SEM Diaries - 12

Looking at things from a different angle

Jeremy Poole

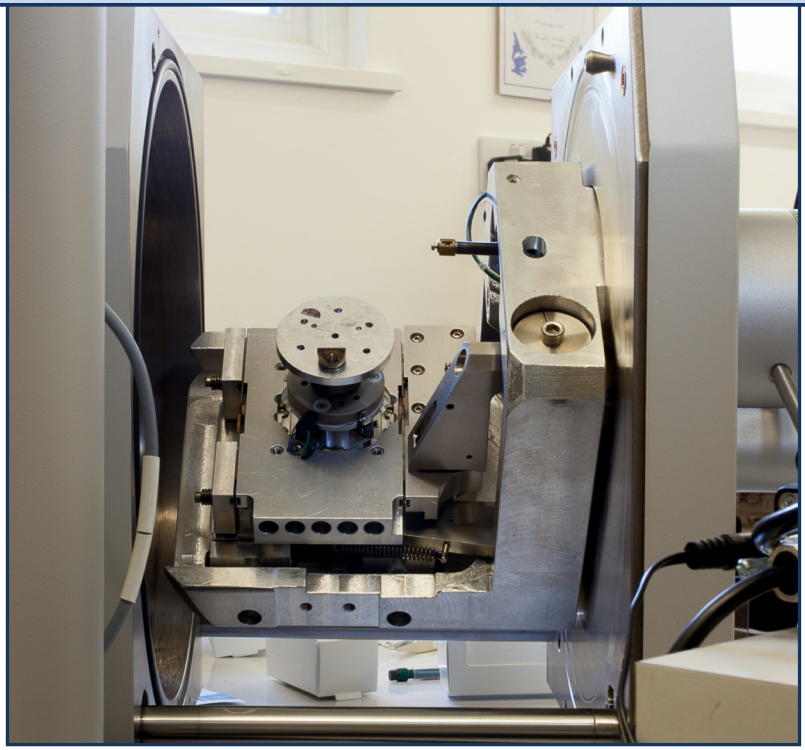


Fig. 1: The open chamber of my SEM with the stage tilted at 45° to the vertical

By the time this edition of SEM Diaries is published I shall have had my SEM up and running for almost exactly two years. Quite long enough to have become an expert, you may think, in both the operation of my instrument and also in the preparation of specimens for it. Well, perhaps so, but in reality I am still learning.

Perhaps there is an infinite amount of knowledge to be gleaned, or maybe my background in light microscopy is to blame for my only recently exploiting my tilting stage, since I have so far tended to treat my

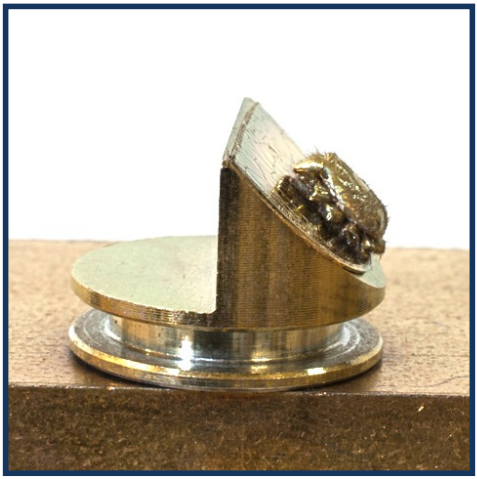


Fig. 2: Spider head mounted on the 45º face of a sloping stub. A 90º (vertical) face is also available on the same stub.

SEM specimen stubs the same way as I do slides. In other words they are clipped securely to the stage in a horizontal position and viewed from above. The furthest I have tended to deviate from this convention with my SEM is to mount the specimen on a sloping stub (usually 45º) so that, although the bottom of the stub is horizontal, the overall result is to view the specimen at 45º to the plane of the stage. Stubs are available where it is possible to mount the specimen effectively vertical on the stub. I have yet to try this, though I have some suitable blank stubs.

But why stick at 45º or 90º? My SEM has a multi-axis stage, which can be moved in X, Y, and Z linear positions and rotated about its centre. These movements can all be carried out using the computer interface or by turning knobs on the front of the chamber door. In addition, the whole stage assembly can be tilted, manually, between -10º and +75º. Here a positive angle denotes a tilt in the direction of the Everhart-Thornley secondary electron detector. Figure 1 shows the stage tilted towards the camera at 45º. Also visible is a stub with a spider head mounted on a 45º face. This stub is illustrated in more detail in Figure 2. The 45º slope of the stub and the 45º tilt of the stage together provide the ability for the electron beam to scan the front of the specimen, as though it had been mounted vertically on a suitable stub.

