

# Cryo-ET data collection by SerialEM

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







### 1. SerialEM tilt series data collection with FastTomo script

### 2. Tomogram reconstruction 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









# SerialEM (David Mastronarde

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



## Defocus Magnification Stage tilt angle





@junliulab

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

## Defocus Magnification Stage tilt angle

View Focus Trial Record

Setup Preview



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Buffer Status

# SerialEM (David Mastronarde

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### 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

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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



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











### 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







# FastTomo: A SerialEM Script for Collecting Electron Tomography Data

Albert Xu, Chen Xu<sup>†</sup> Department of Biochemistry and Molecular Pharmacology & Cryo-EM Core Facility University of Massachusetts Medical School Email: albert.t.xu@gmail.com, <sup>†</sup>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 + ABCDF. SAVEA SEVEACIVE To i 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

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# Tilt series alignment and tomogram reconstruction by IMOD (Etomo)

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

the tracking for fiducial.

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

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

# Dataset

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



![](_page_20_Picture_0.jpeg)

### Etomo interface

![](_page_21_Figure_1.jpeg)

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![](_page_23_Figure_1.jpeg)

# Coarse alignment

![](_page_24_Figure_0.jpeg)

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

![](_page_24_Picture_8.jpeg)

![](_page_25_Figure_0.jpeg)

- 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.

![](_page_25_Figure_4.jpeg)

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Borrelia Project Log	
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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

![](_page_25_Picture_8.jpeg)

![](_page_25_Picture_9.jpeg)

### Final alignment of fiducial

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	Min. # of fiducials (total, each surface): 8,3
	$\bigcirc$ # of local patches (x,y): 5,5
	Compute Alignment View/Edit Fiducial Model
	View 3D Model
	Cancel Postpone Done Advanced
G/Borrelia/Tita	n/20171025/STfiles/temp_for_worksho/Borrelia.edf

![](_page_26_Picture_6.jpeg)

![](_page_27_Figure_0.jpeg)

### 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.

![](_page_27_Figure_4.jpeg)

![](_page_27_Figure_5.jpeg)

![](_page_28_Figure_0.jpeg)

![](_page_29_Picture_0.jpeg)

### 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]

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### 🗙 Borrelia - Etomo

### Options Help

![](_page_30_Figure_7.jpeg)

Tomogram reconstruction

### Save following files: Borrelia.st Borrelia.ali Borrelia.rec Borrelia.rawtlt Borrelia\_fid.xf Other files can be deleted

🗧 😑 💿 📉 Borrelia - Etomo						
<u>F</u> ile <u>T</u> ools <u>V</u> iew <u>Options</u> <u>H</u> elp						
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![](_page_31_Picture_3.jpeg)

![](_page_32_Picture_0.jpeg)

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

![](_page_32_Picture_2.jpeg)

![](_page_32_Picture_3.jpeg)

![](_page_32_Picture_4.jpeg)

IVERSITY

![](_page_32_Picture_6.jpeg)

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![](_page_36_Picture_2.jpeg)

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	Group of 4 items. ID8252. labels 255 to 258			Script 15
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![](_page_36_Picture_5.jpeg)