SerialEM Protocol cryo collection **Fred Hutch** Glacios

What you're starting with:

- Atlases of all of your grids
- Grid you want to collect on chosen
- Screening performed in Leginon so you know what thickness of ice you want to target
- WARP started on your Leginon screening

Insert grid you want to image

- 1. Microscope computer
 - 1. Autoloader tab
 - 2. Click "number"
 - 3. Click "Load"
 - 4. Make sure "Turbo Auto Off" is selected



Prep SerialEM

- 1. Load Settings file
 - Settings -> SerialEMsetting_10cds_ 36kx_nP.txt
- 2. Load Navigator
 - Navigator -> Read & Open -> nav.nav (in your today folder)
- 3. When grid is loaded and ready!!
 - 1. "Open Valves" in Microscope Control box
 - 2. Put in 100um objective aperture



Align grid (62x) to square (155kx)

- Double click on 155 imaging state
- 2. Double click on atlas in Navigator window
- Left click on a noticeable spot on the grid atlas
- 4. Click "Add Marker"
- 5. Click "Go to Marker"

	Navigator: nav_dummy.nav	_ 🗆 X
Label: 1	□ Registration point 1 – Corner point (C) ?	
Color Blue	▼ I Draw	
#1 Not	e: Sec 0 - LMM.st	
Acquire (A)	□ Tilt series □ New file at item □ New file at group	
Set: File Props	Imaging State TS Params Filename Focus Pos	
Add Stage Pos		
Add Points		
Add Polygon	Label Color X Y Z Type Reg. Acq. Note	
Add Marker	1 Blu -38.9 0.7 -19.6 Map 1 Sec 0 - LMM.st	
Move Item	- 2 Blu -38.9 0.7 -0.0 Map 1 Sec 1 - LMM.st	
Undate 7	4 Blu -38.9 0.7 -0.0 Map 1 Sec 2 - LMM.st	-
Go To XX	5 Red -681.7 -581.5 -0.0 Pt 1	
	7 Red -740.7 -640.5 -21.6 Pt 1	
GOTOXYZ	8 Red -796.5 -699.7 -26.7 Pt 1 9 Red -738.3 -753.6 -26.8 Pt 1	
Go To Marker	- 10 Red -559.1 -574.7 -15.6 Pt 1	
Load Map	1 Red -743.5 -523.8 -17.7 Pt 1	
New Map	3 Red -684.1 -462.8 -15.1 Pt 1	
Anchor Map	Red -622.3 -401.2 -12.3 Pt 1 Red -689.8 -347.8 -13.1 Pt 1	
Delete Item	16 Red -748.6 -292.7 -14.5 Pt 1	
Realign to Item	17 Red -807.3 -352.3 -18.3 Pt 1 18 Red -868.0 -297.9 -19.9 Pt 1	
	19 Red -813.3 -236.4 -16.7 Pt 1	
	20 Red -754.1 -178.2 -14.1 Pt 1 21 Red -564.7 -458.3 -12.6 Pt 1	
	22 Red -555.3 -925.4 -31.0 Pt 1	
	100 F 401 7 000 7 7 F 1	

Align grid (62x) to square (155kx)

Sc

- Tasks –> Eucentric-Rough
- 2. Click "View" in Camera
- 3. Find the spot in your new image

N	Set intensity	Shift+I	
	Set Dose Rate		
	Move beam	Shift+M	
	Center Beam	Shift+C	EIN
	Autocenter Beam	Shift+E	DLIM
	Setup Autocenter		um
	Eucentric - Rough		
	Eucentric - Fine	ี	19
	Eucentric - Both		19
	Refine & Realign	الم ورادي	
	Set Tilt Axis Offset		
	Use Trial in LD Refine		
	Walk Up		
	Walkup & Anchor		
	Use View in LD		
	Set Increments		
	Setup Cooker		
	Cook Specimen		
	Assess Angle Range		
	Assess Interset Shifts		
	Revise/Cancel Shifts		
	Reset IS & Realign		
	Set Iteration Limit		
	Use Trial in LD		
	Setup VPP Conditionin	g	
	Setup Wait for Drift		
	Reverse Tilt		
~	Verbose		
	Specialized Options	•	

Align grid (62x) to square (155kx)

