

## EM Sectioning in the LSI (B3 Imaging Suite)

Glass Blades: Sharper than diamond, dulls faster

- Cut that day before use (Addi says they're good for longer since we only need them for facing.)
- Wear gloves when cutting blades! Oil on blade will transfer to sample.
- Cut must be a free break. Otherwise, it will have feathered lines.
- Use metallic tape to create water bath space. To 'catch' slices.
  - o Press tape down, nail polish inside to seal (Away from blade end!)

Microtome: Leica EM

- Angle you should put the knife at: the manufacturer gives a number for diamond blades. For glass, it's  $\sim 2^\circ$
- Rough Trim: use base that holds resin block, regular razor blades to cut (Clean with EtOH first!).
  - o Trapezoid shape, width  $\sim 2/3$  height. Knife hits long side of trapezoid first.
  - o Make sure tissue block is embedded far into metal holder, or it will vibrate.
- Fine trim: Use sharp, double edged razor blades (Clean with EtOH first!)
  - o Make sure edges are smooth, top is relatively flat.
  - o Never use same part of blade twice here.

Leica EM Atlas:

A – Zoom

B – Focus

C – Move View (left/right)

D – Move View (front/back)

E – moves sample up/down

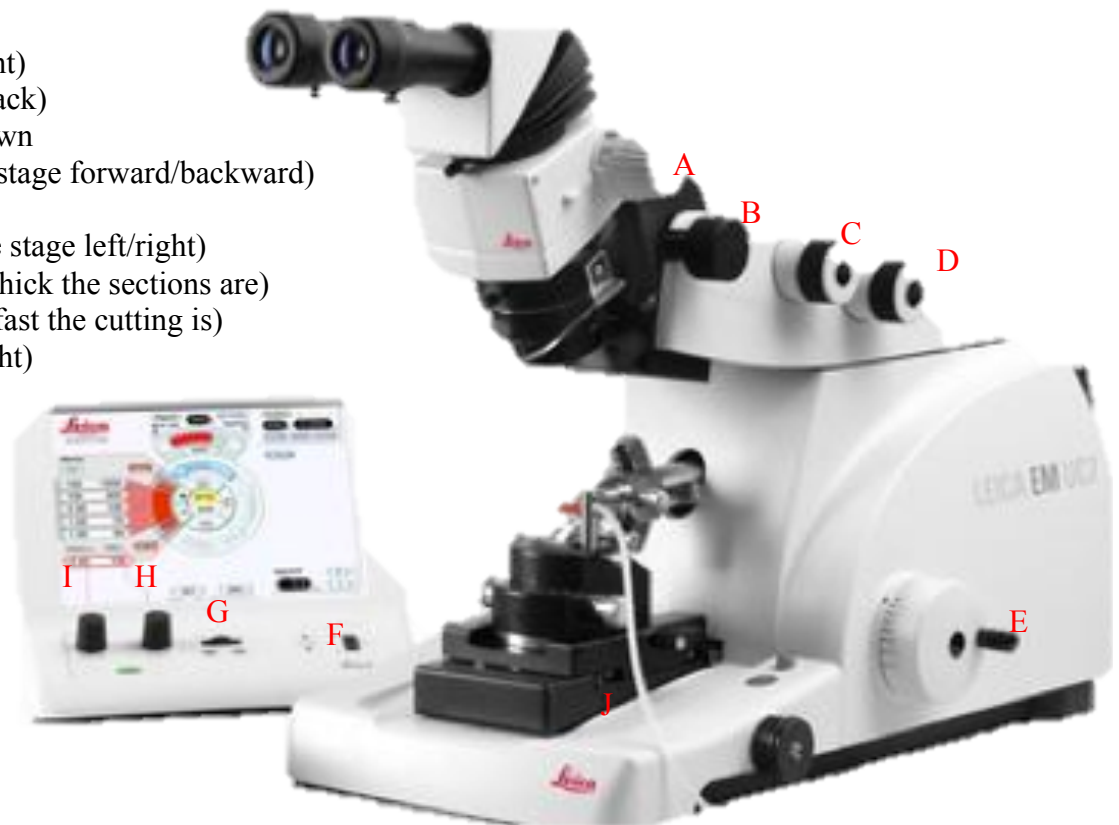
F – N-S wheel (to move stage forward/backward  
and 'Step' Button)

