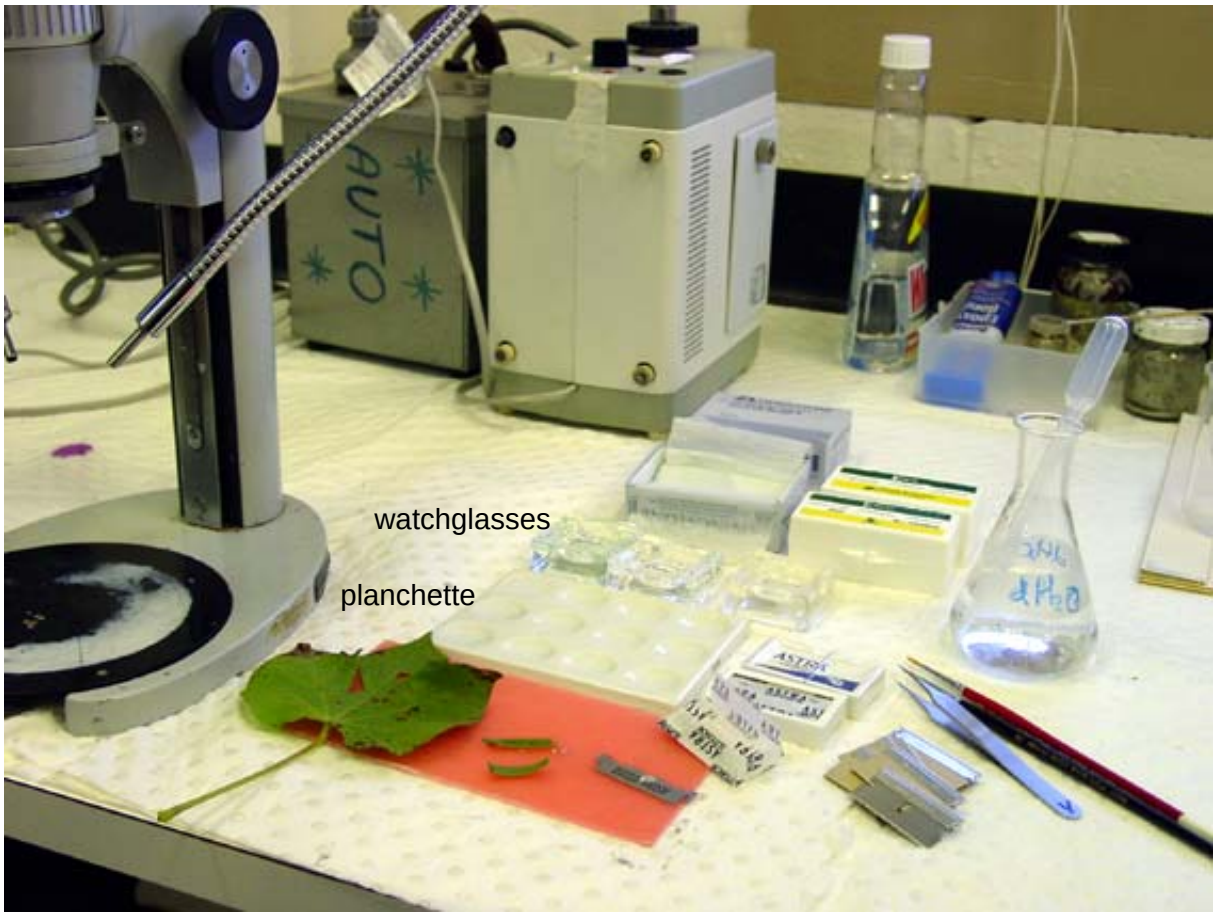
Sectioning unembedded plant tissues

Making hand sections without support material

You need to have at the lab bench:

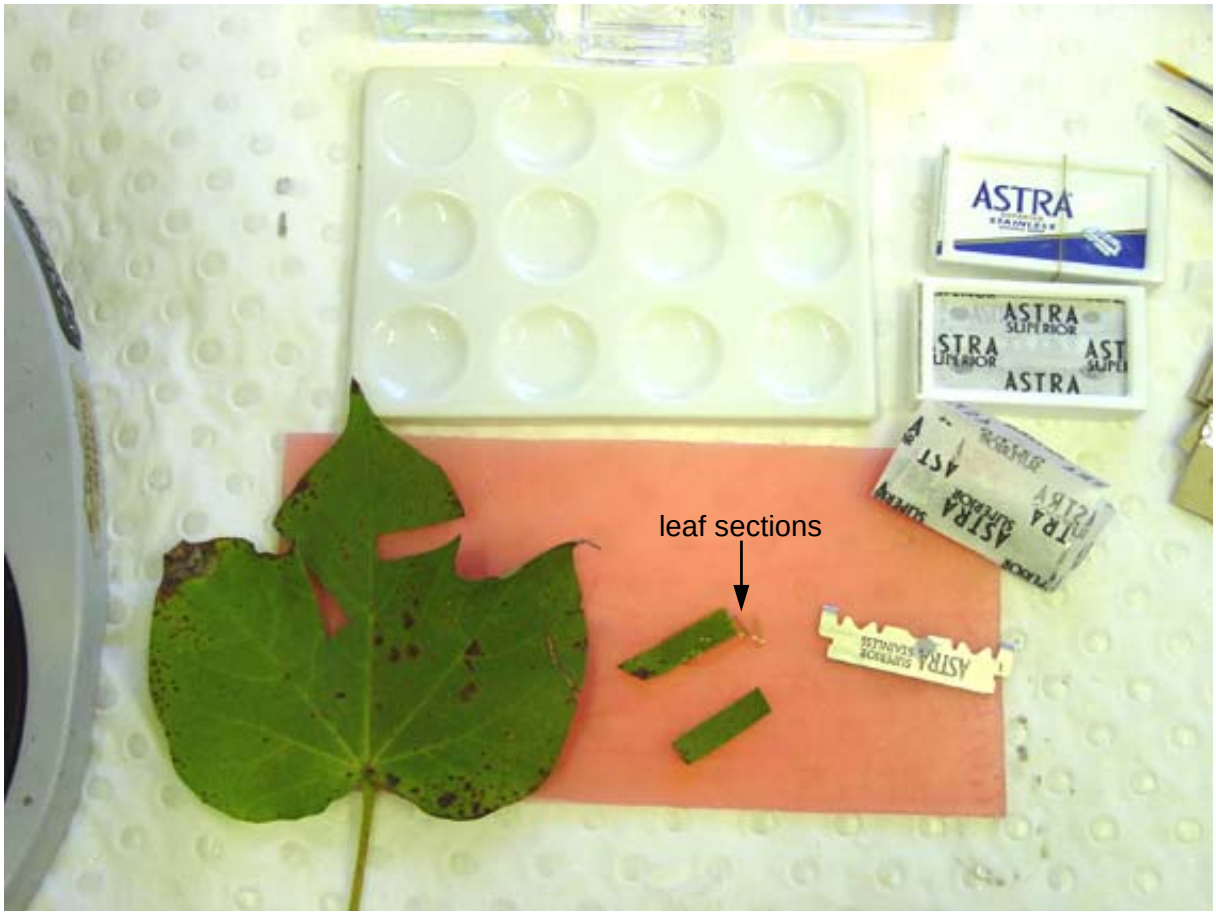
piece of pink dental wax
water - conical flask with plastic or glass pipette, not a wash bottle
watch glasses or planchette with water to put sections in
double-edged razor blades – lots – break in half while in their wax paper wrapping – use lots, stop using a blade as soon as you feel any resistance to cutting the tissue
reasonably fine forceps
fine paintbrush and/or sharpened orange sticks to transfer sections
slides
cover slips
dissecting microscope - doesn't have to be a fancy one
some detergent - 1% Tween-20 or Triton-X-100 – use this if your leaf or other tissue is very hydrophobic, it will help to cover the tissue with water so you don't get air bubbles between tissue and coverslip or slide

See below:



Leaf tissue:

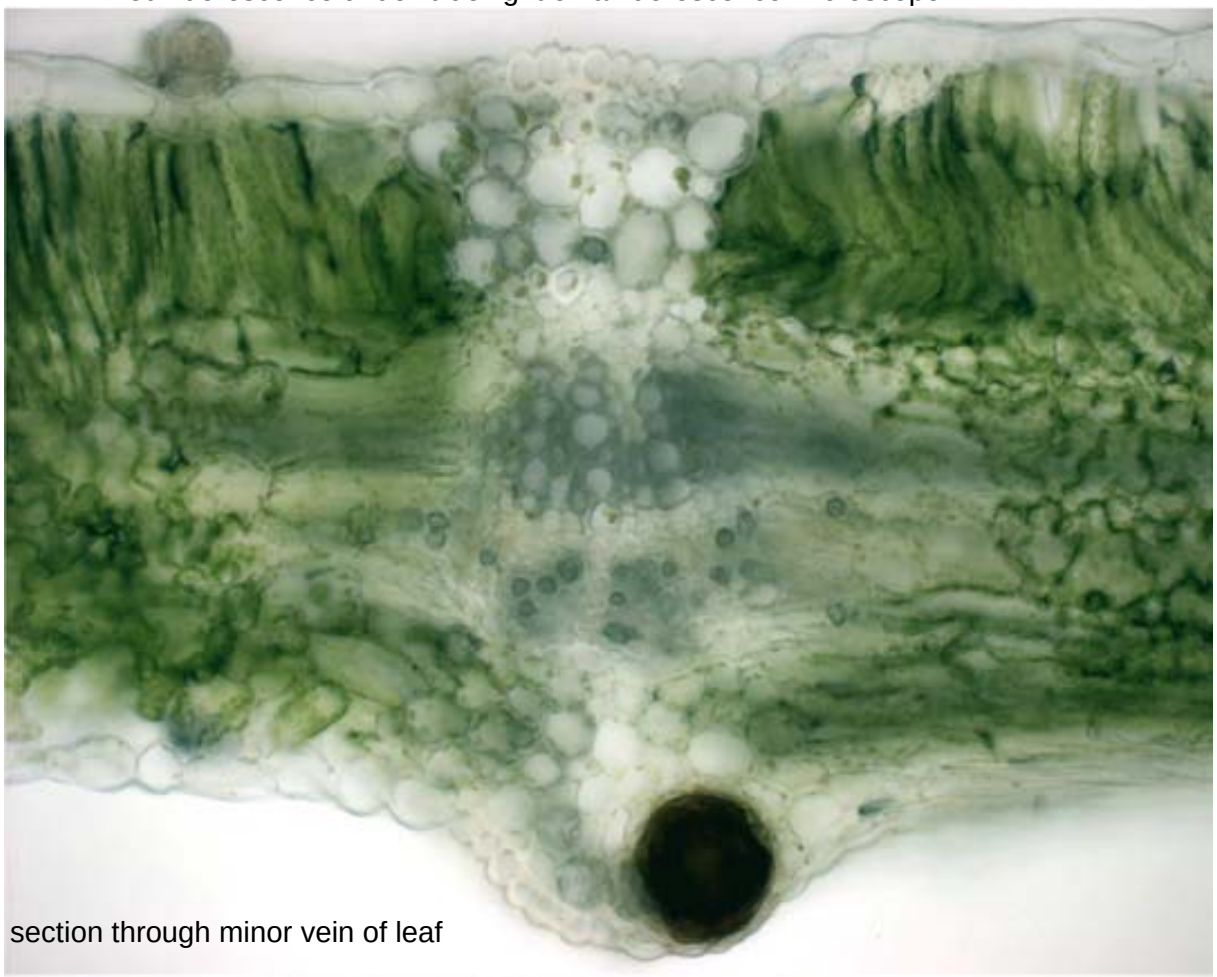
1. Using a sharp razor blade, cut a piece of leaf about 5-10 mm wide and as long as you like. In the example below, a strip was cut that extends from the midrib of the leaf to the edge, but you could also cut along one side of the leaf from tip to base. Include the midrib if desired. Two strips have been cut from the leaf below:



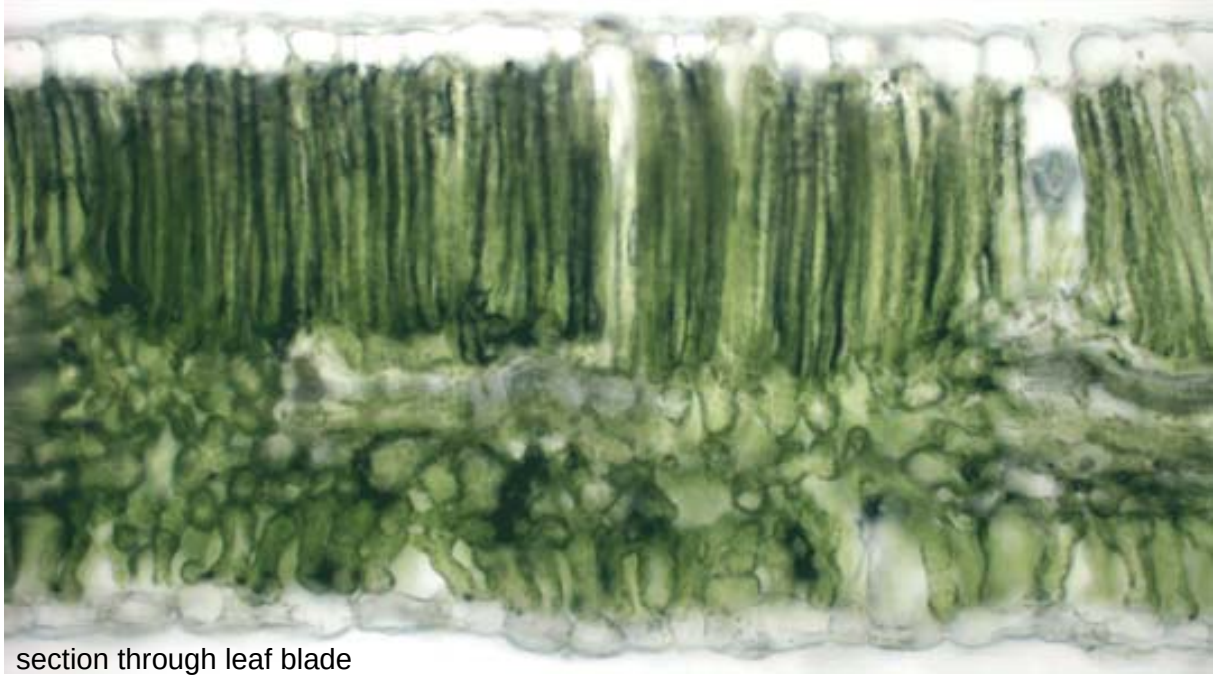
2. Put a drop of water on the pink dental wax over the end of the leaf strip that you want to start cutting sections from.
3. Then cut very fine slices of leaf into the drop of water, holding the leaf down with your left forefinger (if you're right-handed) and cutting each slice with a single cut of the blade from top to bottom. To get really thin slices, watch your cutting on the dissecting microscope, carefully placing the razor blade to get the thinnest sections. Rest the flat of the blade against your non-cutting forefinger (taking care not to cut yourself!).
4. Transfer the sections with forceps, or with the tip of a paintbrush (with few bristles – pull or cut the rest out), from the dental wax into water in a watchglass, or into water in one section of the planchette. Accumulate sections there – cut LOTS of sections.
5. Look at the sections under the dissecting microscope to select the very thinnest, they will be almost transparent. It may be easier to see the thinnest sections if you examine them on a black background. Transfer some of these very thin sections to a SMALL drop of water on a microscope slide.
6. Cover with a coverslip – put one edge of the coverslip into one edge of the drop of water and lower the coverslip slowly with the forceps. If you drop the coverslip onto the sections you are likely to get air bubbles. If the water doesn't extend to the edges of the coverslip, add a SMALL amount of water – don't flood the sections or they will float around.
7. Observe on dissecting or compound microscope. If you need to add more contrast to the sections – more than you can get by closing down the iris diaphragm on the microscope, you can stain your sections. See instructions about staining.

8. Example of hand sections of cotton leaves:

   Palisade mesophyll – long thin cells in upper half of leaf
   Spongy mesophyll – smaller less regular cells in lower half of leaf
   The mesophyll tissue has abundant green chloroplasts, no chloroplasts in epidermis, few in cells around the veins
   Top image is through a large leaf vein, dark spot is a gossypol gland
   Can test chloroplast intactness/chlorophyll abundance in a qualitative way by checking red fluorescence under blue light on a fluorescence microscope.



section through minor vein of leaf



section through leaf blade

Root tissue

Sectioning reasonably large diameter roots, like maize roots, or old lucerne or canola roots, is similar. Fine roots can be sectioned this way as well, as long as you are careful not to squash the roots and if you do the sectioning under the dissector to control section thickness. Try the following:

1. Place the root to be sectioned in a drop of water on the pink dental wax. Get your fingers and the half-razor blade wet, everything should be wet.
2. Cut very fine slices of root into the drop of water, holding the root down GENTLY with your left forefinger (if you're right-handed) and cutting each slice with a single cut of the blade from top to bottom – NO SAWING. It's most important to cut through the tissue with a single, even stroke of the razor blade.

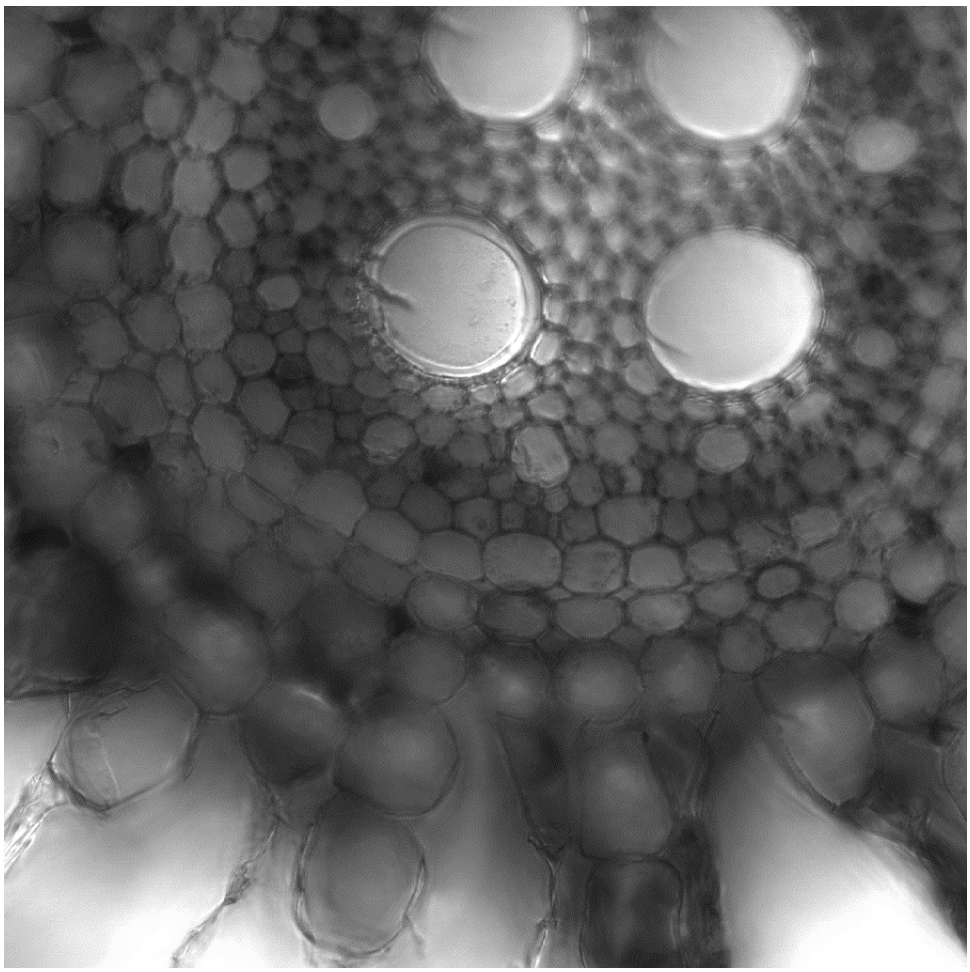
   To get really thin slices, watch your cutting on the dissecting microscope, carefully placing the razor blade to get the thinnest sections. Rest the flat of the blade against your non-cutting forefinger (taking care not to cut yourself!).

