SEM Diaries - 18
Pure Lust

Jeremy Poole

Fig. 1: I discuss the latest Zeiss SEM with their applications engineer (Photo Pam Hamer)

"So, Dr. Poole, how many of these new SEMs do you think you might need for your lab?"

The Zeiss engineer demonstrating the latest £500,000 (+VAT!) SEM from their range at MMC2019 did his best to maintain a serious face. In reality I have known Ken for five years, since my first visit to Microscience Microscopy Congress back in 2014, at which time I was going round bothering the large manufacturers for hints and tips, even before I had bought my own modest instrument.

One reason I enjoy these events is that the exhibitors are unfailingly courteous and helpful even to the likes of me. I was given a 45 minute demonstration slot on the Gemini 2 SEM, despite Zeiss knowing the possibility of their making a sale was precisely zero. They extended the same courtesy to me back in 2014, when I was told to go out into Manchester to find an insect and re-appear at 2 p.m. to see what images they could produce on their Sigma SEM [1].

The new SEMs are packed with features, many of them software based, but in the case of the Gemini it also has some serious "in-lens" detectors, both for secondary and back-scattered electrons. The idea behind these detectors is that they can differentiate between electrons that have originated directly from the sample and those that have bounced off the chamber walls and

other hardware before being detected. This leads to images that are much clearer and of higher resolution, and Ken was able, very easily, to demonstrate the difference.

While the Gemini 2 is a high-end SEM with a Field Emission Electron Gun (FEG), there are "cheaper" models available, from Zeiss and various other manufactures, such as Tescan and Thermo Fisher (the manufacturers of my current SEM). The least expensive Tescan is around £80k. It has a tungsten electron gun and very good electron optics. The "cheapest" Thermo Fisher SEM, with a tungsten electron gun, is their Prisma, which replaces my model in the range. The actual price depends on how many options are purchased, of course, but it starts at around £120k, not much more than the original new price of my own SEM of the year 2005. The model at the exhibition had detectors of all sorts sticking out from many ports, including those for X-rays and cathodoluminescence. (The demonstrator referred to them as his "toys"!)

I mentioned software earlier. The Prisma comes with all sorts of software tools, including a facility enabling engineers to write their own code. One such feature is the ability to generate a 3-D image by taking two exposures, moving the sample in between shots. The two images are combined, having been colourised for viewing with red-blue 3-D "glasses". Another feature is the ability to programme a sequence of images so that the SEM can be left running overnight and the user can return in the morning with views of samples from all directions, and a wide variety of magnifications and so on. A real time saver. The SEM incorporates an auto-focus facility, which is necessary if the stage position and magnifications are to be changed between shots. Seeing this reminded me that I have an auto-focus "button" on my own SEM. I tried this when I got home and it does seem to work, at least for high contrast, low magnification, images.

Shortly before going to Manchester my own SEM developed a fault. The stage lost the ability to rotate under the control of the Windows interface. If the SEM is switched off for any reason, it is necessary to re-calibrate the stage position, so that it can move to any pre-stored locations when asked. This requires running the "home stage" procedure, during which the stage is moved in all directions to its end stops, and is also rotated to calibrate the 0˚ position. While it completed all the linear motions as normal, the stage just continued to rotate "ad infinitum". I booked a service call with Don for just after MMC and also contacted Thermo Fisher to ask them if they would provide me with spares, should Don identify the need for, say, a new PCB. While previously, it seemed, Thermo Fisher did not have a system for taking orders from people without a VAT number, this time they agreed they could do this, against a pro-forma invoice.

While at MMC I mentioned the fault to one of the engineers on the Thermo Fisher stand. He identified the possible cause of the problem immediately, and went so far as to call up the service drawing on his laptop to point out the affected part. Not only that, but he then took me to a stand (not Thermo Fisher) where there was an SEM with a similar stage to mine. He persuaded the demonstrator there to interrupt what he was doing, and to raise the chamber pressure to atmospheric so it could be opened. He was then able to point out the relevant part to look for. The bits he indicated were an optical sensor and a plastic "tongue" that protrudes from the stage and interrupts the light path in the sensor at one particular location. The triggering of this sensor tells the SEM where the zero reference for stage rotation is. Without a reference, the stage would continuously rotate during the home stage procedure, exactly as mine was doing.

As soon as I got home from Manchester I opened up my SEM and located the rele-

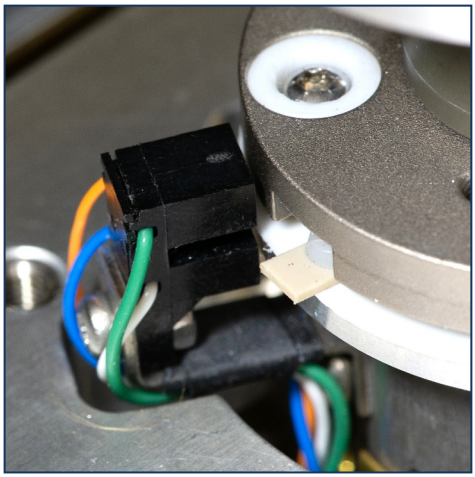


Fig. 2: Re-fitted optical sensor, together with broken tongue for interrupting the light path

vant parts. Sure enough, the plastic tongue that interrupts the light beam in the optical sensor had been pushed out of the way. Also, the optical sensor was sloping backwards and the screw that should have secured it was hanging out loose. Furthermore, there was no sign of the nut, or whatever was used to hold the screw in place - always a worry, when there is delicate rotating machinery in the vicinity.

