Tecnai T12 Operating Instructions

03-12-2009

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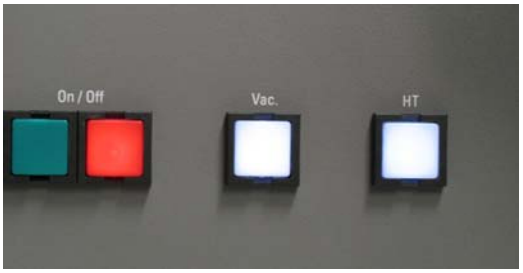
I Start-up (Do not proceed if these conditions are not met)

- Check to see that the **"Vac"** and **"HT"** buttons are lit on the server control panel. If the "Vac" is not lit, do not proceed further and consult with staff. If the "Vac" is lit but the "HT" is not, proceed with these instructions.

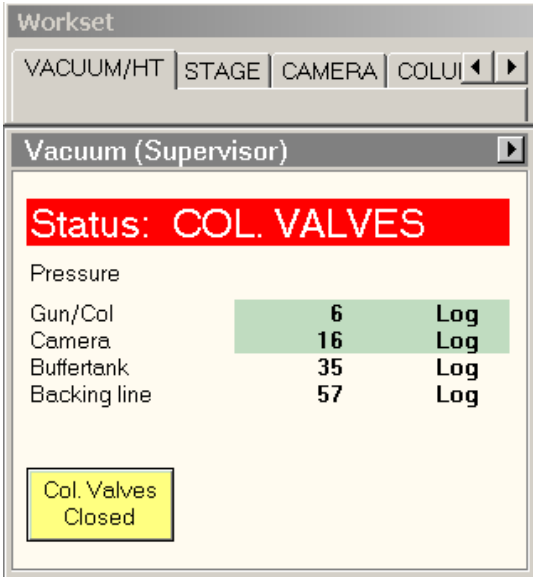


- Log in to the Tecnai computer using your username and password.

- Ensure the Camera Unit (Gatan Multiscan Camera Model 794) is on. Ensure that all three switches on the box are in the "Up" position.

- Launch the Digital Micrograph program; then launch the Tecnai User Interface program.

- Check that the column valve is closed: **Tecnai interface → "Setup" workset → "Column Valves Closed" button is yellow.**

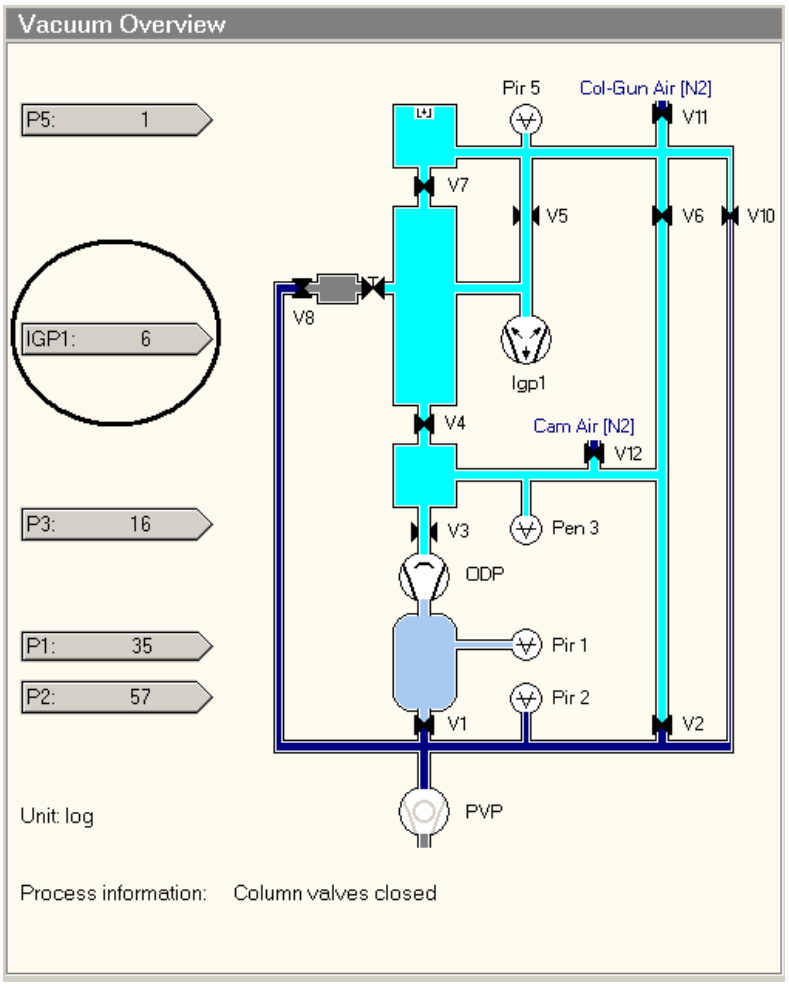


- Look for the holder, it could be either already in the TEM or if you are the first user of the day it could be in the plasma cleaner. See plasma cleaner instructions at the end of this manual.

- Fill LN2 cold trap (make sure viewing screen is covered). The first dewar of the day will last approximately 20 minutes. Subsequent dewars should last for a couple of hours.

Be aware that if the cold-finger has been cold for a while, and is allowed to warm up, the vacuum in the column will deteriorate **seriously** – occasionally to the point of shutting down the microscope. If the Gun/Col vacuum rises above 10 Log then close the column/valves, fill the nitrogen and wait until it reaches 6 again.

