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Dose-efficient tcBF-STEM imaging with real-space information beyond the scan sampling limit

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Tilt-corrected bright-field (tcBF) STEM makes use of almost all electrons in the BF disk to produce coherent STEM images. It has been shown to outperform conventional TEM and energy-filtered TEM (EFTEM) for imaging of thick, dose-sensitive biological specimens [1]. Here, we demonstrate how resolution limits set by the scan step size can be overcome in tcBF-STEM by making use of redundant information in diffraction space. Information transfers up to four times the real-space Nyquist sampling limit are shown in a robust test specimen and a two-fold increase is obtained in a frozen-hydrated apoferritin sample imaged under cryogenic conditions. This allows us to reduce the sampling in real space and still recover information up to a limit set by the probe-forming aperture semi-angle, α.

TcBF-STEM combines images from individual detector pixel located in the center BF disk after correcting for image shifts introduced by probe aberrations. Figure 1a shows a tcBF-STEM image that makes use of electrons scattered within ~4/5 α of a gold test specimen. The correction for the tilt-induced image shifts, both magnitude and direction, is shown in the color overlay of individual pixels in the relevant detector region (Fig. 1a.i). In this detection scheme, each detector pixel is small relative to α resulting in a coherent image while conventional BF-STEM preserves coherency by instead limiting the detection angle to ~1/3 α (Fig. 1b). An illustration of incoherent BF-STEM imaging (IBF) is presented in Fig. 1c by directly integrating over all detector pixels within ~4/5 α without correction for tilt-induced shifts. Radial averages of the FFT amplitudes (Fig. 1d) reflect the higher average image intensity in tcBF and IBF compared to BF, but the IBF amplitude drops off quickly confirming that tcBF outperforms the other two methods.

For dose-sensitive experiments, it is favorable to employ fewer real-space pixels to reduce effects due to the finite detector read-out time. In tcBF, data can be acquired at a larger scan step size and then upsampled by filling in information between scan pixels with redundant information from diffraction space as long as a defocused probe is used. Such information is extracted by determining the image shifts to sub-pixel accuracy. Figure 1e shows an upsampled image with an 8 Å scan step size and a 2 Å pixel size in the reconstructed tcBF image. The FFT in the inset (i) suggests information transfer up to almost 4 Å, however, the large defocus (~900 nm) used here dominates the image. The near zero-intensity lines in the FFT arise from the real-space upsampling process before applying image shifts but these can be further reduced by more optimally filling in information between scan points by increasing camera length or defocus.

A challenge for low-dose tcBF imaging is to determine shifts for low-SNR images formed by single detector pixels. Here we image frozen-hydrated horse spleen apoferritin using an electron microscope pixel array detector (EMPAD) [2] with a total dose of ~77 e-/Å^2 on the full detector and only 0.03 e-/Å^2 in a single detector pixel image. Figure 2a shows that the low SNR prevents accurate determination of the image shifts despite the addition of high-contrast gold beads. One way to improve the cross-correlations is to combine multiple detector pixels at a cost of reducing the detector angular resolution. Figure 2b shows an image reconstructed with detector pixels binned 4-by-4, and a resulting single image dose of 0.5

e-/Å^2. Individual apoferritin particles and 5-nm gold beads are clearly resolved in this case. Image shift patterns reflecting defocus aberrations are now clear (i) compared to Fig. 2a where the shifts are random. However, such binning reduces the effective detector angular resolution and the number of images with distinct shifts needed for upsampling. Fortunately, the image information from individual detector pixels can still be used by fitting the shifts obtained from binned detectors pixels to the probe aberration function [3] and subsequently applying the fitting results to the original detector pixels. Figure 2c is the resulting image after restoring detector angular resolution. Fourier ring correlation (FRC) [4] is used to quantify information transfer for the tcBF images formed with different detector pixel binning and image shifts confirming the benefits of applying accurate shifts. The FRCs of images upsampled by 2 (shown with lines) also agree exactly with non-upsampled images (shown as crosses) in the corresponding frequency range, which underscores that the upsampling technique does not alter any existing low-frequency information.

