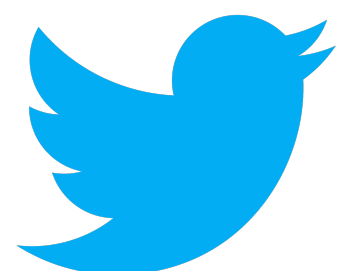




Cryo-ET data collection by SerialEM

Shuaiqi Guo (Phil)
Dr. Jun Liu's Lab
Yale University



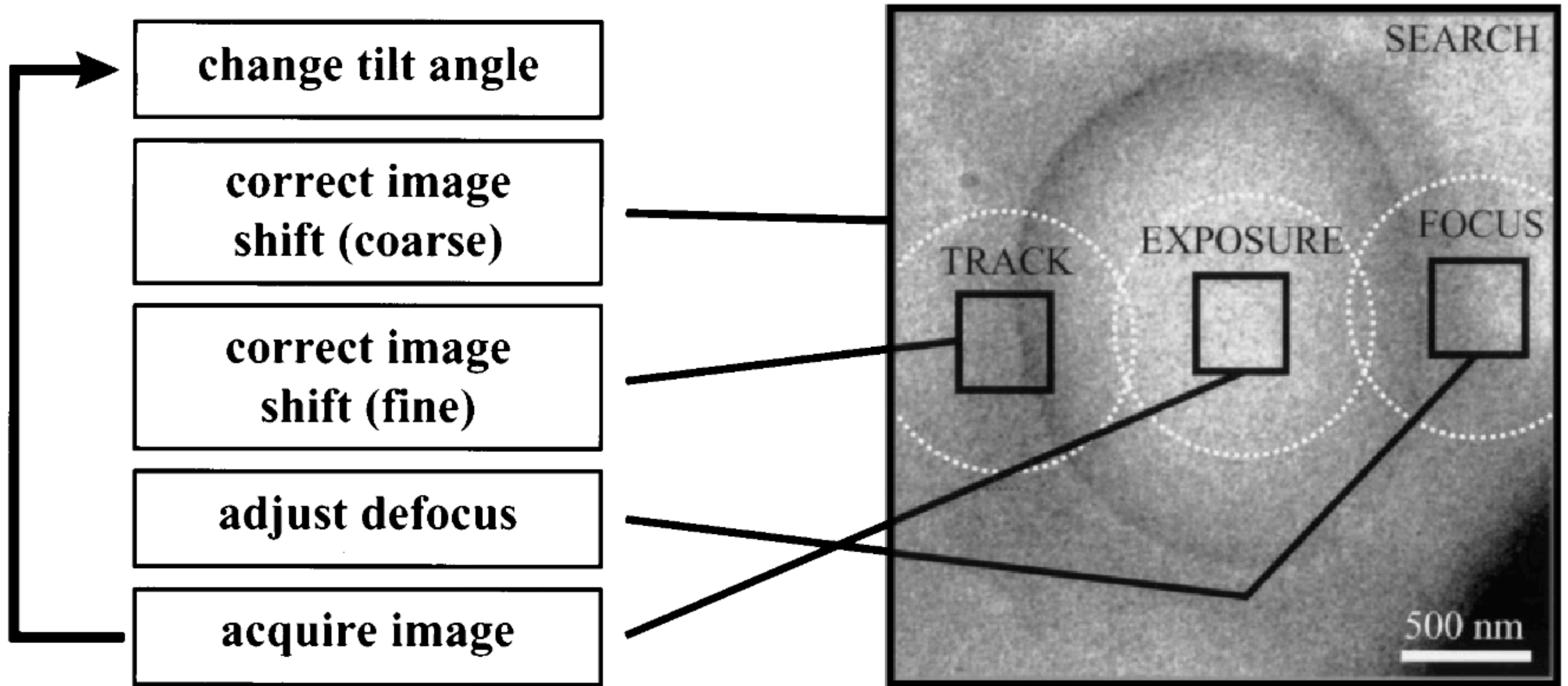
@PhilGuo1
@JunLiuLab



Main goals

1. SerialEM tilt series data collection with FastTomo script
2. Tomogram reconstruction by IMOD

Tilt series data collection scheme



Cryo-ET data acquisition packages

SerialEM

UCSF Tomo

Leginon

FEl tomography

EM-Manu

Automated electron microscope tomography using robust prediction of specimen movements

David N. Mastronarde *

2005

*Boulder Laboratory for Three-Dimensional Electron Microscopy of Cells, Department of Molecular, Cellular, and Developmental Biology,
University of Colorado, Boulder, CO 80309, USA*

Received 5 April 2005; received in revised form 14 July 2005; accepted 20 July 2005

Available online 24 August 2005

**SerialEM provides a flexible interface.
The script capability provides a relatively easy way
to add commands requested by users**

SerialEM (David Mastronarde)



Brookhaven
National Laboratory

The screenshot displays the SerialEM software interface. The central window shows a grayscale electron micrograph of a biological specimen. The interface is divided into several panels:

- Left Panel:** Contains control panels for Buffer Status, Buffer Controls, Image Display Controls, Microscope, Tilt Control, and Camera & Macro Controls.
- Right Panel:** Contains configuration panels for Low Dose Control, K2 Direct Detection, Camera View, and Navigator.

Microscope Panel Data:

0.0355 nA	2300X
Def -40.00 um	IS 0.00 um
Obj 86.41%	VAC Spot 6

Low Dose Control Panel Data:

View: 2300x Sp 6 C2 61.41%
Position on tilt axis: 0.00 um
Maximum area separation: -0.71 um
Additional beam shift: 0.00, 0.00

Navigator Panel Data:

Label	Color	X	Y	Z	Type	Reg.	Acq.	No.
1	Blu	-325.2	-232.6	118.7	Map 1			



@junliulab



SerialEM (David Mastronarde)



Brookhaven
National Laboratory

The image displays the SerialEM software interface. A central window shows a grayscale micrograph of a specimen. A red rounded rectangle is overlaid on the image with the text "Defocus Magnification Stage tilt angle".

On the left side, the "Microscope" panel is highlighted with a red box and contains the following parameters:

- Current: 0.0355 nA, 2300X
- Def: -40.00 um, IS: 0.00 um
- Obj: 86.41%, VAC, Spot: 6

Below the microscope panel is the "Tilt Control" panel, which shows a "Tilt" value of 0.00 and buttons for "Up", "Down", and "To".

On the right side, the "Low Dose Control" panel is visible, showing "Low Dose Mode" checked, "View: 2300x Sp6 C261.41%", and "Continuous update of mag & beam" checked. The "Defocus" is set to -60.00 um. Below this is the "Camera View" panel, which shows "Exposure (s)" set to 0.5 and "Auto Survey" checked.

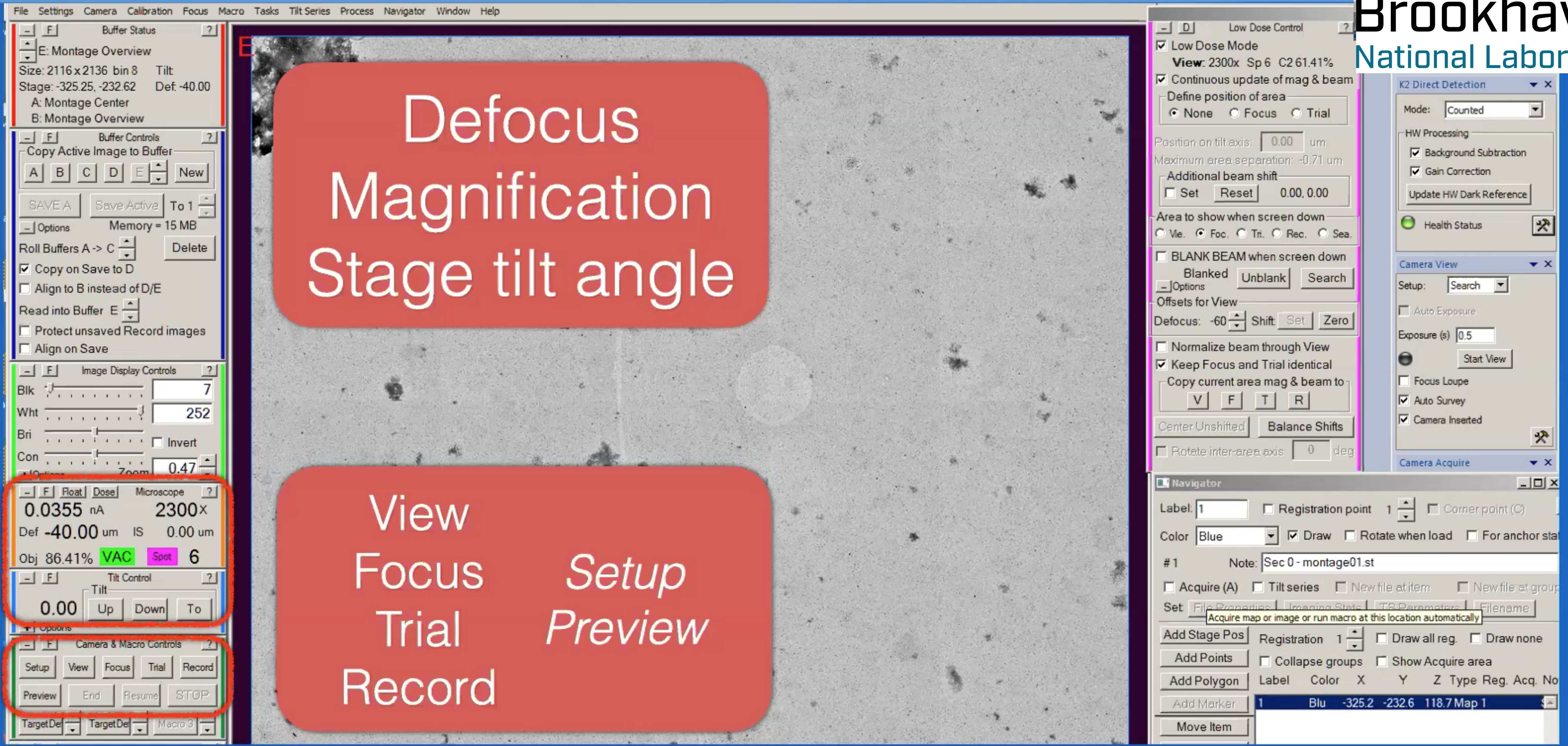
At the bottom right, the "Navigator" panel shows a table of stage positions:

Label	Color	X	Y	Z	Type	Reg.	Acq.	No.
1	Blu	-325.2	-232.6	118.7	Map 1			

SerialEM (David Mastronarde)



Brookhaven
National Laboratory



Defocus
Magnification
Stage tilt angle

View
Focus
Trial
Record
*Setup
Preview*



@junliulab



SerialEM – Navigator



Brookhaven
National Laboratory

File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help

Buffer Status
E: Montage Overview
Size: 2116 x 2136 bin 8 Tilt
Stage: -325.25, -232.62 Def: -40.00
A: Montage Center
B: Montage Overview

Buffer Controls
Copy Active Image to Buffer
A B C D E New
SAVE A Save Active To 1
Options Memory = 15 MB
Roll Buffers A -> C Delete
Copy on Save to D
Align to B instead of D/E
Read into Buffer E
Protect unsaved Record images
Align on Save

Image Display Controls
Blk 7
Wht 252
Bri Invert
Con 0.47
Zoom

Microscope
0.0355 nA 2300X
Def -40.00 um IS 0.00 um
Obj 86.41% VAC Spot 6

Tilt Control
Tilt
0.00 Up Down To

Camera & Macro Controls
Setup View Focus Trial Record
Preview End Resume STOP
TargetDel TargetDel Macro 3

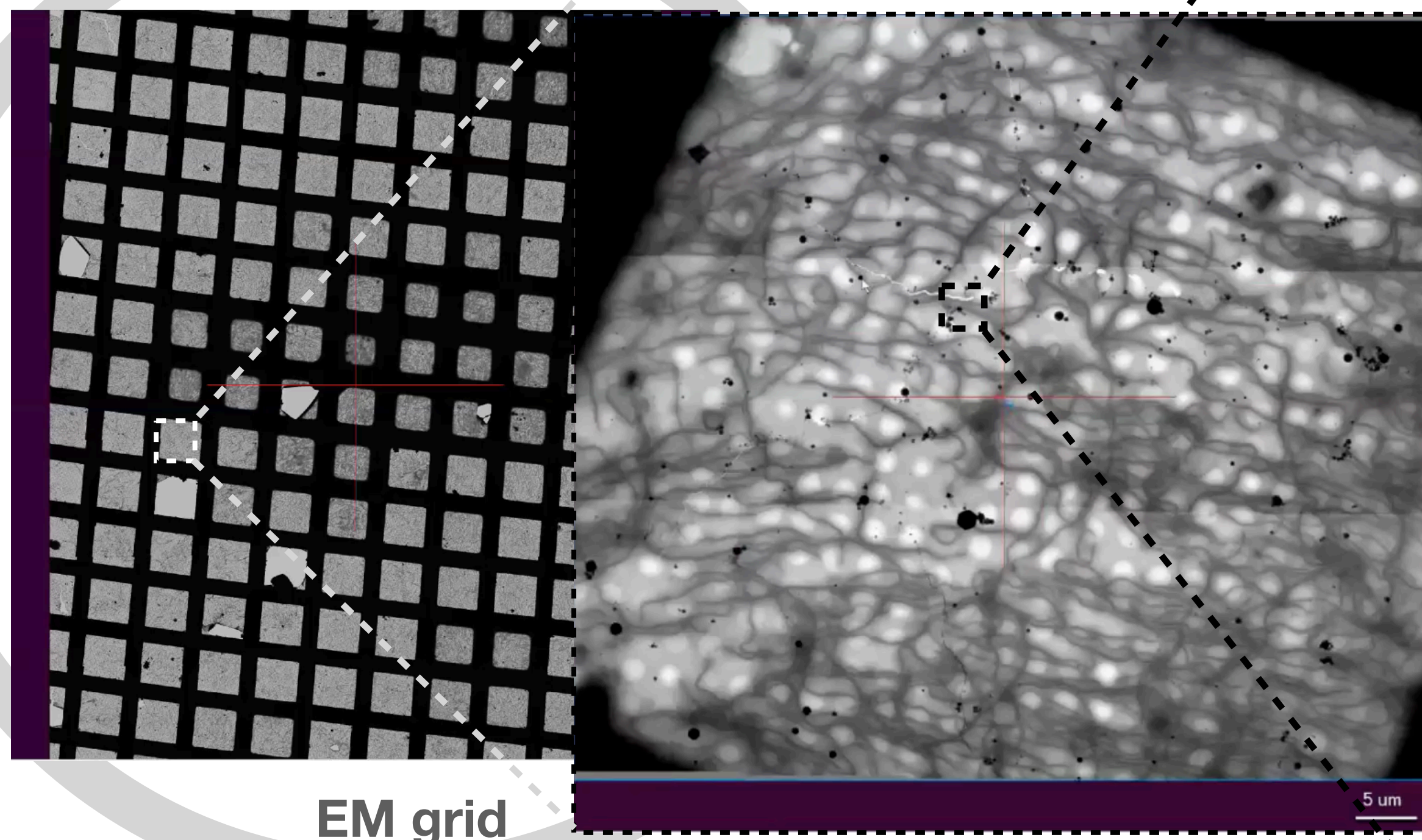
Navigator
Label: 1 Registration point 1
Color Blue Draw Rotate when load For anchor state
#1 Note: Sec 0 - montage01.st
Acquire (A) Tilt series New file at item New file at group
Set File Properties Imaging State TS Parameters Filename
Acquire map or image or run macro at this location automatically
Add Stage Pos Registration 1 Draw all reg. Draw none
Add Points Collapse groups Show Acquire area
Add Polygon Label Color X Y Z Type Reg. Acq. Not
Add Marker 1 Blu -325.2 -232.6 118.7 Map 1
Move Item
Update Z
Go To XY
Go To XYZ
Go To Marker
Load Map
New Map
Anchor Map
Delete Item
Realign to Item

