Tecnai T12 Operating Procedures

I. Initial Procedures

1.) Check that the “Vac.” and “HT” buttons are lit on the microscope control panel (check with the room lights on; the buttons automatically dim when the room is dark). If “Vac.” is not lit, contact the EM staff. If “HT” is not lit, press the button to light it and proceed normally but make a note in the instrument log.

2.) Log in with the username and password created during training. If the vacuum logger is running, close it and log off vacuumuser first.

3.) Sign in to the CharFac reservation system and the paper logbook.

4.) Launch the Tecnai User Interface and Digital Micrograph programs.

5.) Check that the microscope status reads “COL. VALVES” and that the “Col. Valves Closed” button is pressed (buttons appear yellow when active).

6.) Fill the cold trap with LN2. The first Dewar flask of the day should last ~30–40 minutes. Later dewars will each last 2–3 hours. Do not allow the cold finger to warm or the vacuum will deteriorate significantly.
7.) Open the “Vacuum Overview” screen by selecting it from the box in the bottom right corner of the screen. Check that **IGP1 (Gun/Col)** reads ~6 (log units).

IGP1 indicates the vacuum level in the column and gun chamber. It should read 6 when the cold finger is chilled.

**P3** indicates the camera vacuum level. It should read < 25.

**P1** indicates the pressure in the buffer tank, and **P2** the pressure in the backing pump line.

This dialogue box is used to select the vacuum overview (or any of several other information or setup tabs).
II. Accelerating Voltage

1.) If the “High Tension” button is lit and the displayed value reads 120 kV, proceed to Section III.

2.) The “High Tension” will be turned off (grey) for the first user each day. IGP1 must read < 10 before proceeding (see Section I).

3.) If the “High Tension” button is unavailable (grey with faded text), check that “HT” is lit on the microscope control panel (Section I). If not, press it once to light it.

4.) Select 20 kV using the slider in the “High Tension” control, then turn on the “High Tension” button. The emission current (“Emission” in the “Filament” control) will spike and then stabilize to ~0.5–1 µA.

5.) Wait 1–2 minutes before proceeding, and then raise the slider to 40 kV. Continue in 20 kV increments, waiting 1–2 min at each. If the emission current remains high (>1–2 µA), return to the previous step for several minutes.

Note: The HT may switch off and become disabled when reaching 40 kV. If this occurs, simply press the main “HT” console button to re-enable it (see Section I), click the “High Tension” again at 40 kV, and continue.

III. Specimen Loading and Holder Insertion/Removal

Note: the specimen holder, airlock, and compu-stage are made up of delicate, precisely machined components. You should never have to exert significant force during any step of this procedure. Doing so may result in serious damage to the instrument or holder.

1.) Before inserting or removing the sample holder, make sure that the column valves are closed, the objective aperture is not inserted, and the holder has been reset. The stage is reset by using the “Search” tab, “Stage” (flapout), “Reset: Holder” button.
2.) **Sample Holder Removal:**

a.) Reset the sample stage.

b.) **Always keep light pressure on the purple goniometer surface** when removing the sample holder. Pull the holder **straight back without rotating** until it stops moving.

c.) **Rotate** the holder **clockwise** until it stops. This rotation moves the guide pin (see steps 3 and 4) approximately from the 12 o’clock position to 5 o’clock.

d.) **Gently, while keeping pressure on the goniometer,** pull the sample holder back to break the airlock vacuum. This will require a **small** amount of force.

e.) Remove the holder **straight back** out of the column while being careful not to scrape it along the inside of the airlock.

f.) Be careful not to touch the holder o-ring or any part past it with bare hands.
3.) **Specimen Loading:**

**Note:** Never mount magnetic specimen discs in the clamp holder. The clamp spring is not strong enough to prevent the specimen from attaching to the objective lens polepiece.

a.) Place the sample holder in the protective stand.

b.) Remove the sample loading tool from the base of the stand.

c.) Using one hand to prevent the holder from slipping out of the stand, insert the tool into the hole in the specimen clamp and **gently** raise the clamp **straight up** until it stops.

d.) Place the specimen grid into the recess at the end of the holder.

