

Tecnai Biotwin 12

Serial # D271

FEI Computer
Windows XP operating system
FEI Service: (800) 432-1734
website: <http://www.fei.com/products/tem/tecnai/>

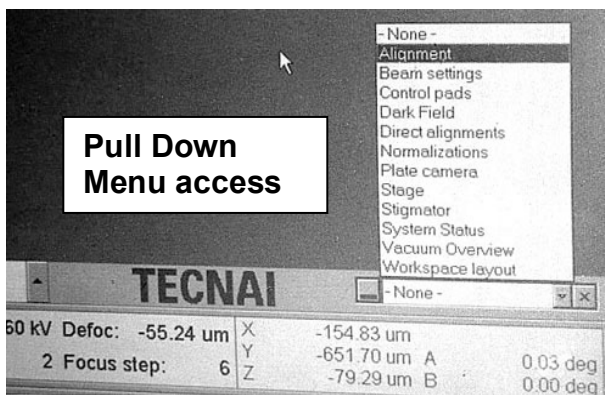
AMT Digital Camera
Dell Precision T3400
Windows XP operating system
AMT (978) 774-5550
Website: <http://www.amtimaging.com/index.html>

June Chan
BMRI staff associate
Office (646) 962-8259
Mobile (212) 316-2217

Setting up your User log on and Desktop

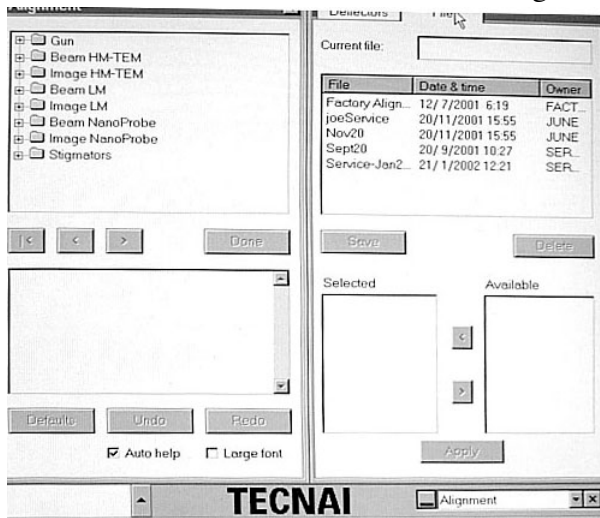
1. Press control, alt, delete to log on. Upon first log on, type your user name and press “enter” to set up your user password. Choose a new password--you will have to remember this password to log on subsequently.
2. Go to My Computer\ C: drive\ tecnai\ Exe\ Peoui. Highlight peoui application and make a shortcut of it (File\ create shortcut), copy it and paste it onto your desktop (right click mouse).
3. Open Peoui by double clicking the shortcut. This controls all aspects of the electron microscope. Always use this shortcut to access the microscope controls after you log on.
4. Make sure your Portable hard drive is correctly formatted for Windows. It must be in FAT32 format if you wish to use it in both mac and PC platforms. Check the format by getting properties (right click) on the drive icon.

Setting up the workspace

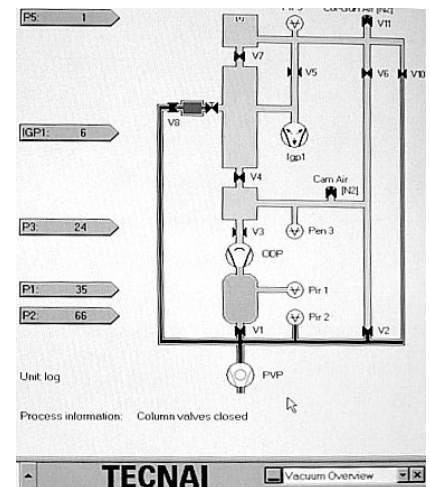


1. Open the pull down menu at the bottom right to access a list of microscope controls. Look at the “Vacuum Overview” to see if you have a good vacuum. Light blue is the best vacuum; light purple and gray is acceptable. The relevant chambers are labeled P5, IGP1 and P3 with a digital readout of the vacuum. You may proceed if the vacuum is good. If not, you can press “Empty Buffer” in the Vacuum page to activate the pump. Wait until a good vacuum is obtained (approx 10 minutes after chamber evacuation).

2. Open the “alignment” menu. Click the arrowhead at the right to access the pull out page with tabs. Choose “File” and pick the most recent file from the list- the microscope will need these alignments to work. If you can’t get a beam, always check the date of the file you selected. Highlight gun and column alignments from the available column and move them into the selected column using < or >. Press “Apply”.

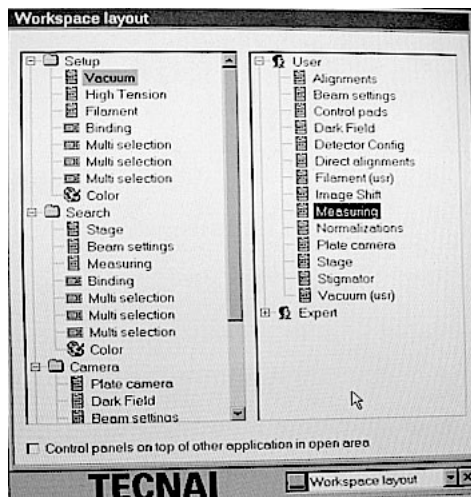


Vacuum Overview



Alignment \File Page

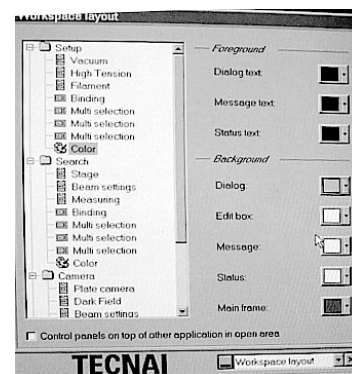
To save an alignment: Type a file name in Current file: Press Save.



Workspace layout

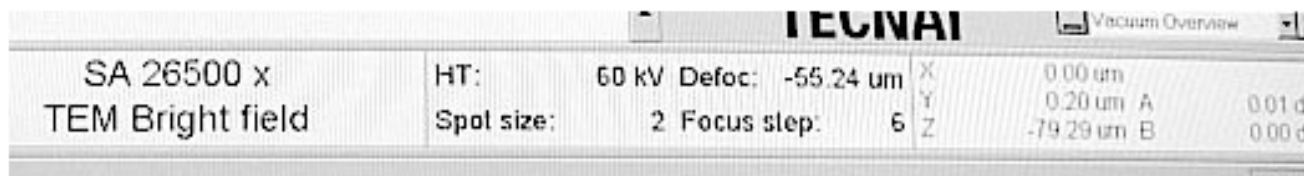
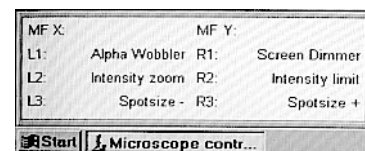
3. You can customize your Tecnai interface using “Workspace Layout”. The default menu is completely customizable so you can place any of the microscope controls into the main pull down tabbed pages. Open the user folder to see which microscope control pages you have access to. Drag the item into the folders in the left window to activate them from the pull down tabs on the desktop. (Only 2 windows can fit per pull down tab). (Change monitor resolution to fit 3 pages: Right click on the desktop, choose display desktop area properties: settings, 1280 x 1024 or 1600 x1200 resolution)

4. You may also rename, copy, or delete page tabs in your workspace; change the color of each page or window; etc using Workspace layout. Right click on the folder or item for options. Open the color palette to select a more suitable color for each item. This is helpful during dark adaptation- the monitor glare can be distracting while looking at the sample. (If the taskbar is too bright, unhighlight the always on top box in taskbar options by right clicking the taskbar, choose properties).



Color setting page

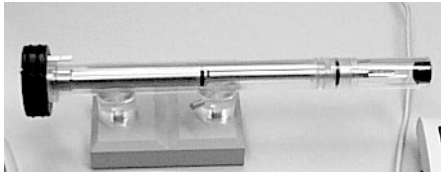
5. The control pad function buttons L1,2,3 and R1,2,3 (bottom left) and Tecnai status information (bottom center and right) can be customized. Drag an item off the status bar to delete it. Right click the mouse button to select and add choices.