G – E-W wheel (to move stage left/right)

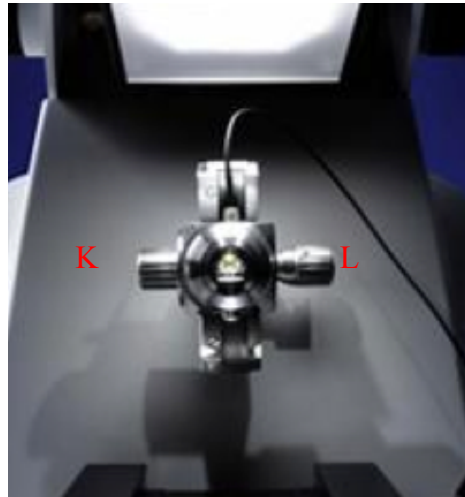
H – Feed (controls how thick the sections are)

I – Speed (controls how fast the cutting is)

J – Stage Swivel (left/right)



K – Block Swivel (up/down)  
L – Block Swivel (rotates clockwise)



At this point, we filled the diamond knife ‘boat’ with water, and let it touch the diamond blades so it wouldn’t repel the water when we started cutting. Set aside, where it WILL NOT BE BUMPED!! (Other users put their diamond knife under a beaker, out of the way.)

- Facing the block: this is where we use the glass blade.
  - o Put the block just above the blade so you can safely adjust the distance between the block and the blade. DO NOT smash the block into the blade.
  - o Line up the knife with the sharpest part (no feather marks in glass).
  - o Change the angle of the block (K) so the shadow is even across the block.
  - o Step button (F): move the block forward by your cutting distance.
  - o Manually: Clockwise motion of wheel (E), hit ‘step’ on the downstroke. At this point, the “Approach” depth should be set at 1 micron.
  - o You can adjust the angle of the knife at this point (J) so that the shadow is even.
- Fill the boat with water: first so meniscus is over the top, so the blade gets wet, then take some off (so meniscus is below tape).
- Thick section to view tissue (0.8 microns, or 800nm).
  - o Clean slide with EtOH, put a drop of water on.
  - o Manually step and cut several sections
  - o Use wire loop to pick them up in a droplet of water, and drop them onto the slide in the loop.
  - o Put on hot plate to dry, dye with toluidine blue (~1min or so, rinse w/ dH<sub>2</sub>O).
- Diamond cutting: adjust angle to 6°
  - o Dry boat with compressed air, load onto stage.
  - o Keep block high, move stage toward block
  - o Change approach to 0.1 microns. In case you cut manually, the blade won’t be damaged.
  - o Fill boat with water as with glass blade.
- Settings: sections should appear silvery, not gold or green (too thick).
  - o Adjust speed and feed: to start speed 0.80, feed 85 nm.
  - o Set the start and stop points using manual hand crank (E) and touch screen.
  - o Then start cutting! Press the green button by the Speed and Feed knobs.
- When you have your sections that you want to load on your grid, stop cutting by pressing the same green button. Wave a stick with chloroform on it over them. The vapors smooth the sections out (careful, not too close to the blade, and don’t touch the water.)
- Clean grid in acetone. Pick up with reverse forceps *on edge* of grid. Sweep under sections in water, pick up sections and wipe bottom along the edge of the boat.
- Cut more sections than you need to pick up. Keep grid away from knife!
- Use eyelash tool to clear away extra sections.

- Static can build up and cause sections to flip over or stick. Use a “static gun” to get rid of this
  - o Aim for the blade as it cuts. Keep a few inches away.
- Clean the diamond:
  - o Move any sections away with the hair tool.
  - o Use foam tool (included with diamond) – *gently* wipe across blade (ahh!!)

Other Notes/Reminders:

- If you want to save your block for later, you can put a gelatin cap on it.
- Speed and Feed (Thickness):
  - o Speed: depends on how hard your plastic is. Adjust with the Speed knob (I).
  - o Feed: Wayne goes by eye. Should appear silvery. Gold or green is too thick. Grey or not visible is too thin. You can adjust using the Feed Knob (H).