e.) **Gently** lower the clamp **straight down** to hold the grid securely. Return the tool to the base of the holder stand.

f.) Retract the holder slightly and turn it upside down. Tap the back end several times, then turn the holder upright and check that the grid has not moved (movement suggests the grid is not properly secured).

g.) Use the microscope to inspect the holder o-ring for debris. Gently remove any debris using a sheet of lens paper.
4.) **Sample Holder Insertion:**

a.) Carefully line the **pin** on the sample holder with the **5 o’clock** position on the goniometer and **gently insert the holder until it stops**. Be careful not to scrape the tip. You should feel some resistance as the holder o-ring seats in the airlock chamber.

b.) The airlock will begin pumping, and the red light on the compu-stage will go on. **Do not move the holder while the red stage LED is lit.**

c.) The pumping time remaining will be visible in the Vacuum Overview window.

d.) Select the specimen holder type (Single Tilt) from the box in the interface. Be sure to click the ↵ button to confirm the selection.

e.) When the **pump times ends** (status reads “COL. VALVES”) and the **red stage LED goes out**, support the purple goniometer surface with one hand and **grip the holder securely** with the other. Slowly **rotate** the holder **counterclockwise** from 5 o’clock to **12 o’clock**.
f.) Gently allow the holder to slide into the microscope column until it stops. Tap the end of the holder to make sure it is securely seated.

IV. Emission Current

1.) In the “Filament” window, set the “Heat To” value to the desired cathode temperature (consult the log sheet for recently used settings). If a change was made, click the button to confirm.

2.) Click the “Filament” button. It will turn yellow and the filament will begin automatically heating to the selected temperature.

Note: The emission “Step” setting controls the bias voltage on the Wehnelt cylinder. A higher step value decreases the bias. This produces more emission current but increases the energy spread and source size of the beam.

V. Alignment

Note: Align the microscope from the top (gun) down. Press F1 at any time for online help with an alignment. Begin with the objective and SA apertures removed. Leave a condenser aperture inserted to avoid specimen damage from high beam intensity.
1.) Finding the Beam

a.) Click the “Col. Valves Closed” button to open the column valves (button turns grey and status becomes “Ready”)

i.) If no beam is visible, try decreasing the magnification (RC “Magnification”) or moving the specimen stage (RC trackball), in case a grid bar is blocking the beam path.

ii.) If these steps fail, see Troubleshooting (Section VIII).

2.) Gun Tilt

a.) Set the microscope magnification (RC “Magnification”) in the 10−60 kx range and set “Spot size” to 3.

b.) Center the beam using the beam shift (LC trackball) and spread the beam (LC “Intensity”) clockwise from crossover to ~3/4 the size of the screen.

Note: If the beam is very asymmetrical, roughly adjust the condenser stigmation (see step 5).

c.) Select “Gun Tilt” from the “Direct Alignments” panel in the “Tune” workspace.

d.) Adjust the gun tilt using the multifunction (MF) knobs (LC & RC) to produce the brightest beam by minimizing the Exposure time. The sensitivity of both MF knobs is controlled by the +/- buttons (LC).

e.) Press “Done” in “Direct Alignments”.
3.) **Gun Shift**
   a.) Select “Gun Shift” from the “Direct Alignments” panel.
   b.) Set the “Spot size” to 9.
   c.) **Converge the beam** to crossover using the “Intensity” (LC) knob and **center** the beam using the **beam shift trackball** (LC)

   Note: if the beam moves significantly when changing the intensity, roughly center the condenser aperture (see step 4.) before proceeding.
   d.) Set the “Spot Size” to 3.
   e.) **Converge the beam** to crossover and **center** the beam using the **gun shift** (MF knobs).
   f.) Repeat steps b–e until the **beam does not move** when changing between **spot sizes 3 and 9**.
   g.) Press “Done” in “Direct Alignments”.
   h.) If the gun shift must be adjusted a substantial amount and it results in a significant change in the measured exposure time at a given spot size, re-check the gun tilt alignment.

