



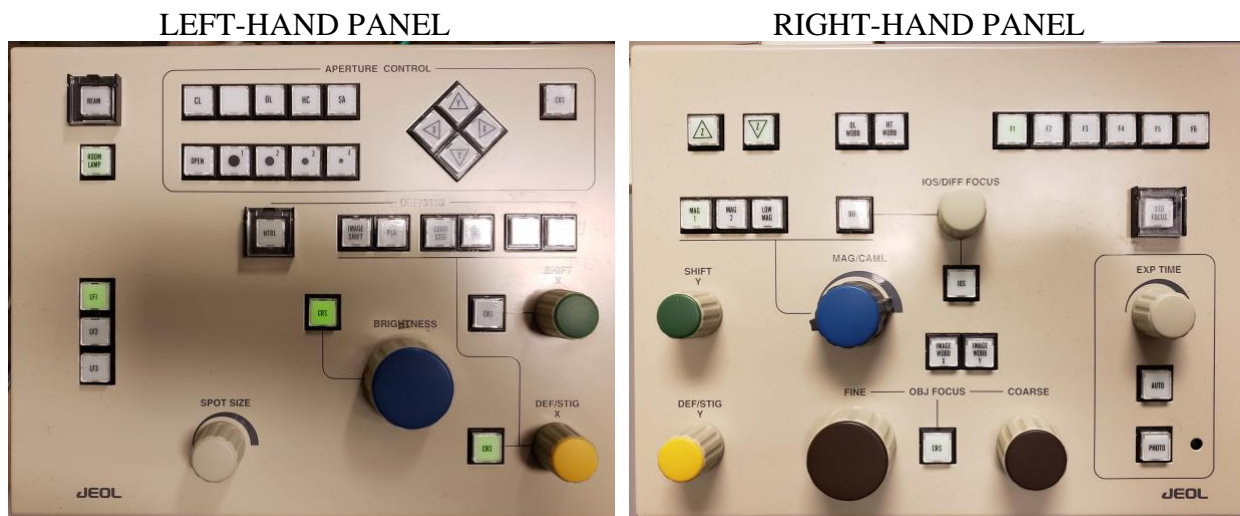
Microscope Usage JEOL JEM-1400
Instrument Location (Room E111 - Cherry L. Emerson Hall)

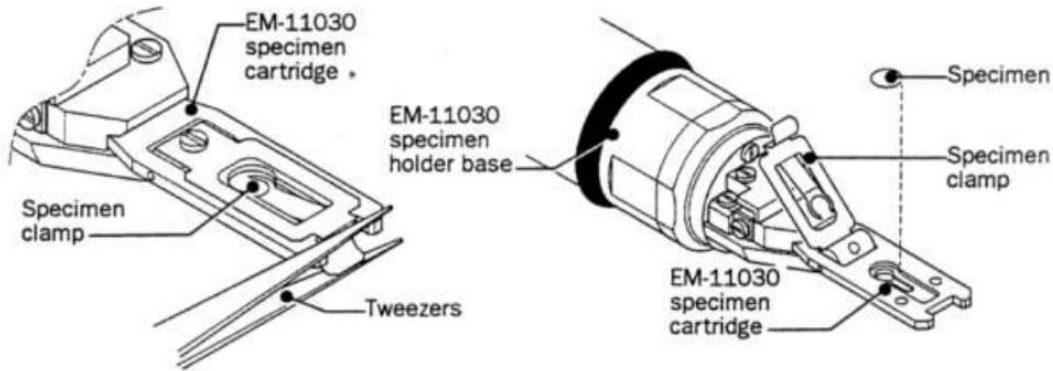
Important

- The protocol below is intended as instruction guidelines for all users. It assumes that the user has gone through training and approval by **Robert P. Apkarian Integrated EM Core (IEMC)** staff.
- This protocol requires knowledge of the controls at the microscope.
- Cryo-EM grid loading and microscope alignments will be carried out by **IEMC** staff unless the user has been properly trained and approved to do it.
- Please contact IEMC staff for assistance in case of any troubles.

Checklist before you start working on the Microscope:

1. Check the main breaker for the microscope on the power supply. This should be ON. If not, please contact IEMC staff for assistance.
2. An accelerating voltage of 80kV is usually selected. Accelerating voltages of 100 kV to 120 kV may be optimal for some samples.
3. Dark current should stay at $44 \mu\text{A} \pm 2 \mu\text{A}$ when high tension is at 80 kV or $66 \mu\text{A} \pm 2 \mu\text{A}$ at 120 kV.
4. The Vacuum should stay at about 13 in the units shown.