   Discard the blade as soon as you feel resistance to cutting, or if the tissue starts to tear during cutting. Don't waste time and tissue conserving razor blades.

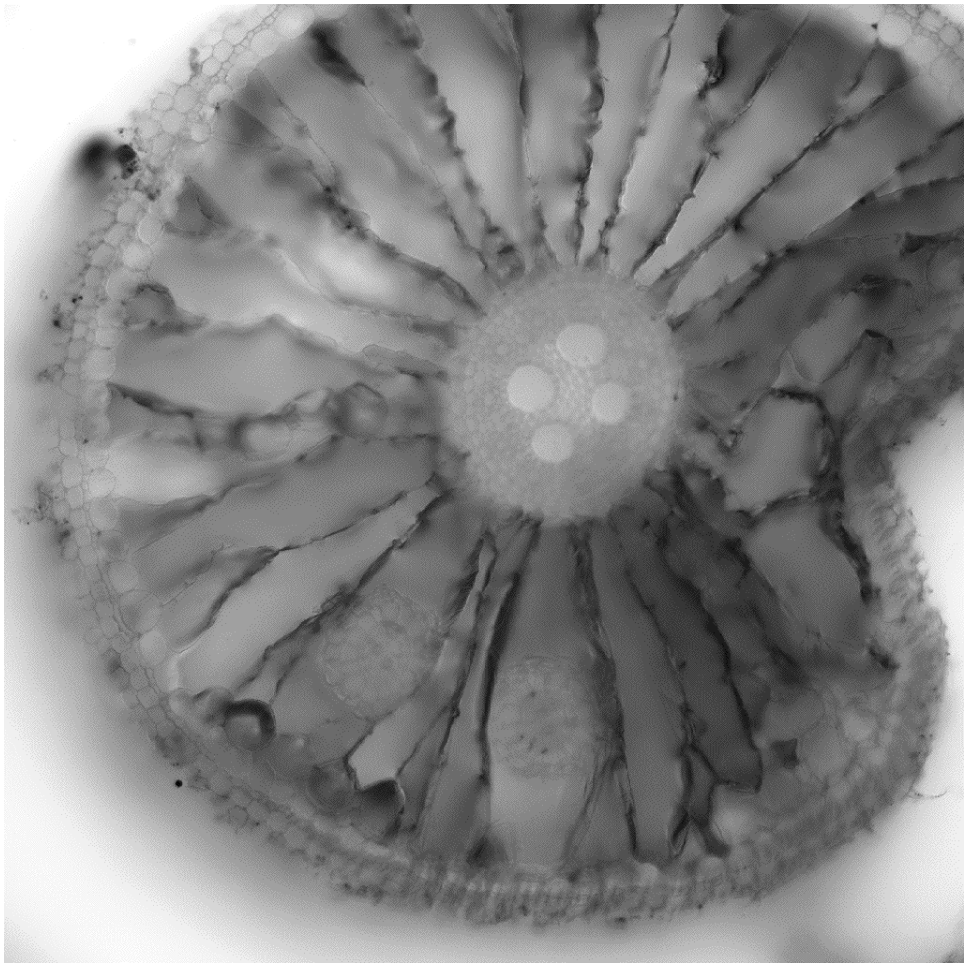
What follows is as described for the leaf sections above:

3. Transfer the sections with forceps, or with the tip of a paintbrush (with few bristles – pull or cut the rest out), from the dental wax into water in a watchglass, or into water in one section of the planchette. If you use a transparent watchglass, it's easier to pick the thinnest sections later on. Accumulate sections there – cut LOTS of sections.
4. Look at the sections under the dissecting microscope to select the very thinnest, they will be almost transparent – this is easy to see if you place the transparent watchglass onto black paper or other black background. Transfer some of these very thin sections to a SMALL drop of water on a microscope slide.
5. Cover with a coverslip – put one edge of the coverslip into one edge of the drop of water and lower the coverslip slowly with the forceps. If you drop the coverslip onto the sections you are likely to get air bubbles. If the water doesn't extend to the edges of the coverslip, add a SMALL amount of water – don't flood the sections or they will float around.
6. Observe on dissecting or compound microscope. If you need to add more contrast to the sections – more than you can get by closing down the iris diaphragm on the microscope, you can stain your sections. See instructions about staining.

The following are hand-sectioned aerenchymous maize and rice and wheat roots. These are unstained sections, observed under brightfield with a compound microscope.



Part of an aerenchymous maize root, hand-sectioned. Section is rather thick because the stained section was imaged on the confocal, for which thin sections are not necessary.



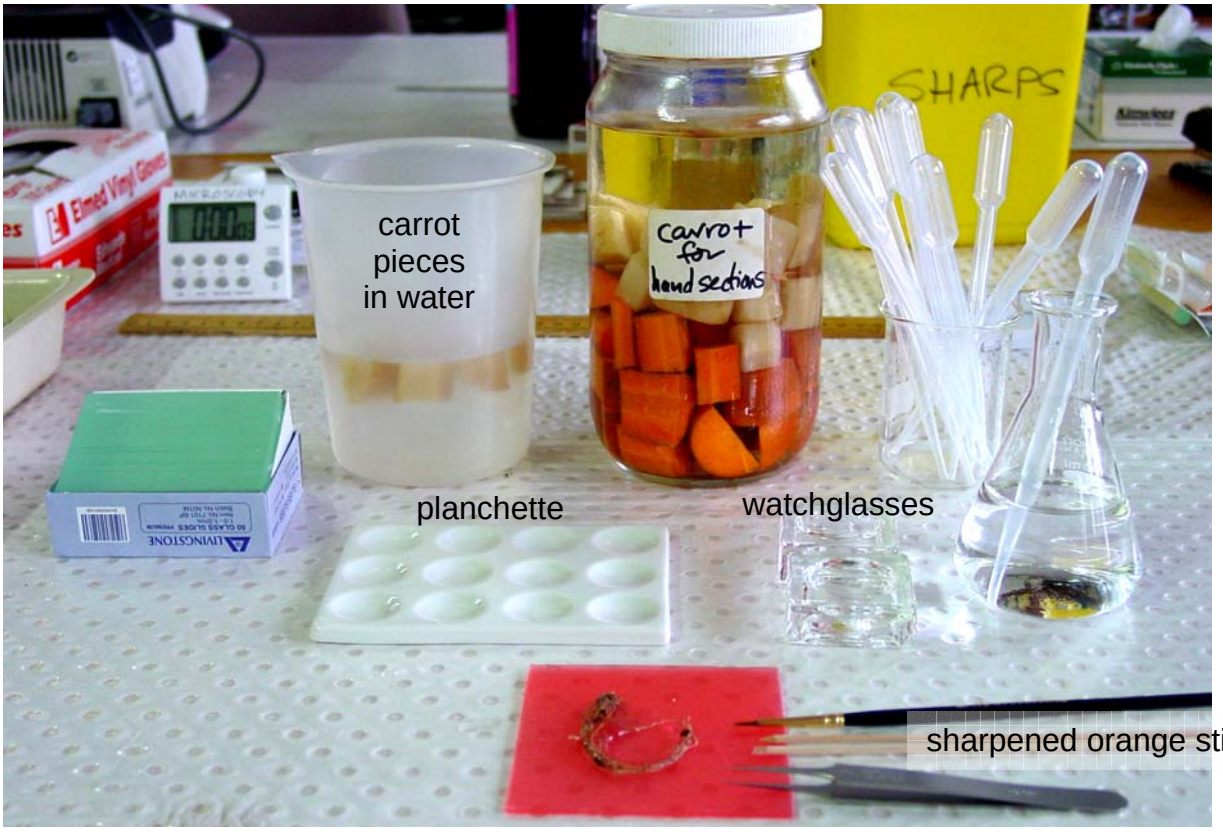
Hand section of aerenchymous rice root which got a bit squashed during sectioning – note interesting phenomenon of lateral roots trapped inside the root cortex.

Using other plant tissues as support tissue to make hand sections

For this you also need:

support tissue for sectioning – pith, carrot or potato – the latter two can be stored in 70% ethanol for many months until required

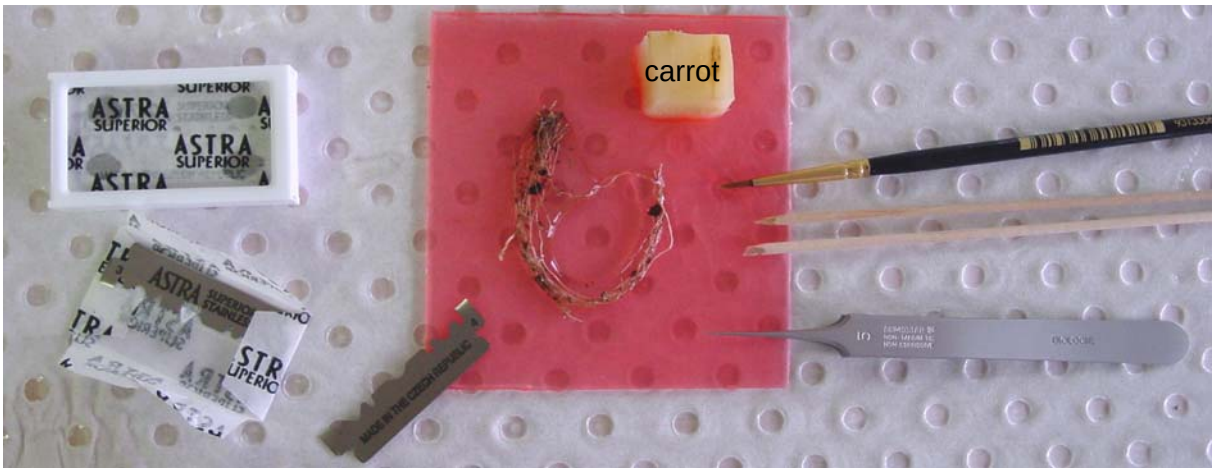
See below:



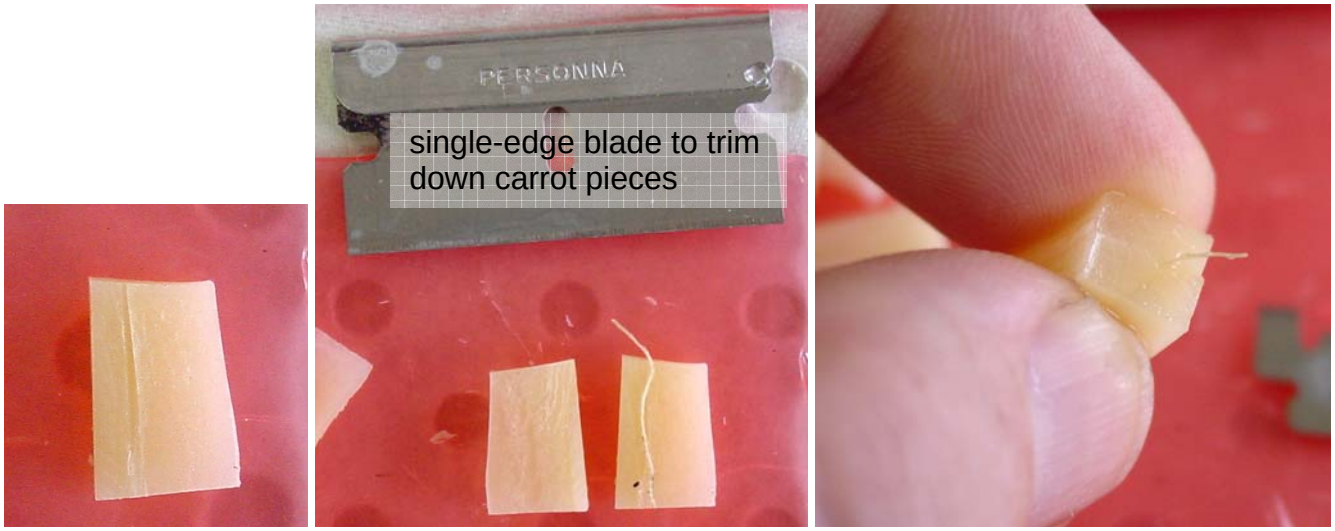
Before starting, take some of the carrot or potato pieces out of the 70% ethanol and soak in water, as above.

1. Place the tissue to be sectioned in a beaker of water, cut out a small piece for sectioning and place in a drop or small pool of water on the pink dental wax. Get your fingers and the half-razor blade wet, everything should be wet.

   Take one of the pieces of carrot from the beaker of water.



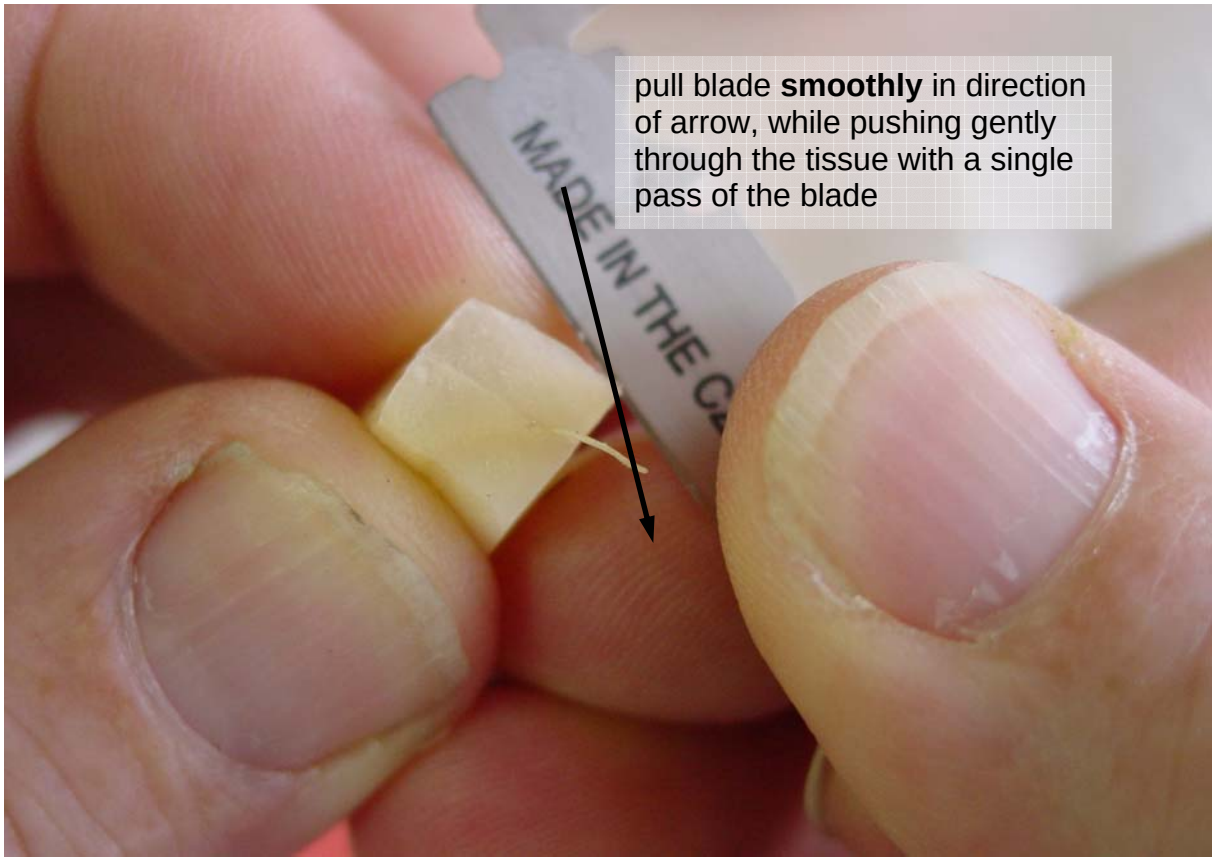
2. Cut out a small block of carrot, then cut it in half. If sectioning a large or soft piece of tissue that might become compressed between the carrot, make an appropriate-sized groove in one of the halves, as shown below, left and centre. Make sure the tissue to be sectioned and the carrot remain wet at all times.



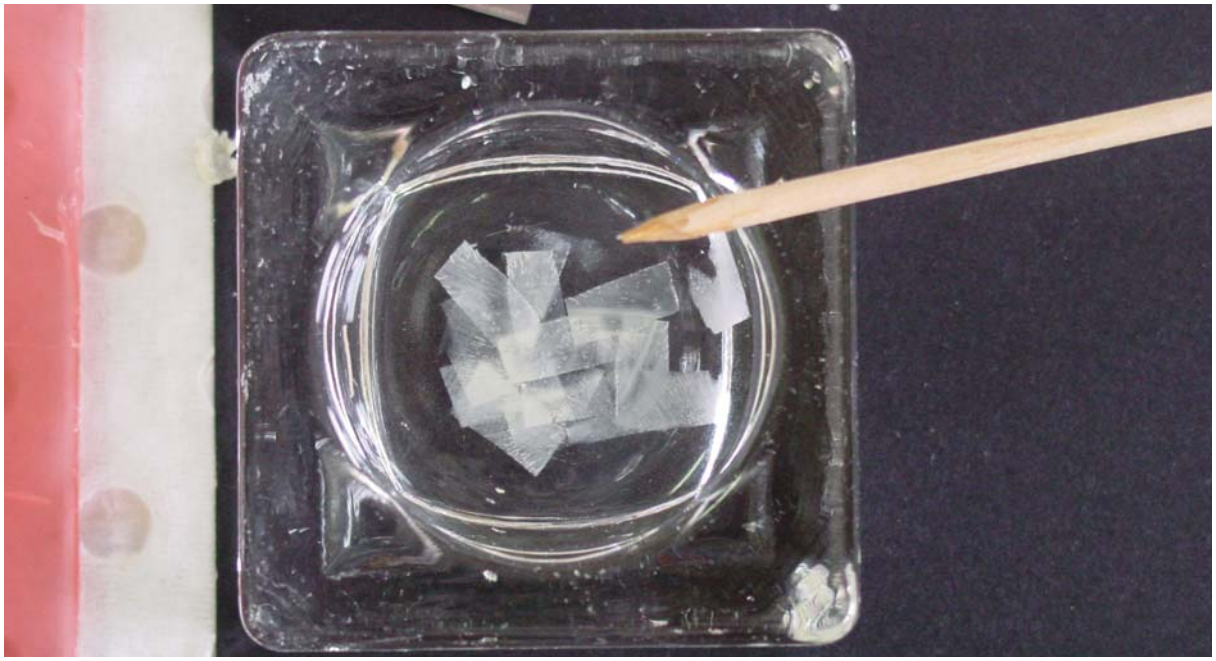
3. Place a piece of tissue in the groove, and sandwich it in with the other half of the support tissue, as above, at right. (The support tissue could be trimmed down a little more.)

4. Make sure your fingers, the carrot and the razor blade are wet. With the tissue in one hand, and the blade in the other, rest your forearms on the edge of the lab bench, and rest the outer edge of your hands on the bench. As when cutting on the dental wax, you want to slice through the plant tissue plus support tissue with one smooth stroke of the blade – **NO SAWING**! To get a smooth, even stroke, it's best to have your hands supported as much as possible – you will also be less likely to wobble and cut yourself.

   You can either pull the blade towards you through the tissue, as shown below, or you can push it away from you. As shown, use your non-cutting forefinger as a guide for the blade. Make sure your non-cutting thumb is below the level of the carrot!



