

STANDARD OPERATING PROCEDURE

JEOL JEM-2100 HR

TRANSMISSION ELECTRON MICROSCOPE

(TEM)



EDS and EELS Chemical analysis
Post column annular dark field detector
Tomography unit for 3D images
In situ heating and cooling: 77 K to 1000 C
ASTAR orientation mapping and precession electron diffraction

Point Resolution:	2.3 Å
Line Resolution:	1.4 Å
STEM resolution:	10 Å
Magnetic imaging resolution:	22 Å

Emergencies

Power failure

The microscope will shut down safely. When power is restored, it is necessary to restart the microscope manually (**see below**).

Cooling Water Suspension

The high voltage power and lens power will shut down, and the vacuum system will go into the protected state. Upon resumption of cooling water, the microscope must be restarted manually (see below).

Emergency Stop

Use the **EM stop** button (in left-hand cabinet) in case of fire or severe flood. Avoid this method of shutdown unless absolutely necessary.

Start-Up Procedure

Preliminaries

Under normal circumstances, the microscope should be switched on and ready for use.

Check to make sure that SF₆ gas pressure > 0.01 MPa (round dial gauge on left-hand unit behind microscope).

Check to make sure that the air pressure is > 0.4 MPa (top round dial gauge behind grill at base of column on left-hand side).

Check to make sure that the gun gas pressure is > 0.3 MPa (bottom round dial gauge behind grill at base of column on left-hand side).

Check to make sure that the column pressure gauge on the sputter ion pump (SIP) power supply is < 2.5×10^{-5} Pa (the rectangular dial gauge on the tall unit behind the microscope).

Make sure that the tiny TEM icon on the taskbar of the left-hand monitor has a rotating blue circle around it.

(If any of these conditions are not met, contact Dr. Karthik Chinnathambi and do not attempt to use the microscope.)

Turn the Gatan camera controller ON and launch the digital micrograph software.

Raising the Voltage

All references to the screen refer to the left-hand monitor unless otherwise stated.

If the “Controller for JEM-2100/HR” software is not currently running, double click on the TEMCON icon on the desktop of the left-hand monitor.

The beam current should be zero and the high voltage (HT) switched off unless the microscope has recently been used, in which case the HT will be left on and beam current non-zero.

If the anti-contamination device heater is in place, carefully unplug it and remove it.

Click on the HT button at the top of the screen.

While you are waiting for the high-voltage to stabilize, fill the anticontamination device (ACD) dewar with liquid nitrogen.

For operation at 100 - 200 kV:

Use the ▲ and ▼ buttons to increase the kV setting to 100 kV. The **Step** option allows you to control the step size of the changes.

Press the HT **ON** button and wait for the voltage to stabilize at 100 kV.

If the desired voltage is greater than 100 kV:

Click on the **More** box to obtain more options.

In the Auto HT section, set the **Target** voltage to the desired value. Again, the step controller will allow you to change the size of voltage steps.

Set the **Step** size to 0.1 and the **Time/Step** to 3.

Press the **Start** button in the Auto HT section. If the target voltage is 200 kV, then this will take about 50 minutes.

Once the HT stabilizes, the **beam current** (top left of screen) should be about half the voltage (in kV) μA (or about 101 μA at 200 kV). *If it is > 105 μA at 200 kV, contact Dr. Karthik Chinnathambi and do not attempt to use the microscope.*

Sample Insertion

Always wear gloves when handling specimen holders.

Single-Tilt Holder

Insert cartridge handling tool into hole at end of holder and open clamp by tilting the tool. Carefully remove the cartridge from the holder with tweezers, lifting it over the retaining pin.

Place the cartridge on the pale plastic holder, locating it on the pin.

Use the screw driver supplied to loosen the copper specimen retainer and rotate it clear.

If a specimen is already loaded (it should not be), remove it.

Place new specimen (facing upward) in the cartridge.

Return specimen retainer to its original position and secure it with the retainer screws.

Open the clamp on the holder again with the cartridge handling tool and re-insert the cartridge, carefully locating it on the pin.

