Microsc. Microanal. 28 (Suppl 1), 2022

Smart Leginon: Fully Automated Cryo-EM Grid Screening for CryoEM using Leginon and Ptolemy

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Single particle cryo-electron microscopy (cryo-EM) has grown by leaps and bounds over the past decade and is now an indispensable technique in structural biology. Automated data collection is an integral part of modern workflows in single particle cryo-EM. Leginon is a modular system for automatically acquiring images from a transmission electron microscope (TEM) that uses a multi-scale imaging strategy for data collection in which images at successively higher magnification are acquired by defining targets on the parent images [1].

An ideal cryo-EM grid contains a rich distribution of monodisperse particles in multiple orientations trapped in a thin layer of vitreous ice. However, it is notoriously difficult to produce ideal grids reproducibly, and in practice, cryo-EM grids often exhibit a wide range of ice and particle quality. Therefore, grids are usually screened to assess whether they are suitable for a full data collection session on high-end microscopes such as the Titan Krios. Unlike typical data collection workflow that attempts to capture all squares/holes in the pre-determined narrow condition on one grid, screening aims to sample a small number but more diverse conditions on many grids. In the Leginon screening workflow, the first step is to acquire an atlas montage at low magnification which provides information about the overall ice quality for the entire grid. Then a few squares are chosen based on the square size, which is a proxy for ice thickness, and higher magnification images are taken to assess the presence of vitreous or crystalline ice, contamination, and variations in ice thickness, among other characteristics. Finally, high magnification images are acquired from regions of ice spanning holes in the substrate so that the particle density can be assessed. The process previously required operator intervention to keep the sampling size small and diverse, adjusting settings of automated template-correlation-based hole finder for different grids as well as grid exchange and organizing the screening sessions. Smart Leginon integrates the Leginon workflow with a machine learning program, Ptolemy [2], to provide a much more adoptable automated square and hole finding and scoring. General Leginon features are also added to relieve all other user intervention mentioned above.

The Smart Leginon square and hole finder interface is shown in **Figure 1**. The inputs required are the maximum number of targets, the number of target groups to sample, a filter “blob” size used by the Ptolemy square finder, and a threshold for the Ptolemy hole finder. The session setup only takes a few minutes, after which it runs completely unattended for as many grids as desired. The session information can also be re-used so that no operator setup is required in future sessions.

To evaluate the performance of Smart Leginon for grid screening, we loaded 11 mouse apoferritin (mApof) gold grids (UltrAu R0.6/1.0) into a Glacios autoloader and set up collection with the parameters as shown in **Figure 2**. Smart Leginon screened all 11 grids automatically. Pre-processing was run simultaneously to provide motion correction and CTF estimation. Users can view the images and metadata using the Appion [3] web browser. The screening time for each grid was ~30 min including grid loading/unloading times. We show grid1 (**Figure 2A/B**) as an example: ALS ice thickness [4] ranged from 32 - 48 nm, CTF estimation resolution ranged from 4 - 8 Å (**Figure 2E/F**) and exceptional particle distribution (**Figure 2C**), all indicating that this grid is ready for data collection.

The ability of Smart Leginon to fully automate grid screening significantly reduces the burden on operator time and increases screening throughput.

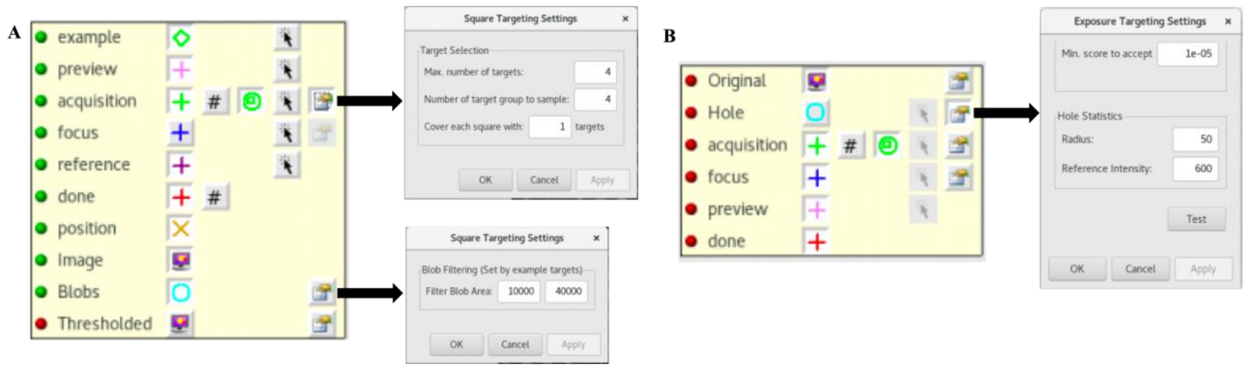

Figure 1. (**A**) Smart Leginon square finder and (**B**) hole finder interface.

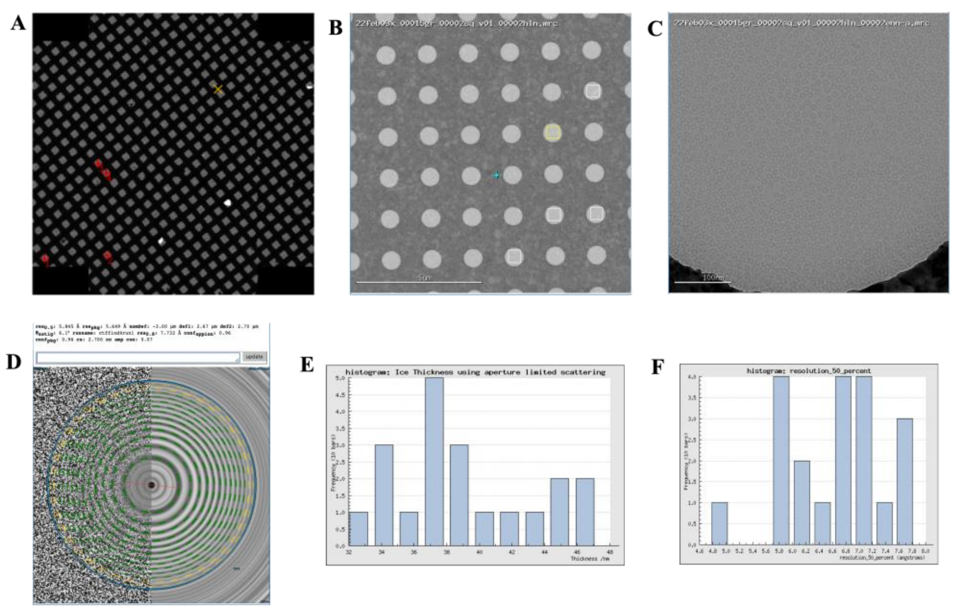

Figure 2. Imaging parameters for autoscreening grids: Glacios TEM, Falcon3-linear mode, 400 ms exposure time, 40 ms/frame, 55.59 $e^{-}/Å^{2}$ total dose, pixel size 1.204 Å/pixel, nominal defocus -3 µm. (**A**) The atlas of grid1 and the best four squares from four groups picked by Ptolemy. (**B**) Five holes randomly selected from all holes identified by Ptolemy. (**C**) Representative motion-corrected cryo-EM micrograph and (**D**) corresponding CTF estimation. (**E**) ALS ice thickness distribution and (**F**) $FSC_{0.5}$ resolution distribution for the set of images from this grid.

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https://doi.org/10.1017/S1431927622005232 Published online by Cambridge University Press