



Diatoms and Microscopy a contrasting combination

The regular patterns of diatoms make them ideal test specimen for evaluating resolution in microscopy. Especially the fine structure of the diatoms *Frustulia saxonica* en *Amphipleura pellucida* are useful in that they permit evaluation of the optical system to the extreme limit of its capabilities.

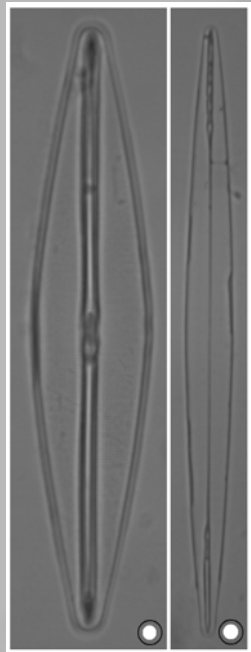
Brightfield illumination

The golden standard in microscopy.

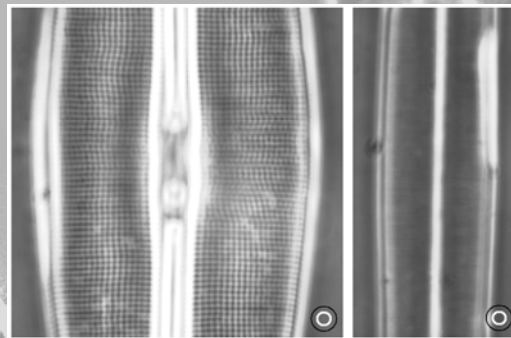
The condenser iris is slightly closed, gaining contrast but compromising resolution.

Frustulia (rhomboides var.) saxonica can just be resolved with a normal 100x immersion objective and dry condenser.

Structure in *Amphipleura pellucida* cannot be detected.



○ Brightfield with reduced condenser aperture



○ Phase Contrast

Phase Contrast

Very strong contrast enhancement, even the lightest of silicified valves are easily spotted.

Bright, obscuring halos are formed around strongly refracting structures.

The striae of *Amphipleura pellucida* cannot be resolved due to the low effective condenser aperture.

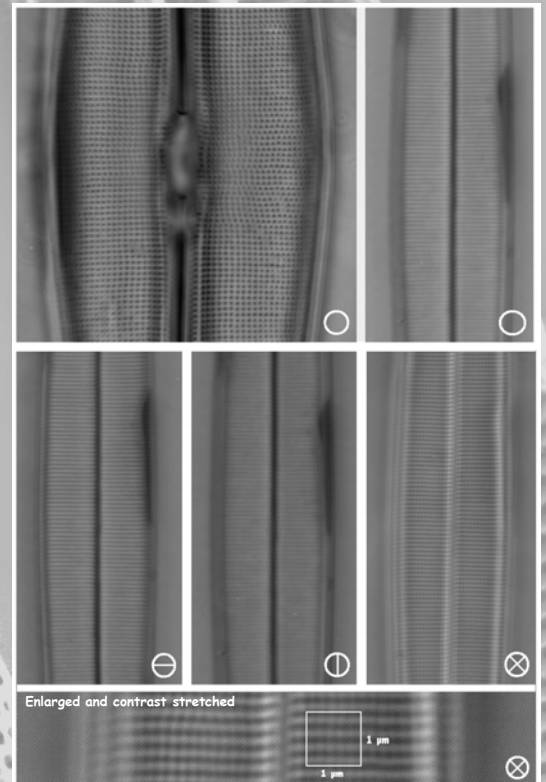
Oblique and annular illumination

Contrast is enhanced by removing part of the illumination cone.

Shadowing effect occurs with unidirectional oblique light.

With annular illumination, the illumination has the shape of a hollow cone. The positioning of the diatom is not critical as there is no directional component.

Polaroid filters can boost the contrast to the extreme limit, but the diatom needs careful positioning relative to the polarisation direction.



Enlarged and contrast stretched

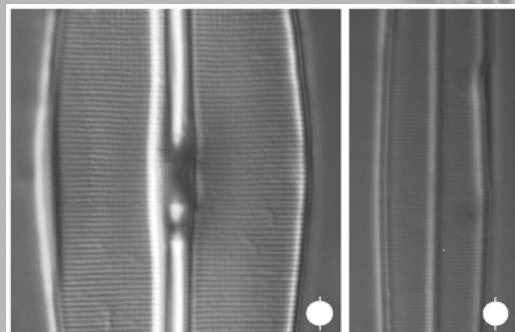


- Annular lighting at the edge of the objective aperture
- ⊖ Annular lighting with polarised light, polarisation direction indicated
- ⊗ Annular lighting, crossed polars

Differential Interference Contrast, ('Nomarski')

Capable of higher resolution than phase contrast, but overall contrast is lower. No halo affect!

The striae of *Amphipleura pellucida* are easily detected, but it needs careful positioning for highest contrast.



○ DIC at full aperture, shear direction indicated

Conclusion

A good quality brightfield condenser should ideally be the start of any identification. If the ultimate in resolution is needed, an oil immersion darkfield (cardioid) condenser is an inexpensive aid to gain the ultimate in contrast from the finest of diatom details.

Technicalities:

The same diatom test-slide from Klaus Kemp (www.diatoms.co.uk) has been used for all images. A Tucsen 1/2' cmos camera was used for imaging at the manufacturers default settings, and images were corrected afterwards for brightness only. Brightfield images were made with an Olympus 60/1.4 objective, Phase Contrast images with a Leitz 100/1.32 Ph NPL Fluotar and DIC images with the same objective on a Zeiss DIC system with oiled condenser. Annular lighting was provided for with an Olympus oil-immersion cardioid condenser DCW 1.2-1.4. A Zeiss 100/1.3 planapo was used with annular lighting, relayed to a Tucsen 1.3Mp monochrome camera via a 1.5x projective.

Poster background: FESEM image of *Amphipleura pellucida*, inside and out. Specimen from the same source as for the test slide. Courtesy of Peter Höbel, Erlangen, Germany.