Data Collection with Leginon Standard Operating Procedure

version 1.0

Authors: Zephan Melville, Carolina Hernandez

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1. Purpose

1.1. Automated data collection of pre-screened grids with Leginon

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
- ❏ Vitrified CryoEM Grids

4. Procedure

The following procedure assumes: 1) microscope alignments have been checked and tuned if needed, 2) a new Leginon session has been started with presets loaded and checked during alignment checks. These items are outside the scope of this SOP. Verify with staff that this has been done.

4.1. Load Data Collection Grid and verify Leginon settings
    4.1.1. Load a grid from the Microscope PC
        4.1.1.1. Microscope PC TEM UI: Autoloader OCX: Click Slot, Click Load (see figure)

    4.1.2. In the Exposure_Targeting node, open the settings (click on [icon] in the top icon bar), ensure that Allow for User Verification is enabled.

    4.1.3. In the Hole_Targeting node, open the settings (click on [icon]), ensure that User Verification and Queue Up Targets are **both** enabled
    4.1.4. In Presets_Manager, click on the exposure preset, enn for counting or esn for super-resolution, and open the edit window [icon]. Enter the desired defocus range (e.g. -0.8 to -1.8 μm)

4.2. Collect Atlas – Whole Grid Overview
    4.2.1. Find the eucentric height of a grid square. (Presets_Manager: Send gr preset using the [icon] icon)
        4.2.1.1. On the microscope PC hand panel: Lower the screen
        4.2.1.2. Move to the center of a square using the hand panel joystick
        4.2.1.3. In Leginon, go to the Z_Focus node and click the icon to simulate a target [icon]. Leginon will automatically find eucentric height.
    4.2.2. Start Atlas collection (Grid Targeting)
        4.2.2.1. In the Grid_Targetting node, click on the settings button [icon]. Enter a label (e.g. g1) and radius for the atlas (e.g. 0.0009 m, which is the maximum size). Click "Apply" then "Ok" to close the settings window. Click calculate [icon], and then publish [icon] to start taking the atlas images.

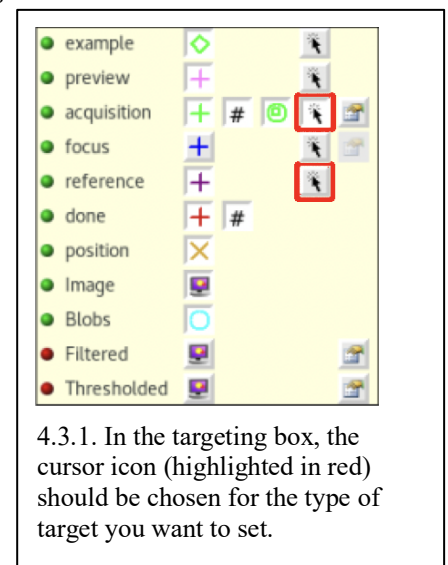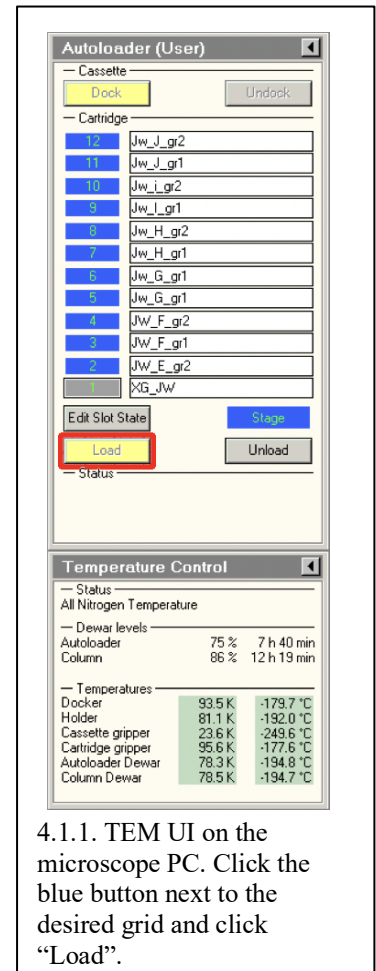4.3. Square Targeting
    4.3.1. In the Square_Targeting node, place acquisition targets
    4.3.2. Select one reference target (vacuum) for aligning the ZLP/energy filter
        4.3.2.1. Also check that in the Align ZLP node Settings that Bypass Conditioner is not enabled
    4.3.3. Insert the objective aperture
    4.3.4. Submit the selected targets by clicking [icon].

