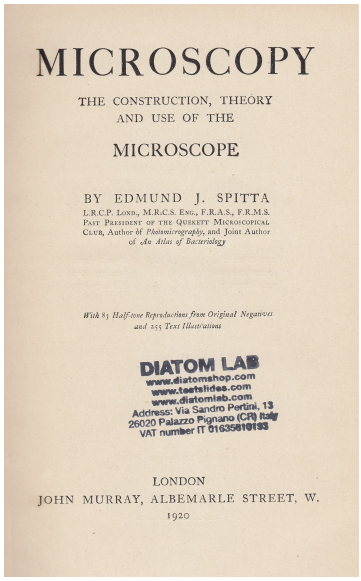
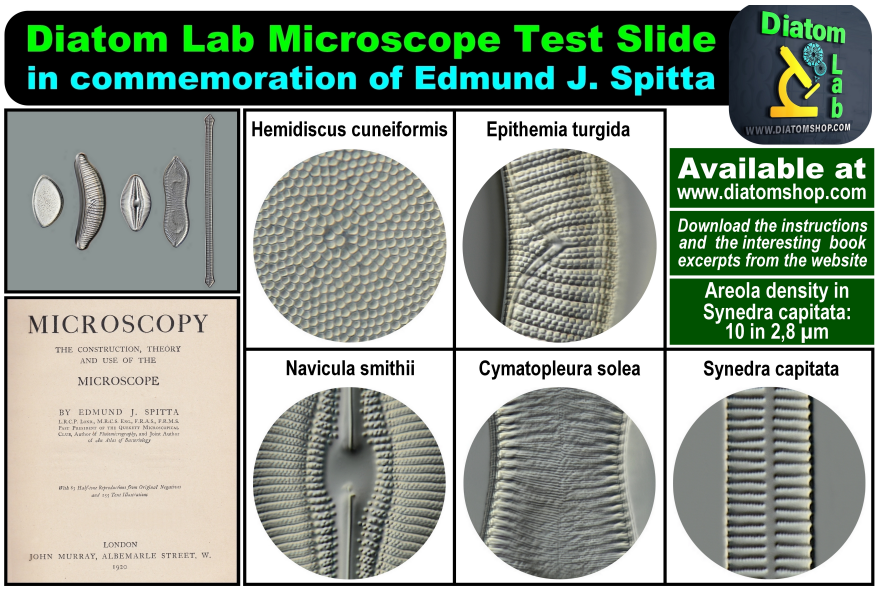
Diatom Lab Microscope Test Slide in commemoration of Edmund J. Spitta (available at www.diatomshop.com)





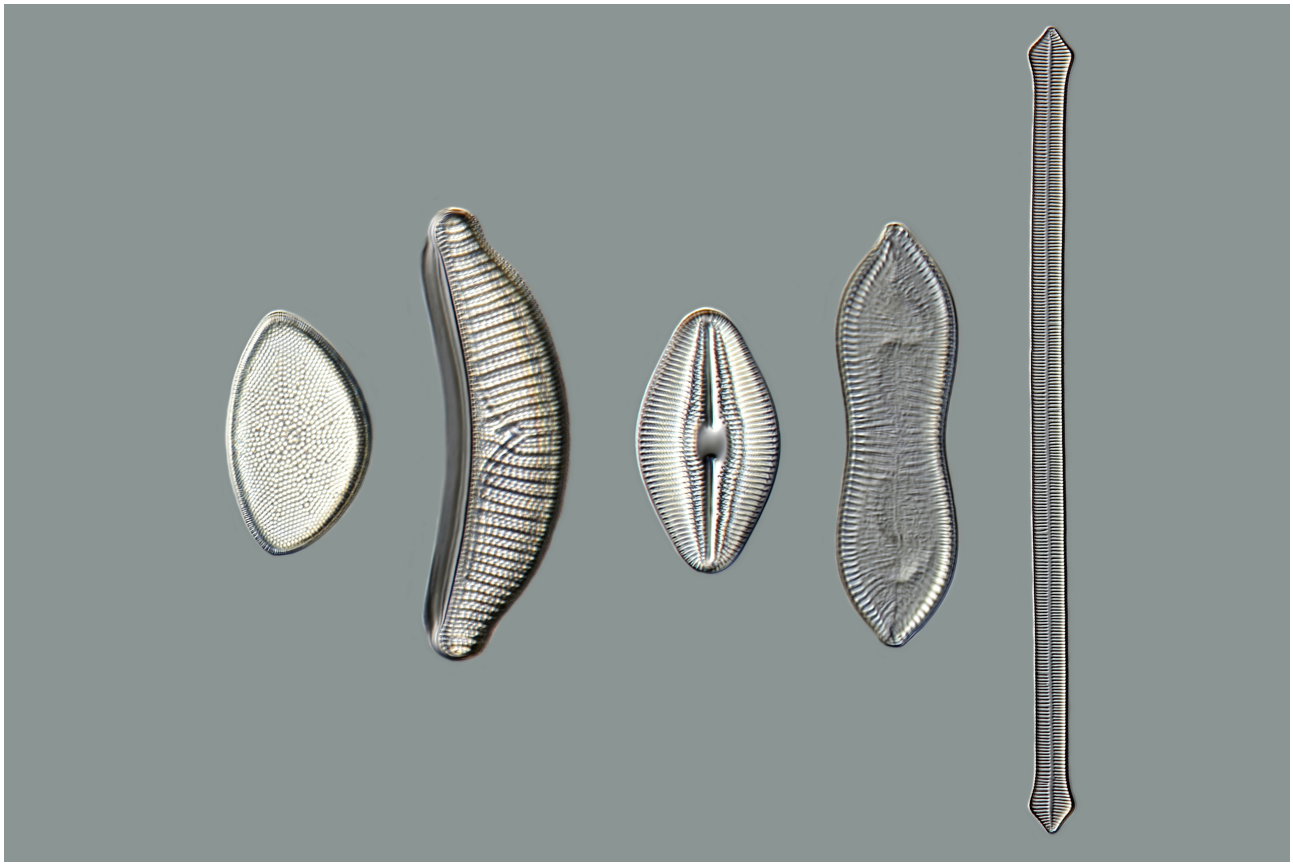
1. Introduction

Edmund Johnson Spitta (1853-1921), well known author of **"Microscopy: the construction, theory and use of the microscope"** and "Photomicrography", joint author of "An Atlas of Bacteriology", was a general medical practitioner who found time to contribute to several branches of microscopy. He was one of the presidents of the Quekett Microscopical Club and a vice-president of the Royal Microscopical Society. His research was mainly focused in the optics of the microscope and photomicrography.
The truly sensational book **"Microscopy: the construction, theory and use of the microscope"** describes – among other things – several microscopic examinations of test diatoms, accompanied by explanatory photomicrographs.
This book has inspired the creation of a new *Diatom Lab Microscope Test Slide* that contains five Diatom species tested and imaged by Edmund J. Spitta in the same work.
To make this new *Diatom Lab Microscope Test Slide* even more complete, these instructions are accompanied both by photomicrographs made using a modern in-house microscope (Zeiss Axio Imager.A2), and by an antique but pristine in-house Zeiss Jena microscope depicted in the same book (the large "Jug-Handle" Stand III D from 1905). You can find some very interesting and useful excerpts from E. J. Spitta, "Microscopy. The construction theory and use of the microscope", John Murray, London, 1920, 3rd edition, below. Happy reading and happy microscopy by Diatom Lab!

