Microtomy

The microscopical study of sections of animal & plant material

Introduction.
Many living things are extraordinarily beautiful. The microscopist has a privileged view and this may be extended and enhanced by looking inside them; this is done by making very thin sections. This is microtomy. Microtomy may also be used to extend our knowledge of how living things are put together and so allow deductions to be made about how they work.

This paper is concerned only with light microscopy. Sections are almost always examined under a light microscope using transmitted light so sections need to be very thin. Thicknesses of 8-10 µm are appropriate for biological sections and 12-20 µm for botanical sections. For geological specimens, 30 µm is usual.

Before living things are sectioned, they must first be fixed (killed) and then preserved. FAA (formol-acetic-alcohol) is usually used for plant material; Bouin's fluid for animal. It must penetrate the tissues and some subjects, seeds for instance, are designed to exclude any penetration so may need piercing to allow the liquid access. Specimens may be held in FAA indefinitely but zoological specimens will harden if left too long in Bouin's fluid – after fixation these must be thoroughly rinsed in 70% alc. and then stored in this medium or, better still, a mixture of 70% alc. plus some glycerine.

Before embedding, subjects must be dehydrated by being 'passed through the alcohols'. From FAA (which is 70% alcohol), transfer to 70%, then to 90%, then to absolute alcohol and, finally to a further bath of abs alc. Specimens must then spend 12-24 hours in Histoclear (or xylene) which are miscible with alcohol; wax is not.

Embedding.
To allow specimens to be very thinly sliced, they must first be embedded, usually in wax.

[The simplest support can be provided using a carrot. This is first cut into a cylinder to fit closely into the cutting instrument or microtome. The carrot is then split longitudinally and a small 'V' taken out of the two flat faces to accommodate the specimen.]

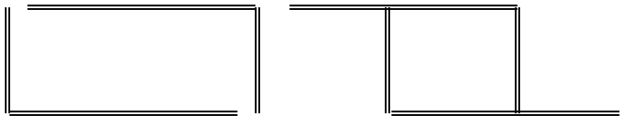
A suitable embedding wax should be solid up to its melting point and liquid thereafter. For this the wax must be homogeneous. Candle waxes are unsuitable; petroleum derivatives, they are mixtures so their constituents melt at different temperatures. A variety of microtomists' waxes, each with a different melting point, are available. The wax chosen will be determined by its purpose. Higher melting points may be appropriate in hot countries or centrally heated rooms. The Iceni Microscopy Study Group (IMSG) uses one with a melting point of 56°C; it is obtainable from several suppliers including Poth Hille.

Specimens need to be well supported against the cutting blade. If sectioning a specimen in which the material is all inter-connected and there are no large cavities, say, a piece of muscle tissue, there would probably be no need to infiltrate with wax before blocking out; it could go straight into a wax block. With something like a flower bud, however, a transverse section would have numerous small parts that are not joined together; to keep these correctly positioned in relation to one another, all the spaces between the elements need to be filled with wax - so infiltration would be essential. As a general rule, if in doubt, infiltrate.

To ensure thorough infiltration, soak specimens in molten wax for 24 hours. Any small pot will do. While

the temperature may more easily be monitored and controlled using a small thermostatically controlled oven, Marson recommends a hot-plate. The wax should be held only a few degrees above its melting point or the specimens will cook. Specimens are then "blocked out" - transferred from their pot of molten wax into a wax block to be sectioned.

For mechanical microtomes, the exact size & shape of the embedded block isn't important. Choices include little cooking foil cups, ice-block trays, origami paper boxes, coverslip boxes lined with shiny magazine paper and Leuchart's embedding moulds - see below. These may be conveniently made from brass strips 2" x 1" x ¾".



The block mould is put onto a cold stone or glazed tile. Have ready a pair of forceps warmed in a flame and some wax melted separately in a small beaker. Pour the wax into the cavity - it will at once begin to cool and set.

Using the hot forceps, quickly take your specimen from the infiltrating wax and hold it in position so that, as the wax in the mould sets, the sections wanted will be seen when the bock is afterwards sliced. The surface to be cut should face downwards. Some waxes tend to crystallise when allowed to cool at room temperature so, when the wax is partially set, plunge both plate and mould into cold water.

Including a small paper strip in the top (back) of the block usefully identifies the specimen and shows which end should not be cut. Ernie Ives recommended storing plugs in 10% glycerine.

