### Cryo-EM Course at the Laboratory for BioMolecular Structure (LBMS)

# Cryo-ET data collection and Day 1-Juesday 14 June 2023 demonstration Liguo Wang (BNL) Introduction to LBMS David DeRosier Uthe evolution, deficiencies, and promise of cryo-electron (Brandeis)rtment of Microscopy Chen Xu (Umess) Introduction to electron microscopes and cameras MICrobial Sciences Institute Tamir Gonen (UCLA) AlcroED? theory application and available software

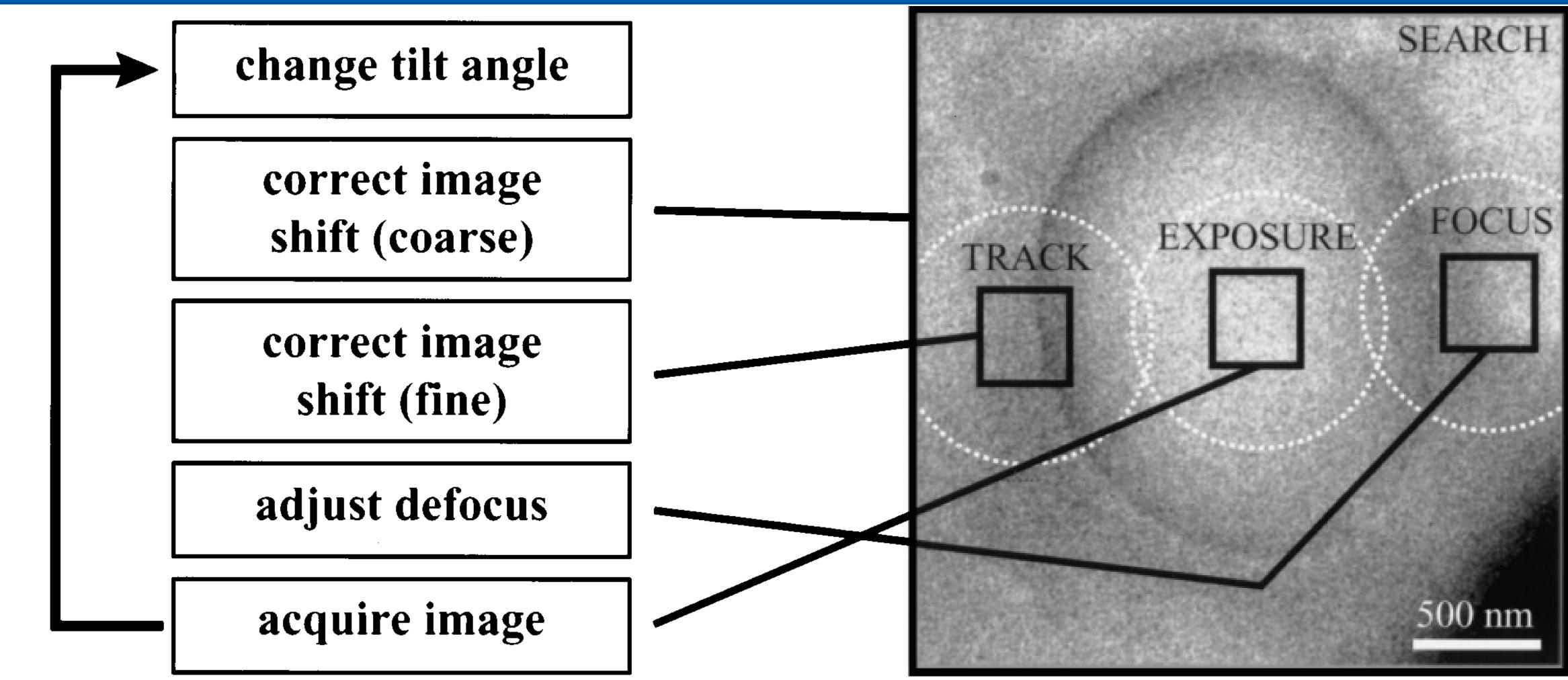
- Liguo Wang (BNL) Introduction to negative staining and cryo-electron micro
  - Coffee break
- uchin Lu (DNU)

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### Cryo-ET data collection





### Many cryo-ET data acquisition packages

UCSF Tomo Leginon FEI tomography EM-Manu





### SerialEM





### 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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### Seria EM

