EMORY UNIVERSITY

Robert P. Apkarian Integrated Electron Microscopy Core

Emory Integrated Core Facilities

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

Important

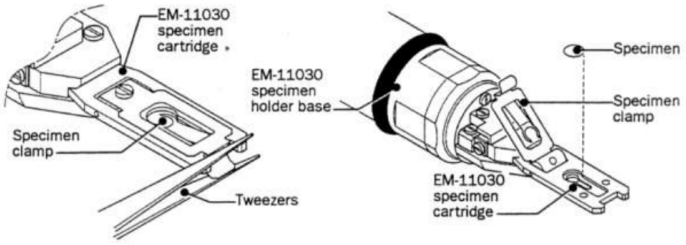
- The protocol below is intended as instruction guidelines for all users. It assumes that the user has gone through training and approval by **Robert P. Apkarian Integrated EM Core (IEMC)** staff.
- This protocol requires knowledge of the controls at the microscope.
- Cryo-EM grid loading and microscope 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 hardware or software issues.

Checklist before you start working on the Microscope:

- Check the main breaker for the microscope on the power supply. This should be ON. If not, please contact IEMC staff for assistance.
- Check the camera temperature controller. It should read -25C.
- Check that the Anti-Contamination Device (ACD) has been filled with LN2.
- An accelerating voltage of 80kV is usually selected. Accelerating voltages of 100 kV to 120 kV may be optimal for some samples.
- In the Illumination System area of the software, the beam current should stay at 44 µA +/- 2 µA when high tension is at 80 kV or 66 µA +/- 2 µA at 120 kV.
- The Vacuum (penning gauge) should stay at about 13 or less in the units shown.

LEFT-HAND PANEL

RIGHT-HAND PANEL



No Gap between Clamp and Holder

Wrong – Gap is present – DO NOT INSERT

TO INSERT THE SAMPLE INTO THE MICROSCOPE:

Checklist:

- The last user should have left the microscope at a magnification of 30Kx.
- All stage coordinates (x, y, and z) in the stage panel must be close to 0.0. This is very important and a value of 1.0 is already not acceptable.
  - If coordinates are not zero: **Reset the stage** by selecting **Stage Neutral** in the **Stage Panel.**
  - If the **Stage Panel** is not visible: select **Control** (C) on the menu and then **Stage**.
- **Lower the screen (press F1 on the right-hand panel)**
- **The filament must be OFF. If not, turn OFF the filament**
- **Make sure that the objective lens aperture (OLA) is REMOVED.** In case the OLA was left inserted, please record this in the logbook.
- **Check that the plug has been removed**

Procedure:

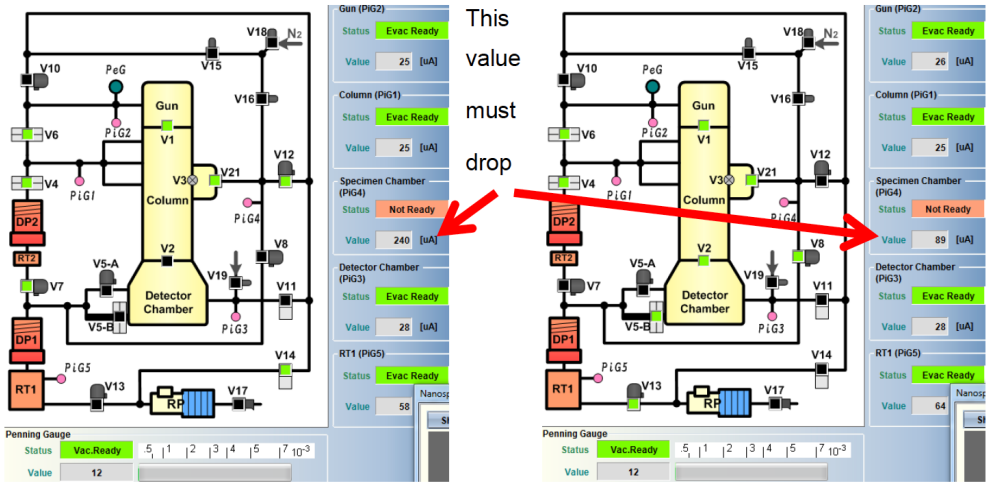
1. Insert the sample holder into the compustage by aligning the pin to the opening and push until it stops and you hear a valve open. The plastic flap of the holder is now facing up.
2. Change the Pump/Air Switch to the PUMP position by gently pulling the metal switch.
3. **DO NOTHING ELSE, except watch the vacuum values on the computer software go down as the vacuum is built -- WAIT FOR THE GREEN.**

If you don't wait for the green and turn the holder immediately the column vacuum will collapse and the TEM vacuum has to be recovered which takes > 1 hour!

