Quanta 250 ESEM Quick Guide

A. LOGGING IN

1. Sign in on the paper log.

2. Turn on both monitors.

3. Press the **Computer** button on the left of the **Magic Switch box** A1 to access PC #2 (left monitor).

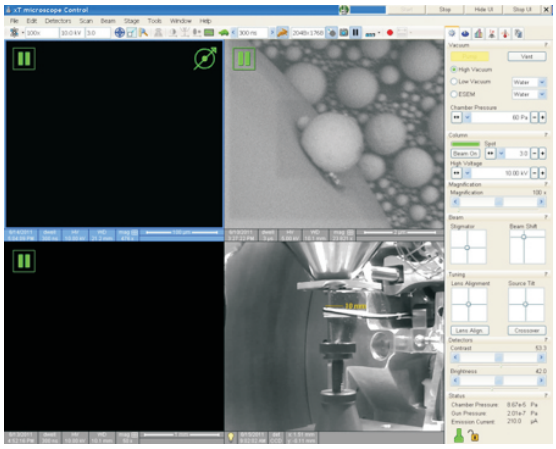
4. Log into the PC using your VUnet ID and password.

5. Open the "User Data" folder on the desktop and create a folder for your imaging session.

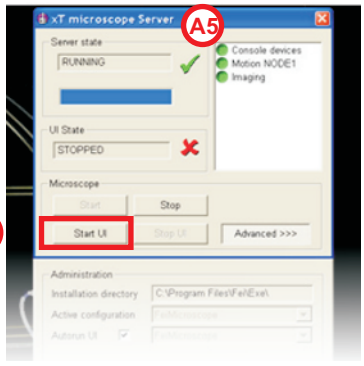
6. Change the Magic Switch to the MPC #1 (right monitor.) If necessary, log in to the MPC as "user", password "user".

7. If the xT Microscope Server software is running minimized at upper right of desktop A2, double click on the blue bar to fully open the server window.

If the xT software is **not** running, double click on the xT Microscope Server icon on the desktop to open the window. Click "Start" and wait until the blue progress bar is full and the server state is "Running".



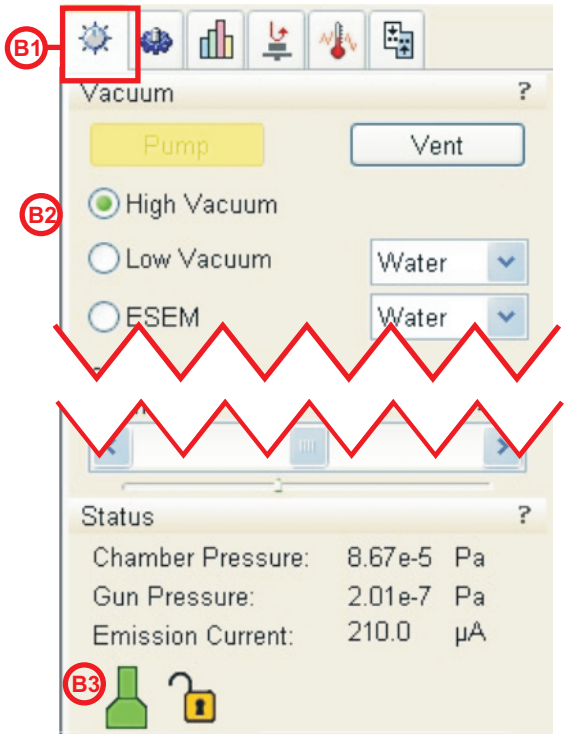
8. If "Quad View" A3 is not running, click on "Start UI" A4 (User Interface.) If the Quad View window is visible, minimize the xT Microscope Server by double clicking on the blue title bar A5 at the top of the window.



