

Benefits and drawbacks of dual-axis STEM tomography

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Electron tomography, which has become a mainstay of biological microscopy, has more recently been employed in the physical sciences to image the fine three-dimensional (3D) structure of materials such as catalysts, quantum dots and semiconductor devices.

Unlike related methods, such as X-ray tomography, the range of tilt angles from which data can be gathered is limited in electron tomography because of the limited tilt available in most transmission electron microscopes and the likelihood of holder shadowing at high tilt. This results in a truncation of data which is best represented in reciprocal space as a ‘missing wedge’ of information, as illustrated in Figure 1(a). This missing information can lead to significant artefacts in the reconstructed tomogram. These are particularly noticeable in any reconstruction in a direction parallel to the optic axis [1].

By summing two tilt series recorded from the same volume of interest, whose tilt axes are mutually perpendicular, the ‘missing wedge’ of data is reduced to a ‘missing pyramid’; see Figure 1(b). Combining the two sets of data can be achieved by summing the (Fourier transforms of) individual tomograms computed from each tilt series in turn, or by reconstructing a single tomogram using both data sets simultaneously. Previous work on bright field TEM series has shown how artefacts can be reduced and the resolution of the reconstruction improved [2,3].

Here we adapt these ideas to a dual tilt series of STEM HAADF images. The example shown is taken from a ‘backbone’ of magnetite crystals found inside certain strains of magneto-tactic bacteria. Two tilt series were recorded, with tilt angles ranging from -72° to $+72^\circ$ in one case, and -64° to $+60^\circ$ in the other, at a 2° interval. In both cases only projections in the range -64° to $+60^\circ$ were used. When slices from the crystal reconstructions are compared from a single-axis tomogram and the combined, dual-axis tomogram (Figure 2), the improvement is clear.

However, before the tomograms can be combined, they must be accurately aligned to one another. Small shifts made as the sample is rotated between tilt series become large discrepancies when the tomograms are computed. Most 3D tomographic alignment schemes rely on gold markers [2,3]; when these are not present, however, it is still possible to use an iterative method to align the two tomograms. Cross-correlation to correct for shift and rotation is performed along each axis in turn, iterating until the best fit is found.

When STEM images are used to create the tomograms, there is an additional challenge in that any scan distortion in the original images is transferred to the computed tomogram, resulting in an overall distortion which prevents simple alignment, see Figure 3(a). Corrections must be made to the original images, using the zero tilt images from each series as a guide. In principle, the two zero tilt images should be identical if all corrections are made. Using the combined corrections, including the retrieved scan distortion, new tomograms are calculated and a much more accurate alignment is possible, as seen in Figure 3(b) [4].

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2. P. Penczek, M. Marko, K. Buttle, and J. Frank, *Ultramicroscopy*, **60** (1995), 393.
3. D.N. Mastrorade, *Journal of Structural Biology*, **120** (1997), 343
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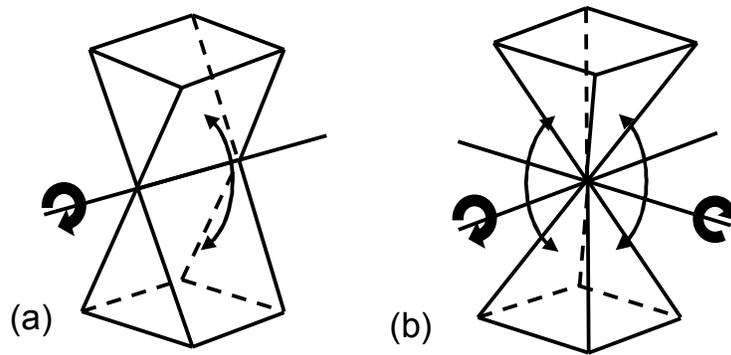


Figure 1. (a) Schematic in reciprocal space illustrating (a) the ‘missing wedge’ of information from a single-tilt series and (b) the ‘missing pyramid’ from a dual-axis series.

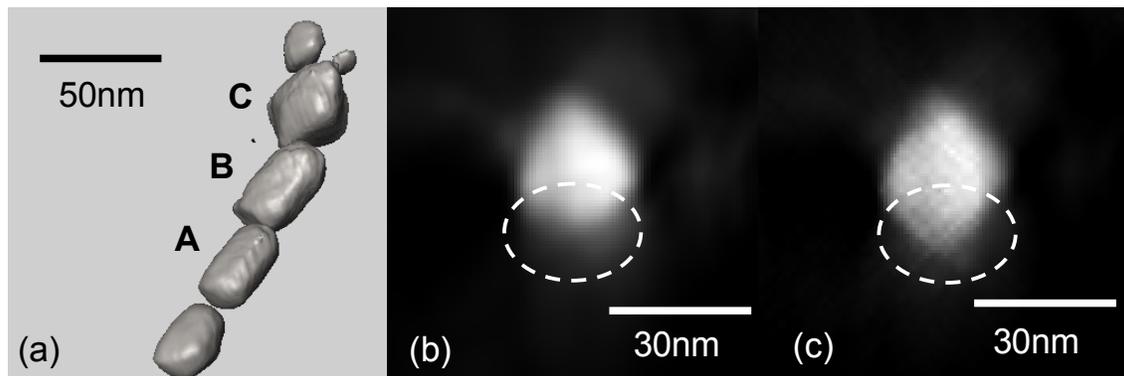


Figure 2. (a) A surface render representation of a reconstruction from a series of magnetite nanocrystals from magnetotactic bacteria. Single pixel slices through crystal B taken (b) from a single tilt series reconstruction and (c) from a dual-axis reconstruction. The slice in (c) shows an improvement in the reconstruction of the lower half of the nanocrystal due to the increased information from a dual-axis series.

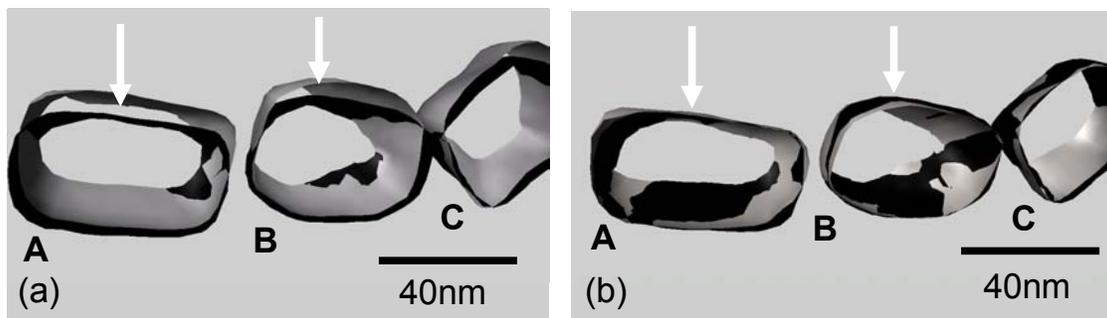


Figure 3. (a) This shows the alignment of two single-axis reconstructions following correction for shift and tilt offset but without any correction for scan distortion. (b) The same two reconstructions superimposed after correction for scan distortion. The arrowed gaps in the crystals seen in (a) is considerably smaller in (b).