A naturalist colleague has a difficult time accepting the legitimate word ‘genitalia’ for animal reproductive organs (insects are animals!). Just about every moth species has two sexes, males and females; a very few species are parthenogenetic having only females. Moth genitalia are distinctive for each species and thus are useful for identifying an individual moth to a species and for showing phylogenetic relationships, although the latter is being usurped by DNA analysis.

MALE

The genitalia have a basic design and occur at the extreme posterior tip of the abdomen. It is a spherical structure (genital capsule) having a pair of conspicuous valves laterally, a dorsal hook, and a ventral plate. Suspended through the center of the sphere in a anterior-posterior orientation is the phallus (aedeagus) which functionally is equivalent to the mammalian penis; sperm transfer in moths is internal.

FEMALE

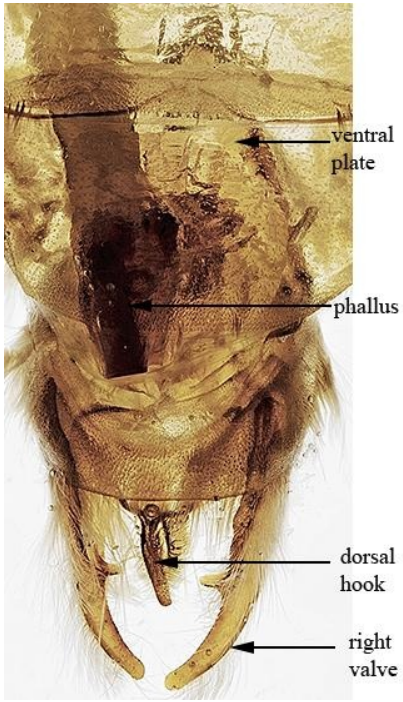
Genitalia are simpler than those of males, a posterior opening leading to a tube which ends in a sac into which the male deposits sperm.

Standard practice is to illustrate the genitalia in a reversed orientation *i.e.*, posterior is shown as anterior and ventral is shown as dorsal; and to move the phallus to the side. Thus, the most posterior hook of the male genital capsule is shown at the top of a genital image, the pair of valves point upwards, and the phallus also points upwards. Such a view bears little resemblance to how the genitalia look in a live moth but as long as every species has the genitalia displayed in this standard orientation comparison between and within species becomes possible.

In females the genital opening is displayed at the top and the sac to hold the sperm displayed at the bottom; in real life the genital opening is posterior and the sac is closest to the head.

‘In use’ most of the male’s genitalia is to hold onto the tip of the female’s abdomen. The phallus consists of a somewhat rigid tube with an internal sac that can be everted and greatly expanded by blood pressure; this part of the phallus enters the female’s reproductive system and delivers a packet of sperms. From this basic design, evolution has run wild.

Dorsal view of abdomen of male *Noctua pronuba*



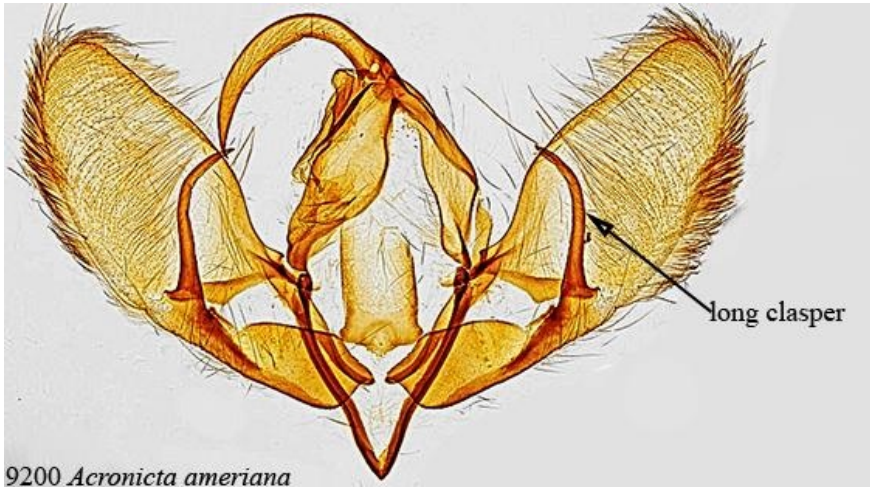
Dorsal view of male genital capsule

Genital capsule of male *Noctua pronuba* mounted (flattened) in the standard reverse upside-down orientation and with the phallus moved from within the capsule

The valves

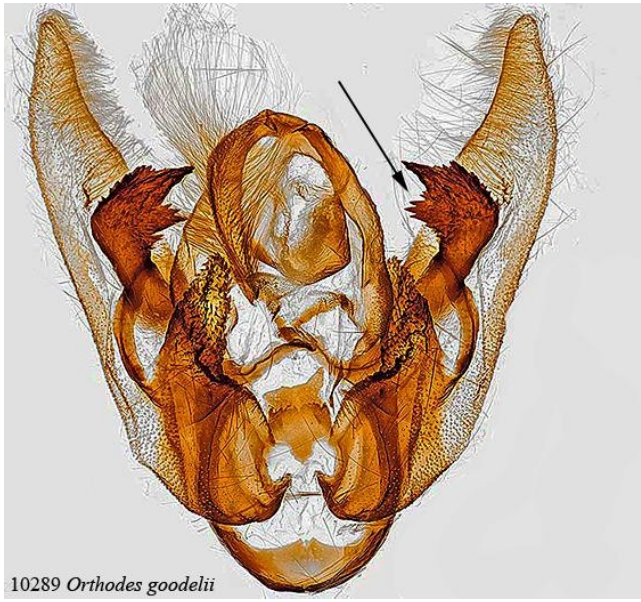
I am assuming that the valves originally were a pair of simple lateral flaps that grabbed the sides of the female's abdomen e.g. *Bomolocha baltimoralis.* The gripping power of the valves could be increased by the development of a spike (clasper), e.g. *Acronicta americana.*

8442 *Bomolocha baltimoralis*

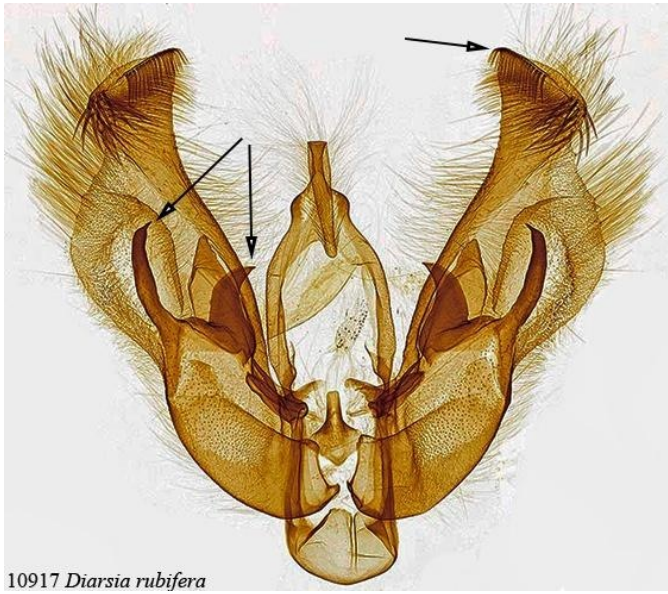


9200 *Acronicta ameriana*

Why stop at a simple one-pointed clasper when a serrated edge, e.g. *Orthodes goodelii* or a double-clasper and some hooks on an expanded tip e.g. *Diasia rubifera* would function better?



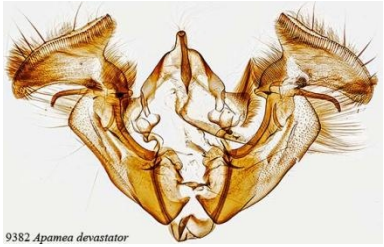
10289 *Orthodes goodelii*

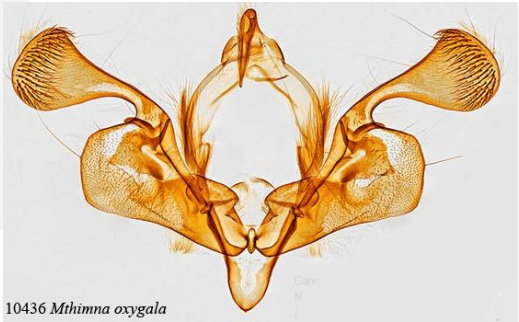


10917 *Diarsia rubifera*

An alternative to a clasper would be to increase the surface area of the tip of the valves, maybe turn then into ‘fingers’ as in *Melanchra pulverulenta* or into a single expanded pad as in *Mthimna oxygala. Apamea devastator* opted for expanded tips to the valves and large hook-tipped spines. *Prodoxus decepiens* fancied a ‘caveman club’, a long shaft expanded at the tip and studded with spikes. *Lacinifolia renigera* turned the apex of the valve into a spike and lengthened the clasper to mate it with the tip of the valve.

10294 *Melanchra pulverulenta*

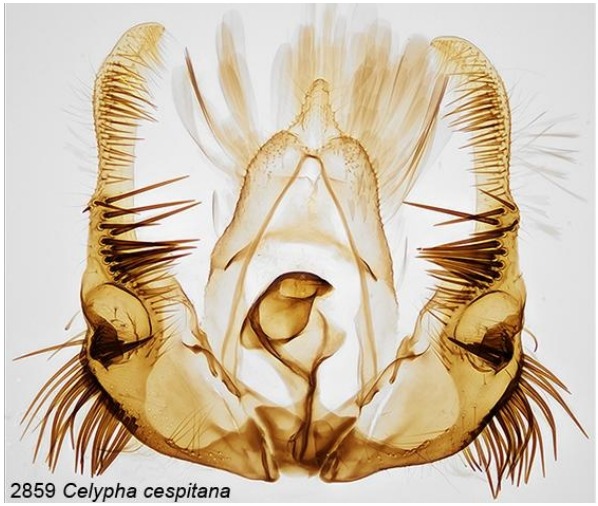

9382 *Apamea devastator*


10436 *Mthimna oxygala*

0200.1 *Prodoxus decepiens*

10397 *Lacinipolia renigera*

Some of the small moths (the micros) seem to go to extremes in the structure of the valves, especially as their only function appears to be gripping the female’s abdomen. *Celypha cespitana* arms the tip of the valves with numerous spines and about mid-way on the valves are truly viscous large spines. *Proteoteras aesculana* has indecent-sized spines on the expanded tips of the valves and some massive spines mid-way down.


2859 *Celypha cespitana*

3230 *Proteoteras aesculana*

As there are over 10,000 North American moth species, all of which can be identified by external features (often colour), perhaps it is not surprising that there is an equal number of variations in the structure of the male genitalia. Below is a sampling.

5226 *Palpita magniferalis*

6258 *Alsophila pometaria*

7139 Cyclophora pendulinaria

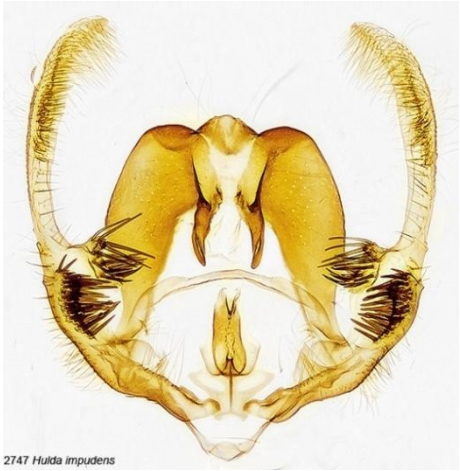
2827 *Olethreutes ferriferana*

6570 *Aethalura intertexta*

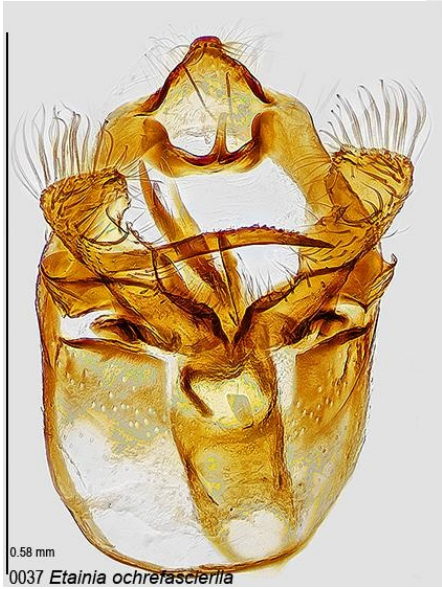
6582 *Iridopsis vellivorata*

1256 *Coleophora tiliaefoliella*

0485 *Bucculatrix solidaginiella*


2747 *Hulda impudens*

0296 *Stenoptinea auriferella*



0037 *Etainia ochrefascierlla*

89 *Dichomeris ochripalpella*

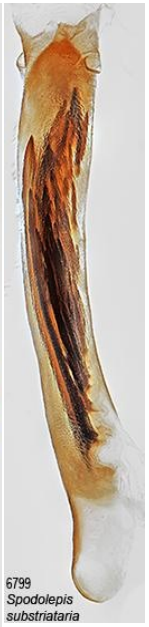
0733 *Phyllonorycter argentifimibriella*

The Phallus

The modifications to the valves might be thought of being masochistic towards the females but compared to the 'ornamentations' on the vesica (part of the phallus) they seem benign. Recall that the vesica is the 'organ' that is inserted into the female's genital tract where it is greatly expanded. What more would you need than a simple tube for inserted sperm? Few moths have such a simple tube, many have opted for incorporating one or more spines on the vesica. Moths are relatively small and proportionally the phallus is also small. It is possible to insert a very fine capillary into the base of the phallus and evert the vesica, thus getting a clear view of the 'armaments' (years ago a company offered a "moth phallus-vesica everter" for $4,00.00 – I never did buy it). I have managed to inflate the vesica in one moth *Leucania multilinea*, which has only a minor amount of 'armament'. However, a similar species in the same genus – *Leucania commoides* – has two large spines in its vesica.

Left: Male genitalia of Leucania multilinea showing complex genital capsule, phallus (moved from within genital capsule), and the everted vesica with an anterior spine and a row of minor lateral spines. This entire everted vesica enters into the female's genitalia. **Right:** larger spikes in the vesica of *Leucania commoides*

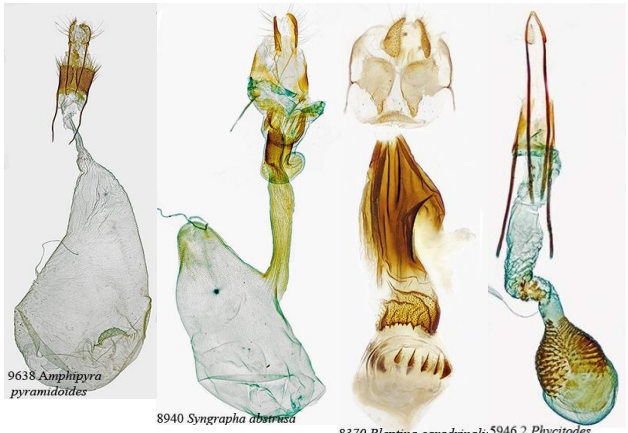
There is a limited space in the phallus but some species fill it with spines. Males have taken two routes: have a very few large spines as in some *Leucania* species or have many smaller, and not so small, spines. Sharp-pointed studs is favoured by some species



Females

After seeing all those viscous vesica armaments one could almost feel sorry for female moths. The simplest female genitalia is a posterior opening leading to a thin-walled tube that leads into a thin-walled sac where the male deposits a packet of sperms., eg., *Amphyra pyramidoides.* Perhaps a modification to protect from aggressive males is to thicken the walls of the tube. However, females are not completely inoffensive, many include a battery of spines in the sac; the function of which is most likely to break open the sperm package .

When spines are at the base of the tube their function to break open the sperm package seems unlikely. *Dioryctria zimmermani* has a strengthened tube leading to a battery of spines before to the sac. *Neogyractis slossonalis* has 5 large spines at the end of the tube 'protecting' the opening to the sac. The prize, however, must go to *Horisme intestinalis* females. The bottom of the tube has a chicane curve bordered with an opposing pair of 'jaws' with many teeth, once the male gets past these he is confronted with a sac whose walls are fully spined. It's a miracle that the eggs ever get fertilized!



The above essay is one of fact and speculation. The genitalia images are fact; the speculation is just that – conjecture without evidence.

Full-sized images and the associated moth images (North America) can be seen on BugGuide and Moth Photographers Group. Simply enter the species # on the search window.

Genital images of UK and European species here: mothdissection.co.uk

Comments to the author Anthony Thomas are welcomed,
email – at5535587 AT gmail DOT com

Published in the January
2022 edition of *Micscape* magazine.
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Just got your microscope? Had one for a while? Go on a journey of adventures which will help you enjoy using your new instrument with joy, excitement, and wonder.