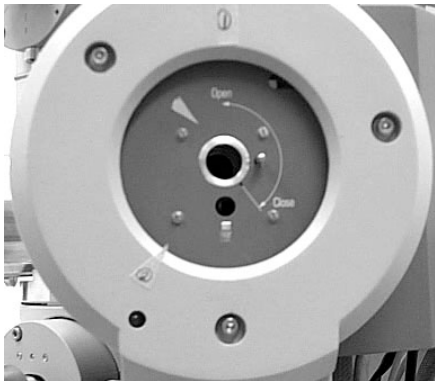
Caution!

Be very careful when accessing pull down menus and opening windows to view their contents. An inadvertent click of the mouse can disable functionality of the microscope- especially in the stigmators window. Many of the alignments are set up already. Do not access these settings and change them in any way unless you know what you are doing. Always write down or remember the default settings in case you need to restore them to a previous working state.

Loading and removing a specimen



1. Take the plexiglass cover off of the end of the specimen arm holder. Use the lifting needle to lift the clamping ring and place your grid in the center of the holder. Close the clamping ring and remove the specimen arm from the holder. **Check the o-ring to ensure it is free from dust or lint. You will not achieve a good vacuum if a piece of dust is on the o-ring.**



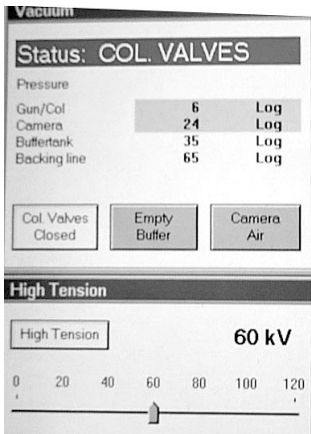
2. Insert the arm into the column with the taped arrow pointing towards “1”. The red indicator light will be lit. The arm will be halfway inserted.

3. Select specimen holder “Single tilt” from the monitors main Tecnai status screen and click on the enter symbol to activate it.

4. After the red light is extinguished, turn the specimen arm arrow counterclockwise toward “2” and gently insert the arm fully into the column.

Remove the specimen from the column by turning the specimen arm clockwise towards “1” and pulling the specimen arm completely out of the column. Removing the specimen will automatically close column valves. Always store the specimen arm in the plexiglass holder to prevent contamination.

Working with an electron beam



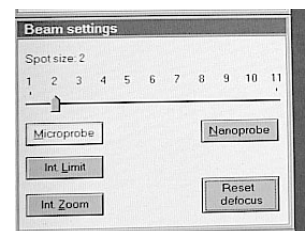
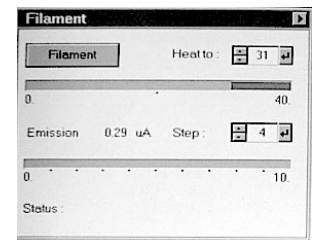
1. Use the main setup tab page to access the Vacuum window. Click “Col. Valves Closed” to open the specimen into the column. The status changes to read “Ready”. This assumes that the column is under a good vacuum (light blue or purple color indicator). See vacuum overview page to verify the column readiness.

2. Click on the “Filament” button to turn on the filament. The blue bar graph shows the filament status. Check filament saturation by focusing the beam into a small dot, lower the filament number until the filament image is seen, and increasing the filament until the image just disappears. Do not increase the filament setting above saturation- as this will cut down the filament life.

3. The Emission step is usually set to 4. The emission indicator bar shows microamps (usually between 10-20) readings. Do not change these settings unless the beam is unstable or too dim.

