SEM Diaries - 15

It's not all about making images (but a bit on that as well)

Jeremy Poole

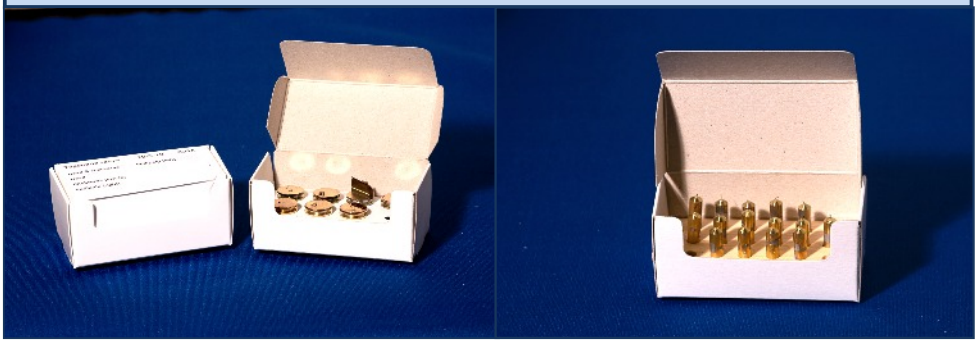


Fig. 1: From Left - closed stub box showing printed label, open box showing stubs inside, box fitted with wooden base for the retention of 3/16" rods for the rotating stub holder.

When I started out in Electron Microscopy, David Spears advised me that I would spend more time in front of my PC than I would in front of my SEM. I think that what he had in mind was that I would (or even should) be spending significant time colouring my images, which is a long process to do well.

Well, this period I have certainly spent a great deal of time in front of my PC carrying out SEM-related activities, but only a small amount of that was spent colouring images. Rather, I decided that it was time to take control of my stubs - in other words, catalogue them going all the way back to January 2016 - so that I have some possibility of locating stubs of interest at a later date. I have also taken significant steps in realising my ambition to build a "Spider Website" (no pun intended) to display my micrographs of spider parts in a way that may be of interest and use to other arachnologists.

Stub Database

So, first to the stubs. I mostly use aluminium stubs of 12mm diameter with a flat upper surface, to which I stick my specimens, either using double sided sticky carbon pads, or else a silver-based conducting glue. Latterly I have been using pre-numbered stubs, as described in SEM Diaries - 13, which make identifying the subject of the stub a lot easier, provided I update my catalogue of stubs at the time I make the mount. I also use stubs that slope at 45° or 90° and are not pre-numbered (see SEM Diaries - 12). The stubs are stored in cardboard boxes that are punched to hold eight stubs per box. These are delivered packed flat and need to be folded to end up with a rigid box in a similar way to the slide boxes that are used for PMS circuits.

Prior to using numbered stubs I simply kept a written record of what I made stubs of in a log book, and scribbled some shorthand on the lid of the box where the stub was stored. Unfortunately, I tended not date the boxes, so cross-referencing them to my notebook was difficult. In fact, my "stub management" was effectively non-existent and my boxes of stubs piled up in the drawer I had allocated to their storage.

Regular readers of this column will know that when making micrographs of, for example, the feet of spiders I like to mount the specimen in the end of a small rod that fits into a custom made holder that permits the rod to be rotated to obtain the desired orientation of the tarsal claw, for example. (See SEM Diaries - 4 for details of the first version of the rotating stub, and SEM Diaries - 11 for pictures of tarsal claws.) On my new website I have elected to show the tarsal claw as one of the features of each spider illustrated on the site, so I have needed to manufacture new rods for mounting the tarsals and take in hand the cataloguing and storage of these rods as well. I have also recently designed and built a "Mark 2" rotating stub holder. Photographs and more details of this are provided later in this article.

