Data Collection Speedups in Leginon

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Cryo-EM has rapidly transformed into the tool of choice for determination of high-resolution structures and dynamics of biologically important molecules, sub-cellular organelles, and viruses. The hardware advancements in electron microscopes and image recording devices coupled with the software advancements in image processing have made determination of near-atomic resolution structures by cryo-EM almost routine for well-behaved samples. Given the high instrumental, operational and maintenance costs associated with this technology, it is important to increase overall throughput and further accelerate user research and turnover.

Leginon [1] is an automated system that uses a multi-scale imaging strategy to acquire images from a transmission electron microscope (TEM). Images at each higher magnification are acquired by defining targets on the parent images. Table 1 lists the presets and corresponding magnifications that have been typically used for data collection at SEMC. One of the most time-consuming steps in Leginon is the determination of eucentric height (Z-focus), which takes about 2 minutes to complete and is performed every time the stage is moved to a new area of a square. Using a lower magnification (940x instead of 2250x) for acquiring the square images provides a larger field of view and reduces the number of times that the Z-focus is determined. In Figure 1 we provide an example where we can collect 167 square targets vs. only 49 square targets by implementing this lower magnification. This strategy eliminates 118 Z-focus cycles corresponding to ~4h of time.

Using beam-image shifts, rather than stage shifts [2], to move to a selected hole target improves targeting speed and accuracy. To maximize the number of hole targets accessible by beam-image shift for each stage movement, we reduced the magnification of the hole image from 3,600x to 2,250x and used extended beam-image shift of up to 13 µm to image all the hole targets. Figure 2 shows an example of using this strategy where ~130 targets/stage movement can be acquired using a hole magnification of 2,250x versus ~50 targets/stage movement at a magnification of 3600x. The sample was prepared on an UltrAuFoil R0.6/1.0 grid. Implementation of extended beam-image shift resulted in a collection of 420 movies/hour vs. 303 movies/hour were collected using the previous settings. This represents a ~40% increase in throughput.

Some of the aberrations, such as coma and astigmatism, arising from beam-image shifts are corrected in Leginon during data acquisition but very high beam-image shifts need further correction using post-processing software. As a proof of principle, we acquired images of mApOF at 0.844 Å/pixel and compared the final map quality as a function of the degree of beam-image shift. The images were sorted into three groups (all images; images with beam-image shift < 7 µm; images with beam-image shift >7µm) and processed independently in Cryosparc [3]. As shown in Figure 2, high beam-image shift (>7µm) does negatively impact the overall final resolution prior to software correction. We used the
Tiltgroup Wrangler program [1], integrated into Appion [4], to sort the images into 99 groups based on their beam-image tilt X/Y values. After grouping, global CTF refinements were performed for each group of particles which resulted in a map resolution Nyquist, even for the highest beam-image shift values.

We conclude that the implementation of low square magnification Z-focus determination in conjunction with extended beam-image shift targeting in Leginon results in significantly improved data-collection throughput without compromising the quality of the data.

<table>
<thead>
<tr>
<th>Preset</th>
<th>Magnification (old settings)</th>
<th>Magnification (new settings)</th>
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</thead>
<tbody>
<tr>
<td>gr</td>
<td>1550x</td>
<td>1550x</td>
</tr>
<tr>
<td>sq</td>
<td>2250x</td>
<td>940x</td>
</tr>
<tr>
<td>hln</td>
<td>3600x</td>
<td>2250x</td>
</tr>
<tr>
<td>enn</td>
<td>81,000x (~1.1 Å/pix)</td>
<td>81,000x (~1.1 Å/pix)</td>
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<tr>
<td></td>
<td>105,000x (~0.8 Å/pix)</td>
<td>105,000x (~0.8 Å/pix)</td>
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**Table 1.** The presets and corresponding magnification that are typically used for data collection at the Simons Electron Microscopy Center.

![Figure 1](https://doi.org/10.1017/S1431927622005220) Published online by Cambridge University Press

*Figure 1.* Multi-scale targeting for (A) previous typically used magnifications and (B) new settings described here that improve throughput.
Figure 2. Imaging parameters for data collection: Krios, K3, counting mode, dose rate 20 e⁻/pixel/s, 1.6 s exposure time, 40 ms/frame, 44.90 e⁻/Å² total dose, pixel size 0.844 Å/pixel, nominal defocus -0.8 to -2.5 μm. (A) Representative 2,250X hole magnification image, motion-corrected high magnification movie, and corresponding ctf estimation. The FSC₀.₁₄₃ resolution for all particles, particles with beam-image shift >7 μm and particles with beam-image shift < 7 μm before (B) and after (C) grouping and global CTF refinement.

References: