1. Setup a Leginon Session

- **A.** Go to the microscope computer. Under the "Autoloader" tab, make sure the cross-grating is loaded (usually position 1 in the cassette). If it is not do the following:
- Make sure the column valves • are closed ("Col Valves Closed" button should be yellow)
- Make sure the objective is out. • Go to the "AAM" tab or select "Apertures" on the right side menu ("Objective" button should be gray).
- Select "insert screen" in the • TEM User Interface to make sure the screen is inserted, not retracted.
- Click the number position the cross-grating is in and click the "Load" button.



For NCCAT:

- Startup the NCCAT Leginon Client on desktop of Microscope Computer
- On the Leginon computer open a terminal and type in: nccatleginon
- if the user is not there in the • terminal type in: /beegfs/sw/bin/change_user.s h <username>; nccatleginon

For EMG:

- Startup the **EMG Leginon** Client on the desktop of Microscope Computer
- On the Leginon computer open a terminal and type in: betaleginon
- if the user is not there in the terminal type in: /gpfs/sw/bin/change user.sh <username>; betaleginon





C. Select "Create a new session", then click "Next>"



D. Select a project, then click "Next>". If there is no project listed, please submit a project here for NCCAT <u>https://nccatdeon.nysbc.org</u> OR here for EMG <u>https://deon.nysbc.org</u>

~~~	Leginon Setup		×
LEGINON	Select the project this session will be associated with, Project: SEMC - Glacios Testing then press the "Next" button to continue.	·	

 E. Please do not change the given session name. For holder, choose "Glacios-X1",then click "Next>".



F. Enter a description of your session (ex: Glacios – ApoF ), then click "Next>".

					L	eginon		-	• ×	
File	Application	Launcher	Node	Events	Others	Help				
					Legi	ion Setup		_		
P		A				Please enter an optior	al description.			
						Description:				
	•									
	GIN	014				Then press the "Next"	button to continue.			
LE										
		-		-	-	_	< Back	vext >	Canc	:e

G. If the user is on NCCAT, make sure the directory is /beegfs/leginon/username. If the user is on EMG, make sure the directory is /gpfs/leginon/username, then click "Next>"

					Le	ginon		-		×
File	Application	Launcher	Node	Events	Others	Help				
					Legin	on Setup				
	$\sim$									
\$		A								
1				Sele (A si	ct the dir ubdirector	ectory where image ry named after the	es from this see session will be	sion will be st created for yo	ored. u)	
		$\leq$		Imag	ge Directo	ry: /beegfs/legino	on/ldap/	Browse		
/				The	n press th	e "Next" button to	continue.			
c	GIN	04								
-										
							Death			<i>c</i>
							< Back	Next >		Canc

 H. Click "Edit". Select "glacios-9953129" from the dropdown menu, then click + sign to add it. Click "OK", then click "Next>".



I. Check that the C2 size is set to 70, then click "Finish".



- L. In the KPresets_Manager
   Node, click the load preset icon
  - TEM = "Glacios"
  - Digital Camera = "Falcon3"
- **M.** Click "**Find**". Select the most recent session. Select all preset (gr, sq, hl, fan, fcn, enn, and tune), then click "**Import**".

		Import Prese	te.	