I ordered a replacement nut, from eBay rather then Thermo Fisher, and when this arrived I was able to re-fix the sensor (Figure 2). Despite this, the fault was not fixed. It transpired that the plastic tongue had not only been pushed out of the way (as it is designed to do) but also it had been damaged, as can just be seen in the photo. Sticking a bit of plastic tape onto the tab demonstrated that the tab was indeed too short, and I was able to persuade a friend to 3-D print a replacement tab for me. When this arrived and was fitted all was well. I do worry about the nut falling off into the mechanism, though!

In "SEM Diaries - 17" I mentioned my impending attendance at the annual get-together of the British Arachnological Society. Well, this duly took place, including a day's field trip to a nature reserve, in pouring rain. One problem with gathering samples in the rain (apart from getting wet, of course) is that it is difficult or impossible to use a sweep net. The net just get waterlogged and very heavy. The specimen collection technique of choice under such conditions seems to be the "vacuum sampler". Two of those present took such devices out, and came back with a most impressive list of species. A vacuum sampler is no different from a leaf blower, set up to act as a "sucker" rather than a blower. In order to prevent the specimens passing through the macerator and into the conventional collecting bag that way, a special net is fitted to the vacuum tube, which filters out the spiders and debris before they reach the macerator. All that is required is a few seconds of operation, sweeping the vacuum nozzle over likely spots. The collecting net is then emptied onto a white sheet or tray and the collected spiders are extracted using a pooter.

On the Sunday evening I gave a short talk on my spiders website, and this seemed to go down very well, with plenty of compliments and promises to provide me with samples. Two months later, as I write this, no-one has been in touch to offer anything! One outcome of the meeting, though, has been that an academic from Manchester has asked if he can use some of my micrographs for display at an exhibition on "bugs", to be held at the Manchester Museum from Mid November 2019 to April 2020. Of course, I said yes.

Following a demonstration of a vacuum sampler in 2018 I had already bought a leaf blower, but I had not quite got round to finishing making a collecting bag for it by the time of the 2019 weekend. At the meeting, one of the users of these devices told me where to purchase a ready-made bag, so I ordered a couple of these and am now up and running. It is amazing what you can collect in a very short time. I have had a few sweeps over leaf litter and

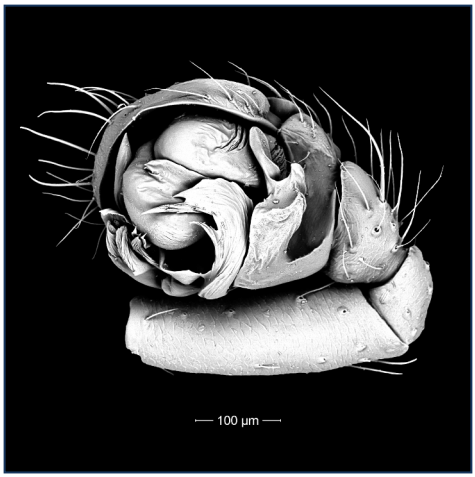


Fig 3. Male pediapalp of *Tenuiphantes flavipes*

among ivy. I came up with many tens of small linythiids (money spiders) and a few larger specimens. Sadly, almost all of the linythiids were of the same species. This did mean, however, that I could have several attempts at making micrographs of their male sexual organs (Figure 3).

The linythiids have a bit of a reputation for being difficult to identify to species level. The process requires one to count the spines on the legs and also to work out exactly where a particular sensitive hair (the trichobothrium) is on meta-tarsus I. (The trichobothrium is of the order of 1 µm in diameter, so it is not easy to locate using a stereo microscope. The preferred technique is to tap the side of the watchglass or staining block containing the spider and look out for a disturbance in the surrounding fluid - 70% alcohol normally.) Having made the necessary measurements and counted the relevant spines, one then refers to one of four tables in the reference to be pointed to the illustrations of the genitalia of possible candidates. I determined that it was high time for me to embrace this, and have now had some success with the technique.

Some genera of the Linythidiidae have several forms of epigyne within individual species, and even then it is not always possible to identify them definitively. Sometimes it is necessary to dissect out or fold back the epigyne to examine it from "underneath". For the first time I had a go at doing this, on what proved to be *Erigone atra*. Observed under the light microscope it was clearly from that species, although once it had been prepared for the SEM one of the key identifying features was no longer visible. The dissected epigyne was about 0.4 mm across at its widest point, so passing it through various baths of alcohol without losing it proved interesting. It was not possible to use the fine gauze containers described in SEM Diaries - 16, as it passed straight through the mesh.

