Sectioning: some ideas that might help

Some years ago I watched David Hill cut sections of a lichen apothecium, I think it was a *Lecidella*. With his rock-steady hand he produced a series of sections as if he was slicing Parma ham in a delicatessen, every slice thin and perfect. It was an uplifting sight but I must admit I felt slightly despondent. Could I ever hope to match this standard? Probably not, I'm afraid, with my not so steady hand, but I hope the following observations may help some of you who like me struggle to produce useful sections.

Of course the first factor is practice. Sectioning lichen thalli and apothecia is a skill and like any skill improves with repetition. For many years my work and family kept me too busy to do much lichenology but since my retirement a couple of years ago I have spent many more hours at the microscope and my skills have improved. But I know that I will never attain the level of skill demonstrated by those who spend their lives working on lichens. I have, however, spent some time working on a couple of techniques that may help.

The first tip, and this is not original (I believe bryologists do this for cutting leaf sections), is to use a straight edge for cutting against. You could use the short end of a steel ruler or any other straight edge. I have found that the short side of a microscope slide works well for me and it's easier if the edge is bevelled. Bevel the edge on a fine carborundum stone. Don't use a coarse stone as the edge will fracture irregularly and don't grind away too much. You must leave part of the original edge (about a third) as the cutting guide. The advantage of a glass slide over a metal guide is that you can see through the glass and this helps with positioning it.

The bevelled slide is particularly useful for cutting sections of foliose lichen thalli. You could of course use the traditional method of inserting a piece of the thallus into a slit in a piece of tissue such as a sliver of carrot and then slicing the carrot. However, I have obtained excellent results with my glass slide. Moisten the item to be sectioned and place it on a microscope slide. Place your cutting edge on top and cut away the first piece to get a clean edge. Then slightly nudge your straight edge, sometimes just relaxing the pressure is enough, and slice again. Repeat until you have a number of sections. Under the dissecting microscope remove the sections that are clearly too thick, add a drop of water if necessary and the cover slip.

I make no claims of originality in suggesting the use of a straight edge for cutting but my second technique is, I believe, my own invention. A year ago, I was attempting to cut sections of perithecia of *Phylloblastia inexpectata*, quite a common lichen in Essex on leaves of *Prunus laurocerasus*. I found that however sharp my blade it dragged the perithecium along the shiny leaf surface instead of cutting into it. I began to experiment with adhesives to fix the perithecia in position and this led to my discovery of Gum Arabic and a potentially useful new technique.

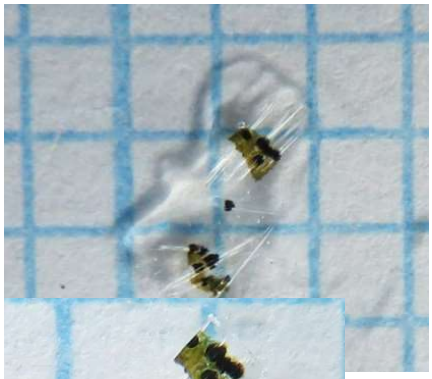
Gum Arabic is a naturally occurring gum, exuded by at least two African species of *Acacia* and one of the exports of Ethiopia. It is a safe product, in fact you will have eaten it as it is a constituent of foods such as ice cream. It is sold in artists' supplies shops in small bottles, for use by watercolour painters. I have tried it from two sources. The first supplier was the up-market Cornelissons in London and I found this gum was fine to use as supplied. My other supplier was the well known firm of Winsor & Newton and their gum was far too runny. For this technique the gum needs to have the consistency of Golden Syrup. I poured the contents of my 75ml Winsor & Newton bottle into a beaker which I covered with a clean handkerchief to keep out dust. After about three days in my boiler cupboard the volume had reduced to about 30ml and the viscosity was perfect.

This technique works well for sectioning small things and anything that you can't manage to hold firmly in position. I have used it for sectioning pyrenocarps and for cutting sections of lobes of tiny *Leptogium* species. You could use it for tiny globose apothecia which become detatched from their thallus as soon as you touch them. My friend Pat Cavanagh obtained excellent longitudinal sections of a *Pseudephebe* species the very first time she tried this method.

The first step is to put a small blob of Gum Arabic onto a microscope slide. I find a small watercolour brush is useful here. Then place your specimen, perhaps a cluster of perithecia or a tiny lobe, in the gum, submerging it with the brush or a mounted needle and orientating it into the best position for cutting sections. Try to keep the blob of gum as small as possible; you will find the right amount with a little experience.