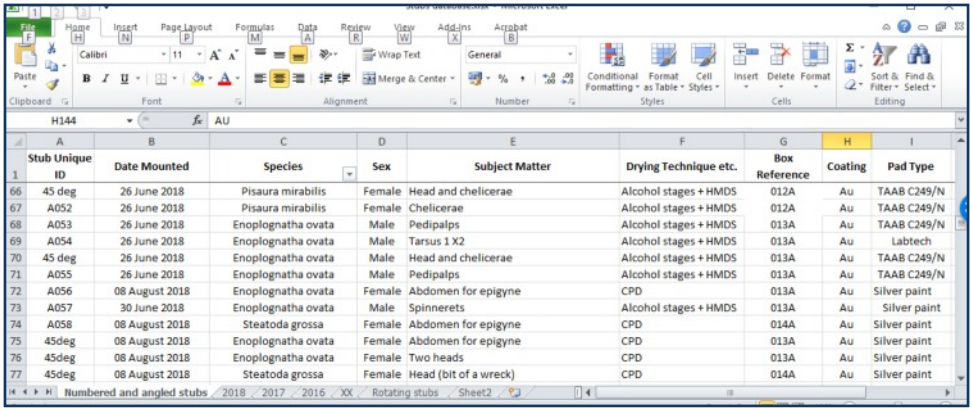
Well, to cut a long story short, I now have a catalogue of all my 523 stubs, plus an additional 30 or so rods with tarsals embedded in their ends. The detailed contents of each stub box is also identified on a stick-on printed label on the lid of each box. I can search the catalogue (implemented in Excel) against species or feature, and hopefully home in on a small number of stubs, should I wish to use these to make new images.

Figure 1 shows the various items mentioned in this ramble: boxes of stubs (closed and open), and rods in a wooden holder placed in a stub box. Figure 2 is a screen grab of my spreadsheet for pre-numbered stubs, which also identifies the 45° stubs, which are not uniquely numbered (despite the column heading!). I need to rely on common sense to identify the subjects of these, although they are catalogued uniquely to box number so there is limited choice.

Spider Website

The design and completion of the stub catalogue took little more than a couple of days, including the typing of the labels for the boxes. On the other hand, the implementation of the website has taken up a great deal of time, and the site remains "under development".

My "vision" for the website is to provide a web page for each species of British spider



	Stub Unique ID	Date Mounted	Species	Sex	Subject Matter	Drying Technique etc.	Box Reference	Coating	Pad Type
66	45 deg	26 June 2018	Pisaura mirabilis	Female	Head and chelicerae	Alcohol stages + HMDS	012A	Au	TAAB C249/N
67	A052	26 June 2018	Pisaura mirabilis	Female	Chelicerae	Alcohol stages + HMDS	012A	Au	TAAB C249/N
68	A053	26 June 2018	Enoplognatha ovata	Male	Pedipalps	Alcohol stages + HMDS	013A	Au	TAAB C249/N
69	A054	26 June 2018	Enoplognatha ovata	Male	Tarsus 1 X2	Alcohol stages + HMDS	013A	Au	Labtech
70	45 deg	26 June 2018	Enoplognatha ovata	Male	Head and chelicerae	Alcohol stages + HMDS	013A	Au	TAAB C249/N
71	A055	26 June 2018	Enoplognatha ovata	Male	Pedipalps	Alcohol stages + HMDS	013A	Au	TAAB C249/N
72	A056	08 August 2018	Enoplognatha ovata	Female	Abdomen for epigyne	CPD	013A	Au	Silver paint
73	A057	30 June 2018	Enoplognatha ovata	Male	Spinnerets	Alcohol stages + HMDS	013A	Au	Silver paint
74	A058	08 August 2018	Steatoda grossa	Female	Abdomen for epigyne	CPD	014A	Au	Silver paint
75	45deg	08 August 2018	Enoplognatha ovata	Female	Abdomen for epigyne	CPD	013A	Au	Silver paint
76	45deg	08 August 2018	Enoplognatha ovata	Female	Two heads	CPD	013A	Au	Silver paint
77	45deg	08 August 2018	Steatoda grossa	Female	Head (bit of a wreck)	CPD	014A	Au	Silver paint

Fig. 2: Screen grab of the database for the numbered stubs and recent 45° stubs

for which I have micrographs. The page should show between six and nine images of key features of a species that would normally include, but not be limited to:

- The head view showing the eyes and chelicerae from the front
- A more detailed view of the chelicerae seen from behind to illustrate the fangs and teeth
- The epigyne and pedipalp (female and male sexual organs respectively)
- The spinnerets
- A tarsal claw.

