Single particle CryoEM structures using iDPC-STEM from 4D-STEM detectors at near-atomic resolution

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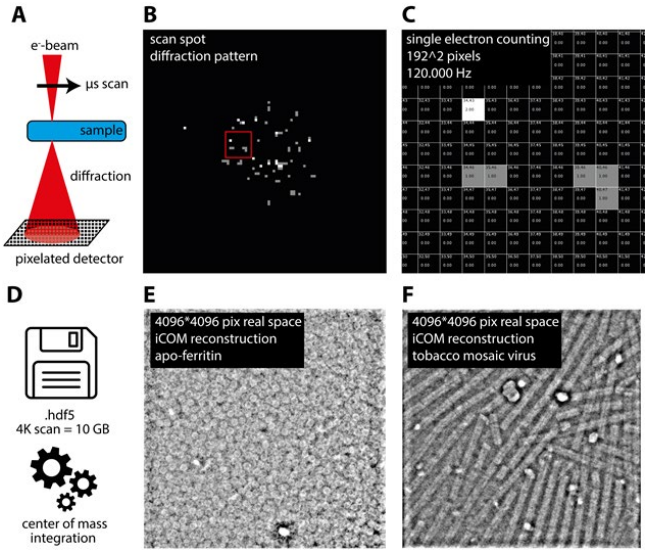
CryoSTEM can image frozen-embedded biomolecules in focus without an oscillating contrast transfer function, enabling complete information transfer in each image. Using integrated differential phase contrast (iDPC)-STEM, one can obtain three-dimensional reconstructions of proteins using single particle analysis. We have recently demonstrated how iDPC-STEM can be used to reconstruct protein structures to near atomic resolution (Lazic et al. Nat.Meth. 2022), depending on the chosen convergence semi angle (CSA) of the incident beam. During scanning the electron dose rate is orders of magnitude higher compared to parallel illumination as the dose is distributed in a very small area (Ang2) and for a very short time (50 ns – 10 µs). We compare beam damage of conventional TEM and iDPC-STEM on the same frozen-embedded protein sample. We will demonstrate how this technique can be further applied to a variety of test samples and how alternative ptychographic image reconstruction methods such as single side band (SSB) can be used to further enhance the image information.

iDPC-STEM using segmented detectors is an approximation for integrated center of mass (iCOM) reconstructions. Therefore, we recorded pixelated 4D-STEM data of frozen-embedded protein molecules with a 300 kV Titan Krios G4i equipped with a fast pixelated 4D-STEM detector (Dectris Arina), derived the corresponding iCOM images and reconstructed protein structures to near-atomic resolution.

Graphic:



Keywords:

CryoEM, iDPC-STEM, 4D-STEM, SPA