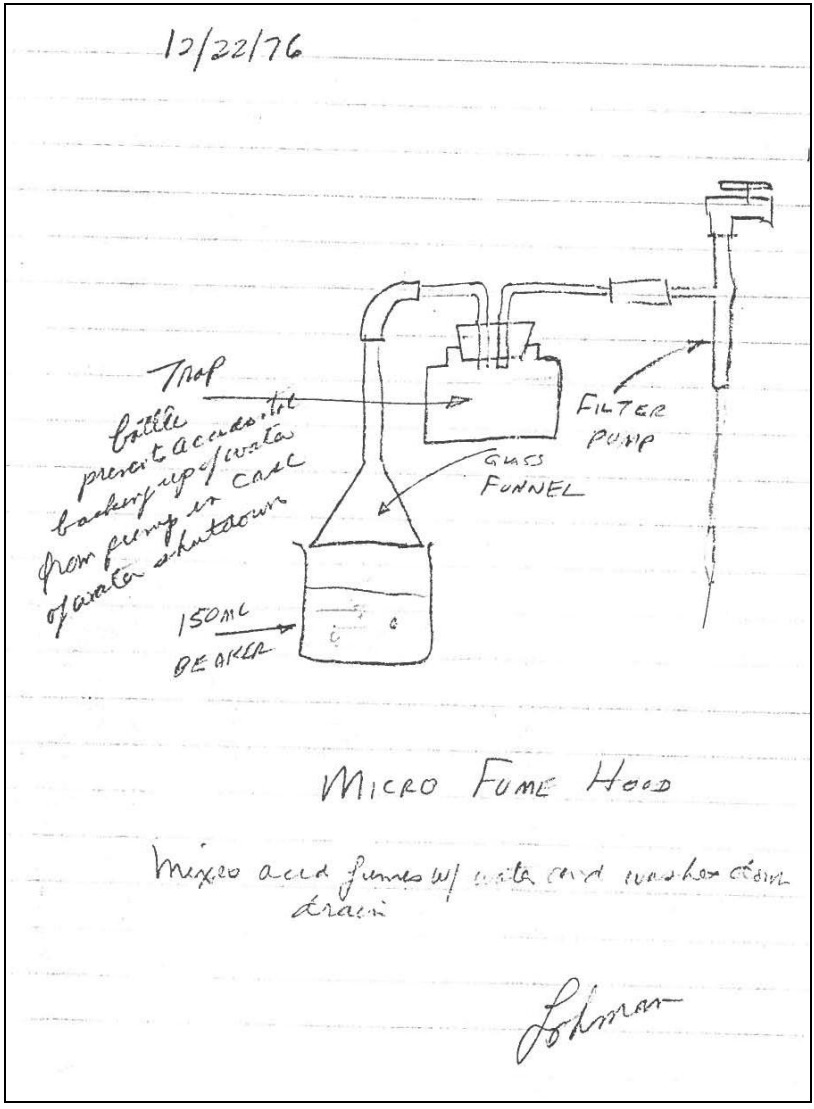


Figure 73.

Hendey describes a very neat and compact apparatus which allows additions of chemicals and extractions of fluids during cleaning operations, and which disposes of fumes. It is based on the use of a water pump and the ingenious use of standard laboratory apparatus. His apparatus designations are those of British suppliers but similar setups with apparatus obtainable through other suppliers may be devised. The primary advantage of his system is that during acid treatment the reaction flask is provided access for the addition of acid, water and bleaching chemicals without disconnection of any apparatus and with continuous fume disposal.

4.3.2.2. Treatment with acids is variously accomplished by many diatom workers. In the following paragraphs several of the methods successfully used will be described. Col. Wm. D. Fleming, the inventor of the diatom mountant Naphrax, and an experienced worker with diatoms, uses the following method:

4.3.2.2.1. Preliminary Treatment

With recent material if there is much water, filter it through the smallest filter paper convenient and drain dry. Then treat the material and filter paper together.

In an Erlenmeyer flask of proper size, cover the material with an equal volume of concentrated nitric acid and bring to a boil. Fumes will be generated and precautions should be taken to handle them without danger by performing this action in the open or by the use of proper apparatus to confine them.

Allow the material to cool and stand overnight. Dilute with water, allow to settle and decant the supernatant fluid, discarding it. Repeat this washing several times. The object of the nitric acid treatment is to remove calcium or other salts which might be precipitated or hardened by subsequent treatment with sulfuric acid.

4.3.2.2.2. Recent Material; freshwater or marine

Drain water from the above washing as completely as possible. This can best be done by suction with a filter pump and a glass tube drawn to a moderate point. This drainage by suction applies to all subsequent processes.

With the material in a generous sized flask (the Kjeldahl type in Figure 74) of 500 ml. capacity, add an equal amount of sodium dichromate. The material will include the debris from the filter paper if such was used. Then add, cautiously, concentrated sulfuric acid to at least equal the combined bulk of material and dichromate. When effervescence has died down, bring to a boil, boil gently for a few minutes and allow to cool completely. When completely cool, pour slowly into a larger volume of water and allow to settle (several hours or overnight or use a centrifuge). Decant the upper water and remove the rest of the water by suction as completely as possible. Repeat this washing and draining until the washings are no longer acid. In this and all other processes, tap water may be used for all washing except the final one.

When washing is complete, examine a sample under the microscope. If all extraneous material has been destroyed except sand and other siliceous matter, keep wet until final cleaning by sieve as below. However, if destruction of foreign matter such as algae or other organic matter is not complete then continue on with a more severe acid treatment as follows.

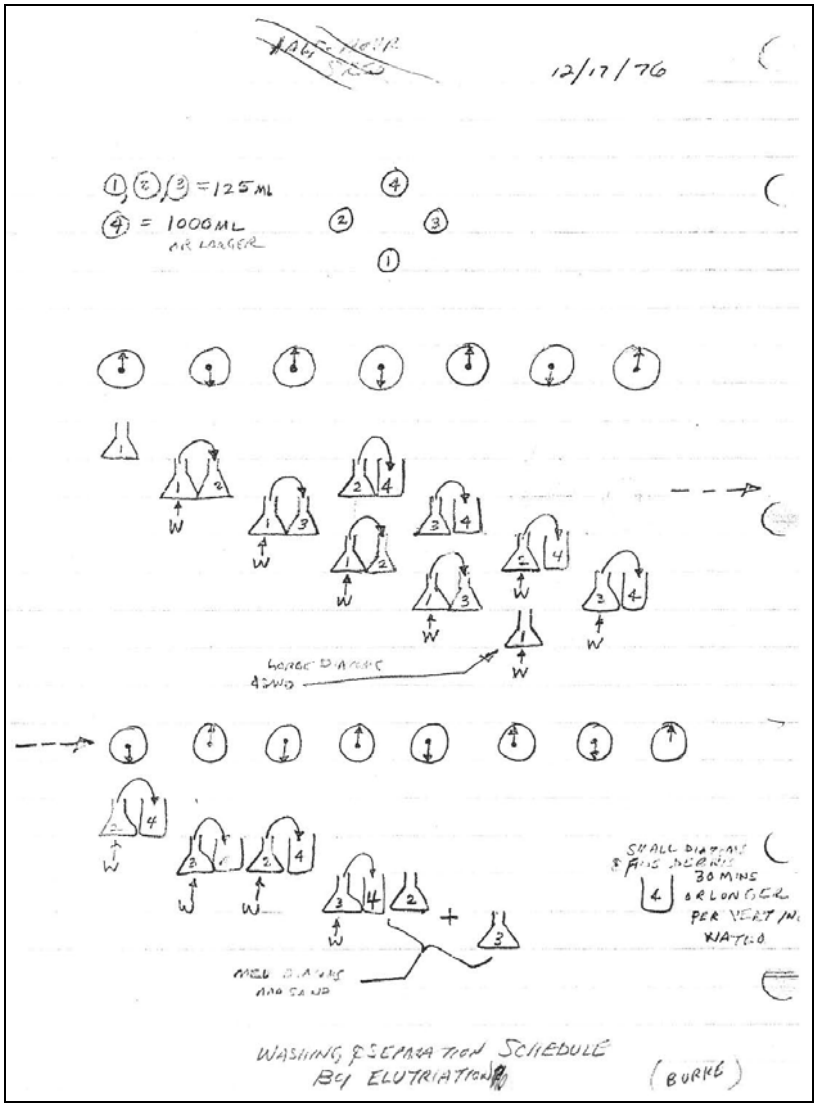


Figure 74.

4.3.2.2.3. Fossil Material

Transfer the washed material to the Kjeldahl flask and remove as much water as possible by suction. Add concentrated sulfuric acid slowly and cautiously until the flask is about half-full. Heat the flask slowly and boil until copious white fumes arise. The boiling is best done with the flask inclined about 50 degrees. Set the flask upright and allow it to cool slightly. Drop a very few crystals at a time, of sodium chlorate, down the neck of the flask, keeping up the suction of air by the Richards pump. Bring to a boil after each addition. Continue adding chlorate until all dark color disappears and contents are white or pale yellow. If the dark color persists try adding sodium nitrate, a few grains at a time, in the same manner as the sodium chlorate was added. Finally, cool thoroughly and pour contents slowly into a large

volume of cold water. To the material remaining in the flask add cold water very cautiously and slowly until sputtering ceases and then in larger amounts, rinsing into the water with the rest of the material. Wash by decantations until neutral.

A sieving step in the diatom cleaning process serves two purposes. It grades the diatoms into two sizes; those that pass through the mesh, and those that are retained. But, much more important, it removes the larger diatoms from the fine silt and other particles that remain from the acid treatment. Larger detritus, such as grains of sand, can be pushed aside by a picking fiber, but if the fine detritus is allowed to dry on the diatom frustules it is very difficult to remove. Therefore, a 325 mesh sieve which has openings of approximately 44 micrometers is used in this step.

First, it must be certain that the material is washed free of all acid. For safety, add a little sal soda to the final wash, Then decant the material slowly onto the sieve suspended in a funnel over a large bottle. Rinse the remaining material onto the sieve. Then wash all of the material on the sieve with a fine jet of water from a wash bottle, continuing until examination of a sample with the microscope shows the diatoms are entirely clean. Then, unless there is so much large foreign matter as to require removal by other means, the material is washed-off the sieve into a vial, using distilled water. The water is decanted after the diatoms have settled in the vial and replaced by either alcohol or acetone for preservation.

The material passing the sieve remains for purifying. This can be done by repeated settling and decanting, preferably in a tall vessel with straight sides. The time of the settling before each decantation is determined by examination of the sediment and supernatant with a microscope. The goal is to have the small diatoms settle while the contaminants remain in suspension. Since the diatoms always carry down detritus, this becomes a somewhat tedious, but necessary process.

If there is much large sand or other detritus remaining after the sieving it may be at least partially removed by “troughing”. (Figure 75)

For this, a piece of plastic, such as lucite, which can be softened by heat, and about 6 inches by 24 inches by $^{1}/_{4}$ inch, is softened in an oven at about 200°F and formed into a trough by bending over a wooden mandrel and then cooled by a spray of cold water. This is fastened, with the assistance of ring stands and clamps, at a slope of about 30 degrees to the horizontal. The lower end is positioned above a beaker or other suitable receptacle. The suspension of diatoms and sand or other insoluble detritus from the sieving, is poured slowly down the trough. The diatoms tend to swirl into the water and be carried all the way down the trough and into the receptacle, while the sand and other large detritus tend to fall to the bottom and remain in the trough. However, some large diatoms always tend to sink with the detritus, so material remaining on the trough should be examined microscopically before discarding. As before, the water with the treated diatoms should be replaced with alcohol or acetone for preservation.

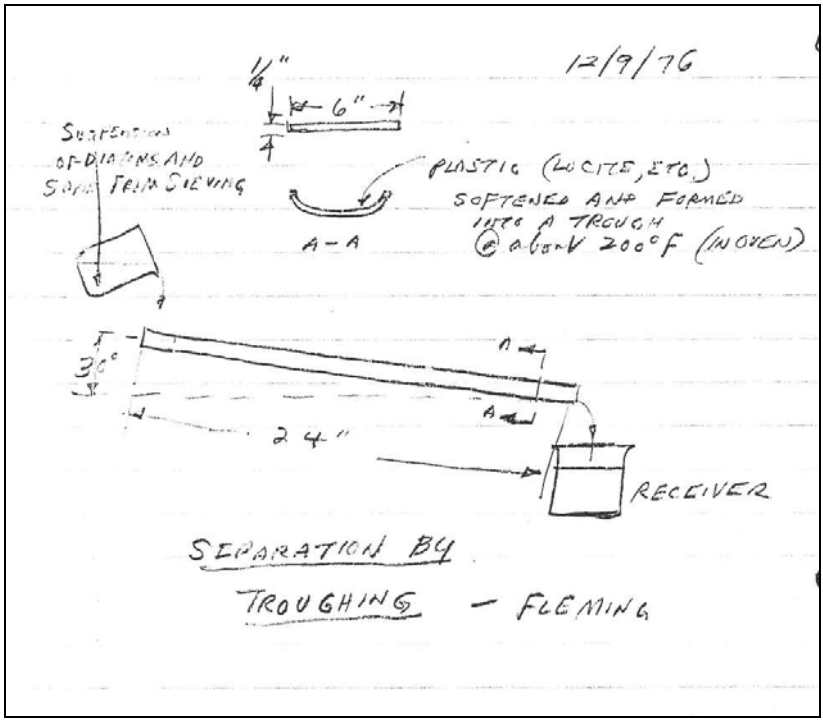


Figure 75.

4.3.3. Procedure for the Permanganate Method

The cleaning of diatoms by the use of potassium permanganate is not new, having been described by Swatman. In recent years this method has been adopted by a number of workers. It is a method as effective as any other for cleaning freshly gathered diatoms and avoids the use of boiling acids. However, it should not be used to clean very weakly silicified diatoms as are found in marine plankton.

Cecil Charles Swatman (1884 – 1958)

The following method is that recommended by Hendey:

Equipment and Apparatus Required

100 ml. Erlenmeyer flasks
100 ml. beakers
9 - 10 mm. diameter Petri dishes
Small nylon sieve about 8 mm. dia. having a 1.0 to 1.5 mm. mesh.
152 x 19 mm. test-tubes, with lip
Test-tube rack
Glass rods
Rubber-bulb pipettes with a 25 ml. reservoir and the lower part about 12 mm. in length.
Hydrochloric acid (dilute) (one vol. to 2 vols. of water).

Sulfuric acid (dilute) (one vol. to 2 vols. of water).
Oxalic acid, saturated solution.
Strong solution of ammonia.
Potassium permanganate, saturated solution (1 part in 18 parts distilled water).
Wash bottle.
Distilled water.

The first step, as in the other cleaning methods, is to get rid of as much as possible foreign matter and detritus by sieving, decantation, etc.

To the separated material, add 10 ml. of dilute hydrochloric acid. If it effervesces, continue adding it until it ceases, and pour off the acidic water. Wash the residue with distilled water by repeated decantations. After the final washing, the volume should be about 10 milliliters.

Next add 2 ml. of dilute sulfuric acid, followed by 10-15 ml. of potassium permanganate solution, the total volume now being about 25 to 30 ml. of liquid with the included diatom material. Allow this to stand overnight. During some part of the standing time the flask should be agitated from time to time. If the color of the fluid shows a tendency to turn brown, add more potassium permanganate until a purple color persists.

After standing overnight the precipitate of manganese oxide and the excess potassium permanganate is removed by adding 10 ml. of oxalic acid solution. Gently warm the bottom of the flask by holding it between the hands until a faint effervescence is noted. When the reaction has commenced the flask should be stood (as a precaution) in the lid of a Petri dish, and further quantities of oxalic acid solution added until all color disappears. (Caution: overheating of the flask will cause a very vigorous reaction and spilling over).

Allow the flask to stand 10–12 hours in an inclined position to let the diatoms collect at the lowest point. The supernatant is decanted, except for about 20 ml. The remaining 20 ml. or so, containing diatoms, is placed in test-tube and the level made up to about $^{2}/_{3}$ capacity with distilled water. These are allowed to stand for an additional 6 hours. The supernatant is drawn off with a pipette and the residue washed by repeated settling and decantation for two days at four hourly intervals.

To prevent clumping of the diatoms, 1-2 ml. of strong ammonia is added to the final washing and vigorously shaken. If the liquid develops a faint orange or brown color, presence of iron is indicated. Remove the iron by adding a little hydrochloric acid, and the iron hydroxide formed will pass into solution giving a pale yellow color. Continue washing with distilled water until all color is dispelled. The diatoms are now ready for storage or mounting.

The advantages of the permanganate method over the acid method are fairly obvious. There is no boiling of concentrated acids with accompanying poisonous and corrosive fumes, thus allowing operations to be carried out indoors without special fume disposal apparatus. Furthermore, the procedure may be broken off at any time for a week or more with no ill effects, or loss or damage, to the specimens.

It is especially advantageous when a large number of samples must be cleaned as each phase of the procedure is of short duration {only the intervals between them being long).

If the sample is a small one, free from vegetable matter and calcareous material, the time for cleaning can be considerably shortened. Instead of about three days, a few hours only may be required. For instance, the permanganate/oxalic acid treatment can be accomplished in two to three hours, and the washing could be by centrifuge, thereby obtaining a cleaned sample rather quickly.

4.4. Other Cleaning Methods

There are literally dozens of methods for cleaning diatoms described in the literature. Most of them are variations of the boiling acid treatment. However, among the many described are some that have been devised to solve special problems involving the matrix in which the diatom material occurs, or to protect against the delicacy or scarcity of the diatoms themselves. A selected group of these methods is included in the following.

4.4.1. Mud Gatherings

This is a particularly vexing problem with some diatom gatherings and deserves some special attention. The diatom content of such gatherings may run as low as 1/1000 or less and the efficient separation of the diatom frustules from the mud and other undesired debris is mandatory.

The first step is to break down the stiff mud into a thin sludge and get rid of twigs, leaves, worms, snails, etc. The use of a 20 mesh sieve in this step is of great assistance.

Next, get rid of as much clay and fine sand as possible. This may be done by placing the material in a shallow dish with water, working it into a suspension. Then allow the material to settle to the bottom. Gently rock the dish to and fro to bring the diatoms into suspension, but leaving the mud and debris on the bottom. Pour off the supernatant fluid with the diatoms. This is repeated as necessary.

Another way to accomplish this primary separation is to use a 200 to 300 mesh sieve and a large basin of water. The sludge is squirted on the tilted sieve. Larger diatoms will be retained on the sieve for further treatment. That material is placed in a bottle or jar and agitated with water, allowing coarse sand to settle after each agitation and the supernatant poured off. This is repeated several times.

After this preliminary separation the material obtained is primarily diatoms, animal and plant debris, some sand and clay, mud pellets, occasionally mica, and some carbon.

Proceed at this point with chemical cleaning by treating the material with strong nitric acid (fumes!) until red fumes are no longer evolved. Dilute and allow to settle, pouring off the dark liquid which contains much organic matter in solution. After washing by multiple settling and decantation, all water is drawn off and the residue

further treated by a strong oxidizing agent such as concentrated sulfuric acid, chromic acid, or potassium permanganate, until all organic matter is destroyed. Wash free from acids or other reagents by decantation.

It will be noted that the primary difference between cleaning mud gatherings and others, is the extra care in primary mechanical separation and the use of nitric acid. Mud gatherings are more liable to contain materials that would subsequently be hardened or precipitated by sulfuric acid, than other types of gatherings.

If mud pellets persist after the preliminary separation steps, they may be broken up by the use of dilute ammonia. It is added to the suspension and shaken vigorously at intervals for 24 hours or longer, the fine material being sieved away. This of course, should be done before proceeding with chemical cleaning.

If mica and carbon is present to a great extent it can be gotten rid of by fusing the material in a silica crucible with potassium bisulfate for about 10-15 minutes at a full red heat. The carbon is gradually burned away and the mica is decomposed. The diatoms are not ordinarily injured.

4.4.2. Cleaning Very Delicate Forms

There are certain types of gatherings which contain diatoms of a very delicate nature. Care needs to be exercised in those cases to prevent partial or complete destruction of the frustules. *Rhizosolenia lauderia* for instance and a number of other marine planktonic forms may be harmed if treated in the normal acid way. A gentle method of cleaning marine forms described by Cassie and attributed to van der Werff (1955) is as follows:

Una Vivienne Cassie
(Cassie-Dellow)
New Zealand

First the material is centrifuged or otherwise separated and washed in distilled water several times.

It is then placed in a porcelain evaporating dish and flooded with 37% hydrogen peroxide and covered with a glass plate. After standing for five minutes, 1 milligram of powdered potassium dichromate, or potassium permanganate, is carefully added. After a few minutes bubbles are formed and then a more vigorous reaction takes place, the temperature rising to about 80°C. The gases emitted are not dangerous and no heating is necessary. If any precipitate (of hydrated manganese dioxide) after the manganese reaction occurs, it can be dissolved by adding some 10% acetic acid solution.

Richard B. Hoover
b. 3rd January 1943
A NASA scientist who inventoried the Henri van Huerck collection and also claims to have found fossilized micro-organisms in meteorites.

The material is allowed to settle, is washed with distilled water and repeated decantations, and then examined on a coverglass.

In the case where extremely small amounts of diatoms are available and great care must be exercised to prevent loss, a method used by Richard B. Hoover is of interest:

The diatoms are placed in a concavity microslide and 37% hydrogen peroxide is dropped on them while observing with the microscope. The material is then heated very carefully and slowly and transferred to the microscope stage for observation. This procedure is continued until the diatoms are clean. It involves no poisonous or corrosive fumes and can be a very gentle procedure suitable for small amounts of diatoms. However, only diatoms already comparatively clean are susceptible to this treatment. Very fresh diatoms in small quantity or museum exsiccati (dry) collection specimens needing additional cleaning before mounting, are considered appropriate for the method.

Paul Sydney Conger
(1897 – 1979)
Botanist

Some diatoms have been found to be so delicate that they challenge the ingenuity of the diatomist in obtaining a permanent preparation. For instance, Conger (1964) describes *Achnanthes subhyalina* Conger, an extremely delicate marine pennate. The frustules are exceedingly delicate and gossamer-like not at all amenable to conventional microscopical preparation methods. The diatom disappears in strong acid but can withstand dilute hydrochloric and sulfuric acids. It is very slightly silicified and destroyed by incineration.

Thallassiophysa rhipides Conger; Gen. et sp. nov. was described and published in Smithsonian Misc. Collections vol. 122 no. 1415, July 1954. Dr. Conger published the original description and drawing from temporary water mounts, as all attempts at other mounting had caused it to collapse. Col. Wm. D. Fleming subsequently devised a method of mounting this diatom which has been published by me in *Microscopy* vol. 33, part 3. His method is as follows:

1. The collection, in the original sea-water, is placed in a small upright narrow tube of about 5 x 75 mm. allowing the diatoms to settle to the bottom in each addition, and decanting the supernatant fluid until the entire collection is at the bottom of the tube.

2. Approximately 20 mm. of the supernatant sea-water is pipetted off and replaced with distilled water carefully, to avoid any mixing.

3. After allowing this to stand (vertically) for one week until diffusion has mixed the two, the process of pipetting off the supernatant water is repeated at weekly intervals for two months.

4. Next, assuming most of the salt to be removed by process, a small amount (5 to 10 mm.) of the top fluid is removed. This is very carefully replaced with acetone, allowing it to flow down the sides of the tube to avoid mixing.

5. After allowing this to stand for one week, another small amount (5 to 10 mm.) of the supernatant fluid is again pipetted off and replaced with acetone. This procedure is repeated every week for approximately two months. After

this, larger and larger amounts are pipetted off the top fluid until the tube contains practically 100% acetone mixed with diatoms.

6. A weak solution of Naphrax in acetone (Naphrax is soluble in acetone) is added at a rate of one or two drops every two or three days, allowing it to diffuse down gradually, and pipetting off the top to keep the tube approximately three-quarters full.

7. From this step on, the tube is kept stoppered, but covered with a paper cap to allow evaporation to aid the concentration. During the addition of the Naphrax in acetone, the tube is agitated to bring the diatoms into suspension.

8. Finally, when the fluid in the tube shows considerable viscosity, a drop of the suspension is placed on a coverglass and immediately covered with a watch glass-and allowed to dry down. When a drop of regular Naphrax solution for mounting is added, the cover is picked up with a microslide and inverted, and allowed to harden. The strew is then finished in the normal manner.

The process is lengthy, taking about one year. However, it requires only a few minutes attention at any one time, and has resulted in an extreme delicate diatom being permanently mounted in a high refractive index medium for examination and study. Other diatoms too fragile to withstand conventional methods of preparation should respond to the treatment as well.

4.5. Notes and Techniques

The procedures for cleaning described are, of course, subject to individual modifications in regard to equipment and apparatus used, duration of specific treatments, types of chemicals, and the condition, or types, of material treated. In the following paragraphs some brief comments are supplied which apply, in some cases, to all methods of cleaning, and in other cases will provide helpful information on technique.

Sometimes, instead of boiling in concentrated sulfuric acid, cover the material with cold concentrated acid and let stand a day or so, giving it a whirl at times. Then dilute and examine. With some material, this treatment will suffice, and thereby avoid the fume disposal problem.

When diatom samples (especially some fossil gatherings) contain no organic matter, the usual treatments with nitric and sulfuric acids may not be necessary.

While some methods admit the use of tap water, it must be used with caution. Carbonates and other contaminating matter may prove to be a problem with the use of some tap water. Use distilled or de-ionized water, when in doubt, in all cleaning operations.

A saturated solution of potassium permanganate (5 to 10 ml.) and dilute sulfuric acid ($^1/_2$ ml.) will often times be sufficient to clean even quite large amounts of organic material, according to Lohman. The resulting black residue of manganese dioxide

can be cleared (removed) by one or two ml. of sulfurous acid, or a small amount of powdered sodium bisulfate.

For diatom sample material that contains no calcium carbonate, boiling in hydrochloric acid is unnecessary.

Preliminary soaking of material in distilled water helps in breaking down masses, lumps, and hard pieces.

An ultrasonic cleaner may be used in breaking up material. It should be used sparingly for short periods only, and frequent examination of the material made with the microscope. If used for any length of time, large centric diatoms especially, may be broken up.

A mechanical stirrer with a coated magnet is useful in breaking up and dispersing rather soft material.

Keeping the material being cleaned under liquids almost continuously is an excellent way to limit contamination, as dry material can be transported for considerable distances by air currents. Many cases of questionable occurrences of diatoms can be traced to such contamination according to Lohman. Methods used for maintaining sterile cultures should be employed in handling diatoms to prevent contamination.

In cleaning fossil diatoms embedded in limestone clean first with hydrochloric acid, and if organic matter is present, follow with boiling in nitric acid, and then with sulfuric acid if cellulose is present. If only hydrochloric acid is required, then addition of potassium dichromate is not necessary. If diatoms are embedded in clay, clean in 37% hydrogen peroxide. This will usually break the clay up into a more homogeneous mixture.

In all marine gatherings the salt should be washed out first before proceeding with the cleaning. A centrifuge, or a method of repeated decantations may be used in the washing process, although the latter is preferable.

The use of a centrifuge for washing and cleaning of diatom material is a valuable time saver. However, a centrifuge sometimes fails to remove traces of acids and other reaction products that penetrate minute cavities of diatoms, according to Hendey. The speed of washing is not as important as the time allowed for acids to diffuse out from the valve structure. Remaining acids sometimes leach out in the final mount, reacting with the mountant and forming precipitates, or otherwise interfering with the resolution obtainable. This is particularly true of synthetic mountants which are often very intolerant of the presence of water or other chemicals.

Diatomite has a great capacity for absorbing and retaining various substances. Persulfate of iron is one of them. The use of a caustic soda solution in such cases will precipitate flocculent iron hydrate.

A brown precipitate of manganese oxide in some potassium permanganate treatments may be reduced to a colorless solution by the addition of oxalic acid in solid form.

During a multiple decantation washing process it is an advantage to examine the suspension by taking samples with a pipette from about three levels and examining with the microscope. This allows the process of sedimentation to be checked.

Sodium hydroxide should be avoided in treating very small or delicately structured diatoms. Although perhaps cleaner preparations result, the sculpture and markings may be adversely affected.

A mixture of sulfuric and nitric acids, with the addition of a little potassium nitrate if the material is very dirty, is sometimes quite sufficient for certain kinds of freshwater diatom study according to Carter.

Shales containing diatoms, which often are difficult to disintegrate, may be broken up by protracted boiling in concentrated sulfuric acid in the presence of copper sulfate. The carbon, which is thus diffused through the acid is decomposed by the addition of potassium chlorate.

To minimize drying, spilling, or loss of specimens in washing, cleaning, and changing of fluids and/or reagents, perform all operations within one container. Add material with some force with a pipette for thorough mixing, and carefully remove supernatants with a pipette after appropriate settling. This method is particularly advantageous when small quantities of material are involved and the containers are test-tubes, vials or small flasks.

For electron microscopy examination the cleaning methods for diatoms are the same as for light microscopy. The further preparation and mounting are different however. The mounting technique for electron microscopy (EM) and scanning electron microscopy (SEM) are themselves different and will be treated briefly in a later chapter.

CHAPTER 5.

5. WASHING, SEPARATION, AND STORAGE

After the diatom frustules have been cleaned by the most appropriate process, there exists the necessity to thoroughly wash them free of acids and other reagents used in the cleaning process, and to separate them as much as possible from remaining insolubles. The separation of the diatoms themselves into various sizes or size groups is also very desirable for many types of permanent preparations. The storage of cleaned diatoms must be considered for that material which will not be immediately utilized in microscopical study and/or must be retained for long periods of time as reference material for future study and comparison.

Under certain conditions the washing and separation may be carried out simultaneously. At other times, the separation of the frustules from remaining insolubles is very difficult, and the separation or grading of sizes may require ingenious or tedious methods to be employed.

5.1. Settling and Decantation

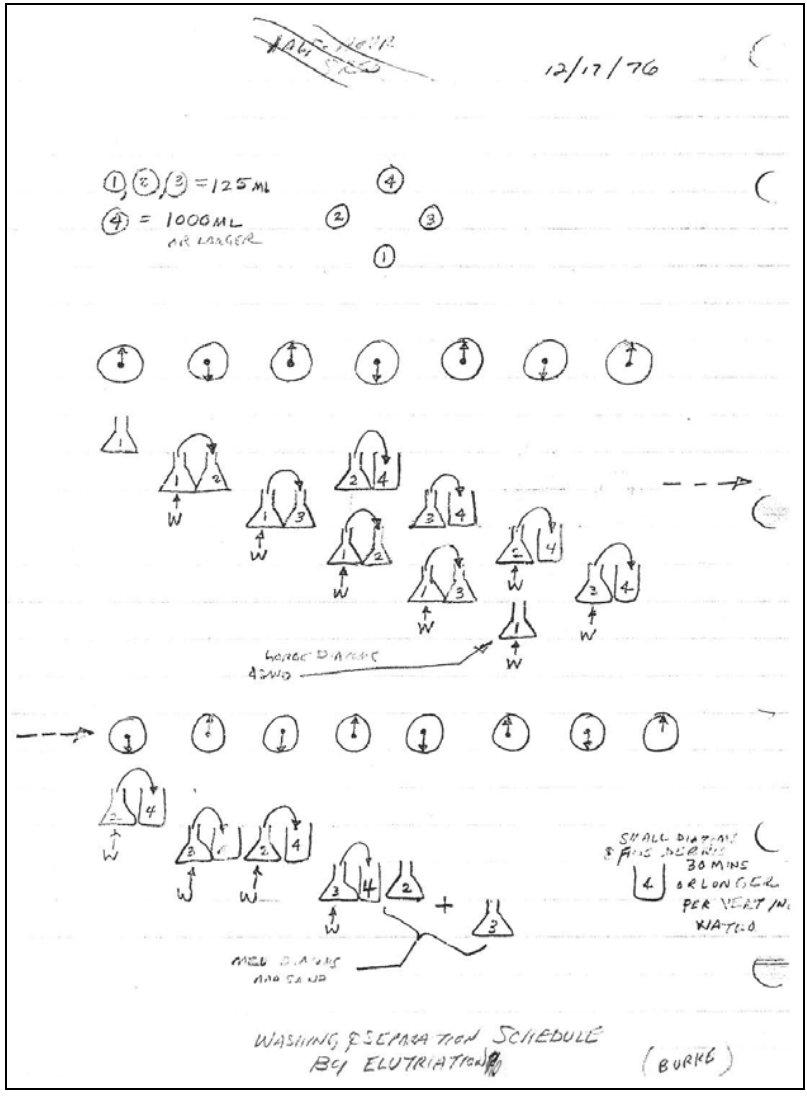
The washing process, as described in the previous chapter, is usually best carried out by repeated settlings and decantations with distilled water. After the treated suspension has completely settled to the bottom of the container, the supernatant washing water is poured off and discarded, more fresh added to the residue, stirred into suspension and again allowed to completely settle, and so on. Settling may be considered complete if the time is equivalent to about l hour per vertical inch of water. In that time all diatoms should have settled to the bottom. The supernatant water should no longer be acidic prior to the final decantation. At this point the diatom residue is considered to be free of chemical reagents which at a future time might possibly interfere with mountants in permanent preparations. also at this point, if other steps have not been taken, the residue will consist of diatoms and other insolubles, largely composed of sand grains and other silica remains.

A method that is quite successful in separation of diatom frustules from other insolubles is that of timed settlings and decantations. The principal mechanism of the method is based on the sinking rate of diatom frustules versus the sinking rates of the accompanying constituents in the residue. Also, larger diatoms sink at a faster rate than smaller ones, and therefore separation of sizes may be accomplished on the same basis.

Before attempting separation of diatoms from sand grains it is advisable to grade the diatoms and debris through various sieves such that diatoms and sand grains are of about the same size. This provides for much easier separation later, for if this is not done, then considerable fine sand will accompany the larger diatoms and they will settle at the same rate, making separation by settling very difficult.

Elutriation by settling is carried out on a time-interval schedule. The time may be varied according to the constituents of the residues to be separated. Determination of

proper times for settling is verified by examination of samplings of the residues and suspensions with the microscope.



Copy of Figure 74.

A schedule similar to that described by Burke is illustrated in Figure 74 (copied above). Three 125 ml. Erlenmeyer flasks and a 1000 ml. or larger Erlenmeyer flask are required. They are arranged and numbered according to the diagram. A time of ten minutes to the vertical inch of water is selected as being appropriate for most aggregates of cleaned material and debris, although as mentioned previously, longer or shorter times may be better for specific mixtures. For instance, for large forms five minutes to the vertical inch of water may be used, and for the small forms fifteen or twenty minutes may be required. The other times in the process are adjusted proportionately.

Referring to Figure 74 flask no. 1 with the material is filled with distilled or filtered water to a height of 3 inches, shaken to bring the material into suspension, and allowed to begin settling on the hour. At half-past the hour the supernatant, with whatever suspension remains, is carefully decanted into flask no. 2. Flask no. 1 is then replenished with water to the 3 inch level, shaken and allowed to settle for another half hour, after which the supernatant is carefully poured into Flask no. 3. This is continued, alternately pouring off into Flask numbers 2 and 3 every half hour, until only larger diatoms and sand remain and the Flask (no. 1) clears in a half-hour of settling. The washing of Flask no. l is then complete.

The pourings from Flask no. 1 have been received in Flask no. 2 on the half hour and in number 3 on the hour. On the half-hour, that is after one hour of settling, the supernatant from Flask no. 2 is carefully decanted into Flask no. 4, replenished with water to the 3 inch level and allowed to settle for another hour and so on. Flask no. 3 is treated in the same manner, on the hour. In other words the flasks in the second row (no. 2 and no. 3) are assigned a settling time of 20 minutes to the vertical inch of water (or one hour) and every hour are poured off into the large Flask (no. 4). After the decantation from the first Flask (no. l) has ceased the pourings from the second row (no.2 and no. 3) are continued until they settle clear in the allotted time (one hour). Their residues contain the medium sized diatoms, and after final decanting they are combined.

Their decanted supernatants, contained in the number 4 large flask, consist of the very small diatoms and fine debris. After the pourings from Flasks no. 2 and no. 3 have ceased the suspension in the large flask is allowed to settle for a time equivalent to 30 minutes to the vertical inch of water. No additional water is ever added to the contents of Flask no. 4, and after settling for the allotted time the supernatant is poured off and discarded, retaining the residue.

Filtered tap water may be used in this process, but at least the two final steps in each case should be carried out with filtered distilled water to prevent any unwanted chemical accumulation with the cleaned and separated material.

Burke suggests separate time cards at each flask to assist in keeping the proper sequence. An audible timer, such as is used in photographic processing is also helpful. Large sand grains in Flask no. 1 may be separated by several subsequent settlings, allowing 1 minute to the vertical inch of water, retaining the pourings with the diatoms and discarding the heavier sand after microscopical examination to make sure it contains no large forms.

Many well-to-be-desired diatoms may be lost in attempting separation from other diatom sizes, or from sand or other particles, if extreme care is not taken. If the mix consists almost wholly of sand, attempts to clean or separate may result in the loss of nearly all diatoms. In the latter case, the separation of the diatoms may be best carried out by the use of a mechanical finger, to be described later.

If the aim of the diatom work is scientific research, rather than just adding material to a collection, then it is most essential to take great care in the cleaning and separation process, and to avoid all chances of contamination. Frequent microscopical examination of material in various stages of cleaning and separation is an excellent form of quality control in this regard.

In this described washing/separation process Burke recommends that if it must be suspended for any reason, hydrogen peroxide should be added to the water. He also indicates that postponement of operations longer than overnight is not advisable.

5.2. Troughing

Separation of diatoms from debris that is essentially heavier than the cleaned frustules is sometimes easily accomplished by troughing as previously described in the cleaning method of Fleming.

An actual trough-shaped device is not absolutely necessary, as the same means may be used using a long flat piece of glass. The glass plate should be at least two feet in length, tilted at about 50 degrees from the horizontal. Pouring the diatom/sand mix slowly onto this plate will cause the sand grains to settle and cling to the glass, and the greater portion of the diatoms to run off into a receptacle. As with any procedure based on a sinking rate, this process will work best if the diatoms and sand have been graded previously through the use of sieves.

5.3. Diffusion

Another method of separating diatoms from unwanted detritus, described by Bastow (1960) in The diatom flora of Sudan. *Journal of the Quekett Microscopical Club* Series 4, Volume V, 1958-1961 Page 236), is by diffusion. The principle of separation in this instance is partly based on the surface tension of a fluid contained in a vessel, which is controllable by the area of the surface, and partly on the laws of diffusion of liquids, The diffusion of the latter can be adjusted by varying their strengths. In the method described, alcohol is used as the diffusing liquid, on water.

Rev. Richard Fraser Bastow, F.R.M.S.
b. circa 1888
Manchester
d. 1st October 1960
Addenbrookes Hospital, Cambridge

If pure alcohol is very gently placed on the surface of water, diffusion does not seem to begin for a long period of time, and it will be several hours before complete diffusion will have taken place. By previously diluting the strength of the alcohol with water, diffusion takes place more readily. A typical procedure is as follows:

The cleaned diatomaceous material is well shaken in a 50 per cent solution of alcohol and placed very gently on the surface of water in the neck of a small (100-125 ml.) Erlenmeyer flask and allowed to diffuse for one minute. The diffused alcohol/diatoms are then picked up from the surface with a pipette and very gently placed on the surface of water in the neck of a second flask and allowed to diffuse for 2 minutes. The process is repeated for a third flask allowing diffusion for 3 minutes, and so on until the alcohol/diatom mix can no longer be picked up with a pipette. All flasks are then allowed to settle, the water poured off, and the residues containing the settled diatoms recovered. This method is best carried out with small quantities of material to be separated.

5.4. Heavy Liquids

Heavy liquids may be resorted to in separating diatoms. Mixtures of bromoform and absolute ethyl alcohol have been used. The mixture is prepared to the required density using a pycnometer, or a refractometer, to determine the specific gravity. Liquids of known specific gravity are also of use in the method.

Separation is effected either by the use of a centrifuge, or in a settling technique.

When quartz is the major constituent to be removed, which is often the case, a liquid having a specific gravity of 2.4 will float diatoms but allow the quartz to sink. A combination of liquids having a specific gravity of 2.4 has a refractive index of 1.555 for the D line. Samples may be placed in a burette and the settled sediments drawn off.

To separate diatoms (specific gravity about 2.l) from sponge spicules (sp. gr. 2.036) and minerals (sp. gr. more than 2.4) the following is recommended (see Figure 76):

Cadmium borotungstate (sp. gr. 2.2) is placed in the bottom of each tube to be used in a centrifuge. Above that a layer of a solution of the same salt (sp. gr. 2.0), then a layer of sp. gr. 1.8, and finally the suspension of diatoms and debris in water. Centrifuge at 4000 rpm. After centrifuging the diatoms are removed by a J-shaped pipette with a rubber bulb, and washed in distilled water. The method is attributed to R. Leboime in an article in *Microscopie* (circa 1949), a publication of La Societe Francaise de Microscopie.

Rene Leboime

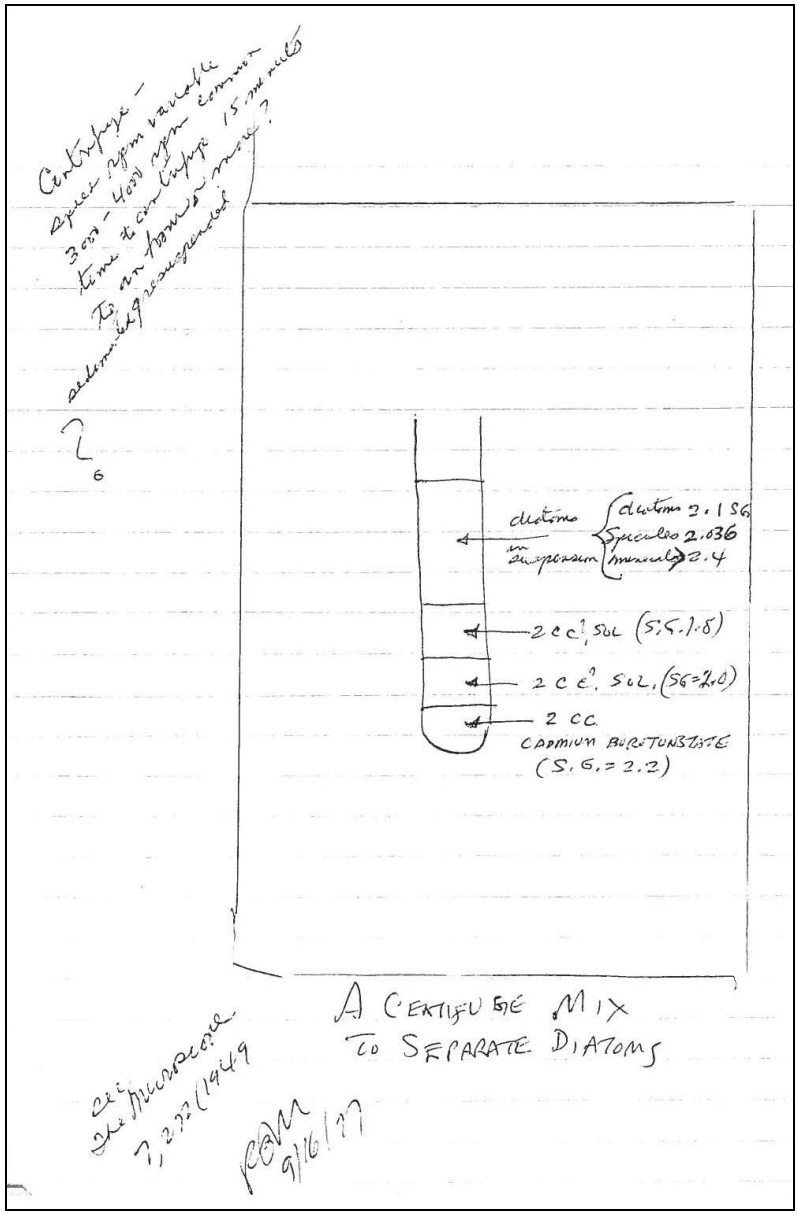


Figure 76.

5.5. The Centrifuge

The centrifuge can be very useful to the diatomist in cleaning and separating diatoms. The type of centrifuge need not be elaborate, even a simple hand-operated model being of considerable use.

In sedimentation, the solid material settles in the liquid at a rate dependent upon the diameter of the particles (assuming them to be spherical), the difference in densities

between the solid and liquid, and the viscosity of the liquid. This relationship was expressed mathematically by Stokes, thus, where g is the acceleration due to gravity:

$$Rate\ of\ fall\ of\ particle = \frac{2g}{9} x \frac{(radius\ of\ particle)}{(coefficient\ of\ viscosity)} x\ difference\ in\ densities\ of\ solid\ and\ liquid$$

Therefore, in a given case the larger particles of solid settle more rapidly than the smaller, and that in two parallel cases, the greater the differences in densities of the solid and liquid, and the less the viscosity of the liquid, the more rapidly sedimentation takes place.

Sir George Gabriel Stokes.
b. 13th August 1819
d. 1st February 1903
Mathematician and Physicist.

Note that the former recommendation to size diatom and other detritus by selective sieving before settling and decantation, is substantiated by these concepts. Also, it is seen that the use of heavy liquids and other variations of sedimentation procedures are founded on the same fundamentals.

In centrifuging, the machines in use are designed to subject the material which is treated to the action of centrifugal force. That centrifugal force exerted on the suspended diatoms and other particles is many times the acceleration due to gravity alone, and thereby hastens the "sedimentation" process considerably.

The centripetal force (F) which is equal and opposite to the centrifugal force, varies directly as the square of the velocity (V) and inversely as the radius (r) of the circle of motion as:

$$F = \frac{W}{g} x \frac{V\ (ft.\ per\ \sec)}{r\ (\text{in}\ ft)}$$

The weight W in the above equation includes, in the case of the centrifuge, all of the effective weight at the end of the arm at which the sedimentation tubes are located. The weight of the arm itself and its effect at the radius and the weight of the sedimentation tubes and its contents, are all involved in this term.

By selecting fluids of differing viscosity, the velocity of rotation, and duration of the treatment, considerable latitude in the size of particles and the time in which they are sedimented, is effected.

For instance, if sedimentation of specific diatoms takes 10 minutes per inch of vertical fluid in a static settling (at 1 gravity), then that rate can be increased by using a centrifuge for a time much shorter by the proportion of the increased g-force exerted.

The radius of the particle to be sedimented directly affects the time it takes it to fall a specified distance in the suspension fluid. Extremely small particles are very

difficult to separate and very high velocity centrifuges are necessary for those cases. Fortunately, diatoms are of sizes, even at their smallest, as to be easily centrifuged. For practical centrifuges generally available, speeds of 3000 to 4000rpm are common, with the g-force in the range of 1500 to 2000 times that of gravity. Hand-operated centrifuges develop a shaft speed of about 2500rpm.

Dependent upon the factors included in the equations above, and the purpose of the centrifuging operation, times for the process vary from but a minute or two to as much as an hour or more.

Centrifuging can be used to separate specific sized or weighted particles, two immiscible liquids, and in simple washing operations. If the sediment is thrown out from the liquid in which it is suspended, the liquid can be withdrawn and the sediment recovered from the bottom of the tube.

For instance, in a diatom washing operation, the material is suspended in distilled water and centrifuged. The supernatant water is drawn off with a pipette, more water added and the settled material stirred or shaken into suspension. It is then again centrifuged. The process is repeated as many times as is necessary to obtain a clean sediment. Usually two or three washings by this means is sufficient. The time is but a fraction of that required in static settling. In this cleaning process, enough time is allowed at the particular speed to obtain a clear liquid with all sediments at the bottom.

In separation of diatoms from unwanted particles of sand, etc., that are heavier, the time (at the particular centrifuging speed) is allowed such as to sediment these heavier particles to the bottom, allowing the diatoms still in suspension to be drawn off with the supernatant fluid. The reverse might be true in separating diatoms from lighter undesirable elements.

In grading diatoms of one size from another, the differences in diameters and/or other dimensions affect the centrifuging times for separation, and one size will be recovered in suspension and the other in sediment form. Combinations of various fluids as previously described can be devised for specific applications.

In operating any centrifuge, it is important that the tube loads be balanced, or unequal forces will be set up during operation that can damage the apparatus itself, or become a safety hazard to the operator.

Ordinarily, sedimentation tubes with conical lower ends are used in centrifuging. However, in some cases test-tubes can be used if the proper size. In other cases in working with very small quantities of diatoms, the standard tubes are too large. Very small tubes (of glass tubing) can be used. They are centered and held in the aluminum carriers with perforated corks. In this manner very small quantities of diatoms needing washing can be accommodated. The cleaned material may be recovered by cutting the tubing with a file and breaking it off just above the sedimented diatoms.

A method of removing very minute amounts of suspended diatoms from a large volume of liquid is accomplished as follows. A small separation funnel with a stop cock is fitted to a centrifuge. After being whirled, the suspended matter is forced into the cavity in the stop cock. A quarter turn of the stop cock completely cuts off

sediment from the liquid and the latter is poured off. The stopcock is then removed and the small quantity of liquid and sediment recovered.

5.6. Manipulation

Diatoms may be separated from unwanted debris, sorted and selected for type and size, and otherwise treated, including washing, by manipulating them as individual specimens under the microscope. In spite of their small size, this type of activity is not uncommon in working with diatoms. A number of specialized devices are employed to selectively separate them and prepare microslide mounts. Manipulation of diatoms in either case is quite similar, and it is appropriate at this point to discuss ways and means of accomplishing these delicate tasks.

5.6.1. The Bristle

The bristle is the actual tool to come into contact with the diatom that is to be manipulated. It is the part of the manipulator that actually pushes, pulls, lifts, and otherwise contacts the diatom or parts of diatoms. It must have the proper dimensions and physical characteristics to successfully be employed in these operations. It should be of small cross-section, flexible, tapered, and with a tip radius of curvature that is preferably not greater than about one-fifth the largest dimension of the smallest diatom to be moved.

There have been a variety of bristle materials found to be satisfactory. Hairs, both human and animal, have desirable characteristics, and many types have been used. Some of those which have been recommended and used are a “hair from a mole on a dog's jowl”, a “pig's eyelash”, a “tiger's whisker”, a “hair from the neck of a fox-terrier”, a “hair from the neck of a cow”, and a “cat’s whisker”. Hairs, split or otherwise, from shaving brushes, clothes brushes and other such household items have also been used with success. Although some of these may appear to be amusing, they all have the proper diameter, taper, and degree of flexibility to make excellent tools for manipulating diatoms.

The bristle should taper fairly rapidly toward the point and preferably be short rather than long. This latter attribute is an advantage when it is turned to examine a diatom as less focusing of the microscope is necessary. Hairs may be sharpened on an oil stone, and various degrees of taper, smoothness, and sharpness obtained thereby.

Hairs have a predilection to oiliness, and they are susceptible to attack by chemicals and are affected by moisture. They also are greatly variable in size. In spite of these shortcomings, many diatomists successfully use hairs as bristles.

However, many other materials have been tried as well. Among them are wire, plastics, cactus spines, and glass. Plastics are attractive because of their flexibility, ease of fabrication and sharpening, and low cost. Glass, drawn into fine tapered threads is, perhaps, the most useful of all and is extensively used in even the most sophisticated micro-manipulators.

Some workers prefer a bristle made from a fine tungsten wire. It has the advantage that it can be straightened so the tip does not wander out of focus as it is rotated. An extremely fine tapered point from 1 to 10 micrometers is readily obtained by dipping the extremity momentarily in fused sodium nitrite kept at the boiling point in a half-inch metal capsule, quenching in water and wiping. A suggested wire size is no. 20 or 22 AWG, or 0.07 mm. diameter. Alternatively the end of the tungsten wire may be heated red hot and delicately stroked on a sodium nitrite stick. An electrolytic method is to use tungsten wire as electrodes of a cell. Place only the tips of the tungsten wires in a potassium hydroxide (KOH) solution and apply a low voltage from a variable-voltage transformer. Microscopical examination of the wire will determine when the tips are properly tapered and sharp.

The making of glass fibers for use as bristles is the basic procedure of drawing out glass rod, over a flame, to the required thickness and taper. These are then attached to the actual manipulator, handheld or otherwise, with beeswax or a suitable cement. The following describes a procedure wherein the glass fiber is attached to steel rod to be either hand-manipulated or attached to the controls of a mechanical finger.

Start with a fine glass rod and draw it out over an alcohol flame to about l mm. diameter, melting the end of it onto a platinum wire (20 to 22 AWG) which has been previously hard-soldered to the steel rod. Hold this pair (glass rod and wire) higher and higher over the flame, drawing it out until the glass parts with a perceptible tug. The ideal picking fiber is drawn out to not too fine a diameter, and then suddenly comes to a short and very fine point. The very fine end should not be too long or it will be uncontrollable. Considerable practice is required to obtain an ideal fiber.

In another method two different sizes of flames are used. Gas flame is best, but alcohol will be satisfactory. One flame is of an ordinary size with an ordinary wick. The other is as small as it can be made (when gas is available a small hypodermic needle bent into a right angle will be very good). With such a small flame as the latter, a supply of air is not needed, and would be too hot. With an alcohol flame it is difficult to get a small wick and one will have to be improvised.

The larger flame is used to melt a bead of glass on to the tip of the platinum wire (using a glass rod about 2 or 3 mm. in diameter). Then the combination is transferred to the small flame and the glass tip is drawn out in short successive steps until the end of the fiber is as small as possible, but keeping the final tip as short as possible. As with the making of tungsten bristles, the desired taper and sharpness is controlled by examination with the microscope.

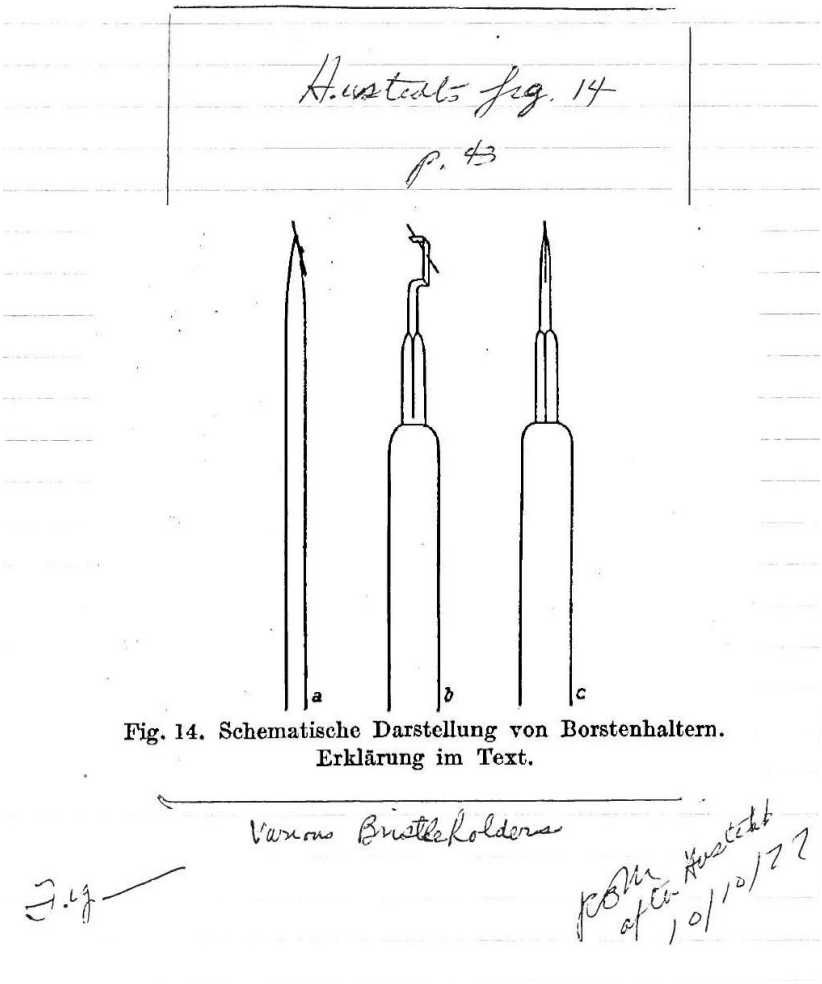
Plastic bristles may be may be made from thin strips of sheet plastic. The proper taper and tip size is obtained by sharpening the end on a very fine sandpaper and emery cloth. They are easily made, do not break as easily as glass fibers, and are very economical. Although making tips as fine as those with tungsten wire or glass is not possible, many working diatomists prefer them.

