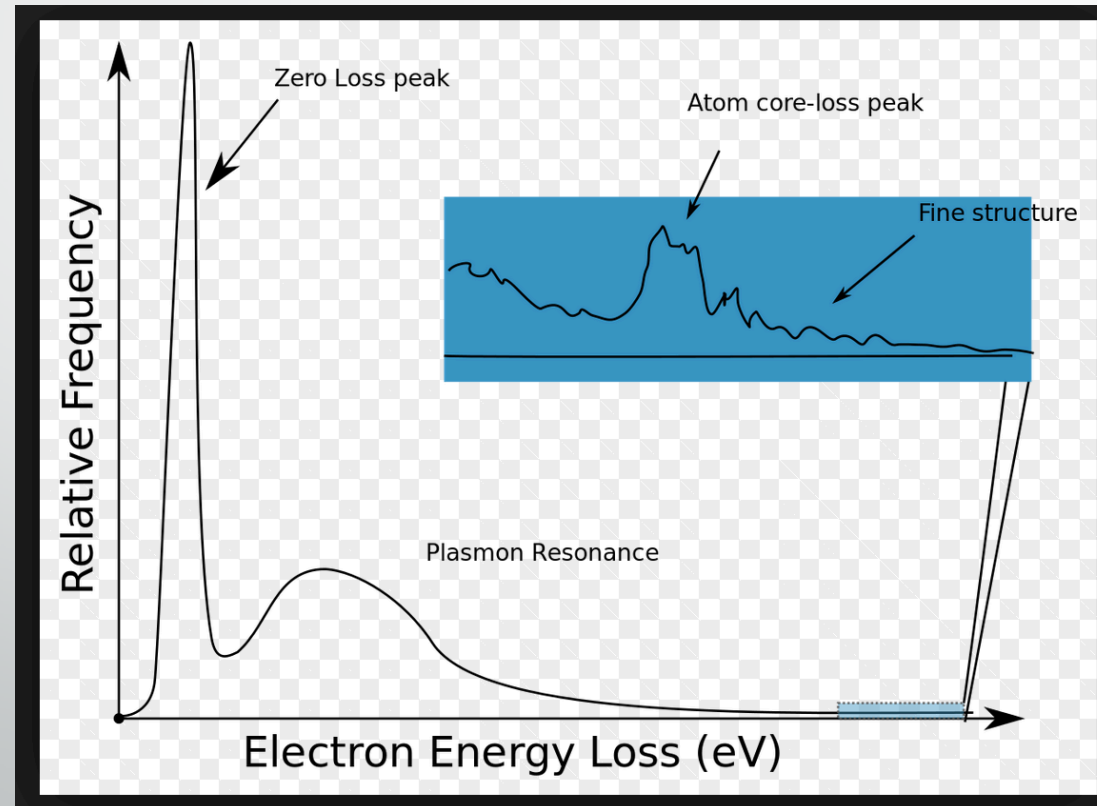


Routine Determination of Ice Thickness in Cryo-EM Samples

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Energy Filter Separates Images into Energy Loss Spectra



Thickness can be determined by integrating entire spectrum and zero loss peak:

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

Λ : mean free path for inelastic scattering through ice

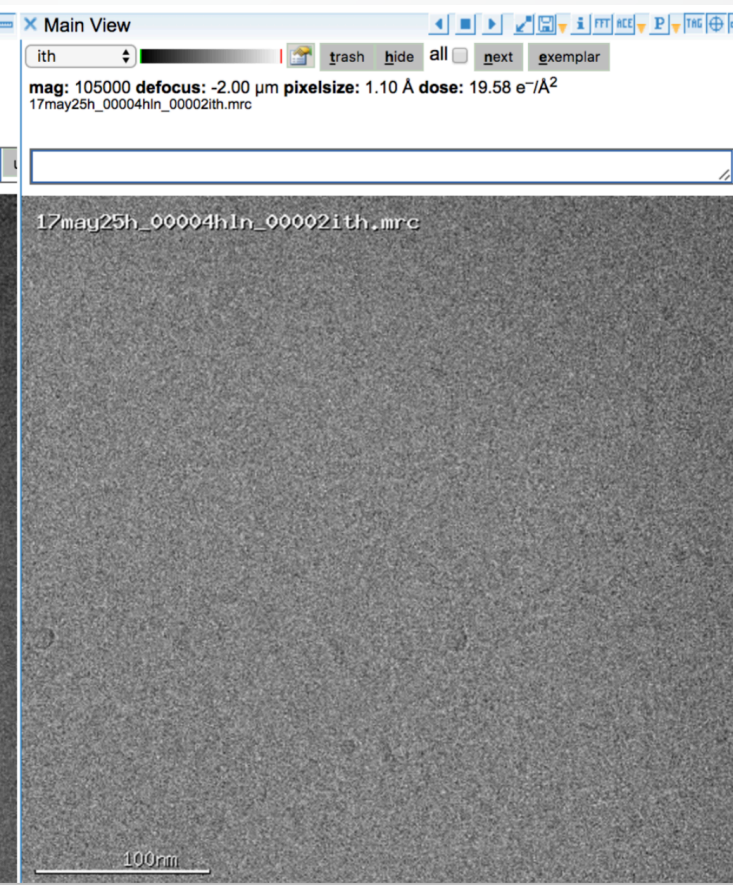
Image Source: Wikipedia

Take images with and without slit to get I_{zlp}
