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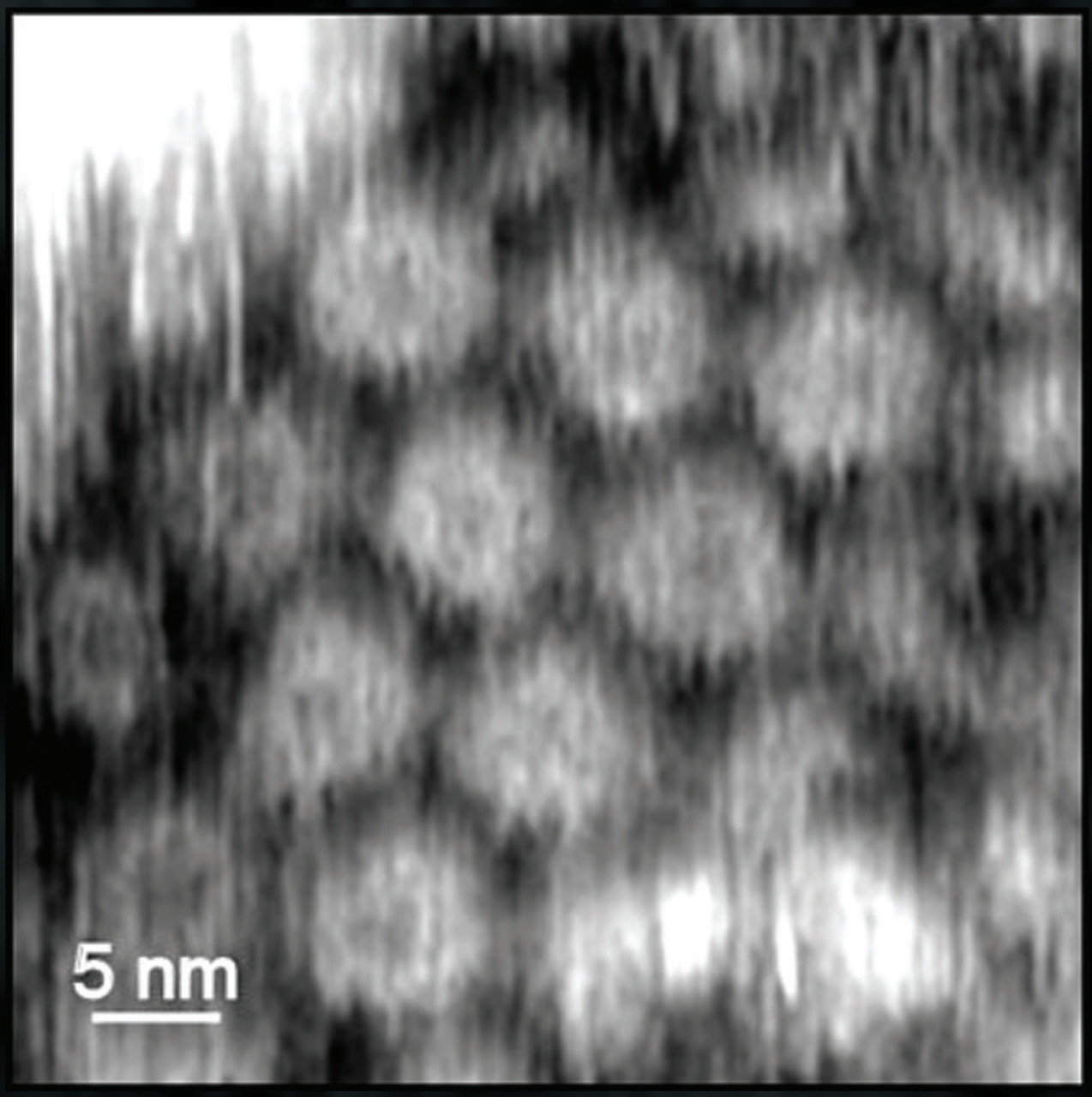
MICROSCOPY AND ANALYSIS



INVESTIGATING **MOSAIC** VIRUSES WITH **AFM**

Diatoms: the best microscopic objects to check, set and compare optical microscopes and contrast techniques

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BIOGRAPHY
Stefano Barone is the founder and owner of Diatom Lab, the scientific laboratory company that specializes in test slides for microscopy, micromanipulation of diatoms, radiolarians and other microscopic objects, plus microscopy imaging services. He has greatly improved the mounting and micromanipulation techniques of diatoms and radiolarians, thanks to the invention of new state-of-the-art techniques, instruments and proprietary materials, in order to meet the requirements of modern high performance microscopes. Stefano Barone has also been a publicist, nature photographer and science popularizer for more than twenty years.

ABSTRACT
No microscopic objects are more suitable and accurate than test diatoms for testing microscopes and compare microscope models, objectives, condensers and contrast techniques with each other. Optics have greatly improved over the centuries, many of the smallest structures of diatoms were already resolvable in the eighteenth century albeit with a reduced quality, but some structures can only be resolved by means of the most modern and performing optics and contrast techniques.

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INTRODUCTION

Charles Darwin in his renowned publication *On the origin of species* stated "Few objects are more beautiful than the minute siliceous cases of the diatomaceæ: were these created that they might be examined and admired under the higher powers of the microscope?"[9]. In fact diatom frustules, thanks to their innumerable wonderful forms and their tiny details (striae, areolae, pores and so on), are among the most intriguing and mesmerizing objects to observe under a microscope (figure 1). And since the appearance of this instrument, some species (called test diatoms by microscopists) have been and still are used to test optics, because striae and areolae distances in a same species collected in a same area are constant regardless the size of the frustule. In other words test diatoms are extremely valid and accurate to quantify resolution of optical microscopes, defined as the smallest distance between to points on a specimen that can still be distinguished by the observer or camera system as two separate entities. It follows that test diatoms are also the elite tools to compare microscope models, objectives, condensers and contrast techniques with each other.

