Sectioning on the Leica UC7 Ultramicrotome

Shannon Modla

Bio-Imaging Center

Delaware Biotechnology Institute

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Both diamond and glass knives can be used to obtain ultra-thin sections of epoxy-embedded material for viewing with the TEM. However, due to the expense and added care one must use when sectioning with a diamond knife, beginners should first learn to section with glass knives.

Part 1. Checking Glass Knives

1. View the glass knife with a dissecting microscope or using the binoculars of the ultramicrotome.

2. Examine the knife edge for any imperfections, whiskers, knicks, or contamination (Fig. 1A).

3. The sharpest edge is on the left side of the knife (Fig. 1A). This area is highlighted by a stress line that starts in the corner and arcs down toward the heel. The usable knife edge (Fig 1A) extends from the left side and up to where imperfections occur on the right side of the knife edge. The area where the stress line contacts the knife edge (far left corner) should be avoided.

4. Of the two knives created by bisecting the glass square, one knife is generally of higher quality than the counter piece. When the two knives are viewed as a pair, the knife with the larger heel is sharper and more durable than its counter piece. Leica recommends using glass knives with a 1 mm heel for thin sectioning of resin blocks.

5. Store good knives in a knife holder box to prevent dust and dirt from contaminating the knife edge. A cheap and effective knife box can be made by sticking down double-sided tape to the bottom of a pasteboard cryobox. The knives can be held upright by tacking their bottoms to the tape.

6. Discard unwanted knives into a sharps container.

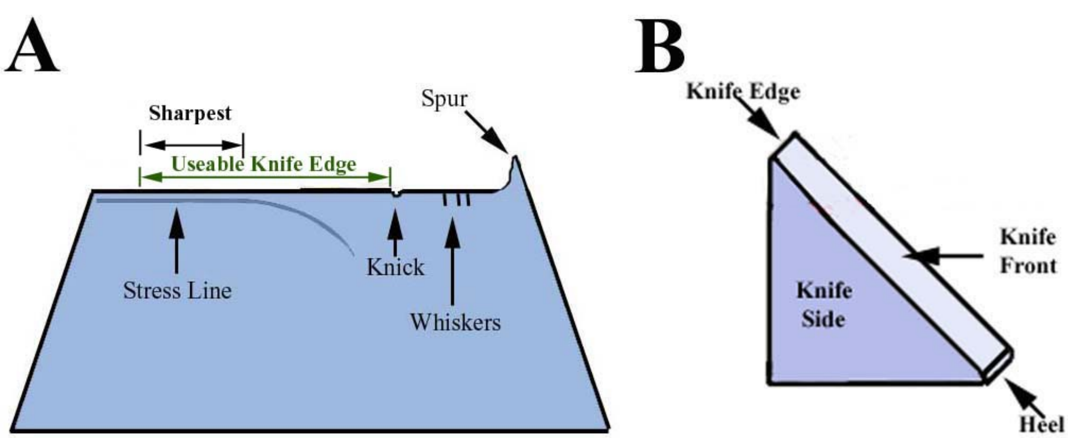


Figure 1A-B. A. Diagram showing knife imperfections and cutting areas of a glass knife. B. Features of a glass knife.

Part 2. Making Boats for Glass Knives

When cutting a sample embedded in epoxy resin on the ultramicrotome, sections are floated onto water. For this to occur, a boat that will hold water must be attached behind the knife edge.

<u>Supplies needed:</u>
Glass knives
Shiny silver tape
Scissors
Nail polish
Razor blades

1. Remove the outermost layer of silver tape to expose a clean portion of tape that is free of fingerprints on the adhesive surface.

2. Using scissors, cut a 3-4 inch strip of tape. This is more tape than is needed to make the boat, but extra is cut so that the tape can be gripped by the two ends without getting body oils on the middle of the cut strip. Avoid touching the portion of the tape that will be against the knife side or on the inside of the boat.

3. Without touching the knife edge, carefully stick the middle portion of the tape to the knife side adjacent to the knife edge (Fig. 2A). The tape should be perfectly aligned with the knife edge — tape should not extend too far above or below the knife edge. Otherwise, the boat may not hold water properly. Make sure the strip of tape is parallel to the bottom of the knife and press firmly to stick the tape to the knife side.

4. Holding the opposite end of the tape, wrap the strip of tape around the knife front and stick it to the opposite knife side as described in step 3 (Fig. 2B, C). The tape should not be too tight or too loose — it should form a U-shape behind the knife edge.

5. With a razor blade, remove excess tape from the back of the knife by applying a single smooth stroke from the bottom of the knife toward the knife edge (Fig. 2D). Be careful not to damage the knife edge.

6. Seal the bottom of the boat to the knife front using nail polish (Fig. 2D, 3). Ensure the nail polish is completely dry before using.

7. Boats and knives can be stored for several days to weeks in a knife holder box. The knife edge should never be touched. Although knives can be stored for a period, the best results will be obtained when using newly made knives.

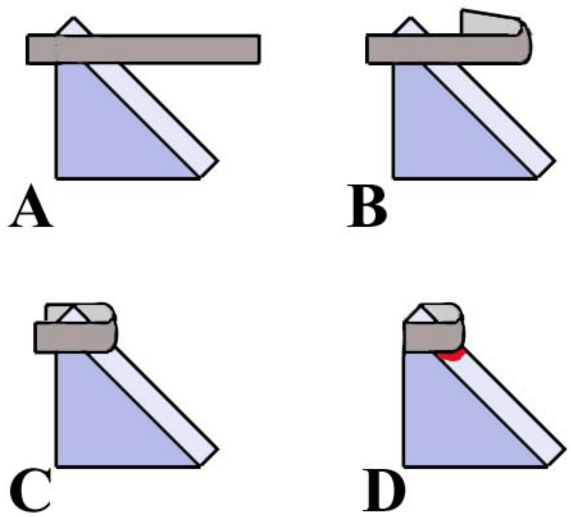


Figure 2A-D. Diagram illustrating how to make a boat from silver tape.

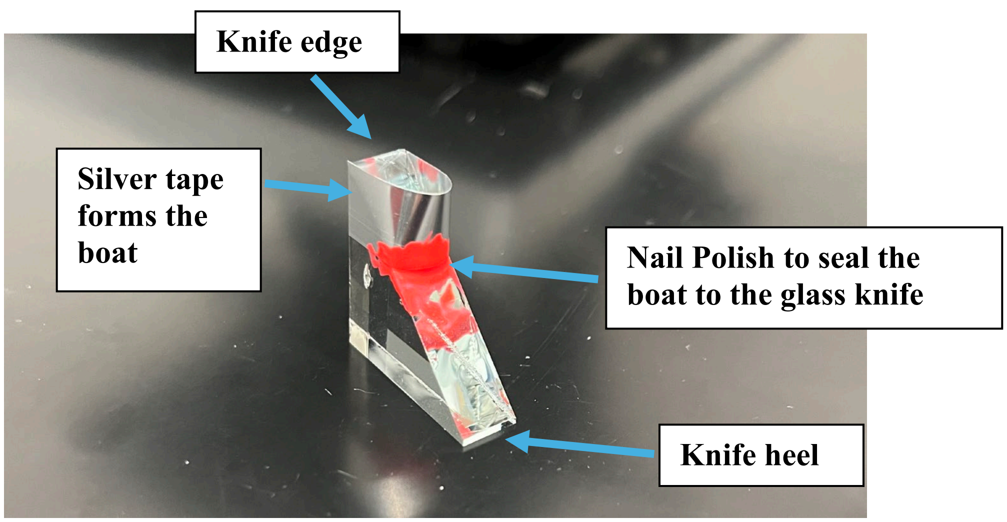


Figure 3. Glass knife with boat.

