ULTRATHIN SECTIONING
For conventional TEM it is usual to examine sections of 50-70 nm thick. Only with sections this thin is it possible to obtain sharply focused images at high magnification. In fact, for high magnification (>30,000x) it is preferable to examine even thinner sections provided that there is still sufficient contrast present in the specimen.

Trimming blocks
1. Place the block into the specimen holder and tighten the clamping screw. Make sure that the block is firmly fastened into the holder as lose blocks can cause problems when sectioning (see below).

2. The block should be trimmed to a trapezoid shaped pyramid with the section area being as small as possible to include the part(s) of the specimen of interest. There are several ways to achieve this and two ways are outlined below.

A. The block in the specimen holder is mounted onto the arm of the microtome and the arm put into the locked position. Using a clean razor blade and looking down the binoculars trim away the excess resin from each of the four sides of the block to obtain a trapezoid shaped pyramid. Initially leave a margin of resin around the specimen. Once a trapezoid shape has been achieved use a dry glass knife to trim the front surface of the block until you start cutting the specimen. At this stage semi-thin sections can be cut (see below) to allow you to access the specimen and locate particular parts of the block. The four sides of the block can then be carefully trimmed down again using a new clean razor blade so as to obtain the smallest possible block face that includes only the area(s) of interest. To obtain ribbons of sections make sure that the top and bottom of the block are parallel.

B. The block may also be trimmed using a new glass knife with the knife and block being rotated simultaneously to obtain a trapezoid.

Semi-thin sectioning
Semi-thin sections can be cut using either a dry glass knife or a knife with a liquid-filled boat.
1. Cut semi-thin (0.5-1 µm) section(s) and remove the sections from the boat with either a clean wire loop or mounted eyelash.
2. Transfer the section(s) to a drop of water on a clean glass slide - the section should float in the drop. (Pre-treating the slides with 0.5% gelatin in distilled water and letting them dry prior to use will make the section adhere better to the slide.) Place the slide on a low temperature hot plate - this will help expand and flatten the section and will make the section adhere better to the slide than if the section was allowed to dry an ambient temperature.
3. The sections can be viewed either directly with a phase contrast microscope or stained with toluidine blue, or methylene blue (see below). For a temporary slide, mount cover slip with glycerol (removable with water) or, for a permanent slide, seal the edges of the cover slip with nail varnish. (Most solvent-based mountants cause the stain to bleach out – often with 24 h - and are not recommended.)

Cutting ultrathin (50-70 nm thick) sections
1. Place the knife (glass or diamond) in the knife holder and check that the holder is tightly fastened and at a clearance angle of 6º.

2. Advance the knife/knife holder by hand to within ~1 mm of the block and clamp the holder in position.

3. With the specimen arm in the upper, locked position, adjust the position of the block so that the top of the block is positioned just below the knife-edge. This sets the end of the cutting stroke (when the motor cuts-in again to initiate another cutting cycle) and by default also sets the beginning of the free-fall cutting stroke (this is ~4 mm above the knife edge).

4. Advance the knife stage using the wheel at the front of the microtome until only a thin slit of reflected light is visible on the specimen face. This slit of light should be of the same thickness along the whole face of the block – this means that the block face and the knife-edge are parallel in the left-right direction. Make any necessary rotational or lateral adjustments to make this slit of light parallel (remember that with a glass knife, the left-hand side of the knife-edge is the sharpest part of the edge).
Remember that this may not apply for the vertical movement so carefully move the block up and down in front of the knife and watch the slit of light to see if it changes in thickness.

5. Once you have aligned the block face to the knife, continue to advance the knife until the thin slit of light begins to appear yellow and/or disappears. The knife is now within approximately 1 µm of the specimen face.

7. Fill the knife boat with either filtered distilled water or 10% ethanol so as to obtain a silvered reflection off the surface of the liquid. Make certain that the liquid 'wets' the edge of the knife particularly along the part that you are using to cut.

8. Either cut individual sections by hand or activate the motor and begin cutting continuous sections. Adjust the section thickness settings until you obtain pale gold to silver sections.

Section pick-up
1. Generally pale gold to silver sections will expand without any problems when using 10% ethanol in the knife boat. However, if the sections do not appear to expand they can be expand with either chloroform or acetone vapour. Use either a thin, wedge-shaped piece of filter paper or a cotton swab soaked in the solvent and gently wave it over the sections. Be careful not to over-expand the sections as this can lead to damage (cracks and/or loss of material from the section).

2. The easiest way to collect the sections on to a grid is to use uncoated grids and carefully lower the grid, dull side downwards, onto the sections. You must lower the grid parallel with the surface of the boat liquid or else the sections will be bunched-up onto the side of the grid by surface tension effects. If you lower the grid down slowly over the sections you should be able to see the sections through the grid mesh and be able to place the grids into the centre of the grid.
Depending on how big the sections are between 3-10 sections can be collected onto each grid. It is better to have a few good sections on a single grid and collect a number of grids, than to try to put large numbers of sections onto 1 or 2 grids.

3. Place the grid onto a piece of filter paper with the sections facing upwards. If you use uncoated grids then the liquid will be absorbed through the grid mesh and you should see the sections on the grid.

