Cryo-ET data collection by SerialEM

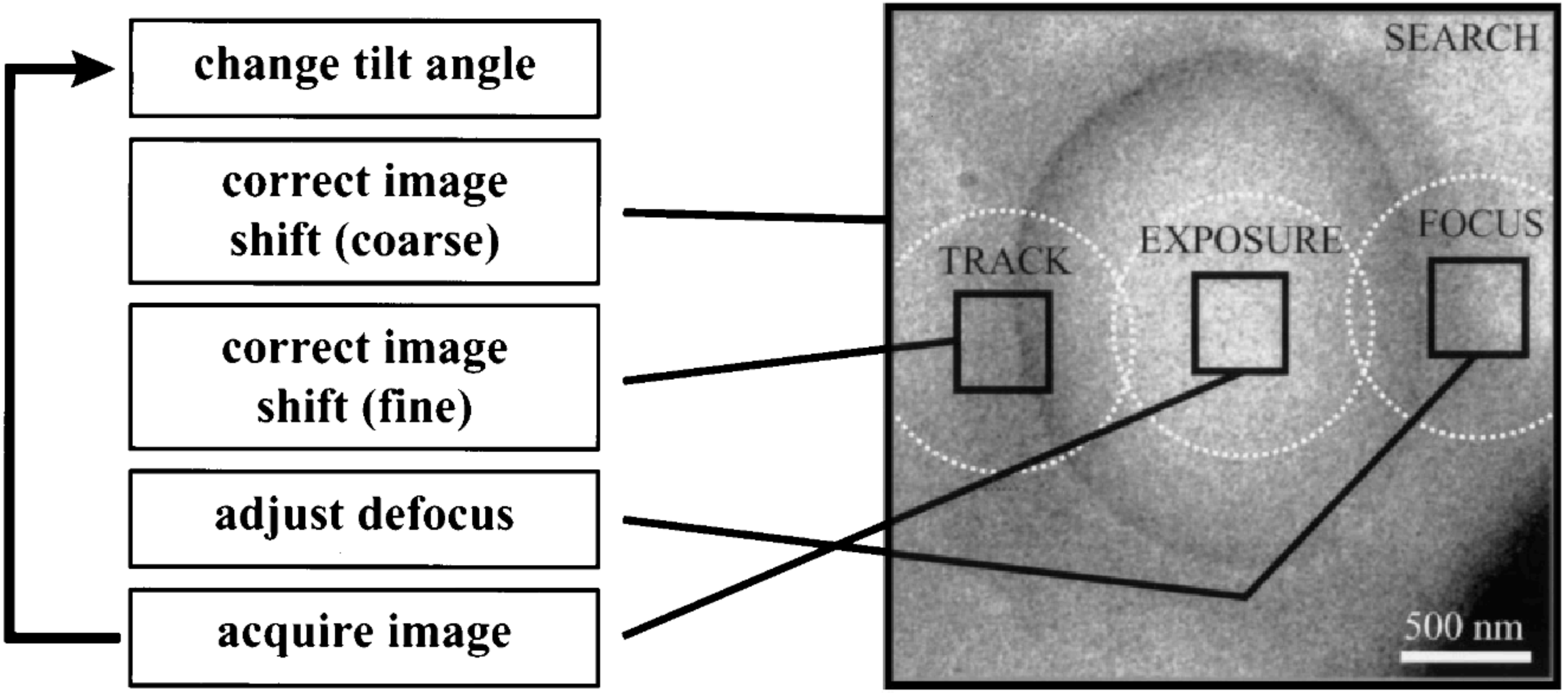
Shuaiqi Guo (Phil)
McGill University
June 13 2024

Main goals

1. SerialEM tilt series data collection with FastTomo script

2. Tilt Series alignment by IMOD

Tilt series data collection scheme



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

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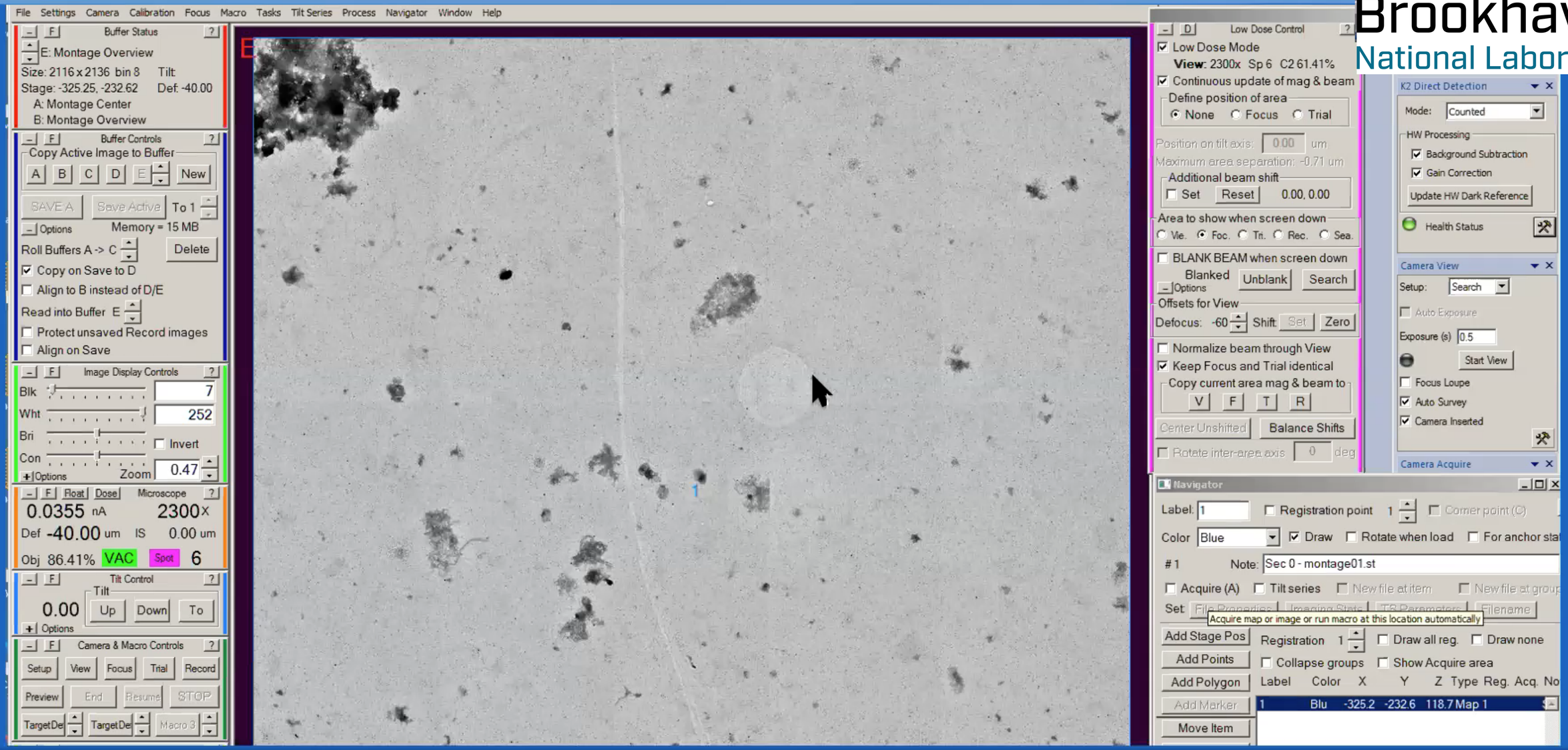
Available online 24 August 2005

