

## Joint Ptychographic Tomography of Frozen Hydrated Proteins

Georgios Varnavides<sup>1,2</sup>, Dr. Yue Yu<sup>3</sup>, Berk Küçükoğlu<sup>4</sup>, Dr Stephanie Ribet<sup>2</sup>, Dr Mary Scott<sup>2,5</sup>, Dr Henning Stahlberg<sup>4</sup>, Dr Colin Ophus<sup>2</sup>

<sup>1</sup>Miller Institute for Basic Research in Science, University of California, Berkeley, USA, <sup>2</sup>National Center for Electron Microscopy, Lawrence Berkeley Laboratory, Berkeley, USA, <sup>3</sup>Chan Zuckerberg Institute for Advanced Biological Imaging, Redwood City, USA, <sup>4</sup>Laboratory of Biological Electron Microscopy, Institute of Physics, SB, EPFL, Lausanne, Switzerland, <sup>5</sup>Department of Materials Science and Engineering, University of California, Berkeley, USA

Single particle analysis (SPA) of frozen-hydrated proteins using cryogenic electron microscopy (cryo-EM) enables the three-dimensional structure determination of biomolecules with ångström resolution. Despite the remarkable advances enabled by cryo-EM SPA, the technique requires extensive data acquisition and processing and suffers from size limitations. Cryo-EM techniques are limited for very large biomolecules and very small proteins, due to the presence of multiple-scattering and poor contrast arising from the low electron fluence necessary to prevent sample damage respectively.

Scanning transmission electron microscopy (STEM) techniques have traditionally not been applied to the study of biological samples, due to the high fluence requirements of the most easily interpretable imaging modality using high angle annular detectors (HAADF). However, considerable efforts have recently been employed to apply phase-contrast STEM methods to study biological structures [1,2,3]. Among these techniques electron ptychography, where one iteratively reconstructs the scattering potential using a set of converged beam diffraction patterns (4D-STEM), stands out due to its high dose-efficiency and relaxed sampling requirements [4].

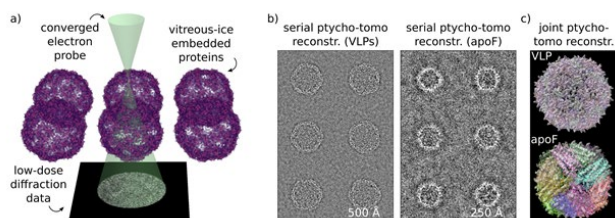
Cryogenic electron ptychography has recently been used to obtain sub-nanometer resolution of apoferritin samples using a relatively small number (~11,000) of high signal-to-noise reconstructions [5]. This “serial” approach, where one uses the 4D diffraction datasets to reconstruct 2D projection images which are then subsequently used to reconstruct a 3D volume using standard cryo-EM methods, is not maximally dose efficient. In this talk, I will propose an alternative technique we term “joint” ptychographic tomography SPA, where the 3D volume is reconstructed directly from the 4D data. This has multiple advantages over 2D projection-based techniques: first, nonlinearities arising from multiple scattering in the sample can be accurately modeled; second, it enables 3D regularization directly which can more effectively fill-in information from missing projection directions; and finally, it can more accurately capture amplitude and phase variations of the scattering potential.

Figure 1 illustrates the technique on small (1728 particles) simulated datasets of virus-like particles (PDB ID:1dwn) and apoferritin (PDB ID: 8rqb).

Representative reconstructed micrographs are shown in Fig. 1b for the two proteins using electron fluences of  $45\text{e}/\text{\AA}^2$  and  $35\text{e}/\text{\AA}^2$  respectively. These are used to reconstruct 3D volumes “serially” using the commonly used SPA software cryosparc with and without imposing symmetry. Alternatively, the volumes can be directly reconstructed using our joint ptychographic-tomography implementation in the open-source software py4DSTEM [4]. The resulting 3D maps, together with the respective docked models are shown in Fig. 1c.

We estimate that, for properly oriented poses, joint ptychographic-tomography offers a 10-20% improvement in resolution, as assessed by gold-standard Fourier shell correlation. Finally, we illustrate how joint ptychographic tomography can be used to estimate the unknown tilt orientations directly from the 4D data and show progress towards experimental results.

#### Graphic:



#### Keywords:

single-particle analysis, ptychography, tomography, phase-retrieval

#### Reference:

- [1] L Zhou, J Song, J Kim, et al. Nature Communications (2020), DOI: 10.1038/s41467-020-16391-6
- [2] I Lazic, M Wirix, M Leidl, et al. Nature Methods (2022), DOI: 10.1038/s41592-022-01586-0
- [3] Y Yu, K Spoth, D Muller, and L Kourkoutis, Microsc. Microanal. (2020), DOI: 10.1017/S1431927620019169
- [4] G Varnavides, S Ribet, et al. arXiv:2309.05250 (2023), <https://arxiv.org/abs/2309.05250>
- [5] B Küçükoğlu, I Mohammed, RC Guerrero-Ferreira, et al. bioRxiv (2024), DOI: 10.1101/2024.02.12.579607