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Approaching Tissue Ultrastructure in 3D

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There are several methods to explore tissue ultrastructure in 3D. They differ in their mode of sampling the third dimension – continuous sampling in classical electron tomography (ET) based on recording of tilt series [1] in a transmission electron microscope (TEM) versus discrete sampling in physical sectioning methods such as array tomography (AT) [2] and serial blockface [3] or focussed ion beam [4] scanning electron microscopy (SBFSEM or FIBSEM). The size of volume, which can be addressed with reasonable effort also varies considerably.

Using ET from a single tilt series volumes can be reconstructed with lateral dimensions of around 5x5 μm^2 with typical voxel sizes of 1-4 nm and a resolution not better than 5 nm. This is just the size of a small eukaryotic cell and even stitching of many tomograms will not easily lead to tissue dimensions. Since section thickness is a severe limitation in TEM we used the Argonne C_s/C_c-corrected Titan to record tilt series of resin-embedded mouse muscle. We could reconstruct volumes with about 4nm resolution from up to 1µm thick sections (Fig. 1, visibility of actin filaments).

AT on the other hand is similar to the classical serial sectioning approach. However, sections are placed on silicon wafers (Fig. 2A) and ultrastructural data are recorded in a SEM (Fig. 2B). Here the z-resolution depends on the thickness of the sections, typically 50-200nm, resulting in non-isotropic voxels of e.g. 5x5x50 nm leading to "steps" in the reconstructed volume (Fig. 2C), here that of an isolated Zebrafish immune cell. In that case this is not a problem since the aim was to create an organelle inventory of an unknown cell type and not to obtain a full macromolecular atlas of the cell.

If higher z-resolution is required there is still the possibility to use FIB on sections. After imaging the surface of a section (Fig. 3A) a FIB-stack was created through part of such a Zebrafish immune cell. After reconstruction the volume can be resampled in xy and virtual slices from deeper inside the section can be displayed (Fig. 3B). Volume rendering shows an "isotropic", smooth appearance of small intracellular features (Fig. 3C). With such a combination of AT and FIBSEM the distinction between continuous and discrete sampling can gradually be obliterated.

Along these lines we would like to propose a hierarchical workflow "to find the needle in the haystack" without the need to know *a priori* where exactly the needle is located: Certain cells or rare events inside a tissue are preselected on an array of sections by some light microscopic modality. They are then imaged in normal SEM mode with relatively large z-steps. This can be followed by FIBSEM analysis of special regions of interest defined in the first round of SEM images.

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[5] The authors acknowledge C. Bartels, I.V. Röder, R. Rudolf, and C. Grabher for technical support and samples; data management in this project is supported by the LSDMA (Large Scale Data Management and Analysis) BMBF/Helmholtz Project.

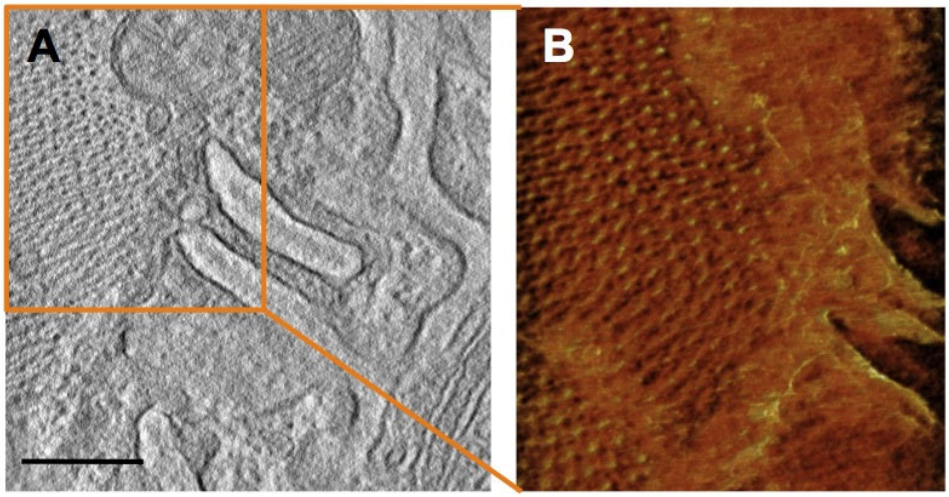

Fig. 1 Electron tomography of thick sections: Tilt series of up to 1µm thick sections were recorded in a C_s/C_c-corrected TEM at 300keV and reconstructed with IMOD. (A) virtual slice through reconstructed volume, (B) volume rendering in AMIRA. Scale bar 1 µm.

