

## Electron Ptychography of Single Biological Macromolecules

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Due to the advent of direct electron-counting detectors and improvements in algorithms, cryo-electron microscopy (cryo-EM) has rapidly developed into a mainstream technique for solving the structures of biological macromolecules. The key to improving the resolution even further lies in measuring more effectively the phase shift which the molecules impart on the high-energy electron wave.

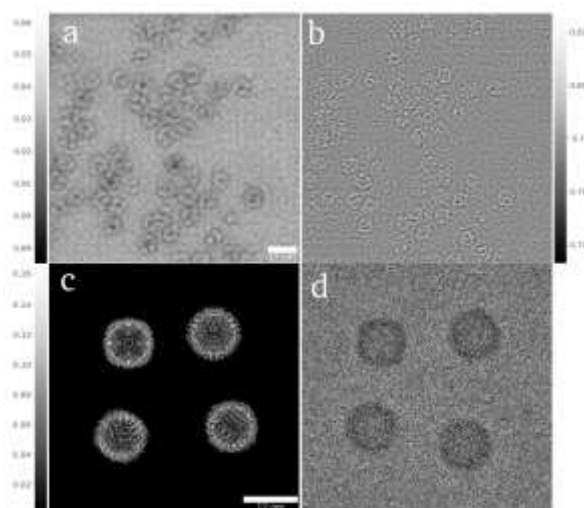
In a previous paper [1] we have shown that, with the help of multi-slice simulations, electron ptychography in combination with Bayesian reconstruction algorithms provides up to two orders of magnitude improvement in signal-to-noise ratio at 3 Å resolution compared to Zernike-type phase contrast and phase contrast from defocus aberration. Therefore, ptychography may become a more efficient alternative to conventional phase contrast imaging methods in cryo-EM. Recently Pennycook et al. [2] showed that single side-band electron ptychography with a focused probe is less affected by partial coherence than HRTEM, and might, therefore, extend the resolution limits of current methods. The additional amenity of having immediate online feedback from the STEM images makes this method highly attractive, once fast enough detectors become available.

So far, however, the high beam current of the focused electron beam and the relatively low frame rate of the currently available detectors do not permit to perform focused-probe ptychography experiments at resolutions relevant for single-particle cryo-EM. Here we experimentally demonstrate electron ptychography with a defocused beam on apo-ferritin molecules on an ultrathin carbon grid at room temperature. The experiment was performed on an FEI Tecnai F20 microscope equipped with a custom bottom-mounted Medipix3 electron counting detector with 512x1536 pixels and a maximum frame-rate of 2 kHz. We selected a condenser aperture with 70 μm diameter, which resulted in a convergence semi-angle of 7.4 mrad and a diffraction-limited resolution of 1.69 Å at an electron energy of 200 keV. We chose a defocus of 800 nm, resulting in a real-space probe diameter of ~11.5 nm. We scanned the sample in a grid of 64x64 positions with a probe overlap of 70%. The lowest beam current with standard microscope alignments was 2.8 pA. Therefore we chose an exposure time of 2 ms to reach an accumulated dose of ~40 e<sup>-</sup> / Å<sup>2</sup>. For the reconstruction, we have developed a Bayesian reconstruction algorithm to properly account for the noise statistics of the detector [3]. We use stochastic gradient

descent with Nesterov momentum to alternatively optimize the maximum-a-posteriori estimate of the transmission function and the estimate of the probe function, given the set of noisy diffraction patterns. As a prior for our Bayesian reconstruction method, we choose the transmission function to have high probability if it is close to the output of the denoised image of the current estimate. For denoising, we use the sparsity-enforcing BM3D algorithm [4]. Fig. 1 shows the reconstruction after 50 iterations. One can make out the apo-ferritin particles in the phase image, although the background from the carbon substrate is quite strong. Because the experiment was performed at room temperature, the particles are expected to have undergone significant radiation damage, although the shape of some particles resembles the known structure. It can also be seen that the reconstructed phase shift of 0.08rad is only half of the expected maximum phase shift of 0.16rad. This may be due to the inhomogeneous background of the substrate, or mass loss due to radiation damage. The dose rate used in this experiment was  $\sim 550 \text{ e}^-/\text{\AA}^2/\text{s}$ , while typical single-particle cryo-EM experiments use between 1-2  $\text{e}^-/\text{\AA}^2/\text{s}$ . Future experiments with a cryo-transfer holder will allow answering the question if the high dose rate used in cryo-ptychography experiments has detrimental effects on the resolution of the final reconstruction.

#### References:

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**Figure 1.** Ptychographic reconstruction of apo-ferritin molecules on ultra-thin carbon at an accumulated dose of  $40 \text{ e}^- / \text{\AA}^2$ . a) The amplitude of the reconstructed transmission function, b) the phase of the reconstructed transmission function. c) Phase maps of apo-ferritin molecules simulated for 200keV for visual comparison with the reconstruction. d) simulated phase plate image with a perfect Zernike phase plate at an accumulated dose of  $40 \text{ e}^- / \text{\AA}^2$ .