

## The NCI National Cryo-EM Facility

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Over the last several years, the field of cryo-EM has undergone a resolution revolution. The data generated by cryo-EM imaging is now at high enough resolution to directly generate atomic models for proteins and other biological macromolecules. This puts cryo-EM at the same level with other high-resolution structural biology modalities such as X-ray crystallography and nuclear magnetic resonance spectroscopy.

This resolution revolution has been driven by several factors, including advances in microscope and camera technology, as well as improved computational methods for analyzing single particle cryo-EM data. Access to the latest microscope and detector technologies, which can be prohibitively expensive for many institutions is critical for acquiring the best quality data, and generating the highest resolution structures by this method. The NCI has created the National Cryo-EM Facility (NCEF) to meet the needs of cancer researchers in academic labs who do not have adequate access to these instruments. NCEF currently houses two Titan Krios microscopes, each equipped with a phase plate, a Falcon 3EC direct detector and a K3/K2 Summit direct electron detector at the end of a Gatan imaging filter. Automated imaging on the K2 and K3 cameras is performed using either Latitude S (Gatan, Inc.) or SerialEM software.

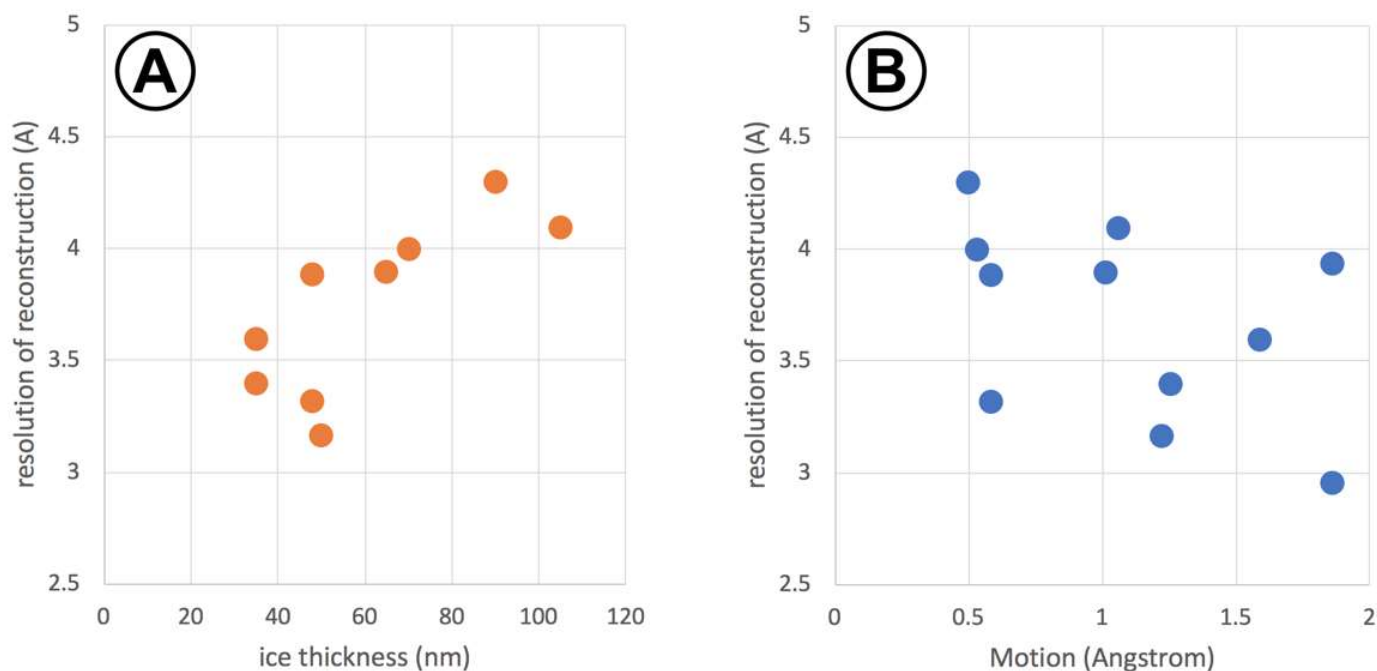
The queue at NCEF is designed to minimize wait time for users, optimize imaging time, and maximize efficiency. To minimize wait time, only one user project from each lab is in the active queue at any one time and data collection is restricted to 48 hours, unless technical problems with the microscope lead to loss of data collection time. Users are encouraged to ship their samples rather than visit in person, so travel coordination can be reduced, and the queue is as flexible as possible in case of instrument issues. Data runs are set up with the users directly engaged via video conferencing. Remote access to images of the data being collected and on-the-fly feedback on imaging during the run will soon be available to users. NCEF has collected data for 50 independent research groups across the US, and over the calendar year of 2018 has used 83% of the time for user data collection, with 5% for testing purposes and technical development, 8% for cryocycles and other preventive maintenance. Carefully planned scheduling of cryocycles and preventive maintenance enabled us to keep the downtime from instrument problems to <4%. Tracking the time that the column valve stayed open showed that the imaging time was > 95% over the time period that the microscope was useable for data collection.

One of the continuing projects at NCEF is to use the general characteristics of the data collected on various projects to better understand trends such as the influence of sample quality on data quality as well as the parameters that affect optimizing collecting the highest quality of images from a given sample. Beyond the typical image parameters, NCEF estimates the average ice thickness of the samples that are imaged. As publications that report structures determined at NCEF are published, we noticed a measurable correlation of reported resolution with ice thickness, with thinner ice clearly correlated with higher resolution. There was no obvious correlation between the extent of motion in the movie frames and the

reported resolution, indicating that the software of motion correction available to most users is good enough to compensate for beam-induced and stage motion that occurs during data collection [1].

#### References:

[1] This project has been funded with Federal funds from the Frederick National Laboratory for Cancer Research, National Institutes of Health, under contract HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products or organizations imply endorsement by the US Government.



**Figure 1.** Correlation between ice thickness and reported resolution of final reconstruction in papers published using data collected at NCEF in the last 12 months. (A) published final resolution of reconstruction plotted over the peak of the ice thickness distribution of the corresponding data collection run and (B) published final resolution plotted over the average motion of the first frame of the corresponding data collection run.