

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

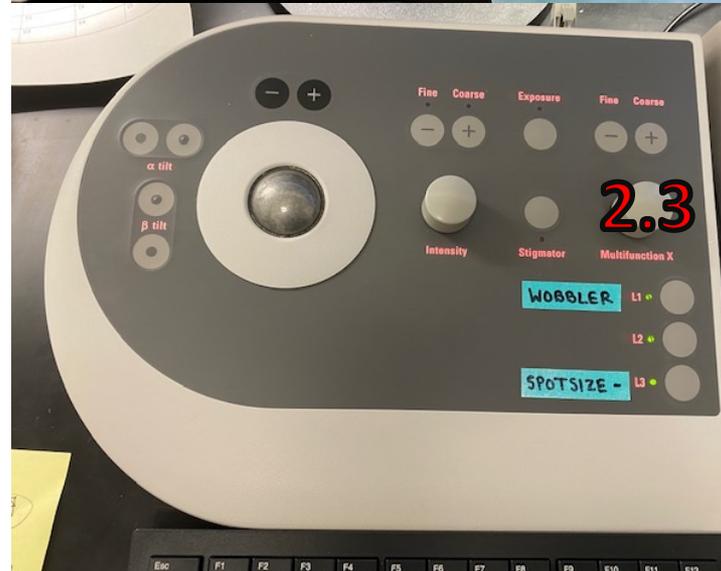
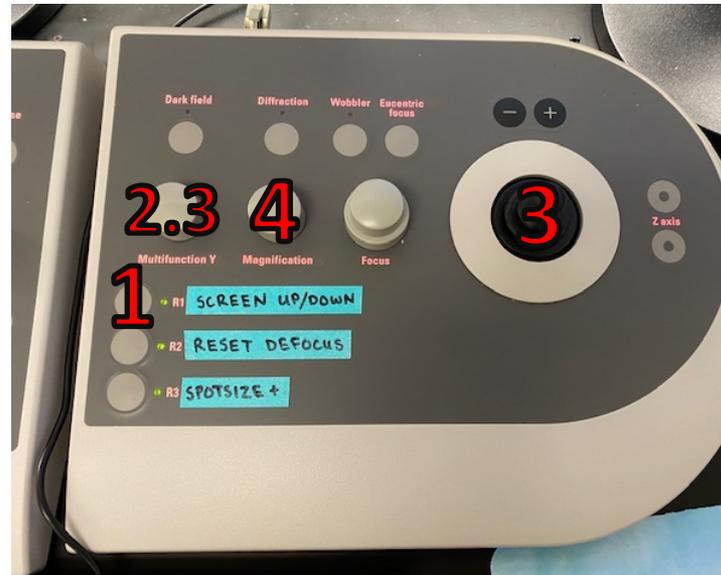
The screenshot displays the TEM User Interface with several panels and controls:

- Setup Panel:** A red box highlights the 'Setup' tab. Below it, a 'Col. Valve Closed' button is highlighted with a red box and a large red '4' next to it.
- Vacuum (Supervisor) Panel:** Shows 'Status: Busy' in red. Parameters include Accelerator (1 Log), Column (5 Log), Detection Unit (12 Log), Nitrogen level (90%), Turbo (Full speed (100.0%)), and Airlock cycle time remaining (02:10).
- High Tension Panel:** Shows 'High Tension' set to 120 kV. A 'Free high tension' checkbox is present.
- Filament (Expert) Panel:** A red box highlights the 'Filament' button with a large red '1' next to it. It includes a 'Set to:' field (40), a 'Mode:' dropdown (Normal), and a 'Status: 10:37 mins remaining' indicator.
- Apertures Panel:** Shows 'Condenser 2' set to 100 and 'Objective' set to 70, both with 'Adjust' buttons. Large red numbers '3.1' and '3.2' are overlaid on the right side of this panel.
- Condition Panel:** Shows 'Measured HT: 120.1 kV' and 'IGFa' with a '1 Log' indicator.
- Readouts Service Panel:** Shows 'Filament' current (0.886 A) and power (0.241 W), 'Bias Voltage: 450,247 V', and 'Emission: 0.000 uA'.
- Bottom Panels:** Includes 'Apertures' (Natural, Linear, High), 'Beam shift X', 'Beam shift Y', and 'Screen lift' controls.

