

SEM Diaries - 20

Five Years and Counting

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Fig. 1: The SEM as it was at the end of 2019. The right hand screen is connected to the small PC on the floor between the SEM and the control desk and is used to operate the Backscattered Electron Detector protruding from the right of the chamber.

When I said in Diaries - 19 that not a lot had happened in the period covered, I had somehow overlooked the fact that I had increased the computing power and ease of use of my laboratory quite significantly. What is

more, the fact that 2019 was the 50th anniversary of the first moon landing had given me reason fancifully to compare my current laboratory computing arrangements to those in the "Firing Room" at Cape Canaveral.

The two left hand screens in Figure 1 are connected to the microscope controller, which is the large (Windows XP) PC under the desk. The right hand screen is connected to the smaller PC lying up against the left hand side of the column unit. The small PC is a Windows 10 machine that I bought when I was given updated software for my Backscattered Electron Detector (BSED), which can be seen projecting from the right hand side of the SEM chamber. The display on the screen is the revised user interface of the BSED. The main PC is isolated from the internet and any networks, because it does not have any up to date virus protection, but the small PC is connected to the internet, and to my other PCs via DropBox, and having this alongside my SEM displays helps productivity significantly. For example, I can call up my database of stubs and update it easily, when making or searching for stubs. Since that photo was taken I have incorporated a USB switch so that I can control both PCs from the same keyboard and mouse.

Not visible in the photo there is a further PC, with two screens that I use for controlling my WeMacro stacking stand, and storing and displaying the resultant images. I am trying to get into the habit of photographing my specimens before they are dissected and again once on stubs but prior to sputter coating. This is to provide evidence of the true colour of the subject matter should I ever wish to take artificial colouring of images seriously.

In stark contrast to Figure 1, Figure 2 shows the single screen configuration of my SEM at the time of delivery.



Fig. 2: The SEM following commissioning, in an empty lab - Feb. 2016.
(Photo Don Ashcroft)

It is worth pointing out that the additional PCs are not high specification devices. I bought them, secondhand, from a company called EncorePC for not much more than £100 each including the monitors. They are, however, loaded with Windows 10, and the BSED PC came with a solid state drive (SSD). I have since converted the other PC to run with an SSD as well.

A Sick SEM

As I write this, my SEM is sick. Very sick.

The SEM has built-in diagnosis facilities, which have directed Don in the general direction of the issue, in the EHT area, and we think we have located a faulty board. There is no guarantee, however, that replacing this board will not then identify more faults further up the chain. Fingers crossed for third time lucky! The cost of calling in the official FEI service engineers is very high, as is the cost of an exchange EHT unit should that need replacing. Ironically, many of the older models of SEM are easier to maintain, as they use simpler circuitry and fewer, if any, custom integrated circuits.

I am suffering from withdrawal symptoms as we wait to decide on the next step to take. I have 27 new stubs awaiting imaging before I can upload the results onto my spider website, which has not been updated for quite a while now.

First Five Years - Looking backwards, and forwards

This being the 20th issue of SEM Diaries, marking five years of my committed interest in scanning electron microscopy, it seems a good time to look back on the highs and lows of my life with an SEM, and bring you up to date with future plans.

Getting Started

I often wondered what Don thought when this would-be amateur electron microscopist first made contact - in fact I finally asked him when I last saw him. Diplomatically, he replied that it was clear I had done my homework and knew what I was talking about, so he did not doubt I could make a go of it. I would not be surprised, however, if he did wonder at times, especially during the eight months over which he had to store my SEM while my laboratory was being built. At least I had paid for it!

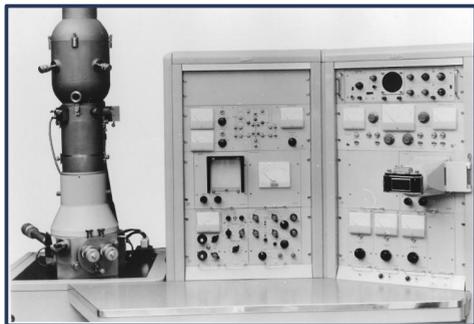


Fig. 3: The Cambridge Scientific Instruments Stereoscan Mk I (1965)

Of course, though a key player, Don was only one of the many people who had contributed to my getting even that far. In fact I can trace my interest in scanning electron microscopes all the way back to 1968, when I received a demonstration of the Cambridge Stereoscan (Figure 3) from Prof. Charles Oatley, who had directed a succession of students from the late 1940s onwards in research leading to the first commercial SEM and beyond. My interest aroused, I enquired of the price and was told it cost £100k. To put this in context, my first salary on graduating three years later was under £1k, and my first flat in London cost me less than £10k in about 1976.

