Ptolemy application in Leginon

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Ptolemy is a tool for multi-grid **autoscreening** implemented in Leginon as an application. The method was developed by Paul T. Kim et al in 2023 which provides a pipeline to automate low- and medium-magnification targeting using a suite of purpose-built computer vision and machine-learning algorithms. Here at FSU/BSIR we have done comprehensive research on different type of grids: Chameleon, carbon and Au, and all gave us reasonable and efficient results.

Link to Ptolemy application in Leginon and the paper:

https://emg.nysbc.org/projects/leginon/wiki/Multi-grid_autoscreening

https://journals.iucr.org/m/issues/2023/01/00/pw5021/index.html



Ptolemy usually runs by two steps: the first step is to manually set up proper parameters for a new type of grid (or the first grid of the whole batch); the second is to run autoscreening for the rest of grids by loading the setting from the first step or session.

Or, user can skip the first step and start autoscreening directly by loading previous successful autoscreening sessions for the same type of grids. Here are few typical successful sessions:

Chameleon grid: 24may03gCarbon grid (2/2): 24may06dAu grid (1.2/1.2): 24may06g

Step 1: Setup for a new type of grid

- 1. Run command "su username" on Maia. ptolemy is only installed on Maia.
- Run command "module load ptolemy".
 Or, check if it has been loaded by "module list", and make sure ptolemy is the only module loaded, unload other module if they exist.
- 3. Run command "start-leginon.py"
- 4. Leginon \rightarrow Application \rightarrow MSI-Ptolemy2 (find it from the "Show All" list)

Run Application ×				
Application: M	SI-Ptolemy2	•	Show All	
main:	maia 🔹			
camera:	apollo-fsu 🛛			
scope:	titan5233191(•			
		Run	Cancel	

- 5. Disable automation
 - Square_Targeting setting: uncheck "Enable auto targeting"
 - ➢ Hole_Targeting setting: turn on "user verification"
 - Exposure_Targeting setting: turn on "user verification"
- 6. Setup script location
 - > Square_Targeting →Blobs setting •Blobs
 →File entry: /usr/local/ptolemy/sq_finding.sh



➢ Exposure_Targeting → Hole setting •Hole
 → Shell Script to source: /usr/local/ptolemy/hl_finding.sh

Exposure Targeting Settings ×				
External hole finding with score Shell Script to source				
/usi	r/local/ptolen			
Script output key to threshold on				
	score			
Min. score to accept	0.1			

7. Collect a fresh gain

It is important since it might affect the intensity read for Square_Targeting.

- 8. Collect Atlas
 - ➢ Load one grid
 - Send Z to around -80µm via Microscope GUI or Simulate Z focus in Leginon (make sure hl image is not fully black)
 - Collect Atlas
- 9. Set up threshold to select the ice thickness of interest
 - Once Atlas completes Ptolemy will find and display all squares with blue blobs on Atlas image at Square_Targeting.
 - Move mouse to blue blobs to read information for different ice thickness of squares. Here are three square sizes from small to big for a carbon grid.

Blobs	1227,835	576,11097	309,166
Size	20420.1	22857.4	48475.9
Mean	10054	11316.8	11707.8
Score	0.782416	0.833721	0.356901

- For carbon grid and Au grid, better to select proper ice thickness by size; for Chameleon grid, better to use Mean since the size are all the same between with and without ice.
- Square_Targeting \rightarrow Thresholded : Size 22000 26000 \rightarrow Find Squares



> Here are typical parameters for different grids:

Chameleon grid	: Mean	12200	12600
Carbon grid	: Size	20000	26000
Au grid	: Size	20000	26000

10. Set up square number for screening

User can set square targets number here, the example is 4 squares Square_Targeting \rightarrow acquisition setting \rightarrow Max. number of targets: 4



Here are few examples:



11. Submit squares targets and Hole_Targeting

Hole_Targeting setup is still the same for data collection, step by step following the settings of Template \rightarrow Threshold \rightarrow Blobs \rightarrow Lattice \rightarrow acquisition, but here is much less strict since we only need to separate the hole targets and no need to follow the grid geometry exactly. Here is an example for carbon grid (2/2).

Hole Targeting Settings	×			
Template Correlation				
Use cross · correlation			Hole Targeting Set	tings ×
Fill image values below 3000 with mean be	fore correlation		Dish finding	
 invert template for correlation Template Filename 			Border:	10
Original Template Diameter 16				
Final Template Diameter 32			Max. blobs:	100
Multiple Hole Template Number of holes in template		Hole Targeting Settings ×	Max. blob size:	100
Hole lattice spacing 50 pixels		Threshold	Min. blob size:	0
Hole lattice angle -46 degrees		Threshold = mean + A * stde -	Min. blob roundness	: 0.1
low Pass Filter (Phase Correlation)		A: 1.8		
Sigma:	1			Test
		Test		rese
	Test	OK Cancel Apply	OK Cancel	Apply
ОК	Cancel Apply	OK Cancel Apply		· +F -7
	Ice Thio	ckness Threshold	rungs	
	Min. m	nean:		0
	Max. m	nean:		10
	Min. st	tdev.:		0
	Max. st	tdev.:		10
	Focus	hole selection:	C	enter •
			Focus offset x:	0 y: 0
	Target	Template		
Hole Targeting Settings	× Use t	target template	Focus Target Template	
Lattice Fitting	Appty	y ice thickness threshold on template-convolved acqui: Focus Template Thickness	siton targets Relative target: (0, 0)	
(Set spacing to 0 to accept all blobs)		Use focus template thickness and limit to one focus	target Add Edit Delete	Auto Fill
Spacing:	250	Stats. radius:	10 Acquisition Target Temp	late
Tolerance:	0.1	Min. mean thickness:	0 Relative target: (0, 0)	
Extend Lattice	off	Max. mean thickness:	10 Add Edit Delete	Auto Fill
Extend Lattice	UII ·	Min. std. thickness:	0	
Hole Statistics		Max. std. thickness:	1	
Radius:	15 Acquisi	ition Target Sampling		
Reference Intensity:	500 Sample	subset of the acquisition targets		4
	Test			
	Clear t	argets	Te	st targeting
OK Cancel	Apply		OK Ca	ncel Apply