- 1. Left click on the same spot in your new image
- 2. Navigator -> Shift to Marker
- Select atlas in Navigator window and click "Update Z"
- 4. Repeat on a few different spots to make sure these are now aligned

igator Window Help		
Open		
Read & Open		
Merge File		
Save		
Save As		
Close		
Montaging & Grids		
Options •		Navigator: nav_dummy.nav
Transform Items	Label: 1	Registration point 1 Corner point (C)
Undo Transformation	Color Blue	Draw Rotate when load For anchor state
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Shift to Marker	C Acquire (A)	☐ Tilt series ☐ New file at item ☐ New file at group
Undo Last Shift	Set: File Props	Imaging State TS Params Filename Focus Pos
Align with Rotation	Add Stage Pos	Registration 1 📥 Draw: 🗆 All reg. 🗆 None 🔽 Labels
New Map from Image	Add Points	Collapse Show Acquire Edit mode Edit Focus Label Color X Y Z Type Reg. Acg. Note
Import Map	Add Marker	1 Blu -38.9 0.7 -19.6 Map 1 Sec 0 - LMM.st
Rotate Map	Move Item	Blu -38.9 0.7 -0.0 Map 1 Sec 1 - LMM.st 3 Blu -38.9 0.7 -0.0 Map 1 Sec 2 - LMM.st
Adjust for Backlash	Update Z	4 Blu -38.9 0.7 -0.0 Map 1 Sec 3 - LMM.st 5 Red -681.7 -581.5 -0.0 Pt 1
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backlash Settings	YZ YZ	8 Red -796.5 -699.7 -26.7 Pt 1 9 Red -738.3 -753.6 -26.8 Pt 1
Open Imaging States		- 10 Red -559.1 -574.7 -15.6 Pt 1
Set Map Acquire State	New Map	12 Red -619.4 -519.3 -15.4 Pt 1
Restore State	Anchor Map	13 Red -684.1 -462.8 -15.1 Pt 1 14 Red -622.3 -401.2 -12.3 Pt 1
Acquire at Items	Delete Item	15 Red -689.8 -347.8 -13.1 Pt 1 16 Red -748.6 -292.7 -14.5 Pt 1
End Acquire	Realign to Item	17 Red -807.3 -352.3 -18.3 Pt 1
List Files/Series/States		19 Red -813.3 -236.4 -16.7 Pt 1
Delete Item		20 Red -734.1 -176.2 -14.1 Ft 1 21 Red -564.7 -458.3 -12.6 Pt 1
Realign to Item		22 Red -555.3 -925.4 -31.0 Pt 1
Force Center Alien		
The Cooling in Poolign		

- Open up atlas of interest by double clicking on item in Navigator window
- 2. Click "Add points"
- Left click to add points in squares that you want to collect maps of
- 4. Check "Collapse group" so you can see how many you're selecting
- 5. Check "Acquire" to add A next to all of these points

 File -> New Montage -> correct settings -> save in "setup" folder as MMM.st

Montage Setup	File Properties
Magnification: 155 Pixel size: 156 nm Number of pieces in X: 1 Y: 1	Save non-float data as When saving 16 bit data C Bytes @ Truncate above 32767 © Integers C Divide by 2 C Subtract 32768
Piece size in X: 5760 Y: 4092	Percent of pixels to truncate converting to bytes As black (0): 0.2 As white 0.2
Overlap in X: 970 Y: 970 Reset ric Minimum overlap: 10% and 0.5 micron ati Total Area: 5760 x 4092 pixels Update ati 901.0 x 640.1 microns Update	Save in extended header Tilt angle Intensity Stage position Magnification
Move stage instead of shifting image	 Exposure dose
Skip pieces outside Navigator item	Maximum number of sections: 360
Do full rectangle; ignore list of pieces to skip	Save extra information in a '.mdoc' metadata file
Ask about making map after each montage	Save images to
 ☐ Use Montage Mapping, not Record parameters ☐ Use View parameters in Low Dose mode ☐ Use Search parameters in Low Dose mode 	 MRC stack file TIFF file (one image per file) Series of TIFF files listed in an Autodoc file JPEG file (one image per file)
Use continuous mode with settling factor 0.5	Type of compression in TIFF file
OK Cancel ?	Skip this dialog in future (re-enable in File menu) OK Cancel ?