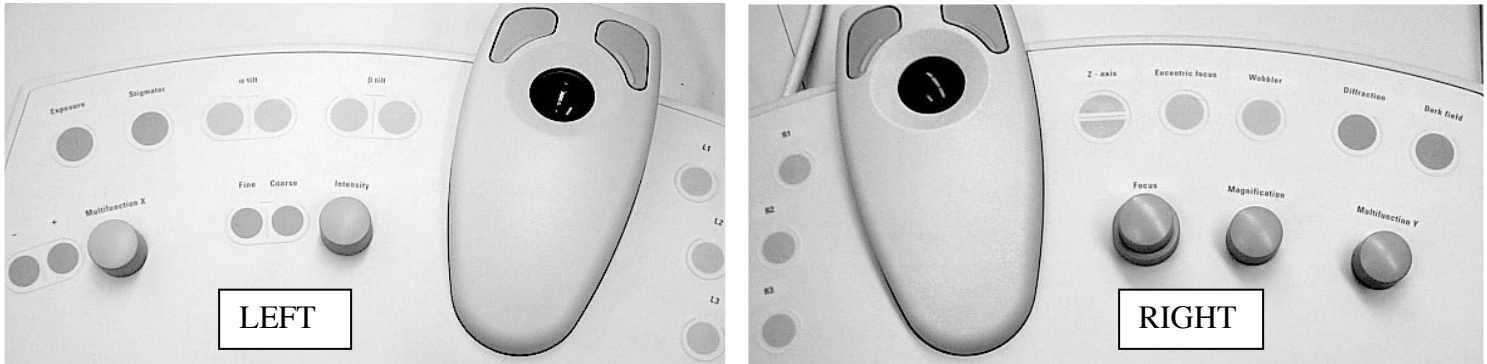
4. Make sure the High Tension is set to 60 KV. The HT is always on (button is yellow). Do not turn off the HT or change the 60 KV setting.

5. Check that the spot size (under Beam settings window) is set to 2. You can use spot size 1 to get a brighter beam. The default beam setting is Microprobe (yellow). Intensity Limit, Intensity zoom, Reset Defocus buttons function as on the CM10.



Working with control pads

1. The left control pad centers the beam (trackball) and controls the beam intensity.



2. The right control pad moves the specimen (trackball); focuses the image (inner knob); selects the focus step (outer knob); and magnification.
3. The right hand trackball can move the specimen smoothly on its own or through direct tracking. Press the two upper buttons together simultaneously (chord) to toggle this function on or off. To stop automatic specimen movement, press either the right or left button. The trackball dictates direction and speed of tracking. Direct tracking speed is increased or decreased by pressing the right or left button.
4. The small function buttons L 1, 2, 3, and R 1, 2, 3, are customizable- see main window-bottom left. Right click the mouse button to see your options. Do not repeat functions. Toggle “Screen dimmer” button to cut out monitor glare. Ignore the screen indication of shift+esc. To restore the window, press R2 again. Use “alpha wobbler” for eucentric height adjustment.
5. The remaining buttons are labeled with their functions. Right: Exposure, Stigmator, a tilt, b tilt, Multifunction x. Left: Z axis, Eucentric focus, Wobbler, Diffraction, Darkfield, Multifunction Y. You would not normally need to use most of these buttons except for Z-axis during eucentric height adjustments.
6. You can use the Control pad window to activate the backlit buttons and dim out the emergency buttons. Remember to reset the dimmer and turn off the backlights when you are through for the day. The control pad lights may burn out if you forget to turn them off.

Similarity to CM10 EM

There are only a few features that are similar on the Tecnai to the CM10. One is the condenser and objective aperture insertion into the column. The handle faces left when the apertures are inserted. Align the apertures using the inner knob and side knob on the column. Select them using the inner knob. Normally, these are never adjusted- as they can easily be knocked out of alignment.

The default condenser aperture sizes are: 1) 30 um, 2) 50um, 3) 100um, and 4) 200um.

The default objective aperture sizes for the BioTwin are: 1) 20um, 2) 40um, 3) 70um, and 4) 100um.

Remember: the combination of aperture sizes selected can alter the contrast of the final image obtained. We use a combination of a 200um condenser aperture and 40-100um objective aperture on the Tecnai.



Condenser and Objective Aperture handles face left



The eyepieces, small screen, and arrow pointer are exactly as they are on the CM10. Twist and insert the pointer into the column and focus each eyepiece using the small screen. Flip the small screen into view using the pull handle on the left. The main screen does not lift up as on the CM10.