6. Collect the sections plus carrot in a watchglass, or in water in one section of the planchette. Accumulate sections there – cut LOTS of sections, see below. It's much easier to see the tissue and sections against a dark background – here, it's a piece of black paper, but black plastic is good too, and doesn't fall apart when it gets wet. If necessary, you can place the pink dental wax on a black background to detect small tissues, e.g. fine roots, more easily.



7. Look at the sections under the dissecting microscope to select the very thinnest, they will be almost transparent. It may be easier to see the thinnest sections if you examine them on a black background. Transfer some of these very thin sections to a SMALL drop of water on a microscope slide.
8. Cover with a coverslip – put one edge of the coverslip into one edge of the drop of water and lower the coverslip slowly with the forceps. If you drop the coverslip onto the sections you are likely to get air bubbles. If the water doesn't extend to the edges of the coverslip, add a SMALL amount of water – don't flood the sections or they will float around.
9. Observe on dissecting or compound microscope. If you need to add more contrast to the sections – more than you can get by closing down the iris diaphragm on the microscope, you can stain your sections. See instructions about staining.

Using support tissue in a hand microtome to make hand sections

For this you also need:

support tissue for sectioning – pith, carrot or potato – the latter two can be stored in 70% ethanol for many months until required
hand microtome
cork borer – same ID as hand microtome

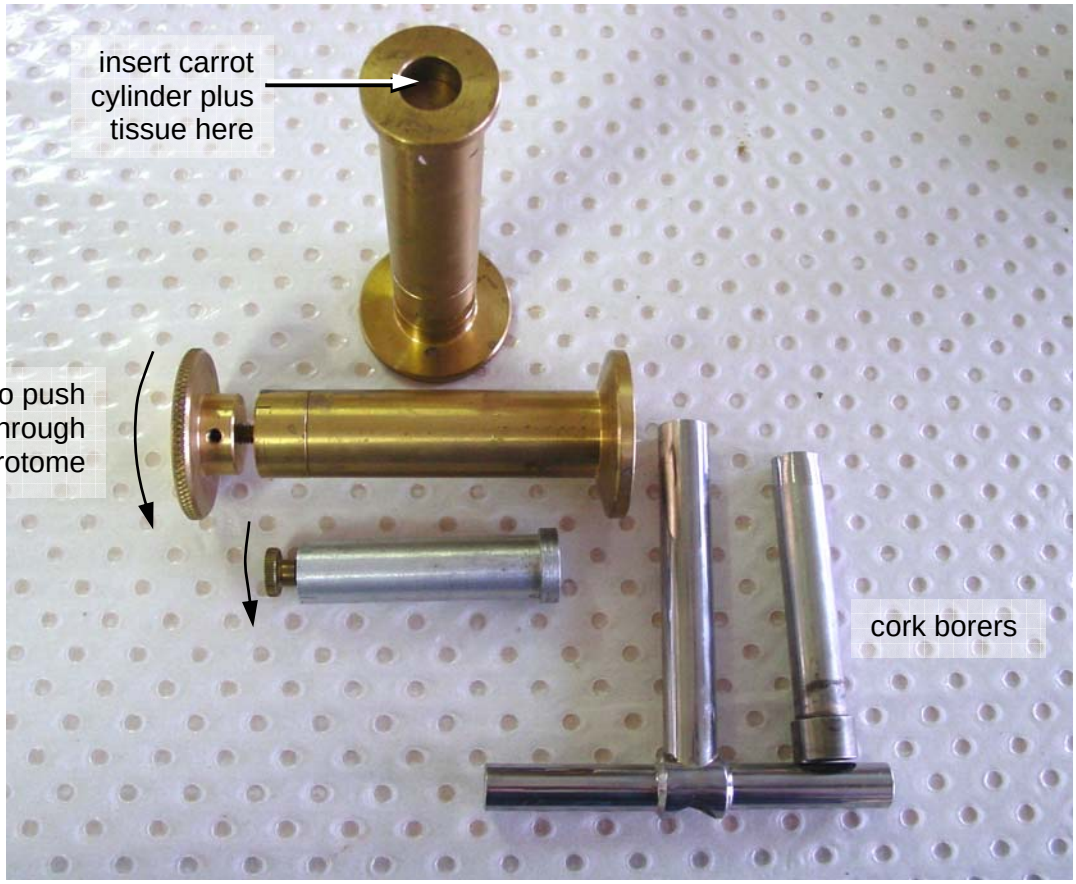
Before starting, take some of the carrot or potato pieces out of the 70% ethanol and soak in water.

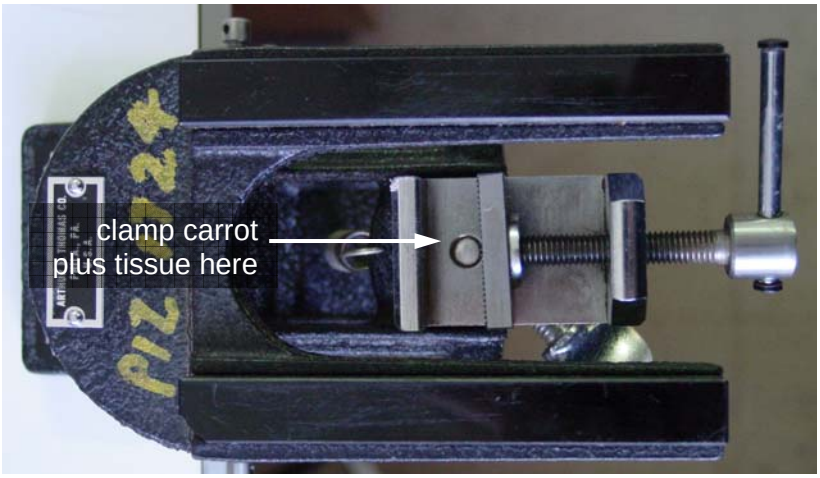
1. Place the tissue to be sectioned in a beaker of water, cut out a small piece for sectioning and place in a drop or small pool of water on the pink dental wax.

2a. This is a hand-held microtome. The tissue is squashed into the top hollow, and the blade pulled across the top to cut off protruding tissue. Section thickness is determined by how much you raise the tissue by turning the knurled knob at the base of the microtome.

Make appropriate-sized carrot pieces using a cork borer of the same diameter as the hand microtome, then cut the resulting carrot cylinder in half, lengthwise.

If the tissue is large or liable to be squashed, make a groove in one of the carrot halves, place the tissue between, then push the carrot cylinder plus tissue into the hand microtome.





2b. This hand microtome attaches to the bench, as shown above. Here, the tissue is clamped into the top, and you slide a blade across to cut off protruding tissue. Section thickness is determined by raising the tissue with the knurled knob at the bottom.

Prepare the carrot support tissue exacty as for free-hand sectioning. Cut out a small block of carrot, then cut it in half. If sectioning a large or soft piece of tissue that might become compressed between the carrot, make an appropriate-sized groove in one of the halves. Make sure the tissue to be sectioned and the support tissue remain wet at all times.

Place a piece of tissue in the groove, and sandwich it in with the other half of the support tissue. Clamp this into the hand microtome.

3. Make sure your hands, the support tissue and the blade are wet. A stiff blade works best for both of these hand microtomes, either an old-fashioned shaving razor or a sharpened old microtome blade is best, but a reasonably inflexible disposable blade works OK. The blade must be sharp.

4. Cut sections by either pulling the blade towards you through the tissue, or pushing it away from you. In either case, the tissue must be cut with a single, smooth, even stroke.

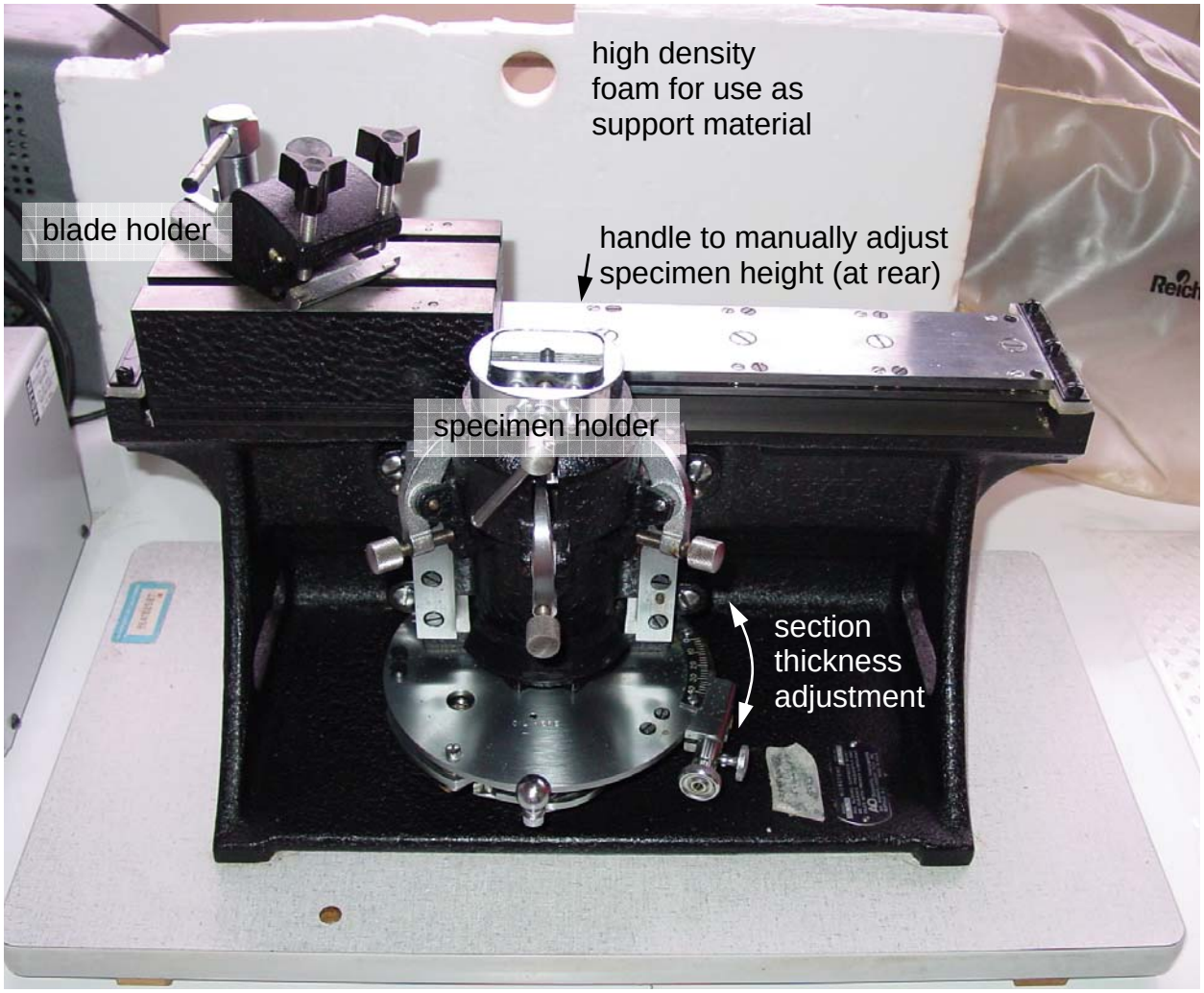
6. Collect the sections plus support tissue in a watchglass, or in water in one section of the planchette. If you use a transparent watchglass, it's easier to pick the thinnest sections later on. Accumulate sections there – cut LOTS of sections.

