

A. Startup

- a. **Make sure** to have signed up on **iLab**.
- b. Ensure the switch on the SIP unit is down, to turn the light from **SIP-red** to **ACD-green**.
 - i. If the switch was on the **RED** SIP setting, you must let the microscope pump 1-2 hours to allow the SIP to cool before use.
- c. Fill anti-contamination dewar with liquid nitrogen. Liquid nitrogen is needed for improved vacuum and reducing the chance of contamination depositing on the sample.
- d. Ensure the BAKEOUT switch is off.
- e. Press and hold the ACD button to turn on the SIP.
 - i. After 10 minutes of SIP power with the ACD cold, the SIP gauge (Figure 1) should read below the green tab in the 10^{-4} range.
- f. Turn up the CRT brightness.
 - i. Optional: Panel lights, overhead lights, and sub cabinet fluorescent lights can be also used for room brightness.
- g. Make sure the camera water circulator (behind computer near far wall) is on and turn cooling on.
 - i. The cooling water must be set to 20°C.
- h. Turn the GATAN Multiscan Camera Model 794 **control box** and Peltier **cooler water supply** on.
- i. Load sample rod with sample.
- j. Check O-ring for dust and clean if necessary. Use dissecting scope to better see O-ring.
 - i. Align pin on sample exchanger and press in until you hit pin stop. You will hear a click and hissing which is the pre-pump engaging.
 - ii. **Wait for 4 pumping cycles**
 - iii. Rotate clockwise until you feel the second pin stop and guide rod to be fully in.
Be careful to keep a grip on the holder while it goes in and keep an eye on the sputter ion pump (SIP) readings (gauge in Figure1)



Figure 1: SIP power unit. Power light (black arrow), ACD vs SIP selector light and switch – up SIP - down ACD (white dotted box), SIP vacuum gauge (white dashed box).

B. Optional: Raising the Accelerating Voltage (HT) to 200kV (Can be done simultaneously with A, but requires significant re-alignment to operate at 200kV instead of 100kV.)

- a. Check to see if the accelerating voltage on page 1 reads 200 kV, the HT button under the filament knob is depressed, and that the dark current is nominally 100. If yes, continue to C.
- b. Check the vacuum gauges. SIP gauge to the right of the HT tank should show below back line (at worst) and optimally below the green line. Penning gauge should be on L in the green. PAGE-3 of the monitor should show PI1 = ~25, PI2 = ~25, PI3 = ~35, and PI4 = ~115.

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- i. If the SIP gauge is not reading and no large red light is showing (but either SIP selector lights are on, Figure 1), check to see if the BAKE OUT button is depressed.
- ii. Release the BAKE OUT button (if needed)
- iii. Hold the ACD HEAT button for 5 seconds to turn off the ACD cycle. The SIP gauge should come back on.
- iv. If the SIP gauge isn't reading and the vacuum valves are cycling, turn on the ACD button with the SIP selector switch flipped UP for 5 minutes. Then turn off the ACD cycle and see if SIP vacuum recovers.
- v. If the vacuum gauges are not reading properly otherwise, contact SRNML staff.
- c. Set the accelerating voltage to 100kV with the ACCEL VOLTAGE paddle selector.
- d. Turn on the HT button
 - i. Current should read ~50uA at 100kV.
- e. Bring up the HT to 160kV with single clicks per second of the HT lever, stopping for 1 minute every 20kV.
- f. Continue to 180kV stopping every 10 kV for 1 minute.
- g. Continue to 190kV stopping for 2 minute every 5kV.
- h. Continue to 200kV stopping for 1 minute every 2kV.
- i. Watch the current reading, if the current is stable at 100uA, you may continue. If not, wait until current is stable, or contact SRNML staff.

WARNING: Hearing a puff and valve clicks coming from the HT tank behind the column means the HT current is spiking. Reduce HT voltage and wait. Contact SRNML staff.