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VARIATIONS FROM OCULARS

It all began when I started looking for a new 5x ocular to take pictures with a wider field of view than with a standard 10x. I did have a 5x that gave me reasonable results, but with a certain amount of distortions at the edges. A search on eBay produced a likely candidate: an Olympus 5x that was actually recommended for photography.

When it arrived I immediately made some tests, comparing my old 5x with the new arrival. I was somewhat taken aback, not by the image quality but by the resulting magnification. Used in conjunction with the 4x objective, its theoretical field of view should be double that of the field seen with the 10x ocular. The 10x gives me a field of view of 2.8 mm; a 5x ocular should give me 5.6 mm, but the Olympus gave me a 3.2 mm, or a mere 1.15x wider field that the 10x. In other words, the magnification went from 40x to 34.78x, a far cry from the anticipated 20x… So I was left to ponder and investigate.

Images produced by various oculars used on a given microscope can vary greatly not only in the intrinsic quality of the images but also in final magnification. To get the purported magnification, one should ideally use oculars and lenses of the same brand as the microscope. Photographers should also use camera adapter designed for a given microscope. Any variations can cause slight (or not so slight!) variations of the magnification. Microscope optical tubes can be of various lengths; most are of 160mm, a length that can be found engraved on microscope lenses. More recent microscopes may also be “infinity corrected”, and their lenses bear an infinity symbol (∞); those lenses must be used with the right microscope. Lenses screw on the turret and have a standard thread. Oculars may have a standard diameter, but as far as I can tell, they don’t necessarily fit the same way on top of the optical tube, especially those of the trinocular tubes used for photography. That is why we should be very circumspect when precise magnifications must be included with the pictures being made. This can be a problem with amateurs such as myself, who work with different microscopes equipped with parts of mixed origins.

In the following pages we can see the effects produced by four oculars used on the same Zeiss Standard microscope. Both the oculars and lenses are from different brands. All pictures were shot with the same objective and the same camera adapter, so all variations come from the oculars themselves.

You may notice some differences in tints between those pictures, even though they were all shot with exactly the same color balance settings. As with photographic lenses (in fact, with any optical instruments) the lens elements chosen by the optical engineer will have an effect on the color rendition. Photographers will often refer to some lenses as being “warm” or “cold”, a reference to their color cast. In our examples, the two 5x show an image that is “warmer”, or more yellowish. As for the very last image, it has a bit more chromatic aberrations. These defects can be corrected in post-production.

But why should oculars give different magnifications? Below we see four oculars, first the 10x I normally use for photography, followed by three 5x. Their “lips”, the part that will sit against the camera adapter, are not all at the same height. That is already one reason why not all 5x oculars will provide identical magnifications on a microscope tube designed for a different set of optics. And we don’t even see their internal design, which may add to the problem. So my 5x Olympus ocular mounted on the trinocular head of my Zeiss Standard gives an image that is much bigger than its theoretical magnification. On top of it, different camera adapters can also influence the resulting magnification, depending on the length and design on the adapter itself. That’s a subject I covered in a past Micscape article (http://www.microscopy-uk.org.uk/mag/artjul20/ca-magnify.pdf).

I may be wrong in my assumptions, but after much research and testing, I can see no other reasons for the discrepancies. If anyone has other explanations I would appreciate their input.

Comments to the author Christian Autotte are welcomed, email:
cautotte214 AT gmail DOT com
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www.micscape.org

Topical tip. Interchanging microscope maker's photo-adapters.

by David Walker, UK

In a November 2021 Micscape article I presented the case for tolerating vignetting in a digital camera if the total visual field from the microscope was captured. With the recent high megapixel count of consumer cameras, a crop may still provide sufficient pixels for an objective's full resolution. Many subjects also fit a circular field better than a rectangular crop.

The example used a Nikon Eclipse 800 stand which my brother Ian and I are slowly building up (shown left below with Sony NEX 5N APS sensor body with a 38mm to C-mount adaptor). Although the vignetting is acceptable for many subjects that essentially fill the visual field, a feature that is missed, present on our Zeiss Photomicroscope III, is an Optovar. Smaller subjects such as diatoms may not fill the field well without resorting to a possibly unwanted step up to a higher mag immersion objective. An Optovar can be useful in this case especially if the objectives are high NA examples of their mag.

Understanding the photo system options of the Nikon Eclipse 800-1000 range remains a tad baffling, for me at least, especially for consumer APS or full frame sensors; many pro' photo systems primarily support dedicated small sensor microscope cameras. The Nikon ergo head has a 38 mm ID photo port which seems fairly standard across many maker's systems. In the past I'd purchased Leica's adapter (#10446175 2.5x) for my S-series stereo to take a full frame DSLR body. As remarked in a previous article this was a very impractical solution 葉 all, cumbersome and vibration prone.

The Leica stereo photo adapter uses the same 38 mm port so out of interest I tried this adapter on the Eclipse 800 port as shown below using a Canon 600D APS sensor camera. This is the body I use on a Zeiss

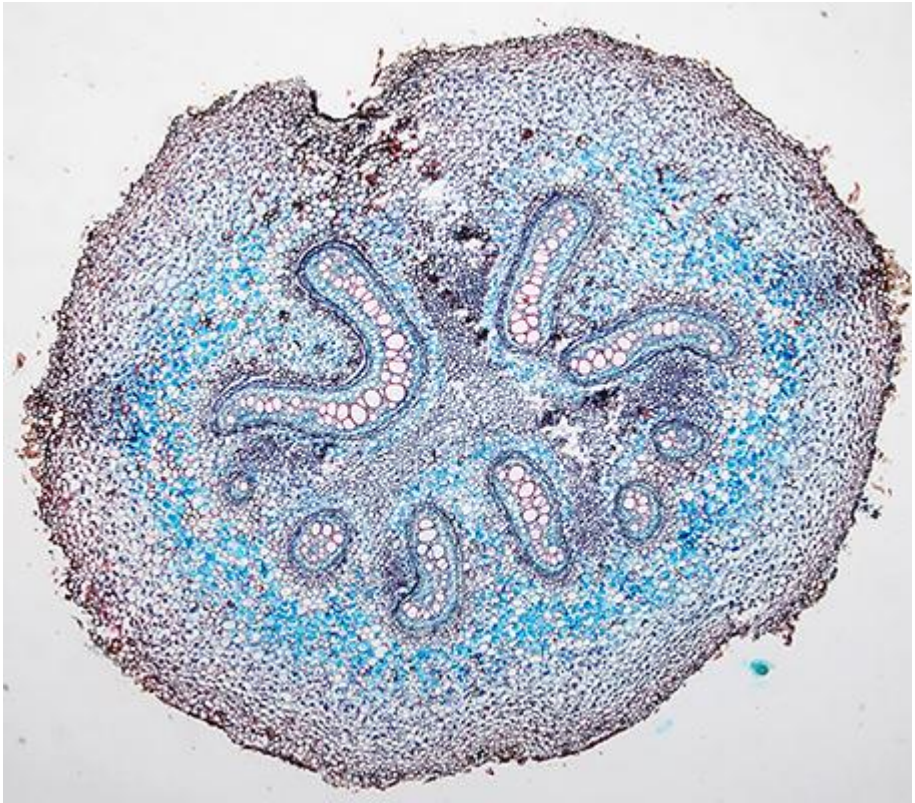
PMIII. A simple try it and see as was uncertain if the photo port of the Leica stereo used finite based projection optics as the Nikon Eclipse is infinity based.

A useful test subject for a photomicro' setup is a micrometer scale to show any non-planarity or aberrations. Planarity was very good for the 600D APS sensor with only slight chromatic aberration near the edges if pixel peep at 1:1. A similar amount of CA that tolerate on the PMIII as unseen at typical image sizes used. At the time of testing I briefly owned a used Canon 5D Mk II body with full frame 35 mm sensor but that gave marked CA and softer towards the edges with vignetting at the corners, so unsuited.

Although the 600D has a cropping effect, capturing ca. 60% of the max. that can be expected for a 35 mm format, this as remarked above was potentially useful if seeking a little extra mag in the absence of an Optovar on the Nikon. (The crop of course being a modest digital zoom rather than the optical action of the Optovar). The Leica adapter has a limited travel adjustment to set parfocal on a stereo. When set optimally on the Nikon, a low mag 2.5X objective required a quarter coarse focus turn and a 10X a fine focus turn to be parfocal with visual. This was not critical as use Live View on a PC for focussing and to exploit vibrationless exposures with the all electronic shutter in this mode.

So the Leica adapter will have a useful role on the Nikon Eclipse and avoid purchasing often quite expensive and hard to source photo adapters.

The ergo head with 38 mm ID lensless port on Nikon Eclipse 800.
Left - Sony NEX 5N (APS sensor) attached using a C-mount adapter provides vignetted full visual field when required.
Right - Leica adapter gives vignette free image on a Canon 600D (APS sensor) with a useful Optovar crop factor when required. It is unsuited for a full frame sensor.



Brake fern, Pteridium, T/S rhizome (Biosil slide). Nikon 2/0.10 objective.
Left. The Sony setup allows a full capture of the section,

cropped from full visual field. Right, the Canon setup with Leica adaptor provides a crop / Optovar type option, particularly useful for e.g. diatoms at higher mag that do not fill the field.

Comments to the author David Walker are welcomed.

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Amazing exploration of a single plankton sample

JM. Cavanihac - France

Often when we collect marine plankton samples, it's a bit like a fisherman who one day does not bring back any fish and another day takes lot of them!

This article wants to illustrate several things:

1 - It is not necessary to have sophisticated and / or expensive equipment (plankton net, boat ... bathyscaphe?) to collect plankton: a small jar (jam ...) is sufficient

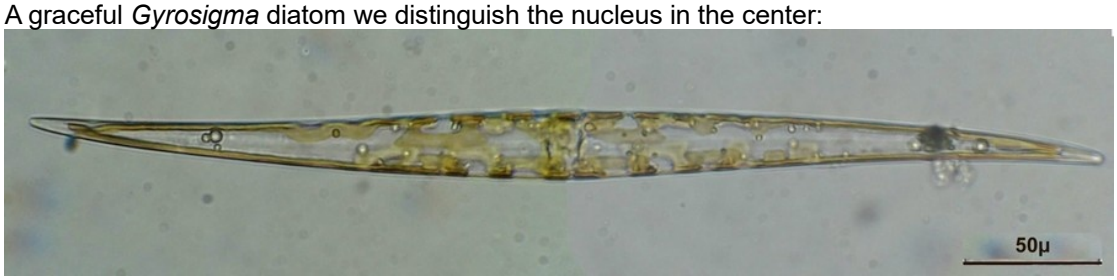
2 - For those new to microscopy who are looking for interesting things to see and don't always have ideas when they get their first microscope, those are hours of sightings guaranteed! (This same approach is also valid in fresh water.)

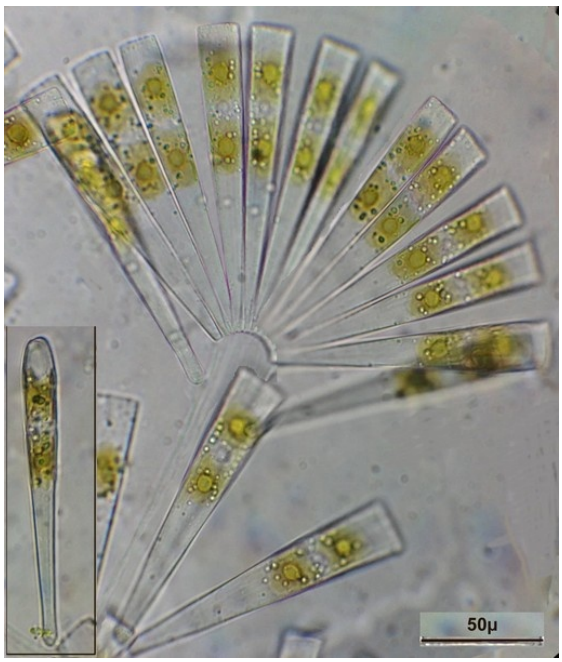
3 - After observation and taking pictures (do not think that expensive equipment is needed - all the images are taken with a mobile phone (not even a smartphone!) We can then spend some exciting moments looking on web sites for the names and particularities of the found species.

What is the origin of this exceptional sample named ECH1910? (19/10 being the date)

I am fortunate to live in a town located between the sea and a large salt pond (19 km x 8 km and average depth 5 m). The pond communicates with the sea by channels that cross the city and allow the exchange of water. So it was at the edge of one of these channels that this extremely rich sample was taken, on a rope soaked in water. The collection in a jam jar is obtained by scraping the rope several times with the lid of the jar and diluting the result in 20 ml of sea water. Here is the 1-meter-long line taken out of the water for the occasion: note a bush of bryozoans at the left side of the picture:

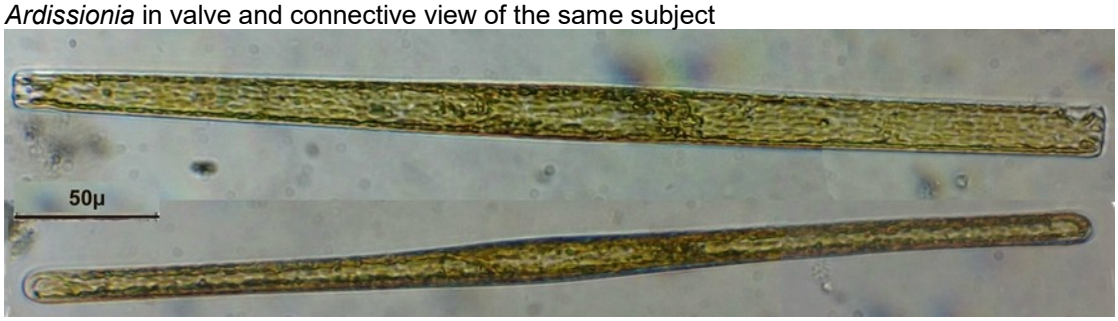
We find a lot of things already known: copepod cyclops, caprella, various common small-sized diatoms, such *Navicula, Cocconeis, Thalassionema, Coscinodiscus*…subjects which will not be presented here but we will see some of the less common, even rare. Note that the images re taken with a 5 Mpx cell phone held behind the occular lens!
A graceful *Gyrosigma* diatom we distinguish the nucleus in the center:



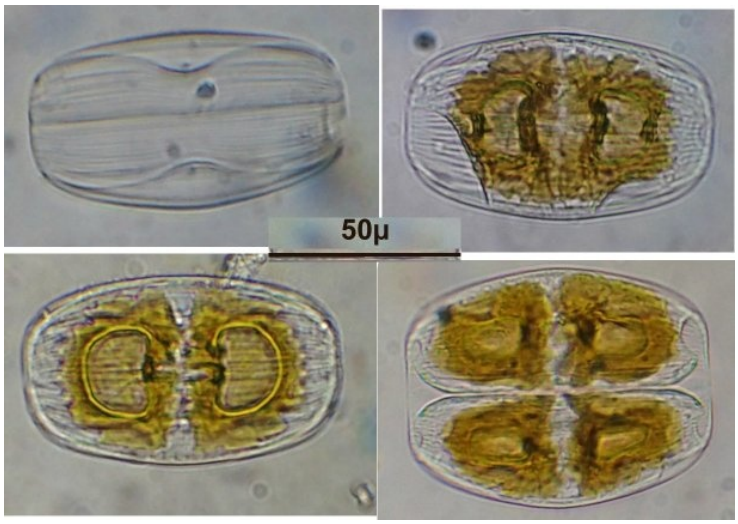


Another but colonial diatom:
Limophora flabellata: diatoms fixed on their peduncle in valve view, and lower left, inset, a diatom in connective view. The club shape explains that from a valve view it is difficult to see clearly both the center and the extremities which are not at the same level in focus

Ardissionia in valve and connective view of the same subject

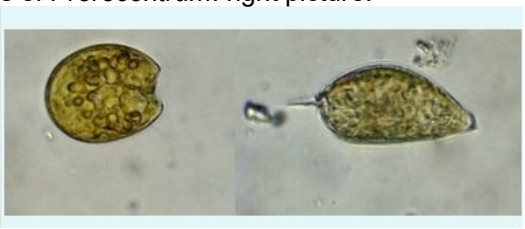


Cylindrotheca

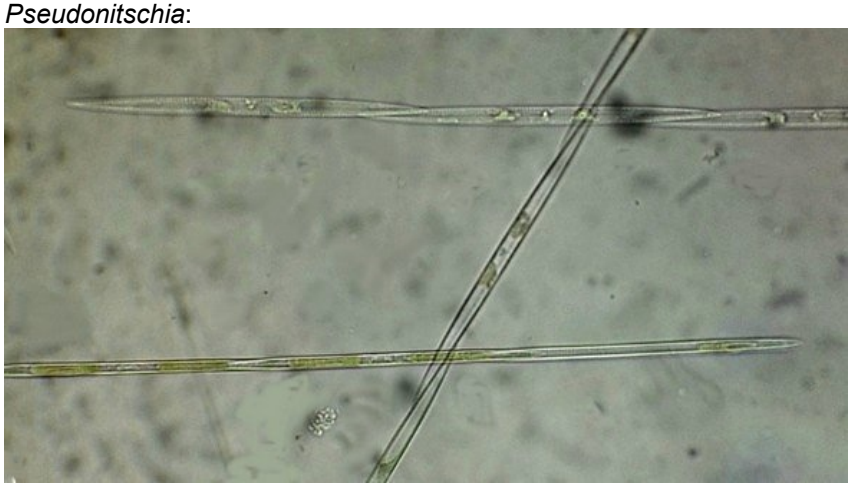


Amphora laevis: Top left empty frustule, bottom right start of division: we see the two daughter cells.