4.1.1. TEM UI on the microscope PC. Click the blue button next to the desired grid and click "Load".

4.3.1. In the targeting box, the cursor icon (highlighted in red) should be chosen for the type of target you want to set.
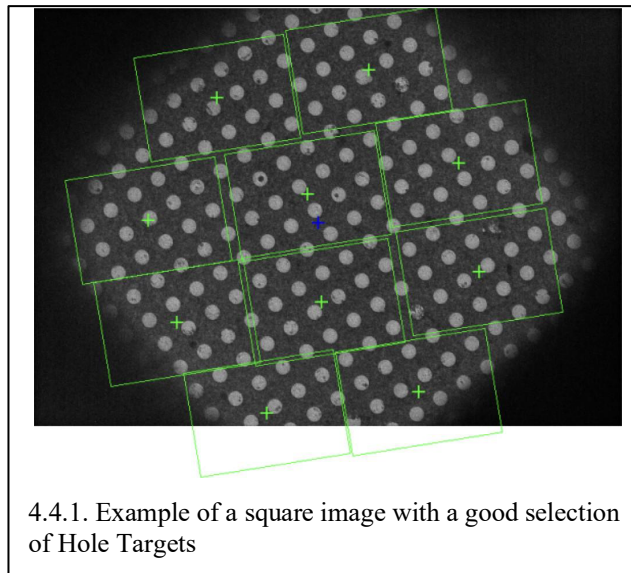
4.4. Hole Targeting

4.4.1. In the Hole_Targeting node, manually select Acquisition and Focus targets for each square (see figure 4.4.1)

4.4.1.1. Only one Focus target is required per square and can be anywhere over the square

4.4.1.2. Do not overlap acquisition targets.

4.4.1.3. Because "Queue up targets" has been enabled in this node, the microscope will take all submitted square targets before processing selected hole targets. Thus, for each square image, you can set and submit targets using the ▶ icon as they are being acquired.

4.4.2. Once you have submitted the acquisition and focus targets for every square, submit the queue using the 🔍 icon.

4.4.1. Example of a square image with a good selection of Hole Targets

4.5. Exposure Targeting - here we will use thresholding to allow automatic exposure targeting

4.5.1. When you have an image in the Exposure_Targeting node, activate the ruler 📏 and measure the diameter of a hole (left click and drag the mouse)

4.5.2. Open the **Template Settings (see figure)**: Input the final template diameter of the hole (add ~20% to the measure distance), then click "Test"

4.5.2.1. With the correct diameter, there should be a sharp, white dot at the center any good hole

4.5.3. Open the **Threshold Settings**: Input 1.0, Test

4.5.3.1. 1.0 is a good default and works well for gold, lower values (e.g. 0.6) may be required for thicker ice on carbon grids.

4.5.4. Open the **Blob Settings**: Border 10 or 20, Max blobs of at least 100, Max blob size of at least 10,000, Min blob size 50, Blob roundness 0.6.

4.5.4.1. These are general defaults. The maximum number of blobs needs to be high enough that you aren't eliminating holes, same with the maximum blob size. The minimum blob size and blob roundness are useful to discard false positives, especially with a lower threshold.

4.5.2 Click the settings button (top figure, hand holding a card, highlighted in red) to open the settings dialog (bottom figure) for each step of the automation process. You will check the settings for each step, starting from top to bottom.

4.5.5. Measure the distance between the centers of two adjacent (not opposite) holes using the ruler (📏).

4.5.6. Open the **Lattice Settings**: Input the distance as the Spacing
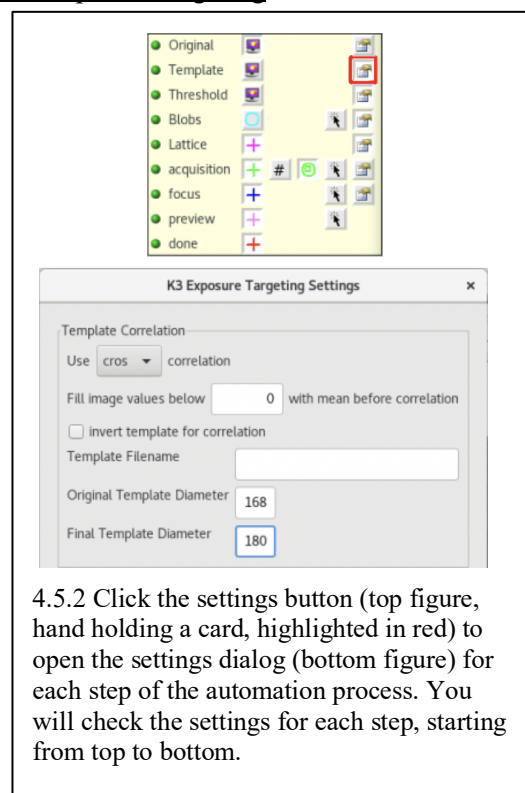
4.5.6.1. A Tolerance of 0.25 is a good default but you may need to raise it for more unusual grids, especially for carbon grids with low contrast between the carbon and the holes. 0.45-0.6 is a reasonable "high value" range.