C. Saturate Filament

- a. Ensure the HT button is on, if not reference part B (c-d for 100kV).
- b. Filament is set to pre-heat (~#3 on scale)
 - i. Dark current should be 50 uA (100uA for 200kV).
- c. Turn up knob in ½ steps and wait 30 sec.
- d. Repeat until you reach the saturation stopper position
 - i. When beam current is 51uA (110 for 200kV) a beam should be visible on the phosphorus screen.
- e. The hot current should be ~53uA when the beam is fully saturated.
 - i. Contact SRNML staff the current readings are abnormal (outside of ±2uA from normal dark/hot current readings)
- f. Go to 10-40kX magnification using the selector switch; depending on sample size.
 - i. Optional: It helps to go to focus (minimum visible sample contrast) using the z-height knob (Figure 6) before alignment.

D. Gun/Condenser Alignment

- a. For brightest beam, use spot size 1L, for lowest beam dose spot size 5L
- b. At spot size 5L go to crossover with the brightness knob. Center spot with BEAM SHIFT X&Y on the top front left and right panels.
 - i. Spot size information on page-1 on CRT
- c. Use spot size switch to go to 1L, go to crossover with the brightness knob. Center spot with GUN ALIGN SHIFT X&Y (seen in Figure 2).
 - i. **NOTE:** By changing the spot size, the condenser stigmation will have to be readjusted. This does not affect the gun alignment but should be done after the alignment is finished when using the spot size you wish to image with.
- d. Repeat step a and b until crossover does not wander when changing the spot size.
 - i. **NOTE:** There is significant hysteresis in the 2nd condenser lens (BRIGHTNESS knob). To mitigate this, try to approach crossover from same direction each time while adjusting some condenser system alignments.
- e. Go to spot size 3L (or your preferred spot size for proper beam brightness)



Figure 2: Lower front panel GUN ALLIGN - SHIFT and TILT knobs. The GUN SHIFTS are boxed on top, GUN TILTS are dashed boxed on bottom.

E. Align Condenser Aperture

- a. Expand the beam going counterclockwise (weak side) and center the brightness with the X&Y Condenser Aperture Knobs (Figure 3).
 - i. The side of the aperture has two silver knobs. Use the wider one more distant from the column.
 - ii. The thin long knob is used to switch between the two rows in the aperture strip.
 - iii. **CAREFUL!** It is very easy to lose and unscrew the knobs of any aperture. Only use slow gentle movements when centering apertures. If you lose the aperture or beam, contact SRNML staff for help.



Figure 3: Condenser aperture knob assembly. Fine adjustment X and Y knobs are shown. To change between aperture strips (chart behind assembly on column), the thin long thin knob is used.

- b. Repeat step D) gun/condenser alignment.

F. Filament Alignment

- a. Go to crossover and desaturate the beam until a filament image appears.
 - i. This can be done more quickly than saturating the filament; desaturation to ~4-5 on the filament knob is typically required.
 - ii. The desaturation current should be ~50-51 uA (110-112 uA for a tungsten filament, 101-105 for a LaB₆ filament at 200kV). It is indicative of an aged filament for the filament image to also be visible when fully saturated.
- b. Adjust the BRIGHTNESS knob to find sharpest image (use small screen and binoculars)
 - i. **NOTE:** This must be done without bright 16 selected for fine controls.
- c. Hit COND STIG deflector button, Figure 4.
- d. Adjust condenser stigmatism with DEF X&Y to make the sharpest image.
 - i. **NOTE:** Switching Bright 16X back on will help for stigmatism adjusting.
- e. Center filament image within the bright spot with GUN ALLIGN TILT (Keyboard drawer, Figure 2) until the crossover image appears as in Figure 5.

- f. Iterate until satisfied with the gun tilt alignment and re-saturate before repeating step **D. Gun/Condenser Alignment.**



Figure 4: Location of the COND STIG button on the top left panel, white box.

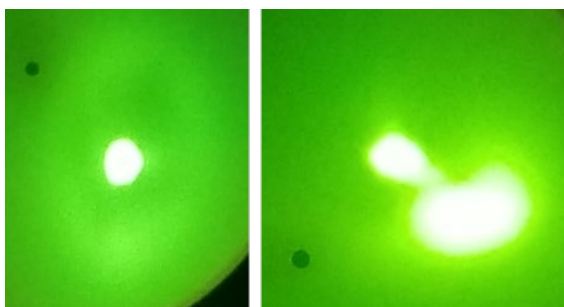


Figure 5: Left shows the optimal GUN TILT alignment (cond. ap. removed, 112-114 beam current), right shows a misaligned GUN TILT.