Small screen handle

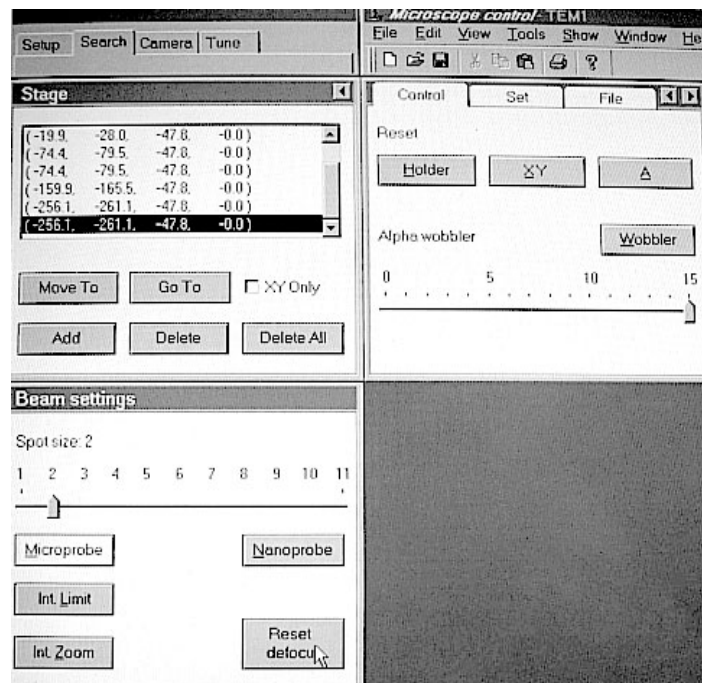
Align the specimen to eucentric focus

Select a sample area to be photographed. Pull out the stage control page (see picture below). Click the alpha wobbler button. The specimen will move from side to side. Use the Z axis upper and lower buttons to minimize the specimen movement. (The Z axis controls are on the right control pad). You can monitor the z axis readout at the bottom right screen. Eucentric focus is obtained when the object in the center does not move outside the large circle on the phosphor screen at medium magnifications.

Remember to set eucentric height at the beginning of your session if you plan to store coordinates. When you move to a different part of the grid the eucentric adjustment shouldn't change much. You can set eucentric height manually in the set page and select just the x and y coordinates in the stage controls to move to new stored coordinates.

You may use the set page to specify the correct z axis. Type in the coordinate and press go to instead of using the z axis buttons on the right control pad.

Store and recall coordinates

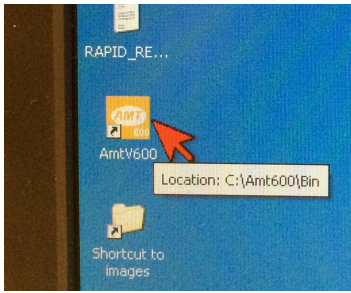


The Stage page (under Search tab) contains a coordinate storage and recall system similar in function to the CM10's XY control. There is an "add" button that adds a coordinate to the list. There are "move to" (resets to 0,0 first) and "go to" (moves directly) buttons to recall the coordinate that is highlighted. Use the pull out arrow to access more functions. You can save the coordinate list as a file for future reference (File page).

In the Control Page the Reset buttons reset (zeros) the holder (XYZ), XY and A (alpha tilt) when pressed. The wobbler button is used to adjust eucentric height. The angle of tilt is indicated on the 15 degree scale.

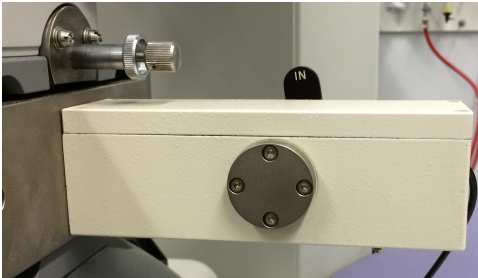
The "Set" page allows the user to specify coordinates. Type in a number and press "Go to".

Enable page controls the stage goniometer. If the specimen is stuck or the red indicator light stays on, try resetting the goniometer enable checkbox to reset the stage. This is a good place to troubleshoot locked control pads, too.

