This document provides a description of the equipment I typically use to take my diatom images. I plan on doing another one looking at how I process my images when stacking. I'll concentrate on my standard setup here rather than discussing my deep UV work (which requires very special objectives and condensers).

The microscope itself is an Olympus BHB from sometime in the later 1970s or early 1980s. It is shown setup for imaging in Figure 1 (although to be clear I do not do my imaging on the floor).

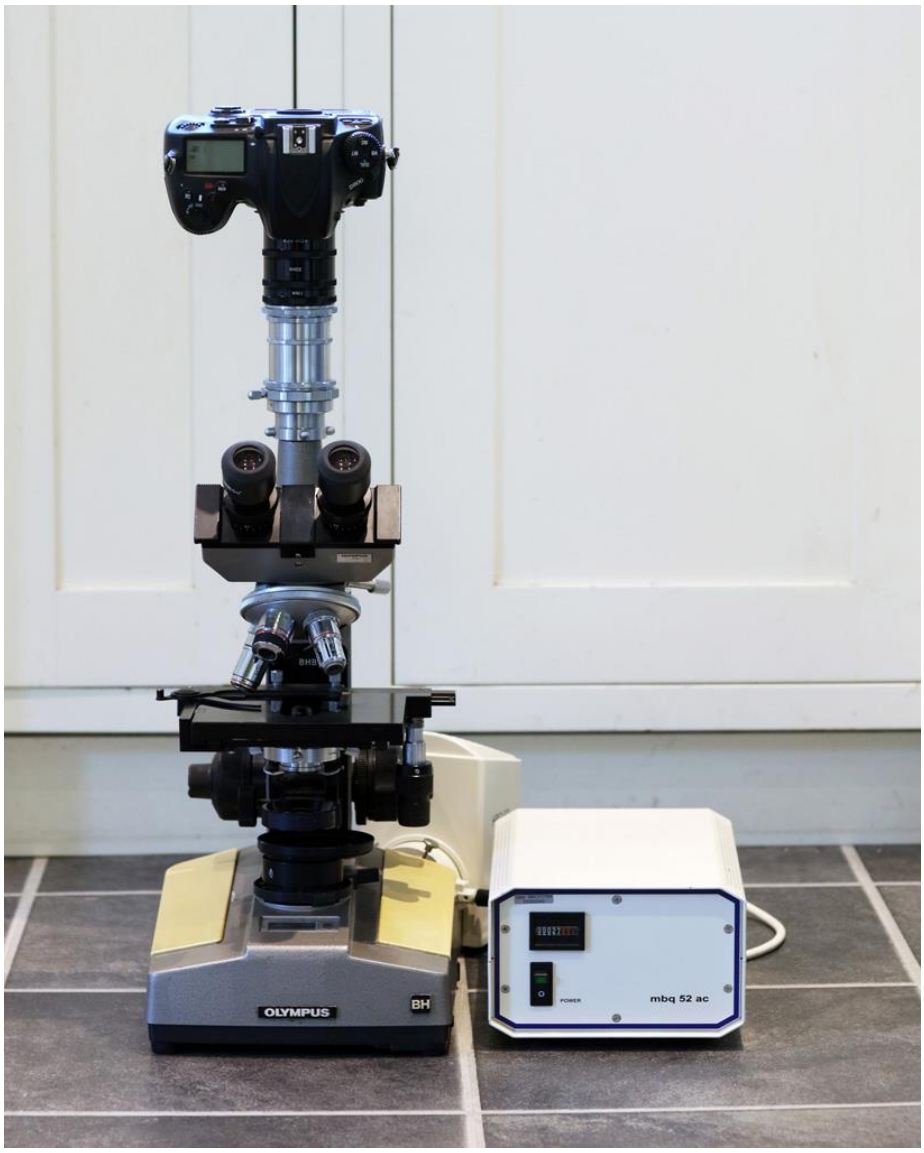

Figure 1. Olympus BHB microscope.

