

TEM Preparation

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Aims: This covers

- basic safety issues
- computer login system
- negative film developing
- negative film scanning and image processing
- sample preparation equipments

I. Basic safety issues

Safety education is mandatory for all TEM users. Only those who receive safety training and sign the liability agreement can use EPIC facilities. Please do not touch or use anything in EPIC if you do not know the consequence of your actions. You should ask EPIC staff first if you are not sure about the consequence of your actions.

What You Should Know Before an Emergency Occurs

1. KNOW the established emergency procedures for this building and work area.
2. KNOW two means of egress from your area.
3. KNOW the locations of fire alarm pull stations.
4. KNOW the locations of portable fire extinguishers and how to use them.
5. KNOW the location of EPIC Safety Desk Book and how to find information in it.
6. KNOW the location of Materials Safety Data Sheets (MSDSs) and how to read it.
7. KNOW the location of the nearest first aid kit and know how to use it.

What You Should Read Before Using EPIC Facility

1. NU Emergency Response (<http://www.northwestern.edu/research-safety/emerg/index.htm>)
2. NUANCE Emergency Response Procedures (<http://epic.ms.northwestern.edu/emergency.asp>).
3. NU Safety Plan (<http://www.northwestern.edu/research-safety/index.htm>).
4. NU Chemical and Biological Safety in Laboratories (CBSL) (<http://www.northwestern.edu/research-safety/biosafe/index.htm>, <http://www.northwestern.edu/research-safety/chem/index.htm>).
5. NU Hazard Communication Program (<http://www.northwestern.edu/research-safety/hazcomm/hazcomm-toc.htm>).
6. NU radiation safety (<http://www.northwestern.edu/research-safety/rad/radwor/index.htm>)
7. COOK Hall Safety Plan.

8. EPIC Facility Rules.

- Please read and understand departmental safety plan (posted on an EPIC notice board). Know what users should do if an emergency occurs, Know users responsibility and Cook Hall emergency evacuation plan.
- Please read and understand EPIC facility rules (posted on an EPIC board).
- No SMOKING, FOOD, or DRINK AT ANY TIME in EPIC labs
- Please read and understand TEM and Dark Room safety rules (shown below):

Safety in TEM and dark room areas (Draft)

TEMs are complicated high voltage facilities. You need to treat them cautiously while using them. You MUST NOT touch anything you do not know. You should always ask staff if you are not confident to use the function of related facilities. Before using TEMs, you must get a safety training and sign a form to release and waives any and all claims whatsoever against Northwestern University, its trustees, officers, employees and agents from any liability or any loss, cost, damage, expense, injury or death arising from or in any manner connected with or related to your use of the facilities and equipment.

TEMs:

Your personal safety:

- a) High voltage: Stay away from the high voltage generator and cables to avoid electric shock. The voltage is 200,000 V (compared to a normal voltage of 120 V for your home appliances)*
- b) Radiation: Radiation is shielded in TEMs and is within the safe limit if the facilities are used properly. Please understand that it can not be completely avoided, especially at some weak points or when the facilities are not used properly. Please do not go behind the TEMs when their filaments are energized. Please do not take the condenser aperture out completely without permission from the staff. Please do not use TEM facilities if you are pregnant.*
- c) Liquid nitrogen: You must not leave when you are getting LN2 from the big tank. You must deal with LN2 carefully: avoid injury by LN2 when you hook the cold finger Dewar on TEMs and avoid falling off from the stand when you pour LN2 into the cold trap.*
- d) Eye protection: Try to use a weaker beam if possible and avoid damage to your eyes*
- e) Avoid catching cold: Put enough cloth (especially trousers) on. TEM rooms are cooler than other rooms in summer.*
- f) Avoid fatigue: Take a break every 2 or 3 hours.*

TEM safety:

- a) Rules for TEM: Read and follow the operation instruction carefully, especially the notes.*

- b) *Vacuum: Make sure that vacuum is always good; Follow rules when you load/unload specimen and change negative films. Objective aperture must be out when loading/unloading samples.*
- c) *Liquid nitrogen: Fill cold trap/figure with LN2 at the beginning of your experiment and top up them every two hours during your session*
- d) *Filament: Do not over-saturate the filament.*
- e) *Make sure that your sample is set at the eucentric height when loading the Obj aperture into the column. Do not change the sample holder height when the Obj aperture is in the column. Never adjust the height of the Obj aperture.,*
- f) *If you are not confident of the consequences resulted from your action, please ask staff before your action*

Dark rooms:

Please read all standard safety rules for chemical and electrical laboratories (by the note board).

