

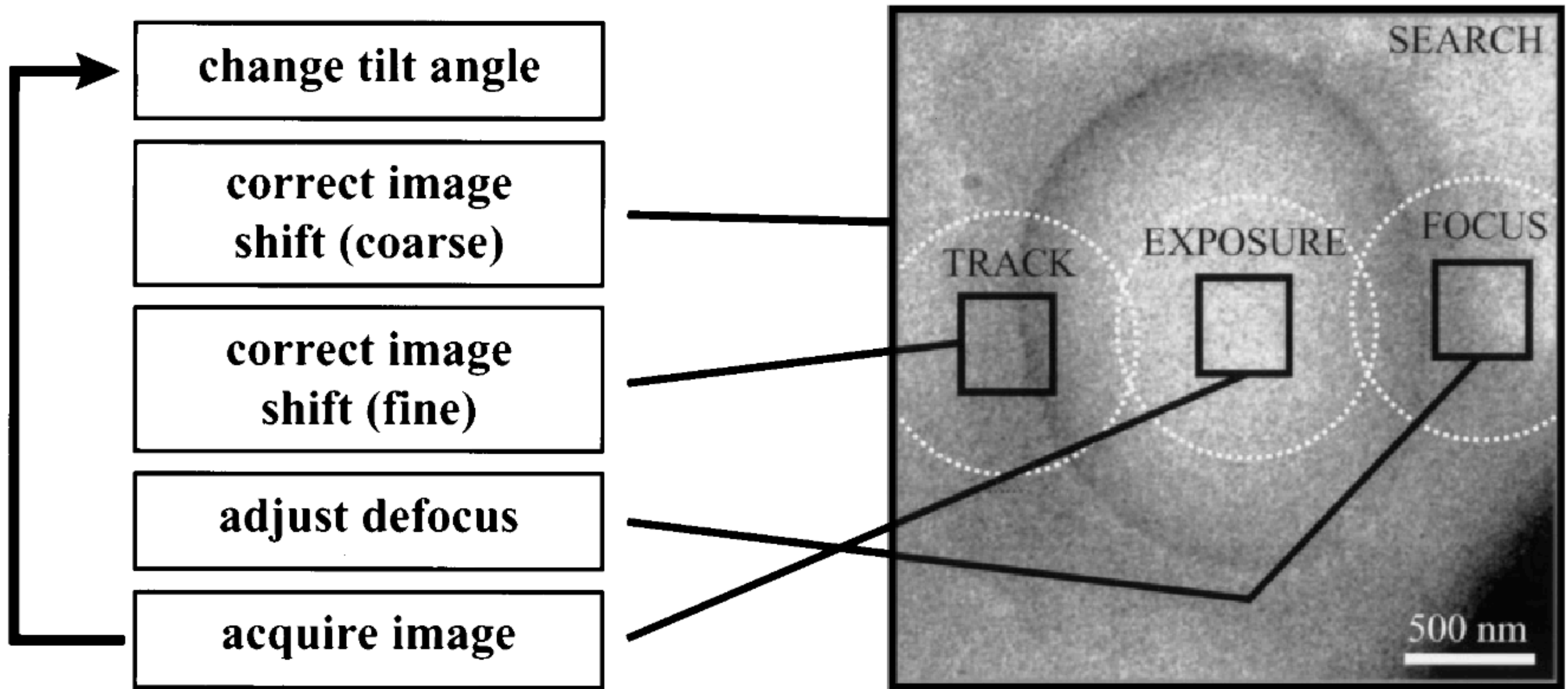
Cryo-ET data collection by SerialEM

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McGill University
June 13 2024

Main goals

1. SerialEM tilt series data collection with FastTomo script
2. Tilt Series alignment by IMOD

Tilt series data collection scheme



Cryo-ET data acquisition packages

SerialEM

UCSF Tomo

Leginon

FEI tomography

EM-Manu

Automated electron microscope tomography using robust prediction of specimen movements

David N. Mastronarde *

2005

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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 image displays the SerialEM software interface, which is used for automated electron microscopy data acquisition. The central window shows a grayscale micrograph of a sample. The interface is divided into several panels:

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

Microscope Panel (Left):

- Label: 1
- Def: -40.00 μm
- Obj: 86.41% VAC Spot 6
- 2300X
- 0.0355 nA

Low Dose Control Panel (Right):

- Low Dose Mode: ☒
- View: 2300x Sp 6 C2 61.41%
- Continuous update of mag & beam: ☒
- Define position of area: ☒ None ☐ Focus ☐ Trial
- Position on tilt axis: 0.00 μm
- Maximum area separation: -0.71 μm
- Additional beam shift: ☐ Set ☐ Reset 0.00, 0.00
- Area to show when screen down: ☐ Vie. ☒ Foc. ☐ Tri. ☐ Rec. ☐ Sea.
- BLANK BEAM when screen down: ☐ Blanked
- Offsets for View: Defocus: -60 Shift:
- Normalize beam through View: ☐ Keep Focus and Trial identical: ☒
- Copy current area mag & beam to:
- Center Unshifted
- Rotate inter-area axis: 0 deg

K2 Direct Detection Panel (Right):

- Mode: Counted
- HW Processing: ☒ Background Subtraction ☒ Gain Correction
- Health Status: ☒

Camera View Panel (Right):

- Setup: Search
- Auto Exposure: ☐
- Exposure (s): 0.5
- Focus Loupe: ☐
- Auto Survey: ☒
- Camera Inserted: ☒

Navigator Panel (Right):

- Label: 1 ☐ Registration point 1 ☐ Corner point (C)
- Color: Blue ☒ Draw ☐ Rotate when load ☐ For anchor stat
- #1 Note: Sec 0 - montage01.st
- Acquire (A) ☐ Tilt series ☐ New file at item ☐ New file at group
- Set:
- 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. No
- Add Marker: 1 Blu -325.2 -232.6 118.7 Map 1
- Move Item

SerialEM (David Mastronarde)



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The image displays the SerialEM software interface. A central micrograph shows a grayscale image of a sample. Overlaid on the micrograph is a red rounded rectangle containing the text: Defocus, Magnification, Stage tilt angle. The interface includes several panels: Buffer Status (top left), Buffer Controls (middle left), Image Display Controls (bottom left), Camera & Macro Controls (bottom left), Low Dose Control (top right), K2 Direct Detection (middle right), Camera View (bottom right), and Navigator (bottom right). The Navigator panel shows a list of acquisition points with columns for Label, Color, X, Y, Z, Type, Reg, and Acq. No.

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

Con 0.47

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

Low Dose Control

Low Dose Mode

View: 2300x Sp 6 C2 61.41%

Continuous update of mag & beam

Define position of area

None Focus Trial

Position on tilt axis: 0.00 um

Maximum area separation: -0.71 um

Additional beam shift

Set Reset 0.00, 0.00

Area to show when screen down

View Foc Tri Rec Sea

BLANK BEAM when screen down

Blanked Unblank Search

Options

Offsets for View

Defocus: -60 Shift Set Zero

Normalize beam through View

Keep Focus and Trial identical

Copy current area mag & beam to

V F T R

Center Unshifted Balance Shifts

Rotate inter-area axis 0 deg

K2 Direct Detection

Mode: Counted

HW Processing

Background Subtraction

Gain Correction

Update HW Dark Reference

Health Status

Camera View

Setup: Search

Auto Exposure

Exposure (s) 0.5

Start View

Focus Loupe

Auto Survey

Camera Inserted

Camera Acquire

Navigator

Label: 1 Registration point 1 Corner point (C)

Color Blue Draw Rotate when load For anchor stat

#1 Note: Sec 0 - montage01.st

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

Set File Properties Imaging Stats 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 No

Add Marker 1 Blu -325.2 -232.6 118.7 Map 1

Move Item

SerialEM (David Mastronarde)



Brookhaven
National Laboratory

The image shows the SerialEM software interface. The central window displays a grayscale microscopy image of a sample. Overlaid on this image are three red rounded rectangles containing text:

- Defocus Magnification Stage tilt angle** (top rectangle)
- View Focus Trial Record** (bottom-left rectangle)
- Setup Preview** (bottom-right rectangle)

The interface includes several panels:

- Buffer Status:** Shows montage overview, size (2116 x 2136 bin 8), tilt, stage coordinates, and defocus (-40.00).
- Buffer Controls:** Includes buttons for copying images to buffers (A, B, C, D, E) and saving them.
- Image Display Controls:** Features sliders for brightness, contrast, and zoom, along with checkboxes for invert and auto exposure.
- Microscope:** Displays current parameters: 0.0355 nA, 2300X magnification, -40.00 um defocus, 86.41% objective, and VAC/Spot settings.
- Tilt Control:** Includes a tilt slider and buttons for up, down, and to.
- Camera & Macro Controls:** Contains buttons for Setup, View, Focus, Trial, Record, Preview, End, Resume, and STOP.
- Low Dose Control:** Includes checkboxes for Low Dose Mode, Continuous update of mag & beam, and Define position of area (None, Focus, Trial).
- K2 Direct Detection:** Includes a Mode dropdown (Counted) and checkboxes for Background Subtraction and Gain Correction.
- Camera View:** Includes a Setup dropdown (Search) and checkboxes for Auto Exposure, Focus Loupe, Auto Survey, and Camera Inserted.
- Navigator:** Includes a Label dropdown (1), checkboxes for Registration point and Corner point, and a table for stage positions.

SerialEM — Navigator



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The image displays the SerialEM Navigator software interface. The central window shows a grayscale micrograph of a sample. On the left, there are several control panels: Buffer Status, Buffer Controls, Image Display Controls, Microscope, Tilt Control, and Camera & Macro Controls. The right panel, titled 'Navigator', is highlighted with a red border and contains various controls for managing the acquisition. An orange arrow points to the 'Add Points' button in the Navigator panel. Below the buttons is a table with columns for Label, Color, X, Y, Z, Type, Reg, Acq, and Note.

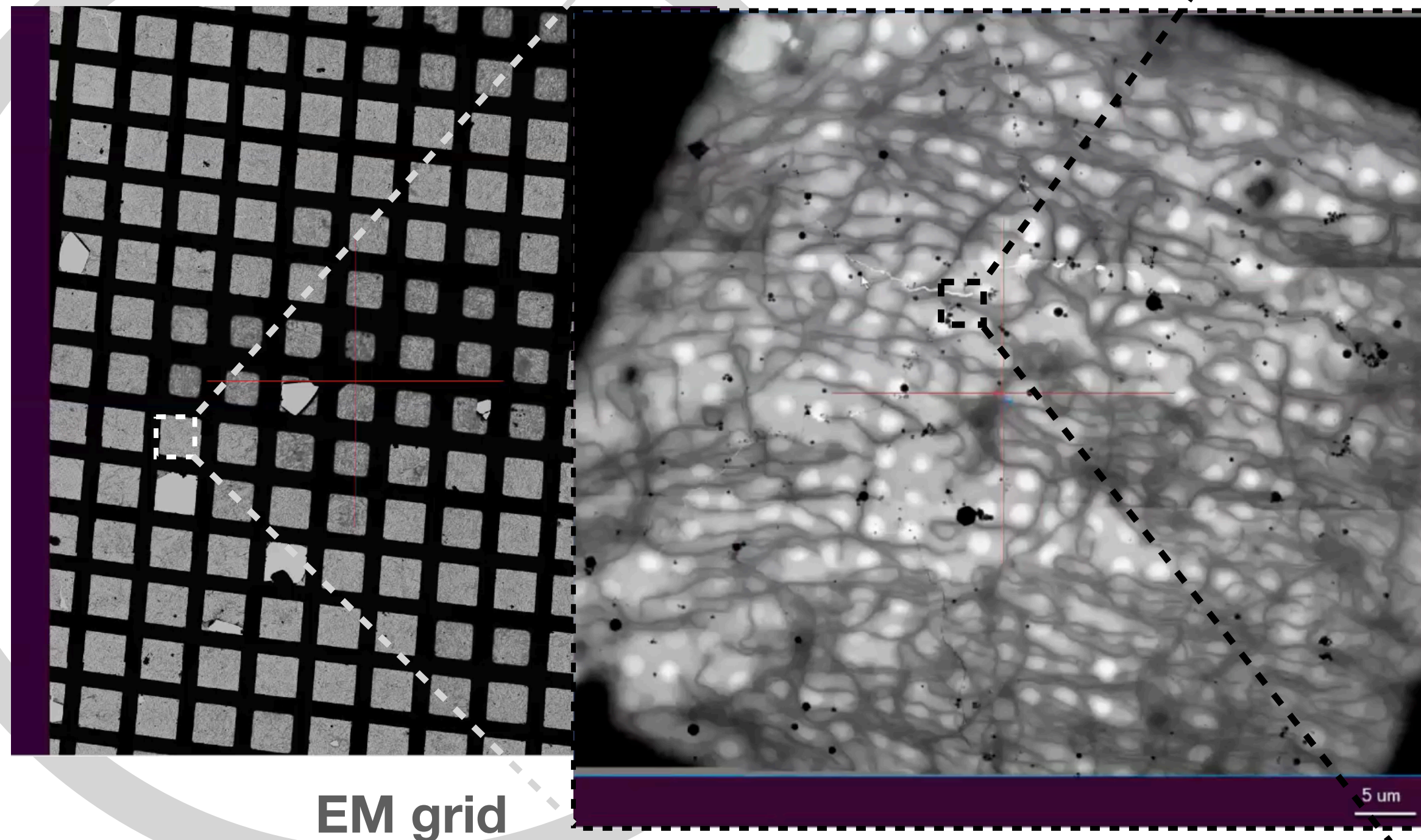
Label	Color	X	Y	Z	Type	Reg	Acq	Note
1	Blu	-325.2	-232.6	118.7	Map	1		