		import Prese	G	
Instrument TEM Digital Camera	Glacios Falcon3		Preset Parameters     TEM:     Magnification:     Defocus:     Random Defocus Range:	Digital Camera: Energy filtered: Energy filter width:
Session         Ler           n20nov17c         3.8           n20nov09g         17.           n20nov09f         0.4           n20nov09b         3.7           n20nov04b         24.	ng User Mahira Aragon 8 Anjelique Sawh Anjelique Sawh Anjelique Sawh 9 Mahira Aragon		Spot size: Intensity; Image shift: Beam shift: Diffraction shift: Energy filtered: Energy filter width: Skip when cycling: Preset	Dimension: Offset: Binning: Exposure fime (ms Pre-Exposure (s): Dose (e/A^2): Save raw frames:
		Limit sessions to last 20 days	gr sq Find fan	Import Done
		Ener	rgy filter width: Save raw frame	5:

# 2. Saving Vacuum and Carbon Location

			Leginon: n20nov25b			_ 0	×
File Application Launcher	Node Events Other	Help					
	2 ·						
KPresets_Manager Beam_Tit_Image Grid_Targeting Grid	Levi Time Me (1) 10:41:21 AM Nav (1) 10:41:21 AM Pre	ssage igation unlocking set changed to "sq"				_	j
Square Targeting     Square     Square     Square     Square     Prote     Proteix     Proteix     Proteix     Spontracting     Proteix     Spontracting     Proteix     Proteix	Presets (Cycle Order) 9 59 59 59 50 50 50 50 50 50 50 50 50 50 50 50 50	Most Recent Calibrations Most Recent Calibrations Press State Modeled stage Modeled stage (may on Presst Parameters Presst Parameters Press	V) Glacios 2010 - 0.0002 None 5 1.0658877528 (1.2596e-07, 1.2144e-06) (0, 0) No None No	2020-0 2002-0 None x: None Digital Camera: Energy filtered: Energy filtered: Energy filtered: Energy filtered: Binning: Pro-Exposure (ns) Pre-Exposure (s): Dose (eA/2; Save raw frames:	5-15 24-54-16 5-11 10:21:57 3-13 16:22:37 y; None y; None 10:24 x:10:24 10:24 x:10:24 10:24 x:10:24 0,0 0,0 4 x 4 200.0 0,0 N/A No		

- B. On the Microscope computer, lower the screen (hand panel R1 button) Try to move to an empty square using the stage track ball on the hand panel.
- C. Under the "Search" tab type in the label vacuum or empty, then click "Add". Repeat this for a carbon square, label it carbon.



## 3. Eucentric Height/Focus

- A. Must be on carbon area. If not, on the Microscope computer under the "Search" tab, select the label for the carbon square, then click "Go".
- B. Go to the Z_Focus Node, then click the simulate target icon



- C. Select the manual focus icon to verify zero focus.
- Type in 0 m then click M to set the defocus to 0 m.
- If the FFT is not zero at this stage, click and hover your mouse over the first zero to take note of the value. Left click on the first zero, then use + or – buttons next to 'Defocus' to increase or decrease the focus respectively. Repeat until you are at focus, as in the image on the right.
- Once at zero defocus, click the reset defocus icon . Then click to close the window.



## 4. Beam tilt pivot point and rotation centering

- A. Must be on carbon area. If not, on the Microscope computer under the "Search" tab, select the label for the carbon square and click "Go". Must be at Z height. If not refer to step 3.
- B. Go to the *KPresets_ Manager* Node, select the *tune* preset and click .



C. Put the screen down (R1 button on hand panel). On the Microscope computer, under the "Tune" tab, in "Direct Alignments" check pivot point X, pivot point Y, rotation center, and beam shift to center beam.



# 5. Objective Centering

- A. Must be on carbon area. If not, on the Microscope computer under the "Search" tab, select the label for the carbon square and click "Go". Must be at Z height. If not refer to step 3.
- B. Go to the KPresets_Manager Node, select the tune preset and click the send to scope icon M.



 C. On the Microscope computer, lower the screen by pressing R1 button on the hand panel. Next click the "Diffraction" button on the hand panel.



- D. Under the "AAM" tab insert the objective, by clicking "Objective" button (will turn yellow)
- To adjust click "Adjust" button (will turn yellow), use MF X & MF Y on the hand panels to center the objective, when done click "Adjust" button again (will turn gray).
- Make sure to click the "Diffraction" button on the hand panel to exit diffraction mode.



# 6. Coma Correction

- A. Must be on carbon area. If not, on the Microscope computer under the "Search" tab, select the label for the carbon square and click "Go". Must be at Z height. If not refer to step 3.
- B. Go to the *Beam_Tilt_Image* Node and select the simulate targe icon

. Select select next to "**Tableau**" to view the Tableau Image.



C. To adjust select the crosshairs and the cursor , click toward the more defocused image until the top and bottom images are symmetric and the left and right images are symmetric.



#### 7. Leginon Gain Reference

- A. Must be on vacuum area. If not, on the Microscope computer under the "Search" tab, select the label for the vacuum square, then click "Go".