Personal safety:

- a) *You must follow instructions and rules for dark rooms*
- b) *Wear gloves AT ALL TIMES*
- c) *Avoid chemicals spills. Wash your eyes immediately for 15 minutes with plenty of clean water if chemicals reach your eyes.*
- d) *Avoid electric shock*
- e) *Pay attention to fire and other alarms (which are hard to hear because the door is closed). Follow the routine in map to evacuate.*
- f) *Keep the door room tidy and clean*

The form you need to sign (posted on the board):

I certify that I am trained on safety for the use of the TEM facilities and dark rooms. I sign below to release and waives any and all claims whatsoever against Northwestern University, its trustees, officers, employees and agents from any liability or any loss, cost, damage, expense, injury or death arising from or in any manner connected with or related to your use of the facilities and equipment.

<i>Department</i>	<i>Advisor</i>	<i>Name</i>	<i>Signature</i>	<i>Date</i>

II. Computer login system

Know how to reserve, cancel and log in/out TEM sessions

Reserve TEM session:

1. Find the (EPIC:TEM facility) login window from desktop. If not, click Login32 from desktop

2. Click Access\reservation from the window
3. Import Name: MSE460, Account : 0000-000-0000
4. Select an instrument: H 8100TEM
5. Select Date and hours
6. Click OK
7. Select a password you can easily remember. You need it to cancel, login/out your session.
8. Clicking Access\login to see if you have reserved your session.
9. Click cancel to exit

Cancel TEM session:

1. Click Access\login in the login window
2. Click the session you want to cancel (you must do it 24 hours in advance, please plan ahead)
3. Click delete
4. Input the password you selected when you reserved your session
5. Click Access\login to check if the session is deleted.

Login TEM:

1. Click Access\login in the login window
2. Click the session you want to login
3. Click OK
4. Input the password you selected when you reserved your session
5. Your name should appear behind the instrument you are going to use. If not, redo it or ask.

Logout TEM:

1. Click the instrument you just used in the login window
2. Click Access\logout
3. Input the password you selected when you reserved your session
4. Answer the questions and click OK
5. Your name should disappear from the window. If not, redo it (think about password?) or ask.

III. **Rules and Instructions for dark room**

1. Before Developing:
 - a) Turn on the switch marked with “MASTER TANK”, in which the developer and fixer tanks are suited, counter-clockwisely by 1/6 turn.
 - b) Turn on the switch marked with “RINSE WATER” counter-clockwisely by 1/6 turn.
 - c) Turn on the switch marked with “WASH” counter-clockwisely by 1/6 turn.

- d) Do not adjust the water temperature. It has been set between 60-75°F. Please keep an eye on the instrument, if there appears to be a problem with the water, please contact the EPIC staff.
- e) Turn on the Main valve of the nitrogen cylinder. Do not adjust the pressure.
- f) Push down the switch to the “CYCLE” stage on the Agitation Timer Control. Please do not adjust time for burst functions

2. During Developing:

- a) Turn on dark room safety (red) light. This switch will also turn on “IN USE” light indicating that the dark room is being used.
- b) Load your films on the Lucite developing rack and start developing.
- c) Please follow the guidelines:

“FOR OPTIMAL DEVELOPMENT OF NEGATIVES”.

- i. Develop in D-19: 4-5 minutes. Drain developer for 1 min.
- ii. Rinsing Time: 1-1.5 minutes.
- iii. Fix in Rapid Fixer: 4-5 minutes. Drain fixer for 1 min.
- iv. Washing Time: 30 minutes.
- v. Soak in Photo-Flo: 1-2 minutes. Drain solution for 1 min.
- vi. Drying Time: 20-30 minutes.