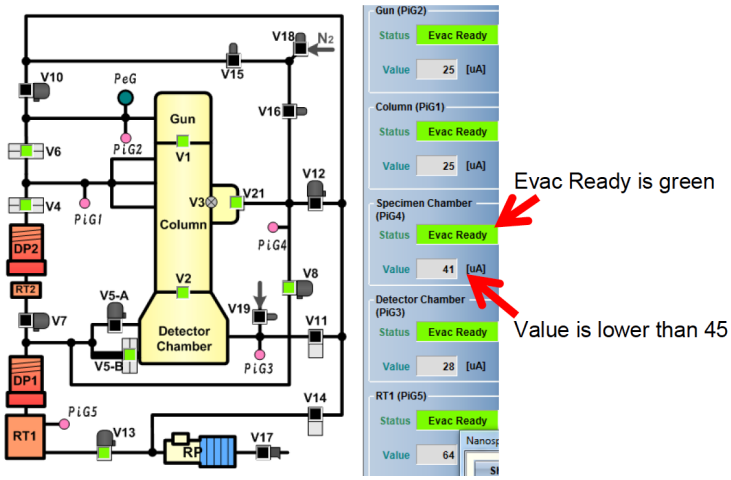
4. WAIT FOR THE VACUUM VALUES AND THE GREEN LIGHT

a. **Watch for** the vacuum in the specimen chamber to drop (to around 45 µA). While this is happening, a window will appear on the computer to select the inserted holder. As you wait for the vacuum, select the holder being inserted.

b. If values are not dropping within 10 seconds it means the holder is not completely in. Press a bit harder on the holder.



c. **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 be ON (the yellow light is still ON).

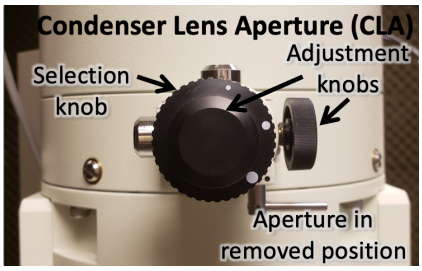


5. Keep control of the holder during this step. Do not let it be pulled into the column freely at any moment or the tip may be damaged.
    a. **Gently** turn the holder clockwise (short turn). As the holder goes into the microscope (short distance), hold onto it to guide it into the column and control the speed of insertion, **do not pull the holder**, just let it go into the microscope as you hold it.
    b. Turn clockwise again (long turn) for a final insertion (long distance). 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.
6. 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.
7. 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.

1. Change the magnification to 20kx.
2. Up to step 19, these alignment steps can be performed with or without a sample inserted.
3. **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 I on the menu and then Alignment Panel (A).
4. 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-clockwise. 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).
5. 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).
6. Repeat steps 14 and 15 until the beam stays centered.
7. Turn all alignment buttons off

CONDENSER LENS APERTURE CENTERING



1. Select the desired spot size according to the sample type you will be imaging. Do not use Spot 1 for imaging. Use Spot 1-3 for room temperature TEM and Spots 3-5 for Cryo-TEM.
2. Making sure that no alignment option is selected, condense the beam to a spot and center it.
3. Check that the Condenser Lens Aperture (CLA) is inserted. If not, insert it by moving the lever to the left. The middle size, 200 µM aperture is normally selected, please don't change it to the other sizes (100 and 300 µM).
4. Expand the beam as wide as possible, to where you can still see its edges on the fluorescent screen. Center the physical aperture manually using the CLA adjustment knobs (see picture).

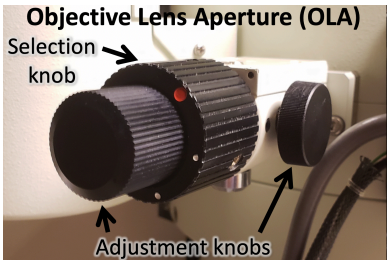
CONDENSER ASTIGMATISM CORRECTION

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

EUCENTRIC HEIGHT – to be done for each new grid and then for every new square:

1. The eucentric height, the height of the specimen at which its image does not move laterally as a function of specimen tilt, 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.
    a. Find a noticeable, contrast-rich feature on the square.
    b. Use the trackball (stage) to center the contrast-rich feature on the screen.
    c. Using the Stage Controller (-TX and +TX), tilt the stage between 5 and 10 degrees and watch the feature.
    d. 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 to zero by clicking on the Tilt X neutral button. Only adjust Z height when the stage is tilted.
    e. Alternatively, press the Image Wobbler, X or Y. As the image wobbles press the Z-up or Z-down button until the wobbling stops.
2. Press the **Standard Focus** button located on the right-hand panel.

