Cryo-ET data collection by SerialEM

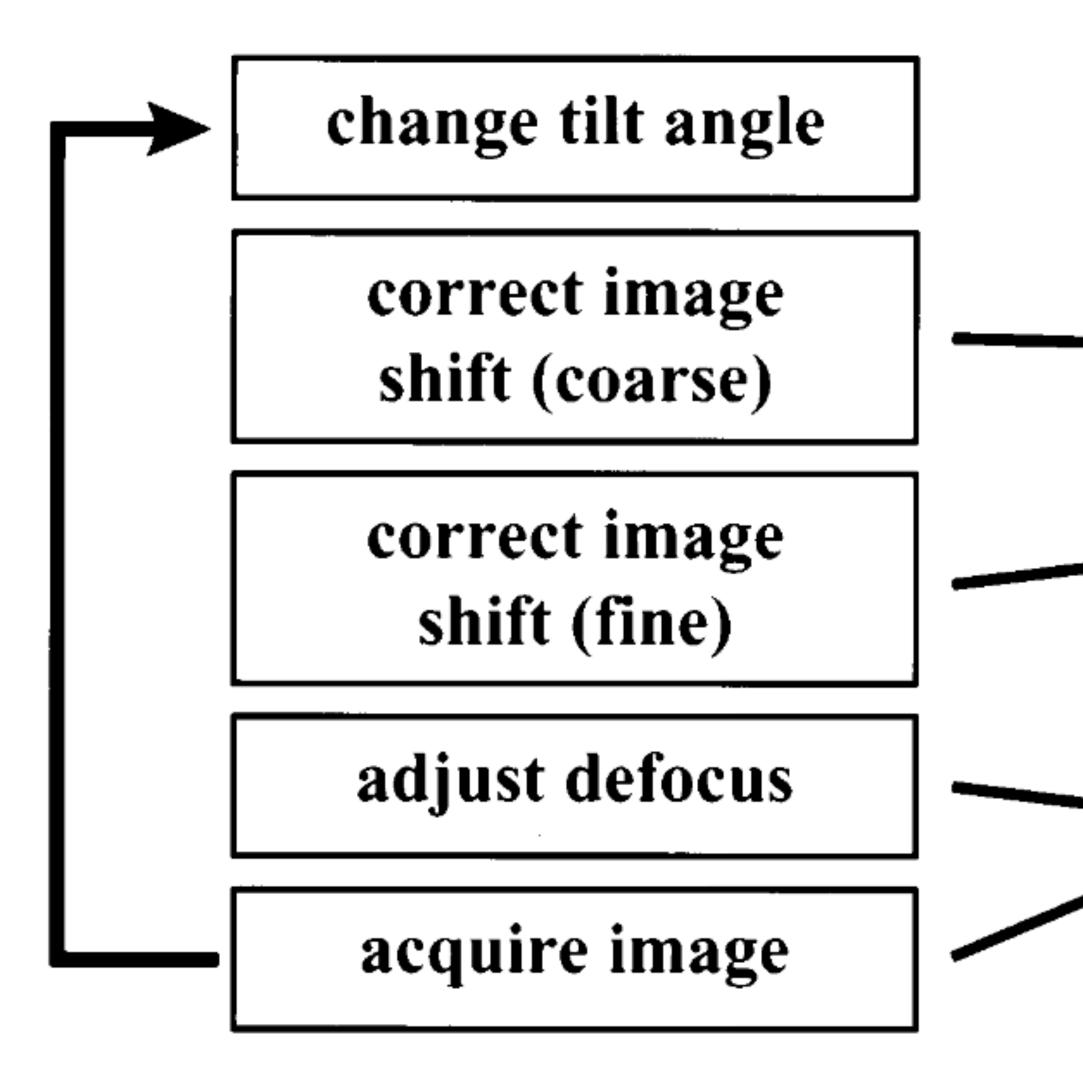
Shuaiqi Guo (Phil) McGill University June 13 2024

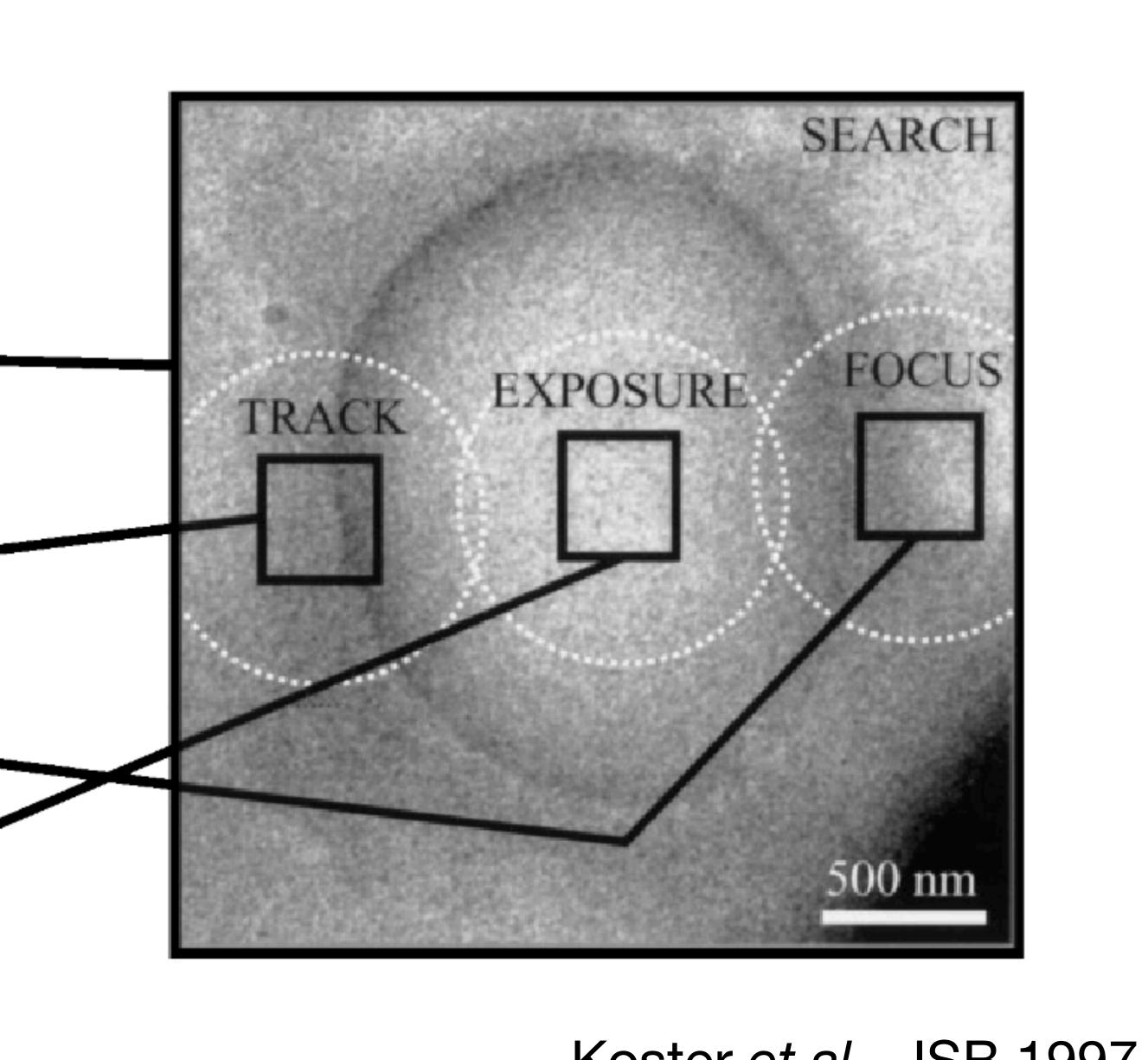


1. SerialEM tilt series data collection with FastTomo script 2. Tilt Series alignment by IMOD

Main goals

Tilt series data collection scheme





Koster et al., JSB 1997

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*

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

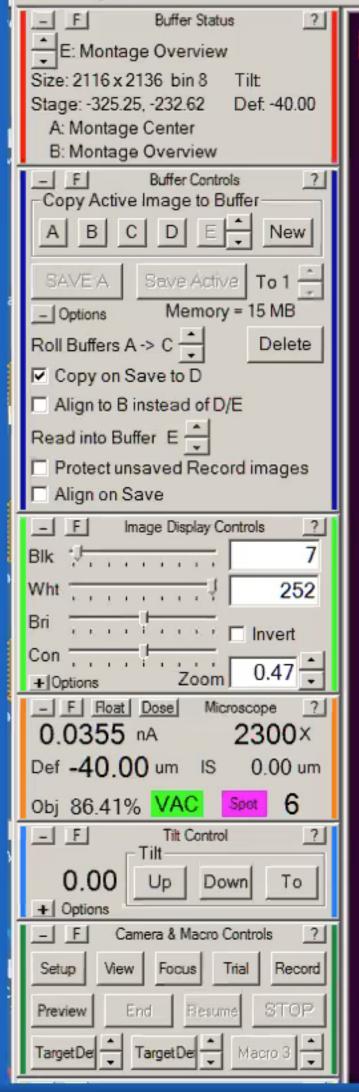
SerialEM provides a flexible interface. The script capability provides a relatively easy way to add commands requested by users

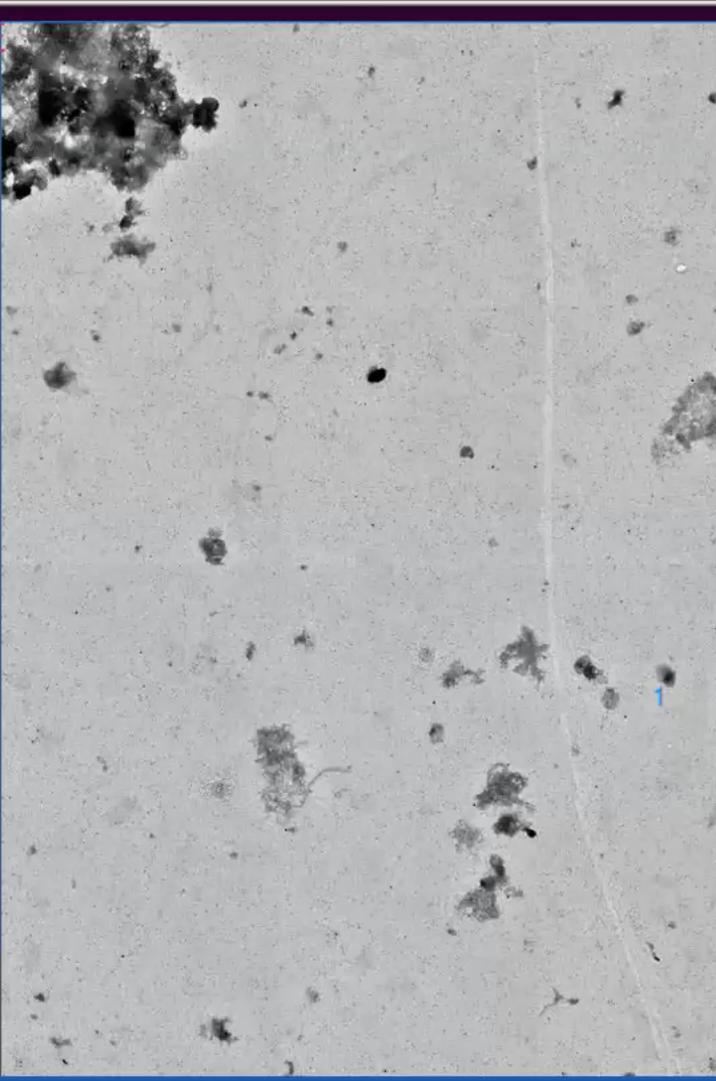
2005





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









	Mastro	narde
		 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 None ○ Focus ○ Trial Moximum area separation: -0.71 um 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 Unblank Search □ Options ○ Offsets for View □ Defocus: -60 → Shift. Set Zero □ Normalize beam through View □ Auto Exposure Exposure (s) 0.5 □ Start View □ Focus Loupe □ Auto Survey □ Comera Unshifted Balance Shifts
		□ Rotate inter-area axis 0 deg Camera Acquire □ Mavigator □ □ □ □ Label: 1 □ Registration point 1 □ □ Color Blue □ □ □ □ □ □ #1 Note: Sec 0 - montage01.st □ □ □ □ □ #1 Note: Sec 0 - montage01.st □ □ □ □ □ #1 Note: Sec 0 - montage01.st □ □ □ □ □ Acquire (A) □ □ □ □ □ □ □ □ Add Stage Pos Registration 1 □ □ □ □ □ □ □ □ Add Points □
C. Plant a bet		Move Item

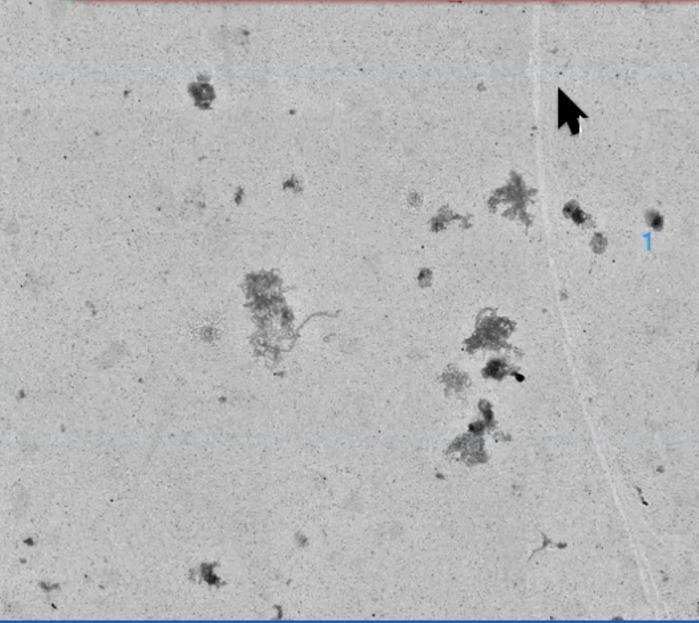




File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help



Defocus Magnification Stage tilt angle





@junliulab

rid Mastro	narde
	 □ Low Dose Control ○ Low Dose Mode View: 2300x Sp 6 C2 61.41% ○ Continuous update of mag & beam ○ Perine position of area ○ None ○ Focus ○ Trial ○ Pesition on tilt axis: 0.00 um ○ Area to show when screen down ○ Vie. ○ Foc. ○ Tri. ○ Rec. ○ Sea. ○ BLANK BEAM when screen down
	Blanked Unblank Search 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
	Image: Camera Acquire Image:
	Acquire map or image or run macro at this location automatically Add Stage Pos Registration 1 Image: Draw all reg. Image: Draw all reg. Add Points Image: Collapse groups Image: Show Acquire area Add Polygon Label Color X Y Z Type Reg. Action Add Marker 1 Blu -325.2 -232.6 118.7 Map 1



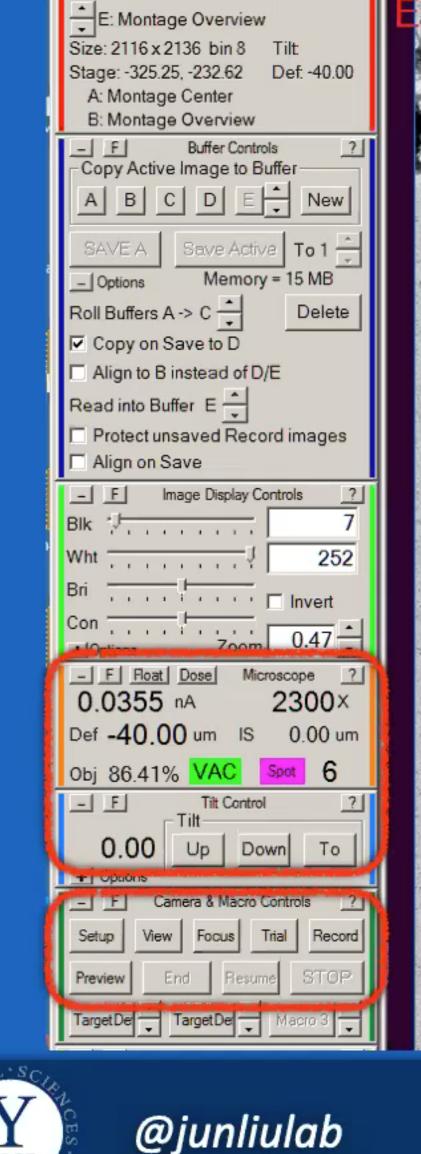


File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help

Defocus Magnification Stage tilt angle

View Focus Trial Record

Setup Preview



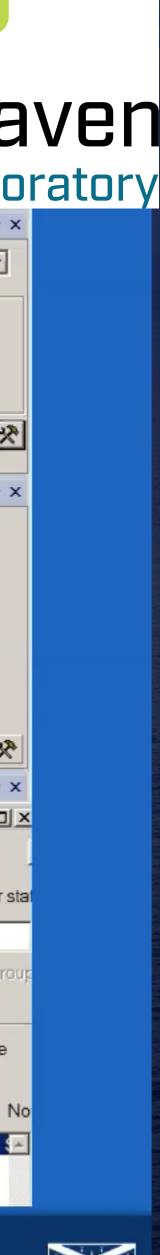
- F

Buffer Status

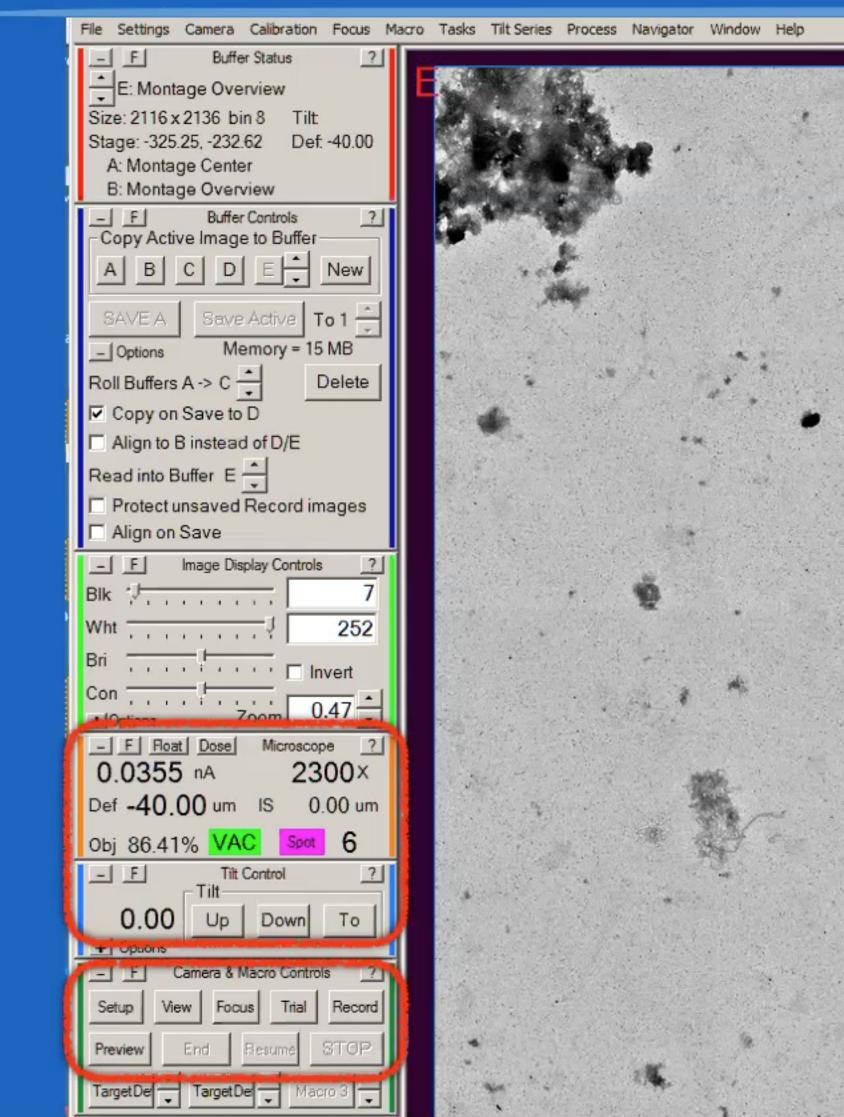
SerialEM (David Mastronarde

	Эх Т				****
				* *	
	*	40. *			
	ж. Т				
		di Ma		-	
				4.	
		۰.			
·*****					
			*		
		3			
and the second se			1.00		and the second

