Grid Screening with Leginon Standard Operating Procedure

version 1.0

Authors: Anjelique Sawh-Gopal, Mahira Aragon, Christina Zimanyi

Approved Date: 1/30/2023

1. Purpose:

1.1. Operation of Leginon software to screen cryoEM grids in a transmission electron microscope (TEM)

2. Definitions:

2.1. **TEM UI** is the microscope user interface located on the microscope computer.

2.2. **Leginon** is a system designed for automated collection of images from a TEM and run from a different computer than the microscope. You will interact with two computers: 1) the **microscope computer** that has the direct microscope control software. 2) the **Leginon computer** – a separate computer running the Leginon software. Pay attention to which computer you are running which action from.

2.3. A Leginon **session** is an execution from start to finish of a Leginon application. All data (images, results, etc.) created by Leginon are associated with a session.

2.4. A Leginon **application** is an image acquisition process that is built from several 'nodes'. An application defines your preferred scheme for how to acquire images. An application definition includes which nodes to use, how they are connected, and where they are running.

2.5. **Nodes** are the building blocks of Leginon applications and are defined for specific tasks. For instance, an "Acquisition" node is designed to acquire images when it receives targets from another node, which is typically a 'TargetFinder' node. Nodes can "publish" the data they create. This means they are making their data available for other nodes to use. The other nodes can "research" to find a specific item of data. Nodes may communicate with each other by generating "**events**".

2.6. The Leginon **manager** is the master of all nodes in an application. Its existence is usually transparent while running a session, but it is responsible for starting up the application with all its nodes and event bindings. It works behind the scenes to ensure events are properly distributed throughout the system.

2.7. A **preset** is a piece of data that encapsulates the state of an instrument. At any time, the current state of an instrument (magnification, image shifts, camera settings, etc) can be recorded for later use. There is a particular class of Node called the PresetsManager which maintains a list of presets for the current Leginon session. The Leginon PresetsManager allows for an unlimited number of presets to be used (like several search presets at different magnifications, or multiple exposure presets at different defocus).

2.8. A **Target** is a location where an image will be acquired. Targets are often selected from existing images (using a TargetFinder node). Acquisition nodes are responsible for interpreting Targets and then acquiring images of them.

3. Supplies & Equipment

- ❑ A TEM with a dedicated Leginon computer

4. Procedure

4.1. <u>Software and Microscope checks</u>

4.1.1. Before screening, microscope alignments should be checked and tuned if needed. This is outside the scope of this SOP. Verify with staff that this has been done.

4.1.2. Set up a Leginon session

4.1.2.1. Startup the **NCCAT Leginon Client** by double clicking the icon on the desktop of microscope computer (Figure 1).

4.1.2.2. Log in to the Leginon computer. Open a terminal and run: **nccatleginon**

4.1.2.3. Select [create new session] → [next]

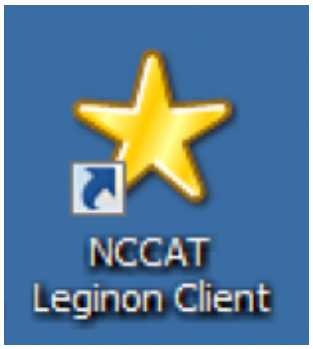

Figure 1. Click on the "NCCAT Leginon Client" icon on the **microscope computer** desktop before launching Leginon from the Leginon computer.

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4.1.2.4. Select holder (*microscope dependent* - ask NCCAT staff) → [next]
4.1.2.5. In session description, type "microscope name - NCCAT proposal ID – other desired information about sample" → [next] [example: Glacios1-NCCAT-TP1-CZ200215)
4.1.2.6. Select your project from the drop down → [next]
4.1.2.7. Image directory (use default) → [next]
4.1.2.8. Connect to clients (*microscope dependent*). After selecting the client from the drop down click the [+] button. Then you will see the client in the box below. Then click → "next"
4.1.2.9. C2 size = refer to the aperture tab on microscope PC, make sure it's correct → "finish"

4.1.3. Once you have started Leginon, open an *application*
4.1.3.1. Application → Run → Application for that microscope (ask staff if unsure)
4.1.3.2. Main = *microscopename*leginon
4.1.3.3. Scope = *microscope name*
4.1.3.4. Select [Run]

