FEI CM200F Standard Operating Procedures

1 Begin the session: Turn on Panel/Monitor

a **Sign** in the logbook.

b Panel Dim- push knob. Data Dim-rotate clockwise to bring up intensity.

c Go to vacuum page.

P1~33	P3~30 or less (zero is less than 30)
P2~51	IGP≤15 10 is better, very important for cryo samples

d Check that high tension is on and set to 200kV. Check Parameter page for actual HT value. If not on, turn HT on. Wait for it to reach 200kV.

e Verify FEG is in Operate mode. Select MODES, then CONFIGURATION.

f If in **Standby** mode then:

1) Select DISPLAY
2) Select preset extractor (get error message)
3) Press Reset
4) Select preset extractor (now voltage extraction works fine). Message reads: Presetting Extraction Voltage Please Wait. Wait until extraction voltage is reached. Extraction voltage will be about 3.8kV. Now the FEG is in operate mode.

g **If FEG is OFF, call help.** *If I can not be found, sign into the logbook and note the problems, then use the computer to send an email to manager stating the problems.--Then take day off.*

h **Put liquid nitrogen into the anti-contamination dewar. (*Always wear safety glasses when handling liquid nitrogen.*)**

2 Choose Settings:

a Go to Modes: select TEM low dose.

b On parameter page: set Gun Lens to 3 for studying robust samples with large spot size; Set the Gun lens to 5 if samples are electron beam sensitive and you will use a small (5-9) spot size. Conditions for resin sections or negatively stained samples typically are Gun Lens 3, and cryo specimens are more beam sensitive and use of Gun Lens 5 is better.

Always Close Gun/Beam Valve--Before changing samples

3 Load/Insert the specimen:

a **Check that goniometer/stage is at zero; if not zero, set it to zero. With:** Compustage:Compucntral:Reset Holder.

b Bring up the vacuum status page.

c Turn on the airlock pump. (Left side of column, see photo)

d Load specimen into holder.

e Make sure that the specimen holder's o-ring is clean.

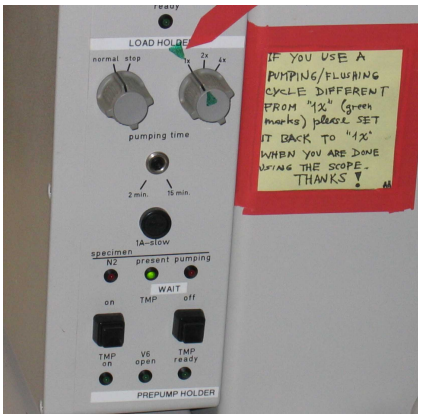
f Wait for the TMP ready light on pump controller, lower right corner. (TMP = turbo-molecular pump)

g Insert specimen holder and Select sample holder prompt comes up; choose NoCumpustage ß-tilt. Press READY to return to vacuum page.

h Wait 2 minutes for the ready light on top of the control box.

i Carefully rotate the holder counter-clockwise until it stops, then guide it straight into the column. IGP level should remain below 40 during this process.

j Then shut off the TMP.



Prepared by Roseann Csencsits, PhD. Life Sciences Division, Lawrence Berkeley National Laboratory 2011-05-11

FEI CM200F Standard Operating Procedures

4 When IGP≤15- Open Gun/Beam Valve – SLOWLY-counterclockwise

5 General Alignment Notes:

a Press the STANDARD FOCUS BUTTON.
b Focus and align beam and condenser aperture.
c Put specimen at the eucentric height. Use stage α-wobbler found on compustage page.
d Focus the image to the minimum contrast condition. *The in-focus objective lens current for the eucentric condition is approximately achieved by pressing the standard focus button.*
e Enter the Alignment page on the CM200 display by depressing the Align button. This button is found on the lower right side of the console; The group of buttons are: STG, DF, ALG, stigmators, dark field, alignment.
f Select and adjust the beam coil pivot points for x and y independently using the multi-function knobs. Do at 15kx or higher.
g Adjust the current center ("Rotation Center"). Do at as high a magnification as you will work. Depress the Align button to return to the Low Dose TEM menu.
h To adjust the stigmators, depress the STIG button and the stigmator adjustment page comes up. Tweak the stigmators as necessary. Note: the objective stigmators come up as default. Condenser Astigmatism correction is easiest at very high mag, over 100kx, simply easier to observe oval beam at high magnification.

6 General Use Notes:

a Cover viewing port when not looking in it, and **always** when handling liquid nitrogen.
b Low dose mode - align in EXPOSE mode. In SEARCH or FOCUS, the reset button on lower left will reset the beam to EXPOSE beam shift condition.
c For Tomography the microscope must be in SEARCH mode. This allows control of the TEM by the software.
d Gatan Camera – saturates at ~50,000 counts/pixel, do not exceed this value.
e For low-dose cryo work-- focus in Focus mode, then blank and press expose to record an image on film.
f Return to Low Dose page from Modes page, press TEM LOW DOSE **two** times.
g **Reset goniometer/stage to zero before removing or inserting samples.**

7 Removing the specimen:

a **Check that goniometer/stage is at zero; if not zero, reset holder.**
Compustage:Compucntral:Reset Holder.
b Bring up the vacuum status page.
c Grasp end of holder with right hand.
d Press against the green central part of the goniometer with your left thumb and index finger.
e While observing the IGP vacuum reading, withdraw the sample holder until it stops. Rotate the holder clockwise until it stops. The holder will stay in this position to let you change your grip on it here. If the vacuum level degraded above 50, make a note in the logbook.
f With both hands, uniformly pull the holder, from the goniometer, until it is released.
g Remove your sample from the holder.

8 Changing film:

a If film was used, change film box and reset the film counter to 50.
b To vent the camera, press CAM AIR once to vent the camera.
c Remove camera cover.
d Using gloves remove the film box assembly and insert a new one.
e Replace camera cover.
f Hit CAM AIR once to pump the camera.
g Wait for camera vacuum to return to READY, P_3 <65.

FEI CM200F Standard Operating Procedures

9 Before you leave for the day:

a Close Gun/Beam valve.

b Cover Viewing Screen.

c **Check that goniometer/stage is at zero; if not zero, set it to zero.**

d **If you want to keep your sample: remove it from the holder.**

e Put FEG in Standby.

f Turn HT OFF.

g If film camera was changed, wait for camera vacuum to return to READY, P_3 <65 and TMP has completely shut off, including the "winding down sound" before starting the Cryo Cycle.

h Remove the liquid nitrogen dewar from the copper "pig-tail". Pour excess liquid nitrogen into **styrofoam box**, not trash bin.

i Go to vacuum page, choose Cryo. The cryo cycle should be set to 4 hrs; with the 4 hr cycle the use of a hair dryer is not needed.

j After TMP has completely shut off, including the "winding down sound", press Cryo-start.

k Pull out the PANEL DIM switch.

l Rotate the DATA DIM knob counter-clockwise to the stop.

m Log off the computer.

n Sign out of log book.

o Turn off ALL room lights.

p If you used the cold stage, let it warm to room temperature in cryo transfer station or pumping station--ONLY PUMPING ROD until at room temperature.