ROUTINE START-UP PROCEDURE

1. Log-in
   - Record initial parameters: Beam current (L1~94), IGP Pressure (right console door~2.4×10⁻⁵), P3 (~26) and P5(~127) pressure (Page3), and Filament Hours (L2).

2. High Voltage Generation
   Step 1
   - If the HT is on and at 180 kV go to Step 2.
   - Check that the system is not in ACD (Anti Contamination Device) heat mode (ACD light off (L2)) and ACD heater is withdrawn from trap. If the system is in ACD heat mode, press and hold the ACD heat button for several seconds and then remove the ACD heater (Caution: it may be hot!).
   - Check on Page 1 that HT is set at 180 kV.
   - Press HT button (L1). Microscope will ramp up to 180 kV.
   - Wait for green light to stop flashing. Dark current at 180 kV is 93 uA.

   Step 2
   - LOAD HTUP ←ENTER
   - RUN ←ENTER
   - It will say “START HT (kV)” enter “180” ←ENTER
   - It will say “FINAL HT (kV)” enter “200” ←ENTER
   - It will say “HT STEP” enter “1” ←ENTER (this means 0.1 kV – always use 100 V)
   - It will say “TIME DELAY” enter “10” ←ENTER

   The microscope will ramp from 180 to 200 kV in 0.1 kV increments over 10 minutes. Do not shorten this time to “hurry up” your session. All this does it give you an unstable filament.

   Dark current at 200kV is ~104 uA.

3. Fill the cold trap with LN2 while waiting for the microscope voltage to ramp up
   - Be sure the viewing screen cover is on before filling trap. The trap will boil very violently when its temperature approaches that of the l-N₂, and the N₂ may boil out of the top of the dewar. You may want to fill part way and allow the trap to cool, then after loading the sample top it off.

   Caution: The dewar should be re-filled every 4-6 hours. If you fail to do this, the vacuum will gradually degrade as the ACD warms. This will: (a) contaminate your sample and (b) may cause ion pumps and HT to turn off.

4. Turn the ion pump meter to the 10⁻⁵ range.
   “Good” vacuum is about or better then 2×10⁻⁵ Pa.

5. Load the sample
   - Use Gloves! Load your sample in upside down, as holder rotates a total of 180° during insertion.
   - Carefully check the o-ring and sample area for dust and lint.
6. Insert the holder
- Check that the stage has been neutralized or “zeroed” on page 1.
- Insert the holder into the goniometer. Align the pin on the rod shaft so that it slides into the slot and give it a bit of push to engage. Once the holder is in the goniometer, toggle the switch to “Pump”. You will hear a series of clicks as the vacuum engages.
- Look at Page 3 on the LCD. The goniometer gauge is number “4” on the top right of the page. It will start at about 240-250 uA. The roughing pump will bring it down to about 170 uA, then the diffusion pump will kick-in and the vacuum will drop quickly until it hits between 37 and 40 uA.
- The green light will kick on at about 37 uA.
- Wait 30 to 40 more minutes. This is necessary to maintain a clean vacuum in the column.
- Turn the specimen rod about 1/10 of a turn (clockwise), and it will go into the microscope about 1/2 of an inch.
- Pause. Take your hand off the holder. This keeps you from crashing the vacuum. Wait to hear valve clicks.
- Rotate the rod to the right (i.e. clockwise) until it stops. The vacuum will pull the holder into the microscope. Hold on the rod tightly, and let it slide in slowly – this prevents crashing into the holder into the piezo-stage. Be certain that the pin on the right hand side engages as you put the holder in. The vacuum is strong – do not have your fingers between the front end of the motor housing and the column.
- Wait for V2 to open (solid green not outlined)
- Check IGP to make sure <3.0x10^-5

7. Generate the beam
- Make sure IGP (P4) is below 37 before you continue this step.
- Set the filament knob to stop position and press Filament Switch to On. It will take a minute or so for the filament to reach saturation. You may simply turn the filament on and off using the Filament Switch button when changing samples etc. When the cathode is ready, the emission meter will change from 104 to ~108 uA
Caution: You cannot generate a beam without the sample holder inserted.

