3D in SEM, (S)TEM, Ion Imaging, incl. FIB-SEM and SBF-SEM

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Finding the needle in the haystack: Hierarchical imaging workflow combining array tomography with FIB-SEM

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Problems in cell or developmental biology often ask for ultrastructural characterisation of a small volume such as a rare event or a specialized substructure inside a large bulk specimen. We propose an intelligent workflow consisting of hierarchical imaging cascades, potentially also relying on different imaging modalities for different resolution ranges. Based on array tomography (AT) [1] this allows a stepwise zooming in to a structure of interest from light microscopy via conventional SEM to FIB-SEM.

First we used this approach to characterize rare immune cell populations of unknown function from different hematopoietic organs of Zebrafish isolated by fluorescence activated cell (FAC) sorting on the basis of an XFP-reporter gene. Since only e.g. about 30 000 of these cells can be recovered from the spleens of five adult fish we concentrated them by pelleting in agarose followed by conventional aldehyde fixation and embedding in epoxide (Figure 1a). Long ribbons of serial sections were deposited on silicon wafers (Figure 1b), inspected in a reflected light microscope (rLM) for interesting cells (Figure 1c), which were then imaged in a FEG-SEM (Figure 1d). Images were aligned in Fiji and segmented in Amira. Using these 3D reconstructions we could define an inventory of organelles.

For functional characterization, isolated cell populations were co-cultured with human tumor cells. Analysis of such co-cultures by the described multimodal hierarchical AT (hAT) revealed immunological synapses between fish immune cells and human target cells (Figure 2a). To further characterize the contact region between the cell pair with its typical arrangement of organelles we did FIB-milling (Figure 2b) on selected sections to analyze at higher z-resolution only those regions of interest that enclosed centrosomes, Golgi complex, and other membrane-bound organelles (Figure 2c).

Next we used hAT to identify a rare structure – the neuromuscular junction (NMJ) – within a large tissue block. Tibialis muscle from mouse was chemically fixed, embedded, and serially sectioned as described above. In a single cross-section containing hundreds of muscle cells usually only a few cells exhibit part of an NMJ (circle in Figure 3a). Once an NMJ was found it was imaged in xy on the surface of the section which in this case was nominally 1µm thick (Figure 3b). Then FIB-stacks were recorded from a 10µm x 10µm x 1µm volume of interest with 10nm slice thickness and 5nm lateral pixel size. Figure 3c shows several images of such a stack with one postsynaptic fold on the left and actomyosin filaments on the right. Currently we are recording more stacks from the same region of interest in consecutive sections. Fusion of individual stacks into a larger 3D volume will allow to observe the convoluted network of the postsynaptic folds at a resolution that allows unambiguous tracking of the membranes.

A combination of hAT with FIBSEM is a good approach whenever it is not necessary for a given problem to create a quasi-native molecular atlas of a cell or a total wiring diagram of a brain as needed in many connectomics approaches. In many cases a mere inventory of organelles or the architecture of just one, however rather large, synapse such as the NMJ might be sufficient. Generally, hAT can help to extend AT – with its non-isotropic voxels resulting from discrete z-sampling – to classical electron tomography with its more isotropic and continuous sampling.

1. K.D. Micheva and S.J. Smith, Neuron 55 (2007), p. 25.
2. We acknowledge the German Federal Ministry for Education and Research, project NanoCombine, grantnos. FKZ: 13N11401 and FKZ: 13N11403 for financial support.

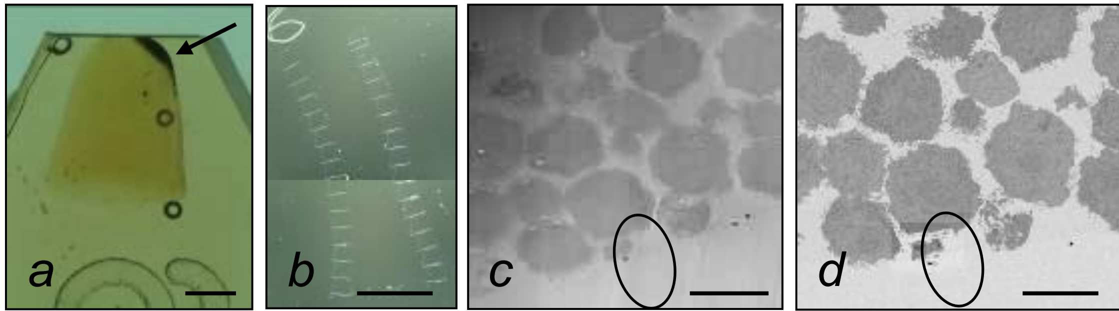

Figure 1. Preparation of FAC-sorted immune cells from Zebrafish for array tomography: a) agarose enrobed cell pellet (arrow) embedded in epon block, b) serial sections on silicon wafer, c) pre-selection of cells in reflected light microscope, d) imaging sections in SEM; scale bars: 1mm (a), (b), 10µm (c), (d)