Find targets for tilt series data acquisition

1. Obtain 175 X full montage to survey the grid

2. Obtain multiple small montages (2250X) with many target cells

3. add targets in the small montages for tilt series acquisition

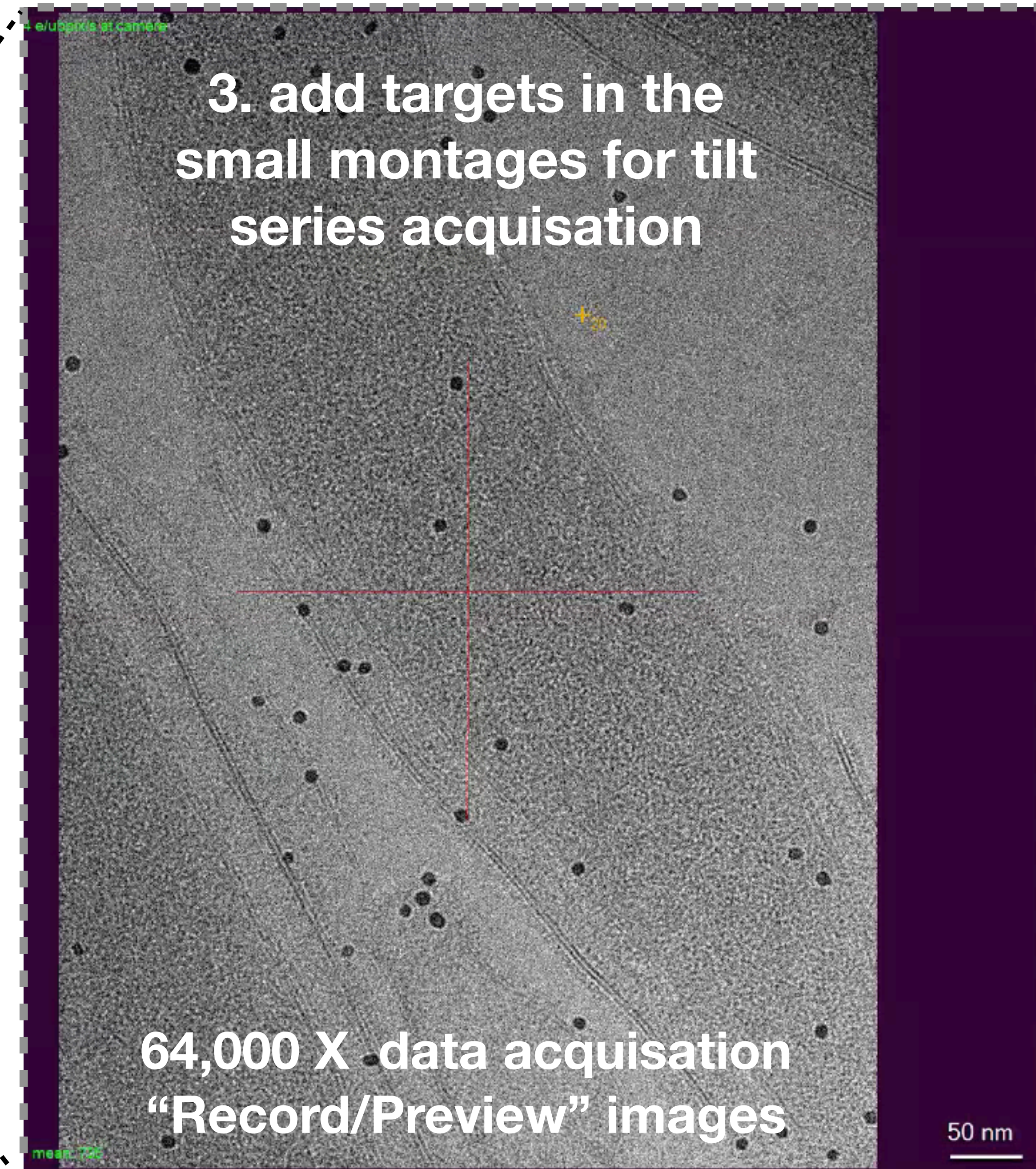


EM grid

5 μm

6 x 6 "Search" images

5 x 3 "View" images



64,000 X data acquisition "Record/Preview" images

50 nm

File Settings Camera Calibration Focus/Tune Script Tasks Tilt Series Process Navigator Window Help

Buffer Status
A: Saved to File, sec 1
Size: 1904 x 2822 bin 2 Tilt: 0.00
Stage: 305.35, -460.19 Def: 0.00
B: Saved to File, sec. 0
C: UNSAVED, Search

Buffer Controls
Copy Active Image to Buffer
A B C F New

Image Display Controls
Blk 0
Whi 1019
Bri Cross
Con Invert
Options Zoom 0.56

Microscope
-0.0000 nA 175 LM
Def 0.00 um IS 0.00 um
IA 718.40 um VMC Spot 8

Microscope Control
Tilt Control
0.00 Up Down To

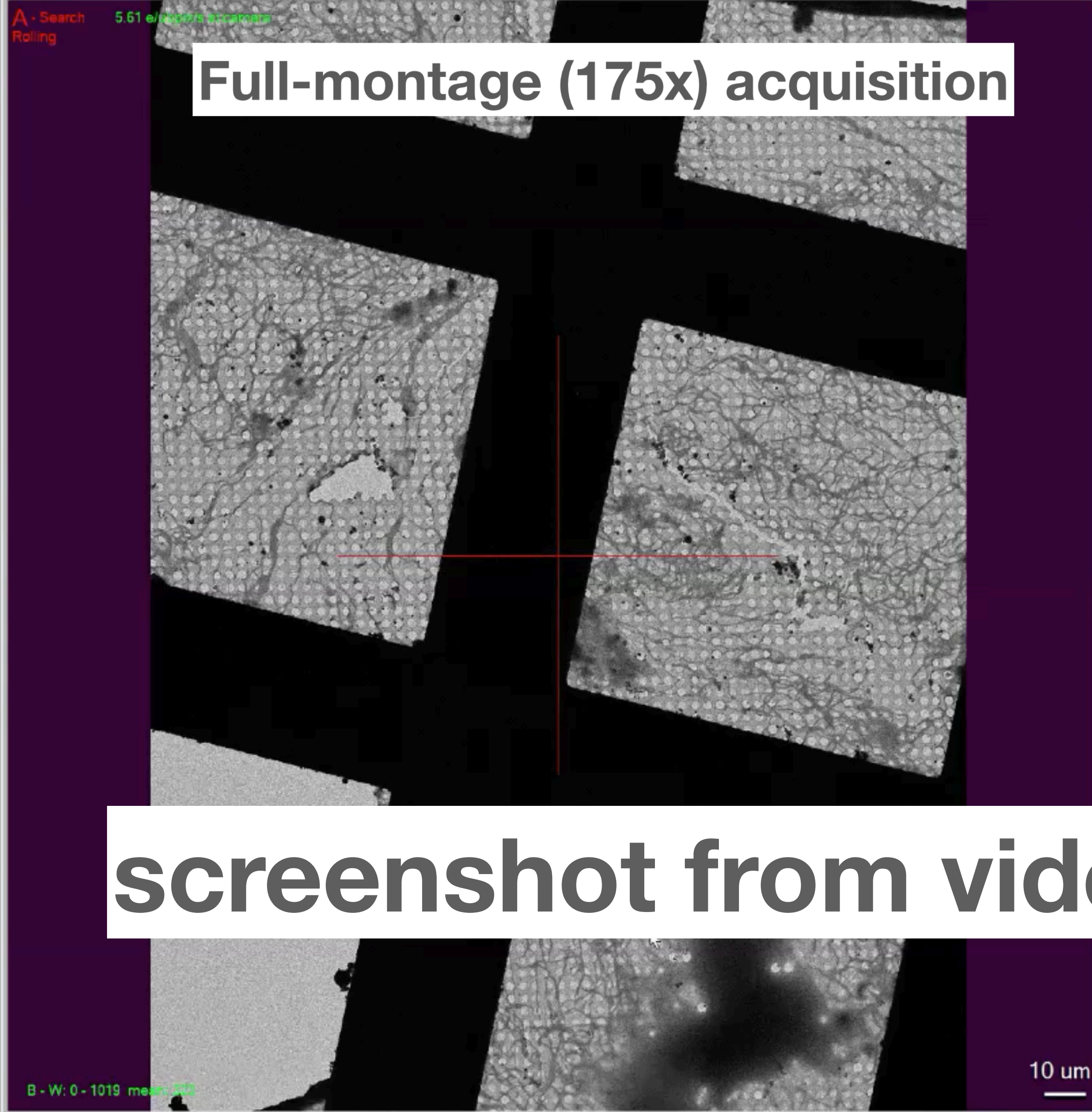
Camera & Script
K3
Swap View Focus Tilt Record
Pause Search Pause STOP

Image Alignment & Focus
Align to P To Marker Clear
Reset mega shift Autofocus
Options Def target = -4.00 um
Move stage for big mouse shifts
Set Threshold Shift
Correct backlash in stage moves
Center image shift on tilt axis
Adjust mega shift between megs
Trim dark borders in Autoalign
Set Autoalign Trim Fraction

Low Dose Control
Low Dose Mode
Search: 175x Sp: 8 W: 718.40u
Continuous update (see tooltip)
Define position of area
None Focus Trial
Position on tilt axis: -2.00 um
Maximum area scan fraction: 1.00 um
Go to: View Focus Tilt Rec Search
Additional beam shift (and DF) shi
Set Reset Uncalibrated
Offsets for: View Search
Defocus: -100 Shift Set Zero
Blanked Unblank
Options
BLANK BEAM when screen down
Normalize condenser lenses
Keep Focus and Tilt manual
Copy current area settings to
Center Unfilled Balance Shifts
Rotate inter-ered axis 0 600

Montage Controls
Start Pause 0
Options Current: 2

Full-montage (175x) acquisition



screenshot from video

Navigator

Label Flag: Has: Has: Corner point:

Color Red Draw Rotate when load For anchor: none

Note

Acquire (A) Tilt series New file: none New file as group

Set File Props Imaging Data TS Params Filenames Focus Pos

Add Stage Pos Registration 1 Draw: All reg None Labels

Add Points Collapse Show Acquire Edit mode Edit Focus

Label	Color	X	Y	Z	Type	Reg.	Acq.	Note

Buttons: Add Polygon, Add Marker, Move Item, Update Z, Go To XY, Go To XYZ, Go To Marker, Load Map, New Map, Anchor Map, Delete Item, Realign to Item

Scripts

- PrepMMM no2
- autoun
- CycleTargetDetector
- PrepMMM2
- MyPlans
- Script 6
- autoun-multi
- e-Align-vpp
- SimpleFocus
- Z-checker-align-FR
- shot-DS-Tomo
- FastTomocast
- FastTomoWideCell
- Z
- Z-checker-align-2
- Script 16
- Script 17
- Script 18
- Script 19
- Script 20
- Script 21
- Script 22
- Script 23
- Script 24
- FastTomo
- OpenFile
- CloseFile
- Script 25
- Script 26

Log: log1.log

```

Error above tolerance, retaking shot
15 frames were saved to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.13.43.tif
Saved Z = 22, -33.00 degrees
15 frames were saved to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.13.47.tif
Saved Z = 23, -36.00 degrees
15 frames were saved to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.13.52.tif
Saved Z = 24, -39.00 degrees
Error above tolerance, retaking shot
15 frames were saved to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.13.56.tif
Saved Z = 25, -39.00 degrees
15 frames were saved to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.14.01.tif
Saved Z = 26, -42.00 degrees
15 frames were saved to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.14.07.tif
Saved Z = 27, -45.00 degrees
retaking shot
to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.14.11.tif
agrees
to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.14.15.tif
agrees
to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.14.59.tif
agrees
to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.15.07.tif
agrees
to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.15.12.tif
agrees
15 frames were saved to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.15.17.tif
Saved Z = 33, 36.00 degrees
15 frames were saved to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.15.22.tif
Saved Z = 34, 39.00 degrees
15 frames were saved to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.15.27.tif
Saved Z = 35, 42.00 degrees
15 frames were saved to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.15.32.tif
Saved Z = 36, 45.00 degrees
15 frames were saved to X:\DoseFractions\20210613\Jun(G205SC)\Jun14_10.15.37.tif
Saved Z = 37, 48.00 degrees
----- end of dose symmetric TS -----
TS time: 327.25
total time: 327.25
  
```


Obtain multiple small montages (2250x)

screenshot from video

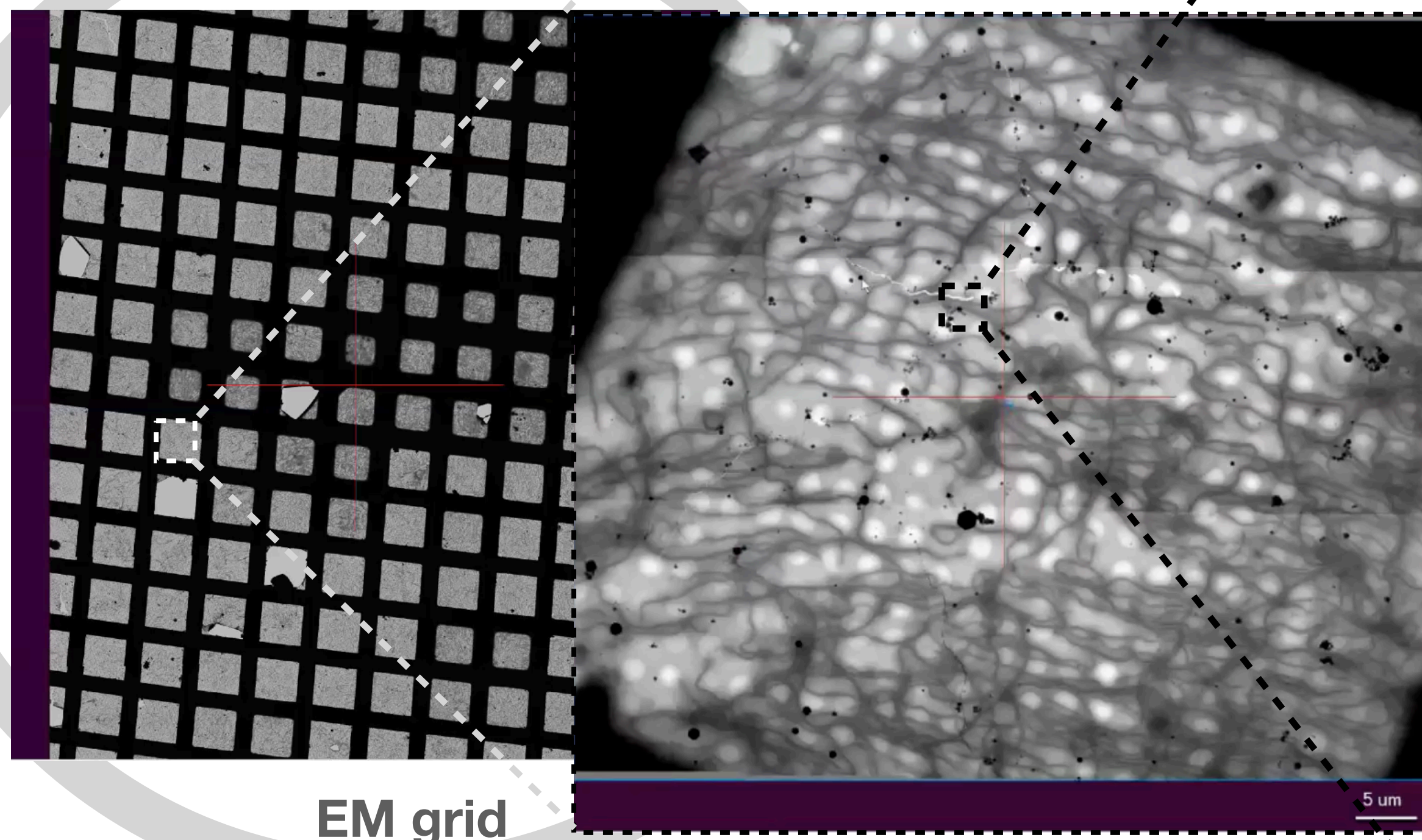
The screenshot displays the SerialEM software interface. The central window shows a grid of micrographs with a red crosshair. The left sidebar contains several control panels: Buffer Status, Montage Overview, Buffer Controls, Image Display Controls, Microscope Control, Tilt Control, Camera & Script, Image Alignment & Focus, Low Dose Control, and Montage Controls. The right sidebar shows a Navigator window with a list of items and a Scripts window with a list of scripts. The bottom window shows a Log window with a detailed log of the acquisition process, including saved Z-positions, frame counts, and alignment statistics. A scale bar of 50 μm is visible in the bottom right corner of the micrograph grid.

