doi:10.1017/S1431927621001501
Microsc. Microanal. 27 (Suppl 1), 2021

Routine collection of 10,000 direct detector movies a day using Leginon

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The growth of single particle cryo-electron microscopy (cryo-EM) over the past decade has now established it as a useful technique for structural biological research. The hardware developments in electron microscopes and image recording devices coupled with the software developments 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 becomes necessary to increase the overall throughput that could further accelerate throughput of users and research projects.

To address this challenge we have set a goal to establish a workflow to allow data collection of over 10,000 direct detector movies a day. The test sample used for these studies was mouse apoferritin (mApof) at a concentration of 8 mg/mL blotted on a UltrAuFoil R1.2/1.3 and vitrified in liquid ethane. Micrographs were collected in "counting mode" on a Gatan K3 camera mounted on a Titan Krios (G2i) operating at 300 kV [1]. Imaging conditions were: exposure time 600 ms/movie, frame rate 20 ms, dose rate 12 e-/pixel/sec, total dose 42 e-/Å2. A total of 10,691 high magnification images were collected in a continuous 24 h period excluding setup time of 1 h.

The high throughput collection (445 images/h) can be attributed to three main developments:

1. *Gatan K2 camera upgraded to K3 camera.*

The K3 has a higher DQE, larger field of view (24 megapixels, 5,760 x 4,092) corresponding to 1.6x increase over the K2 camera, and faster readout rate (1,500 full frames per second) or 3.75x speedup relative to the K2 camera) [2].

2. *Improving speed of automated data collection software package Leginon*[3].

In addition to using beam image-shift targeting (Figure 1A) to acquire data we also minimized the overhead of processing time (Figure 1B) in Leginon as described in [4-5]. The Leginon Client on the camera computer was modified to produce an 8x8 pixel image instead of returning the full 24 megapixel image array. This compressed 8x8 image preservesthe mean and standard deviation of the full array, and thus can be used for calculating ice thickness [6] or to detect changes in beam intensity. In addition, Leginon skips dark and gain correction on images of these compressed arrays, further reducing processing time, while still recording the associated dark and normalization images so that movies can be gain corrected after data collection.

3. *Data acquired at a higher magnification (pixel size: 0.4034 Å/pix).*

At sub-angstrom pixel sizes we can use a smaller illumination area and implement multi-hole targeting (Figure 1A). To optimize the data collection efficiency, beam-image shift of up to 7 μmwas used resulting in the acquisition of 64 high mag images per stage movement (Figure 1A). Aberrations, especially coma, arising from image shift targeting were corrected in Leginon [3] and can also be further corrected in Relion [7].

To assess the effects of the large image-beam shift reconstructions were calculated using both the full dataset as well as a restricted set of images corresponding to only moderate image shift. The resultant maps normalized for particle number have comparable resolutions. For the entire dataset of 10,691 high magnification images, a ~1.67 Å structure of apoferritin was obtained (Figure 1D, 1Eand 1F). This result demonstrates that atomic-resolution structure determination can be achieved by single-particle cryo-EM within a day of automated data collection and that 10,000 images a day is feasible. The high throughput collection strategies will be of interest to researchers wanting to routinely use cryoEM as their structural biological method of choice.

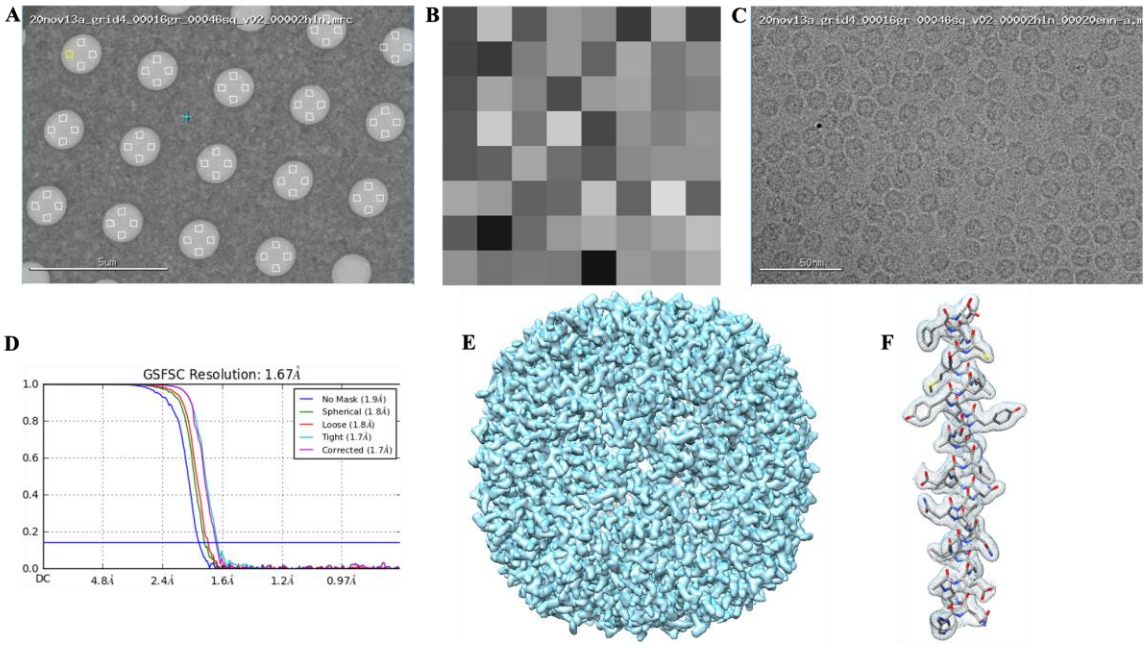

Figure 1. (A) Image shift with 16 holes (4 shots per hole) per stage shift provides ~10,000 high magnification image per 24 hours. (B) Representative8x8 pixel compressed image. (C) Representative motion-corrected cryo-EM micrograph. (D) Gold standard FSC of mApoF indicating a resolution of 1.67Å. (E) Isosurface representation of the mApoF map. (F) An α-helicalsegment from one subunit (PDB: 6v21) is shown docked into thecorresponding region of the reconstruction.

References

[1] This work was performed at the Simons Electron Microscopy Center and National Resource for Automated Molecular Microscopy located at the New York Structural Biology Center (NYSBC), supported by grants from the Simons Foundation (349247), NYSTAR, and the NIH (GM103310). We thank the Columbia University Cryo-Electron Microscopy Center for access to time on their instrument housed at NYSBC.

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https://doi.org/10.1017/S1431927621001501 Published online by Cambridge University Press

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https://doi.org/10.1017/S1431927621001501 Published online by Cambridge University Press