CM200 Basic Operation Procedures

CM200 Microscope Procedures

A. Daily start-up procedure:

1. Log onto the computer with your *onepurdue user name & password*
2. Turn on the "Room In Use" lights.
3. Start a logbook entry
   a. log *user name, date, start time, sample description, and cryo or negative stain*
   b. Note room temp and humidity in log book
   c. Note vacuum levels in log book, P1, P2, P3, & IGP
   d. For the safety of the FEG, P3< 40 and IGP < 20 before opening the gun valve and doing microscopy
4. **Always** add liquid nitrogen to the anti-contaminator dewar
   a. Cooling the anti-contaminator is mandatory for **ALL USE!**
      i. It helps maintain clean apertures
      ii. It keeps heavy metal stains and plastics from depositing on inside lining of the column
      iii. It prevents "gunk" related astigmatism
   b. Allow the instrument to cool at least 30 minutes.
5. Check the status of the high tension (HT)
   a. If it is **ON** (light is green), proceed to step 6.
   b. If it is **OFF**, check the status of the *vacuum*. If it reads "ready" proceed to next step. If reads anything else wait 10- 30 minutes
   c. If it does not change to "ready" check with Valorie or Zhenguo.
   d. DO NOT PROCEED until the vacuum is in the "ready" state, no matter the IGP reading.
6. To turn on the HT
   a. In "Parameters" set the HT to 200kV
   b. Press the HT button.
      i. The light should go green
      ii. Wait for the HT will slowly ramp up to 200kv.
7. Recheck and log IGP value.
8. Check the status of the filament.
   a. Go to the *configuration page,* then the push *display*
   b. If it is in "Standby" press the "preset extraction voltage" button.
      i. You may get an error message stating the present "*extraction voltage routine has aborted*". This is normal.
      ii. Press the "reset" button on the lower left below the screen and press the "preset extraction voltage" button again.
      iii. Wait until the extraction voltage reaches 3.81kV and the "Presetting…" message disappears from the screen.
      iv. SLOWLY ramp up the filament knob until it reaches desired extraction voltage--currently using 4.2kV. (This value may change as the filament gets older).
      v. Press "Extr Limit" to lock the filament at 4.2kV for safety.
   c. If the filament status is "operate"
      i. check that your extraction voltage is at 4.2kV.

ii. If not, follow 8b above to ramp it to that level.
iii. If you get any other type of FEG status, contact Valorie or Zhenguo.

9. Insert a RT sample
   a. Insert the RT holder with the pin lined up with the mark on the face plate of the goniometer. This lines the pin up with the slot in the airlock
   b. Do not bang the pin into the face of the goniometer or scrape it along the sides going in!
      i. You can break off the pin.
      ii. You can scratch the float tube.
   c. Goniometer Prepump- the Pre-pump time is set to **60 seconds**.
      i. Do not shorten the pre-pump time,
      ii. Not even for the RT holder
   d. Holder sensor
      i. The red light on face of the goniometer (lower Left) will go on when the holder makes contact with the sensor.
      ii. You should hear the rotary pump come on. DO NOT ROTATE holder at this stage. You will crash the vacuum.
      iii. When the red light goes out, *rotate the holder counterclockwise* until the vacuum starts to pull it in. DO NOT release the holder.
      iv. *Firmly grip the holder* and guide it gently into the scope
      v. If the red light and pump do not come on, DO NOT INSERT THE HOLDER INTO THE COLUMN. You will crash the vacuum!
      vi. Take it out and try again (removal see C2 below)
      vii. If it still doesn't work, get help from your senior person.
10. For cryo-holders, see the CM200 Cryo procedure.
11. SLOWLY open the gun valve (V7). Opening the valve too quickly can cause the HT to shut off.