7. Look at the sections under the dissecting microscope to select the very thinnest, they will be almost transparent – this is easy to see if you place the transparent watchglass onto black paper or other black background. Transfer some of these very thin sections to a SMALL drop of water on a microscope slide.
8. Cover with a coverslip – put one edge of the coverslip into one edge of the drop of water and lower the coverslip slowly with the forceps. If you drop the coverslip onto the sections you are likely to get air bubbles. If the water doesn't extend to the edges of the coverslip, add a SMALL amount of water – don't flood the sections or they will float around.
9. Observe on dissecting or compound microscope. If you need to add more contrast to the sections – more than you can get by closing down the iris diaphragm on the microscope, you can stain your sections. See instructions about staining.

Sliding or sledge microtome sectioning of fresh or fixed tissues

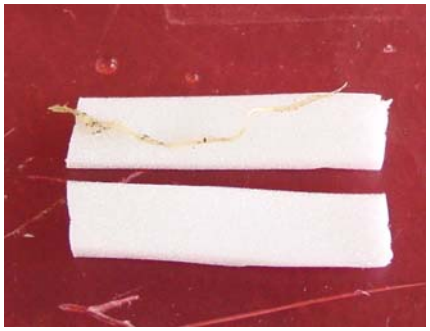
Materials:

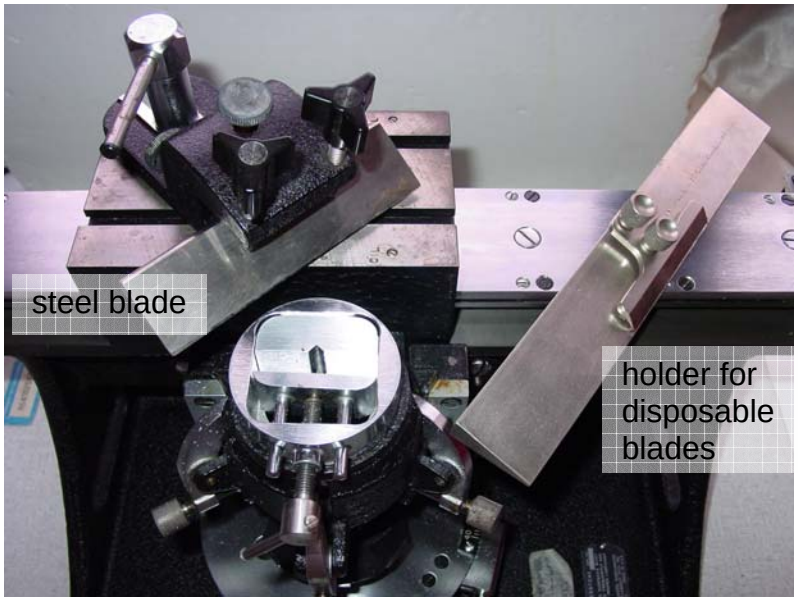
support material, for small tissue pieces – the best we've found is high density foam, which is very good for soft tissues
hard tissues, like woody roots or stems, may not need supporting material
sharpened microtome blades or disposable microtome blades
water - conical flask with plastic or glass pipette, not a wash bottle
watch glasses or planchette with water to put sections in
reasonably fine forceps
fine paintbrush and/or sharpened orange sticks to transfer sections
slides
cover slips
dissecting microscope - doesn't have to be a fancy one
some detergent - 1% Tween-20 or Triton-X-100 – use this if your leaf or other tissue is very hydrophobic, it will help to cover the tissue with water so you don't get air bubbles between tissue and coverslip or slide



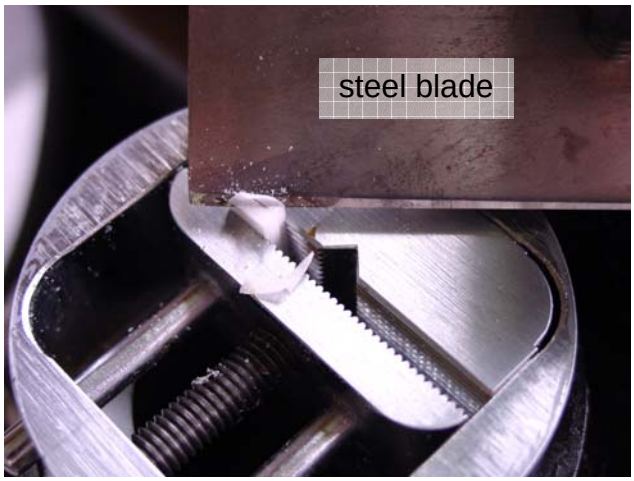
1. Place the tissue to be sectioned in a beaker of water, cut out a small piece for sectioning and place in a drop or small pool of water on the pink dental wax.
2. Cut out a small block of support material, then cut it in half. If sectioning a large or soft piece of tissue that might become compressed between the support material, make an appropriate-sized groove in one of the halves (note that since this material is somewhat compressible, it doesn't squash the tissue as much as the carrot). Make sure the tissue to be sectioned and the support material remain wet at all times.

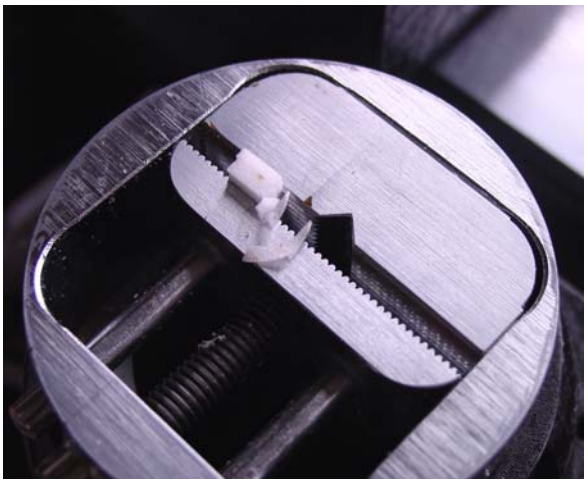
3. Sandwich your tissue between the two halves of the support material.





4. Place the sandwich into the specimen holder on the microtome and tighten until the material is held firmly, but without compressing the plant tissue unduly.





5. Adjust specimen height until it is just below the blade.
6. Pull the blade to the right (in this case) and increase specimen height manually until the blade is cutting the tissue plus support material. Push the blade back to the left before the next cutting stroke.

   A more ergonomic way to section is to stand with your right side towards the bench, and pull the blade towards you and push away from you. Best to avoid back strain if you have much sectioning to do!
7. Adjust section thickness as desired, this microtome sections up to 50 µm thick, and down to 5 µm or so depending on density of tissue being sectioned.
8. Collect the sections with forceps, or with the tip of a paintbrush (with few bristles – pull or cut the rest out), or with a sharpened orange stick into water in a watchglass, or into water in one section of the planchette. If you use a transparent watchglass, it's easier to pick the thinnest sections later on. Accumulate sections there – cut LOTS of sections.
9. Look at the sections under the dissecting microscope to select the very thinnest, they will be almost transparent – this is easy to see if you place the transparent watchglass onto black paper or other black background. Transfer some of these very thin sections to a SMALL drop of water on a microscope slide.
10. Cover with a coverslip – put one edge of the coverslip into one edge of the drop of water and lower the coverslip slowly with the forceps. If you drop the coverslip onto the

