Implementing 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 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. At the center, we bring the most current best practices to assist researchers access new technologies and accelerate their research. In particular, a significant bottleneck to the generation of high-resolution structures is sample preparation. The method of preparing vitrified samples by blotting followed by plunging into liquid ethane or propane has changed little in the past couple of decades apart from the development of automated blotting devices. This technique takes expertise to master and can produce a range of ice thicknesses. 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 should be abandoned. Towards that goal, we have implemented a way to measure ice thickness directly into our data acquisition and processing workflow. In addition, to aid with the reproducibility of this technique NCCAT has access to a new type of grid that is essentially "self-blotting" and can be used in conjunction with a robotic device that dispenses small volumes (tens of pL) of a sample using a piezoelectric nozzle. When small volumes of sample are applied to these grids it is rapidly wicked away, leaving behind a thin film that is then rapidly vitrified, which may assist with samples intractable by traditional methods. Taken together we aim to lower the barriers of access and cross-train biomedical researchers to broadly utilize cryoEM techniques.