Part 3. Buttons and Knobs

1. Binoculars
2. Magnification knob
3. Focus knob
4. Knob to tilt binoculars (Note: This is to adjust the reflection of the water in your boat and not intended for ergonomic adjustments)
5. Knob to move the binoculars forward and backward
6. Cutting wheel
7. Stage guides with knife holder
8. Cantilever arm with chuck and segment arc
9. Allen key

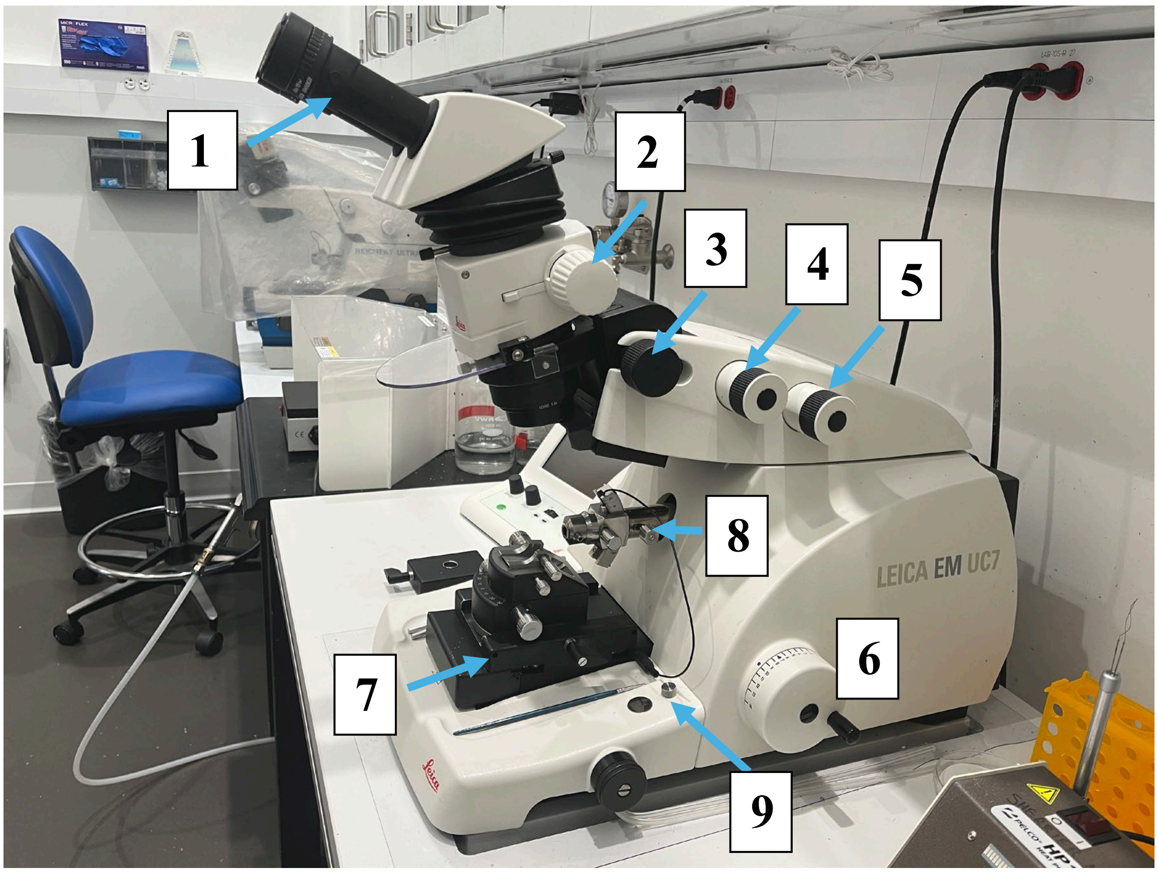


Figure 4. Labeled parts of the Leica UC7 Ultramicrotome.

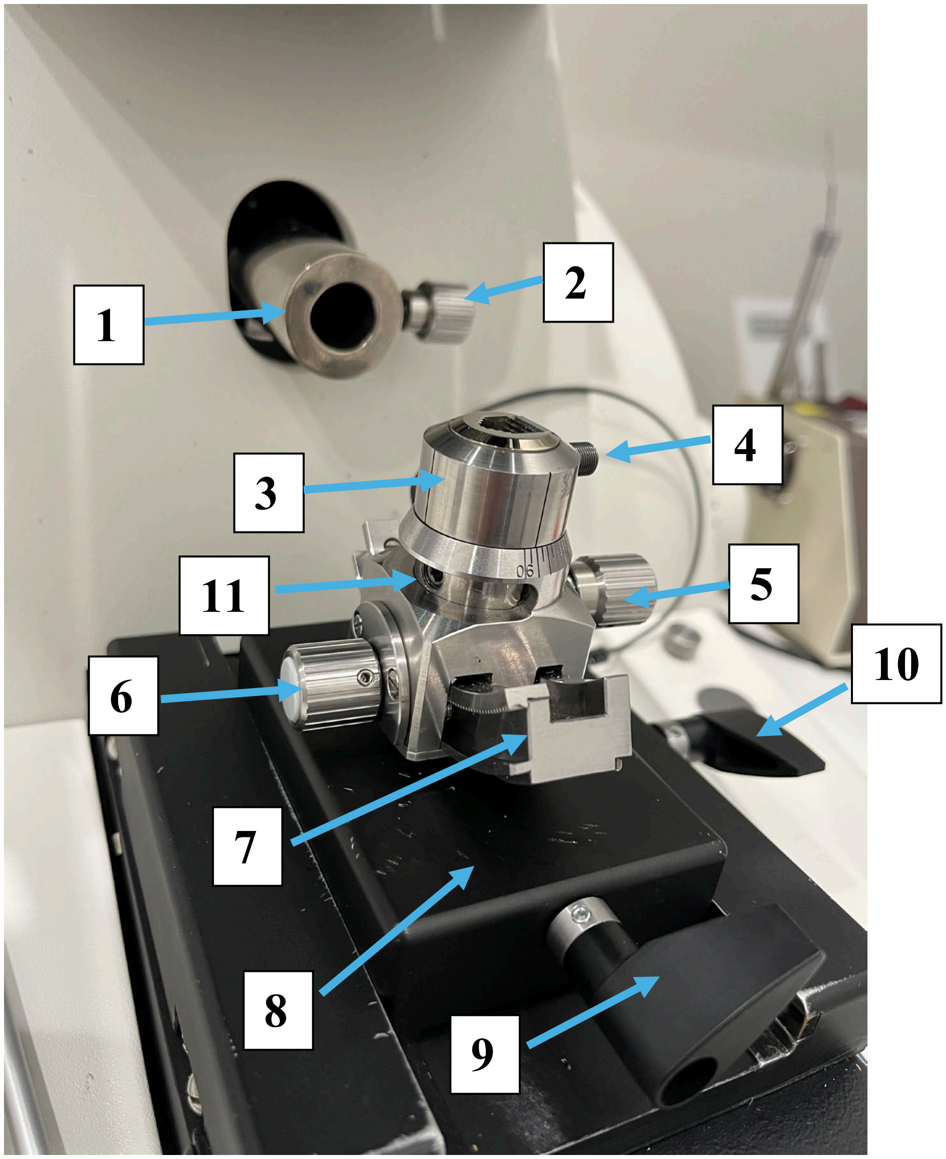


Figure 5. Labeled parts of the chuck and trimming block.