2005

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

SerialEM (David Mastronarde

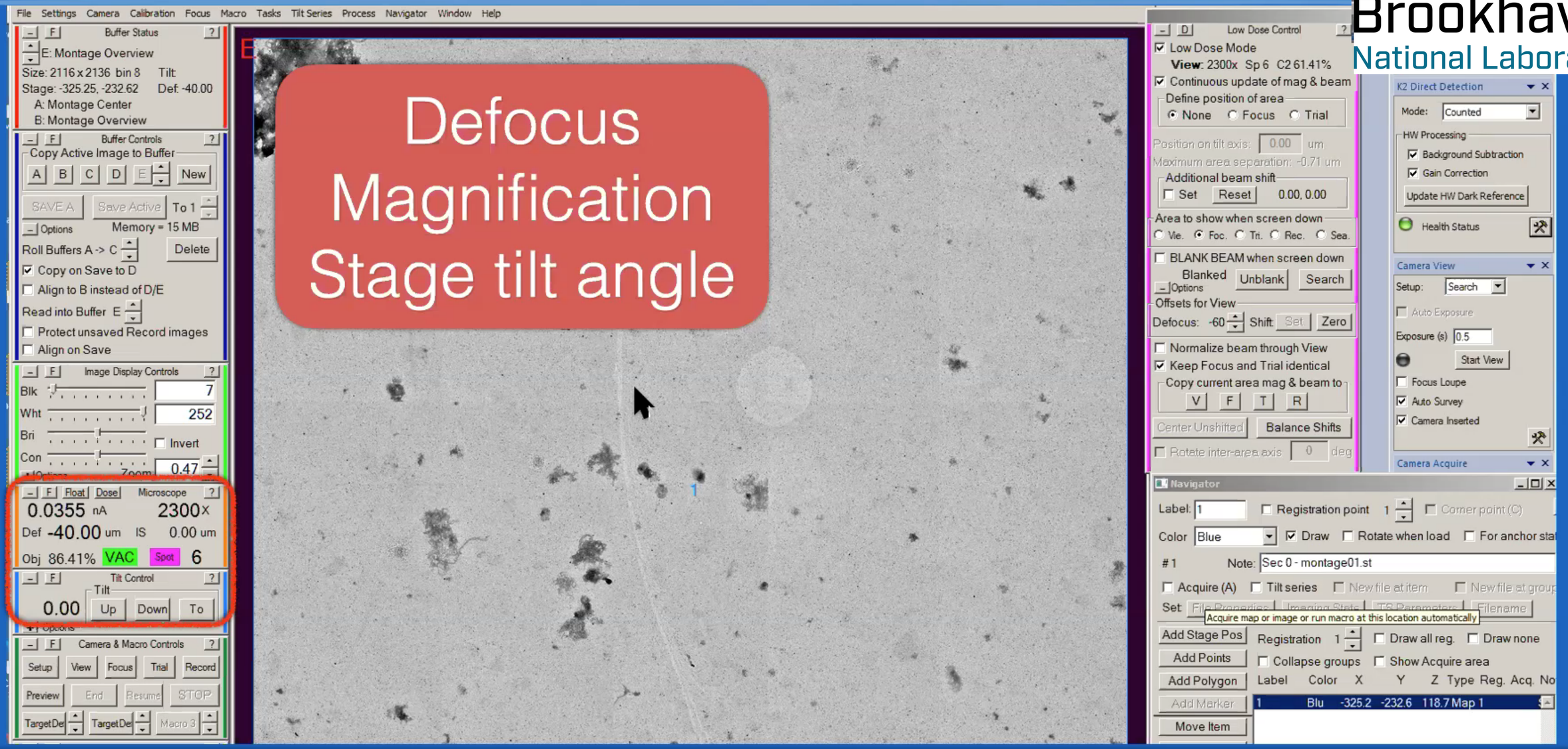


Brookhaven National Laboratory

@junliulab

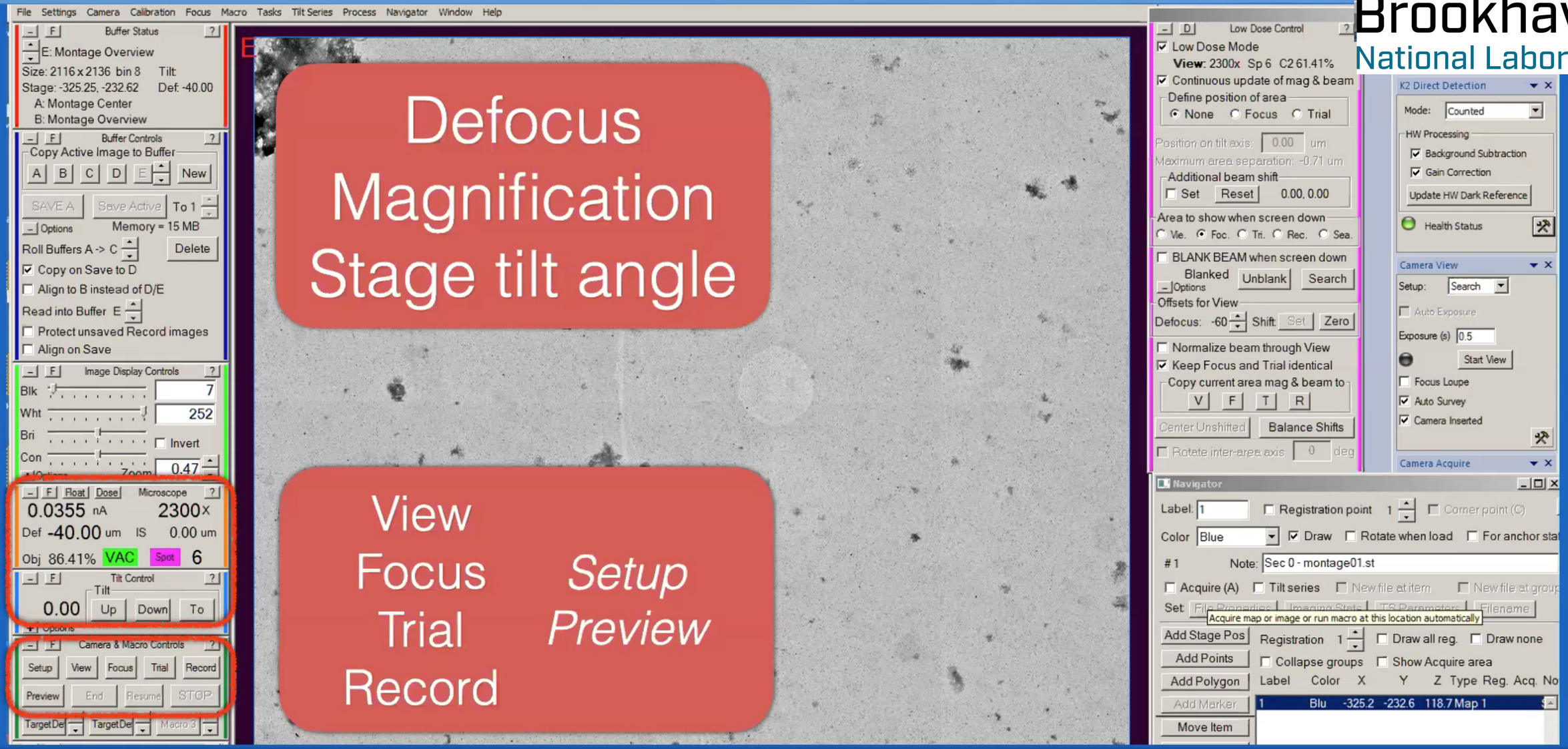
SerialEM (David Mastronarde

Brookhaven National Laboratory

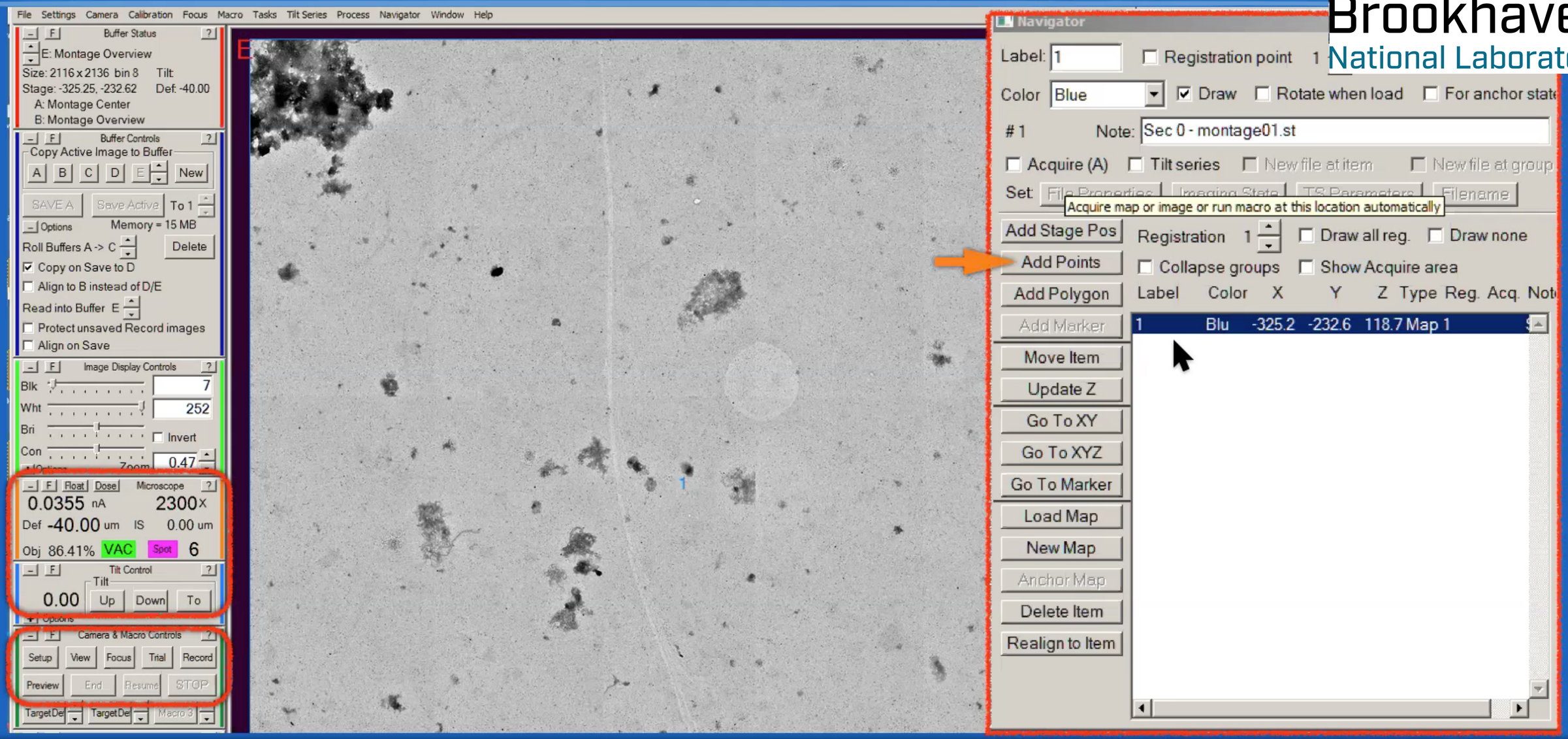


SerialEM (David Mastronarde

Brookhaven National Laboratory



SerialEM — Navigator



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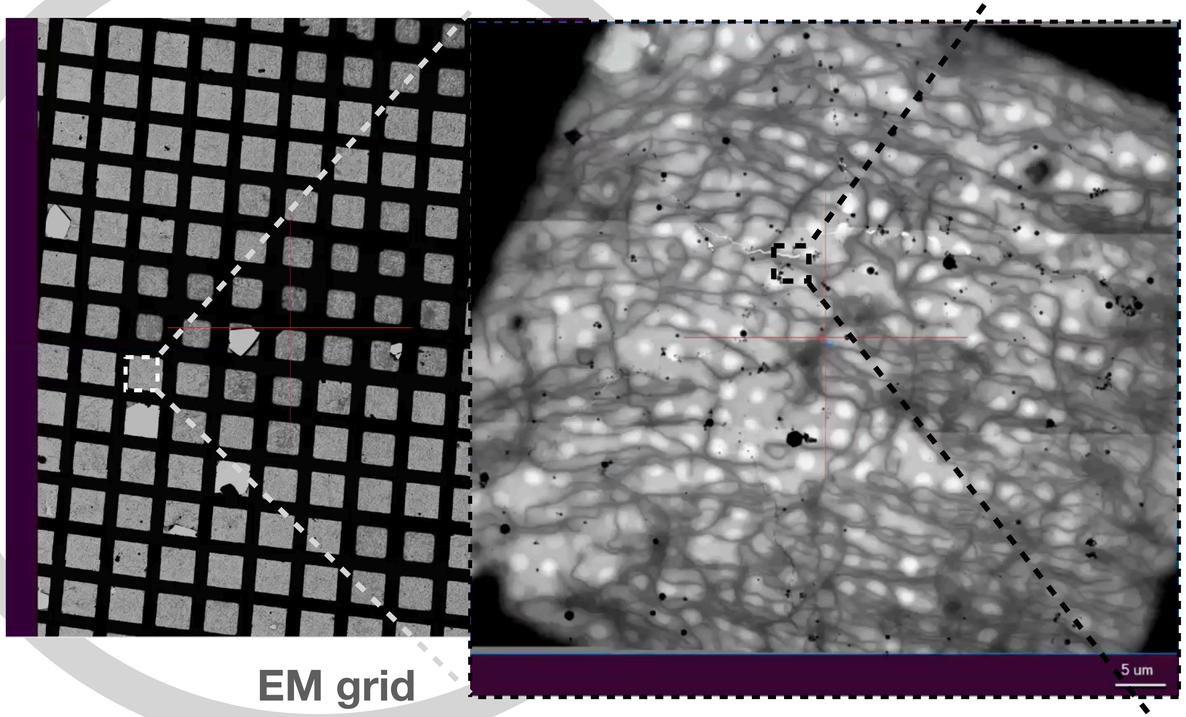
Find targets for tilt series data acquisation