Double Tilt Beryllium Holder

Use the screwdriver supplied to loosen the screws on the copper retainers.

Very carefully lift them from the pins and rotate them clear of the beryllium wing-shaped sample retainer.

Gently lift the sample retainer out and place it down on a clean part of the holder.

If a specimen is already loaded, remove it.

Insert new specimen (facing upward) in the holder.

Replace the beryllium sample retainer over the specimen.

Carefully maneuver the copper retainers back over the beryllium sample retainer and gently screw them down.

Ensure the sample is being held securely in the holder by gently tapping – DO NOT HIT THE HOLDER.

Inserting Specimen Holder into Microscope

See diagram.

Ensure that there is no dust or lint on the holder O-rings. If there is, wipe gently with lint-free tissue.

Ensure that there is sufficient vacuum grease on the O-rings (they should be shiny but have no noticeable white traces of grease). If excess grease is present, wipe it away with lint-free tissue.

Align the specimen holder guide pin with the guide groove in the goniometer. Push the holder into the goniometer until it stops.

Holding the specimen holder firmly in place, flip the goniometer switch to PUMP. *The yellow light should appear as evacuation commences.*

Wait a few minutes for the green light to appear. **DO NOT ROTATE THE HOLDER YET.** If using the double tilt holder, place the y-tilt control cable on top of the goniometer for support. Do not let the cable dangle.

Turn the holder clockwise until it stops and insert the holder as far as it will go (not far).

Turn the holder again clockwise until it stops and insert the holder fully into the column until it stops. *The yellow light on the goniometer will go out at this point.*

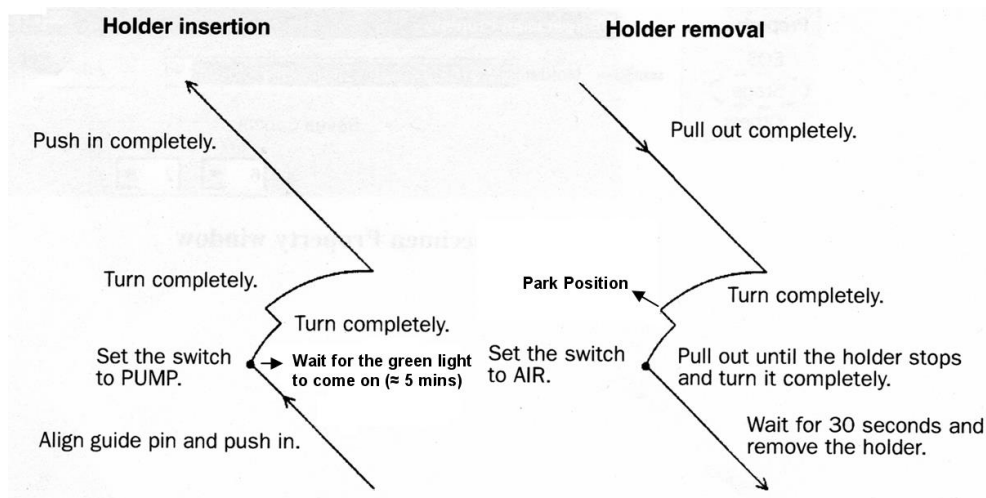
If using the double tilt holder, plug the y-tilt control cable into the TL-Y socket on the goniometer.

Select the appropriate specimen holder on the top right of the Controller for JEM-2100/HR main window:

Single-tilt holder, select EM-21010//21020: Single Tilt Holder.

Double-tilt holder, select EM-31640: Specimen Tilting Beryllium Holder.

Ensure that the SIP vacuum gauge recovers to $< 2.5 \times 10^{-5}$ Pa before continuing.



Filament Ramping

Make sure the **Normal Setup** button is selected in the Filament Control Speed.

The filament should be set to ramp to some percentage (typically 60% - 70%) less than the maximum limit. Do not alter this number or the filament maximum limit.

Press the filament ON button in the High Voltage Control window (or the **Beam** button on the top left of the left-hand control panel).

