Diatom Test Slide *version 3.0* Instruction Manual

Test your microscope! Venture into the Micro and Nano structures!

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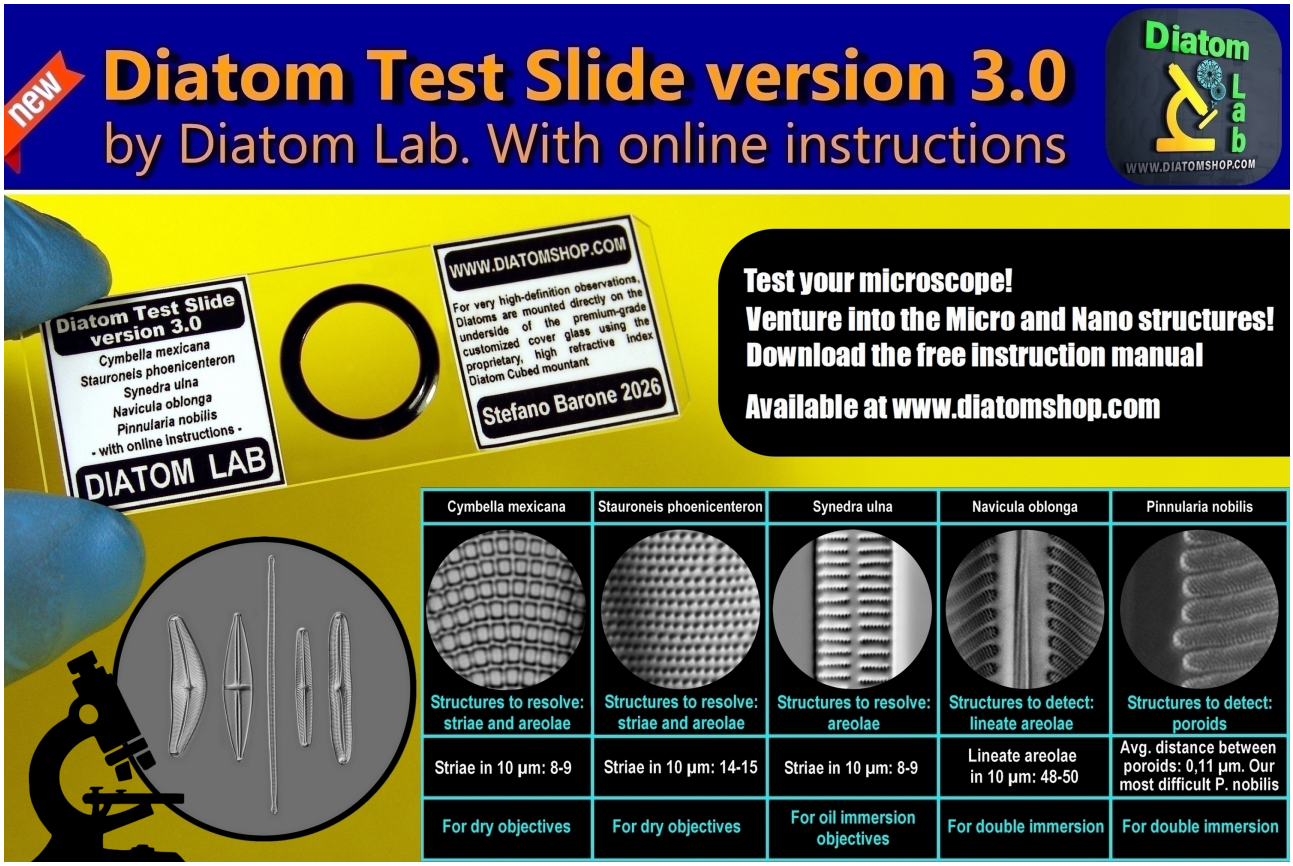


PREMISE:

Version 3.0 was created for the following reasons:
1) while the version 2.0 generally required dry objectives of at least 20x to resolve the less difficult areolae (this is the case of *Stauroneis phoenicenteron*), the new version 3.0 includes *Cymbella mexicana* to accommodate users wishing to experiment at lower magnifications: in fact, *Cymbella mexicana* areolae can be resolved using a high-quality 10x objective;
2) a subsequent step forward was to replace *Gyrosigma reimeri* with *Synedra ulna*, as the areolae of the latter are more distinct.

In any case, after ten years it was almost necessary to introduce something new.

A) This microscope test slide contains five cleaned, selected and micromanipulated Diatom species with areolae (an areola is one of the pores in a row that forms a stria on a valve. The plural is areolae), striae (a stria is a row of areolae. The plural is striae) and poroids (small holes on the diatom valve) that can be resolved or detected through a light microscope. The **resolving power** of a microscope is measured by its ability to differentiate two lines or points in an object;

B) “The use of Test Diatoms to check the quality of objectives is only one aspect of their use. The Test Diatoms should be also used to make sure that the whole system of the microscope is working well. That is the illumination as well as the combination of objective and eyepiece. It should become a habit to use a test slide every time the microscope is set up. In this way one ensures that what one sees is really there, not false and blurry images, and that one is missing nothing which should be visible with the optical system being used. Diatoms fill this requirement better than any other object” (from a classic N.B.S. leaflet).

C) **The Diatom Test Slide version 3.0 is extremely useful to:**
- Evaluate and compare microscope models, objectives, eyepieces, condensers, light sources, optical filters and microscope cameras. They are perfect for testing techniques such as bright field, oblique illumination, differential interference contrast (DIC), phase contrast, dark field, polarized light, Rheinberg illumination and Hoffman modulation contrast;
- Quantify resolution (the smallest distance between two points on a specimen that can be distinguished as separate entities) in optical microscopes;
- Practice using your microscope at its highest level of performance;
- Examine variations in contrast and resolution by regulating the condenser aperture diaphragm;
- Understand the importance of the correction collar for minimazing spherical aberration.

D) **Our diatom test slides are trusted by major microscope manufacturing companies for quality control and customer demonstrations.**

E) **Guaranteed Mounting:** all micromanipulated diatoms are fixed directly to the underside of the optical-quality cover glass (rather than the microscope slide itself) for maximum resolution and contrast. The reason: objective performance drops significantly as the specimen distance from the cover glass increases (microscope objective lenses are designed to be optimally corrected for objects located immediately below the cover glass).

F) Diatoms are mounted in Diatom Lab’s proprietary high-refractive-index ***Diatom Cubed* mountant**. It is unalterable over time and has an R.I. > 1.7, ensuring maximum optical performance. Diatoms are attached to the underside of the cover glass by means of Diatom Lab's unique ***NANO-ADHESIVE***, which is invisible even in phase contrast, darkfield illumination, differential interference contrast (DIC) and oblique illumination! This ensures all the finest Diatom details such as areolae, poroids, striae and so on, are always perfectly clean, regardless of the technique used, with the additional benefit that there are no traces, stains or swipe-marks caused by fixatives typically seen on some older preparations. Our *NANO-ADHESIVE* is also invisible to scanning electron microscopes (SEMs), and is therefore used to create a clean background for our SEM specimen preparations

G) It is a **standardized** microscope test slide that allows different laboratories and microscopists to compare their results. Each slide is manufactured to identical

specifications. Each diatom sample contains specimens collected from the same location, depth, and time.

H) **Not all *Pinnularia nobilis* diatoms are identical! For the Diatom Test Slide version 2.0 and 3.0, we selected the most challenging *Pinnularia nobilis* sample from our collection! Measurements performed via Scanning Electron Microscopy (SEM) confirm that poroid distances and dimensions vary among *Pinnularia nobilis* specimens worldwide.**

TECHNICAL SUGGESTIONS:

(µm = micrometer; um = nanometer)

Diatom number 1

Species: *Cymbella mexicana* (Ehrenb.) Cleve 1894
Striae in 10 µm: 8-9
Structures to resolve: striae and areolae
Suggested techniques: dry objectives in Bright field, Oblique illumination, Darkfield illumination, Phase contrast, Differential interference contrast (DIC)

Diatom number 2

Species: *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg
Striae in 10 µm: 14-15
Structures to resolve: striae and areolae
Suggested techniques: dry objectives in Bright field, Oblique illumination, Darkfield illumination, Phase contrast, Differential interference contrast (DIC)

Diatom number 3

Species: *Synedra ulna* (Nitzsch) Ehrenberg 1832
Striae in 10 µm: 8-9
Structures to resolve: areolae
Suggested techniques: oil immersion objectives in Bright field, Oblique illumination, Darkfield illumination, Phase contrast, Differential interference contrast (DIC).