4.) **Centering the Condenser Aperture**
   a.) Select the desired **condenser aperture** (4 is largest; 1 is smallest) using the large, outer knob on the aperture.
   b.) **Converge** the beam to crossover (LC “Intensity”) and **center** the beam using the **beam shift trackball** (LC).
   c.) **Spread** the beam (“Intensity” clockwise from crossover) and **center** the illuminated area using the **X and Y knobs on the aperture** (not MF knobs)
   d.) Repeat steps b and c until the beam spreads evenly across the screen.
5.) **Condenser Astigmatism**

Note: Condenser lens astigmatism should be corrected when the beam does not expand in a symmetrical, circular fashion when the “Intensity” knob is adjusted. Astigmatism must be corrected separately for each spot size that is used.

a.) **Converge** the beam to crossover (LC “Intensity”) and **center** it with the **beam shift** (LC trackball).

b.) Select “Condenser” on the “Stigmator” control panel (the panel can also be brought up by pressing the “Stigmator” (LC) button).

c.) **Spread** the beam and use the MF knobs to make the beam as **circular** as possible.

d.) Select “None” on the “Stigmator” panel (or press the LC “Stigmator” button) to end stigmator control.

6.) **Specimen Height Adjustment**

Note: all alignments beyond this point depend on the objective focus. To ensure proper alignment, both the specimen and objective lens focus must lie on the **eucentric plane** of the microscope. This step will position the specimen.

a.) Find a point of interest on the specimen using the stage trackball (RC).

b.) Activate the “**Alpha wobbler**” (LC button L2, by default). The stage will begin rocking through a tilt range of +/- 15°.

c.) **Minimize** the specimen movement by adjusting the “**Z-axis**” control buttons (RC). These buttons are pressure sensitive; pressing harder = faster change.

d.) **Deactivate** the “Alpha wobbler”.

Alternatively: the eucentric height can also be set by using the “**Eucentric focus**” button (RC) to focus the objective lens on the eucentric plane. The specimen can then be brought into focus using the “**Z-axis**” buttons. When it is in focus, it is located at the eucentric plane of the microscope.
7.) **Beam Tilt Pivot Points**

   a.) **Focus** (RC “Focus”) the specimen to **minimum contrast**.

   Note: the “Focus” knob has two parts. The smaller, inner knob changes the focus. The larger, outer knob adjusts the “Focus step” i.e., how much the focus changes with each movement of the inner knob.

   b.) Move to a **non-beam-sensitive area** of the sample. **Converge** (LC “Intensity”) the beam to crossover and **center** it (LC trackball).

   c.) Select “**Beam tilt pp X**” from “Direct Alignments”.

   d.) Use the **MF knobs** to **superimpose** the two **beam spots** and minimize the beam movement.

   e.) Press “**Done**” in “Direct Alignments”.

   f.) Repeat steps b–e for “**Beam tilt pp Y**”.

8.) **Beam Shift**

   a.) **Converge** the beam to crossover (LC “Intensity”) and **center** it using the LC trackball.

   b.) Select “**Beam shift**” from “Direct Alignments” and use the **MF knobs** to **re-center** the beam on the screen.

   c.) Press “**Done**” in “Direct Alignments”.

9.) **Rotation Centering**

   Note: Rotation centering is the most important alignment for high-resolution work on this microscope. It should be done carefully and at or above the magnification that will be used for imaging.

   a.) Find a suitable area of the specimen and **focus** (RC “Focus”) to **minimum contrast** at a magnification (RC “Magnification”) **above 100kx**.

   b.) Select “**Rotation center**” from “Direct Alignments”. Use the **MF knobs** to **minimize image movement**. The center of the image should pulse in and out.
of focus, but there should be little or no lateral movement. The amplitude of the image wobbler can be controlled by the focus step knob (outer ring of the RC “Focus”).

c.) Press “Done” in “Direct Alignments”.

Note: If the beam moves appreciably while the rotation center alignment is active, readjust the X and Y pivot points and beam shift (steps 7 and 8) and repeat.

Note: The binoculars can be used to more accurately focus and perform alignments, particularly the rotation center. To use the binoculars, gently raise the small viewing screen (lever, left side of column base). The eyepieces of the binoculars can be focused by rotating. Inserting the beam stop (knob, just above viewing chamber on right side of column) can aid in focusing the binoculars: adjust each eyepiece until the shadow of the beam stop appears sharp.

