

## Ptychographic Single Particle Analysis for Biological Science

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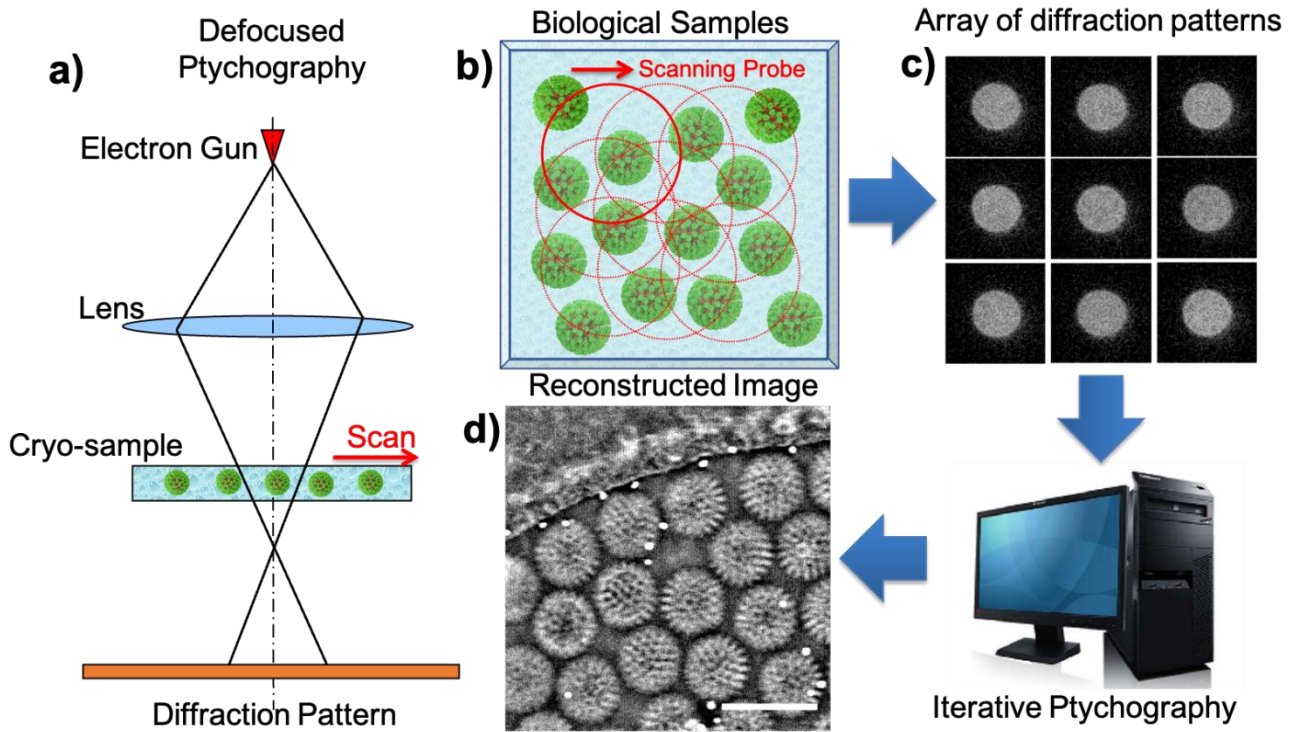
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Cryo-electron microscopy (cryo-EM), has attracted significant efforts to develop techniques for the high resolution three-dimensional structure characterization of biological samples in a close-to-native state at high resolution, including single particle analysis[1] and tilted tomography[2]. However, unstained biological samples embedded in thin vitreous ice are essentially pure phase objects that are extremely radiation sensitive[3], and consequently images of these have low signal-to-noise ratios[4] and low contrast[5,6]. To counter the latter, cryo-EM relies on the use of phase contrast imaging at high defocus to improve information transfer at low spatial frequencies at the expense of higher spatial frequencies[7]. Therefore, it is important to develop ways to improve resolution and contrast without damaging samples, including the use of direct electron detectors and novel phase plates[6]. 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[8,9].

