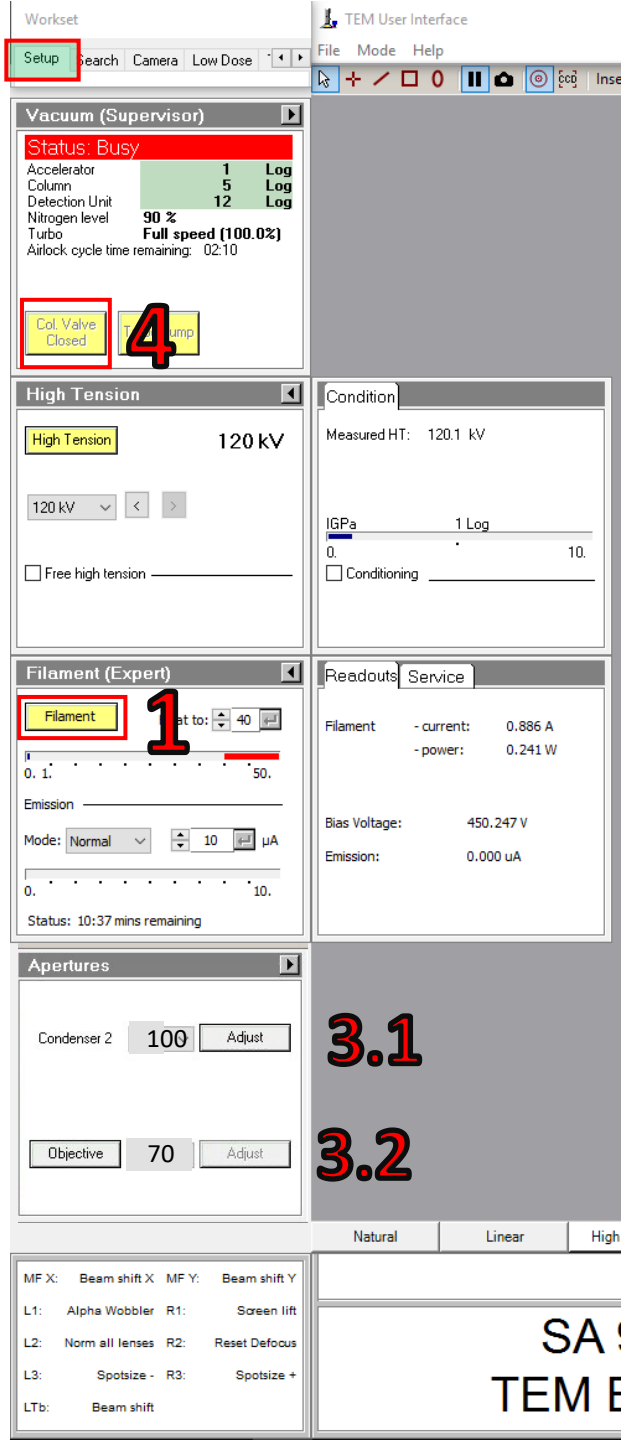
TIA Protocol
Fred Hutch
Talos

Start Up

Start on 'Setup' tab

1. Click 'Filament' to turn on filament
    1. Will turn yellow and take ~13min
2. Insert specimen
    1. Staff will do unless you have been trained
3. Set apertures
    1. C2 = 100 um (dropdown)
    2. Obj = 70 um (dropdown)
4. Click "Col. Valve Closed" to open valves
    1. Will turn grey



