SEM Diaries 6 - The Mysterious Case of the Imploding Tick

Jeremy Poole

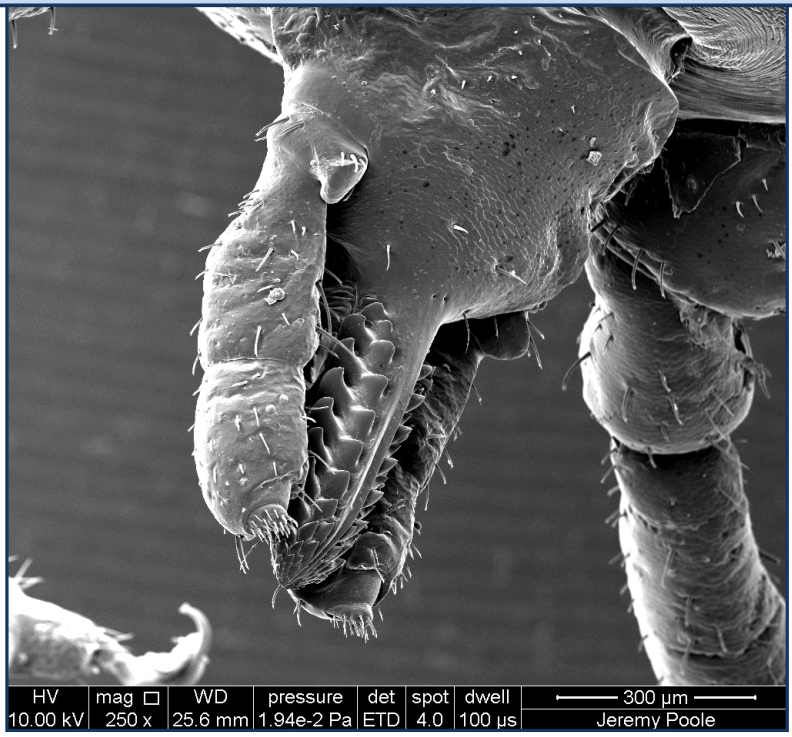

Fig. 1: Mouthparts of the tick, showing the barbs that latch into the prey

Many microscopical friends (in interest rather than stature) are aware of my interest in arachnids, which includes mites and ticks as well as the spider family. Thus it was no great surprise to receive an email from a regular contributor to BP advising me to look out for a package he had posted to me containing a tick from a hedgehog. That same morning the package arrived, which I duly unwrapped, wondering to myself what particular preservative he had used.

I was more than a little surprised, therefore, to find an extremely bloated tick still very much alive, crawling round in a transparent inner container. This creature had obviously eaten well, as it bore a close resemblance to "Mr. Blobby" with legs and jaws sticking out of a balloon-like body about the size of a large almond.

I kept this creature on my bench for a couple of days, still in its inner tube, before finally consigning it to my container of acetone at -20 Celsius. The next day I put it through my standard critical point drying routine, mounted it on its back on a 1" stub, sputter coated it and consigned it to the chamber of the SEM.

My plan was to image its mouthparts as best I could before homing in on its feet, to compare these with the feet of a red velvet mite I had recently studied. Well, I succeeded reasonably well at the first objective (Figure 1) but before I could home in on any of its feet the image disappeared from my screen, to be replaced by darkness with white specks on it, resembling a night sky. Try as I might I could not restore a vestige of a picture, so there was no option but to vent the system and inspect the stub.

The sight that greeted my eyes is reproduced in Figure 2. The "balloon" of the body had collapsed in on itself and the edges of the body had curled round as shown. Obviously, some major change of shape and state had occurred!

In retrospect it is not unreasonable to assume that the cavity that originally contained the hedgehog blood must have contained either some residual acetone or blood, or else CO_2 from the critical point dryer.

Well, by putting a grid into the centre stage position and focusing on that, I managed to coax an image out of the SEM - indeed for a day after this interesting event I was obtaining quite decent pictures. Soon, however, I detected significant astigmatism and it became impossible to obtain a sharp image at magnifications much in excess of x100. All sorts of questions went through my head. Had something gone wrong with the focusing coils? Had a power supply gone down? Had some machinery with a large magnetic field been installed at the depot the other side of the fence?!!! And, worst of all, how was I going to demonstrate the SEM at the weekend party I have laid on for the contractors who contributed to the construction of the laboratory? Time for another service visit, I guessed.

Fig. 2: The imploded tick, still fixed to its blob of conducting glue

With the SEM effectively out of action while I waited for the day of Don's visit I decided I would set about other jobs around the lab, and in particular I wanted to add a second monitor to the PC that controlled the SEM. This, connected in "screen extension" mode, would permit me to view micrographs taken earlier alongside a "live" picture from the SEM. I had previously set up several office PCs in this mode, so I was pretty confident of success. My attempts not only failed to set up the two monitors in the way I wanted, but I also managed to truncate the right hand side of the SEM's user interface, rendering some controls inaccessible. I had not managed to resolve this problem by the time of Don's service visit, and nor was he any more successful in doing the same.

