

# 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

		Indexe 7 served	A REAL PROPERTY AND ADDRESS OF ADDRE
2 4 4	Provident The	1	10 X 12
14 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	11 - 1 - 1 - 1		and all the second s
	A STATE OF A		2019 C
-7.5 - 2, "7	1	한 번 개조들은 문제	and the second states of the
		State State State	5
	WARE Ser - J	and the second second	State of the second sec
The second second	and the set		20
		1-12 11 2 x 1 1 1 1	
10 M	The second s	7	In the second second second
	AND A REAL PROPERTY OF		1
	LL & Church	Wards in the state	A CONTRACTOR OF THE OWNER
1 40 H H H	the share is a share	and the second second	A CONTRACTOR
N. 2 2 3 3 3 10	Tala and a set of the	4 4 4 1 1 1 1 1 1 1 1	11 A A A A A A A A A A A A A A A A A A
the second second	Night I and	the fail of the second s	I FLERE A SALES
	2.19.2	The Las The Las	
5 1 1 1 1 1 1 1	And the second second second	and the statements	56
Charles and the second	1	and a second second second	100 C
the second second		apart - la terre	
The set of the		A state of the state	The second se
a little support	1. 1. 29	the state of the s	All a start and a start and
	10 L	and the second s	and the second second
Service and service		1 1 m m m m	A Contract of the
		and the second second second	A
WAR S E. S WE !!	1 11月期 日第二月一		a the second
The set is a strate to	the second se	11 TR	
		and so that is a set of the	
	and the second second	17 - 1 - 10 - 11 - 1. I	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
ALR.	the second second		The second second second
and the second second	A - Carton - The second	the second second second	the state of the state of the state of the
and the second se			· · · · · · · · · · · · · · · · · · ·
the state of the second		and the second sec	the second second
	-21, 51		The second second
	- 12		A LEAST AND A LEAST A LEAST
F	1. JAN 11 = 2 1 1		
		10	and the second sec
States and States	a the same the same	The second second	The state of the state
		Ser.	the second
19 1 1 1 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		and the second second second	
All in the second	THE A SHE AND A REAL		N 12 1 1 2
All the second second			and the stands
the structure in the state	And a series that a	a) and a final	All and a second second
	and the second second	the state of the s	· 12
En -		1 3 3 A A A A A A A A A A A A A A A A A	A CARLES AND A CARL
CEEP	10. J. T.		2 24 - 2 2 1 1 2
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	and a local set	1	
- 1 al - 17 - 5	1	the second states	
The second second second		a - to a long the second	a general sector of a sector
			· · · · · · · · · · · · · · · · · · ·
The set Bas is in		A MARKED STATE	1 10 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
and the second second			12 U. C. C. C. L.
and the second sec	and the second	A State of Constant	The second second
A CARLER CARLES	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	and the second second second	and the second
ALS C.	N 5- 8 11 12		A
Arrian - Day C. F.	and the second sec	13 31 +	A . All a R. AN
1 10 10 10 10 140		100 m X 2.	
and a series of the	" By the "- again		and the second second
A STATE		1	15 N. F. S. R 18
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	a launa		2 7 7 7 7 7 7 8
	16. 19	ALL FULLY ALL FOR	
T 107	ST THE R ST IN C.		1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1
			and the second sec
100	at an in the state		14 - 14
and the second second	and and	Carrier Martin	
		The second state of the	MALL PARTY MALL
and some it.	and the second and	1 1 1 1 L 1	1
THE PATE DATE	A MIL I WAY		1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
And the second s	The Part Street	ALE ALE A	
And the second sec			the second se
		1 3	1 S
Contration - 114	191		
S. W. There and	State State	Sec. 1883. 3	
	and the second	No.	
		3 2. S. C.	
		3	
		*	
		*	