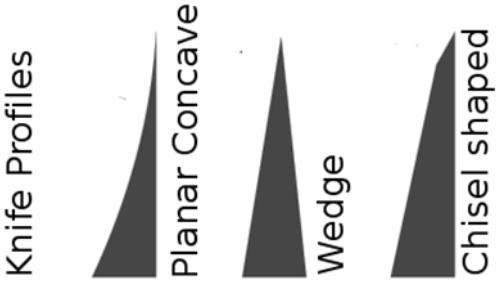
[Using a cut-throat razor and a hand held or bench microtome, sections can be cut, slicing diagonally, but it is difficult to achieve constant thicknesses - or thinness!]

Mechanical microtomes allow many slices of a pre-determined thickness to be made. Such machines come in a wide variety of shapes and sizes. All have extremely sharp blades that must be frequently (and correctly) sharpened. Such dangerously sharp blades should only be moved in their box.

When very thin sections are cut using a mechanical microtome, the heat generated by each cut warms the trailing edge of the previously cut section just enough to stick it to the next, producing a ribbon of sections. This allows several sections to be mounted side by side so that, conveniently, progressive views of the internal structures may be seen all on one slide.

The IMSG has sledge, rotary, bench and hand microtomes.

Sharpening blades.
Blade profiles may be double-concave, planar-concave, wedge or chisel. It is important to know exactly how to sharpen each of them. Concave blades have small screw holes in their backs and need sharpening with a 'back' - see Ernie Ives's book (Further reading 3).



A suitable grinding medium for sharpening, aluminium oxide 3µm, is obtainable from Kemet - but this powder is a respiratory hazard so should be mixed 1:1 with '3 in 1' oil & paraffin (or oil mixed 1:2 with white spirit) before use. Brasso or Silvo are used for the final polish.

Each grinding medium should have its own glass pane), labelled for that grade alone. Glass plates need good support on, say, a piece of kitchen worktop.

Diamond paste should not be used with a glass plate but a wooden board, usually beech. Both diamond and glass are so hard, diamonds becomes embedded in the steel.

Starting with the concave side, sharpen with an elliptical action of some 20 circles for each side. Finish by drawing the blade off the glass length-wise and wipe clean. The need continually to sharpen blades can be avoided by using suitably supported surgeons' skin graft knife blades. Micscape recommends using a jig.

The ground edge (easily seen in good light) should not be wider than about $^{1}/_{16}$" (1.6 mm). If it is, re-grinding is needed - or a new blade. If the blade has a flat side, this should be towards the block.

Procedure.
Before starting to cut, pare back the wax block to where wanted, perhaps using a fine saw.

To fix the block with the specimen to the microtome, heat a table knife in a spirit flame and put the hot blade between holder and block; when both are slightly melted, withdraw the blade - and then use it to make a fillet to ensure the block is well joined and supported.

Spare Cambridge rocker holders may be made from copper tube with a disc of zinc fly-screen soldered onto one end and that end dipped into beeswax.

Slide Cleaning.
Slides and cover slips often come from the manufacturer with dust, grease and small glass shards so need washing before use. This is best done using an old toothbrush dipped first into a standard cleaning fluid (50:50 methylated / water mixed with a small dash of washing-up liquid), then with scouring powder or 'Jif'. Finish by wiping with a soft cloth (such as a clean old handkerchief) until dry and free of abrasive, polish with chamois leather and put back into their vacuumed box ready for use. Mark the box "Clean slides".

Haupt's Adhesive for attaching a section to a slide
From The Microtomist's Formulary & Guide by Peter Gray; Constable, 1954.
Dissolve 2g gelatine in 100 ml de-ionised water;
To prevent fungal growth in storage add 2g Phenol (or a crystal or 2 of thymol or 2 or 3 drops of cedar-wood oil)
Add 15 ml Glycerine and mix thoroughly.
Filter (e.g. through coffee filter paper or muslin).

Ernie Ives successfully substituted Pearl (Scotch) glue for the gelatine. 2g of this are placed in a small container and just covered with cold water; after a few hours the water will have been absorbed and the material is then ready for making the adhesive.

Other options are Isinglass, egg albumin (egg white) or, if nothing else is available, plain saliva.

Have ready on a hotplate a deepish (50+ mm) dish of warm water at a little below the melting point of the wax, say 40-48°C. If not using frosted slides, decide which is to be the top side of the slide and mark it; this can be done with or a tiny scratch in one corner using a diamond cutter. Prepare enough thoroughly cleaned slides and add adhesive to them. If Haupt's Adhesive is to be used, use a little finger to add a small dab towards the centre; this is then smeared across the central section using the back of the hand. Before adding your cut sections, 'tan' the adhesive with a drop of 2-5% Formalin in a watch glass.
As the microtome cuts each section, or a ribbon of sections, it should be picked up using a small artist's paint brush and floated, convex side down, onto the warm water surface to unroll and flatten.

Push a slide prepared with adhesive into the water at an angle of 30° and move it under the section to be mounted. Raise the slide and capture the section. It may have to be encouraged with the paint brush. If the section needs moving, pulling it is less likely to damage it than pushing.

Specimens often expand once on the slide so allow for this. Sometimes, sections turn over; the top surface will be dull and the bottom one shiny.

Remove the slide with its section from the water and blot it very carefully. Suitable blotting paper is that used to pack slides in boxes or ordinary stationer's. Blot using a single firm roll of a finger. After blotting, the paper needs to be carefully peeled away.

Warm the slide in a flame to ensure it is absolutely flat. It may be stored indefinitely in this condition.

Before the section can be stained, all wax must be removed. This is done by carefully lowering it into Histoclear (or xylene) for some 30 minutes. Coplin jars are ideal for this as they hold the slides vertically; almost as good are 5 slide transport boxes ("mailers") held vertically, perhaps in a rack. A second and third de-waxing in fresh Histoclear (or xylene) is advised.

Staining.
To allow details to be seen, specimens are usually stained before final mounting. Different stains may be taken up by different parts of the specimen and so provide pleasing contrasts. Experimentation may be necessary. Time in a stain depends upon how it is made up.

Ernie Ives recommended the following staining processes for botanical material:

Astra Blue and Safranin.
Immerse in aqueous Astra Blue stain for 15-30 minutes; rinse in water to remove surplus stain; drain and mop up surplus water with tissue.
Immerse in Safranin for 20 mins; rinse, drain and mop.
Dip in Meths, reverse slide and dip again; drain and mop.
Using a pipette, flush once with abs. isopropanol.
Dip in abs. isopropanol, reverse slide and dip again.
Clear in clean Histoclear (or xylene) for a few minutes.
Mount in Canada balsam or other resin mountant.

Safranin and Fast Green.
Immerse in aqueous Safranin for a minimum of 20 minutes; rinse in water to remove surplus stain; drain and mop up surplus water with tissue.
Differentiate: dip in Meths - watch under microscope - reverse slide and dip again; drain and mop.
Immerse in Fast Green for 2½-3 minutes; rinse, drain & mop.
Using a pipette, flush once with abs. isopropanol.
Dip in abs. isopropanol, reverse slide and dip again.
Clear in clean Histoclear (or xylene) for a few minutes.
Mount in Canada balsam or other resin mountant.

Fast Green.
This formula is Ernie Ives's modification from an old Fast Green in clove oil stain:
Cellosolve 150 ml *[Obtainable from Sigma Aldrich]**
Isopropanol abs. 150 ml
Terpineol 150 ml *[This is **not** turpentine!]*
Fast Green FCF 0.25 g
Mix liquids and add stain. Leave for 1 day to dissolve.

This is much slower working than Fast Green in cellosolve alcohol alone and, being weaker, gives a lighter green colour but is much easier to control.

* Be careful, there are several 'cellosolves'. The first one commercially available was 2-ethoxyethanol and it is generally accepted that it is this which is meant when the word 'cellosolve' is used.

Toluidine Blue is another possibility.

Coplin Jar and 'Mailer'

Over-staining may be corrected using dilute alcohol (5-10%) or Acid Alcohol in a Coplin jar for c.2 minutes; if satisfactory, wash in water. Neither will work with all stains.

Ernie Ives preferred rectangular cover-slips and used a No 3 brush to 'ring' them with Humbrol paint. For ordinary ringing, once the slide was dry, he used a scalpel to clear the top of the cover-slip. This left a meniscus clinging to the edge of the cover-slip.
For 'best' he used a slide on edge to leave a slide's width overlapping the cover-slip top and edge.

Further reading.
1. "Practical Microscopy" (Booklet 14). Eric Marson. Obtainable from Brunel Microscopes.
2. "Practical Section Cutting and Staining". E.C. Clayden. Churchill. 1955.
3. "A Guide to Wood Microtomy". E. Ives, 2001.

Members of the IMSG at work on 1st September 2012

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