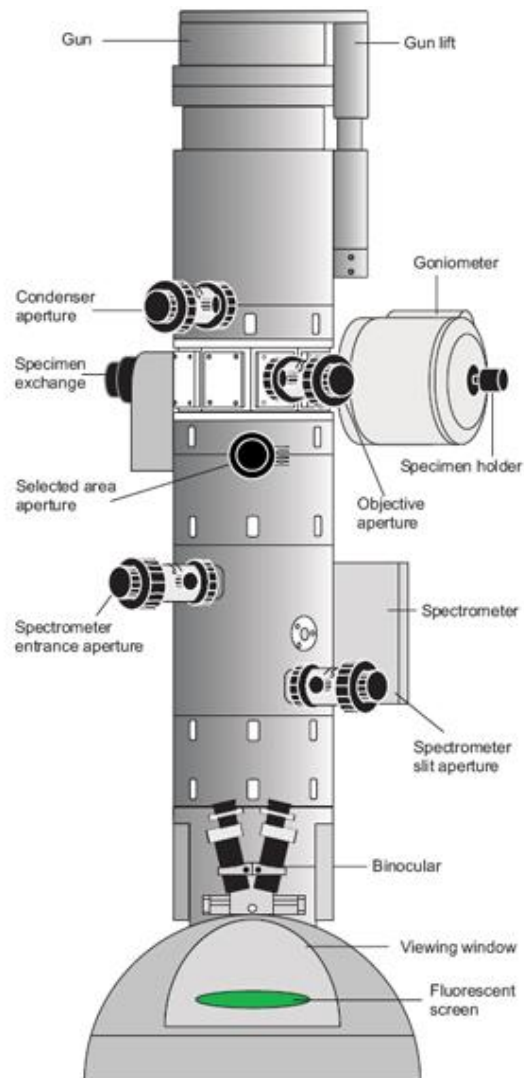


Libra 120 TEM Quick-Start for Basic Imaging



1. Sign in on the log sheet
2. Log on to WinTEM using your user name and password. If any errors are present in the EM server notify Shannon, Debbie, or Kirk.

3. If you are the first TEM appointment of the day, fill the liquid nitrogen anti-contaminator:
 - a. Wear protective cryogloves and face shield
 - b. Be careful not to grab the high voltage cable coming from the microscope
 - c. Fill the anti-contaminator half-way with liquid nitrogen. Wait 15 min to allow liquid nitrogen to stop boiling. Fill to top. Wait 15 min and fill to top again.
 - d. Once anti-contaminator is filled, the microscope can be operated for 8-10 hr without needing to be refilled.

4. Check the Vacuum Status
 - a. Under the **VACUUM TAB**, you will see vacuum readings for the gun, column, and viewing chamber of the microscope. If the vacuum in these compartments is good, they will appear green.
 - b. Verify the following vacuum readings are above:
 - i. Gun: 6×10^{-7}
 - ii. Column: 1×10^{-6}
 - iii. Camera: 1×10^{-5}
 - c. Make sure the column valves are **CLOSED**

5. Ramp up the Filament
 - a. Under the **GUN TAB**
 - i. Check that the HT is on and set to 120kV
 - ii. Ramp up the filament by clicking **Filament ON** button. The filament will ramp up from 1.8A to approximately 3.0 A (The exact value will vary depending on the age of the filament and the setting made by the service engineer).
 - iii. Adjust the emission current to read between 3 and 6 μA . An emission current from 3-6 μA is used for basic imaging of biological samples. Higher emission currents may be necessary for energy filtered

imaging (12-15 μ A) and lower emission currents for cryo low dose imaging (<1 μ A).

6. Sample Removal and Insertion

- a. Under the **VACUUM TAB**, make sure the column valves are **CLOSED**. Valves **MUST** be closed whenever a sample is removed or inserted to protect the gun.
- a. Removing sample rod:
 - i. Slowly pull the rod back until it stops
 - ii. Rotate counterclockwise until it stops. You will hear a click and hissing noise as the sample exchange is purged with N₂ gas.
 - iii. Wait 15s
 - iv. Rotate counterclockwise until it stops and slowly pull the rod out of the microscope. Place the rod in wooden container.
- b. Loading grids
 - i. **Do NOT touch the sample rod anywhere past the O-ring with bare fingers! If in doubt, wear gloves!**
 - ii. Use the special screwdriver device to remove the holding bow.
 - iii. Insert grids and center in depressions. The sample holder can accommodate 2 grids at one time.
 - iv. Replace holding bow and tighten with screw driver device. The special screw driver device is designed to not allow over-tightening of the screw.
- c. Inserting sample rod
 - i. Insert the rod so that the pin on the rod is aligned with the notch located on the microscope. Be careful not to bump the sapphire tip on the end of the sample rod.
 - ii. Insert the rod into the microscope until it stops and rotate clockwise. The red STOP light will turn on and you will hear the pump start to evacuate the sample exchange.

- iii. Wait until the red stop light turns off! **Do NOT insert the rod while the red STOP light is illuminated.**
- iv. Pull the rod back about 1 cm, rotate clockwise until it stops and SLOWLY guide the rod into the microscope.

d. To change specimen position

- i. The grid position can be changed by using the knobs located on the left side of the column opposite the sample exchange.
- ii. Unlock by turning the small knurled ring counterclockwise
- iii. Move grid position by rotating large knurled ring
 - 1. Rotating to the counterclockwise stop moves to the inner rod position
 - 2. Rotating to the clockwise stop moves to the tip position
- iv. Lock the position by turning the small knurled ring clockwise

7. Finding Sample Using LOW MAG Feature

- a. Under the **VACUUM TAB** open column valves. The beam should appear on your screen. If no beam is present, try using the joystick to move the stage—you may be on a grid bar.
- b. Adjust the illumination with Brightness knob
- c. Press **MAG/LOW** button on right control panel.
- d. Remove the objective aperture. A low mag overview of your sample should appear on the viewing screen.
- e. Use the joystick to find a region of interest.
- f. Insert objective aperture to the first click-stop. The objective aperture should always be inserted before returning to Mag mode.
- g. Press **MAG/LOW** button to return to the normal Mag mode.