2. The five species present in the *Diatom Lab Microscope Test Slide in commemoration of Edmund J. Spitta*

All selected Diatoms are micromanipulated and mounted by Stefano Barone directly on the underside of a custom optical quality cover glass for maximum resolution and contrast.
High refractive index mountant: Diatom Cubed. Innovative, proprietary Diatom Lab Technology and Materials.

Diatom number 1: ***Hemidiscus cuneiformis*** Wallich 1860
Diatom number 2: ***Epithemia turgida*** (Ehrenberg) Kützing 1844
Diatom number 3: ***Navicula smithii*** Brébisson, 1856
Diatom number 4: ***Cymatopleura solea*** (Brébisson) W.Smith 1851
Diatom number 5: ***Synedra capitata*** Ehrenberg, 1836
(Synonym) ***Synedra ulna f. capitata*** (Ehrenberg) Skabichevskii, 1960
(for measured areola density read Chapter 3)



The microscope test slide imaged by a dry lens. Zeiss Axio Imager.A2; Zeiss Plan-Apochromat 40x/0.95 Korr ∞ /0.13-0.21 M27; Zeiss Achromatic-Aplanatic universal condenser 0.9 H D Ph DIC; Zeiss VIS-LED; Differential Interference Contrast (DIC).

3. Useful objectives and techniques to resolve each Diatom

	Hemidiscus cuneiformis	***Epithemia turgida***	***Navicula smithii***	***Cymatopleura solea***	***Synedra capitata****
Bright field	from 10x to 100x Oil	from 20x to 100x Oil	from 40x to 100x Oil	from 40x Oil to 100x Oil	90x Oil -100x Oil, better in double immersion
Oblique illumination	from 10x to 100x Oil	from 20x to 100x Oil	from 40x to 100x Oil	from 40x to 100x Oil	90x Oil -100x Oil, better in double immersion
Phase contrast	from 10x to 100x Oil	from 20x to 100x Oil	from 40x to 100x Oil	from 40x Oil to 100x Oil	90x Oil -100x Oil, better in double immersion
Darkfield	from 10x to 100x Oil	from 20x to 100x Oil	from 20x to 100x Oil	from 40x to 100x Oil	from 60x Oil to100x Oil
Oil immersion darkfield (Oil Darkfield Condenser)	Any Oil lens	Any Oil lens	Any Oil lens	from 40x Oil to 100x Oil	from 60x Oil to100x Oil
DIC	from 10x to 100x Oil	from 20x to 100x Oil	from 20x to 100x Oil	from 40x to 100x Oil	from 60x Oil to100x Oil, better in double immersion

*** Measured areola density in *Synedra capitata*** Ehrenberg, 1836 that belong to this sample **: 10 in 2,8 µm**

(This table is generic and may vary based on lens and condenser characteristics - such as their numerical aperture.)

4. Microscopes used for imaging this microscope slide at Diatom Lab

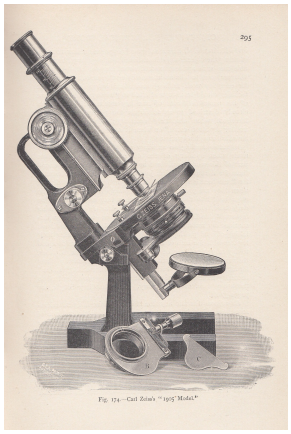
1) Zeiss Axio Imager.A2 in transmitted light microscopy.
Objectives: Zeiss Plan-Apochromat 40x/0.95 Korr ∞ /0.13-0.21 M27; Zeiss Plan-Apochromat 63x/1.4 Oil DIC ∞ /0.17 M27.
Condenser for dry observations: Zeiss Achromatic-Aplanatic universal condenser 0.9 H D Ph DIC.
Condenser for double immersion microscopy: Zeiss Achromatic-Aplanatic condenser 1.4 H D Ph DIC.
Illumination Unit: Zeiss VIS-LED (color temperature 5600 K)

2) Antique, pristine Zeiss Jena “Jug-Handle” Stand III D from 1905 (serial number 41618).
Equipped with three Zeiss Jena objectives, found in its original box: A (equivalent focal length 15 mm; numerical aperture 0,20); D (equivalent focal length 4,2 mm; numerical aperture 0,65); 1/12” Homog. Immersion (equivalent focal length 1,8 mm; numerical aperture 1,25. Zeiss Jena produced also a 1/12” lens with numerical aperture 1,30). An additional Koristka Apochromat 1,5mm Oil immersion (numerical aperture 1,30) objective was used to resolve *Synedra capitata* Ehrenberg, 1836.
Abbe illuminating apparatus (N.A. 1,40 regarding this Stand) for central or oblique illumination.
Zeiss Jena microscope lamp equipped with iris diaphragm.

Stefano Barone at Diatom Lab, next to some of his modern research microscopes (Zeiss Axio Imager.A2 in front)

Zeiss Plan-Apochromat 63x/1.4 Oil DIC ∞ /0.17 M27 and its DIC slider

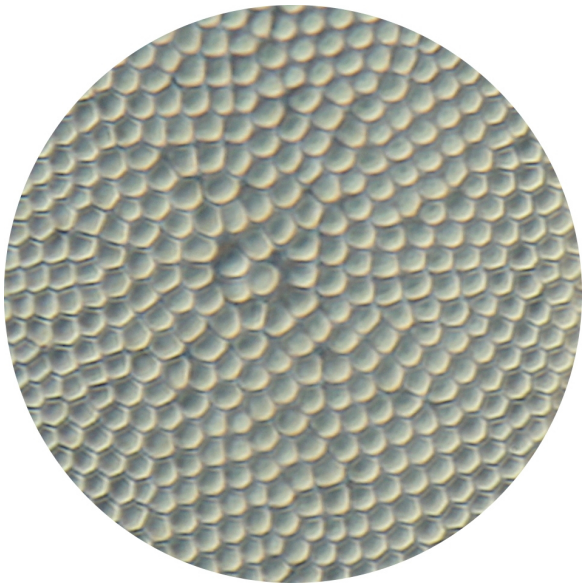
The pristine Zeiss Jena Jug-Handle” Stand III D from 1905 (serial number 41618)


The Zeiss Jena “Jug-Handle” Stand III D in the book written by Edmund J. Spitta

5. The five Diatoms imaged by the Zeiss Plan-Apochromat 63x/1.4 Oil DIC ∞ /0.17 M27

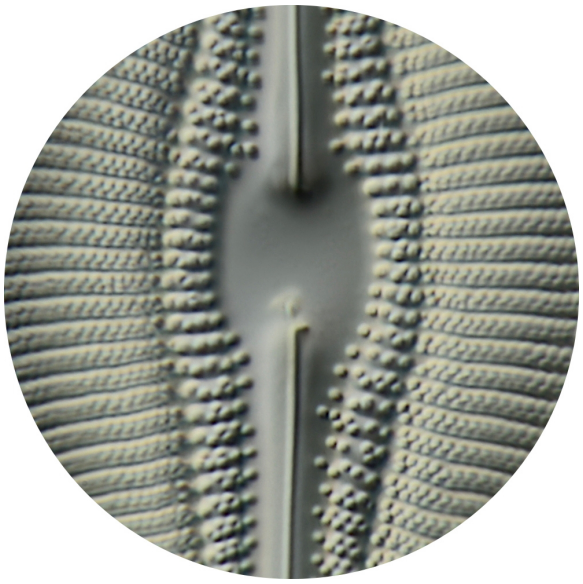
Microscopy technique: Differential Interference Contrast (DIC). Zeiss Plan-Apochromat 63x/1.4 Oil DIC ∞ /0.17 M27 and Zeiss Achromatic-Aplanatic condenser 1.4 H D Ph DIC. Zeiss VIS-LED has been used as excellent light source, but if you use UV light (with necessary protective devices as UV light is dangerous - consult specialized literature) you will probably get even higher resolution.