	BIUUKIIć
Low Dose Control ✓ Low Dose Mode	
View: 2300x Sp 6 C2 61.41%	National Labo
Continuous update of mag & beam	K2 Direct Detection 🔹 🗙
Define position of area	Mode: Counted
None O Focus O Trial	
Position on tilt axis: 0.00 um	HW Processing Background Subtraction
Maximum area separation: -0.71 um	Gain Correction
Additional beam shift Set Reset 0.00, 0.00	Update HW Dark Reference
Area to show when screen down	
O Vie.	Health Status
E BLANK BEAM when screen down	Camera View 💌 🗙
Blanked Unblank Search	Setup: Search 💌
Offsets for View	Auto Exposure
Defocus: -60 - Shift Set Zero	Exposure (s) 0.5
Normalize beam through View	
Keep Focus and Trial identical	Start View
Copy current area mag & beam to	Focus Loupe
VFTR	Auto Survey Camera Inserted
Center Unshifted Balance Shifts	Camera Inserted
Rotate inter-area axis 0 deg	Camera Acquire 🔹 🗙
🔜 Navigator	
Label: 1 E Registration poi	oint 1 📑 🗖 Comer point (C)
	Rotate when load For anchor st
#1 Note: Sec 0 - montage01	1.st
🗖 Acquire (A) 🗖 Tilt series 🗖 🕅	Vew file at item 🛛 🗖 New file at gro
Set File Properties Imaging State Acquire map or image or run macro a	at this location automatically
Add Stage Pos Registration 1	Draw all reg. Draw none
Add Delines 1	s 🗂 Show Acquire area
Add Polygon Label Color >	X Y Z Type Reg. Acq. N
Add Marker 1 Blu -32	25.2 -232.6 118.7 Map 1 🕴
Move Item	





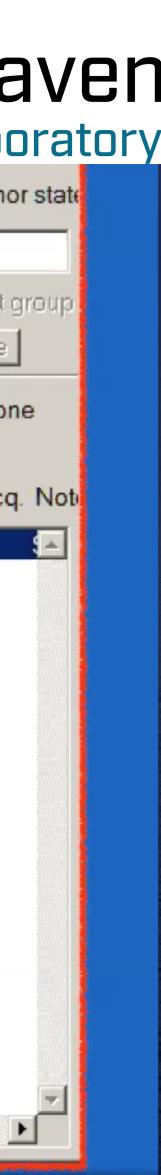






SerialEM - Navigator Brookhaven Navigator National Laboratory Registration point Label: 0 Color Blue Note: Sec 0 - montage01.st #1 □ Acquire (A) □ Tilt series □ New file at item New file at group Set File Properties Imaging State TS Parameters Filename Acquire map or image or run macro at this location automatically Add Stage Pos Registration 1 - Draw all reg. Draw none Add Points Collapse groups C Show Acquire area Z Type Reg. Acq. Noti Add Polygon Label Color X Blu -325.2 -232.6 118.7 Map 1 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

4

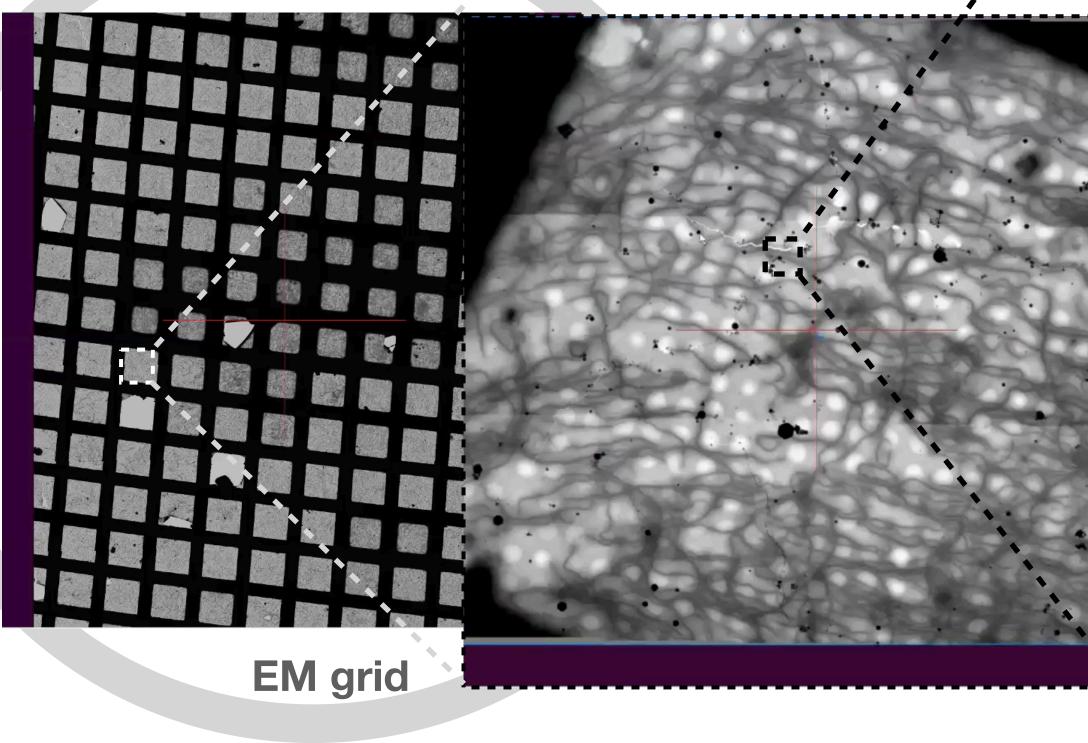




Find targets for tilt series data acquisation

1. Obtain 175 X full montage to survey the grid

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



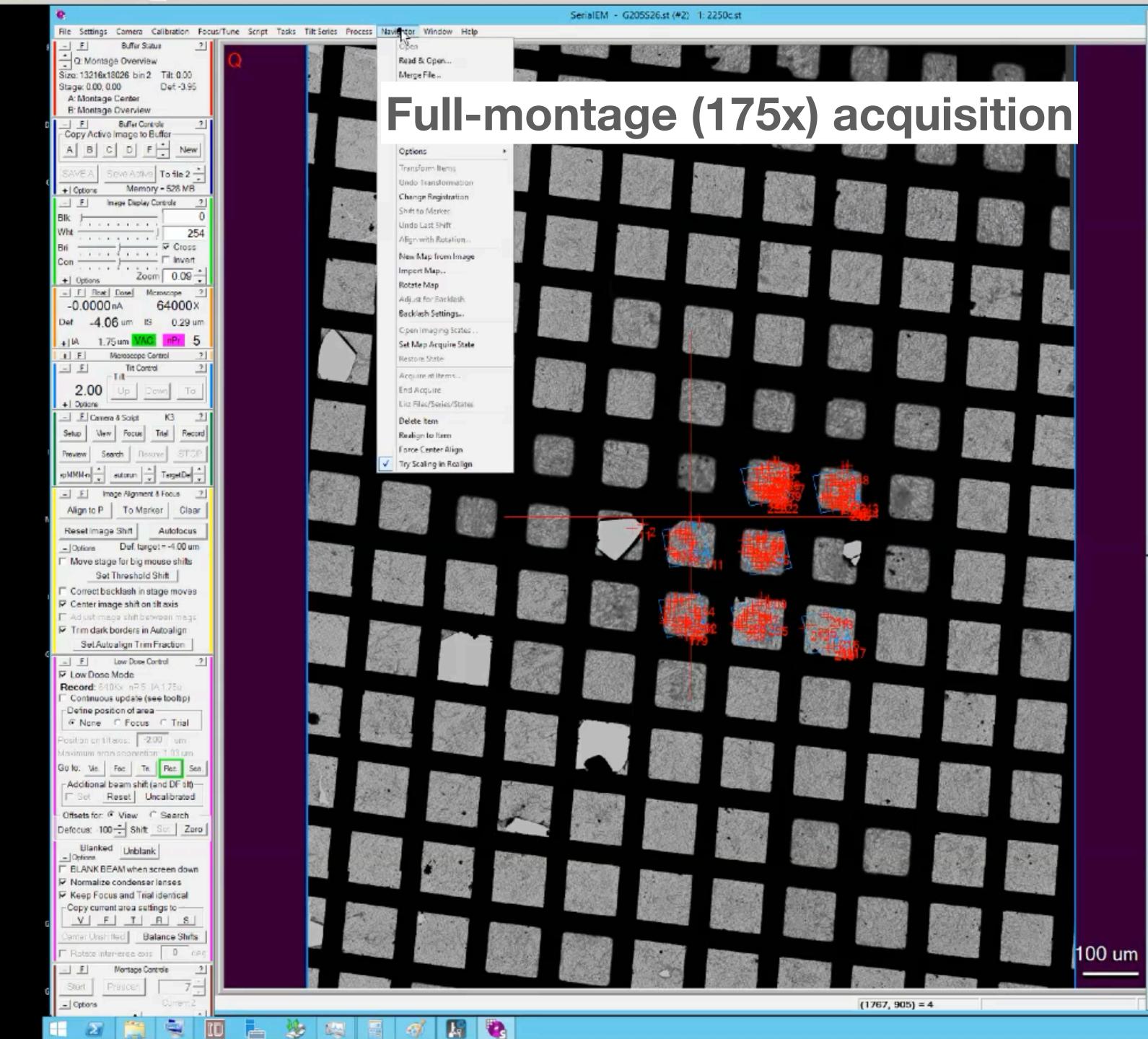
6 x 6 "Search" images

5 x 3 "View" images

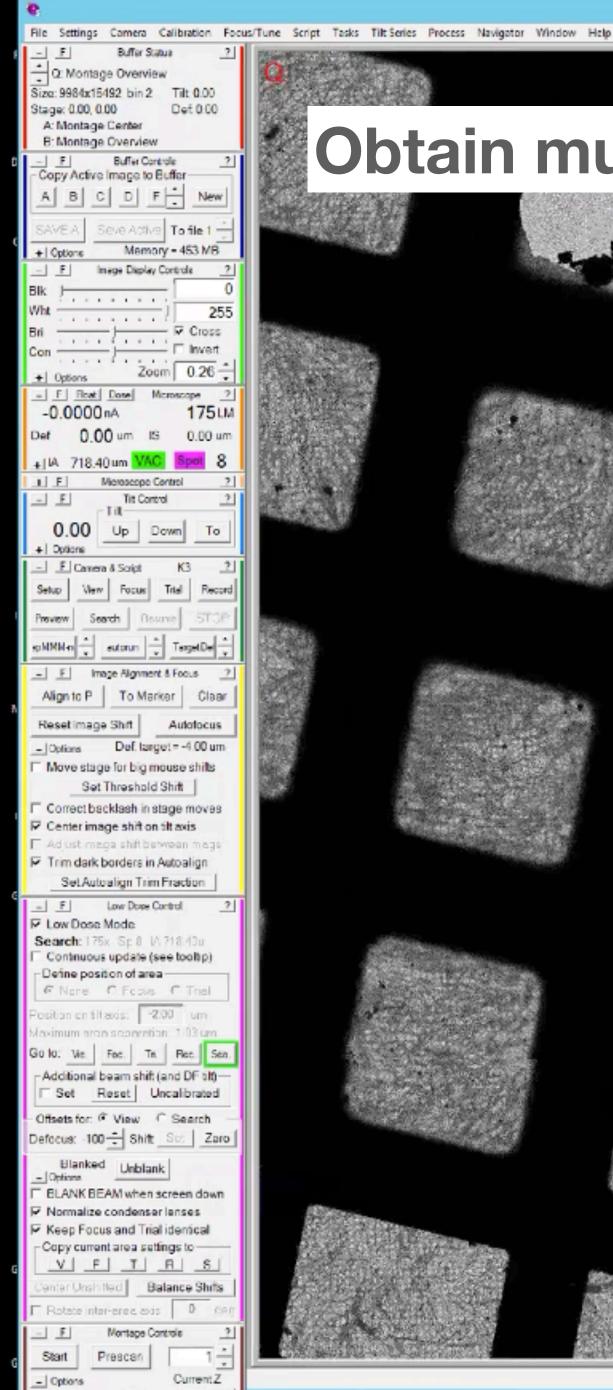
3. add targets in the small montages for tilt series acquisation

