# SEM Diaries - 21

## Compucentric, or plain Eccentric?

Jeremy Poole

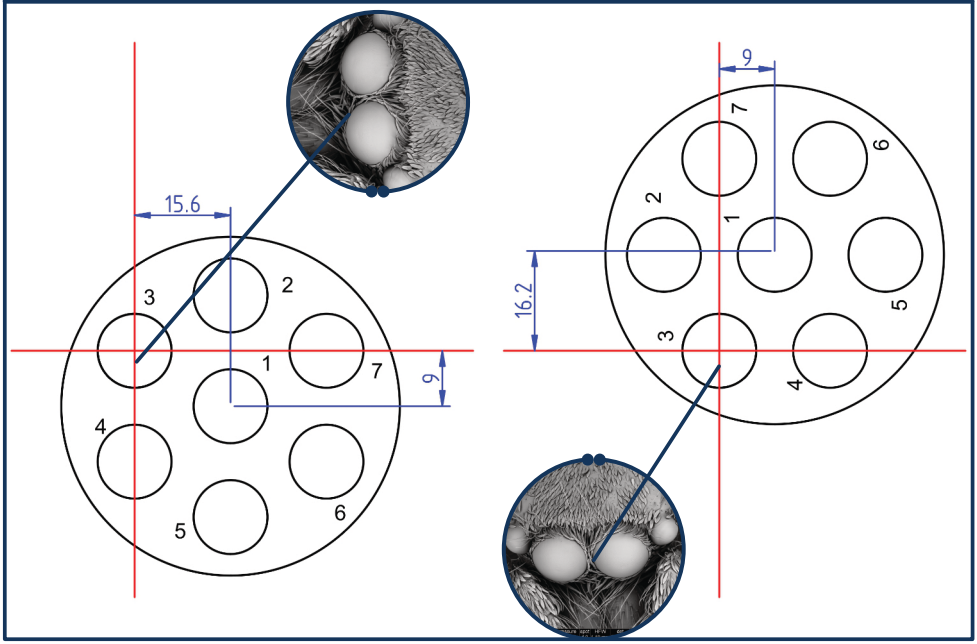


Fig. 1a: Stub 3 located directly under the electron gun. Fig. 1b: The stage has been rotated through 90° and then the X and Y position of the stage have been updated to bring stub 3 once more under the electron gun.

First, to all those who enquired of the health of my SEM following my mention of its not working in the last edition of "Diaries", you will both be glad to hear that is it now up and running again - albeit not before I had stumped up the cost of a small car for an exchange high voltage power supply assembly.

The down time, although frustrating, was not entirely wasted however. I used it to talk to manufacturers to ask if they might be interested in selling me a new SEM. Two of the three whom I approached were understandably enthusiastic, but I am still waiting to hear from the third! There was no contact number on their website. Instead I had to fill in an enquiry form. Perhaps I forgot to press the "Submit" button. But, sorry Thermo Fisher, you are too late now! I also spent some of the down-time making new stubs of spiders and other creatures, ready for when the SEM was up and running again.

When the new power supply had been installed I was able to set about immediately imaging this latest bunch of stubs. I was especially keen to do this as it had been months since I had updated my spiders website, and I had come across a spider new to me in my garden just before Christmas.

During this imaging exercise I was hit with two minor frustrations. The first was

that when I rotated my stage to align a specimen on the screen - for example to orientate a head with the eyes at the top and the fangs at the bottom - the specimen would disappear from the field of view and I would then have to randomly move the stage in the X and Y directions until I could re-locate the rotated specimen.

When I say I rotated my stage, what I actually did was use a technique called "compucentric rotation". For example, if my spider head is pointing sideways and I want to rotate it through, say, 90° to achieve the required alignment, I click on a circle superimposed on the image, in a position representing by how much I would like the specimen to be rotated. A few whirring noises follow as the creature goes rushing out of the field of view to reappear in an orientation nearer to my liking. If necessary, I repeat the operation to fine tune the orientation. Why all this rushing about the countryside, you may ask? Well, unless I am using the central hole in my seven-stub stage, rotation of the stage is bound to move the specimen out of the field of view, and the X and Y coordinates of the stage position need to be adjusted to compensate for this.

Figure 1 should make this clear. Assume that I have a spider head on a stub in position 3, and suppose its fangs are to the left of the image and its eyes to the right. I need to rotate the image through 90° anti-clockwise. Simply rotating the stage by 90° would move position 3 to a location somewhere between positions 4 and 5 in Figure 1a. So, in addition I need to move the stage upwards (in the diagram) by (9+16.2 mm) and to the left by (15.6-9 mm) to the position shown in Figure 1b to once more bring stub 3 under the electron beam. Now, fortunately, I do not have to sit down with Excel and calculate the new co-ordinates every time. The SEM will do it for me and complete the required movement automatically - or that is the theory. My problem was that, although the stage would rotate to the new required angle, it was not so good at navigating to the precise X and Y coordinates needed to bring it back into view - and it was driving me nuts!

My other problem was in setting up the "stigmator". At high magnifications, for example x5k to x100k it is really important that the cross section of the electron beam is circular rather than elliptical, and there are some special coils built in to the column to control this. Adjusting these is a bit of an iterative process and there is a semi-automated procedure to help with this. It was while looking up the instructions on using this procedure that I discovered that was a way to calibrate the compucentric rotation hardware and software. I followed this through and found I could then use a significantly higher magnification while rotating and keep the subject of interest in view - success!