9. Log in to the User Interface using the (user/user) login.

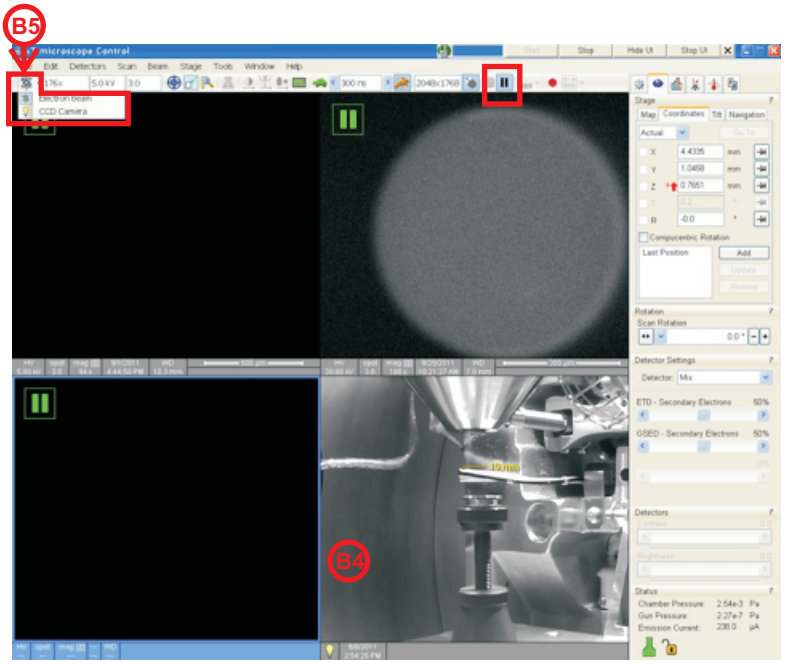
B. SEM STARTUP AND SAMPLE INSERTION - HIGH VACUUM MODE

1. On the **Beam Control** B1 tab, make sure High Vacuum B2 is selected.



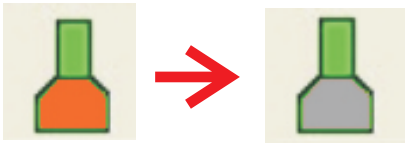
2. Check vacuum status on the Beam Control tab, using the **vacuum icon** B3 at the bottom left of the tab. If the entire icon is not green, call EM Staff – **Do Not Proceed!**

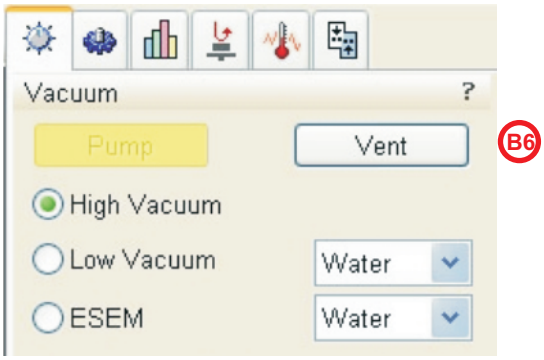
3. Click on the lower right quadrant B4 of the Quad View window to select it, then click the Pause/Unpause button on the top toolbar for a live image of the inside of the chamber. If the lower right quadrant is still not showing a live image, click on the window, then set the detector to "CCD" for that window using the button B5 on the top toolbar. If you are unable to get an image of the chamber, call EM Staff – **Do Not Proceed!**



Windows with a ⏸ icon are paused. Windows without that icon are live.

4. Click **Vent** B6 at the top of the Beam Control tab. The chamber will vent with nitrogen gas for two minutes. During this time note that the vacuum icon remains green at the top (FEG column) but is orange in the chamber area at the bottom. When venting is complete the chamber area turns dark gray.