Find targets for tilt series data acquisition

1. Obtain 175 X full montage to survey the grid

2. Obtain multiple small montages (2250X) with many target cells

3. add targets in the small montages for tilt series acquisition

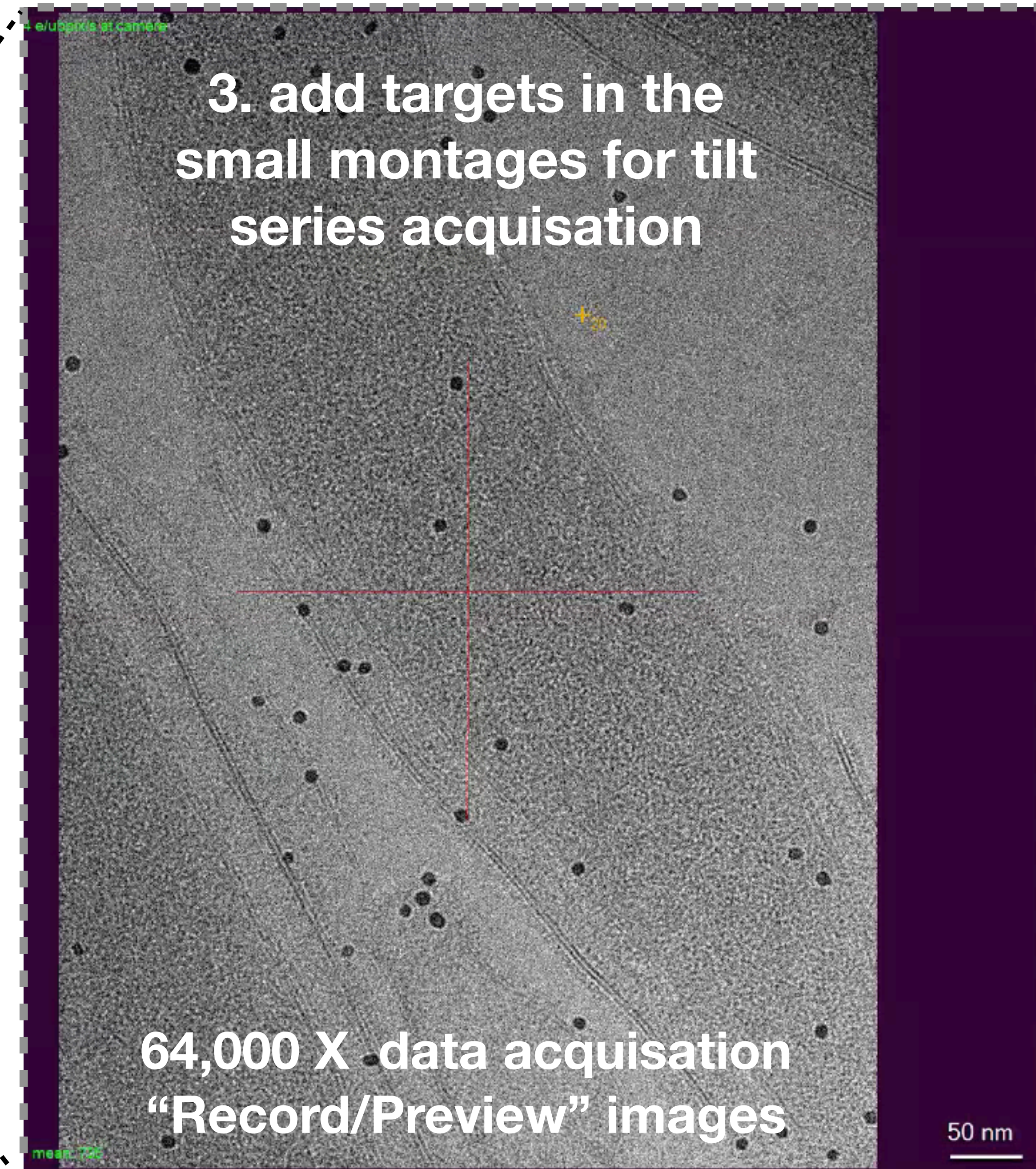


EM grid

5 um

6 x 6 "Search" images

5 x 3 "View" images



64,000 X data acquisition "Record/Preview" images

50 nm

Aligning "Preview/Record" beam with "View"

The screenshot displays the DigitalMicrograph software interface. On the left, a vertical diagram of the microscope system shows the electron path from the 300 kV source through the EFTEM and EF-CCD detectors. The main window shows a dark field with a "GIF Tuning" dialog box in the center, which has a "ZLP / ignore" checkbox and a "Fine alignment" slider. The right-hand side contains control panels for "BioQuantum K3" (Primary energy: 300.0 keV, Slit: 20.0 eV) and "EF-CCD Camera" (Exposure: 0.407 s). At the bottom, an "Output" window shows a log of system parameters and error messages.

```
Output | Image Browser | Script Debugger
Welcome to DigitalMicrograph. 6/11/2021, 12:44:06 PM
SerialEMCCD framealign : Error 126 occurred trying to load C:\ProgramData\Gatan\Plugins\...\FrameGPU.dll
SerialEMCCD framealign : GPU is not available, could not load FrameGPU.dll

ELP Offset = -20 eV.
ELP Offset = -2 eV.
ELP Offset = -7 eV.
ELP Offset = 5 eV.
ELP Offset = 0 eV.
ELP Offset = -1 eV.
ELP Offset = 0 eV.
```

screenshot from video

FastTomo: A SerialEM Script for Collecting Electron Tomography Data

Albert Xu, Chen Xu[†]

Department of Biochemistry and Molecular Pharmacology & Cryo-EM Core Facility

University of Massachusetts Medical School


Email: albert.t.xu@gmail.com, [†]Chen.Xu@umassmed.edu

***Abstract*—FastTomo is a SerialEM script for collecting tilted specimen images in transmission electron microscopes to be further used in tomographic reconstruction. It achieves a speedup over conventional tracking methods by minimizing the usage of off-target tracking shots, and instead applies proportional control to the specimen images. Movement in the Z coordinate is estimated prior to each tilt series in a separate calibration routine. Overall, this method is fast and reliable when the field of view is at least 1 um, and can tolerate minor errors in setting eucentric height. The implemented tilt series schemes include the unidirectional, bidirectional, and dose-symmetric schemes.**

* Author: Albert Xu <albert.t.xu@gmail.com>
* Date Created: May 22, 2020
* Last Modified @ChenXu: June 13, 2022

FastTOMO script

*/

scheme = 1 

- # 0 = bidirectional
- # 1 = dose-symmetric
- # 2 = unidirectional

runOnNavItem = 0


set to 1 to run on highlighted navigator point, and when using Acquire at Items

Debug = 0

verbose output for debugging

shot = R

low dose beam to use for saving data

usePrevCalib = 0 

skip calibration and use most recent parameters if they exist

tolerance = 0.4

redo a shot if the current frame is off target (0.5 = more than 50% off screen)

eucentricity_option = -1

1 = rough, 2 = fine, 3 = rough & fine, 4 = calls the script named Z, -1 = using autofocus

multiRecord = 1

take more than one R shots along tilting axis

multiR = { 3 6 }

R shift 3 and 6 um, can be more than 2 here.

dose-symmetric settings

startAngleDS = 0

endAngleDS = 48

stepSizeDS = 3

groupSizeDS = 8

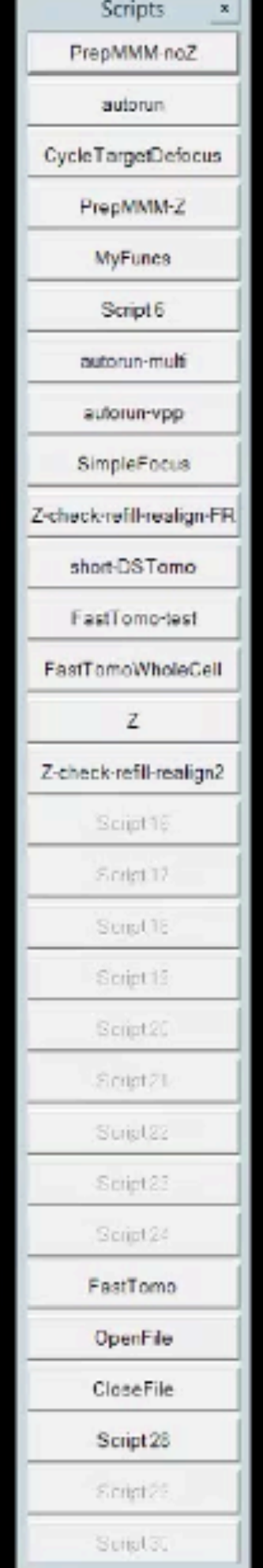
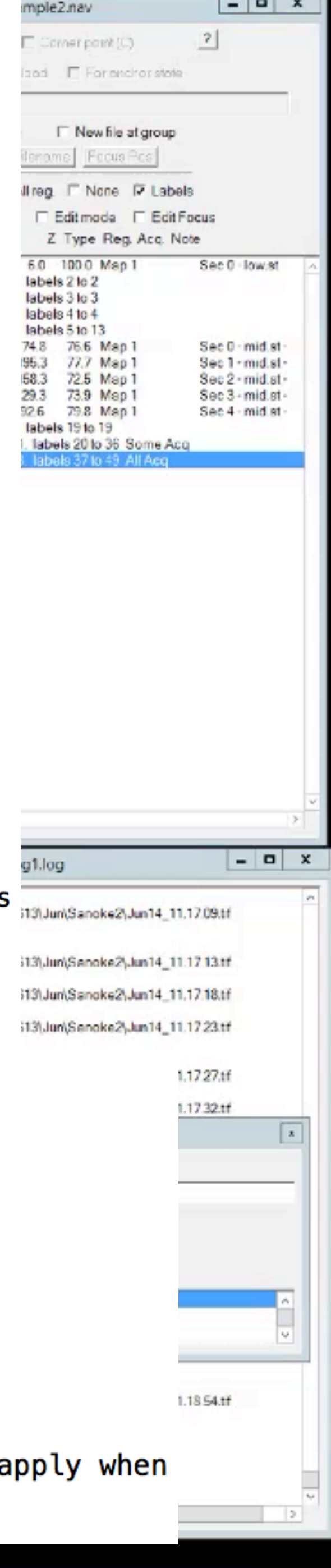
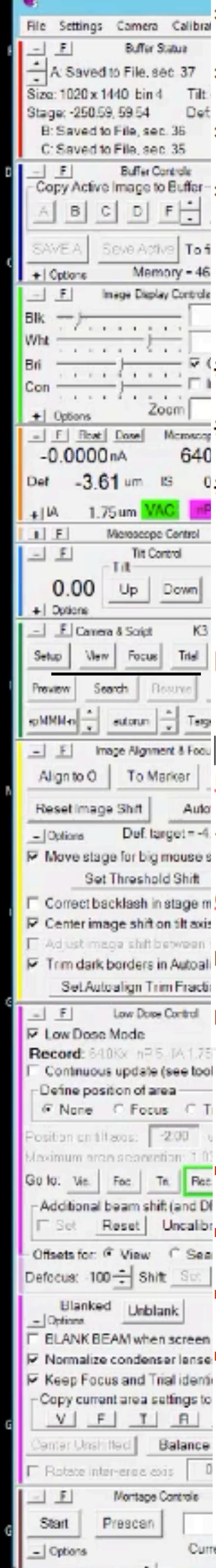
number of tilts before switching sides, 1 = original Wim Hagen scheme

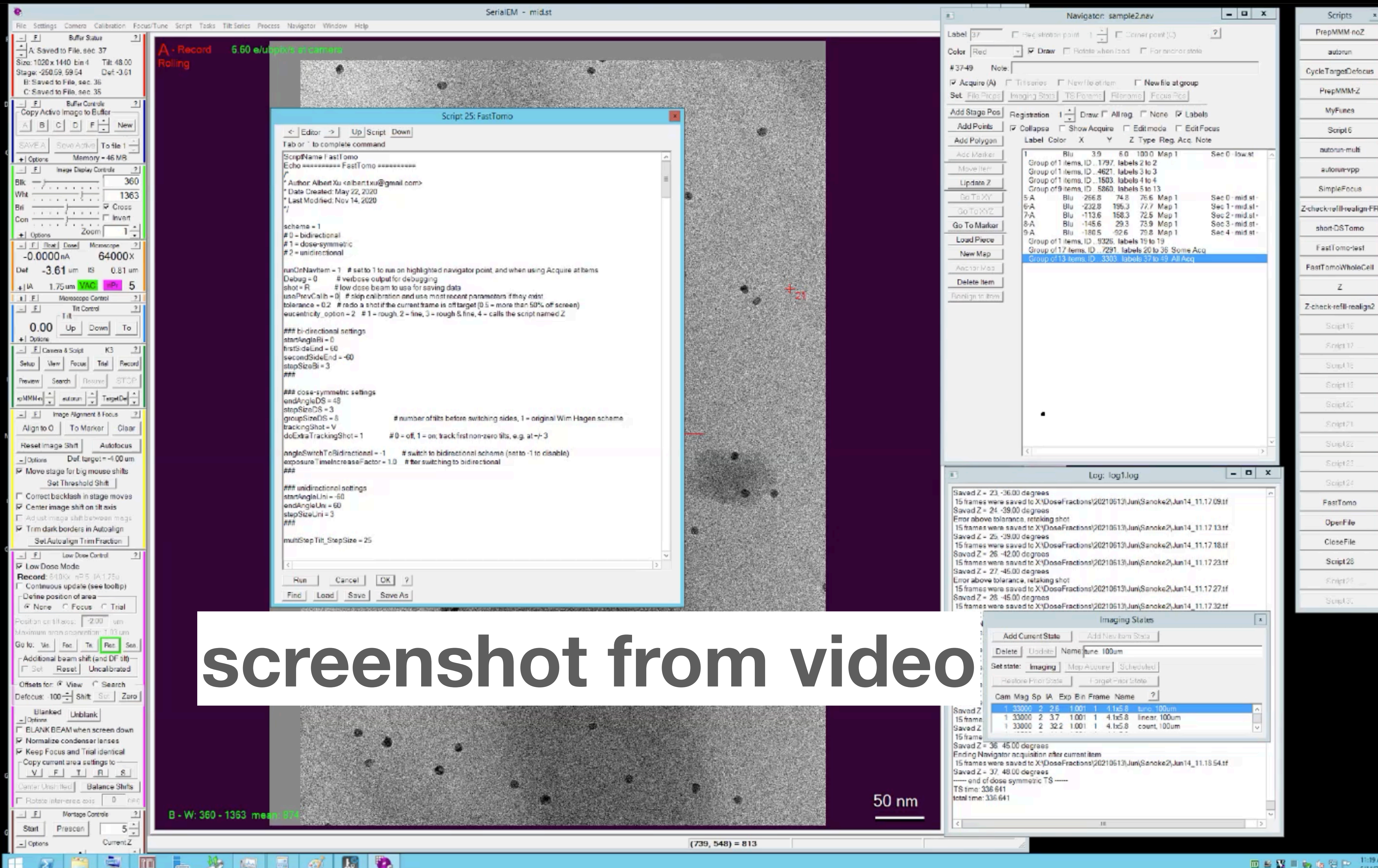
trackingShot = V

can also be set to V

doExtraTrackingShot = 1
startAngleDS is non-zero

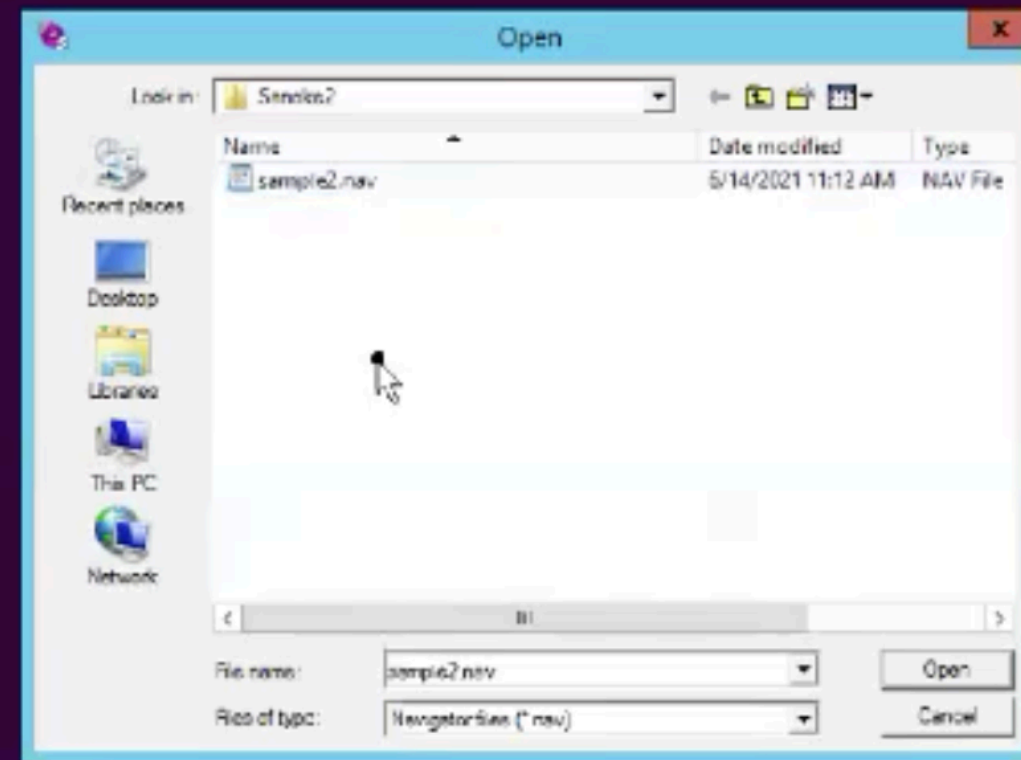
0 = off, 1 = on; track first non-zero tilts, e.g. at +/- 3; does not apply when





screenshot from video

Use the "Dummy SerialEM" to add more targets without stopping data collection



screenshot from video

Navigator: sample2.nav

Label: 20 Registration point: 1 Corner point (C): ?

Draw: Rotate when load For another state

Draw: All reg None Labels

Registration: 1 Draw: Show Acquire Edit mode Edit Focus

Color	X	Y	Z	Type	Reg.	Acq.	Note
Red	342.8	376.5	100.0	Pt	1		hole
Red	564.3	-185.6	100.0	Pt	1		bad
Off	-266.9	75.2	100.0	Pt	1		
Off	232.9	195.6	100.0	Pt	1		
Off	113.6	158.7	100.0	Pt	1		

Navigator

Label: Registration point: Corner point (C): ?