- Template: Hole Lattice angle can be any value here since grid is loaded at random orientation, and it doesn't affect the final result.
 - Lattice: Spacing 250 has been tested to work the best for all kind of grids
 - Acquisition: at the bottom there is a special setting of Ptolemy to allow user to select hole target number for each square, the example is 4 hole targets.

12. Submit hole targets and Exposure_Targeting

Exposure_Targeting is much simpler here since Ptolemy will find all holes on the image automatically, only needs to set up the Hole and acquisition as below.

Exposure Targe	eting Settings	Exposure Targeting Set	ttings ×
External hole findi Shell Script to so	ng with score urce	Ice Thickness Threshold Min. mean: Max. mean: Min. stdev.:	0 10 0
	/usr/local/ptoler	Max. stdev.:	1
Script output key	to threshold on	Focus hole selection:	Center • Focus offset x: 0 y: 0
Min. score to acce	ept 0	Target Template Use target template Apply ice thickness threshold on template-convolved acquisito Focus Template Thickness Use focus template thickness and limit to one focus targ Stats, radius;	Focus Target Template In targets Relative target: (0, 0) get Add Edit Delete Auto Fill 10 Acquisition Target Template
Hole Statistics		Min. mean thickness:	0 Relative target: (0, 0)
Radius:	40	Max. mean thickness: Min. std. thickness: Max. std. thickness:	IO Add Edit Delete Auto Fill 0 1
Reference Intensi	ty: 600 Test Cancel Appl	Acquisition Target Sampling v Use subset of the acquisition targets Sample Maximal of Clear targets	6 Test targeting OK Cancel Apply

- Hole: Radius should set less than the real hole radius Reference Intensity: 6000
- Acquisition: Focus hole selection: Center
- User can set exposure acquisition target number here, the example is to only 6 holes (green ones)



- 13. Enable automation
 - Square_Targeting setting: check "Enable auto targeting"
 - Hole_Targeting setting: turn off "user verification"
 - Exposure_Targeting setting: turn off "user verification"
- 14. Quite Leginon

Step 2: Autoscreening

1. Run command "autoscreen.py" in terminal.

xfu2@maia:~\$ autoscreen.py
gridhook.cfg does not exist. Grid Management Hook disabled
remote.cfg does not exist. Remote disabled
Enter autoloader cassette-grid mapping filename (leave it blank to use gui):
List comma-separated slot number to screen, i.e., 1,11,12: 9,10
Full workflow or atlas only (full/atlas): full
Enter an old session name to base new sessions on: 24may06b
Enter Z stage height to return to in um (default: the old sessionvalue -104.4): -104.4

- Follow the questions step by step.
- > The example is to only screen two grids on cassette slot 9 and 10
- > Always do full workflow unless you only need to collect Atlas
- The old session is the session from step 1 or any previous successful session of the same type of grids
- Z stage height should be any number around -80 or the number suggested from the old session as in the example, but some value has to be typed in, can Not be blank.
- 2. Continue optimizing the parameters during autoscreening if needed Disable certain automation
 - Square_Targeting setting: uncheck "Enable auto targeting"
 - Hole_Targeting setting: turn on "user verification"
 - Exposure_Targeting setting: turn on "user verification"
- 3. Terminate certain steps if needed Abort at Node of Square, Hole or Exposure
- 4. Time cost

~1hr for 4 squares X 3 hole targets X 20 exposure targets

	Chameleon	Carbon (2/2)	Au (1.2/1.2)
Session	24may03g	24may06d	24may06g
Square_Targeting \rightarrow	Mean 12200	Size 22000 26000	Size 22000 26000
Thresholded	12600		
Hole_Targeting	3000,168,42,1,37,	3000,168,32,1,50,	1000,168,32,1,46,
\rightarrow Template	0	0	0
Hole_Targeting	1.8	1.8	1.8
→Threshold			
Hole_Targeting	10,1000,1000,0,0.	10,1000,1000,0,0.	10,1000,1000,0,0.
→Blobs	1	1	1
Hole_Targeting	250,0.1,15,5000	250,0.1,15,5000	250,0.1,15,5000
→Lattice			

5. Here is a table of input examples for Chameleon, carbon(2/2) and Au(1.2/1.2) grid

Hole_Targeting	0,10,0,10,center	0,10,0,10,center	0,10,0,10,center
\rightarrow acquisition			
Exposure_Targeting	score,0.1,40,6000	score,0,40,6000	score,0.1,40,6000
→ Hole			