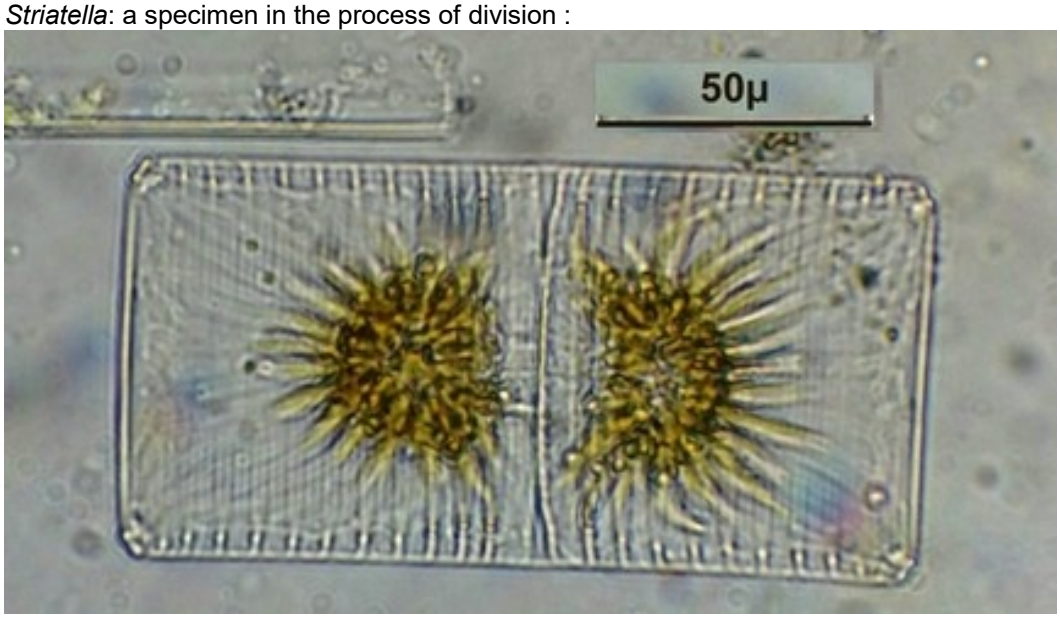
Dinoflagellates: *Ceratium* left side and two varieties of *Prorocentrum*: right picture:



Pseudonitschia:

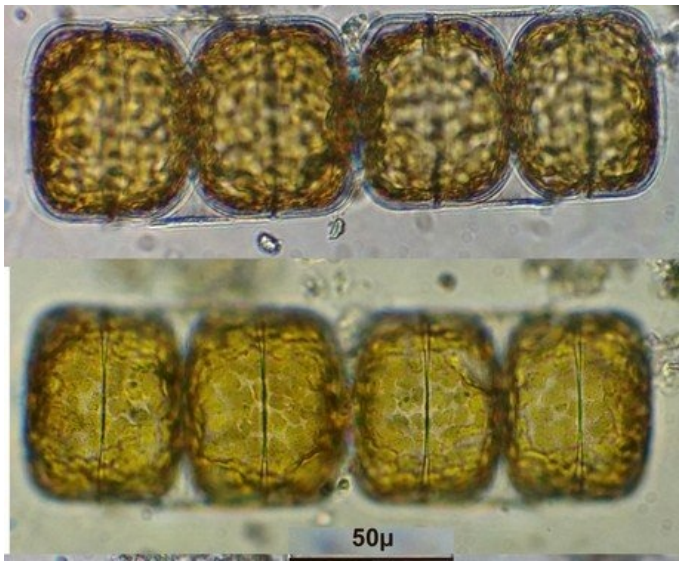


Striatella: a specimen in the process of division :



Rarer (she gave me trouble identifying it!) *Thalassothrix* 1.3 mm long!: bottom detail of the tip at the start of the division:

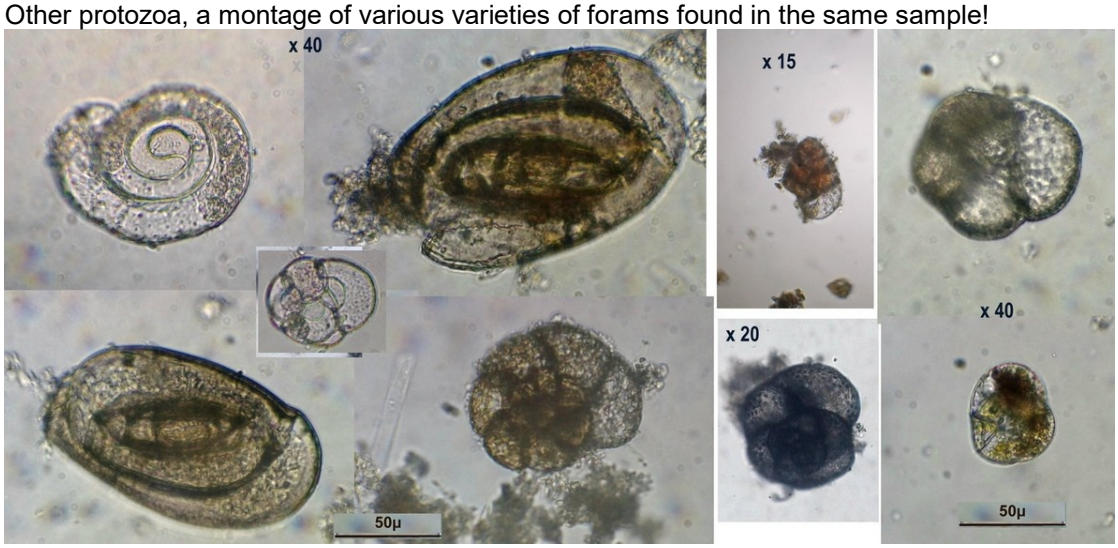
Another unusual diatom! *Toxorium undultatum* in connective view :



A variety of *Melosira*, two dividing cells (at two levels of focus):

Let's move on to the protozoa: *Vorticella* with two levels of focus and on the right a rare image seen from above!

Other protozoa, a montage of various varieties of forams found in the same sample!



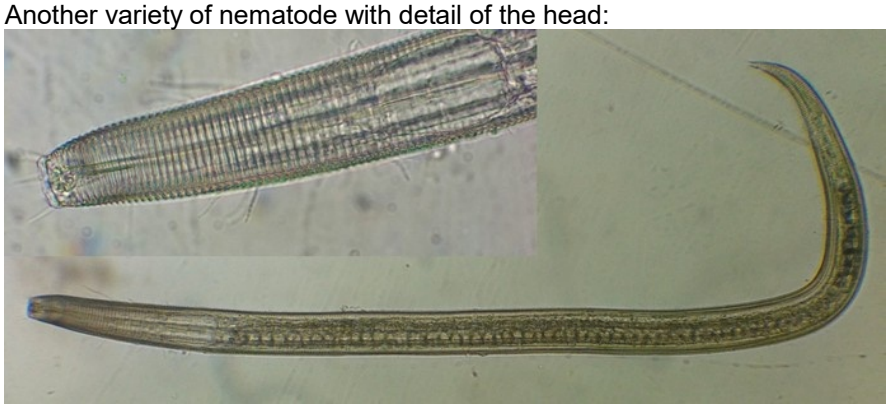
A nice *Loxophyllum*:

Another who made me look for a long time for his identification, and difficult to take picture because quite fast (therefore picture is a little blurry): *Epiclintes* (X15 objective) :

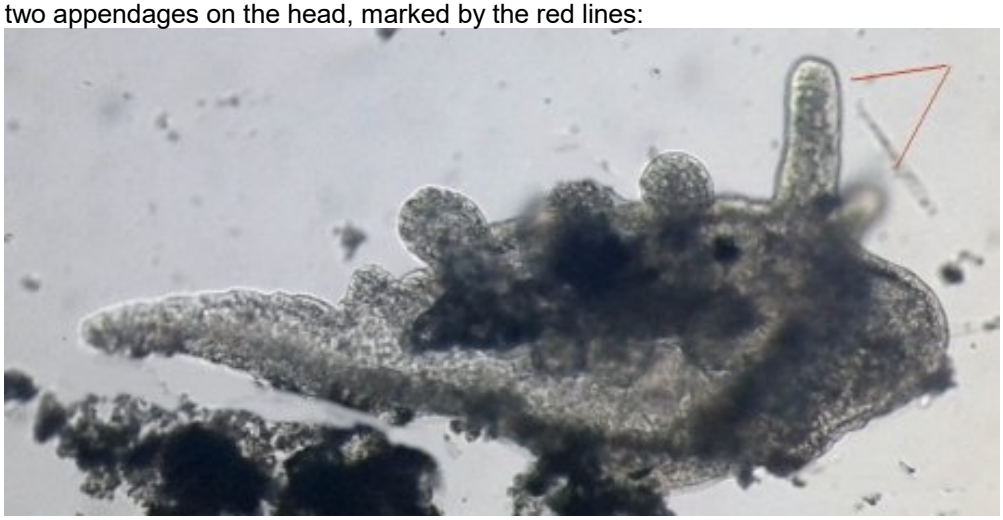
A rare protozoan difficult to identify: *Gastrocirrhus*: On the right image we can see the large cytostome which has a notch with the cilia in the center of the image :

Several nematodes of which here are two varieties: details of the head showing the teeth :

Another variety of nematode with detail of the head:



Another rare subject (in this size!) A nudibranch also nicknamed "sea hare" because of the two appendages on the head, marked by the red lines:



A species of marine worm: Serpulidae, the tentacles of which can be seen emerging from the calcareous tube that it builds; inset detail of the conical stopper which is used to seal the tube. Picture taken X10 on inverted microscope :

A Child's Garden of Micro-Wonders

Richard L. Howey, Wyoming, USA

In relation to microscopy, I am still a child in some respects; I have boundless curiosity and I keep asking "Why?" Unfortunately, I no longer have that seemingly endless reservoir of frantic energy that children have. When I think of microscopy, I think of that marvelous remark of Nietzsche: "The maturity of man–to have regained the seriousness that one had as a child at play." My pursuits can be fun, even exhilarating and I can be looking through a microscope and suddenly blurt out loud "Oh, WOW!" and not feel the least embarrassed that I am talking to myself.

Children, beginners, and child-like amateurs such as myself are sometimes frustrated by the complexity and difficulty of preparing specimens for observing under the compound microscope. It is highly discouraging when one spends ½ hour or more preparing a slide and then finds that it is either boring or requires different techniques to get a suitable slide. It is, of course, essential that one learns the basic techniques of making an adequate

preparation; otherwise one is simply wasting time. This essay is designed to provide some simple suggestions regarding specimens and techniques that with a bit of care will provide interesting material to observe and study. We'll limit ourselves to 5 basic illumination techniques, all of which are easily available to the amateur: 1) brightfield, which is the standard system for observation, 2) oblique, which brings light to the specimen at an angle and produces interesting contrast, with some specimens, a quasi-3-dimensional effect; 3 darkfield, which can provide stunning contrast for certain types of specimens; 4) polarization, which structures the light beam in such a way that in certain types of specimens (called birefringent), different angles and densities show up in different colors; and finally, 5) Rheinberg, which uses combinations of colored filters to produce remarkable color contrasts. The best part is that you can try out all of these methods with relatively simple and inexpensive apparatus and then if you really get hooked, you can invest in some special high quality accessories.

1) Brightfield

This what most people think of as the primary method of optical microscopic examination and with the development of all kinds of new high-tech specialized techniques, traditional brightfield microscopy is often underrated and relegated to the

status of a poor cousin. In microscopy in general, two major problems are contrast and resolution of detail. Naturally, much of this depends upon the quality of the optics–that's inescapable, so the real issue is to have good (not necessarily, by any means, state-of-the-art) lenses and to learn how to tweak them to get the best performance.

The traditional methods for enhancing contrast and resolution for brightfield involve stains and filters. The standard filter is a blue daylight filter. These substage filters are inexpensive and effective. Less often a green filter is recommended. Traditionally, such filters are usually reserved for phase contrast work, but since the eye is especially sensitive to green, such a filter can, with certain kinds of specimens, significantly enhance contrast. There is a bewildering variety of filters and many of them are quite expensive, some of them costing hundreds of dollars each. Nonetheless, experimenting with filters can be pleasing, enlightening, and microscopically valuable. My recommendation is to buy a "book" of plastic filters from a supply company. I originally bought mine from Edmund Scientific and they come in 2 sizes and you can also find them at Adorama Camera which carries small size 1 3/4" x 3" for $3.95 plus shipping, but they say that their shipping price applies for an order up to $50, so you can order 10

books of filters and have a nice stock and only pay $6.84 shipping. Then, you can convince your friends how rare and difficult these are to obtain and sell the extra ones to them for $15 each. (Your enemies you can charge more.)

However, you might prefer the convenience of having 2 sizes and Edmund Scientific has 1 ½" x 3 1/4" for $7.95 for 100 different colors and 3" x 5" for $39.95 plus shipping. You can see them here.

These are considerably more expensive than when I bought them quite a number of years ago, but apparently inflation is inescapable. Nonetheless, in the long run, investing in such a "book" of filters is a bargain. "Trust me. Would I lie to you?" as Richard Nixon once asked. However, if you are a conservative experimenter (is that an oxymoron?) and want to start with just a few colors, you can go to a university bookstore or office supply company and buy a few of those colored plastic holders that students use to put their essays in for submission hoping that the cheerful colors will blind the professor to the appalling spelling, atrocious grammar, and the utter lack of content of their writing. When I was still teaching, there was an administrative rule that final papers and exams had to be retained for a calendar year in the event of a grade challenge by a student. As a consequence, I accumulated hundreds of these vinyl sheets. The

only problem is that these are very thin, generally of poor quality, and have little color saturations, which means that if you want to use them as filters, you will need to cut 2, 3, or 4 of any particular color and stack them to get any satisfactory color saturation.

Another approach is possible if you have some old camera filters lying around or you might be able to find some at a yard sale. The problem with these is that they are usually mounted in glass and of a size that will probably not fit your microscope filter holder. In the long run, my recommendation is to purchase a “book” of the plastic filters; these are thicker than the essay covers, but still can easily be cut to size. These can also be used to make Rheinberg filters which we shall discuss later.

Staining is an art and a challenge. Most of these dyes were originally developed for the textile industry and there are literally thousands of them, hundreds of which have been tried in microscopical applications. Many of these dyes are difficult to obtain; some are extremely expensive and many are toxic in their concentrated, powdered form or in strong solutions–some being suspected or proved carcinogens and/or mutagens. The best and easiest approach for those with limited experience is to buy a few stains from a scientific supply house already prepared in solution in suitable concentrations. Some companies even offer sets of 5 or 10 solutions

selected for bacteriological studies, protozoa, or plant materials. This may seem like an expensive option at first, but when you consider that to make your own solutions, you need 1) a balance that weights to 0.01 grams, 2) distilled water, 3) dropper bottles, 4) weighing papers or boats, 5) funnels and filter papers to remove sediments and/or precipitates. Then there are those potential, hidden unanticipated costs which might ensue when, at the very moment that you are weighing out the powdered stain, someone comes into the room and turns on the fan, and, caught off guard, you inhale some of the dust and your face and hands take on a vivid blue, green, orange or brilliant crimson hue and then you pay $1,500 for the ambulance which hands you off to the Emergency Room where they try to detoxify you, keep you overnight for observation, send you home with a bottle of pills and a bill for $14,250.17. (You'd think they could knock off the 17 cents!) So, you see, it really is cheaper to buy the solutions already prepared.

