Operating Procedures for TEM2
FEI Tecnai G^2 Twin

Note: Do not press the buttons on the TEM. This will turn the TEM off and will take hours to bring it back up. **Please do not reboot the TEM computer.** This will turn off the vacuum and the high tension**. If you are having problems with the TEM, please find a SMIF staff member immediately.**

Before You Start

1) Log usage into the CoreResearch@Duke.

2) Go to the ***Setup* tab** in the TEM software (left monitor)

- a) The *Status* bar at the top should read ***COL. VALVES***.
  - i) If it reads *CRYO CYCLE* or *COL. COND*, a cryo cycle is running and it has to complete before the TEM can be used. Do not stop the cryo cycle prematurely.
  - ii) If there something other than the above *or READY* in the *Status* bar the TEM is not operational. Please contact a SMIF staff member.

3) Make sure the *Col Valves Closed* button is yellow (meaning the column valves are closed)

4) Check that the vacuum values are green and within the range listed below:

- **a) Gun: ≤ 25 log**
- **b) Column: ≤ 15 log**
- **c) All vacuum values should be green**

If any of the vacuum values are red or out of range, contact a SMIF staff member

5) Fill LN2 into the cold finger dewar. The LN2 needs to be refilled before and after each use
TEM2_Liquid Nitrogen video

6) Check the Camera Monitor

- a) Log into camera computer (Right-hand monitor, mouse, keyboard)
  - i) Username: VALUEDGATANCUSTOMER, Password: $admin
- b) Open the camera software: GMS3 DigitalMicrograph
  - i) Click *OK* to popup about Windows real-time protection (ignore the message)
- c) Check the ***Camera Monitor* on the left side of the software**
  - i) Click the dropdown arrow to view the camera status
  - ii) The **camera temperature should be -5°C** (+/- 0.2) and the **Health Status light should be green** and not blinking. If either of these are off, contact SMIF staff.

Setting Up the TEM

1) **Go to the *Setup* tab** in the TEM software and make sure that:
   - **a)** ***Col Valves Closed* button is yellow** and *Status* reads "Col. Valves"
   - **b)** **Gun: ≤ 25 log**
   - **c)** **Column: ≤ 15 log**
   - **d)** **All vacuum values should be green**

2) **Check the *High Tension* setting (kV)**
   Note: It is important to change the kV before ramping up the Heat to # when turning on the TEM.
   - **a)** *High Tension* button should be yellow. If it is not yellow, click *High Tension* to turn it on.
     - i) If the button does not go yellow and says "High Tension Unavailable", check to see if the HT button on the TEM is lit. If it is not lit, ask a SMIF staff member to turn it on.
   - **b)** Change the High Tension setting (80-200kV) if needed in the drop down menu. Wait 1 minute.

3) **Check the *Filament* and *Heat to #***
   - **a)** First, adjust the ***Heat to #* 19** and click the enter button to set it.
     - i) The standby setting for the *Heat to #* is 15 in between reservations.
     - ii) The *Step #* is set to 1. Do not change Step #.
   - **b)** If the *Filament* button is grey, click it to **turn the filament on (yellow = ON)**
   - **c)** **Wait for the filament to finish warming up** to the set *Heat to #*
     - i) Wait for the countdown timer to finish (3-10 minutes) before opening the column valves.
     - ii) The emission current should read ~ 4 - 9 μA once the filament has reaches *Heat to #* 19.

   TEM2_turningOnFilament (part1) TEM2_turningOnFilament (part2)

4) **Adjust the *Spot Size* to 3** (or desired spot size)
   - **a)** Spot size 3 is recommended. It is higher resolution (but less bright) than Spot 1 or 2.
   - **b)** Spot size 1 is recommended for 80kV settings. Spot size 1 is larger, providing more current but lower resolution.

Check the Beam

Verify that you can see a beam on the phosphor screen before unloading the holder

1) Confirm the **vacuum values are in the correct range** and the **filament has finished warming up**.

2) **Click the *Col Valves Closed* button** (button will turn grey) in the *Setup* tab to unblank the beam.
   - a) With the room lights off, you should see the beam on the phosphor viewing screen.
   - b) To turn on the control panel lights: Go to the *Tune* tab → *Control Pads* box → Click the *Fluorescent Background Light* button. (Please turn them off at the end of your session)

3) **Center the beam****
   - a) Adjust *Magnification* to SA 10,000X
   - b) Reduce the size of the beam with the *Intensity* knob (counterclockwise) on the left panel.
   - c) Bring the beam to the center of the screen with the track ball (also on the left panel).
   - d) Spread the beam with the *Intensity* knob (clockwise) so that it covers the entire viewing screen.