Optics have greatly improved over the centuries, many of the smallest structures of diatoms were already resolvable in the eighteenth century albeit with a reduced quality, but some structures can only be resolved or detected by means of the most modern and performing optics. It can be argued that diatoms have forced microscope manufacturers to make progress, in fact Smith, J. Edwards wrote in 1885: "Luckily, there were those who would at times 'fight objectives,' play with diatoms, etc., and in response to their demands the optician increased his angles and working force of the object-glasses. To meet this in turn called for the construction of condensers of greater angle; until finally it occurred that the aperture of the objective had arrived at proportions to which the condensers did not satisfactorily respond. Something had to be done, and something was done, for necessity is the mother of invention; in the same book the author states forcefully "When we say that diatoms are the most convenient objects over which to study the adjustment of the objective, we mean it, and thereto attaches greater force than the casual reader may suppose."[12]

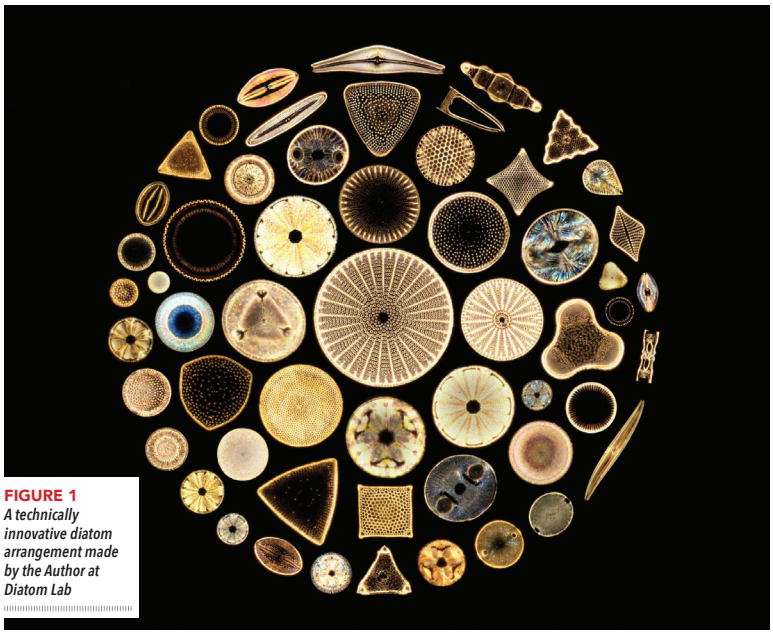

FIGURE 1
A technically innovative diatom arrangement made by the Author at Diatom Lab

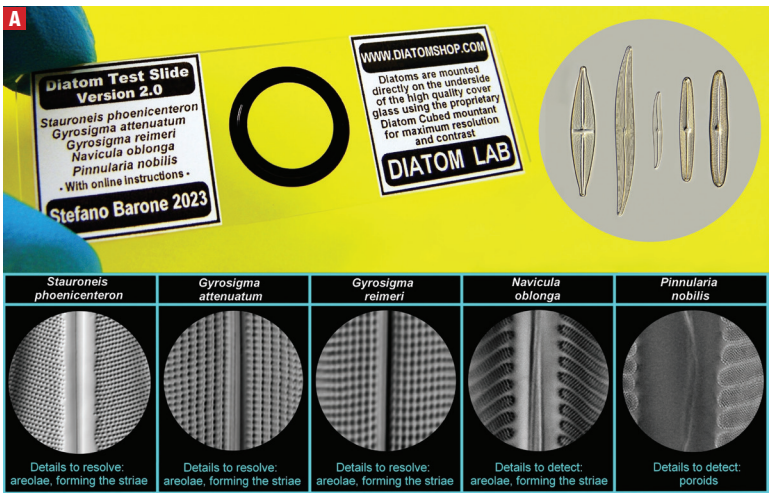

FIGURE 2A, *top, The Diatom Test Slide Version 2.0 by Diatom Lab with its five selected, micromanipulated diatoms at low magnification and their resolved details;*

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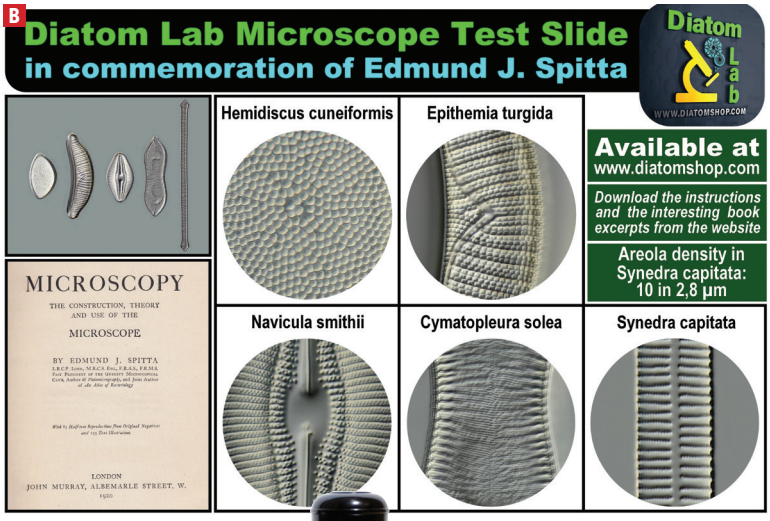

FIGURE 2B, *right, The Diatom Lab Microscope Test Slide in commemoration of Edmund J. Spitta with its five selected, micromanipulated diatoms at low magnification and their resolved details.*

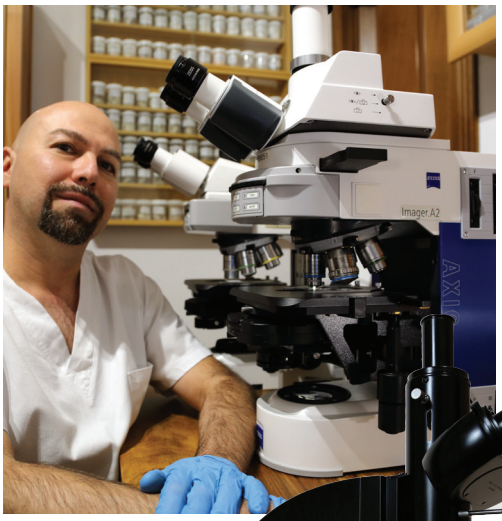

FIGURE 3, *Stefano Barone in a corner of Diatom Lab, next to his Zeiss Axio Imager.A2 research microscope.*

FIGURE 4, *above, The legendary Nikon Model H field microscope, produced in the 1960s. From the Diatom Lab collection*

FIGURE 5, *right, This impressive Leitz Ortholux I black enamel microscope was produced around 1967. Below: A cased complete Leitz phase contrast set with Heine condenser, usable with the Leitz Ortholux I microscope. From the Diatom Lab collection*

FIGURE 6, *above left, The Zeiss Jena Lumipan catalog and a cased complete Zeiss Jena phase contrast set for the Zeiss Jena Lumipan. From the Diatom Lab collection*

FIGURE 7, *above, A Zeiss Jena black enamel L Wd E microscope produced in 1941, equipped with the extremely rare Zeiss Jena ocular revolver. and, above right, two Zeiss Jena Siedentopf cardioid condensers for dark field illumination. From the Diatom Lab collection*

This work is a journey into the past up to the present day, along with a set of experiments to understand how microscopy has evolved, thanks to a small part of the many antique, vintage and high-end modern microscopes kept at Diatom Lab.