SA
TEM E

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



Works: 2.1 Low Dose

Setup

Low dose

Low Dose off

Search Focus Exposure

TEM SA 28000x Spot 6 Int 44.68 x 0.0 um y 0.0 um

TEM SA 28000x Spot 6 Int 42.30 1.93 um 1.6"

TEM SA 92000x Spot 5 Int 41.39 1.0 s

Expose Focus Series

Expose Series

CCD Integration time (s) 1.0

Wait (s) after CCD in 5

Stigmator

Condenser Objective

None

Step size: 3

x y

Direct Alignments

Gun Tilt
Gun Shift
Beam tilt pp X
Beam tilt pp Y
Beam shift
Center CCD spot size
Rotation center

Done

Auto help

MF X:	Beam shift X	MF Y:	Beam shift Y
L1:	Alpha Wobbler	R1:	Screen lift
L2:	Norm all lenses	R2:	Reset Defocus
L3:	Spotsize -	R3:	Spotsize +
LTb:	Beam shift		

Set Eucentric (Z) Height

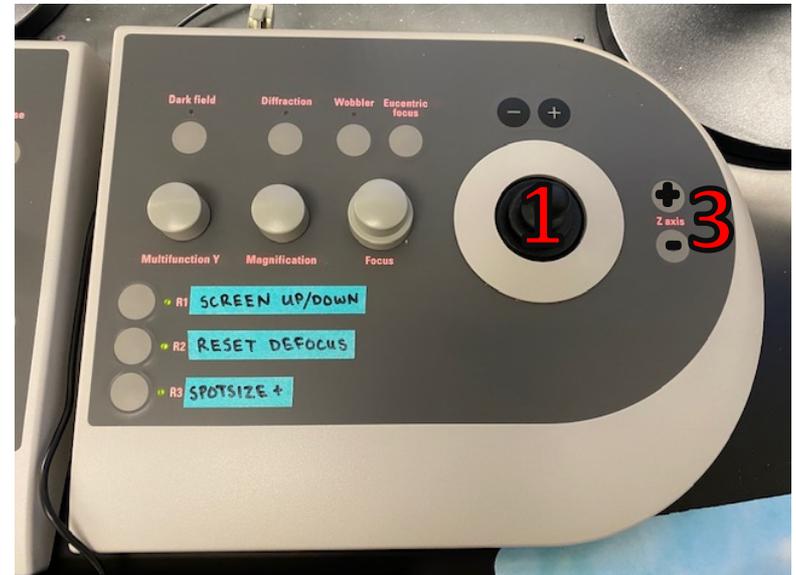
1. Move stage to center a feature (joystick, handpanel)