Not all *Synedra ulna* diatoms are identical. For the Diatom Test Slide version 2.0 and 3.0, we selected the most challenging Synedra ulna sample from our collection!

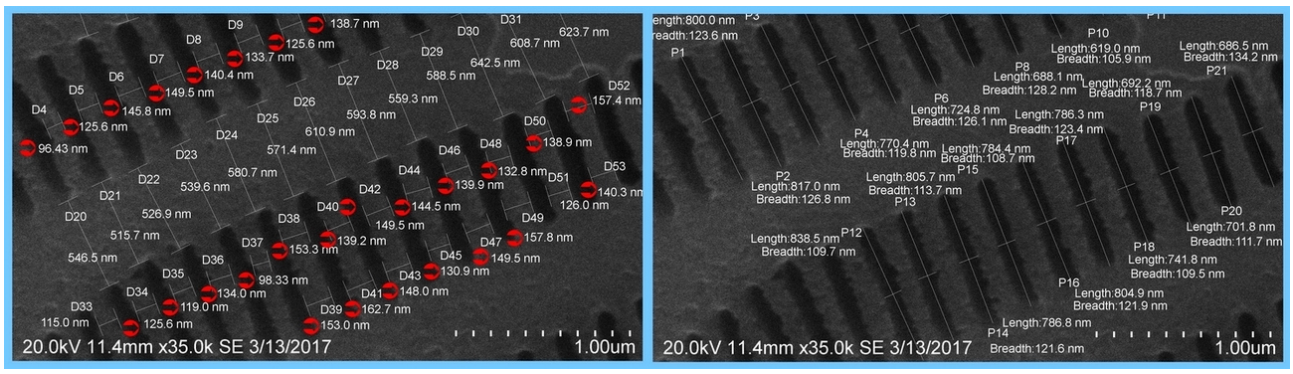
In some cases, the use of excellent dry microscope objectives (such as a plan-apochromat 40x/0.95 iris), combined with several optical contrast methods, permits the detection and even imaging of these areolae.

Diatom number 4

Species: *Navicula oblonga* (Kützing) Kützing
Structures to detect: lineate areolae (lineolae), forming the striae
Lineate areolae (lineolae) in 10 µm: 48-50, see Scanning Electron Microscope (SEM) measurements below
AVERAGE DISTANCE BETWEEN areolae (lineolae): 0,14 µm, see Scanning Electron Microscope (SEM) measurements below.

The theoretical limit of resolution of most light microscopes is ~0.2 µm; however, these areolae (lineolae) can be detected and imaged using the methods described below, made possible by our proprietary materials and advanced techniques.

Recommended microscope objectives: Oil-immersion 63 or 100x objectives having a good or excellent numerical aperture (starting from 1,2; better: 1,3 or 1,4)
Suggested techniques: Double immersion (= Oil immersion objective and Oil immersion condenser) and:
Polarized light (the polarizers should be oriented perpendicular to each other = maximum level of extinction);
or Circular oblique illumination (C.O.L.) with polarized light;
or Darkfield illumination using an immersion dark field condenser (better 1,2/1,4);
or Differential interference contrast (DIC);
or UV illumination: in this case highly specialized laboratory facilities are required. UV radiation is hazardous to the eyes and necessitates appropriate personal protective equipment (PPE), specialized accessories, and dedicated cameras. Always refer to your instrument's operating manual.
or some variants of the Differential interference contrast (such as AVEC-DIC, the Allen Video-enhanced Contrast);
or IRC (Interference reflection contrast technique



Scanning Electron Microscope (SEM) measurements of our *Navicula oblonga* diatoms:
- average distance between lineate areolae (lineolae): 0,14 µm;
- average lineate areolae (lineolae) dimensions: 0,12 µm X 0,74 µm.

In the SEM images all measurements are in nanometers (nm). Several specimens of the same sample were analyzed (images and measurements courtesy of Professor Benjamin Dattilo and Professor Anne Argast, Argast Family Imaging and Analysis Lab, Purdue University Fort Wayne, Indiana, USA.