10.) **Objective Aperture Centering** – Skip if no objective aperture is desired.

a.) Enter diffraction mode (RC “Diffraction”) and **center the direct-beam spot** (central diffraction spot) using the MF knobs.

b.) **Select and insert** the desired **objective aperture** (see aperture figure in step 4). The aperture should be visible in the diffraction plane.

c.) **Center** the aperture around the **central spot** (aperture X and Y knobs).

d.) **Deselect** “Diffraction” to return to imaging mode.

Note: If the aperture is not visible in the diffraction pattern, do not randomly adjust the aperture, as this can further lose it. Remove the aperture and return to imaging mode. Decrease magnification to low mag. mode (“LM” visible by magnification value) and reinsert the aperture. It should be visible in the image plane and can now be roughly centered. Return to the desired magnification and repeat steps a–d.

11.) **Objective Astigmatism**

a.) Select an appropriate **magnification** (50–100kx is a good starting point) and an amorphous region of the sample (support film or ion damaged region).

b.) Objective astigmatism is most easily corrected using the **CCD camera** and online FFT functionality (see Section VI for more details on the CCD camera). Begin collecting “search” images on the camera. In Digital Micrograph, select “Process” → “Live” → “FFT” to show a real-time fast Fourier transform of the image.
c.) Press the LC “Stigmator” button or click on “Objective” in the “Stigmator” window.

d.) Use the MF knobs to adjust the objective stigmator. The goal is for the rings in the FFT to **appear circular**, not elliptical or hyperbolic. The rings will grow larger and astigmatism will be more apparent when closer to focus. Adjust the focus so that the rings are large enough to be clearly visible but are not hyperbolic. As astigmatism is corrected, move closer to focus (larger rings) to fine tune the stigmator settings.

e.) Click “None” in the “Stigmator” window or press the LC “Stigmator” button.

Note: Objective astigmatism should be checked periodically throughout a session. It can be changed by changes in magnification mode, spot size, Z-height of the sample, or objective aperture size or position.

Note: Each stigmator has three registers available. Clicking on a register selects among them. These can be used to store stigmator settings for different conditions, such as different objective apertures. They can also be used to store a backup before adjusting the astigmatism. One register can be copied to another by right clicking.
VI. Camera Control and Imaging

1.) **Camera Operation**

a.) The CCD camera is operated by the “**CCD/TV Camera**” panel, found in the “**Camera**” tab of the microscope interface.

b.) Click “**Insert**” to extend the CCD camera into the microscope.

c.) **Lift** the phosphor **screen** (LC button L1) so the beam can reach the camera.

d.) There are three camera modes:

- **“Search”** is a high refresh rate, lower resolution mode used for viewing and focusing on the specimen in real time.

- **“Preview”** is a slower, higher resolution mode that uses half the CCD area to preview the final acquired image quality.

- **“Acquire”** is used to capture a single image from the whole CCD detector.

Note: The parameters for any of these settings can be configured using the “**Settings**” flap-out panel on the “**CCD/TV Camera**” panel.
2.) **Saving Images**

Images should be saved to the support computer, never to the microscope PC. **Any files on the microscope PC are subject to deletion without warning.**

a.) Save files to the location `\Rem30271\T12_DataFileDump\<user directory>`

This can typically be found in “My Network Places”. The username and password are written on the corner of the right monitor.

b.) Files can be accessed remotely (instructions in Addenda).

c.) Please delete files from the support PC once transfer to another location has been verified. Any **files older than 1 month are subject to deletion.**

3.) **Dark and Gain References**

If consistent artifacts are present in acquired images, a new dark reference or gain correction may be necessary. Follow these steps:

a.) Verify that the “Bias corrected” and “Gain corrected” boxes are checked for “Acquire” mode in the “General” flap-out panel of “CCD/TV Camera”.

b.) If both boxes are checked, first try preparing a **new dark reference** (otherwise known as bias reference) by clicking the “Clear Bias” button on the “Bias/Gain” flap-out panel. (This can also be done by selecting “Camera” → “Remove Dark References” in Digital Micrograph.)
c.) If artifacts persist, a new gain reference must be collected.