Color: Red Draw: Rotate when load For another state

Note:

Acquire (A) Tilt series New file at item New file at group

Set File Props Image Props TS Params Filtername Focus Pins

Add Stage Pins Registration: 1 Draw: All reg None Labels

Add Points Collapse Show Acquire Edit mode Edit Focus

Add Polygon Label Color X Y Z Type Reg. Acq. Note

Add Marker

Move Item

Update Z

Go To XY

Go To XYZ

Go To Marker

Load Map

New Map

Anchor Map

Create Item

Realign items

SerialEM - B0221.st (#2) 1: mid.st

DUMMY SerialEM

File Settings Camera Calibration Focus/Tune Script Tasks Tilt Series Process Navigator Window Help

Buffer Status: A: Empty B: Empty C: Empty

Image Display Controls: Blk, Wht, Bri, Con, Zoom: 1

Tilt Control: -68.88

Image Alignment & Focus: Def. target = 0.00 um

Low Dose Control: Continuous update (see tooltip) Define position of area: None, Focus, Trip

Message Controls: EFTSM Mode: OFF

Filter Control: Filter (str in): Slit width: 10 Zero Loss: Loss: 0.0

Ready

DOING 25: FastTomo

Tilt series alignment and tomogram reconstruction by IMOD (Etomo)

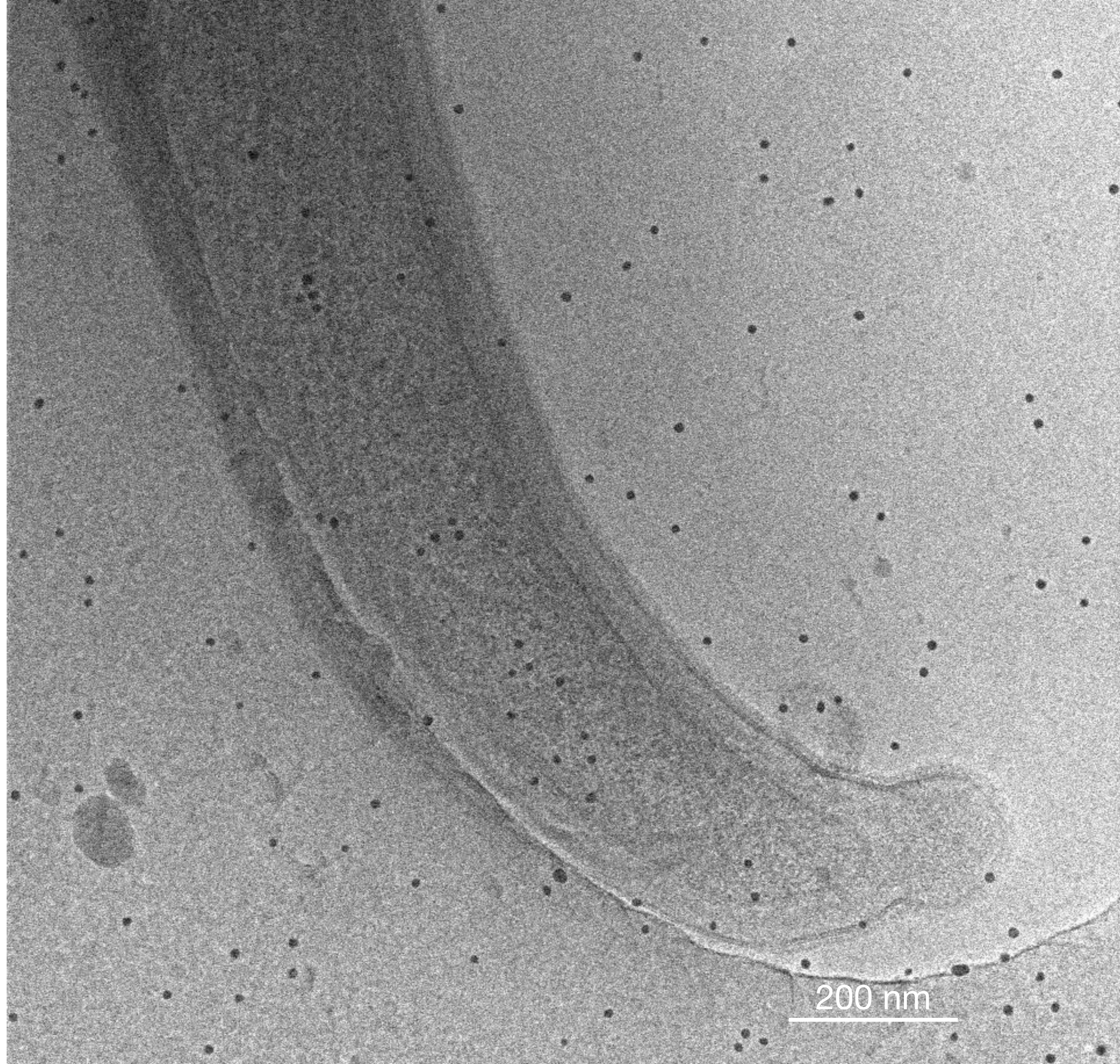
- 1. Motion correction for tilt images.**
- 2. Stack the tilt images to get the motion corrected tilt series.**
- 3. Preprocess the tilt series.**
- 4. Manually pick several fiducial gold (10 nm) and let the software do the tracking for fiducial.**
- 5. Align the tilt series based on the tracking result.**

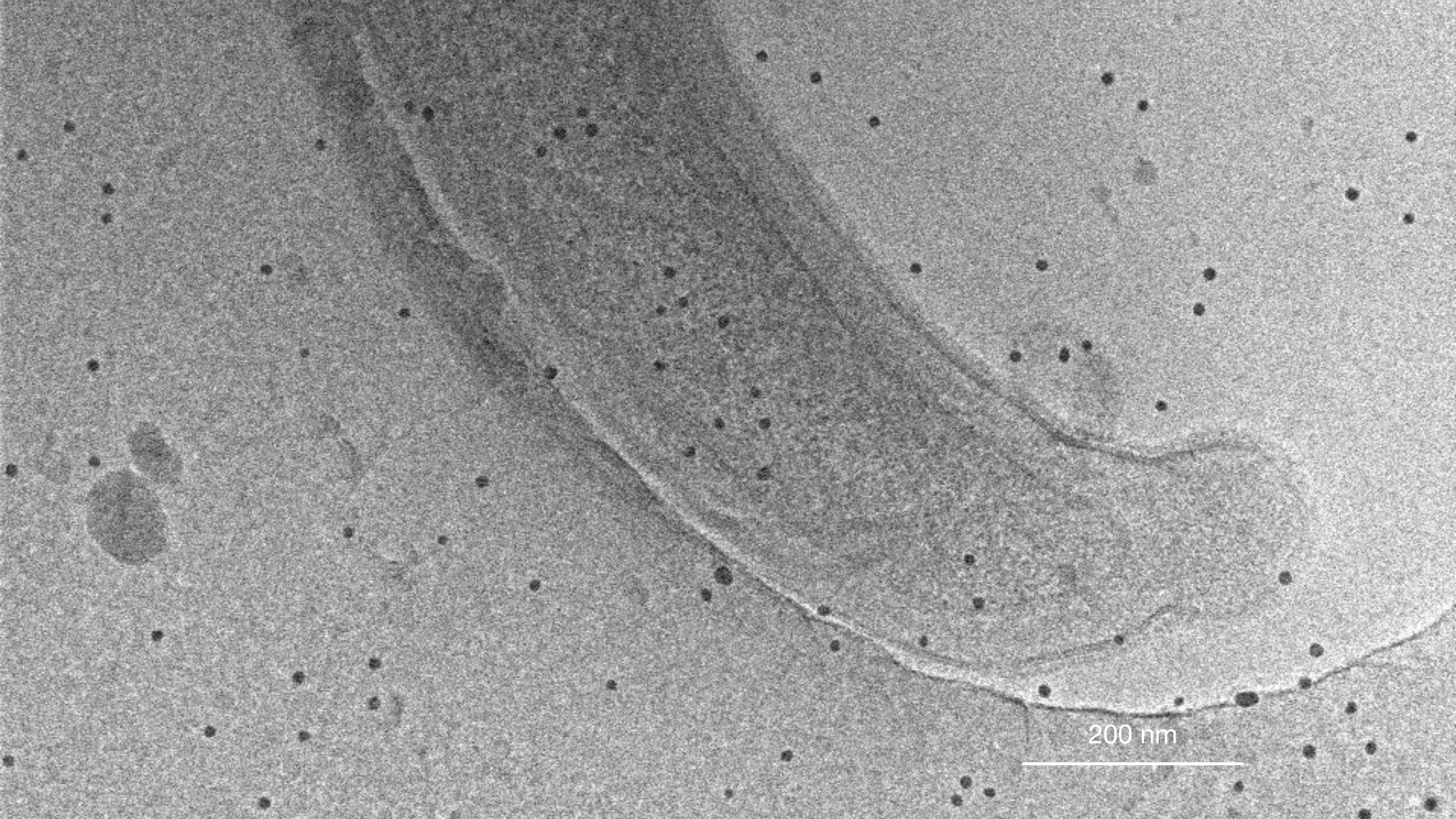
More details about etomo:

<http://bio3d.colorado.edu/imod/doc/etomoTutorial.html>

Dataset

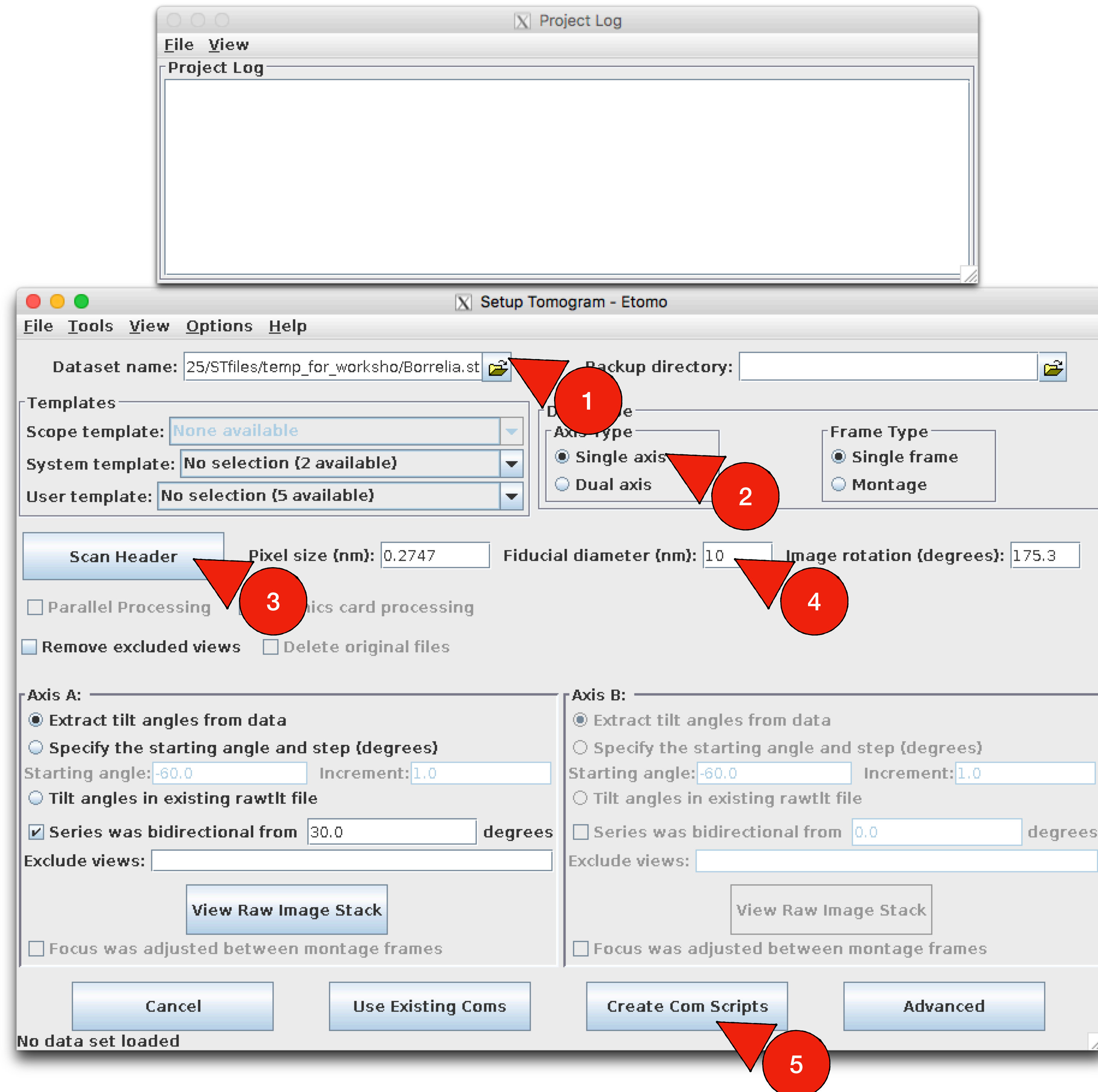
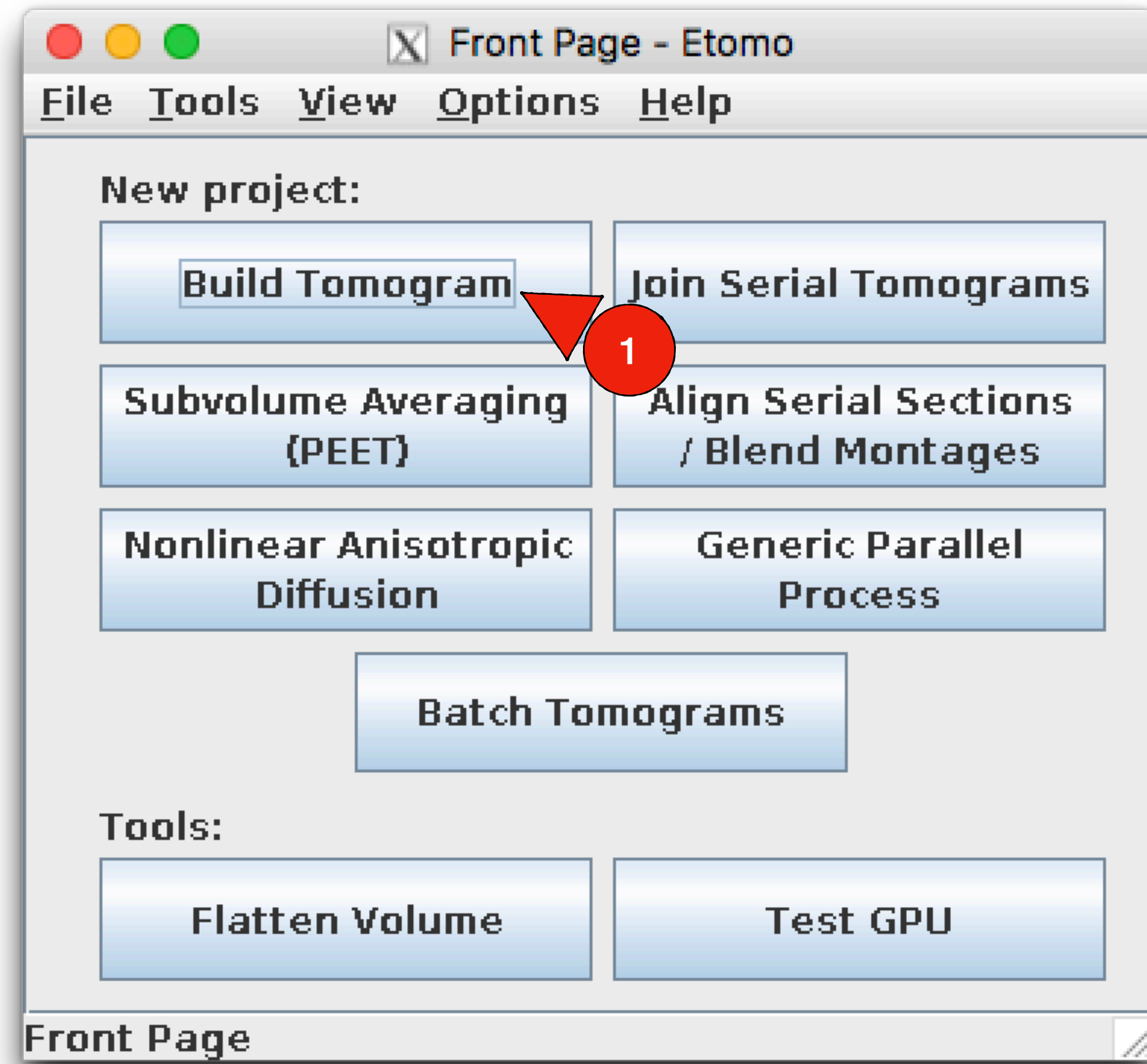
- *Borrelia burgdorferi*
- Collected by Titan Krios
- $-51^{\circ}:3^{\circ}:51^{\circ}$
- $2.747 \text{ \AA}/\text{pixel}$