NOTE: For some experiments which you may do in the future, for instance weak-beam, it is possible to increase the sensitivity by developing for longer times. While you don't want to do this for these labs, it is worth remembering this for the future.

3. After Developing:

- a) Turn off the “RINSE WATER”
- b) Turn off the “MASTER TANK”.
- c) Turn the switch from “cycle” to “off” on the Agitation Timer.
- d) Turn off the Main valve of the nitrogen cylinder.
- e) Replace lids on developer, fixer, and Photo-Flo.
- f) When the washing time is over, turn off all water switches.
- g) Clean the dark room before leaving. This includes wiping up spills, putting things in order, and removing your equipment.
- h) Be sure to turn off safety and “IN USE” lights.
- i) Fill in the log-sheet for film developing.

If you have any questions or concerns, please contact the EPIC staff.

IV. Negative film scanning and image processing

1. Make sure that Microtek Scanner (SPF room) is ON.
2. Place your negative film within the scanning area of the template. Please DO NOT scratch the window glass and keep the glass clean.
3. Start **Adobe Photoshop 7.0** from desktop

4. Start Microtek ScanWizards5 from the photoshop software (**click File\Import\ScanWizards5**)
5. Select **Negative film** from Microtek ScanWizards5-advanced control panel.
6. Select scanner setting parameters: Type: **Gray scale**; Resolution: **300 ppi** (which is recommend. It is possible to select 150-1200ppi, the higher the resolution, the bigger the file)
7. Click **Overview** to see the whole area
8. Select scanning area defined by **dashed lines**
9. Click **Scan**
10. **Close** the Microtek ScanWizards5 software
11. Process the scanned image in Adobe Photoshop (brightness, contrast..)
12. **Save** the image in your directory under users (please create one directory if you do not have one)
13. **Copy** your files into your own disk (floppy disk/zip disk/CD-R)
14. **Close** the Photoshop software

Note: Of course, many of you have scanners in your group laboratories which you can use. With some of these you can adjust the scanning range, which can be very useful. A precision scanner is available in Prof. Marks group in the Catalysis Center for quantitative work.

- V. Introduction to sample preparation equipment
- Cutting: wheel saw, wire saw, ultrasonic cutters...
 - Polishing: mechanical polishing, electro-chemical polishing...
 - Dimpling
 - Ion milling: PIPS, IBT
 - Microtome

Basic H 8100 TEM Alignment and Operation

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Dear users,

To help you have a smooth and successful TEM session, I wrote up this basic operation procedure. Systematic theoretical training (TEM courses) plus hands-on practical training may be the best way to understand the details of this operation instruction for H-8100 TEM. Please make sure that *you follow what I told you*. There are some **notes** in this instruction. Please read and follow them carefully. Please remember the golden rule in TEM rooms: **ASK before you touch the TEM** if you do not know the consequence of your action. You would not be blamed when you made a mistake if you could inform me what really happened soon. You are welcome to make comments on this instruction.

Jian-Guo Zheng / revised on Jan 2004

Aims: This instruction to familiarize you with basic TEM alignment and operation. It 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 and develop negative films

I. TEM structure, functions and knobs

1. Electron gun, Wehnelt unit, Anode: HV, READY/OFF, FILAMENT, BIAS
2. Gun gun beam deflector coils: GUN TILT, GUN HORIZ
3. Condenser lens coils, C1 and C2: SPOT SIZE, BRIGHTNESS
4. Condenser aperture: 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
8. Specimen Holder
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, P1 and P2
17. Viewing screen
18. Camera: PHOTO, FEED

II. TEM startup

- Log in the computer and check the comments in the TEM log book. Enter your name on the log book before any action on the microscope.
- Understand the vacuum system:
 - Two major pumps: DP-1 and TMP.
 - Three rotary pumps: RP-1, RP-2, RP-3.
 - Vacuum meters: PE, PI-1, PI-2, PI-3, PI-4
 - The valves are open when they are lit (red).
 - Cold trap is above the TMP for improving vacuum in Gun and Column.
 - Cold finger is in the sample area to improve vacuum around the sample and reduce sample contamination.