To prepare a gain reference:

i.) Move to an empty location, such as a large hole in the support film. This empty region must cover the entire CCD field of view.

ii.) Spread the beam until it illuminates at least half of the viewing screen.

iii.) Lift the screen and click “Gain Acq.” in the “Bias/Gain” flap-out panel. (Or select “Camera” → “Prepare Gain Reference” in Digital Micrograph.)

VII. End of Session

1.) Leave the microscope in the standard condition for the next user:

a.) Remove any objective or SA aperture; leave condenser aperture inserted (in, out, out).

b.) Leave the column valves closed.

c.) Place the viewing screen down; cover the window with the rubber mat.

d.) Switch the filament off (“Filament” button in the “Filament” panel).

e.) Leave the magnification in the SA range (preferably 2400×). This is essential to maintain stable objective lens current and prevent thermal drift for the next user.

f.) Remove the sample holder (be sure to reset the stage first), remove your sample, and return the holder to the microscope. See section III for details.

g.) Check the reservation system to see if another user is scheduled after you.

h.) If you are not the last user of the day, fill the LN2 dewar.

i.) Sign out of the reservation system and leave any relevant comments. If running the cryo cycle, please indicate this in the comments.
2.) If you are the last user of the day:

a.) **Remove the LN2 dewar**, return the LN2 to the large dewar, and place the microscope dewar **upside-down on its stand**. Place a towel below the cold finger on the microscope to collect condensing moisture.

b.) **Run the cryo cycle**, located in the flap-out panel from the “Vacuum” control panel. Standard settings are “Duration” = 300 min, “Start after” = 10 min.

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<tr>
<th>Status: COL. VALVES</th>
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<tr>
<td>Pressure</td>
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<td>Backing line</td>
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Log off your user account and log in as the vacuum monitor: username = “vacuumuser”, password = “vacuum”

c.) The Vacuum Logger software should start automatically. Click “Log” → “Start” to begin logging the vacuum levels. **Create a new file using today’s date** (mm-dd-yyyy) as the file name (include zeros in the day/month).

VIII. Troubleshooting

1.) **No beam is visible**

There are several possible causes for no beam being visible. Try the following steps until a beam can be found:

a.) Make sure that no objective or SA aperture is inserted to block the beam.

b.) Decrease the magnification (RC “Magnification”).

c.) Move the specimen stage (RC trackball) in case a grid bar or other non-electron-transparent region is blocking the beam path.
d.) Check that the C2 lens (LC “Intensity”) is not severely under- or overfocused, spreading the beam to a point that it is too dim to see. The optimal C2 setting will vary with magnification, but is typically 30–60%.

e.) If no beam is still visible, try loading a previously saved gun alignment file. Alignments can be loaded through the “Alignments” control panel, typically founding under the “Align” tab. Open the flap-out menu, go to the “File” tab, and select a saved alignment file. Select “Gun” from the “Available” list by either double clicking or using the < button, then click apply. If desired, alignments for other systems/modes of the microscope can also be loaded by moving the appropriate labels to the “Selected” list and pressing apply.

2.) **One of the control pads (or z axis or tilt buttons) is unresponsive**

Re-initialize the pad by unplugging the USB cable from the back of the PC and re-inserting it. The right plug connects to the right panel, left to the left. **Always note that this occurred in the instrument log.** Reinitializing the panels may randomly activate functions on that panel, so check for changes in spot size, diffraction mode, etc. after plugging it back in.
3.) **The objective or SA aperture cannot be found**

Do not randomly turn the knobs to look for it! This not only makes it even harder to eventually find, turning too far in either direction can also cause damage to the aperture mechanism (and has!)

For the SA aperture, reduce the magnification, as it may be located outside the field of view. If that doesn’t work, try centering a larger aperture first.

For the objective aperture, you can likewise reduce the camera length and try a larger aperture. If it is still not visible, exit diffraction mode and reduce magnification to the “LM” range. The aperture should be visible in the image now, and centering it will bring it near enough that it should be visible in the back focal plane when you return to “SA” mode diffraction.