MATERIALS AND METHODS

Although Diatom Lab produces and offers several microscope slides of test diatoms, for this work two products have been used: Figure 2a shows the Diatom Test Slide version 2.0 – that contains: *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg 1843; *Gyrosigma attenuatum* (Kützing) Rabenhorst 1853; *Gyrosigma reimeri* Sterrenburg, 1994; *Navicula oblonga* (Kützing) Kützing and *Pinnularia nobilis* (Ehrenberg) Ehrenberg 1843 – and the Microscope Test Slide in commemoration of Edmund J. Spitta (figure 2b) – that contains *Hemidiscus cuneiformis* Wallich 1860; *Epithemia turgida* (Ehrenberg) Kützing 1844; *Navicula smithii* Brébisson, 1856; *Cymatopleura solea* (Brébisson) W.Smith 1851; *Synedra capitata* Ehrenberg, 1836 Synonym: *Synedra ulna f. capitata* (Ehrenberg) Skabichevskii, 1960.

Among all these species, the following have been selected for the experiments in question:

1) *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg 1843. Details to resolve: areolae (an areola is one of the pores in a row that forms a stria on a valve. The plural is areolae), forming the striae (a stria is a row of areolae. The plural is striae). Number of striae in 10 µm: 12-15 longitudinal;

2) *Gyrosigma reimeri* Sterrenburg, 1994. Details to resolve: areolae, forming the straie. Striae in 10 µm: 18-22 longitudinal;

3) *Navicula oblonga* (Kützing) Kützing. Details to detect: areolae (lineolae), forming the striae. Areolae (lineolae) in 10 µm: 48-50. Average distance between (lineolae): 0,14 µm;

4) *Synedra capitata* Ehrenberg, 1836. Measured areolae density: 10 in 2,8 µm;

5) *Pinnularia nobilis* (Ehrenberg) Ehrenberg 1843. Details to detect: poroids (small holes on the diatom valve). Average distance bewteen poroids: 0,11 µm.

As is the case with all Diatom Lab innovative microscope slides of selected, micromanipulated diatoms, specimens are fixed directly to the underside of the custom optical quality cover glass using the proprietary Nano-adhesive and Diatom Cubed high refractive index mountant, to ensure maximum optic performances.

The following microscopes were selected:

A) Zeiss Axio Imager.A2 high-end modern research microscope for transmitted and reflected light. For the occasion it was equipped with Zeiss Achromatic-Aplanatic condenser 1.4 H D Ph DIC, Zeiss Achromatic-Aplanatic universal condenser 0.9 H/0.8-0.9 DF with Darkfield attachment 1.2-1.4 Oil

FIGURE 8 *An antique Koristka microscope, large model IIC with wide tube ideal for photomicrography. Appeared in a Koristka catalog published in 1908.* From the Diatom Lab collection

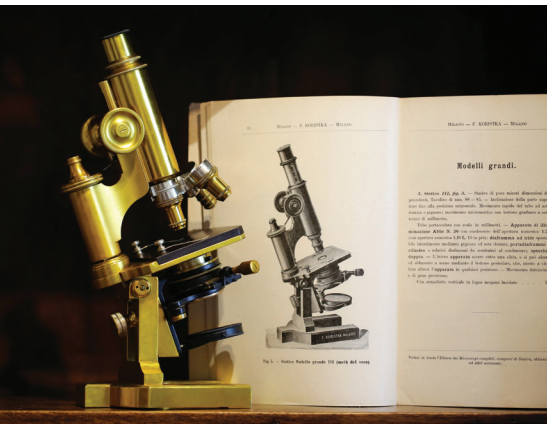

FIGURE 9, *above, An antique Koristka microscope, large model III. Appeared in a Koristka catalog published in 1894 and, below, two antique Koristka paraboloid condensers for dark field illumination, with three funnel inserts.* From the Diatom Lab collection

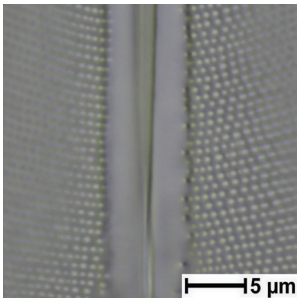

FIGURE 10 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, with clearly visible striae and areolae. Technique: simple (without any boosters) bright field. Microscope: Zeiss Axio Imager.A2. Lens: Zeiss Plan-Apochromat 63x/1.4 Oil DIC ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic condenser 1.4 H D Ph DIC.*

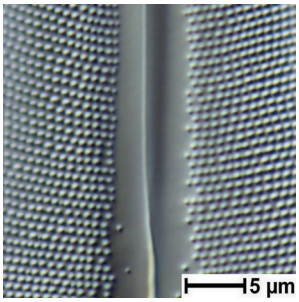

FIGURE 11 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, with clearly visible striae and areolae. Technique: differential interference contrast (DIC). Microscope: Zeiss Axio Imager.A2. Lens: Zeiss Plan-Apochromat 63x/1.4 Oil DIC ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic condenser 1.4 H D Ph DIC.*

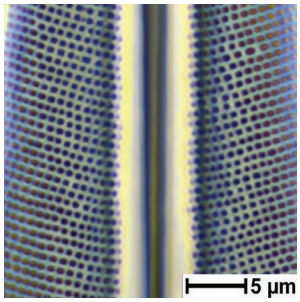

FIGURE 12 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, with clearly visible striae and areolae. Technique: phase contrast. Microscope: Zeiss Axio Imager. A2. Lens: Zeiss Plan-Apochromat 63x/1.4 Oil Ph3 ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic condenser 1.4 H D Ph DIC.*

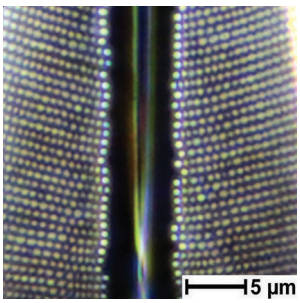

FIGURE 13 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, with clearly visible striae and areolae. Technique: dark field illumination. Microscope: Zeiss Axio Imager. A2. Lens: Zeiss Plan-Neofluar 63x/1.25 Oil Iris ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic universal condenser 0.9 H/0.8-0.9 DF with Darkfield attachment 1.2-1.4 Oil*

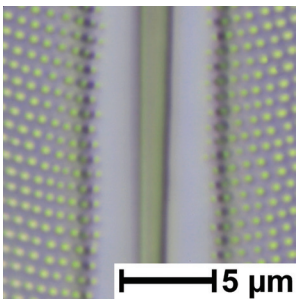

FIGURE 14 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, with clearly visible striae and areolae. Technique: simple (without any boosters) bright field. Microscope: Zeiss Axio Imager.A2. Lens: Zeiss EC Plan-Neofluar 100x/1.3 Oil ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic condenser 1.4 H D Ph DIC.*

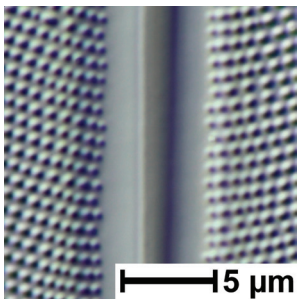

FIGURE 15 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, with clearly visible striae and areolae. Technique: differential interference contrast (DIC). Microscope: Zeiss Axio Imager.A2. Lens: Zeiss EC Plan-Neofluar 100x/1.3 Oil ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic condenser 1.4 H D Ph DIC.*

