UNCC JEOL 2100 MANUAL

Quick check list

1. Fill the reservoir with LN2
2. Raise HT to 200kV
3. Insert specimen holder into TEM (*Insert holder in airlock, set air/pump switch to pump (yellow light turns ON), when green light turns ON, rotate fully clockwise in two stages and push in completely*)
4. Generate electron beam by clicking Filament ON
5. Insert the largest condenser aperture into the column, center it and correct for astigmatism
6. Adjust eucentric height (*click STD focus button and use Z buttons to focus*)
7. Correct gun shift at spot size 1 and condenser shift at spot size 5. Iterate.
8. Adjust condenser lens deflection coils by adjusting Tilt X and Y and shift X and Y.
9. Center voltage axis (*click wobbler HT. Image should expand and contract around the center. If not, click BRIGHT TILT switch, adjust DEF/STIG so that image expands and contracts around the center of the fluorescent screen*)
10. Correct for objective lens astigmatism.

1. Starting up

1. Sign the Log sheet. Check to see if there are any issues with the microscope before you continue (*check chiller temperature = 70, water flow to and from the TEM: DP~48, OL~38, CL~20 & Gate~60, & SIP pressure~5.8 x 10^{-5} Pa,* ***Column pressure ~5x10^{-4}***).
2. Fill the anti-contamination device (ACD) liquid nitrogen (LN2) tank with LN2. The ACD greatly reduces specimen contamination due to electron beam irradiation. **Put on protective clothing!**
3. Ramp up the HT to 200kV

- Click HT ▼ or ▲ button in the **High Voltage Control** window so that HT reads 120 kV
- Click HT **ON** to turn on the accelerating voltage. Wait for voltage to stabilize at 120 kV (~60 μA). Increase the HT to 180 kV at a step voltage of 10 kV (160 kV (80 μA).
- 180 – 200 kV use the **Auto HT Function**: (**Do not change settings**). Click Auto HT **start** in the **High Voltage Control** window). Load your specimen into the holder while HT is rising.

2. Loading Specimen onto the Specimen Holder

1. Put on some gloves to limit contamination in the TEM.
2. There are 4 different types of holders for this TEM-2 JEOL (Single & double tilt) and 2 Gatan (Double tilt and Cryo).

➢ **Single Tilt Holder**

1. Insert the cartridge handling tool into the hole in the cartridge clamp at the end of the specimen holder, draw up the clamp, and then remove the cartridge from the holder.
2. Loosen the specimen retainer screws, rotate the specimen retainer.
3. Place the TEM grid with the specimen side facing upward in the specimen cartridge.
4. Return the specimen retainer to the original position and secure it with the specimen retainer screw.
5. Insert the cartridge handling tool into the cartridge clamp hole at the end of the specimen holder and draw up the clamp.
6. Attach the cartridge to the holder so that the guide hole on the cartridge aligns with the guide pin on the clamp, then clamp the cartridge by returning the tool to the clamping position. Confirm that the cartridge is firmly secured in the clamp (there should be no gap between the cartridge and the clamp.
7. Now turn the specimen holder upside down and tap on it to make sure the sample is secure and does not fall out
8. Check for debris on the O-Ring. If the O-Ring is dirty use a Kleen wipe and clean the O-Ring.

➢ **Gatan Double Tilt Holder**

1. There is a small tool used to remove the top cover hex screw on the sample holder.
2. Use the small tool "wrench" to unscrew the small screw on the top of the sample holder. Place the wrench and screw in a safe place.
3. Then turn the sample holder upside down and let the washer fall out.
4. Turn the sample holder back upright and load your TEM sample grid to the sample holder with the copper side up.

5. Now place the washer back in the sample holder. Notice that it goes in a specific orientation.
6. Then place the screw back on top and using the wrench, screw it back in. Make sure that screw is all the way in and does not extend above the sample holder.
7. Now turn the sample holder upside down and tap on it to make sure the sample is secure and does not fall out.
8. Check for debris on the O-Ring. If the O-Ring is dirty, clean the O-Ring.

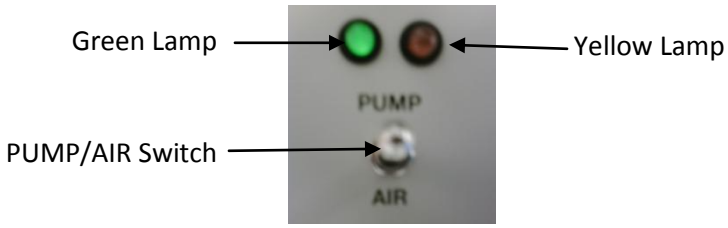
Cryo Transfer Holder

Refer to Cryotransfer system and dry pumping station manuals.

