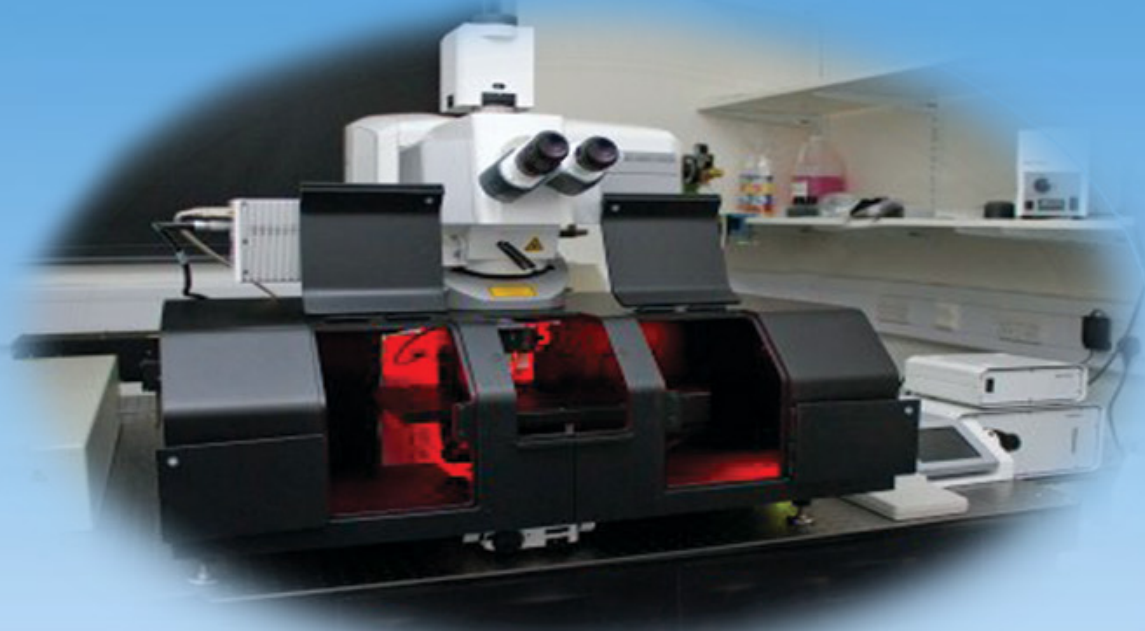


An SEM at Home — from Fantasy to Realisation, over Half a Century

Dr Jeremy Poole



Since seeing my first SEM image at the age of 19, I had wanted to own my own instrument. Until very recently, this was pure fantasy, but a coming together of happy coincidences, and the generosity of experts with their knowledge, has led to this becoming a reality. This article describes my journey to becoming an amateur electron microscopist, nearly fifty years after my first encounter with an SEM, and provides practical advice for anyone wishing to follow a similar path.



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Gestation of the Project

My first encounter with an SEM occurred in September 1968 when, as a freshman at Cambridge University, I went on a tour of the Engineering Laboratories, as part of my induction to that department. The one thing that stood out for me from that tour was a demonstration of an SEM by the then Head of Department, Professor Charles Oatley, "Father of the Modern Scanning Electron Microscope" (Everhart 1996). The image was of the compound eye of an insect and the three dimensional effect for which the SEM is known was very obvious. This was the moment that my fantasy of owning an SEM was born.

I started to become interested in light microscopy and also arachnology in about 1997. A few years

later I purchased a book on spider anatomy that contained a large number of scanning electron micrographs (Foelix, 1996) and later still a book of coloured scanning electron micrographs of "tiny creatures" by David Spears (Spears et al, 2007). Also, during that period, I was able to view the SEMs at the Natural History Museum on two occasions while attending annual exhibitions of the Quekett Microscopical Club (QMC). I really, really, wanted my own SEM!

It was at a QMC "Plankton Weekend" a few years ago that I first met David Spears, author of the second book mentioned above, and discovered that, in addition to owning the SEM that he used for his business of natural history photography, he lived within an hour's drive of my home. Furthermore,