Which stain will be useful to you depends, of course, on not only the type of object or organisms, but also the particular structures you are trying to differentiate. To begin with, there are some items around the household that will work fairly well for certain specimens. For example, micro-paleontologists frequently use food coloring on the

shells of foraminifera (forams) to increase the visibility of pores, spirals, etc. that may not be readily observed on what is often a bright, white surface. Naturally, biological stains can be used as well, but food coloring is cheaper and easier to obtain. Sometimes the biological dyes have a distinct advantage in that they tend to produce a more intense and vivid stain.

This same technique can be applied to fragments of the tests (shells) of sand dollars and other echinoids which have been bleached white to remove the spines.

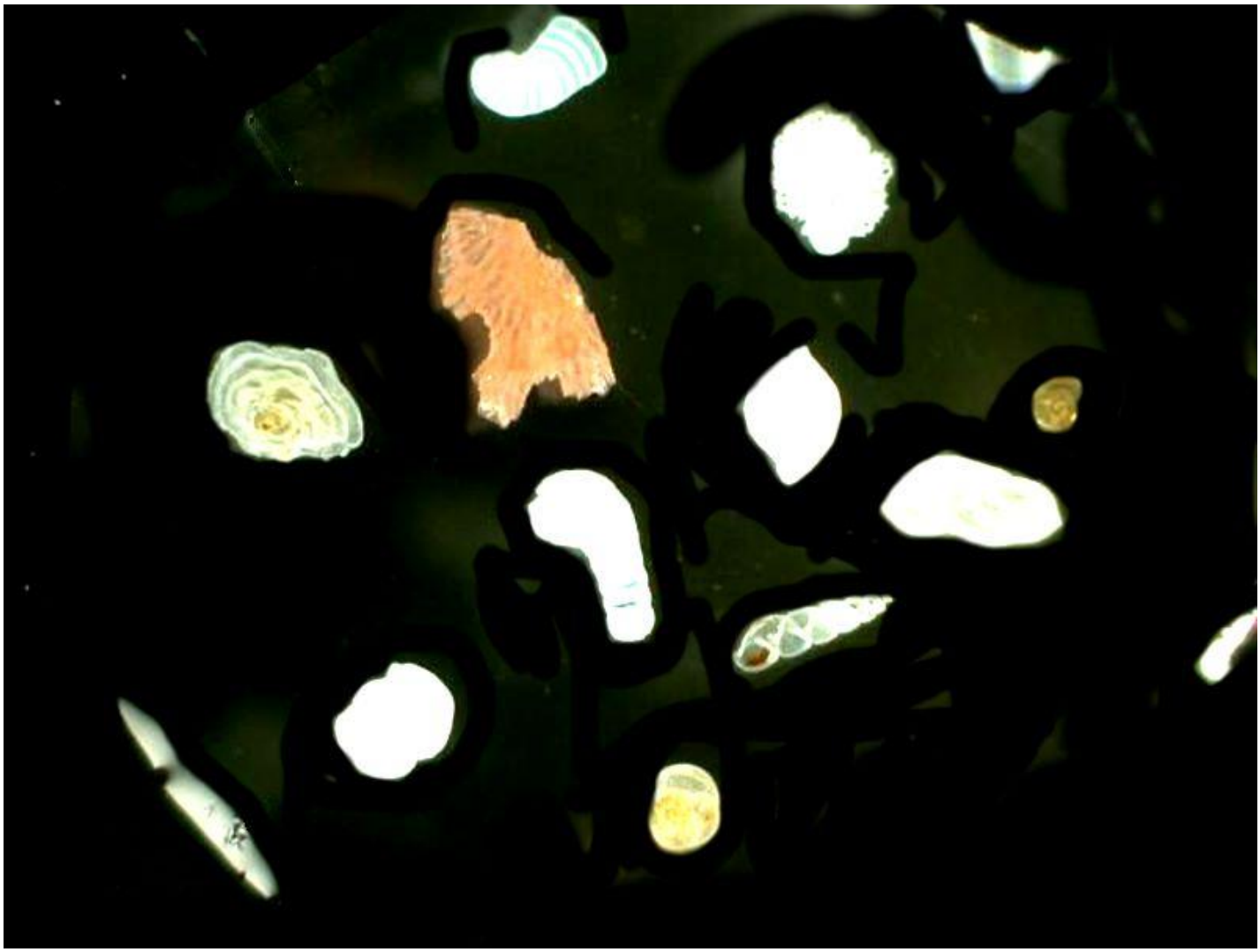
Trial and error will tell you which stains provide the best contrast.

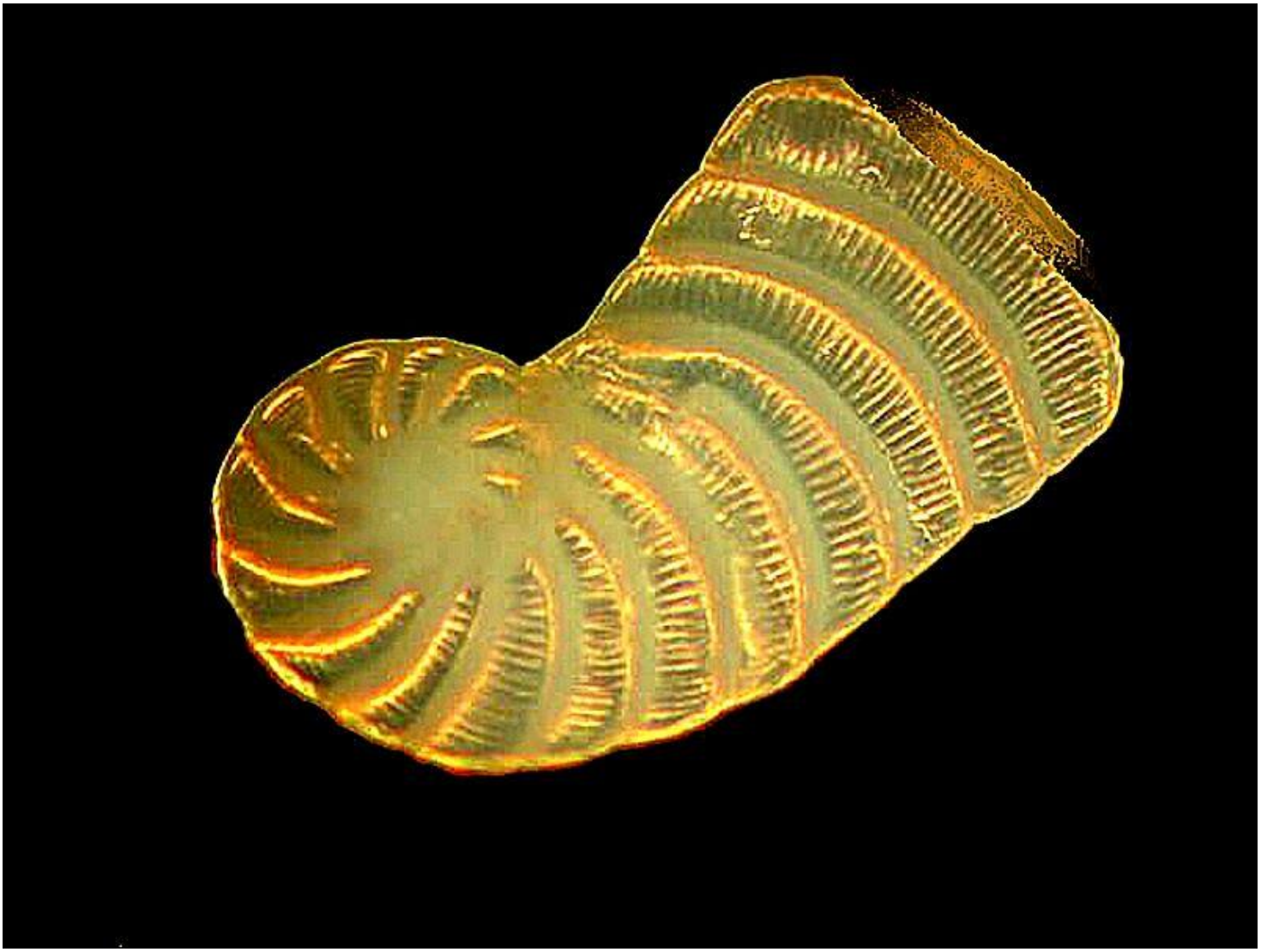
Almost everyone uses a ball point pen these days when they're forced to sign their names and can't use their cell phone to text it (yet). However, if you're as old as I am, you may have some bottles of ink around and it can be had in a variety of colors–black India Ink, blue green, purple, and red being the most popular. Where on earth does one buy ink today in an electronic culture? I suggest trying a shop that sells artist supplies. You'll also want a packet of brushes to apply the inks and stains, but don't buy them from the art store; they'll try to sell you camel hair sable, or Peruvian agouti hair brushes at an exorbitant price. Go to a discount store with a

craft department and you can find packets of inexpensive, but quite serviceable brushes.

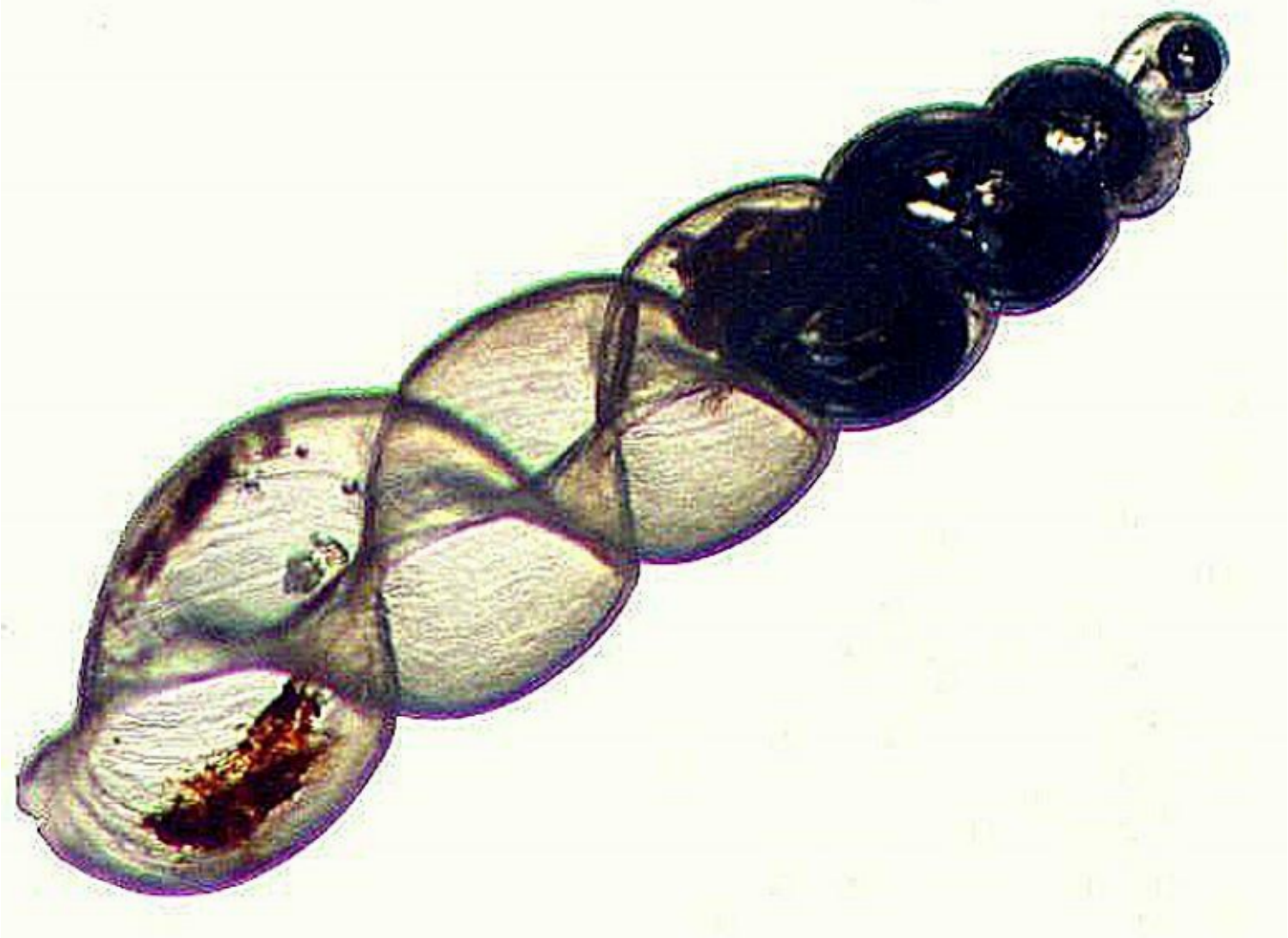
For certain types of forams; namely, those which are semi-opaque–that is, you can see some light through these, but not enough to clearly see the internal chambers–one can use a drop of mineral oil, cedar oil, or immersion oil to enhance the contrast and reveal some of the inner structure of the shell.

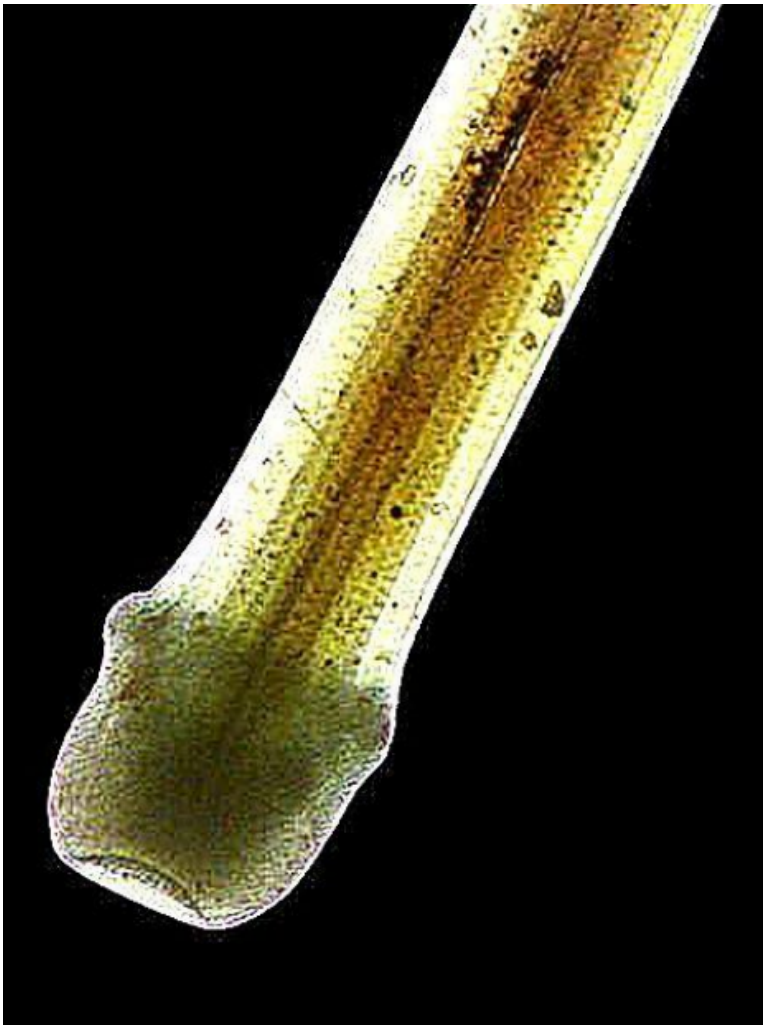
First 4 foram images using oil to enhance contrast.





Next 3 images where the oil has been applied to other organisms' calcareous shells. First, a tiny snail shell where the spirals are nicely visible, secondly, a portion of a bryozoan colony showing the internal chambers which were inhabited by the zooids, and thirdly, a portion of a sea urchin spine which very nicely shows the crystalline character of the spine.





Staining presents many possibilities with such a wide variety of specimen materials that it isimpossible to cover such a vast field in a short essay,but I will try to briefly cover a few basics. The firstrule is to stick with the classic, tested, tried and truereagents that can be readily obtained from a supplyhouse. From my point of view, it's desirable to haveMethylene Blue (a multi-purpose, polychromaticstain); a good hematoxylin stain, such as, Delafields's or Harris (preferably the versionwithout mercuric oxide); Alizarin Red S (which hasan affinity for calcium), so is very useful fordifferentiating calcareous material; Janus Green Bstains mitochondria; Neutral Red is a good contraststain and reveals Neutral Red granules in some ciliates, such as Paramecium.

Many aquatic invertebrates are eithercontractile or distort rapidly when fixation isattempted. Protists are particularly problematic andmy three favorite relaxants are 10% methyl alcohol,1% Magnesium sulfate (Epsom salts) and 2% to 3% Copper acetate. Methyl alcohol is easily obtained ata hardware store, Epsom salts at a drugstore, andsome biological supply houses will sell smallquantities of copper acetate to individuals even ifyou are not associated with a university or researchinstitution or if you know a local pharmacist, he orshe might be willing to order a small quantity foryou. I especially like Copper acetate as it allowsquite delicate control and a gradual paralysis withminimal distortion. When reactivity is minimal, thena fixative needs to be added.