1. Obtain 175 X full montage to survey the grid

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

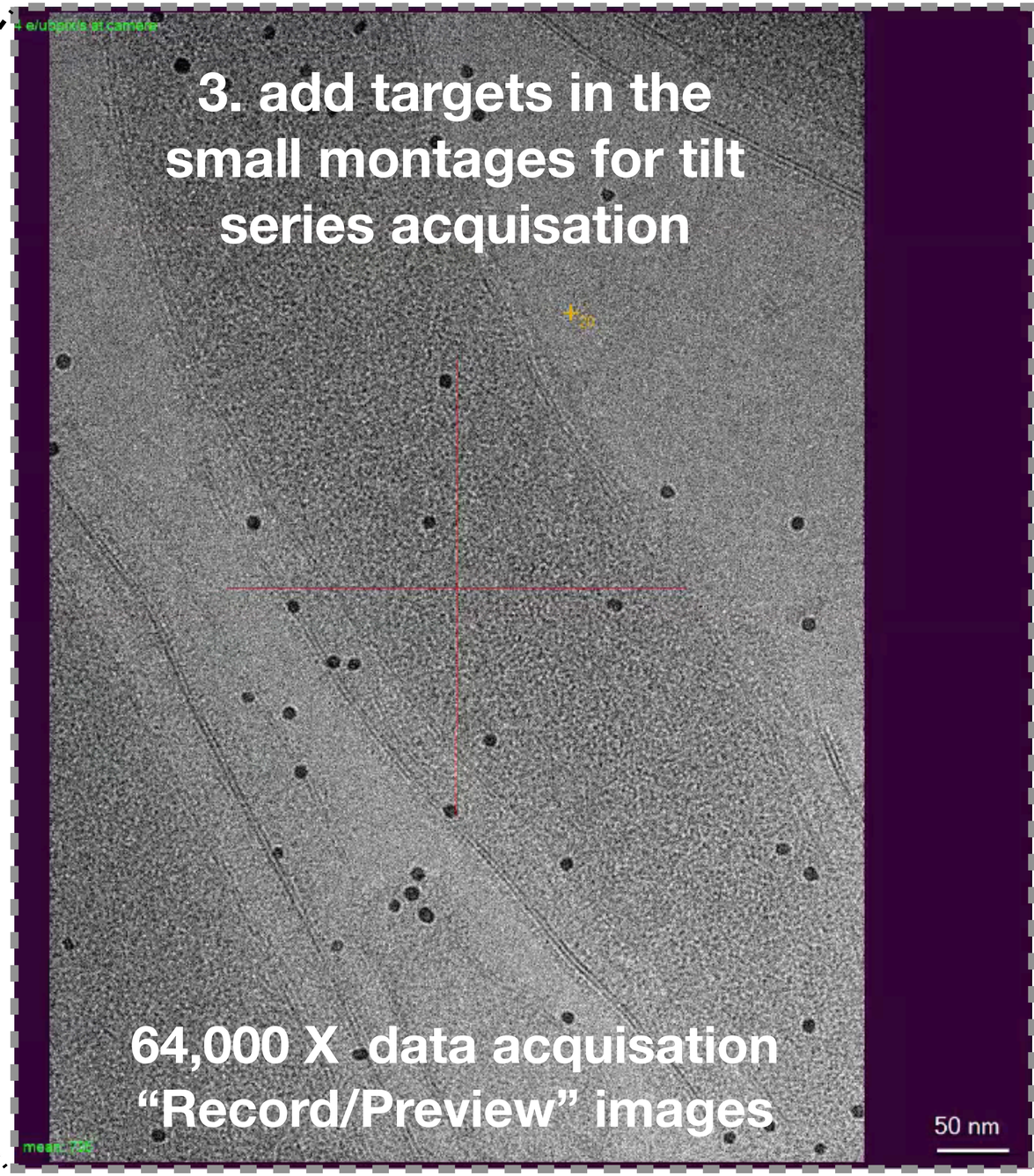


EM grid

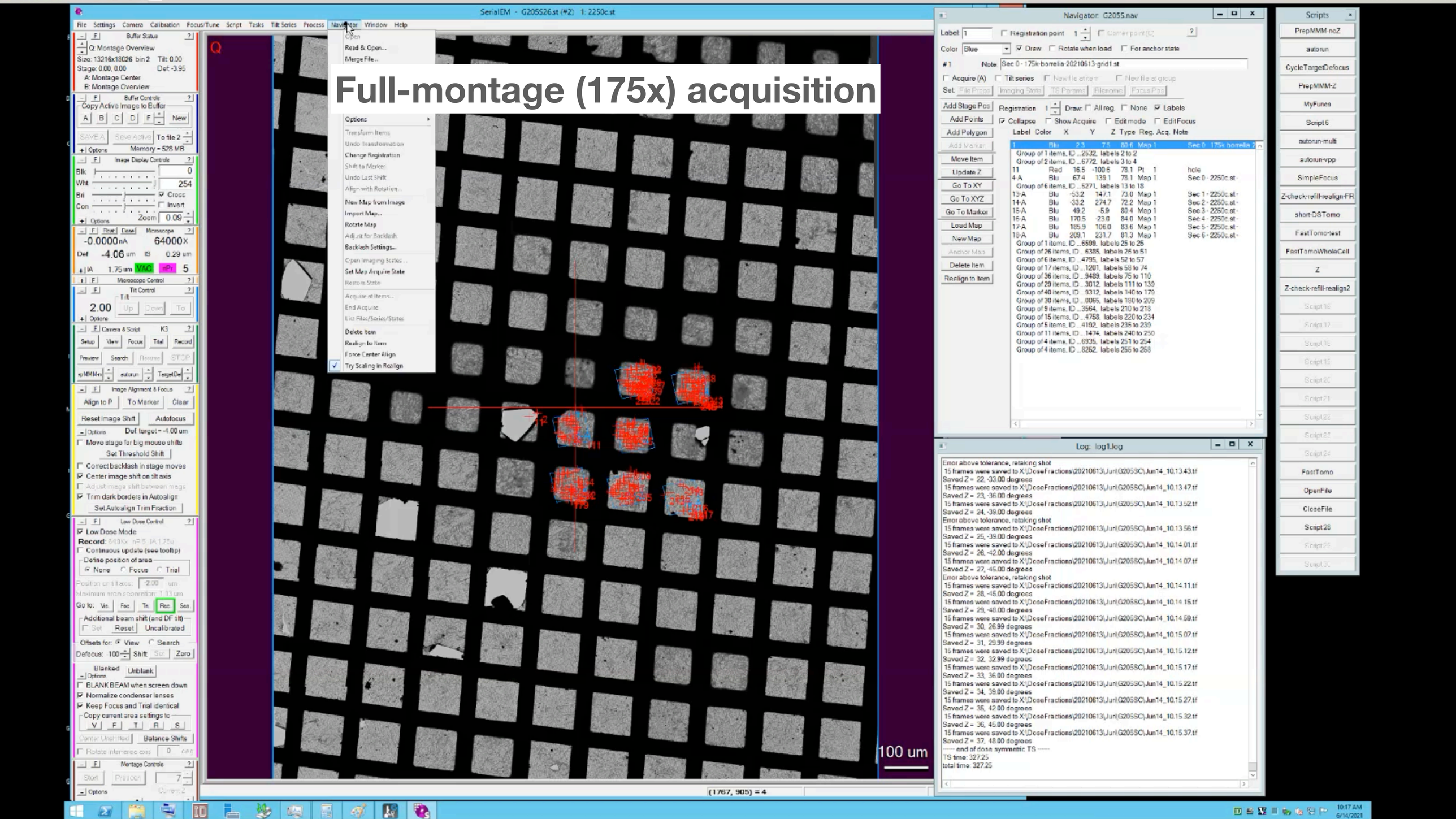
6 x 6 “Search” images

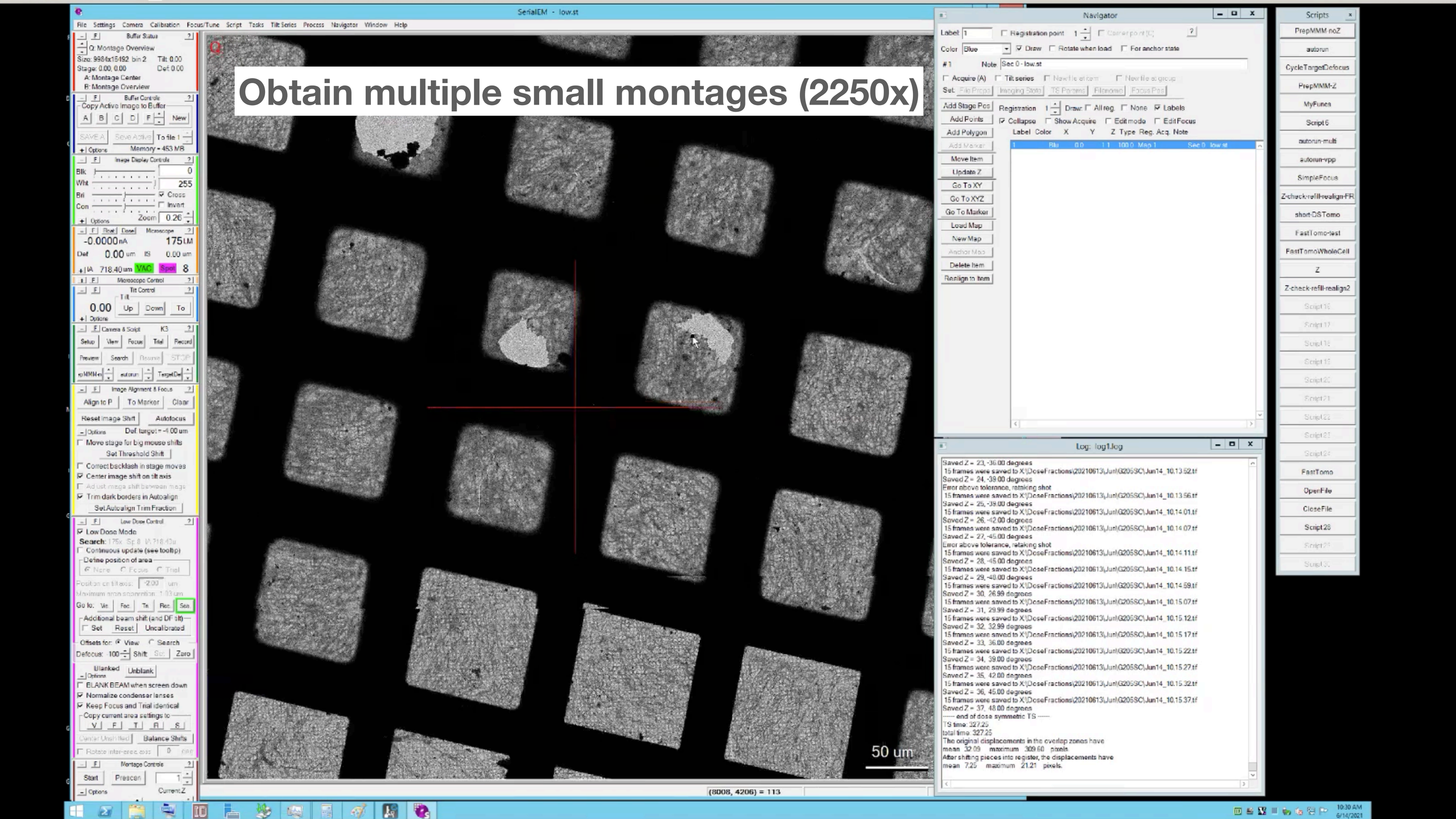
5 x 3 “View” images

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



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

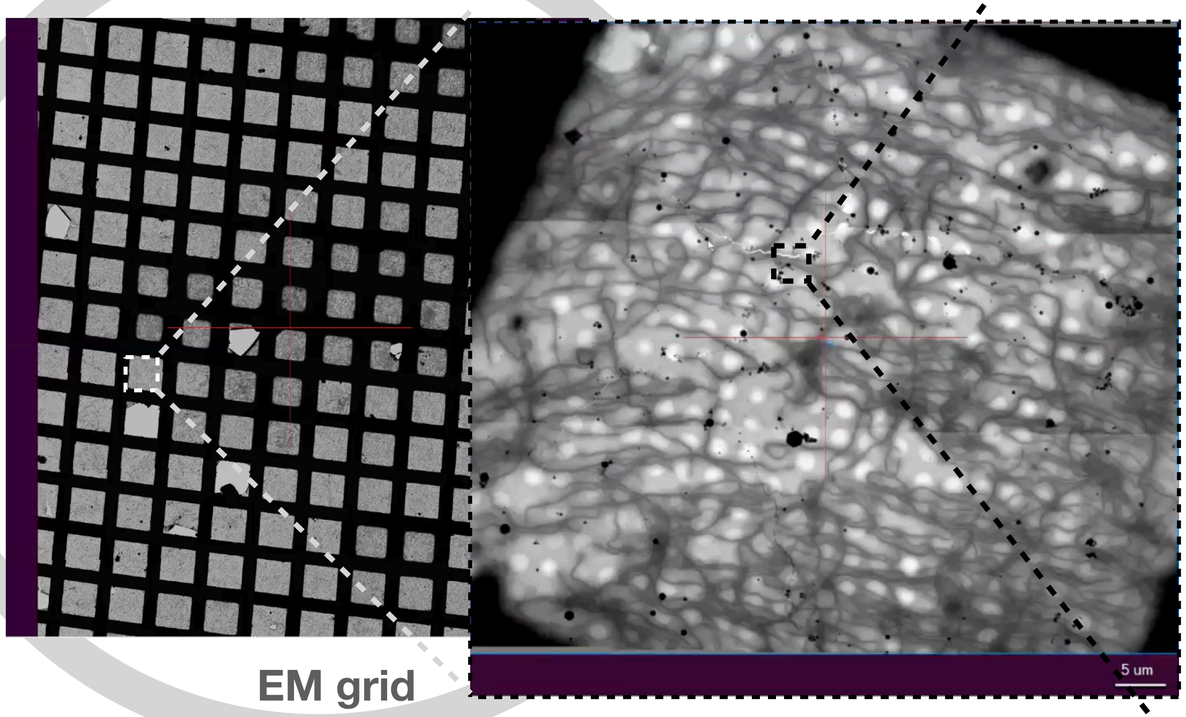

Full-montage (175x) acquisition
SerialEM - G205S26.st (#2) 1: 2250c.st
Navigator: G2055.nav
Log: log1.log
Scripts
100 um


Obtain multiple small montages (2250x)
SerialEM - low.st
Navigator
Log: log1.log
Scripts
50 um

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

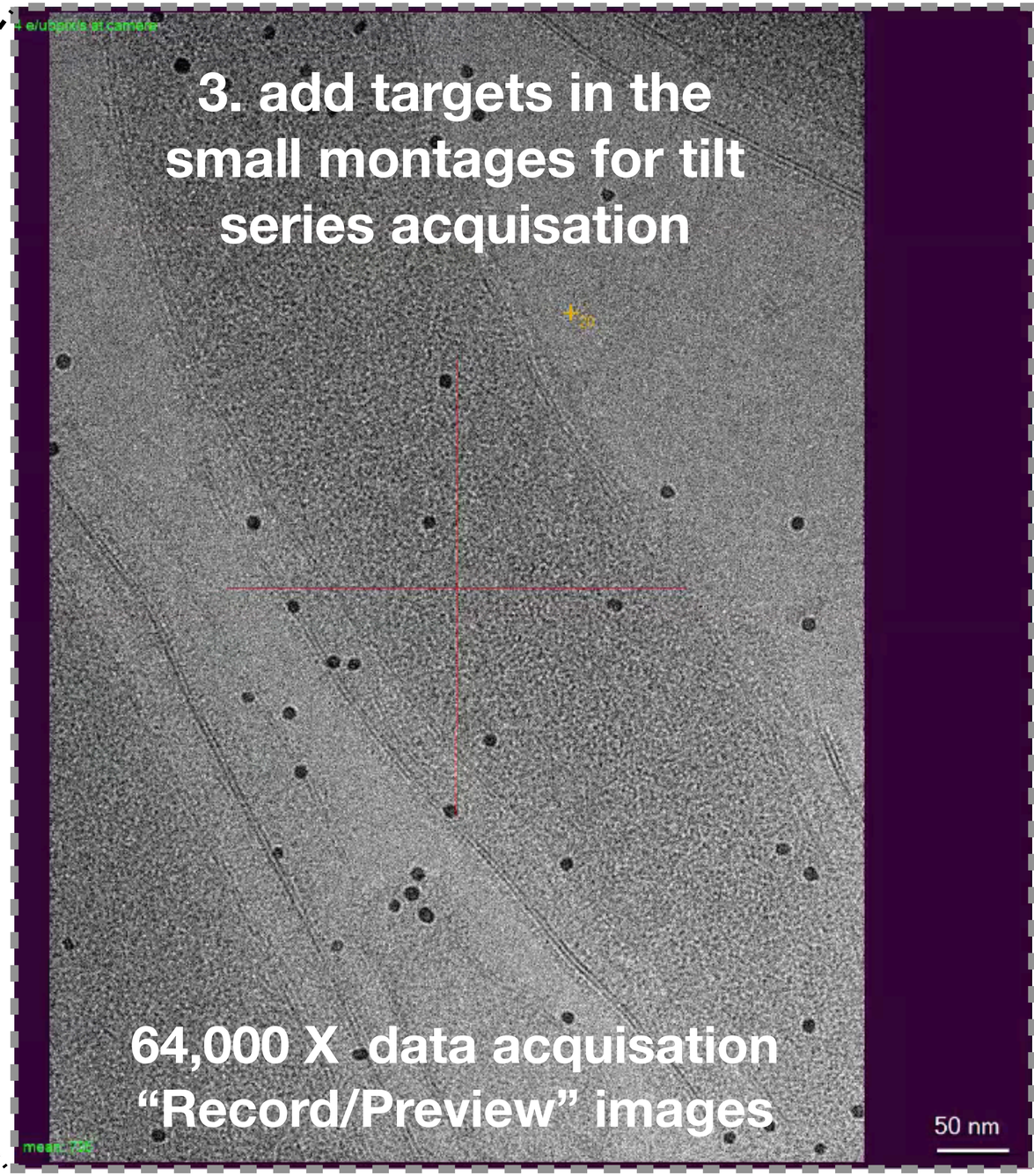


EM grid

6 x 6 “Search” images

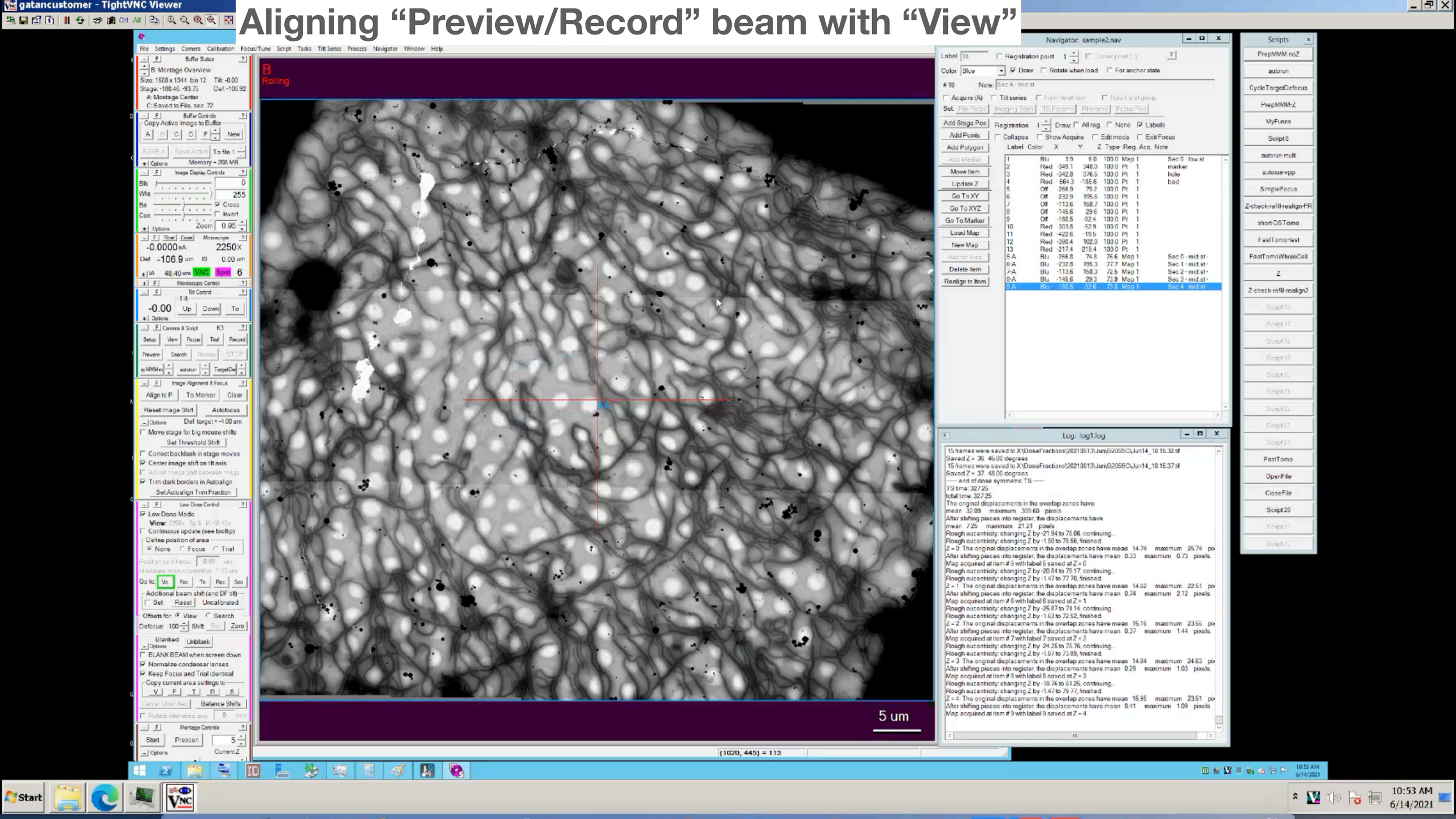
5 x 3 “View” images

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



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

Aligning “Preview/Record” beam with “View”