The difference in appearance of the resulting micrographs of the same specimen, without and with the 45º tilt is quite marked, as is illustrated in Figure 3.

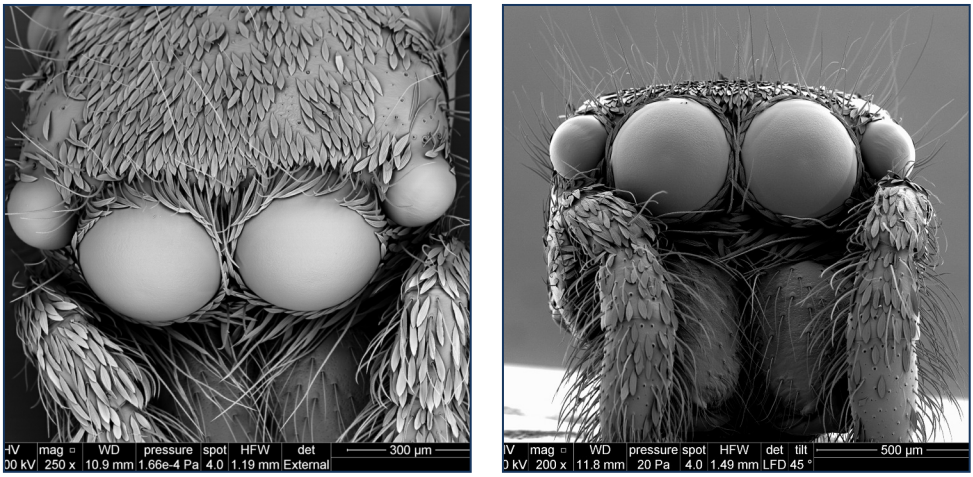


Fig. 3: Head of *Salticus scenicus* spider (on a 45º stub) imaged with a stage tilt of 0º (left) and 45º (right). The (screen) magnification used was x250 (left) and x200 (right)

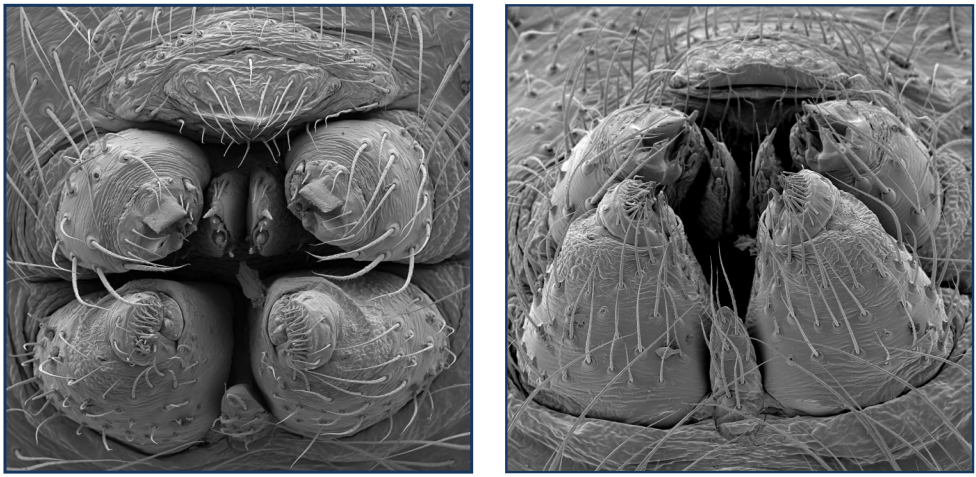


Fig. 4: Spinnerets of *Steatoda bipunctata* on a flat stub with 0º tilt (left) and 45º tilt (right)

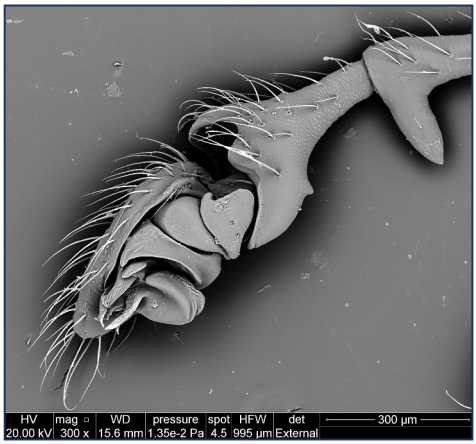
It was only when I dissected out the spinnerets of a spider at rather a rakish angle that I discovered the potential of imaging that part of spider anatomy at different tilt angles. Figure 4 shows the difference between one "spinnerets stub" imaged without tilt and then tilted at 45º. The images are very different from each other. Of course, the choice of angle should be determined by the scientific or artistic goal from taking the micrograph.