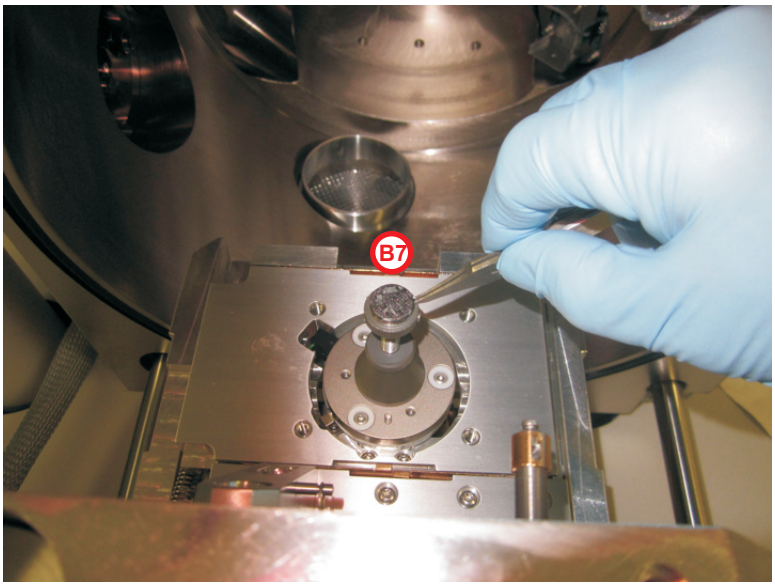




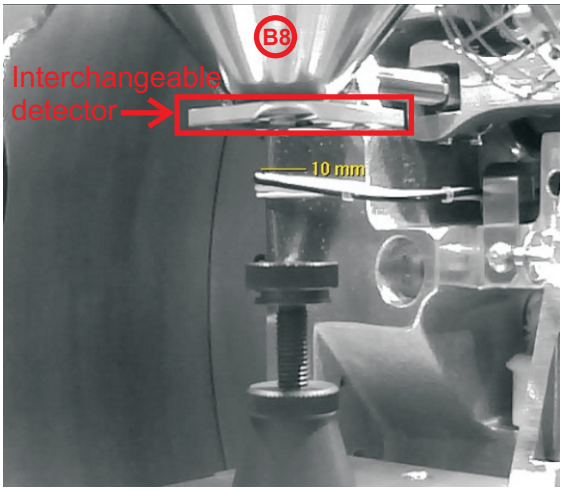
5. Once venting is complete, watch the chamber view on the monitor while GENTLY opening the chamber door using **BOTH HANDS**. Ease the door to a stop at the full open position.

PUT ON GLOVES.

Always wear gloves while touching anything inside the chamber. Do not introduce a sample into the chamber that has been handled without clean gloves. Introduction of finger oils into the specimen chamber will damage the ability of the SEM to control and maintain vacuum.

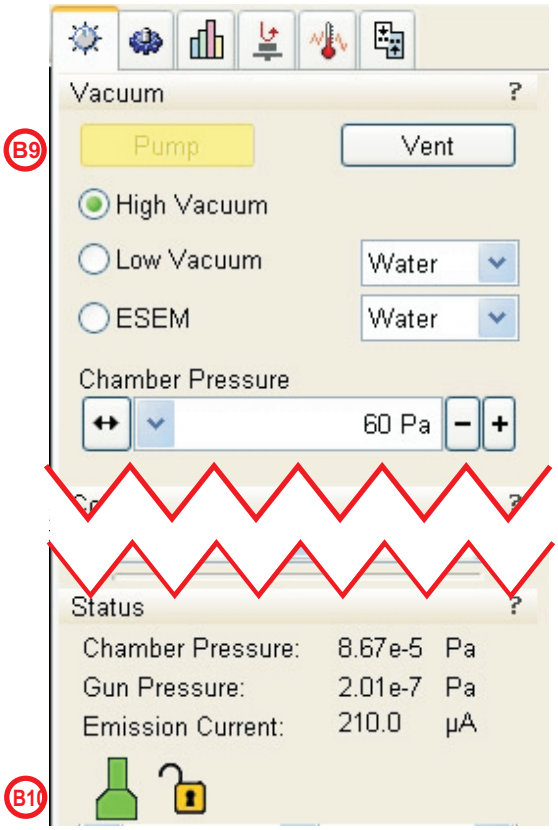


6. While wearing gloves (provided), insert the sample stub on the specimen holder (B7) using curved forceps, and tighten the set screw with the small Allen wrench. **Do not over-tighten.**



7. Watching the live chamber view on the monitor, gently slide the chamber door closed while visually ensuring there is no chance of sample contact with the bottom of the pole piece (B8) of the microscope.

8. Hold the chamber door in the closed position with one hand. Using the other hand, click on the “Pump” (B9) button and watch that the chamber vacuum begins to form.