_ D Low Dose Control ?	
✓ Low Dose Mode	National Laho
View: 2300x Sp 6 C2 61.41%	
Continuous update of mag & beam	K2 Direct Detection 🔷 🗸
C Naca C Facua C Trial	Mode: Counted
I None O Pocus O mai	
Position on tilt exis: 0.00 um	HW Processing
Maximum area separation: -0.71 um	I Background Subtraction
Additional beam shift	Gain Correction
☐ Set Reset 0.00, 0.00	Update HW Dark Reference
Area to show when screen down	Health Statur
O Vie. O Foc. O Tri. O Rec. O Sea.	
E BLANK BEAM when screen down	Camera View 🗸 🗸
OptionsOptionsOptions	Setup: Search 💌
Offsets for View	Auto Exposure
Defocus: -60 - Shift Set Zero	
Normalize beam through View	Exposure (s) [0.5
Keep Focus and Trial identical	Start View
Copy current area mag & beam to	Focus Loupe
V F T R	Auto Survey
Center Unshifted Balance Shifts	Camera Inserted
	*
E Rotate inter-area axis V deg	Camera Acquire 🔷 🗸
🔜 Navigator	
Label: 1 🗖 Registration po	int 1 📑 🗖 Comer point (C)
Color Blue	Rotate when load For anchor s
#1 Note: Sec 0 - montage01	l et
F Assuing (A) E Till series E bl	
Acquire (A) To Tilt series TON	ew file at item
Acquire map or image or run macro	at this location automatically
Add Stage Pos Registration 1	Draw all reg. Draw none
Add Points Collapse groups	Show Acquire area
Add Polygon Label Color 2	X Y Z Type Reg. Acq. N
Add Marker 1 Blu -32	5.2 -232.6 118.7 Map 1
Move Item	





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



## Defocus Magnification Stage tilt angle





@junliulab

vid Mastro	narde V
	<ul> <li>Low Dose Control</li> <li>Low Dose Mode</li> <li>View: 2300x Sp 6 C2 61.41%</li> <li>Continuous update of mag &amp; beam</li> <li>Define position of area</li> <li>None</li></ul>
	Blanked       Unblank       Search         _Options       Offsets for View         Defocus:       -60 ÷       Shift       Set       Zero         Normalize beam through View       ✓       Auto Exposure         ✓       F       T       R         Center Unshifted       Balance Shifts       ✓       Camera View
	Image: Protected interval exacts       Image: Protected interval exacts <td< th=""></td<>
	Add Stage Pos       Registration       1       Image or run made of at this location automatically         Add Stage Pos       Registration       1       Image or run made of at this location automatically         Add Points       Image or run made of at this location automatically       Image or run made of at this location automatically         Add Points       Image or run made of at this location automatically       Image or run made of at this location automatically         Add Points       Image or run made of at this location automatically       Image or run made of at this location automatically         Add Points       Image or run made of at this location automatically       Image or run made of at this location automatically         Add Points       Image or run made of at this location automatically       Image of at this location automatically         Add Points       Image or run made of at this location automatically       Image of at this location automatically         Add Points       Image of at this location automatically       Image of at this location automatically         Add Polygon       Label       Color       X       Y       Z       Type Reg. Act         Add Marker       Image of at this location automatically         Add Polygon       Label       Color





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

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 Irial	House, Counted
Position on tilt axis: 0.00 um	HW Processing
Maximum area separation: -0.71 um	Gain Correction
Additional beam shift	Undate HIV Dark Reference
O Vie.	🖯 Health Status 😤
BLANK BEAM when screen down	Camera View 🔻 🗙
_Options Unblank Search	Setup: Search 💌
Offsets for View	Auto Exposure
Defocus: -60 Shift Set Zero	Evoques (a) 0.5
Normalize beam through View	
Keep Focus and Trial identical	Start View
Copy current area mag & beam to	Focus Loupe
	Auto Survey
Center Unshifted Balance Shifts	
Rotate inter-area axis 0 deg	Camera Acquire 🔹 🗙
🔜 Navigator	
Label: 1 E Registration poi	oint 1 📑 🗖 Comer point (C)
Color Blue 🔽 🔽 Draw 🗆	Rotate when load T For anchor st
#1 Note: Sec 0 - montage01	1.st
🗖 Acquire (A) 🗖 Tilt series 🗖 🕅	New file at item 🛛 🗖 New file at gro
Set File Properties Imaging State	at this location automatically
Add Stage Pos Registration 1	Draw all reg. Draw none
Add Points Collapse groups	os □ 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











### 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

### PrepMMM-n 2 Corner point (C) Isad 🛛 🗖 For enciror state autorun Gycle TargetDe □ New file at group PrepMMMlenome | 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
οZ
1
focus
z
_
н
<u>۹</u>
lign-FR
mo
est
leCell
align2
-
-
_