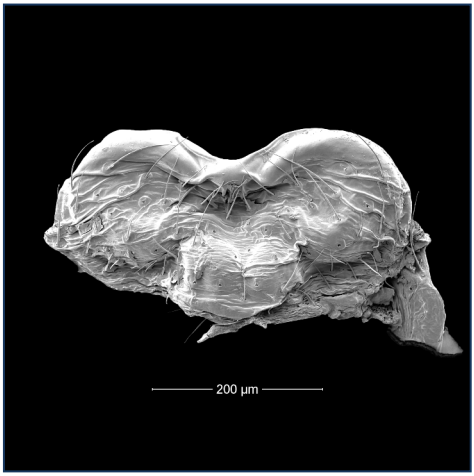


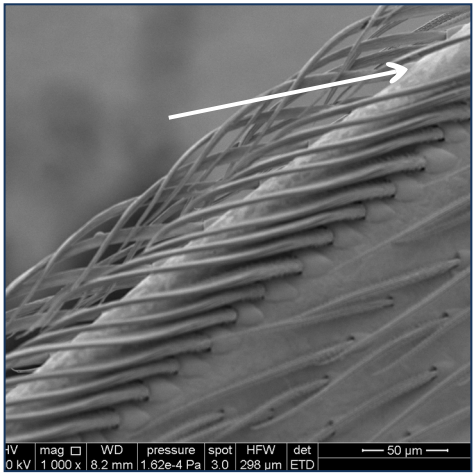
Fig. 4: Underside of the epigyne of *Erigone atra*

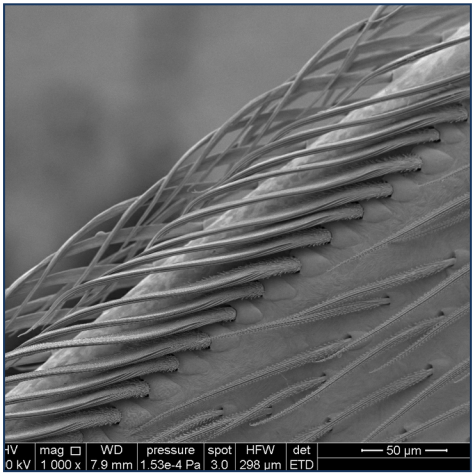
One attribute that an SEM is known for is its extremely large depth of field, compared with light microscopes, for a given magnification. However, even an SEM image is not always sharp throughout as I discovered with an otherwise pleasing image that I made recently. This is of the calamistrum (or comb) on the hind legs of the spider *Amaurobius ferox*, which it uses to tease out the very fine silk from its cribellum. (See SEM Diaries - 15 for an image and description of a cribellum.)

Figure 5 appears quite out of focus, but there is one "tooth" of the comb at the top right that is sharp. Figure 6 shows a much more pleasing image, with the hairs in the foreground being very sharp and much of the rest of the image is acceptable. However, the sharp hair in the top right of Figure 5 is but a blur in Figure 6, and the "teeth" of the comb in the background are certainly not sharp. I made a stack of 20 images, slightly adjusting the focus between shots, and the result, combined using Helicon Focus, is shown in Figure 7. I hope you will agree that stacking has its place, even with SEM images!

Reference

1. Poole, Jeremy. MMC Exhibition at Manchester. Balsam Post Issue 105, October 2014





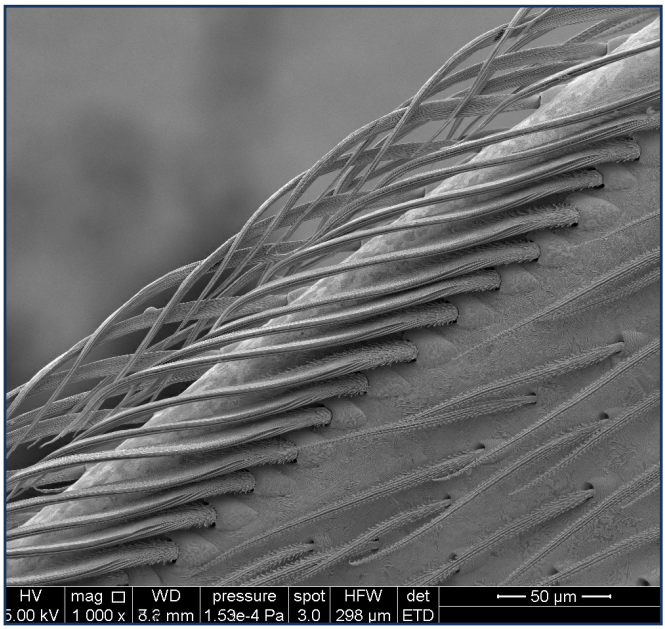


Figure 5 (top left): Showing single comb tooth in focus (arrowed)

Figure 6 (above): A pleasing image, but with comb teeth in the background less sharp than those in the foreground.

Figure 7 (left). Combined stack of 20 images showing sharpness from front to back (apart from the "tastefully blurred" background)

Field width in each image is 0.3 mm. Apart from the stacking itself, no post processing has been carried out on any of these images.