4) **Close the column valves** by clicking ***Col Valves Closed*** in the setup tab (button will turn yellow)

**Note: if you do not see a beam, try these steps in order:

a) Check that the filament is at a *Heat* value of 19
b) Go to a lower magnification (M or LM range)
c) Rotate the *Intensity* knob counterclockwise to reduce the beam size
d) Slightly move the *Beam Shift* trackball in case the beam is shifted off of the screen

***If these steps do not work to find the beam, contact SMIF staff*

TEM2_beam check

Removing the Sample Holder

Note: ***Wear gloves*** when handling the sample holder. Never touch the brass part of the sample holder, even with gloves on. At the end of the session, replace the sample holder into the TEM.

1) Go to the *Setup* tab and **confirm the vacuum values are in the correct range**
2) Make sure the ***Col Valves Closed*** **button is yellow**

3) **Reset the sample holder position**
   a) Go to the *Stage* tab → *Stage*[2] box (if it is hidden, click the arrow button to flap out the *Stage*[2])
   b) Under the *Control* section, find the **Reset → Click *Holder* button**.
      i) This will reset the stage to XYZ=0 and tilt (alpha)=0.
      ii) You must reset the position before removing the sample holder from the TEM

4) Go back to the ***Setup*** **tab** and keep an eye on the vacuum values (Gun < 25 log and Col <15 log).

To remove the sample holder from the TEM:

1) Place two fingers on blue plate of the goniometer and grasp the holder with your other hand.

2) Gently pull the sample holder straight out, without rotating, until it stops. Do not let go.

3) Rotate the holder clockwise, without pulling, until it stops. Now let go of the holder.

4) Check that the pressure values are in range before continuing

5) Again place two fingers to support the blue plate and grasp the holder

6) Gently pull the holder straight out of the TEM.

7) Place the sample holder into the protective receptacle.

To load a grid onto the sample holder:

1) Gently raise the sample clamp with the pin tool. The clamp is delicate – *work slowly!*

2) Place a new grid in the sample holder. Be careful to not scratch the holder with the tweezers.

3) Gently lower the sample clamp with the pin and place the pin back on the protective receptacle.

4) Remove the sample holder from the receptacle and carefully walk it back to the TEM.

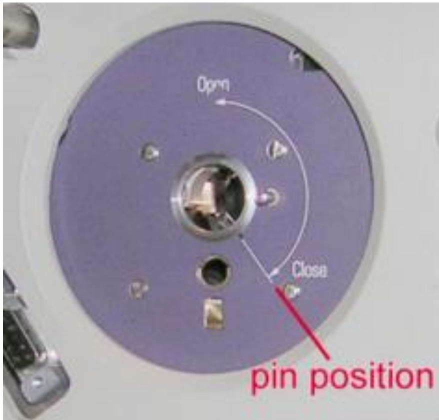
   TEM2_sample loading

Inserting the Sample Holder

Note: ***Wear gloves*** when handling the sample holder. Never touch the brass part of the sample holder, even with gloves on. At the end of the session, replace the sample holder into the TEM.

1) Go to the *Setup* tab and confirm the vacuum values (Gun <25, Col <15)
2) Confirm that the column valves are closed (button is yellow).
3) Confirm the holder position has already been reset (this should be done before removing holder)

To insert the sample holder into the TEM:



1) Locate the small pin on the shaft of the holder (near the sample end)

2) Align the small pin with the white line mark on the plate at the 5 o'clock position next to "Close"

3) Gently insert the holder straight in, with the pin aligned, until it stops.

   a) Let go of the holder. Do not rotate.

   b) The red light will turn on and the pump will start.

4) Select the holder (Single Tilt) in the TEM software notifications bar. Click the enter button.

5) Wait for the airlock pump timer to finish (3 minutes) before continuing

Note: You may feel a 'false stop' at first as the o-ring slides into place. If you hear air leak around the holder, then gently push the holder straight in *just slightly more* until it stops. Otherwise, do not touch the holder while the airlock pumps.