FastTomo: A SerialEM Script for Collecting Electron Tomography Data

Albert Xu, Chen Xu†
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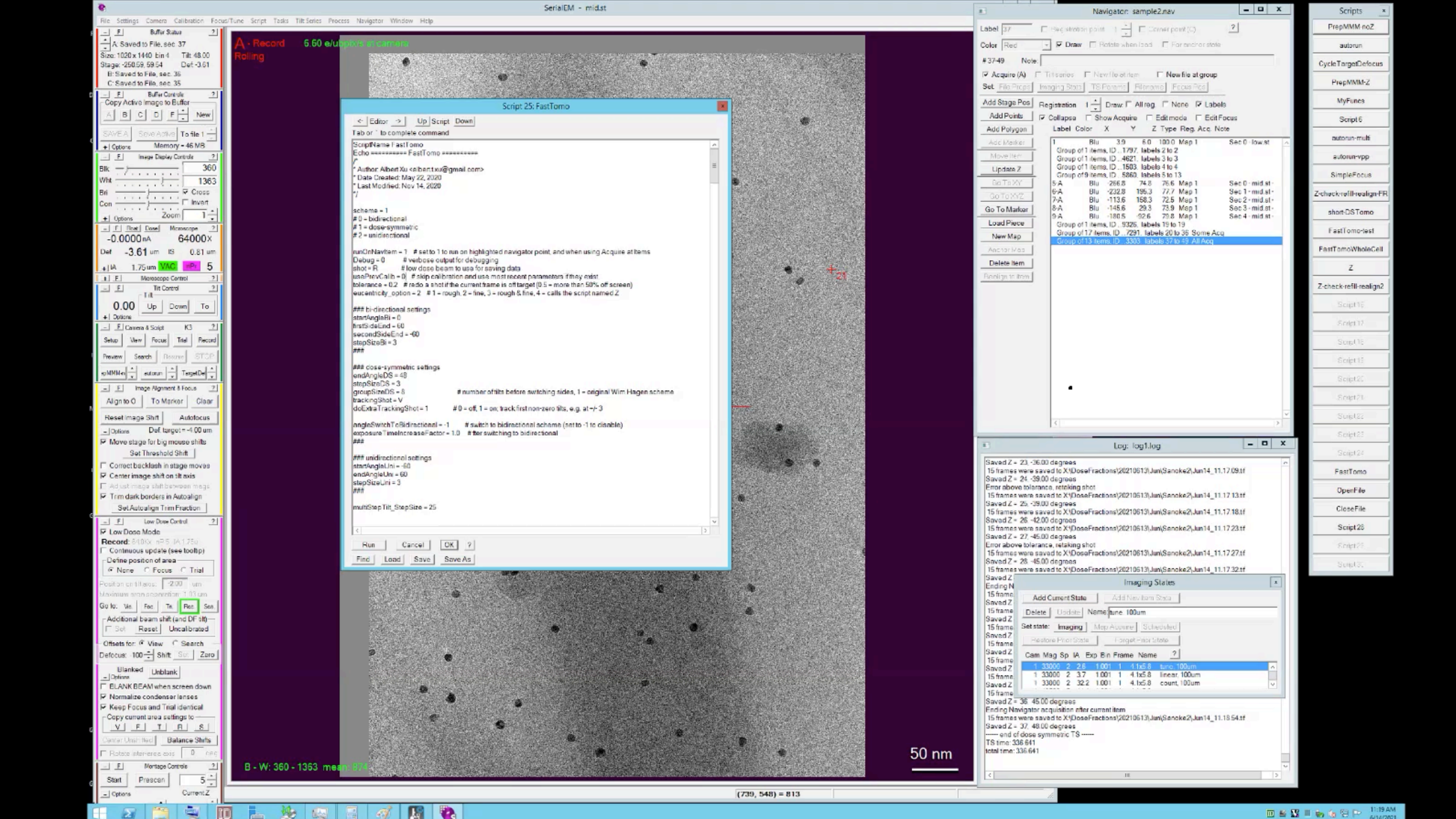
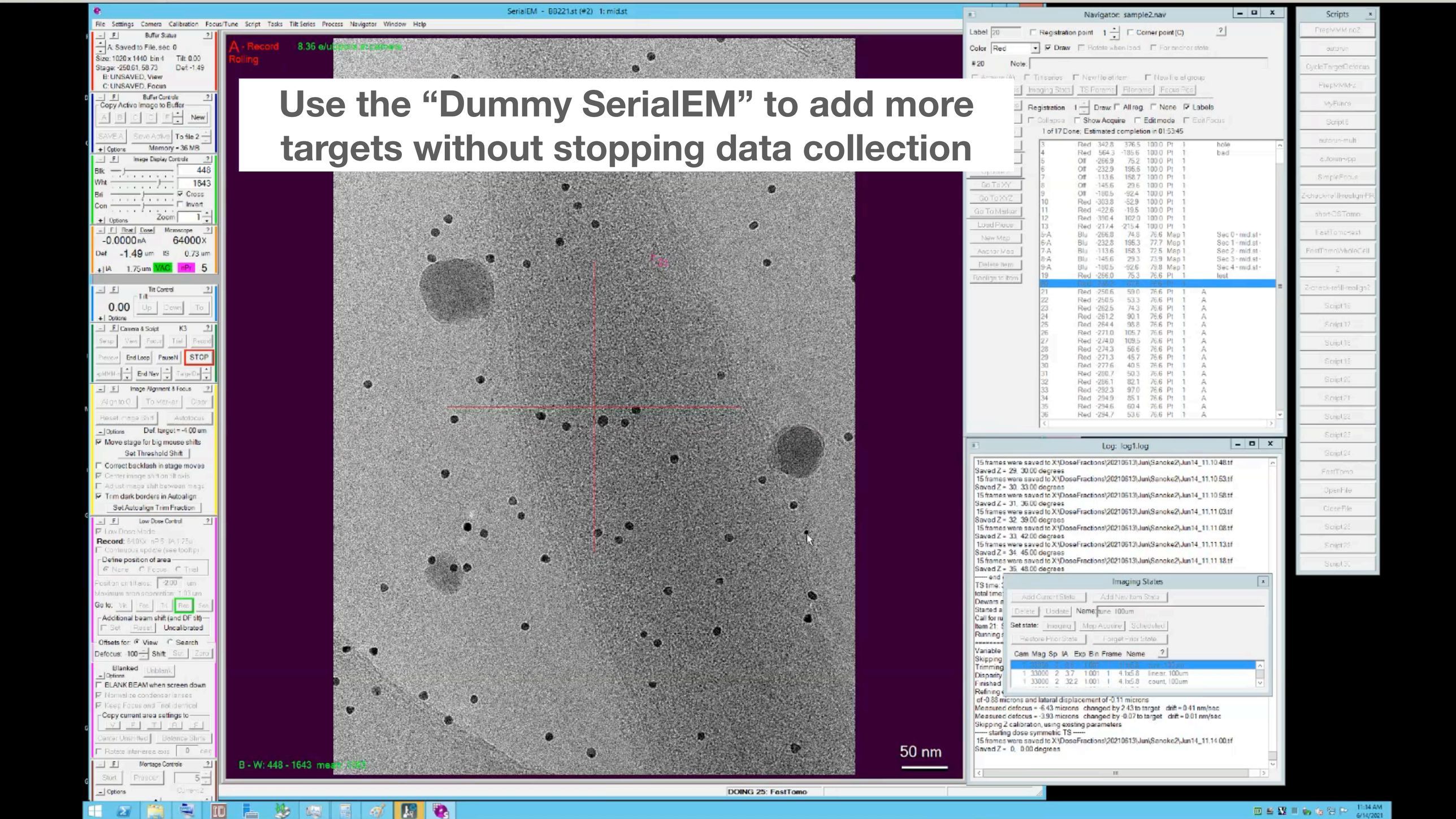
University of Massachusetts Medical School
Email: albert.t.xu@gmail.com, †Chen.Xu@umassmed.edu

***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.**

FastTOMO script

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

Debug = 0                 # verbose output for debugging

shot = R                  # low dose beam to use for saving data

usePrevCalib = 0          # skip calibration and use most recent parameters if they exist

tolerance = 0.4           # redo a shot if the current frame is off target (0.5 = more than 50% off screen)

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

multiRecord = 1           # take more than one R shots along tlting axis

multiR = { 3 6 }          # R shift 3 and 6 ums, can be more than 2 here.

### dose-symmetric settings

startAngleDS = 0

endAngleDS = 48

stepSizeDS = 3

groupSizeDS = 8                 # number of tilts before switching sides, 1 = original Wim Hagen scheme

trackingShot = V                # can also be set to V

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


SerialEM - mid.st
A - Record
Rolling
5.60 e/u/pix/s at camera
Script: 25: FastTomo
ScriptName FastTomo
Echo ========== FastTomo ==========
Navigator: sample2.nav
Log: log1.log
Imaging States
Scripts
PrepMMM noZ
autorun
CycleTargetDefocus
PrepMMM-Z
MyFuncs
Script 6
autorun-multi
autorun-vpp
SimpleFocus
Z-check-refill-realign-FR
short-DSTomo
FastTomo-test
FastTomoWholeCell
Z
Z-check-refill-realign2
FastTomo
OpenFile
CloseFile
Script 28
50 nm


Use the “Dummy SerialEM” to add more targets without stopping data collection
SerialEM - BB221.st (#2) 1: mid.st
Navigator: sample2.nav
Scripts
Log: log1.log
A - Record
Rolling
B - W: 448 - 1643
50 nm
DOING 25: FastTomo

Tilt series alignment and tomogram reconstruction by IMOD (Etomo)

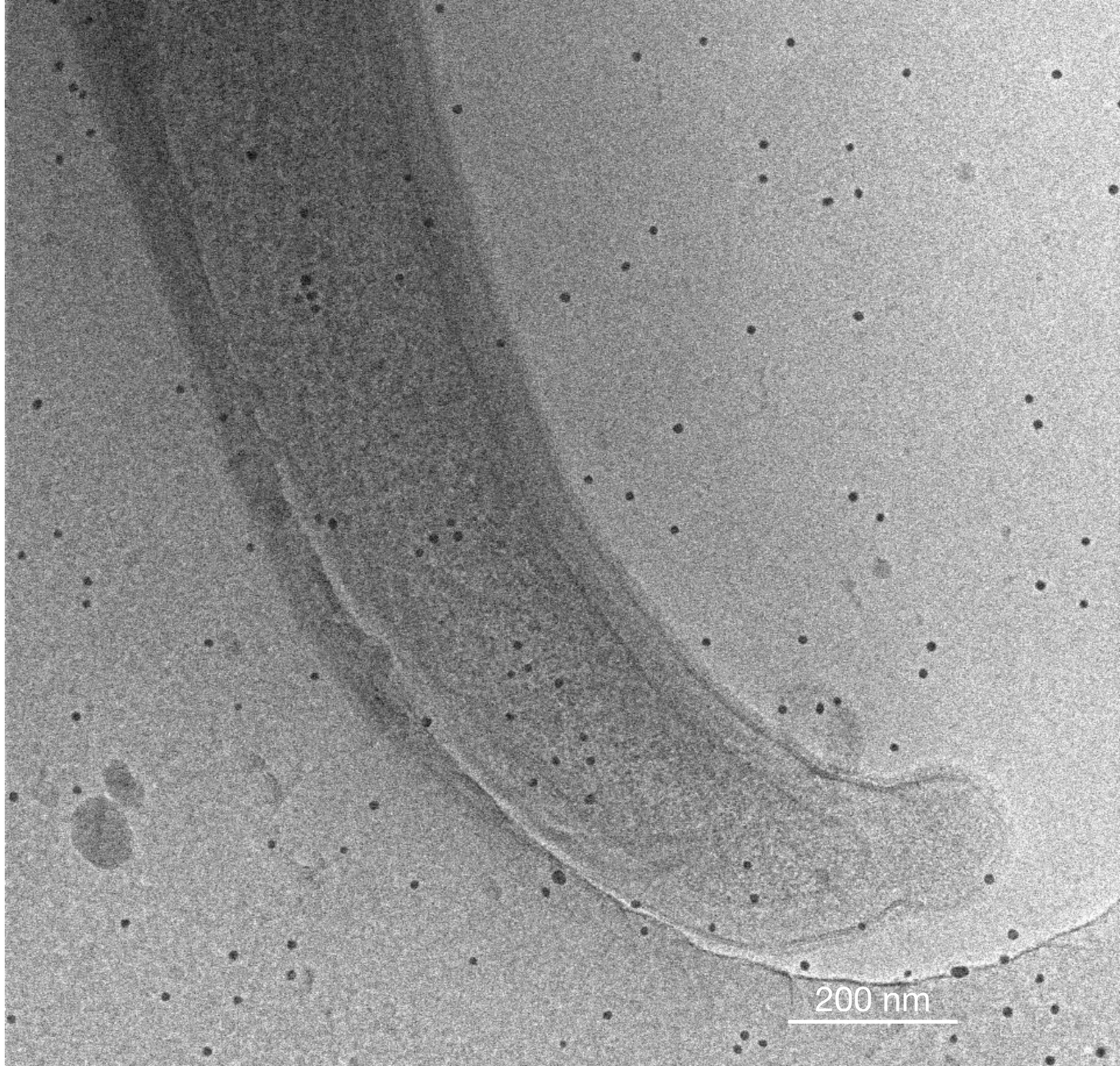
1. Motion correction for tilt images (MotionCorr2 or 3).
2. Stack the tilt images to get the motion corrected tilt series (IMOD).
3. Preprocess the tilt series (ETOMO).
4. Manually pick several fiducial gold (10 nm) and let the Etomo do the tracking for fiducial.
5. Align the tilt series based on the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

