SEM Diaries - 10

Butterfly Scales and Diatoms

Jeremy Poole

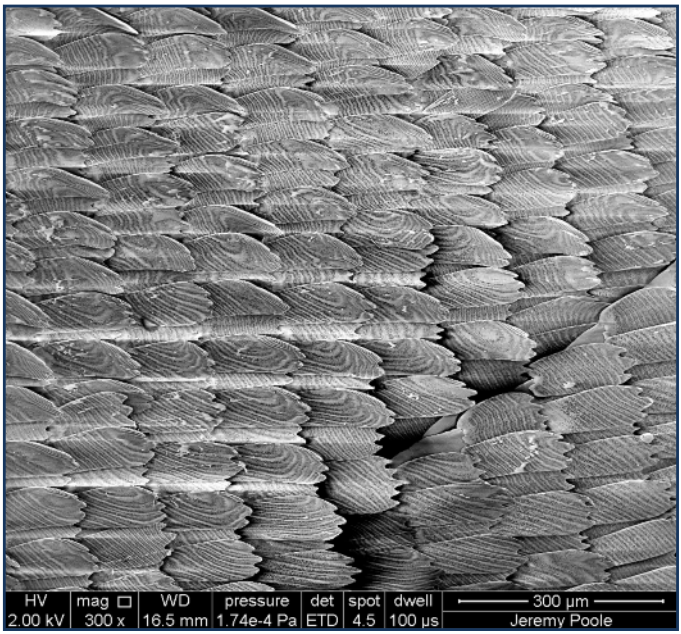
A butterfly wing that I have had in my collection of samples for a number of years

Many years ago I acquired some butterfly wings. I think they came from NBS. Apart from using a few fragments to make balsam mounts of scales, (long since consigned to the bin) these have remained stored in a polythene bag ever since I acquired them.

Now, many of you will know that the colouration of the wings of some butterflies arises from a physical diffraction effect rather than pigmentation. The same "structural colouration" appears on peacock and humming bird feathers and other creatures. I decided to investigate the wing material I have in my possession, and at the same time explore the high resolution capability of my SEM.

Having looked into the mechanism of colour production (basically an optical interference effect similar to that seen using light and a diffraction grating) I have decided not to attempt to explain it here. In fact, I am not even dead sure that the sample I have been using is from a morpho butterfly (the type showing this colouration effect) or that I am inspecting the top or bottom surface. Thus I shall provide you with the micrographs and rely on you, the readers, to inform me!

The micrograph shown top-left, on the following page, although a very unsatisfac-