When the timer ends and the red light turns off:

6) Gently rotate the holder counter-clockwise (~120°) until the vacuum starts to pull the holder in.

7) Let the vacuum gently pull the holder into the TEM with your hand guiding it until it seats all the way into the TEM.

   a) **DO NOT let go or pull back on the holder as the vacuum pulls it in**

   b) The holder should seat into the TEM with the larger pin fitting into the slot at 6 o'clock.

8) Check the Column pressure and Gun pressure (in the *Vacuum* box).

   a) If the Column pressure is greater than 15 log and/or the Gun pressure is greater than 25 log, wait until they return to 15 and 25 before opening the Column Valves.

   b) If the pressures are out of range and do not correct themselves, contact a SMIF staff member.

Note: If the Column, Camera and/or Gun Pressure read 99 and there is a red line through it, please contact a SMIF Staff Member immediately.

Find Your Sample for TEM Imaging

1) Confirm the vacuum values are in the correct range (**Gun ≤ 25 log**, **Col ≤ 15 log**, all green).
2) Open the column valves: Click the *Col Valves Closed* button (it will turn grey) in the *Setup* tab
3) If you do not see a beam, try these in order until you find the beam:
   a) Move the stage XY with the joystick
   b) Go to a lower magnification (M or LM range)
      i) At low-mag you need to remove the objective aperture (turn switch to "out")
   c) Rotate the *Intensity* knob counterclockwise (try clockwise if this doesn't work)
   d) Check that the filament is at a *Heat* # of 19
   e) Slightly move the *Beam Shift* trackball in case the beam is shifted off of the screen
   **If these steps do not work to find the beam on your sample, contact SMIF staff*
4) Once you find your sample, perform the alignments according to the steps below.

Condenser Aperture Alignment and Stigmator Correction

Note: The condenser (C2) aperture is the top aperture. The usual setting is C2 aperture #4.

To align the C2 Aperture:

1) Change *Magnification* to SA 22,000X
2) Reduce the size of the beam to a small spot using the *Intensity* knob (counter-clockwise)
3) Bring beam to the center of the screen with the track ball.
4) Increase size of the beam by turning the *Intensity* knob clockwise to fill about ¾ of the screen
5) Adjust the C2 Aperture knobs on the TEM to center the beam on the viewing screen

To correct the condenser stigmator:

6) Decrease size of beam to about the size of the medium circle on the screen.
7) Look at the shape of the beam. If it is non-circular, you need to correct it with the *Stigmator*
8) Go to the *Tune* tab → *Stigmator* → Click *Condenser*
9) Use the *Multifunction X&Y* knobs to make the beam shape as circular as possible.
   a) Check the roundness of the beam by turning the *Intensity* knob both directions.
10) Click *None* to deselect the function when finished
11) Adjust the *Intensity* knob clockwise to expand the beam to the edge of the viewing screen

TEM2_alignments

Objective Aperture Alignment

Note: This is the middle aperture. The usual setting is Obj aperture #4, you can also use 1-3.

1) Change *Magnification* to SA 10,000X
2) Adjust the *Intensity* knob to expand the beam to the edge of the viewing screen
3) Center the beam with the *Beam Shift* track ball.
4) Use the joystick (XY) to bring sample into view on the screen.
   a) The objective aperture alignment needs material (carbon film or sample) in beam path.
5) Press the *Diffraction* button on the right panel.
   a) If needed, you can adjust the camera length using the *Magnification* knob to make the diffraction image larger/smaller.
6) Adjust the Objective Aperture knobs on the TEM to center the halo around the bright spot
   Note: The bright spot may not be centered relative to the viewing screen
7) Press the *Diffraction* button again when finished to return to imaging mode.

Focusing the Z Axis to the Eucentric Height

Note: The Z position of the stage needs to be corrected each time a sample is placed into the TEM to ensure you are at the eucentric height on your sample.

1) Press the *Eucentric Focus* button on the control panel (resets focus to a default value)
2) *Magnification*: SA 10,000X and expand the beam to the edge of the viewing screen
3) Use the joystick to center a landmark on your sample in the middle of the viewing screen
4) Go to the *Stage* tab → *Stage²* window (may need to click flap-out arrow) → *Control* tab
   a) Locate the *Set Alpha* button and set value box. Type in -20° into the box.
5) Click the *Set Alpha* button (it will turn yellow).
   a) The stage and image will tilt. **Do not use the joystick while the stage is tilted**
   b) Use the *Z axis* buttons (control panel) to bring the landmark back to the center of the screen.
6) Click the *Set Alpha* button again to turn off the tilt (it will turn grey).
   a) If the landmark does not return to the center at 0 tilt, use joystick to center landmark again.
7) Repeat steps 6-7 until the landmark remains in the center of the screen with tilting/un-tilting
8) Make sure that the *Set Alpha* button is grey and the stage is not tilted before you proceed.