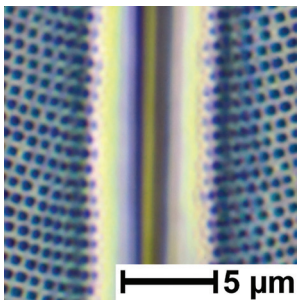

FIGURE 16 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, with clearly visible striae and areolae. Technique: phase contrast. Microscope: Zeiss Axio Imager. A2. Lens: Zeiss EC Plan-Neofluar 100x/1.3 Oil Ph3 ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic condenser 1.4 H D Ph DIC.*

and Zeiss VIS-LED illumination unit (figure 3);

B) Nikon Model H field microscope, serial number 43046 (the invoice sent to the first owner dates back to 1979) (figure 4) ;

C) Leitz Wetzlar Ortholux I black enamel microscope, serial number 717883 , manufactured around 1967, (equipped with Leitz Heine condenser and Pv objectives (figure 5);

D) Zeiss Jena Lumipan black enamel microscope, serial number 337858, produced in the Fifties (figure 6). Equipped with its phase contrast set and several other lenses.

E) Zeiss Jena L Wd E black enamel microscope, serial number 283531, manufactured in 1941 (figure 7), equipped with Zeiss Jena Aplanatic condenser N.A. 1,4, Zeiss Jena Siedentopf 1.0 Cardioid condenser for darkfield illumination and Zeiss Jena separate lamp with field diaphragm for Köhler illumination;

F) Koristka brass microscope, Large model IIc – appeared in a Koristka catalogue published in 1908 – (figure 8) equipped with Abbe illuminating apparatus N.A. 1.40 and separate lamp;

G) Koristka brass microscope, Large model III – appeared in a Koristka catalogue published in 1894 – (figure 9, equipped with Koristka paraboloid condenser for darkfield illumination and separate lamp.

All vintage and antique instruments kept at Diatom Lab comes from unsold stocks or are at least in perfect condition, to ensure the accuracy of the tests.

Immersion oils were selected according to the makers notes: Zeiss Immersol 518 N for Zeiss Axio Imager. A2; anisol for Nikon Model H and cedar wood oil for all other models.

All the photomicrographs presented here do not show the entire field of view visible under the microscope, but only a portion of the diatom to better illustrate this work: the microscopy methods and the optics used are fully listed in the description of the images. Furthermore, although *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg 1843 can be easily resolved using dry objectives and dry condensers, for this work it was decided to observe it in oil immersion to maximize detail magnification and above all to give an extra chance to vintage and antique optics. All condenser top lenses have been oiled to fully benefit from their high numerical aperture (they are all immersion types), except that of Nikon Model H that is not meant for this type of procedure.

RESULTS

ZEISS AXIO IMAGER.A2 (PHOTOMICROGRAPHS NUMBERED FROM 10 TO 26)

The high-end, modern Zeiss Axio Imager.A2 research microscope not only guaranteed the best results in terms of resolution, contrast and sharpness of the diatom details (figures from 10 to 29), but it was also the only microscope capable of resolving the first four aforementioned species used for the experiments with excellent results without any additional booster (for example blue filter, oblique illumination) and detecting/imaging the poroids of *Pinnularia nobilis* (Ehrenberg) Ehrenberg 1843 in double immersion (= oil immersion objective and oil immersion condenser 1.4 N.A.) either with polarized light or with immersion dark field (look at the last photomicrograph of Figure 2a obtained in immersion dark field). Several *Pinnularia nobilis* (Ehrenberg) Ehrenberg 1843 that belongs to the same sample used for the Diatom Test Slide version 2.0 have been measured under SEM to find out that the average distance between poroids is 0,11 µm: the theoretical limit of resolution of most light microscopes is 0,2 µm, but these poroids can be clearly detected (and also imaged) thanks to the specimens fixed directly to the underside of the custom optical quality cover glass, the proprietary Nano-adhesive, the Diatom Cubed high refractive index mountant and the two aforementioned microscopy methods using high quality optics. For example it is known that dark field illumination permits to detect and image the presence of microscopic objects that are not visible in bright field, even placed at the resolution limit or even below it[8]. Also DIC (and I add some of its variants, such as AVEC-DIC, the Allen Video-enhanced Contrast) "can provide sufficient contrast to detect objects smaller than the resolving power of the microscope. DIC has been used to visualize single microtubules – protein polymers that are 0,025 microns in width, or almost one-tenth the limiting resolution of the microscope"[11].
By examining the photomicrographs, we can notice that "simple" (= without any booster such as oblique illumination) bright field in Zeiss Axio Imager.A2 gives even better results

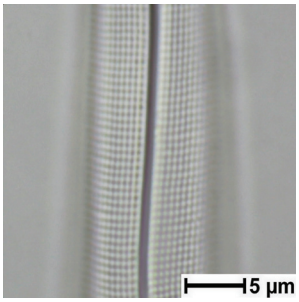

FIGURE 17 *Photomicrograph of Gyrosigma reimeri Sterrenburg, 1994, with clearly visible striae and areolae. Technique: simple (without any boosters) bright field. Microscope: Zeiss Axio Imager.A2. Lens: Zeiss Plan-Apochromat 63x/1.4 Oil DIC ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic condenser 1.4 H D Ph DIC.*

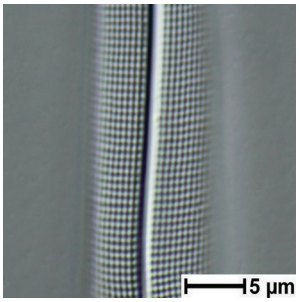

FIGURE 18 *Photomicrograph of Gyrosigma reimeri Sterrenburg, 1994, with clearly visible striae and areolae. Technique: differential interference contrast (DIC). Microscope: Zeiss Axio Imager.A2. Lens: Zeiss Plan-Apochromat 63x/1.4 Oil DIC ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic condenser 1.4 H D Ph DIC.*

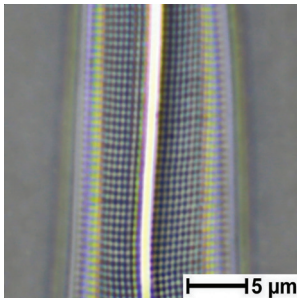

FIGURE 19 *Photomicrograph of Gyrosigma reimeri Sterrenburg, 1994, with clearly visible striae and areolae. Technique: phase contrast. Microscope: Zeiss Axio Imager.A2. Lens: Zeiss Plan-Apochromat 63x/1.4 Oil Ph3 ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic condenser 1.4 H D Ph DIC.*

