**TEM sectioning**

List of items for sectioning

These are the items needed for sectioning:

* Slides
* Grids
* Petri dishes
* Grid forceps
* Glass stip for making glass knives
* Box of glass kinives with double sided sticky tape
* Silver tape for boats
* Nail polish and dental wax for sealing boats
* Filter paper
* Eyelash on a stick
* Orange stick
* Single edge razor blades
* Double edge razor blades
* Toluine blue stain
* Distilled water in a wash bottle
* Distilled water in a beaker
* 95% EtOH in wash bottle
* Grid log sheet
* Grid holder
* Uranyl acetate and lead stain
* Pipettes
* 2 washing beakers containing de-ionised water
* NaOH pellets
* Long cover slips
* Permount
* Parafilm
* Demo blocks

Mounting of serial sections

**Materials**

* Copper wire cut into about 4cm long pieces (#22 wire, acetone cleaned).
* 0.5% solution of Formvar kept water free in molecular sieve (Fisher Scientific, type 3A, grade 564). Formvar made up in ethylene dichloride or chloroform can be used.
* Glass slides and other glassware necessary for Formvar coating slides.
* Small blocks of balsa or styrofoam.

**Procedure**

1. The copper wire is bent to form a loop at one end. The loop should be about 4mm in diameter. The remaining shank is used as a handle so the loop can be manipulated. The loop should be flat and free of irregularities.
2. Formvar films are prepared on glass slides by the dip method and air dried. The film is scored to produce 6 x 6 mm squares which are floated off on distilled water. These squares are picked up on the loops and excess water drained off by blotting the base of the loop with filter paper. The loops can be conveniently stored in an upright position, poked into a balsa or styrofoam block and covered by a glass beaker or bell jar.
3. A ribbon of sections floating on the surface of the water in a Dupont knife boat is picked up from underneath with the Formvar coated loop. Successive ribbons are kept in order on the block until a series is completed.
4. The sections are mounted on single-hole “slot” copper grids (2 x 1mm) under a dissecting scope. The grids are placed dull side up on a rotatable peg (eg: Ladd Cat. No. 1025 or a homemade one) and the loop centered over the grid with the ribbon of sections orientated parallel and in the center of the slot grid. The loop is drawn over the grid. Here you can affix the handle of the loop to the stage of a compound microscope with tape and simply lower the stage. This will allow the loop even passage over the peg. After the Formvar and ribbon of sections are affixed to the grid, the grid is passed over water vapor to stretch and firmly attach the Formvar. Usually the vapor from a small beaker of water warmed over a bunsen burner is adequate. The grids are then stored in LKB-type grid boxes. The grids should be stored on end and not in contact with other grids or objects.
5. Staining of sections is by the usual methods employed for your material. Grids should be thoroughly dry before carbon coating. Placing the grids in a desiccator overnight is a good practice. If grids are to be transported, for example, to a HVEM laboratory, they should be coated on site before use. Carbon coating tends to make the Formvar membrane very brittle.
6. Care must be taken during the carbon coating to prevent breakage of the film. Grids should be coated on a holder so air can move freely above and below to offset and pressure differences during pumping of the evaporator. The carbon electrodes should be shielded to prevent sputter damage to the films and the carbon rods should be baked dry and degassed before use.

Rough trimming by hand

1. Clamp the speimen block into an appropriate holder. Place the holder with block into a stable holder under a dissecting microscope.
2. Cut into the specimen by making a series of slices with a new, double-edged razor blade.
	* Break in paper wrapper and clean with 95% EtOH and kimwipes to remove oils.
	* Trim block parallel to the tabletop and should stop just before the desired area in the specimen is reached (as shown in Fig.1-A).
	* The razor cuts should be as thin as possible to prevent removal of large chunks from the specimen block by haphazard fracturing rather than cutting.
3. Trim resin from top to just reach specimen (Fig.1-A). Begin making a series of razor cuts to a depth of 1 to 2 mm (thin slices) and at a 60 degrees angle relative to the desk top along one side of the block (Fig.1-B). – Base of trapezium.
4. Rotate the specimen block 180 degrees and begin a second series of 60 degrees cuts until the side of the specimen is reached (Fig.1-C). – top of trapezium:
	* These cuts will generate a narrow specimen platform on the top of the pyramid. The platform should be wide enough so that no important areas of the specimen are cut off, but not so wide as to cause sectioning difficulties.
	* When in doubt, do not trim away any areas until a survey section has been examined in the light microscope.
5. Rotate the specimen block and make a third and fourth series of side cuts a 60 degree angle to generate a trapezoid-shaped pyramid top (Fig.1-D and 1-E).