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



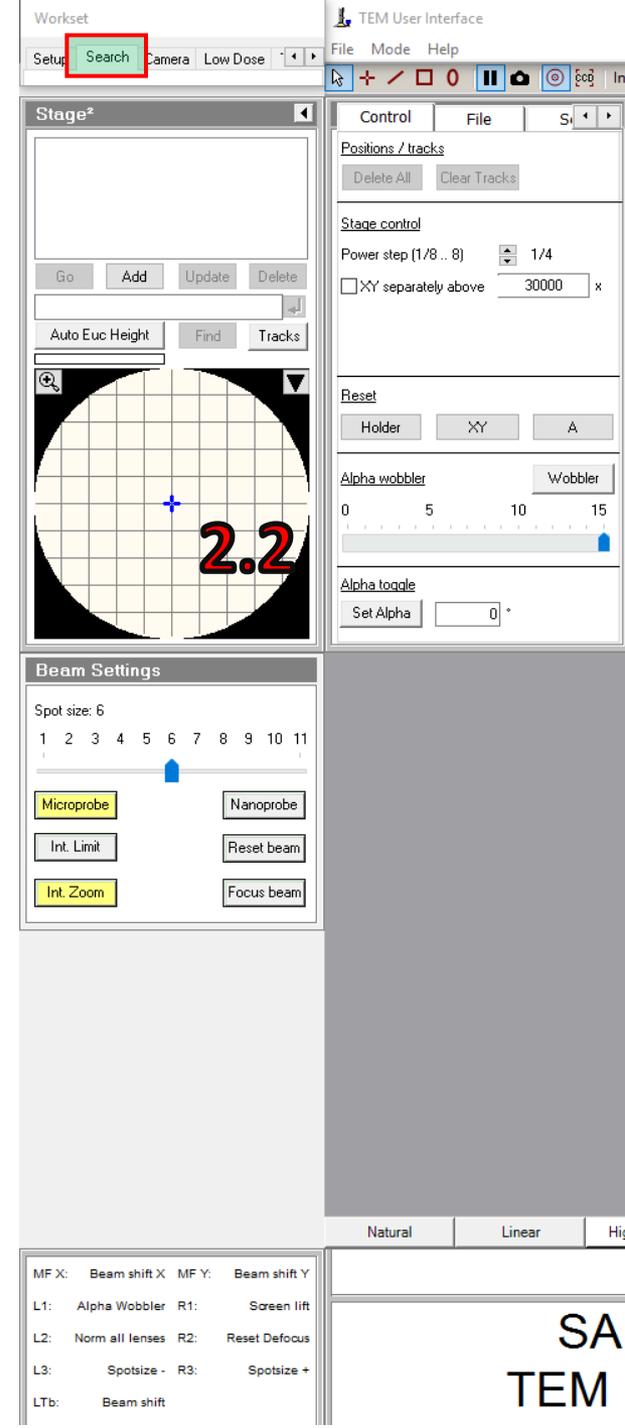
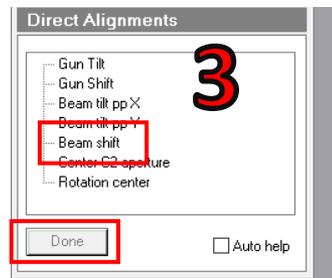
2. Move stage

