MeasureIce: Accessible Ice Thickness Measurement for Single Particle Cryogenic Transmission Electron Microscopy

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The development of direct electron detectors and automated cryo-EM instruments has catapulted single particle cryo-EM from a niche field to a widely used characterization technique in structural biology [1]. Advances in microscope automation and data processing mean that it is often possible to go from a vitrified sample to a few Ångstrom resolution structure in a few hours [2]. However, the process of cryo-EM sample optimization remains an often time consuming and tedious process where multiple samples are prepared and screened in a cheaper, low-end TEM to gauge the presence of a pure and sufficiently concentrated protein sample in thin (< 50 nm), vitreous ice [3]. Thin ice is particularly important since the amorphous atomic structure of the ice is imprinted on the electron wave-front, resulting in a lower effective signal-to-noise ratio in the images [4]. This means thick ice makes 3D reconstruction of the particle impossible in the worst case and deteriorates eventual model resolution in the best case.

To speed the preparation of optimal cryo-EM samples we have developed MeasureIce, a software that enables on-the-fly ice thickness measurement on a basic screening TEM by an inexperienced microscopist without requiring time-consuming experimental calibration the TEM [5]. The basis of the approach is the aperture-limited scattering (ALS) technique where in-focus TEM images are recorded with the objective aperture inserted, see Fig. 1(a) [6]. Thicker ice scatters more electrons to high angles where they are blocked by the objective aperture in the back focal plane so regions with thicker ice appear darker in these images. The advantage of this technique is that ice thickness can be measured directly from single low (< 10,000 x magnification) images on a basic screening TEM without expensive add-ons such as an energy filter. We by-passed the need to experimentally calibration the relationship between TEM image intensity and ice thickness by instead simulating look-up tables using multislice simulations of a realistic ice model generated by the GROMACs molecular dynamics package [7] coupled with recent measurements of the inelastic mean free path of electrons in water [8]. This approach agrees well with independent experiment measurements (achieved via the ice channel approach, see [9]) for different sized objective apertures and electron beam accelerating voltages, see Fig. 1 (b).

The code for simulating ice thickness – image intensity look-up tables and for an easy to use GUI, see Fig. 2, that can be installed in microscope control and support PCs for on-the fly ice thickness measurement is hosted on GitHub [9]. We will discuss examples were MeasureIce has assisted in screening of grids before final data acquisition on a Titan Krios cryo-EM and ongoing work to implement the approach within software tools such as serial-EM for more streamlined and automated approaches to cryo-EM grid screening. Finally, as a demonstration of the benefits of quantitative ice thickness we present the reconstruction of an Equine apo-ferritin standard prepared on gold quantifoil to

1.9 Ångstrom resolution from foil holes determined by MeasureIce to be < 15 nm an improvement on the previous best 2.1 Ångstrom resolution reconstructions of this molecule [5, 10].
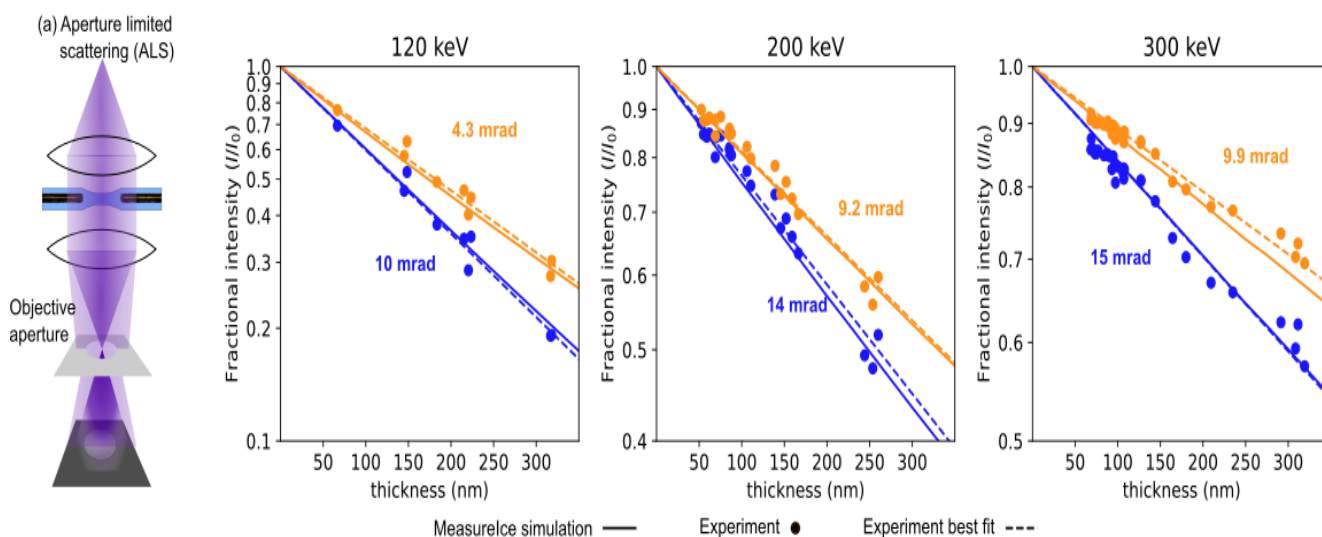