Magnesium sulfate is another exceptionally useful reagent which can be applied to bothfreshwater and marine invertebrates. It has theadditional advantage that the commercial drugstorevariety is as good or better for our purposes than the refined chemical; it's cheap, it's easy to take alongon field trips, and it works from hydroids topolychaete worms to echinoderms and ascidians.

As for methyl alcohol, it's a technique to get the organisms totally drunk and this stuff is poisonous, so don't drink it–don't even spill it on your skin. This reagent also has the advantage of allowing fine-tuning the effects somewhat by adjusting the concentration.

So, for brightfield illumination, experiment with filters and stains to enhance contrast and reveal detail.

Next, let's briefly consider oblique illumination. There are special substage condensers that were made specifically for oblique illumination, but they were rare and expensive. For starters, you can use a stiff, heavy sheet of black construction paper. If your condenser has a filter holder, you can cut a circle of the paper that will fit neatly into the holder. You can use a compass to draw a line along which you can cut smoothly. Some compasses allow you to replace the pencil with a small, sharp blade which means that you can quickly cut a circle with a clean edge. The solid circle will, of course, not let any light through, so now you get to experiment. You can cut out a wedge, rather like a slice of pie, from the disk.

Another pattern that is commonly used is created by cutting a small arc along one edge.

If you consult some older textbooks on micro-technique, you will find drawings of traditional oblique stops. You should experiment with both size of cuts and position.

Another possibility that is more high-tech can also give some nice results. If you have a microscope with a phase condenser then, with a bit of practice, you can get some fine contrast simply by throwing the condenser out of phase and you don't even have to be using phase objectives; in fact, it's preferable not to.

Use a phase telescope or a Bertrand lens to make sure that the rings in the phase turret are properly centered, then very slowly rotate the turret to move the ring off-center. A very small movement can produce a dramatic shift. The full field will not be evenly illuminated and this, when properly "misaligned" will produce a pseudo-3-dimensional effect as well as enhancing contrast.

The third approach is darkfield which, I will warn you in advance, is addictive. A minordrawback is that it takes a fair amount of practice,patience and persistence to get good digital imageswith darkfield. For your first-time trial, I recommend

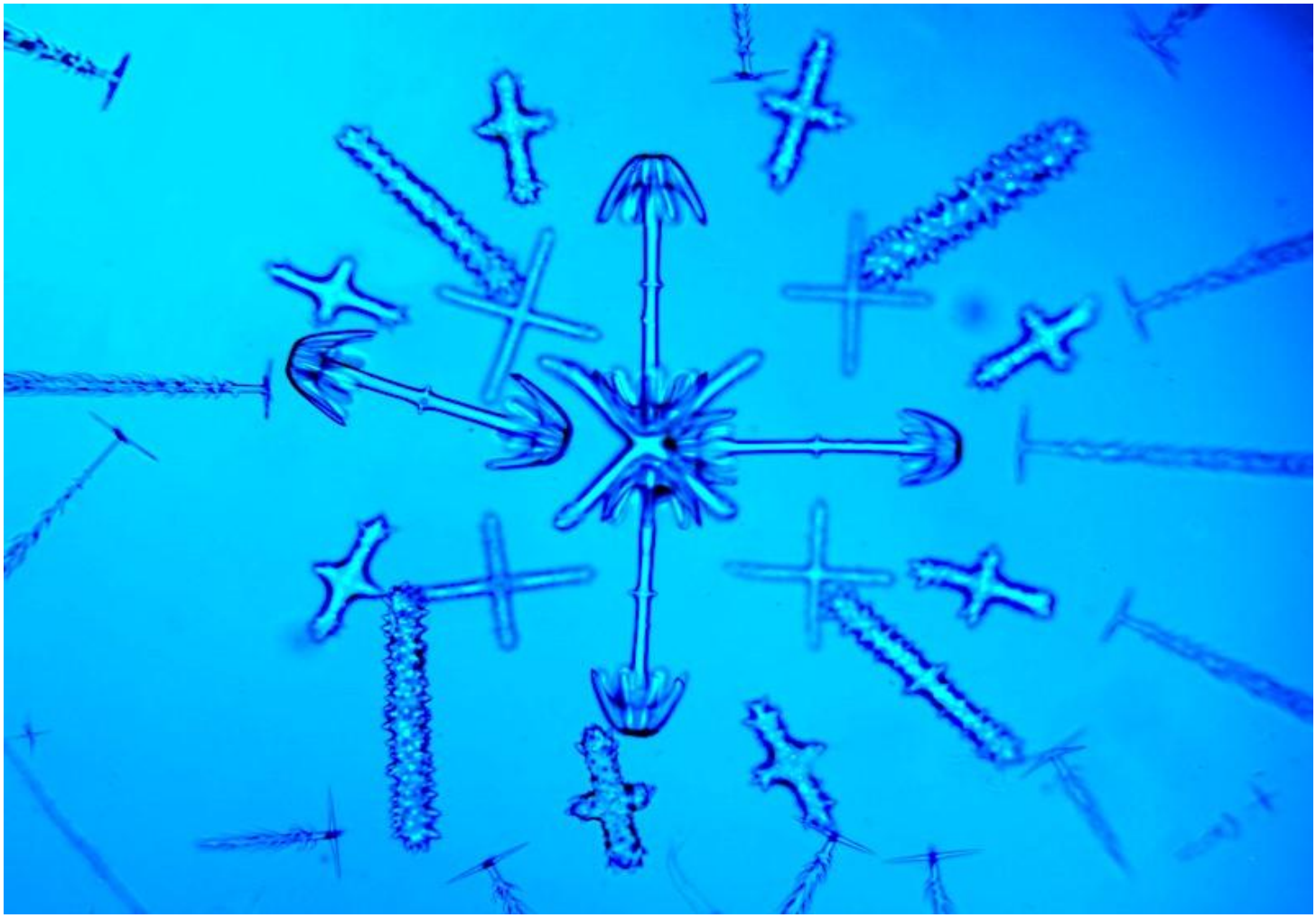
taking a drop of a rich culture of *Paramecium* which should always be kept on hand, since it is such a wonderful and mysterious organism and is easy to culture. Again, you can make your own disks using construction paper and a sheet of sturdy, clear plastic. Cut the black center disk and glue it to a plastic circle you have cut. Each objective will require a different diameter disk. Again, older books on micro-technique will often provide details. The basic concept is quite simple; you want the disk to produce a black background so that the remaining light comes in at angles such that the specimens appear to glow and glimmer against the black background, especially when they are in motion. To get the best results you will likely have to engage in a fair bit of trial and error.

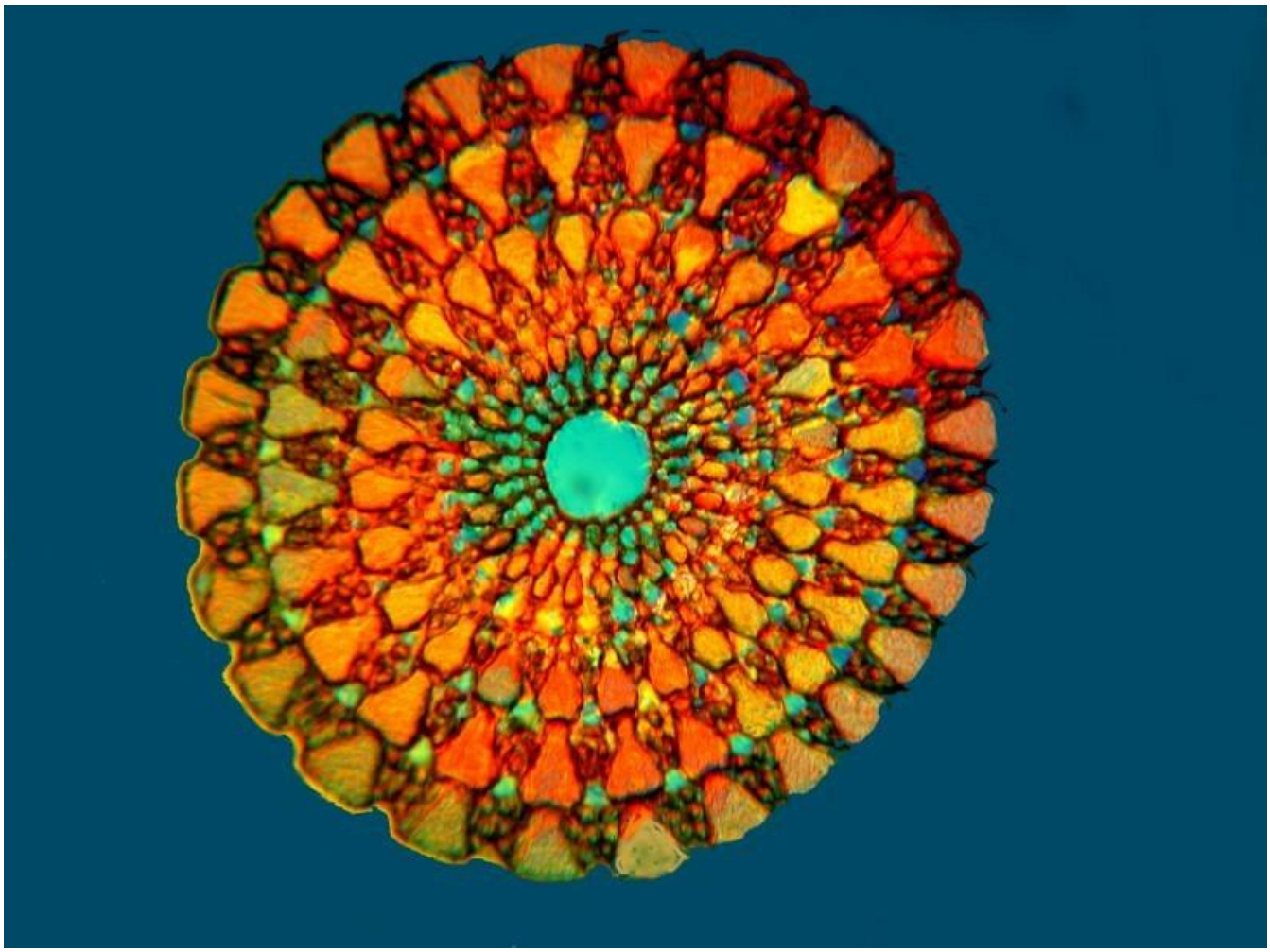
If you are fortunate enough to have an advanced microscope and the luxury of a separatedarkfield condenser, then you really are a candidatefor serious addiction. Such a condenser from a majormanufacturer is a minor technical work of art andwhen properly aligned and adjusted can produceocular intoxication. It is a tad messy to use; you haveto place a drop of immersion oil on the top lens ofthe condenser and then slowly raise it until the dropcomes into contact with the bottom of the slidewhich has your specimen on it. The oil drop needs tobe big enough so that when you make contact, it fills

the field of view and doesn't contain any air bubbles, but not so large that it runs over the lens and down onto the condenser. In any case, after you finish your session for the day, you need to remove the oil from the condenser. There is considerable disagreement among the experts regarding what is a safe and effective way to do this. Most agree that alcohol is NOT a desirable cleaning agent for lenses. It is an aggressive solvent and over time can dissolve the cement that holds the lenses in place. The debate about the use of solvents, such as, acetone, toluene, xylene has been intense and there is a wide range of opinion. Two points of agreement are 1) if you use such a solvent, use as little as possible, and 2) apply it and clean and then remove as quickly as possible. There are some less aggressive commercial solutions which can be purchased and you may wish to try one of those. Some microscopists remove only the excess oil with a cotton swab and store the condenser in a dust-free plastic case. The risk with this method is that over time, oil may seep down into the other lens elements producing problems that usually can be dealt with only by a professional technician.

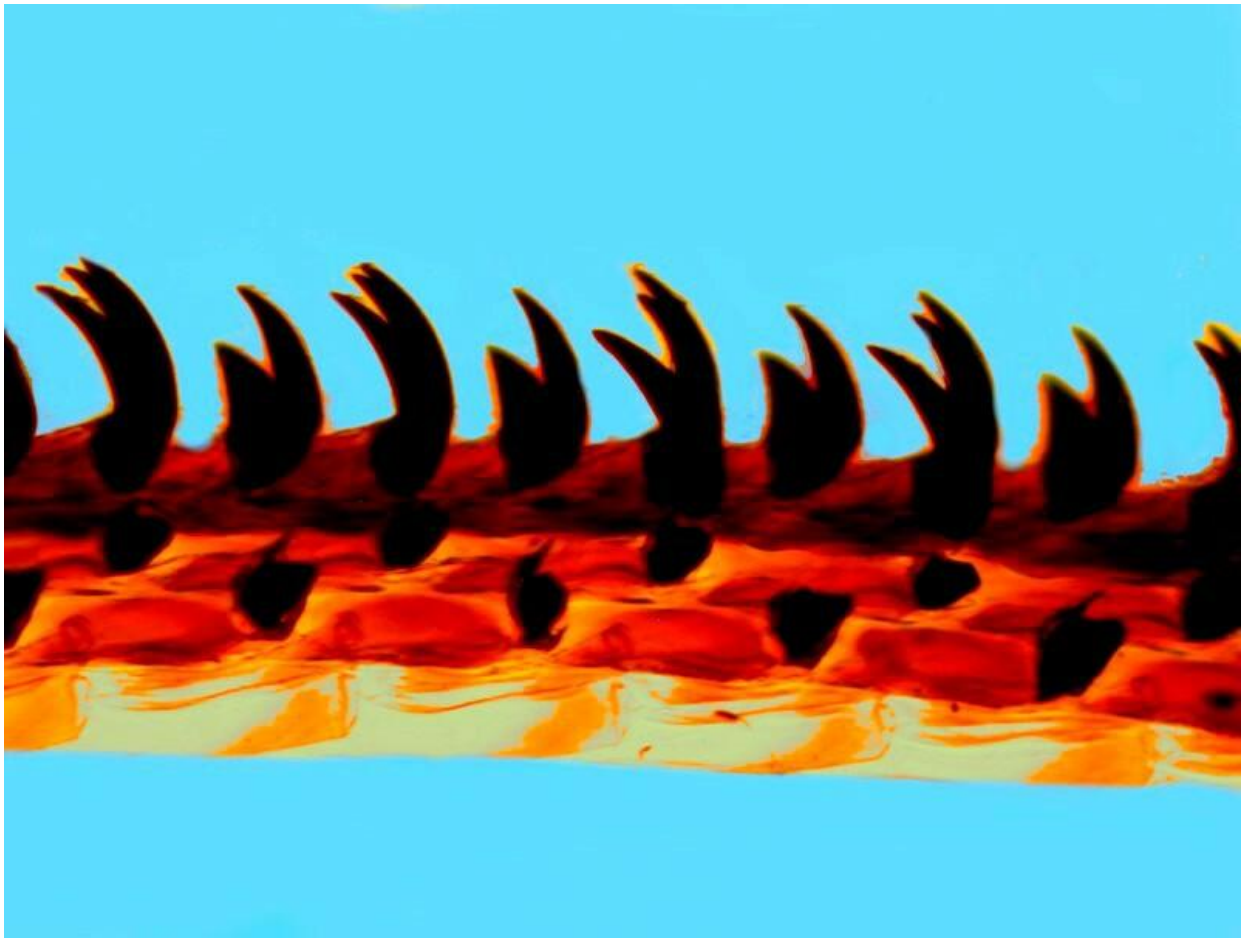
In the late 19th Century, Julius Rheinberg developed a wonderful contrast method usingcolored filters. Several articles on Rheinbergillumination have already been published on

Micscape and so I will limit my discussion to just a few remarks and a few images to illustrate the technique. (If you do a search on Micscape for Rheinberg, you will find a number of helpful articles.) Today, we are in the very nice situation of having access to a wide variety of colors in plastic sheets which can easily be cut and sized to fit almost any compound microscope. It just now occurred to me in writing this that I have never thought of trying to apply Rheinberg to a stereo-dissecting microscope .So, I cut some disks from a few of those cheapstudent essay folders and did a quick trial. Not veryencouraging. I will try once more later with adifferent color combination, but I suspect that theresults will be indifferent at best. In the meantime,here are 3 Rheinberg images taken with a compoundmicroscope. This first one is a strew of siliceousspicules from a glass sponge of the genus*Hyalonema*. Because they are composed of silica,they would appear as simply transparent withbrightfield illumination.





