A framework for markerless alignment with full 3D flexibility

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Electron tomography is a powerful tool for investigating the three-dimensional (3D) morphology and inner structure of nanomaterials, based on a tilt series of projection images acquired with an electron microscope [1]. Before a reconstruction algorithm can be applied to compute a 3D image from the set of 2D projections, *alignment* must first be carried out to correct for geometrical changes (e.g., sample shifts, rotations, magnification changes) that occurred during acquisition.

If the sample has been treated with fiducial markers, such as gold beads, the unknown geometrical parameters can be accurately determined by tracking the markers as the sample is rotated and combining the information from a large number of marker positions. Markerless alignment, which is solely based on the structure of the sample itself, is much more challenging, but also highly useful, as markers can interfere with the structure of interest and may induce reconstruction artefacts.

In most software packages for electron tomography, markerless alignment is performed by maximizing the cross-correlation between consecutive images in the tilt series [2]. As noted by several authors [3-5], this approach has many drawbacks. In particular, it suffers from accumulation of alignment errors throughout the tilt series and does not lead to a combined set of geometrical parameters for which the measured projections fit optimally with the reconstructed image. More importantly, alignment methods based on shifting, rotating and magnifying the individual projection images are intrinsically incapable of correcting geometrical distortions caused by slight 3D variations of the tilt-axis position. Such variations occur frequently in practice, in particular when using full-rotation tilt holders.

We have developed a software framework for automatic markerless alignment of projection images that can compute a full 3D set of geometrical alignment parameters from the tilt series. The parameters that can be recovered include sample shifts, tilt axis changes, detector tilts, and magnification changes. By combining advanced numerical algorithms with highly parallelized GPU operations [6], an implementation was constructed that can optimize the correspondence between the reconstructed image and the aligned tilt series, taking all these parameters into account, in reasonable time (a few hours for a moderately sized 3D dataset).

The algorithm was successfully applied to the alignment of several challenging experimental datasets (see Figs. 1-2) that could not be properly aligned by taking only shifts and rotations of the projection image into account, revealing significant changes in tilt-axis orientation during acquisition. Besides leading to optimized reconstruction quality, the algorithm also yields valuable diagnostic information about the different 3D geometrical distortions that occur during acquisition, and can therefore be used as a calibration tool.

References

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[7] KJB and FB acknowledge support from the Netherlands Organisation for Scientific Research (NWO), programme 639.072.005. JDB, WJP and JS thank the IBBT for financial support.
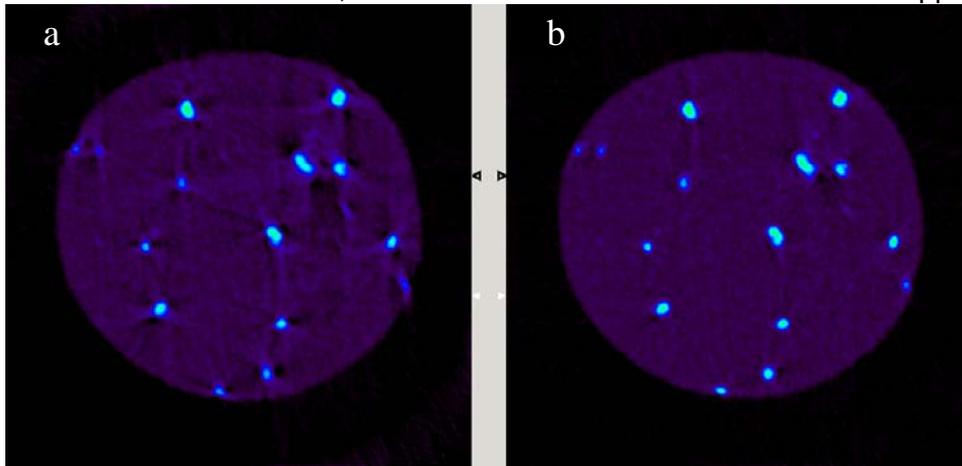
Figure 1. *This figure shows an xz-slice, orthogonal to the tilt axis, through a 3D reconstruction of a Si needle shaped sample that contains Pb precipitates. The series was acquired using on axis-tilt tomography [N Kawase et al, Ultramicroscopy **107** (2007) p. 8. E Biermans et al, Nano Letters **10** (2010) p. 5014.]. (a) cross-correlation alignment; (b) new fully 3D alignment result.*
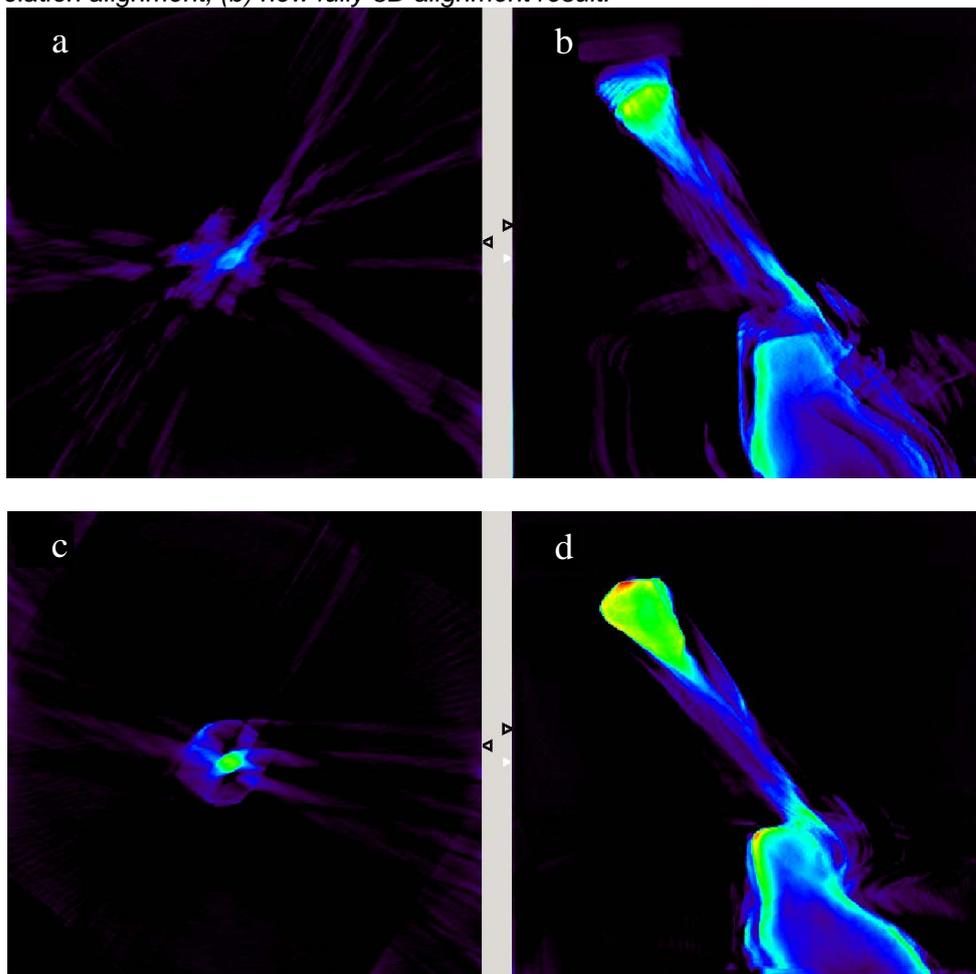

Figure 2. *This figure shows orthoslices through a 3D reconstruction of a carbon nanotube grown from a catalytic nanoparticle. The nanotube was directly planted on the needle tip, causing severe alignment problems. The ; (a) cross-correlation alignment, xz-slice; (b) cross-correlation alignment, xy-slice; (c) new fully 3D alignment result, xz-slice; (d) new fully 3D alignment result, xy-slice.*