Find targets for tilt series data acquisition

1. Obtain 175 X full montage to survey the grid

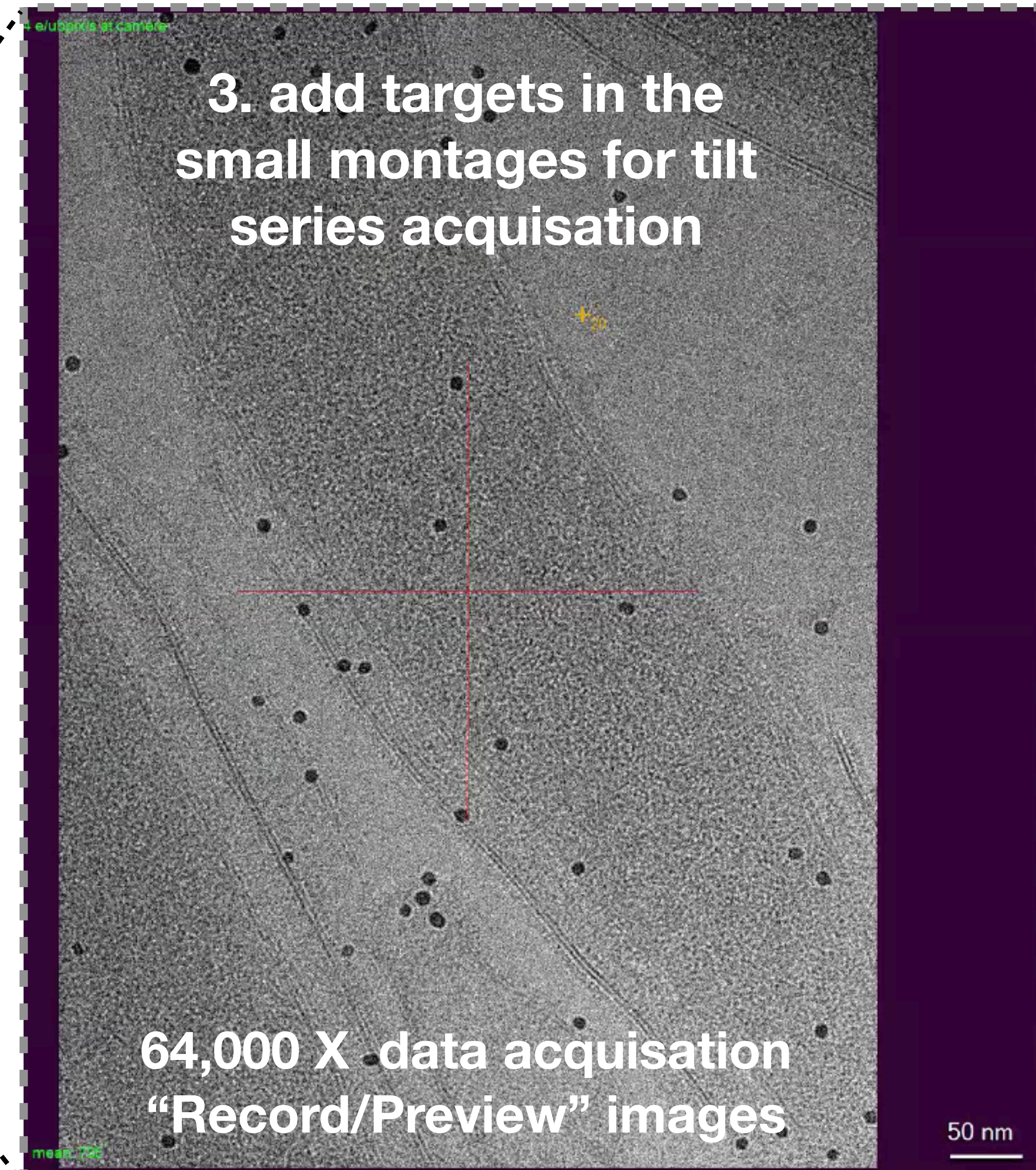
2. Obtain multiple small montages (2250X) with many target cells

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



6 x 6 “Search” images

5 x 3 “View” images



64,000 X data acquisition
“Record/Preview” images

Full-montage (175x) acquisition

SerialEM - G205526.st (#2) 1: 2250c.st

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

Buffer Status
Q: Montage Overview
Size: 13216x18026 bin 2 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 = 528 MB

Image Display Controls
Blk: 0
Whi: 254
Bri: 0
Con: 0
Zoom: 0.08

Microscope
-0.0000 nA 64000X
Def: -4.06 um IS: 0.29 um
W: 1.75 um VAC nPr: 5

Microscope Control
Tilt Control
2.00 Up Down To
Options

Camera & Script K3
Setup View Focus Tilt Record
Preview Search Resume STOP
pMMM: autoun TargetDef

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: 6400X nPr: 5 W: 1.75 um
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: Ws Fac Tr Rec Sen
Additional beam shift (and DF stt)
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 identical
Copy current area settings to
V F T R S
Center Unshifted Balance Shifts
Rotate interference axis 0 nPr
Montage Controls
Start Preset 7
Options Current: 2

Options
Transform Items
Undo Transformation
Change Registration
Shift to Marker
Undo Last Shift
Align with Rotation...
New Map from Image
Import Map...
Rotate Map
Adjust for Backlash
Backlash Settings...
Open Imaging States...
Set Map Acquire State
Restore State
Acquire all Items...
End Acquire
List Files/Series/States
Delete Item
Realign to Item
Force Center Align
Try Scaling in Realign

Navigator: G2055.nav
Label 1 Registration point 1 Camera point 1
Color Blue Draw Rotate when load For anchor state
#1 Note: Sec 0 - 175k-borelta-20210613-grd1.st
Acquire (A) Tilt series New file or item New file as group
Set: File Props Imaging State TS Params File Props Focus Props
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
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

Label	Color	X	Y	Z	Type	Reg.	Acq.	Note
1	Blu	2.3	7.5	80.6	Map 1			Sec 0 - 175k borelta-20210613-grd1.st
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	PI 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: 4182, 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								

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
autoun
CycleTargetDefocus
PrepMMM-Z
MyFuncs
Script6
autoun-multi
autoun-vpp
SimpleFocus
Z-check-refill-realign-FR
short-DSTomo
FastTomo-test
FastTomoWholeCell
Z
Z-check-refill-realign2
Script16
Script17
Script18
Script19
Script20
Script21
Script22
Script23
Script24
FastTomo
OpenFile
CloseFile
Script26
Script27
Script28

100 um

(1767, 905) = 4

10:17 AM 6/14/2021

Obtain multiple small montages (2250x)

SerialEM - low.st

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

Buffer Status
Q: Montage Overview
Size: 998x15192 bin 2 Tilt: 0.00
Stage: 0.00, 0.00 Def: 0.00
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 1
Options Memory = 453 MB

Image Display Controls
Blk 0
Whi 255
Bri 0.00
Con 0.00
Zoom 0.26

Microscope
-0.0000 nA 175.0 μm
Def 0.00 μm IS 0.00 μm
W 718.40 μm YAC Spot 8

Microscope Control
Tilt Control
0.00 Up Down To

Camera & Script
K3
Setup View Focus Tilt Record
Preview Search Resume STOP
epMMN+ autoun- TargetDef

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

Low Dose Control
Low Dose Mode
Search: 175x Sp 8 W 718.40 μm
Continuous update (see tooltip)
Define position of area
None Focus Trial
Position on tilt axis: -2.00 μm
Maximum area proportion: 1.03 μm
Go to: Me Foc Tr Rec Sen
Additional beam shift (and DF off)
Set Reset Uncalibrated
Offsets for View Search
Defocus: 100 Shift Set Zero
Blanked Unblank
BLANK BEAM when screen down
Normalize condenser lenses
Keep Focus and Trial identical
Copy current area settings to
V F T R S
Center Unshifted Balance Shifts
Rotate inter-area axis 0.00
Montage Controls
Start Prescan 1
Options Current Z

Navigator
Label 1 Registration point 1 Camera port [C]
Color Blue Draw Rotate when load For anchor state
#1 Note Sec 0 - low.st
Acquire (A) Tilt series New file or item New file as group
Set File Props Imaging Stats TS Params 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
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

Log: log1.log
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
Error above tolerance, retaking shot
15 frames were saved to X:\DoseFractions(20210613)\Jun(G205SC)\Jun14_10.14.11.tif
Saved Z = 28, -45.00 degrees
15 frames were saved to X:\DoseFractions(20210613)\Jun(G205SC)\Jun14_10.14.15.tif
Saved Z = 29, -48.00 degrees
15 frames were saved to X:\DoseFractions(20210613)\Jun(G205SC)\Jun14_10.14.59.tif
Saved Z = 30, -26.99 degrees
15 frames were saved to X:\DoseFractions(20210613)\Jun(G205SC)\Jun14_10.15.07.tif
Saved Z = 31, -29.99 degrees
15 frames were saved to X:\DoseFractions(20210613)\Jun(G205SC)\Jun14_10.15.12.tif
Saved Z = 32, -32.99 degrees
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
The original displacements in the overlap zones have
mean 32.09 maximum 309.60 pixels
After shifting pieces into register, the displacements have
mean 7.25 maximum 21.21 pixels

Scripts
PrepMMM noZ
autoun
CycleTargetDefocus
PrepMMM-Z
MyFuncs
Script6
autoun-multi
autoun-vpp
SimpleFocus
Z-check-refill-realign-FR
short-DSTomo
FastTomo-test
FastTomoWholeCell
Z
Z-check-refill-realign2
Script16
Script17
Script18
Script19
Script20
Script21
Script22
Script23
Script24
FastTomo
OpenFile
CloseFile
Script26
Script27
Script28

50 μm

(8008, 4206) = 113

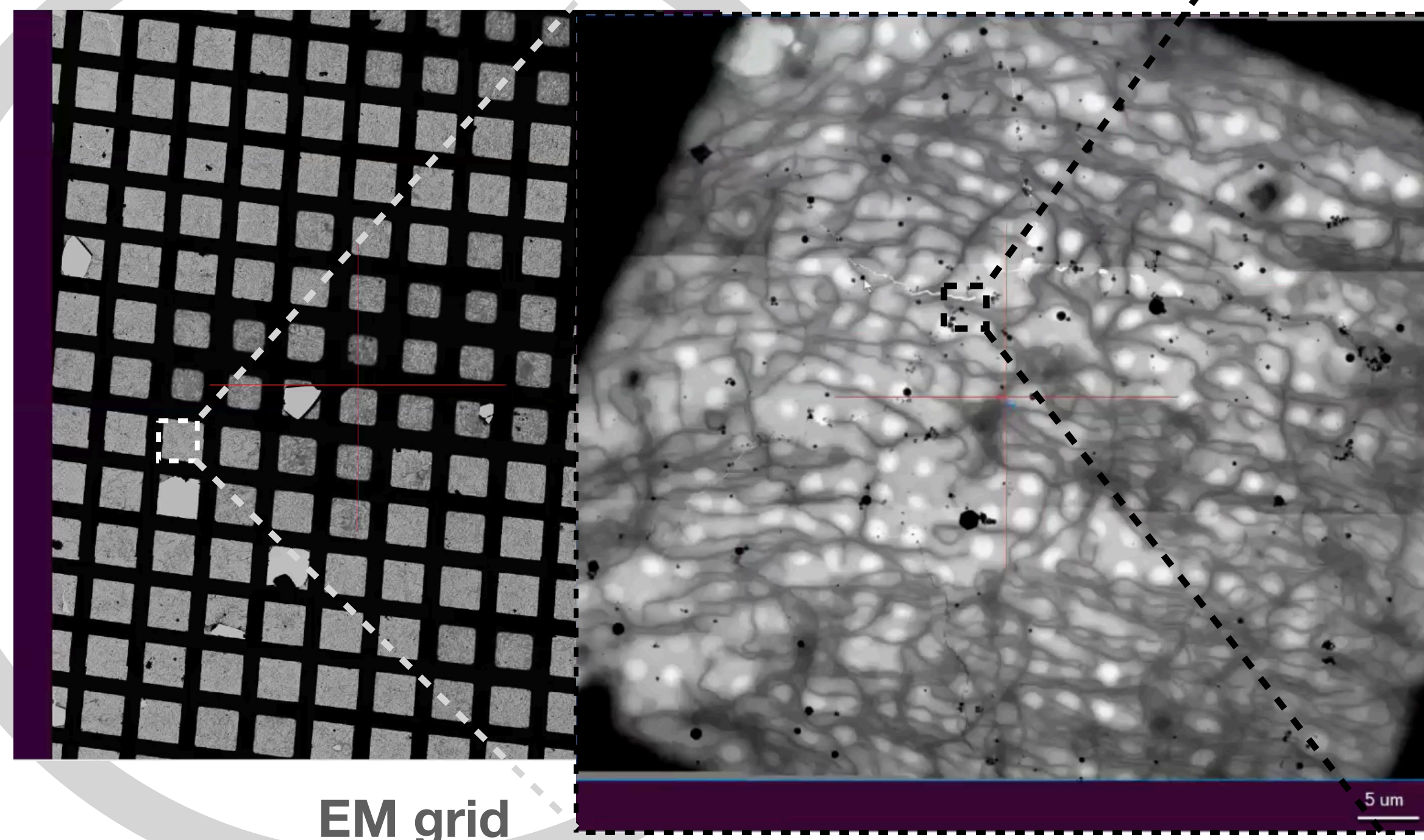
10:30 AM 6/14/2021

Find targets for tilt series data acquisition

1. Obtain 175 X full montage to survey the grid

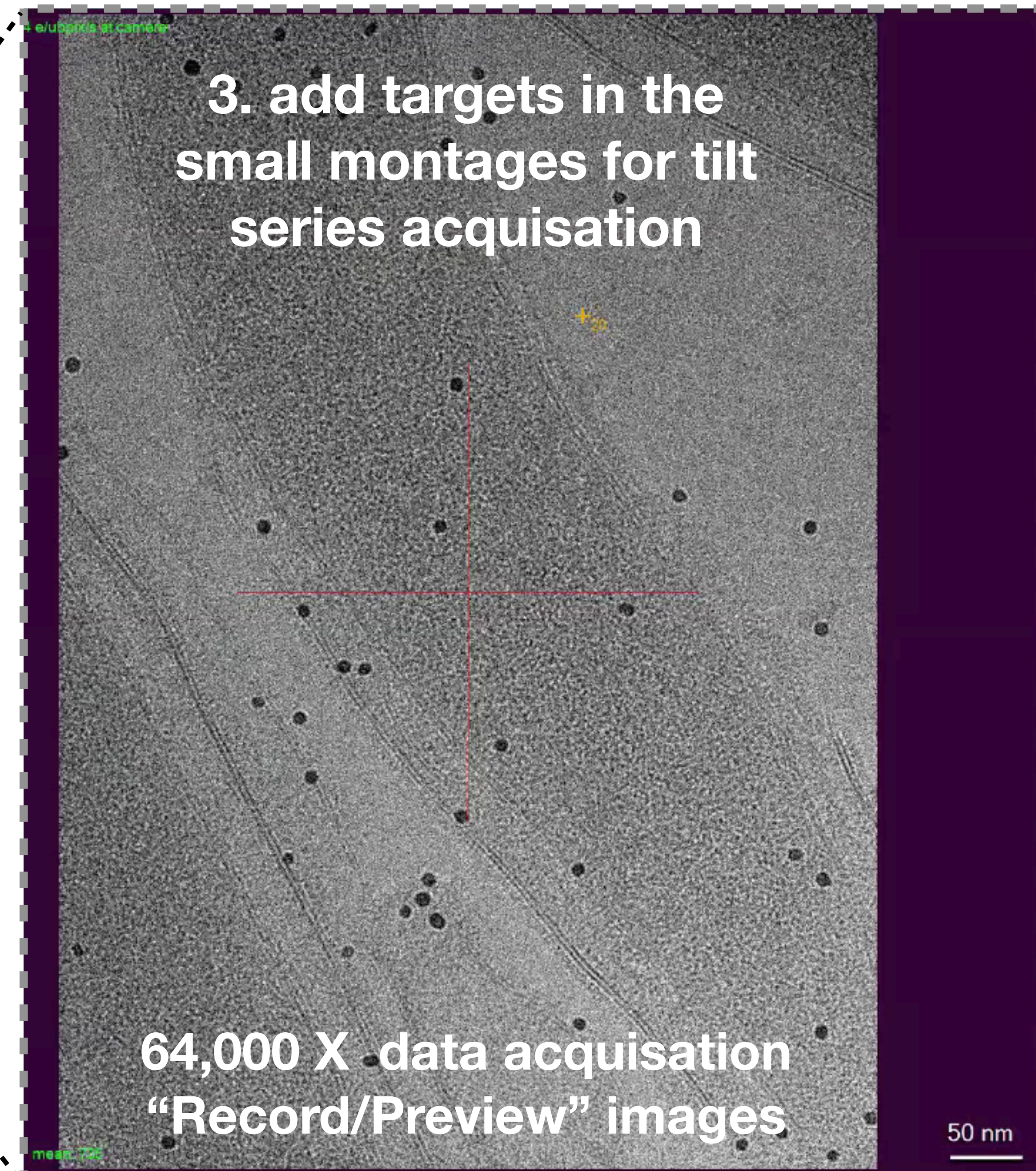
2. Obtain multiple small montages (2250X) with many target cells