1. Joystick
2. Can track movement in Search tab



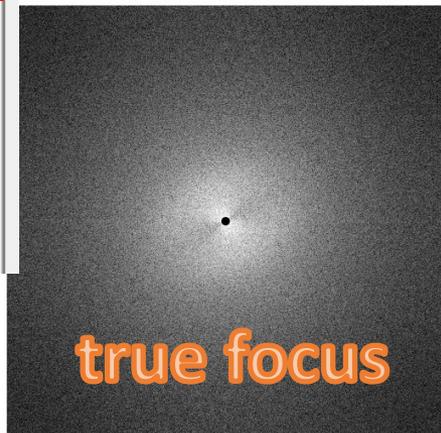
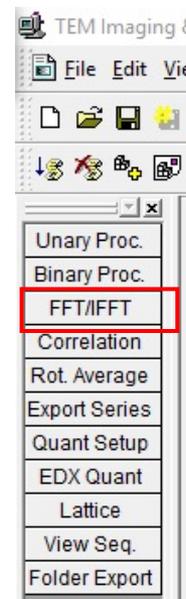
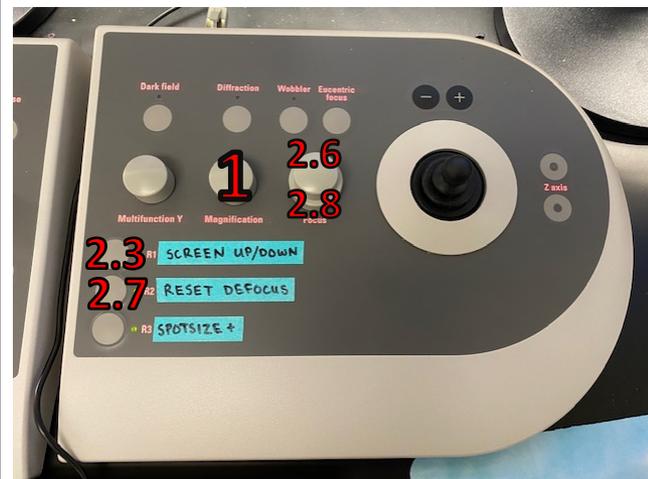
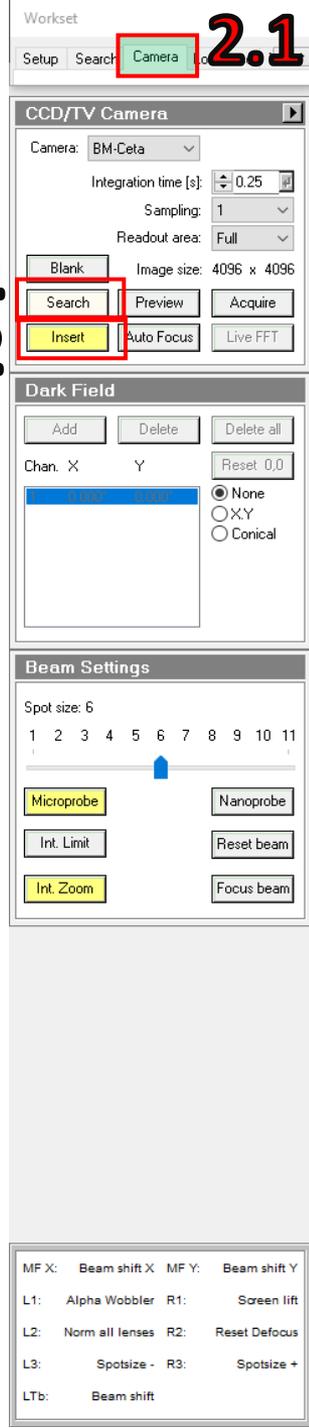
3. Center beam

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)



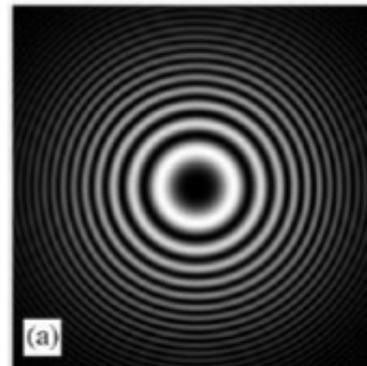
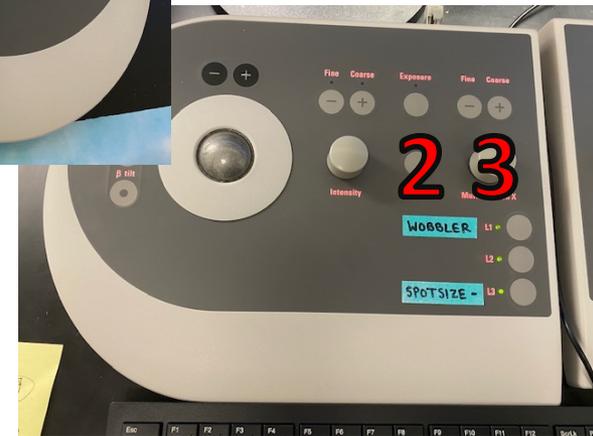
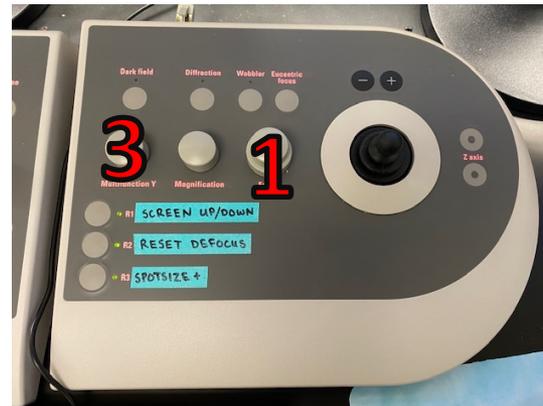
2.5