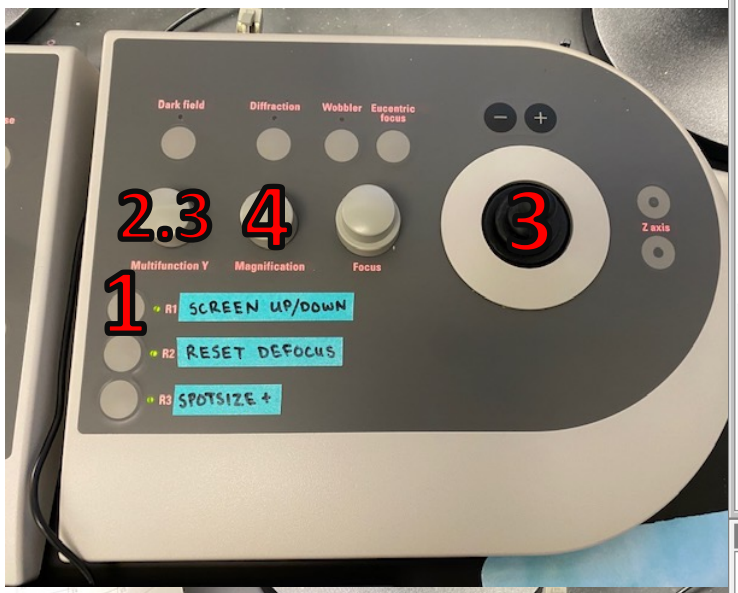
Find Beam

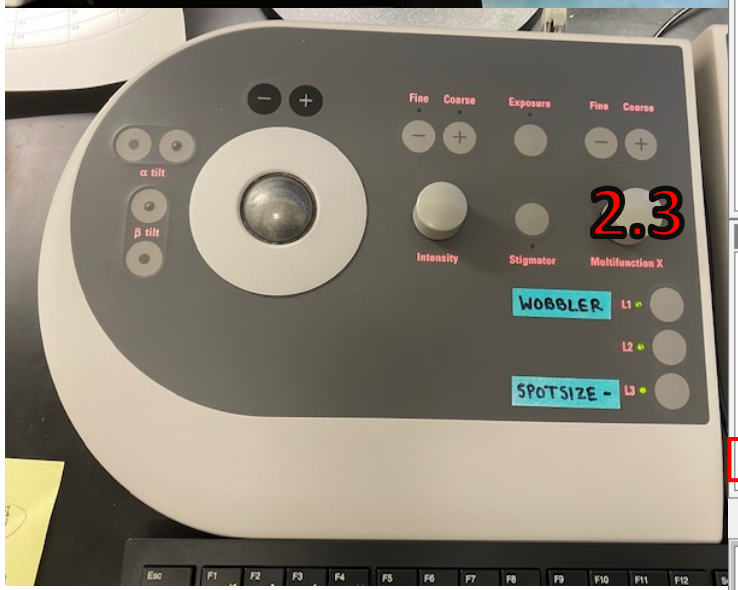
1. Insert Flu screen (R1, handpanel)

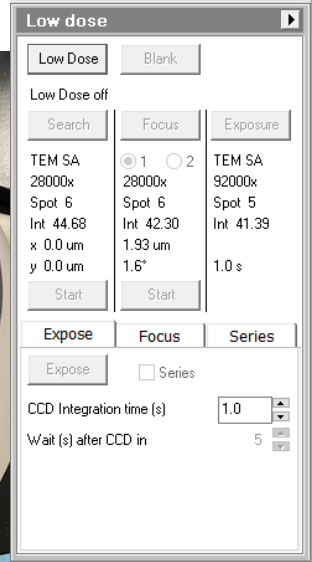
2. Center beam
   1. Click ‘Low Dose’ tab
   2. Click Beam Shift in Direct Alignments
   3. Multifunction X/Y (handpanel) to center
   4. Click Done

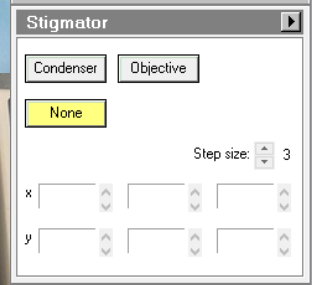
3. Move stage away from ROI (joystick, handpanel)

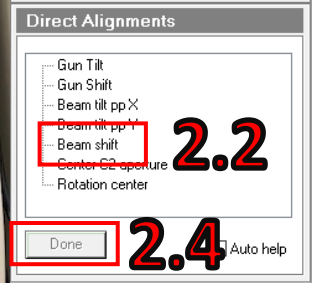
4. Go to magnification 11000
   Counterclockwise = lower
   Clockwise = higher