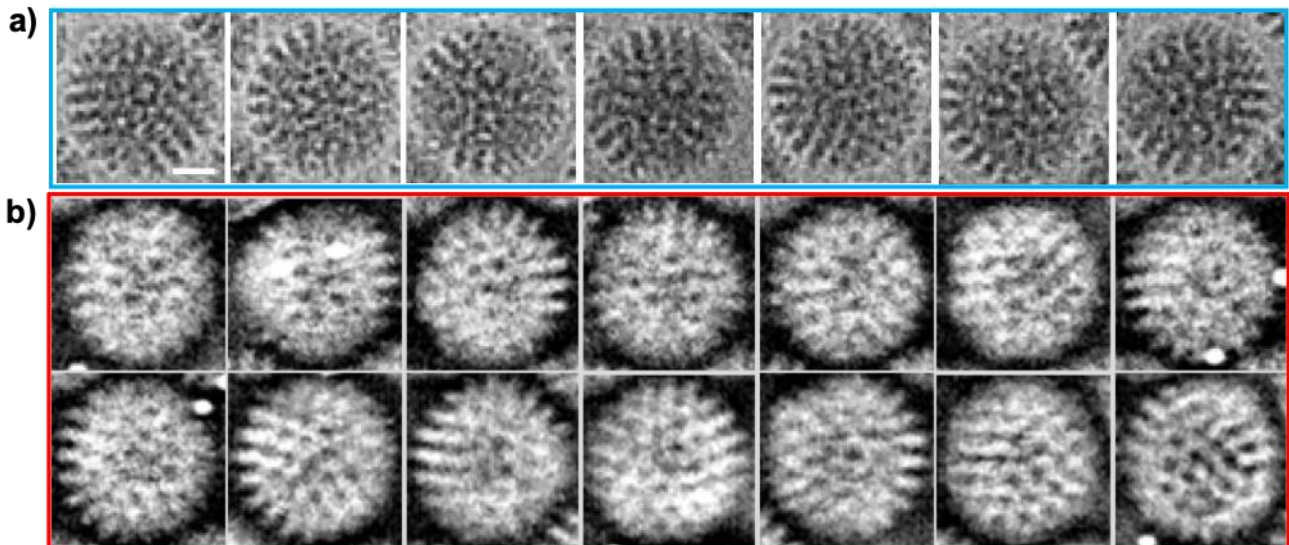
An alternate technique is electron ptychography as shown in Fig. 1, where a probe, defocused by several tens of nm's is scanned over a specimen with highly overlapping probe positions. This approach has demonstrated its potential for super-resolution imaging[10,11], high-contrast light-element detection[12], low dose imaging[13] and three-dimensional imaging[14,15]. The development of advanced direct electron detectors dramatically increases the signal-to-noise ratio and improves the dose efficiency of ptychography[13] making it applicable to low dose biological imaging with high contrast and signal-to-noise ratios[16].

Here, we experimentally demonstrated low-dose ptychography with a defocused probe for biological imaging in a cryo-state in a scanning electron diffraction mode as shown schematically in Fig. 1(a and b) using a JEOL ARM300CF. Ptychographic diffraction datasets in Fig. 1(c) were acquired on a direct electron detector with the probe rastered across the samples. Rotavirus double-layered particles (DLPs) with a diameter of 76.5 nm were used as a typical example for the demonstration of this method under cryo conditions. The ptychographic phase of the rotavirus DLPs was reconstructed at a dose of 23 e/A<sup>2</sup> as shown in Fig. 1 (d). At this dose, the phase image shows a strong contrast in the virus particles, where both the capsids of viral protein (VP) and channels between these can be clearly seen. These features are consistent with those observed using conventional defocused TEM images[Fig. 2 (a)]. The ptychographic phase preserves low spatial frequency information, facilitating the recovery of these important molecular features. Importantly by altering the probe convergence angle the ptychographic transfer function can be "tuned" to recover specific frequency ranges including better transfer of both low and high spatial frequencies when compared to conventional phase contrast imaging. We will show that it can be applied to the micron sized fields of view that are required for either imaging many identical particles for single particle analysis[Fig. 2 (b)] or capturing multiple biological

ultrastructures in a cellular context. Finally, we will show that ptychographic reconstruction can also be readily combined with single particle analysis and tomography to yield 3D reconstructions of biological samples.



**Figure 1.** Schematic diagram of workflow used for cryo-ptychography. (a) Optical configuration; (b) Schematic biological samples at a cryo-state; (c) Array of diffraction patterns as a function of probe positions; (d) Reconstructed phase of rotavirus double-layered particles. 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

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