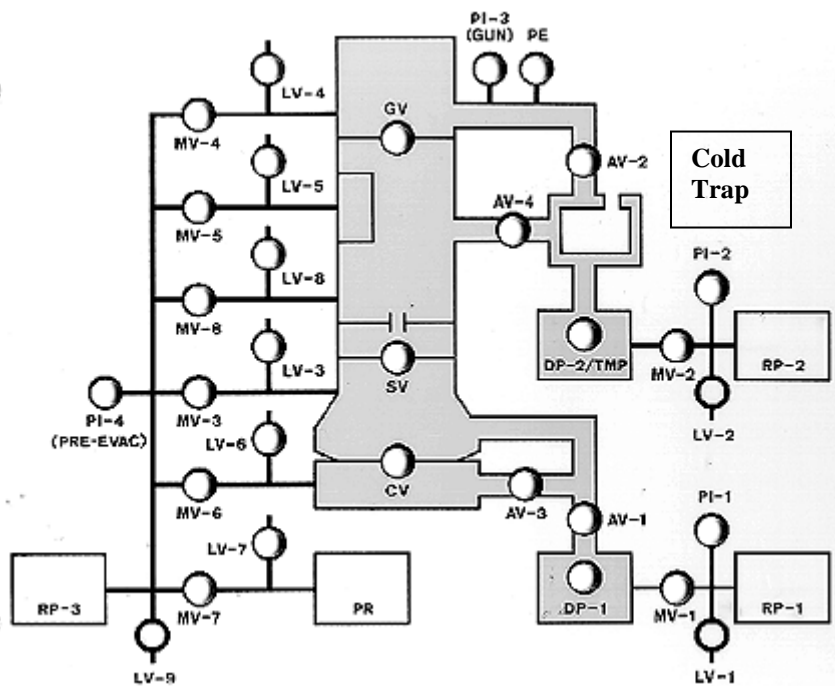


Diagram 1: H 8100 vacuum system

- Make sure that TEM vacuum is good. At the vacuum state (right panel), **GUN**, **COL**, and **CAM** indicators should be at **VAC** position (all are **GREEN**), and **SPEC** indicator is at **CLOSE** position (**YELLOW**).
- Get liquid nitrogen from a big LN tank in EPIC and fill the LN log book which is at the big tank.
- Fill out cold trap on TEM with liquid nitrogen first and cold finger then.
 - Note:* a) Liquid nitrogen may hurt you if careless. Wear gloves if necessary.
 - b) **Always** top up cold figure/trap with liquid nitrogen at the beginning of your session.
 - c) LN in the cold finger Dewar lasts for about **2.5** hours. Please make sure that there is LN there during your whole session, this mean 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**
- Turn on the panel light and turn up the data monitor if necessary.
- Make sure that TEM is set at standby positions:
 - a) **Objective and selected area apertures are out** (position 0) and condenser aperture in at position 1.
 - b) Filament: off; HV: on at 200kV
 - c) Specimen position: X=0, Y=0; specimen tilts: 0
 - d) Bright/Dark-field selector set at Bright field.
 - e) Microscope is at Zoom mode.
 - f) Mag: 50K-100K
 - g) Unexposed film number: 21
 - h) Spot size: Micro No. 3

Note: **Objective aperture must be out during sample holder loading/unloading.**

(8) Turn off HV by press **READY/OFF** twice and unload the sample holder from the TEM.

Note: Normally, a sample holder stays in TEM. It is situated at **position A** (see the following diagram). Follow step 5 to take it out.