Make sure your beam is not astigmatic

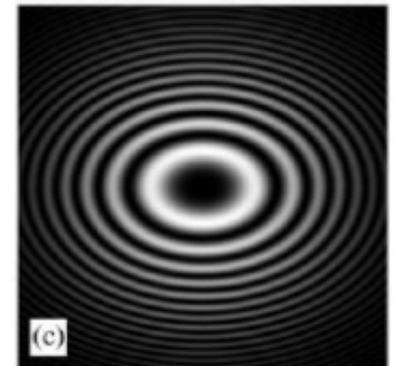
1. Are your Thon rings circles or ovals??

If they are ovals:

1. Turn Focus knob (right hand panel) until defocus is $\sim 0.3\mu\text{m}$
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)



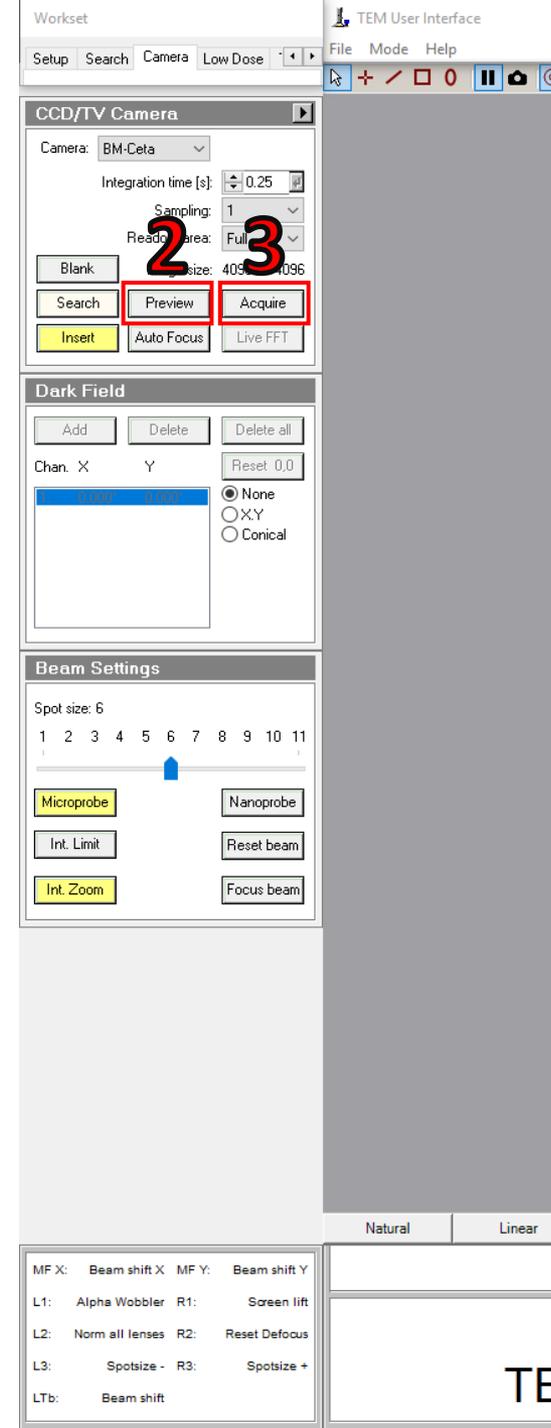