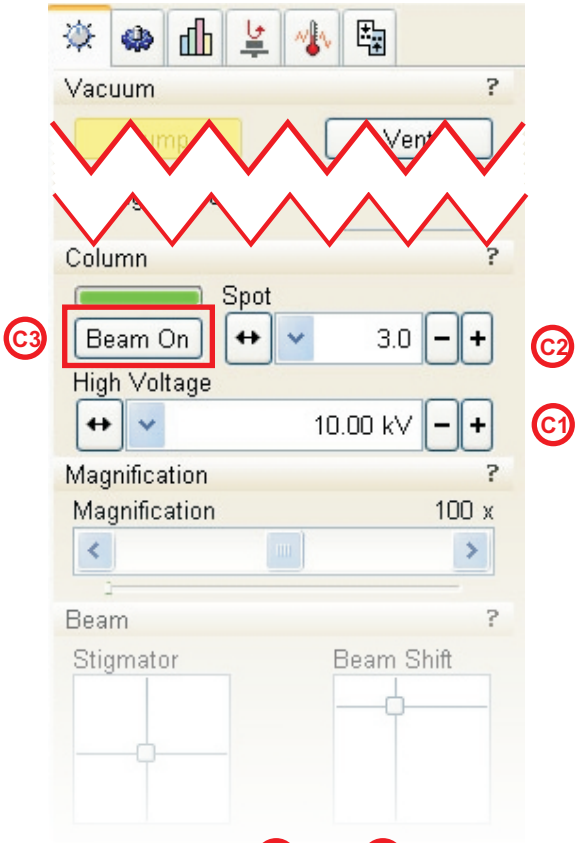
9. Monitor the vacuum icon (B10). W hen the entire icon is green, high vacuum has been reached.

C. SEM OPERATION - HIGH VACUUM MODE

1. On the Beam Control tab, select the desired accelerating voltage (C1) from the drop down menu, or enter a specific voltage value. For sputter coated biological samples, 5 to 10 kV is a good starting range.

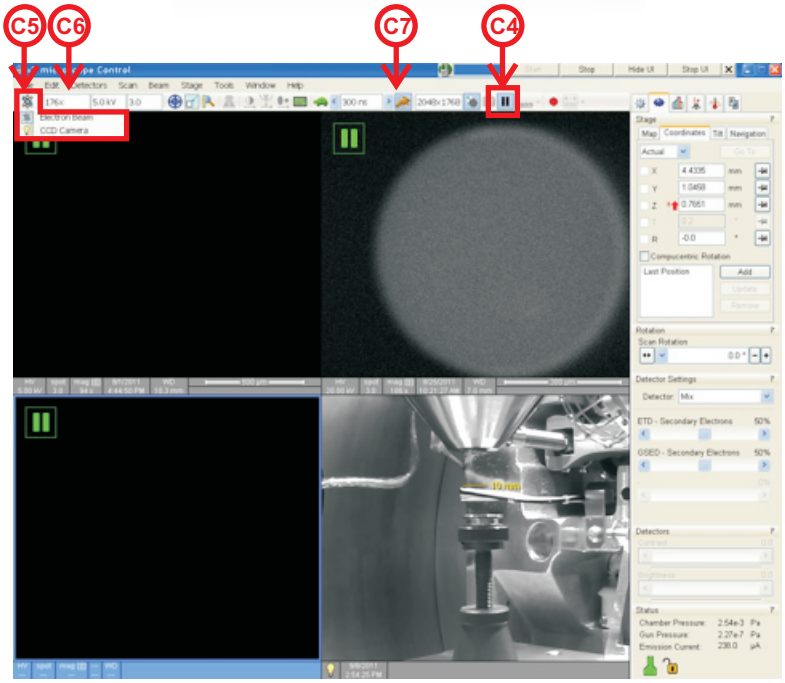
2. On Beam Control tab, select the desired spot size. (C2) Spot size 3.0 is a good value for initial imaging.

3. Click “Beam On” (C3). This initiates electron emission and scanning of the resulting electron beam.



4. Click inside the upper left quadrant, then click the Pause/Unpause button (C4) on the top toolbar to get a live image. Ensure that this window is set to “electron beam” (C5) on the top toolbar.

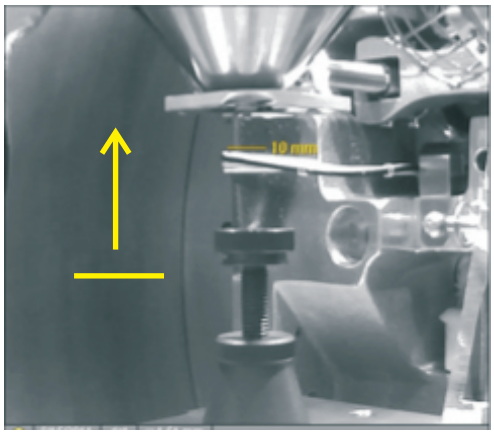
5. Adjust to a low magnification setting: 50x--100x (C6). Adjust scan speed (”dwell time”) (C7) to obtain a fast scan. Obtain rough focus and get oriented on the sample surface.



