MSE 460 TEM Lab 2:
Basic Alignment and Operation of Microscope

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Aims: The aim of this lab is to familiarize you with basic TEM alignment and operation. This lab covers

I. TEM structure, functions and knobs
II. TEM startup
III. Obtain a good electron illumination
IV. Set the sample at eucentric height
V. Condenser lens alignment
VI. Beam tilt purity
VII. Objective lens alignment (Voltage center)
VIII. Image focus and astigmatism correction (Fresnel-fringe method)
IX. Selected area electron diffraction (SAED) including focus and center diffraction pattern.
X. Simple bright/dark field imaging
XI. TEM shutdown

TEM: JEOL-2100F TEM

Sample: Au nanoparticles on carbon supporting film

I. TEM structure, functions and knobs

1. Electron gun, Anode: BEAM, HV/FILAMENT, BIAS
2. Gun beam deflector coils: GUN TILT, GUN HORIZ
3. Condenser lens coils, C1 and C2: SPOT SIZE, BRIGHTNESS
4. Condenser aperture: CL, Illumination angle
5. Condenser Stigmator: COND STIG
6. Beam displacement compensating coil: B.T. Align X and Y
7. Condenser lens beam deflector coils: Beam tilt and shift: BEAM TILT, BRIGHTNESS CENTERING, BRIGHT/DARK field, WOBBLERS
8. Specimen Holder: Trackball & PIEZO switch
9. Objective Aperture: Bright/dark field, back focal plane (diffraction pattern)
10. Objective lens stigmator coil: OBJ STIG
11. Objective lens coil, OBJ: FOCUS
12. Selected Area Aperture: image plane, SAED
13. Intermediate lens stigmator coil: INTER STIG
14. Intermediate lens system, I1: DIFFRACTION SPOT

15. Intermediate lens beam deflector coil: Diffraction pattern alignment: INTER ALIGN
16. Projector lens system, PL and PLA
17. Viewing screen
18. Camera: RIO

II. TEM startup

(1) Log in the computer.

(2) Understand the vacuum system:
Rough evacuation: oil rotary pump and oil diffusion pump.
Main evacuation: Ion pump.
Vacuum meters: Pirani gauges PiG1-PiG5 and Penning gauge
The valves are open when the corresponding valve lamp is lit (green) in the vacuum pump window in the figure below.
Cold trap is above the main evacuation pump for improving vacuum in Gun (PI2) and Column (PI1). Cold finger, if present, would be in the sample area to improve vacuum around the sample and reduce sample contamination.

In Diagram 1:

① Pirani gauge status

- GUN/PiG2: Gun chamber PI2) status and gauge reading
- Column/PiG1: Column (PI1) status and gauge reading
- Specimen/PiG4: Specimen chamber (PI4) status and gauge reading
- Camera/PiG3: Camera chamber (PI3) status and gauge reading
- RT/PiG5: Reservoir 1 (PI5) status and gauge reading

② PEG1: Status of the Penning gauge; as pressure decreases, status changes from OFF → Evac. Low → Evac. High → Vac. Ready

