SEM Diaries - 29

Foraminifera and related specimens - and AI

Jeremy Poole

Fig. 1: *Oolina borealis*

Foraminifera make good subjects for SEM, especially if the user is in a lazy mood. Once collected, cleaned and separated from contaminants such as sand, they require no special treatment for SEM, other than the almost universal sputter coating - no taking through increasing strengths of alcohol, and no critical point drying. In the case of the forams I have been imaging recently I did not even need to do the collection or cleaning, but was able to work on samples provided to me by various members of the PMS. Almost all that I had to do was to lower the specimen onto a stub with a sticky pad on it, sputter coat it and load it into the chamber of the SEM. Unlike diatoms, forams are large enough to handle (carefully) with forceps, yet small enough to permit more than 20 to be mounted on the same stub. They are quite fragile, though. On a number of occasions I have wanted to slightly change the orientation of a foram on its sticky tab, and given it what I would say was a gentle "ease" with the tip of my forceps.

Fig. 2: Stub containing 17 different forams. The orientation is provided by the straight chord of the sticky tab at the top of the photo.

Almost always this has caused it to disintegrate in front of my eyes.

Another advantage of foraminifera over diatoms is that they are easy to find on the stub. Many is the time when I have wanted to return to a particular diatom to produce another image, only to find that I just cannot locate it, even though I know it is there "somewhere". With forams I have developed a system by which, having laid out a number of them on a stub I photograph the stub, prior to sputter coating, and attach a reference to each specimen on the stub using Adobe Photoshop. I thus have a "map" of the stub showing the location of each individual foram with its reference (Figure 2). I can then easily return to the stub at a later time and locate a particular specimen that I might want to image once more. In addition, the reference, including the stub number can be captured in the file name of the image and tagged in my Adobe Lightroom catalogue, with the species.

When I first started looking at forams with a stereo microscope, the samples I had seemed only to contain genera such as *Elphidium* or *Ammonia* (Figures 3 and 4). It has only been recently that I have managed to extend my canvas to include types such as the *Oolina borealis* shown in

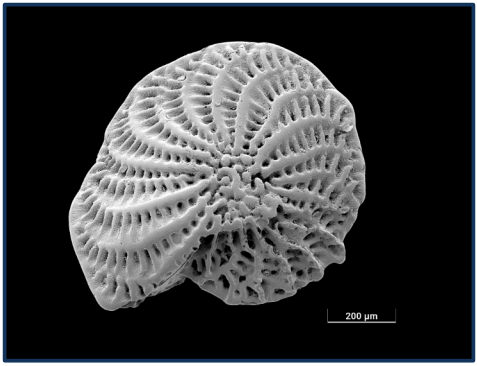


Fig. 3: *Elphidium crispum*

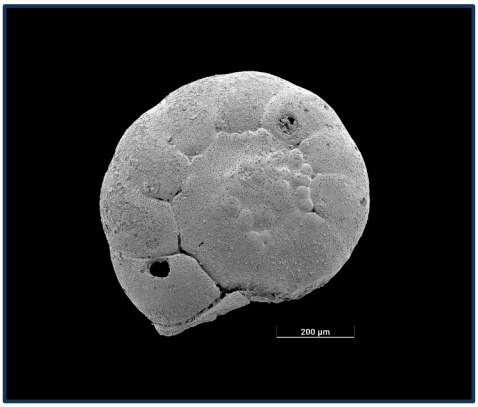


Fig. 4: *Ammonia falsobeccarii*

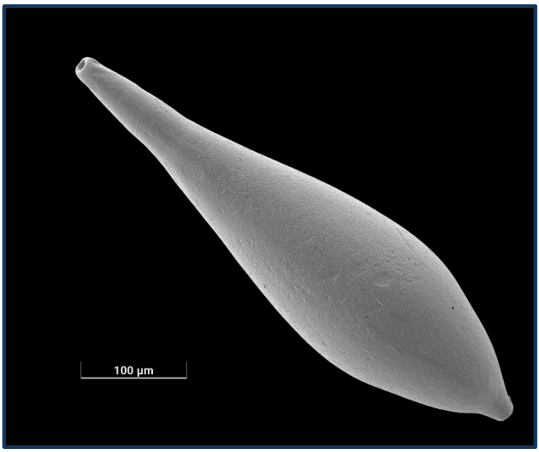


Fig. 5: *Hyalinonetrion clavatum*

Figure 1 or *Hyalinonetrion clavatum* (Figure 5).