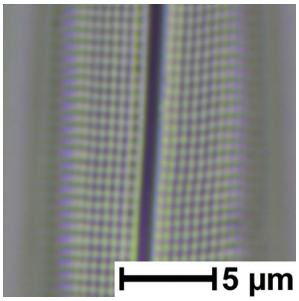

FIGURE 20 *Photomicrograph of Gyrosigma reimeri Sterrenburg, 1994, with clearly visible striae and areolae. Technique: simple (without any boosters) bright field. Microscope: Zeiss Axio Imager.A2. Lens: Zeiss EC Plan-Neofluar 100x/1.3 Oil ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic condenser 1.4 H D Ph DIC*

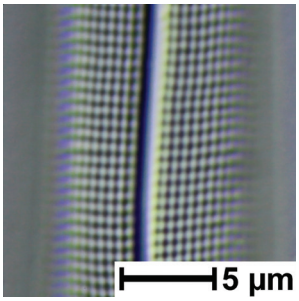

FIGURE 21 *Photomicrograph of Gyrosigma reimeri Sterrenburg, 1994, with clearly visible striae and areolae. Technique: differential interference contrast (DIC). Microscope: Zeiss Axio Imager.A2. Lens: Zeiss EC Plan-Neofluar 100x/1.3 Oil ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic condenser 1.4 H D Ph DIC*

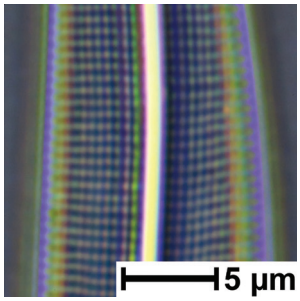

FIGURE 22 *Photomicrograph of Gyrosigma reimeri Sterrenburg, 1994, with clearly visible striae and areolae. Technique: phase contrast. Microscope: Zeiss Axio Imager.A2. Lens: Zeiss EC Plan-Neofluar 100x/1.3 Oil Ph3 ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic condenser 1.4 H D Ph DIC*

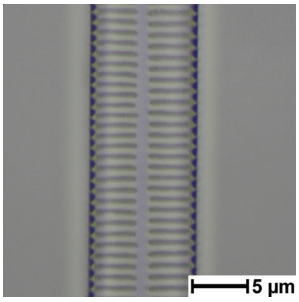

FIGURE 24 *Photomicrograph of Synedra capitata Ehrenberg, 1836, with clearly visible striae and areolae. Technique: simple (without any boosters) bright field. Microscope: Zeiss Axio Imager.A2. Lens: Zeiss EC Plan-Neofluar 100x/1.3 Oil ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic condenser 1.4 H D Ph DIC*

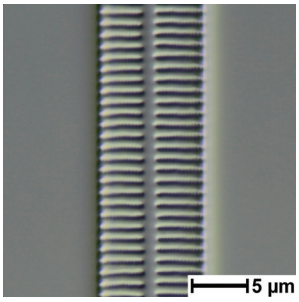

FIGURE 25 *Photomicrograph of Synedra capitata Ehrenberg, 1836, with clearly visible striae and areolae. Technique: differential interference contrast (DIC). Microscope: Zeiss Axio Imager.A2. Lens: Zeiss EC Plan-Neofluar 100x/1.3 Oil ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic condenser 1.4 H D Ph DIC*

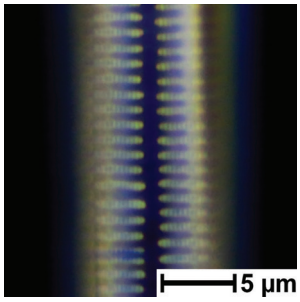

FIGURE 26 *Photomicrograph of Synedra capitata Ehrenberg, 1836, with clearly visible striae and areolae. Technique: dark field illumination. Microscope: Zeiss Axio Imager. A2. Lens: Zeiss Plan-Neofluar 63x/1.25 Oil Iris ∞ /0.17 M27. Zeiss Optovar (magnification changer) 2,5x. Condenser: Zeiss Achromatic-aplanatic universal condenser 0.9 H/0.8-0.9 DF with Darkfield attachment 1.2-1.4 Oil*

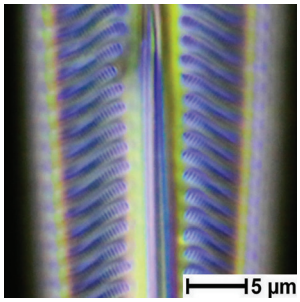

FIGURE 23 *Photomicrograph of Navicula oblonga (Kützing) Kützing, with clearly visible lineolae (areolae). Technique: dark field illumination. Microscope: Zeiss Axio Imager. A2. Lens: Zeiss Plan-Neofluar 63x/1.25 Oil Iris ∞ /0.17 M27. Condenser: Zeiss Achromatic-aplanatic universal condenser 0.9 H/0.8-0.9 DF with Darkfield attachment 1.2-1.4 Oil*

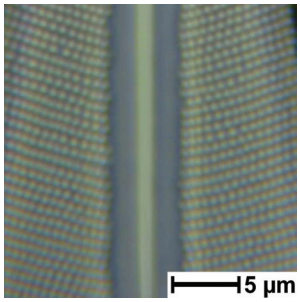

FIGURE 27 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, with discreetly visible striae and areolae. Technique: simple (without any boosters) bright field. Microscope: Nikon Model H field microscope. Lens: Nikon Achromatic 100x/1.25. Condenser: Abbe double-lens type, 0.9 N.A*

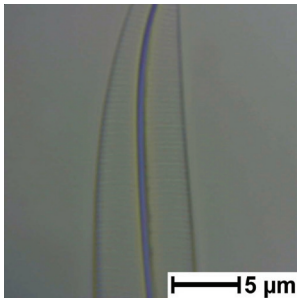

FIGURE 28 *Photomicrograph of Gyrosigma reimeri Sterrenburg, 1994, striae are not very visible, while areolae are not noticeable. Technique: simple (without any boosters) bright field. Microscope: Nikon Model H field microscope. Lens: Nikon Achromatic 100x/1.25. Condenser: Abbe double-lens type, 0.9 N.A*

(figures 10, 14, 17, 23, 27) than oblique illumination obtained with the other microscope models (!) and we know that oblique illumination enchance contrast and resolution. The Axio Imager.A2 can easily resolve the areolae (lineolae) of *Navicula oblonga* (Kützing) Kützing in immersion dark field (figure 23) without any boosters (color filters, polarization), the same results can be obtained in double immersion DIC: several *Navicula oblonga* (Kützing) Kützing that belongs to the same sample used for the Diatom Test Slide version 2.0 have been measured under SEM to find out that the average distance between poroids is 0,14 µm, and here the same observations discussed above with reference to *Pinnularia nobilis* (Ehrenberg) Ehrenberg 1843 also apply. The excellence of DIC (figures 11, 15, 18, 21, 25), ultimate phase contrast (figures 12, 16, 19, 22) and modern immersion darkfield (figures 13, 23, 26) is also evident here, moreover Axio Imager.A2 is well known for its Innovative IC²S infinity system for considerably more contrast in all established contrasting techniques.