3. Inserting Specimen Holder in the TEM Column

Confirm that the accelerating voltage is 200 kV (100 μA ±10%)

1. Align the specimen guide pin with the guide groove on the TEM goniometer. Push the holder into the airlock system until it stops and set the PUMP/AIR switch to pump. *(The yellow lamp lights up)*



2. When the green lamp lights up, turn the holder a bit clockwise, push the holder until it stops, turn it fully clockwise, you will feel that the vacuum is trying to pull the holder in. Gently let the holder slide into the TEM **using the Thump of your left hand** until it stops. **Do not let go of the holder while it is going into the TEM column.** If it goes too fast the holder and/or the stage can be damaged.
3. Select the holder you are using on the right main screen of the TEM Controller window. For the Gatan Be Double Tilt holder and Cryo holder select "Single Tilt Holder".

4. Electron Beam Generation

1. Remove all apertures from the beam path.
2. Click Filament **ON** in the **High Voltage Control** window or press the Beam Switch on the left Control Panel to generate the electron beam. *(If the reading of the beam current does not change when you click the Filament ON, the filament has burnt out)*
3. If you see the electron beam go to Step **5**. If you cannot see the beam, select TEM mode, Spot size 2, $\alpha = 3$, select MAG2 on the right Control Panel.
4. Turn BRIGHTNESS knob to see if the electron beam appears on the fluorescent screen. Adjust the BRIGHTNESS knob and the SHIFT X and Y knobs to make the beam bright. If you cannot find the beam, the specimen or grid may be in the way-try moving it around. If you still cannot find the beam, GET HELP.

Aligning the TEM

5. Condenser lens (CL) Aperture

1. Select MAG1 and a suitable magnification, e.g. 40kx.
2. Insert the largest CL aperture into the column and center it as follows:
   a. Obtain the smallest electron beam using the BRIGHTNESS knob and center it using the SHIFT X and Y knobs.
   b. Slowly widen the electron beam using the BRIGHTNESS knob. If the beam moves off the screen center as you widen it, center it using the aperture knobs.
   c. Adjust the aperture knobs so that the beam expands and contracts coaxially when you turn the BRIGHTNESS knob back and forth around the focus position.
3. Insert the desired CL aperture (150 μm, 70 μm, 50 μm and 10 μm) and center it.

6. Correcting CL Astigmatism

If astigmatism is present in the CL, the beam, when brought to a focus on the screen, is elongated. Correct astigmatism of the CL lens to make the shape of the electron beam spot round.

Fig. 2 Left Control Panel

1. Focus the beam using the BRIGHTNESS knob.
2. Press COND STIG switch.
3. Slowly turn the BRGHTNESS knob back and forth around the focus position and adjust the DEF/STIG X and Y knobs so that the shape of the electron beam spot becomes round just before and after the focus position.
4. Press the COND STIG switch to deselect it.

7. Adjust Eucentric Height (Wobbler)

1. Locate the specimen *(or carbon network on grid)* screen at a magnification of 40Kx.
2. Press the STD FOCUS switch on the right Control panel.
3. Press IMAGE WOBB X knob
4. Use the Z height controls to focus the image (minimum contrast).
5. Deselect the IMAGE WOBB knob
6. After this alignment all coarse focusing should be done with the Z height adjustment.

Fig. 3 Right Control Panel

8. Gun Shift and Condenser Shift Correction

1. Select the MAG mode and set the magnification to 40kx.
2. Set the SPOT SIZE knob to 1 and focus the beam using the BRIGHTNESS knob.
3. Click DEF Select **Gun** in the **Alignment Panel for Maintenance** window.
4. Center the beam on the fluorescent screen using the SHIFT X and Y knobs.
5. Click DEF Select **Gun** to deselect it.
6. Set the SPOT SIZE knob to 5 and focus the beam using the BRIGHTNESS knob.
7. Press the BRIGHTNESS TILT switch or click the DEF Select CLA in the **Alignment Panel for Maintenance** window.
8. Center the electron beam on the screen using the SHIFT knobs.
9. Click DEF Select CLA to deselect.
10. Repeat steps 2 to 6 above until the electron beam stays at the center of the fluorescent screen when you change the SPOT SIZE.