Figure 1. (a) The aperture limited scattering (ALS) approach to quantitative ice thickness measurement where the TEM objective aperture blocks electrons scattered by the ice making thicker regions of ice darker. The MeasureIce approach simulates look up tables which relate image intensity to ice thickness (b), which compare well with experimental benchmarks obtained using the "ice channel" approach (Experiment).
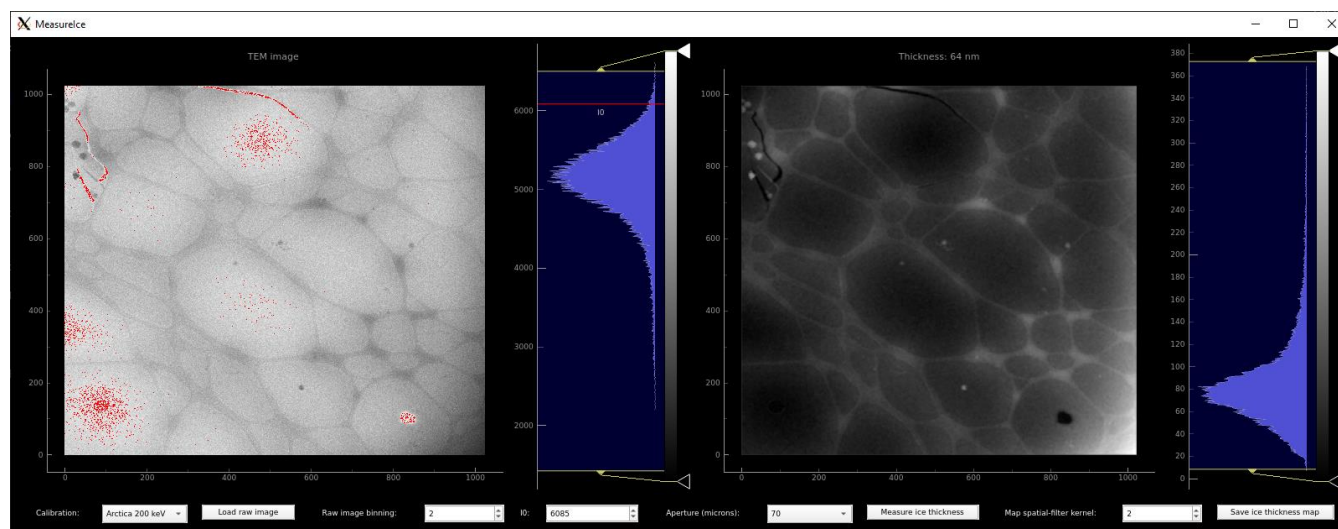

Figure 2. The MeasureIce GUI for measuring ice thickness from TEM images using pre-simulated look up tables. The raw image is loaded and displayed on the left, the user must input the reference intensity I0 (ie. the image intensity measured in the absence of sample in the beam path). The generated ice thickness map is shown on the right and ice thickness can be read off by hovering the mouse pointer over different parts of the thickness map.

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