8. Alignments for General Users for Basic Imaging

- a. Centering the objective aperture
 - i. The objective aperture should be inserted to the **first click-stop** (90 μ m aperture)
 - a. 90 μ m aperture= first click-stop
 - b. 60 μ m aperture= second click stop
 - c. 30 μ m aperture=third click-stop
 - ii. Go to 12,500X magnification
 - iii. Press **IMG/DIFF** button on the right control panel to go into diffraction mode. Decrease the illumination with brightness knob.
 - iv. Focus the diffraction spot with the focus knob
 - v. Center the objective aperture around the bright diffraction spot.
 - vi. Press **IMG/DIFF** button on the right control panel to return to imaging mode.
- b. Adjust the Eucentric Height (Mechanical Focusing)
 - a. Setting the eucentric height will mechanically move the stage in the Z direction to a position that is calibrated for the objective lens of the microscope. **The eucentric height must be checked on every sample and at different locations within the same sample. If the grid is very bent, repeat this alignment often.**
 - i. Go to a magnification that is several times higher than you will be imaging
 - ii. Press **Cal** button on right control panel
 - iii. Press **FOC AID** on right control panel. The Focus Panel should say **MECH**.
 - iv. Minimize the image shift on the viewing screen with the focus knob
 - v. Press **FOC AID**. The Focus Panel should now say **AUTO**.

9. Using Digital Micrograph to take an image

** A note about the CCD Camera. The CCD camera is a very sensitive and expensive piece of equipment. It **IS** possible to damage the camera by exposing it to a very intense beam! Exercise caution when using the camera! Before exposing the camera to the beam **ALWAYS** minimize the brightness!

- a. Check the temperature of the CCD camera by looking at the white box under the table. The camera should be -25.0°C to obtain images with a good signal-to-noise ratio.
- b. If not already opened, start Digital Micrograph (program that controls the camera) by double clicking the icon on the right computer monitor. Click OK to the message "Please retract the slit". Keep the Results window open.
- c. Because the CCD is in a bottom mount position, images appear much more magnified with the camera than on the viewing screen. What is seen by the CCD is only an area about 1 inch^2 around the small hole in the focusing screen on the TEM so adjust magnification accordingly.
- d. Cover the viewing screen of the microscope.
- e. Press the **M8 hotkey** on the left control panel to lift the viewing screen
- f. In the **Camera View** box, press **Start View** button in Digital Micrograph to get a live, low resolution image in **Search Mode**
- g. By moving your pointer over different regions of your image, you will get a reading of the pixel values. The dynamic range of the camera is from 0 to 64,000. **The maximum pixel value (brightest areas) should fall between 10,000 to 30,000 in the final image.** Adjust brightness, exposure time and/or binning accordingly.
- h. Focusing the image and capturing an image:
 - i. While pressing the Alt key, draw a box over a portion of your image using the dotted box tool
 - ii. Under the **Process** menu go to **Live** → **FFT**. A live FFT display of the region contained by the box will appear alongside your image to aid in focusing.

- iii. Use the focus knob to focus the image.
 - iv. As a general rule, images tend to be taken as a slight underfocus because the image has better contrast than when it is taken in true Gaussian focus.
- i. If the image is acceptable, in the **Camera Acquire** box, click **Start Acquire** to capture an image.
 - j. Saving images using a series name:
 - i. Go to **File** → **Global Info**
 - ii. Under **Saving** → **Save Numbered**
 - iii. Create a folder to save images. Images are saved in a folder named by the principal investigator or user and in a secondary folder labeled with the date of image acquisition.
 - iv. In **Building Using String** box, type a root name to identify your images.
 - v. In **Next Index** box, type 1 so the first image is numbered 1.
 - vi. Exit **Global Info**
 - vii. Save image by clicking **Save Numbered** button at top of the screen. The image will automatically be saved in a Gatan .dm3 format in the designated folder
 - k. To convert from .dm3 to tif format:
 - i. Go to **File** → **Batch Convert** → **Choose folder** → **Save as Tif Format**
 - l. Transferring data off the computer
 - i. **To prevent viruses from getting on the TEM computer, do NOT place a personal USB Memory Stick into either computer in the TEM room!**
 - ii. **Use a BioImaging portable drive or Netdrive to put images on a server**

10. Shutting Down

- a. At the end of your session, make sure the settings of the microscope are the following:
 - i. The microscope should be in **MAG Mode** and **IMG Mode**.
 - ii. Center Stage. Do not move the joystick while the microscope automatically centers the stage. **Do not try to remove the sample while the stage is BUSY! Wait until the Stage is IDLE before continuing.**
 - iii. Make sure the objective aperture is inserted to the first click-stop (90 μm) and centered (See 8a above)
 - iv. Reduce Illumination Angle to the minimum value (40 μrad)
 - v. Reduce magnification to 4000X
- a. Under **VACUUM TAB**, close column valves.
- b. Remove sample from microscope and insert the empty sample rod (See 6 above).
- c. Under **GUN TAB**, put the filament into standby by clicking the **STANDBY** button. The filament will ramp down.
- d. Sign out on the log sheet.