5.6.2. The Hand-held Bristle

The simplest method of manipulating diatoms is by the use of a hand-held bristle. In order to do this successfully the bristle is fastened to a handle of wood, glass or

metal. The handle must be of a length to fit comfortably in the fingers of the operator and have the size and balance necessary for very delicate movement.

Hustedt recommended using a cactus needle or pig's eyelash as a bristle (he advised defatting the pig‘s eyelash through the use of sulfuric ether). Figure 77 illustrates the various types of bristle holders he describes. That at Figure 77a is a water-color brush handle. The bristle is fastened at an angle with glue to the handle. A very thin wooden wand (with the bristle) can be fastened into a pencil set into a crayon as at Figure 77c. Alternatively, after the graphite lead of the pencil is removed, in its place a heavy wire may be inserted. The wire is flattened at the end and formed into a “U” shape. A fine hole is drilled at an angle through the flat U-shaped portion. The bristle is then passed through the holes, being fastened with glue at the rear hole. The pencil then is fastened into a crayon. Details of this arrangement are at Figure 77c. The crayon lends sufficient bulk and weight to the assembly to provide the balance necessary for delicate maneuvering of the bristle. The bristle in any case should only protrude 2 or 3 mm. beyond the end of the holder. It should be examined under the microscope to determine that it has not been broken or split during the assembly.



Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 14)

Figure 77.

Although the use of such a hand-held bristle is possible, it takes a great deal of practice to successfully pick diatoms with it. Also, with very small diatoms, as even the pulse is transmitted to the picking fiber, making it very difficult to make exacting movements. In spite of these shortcomings, the diatomist should have at least one hand-held bristle. It will be found extremely useful at times, either by itself, or used in conjunction with a mechanical finger.

For those who do not wish to make their own holders, commercially available ones are to be found in a variety of forms. Ordinary pin-vices, used in different crafts, are excellent holders for various sized shafts that could ultimately contain a suitable bristle. Commonly available inoculating needle holders are excellent for tungsten wire bristles, and dissecting-needle holders are also very useful. The electronics

industry has produced a complete range of micro tools and micro tool sets that can be used to advantage in long duration precision work, and that are adaptable to working with diatoms.

5.6.3. The Mechanical Finger

The logical solution to problems of tremor and precise movements in hand-held bristles is to substitute mechanical controls for the hand. The mechanical finger is the result and is the first true forerunner of modern micromanipulators. The diatomist does not, fortunately, need all the sophistication of a modern micromanipulator in his work. The mechanical finger has evolved into several different forms, all of which are successful. Its fundamental form consists of a bristle, bristle-holder, support, and motion control. The finger may be attached to the microscope, or be separately supported. The controls for motion may be separate and distinct from the microscope or utilize the microscope controls themselves, in whole or in part, to provide relative guidance to the bristle.

In the following paragraphs a number of different mechanical fingers will be described along with their shortcomings and/or advantages. Choice as to the best form is left to the individual. Personal preference, mechanical dexterity, and the frequency or constancy of use of such a device, all play an important part in its selection. Some diatomists prefer one type of mechanical finger to the exclusion of all other designs, while to some, several types seem satisfactory. As in any skill of this type, experience alone will dictate much in the way of individual preference.

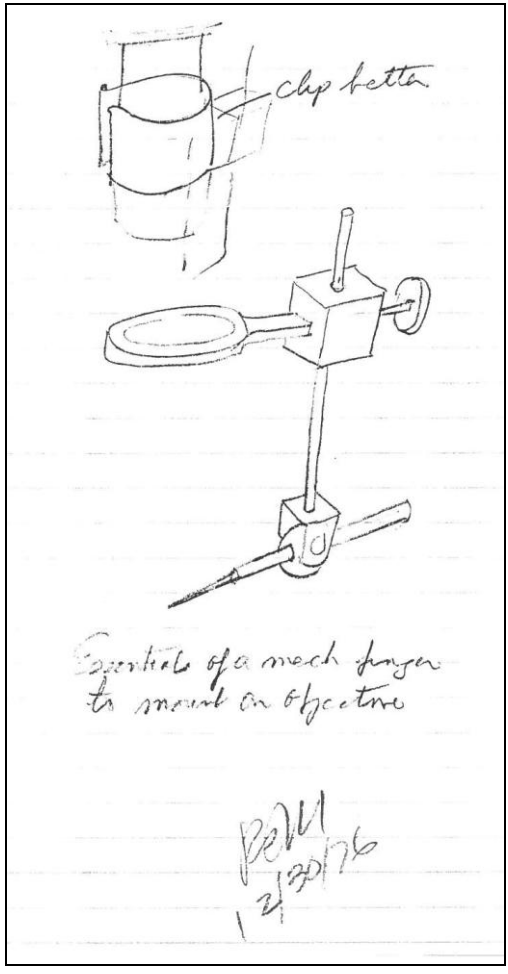


Figure 78.

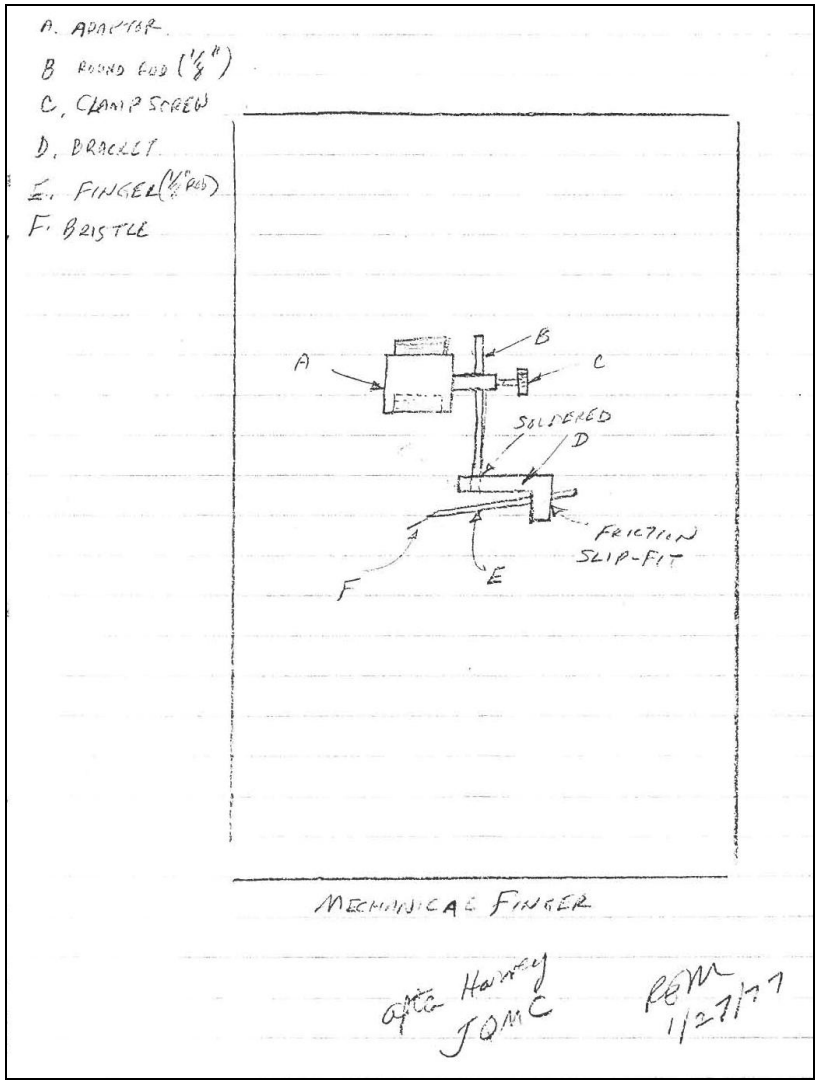


Figure 79.

A simple and effective design for a mechanical finger is illustrated in Figures 78 and 79. The precision controls for operation of this finger are entirely those of the microscope. The focusing mechanism and mechanical stage controls furnish all the motions necessary in three axes. It is noted that the finger is attached to the nosepiece or tube of the instrument and is therefore adjustable in the "Z" axis with the coarse or fine focusing adjustment. The X-Y movements are taken care of by the relative motions of the mechanical stage. There are both advantages and disadvantages to such an arrangement. The main advantages are the extreme simplicity of the mechanism and the very precise adjustment in the Z axis. Another advantage is that upon being properly adjusted initially, the diatom being manipulated is always in focus and viewed by the diatomist, even when it is lifted from the microslide. A disadvantage in fastening this finger to the nosepiece is that

it often effectively increases the mechanical tubelength by the thickness or length of an intermediary mechanical connection. Although this difference in mechanical tube length may be minimal, the necessity to unscrew and screw the objective to effect removal or installation is troublesome at times. This disadvantage can be easily taken care of by providing a spring-clip fastening which fits the barrel of the objective. Another more serious disadvantage of this style of mounting for a mechanical finger is that the field of view is lost in the plane of the stage every time a lifting or transfer operation is performed. A further disadvantage is common with all fingers utilizing the mechanical stage for relative XY-movements. That disadvantage is the movement of the object-specimen site. For some types of micromanipulation activity this may be very undesirable. However, in the manipulation of diatoms it is a minor inconvenience at times, and has the advantage of speeding up various transfer operations. In spite of this shortcoming, after some practice, all of the necessary operations in selecting, cleaning, and mounting diatoms are very effectively accomplished. This attached arrangement permits a very great simplification in the construction of the finger in that all that is required is some means for setting up initial adjustments for angle, etc., of the bristle for the particular situation; other adjustments being "built-in". A variation of this simplest of mechanical fingers frees the focusing mechanism of the microscope for strictly optical duties. The finger is mounted on the edge of the stage or another part of the microscope stand with brackets and/or clamps, or is on a separate mount from the microscope. The X-Y motions are-still furnished by the mechanical stage, but the Z-plane motion (up and down) is furnished by a fine screw or other form of vertical control. One such device of this style is typified by a design described by S. H. Meakin in 194O. Figures 80 and 81 illustrate the construction features at reduced scale (original notebook portrayed these at full scale). Many of these mechanical fingers have been constructed and are currently in use by diatomists in various parts of the world, testifying to the excellence of design. A brief description, referring to the illustrations, is in order.

Samuel Henry Meakin (1876-1955)

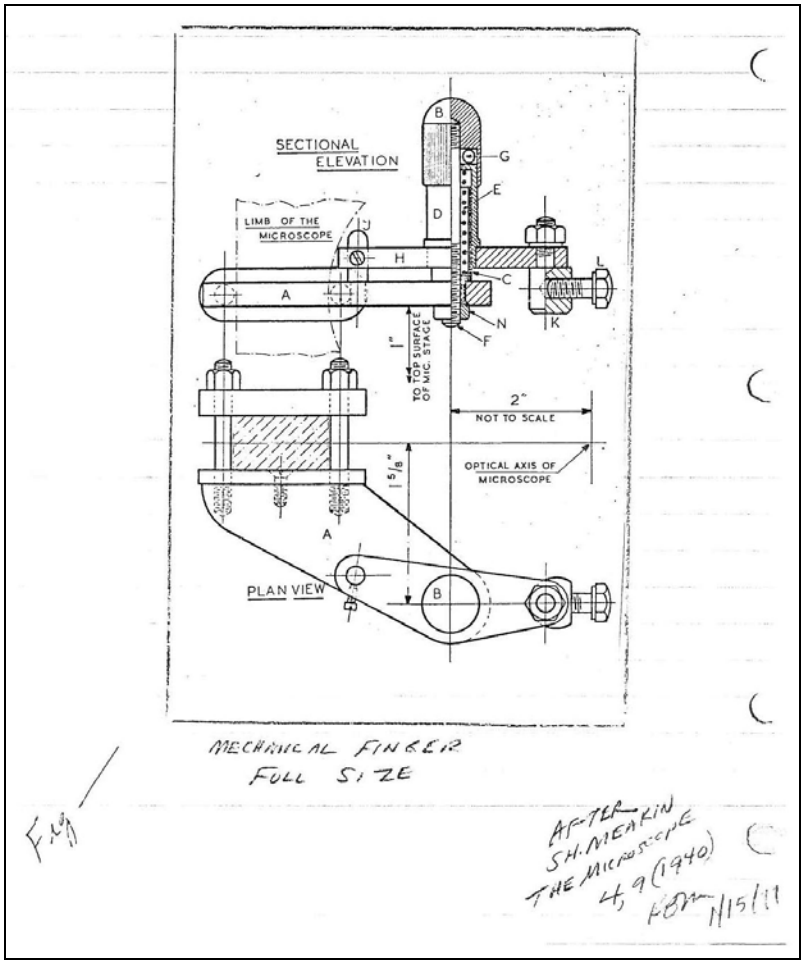

SECTIONAL ELEVATION
LIMB OF THE MICROSCOPE
B
G
E
D
J
H
C
L
A
N
K
F
1" TO TOP SURFACE OF MIC STAGE
2" NOT TO SCALE
OPTICAL AXIS OF MICROSCOPE
1 5/8"
A
PLAN VIEW
B
MECHANICAL FINGER
FULL SIZE
Fig
AFTER SH. MEAKIN THE MICROSCOPE 4, 9 (1940)
11/15/11

Figure 80.

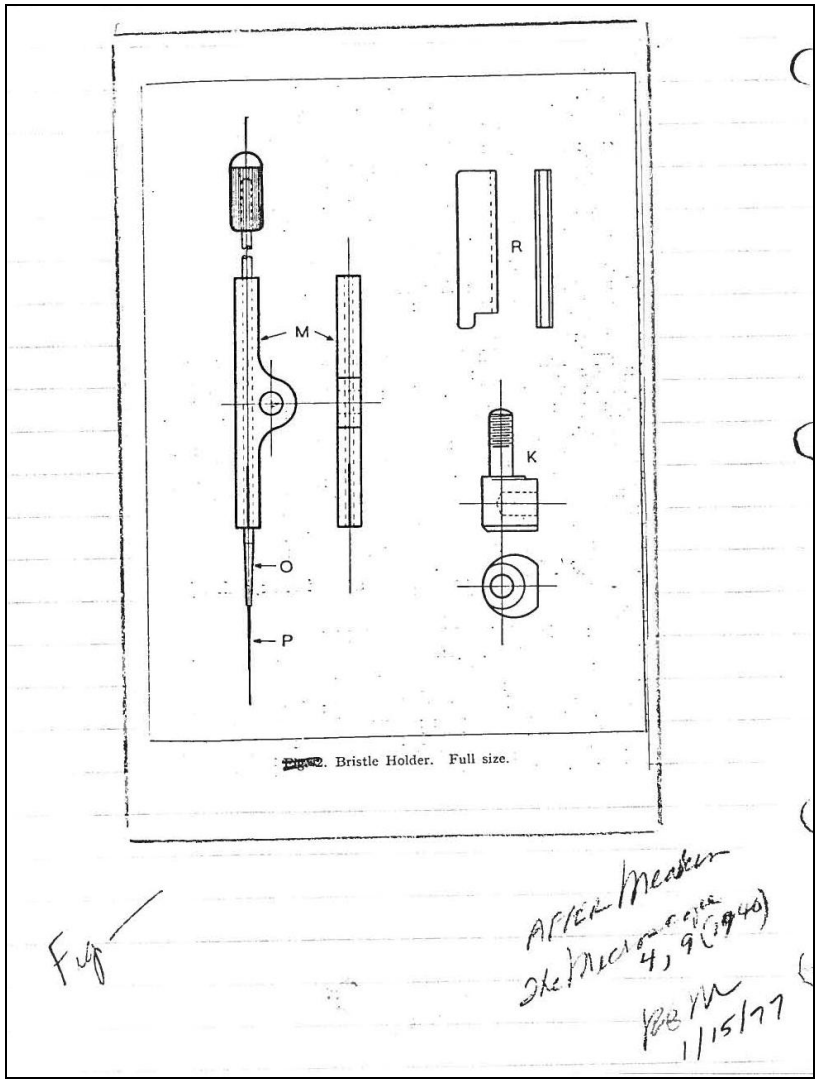


Figure 81.

The finger consists of a main support A, of $^{1}/_{4}$ inch brass or aluminum securely fastened to the limb of the microscope. An extending arm H is controlled vertically by knob B and guided by pin J, which prevents any turning of the arm during vertical adjustment. A set screw is provided to take up any possible slack due to long wear.

The turned barrel C is securely fastened to support A. It is tapped at its bottom end with a $^{3}/_{16}$ inch Whitworth thread (or similar thread). Sleeve D slides on barrel C with a very close fit to prevent any wandering at the bristle point. The sleeve should be lubricated by a heavy grease made by melting equal parts of beeswax and Vaseline. Spindle F is permanently screwed into knob B and threaded to travel through barrel C.

A number of hard steel balls of l/8 inch diameter (G) provide smooth turning for spindle F. Sleeve D is spring-loaded upward by spring E and moved upwards and downwards by turning spindle F. Sleeve D is fitted to the hole in arm H and fastened securely to it. At the end of arm H a hole is drilled into which the adjusting block K is fastened. The bristle holder M (also of $^{1}/_{4}$ inch brass or aluminum) is gripped securely to block K by set screw L. The attitude of the bristle holder may be adjusted and fixed in angle by loosening and tightening screw L. Block K is adjusted once to bring the bristle point to the center of the microscope field and then set permanently. Nut N is tight on the end of spindle F to limit the vertical travel of H to about $^{3}/_{16}$ inch and to prevent the bristle from coming up against the objective. The bristle holder O is made of no. 12 gauge brass wire. Its length is selected to fit the particular microscope it is to be used with, about 4 to $4^{1}/_{2}$ inches. Its end is tapered to more easily be guided through the hole of M. The end of M is cross-slit at 90 degrees for about $^{1}/_{2}$ inch to effect an end press-fit for the bristle holder, which will slide rather stiffly, but smoothly, in the hole in M.

Piece R in Figure 81 is a form of limiting lock to prevent damage to the bristle when not in use. In that condition the bristle is withdrawn into the guide O and the piece R placed between the top end of M and the knob on the bristle holder. This will prevent accidental emergence of the bristle and possible breakage.

Any type of bristle may be used, according to the preference of the diatomist user. A final and very sharp point may be obtained according to Meakin with hair or plastic bristle, by placing a very finely ground glass, of the size of a microslide, on the microscope stage. It is then wetted and the stage racked back and forth, at the same time revolving the bristle, the point of which rests on the ground glass. He also suggests that this is an effective way to clean the bristle from time to time, the tip being revolved on a water-moistened microslide.

Glass bristles can be cleaned more thoroughly by washing them similarly in a drop of xylene.

The preceding arrangement provides very precise and repeatable motion control through the fine-screw spindle. However, there is a lack of “feel” which some diatomists claim is required for increased confidence and long-term use.

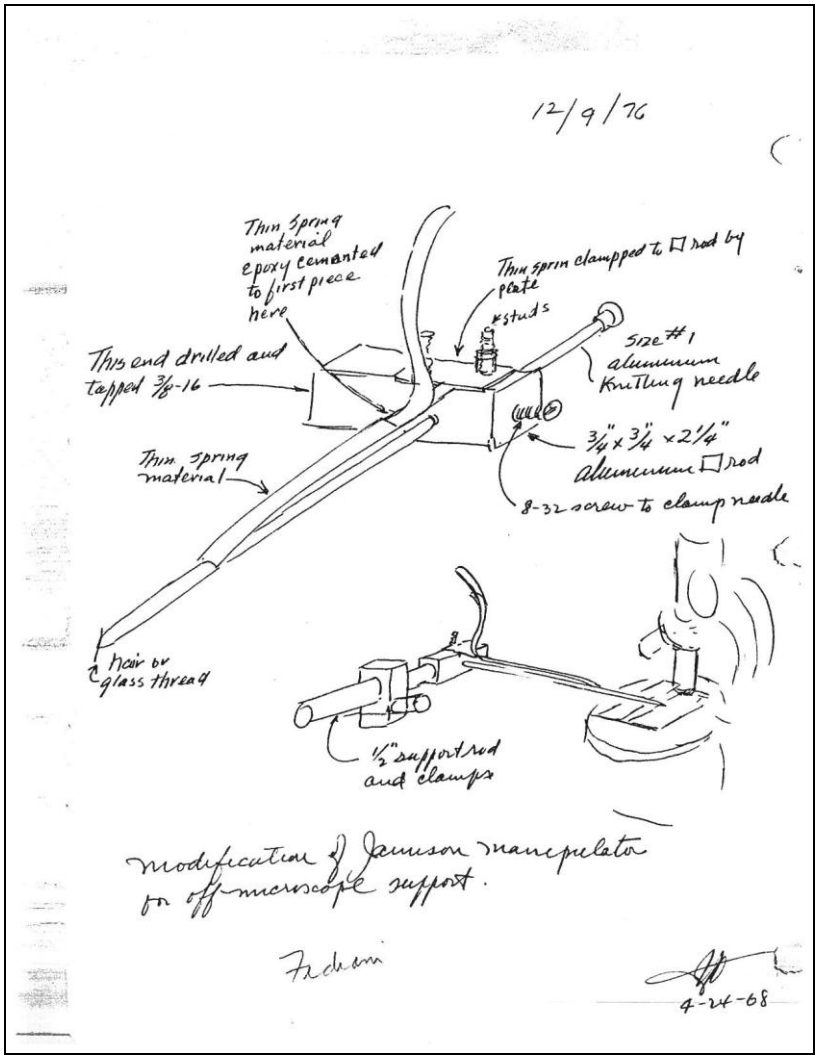
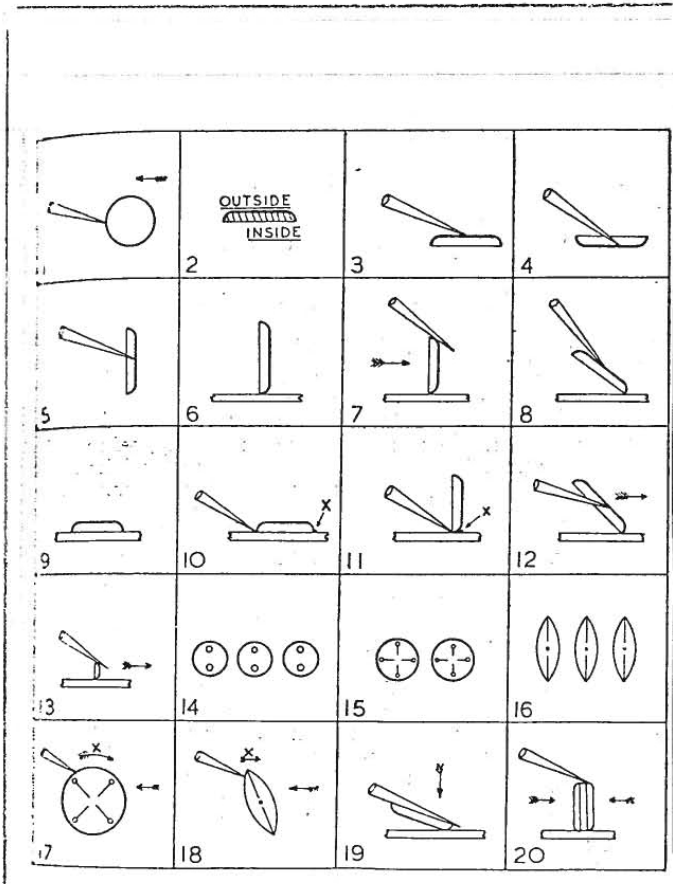


Figure 82.

A design which provides a modicum of "feel" to the vertical motion is that which incorporates the weak-spring on strong-spring principal. An article by Jamieson describes the construction of such a spring controlled finger. Figure 82 illustrates variations of Jamieson's design. The aluminum knitting needle being deflected downward very slightly by a rather large motion of the flat spring controlled by a finger of the operator. Thus a direct correlation between a motion of the finger and the movement of the bristle is established. Designs for mechanical fingers in many variations other than those described and illustrated here, are referenced in the Appendix.

5.6.4. Technique

The mechanical finger is used by the diatomist in conjunction with the microscope in selecting, sorting, picking up, putting down, turning over or otherwise orientating, aligning, washing, cracking and removing girdles, separating valves, and rotating, diatoms of various shapes and sizes. Microscope magnification usually employed for this activity is approximately 100X. A 10X objective and 10X ocular being a common combination. Less magnification results in difficulties in visual selection, at least with the smaller diatoms, and greater magnification allows too little in the way of working space between the objective and focusing plane. All of these operations involve basic maneuvers which are easily learned and perfected by practice. The diatomist should practice the various operations illustrated and described herein with large diatoms at first. Specimens of *Coscinodiscus*, or of *Pinnularia* are excellent practice material.



Diagrams showing manipulation of diatoms with Mechanical Finger

after Meaker
The Microscope
4,53 (1940)
RBM 11/5/77

Figure 83.

Figure 83 illustrates various maneuvers. Diatoms will adhere to the bristle as it is lightly touched to them if they are free on the microslide. If they are not clean, or are

otherwise contaminated with residues, or the microslide surface itself is unclean, the diatoms may adhere strongly to the glass. This often is the case where tap water has been used and a carbonate film has been developed which, along with other residues, may create a cement-like hold on diatoms. If this is the case, and the diatom selected for maneuvering is sticking slightly to the glass and will not lift with the bristle, use the following technique: Refer to Diagram 1 of Figure 83. Using the mechanical controls rack the stage in the X-direction until the point of the bristle touches the microslide surface near the edge of the diatom. Then move the stage very slowly in the direction indicated by the arrow, forcing the diatom against the bristle point and freeing it from the microslide surface. If it is stuck too firmly, the diatom will break. Under the latter condition, it will be necessary to re-wash the diatoms and apply them again to a microslide. However, the maneuver in Diagram 1 usually is successful in freeing slightly stuck diatoms. If the first diatom breaks under such conditions, try another or two before deciding it is necessary to re-wash and spread a new batch. Some diatoms, especially a single round frustule resting on its outer surface, may stick more tenaciously than others of its kind, in a slightly different orientation or attitude, than one of a different species having a different shape.

When the diatom is free, raise the bristle slightly, rack the stage to bring the diatom under the bristle, lower the bristle until it touches the diatom and raise the latter from the microslide surface. The diatom now being on the bristle, it is ready to be examined by focusing on it and turning the bristle, or maybe transferred to a new location on the same microslide or to another, dependent upon the purpose of the operation. Lowering the diatom to the new location/surface until it touches is generally all that is necessary for it to be released from the bristle at that point. As long as no gusts of air or other disturbing vibrations are introduced, the diatom will adhere to the bristle almost indefinitely. It may be held a convenient distance above the stage surface to allow relative movements beneath it, or for exchanging microslides.

As the diatom is inspected, and it should be in each case, if it is found to be fixed to the bristle point as in (Figure 83 Diagram 3) it is outer surface uppermost, and in (Figure 83 Diagram 4) inner surface uppermost, and in (Figure 83 Diagram 5) edgewise, all in relation to the surface from which it was picked, or is to be laid upon. Dependent upon the purpose of the manipulation, these orientations may be important, especially in various types of selected mounts, to be described in a succeeding chapter. If the purpose is to merely select and store for future mounting, the orientation of the diatom on the bristle before release to the new surface is rather unimportant. The remainder of the diagrams in Figure 83 relate to orientating, moving, aligning and placing diatoms for permanent mounts. The horizontal arrows in each case represent the relative movement-direction of the mechanical stage. The letter x indicates the accumulation point of a fixative, due to motion of the diatom in it on the surface of a microslide when permanent mounting operations are in progress. These latter indications may be disregarded in purely maneuvering operations. The diagrams are largely self explanatory, and where appropriate will be referred to in a section devoted to mounting diatoms. Figure 83 Diagram 20 indicates pressure applied with the bristle and a racking back and forth of the stage to effectively crack the girdle loose from the two frustules - often desirable in certain types of selected mounts.

Use of the bristle against the edge of the microslide to free the diatom for releasing it (it will generally adhere to the edge at that point) and re-picking it up in a different orientation relative to the bristle point, is a common maneuver easily learned. Pushing the diatom through a minute drop of water with the bristle point will sometimes provide additional cleaning of the particular frustule, or float off persistent undesired particles. The use of an auxiliary hand-held bristle to assist in some operations is of great convenience.

The basis of all operations is that the bristle is used in vertical (Z-axis) and rotary motions only, and the relative X-Y motions are effected by the mechanical stage, sliding the glass surface of the microslide beneath the diatom. With some practice, the basic movements are easily learned, and in time quite sophisticated maneuvers accomplished using these simple related movements of bristle and stage.

This brief description of mechanical fingers, their types, construction, and basic techniques, has been introduced at this point as a basis for describing certain selection and storage methods treated in the next section of this chapter. The further employment of the mechanical finger in mounting selected diatom slides is included in another chapter.

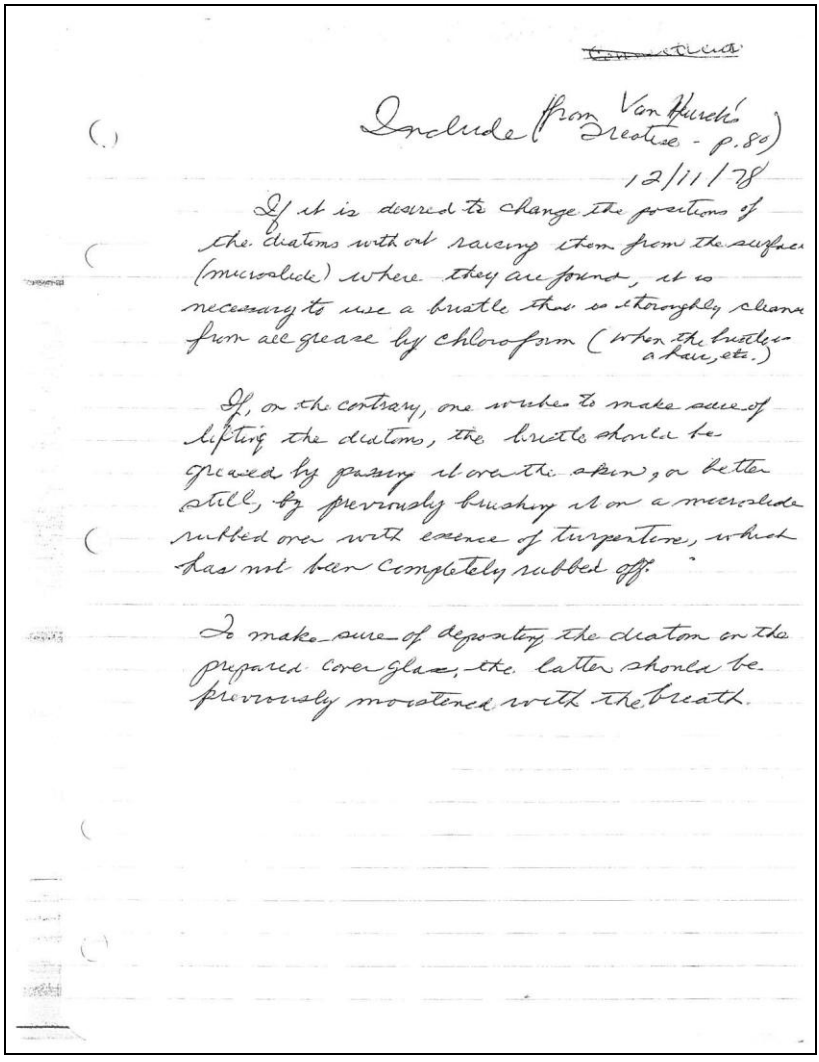

Include (from Van Heurck's Treatise - p. 80)
12/11/78
If it is desired to change the positions of the diatoms without raising them from the surface (microslide) where they are found, it is necessary to use a bristle that is thoroughly cleaned from all grease by chloroform (when the bristles are a hair, etc.)
If, on the contrary, one wishes to make sure of lifting the diatoms, the bristle should be greased by passing it over the skin, or better still, by previously brushing it on a microslide rubbed over with essence of turpentine, which has not been completely rubbed off.
To make sure of depositing the diatom on the prepared cover glass, the latter should be previously moistened with the breath.

Figure 84.

5.7. Storage

After diatoms are cleaned, there is usually a requirement to store the material in one form or another for future reference and study. Much of diatom storage is in bulk, dry or liquid, and some in a semi-selected form on microslides.

5.7.1. Dry Storage

Cleaned material may be stored dry on small pieces of plastic, mica, or glass. If so, they should be protected from contamination. A method that is satisfactory, is to use small enough pieces of the substrate material to fit into the rectangular wells of

cardboard micropaleontological slides. They in turn can be covered with a standard sized microslide as protection against dust and other contamination. Small square or circular coverglasses fit the purpose admirably. A few crystals of thymol in the container will prevent fungus from forming.

Storing very small quantities of cleaned diatom material dry in concavity microslides is also possible. A coverglass is tacked in position with one or two minute spots of an appropriate cement as a protecting cover.

Bulk dry storage in small vials is perhaps more prevalent in the United States than in other countries. It has the advantage of being a relatively maintenance-free storage system. But, the difficulty of keeping the relatively large bulk of material really dry, and a tendency of such material to settle and pack into the vial is very troublesome. In retrieving dry material from a vial, some difficulty may be experienced for the same reason.

5.7.2. Liquid Storage

This method of storing diatom material is by far the most satisfactory, and nearly universally used, especially if a relative bulk is involved.

Either distilled water or alcohol, is used as a storage liquid. When storing cleaned diatoms in distilled water, add several drops of 5% formalin to serve as an inhibitor to the growth of algae or fungi. Unless the storage vials are hermetically sealed (that is very unlikely) the fluid will evaporate in a surprisingly short time. Therefore it is necessary to examine storage vials periodically, and add additional fluid, or better, pour off the old suspension fluid and replenish with a fresh supply. This type of maintenance minimizes contamination with spores, etc. which enter during opening and closing the vials. Replenishment of the suspension fluid at regular intervals with a shaking of the contents also helps prevent matting of the diatoms. When diatoms adhere in bunches over a period of time it is very difficult to effect separation.

instead of the distilled water/formalin storage fluid, diatoms may be stored in a part-water part-alcohol solution. The percentage of alcohol is not critical. A 50% to 70% solution of ethanol is satisfactory. Remarks above on maintenance of such stored material also apply in this case.

A 5% solution of carbolic acid in water has been used as a medium to store diatoms in the past. Since the acid does not mix readily with water, globules of acid may remain undissolved. Heating the mixture may solve this. Diatoms may also be stored in hydrogen peroxide or acetone. However, alcohol or distilled water is used by most diatomists.

5.7.3. Store Slides

Store slides are microslides onto which have been placed selected diatoms that are eventually destined to be used in making permanent mounts of individually selected, or groups of selected, diatoms.

Before store slides can be prepared it is necessary to prepare a "spread" from cleaned diatom material. The following paragraphs describe the necessary steps to be taken.

Transfer the cleaned diatom material, either from a recent cleaning or storage vial, to a large clean tube and fill with distilled water containing 2% (or less)formalin. The latter should have been carefully filtered before use. Allow the diatoms to settle to the bottom, pour off the water, refill and repeat the operation three or four times. This washing by settling is to get rid of microscopic particles of silica which seem to accumulate when diatoms have been stored for a time and then agitated.

Glass microslides should then be cleaned in preparation for their subsequent use as store and spread-slides. The required cleaning of microslides varies as does the quality of slides used, and their condition prior to cleaning. Most commercially available microslides are now furnished in a "pre-cleaned" condition and the cleaning of them may only require that they be washed thoroughly in a soap or detergent and wiped carefully just before use. If there is any doubt as to the cleanliness of slides then a more thorough cleaning may be obtained by chemical means. Some chemical cleanings recommended may leave the slides (and coverglasses) hygroscopic, making it necessary to wipe them free of moisture from time to time. Microslides and coverglasses can be cleaned thoroughly by soaking in a solution composed of 25 parts distilled water, 3 parts concentrated sulfuric acid and 2 parts potassium dichromate or potassium bichromate. This and similar mixtures have been used for many years in successfully cleaning laboratory glassware. The microslides and/or coverglasses should be soaked for at least several hours, and after the solution is decanted, washed repeatedly in distilled water until all traces of the soaking solution are removed. They then should be stored in 50% alcohol. When needed they are removed from their containers with forceps and wiped dry with a linen cloth. This treatment will assure that suspended diatoms dropped on the glass surface will spread evenly (to the edge in the case of coverglasses). If, after cleaning, the microslides and/or coverglasses are stored in distilled water, then washing them immediately in alcohol before using will provide the same advantage of an even spreading of the dropped diatom material.

Bacteriologists would 'flame' the slides to dry and sterilize them after removal from alcohol.

The tube or vial of clean diatoms should be agitated to bring diatoms into suspension and a small amount picked up with pipette. Six or seven drops of the suspension are then allowed to fall on the clean microslide from a height of about 2 inches. If the slide is clean and the diatom suspension pure, an oval "spread" of about $1^1/_2$ by $^3/_4$ inches will result, that will not run off the slide.

If the diatoms are fairly large, dry with gentle heat. Very small diatoms should be dried at room temperature in a dust-proof place for 2 or 3 days. If the latter are dried too rapidly they will adhere to the glass, making picking difficult if not impossible. More details on methods, technique, and special apparatus for drying diatoms will be found in the chapter devoted to mounting.

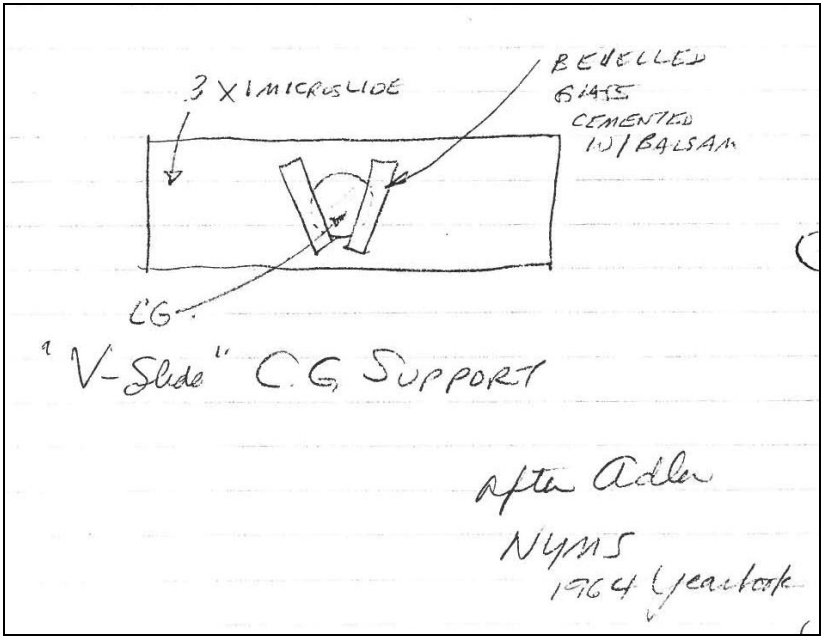


Figure 85.

Instead of spreading diatoms on microslides, they are often advantageously spread on coverglasses. Adler has described a "V"-slide coverglass support for handling of coverglasses for this purpose. An illustration is provided in Figure 85 that is self explanatory. Instead of dropping the suspension on a microslide it is dropped on a clean circular coverglass and fitted into a "V-slide" which can then be handled conveniently in subsequent transfer operations to a store slide.

All spreads should be labeled and dated. As complete information as it is possible to include in the label space should be provided. A number system will allow reference to file cards with more complete information, and for the purpose of maintenance records of such storage.

The spread slide (or coverglass) contains clean diatoms in all manner of physical condition, with debris and possibly sponge spicules, radiolaria, etc. The diatoms on the slide may be whole frustules, broken or cracked, in separated valves, girdles, etc. The next step provides for picking and storing the desired material for future mounting. The most perfect diatoms, or those of particular interest, or their parts, may be selected and stored for future treatment.

Essentially, the procedure is to pick, with the aid of a bristle (usually manipulated by a mechanical finger), and transfer diatoms to a store slide (or coverglass). It is a tedious activity at best, and a number of techniques and special apparatus have been devised to accelerate the process.

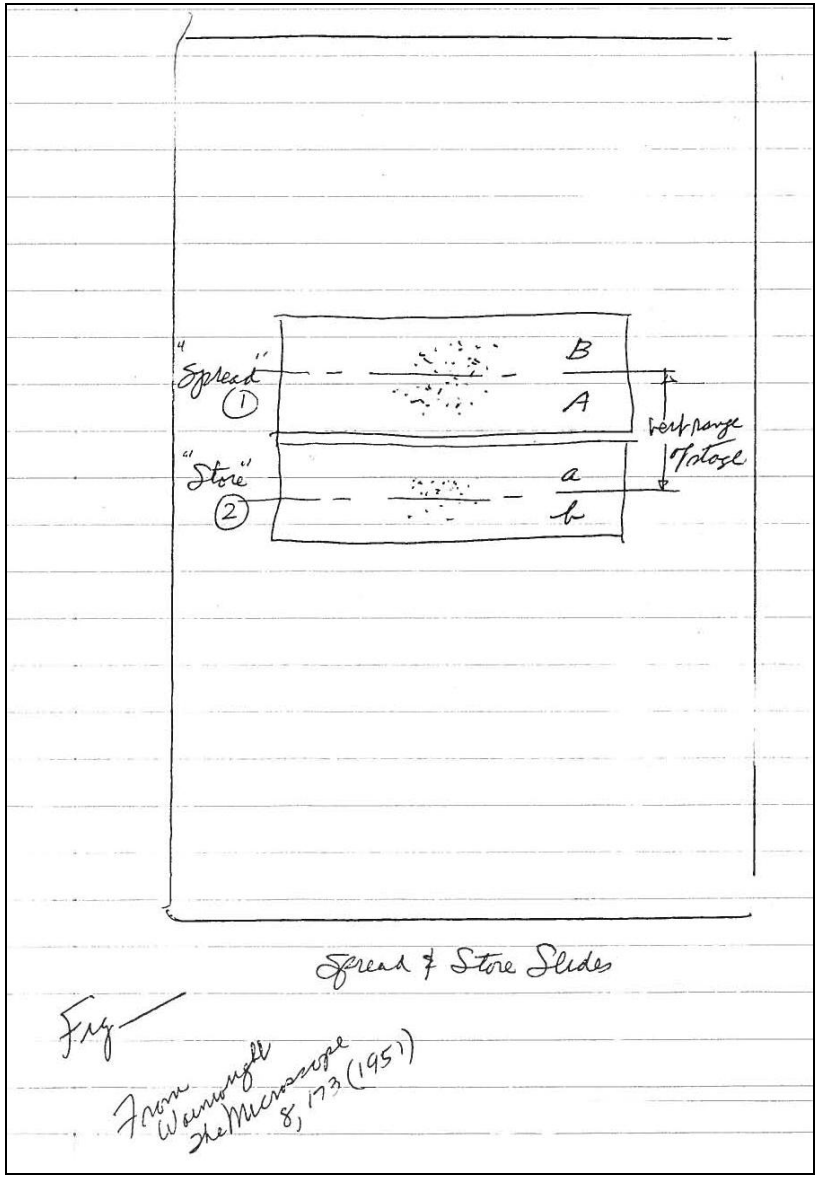


Figure 86.

Carrying the spread and store slides together on the microscope stage, while selecting from the spread is a great advantage. Wainwright (1951) described a simple technique in selecting diatoms from spread slides, involving only a slight movement of the mechanical stage. The scheme can also be used in making selected permanent slides. The range of vertical (Y-direction) movement of the average mechanical stage is about one inch. Referring to Figure 86 the procedure in making transfers from spread to store slide is as follows. Transfer A to a, invert 1 and transfer B to a. Then invert 2 transfer B to b and so on. The store slide receives only those diatoms considered suitable for mounting.

One final note on this procedure. When examining and selecting diatoms from the spread slide and/or from a store slide, and thereby necessarily working with uncovered specimens, an additional precaution will be of advantage. A cover glass of either 0.18 mm. or 0.17 mm. thickness is used (dependent upon the objective correction), being fastened with an adhesive to one end of a paper tube which can be slipped over the objective. This will be found to be an advantage in providing better resolution for objectives with a numerical aperture of more than 0.3. Although much of the selecting and transferring may be carried out with a 10X objective of 0.25 N.A., it is at times advantageous to examine a diatom with a high-dry objective for identification or other purposes. It is for this latter situation that the coverglass is recommended.

A number of unique and specialized devices are described in the chapter on mounting which facilitate rapid and exacting transfers of diatoms from one location to another.

5.8. Notes and Techniques

As in the cleaning process there has grown, over the years, a voluminous literature of ingenious methods and apparatus to deal with washing, separating, and storing diatoms. A good part of the literature is referenced in the Appendix. In the following paragraphs, a brief selection is presented that supplements the preceding material of this chapter.

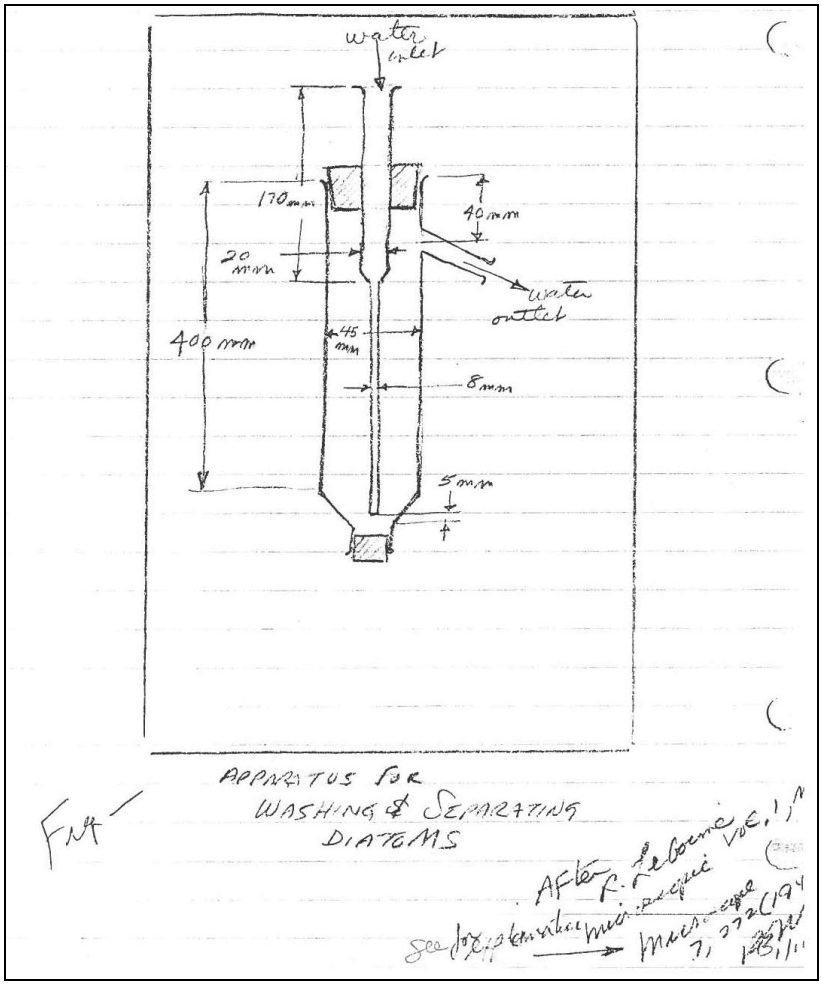


Figure 87.

Figure 87 illustrates an apparatus for washing and separating diatoms after R. Leboime. The diatomaceous material to be treated is placed in the 45 mm. diameter tube and water is supplied at a rate of about 7 cc. per minute. The water fills the large tube and leaves through the sloping outlet. The turbulence created by the continuous flow cleanses the diatoms in about 2 or 3 days. The method may be accelerated by increasing the flow of water to 10 or 15 cc. a minute. Diatomaceous earth, previously broken up into a powder or sludge is particularly susceptible to such treatment.

When diatoms stick to a spread slide and become difficult to pick, breathing slightly on them with moist breath sometimes frees them.

A diatom may be cleaned by breathing heavily on a clean microslide with moist breath. The diatom is pushed through the accumulated moisture with the mechanical finger bristle. turning it over if necessary. If there is difficulty in lifting a diatom, push it to the edge of the microslide and lift with the bristle from underneath. If it

refuses to leave the bristle, lower it to be just off the glass and gently brush it off with a spare bristle in a hand-held holder.

Effective separation of diatoms from sand grains can be accomplished by swirling. A small amount of the material is placed in distilled water in a beaker. The beaker is moved about an imaginary center creating a small vortex which by its action deposits heavier sand grains etc., in a little mound in the center bottom. Pour off the supernatant quickly after about a 30 second period.

For larger forms separate by boiling material in water to which soap has been added. Flocculent material will become suspended and larger diatoms will settle. However, small diatoms may become entrapped and not settle.

Any remaining (after cleaning) carbon, soot, coal dust, etc., can be removed by gentle fusion of cleaned diatoms with pure potassium nitrate in a small crucible. The melt is dissolved in warm water, sifted, and finished as usual.

Fine sand remaining after cleaning can be separated as follows.

Leave about $^{1}/_{2}$ to $^{1}/_{3}$ inch of water above the diatoms in a jar. Turn the jar on its side and very gently swing it to and fro, moving it about a half inch each way. The diatoms will swing with the water, while the sand will accumulate at the nodes of the wave action. With one gentle but quick movement, pour off the suspended forms into a small bottle.

Retain the sand for further inspection. This procedure is a variation of “swirling” as explained above.

END OF PART II

PART III

STUDY METHODS

CHAPTER 1.

1. INTRODUCTION

The study of diatoms requires the usual activities associated with the study of any life form. Observation, examination, identification, interpretation and conclusions are all dependent upon a basic familiarity with the subject and upon the techniques used to yield meaningful results. The learning process involved in the study of diatoms includes familiarity with the literature and it's special terminology. It also requires certain technical knowledge and ability to measure, count, illustrate and otherwise describe diatoms and their characteristics. Diatoms, which are probably among the very smallest structures exhibiting a defined pattern, are studied almost exclusively with the microscope. At present, as in the past, a great proportion of that study is by the use of the light microscope. It is inconceivable that a diatomist would not be an accomplished microscopist. It is essential, in this very narrow field of study, that the diatomist be completely familiar with the basic fundamentals, operation, and limitations of the instrument that provides nearly all of the information he obtains about his subject. It is the purpose of this section to provide the methods and techniques for the study of diatoms with the microscope. In general, they may be applied regardless of the specialized scientific field connected with diatom study. Some techniques or methods of study; included in these chapters, are those used within certain scientific disciplines, but which in most cases could be applied in other areas of study as well, if not across the board. In addition a number of techniques are described that are used in special areas to provide the student an overview of the wide range of methods used in the study of these important plants. It would be extremely presumptuous for one author to attempt to set out the procedures for the various scientific fields in studying diatoms. Each of those areas requires the knowledge and experience of an expert. However, a brief and admittedly superficial, discussion of diatom study in several major scientific fields is provided in these chapters. It is included mainly as an attempt to indicate the very wide range of diatom study application, and to stir the interest of the student in taking up a specialized area of study.

1.1. Literature

Literature on the diatomaceae is voluminous, and almost any and every aspect of diatom study is represented. The majority of the material, especially that of the past, is devoted to the description and identification of the diatom frustule, upon which classification is largely based. The biology and ecology of diatoms has, only in the comparatively recent past, been treated extensively in larger works. Likewise, the

importance of diatom research in connection with geological and paleontological problems, has only recently been accented by workers in those fields, and now appears with increasing frequency in the subject literature.

The beginning diatomist needs, primarily, works devoted to description and identification, and secondly those treating the biological and other aspects of diatom study. It is the purpose of this section to provide a brief description of the literature recommended for these purposes. As the uninitiated will not be familiar with the various works, or of sufficient experience to evaluate the suitability of one work over another, an attempt to do so will be made here. The reasons for such selections will be set out in considerable detail, so that the beginner will have a basis for making future literature selections of his own.

For the beginning diatomist, the most important attribute of a work of reference for identification purposes is the number of figures or plates, and the quality of illustration. Regardless of the language of the reference, whether it be English, French, German, or another, the illustrations are most important and they will be identified in a common Latinized scientific nomenclature. The greater the number of figures and/or plates the better, as there are literally many thousands of diatoms and the fewer references necessary to be handled and searched the easier and faster an identification can be made. A few reference works rely almost entirely on the illustration to describe the diatom and, if the quality of illustration is superior, the identification may be made almost on a comparison basis with what is seen in the microscope. However, these types of references are in the minority, most illustrations being accompanied by a written description as well. As is often the case, the reference work is in a foreign language, especially insofar as an English speaking student is concerned. Diatom literature appears in many languages including but not necessarily limited to English, German, French, Swedish, and Russian. The preponderance of references in the German language with English running a poor second and with perhaps French or Swedish in third place. Russian-language publications in the diatom literature are growing at a comparatively rapid rate. About a third of the extant taxonomic on diatoms is in the German language and about 20% (one fifth) in English.

Therefore an English speaking student, with some familiarity with the German language, has ready access to more than 50% of the information, no mean advantage in the study of such an extensive literature.

Fortunately, the descriptions associated with figures, or other illustrations of diatoms are presented in a more or less standard format of presentation. Even though one is not familiar with a foreign language it is not difficult to follow most of the brief descriptions. A small dictionary for the language in question is sufficient to make the descriptions clear, and after some experience, one becomes quite proficient in a number of languages (without reference to a dictionary) insofar as diatom description is concerned. Text material, however, is quite another matter and reading and understanding that type of material requires more than “dictionary” knowledge of the particular language. English-speaking students who will obtain e reading knowledge of German, are actually quite well equipped to avail themselves of the mainstream of diatom publication. Other languages can be dealt with on a dictionary

basis for the most part (insofar as identification and description are concerned), including Russian.

Some familiarity with the Cyrillic alphabet, a small Russian/English dictionary, and a little experience, will enable the student to sample some of the content of these important contributions.

There is still, after many years of publication only one comprehensive work in the English language on the diatomaceae. That reference is *A Treatise on the Diatomaceae* by Henri Van Heurck translated by Wynne E. Baxter. It contains introductory remarks on the structure, life history, collection, cultivation, and preparation of diatoms in addition to a description and figure typical of every known genus, as well as a description and figures of every species found in the North Sea and countries bordering it, including Great Britain and Belgium. The illustrations are comprised of about 2000 figures, some of which are in the text but mostly in 55 plates at the end of the book. Each plate is accompanied by a facing legend-list of the figures contained thereon, but descriptions are referred back into the text. This scheme requires some constant flipping back and forth from text (which page(s) are referenced in the legend) to plate figures when studying a particular diatom.