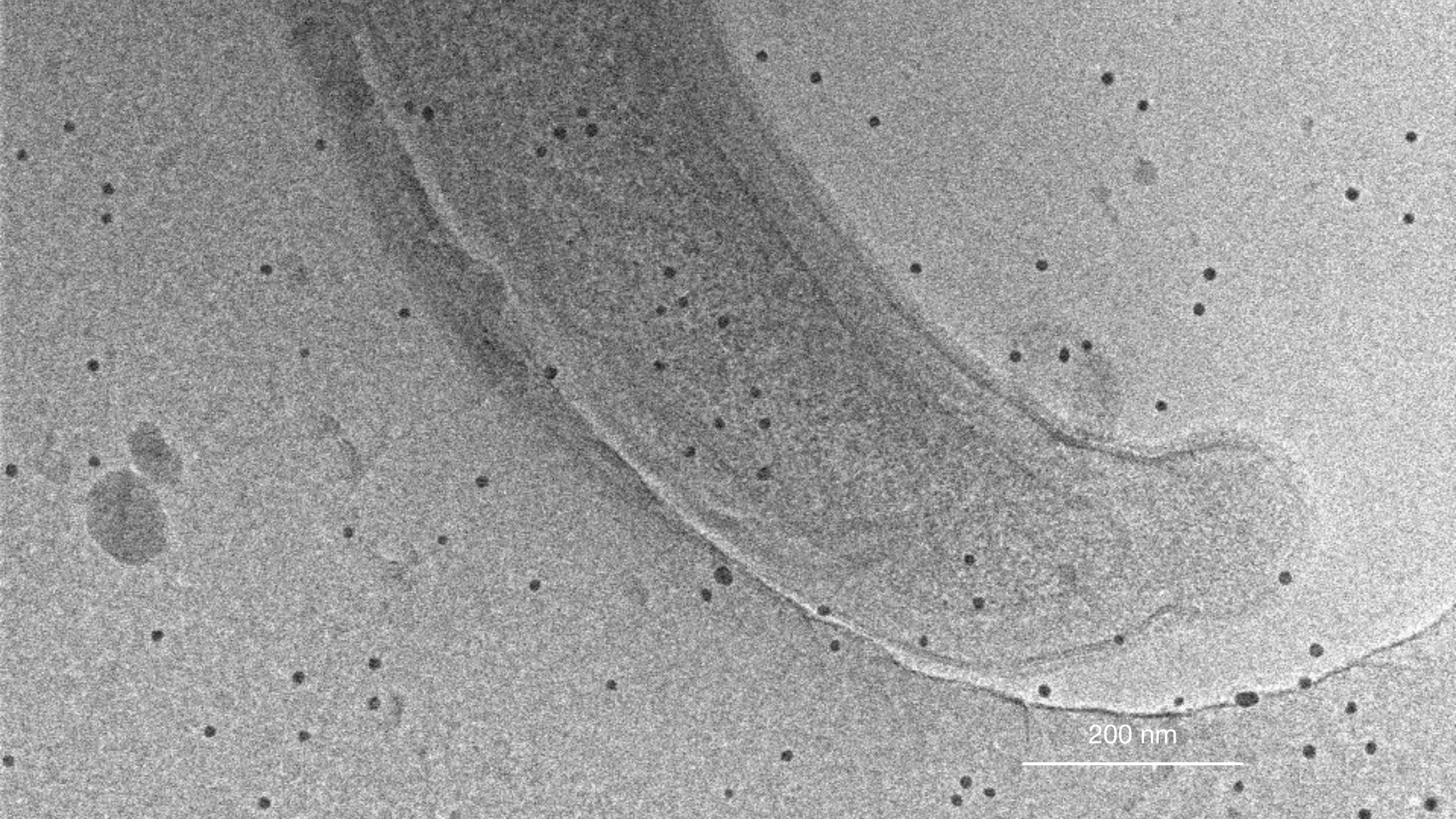
More details about etomo:

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

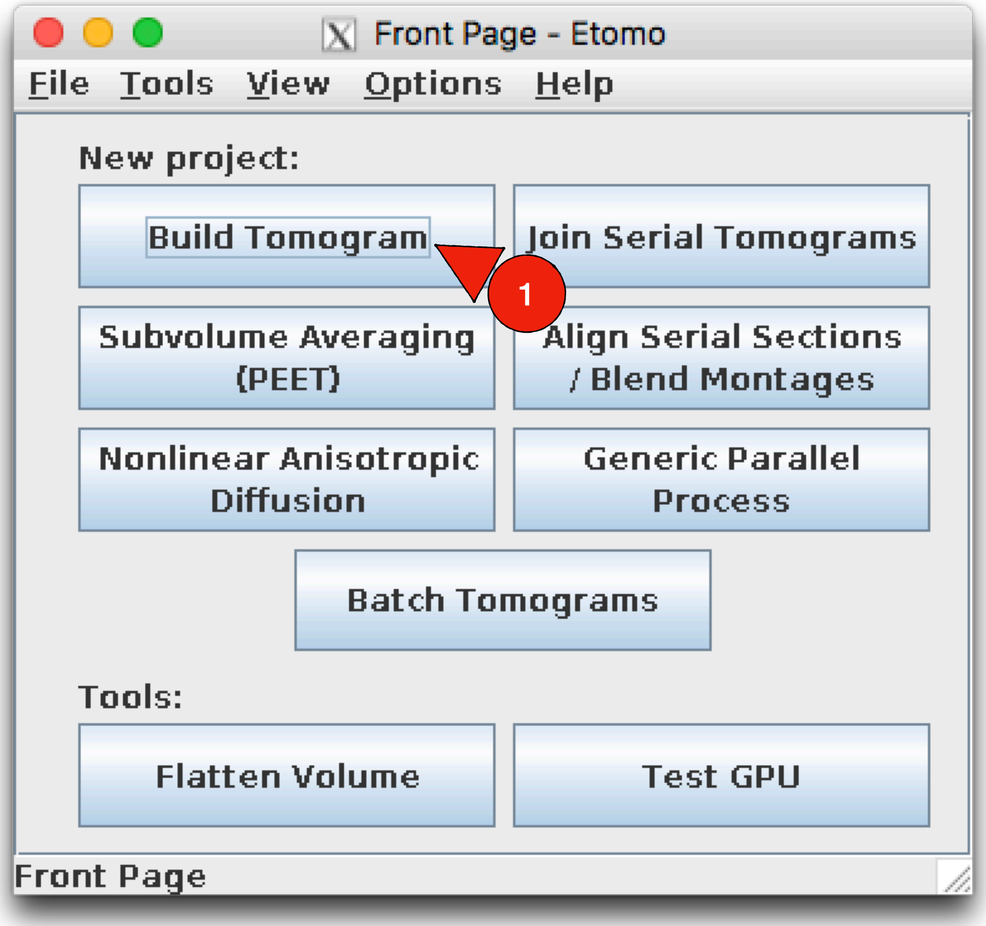
Dataset

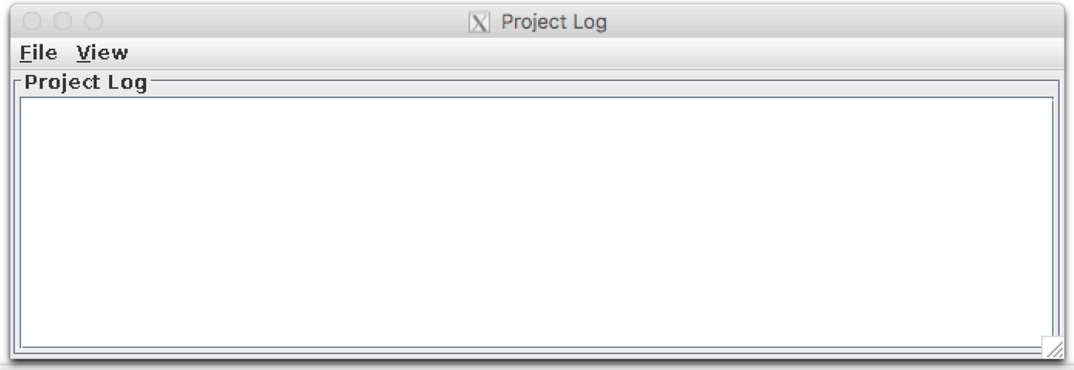
- *Borrelia burgdorferi*
- Collected by Titan Krios
- -51˚:3˚:51˚
- 2.747 Å/pixel