4.1.4. Upload presets (see Figure 2)
4.1.4.1. Preset manager → "import presets from another session"
4.1.4.2. TEM = *microscope name*; Digital Camera = *camera name* (select the detector that suits your session's needs. If you don't know, ask a staff member)
4.1.4.3. [Find] presets from past 20 days; select desired preset (usually from the most recent user)
4.1.4.4. Highlight all the presets [ctrl+A] (GR, SQ, SQLM, HLN, FAN, FCN, ENN) and [import] → [done]

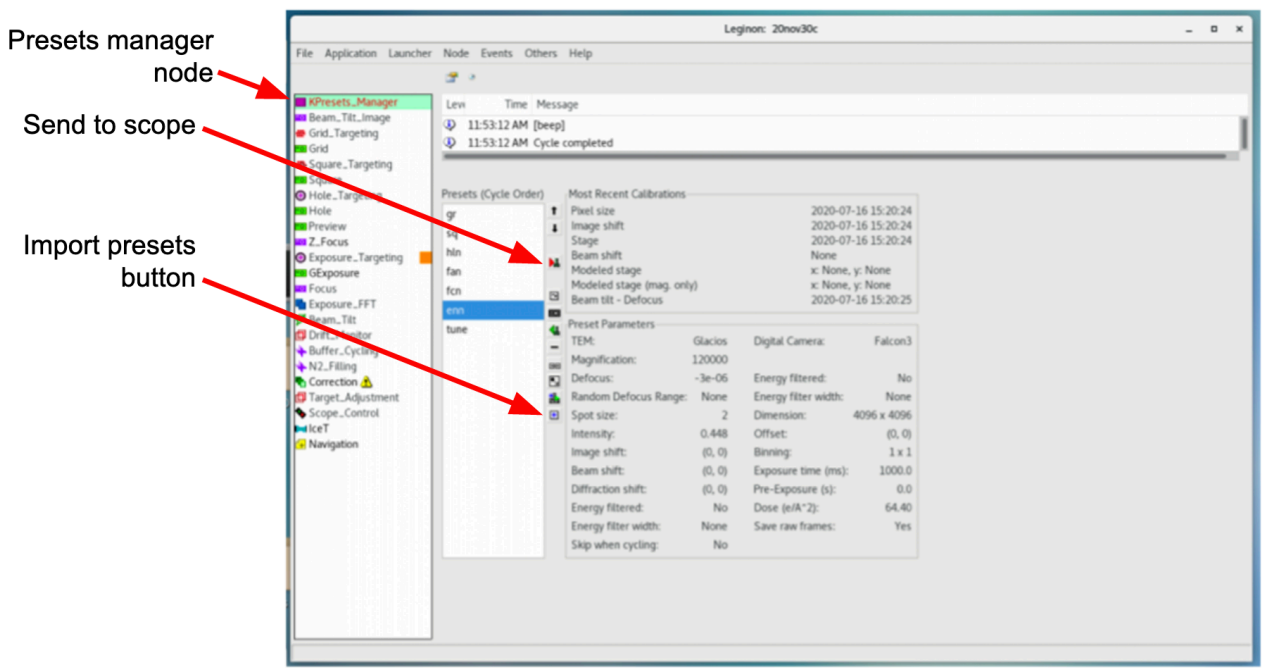

Figure 2. Leginon interface with Presets Manager node, "import presets" and "send to scope" buttons highlighted.

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4.2. Load grids and Find Eucentric Height/Focus

4.2.1. Go to the TEM UI on the **microscope computer** (Figure 3):

4.2.1.1. In the TEM UI , the button colors are status indicators:

Yellow = true, grey = false, orange = in progress

4.2.1.2. Make sure the column valves are closed: "Col Valves Closed" button yellow.

4.2.1.3. Check that the objective is out: Select "Apertures" on the lower right side menu. "Objective" button should be gray.

4.2.1.4. Select "insert screen" in the TEM User Interface or click R1 on the hand panel, to insert the screen. Green text in the image window will confirm if the screen in inserted or retracted.