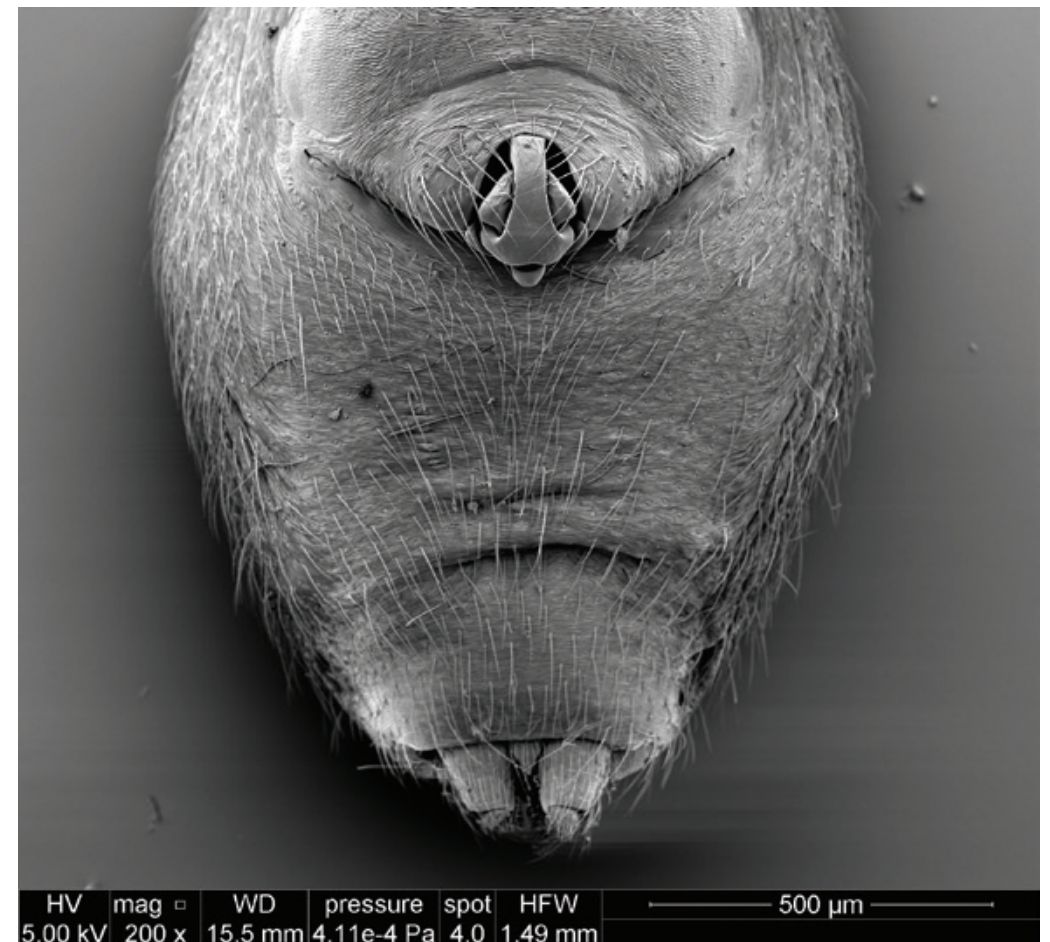


Figure 1. Epigyne (female sexual organ) and spinnerets of spider *Lepthyphantes tenuis*.

David advised me that small SEM's could be purchased for "only" £50k or so. While even this figure is a significant amount of money, it was not totally out of the reach of a single man with no family to support. If colleagues could spend their pension lump sums on sports cars or world cruises, why not spend mine on an SEM?

The next milestone was a visit to the Manchester Microscopy Congress in 2014. I spent a couple of days there, attending talks in the RMS Learning Zone, and visiting stands of SEM manufacturers as well as suppliers of pre-owned instruments. I initiated contact with the sellers of one particular desktop SEM and also chatted with the demonstrator at the Zeiss stand. On learning that my particular subject interest was arachnology he suggested that I go out into Manchester at lunch time to catch a creature, and we could then spend an hour looking at it in the afternoon. I did explain that I could not afford to buy a Gemini SEM, but this did not seem to be an issue. Thus, at 2pm, armed with a couple of squashed ants, I returned to the Zeiss stand, and within 15 minutes was viewing the eye of an ant at X750 magnification. I was surprised that the Gemini could produce an image without the specimen being sputtered with gold, or even dried. This was when I was first introduced to the Low Vacuum mode of modern SEMs. I left MMC2014 with the firm view that I would definitely be purchasing an SEM and started to follow up the leads I had been given at the exhibition.

I had initiated a dialogue with the supplier of desktop SEMs I had spoken to at mmc2014, with the idea of buying their bottom-of-the-range instrument, or an ex-demo version of something similar, when I followed up a lead provided by Quorum as to a possible supplier of pre-owned specimen preparation equipment. A phone call with Don Ashcroft of Tron Tech advised me that they should be able to help me with a Critical Point Dryer (CPD) and a sputter coater, but what, as a matter of interest, was my SEM and what subjects would I

want to view with it? There followed a conversation lasting more than half an hour in which Don Ashcroft described the various pre-owned SEMs that his company had available, including one with a low vacuum mode, and suggesting that I might like to come and view them. To cut a long story short, I viewed a number of SEMs offered by Tron-Tech, and in April 2015 I put down a hefty deposit on an FEI Inspect S50 tungsten SEM (with low vacuum mode). To be honest, I had identified this as the instrument I wanted within 15 minutes of entering their show-room.