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



6 x 6 “Search” images

5 x 3 “View” images



64,000 X data acquisition
“Record/Preview” images

Aligning "Preview/Record" beam with "View"

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

Buffer Status
B: Montage Overview
Size: 1508 x 1344 bin 12 Tilt: -0.00
Stage: -180.46, -93.75 Def: -106.92
A: Montage Center
C: Saved to File, sec. 72

Buffer Control
Copy Active Image to Buffer
A B C D F New
SAVE A Save Active To file 1
Options Memory: 208 MB

Image Display Controls
Blk: 0
Whi: 255
Bri: Cross
Con: Invert
Options Zoom: 0.95

Microscope
-0.0000 nA 2250 X
Def: -106.9 um IS: 0.00 um
+JA 48.40 um MAG Spot 6

Microscope Control
Tilt Control
-0.00 Up Down To
Options

Camera & Script K3
Setup View Focus Tilt Record
Preview Search Resume STOP
PrepMM noZ autoun TargetDef

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 mag shift between maps
Trim dark borders in Autoalign
Set Autoalign Trim Fraction

Low Dose Control
Low Dose Mode
View: 2250x Sp. 6 JA: 48.40u
Continuous update (see tooltip)
Define position of area
None Focus Trial
Position on tilt axis: 2.00 um
Maximum area acquisition: 1.03 um
Go to: Vis Fac Ts Rec Sen
Additional beam shift (and DF tilt)
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 identical
Copy current area settings to:
V F T A S
Center Unshifted Balance Shifts
Rotate interference axis 0 PRG

Montage Control
Start Prescan 5
Options Current Z

Navigator: sample2.nav
Label 18 Registration point 1 Corner point (C)
Color Blue Draw Rotate when load For anchor state
18 Note: Sec 4 - mid st
Acquire (A) Tilt series New file of item New file of group
Set File Props Imaging Stats TS Formats Reroms Focus Rec
Add Stage Pos Registration Draw All reg None Labels
Add Points Collapse Show Acquire Edit mode Edit Focus
Add Polygon Label Color X Y Z Type Reg. Acc. Note
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

Label	Color	X	Y	Z	Type	Reg.	Acc.	Note
1	Blu	3.9	6.0	100.0	Map 1			Sec 0 - low st
2	Red	-349.1	348.0	100.0	Pt 1			marker
3	Red	-342.8	376.5	100.0	Pt 1			hole
4	Red	564.3	-185.6	100.0	Pt 1			bad
5	Off	266.9	75.2	100.0	Pt 1			
6	Off	232.9	195.5	100.0	Pt 1			
7	Off	-113.6	158.7	100.0	Pt 1			
8	Off	-145.6	29.6	100.0	Pt 1			
9	Off	-180.5	-92.4	100.0	Pt 1			
10	Red	303.8	-52.9	100.0	Pt 1			
11	Red	-422.6	-19.5	100.0	Pt 1			
12	Red	-390.4	102.0	100.0	Pt 1			
13	Red	217.4	-215.4	100.0	Pt 1			
5 A	Blu	266.8	74.8	75.6	Map 1			Sec 0 - mid st
6 A	Blu	-232.8	195.3	77.7	Map 1			Sec 1 - mid st
7 A	Blu	-113.6	158.3	72.5	Map 1			Sec 2 - mid st
8 A	Blu	-145.6	29.3	73.9	Map 1			Sec 3 - mid st
9 A	Blu	-180.5	-92.6	75.8	Map 1			Sec 4 - mid st

Log: log1.log
15 frames were saved to X:\DoseFractions\20210613\Jun(G20SSC)\Jun14_10 15 32.1#
Saved Z = 36.45.00 degrees
15 frames were saved to X:\DoseFractions\20210613\Jun(G20SSC)\Jun14_10 15 37.1#
Saved Z = 37.48.00 degrees
----- end of dose symmetric TS -----
TS time: 327.25
Total time: 327.25
The original displacements in the overlap zones have
mean: 32.09 maximum: 309.60 pixels
After shifting pieces into register, the displacements have
mean: 7.25 maximum: 21.21 pixels
Rough eucentricity: changing Z by -21.94 to 75.06, continuing..
Rough eucentricity: changing Z by -1.50 to 75.56, finished.
Z = 0 The original displacements in the overlap zones have mean: 14.74 maximum: 25.74 pixels
After shifting pieces into register, the displacements have mean: 0.33 maximum: 0.73 pixels.
Map acquired at item # 5 with label 5 saved at Z = 0
Rough eucentricity: changing Z by -20.84 to 75.17, continuing..
Rough eucentricity: changing Z by -1.47 to 77.70, finished.
Z = 1 The original displacements in the overlap zones have mean: 14.62 maximum: 22.51 pixels
After shifting pieces into register, the displacements have mean: 0.74 maximum: 2.12 pixels.
Map acquired at item # 6 with label 6 saved at Z = 1
Rough eucentricity: changing Z by -25.87 to 74.14, continuing..
Rough eucentricity: changing Z by -1.63 to 72.52, finished.
Z = 2 The original displacements in the overlap zones have mean: 15.16 maximum: 23.55 pixels
After shifting pieces into register, the displacements have mean: 0.37 maximum: 1.44 pixels.
Map acquired at item # 7 with label 7 saved at Z = 2
Rough eucentricity: changing Z by -24.25 to 75.76, continuing..
Rough eucentricity: changing Z by -1.87 to 73.89, finished.
Z = 3 The original displacements in the overlap zones have mean: 14.84 maximum: 24.63 pixels
After shifting pieces into register, the displacements have mean: 0.29 maximum: 1.03 pixels.
Map acquired at item # 8 with label 8 saved at Z = 3
Rough eucentricity: changing Z by -18.76 to 81.25, continuing..
Rough eucentricity: changing Z by -1.47 to 79.77, finished.
Z = 4 The original displacements in the overlap zones have mean: 15.95 maximum: 23.51 pixels
After shifting pieces into register, the displacements have mean: 0.41 maximum: 1.09 pixels.
Map acquired at item # 9 with label 9 saved at Z = 4

Scripts
PrepMM noZ
autoun
CycleTargetDefocus
PrepMM-Z
MyFines
Script 5
autoun-multi
autoun-vpp
SimpleFocus
Z-check-refill-realign-FR
short-OSTomo
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

(1020, 445) = 113

10:53 AM 6/14/2021

FastTomo: A SerialEM Script for Collecting Electron Tomography Data

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***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

*/

scheme = 1

0 = bidirectional

1 = dose-symmetric

2 = unidirectional

runOnNavItem = 0

Debug = 0

shot = R

usePrevCalib = 0

tolerance = 0.4

eucentricity_option = -1

multiRecord = 1

multiR = { 3 6 }

dose-symmetric settings

startAngleDS = 0

endAngleDS = 48

stepSizeDS = 3

groupSizeDS = 8

trackingShot = V

doExtraTrackingShot = 1

startAngleDS is non-zero

FastTOMO script

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

verbose output for debugging

low dose beam to use for saving data

skip calibration and use most recent parameters if they exist

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

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

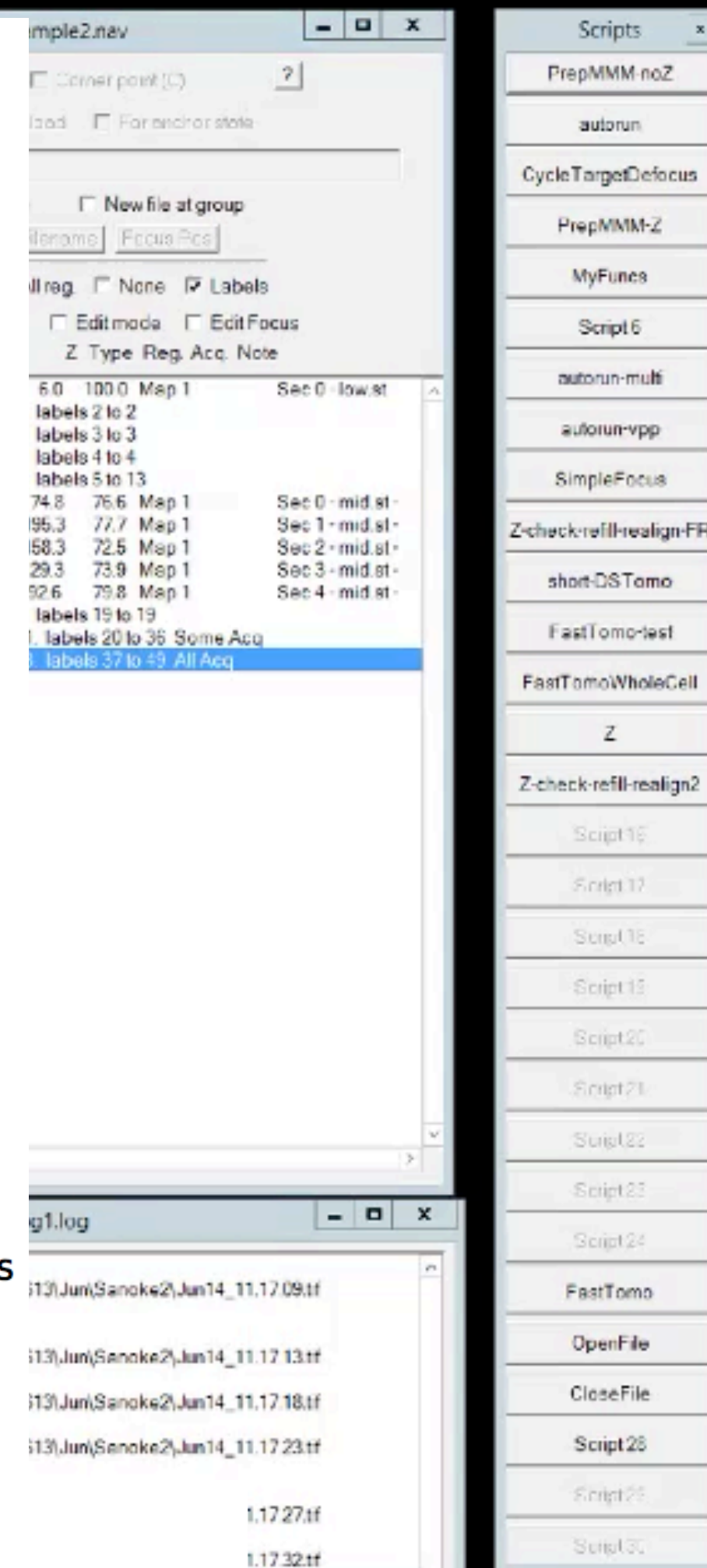
take more than one R shots along tilting axis

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

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

can also be set to V

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



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

Tilt series alignment and tomogram reconstruction by IMOD (Etomo)

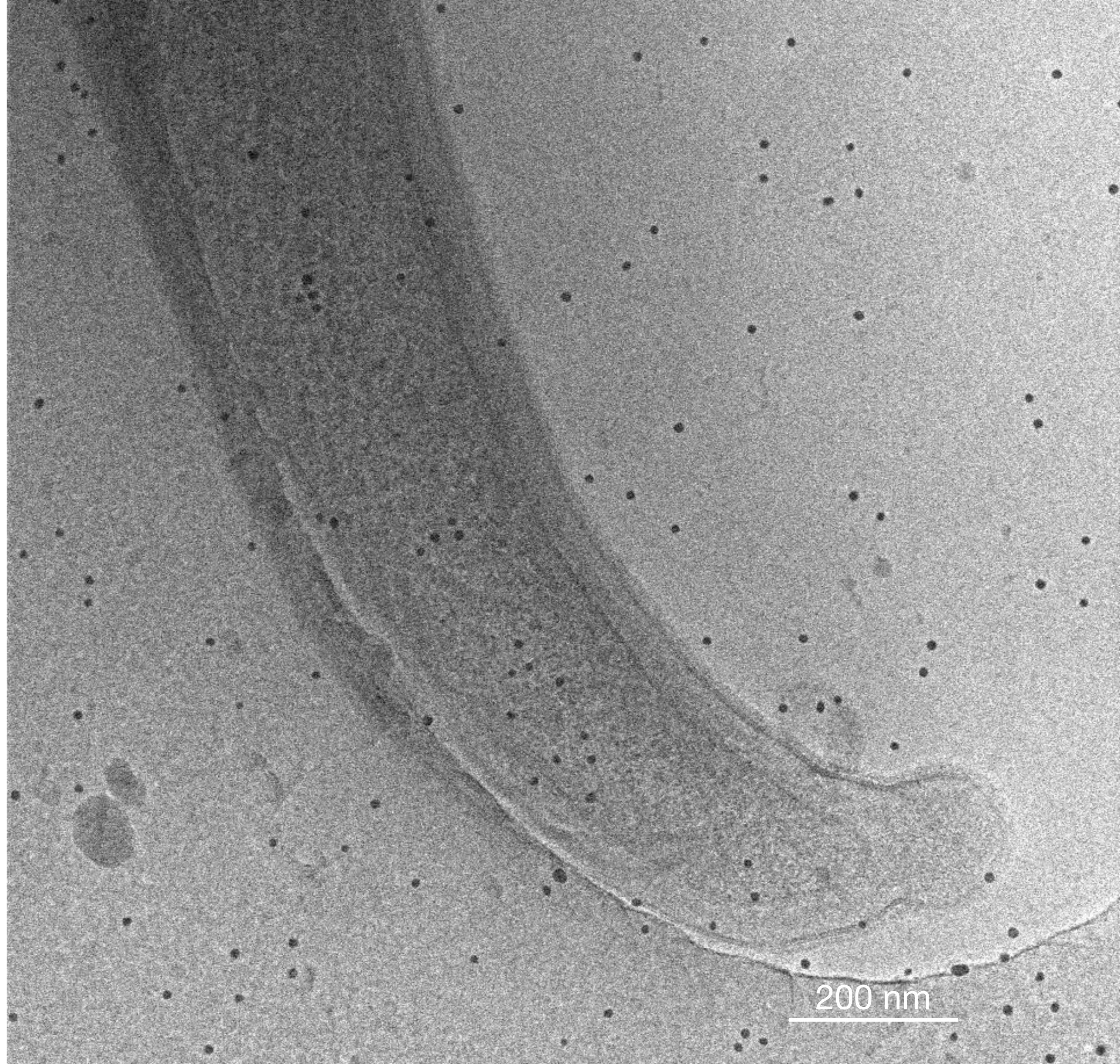
- 1. Motion correction for tilt images (MotionCorr2 or 3).**
- 2. Stack the tilt images to get the motion corrected tilt series (IMOD).**
- 3. Preprocess the tilt series (ETOMO).**
- 4. Manually pick several fiducial gold (10 nm) and let the Etomo do the tracking for fiducial.**
- 5. Align the tilt series based on the tracking result.**