The figures in the 35 plates are not of the best quality and, in general, are too small. Text figures are as a rule, larger, better drawn, and easier to use.

As this work is a translation of one by Van Heurck in 1896, it is considerably out of date in many respects. This is particularly true of some of the biological concepts, cleaning techniques, and in taxonomy which is under constant revision. However, despite these shortcomings, it remains unique in diatom literature in its scope of treatment within the confines of a one-volume reference.

Wynne Edwin Baxter FRMS, FGS LL.B (b. 1st May 1844 Lewes, Sussex d. 1st October 1920 in Lewes, Sussex).
English Lawyer, Coroner, Translator, Antiquarian and Botanist.
Conducted the inquests on three victims of Jack the Ripper in 1888.

Since this was written a number of more comprehensive publications have found their way to market and with the advent of the internet ready access to diatom databases has become available to all. Printed publications are still, however, an expensive luxury.

It is particularly useful as a first reference for the amateur diatomist, providing a broad base of information on diatoms in general, and as a useful, though limited, identification source. It is recommended as a first book of reference for the beginning amateur diatomist.

The above is still true and is still used as a reference by many.

From the standpoint of the beginner, the next most useful diatom reference is *Die Susswasser-Flora Mitteleuropas* by Friedrich Hustedt. Although this little volume is in the German language and its content is restricted to the freshwater diatomaceae, it is highly recommended. Often the beginning diatomist has easier access to freshwater diatoms, and he requires very good illustrations for identification purposes. This book fills those needs admirably. The illustrations (875

of them) are included in the text, usually adjacent to or near the description, are large, and of excellent quality. Hustedt is renowned as one of the most prolific and authoritative contributors to diatom literature, and his drawings and illustrations are meticulous and dependable. For those with a reading knowledge of German, the first part of the book contains a very excellent brief treatment of diatoms in general. Their biology, morphology, classification, culture, and preparation for study are well covered, in a general way, in the first 80 pages. The remainder of the work is devoted to the illustration and description of freshwater diatoms. To the beginner it may seem that a work on freshwater diatoms of Central Europe would be useless in identification of diatoms in the United States for instance. However, as pointed out earlier in this book, the diatom in general is ubiquitous and although there are definitely certain ones appearing in only certain localities, that is the exception rather than the rule. The identification of a *Cymbella* sp. from a source in the United States is oftentimes just as easily done from the Hustedt reference as from a work directly related to the United States. Of course, regional considerations are of some importance to a specific diatom research project, and regional or local references are then indispensable. However, for general identification, diatomists at all levels utilize worldwide references as a means of identification.

Frederick Beatson Taylor (b. circa 1851 Dinapur, India, d. 22nd January 1931 Bournemouth)

Another work the beginner should have is *Notes on Diatoms* (1929) by F. B. Taylor. Although this little book was privately published essentially for the amateur, it contains material to be found nowhere else in such compact form. It is long out of print and difficult to obtain, but should be at the right hand of every amateur. The usefulness of the work is evidenced by its continuing appearance in the bibliography of many contemporary professional scientific papers. It is basically an introduction to the study of diatoms, providing in one small volume the essentials of the subject. It is not a book meant to be used primarily for identification purposes, although there are five figure plates. The first four plates are line drawings of rather poor quality and the fifth photomicrographs of good quality. The usefulness of the volume is largely in its extensive notebook approach and exposition of gem-like information immediately useful and pertinent, especially for the beginner. It is highly recommended.

Adolf (Adolph) Schmidt
Born 12.8.1812 Berlin
Died 25.6.1899
Aschersleben

Friedrich Fricke
Born 25.3.1863
Bremen
Died 20.1.1926
Bremen

George Ferdinand Otto Müller
(1837 – 1917)

For purely identification purposes, for the diatomist at any level, the outstanding reference is Schmidt's *Atlas der Diatomaceenkunde*. The most important single reference of its kind, the number, size, and quality of the illustrations are in themselves sufficient justification for its acquisition and use. The long list of famous diatomists who have contributed to it provides an authoritative stature to this atlas not equaled by any other. This monumental work was initiated by Adolf Schmidt, a Canon of Aschersleben, in 1874. It was continued by his son Martin Schmidt, Friedrich Fricke, Heinrich Heiden, Otto Müller, and up to 1959 by Friedrich Hustedt. Others who assisted in the preparation of this work included such other

famous diatomists as Grundler, Grunow, Janisch, Weissflog, Witt, and Cleve.

Otto N. Witt
(circa 1852 – 1915)

Eugen Weissflog
(1822 – 1898)

There are a total of 469 Plates numbered 1-480 (plates 421-432 were not issued). The size of the plates is folio (approximately $8^5/_8$ inches by 12 inches to the figure-including border). In only the first 212 plates (prepared by A. Schmidt) there are 9000 figures. The figures are large magnifications using approximately from 600 diameters to 1000 diameters, and as a rule excellent for identification purposes. The figures stand by themselves with no additional description being furnished. However, legends accompanying each plate occasionally contain comments or remarks on various details of the figures. Originals of the Atlas are virtually impossible to obtain. Photographic copies have been made in the past by individuals and are very useful. Even photo-copies reduced to one-half size (one-fourth the area) have been made and are very useful even with the size reduction, as the original illustrations are very ample. Anyone seriously engaging in the study of the diatomaceae should have the Schmidt atlas in one form or another. Recently, full-sized facsimile copies have become available, but they are very expensive, costing hundreds of dollars.

Carl Janisch
(1825 – 1900)

Albert Grunow
(1826 - 1914)

Indices to the Atlas have been made, the first one by Fricke covers only the first 240 plates. A comprehensive index has been prepared by Hanna, *Index to Atlas der Diatomaceen-Kunde*, and covers all existing plates of the atlas. It also contains a very useful index to the genera of diatoms illustrated in the Atlas, by plate number. This Index should be in the possession of all who use Schmidt's atlas.

Gottlob Ludwig
Rabenhorst
(1806 – 1881)

A very important reference for the diatomist is *Die Kieselalgen* von Friedrich Hustedt, Band VII of Dr. L. Rabenhorst's *Kryptogamen-Flora von Deutschland, Osterreich und der Schweiz*. Hustedt's work is uncompleted, in three parts, comprising 920 pages in part 1, 845 pages in part 2, and 816 pages in part 3. His death in 1968 terminated work on this important contribution to the literature. Illustrations (numbering 1788) are included in the text rather than in separate plates, as is the case with most such works. They are large and excellent. Hustedt's work on the very small diatoms, especially on the *Fragilaria* and similar sized genera, is very important and well represented in this reference. Indices are provided for parts 1 and 2, but not for part 3, which is entirely devoted to the *Navicula*. Much of the illustration in this latter part has been enhanced by the use of photomicrographs.

Of the older references, one which is of extreme usefulness in identification, is *Synopsis des Diatomees de Belgique* by Henri Van Heurck (1880-1885), containing 141 plates. The figures are numerous and well executed, but this reference is very difficult to obtain and then at a high cost. Copies of the plates only are sometimes available and are useful by themselves.

Hippolyte (b. circa 1851) and Maurice (b. circa 1853) Peragallo

Diatomees Marines de France by H. et M. Peragallo is very valuable to the beginner as well. It is in two parts - a text and an atlas of 137 plates. The atlas can be used by itself as it contains the figures on plates, accompanied in each case by a facing legend page. The text is cross referenced to the atlas and provides word descriptions of the figured diatoms. In French, originals are difficult to come by, but reprints of this important work appear from time to time, although they are relatively expensive. Illustrations are excellent.

Of the modern or more recent contributions to diatom literature, *The Diatoms of the United States* by Patrick and Reimer is highly recommended. Two volumes have thus far been published with one or two more to follow. The illustrations (64 plates in volume 1 and 28 plates in volume 2) are excellent, being executed with an attention to detail that is remarkable.

For quality of illustration and ease of use, *Diatomaceen van Nederland* by A. van der Werff and H. Huls is outstanding. Each diatom treated in this work is on a separate one-sided page complete with one or more superb line-drawn illustrations. Also, in this reference there are more girdle views and other viewing aspects of diatom frustules than are ordinarily encountered in most of the literature. There is no index provided, however it is not difficult to compile one.

At this point it would be amiss to not at least mention one of the newest diatom references. *The Diatom Flora of Southern Africa* by Schoeman and Archibald which has only recently made its appearance Only the first few plates, of a projected large work, have been thus far completed. It promises to be an outstanding reference for the diatom worker. The most notable feature of the presentation is the very liberal use of illustrations, and in particular the large number of TEM and SEM micrographs. The latter enhance and reinforce the other accompanying descriptive material which includes a comprehensive description including comments on and distribution of the diatoms in South Africa. A list of references also accompanies the material. Illustrations include line drawings, bright and darkfield light microscope photomicrographs, as well as photomicrographs made with the aid of phase contrast microscopy, in addition to the electron micrographs mentioned previously. Some diatoms are described by as many as 18 such illustrations, all of the highest quality. The format is on large (approximately 8½ x 12 inches) pages of high quality glossy paper, each set exclusive to each diatom described.

In the identification of diatoms, as can be seen by the described references, a great number of plates, figures, and word descriptions are necessarily referred to. In even a modest diatom reference library, the figures run into the many thousands, and considerable time can be spent searching and comparing appearances under the microscope with those of the literature. To assist in shortening the search time, use of the indices of individual works is necessary, However, some works have no index, and the very large number of separate references in itself poses a problem. Comprehensive lists of diatoms alphabetically by name, have been prepared that indicate sources of descriptions by page and illustrations by plate and figure number. The most well known of these lists is the one prepared by Frederick Wm. Mills entitled *An Index to the Genera and Species of the Diatomaceae and their Synonyms*. Completed in the 1930's it has been out of print for many years and is considerably outdated. However, if a copy of Mills Index can be obtained, it will be found to be of very great assistance in diatom identification work.

Frederick William Mills, F.L.S., F.R.M.S. (1868-1949).

Currently in preparation is the monumental *Catalog of the Fossil and Recent Genera and Species of Diatoms and their Synonyms* by Sam L. VanLandingham. To be composed of eight volumes plus an Addenda it will be the most comprehensive catalog of the sort to ever be published. VanLandingham has revised and updated Mills listings, made corrections and additions to it and carried on listings from where Mills left off. His work is not merely a revision of Mills Index, but such an improvement in the listing of taxa and their synonyms from 1786-1964, and in the inclusion of more than 4000 omissions, that it certainly can be considered an independent and superior work. When completed, the *Catalog of Diatoms* will be the most useful single tool of the diatomist, and can be considered one of the most important and far reaching contributions to diatom literature of recent times.

Sam Leighton VanLandingham (b. circa 1935)

In addition to identification of diatoms, which subject the former references largely deal with, the biology and ecology of this algal form are of interest to the beginning student. Unfortunately, the literature of the past in this respect has been very widely dispersed in hundreds of individual papers; and articles, in as many publications, making it difficult to gain any kind of consolidated view of the subjects. The previous references discussed, do in many cases, include an abbreviated introduction to these subjects, although their mainstream of effort is floral listings for specific localities.

For a good background on current thought on diatom biology, *The Biology of Diatoms* edited by D. Werner is recommended. It is a one volume work, in English, covering the various aspects of diatom biology in separate chapters, each written by a specialist. The range of subjects includes growth and culture, ultrastructure, metabolism, photosynthesis, nutrition, biochemical composition, movements, sexuality, and the ecology of freshwater and marine forms.

Dietrich Werner b. 1938

For those with a reading knowledge of German, *Der Okologie der Diatomeen in Binnengewassern* by Cholnoky is one of the best one volume treatments of freshwater diatom ecology. Although devoted to African locales, the treatment, including techniques and many of the conclusions, is applicable and pertinent, in a general sense to any ecological study in any part of the world.

Astrid Maria Cleve-Euler
(1875 – 1968)

Two other works for identification purposes should be obtained by the beginner if possible. The first is the monumental *Die Diatomeen von Schweden und Finnland* by Cleve-Euler. Although many of the illustrations in this publication are not of the best quality, the large number of them makes this an important work.

The drawings and photomicrographs are from many different sources and the quality varies from poor to excellent. There are more than 1500 figures in many plates, grouped into various sections. The legends for the plates or figures are not located adjacent to them, but a long list of names immediately precedes the figures. The actual word description and other information for the illustrations is in a separate part of the text. This latter information is in German and in most cases very sketchy. Multiply this arrangement by 4 different sections and the use of this work becomes troublesome, and even difficult in some cases, to use.

The other reference of note is in *Introductory Account of the Smaller Algae of British Coastal Waters* by N. Ingram Hendey. This particular work is outstanding in its excellent illustrations, mostly photomicrographs. There is no other work, in this author's opinion, that provides such high-grade photomicrographic illustrations. They are superior in resolution and contrast to any others generally available, and appear in 45 plates at the end of the book. This work also contains some excellent introductory material in addition to the usual taxonomic information relating to the plate/figure illustrations.

With the preceding volumes at his disposal the beginning student of diatoms is well equipped. There are other references, many of them, that are also very desirable, but these given will provide sufficient working references for a beginning student and around which to build a more complete diatom reference library. A listing of these and other selected reference works is included in the Appendix.

In the use of diatom literature it will be immediately apparent that there are many synonyms of diatom names. VanLandingham's Catalog will assist in sorting out the hundreds and hundreds of these confusing entries. Many references list the major synonyms at the beginning of each taxonomic description, along with the original reference with which it is associated, providing a kind of cross reference to other literature on the same diatom, although it may have been named quite differently.

Translation of diatom descriptions is aided considerably by the use of appropriate language dictionaries. The small limited-sized dictionaries are, for the most part, satisfactory. An outstanding dictionary for the German language that is highly recommended is the German-English Science Dictionary by De Vries. This little book is of great assistance, containing many scientific terms, and has a section at the beginning devoted to notes for translators. One final comment on the literature applicable to the beginning student. It is highly recommended that any student of

diatoms become acquainted with frustular morphology to as great an extent as possible through the SEM micrograph. Although most of diatom study will be via the light microscope, the SEM micrograph provides a dimension, an overall viewpoint, on diatom microstructure that is otherwise easily misinterpreted by light microscope observation, especially by a beginner. If diatomists of 50 or 100 years ago had had the advantage of what the SEM micrograph reveals about structure, many misinterpretations, promulgation of errors, and bitter disputes could have been avoided. There is a growing use of the electron microscope in diatom research and many recent publications are replete with electron micrographs, both TEM and SEM. Dr. W. W. Wornardt has published a number of papers on diatom research with the SEM, and the many micrographs he has produced are excellent familiarization material for the beginning student.

Walter W. Wornardt
(b. 1934)

CHAPTER 2

2. MICROSCOPE EQUIPMENT

In such a limited space it is not possible to provide an extensive treatment of microscopical theory, technology, and practice. However, the light microscope is so important to diatom research that at least some fundamental facts concerning it in both theory and practice need emphasis. Also, the equipment requirements for satisfactory diatom study are in need of delineation. The importance of proper objective selection, fundamental adjustments, and the wide range of techniques now available with this instrument cannot be over-emphasized if satisfactory work with diatoms is to be accomplished. The proper interpretation of microscopical images is intimately associated with an adequate knowledge of microscope optical performance including optical artifacts, and component limitations. Improper adjustment of the instrument can lead to false or confusing images that are incorrectly interpreted by the observer. The human eye is easily fooled, and the interpretation of what is seen with the microscope is dependent entirely on knowledge of the subject and the capabilities and limitations of the instrumentation.

2.1. Objectives

2.1.l. Basic Information

The objective of a microscope determines the resolution capability of the instrument. It's degree of correction for color and other lens aberrations determines the excellence of performance in providing maximum resolution with accompanying sharpness and contrast in the microscopical image.

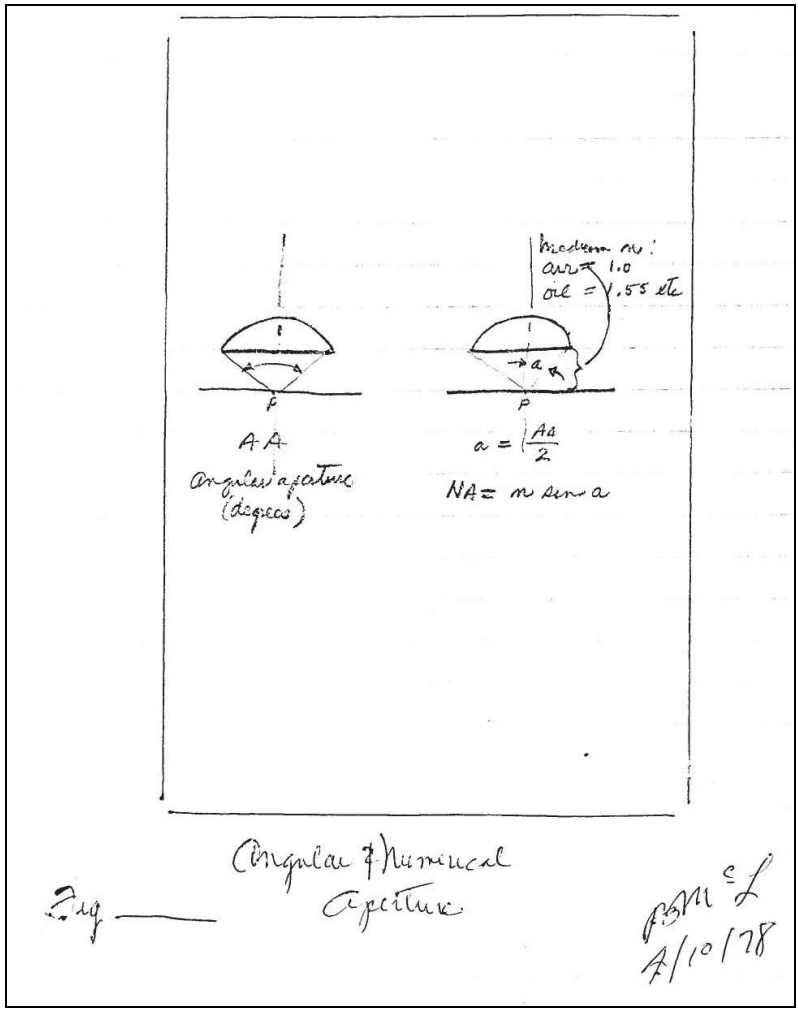


Figure 88.

With monochromatic lighting conditions the resolution of a lens is:

$$d = \frac{1.22C\lambda}{N.A}$$

Where

d = separation distance of two self-luminous points in the object plane.

C = a factor varying between a minimum of 0.4 and 1.0 depending on such factors as the correction of the objective, and the individual capacity of the observer to detect minute differences in intensity.

Lambda (λ) = wavelength of the illuminating light.

N.A. = numerical aperture of the objective.

1.22 = a constant.

If objectives and condensers of the highest. perfection are used the factor C can be assumed to be slightly more than 0.4. If this becomes the case, then the equation reduces to

$$d = \frac{0.5\lambda}{N.A} \text{ or } d = \frac{\lambda}{N.A.}$$

and the minimum separation distance to be resolved with light of approximately 0.55 micrometer wavelength (λ) and an objective of 1.25 N.A. is about 0.22 micrometer. Objectives of slightly higher N.A. (1.40) and light of somewhat shorter wavelength can provide a resolution figure of somewhat less than 0.2 micrometer which is the generally accepted limit for the visual light microscope.

It is seen then that the two factors determining resolution are the N.A. of the objective and the wavelength of light used. White light is that normally used in examining most microscopical objects, especially when they are stained and/or color is an important part of the image interpretation. For a given wavelength of light (λ) the objective with the highest N.A. will provide the greatest resolution (separation of points in the object plane).

While mere separation of points in the object plane is dependent upon only the two factors above (λ and N.A.), the characteristics of the points (sharpness, color, symmetry, etc.) are dependent upon the degree of correction the objective has received for chromatic, spherical and other lens aberrations. In other words, two objectives of the same N.A. (and under the same lighting conditions) will have the capability of providing separated images of two points in the object plane spaced the same distance. However, a poorly corrected objective will provide a fuzzy, distorted image of the points, while a highly corrected one will show the points sharply and well defined one from the other. For the best image microscopically then, one should choose objectives with a high numerical aperture which have been highly corrected for aberrations. What is a "high" N.A., and how does one tell how highly corrected an objective is?

Numerical aperture (N.A.) is the most important optical parameter of the objective. It is the determining factor for the relative brightness of the intermediate image (that formed by the objective itself) and is valid for any medium of given refractive index, adjacent to the objective. The brightness of the image formed by an objective at a given magnification increases with the angle of the cone of light collected by the objective. The numerical aperture mathematically is *n* sine *a*, expressing a value obtained by multiplying the sine of one half the angular aperture (*a*) by the refractive index of the medium between the front lens of the objective and the coverglass. With considerations involving physical optics, which are beyond the scope of this treatment. It can be rigorously shown that the magnitude of the smallest detail which can be reproduced in the image formed by the objective, varies inversely as the N.A.

Objectives are designed for image formation under two general conditions. The first, where air is adjacent to the front lens, is called a dry system. The second, wherein

the objective is designed for immersion into a medium of higher refractive index than air, is called an immersion system.

The N.A. of any dry system is always smaller than 1.0. The highest N.A. of commercially produced dry systems is 0.95. The range of the N.A. of dry system objectives used by most microscopists, is from about 0.05 to 0.65. A high N.A. for a "dry" objective would be from 0.65 upwards to the commercially practical limit of 0.95.

Immersion system objectives run as high as 1.40 N.A. but that is indeed exceptional and most available are from 1.25 to 1.30.

The corrections applied in the design of objectives are largely associated with spherical aberration and color. That is, the design is such as to attempt to provide the same focusing characteristics for light across the visible spectrum. Practical design falls short of this ideal, but is applied to produce three generally available objective design types; the achromat, semi-apochromat, and the apochromat. It is pertinent to briefly state what degree of correction is applied in these cases as a basis for eventual objective selection.

2.1.2. Types

2.1.2.1. Achromat

The achromatic objective is corrected for chromatic aberrations at two wavelengths, one in the red and one in the blue, and is fully corrected for spherical aberration at only one wavelength in the yellow-green (D line). At other wavelengths in the visible spectrum this correction for spherical aberration is good but not complete. Field curvature is present.

2.1.2.2. Semi-apochromat

Fluorite lenses are combined with glass to a limited degree to obtain a compromise between the achromat and apochromat in performance. Generally they are also corrected for chromatic and spherical aberration. Field curvature is present.

2.1.2.3. Apochromat

The apochromatic objective has several fluorite lenses in combination with glass lenses, achieving correction for chromatic aberrations at three wavelengths, in the red, green and blue respectively, and for spherical aberration throughout the visible spectrum to a greater degree than with the achromat. This type of objective is usually higher in numerical aperture than those of corresponding magnification in the achromats. Curvature of field is present. It is to be noted that apochromats are not well corrected for lateral color, and therefore require special oculars to compensate for that shortcoming.

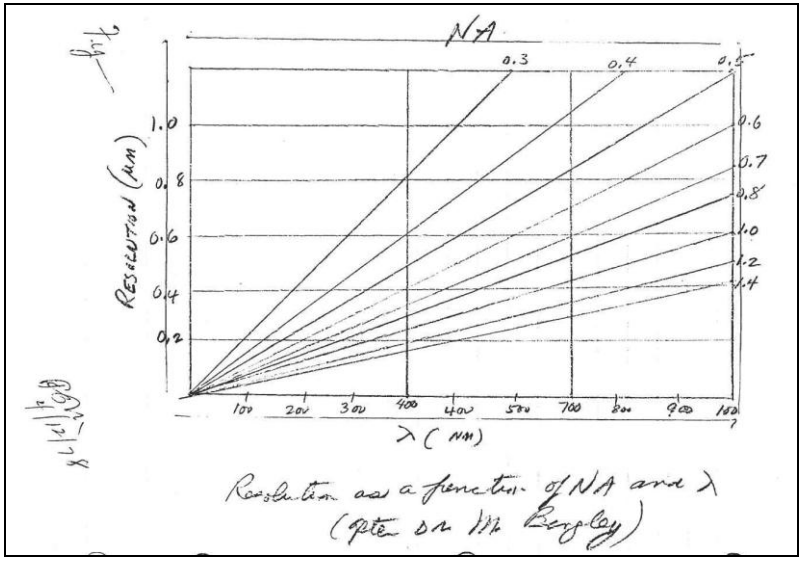


Figure 89.

Referring to Figure 89 the relationships of resolution, numerical aperture, and the wavelength of light used are shown. Since much of the work with the light microscope on diatoms is the examination of the frustule, color in the image is of minor importance. Considerable advantage is gained by using monochromatic light. If light in the yellow-green is chosen (for instance) to illuminate the subject matter, an achromatic objective, which costs but a fraction of an apochromat, can be used to yield nearly the same resolution (N.A. being the same) and quality of image. The latter factor, at and around the "design center" of the achromat, is very nearly equal to that of the apochromat. Most objectives are designed, insofar as the basic index for ray tracing and specification of focal length, at or near the Fraunhofer "D" line. The peak of the visual brightness curve also occurs near this point in the spectrum. Therefore, a filter which would provide yellow green light for illumination will be of considerable importance for yielding maximum resolution and quality of image with an attendant benefit to the human eye. More of this later in choosing filters for diatom work.

From Figure 89 it is also evident that as the wavelength of the illuminating light (λ) becomes shorter (more towards the violet end of the spectrum) the resolution improves (for a given N.A. of an objective). Here again, the importance of the illumination wavelength used is emphasized. The advantages of illumination of diatoms in the near ultraviolet will be discussed later.

Of course, there are conditions under which white light, or some wide band of light frequencies, are particularly useful in diatom work. Those conditions might be interpretation of living diatoms and their contents, or in the studying of refraction and diffraction effects of diatom frustular microstructure, or in the interpretation of stained diatoms . In those cases the more expensive apochromats are unexcelled in quality of image. For certain kinds of high resolution microscopy in the study of

diatoms there is no substitute for the most highly corrected objectives one can obtain.

However, with some knowledge of objective design and performance, a very great preponderance of diatom research may be accomplished with the much more economical achromatic objectives.

One more factor is extremely important in selecting objectives. That is the matter of mechanical tubelength for which the objective is corrected. The two most common are 160 mm, and 170 mm. Some specialized objectives are made to work at other tube lengths, but these two are by far the most commonly encountered, with most being 160 mm. E. Leitz until very recently, made all of their objectives to work at 170 mm. tubelength. The tubelength is usually marked on the objective barrel in some way. The importance here is that the various aberrations which are corrected in design, are corrected for one tubelength only. If an objective is used with a tubelength different than for which it is designated, the quality of the primary image will be adversely affected, primarily in sharpness due to a resultant under or over correction of spherical aberration.

Table 5

TABLE

EQUIV. FOCUS		TYPE				NA	APPROX INITIAL MAG (16 mm TL)	WRKG DIST IN mm	ACTIVITY
MM.	INCHES	DRY	OIL	ACHRO	APO				
32	1¼	✓		✓		0.10	5X	30	SEARCHING, MANIPULATION, MOUNTING
25	1	✓			✓	0.15	7X	18	SHOWING COLORS (w/ HIGH PWR OCULAR)
16	⅔	✓		✓		0.25	10X	8	SEARCHING, SORTING MANIPULATION, MOUNTING, DARKFIELD
8	⅓	✓		✓		0.50	20X	2	SORTING
4	1/6	✓		✓		0.65	40X	0.55	STUDYING FORMS, DARKFIELD
4	1/6	✓			✓	0.95	40X	0.20	STUDYING SMALL FORMS, CORRECTION COLLAR ADJ.
3	⅛	✓			✓	0.95	60X	0.13	STUDYING SMALL FORMS, SECONDARY DETAIL
2	1/12		✓	✓		1.25	90X	0.11	SECONDARY AND OTHER FINE DETAIL
1.5	1/17		✓		✓	1.30	120X	0.08	" " " MAX. MAGNIFICATION

2.1.3.For the Study of Diatoms

The microscope is used in all aspects of diatom study from preliminary examination to detailed study of temporary or permanently mounted specimens. Table 5 suggests what magnifications, and in some cases what type of objectives are most useful in various activities.

A battery of eight or nine objectives will serve the diatomist well, although fewer than that will be satisfactory if selected judiciously. For instance, the 5X searching and manipulation objective is desirable but not an absolute necessity. The 10X lens can serve for searching, manipulation and mounting, although considerably less

working distance is available. It will be found that the 10X objective (with appropriate 10X oculars) is the most versatile for the diatom worker. The darkfield indicated in the table is the simplest arrangement, using a stop beneath the substage condenser. More complete information on darkfield using specialized apparatus will be dealt with later. The 40X objective serves as the single most important objective for general examination of diatoms. It is used to examine and/or study large and small forms and general features of most diatoms, and much in the way of identification work is accomplished using this lens.

For detailed study of very small structure and the analysis of smaller or more delicately sculptured diatoms for identification, an oil immersion objective is mandatory. The highest magnification obtainable with the light microscope is of use in diatom study. More of this later when techniques and application of the instrument are covered.

As a minimum then, a 10X, 40X, and oil immersion objective of 90X or 120X should be available to the working diatomist. For best work the 40X should be an apochromat as should the oil immersion lens. However, if monochromatic light (yellow-green) is used, achromats will prove quite satisfactory. If the microscope used is a binocular instrument the 40X objective should be equipped with a correction collar if possible.

2.2. Substage Condensers

To obtain the ultimate in performance from any objective, the importance of the substage condenser cannot be overemphasized. This is particularly important when high N.A., highly corrected objectives are used. If a substage condenser cannot fill the objective completely with light, or it is in itself producing faulty illumination, then the most advanced objective cannot produce an image to the degree of which it is capable.

There are two generally available classes of condensers with which most microscopes are equipped. We will consider these two here in light of their use in diatom study. Other specialized substage condensers will be dealt with in following discussions on special techniques and illumination methods.

2.2.l. The Abbe Condenser

Ernst Karl Abbe
b. January 23rd, 1840
d. January 14th, 1905

Considerable aberrations are associated with this condenser, not the least of which is spherical aberration. Since it is not corrected for spherical aberration, the Abbe is unsuitable for very critical work with the microscope. It also suffers from chromatic aberration. In spite of these defects, it performs adequately for many types of routine work with the microscope, and has the advantage of being reasonably priced because of its simple construction (a plane-convex hemispherical upper element and a biconvex lower element). It will provide adequate service for most diatom study wherein achromatic objectives are in use, and its performance is much

improved by the use of yellow green light as well. With proper adjustment (to be covered later) and the proper wavelength of monochromatic light a microscope equipped with achromats and an Abbe condenser will serve adequately for most diatom work.

2.2.2. Corrected Condensers

The shortcomings of the simple two-lens Abbe have been overcome in a number of other designs. The best of these are the achromatic-aplanatic condensers, which incorporate a much more complex arrangement of lenses (5 or 6 elements) to accomplish their purpose, and therefore are correspondingly more expensive. A truly aplanatic-achromatic condenser is corrected spherically for the rays away from the axis, as well as the axial rays, and correction for chromatic aberration is such that it can be used with higher performance objectives such as the apochromats.

For critical work with diatoms, wherein interpretation of structure near the limit of resolution is necessary, the use of a corrected substage condenser in conjunction with apochromatic objectives is called for. The corrected condenser is practically a necessity in color photomicrographic work.

2.3. Oculars

Oculars are generally of three different types. The Huygenian, the most commonly used, the Ramsden, and compensating. All are generally available to the microscopist. The Huygenian design utilizes two lenses made of crown glass with a field stop located at the primary focal point of the eye lens which lies between it and the field lens. It is this field stop location where cross hairs or reticles are mounted. Reticles used with Huygenian oculars should be confined to the center of the field to minimize distortion effects. The Huygenian design as a whole is corrected for lateral chromatic aberration but the individual lenses are not, so that reticles seen through the eye lens alone may show considerable distortion and color. The design allows for some spherical aberration, astigmatism and a rather large amount of longitudinal color and pincushion distortion. As a general rule, the eye relief (the distance between the top of the eye lens and the exit pupil) is comparatively shortly ranging, between 6 and 9 mm.

The Ramsden design has more lateral color than the Huygens, but the longitudinal color is only about half as great. It has about one-fifth the spherical aberration, half the distortion and no coma. It also has the important advantage of about 50% greater eye relief than the Huygens. It is especially superior when using reticles for measurement purposes.

Christiaan Huygens, FRS (14th April 1629 – 8th July 1695) Dutch Mathematician, Astronomer, Physicist

Jesse Ramsden FRSE (6th October 1735 – 5th November 1800) English Astronomical and Scientific Instrument maker

Compensating oculars are usually based on either the Huygenian or Ramsden design, dependent upon the magnification. Low power compensating eyepieces follow the Huygenian design, and the high power ones

the Ramsden design. The important feature is that the eye lens is a doublet providing correction for lateral color by causing red and blue rays to emerge parallel so that they unite in a single color-free image on the retina of the eye.

With low power objectives (achromats) the Huygenian ocular performs well and because of its inexpensive design is the most used. However, when high powered achromatic objectives are in use, the compensating oculars can produce an effectively better image resolution-wise because they can correct for some residual color aberrations present in those objectives. For the apochromats, it is mandatory that a compensating ocular be used as in its design it is left to the compensating ocular to correct for lateral color aberration.

2.3.1. Monocular or Binocular

For diatom work a monocular microscope is satisfactory in most cases, and has definite advantages under certain conditions.

Using a monocular that is equipped with an adjustment for tubelength, very delicate refinements (to be covered later) can be made to minimize spherical aberration in the primary image, providing that extra sharpness required in interpretation of delicate diatom structure. The tubelength adjustment can be a sliding sleeve tube calibrated, or not, in millimeters. Very elegant old microscopes of the turn of the century (particularly English makes) were provided with a rack and pinion adjustment of this feature. The latter style of instrument is ideal for diatom work. The greatest advantage of the monocular with provision of tubelength adjustment is that considerable variation in coverglass thickness (or equivalent coverglass thickness) can be accommodated and the resulting variation in spherical aberration corrected for easily and inexpensively. The disadvantage of a monocular is that for long observing periods it is a bit tiring to use.

The binocular microscope is much more comfortable for long-term viewing and is preferred by many workers. However, tubelength adjustment for a binocular is rather impractical (although it has been provided for in some past designs) and therefore that avenue for spherical aberration correction due to coverglass (or equivalent coverglass) thickness, is usually closed to the user. Correction is usually made, in the case of a binocular microscope, by the use of expensive special “correction collar” equipped objectives. As the sensitivity of an objective to coverglass thickness (or equivalent) variation increases with numerical aperture, one such correction collar equipped objective with an N.A. of 0.65 to 0.85 will be sufficient for binocular microscope users.

2.4. Stage Facilities

Microscopes are variously equipped with facilities for supporting the microslide, and for fixing it in location or moving it about under the objective. The simplest of these arrangements is a pair of spring metal stage clips, which under spring tension grip the microslide, and yet still allow it to be moved, by lateral finger pressure, in a gliding movement in any direction.

Excepting for very casual examination, diatom work in general requires the use of a mechanical stage. By this means the microslide with the specimen(s) can be moved in a systematic fashion for search and examination more suited to detailed study. The mechanical stage should be calibrated so that an accurate means is provided for setting and resetting to specific fields within the specimen area.

2.5. Illumination

Most modern microscope stands are equipped with built-in illumination. For convenience, compactness, and routine work, even the simplest of a built-in source of illumination has its attractions . However, many built-in illumination sources are fixed or severely restricted in adjustment. The simpler stands are provided with only a rough compromise of adequate illumination, and even the most advanced are limited greatly in flexibility. If a stand with built-in illumination is to be used for diatom work, it should at least be capable of providing Köhler illumination as a minimum. This type of lighting requires that the source have an adjustable focus and that a field diaphragm of variable aperture is provided.

August Karl Johann Valentin Köhler
b. 4th March 1866
d. 12th March 1948
Worked for Carl Zeiss AG.

For extensive diatom work the best lighting arrangement is provided by a separate research-quality illuminator. It will provide for the best illumination under almost any desired conditions and is flexible enough in its capabilities to provide almost any special illumination required. Exceptions to the latter are requirements for true darkfield and near ultraviolet illumination which usually demand; special very high intensity lamps.

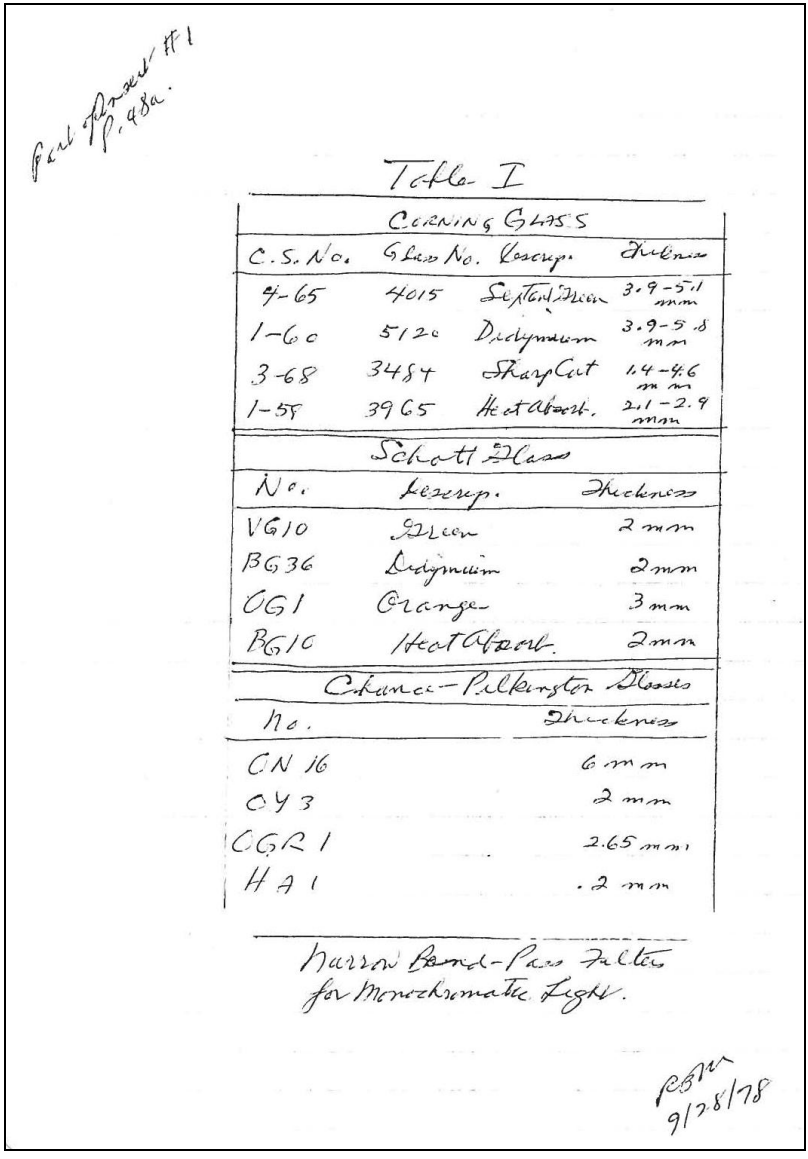
1. Monochromatic Light

For maximum resolution of very fine detail the diatomist should make it a practice to use and get used to, monochromatic light. His subject matter in most cases is colorless and the rendition of color, or interpretation of colored images, is of minor or special concern with only certain types of studies. For examining, analyzing, and interpreting images of the diatom frustule, more accurate information can be obtained by monochromatic light than with any other.

Wratten filters were originally the product of Frederick Charles Luther Wratten – an English inventor.
b. 1840
d. 8th April 1926

As mentioned previously, for over-all improvement of optical element performance and for the eye, a yellow-green light is most desirable. A Wratten No. 15 and No. 58 combined, will provide a yellow-green light that is very satisfactory. Other green light is beneficial too, and Hendey has recommended a Wratten No. 66 green filter. The Wratten filters, although providing a green light that is very beneficial, have comparatively broad transmission bands. For a much greater improvement in lessening eye fatigue, improving the response of achromatic objectives, Abbe substage condensers, and the visual acuity of the eye itself, a more nearly monochromatic source of light than provided by simple filters or combinations as above is required.

Table 6



Part of Appendix #1
p. 98a.

Table I

Corning Glass			
C.S. No.	Glass No.	Descrip.	Thickness
4-65	4015	Septentrion	3.9-5.1 mm
1-60	5120	Didymium	3.9-5.8 mm
3-68	3484	Sharp Cut	1.4-4.6 mm
1-58	3965	Heat Absorb.	2.1-2.9 mm

Schott Glass		
No.	Descrip.	Thickness
VG10	Green	2 mm
BG36	Didymium	2 mm
OG1	Orange	3 mm
BG10	Heat Absorb.	2 mm

Chance-Pilkington Glasses	
No.	Thickness
CN 16	6 mm
OY 3	2 mm
OGR 1	2.65 mm
HA 1	.2 mm

Narrow Band-Pass Filters for Monochromatic Light.

PBM 9/28/78

Col. Wm. D. Fleming (1966) a diatomist of long experience and an accomplished microscopist, has described a narrow band-pass filter for nearly monochromatic light. His recommendations provide for the use of glass filters, instead of dyed gelatin ones to take advantage of their availability in narrow band-pass form, higher transmission levels, and permanency . His monochromatic filter is composed of a combination of three filters forming a very narrow pass-band between 550 and 560 nanometers, the shape and boundaries of which are largely dependent upon a Didymium glass filter. This latter filter has a number of high transmission bands bordered by others of low-transmission. One of the high transmission bands lies in the desired region of 550 nanometers. He used an orange filter to cutoff regions above the desired frequency and a green glass to cut those off below. Combinations of filters recommended are in Table 6. In each case it will be noted that a heat absorbing glass is included. Because with combination filters such as these, considerable light-loss is experienced, comparatively high intensity lamps are required with consequent protection against heat. The heat absorbing glass is usually spaced from the other members of the filter combination on the lamp side. The three glass filters were combined by Col. Fleming into a sandwich with the least stable Didymium glass in the center for maximum protection. However, the combination need not be so packaged, especially if the individual units are to be used separately or otherwise.

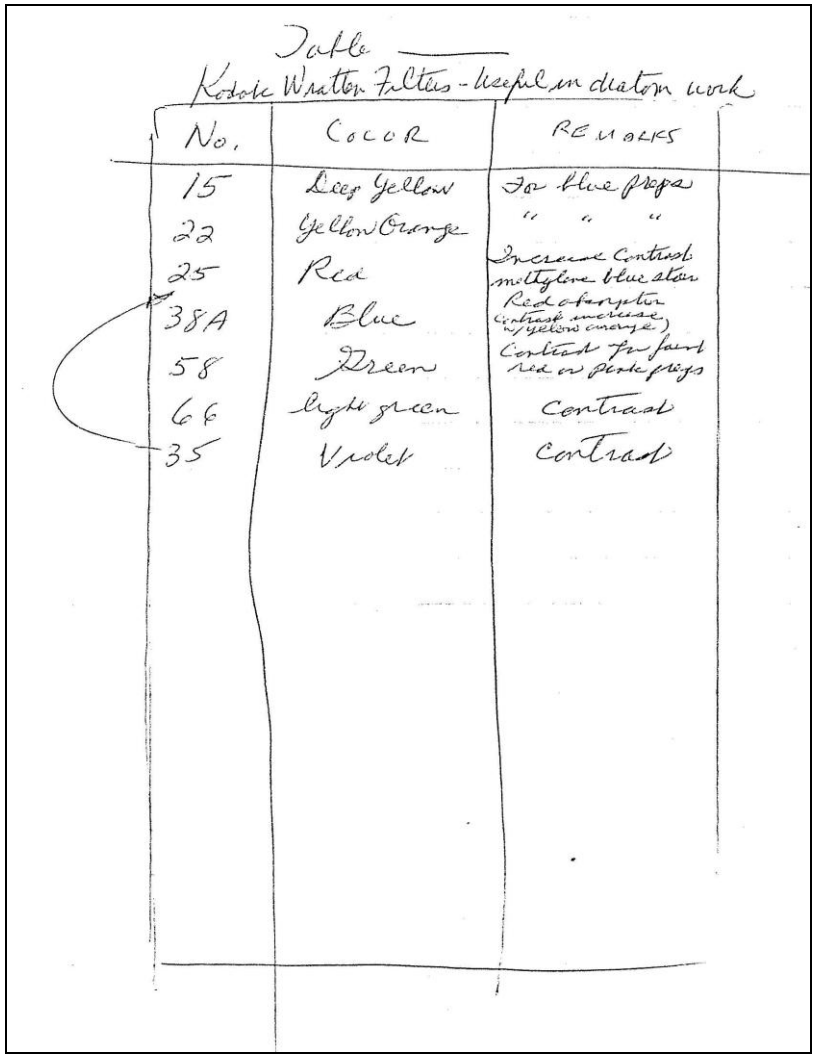
Didymium - *Greek: two element.* A composition of the elements Praseodymium and Neodymium.

Strict adherence to these exact combinations is necessary to gain a very sharply peaked transmission curve at 550 nanometers. However, some deviation in the selection of the orange or green filters may be made, and still retain a very respectable narrow band-pass characteristic. For instance a Corning number 4010 green could be used instead of the 4015 with very little difference, or a number 3486 used instead of the 3484. For instance the transmittance of number·3484 is 37% at the cut between 527 and 544 nanometers while number 3486 has the same transmittance at the cut between 513 and 527 nanometers. The author has used such deviant combination with excellent results. The diatomist cannot really appreciate the advantages of such a narrow band-pass combination until it is experienced in use. For much analysis of the diatom frustule this type of arrangement is highly recommended.

Corning Inc. - an American glass manufacturer. Known as Corning Glass Works prior to 1990

For highest resolution and contrast a blue filter is best. The Wratten 45 and 45A are recommended. The latter has been designed for the highest resolving power in visual microscopy. The 45 has a somewhat broader transmission pass-band, but a higher transmission level than the 45A.and is also excellent for improved resolution. Filters to provide improved contrast, and for photmicrographic purposes are treated elsewhere in this section.

Table 7



Table

Kodak Wratten Filters - useful in diatom work

No.	Color	Remarks
15	Deep Yellow	For blue preps
22	Yellow Orange	" " "
25	Red	Increase Contrast methylene blue stain
38A	Blue	Red absorption (contrast increase, yellow emerge)
58	Green	Contrast for faint red or pink preps
66	Light green	Contrast
35	Violet	Contrast

The diatomist will find that a green filter will be his most used accessory, providing best performance and restful lighting Over long periods of visual observation at the microscope. Other lighting colors and/or combinations and techniques are covered in succeeding chapters.

2.6. Adjustment

Resolution of diatoms is dependent quite as much on the skill of the microscopist as on the quality of the instrument he uses. The keynote of obtaining the best from a microscope is proper adjustment. A high quality instrument adjusted improperly will

perform in an inferior manner. As the light microscope is ordinarily used in the "brightfield" mode, diatoms being examined in transmitted light, the procedure for obtaining the utmost in resolution by that method is provided. Other illumination methods requiring special adjustment procedures will be included as necessary. For best results by transmitted light, whether for visual observation or for photomicrography, Köhler illumination is used.

The Köhler system requires a lamp with a small concentrated filament, a large adjustable condenser lens, and an iris diaphragm close in front of the lens, which is used as the actual light source. That is to say, the image of the area surrounded by the diaphragm on the illuminator (called the field diaphragm) is ultimately focused into the object plane as a self-luminous source, at least insofar as the brightness and evenness of the illumination are concerned. The steps in practical adjustments that are necessary to attain this condition are:

(1) A selected diatom slide is placed on the stage of the microscope and the diatoms focused. This is done without particular adjustment of the light source. It is merely adjusted (if necessary) at this point, to get sufficient illumination of the diatom to permit this preliminary focusing.

(2) Close the field iris completely, or almost completely, and focus the image of it in the object field by racking up or down the substage condenser. If an Abbe condenser is used, the edges of the field iris will be somewhat indistinct. With a corrected condenser the image of the iris will be sharply defined. This adjustment is made as the image is observed, of course, with the ocular in place.

(3) The field condenser (on the lamp) is focused such that an image of the lamp filament appears in the plane of the substage condenser iris. This adjustment can be made while looking at the back focal plane of the objective with the ocular removed, and the field iris wide open. Alternatively, an adjustment close enough for the purpose is to focus the field condenser such that an image of the lamp filament appears on the plane surface of the substage mirror. This is aided by the use of a piece of tissue or other opaque surface being held there momentarily for that purpose.

(4) Center the focused image of the field diaphragm in the field of view by tilting the mirror.

(5) Open the field iris until it just delimits the field. At this point the area of light enclosed by the field iris is essentially a self-luminous source in the object plane. Köhler illumination is thus obtained.

In step 3. above, it was assumed that the illuminator was separate from the microscope stand. Instruments with built-in illuminators simplify the adjustment somewhat, as the lamp is very close to the focused position and there is no substage mirror involved.

The adjustment in that case is essentially the same however, and involves making sure the lamp iris is in focus in the object plane. Sometimes the built-in illuminator requires substage condensers to have an "auxiliary" lens just below them to image the field diaphragm in the specimen plane without racking the condenser down so far as to sacrifice aperture.

In the steps above the substage iris, associated with the condenser is wide open. Also, the steps above are given for the case of dry objectives. With immersion objectives a few other adjustment details must be taken into account.

Preliminary to making final adjustments for Köhler illumination with an oil immersion objective:

(a) Place a drop of immersion oil on the top substage condenser lens.

(b) Place the selected diatom slide (with an ink ring) in place and rack the condenser far enough up so that oil contact is made with the bottom of the specimen slide.

(c) Focus on the ink locating-ring with a 40X dry objective, then swing it out of position with the rotating nosepiece.

(d) Place a drop of immersion oil on the microslide coverglass and swing in the oil immersion objective.

(e) Focus, with the fine adjustment, on the ink ring. If the diatom is not in the field of view, a slight movement of the mechanical stage should find it without focusing and risking damage to the object or coverglass.

(f) When it (the diatom) is in view, re-touch the fine focusing adjustment to bring it into focus. Now step (3) above, and succeeding ones, can be taken to adjust for Köhler illumination.

With the substage condenser iris wide open there is (in most cases) a lack of contrast, reducing visibility of the diatom(s) in the field of view. With objectives in use which have numerical aperture low enough that the condenser can more than adequately fill their apertures with a cone of light, there is additional glare. As the design of lenses is on a spherical bias, the maximum aperture of an objective does not correspond with its maximum corrected operating condition because of off-axis and edge effect aberrations. To accommodate for these various conditions the substage condenser iris is closed down to:

(1) At least limit the cone of light presented to the objective so as to be within its numerical aperture.

and

(2) Further closed down to reduce glare and allow the objective to operate within its maximum corrected aperture.

There is some point of substage iris closure that has the most beneficial effect. When the N.A. of the illumination has reached the value at which the smallest resolvable detail is still reproduced, with slightly increased contrast, optimum image quality will prevail. Some handbooks and microscope instructions provide various "rules of thumb" whereby the substage iris diaphragm is closed down to provide $^{2}/_{3}$, $^{3}/_{4}$ or some fraction of the full aperture of the back focal plane of the objective (as observed with the ocular removed). Regard these settings as guides only and make substage iris adjustments with maximum quality of image in mind for the particular diatom(s) being viewed.

Incidentally, the back focal plane of the objective can easily be observed without removing the ocular. View it alternatively by magnifying the Ramsden disc (eye point) of the ocular with a common 10 or 15 power hand lens.

For the resolution of very fine diatom detail this form of illumination is best. It is assumed that the optical components of the microscope are in proper alignment. The alignment of the objective with the ocular is no problem, but a substage condenser can be out of alignment and its centration should therefore be checked. As most conditions of microscope illumination require centration of the substage condenser, it is pertinent here to indicate how that condition can be checked and/or adjusted. It is particularly important when using the microscope for critical work, as is often the case in the resolution of extremely fine diatom detail. There are several ways in which this condition can be checked.

(1) The center of the top lens of the condenser can be marked with an ink dot and centering accomplished by making mechanical adjustments to bring it into alignment with the optical axis.

(2) If the substage iris is attached to the condenser (which is often the case) the centering can be accomplished by making adjustments while observing the image of the closed iris with a low powered objective.

(3) The back focal plane of the objective may be observed through a "pinhole" cap over the tube of the microscope. (It is simply a rimmed disc that fits snugly over the tube with an accurately centered pinhole).

(4) The Ramsden disc of the ocular may be magnified with a 10X magnifier.

(5) When available, of course, a Bertrand lens in the body tube is an ideal way to view an enlarged image of the objective back focal plane. This accessory is usually available only in polarizing microscopes used in petrographic and mineralogical fields. However, there is certainly no restriction on a diatomist using this style of instrument.

Emile Bertrand
(1844 – 1909)
French Mineralogist

In the above, it is assumed that there are centering adjustments available for the condenser. This is usually the case with the more sophisticated stands. However, with simple "sleeve" type fittings for the condenser, centering may be more difficult of accomplishment. Sometimes there are screws to provide some adjustment even on sleeve-mounts, or paper or metal shims can be placed to maintain its centered position, if sufficient space is available.

2.6.1. The Binocular Head

Most adjustments of the light microscope are described as if the instrument were a monocular. With a microscope equipped with a binocular head there are a few items needing attention if critical diatom work is to be accomplished.

(i) Unless both eyes of the diatomist are known to be perfect, each tube of the binocular should be tested with the relevant eye. In making such adjustments a black card or cap over the unused tube is much better than closing the eye.

(ii) To check the differences in oculars, interchange them. If any difference is noted, they should be appropriately marked and used in their designated locations only.

(iii) Registration of the two fields of view should be checked (for each ocular) to minimize eyestrain. A focused circular pattern in one ocular should show up in the other ocular in an identical position in that field. A large species of *Coscinodiscus* makes a good subject.

(iv) In (iii) above a very objectionable prism error is indicated if the images appear relatively high or low (skewed) or divergent.

(v) Precisely adjust the interpupillary distance. This is most easily done by using low power oculars with a Ramsden disc approximately the eye pupil diameter (about 3 mm.). Improper adjustment here will definitely reduce resolution of the image in the eye.

2.6.2. Accessories

An accessory that is most important in diatom work is an ocular reticle for making measurements of diatom dimensions and counts of striae (per 10 micrometers) on the valve(s). This activity is an essential one in identification and analysis of diatom specimens.

2.6.2.1. The Reticle

There are many types of eyepiece reticles available. One very useful for the diatomist is a straight line scale 5 mm. in length divided into 100 parts. The scale is usually photo-engraved on a transparent glass disc of 21 mm. diameter and

approximately 1.5 mm. thick. The glass disc is placed in the eyepiece of a microscope such that it appears superimposed on the field of view. If a binocular-head instrument is used one such reticle only is needed, and may be placed in either ocular.

In a Huygenian ocular, the focal plane lies within the tube between the eye lens and the field-lens at the diaphragm position. This is where the reticle is placed. The reticle is inserted by unscrewing the eye lens cap from the ocular tube and placing the reticle so it rests directly on the diaphragm. To protect against reticle movement, a retaining ring of split spring-wire is often used, located so as to press the glass disc firmly against the diaphragm surface.