The Laboratory

So, I had committed myself to half a tonne of SEM, with nowhere to put it. There was no way it would get through the front door of my house, even if I had room indoors for it. I had previously started to research the planning regulations for "sheds" in one's garden and discovered that fortunately, provided the erection does not exceed a certain floor area and specified height (and conforms with a number of other requirements), there is no need to apply for planning permission. I therefore drafted a specification and sought a quote from my local builder.

The table and photographs on the following pages summarise the design of my laboratory, which may be of assistance should any reader wish to follow my example and replicate this project.

The builder was instructed in early July 2015, but the laboratory was not completed until January 2016, much to the despair of Tron Tech who had volunteered to look after my SEM until they could deliver it.

Specimen Preparation Equipment

With cost in mind, I spend some time considering whether or not to buy a CPD or a sputter coater. If I can view an ant's eye in low vacuum mode without dehydration or gold sputtering, could I get away without such equipment altogether, given that my

Topic	Designed solution / consideration
Size	The floor area is 5m x 4m and the height to the top of the pitched roof is less than 4 metres.
Construction	The building is of timber construction, with dry lining on the interior surfaces, and tongued and grooved timber cladding on the exterior. It is insulated with 75 mm of sheet foam material in the walls and under the concrete floor, and with adequate rock wool in the roof space. The roof is tiled.
Doors and Windows	There are double doors at one end, wide enough to permit the SEM to pass through them. Two windows, glazed with frosted glass, provide some daylight.
Benches	There are two main benches. The tops are 900 mm front-to-back and two metres long, intended as "breakfast bars". They have a marbled appearance that hides mess, but at the same time makes it very difficult to find specimens that might have sprung out of tweezers while being transferred to a stub!
Water	Cold water is piped to the laboratory from the house. An electric water heater provides hot water. One bench is fitted with a small epoxy sink and swan neck taps. It was fortunate that the main sewer for my property passes under the site of the laboratory. A T-joint was made with this for the waste from the sink, which is used mainly for washing up coffee cups and to carry away cooling water from the CPD. Fortunately, none of the chemicals I use to prepare specimens for the SEM is particularly dangerous, unlike those for staining TEM specimens, which would need great care in disposal.
Storage	Some kitchen units provide storage for books, more light microscopes and boxes of stubs, as well as an additional work surface. A couple of desk plinths contain specimen mounting materials, stationery, tools and spares.
Electricity	Mains electricity is fed to the laboratory via a buried armoured cable from the main switchboard in my house. The main breaker is rated at 50 Amps. The power requirement of the SEM is less than 3 kVA, although it is powered via a 30 Amp socket. I have a watt-hour meter installed to monitor consumption. Three 58W fluorescent tubes provide adequate lighting, and there are several ring mains and an intruder alarm.
Air conditioning	There is a 5kW air conditioner, which doubles up as a heat pump in cold weather. This maintains the temperature of the laboratory at 20 Celsius. My SEM has a turbo pump. If an SEM uses a diffusion pump, then a water cooler will be required and this would be best installed outside the main laboratory (but protected from frost).
Vibration reduction	The floor of the laboratory is generally 150 mm thick concrete, with reinforcement. However, where the SEM column is located the concrete depth is increased to an arbitrary 600 mm. Despite the laboratory being 20 metres from a lorry depot and less than 100 metres from a railway line, the only time I have noticed vibration effects is as a result of heavy footsteps near the SEM while it was operating at high magnification.
Flooring	The concrete raft was coated with a latex screed before industrial grade vinyl flooring 2.5 mm thick was laid.
Computing etc	A desktop PC provides access to the internet and to key shared files via Dropbox. The internet connection is via a buried CAT5 cable connected to the router in my study.
Flammable liquids	Chemicals such as acetone, hexamethyldisilazane (HMDS), alcohol and solvents for mounting materials are stored in a suitable metal cabinet under one of the benches.
Gases	I use liquid CO ₂ for the CPD and Argon for the sputter coater. See below for further details on obtaining gases. These cylinders are firmly attached (by webbing straps) to uprights of a bench.
Refreshment and Entertainment	There is a kettle for tea and coffee making. A small fridge-freezer is installed for storing milk and also for maintaining acetone at -20 C (for rapid dehydration of specimens). A mini sound system provides relief from boredom during long exposures.



Figure 2. Exterior view of the laboratory



Figure 3. Wide-angle view of the interior of the laboratory

SEM has a low vacuum mode? Alternatively, could I use HMDS for dehydration, avoiding the need for a CPD, even if I did need a sputter coater? I drafted an email to the RMS for advice on drying using HMDS

vs. CPD, and within three hours I received a detailed reply from Pippa Hawes of the Pirbright Institute.

I eventually elected to purchase a pre-owned sputter coater from Tron Tech. I also purchased a

brand new Quorum CPD. Although Tron Tech had a number of used CPDs they were of an old style with a window thickness of $\frac{1}{2}$ ", compared to the 1" of the more modern version, and these were no longer regarded as safe.