Scripts

= 🗆 X

mple2.nav





×	ĺ
Z	ſ
	ĺ
ocus	1
	i
	i
	ĺ
í	i
	Ì
	i
FD	
gin n	
0	
st.	
Cell	ļ
lign2	
	ĺ
	Ì
	Ì
	i
	1
-	ļ

8					
File	Settings	Camera	Calibration	Focus/Tu	ne
	F	R.Mar S	sha	211	-
	T-Minor		200000		
- tue	FI	Biffer	Childrenion	21	ine.
	A: Empty			~	
Siz	e:		Tilt		
Sta	ge.		Def.		
	Empty :				
1.1	F	Buffer C	ortrois	21	
1	F	mage Diepk	ey Controle	21	
Blk	1			_	
Whi	1.101	111			
Bn	a statu		- Gros	is. A	
List		7	iom 1	-	
-	Dolions at Tauncat	ion L Ba	dana Fract	-	
	Extra info		Autozoom	ion	
₩.	Antialias f	Itering for	700m < 1		
1 ±	F Foat	Dose	Moroscope	21	
+	F	Marascepe	Control	2	
	-	Tilt	( BOI	-	
-6	88.88	.Qp:	Dawn	0	
+	Cotions .			21	
	- Carren	Econor	Trail Br	med	
		1 10000			
Pre-	view Se	arch B	esune ST	OP I	
So	1011	Solpt 2	Soript 3	-	
-	F In	age Aignm	ent & Focus	2	
A	lighte D	TOM	arker Clé	ser i	
B	esetimao	e Shift	Autoriace	15	
+	Options	Def. ta	rget = 0.00 u	m	
-	F	Loss Dose	Control	2	
<b> </b>	Low Dose	Mode			
E C	Continuou	s update	(see too tip)		
C	efine pos	ition of an	ea		
0	F None	C Fagu	s 🦿 (ria	- 1. <b> </b>	
Pre	ition pri fil	6/15	0.00 Um		
Ga	a the I	and La	a lea la	Saul	
	dditional	hearnahi	in Nice	1994	
F	Sel 1	tesat			
of	Sets for	* View	C Search		
Def	ocus:	÷ Shi	t Sel Z	eru -	
		Bire	+ 1		
-	Ciptions	Diar	K		
-	BLANK BE	condens	n screen dor ter lenses	NTI I	
1	Keep For	us and Tr	ialidentical	_	
- C	opy curre	nt area s	etings to		
	V. F		<u> </u>		
Ce	nter unsh	itte c	La ance Shi	27	
<b></b>	Bolote inte	er area a	4is 0	363	
+	F	Montage Resc0	Controla	21	
	ETEMM	bole OF	F Ceine?	100	
<b>F</b> _ (	itier (slit e	) Slitw	idth 10	-	
F 2	Cero Losà	L	0.0	5-1 I	
Adi	ustmente	0.0	Net@%set	0.0	

4 Options

Ready

- Options

Script Tasks Tilt Series Process Navigator Window Help

ript Tasks TiltSeries Process Navigator Window Help

DUMMY SenalEM

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

¢.		Open		x
Look in Pecert places Desktop Lbranes	Name	-	Cute modified	Type NAV File
This PC	< Ris name: Riss of type:	Bi sampie2 nev Neugetorfiles (* nev)	•	> Open Cancel

# screenshot fi

Ŀ

61

🏂 🕰 🗟

10

2

abel 20

- 0 X

Navigator: sample2.nav	= 🗆 X	Scripts
egistration point 1 🕂 🗆 Corner point (C)	2	PrepMMM no2
Draw CRotate when Izad C For anchor state		autorun
		OycleTergetDelocus
a State   TS Porems   Floridam   Focus Ros		Piepkovikiez.
ation I - Draw F Allreg, F None F Label	-	WyEutos
apsa - E Show Acquire E Editmode E Edit F	icus	Script6
of 17 Done; Estimated completion in 01:53:26	hala	sutorun-mubi
Red 542.5 576.5 1000 Pt 1 Red 564.3 -185.6 1000 Pt 1 Off -266.9 75.2 100.0 Pt 1	bad	a.foiuh-vpp
Off -232.9 195.6 100.0 Pt 1 Off -113.6 158.7 100.0 Pt 1		SimpleFools
E Na	vigator	- 0
Labet Pegistratum point - 👘 🛙	Comergion (C)	7
Color Red P Draw E Rolata when is	ad 🗍 For enchor state	
Note.		
Set Fla Propa [Imaging Store] TS Porces [Fil	enome Encus Pha	
Add Stage Pos Registration 1 Draw T Al	Ireg T None T Label	8
Add Points   Collapse   Show Acquire	Editmode Edite	jous.
Add Polygon Label Color X Y	Z Type Reg. Acq. No	ste
Mave Rem		
Update Z		
GoTaXY		
Go To XYZ		
Load Map		
New Map		
Assher Mep		
Delete hem		
Healignanitem		
<		
3 microns c		×.
ating existing		>
X\DoseFractions\20210613\Jun\Sanoke2\Jun14_11.	14.00.tf	
ference by 20 20	_	
	~	



