Improved visualization of intracellular morphology by tomography of thick sections using a spherical and chromatic aberration corrected TEM

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For the imaging of thick samples – and in particular for electron tomography - inelastically scattered electrons have in the past mainly been considered as disturbing background and thus were rigorously filtered out. However, with the availability of chromatic and spherical (Cc/Cs) aberration correction of the TEM lenses [1] the inelastically scattered electrons are now also focused into the elastic image plane and can contribute to image formation in a productive manner. Preliminary experiments at the TEAM Cc/Cs corrected TEM prototype [2] indicate, that high resolution image information is also available in the inelastic image. First examples for materials science samples have been published recently [2]. One working hypothesis for this high resolution information is coherent multiple scattering, which leads to an elastic high resolution image formed by inelastically scattered electrons.

The inclusion of all inelastically scattered electrons is therefore beneficial in several ways. For tomography its immediate effect is an increased image signal that allows the imaging and reconstruction of thicker samples. We demonstrate this here with plastic section tomography of biological material. At 80 kV a section of 300 nm thickness would – imaged in a non-aberration corrected TEM - be more or less featureless, since the overlay of defocused inelastic images would smear out information. Or – when using zero-loss filtered imaging – the image intensity would rapidly fall off for higher tilt angles. In Fig. 1 we show typical images recorded at the TEAM Cc/Cs corrected TEM and the intensity fall-off as a function of tilt angle. The amount of detail that is visible in figures a-c is remarkable.

We have then used 60 views at different angles to reconstruct the slice in 3D. The reconstructed area (Fig. 2a) shows a section through mouse muscle tissue and was chosen for its richness in structural features. Collagen filaments can be seen in the lower left corner and several undulating membranes in the centre part. Within the boxed area an apparently free standing vesicle is visible. Fig. 2b shows crossections of the vesicle perpendicular to Fig. 2a. The consecutive slices through the vesicle allow an estimate of the thickness up to where structural information was obtained. Based on the pixel size we judge the vesicles height to be in the range of 300 nm.

[1] M. Haider et al., Ultramicrocopy 108, 167-178 (2008)
[2] B. Kabius et al., Journal of Electron Microscopy, submitted