Obtaining Gases

I approached BOC with a view to purchasing CO₂ and Argon for my preparation equipment, and possibly liquid nitrogen should I ever wish to go down the route of cryo-preparation, or alternatively EDX. I received a quote, asking me to provide a company name and VAT number, on receipt of which the gases could be delivered. When I stated that I was a private individual, I was told that

they would not deliver gases to people such as me, although I could collect them from a supplier. Under no circumstances would they supply liquid nitrogen, in case I used it for dangerous party tricks. —

Fortunately, I have a friendly supplier only 5 miles away, so I am able to collect cylinders of CO₂ and Argon when required, saving a hefty delivery charge. (There are guidelines for the transport of gas cylinders in motor vehicles, of which one should be aware). It should be pointed out that when procuring gases there is a separate monthly charge for the hire of the cylinders. This charge significantly exceeds the cost of the gas itself, at least at the rate that I use it.

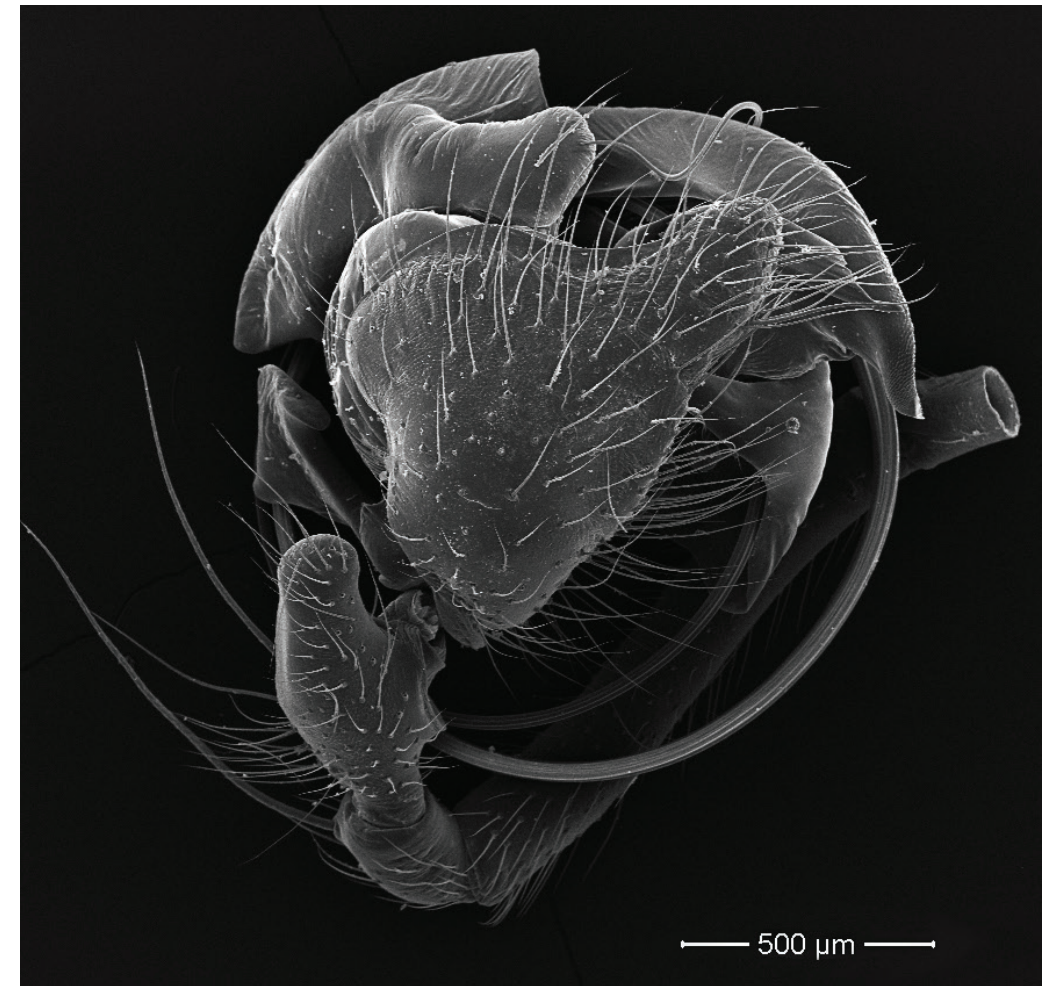


Figure 4. Pedipalp (male sexual organ) of spider *Labulla thoracica*. This spider is comical to look at, as its pedipalps are nearly a quarter the size of its body

