Towards More Efficient Use of Electrons: Demonstrating Cryo-4D-STEM Phase Imaging Techniques on Thick and Thin Biological Specimens, from Organelles to Proteins

Yue Yu, Stephanie M Ribet, Georgios Varnavides, Colin Ophus, David A Muller

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Meeting-report

Towards More Efficient Use of Electrons: Demonstrating Cryo-4D-STEM Phase Imaging Techniques on Thick and Thin Biological Specimens, from Organelles to Proteins

Yue Yu[1,*], Stephanie M. Ribet[2], Georgios Varnavides[2,3], Colin Ophus[2], and David A. Muller[4,5]

[1]*Chan Zuckerberg Institute for Advanced Biological Imaging, Redwood City, CA, USA*
[2]*NCEM, Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*
[3]*Miller Institute for Basic Research in Science, University of California, Berkeley, CA, USA*
[4]*School of Applied and Engineering Physics, Cornell University, Ithaca, NY, USA*
[5]*Kavli Institute at Cornell for Nanoscale Science, Cornell University, Ithaca, NY, USA*

*Corresponding author: yue.yu@czii.org

Rapid 4D-STEM data acquisition has simplified the implementation of powerful phase imaging techniques, impacting material science studies, and finding applications in structural biology research. For example, with single particle analysis (SPA) on purified and isolated biomolecular complexes, (integrated) differential phase contrast (iDPC-)STEM demonstrated the first map at near-atomic resolution with a STEM technique [1]. Furthermore, recent demonstrations have shown that SPA with ptychography, can resolve protein structures at a sub-nanometer level [2]. However, for thin, single-particle type of specimens, energy filtering TEM (EFTEM) with the state-of-art instrumentation can routinely determine macromolecular structures at atomic and near atomic resolution. In this study, we demonstrate two 4D-STEM phase imaging techniques applied to diverse biological specimens: tilt-corrected bright field (tcBF-)STEM [3, 4] (also known as parallax [5]) and ptychography.

For a thick mitochondrion specimen, we present a comparison between tcBF-STEM and EFTEM. This reveals enhanced contrast and a clearer resolution of the double-layer membrane in the thick regions of the sample. In tcBF-STEM, each pixel within the bright-field (BF) disk functions as a virtual coherent detector with a small collection angle. The resulting virtual BF images exhibit shifts depending on the probe aberration function, and these shifts are measured and corrected on a pixel-by-pixel basis. We believe where tcBF has an advantage over EFTEM is in thick samples. It allows a coherent image to be retained and uses almost all the forward-scattered incident beam -i.e. a similar dose efficiency to TEM. The post-specimen lenses for (EF)TEM are the image-formation lenses so chromatic aberrations there degrade the image resolution. However, for tcBF-STEM, the post-specimen lenses simply transfer an image of the diffraction pattern so chromatic aberrations result in a small loss of angular resolution instead -i.e. a reduction in coherence.

To compare the performance of tcBF-STEM with EFTEM on thick samples, we image the same area in a mitochondrion in succession using the two methods at 300 kV. Comparison between tcBF-STEM (Fig.1 a and b) and EFTEM (Fig.1 c and d) on a mitochondrion specimen shows similar resolution of membrane bilayers at the leading edge. However, in thicker regions, tcBF-STEM demonstrates clearer membrane resolution (orange arrows) compared to EFTEM. Average line profiles (Fig.1 e) of the solid-line boxed areas illustrate the intensity variation across a membrane double layer. More data were acquired at various defoci, doses, and acquisition orders, and similar trends were observed.

Additionally, to quantitatively assess the current performance of tcBF-STEM and ptychography for molecular structure analysis, we conducted analyses on thin, single-particle type samples of the purified hydrated vitrified coat protein of bacteriophage PP7 particles (virus-like particles, VLPs). These analyses were carried out under various instrumental and experimental settings, as shown in Fig. 2. Notably, employing tcBF-STEM on 789 VLP acquired from 20 micrographs, we achieve a 7.03 Å nominal resolution with a B-factor of 351 Å^2. Similar results are also observed with ptychography on the same sample. Overall, both tcBF and ptychography were observed to reach sub-nanometer nominal resolution despite large B-factors, which might be due to the presence of underlying specimen motion that needs to be addressed [6].