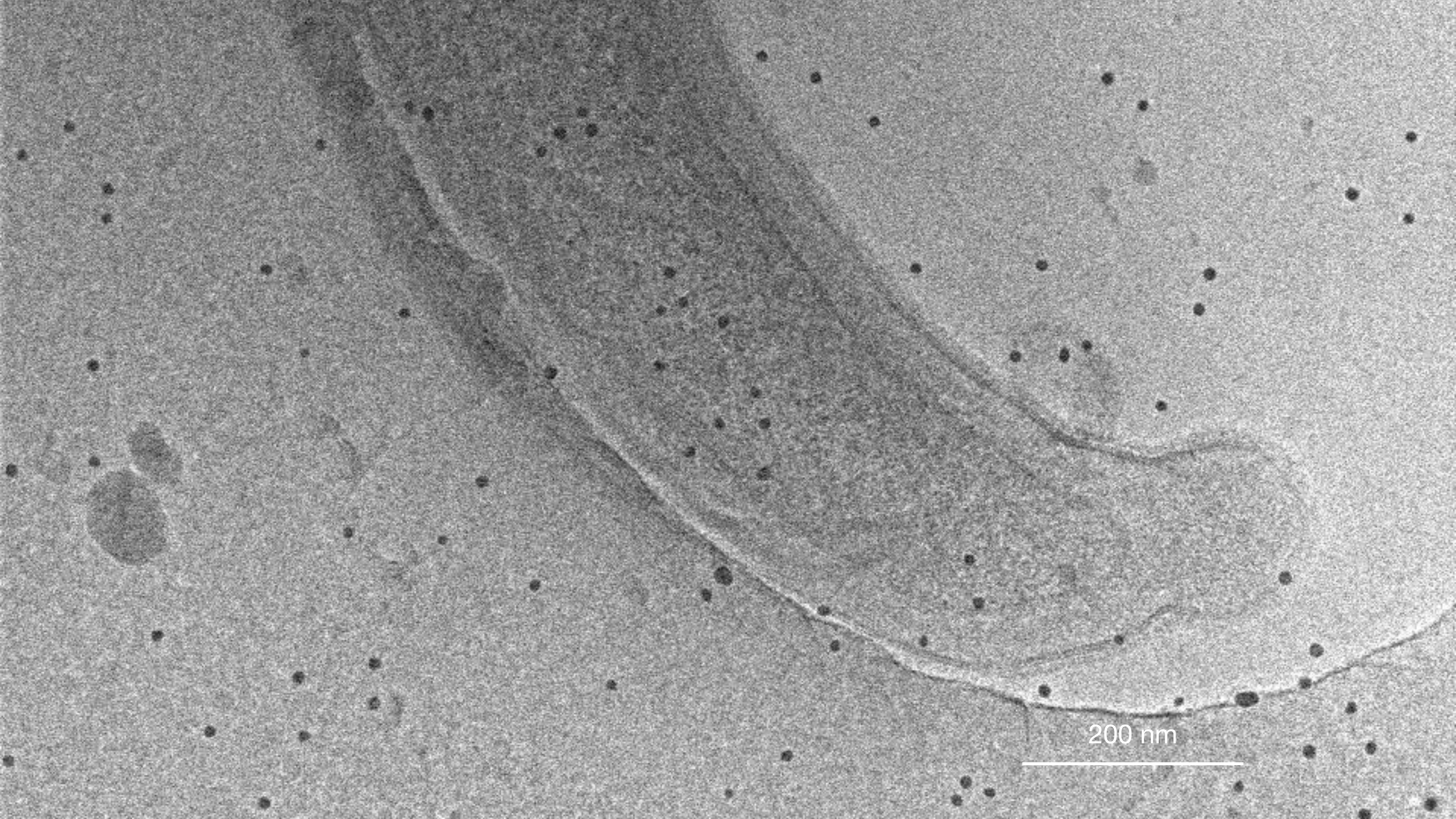
More details about etomo:

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

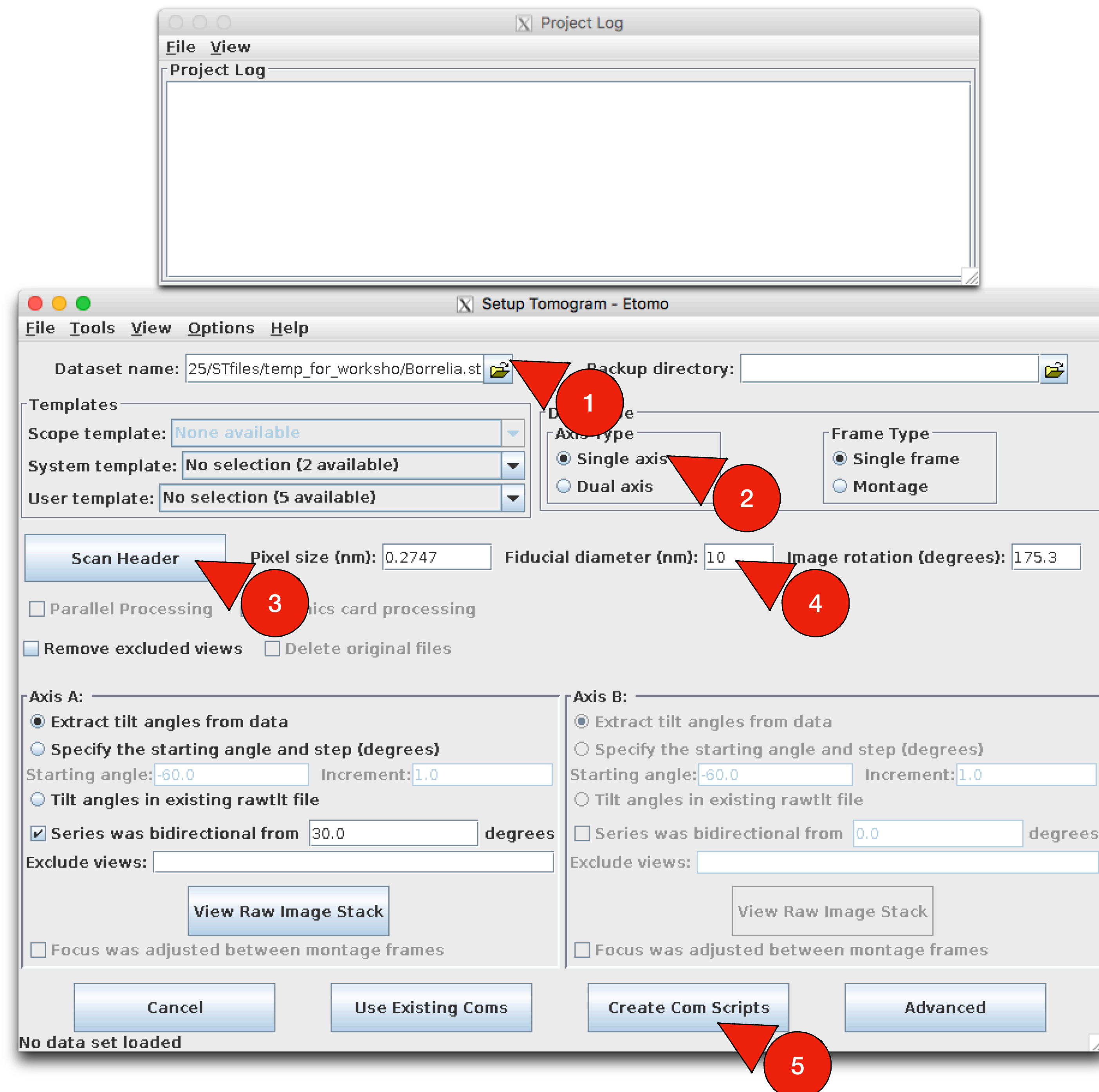
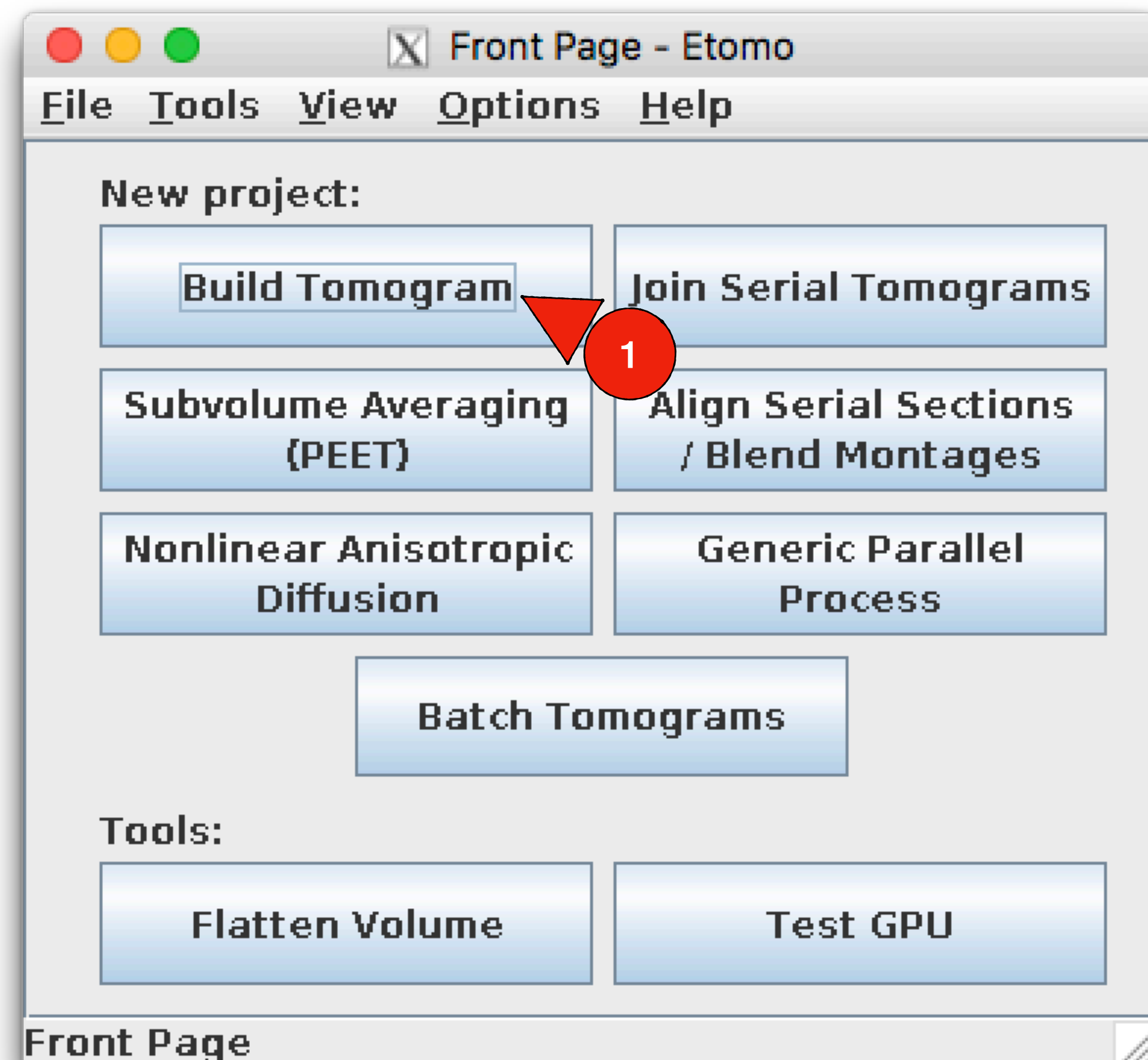
Dataset

