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High Resolution Cryo-TEM Single-Particle Averaging Reconstruction with Beam-Image Shift

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Automated data acquisition is now used widely to facilitate single-particle averaging (SPA) approaches to reconstructing three-dimensional (3D) volumes of biological samples preserved in vitreous ice and imaged in the transmission electron microscope (cryo-TEM) using low–dose methods. Automation is highly desirable as very large numbers of particle images are required to overcome the very low signal-to-noise ratio of these images; typically from 1000-10000 images may be required for a single reconstruction.

In the interests of efficiency, all popular automated data acquisition software [1-3] employ some degree of beam-image shift to facilitate quick and accurate targeting to a desired area within +/- 0.1 μm accuracy. Using pure stage movement requires multiple iterative attempts to reach the target accurately and also may require long relaxation or settling times to achieve stability; both of these factors reduce efficiency of data collection. However, it is well known that beam-image shift induces beam-tilt and thus, in a system that its optical aberration is dominated by spherical aberration constant *Cs*, introduces a structure phase error. Given that $\pi/4$ phase error is considered as the worst acceptable in describing a wave at a given frequency[4], for a given beam tilt θ, electron wavelength λ, and spherical aberration constant *Cs*, this phase error limit can be used to derive the limit of resolution that is achievable [4].

$$(8\ \theta\, C_s\, \lambda^2)^{1/3} \qquad (1)$$

In this study, we performed cryo-TEM SPA on a T20S proteasome sample using a wide variety of different beam-image shifts and beam tilts from 0 to 10 mrad. We examined the $FSC_{0.143}$ values of maps reconstructed under these conditions, and also specifically focused on water density peaks in the 3D map (Figure 1). We concluded that maps generated with beam tilts do have the resolution as calculated by the FSC and have undistorted density features when resolution is comparable. We also found that Eq. 1 does not limit the resolution of 3D reconstruction from SPA as much as expected. We believe the SPA approach avoids this problems because the method itself minimizes the phase error by sampling all orientations across different images.

References:

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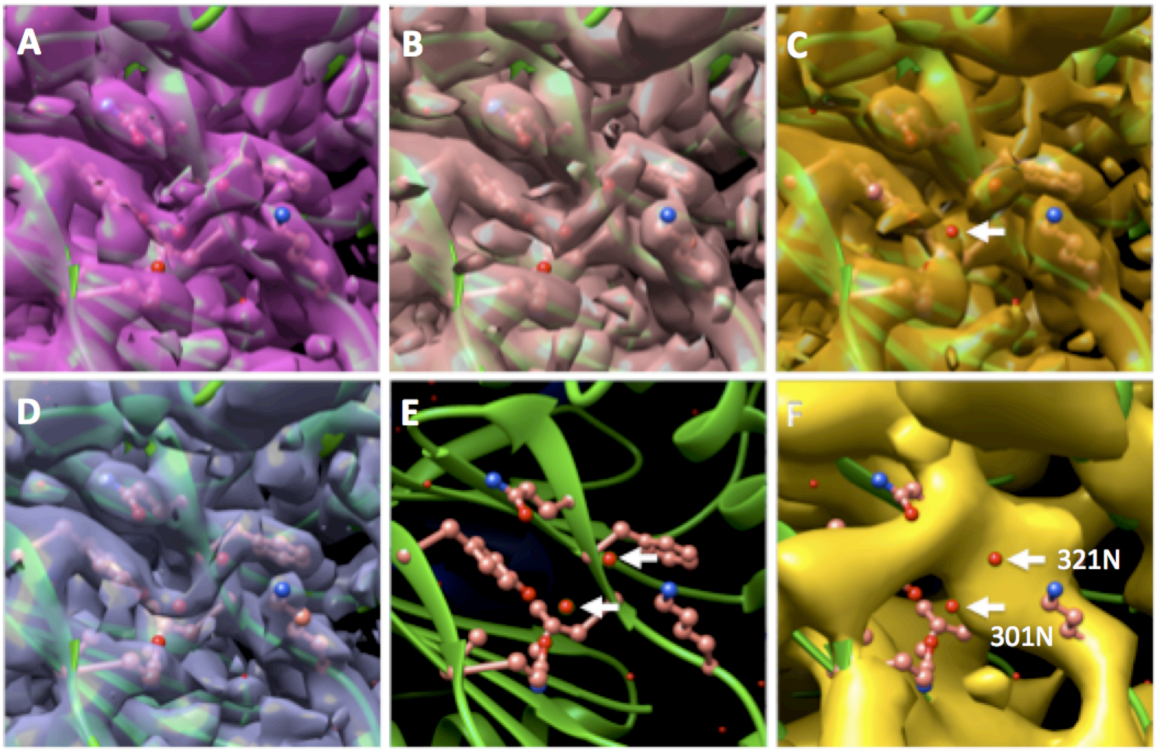

Figure 1. Water density as criterion of the phase correctness of the density maps. A,B,C are from the same cryoEM grid and the same experimental session but with different beam tilts applied in each case. D is the map from EMD-6287, and E shows the PDB modeled from D. For Panel A, B, C, D, F the estimated beam tilts are 0, +/-0.5, +/-1.33, 0, +10 mrad respectively. The white arrows show the water model we tracked in the maps. The arrow in C points to the 301N water that was not covered by the density at the contour level. Surface contours are thresholded using the UCSF Chimera default where 1% of voxels are above the threshold level. The $FSC_{0.143}$ resolutions are A: 2.7 Å, B: 2.9 Å, C: 3.2 Å, D: 2.8 Å, and F:5.6 Å

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