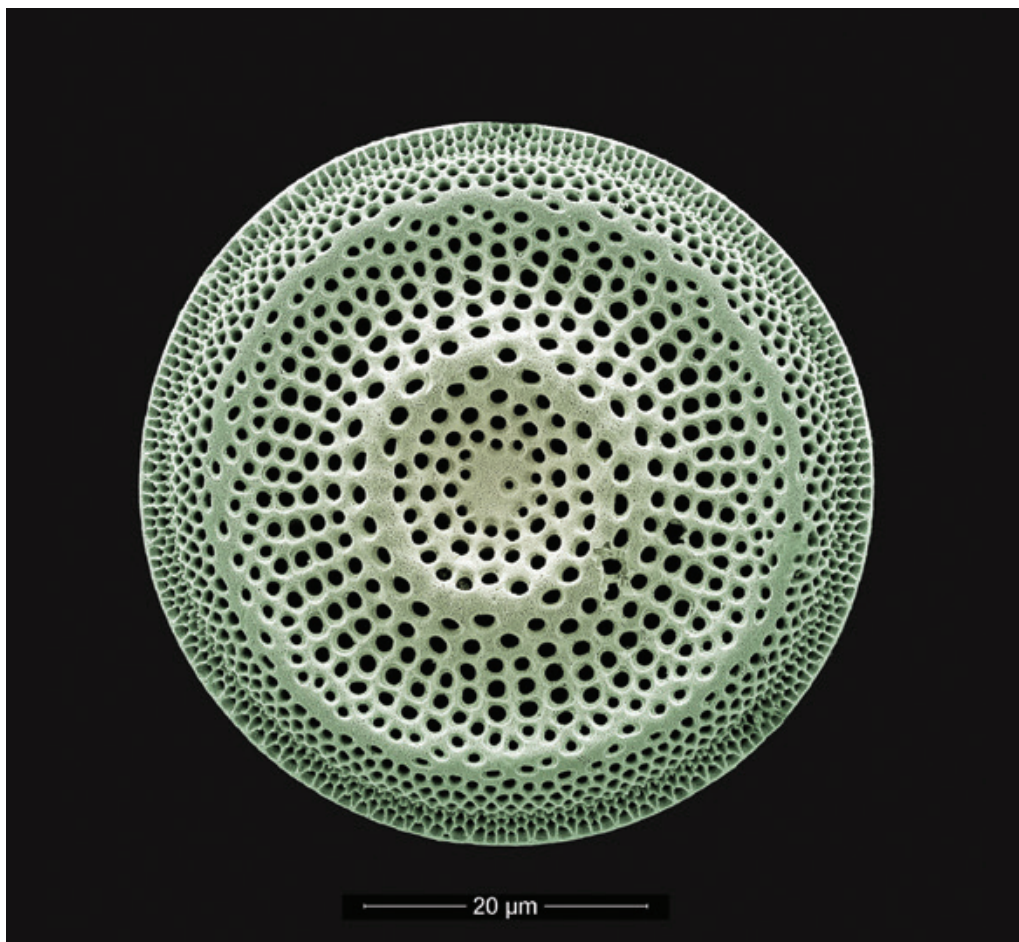


Figure 5. A marine diatom, with simple artificial colouring.

I followed up the liquid nitrogen option with BOC and finally had a very friendly chat with the man responsible for deliveries in my area. He did undertake to provide this if and when I eventually need some. He was also able to give useful advice on storage and ventilation. The secret to receiving good service from BOC is to keep in touch with their "Customer Care Department".

Another company that might be able to help is "Air Products".

Operating as an "Individual"

I mentioned that BOC will not deliver gases to individuals. I have also encountered difficulty with obtaining supplies from other companies. I even found it difficult to register ownership of my SEM with FEI (now Thermo Fisher) because their

registration internet page does not accept hotmail addresses! I contacted FEI for a quotation for some O-rings. When I tried to order these, however, I came up against the problem that I did not have a VAT number. Another company who have refused to supply is Merck Millipore.

Sometimes this can work to one's advantage. I required a new seal for my CPD and contacted Quorum. They decided they would supply that free of charge, as it was easier than setting up an account. Sadly they did not take such a generous view when I came to purchase a sputter coater from them, although I was able to make the purchase!

I am happy to report that I have had no such problems in dealing with suppliers such as Agar Scientific, TAAB, EMR or Labtech, all of whom accept credit card payments.