9. Adjusting CL Deflection Coil

A. Adjusting the Tilt X and Y

This adjustment consists of adjusting the ratio of the currents in the 1st and 2nd CL deflection coils so that the electron beam spot remains stationary when you tilt the electron beam.

1. Focus electron beam using the BRIGHTNESS knob
2. Press BRIGHT TILT and center the beam using the SHIFT X and Y knobs
3. Press BRIGHT TILT to deselect it when done
4. Click Compensator **Tilt** in the **Alignment Panel for Maintenance** window.

5. Click the Wobbler **Tilt X** in the **Alignment Panel for Maintenance** window. Unless the ratio of the currents is adjusted properly, the electron beam spot splits into two parts in the X direction.
6. Unify the split spot using the DEF/STIG X knob *(when the beam spot moves off the screen, lower magnification and continue adjustment).*
   If the beam spot splits into two parts in the Y direction, click Compensator **Angle** in the **Alignment Panel for Maintenance** window, then unify the split spot using the DEF/STIG X knob. After unifying the spot, click the compensator angle to turn it off.
7. Click the Wobbler **Tilt X** button in the **Alignment Panel for Maintenance** window to turn it off.
8. If the beam is shifted from the screen center, press BRIGHT TILT and then center it using the SHIFT X and Y knobs.
9. Press BRIGHT TILT to deselect it.
10. Click the Wobbler Tilt Y button in the **Alignment Panel for Maintenance** window. Unless the ratio of the currents is adjusted properly, the electron beam spot splits into two parts in the Y direction.
11. Unify the split spot using the DEF/STIG Y knob. (If the beam moves off screen, lower magnification and continue adjustment)
    If the beam spot splits into two parts in the X direction, click Compensator **Angle** in the **Alignment Panel for Maintenance** window, then unify the split spot using the DEF/STIG Y knob. After unifying the spot, click the compensator angle to turn it off.
12. If the beam is shifted from the screen center, press BRIGHT TILT and then center it using the SHIFT X and Y knobs.
13. Click the Compensator **Tilt** button to turn it off

B. Adjusting Shift X and Y

This adjustment consists of adjusting the ratio of currents in the 1st and 2nd CL deflection coils so that the caustic spot tilt remains unchanged when you shift the caustic spot.

1. Press the SA DIFF switch.
2. Turn BRIGHTNESS knob fully clockwise.
3. Adjust the DIFF FOCUS knob to obtain a caustic spot on the fluorescent screen.
4. Click Compensator **Shift** in the **Alignment Panel for Maintenance** window to turn it on.
5. Click wobbler **Shift X** in the **Alignment Panel for Maintenance** window to turn it on. Unless the ratio of the currents in the 1st and 2nd deflection coils is adjusted properly, the caustic spot splits into two parts in the X direction.
6. Unify the split spots using the DEF/STIG X knob. (When the caustic spot moves off screen, shorten the camera length and continue adjustment).
   If the electron beam spot splits into two parts in the Y direction, click Compensator **Angle** in the **Alignment Panel for Maintenance** window, then unify the split spot using the DEF/STIG X knob. After unifying the spot, click Compensator **Angle** to turn it off.
7. Click Wobbler **Shift X** in the **Alignment Panel for Maintenance** window to turn it off.
8. If the caustic spot is shifted from the screen center, press BRIGHT TILT and center it using the DEF/STIG X and Y knobs.
9. Click Wobbler Shift Y in the **Alignment Panel for Maintenance** window.
   Unless the ratio of the currents in the 1st and 2nd deflection coils is adjusted properly, the caustic spot splits into two parts in the Y direction.

10. Unify the split spot using the DEF/STIG Y knob.
    If the electron beam spot splits into two parts in the X direction, click Compensator **Angle** in the **Alignment Panel for Maintenance** window, then unify the split spot using the DEF/STIG X knob. After unifying the spot, click Compensator **Angle** to turn it off.
11. Click Wobbler **Shift Y** in the **Alignment Panel for Maintenance** window to turn it off.
12. Click Compensator Shift Y in the **Alignment Panel for Maintenance** window to turn it off.
13. If the caustic spot is shifted from the screen center, press BRIGHT TILT and then center it using the DEF/STIG X and Y knobs.
14. Press MAG1 to return to TEM mode.

10. Centering the Voltage Axis

Adjust the TEM optical so that the image stays at the center of the fluorescent screen even if you change the accelerating voltage.

