SEM Diaries - 16
HEXAMETHYLDISILAZANE

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Fig. 1: Small container of Hexamethyldisilazane and stainless steel mesh "saucer"

No, that title is not a swear word, nor (heaven forbid) an abbreviation. In fact it is the name of a chemical I have been using quite a lot recently.

When a specimen is imaged in the SEM it needs to be thoroughly dry, at least for the high vacuum mode of operation, or any residual water vapour will interfere with the electron beam. It is common to displace any water during preparation with a volatile organic solvent such as acetone or alcohol, but when that solvent evaporates from the specimen the resulting surface tension can significantly deform its shape.

There are two ways round this problem. One is to use a "Critical Point Dryer" (CPD) as described in the first issue of SEM Diaries. This involves displacing the organic solvent with liquid CO_2 in a pressure vessel, and raising its pressure and temperature to the "critical point" which is where the properties of liquid and gaseous CO_2 are identical (around 1050 p.s.i. and 31.5 C). A simpler alternative for non-critical applications is to use an organic solvent that has a relatively low surface tension. This is where hexamethyldisilazane (henceforth referred to as HMDS) comes in.

The advantage of using HMDS is that it is relatively quick and simple. Among its disadvantages are its cost, at about £300 per litre, and its smell, which is not pleasant!

My protocol is to pass the specimen through a number of baths of iso-propyl alcohol and then finally through a bath of HMDS, before removing the specimen from the bath and leaving the HMDS to evaporate at room temperature. In theory it is best to use increasing concentrations

of alcohol through the bath sequence, but since my specimens are quite robust I just use 100% in each of my four baths. Obviously each bath becomes slightly diluted as it removes water from the specimen. (I mentioned my process in passing to David Spears when I last saw him, and I was surprised but gratified to learn that he himself likes to use HMDS in preference to his CPD when he can.)

So, why all this activity with HMDS? Well, since I advised readers of the launch of my new "spider" website in the last issue of SEM Diaries I have contributed an article on it to the British Arachnological Society. I am keen to get electron micrographs of as many species as I can before the next issue of their Newsletter hits the doormats. Although it has not exactly been the best season for collecting spiders I have managed to raid my preserved specimens and added a few more species to my collection of micrographs, and by the time you read this, hopefully to my website as well.

In preparing my specimens for mounting on stubs, I have two choices. I can either desiccate the whole spider and then dissect out the desired parts, or I can carry out the dissection under water or dilute alcohol and desiccate the resulting anatomical features. These days I normally use this latter order since it reduces the chance of parts flying off when they are cut, as has been described in SEM Diaries - 7.

Each spider should yield up about 13 parts of interest. These are: 8 legs, 2 pedipalps (male or female), spinnerets, head with chelicerae (or alternatively just the chelicreae) and epigyne (females only). These are dissected in the alcohol in which they were stored and then placed in the first bath of neat alcohol.

Since I use five baths (including the HMDS) the idea of transferring each of these fragile parts individually between baths using tweezers is not very appealing, not least because of the high likelihood that some part or other might be damaged by the tweezers at some stage during the routine, or even mislaid.

Taking a cue from the late great Eric Marson, of Northern Biological Supplies fame, I decided that what was needed was a container with a perforated bottom that could be moved between baths without having to handle the specimens at all. While Eric used glass tubes with stocking material lashed to the bottom of them, I managed to find some fine stainless steel mesh material which could be cut up and folded to make saucer-like containers (grids) that fitted the small (5ml) plastic pots that I use for my baths. A grid, together with a pot containing another grid, are illustrated on the frontispiece (Figure 1).

Now, one of my favourite spiders is the Linythiid *Labulla thoracica* (Figure 2). I like it for no other reason than that the sexual organs of the male are so totally out of proportion to the rest of the spider. The business end of its pedipalp is around 1.5 mm across its widest part, and this is for a spider whose overall length (head to spinnerets) is about 5 mm. One only has to view that in a human context to realise how impressive that is (if you are impressed by that sort of thing!).

The structure of the pedipalps resembles a flat disc, with a "hose pipe" coiled around it. Ironically, it took me quite a while to find this in the Field Guide [1], as the view

Fig. 2: The impressive pedipalps on the male *Labulla thoracica* (arrowed)

