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Improving Cryo-EM Ice Thicknesses Workflows on the Chameleon Sample Preparation Device

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Sample preparation for cryo-EM imaging involves suspending macromolecules in a film of vitreous ice that is simultaneously thin enough for electron transmission and thick enough to embed the molecule of interest. For most macromolecules, a 20-100 nm thick ice layer is ideal [1]. On a chameleon sample preparation device [2], picolitre droplets of the sample are dispensed onto a glow discharged nanowire grid as it flies past on the way to plunging into liquid ethane. Excess liquid is wicked away by the nanowires during the travel time, leaving behind a thin film of liquid suspended across the grid foil that is then vitrified in liquid ethane [3]. A video of the wicking process is made available for the user to examine, and the grid accepted or rejected based on the user's experience of likely outcomes in the TEM.

The current sample preparation workflow starts with the preparation of multiple grids using a range of grid-making conditions. The grids are then screened in a TEM and different grid squares imaged to find suitable ice thicknesses containing the sample of interest. This information is taken back to the sample preparation stage and the process repeated iteratively until a grid with ideal ice thickness and sample concentration is obtained. This process is time consuming and the results sometimes unpredictable.

Here, we present our efforts on the chameleon to (i) obtain more desirable and consistent ice thicknesses, and (ii) to improve ice thickness estimations from the chameleon videos. Fundamental to our efforts is the ability to measure ice thicknesses with aperture limited scattering in a TEM [4], and the storage of this ice thickness information in the Leginon database [5]. By then mining the database, we can analyze ice thickness data from the grids of interest. With this, we first benchmark the performance of our chameleon plunge freezing device for its ability to produce consistent, reproducible, and desirable ice. We vary grid-making parameters systematically to characterize their contributions to the resulting ice thickness measured in a TEM. Second, we correlate TEM ice thickness information with videos captured by the chameleon during plunging. By tying together information from the TEM to the chameleon videos, we may be able to better estimate the likely ice thickness outcomes at the sample preparation stage prior to clipping and inserting the grid in the TEM.

Our work leverages data in the Leginon database to characterize ice thickness workflows on the chameleon. We suggest ways in which sample preparation workflows on the chameleon and other plunge freezing devices might be made better, cheaper, and faster.

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