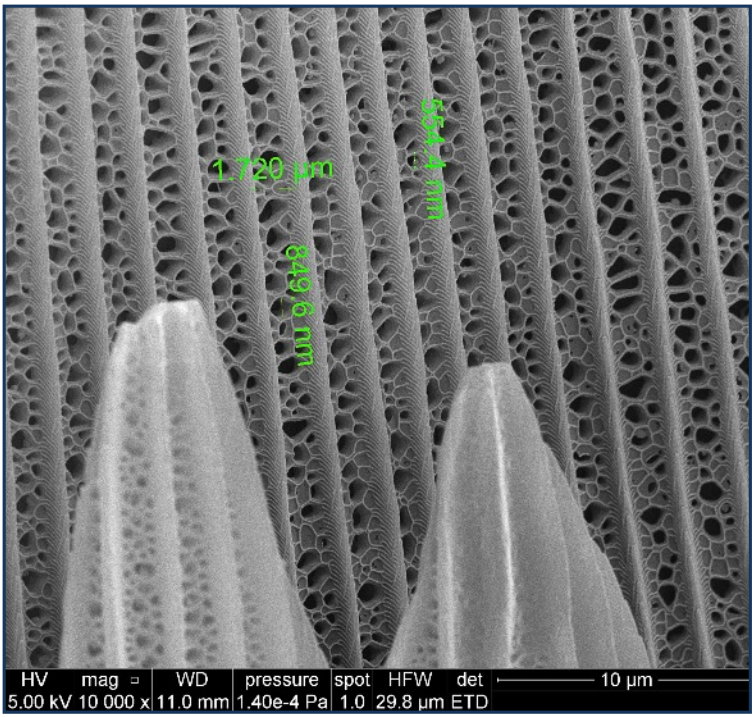


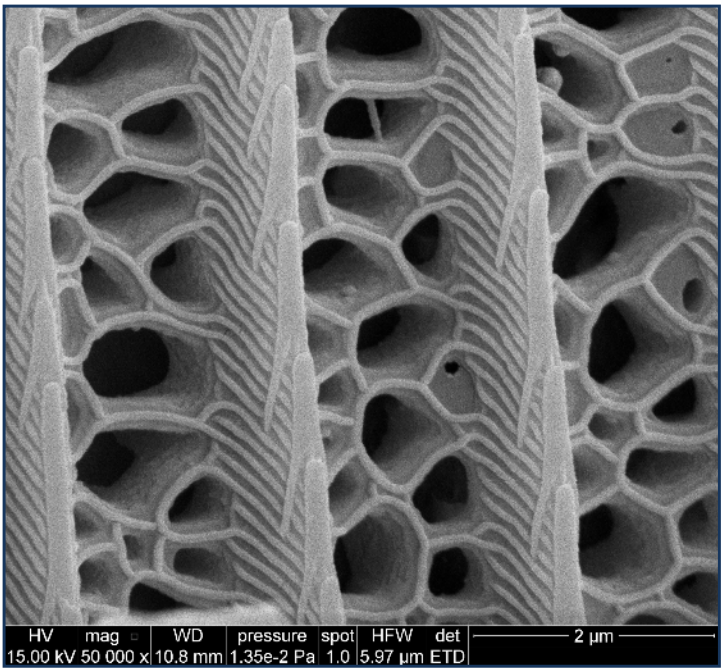
This shows some interesting structural details. The specimen is rotated through -90° compared to the previous micrograph, so the "spikes" at the bottom are the "trailing edges" of scales. These lie on a ribbed structure (of the butterfly scale beneath it) separated by what look like cells (i.e compartments). The ribs themselves have striations on them. All these features can be seen more clearly on the following micrographs.

Butterfly wing captured at magnifications of X300 left, and x10,000 below

tory image, illustrates well an incidental effect of imaging, which is the generation of "Moiré patterns". This is not the same interference process as that producing colouration, as the geometry is on a different scale. The magnification setting of the SEM was x300, but this means little unless the image is scaled so that the diagonal of the image, including the data bar, is 41 cm.

The lower image is captured with a magnification setting of x10,000.





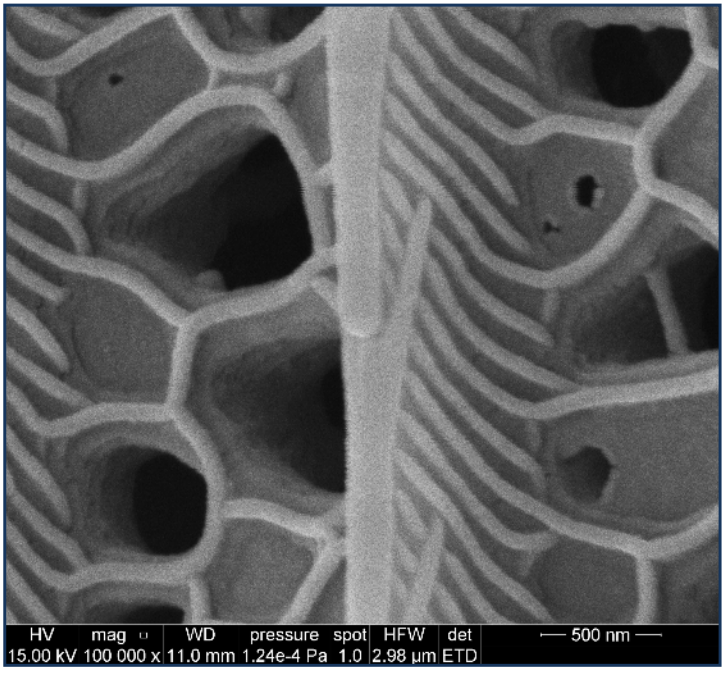
same clarity. My guess is that as I had been blasting the specimen with a beam energy of 15keV, I was almost certainly causing damage to the specimen, the results of which might have polluted the chamber, and in particular the final aperture.

Butterfly scale captured at magnifications of X50,000 left, and x100,000 below

The dimensions of these features, read across from the illustration on the previous page are:

Large cell diameter is 850 nm, smaller cells around 550 nm and spacing of ridges approx. 1.7 µm. The spacing of the close striations on the ridges is around 80-110 nm.

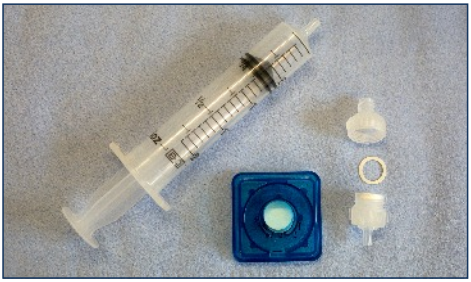
You may wonder why I did not include the dimensions on the micrographs with the higher magnification. The plain reason is that I forgot at the time! I did go back and attempt to re-image at x50,000 but I could not get the



At the Langton Matravers joint PMS/QMC spring meeting, Klaus Kemp kindly provided me with a small quantity of cleaned marine diatoms for me to attempt to image in my SEM. The material was supplied in liquid suspension in a small tube, so the challenge was to find a way to go from a liquid suspension to material that was dry, secure (not likely to blow away) and sputtered in gold, suitable for imaging in the SEM. I had previously asked Dave Spears how he imaged specimens such as blood, and the reply was to filter a suspension of the material through very fine filter paper (under pressure) and then put the filter paper through a drying process, such as with the critical point drier. Dave also made it clear that the material needed to be very dilute if the cells were not to appear as a large heap.

I therefore ordered 100 off 13 mm diameter filter papers, together with special holders permitting the suspension to be pumped through the filter paper under pressure, using a syringe (also purchased). Like most consumables associated with electron microscopy, these items were ridiculously expensive, with the papers costing about £150 per hundred and the filter holders around £12 each. The syringes were about £100 for 12.

