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Rapid Tilt-series Methods: The Future of Cryo-ET

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Cryo-electron tomography (Cryo-ET) has become an indispensable imaging technique to reveal the structures of biological complexes and the architectures of cells in a nearly native frozen-hydrated state. In Cryo-ET, a tilt-series is collected by tilting the sample around a tilt axis, acquiring 2D images at each discrete tilt angle. This tilt-series is then used to computationally reconstruct a 3D tomogram. One of the major challenges in Cryo-ET is throughput; a typical tilt-series takes an average of 30-40 minutes to collect, where the time is mostly spent on tracking tasks that ensure the target remains centered in the field of view (FoV).

We recently developed methods[1] that significantly improve the speed of tilt-series acquisition by skipping all tracking of the target during acquisition and showed that a tilt-series can be acquired in less than 5 minutes using serialEM software[2] on a Titan Krios equipped with a K2 direct electron detector and energy filter. While our continuous-tilting method is limited by current stage technology to ~4nm resolution, the fast-incremental single exposure (FISE) method can achieve sub-nanometer resolution, as shown more recently[3]. Since current cryoholder technology was not designed with the intent to collect rapid tilt-series in mind, some inherent factors affect the quality of the data. These problems include FoV loss due to movement along the x-axis and y-axis during acquisition, large changes in defocus due to stage movement along the z-axis, and mechanical drift immediately after stage tilting, prior to exposing the target.

Using Gatan's latest K3 summit direct electron detector, the time per tilt series has now been reduced to less than 4 minutes. We have also analyzed the stage behavior during FISE acquisition and developed solutions to the challenges mentioned above. To resolve eucentricity loss along x and y, we find that a robust measurement of eucentric height, combined with the collection of a calibration tilt-series acquired on the same gridsquare as other targets, is currently the best way to minimize FoV loss (Figure 1A). We were also able to resolve eucentricity loss in z, exhibited by large defocus changes throughout a FISE tilt-series (Figure 1B). We find our stage behaves predictably enough to simply apply shifts averaged from fitting the contrast transfer function (CTF) measured during previous FISE acquisitions. We have also analyzed mechanical drift immediately after stage titling (Figure 2A and 2B) and found that some delay may be necessary depending on project resolution goals. We describe how we tested our own Titan Krios at Caltech so others can replicate. Finally, we give other users interested in FISE suggestions for how long to delay before exposing the target based on their resolution goals.

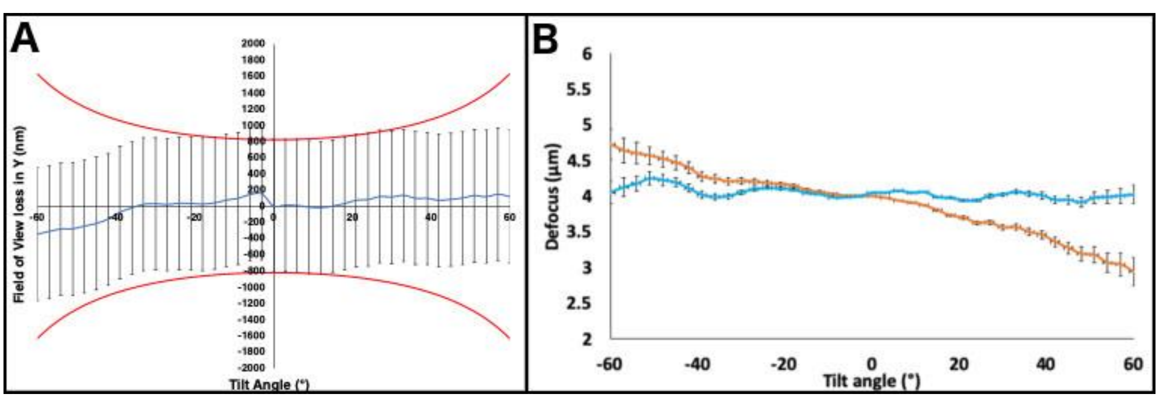

Figure 1. (A) Eucentricity loss in Y after correction overlayed over the field of view. (B) Eucentricity loss in Z before correction (orange) and after correction (blue).

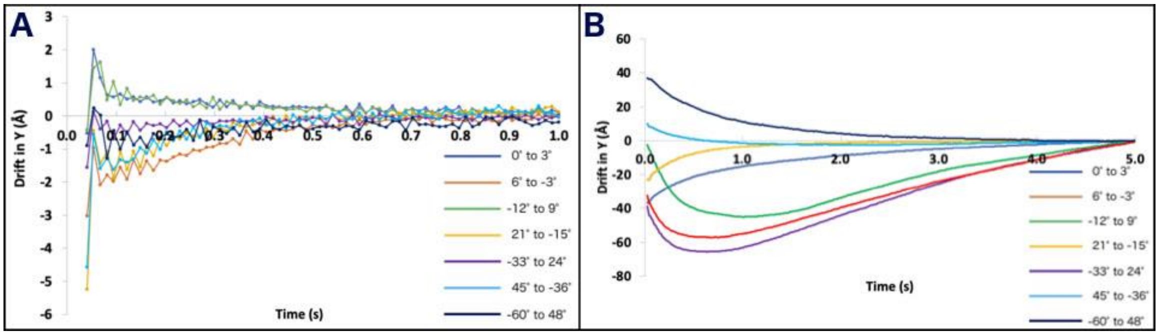

Figure 2. Mechanical drift observed immediately after common stage tilting operations (inset) in a FISE tilt-series, measured by motion-correction of frames. (A) Drift relative to the previous frame (B) Drift relative to the first frame.

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