G. Optimum Objective Focus

- a. Make sure objective current is around 4.8V (7.37/7.38 for 200kV) with the Obj. focus knob.
 - i. Lens voltage values can be found on Page-4 on CRT.
- b. Adjust Z-height knob (located on SEG below specimen rod handle, Figure 6) to find true focus (**minimum contrast**) on an object of interest.



Figure 6: Z-height adjustment knob. Used for focus.

H. **Optional:** Current Center Adjustment (only for large bright tilt misalignments)

- a. Focus on an object in the sample, you must be at a **mag lower than 40kX** for OBJ WOBBLER to wobble.
- b. Center the crossover spot on the screen using the BRIGHTNESS and BEAM SHIFT knobs and spread the beam.
- c. Hit OBJ Wobbler, Figure 7.



Figure 7: OBJ wobbler button for current center adjustment.

- d. Adjust DEF X&Y in BRIGHT TILT mode (seen to the right of COND STIG in Figure 4) to move the rotation center of motion to the crossover position (optic axis of the microscope)
- e. Turn off objective wobbler and re-center the crossover spot to the middle of the screen.
- f. Iterate a-e until no change is noticed.

I. Image Wobbler Balance

- a. Go to crossover and center the beam.
- b. Hit IMAGE X wobbler (Figure 8) and adjust X&Y IMAGE WOBBLER ADJ, the second and third knobs from the top left on the front right keyboard panel (Figure 9). The speed of wobble can be changed with the top left most knob of the row.
- c. The two spots produced by the wobbler should coincide and (mostly) disappear when IMAGE WOBBLE ADJ is properly aligned.
- d. Stop the IMAGE X wobbler with a 2nd press.
- e. Repeat for IMAGE Y wobbler using X&Y IMAGE WOBBLER ADJ Y dials underneath the X dials.
- f. Iterate a-e until desired alignment is achieved.
- g. Repeat step D) Gun/Condenser Alignment (if necessary).



Figure 8: Front right panel showing the Image Wobblers.



Figure 9: IMAGE WOBBLE ADJ knobs on front left panel under the keyboard - Top row X, Bottom row Y.

J. Optional: Refill Anti-contamination Dewar with liquid nitrogen (LN₂)

K. Voltage Center Adjustment

- a. Go to 40kX using the mag selector switch.

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- Find a multi-directional object (a corner of a particle, or kink in the lacy carbon works well) and center both crossover with the BEAM SHIFT knobs and this object with the stage translate wheels.
- Spread the beam counterclockwise.
- Hit HT WOBBLER, Figure 10.



Figure 10: Accelerating Voltage wobbler used for bright tilt.

- e. With the BRIGHT TILT button depressed, adjust DEF X&Y knobs to minimize object motion translation. The object should look like it is going through focus, without translation.
- f. Depress HT WOBBLER, go to crossover, and center beam using BEAM SHIFT X&Y knobs.
- g. Repeat step K until object does not translate around and there is no change in beam centering.

L. Open the GATAN Digital Micrograph software on the GATAN MSC-794 computer

- a. Optional: In the menu bar go to CAMERA → INSERT CAMERA
 - i. You should hear a puff of air as the camera is inserted into the viewing area
 - ii. The software will ask you to insert when you start view if you do not do this step.
- b. In the menu bar go to CAMERA→SEARCH. Make sure AUTO SURVEY is checked
 - i. EXPOSURE time should be set to a reasonable value. ~0.2 sec should be fine
 - ii. By clicking SEARCH the camera will auto insert.
- c. In the menu bar go to PROCESS → LIVE → FFT
 - i. A live FFT window should appear next to the image window
- d. Push the Lift Screen button (above panel light knob. L shaped) to lift the screen **WHEN FOCUSED ON SPECIMEN.**

WARNING: Large stage movements, brightness changes, mag changes, beam shifts, and z-stage focusing should **ALWAYS** be done on the phosphorous screen.

