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IT-13-P-1484 Finding the needle in the haystack: FIB-SEM combined with array tomography to achieve higher Z-resolution in selected areas

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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) [1,2] this allows a stepwise zooming in to a structure of interest from light microscopy via conventional SEM to FIB-SEM.
As a first example we studied a mixed population of cells, a coculture of human tumor cells with immune cells isolated from Zebrafish. Ribbons of serial sections from chemically fixed, epon-embedded cell pellets were placed on silicon wafers and inspected in a reflected light microscope (Fig. 1a). Cell pairs consisting of a large tumor cell and a small fish cell (circle in Fig. 1a) were then imaged in a FEG-SEM (Fig. 1b) revealing immunological synapses between fish immune cells and human target cells. To further characterize their contact region we applied FIB-milling to selected sections to analyze at higher z-resolution only those regions of interest that enclosed centrosomes, Golgi complex, and other membrane-bound organelles (Fig. 1c).
Next we used our approach 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. In a single cross section containing hundreds of muscle cells usually only a few cells exhibit part of an NMJ (circle in Fig. 2a). Once an NMJ was found it was imaged in xy on the surface of the section, which in this case was nominally 1µm thick (Fig. 2b). Then FIB-stacks were produced from a 10µm x 10µm area with 10nm step size. Figure 2c shows several images of such a stack with one postsynaptic fold on the left and actomyosin filaments on the right. After alignment the 3D volume can be resliced in xy (Fig. 3a) or volume rendered (Fig. 3b).
Currently we are recording more stacks from corresponding regions of interest in consecutive sections. Fusion of individual stacks into a larger 3D volume allows observing the convoluted network of the postsynaptic folds at a resolution that allows unambiguous tracking of the membranes.
A combination of AT with FIB-SEM 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 as needed in brain connectomics approaches. In many cases the region of interest is small enough to be amenable to analysis by FIB-SEM.

[1] Micheva and Smith (2007), Neuron 55, 25
[2] Wacker and Schröder (2013), J Microscopy 252, 93

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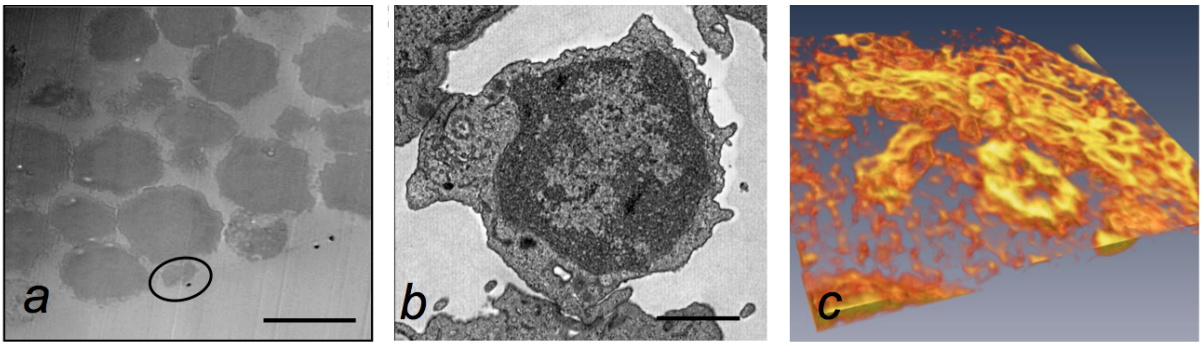

Fig. 1: (a) Immunological synapse between Zebrafish immune cell and human cancer cell preselected in reflected light microscope; (b) imaged in SEM (Zeiss Ultra); (c) volume rendering of Golgi complex and centrosome in Amira, scale bars: 10µm (a), 1µm (b)

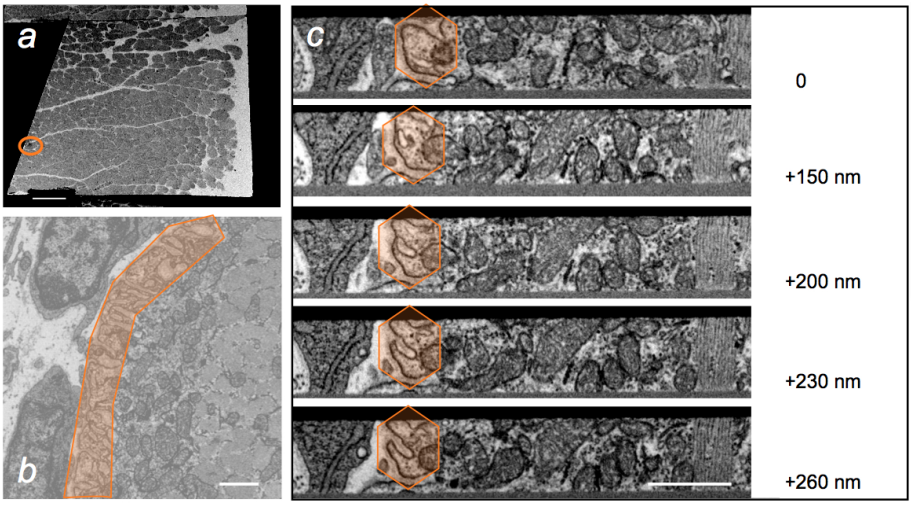

Fig. 2: Imaging of NMJ (Zeiss Auriga): (a) overview of a cross section from mouse leg muscle, circle shows muscle cell containing part of an identified NMJ; (b) postsynaptic folds (orange overlay) imaged on surface of 1µm thick section; (c) postsynaptic folds (orange) in FIB-stack; scale bars: 100µm in (a), 1µm in (b), (c)

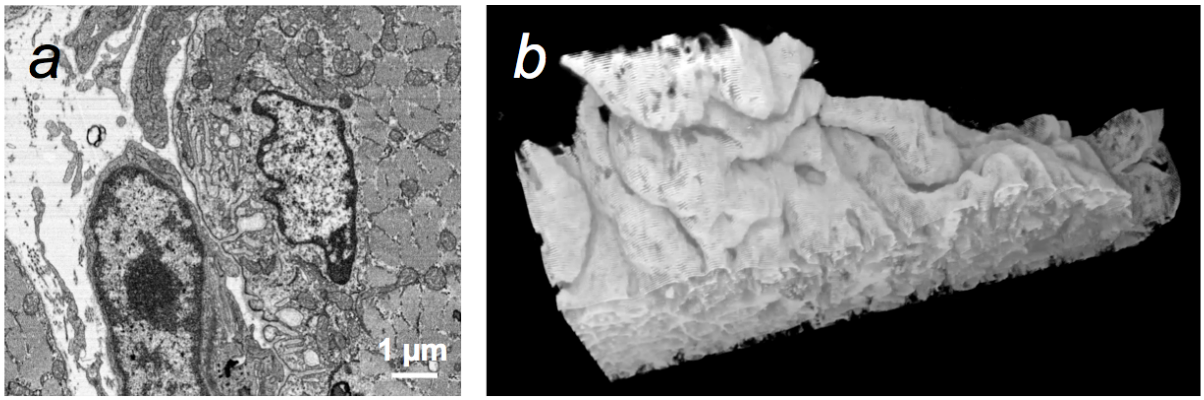

Fig. 3: 3D reconstruction from FIB-stack: (a) stack resliced in xy; (b) volume rendering in Chimera