With a Ramsden ocular, the focal plane lies just beyond the field lens and therefore the reticle must be placed in the same location. These oculars are usually constructed such that the eye lens and field lens can be removed as a unit cell from just above the field lens diaphragm location. To insert the reticle, the lenses are removed from the tube and the reticle placed on the diaphragm.

The reticle must be placed in either case (above) such that the scale reads from left to right and right side up. If the reticle is sandwiched between two pieces of glass or covered with another piece of glass, the additional thickness may be such as to disallow proper focus on the reticle by the eye lens. This sometimes can be remedied by moving the diaphragm in the ocular tube to bring the reticle into focus. The direction to move the diaphragm can be determined by gradually unscrewing the eye lens while viewing the reticle scale. If it gets clearer, then the diaphragm needs to be moved away from the eye lens, and if it becomes more blurred it needs to be moved toward the eye lens. Unless eyeglasses are used when viewing through the microscope, the adjustment should be made with eyeglasses removed.

If it is not possible to move the diaphragm to obtain a clear focus on the reticle scale, adjustment of the eye lens by screwing it out may be resorted to if (a) that is indeed the proper direction and (b) if enough thread is available. When reticles are to be used for measuring, it is essential that both images be in perfect focus. A focusing ocular especially constructed for the use of reticles is recommended.

The magnification of the ocular has no relationship to the measurement capability of the reticle. It only serves to magnify the reticle so that it can be seen clearly and also serves as one factor in the total magnification of the microscope. The reticle is not measuring the specimen itself but its magnified image. For instance, a microscope with a 10X objective and a 10X ocular has a total magnification of 100X. The image at the reticle is 10X the real diatom size (this is what the reticle is measuring) and it is then magnified by a factor of 10X with the ocular. With a given reticle in an ocular, every time the objective magnification is changed the reticle is a measuring different sized image. Therefore, calibration of the reticle for different objectives magnifications is necessary.

2.6.2.1.1. Calibration of the Reticle

There are a number of factors of consideration in using the average microscope which dictates the necessity of calibration of an eyepiece reticle against some

standard, including the changing of objective powers as mentioned above. The quoted figures of magnifications of objectives are only approximate. They refer to a theoretical working distance which may not correspond to that in the microscope. Therefore, although the quoted magnification may be used in selecting appropriate ocular reticles, it should not be used in place of calibration.

Microscopes with variable drawtubes cause an increase in the magnification of the image from the objective as the drawtube is extended. Adjustment therefore, of the length of the drawtube, will invalidate a previous calibration of a reticle scale made at some other drawtube setting. Using oculars that are not designed for the microscope stand in use can also produce a "drawtube" effect that invalidates a previous calibration. Although the magnification of the ocular may be the same from case to case the same may not necessarily be said of the location of the lower focal plane below the upper rim of the tube. As the lower focal plane of eyepieces varies considerably, (as much as 4 or 5 millimeters) it has the same effect as varying the drawtube by the same amount. When a binocular head is used there is usually provided some adjustment of eye-separation (interpupillary) distance and for individual eye (diopter) adjustments. These settings also should be noted and remain unchanged for a given reticle calibration.

For these reasons calibration of an eye piece reticle holds only for the same optical and mechanical conditions from case to case. It is necessary that calibration be performed for each objective to be used and/or objective/ocular combination.

The ocular scale is calibrated using a stage micrometer. The latter is in the form of a microslide with a known scale-interval (0.01 mm. divisions are common) ruled on it and protected by a coverglass. Calibrate by the following:

(1) Place the stage micrometer on the microscope and adjust its position to view the scale.

(2) The value of the ocular scale interval is obtained simply by dividing the value of the stage micrometer interval by the number of ocular scale intervals which it subtends in the image.

In aligning the stage micrometer and ocular micrometer scales, it is often found that the lines on the two scales coincide at two or more points. The relationship between the reticle and stage micrometer will be that of the respective number of divisions on each between the points of coincidence. If coincidence occurs at only one point, the value of each reticle division must be estimated by eye off the stage micrometer scale.

Once the calibration is determined, the stage micrometer is not used in taking measurements. It is reserved for the calibration role only and the calibrated ocular micrometer is used in the measuring activity.

2.7. Summary

For work with diatoms, the minimum equipment required is:

(1) A light microscope stand with either a monocular or binocular head.
(2) A battery of at least three objectives; 10X, 40X, and 100X oil immersion; the high-dry and oil immersion being apochromats if possible.
(3) A highly corrected substage condenser.
(4) Ocular(s) of 100X, compensating if possible.
(5) A research-grade illuminator.
(6) A green filter.
(7) An eyepiece reticle and stage micrometer for calibration.

CHAPTER 3.

3. MICROSCOPICAL TECHNIQUES

3.1. Introduction

Techniques with the light microscope in diatom study, range across the spectrum from brightfield to the use of the most modern and advanced phase contrast equipment. Before the advent of the more sophisticated illumination technology of today, diatomists were using many ingenious variations of ordinary bright and darkfield illumination to improve the resolution and enhance contrast of diatom images.

Although many of them are comparatively simple in comparison to modern and recent sophisticated microscopic techniques, they are nevertheless, in many instances, very effective. For the diatomist with limited resources these techniques are invaluable and will be described herein. For the diatomist who is fortunate enough to possess, or have access to, the most modern up-to-date equipment, the basic principles and advantages of their use in diatom study is also included. In the following, especially as regards brightfield illumination, it is assumed that proper attention has been paid to accurate alignment and adjustment of the microscope optical train. In this way only, can critical images of diatoms be obtained. A great and very real danger with uncritical images, is that, while wholly false diffraction effects may be mistaken for genuine detail, real structures of an unexpected or complex character may quite as easily be overlooked or doubted, thus limiting the effectiveness of the microscopical examination.

3.2. Brightfield

As this form of illumination is the most commonly used, the variations and techniques of its use are most numerous.

3.2.1. Magnification

The matter of magnification possible or “permissible” is of importance. The “rule of thumb” that many users of the microscope diligently adhere to; namely that the total magnification of the microscope (the objective magnification multiplied by the ocular magnification) should not exceed 1000 times the numerical aperture (N.A.) of the objective needs examination, especially as regards diatom work.

The ideal human eye, in rare cases, under optimum conditions of illumination and contrast, can distinguish objects subtending less than 40 seconds of arc (visual angle), or about 40 micrometers lateral separation at a normal accommodation distance of 250 mm. However, the dependence of the eye on the quality of illumination and contrast for maximum resolution is very great. Under optimum conditions of contrast, the good or average eye is only about one-fourth as good at resolving as the “ideal eye”. Assuming the average eye to be capable of resolving

objects separated by 160 micrometers under optimum conditions, the magnification required by the microscope optics is of interest.

Assuming a wavelength for the illuminating light of 500 nanometers and an N.A. of the objective to be 1.25, the nearly maximum resolution is 0.20 micrometer. The total instrument magnification required for the eye to distinguish that separation at its 250 mm. accommodation distance(as it is at the ocular) is:

$$\frac{160}{0.20} = 800X$$

It is evident that the magnification required is less than 1000 x N.A. (1000 x 1.25). However, it is not at all exaggerated to say that the usual contrast conditions with a light microscope are less than optimal. The eye, under such instrument conditions, at the lower limit of contrast, which is quite often the case in diatom examination, can resolve separations only about 4 times as great as under optimum conditions. That means that for the example we have been discussing, the total magnification would have to be 4x800 or 3200 diameters. This is much greater than 1000 x N.A. (1250X), being more than twice that dictated by the "rule". The "rule" was based, by Abbe, on the resolution of certain periodic structures under optimum contrast conditions. This basis has been forgotten, overlooked, or misinterpreted by many microscope users over the years looking for a "cookbook" set of rules with which to operate their instruments. The result of using magnifications greater than 1000 times the N.A. was, horror of horrors, to obtain an image with "empty" magnification. The word "empty" being meant to indicate that although the image becomes larger there is no additional detail resolved and revealed.

It is apparent that the 1000 x N.A. rule is not, or has not been, usually interpreted properly as was intended by Abbe. Even in the heyday of light microscopy immediately following Abbe's enormous contributions to microscopical theory and design, many microscopists, especially diatomists, recommended magnifications greater than 1000 x N.A. for superior results under various conditions. For instance, A. A. C. Eliot Merlin, a well known English microscopist, in 1915 provided photomicrographs in the *Journal of the Quekett Microscopical Club* which proved that with a magnification of 2150 diameters there was no excess of "empty magnification", when employing a good objective lens of 1.40 N.A. He also produced very good photomicrographs of secondary structure in diatoms at 2900X and 5500X (the former of *Navicula Smithii* and the latter of *Coscinodiscus asteromphalus*).

Augustus Alfred Cornwallis Eliot Merlin
(1860 – 1946)
H.M. Consul for Thessaly

Van Duijn in a series of articles in *The Microscope* recommends more common and frequent use of high power magnification (greater than 1000 x N.A.) to improve the visibility of microscopical detail and to increase awareness and proper interpretation of optical artifacts.

Visibility is that quality of a microscopical image that enables the observer to "see" resolvable detail. Insufficient contrast for instance, will prevent detail that is resolved from being visible. An important aspect of diatom frustular examination has to do with the counting of striae and puncta. As the puncta may number as many as 50 in 10 micrometers (on *Amphipleura pellucida* for instance), any means whereby the count can be made more easily or accurately by the diatomist, is useful. A very high power eyepiece can be of enormous assistance in this case, although by the "rule" only "empty" magnification results. A very high power eyepiece that has been found to be very useful in the counting of puncta and striae is a 25X Huygenian topped with a 25X orthoscopic and held together with a connecting collar. This gives a magnification as the sum of the two (50X) and the eye point is equivalent to the orthoscopic one which is on top. Used with a good oil immersion objective of 1.30 to 1.40 N.A. this combination will provide good visibility of actual dots on *Amphipleura pellucida*.

A further advantage of high power magnification in the study of diatoms is the increased ability to discern between optical artifacts and true structure. "Optical membranes" imaged as the result of diffraction effects can be construed, especially at lower total magnifications, as walls, membranes, etc. in the frustule, or contribute to an indistinctness in the image of true structural members as to make them difficult of analysis. Increased magnification will separate the diffraction images far enough to reveal them for what they are. As the microscope is adjusted either side of focus the number and position of such artifacts will change, wherein true structural members do not.

3.2.2. Contrast

Contrast is the degree of difference in tone, brightness or color from point to point or from highlight to shadow in an object or image. Visibility of detail, as mentioned previously, is bound intimately with contrast.

In the various methods of improving visibility through contrast, some assist in improving resolution, and some act to decrease it. At lower powers of magnification, sufficient contrast can usually be easily attained that still allows maximum (or at least sufficient) resolution of detail with the optics employed. However, at high magnifications where extreme resolution of very minute diatom structure demands the most from the optical system, methods of improving contrast are complex or difficult to accomplish.

In the majority of cases diatom work is with specimens of a single color (colorless) and the required improvement in visibility can be obtained through simple intensity contrast.

Improvement of intensity contrast is accomplished in a number of ways. Flare, from internal reflections within lens systems and their supporting mechanical structures can be greatly reduced by coating of lens surfaces, reducing the number of lens to air surfaces, and in the judicious design and placement of stops and blackening of all metal surfaces.

Whenever possible, use objectives and other optical elements for the microscope which have been anti-reflection coated. Coated lenses usually exhibit a purplish hue when examined by reflected light.

In practice, if provisions for darkening the inside of the microscope tube have not been made, it should be lined with “coffin paper” or velvet, or painted flat-black.

A stop placed in the tube just below the ocular position is also of assistance in reducing the effect of flare.

Glare, which occurs in the object space, comes from light scattered from various glass surfaces, such as the microslide and coverglass, and from the mountant. Also, re-reflection from the shiny objective barrel underside can contribute to glare.

One remedy to reduce glare is to decrease the angle of the rays entering the objective by stopping down the substage condenser, and using the condenser without immersion (because total reflection will occur before wide angle rays have entered the slide). With an oil immersion objective and homogeneous immersion, no glare will occur. Blackening of the lower horizontal metal surface of the high dry objective will sometimes be found helpful.

Visibility not only depends upon the contrast between structural details among themselves, but also on the contrast between the whole structure (diatom) and the total background area. At low illumination levels, best contrast sensitivity of the eye is obtained when the diatom structure to be resolved appears brighter than its surround (more of this later in relation to darkfield illumination). In brightfield illumination the contrast sensitivity is improved as the surround (background or field) is brighter. Therefore high illumination levels in ordinary brightfield are advantages from that standpoint, and should be a matter of practice rather than the exception it is, in most cases. The large area surrounding the detail to be observed, at the center of the field, forms a so-called sensitizing field which determines the contrast sensitivity of the eye. The latter decreases very rapidly as the illumination level of the surround decreases.

3.2.3. Filters

Improvement in the microscopical image of diatoms can be obtained in a number of ways by the use of filters. The use of green, yellow-green, and blue filters has been previously recommended as means for increasing resolution of detail. These filters, furnishing a monochromatic illumination of the subject are suitable for examination of the cleaned diatom frustule, but not best for the examination of living or stained specimens. Living and/or stained specimens can often be viewed to advantage by the use of color filters. This type of filter modifies the white light source to pass only light of specific or limited coloration that is then used for specimen illumination. By this means contrast, and resulting visibility, can be greatly improved. The contents of living diatoms are revealed in various shades of green, yellow, brown and gold, while stained diatoms (in part at least) may be colored blue, red, orange, or other colors dependent upon the stains used.

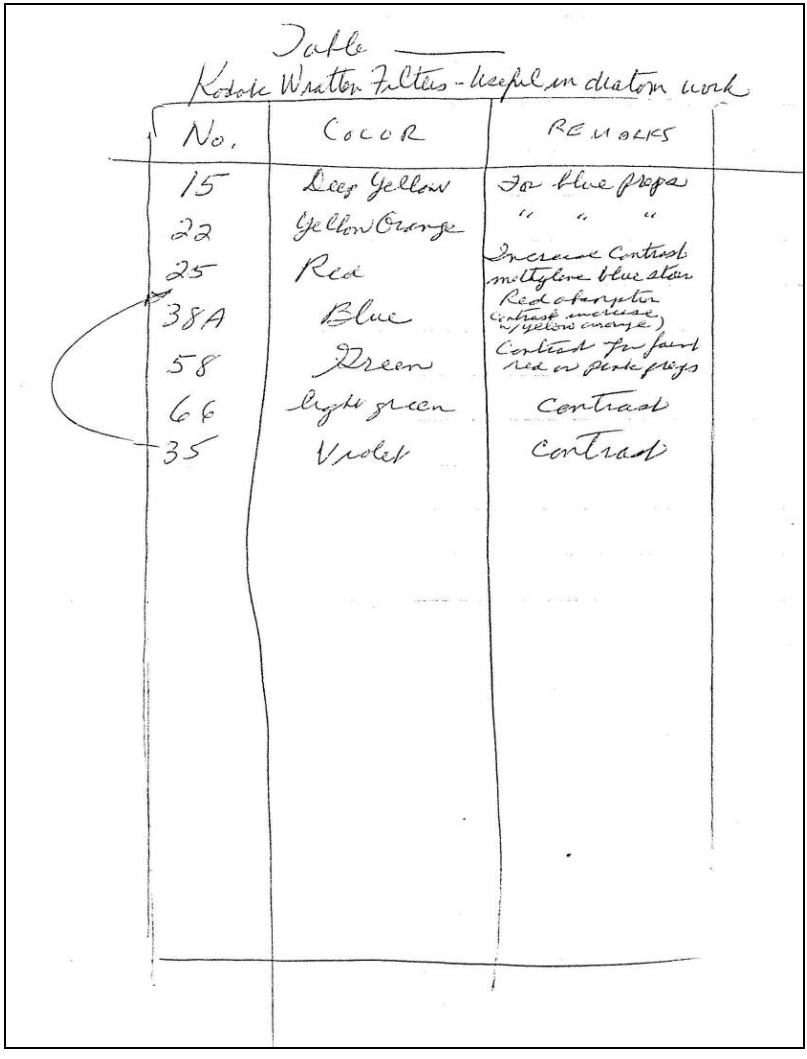
In selecting contrast filters, a filter is chosen that is complimentary in color to the object color. For a color to be rendered as dark as possible requires that it be viewed by light which is completely absorbed by the color - that is, by light of wavelengths comprised within its absorption band.

The best method of determining the contrast required is to examine the diatom visually with the microscope, first by a combination of filters transmitting as completely as possible the wavelength absorbed by the particular preparation, and then by other filters transmitting light less completely absorbed until the degree of contrast obtained is satisfactory to the eye. As a general guide :

Use for blue colored preparations a red, yellow or orange filter.
Use for green colored preparations a magenta filter.
Use for red colored preparations a green filter.
Use for yellow colored preparations a blue filter.
Use for brown colored preparations a blue filter.
Use for purple colored preparations a green filter.
Use for violet colored preparations a yellow or green filter.

For instance, a blue filter will materially darken a yellowish color or, if the object is blue, a yellow or red filter will make it stand out.

Table 8



Table

Kodak Wratten Filters - useful in diatom work

No.	Color	Remarks
15	Deep Yellow	For blue preps
22	Yellow Orange	" " "
25	Red	Increase Contrast methylene blue stain
38A	Blue	Red absorption (contrast increase, yellow emerge)
58	Green	Contrast for faint red or pink preps
66	Light green	Contrast
35	Violet	Contrast

Kodak Wratten filters that are useful in diatom work are shown in Table 8 reproduced above.

Polarizing filters are also sometimes useful in the study of diatoms. Although the silica of the frustule in general is not birefringent and will not usually produce contrasting colors, it is of real advantage to use a polarizing filter in the "analyzer" position (at the eyepiece). Some reflected light from glass surfaces etc. appearing in the image to the eye is plane polarized by virtue of its production (reflection, scattering, etc.) and, if the "analyzer" is oriented properly, that light appearing as glare in the image can be suppressed or eliminated, thus improving the contrast in the final image. Simple Polaroid-film filter material is quite satisfactory for the

purpose. A simple polarizing cap can be applied over the top of the ocular, or a disc of the material laid on the eyepiece diaphragm. A number of workers claim this simple accessory materially assists in improving the visibility of delicate striae.

3.2.4. Black and White - Dot Focus

In brightfield illumination especially, and most noticeable when examining diatoms, there is a phenomenon that always puzzles beginners. This is the changing of dot-like appearances in the image from black to white and vice versa as the focus of the microscope is varied slightly. What is being seen? Should the focus remain on black, or white, as the best point for resolution? These questions and related ones were discussed and argued pro and con by leading diatomists and microscopists over a period of many years, Now that most diatomists are familiar with the nature of the "dots", "pearls"," beads", and other fancied manifestations of puncta, it may seem strange that so much attention and controversy was associated with these appearances. Experienced diatomists equipped with the knowledge of many decades of research and the revelations of the electron microscope, particularly the SEM, are rather blasé toward these "elementary" considerations. The beginner, on the other hand, is in some respects, in the position of our diatomist forerunners. He sees the same images that they did and, lacking experience, asks the same questions. Therefore, it seems appropriate in this elementary treatment of the subject, to include these considerations.

For nearly twenty years (1889 to 1907) considerable controversy raged in various journals regarding whether the black or white dot focus was to be accepted as the correct microscopic image. Many famous men and authorities on microscopical optics and techniques aired their opinions and theories pro and con over the question.

Such men as A. A. C. Eliot Merlin, J. Rheinberg, and E.M. Nelson, devoted a great deal of attention to the matter.

Julius H. Rheinberg
(1871 – 1943)

Edward Milles Nelson
(1851 – 1938)

At that time a cardinal factor in the problem of determining diatom structure was the perplexing alternation between white dots and black dots, which in most diatoms accompanies slight changes in focus. In the very finely structured diatoms the situation is complicated by the diffraction-grating effect arising from regularly repeated fine structure.

Arguments for black-dot focus and for white-dot focus were advanced in the various Journals without any definite conclusion being reached as to which should be considered correct. For a given separation of puncta, the optics of the microscope (namely the objective) must be of a capability as to resolve them (separate the puncta). The very finely marked diatoms such as *Amphipleura pellucida* challenge the optics and best techniques of the microscopist to resolve them into simple separated dots. The black- and white-dot manifestation in an image of such finely marked structure is merely evidence that the resolution capability of the optics has failed to reveal individual puncta structure. The dot-alternation originates in such

halo effects as may be seen and studied with any simple silex fragment. *Navicula lyra* is one diatom which shows the white dot, black dot phenomenon in a very typical manner. It was shown clearly that the correct focus is what is termed "ring-resolution". This obtains when the puncta appears as a white spot rimmed by a black ring of definite width and separated from others of its kind by a white background (all of this in brightfield illumination). Perfect ring-resolution requires at least twice as much optical resolution capability as dot resolution of the same structure. In dot resolution each punctum is treated as a single object. But, for ring-resolution each annulus ranks as two objects because each side of the ring must be kept clear, both from the opposite side of the same ring and from the adjacent ring. The number of discontinuities resolved is doubled. That, in many cases, the optics of the light microscope are incapable of resolution to this degree results in the white and black-dot phenomenon. With this in mind, we can consider either the white-dot or black-dot focus to be correct, and that it is in either case an indication of resolution limitation of the microscope optics.

3.2.5. Coverglass Thickness and Spherical Aberration

Critical examination of the diatom frustule in particular, requires all distortion to be reduced to a minimum. Use of monochromatic illumination will, to a great degree, improve the performance of the microscope resolution-wise. An important factor in presenting a sharp, clear image to the observer, is the reduction of spherical aberration. Fortunately, there is something the microscopist can do to keep this aberration to a minimum . Often neglected in many fields, even in critical work at high magnifications, it is of sufficient importance to the diatomist to warrant emphasis here.

Spherical aberration produces a blur or fuzziness of images in brightfield and produces halos in darkfield. Objectives for use with covered objects are designed to be used with a specific thickness of coverglass. The two most common design thicknesses are 0.17 and 0.18 mm. From the designers point of view, the coverglass is regarded as a plano-parallel plate interposed between the objective and object. The computed thickness of the plano-convex front element of the objective is reduced in design by the assumed thickness of coverglass, and aberrations accounted for on that basis. Many (if not most) high-dry objectives are marked as to the coverglass thickness for which they are corrected. Use of the objective with its designed thickness of coverglass will minimize spherical aberration to the designed limitations. The use of covers thicker or thinner will allow spherical aberration of greater than the designed minimum to occur and result in consequent degradation of image sharpness.

The best practice is to use the proper thickness of cover and mount the diatom to be in contact with the cover. If the diatom is mounted such that it is not in contact with the cover, or on the microslide, then the effective thickness of the coverglass is increased by the distance of separation between diatom and cover and the refractive index of the mounting medium. If the mountant is the same refractive index as glass (1.515 approx.) the effective coverglass thickness is then the thickness of the actual cover plus the thickness of the layer of mountant between its underside and the

diatom. If a mountant of higher refractive index than glass is used (1.60 for instance) then the optically denser medium acts as an even thicker layer of "glass" between the actual cover and the diatom. This effects equally the so-called homogeneous immersion objectives wherein they are designed to work through glass and Canada Balsam (or a mountant with a refractive index equal to glass). Under such conditions, if no steps are taken for correction, spherical aberration in the image, with resultant loss of sharpness, will result.

There are two ways in which spherical aberration introduced in this way can be compensated for . The first is to adjust the tubelength of the microscope accordingly and the second is by the use of a "correction collar" objective. Older microscopes are usually constructed such that the tubelength may be adjusted. If the effective coverglass thickness is too great, the tubelength of the microscope needs to be shortened from its normal length to compensate for the spherical aberration. If the effective coverglass thickness is too thin, then the tubelength is lengthened. As the diatomist may not know whether the effective cover thickness is too thin or too thick, the following procedure will provide proper adjustment:

(1) Focus on a small puncta or dot on the diatom to be resolved with the tubelength set at its normal position.
(2) If, on focusing slightly above and below this point, the appearance of the puncta is the same, the tubelength is proper for the effective coverglass thickness.
(3) With improper tubelength, one side of the focus will reveal a dark ring around the object (particle, dot, puncta) and on the other side the particle will dissolve into a foggy image.
(4) If the dark ring persists on downwards focusing, the tubelength should be increased, as the effective cover is too thin.
(5) If the dark ring persist on upwards focusing the tubelength should be shortened, as the effective cover is too thick.
(6) After several such adjustments, it will be found that the ring becomes less and less defined on the one side and begins to appear on the opposite side of the focal point until a stage is reached when dark rings, now comparatively faint, have become equally apparent on both sides of the focus. The correction is complete, and spherical aberration due to deviation from designed coverglass thickness is minimized.

It is apparent that more often the effective coverglass thickness will be too great, especially if the diatom is mounted on the microslide instead of the cover, the requirement then being to shorten the tubelength. Microscopes with adjustable tubes usually provide less adjustment in the shortening direction than the lengthening direction, and therefore create additional problems in correcting for this error, since possibly not enough shortening of the tube can be accomplished. A deviation in thickness of 0.01 mm. from the recommended cover thickness (0.17 or 0.18mm) will produce a very noticeable spherical aberration when using a high dry objective with an N.A. of 0.65. The amount of tubelength change is about 10 mm. for each 0.0l mm. departure from ideal coverglass thickness.

The second means for making adjustment to offset introduced spherical aberration from effective coverglass deviation, is to use an objective equipped with a correction collar. These objectives are expensive, but if no tubelength adjustment is possible with the microscope stand in use, they are indispensable in achieving maximum resolution and sharpness in the image. This type of objective employs a convenient mechanical device whereby the distance between the front lens and remaining lens parts of the objective can be delicately and continuously varied. Optimum image quality is obtained when the figure corresponding to the effective thickness is opposite an index mark on a knurled ring. The procedure for making such adjustment is the same as tubelength adjustment excepting the correction collar is adjusted for the best image. The correction collar objective has three advantages over the tubelength adjustment. One, it can be adjusted while the diatom is under full observation; two, it accomplishes the correction for spherical aberration with far less disturbance of other objective corrections; and three, it allows the magnification to remain practically unchanged. In regard to the latter for instance, the magnification with an objective of nominal 40X will only change between the limits of approximately 39.6 to 40.6X for a coverglass thickness change of from 0.14 to 0.20 mm. respectively. With tubelength correction for similar coverglass deviation the magnification will vary from approximately 35X to 45X respectively. Correction collar objectives are usually high dry fluorites or apochromats with an N.A. of 0.85 to 0.95, making them very superior in performance to the usual achromat, or even apochromatic objective. For very critical work with the diatoms, they are well worth obtaining. One such objective in the battery used by the diatomist will increase his capability for critical work considerably.

The resolution of a fine *Pleurosigma* or similar diatom for instance with a high-dry objective without proper adjustment for effective coverglass thickness varying as little as 0.02 mm. from the ideal, is very difficult; if not impossible. Therefore, it is most important that this aspect of microscope adjustment be taken into full account by the practicing diatomist.

3.3. Darkfield

The advantages of darkfield illumination are not put to use, or even realized, by most of those who use a microscope. This method of illumination has been largely neglected through unfamiliarity with it, a firm belief that it renders inferior results unless expensive equipment is used, and ignorance of its variations and/or flexibility. Hendey recommends good darkground with a 10X or 20X objective and indicates that more is to be seen by this method than any other. Many early diatomists used darkfield illumination in a number of variations. However, because of the beliefs of many modern workers as indicated above, and with the advent of phase contrast and other sophisticated systems, it has fallen into disuse. For the diatomists lacking the most advanced means available, and on its own merits, it remains a valuable tool in diatom research.

3.3.1. Principles

Simple darkfield can be obtained by blocking all central rays of light emanating from the substage condenser from entering the objective. This is easily accomplished by the use of opaque stops in the filter carrier of the substage condenser assembly. The only light (ideally) which enters the objective under this condition, is that which is scattered by the specimen because it is illuminated by a hollow cone of light of too large an angle to permit the direct beam to enter the objective.

The size of the stop required varies with the numerical aperture of the objective in use, the larger that being, the larger the stop required.

For providing light that is restricted from entering the objective, and yet of such angularity as to provide a high proportion of light scattered from the object, the diameter of such stops should be about 10% greater than an opening of the substage iris diaphragm which corresponds to full aperture illumination of the objective in use, This can be easily be determined by adjusting the iris and observing the back focal plane of the objective. The measured iris opening is increased by 10% and a stop diameter selected to equal that figure. Stops are commercially available, made from blackened metal, but they are also easily handmade, pieces of thin clear plastic or glass of a diameter suited for insertion in the filter carrier are excellent. The stop can be made of cutout black (opaque) paper of the proper diameter, as determined above, and fastened to the disc. Alternatively, a circular opaque disc may be made by applying india ink or black paint, with the aid of a turntable, to the plastic.

A somewhat “blacker” field results when using these simple stops by oiling the condenser to the bottom of the microslide during observation.

This simple method of obtaining darkfield is useful and adequate at low and medium magnifications (using up to about 40X, N.A. 0.65 objectives). The field remains quite dark, and the diatoms are illuminated by marginal rays at such an angle as to appear self-luminous in the dark field, providing excellent contrast. If the size of the stop is too large, the illumination is dim, and if it is too small the field (background) is not very “dark”.

If the illumination level from the lamp is adequate, one stop instead of several, may be all that is necessary for general use, A stop made for the objective with the highest numerical aperture will suffice for all lower numerical aperture objectives. When the stop is too large for those of lower numerical aperture, the effect is to dim the “self-luminous” diatom(s). However, increasing the light level at the illumination source will offset that effect.

The resolution with this simple method of darkfield illumination is less than that obtainable by brightfield, However, the increased contrast which presents the diatom as a bright object in a darkfield is of great advantage, and structure interpretation in some cases actually is enhanced. Since the image of the diatom is being produced by scattered illumination in the case of darkfield, the surfaces of the frustule are emphasized at the expense of interior details. Therefore, in most cases where interpretation of the frustular surface is of importance, this form of lighting is very useful. Interior details however would be better examined with brightfield

illuminating methods. This form of simple darkground illumination has been found to be most useful in searching for diatom genera such as *Pleurosigma*, and in fossil marine spreads such genera as *Asterolampra*, *Hyalodiscus*, *Actinocyclus*, and *Actinoptychus*.

3.3.2. Variations

Darkfield can be used with monochromatic light with attendant advantages as in "white" light. The object will be of the same color as the filter used (green for instance) on a black field.

A variation of darkfield that was more popular many years ago is Rheinberg illumination. This method uses colored discs placed in the substage filter carrier of the microscope. The center of such a disc, instead of being opaque, is of a colored light-transmitting material and the annulus is clear or colored. The color of the central portion, which is of the same size as the stop for simple darkfield as described above, determines the color of the "field" and the annulus or surrounding-ring color determines the coloring of the object. If a disc with a central red stop and blue surround is used for instance, a diatom will appear to be colored blue in a red field. This form of "illumination staining" can furnish some beautiful effects and useful contrast in some cases. Selection of proper contrasting color; can improve visibility of detail on some diatoms. Direct contrast presented to the eye by the object (diatom) in one color lying on a background of another, furnishes a means of differentiating that is of great advantage when examining the contour of a diatom. It is hardly questionable that the transition in hue from one color to a strongly contrasting one assists in a more vivid realization of the disposition and relative thickness of different parts of a diatom than results from examining the "black and white" one obtains with ordinary darkfield illumination.

To obtain resolution equal to that of brightfield illumination, darkfield must be obtained with a special darkfield condenser. The reflections inherent in the glass surfaces of a conventional condenser prevent critical work with maximum resolution from being possible. Specialized darkfield condensers are designed such that resolving power, at high magnification, can be obtained equal to that by brightfield methods. These condensers are of the reflecting types wherein the optical paths of light are formed via reflecting mirror surfaces in rather than through refracting lenses. This provides for absence of chromatic aberrations over the spectrum and excellent spherical correction.

Instructions for the adjustment and use of darkfield condensers usually accompany them and should be followed carefully for best results. Since the centration of the illuminant is very important in darkfield work the condensers are often furnished with built-in pre-centered illuminators. Also, since the numerical aperture is high, immersion media between the darkfield condenser and microslide is always used. Cedarwood oil is a common medium, although newer ones that have improved characteristics such as non-drying, nonfluorescence, etc., are now available too. Diatoms to be examined by such means should always be mounted in a medium and provided with a coverglass. When using oil-immersion objectives with a numerical aperture greater than 1.0, the objective aperture must be reduced in some way.

Funnel stops inserted in the back of the objective are commonly used to reduce the N.A., special, and high-priced, objectives can be obtained with adjustable iris diaphragms for the purpose.

The tip of the focus of the hollow cone of light from a darkfield condenser is very fine, being formed from a very high-angular aperture so that the focus position is sensitive to the location of the object plane, which is determined by the thickness of the microslide upon which the diatom is mounted. Therefore the proper thickness of microslide must be used, and is usually stated by the manufacturer. It is commonly engraved on the condenser barrel or mount, or included in the instructions, and is usually 1.15 to 1.25 mm., or in the case of a paraboloid for instance, might range from 1.35 to 1.6 mm.

Intense illumination for darkfield work is either furnished by built-in lamps, as stated previously, or by the use of carbon-arc, ribbon filamented, or compact-coil filament, high intensity lamps. Special care in lamp adjustment, especially centration, in the types not built-in, is extremely important for superior work.

3.4. Vertical Illumination

For purposes of this discussion the term "vertical illumination" will be taken to include so-called "top lighting" which is not actually within the technical meaning "vertical".

Vertical illumination has perhaps, not been utilized to the extent possible in diatom research. Probably the major obstacle in using such lighting has been the special equipment necessary.

3.4.1. Principles

Vertical illumination is a special case of incident lighting wherein the illuminating rays are caused to impinge on the subject coaxially with the axis of the microscope optics from the viewing direction. This is usually accomplished with an illuminator mounted to direct light normal to the optical axis, into the microscope tube, where it is reflected by a 45 degree half-mirrored surface on an annular mirror.

The light travels through the objective to the object and the light reflected from the latter proceeds back up through the objective, through the interposed mirror, and thence to the intermediate image plane to be magnified by the ocular as usual.

Microscopes used to examine metals and other opaque specimen materials are equipped with properly designed vertical illuminators, and if available to the diatom researcher, might be of considerable aid in improving visibility of certain surface detail.

With vertical illumination it is claimed that the slits in *Pleurosigma angulatum* are visible with a 120X objective and a 10X ocular, and that the slits in *Pleurosigma decorum* can be seen on a membrane separate from the primary structure (the direction of the latter slits is at right angles to the raphe). These claims can be, verified by the use of commercially equipped vertical illumination. Substitute or

makeshift vertical illumination of a temporary nature is not nearly so satisfactory in performance. Any diatomist who has access to a metallurgical microscope should take advantage of its special vertical illumination capabilities.

Incident illumination of the general “top lighting” type has been neglected also, excepting for very low magnifications of large specimens, and then generally has been restricted for use with stereomicroscopes. However, top lighting, alone or in conjunction with brightfield and/or darkfield illumination, can provide additional information regarding surface details of the frustule that are lost in conventional illumination. The diatomist, particularly the beginner, can benefit greatly by getting as many varied views of his subject as possible, increasing his familiarity with shapes, construction, and details. Examination of diatomaceous material with top lighting can oftentimes supply at least some additional information not otherwise available. No diatomist should pass up the chance to examine at least the larger diatoms with the use of a stereomicroscope at from 40X to 50X to 100X or 200X. The revelation of detail and three dimensional effect is an education in itself and will equip the future researcher admirably with comparison and judgmental information to be gained in no other way with the possible exception of the SEM.

3.5. Oblique Illumination

3.5.1. Principles

In ordinary brightfield and darkfield illumination, the light source is concentric with the optical axis and the full benefits of these forms of lighting are obtained in only that way.

If the position of the light source is such that it is caused to enter the substage condenser off-axis, oblique illumination is obtained. Many of the older microscope stands provided for the substage mirror to be swung on an arm to positions at varying angles (up to 90 degrees) off-axis and thus reflect light from the illumination source at an angle into the substage condenser. Effects produced by this means are similar to oblique illumination but not in all respects.

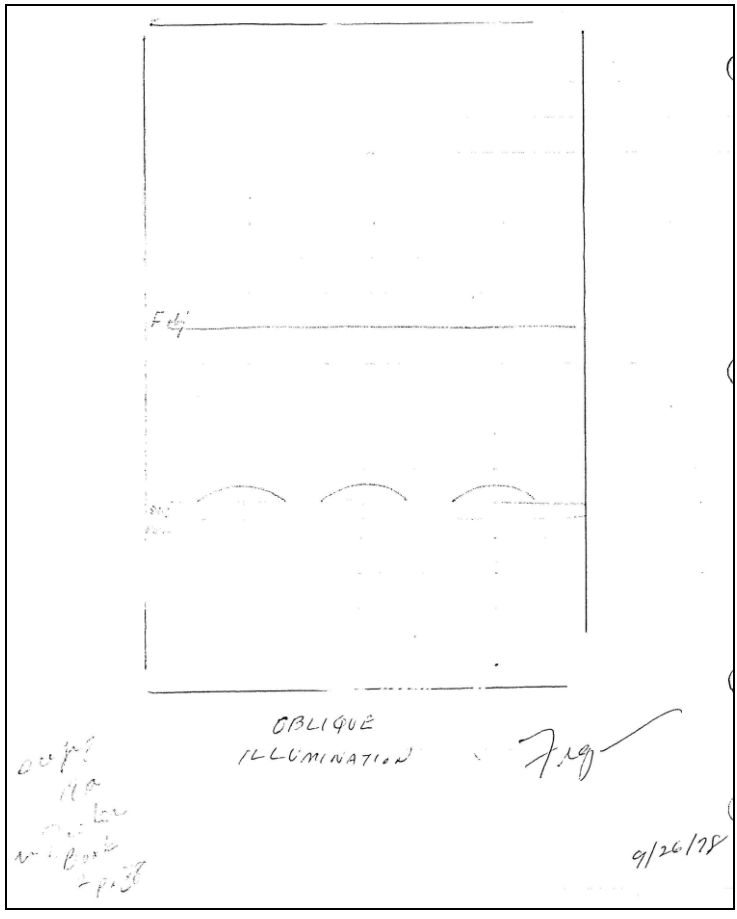


Figure 90.

Oblique illumination in this discussion is meant to be unidirectional oblique illumination as opposed to unidirectional axial illumination. That is (referring to Figure 90) the light admitted to the substage condenser is parallel but off-axis, to the optical axis, and enters the condenser off-axis, and in its turn actually produces an oblique illumination of the diatom in the object plane. A means of introducing light off-axis is to laterally displace the closed-down aperture stop of the substage condenser. In order to make lateral displacements of the aperture diaphragm of the condenser possible, Abbe designed the so-called Abbe Illumination Apparatus. It is provided with facilities for laterally displacing the (nearly closed) iris diaphragm, and for rotating it around the optical axis to change the azimuth of the oblique illumination.

When the closed aperture stop is displaced in the lower focal plane of the condenser, its images in the back focal plane of the objective also undergo lateral displacements. It can be rigorously proven mathematically that under conditions of oblique unidirectional illumination it is possible to resolve finer periodic structures than by unidirectional axial illumination. Although it is beyond the scope of this discussion to include that proof, it suffices to say that:

(1) By the introduction of off-axis unidirectional illumination, the separation of the zero-order maxima and the first-order maxima (which are sufficient to reproduce the periodic structure in the image plane) can be increased to the full diameter of the objective aperture stop. This means increased capability to resolve periodic structure by a magnitude of 2 to 1. The latter obtains when the interference maxima (zero and first-order) in the back focal plane of the objective are separated by a distance equal to the diameter of its aperture stop.

(2) Diatoms, fortunately, present many cases of periodic structure that are resolvable under such conditions.

(3) That, although the periodic structure may be resolved under such conditions, the fidelity of the image produced is not complete.

(4) That there is an azimuth angle of the oblique illumination most favorable to the direction of the linear periodic object structures to be resolved.

The diatom worker can take advantage of this type of illumination without the highly specialized, and now nearly unobtainable, Abbe Illumination Apparatus. The position of the holder of the substage condenser approximates closely the lower focal plane, and simply constructed stops inserted at that point are satisfactory. The stops may be in the form of metal, cardboard, or plastic discs of such a diameter as to fit the filter carrier. The metal or cardboard ones can have the apertures cut-out of the material to admit the off-axis illumination or, in the case of the clear plastic ones, opaque ink or paint applied in such a manner as to provide the aperture. Figure 91 shows suggested patterns of the stops.

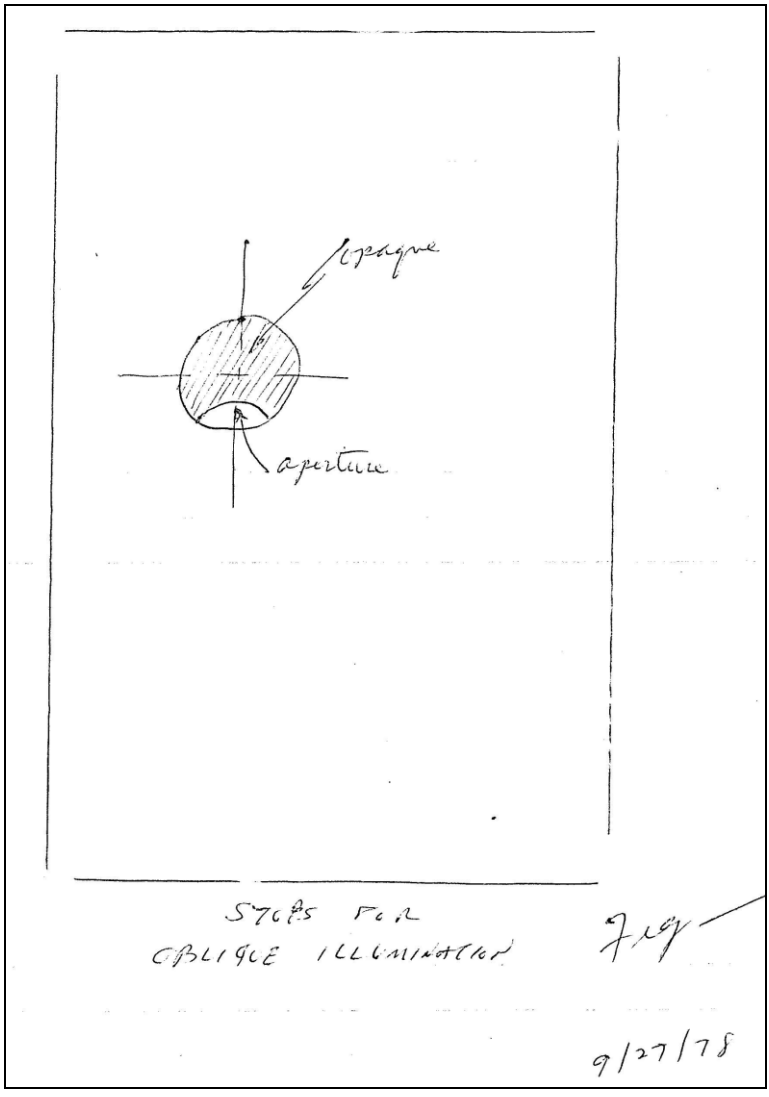


Figure 91.

The eighth moon-shaped aperture in the opaque stop may be rotated about the optical axis for best effectiveness. In this type of illumination the relative position of the off-axis aperture and periodic structure to be resolved is of importance. The azimuth movement of the aperture in the filter holder is effected by finger manipulation, and the exact position of the aperture checked by observing the back focal plane of the microscope objective. If, for instance, the aperture appearance in the back focal plane is as in Figure 91 then periodic structure, such as lines (fused dots) etc., transverse to this will be made much more distinct. (it must be remembered, in the case above, that the crescent moon-shaped aperture itself is actually at the top or opposite side of the condenser, it's lower portion being covered up). As the aperture is rotated about the optical axis, linear periodic structure transverse to its position will be found to be enhanced in distinctness. In the case of

a periodic structure of puncta, an azimuth position can be found for the aperture that resolves them into dots which are distinctly separate in even such difficult cases as the diatom *Amphipleura pellucida*. In circumstances where extremely finely marked diatoms (as above) are under examination, the use of monochromatic light of yellow-green or blue will be of great assistance with this technique.

The resolution of lines into dots by the proper positioning of the oblique aperture must be done very carefully, or the effect may be missed. The advantages of oblique illumination in resolving fine detail are more apparent when high-power, high N.A. objectives are in use. The actual shape of the aperture opening, whether it is to be made from the large arc of a small circle or a small arc of a large one is a matter for experimentation. A selection of several such stops on hand will provide suitable variations for trial.

The actual appearances of object details illuminated by this method will vary somewhat, dependent upon the size and shape of the off-axis aperture. This is related to how important the outer and central zone of the diffraction spectrum are in forming a true image of the particular periodic structure. Some types of structure, for better fidelity, may require more of the central zone than of the outer, or vice versa, and the shape and extent of the stop aperture therefore plays a major part in that formation.

In addition to improving the resolution of periodic structure, this type of illumination can also enhance or change the appearances of complex non-periodic structure to give new insight to their shape and relationship to other structure. For instance, under oblique illumination a “plastic” effect is produced, which if interpreted properly, provides a nearly three-dimensional picture of the diatom frustular surface. The central areas of diatom valves are seen to be thickened and the form of “ribs” and “lipped” structures takes on new meaning. However, it is again emphasized, that this form of illumination, while providing twice the resolution capability of axial illumination, must be used with care. The examination and analysis of a diatom should not rely on oblique illumination alone in any case. The main examination and overall conclusions must be based on axial brightfield and/or darkfield illumination methods. Oblique illumination should be reserved for the resolving of difficult fine structure of a periodic nature and, as an adjunct to the analysis of other complex non-periodic structure.

3.6. Phase Contrast

Contrast in the microscope image can be improved by taking advantage of the optical path (refractive index times thickness) differences within the specimen which result in phase differences between the light waves transmitted by various portions of the specimen. Phase contrast is especially useful in the examination of living material with poor contrast, and provides the advantages without staining of the specimen. It has a considerable advantage over darkfield in that internal specimen details are contrasted, whereas in darkfield the contrast is largely applicable to whole specimen versus the background and/or or its surface details.

The theory and adjustment of phase contrast equipment is beyond the scope of this brief treatment. Those who use, or have access to, such equipment, might benefit by a few observations regarding the system with regard to diatoms however.

First, the method of illumination of phase contrast is Köhler illumination. Although white light can be used, the best results are obtained using monochromatic light of the same wavelength as used for the original calibration of the phase plates. This provides the contrast benefits of a full phase shift effect. Diatoms, particularly the examination of living specimens, are benefited greatly by this form of illumination. The light used in this instance is in the green portion of the spectrum, furnishing attendant benefits to the optics as mentioned previously.

There are two major manifestations of phase contrast generally obtainable, positive and negative, sometimes referred to as dark contrast and bright contrast, respectively. In dark contrast (positive) the results obtained have a similarity to the usual haematoxylin·stained brightfield image. This type of phase contrast provides greater enhancement to contrast and graded variations.

Where there is a choice of observing modes the following may be used as a guide in examining diatomaceous material:

(1) Dark-low-low
Since this mode of phase contrast is designed to furnish the strongest dark contrast having major differences in refractive indices, the subject is dark against a light background. Living diatoms are advantageously examined in this type of contrast.
(2) Bright-medium
Similar to darkfield, this contrast is especially suited for visual examination of the cleaned diatom frustule and/or valves.
(3) Dark-medium
This mode is best for image contrast for specimens with small phase differences. Material appears relatively dark against a bright background. Examination of strews, especially incinerations, benefit by this mode.

The mounting medium for phase contrast is very important, especially in the examination of diatoms which have been fixed without staining. For both permanent and living specimen observation the mounting media with the proper refractive index can be found without difficulty, that with the optical properties of diatoms produces the best results. Special mounting media for the purpose, are available commercially such that there is a wide range of choices to suit most specimen conditions. Such media are furnished in both non-hardening and hardening forms.

Optically perfect slides and coverglasses should be used. The diatomist will, in ordinary mounting of his subjects, adhere to such practice. However it is very important in phase contrast because any unevenness, pits, cracks, striae, bubbles, etc., will be starkly apparent in a total phase image and interfere with features of the specimen image.

It is to be noted that phase contrast cannot equal ordinary brightfield methods in the resolution obtainable. However, the improvement in contrast provides a visibility condition that, at least in some instances, is far superior in gathering total information microscopically. The diatomist with this means at his disposal, should take advantage of it whenever possible.

3.7. Differential Interference Contrast (DIC)

To obtain a superior variable contrast image, and one in which the inhomogeneity of the surround plays a minimal part, differential contrast (DIC) is very valuable. Again, this special method has not been exploited by the diatomist nearly as much as it should be. It does require a specially equipped microscope, not available to all workers. A specific practical system in wide use generally is Nomarski differential interference contrast. In this method incoherent illumination at high N.A. is useable which results in exceptional image brilliance and with resolution almost twice as great as in phase contrast. In DIC systems the design is directed toward variable contrast capability at maximum resolution rather than measurement capability as in ordinary interference contrast. Adjustment of beam-splitters and combiners (with respect to the optical axis) accomplishes the variable contrast feature.

Georges (Jerzy) Normarski (1919 – 1997) Polish Physicist

In making these adjustments, different interference colors are produced which is used for effecting different color contrasts. If the background is made dark, an interference image similar to the bright contrast in phase contrast microscopes is obtained. When the background is made gray, slightly lessened from the darkest, the image will show a gray "hypersensitive" color, giving the highest contrast. This appears in a relief image of the Phase difference distributed over the entire specimen.

When the background is made a sensitive purple, the interference color of the specimen will depend upon the inclination of the optical thickness when the diatom has a difference in level.

The adjustments of the particular beam splitter (or combiners) to accomplish the above effects are sometimes termed "compensation adjustments". They give rise to high contrast in the case of specimens of small phase differences.

The advantages of the DIC systems is to provide excellent variable color contrast at maximum light microscope resolution capability. Diatomists can use this to great advantage in the interpretation of the diatom frustule. Color effects can be produced (in Nomarski) whether there is an object in the light path or not. Therefore the advantages of optical "staining" on a variable basis is available. As optimum contrast is achieved in the case of structures running diagonally to the field of view, a rotatable microscope stage is of considerable assistance.

CHAPTER 4.

4. OBSERVATION AND INTERPRETATION

4.1. Introduction

The study of diatoms, as the study of any other natural subject, involves the interpretation of what is observed during and after the recording of facts. As the study is carried out predominantly with the light microscope, the determination of what are facts and what are not, relative to diatoms, is complicated by not only what appears in the microscopic field, but how the appearances are imaged in the eye of the observer. The adequacy of the light microscope for diatom study, and the importance of proper observation and interpretation is exemplified by a comment by T. F. Smith in the *Journal of the Quekett Microscopical Club*

Thomas Field Smith
b. 27th May 1840
d. 7th October 1916
bu. Wandsworth Cemetery
A House Builder.

> "I think the opinion of the majority of us who have given their minds to the study, has crystallized into the form that diatoms are built up mostly, of perforated plates of silex, and may consist of one or more layers; combining this strength with lightness and economy of materials".

This remark was made in the year 1888! The general consensus on diatom structure has not changed appreciably in the nearly 100 years following that statement, despite the very great advantages of the electron microscope.

4.2. Diatoms and Associated Material in the Field of View

If live diatom material is being observed with the microscope, much other microscopic material, both living and non-living often appears in the field of view to the observer. Dependent upon whether the specimen material has been taken from a marine, brackish, or freshwater environment, the nature of associated material may vary greatly.

The sand grains, common algae and more active protozoans are easily recognized for what they are. To the uninitiated a few things are sometimes easily confused with the diatoms. Among these are sponge spicules, holothurian elements, shelled or thecate amoeba, and the desmids. It is imperative that the beginning diatomist be able to recognize diatoms and to differentiate, at least, them from other forms he may confuse them with. With living or fresh material even the beginner realizes there may be many forms appearing in the microscope field, and is therefore on guard, against making assumptions as to the nature of the items he is identifying.

In uncleaned material some spicules appear dull (not glassy at all) and these together with other calcareous artifacts can be misleading.

However, even after the most vigorous cleaning of diatomaceous material, there often remains sponge spicules, holothurian elements, radiolarians, silicoflagellates, and they remain to be differentiated from diatoms in strewn preparations.

The sponge spicules are very diverse in form, many different ones perhaps belonging to the same sponge. They are solid, sharpened at the ends, very glassy in appearance and usually a narrow channel can be detected running concentric to the structural elements. Radiolaria tests are, in the main, very open structures, given to delicately constructed open networks of silica in globular, cylindrical, and other inflated shapes. Openings are usually large in comparison to the lace like silicate members. The holothurian elements commonly encountered are in the form of plates, flat and/or curved, the thickness being only a fraction of transverse dimensions. They occur in various and diverse shapes, mostly circular or elliptical. Openings are large in comparison with their size. The combination of openings and shape often convey the impression of "wheels" or other fanciful forms.

4.3. Appearances

Simon Henry Gage
b. 20th May 1851
d. 1944
Professor of Anatomy, Histology and Embryology

Gage (1936) says "appearances which seem perfectly unmistakable with a low power may be found erroneous or very inadequate with high power; for details of structure which cannot be seen with a low power may become perfectly evident with a higher power or a more perfect objective. On the other hand, the problems of microscopic structure become more and more complex with increased precision of investigation and more perfect optical appliances, for structures that appeared intelligible with a less perfect microscope-may show complexities in their details of structure with the more perfect microscope which open up an entirely new field for interpretation".

Because of the nature of diatoms their study demands the ultimate performance from the microscope optics, and the utmost skill of the diatomist. Gage's words are particularly applicable to that study. The diatomist must not allow any false appearance to mislead his judgment and observation of the factual appearance in the image presented to his eye. Those false appearances that can be avoided or eliminated, should be. Those that cannot must be recognized for what they are.

In order to get a correct visualization of any body, one must experience more than one dimension, - two for plane surfaces, three for solids. So, for microscopic objects, one must examine more than one face. This is particularly true for diatoms. Many professional and amateur slides show only one or two valves, or, if more, then a number of duplicate specimens all showing only one face of the valve.

It is important that the inside and outside of the diatom valve, as well as the zonal aspect, be examined, or the diatomist will obtain a very inadequate and often erroneous conception of the true form.

Where material can be examined before mounting and new mounts prepared, the judicious use of a mechanical finger to examine the diatom frustule in all its aspects

should not be passed up, and a mount prepared that will show the salient features and provide a complete true visual reference to the specimen. When diatoms already mounted on microslides are being examined, or when it is not possible or feasible to manipulate them for a three dimensional view, proper interpretation of the fixed diatom appearances will do much to clarify the actual structures. This can be accomplished by selective focusing of the outline and details of the frustule or valve, as the case may be; the general rule is that objects highest up come into focus last in focusing up, first in focusing down. By examining a diatom with at least a high N.A. high-dry objective, and/or an oil imm. objective, in a systematic manner a mental image of its surface variations, thicknesses, etc., may be built up by optical section.

The distinctness of outline of diatoms and their various features depends, as with other microscopic objects, on the difference between the refractive index of the diatom and the medium in which it is immersed. (See "Index of Visibility" in Part I Chapter 7). Therefore it is important that delicate outlines, striae etc., be viewed with the diatom immersed in a medium with a high refractive index. The R.I. should be at least 1.60 or more. Observations of diatom outline and structural detail in water are very poor, and identifications made on such preparations, excepting in very obvious cases, are suspect.