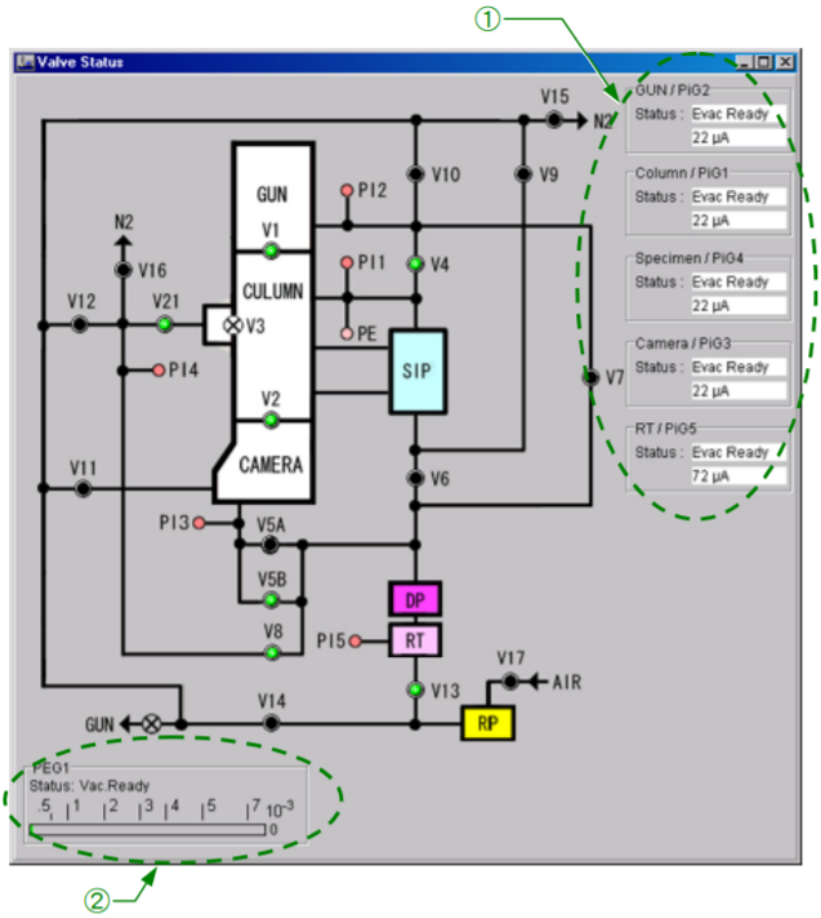


Diagram 1: 2100F vacuum system & valve status window

(3) Make sure that TEM vacuum is good. The vacuum meter (left-bottom rack) should read <3 x 10^{-5} Pa with blue scale. PIG1, PIG3, and PIG5 should read "Evac. Ready" as should PIG4 if the sample plug is in.

(4) Get liquid nitrogen from a big LN tank.

(5) Fill out cold trap on TEM with liquid nitrogen first. (If present, then fill the cold finger chamber.)

Note: a) Liquid nitrogen may hurt you if careless. Wear gloves please.

b) **Always** top up cold finger/trap with liquid nitrogen at the beginning of your session.

c) LN in the cold finger Dewar lasts for about **6** hours. Please make sure that there is LN there during your whole session; this means you may need to top up the cold finger with LN during your session. **Please remember that poor vacuum will occur when LN in cold trap/finger runs out, and is very bad to the microscope when the filament and HV are on.**

d) Wait a minute for the LN2 to cease boiling and then top off the LN2 again to ensure the Dewar is full.

(6) Make sure that TEM is set at standby positions:

a) **Objective and selected area apertures are out** ("open").

b) Beam/Filament: off; Acc. (HV): 200kV, Emission ~ 180 μA, Spot Size = 1, Alpha = 3
c) Specimen position: X=0, Y=0, Z=0; specimen tilts TX = 0
d) Bright/Dark-field selector set at Bright field.
e) Microscope is at MAG1 mode.
f) Mag: 50K-100K

Note: **Objective aperture must be out during sample holder loading/unloading or it will break!!**

(8) Unload the sample plug/holder from the TEM.
Note: A sample holder or plug may stay in TEM. Follow these 5 steps to take it out.