1. Cantilever arm
2. Cantilever arm set screw: Secures chuck into the cantilever arm
3. Chuck: Holds specimen block
4. Specimen block clamping screw: Secures specimen block into chuck with an allen key
5. Knob that rotates the chuck
6. Knob that tilts the chuck along the segment arc
7. Segment arc
8. Trimming block
9. Locking lever 1: Secures chuck/segment arc to the trimming block
10. Locking lever 2: Secures trimming block between the stage guides
11. Locks chuck into the segment arc with the allen key

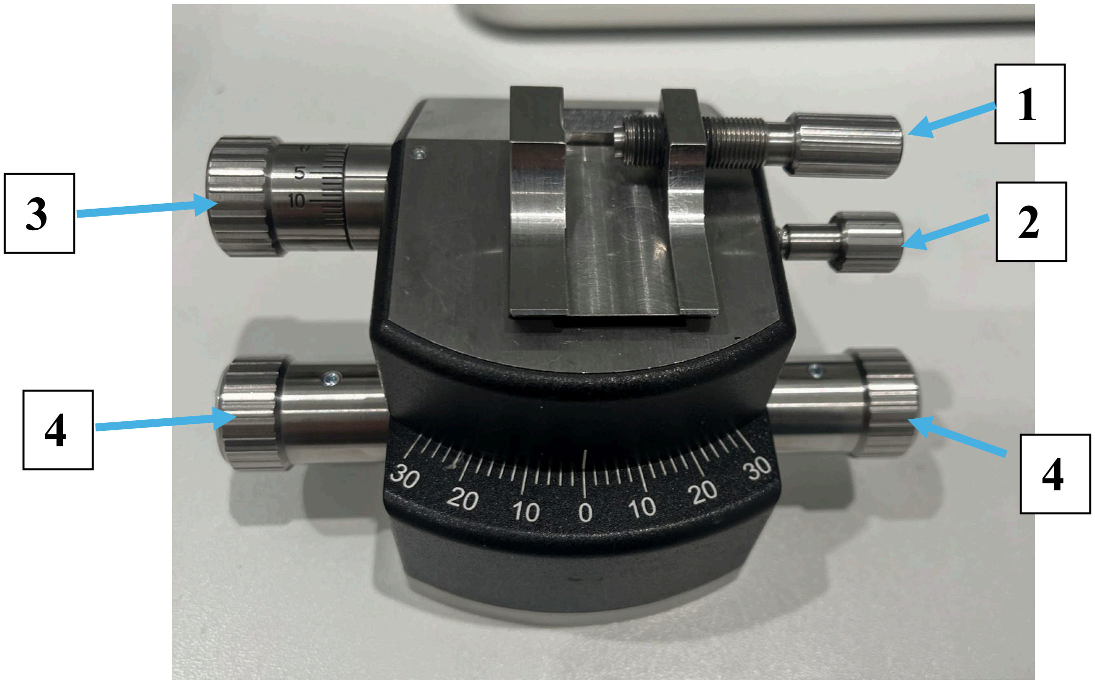


Figure 6. Labeled parts of the knife holder.

1. Clamping knob: Secures knife into knife holder
2. Clearance angle locking screw
3. Clearance angle adjustment knob
4. Knife carrier pivot/tilt control knobs

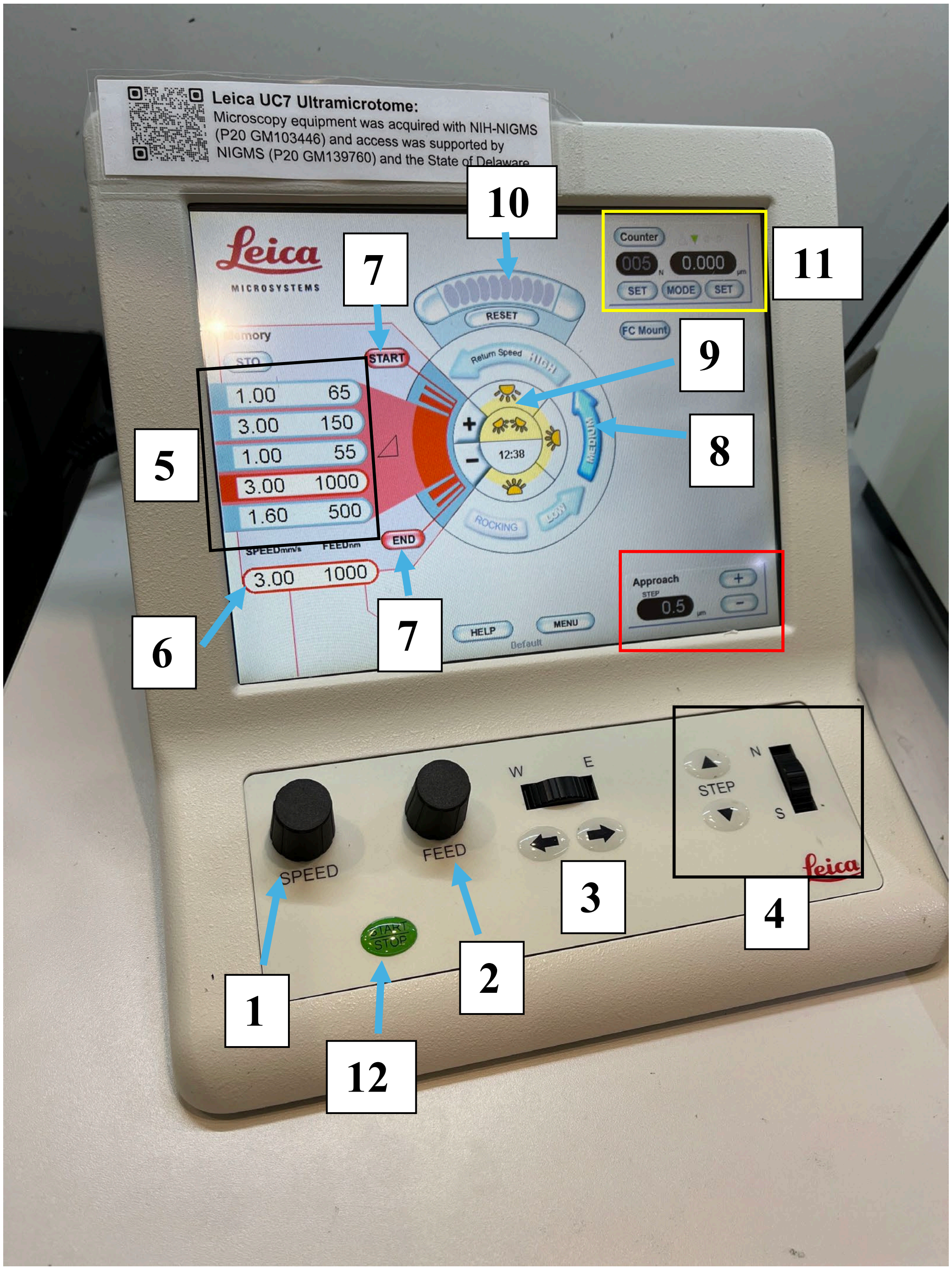


Figure 7. Labeled touch screen controller

1. Knob that sets the cutting speed
2. Knob that sets the section thickness (increment by which the cantilever arm advances with each cutting stroke)
3. Moves the knife laterally left and right
4. Moves the knife forward and backward. The step arrows allow you to do a fine advance or retraction of the knife based on the value in the "Approach" box on the

screen (red box). The roller wheel allows you to do a coarse advance or retraction of the knife.