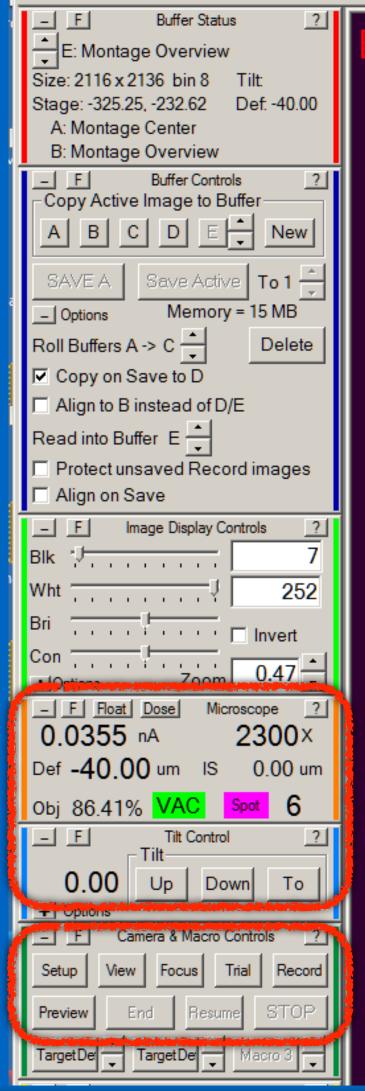
SerialEM provides a flexible interface. The script capability provides a relatively easy way to add commands requested by users.



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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



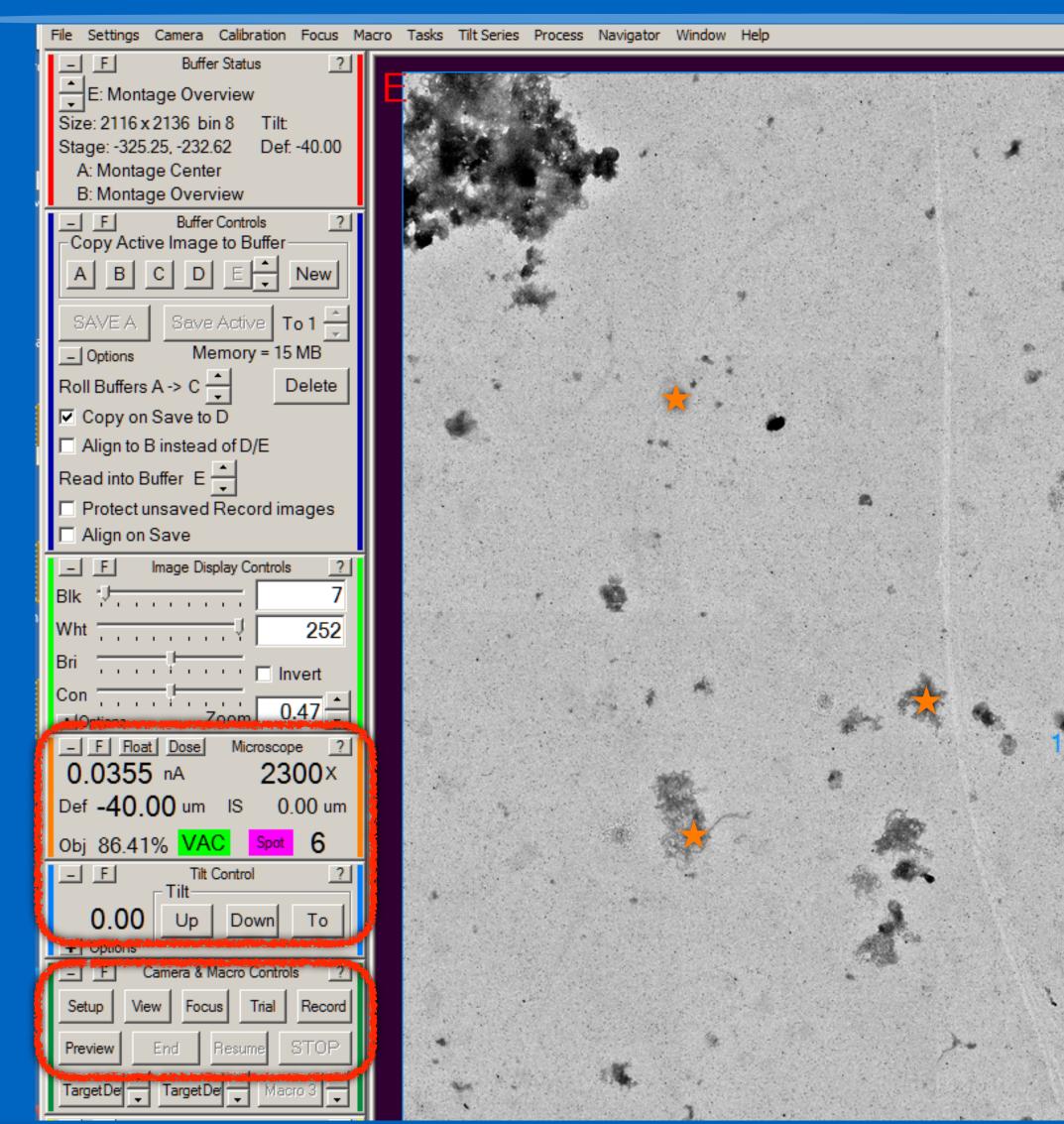
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## SerialEM (David Mastronarde)

