Making permanent slides of delicate liverworts

David Copestake shares decades of work unlocking the secrets of slide making

It is very difficult to make good stained mounts of the more delicate liverworts in a permanent resin mountant such as Canada balsam. Here is a method which although being quite complicated, is guaranteed to give good and beautiful results with the most delicate of small species. I have developed this in the past few years from odd methods and references in various old books.

First of all collect your specimens and wash loosely, cutting off small clumps and examining under the stereo microscope. Cut off a few of the best shoots and place in another small dish of water, then wash carefully with two small acrylic paint brushes (nos 3 & 4 are useful) and remove all the mud and debris you can. Place in fresh water and move around gently.

Fixing

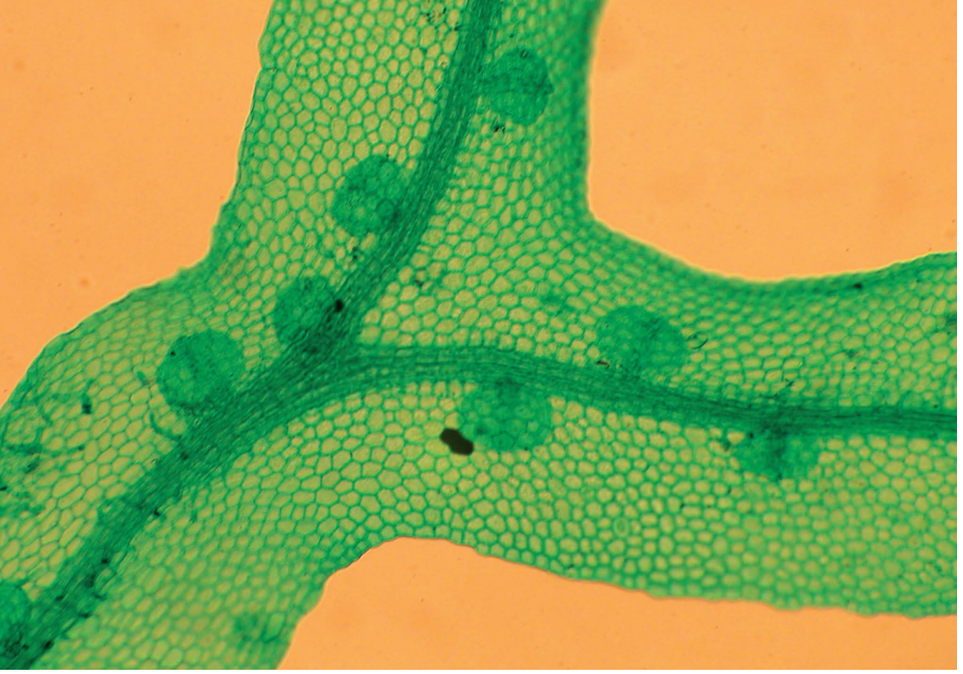
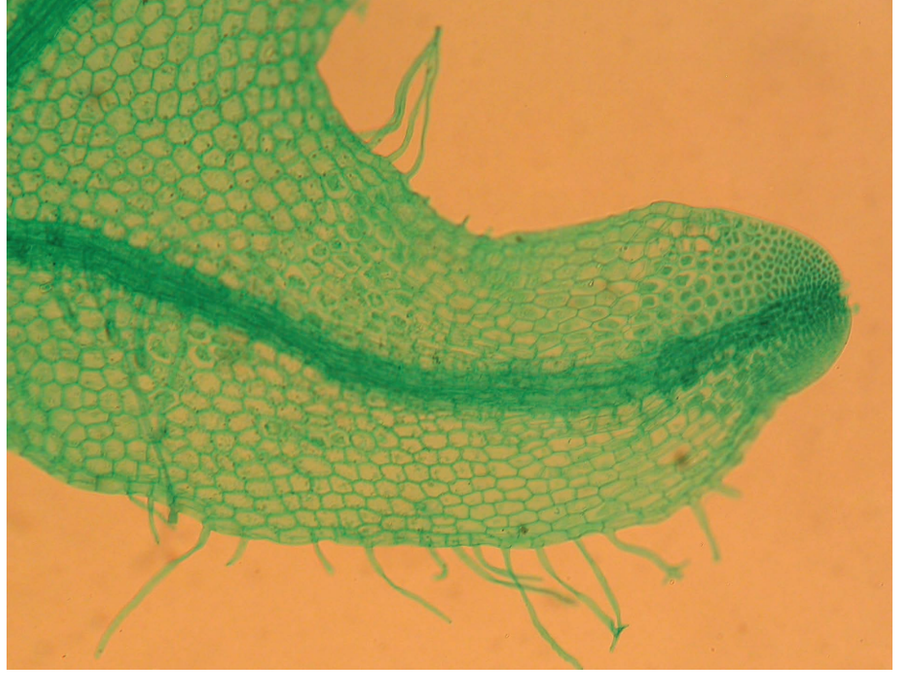
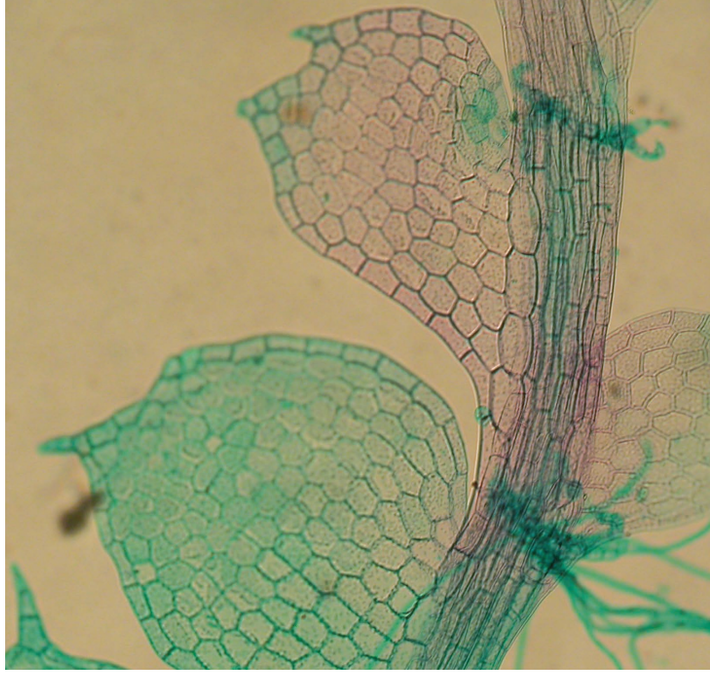
It is best to use Chrome-acetic fixative of the medium strength. Place the specimens in it for two or three days and use the solution only once. The formula is:-

