

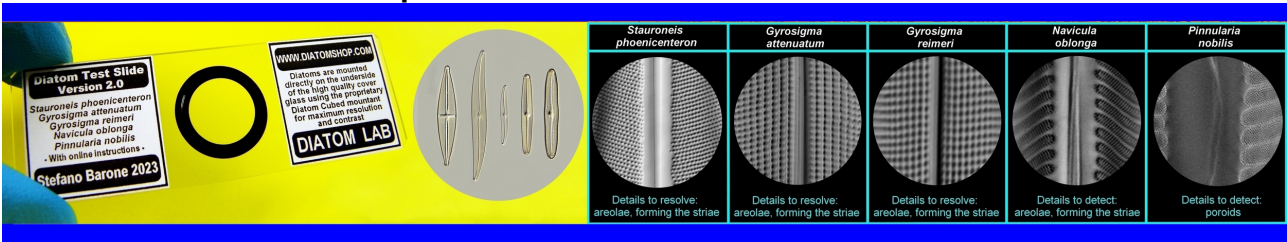
Diatom Test Slide version 2.0 Instruction Manual

(Updated edition)

Venture into the Micro and Nano structures

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www.diatomshop.com www.testslides.com www.diatomlab.com



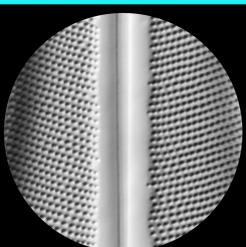
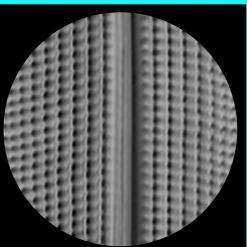
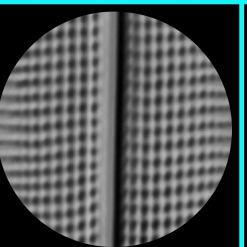
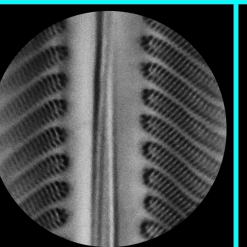
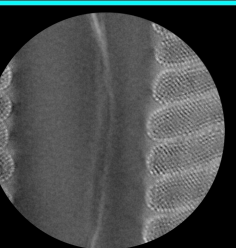


Since the appearance of the microscope, some Diatom species (called Test Diatoms by microscopists) have been and still are used to test optics, because striae and areolae distances in a same species collected in a same area are constant regardless the size of the Diatom frustule (an areola is one of the pores in a row that forms a stria on a valve. The plural is areolae. A stria is a row of areolae. The plural is striae).

In other words Test Diatoms are extremely valid and accurate to quantify resolution of optical microscopes (defined as the smallest distance between two 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 evaluate and compare microscope models, objectives, eyepieces, condensers, light sources, optical filters, microscope cameras and contrast techniques with each other. They can be considered as extremely accurate and "merciless" tests for the microscope and its accessories.

Test Diatoms are always available at www.diatomshop.com, the online store of Diatom Lab (Stefano Barone, founder & owner of Diatom Lab).

 <h1 style="text-align: center;">Diatom Test Slide</h1> <h2 style="text-align: center;">Version 2.0 by Diatom Lab</h2> <p style="text-align: center;">In DIATOM CUBED mountant. In order to give you the best conditions for resolution and contrast, Diatoms are attached to the UNDERSIDE of a properly thick cover glass having high optical quality</p> 				
<i>Staurois phoenicenteron</i>	<i>Gyrosigma attenuatum</i>	<i>Gyrosigma reimeri</i>	<i>Navicula oblonga</i>	<i>Pinnularia nobilis</i>
				
Details to resolve: areolae, forming the striae	Details to resolve: areolae, forming the striae	Details to resolve: areolae, forming the striae	Details to detect: areolae, forming the striae	Details to detect: poroids
Striae in 10 µm: 12-15 longitudinal	Striae in 10 µm: 13-17 longitudinal	Striae in 10 µm: 18-22 longitudinal	Areolae (lineolae) in 10 µm: 48-50	Average distance between poroids: 0,11 µm (New more difficult sample)
For DRY objectives	For DRY and OIL immersion objectives	For OIL immersion objectives	For DOUBLE IMMERSION: to detect areolae, read the ONLINE INSTRUCTIONS	For DOUBLE IMMERSION: to detect poroids, read the ONLINE INSTRUCTIONS
<h1 style="color: green;">Available at www.diatomshop.com</h1> <p style="text-align: center;">To download the PDF INSTRUCTION MANUAL, visit the Price list page</p>				

PREMISE:

A) This microscope test slide contains 5 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.