5. User-defined presets for cutting speed and feed that are stored into the memory for convenience.
6. Current cutting speed and feed
7. Start and End. These buttons allow you to define the cutting window. The cutting window is that location where the block passes in front of the knife and a section is cut. The cutting speed must be lower for the cutting window than during the return, so it is important to set the cutting window properly.
8. Return speed settings. You can set the return speed to be slow, medium, or high. This is how fast the cantilever arm moves before it returns to the cutting window.
9. Lights. You can turn on or turn off various lights and adjust their brightnesses. There is a light that shines through the trimming block, a light that shines through the specimen block when it is mounted in the cantilever arm, and there are lights that illuminate the entire stage from above.
10. Reset will reset the cantilever arm. The cantilever arm has a limited feed and eventually it must be reset and retracted. The amount of feed used is displayed in the oval shapes above the word ‘reset’. As the cantilever arm advances a certain distance, each of those ovals will fill and turn red. When all the ovals are red, you have run out of feed.
11. This lets you select different sectioning modes. There are several different modes available: Counting, Count Down, E-W Measurement, and AutoTrim. I most frequently use the default mode, Counting. This will count how many sections were cut and show you how many microns you have cut into the specimen. To toggle through the modes, press mode. The other handy mode is Count Down. This mode allows you to set a depth in microns, and when you start the ultramicrotome, the machine will cut until it has reached that defined depth and then automatically stop. This mode is convenient when you want to systematically sample sections through the specimen.
12. Start/Stop Button: Starts or stops the automatic cutting of the ultramicrotome.

Warning Sounds

a. Sounds when cantilever arm is left in the bypass position. Rotate the cutting wheel clockwise until the cantilever arm is in the cutting position.
b. Sounds when the cantilever arm has reached its limit. Retract the cantilever arm by pressing reset on the touch screen controller.

Part 4. Trimming Trapezoids

Tissue samples that are embedded in a resin must be trimmed into a shape that is conducive to sectioning. The shape most often used is that of a trapezoid block face with sloping sides. This shape has several advantages: makes orienting the block to the knife easier, minimizes compressive forces on the sample as it is sectioned, and facilitates serial sectioning. The exact method for trimming a block can vary depending on the sample and goal of the project, but the below pointers give a general guide.

1. Obtain a box of razor blades. Single-edge razorblades (Fig. 8A) or double-edge razorblades (Fig. 8B) may be used, but I prefer double-edge razor blades for most samples. The double-edge razor blades are much sharper and give clean cuts. NOTE: Both types of razorblades are sharp and care should be taken so as not to cut yourself.

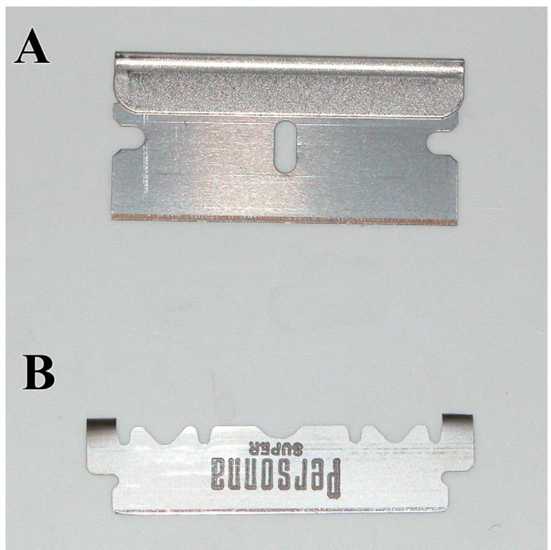


Figure 8. A. Single-edge razorblade. B. Double-edge razorblade (snapped in half).

2. Tighten the sample into the chuck and secure the segment arc into the trimming block on the ultramicrotome stage. Lock the trimming block between the stage guides. You do not want your sample block moving around while you trim. The type of chuck used depends on whether the sample was embedded in flat molds or in a round BEEM capsules (Fig. 9).

3. If the sample is not at the top of the block, remove excess resin from the surface until you reach the tissue (Fig. 10A, B). Grip the razor blade by the sides so that the sharp edge is facing toward the front of the ultramicrotome. Make thin slices parallel to the ultramicrotome stage and stop just before the desired area in the specimen is reached. If the sample was treated with osmium tetroxide, it will appear black and be easy to see. Try to make the surface of the block as flat as

possible—rotating the specimen block while looking at it through the binoculars will help to determine if the surface of the block is flat.

4. Now trim a trapezoid. The top and bottom sides of a trapezoid are parallel with the bottom side being longer than the top. The two sides of the trapezoid are slanted, and the two bottom corners should preferably make equal angles with the long bottom side (Fig. 11).
   a. Using a razor blade, make a series of thin cuts along one side of the block to a depth of several millimeters and at a 45-60° angle relative to the table top along one side of the block (Fig. 10C). This will form one of the parallel sides of the trapezoid. These cuts should be made with the edge of the razor blade angled toward you (this allows more control when trimming).
   b. Rotate the specimen block 180° and repeat step 4a to form the opposite parallel side (Fig. 10D). Make sure the two sides are parallel as this will allow a straight ribbon of sections to be formed once sectioning begins.
   c. Rotate the specimen block 90° and make a series of cuts at about a 60° angle to generate one of the slanted sides of the trapezoid (Fig. 10E).
   d. Rotate the specimen block 180° and repeat step 4C to make the opposite slanted side (Fig. 10F). In the end, the trapezoid should have sloping sides of a 45-60° angle with a relatively flat surface (Fig. 11 and 12), and it should contain the region of interest of the specimen.

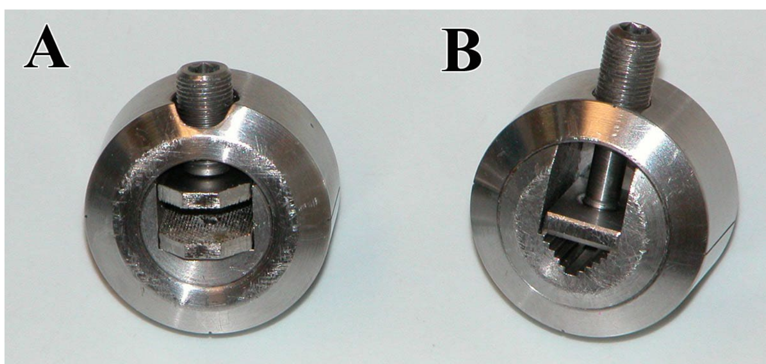


Figure 9. A. Chuck for samples embedded in flat molds B. Chuck for samples embedded in round BEEM capsules.

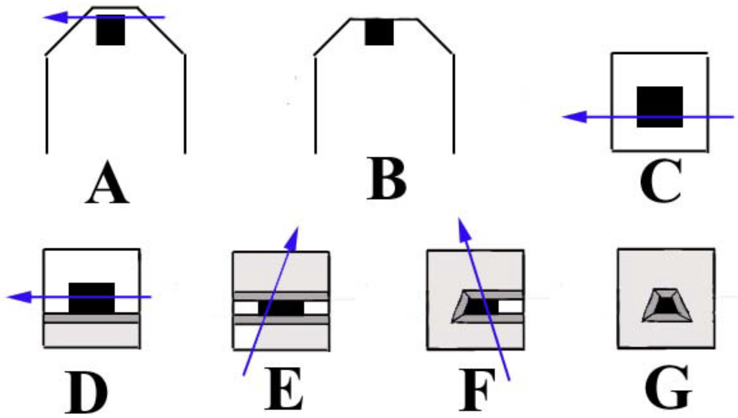


Figure 10A-G. Steps to trimming a trapezoid from a specimen block.

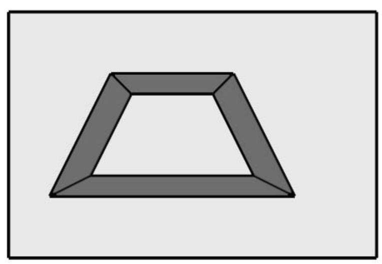


Figure 11. Illustration of a trapezoid as seen from above.

