Hand lenses and microscopes part 2: choosing a microscope

Following the introduction to hand lenses and microscopes in FB106, **Sharon Pilkington** discusses the points to consider when buying a compound microscope.

Compound microscopes are used for examining slide preparations of very thin/flat objects, sections of larger objects, or objects that are very small. Even at their lowest magnifications they have little depth of focus and are therefore unsuitable for examining whole objects with any degree of success.

Slide preparations are illuminated from below and have to be thin enough to be transparent – a moss leaf that is only one cell thick is ideal.

Compound microscopes have eyepieces and usually a rotating turret of different objective lenses, a platform known as the stage for holding/moving the slides and some form of under-stage lighting such as a mirror or a bulb. A typical magnification range may extend from x40 up to x1,000.

If you're new to the world of high power microscopes then the options open to you can be bewildering, but it may be worth choosing a relatively basic and low-cost model to start with. It should suffice for most identification tasks and help you gain familiarity with techniques until you are ready to take your bryology on to the next level. A basic 'student-type' microscope will cost £100–£200. Expect to pay £300–£500 for more

▷ Trinocular compound microscope. *I. Atherton*

advanced scopes (with better illumination and optical quality). The best microscopes (typically used by researchers and bryological professionals) will cost £1,000 or more to purchase new. Bargains can also be had by scanning the second-hand market for older, good-quality microscopes and university cast-offs.

KEY FACTORS TO CONSIDER WHEN CHOOSING A HIGH-POWER MICROSCOPE

Magnification. A mistake that many beginners make is to choose a microscope with higher magnification than they need. Most compound microscopes have a magnification range of x40 to x1,000, but as image quality and field of view decrease greatly with higher magnifications, it is usually the lowest magnifications that are the most useful (x40 to x400).

Monocular or binocular eyepieces. Monocular microscopes are cheaper and offer the same optical quality, but if you plan to spend a lot of time poring over slides, do consider buying a binocular version as you will find it far more comfortable to use. Some microscopes offer a trinocular head for little or no extra cost and this is a useful option if you are likely to want to take photographs of your specimens.

Illumination. Good under-stage illumination is extremely important, and together with good

optical integrity, can make the difference between seeing certain cellular features (such as cell papillae) or not. Low-cost scopes typically have relatively basic (but functional) halogen or LED bulbs, while more expensive models come equipped with more advanced lighting. Older models may have an under-stage mirror to reflect light from an external source. Whatever your budget, make sure your microscope is equipped with an adjustable sub-stage condenser to allow you to control the amount of light reaching the slide.

Tip: Halogen microscope bulbs can be very expensive. Extend their life by turning on at low power and gradually increasing to full power when warm. Apply the reverse process when switching off.

When identifying bryophytes using keys, it is essential to accurately measure the dimensions of leaves, their cells and other characteristics. The easiest way to do this is to replace one of the eyepiece lenses with a measuring eyepiece, which is essentially a miniature ruler, visible over the slide image. This will need to be calibrated with a graticule slide, which can be purchased, or borrowed from the BBS Librarian. Make a note of how many micrometres each division equates to for each objective lens, or prepare a sheet of measurements for at-a-glance reference.

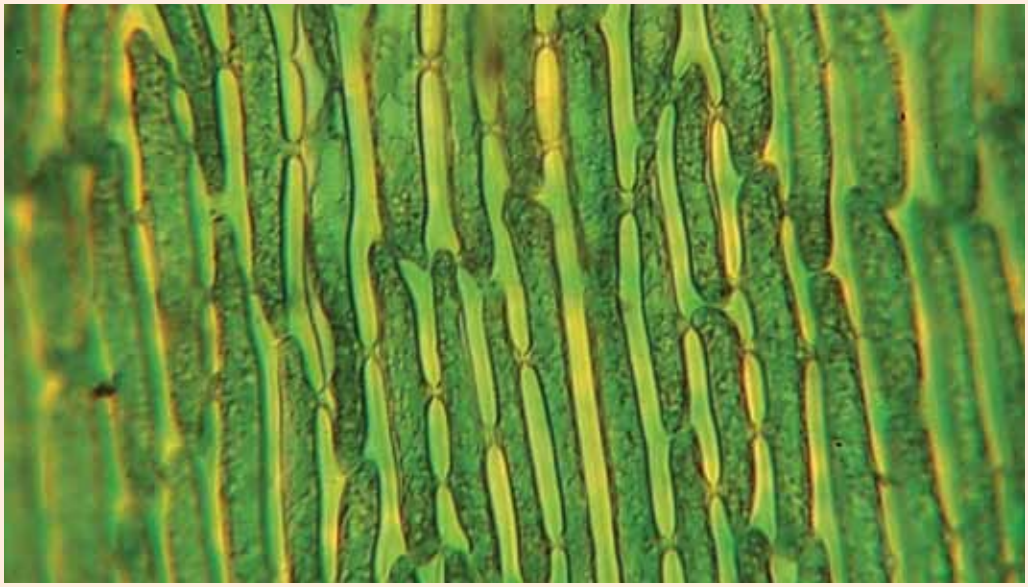
SLIDE PREPARATION

Everyone has their own preferences about dissecting equipment, but as a minimum I would suggest the following:

- fine dissecting forceps (no. 3): two pairs, for removing leaves and other structures from plants (fine-tipped dissecting needles or mounted pins can also be used);
- a fine watercolour paintbrush for gently cleaning mud and debris from specimens when held in water;
- double-edged razor blades for cutting sections;
- a small plastic pipette/dropper for placing a few drops of water on slides.

Some of these items are available from the BBS Librarian.

▽ Leaf cells of *Dicranum bonjeanii* stained with 1% Methylene Blue to highlight the pores in the cell walls (x200). *S. Pilkington*



Tip: Finely pointed dissecting forceps are easily damaged and the tips break off easily if they are dropped or handled roughly. When not in use, protect the points with plastic guards, or make your own protectors by cutting 5 mm thick sections of wine bottle corks and sticking the tips into these. With care, forceps can also be sharpened by gently rubbing the points with fine sandpaper.

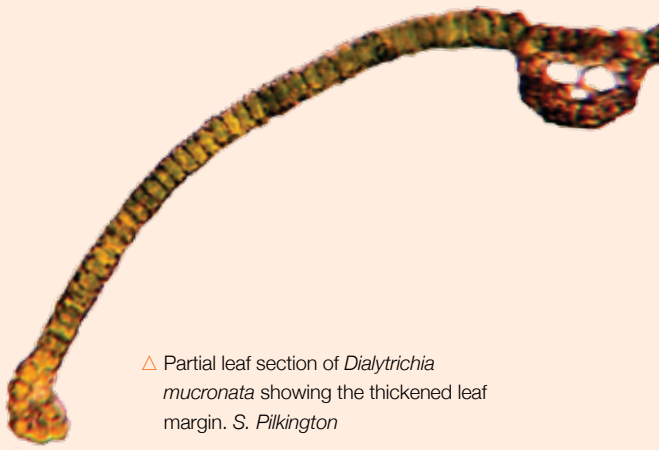
When mounting specimens on slides, mount in a few drops of water on a standard flat glass slide and gently place a coverslip on top. Cavity well slides can be useful for mounting slightly thicker specimens such as capsule sections and small leafy liverworts, and for viewing auricles *in situ*. Slides and coverslips can be re-used many times as long as they are cleaned after use. Water is often sufficient but over time greasy residues can accumulate and these are best removed by rubbing gently with a tissue soaked in a non-toxic solvent such as IPA (isopropyl alcohol) which is widely available.

Tip: Experiment with stains to bring out hard-to-see microscopic features such as pores and papillae. Small quantities of various botanical stains are available as solutions or dry powders from suppliers of laboratory equipment – for starters, try Methylene Blue, Fast Green and Gentian Violet.

PREPARING STEM AND LEAF SECTIONS

Sometimes thin cross-sections of tissue have to be examined under the compound microscope. For example, the structure of the midrib must be examined in some *Campylopus* and *Grimmia* species, whilst cross-sections of thalloid tissue are important in the accurate identifications of *Riccia* and other thalloid liverworts. Inevitably, sectioning is fiddly and, whilst techniques vary, sharp razor blades, a steady hand and lots of patience are essential!

Having experimented with various techniques over the years I have settled on one that works very well for me (I am right-handed) using a dissecting microscope and two pairs of fine forceps. After stripping off a whole leaf (or other structure), I place it in a few drops of water left of centre on a standard glass slide. After soaking it for a few moments I remove it from the water and transfer it onto the slide about 1 cm to the left of the drops. It is important that the leaf is thoroughly hydrated but not floating in water.



△ Partial leaf section of *Dialytrichia mucronata* showing the thickened leaf margin. *S. Pilkington*

Having aligned the leaf midrib with the long axis of the slide, I then lay a second glass slide gently over the leaf, in the same orientation as the first, leaving a bit of leaf sticking out toward the drops of water. The second slide should lay flat over the first and by applying gentle pressure to it with a finger from my left hand it can be moved firmly but gently over the leaf (the dampness of the leaf provides a certain amount of traction). Taking a sharp razor blade I cut vertically downwards along the right-hand edge of the top slide to remove thin slivers of leaf section. Pulling the top slide gently back exposes more leaf, and multiple sections can be cut, the aim being to make them as thin as possible. After each section is made, it can be transferred immediately to the pool of water to the right with a sweeping movement of the blade. Make multiple cuts, apply a stain if required and a coverslip, and examine under high power.

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