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



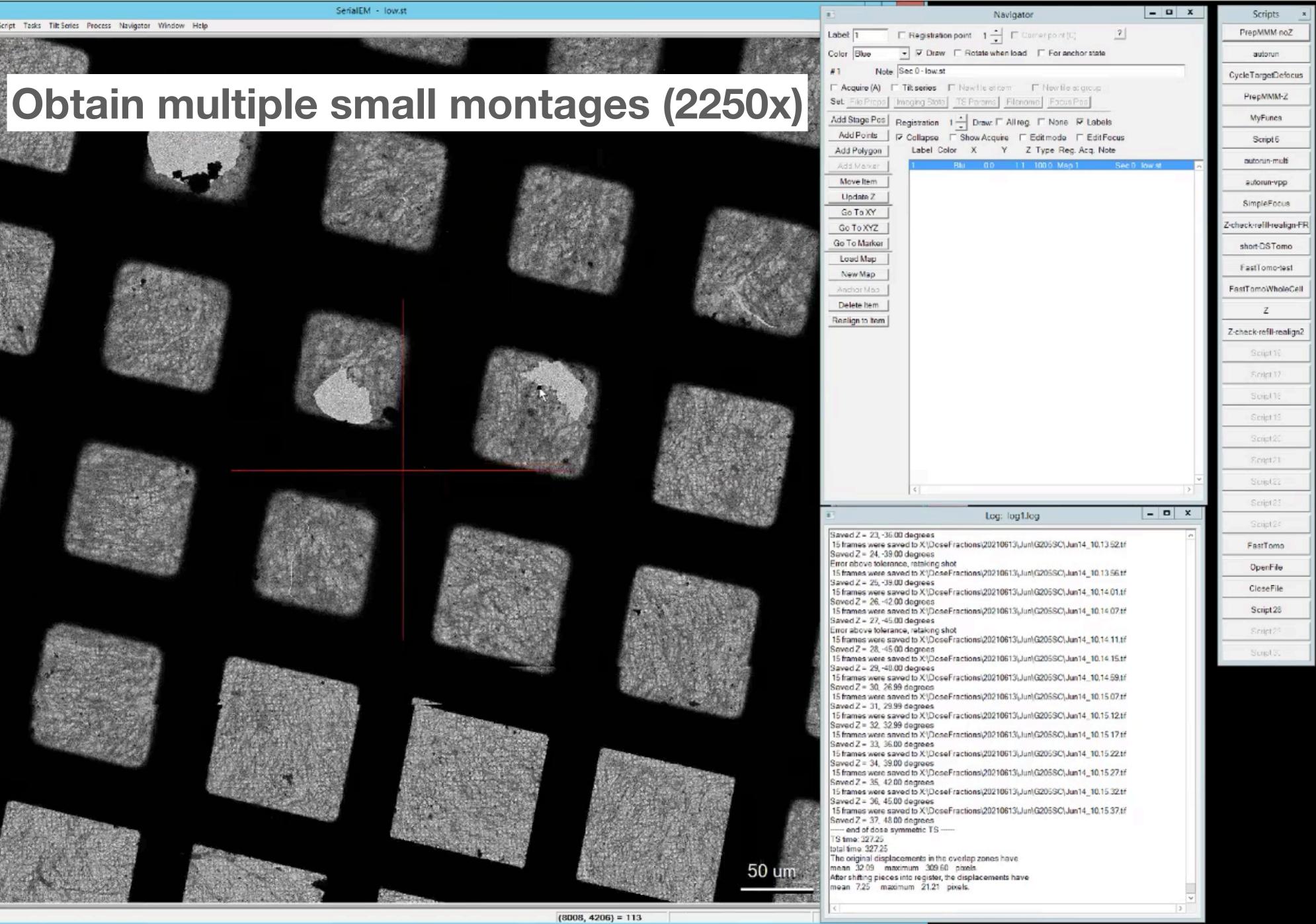


	Navigator: G2055.nav	- • ×	Scripts ×
Label 1 Fieg	gistration point 1 🕂 🗐 Converpont (C)	2	PrepMMM-noZ
Color Blue 🔹 🗟	Drew 🗆 Rotate when load 👘 For anchor state		autorun
	- 175k-borrelia-20210613-grid1.st		Cycle TargetDefocus
	nnies FNawfleatham FNawfleatgroup g Stote TS Parama Filenome Focus Pos		PrepMMM-Z
	ation 1 - Draw: TAllreg. T None P Label	-	MyFunes
Add Points IZ Colla	apse T Show Acquire T Editmode T Edit F		Script6
Add Polygon Lat Add Marker 1	bel Color X Y Z Type Reg. Acq. No Blu 23 75 806 Map 1	Sec 0 175k borrelia 2 a	autorun-multi
G	roup of 1 items, ID2532, labels 2 to 2 roup of 2 items, ID6772, labels 3 to 4	Sec 0 Tras coneta 2 a	autorun-vpp
Update Z 11 4 A	Red 16.5 -100.6 78.1 Pt 1	hole Sec 0 - 2250c.st -	SimpleFocus
Go To XY G Go To XYZ 134	roup of 6 items, ID5271, labels 13 to 18	Sec 1 - 2250c.st -	Z-check-refil-realign-FR
Go To Marker 15-	A Blu 49.2 -5.9 80.4 Map 1	Sec 2 - 2250c.st - Sec 3 - 2250c.st -	short-DSTomo
Load Map 16/	A Blu 185.9 106.0 83.6 Map 1	Sec 4 - 2250c.st - Sec 5 - 2250c.st -	FastTomo-test
	roup of 1 items. ID6599. labels 25 to 25	Sec 6 - 2250c.st -	FastTomoWholeCell
G	roup of 26 items, ID6385, labels 26 to 51 roup of 6 items, ID4795, labels 52 to 57 roup of 17 items, ID1201, labels 58 to 74		
Realign to item G	roup of 36 items, ID		Z
G	roup of 40 items, ID		Z-check-refil-realign2
G	roup of 9 items. ID 3564. labels 210 to 218 roup of 15 items. ID 4758. labels 220 to 234		Scipt 15
G	roup of 5 items, ID 4192, labels 235 to 239 roup of 11 items, ID 1474, labels 240 to 250		Soriet 17
G	roup of 4 items, ID6935, Tabels 251 to 254 iroup of 4 items, ID8252, Tabels 255 to 258		Sup(15
			Script 15
			Script20
			Script21
4		>	Suipt22
		- 0 X	Script22
	Log: log1.log		Script24
Error above tolerance, reta 15 frames were saved to X Seved Z = 22, -33,00 degre	C(DoseFractions)(20210613)(Jun)(G205SC) Jun14_10.13	1.43.tf	FastTomo
	C\DeseFractions\20210613\Jun\G205SC\Jun14_10.13	3.47.tf	OpenFile
	C(DoseFractions)(20210613)(Jun)(G205SC) Jun14_10.13	152.tr	CloseFile
Error above tolerance, reta		356.tf	Script 28
	ClDoseFractions\20210613\Jun\G205SC\Jun14_10.14	1.01.tf	Sicript 25
	C\DeseFractions\20210613\Jun\G205SC\Jun14_10.14	07.tf	Sciet30
Seved Z = 27, -45.00 degre Error above tolerance, reta	along shot		
Saved Z = 28, -45.00 degree	C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14		
Saved Z = 29, -48.00 degre	C\DeseFractions\20210613\Jun\G205SC\Jun14_10.14 ees	15.tf	
Saved Z = 29, -48.00 degra 15 frames were saved to X Saved Z = 30, 26.99 degra	C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees	15.tf	
Saved Z = 29, -48.00 degre 15 frames were saved to X Saved Z = 30, 26.99 degre 15 frames were saved to X Saved Z = 31, 29.99 degre	C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees	59.tf	
Saved Z = 29, -48.00 degre 15 frames were saved to X Saved Z = 30, 26.99 degre 15 frames were saved to X Saved Z = 31, 29.99 degre 15 frames were saved to X Saved Z = 32, 32.99 degre	C\DoseFractions\20210613Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613Jun\G205SC\Jun14_10.14 aes C\DoseFractions\20210613Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613Jun\G205SC\Jun14_10.15 aes	15.tf 59.tf 507.tf 5.12.tf	
Saved Z = 29, -48.00 degre 15 frames were saved to X Saved Z = 30, 26.99 degre 15 frames were saved to X Saved Z = 31, 29.99 degre 15 frames were saved to X Saved Z = 32, 32.99 degre 15 frames were saved to X Saved Z = 33, 36.00 degre	C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees	15.tf 59.tf 507.tf 5.12.tf 5.17.tf	
Saved Z = 29, -48.00 degre 15 frames were saved to X Saved Z = 30, 26.99 degre 15 frames were saved to X Saved Z = 31, 29.99 degre 15 frames were saved to X Saved Z = 32, 32.99 degre 15 frames were saved to X Saved Z = 33, 36.00 degre 15 frames were saved to X Saved Z = 34, 39.00 degre	C\DoseFractions\20210613Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613Jun\G205SC\Jun14_10.15 ees	15.tf 59.tf 507.tf 5.12.tf 5.17.tf 5.22.tf	
Saved Z = 29, -48.00 degree 15 frames were saved to X Saved Z = 30, 26.99 degree 15 frames were saved to X Saved Z = 31, 29.99 degree 15 frames were saved to X Saved Z = 32, 32.99 degree 15 frames were saved to X Saved Z = 33, 36.00 degree 15 frames were saved to X Saved Z = 34, 39.00 degree 15 frames were saved to X Saved Z = 35, 42.00 degree 15 frames were saved to X Saved Z = 35, 42.00 degree	C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees	15.tf 59.tf 07.tf 512.tf 517.tf 522.tf	
Saved Z = 29, -48.00 degree 15 frames were saved to X Saved Z = 30, 26.99 degree 15 frames were saved to X Saved Z = 31, 29.99 degree 15 frames were saved to X Saved Z = 32, 32.99 degree 15 frames were saved to X Saved Z = 33, 36.00 degree 15 frames were saved to X Saved Z = 34, 39.00 degree 15 frames were saved to X Saved Z = 35, 42.00 degree 15 frames were saved to X Saved Z = 36, 45.00 degree 15 frames were saved to X Saved Z = 36, 45.00 degree 15 frames were saved to X Saved Z = 36, 45.00 degree	C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees	15.tf 59.tf 507.tf 512.tf 517.tf 522.tf 527.tf 532.tf	
Saved Z = 29, -48.00 degre 15 frames were saved to X Saved Z = 30, 26.99 degre 15 frames were saved to X Saved Z = 31, 29.99 degre 15 frames were saved to X Saved Z = 32, 32.99 degre 15 frames were saved to X Saved Z = 33, 36.00 degre 15 frames were saved to X Saved Z = 34, 39.00 degre 15 frames were saved to X Saved Z = 35, 42.00 degre 15 frames were saved to X Saved Z = 36, 45.00 degre 15 frames were saved to X Saved Z = 37, 48.00 degre	C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees	15.tf 59.tf 507.tf 512.tf 517.tf 522.tf 527.tf 532.tf	
Saved Z = 29, -48.00 degre 15 frames were saved to X Saved Z = 30, 26.99 degre 15 frames were saved to X Saved Z = 31, 29.99 degre 15 frames were saved to X Saved Z = 32, 32.99 degre 15 frames were saved to X Saved Z = 33, 36.00 degre 15 frames were saved to X Saved Z = 34, 39.00 degre 15 frames were saved to X Saved Z = 35, 42.00 degre 15 frames were saved to X Saved Z = 36, 45.00 degre 15 frames were saved to X Saved Z = 36, 45.00 degre 15 frames were saved to X Saved Z = 37, 48.00 degre end of dose symmetri TS time: 327.25	C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees	15.tf 59.tf 507.tf 512.tf 517.tf 522.tf 527.tf 532.tf	
Saved Z = 29, -48.00 degre 15 frames were saved to X Saved Z = 30, 26.99 degre 15 frames were saved to X Saved Z = 31, 29.99 degre 15 frames were saved to X Saved Z = 32, 32.99 degre 15 frames were saved to X Saved Z = 33, 36.00 degre 15 frames were saved to X Saved Z = 34, 39.00 degre 15 frames were saved to X Saved Z = 36, 42.00 degre 15 frames were saved to X Saved Z = 36, 45.00 degre 15 frames were saved to X Saved Z = 37, 48.00 degre 15 frames were saved to X Saved Z = 37, 48.00 degre end of dose symmetri TS time: 327.25 total time: 327.25	C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees	x 15.tf 59.tf 507.tf 512.tf 522.tf 527.tf 537.tf	
Saved Z = 29, -48.00 degre 15 frames were saved to X Saved Z = 30, 26.99 degre 15 frames were saved to X Saved Z = 31, 29.99 degre 15 frames were saved to X Saved Z = 32, 32.99 degre 15 frames were saved to X Saved Z = 33, 36.00 degre 15 frames were saved to X Saved Z = 34, 39.00 degre 15 frames were saved to X Saved Z = 35, 42.00 degre 15 frames were saved to X Saved Z = 36, 45.00 degre 15 frames were saved to X Saved Z = 37, 48.00 degre end of dose symmetri TS time: 327.25	C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.14 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees C\DoseFractions\20210613\Jun\G205SC\Jun14_10.15 ees	15.tf 59.tf 07.tf 5.12.tf 5.17.tf 5.27.tf 5.32.tf 5.37.tf	



.

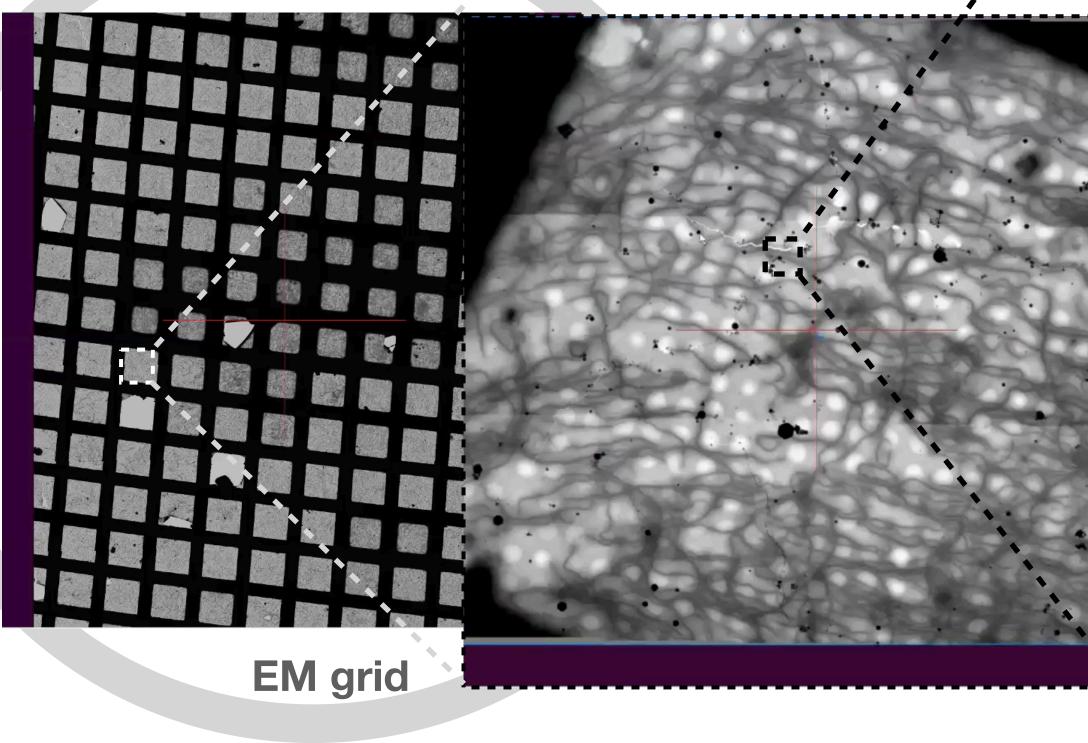
64



Find targets for tilt series data acquisation

1. Obtain 175 X full montage to survey the grid

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



6 x 6 "Search" images

5 x 3 "View" images

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

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



🚾 gatancustomer - TightVNC Viewer

Aligning "Preview/Record" beam with "View" File Settings Camera Calibration Focus/Tune Script Tasks Tilt Series Process Navigator Window Help

占 😕 🚳 🗐 🛷

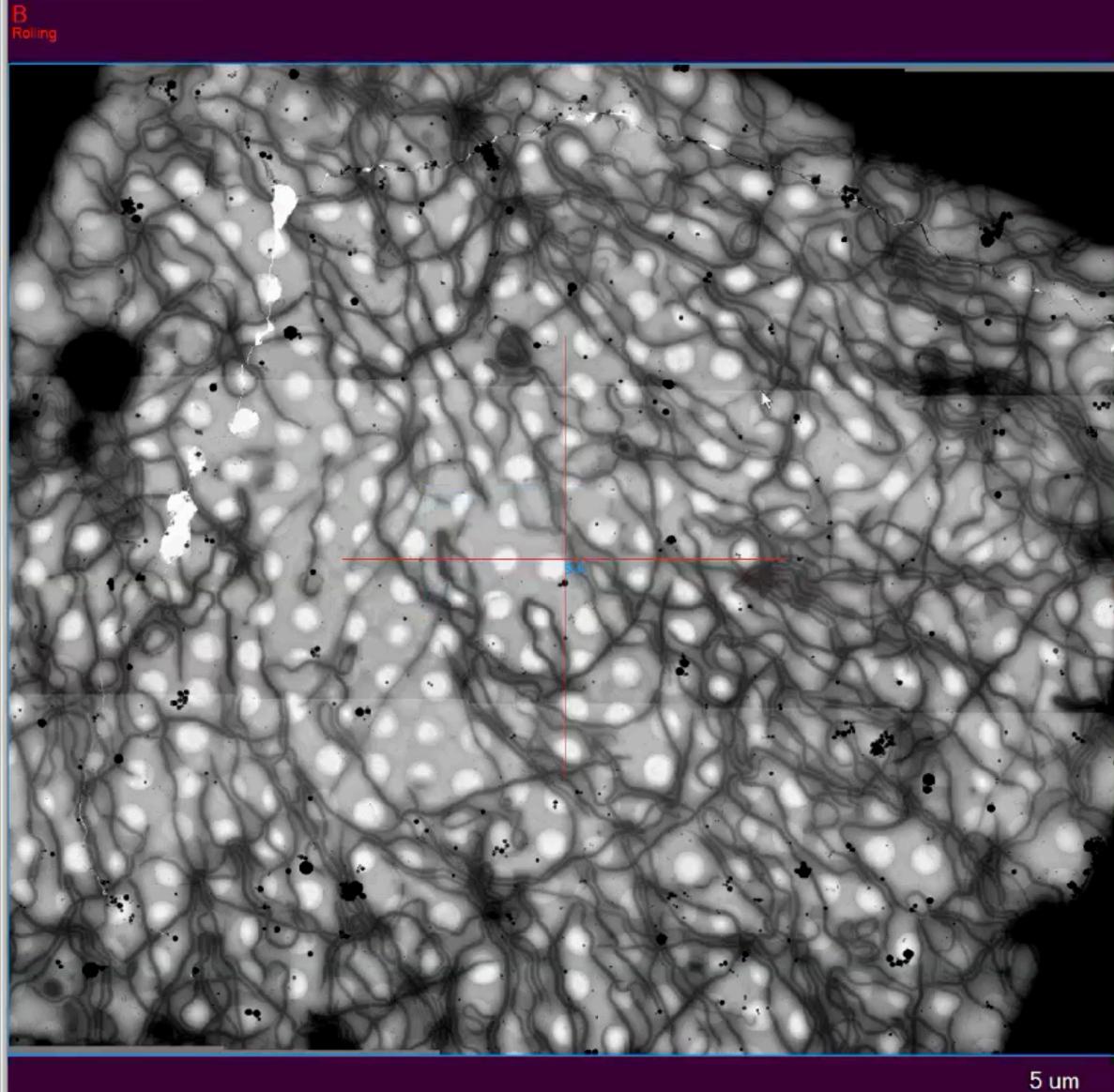
- F Buffer Status B: Montage Overview Size: 1508 x 1344 bin 12 Tilt -0.00 Stage: -180.46, -93.75 Det -106.92 A: Montage Center C: Saved to File, sec. 72 Buffer Controle - F Copy Active Image to Buffer-A B C D F New SAVEA SEVE ACTVE To file 1 Memory - 208 MB + | Options - F Image Display Controls Blk)-1.1.1.1.1.1.1.1 Wht -255 Cross Con _____ Ortens Zoom 0.95 + Options E Rost Dose Mcroscope ? 2250× -0.0000nA Det -106.9 um IS 0.00 um + 1A 48.40 um VAC Spot 6 Microscope Centrol I F - F Tit Control Tit -0.00 Up Down To + Options - E Camera & Script K3 View Focus Tital Record Setup. Preview Search autorun 📩 TargetDe 🛓 RIMMIN + - F Image Alignment & Focus ? Align to P To Marker Clear Reset image Shift Autotocus Def. target = -4.00 um - Options Move stage for big mouse shifts Set Threshold Shift Correct backlash in stage moves Center image shift on tilt axis Adjust mage shiftbetween mage Trim dark borders in Autoalign SetAutoalign Trim Fraction - F Low Dose Control V Low Dose Mode View: 2250x Sp 6 1A 48.40a Continuous update (see tooltp) Define position of area -@ None C Focus C Trial ositon en til acos: 200 um stion: 1.03 um Go to, Vie Foc. Tn. Rec. Sen. -Additional beam shift (and DF tilt)-Set Reset Uncalibrated Offsets for: @ View C Search Defocus: 100 ÷ Shift Sot. Zaro Blanked Unblank - Options ELANK BEAM when screen down Vormalize condenser lanses Keep Focus and Trial identical Copy current area settings to -V F T R S Rotate inter-erectionis 0 na Montage Controle - F Start _ Options Prescan 5 Current Z

