doi:10.1017/S1431927618004981
Microsc. Microanal. 24 (Suppl 1), 2018

Routine Determination of Ice Thickness by Energy Filtration

William J. Rice[1], Anchi Cheng[1], Alex J. Noble[1], Edward T. Eng[1], Laura Y. Kim[1], Bridget Carragher[1,2] and Clinton S. Potter[1,2]

[1.] National Resource for Automated Molecular Microscopy, Simons Electron Microscopy Center, New York Structural Biology Center, NY.
[2.] Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY.

The study of protein structure by cryo-electron microscopy (cryo-EM) has advanced to the point where structures of 4 Å or better resolution 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 and SerialEM, has been in the field for over a decade [2,3]. With these advances, particle numbers in the range of 10^5-10^6 are now routinely obtained. Despite these instrumentation improvements, sample preparation remains a significant bottleneck to the generation of high resolution structures. Although some new approaches are under development [4,5,6], 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 automated blotting devices. This technique takes some expertise to master and can produce a range of ice thicknesses. Moreover, it was recently demonstrated that most particles adhere to one or both air-water interfaces, which can produce a wide range of defocus if the ice is significantly thicker than the protein size [7]. The ability to determine ice thickness routinely during screening or data collection would provide a helpful guide to deciding which areas to image or indeed whether further imaging on a grid should be abandoned.

Several techniques are available for measuring ice thickness. Collecting a tilt series of an area and calculating a tomogram is perhaps the most accurate but also the most time consuming [7]. A second technique which can produce more direct results but requires tilting the stage twice and burning a small hole is also not easily incorporated into a high throughput automated workflow [8]. For microscopes equipped with an energy filter, a more direct way is to compare intensities of images taken with and without the slit inserted [9]. The thickness can be directly calculated using the following equation:

$$d = \Lambda \ln \frac{I}{I_{zlp}}$$

where d is the thickness, Λ is the mean free path for inelastic scattering, I is the total image intensity, and I_{zlp} is the intensity of the zero-loss peak energy filtered image. The main parameter which needs to be determined is the mean free path, which will depend on the voltage used as well as the objective aperture diameter.

We determined the mean free path for inelastic scattering on our Bioquantum Titan Krios electron microscopes by collecting total and zero loss images over several holes, then collecting tomograms over these same holes and measuring ice thickness. For imaging at 300 keV and a 100 µm objective aperture, we determined the mean free path for inelastic scattering to be 395 nm (Figure 1). Using this value, ice thickness can be readily determined under these imaging conditions. We have implemented this technique directly into our Leginon workflow and the results are automatically displayed in the Appion session summary (Figure 2).

For microscopes without an energy filter, we also added an estimate for determining ice thickness based on scattering outside of the objective aperture (Figure 2). For this we needed to characterize the scattering outside of the objective aperture for each microscope and imaging condition using similar tomographic techniques [10].

References:

[1] L.A. Earl *et al*, Current Opinion in Structural Biology **46** (2017), p.71.
[2] C. Sulloway *et al*, Journal of Structural Biology **159** (2007), p. 335.
[3] D. N. Mastronarde, Journal of Structural Biology **152** (2005), p. 36.
[4] M. Adrian *et al*, Nature **308** (1984), p. 32.
[5] V. Dandey *et al*, Journal of Structural Biology (2018), in press
[6] X. Feng *et al*, Structure **25** (2017), p. 663.
[7] A. Noble *et al*, bioRxiv 230276 (2017).
[8] I. Angert *et al*, Ultramicroscopy **63** (1996), p.181.
[9 R. Brydson in "Electron Energy Loss Spectroscopy" (Taylor & Francis, New York) p.100.

[10] This work was supported by grants from the Simons Foundation (SF349247), NYSTAR, NIH (GM103310, OD019994-01) and the Agouron Institute (F00316).

Fig. 1

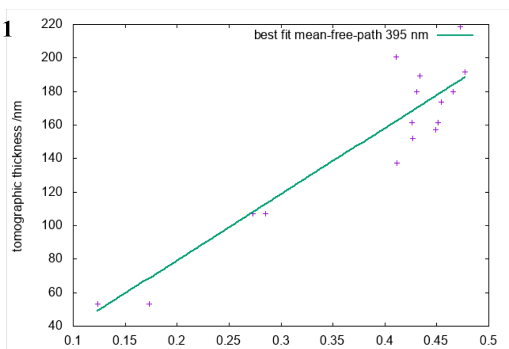

Fig. 2a

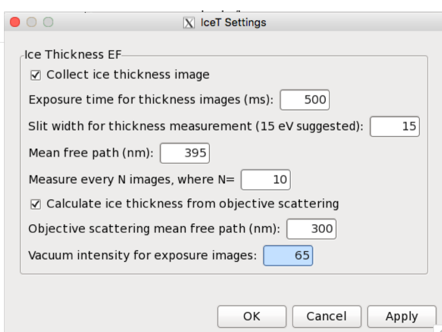

Fig. 2b

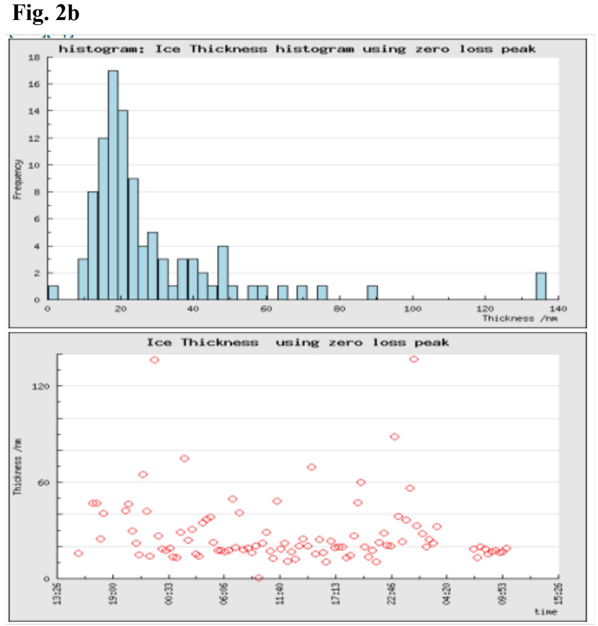

Figure 1. Plot of thickness, as determined by tomography, versus log I_0/I. The green line shows a least squares best fit with slope 395 nm.

Figure 2. (a) Leginon control panel for measuring ice thickness and (b) Appion summary of results from a typical session. The user can control how often thickness is measured as well as all required parameters. The summary shows a histogram and a plot of thickness over time.

https://doi.org/10.1017/S1431927618004981 Published online by Cambridge University Press