

## Thick Sectioning

1. Place block into block holder and tighten with the special pin.
2. Trim off excess plastic from the top surface of the block using a razor blade. Trim the sides near the top surface at an angle to produce a pyramid shape with a flat top surface (the cutting surface).
3. Place block holder with the block into the microtome using the special pin.
4. Mount your Histo knife onto the base and tighten. Most diamond knives have a cutting angle of 6 deg. so check that this is set properly.

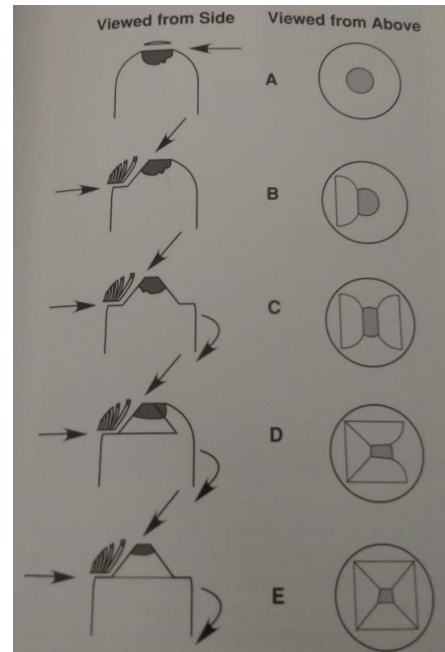


Image 1: Trimming block (Bozzola & Russell, 1992)

5. Line up the edge of the knife parallel to the block face using the bottom light to create an even line of light onto the block face. The image of the light should be even left to right and top to bottom as you move the block by hand (wheel on right) up and down. To achieve this, use the appropriate dials to move the knife holder right and left and the block holder up and down.
  - a. If the light reflection is angled this means that one side of the block is closer to the knife than the other. You then have to move the knife angle till the reflection is a flat line.
  - b. If the light reflection is narrower toward the top of the block and larger at the bottom this means then top is closer than the bottom. You then have to adjust the block angle so the size of the reflection is the same all along the block as you move it up and down.

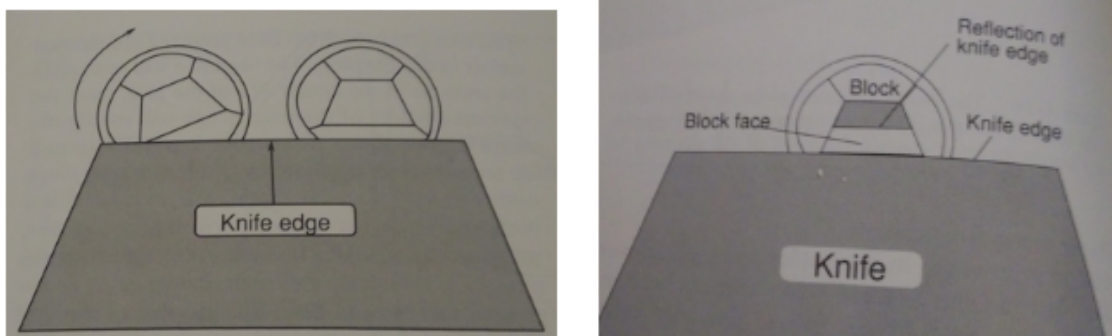


Image 2: Aligning the Block (Bozzola & Russell, 1992)

6. When the knife is close enough to the block face, fill the boat of the knife with water to the edge of the blade. Turn off bottom light. Turn on top light.
7. On the control panel set the cutting thickness using the "Semi" button to 0.5 microns (standard thickness but can increase if desired) and the speed between 1-5. Or faster if you are impatient!
8. Set the cutting window by manually moving the bottom edge of the block face just above the edge of the knife and pressing the "top" setting. Move the block so that the top of the block face is now below the edge of the knife and press "bottom" setting.
9. Press the green button to start the machine sectioning.
10. The sections will float onto the top of the water in the boat of the knife. Prepare a glass microscope slide with a drop of water on top. When you see consistent sections, pick them up using a wooden stick to lift them up from underneath the sections. Roll the sections onto the drop of water so they are floating on top of the drop. Place the microscope slide with the sections onto a warm hot plate and let the sections/water dry completely.
11. Remove the slide and add several drops of 1.0% Toluidine Blue stain on top of the dried sections. Place back onto the hot plate to warm until the edges of the stain dry. Rinse off with dH<sub>2</sub>O, dry, and view under the Light Microscope.