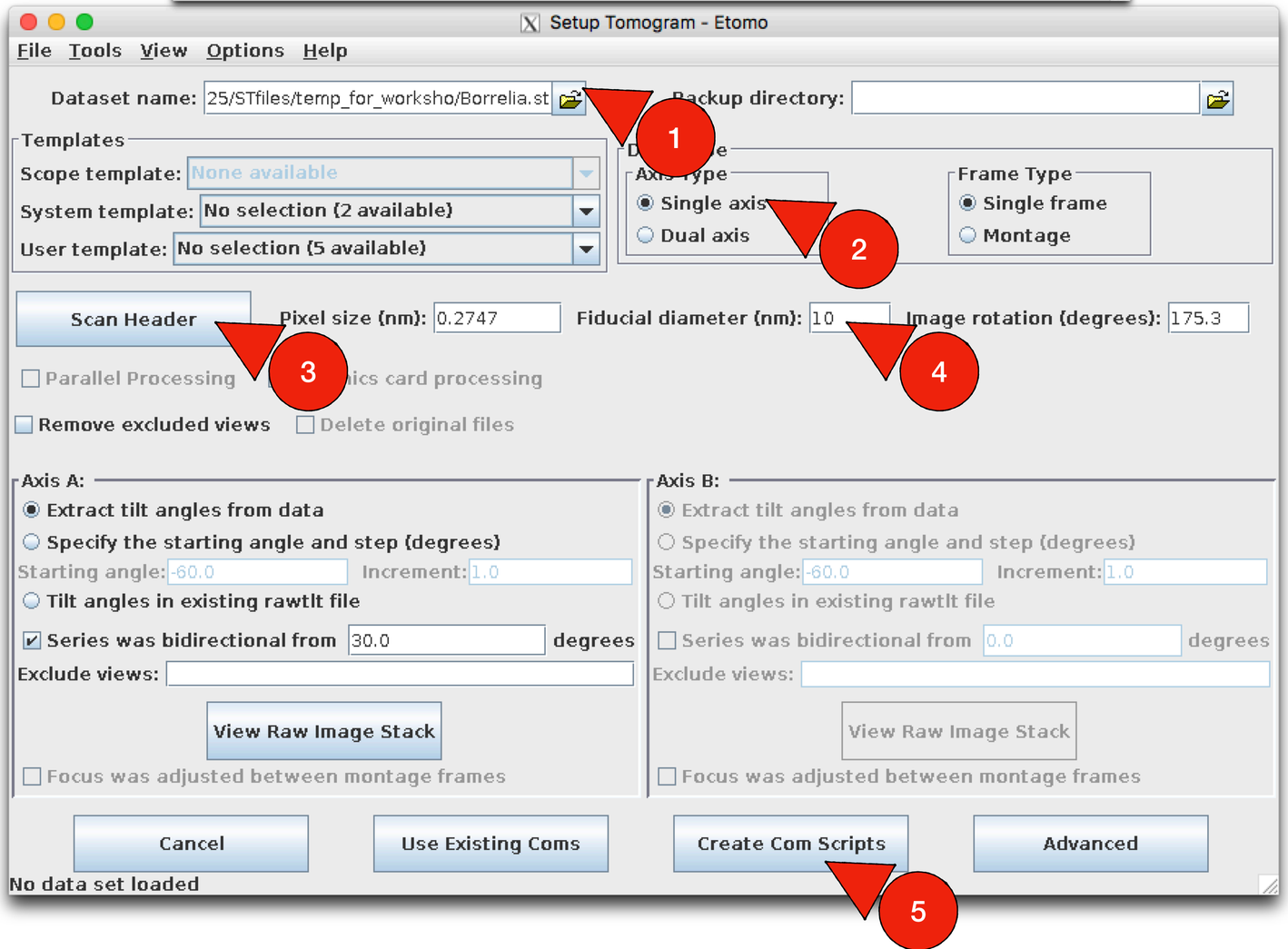



200 nm

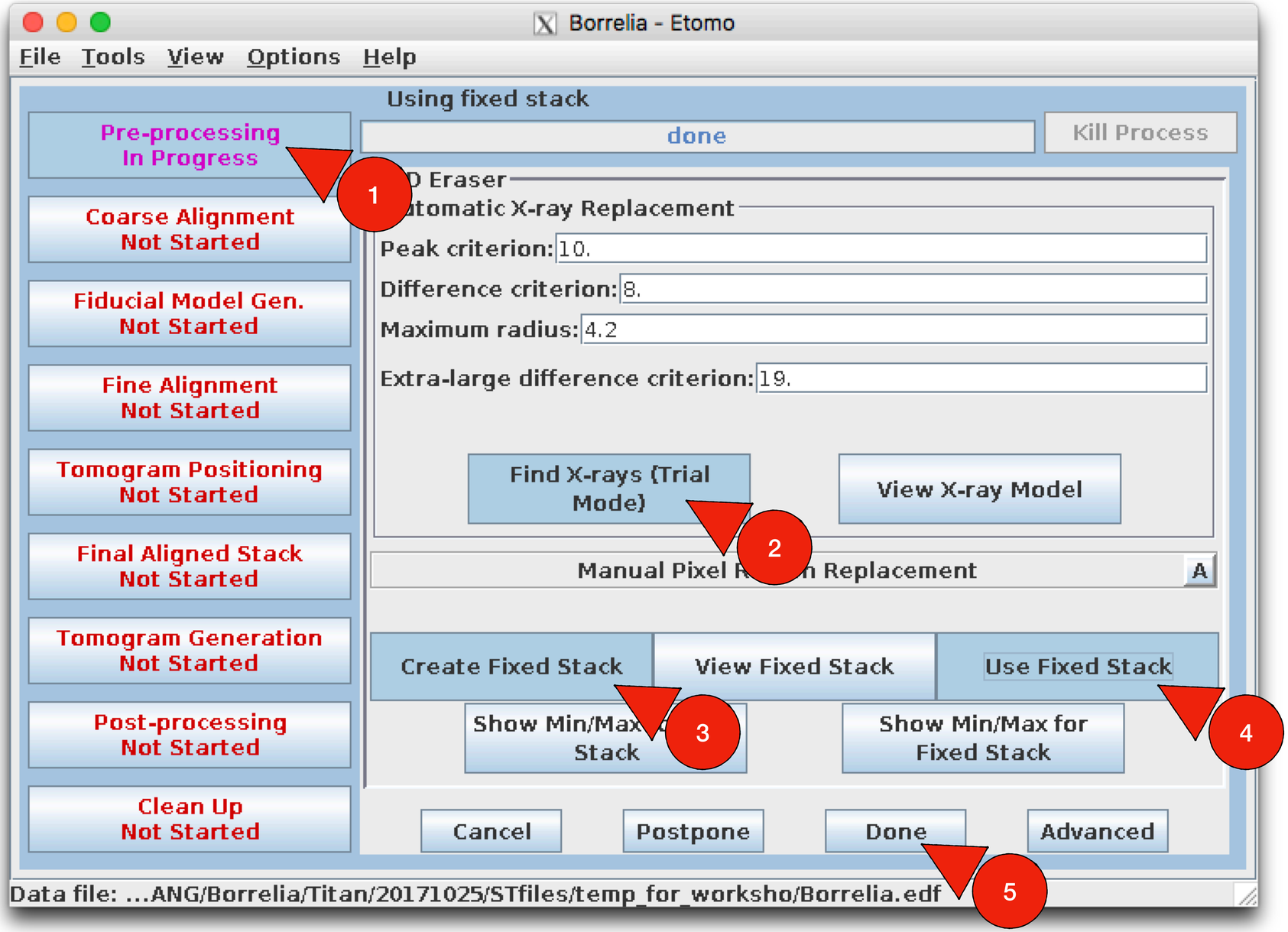
Etomo interface



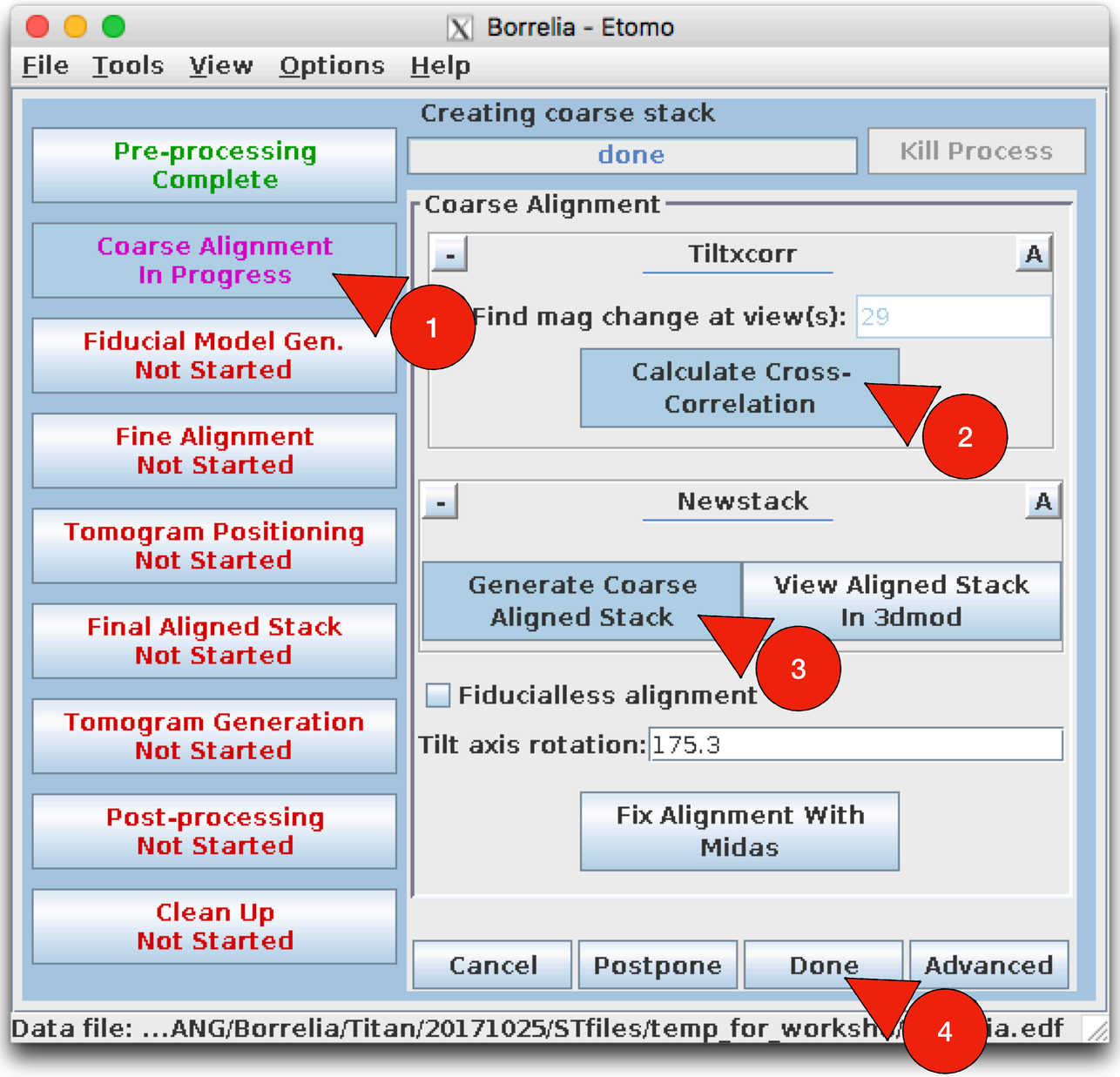


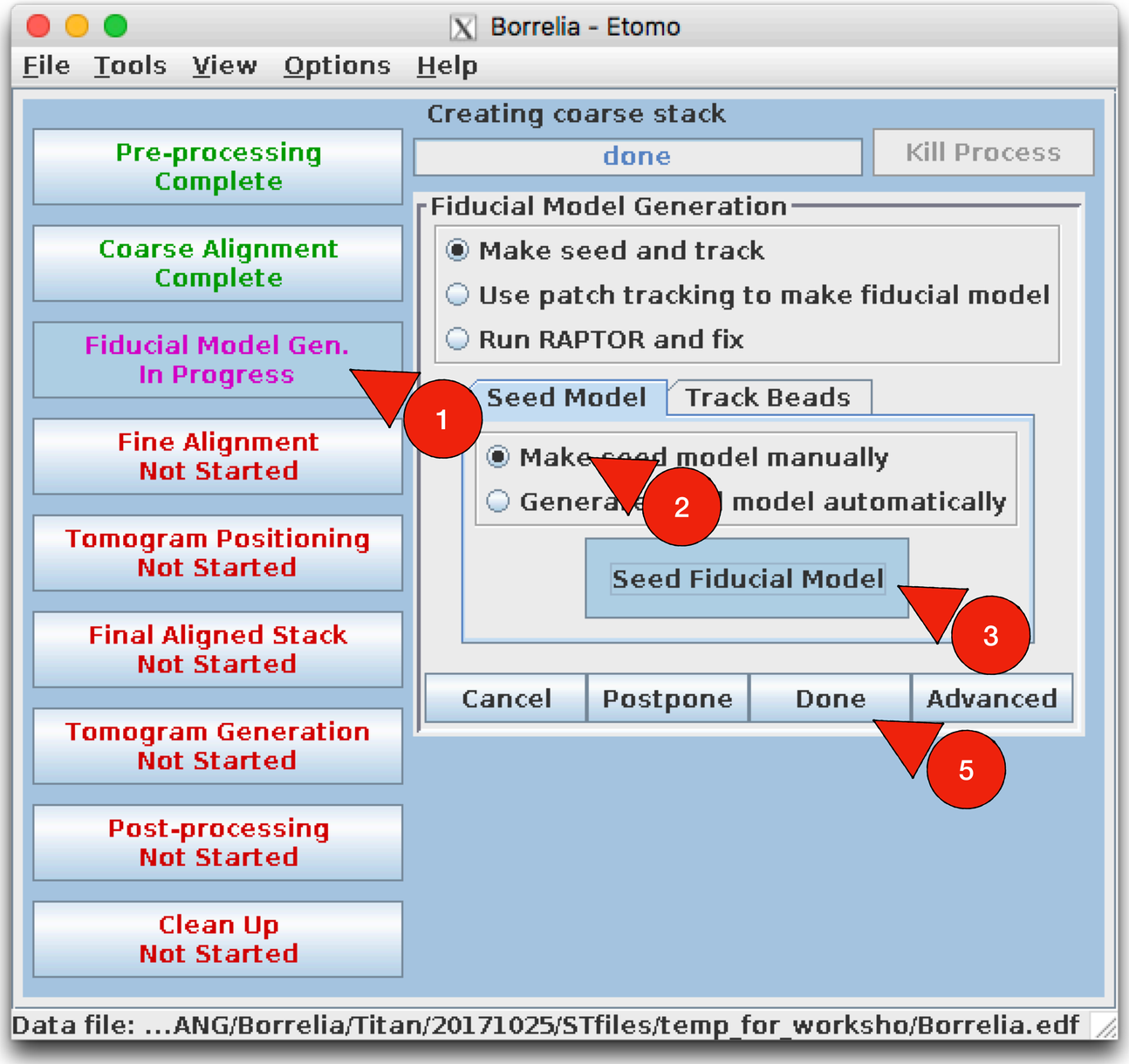


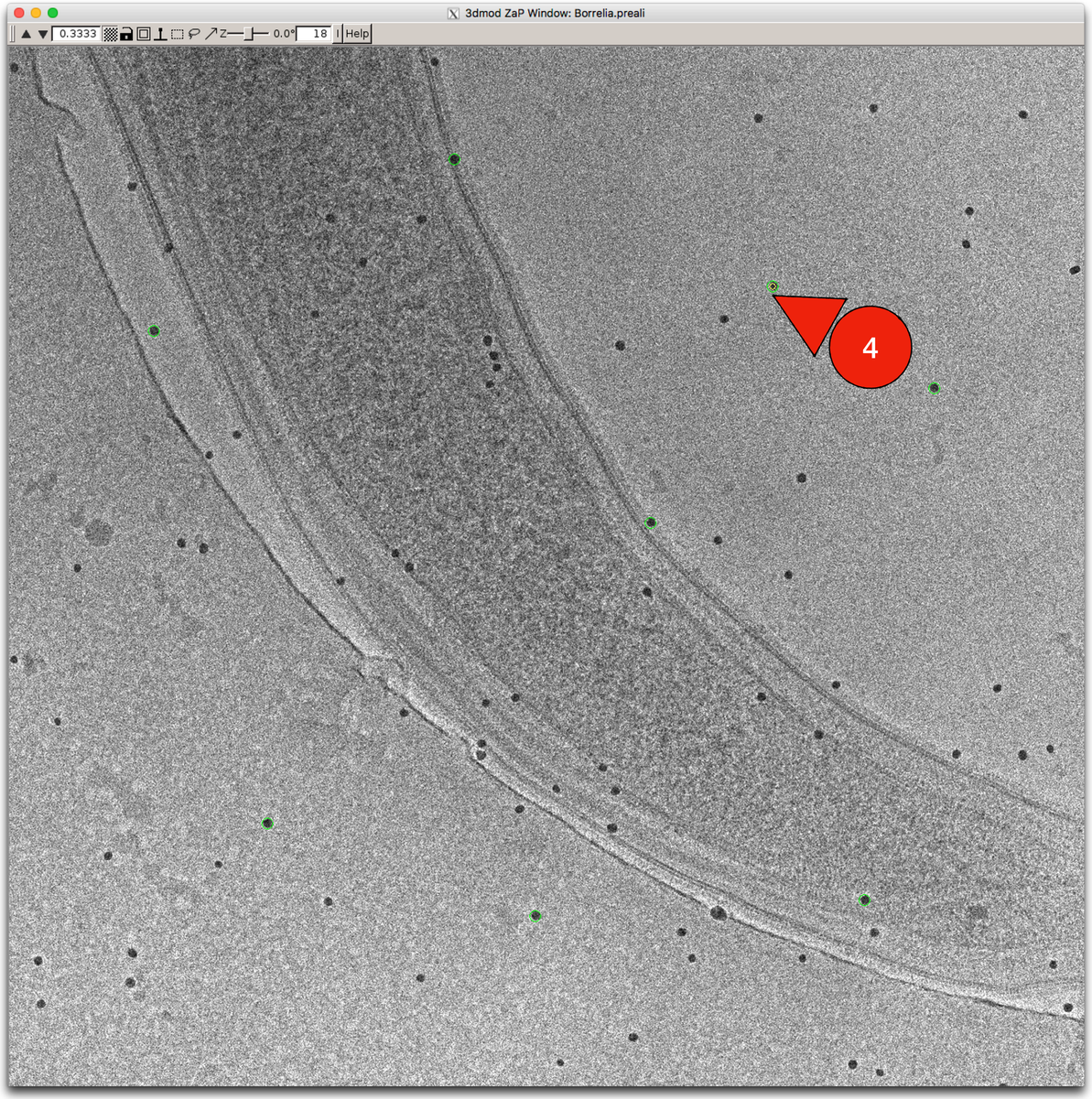
Pre-processing



Coarse alignment

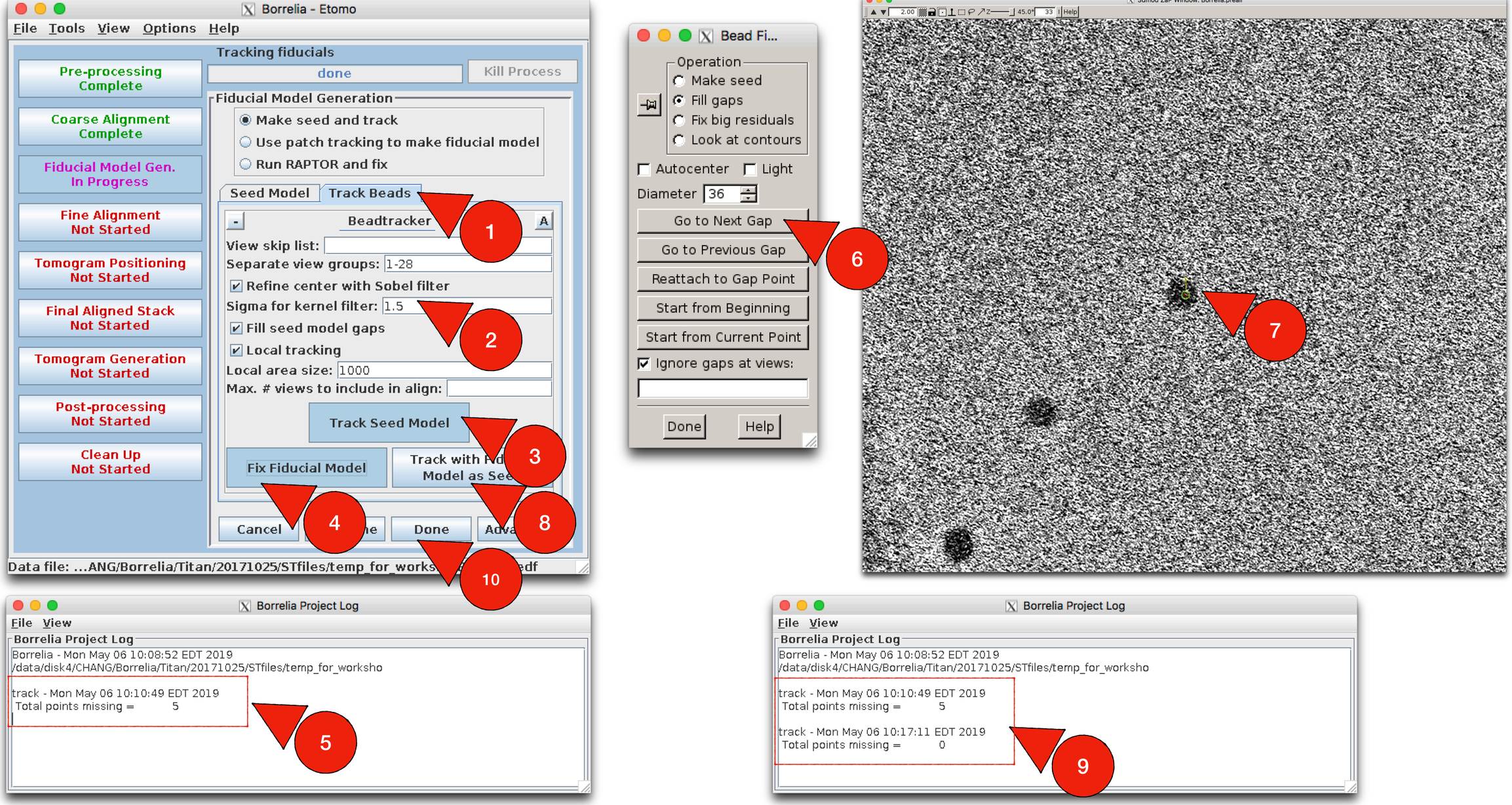