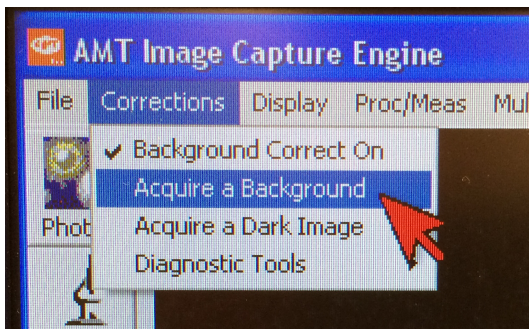


Using the AMT camera (See AMT Camera Manual for details)

1. Turn on the AMT monitor using the power button on the surge protector. Double click the AMTV600 software icon on the AMT computer desktop.
2. Toggle the camera sensor into the path of the electron beam. The black IN bar tells you the CCD is in place. Flip the toggle switch that pushes the **digital camera IN** -(handle turns up).

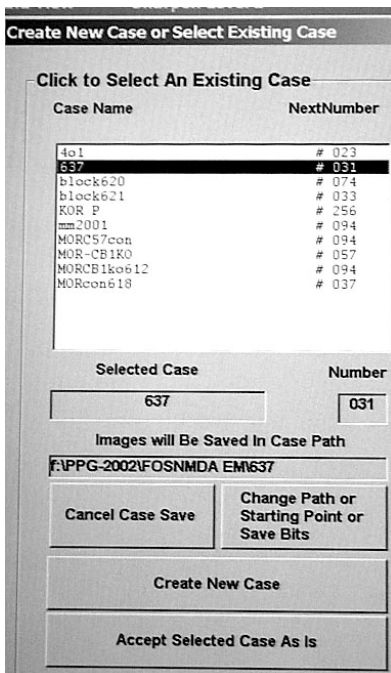


3. Remember to store a blank area to check the background image. Move to an area that is free of thin sections before you collect a background.



4. Users can assume the “**background correct on**” is saved in the software memory and will be good for capturing subsequent images. This means that the CCD camera chip’s faults and imperfections are subtracted from your final image. If you wish to collect a new background, click Acquire a Background, set the intensity so the histogram is centered and follow the instructions on screen. Make sure the room is dark and no extraneous light enters the microscope during the capture. Background Correct is stored when you are finished.

5. Images will have a direct magnification and calibration bar on the print. Any additional information is available by checking the show box on the capture window.

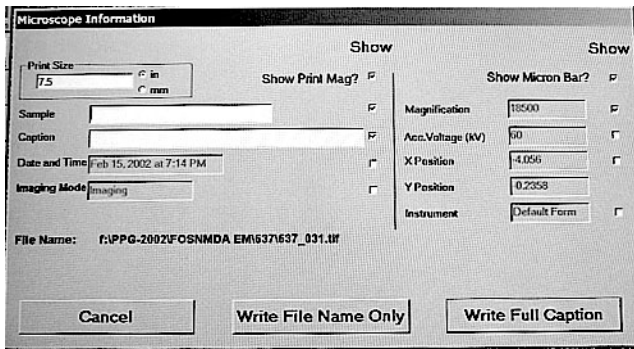


Using Cases

1. Open the Menu File- Open/Close Case- **Open Case**. The window allows you to choose an existing case or **set up a new case** in a folder.
2. You must open File- Open/Close case- **Open case** and highlight the case you just created. Choose “**Select case as is**” to begin storing numerically sequenced images to the selected folder.
3. Remember to **focus the image**. Focus your image with the histogram centered, beam centered, even intensity, no image movement.
4. Press “**Click for Live Image**” to view the EM image on the screen. Frame your shot, focus, adjust the intensity of the beam to **position the histogram** to right of center and “**Click for Final Image**” to capture the image.

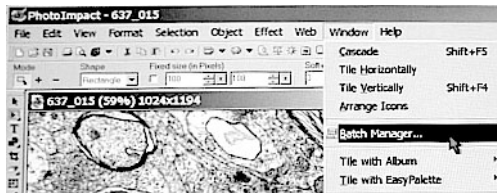
5. Click on the case icon to annotate the image. Note: What you type appears as a caption on the image. It is not the file name. A magnification bar will be printed on the image. You can also set the size of the working print (7.5 inches wide recommended). Select Write full caption.