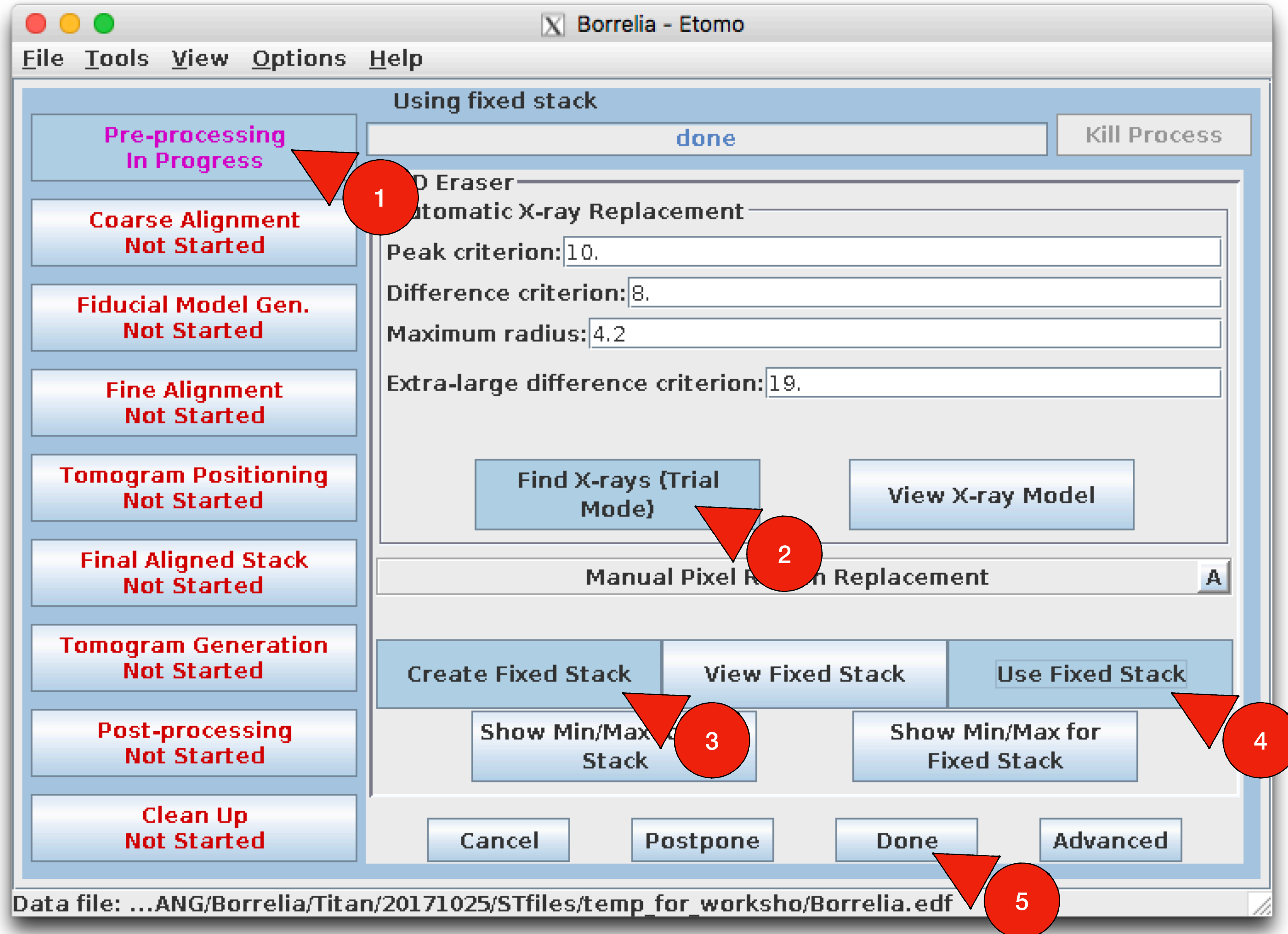


200 nm

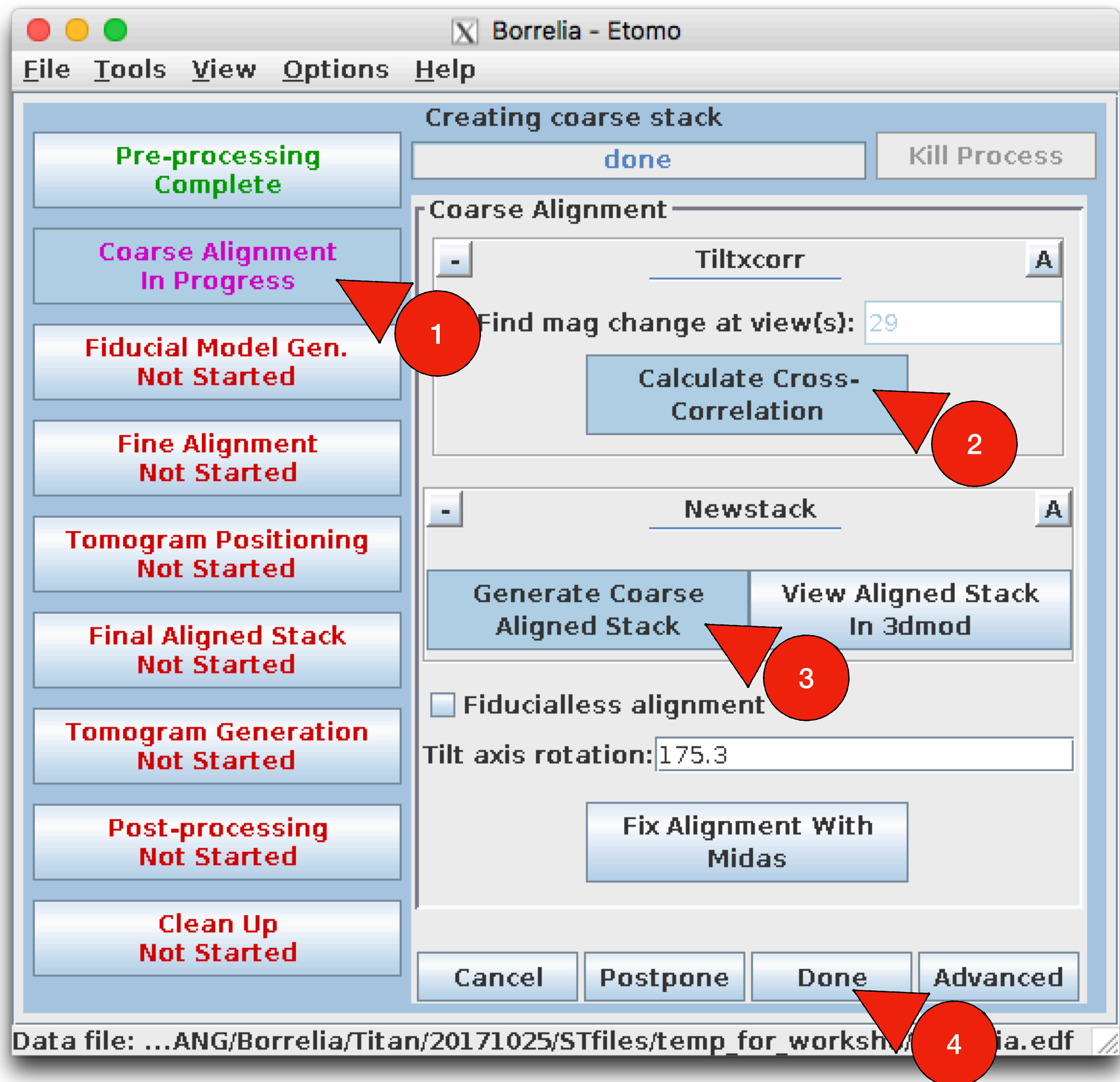
Etomo interface

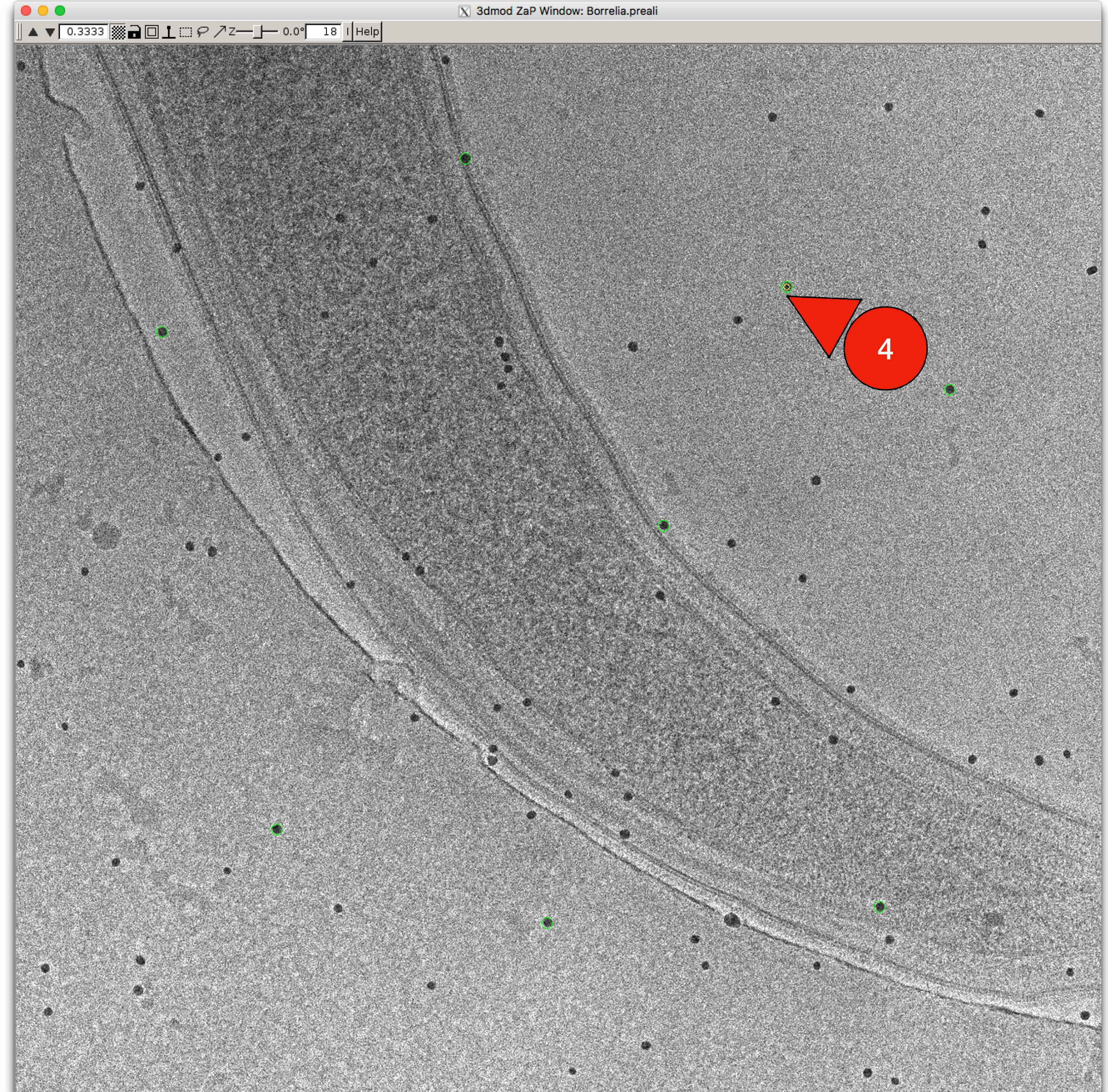
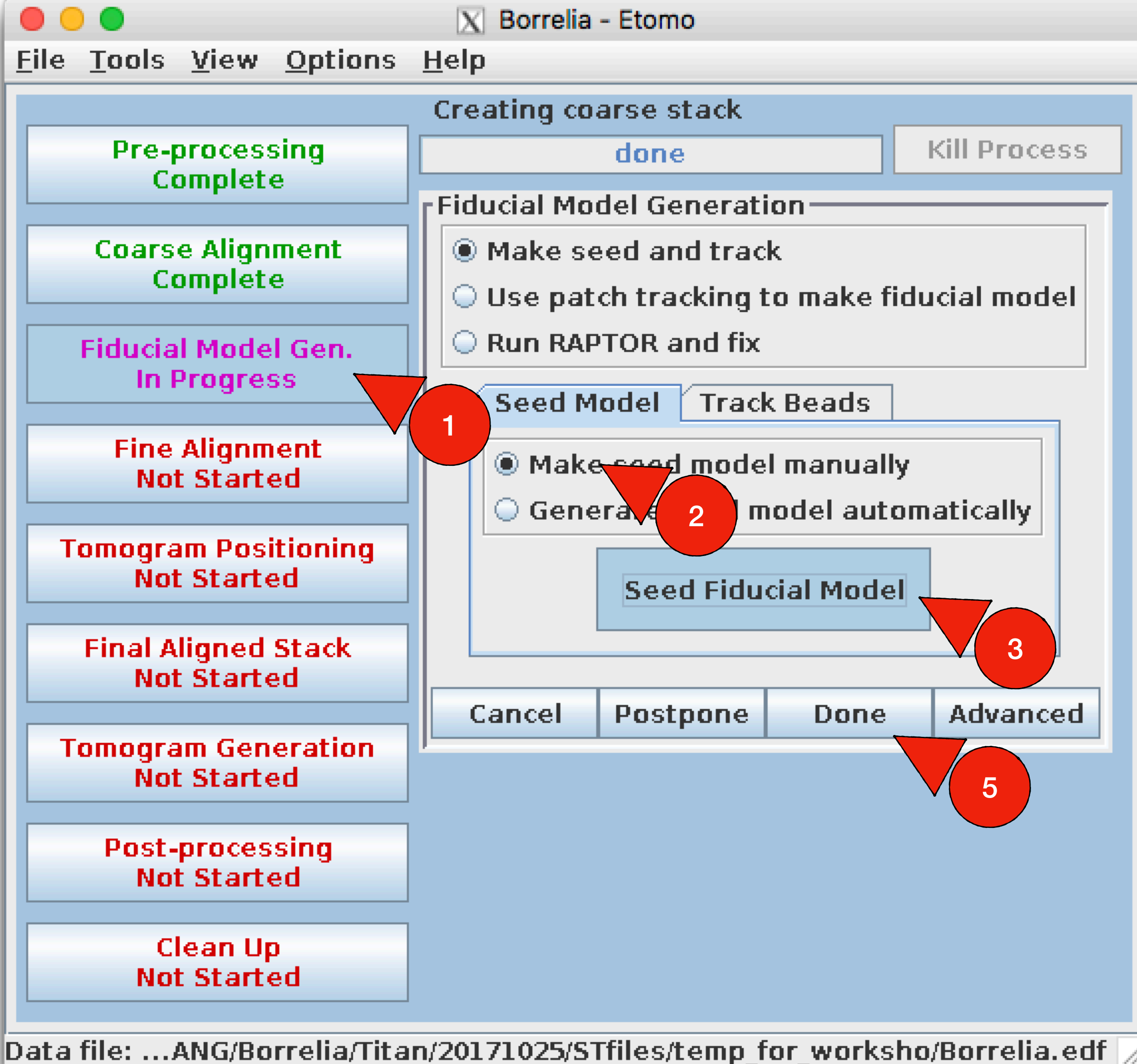