good



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

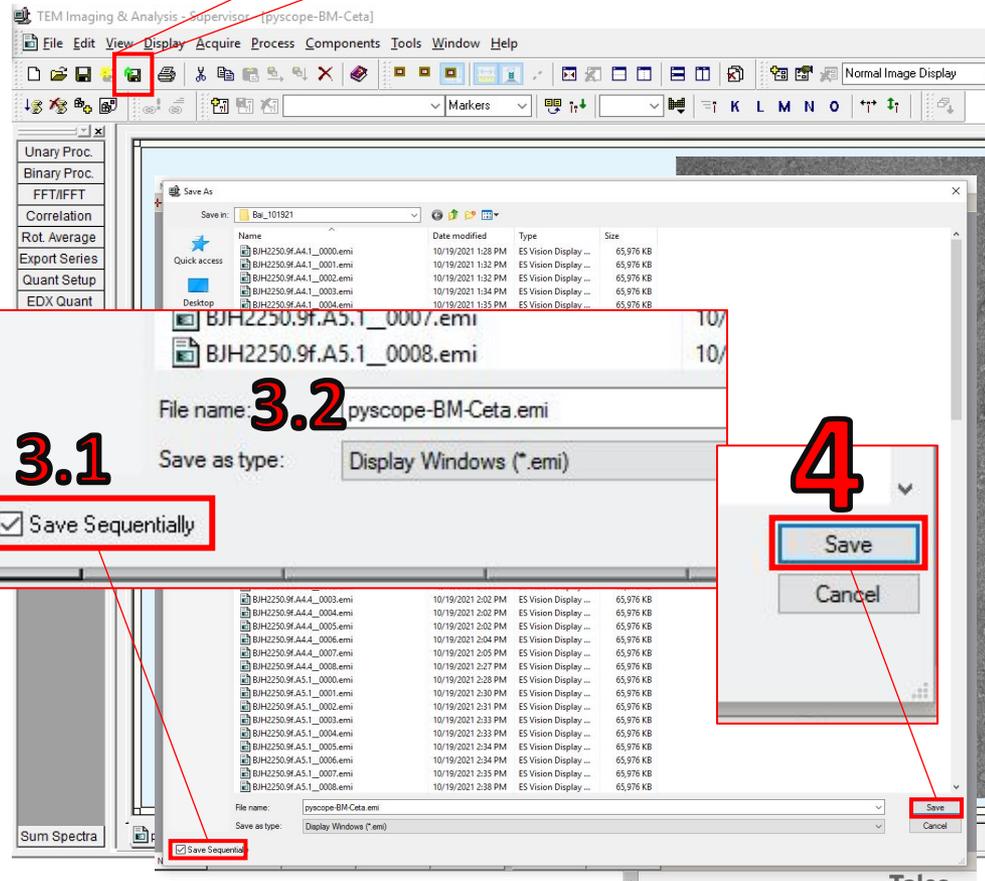
1. Save Sequentially should be
checked

2. Enter file base name:
SM_2345_3

4. Click Save – TIA

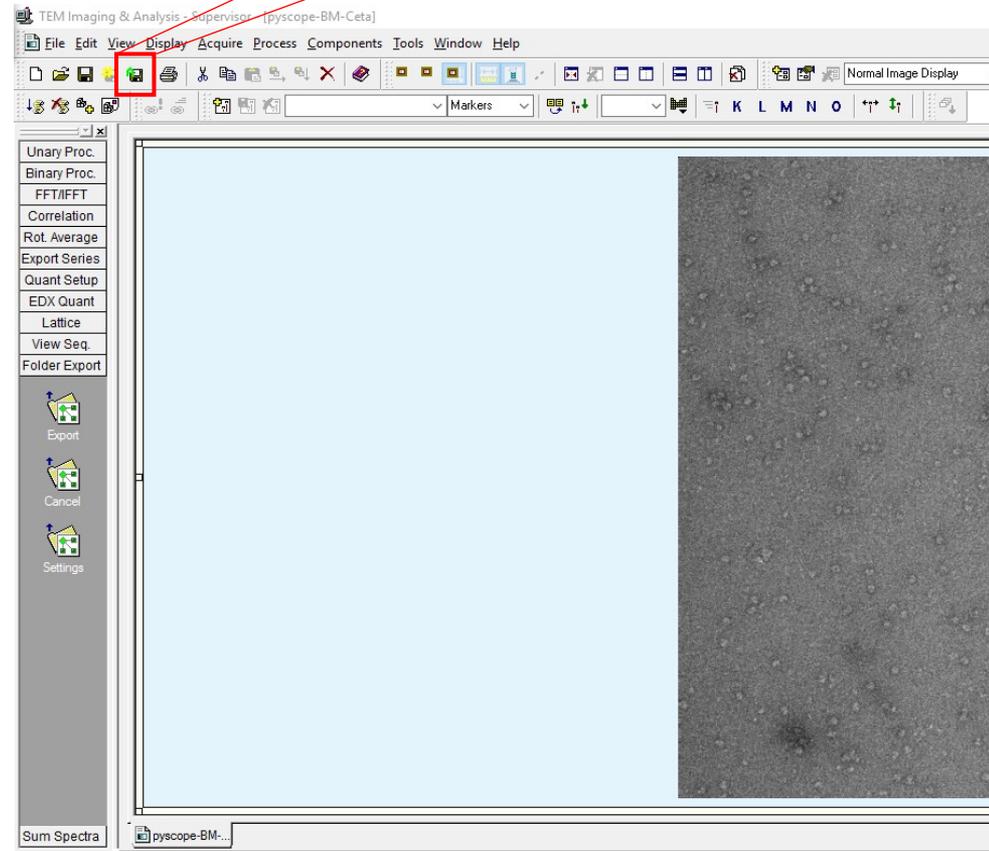
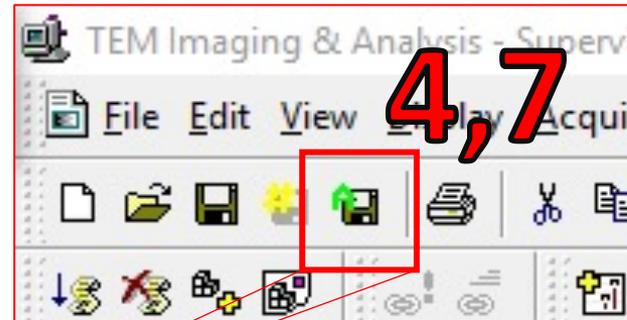
1. Double check that the
images are saving