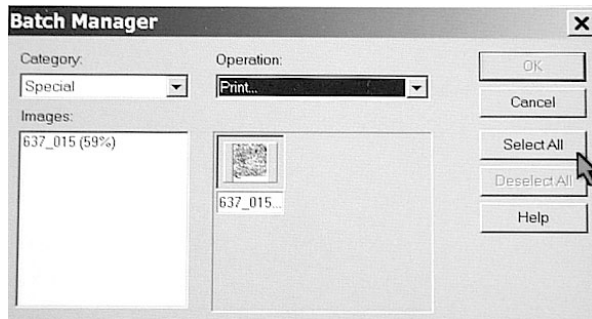


6. **Optional:** Batch print a folder of AMT images using batch print function in PhotoImpact. Open the PhotoImpact program. Open all the images by selecting the folder, highlight the first image and hold the shift key down while selecting the last image. Wait for the images to open on the screen.

Click Window\Batch manager to access the batch print functions. Click Select all. Category: File; Operation: Print; Click OK. Choose printer properties, Select Custom EM settings: (720 dpi, microweave on, high speed on).



7. Choose Operation: Close; Select all to close all the files when you finish printing the folder.

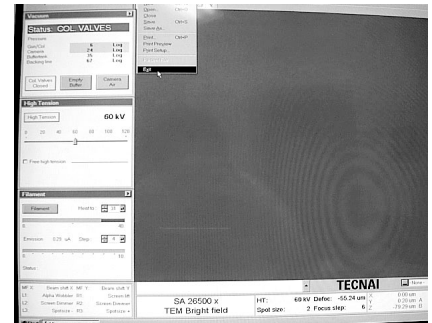


8. Disconnect external drives by using the remove/disconnect icon at the lower right of the windows desktop. Exit PhotoImpact software and AMT camera software.

9. Use the toggle switch to remove the AMT camera out of the column.

Finishing up:

1. Click filament to turn off the filament.
2. Close column valves.
3. Leave the High Tension ON! (Yellow box)
4. Remove the specimen from the column by pulling the arm out halfway, then turn the arrow clockwise towards “1” and pulling the specimen arm completely out of the column. This will close column valves if you didn’t do so earlier. Place the specimen arm back in the plexiglass holder. Remove your grid.
5. Exit the Tecnai microscope controls using File Exit or close the microscope control window. Save your settings unless something drastic happened during your session that you don’t want to keep.
6. Go to the windows start menu and select Shut down. Select the Log on as a different user option.
7. Shut down AMT and PhotoImpact programs.



Tecnai options:

We have an optional rotation specimen arm that is capable of turning the specimen on a flat plane. Please read the manual for specific cautions on using the rotation holder.

AMT computer management:

We strongly suggest that you store your images on external drives and that you archive your images to CD ROM or other formats for future use. Images left on the computer hard drives will be deleted as necessary.

Case names are stored in a file named AMTcase at the root level of the C: drive. Always backup the AMTcase file before you edit case names. Please be careful and delete the entire line. Save the new case file as AMTcase.

Please log all error messages and problems with the system in the Tecnai log book. This will document problems and aid in repair and troubleshooting recurring problems.

Comparison of microscope magnification ranges available to users:

CM10 screen mag range				Tecnai screen mag range		
LM stereo	LM zoom	stereo	zoom	LM	MAG	SA
18	20	620	620	19.5	310	1050
22	25	800	800	24	610	1250
27	30	940	1100	27.5	790	1700
35	40	1100	1450	35		2550
47	53	1250	1950	44		4200
58	65	1650	2600	53		6000
75	85	2400	3400	71		8200
94	105	3400	4600	89		9900
115	130	4400	5800	100		11500
150	170	6200	7900	130		16500
190	215	8700	10500	170		20500
230	260	12500	13500	205		26500
310	350	16000	19000	330		43000
380	430	24500	25000	410		60000
460	520	31000	34000	490		87000
530	600	41000	46000			105kx
		64000	64000			135kx
		94000	92000			165kx
		130kx	130kx			220kx
		180kx	180kx			300kx
		245kx	245kx			
		340kx	340kx			
		450kx	450kx			

SA=selected aperture