and I

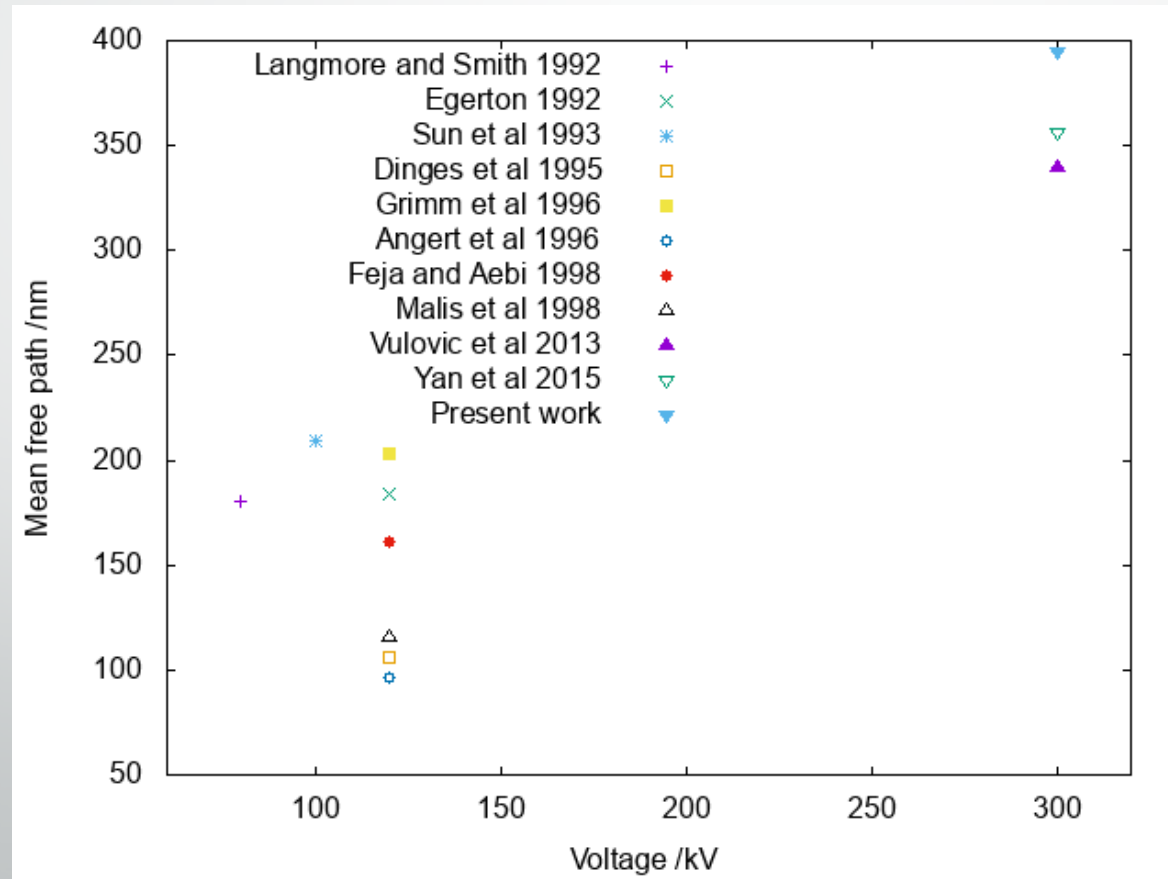
15 eV slit



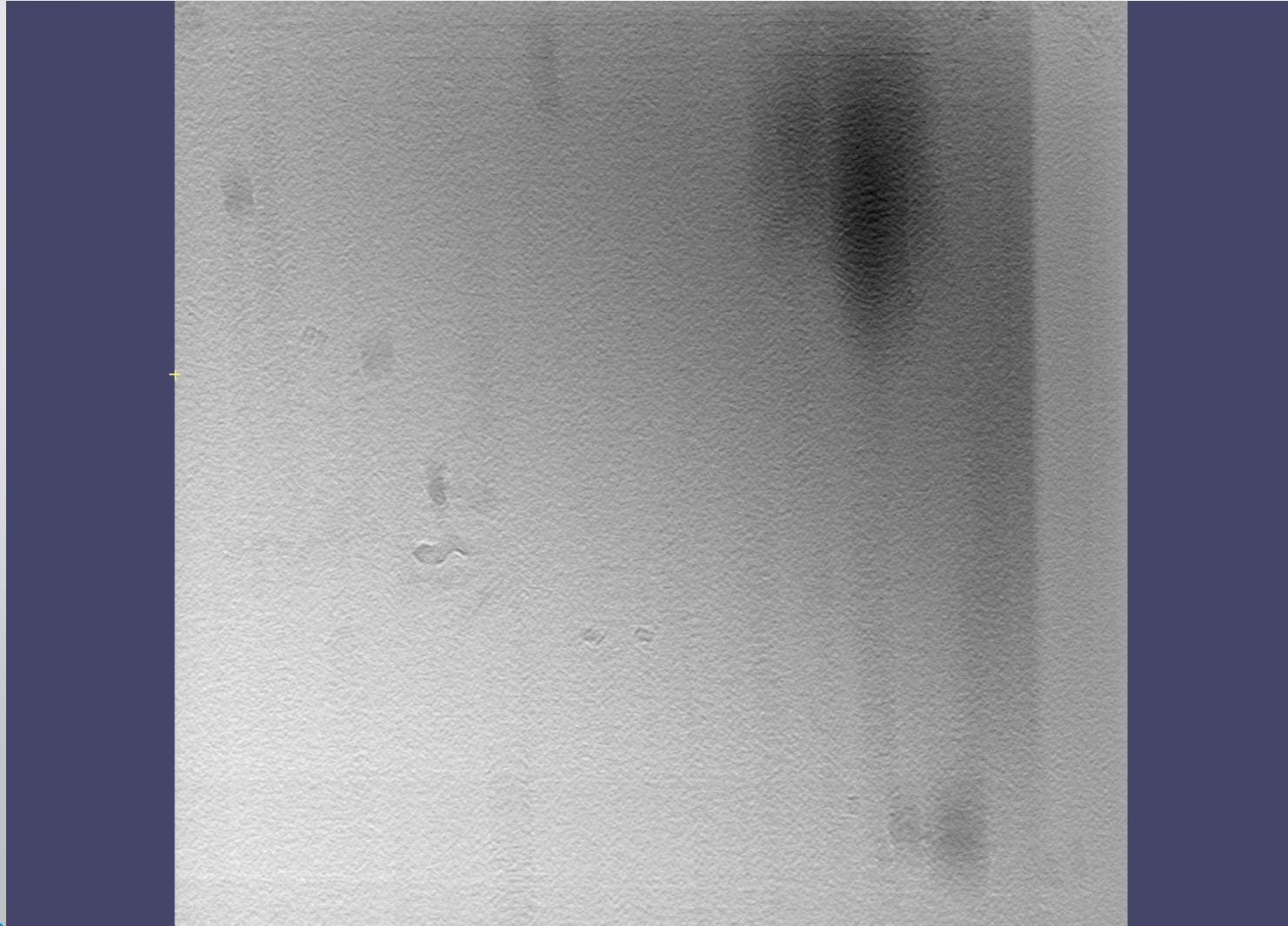
No Slit



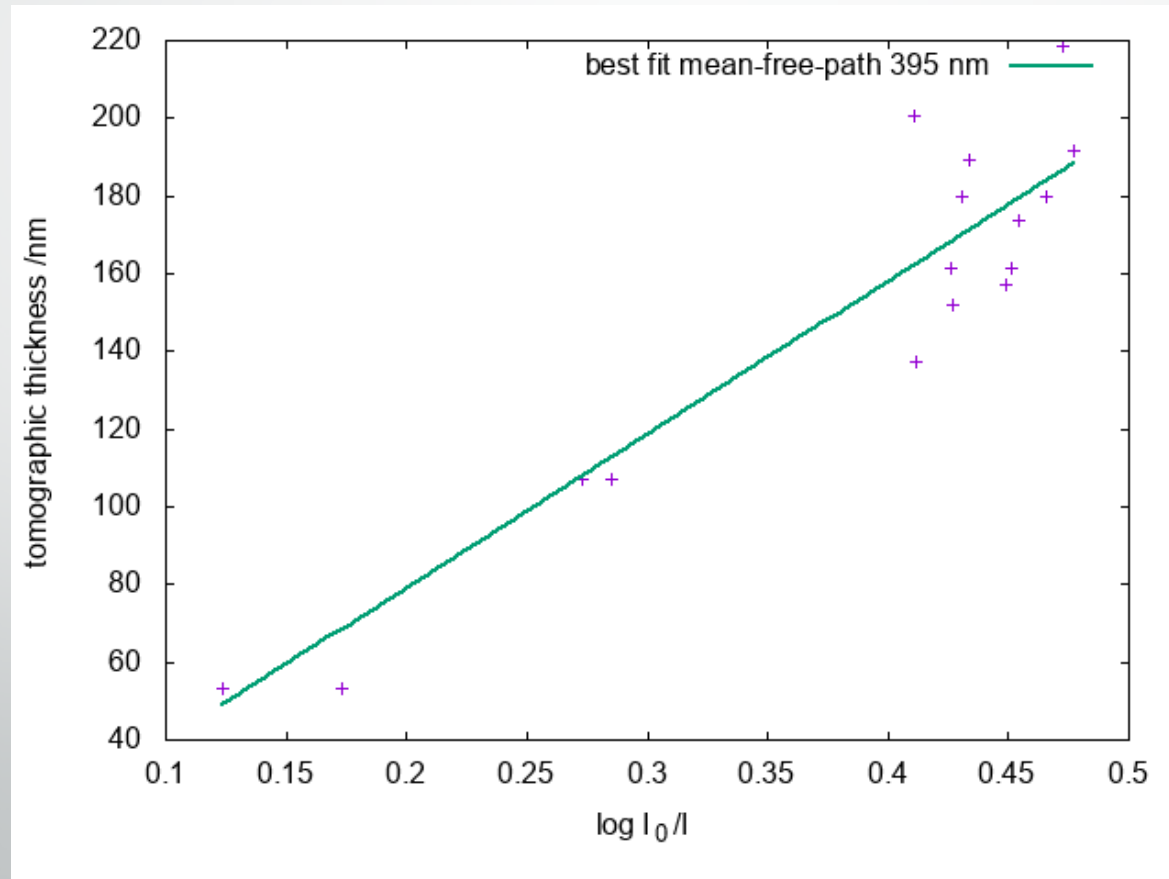
Literature values for Λ vary widely, mostly measured at low voltage