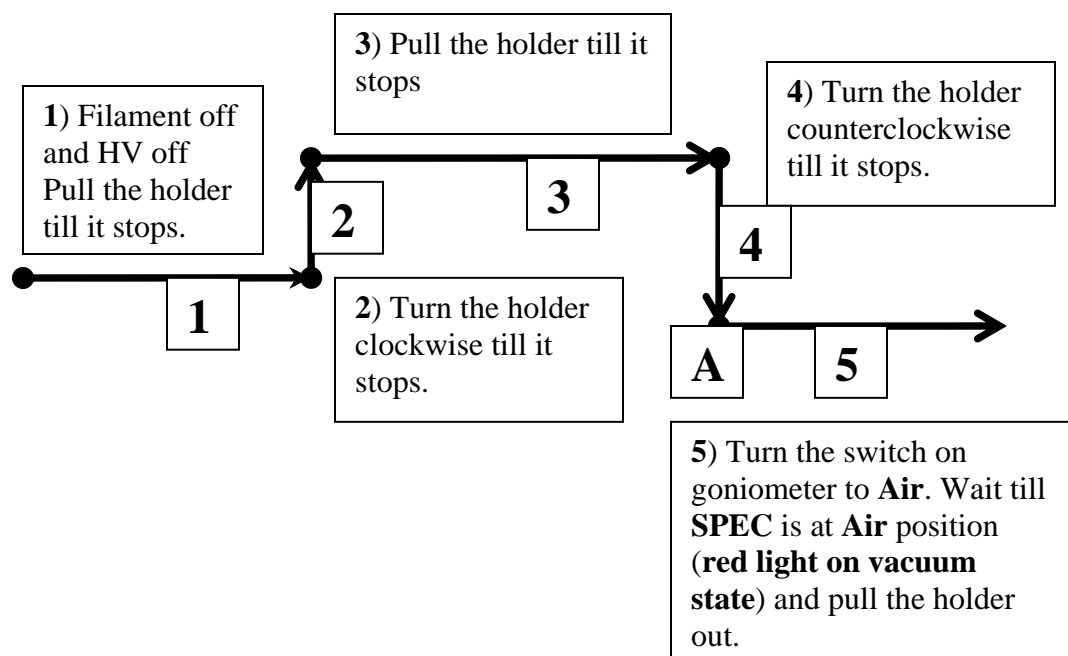


Diagram 2: Sample holder unloading procedures

(9) Choose a right sample holder, **wear dust-free gloves**, load your sample into the holder (use a good tweeze and a sucker) and make sure that the sample is securely seated in the holder and black **O-ring** on the holders 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 is 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!).

(10) Please make sure that PI-4 light (see the vacuum diagram in the last page) is lit.

(11) Follow the route indicated in the diagram below to load your sample holder into the TEM. Sample holder goes into the TEM horizontally along the horizontal

arrow, is turned clockwise when the arrow is up and counter-clockwise when the arrow is down.

Note: a) Make sure that **HV** 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;

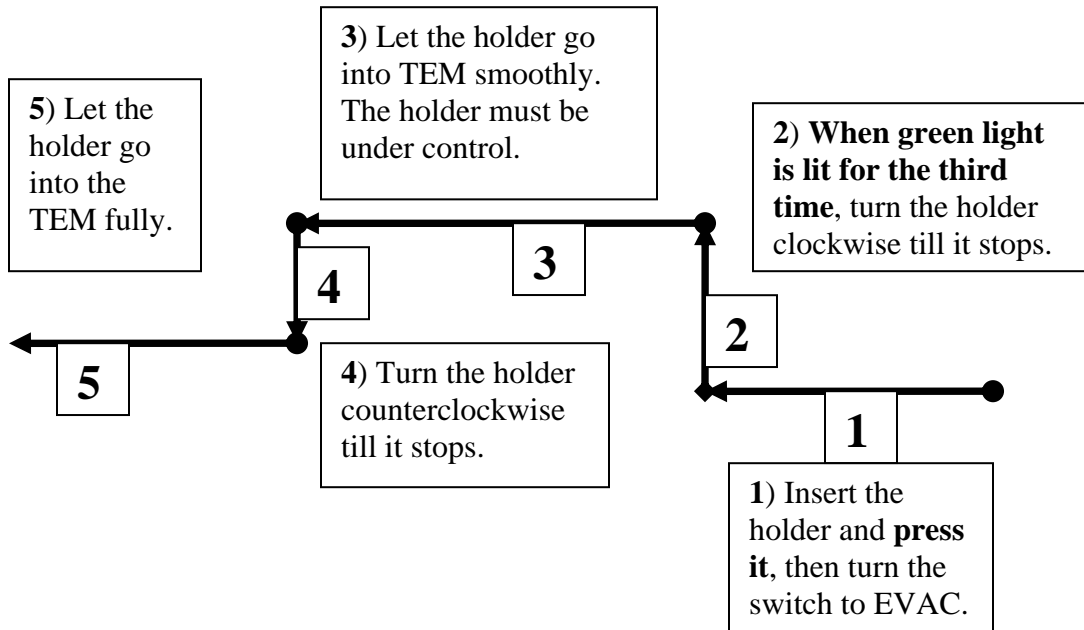
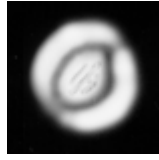


