Cryogenic Electron Ptychographic Single Particle Analysis (Cryo-EPty SPA)

Xudong Pei¹, Liqi Zhou¹, Chen Huang², Mark Boyce³, Judy S. Kim²,⁴, Emanuela Liberti², Takeo Sasaki⁵, Peijun Zhang³,⁶, David I. Stuart³, Angus I. Kirkland²,⁴ and Peng Wang⁷,*

¹College of Engineering and Applied Sciences, Nanjing University, Nanjing 210093, China. ²The Rosalind Franklin Institute, Harwell Science and Innovation Campus, Didcot, OX11 0FA, UK. ³Division of Structural Biology, Welcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, UK. ⁴Department of Materials, University of Oxford, Parks Road, Oxford OX1 3PH, UK. ⁵JEOL Ltd., 3-1-2 Musashino, Akishima, Tokyo 196-8558 Japan. ⁶Electron Bio-Imaging Centre, Diamond Light Source, Harwell Science and Innovation Campus, Didcot OX11 0DE, UK. ⁷Department of Physics, University of Warwick, Coventry CV4 7AL, UK.

peng.wang.3@warwick.ac.uk

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Cryo-electron microscopy (Cryo-EM) is an advanced technique for obtaining high-resolution, three-dimensional images of various biological samples in their natural, frozen-hydrated state. However, conventional cryo-EM images can be affected by low signal-to-noise ratios and low contrast due to the sensitivity of vitrified biological samples. To address these issues, cryo-EM single particle analysis typically records images at high defocus, but this method reduces the accuracy of high-frequency information. Our new technique utilizes cryo-electron ptychography (Cryo-EPty) [1], as depicted in Figure 1a, that is a variant of this method that uses scanning ptychographic diffractive imaging [2]. In Ptychography, the probe is scanned over the specimen in overlapping positions, using a defocused probe. As the full diffraction pattern is captured, this technique is highly efficient, especially when data is recorded using direct electron detectors that produce high signal-to-noise ratios at low electron doses [3]. This paper presents a new 3D SPA technique that is based on Cryo-EPty SPA and demonstrates its ability to restore 3D information from a single sample. The experimental Cryo-EPty SPA datasets (Figure 1b) [1] were used to reconstruct the ptychographic phase of rotavirus double-layered particles (DLPs) at a dose of 22.7 e/A². The particle-picking procedures developed for Cryo-EM SPA can be directly applied to this phase, producing a stack of particle phases that are coordinated by position, as shown in Figure 1b. A 3D density map of rotavirus DLPs (Figure 1c) was then reconstructed using 300 particles from this stack of phases. We believe that the combination of Cryo-EPty and SPA has the potential to produce high-resolution 3D reconstructions of biological samples [4].

Figure 1. (a) Schematic optical configuration diagram of the workflow used for cryo-ptychography; (b) Many instances of the viral particles for single particle analysis can be extracted from reconstructed ptychographic phases, scale bars: 20 nm. (c) 3D map corresponding to the particle instances.

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