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



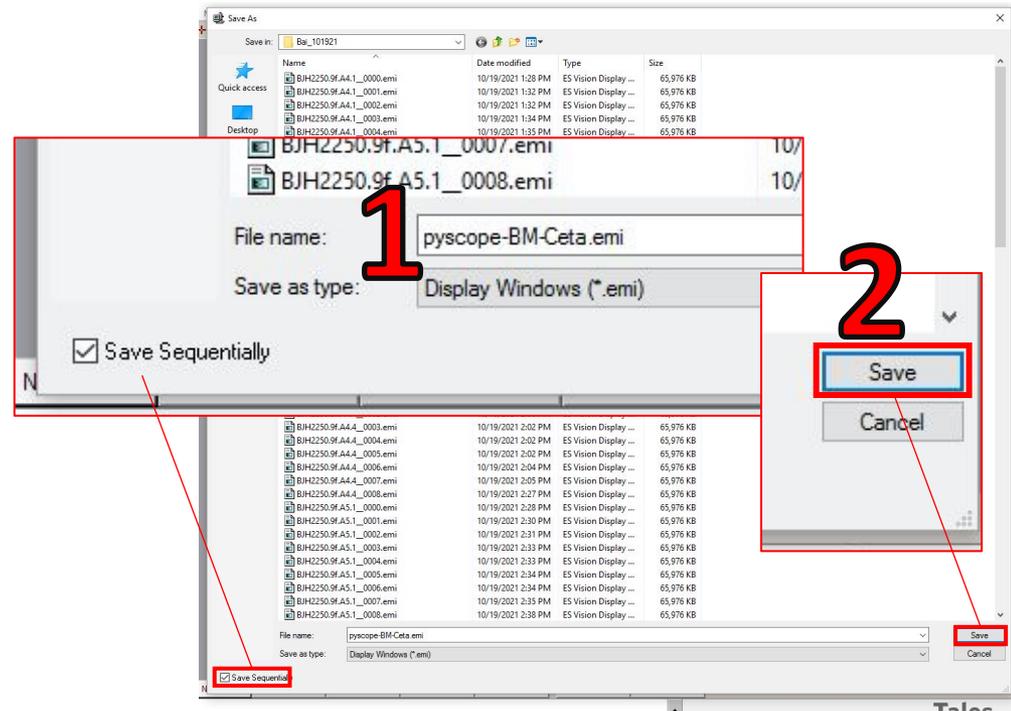
New Sample

1. Change name of the sample

CHANGE_####.emi

2. Click “Save”

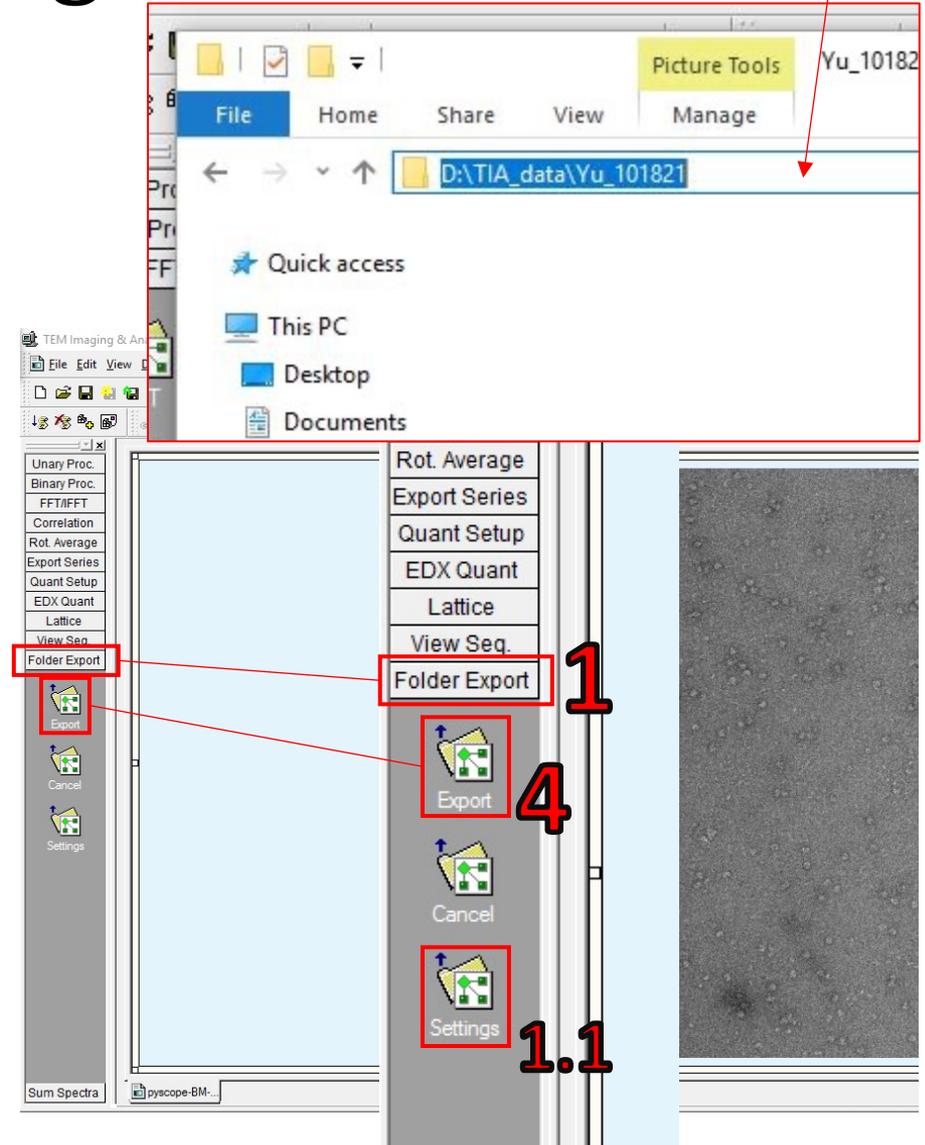
1. Double check that the images are saving
2. Continue acquiring until you're finished with this sample



Batch convert images

Click in this bar
2.1

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