Alternate method for adjusting Z axis (using the Wobbler):

1) Follow steps 1-4 above to find a landmark on your sample at 10kX
2) Go to the *Stage* tab → *Stage²* box → *Control* section → Click the *Wobbler* button (turns yellow)
   a) The stage will tilt between positive and negative tilt. The ± Alpha value is indicated by the slider.
3) Use the *Z axis* buttons on the control panel to minimize the amount of image shift.
4) When finished, click the *Wobbler* button again to stop tilting.

Imaging Samples with the Gatan OneView camera

1) Use the joystick to center the sample area of interest on the viewing screen. The 4-cm circle on the view screen is the approximate area that will show up on the camera.

2) **Close the column valves** (button will turn yellow)

3) **Insert the camera** into the beam path by clicking on the one view camera icon in the camera software (lower left in beam diagram). Live view will start.

   a) Camera automatically retracts if inactive for 10min. If so, follow steps 2-3.

4) **Click the blue TEM Imaging** button on the right side of the screen to open the imaging controls.

   a) The settings should be: Normal, I (imaging), select Resolution (4k for most).

5) **Click *View* button** to start live image again (turns blue).

6) **Open the column valves** only if camera pressure is decreasing (or Camera <35 Log)

Important! *Before you raise the viewing screen:*

7) Make sure the intensity of the beam is not excessively high by **adjusting the *Intensity* knob until the beam is at the outer edges of the viewing screen** (past the bent lines).

   a) The image should be bright, but the beam should not be too small, as this can damage the camera sensor.

8) **Do not adjust the magnification or intensity without the screen being down,** as these can cause a very bright spot that could damage the camera sensor.

After you check the intensity on the viewing screen:

9) Press the *R1* button on the control panel to raise the screen

   a) You can also click the picture of the phosphor screen in DigitalMicrograph to raise/lower it
   b) Now you are working on the camera computer (larger monitor on right).

10) Adjustment to focus can be made using the *Focus* knobs on TEM control pad.

   a) Focus the sample with the focus knob on the right panel. The top knob focuses, lower knob changes focus step size. The smaller the focus step #, the finer the focus.
   b) Do not use focus step > 5

*** At low magnifications (< 3kx) and high magnifications (> 280kx) remove the objective aperture**
Note: For very beam-sensitive samples, always leave objective aperture in.

*** At high magnifications > 100kx, the objective stigmator may need to be corrected:**

To correct the objective stigmator: Go to high magnification >100kX, center over carbon film

1) In DigitalMicrograph, go to Process→Live→FFT

   a) This brings up the fast Fourier transform of the live image. The rings in the FFT are useful for focusing and correcting objective astigmatism (the rings will be non-circular if astigmatic)

2) Under-focus the image (turn *Focus* counterclockwise) until you see the shape of the rings in FFT

3) In the TEM software, go to the *Tune* tab → S*tigmator* → Click *Objective*

4) Adjust the *Multifunction X&Y* knobs until the outer ring in the FFT is as circular as possible

5) Click *None* to deselect the function when finished

Capturing and saving images:

Note: Under Dataset you can set up the auto-naming settings for the image (information for specimen is located under File/Global Info/Session Info) and/or setup auto-save (change path to your folder). Do this prior to capturing images.

a) If you change the specimen name or enable auto-save, please return it to the original settings (no specimen name, no auto-save) when you are finished.

*** Download free offline version of DigitalMicrograph for opening .DM4 files and .DMW workspaces: https://www.gatan.com/installation-instructions*

1) **To collect an image, click the *Capture* button** below the *View* button
   a) Adjust the exposure time as desired (automatic is recommended)
   b) Drift correction is recommended, especially if working at high magnification

2) **The captured image will be viewable under the *TEM(1)* tab at the top of the screen.**
   a) The *VIEW* tab will take you back to the live view image.