4.2.1.5. Click the number position the desired grid is in and click the "Load" button.

4.2.1.6. When the "Load" button turns yellow, the grid is successfully placed on the stage

4.2.2. With the screen inserted, open Column Valves

4.2.3. In Leginon, from the Presets_Manager node, click on the GR preset and send it [to scope]  (Figure 2)

4.2.4. Using the stage movement joystick (right hand panel), move to a square with fully intact support film. To reduce exposure of a grid square you may want to image, chose one that is less nice, perhaps with some surface contamination.

4.2.5. Go to the Z_Focus Node on the Leginon computer, then click the simulate target icon 

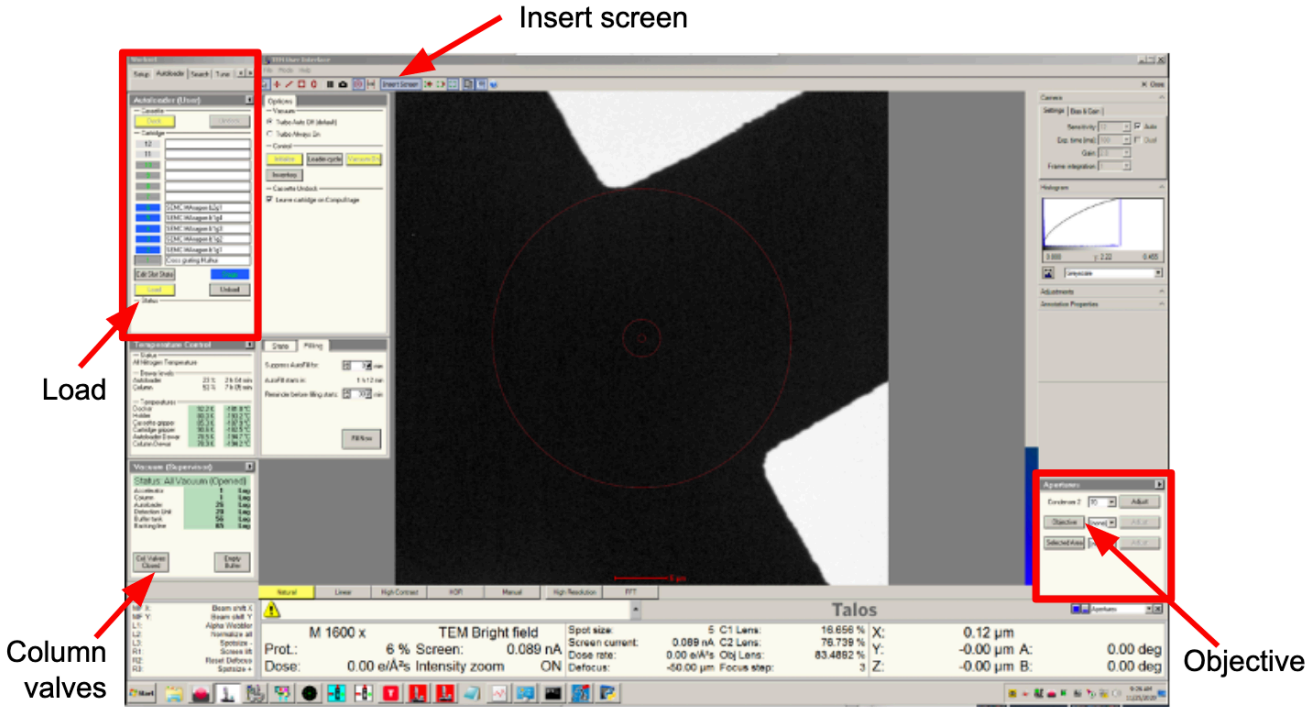

Figure 3. The TEM UI window is shown with the relevant buttons in the SOP highlighted.

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4.3. Low magnification imaging: Take an Atlas

4.3.1. Go to the Grid_Targeting Node (Figure 4). Click on the settings icon (above the log message box): .

4.3.2. Enter a label for the grid (generally your box # and grid position in that box, for example Grid 2 in box 1 is B1G2, etc) and the radius. Click OK.

