

High-Z Nanoparticle Tagging in Cryo-STEM for Localisation in Cryo-ET: Theory and Damage

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Background:

A key issue with the established method of cryo-electron tomography (Cryo-ET) often lies in the challenge of accurately locating complexes or proteins of interest within the crowded cellular environment of the generated tomograms. This project aims to use cryo-scanning transmission electron microscopy (STEM) to highlight regions of tomograms containing the protein of interest by tagging with a minimally-sized heavy atom nanoparticle for downstream processing. A fine balance must be achieved between resolution, signal-to-noise ratio (SNR), depth of focus, and damage to the lamella.

Methods:

A scan generator offers alternative (non-raster) methods of scanning the beam to offset damage and allow higher electron fluences to be used without compromising ice quality. Small raster and interleaved scans (< 200 nm) were compared in the same quantifoil hole of vitreous ice, using an equal overall electron fluence and flux.

Analysis of elastic scattering cross section theory yields an approximate minimal size of nanoparticles for detection in STEM of vitreous amorphous specimens. Single particle-like sample preparation was employed to freeze varying sizes of gold nanoparticles (0.8-4 nm) in different thicknesses of ice. These were subsequently exposed to STEM to assess a drop-off in SNR with increasing collection angles using an annular dark field detector.

Results:

Scanning in an alternative fashion using long dwell times demonstrated a significant reduction in mass loss. Raster scanning appeared to be marginally better than interleaved scanning when using shorter dwell times (20 μ s), as evidenced by greater loss of intensity in the scanned areas, normalised to reference areas taken within the same hole of vitreous ice. However, using longer dwell times (250 μ s or 500 μ s) reversed this effect, showing raster scanning to be significantly more damaging than interleaved, melting the ice

completely in thin samples for raster scanning, whilst maintaining the ice intact using an interleaved sequence.

Conclusions:

These findings provide valuable first steps toward optimizing cryo-STEM imaging for detecting nanoparticles and correlating these findings with in-situ Transmission Electron Microscopy (TEM).

Keywords:

STEM, Cryo-ET, Nanoparticle, ADF