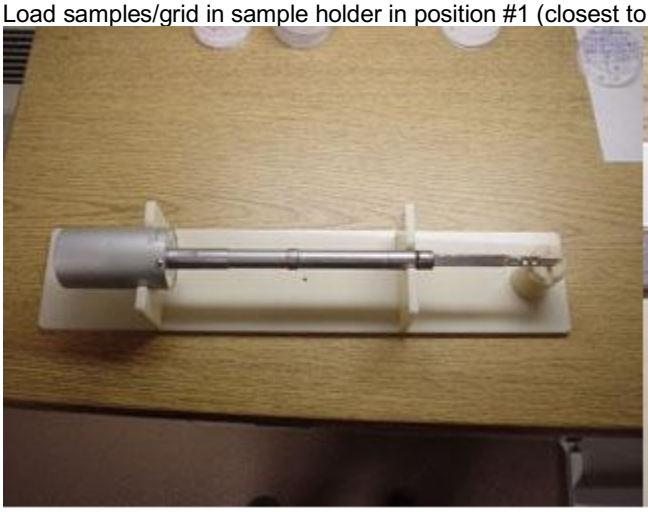
OPERATING INSTRUCTIONS FOR JEOL 1200EX

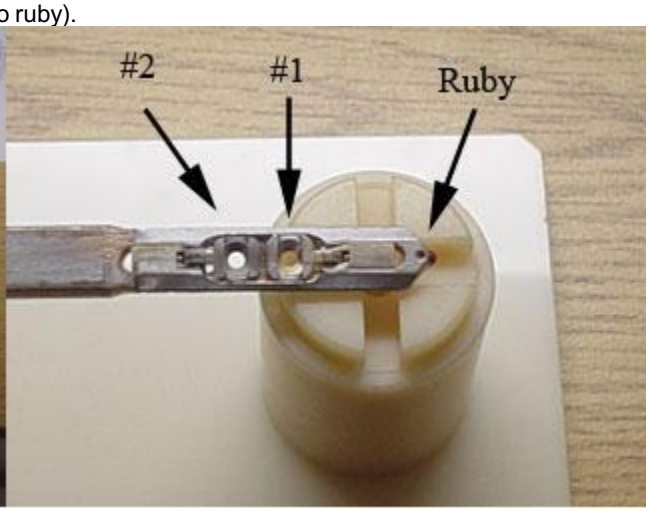
Check for the following:

1. **Green Ready** lamp is lit.
2. **Beam Current** reads 000 or **047**. (If Beam Current is blank, open the upper left panel and flip the lens power supply switch to ON and the accelerating voltage switch to operate).
3. Accelerating Voltage is at **80.0 kV**
4. Mag is at **3,000x**
5. **Sign in on sheet on left:** Name, PI, filament time (read number on left top panel)

Load Sample:

1. Load samples/grid in sample holder in position #1 (closest to ruby).





   a. Use the back of a forceps to flip open the latch and place the grid with the sections face up over the hole Flip down the latch to secure the grid.
2. Align the pin on the sample holder with the notch at the insertion hole on the column of the scope.
3. Insert fully without turning. Firmly press the sample holder toward the column until you hear the vacuum pump engage AND the **red lamp lights up** next to the insertion hole. **WAIT until the red light turns OFF** and the pump recycles.
4. Turn 1/4 turn clockwise and control sample holder speed as it is pulled into column by the existing vacuum.

5. Depress the HT **button.** Wait until **Beam Current** stabilizes @ 047.
6. Slowly increase the filament knob to the metal stopper (this has been set at the saturation point for the filament).
7. Condense the beam on the viewing screen with the **brightness** knob (counter-clockwise).
8. Center beam with **SHIFT X/Y** knobs if necessary.
9. Spread the beam to the edge of the screen using the brightness knob.
10. Focus the image using the **image x wobbler.**

Imaging software, taking and saving pictures:

1. Double click on the orange **AMT icon** to open camera software.
2. Set up a "Case' folder for saving images (File/case study/open new case OR continue previous case).
3. Push green **screen lift** button marked "L" on (Right Hand panel).
4. "Click **for live image'** in the top right corner of AMT software to view the specimen.
5. Adjust light with the **brightness** knob so the histogram is centered (red peak in the middle).
6. Move your grid/sample by using the 2 large knobs (x-y motion) on either side of the column.
7. Change the magnification using the selector switch: right to increase and left to decrease.
8. Focus the image with the focus knobs while using the image **x wobbler.** Turn off the wobbler before taking your image.
9. Click for "final image" to acquire an image. Once it's ready you can read 'final image' in the top left corner.
10. Click on "Case" to annotate (which includes typing in magnification each time) and save the image to your folder using "Save with Caption."
11. Click on "live image" to continue viewing your sample.

For more details on CCD Imaging and settings refer to AMTs "Everyday Procedures for CCD imaging" manual.

Remove Sample:

1. Turn the brightness knob **clockwise** until the screen is dim.
2. Set the filament to **zero.**
3. Pull the sample holder straight out to the stop.
4. Turn 1/4 turn counterclockwise.
5. Pull straight out to remove from the column.
6. Remove your grid and place the holder back into the column- **without pushing in to start the pump.**

Standby:

1. Set Magnification to 3000x.
2. Turn the brightness knob **clockwise** until the screen is dim.
3. **Turn Filament** knob set to **zero.**
4. Remove the sample.
5. Make sure the accelerating **voltage** is at 80.0 KV.
6. Depress the **HT button** (beam current reads 000).
7. Put the cover on the viewing window.
8. **Sign out by writing down new filament time.**

Troubleshooting:

1. If you notice a dark corner on your image-this could be that the light is not centered after changing magnification. Lower screen and re-center the beam using ‘shift X’ and ‘shift Y’ knobs.

2. Occasionally the microscope “freezes” and you cannot move around or change magnification. Flip the “reset” switch found in the lower right small panel (just above your right leg!). After a few moments everything will reset, the beam will come on and you can continue working.

3. If your sample is a larger piece of tissue or a type of tissue that is harder to infiltrate the edges of the tissue will look better than the middle. The middle can be poorly fixed or the plastic might not have polymerized evenly throughout the tissue.