- B. Go to the *KPresets_Manager* Node, select the *enn* preset, then click .



- C. In the Correction Node, select the settings icon[™] and input the following information:
- TEM = "Glacios"
- Camera = "Falcon3"
- Select the dimensions "4096 x 4069 bin 1"
- Exposure time = **1000** ms
- Exposure time per Frame = **40** ms
- Images to combine = 10
- Click "OK"
- D. Select "Dark" and "Both Channels", then click the camera icon
- E. Once it is done select
  "Bright" and "Both
  Channels", then click
- F. Once it is completed select
   "Corrected" and "Both
   Channels", then click .

File Application Launche	Node Events	Correction Settin	ngs ×	
File Application Launche Presets_Manager Beam_Titt_Image Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid Grid	Node Events Dark Dark I Message Plan Edit 0 Bad rows 0 Bad columns 0 Bad potets Despike: Off Clear Bad Pixe Grab From Im	Correction Setti Image Correction Instrument TEM Glacios • Digital Carrera Falcon3 • Carrera Configuration 4096 x 4096 bin • Custom Dimension: 4096 x 4096 Offset: (0, 0) Binning: 1 × 1 Exposure time: 1000 ms Carrera with Movie Mode Save frames Exposure time per Frame: 40 ms Readout delay: 0 ms Readout delay: 0 ms Dimension: 1000 ms	gg x Reference Creation Images to combine: 3 Combine method: averag v Save all images	
		Frame-Aligning Camera Only Align frames C-correlation filter: None	OK Cancel Apply	



#### 8. New Dose for exposure

- A. Must be on vacuum area. If not, on the Microscope computer under the "Search" tab, select the label for the vacuum square, then click "Go".
- B. Go to the KPresets_ Manager Node Select the enn preset and send to scope ➡.
- **C.** On the Microscope computer, lower the screen (hand panel **R1** button) to confirm that the beam is present and centered.

KPresets_Manager	Levi Ti	ime Messi	ige				
Beam_Tilt_Image	3 11:53:12 /	AM [beep]					
Grid_Targeting	11:53:12	AM Cycle	ompleted				
Grid				_			_
Square_rargeting							
Hole_Targeting	Presets (Cycle	Order)	Most Recent Calibrations				
Hole	or	1	Pixel size		2020-0	7-16 15:20:24	
Preview	5	4	Image shift		2020-0	7-16 15:20:24	
Z_Focus	blo		Stage		2020-0	7-16 15:20:24	
Exposure_Targeting	101	<b>M</b>	Beam shift Modeled stage		None v: None	ur blong	
GExposure	fan		Modeled stage (mag. on	v)	x: None	v: None	
Exposure FET	fcn	6	Beam tilt - Defocus	.,,	2020-0	-16 15:20:25	
Beam_Tilt	enn	•	Dracat Daramatarr				
Drift_Monitor	tune	41	TEM-	Glacion	Digital Camera:	Ealcon3	
Buffer_Cycling		-	Magnification	120000	Longital Camera.	racons	
N2_Filling		98	Pagnincation.	220000	Farmer filment		
Correction 🔥		<b>B</b>	Derocus:	-3e-06	Energy filtered:	NO	
arget_Adjustment		2	Random Derocus Range:	None	Energy fitter width:	None	
lceT		•	Spot size:	2	Dimension:	4036 X 4036	
<ul> <li>Navigation</li> </ul>			intensity:	0.448	Offset:	(0, 0)	
-			image snift:	(0, 0)	Binning:	1×1	
			Beam shift:	(0, 0)	Exposure time (ms):	1000.0	
			Diffraction shift:	(0, 0)	Pre-Exposure (s):	0.0	
			Energy filtered:	No	Dose (e/A^2):	64.40	
			Energy filter width:	None	Save raw frames:	Yes	
			Skip when cycling:	No			

D. Select the camera icon ■ to take a dose (~65 e/A^2), then click "YES".



9. Ice Thickness

- A. Must be on vacuum area. If not, on the Microscope computer under the "Search" tab, select the label for the vacuum square, then click "Go".
- B. Go to the *Exposure* Node and click the simulate target icon
  OR go to the *Navigation* Node, select the *enn* preset, click and then click to take an image. After the image is taken, note down the "Mean" value.

