2662
doi:10.1017/S1431927619014041
Microsc. Microanal. 25 (Suppl 2), 2019

Best Practices at the National Center for CryoEM Access and Training

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The mission of NCCAT (National Center for CryoEM Access and Training) is twofold: to provide nationwide access to advanced cryogenic electron microscopy (cryoEM) technical capabilities, and to assist users in the development of cryoEM skills needed for independent research. NCCAT provides access to state-of-the-art equipment required to solve structures to the highest possible resolution using cryoEM methods. Our categories of access include: 1) access to high-end cryoEM instrumentation, 2) access to sample preparation and screening microscopes, and 3) embedded training to train scientists to become independent cryoEM researchers, or 4) training individuals who are responsible for managing, leading or training at their own facilities.

At the center we aim to use the most current best practices to assist researchers with access to new technologies and accelerate their research. The study of protein structure by cryoEM has advanced to the point where near-atomic resolution of suitable specimens are now commonly obtained, thanks largely to improvements in detectors, instrument stability, and image processing software [1]. Software for automated data collection, such as Leginon, has been in the field for over a decade [2] and is now used widely to facilitate single-particle averaging (SPA) approaches to reconstructing three-dimensional (3D) volumes of biological samples. Large numbers of particle images may be required to overcome the very low signal-to-noise ratio of cryoEM images; typically, from 1000-10000 images may be required for a single reconstruction. In particular, for cases where sufficient sample optimization is not possible, due to the source material being precious or limiting, it is imperative to exhaustively collect on available samples to push research projects forward. NCCAT offers beam-image shift modes to allow maximal numbers of high resolution cryoTEM SPA images to be obtained within a data collection session [3].

Leginon has the ability to minimize the coma and astigmatism introduced during this mode of data collection with image shifts >15µm from the optical axis. From our internal tests we can acquire an image every ~20s and still readily reach sub-3Å resolutions for single particle reconstructions using a Krios/K2 configuration [Figure 1]. We anticipate a further increase in throughput when using the new K3 cameras.

While instrumentation has greatly improved, sample preparation remains a significant bottleneck to the generation of high-resolution structures. The method of preparing vitrified samples by blotting followed by plunging into liquid ethane or liquid propane has changed little in over 20 years apart from the development of semi-automated blotting devices. This technique takes expertise to master and can produce a range of ice thicknesses and outcomes. To improve the reproducibility of vitrification, NCCAT provides access to a new method for making cryoEM grids. This method uses a commercial version (Chameleon from TTP Labtech, Inc.) of robotic device, called Spotiton [4,5], to dispense small volumes (tens of pL) of sample using a piezo electric nozzle, onto specialized grids that are essentially

"self-blotting" [6]. The copper surface of the grids is covered with nanowires which act to rapidly wick away sample, leaving behind a thin film that is then rapidly plunged into liquid ethane to achieve vitrification. A side benefit of this approach is that some of the deleterious effects of vitrification caused by samples contacting the air-water interface appear to be ameliorated, presumably as a result of reducing the dwell time of the sample in the thin liquid film formed just prior to vitrification.

It was recently demonstrated that most particles adhere to one or both air-water interfaces, which makes accurate determination of CTF challenging if the layer of vitreous ice is significantly thicker than the protein diameter [7]. The ability to determine ice thickness routinely during screening or data collection provides a helpful guide to deciding which areas to image or indeed whether further imaging on a grid is advisable. We have implemented routine ice thickness measurements, using either an energy filter or scattering outside the objective aperture, into our Leginon workflow and the results are automatically displayed in our web-based session summaries [8].

Taken together, NCCAT's utilization of these technologies and best practices lower the barriers of access for biomedical researchers to make use of cryoEM, and raise the capacity and competency of our users to incorporate these techniques into their own research programs [9].

References:

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[9] This work was performed at the National Center for CryoEM Access and Training (NCCAT) and the Simons Electron Microscopy Center located at the New York Structural Biology Center, supported by the NIH Common Fund Transformative High Resolution Cryo-Electron Microscopy program (U24 GM129539), and by grants from the Simons Foundation (SF349247) and NY State.

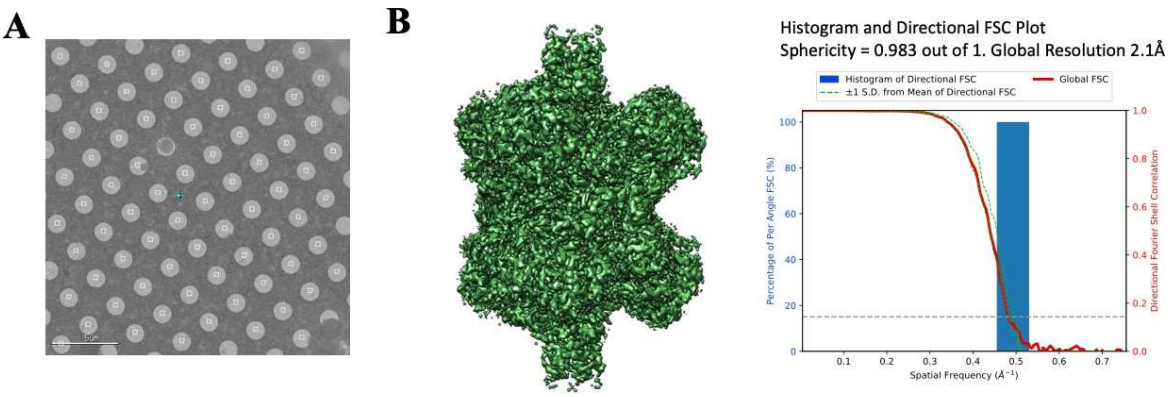

Figure 1. Example of 15 µm image shift data collection. A) Image shift with 70 holes per stage shift provides a high magnification image every 20 seconds or ~4250 images per day. B) Resulting cryoEM reconstruction and 3D FSC of GDH indicating a resolution of 2.1Å.