(S)TEM Tomography

Tomography is a technique that employs a series of images successively recorded from an object at different tilt angles with respect to the electron beam in order to obtain that object's three-dimensional structure using a back projection technique. The images can be recorded in a Transmission Electron Microscope (TEM) or a Scanning Transmission Electron Microscope (STEM). JEOL have adopted SerialEM (Boulder laboratory for 3D electron microscopy of cells BL3DEMC) to either modality making tomography an accessible, efficient and easy technique for all aspects of scientific, industrial and medical research. (Fig. 1).

The modality of imaging depends primarily on the object being studied. For example, structures that show strong diffraction effects, very common in Materials, tend to be studied most effectively in STEM mode using images acquired on a High Angle Annular Detector (HAADF). Conversely, specimens in Life Sciences, characterized by an abundance of low Z elements and thus fairly devoid of diffraction effects, tend to be studied mostly using TEM bright field. SerialEM tomography can be applied to all TEMs in the JEOL line---up thus providing a solution for every aspect of microscopy that targets 3D structures.

Tomography is applicable to a wide variety of samples, such as ranging from beam---resistant FIB lift---out to frozen---hydrated specimens. A low---dose option in SerialEM tomography allows specimens to be imaged whilst in their native state, i.e. after vitrification. Entire tomographic series can be obtained without visibly damaging the sample (Fig. 2). JEOL USA's choice for tomography includes processing using IMOD (also available from the BL3DEMC).

The low---dose option also allows the use of extremely high magnification for imaging, as is the case for instance in STEM mode (Fig.3). The ability to perform critical steps in tomography, such as tracking, focusing

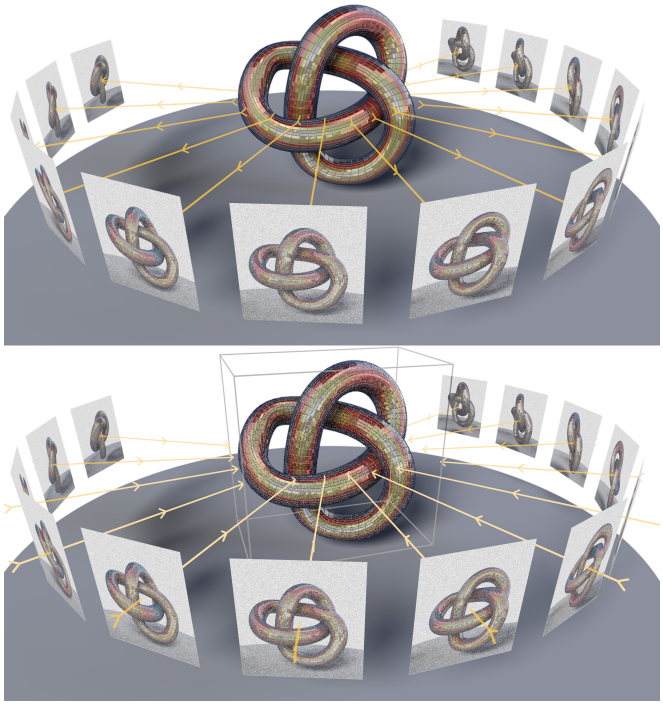

Fig. 1: Schematic illustration of tomography and the reconstruction process. The 3D object gets projected in the (S)TEM yielding images (top panel), which can be recombined using a back-projection technique (inverse Radon transform) resulting in a 3D reconstruction of the original object (bottom panel).

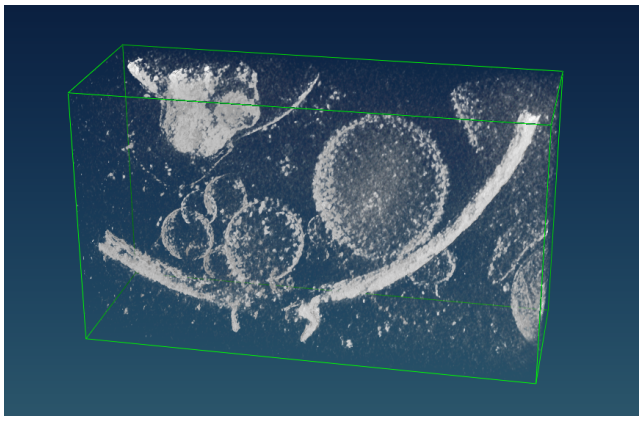

Fig.2: 3D rendering in chimera of frozen-hydrated vesicles coated with viral proteins after tomography in a JEM-3200FSC at 300 kV and 20 eV zero-loss imaging. Processing included NAD filtering (Sample courtesy of B. Russin, Northwestern U.)

and recording the final image, at different magnifications, ensures the operator that the object of interest remains on the camera/detector under all conditions as the sample is being tilted.