File Application Launcher Node Events Others Help Presets-Magger end, Trageting end, Trageting end, Trageting end of the stage of		Leginon: n20nov25b	-	•	×
Image: Separation of the second se	File Application Launcher	Node Events Others Help			
RPrests.Navgr Gold. Targeting Gold.     9:50:57 AM Bone with simulated target, status: ok (repeat will not be honored)       Gold.     9:50:57 AM processing processing complete       Sigure     9:50:57 AM processing processing processing complete       Sigure     9:50:57 AM processing procesing procesing processing processing processing processing processin		☆ > = = ● % ② D 型			
	KPresets_Manager Beam. Titt_Image Grid, Targeting Square O Hole. Targeting Hole Prevew Z_Focus Exposure_Targeting Exposure_Targeting Exposure_FT Beam. Titt Durit_Monitor Buffer_Cycling Correction Correction Surget_Adjustment Scope_Control Market Nz_Falling	Image       Image <td< th=""><th></th><th></th><th></th></td<>			

C. Go to *lce_T* Node and select the setting icon . Enter the "Mean" value in the space next to "Vacuum intensity for exposure images". Click "OK".



- A. In the *KPresets_Manager* Node, make sure that *fan, fcn* and *enn* presets have:
  - Image shift: (0,0)

File Application Launcher Node Events Others Help

- Beam shift: (0,0)
- B. Must be on carbon area. If not, on the Microscope computer under the "Search" tab, select the label for the carbon square and click "Go". Must be at Z height. If not refer to step 3.

 C. Go to the Navigation Node. Select the sq OR hln preset then click . Select "stage position", then click . Select + and i to center

over a feature.

KPresets_Manager	Leve Time	Message					
Beam_Tilt_Image	11:53:12 AM	[beep]					
Grid_Targeting	D 11-53-12 AM	Cycle completed					
Grid	ALL DOLLE PAPE	afere compressed	_				
square_rargeting							
A Hole, Targeting	Presets (Cycle Orde	er) Most Recent Calibration					
Hole	ar	t Pixel size		2020-0	7-16 15:20:24		
Preview	gi gi	Image shift		2020-0	7-16 15:20:24		
Z_Focus	sq	Stage		2020-0	7-16 15:20:24		
Exposure_Targeting	hln	Beam shift		None			
GExposure	fan	Modeled stage		x: None	, y: None		
Focus	fcn	Modeled stage (mag. or	ily)	x: None	, y: None		
Exposure_FFT	enn	Beam tilt - Defocus		2020-0	/-16 15:20:25		
🖻 Beam_Tilt	tune	Preset Parameters					
Drift_Monitor	conc.	TEM:	Glacios	Digital Camera:	Falcon3		
Buffer_Cycling		Magnification:	120000				
Correction		Defocus:	-3e-06	Energy filtered:	No		
Tarnet Adjustment		Random Defocus Range	None	Energy filter width:	None		
Scope_Control		Spot size:	2	Dimension:	4096 x 4096		
IceT		Intensity	0.448	Offset	(0, 0)		
Navigation		Image chift:	(0, 0)	Dinning	(0, 0)		
		Image shift	(0, 0)	Evenesure time ()	1000.0		
		Beam shirt:	(0, 0)	Exposure time (ms)	1000.0		
		Diffraction shift:	(0, 0)	Pre-Exposure (s):	0.0		
		Energy filtered:	No	Dose (e/A^2):	64.40		
		Energy filter width:	None	Save raw frames:	Yes		
		Skip when cycling:	No				
File Application Launcher No	ide Events Others Help	ge position 🔹 😥 🚍 🔳	Leginon:	n20dec02c			_ 0 X
File Application Launcher No KPresets_Manager Beam_Titt_Image Gind_Targeting Square_Targeting Square_Targeting	events Others Help hin • M stage events Time Message 1:31:50 PM Acquired 1:31:48 PM Acquiring	ge position	Leginon:	n20dec02c			- • ×
File Application Launcher No Revents_Manager Beam.Titt_Image Gidd_Targeting Gidd_Supering Square Hole_Tropeting Hole Very Very Very Very Very Very Very Ver	de Events Others Help Thin • M • stag evi Time Message 1:31:50 PM Acquiring 22		Leginon:	n20dec02c			_ + + ×
