SEM Diaries - 19

A Quiet Period

Jeremy Poole

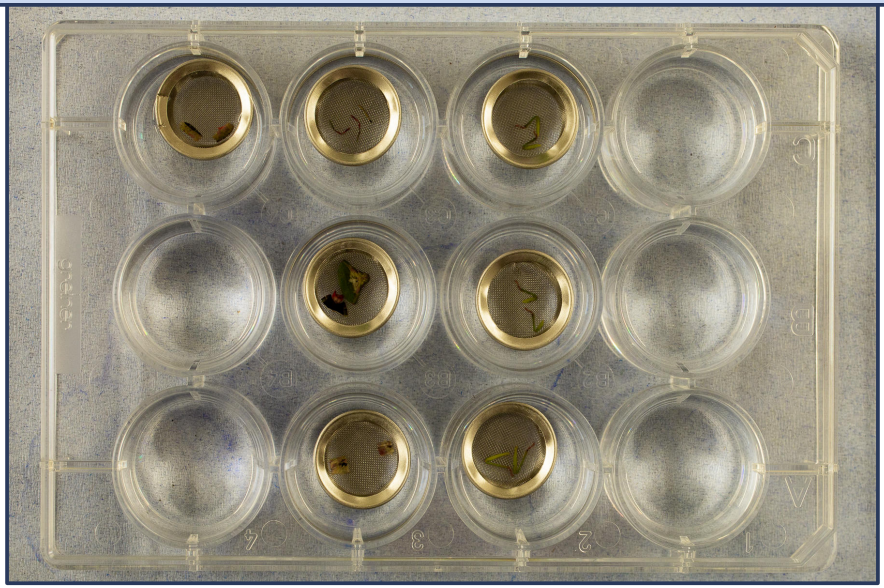

Fig. 1: Specimens at different stages of dehydration in pipe filters, immersed in alcohol

For various reasons, the last couple of months have not left me much time to work seriously on the SEM. As I write this, I have seen the light at the end of the tunnel - indeed in mid November I dissected a green shield bug (*Palomena prasina*), and a selection of anatomical features, going through the desiccation process, can be seen in Figure 1. The tray contains 12 cavities, each containing neat iso-propanol and the specimens are retained within pipe filters as suggested by Mark Burgess in a letter in the January 2020 issue of Balsam Post (BP126). They are moved progressively through each column, from left to right, before being immersed in HMDS in one column of a similar tray. (The wonders of HMDS were described in SEM Diaries - 16.)

The filters are a 1.2 cm diameter option supplied by Mentin and available from Amazon, and are almost perfect for the job. Ideally they could be slightly larger in diameter, and have a vertical lip to ease lifting with tweezers, but they work very well as they are. The gauze mesh is much finer than on my home-made version, which means that no specimens I am likely to dissect will be able to escape (other than by floating out of the top if I fill the cavities too full!).

Most of my work with spiders, and with insects such as the shield bug, is done at relatively low magnifications, compared to what my SEM is capable of, so it is always "fun" to explore the potential of the higher magnifications when suitable material presents itself. Unfortunately, this is not simply a case of selecting a higher magnification and letting the instrument

do the rest. As the magnification increases it is necessary to select a smaller spot size, which in turn introduces more noise, making focusing more difficult. It is also necessary to increase the acceleration voltage (which also reduces spot size) and then one needs to adjust the "stigmator", which is a control designed to ensure that the beam is not elliptical in cross-section. Overall, a high level of skill is demanded of the operator.

Although my SEM is specified to operate at magnifications up to x1,000,000, that part of the specification is a joke. The width of the display on the instrument is around 300 mm. Thus, at a magnification of a million times this would represent 300 nm. The best resolution of the SEM is specified as 3 nm, which corresponds to the smallest spot size at the highest voltage. Thus, there are only 100 "resolution units" across the width of the screen, even though the normal capture mode uses 1024 pixels in the horizontal direction. Focusing with such coarse resolution is next to impossible, and the noise floor is very high. The highest magnification that I have actually used to create decent images is x100,000 and experts who have seen those micrographs say that they are "very good for tungsten", where tungsten refers to the type of electron source on the SEM. (See, for example, the micrograph of a butterfly scale at the bottom of the third page of SEM Diaries - 10.)

So, what was the material that I was imaging to make use of higher magnifications? It was the light microscopists' go-to material for testing out their microscope set up - the diatom!