The Filament window appears. While it is open you cannot access any other windows.

Alignment Procedure

Finding the beam

The last user should have left the microscope at a magnification of about 50k and with all the apertures out. If you can't find the beam, the most likely reason is that your sample is in the way. Try moving it around. **DO NOT** move apertures or use beam shift knobs to find the beam!

If you can't find the beam, try:

1. Check that the beam current has reached its normal value ($\frac{1}{2}$ kV \pm 10%).
2. Check that all the apertures are out.
3. Check that the spot size is set to 1 (use **SPOT SIZE** knob on left-hand panel).
4. Decrease the magnification and spread the beam.
5. Go to **LOW MAG** mode (button on right-hand panel) and spread the beam with the **BRIGHTNESS** knob (on left-hand panel).
6. Move your sample around with the trackball.
7. If you still can't find it, turn off the filament and remove the sample holder to the park position. Turn on the filament again and repeat the above steps to find the beam. If you find it, center it, then shut off the filament, re-insert your sample, and begin again.
8. Try loading a previous alignment (Maintenance - Alignment - Load Alignment File)
9. If you still can't find the beam, get assistance from Dr. Karthik Chinnathambi.

Gun Alignment

Find a hole in the sample.

For initial alignment, set the α -selector to 3. In general, for magnifications less than \approx 100k, $\alpha = 3$ is appropriate. Setting 2 is recommended for magnifications above 100k, and setting 1 is recommended for magnifications of 400k and above.

Condenser Aperture Centering

Insert a condenser aperture.

Set the magnification to 100k.

Focus the beam with the **BRIGHTNESS** knob and center it with the **SHIFT** knobs.

Turn the **BRIGHTNESS** knob clockwise. If the illumination shifts, re-center it with the condenser aperture **X** and **Y** knobs.

Repeat until the illumination no longer moves off center.

Gun Tilt Correction

Focus the beam.

Press **F4** on the right-hand panel and use the **DEF/STIG** knobs until the beam is circular and as small as possible. It may help to watch the current density value - the point is to maximize the current density (brightness) on the screen.

Press **F4** again to de-select the gun assignments.

Condenser Astigmatism Correction

Press the **COND/STIG** button (left-hand panel) and use the **DEF/STIG** knobs to sharpen the emitter image. It may help to slightly desaturate the filament or increase the spot size, keeping the image as small and circular as possible.

The alternate method is to spread the beam to about an inch in diameter and make it as circular as possible using the **DEF/STIG** knobs. No need to desaturate the filament.

Press the **COND/STIG** button again to shut it off.

Condenser Lens System Alignment

Set **SPOT SIZE** (knob on left-hand panel) to 5

Focus the beam and center it using the beam **SHIFT** knobs.

Change **SPOT SIZE** to 1

Focus the beam and center it using **GUN SHIFT** (press **F4** on right-hand panel then use **SHIFT** knobs).

Repeat these steps until the beam remains centered. Finish with **SPOT SIZE** set to 1 and the beam spread on the sample.

Condenser Lens Deflection Coil Adjustment (Tilt Purity)

Focus the beam..

Click on **Tilt** in the Compensator portion of the Alignment Panel for Maintenance window.

Click on **TiltX** in the Wobbler portion of the Alignment Panel for Maintenance window.

Unify the resulting two spots with the **DEF/STIG X** knob (left-hand panel). This is sometimes easier with a slightly desaturated filament.

Click on **TiltX** again to turn it off.

Click on **TiltY** in the Wobbler portion of the Alignment Panel for Maintenance window.

Unify the resulting two spots with the **DEF/STIG Y** knob (right-hand panel). This is sometimes easier with a slightly desaturated filament.

Click on **Tilt** in the Compensator portion of the Alignment Panel for Maintenance window to turn it off.

Shift Purity Adjustment

Press **SA DIFF** (right-hand panel) and set the camera length to 200cm with the **MAG/CAM L** knob on right-hand panel.

Turn the **BRIGHTNESS** knob fully clockwise.