Since that time I was always interested when I came across images created with an SEM in text books, and after taking up microscopy as a serious hobby in my 50s this interest took off, especially after viewing modern SEMs at microscopy congresses and at the Natural History Museum. But, if an SEM cost £100k in 1968, how much would it cost to own one 50 years later?!

Another key player in my journey is David Spears, whom I met at a QMC Dale Fort weekend at which he contributed a lecture entitled something like “Big Views of Tiny Creatures”. I had already purchased his book of SEM and light microscope images and was quite surprised to find myself in the same room as the author! Dave told me that a desktop SEM could be purchased for “only” £50k, still a large amount of money but considerably less than the £100k plus 50 years inflation that I had imagined! Plus, I now had access to someone who could teach me the basics of preparing spe-

cimens, not to mention demonstrate his own SEM to me.

Things rather took off after that, with visits to two successive Microscience Microscopy Congresses, in 2014 and 2015 where I initiated contact with suppliers of SEMs, both new and pre-owned, and of sample preparation equipment. In fact it was the Commercial Manager of Quorum, who make sputter coaters and critical point dryers (among other things), who introduced me to Don Ashcroft of Tron-tech who eventually supplied and occasionally maintains my pre-owned SEM.

The Failures

I would be a liar if I were to say that everything has gone swimmingly since my SEM was commissioned. My initial attempts at making stubs and imaging them did, as one might expect from a novice, leave something to be desired. I had one spectacular failure when attempting to image the mouthparts of a tick (still attached to its blood-filled and voluminous body). While framing a picture the screen suddenly went grey, and I could do nothing to recover an image. It transpired that, although I had nominally dehydrated the tick, the size of its body meant that my process had been inadequate with moisture trapped within its abdomen. Either that, or air had become trapped inside, and under vacuum the air had caused the body to expand and eventually explode. The next day I managed to coax an image out of the SEM, at least at low magnifications, but even then the image quality was very poor. Too bad I had invited the contractors involved in assembling my laboratory to come and view the SEM in a few days time.

Don could not get to me in time to rectify this before that party, but fortunately he managed to visit to clean the column (and teach me how to do it) prior to my party for friends and neighbours.

I suffered another disaster when experimenting with the settings of the video card in the SEM controller. As shown in Figure 2, the SEM came with a single screen, although the video card had two display port outputs. I had previously set up other PCs to run in a dual screen configuration, so I was confident I could do that for my SEM. To cut a long story short, the control panel for my video card turned out to be a lot more complicated than anything I had come across before, and at some stage I

managed to change the size of the displayed font. The net result was that the part of the screen area used to control the SEM ended up extending beyond the edge of the screen and became un-usable. I was due a visit from Don, but I could not fix the issue before he arrived - and nor could he. I eventually found the wrong turning I had made on the control panel and successfully incorporated a second VDU, which has proved extremely useful.

Other failures have usually been connected with specimen preparation. I am becoming immune to the disappointment that arises when one takes great care in the dissection and dehydration of a specimen for it only to flip over and land its key feature in a puddle of glue. This has happened frequently. Another frequent occurrence is for a specimen to go missing - normally by flying or falling out of the tweezers as it is moved from under the stereo microscope to the stub. My bench top has a sort of marbled pattern - ideal camouflage for small bits of spiders. In fact I have now laid out white paper over the area of the bench where I do my mounting and this has helped greatly in locating missing specimens.

Projects

I mentioned in Diaries - 19 that I am part of a team studying a bug using our combined knowledge and capability. Hopefully this will be the subject of an article in BP at some stage, although the current report on the project so far would take up a whole issue! I enjoy projects such as this. Not

only does it provide interaction with other people, rather than just working away on one's own in an ivory tower (or in my case a wooden laboratory), but also the shared knowledge and discussions on interpretation of some features can lead to a result that is greater than the sum of its parts.

I am providing the SEM images for the project, while others provide images from real life, or of preserved specimens using light microscopy. Yet others are attempting to produce sections! I am purposefully not giving too much away about the project at this stage, but we shall announce it to the world in due course, I am sure.

