Standard Operating Procedure for Ultramicrotome

Careful sample preparation is critical to obtain good results. Sample dimensions should preferably be <1mm square before sectioning your material. Please review the information on the Leica EM UC6 Operation manual (for RT sectioning) or the FC6 manual (for Cryo sectioning) and the information on "Ultramicrotomy: Common problems and Mistakes" that is located in the ultramicrotome room. Please do not take the original information with you, but you are welcome to make a photocopy.

Basic Set-up for RT Sectioning

***Both glass and diamond knives are very sharp. Exercise extreme caution when handling them to avoid being cut.**

1. Remove sample chuck from segment arc and insert sample, loosely tighten with wrench
2. Insert glass knife into knife stage if fine trimming of a blockface is needed
3. Remove glass knife and insert diamond knife after trimming
4. Turn on the overhead lighting by pressing the down arrow in the center of the touch panel display
5. Fill diamond knife boat with water to a point when the surface reflects silver and the water is evenly aligned with the knife edge (you can view this through the binoculars)
6. Select a cutting window by putting the lower edge of your sample at the knife edge and pressing the start button, then the upper edge of your sample at the knife edge and pressing the end button
7. Very carefully move the sample close to the knife edge without touching it by adjusting the coarse control with the black track wheel (left/right and forward/backward)
8. Select a speed and thickness setting from the memory settings along the left side of the touch screen panel
9. Press run/stop to begin or pause cutting
10. Once several sections have been made at thicknesses of <100nm, collect the sections with a loop and place them onto a TEM grid
11. Let the grids dry and store them in a TEM grid box until viewing
12. Turn off room light and shut door when done (touch panel display will go to a screen saver)

Basic Set-up for Cryo Ultramicrotomy

***Do not under any circumstances remain in the ultramicrotome room with the door shut when cryo sectioning. This can cause suffocation due to a drop in Oxygen levels in this confined area when Liquid Nitrogen is being used.**

1. Remove plates on ultramicrotome housing
2. Mount cryo chamber
3. hook up dewar system and start pumping
4. Insert sample, then knife
5. cool to desired temperature
6. Hook up static ionizer (on, green and on screen 7-10)
7. For shutdown, remove sample in chuck
8. Remove and clean knife (let it come to RT first)
9. Heat (press) and open clamp (30-40 minutes)
10. Detach hose to LN2 dewar, remove pump, store (let this come to RT first unless sectioning again the next day)
11. Cap extra LN2
12. Turn off lights
13. Turn off static ionizer

*If returning to section again the next morning, insert sample, insert knife, and press start to cool