-

Σ

Start

10

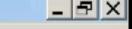


(1020, 445) = 113

ew	r -	Navigator campl	D may		- 0	x
hal 19	E Reader	Navigator: sample		2		
lor Blue		w Rotate when load				
	Sec 4 - mid		1 Por anchor su	a bel		
		Newfile atidem	Newfie et aros			
		3 TS Forema Filenon				
dd Stage Pos	Registration	1 - Draw: T All reg.	E None P La	bels		
	Collapse					
Add Polygon	Label (Type Reg. Acq.			
Add Marker	12		100.0 Map 1 100.0 Pt 1	Sec 0 marke		-
Move Item	3 4		100.0 Pt 1 100.0 Pt 1	bad		
Go To XY	5		100.0 Pt 1 100.0 Pt 1			
Go To XYZ	7 8	Off -113.6 158.7				
ao To Marker	9		100.0 Pt 1 100.0 Pt 1			
Load Map	11	Red -422.6 -19.5	100.0 Pt 1 100.0 Pt 1			
New Map	12	Red -217.4 -215.4	100.0 Pt 1	5-1-5		
Delete item	5-A 6-A	Blu -266.8 74.8 Blu -232.8 195.3		Sec 1	mid.st - mid.st -	
celign to Item	7-A 8-A	Blu -113.6 158.3 Blu -145.6 29.3	72.5 Map 1 73.9 Map 1	Sec 3	mid.st- mid.st-	
	9 A	Blu 180.5 -92.6	79.8 Map 1	Sec 4	mid st -	
	6					4
	<					× ×
		Log: log1.log	-		- 0	*
Saved Z = 36, 45	saved to XND	oseFractions\20210613\Ju	n(G2055C\Jun14_		- 0	·/
Saved Z = 36, 45	saved to XND 5.00 degrees saved to XND		n(G2055C\Jun14_		- 0	·/
Baved Z = 36, 45 15 frames were s	saved to XND 5.00 degrees saved to XND 8.00 degrees	oseFractions\20210613\Ju oseFractions\20210613\Ju	n(G2055C\Jun14_		- 0	·/
Saved Z = 36, 45 15 frames were s Saved Z = 37, 48 end of dose : 15 tme: 327.25 otal tme: 327.25	saved to XND 5.00 degrees saved to XND 8.00 degrees symmetric TS	oseFractions\20210613\Ju oseFractions\20210613\Ju	n(G2055C\Jun14_		- 0	·/
Saved Z = 36, 45 15 frames were s Saved Z = 37, 48 end of dose : 15 time: 327.25 otal time: 327.25 The original displ nean, 32.09 m	saved to XND 5.00 degrees saved to XND 8.00 degrees symmetric TS Nacements in naximum 309	oseFractions\20210613\Ju oseFractions\20210613\Ju s the overlap zones have 60 pixels.	n(G2055C),Jun14_ n(G2055C),Jun14_		- 0	·/
Saved Z = 36, 45 15 frames were s Saved Z = 37, 48 	saved to XND 5.00 degrees saved to XND 8.00 degrees symmetric TS Nacements in naximum 309 ses into regists aximum 21.2	the overlap zones have by pixels or, the displacements have pixels or, the displacements have pixels	n(G205SC),Jun14_ n(G205SC),Jun14_		- 0	·/
Saved Z = 36, 45 15 frames were s Saved Z = 37, 48 	saved to XND 5.00 degrees saved to XND 8.00 degrees symmetric TS lacements in naximum 309 les into registr aximum 21.2 ity: changing 2 ity: changing 2	the overlap zones have 60 pixels r, the displacements have 1 pixels 2 by -21.94 to 78.06, continu 2 by -1.50 to 75.56, finished	n(G205SC\Jun14_ n(G205SC\Jun14_ ing	10.15.37.tif		x
Saved Z = 36, 45 15 frames were s Saved Z = 37, 48 	saved to XND 5.00 degrees saved to XND 8.00 degrees symmetric TS lacements in naximum 309 les into registe aximum 21.2 ity: changing 2 al displaceme les into registe	the overlap zones have 60 pixels 7, the displacements have 7 pixels 7 by -21.94 to 78.06, continu 7 by -1.50 to 75.56, finished 7, the displacements have 8 by -21.94 to 78.06, continu 9 by -1.50 to 75.56, finished 1 by -1.50 to 75.56, finished 1 by -1.50 to 75.56, finished	n(G205SC\Jun14_ n(G205SC\Jun14_ ing we mean 14.74	meximum	25.74 p	×
Saved Z = 36, 45 15 frames were s Saved Z = 37, 48 	saved to XND 5.00 degrees saved to XND 8.00 degrees symmetric TS lacements in naximum 309 les into regists aximum 21.2 ity: changing 2 ity: changing 2 al displaceme les into regists item # 5 with la ity: changing 2	the overlap zones have 60 pixels ar, the displacements have 1 pixels 2 by -21.94 to 78.06, continu 2 by -1.50 to 76.56, finished nts in the overlap zones have abel 5 saved at Z = 0 2 by -20.84 to 79.17, continu	n(G205SC\Jun14_ n(G205SC\Jun14_ ing we mean 14.74 mean 0.33 ma	meximum	25.74 p	×
Saved Z = 36, 45 15 frames were s Saved Z = 37, 48 end of dose : 15 tme: 327,25 otal tme: 327,25 The original displ near, 32,09 m ther shifting piece Rough eucentricit 2 = 0: The original Mer shifting piece Mer shifting piece Me	saved to XND 500 degrees saved to XND 800 degrees symmetric TS lacements in naximum 309 res into regists aximum 21.2 ity: changing 2 ity: changing 2 ity: changing 2 ity: changing 2 ity: changing 2 ity: changing 2 al displaceme	the overlap zones have 60 pixels. ar, the displacements have 1 pixels. 2 by -21.94 to 78.06, continu 2 by -1.50 to 78.56, finished aris in the overlap zones have abel 5 saved at Z = 0 2 by -20.84 to 79.17, continu 5 by -1.47 to 77.70, finished aris in the overlap zones have	n(G205SC),Jun14_ n(G205SC),Jun14_ ing ive mean 14.74 imgan 0.33 ma ing ive mean 14.62	mædmum edmum 0.7.	25.74 pi 3 pixels. 22.51 pi	×
Saved Z = 36, 45 15 frames were s Saved Z = 37, 48 end of dose : 15 tme: 327,25 otal tme: 327,25 The original displ near, 32,09 m Wer shifting piece near, 7,25 ma Rough eucentricit 2 = 0: The original Wer shifting piece Map acquired at i Rough eucentricit Rough eucentricit	saved to XND 500 degrees saved to XND 800 degrees symmetric TS lacements in naximum 309 res into regists aximum 21.2 ity: changing 2 ity: changing 2	the overlap zones have the overlap zones have 60 pixels ar, the displacements have 2 by -21.94 to 78.06, continu 2 by -1.50 to 78.56, finished ms in the overlap zones have abel 5 saved at Z = 0 2 by -20.84 to 79.17, continu 2 by -1.47 to 77.70, finished ms in the overlap zones have abel 5 saved at Z = 1	n(G205SC),Jun14_ n(G205SC),Jun14_ ing we mean 14.74 mean 0.33 ma ing we mean 14.62 mean 0.74 ma	mædmum edmum 0.7.	25.74 pi 3 pixels. 22.51 pi	×
Saved Z = 36, 45 Is frames were s Saved Z = 37, 48 end of dose : IS time: 327,25 otal time: 327,25 The original displ near, 32,09 m der shifting piece near, 7,25 ma Rough eucentricit Cough eucentricit Rough eucentricit Rough eucentricit Rough eucentricit Rough eucentricit Cough eucentricit Rough eucentricit Rough eucentricit Rough eucentricit Rough eucentricit Rough eucentricit Rough eucentricit Rough eucentricit	saved to XND 500 degrees saved to XND 800 degrees symmetric TS lacements in naximum 309 res into regists aximum 21.2 ity: changing 2 ity: changing 2	the overlap zones have 60 pixels ar, the displacements have 2 by -21.94 to 78.06, continu 2 by -21.94 to 78.06, continu 2 by -1.50 to 78.56, finished ms in the overlap zones have abel 5 saved at Z = 0 2 by -20.84 to 79.17, continu 2 by -1.47 to 77.70, finished ms in the overlap zones have abel 6 saved at Z = 1 2 by -25.87 to 74.14, continu 2 by -1.63 to 72.52, finished	n(G205SC),Jun14_ n(G205SC),Jun14_ n(G205SC),Jun14_ ing we mean 14.74 ing we mean 14.62 ing we mean 0.74 ma	mædmum edmum 0.7. mædimum 2.12	25.74 pi 3 pixels. 22.51 pi 2 pixels.	×
Saved Z = 36, 45 15 frames were s Saved Z = 37, 48 end of dose : 15 tme: 327.25 otal tme: 327.25 The original displ near, 32.09 m Atter shifting piece Near, 7.25 ma Rough eucentricit Rough eucentricit 2 = 0: The original Atter shifting piece Map acquired at i Rough eucentricit 2 = 1: The original Atter shifting piece Map acquired at i Rough eucentricit Rough eucentricit Rough eucentricit Rough eucentricit Rough eucentricit Rough eucentricit 2 = 2: The original Mar shifting piece	saved to XND 5.00 degrees saved to XND 8.00 degrees symmetric TS lacements in naximum 309 res into regists aximum 21.2 by changing 2 id displaceme les into regists item # 5 with li by changing 2 al displaceme les into regists item # 6 with li by changing 2 al displaceme les into regists item # 6 with li by changing 2 al displaceme les into regists	eseFractions\20210613\Ju eseFractions\20210613\Ju seFractions\20210613\Ju seFractions\20210613\Ju ser. the overlap zones have 0 pixels or, the displacements have 1 pixels 2 by -21.94 to 78.06, continu 2 by -1.50 to 75.56, finished ints in the overlap zones have abel 5 saved at Z = 0 2 by -20.84 to 79.17, continu 2 by -1.47 to 77.70, finished ints in the overlap zones have abel 6 saved at Z = 1 2 by -25.87 to 74.14, continu 2 by -1.63 to 72.52, finished ints in the overlap zones have abel 6 saved at Z = 1 2 by -25.87 to 74.14, continu 2 by -1.63 to 72.52, finished ints in the overlap zones have abel 6 saved at Z = 1 2 by -25.87 to 74.14, continu 2 by -1.63 to 72.52, finished ints in the overlap zones have ar, the displacements have	n(G205SC),Jun14_ n(G205SC),Jun14_ ing we mean 14.74 imgan 0.33 ma ing we mean 14.62 imgan 0.74 ma ing	mædmum edmum 0.7. mædmum 2.12 mædimum	25.74 pi 3 pixels. 22.51 pi 2 pixels. 23.55 pi	
Saved Z = 36, 45 15 frames were s Saved Z = 37, 48 end of dose : 15 tme: 327.25 otal tme: 327.25 The original displ near, 32.09 m Atter shifting piece Rough eucentricit 2 = 0: The original Atter shifting piece Map acquired at i Rough eucentricit 2 = 1: The original Atter shifting piece Map acquired at i Rough eucentricit 2 = 2: The original Mar shifting piece Map acquired at i Rough eucentricit 2 = 2: The original Mar shifting piece Map acquired at i Rough eucentricit 3 = 2: The original Mar shifting piece Map acquired at i 3 = 2: The original	saved to XND 5.00 degrees saved to XND 8.00 degrees symmetric TS lacements in naximum 309 res into regists aximum 21.2 ity: changing 2 id displaceme les into regists item # 5 with li ity: changing 2 ity: changing 2 al displaceme les into regists item # 6 with li ity: changing 2 al displaceme res into regists item # 7 with li ity: changing 2	eseFractions\20210613\Ju eseFractions\20210613\Ju seeFractions\20210613\Ju seeFractions\20210613\Ju seer, the overlap zones have 1 pixels 2 by -21.94 to 78.06, continu 2 by -21.94 to 78.06, continu 2 by -1.50 to 78.56, finished ints in the overlap zones have abel 5 saved at Z = 0 2 by -20.84 to 79.17, continu 2 by -1.47 to 77.70, finished ints in the overlap zones have abel 6 saved at Z = 1 2 by -25.87 to 74.14, continu 2 by -1.63 to 72.52, finished ints in the overlap zones have abel 7 saved at Z = 2 2 by -24.25 to 75.76, continu	n(G205SC)Jun14_ n(G205SC)Jun14_ ing we mean 14.74 imgan 0.33 ma ing we mean 14.62 imgan 0.74 ma ing we mean 15.16 imgan 0.37 ma	mædmum edmum 0.7. mædmum 2.12 mædimum	25.74 pi 3 pixels. 22.51 pi 2 pixels. 23.55 pi	×
Saved Z = 36, 45 15 frames were s Saved Z = 37, 48 	saved to X:)Dr 5.00 degrees saved to X:)Dr 8.00 degrees symmetric TS lacements in naximum 305 les into regista at displaceme les into regista item # 5 with la ity: changing 2 at displaceme les into regista item # 6 with la ity: changing 2 at displaceme les into regista item # 6 with la ity: changing 2 at displaceme les into regista item # 7 with la ity: changing 2 at displaceme les into regista item # 7 with la item # 7 with la	the overlap zones have 60 pixels ar, the displacements have 20 pixels ar, the displacements have 20 pixels ar, the displacements have 20 pixels 20	n(G205SC)Jun14_ n(G205SC)Jun14_ ing we mean 14.74 mean 0.33 ma ing we mean 14.62 mean 0.74 ma ing we mean 15.16 mean 0.37 ma ing we mean 14.84	mædmum edmum 0.7. mædmum 2.12 mædmum 1.44 mædmum 1.44	25.74 pi 3 pixels. 22.51 pi 2 pixels. 23.55 pi 4 pixels. 24.63 pi	×
Saved Z = 36, 45 15 frames were s Saved Z = 37, 48 end of dose : 15 tme: 327.25 otal tme: 327.25 The original displ near, 32.09 m ther shifting piece Rough sucentricit Rough sucentricit 2 = 0: The original ther shifting piece Map acquired at i Rough sucentricit Rough sucentricit	saved to XND 500 degrees saved to XND 800 degrees symmetric TS lacements in naximum 309 es into registe aximum 21.2 ity: changing 2 ity: chang	baeFractions\20210613\Ju baeFractions\20210613	n(G205SC)Jun14_ n(G205SC)Jun14_ ing we mean 14.74 mean 0.33 ma ing we mean 14.62 mean 0.74 ma ing we mean 15.16 mean 0.37 ma ing we mean 14.84	mædmum edmum 0.7. mædmum 2.12 mædmum 1.44 mædmum 1.44	25.74 pi 3 pixels. 22.51 pi 2 pixels. 23.55 pi 4 pixels. 24.63 pi	×
Saved Z = 36, 45 15 frames were s Saved Z = 37, 48 end of dose : 15 trme: 327,25 otal trme: 327,25 The original displ near, 32,09 m ther shifting piece Rough eucentricit Rough eucentricit 2 = 0: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 2: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 2: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 3: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 3: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 3: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 3: The original	saved to X:)Dr 5.00 degrees saved to X:)Dr 8.00 degrees symmetric TS lacements in naximum 309 les into regists aximum 21.2 ity: changing 2 at displaceme les into regists item # 5 with la ity: changing 2 at displaceme les into regists item # 6 with la ity: changing 2 at displaceme les into regists item # 7 with la ity: changing 2 at displaceme les into regists item # 7 with la ity: changing 2 displaceme les into regists item # 8 with la ity: changing 2 displaceme les into regists item # 8 with la	baeFractions\20210613\Ju baeFractions\20210613	n(G205SC\Jun14_ n(G205SC\Jun14_ n(G205SC\Jun14_ mean 14.74 mean 0.33 ma ing we mean 14.62 mean 0.74 ma ing we mean 15.16 mean 0.37 ma ing we mean 14.84 mean 0.29 ma	mædmum edmum 0.7. mædmum 2.12 mædmum 1.44 mædmum 1.44	25.74 pi 3 pixels. 22.51 pi 2 pixels. 23.55 pi 4 pixels. 24.63 pi	×
Saved Z = 36, 45 15 frames were s Saved Z = 37, 48 end of dose : 15 time: 327,25 otal time: 327,25 Ine original displ near, 32,09 m ther shifting piece near, 7,25 ma Rough eucentricit Rough eucentricit 2 = 0: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 1: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 2: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 3: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 3: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 3: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 3: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 4: The original	saved to X:)Dr 5.00 degrees saved to X:)Dr 8.00 degrees symmetric TS lacements in naximum 305 les into registr aximum 21.2 ity: changing 2 at displaceme les into registr item # 5 with li ity: changing 2 at displaceme les into registr item # 6 with li ity: changing 2 at displaceme les into registr item # 7 with li ity: changing 2 at displaceme les into registr item # 7 with li ity: changing 2 at displaceme les into registr item # 8 with li ity: changing 2 at displaceme les into registr item # 8 with li ity: changing 2 at displaceme les into registr item # 8 with li ity: changing 2 at displaceme	baseFractions\20210613\Ju baseFractions\20210613\20210613\Ju baseFractions\20210613\20210613\20210613\Ju baseFractions\20210613\20210613\20210613\20210613\Ju baseFractions\20210613\20210613\20210613\20210613\20210613\20210613\20210613\20210613\20210613\20210613\20210613\20210613\20210613\20210613\20210613\20210613\20210613\20210613\2021061200613\2021000000000000000000000000000000000	n(G205SC\Jun14_ n(G205SC\Jun14_ ing we mean 14.74 mean 0.33 ma ing we mean 14.62 mean 0.74 ma ing we mean 15.16 mean 0.37 ma ing we mean 14.84 mean 0.29 ma ing we mean 15.95	mædmum edmum 0.7. mædmum 2.12 mædmum 1.44 mædmum 1.00 mædmum 1.00	25.74 pi 3 pixels. 22.51 pi 2 pixels. 23.55 pi 4 pixels. 24.63 pi 3 pixels. 23.51 pi	
Saved Z = 36, 45 15 frames were s Saved Z = 37, 48 end of dose : 15 trme: 327,25 otal trme: 327,25 The original displ near, 32,09 m ther shifting piece Rough eucentricit Rough eucentricit 2 = 0: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 1: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 2: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 3: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 3: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 3: The original ther shifting piece Map acquired at i Rough eucentricit 2 = 4: The original ther shifting piece	saved to X:(D) 5.00 degrees saved to X:(D) 8.00 degrees symmetric TS lacements in naximum 309 les into regists atimum 21.2 ity: changing 2 atisplaceme les into regists item # 5 with la ity: changing 2 atisplaceme les into regists item # 6 with la ity: changing 2 atisplaceme les into regists item # 7 with la ity: changing 2 atisplaceme les into regists item # 7 with la ity: changing 2 atisplaceme les into regists item # 8 with la item # 8 wit	beeFractions\20210613\Ju beeFractions\20210613\Ju beeFractions\20210613\Ju beeFractions\20210613\Ju beer the overlap zones have 60 pixels ar, the displacements have 1 pixels 2 by -21.94 to 78.06, continu 2 by -2.94 to 78.06, continu 2 by -2.94 to 77.0, finished ints in the overlap zones have abel 5 saved at Z = 0 2 by -20.84 to 77.0, finished ints in the overlap zones have abel 6 saved at Z = 1 2 by -25.87 to 74.14, continu 2 by -1.63 to 72.52, finished ints in the overlap zones have abel 7 saved at Z = 2 2 by -24.25 to 75.76, continu 2 by -1.87 to 73.89, finished ints in the overlap zones have abel 8 saved at Z = 3 2 by -18.76 to 81.25, continu 2 by -1.47 to 79.77, finished	n(G205SC\Jun14_ n(G205SC\Jun14_ ing we mean 14.74 mean 0.33 ma ing we mean 14.62 mean 0.74 ma ing we mean 15.16 mean 0.37 ma ing we mean 14.84 mean 0.29 ma ing we mean 15.95	mædmum edmum 0.7. mædmum 2.12 mædmum 1.44 mædmum 1.00 mædmum 1.00	25.74 pi 3 pixels. 22.51 pi 2 pixels. 23.55 pi 4 pixels. 24.63 pi 3 pixels. 23.51 pi	
Saved Z = 36, 45 Is frames were s Saved Z = 37, 48 	saved to X:(D) 5.00 degrees saved to X:(D) 8.00 degrees symmetric TS lacements in naximum 309 les into regists atimum 21.2 ity: changing 2 atisplaceme les into regists item # 5 with la ity: changing 2 atisplaceme les into regists item # 6 with la ity: changing 2 atisplaceme les into regists item # 7 with la ity: changing 2 atisplaceme les into regists item # 7 with la ity: changing 2 atisplaceme les into regists item # 8 with la item # 8 wit	beeFractions\20210613\Ju beeFractions\20210613\Ju beeFractions\20210613\Ju beeFractions\20210613\Ju beer the overlap zones have 2 op pixels ar, the displacements have 1 pixels 2 by -21.94 to 78.06, continu 2 by -2.94 to 79.17, continu 2 by -20.84 to 79.17, continu 2 by -20.84 to 77.10, finished ints in the overlap zones have abel 6 saved at Z = 0 2 by -25.87 to 74.14, continu 2 by -1.63 to 72.52, finished ats in the overlap zones have abel 7 saved at Z = 2 2 by -24.25 to 75.76, continu 2 by -1.87 to 73.89, finished ints in the overlap zones have abel 8 saved at Z = 3 2 by -18.76 to 81.25, continu 2 by -1.47 to 79.77, finished ints in the overlap zones have abel 8 saved at Z = 3 2 by -18.76 to 81.25, continu 2 by -1.47 to 79.77, finished ints in the overlap zones have abel 8 saved at Z = 3 2 by -1.87 to 79.77, finished ints in the overlap zones have abel 8 saved at Z = 3 2 by -1.87 to 79.77, finished ints in the overlap zones have abel 8 saved at Z = 3 2 by -1.87 to 79.77, finished ints in the overlap zones have abel 8 saved at Z = 3 2 by -1.87 to 79.77, finished ints in the overlap zones have abel 9 saved at Z = 3 2 by -1.87 to 79.77, finished ints in the overlap zones have abel 9 saved at Z = 3 2 by -1.87 to 79.77, finished ints in the overlap zones have abel 9 saved at Z = 3 2 by -1.87 to 79.77, finished at the displacements have	n(G205SC\Jun14_ n(G205SC\Jun14_ ing we mean 14.74 mean 0.33 ma ing we mean 14.62 mean 0.74 ma ing we mean 15.16 mean 0.37 ma ing we mean 14.84 mean 0.29 ma ing we mean 15.95	mædmum edmum 0.7. mædmum 2.12 mædmum 1.44 mædmum 1.00 mædmum 1.00	25.74 pi 3 pixels. 22.51 pi 2 pixels. 23.55 pi 4 pixels. 24.63 pi 3 pixels. 23.51 pi	

Scripts. PrepMMM-noZ autorun CycleTargetDefocus PrepMMM-Z MyFuncs Script 6 autorun-multi autorun-vpp SimpleFocus check-refil-realign-Fi short-DSTomo FastTomo-test FastTomoWholeCell Z Z-check-refil-realign/ Scipt 18 Soriet 17 Sup[18 Script 19 Script20 Script 21 Sunjal 22 Script 23 Script24 FestTomp OpenFile CloseFile Script 28 Script29 Script30