4.5.6.2. Grids with especially low contrast can also make use of the Extend Lattice option by setting it to full. This will extrapolate based on the Lattice that Leginon is able to determine and should not be used unless necessary as it can also result in collecting on carbon if Leginon doesn't find the right anchor points for the lattice.

4.5.6.3. The Radius can be set to the actual radius or lower (as low as 30) in order to collect on holes near the edge of the hole target image.

4.5.6.4. The Reference Intensity only needs to be high enough that the mean thickness of the holes does not display as "0" when highlighted but it can be useful to set a consistent value (e.g. 2x the mean intensity) to make it easier to discern holes where the ice is too thin or too thick.

4.5.7. Set up the **Acquisition Settings** for automated exposure targeting.
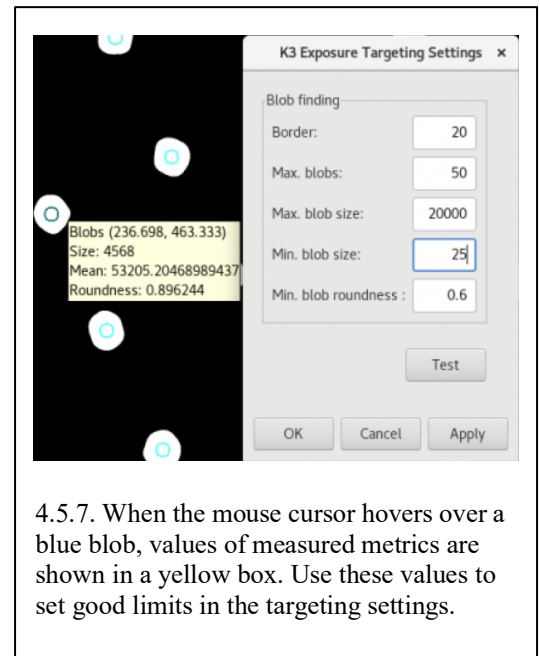
4.5.7. When the mouse cursor hovers over a blue blob, values of measured metrics are shown in a yellow box. Use these values to set good limits in the targeting settings.

4.5.7.1. Evaluate the following parameters for different holes by hovering over blobs with the mouse cursor (see figure).

4.5.7.2. Set parameters for the following:

Parameter	Purpose	Useful reference values
Min Mean	Set a lower limit on mean ice thickness to exclude empty or too thin holes	0.5 or higher
Max Mean	Set an upper limit on mean ice thickness, to exclude ice that is too thick	0.8 ± 0.04
stdev	Standard deviation of the intensity across the hole. Setting an upper limit is useful for eliminating holes that are cracked or have significant ice contamination.	A value between 0.13 and 0.16 often works well on a K3.

4.5.7.3. The target template is used to set acquisition targets relative to the lattice

4.5.7.4. The primary window is the Acquisition Target Template, which can be used to set one or more targets relative to the lattice, depending on whether or not multi-shot targeting or the use of an offset is desired, otherwise it is simplest to set a single target with no x or y offset (0,0)

4.5.7.5. The Focus Target Template is used to automatically place the focus target

4.5.7.5.1. The simplest method of setting a focus target is to measure the distance between the centers of two holes that are opposite one another (not adjacent)

4.5.7.5.2. With the Autofill option, you can input the radius (half the total distance) and the angle and focus targets will be automatically placed

4.5.7.5.3. While this will place multiple targets, only the one closest to the center will be used

4.5.7.6. Submit targets

4.5.8. Inspect settings and start Automated collection

    4.5.8.1.   If the exposure targeting settings are consistent for the first few hole images, you can turn off User Verification in the Exposure_Targeting node settings.

    4.5.8.2.   In Presets_Manager, enable Error Notification (clock icon:)

    4.5.8.3.   To speed up the collection, open the Settings for the exposure preset and enable "early return", listed as "Save 8x8 stats image"

4.6. <u>Shutdown</u>

    4.6.1. After data collection is complete

    4.6.2. In Preset Manager, uncheck Early Return

    4.6.3.In Exposure Targeting, check User Verification

    4.6.4. Close Leginon

    4.6.5. Close column valves and load grid 1, the cross grating.

5. **Chemicals:** N/A

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

7. **Appendix:** N/A