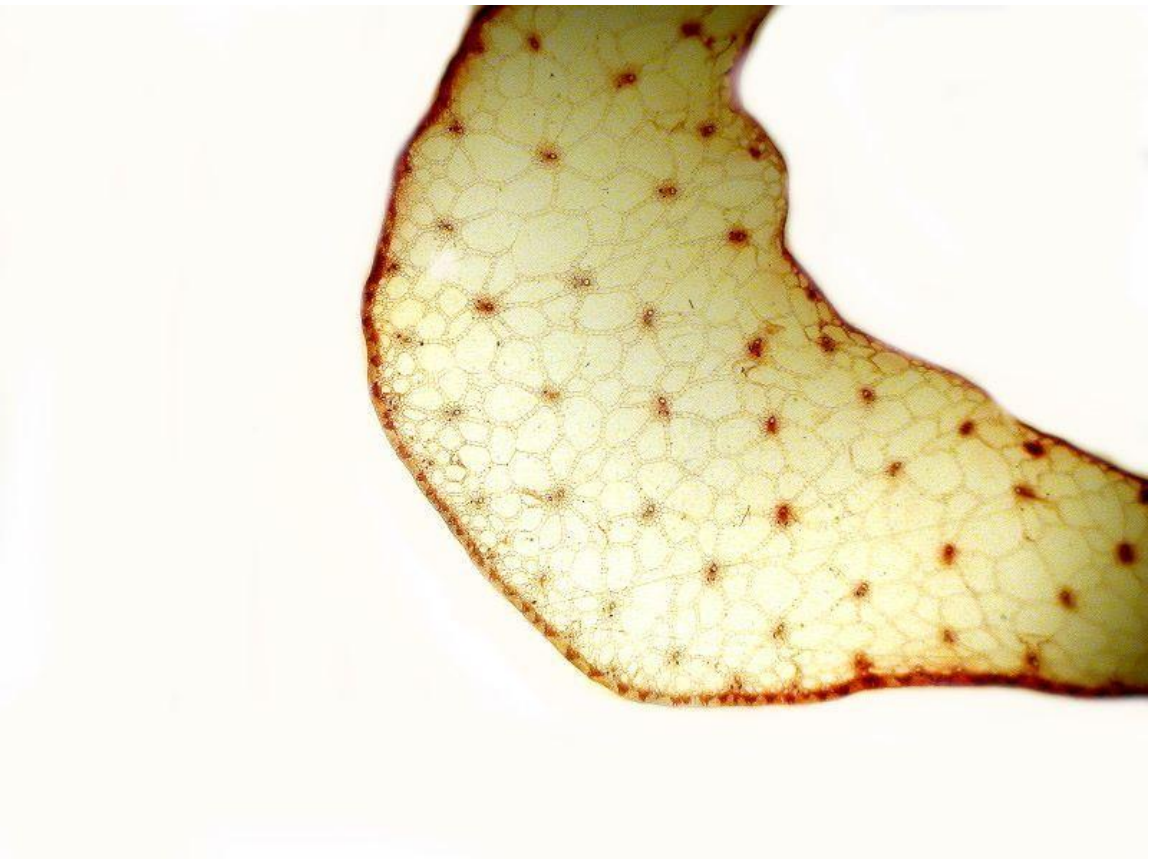
This second image is a cross section of the spine of a sea urchin. Since it is calcareous, it would be colorful with polarized light, but it is even moreso with Rheinberg.

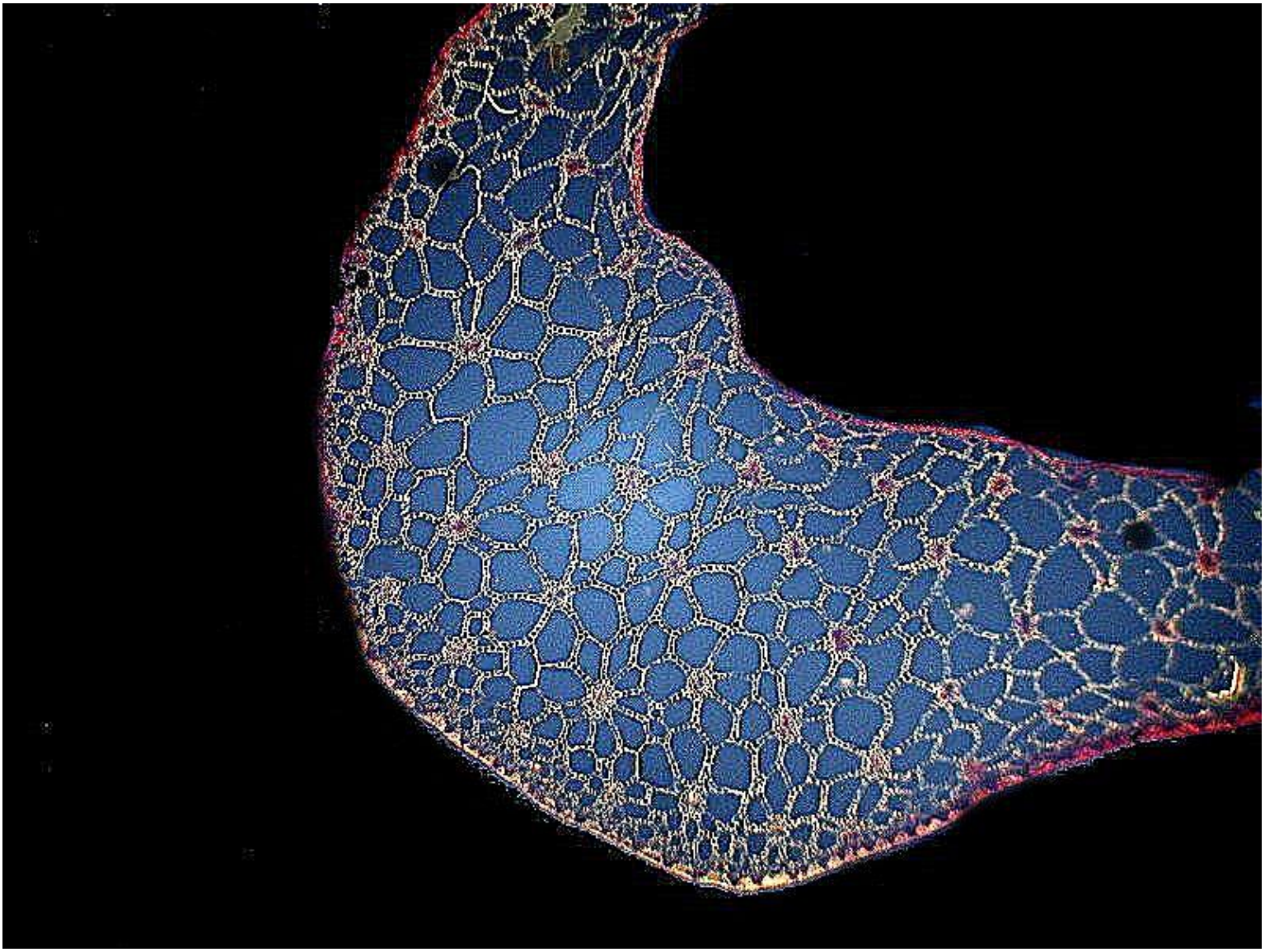


The third image is a section of the radula (tongue/tooth apparatus) of a snail. Using Rheinberg shows up detail that would not be as readily visible in brightfield.

Regarding polarization, most of you are already familiar with the basic principles and again, several articles on polarization have already been published on Micscape. As a consequence, I shall only give a brief oversimplified description and a

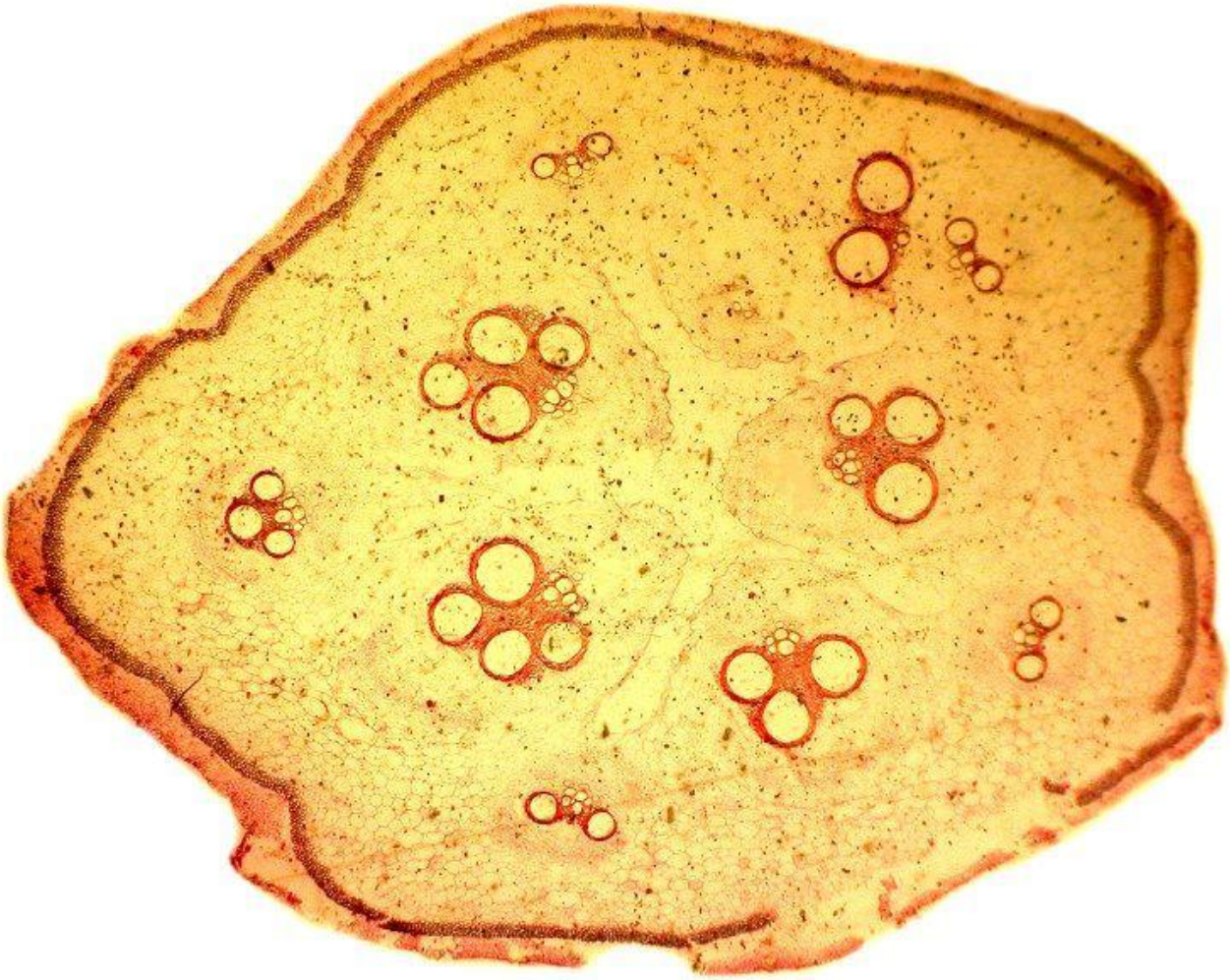
few images. When light passes through a condenser,it comes out at a wide variety of different angles. Putting a polarizer at the substage in the light path transforms the light beam so that all of the rays are now parallel to each other. When the light now passes through certain kinds of specimens, which are described as being birefringent, differences in density, thickness, and angle bend the light waves at different angles and when these light rays pass through an analyzer, these differences show up in different colors. The difference between a specimen observed with ordinary brightfield illumination and polarization can be striking. Here are 2 examples. The first is a cross section of a pine needle with brightfield and the second is with polarized light.

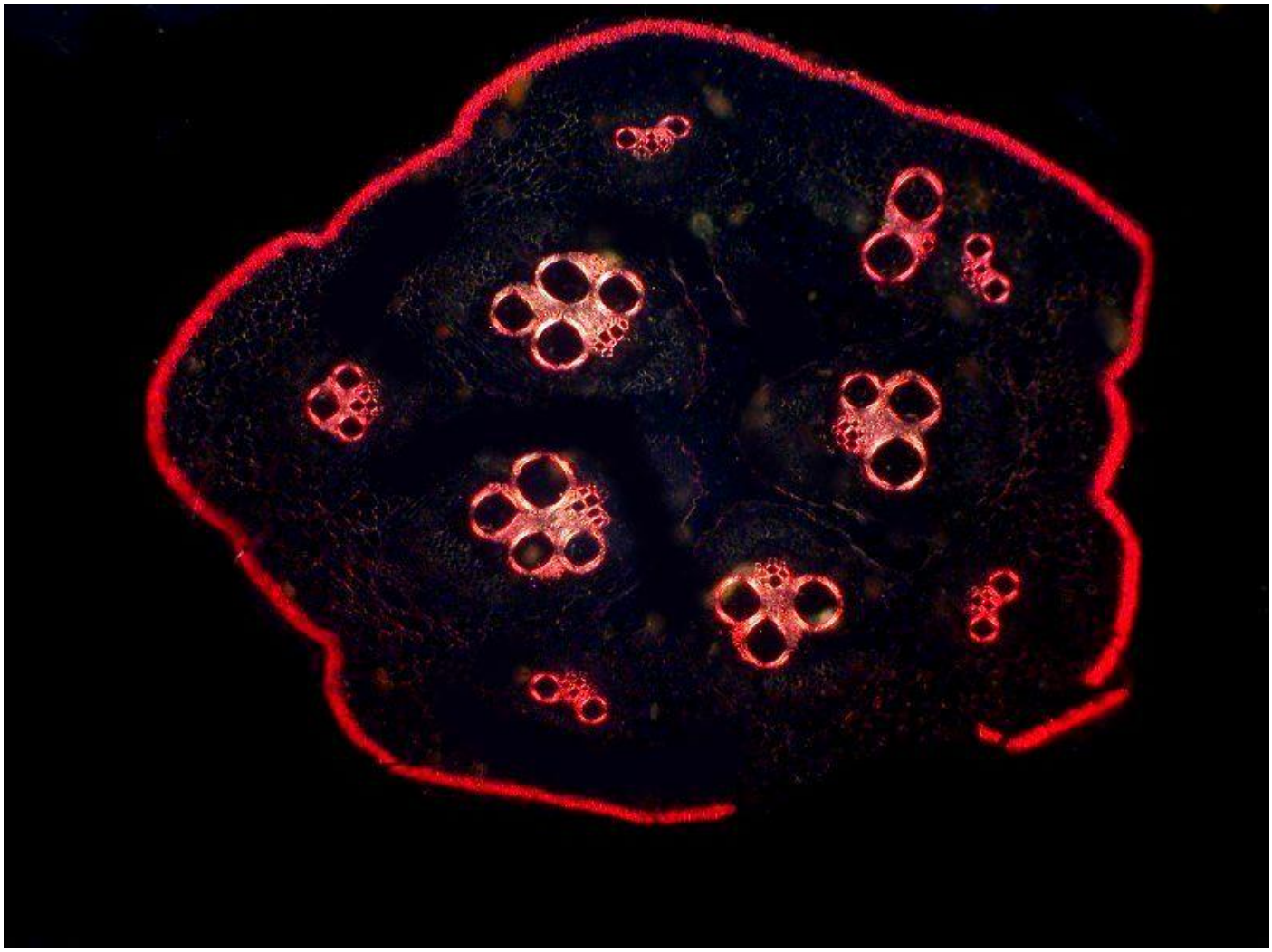




The second example comes from the genus *Cucurbita* which consists of the gourds and pumpkins. This was taken from a 19th Century slide and there was no identification beyond the genus, so I can't tell you anything more about it.

The first image is with brightfield and the second with polarization.





[Note: Even though the polarizer (substage) and theanalyzer (above the specimen and objectives) maybe made from the very same sheet of polarizingmaterial and thus are, for all practical purposes,identical, the distinction is nonetheless of vitalimportance. Ideally, the polarizer and analyzershould both be of a very high quality, but if forfinancial reasons, you find it necessary to take cost-cutting measures, then make the sacrifice in terms of the polarizer; it is essential that the analyzer be ofhigh quality. Even an inexpensive polarizer willgenerally provide reasonable parallel alignment, but once the light has passed through the specimen andthe objectives, it is crucial to have high qualitymaterial that will translate the optical informationresulting from density, thickness, and angular shiftswith precision.

If you want to do a simple test to make certain that you have good quality polarizing material, youcan take two filters (or cut them from a polarizingsheet) and hold them up to an ordinary incandescentlight bulb so that you can view its filament. Rotateone of them until the polars are crossed. If you getextinction, that is, if the field is consistently dark,then you have good filters; on the other hand, if youget a purplish image of the filament of the bulb, thenyou know that the polarizing material you have is ofmediocre quality.