In strewn mounts, where the diatom specimens are so plentiful as to overlap or overlie one another, occasionally striking moiré effects are observed. In very crowded specimen strews these patterns may confuse the actual structure being observed, to the point of misinterpretation. These images are the result of the superposition of two or more periodic patterns. Similar effects are sometimes (rarely) evident from one diatom wherein an entire frustule is being observed that is tilted and in which the patterns of striae of both valves are sufficiently in focus such as to combine.

The term moiré derives from a form of textile (usually silk) that has a 'rippled' appearance.

4.3.1. Puncta

Puncta, while mostly within the range of the light microscope, are subject to misinterpretation. The extreme of misinterpretation is either when insufficient resolution is available to separate individual puncta and "lines" or striae are the interpretation, or when they are so small as to be beyond the resolution capability and thereby invisible and assumed to be not present.

The appearance of puncta as "dots" or "beads" is, of course, a manifestation of the limitation of the resolving capability of the microscope optics. Their actual structure is that of a hole in a flat, raised or sunken surface. Regular "beaded" formations are usually depressions or cavities pierced at the top and/or bottom of each by an elliptical or circular hole or aperture. The resolution of a puncta into a circular (or elliptical) edge which bounds a hole in a silicate membrane of some type has been discussed before, and will be interpreted as such when the microscope optics are sufficient as to image the details in true form. If brightfield illumination alone does not reveal true structure, then other illumination techniques can be sometimes

employed such as darkfield, oblique, etc., to enhance or substantiate the proper interpretation.

The true nature of the "beads" on the surface of diatom valves was demonstrated long before the SEM and its advantages were available to the diatomist. Diatoms were examined in liquids of various refractive indices, and the appearances of these features observed as the microscope was focused. On raising the tube of a microscope an object will show a bright center if its refractive index is greater than that of the liquid in which it is immersed, and a dark center if its R.I. is less than that of the liquid. On lowering the tube (from exact focus) the opposite effect will be produced. It was found that the so-called beads assume the refractive index of the medium (being filled by it) and are therefore cavities.

Since the puncta present characteristics that are very useful, specifically, generically, and ecologically, the proper interpretation of their true nature, number, size, direction and coarseness of distribution is very important.

To determine the direction (or attitude) of the striae (unresolved puncta rows) with relation to the center-line of the diatom valve is sometimes difficult. To facilitate this observation, Hustedt recommends:

> (1) Bring the end of the valve into the axis of the microscope.
> (2) Follow the striae from the real surface of the valve downwards, the light being absolutely centric.
> (3) They (the striae) may be perpendicular to the median line (the imaginary longitudinal line in the middle of the valve that is often, but not always, identical with the raphe.
> (4) If the mirror of the microscope is inclined in a direction parallel to the longitudinal line, the striae will appear to be convergent at the ends.
> (5) Returning the mirror to incline it in the opposite direction will cause the striae to become first parallel (perpendicular) and then become divergent at the ends.

If diatoms of different structure are examined, there will be species which will show the change of direction corresponding to the change of direction of the light. These remarks place great emphasis on absolute centration of the lighting when examining such features as might have their apparent symmetry and/or geometry affected otherwise. While oblique lighting, and other deviations from normal centric illumination have great utility in enhancing certain information gained from the image, they are not suited to this type of observation and previous cautionary comments regarding such are applicable.

4.3.2. Cingula

Beginning diatomists will often notice forms having the exact shape or outline of a diatom, but will not be able to see any markings inside the outline. These shapes are often the girdles which bind the valves of the frustule together, and which have

become detached. Sometimes there is a gap or space in the outline where the ends of such detached girdles are broken or have a natural separation. Details of the separation should be studied, if possible, under high magnification to determine their shape and/or their method of joining or fitting together.

4.3.3. Broken and/or Fractured Surfaces

Diatoms, especially in strewn preparations of a fossil nature, sometimes are broken, cracked, or otherwise fractured. These particular features are valuable sources of special information, often unobtainable in any other way with the light microscope. Diatoms, such as specimens of *Coscinodiscus*, having holes or pieces broken out of the outer valve surface, will reveal the details of the inner, or pore, membrane. If such a valve is broken to show an extended edge, then often the relationship and structural details of the outer membrane, locular walls, and inner membrane are revealed. These observational advantages are more pronounced with the non-pennate diatoms, as the distinction between the inner and outer surfaces of the pennate group is not as marked. However, any broken forms should be examined very carefully at the highest useable powers to extract as much information as possible on such secondary structure.

If such broken diatoms are immersed in a viscous, fairly high refractive index liquid mountant, and the coverglass moved appropriately (with the eraser end of a pencil for instance), the diatoms can be positioned to observe broken valves at many different angles.

In observing fractures and/or edges, the microscopical images are bounded by an umbra, coma, or undefined margin The umbra tends to blot out all finer structure in its immediate vicinity. Thus the umbra of a primary blots out (either partially or wholly) the image of the secondary. For example, the postage-stamp fracture in a specimen of the diatom *Triceratium favus* is not so easily seen as it might be because of the umbra of the large primary structure. On the other hand, there is no similar difficulty experienced with the postage-stamp fracture of *Pleurosigma angulatum* or *Navicula rhomboide*s as there is no coarse primary present. So, it is evident that dependent upon the diatom structure itself, the advantages of such observations vary considerably.

4.3.4. Color

The beginning diatomist, although usually aware that the material of which diatoms are made, is colorless, is curious about the appearance of coloration of certain diatom specimens.

Diatoms owe their “color” to the diffraction of light by their minute structure. They change “color” from violet to red and finally lose it altogether, as the power, or rather the aperture of the microscope objective is increased. For instance, with dark ground illumination and a low-power objective (10X) of aperture 0.25-0.30 N.A., a slide containing various species of *Pleurosigma* having different degrees of fineness of structure will show differing hues. The coarser forms will appear ruddy, those a

little finer greenish, and those still finer, blue, and if even finer, violet or indigo. If the objective is now changed to one with a greater N.A. of say 0.4, those that were ruddy will be colorless, and the structure that gave rise to the color resolved, those that were green will be ruddy, and those that were blue, will have become green, and so on. If a lens of greater aperture is employed, those that were originally green will become colorless and structure resolved, and the colors of the others will be lowered a step in the gamut.

Diatoms that are most notable in showing the color phenomena include many forms of *Actinocyclus, Actinoptychus, Auliscus, Climacosphenia, Lepidodiscus,* and *Pleurosigma*. Taylor includes a list (as follows) of diatoms showing such colors, with their localities:

Actinocyclus: from San Francisco, Yarra River, Panama, Japan Oysters, Mauritius, Indian Ocean, Cuxhaven.
Actinodiscus: From Oamaru, Yedo, Barbados, Atlantic City, Ananino.
Actinoptychus: from San Diego, California, Santa Monica, Calif., Atlantic City, Oamaru, Japan Oysters, Campbell Is., Madagascar, Cuxhaven, Bory, Szacal, Szent Peter, Ananino, Simbirsk. Especially *A. Ezontugii, Gruendleri, hexagonus, undulatus, spinifer, vulgaris, trilingulatus, heliopelta*.
Auliscus: from Oamaru, Santa Monica, Pisagua (Peru), Brunn. Especially *A. normanianus, hardmanianus, speciosus, oamaruensis*.
Asterolampra: from Brunn.
Cerataulus: from Cuxhaven, Mexillones, Gulf of Carptenaria.
Cocconeis: from Cape Verde Islands.
Entopyla: from Pisagua, Walfisch Bay.
Grammatophora: from Cape Verde Islands .
Grayia: from Maryland, Santa Monica, Redondo Beach.
Heliopelta: from Atlantic City, Nottingham, Maryland, (Heliopelta is now considered an invalid genera and is replaced by *Actinoptychus*)
Hyalodiscus: from Santa Monica, Santa Cruz, San Francisco, Oamaru, Yarra River, Port Louis, Mauritius, Kerguelen Is., New Hebrides, Sandwich Is. (Hawaii), Red Sea, Bory, England.
Hydrosera: from Bory, Indian Ocean, Mauritius, Madagascar, Hobart, Gulf of Carpentaria.
Lepidodiscus: from Anino, Archangelsk, Simbirsk,
Melosira: from Oamaru, Walfisch Bay, England, Bory, Szent Peter, Simbirsk.
Nitzschia: from Amherst (Burma), Cameroons, Caspian Sea, Adriatic.
Pleurosigma: from Puget Sound, Samoa, New Hebrides, England.
Polymyxus: from Para River, Cameroons.
Rhizosolenia: from Aragura Sea, Hongkong, etc.
Rutilaria: from Sendai, Santa Monica, Hungary.
Stauroneis: from England, United States.
Terpsinoe; from Mobile, Ala., Demerara River.
Triceratium: from Oamaru especially *T. lineatum*

Color effects seem much more emphasized in darkfield because of the increased enhancement of the colors by contrast. They also exist in brightfield, perhaps to a lesser marked degree to the eye. The color observed is not that attributable to microscope optics, which manifests itself in an entirely different manner. It is the result, as mentioned before, of the diffraction of white light by the diatom structure. If monochromatic light is utilized the colors are not presented. Although many diatoms are very typically colored, especially *Pleurosigma*, *Actinoptychus*, etc., when using white light, the effect is so dependent upon the actual color of the light source, the centration or obliquity of the light to the optical train, and the general chromatic corrections applied to the microscope optics, that it has seldom been utilized as a firm factor in diatom description. Although a rigid setup of standardized optics and specific adjustments of the microscope could be devised to obtain repeatable color indications within limits to describe certain diatoms, the benefits gained would be minimal compared with the effort.

However, although information not specific enough for a formalized written description may be gained, the coloration of certain diatoms does contribute to their recognition especially in strewn preparations. The criteria by which we recognize certain objects, their "obviousness", includes such color phenomena, as well as shape and other visual impressions. For instance, *Actinocyclus moniliformis* is distinguished, in part at least, by its strong iridescence, and by its sharply defined zones of color under low-powered observations, from *Actinocyclus ehrenbergii*. Color could very well be dependent, in some cases, on the condition of the valve-whether it consists of more than one plate for instance, or whether there is a significant spacing between layers etc., which affects the degree of light diffraction.

4.3.5. First Impressions

A quick glance at a horse and a dog enables us to tell them apart and for what they are. An equally cursory glimpse of a small dog and a fox is sufficient to differentiate between them. The ability to make such distinctions is based on our knowledge of familiar objects, and on certain factors relating to a visual impression of shape, size, symmetry, texture, color, and other "obvious" features. After some experience in diatom study, the various genera and sometimes species, are very obvious and easily identified, as familiarity with them increases.

At the outset, a good start in making these distinctions can be made by considering certain prominent, or dominant, features of the various genera. Chapter I of Part I of this book contains a listing of morphological features best exemplified by certain genera of diatoms. Study of this list, and/or reference to it when examining strews will, in many cases, provide a valuable clue to specific diatom identification. The arched and/or saddle-shaped genus *Campylodiscus* for instance, is in no way confused with the radial undulated and circular form represented by *Actinoptychus*. Nor is the latter easily confused with *Arachnoidiscus* although both are circular. Wherein some of these major physical appearances are common to two or more genera, then a fairly obvious secondary or minor feature, usually suffices to differentiate them and so on.

The identification of the pennate diatoms is generally by outline of the frustule, its length and breadth, and by structure and markings of the valve surface. The centrics, being mostly circular are identified at first glance by valve surface structure and markings, prominences, and edge characteristics.

4.4. Special Preparations

In previous portions of this book a number of methods of preparing diatoms for study have been detailed. Most were for the staining and/or other preparation revealing the contents or parts of the contents of the frustule, or for the study of the whole frustule or valves in strewn or selected mounts.

Certain special preparations, designed to illustrate specific features, construction, or other truths about diatoms are not as commonly made. In fact, with the advent of the electron microscope (TEM or SEM) the need for some of them has become completely unnecessary.

However, the diatomist who has access only to the light microscope can benefit by these special preparations in gaining first-hand insight into diatom ultrastructure.

They, in some cases, will be of considerable practical use, and in any event are academically interesting and will serve to provide an excellent supplement and confirmation to "known" information via the electron microscope.

Studying broken diatom frustules to gain information on ultrastructure is indeed valuable and has the advantage of no special preparation being necessary. However, the types, locations, and positions, or orientations, of the fractures, are of random occurrence and of a haphazard or fortuitous design.

The desire for specifically located sections or, at least smooth regular ones, must be the result of special preparations. A number of the older diatom researchers, among them Otto Muller, W. Prinz, and E. van Ermengen, J. D. Cox, C. Houghton-Gill, Julien Deby, and Dr. J. H. L. Flögel, used special preparations in studying the diatom frustule. Their pioneer work did much to advance the correct concepts of diatom structure, much of which is valid now more than one hundred years later.

Charles Haughton Gill
(not as is commonly used Houghton-Gill)
b. 1841
d. 21st February 1894

Émile Pierre-Marie van Ermengen
(1851 – 1922/1932)
Belgian bacteriologist

Dr. Jacob Dolson Cox
b. 27th October 1828
d. 4th August 1900
Governor of Ohio
Secretary of the Interior
President of Toledo and Wabash Railroad Co.
Dean of the Cincinnati Law School

Prinz and van Ermengen based their conclusions on sections of diatoms found in the Cementein of Jutland. Gill employed chemical solutions in a double decomposition method to examine cavities in the frustule, and Flögel made sections of selected diatoms to disclose frustular wall structure and membrane relationships. Most of the methods employed by these diatomists are well within the capabilities of modern students who have the interest and patience to perform them. A few of the more interesting of these special

preparations are described in the following paragraphs.

4.4.1. Diatom Sections

4.4.1.1. Cutting

A somewhat unusual method of preparing sections of diatoms was described by Pelletan. Instead of the usual grinding procedures common in rock sections, he used an ordinary microtome to cut them.

Julien Deby
(1826 – 1895)
Belgian diatomist

He cut sections of fresh material, and was able to verify the arrangement of internal diatom features, such as the endochrome, in certain diatom species. Freely translated from the French, the essentials of his method are as follows:

Johann Heinrich
Ludwig Flögel
(sometimes Floegel)
b. 10th June 1834
d. 25th January 1918
Lawyer and Naturalist.

Jules Pelletan
(1833 – 1892)

First, a quantity of the diatom material to be sectioned is cleaned as completely as possible. Material in which the sections are to reveal internal structure must not be treated in acids of course, and recourse to only simple washing, sieving, and settling methods can be tolerated. The removal of all impurities; particularly sand grains, even the smallest, is necessary, as the latter will tear the sections to pieces and chip and/or rapidly dull the cutting knife. Of course it is clear that the most lightly silicified forms are most susceptible to this method of sectioning. The use of pure collections of diatoms free of debris, is recommended.

The cleaned diatoms are placed in a quantity of dense gum arabic to which is added a small quantity of glycerine to keep it from becoming too brittle through drying. The diatoms will stay suspended in this thick solution.

A large drop of the suspension on the tip of a glass rod is applied to a small piece of cork or the pith of an elder tree. It is then left to dry, being covered to protect it against dust. When it is dry a second or even a third drop may be applied over it, but it is not advantageous to make the preparation too thick.

When the enclosing mass on the cork is well dried, it is sometimes helpful, if the diatoms are sufficiently well silicified, to harden it further by immersion in alcohol at about 82 degrees for 24 to 48 hours. When it is sufficiently hard, such that it cannot be dented by the fingernail, it is possible to section it as histological sections are.

Instead of the gum arabic solution, one can use, at a gentle heat, a solution of gum and gelatin, or celloidin, both of which give good results and permit the use of small blocks holding a larger number of diatoms in suspension. However, the sections obtained appear less satisfying and are more difficult to treat later on.

Hand cutting of such material is unsatisfactory. The movement of a razor provided by hand is not rapid or strong enough, as the diatoms that the blade meets have sufficient time to see-saw or otherwise shift position under knife pressure because their cutting resistance is much greater than the surrounding mass. It is essential to use a microtome. It should be one in excellent condition with a smooth regular action. (gliding sledge type is perfectly satisfactory). The knife should be one of a hard temper as it will be quickly enough dulled, and need to be sharpened often.

It is possible to actually produce fine enough cuts that would provide multiple sections of the larger diatoms. However, it is not necessary to obtain very thin sections, but to only provide cuts through the frustule.

The small piece of cork, or elder tree pith, on which are the gum encased diatoms, is placed in the microtome specimen carrier, taking care not to crush it. The diatom drop should have been placed on the cork (or the cork oriented) in such a manner as to allow transverse cuts at the surface, not parallel or tangential ones, as they are much less successful. With this orientation of cut, the heavy blade goes through the gum drop and supporting cork in one swift motion. The diatoms between the edge of the blade and the cork have sufficient time to be displaced by the traversing cutting edge. A cut of one-hundredth of a millimeter in thickness, or even greater is sufficiently thin.

The sections thus made, contain a part of the cork or elder pith and a part of the diatomaceous gum suspension. These are placed in a watch glass with a little water which rapidly softens the gum, and the cork is removed easily. The gum suspension is placed beneath a cover on a microslide and examined with the microscope. It is apparent that many diatoms have not been cut at all, but that others have with parts of the frustules in all attitudes. The two surfaces of the section show the same result.

It is in this form (the gum section) that the constituent elements of the diatom cell are studied as the frustules maintain their positions. If additional water is added sufficient to dissolve the gum the frustules, whole and sectioned alike, will become free. The desired sections can be retrieved and stained by an ammoniacal solution of picrocarminate to reveal the protoplasm, nucleus, and endochrome, leaving the oil globules colorless. For the best results, the diatoms contents are fixed by the application of osmium tetroxide before the embedment in the gum and sectioning take place. Pelletan recommended that the subject diatoms be suspended for an hour in water into which a few drops of 2% solution of osmium tetroxide are added. (He reports that penetration of the silica frustule is too slow by vapor exposure to the acid).

The fixing of the diatoms in this way does not hinder the staining of the sections, although it may take place at a slower rate. The oil globules will be colored intensely black by the osmic acid. After the staining the material is removed from the water and placed in glycerine for microscopic examination.

Commenting on other methods of embedding the diatoms, Pelletan indicates that the processing, especially that using celloidin or other such materials is inferior in results. He mentions that excessive use of alcohol in such cases, to harden the frustules, affects the color of the endochrome and the oil globules. Also, the use of celloidin or similar embedding materials produces problems in mass cohesion and

sections have a tendency to crumble, dry, or shrink. (In the use of modern embedding materials, this may not be the case.) He recommends the simple gum embedment and sectioning procedure described for best results.

Unfortunately, sectioning of the diatom frustule (or valves) by cutting in a microtome, as described, is not always applicable as many diatoms are very heavily silicified and will not be cut by the knife.

Hustedt describes a similar simple process for the sectioning of diatoms. A water drop rich with a suspension of diatoms, is mixed with gum (presumably gum tragacanth) and applied to the surface of a small piece of elder pith and allowed to dry. Then, avoiding any moisture whatever, delicate cuts with a razor are made through the dried material that are subsequently mounted on a microslide in Canada Balsam.

From the outset correctly oriented sections can be obtained if the diatoms are in advance properly positioned in the gum. A thin coating of collodion is applied to a part of a microslide and after it has dried completely, a drop of the thick gum solution is applied to it. A trace of water suspension of diatoms is then applied to the gum drop and mixed in. With the help of a very fine needle, and under the microscope, a diatom is positioned in the proper attitude, near the edge of the gradually drying gum drop, in which it will remain.

As the diatom(s) will become completely enclosed by the gum drop it may be necessary to remoisten it from time to time while positioning is being performed. Finally the upper surface of the gum drop (with diatom positioned) is covered with a thin layer of collodion and then the entire preparation lifted off of the microslide. It can then be properly sectioned in the desired orientation.

The positioning and sectioning of stained material from the alcohol step can be accomplished in the same way. It is not recommended that dry diatom material be introduced into the gum, as frequent air bubbles interfere with the sectioning.

To properly analyze the sections one must be very careful, as with the thinly silicified walls slight displacement of the individual parts against one another easily occurs. This means that the position relationships in section do not, in all details, absolutely correspond to reality.

4.4.1.2. Grinding

Grinding of diatom sections has been accomplished in various ways. The most obvious method is to grind rock sections containing diatom frustules thin enough to allow transmitted light to be used in examination and study. The procedure is the same as would be followed with the grinding of rock or mineralogical sections. Prinz and Van Emengem examined diatoms of the Cementstein of Jutland, and the London clays, by such means. Mon. J. Deby was able to compose a tough stony-like embedding material made with the chlorates of zinc and magnesium mixed with oxides of the same metals. He sectioned the “artificial stone” into which diatoms had been suspended, by grinding.

The grinding of rock materials containing diatoms is comparatively simple and well within the capabilities of the average worker. First, a small chip or piece of the diatom-containing rock is selected, preferably with one fairly flat side, and fastened to a microslide, flat side down. Canada Balsam has been used as a cement with success, although a more modern material easier to use is Lakeside 70. This hard tough cementing material penetrates voids easily, has a very strong adherence to glass, melts at a low temperature, and hardens rapidly. In any event, after the small specimen piece has been cemented to the microslide, the grinding process is begun.

Using a rather coarse grinding compound of 120 grade carborundum, the piece is ground flat on an iron lap. The piece is then ground smoother with finer abrasives; such as 3F carborundum, on a copper lap, and finally with 600 grade compounds on a plate glass and cloth lap. The grinding compounds are used in a slurry formed with water. The grinding is accomplished by a rotary motion of the microslide holder without too much pressure. After the top side is finished it is carefully removed from the microslide by application of the cement solvent, inverted and re-cemented on a clean microslide. This side of the specimen piece is ground smooth using the same procedure as on the first. In this step, as the slice becomes thinner, frequent checks under the microscope will reveal when the proper thickness is reached. When the section is deemed to have the desired thickness it can be immediately made into a permanent preparation by application of mountant and cover glass (if Canada Balsam was used as the cement). If the cement used was Lakeside 70 or if higher refractive index mountants than Canada Balsam is desired in the final mount, then it will be necessary to remove and clean the cemented section and remount it on a new microslide. The very thin section on the microslide is placed in a Petri dish with enough cement solvent to cover it and the section allowed to loosen and float off. It is then retrieved by a section lifter, forceps, or other means, and mounted by normal procedure.

Hustedt recommends using a hone, or emery paper, to initially flatten and smooth the first side of the chip before fastening it to the microslide. He further indicates a finer grinding and complete polishing of the initial side with leather or cloth This method would then complete in one step the smooth grinding of one side of the specimen. Following this he proceeds as above, using Canada Balsam as the cement, and grinds smooth and polishes the upper surface. Removal of the completed section is dependent upon whether the final mount is desired to be in Canada Balsam or in a higher refractive index medium. For the former, it suffices to add additional Canada Balsam and coverglass. For the latter, the section is removed as before and then mounted in the desired medium. Frequent microscopical examination during the final grinding and/or polishing, not only reveals the thickness necessary to transmit light, but can also be useful in determining when a particular feature such as membrane septa, wall, raphe, etc. of a frustule has been revealed in satisfactory section. Careful analysis at this point and subsequent observations during continuing grinding can in itself be of inestimable service in building up a complete picture of internal structure. A series of sketches, drawings, or photomicrographs during this process will be an important record of such observations for future reference and comparison.

Diatoms purposely embedded in an artificial hard material may also be sectioned in a similar manner to those in a rock matrix. The advantages are, of course, that the diatomist can choose the diatom type(s) he wishes to section, and with certain techniques, even orient, and otherwise position, individual frustules to obtain pre-determined sectional views. This approach is perhaps the most valuable to the researcher. When a strew is examined the diatoms lying in their natural attitudes throughout, generally are thin enough to exhibit views in longitudinal cross-section. However, the views of diatoms through sections normal to their long dimensions are rarely seen, so the selective placement of such and subsequent grinding to reveal these sections is highly valuable. For instance, the cross-sections of forms such as *Nitzschia, Hantzschia, Pinnularia, Synedra,* etc., are not ordinarily viewed and the views of transverse (diametral) sections of centric forms are not either.

Before going on to the methods of accomplishing such sections, it is of interest to examine various methods of accomplishing random sections in artificial hard media:

The simplest approach to the latter is by the use of Canada Balsam. If a sufficiently pure and porous piece of diatomaceous earth is available, it is normally too friable for the purposes of grinding. Therefore some sort of binder to retain diatoms in position is necessary. Hustedt and others have used Canada Balsam as the binding agent. The diatomaceous mass (a small piece from 5 to 10 millimeters in the largest dimension) is soaked in a thinned Canada Balsam preparation for 12 to 24 hours. The balsam diatomaceous mass is placed on a microslide and allowed to harden (heat may be applied to drive off the excess solvent). Then the mass is ground with a fine grinding compound and topped with a coverglass for final examination. Because there are many forms in many different attitudes and positions in such a mass, the grinding process will usually produce sections of sufficient variety to satisfy at least a cursory examination.

Diatom specimen material in liquid (either fresh or in storage) may be likewise treated. It is first passed through a series of alcohol stages until no trace of water remains, then into xylene and finally into balsam, which is allowed to harden and treated as before to obtain sections.

The methods of obtaining random sections of diatoms are simple and easy to accomplish, but often more precise sections are desired. This is accomplished by orienting a single diatom on a microslide with a mechanical finger, and a mount prepared with a gum-tragacanth or similar adhesive.

If a stereomicroscope is available with a magnification of about 40 to 100 diameters, the selection, placement, and subsequent checking of the grinding in process, is made very easy. However, with some care, the same techniques may be applied using a conventional microscope at about 100X. An objective of 10X magnification (with a 10X ocular) is sufficient and provides adequate working distance.

The diatom is first selected from a store slide (for instance) and by use of a mechanical finger transferred to a microslide that has been previously prepared with a mucilage for fastening it in place. It is then oriented by mechanical finger, fixed into place, and the embedding material to be used, added. If Canada Balsam is used Hustedt recommends that it be in a dry powder form. Before it is applied he recommends flooding the positioned diatom with ether, then applying the pulverized

balsam. The ether dissolves the balsam into a solution which penetrates the cell completely. Warming this preparation, at no more than 50 degrees, allows the balsam to thicken to the point, which after cooling, is hard enough for the grinding process.

The grinding is accomplished using the surface of a coarsely ground glass plate with the aid of ordinary olive oil and gentle pressure. Finishing is on a finely ground glass surface, and polishing on a smooth piece of glass. During the grinding, control is exercised by applying benzene to the ground surface (to free it of oil) and examining with the microscope.

4.4.1.3. Etching

Aside from cutting and grinding, another method for revealing diatom structure is by etching with hydrofluoric acid. As this acid attacks silicates (including glass) it must be used with great care and ordinarily in very dilute form for the purpose. While observing with the microscope, a very dilute solution of hydrofluoric acid is applied to gradually dissolve the frustule (or valve). Protection of the microscope objective glass from attack by fumes of the acid is necessary and can be accomplished by a very thin film of mica fastened over it. This procedure is only practical with comparatively large diatoms wherein the attacking acid etches away specifically moistened surfaces or features, or those that are considerably thinner than others. Bailey, according to Taylor showed that large spots on *Isthmia*, which look like granular projections, are really thin portions of the cell-wall, like panels of a door, by a similar process.

The silica frustule of large diatoms such as *Gyrosigma balticum*, *Pleurosigma angulatum*, and *Amphora alata* have been dissolved away by immersion for 24 hours in 4% hydrofluoric acid (after fixing) and the cytoplasm examined.

Hustedt also mentions corrosion methods. In diatom collections it is frequently found that certain rib-like thickened parts are completely preserved and that thinner membrane parts on the other hand have disappeared. The thinner membrane layers are more easily destroyed in nature and account for the condition. The susceptibility of these thinner members of the diatom frustule to chemical influence provides an advantageous means of examining many structural relationships.

One method to take advantage of this is to expose the diatoms to be investigated to a hot solution of sodium carbonate, or even better, of potassium hydroxide. The application of the chemical is observed and controlled under the microscope so that the corrosive action can be interrupted at the proper moment to reveal certain key membrane structure. In this way many worthwhile contributions to the clarification of diatom structure can be made, especially if the frustule (or valve) is properly oriented. Which orientation to select of course is dependent upon the requirements of the investigator.

It is emphasized that the proper manipulation of the microscope fine adjustment during such observations is of primary importance. Proper attention to it permits, after all, many insights into, and the correct comprehension of, the observed details.

4.4.1.4. Chemical Feature-Enhancement

In addition to the means described above to obtain sections or other dissected views of diatom structure, there are ways by which diatom structural features may be enhanced so that they are contrasted with other structure, or, for purposes of revealing their true structural nature by inference rather than direct observation. These means largely entail the use of chemicals acting upon, or in conjunction with, the structure to provide visual effects which infer, upon proper interpretation, certain facts about frustule structure not apparent, or difficult of analysis, by direct observation. Many of these methods were used fifty or a hundred years ago to answer questions about diatoms which are now answered and very graphically illustrated by use of the various modes of electron microscopy. Nonetheless, it is important that these rather simple, but ingenious, methods not be lost to the diatom worker. For those who work alone, with limited facilities, or for simplified and straightforward means of demonstrating various facts about these minute plants, the old methods are very valuable. Some of them will be described here.

4.4.2. Contrast Improvement

Diatoms placed in a sugar-water solution to which is added some concentrated sulfuric acid, will be stained a dark brown.

When diatoms have been soaked for a long period of time (several days) in a mercury salt (such as mercuric nitrate), the substance of the silica itself takes on a slight grayish stain and reportedly renders improved definition by contrast.

If clean diatoms are soaked in a solution of mercuric nitrate until their hollows are filled with it, and then are immersed in ammonium sulfide, a double decomposition takes place (double decomposition is a simple exchange of radicals of the two substances concerned) whereby black insoluble mercury sulfide is produced and left in the minute cavities in which it was formed. Careful levigation with water will free the charged diatoms from the greater part of the loose and unconfined sulfide and leave them clean enough for examination. C. Houghton·Gill in the *Journal of the Quekett Microscopical Club* for 1890 described this method to demonstrate the hollow nature of many of the “markings” on the diatom valve.

Another method to accomplish a coloring of the interiors of alveoles and/or other cavities is described in Van Heurck. The diatom valves are soaked in ferric chloride and then treated with a solution of potassium ferrocyanide, thus producing deposits of Prussian blue, which are fixed in the interior of alveoles, while the exteriors can be cleaned by washing.

The means described above to chemically enhance contrast and structural details are, of course, only representative. An ingenious worker, with a minimum of chemical knowledge, can certainly devise alternative similar methods to accomplish the same results. The use of fluorescent stains now available in profusion should be a fertile field for investigations using the fluorescence microscope.

4.5. Observation and Notes

One of the most important activities, if not the ultimate activity, of a diatomist is to observe the diatom(s) and record his observations for a definite purpose. That observing process and the recording of data resulting from it may take the form of written notes, drawings, and photomicrographs. The latter two of these will be taken up in greater detail later on. At this point an important factor in any investigation involving diatoms is identification. The identification is made from observations through the microscope and reference to existing literature. What to observe and what and how to record information to identify diatoms is described in the following paragraphs. The procedures and methods described are, of course, not hard and fast rules to be followed blindly. They will vary according to the experience and inclinations of the individual. They do describe a way to go about the task of identification however, that is straightforward, workable, and avoids pitfalls sometimes encountered by the beginner.

Identification requires the observing and/or recording of minute detail of the diatom frustule. When examining such very minute objects with objectives of the highest powers, employing large axial cones of light, slightly averted vision may be of great assistance in steadily holding faint and difficult details. The utility of averted vision is well known in telescopic observations. In some cases, faint diatom structure which can be certainly held with averted vision, becomes absolutely invisible when viewed directly.

Of first importance, unless the diatom can be identified on sight as a species, is a sketch that should be made on a paper pad or notebook, of its salient features. This sketch is just that, and not a drawing (treated later) that one would make for illustration of a diatom. The sketch is made as word notes are in a concise form, mainly to record data and reinforce the memory of the observer. The literature search in identification of a diatom can be very time consuming. If an adequate sketch has been made, along with appropriate notes, constant microscopical re-examination is avoided. As the references are numerous and, many times, of large format, it is not usual to have them handy at the microscope observing position without undue clutter and interference. An even more important reason for the sketch is that it serves as a sharpener of the visual senses of observation. The necessity of actually sketching or drawing out individual features requires a certain concentration on the object such as to improve the quality and quantity of visual data obtained.

The sketch should be made with a soft pencil so that it can easily be corrected and adjusted if necessary. No necessity exists for the use of any drawing aids. An outline of the diatom is drawn as best the observer can make. If the diatom is circular, a simple pencil-compass can be used, but even that is not essential. A perfect circle is not necessary to remind one that the form is circular.

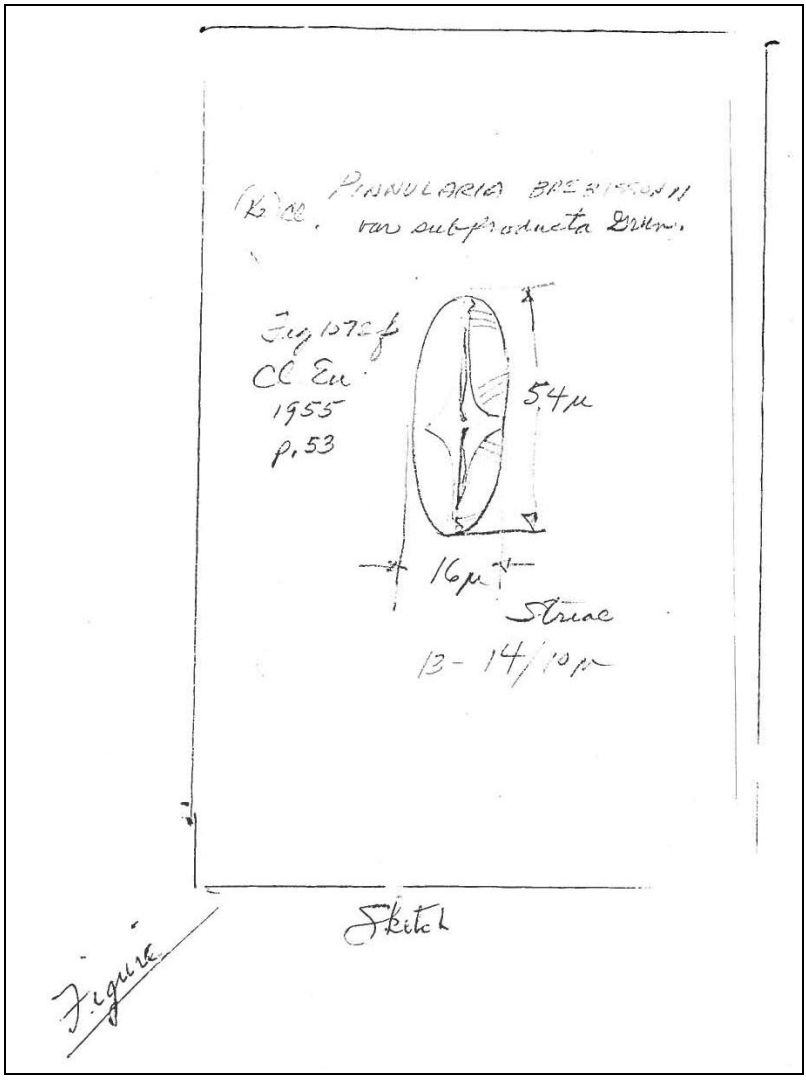


Figure 92.

The identification of the pennate diatoms is generally by (1) the outline of the frustule in valve view, (2) the length and breadth, and (3) the structure of the valve surface. After the outline is drawn a representation of the axial and central areas formed by the striae (puncta) is sketched in. Merely an outline of this area is sufficient. Within that outline some indication should be made of the attitude or orientation of the striae at the center and ends of the valve depicted. A few lines suggesting this are all that is required. The raphe should next be drawn in with attention paid to whether it is straight or curved and its nature at the central nodule and polar nodules. The valve should be measured in length and breadth (in micrometers) and such dimensions applied to the figure. The striae counted in 10 micrometers should be recorded and the number of puncta in 10 micrometers if that

is appropriate. If the raphe is evidently complex that should be sketched in, or at least recorded in a note alongside the sketch. See Figure 92.

If the diatom is a circular one (not a pennate) the items appropriate to sketch in are an indication at least of the surface markings -- whether they take the form of dots, alveoles etc., and a small "pie-shaped" segment sketched to show their distribution over the surface with added notes if necessary. Any protuberances or other features should be roughly located and sketched also. If there are observable differences at the edge and center they will be of particular importance and require recording. The diameter should be recorded or indicated in micrometers.

All of this usually takes less time than it does to tell about it. A few minutes usually suffices to make a sketch with indicated measurements and word-notes. Alongside the sketch some notation should be made of the mechanical stage setting so that location or re-location of the diatom is assured.

With the sketch and notes appended to it the search for the identification can begin. If the worker is completely inexperienced, some help can be gained by referring to the "keys" in the various references at hand. The worker should, at least, be able to recognize whether the form is pennate or centric. To determine what genera is represented is not too difficult even with little experience. The list of diatom genera exemplifying certain distinctive characteristics in Chapter 1, Part I, of this book will be helpful. Also, if the diatom is in a strewn preparation the indicated mode or manner of growth (if retained) will sometimes be a clue to its genus. A partial listing of such is included in Chapter 2, Part I. The more experienced worker will usually have no trouble assigning a generic name to the form he is observing. It is at this point that the identification process becomes more difficult.

When the genus has been determined (or assumed) the next step is to locate illustrations in the available references pertaining to it. A listing such as Mill's Index or VanLandingham's Catalog is then consulted. They list diatoms by genera and species with indicated references, page numbers and plate and figure numbers. From the listing, figures, in the references available to the diatomist, are consulted as to appearance which resemble the diatom to be identified. There may be several such figures which resemble very closely the diatom in question. Then accompanying data and descriptions are consulted, comparing it with the notes made during observation. The length and breadth of a pennate form, for instance, that falls within the range of length and breadth dimensions given in the reference is a beginning toward specific identification, but certainly not complete. A specific identification is complete when the valve outline, dimensions, striae and/or puncta count, striae and/or puncta and orientation, raphe type, polar and central nodule characteristics, all match, or fall within limitations, in the reference description. Of the characteristics which might mostly vary from the referenced limits are the length and breadth dimensions. The other characteristics are much more stable. With this procedure it is possible to identify most diatoms encountered. The positive identification of course requires a good set of references. The more extensive the references, the more species and varieties that may be identified. A similar procedure is applicable to the centric forms.

It is to be emphasized that some variations from descriptions are very often encountered, and that the beginner should not hesitate to identify a form because of non-exactly matching characteristics. He also should not be tempted to generate a new species on the strength of slight differences, and even not on rather large size differences. Also, the temptation to generate a new variety because of minor deviations from the described form is to be resisted. There are entirely too many instances of "species" and "varieties" having been established on the basis of superficiality, and it is far better to identify within the species framework (at least for a beginning student) of a genera, than to promulgate the constant expansion of "varieties".

The latter term is highly overworked, even by experienced diatomists, and some considerable evidence has accumulated for years as to doubt the actual validity of that taxonomic differentiation.

It will be found that the drawings and/or other illustrations in the references vary, as to accuracy and quality of presentation. Unless the identification is beyond doubt on the first such comparison, others (along with descriptions) should be used to confirm or strengthen the identification as necessary, and as far as the breadth of references will allow.

In modern references dimensions and other measurements are expressed in SI units (Le Systems International d'Unites) introduced in 1960. The common unit for measuring diatoms is the micrometer (10^{-6} meter), formerly termed the "micron". However, especially in some of the older references, different units of measurement were used. For instance, Van Heurck and Peragallo used the term "centiemes de millimeter" (l c.d.m. is $^{1}/_{100}$ mm.) which is equivalent to 10 micrometers. In most cases, the older authors state precisely which units were used, or a scale denoting the units is appended to the figures.

With these it is a simple matter to convert back and forth between their units and the micrometer. For instance, Van Heurck describes the length of *Stauroneis phoenicenteron* Ehr. as being 10 to 17c.d.m. and the striae about 14 in 1 c.d.m. In SI units this converts to 100 to 170 micrometers in length and a striae count of about 14 in 10 micrometers.

Because of the necessary extensive use of older references in diatom research and identification, it is important that the student is aware of the differences between antiquated units of measurement and SI units. The c.d.m. used by Van Heurck and Peragallo (and some others) is easy of conversion since it is merely a direct relationship to the millimeter. When other units, such as the inch, are converted to SI units significant differences can arise dependent upon whether it is for example a Paris inch or an English inch.

These and other antiquated units have been used by the elder diatom workers in their publications. Schoeman and Archibald have discussed the ramifications of such conversion differences between antiquated units and SI units. Some of the older authors very specifically indicated which units were used, but others did not.

Frederich Traugott Kützing
(1807 – 1893)

Kützing, for instance, used the Paris lignes (1'''

equals “linea parisiens”) in his Species Algarum. Rabenhorst used both Paris lignes and English inches, and most of the English authors used English inches. The two most commonly used units by the older authors were the Paris ligne and English inch. Other units used were the Paris inch or Zoll (1’’’) and Schumann (1869 etc.) employed his own units symbolized by T and R (T is the length of a frustule in thousandths of a Paris ligne, and C is the number of striae in a hundredth of a Paris ligne).

Table 9

Antiquated Unit	**S.I. equivalent**
English Inch (1”) (0.001” = 25.4µm)	25.4mm
Paris Inch or Zoll (1’’’)	27.07mm
Paris ligne (1’’’) (0.01’’’ = 22.56µm)	2.256mm
Schumann’s T ($^{1}/_{1000}$’’’)	2.256µm
Schumann’s R ($^{1}/_{100}$’’’)	22.56µm
1 c.d.m. ($^{1}/_{100}$mm)	10.0µm

Table 8 indicates these older antiquated units with their SI equivalents. It will be noted that the Paris inch or Zoll, the Paris ligne, and the Schumann T and R are all indicated by the symbol 1’’’. This makes it difficult to determine what units were used by some elder authors wherein they used the symbol, but did not indicate the unit.

In identifying diatoms and comparing the measurements (in micrometers) with the older figures and texts it behooves the student to determine, if he can, what units were in use. In length and width measurements the conversion differences are not so large. However, in the indications of the density of transapical striae and punctae, the differences can become a considerable proportion of the total count and therefore contribute to confusion and difficulty of identification.

Careful study of the pennate diatoms to be identified cannot be overemphasized. Some of them have no raphe, some have a raphe on one valve only and some have a raphe on both valves. Dependent upon the orientation of the frustule, or which valve (sometimes only a single one) is being observed, an erroneous conclusion can be reached. If possible any diatom should be examined from all sides. Additionally, some of these forms are curved in a plane normal to the regular viewing axis and the curvature not evident unless careful focusing observations are made.

In the centric diatoms particularly, the nature of the inner and outer surface may be quite different and at very high magnifications with an oil immersion objective the differences are evident. It is important to know in these cases which surface of a single valve, for instance, is in focus when noting details.

Most diatoms encountered are, with even minimum experience, fairly easily identified as to genus. There are a few however, that present some difficulties, particularly to the beginner, and sometimes to the more experienced worker. Notable

among these are the genera *Nitzschia* and *Hantzschia*. Their appearance, at least on a superficial basis, is very similar, and there is very little in the way of "obviousness" that differentiates between the two. Reference to the part of this book treating diatom symmetry shows that the major difference lies in the positioning of the raphe keels in relation to the rest of the frustule.

In *Hantzschia* the keels of the two valves are opposite each other, whereas in *Nitzschia* the keels are diagonally opposite each other. Other distinctions are that *Hantzschia* is generally arcuate with rostrate ends, while *Nitzschia* cells are generally linear or gently curved. The attitude in which these two genera are found lying in a strewn preparation, for instance, makes these distinctions somewhat difficult to discern. Also, the genus *Nitzschia* is quite complex, and has been divided into many groups relating to shape and markings. Before positive identification is made a very careful study of these two genera is in order.

Two other genera are sometimes difficult to separate without experience and, sometimes, special knowledge of the ecology of the specimens. These are the *Fragilaria* and *Synedra*. Both are quite often narrow, linear, more or less needle-like in shape. The *Fragilaria* are united in ribbon-like bands, the fasciae breaking up easily. *Synedra* cells are solitary or in tufted colonies. Because in some strewn preparations the colonial aspect of either of these genera may not be apparent they are difficult to differentiate when they are of similar size. A clue to their identity is that the large cells as such are usually *Synedra* and the smaller ones are more likely to be *Fragilaria*. Also, the valves of *Fragilaria* can be oblong or elliptical with sinuate margins, whereas *Synedra* are nearly always linear to linear-lanceolate. The similarity of the two genera has promoted the thought that they may be combined into one genus. This has not been done as yet.

Of importance in diatom identification and description are some observations useful to the identifier, although very few, if any are used in written descriptions. They are:

(1) Visibility

The visibility of the particular diatom in relation to the medium in which it is observed. Some diatoms are more "visible" (usually due to being more heavily silicified) and easily distinguished in Canada Balsam. With others, the highest refractive index media are necessary as mountants: before they become "visible".

(2) Color

In both transmitted light and in darkfield modes certain diatoms exhibit rather constant color characteristics at particular magnifications and within limited objective numerical apertures.

(3) Contrast

Closely related to visibility, some diatom outlines and features seem "blacker" or more heavily accented, while others in a given mounting medium may appear faint or diaphanous.

Observe if possible a number of diatoms in a series rather than a single specimen, taking into consideration variations in size and appearance. Averaging dimensions and describing limits, rather than absolutes, is usually more indicative of a species. Many diatom descriptions have been formulated on particular specimens, without allowance for the range of variation usually present. Translucent or diaphanous characteristics should be viewed with caution and confirmed (or denied) by additional observations or varied techniques.

In fossil diatoms especially, the state of preservation of the diatom frustule may vary considerably from specimen to specimen. This has led on occasion to the naming of a new variety or even a new species where the specimens under examination represented but a single taxon. In the determination of species, condition of the specimen, in any case, presents an important factor for consideration.

CHAPTER 5.

5. DRAWING DIATOMS

5.1. Introduction

This chapter covers the various aspects of drawing diatoms as seen with the light microscope. The drawings discussed are not the "sketches" of the previous chapter, but are ones made as illustrations to scientific observation. As such they may substantiate and visually reinforce the description of the diatom, even to the extent of practically standing alone for that purpose, They accompany scientific reports and/or become an important inseparable part of floral listings, and in many cases, the most important reference for identification of similar diatoms by others.

With diatoms, good illustrations furnish information on detail that even the most expressive language would have difficulty in describing.

Drawings should, with the help of modern drawing apparatus, be rendered as exactly in appearance to the actual diatom as they can be. Actual specimens are difficult, if not at most times impossible, of comparison on a side-by-side basis. Drawings however, especially when they are good ones, can be easily compared in that manner.

Every diatom collection, in the form of mounted slides of specimens, should be reinforced with illustrations serving to detail variations in diatom forms, varieties and peculiarities. Its value, so enhanced, will be much greater scientifically than otherwise.

What size of drawing needs to be prepared, what to show in the drawing, and how to go about it, are the main subjects of this chapter. However, before the more practical aspects of this task are treated it is appropriate at first to discuss the purpose or intent of such illustrations.

One thing to be considered is whether the drawing of a diatom is to indicate how the frustule is constructed or, how it appears under the microscope. One concept involves details of structure and design not necessarily visually apparent, and the other is a graphical representation of form and visible features.

Also, we must consider whether the drawing is to represent what can be seen by any and all microscopic means, or just what can be seen by limited or ordinary means.

We must subscribe to the idea that a drawing of a diatom is a graphical representation of form and visible features under ordinary conditions. Ordinary conditions are those visual results obtained by the light microscope in the transmitted light mode. Any other feature or representation on the drawing should be definitely noted in relation to special lighting, or other enhancement, which has brought it out. For instance, if the puncta can only be resolved using oblique lighting and otherwise have the appearance of lines or striae, then the ordinary representation would be the latter. If the puncta are represented in the drawing, under this

condition, then the special lighting required should be so noted, preferably alongside the figure, or appended to the figure or plate legend, or included in the description.

Secondary structure, such as that in many genera of *Coscinodiscus*, should not be indicated unless it can be seen at one magnification. If it requires higher magnification than is indicated on the drawing to see that structure microscopically, then it should be so indicated for any figure which shows it.

5.2. Magnification

In existing literature the figures of diatoms appear at many different magnifications, ranging generally from 400X to 1000X. The magnification considered here is the total magnification of the subject diatom as represented in a drawn figure. For instance, if the microscope optics provide a 600X magnification of the diatom visually and the drawing made is directly scaled to that magnification then a direct measurement, on the drawing, of any dimension will be 600X the same actual dimension of the diatom itself. The drawing is made at 600X microscope magnification and represented at unity scale. If the same observational magnification is used, but the drawing enlarged to double the dimensions then the drawing is at two times scale and the magnification of the drawing, representing the diatom, is 1200X. In other words, the total magnification represented by a drawing is the magnification of the microscope times the scale of the drawing. Similarly, the drawing is reduced to half-scale, in the example above, the total magnification of the drawn illustration 300X.

To simplify drawn illustrations, all drawings and/or illustrations could be made at unity scale. However, because at for instance 1000X optical magnification, a drawn figure of some diatoms may be very small, or others may be very large, if drawn to unity scale, this simplification is not always desirable.

In a plate of figures representing a number of different diatoms, it may be necessary to increase the drawing scale for some and decrease it for others to adequately represent them, and at the same time utilize the plate area effectively.

In making observations to illustrate the diatom with a drawing a number of optical magnifications might be used. One, of magnification at which the entire diatom, in the particular desired view, can be included in the field of view of the microscope to obtain outline. Next a higher magnification, may be necessarily employed to adequately detect the relationship of smaller details and/or structure, and even a higher magnification to determine fine structure. These observations, at various magnifications will be illustrated in a single drawing depicting the diatom. If this is the case, then the diatom drawing, or figure, should be indicated as having total magnification indicative of the highest optical magnification used. This is necessary so that future workers referring to the figure are aware of the magnification (optical) used to obtain the finest detail illustrated. A method of indicating this regardless of what the drawing scale is used, is to include alongside each drawn figure a length of line that represents 10 micrometers at the optical magnification used. Therefore if an enlarged or decreased size of the drawn figure is used in illustration, the short scale line represents ten micrometers in either case.

The question as to whether to actually draw details observed with the microscope at unity scale, or an enlarged or reduced scale is answerable in a number of ways, depending upon the method of drawing used, and the ultimate use of the drawing. If the latter is for the personal files only of the observer that is one thing. However, if the drawing is to be submitted for publication many other factors involving printing, reproduction, and space available are factors to be considered.

Generally speaking, for most diatoms, the magnification of the drawn illustration (optical magnification times the scale) should be at least 900X or even double that for really good representation. Reduction (in illustration) to about 600X is permissible where it is necessary to incorporate more drawings per plate in a particular case to reduce space required and the cost of reproduction. If at all possible the illustration size should be the same as, or near, magnifications which can be compared with other good illustrations, especially those relating to the particular subject matter being treated.

In the representation of striae and/or puncta the number in ten micrometers can be fairly accurately represented when the magnification of the drawing is 800X and they count less than 20 in 10μm. If they are more than 20 in 10μm. it is nearly impossible to represent the markings on figures of magnifications of 800X. In those cases either the complete drawing must be enlarged, or a separate scaled part, pertaining to the striae and/or puncta, appended to it. The latter is not an uncommon method and many such representations are found in extant literature.

Another consideration is deserving of comment. If the diatom drawing is to be reproduced, especially for publication, it is well to consider making the drawing at least 2 or 5 times the size it will be in final presentation. By this means small imperfections of drawing can be reduced to unnoticeable proportions in the published figure. It must be remembered, however, that the weight of lines used must be enough for the reduction desired, otherwise the finest details are likely to be lost. Original drawings made at 2000X to 4000X are recommended.

Magnifications, where they are large, can be rounded off when making comparisons. Instead of 1020/1, use 1000/1, as the small difference in this case is without practical significance.

There are a great many combinations of objectives and oculars that provide the same, or essentially the same magnification. A high-powered objective with a low-powered ocular or vice versa in many cases will result in the same magnification. However, dependent upon the size of the diatom or what aspect of it is under examination there is usually a best combination of objective and ocular. It is advisable for the diatomist to make a table, or chart, indicating the magnification numbers of the various combinations at his disposal. For the drawing of diatom habit illustrations a low-power objective with a high-powered eyepiece will be found most useful. On the other hand, for the portrayal of fine structure the highest-power objectives are indispensable. Choose a field of view as great as possible, commensurate with desired magnification, to render fine detail.

5.3. Methods of Drawing

There are a number of methods of drawing diatoms. They are:

(1) Free-hand drawings.
(2) Camera lucida drawings.
(3) Projection drawings.
(4) Line drawings on the back of photographs, and on various types of chemically reproduced printings such as Ozalid, Xerox, etc.

5.3.l. Free-Hand Drawing

This the simplest method as there is no special apparatus required, only pencil, pen and paper. However, the method does require some natural ability to draw, or in lieu of that, some practice in using a definite plan of drawing (with cross-section [graph] paper or other lines for guidance).

Everyone is familiar with drawing short line intervals on a grid background and ultimately connecting them to form a completed figure. Feature by feature, and point by point, the diatom details can be drawn on a squared-grid format. The grid provides the guide necessary to arrange the diatom details in their proper proportions and relative positions. The size of the grid intervals on the drawing paper will determine the size of the final product.

Those with some natural ability to draw will need less guidance. A rectangle, square, or other convenient form is sketched in pencil on a piece of drawing paper which denotes the limits of the diatom extremities. A rectangle for instance with lightly sketched in center lines is useful for drawing the *Navicula*. The length to width ratio of the rectangle is selected to suit the diatom being portrayed, and the outline sketched in each quadrant of the center-line divided rectangle. Other features may then be added in relative position.

A very short step away from entirely “free-hand”, drawing can be accomplished by those of lesser natural ability with the use of an ocular reticle with a squared-net pattern. The reticle is placed in the eyepiece at the point where the aerial image of the diatom is focused by the objective, and thus the net-pattern appears superimposed simultaneously to the observers eye. The image of the specimen appears to be overlaid with a system of cross lines in a squared-grid configuration. If the drawing paper on which the illustration is to be made is furnished with a similar system of lines, it is quite simple to fill in the figure. If the lines on the paper are in pencil they can be erased after they have served their purpose. Alternatively, a transparent drawing paper backed with cross-section paper can be used. Also, there are commercially available special drawing papers which have printed square grid lines that do not reproduce photochemically. The finer the net in the ocular, the closer and closer the lines between intersections approach straight lines, and the easier the variations in the diatom image line form can be followed.

In drawing diatoms for illustration a finely pointed pencil with hard lead should be used. Pencils, such as are used by draftsmen, with hardness of 3H or 4H are

appropriate. Mechanical drawing pencils that use leads of a half-millimeter in diameter are excellent. The very fine lead requires very little in the way of pointing, and the hard material is less easily blunted and permits very fine lines with minimum separation to be drawn (as striae etc.). Pressure on the pencil should be light and the lines drawn very finely. After the figure is completed in pencil, it is finished using India ink. India ink is recommended because of its intensity, and excellence of reproduction by most any means. Various types of inking pens can be used. For fairly long straight lines in the figure (especially if the drawing is comparatively large) a fine draftsmen's ruling pen used with a straight edge is good. With very large figures the ruling pen and a French curve can be used for most curved lines. However, usually the drawing is too small as to permit the use of these tools, and more or less "freehand" use of other types of pens is required. A popular draftsman's pen is that now available in a "fountain pen" or "reservoir" type. A common one is the Koh-i-noor brand "Rapidograph" made in Germany and available where drafting supplies are sold. Various sizes of tips are available from coarse to very fine. These pens are susceptible to clogging, and frequent cleaning is necessary for successful use of them. A much simpler type of inking pen is the "crow quill" which is not a "fountain pen" type but uses individually sized points that are inserted in a wooden holder. The points can be obtained in varying sizes from coarse to very fine and some are available in a "reservoir" type. These pen points are easy to use and maintain.