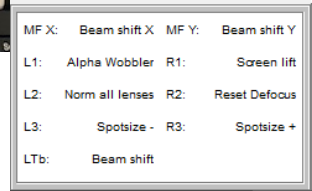






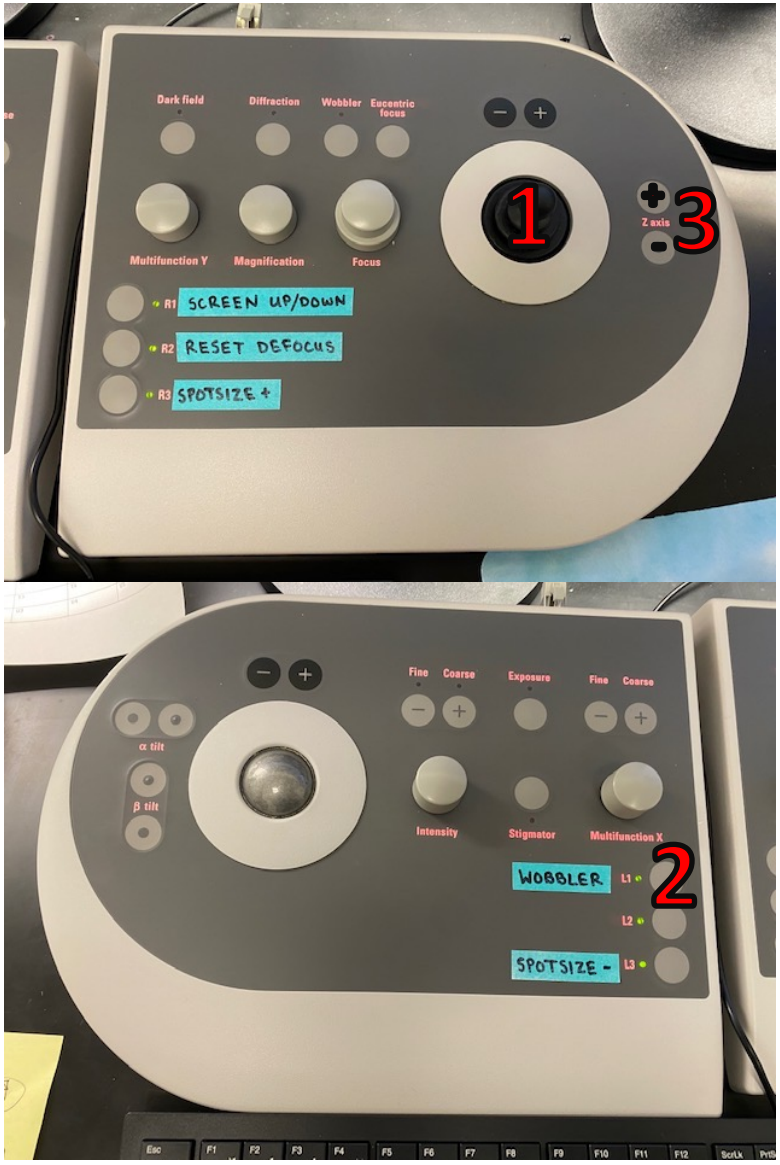






Set Eucentric (Z) Height

1. Move stage to center a feature (joystick, handpanel)
2. Click Wobbler (L1, handpanel)
3. Adjust Z (+/-) until feature does not move side to side
    1. Mesh usually ~0
    2. Slot usually ~80



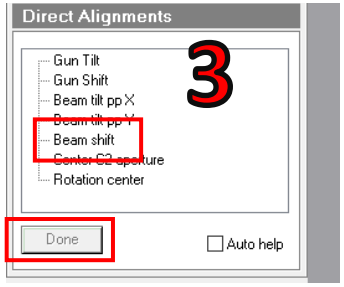
Find ROI

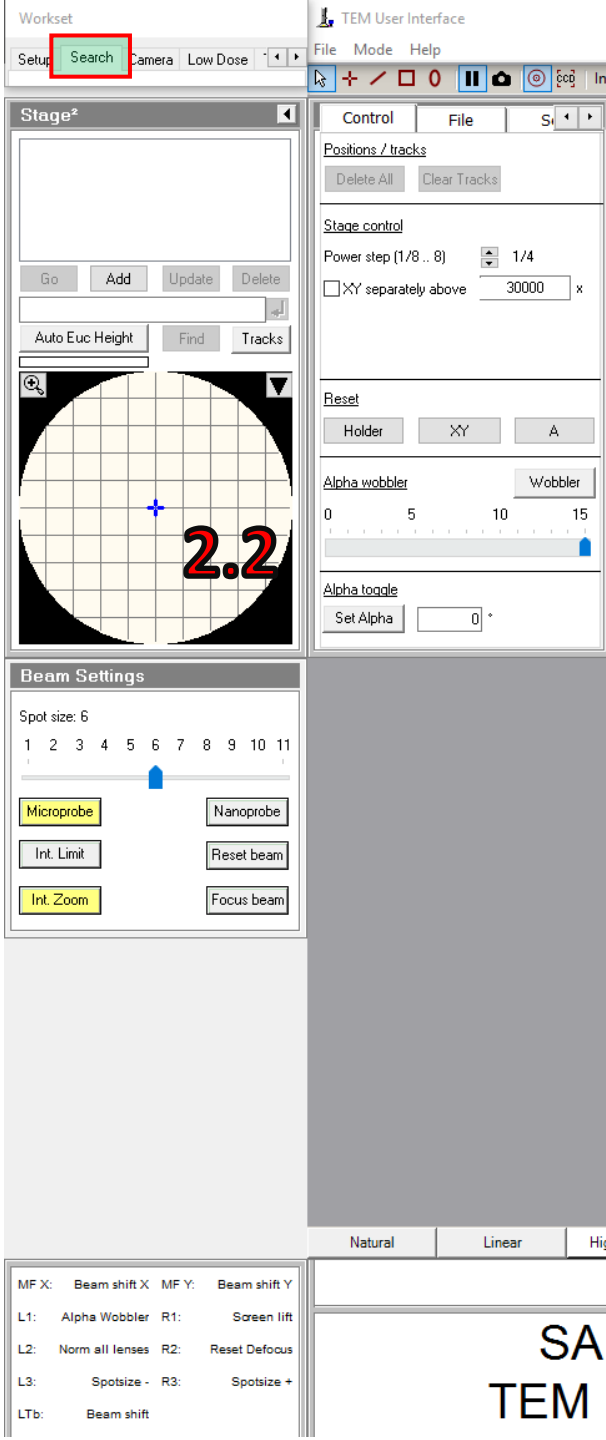
1. Zoom to desired magnification
   1. Use magnification knob
   2. If you move from LM to SA press L2 (handpanel) to normalize lenses