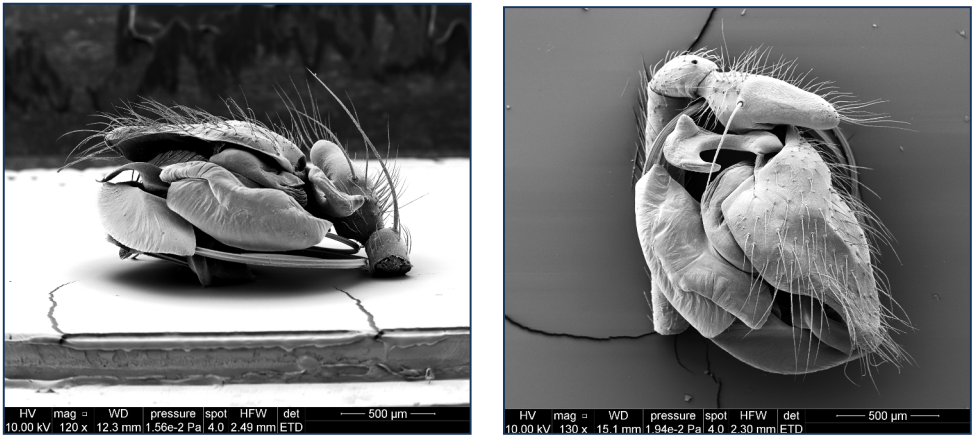

Fig. 3: Two views of male pedipalp of *Labulla thoracica.* On the left is the left pedipalp viewed from the side, as is shown in the references. The right hand micrograph is of the right pedipalp viewed from underneath. Scale bar 500µm.

in that volume is edge on, and therefore less impressive. As part of my recent capturing of images of this creature, I took micrographs from two different angles, which are reproduced in Figure 3. For my website I like to black out any distracting background. I am not looking forward to doing this (if I actually attempt it at all) on account of the myriad hairs around the subject in both views.

EM-UK

I was able to attend "EM-UK" at Warwick University in early January 2019. This is an annual event, organised by the Royal Microscopical Society, which brings together members of the electron microscopy community in the UK (both academics and suppliers) to discuss matters of common interest.

I was possibly a bit out of place at a meeting aimed at academics, although it made me nostalgic for the days when I carried out university research! It did, however, give me the opportunity to catch up with two of my suppliers, including the vendor of the "dodgy" filaments that I described in SEM Diaries - 14.

While many of the lectures were of general interest to me, two stood out to grab my attention. The first was entitled "Unusual Sample Preparation for TEM". In this the lecturer described an exercise he carried out with two 17 year old work experience students. The object of the exercise was to collect water samples, stain them (with uranium salts) and image them on a transmission electron microscope. A wide variety of viruses were discovered, even in the drip tray from the water cooler in the Junior Common Room. This exercise was used as the basis for a "Citizen Science" project, called "Virus Hunters", which invited schoolchildren to send in water samples and to help analyse the images obtained from a transmission electron microscope [2].

The second lecture was on the status of technicians at universities and an initiative being undertaken to enhance the respect in which they are held. Whilst my recollections from school days was that the lab technician was the person who topped up the reagent bottles in the chemistry lab and washed up the beakers and pipettes, the role of a technician in a university or

research laboratory (or even schools) these days is much more skilled.

The problem is that the original stereotype still exists, and is reflected in salaries and job progression opportunities. In reality, someone in charge of an electron microscopy laboratory, who might well be classed as a technician, will almost certainly be a graduate or of graduate level, and know much more about the instruments in his or her charge than the people they report to. Similarly, they would be perfectly capable of carrying out research to PhD level and possibly beyond.

An initiative called "The Technician Commitment" [3] has been set up to tackle these issues in four key areas:

- Visibility - within their organisation
- Recognition - professional registration
- Career Development - through a defined pathway
- Sustainability - to ensure a flow of new recruits to provide required skills

Nearer Home

In SEM Diaries - 13 I mentioned that I now use numbered laser-engraved stubs on which to mount my specimens, to aid traceability and identification. I also mentioned that on delivery my 1000 stubs required to be sorted manually into numerical order. Well, in January 2019 I placed one of my compartmentalised boxes on my office chair in the laboratory, having extracted a stub of some sort. A while later I heard a crash.

The open box had fallen off the seat and spread numbered stubs all over the lab floor and under my desk. Thus I had to both clean and then sort approximately 500 stubs once more, before they could be put away in their correct locations.

It had been about 15 months since my service engineer, Don, had visited when I heard a grinding noise from the mechanism that rotates my stage. This noise soon went away and I had the dilemma of whether to call in Don (who might not find anything when he did visit) or to wait until the noise persisted and Don would have something to get his teeth into (so to speak).

Well, since running my SEM Lab is something of a lonely occupation I decided on the former approach, and Don duly arrived. He soon located the source of the problem and carried out the necessary adjustment, so we spent much of the rest of the day carrying out routine maintenance including changing the oil in my rotary pump and topping up the distilled water bottle for my low vacuum mode.

Don was able to confirm that my instrument was working well, and I even learnt a few more features of the computer interface that I did not even know existed! All in all this was a very worthwhile visit, but hopefully I shall not need another for some time yet.

References

1. Roberts, M.J. Spiders of Britain and Northern Europe. Collins Field Guide, Harper Collins, London 1996
2. https://warwick.ac.uk/fac/sci/lifesci/outreach/virushunters/
3. http://technicians.org.uk/techniciancommitment/