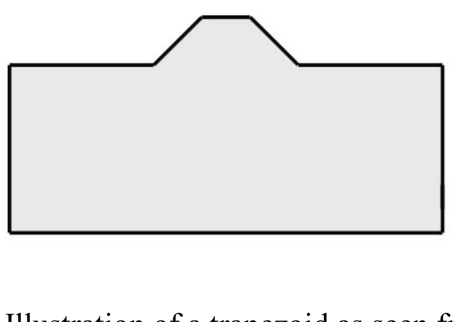


Figure 12. Illustration of a trapezoid as seen from the side.

Part 5. Facing the block with a glass knife

Facing the surface of the trapezoid will form a smooth, reflective surface that will make it easier to align the trapezoid with the knife. Facing should always be done with a glass knife.

1. Adjust the tilt angle on the segment arc to 0°.

2. Insert the segment arc into the cantilever arm and secure it in place with the set screw.

3. Place the knife carrier between the guides of the ultramicrotome stage and secure it into place with the locking lever. The knife carrier should be set back away from the sample so as not to ram the knife into the block.

4. Place a glass knife into the knife holder and secure it in place with the set screw. A boat does not need to be attached to the knife for facing the block.

5. Set the clearance angle of the knife to 4-6°. Loosen the clearance angle locking screw. To decrease the clearance angle, press down on the wedge-shaped base supporting the knife until the desired angle is reached. To increase the clearance angle, rotate the clearance angle knob counterclockwise. Retighten the clearance angle locking screw once the clearance angle is set. **Note:** The value of the clearance angle is that number read from the clearance angle knob only when the knob is fully turned counterclockwise to its stopped position.

6. Set the knife tilt angle on the knife carrier to 0°.

7. Adjust the cutting wheel so the block face is in front of the knife. Unlock the knife carrier and manually approach the block until the knife is 3-4 mm from the block face. Relock the knife carrier. Extreme care should be taken so as not to accidentally ram the knife into the specimen block.

8. Using the course advance, cautiously approach closer to the block. Once again, do **NOT** ram the knife into the block. A shadow/reflection of the knife edge on the block face can be used to gage the distance between the knife and the block. The thinner the shadow/reflection, the closer the knife edge is to the block.

9. Rotate the specimen block until the long, bottom side of the trapezoid is parallel with the knife edge.

10. Move the knife laterally so that the left or middle of the knife edge is in front of the specimen block.

11. Advance the sample toward the knife in 0.5-1.0 µm increments using the approach step function on the control panel. Turn the cutting wheel clockwise through a cutting stroke.

12. Continue this process of fine advance with the approach step function followed by a complete turn of the cutting wheel (clockwise only!) until you observe sections cut from the block face. Continue cutting sections until a complete trapezoid-shaped section is cut from the surface of the block. The number of cutting strokes required to obtain a full section will depend on how flatly the surface of the trapezoid was trimmed.

13. The surface of the trapezoid should now be completely flat with a mirror-like quality. The trapezoid may need to be placed back into the trimming block and retrimmed using a fresh razorblade. Make sure the top and bottom sides are parallel.

Part 6: (Optional) Refining the top and bottom of the trapezoid with a glass knife

In some instances, such as when cutting serial sections, it is necessary to make the top and bottom sides of the trapezoid perfectly parallel and not ragged. Since this can be difficult to do by hand, the top and bottom sides can be more precisely trimmed with a glass knife.

1. Place the specimen block into the chuck. Rotate the chuck such that the top side of the trapezoid is perpendicular to the 90° tick mark on the chuck holder. Once you've reached this position, secure the block into the chuck.

2. Insert the segment arc into the cantilever arm and the knife into the knife holder.

3. Rotate the block exactly 90° using the tick marks on the chuck holder as a guide.

4. Tilt the knife carrier so that the right edge of the upper part of the knife carrier is aligned with the 10° mark on the left side of the lower part of the knife carrier.

5. Slowly approach the top side of the trapezoid by moving the stage forward with the course advance.

6. Shave thin sections off the top side of the trapezoid by rotating the cutting wheel and approaching the block in very small increments using the stage course advance.

7. When one side is completely trimmed, back away the knife. Rotate the block **exactly** 180° and repeat steps 5-6 to trim the bottom side of the trapezoid.

8. If the glass knife has a good, straight edge, the top and bottom sides of the trapezoid should be crisply cut and perfectly parallel.

Part 7. Thin sectioning with glass knives

Before beginning to thin section, the specimen block should be securely mounted into the chuck and the segment arc should be secured to the cantilever arm. The knife holder should be set back away from the sample so as not to ram the block into the knife.
If the specimen block, segment arc, knife, or knife holder are not firmly secured, you will get vibrations in your sections. Vibrations cause bands of alternating thick and thin areas in your sections that appear parallel to the knife edge, and this artifact is called chatter.

A. Filling the boat

1. Insert a fresh glass knife with a boat into the knife holder and secure it with the set screw.

2. Using a syringe, fill the boat with filtered, clean water (ie. de-ionized/distilled/RO/Milli-Q) until a positive meniscus is visible above the level of the boat. A single drop of filtered Tween-20 solution (1 drop Tween 20 in 100 ml de-ionized water) may be added to the boat to reduce the surface tension (This helps to keep the edge of the knife wet). The boat should always be filled from the **back** to avoid damaging the knife edge.

3. Using a 1 cc syringe with a needle, withdraw water from the **back** of the boat until the water level is slightly below the level of the boat while still leaving the knife edge wet. Be careful not to poke a hole in the boat! The correct water level is achieved when the water near the knife edge has a uniformly silvery surface. If the water level is lower than the knife edge, a gray crescent adjacent to the knife edge will be visible. The water level should be checked periodically as it will naturally become lower due to evaporation. Improper water levels can cause sectioning problems. If the water level is too high, water can jump from your knife to the block face. If the water level is extremely low, the water will retract from the knife edge and the knife edge will not be wet.

B. Aligning the block to the knife and thin sectioning

If the block face is properly aligned with the knife edge, a full section can be rapidly obtained with minimal cutting strokes. Four alignments must be made: specimen rotational alignment, knife lateral alignment, knife tilt alignment, and specimen tilt alignment. When using glass knives, these alignments can be made by observing a shadow or reflection that is cast onto the block face by the knife. When using diamond knives, a line of reflected light is cast onto the block face.
Important Note: The knife should always be moved back, away from the block, during any change in alignment to avoid damaging the knife edge. Once you have tweaked an alignment, reapproach the knife to the block face.

1. Adjust the cutting wheel so the block face is in front of the knife. Unlock the knife carrier and manually approach the block until the knife edge is 3-4 mm from the block face. Relock the knife carrier to secure it between the stage guides. Extreme care should be taken so as not to ram the knife into the specimen block.

2. Using the course advance wheel cautiously move the knife closer to the block. Once again, do **NOT** ram the knife into the block. As the knife approaches the block, a shadow or reflection of the knife edge will be projected onto the block face when viewed through the binoculars. The knife must be fairly close to the block face to see this shadow/reflection. Don't expect to see it if your knife is very far from the block face. The thickness of this shadow/reflection can be used to estimate the distance between the block face and the knife edge: the larger the shadow/reflection, the greater the distance between the knife edge and block face; the smaller the shadow/reflection, the closer knife edge is to the block face.

3. Specimen rotational alignment (Fig. 13):

    a. Rotate the specimen block until the long, bottom edge of the trapezoid is parallel with the knife edge.