Here are the useful details. Images are reduced in quality to facilitate loading.

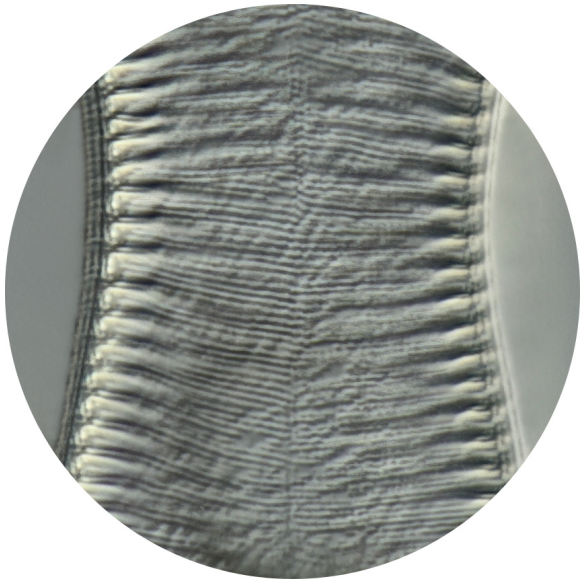


Hemidiscus cuneiformis Wallich 1860

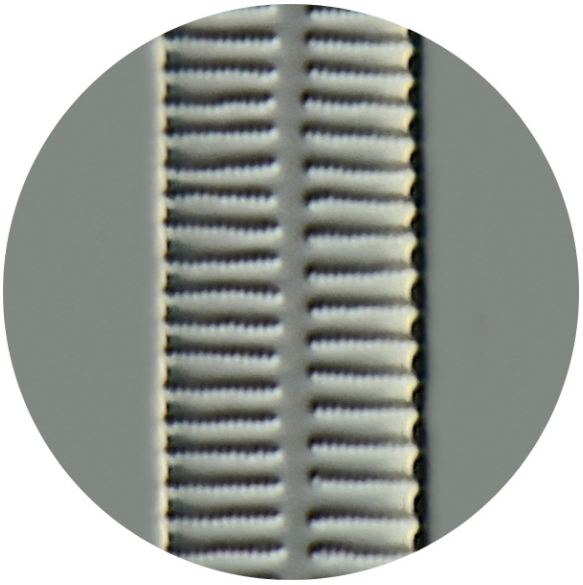
Epithemia turgida (Ehrenberg) Kützing 1844



Navicula smithii Brébisson, 1856



Cymatopleura solea (Brébisson) W.Smith 1851



Synedra capitata Ehrenberg, 1836

6. Interesting excerpts from E. J. Spitta, "Microscopy. The construction theory and use of the microscope", John Murray, London, 1920, 3rd edition

Diatom number 1: *Hemidiscus cuneiformis* Wallich 1860

The second diatom—Hemidiscus cuneiformis—which is also readily obtainable, is much more difficult. It has seemed impossible to obtain a resolution into dots, similar to that shown in Fig. 2, Plate XVI., with less aperture than ·3, a faint suspicion only being seen with N.A. ·24. It seems imperative also to use slightly oblique green light, and it should be borne in mind that oblique light is best obtained with such low powers as an inch by a suitable shifting of the mirror, rather than by employing the usual substage arrangements for higher powers. The blue light arrangement above mentioned renders the seeing far cleaner and more distinct.

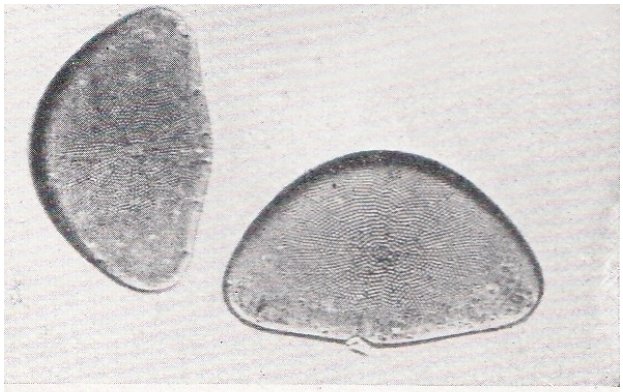


Fig. 2.

Fig. 2.—HEMIDISCUS CUNEIFORMIS. A very difficult diatom to resolve. Green light is better than white, but blue illumination is far superior. Zeiss 1-in. apochromat × 270. See page 407.

Diatom number 2: *Epithemia turgida* (Ehrenberg) Kützing 1844

Epithemia turgida is a test-object in which the dots are very plainly visible; but their exact shape in their doubly arranged rows is a matter we have never been able to be quite certain about. When slightly *out of focus*, as in the right-hand portion of Fig. 2, Plate XI., they look circular, whereas in the centre of the specimen where they look sharp, they appear almost triangular; but at a third plane, as shown in the left-hand end of the photograph, where they are just going out of focus, they appear for the most part round! After paying some attention to this matter, we lean towards the opinion that they should be always shown as circular, although it is difficult to explain their triangular appearance in the central portion. A photograph

showing the three effects has been chosen to better explain the text. The neatness with which these effects can be dis-severated and the general sharpness of the whole afford a guide as to the excellency of the objective. The photo-micrograph was taken with an exceedingly fine Leitz 2-mm. apochromatic. See explanation to Plate.

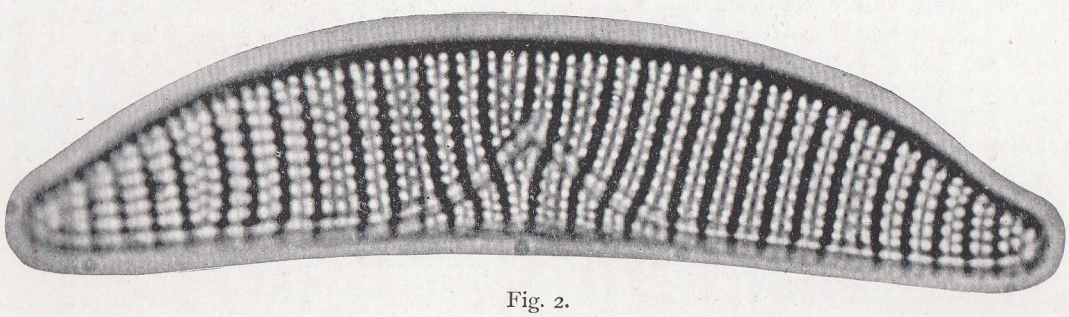


Fig. 2.