ROUTINE ALIGNMENT PROCEDURE

8. Find a hole/thin area in the sample
   • Go to Page 1 on the CRT.
   • Be sure that the microscope is in the TEM mode under PROBE CONTROL (L1).
   • Use LOW MAG mode as needed. Be sure to remove objective aperture in LOW MAG.
   • Find the hole/thin area of your sample. Center this region in the middle of the viewing screen with sample translates.
   • Go back to MAG 1 (regular observation mode). A convenient magnification to begin the alignment is around 5kx.

9. Insert and center CL aperture (if the previous user had to remove it)

10. Find the eucentric height position
    • Turn the FOCUS knob to set the appropriate objective lens current (i.e. to set a “DV” parameter at +0 value).
    • Press the MAG 1 button to set the Focus value to 0.
    • Bring your sample into focus by changing the Z-height. If the Z-height is changing too quickly, you can toggle the CRS button on the sample translates off.

Caution:
(1) The optimal performance of this microscope is obtained when the objective lens current is at a certain fixed value for a given operating voltage. This corresponds to a “DV” parameter (displayed on Page 1 of the LCD) equal +0.
(2) You should be able to focus to better than 500nm using the z-drive. If you operate the scope far from DV = 0, the magnifications will not be correct and the system will not perform well. When the specimen height changes due to translating or tilting the sample, use the z-drive to refocus the image.

11. Set desired spot size (L1), alpha (L1), and condenser aperture.

12. Center the filament emission

Caution: You rarely need to do this alignment, as it seems to always be correct.

use Method A:
• Slightly desaturate the filament emission. Remember that the filament changes emission slowly, so be a bit patient after turning the filament down.
• Use the GUN DEFLECTOR X-Y knobs to center the filament image.
If you find that the beam is off the optic axis on other spot sizes, hit the SPOT button underneath the DEFLECTOR set on the keyboard front panel (R2), and use the SHIFT X-Y knobs to center the beam on the optic axis. This will cause all five spot sizes to be aligned at the same point.

or **Method B:**

- Removing the small screen and focusing the illumination on the center of the large screen.
- Read the beam current on Page 1 of LCD.
- Depress GUN multifunction (R2) and use DEFLECTOR X-Y knobs (lower two multifunction knobs) to maximize the current.

### 13. Gun centering

- Toggle the SPOT SIZE to smallest size, number 5 on the LCD.
- Condense beam to crossover and center with X and Y BEAM SHIFT knobs.
- Toggle the SPOT SIZE back to largest size, number 1 on the LCD.
- Turn on the GUN DEFLECTOR button (R2). Center the beam with the GUN SHIFT X-Y knobs. You may use a coarse (CRS) button associated with this knobs.
- Repeat steps 1 to 4 until the beam stays on the optic axis (black dot) as you change spot size.
- Increase the magnification to 50 kx. Center the beam on the optic axis.

### 14. Check the CL aperture centering

- Condense the beam to a point and center the beam with the X and Y BEAM SHIFT knobs on the optic axis.
- Spread the beam to a size slightly smaller than the screen in the over-focused (the edge of the beam is the image of condenser aperture).
- Center CL aperture using the mechanical alignments.
- Repeat steps 1 to 3 until aperture is in alignment.

### 15. Condenser stigmation

- Toggle the COND STIG button (L1).
- Correct the stigmation of the beam by adjusting the X and Y deflectors until the beam is round on both sides of crossover. You may also slightly reduce the filament heating to get a filament image and sharpen it by adjusting the stigmators.
- Toggle the COND STIG button off when you are done so you don’t accidentally mess it up.

