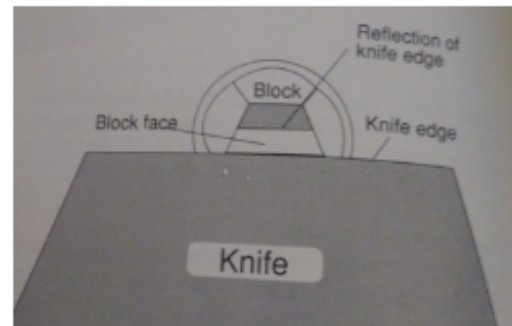


## Thin Sectioning with the Leica EM UC7 Microtome

1. If you just cut thick sections, backup your histo knife and take it out using the special pin key. Put in your thin sectioning knife and tighten it in place with the pin key.
  - a. If you aren't going to take thick sections follow the instructions of the thick sectioning manual, and just take thick sections until the surface of your block is smooth, and sections coming off seem even, and then stop and put in your thin sectioning knife.
2. Approach your sample slowly making sure the reflection on the block surface is still flat and even in size from top to bottom. If it is not, adjust accordingly:

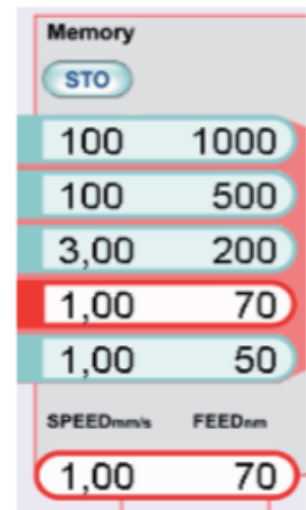
- a. If the light reflection is angled this means that one side of the block is closer to the knife than the other. You then have to move the knife angle till the reflection is a flat line.
- b. If the light reflection is narrower toward the top of the block and larger at the bottom this means the top 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.



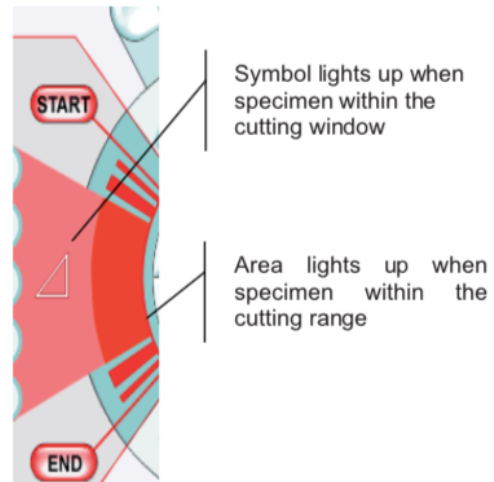
(Bozzola & Russell, 1992)

3. 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 the bottom light. Turn on the top light. Adjust the water level until it is completely flat and even with the edges of the boat. You spot this by looking at the light reflection on the water. You first overfill the boat and you will see a rectangle of light in the middle. As you remove water this should spread all the way to the edges and be even. Use the eyelash tool to make sure the water totally covers the diamond blade.

4. On the control panel set the cutting thickness using the touch screen buttons in this picture →  
Standard thickness for thin sections is 80nm (can be changed if desired, it is hard to go below 50nm). The cutting speed should be 1.00 mm/s, you can increase this if you want to go faster, but it is not recommended. The one highlighted in red, and appearing in the red oval below is the one you have selected. You can also manually adjust the thickness setting with the feed knob and the speed with the speed knob.



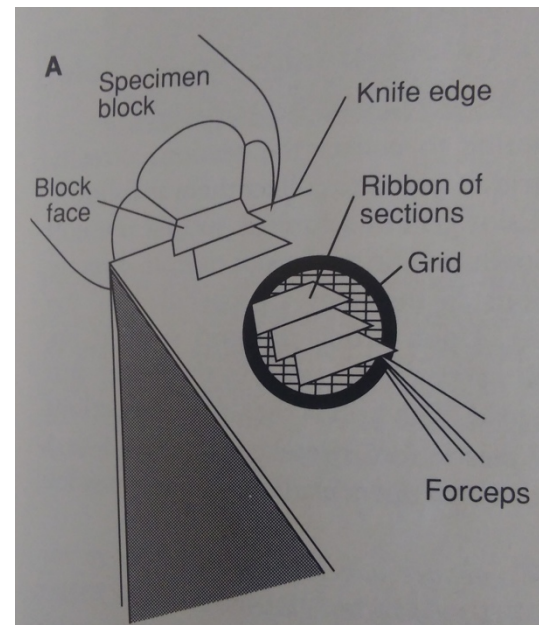
- Set the cutting window by manually moving the bottom edge of the block face just above the edge of the knife and pressing the “START” button on the touch screen. → Move the block so that the top of the block face is now below the edge of the knife and press “END”. The triangle lights up when the specimen is in the cutting window.



- Press the green START/STOP button on the control panel (not the touch screen) to start the machine sectioning.
- The sections will float onto the top of the water in the boat of the knife. Cut a few sections, then hit the START/STOP button to stop the cutting.
- Pickup a grid with your forceps, make sure the top of the grid is facing up. For mesh grids the top looks pinker and duller and the back looks shinier and is more of a copper color. For slot grids the top has 2 dots on one side and the back has a square notch on it.



- Hold your forceps in one hand and an eyelash tool in the other hand. Put the grid into the water at an angle near the section you want to pick up. Without poking it use the eyelash tool to coax the section to stick to the grid. Once the edge of the section seems stuck to the grid pull the grid slowly out.
- Dab the back of the grid on a clean piece of filter paper and place the grid in your dish. You should take at least 2 grids per sample so you have a backup. If your sections are small you can also fit more than one section on a grid.
- Once you are done, backup your knife and remove it. Dump the water out of your knife boats and put them in your boxes. Put all tools back where you found them and turn off the microtome.



(Bozzola & Russell, 1992)

#### Citations:

Bozzola, J. J., & Russell, L. D. (1992). *Electron microscopy principles and techniques for biologists*. Sudbury, Mass.: Jones and Bartlett.