After the drawing is completed in ink, all pencil lines remaining should be erased, and a fine straight-line interval representing 10 micrometers is drawn near the figure.

5.3.2. Camera Lucida Drawings

The camera lucida is an optical apparatus for superimposing, or combining, two fields of view in one eye.

Used with a microscope, it causes the magnified virtual image of the object to appear as if projected upon the table or drawing board, where it is visible with the drawing paper and pencil by the same eye and in the same field of vision. In other words the microscope image appears as if it were a picture on the drawing paper.

One form of this instrument employs a mirror to reflect a drawing surface into a specially designed prism. The prism is in the form of a cube made up of two right-angle prisms, the 45 degree surface where they are joined is silvered except for a circular central area. The prism is placed close to the ocular of the microscope and the observer looks through the prism into the microscope.

As the mirror, after being properly adjusted, reflects the drawing surface into the silvered portion of the prism surface, it appears as though it were coincident with the microscope image (this description is that of the Abbe camera lucida which is probably the most common of the attachable types of drawing accessories). The camera lucida has taken on a number of forms, but the general principles as explained above are the same.

Superficially, the advantages seem very great, as all the observer must do is to trace the "picture" produced on the paper, watching the pencil point do its work.

However, the successful use of a camera lucida involves considerable careful adjustment and a realization of its limitations, as well as its advantages.

The first adjustment (after the instrument is attached to the microscope) involves the correct positioning of the prism in relationship to the microscope eyepiece. The prism is adjusted vertically over the center of the ocular so that it accommodates the particular eye point distance of that unit. The microscope, with an object slide in place on the stage, is first focused (with the prism in place) sharply on the specimen. Then the prism is adjusted vertically over the eyepiece such that a full field of view is observed, and locked into position.

The mirror needs adjustment horizontally, and its distance from the prism and the drawing surface are determinants as to the size of the final drawing. The mirror must be extended, in any event, such that (if possible) the stage of the microscope does not extend into the drawing field of view. In modern versions of the Abbe Camera lucida there is usually a scale on the mirror-arm to facilitate adjustments. The Abbe camera lucida was designed for use with a vertical microscope. On a vertical microscope, if the camera lucida mirror is set at a 45 degree angle, the axial ray is normal to a horizontal drawing surface, and a drawing made under that condition is in true proportion and not distorted. The stage of many microscopes extends so far out at the sides; that if it is to be eliminated from the drawing field of view, the mirror must be adjusted to an angle of from five to ten degrees less than 45 from the horizontal. But, this prevents the axial ray from forming an angle of 90 degrees with the drawing surface and thereby introduces distortion. If the angle of the mirror is 40 degrees, the axial ray will make an angle of 100 degrees with the horizontal drawing surface, not 90 degrees. Therefore, the drawing surface must be tilted toward the microscope at an angle of 10 degrees. This means that for every degree the mirror is set less than 45 degrees, the drawing surface must be tilted 2 degrees toward the microscope (see Figure 93).

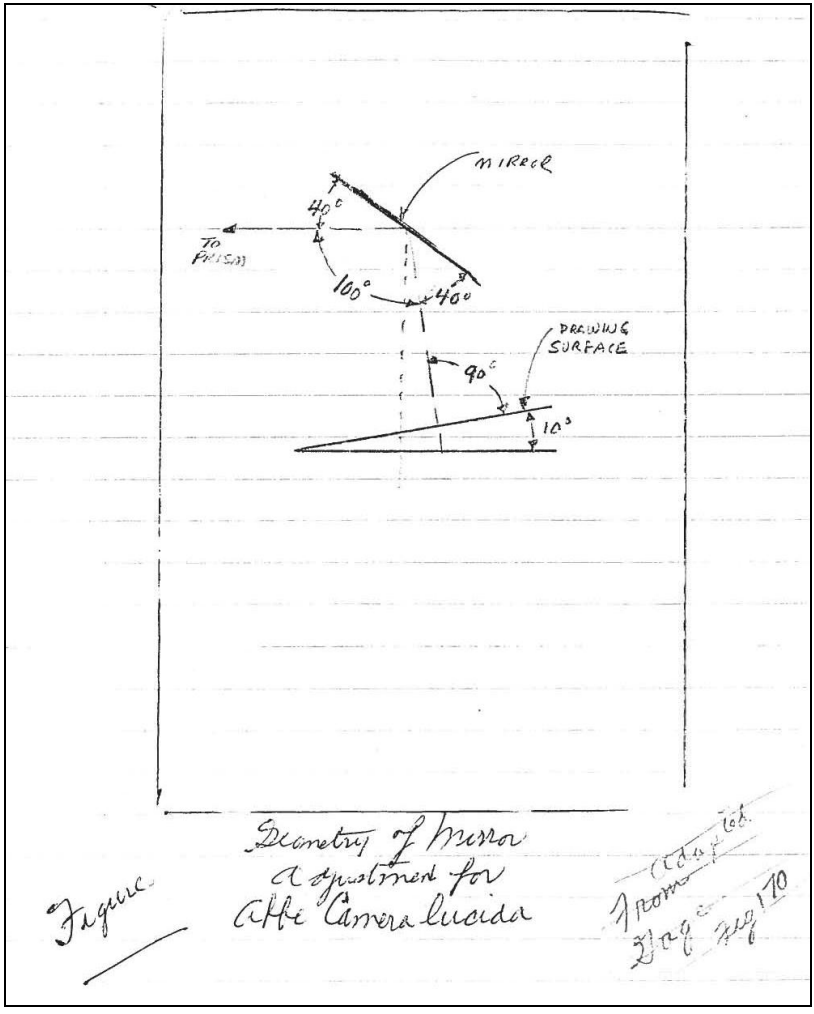


Figure 93.

When the drawing board is so elevated further adjustment of mirror position is necessary so that there is no front-to-back distortion of the image. This is accomplished by making sure that the edges of the mirror are in planes parallel to the edges of the drawing board.

In checking for distortion a large centric diatom form, such as *Coscinodiscus*, can be used. Set up the apparatus as described above and trace the outline of the diatom on the paper. Then check it for circularity using the eye or a compass. Alternatively a squared-net reticle in the eyepiece can be used. Corners of several of the squares in the net are located in the drawing paper and then connected using a straight edge. Distortion will immediately be apparent in asymmetrical squares.

As most microscopes are not used vertically, but are tilted in attitude for comfortable viewing, or have an angled head for the oculars an adjustment of the drawing apparatus is necessary for that reason also. The drawing surface (board) is tilted at

the same angle as the microscope is. This effectively satisfies the requirement to keep the axial ray of the image at right angles to the drawing surface.

In short then, to prevent distortion in the image on the drawing surface, the latter must be tilted twice as many degrees as the mirror is less than 45 degrees to the horizontal, the mirror edges must be parallel to the drawing board edges, and the latter must be tilted at the same angle as the microscope.

Successful use of the camera lucida requires proper adjustment of lighting. The eye, with the use of such apparatus, is receiving light from the microscope optics, and light from the drawing surface via the mirror and prism simultaneously. It is necessary that they should be of approximately equal intensity, or the brighter one will swamp the other out and the two will not be seen with equal distinctness. For instance if the drawing surface is too brightly illuminated, the pencil will be seen distinctly, but the microscopical image will be faint. It is far better to regulate the light at the drawing surface than to tamper with illumination adjustments at the microscope for this purpose. Variable intensity lamps can be used and/or the distance of the drawing surface illuminant can be adjusted. A very convenient means for balancing illumination is often incorporated in modern camera lucidas. Between the mirror and the prism there is located an adjustable set of polaroid discs or several neutral density filters. Adjustment at this point allows the intensity of light from the drawing surface to be easily balanced with that issuing from the microscope.

The final success and satisfaction from using a camera lucida is only attained when the limitations of such apparatus are realized and it is used accordingly. The idea that one merely has to “trace” a specimen object, as easily as if a tracing were made through transparent paper, should be abandoned. First, some experience is necessary in guiding the pencil along an outline of the image. There is a difference from ordinary tracing as the eye is not looking directly at the surface being drawn upon. This spatial difference, despite the optical superposition of the images, is vaguely disturbing and must be overcome by experience.

Another misconception is that the entire diatom figure is drawn using the apparatus. That is usually not the case at all unless the diatom is very simple in structure and details. The usual procedure is to outline the diatom using the camera lucida and perhaps locate the interior or exterior details to that outline. The finer and finishing details are drawn in without the use of the drawing apparatus by first viewing the microscope image directly and then transferring what is seen onto the outlined and mapped portrayal on the paper. The visibility at best with the apparatus in place, and the act of viewing superimposed images, drastically reduces and/or limits the detail that can be discerned. The greatest value of the Abbe camera lucida, or similar aids, is that they provide assistance in reproducing the proportional structure and detail placement of the diatom. If this is realized and the user does not insist on trying to use the instrument to make complete detailed drawings, he will be much better satisfied with its use and will produce better drawings.

In completing the drawing, if available, a stage micrometer can be placed in the specimen position on the microscope stage and a portion of it drawn as it appears on the drawing surface. If the actual value of the intervals are appended to the drawing it is simple to compute the enlargement of the drawing. This is done by measuring

the drawn intervals and dividing by the actual value. For example if the drawn image of an interval measures 60mm and the actual real interval distance is 0.1 mm. the total magnification of the drawing is 60 divided by 0.1 or 600X.

At this point it might be well to comment on some results of using a microscope for long periods when it is fixed-focused for drawing purposes. Many microscopists do not use a microscope at the same focus for periods longer than a few seconds. These observations are pertinent especially to the drawing activity, however accomplished, because of the comparatively long periods that the microscope must remain on one object at a fixed focus. When using a camera lucida under this condition, it may be noticed that the outline and details of the diatom are becoming more and more difficult to see in comparison with the already drawn lines on the drawing paper. This usually happens as a result of the fine adjustment creeping in position, gradually defocusing the image.

Another cause of this type of effect is noticeable when oil immersion objectives are in use. Especially when the microscope stage is tilted, a very gradual change in visibility (and focus) of the diatom may take place. This is usually due to flow of the immersion oil out of position between the objective and coverglass. Either the microscope stage must be leveled or a higher viscosity immersion oil used to avoid this disturbing effect.

A further cause can be an unnoticeable movement of the microscope stage inasmuch as it may be capable of being tilted, or possibly due to the microslide being gripped in a mechanical stage. Any unequally tightened screws in these members are not without influence as the mechanical movements of the instrument take place, and at very high magnifications become very noticeable. It is incumbent on every diatomist to make sure that every mechanical part of his microscope is properly fastened before beginning lengthy drawing sessions, especially when very high powered magnifications are to be used.

5.3.3. Projection Drawings

A very simple method of assisting the diatomist in making drawings is by projection. If the microscope can be inclined to the horizontal (this can be done in some cases by removal of the binder screw in the base next to the inclination joint), it may be used directly as a projector. A powerful light source, such as a motion picture projector or slide projector lamp, is to be preferred over the conventional microscope lamp. If a carbon-arc lamp is available it will serve the purpose well. The main point is to furnish light of sufficient intensity such that a bright image can be projected to a size suitable for the drawing. This does not mean an image as might be projected for a large audience of a size several feet on a side, but an image filling an area of a few inches on a side (or in diameter).

A translucent screen of ground glass or tracing paper on a glass surface, will be suitable for “back projection”. Outlines and details of diatoms may be accurately portrayed by tracing the projection. For comfort in viewing and drawing the microscope can be inclined at a suitable angle. An easel made from a picture frame

(with the glass intact) may be used. Tracing paper fastened over the glass provides the drawing surface.

When the microscope cannot be conveniently tilted at any angle, it can still be used as a projector by arranging a mirror to reflect the projection to a horizontal (or nearly horizontal) surface. The precautions regarding the relationship of the axial ray of the projection image being at right angles to the drawing surface apply here as in the use of a camera lucida. Unless those precautions are taken a distorted drawing will be the result.

An advantage in drawing from projection is that the diatomist is looking directly at the drawing surface as he works on the illustration. A disadvantage is in the extremely high intensity of light required to provide a suitable projection image for drawing. Also, since this method of drawing is done quite often in a darkened room to obviate the very intense light source required, an additional weak light must be used then so that the drawing surface itself is illuminated from above with sufficient intensity to make the contours already drawn clearly visible.

The distance between the mirror and the ocular can be varied to provide a suitable size to the projected image. However, this distance must be quite great to obtain a sizeable projection on the drawing surface, and then consequently requires an even higher intensity light source.

For the drawing of large objects from a half-millimeter to perhaps 50 millimeters in the largest dimension projection is done using a low-powered objective only. However, the diatomist is concerned with subject matter much smaller and therefore objectives of high power, with an appropriate ocular, and substage condenser as well, are used in the projection arrangement.

The method is simple. Focus and arrange the drawing surface to give the right size and magnification. A portion of a stage micrometer scale is an excellent gauge for this purpose. Then the object slide is put in place and the outlines and details of the diatom are traced, and later finished in India ink using the specimen diatom to check with. The light supplied to the substage condenser should be in parallel rays and the condenser itself well centered.

5.3.4. Other Methods

Drawings, or partial drawings, of diatoms may be made in connection with the various common methods of reproduction available. For instance, a photomicrograph of a diatom may be translated into a line-drawing by tracing directly the photographic image on suitable transparent paper, or the print may be inverted, lit from beneath with the aid of a light-table, and the image traced on the back of the photographic paper, or on a separate sheet of transparent paper. Photographs can be copied by Xerox or similar processes and the resulting image inked over partially or in its entirety. The same can be done with illustrations from photographs or line drawings in extant references on diatoms. The reasons for doing this are varied. It may be desirable to compare in one illustration a diatom from a reference source representation with one just investigated. Or, in some cases, portions only of a referenced illustration pictured alongside a newly described

diatom will serve to emphasize differences and/or similarities and consequently enhance any word description furnished.

5.4. Conventions and Techniques for Detailed Structure

Drawings are composed largely of lines. Lines in drawings may be straight or curved, thick, thin dashed, dotted, double, triple, wavy, faint, or heavily accented. A drawing can represent all that is seen plus all that is known and illustratable.

The electron microscope, particularly the SEM, has revealed that all punctae seem to be perforations, of some type or another, through the cell wall, and has established the presence of modifications of sieve membranes covering these punctae or holes. Many markings described as costae are known to be thin areas - that is sieve membranes either with reticulate sieve-pores or as double or multiple lines of sieve pores. Even though we now know about these ultrastructural details., they are not generally observable with the light microscope. We must limit our illustration of diatom structural detail to what the light microscope reveals to our eye when that is the instrument of observation. A very closely spaced line of link dots on paper appears (if they are close enough) as a solid line. If spaced slightly further apart the line may appear to be roughened and not smooth, and finally as the spacing approaches that which our eyesight can resolve, a separate series of dots is apparent. The smaller the dots are, the fainter the line, roughened line, or dot series appears to be. At some smaller limit in size, no manifestation of the dots can be detected by the eye (no solid line, roughened line, or separate dot series is evident at all). The microscope reveals such diatom ultrastructure to the eye in much the same manner. A smooth, apparently featureless, valve surface may in fact be perforated with minute pores too faint for visual detection, or markings, if visible, may look as if solid lines when in fact they are very closely spaced pores in the valve wall.

Various ways of drawing these appearances are available to the illustrator of diatoms. Almost every conceivable method of representation of various structural details has been used and appears in the extant literature. Most of the drawings have been made by diatomists with no background in art or illustration. Some of them are excellent, because of an inherent artistic ability, and many are poor. The drawing of diatoms has also been done, even in the distant past, by artists or scientific illustrators. The quality of these are, in general, very high. However, the average diatomist is not endowed with artistic ability, nor trained or equipped, to duplicate the excellence of the professional illustrator. Therefore, drawing the details of diatom structure, must ordinarily be done with a minimum artistic background and preferably, using as much in the way of conventionalized representation as possible. In the following paragraphs some comparisons are made among various types of illustrative conventions for the various structural details as practiced by past and present diatomists, with some suggested techniques to follow for the tyro. In addition to this material, a great many good hints can be found by studying the illustrations in well-printed references on diatoms.

The best references for studying the technique of rendering excellent drawings of diatoms are, Hustedt (all works), and the Schmidt Atlas which represents the efforts of a number of excellent illustrators of diatoms. Very highly recommended, and

superior to any others, in this authors opinion, are the drawings in Van der Werff and in Patrick and Reimer.

A particular diatom is drawn by different observers in different ways. Part of the difference lies in the illustrating ability of the observer, part in the capabilities of the diatomist as a microscopist, and the limitations of his instrument, and part in the knowledge of the diatom as possessed by the observer. The latter can, and does in some cases, influence what the illustrator includes and perhaps to what detail it is included in the final drawing.

5.4.1. The Outline

The outline of a diatom should ordinarily be drawn using a solid black line. The weight of the line (the thickness) should be that which best illustrates the visual impression the microscope image makes. Dependent upon the relative curvature of the valve surface and contrast of the image, the outline may range from a very fine to a thick heavy representation. Centric diatoms may reveal the actual wall thickness (in visual section) when the valve surface is being viewed. If this visualization is clear, then the outline will be a double line, the inner of which is usually more lightly weighted than the outer. It will be found, in the majority of cases, however, that the single solid line of outline representation is most appropriate. It is conventional to illustrate the diatom (if a pennate form) with the longest dimension vertical and the least dimension horizontal. If, by necessity, the drawing on paper is made in a "horizontal" aspect, it can be eventually re-oriented to the conventional aspect for illustration purposes.

When showing the valve view and girdle view of a diatom species together in the same figure (and this should be the rule rather than the exception) the two views are customarily shown as being of different sizes. This is indicative that the valve view has been drawn from a different diatom frustule than the girdle view, since it would be very unlikely that both valve and girdle aspects of the same diatom (or of the same sized diatom) would be available under the microscope. This usual distinction applies to centric as well as pennate forms.

In drawing the outline of centric forms that are circular, a compass may be used. Dependent upon the species of course, there may or may not be a necessity for representing the edge with a double line. The double line in that case usually represents an actual protruding abrupt "shelf" or horizontal extension of the valve, rather than a sectional view of a vertical wall.

When representing the same species of diatom in a number of different sizes or to illustrate size extremes, or some minor differences in shape, only one valve and/or girdle view needs be drawn in detail, the supplements required being outlines only.

When the drawing (or illustration) is of such a size as to be very large and would take up a prohibitive space to be completely represented it can be shown in broken view. This can be done when the frustular details are repetitive throughout the structure or are repeated in one or more locations. This type of situation is encountered when drawings are made of species of *Rhizosolenia* for instance.

In these and similar cases, the outlines broken at the center or at some appropriate location where the details are repetitive and continued either side to provide the two outlined ends and their details. In some cases of *Rhizosolenia* only half of the frustule or a short portion of the end is represented.

Outlines that are straight or circular or portions of a circle are quite easy to draw smoothly with straight edge and ruling pen and/or compass. For non-circular lines the use of a French curve can sometimes be used to advantage (if the drawing is large enough). This is especially true of finishing the drawing in ink. If the drawing is large enough, and it has been drawn in pencil with the aid of a camera lucida for instance, the inking of the larger portions of non-circular curves can be accomplished sometimes much more smoothly, using the French curve, rather than trying to ink freehand. The size of the drawing and radius of curvature of various portions of the outline will determine whether the French curve can be used or not. Other "irregular" curve-shapes are available as drafting aids. The patterns for them are laid out in parts of ellipses and spirals or other mathematical curves to facilitate their "fit" with most any curving line.

The weight of line considered to be "light" for diatom outline at 1000X on the drawing paper would be one about l/250th (.004) inch in width; one of medium weight 0.010 inch in width, and one considered to be "heavy" about 0.025 inch wide. Visible outlines should be thick and hidden outlines medium.

Draw the outlines in pencil lightly so as to not "groove" the paper, thus making any changes by erasure easier. Erasing of pencil lines, for changes, or after inking has been completed should be accomplished with a Ruby pencil eraser. If the paper has been grooved by the line, it may be rubbed over with a burnisher or even with the back of the thumbnail. In erasing an ink line, hold the paper down firmly and rub lightly and patiently, with a Ruby pencil eraser, first along the line and then across it, until the ink is removed. A draftsman's triangle, or other hard smooth surface, slipped under the paper gives a good backing surface. When an erasure must be made close to other lines use a draftsman's erasing shield, selecting an opening of the best shape and rub through it, first seeing that both sides of it are clean. Avoid the use of so-called ink erasers, and never scratch out a line or blot with a knife or razor blade.

For freehand drawing of outlines in ink, a very smooth and constant width of line may be drawn in ink with a contour pen (or curve pen) sometimes used in map work. It has a swivel-jointed end allowing easy following of non-circular curves.

5.4.2. The Striae

Striae, as mentioned before, are almost always visual manifestations of very closely spaced punctae (perforations) in the valve surface. The punctae may be arranged in various patterns such as single rows, double or even multiple rows of definite length in straight, angular, or curving paths creating typifying or characteristic patterns relative to the median line and outline of the valve. The rows of punctae may be very closely spaced or regularly or irregularly grouped in these arrangements.

Further, many puncta are not simple holes or perforations, but may be partially occluded with plate-like or dendritic marginal outgrowths or completely covered with multiperforate or reticulate sieve-like membranes. Although most of this ultrastructure is only visible with the electron microscope, the appearance of the puncta as revealed by the light microscope may be affected or modified to some degree by these structural variations. Therefore, although individual puncta and their variations in structure may not be visible in the light microscope the striate patterns resulting may appear variously as narrow to wide "lines" of varying geometric shape and structure. The "lines" may appear to be solid, dashed, striped, or otherwise "ornamented". The representation of these manifestations is the subject of this section. As, in most cases, a word description of the striae, their arrangement and nature, accompanies drawn illustrations it is important that the drawn details correspond to the word picture given. Striae are variously described, in their appearance, as robust, delicate, punctate, lineate, single punctate, doubly punctate, punctate single rowed, punctate double-rowed, punctate triple-rowed, etc., cross-lineate, lineate, costate (*Cymbella*), short marginal dashes (*Amphora*), indistinctly punctate, closely punctate, variable dots, tapered dashes, striae undifferentiated (finely punctate), linear series of dots, finely lineate, finely punctate-lineate, more widely spaced lineate, finely lineate-difficult of resolution, individual decussating puncta, finely divided transversely, fascicles or fascicled, and so on.

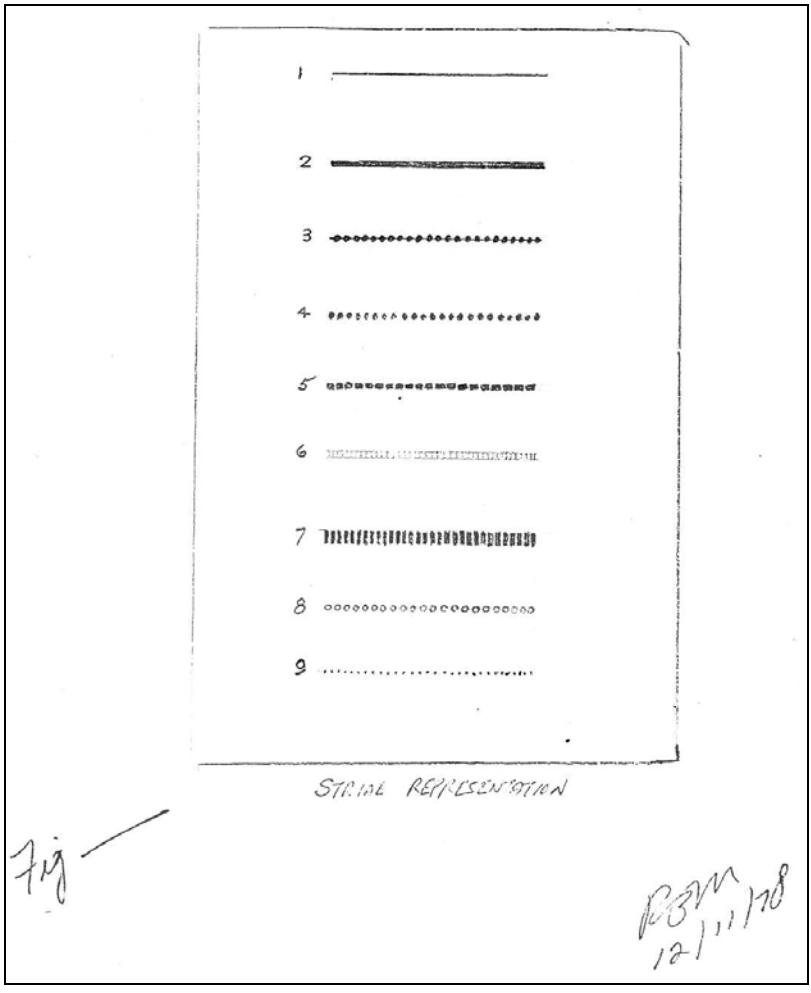


Figure 94.

Figure 94 illustrates some basic symbolization of striae as they may appear under the microscope. Lines l and 2 indicate puncta too close together to be resolved as such at all. Various weights (widths) of lines such as these may indicate single or multiple rows of such punctae. Line 5 indicates that the puncta are so closely spaced as to not be completely resolvable into separate entities, but spaced far enough to be detectable. Again, the width or lateral dimension of such a representation may be used to indicate single or multiple groups or rows of such puncta. Line 4 shows puncta far enough separated as to be completely resolved into dots with definite separations. Line 8 is a similar representation wherein the puncta are actually resolved into perforations as such, and not merely "dots". Line 5 represents a stria that is lineate, or crossed by "lines" themselves. The striae in this case may be groups of very densely spaced puncta separated by narrow spaces (the "lineae"), or costae crossed by short series of very closely spaced punctae, the latter being the lineae in that case. Line 6 may be a representation of the same type of structure in a different light. For example, dependent upon whether the "white-dot" or "black-dot"

focus is used when portraying the diatom, there is both a “positive” and “negative” way in which these arrangements appear.

Line 9 of course is a finer representation of Line 4. All types of variations on these basic patterns can be used in representing what is seen with the microscope.

When drawing striae, in whatever texture they appear, their relative location and aspect to the geometry of the valve outline and center line needs to be accurately portrayed.

5.4.3. The Raphe

The location of the raphe with respect to the center-line of the valve, the location and spacing of the pores of the central nodule, the type of raphe, and the details of the terminal nodules are of primary importance. The raphe of many diatoms, particularly those of smaller forms, are indistinct or devoid of any detail under the microscope excepting perhaps an indication of a thin line. Others, such as in the larger specimens of *Pinnularia*, image considerable detail. The drawing should render the appearance of the raphe as accurately as possible. The spacing and appearance of the pores at the central nodule, whether they are curved or hooked, or terminate in conspicuous dots or swellings, or trail off, are important in the portrayal. No shadings or “artistic” enhancement of the central nodular area should be shown unless the indications are constant under considerable variations of lighting conditions. Often shades and shadows are merely manifestations of environment and/or lighting conditions and not a real attribute of the diatom frustule itself (excepting under those special conditions). The terminal ends of the raphe should be portrayed with the same care as the central ends. In fact, there will in general, be found a greater variation in their shape, direction of curvature, and other details, than those of the central ends. If drawings (of the pennates) are to be successfully used in diatom identification the terminal raphe ends require especially accurate rendering by the illustrator. Any details visible should be portrayed if possible. Merely placing a dot to indicate the end of the raphe is inaccurate and misleading if nothing can be seen excepting a gradual trailing off of the end into the terminal nodule. It best be represented as the latter by a thin line whose weight decreases until it disappears. The direction of curvature is important. Most raphes curve in the same direction in any given valve, but not all.

The detail along the length of the raphe should show (if that is what is revealed microscopically) whether it is filiform, ligmentous, or complex (see Chapter 1.). If complex, the length of the foldings or locations of reversals of the groove should be accurately placed in the drawing. These details are often of very high value diagnostically. Suffice it to say that the written description accompanying such illustrations should be compatible with the graphic portrayal.

5.4.4. The Valve View

Figure 95 illustrates a step by step method of drawing a diatom. Step 1 lay out a very light penciled rectangle of appropriate dimensions with center line. Step 2 draw in

the outline of the valve. Step 3 draw in a very lightly penciled line that includes the hyaline area of the valve (this area is generally outlined by the inner tips or extensions of striae). Step 4 draw in, at the proper angle and attitude, the striae whose inner limits define the outlined area of Step 3 above. The drawing of the striae may take several steps. If they are punctate, it is advisable first to draw a lightly penciled solid-line guide-line illustrating the curvature, angle, and extent of each individual stria. Then, along this line, the dots or small circles representing the punctae are laid out. The penciled guide-line will eventually be erased after inking of the punctate structure has been accomplished. If the area defined by the striae structure is of appreciable width, then that area should be so outlined in its proper proportions in light-pencil before filling in details as in 5 in the figure. Step 6; draw in the raphe and finally include as Step 7 the terminal ends of the raphe. After these steps have been done in pencil, the parts to remain in the illustration are inked in and the lightly penciled construction and guide-lines are erased. This step by step procedure is, of course, only a suggested method. Dependent upon the facilities at hand, and the ability of the illustrator, considerable variation is possible. If a camera lucida is used, then much in the way of construction and guide-lines may be dispensed with, relying on the superposition of the image and drawing paper to provide the proper guidance in proportions and geometrical layout of details.

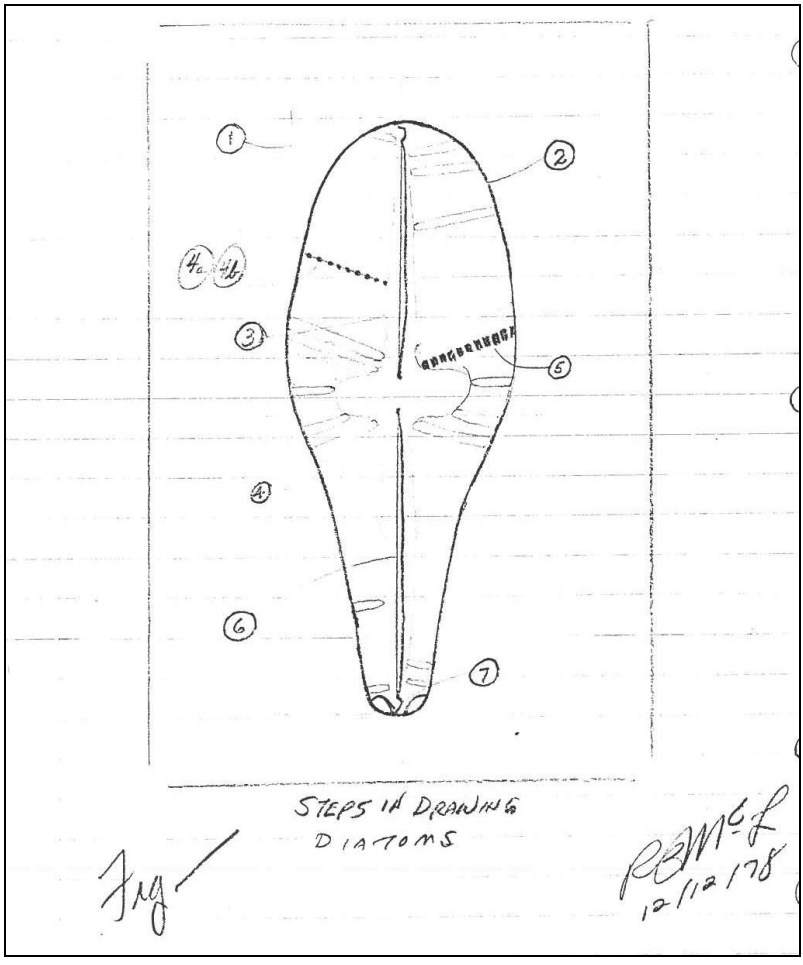


Figure 95.

The drawing of pennate forms is usually more difficult than the centric forms. The latter, mainly circular in outline or of outlines geometrically disposed about a common center are easier of illustration, in the main, with the use of drafting instruments. The circular forms are easily initially, and finally, outlined using a compass (pencil type for initial and inking type for final). The disposition of markings on most (not all) of the centric forms follow a very regular geometric arrangement about the center, if the form is circular. If the form is triangular (as in *Triceratium*) various details are usually disposed about a common center and/or symmetrically arranged with respect to the apices of the triangle.

5.4.5. The Girdle View

In drawings of diatoms the most neglected aspect is that of the girdle view. Probably it is omitted in many cases because diatoms most generally are seen in valve view

and it is that view that most individual detail is observable, and indeed even characteristic.

The zonal aspect, or girdle view, should be made of the whole diatom frustule. If at all discernable, the markings on the girdle bands, apparent thickness of the valves, visible swellings of the central and terminal nodules, and any connecting details such as intercalary bands should be so indicated. For most small forms there will not be much in the way of such detail to draw. Large forms, such as many species of *Pinnularia* however, contain considerable detail to be portrayed in girdle view. Excellent representation of girdle view drawings are contained in Patrick and Reimer and in Van der Werff. Figures in these references should be studied carefully as to the technique employed in illustrating the zonal aspect of diatoms. That technique, in almost all cases, will be found to be simple and straightforward, utilizing simple line representations. With some practice, a diatomist with even minimal artistic ability can produce an acceptable illustration.

5.4.6. The Completed Drawing

The completed drawing of the diatom should be in waterproof black ink (India ink is recommended) as if it were being viewed by the transmitted-light mode. Even though other modes of light microscopy might be used in obtaining detail, such as dark-field or any of its modifications etc., the final representation should be as if viewed in brightfield. This means a black-line drawing on white paper. There should ordinarily be no “shading” or “shadowing” in the drawing. Such representations are often misleading, and/or the product of a special environmental lighting condition not easily repeatable. Or, even worse, they are “artistic” embellishments that do not convey any scientific information regarding the diatom. There are exceptions to this, especially in the portrayal of the central nodule of the larger forms. The genera *Pinnularia* and *Cymbella* for instance, often characteristically display images of these areas that give the impression of a “thickness” or “swelling” and a faint indication of the nodular channel. These indications, being very characteristic of the forms should be portrayed with a minimum of “art”. Study of good references as mentioned above will guide the illustrator in this regard. In Patrick and Reimer, the artist has chosen a finely dotted pattern to give the proper impression. In other works the effects are accomplished with faint curved lines. If any type of shading is found to be necessary, then open-dot shading is recommended. The visual impression of raised and lowered areas is provided by varying concentrations of fine dots. The technique is easily learned, very effective, and acceptable for almost all forms of reproduction for publication. However, it should be avoided wherein it might confuse punctae illustration, and in any event should be kept to an absolute minimum consistent with accurate rendition of observable scientific fact.

CHAPTER 6.

6. PHOTOMICROGRAPHY OF DIATOMS

Digital cameras have substantially simplified photomicrography for the amateur. Together with multi-exposure controllers and stacking software photomicrography is within the means of all diatomists. It provides instant results at no cost, once the camera and suitable display hardware has been acquired. Nonetheless, this chapter still includes some extremely useful insights and as such is included almost unchanged. Some of the featured equipment is no longer available.

6.1. Introduction

The portrayal of diatoms by drawing, in their true natural appearing state, is very time consuming, if not especially difficult. The difficulties arise from the varying abilities of the diatomist-illustrator, and have created thousands of drawn representatives useless for identification purposes. Even the most experienced diatomist, and even one with a highly developed degree of illustrative ability, must take considerable time to produce drawn illustrations if they are to be accurate and useable by others.

It is my own personal opinion that for most cases a drawn figure properly executed, is superior to any other form of illustration. However, the word “properly” indicates that which can be seen by the microscope aided eye (no more). In some cases a very able diatomist is hampered by his inability to accurately and clearly portray certain details although they are plainly visible to him. An inexperienced diatomist, even though an able artist, may portray features fancied through incorrect microscope adjustment or represent optical artifacts as true detail. On the other hand he may omit salient and important features for the same reasons. The ideal condition is to have both abilities in one individual. That is rarely the case and the next best arrangement is to have drawn figures made by a professional scientific illustrator under the guidance of an experienced diatomist.

The illustrations in Patrick and Reimer are examples of this nearly ideal situation and the reason for their outstanding quality and value. However, most diatomists do not have the time, facilities, or proper assistance to accomplish illustrations in this manner.

A very time-saving method of diatom illustration is through photomicrography. Photomicrography has the advantages of speed, a veritable battery of enhancement techniques, and the capability to respond to wavelengths of light not accepted by the human eye. It also is an invaluable time-saver in the portrayal of interior cell details, habit, colonial and diatom associations encountered in ecological studies, and in providing records of individual strews in slide collections. On the other hand, the camera lacks the accommodating ability of the eye and requires some special apparatus and procedures for successful work. Photomicrography is more expensive of accomplishment.

There are a number of good references on photomicrography that provide most of what the diatomist must know to produce acceptable photographic illustrations. This chapter contains information aimed at supplementing such photomicrographic texts, and is directed at the photography of a single subject, - diatoms.

6.2. Photomicrographic Methods

Of the many ways in which a photographic image can be obtained from a microscope only a few of the more commonly available are discussed here. Emphasis will be placed on magnification and the relationship of the microscope and its optics to the camera. In the photography of diatoms it is essential that proper magnification information accompany the final photograph that is to ultimately be used as a prime identification aid.

6.2.1. Cameras of Variable Extension

Reduced to its simplest elements, photomicrography involves only a camera minus a lens, (or a film carrier) connected in a light-tight manner to the microscope ocular so as to allow an image to be projected on the film. There must be some way of focusing the image on the film plane of the camera, for the visual microscope focus is not that which produces a real image.

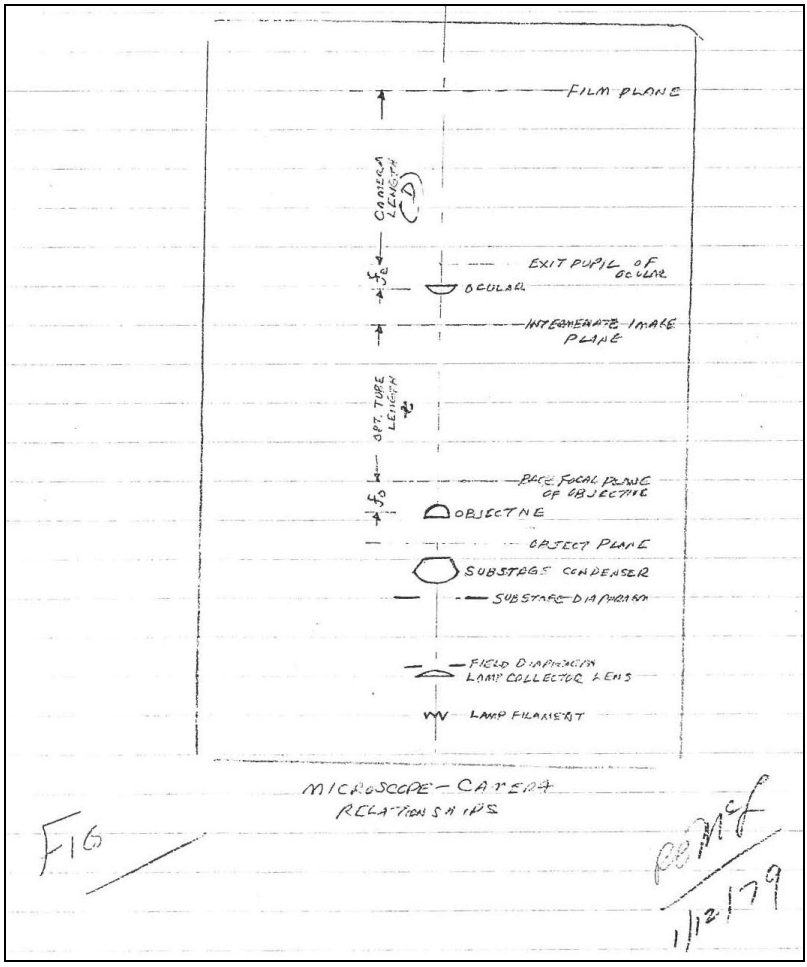


Figure 96.

With this arrangement the magnification of the image on the film plane is dependent upon its distance from the ocular. By varying the distance, it is possible to reproduce within the available film area only portions of the diatom that are of special interest.

The camera is used (without a lens) as a carrier for the film, the image being obtained by direct projection from the microscope ocular. A schematic diagram of such an arrangement is shown in Figure 96. When this arrangement is used, the virtual image (obtained when the eye is placed at the exit pupil of the ocular) is converted to a real image on the film plane by refocusing the microscope with the fine adjustment. When the virtual image is formed at infinity the intermediate image lies in the focal plane of the ocular. In order to have the ocular form a real image, the distance between the intermediate image and the ocular must be increased. This is done by raising the

> Raising the drawtube can have some undesirable effects, such as introducing spherical aberration. It is better to adjust the eye lens of the eyepiece only using a focusing eyepiece.

microscope-tube(with the objective) farther from the object plane. This means that if the microscope is focused visually first and then a camera (without lens) is placed anywhere on the optical axis above the ocular, it will be necessary for the microscope objective to be focused up (or moved further away) to form a real image on the camera film plane. When the distance between the objective and object is increased, the distance between objective and intermediate image is decreased, thereby forming this image at shorter distance with a consequent slight reduction in magnification.

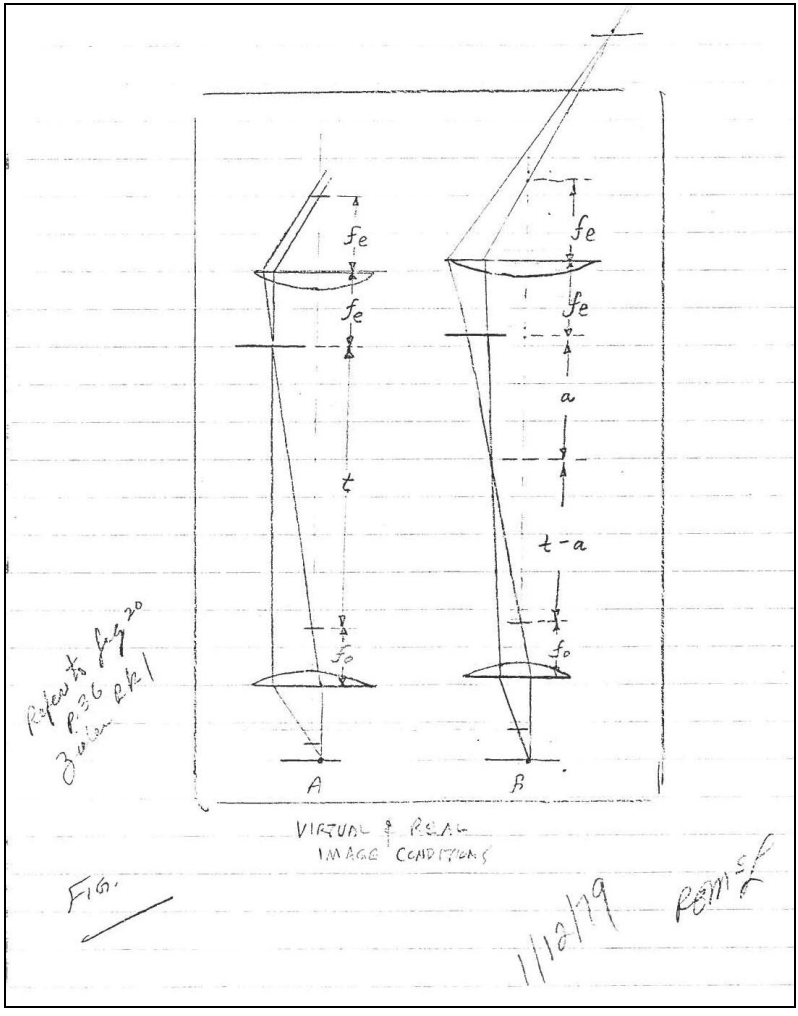


Figure 97.

Referring to Figure 97, the difference between the virtual image production (A) for the eye and real image production (B) for the camera is evident. Since the two schematic drawings are not to scale, and the focal distances of the objective and eyepiece are not in proper proportion, the difference in the location of the intermediate image, compared one with the other, is grossly exaggerated. Actually,

the distance "a" that the intermediate image is shifted down (outside the lower focal plane of the eyepiece) is very slight and reduces the overall magnification very little.

By this refocusing of the image, the optical correction of the image forming system is also slightly derogated. However, if a fairly great distance (10 inches or more) exists between the film plane and the eyepiece, the slight reduction in magnification and the accompanying image quality impairment escapes notice.

Therefore, for practical purposes, the magnification of the image on the film plane can be calculated by:

$$M_f = M_t \frac{D}{10 inches}$$

where
M_f = image on film plane.
M_t = objective power x ocular power.
D = distance of film plane from eyepiece Ramsden disc (in inches). .

Note that if D is less than 10 inches that magnification on the film plane will be less than that obtained visually with the same optics.

With this method of obtaining an image from the microscope on film, and especially when the distance D is 10 inches or more, it is not of great importance to exactly locate the film plane to obtain precise focusing. The reason is that the depth of focus in the image space (at the film plane) is quite great, especially when compared to the focusing range at the microscope objective.

The depth of focus at the film plane varies as the square of the magnification and is expressed by:

Depth of focus = $\frac{\lambda\sqrt{Ma^2 - (N.A.)^2}}{(N.A.)^2} x M_t^2$

Depth of Field (specimen plane) = $\frac{\lambda\sqrt{Ma^2 - (N.A.)^2}}{(N.A.)^2}$

Depth of focus = Depth of Field x M_t^2

Where

λ = wavelength of light
M_d = refractive index between front lens of objective and coverglass.
N.A. = numerical aperture of objective.
M_t = total magnification

Therefore, the focal range, using a 0.3 N.A. objective, (assuming M_d is 1.00) is the depth of field of the objective multiplied by the square of the magnification. In this case the objective depth of field is 0.00588 mm. A magnification of 400 then provides a focal range of 0.00558 (400^2) or 940 mm. This amounts to a distance of 57 inches along which the camera film plane may be placed – still maintaining a sharp image.

with a 0.65 N.A. objective (assuming. M_d is 1.00 and λ is 550nm.) the depth of field of the objective is:

$$= \frac{550x10^{-9}\sqrt{(1.0)^2 x(0.65)^2}}{(0.65)^2}$$

$$= 0.001 \text{ mm.}$$

and, with a magnification of 400, the focal range is then 160,000 x 0.001 or 160 mm. which equals approximately 7.1 inches.

Thus it is evident that there is considerable latitude, even when employing high N.A. objectives, in movement of the film plane without producing any serious change in sharpness of the image. As a result, most any mechanical device for enabling removal and replacement of the camera as a whole need not be of an extremely accurate nature. The reason for this (from Figure 98) is seen to be the very small angle.

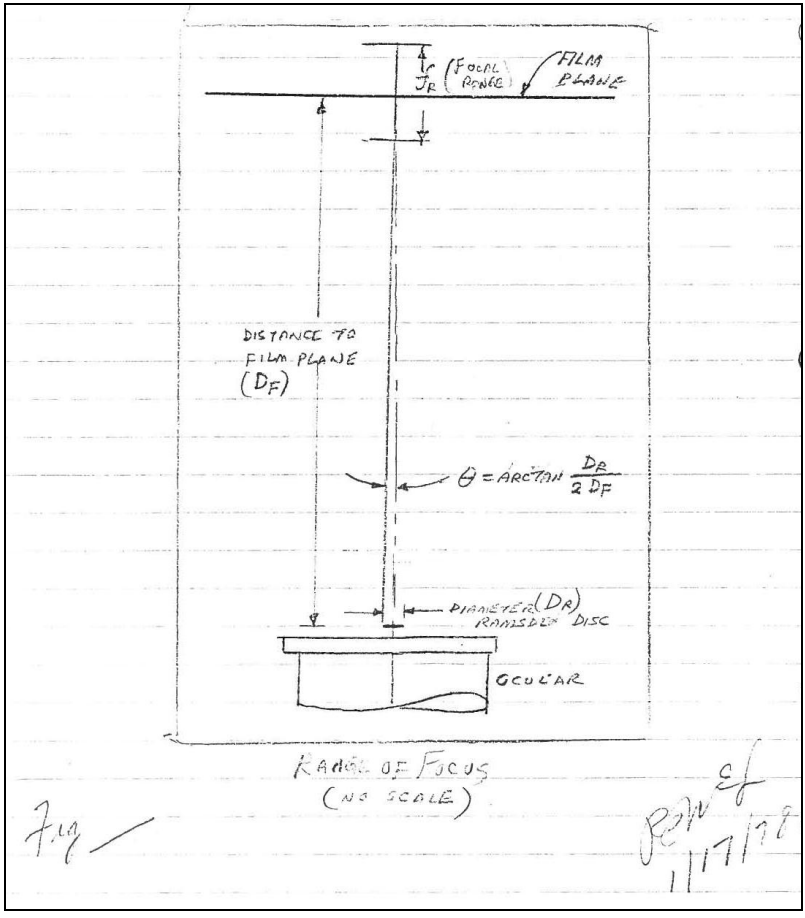


Figure 98.

6.2.2. Attachment Cameras

A very simple method of obtaining a photomicrograph is to focus the microscope visually, clamp its tube in place and center a small photographic camera, with its lens focused for infinity, close over the ocular. If the camera lens is properly centered, an in-focus image will be presented at the film plane. This results because the camera lens accepts the emerging parallel image rays from the ocular (as focused for visual use) and focuses them on the film plane just as if the camera were used to take a picture of a distant object. The resulting picture will be enclosed within a circular border and be reduced in size in proportion to how much closer than ten inches (250mm) the film plane is to the ocular.

Some attachment cameras, using 35 mm. film, are equipped with auxiliary lens with focal lengths of about 3.33 inches. Although there is, in using these cameras, a smaller sized image produced than with extension cameras, there is no loss of object detail. The latter is merely recorded in a smaller area. The magnification in the case of such a camera with a 3.33 inch focal length auxiliary lens, is reduced from the microscope magnification (objective power times the ocular power) in proportion to

that shorter length. For instance with an objective of 10X and an ocular of 10X the visual (microscope) magnification is 100X. On the film plane of such a camera the image will only be

$$100x\frac{3.33}{10} = 33.3X$$

When an enlargement of such an image is made, say with a factor of 6, the total magnification then is 6 x 33.3 or approximately 200X. For every condition of camera film-plane distance, microscope optics combination, and enlargement factor, the total magnification can be calculated. However, to avoid confusion and provide a permanent indication, a photomicrograph or a stage micrometer can be made, magnified and enlarged to the same degree. Then a unit length of this is marked in ink on the subject enlargement.

6.2.2.1. Special Photographic Oculars

6.2.2.1.1. The Projection Eyepiece

Specially designed eyepieces provide focusing capability for the lens component closest to the eye. The focusing mounts are provided with graduated scales and index marks. They can be used visually by setting the eye lens adjustment at "infinity", and the microscope focused for an image. Then a camera is set with a specific extension from the ocular (500 mm. for instance). The eye lens of the projection eyepiece is then set with its index mark at 500 mm., and the image is in focus on the film plane. Alternatively, the camera is in place over the ocular and the image focused on the film plane by adjustment of the ocular eye lens, instead of the microscope fine-adjustment. This provides for the formation of a real image at the film plane without any derogation or diminution of it as may be the case when the microscope is refocused.

6.2.2.1.2. Amplifiers .

Field curvature is an inherent characteristic in lenses designed on a spherical basis. It is manifested by all parts of the field not being in focus simultaneously, as would be the case with a "flat-field".

Visually, the out-of-focus condition across the field is, because of the eye's accommodating ability, of less importance than in photomicrography. Various degrees of field curvature correction can be applied to the optics of a microscope and there are now available, from most manufacturers, a whole family of achromatic and apochromatic objectives so designed. They are generally termed "plan-achromats", "apochromats", etc., and command a premium price.

Prior to the general availability of this type of objective, a series of negative lenses was developed, to be used in place of the ocular for photomicrographic purposes. These lenses are termed amplifiers and have a negative focal length. The Ampliplan by Bausch and Lomb and the Homal by Zeiss are typical. The great advantage of these lenses is that they present a very flat field image at the film plane.

The amplifier is a concave lens, curving the image plane in a direction opposite from that of convex lenses (objective) and the combination thereby produces final real images with less curvature. Although their performance is somewhat limited, they are quite advantageous in diatom photomicrography wherein frustular details of an individual diatom are in focus across the field. They are corrected achromatically to the same degree as compensating oculars and therefore may be used to advantage with apochromatic objectives. They can also be used with achromats, particularly with a green filter. Normally, each amplifier is corrected with only one or at the most, two, objectives. This is because of the great difference in the field curvature of objectives of varying magnifications. So, they are generally furnished in a series to accommodate various objectives that might be used.

These lenses are required, because of the location of their focal plane, to be mounted in a shorter tube (or further down the tube) in a microscope than a normal ocular. Manufacturers of such sets make provisions for this position modification. Amplifiers are not now generally available, but are well worth obtaining for diatom photomicrography.

6.2.2.2 Illumination

Lighting for diatom photomicrography should be provided by a lamp capable of Köhler illumination, and having the same qualities described in Chapter 2 of this section. While the ordinary research illuminator will be satisfactory for general work, high intensity illuminators are preferable in special cases, and to reduce exposure time. As filters and/or combinations of filters with low transmittance are often of great advantage in diatom photomicrography, the employment of high intensity sources is more common than with other subject matter. Also, the finer-grained films ordinarily employed in diatom work are comparatively “slow-speed”, and the high intensity sources will help reduce the exposure time and consequently assist in reducing vibration effects.

6.2.2.2.1. The Carbon-Arc Lamp

Ordinarily regarded by many as an antiquated means of obtaining high intensity light, the carbon-arc offers many advantages to the diatom photographer. It provides a very high intensity light, necessary in overcoming the light loss in the blue filters often used, and is rich in the ultraviolet or near ultraviolet frequencies advantageous in rendering the finest detail of diatom frustules. Some of these old lamps are still obtainable second-hand, and should be procured if possible. They may be operated from either an alternating current (a.c.) or a direct current (d.c.) source, a ballast resistor being used to limit the current to about 4 or 5 amperes.

When an a.c. supply is used, two points of light (one in each carbon crater) are generated and are a bit more difficult to use. Direct current operation is superior, providing a more brilliant single point of light in the horizontal (positive) carbon. The latter is more easily manipulated and focused for illumination purposes. A tube type rectifier such as is used for battery charging may be used to supply direct current from an alternating current power line. If possible such a lamp should be obtained with an automatic feed for the carbons. A mechanical clock-work feed mechanism is quite satisfactory and provides for a continuous carbon spacing of about $^{5}/_{16}$ inch at the normal burning rate. A single winding of such clock-motor drives provides a long duration feed for many sets of carbons. During operation there is a comparatively loud arc-sound and some little sputtering as the carbons burn away. However, the light presented is surprisingly stable in its intensity, and very brilliant. A typical carbon-arc setup is shown in Figure 99. It will be noted that some form of heat protection is necessary, especially if the lamp is used in close proximity to the microscope. Often, with the smaller sized arc-lamps, sufficient protection to filters, microscope and special material, is provided by the interposition of a heat resistant glass. Corning “Aklo” or similar glass in various thicknesses may be used. They ordinarily do not restrict or change the light frequencies emitted by the lamp, but may reduce the intensity because of their less than unity transmittance. In lieu of such heat-absorbing glass, or even in conjunction with it, it may be desirable to interpose a water-cell to dissipate heat. A cell of about 5 mm. thick holding at least 250 cc. of water is suitable for the smaller arc-lamps. The cell is usually placed as far from the lamp as possible in front of the filters.