Di Di Jaw Daga Castral 21
D       Low Dose Control       ?         ✓ Low Dose Mode       Intensity         ✓ View: 2300x Sp 6 C2 61.41%       ✓         ✓ Continuous update of mag & beam       Define position of area         ④ None       ○ Focus       ○ Trial         Position on tilt axis:       0.00       um         Maximum area separation: -0.71 um       ✓       Background Subtraction         ✓ Gain Correction       ✓       Gain Correction         ✓ Position al beam shift       0.00, 0.00       Update HW Dark Reference         ✓ Vie.       ✓ Foc.       ○ Tri.       ○ Rec.       ○ Sea.
□ BLANK BEAM when screen down Blanked Unblank Search Offsets for View Defocus: -60  Shift: Set Zero Exposure (s) 0.5
<ul> <li>Normalize beam through View</li> <li>✓ Keep Focus and Trial identical</li> <li>Copy current area mag &amp; beam to</li> <li>✓ F T R</li> <li>Center Unshifted Balance Shifts</li> <li>Potate inter-area axis</li> <li>✓ deg</li> </ul>
Navigator
Label:     1     Image: Registration point     1     Image: Commer point (C)       Color     Blue     Image: Commer point     Image: Commer point (C)
<ul> <li>#1 Note: Sec 0 - montage01.st</li> <li>Acquire (A) Tilt series New file at item New file at set</li> <li>Set File Properties Imaging State TS Parameters Filename Acquire map or image or run macro at this location automatically</li> <li>Add Stage Pos Registration 1 Draw all reg. Draw nor Add Points Collapse groups Show Acquire area</li> <li>Add Polygon Label Color X Y Z Type Reg. Accord Add Marker</li> <li>Blu -325.2 -232.6 118.7 Map 1</li> </ul>
Move Item











## SerialEM - Navigator

	_ Let Navigator
	Label: 1 🗖 Registration point 1 🚽 🗖 Corner point (C)
	Color Blue 🔽 🗹 Draw 🗆 Rotate when load 🗆 For and
A	#1 Note: Sec 0 - montage01.st
	🗖 Acquire (A) 🔲 Tilt series 🔲 New file at item 🗌 New file a
	Set: File Properties Imaging State TS Parameters Filenam Acquire map or image or run macro at this location automatically
	Add Stage Pos Registration 1 Draw all reg. Draw n
	Add Points Collapse groups Show Acquire area
	Add Polygon Label Color X Y Z Type Reg. Ad
	Add Marker 1 Blu -325.2 -232.6 118.7 Map 1
	Move Item
	Update Z
	Go To XY
	Go To XYZ
	Go To Marker
	Load Map
	New Map
	Anchor Map
	Delete Item
	Realign to Item
• •	





