Advances in Specimen Holder Technology for 3-Dimensional Electron Microscopy

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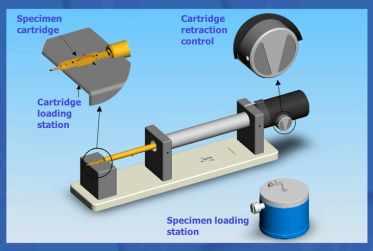
Specimen and Sample Holder Geometry

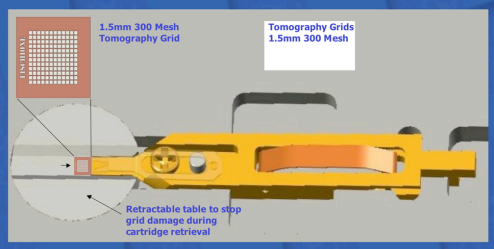
The demand for collecting data over large ranges of tilt in room temperature Transmission Electron Microscopy reaches a mechanical limitation dictated by the combination of specimen thickness, specimen support, pole piece geometry and specimen holder configuration. To obtain high tilt angles in Transmission Electron Microscopes, the pole piece gap should be large and unrestricted. For ultra high resolution materials science applications, this is typically less than 3mm. Hence, high tilt with a traditional 3mm sample geometry is not possible. A new sample holder has been developed to incorporate 1.5mm sample geometry, resulting in more than 70° of tilt. Acquiring a dual-axis tilt series can enhance tomographic imaging by increasing the amount of information obtained from the specimen. Current methodology involves manual 90° rotation of the grid. This manual manipulation often results in sample damage or loss. A new in-situ-rotation holder has been developed, allowing 70° of tilt with initial 360° continuous sample rotation and subsequent accurate 90° increments.

Ultra-Narrow Gap Advanced Tomography Holder

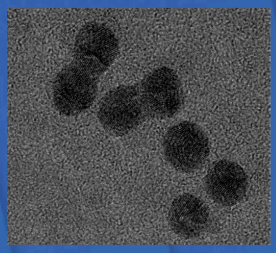
This holder accepts a 1.5mm square/round grid and secures it with a single clamp. A sample loading station and combined retractable specimen table allows easy loading of a 1.5mm Tomography Grid into a specimen cartridge. The specimen table is then retracted and the cartridge removed and placed on the sample holder loading stand, which engages the cartridge with the holder retraction mechanism. The cartridge can then be retracted fully inside the holder to protect the sample from damage during insertion and removal from the TEM goniometer. This results in an unrestricted field of view at tilt angles in excess of 70° without compromising the ultimate resolution of the microscope.



CAD view of specimen holder and loading station

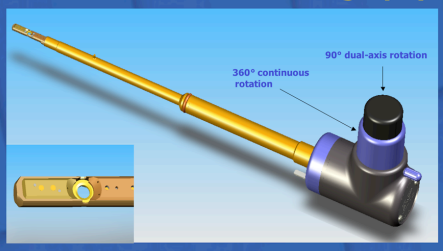


CAD view of tomography grid, specimen cartridge and loading station



TEM bright-field images of 5nm gold islands showing 0.23nm resolution (0 degrees tilt)

Dual-Axis Advanced Tomography Holder

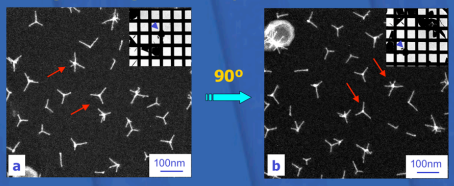


CAD view of dual-axis specimen holder

This holder is for applications, in which TEM tomographic imaging or analysis requires in-plane rotation of the specimen. The fully-jeweled rotation mechanism allows ultra-precise highly repeatable specimen rotation, while maintaining eucentric height. Initially, the specimen can be rotated fully through 360°. Once the specimen is oriented with the goniometer α-tilt axis, a precision mechanism allows 90° in-plane rotation of the specimen. The specimen is clamped by a threaded ring that maximizes the specimen viewing area at high tilt angles. The Dual-Axis Advanced Tomography Holder allows optimal tilt angles in narrow gap pole piece geometries, while maintaining microscope resolution.

Materials Dual-Axis Tomography

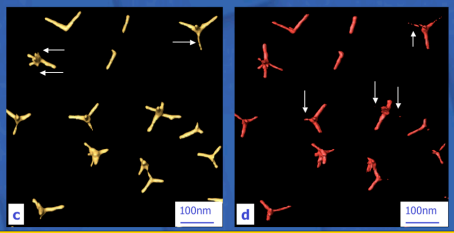
Tetrapods are nanostructures being developed for nanoscale laser light emitting diodes and optoelectronic devices.

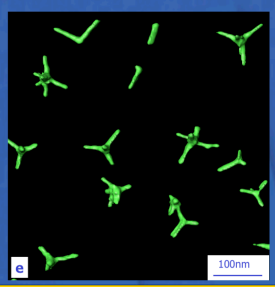


Z-contrast images of CdTe tetrapods in two perpendicular orientations at 0° tilt. Red arrows indicate the same tetrapods. Image (b) has been rotated 90° clockwise with respect to image (a). (Inserted low magnification STEM images show grid bars. Blue arrows indicate the center of the grid. Image (b) has been rotated 90° clockwise with respect to image (a) with an accuracy of better than 1°.)

3-D reconstructions

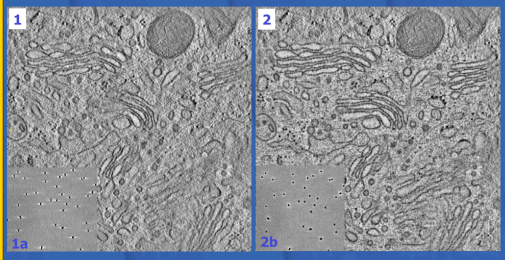
Image (c) shows a reconstruction from the first tilt series. Some arms of the tetrapods are missing or weak as a result of using a single tilt axis, as indicated by the white arrows. (d) is obtained from the perpendicular tilt series. The arms missing in (c) are present in this data set. However, different arms are now missing, again indicated by white arrows. (e) is a dual-axis reconstruction of the two data sets. No arms are now missing. (The tilt axes in (a) and (b) are parallel to the directions of the arrows).





Biological Dual-Axis Tomography

The sample shown below has been prepared by High Pressure Freezing followed by freeze substitution. The image shows a Golgi network. Image (1) is a single tilt series reconstruction tilted from -65 to +65 degrees in 1 degree increments. Image (2) shows the same area reconstructed from two perpendicular single tilt series. Better resolution and structural definition can now be seen. The inset images show reconstructions of both single (1a) and dual-axis (2b) tilt series of colloidal gold particles.



Conclusions

The ability to apply dual-axis tomography to both materials science and biological samples has clear benefits. The limitation of a restricted pole piece geometry can be resolved by using a smaller sample. A square grid shape proves to be ideal to help with alignment of the grid squares with the tilt axis and for easy 90 degree rotation. Dual-axis reconstructions show an enhancement in resolution and in the overall clarity of the reconstruction. In situ rotation facilitates dual-axis tomography.

FISCHIONE INSTRUMENTS

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