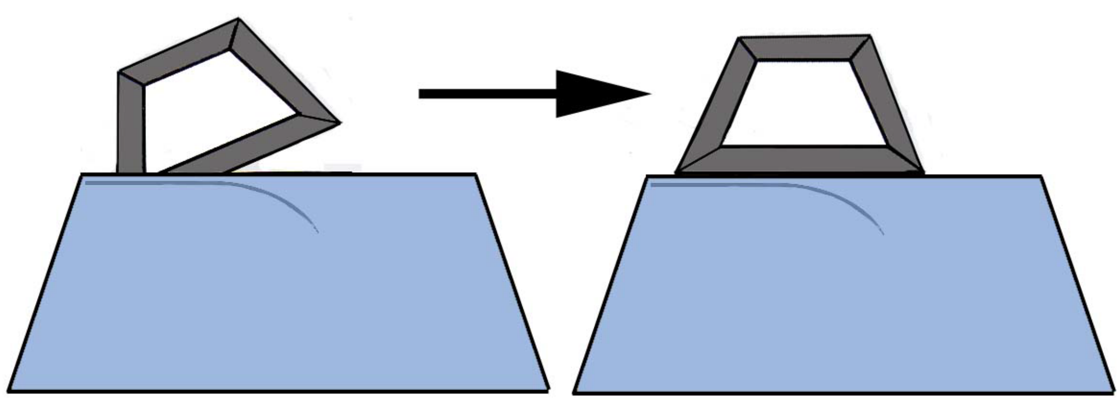


Figure 13. Specimen rotational alignment.

4. Knife lateral alignment:
    a. Move the knife laterally so that the sharpest edge of the knife (usually left) is in front of the specimen block. Avoid areas with knicks and whiskers if present as any knife defects will cause gouges in the block face that appear as vertical lines (these defects are called knife marks).

5. Knife tilt alignment:

a. If the shadow/reflection cast onto the block face by the knife edge is thinner on the right side of the block face than the left, then the knife is closer on the right than the left (Fig. 14A). To correct this, tilt the knife to the left. If the shadow/reflection is thinner on the left side of the block than the right, then the knife is closer on the left than the right (Fig. 14B). To correct this, tilt the knife to the right. Adjust the knife tilt until a shadow/reflection of uniform thickness is seen across the block face.

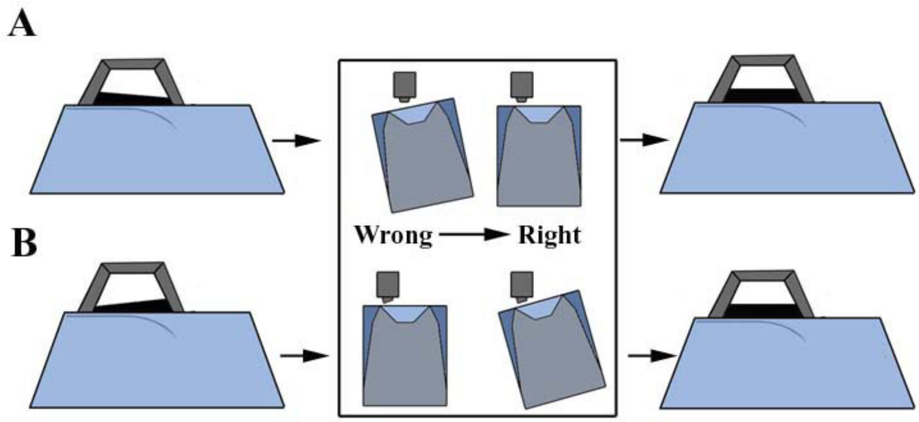


Figure 14A, B. Diagram illustration knife tilt alignment.

6. Specimen tilt alignment
   a. While slowly moving the specimen through a cutting stroke, carefully study how the shadow/reflection line changes as the block face passes in front of the knife edge. If the shadow/reflection line is thinner at the bottom of the trapezoid and becomes thicker at the top of the trapezoid, the top of the block face is tilted further away from the knife than the bottom of the block (Fig. 15A). Therefore, the block must be tilted forward. If the shadow/reflection line is thicker at the bottom of the trapezoid and becomes thinner at the top of the trapezoid, the top of the block face is tilted closer to the knife than the bottom of the block (Fig. 15B). In this case, the block needs to be tilted back. Adjust the specimen tilt until the width of the shadow/reflection line remains constant across the entire block face during a cutting stroke. **Be sure to back the knife away from the specimen after every change in alignment to avoid damaging the knife and then cautiously reapproach the knife to the block!**

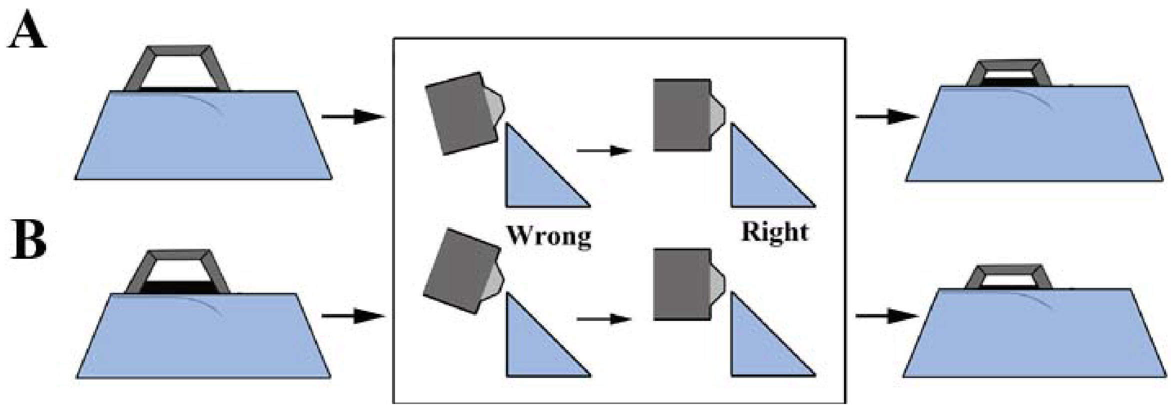


Figure 15A, B. Illustration showing specimen tilt alignment. A. If the block is tilted too far back, the shadow line grows as the block face passes in front of the knife edge. To correct, tilt the block forward. B. If the block is tilted too far forward. The shadow line shrinks as the block face passes in front of the knife edge. To correct, tilt the block back.

7. Set the cutting window. The cutting window is the vertical distance located above and below the knife edge whereby the specimen block passes over the knife edge during the downward cutting stroke of the cantilever arm and a section is cut. Setting the cutting window will allow the movement of the cantilever arm to be slowed to an appropriate speed as it passes over the knife edge. To set the cutting window:
    a. Turn the cutting wheel clockwise until the specimen block is just above the knife edge.
    b. Press Start (See Fig 7 item 7) on the touch screen controller.
    c. Continue to turn the cutting wheel clockwise just until the top of your trapezoid is completely below the knife edge.
    d. Press End (See Fig 7 item 7) on the touch screen controller.

8. Set the sectioning speed to 0.8 mm/sec. The speed may need to be varied depending on the sample characteristics.

9. Approach the block face using the thickness of the shadow/reflection line as a guide. Be careful not to ram the knife into the block face.

10. Choose a section thickness of 150 nm on the touch screen controller.

11. Go through a series of cutting strokes by rotating the cutting wheel until the entire block face is cut and whole sections can be seen floating on the water.

12. Set the section thickness to approximately 65 nm on the touch screen controller to obtain sections with a silver interference color. Press start/stop

on the controller, and the ultramicrotome will automatically start to section. A ribbon of sections should form in the boat, floating off the knife edge. Do not bump the table during sectioning to minimize vibrations.

13. To stop sectioning, press start/stop on the controller.

C. Section Thickness

Although the ultramicrotome has a section thickness control, the true thickness of the sections should be determined from their interference colors. When white light is reflected from the bottom and top of the section, the light will be differentially slowed down depending on the thickness of the section. Consequently, the phase of the light will change. These emerging wavelengths interfere with those reflected from the water's surface, which gives the sections a particular color. The thickness of the sections can be determined with an interference card (Fig. 16) that gives a color spectrum with the corresponding section thickness.