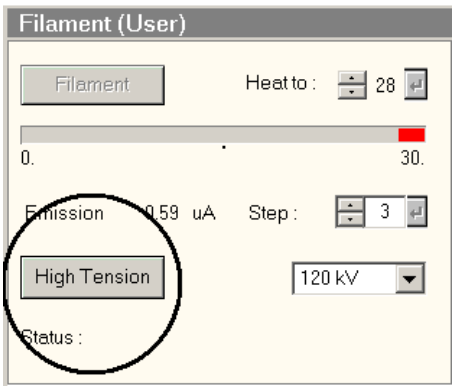
- Activate **"Vacuum Overview"** in the Tecnai interface (lower right portion of the screen). Check to see that **IGP 1 vacuum level is Log 6 (90 x 10^{-9} Torr).** If you are the first user of the day, the vacuum may be higher. With the addition of LN2 the vacuum will improve over the course of an hour.



II Accelerating Voltage

The **"High Tension" (HT)** should be on at 120 kV

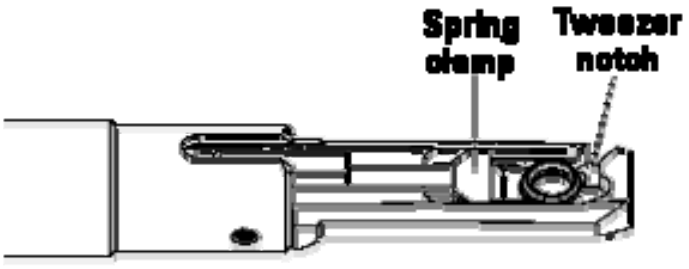
If you are the first user of the day, the **"High Tension"** in the Tecnai User Interface will be off (button is grey) and the **"HT"** button on the server control panel may be off (not lit). This is due to the running of the "Cryo Cycle" at the end of the preceding day. If the vacuum levels are fine (see Start Up), engage the "HT" button on the server control panel if necessary. Then select **20 kV** in the Tecnai **"Filament"** window and turn on the **"High Tension" button (turns yellow)**. Step up to **120kV in 20kV increments**. **Wait (approximately 2-3 minutes) until the emission current has stabilized at each step!**



III Specimen Loading / Unloading

- Keep one hand against the black end cap of the holder, making sure it cannot move out of the cover tube.

- Fit the tool (stored in one of the holes in the supports of the cover tube) into the hole in front of the clamp. Then gently lift the clamp straight up to its fullest extent.

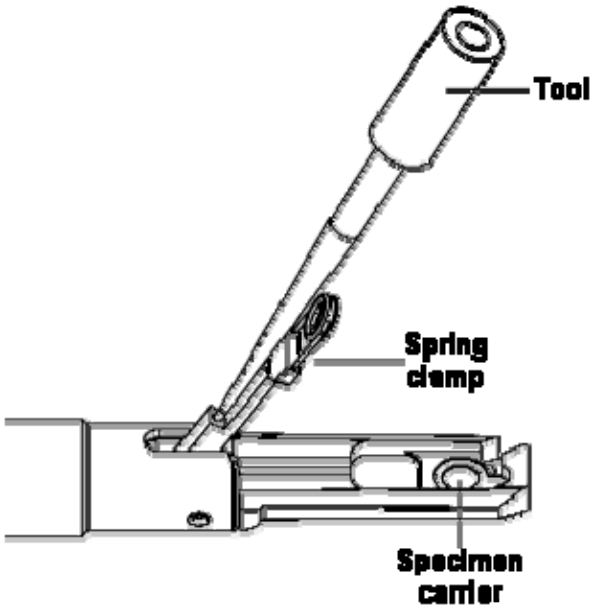


- Place the specimen in the circular recess of the specimen-holder tip.

- Gently lower the clamp with the tool straight down onto the specimen. Make sure the specimen remains correctly in position. Return the tool to its stored position.

- Retract the holder slightly in the cover and turn it upside down. Tap the cap at the end a few times. Turn the holder back and check that the specimen has not moved (movement is a sign that it isn't clamped properly).

- Check the o-ring for debris and carefully remove with a tweezers.



Note:
Be careful not to touch any part of the specimen holder between the tip and the o-ring with bare hands.

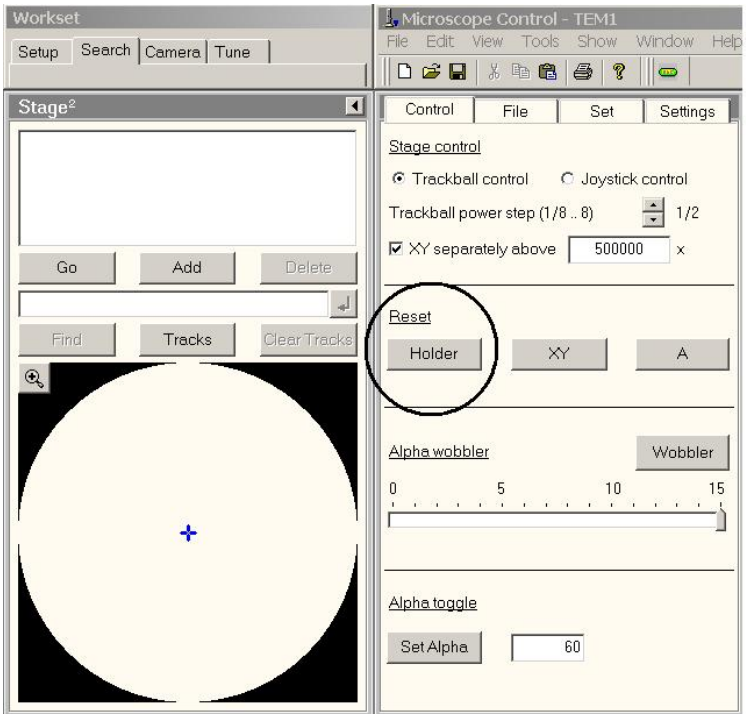
Never mount magnetic specimens (disks) in the single-tilt holder. The clamp is normally not strong enough to prevent the specimen from flying out due to the objective-lens magnetic field and sticking to the objective-lens pole pieces.

