

Experimental Assessment of Cryo Single Particle Data Acquisition by “Beam-image Shift”

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Cryo-EM data collection traditionally has been a low throughput process. Stage movement during data collection is a significant bottleneck that causes prolong wait time and excessive drift. Multi-hole imaging through Image Shift (IS) has been proposed to circumvent this bottleneck (Cheng et al. 2018). Since, during multi-hole data collection, the electron beam needs to shift up to 2.5 μm , excessive coma is expected to be introduced. Although coma vs. image shift calibration can help reduce coma, it is unclear if the remaining coma will deteriorate the image quality and final reconstruction resolution.

The purpose of the work is to check the impact for the final 3D reconstruction resolution between imaging on a single hole with multi-shot, and on multi-hole with multi-shot. We use Beta-galactosidase as a testing specimen. Krios at 300kV with K3 direct detector was set up with routine alignment and Coma-free calibration was carried out using SerialEM. Quantifoil R1.2/1.3 gold grids were used in which there were image shifts less than 0.5 μm for single-hole (3 images per hole) collection and image shifts up to 2.5 μm for multi-hole collection. The motioncor2, CtfFind and Relion were used in data processing. The data was split into two groups, the central hole groups (with small shifts) and the non-central hole group (with large image shifts). Data from central hole groups (370K particles) generate a 2.47 \AA resolution reconstruction with Relion CTF-refine (including defocus and beam tilt) and 2.54 \AA (including defocus only); non-central hole group (500K particles) generates 2.44 \AA (including defocus refinement only) and 2.40 \AA (including both defocus and beam tilt) reconstructions. Justified by the difference in the number of particles, two groups achieved very similar resolutions, indicating that using the multi-hole data collection scheme can still achieve very high resolution.

It demonstrates experimentally that the implementation of both “multi-shot” and “multi-hole” scheme can greatly speed up data collection without compromising reconstruction quality.

References

Cheng A, Eng ET, Alink L, Rice WJ, Jordan KD, Kim LY, Potter CS, Carragher B., (2018), High resolution single particle cryo-electron microscopy using beam-image shift, *J Struct Biol.* 204:270-275