Pre-processing



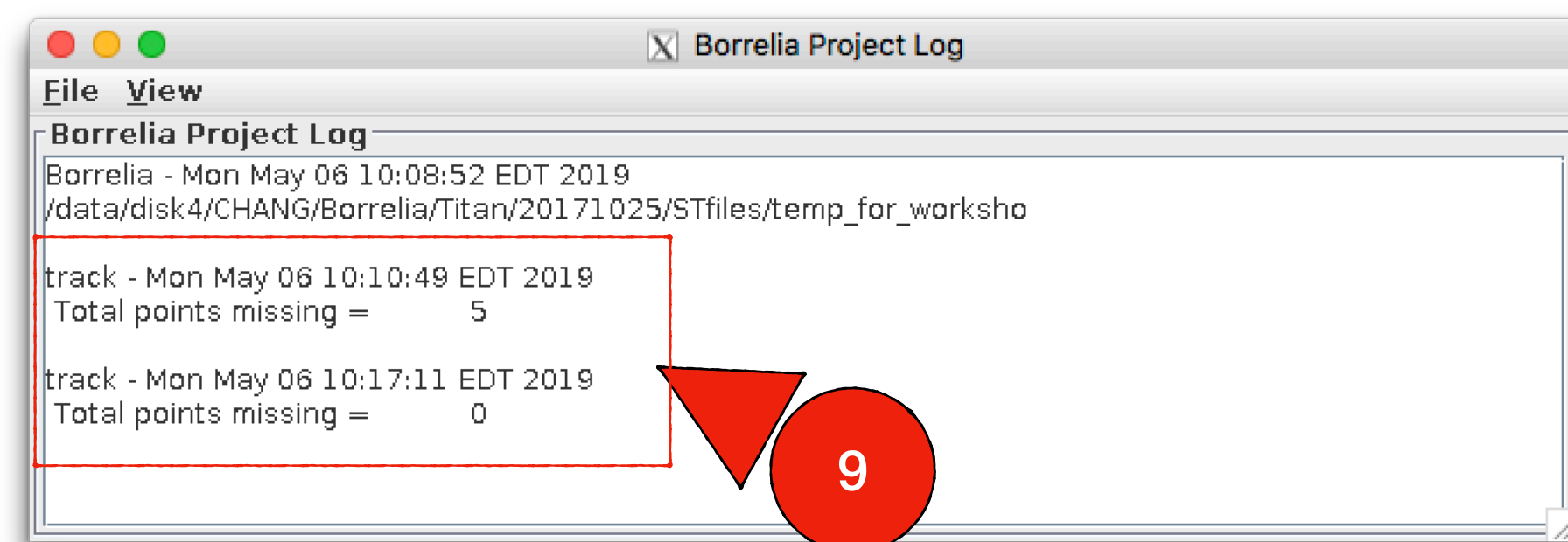
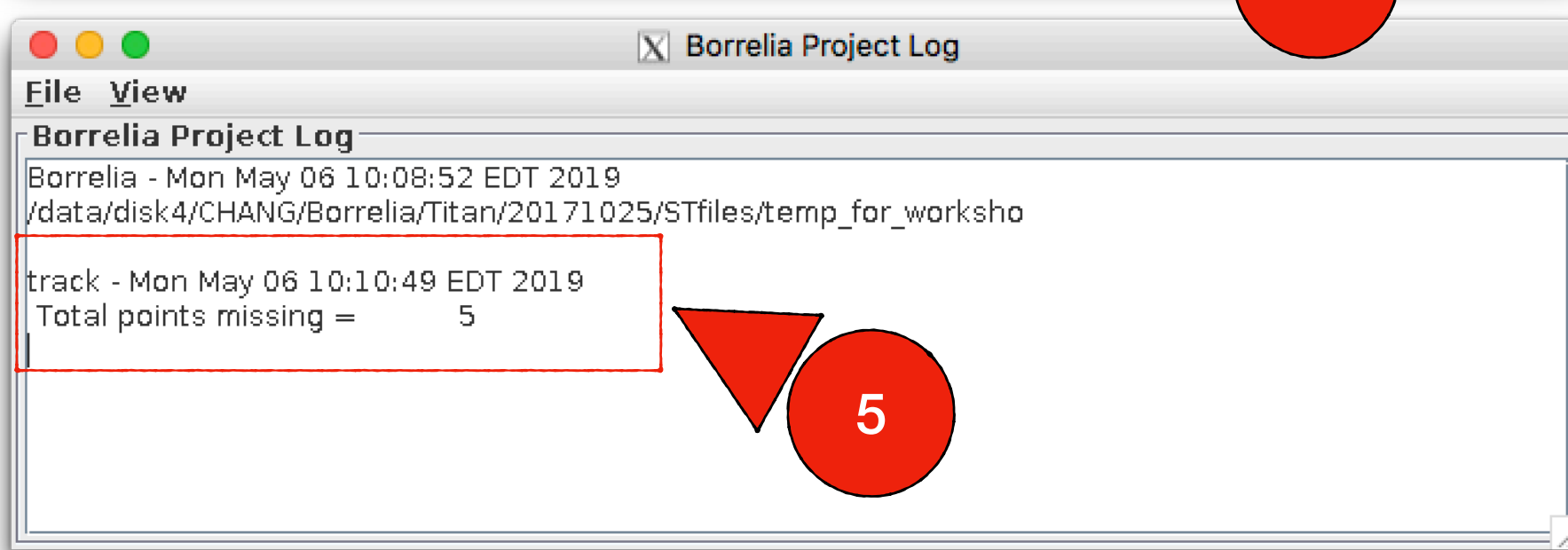
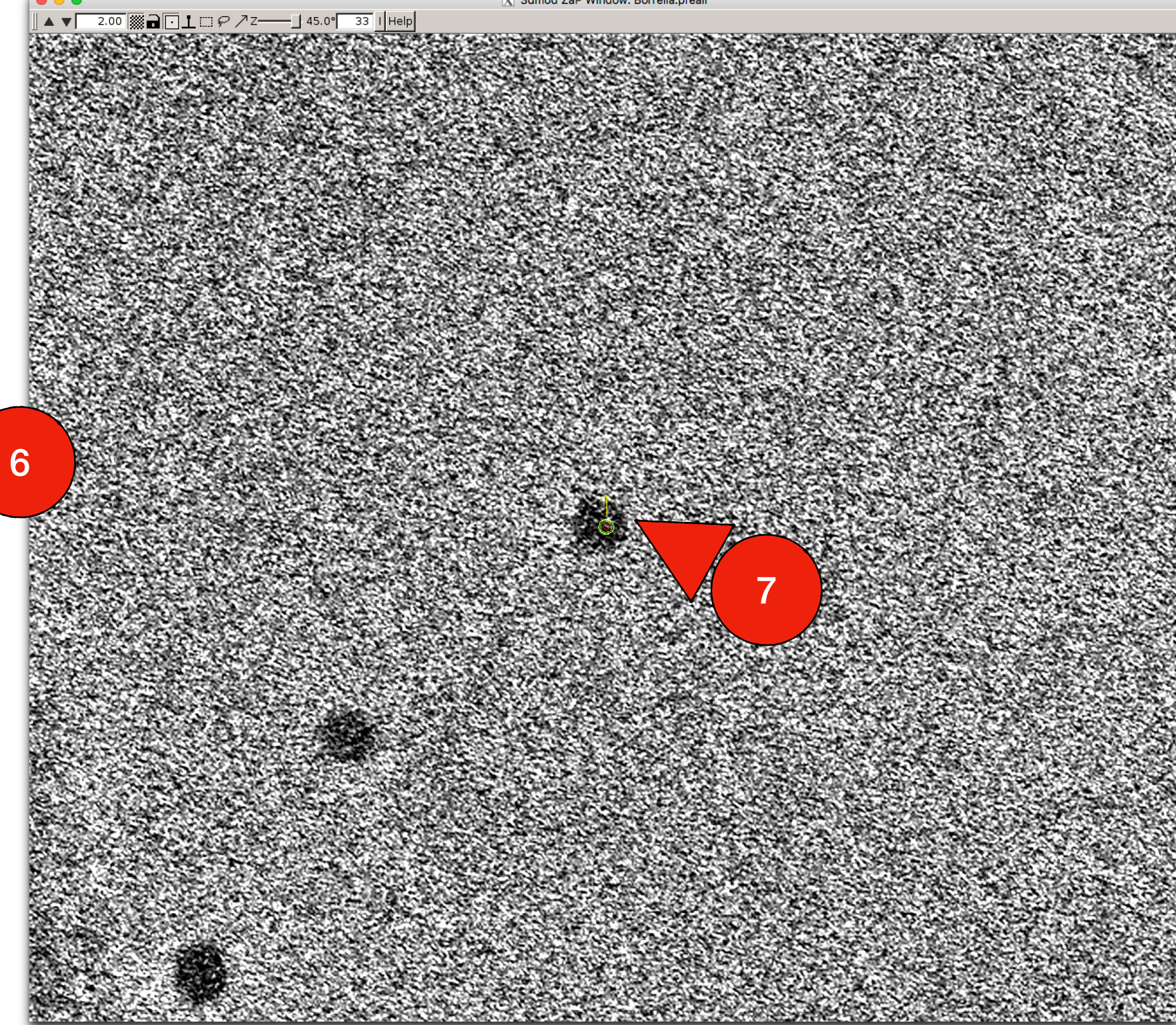
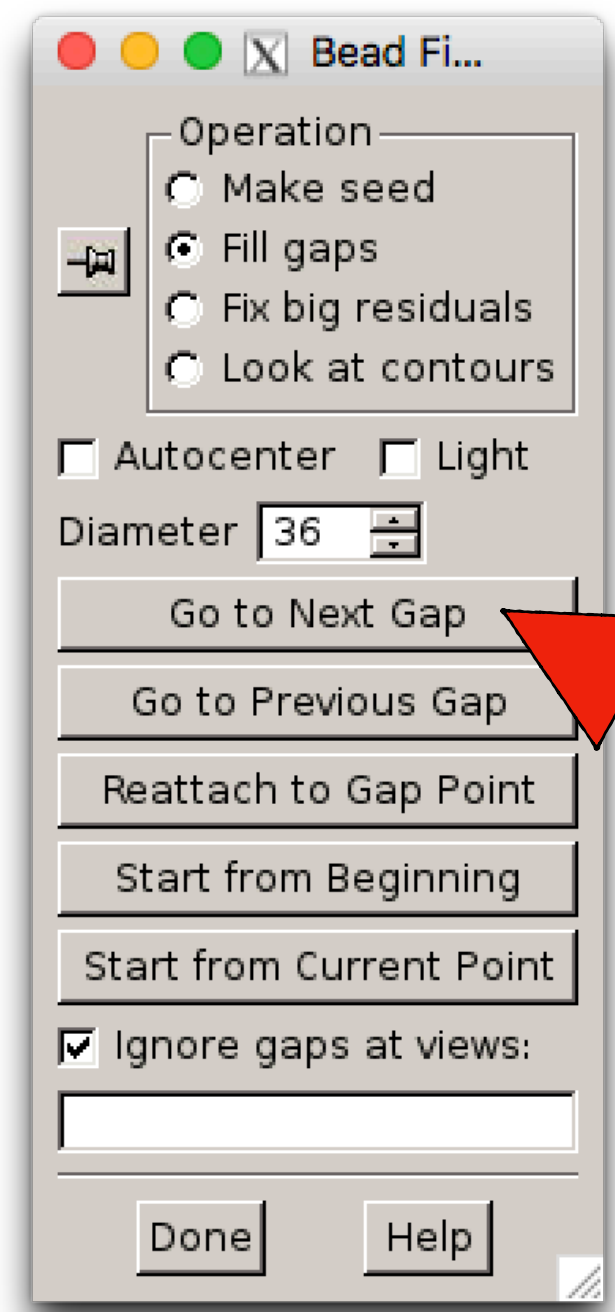
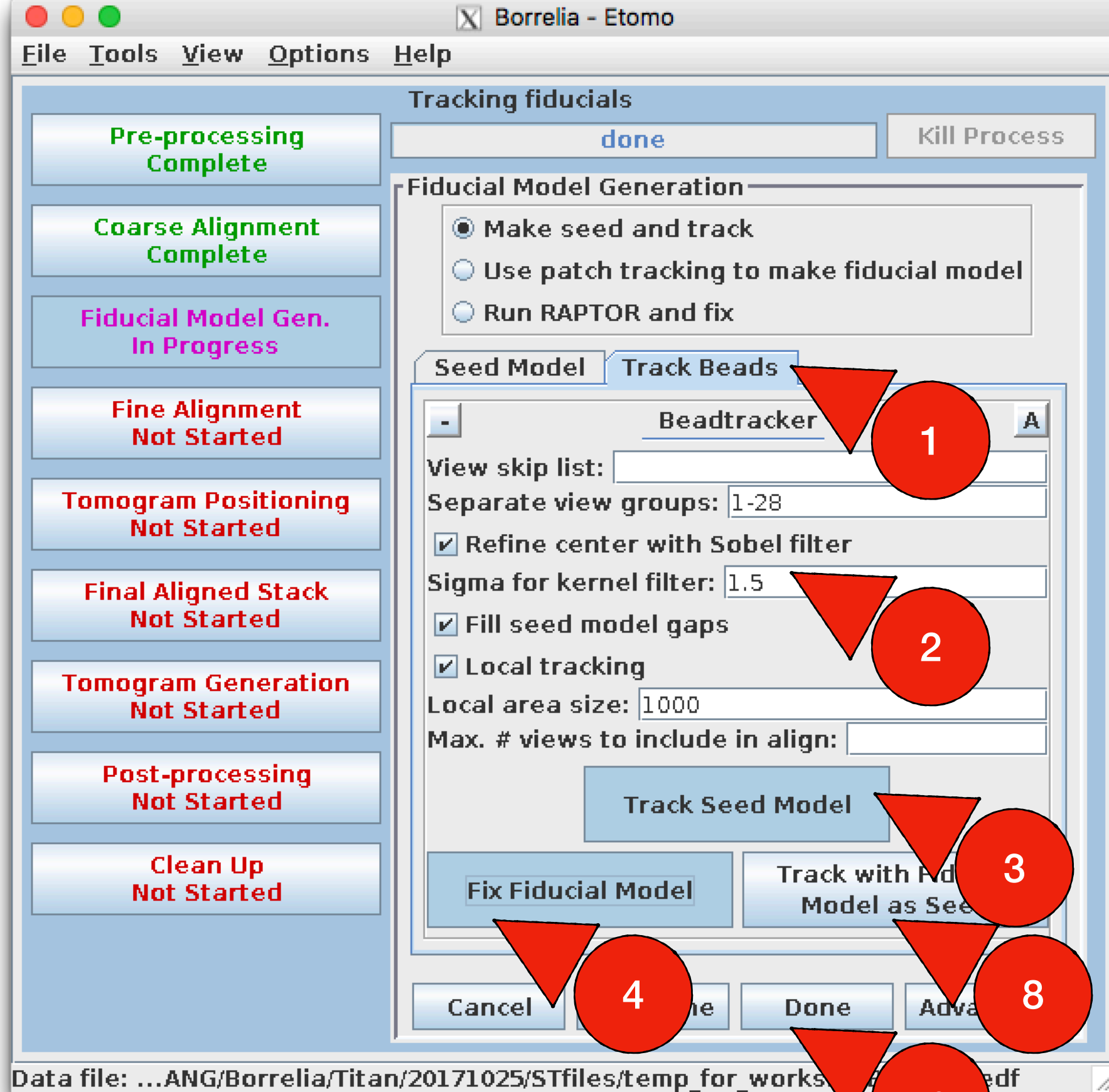
Coarse alignment