🔟 🛎 🌇 🗏 🍖 🕼 🕾 🏱 👘 10:53 AM



* 🚺 🕩 😼 🛅 10:53 AM 💻

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> File Settings Camera Calibrat - F Buffer Status A Saved to File. sec. 37 * Date Created: May 22, 2020 Size: 1020 x 1440 bin 4 Tilt Stage: -250.59, 59.54 Det B: Saved to File, sec. 36 * Last Modified @ChenXu: June 13, 2022 C: Saved to File, sec. 35 - F Buffer Controle Copy Active Image to Buffer + A B C D F. SAVEA Seve Active To f Memory = 46 + Options - F Image Display Controls scheme = 1Blk ----1........ Wht : (# 0 = bidirectional _ Zoom + Options - F Rost Dose Memory # 1 = dose-symmetric -0.0000nA 640 Det -3.61 um 📧 0# 2 = unidirectional + IA 1.75 um VAG 11 I F Microscope Centrol - F Tit Control Tit-0.00 Up Down runOnNavItem = 0+ Options - E Camera & Script K3 New Focus Trial Debug = 0 Setup # verbose output for debugging Preview Search Resume spinister autorun - Tang shot = R # low dose beam to use for saving data - F Image Alignment & Focu Align to O To Marker # skip calibration and use most recent parameters if they exist usePrevCalib = 0Reset image Shift Auto - Options Def. target = -4 tolerance = 0.4 Move stage for big mouse s Set Threshold Shift Correct backlash in stage m eucentricity option = -1Center image shift on tilt axis 🔽 Ad ust mage shift between Trim dark borders in Autoal multiRecord = 1 # take more than one R shots along tlting axis SetAutoalign Trim Fraction # R shift 3 and 6 ums, can be more than 2 here. I E Low Doos Control MultiR = { 3 6 } F Low Dose Mode Record: 54.0Kx h25 Continuous update (see too) ### dose-symmetric settings Define position of area -@ None C Focus C T -2.00 startAngleDS = 0 Go lo: Vie. Foc. Tr. Rec. Additional beam shift (and DI endAngleDS = 48 Reset Uncalibr Offsets for: @ View C See stepSizeDS = Defocus: 100 - Shift Blanked Unblank ELANK BEAM when screen groupSizeDS = 8 Normalize condenser lense Keep Focus and Trial identi-# can also be set to V Copy current area settings to trackingShot = V VFTR Senter Unshifted Balance doExtraTrackingShot = 1 Rotate inter-erola cois. startAngleDS is non-zero Montage Controls F Start Prescan CurrentZ - Options 🗔 🛷 🖪 🖏 n m

PrepMMM-n 2 Corner point (C) Isad 🛛 🗖 For enciror state autorun Gycle TargetDe □ New file at group PrepMMMlename Focus Pos MyFuncs Il reg. T None 🔽 Labels Editmode EditFocus Script 6 Z Type Reg. Acq. Note autorun-mi 6.0 100.0 Map 1 Sec 0 low.st FastTOMO script labels 2 to 2 autorun-vp labels 3 to 3 labels 4 to 4 labels 5 to 13 SimpleFoci 74.8 76.6 Map 1 Sec 0 - mid.st 95.3 77.7 Map 1 Sec 1 - mid.st -Z-check-refill-real 58.3 72.5 Map 1 Sec 2 · mid.st 29.3 73.9 Map 1 Sec 3 - mid.st short-DSTor 92.6 79.8 Map 1 Sec 4 - mid.st labels 19 to 19 FastTomo-t labels 20 to 36 Some Acq FastTomoWho Z Z-check-refill-re Scipt16 # set to 1 to run on highlighted navigator point, and when using Acquire at Items Soriet 17 Scipt18 Script 13 Script20 Script 21 Suipl22 # redo a shot if the current frame is off target (0.5 = more than 50% off screen) Script23 - 0 X g1.log Script 24 # 1 = rough, 2 = fine, 3 = rough & fine, 4 = calls the script named Z, -1 = using autofocus 13\Jun\Sanoke2\Jun14_11.17.09.tf FastTomo OpenFile 313\Jun\Senoke2\Jun14_11.1713.tf CloseFile 313\Jun\Sanoke2\Jun14_11.17.18.tf Script 28 \$13\Jun\Senoke2\Jun14_11.1723.tf Script25 1.17.27.tf Suipl3. 1.17.32.tf x # number of tilts before switching sides, 1 = original Wim Hagen scheme 1.18.54.tf # 0 = off, 1 = on; track first non-zero tilts, e.g. at +/-3; does not apply when

(31, 21) = 946

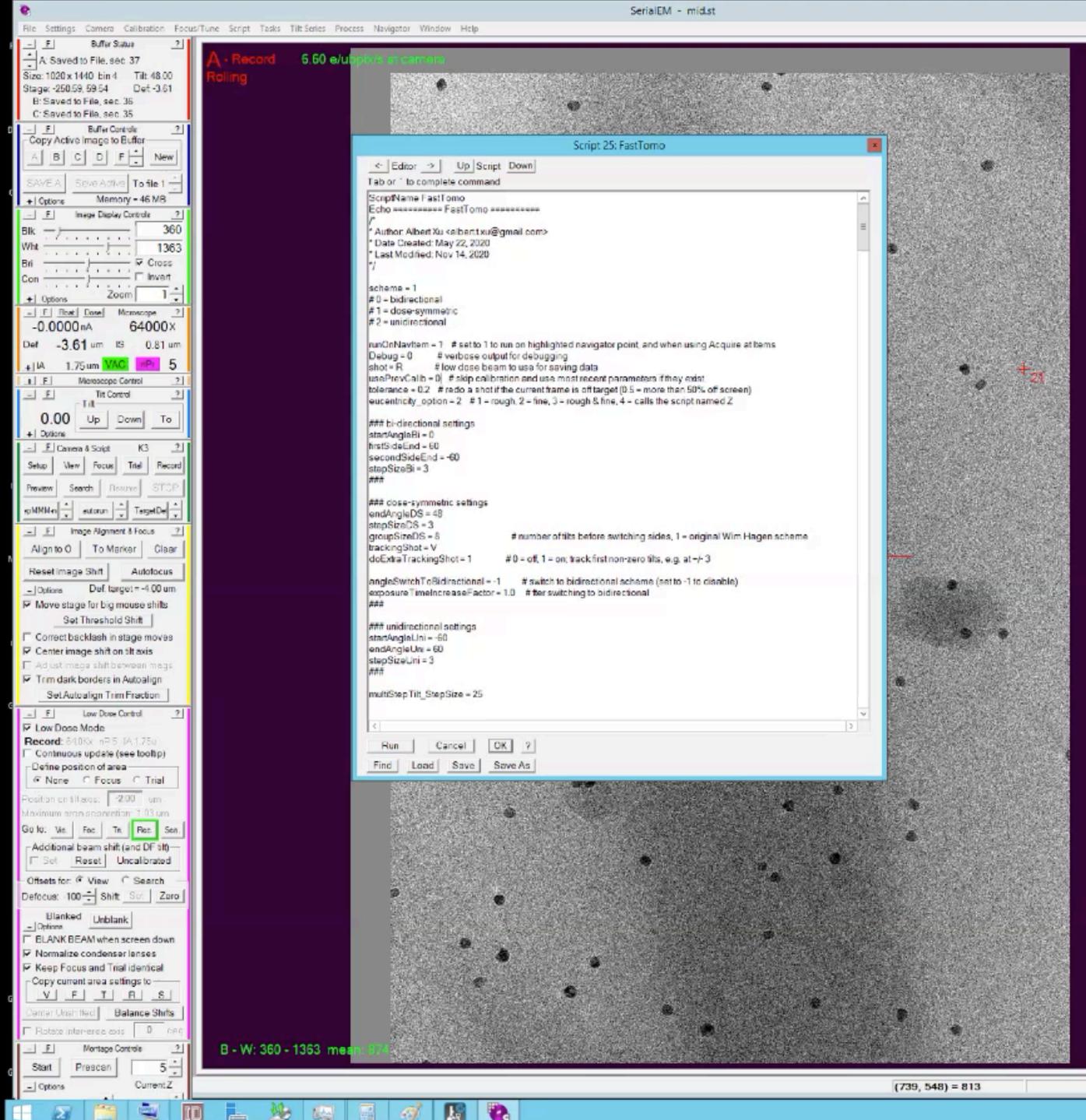
x	Ĩ
οZ	Ì
	1
focus	1
z	1
	1
	1
lti	1
p	1
18	1
lign-FR	
mo	1
est	1
leCell	1
	1
align2	1
	1
	1
	1
	1
	1
	1
	1
	1
	1
,	1
	1
	1
	1

Scripts

= 🗆 X

mple2.nav





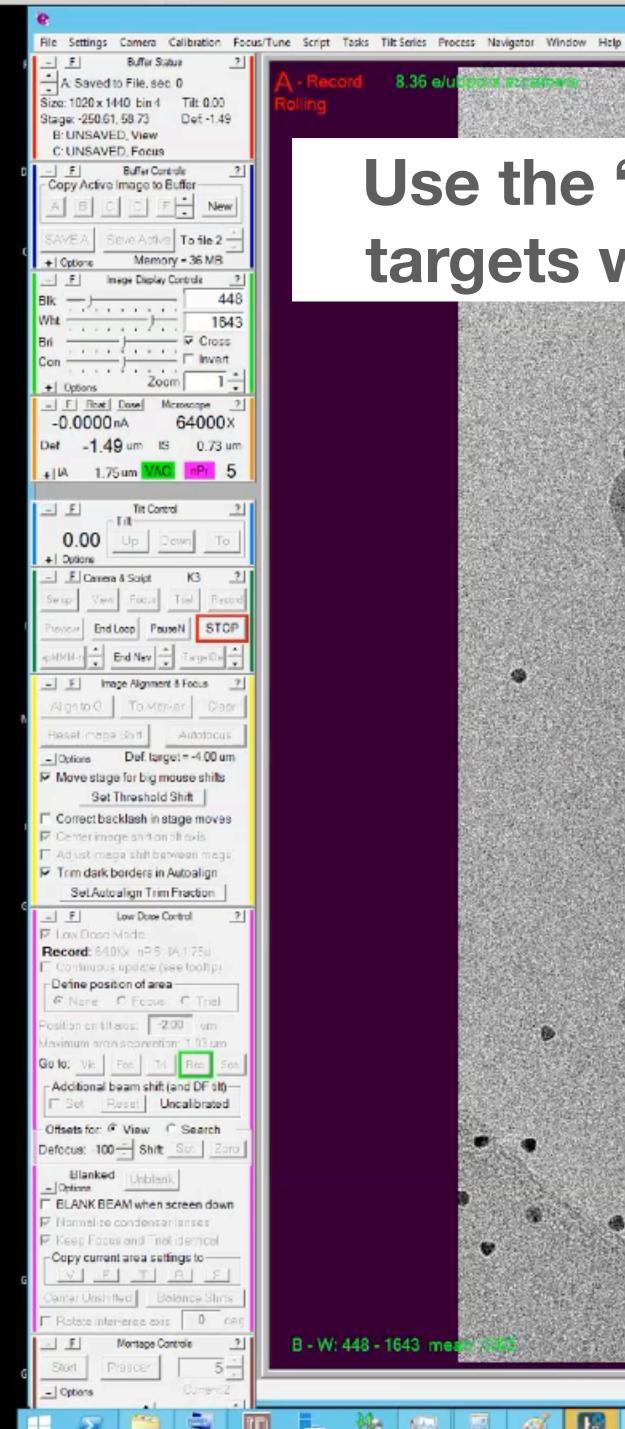
<u>.</u>	Navigator: sample2.nav	- 0 X	Scripts
Label 37	Begistration point 1 🛓 🗖 Corner point (C)	2	PrepMMM-n
Color Red	Draw C Rotate when land C For enciror state		autorun
# 37-49 Note:			CycleTargetDe
	Titseries F Newfle atriem F Newfile at group		PrepMMM-
	aging State TS Parame Riename Focus Ros		MyFunes
a come con a la come	egistration 1 - Draw F All reg. F None F Labels		
Add Polygon	Collapse Show Acquire Editmode Edit Fo Label Color X Y Z Type Reg. Acq. Not		Script 6
Add Marker	1 Blu 3.9 6.0 100.0 Map 1 Group of 1 items, ID., 1797, Jabels 2 to 2	Sec 0 - low.st	autorun-mul
Movelter	Group of 1 items, ID 4621, labels 2 to 2 Group of 1 items, ID 4621, labels 3 to 3 Group of 1 items, ID 1503, labels 4 to 4		autorun-vpg
Go To XY	Group of 9 items, ID . 5860, labels 5 to 13	Sec 0 - mid.st -	SimpleFocu
Go To XYZ	6-A Blu -232.8 195.3 77.7 Map 1 7-A Blu -113.6 158.3 72.5 Map 1	Sec 1 - mid.st - Sec 2 - mid.st -	Z-check-refill-real
Go To Marker	8-A Blu -145.6 29.3 73.9 Map 1 9-A Blu -180.5 92.6 79.8 Map 1	Sec 3 - mid.st - Sec 4 - mid.st -	short-DSTon
Load Piece	Group of 1 items, ID . 9325, labels 19 to 19 Group of 17 items, ID7291, labels 20 to 36 Some Acq	Sec 1 million	FastTomo-te
New Map Anchor Mag	Group of 13 tems. ID 3303. Tabels 37 to 49. All Acq		FastTomoWhol
Delete Item			Z
Boolign to Itom.			Z-check-refil-res
			Script 15
			Script 12
			Sengt 18
			Script 15
			Script20
			Script 21
	<	×	Surjal 22
	121		Script23
1	Log: log1.log	- 0 X	Script 24
	ed to X3DoseFractions320210613\Jun(Sanoke2\Jun14_11.1	7.09.tf	FastTomo
Saved Z = 24, -39.0 Error above toleran	ce, reteking shot		OpenFile
Saved Z = 25, -39,0			CloseFile
Savad Z = 2642.0			Script28
Saved Z = 27, -45.0		72311	
Error above toleran 15 frames were say Saved Z = 28, -45.0	ed to X\DoseFractions\20210613\Jun\Sanoke2\Jun14_11.1	7 27.tf	Script25
	red to X:\DoseFractions\20210513\Jun\Senoke2\Jun14_11.1	7.32.tf	Sumplat.
Ending N	Imaging States	3	
Saved Z Aod	Current State Add New Item State		
Saved Z Delete			
15 frame. Set state Seved Z			
IS mame	And So IA Exp Bin Frame Name ?		
15 frame Saved Z	/ag Sp IA Exp Bin Frame Name		
15 frame 1 3	3000 2 3.7 1.001 1 4.1x5.8 linear, 100um 3000 2 32.2 1.001 1 4.1x5.8 count, 100um		
15 frame	tere a de contra de la contra de		
Savad Z = 36, 45.0	0 degrees cquisition after current item		
15 frames were say	ed to X3/DoseFractions)/20210513).Jun(Sanoke2).Jun14_11.1	8.54.tf	
Saved Z = 37, 48.00 end of dose sy			
TS time: 336.641 total time: 336.641			
		2	
<	Ш	>	

50 nm

_

pts ×
MM-noZ
run
etDefocus
MM-Z
unes
pt6
n-multi
n-vpp
Focus
Hrealign-FR
STomo
mo-test
WholeCell
2
fil-realign2
at 16
ni 12
p(18
pt 15
pt25
pt.21/2
p1,22
rt23
st.24
omo
File
eFile
pt 28
nt 25
at St.

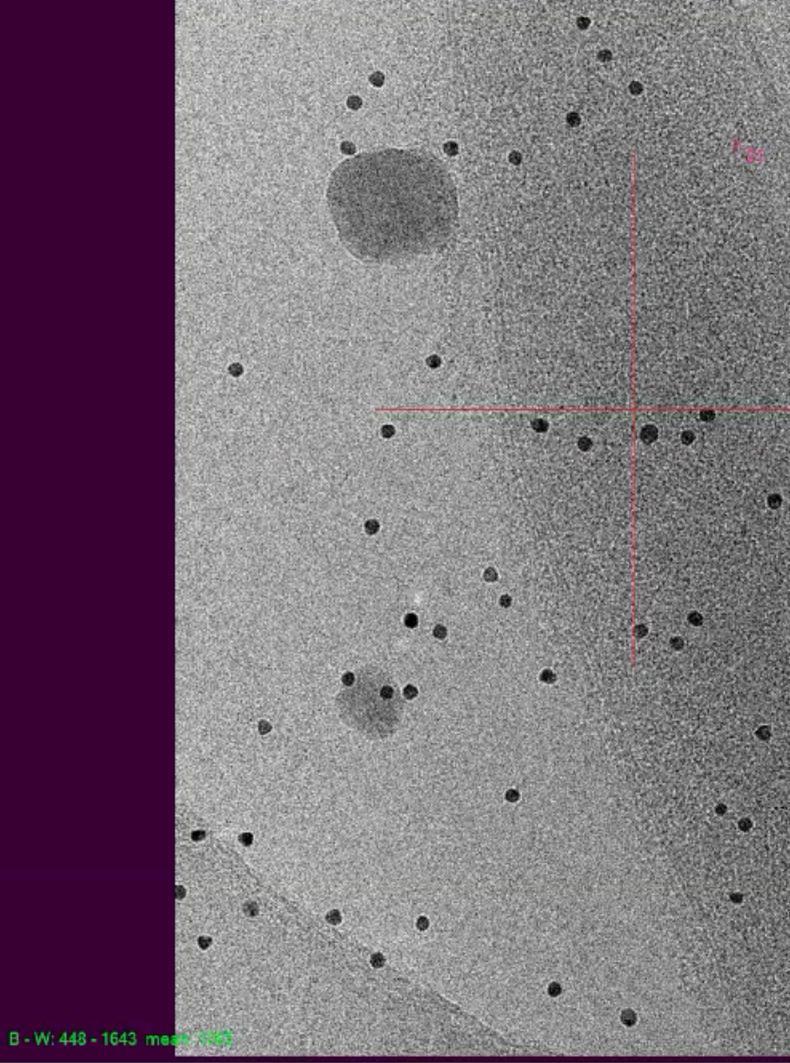




Use the "Dummy SerialEM" to add more targets without stopping data collection

8.36 e/u

SerialEM - BB221.st (#2) 1: mid.st



Label 20 Color Red #20 Red 342.8 Red 564.3 OIL -266.9 Off -232.9 Off 1136 Go To XY Off 145.6 OIL -180.5Go To XYZ Red -303.8 Go To Marke Red -422.6 Red -390.4Load Piece Red -217.4 Blu -266.8 New Map Blu -232.8 Anchor Mag -113.6 Blu Blu -145.6 Delete Item Blu -180.5 -266.0 Realign to Item Red -250.6 Red -250.5 Red -262.5 Red -261.2 Red 264.4 Red -271.0 Red -274.0 Red -274.3 Red 271.3 Red -2776 Red -280.7Red -286.1 Red -292.3 Red 294.9 Red -294.7 Saved Z = 29, 30.00 degrees Seved Z = 30, 33.00 degrees Saved Z = 31, 36.00 degrees Seved Z = 32, 39.00 degrees Saved Z = 33, 42.00 degrees Savad Z = 34, 45.00 degraes Saved Z = 35, 48.00 degrees ----- end (TS time:

50 nm

- 🗆 X Navigator: sample2.nav Scripts 2 E Registration point 1 - E Corner point (C) P Draw E Rotate when I and E For enciror state autorun cleTargetDeioru Titisenes F Newfle at dem F New Field group FrepMMkH. s Imaging Stata TS Parama Filenama Facula Pos WA/EUnca Registration 1 Draw FAllreg FNone FLabels Collapsa T Show Acquire T Editmode T Edit Facus Script6 1 of 17 Done; Estimated completion in 01:53:45 Butorun-multi 376.5 100.0 Pt hole -185.6 100.0 Pt bad. autorun-vpp 75.2 100.0 Pt 195.6 100.0 Pt 158.7 100.0 Pt SimpleFoous 100.0 Pt 29.6 -92.4 100.0 Pt eckere i llerealigne -52.9 100.0 Pt 19.5 100.0 Pt short-CSTomo 102.0 100.0 Pt -2154 100.0 Pt FastTomp-tast 74.8 76.6 Map 1 Sec 0 - mid.st -195.3 77.7 Map 1 Sec 1 - mid.st estThmoWholeCe 158.3 72.5 Map 1 Sec 2 · mid.st · 29.3 73.9 Map 1 Sec 3 - mid.st --92.6 79.8 Map 1 Sec.4 - mid.st-75.3 76.6 Pt eck-refil-realing 59.0 76.6 Pt 53.3 76.6 Pt Sciint Ti 74.3 76.6 Pt 90.1 76.6 Pt Script 17 76.6 Pt 98.8 105.7 76.6 Pt 109.5 76.6 Pt Sunat 16 76.6 Pt 56.6 76.6 Pt 45.7 Script 15 40.5 76.6 Pt 76.6 Pt 50.3 Script20 82.1 76.6 Pt 97.0 76.6 Pt 85.1 76.6 Pt 1 Script21 Red 294.6 60.4 76.6 Pt 1 53.6 76.6 Pt 1 Striat 22 Script23 - 🗆 X Log: log1.log Script24 15 frames were saved to X3DoseFractions)20210613(Jun(Sanoke2)Jun14_11.10.48.tf EnstTomo 15 frames were saved to X1DoseFractions\20210613\Jun\Senoke2\Jun14_11.10.53.tf OpenFile. 15 frames were saved to X3/DoseFractions)/20210513(Jun/Senoke2)Jun14_11.10 58.tf CloseFile 15 frames were saved to X3DoseFractions/20210613(Jun/Sanoke2)Jun14_11.11.03.tf Script25 15 frames were saved to X3/DoseFractions)/20210613(Jun/Senoke2)Jun14_11.11.08.tf 15 frames were saved to X3/DoseFractions/20210613/Jun/Sanoke2/Jun14_11.11.13.tf Scint?? 15 frames were saved to X3/DoseFractions3/20210613/Jun/Senoke2/Jun14_11.11 18.tf Suipt50 Imaging States total time: Add Current State Add New Item State Dewars a Started a Delete Update Name;tune 100um Call for ru Item 21: 5 Set state: Imaging Mep Account Scheduled Runnings Restore Prior State Forget Prior State Variable Cam Mag Sp IA Exp Bin Frame Name Skipping Trimming 33000 2 3.7 1.001 1 4.1x5.8 linear. 100um Disparity 33000 2 32.2 1.001 1 4.1x5.8 Finished Refining 4 of -0.88 microns and lateral displacement of -0.11 microns Measured defocus = -6.43 microns changed by 2.43 to target drift = 0.41 nm/sec Measured defocus = -3.93 microns changed by -0.07 to target drift = 0.01 nm/sec Skipping Z calibration, using existing parameters ---- starting dose symmetric TS -----15 frames were saved to X\DoseFractions\20210613\Jun\Sanoke2\Jun14_11.14 00.tf Saved Z = 0, 0.00 degrees

Ð

83.



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

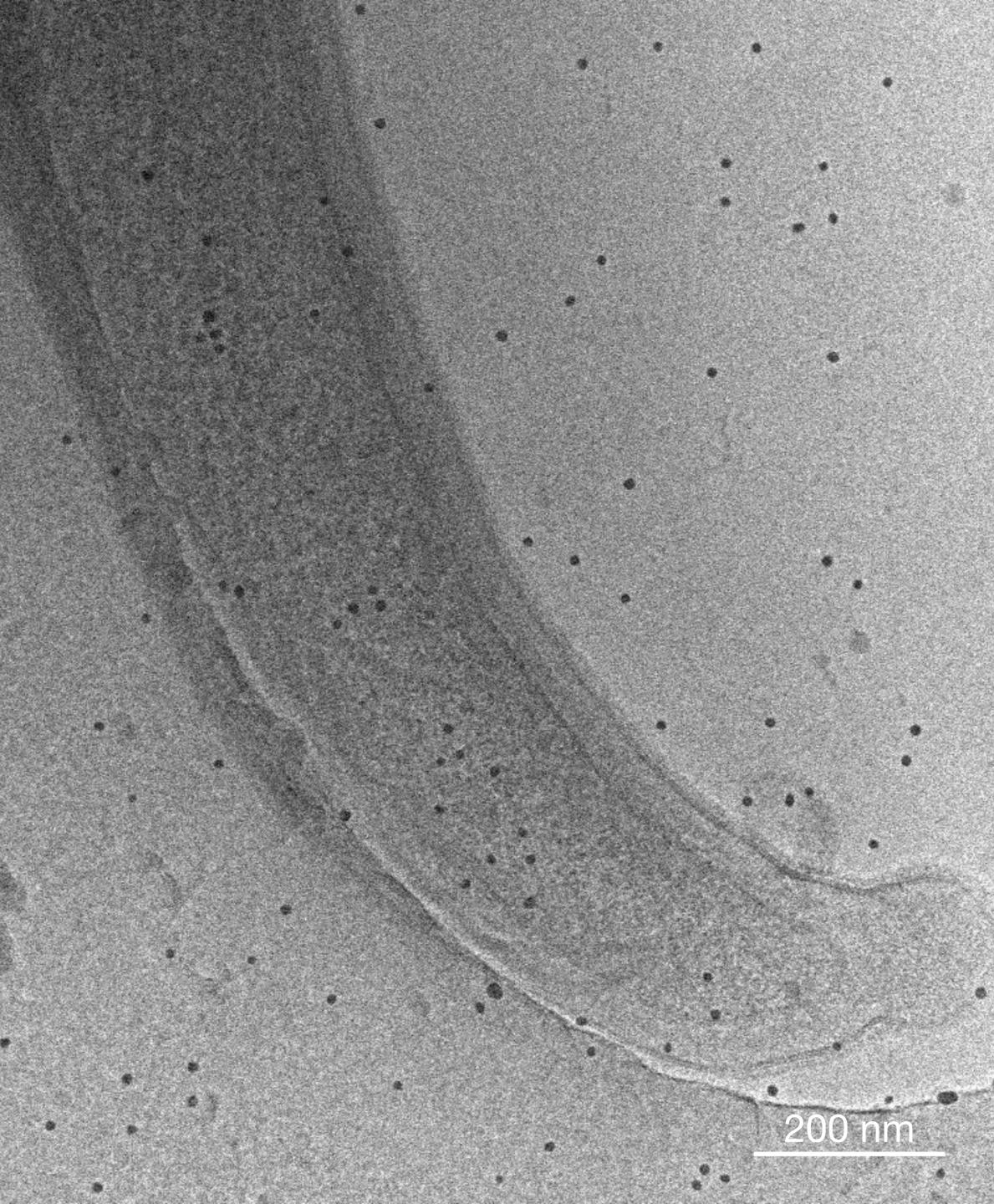
tracking for fiducial.

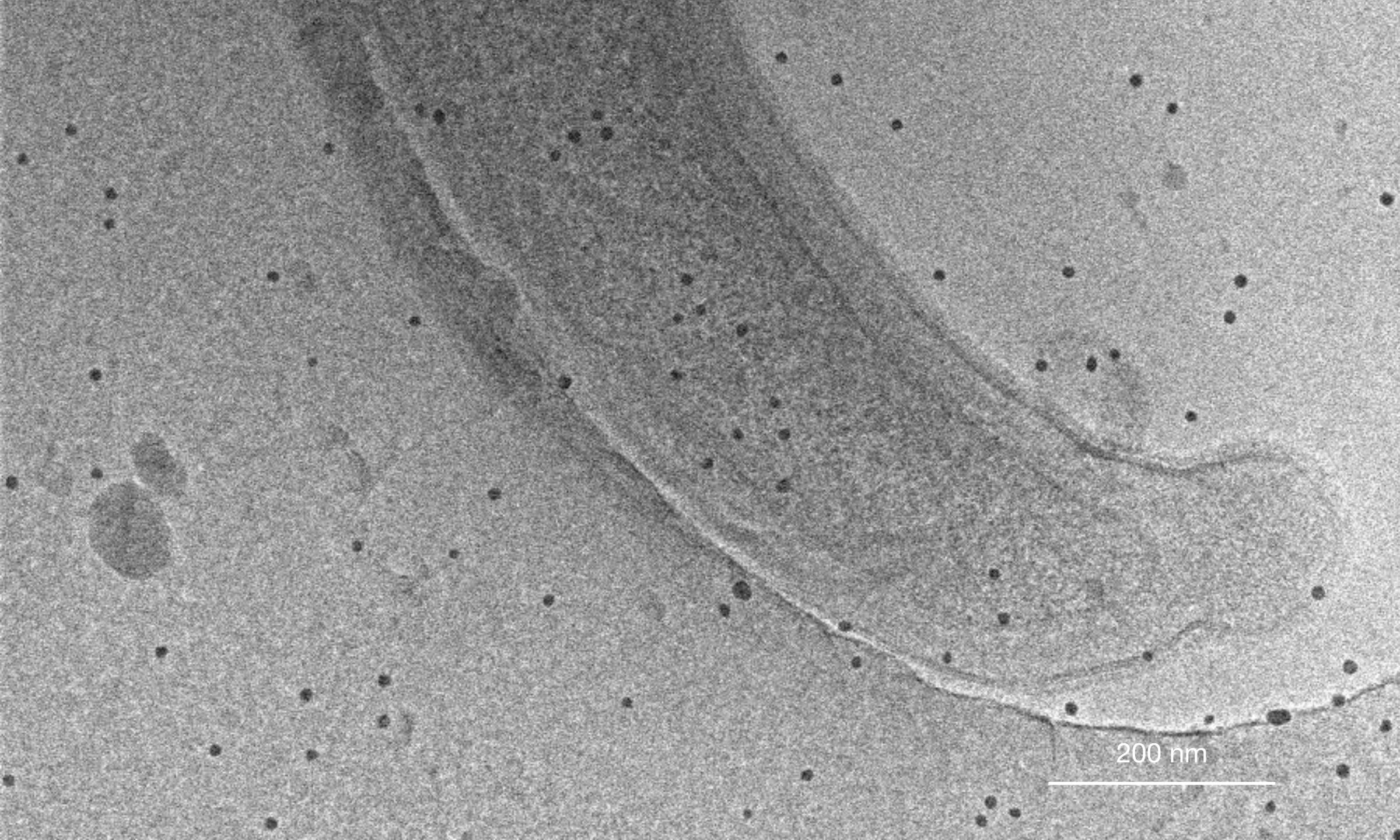
5. Align the tilt series based on the tracking result.