IV Sample Rod Removal / Insertion

The specimen airlock of the CompuStage and the specimen holder consist of fine, high-quality mechanics. **If considerable force is needed for any manual actions on the holder or CompuStage, it is a sign of something being wrong. It should never be necessary to exert strong force and doing so may well result in damage to specimen holder or CompuStage.**

Before both removal and insertion, ensure that the:

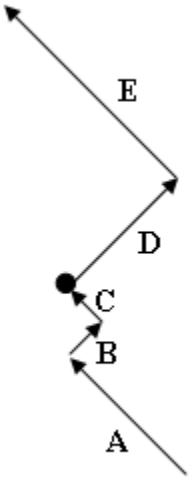
- Column valve is closed **("Column Valves Closed" is yellow)**;
- **Red sample exchange light** on the compustage is **off;**
- Stage is at the home position: **"Search" workset → "Stage" → "Reset Holder".**



Compustage showing 5, 6, and 12 o'clock positions

Pin on Sample Holder

Sample Rod Insertion

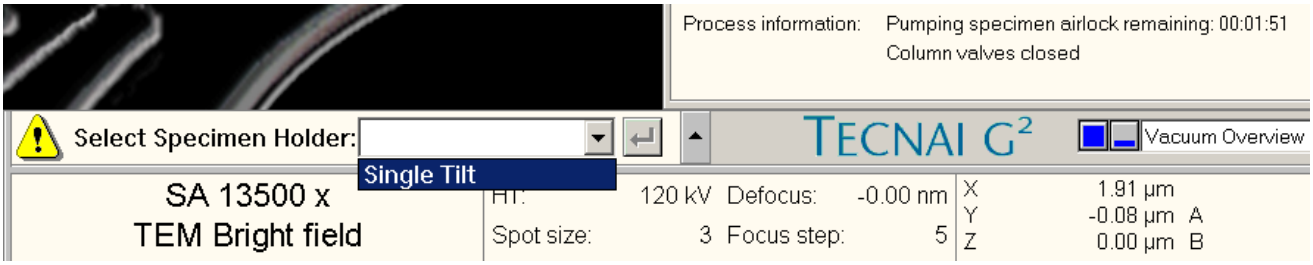


A: Carefully insert the holder, with the **holder pin at the 6 o'clock position**, as far as it will go into the compustage. Be careful not to scrape the tip.

B: **Rotate** (while gently pressing inward) **the holder pin counter-clockwise from the 6 to the 5 o'clock position**

C: At the 5 o'clock position the holder will go into the compustage about an inch further. **Stop.**

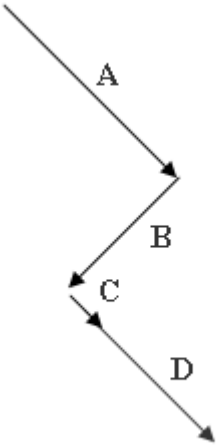
•: The prepump will start (**red light on the compustage will turn on**). Select the Specimen Holder you are using on the Tecnai monitor interface and activate it with the (↵) button. The remaining pumping time for the specimen exchange is shown on the "Vacuum Overview" screen.



D: When the pumping cycle ends (**red light on the compustage turns off**), **support the purple surface of the goniometer** with one hand and with the other **rotate the holder pin counterclockwise from 5 o'clock to 12 o'clock. Do not let go of the holder at this point!**

E: Gently guide the holder into the column. Tap the end of the holder to ensure stabilization.

Sample Rod Removal:



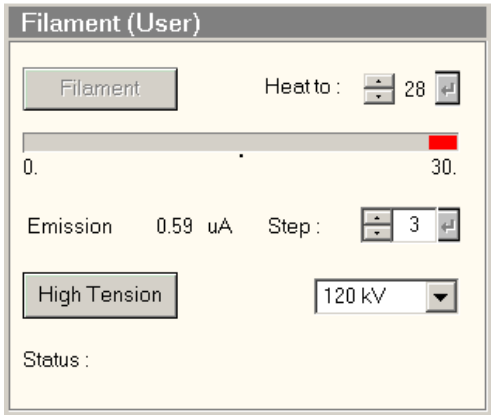
A: **Support the purple surface of the goniometer with one hand**. **Pull the sample holder straight back as far as it will go.** (**<u>do not rotate</u>**)

B: **Rotate (<u>do not pull</u>) the holder pin clockwise from the 12 to 5 o'clock position** – ie. as far as it will go.

C: Stabilize the hand holding the sample holder and pull the holder back (about an inch) to release it from vacuum.

D: Gently guide the holder out of the column taking care not to scrape the tip along the inside of the goniometer.

V Generating Emission Current



Set the "Heat to" button to its maximum value and activate it with the **(↵)** button. Click "**Filament"** to turn on the T12's LaB_6 filament.