Use **DIFF FOCUS** knob (right-hand panel) if you cannot see the edge of the beam.

Use the **DIFF FOCUS** knob (right-hand panel) to reduce the caustic spot. You may find it useful to then underfocus slightly.

If the caustic spot moves off the screen center as the **BRIGHTNESS** knob is turned, center it using the **DEF/STIG** knobs (but make sure the **PLA** button is illuminated).

Click on **Shift** in the Compensator portion of the Alignment Panel for Maintenance window.

Click on **ShiftX** in the Wobbler portion of the Alignment Panel for Maintenance window.

Unify the resulting two spots using the **DEF/STIG X** knob.

If the spot moves off-screen, reduce the camera length and continue adjusting

If the spot splits into two in the Y direction, click on Compensator **Angle** then unify the spot with the **DEF/STIG X** knob. Click on **Angle** again to turn it off.

Click on **ShiftX** again to turn it off.

Click on **ShiftY** in the Wobbler portion of the Alignment Panel for Maintenance window.

Unify the resulting two spots using the **DEF/STIG Y** knob.

If the spot moves off-screen, reduce the camera length and continue adjusting

If the spot splits into two in the Y direction, click on Compensator **Angle** then unify the spot with the **DEF/STIG X** knob. Click on **Angle** again to turn it off.

Click on **ShiftY** again to turn it off.

Iterate the Tilt Purity and Shift Purity alignments until both are simultaneously aligned.

Voltage Axis Centering

Try to find a pointed spot on the sample and center it

Press **STD FOCUS** button (right-hand panel) and focus on the sample using the **Z** controls. (refer to the focus section for more help with focusing)

Adjust the magnification to about 100k

Finely focus the image using **OBJ FOCUS**.

Select **Maintenance** from the Controller for JEM-2100/HR software menu bar and click on **Alignment**.

Click on the **HT** and **CLA** buttons in the Alignment Panel for Maintenance window.

Adjust the **DEF/STIG** knobs so that the image expands and contracts around the center of the screen.

Click again on **HT** to shut this wobbler off.

Objective Lens Astigmatism Correction

Find the amorphous edge of the sample and increase magnification to $\approx 300k - 500kX$, adjusting the **BRIGHTNESS** knob for optimum viewing conditions and using the **ShiftX** and **ShiftY** knobs to center the beam (ensure that the **BRIGHT TILT** button is illuminated).

Finely focus with the **OBJ FOCUS** knobs.

Press the **OBJ STIG** button (left-hand panel)

Adjust the **DEF/STIG** knobs until minimum contrast is observed in the amorphous region. It may help to slowly turn **OBJ FOCUS** back and forth through focus to observe the variation of phase contrast.

Press the **OBJ STIG** button again to shut this adjustment off.

Objective Lens Astigmatism Correction (*alternate method*)

Find and focus an amorphous region on your sample or focus on the carbon support film in case of grids. Use magnifications 100KX or higher.

Lift the viewing screen and click **start** on the Digital Micrograph software to see the digital image.

Hold the **Alt Key** and draw a square using the **ROI tool** on the live image

Click **Process** → **Live** → **FFT** to get the FFT pattern of the amorphous region. Slightly under or over focus the image using **OBJ FOCUS** to see the FFT rings.

Press the **OBJ STIG** button (left-hand panel) and use the **DEF/STIG X & Y** to make the FFT rings circular.

Press the **OBJ STIG** button again to shut this adjustment off.

Focusing

If an objective aperture is in place, remove it.

For low magnifications ($< \approx 100kX$), focusing can be done easily with the image wobbler.

Press the **IMAGE WOBB X** and/or **IMAGE WOBB Y** button (right-hand panel).

Unify the oscillating image with the **OBJ FOCUS** knobs (right-hand panel). If the image cannot be unified, try correcting for objective astigmatism (above).

Press the **IMAGE WOBB X** and/or **IMAGE WOBB Y** button again to shut it/them off.

For high magnifications ($> \approx 100k X$), focusing is best done with either Fresnel fringes or point images.