If you have or are consideringbuying an older, modular research microscope thathas polarizing capabilities, another thing to watchout for is delamination. If there is only very slightdelamination around the edge, there may not be asignificant problem but, unfortunately, once theprocess has begun, it may often continue to otherparts of the filter and finally radically degrade thequality of the image. When there is even slightdelamination, especially in an analyzer, the best

course is to replace the filter. With some systems this can be a difficult challenge for an amateur and may require professional (expensive) replacement.

Luckily, even with simply good, as opposed to excellent, filters, one can achieve spectacular andsatisfying results, so long as you're not trying to docritical research or win prizes or grants. Certainspecimens can be greatly enhanced by theintroduction of a compensator into the light path. Ifyou are fortunate enough to have a dedicatedpolarizing microscope, then compensators shouldhave come with it. If, however, you have a large,older, modular research microscope with slots thatwill accommodate an analyzer and compensators,they may or may not have been included and topurchase them as accessories, if you can find them,can be quite expensive. There was a company thatmade 2" x 2" plastic squares of material that were 1/4 wavelength and full wavelength at $4 each. Recently, I was checking on these for a friend andfound that they are no longer available. For mypurposes, the full wave plate is the most useful (notto mention the one which tends to give the mostcolorful results). It is also known as a Red 1 plate ora Rot 1 Plate (which is the German designationfound on compensators from companies such asLeitz and Zeiss. Rot is the German word for "red"and rhymes with "note"). If, however, you can't find

any compensators, don't worry. You can get some very nice, if somewhat random results, using the lid (or bottom) of a plastic Petri dish. There are so many different kinds of plastics these days that you may want to experiment a bit until you find a type that gives you the most satisfying results. By tilting the dish at various angles, you can vary the color patterns. I use this make-shift device frequently with my stereo-dissecting microscope and a neat, little polarizing accessory which I discussed in an article recently. The rim of the Petri dish in not a problem in this instance where there is plenty of working distance. With a compound microscope, however, working distance becomes much more of a factor especially with objectives having a magnification greater than 10x. Fortunately, there is an easy solution; cut off the rim of the Petri dish and what you have left is a flat, plastic disk that is quite easy to handle. Here is an example of crystals with and without my Petri compensator. The first image is with polarization only and the second combines polarization and a Petri dish compensator. To my eye, the colors in the second image are subtler and more pleasing.

Experimenting with various types of illumination can be highly gratifying and provide not only enjoyment, but additional perspectives and information that enhance our understanding.

All comments to the author Richard Howey are welcomed.
If email software is not linked to a browser, right click above link and use the copy email address feature to manually transfer.

Editor's note: Visit Richard Howey's new website at http://rhowey.googlepages.com/home where he plans to share aspects of his wide interests.

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Your Horrorscope for 2022

Brought to You by The Malaprop Institute

Under the Auspices of The Malaprop Foundation

Founded and Funded by Mrs. Murgatroyd Malaprop

Richard L. Howey, Wyoming, USA

WARNING: This essay is a bit of pure silliness written in the spirit of foolish optimism that this year should be better for all of us than this last year was; so if you're not in a mood for something silly, you might want to try reading the Oxford English Dictionary instead which should keep you occupied for at least a year.

Note: For those of you not familiar with Richard Sheridan's play The Rivals written in 1775 at the age of 23, he introduced the pompous and linguistically pretentious Mrs. Malaprop who became the epitome of one who misused words in egregious ways and generally abused language. Shakespeare had already used such devices and many writers and comics have

subsequently employed them, but Mrs. Malaprop remains, as it were, the grand champagne.

I'm terribly tickled that I've been selected to provide you with relevations to guide the interstitial intercourse of your journeys through 2022. Since I have had this high honor stowed upon me, I have desiderated at length as to how to streamline all of this and delaminate all the clutter. Many of the signs are outmoded and have virtually no revelance to modernistic life, therefore, I shall replace them. Furthermore, it's the height of preponderosity to have time frames overlapping between monthlies when we already have 12 perfectly good months and twelve signs in the references of signosity. All of this pompous ponderosity may have been suitable for the past old geezerinos but, in this high-trek age, we no longer require such fillosities. Therefore, we shall simply start with January and proceed from there with our own more revelant signs relating to natural and unnatural history of which everything is apart.

January. This month is, as you know, either very cold (Northern HemiSemiDemiSphere) or very hot (Southern HemiDemiSemiSphere). As a consequence, thermalosities are of virtually little use in making predictions. This applies as well to all that talk about the alignment of planetary and moonery bodies; in half the major overpopulation centers, you can't see through the cloud cover at night 87.992% of the time and in the others, you can't see through the smog

97.223% of the time. So, unless your astrolaborer lives on the Space Station, which I don't, then none of this data is revalent, simplistically because it's not available. So, my approach has always been directness, simplicity, and non-complexification. My approach involves both synesthesia and anesthesia. I use Tarrif Cards, large Quartz and Amythyst crystals, Oreo Snack Paks, Absinthe (the original Wormwood version), and Eye of Sir Isaac Newt (just kidding).

To you, my fateful believers, I offer my annual review completely free. For weekly daily forecasts, there is a fee and you can find out what that will be by requesting and completing a financial form available at Malaprop.horrorscope@greed.com. We also have an extensive catalog of items for sale. Hurry, don't let your friends outcrass you.

So, what's in store for January now that we've gotten all of those preluminaries out of the way, except for one; if you wish to make a donation, please use, PreyBuddy and send it to Malaprop.malaprop@malaprop.com.

January will start the year off with a slow progression into other months. Events will take place a day at a time, so refrain from acting preciptatiously, especially if it's snowing or raining or screechingly hot. Under no circumstands should you launch into any new major enterprises; however, minor enterprises could be most regarding. If new creative opportunities come

your way, ignore them, unless they are very good and offer a 7-figure salary. January can be regarded as a startup month–you keep having to get someone to help you startup your car, startup your new business, startup your Electron Microscope. So, clearly, January is a good month for starting up new ventures, unless you're one of those con-genital losers.

Animal for January: Hellgrammite

Motto for January: Give 'em Hell, that is, be aggressional.

Product for Bibulation (i.e., DRINK): Single Malt Scotch

February: This is the shortest month with only 28 miserable days and 29 on leap years which I have decided to eliminate so, from now on February will have 30 days just like all the others, because I plan to make some other adjustments as well. This nonsense of having 31 days, 28 days, is just stupid when we can make 12 months each with a nice round 30 days and then we have years with a nice sane 360 days and a five day vacation. The old rhyme will go, to help out those who are a bit slow: 30 days hath September, April, June, and November and all the rest have 30 including February which also has 30.

So, clearly, February is a month for revision and revolting against old absurdities. A lot of new facts have come to the forefort in this past year.

1) Any large number of people are stupid.

2) An even larger number are ignorant; that is, mis- or uninformed.

3) A colossal number of people are understandably interested only in that which allows them to muddle along making life messianic for the rest of us.

Those in categories 1) or 2) should never be allowed to vote or decide anything involulating pubic policy.

What this boils down to is that if the majority is for something, you had best examinate it thoreauly and septically and the same applies if the majority is against something. So, whenever you are making dscisions be certain to apply your mortal compass.

Animal for February: Tardigrade.

Motto for February: Questionify everything and every body.

DRINK: Cognac

March: The March of Time, March Tunes; One, Two, Three, March; The March Hare; Sousa, The March King; March Madness.

However, in spite of all that, March has never been a very popular month: it's been regarded as rather blandacious, but mostly regarded as just a not very

nice month in the Northern, Southern, or Middle Hemispheres. Well, something dramatic needs to be done to blandish that ora of the Idles of March. So, from now on, March will be known as the Month of Festivals. Religious groups have all kinds of celebrashing days, like St. Waldorf's Day, St. Rottweiler's Day, St. Godiva's Day, St. Mephistopheles Day, and on and on. I propose that each day in March have a festival associated with it. Of course, we must have an International Ice Festival like the magnanimus one in Habin, China. Then for those in the Dessert Areas, we will have an International Camelot Race and Falconry Hunt. For the tropical forest places, we'll need a Mosquito Safari and the one who bags the most mosquitos will win a Noble Prize. International Kangaroo Races in Austria and, as you can see, it will be very easy to fill up 30 days, culminating in the Festival of Fools on April 1.

Animal: March Hair

Motto: You haven't won until everybody else has lost.

DRINK: Rum

April: A month of showers. So let's stop this nonsense about June weddings. If there are going to be bridle showers, they should be in April, which will now officiously become the Month of Showers so, for Godliness' Sake, if you haven't bathed since Christmas, now's the time! Well, there are showers of

gold, snow showers, meatier showers, and cold showers.

So what kind of month is April going to be? Well obviously, it's going to be bipolar, oscillating, indecisive, vacilliatory in extremis (and that hurts when it's in the extremis), upsy-downsy–in other words, not a good month to invest or, on the other hand, perhaps a terrific month to invest. This is a good month for coin-flipping; just make sure it doesn't have two heads or, on the other hand, two tails. Clearly, this is not a good month to read lovelorn columns or to try to date a computer.

Animal: Chameleon

Motto: If it ain't broke, don't fix it; but if you don't, who will?

DRINK: Tequila

May: This, according to poets, is the month of flowers.

The very name of this month betokens indecisiveness; we may have success, we may not; we may have weather, we may not; we may win the lottery, we may not. Clearly, this is the month of procrasstinators. It is a month full of tintintabulation and fury signifying not very much or perhaps a great deal. If you are speculating on the stock market, you should possibly exercise some cautious boldness. Now,

it may seem like May is very like April and possibly that's so in certain respects, but not in others.

The saving grace of May over April is that it is less extreme and violent than April. This is a month in which you can make definitive, tenuous plans for planting gardens, making vacation reversations. I always have reversations regarding vaccations. A nice thing about May is that you can hear the children out playing and screaming and the rintintinabulations of their dogs. Instead of having public playgrounds, we should have caves, where children can pursue these activities unseen and unherd.

Animal: Sloth

Motto: Anything that can happen probably will or won't.

DRINK: Brandy

June: this traditionally is the month of Holy Acrimony. Love emblooms and couples couple. This is a month which can cost parents a small fortune, if they have a lovesick son or daughter and God forbid they have twins. This is the perfect month for lollygrogging and not taking anything too siriusly. June is a mellow month desiduated to strolling, strumming, and stridulating. This is the month in which extremes are to be yeschewed.

Animal: Hummingbird

Motto: Heigh Ho Derrido, While Hegel Haggles.

DRINK: Vodka

July: This is a revolting month. It was in July that the storming of the Bastille transpired and the Yanks celebrated their War of Independence by issuing their Disclaimer.

So, this is the month in which to raise hellions and transformate old ways into new jars or something like that. The weather is untrustworthy and you may see turnados, whoricanes, drenching ranes, or even vulcanic eructions and drought. This is most definitively not a good time to invest in agriculture or insurance stocks.

Animal: Scorpion.

Motto: They shall beat their swords into plowshares, and their spears into pruninghooks.

DRINK: Gin and Tonic

August: Well, old P.S. Elite got it wrong; August is the cruelest month. People want to escape from where they are and end up going to vacation in places which they discover they despicate when they get there, especially when they don't know the linguistics. People living in hot places try to go to cool places; people living in cold places try to go to hot spots and all of them end up unsatiated. However, those who go on cruises on enormous ships migrate into a living

permagatory. On these ships of fools, two days and everyone you meet is repellent or needs some. Staying at home is, of course, not an enculturated option among the young, mature, middle-aged, or elderly. And even when all of this activity is supposed to be relaxipating, it turns out to be more like a game of Rushian roulette.

Animal: Badger

Motto: “Oh, how do you do”, in a tone that says “Stay the hell away from me”.

DRINK: Guiness Stout

September: This is a quite desirous month overall, since it is a transition away from the misereries of summer and a lead-in to Autumn with its grace and gentle declination. Of course, in the lower part of Earth, it is the transition into Spring which, is of course, just silly and all backwards. Anyway, we’ll just move on past nonsensical detailing of that sort. As September prepares us for October with its magical movement into the forests where the elves have been busily painting the leaves, we can start to breathe softly and heavily again. Light winds brush against our cheeks as in the famous painting September Morn. This month touches our heart strings as it leads us into future splendiferousness.

Animal: Monarch Butterfly

Motto: Make every effort to touch others and not just yourself.

DRINK: Sherry

October: Oh, glorious Nature, what a treasure you have heaped upon our heavy brows as a bas relief. Trees shimmying in the wind; leaves of redolent colors ripening on the branches; watching as they gradually turn tan and drift down to their grounding below. Now, of course, you have to rake the damned things up. Nonetheless, it's worth it to have the anesthetic experience. This is the month of delicate sediments

Animal: Peacock

Motto: Nothing can be finer than to be in Octoberina.

DRINK: Marsala or Amaretto

November: This is the month for the tough, sturdy sorts. The weather will be temperestuous and overall quite unpredicatable. This will extend to the stock markets as well, particularly the Tao Jones and the NASQUACK. If you're going to invest, put your money in things that will hold up like steel, concrete, and bridges. Interpersonal relationships could be quite stormy and you may find yourself deeply conflicticated on all frontages. It's best to venture boldly forth while keeping all activity at a minimal.

Animal: Rhinoceros

Motto: When things get tough, hibernate.

DRINK: Irish Whiskey

December: This is the month of festivities and many religious and soccular cerebrations. In some climes, we have shimmering snow and ice cycles. People descorate trees with lights, candy canes, orbaments, and tinsel creating a colorful, cherry environment. All over the globe, individuals try to set aside their differentials and seek out arenas of agreement, so that everyone can indulge in peace and quietude. This also happens to be the month in which many very brilliant individuals have been bourne throughout histerical circumstances. Seek out good companions and avoid confictications. This is a time to buy lavish gifts for yourself and something passable for others, things that look like they cost more than they did.

Animal: Ermine

Motto: Peace on Earth and Google Will to All Men (and, of course, Women, and Others).

DRINK: Martinis or Whiskey Sours

Remember that the above provisions only the bearest outliners of the year and for a very modest cost at the cites listed at the beginning, you can get much more informativation specially designated just for you. So, wishing you the finest New Year, I send my unquantifed greetings.

All comments to the author Richard Howey are welcomed.
If email software is not linked to a browser, right click above link and use the copy email address feature to manually transfer.

Editor's note: Visit Richard Howey's new website at http://rhowey.googlepages.com/home where he plans to share aspects of his wide interests.

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Geometry of *Diploneis crabro*

This article explores the geometry of the outside of two valves of the diatom *Diploneis crabro*. Several optical slices were explored by measuring the depths of the slices downward through the valve from the slice closest to the objective. This is a marine naviculoid genus that was collected in Hawaii in 2015, cleaned in hydrogen peroxide, and mounted in Zrax.

Diploneis crabro is distinguished from other large, constricted (panduriform) *Diploneis* by having biseriate striae near the margin of the valve.

In these two valves, the stria counts fit Lobban et al. (2012) but the sizes are outside the range given. Sims (ed.) (1996 pl 78 fig 6 and pl 79 1-7) on the other hand shows a range of sizes and stria counts that include the morphometrics of these two valves. These sources seem to confirm that *D. crabro* is an accurate identification.

All images were taken with a 100x objective in bright field. Depth measurements were made with the calibrations on the fine focus knob of the Nikon Labophot-2 used here.

Depth is the apparent depth of an optical slice below the high focus. The author had no accurate way to measure the real depth.

High focus is the optical slice closest to the objective.

