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Charging Dynamics in Low-Dose Cryo-TEM Imaging

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Many physical effects limit the quality of images that can be acquired in a Transmission Electron Microscopy (TEM). These effects include stage and beam drift, voltage instabilities, and sample damage. Many of the instrumental effects have been reduced in newer generation microscopes and the combination of fast cameras and data processing algorithms has further minimized detrimental effects to the data quality [1]. However, there is still a predominant degradation in the quality of the first few frames of cryo Electron microscopy (cryo-EM) datasets [2]; despite those being the least damaged frames. This is commonly thought to be due to charging effects which can induce beam-induced motion and defocus changes. The current work investigates the dynamics and magnitude of this effect on vitreous ice samples which has not previously been investigated with conditions typically used in cryo-EM and at fast time scales.

Flash-frozen ice samples were prepared on Quantifoil grids and loaded into a Titan Krios (FEI) 300 keV TEM. Nanoprobe illumination was used to produce a parallel beam smaller than the holes in the Quantifoil hole. In this way, only the ice could be illuminated without exposing the carbon film. The microscope was then set to diffraction mode with a long camera length and a strong diffraction defocus. The central diffraction spot was then recorded on a K2 (Gatan) direct detection camera in counting mode at 40 fps. The electron beam was shuttered before recording the data and the shutter was opened after the movie stared to record. The diffraction spot in each frame of the resulting movie was fit to a circle. The radius of this circle was then plotted against electron dose. Datasets were recorded for various samples and dose rates while keeping the other lens settings constant. The dose rates used were representative of those typically used for cryo-EM data acquisition. The change in the diffraction beam radius is related to the charging of the sample as upon charging, the sample itself acts as an electrostatic lens; further focusing the beam and changing the location of the back focal plane.

Typical charging curves are shown in Figure 1 (recorded with highly diffraction underfocused settings) for the case of the electron beam only illuminating ice. There are three main features present across the charging data sets: an initial rapid charging phase, a semi-constant charge state following the rapid charging, and small charge fluctuations within the semi-constant charge state. The change in radius in the rapid charging phase can be closely fit to a decaying exponential function. The degree to which the initial radius value changes and the value of the semi-constant radius value changes significantly between samples; even between adjacent samples holes on the same grid. However, the magnitude of the initial charging and the semi-constant charge state achieved appear to be linearly related. The dose at which the semi-constant charge state is achieved does not change significantly between dose rates used. The value of this dose averaged over different samples and dose rates is 0.36 ± 0.21 $e^-/Å^2$. It is also important to note that the initial rapid charging occurs each time the sample is illuminated; even if it is the same area and the beam was shuttered for a short time. Thus, no pre-exposures can be used eliminate the rapid charging in a dataset.

For the case of carbon or vacuum that is illuminated, as would be expected, there is no charging observed and only very minor fluctuations. If carbon and ice are illuminated simultaneously, overall beam radius changes cannot be observed until the carbon is just outside the beam area. For longer integrated exposures of ice with a small sliver of carbon exposed, a small difference between the beam diameter about the ice and the carbon can be observed; indicating the presence of some charging gradient at the interface.

The present results support the commonly used cryo-EM procedures of illuminating the carbon along with the ice. However, there may be some region with a defocus gradient near the ice-carbon interface. Due to the wide variability in charging magnitude on different samples, it may be challenging to apply a dynamic defocus correction to cryo-EM movies. We present a preliminary quantitative model to predict and correct for the defocus changes occurring in the valuable first few frames of cryo-EM datasets [3].

References:

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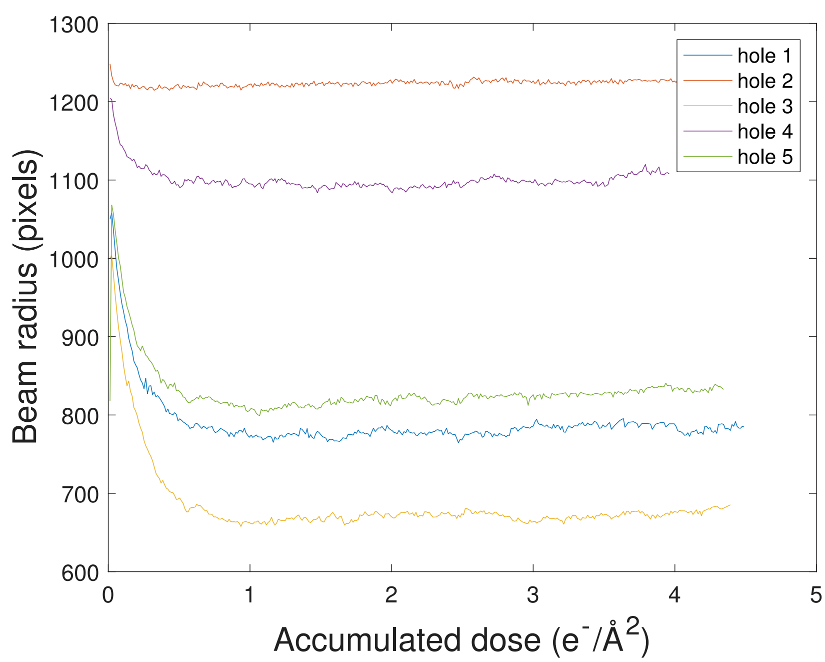

Figure 1. Charging curves for an amorphous ice sample illuminated with a 913 nm 0.48 $e^-/Å^2/s$. Each curve is from a separate ice hole illuminated with identical conditions. The y-scale is the beam radius in pixels measured from the recorded diffraction spot movies.

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