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

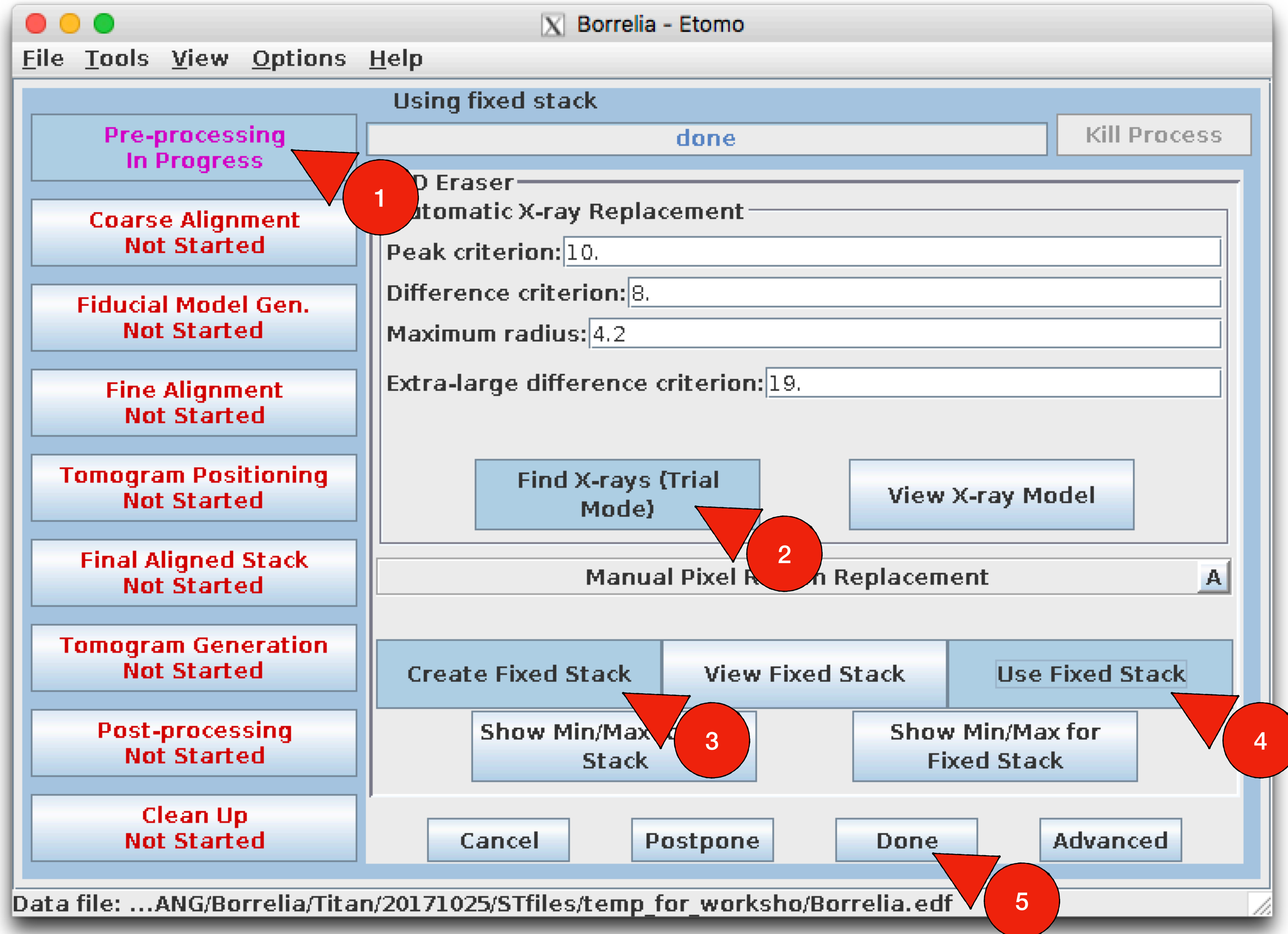




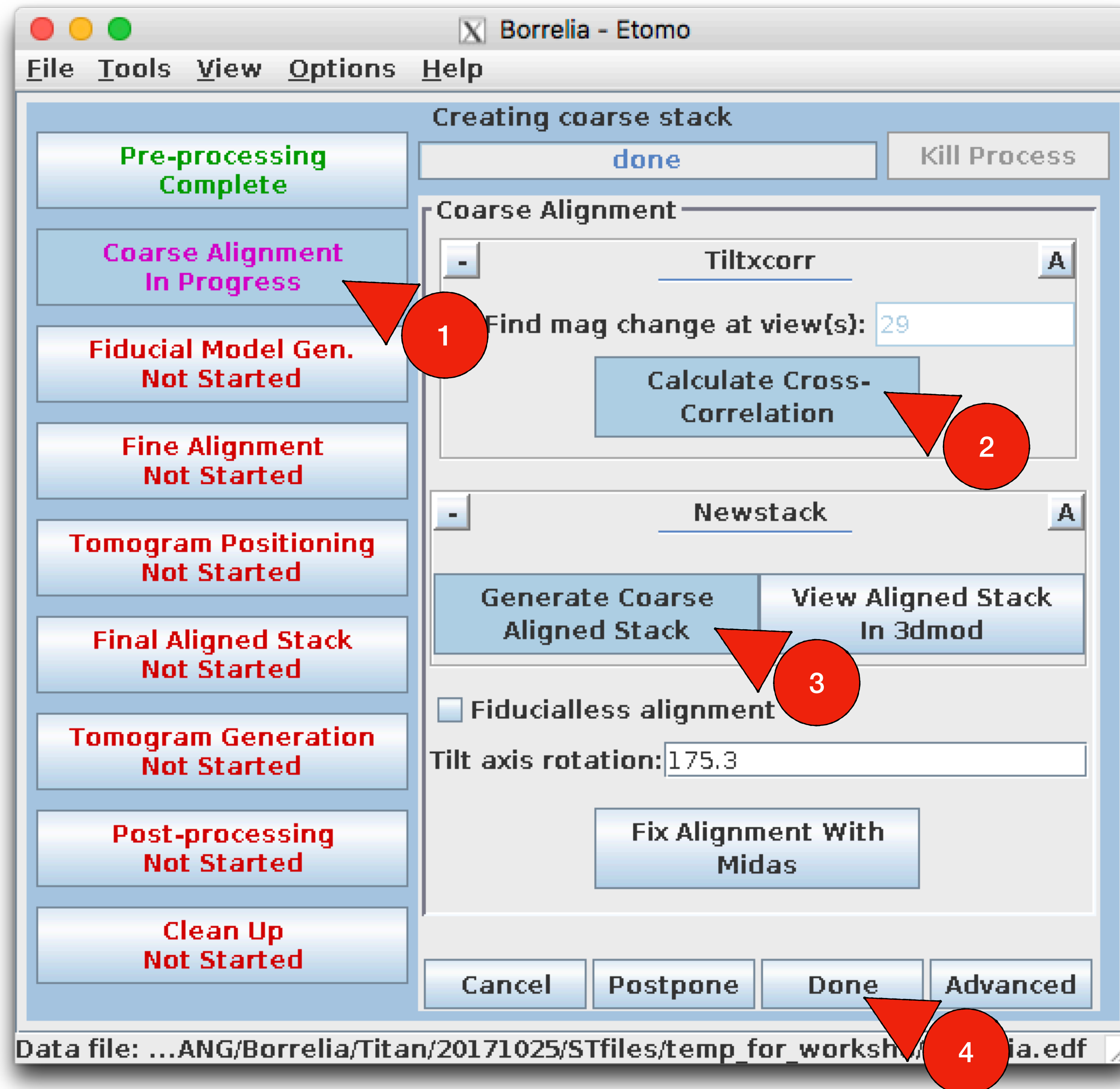
Etomo interface

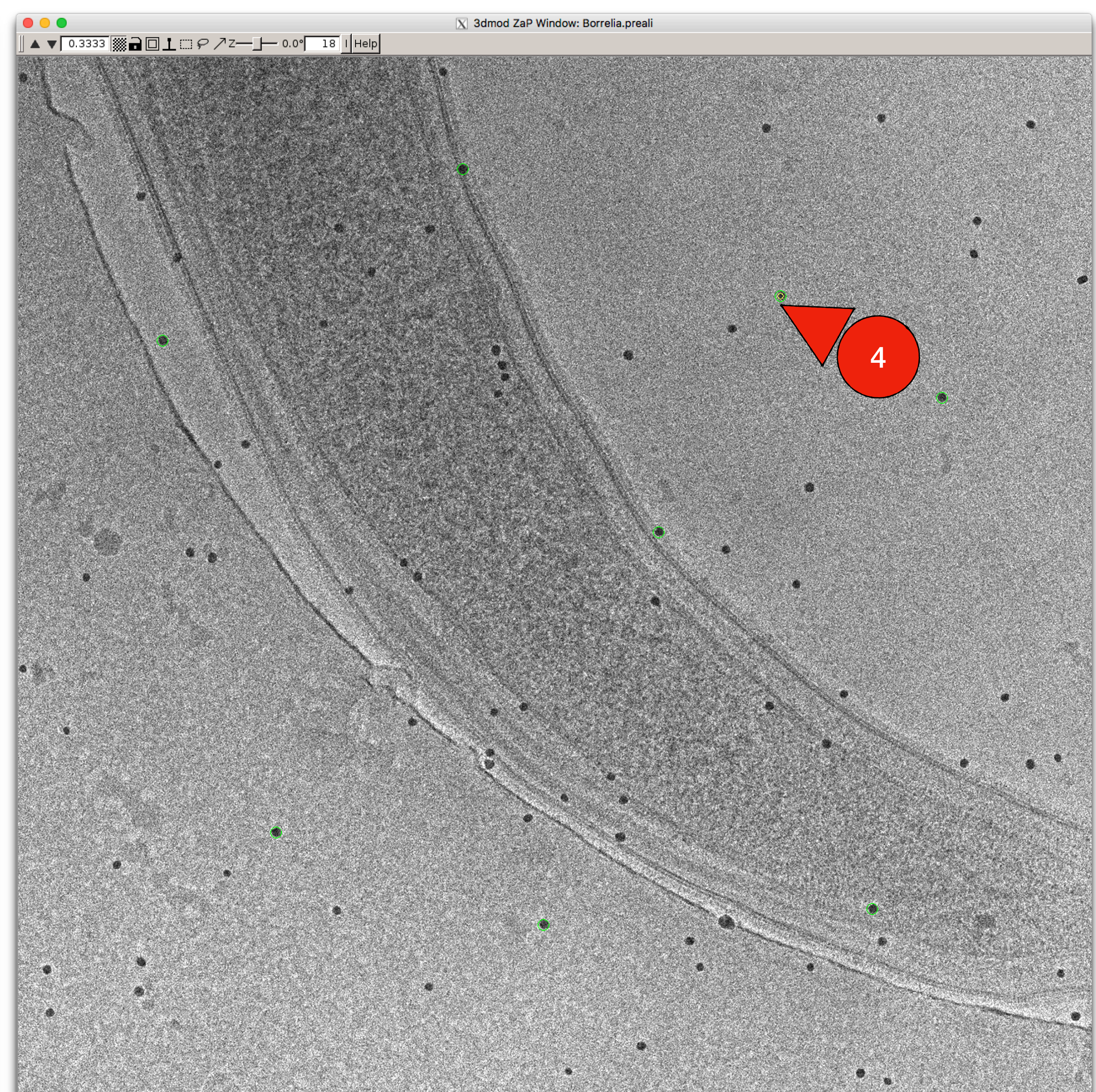
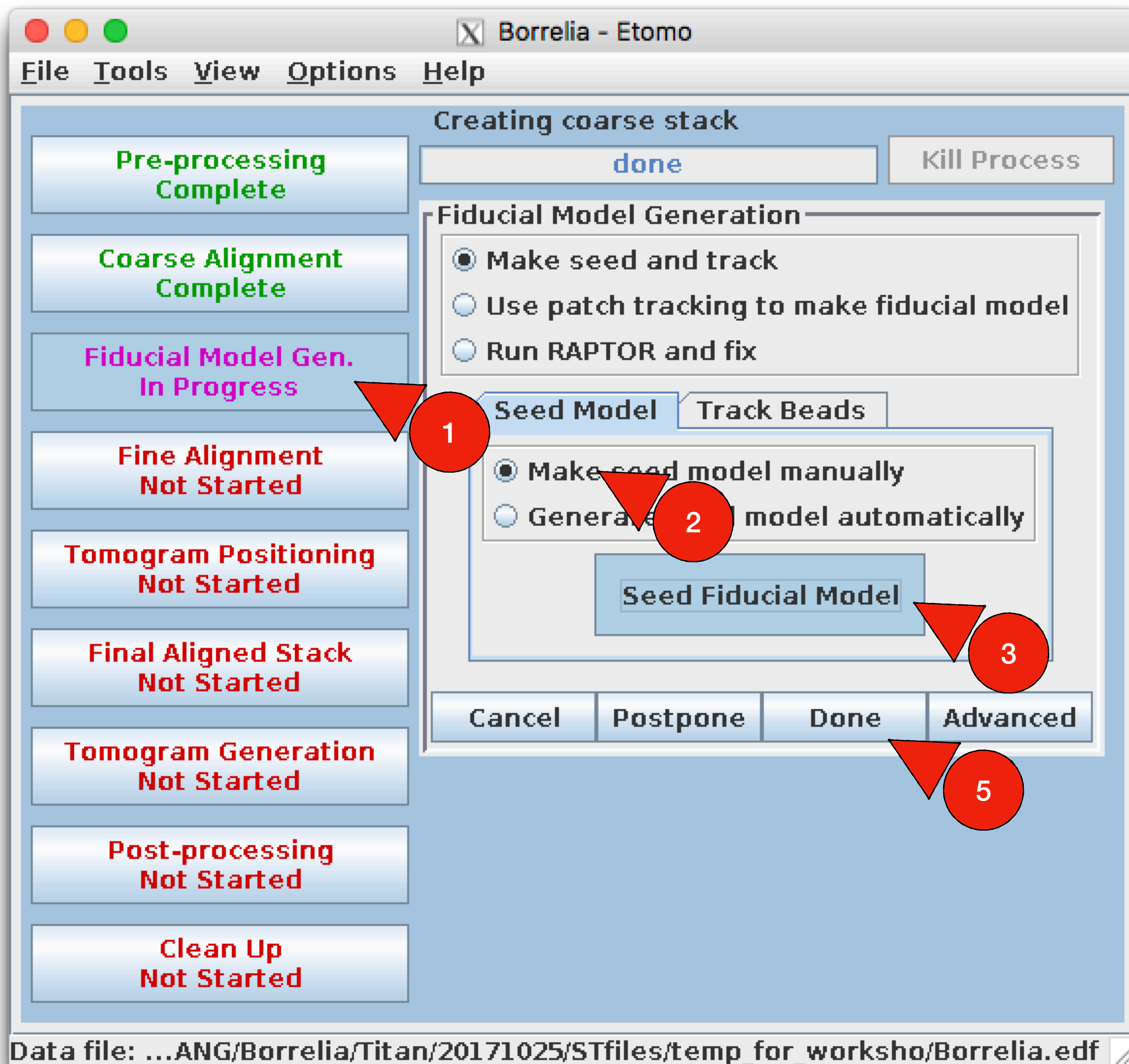


