A marker-free alignment of tilt series images for tomographic reconstruction

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Keywords: tomography, marker free alignment, high resolution tomography

The alignment of a sequence of tilt series images is a crucial factor for a tomographic reconstruction from a tilt experiment in an electron microscope. Analytical as well as algebraic algorithms for the tomographic reconstruction such as backprojection, ART or SIRT, presume the precise knowledge or determination of the projection geometry and image displacements after image recording and prior to the reconstruction.

The most widely used post-acquisition alignment techniques in electron tomography were developed for the biological sciences. They are based on pattern matching by correlation techniques or on the tracking of artificially introduced fiducial markers [6–8] or intrinsic specimen features. However, consecutive cross-correlation of images in the tilt series can only produce an approximate alignment for which errors tend to accumulate leading to a loss of fine detail in the tomogram similar to the effect of the missing wedge.

Tracking of fiducials or landmarks in sinogram is accurate, but the sizes of artificially introduced fiducial markers is typically a few nanometers and hence too big to guarantee an image alignment with the accuracy required for a sub-nanometer tomography. Furthermore, there is little guarantee that fiducial markers can be found in proximity of the detail of interest, even in life science application with lower resolution and larger field of view.

We recently proposed and alternative marker-free algorithm for a refinement of the image alignment that is based on an iterative tomogram feedback [1]. The refinement is done by maximisation of the tomogram contrast in trial tomograms calculated individual image shift vectors. Contrary to reference or marker-based approaches the procedure does not require a model of the three-dimensional object nor does it depend on a pre-identification of objects.

Fig. 1 displays the iterative refinement of the alignment of a tilt series of bright-field images of silver nanoparticles with a gold core for the illustration of the procedure. The contrast of a tomogram sub-volume containing the core-shell particle is a function of the displacement of a single image. The optimization of the contrast with respect to the displacement of each image converges with a few number of iterations. The particular example was taken from a study correlating electron tomography and plasmon spectroscopy [2].

In the second example of a reconstruction of tungsten-disulfide nanotubes in fig. 2 no obvious nodal points can be identified and self-markers cannot be utilised therefore. The cross-correlation alignment does not account for the rotation of the bundle around the tilt axis and approximates a correct sinogram traces only in angular segments, effectively leading to a superposition of non-concentric partial reconstructions and strong artefacts in addition the nominal missing wedge artefacts. The refined alignment leads to correct sinogram traces and a minimization of the artefacts in the reconstruction.

Using simulated phantom data it can be shown that a translational displacement accuracy better than the width of the point-spread function in an individual image can be achieved, competitive with fiducial marker alignment. Besides the assessment of the performance of the algorithm further applications in the field of cryo-electron-tomography and x-ray tomography are shown.
References


Figure 1. Refinement of the alignment in a tilt series of images of a 50 nm big Au-Ag core shell nanoparticles after initial cross-correlation. (a) A single bright-field image of the particle and below a sinogram. (b) Image displacements refined after four iterations of maximising the tomogram contrast. (c) Reconstruction slices in the three principle axes after cross-correlation alignment and (d) after refinement.

Figure 2. Comparison between conventional and refined alignment for the example of a reconstruction of a bundle of WS$_2$ nanotubes. Coloured lines mark the outer shells of two nanotubes. Part (a) displays a sinogram (left part) obtained after conventional cross-correlation alignment. The magnified lower part of the sinogram reveals a lack of correlation with a rotational movement. A tomogram slice of the tube bundle in cross-section is shown in (b). Parts (c) and (d) display a sinogram and a tomogram slice obtained after refinement of the image alignment. The sinogram traces follow the expected projection of a rotational movement and artefacts in the tomogram are minimised.