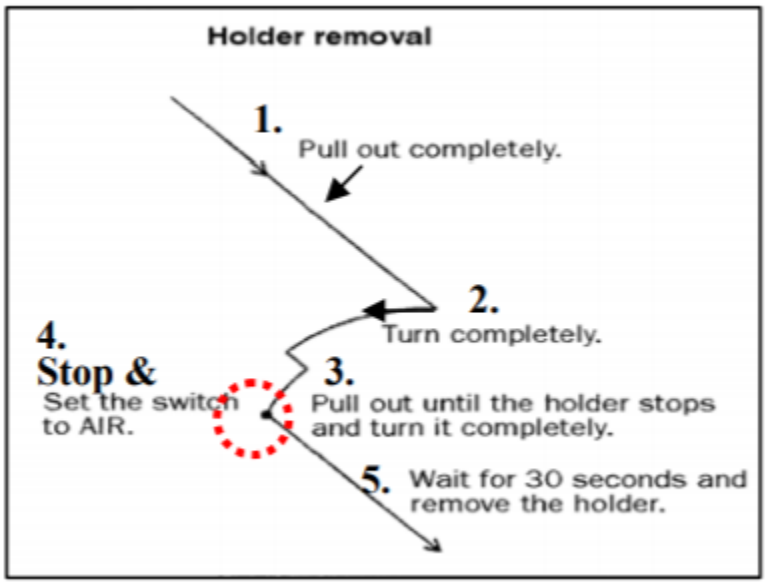


Diagram 2: Sample holder unloading procedures

(9) Choose the right sample holder. (Refer to the operation manual of Fischione 1020 pump station to take out the needed single/double holder.) **Wearing dust-free gloves**, load your sample into the holder (use a good tweezer and a vacuum tweezer) and make sure that the sample is securely seated in the holder and black **O-ring** on the holder is clean. (If not, clean it using a duster or dust-free glove. **Please remember that poor vacuum will cause a lot of problems, for example, beam instability.)**

. Note: a) Your sample must be 3 mm in diameter and not too thick (consider a copper grid as standard sample which is about 100-200 microns thick).
b) Do not breathe on the holder (It is very difficult to pump water vapor in TEM!)
c) For double tilt sample holder, make sure that

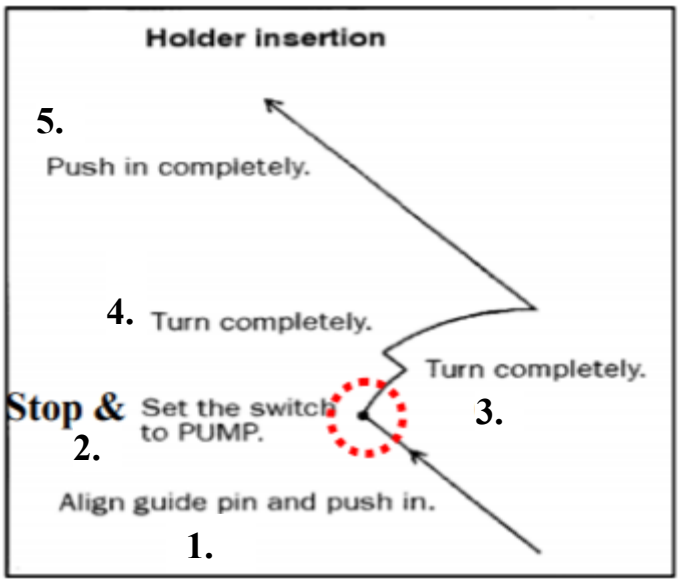
- the sample fixing screw goes into the sample bowl smoothly (If necessary, unscrew it first to make the screw at the level position, then screw it to **avoid thread crossover!**) and
- the screw is finger tight (the bowl will be damaged if it is too tight).

For single tilt sample holder, release the U-shape clamping spring by slightly lifting it (not pushing a pin forward!).

(11) **Follow the route indicated in the diagram on the microscope** to load your sample holder into the TEM.

Note: a) Make sure that the gun is OFF during sample loading/unloading because of temporary poor vacuum.

b) At step 2, turn specimen holder clockwise **only when** the green light is lit, otherwise, you may damage the goniometer.



WAIT for green LED and "Evac Ready" at PiG4 on the monitor before turning in step 3!

Diagram 3: Sample holder loading procedures

12. Wait until the vacuum is below 1.3 x 10^{-5} Pa (see gauge on lower-left panel again) before proceeding.
13. Make sure the correct sample holder is selected on the JOEL PC.