Clicking on any of these images would bring up a larger image in its own 520 px x 560 px window. It would then be possible to navigate to the page for a different species and call up an image of the same feature and compare these two images side by side. I have now managed to implement and upload this and you can see the results so far at:

www.spiders.jeremypoolesem.org.uk

Depending on progress between the time of writing and the time that Balsam Post lands on your doorstep, what you see may or may not still be a development version. Obviously, as I image new species I shall grow the website accordingly.

I built my main SEM website using a package called WebPlus to avoid writing my own code, but it soon became clear that this was not up to the demands of what I wanted to show. Thus I elected to build the spider site from scratch, using a mixture of HTML, CSS and Javascript. If these languages mean nothing to you, never fear! Suffice it so say that I now have about 18 inches of shelf space taken up with references and tutorials for this combination of web-building languages, not to mention a few shortcuts to on-line resources.

It would be reasonable to ask who the target audience of the website might be. My saying "anyone who wants to look at it" is not a very helpful answer, and is in any event the same for every page on the web. A more measured reply would be to say that it is aimed at spider enthusiasts who might want to study features at a magnification and clarity that cannot easily be obtained using a light microscope. I showed a selection of my electron micrographs of spider parts to members of the British Arachnological Society during a field weekend in June, and they were certainly enthusiastic about what they saw. Another practical, if a bit circuitous, reason is to give my work some focus. By keeping a record (i.e. creating a webpage) for each spider species I image I can monitor progress and concentrate on finding spiders that I have not previously imaged, and also ensure each of the key features of a spider has been documented. There is nothing like a target to focus the mind!

Given that there are now well over 650 species of British spider, and my site currently has pages for a mere 14 species, I am unlikely to run out of species to image. The biggest hurdle to overcome will be to obtain the specimens in the first place - and ideally I would need about three specimens of each sex in good condition, for each species!

Rotating Stubs Mark II

In SEM Diaries - 4, I described how I designed and made a special stub, which permitted the alignment of a pedipalp or tarsus to be adjusted so that it can be imaged from various angles. This used a 3/16" diameter aluminium rod fitted though a hole in a flange and retained by an interference fit (Figure 3). Well, this design was extremely crude and it proved difficult to obtain the correct friction in the interference fit to permit the rod to be rotated but retain it in position. I therefore looked into obtaining some spring material to bear down on the rod and hold it in position, but at the same time permitting it to be rotated easily. Normally I would

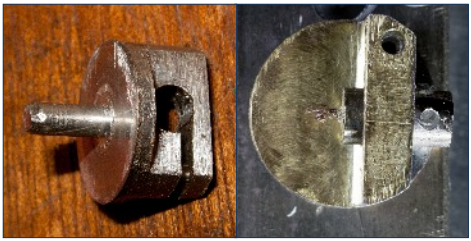


Fig. 3: Two views of the Mk I rotating stub holder.

have used either a discarded band-saw blade or steel band used in holding packing crates together as my source for suitable material, but given that steel is almost certain to have some remanent magnetism built in, I doubted this would work in close proximity to the electron beam. Browsing through the catalogue of one of my suppliers (TAAB) I came across just the thing - a pre-formed copper alloy spring, designed for holding specimens onto an SEM stub, complete with an M2 screw. Thus the Mark II rotating specimen holder was born. The construction of this can be determined from Figure 4.

Because of the length of the spring I had to make the base of the holder 19mm in diameter rather than the 12mm of the original design. This has disadvantages, mainly in how many holders can be put on the stage of the SEM at any one time, and

Fig. 4: The Mk II rotating stub holder, showing the rod retained by a spring clip, and the carbon paste coating on the base of the holder.