Fig. 2.—EPITHEMIA TURGIDA. The double row of irregular-shaped markings in this diatom, when magnified sufficiently, should appear well "lifted out" above the floor; and there should be no fluffiness of the background. The valve is so bent as to make it almost impossible to photograph satisfactorily, and it is not easy to be certain which is the correct focus, for at one plane the dots appear circular, at another hemi-spherical, whilst irregular-shaped ones also can be seen scattered about. The *cleanness* and *whiteness* of the entire object make it a good one to ascertain the type of the colour correction of a semi-apochromat, and the perfection of the correction in an apochromat. See page 386.

Photographed with a Leitz 2-mm. apochromat N.A. 1·35 × 1300.

Diatom number 3: *Navicula smithii* Brébisson, 1856

The third diatom is the well-known Navicula Smithii. It is best to have a few specimens to select from, as this test is one of the most severe we know for the aperture in question. Further, it is better to obtain the diatom mounted in styrax. Much care and time may be required in arranging the light to resolve *any* of the transverse lines, and it has seemed impossible to do so up and down the entire valve at one and the same focus. In the photograph, Fig. 3, Plate XVI., several dots can be seen in each specimen (especially if a hand magnifier be used), but the objective must be a well-corrected one to produce as good results. Oblique blue light renders the seeing some-what easier than when oblique green illumination is employed; but in our experience the specimen is a difficult one to negotiate.

Navicula Smithii.—The true nature of the zig-zag rows of dots was not discovered till the advent of first-class homogeneous objective (Van Heurck). When using a combination that is sensibly perfect, these dots should be very *distinctly circumscribed*, such as shown in Plate X., which was taken with the 1·5 apochromatic by Koristka above mentioned.[1] See explanation to Plate.

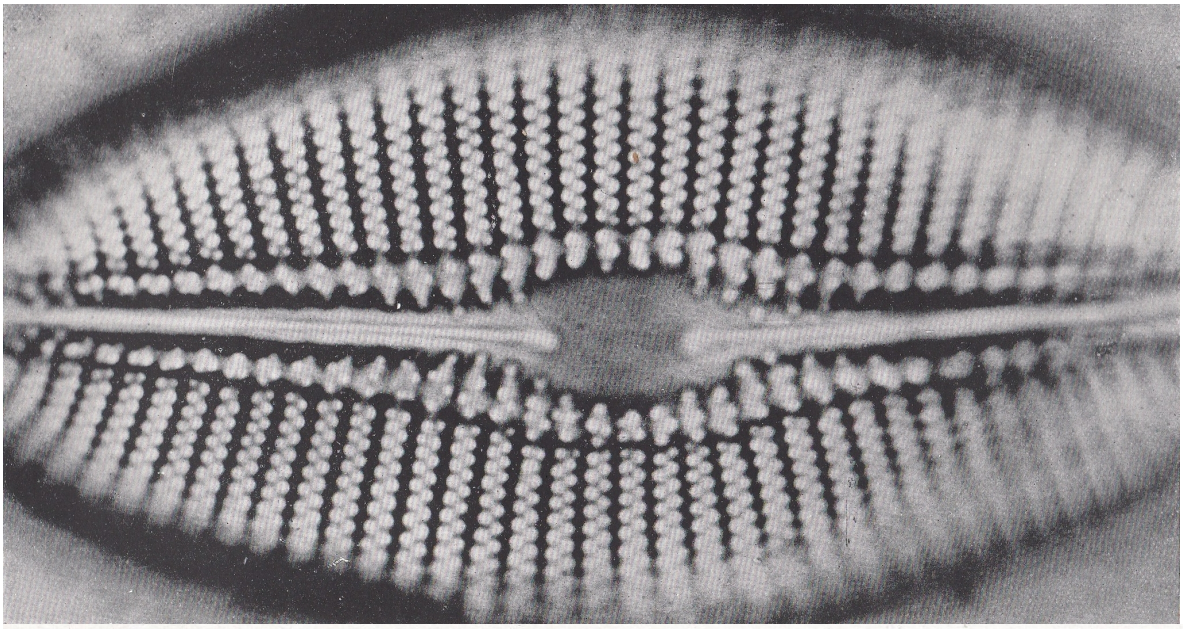


PLATE X.

NAVICULA SMITHII. The double row of circular dots in each costa should be well defined, anyhow in certain portions of the field of view. Van Heurck says: "The exact nature of these markings was not known until the introduction of the homogeneous objective." We have never been able to obtain an objective that will show the dots better than those exhibited in the photograph. A poor semi-apochromat—even with green light—gives an exceedingly foggy image, the dots being perhaps hardly visible. The test, though a severe one, is of a very reliable nature. As the valve is very saucer-shaped, it is impossible to photograph it in its entirety at one plane of focus.

Photographed with a Koristka 1·5-mm. apochromat N.A. 1·40 × 1200 and subsequently enlarged × 2. See page 386, and Plate XVI, Fig. 3.

—NAVICULA SMITHII. This is by far the *most* difficult to resolve into dots of any of the previous test objects for an inch. Slight oblique light (by shifting the mirror) must be used, and it is best to have blue illumination. Zeiss apochromat 1-in. × 270. See page 407.

Diatom number 4: *Cymatopleura solea* (Brébisson) W.Smith 1851

Cymatopleura solea.—In the floor of this little valve, which is very narrow, may be seen, when carefully searched for, if necessary with the aid of oblique green light, a series of closely arranged transverse lines. They appear as if roughly ruled, and engender the belief that they could be broken up into dots, but we have never been able to do so, however. They are very delicate, and are extremely difficult to photograph on account of their great transparency; any diffusion of focus in the objective, and they may be entirely invisible. Fig. 2, Plate XII. (Further details of interest have been found and photographed by Messrs. Herbert & Blakeley respectively.)

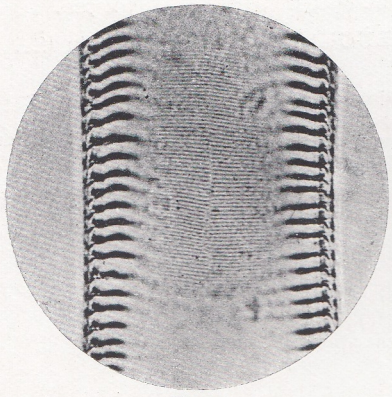


Fig. 2.

Fig. 2.—CYMATOPLEURA SOLEA. A most useful and delicate test-object. If looked at with a three-quarter cone and a ×12 ocular—direct white light—faintly marked transverse striæ should just be visible when employing a fine semi-apochromat or an apochromatic objective. Oblique white light reveals these striations—towards the median line particularly—as abruptly interrupted. Each striation should be so distinctly defined that it can be seen to terminate in rather a *round-shaped extremity*. An inferior combination will most likely fail to show the blunt ends, or perhaps may even fail to show the striations at all, the floor of the valve appearing a foggy desert void of detail. With a three-quarter cone (which is usually necessary) this object is a very searching test, and may enable the microscopist to differentiate between objectives which otherwise appear to perform equally well. See page 387.
Photographed with a Koristka 1·5-mm. apochromat N.A. 1·35 × 1200.

