STANDARD OPERATING PROCEDURE

JEOL JEM-2100 HR

TRANSMISSION ELECTRON MICROSCOPE (TEM)

EDS Chemical analysis
Post column annular dark field detector
Tomography unit for 3D images
Cryogenic temperature control down to 77 K

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 x 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 BSCMC staff and do not attempt to use the microscope.*
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 BSCMC staff 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:

- 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 (½ kV ± 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 BSCMC staff.

**Gun Alignment**

Find a hole in the sample.

For initial alignment, set the $\alpha$-selector to 3. In general, for magnifications less than $\approx100k$, $\alpha = 3$ 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.

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.

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 ≈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 (< ≈ 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 (> ≈100kX), 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 ShiftX and ShiftY 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.*
Scanning Transmission Electron Microscopy (STEM)

Focus the beam with the **Brightness** knob.

Make sure the **BRIGHT TILT** button is illuminated and move the beam to the center of the screen using the **ShiftX** and **ShiftY** knobs.

Click on the **Dialogue** option in the TEMCON menu bar, then select **ASID Control**.

The axis alignment procedure is not normally necessary. If desired resolution cannot be attained, contact Rick Ubic or Steve Letourneau.

1. **Click on the Alignment** radio button in the Function section of the ASID Control Panel window.

2. **Click on the Spot Size** arrow buttons so that an appropriate spot can be obtained.

3. **Click on the Spot Mode** icon near the top of the image Control window.

4. **Move the cross cursor to the center of the screen by clicking on the Center button in the Image Control window.**

5. **Turn the BRIGHTNESS knob fully clockwise.**

6. **Obtain a caustic spot using the OBJ FOCUS knobs.**

7. **Move caustic spot to the center of the screen using DEF/STIG knobs.**

8. **Adjust the BRIGHTNESS knob to obtain an image, press the STD FOCUS button (right-hand panel), then focus the image with OBJ FOCUS.**

9. **Focus the beam with the BRIGHTNESS knob.**

10. **Center this crossover spot using the ShiftX and ShiftY knobs.**

11. **Repeat steps 5-10 until the caustic spot and crossover image coincide with the screen center.**

Open Simple Image Viewer (icon on desktop).

Click on the **MAG** radio button in the Function section of the ASID Control Panel window. If the beam is not in the center of the screen, carry out the axis alignment procedure described above.

Click on the **Scan Mode** icon in the Image Control window.
Click on the **STEI-BF** radio button in the ASID Control Panel window.

Click on the **Spot Mode** icon in the Image Control window.

Remove the condenser aperture to observe the Ronchigram pattern (this is more visible in an amorphous sample).

*If the Ronchigram is small and difficult to see, increase the camera length by using the Camera Length arrow buttons in the Mag/Camera length section of the ASID Control Panel window.*

*If you cannot obtain a Ronchigram, adjust the **OBJ FOCUS** knobs accordingly.*

*If the Ronchigram is off-center, move it to the center by pressing the **PLA** button (left-hand panel) and adjusting the **DEF/STIG** knobs.*

If the Ronchigram is not circular, or if adjusting the **OBJ FOCUS** knobs make it elliptical, press the **COND STIG** button (left-hand panel) and adjust the **DEF/STIG** knobs accordingly. Press the **COND STIG** button again to switch it off.

Insert condenser aperture 3 and adjust it so that its center coincides with the center of the Ronchigram pattern.

Insert an objective aperture and adjust it so that its center coincides with the center of the Ronchigram pattern.

Insert the JEOL camera (left of column above viewing chamber) and cover the viewing window.

To correct for astigmatism:

Slightly defocus a STEM image.

If the image appears unidirectionally blurred, press the **COND STIG** button (left-hand panel) and adjust the image with the **DEF/STIG** knobs.

Refocus the image.

Repeat until no astigmatism is observed.
**Tomography**

Insert the holder and bring up the beam at 3000x. Make sure the beam is spread out but don't go all the way to fully overfocus with CL3.

Acquire a trial image with SerialEM. If what you see looks contrast-rich and has many features, start 'eucentric - rough' from the task menu.

Once this completes, locate your ROI and set the magnification and brightness (you can move the stage and set the brightness all through SerialEM).

Once you have an ROI for tomography run 'eucentric - fine' from the task menu.

Walk up the stage to 53 degrees using 'walk up' from the task menu. This starting angle may be higher if you use a High Tilt Retainer and no in-gap OL aperture in the beam path.

From the 'tilt series' menu choose 'Setup/start' to evaluate your tomography parameters. Make sure that the angles are properly specified and pick your basic increment (1.5 or 2 is perfectly fine, or if you want to get more detail in the tomogram you can pick 1 degrees). Pay attention to the mag and the 'at start and when needed track at magnification'. Rule of thumb here is the latter should probably be ~1/3rd of the imaging magnification.

Keep the beam intensity for the moment constant.

Most biological samples do fine with a focus of -0.35 micron, so do most materials samples. For cryo you need more, say 3-6 microns. If the ROI has features that only show with a lot of defocus, you can add this as an offset to the autofocus (remember that underfocus is negative!).

For initial action you probably should have a trial image in focus before you bring up this window, so 'align to image now in Buffer A' should be on.

Leave tracking unchanged for the moment.

Hit 'GO' and sit back. Remember there are 'End' and 'STOP' on the camera and macro controls panel that will halt the tilt series acquisition.

Once the series is done, terminate it from the 'Tilt Series' menu and close the file and do not leave 90° rotated copies in various buffers.

You can run up the tomogram in etomo.
**End of Session**

Set magnification to ≈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.

Update the hours on iLab calendar.