Pre-processing



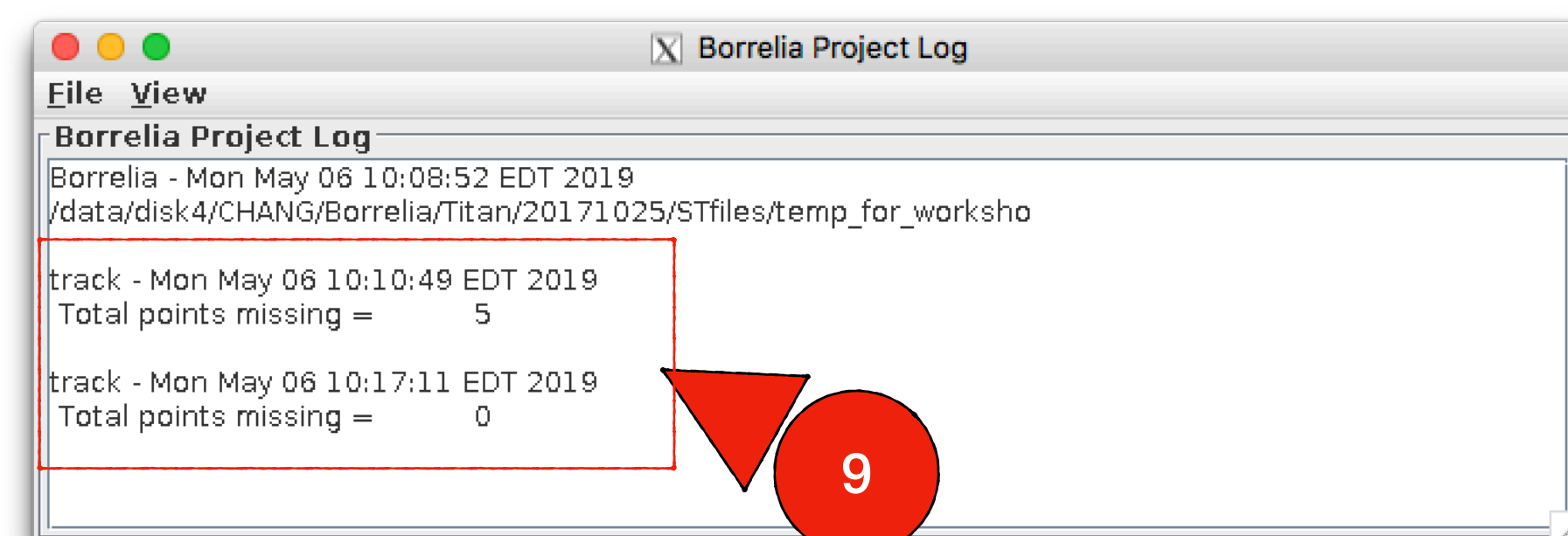
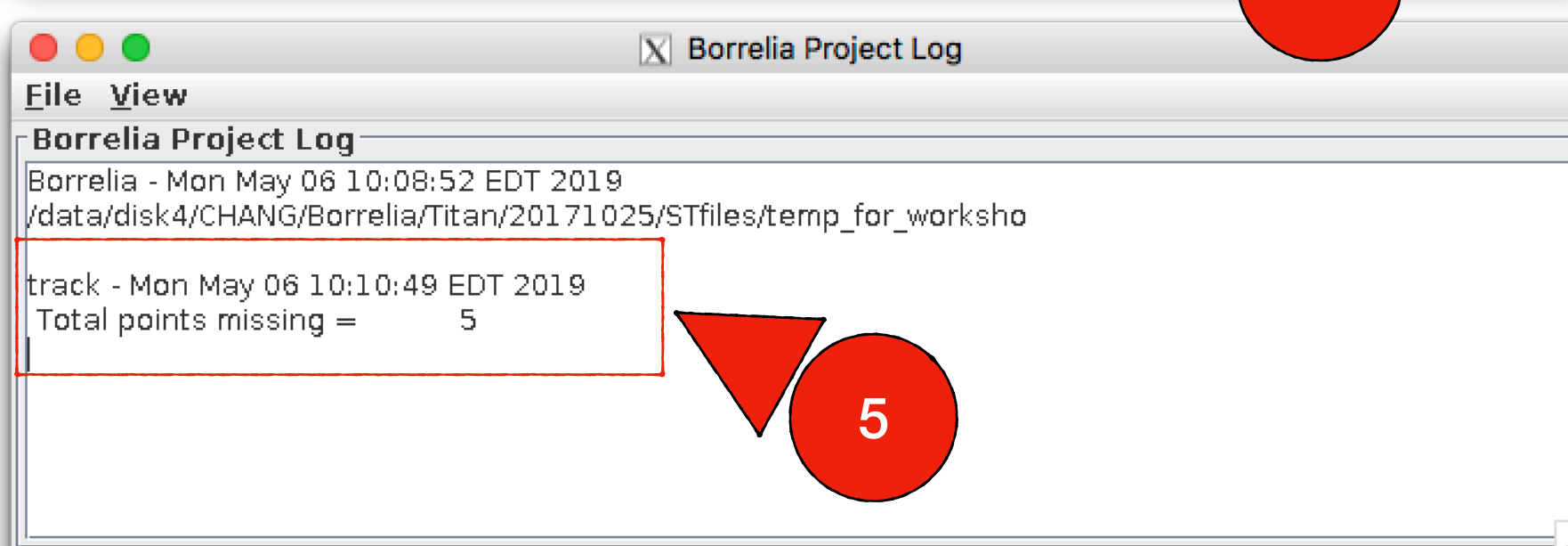
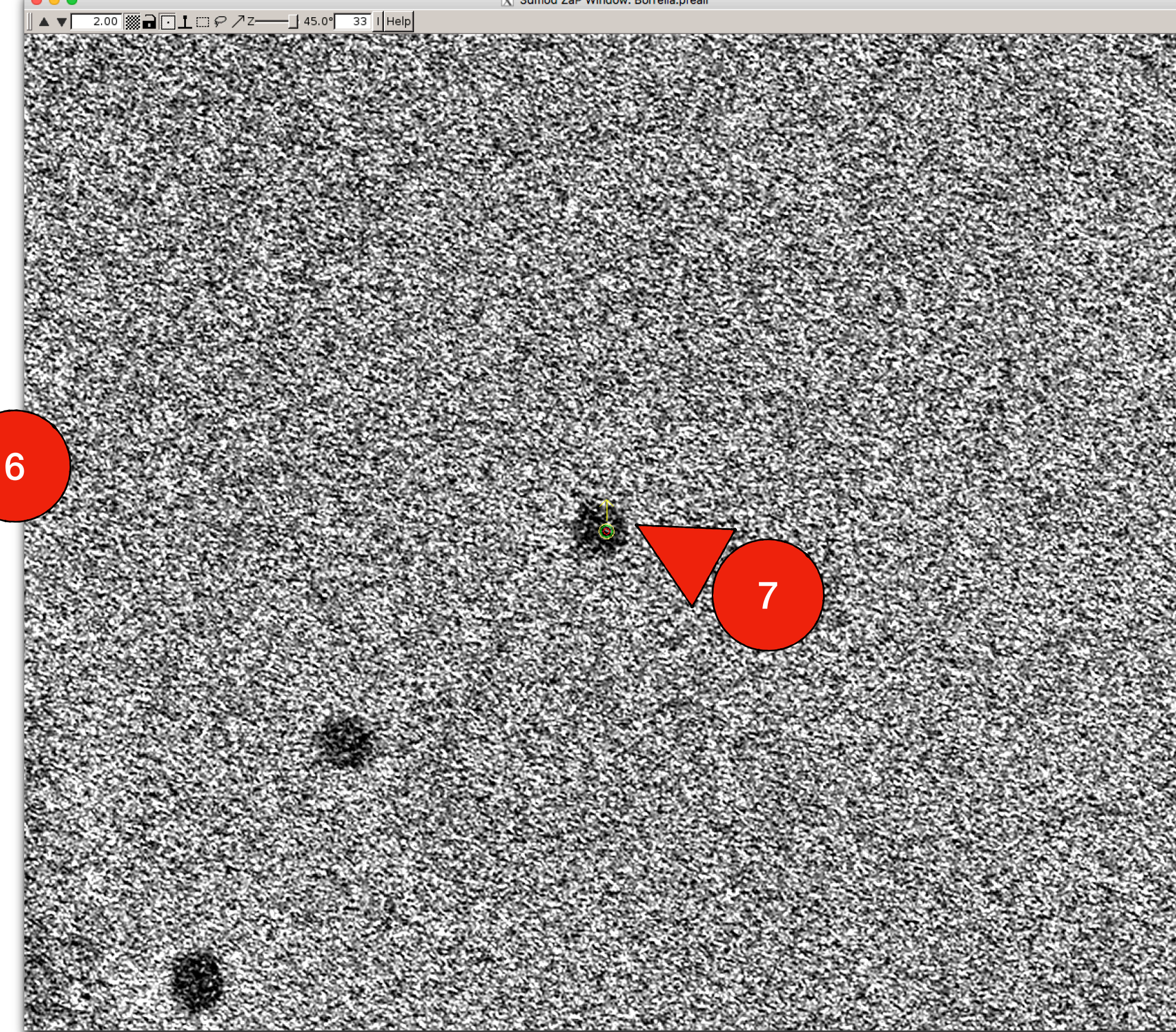
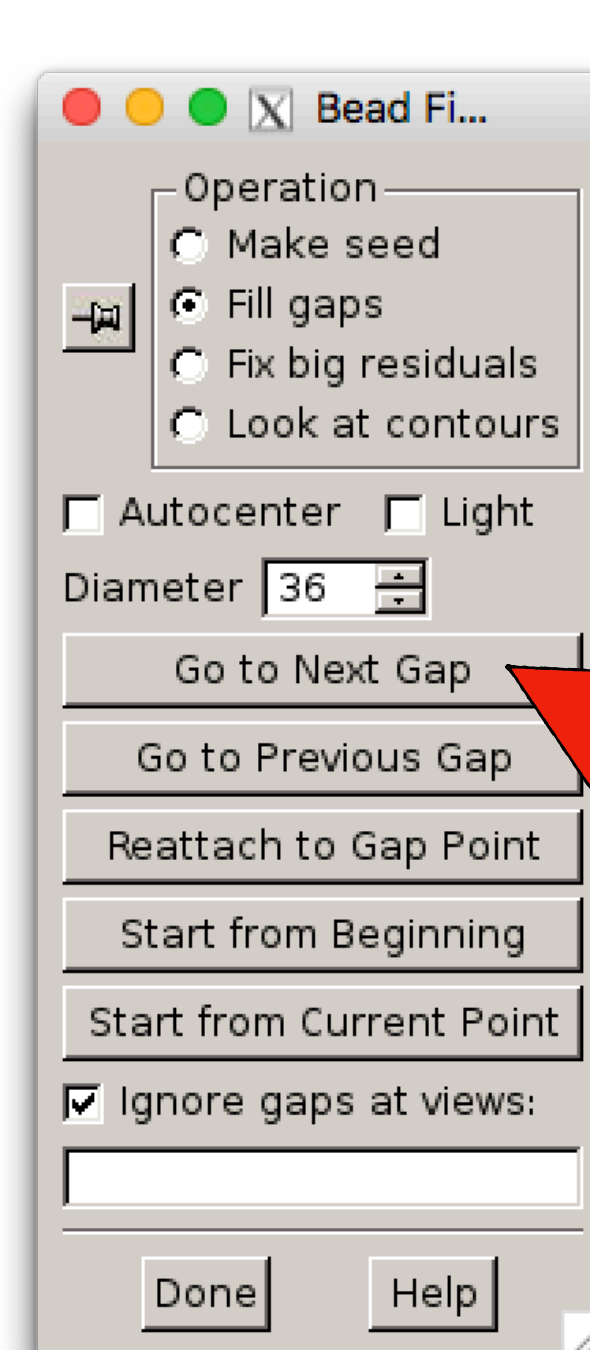
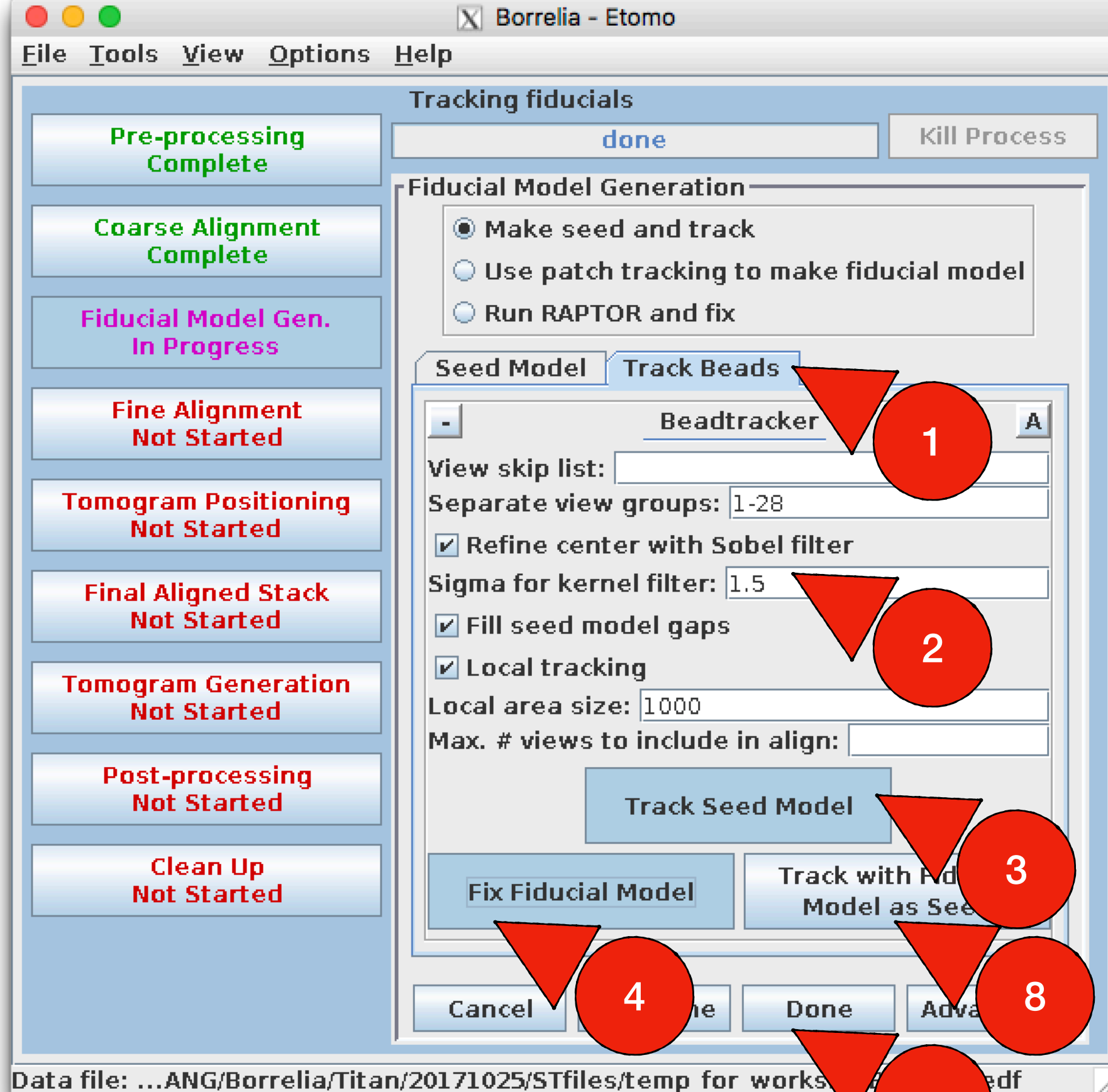
Coarse alignment