The Emission Step setting adjusts the bias voltage on the Wehnelt of the thermionic gun. Decreasing the bias voltage (increasing the step size) increases the total emission current, but at the same time will increase the source size and energy spread of the electron beam. Normal filament emission values for the LaB6 filament lie in the 10-15 uA range. Step sizes of 2 or 3 are commonly used.

Click on **"Column Valves Closed"** (turns grey) to open the column valves.

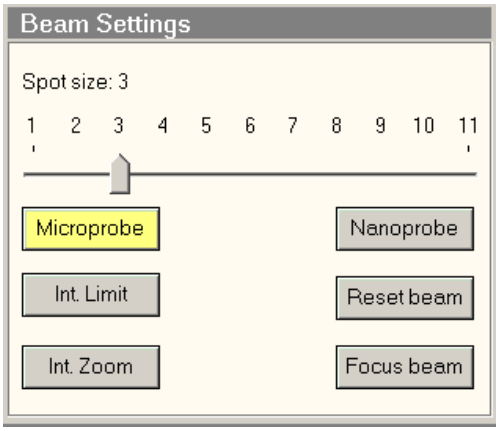
VI Alignment:

Always align the microscope from the top down. Begin with the **Objective and SAD apertures out**; the **Condenser aperture in** to prevent possible damage to your specimen. Pressing F1 will open a help window to give further explanation and assistance at any time. **LC = left console; RC = right console.**

1) Find the beam. If no beam is visible, there are a few "obvious" reasons:

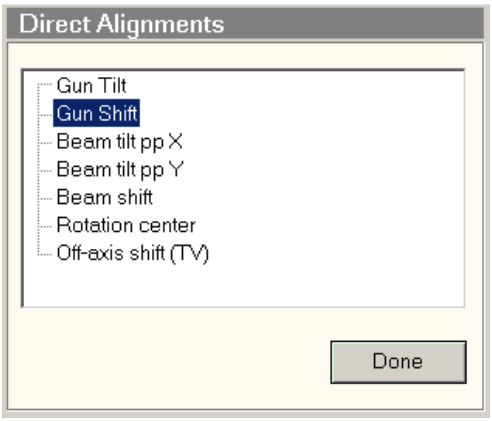
- What you're seeing may be the grid which holds your specimen. If this is the case, then try moving the specimen with the "Track Ball" (RC).
- It could also be that the beam brightness ("Intensity" knob LC) is too far one way or the other from crossover.
- The beam may also not be centered on the screen at the current magnification—try lowering the "Magnification" (RC) to find the beam and center it with "Beam Shift" (Track Ball LC). **Note**: the sensitivity of the magnification adjustment is affected by the lower/larger knob on this control.

2) Gun Tilt:



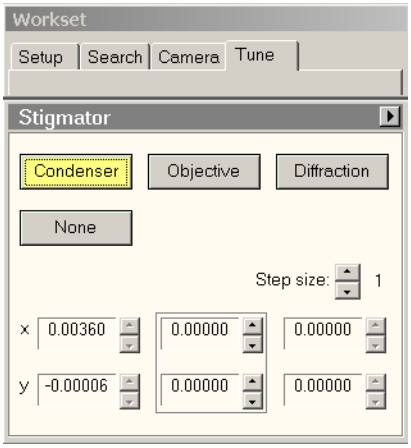
- Set **"Magnification"** (RC) to **10-60k** and **"Spot Size" to 3**.
- **Center** the beam with **"Beam Shift"** (Track Ball LC) and spread the beam on the screen with **"Intensity"** (LC).
- Select "**Direct Alignments**" in the Tecnai workspace menu. Select "**Gun Tilt"**. Adjust the gun tilts using the **"Multifunction (MF)"** knobs (LC & RC) until you minimize **"Exposure time".** **Note:** the sensitivity of the MF knobs is controlled by the +/- buttons (LC).

3) Gun Shift / Spot Size:



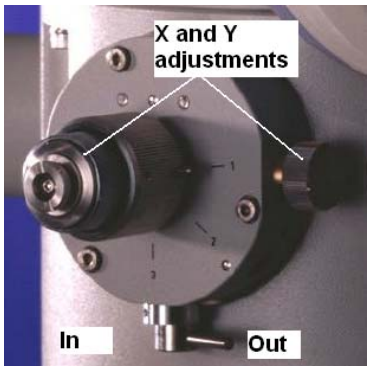
- Select **"Spot Size" 9**. **Converge** the beam to crossover with "Intensity" (LC) and **center** it with "Beam Shift" (Track Ball LC).
- Select **"Spot Size" 3**. Select **"Gun Shift"** in the **"Direct Alignments"** window. **Converge** the beam with "Intensity" (LC) and **center** it using the **"Multifunction (MF)"** knobs (LC & RC). Be aware of condenser lens hysteresis – finish focusing by turning the "Intensity" (LC) in the same direction each time.
- Repeat steps 3 and 4 until the beam does not move when changing from "Spot Size" 9 to 3.
- Check that the filament image is still symmetrical at "Spot Size" 3. Re-adjust "Gun Tilts" if necessary. Deselect "Direct Alignments".

4) Condenser Astigmatism:



- The condenser lens astigmatism should be corrected if the beam does not expand in a circular fashion when the beam is converged and diverged using the "Intensity" (LC) knob. Small amounts of astigmatism will not noticeably affect the imaging conditions. Astigmatism correction needs to be performed for each "Spot Size" you use.
- Select the **"Spot Size"** you require for your session. **Converge** the beam with "Intensity" (LC) and **center** it with "Beam Shift" (Track Ball LC).
- **Under-saturate** the filament to reveal filament structure. Select **"Condenser"** on the **"Stigmator"** window. Use the **"Multifunction (MF)"** knobs (LC & RC) to make the filament structure as sharply focused as possible. **Note**: The binoculars can be used as an aid to more accurate observation and focusing. Introduce the small flip-up screen fully into the beam (lever at left side base of column). Focus the binoculars using the eyepiece adjustment controls. As an aid to focusing, insert the beam stop. When its shadow is sharp, the binoculars are focused.
- Re-saturate the filament.

5) Condenser Aperture Centering:



- **Select the Condenser Aperture** you wish to use (4 is the largest; 1 the smallest). **Converge** the beam with "Intensity" (LC) and **center** it with "Beam Shift" (Track Ball LC) to the middle of the smallest circle on the phosphorus screen. Spread the beam to a size just a little bit larger than the bigger circle and center it around this circle using the X and Y control knobs on the aperture mechanism. Repeat this procedure a few times if it is needed.

6) Eucentric Focus:

- Find your specimen using the specimen "Track Ball" (RC).

- Push the **"Eucentric Focus"** button (RC). Focus the image with the **"Z Axis"** controls (RC) [with crystalline samples, after setting "Eucentric Focus", use "Z Axis" to minimize diffraction pattern]. Alternatively, you can push the **"Alpha Wobbler" (L2)** button in the flap-out tab of the "Stage" window. This will tilt the specimen holder back and forth. You can then minimize specimen movement with the **"Z Axis"** controls (RC). Turn the "Alpha Wobbler" off.

7) Pivot Points

- Focus the specimen to **minimum contrast. Move to a non beam sensitive area of your grid. Converge** the beam with "Intensity" (LC) and **center** it with "Beam Shift" (Track Ball LC).

- Select **"Beam tilt pp X"** from the "**Direct Alignment"** window and then use the **"Multifunction (MF)"** knobs (LC & RC) to super-impose the two spots; repeat for **"Beam tilt pp Y".**

8) Beam Shift

- **Converge** the beam with "Intensity" (LC) and **center** it with "Beam Shift" (Track Ball LC).

- Select **"Beam shift"** from the "**Direct Alignment"** window and then use the **"Multifunction (MF)"** knobs (LC & RC) to center the converged beam on the screen.

9) Rotation Centering:

- Find a suitable area of the specimen and **focus to minimum contrast** at a **"Magnification" above 100kx**.

- Select **"Rotation Center"** from the **"Direct Alignment"** window and minimize the image movement with the **"Multifunction (MF)"** knobs (LC & RC). The amplitude of the image wobble is controlled by the **"Focus"** ring control. Close the "Direct Alignment" window. (If the beam moves appreciably during this process, re-adjust the pivot-points (X & Y), re-center the beam (shifts) and repeat rotation centering).

10) Objective Aperture Centering:

- Press **"Diffraction"** (RC), focus the central diffraction spot, and **center** that spot with the **"Multifunction (MF)"** knobs (LC & RC)**.**

- Insert the **Objective Aperture** and **center** it around the transmitted diffraction spot

- De-select **"Diffraction"** (RC) to re-enter bright-field imaging mode.

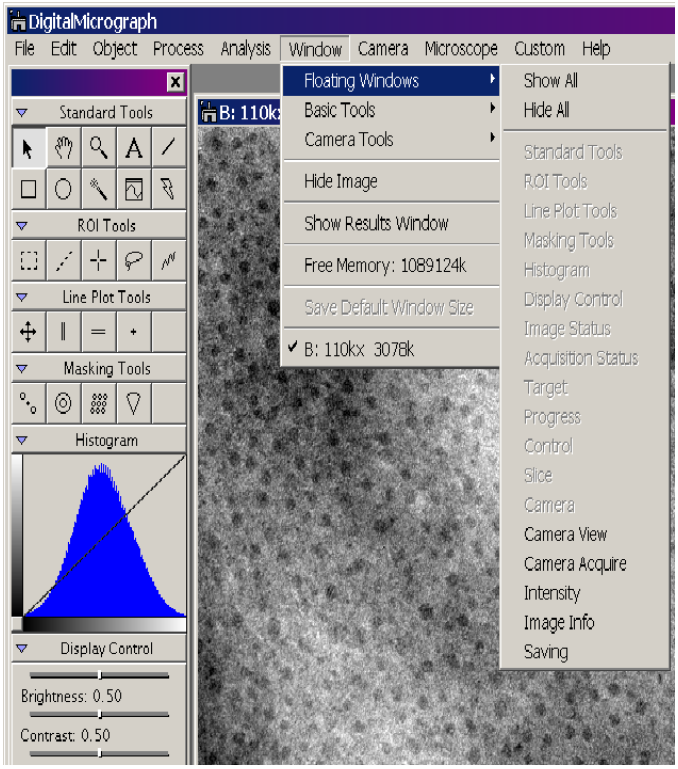
11) Objective Astigmatism:

- This alignment is most conveniently done using the **CCD camera**. Press **"Stigmate"** button (LC) and the **"Objective"** button is automatically selected in the **"Stigmate"** window on the Tecnai interface. Use the **"Multifunction (MF)"** knobs (LC & RC) to correct for astigmatism. Selecting **"Process" – "Live" – "FFT"** in Digital Micrograph will show a Fast Fourier Transform that aids in astigmatism correction. De-select **"Stigmate"** button.