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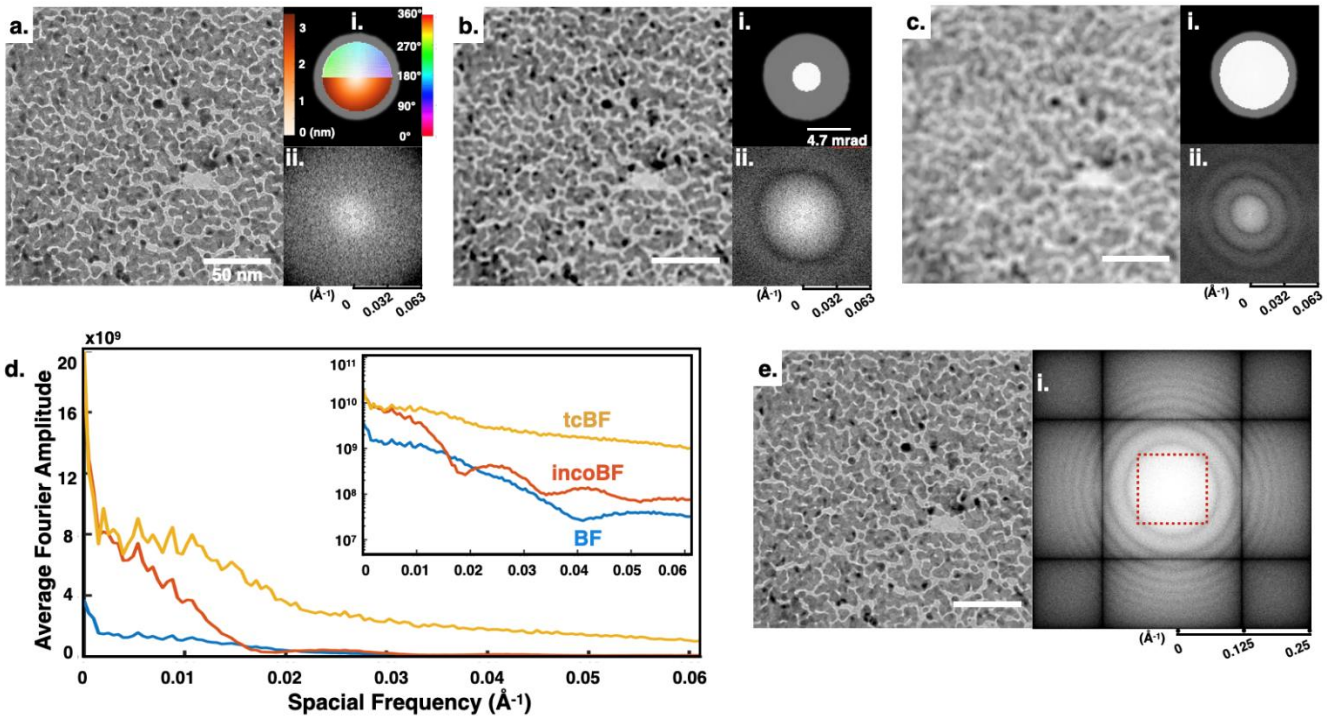

Figure 1. Tilt-corrected bright-field STEM (tcBF-STEM) imaging on a gold test specimen at 300 keV demonstrates resolution improvements compared to incoherent BF and information transfer up to four times the real-space Nyquist sampling limit. (a) 4D-STEM enables reconstruction of a tcBF-STEM image by summing the tilt-corrected images formed by each detector pixel within 4/5 of the convergence semi-angle, α=4.7 mrad. The tilt-induced shift of each image is plotted as a color overlay with the averaged CBED pattern in the inset (i) and the FFT of the reconstructed image is shown in (ii). (b) The conventional BF-STEM image is formed by electrons scattered up to 1/3 α. When electrons up to 4/5 α are included, without correcting for tilt-induced shifts, an incoherent BF-STEM image is obtained (c). (d) Radially averaged FFT amplitudes show increased resolution in tcBF-STEM. (e) Sub-pixel shifts in tcBF allow for further resolution improvements by four-fold upsampling from a scan step size of 8 Å to a 2 Å image pixel size. The red box in (i) indicates the Fourier space limits before upsampling.