III. Obtain a good electron illumination

1. Press **BEAM** to open the gun valve.
2. Turn the **BRIGHTNESS** control (left panel) to crossover, and center the beam with the **BRIGHTNESS CENTERING** knobs (**SHIFT-X** and **SHIFT-Y**).

If you can not see the beam, find it using the following procedure:

a) Make sure that you have logged in the computer and that you are in TEM MAG mode (if not, press **MAG1**)
b) Decrease the **MAG.** or select **LOW MAG** mode
c) Turn **BRIGHTNESS** anticlockwise
d) Make sure **OBJECTIVE APERTURE (OA), HIGH CONTRAST (HC)**, and **SELECTED AREA (SA)** apertures are out.
e) Ask help if you still do not find the illumination.

3. Press **STD FOCUS** and make sure OL current is 4.31
4. Insert and center **CONDENSER APERTURE:**
Change the C2 lens current (**BRIGHTNESS**) so that the beam is going through the crossover and see if the beam center is swinging. If yes, the condenser aperture needs centering:

- Make sure CL-2 is selected (condenser aperture size 2)
- Adjust **BRIGHTNESS** to get the smallest possible beam
- Use **SHIFT X&Y** knobs to center beam
- Spread beam
- Use **CL-2 APERTURE X-Y ARROWS** to center the beam again
- Repeat this process again until there is no beam swinging at the brightness crossover

5. **GUN TILT ALIGNMENT**: off-sample, at ~ 40kx mag
   a) On the standard operation window, select **GUN DEF**. This sets the **SHIFT** knobs to control **gun horizontal** and the **DEF/STIG (deflector/stigmation)** knobs to control **gun tilt.** (Note: selecting **GUN DEF** on the screen is equivalent to pressing the **F4** button on the left-hand panel.)
   b) Perform **GUN AXIS ALIGNMENT:** use the **ANODE WOBBLER** to fix tilt (**DEF X&Y** knobs)
   c) Perform **GUN SHIFT ALIGNMENT:** "1G-5C"; step **down** in **SPOT SIZE** and center beam with **GUN HORIZONTAL (SHIFT** knobs**)**, then step **up** in **SPOT SIZE** and center beam with **BRIGHTNESS CENTERING (**select **CLA**, *then* use **SHIFT** knobs)
   d) Converge the beam to perform **TILT BALANCE ALIGNMENT**: use the **TILT COMPENSATOR** and **TILTX & TILTY WOBBLERS** (one at a time) and adjust the **DEF/STIG** knobs until there is minimal change with the wobble.
   e) Spread the beam and go to **DIFF** mode to perform the **SHIFT BALANCE ALIGNMENT**; repeat step (D) but this time use **SHIFT COMPENSATOR** and **SHIFTX & SHIFTY WOBBLERS**

6. Correct **CONDENSER ASTIGMATISM:**
If the beam does not have good 3-fold symmetry (almost round at lower mag), condenser astigmatism needs correction:

a) Focus **(BRIGHTNESS)** the beam to crossover and center (**BRIGHTNESS CENTERING**) the beam.
b) Open (**BRIGHTNESS**) the beam, select **COND STIG,** and adjust the **DEF/STIG X & Y** knobs until the beam has 3-fold symmetry. Note: it should stay symmetric for different foci (**BRIGHTNESS)**
c) Converge the beam all the way and oscillate the **BRIGHTNESS** knob to either side of crossover.
d) Adjust **DEF/STIG X & Y** knobs to make sure the beam shape does not change when going to either side of crossover.
e) Repeat c) and d) till the open beam is symmetric all the time.
f) Center the beam again (**CLA – SHIFT X & Y**)

IV. Set your specimen at the eucentric height

This is important to all calibration, TEM performance and the safety of the TEM.

a) Set **MAG** to ~20k and use the **TRACKBALL** to center a specimen feature.
b) Make sure you are at **STANDARD FOCUS** and that the beam is converged.
c) Adjust the **Z-HEIGHT up/down arrow buttons** to minimize rings/clouds around the beams, OR turn on **IMAGE X or IMAGE Y WOBBLER** and adjust Z-height until you have minimized the vibration of the sample feature.
d) Now the contrast of the specimen should be minimal (if objective aperture is out).