D. FOCUSING AND Z-LINK PROCEDURE

1. The sample and working distance need to be linked before imaging may proceed.

2. With a live image in the chamber view window, click and drag using the **center mouse wheel** to raise the sample to about the 10mm working distance marked on the screen.



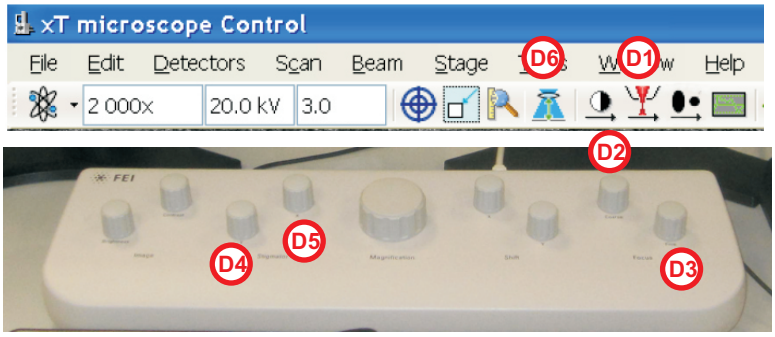
3. Focus on the highest point of the sample surface, ideally at a working distance close to 10mm.

A. Find a feature with sharp edges; do not use an area of special interest to avoid damaging the sample.
B. Use either Auto Focus (D1) on the main tool bar or the Coarse Focus knob (D2) on the Manual User Interface (MUI) to bring the sample into rough focus.
C. Sharpen the focus with the Fine Focus knob (D3) on the MUI. Then adjust the X stigmator (D4), then the Y (D5); repeat, finishing with Fine Focus.

NOTE: The sample MUST NOT touch any detector attached to the microscope pole piece, or any other objects in the chamber. ALWAYS observe the sample by using the live chamber view while moving the stage.

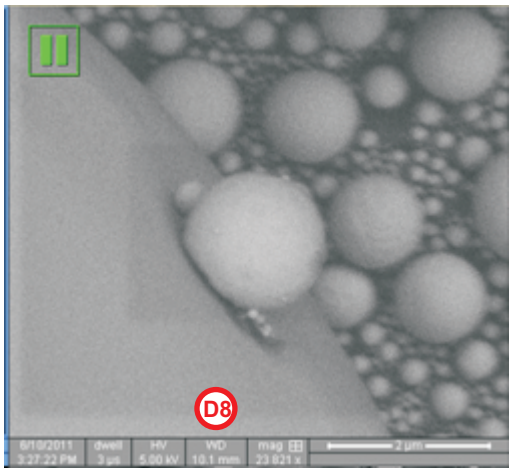
If the image moves as you go through focus, the objective aperture needs adjustment – please call staff for assistance.

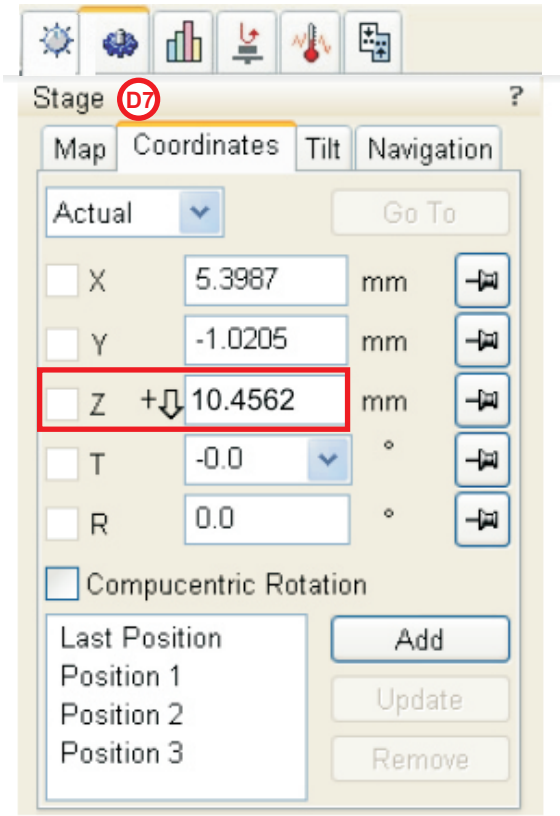
If the image smears with as you go through focus, stigmation is far out of adjustment.