Having been working on forams quite a bit recently I have been becoming more inventive both in how I lay out the specimens and also how I "illuminate" them. Previously I had always laid them out so that they were in the conventional posterior or anterior view. The literature, such as the excellent website foraminifera.eu include lateral views, as do historical drawings from expeditions such as the Challenger ones. Fixing a foram in its edge on the sticky pad on a stub is a little more tricky than laying it flat, but by no means impossible. Indeed, laying them in more random orientations can also convey interesting information, or at least an artistic effect! (Figures 6 and 7).

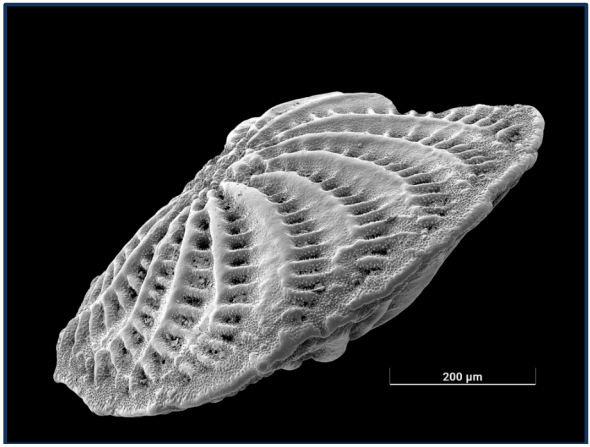


Fig. 7: *Elphidium sp*. In an"in-between" orientation

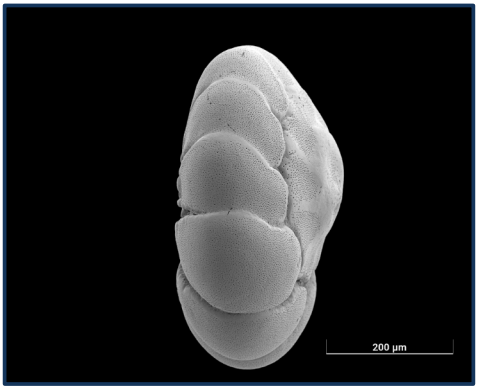


Fig. 6: An *Ammonia sp.* In lateral view

An interesting outcome of using an in-chamber secondary electron (SE) detector in the SEM is that it creates a "modelling" effect. Indeed, this is sometimes called a stereoscopic effect, and led to the name "Stereoscan" for the first commercial SEM. I shall not attempt to describe the physics behind the effect, but the outcome is that the image of the specimen appears as it it were viewed along the axis of the electron beam and "illuminated" by the detector. The detector position is physically fixed, of course, but by rotating the scanning axes you can effectively alter the position of the "illumination". Rotating the scan (beam) also changes the orientation of the image, but this can be countered by rotating the stage in the opposite direction. In fact the SE detector on my chamber is at 45º to the bottom of the image which creates a weird looking image (at least to me) so in normal operation I rotate the scan by 225º. This brings the effective position of the SE detector to the top of the picture, where it was with my FEI Inspect SEM.

Figure 8a illustrates the modelling effect achieved by setting the beam rotation to 270º (illuminated from top left) and Figure 8b (next page) shows the effect with a beam rotation of 180º (illumination from top right). The difference is quite

Fig. 8a: Image with beam rotation of 270º

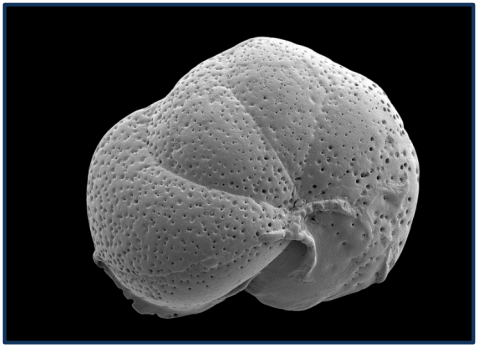


Fig. 8b: Foram “illuminated” from top right (180º)

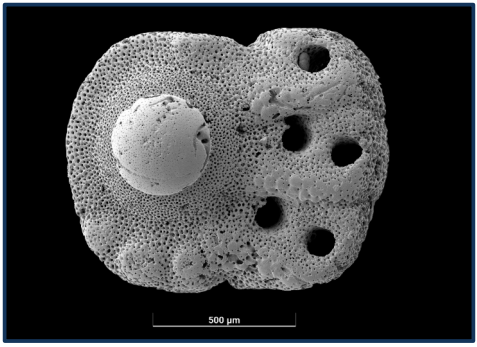


Fig. 9b: Ball joint for echinoderm spine

subtle, but does certainly change the appearance of the image to a useful extent.