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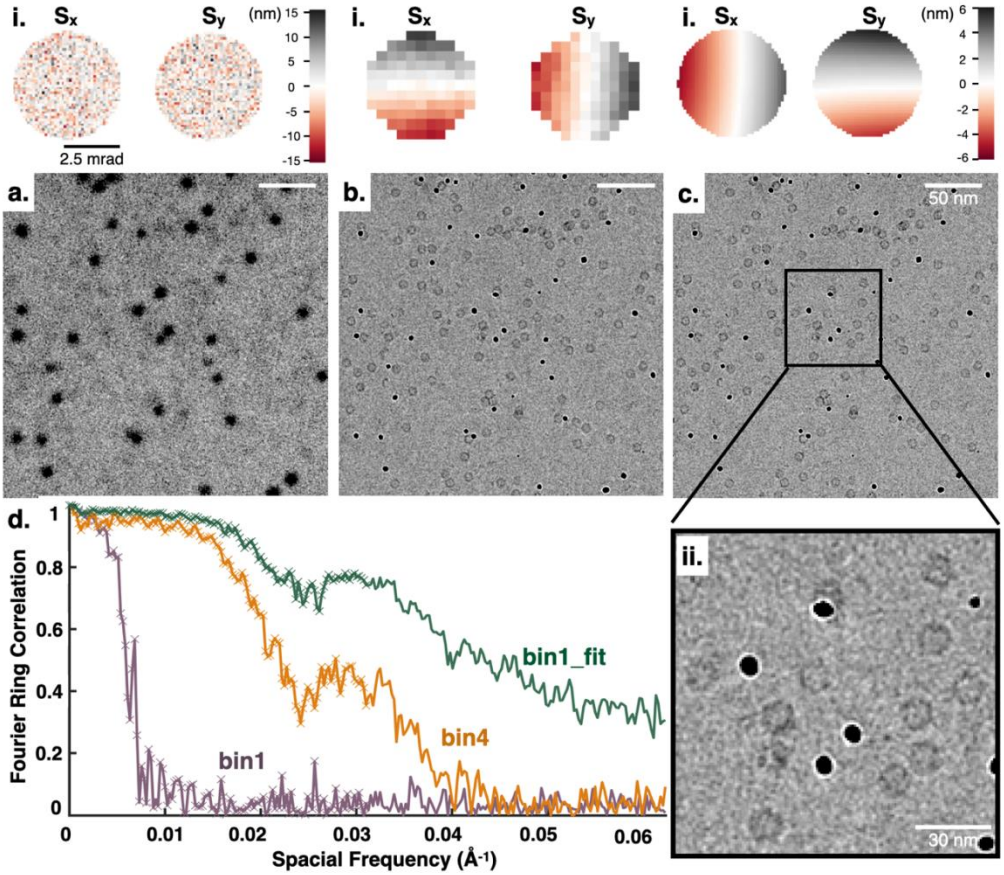

Figure 2. Cryogenic tcBF-STEM imaging of vitrified apoferritin with semi-convergence-angle, α of 3.15 mrad. All images employ detector pixels within 4/5 α and are upsampled by 2. The shift maps resulting from cross-correlation are presented in insets (i). Pixel colors in shift map x (Sx) and shift map y (Sy) indicate the shift distance in the x and y direction, respectively. Due to low dose conditions, cross-correlating images from individual detector pixels fails resulting in image blurring (a). Binning 4-by-4 detector pixels increases the SNR for successful cross-correction (b) and aberration fitting of the shift maps (c) allows accurate shifts to be applied to images from each individual detector pixel overcoming the limitations in (a). The Fourier Ring Correlation (FRC) is shown in (d) for images reconstructed from individual detector pixels (bin1), detector pixels binned 4-by-4 (bin4), and individual detector pixels with shifts determined through aberration fitting (bin1_fit), respectively. The lines represent FRC for images upsampled by 2 and the crosses are for non-upsampled images.

References

[1] K. A. Spoth, et al, Microscopy and Microanalysis 23 (2017), p. 804-805.
[2] M. W. Tate, et al, Microscopy and Microanalysis 22 (2016), p. 237-249.
[3] A. R. Lupini, et al, Journal of Microscopy 263 (2016), p. 43-50.
[4] W. O. Saxton and W. Baumeister, Journal of Microscopy 127 (1982), p. 127–138.

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