M. Objective Lens Stigmatism

- a. Use X&Y Stage Translate wheels to move to a section of amorphous carbon film.
- b. Adjust focus to true focus and mag above 80kX (**you will not see** OBJ STIG changes below 60kX)
- c. Press OBJ STIG 1 mode (the button must be bright, **not** the light above the button) and use DEF X&Y knobs to create symmetric image contrast (OBJ STIG 2 is just a second stigmation setting preset).
- d. In the camera software under Process>Live press the FFT button to view the live FFT of the image, this will allow for better adjustments as per Figure 12.
 - i. If you do not see any CTF rings, try more defocus or a longer exposure time to achieve required visibility.



Figure 11: Objective lens stigmatism button.

- e. Look for image stretching through focus and re-adjust deflectors as necessary to eliminate, Figure 12 shows typical stigmatism on carbon film with additional FFT
 - i. On the FFT window, look at halo around center bright spot and try to make it as circular as possible if stretching is too difficult to see.
 - ii. If no amorphous area of the sample is available, the change in Fresnel fringing at the sample edge can be used to correct for stigmatism.

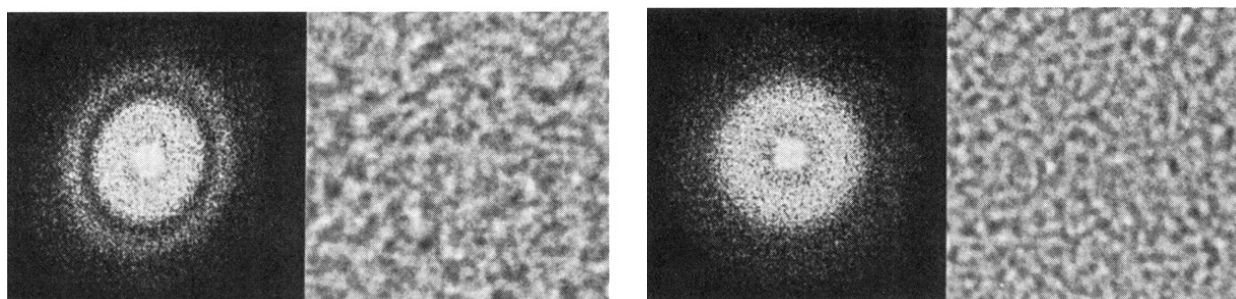


Figure 12: Poor objective stigmatism on carbon film and associated FFT (left), properly stigmatism corrected objective lens (right)

N. Optional: Objective Aperture Alignment

- a. Go to 20kX.
- b. Go to spread the beam counterclockwise fully.
- c. Insert an intermediate aperture (selected area aperture, the lowest on the column to the left of the screen).
- d. Press DIFF button.
- e. Center spot with PROJ ALIGN X&Y knobs (front of keyboard, Figure 13)



Figure 13: Projector Alignment Knobs. Below them are the intermediate lens stigmatism knobs.

- f. Focus spot with DIFF FOCUS knob (to the left of the screen raise L button, Figure 10)
- g. Insert an objective aperture.
 - i. Larger apertures are better for phase contrast; smaller are better for diffraction contrast.
- h. Center objective aperture around central spot using the objective aperture translation knobs on the aperture assembly (seen Figure 14).

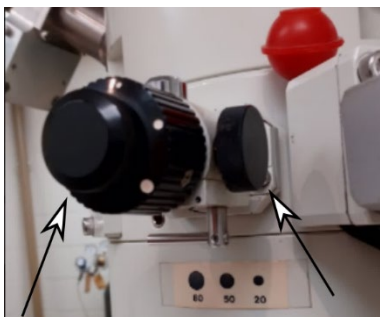


Figure 14: Objective aperture assembly next to the sample stage.

- i. Press MAG 1, remove the intermediate (SAD) aperture, and repeat step **D) Gun/Condenser Alignment** (if necessary).

O. Optional: Selected Area Diffraction Use/Alignment

- a. Find a region of your sample that is crystalline and (preferably) thin.
 - i. Thick regions will show Kikuchi lines in the diffraction pattern and weaker spot pattern intensities.
- b. Remove objective aperture, insert intermediate (SAD) aperture around crystalline object of interest.