Marine Debris

I used the expression “related specimens” in the title of this issue of Diaries, as I have also been imaging marine debris, given to me by another PMS member at the Langton Matravers meeting. The relationship is more that the subject matter is of a similar size to forams and comes from a marine environment, rather than any genetic relationship. The samples contain all sorts of specimens, such as shells of bivalves and echinoderm spines as well as foraminifera.

Figure 9a shows the lower part of an echinoderm spine and Figure 9b shows the “ball” about which it rotates.

Image Processing

Many readers will realise that I use my SEM as much to make interesting pictures as to advance the cause of research, even though I believe that I (working with others) have identified interesting and little known facts connected with shield bugs at least!

An image, as produced by the SEM, can have low contrast and the background can show up all sorts of dirt, cracks, glue marks and other artefacts. Thus my image processing “workflow” involves a number of stages, to arrive at the final picture. (I use the word “Picture”

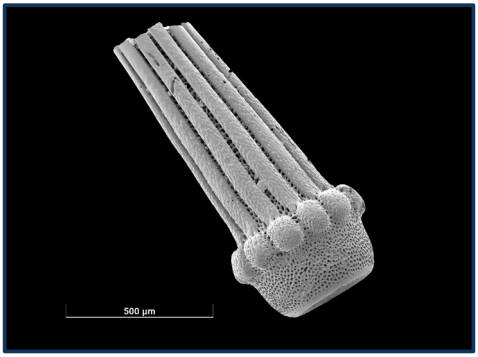


Fig. 9a: Base of the spine of an echinoderm

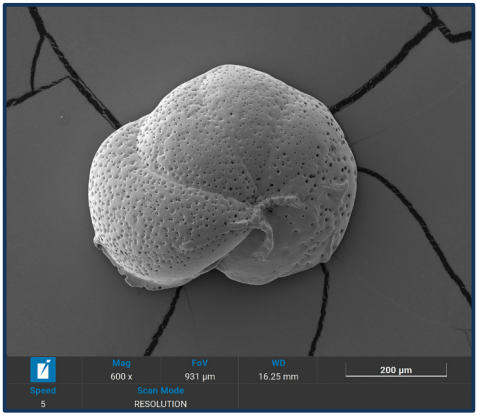


Fig. 10: Image of foram of Figure 8 as saved by the SEM

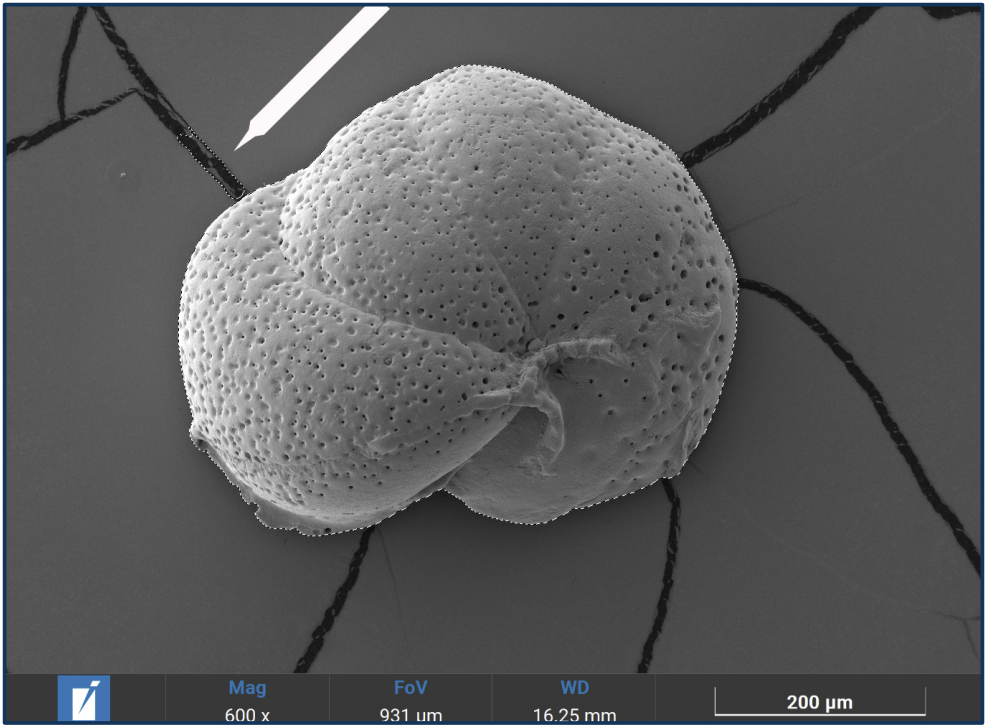


Fig. 11: Foram image from Fig. 10 showing result of the “Select subject” AI tool. The white dots round the foram (and part of the crazing - arrowed) identify the areas selected by the tool.

advisedly, as that is how I see the end result.)