- The objective astigmatism is now set for the specimen at the eucentric height and in-focus at this magnification. It will not be necessary to re-adjust it unless your specimen is magnetic or you

change objective aperture, or if you change magnifications. A good idea is to use each of the 3 different presets for a different magnification. If the image moves when adjusting objective astigmatism please enter this in the log-book.

VII Digital Micrograph



1. Ensure the palettes you wish to work with are open on the left side of the viewing screen: **"Windows" → "Floating Windows"**

2. File Saving
To allow file saving to the remote workstation across the hall: **"My Network" → "Computers Near Me" → "Rem 30271" → enter "username" and "password listed on the bottom of the right monitor " → "T12" → "DataFileDump"**.

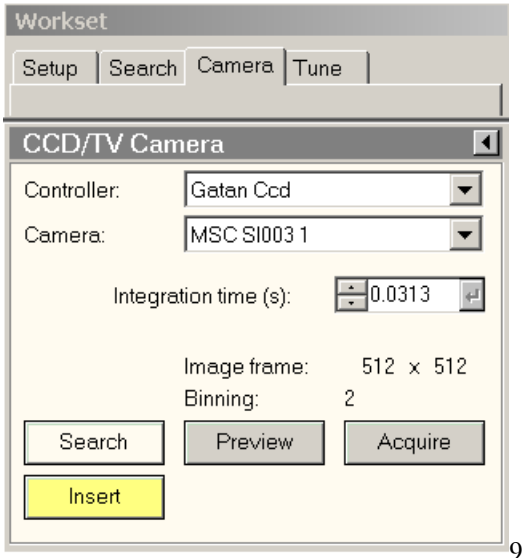
To automatically have the files you generate saved: **"File" → "Global Info" → "Save Numbered"**.

A scale marker should automatically be added to your recorded images. If it is not**: "File" → "Global Info" → "Data Bar" → "Image Mode"** tab → check **"Scale Bar"** and **"Add Scale Marker After Acquisition"**.

<u>Note on file saving:</u> get in the habit of saving your files in the Digital Micrograph Gatan format **(.dm3)**. This will subsequently allow you full access to the significant processing capabilities of that application (a copy of Digital Micrograph is available on one of the computers in Room 22 of CharFac). You may also want to save your files in the **.tif** format. It is recommended that you make duplicates of these files and conduct your image processing on them—leaving the original untouched.

3. Lift the "Viewing Screen" by pressing L1 (LC)

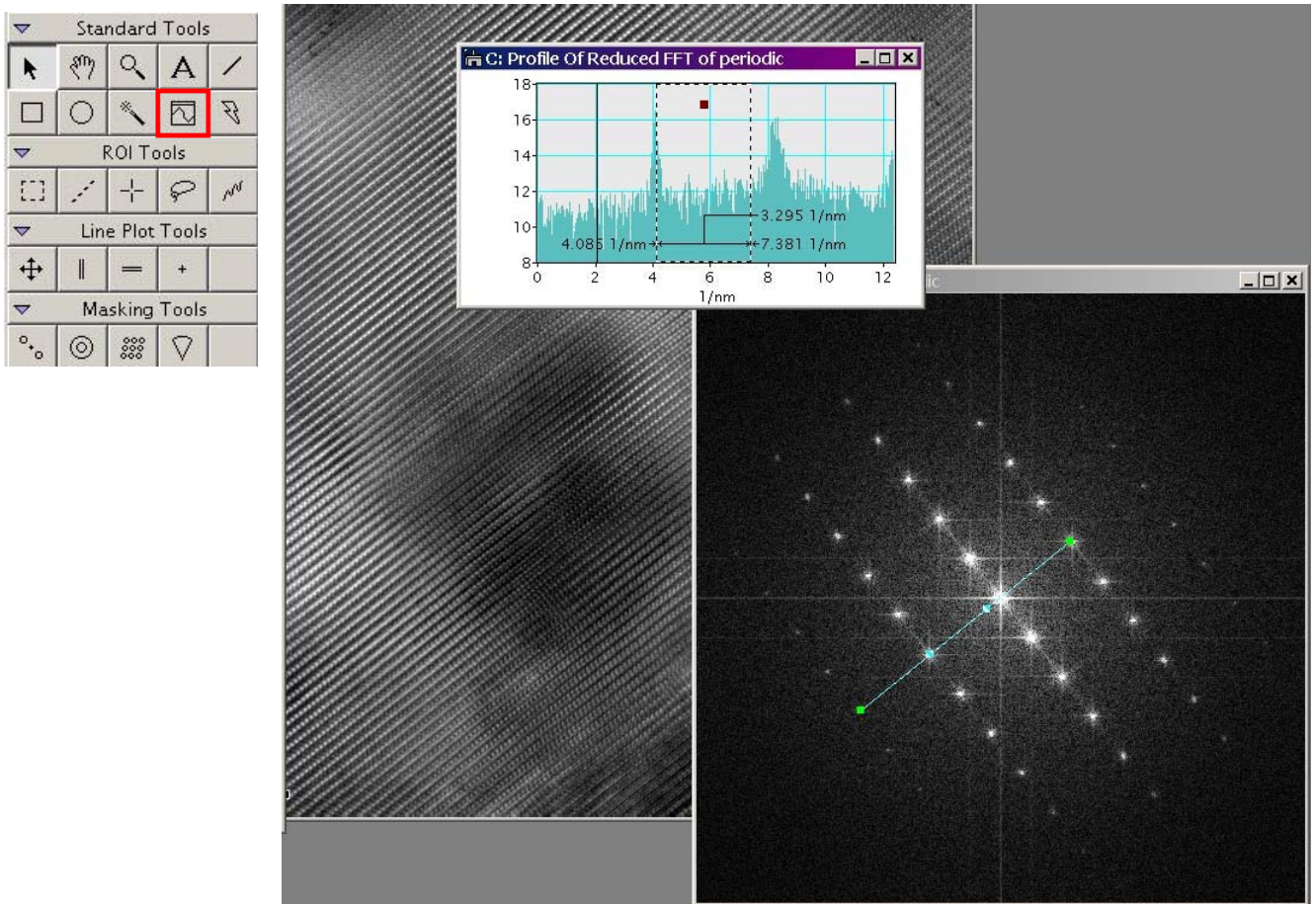
4. Insert the camera: **"Camera" → "CCD/TV Camera" → "Insert"**.