2. Move stage
   1. Joystick
   2. Can track

   movement in Search tab

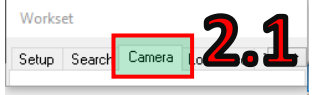
3. Center beam
   1. Same as 'Find Beam' slide

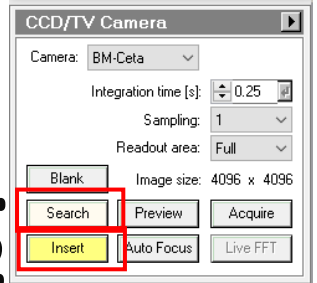


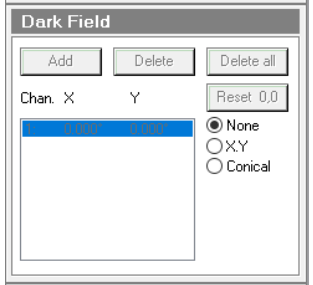


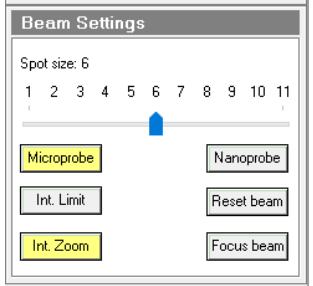
Focus Image

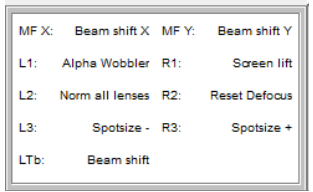
1. Zoom to desired magnification
2. Focus on Camera
    1. Go to Camera tab
    2. Click ‘Insert’ to insert camera (will turn yellow)
    3. Raise Screen (R1, handpanel)
    4. Click Search – Camera tab
    5. Turn on FFT - TIA
    6. Adjust ’Focus’ knob (top, handpanel) to remove Thon rings (true focus)
    7. Reset Defocus (R2, handpanel)
    8. Adjust Focus knob (counterclockwise) and defocus to desired contrast (-2.0 to -4.0 is normal)