### 16. Pivot Point Alignment

- Go to ~80kX and set the objective lens focus value so that DV is the lowest “+0” value (the neighboring value would be “-0”).
- Push the CON DEF ADJ TILT switch (R2) (be sure it is the TILT, not the SHIFT) to activate the multifunction knobs.
- Switch the toggle switch to X. The beam will split into two spots. Use the Shift-X and Def-X multifunction knobs to form a single spot. You will most likely have to adjust the SHIFT knob more than TILT knob.
- Toggle to Y and repeat using the Shift-Y and Def-Y multifunction knobs.
- Deactivate the multifunction knobs and center the toggle switch.
17. **Rotation center correction**
- Find a small feature and focus it at a magnification higher than you plan on using.
- Press WOBBLER HT button (R1) and remove sample motion using BRIT TILT DEFLECTORS (L1).
- If the beam tilts are way off, cancel WOBBLER HT, reduce the magnification, and press WOBBLER OBJ (R2). Use BRIT TILT DEFLECTORS to reduce wobble then proceed to higher magnification and do a proper voltage center.

18. **Align the GIF**
- *Tune the GIF twice if you are the first user of a day*

19. **Correct objective astigmatism**
- Toggle the OBJ STIG button (L1).
- Use the X and Y DEFLECTORS to correct the objective stigmation using an amorphous region of the sample as per the usual method.
- Toggle the OBJ STIG button off when you are done so you don’t accidentally mess it up.

**Caution:** *Next to the stigmator button, there is a switch to select one of six channels where stigmator settings can be stored. Current settings can be viewed on page 7. A good starting point for objective astigmatism is X = 0, Y = -1.62 if your DV value is +0.*

**ROUTINE (EVENING) SHUT-DOWN PROCEDURE**

20. **Remove the apertures (OA and SAA)**

21. **Desaturate the filament**
- switch of the Filament button

22. **Go to Low Mag mode**

23. **With the “CRS” button illuminated, push the “N” or specimen neutral button**
- Check that X, Y and Z specimen coordinates and X and Y tilts are at zero.

24. **Return to Mag mode**
- Set the magnification to 100kx (keeps proper current running through lens).

25. **Remove specimen holder from the column**
- Use gloves.
- Slowly pull the holder out until it stops, rotate counter clockwise to stop.
26. **Remove your sample**
   - Remove your sample and place the holder in its box. This is important!!

27. **Lower HT to 180 kV**
   - HTSET 180 → ENTER

28. **Cold trap refilling/heating**
   - Check the instrument schedule.
   - If there is someone scheduled within 3 hours, fill the cold trap, put the screen cover on and the oculars covers. You can leave the microscope now.
   - If no one is scheduled in the next 3 hours, you must heat the cold trap (ACD).

29. **Turn ion pump to 10 kV setting on right hand lower console**

30. **Shut of HT by depressing HT**

31. **Remove CL aperture**

32. **Put the screen cover on (to protect from stray LN2) and the ocular covers**

33. **ACD heating**
   - Insert ACD heater in the cold trap inserting the two contact pins in their plugs.
   - Depress the ACD heat button.

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**IMAGE CAPTURING USING GIF**
1. Align the microscope in the TEM

2. Open Gatan “Filter control” program
   • Check the Prism energy (for instance, if you are working at 200kV it should be set at 200 keV).

3. Open Gatan “Digital Micrograph” program
   • Once in the DM select the TEM working mode.

4. Switch the GIF button on the L1 console
   • The microscope will switch from the TEM to the GIF mode (magnification will decrease by a factor of about 15)

5. Tune the GIF
   • After switching filament on tune the GIF two times.
   • Use Alt button to access the options in the Tune the GIF command.
   • Adjust beam intensity with the BRIGHNTESS knob if necessary. Confirm by clicking Enter key.

6. Image recording using CCD
   • Check that the DM is in the TEM mode (there are two other modes available: EFTEM and EELS).
   • For live imaging (needed for focusing and/or for correcting OL astigmatism) use the “rabbit” (low-resolution) and/or “turtle” (high-resolution) modes.
   • To capture an image click the "camera" button.