I have modified the microscope by replacing the field lens with a fused silica one. Also, the beamsplitter in the trinocular head has been cut in half and one half replaced with a fused silica block. I built the microscope to allow me to do UV transmission imaging with light down to and below 300nm. The original glass components would have blocked UV below about 365nm, but fused silica allows it to pass. As is, it can now be used with light from about 200nm up to and beyond 1000nm (assuming the objective, condenser and slide are suitable of course). The beamsplitter

originally would have either sent all the light to eyepieces, or split it between the eyepieces and photoeyepiece. The way it is now setup it either sends all the light to the eyepieces, or all the light to photoeyepiece. This is safer for my UV imaging, and as I do my final focus using live view on the camera works fine. I also have 2 yellow filters below the eyepieces which block 99.99% of the light below 400nm as an additional safety measure when using UV, and I wear UV safety glasses as well.

I normally use an Olympus Aplanat Achromat condenser (BH-AAC) for visible light imaging, Figure 2.

Figure 2. Olympus Aplanat Achromat condenser in standard brightfield configuration (left) and setup of oblique illumination (right).

The BH-AAC is a really nice condenser with an NA of 1.4. It can be used for normal brightfield illumination, however the iris can be offset to provide oblique lighting. The iris is on a slider so the extent of 'obliqueness' can be varied, and it can be rotated to adjust the direction the light is coming from. This is especially helpful for thin low contrast diatoms, and can help give a pseudo 3D look to them. I've heard it called "poor man's DIC" and I can see why. Unlike DIC though it can be used with any objective, isn't difficult to setup, and it costs a lot less.

This condenser works well with 450nm light and even 390nm, however it blocks 365nm light. I do use other condensers including a Leitz Heine, a Reichert Neo 1.18/1.42 dark ground, and even a standard Olympus Abbe condenser (it lets 365nm light through, so can be used for brightfield imaging when the BH-AAC cannot).

For objectives I often use Leitz Pl Apo ones, specifically 40x NA 1.00, 63x NA 1.40 and 100x NA 0.60-1.32, as shown in Figure 3.

These are all Plan Apo objectives (flat field of view and apochromatic), and are for use with immersion oil. They are very high quality, and are probably from around the same era as the Olympus BHB microscope. They have high NA's, important for imaging the smallest features in diatoms. Importantly for me, and very surprising for complex Plan Apo obejctives is that they let 365nm light through, so can even be used for UV imaging when needed. The focus point does shift by a bit from that of 450nm which is not a shock given with 365nm they are well outside of how they were intended to be used. The 100x has an adjustable iris which is helpful for darkground imaging.

Figure 3. Leitz Pl Apo 40x, 63x and 100x objectives.

In addition I often use 10x Nikon Plan Apo NA 0.45 and 20x Nikon Plan Apo NA 0.65 objectives. These are not oil immersion objectives and are very sharp and again have a flat imaging field and are apochromatic. I have plenty of other objectives of various makes and types as I am a bit of a lens hoarder. I do have quite a few Olympus objectives, but often do not use them. I'll explain why later.

Next is the photoeypiece. With the Leitz and Nikon objectives I normally use a 2.5x Nikon CF PL one, Figure 4.

Figure 4. Nikon 2.5x CF PL photoeyepiece (left) and Thorlabs 450nm, 40nm FWHM filter (right).

This photoeypiece works well for visible and UV down to 365nm, and I just mount it in the Olympus trinocular port as if it were an Olympus photoeyepiece. It also works well with the Leitz Pl Apo

objectives. The CF photoeyepieces do not correct the image from the objective (unlike the Olympus NFK ones) which probably explains why they work ok with the Leitz objectives as well as the Nikon ones. This flexibility is one of the reasons I use the Leitz and Nikon objectives as opposed the Olympus ones. I do not have a full set of Olympus Splan Apo objectives which is another reason.

On top of the photoeyepiece I put the filter for the specific wavelength of light that I am using. Normally this is a 450nm, 40nm full width half maximum one from Thorlabs. This in combination with the white LED light source gives me plenty of light at 450nm which is great for diatoms. I put the filter on top of the eyepiece for a couple of reasons. Firstly it is a small area so I can use 25mm filters. Next it is the last thing before the light enters the camera. This can be important when using UV or short wavelength light sources as these can cause fluorescence in the slide. If the filter is placed on top of the photoeyepiece this fluorescence can be removed. If the filtering occurs lower down in the setup it is not removed.