NIKON MODEL H (PHOTOMICROGRAPHS NUMBERED FROM 27 TO 28)

The old advertisment for the legendary Nikon Model H field microscope read as follows: "A handy lab to take with you, indoors or out". I think this instrument has the primacy of the lighest travel microscope equipped with an oil immersion objective in addition to the dry ones. Considering the small size of the instrument, the areolae of *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg 1843 (figure 27) are discreetly visible with a simple (= without any boosters) bright field in oil immersion (100x/1.25 oil Achromatic lens). Regarding *Gyrosigma reimeri* Sterrenburg, 1994, (figure 28) striae are not very visible with the same lens, while areolae are not noticeable, but it is a rather difficult diatom which goes far beyond the expectations of a field microscope used to examine samples on site before taking them to the laboratory. The remaining test diatoms cannot be resolved with this model, but we must consider that the optical quality of Nikon Model H is much greater than many other portable microscopes.

LEITZ WETZLAR ORTHOLUX I (PHOTOMICROGRAPHS NUMBERED FROM 29 TO 33)

The Ortholux I, in addition to its solemn design, has the primacy of being the first truly modular microscope in history (having a very large number of interchangeable accessories for the different techniques). For the experiments in question the complete Leitz phase contrast set with Heine condenser was chosen, as the screw-on immersion cap gives a high numerical to 1.40. With this condenser it is more appropriate to refer to a circular oblique lighting (COL) rather than true

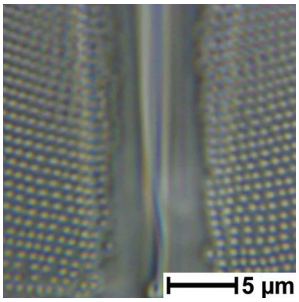

FIGURE 29 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, with visible striae and areolae. Technique: circular oblique lighting (COL). Microscope: Leitz Ortholux I. Lens: Leitz Pv Apo Oel 90x/1.15 n 170/0.17. Condenser: Leitz Heine condenser with screw-on immersion cap (numerical aperture 0.50 to 1.40)*

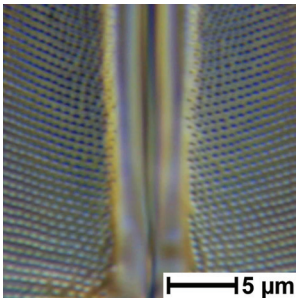

FIGURE 30 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, with visible striae and areolae. Technique: phase contrast. Microscope: Leitz Ortholux I. Lens: Leitz Pv Apo Oel 90x/1.15 n 170/0.17. Condenser: Leitz Heine condenser with screw-on immersion cap (numerical aperture 0.50 to 1.40)*

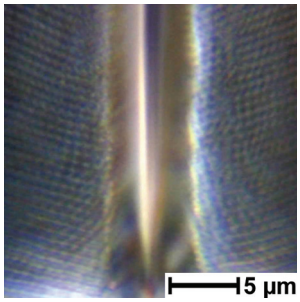

FIGURE 31 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, striae and areolae are not particularly sharp. Technique: dark field illumination. Microscope: Leitz Ortholux I. Lens: Leitz Pv Apo Oel 90x/1.15 n 170/0.17. Condenser: Leitz Heine condenser with screw-on immersion cap (numerical aperture 0.50 to 1.40)*

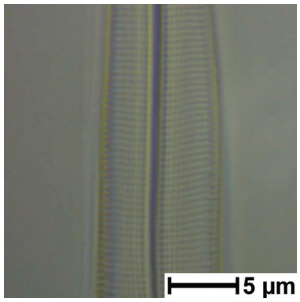

FIGURE 32 *Photomicrograph of Gyrosigma reimeri Sterrenburg, 1994, striae and areolae are not particularly sharp. Technique: circular oblique lighting (COL). Microscope: Leitz Ortholux I. Lens: Leitz Pv Apo Oel 90x/1.15 n 170/0.17. Condenser: Leitz Heine condenser with screw-on immersion cap (numerical aperture 0.50 to 1.40)*

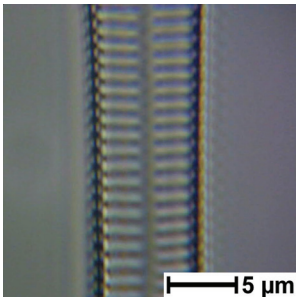

FIGURE 33 *Photomicrograph of Synedra capitata Ehrenberg, 1836, with visible striae, while areolae are blurred in the best case. Technique: circular oblique lighting (COL). Microscope: Leitz Ortholux I. Lens: Leitz Pv Apo Oel 90x/1.15 n 170/0.17. Condenser: Leitz Heine condenser with screw-on immersion cap (numerical aperture 0.50 to 1.40)*

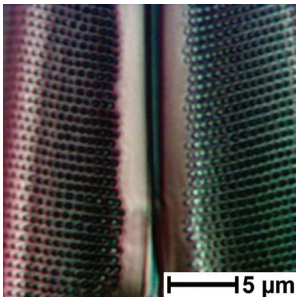

FIGURE 34 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, with clearly visible striae and areolae. Technique: phase contrast and green filter. Microscope: Zeiss Jena Lumipan. Lens: Zeiss Jena Achromat 90x/1.25 Oil Ph 160/0.17. Condenser: Zeiss Jena pancratic condenser with Aplanatic 1.4 part in position*

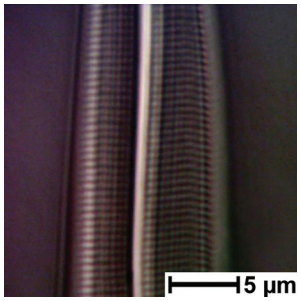

FIGURE 35 *Photomicrograph of Gyrosigma reimeri Sterrenburg, 1994, with discreetly visible striae and areolae. Technique: phase contrast and green filter. Microscope: Zeiss Jena Lumipan. Lens: Zeiss Jena Achromat 90x/1.25 Oil Ph 160/0.17. Condenser: Zeiss Jena pancratic condenser with Aplanatic 1.4 part in position*

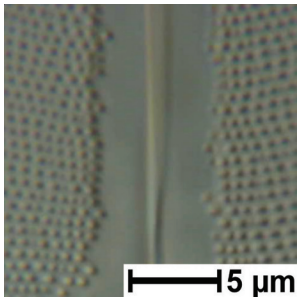

FIGURE 36 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, with visible striae and areolae. Technique: strong oblique illumination. Microscope: Zeiss Jena L Wd E. Lens: Zeiss Jena Apochromat 90x/1.30 Oil 160/0.17. Condenser: Zeiss Jena aplanatic condenser N.A. 1,4*