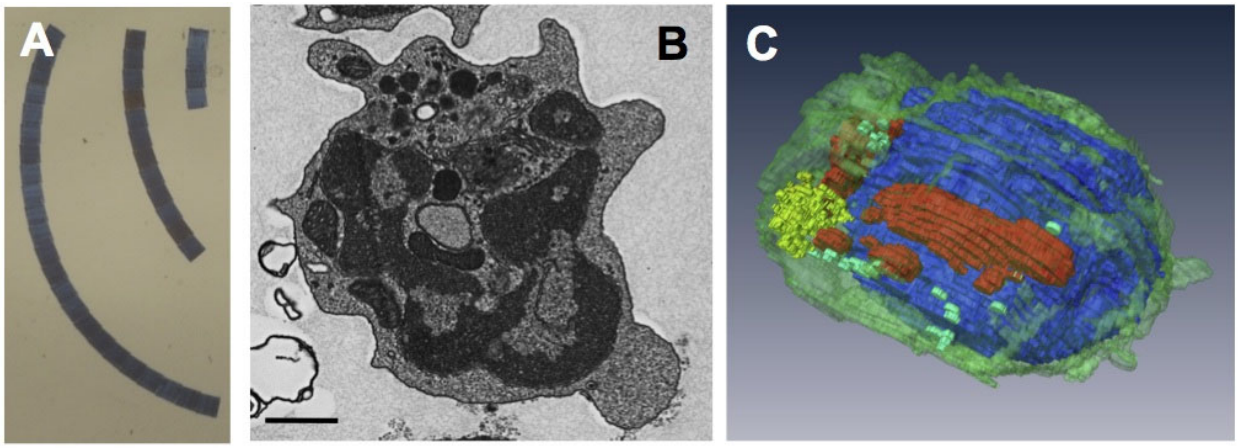

Fig. 2 Use of array tomography to create a cell organelle inventory: (A) 100nmm thick serial sections on silicon wafer, (B) single section imaged in FEGSEM (Zeiss Ultra) at 1.5 keV electron energy, (C) 3D volume segmented in AMIRA (nucleus shown in blue, mitochondria in red, Golgi complex in yellow, lysosomes in cyan, plasmamembrane in green). Scale bar 1 µm.

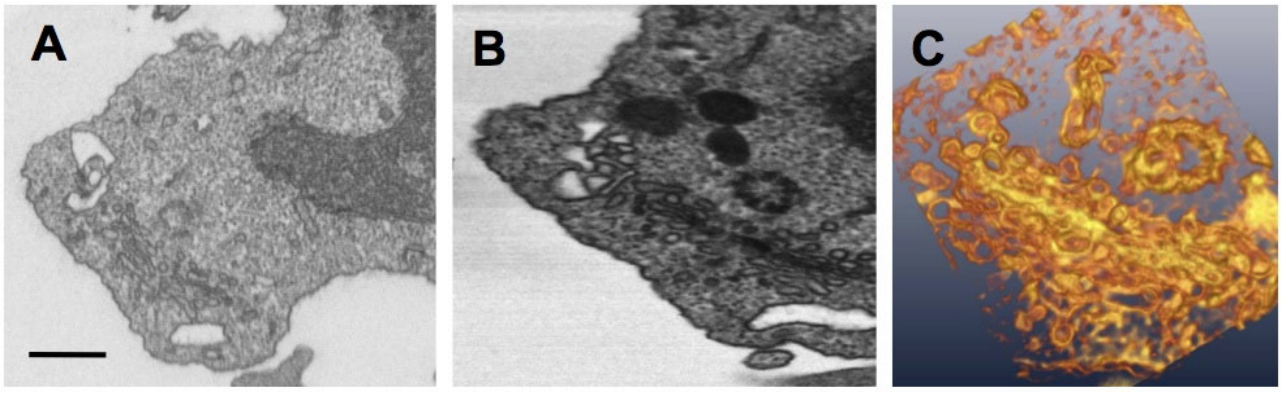

Fig. 3 Use of FIB on sections to zoom in to selected area: (A) reference image = surface of section imaged with FEGSEM, (B) virtual slice through volume reconstructed from FIB-stack (Zeiss Auriga), resampled in xy (C) volume rendering of centrosome and Golgi complex in AMIRA. Scale bar 500 nm.

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