Diagram 3: Sample holder loading procedures

III. Obtain a good electron illumination

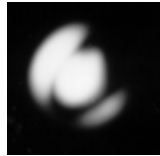
1. Apply HV when vacuum is good. Press the **READY/OFF** button **once** (left panel) and then 200kV. High voltage rises gradually up to 200 kV (watch the **ACC VOLT** on the data monitor). **HV/BEAM** meter indicator (below the data monitor) moves to 200 kV.
2. Turn **Filament** knob clockwise slowly (left panel) to apply filament current (3 minutes) and stop at one division before the maximum. Watch **HV/BEAM** meter and make sure that the emitting beam current is **< 10 μ A**. If the beam current is higher than 10 μ A, turn down the bias till the current is **< 10 μ A**.
 - Turn the **BRIGHTNESS** control (left panel) to crossover, and center the beam with the **BRIGHTNESS CENTERING** knobs (right panel). You should find a normal unsaturated filament image (showing some detail) at Mag. 20k under the filament setting stated above. An unsaturated filament image is shown below



**Fig. 1 unsaturated filament image at crossover
when the gun is aligned properly**

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

- a) Make sure that you have logged in the computer.
 - b) Decrease the **MAG.** or select **LOW MAG** mode
 - c) Turn **BRIGHTNESS** anticlockwise
 - d) **OBJECTIVE APERTURE** and **SELECTED AREA** aperture are out.
 - e) Ask help if you still do not find the illumination.
- Gun alignment: If the unsaturated image has an asymmetric shape as shown below,



**Fig. 2 unsaturated filament image at crossover
when the gun is not aligned properly**

adjust **GUN TILT X** and **Y** (left sub panel) knobs to get a symmetric shape of the filament image as show in Fig.1 and then Use **GUN HORIZ X** and **Y** knobs (left sub panel) to center the image. **Note: make sure that you are adjusting the knobs you want because GUN HORIZ knobs are next to GUN TILT knobs.**

5. 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:

- a) Focus (**BRIGHTNESS**) the beam to crossover, center it using **BRIGHTNESS CENTERING**.
- b) Open (**BRIGHTNESS**) the beam and center it using the **CONDENSER APERTURE X** and **Y** controls.
- c) Repeat a) and b) till there is no beam swinging.

6. Correct **Condenser astigmatism**:

If the beam is not round when it is open, condenser astigmatism needs correction:

- a) Focus (**BRIGHTNESS**) the beam to crossover and center (**BRIGHTNESS CENTERING**) the beam.

- b) Open (**BRIGHTNESS**) the beam and use **COND STIG X** and **Y** knobs to make it round.
 - c) Repeat a) and b) till the open beam is round all the time.
 - d) Center the illumination
7. Get a saturated filament image
- a) Slightly increase filament current till the filament details just disappear at crossover, as shown in Fig. 3.

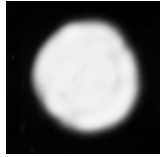


Fig. 3 A saturated filament image at crossover when the gun is aligned properly

Note: Watch filament image when you are increasing the current, and stop increasing filament current when the details in the filament image just disappear (very weak details should be OK as long as the intensity is high enough).

- b) Make sure that the emitting beam current is **< 10 μA** . If the current (read from **HV/BEAM** meter) is **>10 μA** , turn **BIAS** knob counterclockwise so that the current is just below 10 μA .

Note: **During your session, the beam intensity may decrease slightly. You may increase the BIAS to get brighter illumination as long as the beam current is not higher than 10 μA .** Please let me know when you are not happy with the illumination.