Collect Tomograms, measure thickness and plot
against $\ln \frac{I}{I_{zl}}$ to get Λ



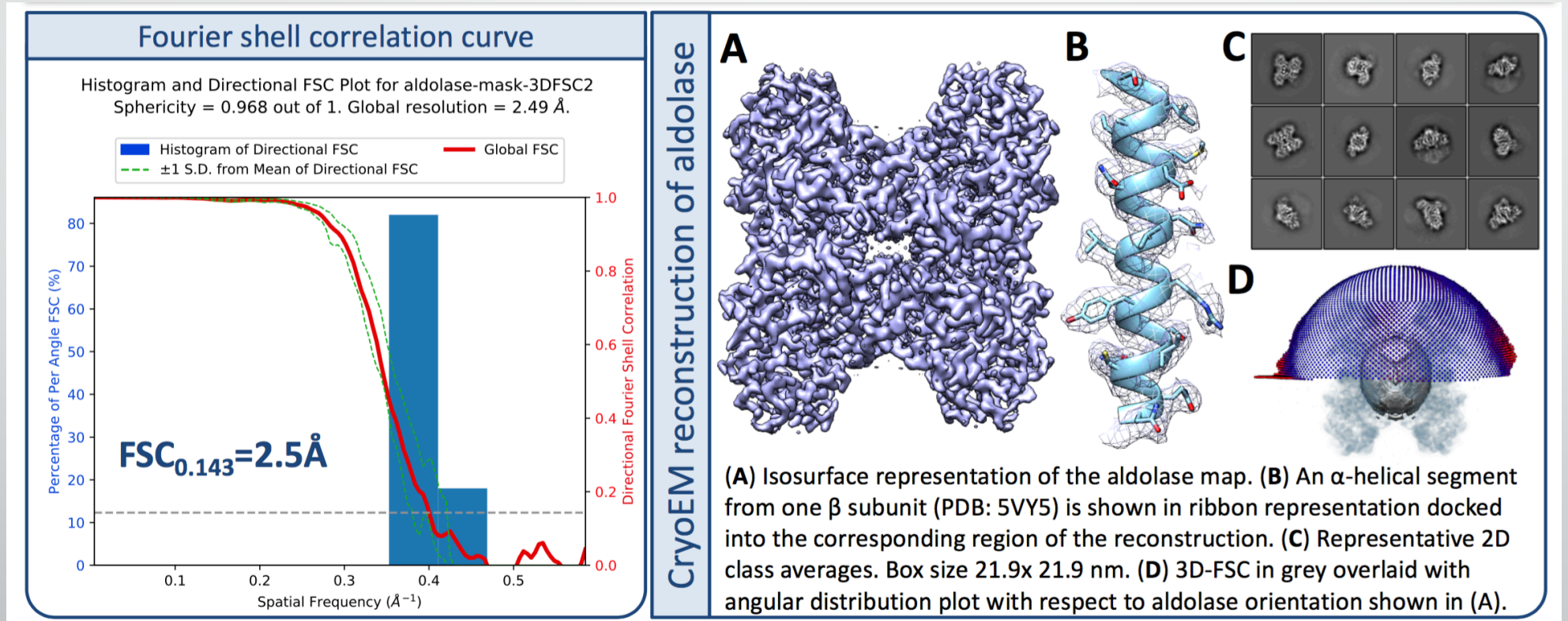
Collect Tomograms, measure thickness and plot
against $\ln \frac{I}{I_{zl}}$ to get Λ