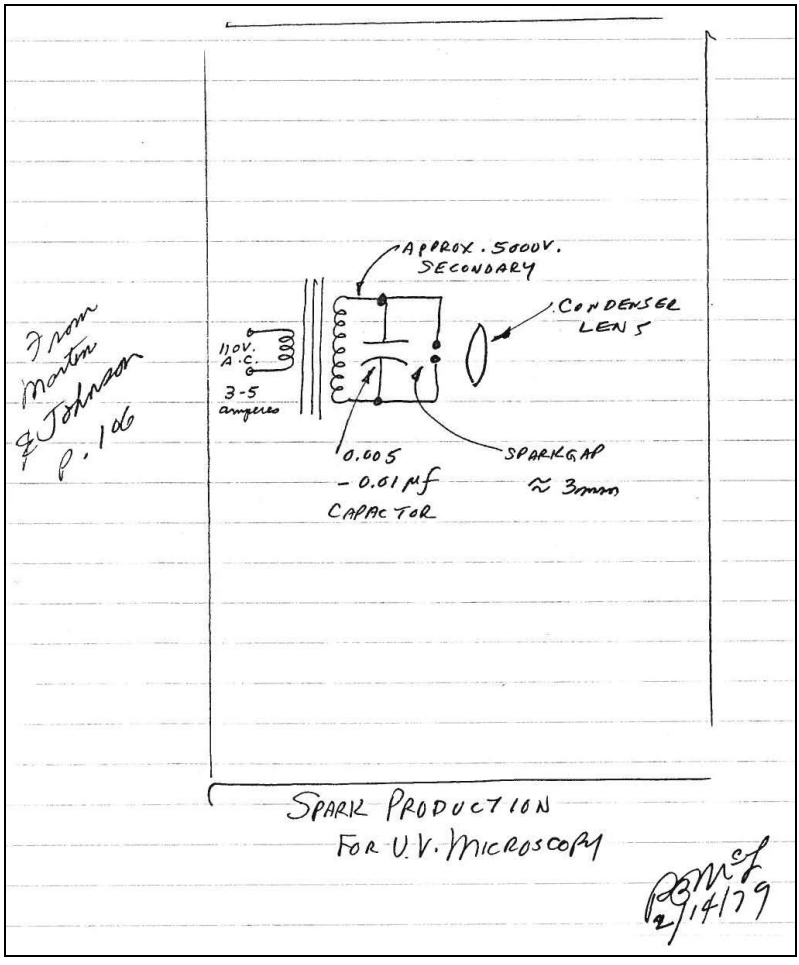


Figure 99.

An outstanding feature in using the carbon-arc is the ability to reproduce the same light quality from one time to another. It is only necessary to know the proper current, voltage, and carbon diameter to affect this very accurate and desirable reproducibility of illumination quality. The "color" temperature of sunlight is 5400°Kelvin. Table 10 indicates the color temperature of the carbon-Arc under various physical and electrical conditions.

Table 10

Amperes	**Anode Diameter**	**Color Temp K.**
4.5 d.c	8 mm.	3645
5 d.c.	8 mm.	3680
10 d.c.	6 mm.	3820
10 d.c.	6.4 mm.	3475

Although the actual color temperature is not of prime importance in black and white diatom photomicrography, if color photography of living specimens is to be done, the carbon-arc is especially useful in that area.

Also, the carbon-arc does emit an excess of ultra-violet radiation just beyond the visible caused by the formation of the gas cyanogen. For color work it is desirable that this excess UV be filtered out, but for diatom photomicrography in the 365nm. region this quality of the arc is very useful.

6.2.2.2.2. Other Sources

Other illuminators of high intensity can be used. Various types of projection lamps of 300, 400, or 500 watts are very useful. A very intense source of light is the tungsten-arc. Although this lamp is unsatisfactory for color work because of the discontinuous spectral illumination from the mercury vapor used to start the arc being mixed with that of the tungsten arc, it is a superior illuminant for black and white diatom photomicrography. It is one of the most intense of the steady concentrated types of lamps available.

If Köhler illumination is to be utilized lamps will require the capability of focusing, and a field diaphragm, so that they can be adjusted properly. Strict Köhler illumination is absolutely necessary for highest resolution. An exception to this is the use of oblique illumination to improve the resolution of certain periodic structure of the diatom valve.

6.2.2.3. Filters

Filter requirements for most photomicrography of diatoms are minimal. For rendering the finest detail in the usual cleaned diatom material, a good blue or violet filter is essential. As this type of color filter has a low transmittance, longer exposures and/or the use of high intensity lighting is necessary. The blue light of 456 nanometers for instance, is a powerful source for the purpose. Although it is deep blue, it can frequently be focused without undue difficulty, and, with apochromatic objectives, affords the highest resolving power that can be visually obtained. Kodak filters for isolation of this blue wavelength are a combination of number 2A plus number 34. The 2A filter is used to absorb any ultra-violet that might be present from the light source (a mercury-arc or a carbon-arc is commonly used). If this filter were not used then possible fluorescence of the mounting medium would derogate the final recorded image quality. A 2B or 2E Kodak filter will equally restrict unwanted ultraviolet. The violet (number 34) filter has a characteristic such that some wavelengths below 400nm. are transmitted and the majority of wavelengths transmitted fall off to nearly zero percentage at 500nm. It has a transmittance of about 62% for 436 nanometers. However its luminous transmittance is only 1.3%, and it therefore appears very dark visually, making the use of high intensity light necessary to focus with it in place.

Other blue filters such as Kodak 45 and 45A may be used for photomicrography. Neither of them would require the UV restriction filter (2A, 2B etc.) as they do not pass ultraviolet. Their maximum transmittance lies in the vicinity of 470nm. and is only between 21% (45A) and 36% (45) at that point. Thus they are not as good for the purpose of rendering detail, and in addition would require longer exposures from a given light source. However, their luminous transmittance of 2.8% (45A) and

5.2% (45) makes them easier to focus with. Corning filter number 5113 has a transmittance of 38% at 405nm, absorbing completely the red end of the spectrum. It does transmit some ultra-violet and would require an UV restrictor mentioned before. At this very short wavelength considerable difficulty may be experienced in proper focusing as the eye is very insensitive in this region of the spectrum.

With these filters the finest detail of diatoms will be resolved with ordinary light. Use of the 365nm. mercury line which, although in the ultraviolet, can be used with visual focusing with the aid of special objectives, is explained later in this chapter.

For the photomicrography of stained or live diatoms the use of contrasting filters in much the same manner as for visual use is recommended. Some knowledge of film characteristics is desirable in choosing contrast filters.

Consultation of photomicrographic references will provide guidance in this area.

6.2.2.4. **Cameras and Film**

6.2.2.4.1. The Camera

There is a very wide range of choice of cameras suited to photomicrographic work including very sophisticated commercial systems that are semi-automatic in operation. To cover all the possibilities in this brief treatment is not possible. Instead, for diatom photomicrography only two types of cameras are treated here. The choice of the two recommended is based on personal experience, flexibility, and economy. One is the 35 mm. single-lens reflex, and the other is the Polaroid Land Instrument Camera model FD-10.

The 35 mm. single-lens reflex camera is recommended as it simplifies focusing the microscope image on the film plane. No auxiliary focusing apparatus, or substitute film plane is required. There is the additional advantage of the shutter in such cameras being at or very close to, the film plane. This reduces vignetting and subsequent reduction of the useful area of a small exposure surface. Also, it employs a film size that is economical to use and which can be obtained in a wide range of emulsion types. The latter consideration is important when it is realized that photomicrography requires considerable experimentation and/or the making of test exposures, and that diatom work involves large numbers of illustrations. Also, processing of 35 mm. film is easily accomplished by the individual worker using small light-tight developing tanks.

There are a large number of makes of this type of camera available on the market today. Most of them will serve the purpose well. However, there are some features that make certain of them more desirable than others for photomicrography. If possible a camera should be selected that has a provision for the interchange of viewfinders.

The Exakta, for instance, allows removal and/or replacement of a number of different types of viewfinders, including special focusing screens and magnifier for photomicrography. In any event, if the ground glass focusing screen can be removed

it can easily be modified temporarily (or permanently) for the more accurate focusing required in photomicrographic work.

Since the camera body will usually be oriented (in a vertical photomicrographic setup) such/that the film plane is horizontal, the so-called eye-level viewfinders are awkward to use. If possible a waist level viewfinder should be used. The latter allows the camera film plane to be horizontal (above the vertical microscope) with the observer viewing the image on a vertically oriented focusing screen. Also, a magnifier is then easily used in making focusing refinements.

The lens of the camera should be removable, as ordinarily the camera is used without it, the microscope optics (objective and ocular) being used to project an image onto the film plane.

The Polaroid Land Instrument Camera is very useful to the diatomist and has become especially so with the advent of Type 105 Positive/Negative film. The advantages of obtaining photomicrographic prints in a one-step operation are greater than the disadvantages of reduced processing capability and increased cost, in using Polaroid films. Many claims of inadequacy for Polaroid films are unfounded in fact and based on prejudicial grounds and lack of experience with it. For that reason, and because the potential for the diatomist is great, considerable information on using this material for the photomicrography of diatoms is presented in this chapter.

The film size for the Instrument Camera is $3^1/_4$ x $4^1/_4$ inches (approximately 8.5 x 10.5 mm.) making originals adequate in size for many illustrative purposes, and convenient for filing and subsequent examination without enlargement. The positive/negative film provides both a positive print and a negative (of the same size) for enlargement if required.

6.2.2.4.2. Film

There is a minimum permissible magnification necessary in photomicrography. This minimum magnification is determined by the “grain” of the film used. Because of the extremely fine markings on diatoms and the use of small format (35 mm.) film this consideration is very important. Grain diameters of films vary from 0.02 mm. for “fast” films to as small as 0.005 for “slow” films. It is advisable in order that the film may resolve the image adequately, to have the magnification such that the smallest distance between two details to be resolved, be approximately ten times the grain of the film. For diatoms this requires a quite fine-grained film. Diatoms with puncta spacings of one micrometer (1μm) are common and many have much finer detail than that, down to the finest resolvable by any objective. Add to this the fact that with 35 mm. film the image would ordinarily be enlarged 2 or 3 times to get adequate illustration size, the minimum magnification on the film assuming an average grain size of 0.01 mm. would be:

$$\frac{0.01 \times 10 \times 3}{\text{Resolution by objective}}$$

For various objectives in "green" light of wavelength 540nm. (Wratten 58 + 15 filters)

Table 11

N.A		Magnification
1.30	necessary magnification factor is 0.30/0.000208	1346X
0.65	necessary magnification factor is 0.30/0.000415	723X
0.20	necessary magnification factor is 0.30/0.00135	222.6X

However, for photomicrography of diatoms usually made in blue light of wavelength 478nm. (Wratten filter 45A) the figures become:

Table 12

N.A.	Magnification
1.30	1652x
0.65	816X
0.20	252x

Note that magnifications required are to maintain a 10:1 detail spacing to grain size plus an enlargement of the negative 3 times. For very fine grained films these required magnifications could be reduced proportionately. Also, note that in each case the "1000 x N.A." rule for "non-empty" magnification is exceeded.

Advances in film design are so rapid it makes the recommendation of specific film types by manufacturer rather difficult. Instead, only the major characteristics desirable in a film for photomicrography will be briefly outlined. Based on that information, the diatomist will be equipped to make selections from various suppliers.

Contrast and resolving power are the most important film properties for photomicrography. Remaining properties to be considered are spectral sensitivity (range of colors), sensitivity (speed), and latitude.

It is nearly impossible to find a film with extreme values in all of these areas of consideration. It is usually the case that films with high available contrast and high resolving power will have a slower speed and less latitude than those in which these latter properties have been emphasized in design.

In general, negative material for photomicrography should be selected first on the basis of contrast, resolution, and other related qualities. Then selection is made on the basis of a film sensitive to the color of the light used. A wide gamut of emulsions is available in 35 mm. format. Films found to be very useful in the past for diatom work include Kodak Panatomic X 35 mm. film, and Micro-file 35 mm. film. Kodak High Contrast Copy film using developer D19 is also highly recommended (1985 addition: Kodak Ektagraphict Technical-Pan).

6.2.3. The Polaroid Land Instrument Camera

6.2.3.1. Equipment Description

This unique camera as purchased is composed of the following items:

(1) Camera with cable release.
(2) Focusing tube.
(3) Eyepiece adapter.

There is no lens with the camera. The camera body is composed of a film-pack holder, and a tapered camera body with a shutter controlled by the furnished cable release. The focusing tube contains a lens at its upper end by which the focused image from the microscope is viewed. The eyepiece adapter is a plastic/metal device that provides for a quick-connect-disconnect of either the focusing tube or camera to the microscope ocular with accurate centration. In operation, the focusing tube is attached to the adapter by merely putting it in place. It is retained in position by a close fit between the barrel of the adapter and a matching sleeve in the tube, and by gravity. No fasteners are involved. When focusing is accomplished, the tube is lifted off the adapter and the camera set in place, being retained there in the same manner. The shutter, which is designed for time exposure only, is operated and the film exposed. The camera is then lifted off the adapter and a procedure followed for developing and printing the photomicrograph. The print is available for inspection in final form within one minute, dependent upon what type of film is being used. Ordinary developing time for Type 107 is 15 seconds, and for Type 105 30 seconds. Complete instructions for use of the camera and films available are with them when purchased.

A few observations from personal experience in using this camera for diatom photomicrography are in order. First, the equipment is designed for quick, easy. and incidental use. The arrangement provided for coupling and decoupling focusing tube and camera leaves much to be desired in the way of stability and repeatability, for extensive and continuous use in diatom work. A simple support for the focusing tube, camera, and adapter which takes all weight off the microscope stand is diagrammed in Figure 100. It is to be noted that the film distance from the ocular is only 8 inches, thus the photographic image will be about 0.8 of the visual magnification. There is no extendable bellows of course, and if greater size in the film image is to be obtained, then either the microscope optics need changing appropriately, or the entire camera (with its fixed-length cone) needs to moved further away from the ocular. This entails some difficulties in focusing with the focusing tube and substitution of the camera. However, this would be a mechanical problem fairly easy of solution. The big drawback is that as the camera body is moved away from the microscope ocular the shutter in the camera body rapidly vignettes the image on the film This means that although a larger image could be obtained in that way, proportionately less of it will be shown and, perhaps more importantly, less of the rather expensive film area utilized.

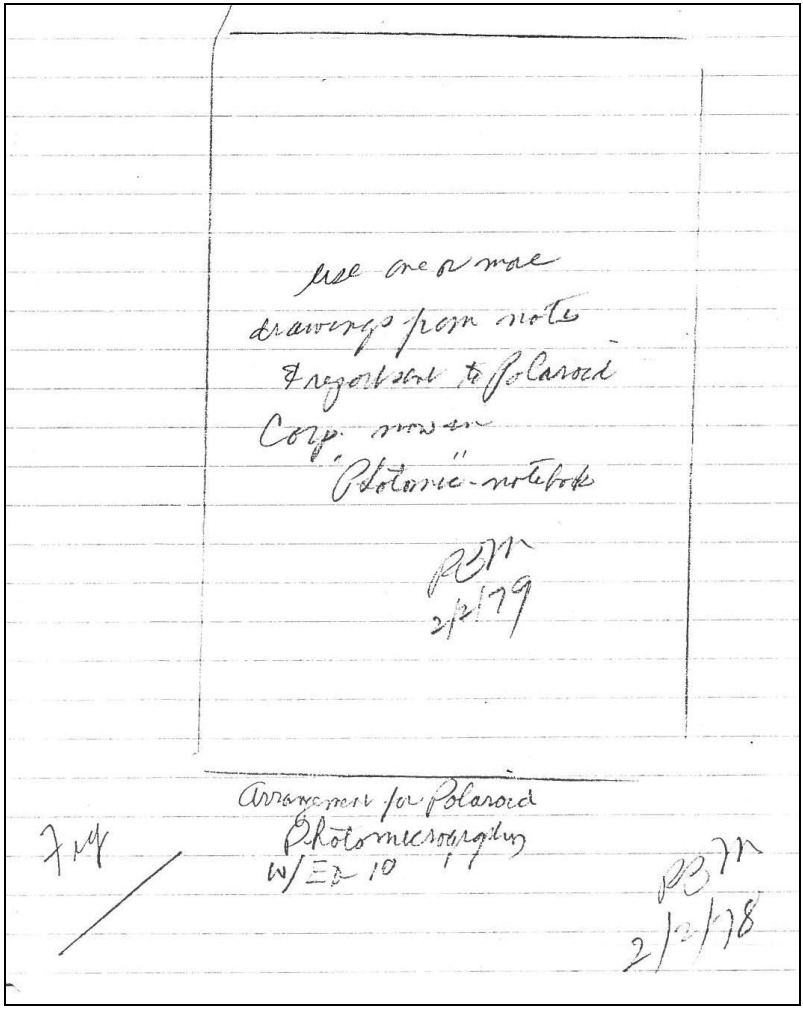


Figure 100.

6.2.3.2. Film

Type 107 film has a speed of ASA 3000, with an accompanying rather coarse grain. The very high sensitivity with even normal lighting does shorten the exposure time excessively. Exposures shorter than a few seconds are very difficult to time or repeat with any accuracy. An exposure time of about 5 to 10 seconds can be accurately timed with a sweep-second hand watch and the shutter operated appropriately. The reduction in light intensity necessary to lengthen the exposure time to this more useable interval can be accomplished by decreasing the illuminating lamp voltage or using a neutral-density filter. A combination of the two will be found to be most appropriate.

The grain of this film does not allow any enlargement excepting with diatoms possessing the coarsest of detail and then only 2 or 3 times at the most. Most diatoms imaged on this film will be satisfactorily represented for record purposes,

wherein their detail is comparatively coarse, or there is no doubt as to the species represented. For best representation an individual diatom should fill the film area as completely as possible.

The recommended developing time for type 107 is 15 seconds at 70°F. For cooler ambient temperatures, longer exposures are required. Information regarding various exposure times is furnished with the film. For diatom work it will be found that development times of double those recommended by the manufacturer will provide a better contrast. For instance, many satisfactory higher contrast images are obtained with developing times of 50 seconds instead of 15 seconds (at 70°F.). Experimentation with various types of diatom microslide mounts will be in order. Dependent upon the mountant used in preparing the diatom slide, different development times will provide differing results. In any event, some quality and contrast control is available to the diatom photographer by this means, and should be utilized.

If enlargement is necessary, it of course must be obtained through the use of the print obtained, as there is no negative in the normal sense. This of course means that very little, if any, control over contrast is left after the initial picture is made. The "negative" material that is ordinarily discarded is of some use to the diatomist.

Although the material has a peculiar brownish color, the image, for many record purposes, is adequate. Many such "negatives" diatom pictures have been allowed to dry, mounted in appropriate albums and are as good today as they were 5 or more years ago. Thus there is the possibility, for the diatomist at least of two "prints" when using Type 107 film.

A more expensive, but much more versatile film for the Polaroid camera is Type 105 Positive/Negative film. It has a much slower speed (lower sensitivity) and very fine grain. The speed is ASA 75, requiring 40 times the exposure or light intensity (as the case may be) as compared with type 107. It also provides in the process a true negative that can be used in producing additional prints and enlargements. See Figure 101.

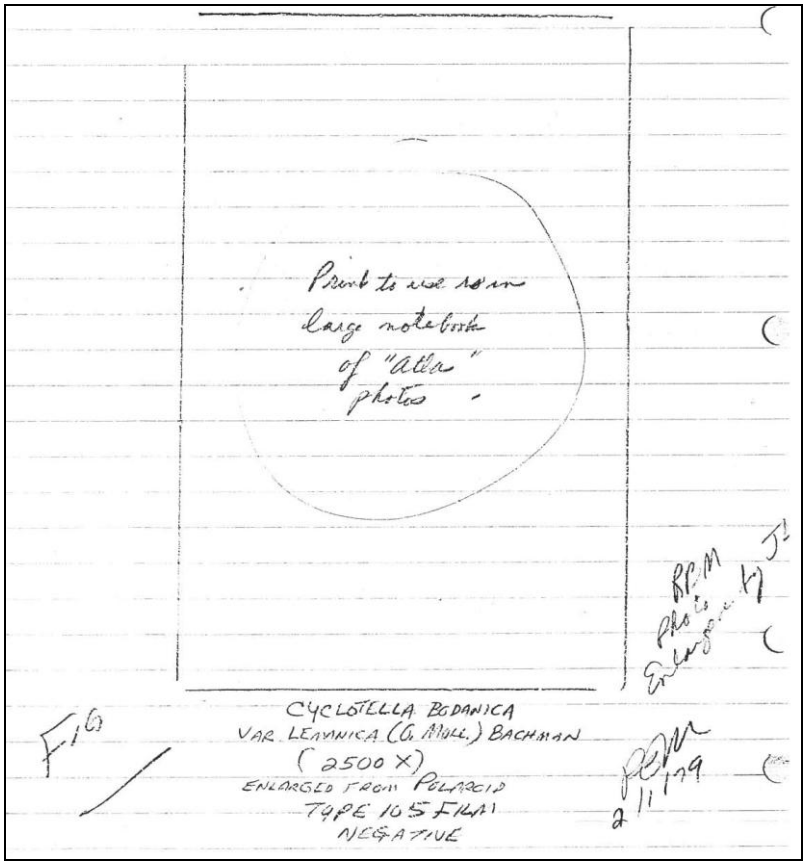


Figure 101.

In each of the above films, only 8 each black and white prints are provided per pack. That makes the cost per print high, and for large numbers of diatom illustrations the cost is great. However, for incidental illustration or for limited numbers of illustrations and since prints are acquired at no additional processing cost, the use of these films is very desirable.

Both Type 107 and 105 Polaroid films are capable of producing diatom structure sufficient for record purposes in all cases, and for identification purposes in most cases. Some exceptions to the latter will be found in diatoms whose detail is so fine as to disappear in the grain of the film. Enlargement of Type 105 to 5X is generally satisfactory without "graininess" becoming an interfering factor. An enlargement of the diatom *Cyclotella bodanica* Eulenstein var. *lemanica* (O. Mull.) Bachman to 2500 diameters on the film was found to be satisfactory.

In this case the diatom is only 34.5 micrometers in diameter but appears to be 8.6 mm. (approx. 5.58 inches) in diameter on the print. It was at a magnification of 800X on the original negative and therefore enlarged more than three times for the large print.

6.2.3.3. Simple Equipment Arrangement

The microscope and illuminator are arranged on an 18 inch hardwood base. The illuminator is at a fixed distance from the microscope, and the FD-10 camera is accommodated on a specially built supporting bracket to allow quickset and removal.

Because the microscope is oriented vertically and is of the focusable body type, precautions are taken to eliminate any focusing change upon placing the FD-10 in position for photography. This is accomplished simply by a supporting framework and a special mounting of the camera (FD-10) adapter assembly.

It will be noted that the microscope tube does not contact the adapter assembly (upon which the FD-10 rests), and therefore no forces due to its weight are transmitted to the focusing mechanism. The entire weight of the adapter and camera is borne by a plexiglas platform supported above and on the hardwood base by a one and a half inch diameter steel column and an associated framework. In this arrangement, the camera adapter assembly becomes an integral part of the microscope set-up, as the three plastic hand screws are removed and the adapter fastened with L-brackets to the plexiglas platform. The microscope ocular is fastened into the adapter in the usual way, as described in the Polaroid instruction manual for the FD-10 Instrument Camera. The height of the plexiglas platform is adjusted such that the microscope tube is free to run vertically within the focusing range and not contact the inside of the adapter bracket or the ocular excepting as a sliding fit over the latter. The ocular then, “floats” in the microscope tube during focusing.

The small deviation in tubelength created by this arrangement produces an inconsequential increase in spherical aberration. In order that the microscope tube-top does not contact the interior underside of the camera adapter, about $^{1}/_{16}$ inch spacing to the tube-top is allowed when adjusting the apparatus. Additionally, during focusing it will be realized that as the objective is moved up and down, the tubelength is changed by that focusing distance. All of this appears unorthodox, and is, but insofar as recording the diatom image on the film is concerned, it does not appear to be a significant factor in affecting sharpness.

Since the hardboard support is of limited size, the distance from the illuminator to the microscope is comparatively close; about eight inches. As the arrangement has been used with Polaroid Type 107 with a speed of ASA 3000, some way of reducing illumination level to the microscope was necessary. Although the illuminator is supplied electrical energy through a transformer with a metered variable voltage control, it was found that the adjustment of the autotransformer to limit the illumination to a workable level was too critical at the lower voltage. Because of the “tapping” action on the autotransformer, at lower voltages, the voltage “bumps” from turn to turn and further, the voltmeter accuracy of reset is reduced too, as reading accuracy is a percentage of full-scale. Reduction of the illumination intensity can be effected easily with a neutral density filter or varied with a Polaroid intensity filter made from two pieces of Polaroid filter sheets.

6.3. Focusing the Image

Focusing with the FD-10 is restricted to the means provided, and at times may be a drawback to its use because of the comparatively coarse-grained "ground glass" used in the focusing tube.

With a 35 mm. single-lens reflex (SLR) camera an interchangeable viewfinder for photomicrography, there is no particular problem. With a 35 mm. SLR camera which has an interchangeable view finder system, it can be improved for focusing (even though not especially designed for photomicrography) if the surface of the "ground glass" focusing screen is available for modification temporarily or permanently. The modification consists essentially of making the ground glass focusing screen more transparent and/or of a finer structure. The transparency of the screen can be improved and the structure made to appear finer by the application of a thin film of oil of anise to it. The image on such a treated surface is brighter and easier to focus more sharply. The oil is easily removed after it has served its purpose.

A still more satisfactory arrangement is to draw a fine cross-line in the center of the ground glass screen with a soft pencil. Fasten a small circular (or square) coverglass in place, over the penciled cross, using Canada Balsam. Now there will be a circular (or square) completely transparent area in the center of the screen traversed by the penciled lines. The image is focused both aerially and on the cross-lines for maximum sharpness. The use of a small 10X hand held magnifier, such as a Hastings Triplet or another corrected type, will greatly assist in obtaining the finest focusing.

6.4. Exposure Determination

This section was never completed.

6.5. Special Methods

Photomicrography is itself, of course, a special type of photography with its particular methods and techniques. Within that framework some special methods have been developed or adapted, over the years by various diatom workers to take full advantage of photographic techniques in illustrating their subject matter. Some of the more notable of these are briefly discussed in this section.

6.5.1. Successive-level Focusing

Critical features of diatoms for identification purposes can be photographed at successive levels of focus starting with the focus on, say, a rim or protruding process and working down successively with exposures at various levels. Each exposure being made to bring out critical features at that focus level. The result will be a series of photographs which are a record analogous to various focusing planes used in visual examination.

Diatoms such as *Stictodiscus californicus*, *Melosira claverga* Grun., *Auliscus hardmanianus* var. *bifurcata*, and others were photomicrographed by W. D. Fleming (1948) in this manner, the diatom being presented in no fewer than eight (8) different focusing planes at 970X magnification. Exposures were made on 35 mm. Eastman Microfile film and developed in D76c formula for 12 minutes at 20°C. The 24 x 35 mm. negative size was enlarged to 5 x 7 inches on paper.

6.5.2. Intense Blue-light

Edmund Johnson
Spitta **(**1853 – 1921)

About 470nm. is the usual limit of visibility, therefore limiting human vision of diatoms illuminated in blue light with its accompanying increase in resolution. Spitta produced diatom photomicrographs of superb quality and definition more than 50 years ago using light of this wavelength. He combined this short wavelength of illumination with oblique lighting to obtain some of the most revealing photographs of diatoms ever made. It is of value to briefly describe his method even though some aspects may be somewhat outdated.

He recommended using a Kodak no. 48 filter (often called a C filter) combined with a no. 38 (the latter suppressing the small amount of red passed by the no. 48) for obtaining light peaked at about 470nm. As a source he used a carbon-arc lamp, He adjusted the position of the lamp and its condenser such that the back focal plane of the objective was filled with light and proceeded, with the use of a suitably shaped diaphragm placed in the filter holder of the substage condenser, to obtain oblique illumination. When such blue light is used to obtain the highest resolution, it necessarily demands the employment of oblique illumination. The opening of the special diaphragm is at one edge and the position of it chosen such that the two diffraction images are in adjacent parts of the field (back lens of the objective).

If the back lens of the objective cannot be filled with light from the source, the light will be in the form of a star-shaped pattern occupying the central and part of the intermediate zones only. If moving the lamp (closer or further away) does not improve the light distribution in the back focal plane of the objective then the problem arises from the substage condenser in not being designed to work with a thick microslide. If this is the case, the star-shaped pattern can be adjusted in its position and azimuth by moving the substage mirror, to emulate a properly located oblique light source, no special oblique-illumination diaphragm being required. Final perfection of the image will result when the specimen is revolved about the optical axis and/or slightly altering the position of the mirror with respect to the azimuth of the oblique light spot. Spitta indicated that this method, in his opinion, yields a much brighter image than that produced by the ordinary method using a diaphragm.

6.5.3. Near Ultra-violet Light

The advantages of the application of ultra-violet over visible light are (l) increase in resolving power, (2) structural differentiation unobtainable, or obtainable only with difficulty, at longer wavelengths. With a C filter, negatives can be made showing

dots in the diatom *Amphipleura pellucida* which are 100,000 to the inch, while these are invisible with a filter of longer wavelength.

It was originally suggested by Kodak Research Laboratories that it might be advantageous to utilize that portion of the ultraviolet spectrum where the wavelength is just sufficient in length to not be appreciably absorbed by optical glass, thus not only saving expense over the use of shorter wavelengths but allowing the use of greater numerical apertures and achromatic corrections. This was successfully developed using the 365nm. spectral line from a high intensity mercury lamp, a set of specially corrected objectives and a pair of filters. The objectives are designed so that the green mercury line with a wavelength of 546nm. and the ultraviolet of 365nm. are simultaneously in focus. This means it is merely necessary to focus the image using the mercury green filter, such as a Wratten no. 62, no. 72A, or a no. 74, substitute the filter 18A which transmits the 365nm. mercury line and make the exposure. The result is a sharp picture of the object revealing greater detail than could be seen by visible light. At the highest aperture and adequate magnification (1.7mm, 1.30 N.A. objective) a very slight, and previously calibrated, adjustment of the fine focus must be made after shifting to the no. 18A filter in order to obtain the best definition. This final calibration is no greater than would have to be made when focusing in green and photographing in strictly blue light with the usual type of achromats or even apochromatic objectives. The maximum theoretical resolution limit with the 365nm. line and an objective of N.A. 1.30 is 0.14μm. as compared with 0.17μm using blue light (λ is 450nm.).

Sandalwood oil is used as the immersion medium since cedarwood oil has too high an absorption. The mounting medium should be kept as thin as possible. For photography in the ultraviolet at 365nm. ordinary materials can be used, although Kodak Ortho-X film (or equivalent) will be found to be faster than the Kodak 40 plate but with less contrast and resolving power.

The special objectives described were available some years ago from Bausch and Lomb. They are no longer manufactured, but can be obtained as second-hand items from time to time. The diatom photomicrographer who is fortunate enough to obtain a set can utilize them to advantage. The objectives are of glass, and a standard Huygenian eyepiece can be used with them. All parts that would ordinarily have to be made of quartz can be of ordinary glass in this method. The objectives provided in the B&L set are three; a 16 mm. (10X) N.A. 0.25, a 6 mm. (28X N.A. 0.65. and a 1.7 mm. (102X) N.A. 1.30 oil immersion type. All objectives have the designation "UV" marked on the barrel.

In performing photomicrography at 365nm. care must be taken that the diatom mountant used and the immersion oil (if used) is of a low absorption. The relative absorption of a 0.2 mm. thick layer of some of the common immersion and mounting media at various light wavelengths is listed below. The absorption of balsam is variable and increases rapidly with the age of the mounted slide.

Unfortunately the table doesn't include the wavelengths associated with the results.

Table 13

Cedarwood Oil	1.51	14	56	100
Sandalwood Oil	1.51	10	32	100
Glycerine	l.46	very transparent throughout		
Balsam	1.52	11	32	100
Styrax	1.58	22	94	100
Hyrax	1.75	11	60	100

6.6. Notes and Technique

Rheinberg illumination is suitable in many cases for emphasizing contrast in diatom photomicrography. Black-and-white or color photographs will benefit using that technique. A set of substage discs for photomicrography was devised by Rheinberg and marketed at one time by Kodak. The colored central discs and colored peripheral rings were supplied in the same mountings as the Wratten Visual "M" filters.

They were thin enough to permit two or more of being inserted into the condenser filter carrier and rapidly changed as required. The central disc-stops determined the color of the background and the peripheral rings determine the color in which the object was seen. The set was comprised of : Central Disc-Stops (1.) Greenish Blue, (2.) Blue, (3.) Green, (4.) Red, (5.) Purple, (6.) White Matte. (7.) Black; Peripheral Ring-Stops; (8.) Red, (9.) Orange, (10.) Blue-green, (11.) Blue; also (12.) Red and Blue Sector-Stop.

Although this set is no longer available commercially, one can easily be made up using plastic or glass discs and colored inks or transparent colored tapers. The red and blue sector stop is particularly useful in emphasizing diatom striae or ridges which are transverse to one another in different colors. It should in those cases, be used with a black central stop. By rotating the sector-stop disc unknown striae can be ascertained.

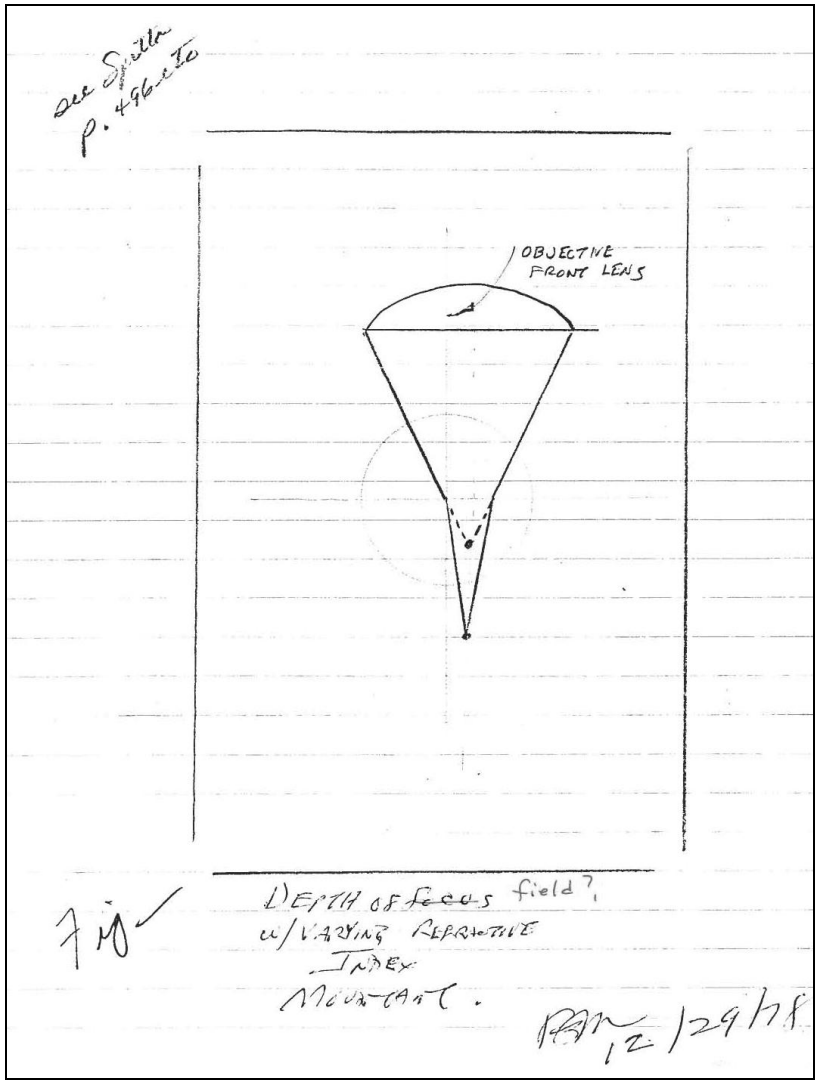


Figure 102.

Depth of focus is increased by the use of denser media in mounting specimens. This occurs since a light ray passing from a rare into a denser medium is always deviated toward the normal, and the point at which the object is in focus is thereby extended from a high to a lower point. See Figure 102.

When photographing diatom specimens with a raphe for instance, the obliquity of the light should be adjusted such that the raphe branches seem to lie on the floor of the valve.

The advantage of a very narrow band-pass filter used in photomicrography is to provide a constant photographic response of the film regardless of the lamp voltage required for adequate light intensity. Otherwise color variation of the lamp

(especially in color work) affects film response, as the voltage is varied to provide different light intensity levels.

When contrast in detail is weak as with many diatoms, exceeding the so-called "1000 X rule" for magnification can be quite useful. The illumination per unit area of image is approximately equal to:

$$B \times \left(\frac{N.A.}{m} \right)^2 \times t$$

Where

B is intrinsic brightness of the source.
N.A. is N.A. of objective used (that is, the portion filled with light by the substage condenser).
m is magnification
t is transmission factor of the optical train.

For instance, all other factors being equal, if the magnification is doubled the image illumination is decreased inversely by the square of the magnification and therefore the exposure time would need to be increased by a factor of four.

The production of ultraviolet light may be effected by an electric spark. A suitable source may be obtained using a high-voltage transformer arranged circuit-wise. An appropriate transformer might be a neon-sign transformer.

The essence of good diatom photomicrography is to know beforehand what the diatom should look like. This means a preliminary microscopical examination using conventional illumination. If not done the procedures in photomicrography to enhance contrast and emphasize certain details may produce a misleading and/or false representation in the final photograph.

If the length of exposure is controlled by the light (lamp) switch, rather than by the shutter release, one possible source of vibration may be eliminated.

Restrict, if possible, photomicrograph magnifications (at least in the final print or illustration) to certain specific values related to direct comparison with extant illustrations in the literature. This makes it much easier to compare striae-count and dimensions.

A carefully maintained record of all data is an integral part of photomicrographic procedure.

In general for weakly contrasted subjects such as diatoms, choose black-and-white emulsions with steep gradation and strong developers. Contrast is always improved through longer development or with developers of more vigorous activity.

If exposures are less than l second the vibration caused by a shutter opening and closing will occur for most of the exposure time. Longer exposures lessen the effect

as it is experienced for only a fraction of the time. Therefore, dependent upon the mechanical setup of the photmicrographic equipment, it may be advantageous to choose longer exposures rather than very short ones.

Photomicrographic apparatus can be mounted in a box of sand or sawdust to dampen outside vibrations.

CHAPTER 7.

7. QUANTITATIVE EXAMINATION OF DIATOMS

7.1. Introduction

In almost any scientific investigation, observation generally leads to the need for quantifying data of one form or another. Morphological, ecological, and biometric studies of diatoms are no exception. There are a number of techniques and principles common to any and all specialized areas of study. In this section is included information relative to techniques in data acquisition on some of the more common diatom areas of investigation. In this age of sophisticated measuring and quantifying devices there are myriads of techniques and methods in use. This section will only treat those basics that can be used by any knowledgeable worker with a minimum of special devices and training.

In the main the subjects discussed in regard to quantification will deal with those actions and techniques that are performed with the light microscope. Other aspects of diatom study such as collection and preparation which affect such quantitative examination will be included also.

7.2. Measurements

In the study of diatoms it is necessary to make linear measurements of length, width, and thickness (depth). Other linear measurements involve the spacing of costae, striae, and punctae. Relative locations and/or sizes of various frustular or valve features also require simple measurement techniques. The study of live diatoms, especially those which have the power of movement, sometimes requires the measurement of distance traveled and the proper correlation of time-intervals with those distances to determine velocity.

Valuable to ecological studies are ratios of length to width, volume, and other combinations of simple measurements. All of the measurements mentioned, and more, can be easily accomplished by very simple means.

The basic device by which linear measurements are accomplished is the ocular micrometer, the description and calibration of which is covered in Chapter 2. The basic unit of measurement is the micrometer (10^{-6} meter). The calibration procedure, previously described, determines the valuation of each interval of the ocular micrometer scale for a particular combination of ocular and objective.

William Benjamin Carpenter
b. 29th October 1813
d. 10th November 1885
President of the Royal Microscopical Society
Registrar of London University.

In older works devoted to microscopy (especially of English origin) the term “lines per inch” was used to express microscopic spacings instead of the now used micrometer. This no doubt originated in the practice of those times in expressing the resolving power of objectives in lines per inch. Since diatoms were used extensively

then to test objectives, it was logical to express certain aspects of diatom structure in the same way. For instance Carpenter indicates in a Numerical Aperture Table that *Amphipleura pellucida* has 95000 striae per inch, *Navicula rhomboides* 78000 to 87000 lines per inch, *Surirella gemma* 64000 to 69000 lines per inch, and so on. This carried over into descriptions of diatoms in some cases, and will be noted occasionally in older diatom descriptions.

7.2.1. Frustule and Valve Dimensions

Length and width dimensions of the diatom frustule or valve are determined by multiplying the number of divisions on the ocular micrometer to include the length (or width) of the diatom image, by the calibrated value of one interval. For instance, if the length of a diatom valve requires 25 spaces (intervals) of the ocular micrometer to include it, then the actual length of the diatom would be 25 times the calibrated length of one interval. It is not necessary to position the diatom such that one extremity lies at one end of the micrometer scale. It is only necessary to know the number of intervals necessary to include the image dimension being measured. Often the diatom to be measured is in an orientation such that its length (or width) is not coincident with the ocular scale. If a rotating stage is available on the microscope the proper orientation for measurement is easily obtained. If not, then positioning the diatom in the x-y directions to get it to the center of the field and rotating the ocular (with the incorporated scale), will accomplish the same thing.

While the principles of such measurements are very simple, it is very difficult to get the exact size of diatoms. This is due to the lack of perfection and uniformity of ocular micrometers and the difficulty in determining the exact limits of the frustule or valve to be measured. A difficulty in calibrating at high powers (often used in diatom work) is the width of the lines of the micrometer. If it is very accurately ruled, half the width of each line belongs to the contiguous spaces, hence one should measure the image of the space from the centers of the lines bordering the space, or as this is somewhat difficult in using the ocular micrometer, one may measure from the inside of one bordering line and from the outside of another, that is from the right side of all the lines or from the left side of all. If the lines are of equal width, this is as accurate as measuring from the centers of the lines. This holds true in either the initial calibration of the ocular micrometer or in its use to measure linear dimensions. See Figure 103.

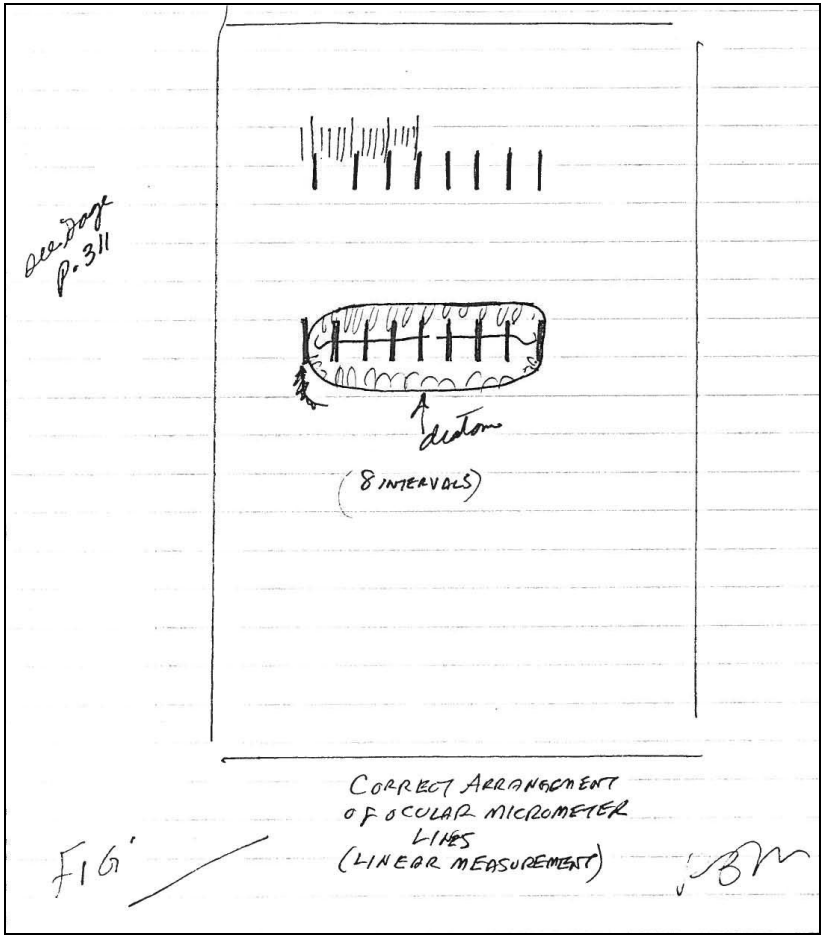


Figure 103.

It is necessary to use an objective of sufficient magnification to enable one to see all the details to be measured with great distinctness. The limit of accuracy or precision is about 0.2µm with the light microscope. Where the diatom is not exactly included by whole scale intervals on the ocular micrometer, there is an additional source of error in estimating just how far into the next interval the diatom reaches on the side not in visual contact with one of the micrometer lines. This deficiency is eliminated when using a filar screw-type micrometer eyepiece, as the calibration drum serves to provide accurate distance determination between lines.

The outline of so-called linear forms may vary considerably, the length being from two to three times, to as much as ten or fifteen (or even more) times the width. The measurements to be made on such valves are length in micrometers from one end of the apical axis to the other, and the width (along the transapical axis) in the same units. The variation in outline from simple to complex generally requires only that the width be measured at the widest point of the valve, and unless otherwise noted, is understood to be measured at that point. If certain forms require other width measurements they are so noted. For instance, the constriction in outline at the

center of *Fragilaria capucina* var. *mesolepta* Rabenhorst is a distinguishing feature of the variety and for that reason would ordinarily be measured in micrometers and especially noted in the description. Similarly a swelling in outline such as with *Synedra rumpens* var. *fragilaroides* is important as a distinguishing feature and should be so noted.

It is not uncommon to find species of *Nitzschia* and *Hantzschia* provided with width dimensions at the center where constricted, and maximum widths at the polar ends of the frustule in extant descriptions.

Certain cymbiform diatom genera, exemplified by *Amphora*, which is quite as often seen in girdle view as valve view should, if possible, have two measurements of "width", one being the usually understood "valve-width" and the other the "width" of the frustule in girdle view, although the latter is really a measurement of valve depth along the pervalvar axis. Van der Werff in his descriptions of *Amphora* includes a measurement in micrometers for the length, width of the valve, and width (depth) along the pervalvar axis. *Cymbella*, on the other hand is described adequately with the maximum width dimension of the valve, as it is very seldom seen in girdle view. The length measured is the linear distance from one "apex" to the other, although the valve may be curved in outline.

Some valves, such as *Fragilaria pinnata* var. *trigona* (Brun & Herib.) Hust. take on a tripolar aspect or shape. It is appropriate in such cases to indicate the linear distance between any two poles measured in micrometers.

Wherein the shape of the valve outline is the primary distinguishing feature of a diatom among others of similar shape a ratio of the length to the width (expressed as a whole number) may be a good additive feature to the description. Many diatoms of the genera *Fragilaria* and *Synedra* benefit by this "shape factor" description.

In the star-shaped colonies of the genus *Asterionella* the diatoms are ordinarily seen in girdle view under the microscope. While length and width dimensions are usually sufficient, sometimes the great difference in size between the two ends requires an added dimensional measurement at the middle of the valve. This may also be applicable in certain species of *Rhoicosphenia* as well. In some *Asterionella* the one end of the valve is inflated so greatly in comparison to the other that it is expressed as a percentage or fraction of the total length.

In discoid diatoms the circular forms (*Coscinodiscus* etc.) are measured for diameter, and where known the depth or pervalvar axis dimension should also be provided. There are particular species and even entire genera that are normally seen in the girdle view. Diatoms appearing thus are species of the genera *Melosira* and *Chaetoceros* for instance. Although in many cases the microscopic aspect seen appears as a square or rectangle, one of the dimensions is a diameter and is so expressed, and the other is the pervalvar axis dimension. The latter is often labeled as the "height" of the frustule by some authors, and is considered an important descriptive feature. The ratio of height to diameter is also provided in many extant descriptions. It is often appropriate to include a measurement of the height of the mantle (see Chapter 1) in diatoms of the genus *Melosira*.

Forms that normally appear in girdle view but that are not circular in valve cross-section (being elliptical or oval) such as *Hemiaulus*, are usually measured in the pervalvar axis only. If the cross-sectional form of the valve is a very flat oval then usually the "polar" axis length is measured as well, *Eucampia* being an example. In the genus *Auliscus* the valve view is almost always a good oval or ellipse and measurements are made for the "major" and "minor" axes of such forms.

Diatoms that are semicircular, or not of a symmetrical design and almost always seen in valve view such as *Hemidiscus*, and *Auricula*, are measured for length and breadth dimensions only.

Basically triangular shaped valves, as in *Triceratium* and *Lithmodesmium* are measured for length between the angles (length of a side) and for depth of the pervalvar axis. These diatoms are often seen in both girdle and valve view.

Some diatoms possess extensions such as spines that are very distinctive, as *Ditylum*. The diameter and pervalvar dimensions are measured along with the length of the spine in such cases.

In *Chaetoceros* with setae or awns, the length of the awns and in particular the spacing of any spines present along them is a desirable measurement. In this same genus the frustules are often seen in colonial chains. The open area between attached frustules (the "fenster" of Hustedt) (foramina of others) frequently appear in characteristic shapes, the dimensions of which (especially ratios) are of descriptive interest. The depth of the valve mantle, while not generally given in so-many micrometers, is frequently expressed as being "deep", or is compared with the depth of the girdle as "equal", "half as great", or as a percentage, thus requiring at least a measurement, although exact figures may not be appropriate in the description. As with other genera of diatoms having similar characteristics, the *Chaetoceros* are almost always seen in girdle view and have very little valve or girdle surface ornamentation, and therefore descriptions are heavily weighted (necessarily so) on dimensions and ratios of dimensions of the frustule and parts of frustules, and in the architecture of colonial arrangements.

The ordinarily very long (pervalvar axis) diatoms of the genus *Rhizosolenia* are measured much the same as *Chaetoceros*. Sometimes, however, the diameter and length (pervalvar axis) dimensions are supplemented by an additional pervalvar axis measurement of the valve. In some cases, the valve assumes a long conical shape as in *Rhizosolenia robusta* Norman ex Pritchard which may reach a length (height) of up to 100 micrometers.

In *Buddulphia*, the shape is often such as to require a dimension for the apical axis, breadth, and pervalvar axis.

7.2.2. Valve Features

Aside from the actual dimensions of the frustule and its major parts, there is a necessity to measure the extent, position or location, and number of various other features and markings on the diatom valve which are important diagnostically, taxonomically, or play an important part in identification.

The diatoms of the genus *Achnanthes*, among others are sometimes supported on a mucous stipe often of considerable length. When describing such diatoms the length of the stipe (if observed) is measured and included as a descriptive feature. This feature, of course, will normally only be observed with live or fresh untreated gatherings.

Hyalodiscus is provided with, by at least some authors (Van der Werff), three measurements of the valve view; an over-all diameter, a diameter of the umbilicus (central area), and a measurement of the breadth of the edge-zone. Other genera of diatoms sometimes requiring measurement of edge-area widths, either in micrometers or as whole number ratios to the total diameter include circular forms in *Coscinodiscus*, etc.

Some diatom genera such as *Roperia* and *Actinocyclus*, often have included in their descriptions, measured diameters of ocelli present on the valve surface, especially if their diameter exceeds 1 or 2 micrometers.

Pinnularia is a good example of the necessity for measurement of hyaline areas of the diatom valve surface. Although the shape of the area may vary somewhat in this particular genus it is often of specific import to know the maximum width of this zone in relationship to the total valve width. Although the actual measurement is not usually expressed as an integer, it is expressed as occupying a specific percentage or fraction of the width. A number of otherwise similar appearing species are identified, at least partly, by this factor.

7.2.2.1. Striae

As defined previously the striae are regular arrangements of puncta (sometimes areoli or alveoli) in straight or curved transverse or radiating lines. In pennate diatoms the striae along a line parallel to the apical axis is measured. However, instead of indicating the distance between each stria in micrometers, the number of striae in 10 micrometers is counted and the spacing expressed in that manner; that is, the number of striae included in a 10 micrometer unit of length. This unit (10 micrometers) is also used in expressing the spacing of costae and individual puncta.

Commonly the striae are counted (per 10 micrometers) in pennate forms along the margin of the valve near the center with supplementary measurements, if necessary, near the apices. However, difficulties arise when the central nodule or central hyaline area extends entirely to the edge of the valve, or the striae are considerably more widely spaced at that point. For instance, *Stauroneis* and *Caloneis* frequently are marked to create such problems. Also, if the measurement of the number of striae per 10µm at the center varies considerably from the number at the two ends of the valve, the number of striae at the center can hardly be called “typical” of the valve. A too low or too high count at the center if reported as the value will provide a false impression of the density of the striae on the described valve. A more realistic value of striae count in such cases would be obtained by making the count from a point near the center of the valve along the raphe towards the nearest end. Of course, if necessary, other supplementary measurements can be made to describe differences occurring in the center or at the extreme ends of the valve. In any event,

the measurement of the number of striae should represent a good average or typical value for the frustule, and the place of measurement on the valve should be appended to it in the written description.