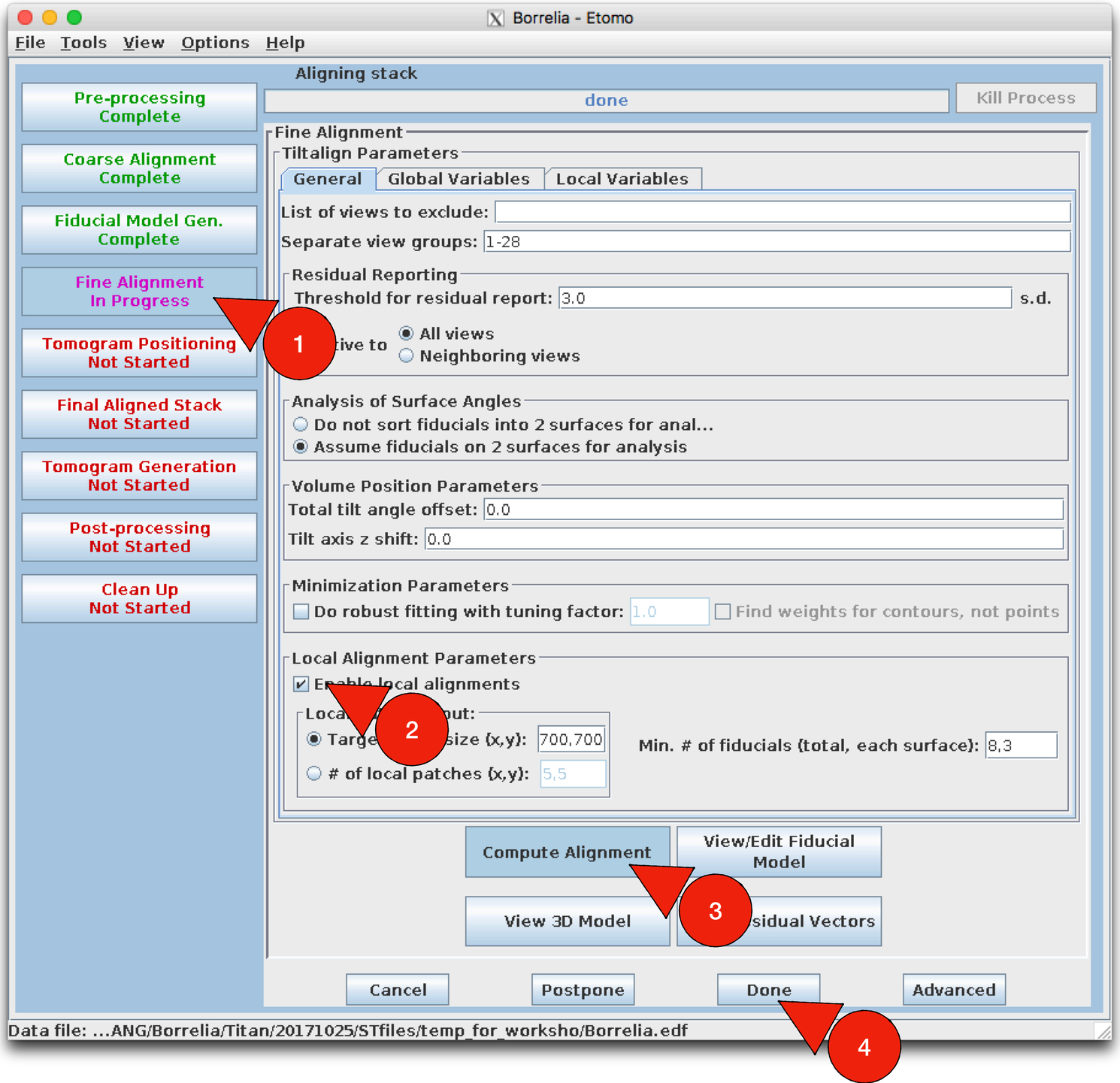
Generate fiducial model

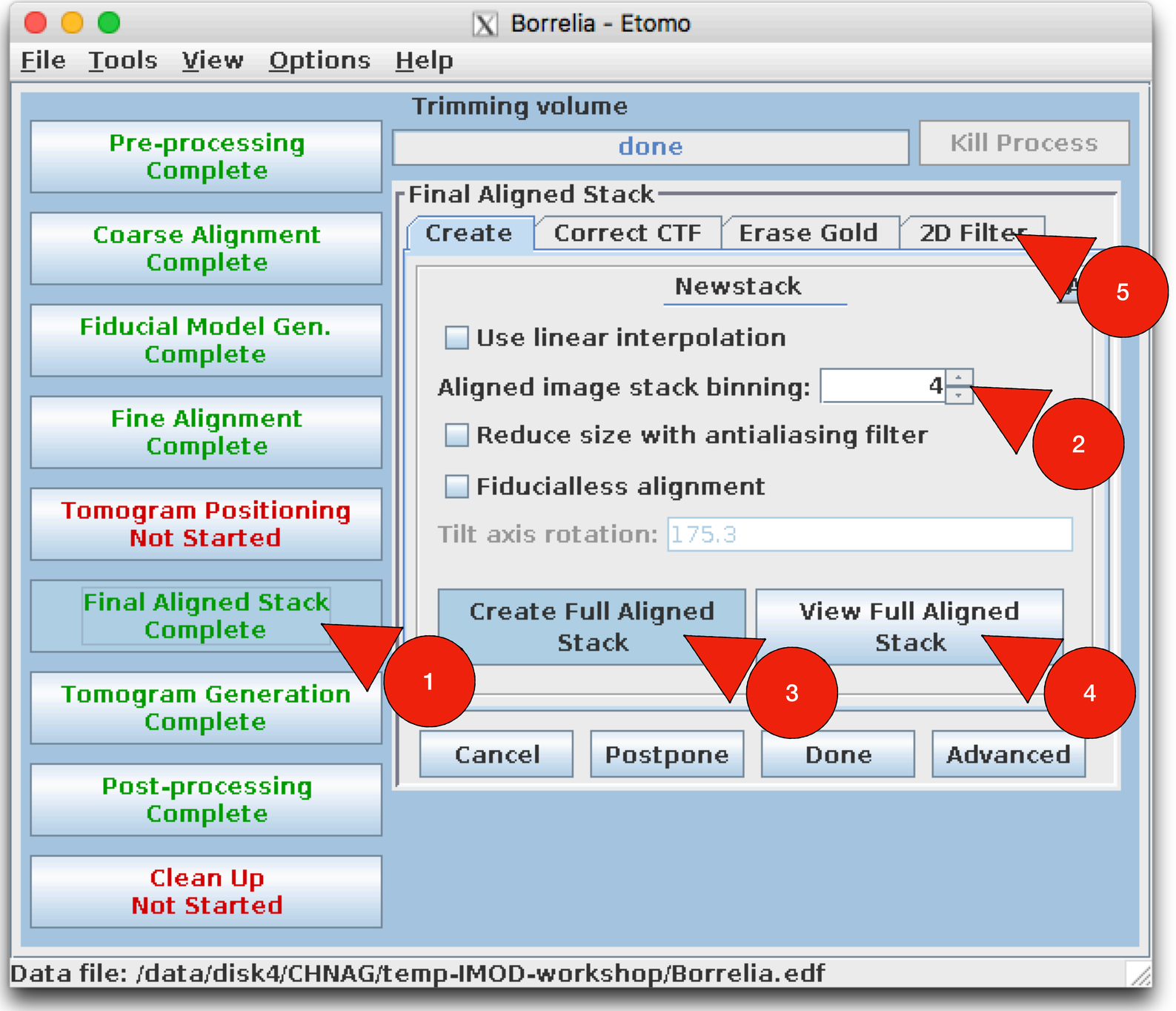
1. **Press mouse middle button to select one gold particle**
2. **Then press “N” in keyboard to create a new contour and then select another gold particle**
3. **Choose 5-10 gold particles in different areas**
4. **Close IMOD and save the model**