5. Camera Modes:
"Search" is a lower resolution, faster refresh rate camera that allows real-time movement about the specimen**; "Preview"** is a higher resolution camera used for fine focusing and objective astigmatism correction**; "Acquire"** will capture an image. The only parameter you should/will need to alter is the "Integration Time" for Acquire. Typical vales for brightfield imaging range from 1-2 seconds.

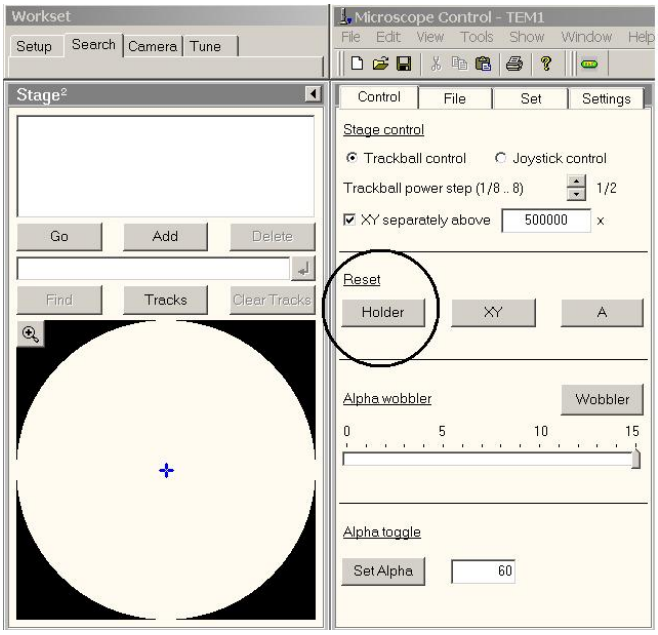
To stop "Search" or "Preview", click on the header bar of the active window and press the "Space Bar".

6. The **"Line Profile tool"** is useful for measurement in the Image or FFT mode. Select the "Line Profile tool" icon and draw a line over the region of interest. A "Profile window" will appear. You can click and drag within this window to establish the endpoints corresponding to the measurement of interest.



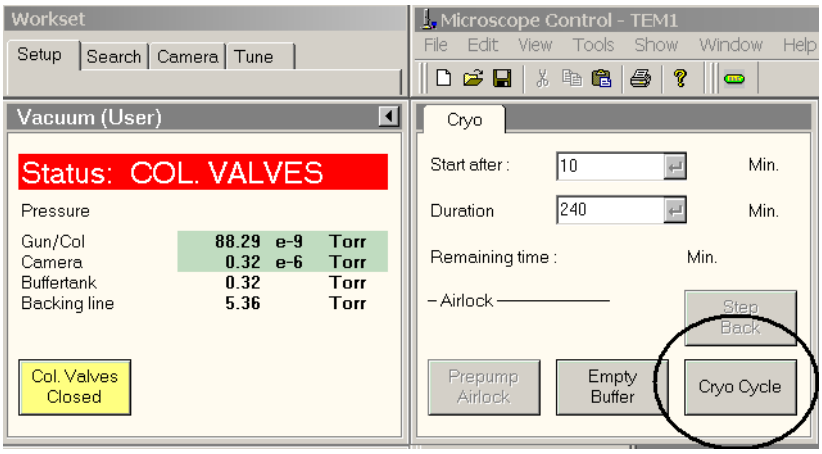
VIII End of Session

Leave the microscope in standard conditions for the next user. This includes:



- Column valve closed: **Tecnai interface → "Set Up" workset → "Column Valves Closed" button is yellow.**
- Stage is at home position: **Tecnai interface → "Search" workset → "Stage" → "Reset Holder".**
- **SA and Objective** apertures **out**; **Condenser** aperture **in.**
- **"Viewing Screen"** is in the **down** position.
- **Filament off.**
- Retract the holder take your **sample out and insert the holder back in to the column.**
- LN_2 Dewar filled for the next user.