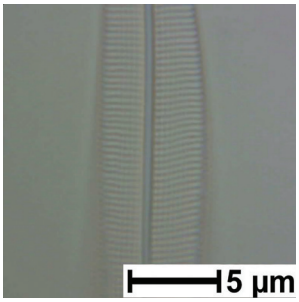

FIGURE 37 *Photomicrograph of Gyrosigma reimeri Sterrenburg, 1994, striae and areolae are discreetly visible. Technique: strong oblique illumination. Microscope: Zeiss Jena L Wd E. Lens: Zeiss Jena Apochromat 90x/1.30 Oil 160/0.17. Condenser: Zeiss Jena aplanatic condenser N.A. 1,4*

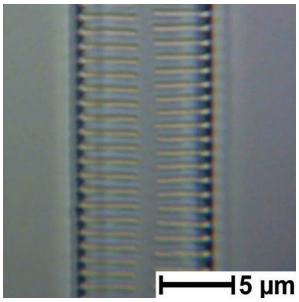

FIGURE 38 *Photomicrograph of Synedra capitata Ehrenberg, 1836, with visible striae, while areolae are very blurred in the best case. Technique: strong oblique illumination. Microscope: Zeiss Jena L Wd E. Lens: Zeiss Jena Apochromat 90x/1.30 Oil 160/0.17. Condenser: Zeiss Jena aplanatic condenser N.A. 1,4*

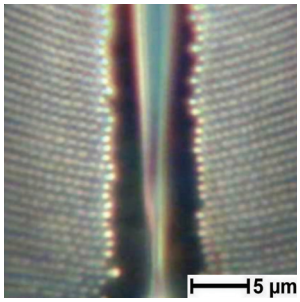

FIGURE 39 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, with visible striae and areolae. Technique: dark field illumination. Microscope: Zeiss Jena L Wd E. Lens: Zeiss Jena Apochromat 60x/1.0 Oil Iris 160/0.17. Condenser: Zeiss Jena Siedentopf 1.0 Cardioid condenser*

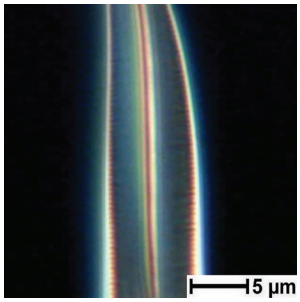

FIGURE 40 *Photomicrograph of Gyrosigma reimeri Sterrenburg, 1994, with striae only visible to the outside, while areolae are not visible. Technique: dark field illumination. Microscope: Zeiss Jena L Wd E. Lens: Zeiss Jena Apochromat 60x/1.0 Oil Iris 160/0.17. Condenser: Zeiss Jena Siedentopf 1.0 Cardioid condenser*

bright field, it also allows observations in phase contrast and dark field. All images have been obtained using the Leitz Pv Apo Oel 90x/1.15 n 170/0.17 lens. While areolae in *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg 1843, are visible in COL and phase contrast (figures 29, 30), dark field illumination gives less results (figure 31). Regarding *Gyrosigma reimeri* Sterrenburg, 1994, striae and areolae are visible only in COL (figure 32), although they are not particularly sharp here. *Synedra capitata* Ehrenberg, 1836, in COL shows visible striae, while areolae are blurred in the best case (figure 33). For *Navicula oblonga* (Kützing) Kützing in the past I obtained unexpected discrete results using Heine condenser in immersion, adding polarization with the polarizers oriented perpendicular to each other (maximum level of extinction).

ZEISS JENA LUMIPAN (PHOTOMICROGRAPHS NUMBERED FROM 34 TO 35)

Defined in Zeiss Jena catalogues as a "large research microscope", the Lumipan first introduced the pancratic condenser (which alone allows three types of observation) and could be provided with one of the first commercial phase contrast sets in history of microscopy. Both images have been obtained using the Zeiss Jena Achromat 90x/1.25 Oil Ph 160/0.17 lens. *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg 1843 in phase contrast shows clearly visible striae and areolae (figure 34), the contrast has been enhanced thanks to the supplied green filter. *Gyrosigma reimeri* Sterrenburg, 1994 shows visible striae and areolae in the same previous conditions abeit with less effectiveness as it is a more difficult test diatom (figure 35). The remaining test diatoms (*Navicula oblonga* (Kützing) Kützing, *Synedra capitata* Ehrenberg, 1836, and of course *Pinnularia nobilis* (Ehrenberg) Ehrenberg 1843) cannot be resolved with this equipment.

ZEISS JENA L WD E (PHOTOMICROGRAPHS NUMBERED FROM 36 TO 42)

Equipped with a Abbe illuminating apparatus with Aplanatic condenser N.A. 1.4, Zeiss Jena Apochromat objectives and compensating eyepieces, this black enamel jewel manufactured in 1941 was one of the best and most expensive research microscopes at the time. The stand kept at Diatom Lab is also equipped with the extremely rare Zeiss Jena ocular revolver.

Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843 shows visible striae and areolae in strong oblique illumination (figure 36) and also in dark field illumination (figure 39) using the Zeiss Jena Siedentopf 1.0 Cardioid condenser. Regarding *Gyrosigma reimeri* Sterrenburg, 1994 striae and areolae are discreetly visible only with strong oblique illumination (figure 37); in dark field striae only visible to the outside, while areolae

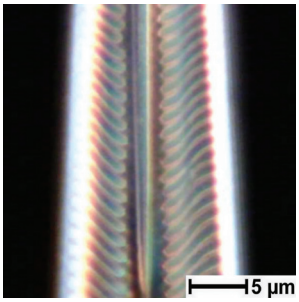

FIGURE 41 *Photomicrograph of Navicula oblonga (Kützing) Kützing, lineolae (areolae) are not visible. Technique: dark field illumination. Microscope: Zeiss Jena L Wd E. Lens: Zeiss Jena Apochromat 60x/1.0 Oil Iris 160/0.17. Condenser: Zeiss Jena Siedentopf 1.0 Cardioid condenser*

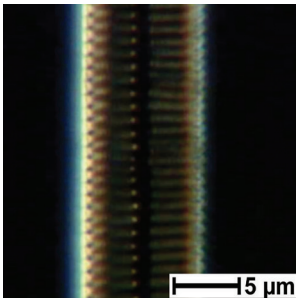

FIGURE 42 *Photomicrograph of Synedra capitata Ehrenberg, 1836, with visible striae, while areolae are blurred. Technique: dark field illumination. Microscope: Zeiss Jena L Wd E. Lens: Zeiss Jena Apochromat 60x/1.0 Oil Iris 160/0.17. Condenser: Zeiss Jena Siedentopf 1.0 Cardioid condenser*