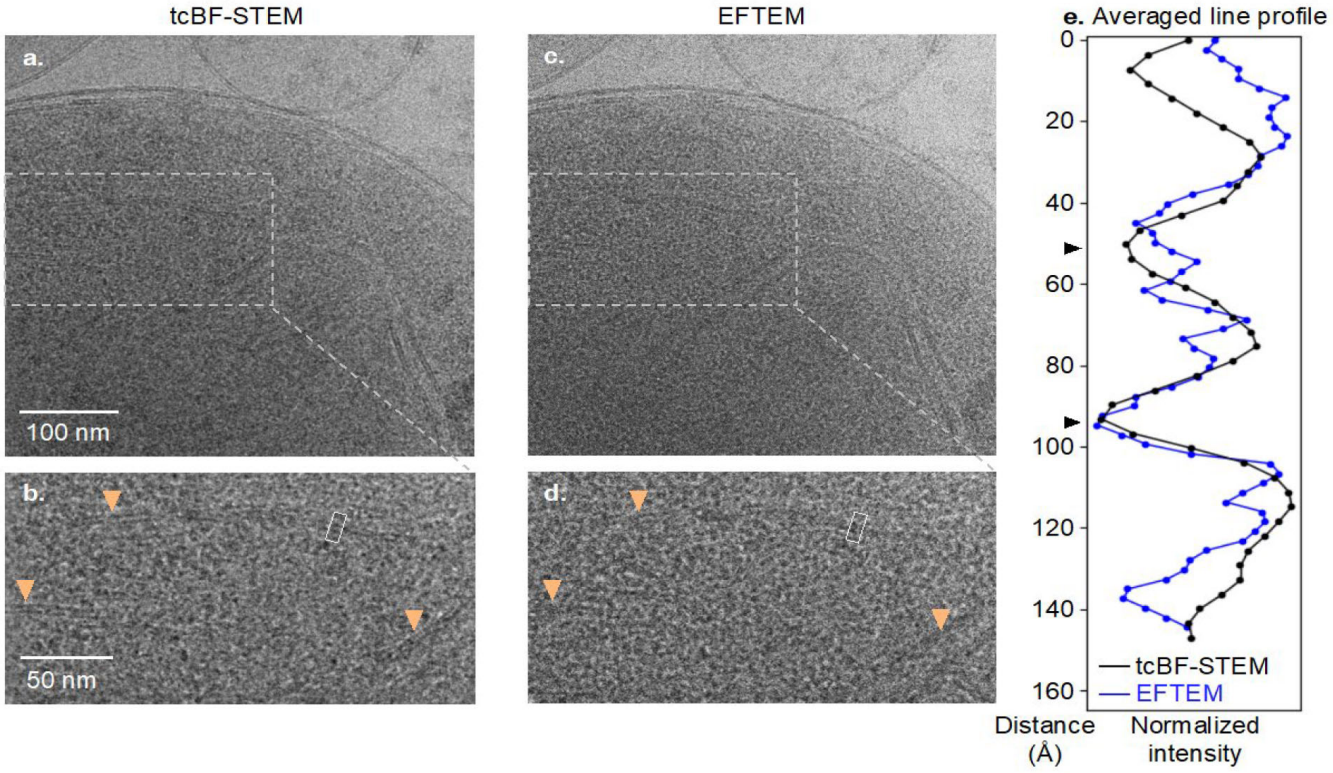

Fig. 1. Comparison of EFTEM and tcBF-STEM on a mitochondrion. The membrane bilayers are similarly resolved in the leading edge of the organelle, but in the thicker region, tcBF-STEM (**a** and **b**) exhibits clearer resolution of the membrane (orange arrows) than EFTEM (**c** and **d**). Average line profiles (**e**) of the solid-line boxed areas depict the intensity variation across a membrane double layer. Each image was acquired with 14 $e^-/Å^2$ dose measured over vacuum. The STEM image was acquired before EFTEM.