Diatom number 5

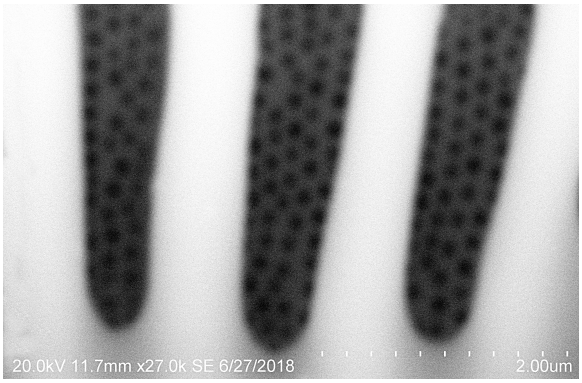
Species: *Pinnularia nobilis* (Ehrenberg) Ehrenberg

Not all *Pinnularia nobilis* diatoms are identical. For the Diatom Test Slide version 2.0 and 3.0, we selected the most challenging *Pinnularia nobilis* sample from our collection! Measurements performed via Scanning Electron Microscopy (SEM) confirm that poroid distances and dimensions vary among *Pinnularia nobilis* specimens worldwide.

Structures to detect: Poroids
AVERAGE DISTANCE BETWEEN POROIDS: 0,11 µm

The theoretical limit of resolution of most light microscopes is ~0.2 µm; however, these pores can be detected and imaged using the methods described below, made possible by our proprietary materials and advanced techniques.

Recommended microscope objectives: oil-immersion 63 or 100x objectives having a very good or excellent numerical aperture (1,3 or 1,4)
Suggested techniques: Double immersion (= Oil immersion objective and Oil immersion condenser) and:
Polarized light (the polarizers should be oriented perpendicular to each other = maximum level of extinction) with possibly oblique illumination;
or Immersion dark field condenser (better 1,2/1,4) with Polarized light (the polarizers should be oriented perpendicular to each other = maximum level of extinction)

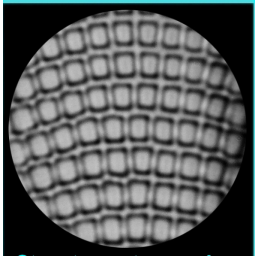
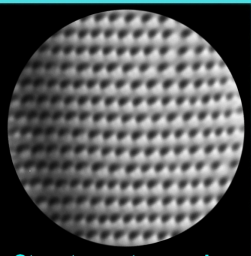
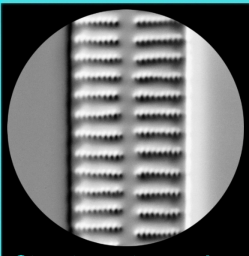
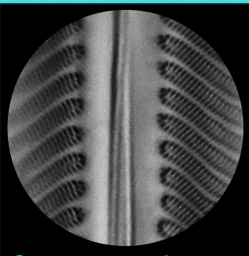
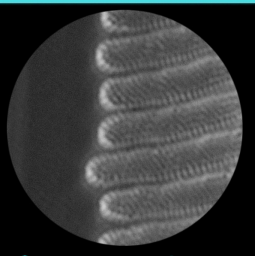


Scanning Electron Microscope (SEM) measurements of our *Pinnularia nobilis* diatoms:
- average diatance between poroids: 0,11 µm.
In the SEM images all measurements are in nanometers (nm). Several specimens of the same sample were analyzed (images and measurements courtesy of Professor Benjamin Dattilo and Professor Anne Argast, Argast Family Imaging and Analysis Lab, Purdue University Fort Wayne, Indiana, USA.

new

Diatom Test Slide version 3.0

by Diatom Lab. With online instructions

Cymbella mexicana	Stauroneis phoenicenteron	Synedra ulna	Navicula oblonga	Pinnularia nobilis
				
Structures to resolve: striae and areolae	Structures to resolve: striae and areolae	Structures to resolve: areolae	Structures to detect: lineate areolae	Structures to detect: poroids
Striae in 10 µm: 8-9	Striae in 10 µm: 14-15	Striae in 10 µm: 8-9	Lineate areolae in 10 µm: 48-50	Avg. distance between poroids: 0,11 µm. Our most difficult P. nobilis
For dry objectives	For dry objectives	For oil immersion objectives	For double immersion	For double immersion

Available at www.diatomshop.com