More details about etomo: http://bio3d.colorado.edu/imod/doc/etomoTutorial.html

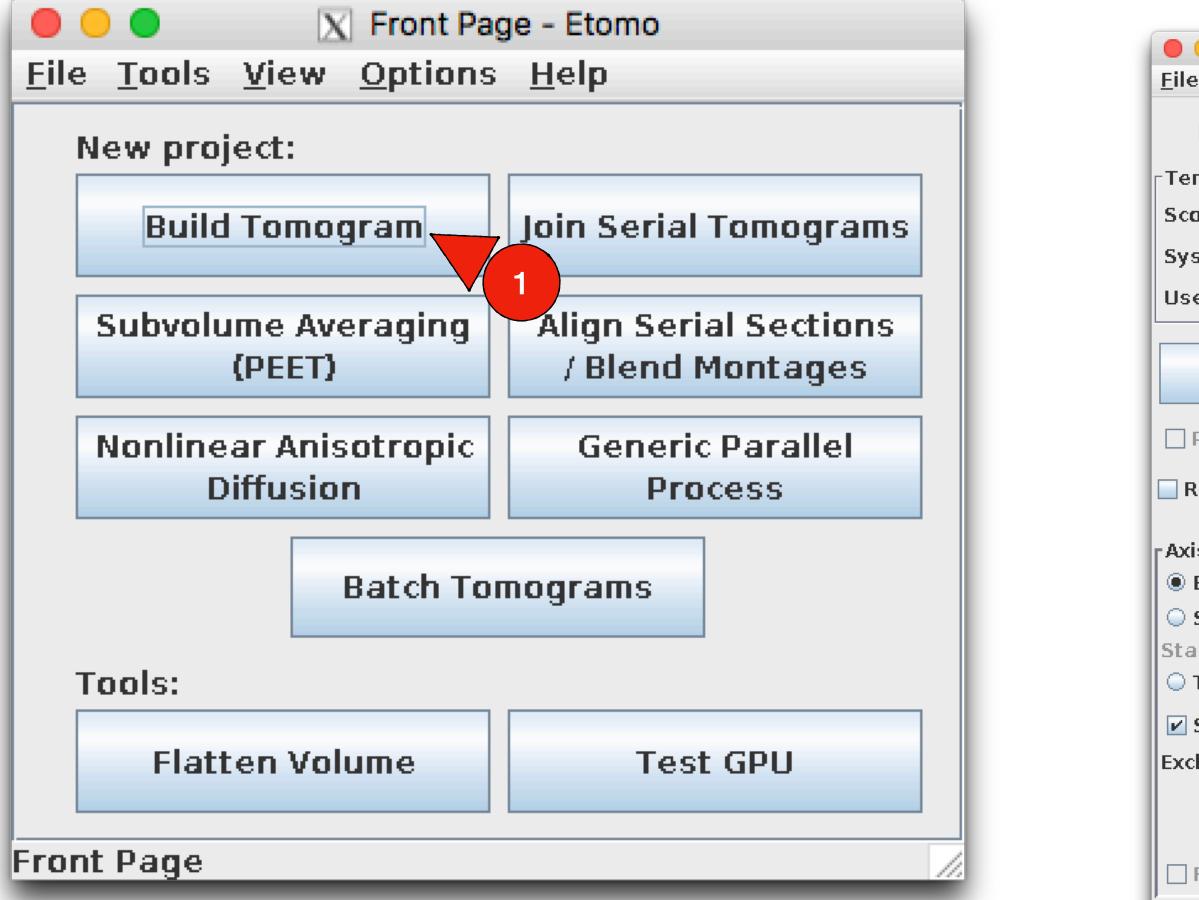
Dataset

- Borrelia burgdorferi
- Collected by Titan Krios
- -51°:3°:51°
- 2.747 Å/pixel





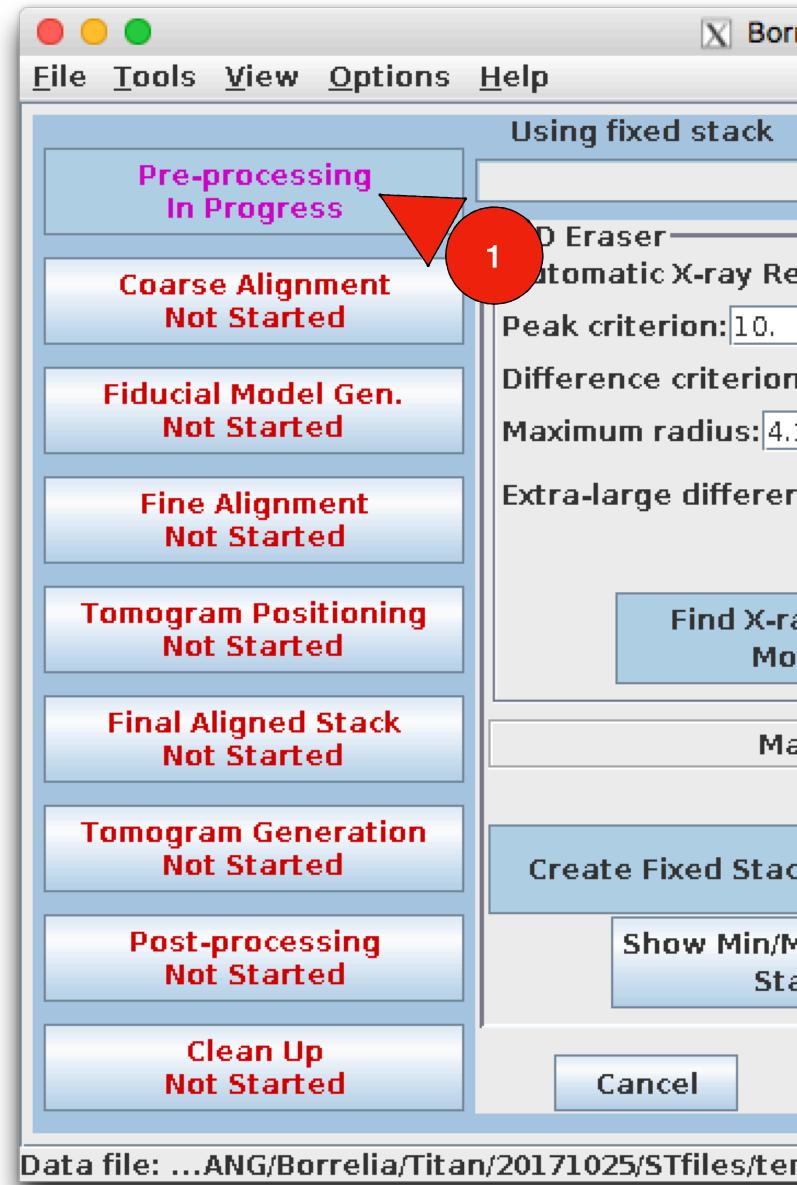
Etomo interface



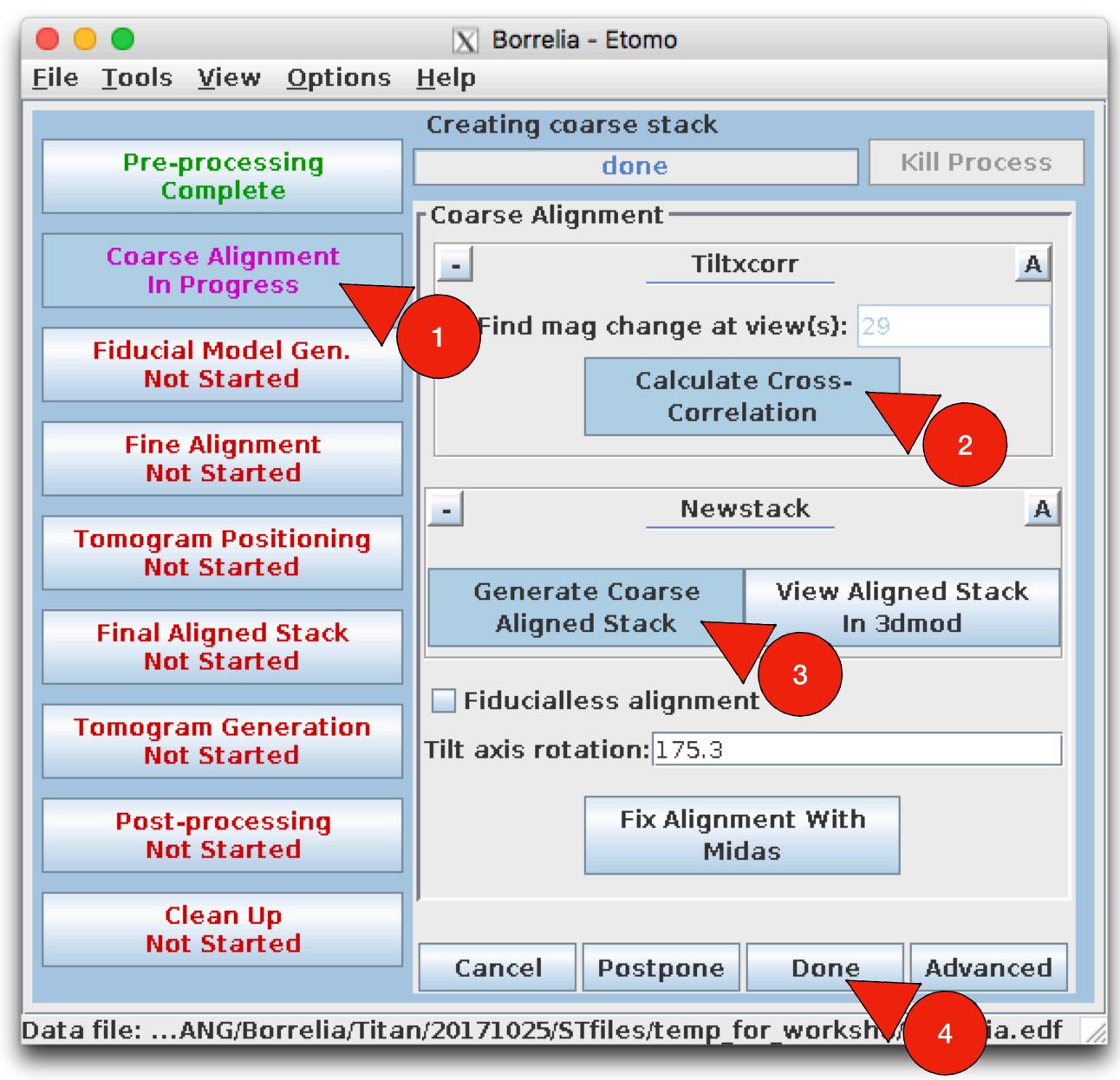
000	X	Project Log	
<u>F</u> ile <u>V</u> iew			
Project Log			
	V Setup T	omogram - Etomo	7/3
<u>T</u> ools <u>V</u> iew <u>Options</u> <u>H</u> elp		Shograni - Etonio	
Dataset name: 25/STfiles/temp_for	r_worksho/Borrelia.st 🖻 👅	Packup directory:	
nplates			
pe template: None available			-Ecomo Tuno
		Axis ype	Frame Type
stem template: No selection (2 a	vailable)	● Single axis	● Single frame
er template: No selection (5 avai	lable) 🔽	Dual axis	O Montage
Scan Header Pixel size	(nm): 0.2747 Fidu	cial diameter (nm): 10 🚤	 Image rotation (degree
Scall freader			
Parallel Processing 3	s card processing		4
	s card processing		
emove excluded views 🗌 Delet	e original files		
s A:		— [Axis B:	
Extract tilt angles from data		Extract tilt angles	from data
Specify the starting angle and st	ep (degrees)	O Specify the startin	g angle and step (degrees)
rting angle: -60.0	ncrement: 1.0	Starting angle: -60.0	Increment: 1.0
Filt angles in existing rawtlt file		○ Tilt angles in exist	ing rawtlt file
Series was bidirectional from 30).0 degree	es 🔲 Series was bidirec	tional from 0.0
	uegree		
lude views:		Exclude views:	
View Raw Image	Stack	Vi	iew Raw Image Stack
Focus was adjusted between mo	ntage frames	Focus was adjuste	d between montage frame
,	5	,,	5
Cancel	Use Existing Coms	Create Com Scrip	ots Advanced
lata set loaded			
			(5)

5):	175.3
	degrees

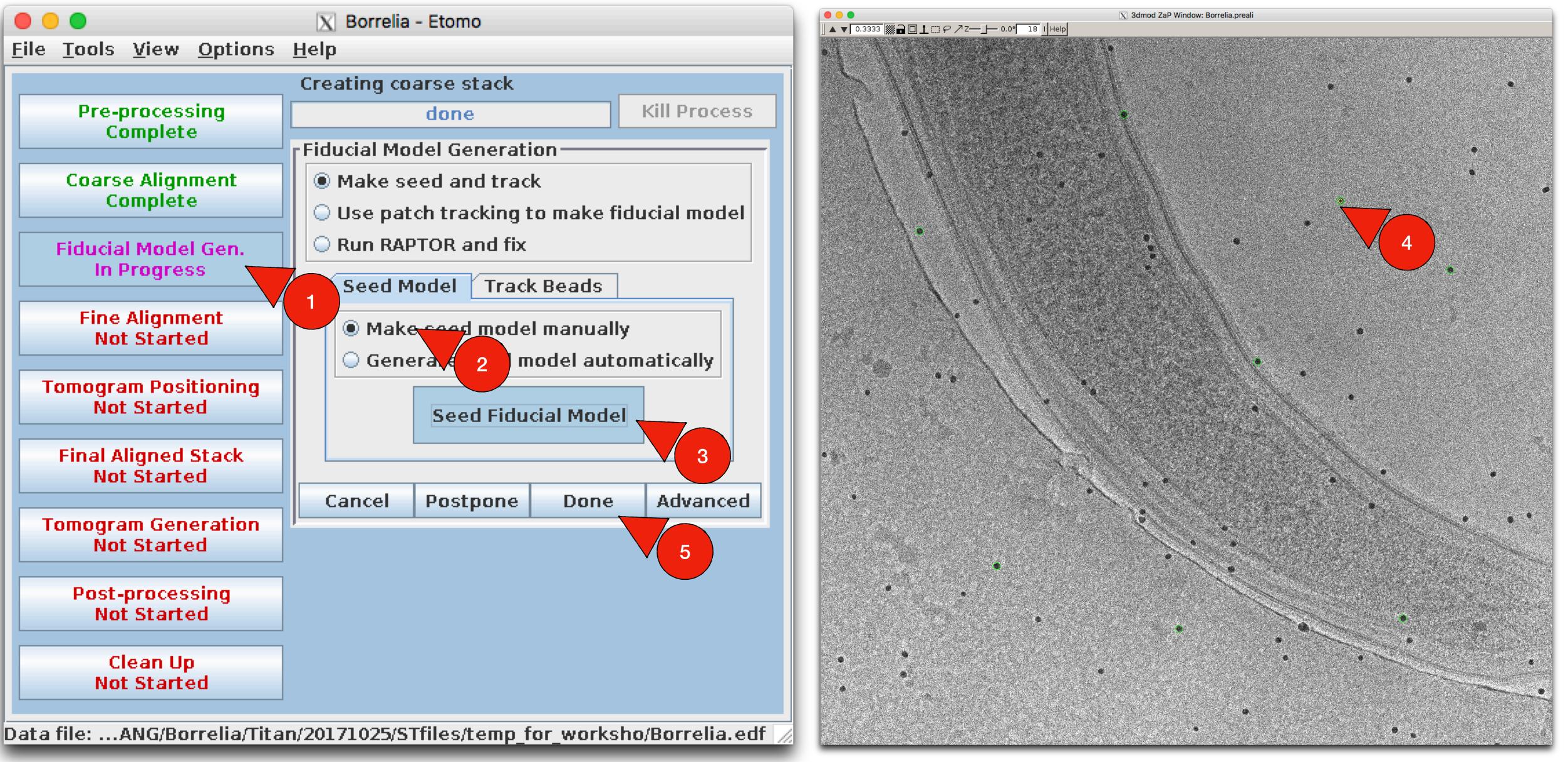
Pre-processing



relia - Etomo
done Kill Process
placement
: 8.
2
ice criterion: 19.
ays (Trial de) 2
nual Pixel A A Replacement
k View Fixed Stack Use Fixed Stack
Show Min/Max for Fixed Stack
Postpone Done Advanced np_for_worksho/Borrelia.edf 5
inp_tot_worksho/bottella.eur



Coarse alignment

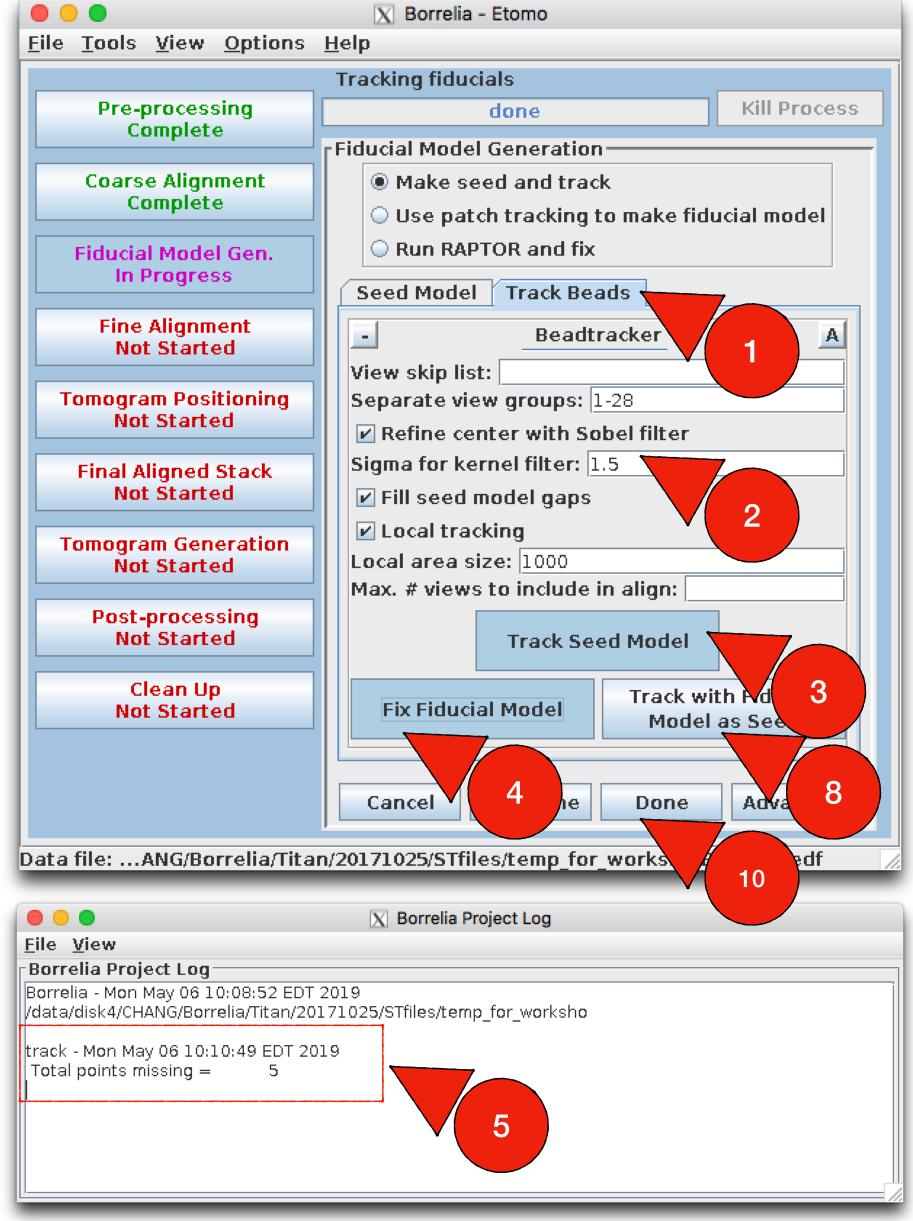


Generate fiducial model

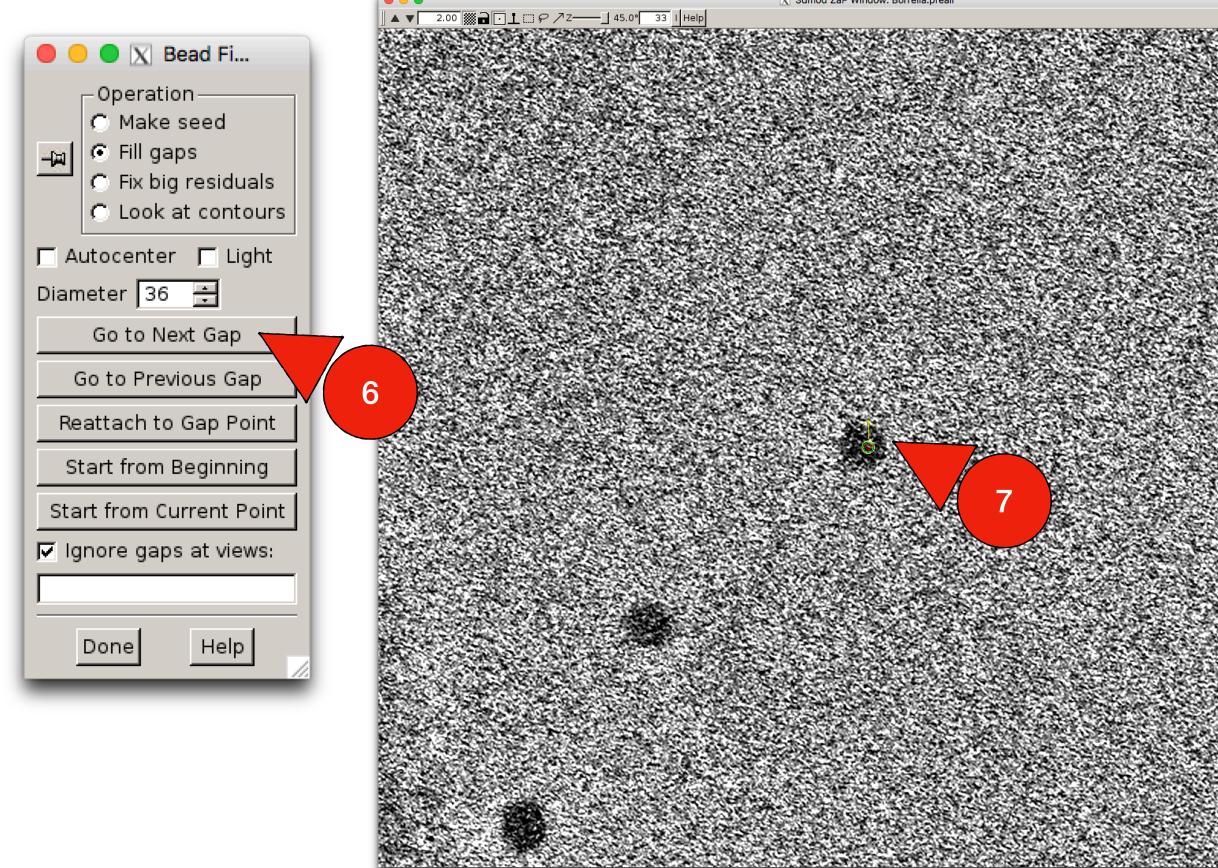
- 3. Choose 5-10 gold particles in different areas
- 4. Close IMOD and save the model

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



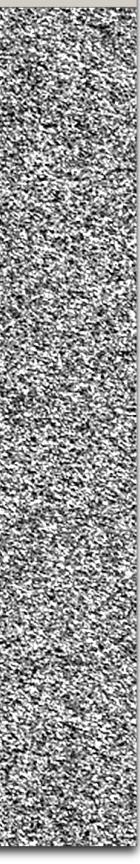


- Track seed model (step 1-3), then check the total missing points
- point at the gold particle position.
- 3. Repeat step 6-7 until you add all missing points; then close IMOD and save the model.



	🔀 Borrelia Project Log
<u>F</u> ile <u>V</u> iew	
Borrelia Project Log	
Borrelia - Mon May 06 10:08:52 EDT 2019 /data/disk4/CHANG/Borrelia/Titan/201710:	
track - Mon May 06 10:10:49 EDT 2019 Total points missing = 5	
track - Mon May 06 10:17:11 EDT 2019 Total points missing = 0	9

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





Final alignment of fiducial

● ● ● <u>F</u> ile <u>T</u> ools	V
Pre- Co	pr on
Coars	е on
Fiducia	n li nc
Fine In	
Tomogra Not	am t S
Final A Not	liç t S
Tomogra Not	am t S
Post- No	pr t S
C No	le t S

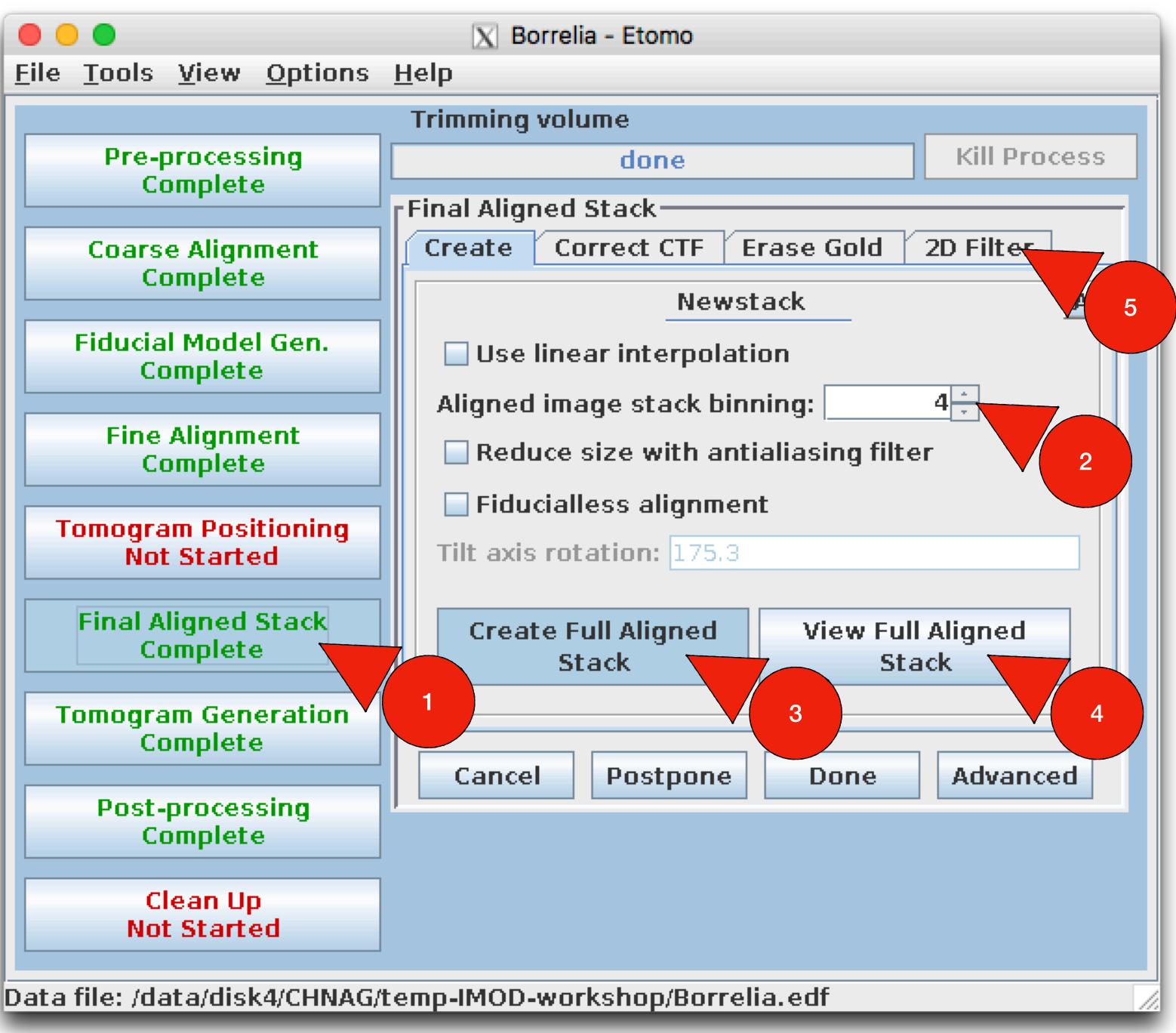
Data file: ...ANG

X	Bo	rrel	ia	-	Etc	mo
---	----	------	----	---	-----	----

ew	Options	Help
	<u>-</u> priono	<u></u>

•	
	Aligning stack
ocessing	done Kill Pro
plete	Fine Alignment
Alignment	Tiltalign Parameters
plete	General Global Variables Local Variables
	List of views to exclude:
4odel Gen.	Separate view groups: 1-28
piere	
ignment	Residual Reporting
ogress	Threshold for residual report: 3.0
Positioning	1 tive to O All views
tarted	Neighboring views
ned Stack	Analysis of Surface Angles
tarted	 Do not sort fiducials into 2 surfaces for anal Assume fiducials on 2 surfaces for analysis
Generation	S Assume nuuciais on 2 surfaces for analysis
tarted	Volume Position Parameters
	Total tilt angle offset: 0.0
ocessing tarted	Tilt axis z shift: 0.0
an Up	Minimization Parameters
started	Do robust fitting with tuning factor: 1.0
	Local Alignment Parameters
	Enable local alignments
	$\begin{bmatrix} Loca \\ 0 \\ Tarred \\ 2 \\ rize (x, y); \\ 700 \\ 700 \\ rize (x, y); \\ 700 \\ 700 \\ rize (x, y); \\ 700 \\ rize (x, y); \\ 700 \\ rize (x, y); \\ ri$
	Targe Size (x,y): 700,700 Min. # of fiducials (total, each surface): 8,3
	\bigcirc # of local patches (x,y): 5.5
	Compute Alignment View/Edit Fiducial Model
	Sign 2D Model 3 sidual Masters
	View 3D Model View Solution View 3D Model
	Cancel Postpone Done Advanced
G/Borrelia/Tita	n/20171025/STfiles/temp_for_worksho/Borrelia.edf