Protocol

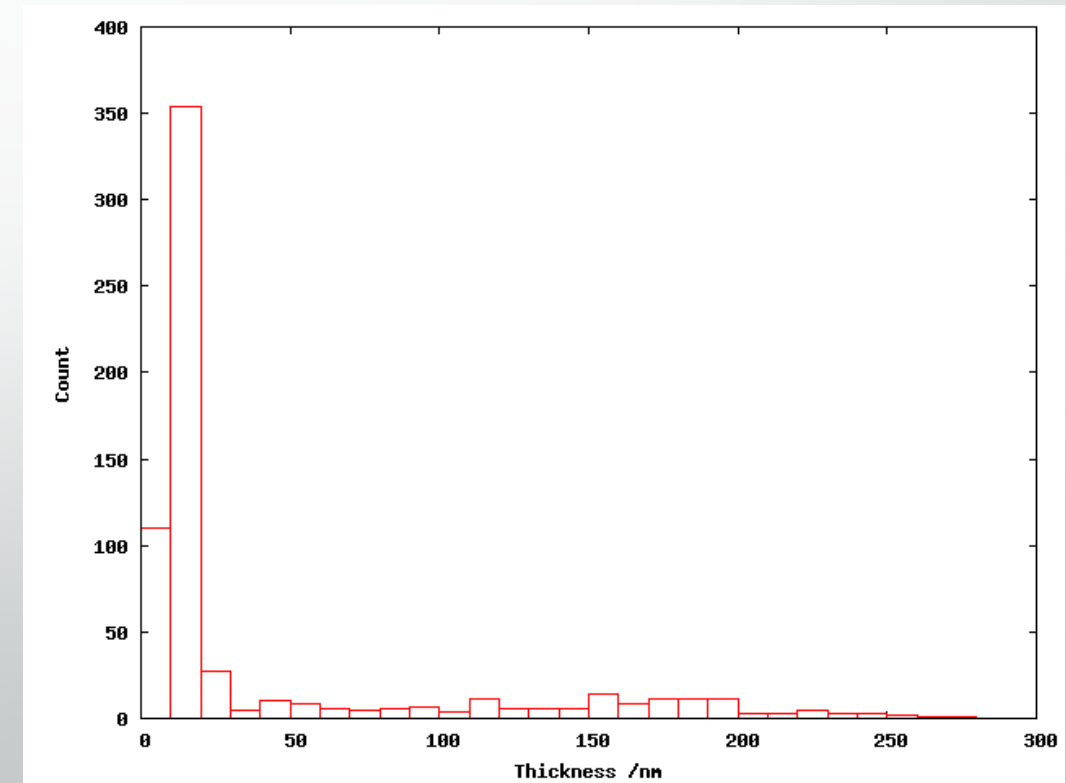
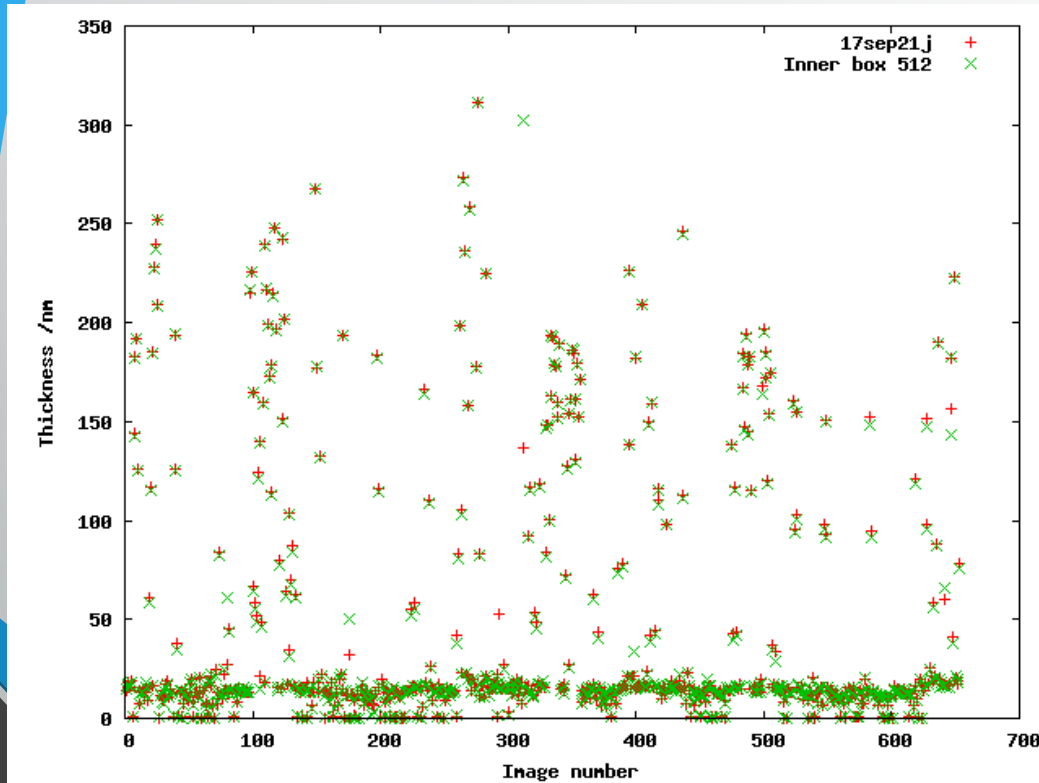
- Take exposure image
- Take short image without energy filter, same area and same beam conditions
- Take short image with energy filter, same area and same beam conditions