TO INSERT THE SAMPLE INTO THE MICROSCOPE:

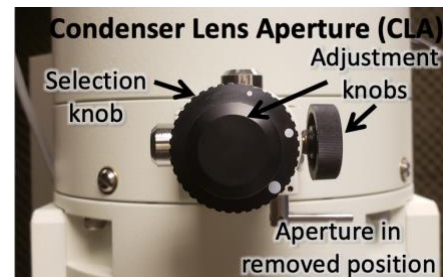
1. The last user should have left the microscope at a magnification of 30Kx.
2. **ESSENTIAL:** Reset the stage by selecting **Control (C)** then **Stage** then **Stage Neutral**. All stage coordinates (x, y, and z) in the stage panel should be close to 0.0. This is very important and a value of 1.0 is already not acceptable.
3. **Lower the screen (press F1 on the right-hand panel), turn OFF the filament and make sure that the objective lens aperture (OLA) is REMOVED.** In case the OLA was left inserted, please record this in the logbook.
4. **Check that the plug has been removed**
5. Insert the sample holder into the compustage by matching the pin to the opening and push until you hear a valve open. At this point, the plastic flap of the holder is facing up.
6. Move the Pump Switch to the UP position and watch for the vacuum value to go down on the specimen chamber (to around 44 μ A). A window will appear on the computer where the holder being inserted may be selected.
7. Wait for the **Evac Ready** message (Green color) on the specimen chamber vacuum indicator on the Vacuum panel. The light over the pump switch will also become green when the vacuum is ready.
8. Keep control of the holder during this step. Do not let it be pulled into the column freely at any moment or the tip may be damaged.
 - a. Turn the holder clockwise (short turn). As the holder goes into the microscope (short distance), hold onto it to guide it into the column and control the speed of insertion, don't pull the holder, just let it go into the microscope as you hold it.



- b. Turn clockwise again (long turn) for a final, longer insertion. Do not let go of the holder until it is completely inserted. The holder's plastic flag slides into its corresponding opening on the compustage.
9. Wait for the vacuum to be stable at ~13 units or below. This is immediate for room temperature but it may take about 5 to 10 minutes for cryo-EM samples.
10. Turn on the filament and wait for the current to reach the set value automatically.

GUN ALIGNMENT: done at 20Kx at the beginning of your session.

10. Change the magnification to 20kx.
11. Up to step 21, these alignments steps can be performed with or without a sample inserted.
12. **ESSENTIAL for the first user of the day:** On the Alignment Panel, click on 'Load.' Select the most recent engineer's alignment file from the AlignmentData directory with the accelerating voltage that you wish to use (80 kV: alignment_80kv.jal; 120 kV: alignment_120kv.jal).
 - a. In case the Alignment Panel is not visible: Select Control (C) on the menu and then Alignment Panel (A).
13. Click on Gun Align and change the Spot Size to 1 (knob on left-hand panel). Condense the beam (make it brighter) by turning the Brightness knob on the left-hand panel counter-clock wise. Center the beam using the green knobs on both panels (because Gun Align is selected, the green knobs are now assigned to Gun Align Shift).
14. Click on Bright Tilt. Change the Spot Size to 5. Condense the beam into a spot with the Brightness knob and center it using the green knobs (now assigned to CL alignment shift).
15. Do steps 13 and 15 a few times until the beam stays centered.
16. Check that the Condenser Lens Aperture (CLA) is inserted. If not, insert it by turning the lever to the left.
17. Expand the beam as wide as possible to where you can still see the edges on the fluorescent screen. Center the physical aperture manually using the CLA adjustment knobs (see picture).
18. Depending on the sample type you will be imaging, select desired spot size for current use and center it.
19. If beam is not round, press COND STIG on in the Alignment Panel. Make the beam round using the Brightness knob to change the size of the beam and the Yellow Knobs (now assigned to CL Stig) to adjust astigmatism.





20. Press CL Stig again to deselect this option (or click on Cond Stig on the computer screen).

EUCENTRIC HEIGHT – to be done at the beginning and then for every new square:

21. The eucentric height may be adjusted with the Z axis buttons (Z-up and Z-down), found on the upper left of the right-hand panel or on the computer screen on the State panel. The speed of change for the Z height buttons can be adjusted in the Stage Panel. It has three settings: >, >>, and >>> for slow, medium, and fast.
- Find a noticeable, contrast-rich feature in the square.
 - Use the track ball (stage) to center the contrast-rich feature on the screen.
 - Using the Stage Controller (-TX and +TX buttons), tilt the stage between 5 and 10 degrees and watch the feature move away.
 - Using the Z axis controls, change the Z height to where the feature comes back to the center of the screen. Bring the tilt angle back to zero by clicking on the Tilt X neutral button (Stage Controller window).
 - Alternatively, press the Image Wobbler, X or Y. As the image wobbles, press the Z-up or Z-down button until the wobbling stops.
22. Press the **Standard Focus** button located on the right-hand panel.

INSERTION AND CENTERING OF OBJECTIVE LENS APERTURE (OLA)

24. THIS STEP SHOULD NOT BE PERFORMED ON THE CAMERA. ONLY ON THE FLUORESCENT SCREEN.

25. Insert OLA, second largest aperture is generally suitable. In the picture on the right, the red dot is selected meaning that OLA aperture is NOT inserted.