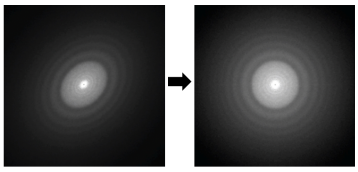
INSERTION AND CENTERING OF OBJECTIVE LENS APERTURE (OLA)



1. THIS STEP SHOULD NOT BE PERFORMED ON THE CAMERA. ONLY ON THE FLUORESCENT SCREEN.
2. Insert OLA, the second largest aperture (60 µM) is generally suitable. In the picture on the right, the red dot is selected meaning that the OLA aperture is NOT inserted. The OLA sizes are 20, 40, 60, and 120 µM.
3. Select Diffraction (right-hand panel) to make the OLA visible.
4. Center the objective aperture (OLA) around the bright diffraction spot, using the knobs on the OLA control area.
5. Press MAG1 to get out of diffraction mode and return to image mode.

OBJECTIVE ASTIGMATISM CORRECTION

1. Find a contrast-rich region on your grid. Start Digital Micrograph. Insert the US1000 camera by clicking on Camera Inserted on the Camera Panel.
2. ON Digital Micrograph – Process – Live – FFT: to get the image of the FFT on the right side of the screen.
3. Adjust the beam brightness by adjusting the Intensity control to be in the center of the green region.
4. Turn on **Obj Stig** on the left-hand panel. Lift the screen with F1 and start the View on Digital Micrograph.
    a. 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:

Checklist:

- Stop the view on Digital Micrograph
- Retract the Gatan CCD camera: Unclick the "Camera inserted" box to retract the camera.
- **Remove the Objective Lens Aperture**.
- Turn the filament OFF.
- Lower the screen (F1).
- Center the stage by clicking **Stage Neutral** on the Stage Window. If the window is not available, select **Control menu --** then **Stage** to open it.

Procedure:

1. The vacuum is quite strong so make sure to put a hand on the compustage to balance.
2. Pull the sample holder out until the first stop and turn it counterclockwise (long-turn).
3. **Very carefully** pull the holder again (short distance) and turn it counter-clockwise (short turn). The goal is to get the Flap to be at the 12:00 position. **DO NOT PULL THE HOLDER ANYMORE**
4. Move the Pump/Air Switch Down to the Air position. Wait for the airlock to be vented
5. Pull the holder out being careful not to hit the tip of the holder against the edge as it exits.

TO END YOUR SESSION:

Checklist:

- Stop the view on Digital Micrograph
- Retract the Gatan CCD camera: Unclick the "Camera inserted" box to retract the camera.
- **Remove the Objective Lens Aperture**
- Turn the filament OFF.
- Lower the screen (F1).
- Center the stage by clicking **Stage Neutral** on the Stage Window. If the window is not available, select **Control menu --** then **Stage** to open it.

Procedure:

1. Remove the specimen holder as indicated above.
2. Insert the blanking plug into the goniometer and switch to Pump.
3. **Wait for all vacuum indicators to be green**. Do not leave until you ensure that all vacuum indicators are back to normal (green).
4. Log your beam time and session parameters in the notebook.

Saving, & Sending Images on JEOL 1400 TEM

To save images in your TEM session by setting up a folder and file name.

- If it doesn't already exist, create a folder with PI's last name within this year's image folder (User_Images_year).
- Within that PI folder, create a folder for today's work; the new folder will have that day's date, i.e. 2021_06_23
- Setting up the "Save Numbered" option in Gatan Digital Micrograph
  i. The program must be open, if not, open it.
  ii. Go to FILE, select GLOBAL INFO.
  iii. Under the Saving area select SAVE NUMBERED.
  iv. BROWSE to User_Images_year, find your PI's folder, and your folder inside.
  v. Select BUILDING USING STRING, and create the name for all your images, i.e., Co1007_1A_specific_sample _name. The separator is an underscore (_) by default. The next Index should be 1 or can be changed to whatever number to continue the sequence. For example, you will end up with Co1007_1_A_secific_sample_name-0001, all the way to …-0009 for 9 images. Co1007 is the grid box number. Number 1 through 20 is the row number for grids and A or B etc. are for the column of the grid, i.e., grid 1A, 1B, or 13D. This way we can go back and find the very grid used for images.
  vi. Make sure that "Save Images in Gatan 3 Format" is selected. This is important! This is usually already selected but double-check. This will allow you to convert images to any format you would like afterward. If TIFF is selected instead, further modifications are not possible.
  vii. Select OK.
- To save each image, select the image and hit Control+Y (Menu File – Save Numbered). The image will be given your string and the next INDEX number.

To batch-convert your images from *dm3 to other formats

- Go to FILE, select BATCH CONVERT, select BROWSE, select your folder, and select OK. Be sure Display is converted to TIF (not JPG or anything else). Check it, sometimes this is changed for special use.
- Sort the images by TYPE.
- Create a new folder within your image folder and put all the TIF images in it, i.e., 2021_06_23_TIF.