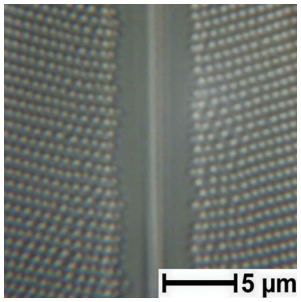

FIGURE 43 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, with visible striae and areolae. Technique: strong oblique illumination. Microscope: Koristka Large model IIc. Lens: Koristka Apochromat 1.5mm/1.30 Oil. Condenser: Koristka/Abbe illuminating apparatus N.A. 1.40.*

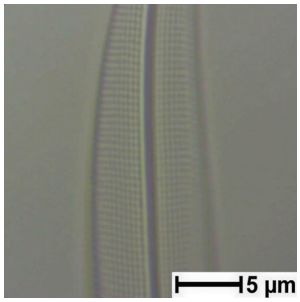

FIGURE 44 *Photomicrograph of Gyrosigma reimeri Sterrenburg, 1994, with striae and areolae visible, though blurred. Technique: strong oblique illumination. Microscope: Koristka Large model IIc. Lens: Koristka Apochromat 1.5mm/1.30 Oil. Condenser: Koristka/Abbe illuminating apparatus N.A. 1.40.*

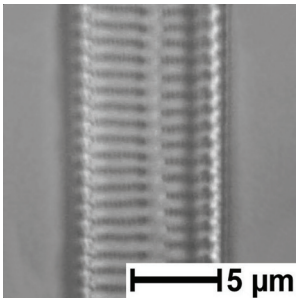

FIGURE 45 *Photomicrograph of Synedra capitata Ehrenberg, 1836, with striae and areolae visible, though blurred. A longitudinal artifact appears along the entire diatom (on the right of the image, this is due to the oblique illumination). Technique: strong oblique illumination. Microscope: Koristka Large model IIc. Lens: Koristka Apochromat 1.5mm/1.30 Oil. Condenser: Koristka/Abbe illuminating apparatus N.A. 1.40.*

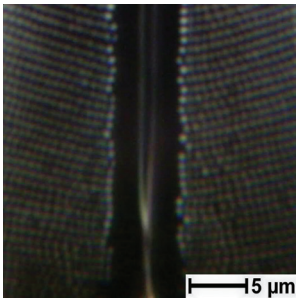

FIGURE 46 *Photomicrograph of Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843, with striae and areolae visible, though blurred. Technique: dark field illumination. Microscope: Koristka large model III. Lens: Koristka Achromat 1/12 /1.30 Oil with Funnel insert placed into the objective to reduce its N.A. for dark field use. Condenser: Koristka paraboloid condenser*

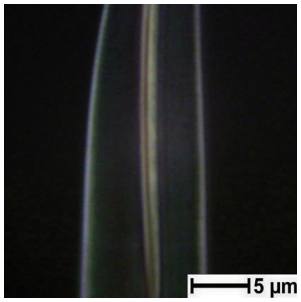

FIGURE 47 *Photomicrograph of Gyrosigma reimeri Sterrenburg, 1994, striae and areolae are not visible. Technique: dark field illumination. Microscope: Koristka large model III. Lens: Koristka Achromat 1/12 /1.30 Oil with funnel insert placed into the objective to reduce its N.A. for dark field use. Condenser: Koristka paraboloid condenser*

are not visible (figure 40). *Synedra capitata* Ehrenberg, 1836 has visible striae in strong oblique illumination, while areolae are very blurred in the best case (figure 38); in dark field illumination the results are not very different (figure 42). Although the Zeiss Jena Siedentopf 1.0 Cardioid condenser was one of the best dark field condensers of the time, it cannot detect the lineolae (areolae) of *Navicula oblonga* (Kützing) Kützing (figure 41) Images from 36 to 38 have been obtained using the Zeiss Jena Apochromat 90x/1.30 Oil 160/0.17 lens, while for images from 39 to 42 the Zeiss Jena Apochromat 60x/1.0 Oil Iris 160/0.17 lens was used.

KORISTKA LARGE MODEL IIC (PHOTOMICROGRAPHS NUMBERED FROM 43 TO 45)

The F. Koristka Apochromat 1.5mm/1.30 Oil was called a "spécialité" by Edmund J. Spitta[12], it has been used for the images from 43 to 45 It was no coincidence that Francesco Koristka was the great inventor of the Semi-Apochromat lenses[10] and founder of the renowned F. Koristka company based in Milan, Italy. Of course this superb antique lens shows the striae and areolae of *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg 1843 in strong oblique illumination (figure 43), while in the case of *Gyrosigma reimeri* Sterrenburg, 1994 striae and areolae are blurred (figure 44). This lens also resolved *Synedra capitata* Ehrenberg, 1836 in strong oblique illumination (figure 45) although striae and areolae look blurred and a longitudinal artifact appears along the entire diatom (on the right of the image, this is due to the oblique illumination).

KORISTKA LARGE MODEL III (PHOTOMICROGRAPHS 46 AND 47)

The Koristka paraboloid condenser for dark field illumination permits to resolve only *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg 1843, that shows visible striae and areolae, though blurred (figure 46). *Gyrosigma reimeri* Sterrenburg, 1994, the second less difficult diatom cannot be resolved (figure 47): in fact, as it is known, paraboloid condensers are less effective than cardioid condensers in some cases. For both images I used the Koristka Achromat 1/12 /1.30 Oil with funnel insert placed into the objective to reduce its N.A. for dark field use

SUMMARY AND CONCLUSIONS

Through this work it is possible to understand how test diatoms are considered by many as the best microscopic objects to check, set and compare optical microscopes of all ages and contrast techniques. Several Diatom Lab customers (laboratories, universities, microscope manufacturers and so on) achieved the expected results shown in the first photomicrographs (images numbered from 10 to 26) using both Diatom Lab microscope test slides on their modern microscopes.

In fact advances in optics (regarding above all microscope objectives, condensers, beam path, illumination systems and contrast techniques) have greatly improved the quality of microscope image and the possibilities of visualizing microscopic details that were once unimaginable under an optical microscope.

But a necessary clarification must be specified: this work wasn't made to cruelly demolish vintage and antique microscopes, otherwise it would not be possible to explain why at Diatom Lab there is also a rich collection of instruments from different historical periods (used for testing old instruments and scientific disclosure), in addition to other indispensable modern ones of course. Quite to the contrary: it is shown that in antiquity up to the 1970s it was possible to observe and resolve many very small structures, in fact most scientific discoveries related to the optical microscopes have been made in the past despite using instruments with much inferior performance.

If we verify how optics have made great strides over time, we can appreciate the present microscopy even more, without forgetting that everything comes from efforts gradually made in the past.

Finally, test diatoms are the microscopist's best friends (and advisors) as they always show the truth, they help with correctly setting the microscopes (helping to understand with extreme precision which settings give the best results) and in the selection of optics: no cross-section or blood smear will perform better than test diatoms!

FIGURE 48, *right, An antique engraving showing a microscopist. From the Diatom Lab collection*

Article, and references available online at: analyticalscience.wiley.com/publication/microscopy-and-analysis