File Application Launcher No Presets, Manager Benn, Titz, Image Geam, Titz, Image Gea	de Events Others Holp P Inn • M • Mage or The Mosage ) 13:50PM Acqueres. - 13:530PM Acqueres. - 13:48 PM Acqueres. - 14:48 PM		Leginox:	n208ec02:			
File Application Launcher No Bean, Titz, Image Gol Gol Gol Holes, Targeting Hole Holes, Targeting Hole Deposite Z Joos Boose, Targeting Holes, Targeting Holes, Targeting Holes, Targeting Holes, Targeting Boose, Targeting Boose, Targeting Soft, Neurosci Soft, Neurosci Soft, Control of Soft, Cont	de Events Others Holp Pinn • M • Stage or Thre Mosage ) 13:56PH Acquires. ac exer 1343.77 mi: 738 mi: 748 correction • Peak •			n2084c02:			
File Application Launcher No. Presets Manager Galan Titz Anager Galan Titz Anager Galan Titz Anager Galan Titz Anager Galan Titz Anager Galan Titz Anager Galan Titz Anager Hole Topologies Comestion State Dirth Movare Market Agenter Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder Norder N	de Events Others Holp Pinn • M • stay externa free Mosage • ) 131:50 PM Acqueres. • 131:43 PM Acqueres. • 131			x204c02:	••		
File Application Launcher No Phrests, Manager Benn, Tit, Intige Genn, Tit, Intige Denner, Intige Denner, Fitt Genn, Fitt Genn, Tit, Intige Denner, Fitt Genn, Tit, Intige Denner, Fitt Genn, Fitt Genn, Fitt Genn, Tit, Intige Denner, Fitt Genn, Tit, Intige Denner, Fitt Genn, Fitt Genner, Fit	de Events Others Holp Pinn • N • star ext The Mosage ) 135.09 M Acquiring. ext 134.377 min • 738 act . 349 Correlation • Peak •				8		
File Application Launcher No. (1) (1) (1) (1) (1) (1) (1) (1)	de Events Others Holp Point Inter Pessage 115:00PH Acqueres 115:00PH Acqueres 115:00P	p potos		x204ec02:			
File Application Launcher No. Presets Manager Benn, Titz, Anager Benn, Titz, Anager	de Events Others Help Pinn • M • Stage Di 1550PH Acqueres Di 1550PH Acqueres 0 1548PH Acqueres 0 1548PH Acqueres 0 100 exer 134377 100 100 100 100 100 100 100 1	p poton 2 = 1		x204ec02:			- 0 X
File Application Launcher No. Generative Advancement Breins That anyon Generative Advancement Generative Advancement Generative Advancement Sparse Transform Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concentration Concent	de Events Others Help Pinn N I I ISSOP ISSOPH Acquered ISSOPH Acquered ISSOPH Acquered ISSOPH Acquered ISSOPH Acquered ISSOPH Acquered ISSOPH Acquered ISSOPH Acquered ISSOPH Acquered Interpretation Correlation Peak +	korgen • A • •	Legions:	x204ec02:			
File Application Launcher No. Generative Advancement Sensen Statumper Generative Advancement Generative Advancement Gener	de Events Others Help P Inn + N = 132 ext Table Acqueters 1 315.07 H Acqueters 1 315	k kopou	Legion:	x204ec02:			
File Application Launcher No. Square: Toyong Square: Square: Toyong Square: Square: Toyong Square: Square: Toyong Square: Square: Toyong Square: Square: Toyong Square: State: Square: Toyong Square: State: Square: Square: State: Square: Square: State: Square: Square: State: Square: Square: Square: Square: Squ	de Events Others Help P Inn A State P Inn A State P Inn A State P Inn A State I 13140 PM Acquires I 13140 PM Acquires I 13140 PM Acquires Corretation Peak P E Events Others Help Enn A State Page I I I I I I I I I I I I I I I I I I I		Legion:	x284ec02:	8		

D. Once centered select the *enn* present then click . Select "stage position", then click
Note the position of the feature in the center of the crosshairs.



E. Select the *hl* preset then click
M. Select "image shift", then click . If the center of the crosshairs is not at the same location as in the *enn* image. Use the to move it to the same location.



