doi:10.1017/S1431927619005671
Microsc. Microanal. 25 (Suppl 2), 2019

Development of Rapid Tilt-Series Collection for Electron Cryo-Tomography

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The main limitation for many electron cryo-tomography (ECT) projects today is low throughput. Recently, we characterized a "high precision" single-axis-tilt cryoholder (Thermo Fisher) for the Titan Krios microscope that uses static arms to improve eucentricity by increasing stage stability. By rolling this stage as a K2 direct electron detector (Gatan) continuously recorded frames, we were able to collect a full tilt-series in seconds, although several minutes were subsequently required to transfer the data off the camera. We found that the stage was eucentric enough to allow us to eliminate tracking and focusing steps during acquisition, dramatically reducing collection time. Unfortunately, we also found that high spatial frequency data was lost due to vibration from the tilt motor in the stage [1].

To combat this loss of information, we developed "fast-incremental tilt-series acquisition." In this scheme, the camera records a continuous movie, but the stage is stopped at discrete tilt angles for exposures. We were concerned that residual drift after each tilt might blur the exposures, so we analyzed the stage shift between frames at each angle (Fig. 1A). We found that the shifts were small, indicating that the stage becomes stable immediately after tilting, eliminating the need for a stage settling delay. Using this fast-incremental scheme, we can collect tilt-series with a range of ±60º and a 3º increment in 3 minutes, while retaining high-resolution information (Fig. 1B) [1]. We are now further reducing tilt-series collection time to ~1 minute with next-generation direct electron detectors that largely eliminate the data-writing latency. We are also developing adaptive holder calibrations to measure shifts in recent tilt-series and then apply them on-the-fly to the next tilt-series. As rapid tilt-series collection generates high volumes of data, improvements in software to align tilt-series accurately and fully automatically will become critical.

Rapid tilt-series collection has profound implications for several cryo-EM applications. The first is cellular ECT and sub-tomogram averaging (STA). The limiting factor in most STA projects is particle number. Recently, a few sub-tomogram averages reached resolutions rivaling those of single particle reconstruction (SPR). John Briggs' group reported a 3.9 Å resolution reconstruction of immature HIV-1 virus-like particles [2], subsequently improved to 3.1 Å [3], as well as a 7.4 Å resolution average of the *Caulobacter crescentus* S-layer [4]. In these cases, the nature of the target (a highly repetitive protein lattice) generated tens of thousands of particles from relatively few cryotomograms; low-copy-number objects in the cell will require orders of magnitude more tomograms, for which rapid collection is key.

Currently, SPR is the best method for solving the high-resolution structure of large macromolecular complexes. The dose fractionation theorem states, however, that for a given dose, a tilt-series provides more information than a single projection [5], provided that the quality of the tilted images is as good as the projection and that the images can be precisely aligned. Hybrid SPR/ECT methods are therefore promising (for example the “TYGRESS” method [6]). In this approach, an initial projection image is recorded for use in the final reconstruction, followed by a fast tilt-series that can be used to identify

damaged particles at the air-water interface, construct initial models, improve particle classification and alignment, increase the accuracy of defocus estimates for per-particle CTF correction, and disambiguate conformational changes from variations in orientation. Until now, however, this idea has been rarely pursued due to the difficulty of obtaining a tilt-series compared to a single projection. A recent study exploring this hybrid approach cited tilt-series acquisition and processing time as a major obstacle [6]. As we develop methods to collect a tilt-series almost as quickly as a projection, we expect a hybrid tomography approach to become standard in SPR.

MicroED is increasingly being used to solve the structures of metabolites and small proteins, including those resistant to forming macrocrystals suitable for X-ray crystallography. Rapid tilt-series acquisition could enable hybrid ECT/MicroED workflows in which a tilt-series is used for direct phase determination. We are currently developing a novel application—ECT of nanocrystals—which we expect to offer an efficient and competitive alternative to current methods of structure determination, potentially allowing structures of proteins of any size that form only small and/or imperfect crystals to be solved to high resolution [7].

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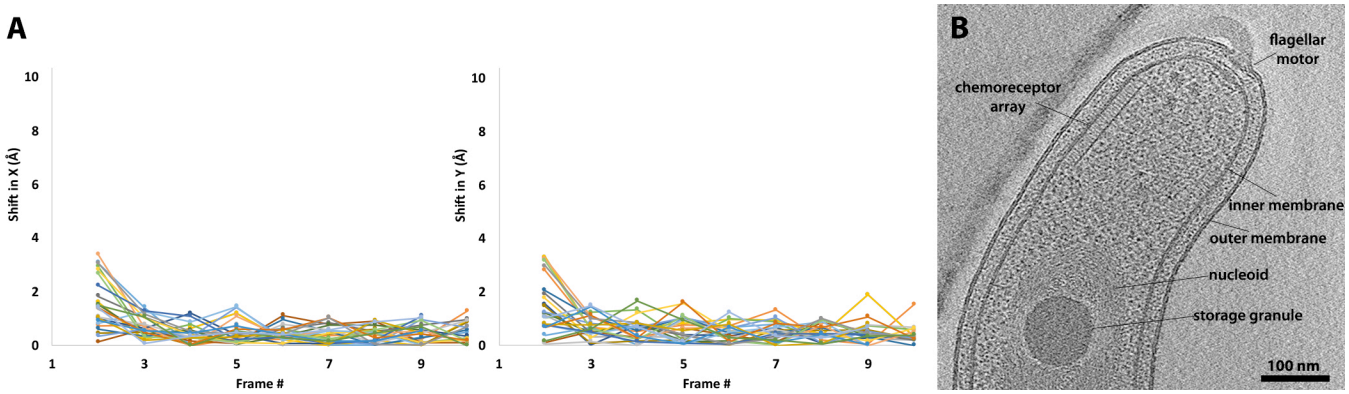

Figure 1. **(A)** Stage shift in x (left) and y (right) between subsequent frames at 10º tilt-angle during fast-incremental tilt-series acquisition. The small values (nearly all less than half a pixel) indicate that no settling time is required after stage movement. **(B)** Central slice through a cryotomogram of a *Bdellovibrio bacteriovorus* cell collected in a few minutes using fast-incremental tilt-series acquisition. Note the high-resolution details such as the individual leaflets of both the inner and outer membranes. This tomogram is available from the Electron Microscopy Data Bank (EMDB) under accession number EMD-9261. For details, see [1].

https://doi.org/10.1017/S1431927619005671 Published online by Cambridge University Press