4. When good focus of the highest point of the sample is obtained, click on the Link icon (D6) in the top toolbar, and follow prompts to link the z position to the working distance.

5. Check the Coordinates tab (D7) at the right side of the screen. The sample position in "z" should closely match the working distance of the lens as noted in the bottom of the image window (D8) of the quad view. The z position is now linked.





E. OPTIMIZING THE IMAGE

1. Find an area of interest by using the joystick or the arrow keys. The arrow keys move the sample approximately one frame distance at the displayed magnification which is very useful for scanning across a sample at relatively low magnification.

2. Double-click on a feature to move the stage to center the feature in the field of view.

3. Within the area of interest, select an area with detail. Increase the magnification to 10x or higher than that desired for the final image.

4. Focus by adjusting the fine focus alternately with x and y stigmation.

5. When you are satisfied with the focus and stigmation, decrease magnification to the desired amount.

6. Optimize contrast and brightness:

   a. Select a slow scan in an active quad.
   b. Click on **Videoscope** (E1).
   c. Reduce the contrast to zero and adjust the brightness level to the lower dashed line (black).
   d. Increase the contrast so that the signal level just clips the upper dashed line (white).
   e. If necessary, adjust the brightness level once more so that the average signal level is roughly in the middle.
   f. The high and low peaks should just clip the dashed lines.

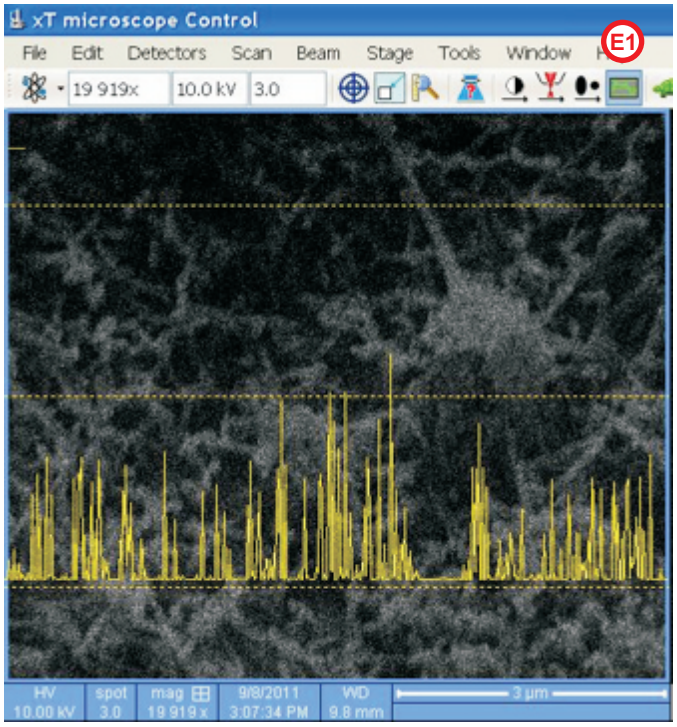
7. To acquire an image, click on the camera icon on the toolbar (E2).

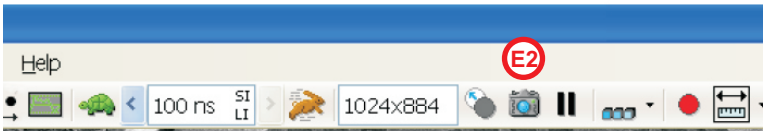
8. When the scan is complete, a window appears, prompting to Save As. Save as an 8 bit TIFF file in your folder in the "User Data" folder on PC #2.