It was while discussing a new SEM with Zeiss, that I came across the use of "compucentric tilt". This is not available on my current SEM, but can be extremely useful. Just as with rotation, if you tilt the stage, this will tend to move the specimen

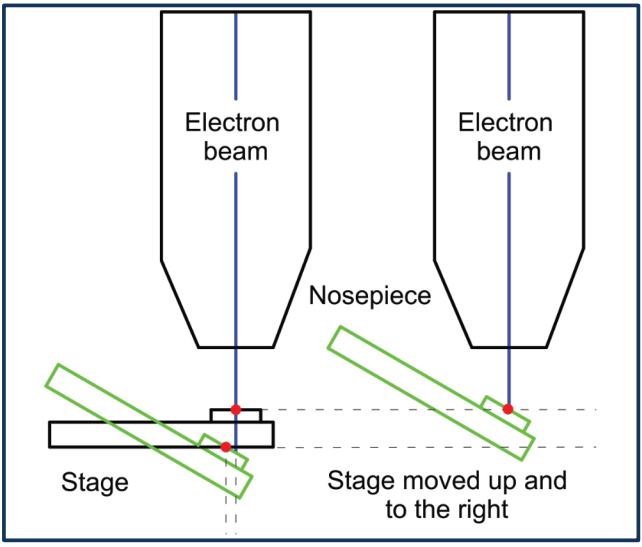


Fig. 2a (left) The stage is tilted and the specimen (red) moves downwards and to the left. Fig. 2b (right): The stage is then moved upwards and to the right to bring the specimen back to its original location

out of the field of view. Furthermore, in this case it will also have the effect of defocusing the image. This is illustrated in Figure 2. Figure 2a shows a specimen on a stage (red dot) with the stage horizontal (black outline) and also tilted down by 30 degrees (green outline). It can be seen that the specimen has moved slightly to the left of the electron beam and quite a way downwards as well. Compucentric tilt will move the tilted stage upwards and to the right to bring the specimen back under the beam, in the original, focused location. This is shown in Figure 2b.

So much for "Compucentric", but where does "Eccentric" come into play? While I am out and about on my Covid-19 exercise periods I am often to be seen on my hands and knees in the grass with a transparent plastic pot in my hand. If that is not strange enough behaviour for a septuagenarian, if people knew that the objects of my interest are hunting spiders, then they would surely give me a wide berth!

The Lycosidae, to give them their formal name, are pretty common, or at least a number of the genus *Pardosa* are. But, they are tricky to catch. They have quite good eyesight and are very fast on their feet. There is no way you could catch them with a pooter, and given that they are ground dwellers it would be difficult to catch them with a sweep net. My vacuum sampler might work, but I feel conspicuous enough already without drawing attention to myself with 95 dB(W) of noise!

My technique is to use a transparent pot about 50 mm in diameter and drop it over the spider to entrap it. This is easier said than done, given their speed and erratic trajectory, often through the roots of long grass. However, if there is a spot of bare earth or tarmac my chances of entrapping one are increased. Having contained the spider under my pot, I then simply slide a piece of card under the pot and the spider, invert the pot with the card in place and transfer the spider to a tube.

The reason that I have been catching these generally common spiders is that the genitalia of both males and females are interesting - or I think so - and make good micrographs (Figure 3).

I did mention at the start of this piece that I have been speaking to manufacturers. Well I have now ordered a new SEM

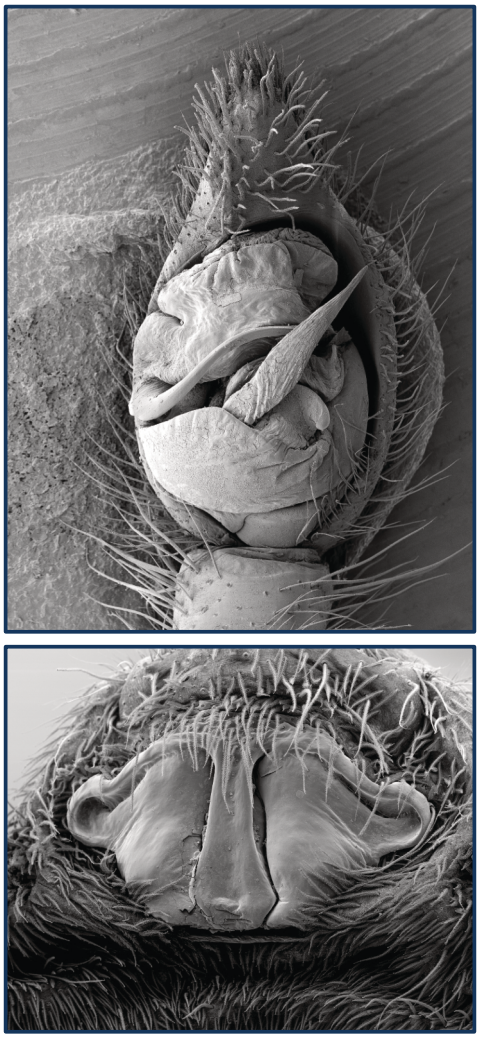

Figure 3: Pedipalp (top) and epigyne of *Pardosa pullata,* a very common hunting spider.

and paid a 25% deposit. Although the Tescan factory (in Brno, Czech Republic) is not closed on account of Covid-19, they are taking longer than usual to make the SEMs because of social distancing. Also, there is always a question mark over whether all their sub-contractors can support them at the appropriate time. I am excited, though.

More on this next time!