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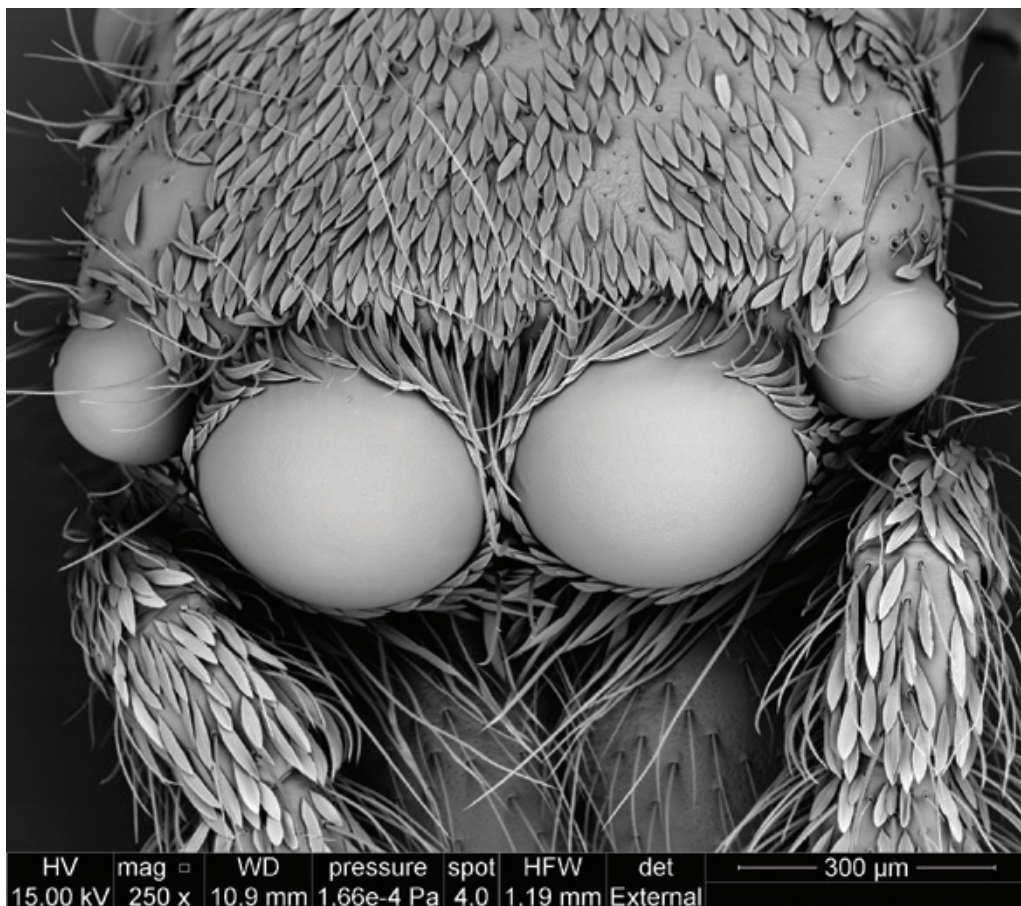


Figure 6. Head of the spider *Salticus scenicus*, imaged using the Deben backscattered electron detector. This micrograph was reproduced on the July page of the 2018 RMS Calendar.

Installation and Use

The SEM was delivered on the 27th January 2016 and commissioned by mid-day the following day. Since I had never used an SEM before, there was a massive learning curve to follow before becoming relatively proficient. This process was greatly helped by a number of individuals:

I received basic training on the operation of the SEM from Don Ashcroft prior to delivery, and again during the commissioning process; Don also arranged for a further day of training from an EM expert from a local university; I attended the RMS Electron Microscopy Summer School at Leeds University; David Spears was also able to provide advice from time to time. I have had occasional maintenance visits from Don Ashcroft which have

always included lively debates on the best settings to use for various specimens. Also, key members of the RMS, including Dick Paden, Pippa Hawes and Kim Findlay have been very supportive of my project.

My main reason for acquiring the SEM was to obtain micrographs of defining features of as many varieties of British spider as I could, and to gather these together in some form of database or publication. I have made a start, but have a long way to go. In fact, my choice of subject matter so far has been somewhat random and opportunistic. It is time to define this project in more detail now that I have plenty of experience with the basics of using the SEM. Obviously I have pursued non-arachnological subjects as well, including insects, pollen and marine life, to mention but three. A selection of my

