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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 instrumentational, 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. Table1 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.

Preset	Magnification (old settings)	Magnification (new settings)
gr	1550x	1550x
sq	2250x	940x
hln	3600x	2250x
enn	81,000x (~1.1 Å/pix) 105,000x (~0.8 Å/pix)	81,000x (~1.1 Å/pix) 105,000x (~0.8 Å/pix)

Table 1. The presets and corresponding magnification that are typically used for data collection at the Simons Electron Microscopy Center.

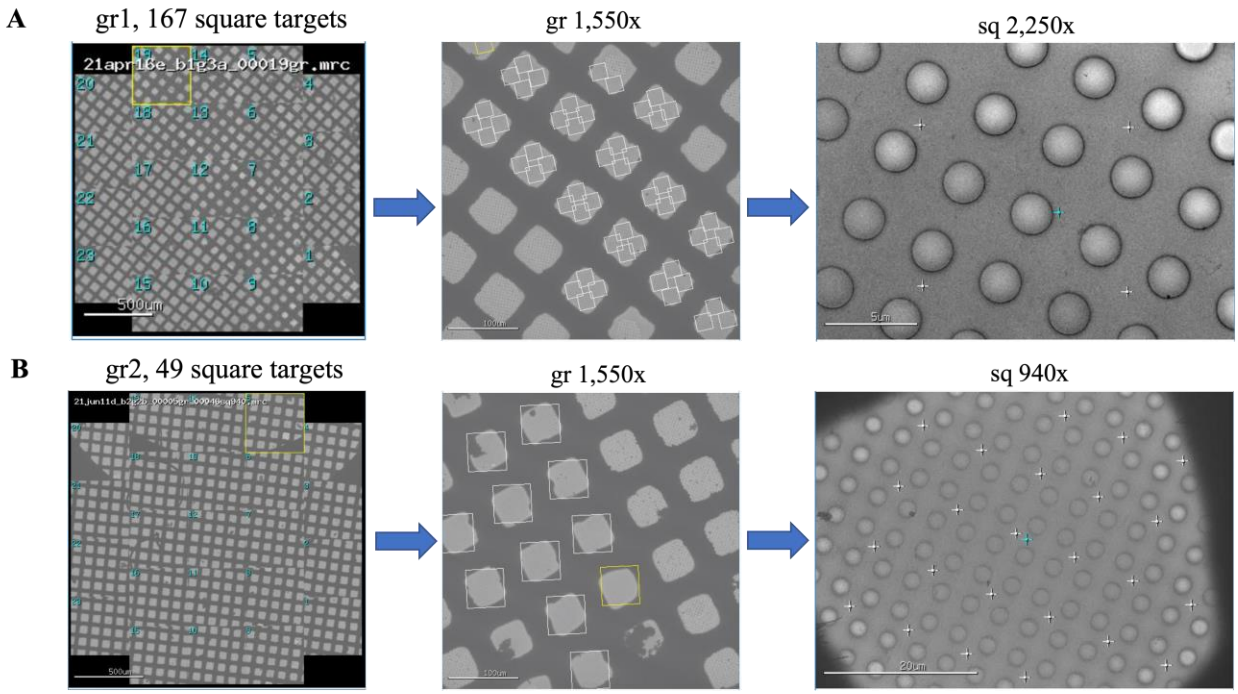

Figure 1. Multi-scale targeting for (**A**) previous typically used magnifications and (**B**) new settings described here that improve throughput.

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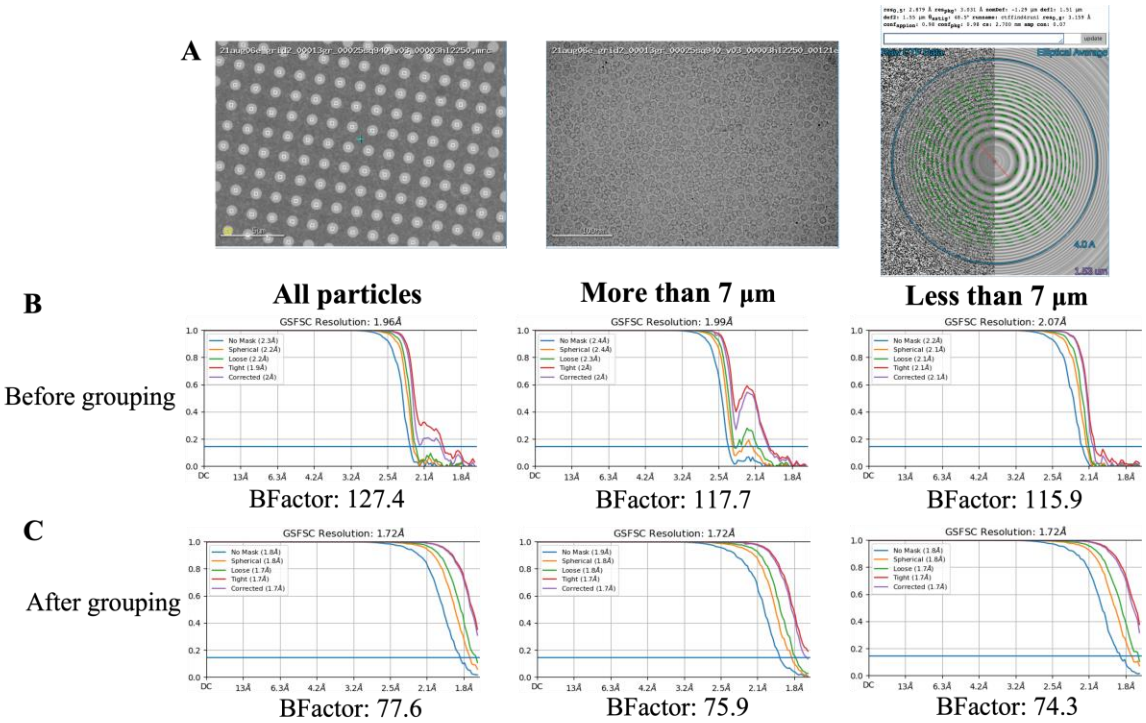

Figure 2. Imaging parameters for data collection: Krios, K3, counting mode, dose rate 20 e^{-}/pixel/s, 1.6 s exposure time, 40 ms/frame, 44.90 $e^{-}/Å^2$ total dose, pixel size 0.844 Å/pixel, nominal defocus -0.8 to -2.5 µm. (**A**) Representative 2,250X hole magnification image, motion-corrected high magnification movie, and corresponding ctf estimation. The $FSC_{0.143}$ 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:

[1] A Cheng et al., Protein Sci **30** (2021), p. 136. doi: 10.1002/pro.3967
[2] A Cheng et al., Journal of structural biology **204**(2) (2018), p. 270.
[3] A Punjani et al., Nature methods **14**(3) (2017), p. 290.
[4] GC Lander et al., J Struct Biol. **166**(1) (2009). PMID: 19263523

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