If you are the last user signed up for the day (You need to check this at the end of your session!):

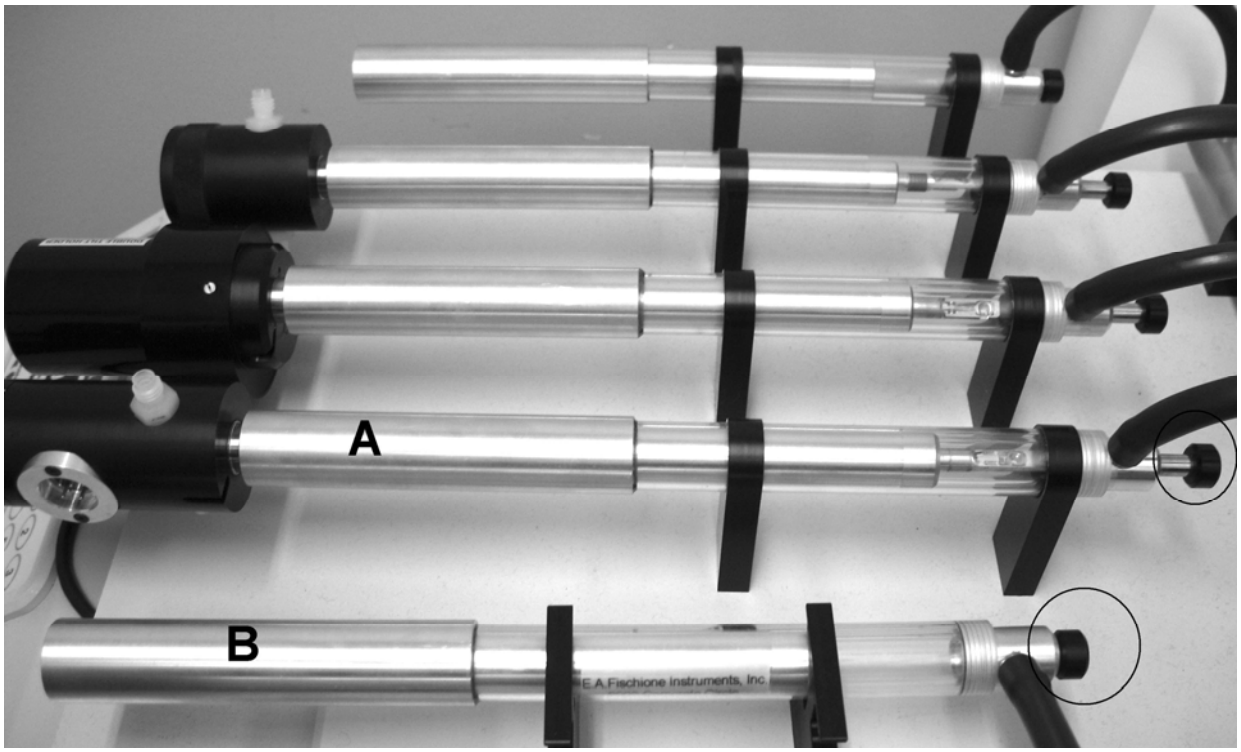


- Turn the **High Tension off.**
- Put the sample holder into an empty port on the plasma cleaner. (See instructions below)
- Use the rubber cork to block the hole on the goniometer. This protects the inside o-ring from dusting.
- Remove the LN_2 Dewar to let the cold finger warm up.
- Run **"Cryo Cycle"**: **"Setup" workset → "Vacc" → "Cryo cycle".**
- **Clos**e the **Digital Micrograph** and **Tecnai User Interface** program.
- **Log out** of the Tecnai computer and your session
- **Log in** as **"vacuumuser" with password "vacuum".** Run the **"Vacuum Logger"** program. **"File" – "New"—use current date as the file name. "Log" – "Start"**

Keeping Holders in the Plasma Cleaner:

Before you remove the T-12 single tilt holder from the plasma cleaner you need to close the valve. It is the little black pin at the end of the port.
Push the valve in to close it. Valve on port B is an example for a closed valve.

After you insert a holder into a port you should open the valve to start the pumping of the holder. Pull the black pin out. Valve on port A is an example for an open valve.



IX "Common" Problems

If the left or right console is not responsive, unplug and replug them in

If either the Digital Micrograph and Tecnai User Interface programs encounter a problem, you may restart them to see if that corrects the problem. **Never restart the computer!**

TEM discussion lists

For the **FEI T12 TEM,** send your email to the following address: **TEM-T12@umn.edu**

For the **FEI Tecnai F30 TEM in Shepherd,** send your email to the following address: **TEM-F30_SHEPHERD@umn.edu**

For the **JEOL 1210 TEM,** send your email to the following address: **TEM-JEOL_1210@umn.edu**

For the **FEI Tecnai F30 TEM (Cryo) in NHH,** send your email to the following address: **TEM-F30_NHH@umn.edu**

Your email will be distributed to everybody on that list. Some examples of using mailing lists are:

- If you won't be able to use your session or you get done early, you can send an email to the list so someone with urgent needs can jump in and use the equipment.
- If the equipment has a minor problem (for major problems please contact Ozan directly --you can also notify the list).
- If you have a TEM related research problem, you can ask the list for an advice.
- If you have found a nice trick to overcome a problem, you can share it with the list.
- If the equipment is down or having problems, Staff will post these to the list so you don't have to come down here to find the equipment down.

To subscribe to a list:

Send an email to the LISTSERV with the SUBSCRIBE command from the umn.edu email address. For email addresses other than umn.edu please contact Ozan. Here is an example:

To subscribe to F30 in shepherd list under my name (Ozan Ugurlu):

- Send email to: LISTSERV@UMN.EDU with no subject
- In the body of the email type: SUBSCRIBE TEM-F30_SHEPHERD Ozan Ugurlu
- A confirmation email will be sent to you from the server.

To unsubscribe to a list:

Send an email to our LISTSERV with the SIGNOFF command. Here is an example:

To unsubscribe from the T12 list:

- Send email to: LISTSERV@UMN.EDU with no subject
- In the body of the email type: SIGNOFF TEM-T12
- A confirmation email will be sent to you from the server.