Test Sample: Rabbit Muscle Aldolase

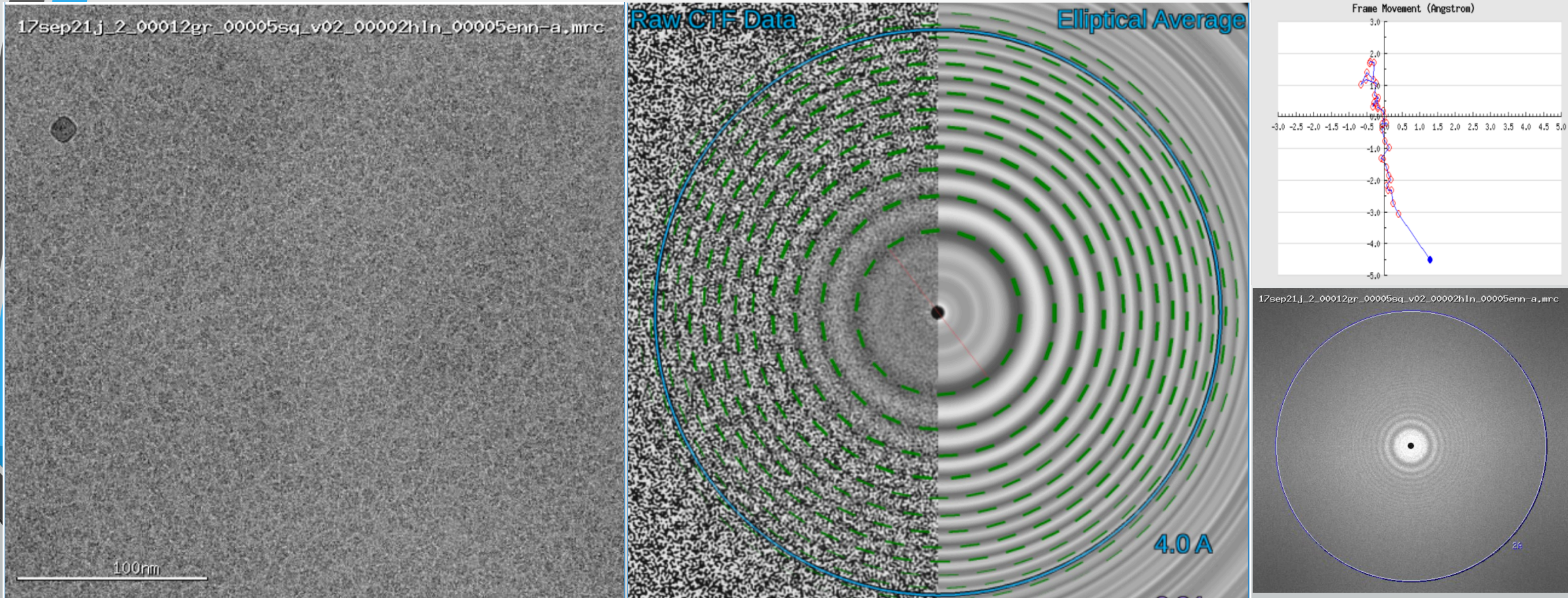


Sample collected on Titan Krios with K2 GIF, 100 μm objective aperture, pixel size 0.855 Å
130,000 particles in final reconstruction