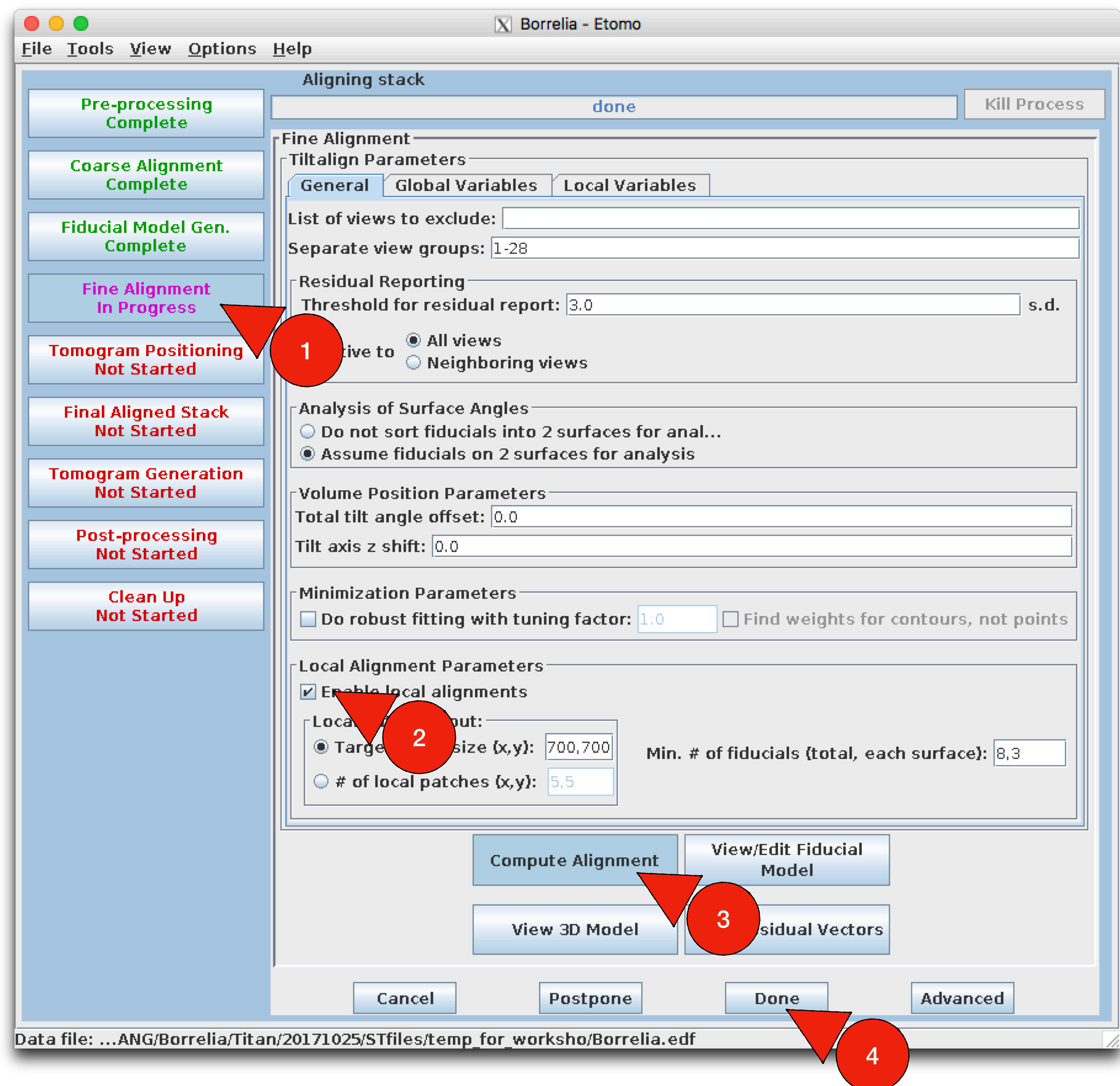
Generate
fiducial model

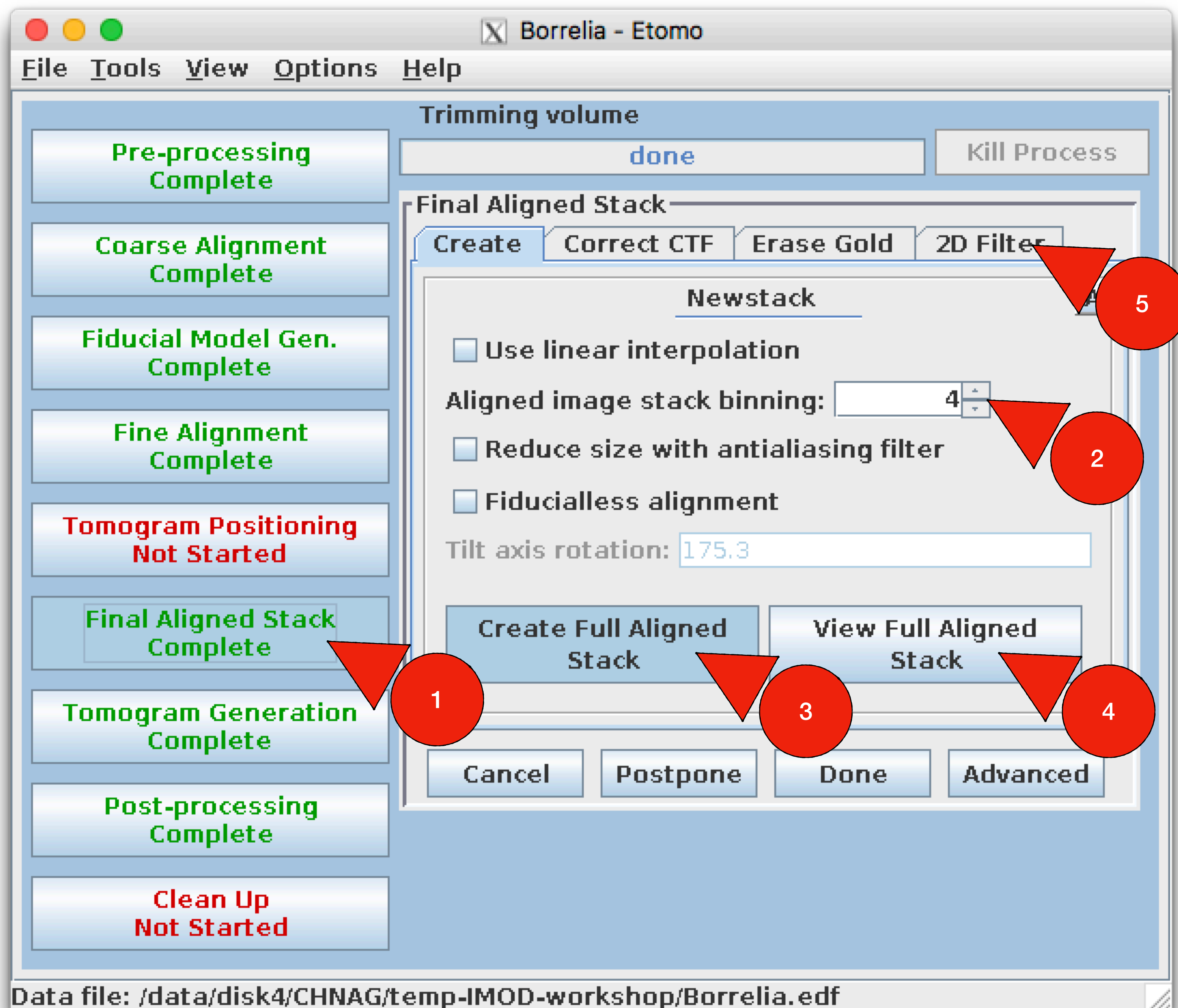
1. Press mouse middle button to select one gold particle
2. Then press “N” in keyboard to create a new contour and then select another gold particle
3. Choose 5-10 gold particles in different areas
4. Close IMOD and save the model



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.

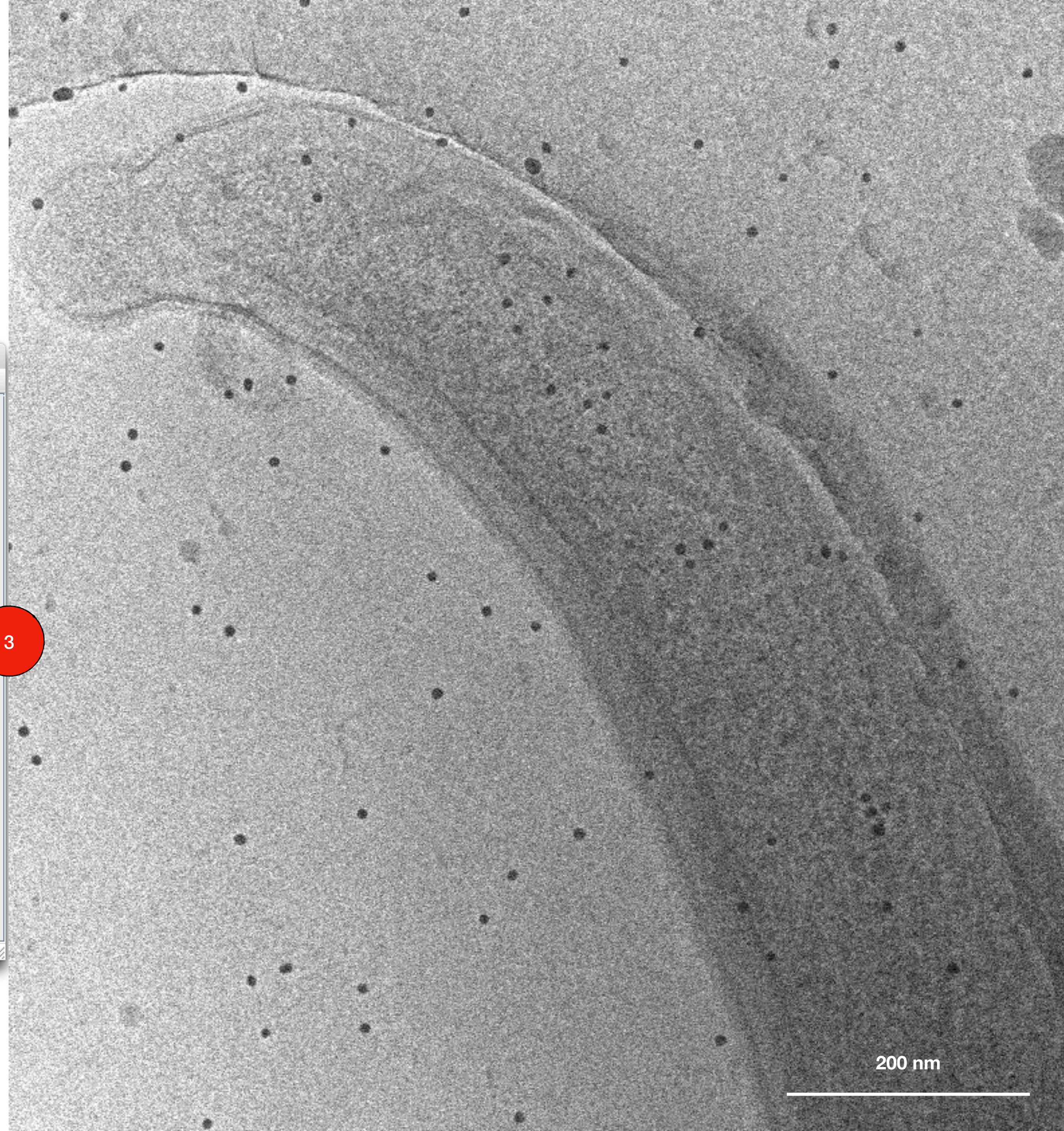
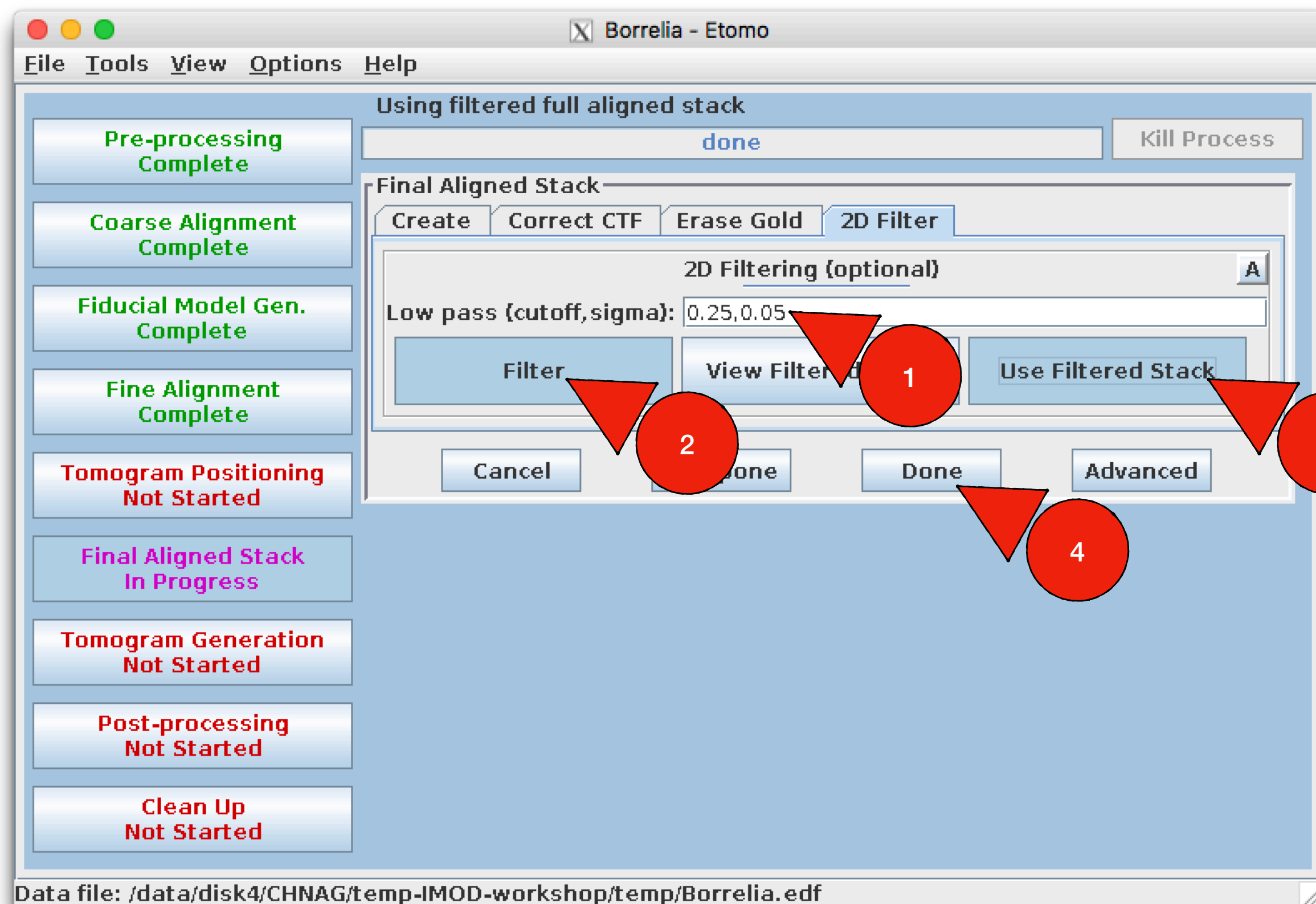
Final alignment of fiducial