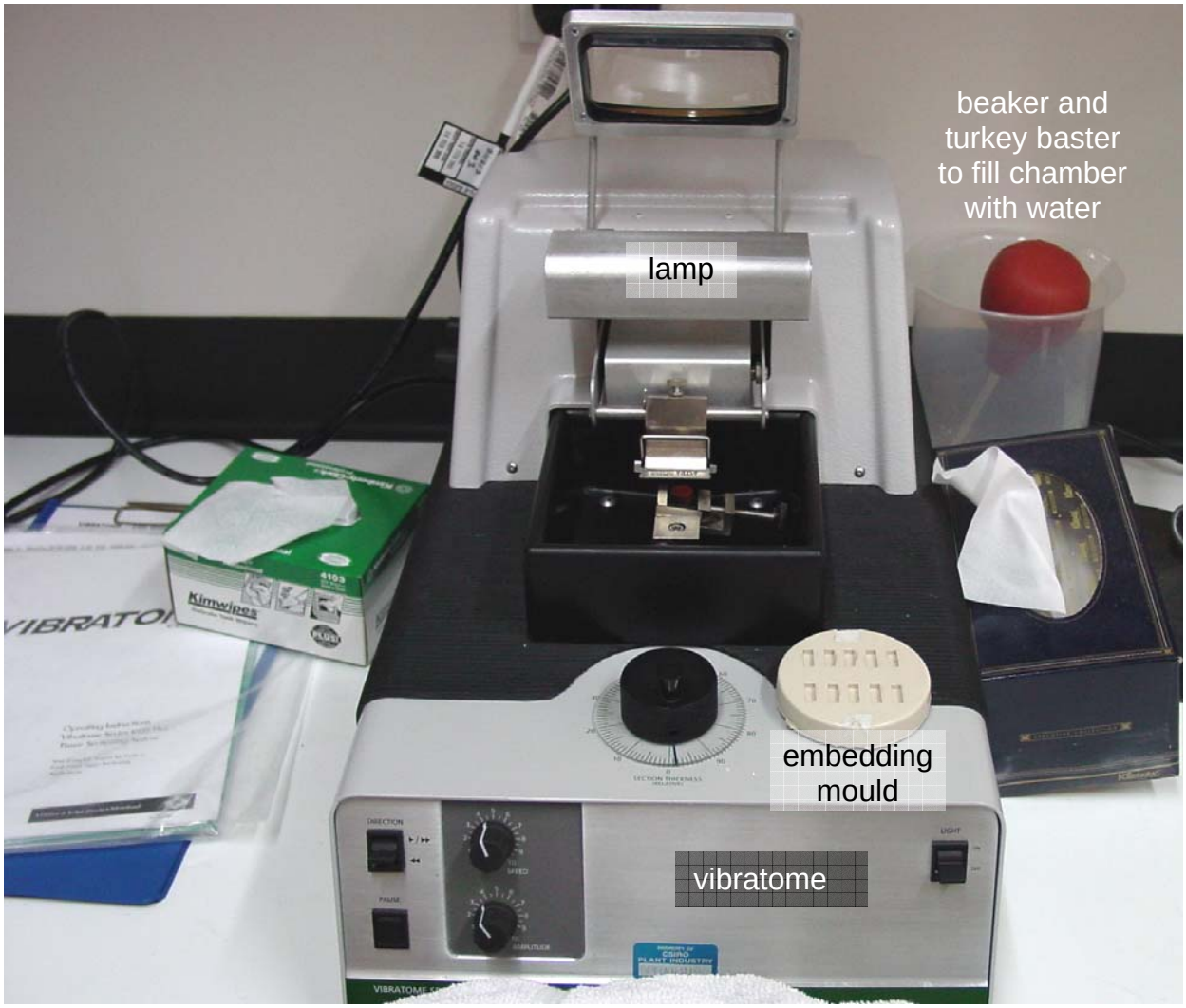
sections you are likely to get air bubbles. If the water doesn't extend to the edges of the coverslip, add a SMALL amount of water – don't flood the sections or they will float around.

11. Observe on dissecting or compound microscope. If you need to add more contrast to the sections – more than you can get by closing down the iris diaphragm on the microscope, you can stain your sections. See instructions about staining.

Vibratome sectioning of fresh or fixed tissues

Materials (this protocol is for **root cross-sections**, but will work for other tissues):

Agarose - ~3% is good for most roots
1-2% for very soft roots
>3% for harder roots and other tissues
regular Progen DNA grade agarose, gels at 35-37°C, melts at >90°C, is OK
embedding moulds, either commercial or homemade (homemade illustrated below)
water bath, set at about 60ºC, depending on % agarose used
Locktite or other cyanoacrylate-based superglue
Sharp razor blades - single-edged for trimming agarose blocks before glueing down
double-edged carbon steel for sectioning
piece of pink dental wax to trim agarose blocks on
water - conical flask with plastic or glass pipette, not a wash bottle
reasonably fine forceps
fine paintbrush and/or sharpened orange sticks to transfer sections
sticky slides
Petri dish with moist filter paper to keep sticky slides while collecting sections

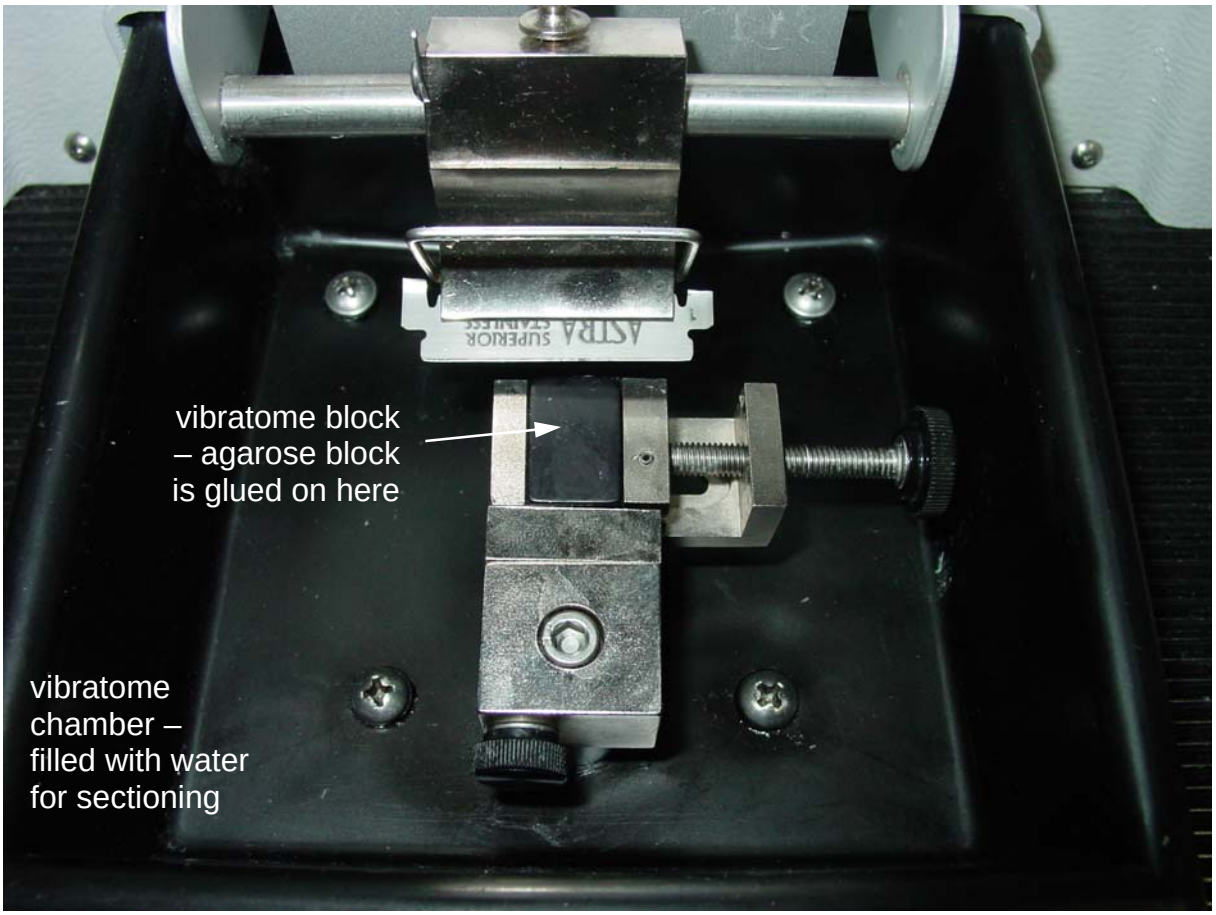


1. Melt agarose in microwave – loosen lid first, and place agarose bottle in a dish of water, and keep it molten in the waterbath. Also place a small flask of water in the bath, and put some plastic pipettes in this – so you can withdraw molten agarose from the bottle without it clagging up the pipette. Place the embedding mould on a support in the bath so it's just above the water surface – to keep it warm.
2. Fill embedding moulds half-way with molten agarose.

3. Dry off root and lay on top of agarose - root must be dry or it will pop out of the agarose during sectioning.

4. Cover root with molten agarose and allow to set firmly. If the root does not stay in the agarose during sectioning, place the embedding mould containing root in molten agarose back onto its support in the waterbath so the agarose can infiltrate a little into the outer layers of the root. Then when it sets, it will then be held better in the agarose.

5. Pop agarose block containing root out of the mould, and trim so that root is exactly vertical (for cross-sections) or horizontal (for longitudinal sections).



6. Glue trimmed agarose block onto black vibratome block with superglue.
Trim block carefully so that top and bottom are parallel before glueing down.
Line up on vibratome block first to check before glueing.

7. Fill vibratome chamber with water or buffer until it comes up to the top of the chuck.
Place vibratome block with sample into chuck and clamp firmly.

8. Insert half of double-edged razor blade, or injection blade, into holder.
Set blade angle to between 10° and 25° in the first instance.

9. Wind chuck up or down so that the top of the agarose block is just below the blade.
Top up vibratome chamber so water/buffer level just touches the blade and covers the specimen.

10. Set amplitude and speed at about the middle to start with.
Advance the blade, check that it does not begin to slice off a large chunk of tissue.

11. Advance the blade until it begins to section through the agarose and tissue.
Use section thickness of 50-200 µm, depending on cell size.

12. Collect each section carefully with forceps and place on a sticky slide (coated with silane, polyethyleneimine, poly-L-lysine or other adhesive material).
Keep slides in a moist chamber until sectioning is finished.

13. Stain the sections!!

Cryostat sectioning of frozen tissues

You can cryo-section fresh or fixed tissues, and tissues are either frozen before placing in embedding compound on the cryostat stub, or are frozen after placing in embedding medium on the stub, which is the more common method. For the latter method, fixed tissues are either rinsed in buffer or water and frozen directly, or they are infiltrated with up to 15% sucrose, and/or coated in molten agar, then frozen on the stub. The sucrose acts as a cryoprotectant to prevent ice crystal damage to subcellular structures, and is only needed if you need to see this level of detail.