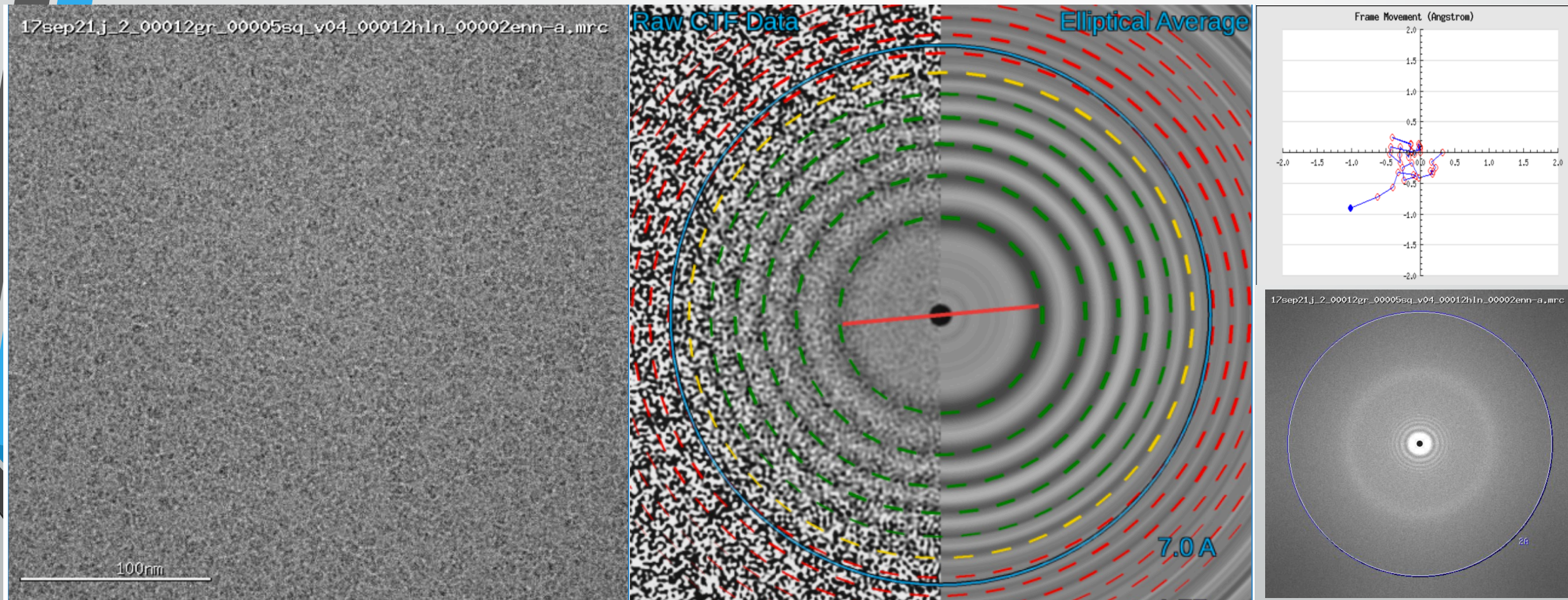
Most images were 15-20 nm thick



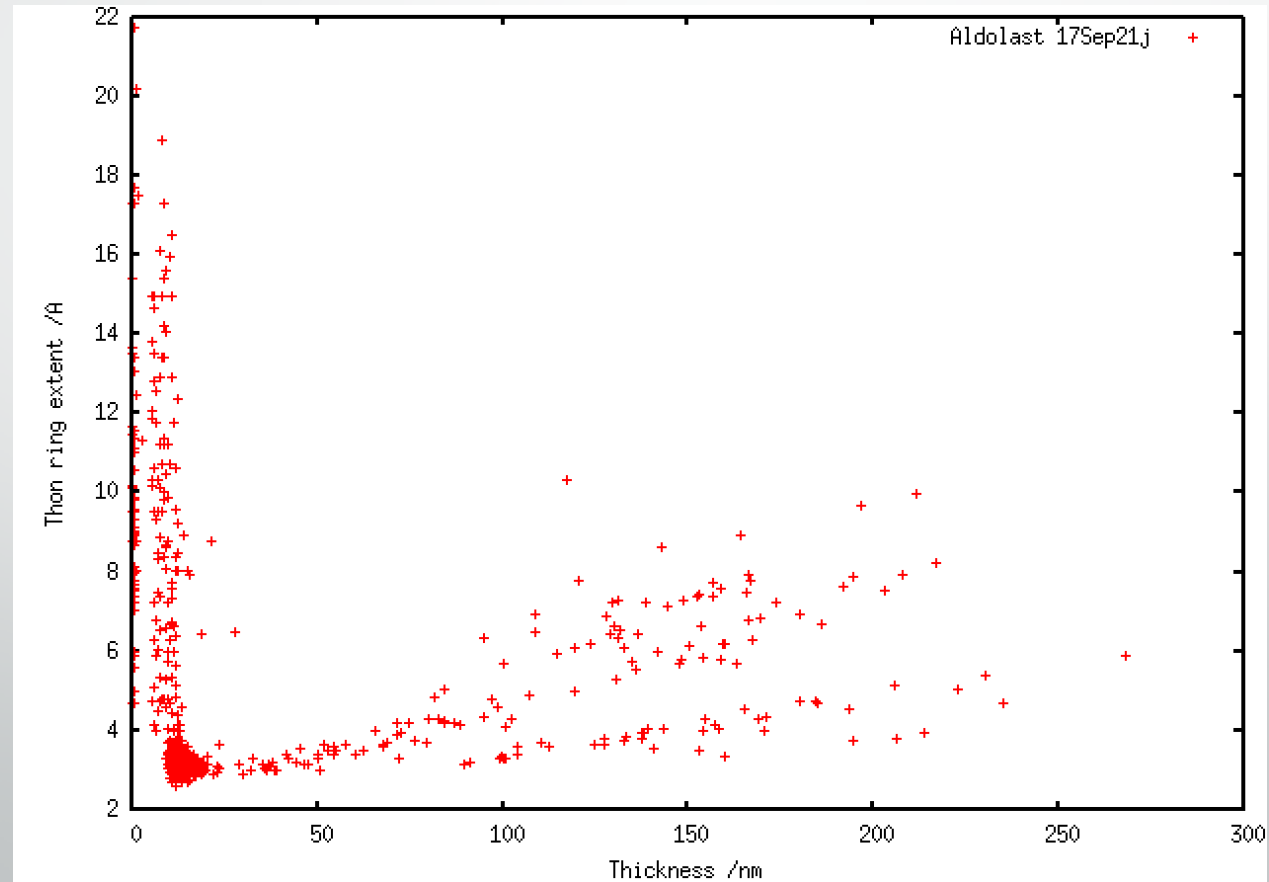
Thickness 17 nm: Thon Ring Extent 2.73 Å
Defocus=-0.94 μm