This microscope test slide is excellent to EVALUATE and COMPARE microscope models, objectives, eyepieces, condensers, light sources, optical filters, microscope cameras and contrast techniques with each other. It can also be used as a TRAINING TOOL or a TEACHING TOOL, for example it is possible to:

- practice using your microscopes at their highest levels!
- examine the variations in contrast and resolution by regulating the condenser aperture diaphragm
- understand the importance of correction collar for minimizing spherical aberration
- examine the variations in resolution by using different wavelengths of light

B) It is a STANDARDIZED microscope test slide, so different laboratories and microscopists who own this product can compare their results from different places, in fact each microscope slide has the same production characteristics:

- **Each Diatom species belongs to a sample collected in the same place / depth / time;**
- In order to give you the best conditions for resolution and contrast, Diatoms are attached / fixed to the **UNDERSIDE** of a properly thick cover glass (and not commonly on the microscope slide!), so there is no space between Diatom frustules and cover glass (although this procedure requires several special manufacturing precautions). Furthermore, the mounting to the underside of the cover glass allows to easily use all oil immersion objectives (even those with very short working distance, such as plan-apochromat 63x/1,4 or 100x/1,4);
- **Diatom Cubed, the PROPRIETARY MICROFILTERED HIGH REFRACTIVE INDEX MOUNTANT** belongs to the same production lot;
- **The CUSTOMIZED cover glass with HIGH OPTICAL QUALITY** has been manufactured in Germany specifically for Diatom Lab.

C) NOT ALL *Pinnularia nobilis* (Ehrenberg) Ehrenberg ARE THE SAME! The MOST DIFFICULT *Pinnularia nobilis* (Ehrenberg) Ehrenberg sample among all the others we keep was chosen for the Diatom Test Slide version 2.0 (measurements were performed using a scanning electron microscope or SEM), in fact not all *Pinnularia nobilis* (Ehrenberg) Ehrenberg in the world have the same poroids measurements!

D) **Since its appearance, hundreds of laboratories, microscope companies and microscopists from all over the world use this slide with profit and satisfaction!**

TECHNICAL SUGGESTIONS:

Diatom number 1

Species: *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg

Striae in 10 µm: 12-15 longitudinal

Details 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: *Gyrosigma attenuatum* (Kützting) Rabenhorst

Striae in 10 µm: 13-17 longitudinal

Details to resolve: areolae, forming the striae

Suggested techniques: dry or oil immersion objectives in Bright field, Oblique illumination, Darkfield illumination, Phase contrast, Differential interference contrast (DIC)

Diatom number 3

Species: *Gyrosigma reimeri* Sterrenburg

Striae in 10 µm: 18-22 longitudinal

Details to resolve: areolae, forming the striae

Suggested techniques: oil immersion objectives in Bright field, Oblique illumination, Darkfield illumination, Phase contrast, Differential interference contrast (DIC)

Diatom number 4

Species: *Navicula oblonga* (Kützinger) Kützinger

Details to detect: areolae (lineolae), forming the striae

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, but these areolae (lineolae) can be detected and imaged by the techniques below, thanks to Diatom Cubed high refractive index mountant.

