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IT-10-O-1637 Combined tilt- and focal series scanning transmission electron microscopy: TFS 3D STEM

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Tilt-series transmission electron microscopy (TEM) tomography is the method of choice to obtain nanoscale three-dimensional (3D) information from samples in biology and materials science. 3D data is acquired by mechanically tilting the sample stage and recording images at each tilt angle. However, the tilt range is limited to $\pm 70^\circ$ for most samples, and the tomographic reconstruction suffers from missing information and a limited resolution on account of the so-called “missing wedge”. Furthermore, the quality of the reconstruction critically depends on the precision of the alignment of the individual images. Alternatively, scanning TEM (STEM) focal series can be recorded avoiding tilting altogether but this method lacks axial resolution [1]. A new recording scheme to obtain 3D data is presented by combined tilt- and focal series (TFS) STEM. This method significantly reduces the two aforementioned limitations of tilt series tomography. The specimen is rotated in relatively large tilt increments, and for every tilt direction, a through-focal series is recorded (Fig. 1). 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 exhibits reduced “missing wedge” artifacts and a higher axial resolution than obtainable using STEM tilt series [2]. Fig. 2 shows that the missing wedge is still present in the TFS but low spatial frequency signal components are now present (arrow) in the central vertical region. Streaks corresponding to the tilt directions are also less pronounced. The TFS reconstruction results in a superior shape representation and tolerates a much smaller number of tilt angles than tomography, which is beneficial for image stack alignment. A further advantageous application of TFS STEM is the imaging of micrometers-thick samples. With TFS it is possible to limit the overall tilt range while obtaining a higher axial resolution than for a tilt series alone.

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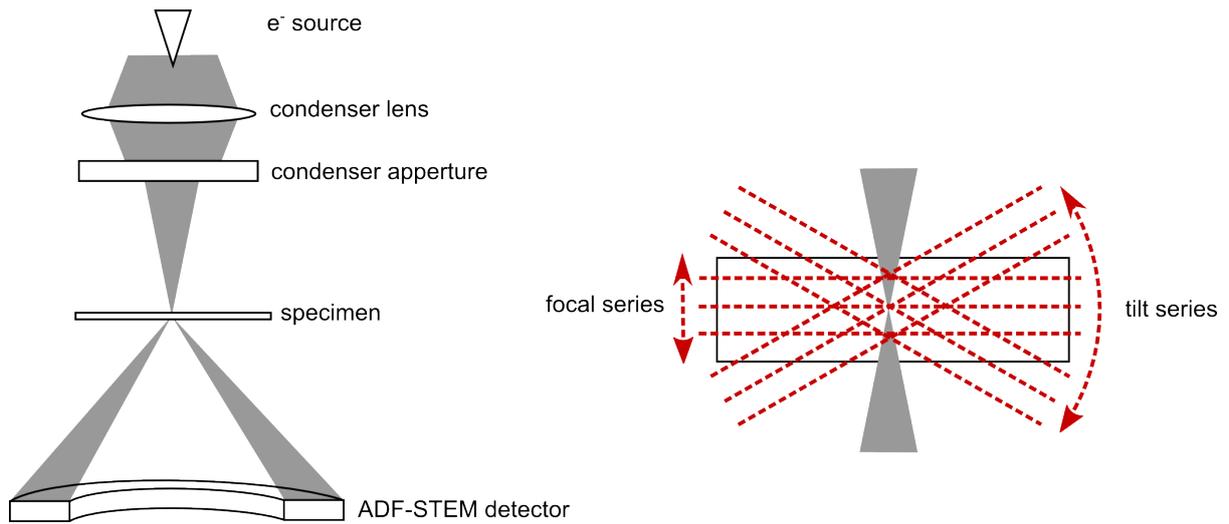


Fig. 1: Schematic views of the TFS recording scheme with STEM. (left) A thin specimen is imaged pixel-by-pixel in dark field mode STEM using the annular dark field detector (ADF). (right) In a combined tilt- and focal series, images are acquired in a through-focal series at each tilt angle. The specimen stage is tilted after each focus series.

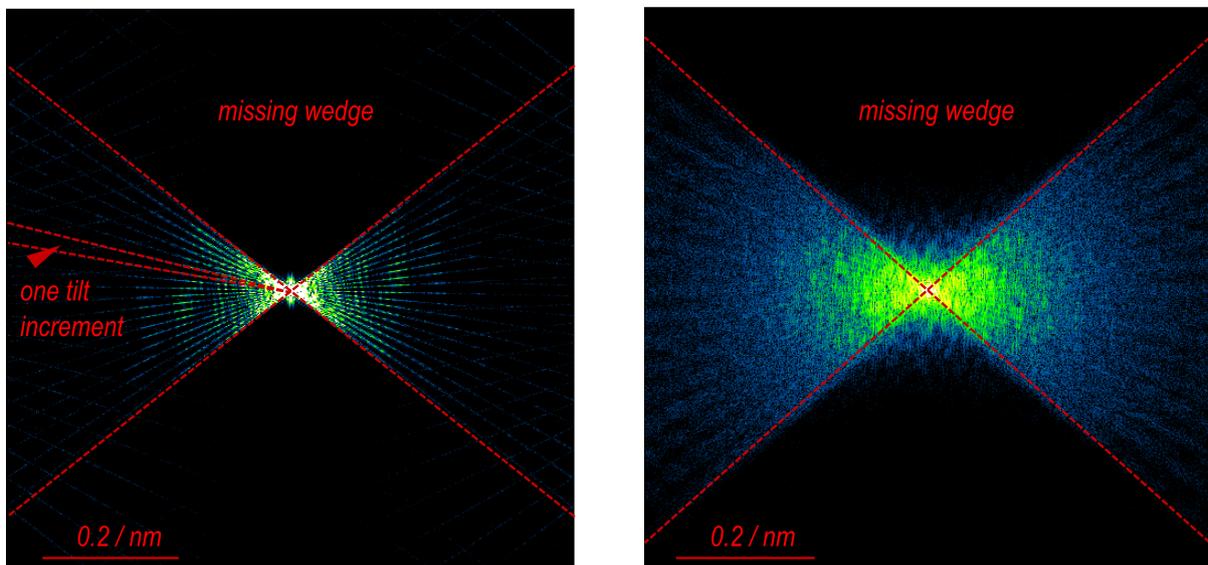


Fig. 2: Comparison of tilt series STEM tomography with TFS STEM. (left) Spatial frequency spectrum (Fourier Transform) of a vertical (xz) slice of the conventional tilt series STEM tomography data. (right) Frequency spectrum of an xz slice of the TFS STEM dataset. The red lines mark the border of the missing wedge. With permission from [2].