(An alternative method of collecting sections is by putting the grid under the liquid in the knife boat and then coming up under the sections with the gird. It is easier to pick-up sections this way using uncoated grids as the liquid will (usually) pass through the holes in the grid. With coated grids the liquid flows over the grid surface and may carry the sections away from the grid as you raise it under the sections.)

4. Allow the grids to properly dry on the filter paper before staining with aqueous uranyl acetate and lead citrate using the standard thin section staining protocol.

TROUBLSHOOTING:
The following is a brief summary of **some** of the difficulties that can occur during sectioning along with a few tips on what to do if these problems occur.

Difficulty wetting the knife-edge:
Fill the boat with liquid (10% alcohol or distilled water) until the water level is a little too high, wait a few minutes, and then carefully remove the excess water.
Often a mounted hair or eyelash can be carefully drawn along the cutting edge of the knife while the boat is full.

For a diamond knife clean the knife-edge with alcohol (100%) and a cleaning rod.

Wetting the block face:
For epoxy resins the main cause of this problem is either filling the boat too full (causing the liquid to be dragged over the knife edge by capillary action) and/or electrostatic charging (low room humidity and or transportation).
Solutions:
i) Lower the water level ever so slightly until you have an even silver reflection over the whole of the liquid surface or at least around the part of the knife-edge you are cutting from.
ii) Dry the block face with filter paper.
iii) Use an antistatic device (gun) to eliminate electrostatic charging.

For methacrylates (LR White, Lowicryl etc.) some of which are hydrophilic and tend to wet the block surface because they attract water.

Solutions:
i) Lower the water level to a concave shape.
Note, however, that lowering the liquid level in the knife boat may cause difficulties with wetting the knife-edge (to combat this just follow the steps outlined above in edge wetting) and you will have difficulties with surface reflection (to overcome this problem it may be possible to adjust the light source to a more appropriate angle).

Chatter:
Chatter is caused by a number of different reasons the most common of which are:
i) screws are not fully tightened (block, block holder, and knife).
ii) clearance angle is too small (may cause friction between the block face and the diamond face).
iii) cutting pressure too great – mainly caused by trying to cut too large a block face and/or poorly embedded specimen
iii) external vibrations.
iv) a faulty microtome.

Solutions include:
Make sure all of the screws are tightened.
Increase the clearance angle by 1-2 degrees (usually cut at a clearance angle of 6º).
Reduce the block size and/or try cutting another specimen.
Change the location of the microtome.
Have the microtome checked by a service engineer.

Section compression:
This occurs normally due to the physical process of section cutting and can be rectified by using chloroform or acetone vapour, but can be a problem under certain conditions.
The main causes and possible solutions are:
The block is too soft – try putting it back in the oven for a few days.
The knife is dull – time to change the knife.
The clearance angle is too big and or the cutting speed is too high - reduce the clearance angle by 1-2º and/or reduce the cutting speed from 1mm/sec to 0.5mm/sec.

Nicks, tears etc to sections:
Damage to the cutting edge of the knife will cause damage ranging from very fine lines through to tears in your sections.
The causes of damage to the knife-edge include:
i) remnant particles in your block from trimming.
ii) hard particles in your block and specimen.
iii) normal use of the knife will all cause nicks.

Solutions include:
Use a new, clean razor blade for the final trimming of the block. This will prevent small, hard bits from attaching to the leading edge of the block and/or damaging the knife-edge.
Many specimens will have ‘hard particles’ in them and it is difficult to counter the problems they cause. One way is to be aware of this problem and use a harder resin mix and/or modify the embedding procedure to try to overcome the problem.
During normal use the knife-edge will become less sharp. So with glass knives replace the knife once scoring on the sections becomes visible.

Staining semi-thick sections for LM
Toluidine blue.
Add 1 gm of toluidine blue and 1 gm of sodium borate to 100 ml distilled or deionized water and allow to dissolve completely. Filter the stain through a Whatman type ?? filter paper. Alternatively, use a 0.2 µm disposable filter.

Azure II –Methylene blue.
Solution A:
0.5 gm of methylene blue in 50 ml distilled water.
Solution B:
0.5 gm of azure II in 50 ml of distilled water.

To make a working solution, mix equal volumes of solutions A and B.

Staining procedure:

1. Place several 0.5-1.0 µm sections on a droplet / individual drops of clean distilled water on a slide. Place the slide on a hot plate (temperature 60-80˚C). For large sections a lower temperature usually prevents or lessens wrinkling. Allow the sections to dry on to the slide (heat fix) for 5-10 min. Too short a time will cause the sections to wash-off during the staining/washing steps.

2. Place drops of filtered stain on to the sections and heat for long enough until a metallic (greenish) dry edge appears around the drop. The actual time will vary depending on the temperature of the hot plate, amount of stain and the type of tissue.

3. Rinse off stain with distilled water and dry.
NOTE: Toludine blue stained sections will change from a pink-purple colour to blue once the sections have dried.
Only the tissue section should stain; if the resin also stains then place the block(s) back into the oven since resin polymerization has not been properly completed.