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TFS: Combined Tilt- and Focal Series Scanning Transmission Electron Microscopy

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The primary method currently used for obtaining insight into the three-dimensional (3D) structure of unique samples from biology and materials science at the nanometer scale is tilt-series transmission electron microscopy (TEM) [1]. A 3D volume is reconstructed from images recorded at many projections obtained by mechanically tilting the sample stage. The tilt range strongly influences the resolution and characteristics of the 3D reconstructions. Therefore, one would ideally acquire tilted images covering the entire angular range of ±90°. However, in practice the maximum tilt range is usually only about ±60 -78° due to mechanical limitations of specimen holders and because the effective thickness of the specimen as seen by the electron beam increases as the section is tilted. The tomographic reconstruction then suffers from missing information and a limited resolution on account of this so-called “missing wedge”. The reconstruction of conventional tilt-series tomography data is particularly difficult because its data acquisition involves a large amount of mechanical movements of the stage. The quality of the tomogram is mostly determined by the precision of the alignment of the individual images. The alternative of recording focal-series scanning TEM (STEM) data avoids the problem caused by the mechanical movements but it lacks axial resolution [2].

We have introduced a new recording scheme for 3D STEM that significantly reduces the aforementioned two limitations of tilt series tomography. In the combined tilt- and focal series (TFS) method, the specimen is rotated in relatively large tilt increments over the possible tilt range, and for every tilt direction, a through-focal series is recorded (Fig. 1). The reconstruction method differs from existing methods such as sequential algebraic reconstruction technique (SART). Both the tilt-series and focal-series data are reconstructed into a 3D tomogram in the same software algorithm. The conical shape of the STEM probe is taken into account via forward- and backward projection operators. The TFS method was demonstrated to exhibit reduced “missing wedge” artifacts and a higher axial resolution than obtainable using STEM tilt series [3]. Fig. 2 shows that the missing wedge is still present in the TFS but in the central vertical region, low spatial frequency signal components are now present (arrow). Moreover, the streaks corresponding to the tilt directions are less pronounced in the TFS data.

Amongst the most promising applications for STEM tomography with TFS are specimens that require a high precision in the representation of the 3D shapes, whereby TFS reconstruction results in a superior shape reconstruction. The combined tilt- and focal series method could potentially present a better method for atomic 3D resolution while it tolerates a much smaller number of tilt angles than tomography. We expect that a further advantage of TFS STEM can be found for the imaging of micrometers-thick samples. The largest problem when imaging thick samples occurs at the high tilt angles, where the effective thickness increases by a factor of 3 (for a tilt angle of 70 degrees), and beam blurring on account of electron scattering severely limits the resolution. With TFS it is possible to limit the tilt range while obtaining a higher axial resolution than for a tilt series alone.

References:
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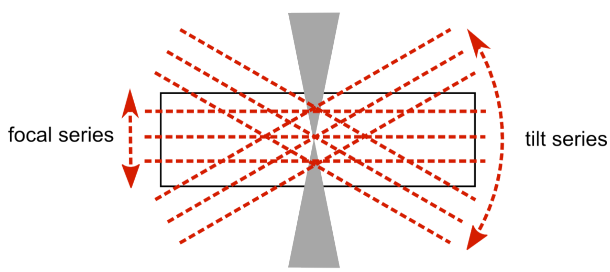

Figure 1. Schematic view of the TFS recording scheme with STEM. A thin specimen is imaged pixel-by-pixel in dark field mode using the annular dark field detector. In a combined tilt- and focal series, STEM images are acquired in a through-focal series at each tilt angle. The specimen stage is titled after each focus series. The TFS reconstruction accounts for the conical beam

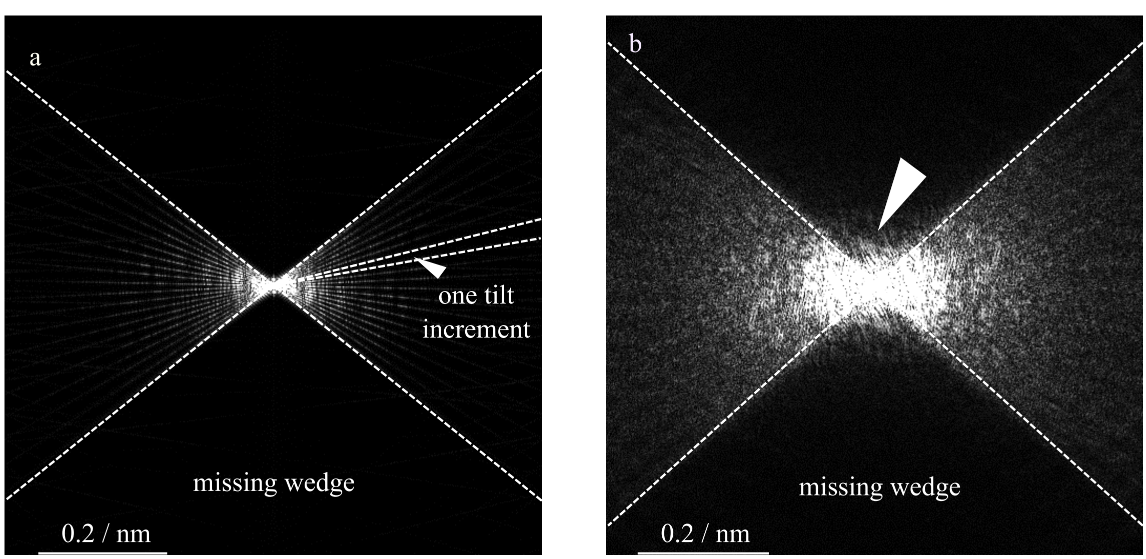

Figure 2. Comparison of tilt series STEM tomography and SART reconstruction with TFS STEM tomography and reconstruction. a: Spatial frequency spectrum (Fourier Transform) of a vertical (xz) slice of the conventional tilt series STEM tomography data. b: Frequency spectrum of a xz slice of the TFS STEM dataset. The white lines mark the border of the missing wedge. With permission from [3].