Another small project has been to image 149 different diatoms, collected in Croatia. Fortunately these were all on the same stub rather than requiring individual mounting, one per stub! Although I am not a diatomist, and imaging 149 different diatoms can take quite some time, especially as setting up the SEM for the high magnifications used is time-consuming, I have still enjoyed this project. It has given me the opportunity to explore the capability of my SEM at higher magnifications and provided me with a library of diatom images, most of which have been identified, at least to genus level (Figures 4 and 5). The SEM is an ideal tool for imaging diatoms. In Diaries - 19 I included several images of diatoms, including two of *Diatoma mesodon*. On Figure 4 (of Diaries - 19) an area of the diatom is shown that has perforations that are 129 nm apart. Their diameter is possibly 1/3rd of that. These

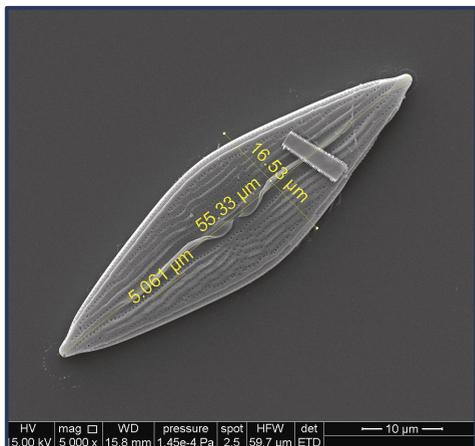


Fig. 4: *Mastogloia erythroa* Grunow 1860

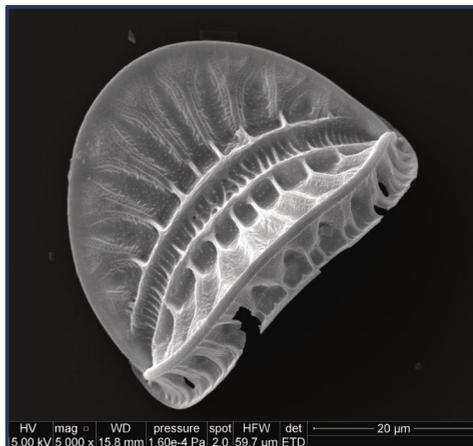


Fig. 5: *Campylodiscus* sp.

perforations are clearly seen, even though it may have taken me some time in setting up the SEM parameters to achieve the image as clear as it is. As most readers will know, the best resolution obtainable on a conventional light microscope is of the order of 250 nm, so without access to an SEM or to a highly specialised diffraction limit-busting light microscope, these details would be invisible. This, of course, is in addition to another feature of the SEM, which is its ability to provide images of great depth of field and with a “three dimensional” appearance.

Just like London buses, a third project arose at around the same time as the other two. This was to image hairs of some mammals, both “current” and extinct. Again, while hairs are not among my main interests in microscopy it was an interesting exercise, and provided the opportunity to liaise with experts, including an electron microscopist, in the United States.

The Successes

Overall I am quietly pleased with what I have achieved to date.

It can be a lonely occupation, working away in my lab on my own and I have felt the need to have a “yardstick” against which to judge my work, just as in my musical endeavours as a cellist and pianist I worked my way up through the grade exams. Or is it more a case of wanting certificates to hang on my lab wall or more letters after my name!

My first target was to attempt to achieve a Fellowship of the Royal Microscopical Society. The criteria for this are ridiculously easy to meet. As well as having been a member for three years, there is only one other requirement to fulfil, selected from three choices. You should have either attended one of the courses laid on by the RMS, or submitted a mini-CV for publication in their members’ journal “*infocus Magazine*” or alternatively had an article printed in that publication. I decided that I would write an article on my experience in setting up my laboratory. I sounded out the Editor of

infocus Magazine at MMC in 2017, who was very encouraging, and my article appeared in the June 2018 issue [1]. Shortly after this I enquired about becoming a Fellow, only to be told that since I paid the reduced subscription offered to retired people, I am not eligible!

Another outlet for images is the annual RMS Calendar. I submitted a micrograph for the 2018 calendar, which was accepted and used for the July page. My image for the 2019 Calendar did not get selected, but I made it as “June 2020” with one of my spider images (Figure 6), although by then the calendar had become an electronic version suitable for display on one’s PC desktop, rather than a paper calendar [2]. Winning a place in the RMS calendar does not bring with it any reward other than the satisfaction that one has been selected, but it does at least get one’s work seen. One positive outcome of the 2018 image was that it brought my work to the attention of Deben who make the backscattered electron detector on my SEM used to produce the selected image, and other SEM add-ons. They were happy to provide me with an updated version of the software for my detector, in exchange for featuring my work in an “advertorial” [3].