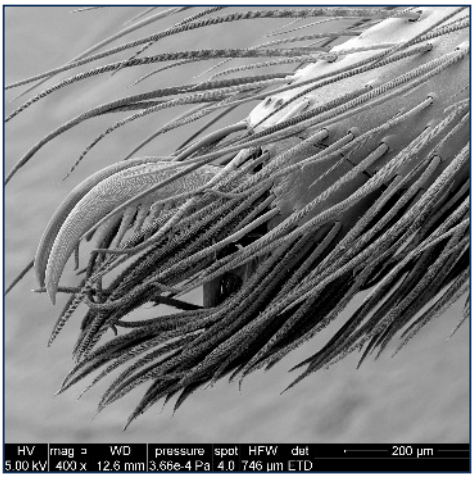


Fig 5: Micrograph of Tarsal claw of *Araneus diadematus,* made using a Mk II rotating stub holder.

also it limits how many can be accommodated on the turntable of the sputter coater. However, these are minor issues compared with the advantage of being able easily to assemble the 3/16" rod onto the holder and then rotate it smoothly by hand. One further refinement I incorporated was to coat the area under the specimen with a carbon paste/glue. Once set, this provides a pleasing background to the specimen (Figure 5). This normally obviates the need for work in Photoshop to eliminate distractions, which can be a long and unsatisfactory process, especially with hairy subjects.

Processing Images

For the website I have adopted a standard format for the images. In arriving at this I take an "original" from the SEM and then process it in various ways. I crop off the data bar at the bottom of the image. This contains quite a lot of information, but most of this is only of interest to other electron microscopists. The one piece of data that is essential is the scale bar, so I use Photoshop to move this onto the body of the image prior to cropping.

By far the most time-consuming activity, however, is to remove distracting backgrounds, and this can often involve making a selection round the outline of the subject (often hair by hair!), and then replacing the un-selected area with a neutral coloured background (normally black). Although this process does not enhance the scientific value of the image it does make a significant improvement to its aesthetic appeal.

In addition to the masking mentioned above I apply "curves" and "levels" adjustments to manipulate the contrast of the image. Very occasionally I may sharpen the image as well.

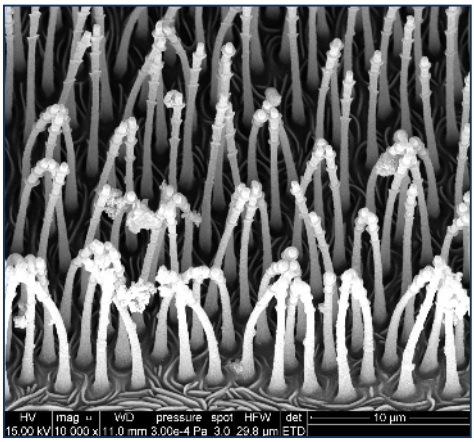


Fig. 6: The monochrome original of the "magical forest" image.

Colouring Images

Artificial colouring of electron micrographs also requires careful selection of parts of an image and having acquired more familiarity of this since I last tried colouring (see SEM Diaries - 8) I decided to give it another go. I elected to work on a high magnification micrograph of the cribellum of a spider called *Amaurobius similis*. The cribellum is an area adjacent to the conventional spinnerets where very fine silk is generated (approximately 15 nm in diameter) from a very large number of individual nozzles. It only exists (at least in a functional state) on a small number of species of spider. I chose an image showing just a few of the multitude of nozzles that make up the cribellum, and made a selection round each of these nozzles. These I coloured a yellowish green. I then reversed the selection and coloured the background of the image purple. Neither of these colours is present in the natural state, but together they create what I think is a pleasing if slightly surreal image, resembling a magical forest. This image is reproduced on the following page (Figure 8). For comparison I reproduce the monochrome original as Figure 6, and a lower mangification image of a complete cribellum, also from *Amaurobius similis*, as Figure 7.