V. Beam tilt purity (compensation)

This is to make sure that beam tilt and shift are independent, that is, the beam can stay at the same place while the beam is being tilted.

1. Find sample at high magnifications >100kx, ensure sample is focused at eucentric height.
2. Center the beam, spread beam, find a recognizable feature by moving the sample and turn on **HV/HT WOBBLER** (right panel or standard operation window on screen)
3. Select **CLA DEF** in the operating window (OR press the **BEAM TILT** button on the left control panel) and use **DEF/STIG** knobs to minimize variation with wobble; image will move in and out, but minimize lateral translation. Turn off the HT WOBBLE and BEAM TILT.

VI. Objective lens alignment (VOLTAGE CENTER)

This is to make sure that the beam passes through the optical axis of objective lens.

1. Find and center a small specimen feature with amorphous structure at **MAG** >20k on the viewing screen
2. Insert the **RIO camera** in GMS3 on the **GATAN PC (middle)** and start a live view.
3. Spread the beam (so as not to damage camera) and press **F1 button** (right control panel) to lift the phosphor screen and see your sample on the camera.

4. Select **OBJ STIG** and adjust **DEF/STIG** X & Y knobs until the Live FFT of the amorphous region is circular. To access the live FFT, click Process > Live > FFT.

VII. Image focus and objective lens astigmatism correction (Fresnel-fringe method)

This is to tell you how to focus your specimen and correct astigmatism of objective lens using Fresnel-fringe method. You may also focus your specimen using wobbler method or minimum contrast method, and correct objective lens astigmatism using amorphous carbon film (which is useful to record a high resolution image).

1. Find a small hole/a particle/ the sharp edge of your specimen
2. Change **FOCUS** and watch the change of Fresnel-fringes at the edge of the hole or the particle. The fringes may be either dark, bright, or disappear, corresponding to OVERFOCUS, UNDERFOCUS and INFOCUS conditions of objective lens, respectively.

a) Underfocus b) In focus c) Overfocus

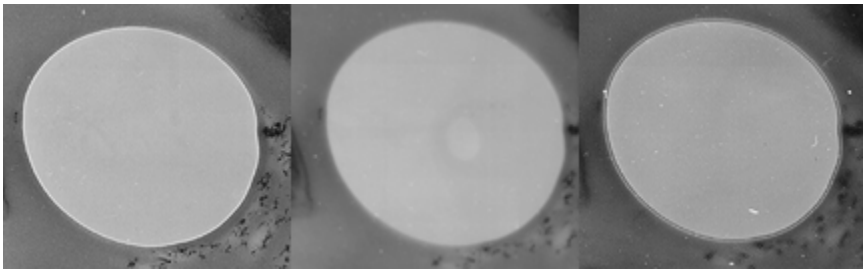


Fig. 4 the specimen was recorded
under different objective lens conditions as indicated above.

3. Without objective lens astigmatism, Fresnel-fringes shown under a defocus condition should have the same contrast and the same width in all directions, as shown in Fig. 4. If not, use **OBJ STIG X and Y controls** to correct the astigmatism.

VIII. Selected area electron diffraction (SAED) including focus and center diffraction pattern.

1. Find and center an interesting specimen area. Spread beam with **BRIGHTNESS** knob.
2. Insert and center a suitable **SA APERTURE** by pressing **SA** and selecting the desired aperture size.
3. Choose a magnification that allows you to position the feature and SA APERATURE easily.
4. Lower viewing screen to avoid damaging the camera.
5. Press **SA/DIFF** (right panel) and spread the beam to have the smallest possible spot.
6. Use **MAG/CAM L** knob to adjust camera length.