1. Check the PrepMMM script (ctrl+left click to open)

	Script 2: PrepMMM	
necessary	 Editor -> Up Script Down Tab or ` to complete command MacroName PrepMMM # Select square targets, setup 1x1 montage at square imaging state Eucentricity 1 UpdateItemZ #Acquire montage M #Make the current image or montage a new Navigator map Newmap ErrorBoxSendEmail Script Stopped! 	
	Add TiltTo ## if you are collecting at tilt	

- 1. Navigator -> Save
- 2. Navigator -> Acquire at items
 - 1. Choose PrepMMM next to "Run script"
 - Check "Close column valves" if you're going to leave the room

A	cquire at Items
Initial Actions af	te Moving Stage
Rough euce	entricity
Autocenter	beam
Realign to it	em
Cook speci	men
Fine eucent	tricity
T Autofocus	Conly at start of group
Run script	MapGrids
Primary Task	
C Acquire ma	p image or montage
C Just acquir	e and save image or montage
Run script	PrepMMM
C Acquire tilt	series
	after: MapGrids
1 Hun Schpt	
Do subset	from index 1 to 380
Skip initial :	stage move to item
Skip Z mov	res in initial move and Realign
Restore sci	ope state after aligning them
Close colu	mn valves at end
Send emai	l at end
GO	Postpone Cancel of

Align square and hole (vie

- 1. Check "Low Dose" in LowDose
- 2. Go to: Rec in LowDose
- 3. Go to: View in LowDose
 - Make sure that defocus on microscope is -80, if not change defocus to -80 with hand panel
- 4. Double click on a square in the Navigator window
- 5. Left click on a feature on the square map
- 6. Click "Add Marker"
- 7. Click "Go to XYZ"

Align square and hole

- 1. Click "View" in Camera window
- 2. Find the feature in your new image
 - If it's not there then Shift + right click and drag the image, to move the stage until you can see it
- 3. Left click on the same spot in your new image
- 4. Navigator -> Shift to Marker
- 5. Repeat on a few different spots to make sure these are now aligned

Make P template

- Shift + right click and drag to move your stage until you are centered over the center of your pattern
 - 1. Centered on a hole for a single, 3x3, or 5x5 pattern
 - 2. Centered in the middle of four holes for a 2x2 or 4x4
- 2. Click "View" in Camera a few times to make sure your stage isn't drifting away from this position

Make a P template

- 1. Click "Setup" in the Camera box
- 2. Select View and set parameters
 - Bin = 8, Area size = encompass P template, Exposure = 2s
- 3. Click "View" in Camera and you should see just the template you want very centered
- 4. Click "P" in Buffer Controls to save the image in buffer P

D Copy Acti	Buffer Controls ?
AB	C D P New
SAVE A	Save Active To
- Options	Memory = 0 MB
Roll Buffers	A -> N 📥 Delete
C Align to	B instead of O
Read into E	Buffer O
Protect	unsaved Record images

Make a P template

- 1. Click "Setup" in Camera again
- Select "View" at the top and set
 - 1. Bin = 8, Area size = full, Exposure = 0.2s

	Camera &	Script		?
Setup	View	Focus	Trial	Record
Pr ew	Searc	h Re:	sume	STOP
<mark>کل</mark> ے	Pre	pMMM	meF	orCoffi

- Click "Add Points" and left click the inner edge of the four corner holes of your X by X pattern
- 2. Click "Stop Adding"

On flat grid, go with 5x5 unless you have a lot of empty/dirty holes that you're going to have to delete

				Na	vigator:	nav_du	nmy.nav	_ □	x
	Label: 1	Registra	ion point 1		orner point	(C)	?		
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	Go To XY	6	Red -817.	3 -353.7	-0.0 Pt	1			
	Go To XYZ	7	Red -740.	7 -640.5	-21.6 Pt	1			
		8	Red -796.	-699.7	-26.7 Pt	1			
	Go To Marker	9	Red -/38.	5 -/53.6	-20.8 Pt	1			
	Load Map	11	Red -743	-573.8	-13.0 Pt	1			
		12	Red -619.	-519.3	-15.4 Pt	i			
	New Map	13	Red -684.	-462.8	-15.1 Pt	1			
	Anchor Map	14	Red -622.	-401.2	-12.3 Pt	1			
		15	Red -689.	3 -347.8	-13.1 Pt	1			
	Delete Item	16	Red -748.	-292.7	-14.5 Pt	1			
	Realign to Item	17	Red -807.	3 -352.3	-18.3 Pt	1			
		18	Red -868.	-297.9	-19.9 Pt	1			