- c) Spread the beam to the whole observation screen.

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 **SPECIMEN TRANSLATION X** and **Y** controls to center a specimen feature.
- b) Set **OBJ** lens current to **5.25 (TEM monitor)** by adjusting **FOCUS** or pressing the **RESET** button on the data monitor.
- c) press **WOBBLER** (right panel) and the feature may vibrate.
- d) Adjust **Thumb-screw** on **GONIOMETER** so that the feature does not vibrate. Now the contrast of the specimen should be minimum if Objective aperture is out.

V. Condenser lens alignment

1. Focus (**BRIGHTNESS**) the beam to the crossover and center the beam with **BRIGHTNESS CENTERING**
2. Change **SPOT SIZE** (left sub panel) to 1 (Note: the monitor will show the probe size **Micro No 1**) and center the beam with **BRIGHTNESS CENTERING**

3. Change **SPOT SIZE** to 4 (that is **Micro No 4** on the monitor) and center the spot with **GUN HORIZ**
4. Repeat b) and d) till the beam remains at the screen center.

VI. 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. Center the beam at spot size **Micro No 3** and turn on **WOBBLER**
2. Set **WOBBLER ANGLE** to maxima (turn the knob fully clockwise)
3. Make the separated spots into one by adjusting **B.T. ALIGN X** knob
4. Turn **WOBBLER ANGLE** three divisions counterclockwise
5. Make the separated spots into one by adjusting **B.T. Align Y** knob

VII. 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 at **MAG 60k** on the viewing screen
2. Turn on **CCD CAMERA MONITOR** (right) and Press **STOP** first and **PHOTO** then on TEM data monitor (left). Viewing screen will be totally lifted without hearing camera noise. The feature will be displayed on the CCD monitor.
3. Turn on **HV MODULATION** in **BRIGHT** field mode (right panel). The image feature may oscillate.
4. Adjust **BEAM TILT** (right sub panel) to minimize the vibration of the feature.
5. Repeat 4) at **MAG 100k** or higher.

VIII. 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) Infocus c) Overfocus

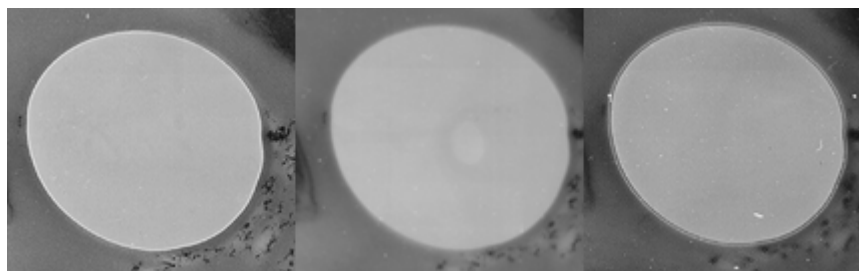


Fig. 4 the specimen was recorded under different objective lens conditions as indicated above.

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** (left sub panel) to correct the astigmatism.

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

1. Find and center an interesting specimen area
2. Insert and center a suitable SA APERTURE
3. Press **SA** (left panel) and focus SA aperture using **DIFFRACTION SPOT** knob (left panel) and binocular.
4. Make sure that the **STOP** light on TEM data monitor is **NOT** lit. If yes, press **STOP** once to cancel it.
5. Press **DIFF** (left panel). If the central spot is not at the viewing center, center it using the **INTER ALIGN X and Y** knobs (left sub panel). If the spot is not round, make it round using **INTER STIG X and Y** knobs (left sub panel).
6. 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.
7. Make sure that the * is at left corner of intermediate lens current **I1** on the TEM monitor. If not, click UP/DOWN ARROW key to cancel any highlighted item on the TEM monitor. Input **5** from keyboard (left panel) and press RETURN.
8. Focus the pattern by adjusting **DIFFRACTION SPOT** (left panel).
9. Record a few SA diffraction patterns using different exposure time.