Tomography is now also being applied in the field of diagnostic imaging, for instance in primary ciliary dyskineasia (see Brink and Carson, MSA Proc. (2010) **16**: 970). Shown is a cilia cross section that has been obtained after applying 3D imaging by tomography. The reconstruction is rotated so as to yield views that are identical to perfect axial cross sections through the cilia (Fig. 4). Note the microtubule doublets in this view indicating the proper orientation of the cilia. A reliable workflow can thus be established that gives quick answers as this reconstruction can be obtained in roughly 30 minutes.

Finally, SerialEM has the ability to capture large-scale montages as exemplified in the work from the Marc lab at University of Utah (Fig.4).

Montages such as these are at the fingertips of researchers through a robust and easy to set up GUI in SerialEM. The montaging feature makes use of either stage-based or deflector-based navigation.

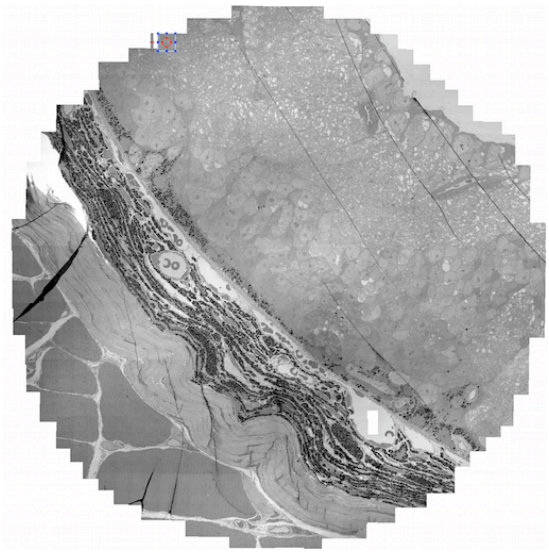

Fig. 5: Montage of mouse retina obtained in a JEM-1400 using SerialEM stage-based montaging. The area measures approx. 0.5 x 0.5 mm and was imaged at a magnification of 5000x. The montage contains circa 1000 images acquired from the 4k x 4k CCD camera.

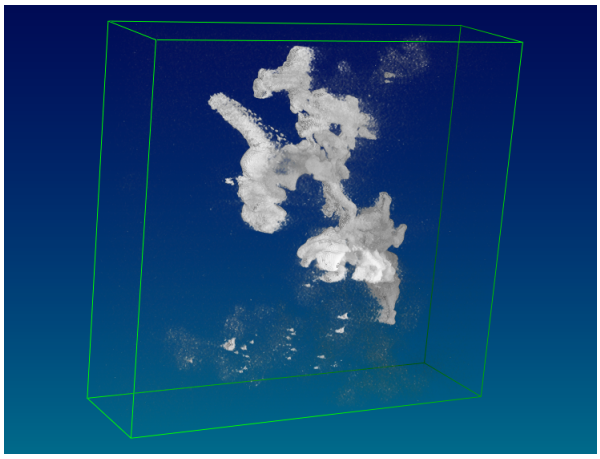

Fig. 3: 3D rendering in chimera of catalytic sample acquired in HAADF STEM mode in a JEM-ARM200CF (Sample courtesy of R. Klie, UIC).

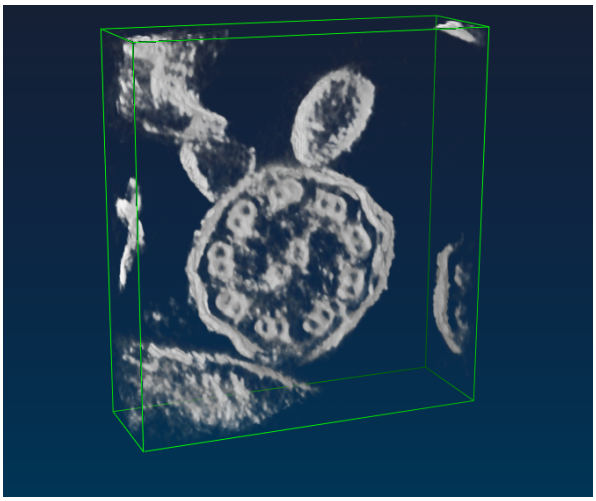

Fig. 4: 3D rendered cilia from sick patient after SerialEM tomography in a JEM-1400 showing the disconnected dynein arms (Sample courtesy of Dr. Carson, UNC).