### 🙀 gatancustomer - TightVNC Viewer

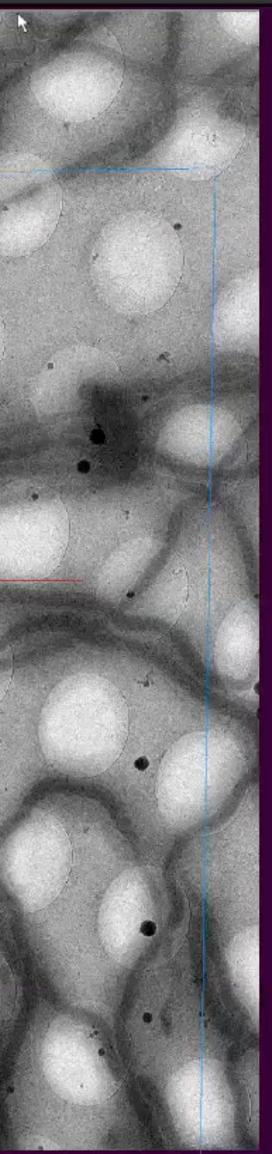
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era Calibration Focus/Tune Script Tasks Tilt-Series Process Navigator Window Help 34.30 e/ A UNSAVED. View Size: 1020 x 1440 bin 4 Tilt 26.99 Stage: 213.71, 79.75 Det -106.92 B: UNSAVED, View C: Saved to File, sec. 29 - F Buffer Controle Copy Active Image to Buffer-A B C D F New SAVEA SeverActive Tofle3-+ Options Memory - 49 MB - F Image Display Controls 860 Blk -- )------Wht -4499 Bri \_\_\_\_\_ → Cross Con \_\_\_\_\_ → □ Invert +| Options Zoom □ \_\_\_\_ + Options - F Roat Dose Mcroscope -0.0000nA 2250× Det -106.9 um IS 0.40 um + IA 48.40 um VAC Spot 6 F Microscope Control Tit Control \_ E 26.99 Up Down + Options - F Camera & Script K3 Setup View Focur Trial Bec End Loop PauseN STOP MRH - Fnd Nev - Targe De - F Image Alignment & Focus Align to P To Merver Reset mage Shift Autolocus - 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 toris Adjust mage shift between mage Trim dark borders in Autoalign Set Autoalign Trim Fraction - F Low Dope Control 2 E Low Dose Made View: 2250x Sp 6 1A 48.40u Continuous updicte (see loolbp) Define position of area C None C Fooles C Trial Position on till acos: 200 um num arca separation: 1.03 um Go to: Vie Fee Tri Ree S -Additional beam shift (and DF tilt)-Set Reset Uncalibrated Offsets for: @ View C Search Defecus: 100 - Shift Set Zen Blanked Unblank ELANK BEAM when screen down inalize configneer langes eep Facus and I not derricel Copy current area settings to -ETRE enter Unshilled Bolonce Shirts Rotate inter-end axis 0 c B - W: 860 - 4499 mean: 2506 - E Montage Controls Start Presce - Options 🤤 🔟 📥 🛬 🗠 🗄 🚿 👪 🇞 2