I described how I prepare cleaned diatoms for imaging in SEM Diaries - 10. There I mentioned the difficulty of selecting just the right dilution of the liquor containing the diatoms so that the diatoms were distributed evenly around the filter paper used to collect them, without overlapping. Well, aware of this difficulty I experimented with various dilutions and various volumes of fluid pumped through the filter papers, and am not sure I am any nearer to finding a good method. The problem is that each suspension of diatoms in its liquor is at a different concentration, depending on the raw material gathered at the site.

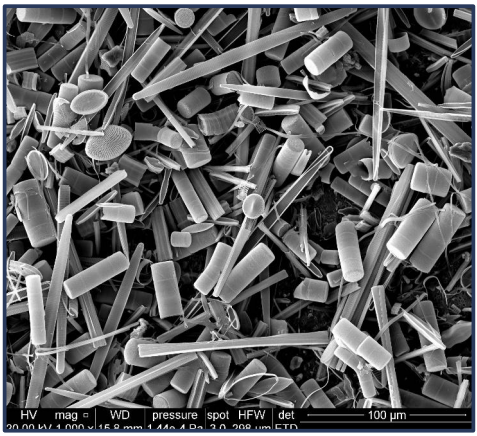

Fig. 2: A strew showing the rich variety of diatom species from Yeddingham Locks. Field Width 300µm

Figure 2 shows a somewhat over-populated strew of various diatoms gathered by colleagues during the Cranedale Microscopists' Weekend, in September 2019. Figure 3 is an image of one of these diatoms at higher magnification, showing the excellent resolution available from the SEM. Figure 4, on the following page shows two images of *Diatoma mesodon* (also known as *Odontidium mesodon*) that I made in September. The left hand image shows several individual diatoms, arranged in various attitudes, which the 3-D effect of the SEM shows up to good advantage. The higher magnification

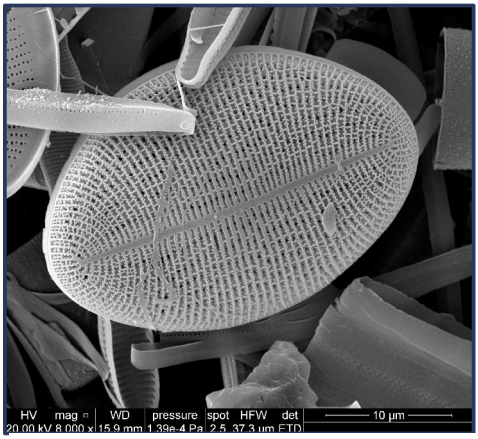

Fig. 3: Detailed view of a single diatom from the strew of Fig. 2. Field width 37 µm

right hand image has dimensions superimposed on it. Each dimension records 10 times the spacing of the holes, so for the holes at the tip of this view the separation is 129 nm, which is clearly resolved in this micrograph, but would be impossible to discern with a conventional light microscope.

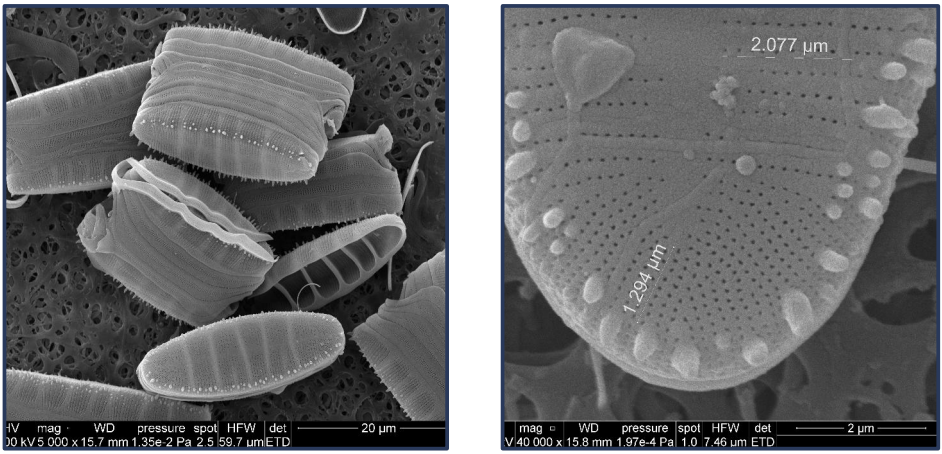

Fig. 4. Left: A cluster of *Diatoma Mesodon* at a magnification setting of x5k.
Right: A detail at x40k. (The magnification is that seen when viewed on the screen of the SEM)