The ultramicrotome and the sectioning process

1. Once specimen block has been trimmed, the holder bearing the block is securely clamped into the arm of the ultramicrotome.
2. Set the block at 0 and the block holder in the vertical position. Make the knife holder at 0.

**Knife advancement**

1. Advance knife to within a few milimeters of the specimen block face by hand without touching the block. Clamp the knife holder in place and any further advancements are made by the ultramicrotome controls.
2. Knife should also be moved laterally to bring a good cutting edge of the knife opposite the block face.

**Specimen orientation**

1. Orient the leading edge of the specimen block parallel to the knife edge and horizontal to the table top.
2. Knife edge must be adjusted so that it is parallel to the specimen cutting face. For this, put the underneath light on so that you can see the shadow between the knife and the block.
3. \*\*Important: Knife should always be moved back away form the block during the specimen orientation procedure to avoid damaging the knife edge.

**Water level adjustment**

1. Knife is mounted in the holder and slightly overfilled with deionised distilled water. Edge of knife should be wetted.
2. Lower the water level using a small syringe until a silver to silver/gray reflection is obtained with the flurorescent light of the microtome. Level should not be so low that the knife edge becomes dry.
3. \*\*Never let sections dry down onto the knife edge: they are difficult to remove once dried.

**Ultrathin sectioning**

1. Set the thickness control to an appropriate point, ultrathin sections are cut and collected in the trough of the knife.

Thickness of sections:

* + Colorless to gray sections = 30-60nm
	+ Silver/gray to silver = 50-70nm
	+ Gold = 70-90nm
	+ Purple = 100-190nm
	+ Blue = 200+nm

**Section retrieval**

1. There are several ways to retrieve sections. Find the one which suits you. Manouever the sections with a clean (95% EtOH) eyelash on a stick.
	* For thick sections
		+ Cut the tip of an orange stick into a wedge to pick up sections. Roll out onto a drop of water on a clean glass slide.
	* For thin sections
		+ Submerge the grid and maneuver it directly under the sections to be collected.
		+ Slowly lift up the grid parallel to the water surface to collect the sections. This only works if there is no static electricity in the grids.

Methods to clear static:

* + - sonicate in acetone and ethanol to clean grids
		- glow discharge
		- use water from the medicine cups with destatic plastic also helps.
1. Gently contact the underside of the grid and the edge of the filter paper, permitting the water to soak into the paper.

Knife making protocol

**Clean glass strip**

1. Rinse in tepid tap water.
2. Put small quantity of Sparkleen on damp paper towel.
3. Wipe glass strip with paper towel + sparkleen once.
4. Wash thoroughly in tap water.
5. Rinse well with deionized distilled water.
6. Wipe off water by sweeping glass with Kimwipes once each side.

**Positioning and breaking glass strip**

1. Place clean glass strip on knife breaker.
2. Place needle edge of glass strip in downward position (i.e. rough side down).
3. Line right end of glass strip up with dot on right side of instrument table top. The dot permits positioning of the 16 inch glass strip in order to break it exactly in half.
4. Score and break glass. The resulting product is two 8 inch glass strips.
5. Remove one strip and store for later use.

**Making glass squares**

1. Make sure:
	* Locking lever is in rear position.
	* Scoring shaft is pushed in and breaking-knob is turned all the way counter-clockwise.
	* Disengage knob is turned all the way counter-clockwise and pulled out to lock it in position.
2. Slide glass strip to arresting stub allowing it to rest against guide plate.
3. Lower clamping head with locking lever until it rests on the glass.
4. Gently swing locking lever all the way down, clamping strip. DO NOT USE ANY UNDUE FORCE IN PRESSING LOCKING LEVER DOWNWARD.
5. Place score selector (11) in position and fork under left end of strip.
6. Pull scoring shaft forward until it stops.
7. Turn breaking knob clockwise until glass breaks.
8. Turn knob counter-clockwise until it stops.
9. Lift up scoring shaft and swing locking lever to rear position.
10. Push scoring shaft to rear position.
11. Remove square with fork.

**Making glass knife diagonals**

1. Set score selector on 25.
2. Take the bottom right hand corner and move it counter-clockwise to make a diamond.
3. Place diamond square on breaking pins.
4. Push disengage knob in and turn clockwise.
5. This is done to move rear glass holder (28) forward to hold glass diamond.
6. Swing locking lever forward to locked position.
7. Place fork under glass square and pull score shaft forward until it stops.
8. Slowly turn breaking knob clockwise to break glass.
9. After breaking return to counter-clockwise stop.
10. Lift score shaft and at the same time swing locking lever to rear position.
11. Push scoring shaft to rear position.
12. Pull out disengage knob to locked position.