

Microscope techniques

Cutting thin sections of bryophyte material

Ken Adams

School of Health and Biosciences, University of East London, Romford Road, Stratford, London, E15 4LZ; ken.adams@virgin.net

Having now sent out several hundred copies of the 1999 update of *Microscope Techniques Part 1* (and had many enquires about the as yet non-existent Part 2!), guilt is beginning to seriously impinge on your librarian's conscience, and despite the fact that there are probably dozens of people who could make a better job of it, he is now seriously contemplating a Part 2.

This would obviously be most valuable to budding bryologists if it consisted of a distillation of the various techniques that bryologists have discovered or invented over the years. It is a pretty frightening prospect when one realises, for example, that many of the techniques used by commercial companies for producing permanent stained sections of delicate plant material were trade secrets, and there are probably precious few, if any, individuals now alive with the skill to reproduce some of them. To kick off the debate in the hope of extracting a plethora of techniques from living bryologists, I describe here a modification of a technique for cutting sections of bryophyte material that I have found useful.

For the budding bryologist, one of the most daunting and frustrating techniques required of them for identification of their quarry, is to cut ultra-thin sections of leaves, stems or thalli. I well remember back in the 1970s, the late Bernard Ward trying to cut sections of *Polytrichum* leaves using a freshly sharpened kitchen knife! Tony Smith, in the 2nd edition of his *Moss flora of Britain and Ireland*, describes and

illustrates a technique attributed to Sean Edwards, involving two microscope slides and half a double-edged razor blade, that involves tilting the blade at an increasingly shallow angle from the vertical, to produce a series of thin sections. Two problems arise with the technique as described. Firstly, double-edged razor blades are not that easy to come by these days (a similar problem has arisen with Mark Hill's 'indelible pencil' for staining *Sphagnum* leaves), and single-edged blades, although still available, are really too thick to control the sectioning adequately. The second problem is that the angle of tilt ensures that none of the sections are truly vertical slices.

The technique can be refined as follows. Throw-away twin-bladed razors are currently in plentiful supply¹. By carefully prising apart the plastic mounts, two very thin, but easily hand-held ultra-sharp blades can be recovered that are ideal for section cutting. In fact I find them easier to hold and use than half a double-edged razor blade. They are slightly thinner than the old double-edged blades, but still stiff enough to use unsupported. Now for the second refinement. This requires the use of a dissecting microscope (as also recommended by Tony Smith). First of all, mount a clean slide under the centre of the low-power field at *ca* 45° to the left-right plane

¹ The razors I currently use are manufactured by Wilkinson Sword. Ideally, if we could obtain several thousand of the blades from the manufacturer before they are mounted we could stock them as items for purchase by members – how much interest would there be?

(near left to away right). Now place a large drop of water on the slide, and then mount your selection of leaves or other bryophyte material in a line across the middle of the slide for sectioning. Finally, add the second slide at right angles as per Sean's technique, carefully trapping the leaves at the level at which you need the sections, and most importantly also trapping a film of water between the slides. Then, if you are right-handed use the second (longest) finger of your left hand (not your thumb as in Sean's technique) to hold the upper slide down tightly onto the leaves, and using a blade in the right hand, slice away the unwanted projecting leaf segments with a gentle (so as not to blunt the blade) horizontal slice. Hold the blade vertically, but with the cutting edge tilted at *ca* 30° towards your right hand, and slide it along the edge of the top slide, gripping the blade between the thumb and second finger, and – doing something you can't do easily with half a double-edged blade – resting your index finger along the top edge to provide extra control. Now comes the innovative bit. Because you have a film of water trapped between the slides, if you flex your left second finger slightly, you can retract the upper slide (observing all the time under the microscope) and expose new tiny projecting segments of the leaves you wish to slice. Keeping your blade in the vertical plane, but with the sharp edge inclined towards your right hand at *ca* 30° as before, you can now slice a beautifully thin section with vertical, parallel sides. With a bit of practice, it is fairly easy to retract the upper slide on its film of water, a potential slice at a time, and go on cutting ultra-thin vertical slices.

Once you have mastered this technique, you might like to try a further refinement that Tim Pyner says he prefers. Instead of using the top slide, use a coverglass. It's a bit more tricky but well worth mastering. Hold the coverglass in place with your left forefinger, with your left thumb held sideways (nail facing right) pressing down on the slide underneath. The thinness of the glass has the advantage of making it easier to

see the projecting leaf segments that you are about to cut, since in the case of a slide, the edge of the glass gives you a double image. It's also easier to trap a film of water under it. Furthermore, if you clean away unwanted bits of leaf you can lift the forward edge of the coverglass slightly with your blade, and pull it forward over the sections all in one operation, the water under it being dragged along with it and serving to suspend the sections.

So have a go! – but try not to overload our Moss Recorder with new vice-county records for *Syntrichia virescens* and *Campylopus* species from all those packets you have been meaning to have a go at, in the 'to be done' shoe box.

The above technique will produce high-quality slices for making drawings or micrographs, but it is also possible to produce sections suitable for identification by simply using your thumb and forefinger. Gripping a bunch of leaves or a shoot between thumb and forefinger, with the soft side of the thumb slightly higher, place a drop of water on the leaves and slice away against your thumb. If you are careful, you can cut a collection of slices, which stick in a film of water at the bottom of the blade, without too much damage to your thumb! On dispersing the sections in a drop of water on a slide, you may find, however, that they are not quite thin enough and insist on lying flat instead of edge on. Don't despair! Pick up the best section with a pair of forceps, and mount it on the edge of a microscope slide in a film of water, as close as possible to the upper face. Now despite the fact that is lying flat on the edge of the slide, if you look at it under the microscope you will see it edge on. 'Cheating' like this is usually sufficient to enable you to observe the cell structure of a costa, and whether a margin is bistratose.

Finally a challenge. Does anyone know how to fix, dehydrate, stain and mount in permanent cement on a slide, a shoot of *Fissidens bryoides* without it looking like a wet rag!