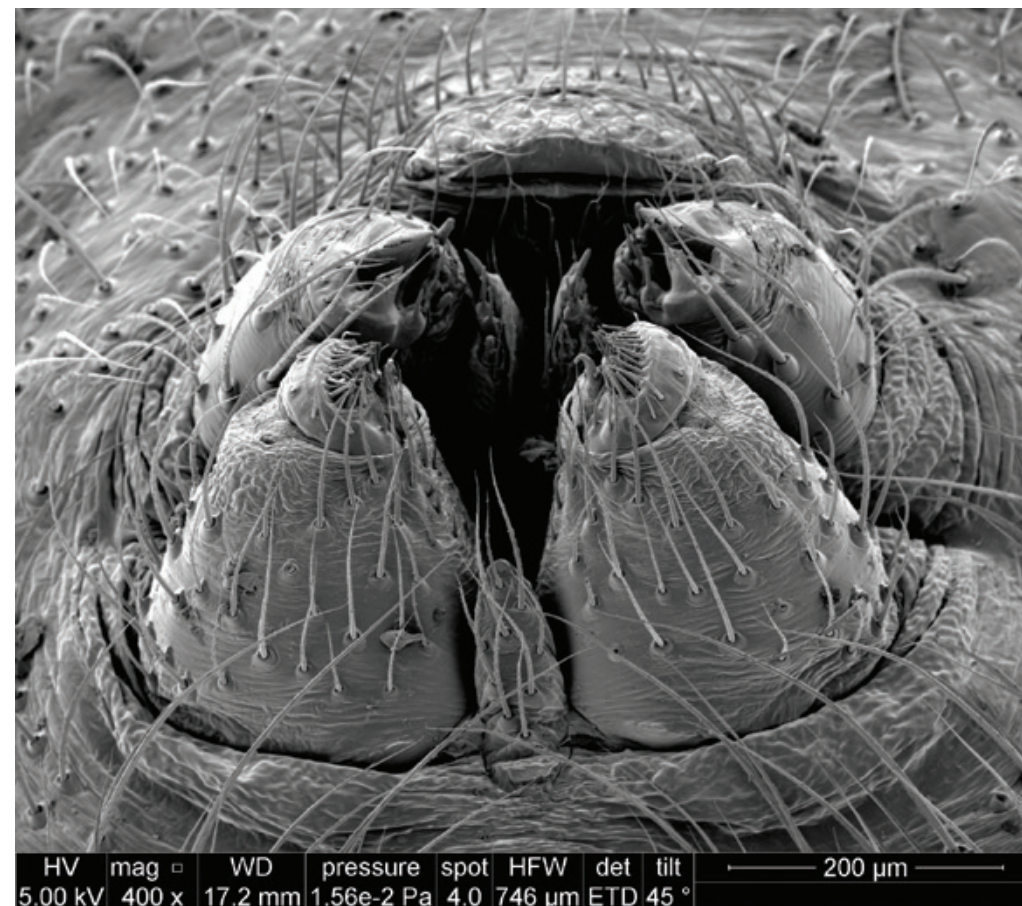


Figure 7. Spinnerets of spider *Steatoda bipunctata*.

electron micrographs is reproduced on these pages. I must be doing something right, as my micrograph of the head of *Salticus scenicus* was selected for the 2018 calendar of the RMS.

Remembering my first impressions on seeing an SEM image I would be very happy to demonstrate the instrument to sixth formers (or even younger students). I have made offers to all the secondary schools in my town, but have not yet received a request for a visit. In some ways this is disappointing, but on the other hand, it saves me the work of preparing a structured demonstration. At the other end of the age range, I have lectured on electron microscopy to my local University of the Third Age science group and also demonstrated my instrument to them, both of which events were very well received.

Despite attending the RMS course on “Enhancing your Image”, which deals with colouring electron micrographs, I have so far made little progress in this area, although I have had a fair amount of practice in making selections in Photoshop® in order to black out distracting backgrounds.

Since 2015 I have been contributing a column to Balsam Post (the newsletter of the Postal Microscopical Society). This series is called “SEM Diaries”, and it recounts progress with the project, and its successes and disasters. These diaries are available on my website, www.jeremypoolesem.org.uk, which also includes some galleries of photomicrographs.

Following publication of the first instalment of SEM Diaries I received an enquiry from a retired

academic, who was “missing” the SEM he used at his university. He was asking for an estimate of costs, and whether it was likely he could install one in his own house. It seems I am not the only individual to have had cravings for his own SEM!

Obtaining a Pre-Owned SEM

I have already mentioned that I obtained my SEM from Tron Tech (www.tron-tech.co.uk), with whom I continue to have a good relationship. In the interests of balance I should mention that there are other companies in the UK that deal in pre-owned equipment. The two known to me are ISS in Manchester (<https://iss-group.co.uk>), and EM Systems Support of Darlington (www.emsys.co.uk).

SEMs are occasionally available on eBay. Purchasing this way seems reckless to me, although if you employ a service company to vet the equipment, and/or undertake to service it on purchase, that might be cost-effective.

Often, when an SEM is replaced, the previous instrument is simply scrapped. It might be possible to obtain agreement to divert such an instrument from a scrap heap to one's home, especially if you work at the establishment where the upgrade is taking place. Manufacturers do, however, prefer to remove used instruments from the market, to encourage customers to purchase new!

Costs

I budgeted £100k for the project and the final price came in at approximately £102k including VAT. This included the SEM, building, preparation equipment, and the laying of services. I could have saved money by carrying out some of the work myself, but preferred to leave all the management and risk to the builder. Anyone wishing to have more detail of the cost breakdown, or any other aspect of the project, is welcome to contact me on jeremypool20@hotmail.com.