Diatom number 5: *Synedra capitata* Ehrenberg, 1836

Synedra capitata.—This diatom is very common, being found "almost everywhere," according to Van Heurck, and its length, he adds, varies from 20 to 50 c.d.m. (see Index). The transverse striæ, about $\frac{1}{20000}$th of an inch apart, are easily seen with a $\frac{1}{12}$th, but to break up these lines into segments, usually about fourteen to sixteen to each line, is somewhat difficult. A finely corrected combination should show these secondary markings as distinctly rectangular in form (as in Synedra crystallina), having their greater measurement lying parallel to the long axis of the valve—that is, transversely to the direction of the striæ. Owing to their unusual shape, some refuse to call them "dots" in the ordinary acceptation of the word. Green light will resolve them fairly well if used strongly oblique; but to see them really well and neatly defined, oblique blue-violet illumination (maximum transmission about 4700 tenth metres)

and a powerful illuminant are necessary. These secondary markings can be made to appear as black or white "effects" according to the focus, and both are shown in the photograph, Fig. 2, Plate XXI. Those in the centre look black, whilst those at one end are white, the two "appearances" in one illustration being, of course, due to the specimen not being quite flat.

We have never been able to resolve this diatom with a $\frac{1}{6}$th, the nearest approach thereto, in one instance only after trying several objectives, apochromatic and others, being a faint exhibition of one or more "dots" near the median raphé.

It should be noted that these secondary markings are formed in this instance by the breaking up of the lines—as usually obtains with most diatoms—and are not due to a resolution of the spaces between them, as some think to be the case with Navicula cuspida.

From what has been said, it will be readily seen that Synedra

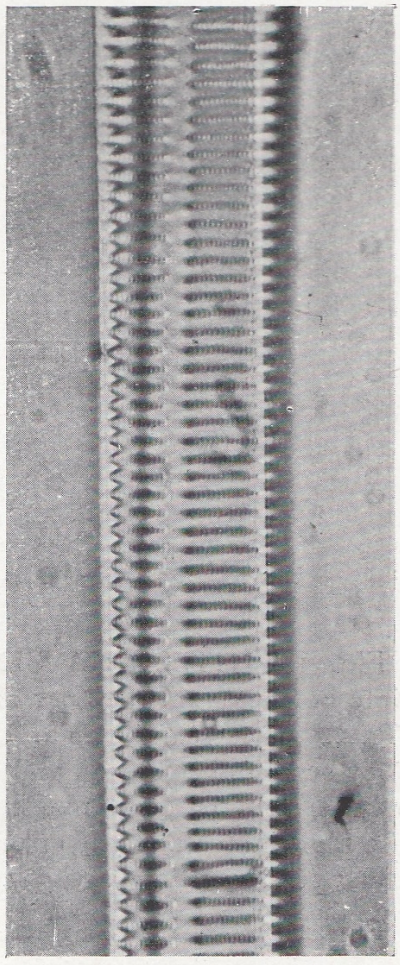
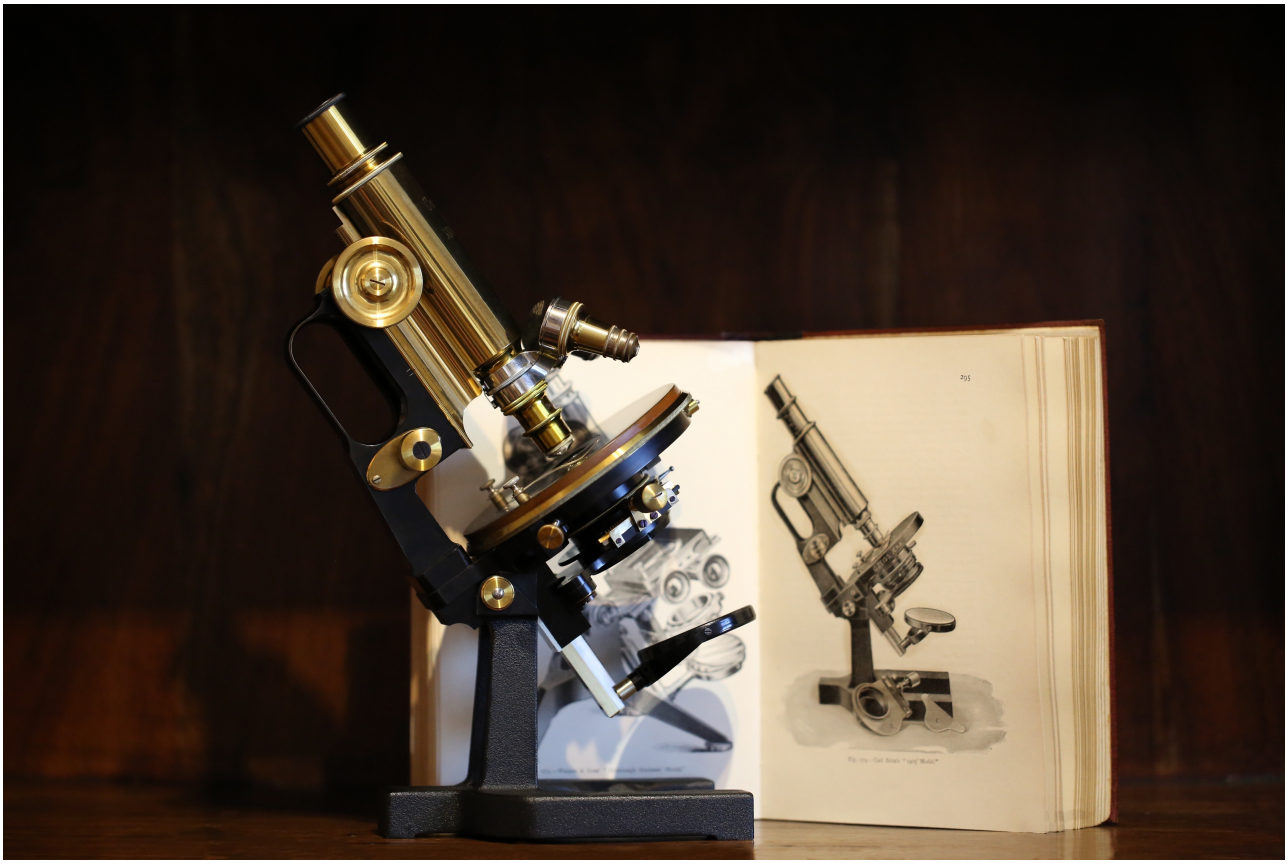


Fig. 2.

Fig. 2.—SYNEDRA CAPITATA. A very difficult specimen to resolve with a $\frac{1}{12}$. Note the black dots (?) are elongated, being much longer with the length of the diatom than they are when transversely measured. Some are shown white and some dark, as this valve is supposed not to be quite flat. Zeiss apochromatic $\frac{1}{12}$ N.A. 1·40 × 1500. See page 388.