Look for the appearance of bright and/or dark Fresnel fringes. If they are very close together, their appearance results in an increase in contrast.

For widely-spaced fringes, use the **Z** control to bring them together. Only use **OBJ FOCUS** knobs to correct for very small fringe spacings.

For very high magnification (> 300k X), find an amorphous section of the sample (typically near the edge) and focus the image using the **OBJ FOCUS** knobs. The point image will have minimum contrast at the point of focus.

Note: The optimum focal condition is not always at exact focus. It is often useful to slightly underfocus samples, especially biological ones, in order to increase contrast.

Obtaining a Selected Area Diffraction Pattern (SADP)

Insert a Selected Area Aperture (*aka* Field Limiting Aperture). It is recommended that the largest one possible which still excludes extraneous parts of the sample be used.

Use the X and Y positioning knobs on the aperture to center it on the region of interest.

Press the **SA DIFF** button (right-hand panel). An image of the back focal plane should appear.

Adjust the **MAG/CAM L** knob (right-hand panel) to the desired camera length (120cm is recommended for viewing but 25cm for recording an image).

The intensity of the pattern can be adjusted with the **BRIGHTNESS** knob.

Adjust the **DIFF FOCUS** knob (right-hand panel) to obtain the sharpest pattern.

Center the pattern by pressing **PLA** on the left-hand console and adjusting position with **DEF/STIG** knobs.

To record the pattern, use a small camera length (≈ 40 cm) and ALWAYS USE THE BEAM STOP to block the central transmitted beam (failure to do this can cause irreparable damage to the camera). See "Recording Images on the Orius SC1000 Digital Camera" below for more information.

Kinematical Bright-Field (BF) Imaging

Obtain an SADP as described above.

Insert an objective aperture and use the X and Y adjustment knobs to center it over the transmitted beam. The smaller the aperture, the more contrast will be in the image, but also the less intensity and the more potential for aberrations.

Press **MAG1** (right-hand panel) to return to image mode.

Recording Images on the Orius SC1000 Digital Camera

Find your region of interest and focus the image.

Digital Micrograph (DM) software is used to acquire and analyze the digital images.

Make sure that CCD camera is inserted ("Camera Inserted" check box should be selected). If not, insert the camera by clicking on check box.

Spread the beam to evenly illuminate the sample and to make sure that the beam is not too bright.

Lift the TEM viewing screen to expose the CCD and click on the "start view" button under camera view on DM to start viewing the live image. Select appropriate exposure conditions to improve the image quality.

Using the keyboard up or down arrow keys will respectively double or halve the exposure time.

Readjust the focus if necessary. Click on the "record" button to capture your image.

Save your image.

Exposing the camera to intense electron beam will permanently damage the CCD. So, it is very important to spread the beam across the screen. Always use higher exposure times and not higher beam intensity to get good quality images.

Do not reduce the magnification or increase the beam brightness significantly when using the CCD. These operations should be performed only using the TEM viewing screen.

You must use the beam stopper to block the direct beam when using the CCD for recording diffraction patterns.

End of Session

Set magnification to $\approx 50,000X$.

Spread illumination to fill the screen.

Double click on the **Stage Neutral** button

Remove all apertures.

Turn off the filament by pressing **OFF** in the Filament section of the High Voltage Control window (press the **HT** button at the top of the screen if this window is not still open).

Wait until filament is completely off before extracting the holder. Do **NOT** remove the holder while the filament is turning off.

Remove sample from holder. DO NOT STORE SAMPLES IN MEC113/113a.

Cover the viewing chamber.

If you are the last user of the day, insert the ACD heater (watch out for excess nitrogen that may pour out of the tube) and plug it in, then turn on the ACD heat by clicking on **Maintenance, ACD & Bake**, and pressing **ON** in the ACD Heat tab. (***This step applies only if the LN2 has been used to cool the ACD.***)

Close the digital micrograph software and turn the Gatan Camera controller OFF.

Update the hours on iLab calendar.