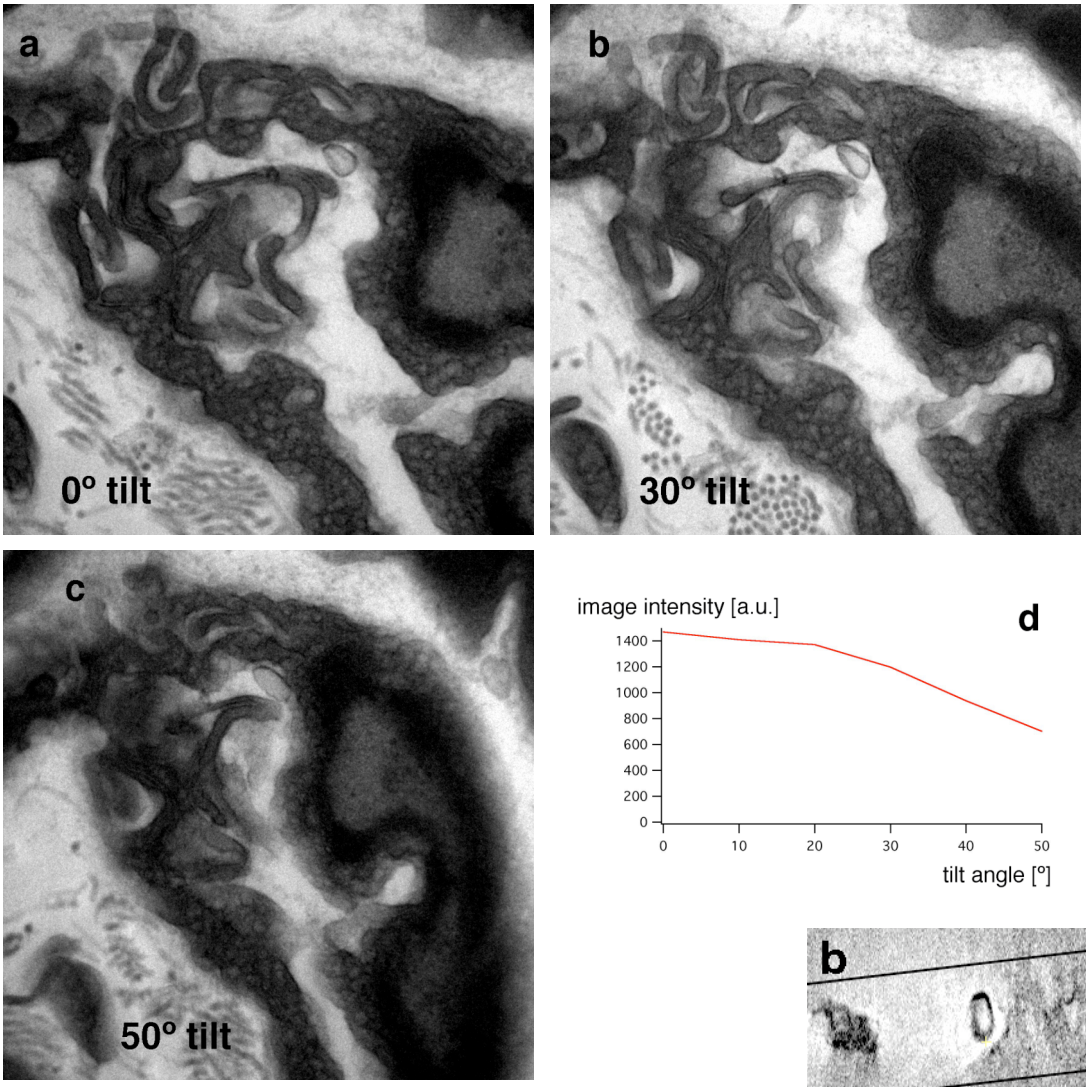


Figure 1. Raw images from a tilt series **a-c** - Images at three tilt angles, recorded with identical electron dose, brightness adjusted to fit display level (8 bit graylevel). **d** - Real image intensity values (12 bit CCD recording) as function of tilt angle.

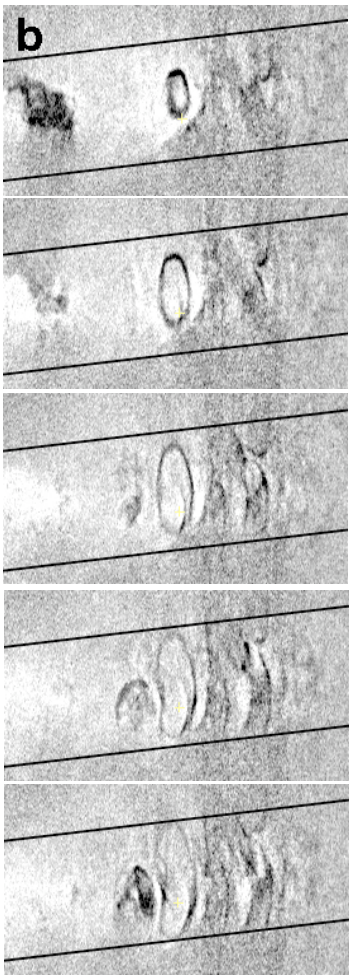
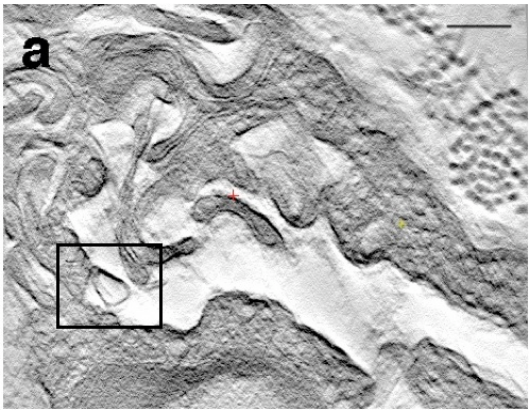


Figure 2. Slices through reconstructed tomogram. **a** - central xy-slice of 24nm thickness through tomogram. (scale bar 500nm) **b** - yz-slice series through tomogram. Shown are adjacent slices of 4.7nm thickness at 15nm distance. Shown is the sectioning of a reconstructed vesicle (box in **a**; section thickness between black lines is about 300nm).



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