10% aqueous chromic anhydride	7 ml
10% aqueous acetic acid	10 ml
distilled water to	100 ml

Always fix more material than you need, for you cannot tell which pieces are best until after staining.

Washing

Wash very thoroughly or the specimens will not stain and differentiate properly. 24 hours in running water is recommended but I find that half a day in running water and half in still is sufficient. I place the specimens in a nylon or stainless steel tea strainer resting on the top of a 250 ml pyrex beaker under a tap which is giving just a very small flow. If you control the flow carefully, the specimens will not wash over the top. For the tiniest specimens, place them in a length of 18 mm test tube open at both ends with fine silk cloth tied over one end with cotton. These are placed in a Buchner funnel supported in a suitable glass beaker, and the water allowed to run into it from above keeping the level about half way up the tubes. These tubes can be moved from one stage of the process to another.

△Examples of liverwort slides prepared using this method. Left, *Metzgeria*. Right, *Cephalozia*.

Clearing in Chloral Hydrate

Suspend the tea strainer over a small basin or container containing a saturated aqueous solution of Chloral hydrate and leave for 30 mins or until the specimens appear clear. (I utilise this procedure as I have the chemical, but I am not sure how essential it is). Wash well over a 250 ml beaker of water.

Staining

The stain used is Harris's haematoxyin, which I make up myself, but it can be bought ready made. (However, it may be advisable to filter it before use) Before use I dip the specimens in 20% IMS (Industrial Methylated Spirit) for a few minutes, then place them in the stain for 20-35 mins. Then back into 20% IMS for a minute or two, rinse off in distilled water and then differentiate in acid water for a few seconds and check with the microscope. Don't get the specimens too pale or too dark either, and it is best to do a few at a time with the microscope. Wash for five minutes and then blue the specimen with tap water to which may be added a few drops of ammonia, then rinse in tap water. If a series of small basins is prepared with the solutions in and labelled, the sieves can be moved quite quickly from one to the other.

Glycerol dehydration

Remove the specimens from the sieves or tubes with great care, and examine under water with the stereo microscope. Select the best ones and place these in a watch glass in 10% glycerol and water. Allow this to evaporate, (I place several watch glasses in an oven at 40 degrees C for a while).

When the glycerol looks quite concentrated, (don't let it get too thick) wash specimens in two changes of 95% IMS.

Counterstaining

Counterstain with a few drops of 0.2% Fast Green in 95% IMS and then place in 100% IMS in a watch glass. Tiny liverwort leaves are very delicate at this stage and must be handled with great care, either with one or two small brushes or with fine forceps at the stem end.

Clearing in Venetian Turpentine

The specimens are now placed in a 10% solution of Venetian Turpentine in IMS and allowed to evaporate. Venetian Turpentine is obtainable from good art suppliers, and I find a 25 ml glass beaker the best container for evaporation. The solution tends to creep up the sides of a solid watch glass and contaminate the dessicator with a sticky mess. Have a depth of about 20 mm. The beakers need to be placed in a dessicator or sealed container with some fused or dry Calcium chloride in the bottom, this prevents the turpentine going milky by ensuring a dry atmosphere. If you have a very dry warm room the Calcium chloride may not be necessary, you can experiment here.

Keep an eye on the beakers and when the solution level has dropped considerably check them for viscosity, it takes several days to evaporate, perhaps a week or so. If the solution is allowed to get too thick, the specimens cannot be removed without breaking them up, it must be fluid enough for easy removal. If it does get too thick, thin it down again with some IMS and re-evaporate. Just a little more fluid than the original density of the turpentine seems to be about right.

Final mounting in Canada or Dammar Balsam

The specimens can now be transferred to balsam on the slide for the final mounting, they are moved straight from the turps to the balsam. On no account try to dip in Zylene, for the whole point of using Venetian Turpentine is to avoid this chemical which will seriously distort your delicate specimens.

I like to mount most specimens in cavity slides where they are not flattened, (Chinese ones are fairly cheap) but exhibit a more natural appearance. This is not the best for photography of course, but enables the microscopist to view the small plants as they are in nature. A second slide can always be made with a flattened specimen for photographs.

Don't forget in all these procedures to keep track of your specimens with labels or numbered containers so that if you are processing various species at once, you do not get them mixed up. I am sure that if you follow this method, you will not be disappointed with the results. Your comments will be welcome.

Happy microscopy

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