X. 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 till 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 ZOOM to see a bright field image (Now the viewing screen is conjugated to the initial image plane indicated in Fig. 5)

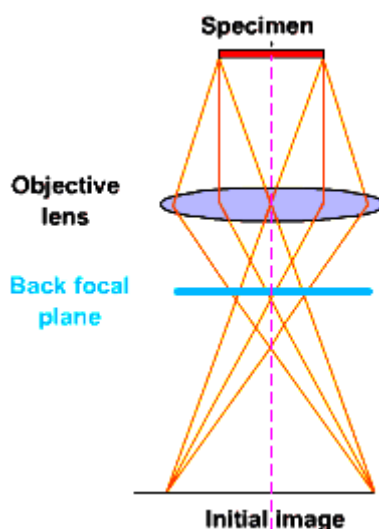


Fig.5 objective lens imaging, back focal plane and image plane

7. Adjust fine focus and OBJ STIG if necessary
8. Adjust BRIGHTNESS so that the AUTO EXPOSURE TIME is between 2-5 seconds.
9. Cover the viewing window
10. Press FEED (camera noise is expected) and wait till the light in the button is lit
11. Press PHOTO to expose the film and record a bright field (BF) image.
12. TO record a dark field (DF) image at a lower magnification (ZOOM) after BF image, go back to step 5, center the OBJECTIVE APERTURE at one of diffraction spots, press ZOOM to see a DF image. To record the image, you may not use the AUTO EXPOSURE. You may have to record a few images with different exposure time.

XI. TEM shutdown and negative film development

1. Make sure that **EDX DETECTOR** is out of the column
2. Take objective aperture out of the column
3. **SPOT SIZE** or Probe size is set to Micro No. 3 and **HV 200kV**.
4. Turn **MAG** down to 6000, center the beam and spread the beam to the whole screen so that the next user can find the beam easily.
5. Turn **MAG** up to **50K-100K** to keep the lenses warm and stable, and prevent any water vapor condensation.
6. Set condenser aperture at position 1 and take SA aperture out of the column.
7. **Slowly** turn the **Filament** current to off (3 minutes)

8. Set specimen shift: X=Y=0, specimen tilts =0
9. Press **READY/OFF** twice to switch **HV** off
10. Take specimen out of the column: follow the diagram 2, step 1-5.
11. Take the specimen out of the specimen holder and check O-ring.
12. Follow diagram 3, step 1 to insert specimen holder to TEM till position A.
13. Change negative films:
 - 1) Open the main valve of nitrogen cylinder **Note**: do not adjust regulator valves.
 - 2) Turn the switch of the desiccator (right sub part) down to AIR position.
 - 3) Press the camera CLOSE and then AIR button
 - 4) Wear gloves.
 - 5) After a few minutes, open the desiccator door, take out the empty receiver, full film magazine and a spare lid, place their on the TEM bench and close the door (it is not necessary to turn the switch to VAC now).
 - 6) When you hear a sharp noise, turn off all lights except the safelight and open the camera door. Hold the receiver handle and take it out (do not hold the cover, you may open the receiver). Unlock the film rail frame, fully pull it out and gently lay it down. Insert the spare lid into the film magazine in the camera and replace it with a full one, lift the frame, align it with the film magazine, insert it into the camera room, make sure that the frame is locked, put the receiver back, pull the receiver handle to make sure that it is properly locked, close and press the camera door while pressing the CLOSE and VAC buttons. You may hear a big noise from a rotary pump and the noise should then go down quickly. Try to open the door and make sure that the door is properly closed.
14. After vacuum is good (camera VAC green light is lit), **turn on HV at 200kV and reset unused film number to 21**
15. **Switch off the nitrogen cylinder main valve**
16. Turn down the monitor and switch off the panel light
17. Make sure that the room is clean and tidy
18. Complete the log book
19. Log out the computer
20. Develop your exposed film in the dark room and reload new films (see rules in the dark room)
21. Open the desiccator door and put the film magazine (with the handle facing the door) and the lid in the lower space and **the receiver in the top space with its handle facing the wall!!** Close and press the desiccator door while turning the switch up to VAC position and waiting for the rotary pump to evacuate the desiccator. Try to pull the door to make sure that it is closed properly.