- 1. Click "Setup" in Camera again
- Select "Record" at the top and set
 - Bin = 1, Area size = full, Exposure = 0.5s, no dose fractionation

	Camera & So	cript		?
Setup	View F	ocus	Trial	Record
Pr ew	Search	Res	sume	STOP
	」	L		

- Select the first corner point you added in the Navigator window (left click on the correct number)
- 2. Click "Rec" in the Low Dose window
- Navigator -> Montaging and Grids
 -> Set Multi-shot
 Parameters

- 1. Check settings in multi-shot panel
- 2. Click "For Corners.." to start
- 3. Click "IS to Navigator Pt"
- 4. Click "Record" in Camera and make sure you don't see any carbon in your image
 - 1. If there is carbon **right click and drag (no shift key!!)** the image until there is none, click "Record" again to check
- 5. Click "Save Image Shift"
- 6. Iterate #3-5 for all four corners – the program will grey out the buttons automatically when you're finished
- 7. Click "OK"

- Click "Reset Image Shift' in Image Alignment and Focus
- Click "View" in Camera and you should be hovering near your last corner point

Correct astigmatism and coma

- 1. Center yourself on carbon (shift+right click)
- 2. Focus/Tune -> Set Target (-0.3)
- 3. Focus/Tune -> Autofocus
- 4. Focus/Tune -> Correct astigmatism by ctf
 - 1. Run again if change is >0.001 in log
- 5. Focus/Tune -> Coma-free alignment by ctf
 - 1. Run again if change is >0.2 in log
- 6. Focus/Tune -> Correct astigmatism by ctf
 - 1. Run again if change is >0.0005 in log

Prepare for collection

- Click "View" in Camera and center yourself over a hole
- Select "Define position of area: Focus" in Low Dose
- Left click to drop focus position (yellow) onto carbon next to the hole

Prepare for collection

- 1. Click "Setup" in Camera and set parameters
 - View: bin = 8, area size = P template exposure = 0.2s
 - Focus: bin = 2, area size= square, exposure =0.2-0.5s
 - 3. Record: bin = 1, area size = full, exposure = 6s dose fractionation ON – frame time: 0.06s Set File Options: (YYYYMMDD_username _sample_grid), Set Folder (X:/SEM_frames)

Pick points to start collecting

- Double click on the square you want to start with (go in order to make it easier for yourself)
- 2. Check "Collapse groups"
- Click "Add Polygon" and outline the area you want to image
- 4. Click "Stop Adding"
- 5. Click "Add Points" and click in the **center** of 5 holes to tell the program spacing
- 6. Click "Stop Adding"

Like this : Near the middle of the square

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Pick points to start collecting

- Navigator -> Montaging & Grids -> Add Grid of Points
 - 1. Enter polygon number
 - 2. Away from focus area
 - 3. Turn acquire on at all points
- 2. Check "Edit Mode" on Navigator window and delete any bad holes
 - 1. Left click on pt to remove and backspace

Nav	igator Window Help	
	Open Read & Open Merge File Save Save As Close	
	Montaging & Grids Options Transform Items Undo Transformation Change Registration Shift to Marker Undo Last Shift Align with Rotation New Map from Image Import Map Rotate Map Adjust for Backlash Backlash Settings Open Imaging States Set Map Acquire State	 Setup Corner Montage Setup Polygon Montage Setup Full Montage Setup Full Montage Setup Super Montage Polygon Super Montage Add Grid of Points Add Grid Jake Lat One Divide Points into Groups Set Group Radius Set Multi-shot Parameters Combine Points for Multi-shots Show Shots when Show Acquire Show Whole Area for All Points Add Circle Polygon
	Acquerrent and a second	Navigator: nav_dummy.nav X Registration point 1Corner point (C)
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	Acque End Label: 1 Dele Real Color Blue Forc #1 Note Try S Acquire (A) Set: File Props Add Stage Pos Add Points Add Points	Navigator: nav_dummy.nav Registration point 1

Pick points to start collecting

- Navigator -> Montaging & Grids -> Combine Points for Multi-Shot
 - 1. In same group as current point
 - 2. Click "Combine Points"
- 2. Navigator -> Save
- 3. Settings -> Save

