Beam image-shift accelerated data acquisition for near-atomic resolution single-particle cryo-electron tomography

Jonathan Bouvette[1], Mario Borgnia[2], Hsuan-Fu Liu[3], Alberto Bartesaghi[4], Roel Schaaper[5], Bradley Klemm[6], Andrew Sikkema[7], Juliana Da Fonseca Rezende E Mello[8], Ye Zhou[9], Xiaochen Du[10], Rick Huang[11]

[1]NIEHS [2]National Institutes of Environmental Health Sciences, [3]Duke University, [4]Duke University, [5]NIEHS, [6]NIEHS, [7]NIEHS, [8]NIEHS, [9]Duke University, [10]Duke University, [11]Laboratory of Cell Biology/CCR/NCI/NIH

jonathan.bouvette@nih.gov

Single-particle Cryo electron microscopy (SP Cryo-EM) has been the method of choice to obtain high-resolution structures by Cryo-EM because of its fast data acquisition schemes and well-developed image processing tools. Cryo electron tomography (Cryo-ET), is the method of choice of in situ imaging by acquiring multiple tilted projections of each area to reconstruct a 3D subvolume of each particle or structure. The need to compensate for errors in targeting introduced during mechanical navigation and tilting of the specimen significantly slows down tomographic data collection to a point where it would be too costly to acquire datasets large enough to achieve high-resolution reconstruction. Combined with the limited toolset for data processing, Cryo-ET cannot consistently reach high resolutions. Solving these limitations would bridge the gap between SP Cryo-EM and Cryo-ET and open the door to in situ structural biology

Here, we introduce BISECT (beam image-shift electron cryo-tomography) protocol for tilt-series acquisition that accelerate data collection speed by up to an order of magnitude. Like single-particle Cryo-EM, we achieve this by using beam-image shift to multiply the number of areas imaged at each stage position and iteratively correct the geometrical constraints during imaging to achieve high precision targeting at each area. Finally, by performing per-tilt astigmatic CTF estimation and data-driven exposure weighting, we improved final map resolution. The method was validated by determining the structure of a low molecular weight target (~300 kDa) at 3.6 Å resolution where density for individual side chains is clearly resolved.

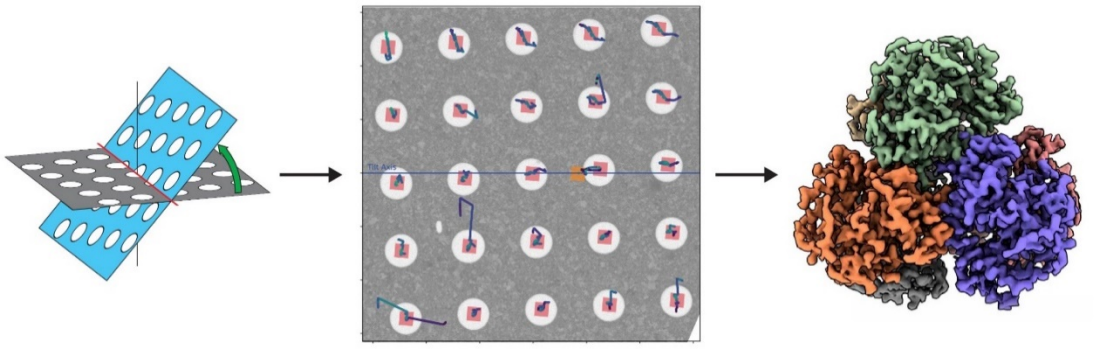

Figure 1