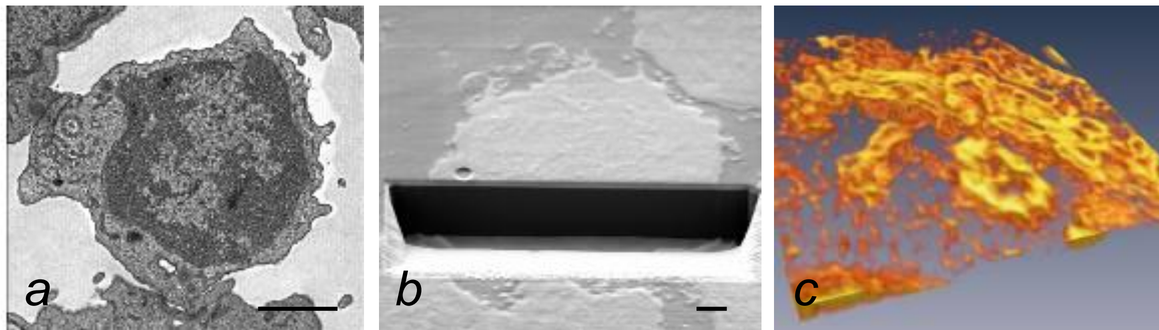

Figure 2. (a) Immunological synapse between Zebrafish immune cell and human cancer cell imaged in SEM (Carl Zeiss Ultra), (b) trench milled into a 200nm thick section by FIB (Carl Zeiss Auriga Crossbeam®), (c) volume rendering of Golgi complex and centrosome in Amira, scale bars: 1µm

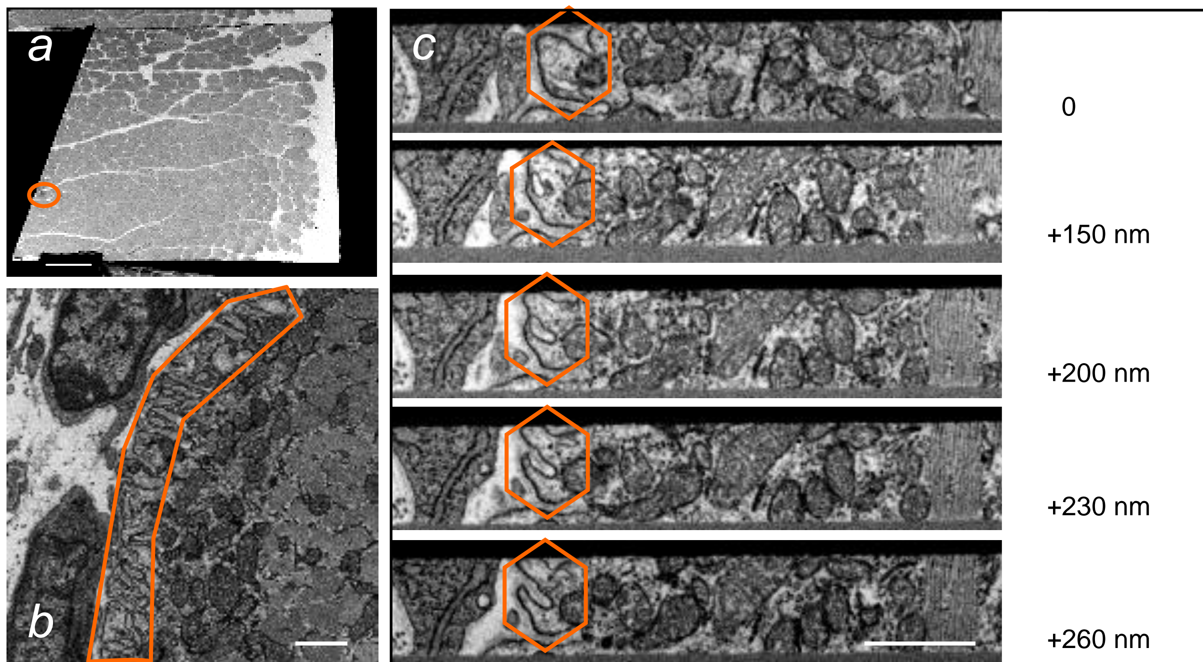

Figure 3. Imaging of NMJ (Carl Zeiss Auriga Crossbeam®): (a) overview of a cross section from mouse leg muscle, circle shows muscle cell containing part of an identified NMJ; (b) postsynaptic folds (orange overlay) imaged on surface of 1µm thick section; (c) postsynaptic folds (orange) in FIB-stack; scale bars: 100 µm in (a), 1µm in (b), (c)