

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

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Cryo-electron microscopy (cryo-EM) is a powerful method for the high-resolution three-dimensional structural characterization of a wide range of biological samples in a close-to-native, frozen-hydrated state [1]. Due to the extreme radiation sensitivity of vitrified biological samples [2], images of these have low signal-to-noise ratios [3] and low contrast [4,5]. Cryo-EM single particle analysis (SPA) relies on the use of conventional phase-contrast images recorded at high defocus to improve information transfer at low spatial frequencies. However, defocusing the image corrupts the information transfer at higher spatial frequencies [6]. In order to surpass this barrier, it is important to develop new high-contrast phase-sensitive imaging modes, such as novel phase plates [5], which have been used to enable in-focus phase-contrast imaging. However, the routine applications of phase plates are still somewhat restricted by signal attenuation at high frequencies, inconsistent fabrication, poor reliability and short working lifetimes due to electrostatic charging [7, 8].

Cryo-electron ptychography (Cryo-EPt) [9] as shown in Fig. 1 is an alternative technique based on scanning ptychographic diffractive imaging [10]. Ptychography uses a defocused probe to scan over a specimen with highly overlapping probe positions. In physical science, this approach has shown great potential in applications such as super-resolution imaging [11,12], high-contrast light-element detection [13], low dose imaging [14] and three-dimensional imaging [15,16]. Moreover, as ptychography utilizes the full diffraction pattern, it is dose-efficient particularly when data is recorded using direct electron detectors, which record high signal-to-noise at low electron dose. This has recently been demonstrated for micrometer wide phase reconstruction of an unstained virus-infected cell at a dose of $27 \text{ e}/\text{\AA}^2$ [9].

Here, we show a new 3D SPA technique based upon cryogenic ptychography (cryo-EPt SPA) and experimentally demonstrate that cryo-EPt SPA under cryogenic conditions can restore 3D information from a single sample. Experimental cryo-EPt SPA datasets (Fig. 1 b) were acquired in a scanning diffraction configuration as shown schematically in Fig. 1 a, in which a defocused probe is scanned over a cryo-sample. Using the ePIE algorithm [17], the ptychographic phase of rotavirus double-layered

particles (DLPs) with 76.5 nm in diameter were reconstructed at a dose of $22.7 \text{ e}/\text{A}^2$ as shown in Fig. 1 c. At this dose, the phase shows strong contrast from the virus particles, where both the capsids of viral protein (VP) and channels are consistent with those observed using conventional defocused TEM images (Fig. 2 a). The particle-picking procedures that have been developed for cryo-EM SPA can be directly applied to the phase. Multiple individual particles can then be sequentially picked from the phase and formed into a positionally coordinated stack of particle phases, as shown in Fig. 2 b, respectively. We will show that using the SPA pipeline, a 3D density map of rotavirus DLPs (Fig. 2 c) can be reconstructed with 300 particles from the stack of particle phases. A single VP6 trimer can be seen in a central slice (Fig. 2 d) extracted across the middle of the 3D map. Importantly by using a larger probe convergence angle the ptychographic transfer function can be “tuned” to recover high spatial frequencies at atomic resolution [9]. We expect that cryo-EPT combined with SPA has great potential to yield high-resolution 3D reconstructions of biological samples [18].

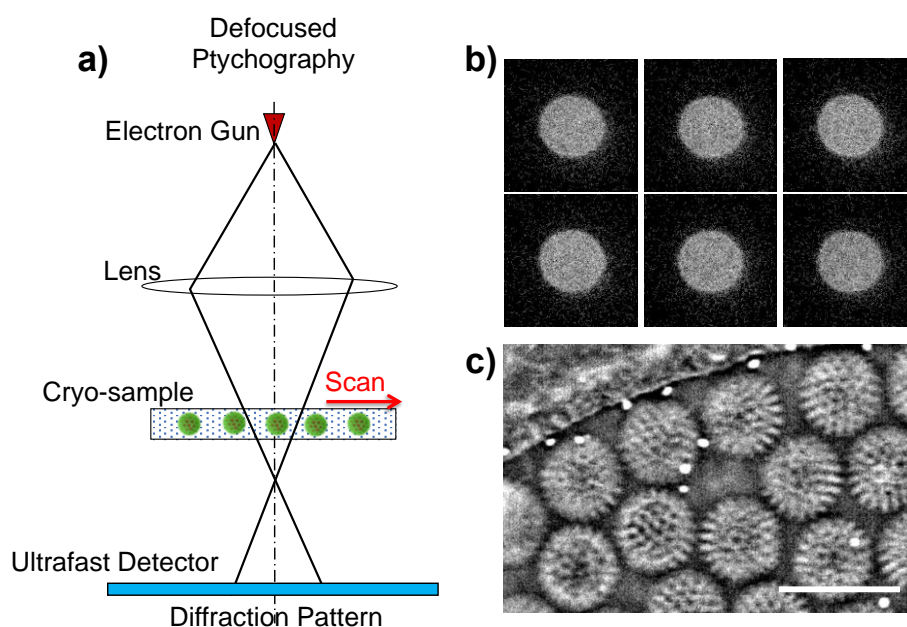


Figure 1 Schematic optical configuration diagram of the workflow used for cryo-ptychography (a); Array of diffraction patterns as a function of probe positions (b); Reconstructed phase of rotavirus double-layered particles (c). Scale bars: 100 nm

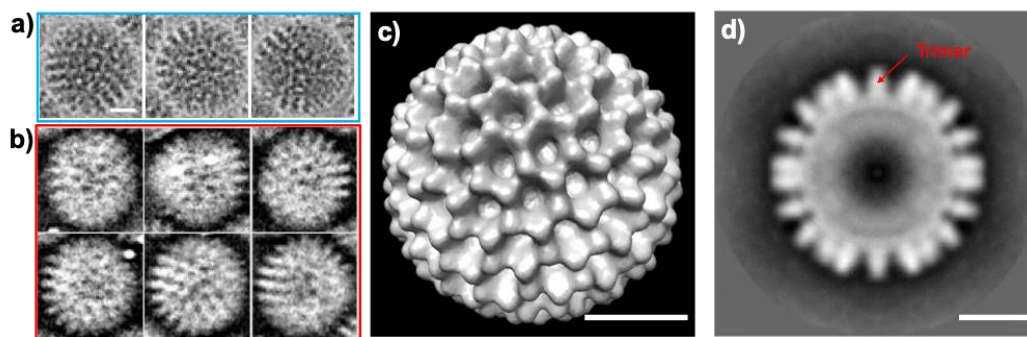


Figure 2 Many instances of the viral particles for single particle analysis can be extracted from (a) TEM images and (b) reconstructed ptychographic phases, scale bars: 20 nm. c) 3D map corresponding to the particle instances and d) central slices extracted from the 3D map, scale bars: 25 nm

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