Novel electron microscopic methods for 3D imaging of subcellular structures

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The main challenge of visualizing subcellular structures in 3D is sample preparation preventing artifact formation. We believe that cryo-fixation protocols are the method of choice to arrest cells at defined physiological conditions to preserve a life-like state. Afterwards we dehydrate these samples at cold temperature (freeze substitution) and embed them in a resin. Different protocols are now available to obtain a 3D dataset. With scanning transmission electron microscopic (STEM) tomography, semi-thin sections up to a thickness of about 1 µm can be recorded in 3D. The resolution is very good in X and Y (in the range of a few nanometers, depending from the structural preservation during specimen preparation), and reasonably well in Z (limited by the missing wedge problem). Eukaryotic cells, however, have a thickness of up to 10 µm or more, therefore, even with STEM tomography only a small portion of the cell can be recorded. Alternative methods are serial sectioning tomography and focused ion beam/scanning electron microscopic (FIB/SEM) tomography. Serial sectioning only requires an ultra microtome and an electron microscope as equipment. The resolution in Z is, however, restricted to the section thickness (about 100 nm). With FIB/SEM tomography the resolution in X and Y can be as good as in TEM images of the same plastic embedded material.