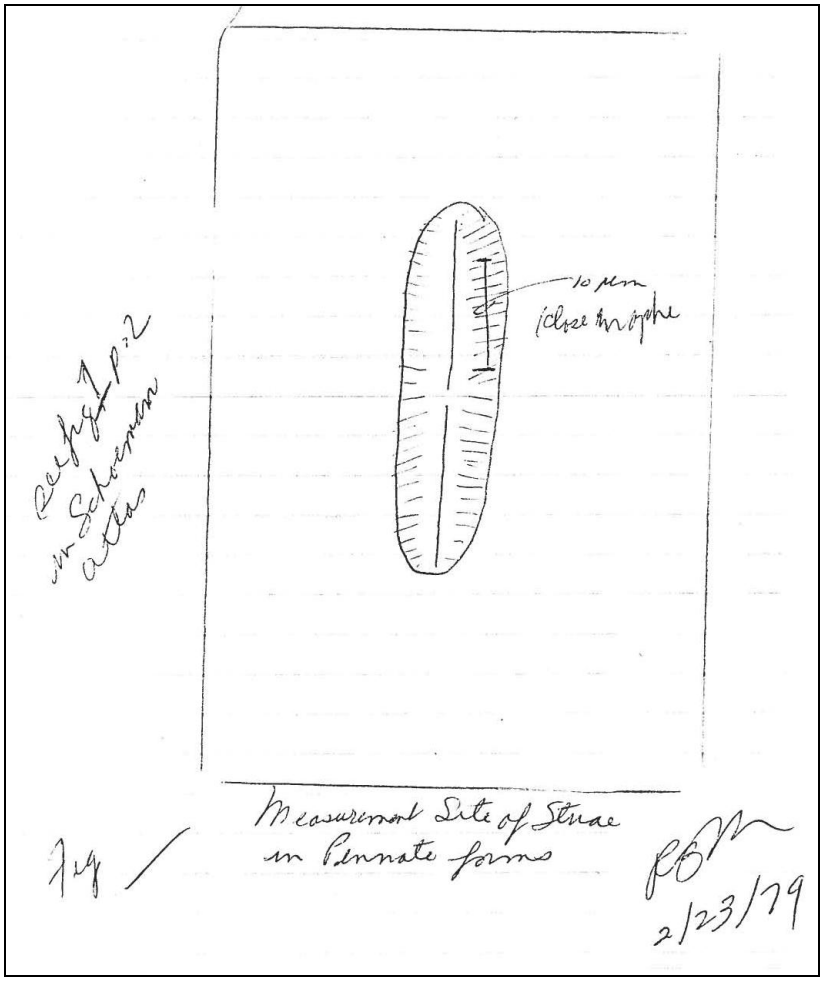


Figure 104.

In circular forms the striae are frequently in radiating lines from the valve center and/or in fasciculate bundles, typified in the genus *Actinocyclus*. Although in general, the actual count of striae is not as important as their arrangement and spatial relationships, counts that are expressed in such cases are for the number of striae at the mid-radial point of the valve surface, unless otherwise noted.

At the edge of such circular forms when present, striations are measured per 10 micrometers and noted in descriptions.

In the genus *Amphora* it may be appropriate to measure the striae on both the ventral and dorsal margins of the valve and express them separately. In some cases striation of *Cymbella* and *Tropidoneis* may require the same measurements.

7.2.2.2. Punctae

The punctae that make up the striae are also measured by counting the number included in a 10 micrometer interval of length. Over the surface of a valve the spacing of the puncta may vary, requiring separate counts for each differing area. In pennate forms the variation may be at the center, ends, or at all three locations.

In circular forms the punctae are counted and expressed along the line of the stria per 10 micrometers. As this number may vary radially, several measurements, or counts, are sometimes necessary. Sometimes there is a separately arranged grouping of punctae at the edge or in a narrow zone around the edge that must be measured and accounted for separately in the description.

7.2.2.3. Costae

Costae are also measured (counted) by the number included in 10 micrometers. Where both costae and striae are combined on the valve, measurements for both are in order. Species of *Denticula* for instance require description in this way. In fact, in this particular genus costae may extended completely across the valve or long and short (some extending across the valve and some not). Separate counts included in 10 micrometers are given in that case. The measurement of many of these types of features involves the measurement of the distance between individual costa for instance (as they are spaced quite widely) and the conversion made to the number that would be included in a 10 micrometer interval. Counts of costae and punctae are sometimes combined in the genus *Gomphoneis*. In *Epithemia* and *Rhopalodia*, wherein the striae are composed of alveoli, the number of alveoli in 10 micrometers and the number of rows between costae are included in the description.

7.2.2.4. Alveoli

The genus *Pinnularia* most typically exhibits the channel-like structures designated alveoli. The alveoli are measured by counting the number in 10 micrometers as with the striae. The width of alveoli in some large forms is of sufficient size to be measured and noted in the written description. Often, in this genus at any rate, the bottom openings in the alveoli, in concert with others, create the impression of a set of double lines near the edge of the valve. Measurement of the separation of these "lines" is sometimes necessary to describe the percentage width they occupy of the total valve width, thus providing another specific diagnostic feature.

7.2.3. Ratios and Volumes

7.2.3.1. Frustules and Valves

While most measurements on diatom valves and/or frustules are made for description or identification purposes, they do at other times serve other purposes. The measured linear dimensions of frustules (length, width and depth) are used in estimating and/or computing frustular volume, or in expressing length to width ratios. Volume figures are useable in many different ways in biological investigations, including photosynthesis and other life processes. Surface area to volume considerations are important in assessing photosynthetic efficiencies, and in questions involving flotation of pelagic forms. Length to width ratios can be used as part of a "form-factor" in studies related to various ecological conditions and geographical distributions.

7.2.4. Velocity

The rapidity with which some diatoms move is studied in connection with light, temperature, and other variables. Distance covered per unit time is velocity. The distance traveled by a moving diatom is easily measured with means formerly discussed. The timing of the movement may be done by means of a stop watch. Alternatively, for long periods of observation, the use of a camera lucida can be very convenient. The Camera lucida is adjusted so that a sweep-second handed watch is visible in the field of observation. Continuous observation of specimen material simultaneously with an elapsed time indication to the nearest second is thus available.

7.3 Counting

7.3.1. Introduction

In many investigations relating to ecological, or geological and geographical questions, it is necessary to count diatoms under the microscope. The number counted being representative of a "population". Although it is certainly beyond the scope and intent of this book to delineate specific methods for any one scientific discipline or area of investigation, there are some basic principles in counting diatoms that will be interesting and informative to the beginning student.

Counting is that process whereby a one-to-one correspondence between a number system and items to be counted is accomplished; in other words, to indicate or name, by units or groups, the total number of units involved.

Since the end result of counting, to find a total number of units, is usually, in microscopical work, only one step in the determination of other information, it is important that the counting be as accurate as possible, and done rapidly to conserve time.

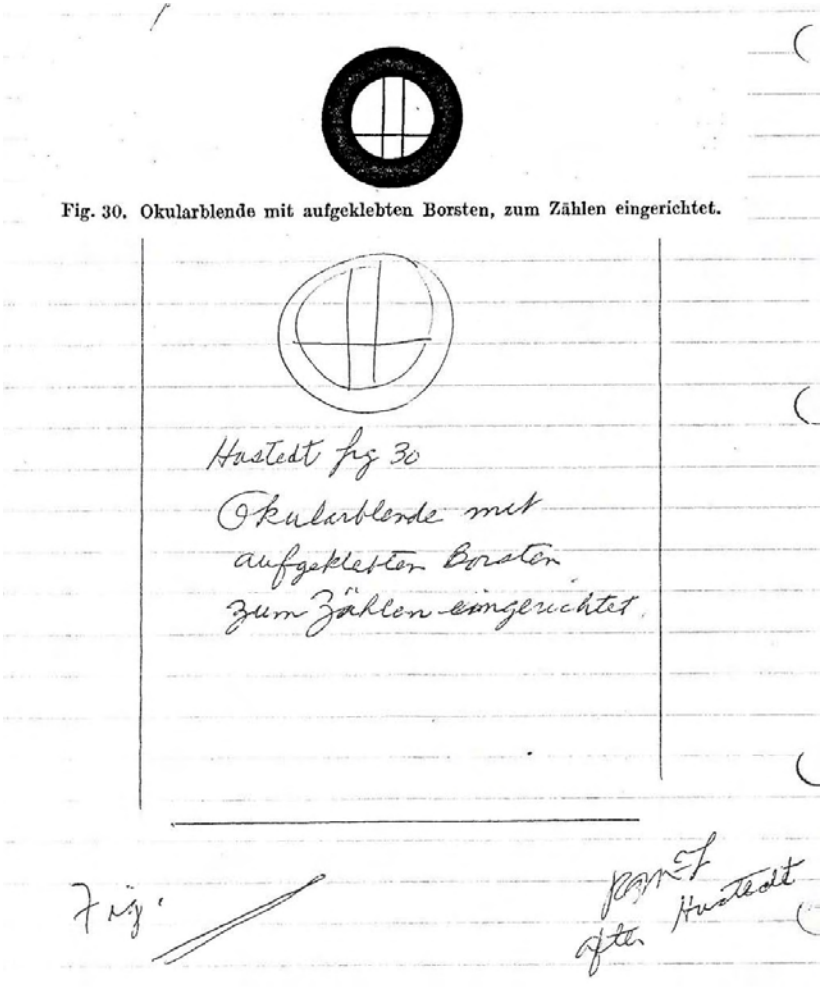
7.3.2. Aids to Counting

7.3.2.1. Counting Plates

Specially marked microslides and/or coverglasses can be of considerable assistance to minimize mistakes such as double-counting or omission of specimens. For instance Kolbe recommends coverglasses to be marked with a system of scratched parallel lines at a distance from each other corresponding to the field of view of the optical system used. The lines can be marked using a “diamond pencil” or carbide-tipped marker. Hustedt also mentions using a system of fine lines marked perpendicular to an edge of a microslide (or coverglass). However, he notes that these types of plates have the disadvantage that different plates must be provided for different magnifications used with the microscope.

7.3.2.2. Reticles

There are many reticle patterns devised for general and specific uses that are designed to reduce the confusion factor in counting large numbers of objects in a microscopic field. The cross-line, squared-grid, and other simple geometric patterns are suitable for diatom work. They ordinarily divide the field into areas which are limited by the pattern lines, thus reducing the count per area, each area acting as a memory to store already counted units. The reticle is placed in the microscope ocular in the same manner as an eyepiece micrometer and appears superimposed on the field of view.



Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 30)

Figure 105.

Hustedt, in order to avoid the inconvenience of the marked plates described previously, recommends a simply constructed counting ocular. Two spaced bristles (or hairs) are fastened from front to back (in the y-direction) and another is fastened perpendicular to them, on the eyepiece diaphragm (refer to Figure 105). Simple hairs or bristles can be so fastened with the aid of Duco cement or a similar adhesive. (The eye lens of a Huygenian ocular is unscrewed and the hairs fastened directly to the diaphragm.) The hairs, just as the lines on any reticle placed at that point, appear superimposed on the field of view. Of course for a given condition, the objective magnification must remain the same. Otherwise, the areas encompassed by the "lines" will be entirely different. Such an arrangement can be calibrated using a stage micrometer.

7.3.2.3. Counting Chambers

In making volumetric analyses, and to provide assurance that the same quantity of sample material is taken for each count, counting chambers are sometimes used. All counting chambers have one attribute in common; that of a chamber of known depth, and sometimes of specific volume. With a known depth between the bottom of a reservoir to be filled with the diatom suspension, and the underside of an applied coverglass, volumes are easily measured.

A useful type for diatom work is the Sedgewick-Rafter Counting Chamber. It is designed primarily for the microscopical examination of water, but also may be used for dust examination as it can be hermetically sealed. It consists essentially of a glass slide 33 mm. x 70 mm. with a 20 mm. x 50 mm. chamber 1.0 mm. deep. All glass construction includes cover supports inlaid in the base. A coverglass 0.5 mm. thick, optically plane on both sides is ordinarily used. A Whipple ocular micrometer disc, consisting of a network pattern of a large square 7.0 mm. on a side subdivided into four smaller ones, each of which is further subdivided into 25 small squares, is quite often used with the Sedgewick-Rafter Chamber.

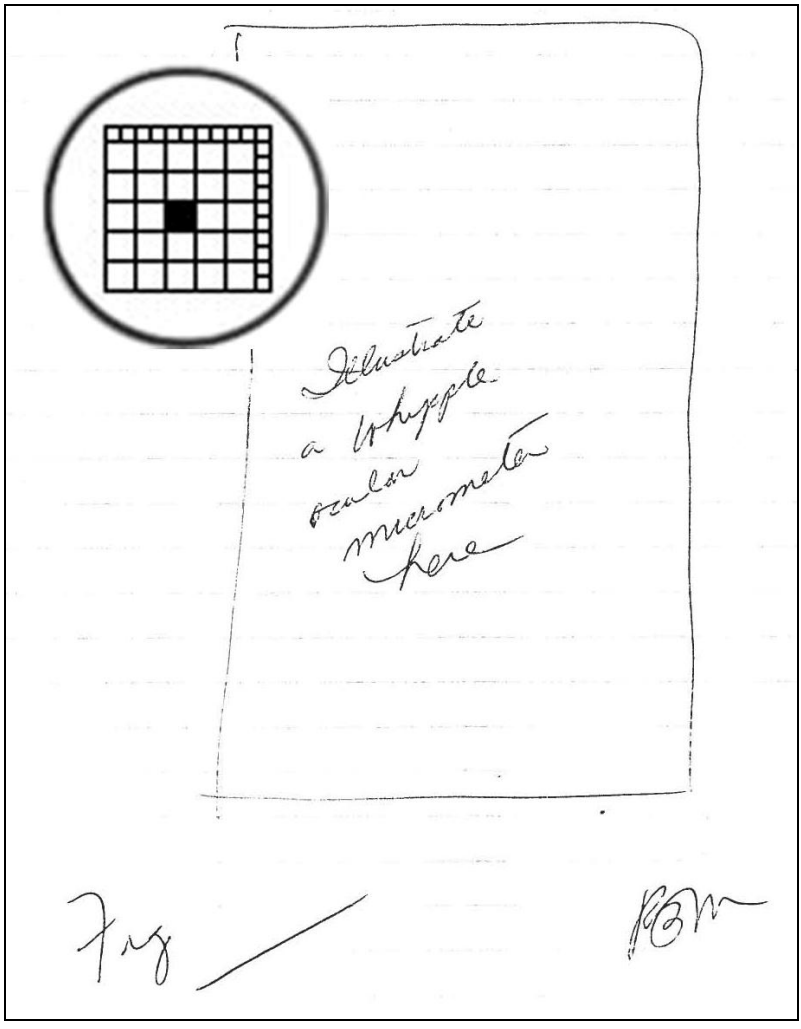
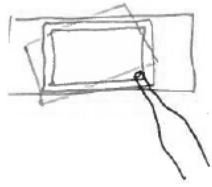


Figure 106.

Other counting chambers such as the Howard (Mold Counting), or even blood counting chambers, may be used for diatom work.

A counting chamber, if its exact depth is to be exploited to the maximum, must be completely full of fluid. To assure that condition, it should be filled to just overflowing before applying the coverglass - allowing a piece of blotting paper to soak up the excess.

In the 'Standard' method for water analysis the cover is laid on the chamber askew and the pipette introduced at the corner and the cover then rotated into its square position.



If the sample being examined is live plankton it is advisable, in order to avoid movement during the count, to apply a killing and/or fixing agent. This can be

accomplished by shifting the coverglass (after the chamber has been charged) to expose the sample in solution and add a drop of fixative. To hasten the killing apply a drop on the other side after which the cover is shifted back into place.

The kind of count made depends on the number in the sample and the type of individuals present. Forms that occur frequently can be counted using only a portion of the chamber, delineated in some way. With the rarer diatom forms it is usually necessary to count through the complete chamber. With very dense concentrations of plankton, it should be diluted in a known proportion prior to charging the chamber to provide more dispersal of specimens. The latter makes species recognition and counting easier of accomplishment.

The Sedgewick-Rafter cell is used in simple water studies to count the number of diatoms present. For the enumeration of plankton diatoms for instance, the following procedure is typical.

(1) Collect water sample from a designated location and depth.
(2) Preserve with a formalin solution.
(3) Concentrate by means of a centrifuge.
(4) Place 1 ml. of the concentrate in a Sedgewick-Rafter cell.
(5) Using a Whipple ocular micrometer, count the diatoms.
(6) With the microscope calibrated for 100X (10X objective and 10X ocular) the field of view, as delimited by the ocular can be adjusted to cover .001 ml. of the concentrate.
(7) The diatoms appearing in ten fields are counted, and from their total, the number per ml., liter or gal. of the unconcentrated sample can be calculated. Quantitative records, for genera and species can be kept, the enumeration being in real standard units or cubic standard units.

With the Sedgwick-Rafter Chamber the maximum workable objective is X20.

Wherein diatom identification must be made during counting, especially at the specific level, the various counting chambers are often inadequate. The furnished coverglass may be quite thick to resist deformation. It may be, in some instances, so thick that the working distance of certain objectives is insufficient to obtain proper focus. Sometimes the floor of the counting chamber is too thick, preventing proper substage condenser adjustment and critical illumination cannot be provided. Counting procedures that are coupled with diatoms difficult of recognition specifically, as is especially true in the very small freshwater forms, is best conducted using standard microslides and coverglasses of selected thickness such that critical illumination and adjustment of the microscope can be accomplished.

7.3.2.4. Ordinary Microslides

Ordinary microslides and conventional coverglasses of a size suited to the investigation may be used in volumetric studies. The major requirement is that a specific known volume is applied so that the count relates to it in some selected

proportion. In most cases a known volume of the fluid suspension is applied, allowed to dry, and a permanent preparation made in the usual manner, taking advantage of a high refractive index mounting medium.

Material centrifuged from a known volume of fluid may need to be further diluted to a thinner constituency in order to facilitate counting. The dilution is made in a predetermined proportion, and then applied in definite amounts with a measuring pipette to one or more (as the case may be) coverglasses. The count on each coverglass is related to its particular proportion of the original volume. It is necessary, for the sake of accuracy, that the pipette used is absolutely free of any residue.

In counting diatoms in marine plankton the required dilution may be greater than with freshwater material. Many *Chaetoceros* for instance, form very thickly constituted chains, making accurate counting difficult, especially if they become matted. Also, the drying of marine material is not always to be recommended, as many species are lightly silicified and change their form under such conditions and become unrecognizable. In any event, it is advisable to determine beforehand the species present in the material to be counted. In that way, during the count there will be little necessity to apply the term "species" in substitution for a specific epithet.

To provide precise amounts of specimen material under the coverglass, that can later be referred to a standard volume, very accurate pipettes are available commercially. Patrick describes a special apparatus for drawing off such exacting and small amounts (a few tenths of a cc. are the usual accommodation under most coverglasses). It consists in part of a column of mercury which can be raised and lowered in a tube. This tube is graduated to a tenth of a cc. By means of a rubber tube it is connected to a disposable pipette. The pipettes are carefully cleaned to assure that they will take up and deliver the same amount. By raising and lowering the column of mercury the same amount is taken up and delivered to each coverglass as a series is prepared.

It is frequently of interest to determine the number of diatoms in a specific weight of a dry sample. The following (after Kolbe) is a simple but effective method for marine sediments.

(1) About 250 grams of air dried sediment, or other dry sample, is treated in hydrochloric acid and decanted.
(2) Distilled water is added to the suspension until a suitable volume (according to the quantity of diatom valves) is reached, usually 50, 100, or 200cc.
(3) Stir the contents vigorously, and while still in motion, extract 0.4 ml. of the suspension using a pipette.
(4) Immediately transfer to a cover glass.
(5) Wash the pipette in distilled water, and spread the washing water onto another coverglass.
(6) After drying at room temperature, mount in the usual way in a high-index mountant.

(7) The total of the valves counted on both coverglasses is finally brought into relation to 1 gram of the dry sample by multiplication with the dilution coefficient.

Hustedt uses a slightly different method. The material is allowed to dry completely and then is incinerated to destroy all other material possible without harming the diatoms. He does not recommend treatment with acids as "weak ones will not completely destroy the foreign matter, and strong ones may attack the more delicate diatom forms".

The dry weight of the silicates remaining is then determined. The incinerated mass is then saturated with alcohol and boiled in water. It is then placed in a very narrow measuring graduate and the volume read off.

It is difficult to determine the volume of diatoms in relation to other plankton, as it depends for its accuracy upon the volume and weight of the entire plankton present. In that case incineration would not be used and the entire mass must be allowed to completely dry. If the latter is not done then the results are of very doubtful worth.

If comparisons of such volumes (diatoms to other plankton) from sample to sample, or locale to locale, are to be made, the difficulties are even further amplified. Many diatoms, especially those provided with long processes (awns etc.) sometimes will not settle completely even after months of standing, but form a light flocky mass. Volume determinations under those conditions are completely illusory and comparison-wise cannot be used unless the same species (not genera) are known to be present in the samples being compared.

Determination of the number of individuals (well preserved cells, species, etc.) in a given volume of water is a straightforward process. It can be accomplished by the use of special counting plates, ordinary microslides, or counting chambers. The microscope of course, should be provided with a suitable mechanical stage in any case.

7.3.2.5. Counting by Fields

The counting of diatoms is ordinarily performed by "fields", usually coincident with the field of view at the microscope ocular. As the ocular field of view is circular, it is often advantageous to divide it into square or rectangular areas by special coverglass patterns (as provided by some counting chambers)or by reticles in the ocular itself.

7.3.2.5.1. Counting Plans

The method of counting diatoms within a specific area depends to some extent upon the shape of the area to be covered and the density of the countable diatoms in the strewn preparation.

The area can be covered in a systematic way, assuming the diatoms to be counted are dispersed in a random fashion. Systematic search of circular, square, or

rectangular areas may be performed in any one or a number of configurations. If the available countable elements are very plentiful (many times the required count) the search need not be over the entire area, but only needs to be composed of a sufficient number of fields to yield the maximum count required.

If this latter condition holds, then the danger of double-counting can be reduced drastically and any clumped (non-random dispersal) areas can be avoided. Despite all precautions to the contrary it is difficult to get an entirely random distribution of diatoms over the entire strewn area of the coverglass. The actual mechanics of preparation often result in some localized non-random and aggregated portions of the "population" in certain areas under the coverglass. For circular covers these conditions often exist at the edges. In squares and rectangles, the corners and edges are points at which the distribution is frequently non-random. These areas should be avoided to assure a more representative count within the population.

For circular coverglasses the center can be temporarily marked with a dot of india ink. This is located under low power and the stage coordinates noted. Draw a circle, denoting the coverglass, on a piece of paper and by knowledge of its diameter and the located center in terms of stage coordinates, lay out a scheme of counting fields.

Square or rectangular areas can be similarly laid out to assure a representative sampling during the counting process. As long as the total available countable elements greatly exceeds the number to be counted, sufficient spacing of the counting fields to preclude double-counting is simple and they can be widely spaced over the area for that reason.

When the density of elements to be counted is such that their number may be only somewhat greater than the total count desired, a nearly-complete coverage search will be necessary. This circumstance will require care in not allowing ocular fields of view to overlap in either the x or y directions.

Knowledge of the diameter of field of view (in millimeters) provides information for setting up the field spacings. For instance, the field of view of a typical 12.5X Huygenian ocular used with a 40X objective is 0.31 mm. This dimension (for spacing) is of sufficient magnitude as to be easily set up on a graduated mechanical stage as, almost without exception, such scales can be read to the nearest 0.1 mm. The field of view diameter is given by manufacturers for various combinations of oculars and objectives, or they supply a "field of view number" which is used to calculate the field of view diameter with a given objective magnification. This number, or index, is a measure for a given ocular that is indicative of how much of the intermediate image, which is limited by the rim of the ocular tube, can in fact be observed. The effective diameter, in millimeters, of the intermediate image is expressed by this number or index. With binocular observation the interpupillary distance of the observers eyes limits the diameter to about 50 mm. The field of view diameter can also be determined by viewing a stage micrometer with the optics that will be used in the counting process.

In accordance with the above, a plan of fields is laid out that in number will accommodate the desired count with minimum danger of overlapping and avoidance of clumped areas. Preliminary examination of the diatom slide preparation will .assist in determining the proper layout. The center of each field is noted by

mechanical stage coordinates, expressing the X-direction first, a comma, and then the Y-direction, to the nearest tenth millimeter (as 67.3, 15.4).

7.3.2.5.2. Conventions

Because diatoms to be counted must ordinarily be identified as well, and because their physical condition may be quite variable, certain conventions are assumed before the counting or census begins. For instance, not all diatoms will be in complete frustules, many being in separate valves. Also, there will be fragments of valves/or only partial views of valves at the edge of the field. Some diatoms will not be in valve view, perhaps being in girdle view, making identification difficult, or at such an orientation as to make positive identification impossible.

In addition, the danger of "double-counting" or counting the same diatom twice from field to field exists, especially where the fields are closely spaced. Also, when a square-net ocular is used to reduce confusion and assist in counting large numbers per field, additional precautions must be taken. What to count relative to the vertical and horizontal lines of the reticle as well as the circular edge of the field, is important to the ultimate accuracy.

A consistent approach is to adopt the convention which might include counting only diatoms that are wholly within the field, and none which touch or are obscured by the field edge. If the diatoms are in a recognizable "whole" form, then one should include the precaution to count only "whole" diatoms and those that are at least half size or larger, disregarding smaller fragments. If this precaution is not taken, error will be introduced by including in the count two halves, or many particles of the same diatom. There are exceptions to the rule of counting only half-size or larger fragments of diatom frustules or valves. In some marine sediment investigations for instance, diatoms of great length-to-width ratios are often encountered. *Thalassiothrix antarctica, Thalassionema nitzscschoides*, and *Rhizosolenia* spp. are examples. In such sediments it is unlikely that more than one half of the valve of such lengthy species would be preserved. In those cases it may be appropriate to count only the end fragments.

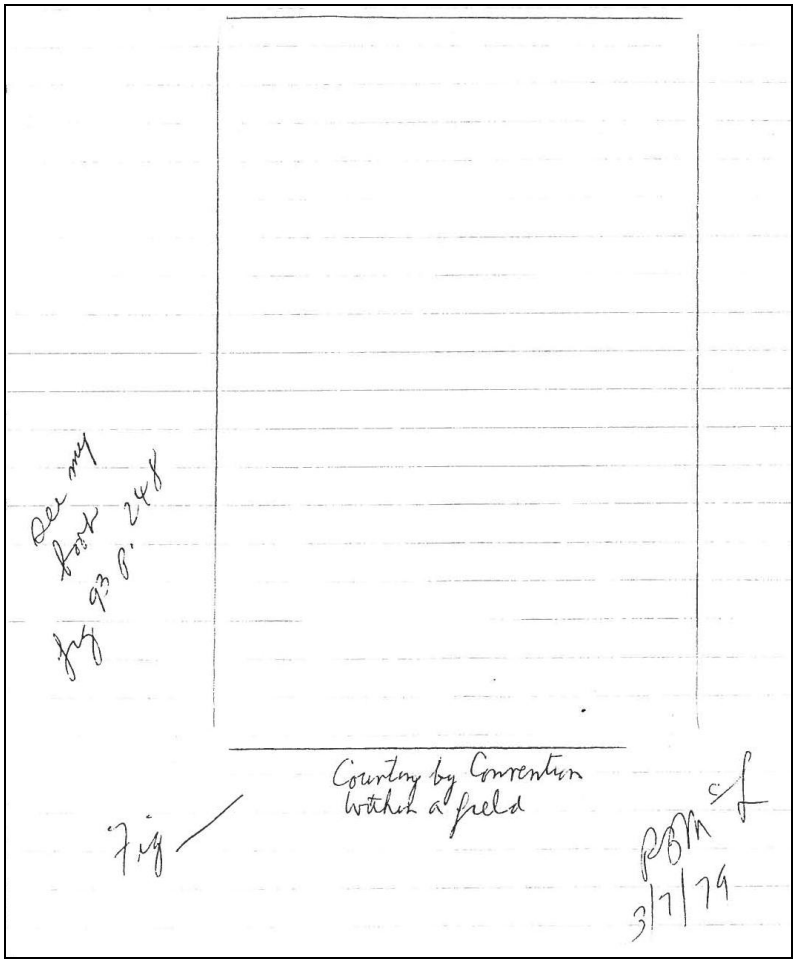


Figure 107.

When the reticle is in place, in order to protect against double counting from one subdivision of it to another, a convention is necessary. For instance, count only diatoms which are within the subdivisions and which touch only the left-side vertical and lower horizontal. Diatoms touching the right-side vertical and upper horizontal are not counted. Refer to Figure 107.

Since diatoms are very often dispersed in a field in separated valve form as well as in complete frustules, it would be very misleading to count both a valve and a frustule as one individual. To provide that the count (for a given species) in a community would be represented by a certain number of valves, or if that number is divided by 2, then so many cells (or frustules), adopt the following convention. Count each valve as a value of 1 and each frustule as a value of 2, not counting intercalary bands or detached girdles.

When difficulties might be experienced in recognizing certain specimens in girdle view, disregard them in the count until working through the preparation (strew slide)

and compiling a species list. Then, from the knowledge of the species present, the girdle views can be more easily identified and counted.

When the count is obtained for each species, as above, and is expressed as a percentage, it represents the relative frequency of occurrence, whether the count obtained represents valves or frustules.

7.3.3. Tabulating the Count

There are many different formats of tabulating counting data, and they will vary, of course, with the intended use of the count. Preformed tables, ruled and divided according to the results desired, should be prepared beforehand. Adaption of business and accounting forms for the purpose is excellent practice, as numerous combinations of columns, sizes and widths are in plentiful supply. Also, the paper is sturdy and will take many erasures without it surface being damaged. Pencil entries are advisable.

It is not possible to prescribe all of the many different layouts for data recording, or to furnish a single form useable in all situations. However, it is of interest to describe a format most commonly used. It is suitable for making a diatom association analysis, a relative frequency of occurrence, or a floral survey. Reference is made to Figure 108.

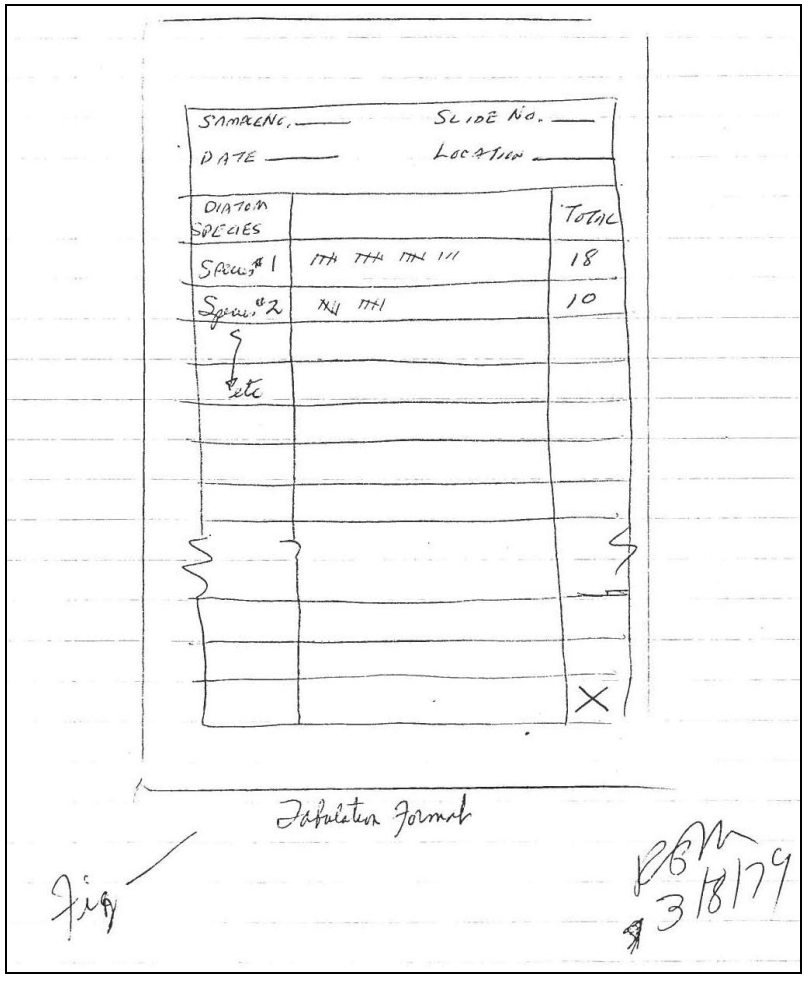


Figure 108.

As the diatoms are identified they are listed in the species column and each occurrence is tallied. At the right a total number of each species is recorded, and at the bottom right the total number of diatoms identified is obtained. The relative percentage of occurrence for each species is then easily obtained using these totals.

7.3.4. Magnitude of the Count

How many diatoms to count and how large a sample must be used is often dependent upon the purpose of the investigation. In order to determine the true species proportions that exist in a statistical population, a large sample size must be used.

If rare occurrences are important, then an even larger sample size is in order so as to ensure their appearance during the counting process. A smaller sample will suffice if only the major constituents are of interest. Just what range of count are we ordinarily

to encounter in this type of sampling? Certainly no less than 200 or 300 counts should be made in any case. If the sample is less than this the importance statistically is very diminished and the accuracy of assessment of a population will be low. A statistical analysis of a diatom population, to be accurate within 1 or 2%, should be based on a count of not less than 400. In recording the presence, and for computing the relative abundance of infrequently occurring forms, a count as high as 1400 to 1500 may be necessary. A cumulative frequency distribution study of the sample will aid in determining the magnitude required. In the study of the population of streams and rivers counts as high as 8000 may be necessary.

When a diatom association analysis is made, species of 1% or less of occurrence are lumped together as "other species", since it has been found (Cholnoky 1968) that relative densities of less than 1% fall into the method range of error. These statistically light densities of certain forms also may be due to the fact that they have been washed in from elsewhere, or otherwise transported into the sample area or location, and are therefore unreliable for any ecological conclusions.

The accuracy of the count may also be affected by the microscope magnification used. If all of the forms to be identified and counted are large a magnification of 100X may be sufficient. However, it is usually found that a magnification 100X results in values (counts) of less than actual because of the usual smaller forms being missed. This is especially true in making counts with a Sedgwick-Rafter Counting Cell. This latter situation would obtain when an enumeration study of diatoms in a water supply were being made, for instance.

In studies involving the identification of species, high-dry or oil immersion objectives, providing microscope magnifications in the order of 400X to 1500X are required.

The relative frequency of occurrence of diatoms in a given population or set of populations, is dependent upon the counting of whole frustules and valves and those at least half size or greater. In some instances in examination of fossil deposits most of the diatoms are so fragmented it is not even possible to count half sizes of frustules or valves. In that case an approximation of diatom abundance at various depths or locations within the area being examined can be made by counting fragments. The number of fragments (of diatoms) are counted in 10 or 20 fields per site and the results expressed semi-quantitatively on a conventionalized basis. For instance, the terms Abundant (A), Common (C), Few (F) and Rare (R) are assigned to counts of greater than 500 fragments per 20 fields of view at 1000X (A), between 60-300 fragments per 20 fields of view at 1000X (C), between 10-60 fragments per 20 fields of view at 1000X (F), and between 0-10 fragments per 20 fields of view at 1000X (R) respectively.

Of course some diatoms are much larger than others and one larger can produce many more fragments of the same size as a smaller one. Also, there can be variations within even one strew. However, the choice of exponentially increasing ranges for each term (A, C, F, & R) does counter these difficulties. Although the method is limited, a real situation is thus described as accurately as possible under the circumstances.

The number of diatoms to be counted (300, 400, 1000, etc.) sets the accuracy as to how well the population is represented. If the sample under investigation possesses a high density of diatoms, few traverses or fields are necessary to attain the predetermined count, and if the diatoms are very sparsely distributed in the sample, a great number of traverses or fields are required . Therefore, notation of the number of traverses or fields required to attain a given count will indicate the relative diatom abundance from sample to sample.

7.3.5. Presentation of the Count

7.3.5.1. Populations

The presentation of the comparable results of counting is often best expressed in graphical form. Curves, formed by connecting directly plotted points representing the magnitude of the count are, of course, linear. However, because of the very wide range of the comparable numbers in usual counts, an extremely large format may be necessary to represent the largest numbers. In turn, the smaller numbers, perhaps only a very small fraction of the larger ones, are difficult to plot on even that very large scale. The actual layout, and perhaps future printing or reproduction of such representations, therefore becomes very troublesome.

Scourfield suggested using logarithmic-lined paper, instead of the usual millimeter paper. The resulting curves do become considerably compressed in this way but are still less clearly represented than is desirable, and so the method has not been widely adopted.

David Joseph Scourfield
b. 20th October 1866
d. 3rd October 1949

Lohman employed so-called sphere-curves. He reasoned that the individual numbers themselves can be related graphically to a body or “population” represented by a three dimensional geometric figure. A three-dimensional figure that can be described by a simple line is either a cube or sphere. The sphere has the quality of containing the greatest volume for a given linear dimension, and the spatial concept of it, represented by a given line, is easier, and it therefore is preferred over the cube.

The abscissa is the "x" coordinate of a point, shown on the horizontal line, with the ordinate, also known as the "y" coordinate, shown on the vertical line. The point (5,8) has 5 as its abscissa and 8 as its ordinate

In the use of such a concept the abscissa of the plotting format serves as the equatorial plane for all spheres, and the radii are introduced as the ordinates.

The volume of a sphere is

$$V = \frac{4}{3}\pi r^3$$

where

v is volume .

r is radius of the sphere

From the above the factor $^4/_3$ pi can be reduced to the number 4.19 (approximately) and the radius of a sphere with a given volume v is then

$$n = \sqrt[3]{\frac{v}{4.19}} or \sqrt[3]{\frac{v}{4}}$$

The number of diatoms counted is related to the "volume" of the "universe" or population. Of course, the number used may be the actual count, but more than likely will be an extrapolated number that represents a given volume of material such as a cubic centimeter, liter, etc.

A convenient unit to use for the radius is the millimeter. If a unit greater or smaller than this is used it must be considered in any calculations made, of course. The number 4.19 can be rounded off to 4 as the added fraction affects the results to a very minor degree. Because of the large numbers involved, computation by logarithms is advisable to reduce errors.

Calculators, either hand-held or on home computers, are more than capable of handling these calculations.

As an example; if the count (or rather the extrapolated number representing the universe) is 1348756 then the radius is

$$\sqrt[3]{\frac{1348756}{4}} = 69.6$$

Using the millimeter as a unit the length of the ordinate is then 69.6 mm., or if the unit is 0.25 mm. the ordinate is 17.4 mm., etc.

On the other hand if the radius is 7.5 mm. the represented population is

7.5^3 x 4 = 1688 (if the unit is the millimeter)

or

$(7.5 \times 4)^3$ x 4 = 108,000, if the unit is 0.25 mm.

Thus it can be seen that through the use of sphere curves one to nine digit numbers are reduced to one to three digits, making the graphical portrayal of them much more compact.

It is not necessary to portray the radii on both sides of the abscissa axis, thus resulting in a diameter, although the illustration can be perhaps made more instructive in that way, an especially striking effect is produced by filling in the areas formed by the sphere-peaks with india ink. (Reference is made to Figure 109).

The axis a-a is the equatorial plane of the spheres, r-r the radii of the different spheres expressing the individual universes or populations.

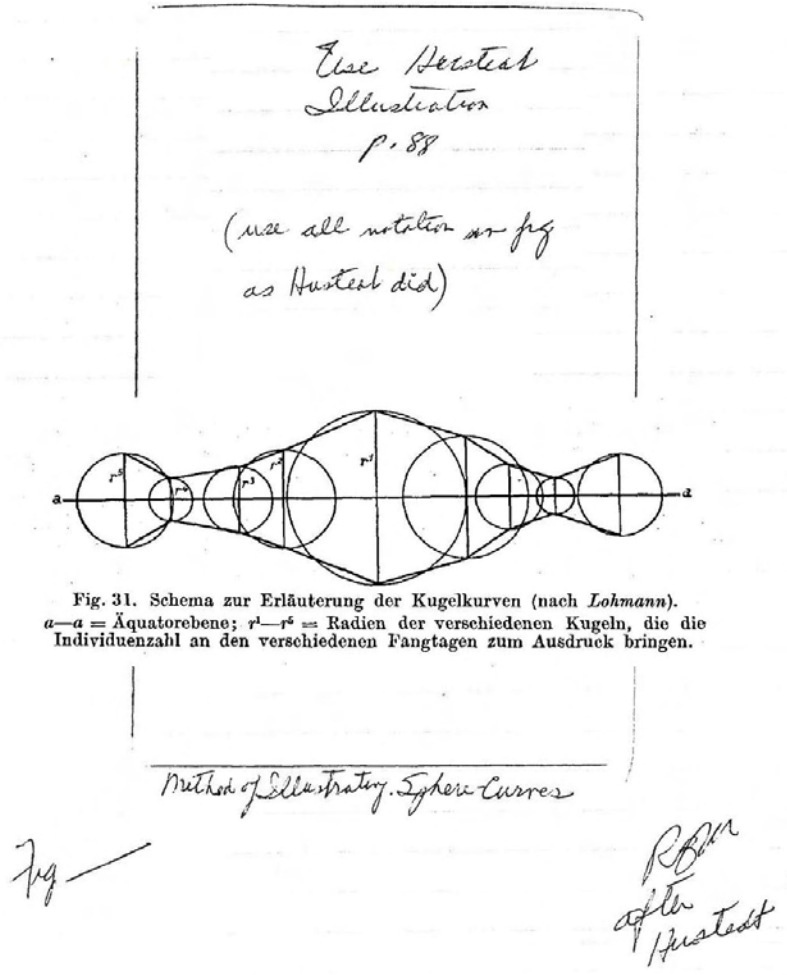


Fig. 31. Schema zur Erläuterung der Kugelkurven (nach *Lohmann*). *a—a* = Äquatorebene; r^1—r^6 = Radien der verschiedenen Kugeln, die die Individuenzahl an den verschiedenen Fangtagen zum Ausdruck bringen.

Friedrich Hustedt: *Vom Sammeln und präparien der Kieselalgen sowie angaben uber Untersuchungs und Kulturmethoden-aberholden* (Fig. 30)

Figure 109.

7.3.5.2. Relative Frequency

Counting diatoms and recording the numbers counted can result in some very useful displays. Each data sheet may represent the counts at different specimen locations, at

specific collecting times, or at the same location during different seasons of the year, or some other geographic or time related ecological condition.

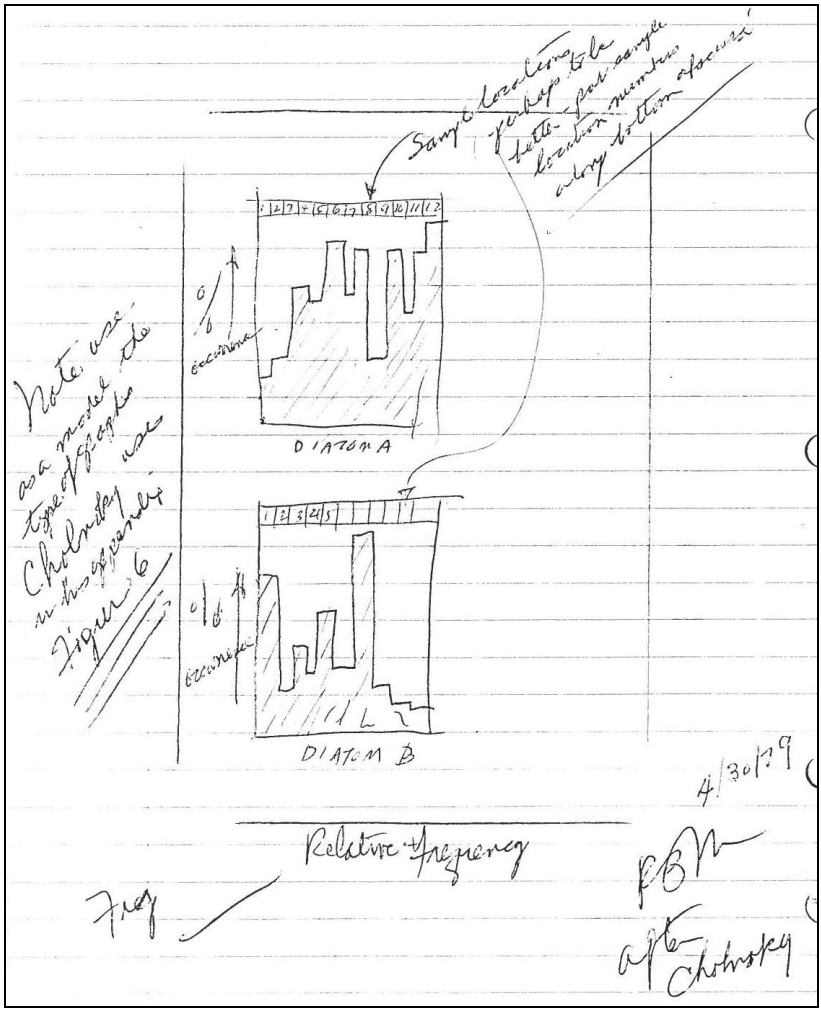
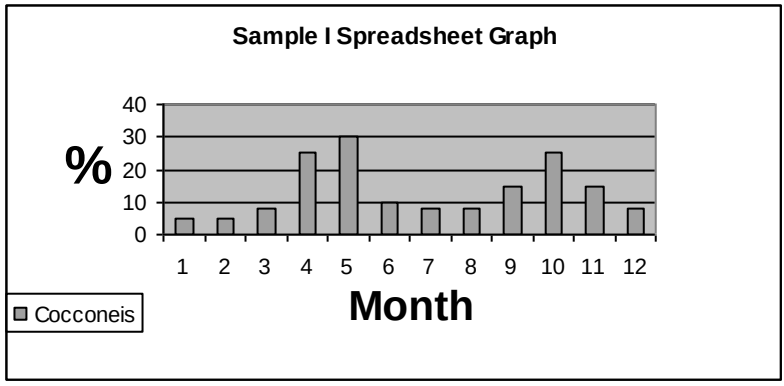
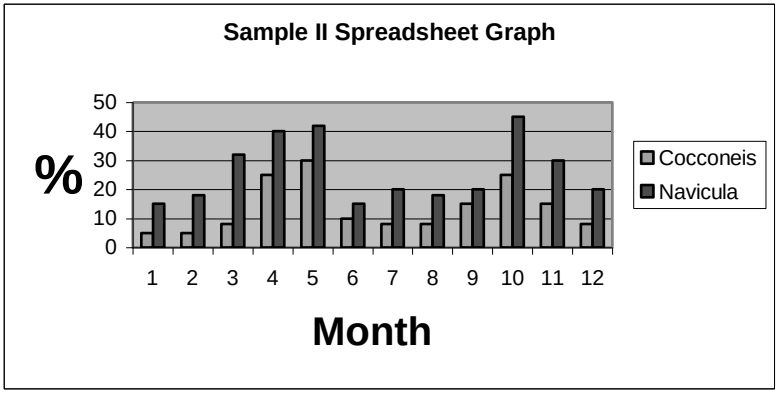


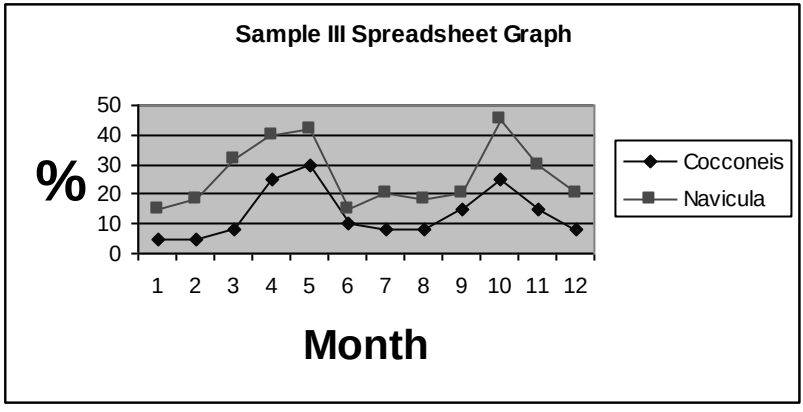
Figure 110.



Sample Graph I



Sample Graph II



Sample Graph III

The relative frequency of a particular diatom or several diatoms at a number of locations along a river might be of interest. A good way to display this type of

information is in the form of a bar graph. The x-axis interval could well represent the different specimen locations and the ordinates the percentage of a particular diatom's occurrence. Referring to Figure 110 an example is shown of a plot of the percentage relative frequency of two specific diatoms at a number of collecting sites, represented by numbers along the-axis.

Most spreadsheet computer programs will produce bar graphs and variations simply, easily and in colour.

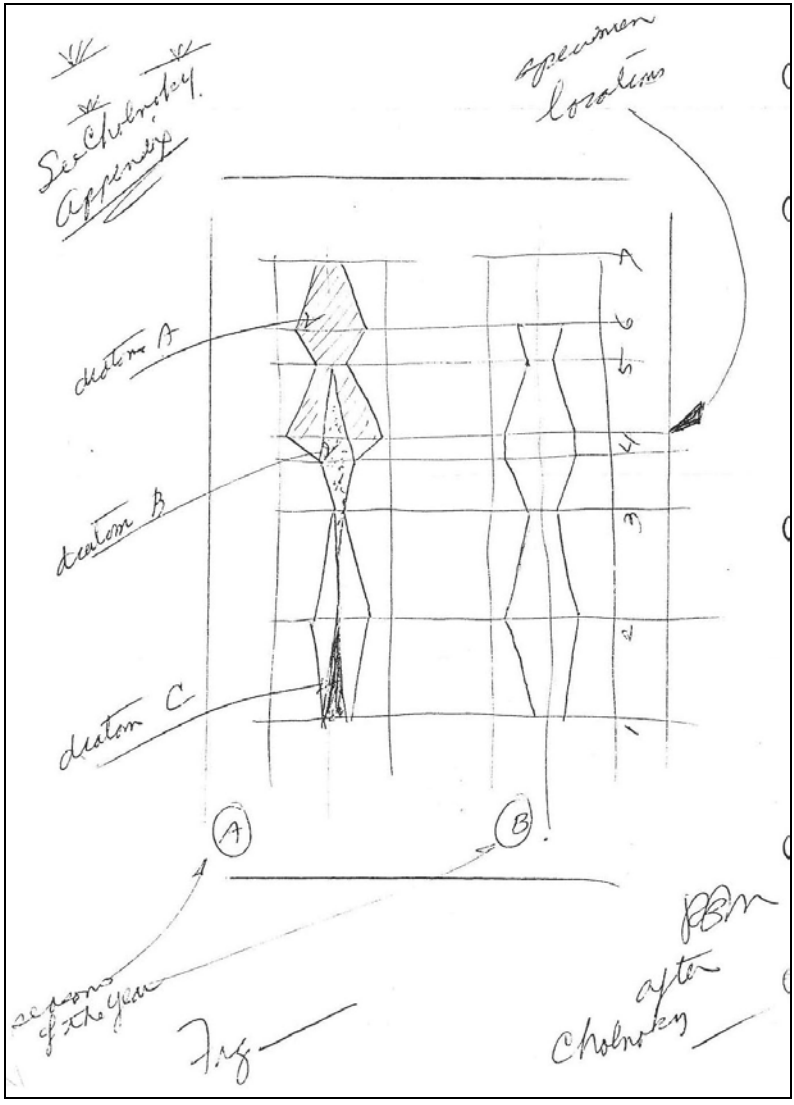


Figure 111.

Cholnoky has illustrated the relative frequency of three different diatoms at a number of locations during two different seasons in the manner illustrated in Figure 111.

The various diatoms under consideration are represented in this case by cross-hatching (diatom A), dots (diatom B), and solid black (diatom C). The width of the figure at each collecting point is scaled to the percentage frequency of occurrence. The seasons are separate plots. This arrangement indicates considerable data and allows a side-by-side seasonal comparison of diatom frequency at specific locations. A similar type of plot might compare the occurrence of a single diatom species with all others (in the same sample) or the same or similar habitat preferences, exemplified by pH etc. The differentiation would in a like manner be indicated by means of different graphical area-symbolization in the plotted figure.

Analysis of diatom populations on a statistical basis is a common research tool. It is beyond the scope of this brief treatment of diatom study to go into detail on statistical analyses. However, statistical studies may be carried out with basic and simple tools and means.

End of Part III

APPENDIX A
Robert B. McLaughlin Bibliography

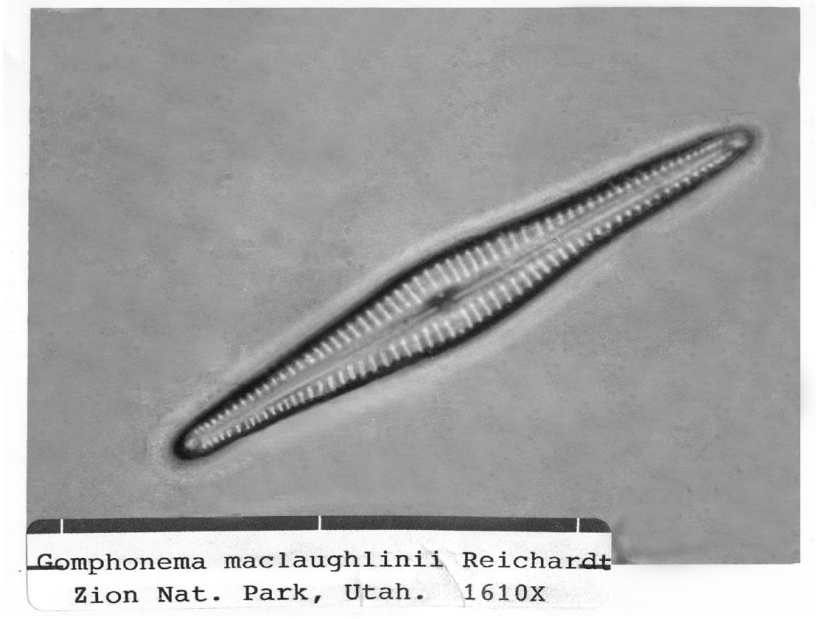
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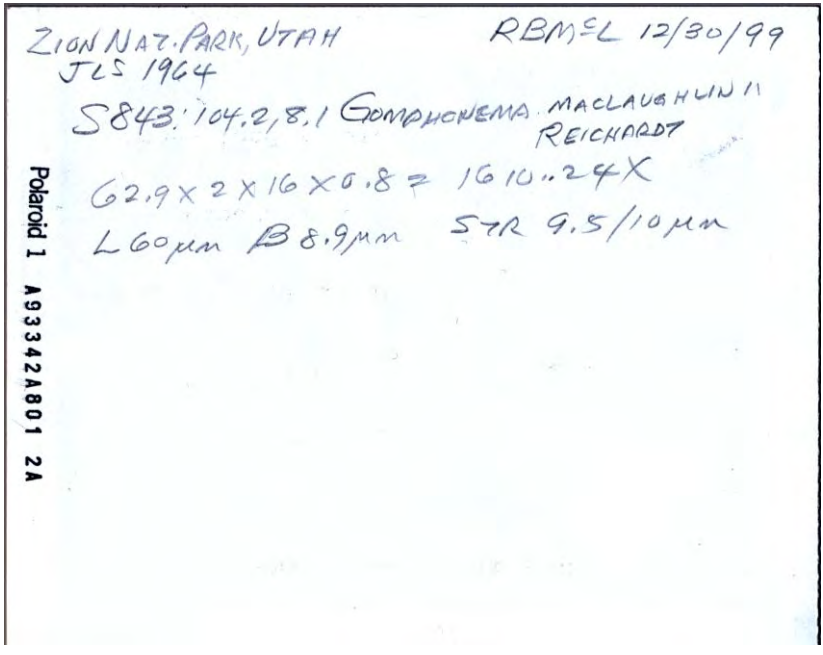
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APPENDIX B

Gomphonema maclaughlinii





APPENDIX C

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End of Volume



Photo by Jorge H. Aigla

Robert McLaughlin in 2000.

Robert B. “Mac” McLaughlin
1922 – 2012

25 years in the writing and another 25 years to publication this ‘tour de force’ is a magnificent achievement of dedication on the part of the author. Were it not for advances in computing and the advent of print-on-demand low cost publishing the manuscript may have never become available to the broad family of microscopists and diatomists, whether professional, academic or amateur.

Though some of the techniques, particularly the photomicrography section, are now dated, the bulk of the material pertains to techniques that are as valid now as they were when the project was first begun some 50 years ago.

It is hoped that by making this work available via the internet it will enthuse others, as the works of Hustedt enthused ‘Mac’.

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