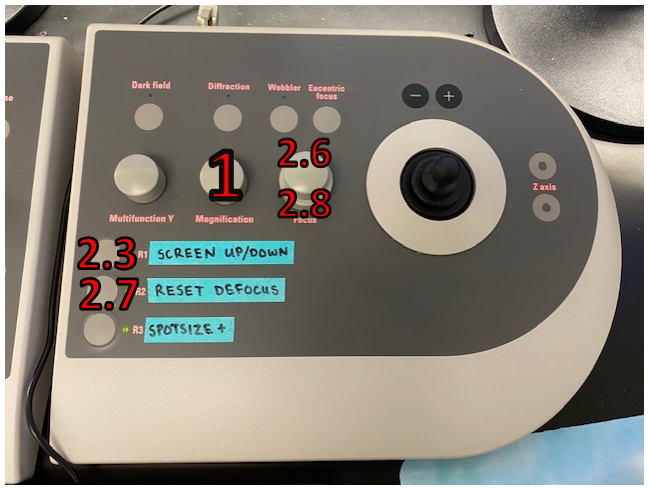


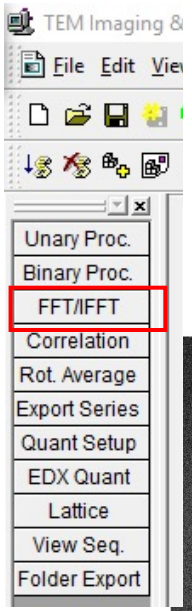


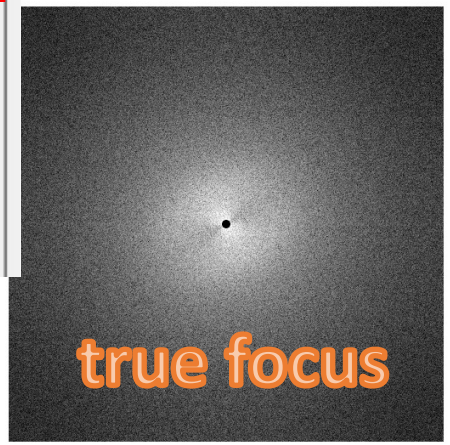






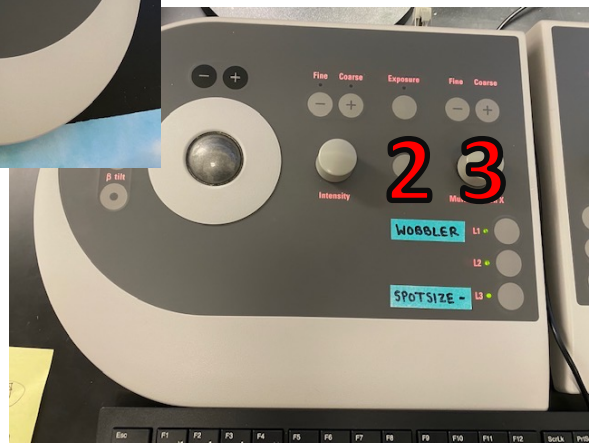






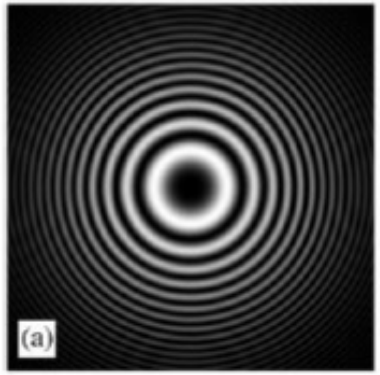
Make sure your beam is not astigmatic

1. Are your Thon rings circles or ovals??

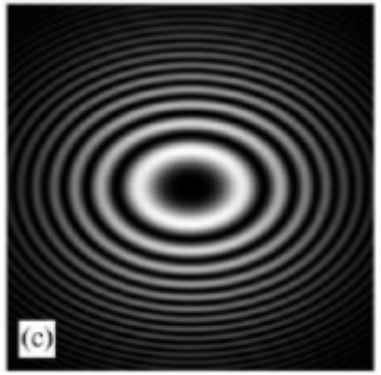


If they are ovals:

1. Turn Focus knob (right hand panel) until defocus is ~0.3um
2. Click Stigmator button (left handpanel)
3. Adjust multi-function X and Y (one at a time) until the Thon rings return to being circular
4. Click Stigmator button again (left handpanel)