26. Select Diffraction (right-hand panel) to make the OLA visible.
27. Center the objective aperture (OLA) with respect to the bright diffraction spot, using the knobs on the OLA control area.
28. Press MAG1 to get out of diffraction mode and return to image mode.

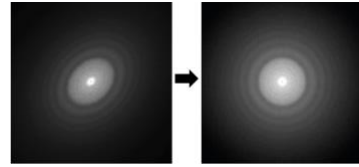
ADJUSTING OBJECTIVE STIGMATION.

29. Find a contrast rich region on your grid. Start Digital Micrograph. Insert the US1000 camera by clicking on Camera Inserted on the Camera Panel.



30. Adjust brightness and turn on **Obj Stig** on the left-hand panel. Lift the screen with F1 and start the View on Digital Micrograph.

1. Use the **Live FFT** (Process – Live FFT) option in Digital Micrograph and adjust the stigmator (yellow knobs) until a true round diffused halo is formed in the FFT.





TO REMOVE THE SAMPLE FROM THE MICROSCOPE FOR SAMPLE EXCHANGE:

31. ESSENTIAL:

- a. Turn the filament OFF.
 - b. Center the stage by selecting **Control (C)** then **Stage** then **Stage Neutral** (the red dot moves to the center of the stage indicating that the stage has been centered).
 - c. **Remove the Objective Lens Aperture.**
32. The vacuum is quite strong so make sure to put a hand on the compustage to balance.
33. Pull the sample holder out until the first stop and turn it counter-clockwise (long-turn).
34. Pull the holder again (short distance) and turn it counter-clockwise (short turn). **DO NOT PULL THE HOLDER ANYMORE**
35. Move the Pump Switch Down to the Air position. Wait for the airlock to be vented (vacuum to a ~200 μ A value)
36. Pull the holder out being careful not to hit the tip of the holder against the edge as it exits.

TO END YOUR SESSION:

37. ESSENTIAL:

- a. Turn the filament OFF.
 - b. Center the stage by selecting **Control (C)** then **Stage** then **Stage Neutral** (the red dot moves to the center of the stage indicating that the stage has been centered).
 - c. **Remove the Objective Lens Aperture.**
 - d. Retract the Gatan CCD camera.
38. Remove specimen holder as indicated in steps 32 to 36.
39. Insert the blanking plug into the goniometer.
40. Log your beam time in the notebook.



SHUTTING DOWN THE JEOL 1400: END OF THE DAY ESSENTIAL TASKS

41. Turn off the filament.
42. Retract the Gatan CCD cameras. Be sure that both cameras are in the **retract position**.
43. Center the stage by selecting **Control (C)** then **Stage** then **Stage Neutral** (the red dot moves to the center of the stage indicating that the stage has been centered).
44. Remove all apertures.
45. Remove specimen holder.
46. Insert the blanking plug into the goniometer.
47. Insert and plug-in ACD heater into the LN2 ACD Dewar and select Control – Maintenance – ACD.
48. When the microscope is shut off at the end of the day, make sure to leave it at 120 kV and a magnification of 30,000 x (30kx).
49. When working with digital micrograph, please do not change the program settings.
50. Conversion of .dm3 files to .tif is done using the ‘convert from display’ option instead of ‘convert from image’.



ALIGNMENT (VOLTAGE CENTERING).

The Objective apertures must be out and the magnification set to 100kx.

48. Spread and center the beam.
49. Activate **HT Wobb**, found on right panel.
50. Turn on **Bright Tilt**.
51. Adjust Wobbling so that it is centered using the yellow knobs.
52. Once achieved, deactivate all buttons used, i.e., **HT Wobb** and **Bright Tilt**.

GAIN REFERENCE FOR THE GATAN CCD CAMERAS: Only to be done if permanent patterns are seen on the images.

53. Find an empty grid square and adjust to a magnification of 20kx.
54. Expand the electron beam so that it is **extremely dim** on the flu-screen.
55. Flip the flu-screen up (using F1 on the right-hand panel) and insert CCD (side mount: Orius or bottom mount: UltraScan).
56. Acquire an image (full 2k frame, 1 s exposure).
57. Select **Analysis** then **Stat** then **Mean & SD**.
58. Reduce or increase the brightness of the image so that the count reads between 2000 and 4000. The value to choose depends on the sample being imaged.
 1. To determine the count read one could position the cursor on the view window and monitor the counts value on the Image Status panel (left side of the Digital Micrograph Window; see Figure).
59. In the Task Bar, select **Camera** then **Prepare Gain Reference**. Enter the average (Mean Counts value) into dialog box and press OK.
60. When the counts become too bright during the gain reference acquisition procedure, the software will ask to expand the beam so that the dose indicator moves to the green intensity zone and to then press Enter.
61. Repeat this procedure until the Gain Reference is acquired successfully.
62. Remember that the beam is now expanded (dim). You will need to condense the beam back to the original brightness after Gain Reference acquisition.