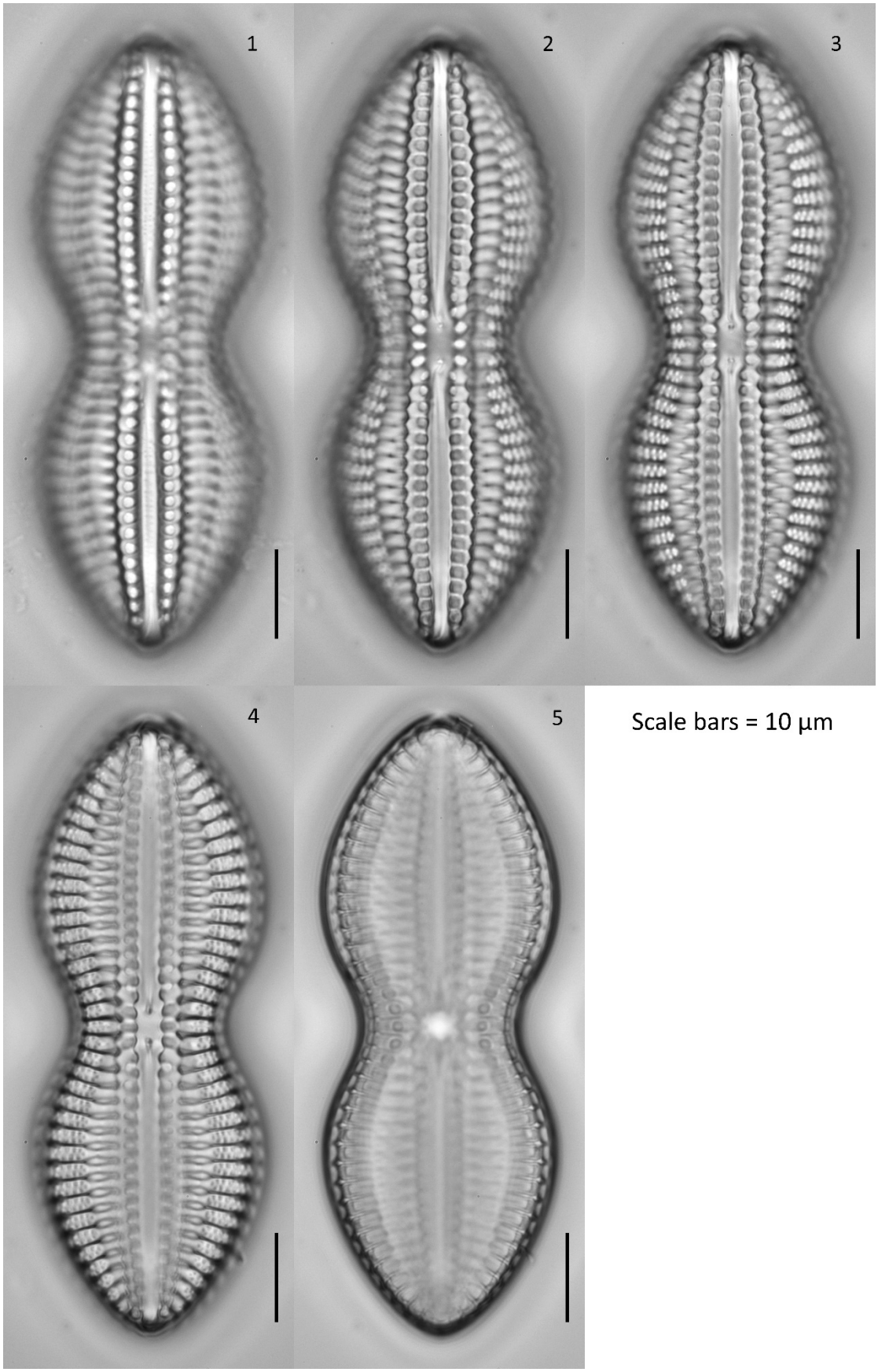
PLATE 1

Figures 1-5

Focal planes of one valve, external view.
Morphometrics: length 70 µm, width 19 µm, striae 6 in 10 µm.
Slide Honaunau-K, Nikon coordinates 352-946, slide catalog # 16.
Image file numbers are in parentheses.

Figure	Depth µm	Note
1	0	High focus on outside of pores in external wall of the longitudinal canal. (8212)
2	1	White spot focus on proximal and distal raphe ends. Distal raphe ends curve toward the secondary side of valve. (8213)
3	2	White spot focus on areolae of striae. (8214)
4	3	Black spot focus on areolae of striae. (8215)
5	5.5	Focus on distal ends of ribs between striae. (8216)

Geometry of *Diploneis crabro*



Scale bars = 10 µm

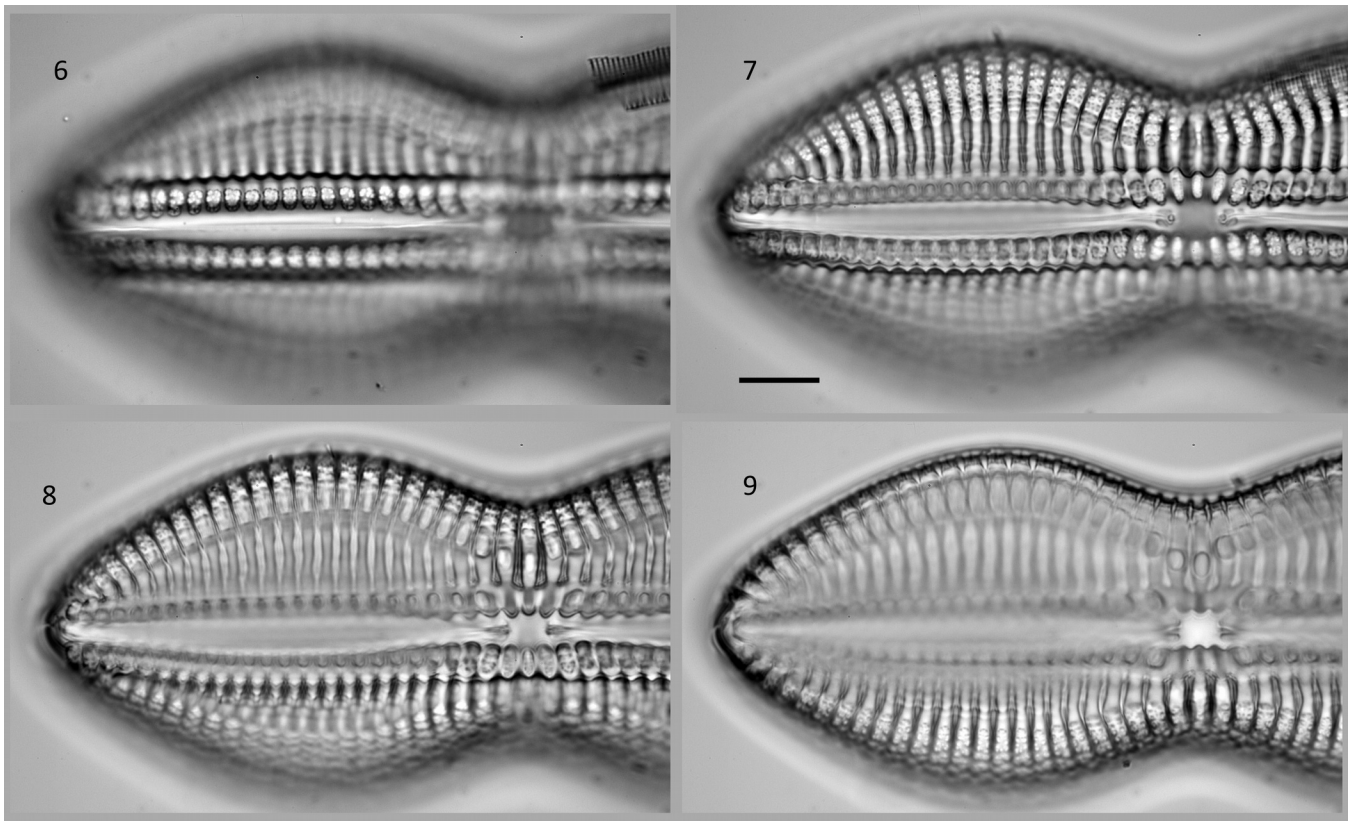
PLATE 2

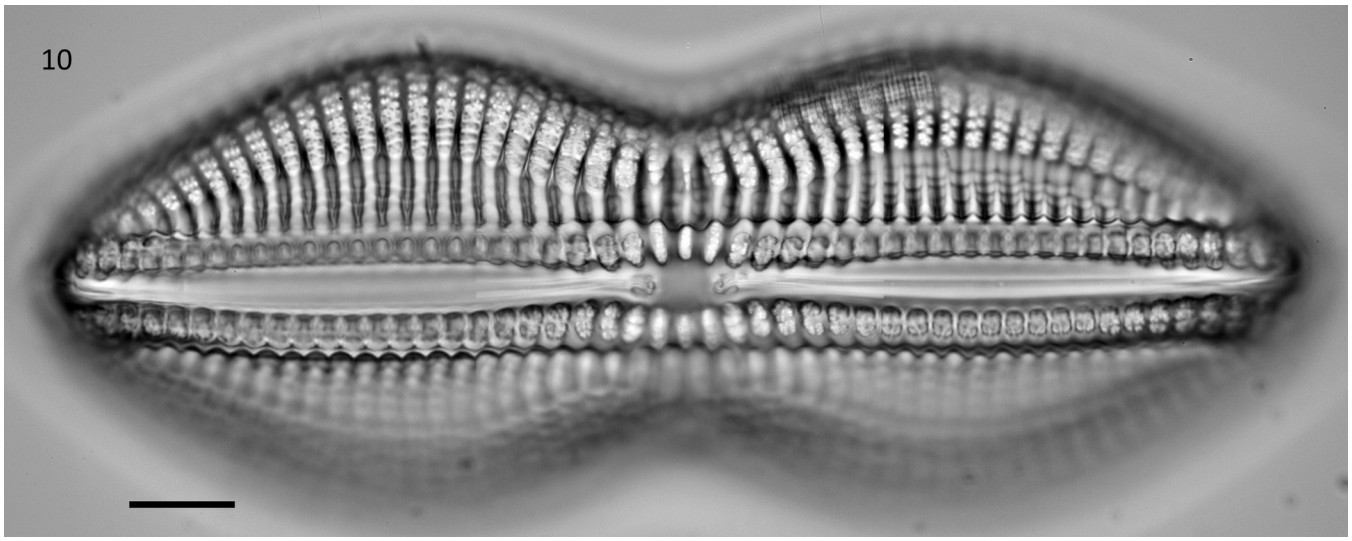
Figures 6-10

Focal planes of one valve, external view. This valve is tipped with the high side at the top of the image.
Morphometrics: length 121 µm, width 31 µm, striae 4 in 10 µm.
Slide Honaunau-K, Nikon coordinates 345-920, slide catalog # 5.
Image file numbers are in parentheses.
Scale bars = 10 µm.

Figure	Depth µm	Note
6	0	High focus on external pores of longitudinal canal. (8222)
7	1.75	Focus on curly proximal raphe ends. (8220)
8	4.5	Focus on edge of central area. (8223)
9	6.25	Focus on distal ends of ribs between striae in middle of the valve. (8224)
10	1.75	Full valve, two images stitched. Focus on curly proximal raphe ends. (8220-8221)

Geometry of *Diploneis crabro*





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Geometry of *Nitzschia coarctata*

This article explores the geometry of the inside of a valve of the diatom *Nitzschia coarctata* by measuring the depths of optical slices downward through the valve from the highest focus point on the valve. This species is a marine nitzschioid diatom that was collected in Hawaii in 2015, cleaned in hydrogen peroxide, and mounted in Zrax.

A simple model may help to visualize the shape of the valve. With a rectangular sheet of paper, make a narrow fold on the long edge to represent the keel. Turn the paper so the keel is on the left. (The raphe slit would be along the left edge of the keel.) On the long axis, gently curve the left side upward and the right side downward. Since this specimen is presenting an internal view, the upward curve on the left is the downward undulation and the downward curve on the right is the upward undulation when viewed externally.

The identification follows Lobban et al. (2012) in their comparison to *N. constricta.* This specimen has a stria count of 10.5 in 10 µm which is in the range for *N. coarctata*. The scanning electron micrographs on plate 59 figs 6-8 of *N. constricta* resemble this species and may be useful for understanding the geometry. Sims (ed.) (1996) pl 182 fig 3 calls *N. coarctata* a *Tryblionella* but this specimen lacks the hyaline central area.

This article was started on 11 Dec 2021 by Rob Kimmich, Salem, Oregon, US and last revised on 20 Dec 2021.

All images were taken with a 100x objective in bright field. Depth measurements were made with the calibrations on the fine focus knob of the Nikon Labophot-2 used here.

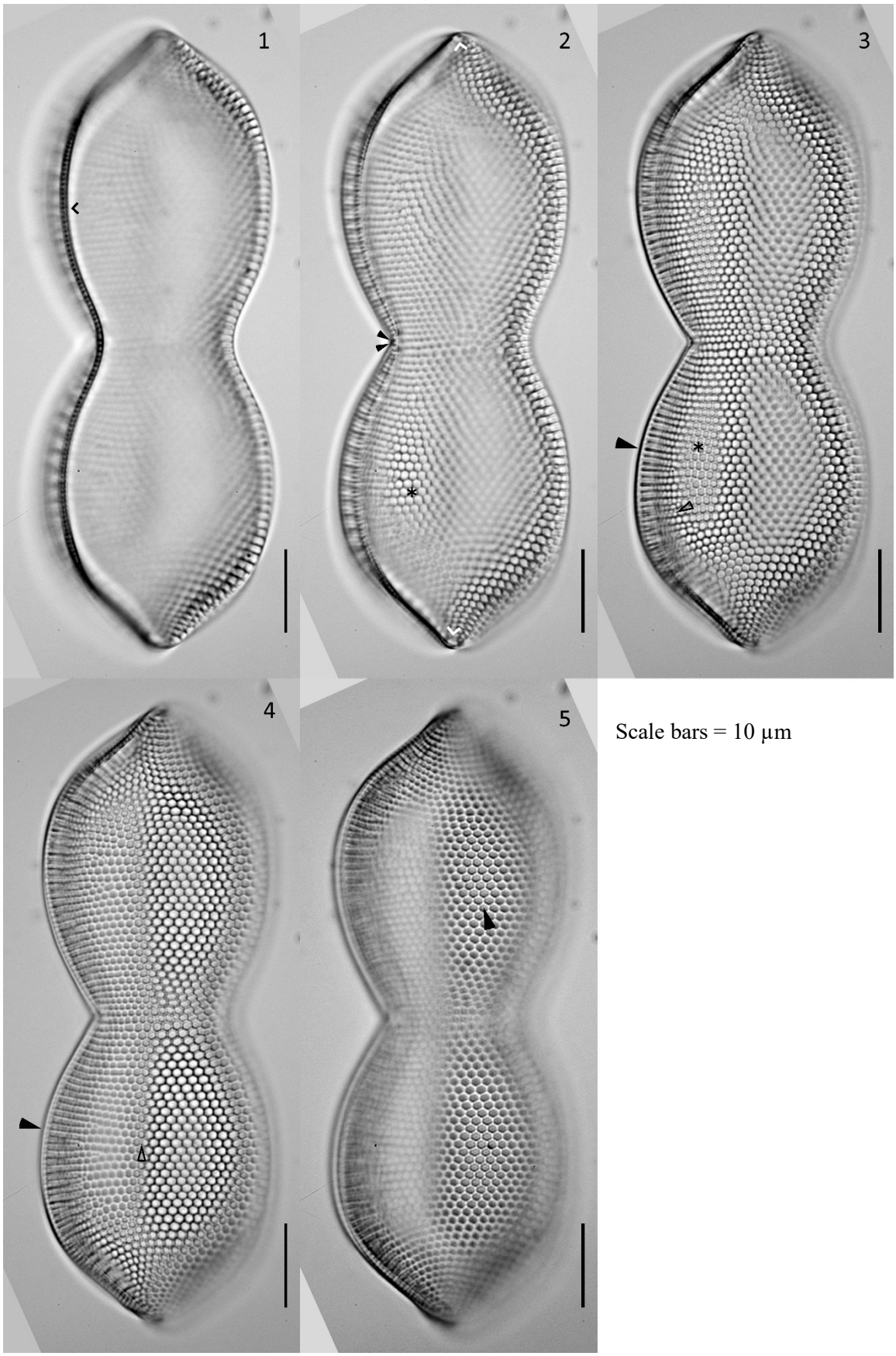
Depth is the apparent depth of an optical slice below the high focus. The author had no accurate way to measure the refractive index to find the real depth.

High focus is the optical slice closest to the slide side of the coverslip.

Figure	Depth	Note
1	0 µm	High focus. **Caret** marks edge of valve. Dark lines on the edge are ends of fibulae.
2	3 µm	**Asterisk** marks the inside of the areolae on inside of the low undulation. **White carets** mark distal raphe ends. **Black darts** mark the proximal raphe ends.
3	4 µm	**Asterisk** marks the outside of the areolae on the outside surface of the valve in the low undulation. These areolae form a network of hexagons [1]. **Solid dart** marks the edge of the keel and a fibula which appears as a dark bar on the keel. **Hollow dart** marks an example of a stria that is biseriate near the base of the keel.
4	4.5 µm	**Solid dart** on the keel marks a fibula which is now white because of the depth change. **Hollow dart** points along the apical fold between undulations.
5	5 µm	**Solid dart** on the right marks the outside of an areola in focus on the high undulation in the hexagon network on the outside of the valve.

1. Lobban et al. (2012) plate 59 figs 6-8 shows this pattern in a similar species, *N. constricta.*

Geometry of *Nitzschia coarctata*



Scale bars = 10 µm

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