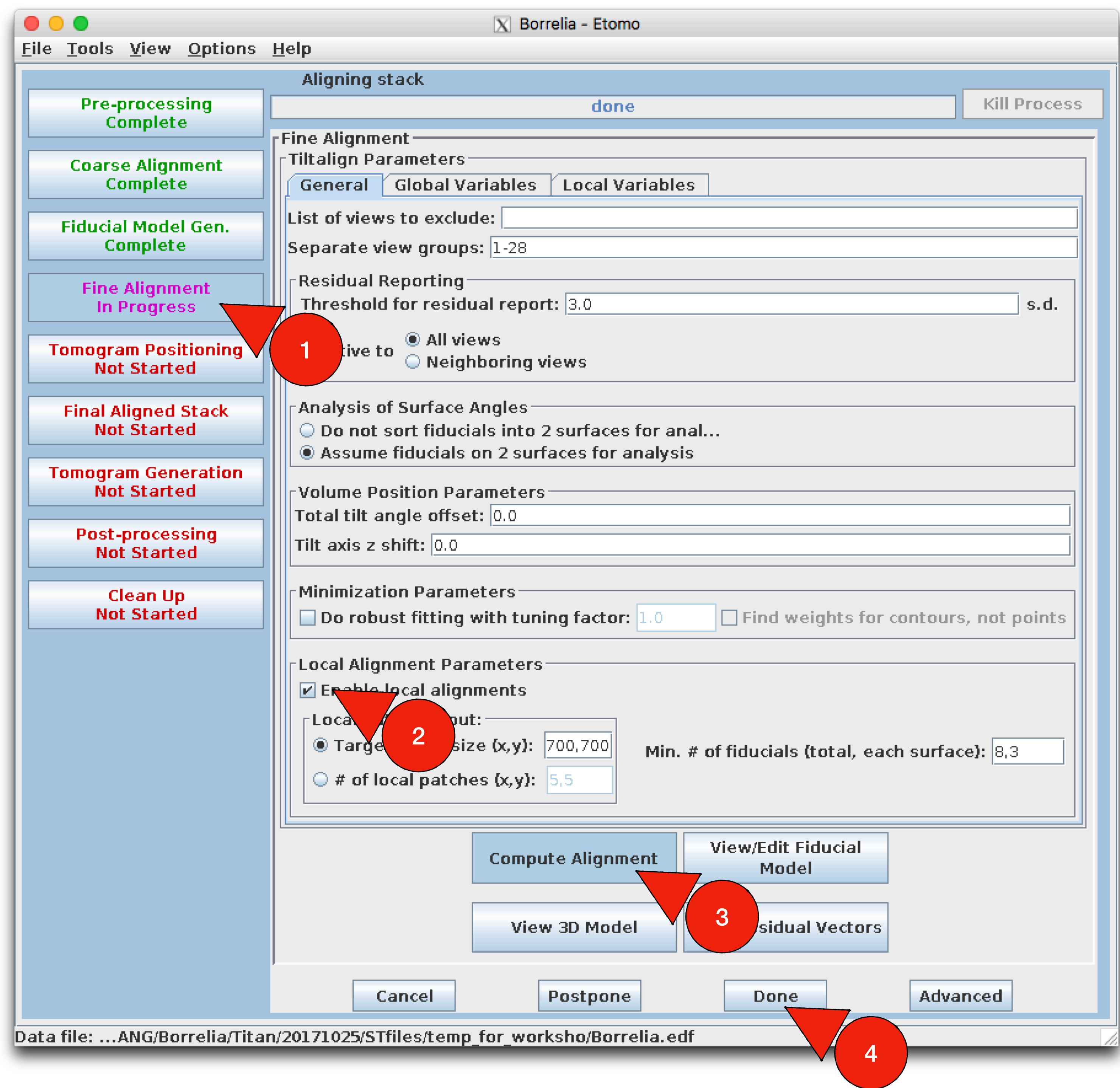
Generate
fiducial model

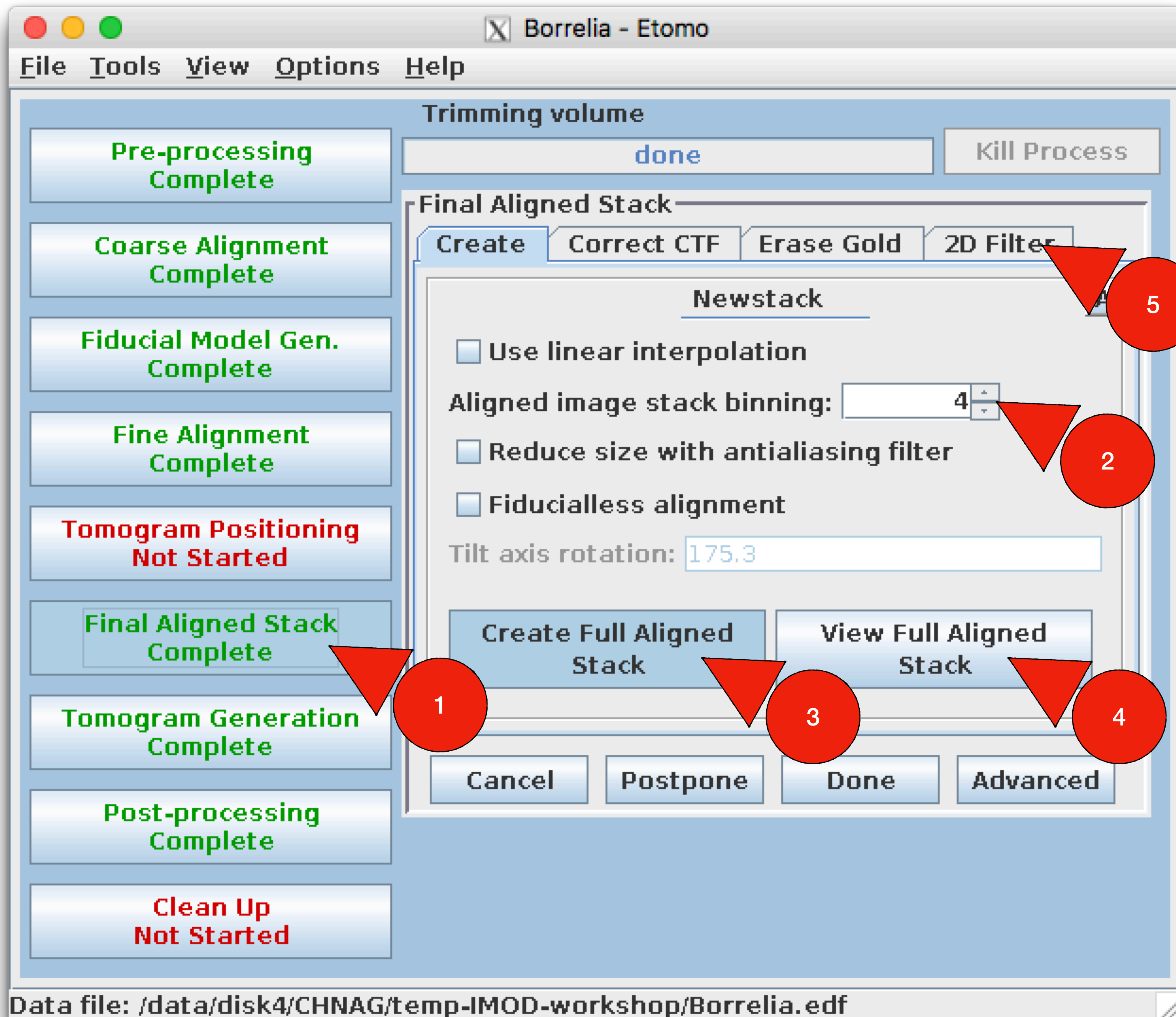
1. Press mouse middle button to select one gold particle
2. Then press "N" in keyboard to create a new contour and then select another gold particle
3. Choose 5-10 gold particles in different areas
4. Close IMOD and save the model



1. Track seed model (step 1-3), then check the total missing points
2. Step 7: press “page up” or “page down” if you see up or down arrow; then press mouse middle button to add a new point at the gold particle position.
3. Repeat step 6-7 until you add all missing points; then close IMOD and save the model.

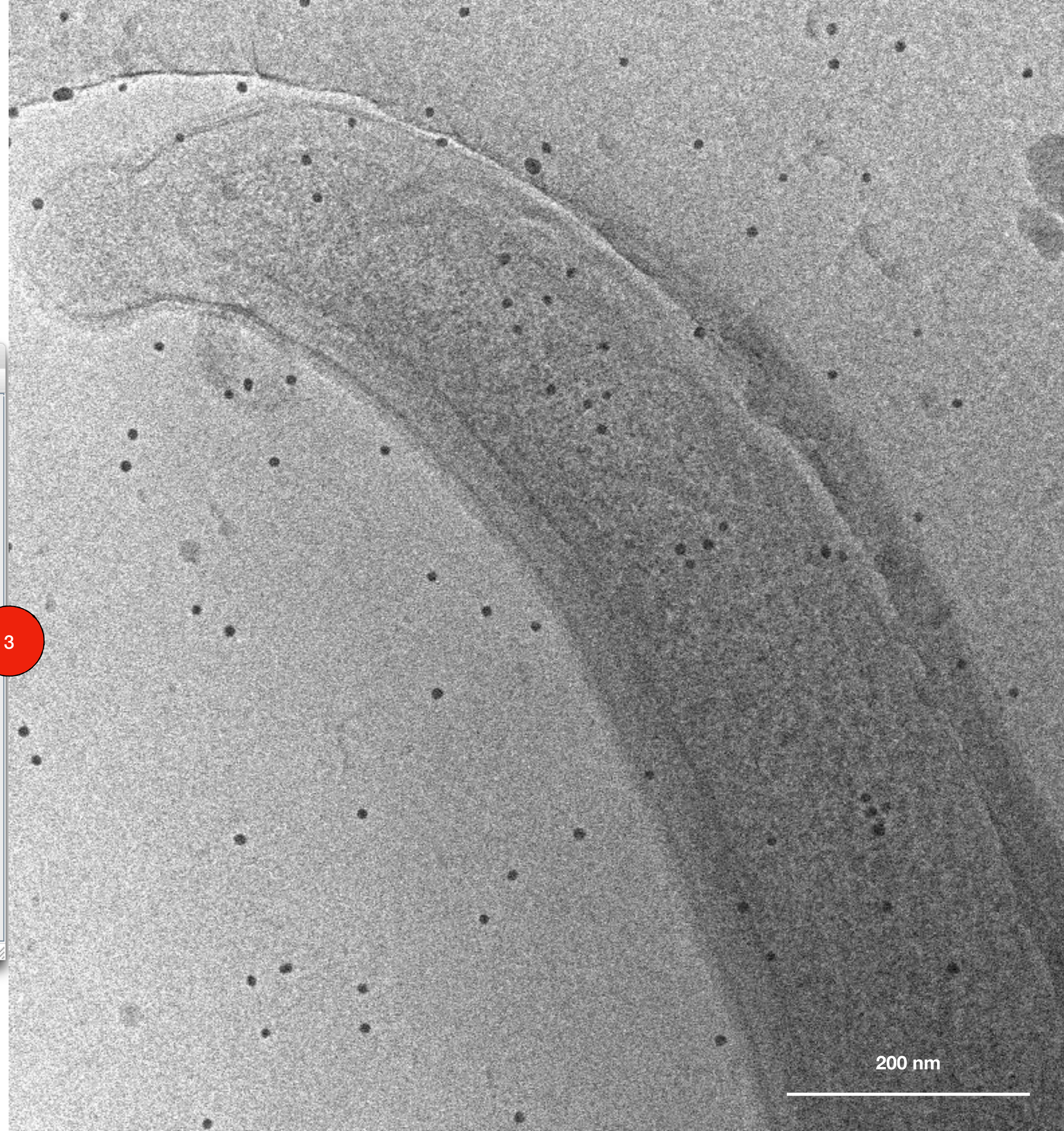
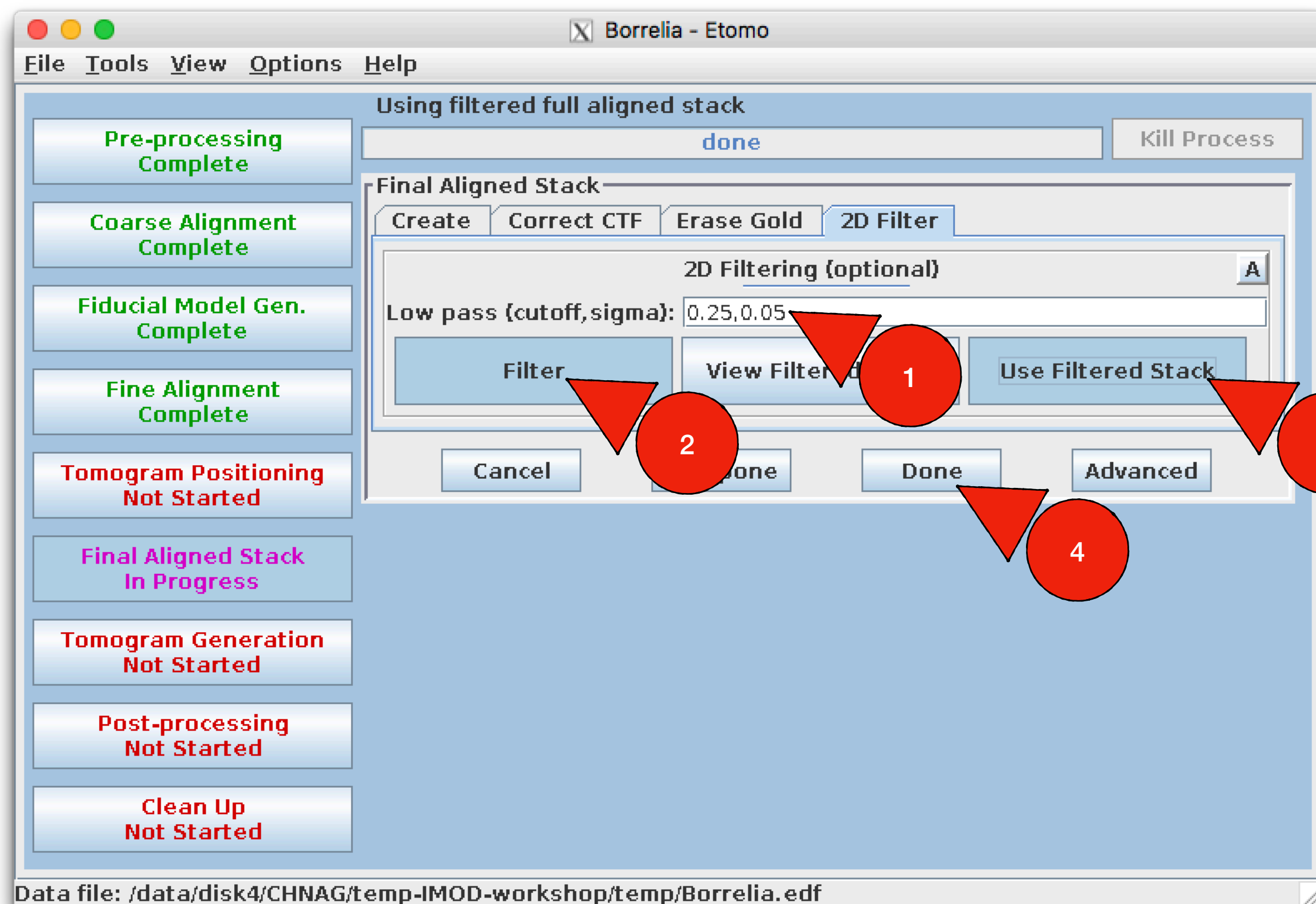
Final alignment of fiducial

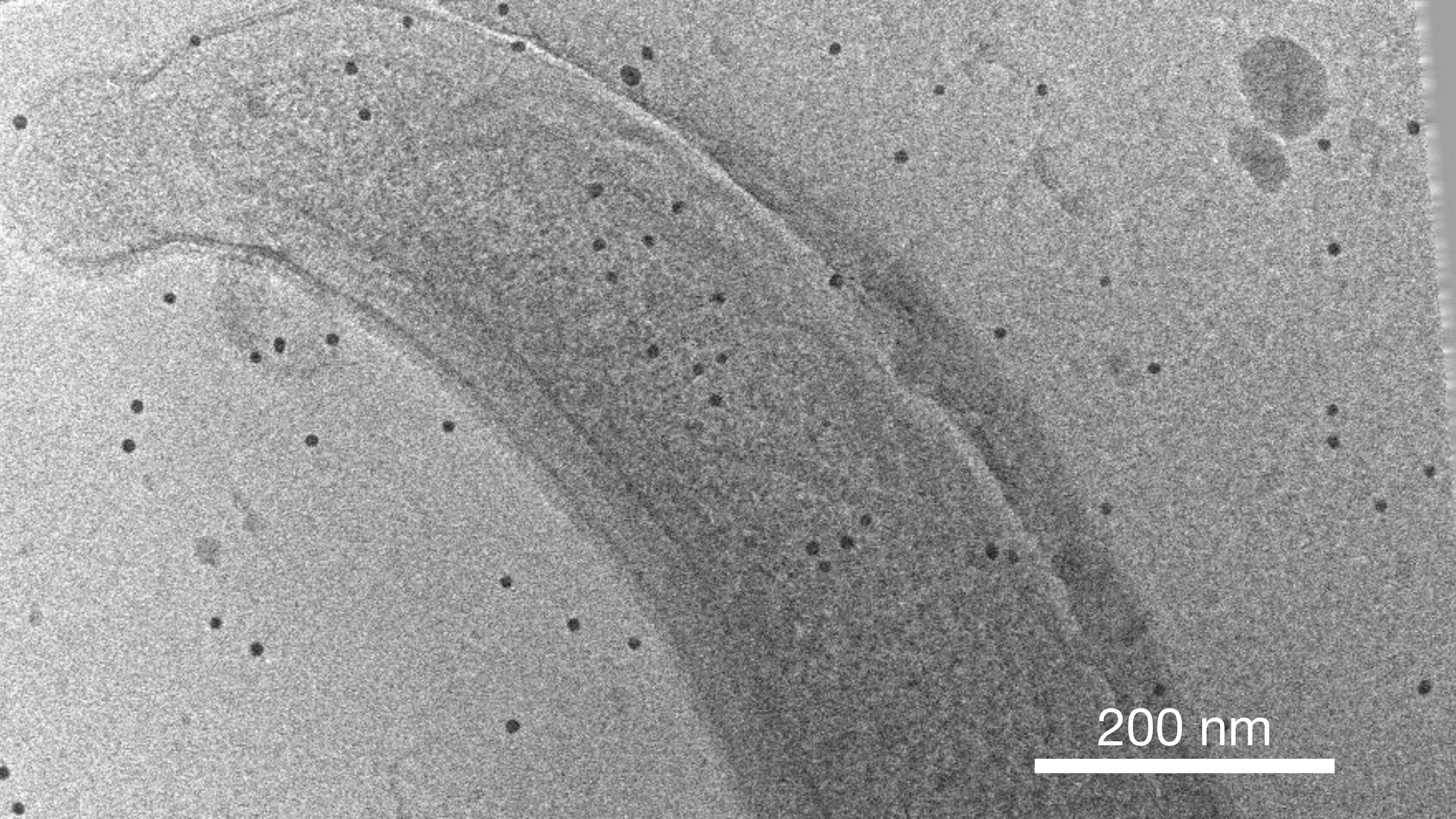




Generate aligned tilt series

The binning number in step 2 determines the binning factor of the aligned tilt series and the reconstructed tomogram. You can change it to any integer you want.





200 nm

Tomogram reconstruction

More details about WBP (weight back projection) and SIRT (simultaneous iterative reconstruction):

[https://en.wikipedia.org/wiki/Tomographic_reconstruction#Back_Projection_Algorithm\[2\]](https://en.wikipedia.org/wiki/Tomographic_reconstruction#Back_Projection_Algorithm[2])

Trimming volume: done

Parallel Processing - tilt

GPU	# GPUs	Load Average	CPU Type	Speed	RAM	OS	Restarts	Finished	Failure
	Used	Max.	1 Min.	5 Min.				Chunks	Reason
<input checked="" type="checkbox"/> localhost	1	3	0.14	1.47				7	
<input type="checkbox"/> frodo	1				AMD 64	2.4	2.0	linux	
<input type="checkbox"/> pippin	0				G5	2.3	5.0	OSX	