good



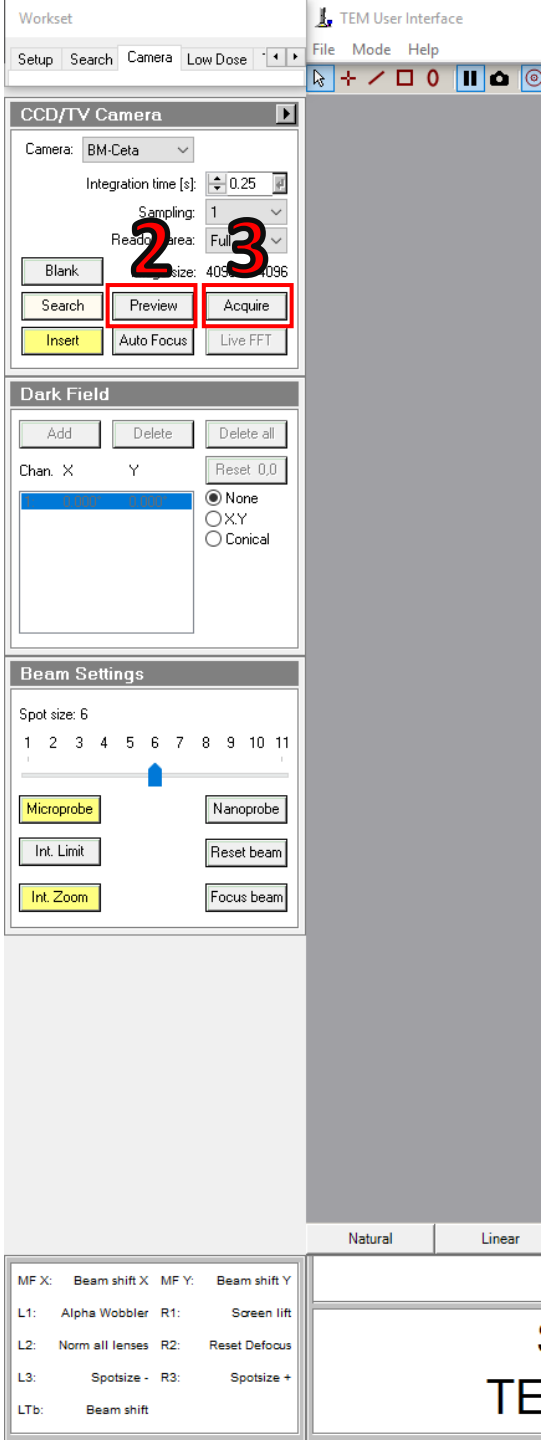
bad

Acquire image

1. Make sure image is a clear defocus

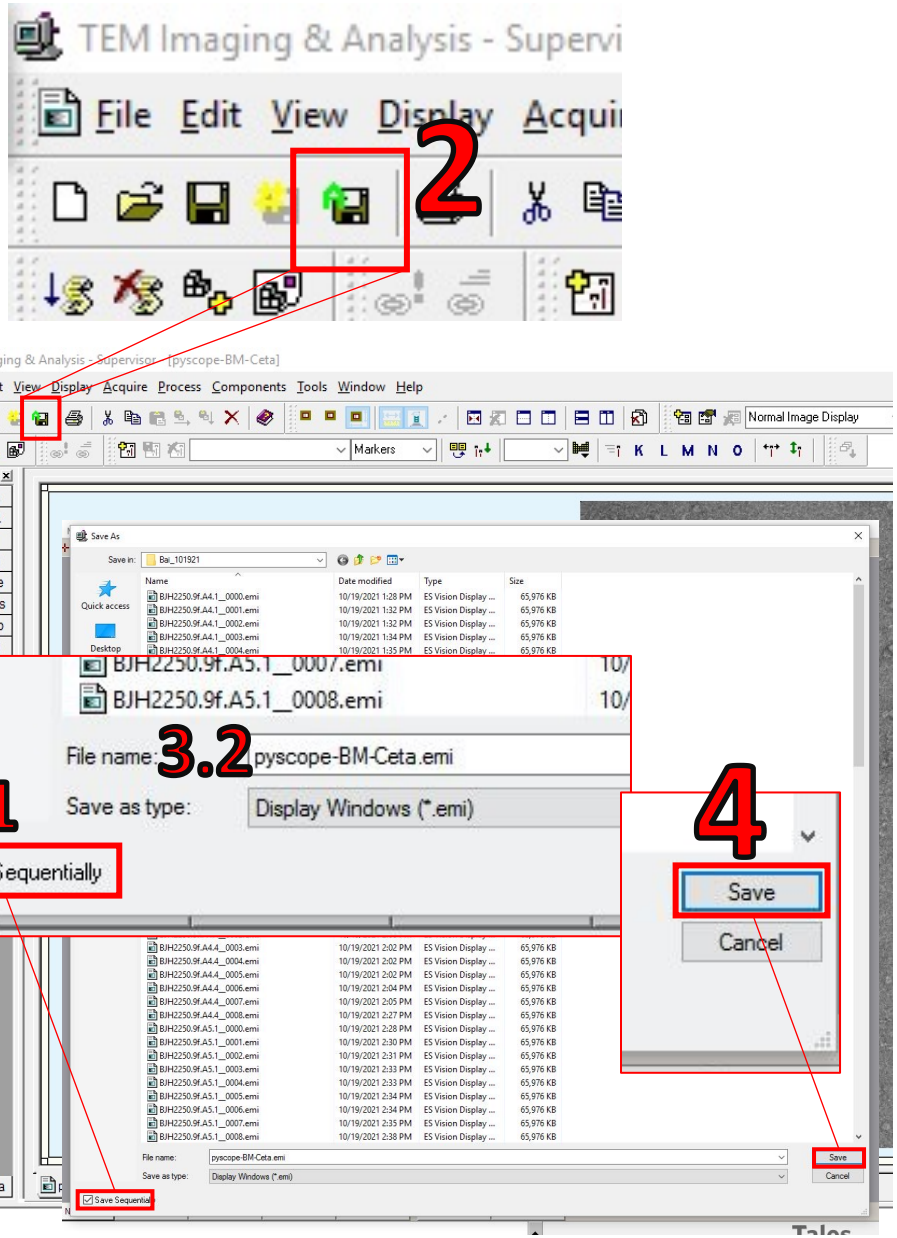
2. Click Preview and refine focus – Camera tab