AM 2021

# 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





### Etomo interface



000	X	Project Log	
<u>F</u> ile <u>V</u> iew			
Project Log			
	V Setup T	omogram - Etomo	7/3
Tools View Ontions Help		Shiografii - Etolito	
Dataset name: 25/STfiles/temp_for	r_worksho/Borrelia.st 🖻 👅	Packup directory:	
nnlates			
na tamplata. Nana availabla			-Esomo Tuno
pe cempiace. Indire available		A Single avie	Gingle from a
stem template: No selection (2 a	vailable)		
er template: No selection (5 avai	lable) 🔽		Montage
Scan Header Divel size	(nm): 0.2747 Eidu	cial diameter (nm): 10	 Image rotation (degree
Scall freader			
Darallel Drocessing 3	e 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 rawtit file
Foriac was bidiractional from 20	dagray	n 🗆 Sarias was bidiras	tional from 0.0
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	Advanced
lata set loaded			
			/ 5

5):	175.3
	degrees

# Pre-processing



relia - Etomo
done Kill Process
placement
:8.
2
ce criterion: 19.
ays (Trial de) 2
nual Pixel A A Replacement
k View Fixed Stack Use Fixed Stack
Iax 3 Ick 3 Fixed Stack 4
Postpone     Done     Advanced       np for worksho/Borrelia.edf     5
np_ror_worksho/Borrella.edr V 3



# 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	) 25/STfiles/temp_for_worksho
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>File Tools y</u>
Pre-pr
Сог
Coarse
Con
Fiducial
Con
Eine A
In Pr
Tomogram
Not 9
Final Ali
Not 9
T
i omogran Not 9
NOL .
Post-o
Not
Cle
Not 9

Data file: ...ANG

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

ew	Options	Help
		<u> </u>

•	<u> </u>
	Aligning stack
ocessing	done Kill Pro
piete	rFine Alignment
Alianment	Tiltalign Parameters
plete	General Global Variables Local Variables
	List of views to exclude:
Aodel Gen.	Separate view groupe: 1.20
piere	Separate view groups. 1-20
ignment	Residual Reporting
ogress	Threshold for residual report: 3.0
Recitioning	All views
tarted	Neighboring views
ned Stack	Analysis of Surface Angles
laneu	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	Tilt axis z shift: 0.0
an Up	Minimization Parameters
started	Do robust fitting with tuning factor: 1.0 Eind weights for contours, not poir
	-Local Alignment Decemptors
	El Casal alignment Parameters
	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





### 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	<u>T</u> ools	⊻iew
	Pre-	proces
	C	omplet
	Coars	e Align
	C	omplet
	Fiduaia	Mada
	Fiducia	omplet
		-
	Fine	Alignn
		unhier
-	Fomogra	am Pos
	Not	t Start
	Final A	linned
	Co	omplet
	Fomogra Cr	am Gen amnlet:
	Post-	proces
	C	omplet
	С	lean U
	No	t Start

### 🗙 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>F</u> ile <u>T</u> ools <u>V</u> iew <u>Options</u> <u>H</u> elp						
	Trimming volume					
Pre-processing	done Kill Process					
Complete Post Processing						
Coarse Alignment	Coarse Alignment Trim vol Flatten Squeeze vol					
Complete	Complete					
Fiducial Model Cap	Get XYZ Volume					
Complete	3dmod Full Volume Range From 3dmod					
	Volume Range					
Fine Alignment	X min: 1 X max: 960					
Compiere	Y min: 1 Y max: 928					
Tomogram Positioning	Z min: 1 Z max: 450					
Not Started	Cooling					
Final Aligned Stack	Scaling					
Complete						
	Scale to match contrast black: white:					
Complete	Find scaling from sections Z min: 150 Z max: 300					
· · · · · · · · · · · · · · · · · · ·	Scaling from sub-area:					
Post-processing	X min: X max:					
Compiere	Y min: Y max:					
Clean Up						
Not Started	Get XYZ Sub-Area					
	From 3dmod					
	Reorientation: Warning:					
	○ None For serial joins, use					
	Swap Y and Z dimensions method for each					
	Rotate around X axis section.					
	Trim Volume 3dmod Trimmed					
Volume						
Cancel Postpone Done						
Data file: /data/disk4/CHNAG/temp-IMOD-workshop/Borrelia.edf						