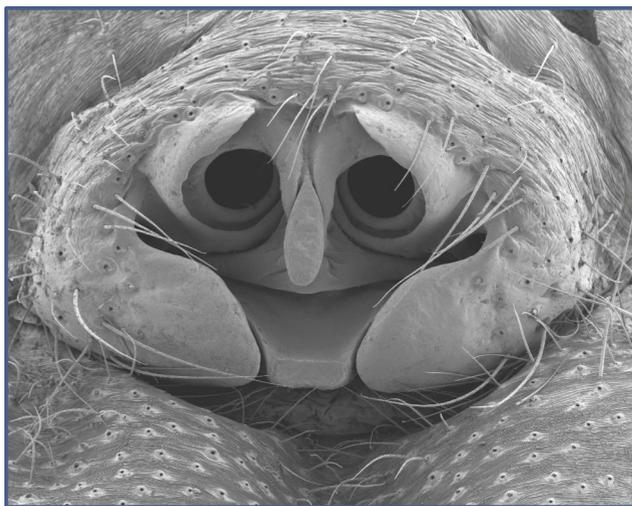


Fig. 6: The image used for the June page from the RMS Digital Desktop Calendar bears a strong resemblance to one of the characters from “Thomas the Tank Engine” (at least I think so!). The subject is the epigyne (female sexual organ) of the spider *Labulla thoracica* (Linyphiidae)

Way back in 2007 I was awarded the Licentiate of the Royal Photographic Society (LRPS). This is the “entry level” distinction of the RPS, roughly equivalent to a good ‘A’ level in photography. I was determined, in 2019, to apply for the next stage up - the Associateship (ARPS). This requires 15 images, of a higher standard than is needed for the LRPS, and also the submission of a “Statement of Intent” to describe why you took the photos you have submitted. Having failed to find a coherent selection of 15 images of wildlife, almost in desperation I assembled a panel of 15 electron micrographs and decided to submit these. The chosen subject area laid down by the RPS was “Imaging Science”. I was advised that in addition to a Statement of Intent I would need to supply sufficient additional information to convince the judges that I understood electron microscopy and the identification of spider species. To cut a long story short, not only was I successful in my application [4], but my work was the subject of a five page spread in the RPS Journal [5]. Now two members of PMS who happen to be Fellows of the RPS are encouraging me to work towards a Fellowship myself.

One final “achievement” in 2019 was providing one of my spider images for an exhibition entitled “Beauty and the Beasts; falling in love with insects” at the Manchester Museum [6]. I had met their curator of arthropods at the British Arachnological Society AGM and showed him some of my electron micrographs there.

Some friends have suggested that I approach a company such as “Athena” with a view to their making posters of some of my images. I have, however, found that pictures of spiders do not make very popular wall hangings! In fact I once had a canvas print of the spinnerets of a spider on display at a meeting and one visitor walked past my display on the opposite side of the room rather than look at my micrograph! Perhaps a soothing coloured image of a diatom might be more acceptable.

When I did the RMS one-day course aimed at teaching the colouring of electron micrographs, we were encouraged to

send examples of our work to the Science Picture Library, who seem to have an insatiable demand for coloured SEM images. Perhaps I shall try that in due course. At least there would be a chance to get some income from the work!

Looking Forward

Much as I enjoy the MMC exhibitions they do generate a certain amount of envy and desire. Many of the instruments on display there are extremely high quality, with an extremely high price tag to match!

At MMC 2019 I had a detailed demonstration of the Thermo Fisher “Prisma” entry level SEM, which is their current model replacing my 15 year old Inspect. It was clear that the quality of images from tungsten emitter SEMs has come on a long way since my instrument was launched. With my current machine being possibly terminally ill as I write this, my mind has started wandering to what might replace it. Watch this space.....!

References

1. An SEM at Home - from Fantasy to Realisation over Half a Century, *infocus Magazine*, 50, June 2019
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3. Electron Microscopy’s very own “Spider-Man”! Deben November 2019
4. www.jeremypoolphoto.co.uk/ARPS-SEM
5. The Spider Man - How Jeremy Poole ARPS turned a passion for arachnology into a Distinction. *Journal of the RPS* Vol 159 No 10
6. <https://mmbeautyandthebeasts.wixsite.com/mmbeautyandthebeasts>

Image Credits

Figure 3 : [https://commons.wikimedia.org/wiki/File:Stereoscan_MK1_\(19515061054\).jpg](https://commons.wikimedia.org/wiki/File:Stereoscan_MK1_(19515061054).jpg)

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