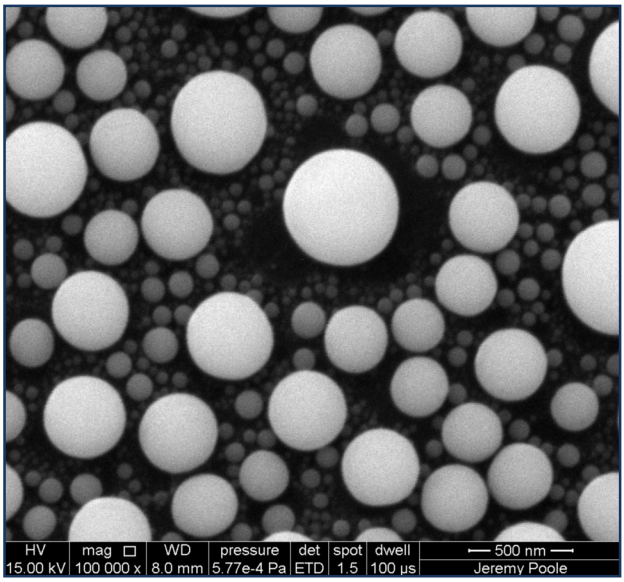

Fig. 3: Tin balls resolution test stub viewed at x100,000. Note the 500 nm scale bar at bottom right.

Well, to cut a very long story short, I managed to resolve the video problem by the time of Don's second visit, during which he carried out a full column clean, complete with the replacement of two apertures, which restored the SEM to full working order. This was demonstrated by the imaging of a tin balls test stub (Figure 3) - the equivalent of the diatom test slide beloved of light microscopists. I was assured by Don that the results achieved are most acceptable for an SEM with a tungsten electron source.

The last three months have not been all doom and gloom in the SEM department, though. In July I attended the RMS Electron Microscopy Summer School. This week-long course, held at the University of Leeds, is aimed at professional users of scanning and transmission electron microscopes, be they in universities, industry or hospitals. Once the students and lecturers had accepted the fact that they had an amateur with an SEM in a "shed" at the bottom of his garden among them, we all got on fine. Fortunately, I had done considerable reading around the subject in advance and had had six months to practice on my own SEM, which meant that I was able to hold my own in most of the lectures and presentations. In fact many of the students were very complimentary of the electron micrographs of spider parts and micro-fossils that I had brought along and showed them during the week.

There are a significant number of electron microscopes at Leeds, both scanning and transmission. In particular there were two different SEM models by FEI, the same maker as my own SEM. These were their Quanta 200 and Quanta 650 models, both of which employ "Field Emission" electron guns (FEGs), which provide significant advantages over the simple tungsten gun of my own FEI Inspect S50. There was also a high specification TEM by the same maker, with a 300 kV acceleration voltage, known as the "Titan", which was reputed to cost £2 million.

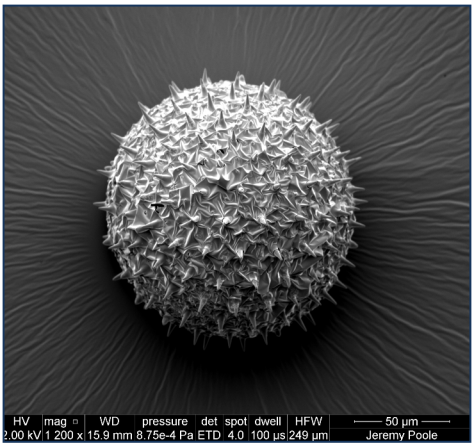

Fig. 4: Hollyhock pollen grain imaged using normal high vacuum mode

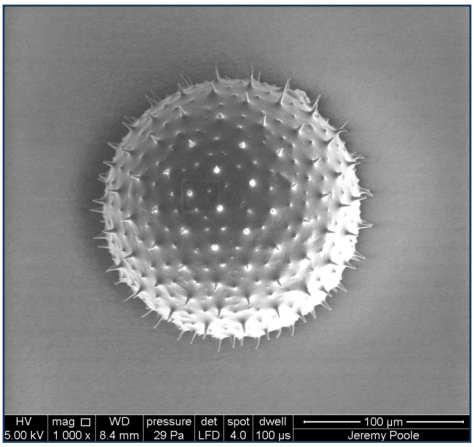

Fig. 5: Hollyhock pollen grain imaged using low vacuum mode. Note the smoother outline

The one negative outcome of this otherwise excellent week was that I went down with a serious case of a mental illness known as "FEG envy". Having witnessed the quality obtained from the FEI Quanta series of SEM, with their field-emission guns, I am no longer satisfied with my own equipment. I am advised by "Dr. Don" that the least expensive cure for this might be a third party FEG add-on for my own SEM - a snip at £30,000 + VAT. I told him I would "think about it". In fact, we might even go on a joint visit to the manufacturers - after all, there is no harm in looking, is there? Meanwhile, Don will be back in about 3 months time to install a back-scatter detector. This has been on my mind for a while, and the RMS course confirmed to me that I should have one, in order to obtain an alternative view of my specimens. Currently my system has a secondary electron (Everhart-Thornley) detector, and also a large field detector used solely for the low vacuum mode.

I mentioned "low vacuum" mode. This is a really clever technique that permits one to image material that is insulating and not sputtered with gold. The specimen chamber is maintained at a relatively low vacuum, such as 20 Pa compared with 10^{-4} Pa in the conventional high vacuum mode. Water vapour is introduced into the chamber and water molecules collide with the electron beam. This interaction ionises the water, and the positive ions migrate towards the negatively charged areas of the specimen, thus neutralising any charging.

I used this to look at some hollyhock pollen. I had already imaged this in conventional mode (Figure 4), but I was wondering if the image was a true representation of the pollen, or if the sample have been damaged by heat generated by the sputter coater or simply drying out. I tried imaging a new sample of pollen from a different hollyhock, using low vacuum mode, and as I suspected this demonstrated a smooth tent-like structure surrounding the pollen grain, with similar spikes to the sputter coated sample (Figure 5).

I have no idea whether the difference is due to a different maturity of the two samples, or if there really is damage in the Figure 4 sample. Whatever the reason, this difference illustrates the need for careful preparation, most suitable choice of imaging mode, and of parameters such as accelerating voltage and spot size.

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