After this I have a set of extension tubes and then the camera. I'll be honest I've never done actual measurements to see exactly what the distance is to the camera from the microscope. I set these up with trial and error, and with the 63x Leitz objective the image on the camera is pretty much parfocal with the eyepieces with 450nm light, so I know the distance is about right. I do occasionally remove one or two of the extension tubes to give me a slightly wider field of view. However this does cause vignetting in the image and will slightly degrade the image quality. I only tend to do it if the diatom is slightly too big to fit in the photo when imaged with the objective I want to use.

For cameras I use a monochrome converted Nikon d850 or d800 for my 450nm or UV images. These have had the bayer filter removed, and was something I got done for my UV imaging work (as it drastically improves UV sensitivity below 365nm). I use ISO 100 to minimise noise. I focus using live view on the back of the camera, and capture the image with self timer and mirror lockup to reduce vibration. I also use a cable release so I do not have to touch the camera.

I do all my focus stacking manually – take a photo, move the stage up, taken another photo and repeat as necessary – and capture the images as JPEGs. The amount of movement for each image depends on the objective and diatom. I'll talk more about focus stacking itself in another article.

A couple of other things to cover. Lighting and to create the scale bars. For lighting I use white LED light I made myself using a broken bulb from the Olympus BHB for 450nm or normal visible light imaging, Figure 5.

I think it was a cool white LED but was just something I bought on ebay. It is mounted to that the LED is roughly where the filament would be for the original bulb. As it is mounted on an old lamp base, it can just be plugged into the normal lamphousing. The leads are then run to an external powersupply (the one in the microscope was broken when I got it, and wouldn't be suitable for the LED anyway). This LED gives plenty of light for brightfield, phase contrast and dark ground illumination. In Figure 1, the light source is actually a 50W Zeiss Mercury Xenon lamp which I use when imaging deeper into the UV. If I am just imaging with 365nm light, an LED torch is fine (I think mine is a 10W or 15W one). This is pushed into the light port on the back of the Olympus BHB and held in place with a 3D printed cylindrical part. Recently I also got a 390nm LED light source and have been doing some experiments with that. This is nice as I can still use the BH-AAC condenser with it (unlike 365nm light) and it offers better potential resolution than 450nm light.

Figure 5. Olympus lamp housing (left), white LED conversion (middle) and original bulb (right).

Final thing to cover here is how I create the scale bars. This is done by using a stage graticule. I have a few by Graticules Ltd which were bought second hand on ebay. When new they are very expensive, but second hand they typically go for around £30-60. I have ones with 10µm divisions and ones with 2µm divisions. The 2µm ones are better when using higher magnification/higher resolution objectives. A 1mm scale with 10µm divisions slide is shown in Figure 6.

Figure 6. A 1mm stage graticule with 10µm divisions.

In use, the graticule is placed on the stage and the imaged with the same setup as the sample. For oil immersion objectives a drop of oil is put on it, but I do not use it in combination with a coverslip. An image is captured and I just measure the number of pixels for a given distance in Photoshop and then use this to manually create a scale bar. This needs to be done for any given combination of objective, photoeyepiece, camera, wavelength and length of extension tube. Varying any one of these will alter the scale. Also changing the wavelength of light will alter the scale slightly as the focusing is different. You can get cheap scale bar slides on ebay, but I have no idea as to their quality, so I stick with second hand ones from a heritage supplier.

Thanks for reading and I hope this is a useful introduction to the equipment I typically use when collecting my images. As you can see my setup is relatively simple with a 160mm finite tube length microscope. I invest in the best objectives I can find though and would recommend this to anyone imaging diatoms. Also, the Aplanat Achromat condenser with its ability to do oblique lighting is very useful, although may be a challenge to adapt to other makes of microscopes.

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