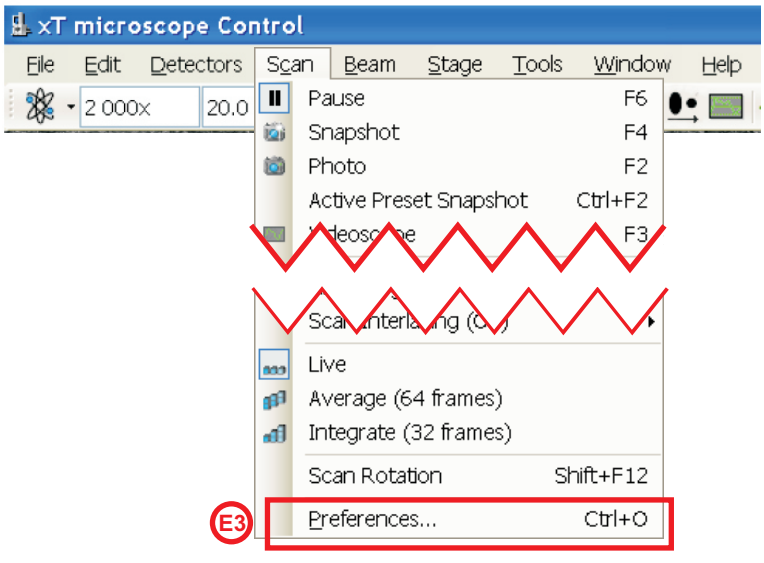
9.To change the Photo-scan parameters: on the Menu bar go to Scan, then Preferences (E3): click on the camera icon at the bottom of the list of scan presets. This will open a list of preset parameters to the right. Edit to change scan speed (dwell time), resolution, line integration or integration.

Remember that focus is always a "three knob" process: fine focus, x stigmation, y stigmation; repeat, and end with fine focus.
For each control, go through focus to establish where smearing or blurring of the image begins on each side of focus: set the control to midpoint.

F5 toggles between Quad View and full screen single frame.







F. SHUT DOWN

1. Adjust to a low magnification setting: 50x - 100x.

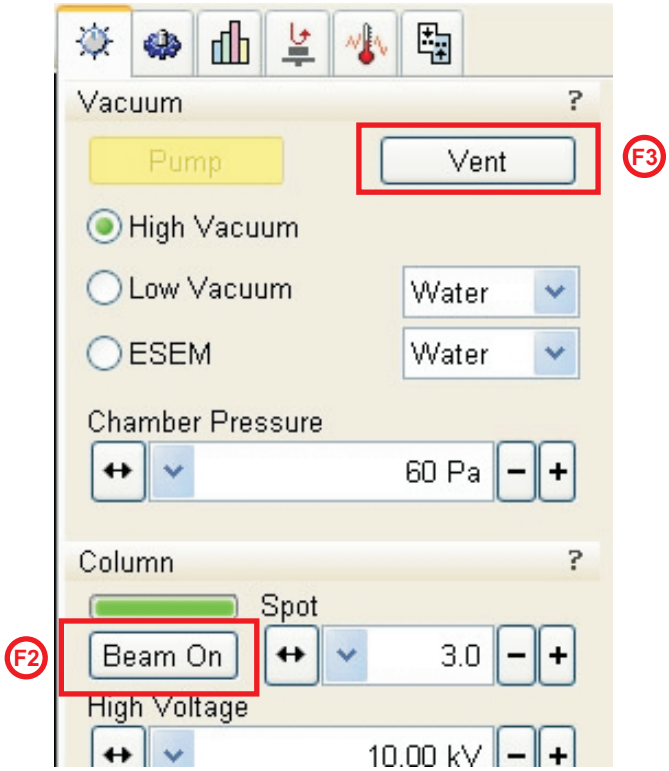
2. Adjust scan speed (“dwell time”) to fast scan. F1

3. Click in the Chamber View window to make it live, then lower the sample to about 15 mm Working Distance.

4.Turn off the electron beam by clicking “Beam On” F2 in the Beam Control tab – the yellow highlight should disappear and the scan should go to black.

5.Pause the scan window.

6. Click on “Vent” F3 in the Beam Control tab.



7. During the 2 minute vent cycle, go to your data in the User Data folder on the PC (#2 monitor). Copy and paste your data to your BlueArc folder.

PUT ON GLOVES.

8. When venting is complete, watch the chamber view on the monitor while GENTLY opening the chamber door using **Both Hands** to control the movement. Open the door to the full open position.