F. Once moved, go to the *KPresets_Manager* Node, select the *hIn* preset, click the edit settings icon , next to "Image Shift" click from scope icon . Click "Save".

			arter	reset not	^	_ 0 ×
File Application Launcher No RPresets.Manager  Color Beam.TitLimage Grid.Targeting Square.Targeting Square Square.Protection	Preset Parameters TEM Magnification Defocus Random Defocus Range	Glacios • 6700 • -0.00015	4	Digital Camera Falcon3 • Energy filtered Energy filter width	4	
Hole 9 Provisov 8 IZ_Focus 9 Oppower 7 Gopoure 16 Dopoure 17 Dopoure 17 Dopou	Intensity Image shift Beam shift Diffr shift Probe Mode Energy filtered Energy filtered Energy filter width Skip when cycling Use Channel 1 correct			Camera Configuration Demension: 1024 x 1024 Demension: 1024 x 1024 Demension: 1024 x 1024 Offset: 1024 Confise: 1024 x 1024 Offset: 00 Binning: 4 x 4 Exposure time: 500 ms Camera with Movie Mode Save frames Exposure time per Frame: 200 ms Readout delay: ms Save fills that image to speed up Save fills that image to speed up Save fills that image to speed up Save All (Sam K3) Frame-All (Sidam K3) Frame-All (Sidam K3) Frame-All (Sidam K3) C-correlation filter. None	a	

G. Select the *sq* preset then click
M. Select "image shift", then click
click
If the center of the crosshairs is not at the same location as in the *hln* image. Use the
to move it to the same location.



H. Once moved go to the *KPresets_Manager* Node, select the sq preset, click the edit settings icon , next to "Image Shift" click from scope icon . Click "Save".



## 11. Test Images

- A. Must be on carbon area. If not, on the Microscope computer under the "Search" tab, select the label for the carbon square and click "Go". Must be at Z height. If not refer to step 6.
- B. Go to the Square Node, click



C. Go to the *Hole_Targeting* Node, select the cursor next to "acquisition". Select one area to image. Select the cursor next to "focus", place a focus target near the acquisition target. Click .



D. Go to the Exposure_Targeting Node, select the cursor next to "acquisition". Select a few areas to image. Select the cursor next to "focus", place one focus target in the center of the acquisition targets. Click to submit.



## 12. Frame Alignment and CTF Estimation

- **A.** For EMG betaleginon session:
- If switching from a nccatleginon session to a betaleginon session.
   Go to <u>nccatdeon.nysbc.org</u>.
- Click "DD Raw Transfer Monitor". Under the section for "Glacios – Ingest4", click "stop". Select "Falcon3-Emg" and click "start".
- Click "Buffer Transfer Monitor". Under the section for "Glacios _EMG – Cn-02", click "start". It should show the word "Active". In the "Glacios_Nccat-Ingest4" section click "stop", it should show the word "Inactive".
- **B.** For NCCAT nccatleginon session:
- If switching from a betaleginon session to a nccatleginon session.
   Go to <u>nccatdeon.nysbc.org</u>
- Click "DD Raw Transfer Monitor" Under the section for "Glacios – Ingest4", click "stop". Select "Falcon3" and click "start".
- Click "Buffer Transfer Monitor". Under the section for "Glacios _EMG – Cn-02", click "stop". It should show the word "Inactive". In the section "Glacios_Nccat-Ingest4" click "start" it should show the word "Active".



- **C.** Open ~5 terminals and ssh the user into ingest04 (~2 for Frame Alignment and ~3 for CTF)
- For NCCAT sessions go to <u>http://nccatweb.nysbc.org</u> OR for EMG sessions go to <u>https://emgweb.nysbc.org</u>
- Select the correct session from the dropdown
- Click "[processing]"



- D. From the Appion menu, choose
   "Select Frame Alignment", then select "MotionCor2".
- Make sure the selected preset is enn (will only appear once enn have been taken). Click "Just Show Command".
- For a betaleginon EMG session only, before pasting the command in a terminal replace /opt/myamisnap/bin/appion with /opt/myamisnap_gpfs/bin/glacio s_test_appion
- Enter the command in the ingest04 terminal.