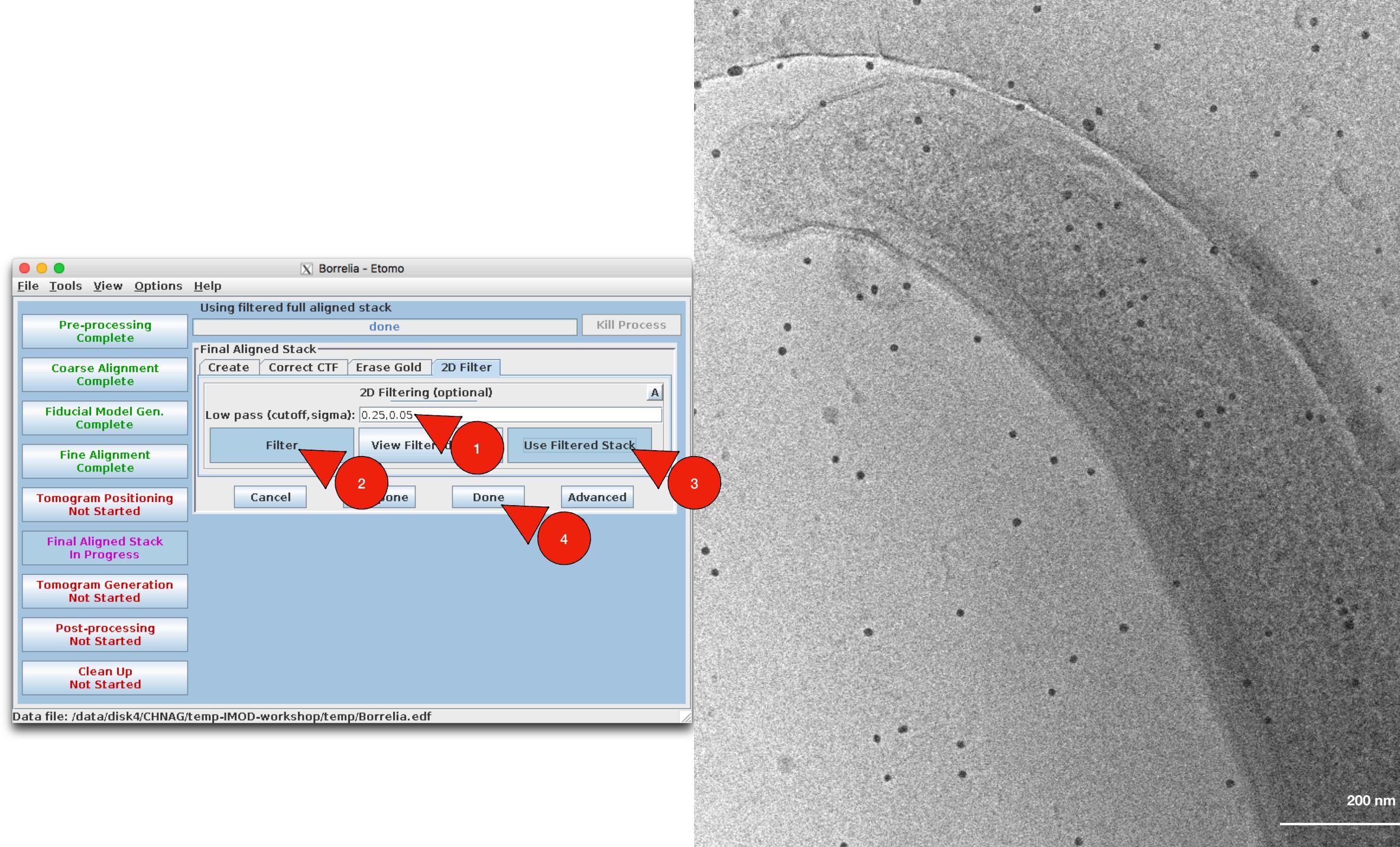


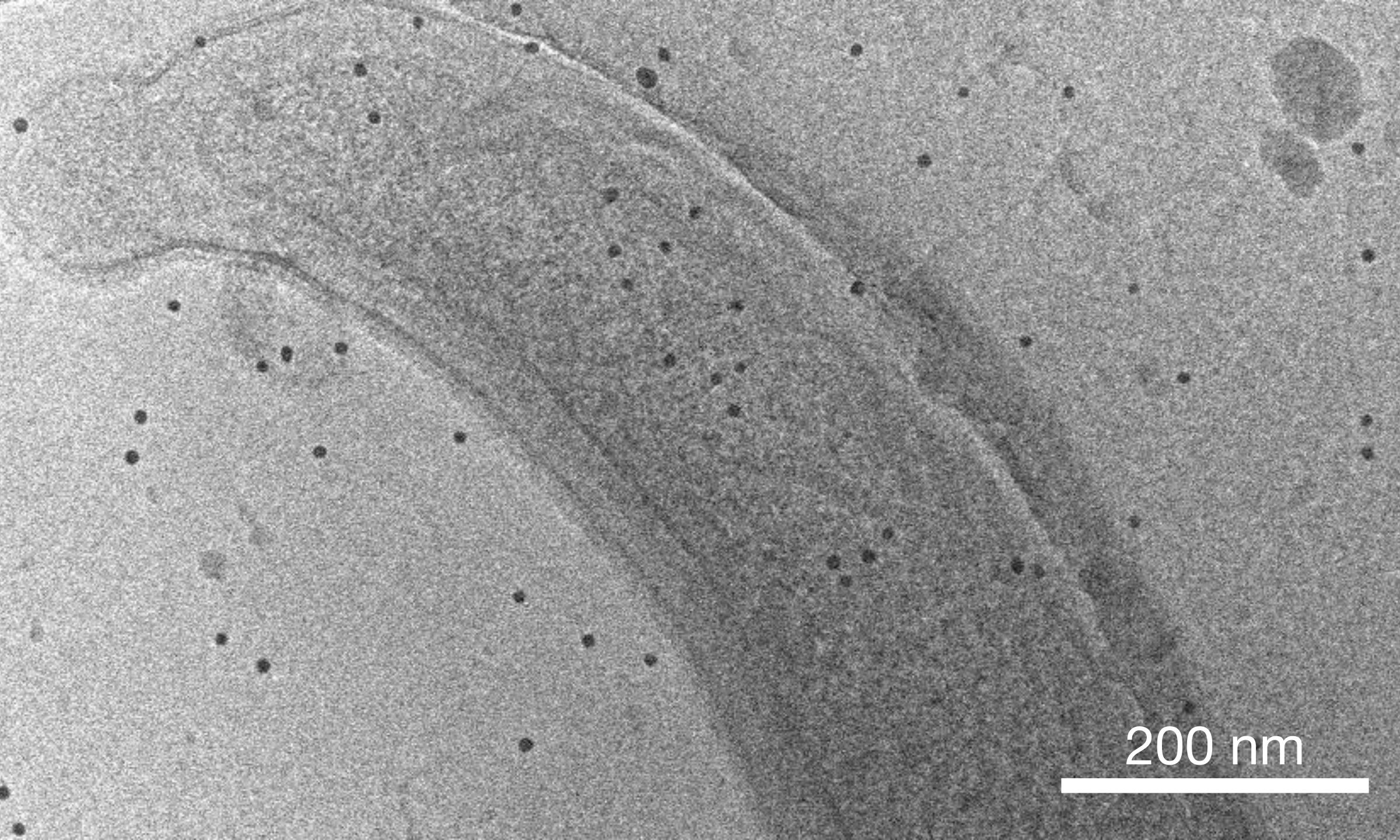
Generate aligned tilt series

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.









Tomogram reconstruction

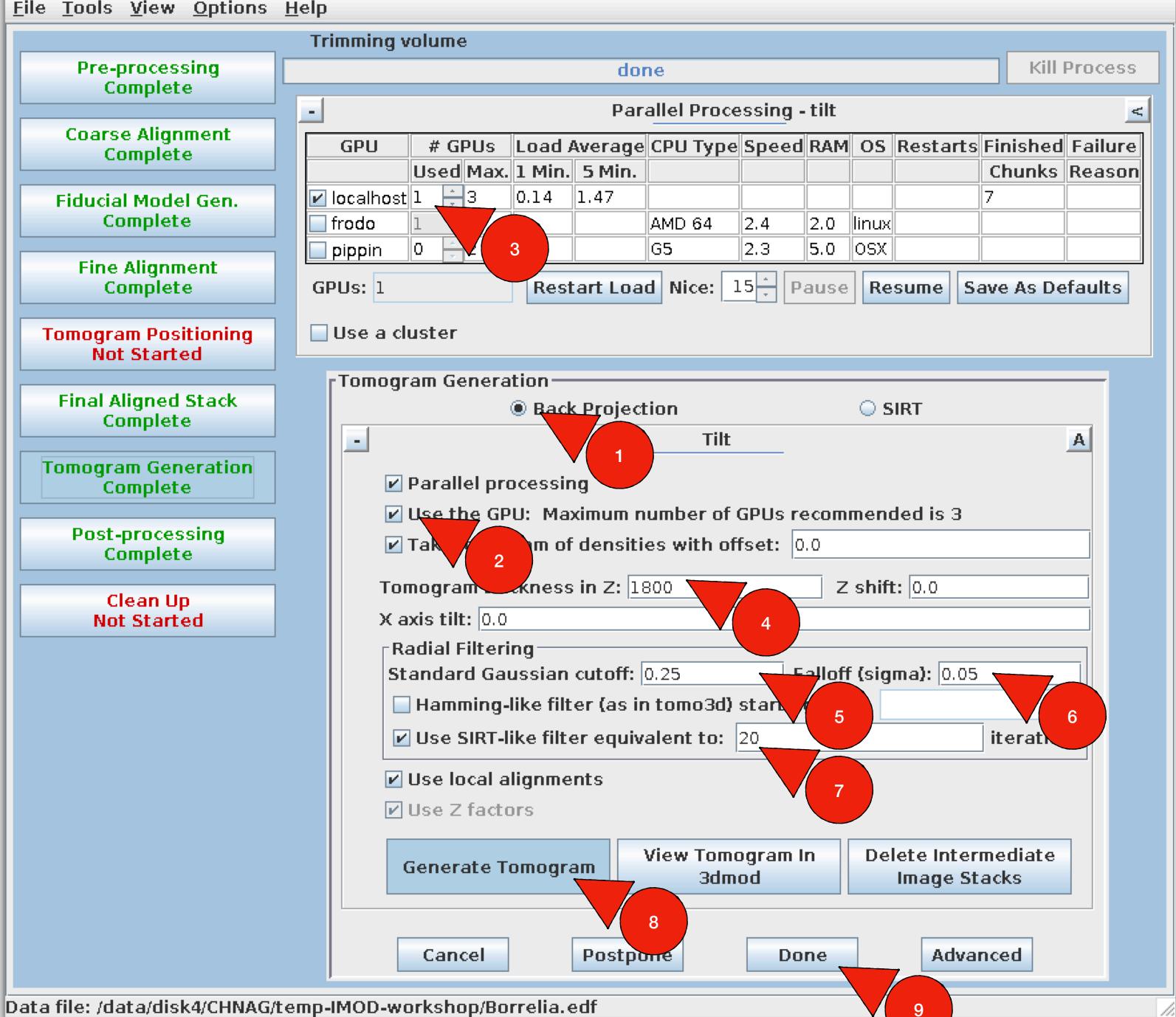
More details about WBP (weight back projection) and SIRT (simultaneous iterative reconstruction):

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

	• •	
<u>F</u> ile	e <u>T</u> ools	⊻iew
		proces
	U	omplet
		e Align
	C	omplet
	Fiducia	l Mode
		omplet
		Alignn omplet
	Tomogra	
	NO	t Start
	Final A	ligned
	Cu	omplet
	Tomogra	am Gen
		omplet
	Dect	
		proces omplet
		lean U _l t Start
	NO	L SLAIL

🗙 Borrelia - Etomo

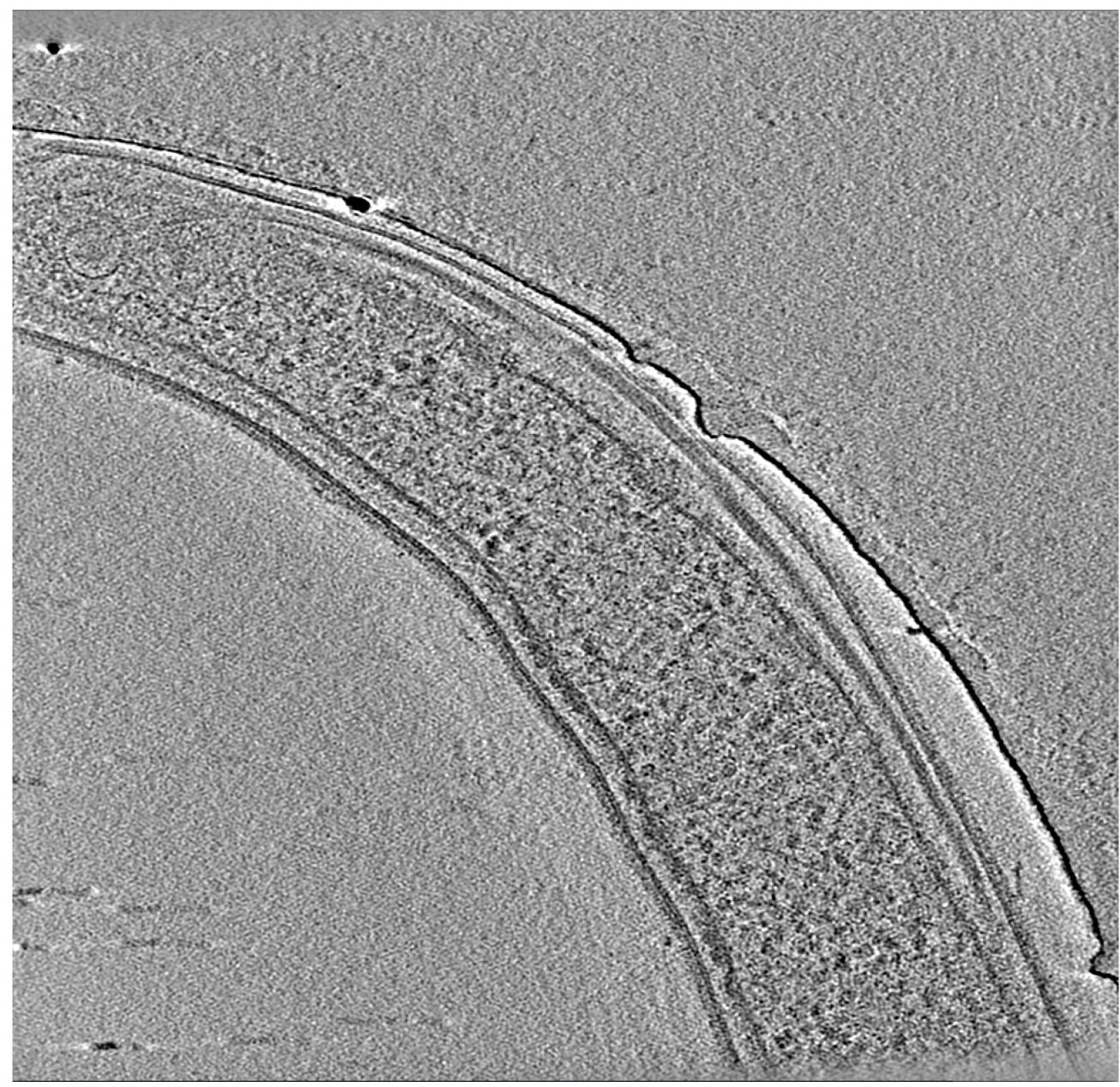
Options Help



Tomogram reconstruction

Save following files: Borrelia.st Borrelia.ali Borrelia.rec Borrelia.rawtlt Borrelia_fid.xf Other files can be deleted

	🔀 Borrelia - Etomo
<u>File Tools View Options</u>	<u>H</u> elp
	Trimming volume
Pre-processing	done Kill Process
Complete	Post Processing
Coarse Alignment	Trim vol Flatten Squeeze vol
Complete	Volume Trimming
Fiducial Model Gen. Complete	3dmod Full Volume Get XYZ Volume Range From 3dmod
	Volume Range
Fine Alignment Complete	X min: 1 X max: 960
comprete	Y min: 1 Y max: 928
Tomogram Positioning	Z min: 1 Z max: 450
Not Started	Scaling
Final Aligned Stack	Convert to bytes
Complete	
Tomogram Generation	
Complete	Find scaling from sections Z min: 150 Z max: 300
Deet erected	Scaling from sub-area:
Post-processing Complete	X min: X max:
	Y min: Y max:
Clean Up Not Started	
	Get XYZ Sub-Area From 3dmod
	□ Reorientation:
	○ None Warning: ○ None For serial joins, use
	Swap Y and Z dimensions the same reorientation
	Rotate around X axis method for each section.
	Section.
	3dmod Trimmed
	Trim Volume Volume
	Cancel Postpone Done
Data file: /data/disk4/CHNAG/	temp-IMOD-workshop/Borrelia.edf



Thank you! **Comments & questions?** shuaiqi.guo@mcgill.ca





Brookhaven National Laboratory