GPUs: 1 Restart Load Nice: 15 Pause Resume Save As Defaults

Use a cluster

Tomogram Generation

Back Projection SIRT

Parallel processing

Use the GPU: Maximum number of GPUs recommended is 3

Take the sum of densities with offset: 0.0

Tomogram thickness in Z: 1800 Z shift: 0.0

X axis tilt: 0.0

Radial Filtering

Standard Gaussian cutoff: 0.25 Falloff (sigma): 0.05

Hamming-like filter (as in tomo3d) starts at: 0

Use SIRT-like filter equivalent to: 20 iterations

Use local alignments

Use Z factors

Generate Tomogram View Tomogram In 3dmod Delete Intermediate Image Stacks

Cancel Postpone Done Advanced

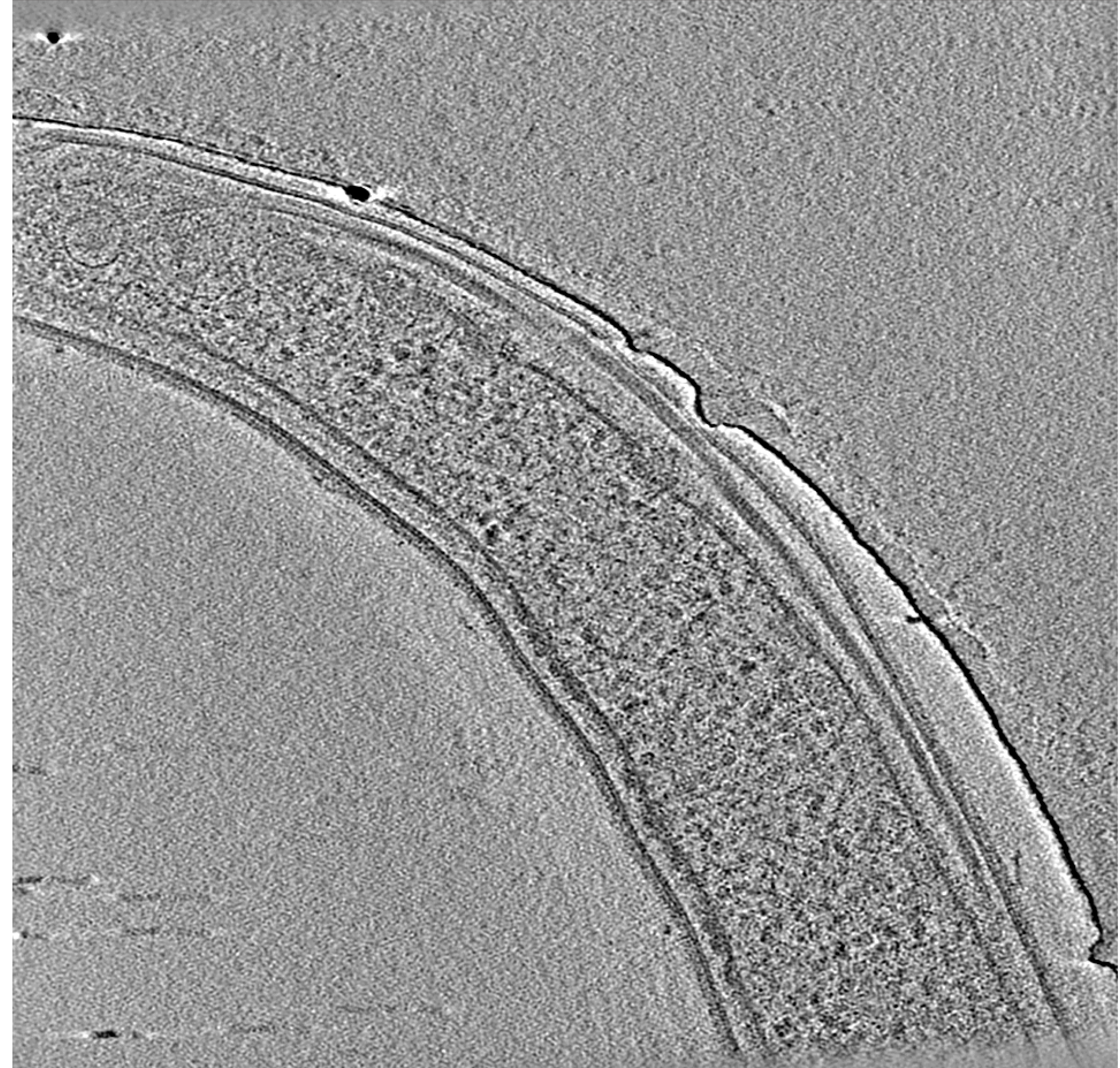
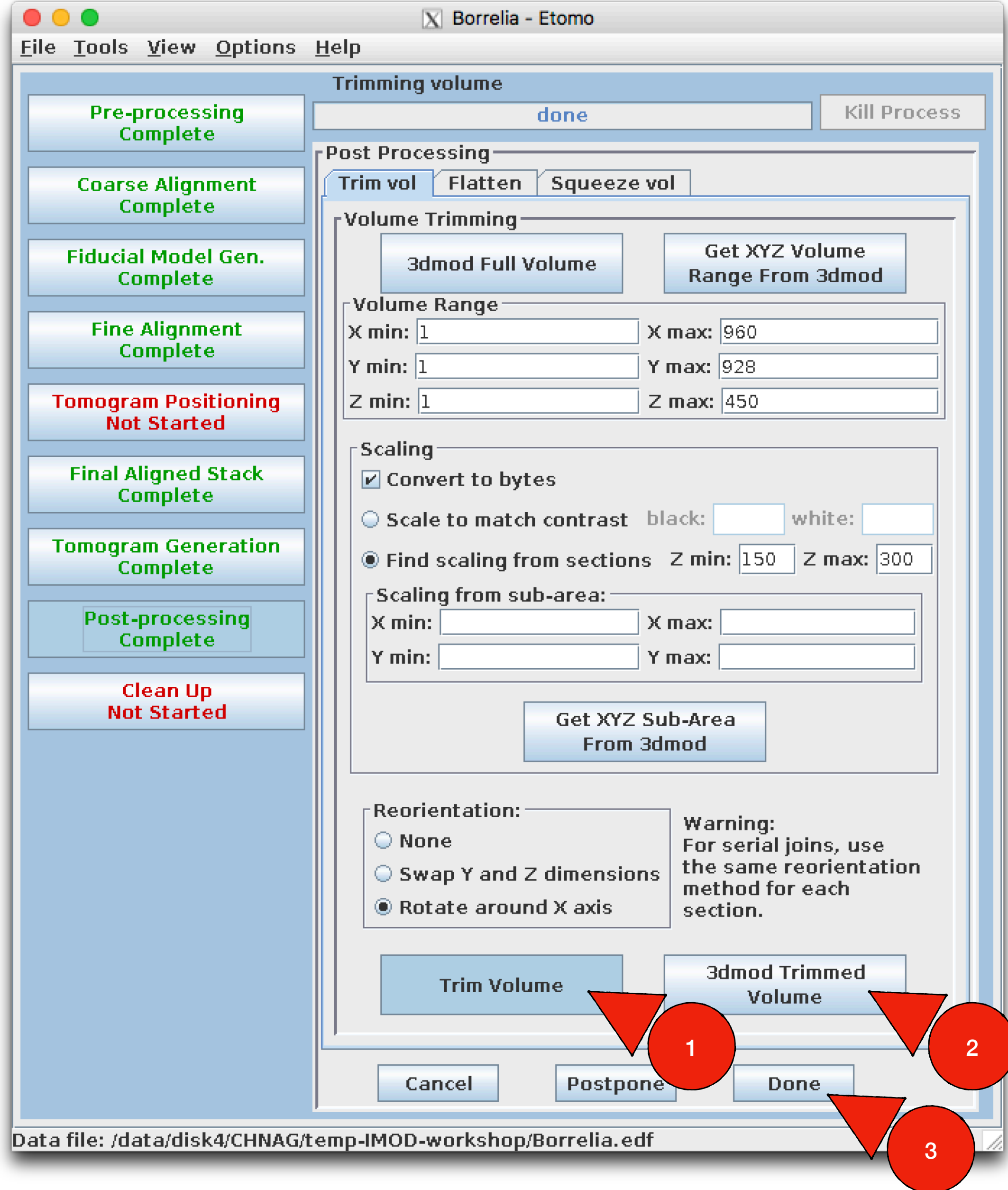
Data file: /data/disk4/CHNAG/temp-IMOD-workshop/Borrelia.edf

Tomogram reconstruction

Save following files:

Borrelia.st Borrelia.ali Borrelia.rec Borrelia.rawtlt Borrelia_fid.xf

Other files can be deleted



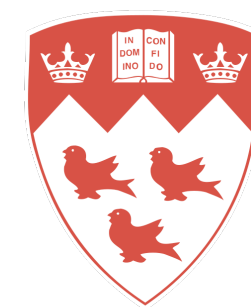


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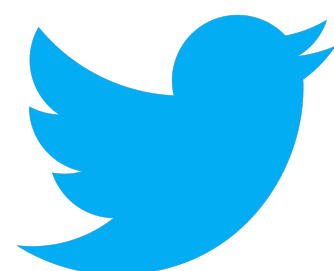
Thank you!

Comments & questions?

shuaiqi.guo@mcgill.ca

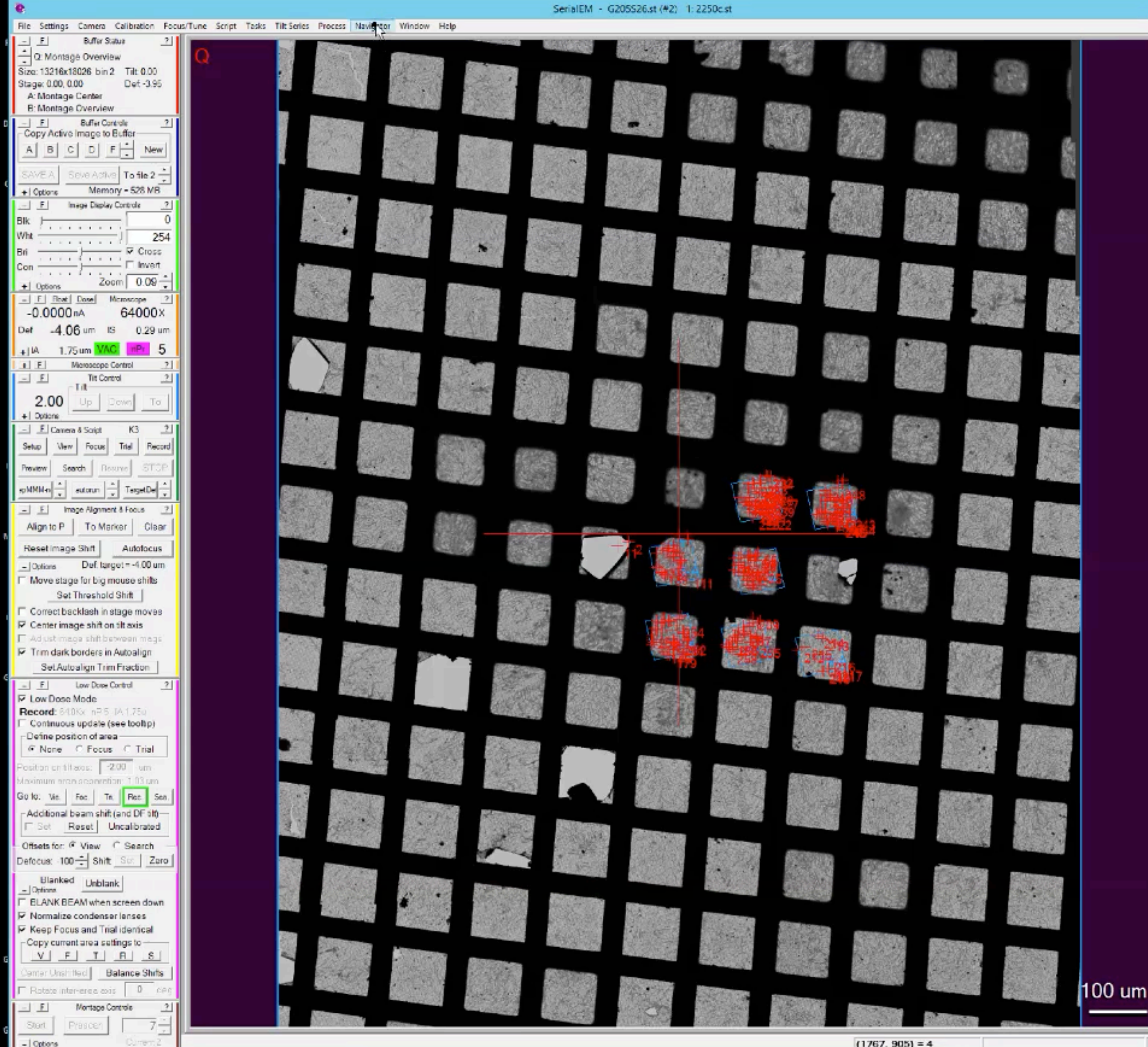


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@JunLiuLab





File Settings Camera Calibration Focus/Tune Script Tasks Tilt Series Process Navigator Window Help

Buffer Status
Q: Montage Overview
Size: 13216x18026 bin2 Tilt: 0.00
Stage: 0.00, 0.00 Def: -3.95
A: Montage Center
B: Montage Overview

Buffer Controls
Copy Active Image to Buffer
A B C D F New

SAVE A Save Active To file 2
Options Memory - 526 MB

Image Display Controls
Blk 0
Whi 254
Bri
Con
Zoom: 0.069

Microscope
-0.0000 nA 64000X
Def: -4.06 um IS: 0.29 um
IA: 1.75 um MAG: nPS: 5

Tilt Control
Tilt: 2.00
Up Down To

Camera & Script K3
Setup View Focus Tilt Record
Preview Search Record STOP
epMMH autorun TargetDe

Image Alignment & Focus
Align to P To Marker Clear
Reset image Shift Autofocus
Options Def. target = -4.00 um
Move stage for big mouse shifts
Set Threshold Shift
Correct backlash in stage moves
Center image shift on tilt axis
Adjust image shift between maps
Trim dark borders in Autoalign
Set Autoalign Trim Fraction

Low Dose Control
Low Dose Mode
Record: 540Kx nPS IA 1.75u
Continuous update (see tooltip)
Define position of area
None Focus Trial
Position on tilt axis: -2.00 um
Maximum area correction: 1.03 um
Go to: Vis. Foc. Tilt. Rec. Set.
Additional beam shift (and DF tilt)
Set Reset Uncalibrated
Offsets for: View Search
Defocus: 100 Shift Set Zero
Blanked Unblank
ELANK BEAM when screen down
Normalize condenser lenses
Keep Focus and Tilt identical
Copy current area settings to
V F T A S
Center Unshifted Balance Shifts
Rotate interference axis 0 deg

Montage Controls
Start Progress 7
Options Current: 2

Navigator: G2055.nav

Label 1 Registration point 1 Camera point (C) 2
Color Blue Draw Rotate when load For anchor state

#1 Note: Sec 0 - 175k-bomelia-20210613-gnd1.st

Acquire (A) Tilt series New file error New file at group
Set: File Props Imaging Slots TS Points Filenames Focus Pos

Add Stage Pos Registration 1 Draw: All reg None Labels
Add Points Collapse Show Acquire Edit mode Edit Focus
Add Polygon Label Color X Y Z Type Reg. Acq. Note

	Blu	2.3	7.5	80.6	Map 1	Sec 0 - 175k-bomelia-2
Group of 1 items, ID ..2532, labels 2 to 2						
Group of 2 items, ID ..6772, labels 3 to 4						
11 Red 16.5 -100.6 78.1 Pt 1						hole
4 A Blu 67.4 139.1 78.1 Map 1						Sec 0 - 2250c.st-
Group of 6 items, ID ..5271, labels 13 to 18						
13 A Blu -53.2 147.1 73.0 Map 1						Sec 1 - 2250c.st-
14 A Blu -33.2 274.7 72.2 Map 1						Sec 2 - 2250c.st-
15 A Blu 49.2 -5.9 80.4 Map 1						Sec 3 - 2250c.st-
16 A Blu 170.5 -23.0 84.0 Map 1						Sec 4 - 2250c.st-
17 A Blu 185.9 106.0 83.6 Map 1						Sec 5 - 2250c.st-
18 A Blu 209.1 231.7 81.3 Map 1						Sec 6 - 2250c.st-
Group of 1 items, ID ..6599, labels 25 to 25						
Group of 26 items, ID ..6385, labels 26 to 51						
Group of 6 items, ID ..4795, labels 52 to 57						
Group of 17 items, ID ..1201, labels 58 to 74						
Group of 36 items, ID ..9489, labels 75 to 110						
Group of 29 items, ID ..3012, labels 111 to 139						
Group of 40 items, ID ..9312, labels 140 to 179						
Group of 30 items, ID ..0065, labels 180 to 209						
Group of 9 items, ID ..3564, labels 210 to 218						
Group of 15 items, ID ..4758, labels 220 to 234						
Group of 5 items, ID ..4192, labels 235 to 239						
Group of 11 items, ID ..1474, labels 240 to 250						
Group of 4 items, ID ..6935, labels 251 to 254						
Group of 4 items, ID ..8252, labels 255 to 258						

Move Item
Update Z
Go To XY
Go To XYZ
Go To Marker
Load Map
New Map
Anchor Map
Delete Item
Realign to Item

Log: log1.log

```
Error above tolerance, retaking shot  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.13.43.tif  
Saved Z = 22, -33.00 degrees  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.13.47.tif  
Saved Z = 23, -36.00 degrees  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.13.52.tif  
Saved Z = 24, -39.00 degrees  
Error above tolerance, retaking shot  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.13.56.tif  
Saved Z = 25, -39.00 degrees  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.14.01.tif  
Saved Z = 26, -42.00 degrees  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.14.07.tif  
Saved Z = 27, -45.00 degrees  
Error above tolerance, retaking shot  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.14.11.tif  
Saved Z = 28, -45.00 degrees  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.14.15.tif  
Saved Z = 29, -48.00 degrees  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.14.59.tif  
Saved Z = 30, 26.99 degrees  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.15.07.tif  
Saved Z = 31, 29.99 degrees  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.15.12.tif  
Saved Z = 32, 32.99 degrees  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.15.17.tif  
Saved Z = 33, 36.00 degrees  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.15.22.tif  
Saved Z = 34, 39.00 degrees  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.15.27.tif  
Saved Z = 35, 42.00 degrees  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.15.32.tif  
Saved Z = 36, 45.00 degrees  
15 frames were saved to X:\DoseFractions\20210613\Jun(G2055C)\Jun14_10.15.37.tif  
Saved Z = 37, 48.00 degrees  
----- end of dose symmetric TS -----  
TS time: 327.25  
total time: 327.25
```

Scripts

- PrepMMM noZ
- autorun
- CycleTargetDefocus
- PrepMMM-Z
- MyFines
- Script 6
- autorun-multi
- autorun-vpp
- SimpleFocus
- Z-check-refill-realign-FR
- short-DSTomo
- FastTomo-test
- FastTomoWholeCell
- Z
- Z-check-refill-realign2
- Script 16
- Script 17
- Script 18
- Script 19
- Script 20
- Script 21
- Script 22
- Script 23
- Script 24
- FastTomo
- OpenFile
- CloseFile
- Script 25
- Script 26