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

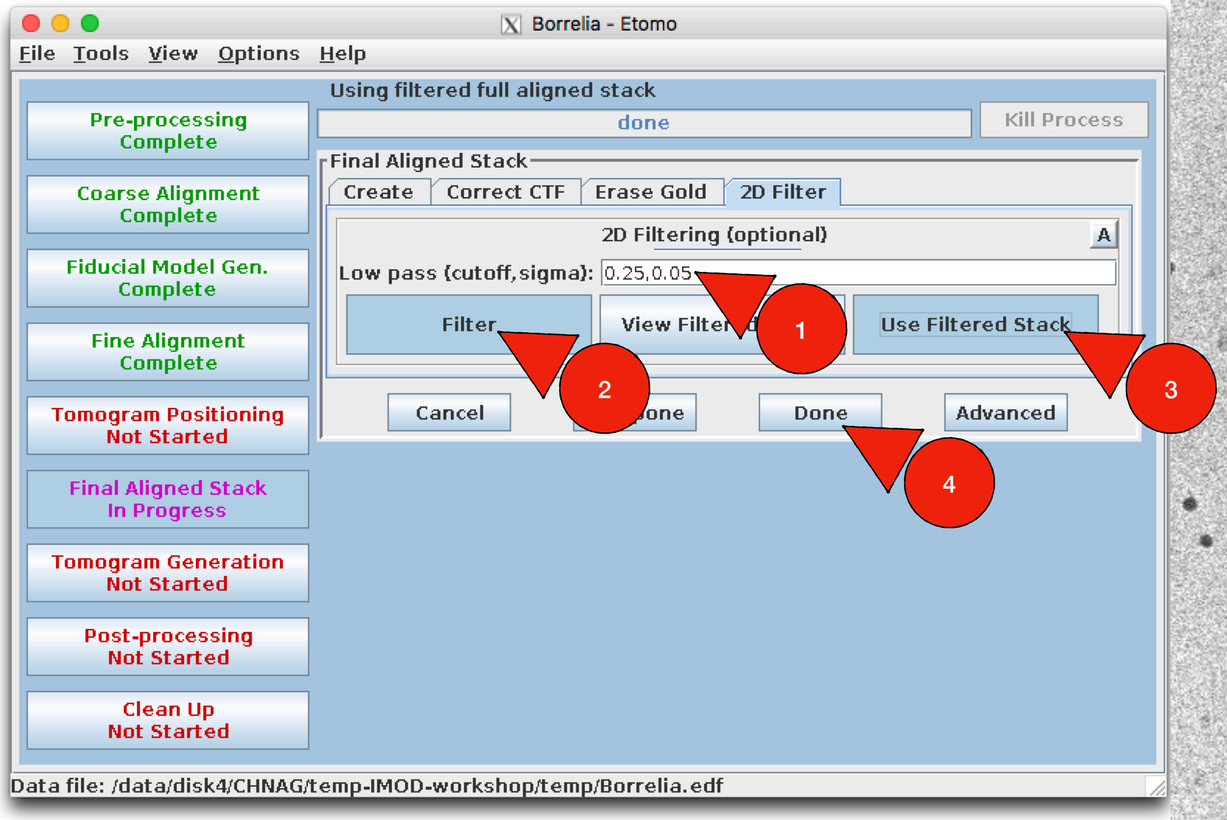
Final alignment of fiducial



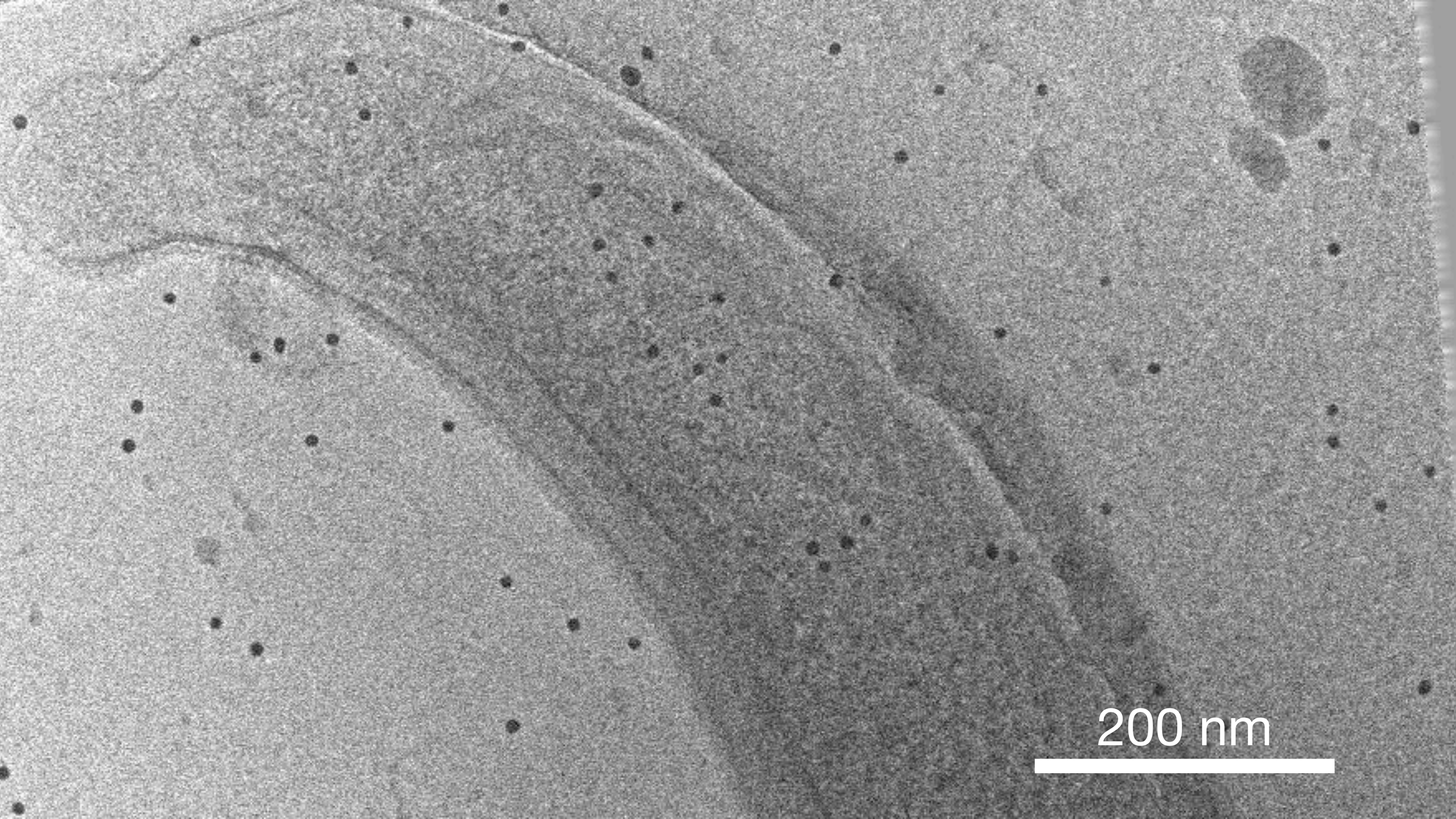


Generate aligned tilt series

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


Borrelia - Etomo
File Tools View Options Help
Using filtered full aligned stack
done
Kill Process
Pre-processing Complete
Coarse Alignment Complete
Fiducial Model Gen. Complete
Fine Alignment Complete
Tomogram Positioning Not Started
Final Aligned Stack In Progress
Tomogram Generation Not Started
Post-processing Not Started
Clean Up Not Started
Final Aligned Stack
Create
Correct CTF
Erase Gold
2D Filter
2D Filtering (optional)
A
Low pass (cutoff,sigma):
0.25,0.05
Filter
View Filter
Use Filtered Stack
Cancel
Done
Advanced
1
2
3
4
Data file: /data/disk4/CHNAG/temp-IMOD-workshop/temp/Borrelia.edf

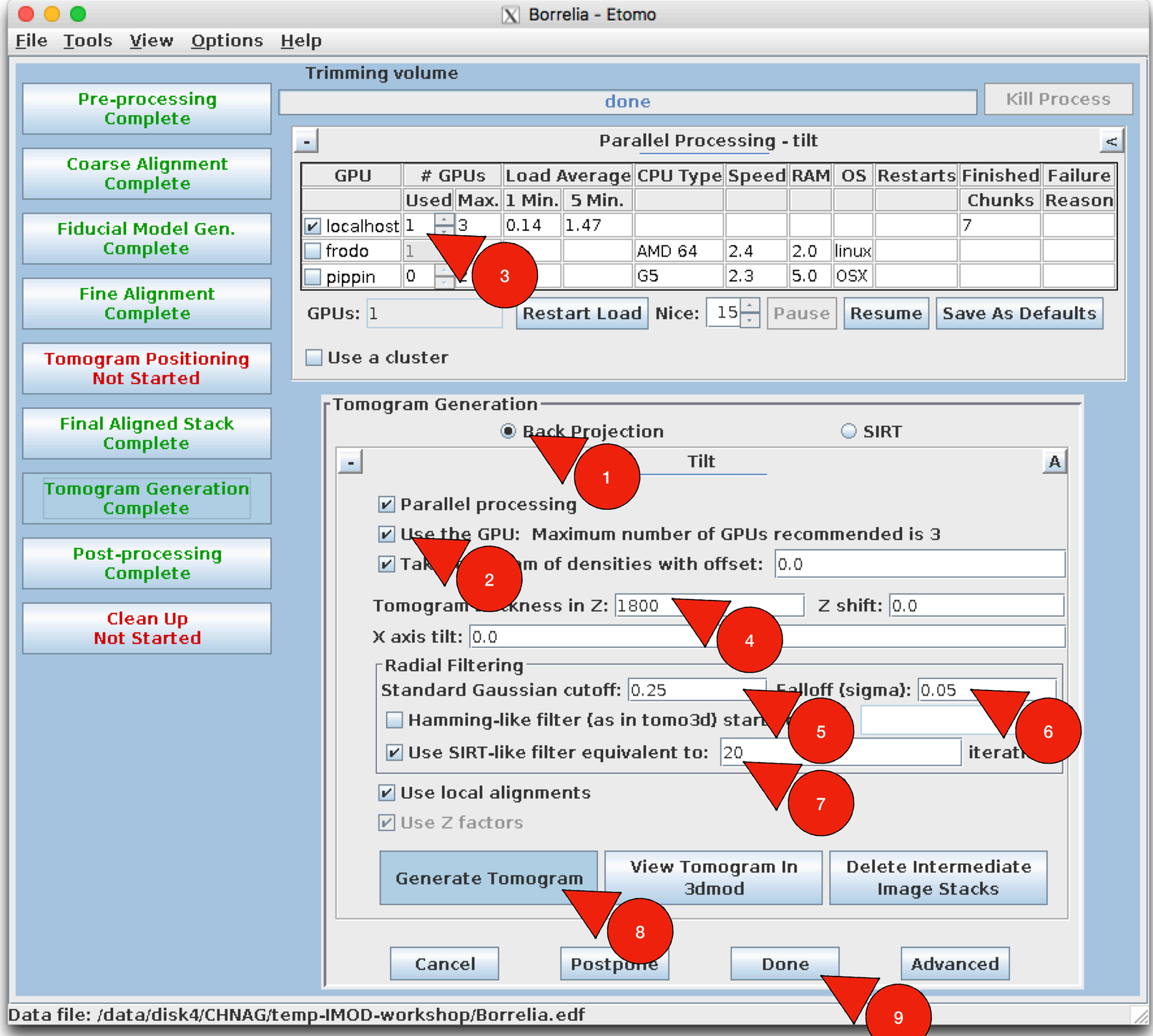
200 nm


200 nm

Tomogram reconstruction

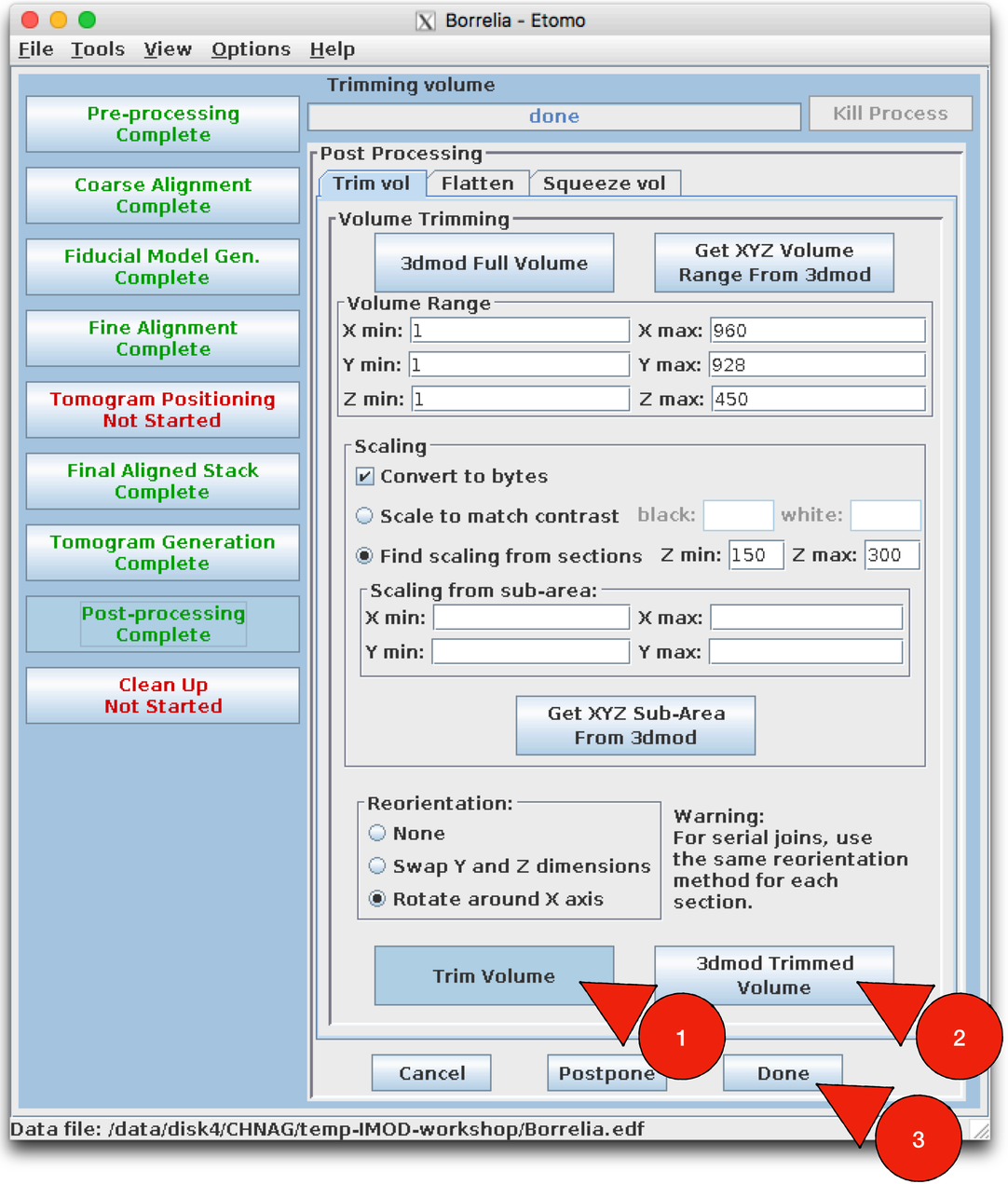
More details about WBP (weight back projection) and SIRT (simultaneous iterative reconstruction):

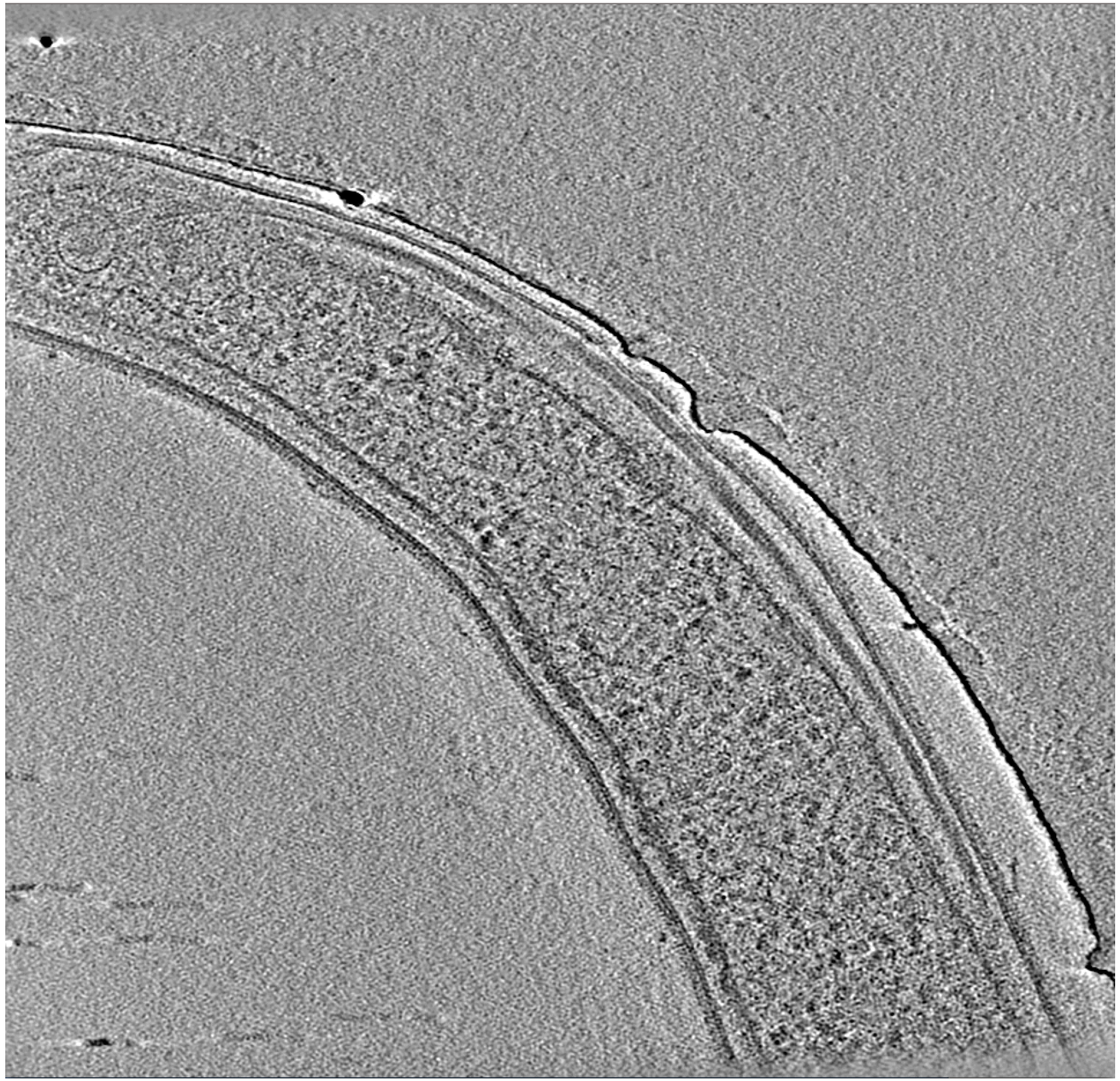
https://en.wikipedia.org/wiki/Tomographic_reconstruction#Back_Projection_Algorithm[2]



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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