- With the same command change **gpuid=0** to **gpuid=1**, and enter the command in another ingest-04 terminal.



y and paste the manacobalgebootc.cor_OCSF by command the a terminat egfs/s/myramisenap/bin/nccatappion makeDDAlignMotionCor2_UCSF py -bin=1 --align --gpuids=0 --dtatframe=0 --MaskCentrove=0 --MaskCentrov=1 --MaskSizecols=1 +-Patchrows=5 --Patchcols=5 --Iter=7 --FrmRef=0 --doseweight --Bft_global=500 --Bft_local=100 --alignlabetes --invrs=1 --unname=ddstack1 --undtr=/beegfs /appion/maragon/n20dec02c=no-refets --continue --parallel --expid=714 --jobtype=makeddrawframestack

- E. From the appion menu, select"Estimate the CTF" then select"CTF Find v4".
- Make sure the selected preset is enn-a (will only appear once Frame Alignment starts). Click "Just Show Command".
- For a betaleginon EMG session only, before pasting the command replace /opt/myamisnap/bin/appion with /opt/myamisnap_gpfs/bin/glacio s test appion
- To the end of the command add the flag "--nproc=5" before entering into the terminal.

Session: N2UNOV25D - G Image Path: /beegfs/leginon/m Hide   Expand   Contract	lacios - Chameleon Apol ^a aragon/n20nov25b/rawdata		Į.	iser Guid
Anishi Anishi Anishi Senghi Lunch Algoree Cabhop Hann Algoree Cabhop Hann Mac Creation Roman Marco Cabhop Selex Anionates Mashing Cell Raintain Cabhonates Taga Denniar Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonates Cabhonat	Flun name           tittedevant           Object diselsky           Deregt inspire/margen/ofdex/zB           State of the Invested.           State of Themsels.           State of Themsels.           The Orbital           The Orbital           State of Themsels.           State Advanced Options	ng	Optional Parameters Optional Adapmetine (num) Optional Adapm	)
Upload particles Upload temptate 2 available Upload temptate stack Upload reconstruction POD to map ENDO to map Upload model coysdMMIC	If you find	Just Show Command		-
Copy and /beegfs rei rui rui	If you find paste this ctffind4 py command int sw/myamisnap/bin/nccati min=50resmax=4det phase_shift=10max_ name=ctffind4run1run set=cenn= a-commitpro	this software useful please cite: o a terminal: appion ctffind4.pyamp fstep=0.1numstep=25 phase_shift=170phas dir=/beegts/appion/mara giectid=25session=n20	contrast=0.07fieldsize=1024 dast=0.05parallel e_search_step=10num_fram gon/n20dec02c+tdr/dtfind4runJ dec02cno-rejectscontinue	e_avg= bestd

# 13. Switch to user's grid and collect an atlas

- A. Go to the microscope computer. Under the "Autoloader" tab:
- Make sure the column valves are closed ("Col Valves Closed" button should be yellow)
- Make sure the objective is out. Go to the "AAM" tab OR select "Apertures" in the right side menu click the "Objective" button (will turn gray).
- Click the position number to be loaded. Then click the "Load" button (will turn yellow once loaded).



B. On the Leginon computer, go to the *KPresets_Manager* Node. Select the *sq* preset then click
M. Then on the Microscope computer, lower the screen (hand panel R1 button). Try to move to a square using the stage joystick on the hand panel. Go to the *Z_Focus* Node, then click the simulate

target icon 🔭

- **C.** Go to the *Grid_Targeting* Node. Click on the settings icon
- Enter a label for the grid and the radius. Largest atlas is 0.009 m (43 targets). Then click
   and then
- Go to the *Square_Targeting* Node to view the atlas.



le Application Launcher RPresets_Manager Beam_TitLinage 6 did_Targeting 6 did_Targeting 6 did_Targeting 6 did_Targeting 8 square 9 Hole_Targeting 8 Hole 1 Preview 8 Z-Focus 8 Exposure_TET 9 Beam_Tit 9 DirtLMontor 8 Buffer_Cycling 0 dirt_Montor 8 Buffer_Cycling 0 dirt_Montor 8 Buffer_Cycling 0 dirt_Montor 8 Buffer_Cycling 1 direction 1 direction