3. Click Acquire – Camera tab



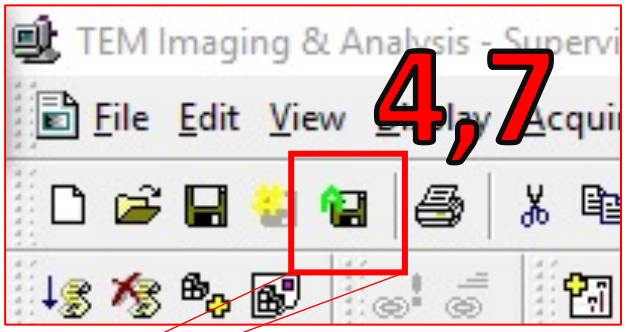
Saving images

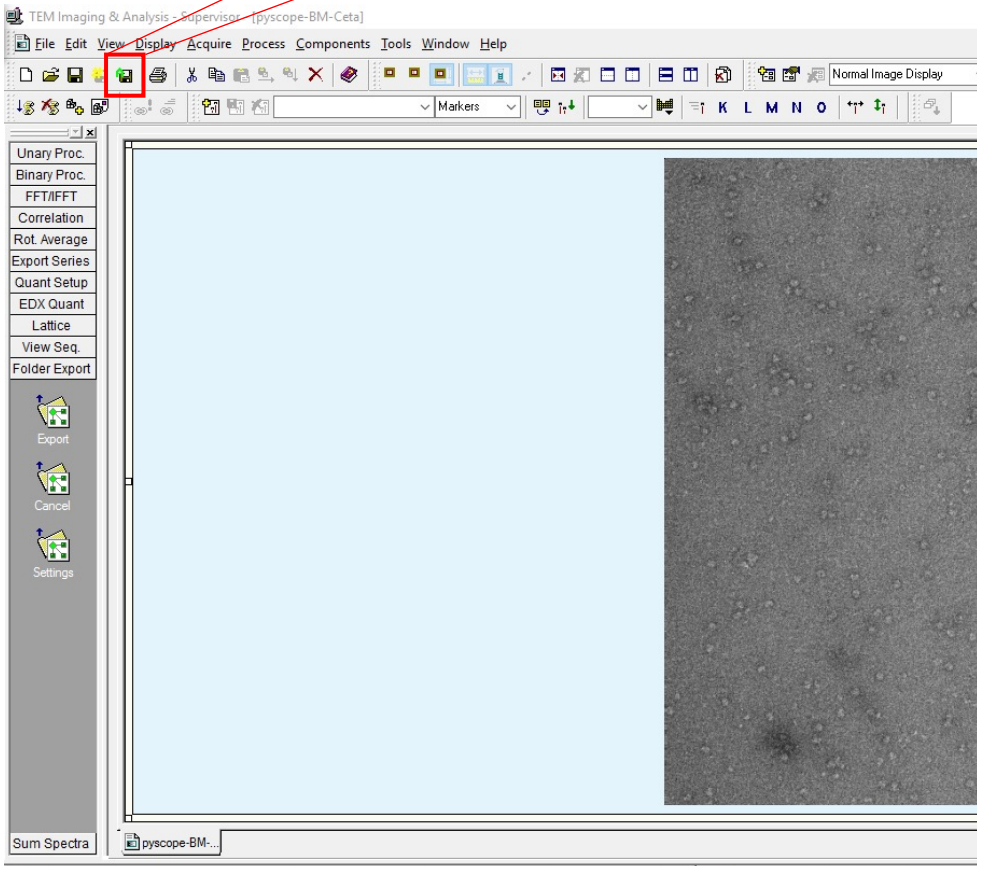
1. Create folder in D:/TIA_data/YYYYMMDD_FHusername
2. Click Autosave - TIA
3. Browse to find the folder you made
    1. Save Sequentially should be checked
    2. Enter file base name: SM_2345_3
4. Click Save – TIA
    1. Double check that the images are saving
    2. Continue moving, focusing, and acquiring until you're finished with this sample



New Sample

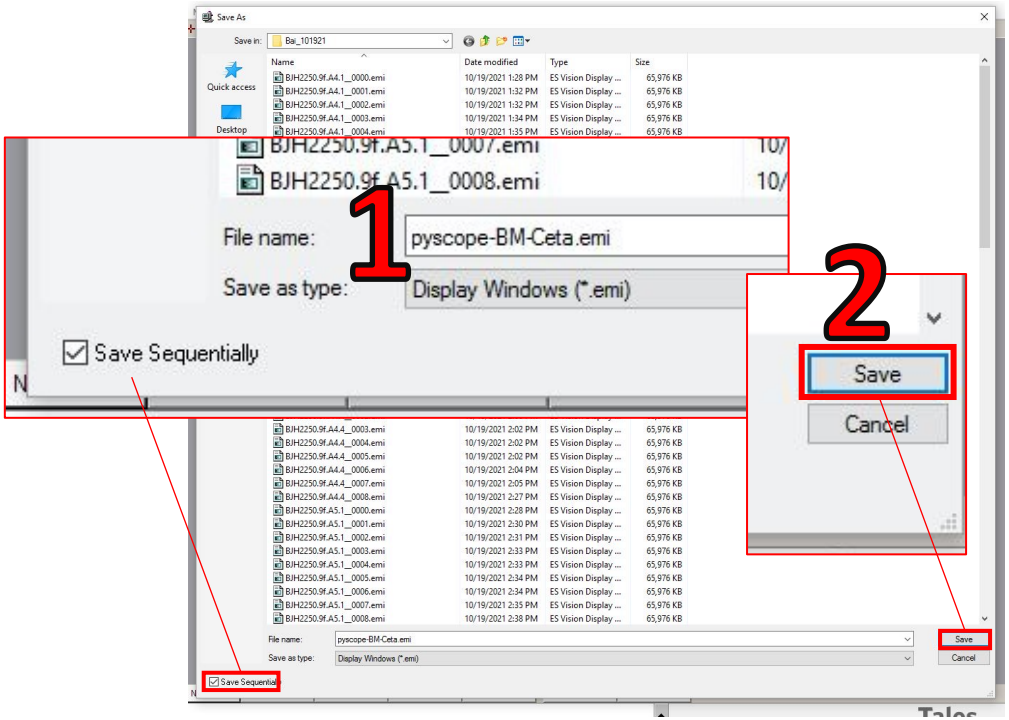
1. Insert new sample (EM staff)
2. Set Eucentric (Z) Height – slide 4
3. Find ROI – slide 5
4. Click “Autosave” so it is unselected
5. Focus image – slide 6
6. Collect image – slide 7
7. Click “Autosave” so it is selected