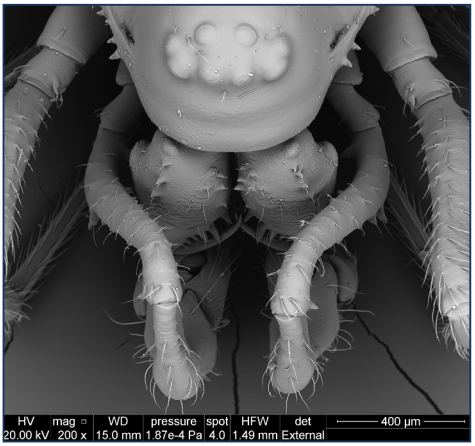
While tilting the stage provides easy adjustment from outside the chamber, there comes a point where it becomes something of a liability, in that it can physically interfere with the final electro-magnetic lens, or the arm of the back-scattered electron detector. When this happens, the ability to move the stage from the windows interface is disabled; the "touch-alarm" is activated. Recovering from this condition requires logging out of the user interface and logging back in - at least it does on my SEM. I am not sure why it has to be that complicated, but it is a good incentive to avoid the condition in the first place!

An alternative to tilting the stage is to use a stub with a built-in tilt arrangement. This does have disadvantages, though. For example, one needs to know at the time of making the stub that it will be subject to tilt, so the correct stub blank can be used. Such stub blanks are expensive, and also there is no easy way of measuring the precise tilt, should one want to. Adjustment of the tilt once the stub is in the chamber requires raising the pressure in the chamber back to atmospheric pressure so that the door can be opened. At least with the tilting stage the angle of tilt is calibrated onto the outside of the chamber door, and tilt can be adjusted at high vacuum. Also, the exact tilt angle can be recorded in the data bar at the bottom of the image (just about visible on the right hand image of Figure 3).

I have it in mind to construct something resembling the tilting stub, but with an 1/8th inch hole in the top, in which can be placed a conventional stub. This would cater for the case when the need to tilt the stub (rather than the stage) becomes apparent after the conventional stub has been made.

Although the winter months are "quiet", in that most potential specimens are hibernating (or dead), I have actually been quite productive recently. Not only has the dis-





Male *Erigone atra* money spider. Left is the male pedipalp - its reproductive organ. On the right you can see the eyes, chelicerae with fangs and the male pedipalps surrounding and tucked under the chelicerae. Both these micrographs were taken with the back-scattered electron detector to avoid charging problems.

covery of the effects of tilt motivated me, but I unearthed quite a few tubes of specimens, some preserved in alcohol and some that have already been through the critical point dryer. In particular I came across quite a few desiccated *Erigone sp*. spiders, which are the little money spiders that you can find hanging from your hair when least expected. The characteristics of the pedipalps, eyes and chelicerae are illustrated at the top of the page.

Because of the small size of these spiders, it is particularly useful to be able to study them under the SEM. Also, for the same reason, they are actually quite difficult to dissect. I recently purchased some "micro scissors" from Labtech which have been a great help in removing legs and pedipalps. I bought two pairs, one with curved blades and the other with straight jaws. The straight bladed pair is illustrated opposite. These cost around £22 plus VAT and carriage.

On a less positive note, I have been having problems with the Low Vacuum mode of my SEM. A while back I selected this mode while the SEM was in a condition when this selection should not be carried out. Unfortunately, the particular lever I moved is not interlocked to prevent such an occurrence. The result was a number of error messages, such as "The vacuum error has occurred", sometimes with the added information "possible leak". Well, sadly, the LV mode is working again. I say "sadly" because until it is really broken rather than intermittent, there is little point in getting the engineer in to rectify it permanently.

That's all for now. My resolution for this year is to gather more specimens than I managed last year. Fresh is best!

Straight bladed micro-scissors (Vannas type)

Jeremy Poole