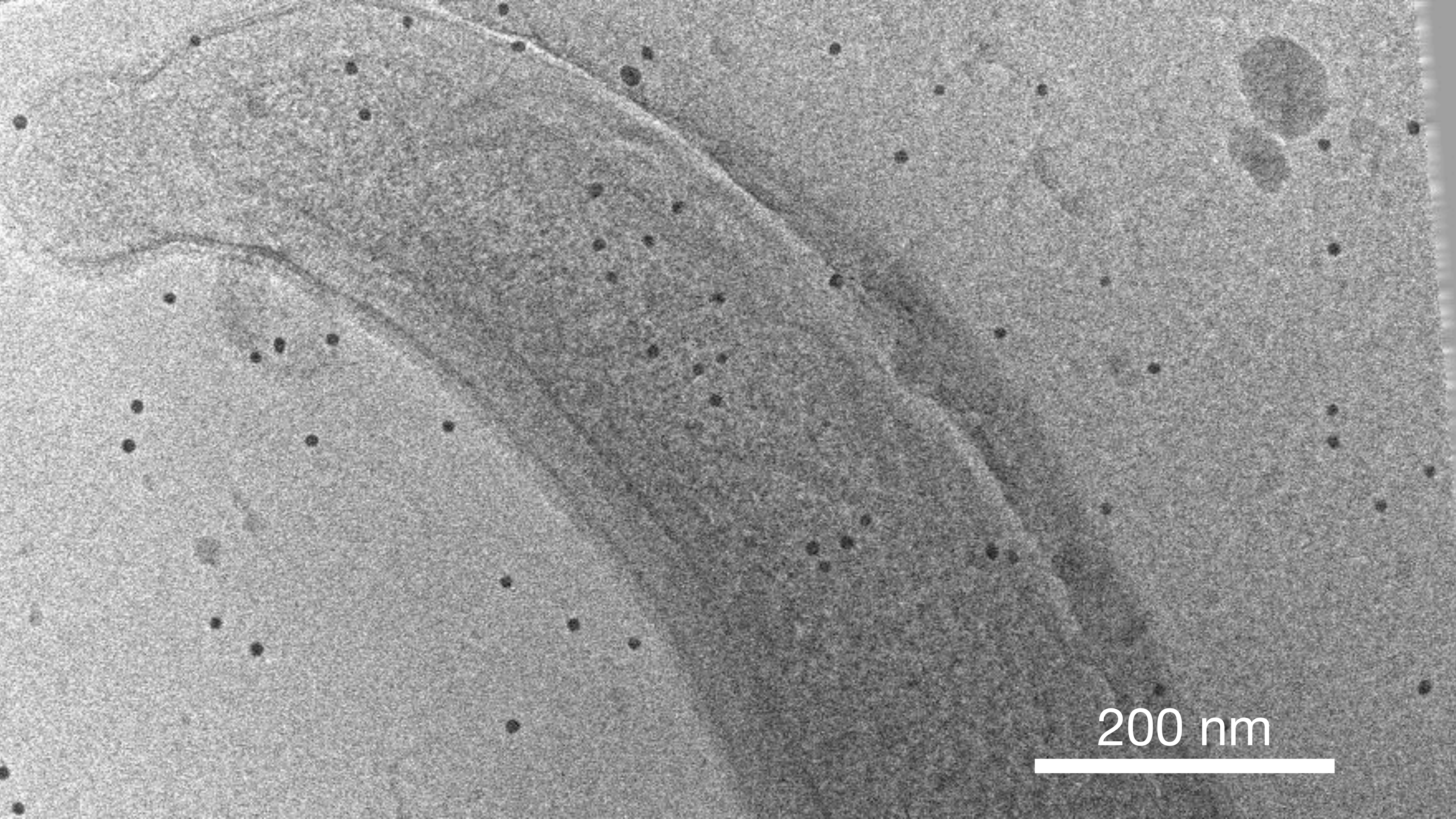




Generate aligned tilt series

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.



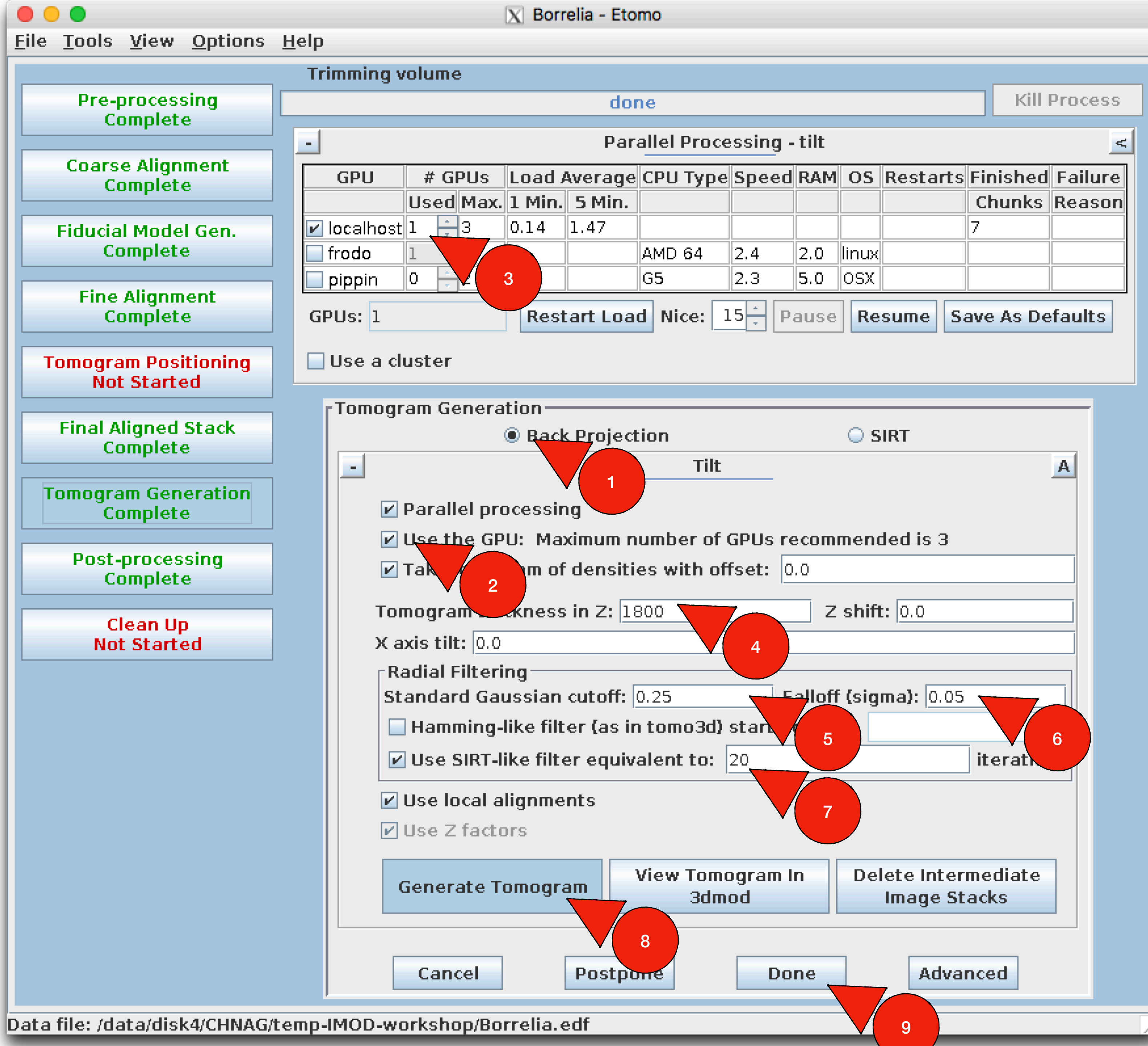


200 nm

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])

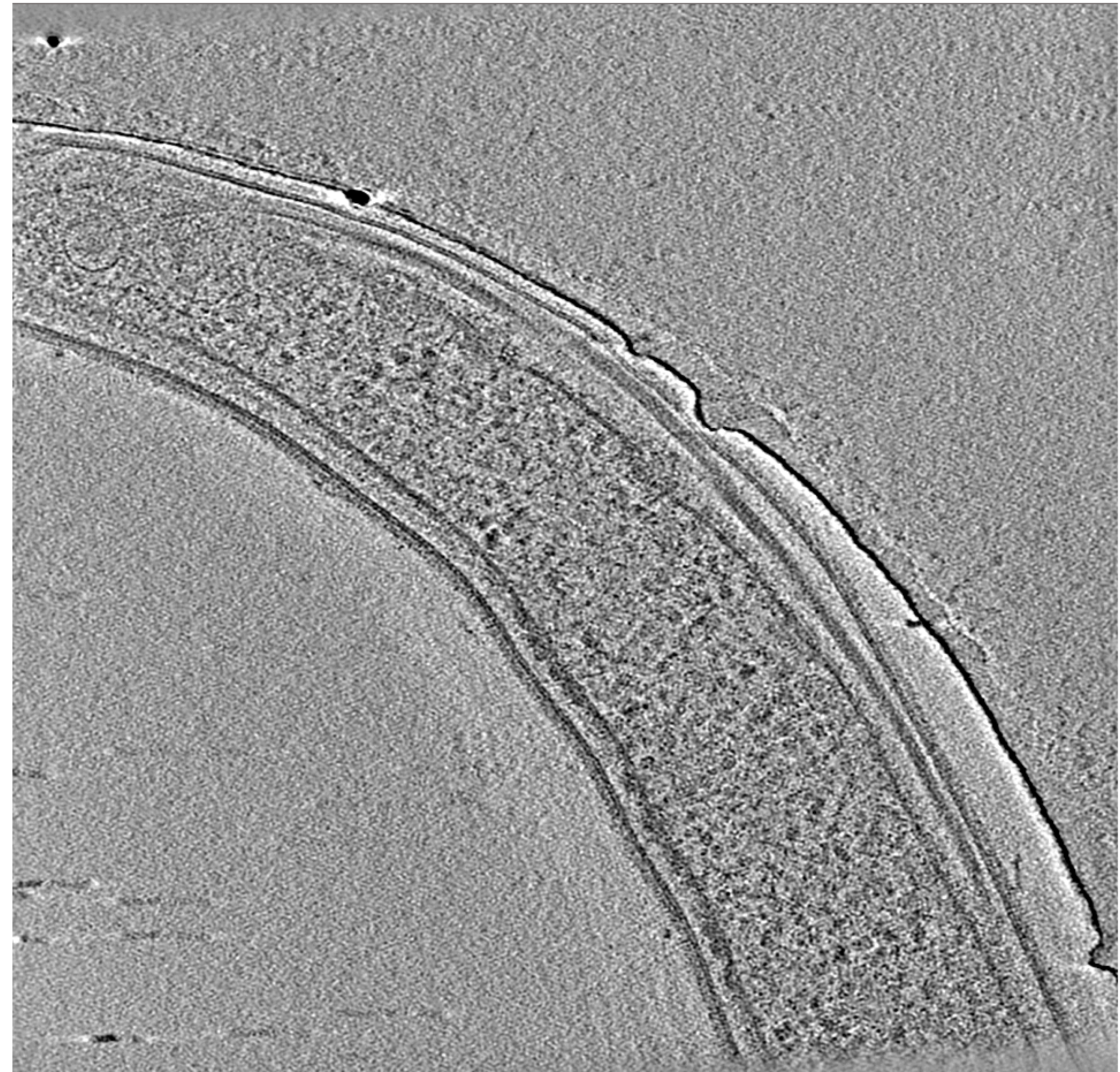
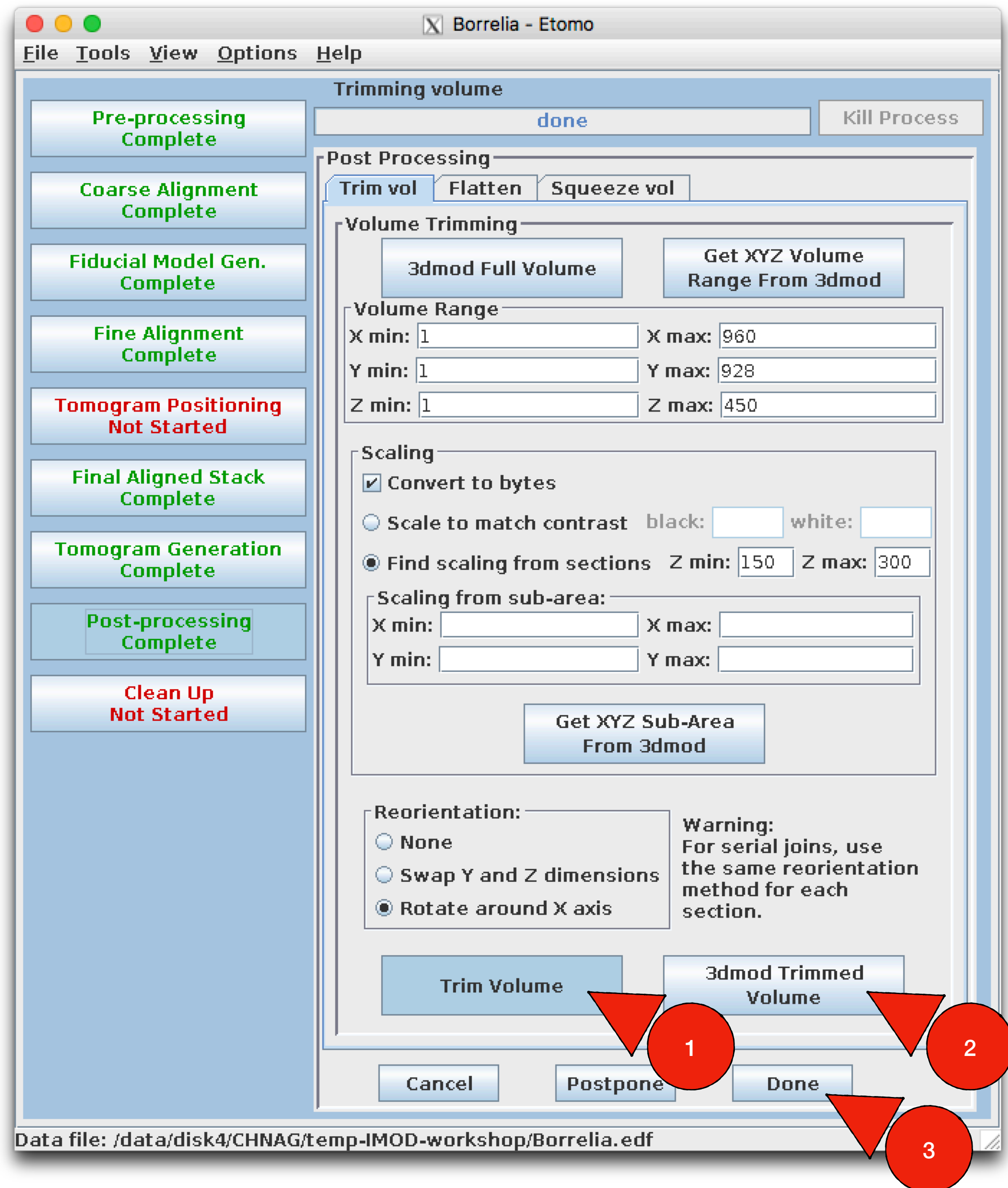


Tomogram reconstruction

Save following files:

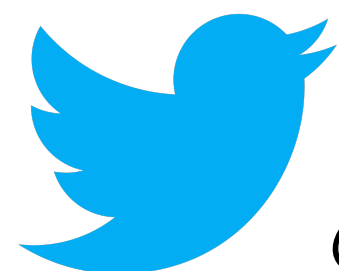
Borrelia.st Borrelia.ali Borrelia.rec Borrelia.rawtlt Borrelia_fid.xf

Other files can be deleted



Thank you!
Comments & questions?

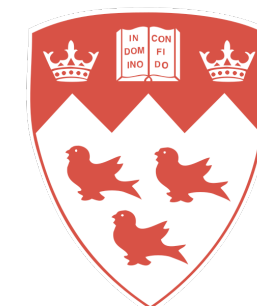
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Brookhaven
National Laboratory



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