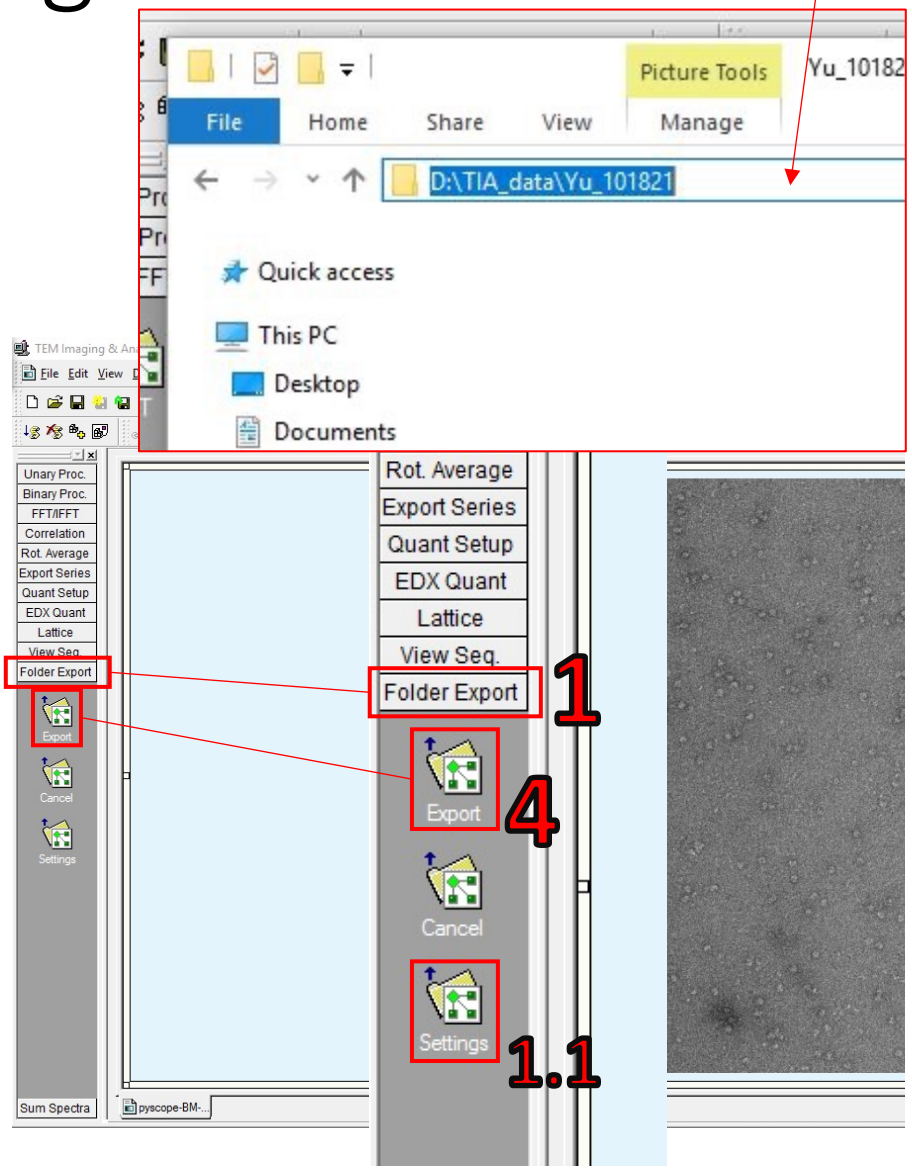
New Sample

1. Change name of the sample CHANGE_####.emi
2. Click “Save”
    1. Double check that the images are saving
    2. Continue acquiring until you’re finished with this sample



Batch convert images

1. Select Folder Export from left sidebar – TIA
   1. Click Settings
2. In File Manager:
   1. Select ”yourFolder” activate and copy the path line from the top left.
   2. Paste into both Source and Target - TIA
3. Select Image Format as “PC tiff with scale marker (full Res)” to include scale bar
   1. Click - OK
4. Click Export



End of Session

1. Camera tab = Click ‘Insert’
    1. Will be grey
2. Search tab = Click ’Holder’
    1. Stage will scroll to 0,0,0
3. Setup tab = Click ‘Col. Valve Closed’
    1. Will be yellow
4. Handpanel = R1 (insert screen)
5. Setup tab = Click ‘Filament’
    1. Will be grey
6. TIA = Click ’Autosave’
7. Remove sample (EM staff)
8. End Kiosk session and logout of iLab