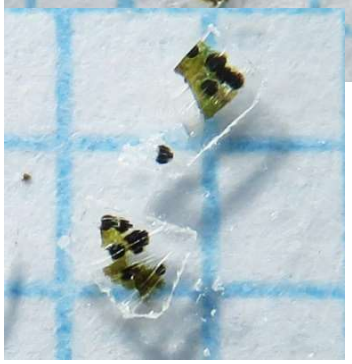
Fig.1: A cluster of perithecia of Porina aenea in Gum Arabic. 2mm square graph paper behind.

Then leave the gum to solidify as the water in it evaporates. You need to leave it until it becomes like a soft plastic in texture. If you are impatient and try to cut into it too soon the gum will stick to the blade. If you leave it too long, say overnight, the preparation can become too dry and rather brittle, in which case breathe on it a few times to soften the gum.



Under the dissecting microscope, using your blade, with or without a guiding straight edge, cut your sections. You will find the plastic texture of the gum gives excellent support. Be sure to cut right through your specimen. If you don't you will later find your sections still joined at the base, resembling a miniature book. One rather good result of immersion in the gum is that it renders the specimen slightly translucent and you can easily see the ostiole of a perithecium for instance.

Fig.2: The perithecia, now set in the hardened Gum Arabic, have been sectioned.



At this stage I cut off any excess gum and discard it, leaving just the areas around the sectioned specimens. Then add a drop of water and wait for the glue to soften and diffuse into it.

Fig.3: Excess Gum Arabic has been cut away and discarded.

Now is the time to have a cup of tea. Resist trying to separate the sections too soon or you will damage them, just let things happen naturally. Leave it at least ten minutes and then you can gently stir your preparation with a mounted needle. This separates the sections and after removal of any chunky pieces the cover slip can be placed.

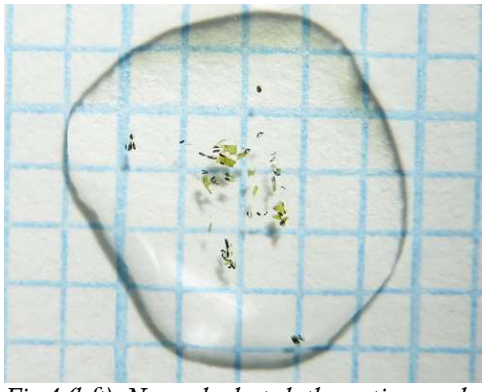


Fig 4 (left): Now rehydrated, the sections and other separated.

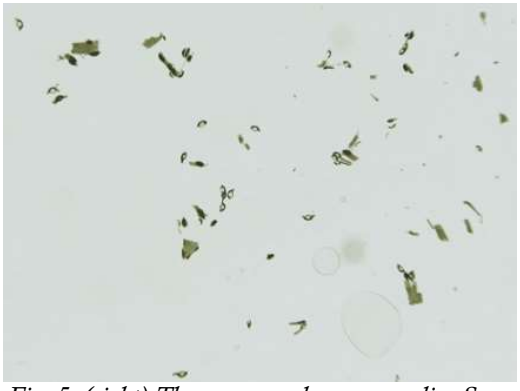


Fig. 5: (right) The same, under a cover slip. Several debris have useful sections are visible.

Gum Arabic is a complex mixture of oligosaccharides, polysaccharides and glycoproteins. The actual chemicals present and their concentrations varies considerably as the gum is collected from

the wild from at least two species of tree. I was concerned that the presence of the gum might affect chemical reactions under the microscope but my crude experiments suggest that there is nothing to worry about when undertaking normal identification work.

Mixing Gum Arabic with bleach solution and with dilute KOH produces no visible reaction. Lugol's Iodine, however, is decolourised. Not being a chemist I can report the observation but not explain it. I tried a couple of tests to see if easily observable reactions were affected when the specimens were mounted in Gum Arabic. I looked at the reaction of granules of *Placynthiella dasaea* with bleach and the reaction of a thallus squash of *Flavoparmelia soredians* with KOH. In both cases the expected reactions were observed. I also cut sections of apothecia of *Lecania cyrtella* using the method outlined above and followed this with treatment with KOH and then Lugol's Iodine, obtaining satisfactorily stained asci. The presence of the gum does slow down the spread of reagents across the slide as it forms an invisible pool of higher viscocity and it makes things easier if you draw off some of the gum with a slip of filter paper, irrigating with water from the opposite side. It also helps if you have kept the amount of gum used as small as possible. My photographs for demonstration purposes show rather more gum than I would normally use.

In conclusion, I would encourage you to purchase a small amount of Gum Arabic and experiment. A small amount will last a lifetime.

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