4.3.2.1. The largest atlas you can take has a radius of 0.0009 m: 43 targets. Keep in mind that with 0.0009 m and 0.0008 m atlases, you can visualize the edges of the grid; however, the microscope cannot image at high magnification at those edges. A radius of 0.0007 m allows full range of imaging from end to end of the atlas. A smaller radius take less time to image. 0.0006 (23 targets) is a common choice but if you know you can get all of the information you need from the center of your grid, you can go smaller.

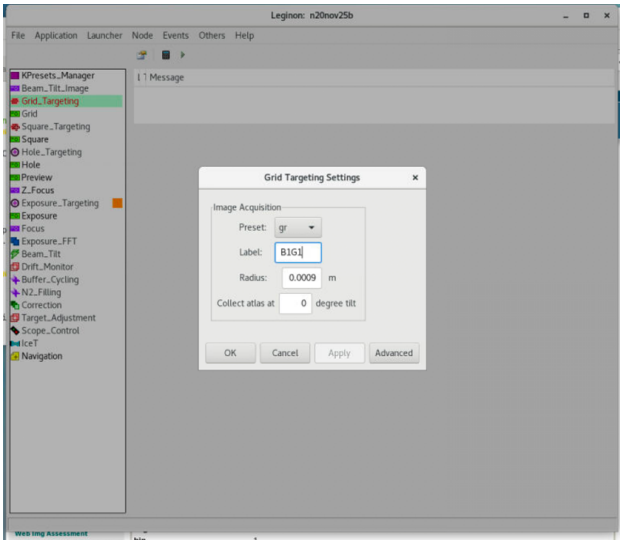

Figure 4. Image of Grid_Targeting node with atlas settings dialog.

4.3.3. Click [calculate atlas]. The number of images that will be taken for that atlas will be shown in the log dialog. If the radius is correct for the atlas size you want, click [publish atlas] to begin taking low magnification images that will be stitched together to create an atlas. If you want to adjust the radius, re-click "settings", type in the new value. Click ok then click "calculate". Check the number is what you want, then click "publish".

4.4. Intermediate magnification imaging: Square Targeting and Hole targeting

4.4.1. Go to the Square_Targeting Node to view the atlas.

4.4.2. Select the cursor button next to Acquisition to select targets (Figure 5)

4.4.3. Choose 3-4 squares of varying sizes (which suggests various ice thicknesses) and set acquisition targets by left clicking them on the atlas image.

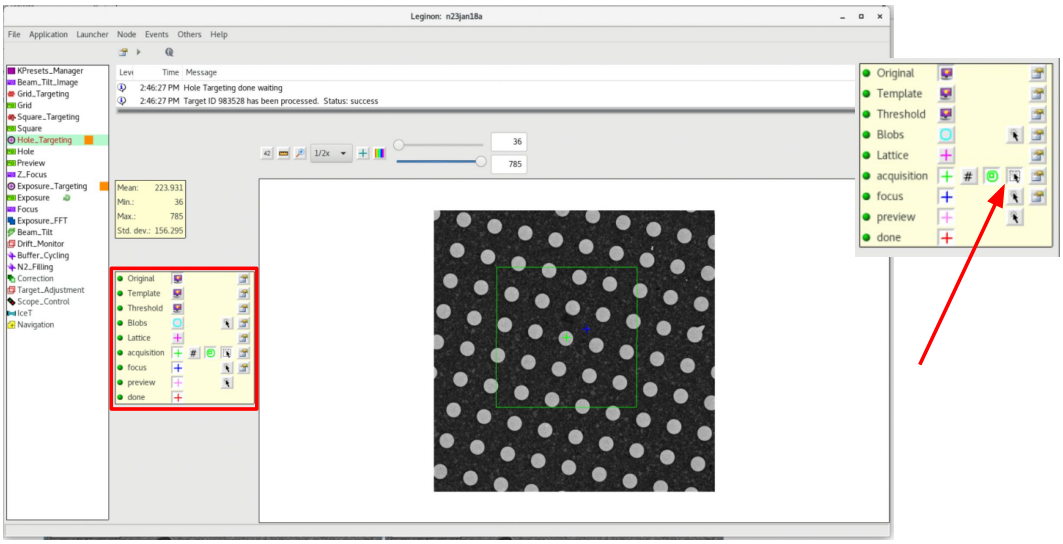

Figure 5. In any targeting node, engage the cursor button (highlighted with the red arrow) to set targets on your image. Acquisition targets appear as green squares, focus targets appear as a blue crosshair. You do not need to set a focus target for square_targeting.