B. Basic User Alignments.

1. Setting specimen height (Z) using α wobbler
   a. Remove the objective aperture by flipping the lever below it to the right
   b. Center the C2 aperture if it is not already centered
      i. Center the beam with the x, y controls
      ii. Rock the intensity control knob to spread the beam above and below crossover
      iii. If the beam sweeps to the sides, the C2 aperture is off-center
      iv. At crossover, use the x, y aperture shifts to bring the beam back to the center
      v. Condense the beam to crossover and recenter with the x, y controls
      vi. Iterate i-iv as needed until the beam spreads smoothly around the center above and below crossover
   c. Set magnification to a low mag in the SA range (i.e. 3400)
   d. Adjust Z height (must repeat for each sample insertion)
      i. Go to *compucontrol* page and press α wobbler button
      ii. Watch a feature on your sample sweep back and forth on the flu screen.

iii. Adjust Z height up or down to minimize sweep using Z joy stick on goniometer control box.
iv. Turn off α wobbler

2. Align Rotational Center
   a. Go to high magnification, 96,000- 200K mag
   b. Push the *align* button to enter align menu
   c. Push the rotational center button on the top R
      i. Using the small flu screen and binoculars, observe the lateral motion of your sample.
      ii. Use the x, y, multifunction knobs to adjust the rotational center until the sample stops moving side to side or up and down and instead appears to pulse in and out towards you.

3. Align Gun Pivot Points (align button still pushed, obj. aperture OUT)
   a. condense the beam on the flu screen to crossover
      i. push the x pivot point button
      ii. use the x, y, multifunction knobs to adjust the x pivot point until the 2 spots converge
      iii. repeat for the y pivot point

   b. Push align button to exit align menu

4. Center and Stigmate Objective Aperture----Since the objective lens forms the last effective image of your sample, these are the *2 MOST IMPORTANT ALIGNMENTS* of all.
   a. *Insert desired objective aperture* by flipping lever back to the left (see B1a above)
   b. Push “zoom” for diffraction mode
   c. Center the shadow of the aperture around the bright diffraction spot. (Easier in a dark room) using x, y aperture shifts
   d. Go to High magnification, > 96k x
   e. Use live imaging mode on the CCD camera (see CCD camera procedures)
      i. Click “Process” in the DM tool bar, and select Live FFT in the drop down menu
      ii. Look at the Thon rings on the FFT. If they are oval but otherwise strong, you have objective lens/aperture astigmatism
   f. Push the stigmate button
      i. select Obj
      ii. Then use the x & y multifunction knobs to correct the astigmatism by making the Thon rings appear round. It is more accurate to do this close to focus.
      iii. Thon rings that are severely truncated in 2 parallel directions indicateDRIFT, not astigmatism. The drift must be corrected before stigmating the objective lens.
5. **Ready to Image!**

C. Shut Down Procedures:

1. **Close the gun valve!**
2. Extract the specimen holder and remove your grid

a. Always watch the IGP vacuum while removing the holder-- if it starts to spike, back up a bit and wait until it comes back down
b. *Slow and easy does it*
c. **After closing the gun valve**, pull the holder straight out until it stops- be sure to hold the goniometer in place with your other hand so you aren't pulling on the O ring
d. Rotate the holder clockwise until it stops
e. Bracing against the goniometer, gently pull the holder out the rest of the way

3. Put the room temperature holder into the microscope & leave it there for the next person.
4. Complete the logbook entry. Be sure and include the *time you finished.* The minimum requirements for entries are posted on the back of the scope room door.
5. Dim the panel lights, log off the computer and turn off the “Room In Use” lights.
6. If you are the last user of the day (Check the schedule!)
   a. *Remove the liquid nitrogen* from the anti-contaminator device.Running a cryo cycle is useless unless the anti-contaminator warms up.
   b. Run a 4 hour cryo-cycle to remove water accumulated on the cold column inner surfaces during use. This process turns off and isolates the IGP (ion getter pump) and allows the diffusion pump to remove any water trapped there.
   c. The cycle will turn the IGP back on when the timer finishes
   d. If you changed the film, NEVER run a cryo cycle until P3 is below 70 and all the valves are open again. When they do open later, it will crash the FEG!

If you finish your session more than 30 minutes early, send an email to the group so that someone else can make use of the time!