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 (it is dangerous for the eyes, it requires the use of special protection devices, accessories and cameras. Please refer to the operating manual of your instruments);

or some variants of the Differential interference contrast (such as AVEC-DIC, the Allen Video-enhanced Contrast);

or IRC (Interference reflection contrast technique)

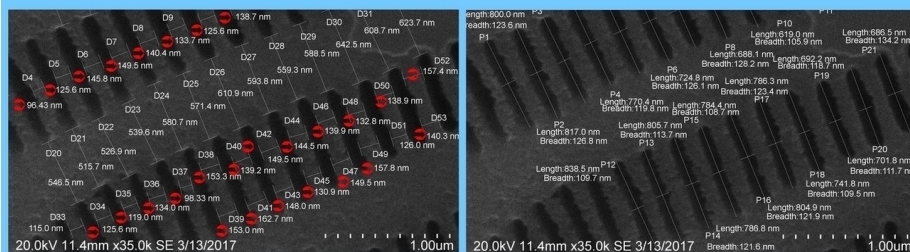
**Scanning Electron Microscope (SEM) measurements of Diatom *Navicula oblonga* (Diatom Lab's sample) which is found in Diatom Test Slide Version 2.0 by Diatom Lab
Microscope slide AVAILABLE AT WWW.DIATOMSHOP.COM**

AVERAGE DISTANCE BETWEEN PORES (LINEOLAE): 0,14 µm (micrometers or microns)
AVERAGE PORE (LINEOLAE) DIMENSIONS: length 0,74 µm, breadth: 0,12 µm
AVERAGE DISTANCE BETWEEN STRIAE: 0,57 µm

The resolving power of a microscope is measured by its ability to differentiate two lines or points in an object: in this case the most difficult (and most interesting) goal is to separate the pores (lineolae) that have an average distance of 0,14 µm

Note that in these SEM pictures all measurements (decimal numbers) are in nanometers (nm). Several specimens of the same sample have been studied

(Image and measurements courtesy of Professor Benjamin Dattilo and Professor Anne Argast, Argast Family Imaging and Analysis Lab, Purdue University Fort Wayne, Fort Wayne, Indiana, USA) All Rights Reserved



Diatom number 5

Species: *Pinnularia nobilis* (Ehrenberg) Ehrenberg

NOT ALL *Pinnularia nobilis* (Ehrenberg) Ehrenberg ARE THE SAME! The MOST DIFFICULT *Pinnularia nobilis* (Ehrenberg) Ehrenberg sample among all the others we keep was chosen for the

Diatom Test Slide version 2.0 (measurements were performed using a scanning electron microscope or SEM), in fact not all *Pinnularia nobilis* (Ehrenberg) Ehrenberg in the world have the same poroids measurements!

Details to detect: Poroids

AVERAGE DISTANCE BETWEEN POROIDS: 0,11 μm

Again, the theoretical limit of resolution of most light microscopes is $\sim 0.2 \mu\text{m}$, but these POROIDS can be detected and imaged by the techniques below, thanks to Diatom Cubed high refractive index mountant.

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)

The most difficult *Pinnularia nobilis* (Ehrenberg) Ehrenberg sample among all the others we keep was chosen for the Diatom Test Slide version 2.0 (measurements were performed using a scanning electron microscope or SEM), in fact not all *Pinnularia nobilis* Diatoms existing in the world have the same poroids measurements!

The Diatom Test Slide version 2.0 groups Test Diatoms ranging from easy to extremely difficult. Since its appearance in 2018, hundreds of laboratories, microscope companies and microscopists from all over the world use this standardized slide with profit and satisfaction!

Available at www.diatomshop.com

Scanning Electron Microscope (SEM) measurements of Diatom *Pinnularia nobilis* (Diatom Lab's sample) which is found in Diatom Test Slide Version 2.0 by Diatom Lab
Microscope slide AVAILABLE AT WWW.DIATOMSHOP.COM

AVERAGE DISTANCE BETWEEN POROIDS: 0,11 μm NEW MORE DIFFICULT SAMPLE

Recommended microscope objectives: oil-immersion 63 or 100x objectives having a very good or excellent numerical aperture (1,3 or 1,4)

Suggested techniques: Homogeneous Immersion System = Oil immersion objective and Oil immersion condenser and: A) Polarized light (the polarizers should be oriented perpendicular to each other = maximum level of extinction) with possibly oblique illumination or B) 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)

Note that in these SEM pictures all measurements (decimal numbers) are in nanometers (nm). Several specimens of the same sample have been studied

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Light microscope image: poroids can be detected in double immersion!

SEM (Scanning Electron Microscope) image courtesy of Professor Benjamin Dattilo, Purdue University Fort Wayne, Fort Wayne, Indiana, USA