1. Press MAG1 switch and obtain an image.
2. Focus the image using OBJ FOCUS.
3. Spread the beam fully across the screen and move a conspicuous object in the image to the center of the screen.
4. Press the HT WOBB switch. The image then expands and contracts periodically.
5. Press the BRIGHT TILT switch.
6. Adjust DEF/STIG so that the image expands and contracts around the center of the screen.
7. Press the HT WOBB switch to turn it off. Deselect the BRIGHT TILT switch.

11. Objective Lens (OL) Astigmatism Correction

To obtain high quality images it is necessary to compensate for OL astigmatism that cause the degradation of images. Perforated amorphous films such as the carbon grid are recommended as test specimens for OL astigmatism correction.

1. Find and amorphous area of the carbon grid and put it on the center of the screen.
2. In MAG1, set magnification to 100kx or higher.
3. Lift the screen.
4. Insert the CCD camera, click start view in Digital Micrograph window to view the image of the amorphous material.
5. In the Digital Micrograph Window, Click Process → Live → FFT.
6. A correct objective stigmatism corresponds to a nice sphere FFT.
7. Click OBJ STIG switch.

8. Adjust DEF/STIG to get a better sphere.
9. Click OBJ STIG switch to deselect it.

12. Taking Digital Image

We use DigitalMicrograph™ for acquiring, visualizing, analyzing and processing digital TEM image data. DigitalMicrograph provides the capability to control and acquire images and data from CCD camera interfaced to a computer.

A. Gain Normalization

It is important to prepare a new gain reference image after the CCD camera has reached the equilibrium temperature (< 5 min) every time the camera is switched ON or before acquiring any important image. Multiple gain references can be collected and assigned to magnification ranges on the TEM by selecting the checkbox "Apply to mag range…." and entering the mag range. Always take gain reference close to mag you want to use.

To prepare gain reference image:

i. Remove specimen from the field of view.

ii. Evenly spread the illumination across the CCD sensor.

iii. Under the camera menu, choose Prepare Gain Reference. Set the intensity value of 5000 – 7000. Set the frames to average to 4.

iv. Then simply follow instructions on the screen.

B. Image Acquisition

1. Insert CCD camera in Digital Micrograph window.
2. Click "Start view" to view the image. Select a suitable exposure time (e.g. 0.065 S). Selecting "Optimized" gives the fastest frame rate with the longest exposure time. Using the keyboard up/down arrows will double or half the exposure time.
3. Focus the image. Use Z knobs first, then the OBJ FOCUS knobs. Clicking on the "Focus Loupe" in the camera view window, allows you to focus the image by using a small area with high speed.
4. To record the final image, click "Start Acquire" button.
5. Save the image in Gatan Format (*dm3).
6.

13. Basic EDX

EDX can be done in the TEM mode or the EDX mode. To analyze smaller areas select the EDX mode and a smaller condenser aperture.

1. Select the area you are interested in and move it to the screen center using the track ball.
2. Open the EDX software by double clicking on INCA.
3. Select suitable spectrum conditions (Process time, Spectrum range, and Number of channels)
4. To acquire a spectrum, click the green “Start Acquisition” function key.

14. ENDING TEM SESSION

A. Removing Holder from TEM

1. Click Filament **OFF** in the **High Voltage Control** window or press the BEAM switch to turn off the electron beam.
2. Click the **OK** button in the message window to turn off the Filament.
3. Double-click the **Stage Neutral** button at the bottom right of the Right main screen in the TEM Controller window.
4. Pull the Holder until it stops, turn it fully counterclockwise, pull it a bit until it stops, and then turn it fully counterclockwise until it stops.
5. Set the PUMP/AIR switch to AIR, wait 30 seconds, and then pull out the specimen holder from the TEM.

B. Returning ACD to Room Temperature

If you are the last user of the day, heat the trap up to ambient temperature. It is necessary to evacuate the microscope that uses an ACD for a long time or to vent the column, drain the liquid nitrogen and heat the trap up to ambient temperature.

1. Put the lid on the viewing window to avoid causing serious damage to it by LN2.
2. Remove the reservoir fill cap. Wear appropriate protective clothing.

3. Insert the heater assembly into the coolant reservoir and insert the heater plug into the HTR socket on the connector box.
4. Select **TEM Controller-Maintenance-ACD & Bake** to open the **Bake Out/ACD Heat** window; then click the **On** button in the **ACD Heat** tab. The evacuation system enters ACD mode (SIP if OFF).

Do not dismount the coolant evaporator during or immediately after heating. The heater is very hot, and there is a danger of burns.