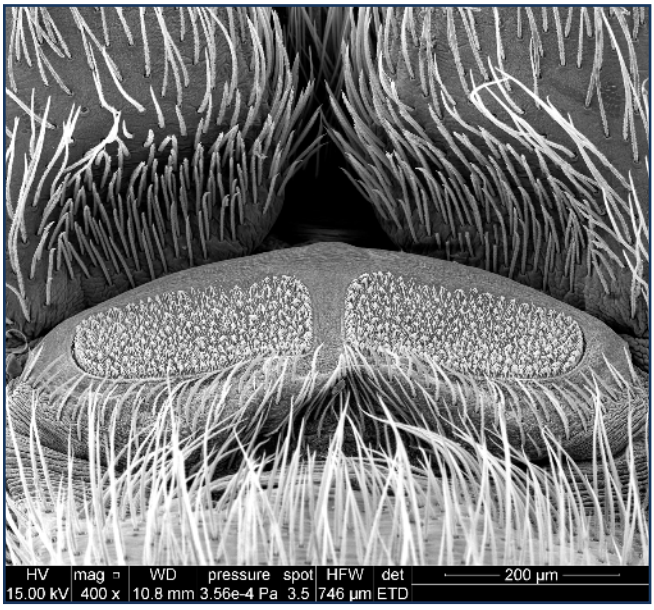


Fig. 7: A complete cribellum at lower magnification.

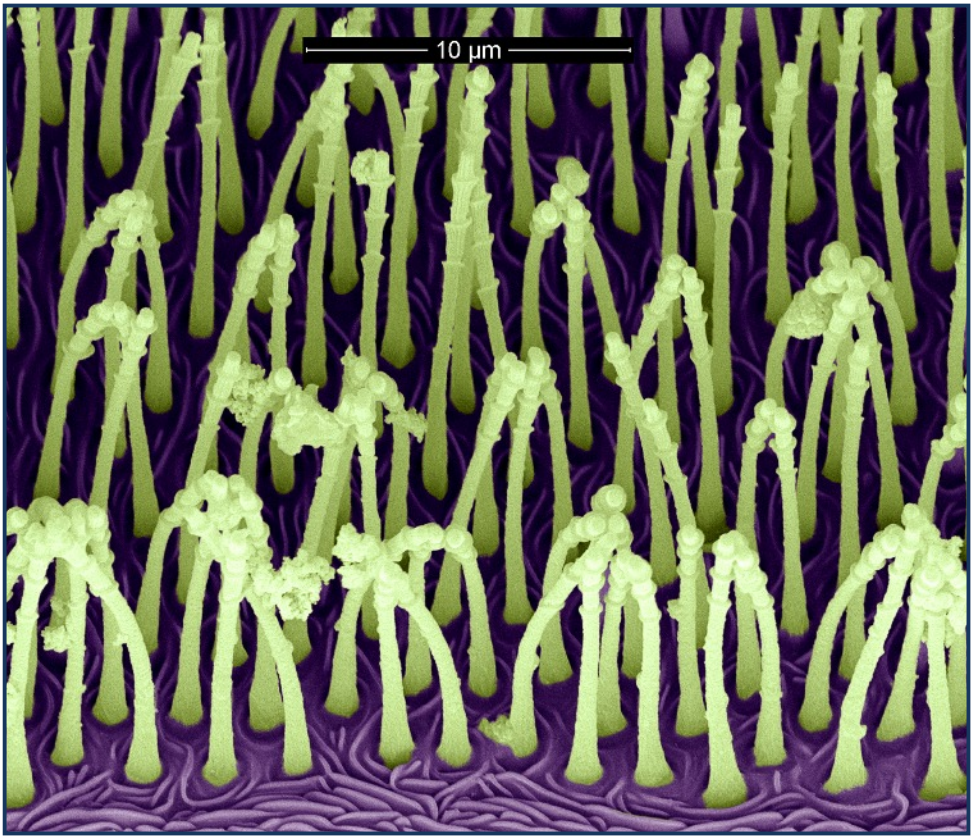


Fig. 8: “A Magic Forest”.
Artificially coloured image of a few of the individual nozzles that go to make up the cribellum of *Amaurobius similis*,