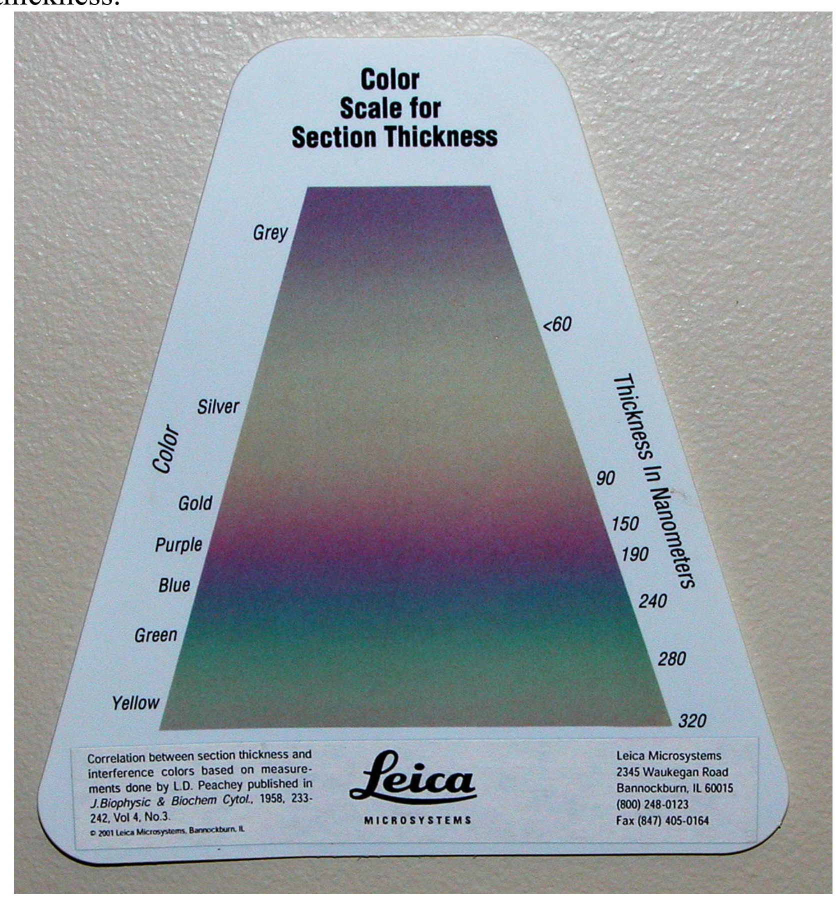


Figure 16. Interference card for determination of section thickness.

Section 8. Thick Sectioning of Epoxy-Embedded Samples

Taking thick (0.5-1 μm) sections of the block can prove useful in a number of instances. For example, determining tissue orientation, finding a particular region within the tissue, or assessing the quality of tissue preservation can all be done by viewing thick sections with a light microscope. Thick sectioning should only be done with a glass knife or a diamond Histoknife, as it will dull the edge of an Ultra diamond knife.

1. Secure and mount the specimen block and glass knife. Fill the boat as described in section 7. Approach the block face with the knife and align the knife to the block face as in thin sectioning.

2. Choose a section thickness of 0.5-1.0 micron on the controller. Go through a series of cutting strokes by rotating the cutting wheel until the entire block face is cut and whole sections appear on the water. To minimize vibrations, use the automatic cutting feature of the ultramicrotome by setting the cutting window and pressing start/stop on the controller.

3. Use an eyelash brush to lift the sections out of the boat and place them on a drop of water on a Colorfrost plus microscope slide.

4. Place the slide on a warming plate set to 50-60˚C. Add a few drops of xylene to the inside of a petri dish lid and place this over the slide. The xylene vapors will help to flatten the sections. Allow the water to completely evaporate.

5. Once the sections have dried, add a few drops of filtered stain to the sections. For epoxy sections, 1% toluidine blue and 1% borax in water works well. Another option is the epoxy tissue stain (a mixture of toluidine blue and basic fuchsin) from Electron Microscopy Sciences.

9. When a dried rim forms around the perimeter of the stain, remove the slide from the warming plate and rinse with distilled water into a waste container. Allow them to dry on the warming plate.

10. To keep slides long-term, mount a No 1.5 coverslip to the slide with Acrytol or HistoChoice® mounting media.

Section 9. Grids and Section Manipulation

What are Grids?

Grids are equivalent to the glass slides used in light microscopy. They are used to support the sections for viewing with the transmission electron microscope. Grids come in many different varieties—the type of grid used depends upon the aim of the research project. The standard grid types are 200-mesh copper grids. Grids can also be made from gold and nickel. The mesh size of the grids may also vary. Grids with small mesh sizes (100 mesh) have more space between grid bars and offer less support for sections. Some grids have an open slot, which provides an entirely unobstructed view of the sections.

Grids will often be coated with a thin support film. A common plastic film is formvar. Grids can be coated with formvar in the lab or they can be ordered already coated. Make a note of when the grids were coated or purchased as formvar will degrade with time.

Upon close examination, the two sides of a grid can be differentiated (Fig. 17). The *dull side* appears bright. The *shiny side* is reflective and appears dark. We routinely use purchased pre-coated grids and place the sections on the shiny side of the grid.

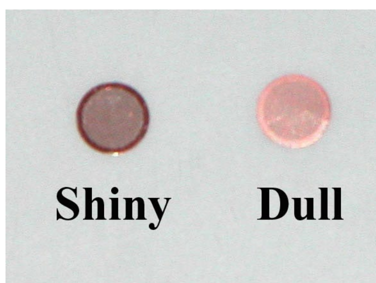


Figure 17. Note the shiny (darker) side and dull (brighter) side of grids.

C. Flattening Sections

When sections are cut on an ultramicrotome, the forces between the block face and the knife lead to compression of the sections. If you were to examine a section cut directly cut by the ultramicrotome, it would look extremely wrinkly on the TEM. Therefore, before sections can be collected onto grids, they must be stretched out or flattened. There are at least two methods for expanding sections: chloroform flattening and heat pen flattening.

1. Chloroform Flattening

a. Place a drop of chloroform on the tip of a filter paper wand (Fig. 18B). To make the wand, glue a wedge of filter paper to a wooden applicator stick.

b. Position the wand over the sections. Be careful not to dip the wand in the water, and do not touch the sections or knife edge with the wand.

c. Watch as the chloroform vapors flatten the sections with the binoculars.

Figure 18. A. Eyelash brush. B. Flattening wand.

2. Heat Pen Flattening

a. Turn on the heat pen (Fig. 19) and turn the knob clockwise to increase the temperature of the filament. The filament of the pen will start to glow orange-red. Caution: it is very hot!

b. Place the hot filament of the pen just above the sections, being careful not to dip it into the water. Do not touch the sections or the knife edge. Sections should not be attached to the knife edge when flattening them with the heat pen since the heat can melt the sections to the knife edge. As the sections begin to flatten, you will see them expand.

c. When finished flattening the sections, return the heat pen to its holder, turn the temperature down, and turn off the heat pen. Do not keep the heat pen on while sectioning. Also, never touch the filament of the heat pen with your bare fingers.