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







IVERSITY



	e	
	File Settings Camera Calibration Foot	us/Tu
	_ F Buffer Status _2	
	Q: Montage Overview	
	Size: 13216x18026 bin 2 Tilt 0.00 Stage: 0.00 0.00 Dot: 3.95	
	A: Montage Center	
	B: Montage Overview	
I	- F Buffer Controle ?	
	SAVEA SevelAdive To file 2	
	+  Options Memory - 528 MB	
	Pite Capta Capta Corecte	
	What 254	
	Bri Cross	
	Con Invert	
	+ Options Zoom 0.09	
Ĩ	E Boat Dose Mcroscope 2	
	-0.0000 nA 64000 X	
	Det -4.06 um IS 0.29 um	
	+   A 1.75 um VAC mPr 5	
	E Microscope Control _2	
	- F Tit Control 2	
	2.00 Up Down To	
	+ Optione	
	- E Camera & Soipt K3 2	
	Setup View Focus Trial Record	
	Preview Search Resure STOP	
	spMMH-n + sutoun + TargetDe	
-	- E Image Alignment & Focus 2	
	Align to P To Marker Clear	
	Resetimons Shift Autobaus	
	Duf tarrat = 4 00 um	
	Move stage for big mouse shifts	
	Set Threshold Shift	
	Correct backlash in stage moves	
	Center image shift on tilt axis	
	Adust mage shiftbetween mage     Tem dark borders in Autoation	
	Set Autoalion Trim Fraction	
1	= F Low Does Control 2	
	F Low Dose Mode	
	Record: 640Kx: nP.5-IA 1.750	
	<ul> <li>Continuous update (see tooltp)</li> <li>Define position of area</li> </ul>	
	@ None C Focus C Trial	
	Positon on tillacos: -200 um	
	Maximum erce accention: 1,03 um	
	Golo. Vie. Foc. Tr. Rec. Sea.	
	Additional beam shift (and DF tilt)-	
	Reset Uncalibrated	
	Offsets for: View C Search -	
	Delecust 100 - Shit Str. 2010	
	- Options Unblank	
	ELANK BEAM when screen down	
	Normalize condenser lenses	
	<ul> <li>Keep Focus and Tinal identical</li> <li>Copy current area settings to</li> </ul>	
	VFTRS	
	Center Unshitted Balance Shifts	
	■ Rotate inter-energia axis 0 ceng	
Î	F_ Montage Cantrole	
	Start Prascen 7	
ſ	- Options Current 2	
μ		