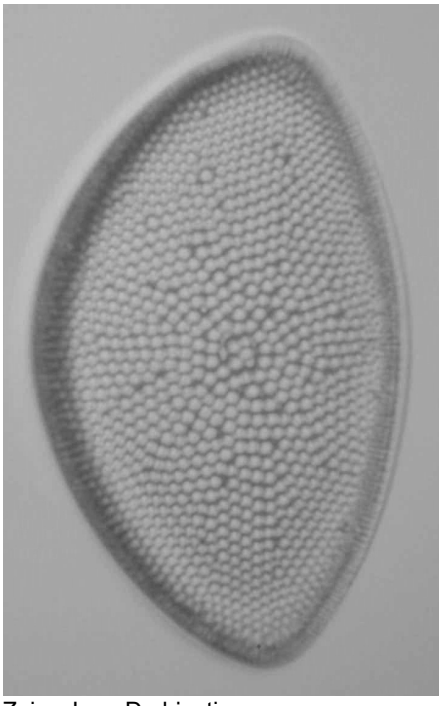
7. The Microscope Test Slide imaged by the Zeiss Jena “Jug-Handle” Stand III D from 1905



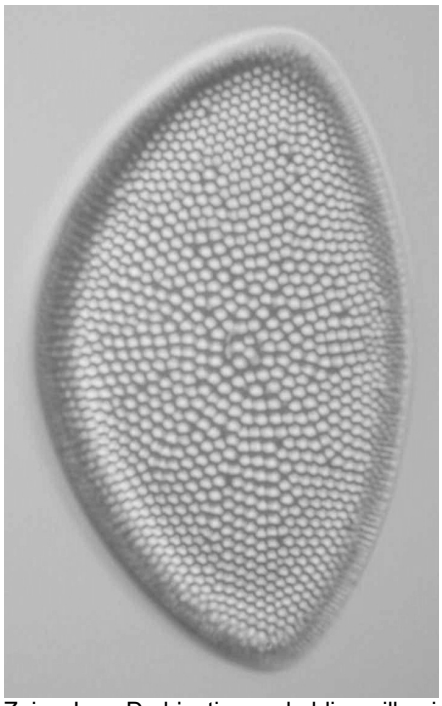
Zeiss Jena A objective (equivalent focal length 15 mm; numerical aperture 0,20) did not resolve any of the five Diatoms, therefore it was discarded fort these tests.

Diatom number 1: *Hemidiscus cuneiformis* Wallich 1860

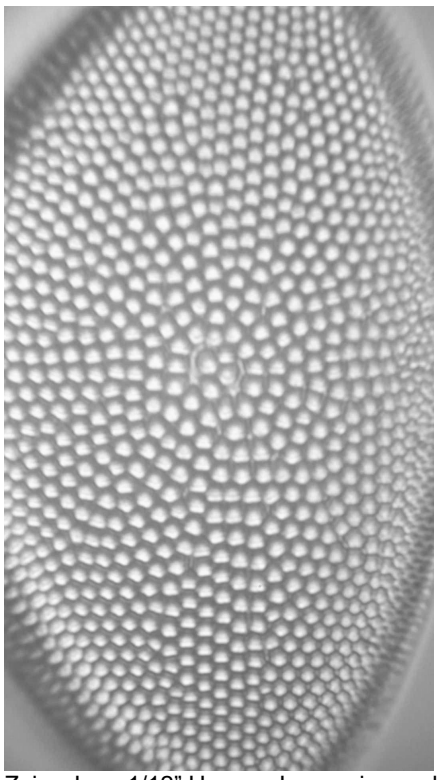
According to Edmund J. Spitta, the “resolution into dots” requires a numerical aperture of at least 0,30, so the Zeiss Jena D objective (numerical aperture 0,65) is more than enough.



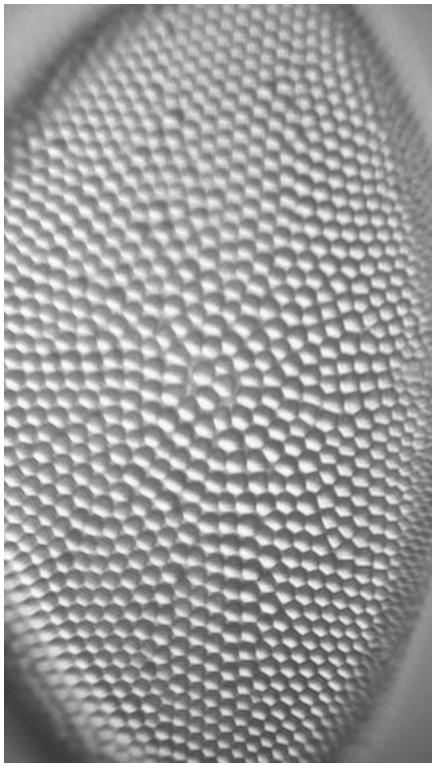
Zeiss Jena D objective



Zeiss Jena D objective and oblique illumination

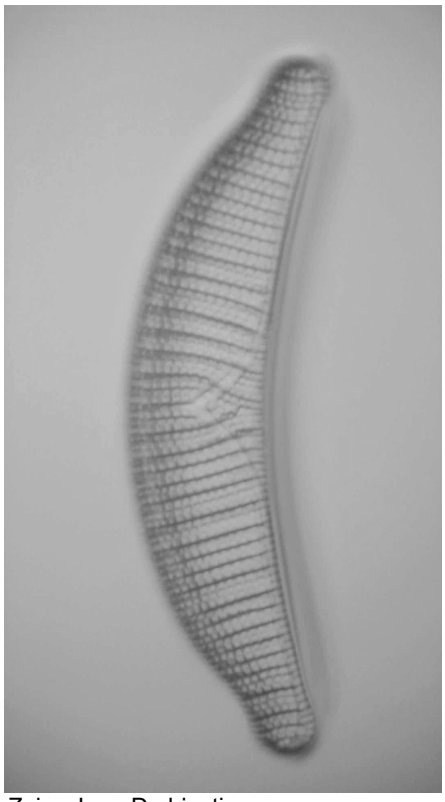


Zeiss Jena 1/12” Homog. Immersion and and oblique illumination

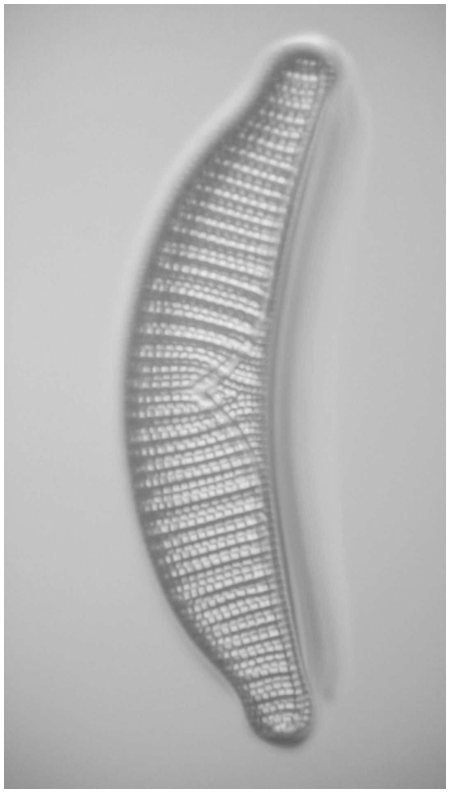


Zeiss Jena 1/12” Homog. Immersion in double immersion (oiled condenser, oiled coverglass) and oblique illumination