In accordance with instructions (I thought) I diluted a quantity of the diatom solution and put the diluted contents into a syringe. I installed a filter paper in the holder, connected the syringe to the holder and pushed. The net result, observed under a stereo microscope, was piles of diatoms, mainly round the edge of the filter paper, in concentrations much too high to isolate single examples. Then the penny dropped - there is no point in diluting a sample if you are then going to press the whole volume of the diluent through the same filter paper. However much additional liquid you use, the same quantity of diatoms will be deposited on the filter paper! With some experimentation I came up with the seeds of a technique that would permit at least some diatoms to settle as individual exhibits, without being heaped upon, or under, other diatoms.

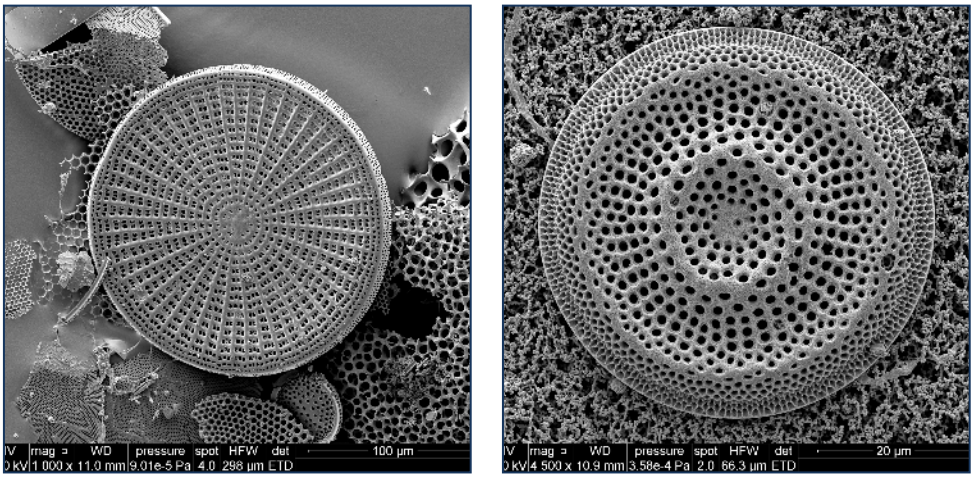


Merk Millipore filter system: Syringe, box of 13mm filters, Swinex filter holder with gasket

Meanwhile, I had determined that the filter papers I had bought were not suitable for use with acetone (which I use as a drying medium in conjunction with the critical point dryer). Well, I thought, exactly how unsuitable would they be? I sacrificed a filter paper by immersing it in a watch-glass containing acetone, and it dissolved before my eyes! (The material of the filter, which has a pore size of 0.45 µm, is cellulose nitrate with cellulose acetate.) I repeated the test (with a new paper) with iso-propyl aclohol (IPA) and determined that this solvent did not dissolve the "paper" in the same way. Fortunately, the silicaceous shells of diatoms are rigid and robust and would survive air drying, so I put a few papers of filtered diatoms in my oven.

I next stuck the dried filter papers directly onto sticky carbon discs on aluminium stubs, sputtered them with gold as normal, and put them on the stage in the chamber of my SEM.

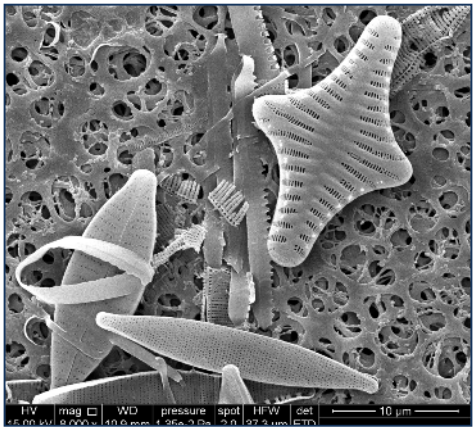
I have previously said that one of the things that makes scanning electron micrographs so appealing is the "wow factor" that one feels when observing them, especially if the 3-dimensional effect is very apparent. So, having navigated to various likely-looking specimens on my stage I was absolutely delighted to obtain



Marine Diatoms. Left: *Arachnoidiscus sp*. diameter approx. 180µm. Right: Species so far unidentified, diameter approx. 50µm. Note the texture of the filter in the background

some great images, two of which are reproduced above, and some more will be appearing on my SEM website in due course.

On return from the microscopists' long weekend at the Cranedale Centre in Yorkshire, armed with a phial of cleaned diatoms from the Gipsey Race, I felt confident that I would be able to produce some great images of that highly rich material, and so it proved to be. Well, not really. I would describe the resulting images as acceptable, but by no means excellent. I alluded to the fact that I was unable to repeat the high magnification images of butterfly scales to the same quality as I had previously obtained, and that I attributed this to contamination of apertures or some other part of the signal chain. At the time of writing this problem has not been resolved. A service visit has been booked! Hopefully, following that visit, resolution and hence sharpness will be much improved.



Diatoms from the Gipsey Race

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