7. If the central spot is not at the viewing center, center it by pressing **PLA** (left panel) and using the **DEF/STIG X & Y** knobs.
8. Open the beam to form a parallel illumination on the sample by turning **BRIGHTNESS** counterclockwise. Diffraction spots will become weaker and smaller. If the central spot is still a disk instead of a spot, diffraction focus is needed.
9. Focus the pattern by adjusting the **DIFF FOCUS** knob and make the center spot as sharp as possible.
10. Use shortest exposure time (0.0399s) to take SAD on RIO camera. Over-exposure may damage the camera! Once short exposure time is set, press **F1** to lift the screen and take a picture of the diffraction pattern. Make sure to adjust brightness of the transmitted spot using **BRIGHTNESS** and **DIFF FOCUS** *before* lifting the screen.
    Attention: A high density beam (the bright transmitted spot) will damage the CMOS.

IX. Simple bright/dark field imaging by shifting objective aperture

You will learn how to use central dark-field imaging in lab 3-4.

1. Find and center an interesting specimen area
2. Make sure that the sample is at eucentric height (see IV)
3. Focus (**BRIGHTNESS**) the beam to the crossover and press **DIFF** (Now the viewing screen is conjugated to the back focal plane indicated in Fig. 5).
4. Center the diffraction pattern if necessary (see IX)
5. Insert **OBJECTIVE APERTURE** (which is situated at back focal plane shown Fig. 5 into the beam by turning the aperture handle clockwise. Note: **One of the 4 apertures should be centered**. If you can not see the one you want to use, do not touch the aperture X and Y controls. You should keep loading other apertures until you see one aperture near the center. Then you may center this aperture first and turn to next one and center it again till the one you want to use.
6. Press **MAG1** to return to image mode to see a bright field image (Now the viewing screen is conjugated to the initial image plane indicated in Fig. 5)

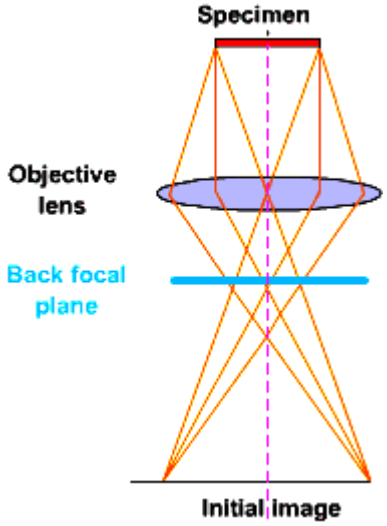


Fig.5 objective lens imaging, back focal plane and image plane

7. Adjust fine focus and **OBJ STIG** if necessary
8. To record a dark field (DF) image at a lower magnification after BF image, go back to step 5, center the **OBJECTIVE APERTURE** at one of diffraction spots, press **MAG1** to see a DF image. To record the image, you may have to use a longer exposure time. You may have to record a few images with different exposure time.

X. TEM shutdown

1. Take objective aperture and/or SA aperture out of the column
2. Return TEM parameters to standard positions (**TEM MAG** mode, Spot size 1, Alpha 3, etc.)
3. Set condenser aperture at position 2 and make sure **again** that all other apertures are removed from the column.
4. Turn off **BEAM**.
5. Set specimen shift: X=Y=0, specimen tilts =0, BEFORE taking the sample holder out of the microscope. You can do this by double-clicking **STAGE NEUTRAL**. You may need to do this multiple times; make sure the specimen position values are really at 0 before proceeding.
6. Take specimen out of the column: follow the diagram on the microscope, steps 1-5.
7. Take the specimen out of the specimen holder and check O-ring.
8. Load the next sample or sample plug into the microscope (if applicable)
9. Return the sample holder to its appropriate position in the Fischione Pumping Station
10. Make sure that the room is clean and tidy
11. If you are the LAST USER of the day (nobody after you in next 12 hours), follow this procedure (images shown in the NUANCE instruction manual):
    1) Plug in ACD Heater
    2) Pull out the ACD&Bake page (TEMCON > Maintenance > ACD&Bake) and **enable ACD heat**.
12. Log out of the computer (NUcore)