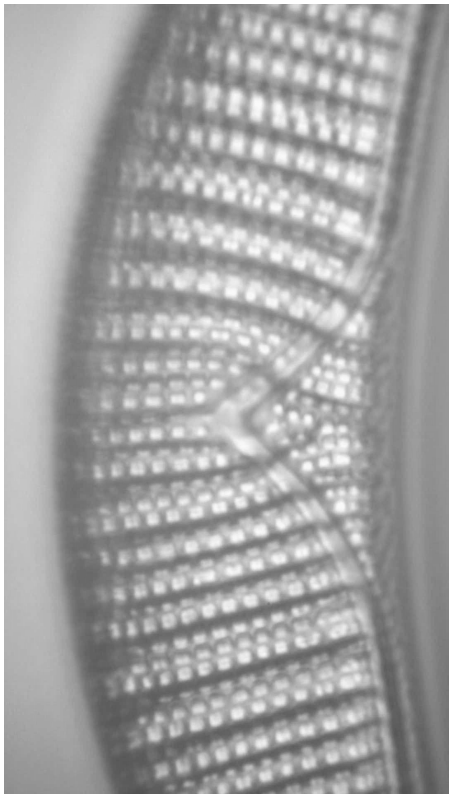
Diatom number 2: *Epithemia turgida* (Ehrenberg) Kützing 1844



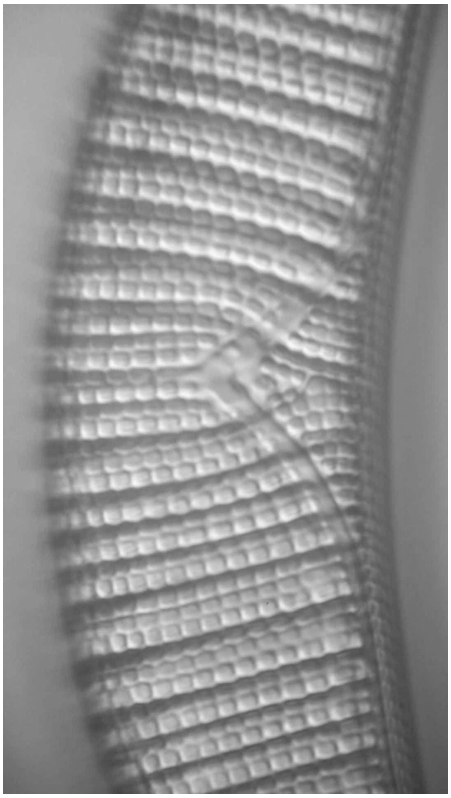
Zeiss Jena D objective



Zeiss Jena D objective and oblique illumination

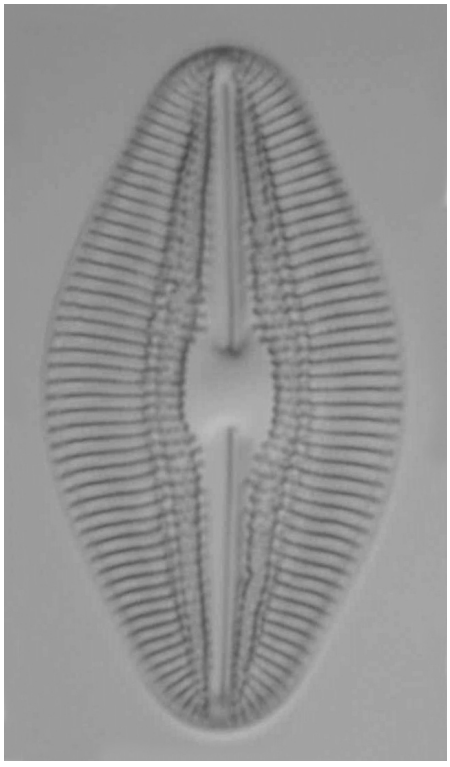


Zeiss Jena 1/12” Homog. Immersion and oblique illumination

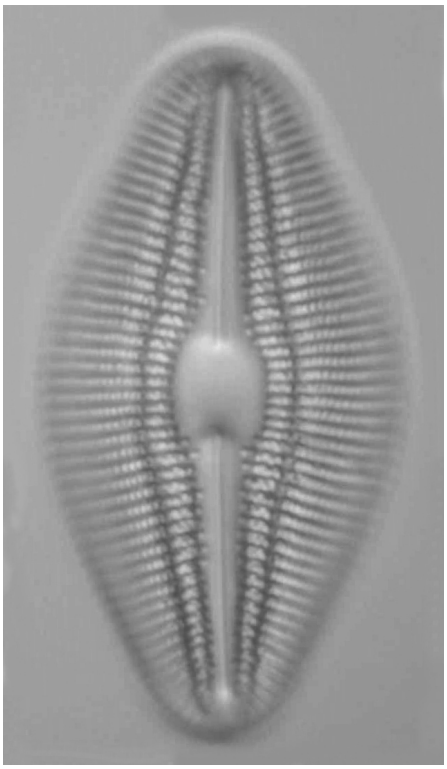


Zeiss Jena 1/12” Homog. Immersion in double immersion (oiled condenser, oiled coverglass) and oblique illumination

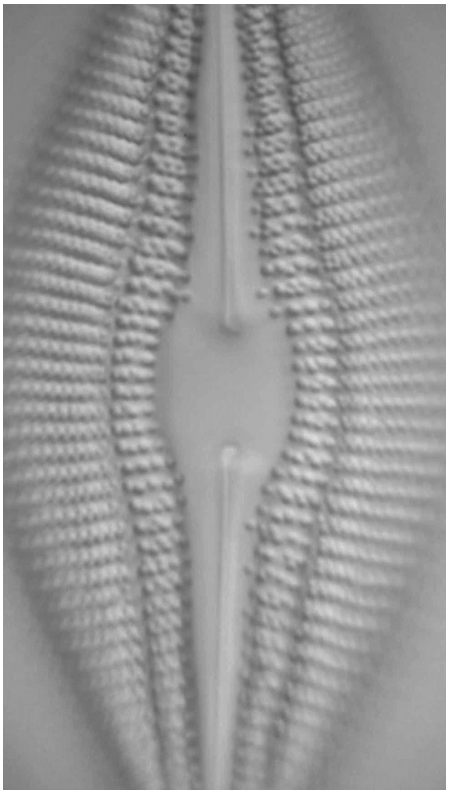
Diatom number 3: *Navicula smithii* Brébisson, 1856