4.) **Images contain black specks, ghosts, or other artifacts**

Make sure that both dark and gain references are being applied to the image. Collect new references if necessary (see section VI, step 3 on pages 15–16).

5.) **The stage or beam shift trackball is unresponsive**

If other controls on the same pad function normally, then try increasing the sensitivity. Each trackball has a sensitivity that can be adjusted by pressing the left (decrease) or right (increase) button above it. The sensitivity at each setting varies with magnification, but in many modes the lowest setting corresponds to essentially zero movement.

6.) **The image shifts when lifting the screen to use the CCD**

The microscope has been set to shift the beam to the off-axis (TV) camera when the screen is lifted. To correct this, use the “Detector Configuration” control panel (usually located under the “Misc.” workspace tab). The “Automatic mode” box should be unchecked and the current “Detector shift” should read “None”.
7.) **The MF knobs do not move the diffraction pattern**

Occasionally, the microscope will stop auto-assigning the multifunction knobs to diffraction shift when entering diffraction mode. Check the lower-left status box to verify that the MF knob assignments read “Diff shift”.

If the MF knobs are assigned to a stigmator, this will override the diffraction shift. Turn the stigmator off to return to diffraction shift. Otherwise, you can manually assign it by right clicking in the “MF X” box and selecting “Diff shift X”. Be sure to right click again and select “None” when finished adjusting the pattern.

8.) **Large amounts of specimen drift are present**

There are several possible causes of specimen drift. The following may reduce it:

a.) Lightly tapping on the end of the sample holder rod can cause it to settle in the goniometer, reducing drift.

b.) Move to a different area of the sample. Damaged regions of a support film will often move as they are subjected to the electron beam. In this case, the sample itself is moving, and viewing a more stable region may reduce or remove the apparent drift.

c.) Avoid moving the stage, when possible. Every stage movement will require a settling time once it completes. Use the image shift function to make small adjustments to the view without moving the stage.

Image shift can be assigned to the multifunction knobs by locating the “Image Settings” control panel (usually found in the “Camera” workspace tab) and clicking the “MF Knobs” button. Click again to return the MF knobs to their previous
assignment. The image shift has three separate registers, like the stigmator controls, which can be used to store different position offsets.

d.) Having the door open, talking, or large groups present in the instrument room will inevitably produce thermal and acoustic variations that will result in specimen drift. Avoid these situations whenever possible.

9.) **Other problems**

If the software is behaving unexpectedly, you may close and restart the user interface and Digital Micrograph. Logging out and back in may also resolve some problems. **Do not restart the PC!**
IX. Addenda

Remote access to saved images

Saved images can be remotely accessed via SFTP. To connect to the file storage, you must use an SFTP client such as WinSCP (Windows) or Fugu (Mac OS X).

The host name is rem30271.charfac.umn.edu, port 22. Be sure to select SFTP as the protocol (regular FTP connections will not be allowed). The username and password to login are the same as those used to save the files from the TEM computer.

TEM Discussion Lists

Signing up for the appropriate TEM mailing lists is strongly suggested. These lists will be used to notify of instrument downtimes, changes in policy, and so forth.

The appropriate mailing list for each instrument is:

FEI T12, Shepherd: TEM-T12@umn.edu
FEI FEGTEM, Shepherd (F30): TEM-F30_Shepherd@umn.edu
FEI Cryo FEGTEM, NHH (NHH F30): TEM-F30_NHH@umn.edu

Messages sent to the mail list will be distributed to everyone on the list. Some uses for the mailing list include:

To subscribe/unsubscribe:

Email addresses at umn.edu can subscribe automatically through listserv@umn.edu. To subscribe non-umn.edu addresses, contact CharFac staff.

Subscribe: email listserv@umn.edu with no subject. In the body, type:

“SUBSCRIBE <list name> <your name>”

For example, to subscribe to the T12 list, I would send “SUBSCRIBE TEM-T12 Jason Myers”. You will receive a confirmation email from the server.

Unsubscribe: email listserv@umn.edu with no subject. In the body type:

“SIGNOFF <list name>”

So to remove yourself from the T12 list, send “SIGNOFF TEM-T12”. You will receive a confirmation email from the server.