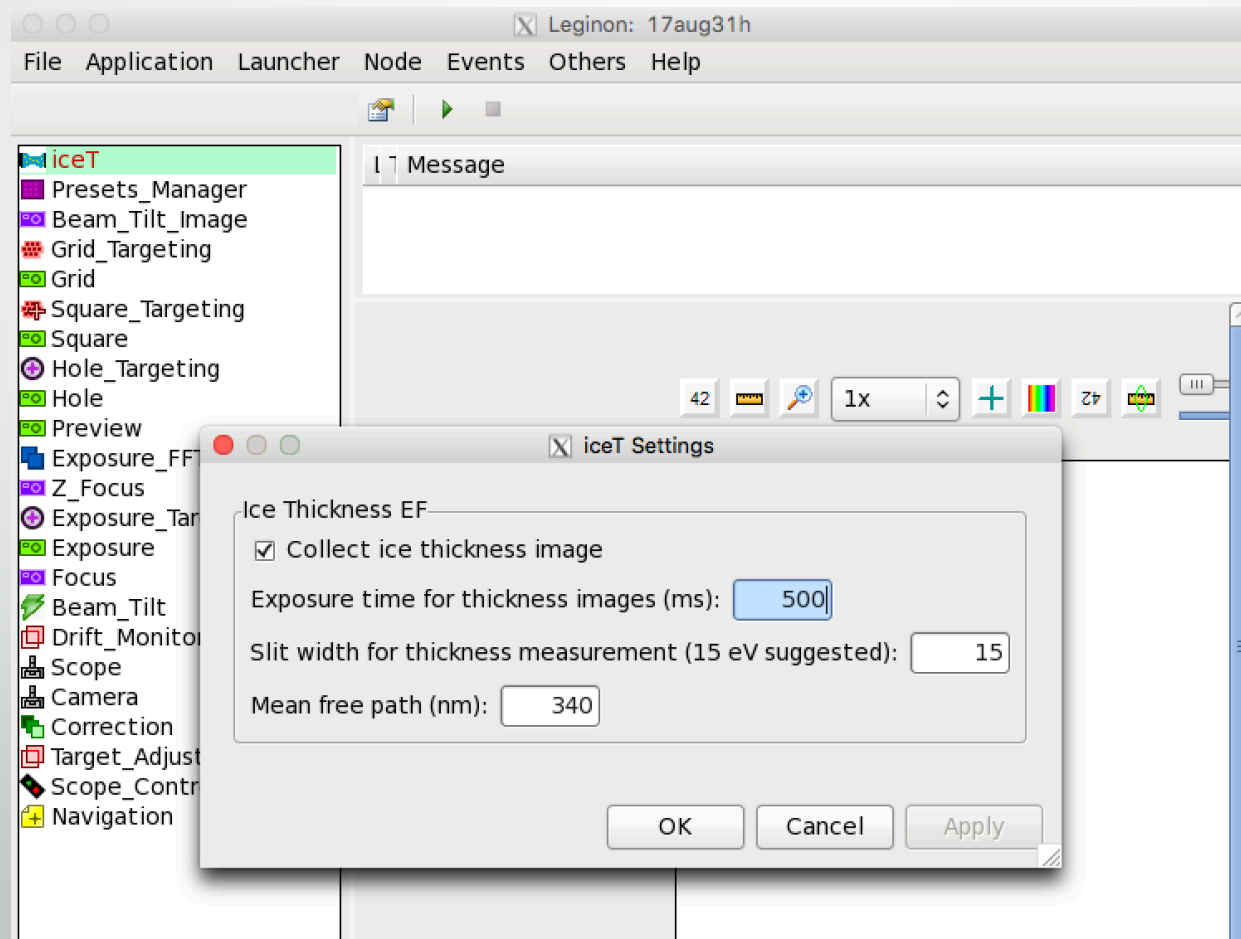
Thickness 125 nm: Thon Ring Extent 6.77 Å.
Defocus: -1.77 μm



Thon Ring Extent vs. Thickness



Integration into Leginon



Conclusions

- Ice thickness is easy to measure with an energy filter
- Thickness measurements can be integrated into standard single particle workflow
- Thickness measurements could be used as a guide during initial screening

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