Investigating Direct Focused Probe Ptychography for Single Particle Analysis

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Single particle analysis (SPA) in transmission electron microscopy (TEM) has been very successful for materials such as proteins. However, achieving atomic resolution for the most beam sensitive materials remains very challenging. Although recent technological advances have pushed the best resolution achieved in SPA to 1.2 Å [1,2], resolutions around 3 Å are the norm for SPA analysis of proteins. Interestingly this is also around the resolution achieved using SPA with a scanning TEM (STEM) technique known as integrated differential phase contrast (iDPC) [3], closely related to the integrated center of mass (iCoM) signal. Both iDPC and iCoM have recently been used to great effect with various beam sensitive materials. However, advances in camera technology have also greatly facilitated electron ptychography, which can provide significantly greater dose efficiency [4, 5].

Recent comparisons to TEM have highlighted potential advantages for ptychography, including the single signed contrast transfer function (CTF) and its robustness to defocus spread [6]. Here we will take this methodological analysis further and investigate the prospect for direct methods such as the single side band method [7] to advance SPA yet further. Direct ptychography avoids the need for iterative solution of the phase and with a focused probe can provide a simultaneous annular dark field (ADF) signal.

In SPA a tradeoff must be made between maximizing the contrast and retaining high resolution details as illustrated in Figure 1 with apoferritin at a dose of 100 e/Å^2. With TEM large defocus values are used to boost the low frequency transfer so that particles can be located, but this brings in strong oscillations in the contrast transfer function (CTF) which limits the ultimate resolution. In contrast the ptychographic CTF can be controlled by adjusting the convergence angle (CA) without the need for aberrations. By reducing the convergence angle, the lower frequencies can be enhanced but the tradeoff is the maximum resolution passed at twice the CA is also reduced.

Recent studies have suggested that significant benefits in the amount of information retrievable before the onset of damage can be had by working at low accelerating voltages [8] where chromatic aberrations are more pronounced and the robustness of ptychography to chromatic aberrations may prove crucial. The images in Figure 1 are simulated at 60 kV and include a 4 nm defocus spread. Calculation of the signal to noise for the single images shows significant benefits for the ptychography, as is apparent visually.

Experimental 50 e/Å^2 STEM apoferritin data were recorded at microsecond dwell time using a Timepix3 [5]. Lower doses are easily reached by reducing the probe current. The simultaneous ADF signal provides strong contrast with this negatively stained sample. This facilitated aligning the data from 13

molecules with template matching and fourfold averaging. Despite the small number of particles included in this simple proof of principle experiment, the features of the ferritin appear relatively strongly. We will present further experimental and theoretical findings investigating how ptychography can expand the possibilities for SPA.
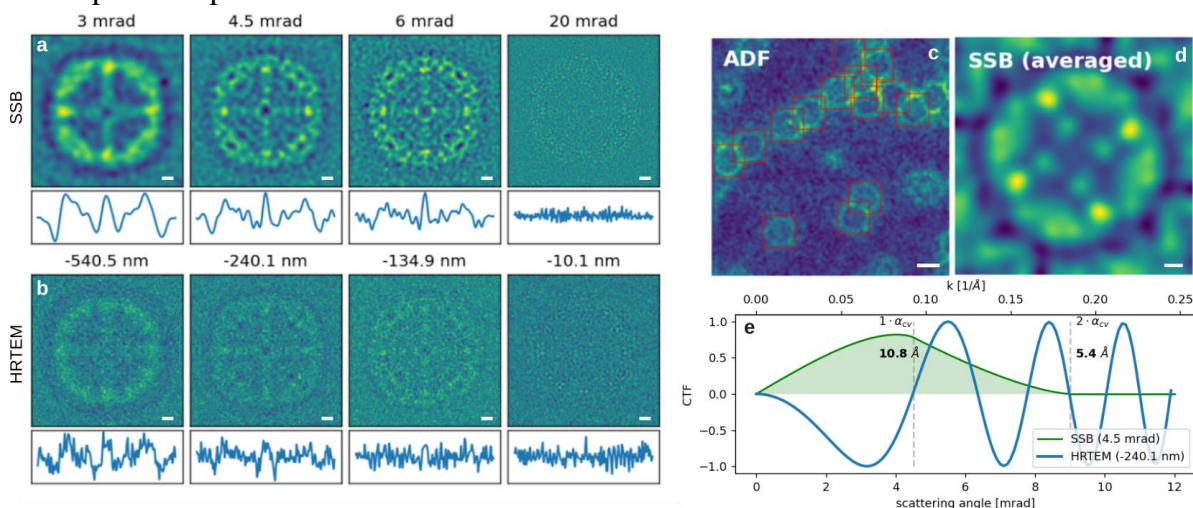
Figure 1. 100 e/Å^2 simulations of Apoferritin at 60kV for (a) SSB ptychography and (b) TEM showing the tradeoff between contrast and resolution for different conditions. The profile along the x-axis is shown below each image. Experimental 200kV 50 e/Å^2 (c) ADF and (d) averaged SSB image of negatively stained apoferritin. The red rectangles in (c) indicate the positions used to average the data for the simultaneously recorded SSB image, which is further 4-fold averaged to increase the signal-to-noise. (e) Calculated CTFs of SSB and TEM for two 60 kV conditions. Scale bars are 1 nm.

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