	Navigator: G2055.nav	- 0	x	Scripts ×
Label 1	Registration point 1 - Conver point (C)			PrepMMM-noZ
Color Blue -	Draw E Rotate when load E For anchor state			autorun
#1 Note So	ec 0 - 175k-borrelia-20210613-grid1.st			CycleTargetDefocus
E Acquire (A) E T Set Ella Propal Line	it series F New Heletitem F New Heletigroup adra State   TS Personal Filenome   Facus Pacif			PrepMMM-Z
Add Stage Pos Pos	gistration 1 - Draw: C Allreo, C None, E Labels			MyFunes
Add Points P	Collapse T Show Acquire T Editmode T EditFocus			Script 6
Add Polygon	Label Color X Y Z Type Reg. Acq. Note	175k hores	10.2	autorun-multi
Moveltem	Group of 1 items, ID2532, labels 2 to 2 Group of 2 items, ID6772, labels 3 to 4	Tox bolle	-	autorun-vpp
Update Z	11 Red 16.5 -100.6 78.1 Pt 1 hole 4.A Blu 67.4 139.1 78.1 Map 1 Sec 0 -	2250c.st		SimpleFocus
Go To XY Go To XYZ	Group of 6 items, ID5271, labels 13 to 18 13-A Blu -53.2 147.1 73.0 Map 1 Sec 1-	2250c.st-		Z-check-refil-realign-FR
Go To Marker	14-A Blu 33.2 274.7 72.2 Map 1 Sec 2- 15-A Blu 49.2 5.9 80.4 Map 1 Sec 3- 15-A Blu 1305 23.0 84.0 Map 1 Sec 3-	2250c.st - 2250c.st -		short-DSTomo
Load Map	17-A Blu 185.9 106.0 83.6 Map 1 Sec 5- 18-A Blu 209.1 231.7 81.3 Map 1 Sec 5-	2250c.st-		FastTomo-test
Andhor Map	Group of 1 items. ID6599. labels 25 to 25 Group of 26 items. ID6385. labels 26 to 51			FastTomoWholeCell
Delete Item	Group of 6 items, ID4795, labels 52 to 57 Group of 17 items, ID1201, labels 58 to 74			Z
Realign to item	Group of 36 items. ID			Z-check-refil-realign2
	Group of 30 items, ID			Script 15
	Group of 15 items. ID 4758. labels 220 to 234 Group of 5 items. ID 4192, labels 235 to 239			Sedet 17
	Group of 11 items, ID 1474, Tabels 240 to 250 Group of 4 items, ID 6935, Tabels 251 to 254			Senal 18
	Group of 4 items. ID8252. labels 255 to 258			Script 15
				Script 20
				Sciet21
			÷	Stried 27.
	<[			Seciet 27
E	Log: log1.log	- 0	x	Scort 24
Error above tolerance 15 frames were sover	, retaking shot d to X\DoseFractions\20210613\Jun\G205SC\Jun14_10.13.43.tf		~	EastTomo
Served Z = 22, -33.00 c 15 frames were served	lagrees d to X\DcseFractions\20210613\Jun\G205SC\Jun14_10.13.47.tif			OpenEda
Saved Z = 23, -36.00 c 15 frames were saved	legrees a to X1/DoseFractions1/202106131,Jun1G2053C1,Jun14_10.1352.tf			CiccoFile
Saved Z = 24, -39.00 c Error above tolerance	legrees , retaking shot Sta XVD see Frantisco 2001/00/10/10/10/10/00/00/10/00/10/00/10/00/0			Seciet 22
Saved Z = 25, -39.00 c	ato A. (Jose Fractions)/20210613/, Juni(52055C) Juni4_10.13.56.ff legrees i to X1Dose Fractions)/20210613/, Juni/62055C3, Juni4_10.14.01.64			Could
Saved Z = 26, -42.00 c	legrees it to X\DoseFractions\20210613\Jun\G205SC\Jun14_10.14.07+f			Souther State
Saved Z = 27, -45.00 c Error above tolerance	legrees , retaking shot			Sugto
15 frames were saved Served Z = 28, -45.00 c	d to X\DoseFractions\20210613\Jun\G205SC\Jun14_10.14.11.tf degrees			
15 frames were saved Saved Z = 29, -48.00 d	to X:\DoseFractions\20210613Jun\G205SC\Jun14_10.14 15.tf legrees			
Saved Z = 30, 26.99 d	tio X (Dese Fractions)20210613(Jun)C2053C(Jun)4_10.1459(tf legrees i to X1Dese Fractions)20210613(Jun)C2058C(Jun)4_10.15.0244			
Saved Z = 31, 29.99 d	legrees it to X1DeseFractions/2021061%, het/G200303, het 14, 10,15,12.44			
Saved Z = 32, 32.99 d	legrees i to X1DeseFractione/20210612/ he/02056/21 he/14 10 15 17 64			
Seved Z = 33, 36.00 d	legrees			
Saved Z = 34, 39.00 d	egrees			
15 frames were saved Served Z = 35, 42.00 d	d to X1DoseFractionsl20210613(Jun)G205SC\Jun14_10.1527.tf legrees			
15 frames were saved Saved Z = 36, 45.00 d	to X:\DoseFractions\20210613\Jun\G205SC\Jun14_10.1532.tf legrees			
15 frames were saved Saved Z = 37, 48.00 d	d to X\DeseFractions\20210613\Jun\G205SC\Jun14_10.1537.tf legrees			
TS time: 327.25	netric TS			
total time: 327.25			>	
<				