### Serial EM Demo



DOING 25: FastTomo

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Color Red	- 🔽 Drav	N E Ba	ricle whi	in lood	E For or	chior	stele	
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Set File Prope								
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Add Points	Collapse				Editmode		EditFocus	
Add Polygon					ion in 00:00	_		
Add Marker	225	Red	44.8	138.9 146.0	73.0 Pt 73.0 Pt	1		
Move Item	227	Red	-56.4	177,0	73.0 Pt	1		
1 Indate Z	- 228 229	Red	-63.5	154.6	73.0 Pt 73.0 Pt	1		
Go Ta XY	230 231	Red Red	-66.5 -68.7	151.7	73.0 Pt 73.0 Pt	1		
Go To XrZ	232	Red	-73.6	171.1	73.0 Pt	1		
Go To Marker	233	Red	-79.9	160.4	73.0 Pt 73.0 Pt	1		
Loed Mep	235	Red	-48.8	137,1	73.0 Pt	1		
NewMap	236 237	Red Red	-56.6	148,9 136,8	73.0 Pt 73.0 Pt	1		
Anchor Mao	238	Red	-56.2	123.0	73.0 Pt	1		
Delete hem	239 240	Red	-44.0	168.4	73.0 Pt 72.2 Pt	1		
Benligs to tem	241	Red	-23.1	270.9	72.2 Pt	1		
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	244	Red	-7.8	307.6	72.2 Pt	î.		
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	240	Red	9.6	287.1	72.2 Pt	i.		
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	250	Red	-29.8	265.7	72.2 Pt 72.2 Pt	i.		
	251 252	Red	180.6	-85	84.0 Pt	1		
	253	Red	179,1	-50.2	84.0 Pt 84.0 Pt	1		
	254	Red	149.4	-5.6	84.0 Pt	1		
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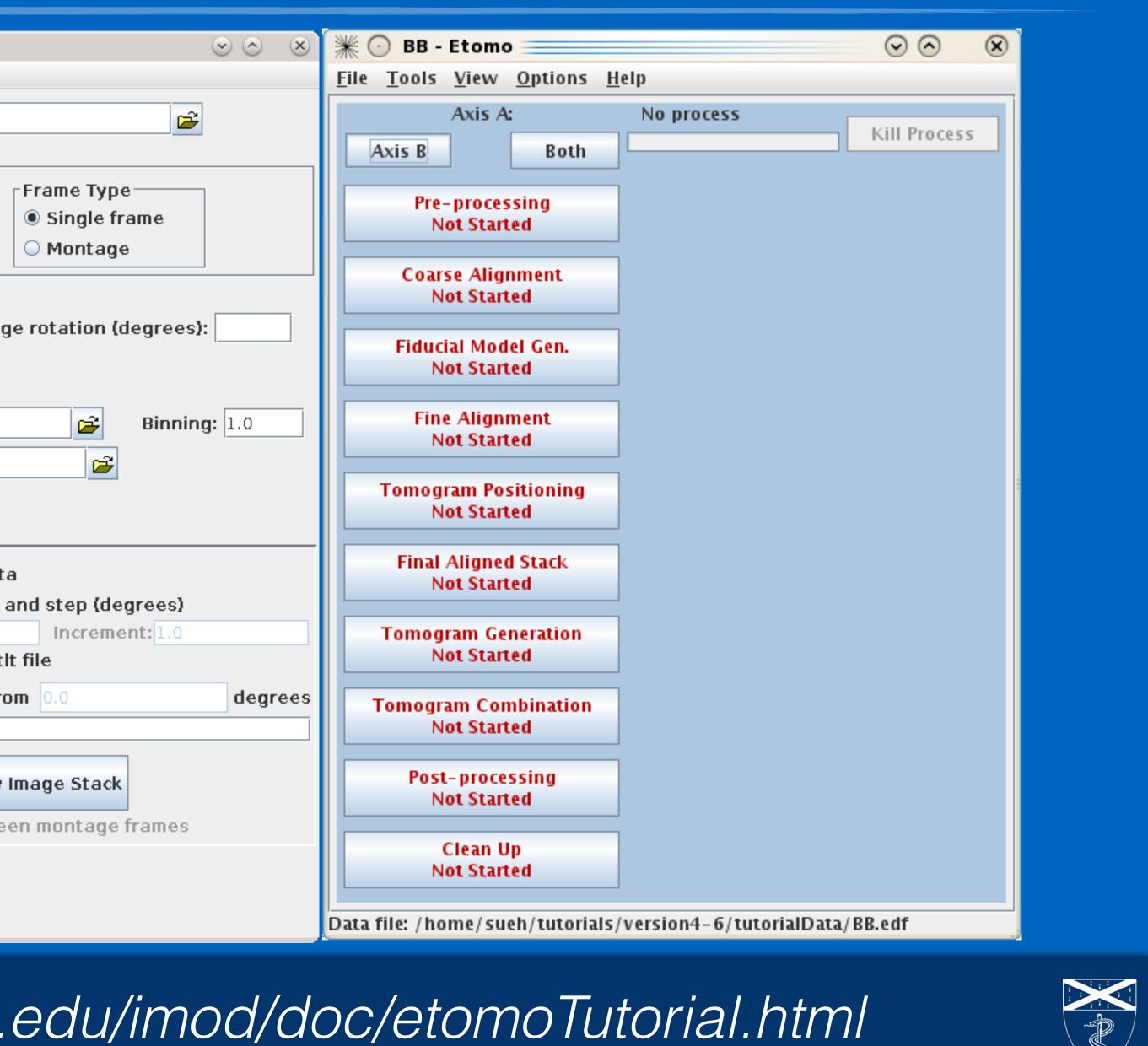
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@junliulab

## Aligning tilt seires (IMOD)



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

### AreTomo -InMrc original\_tilt.mrc -OutMrc aligned\_tilt.mrc

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AreTomo: An integrated software package for automated marker-free, motion-corrected cryo-electron tomographic alignment and reconstruction





## Any questions?