Materials:

TissueTek or other embedding compound (viscous liquid)
fine and coarse paintbrushes to brush debris off blade and frozen tissue – these go into the cryostat before a run
Sharp razor blades - single-edged for trimming agarose blocks before glueing down
single-edged disposable carbon steel for sectioning
water - conical flask with plastic or glass pipette, not a wash bottle
reasonably fine forceps
sticky slides
Petri dish with moist filter paper to prevent sections from drying while collecting sections

Getting started (Note – some of these instructions are specific to our instrument)

1. Turn the cryostat on the night before you plan to use it, or in the morning if you plan to use it that afternoon. It needs a few hours to get down to temperature.

2. When you are ready to section your frozen tissue, make sure you have the complete cryostat toolkit containing forceps, razor blades, sticky slides, small paint brushes, allen key (to undo knife attachment), cryostat blades, water bottle, coverslips and TissueTek. Also have on hand an esky with dry ice if you need to freeze tissue onto a stub outside the cryostat (step 4.)
3. Turn on the light and anti-fog on the cryostat.

Preparing to section

4. You now need to attach your frozen tissue to one of the cryostat stubs using TissueTek. This is a messy job. Try not to drop TissueTek all over the place, and note that it's easiest to clean out of the cryostat while still frozen.

   Put a large dob of TissueTek on a stub (wipe the frost off the stub first). If you can, leave the stub in its holder in the cryostat. If this is too awkward, put the stub on dry ice in an esky.

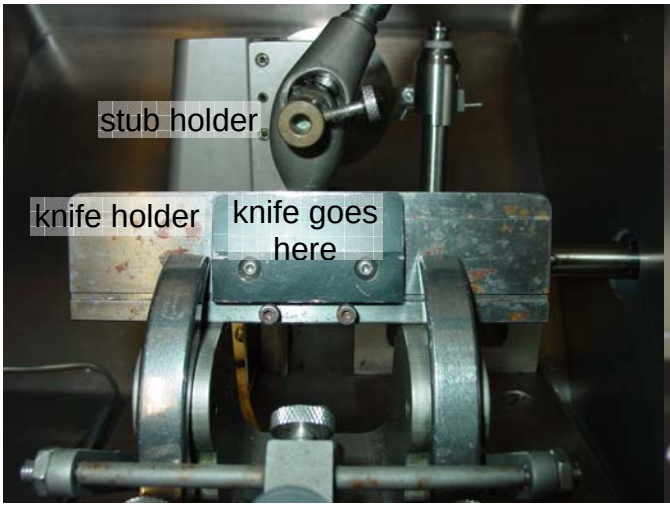
    Cryostat stub – these should be kept cold in the chamber.

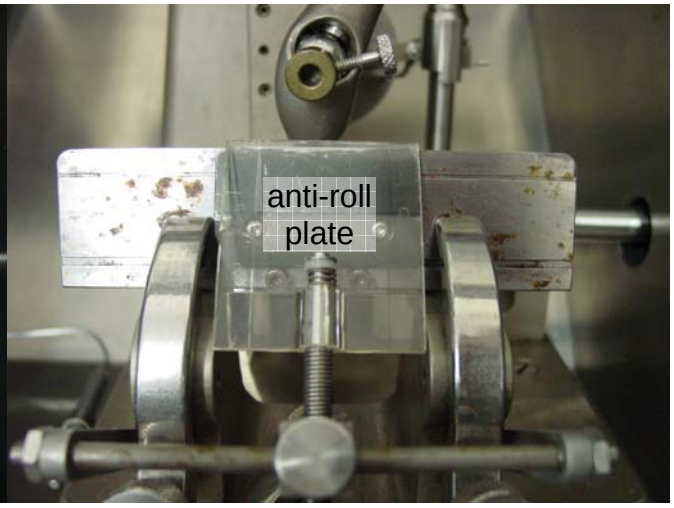
5. Jam your frozen tissue quickly into the TissueTek before it has all frozen, and arrange in the best orientation for sectioning. Leave to freeze completely. You can freeze your tissue more rapidly by plunging into liquid nitrogen.

   Add more TissueTek around the tissue so it is well-supported for sectioning and re-freeze.

6. If you need to use a new knife, remove the knife holder by undoing the two silver clamps holding it in. Then remove the old knife by undoing the two allen nuts, slide old knife out carefully then slide new knife in carefully, making sure the edge of the knife is parallel to the edge of the knife holder and with all of the sharpened edge showing. Take care not to cut yourself on the blades.

   Tighten allen nuts tightly then replace knife holder into cryostat.



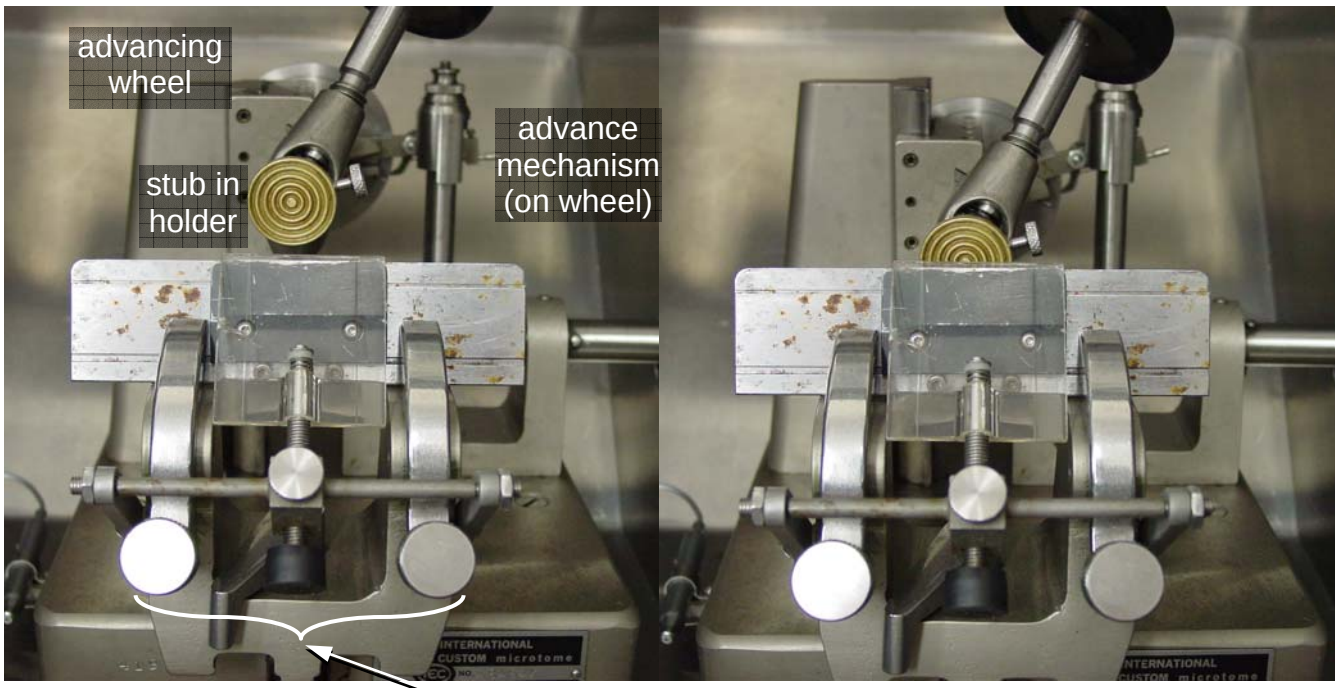


7. Now place the stub plus tissue into the stub holder – this is behind the knife, and the stub is held in place by tightening the knurled brass knob. The specimen can be oriented by undoing the large black plastic handle and rotating the stub holder around until it's in the correct position.

   If the knife is too close to the stub holder to insert the stub correctly, you will need to either retract the knife or retract the stub holder.

   Retract the knife by placing one hand behind the entire knife holding contraption in the cryostat and pushing the silver clamp at the base of this to the left – it is quite stiff, you will need to exert some force. When the clamp is loose, pull the knife holding contraption towards you then re-tighten the clamp.

   Retract the stub holder by turning the advancing wheel right at the back of the device in the cryostat anti-clockwise. You will need to lift the advance mechanism off the wheel before doing this.



8. Once the stub is in the holder and oriented, bring the specimen close to the knife by either unclamping the knife-holding gizmo and moving it closer to the tissue, or by turning the advancing wheel clockwise (with advance mechanism lifted off the wheel).

   Use the advance handle (large stiff wheel on right hand side of cryostat) to raise and lower the specimen as you are positioning the knife close to it.

Sectioning and collecting sections

9. Rotating the advance handle (on right hand side of machine) clockwise will move the tissue up, forward, then down past the knife. If the tissue is close enough, a section of tissue will be cut and stay on the knife edge. If the advance mechanism is positioned on the wheel, the tissue will also move closer to the knife ready for the next section.

   The maximum automatic advance is 8 µm – set the advance mechanism to 8 and make sure the mechanism is contacting the advance wheel.

   Thicker sections can be obtained by advancing the wheel at the back manually, by pushing down on the advance mechanism at any time before cutting the next section. The further you push down, the thicker the section.

   For many plant tissues, especially if not infiltrated in cryoprotectant, thicker sections are better.

10. When you are ready to collect sections, you need to rotate the square plastic shield, which is hanging off the front of the knife-holding device, around so it's resting on top of the knife edge.

    Now, when you rotate the advance handle, the section should slide under the plastic shield and stay flat rather than rolling up. If the section does not do this, use the screw at the back of the shield to move it incrementally forward or backwards until you get good sections.

11. Only cut one section at a time.

    To collect the sections, lower a sticky slide, sticky side down, onto the specimen. When the slide gets near the specimen it will start to melt onto the slide, at which time you can raise the slide and the section should come with it.

    Depending on how you want to process the section, either air-dry onto the sticky slide, protecting from dust under a large petri dish, or allow to dry off slightly, mount in mounting medium (e.g. water) and cover with a coverslip.

12. Once you have finished sectioning, scrape out all frozen TissueTek that you've dropped into the machine, remove specimens from stubs and clean stubs, place stubs back into machine and turn machine off.

    Insert funnel into right hand side of machine with plastic beaker beneath to collect any condensation.

    The next day, wipe out the machine with a towel.