Figure 10 (previous page) is the image of the SEM of Figure 8a as captured by the SEM (before post-processing). Would you blow that up and hang it on your wall? No. I thought not! It is low in contrast, not helped by the grey background, which in any event has craze marks on it. And, what do all those numbers at the bottom mean? Who cares?!

The principle of the image processing that I carry out as a matter of course is, basically, to replace all parts of the frame that are not of the foram (or other subject matter) with a black mask. This is done by making a “selection” around the foram, converting it to a “layer mask” and placing a black layer underneath the layer containing the image. In effect this is equivalent to cutting out the foram outline from a paper print and sticking it on to black card.

I do this in Photoshop, but I am sure other programmes can let you do the same thing, although Photoshop uses Artificial Intelligence (AI) to help you!

Figure 11 shows the same view as Figure 10, but with the Foram selected using the “Select Subject” tool. I have had to blow this up large to show the “marching ants” - a dotted white line - that follow the outline of the foram. You should just be able to see a few marching ants round part of the crazing at the top left, just above the foram (arrowed), which of course needs to be corrected - the work of a moment. At this stage I might also consider tidying up the selection round the edge of the foram at lower left.

Before the AI “engine” of Photoshop was introduced the way to make this selection would have been either to follow the outline with a lasso or pen tool, or else to paint over the area to be selected, or if easier to be removed, using a technique called “select and mask”. I am sure the

Select Subject tool has got better over time. I am not sure if the AI is learning what I do, or whether it is simply a continuous development of Photoshop. (Probably the latter!).

Once the selection has been made, the rest of the process is:

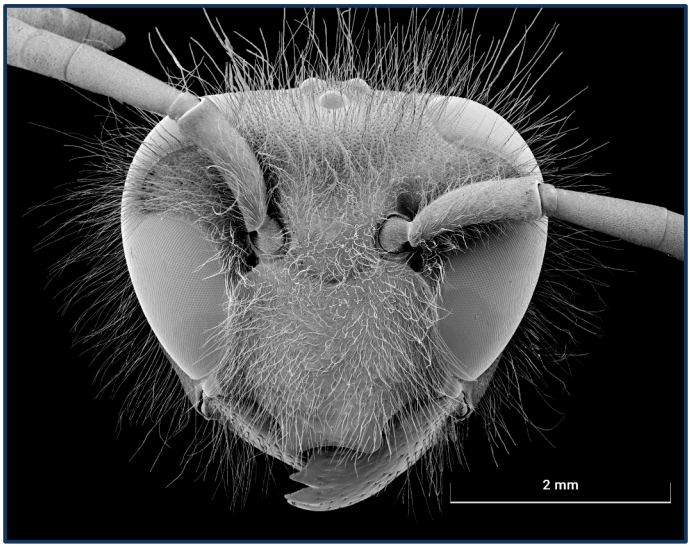
- Save the selection as a layer mask
- create a solid black layer below the layer with the image and layer mask on it
- create a levels adjustment layer above the image layer
- adjust the levels using that layer to improve the subject contrast
- crop the image.

"Phew!" you may think. "That seems like a lot of work." Well, since many of those steps are exactly the same for any image it is possible to create an "action" that carries out almost all of them with one button push. The only two that are carried out manually are the adjustment of the brightness and contrast using the levels layer and the final crop.

I do carry out one other process, not described above, for which I also have an action. That is to move the scale bar from the data bar into the body of the image, but I shall not describe it here.

It may be difficult to believe, but with subjects such as foraminifera, I can carry out the entire process (including moving the scale bar) in the time it takes my SEM to capture the next frame I need. This is 1min and 5 sec.

Sadly, not all subjects are as amenable to this automatic selection. Take, for example Figure 12, of a wasp's head. The Select Subject tool did select the general area surrounding the head, but there was no way it could select round the individual hairs, many of which were of very low contrast against the background. Instead, I did the entire selection manually, which took me more than five hours. (I timed it!).



Fig, 12: Head of *Vespula germanica* processed with the selection made the hard way, which took me over five hours