3) If not using camera for several minutes, lower the screen (*R1)* and click *View* to stop the camera.
   *If you leave room, always lower screen and close column valves to protect sample and TEM

4) **Save all images to User Images folder** on desktop.
   ***Do not save anything to the Capture Data (X:) drive as this is reserved for in situ users.*
   a) **Save each tab as a DigitalMicrograph workspace (highly recommended) which can then be batch converted to 8-bit TIFF files**
      Click on tab. Go to File/**Save Workspace As**/ and choose your folder in the User Images folder.
   b) To convert the workspace files to a batch of TIFF files: Go to **File/Batch Convert and you must use the 'browse' button to find the folder** containing your workspace folder.
      - If you have multiple workspace folders to convert, select 'convert sub-folders'
   c) To save individually, go to File/Save Display As/ and choose your folder and file type.
      - Selecting 'save image as' does not save the correct image data. You must use 'save display'
   d) Move images to your USB drive or upload to your cloud storage when you are finished saving

When you are finished collecting images on the camera:

1) Press *R1* on the control panel to **lower the screen** in place
2) **Stop the camera live view** (click the *View* button in DigitalMicrograph)
3) **Save your images**
4) **Follow shutdown procedures on next page**

Procedures for Leaving the TEM for the Next User

1) Make sure the **viewing screen is lowered**
2) Change ***Magnification*** **to 10kx**.
3) **Center and expand the beam** to the size of the viewing screen.
4) **Close the column valves**
5) **Right click the camera icon on the left side of the DM screen and click *Retract*** to move the camera to its standby position
6) **Close DigitalMicrograph** (save images first)
7) **Reset the holder**
   a) Go to *Stage* → *Stage*[2] → *Reset* → Click *Holder* to reset XYZ=0 and Alpha=0
8) **Remove the sample holder** following the steps on page 3. Remove your sample
9) **Insert the empty sample holder**
10) **Turn off the control panel lights.**
11) **Replace the dust cover** for the viewing screen window.

Next: check SMIF calendar to determine if you are the last user of the day and follow the steps below according to the TEM2 schedule

If you are NOT the last user of the day:

1) Change the ***Heat to #*** **to 15** → **Click the enter button.** Wait for the filament to cool down to 15
2) **Refill the LN2 dewar**
3) Logoff usage on CoreResearch.

If you ARE the last user at the end of the day: Run the Cryo Cycle

Note: If you are the last user, but it is early in the day (before 4PM), please don't start cryo cycle (just follow above steps) and let TEM Manager know. *Please send an email to the TEM manager if you are the last user scheduled but did not run the cryo-cycle for any reason.*

How to run the cryo cycle:

a) **Turn the filament off** by pressing the *Filament* button. Wait for the filament to cool down to 0.
b) **Remove the LN2 dewar** from the cold finger.
   i) Empty any LN2 into the styrofoam cooler
   ii) Place dewar upside down over a paper towel. Place the foam lid next to it.
   iii) Place the black plastic ice bucket under the cold finger.
c) Go to the *Setup* tab → *Vacuum* → *Cryo* tab (may be hidden, click flap-out arrow)
d) **Click *Cryo Cycle*** (make sure the filament is off first)
   i) Button will turn yellow when activated.
   ii) The *Status* bar under the *Vacuum* box will read "Cryo Cycle". Cryo cycle takes ~8 hours.

TEM2_shutdown

Operating Notes

- Apertures – higher #, larger aperture
- Apertures are in if pin is pointing left and out if the pin is pointing right
- Bottom aperture is the *Selective Area Aperture.* It is used for Diffraction.
- Spot size – smaller #, larger spot
- Focus steps – smaller #, finer focus
- Stigmator steps - smaller #, finer adjustments
- Low Magnification and High Resolution imaging - remove the objective aperture.
- Biological samples - set the objective aperture to 4, condenser aperture to 4.
- To get more “light” - use a larger objective aperture and a small spot size.

- TEM computer login: **Username: TEM Users, Password: tecnai**
  - If needed, open the *Tecnai User Interface* and then open the *TIA software*.
  - If the TIA software crashes, please find a SMIF staff member.

- Gatan computer login: **Username: VALUEDGATANCUSTOMER, Password: $admin**
  - If the DigitalMicrograph software (GMS) freezes, open task manager with *crtl+alt+delete* and end the task for “Digital Micrograph”. Do not restart the computer.