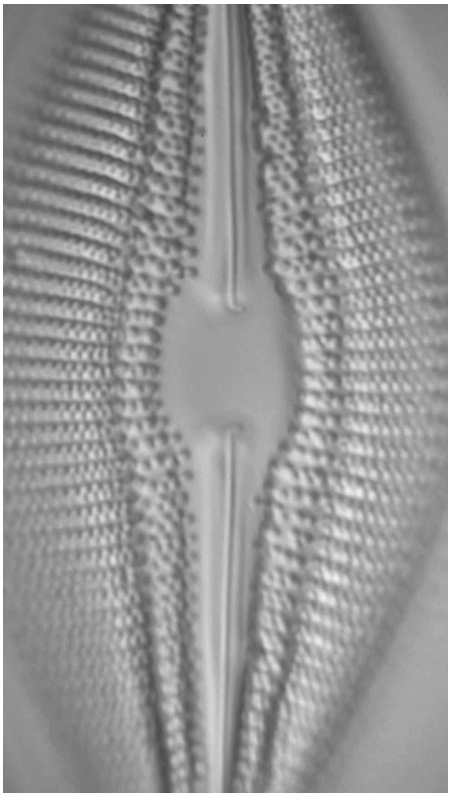
Zeiss Jena D objective



Zeiss Jena D objective and oblique illumination



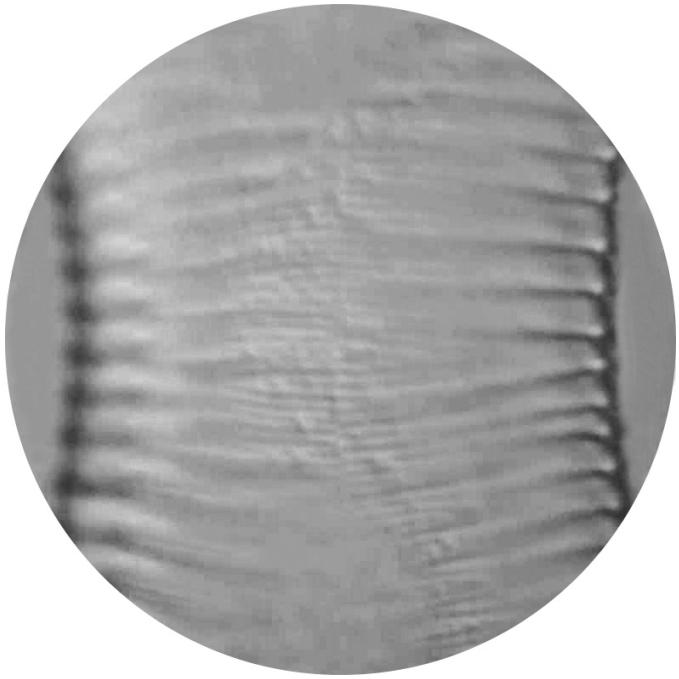
Zeiss Jena 1/12” Homog. Immersion and oblique illumination



Zeiss Jena 1/12” Homog. Immersion in double immersion (oiled condenser, oiled coverglass) and oblique illumination

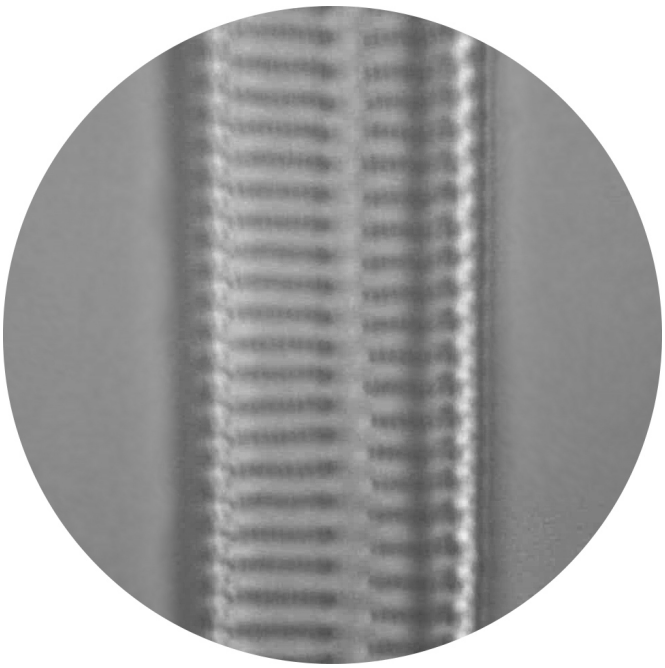
Diatom number 4: *Cymatopleura solea* (Brébisson) W.Smith 1851

The Zeiss Jena D objective fails to resolve this Diatom, both in bright field and oblique illumination. The Zeiss Jena 1/12” Homog. Immersion (N.A 1,25) objective can resolve this Diatom in double immersion and strong oblique illumination. Here is a detail.



Diatom number 5: *Synedra capitata* Ehrenberg, 1836

The Zeiss Jena D objective fails to resolve this Diatom, both in bright field and oblique illumination, of course. Also the Zeiss Jena 1/12" Homog. Immersion (N.A 1,25) objective does not achieve the result, even in strong oblique illumination. In this case a N.A. 1,30 is necessary for antique objectives: the rare Koristka Apochromat 1,5mm Oil immersion with a N.A. 1,30 has highlighted the areolae, by means of a strong oblique illumination. The same Koristka lens was called a "spécialité" by Edmund J. Spitta (see excerpt below): Francesco Koristka was the great inventor of the Semi-Apochromat lenses (see Carlo Koristka, Il microscopio, Hoepli, Milano, 1930) and founder of the renowned F. Koristka company based in Milan, Italy. With a numerical aperture 1,40 used by him the resoult would be even better.



SIGNOR KORISTKA OF MILAN produces apochromats which—after a very extended and severely conducted series of experiments both with the test-plate and with test-objects—are all of *the very highest possible order of excellence*. We have seen, through his courtesy, all his manufactures, and all are equally good. The 1·5-mm, a *spécialité*, with a very long working-distance for so high a power, is an exceedingly fine lens and produces an image so absolutely perfect as to render any distinction between it and that displayed by two apochromatic twelfths—one for the short and one for the long tube—by Zeiss impossible. Comparison with Swift's new apochromatic twelfth should also be made.

8. Conclusion

Diatom Lab *Microscope Test Slide in commemoration of Edmund J. Spitta* is a ***complete test* for all dry and oil immersion lenses from 10x to 100x, in Bright field, Oblique illumination, Phase contrast, Darkfield, Oil immersion darkfield and DIC.**
The five Diatoms can also be resolved by means of vintage or even antique microscopes, **it is not necessary to own very expensive, modern top of the range instruments.**

Although Diatom Lab uses in-house modern, top of the range microscopes and other instruments to guarantee Customers the best quality and accuracy, in the laboratory there is also a rich collection of vintage and antique microscopes for the following purposes:
1) to understand how (and at what quality level) test diatoms, diatoms in general and other microscopic objects were observed throughout history of microscopy;
2) to understand the technical evolution and the optical aberrations of the microscope objectives and accessories throughout history of microscopy. These instruments come from the Nineteenth and Twentieth Centuries and are surprisingly like new: in fact, over the years it has been necessary to find microscopes and accessories from unsold stocks (it was a very difficult undertaking) or at least in perfect condition, to ensure the accuracy of the tests.

The leap into the past through vintage and antique microscopy has several salient aspects:
a) first of all it always generates a strong emotion, especially if it is possible to observe from old but like-new instruments (because we understand how the observations of the past were really like and we can put on the clothes of the ancestors. It's a kind of priceless time machine journey);
b) we realize that many old scientific discoveries have been made despite using instruments with much inferior performances;
c) we understand how much genius, care and passion the past scientists and opticians had in building masterpieces despite having less means than today;
d) we can directly verify how the optics have made great strides over time, in this way we can appreciate the present microscopy even more**.**