NOTE: Do all diffraction adjustments on the phosphorus screen. Only use the camera to collect a diffraction pattern with the beam blocker inserted (Figure 15).

- c. Find object of interest and turn BRIGHTNESS knob **clockwise** until it beeps.
 - i. This creates the most parallel beam, which is necessary for diffraction
- d. Press DIFF button and use selector switch to change effective camera length, L , to desired length. 25-40cm is standard depending on unit cell size.
- e. Use DIFF FOCUS to sharpen central bright spot and make as small as possible.



Figure 15: Location of beam stop next to the viewing window.

- f. Use the INT STIG knobs (Figure 12 below PROJ ADJ knobs) to adjust the stigmatism of the diffraction spots until the spots appear as small and round as possible.
- g. Iterate on e. and f. until satisfied.
- h. Rotate beam stop to block central bright spot. If the central spot is larger in diameter than the beam stop, use Diff Focus to refocus central bright spot to a circle and change camera lengths as necessary.
- i. Lift screen and use GATAN software to image diffraction pattern.
- j. If pattern is not centered, lower phosphor screen and close camera image window. Use PROJ ALIGN dials to diffraction pattern center.

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Note: horizontal translations are not altered, but vertical translations are inverted for the camera. The beam stop can be used as an orientation guide between the phosphorus screen and the digital camera.

P. **Optional: Dark Field (DF) Imaging**

- a. View the diffraction pattern, as per the previous section, of a crystalline region of your sample.
- b. With DIFF mode active, press the DARK TILT button (seen to the right of the COND STIG button in Figure 4).
- c. Move the new central diffraction spot position with X&Y DEF knobs (DARK TILT button must be active) to any diffraction peak that you are interested in.
- d. Select BRIGHT TILT, insert an objective aperture, and center it around the bright central diffraction spot.
- e. Go to MAG 1, remove the intermediate aperture, and condense the beam with the BRIGHTNESS knob until the brightfield image appears.
- f. Press DARK TILT, the image should now be the darkfield shadow of the previous image.

Shut down

- A. Bring the **Filament Heat** knob down over 30 seconds until you hear a click at around the third step.
- B. Remove all apertures and set to largest size.
- C. Set magnification to X20K.
- D. On PAGE-2 center the X&Y Stage Translation wheels.
- E. Toggle Peltier cooler **water supply power** and GATAN Multiscan Camera Model 794 **control box** to off.
- F. Turn off the two switches behind the camera computer on the water circulator.
- G. Turn down CRT and panel lights
- H. Remove specimen rod from TEM and remove grids.

If you are **not** the last user of the day:

- I. Leave the microscope HT button on.
- J. Turn off room lights
- K. Confirm your time use on iLab.

If you **are** the **last user** of the **DAY**

- L. Put the ACD heater in the ACD dewar and connect it to the power supply. **NOTE:** take care as the liquid nitrogen in a full dewar will spray out.
- M. Make sure the ACD heater switch is in the **down** (green ACD) position (Figure 1).
- N. **If using at 200kV:** use the HT ACCEL VOLTAGE paddle to bring the HT down to 100kV, this can be done quickly over a couple minutes.
- O. Depress the HT switch and make sure the beam current is 0.
- P. Press the ADC HEAT button (front left lower panel).
- Q. Turn off room lights.
- R. Confirm your usage on iLab.

If you **are** the **last user** of the **WEEK** (Friday session):

- S. Put the ACD heater in the ACD dewar and connect it to the power supply. **NOTE:** take care as the liquid nitrogen in a full dewar will spray out
- T. Make sure the ACD heater switch is in the **UP** position (red SIP light - Figure 1).
- U. **If using at 200kV:** use the HT ACCEL VOLTAGE paddle down to 100kV, this can be done quickly over a couple minutes.
- V. Depress the HT switch and make sure the beam current is 0.
- W. Press the ADC HEAT button (front left lower panel) and wait for vales to stop cycling.
- X. Press the BAKE OUT button.
- Y. Turn off room lights.

- Z. Confirm your usage on iLab.



Figure 16: Location of the ACD HEAT button and the BAKE OUT button on the front left bottom panel.