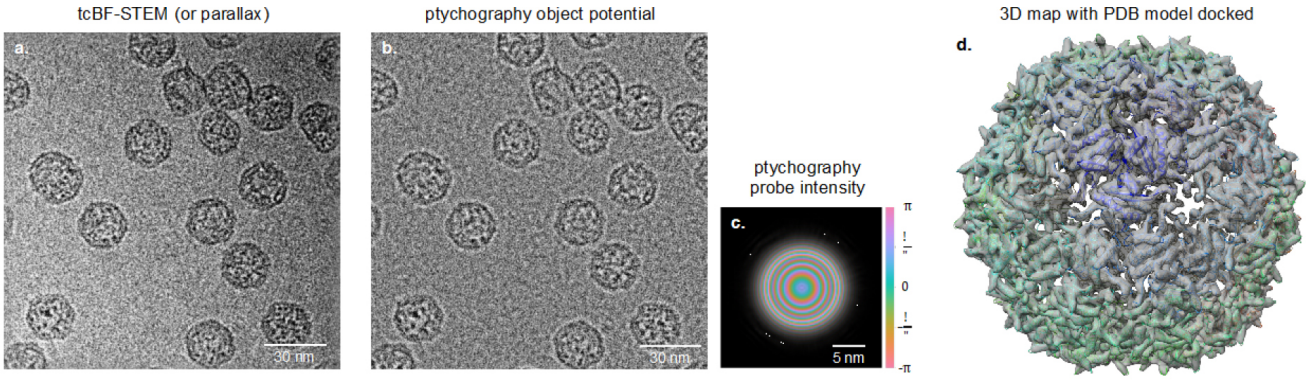

Table e.

Dataset	Microscope	Stage	Camera frame rate	α (mrad)	Step size (Å)	Dose ($e^-/Å^2$)	Detector spacing (mrad/pixel)	Number of particles	Nominal resolution (GSFSC, 0.143 cutoff)	B factor ($Å^2$)
i.	TFS Titan Themis, probe corrected	Gatan side-entry	10 kHz	8	11	45	0.1526	789	tcBF-STEM 7.03 Å	351
ii.	TFS Krios G4	Krios dedicated cryo stage	1 kHz	6.6	10.6	62	0.1426	~ 1400	tcBF-STEM 7.69 Å	529
iii.	TFS Krios G4	Krios dedicated cryo stage	1 kHz	4	13	41	0.0810	~ 1800	Ptychography 7.88 Å	843
									tcBF-STEM 8.53 Å	868

Fig. 2. Single particle analysis (SPA) with tcBF-STEM and ptychography on virus-like particles (VLPs). Phase-corrected tcBF-STEM (**a**), ptychography object potential (**b**), and ptychography probe intensity (**c**) of the same dataset ((iii) in table e). A representative 3D density map resolved with dataset (i) is shown in (**d**) with PDB model 1DWN docked inside. Find a summary of experimental conditions, parameters, and reconstruction results in table (**e**). All samples were prepared from the same batch. The particle has icosahedral symmetry with T=3.

References

1. I. Lazić *et al. Nat. Methods* **19**, 1126–1136 (2022).
2. B. Kucukoglu *et al.* bioRxiv preprint: https://www.biorxiv.org/content/10.1101/2024.02.12.579607v1.full.pdf
3. K. Spoth *et al. Microsc. Microanal.* **26**, 1482–1483 (2020).
4. Y. Yu *et al. Microsc. Microanal.* **27**, 758–760 (2021).
5. G. Varnavides *et al.*, arXiv preprint: https://arxiv.org/abs/2309.05250
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