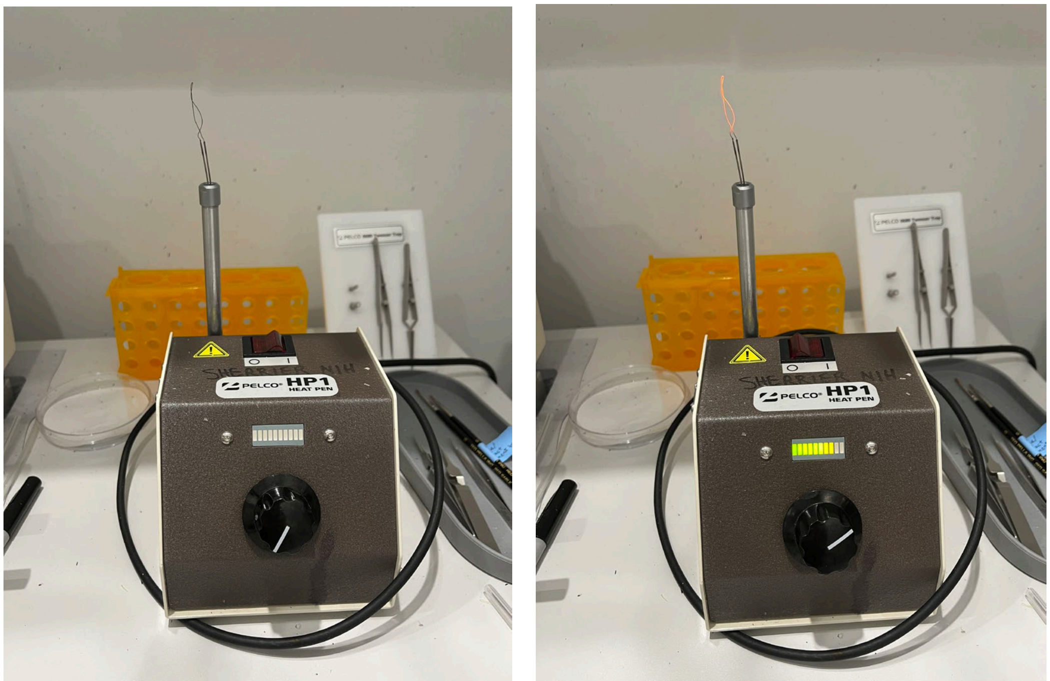


Figure 19. Heat pen. Heat pen in the off position (left) and heat pen turned on (right). When the heat pen is on and the filament is heated and glows orange.

D. Collecting Sections onto Formvar-Coated Mesh Grids

There are different ways to collect sections onto grids. My preferred method is to use the Perfect Loop.

1. Use an eyelash brush (Fig. 18A) to gently move the sections to the center of the boat. If the sections are not in a ribbon, corral them together. Do not poke the sections with the eyelash brush. Simply dip the eyelash in the water alongside the sections and push the sections gently.

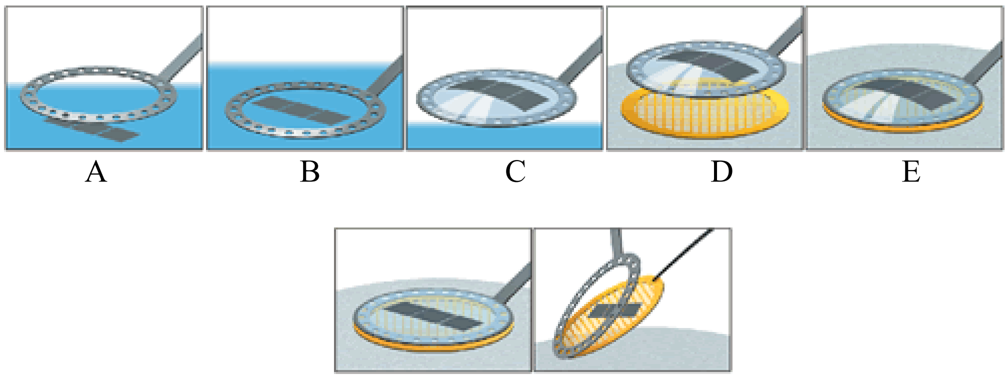
2. Grids should be placed onto filter paper inside a Petri dish. When not using grids, place the cover on the dish to avoid contamination. Position the grid such that the side that will be used to collect sections onto is facing up.

3. Hold the loop above the sections so they appear within the center of the loop. Cut enough sections to fill the entire loop. (Fig. 20A).

4. Slowly lower the loop over the sections and touch the water without breaking the surface tension of the water (Fig. 20B). The sections will be held within the loop by a thin film of water (Fig. 20C).

5. Lower the loop with sections onto a grid (Fig. 20D, E, F). The grid will hold to the loop by surface tension. Wick off excess water by gently touching the edge of the loop to the filter paper.

6. After the loop and grid has air dried, use forceps to gently separate the loop from the grid (Fig. 22G).



A B C D E

F G

Figure 20. A-G. Diagrams illustrating the use of a Perfect Loop

Section 11. Troubleshooting (Taken from Bozzolla & Russel, 1999; Wagner)

Knife cuts every other section.

1. The advance has been set below the capabilities of the cutting edge. Increase the advance until serial sections are cut or use a sharper knife

Failure to cut any sections.

2. Cantilever arm at end of fine advance
3. Dull knife
4. Block too soft
5. Knife or block not secure
6. Negative clearance angle
7. Wet block face
8. Vibrations
9. Temp fluctuations

Thickness variation from one entire section to the next

1. Dull knife
2. Bumping of microtome
3. Drafts or temperature variations
4. Knife or block not secure
5. Block face too large or soft
6. Wrong cutting speed

Wrinkled Sections

1. Block face too large or too soft
2. Dirty or dull knife
3. Clearance angle too great
4. Water level too low
5. Cutting speed too fast
6. Knife not secure

Compressed Sections

1. Block too soft
2. Cutting speed too fast
3. Inadequate expansion-try using chloroform vapors or heat pen to flatten sections

Chatter

1. High-frequency vibrations during sectioning—try a different cutting speed or clearance angle
2. Block too tall with small base
3. Dull knife or soft block
4. Block or knife not secure

Specimen block lifts sections on return stroke.
1. Water level too high
2. Block face dirty, wet, or hydrophilic—If block face is wet, wick dry with clean wedge of filter paper without touching knife edge
3. Clearance angle too small
4. Dirty knife or back of knife is wet
5. Static electricity on block face

Block face gets wet.
1. See a-e above
2. Block face too large
3. Cutting speed too slow

Sections dragged over knife edge.
1. Cutting speed too slow
2. Water level too high
3. Clearance angle too low
4. Block too soft or a ragged edge of trapezoid prevents clean detachment

Sections have holes.
1. Bubbles in resin
2. Incomplete infiltration with resin
3. Hard objects in specimen

Specimen falls out of block.
1. Poor infiltration
2. Block too soft

Sections have striations perpendicular to the knife.
1. Nick in knife edge—Move to a different region of knife edge or change knife
2. Dirt on knife edge
3. Knife damaged by hard region in specimen—Trim block to avoid hard region

Sections do not form ribbons.
1. Top and bottom of trapezoid not parallel—try re-trimming block
2. Water level wrong
3. Cutting speed too slow
4. Static electricity on block face

Ribbon of sections curved.
1. Top and bottom of trapezoid not parallel—try re-trimming block
2. Compression on one side of section

Sections stick to eyelash probe.
1. Dirty eyelash probe
2. Bearing down on sections too much with eyelash probe

Knife edge does not wet.
1. Add a drop of dilute Tween 20 solution to the boat
2. Use saliva to wet knife edge

Sections are hard to see.
1. Water level wrong
2. Illumination wrong
3. Tilt of binoculars is wrong

Sections are hard to move in boat.
1. Contamination in boat water— change water

Sections move away from the grid.
1. Dirty grid

Perpendicular regions with varied interference colors in sections
1. Cutting edge not equally sharp across knife edge—use different part of knife edge or change knife.

Irregular variations in interference colors throughout sections
1. Uneven consistency between specimen and embedding material or within different regions of the specimen—Try to re-trim to include only areas with an even consistency.

Color variations occur in bands parallel to knife edge
1. Low frequency vibrations
2. Knife or specimen not secure
3. Cutting speed too fast
4. Trapezoid needs to be re-trimmed

XII. References

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