9. Remove the sample.

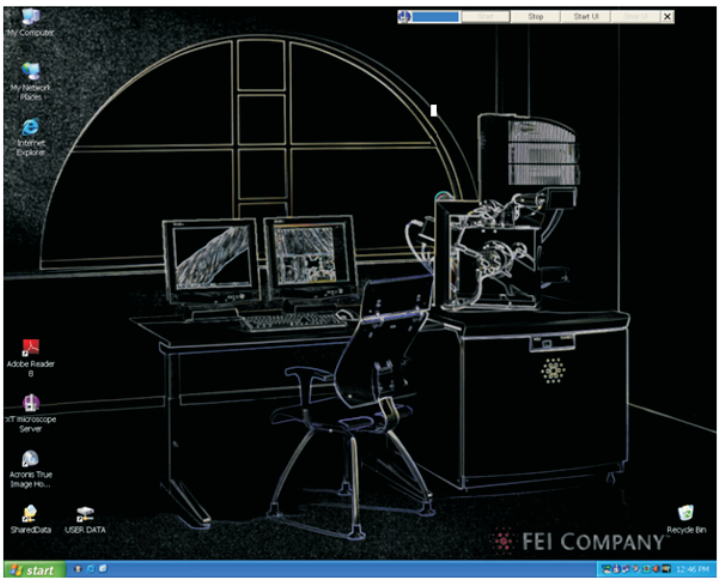
10. Carefully slide the chamber door closed while watching the live chamber view on the monitor to ensure there is no chance of contact with the pole piece or a detector.

Data left in the User Data folder may be deleted at any time without notification.

11. Holding the chamber door in the closed position with one hand, use the other to click the “Pump” button.

12. Monitor the vacuum icon on the Beam Control tab. When the entire icon is green, high vacuum has been reached. If high vacuum is not restored Call EM Staff. The chamber must be left in a high vacuum state.

13. The xT Microscope Server software is running in a minimized state in the upper right of the screen. Click “Stop UI” to close the Quad View user interface. The screen should look like this. ⇨ Do not log off.



14. Switch to the PC (#2 monitor) and log off.

15. Sign out of the paper log sheet. Use “Comments” to note minor issues. Call EM Staff for significant problems or concerns.

USEFUL LINKS

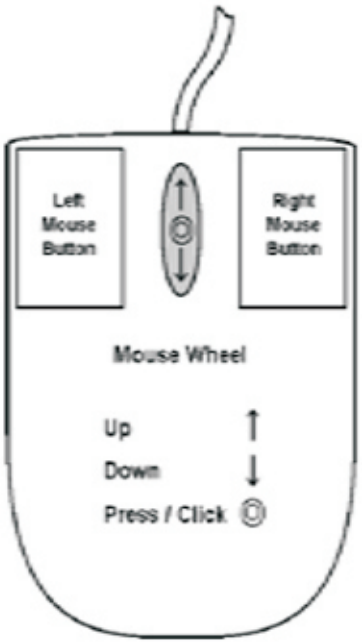
The CISR home page is - https://cisrweb.mc.vanderbilt.edu/CISR/

The equipment scheduling page is - https://cisrweb.mc.vanderbilt.edu/cellimaging/index.php

The CISR staff page is - http://cisrweb.mc.vanderbilt.edu/CISR/site/staff.php

For assistance or equipment problems contact Matthew Stephenson or Mary Dawes at 322-5965 or 343-6691.

The Quanta 250 users manual is located in the User Data folder of PC #2.



USING THE MOUSE

TABLE 4-3 MOUSE BUTTON FUNCTIONS

Key + Button	Function
Click	**Control Areas:** *makes selection* in control areas (single arrow cursor). **On Screen:** press and drag a *selected area* to *zoom in* magnification to fill the imaging area with the selection (selectable in Preferences).
Doubleclick	**Electron imaging:** *moves the selected point* to the middle of the quad. **Optical imaging:** *4 mm marker placement*.
Click + Shift	**On Screen:** press and drag a *selected area* to *zoom out* imaging to fit the selected area.
Shift + click	1. Activates *Beam Shift* (hand cursor). 2. Pauses / Activates all quads when clicking the toolbar pause icon.
Right-click	To *focus* press and move the mouse to the left or right (double ended arrow cursor).
Shift + Right-click	To *correct an imaging astigmatism*, press and move the mouse (four-arrow cursor) to the left / right (X stigmator), or up / down (Y stigmator).
Ctrl + Right-click	Activates *Lens Alignment* (4-arrow cursor with circles).
Shift + Wheel-roll Up / Down	**Fine Control:** rolling the wheel *increases / decreases the magnification*.
Ctrl + Wheel-roll Up / Down	**Coarse Control:** rolling the wheel *increases / decreases the magnification*.
(Ctrl+) Wheel-press	**Electron / Ion imaging:** wheel-press to activate the *TRACK mode* for joystick-like movement over the sample surface. **Optical imaging:** wheel-press to activate the *stage Z - up / down movement (stage Tilt - left / right movement)*, which can be seen live.