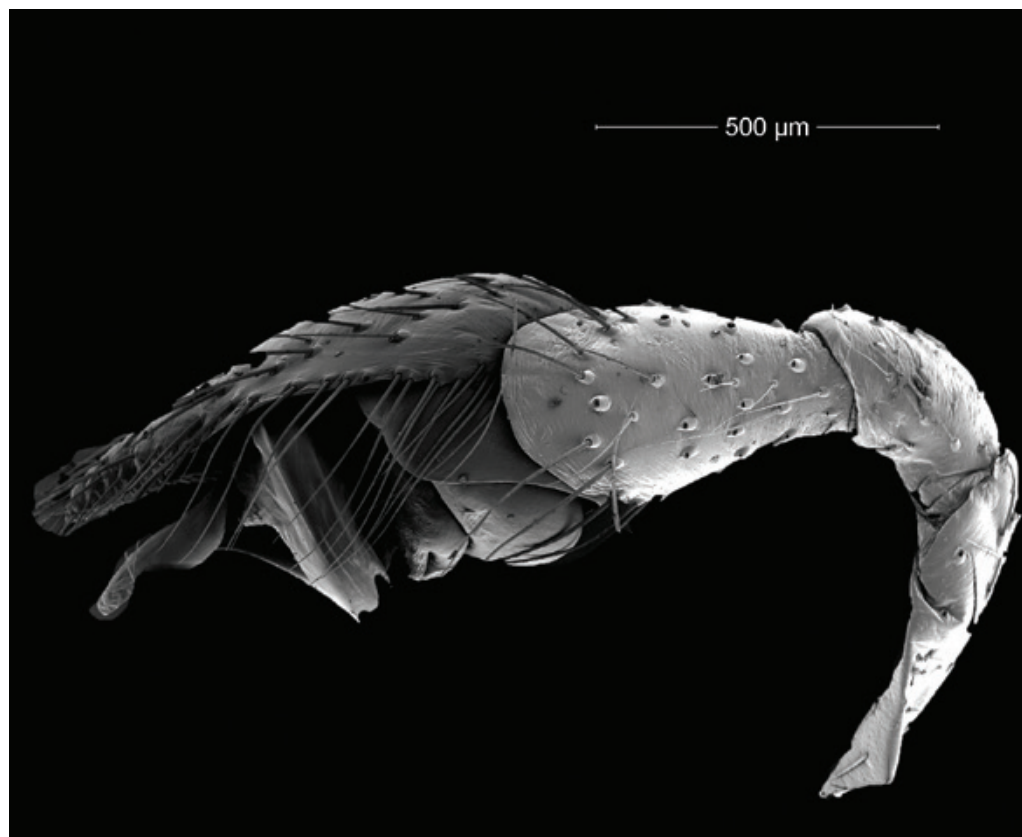


Figure 8. Pedipalp (male sexual organ) of spider *Steatoda grossa* imaged in Low Vacuum mode.

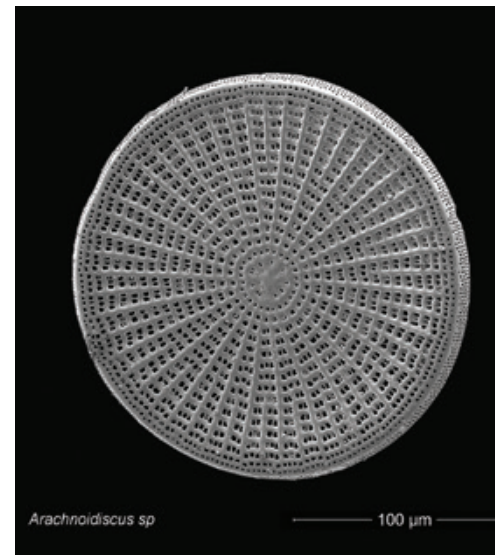


Figure 9. A marine diatom, *Arachnoidiscus sp.*

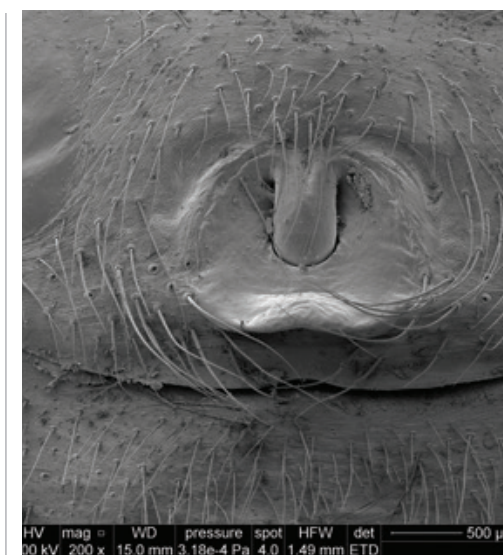


Figure 10. Epigyne (female sexual organ) of the false widow spider, *Steatoda nobilis*.

Not included in the above figure are a Deben Gen5 backscattered electron detector and a replacement Quorum sputter coater, both purchased more recently.

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About the Author

Jeremy Poole studied engineering at the University of Cambridge and later obtained a PhD in acoustics from London University. For most of his working life he worked on naval sonar systems in a variety of roles. His hobbies include microscopy, photography and arachnology, and he is a member of the Quekett Microscopical Club, the Postal Microscopical Society and the British Arachnological Society. Now retired, when not on safari photographing wildlife, he spends much of his time imaging the sexual organs and other key features of spiders on his SEM.