Start collection

- Check the Acq-9Holes...IS script and adjust the defocus range as desired
 - -1.0 and -2.0 common, can lower for higher resolution or raise for harder to see particles
- Navigator -> Acquire at Items
 - 1. Run script: AcqHoles-9HolesCTEM-IS

Start data transfer to WARP

- Double click on "transfer_serialEM.bat"
 - 1. Enter username
 - Enter session name (from Leginon screening – 21sep14f)

Move to DUMMY and pick remaining points

- 1. Open DUMMY serialEM
- 2. Settings -> settingsfile
 - Most likely: SerialEMsettings_10c ds_36kx_nP.txt
- Navigator -> Read & Open -> nav.nav
- 4. Navigator -> Save As -> nav_d.nav

Force Center Align Try Scaling in Realign

Move to DUMMY and pick remaining points

- 1. Pick points the same way that you did before (slides 23-25)
- 2. After the first map,
 - 1. "Add Polygon"
 - 2. "Add Point" in the **center** of one central hole and "Stop Adding"
 - Navigator -> Montaging & Grids -> "Add Grid Like Last One"
 - 4. Check "Acquire" for this group
 - 5. Delete bad holes (Edit Mode)
 - 6. "Combine Points"
- 3. Add points to all squares that you want to collect

	•	Navigator: nav_dummy.nav	
	Label: 1 Registra	tion point 1 📥 🗆 Corner point (C) 🕐	
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T		Rea -817.3 -353.7 -0.0 Pt 1 Red -740.7 -640.5 -21.6 Pt 1	
	GO TO XYZ 8	Red -796.5 -699.7 -26.7 Pt 1 Ped -738.3 -753.6 -26.8 Pt 1	
	Go To Marker 10	Red -559.1 -574.7 -15.6 Pt 1	
	Load Map 11	Red -743.5 -523.8 -17.7 Pt 1	
Na	vigator Window Help		
	Open		
	Read & Open		
	Merge File		
	Save As		
	Close		
	Montaging & Grids	Setup Corner Montage	
	Options v	Setup Polygon Montage	
	Transform Items	Setup Full Montage	
	Undo Transformation	Set Grid Limits Sec 0 - MMM.st Sec 1 - MMM.st	
	Change Registration	Setup Super Montage Sec 2 - MMM.st	
	Shift to Marker	Polygon Super Montag	
	Align with Botation	Add Grid of Points	
	New Man from Image	Add Grid Like Last One Sec 7 - MMM.st	
	Import Map	Sec 8 - MMM.st Divide Points into Groups Sec 9 - MMM.st	
	Rotate Map	Set Group Radius	_
	Adjust for Backlash	Set Multi-shot Parameters Multiple Hole Combiner	-
	Backlash Settings	Combine Points for Multi-shots	-
	Open Imaging States	Show Shots when Show Acquire	le
	Set Map Acquire State	Add Circle Polycon	9
	Acquire at Items	Polygon from Corners Conside current polygon	۰ ۲
	End Acquire	In same group as current point	
	List Files/Series/States		
	Delete Item	Combine Points Undo	?
	Realign to Item	Dianlay multi shat bafara sambi	
	Force Center Align		ne
	ny Scaling in Realign		

Start long-term collection

Save

- 1. In the DUMMY, Navigator -> Save
- 2. Close DUMMY serialEM
- In regular serialEM, Navigator -> End Acquire (wait for the message box to pop up to tell you it 3. stopped)
- Navigator -> Read & Open -> 4. nav d.nav
- Uncheck "Acquire" next to the 5. points that have already collected!
- 6. Navigator -> Save As -> nav.nav
- 7. Navigator -> Acquire at Items
 - Run script: AcqHoles-9HolesCTEM-1.
 - Check "Close column valves at end" 2.
 - 3. Check "Send email at end"

Double check before leaving!!

- Is it aligning to the holes well? If not:
 - increase exposure time in Setup-> "View"
 - Retake P template
- Is it shooting the centers of the holes (no carbon in images)?
 If not:
 - Make sure the hole alignment worked, if not see above
 - Redo IS template step and make sure you are in the middle of the hole when saving
- Did you set the column valves to close at end?
 - You can't actually check this without stopping it, so hopefully you read the earlier slide!!
- Are images being pulled into WARP and processed? If not:
 - Check the CMD prompt and see if anything is running, double click on transfer_serialEM.bat logo on desktop
 - Check that the input directory for WARP is correct