**SOP for Transmission Electron Microscope in the LSI**

**NOTE – you MUST be trained by the Bioimaging facility staff to use the TEM!!!!**

Both the Microscope computer and Camera computer should always be on.

Log into the Microscope computer. The Camera computer should always be logged in.

Open the program “Microscope User Interface”

On the bottom right, use the drop-down menu to open the “Vacuum Overview” screen.

Make sure the column valves are closed (top left box)

Move the high tension (middle left box) to 80 kV.

Turn on the filament (click greyed button – middle left box)

* Emission should be ~5uA (at step 2 for now, will need to run at a higher step as the filament gets used up
* Keep emission between 5-7 uA

Note the Vacuum values in green (top left box):

* Camera at about 24-25.
* Column goes as low as 6. Should be at least as low as camera.
* Gun should be lower than Camera before opening the valves.

Open Column Valves (imaging):

* Change magnification to ~10,000x (11,500x on this machine)
* Intensity should be set to 3 ( Bottom, middle of screen).

Centering the condenser aperture:

* Use the two highest knobs on the tower to adjust so the circle is centered. Make the circle larger (intensity) and make sure it’s still centered with the knobs.
* Note: Always make the beam larger by turning the intensity CW. Smaller by turning CCW. (Better quality images).

Stage control: Under search tab. Make sure the stage is centered:

* click arrow on the top left panel (corner)
* Hit XY reset button

Pull out the sample holder (pull out knob, turn CW until it stops (can sit here), and pull out the rest of the way.

* Put sample holder in plastic tube.
* Use small tool to lift the clamp that holds the gun down.
* Sample side down (shiny side down) Tap to make sure it’s centered.

Put back in, lining up pin with ‘closed’ line. Wait for red light to shut off, timer to run down. Then turn CCW to open, and push in all the way. Tap the end to remove tension

When vacuum on either side of the column and camera (or gun lower), then open column valves.

Turn down magnification and orient sample:

* Search tab, turn on intensity zoom (keeps same brightness at different magnifications).
* Alpha Wobbler (Shortcut button L2): for focusing. Use Zoom + and – (right of scope tower)
  + Adjust so that there is as little movement of images as possible. When it’s good, hit L2 again

Centre objective aperture: Diffraction mode (zoom out to ~4000x)

* Turn large knob to 3
* Make sure aperture is in, focus edges around beam (coarse focus, never above 4 focus step)

Camera: on other computer (press button on white toggle with LED light)

* AmtV601 software for camera
* Tab on box on right side of tower – flip to put camera in
  + Click for live image
  + Quality live (averages across a few frames so the view is less shaky/static-y)
  + Capture: file 🡪 case study 🡪 create new case