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4.4.3.1. To remove individual targets, select the cursor for the respective target type and right click on the crosshairs. To remove all targets, use Control+Shift+Right Click.

4.4.4. Submit these Targets by clicking [submit targets] . As the camera acquires the square images, they will appear in sequenced queue in the Hole_Targeting Node.

4.4.5. Go to the Hole_Targeting Node to view the images of your selected squares.

4.4.5.1. In settings (), ensure that "Allow for user verification of selected targets" and "Skip automated hole finder" are **checked.** If you'd like to utilize the queue feature also check off "Queue up targets".

4.4.6. Select the cursor button for acquisition to select imaging targets.

4.4.7. Choose several targets per square image and set them using left mouse click.

4.4.7.1. Choose 1-2 targets for screening, more targets as desired, if collecting a data set

4.4.8. Select the cursor button for focus to select a focus target.

4.4.9. Place a focus target near the center of your acquisition target using left mouse click (the target will appear as a blue plus sign). This will be the location used by ZFocus node to take correlation images to attain eucentric height.

4.4.10. To remove individual targets, select the cursor for the respective target type and right click on the crosshairs. To remove all targets, use Control+Shift+Right Click.

4.4.11. Once you have all of your desired targets set, click [submit targets] .

4.4.12. If "Queue up targets" was toggled on: repeat for each square selected in Square Targeting. Once targets have been submitted for all square images, click [Submit Queued Targets]

4.5. <u>High magnification imaging - Exposure Targeting</u>

4.5.1. After ZFocus Node is complete and Hole Node generates an image, go to the Exposure_Targeting Node to view your hole images

4.5.1.1. In settings (), ensure that "Allow for user verification of selected targets" and "Skip automated hole finder" is **checked**, and "Queue up targets" is **unchecked**

4.5.2. Select the cursor button for Acquisition to select targets.

4.5.3. Choose several targets per hole image.

4.5.3.1. ~4 targets for screening, maximal targets for collecting

4.5.3.2. You can vary targets by imaging in center and at edge of hole, or even on support film if using carbon to assess particle distribution on your grid. The square that appears when you set the target is a relatively accurate display of the field of view of the exposure image.

4.5.3.3. Select the cursor button for Focus and place the target equidistant between four holes, on support film. This will be the location used by Focus node correlation images to attain eucentric focus. To avoid double exposure of imaging regions, it should **not** overlap with any exposure targets.

4.5.4. To remove individual targets, select the cursor for the respective target type and right click on the crosshairs. To remove all targets, use Control+Shift+Right Click.

4.5.5. Once you have all of your desired targets set, click [submit targets] . Leginon will take these images and load the next Hole Target if you targeted multiple HLNs.

4.5.6. Use the webviewer interface (nccatweb.nysbc.org) to review all acquired images.

4.5.7. Repeat for each hole image selected in Hole Targeting.

4.6. <u>Repeat for each grid you would like to screen</u>

4.6.1. If satisfied with the information obtained during this screening, repeat steps for the next grid.

4.6.2. If you want to target additional squares, go back to step 4.4 and repeat.

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4.7. <u>If you would like to conduct a data collection on a grid, return to Square Targeting</u>
- 4.7.1. For collection lasting overnight, aim to target approximately 50 squares
- 4.7.2. Use Automated Hole Targeting in Hole Targeting
  - 4.7.2.1. In settings, ensure that "Allow for user verification of selected targets" and "Skip automated hole finder" is **unchecked**, and "Queue up targets" is **checked**
- 4.7.3. Once all squares have been submitted, Submit Queued Targets
- 4.7.4. Use Automated Hole Target in Exposure Targeting
  - 4.7.4.1. In settings, ensure that "Allow for user verification of selected targets", "Skip automated hole finder" and "Queue up targets" is **unchecked**
- 4.7.5. In Presets_Manager click the clock icon to Toggle Error Notification (to ensure safety of the scope, this will automatically Close Column Valves when scope is idle for more than 30 minutes).

5. **Chemicals:** N/A

6. **Waste Disposal:** N/A

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