Exploring the Diatom Lab test slide *Pinnularia dactylus var. dariana (A. Schmidt) Cleve 1895 at ca. 400 nm.*

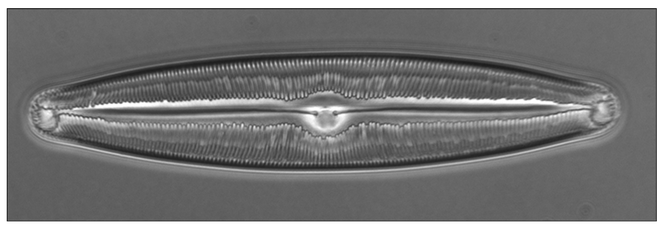
by David Walker, UK

Like many fellow enthusiasts I enjoy on occasions pushing a microscope to its optical limits. It's a good test for a given set of optics, the mechanics, the capabilities of the camera and to some extent the abilities of the user (the latter which I don't claim to have any advanced skill set for!). Prepared slides of diatom frustules are one of the most popular test slides.

Some time ago I treated myself to three single species diatom test slides prepared by Stefano Barone of Diatom Lab but it is only recently that I have started to explore them. From previous slides purchased I knew they were prepared to the very highest standards with the diatom touching the under side of the high spec. coverslip and use a proprietary Diatom Cubed high RI 1.7 mountant. A variety of test slides can be bought on the dedicated www.diatomshop.com website.

This short report is an exploration of one, *Pinnularia dactylus var. dariana (A. Schmidt) Cleve 1895* using exclusively my Zeiss Photomicroscope III with contemporary Zeiss 160 mm tube length optics.

The single species test slides are supplied with a card which provides measurements of the detail (by SEM) to resolve and advice on the techniques for resolving. Included are images of the resolution expected with an optical microscope under optimal conditions and in this case also an SEM image. These cards can be viewed on the website.



Zeiss Neofluar 40/0.75 phase, green filter. A single selected cleaned frustule is presented.
The chambers or alveoli have poroids and it is these that present the challenge to resolve.
Round notes for the Pinnularia genus that 'the poroids are usually invisible in the LM .."
(F E Round et al, The Diatoms, pub. CUP, 1990, p.556. Their SEM image 'd' clearly shows them.)

The suggested techniques include the classics to optimise objective resolution; immersion of a high NA condenser using one or more of crossed polar filters, oblique and ultra darkfield (the latter likely to achieve circular oblique depending on the relative NAs of the objective and darkfield condenser). An additional approach which particularly interests me is using a near UV 400 nm interference filter on the field lens. The 100W quartz halogen bulb emits enough near UV for sensitive cameras to view the image in real time and for image capture, in my case a ZWO ASI178MM astro' type monochrome camera with no sensor filter.

The fine resolution test is potentially tougher than *A. pellucida as* the Diatom Lab info' card notes the pores are ca. 0.16 - 0.19 µm apart, whereas for *P. dactylus* the poroids are 0.072 - 0.089 µm apart (SEM measurements).

I tried the Zeiss 63/1.4 and 100X/1.3 planapos but have never been very impressed with these objectives for diatom dotting. The now dated coating designs and extensive elements required for planapos are not ideal for eking out the maximum contrast from typically weakly contrasted diatoms. My preference is the 100/1.3 Neofluar which with its fewer elements seems to have the edge. Although the Photomicroscope design in itself is not ideal for such studies, there are a number of optical elements between back focal plane of the objective to the camera even without using an eyepiece. A simpler Zeiss stand without Optovar or modern microscope with infinity optics can present the image straight from the back focal plane via the photo port to the camera sensor, as in the Nikon Eclipse Ex00 series for example.

The best image that have achieved to date (below) with the Neofluar 100/1.3 was using the Zeiss 1.2/1.4 darkfield condenser to give circular oblique illumination with crossed polars. Even the sensitive astro' camera required a 5-10 second exposure to capture an image which prevents critical real time imaging at 400 nm. A 410 nm filter instead of 400 nm provided a little more light as being just in the visible. There's poroids evident but not as crisp and with a higher contrast as hoped. Focus was critical and the fine focus of the Zeiss stand was struggling somewhat. Strong conventional oblique was tried but found artefacts of the poroids were created rather than true detail.

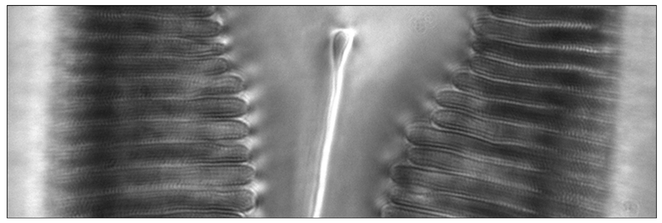


Image crop. Slight tonal balance adjustment.

Comments to the author David Walker are welcomed.

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