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

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Microtome and Focus Ion Beam for serial block face scanning electron microscopy: tools to explore the three dimensional ultrastructure

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The study of biological systems together with the understanding of their fine molecular mechanisms requires new microscopy and image processing approaches. In particular, it is often necessary to image the same biological sample at different scales, ranging from a few millimeters down to a few nanometers. To achieve this goal, different techniques of light microscopy associated with electron microscopy need to be combined. Recently, both serial block face techniques, microtome as well as FIB serial block face SEM, have opened the field of 3D EM to new dimensions, extending the field of view in the 3 dimensions. Neuroanatomy is typically a field where this approach is crucial to better understand how physical interactions between cells contribute to inputs processing.

Correlation of light and electron microscopy require the acquisition of threedimensional datasets at the light and electron microscopy level. To obtain a 3D volume at the ultrastructural level that is covering a field of view compatible with the field of view obtained by light microscopy, samples are processed using an SEM containing a microtome inside the vaccum chamber (Denk and Horstmann 2004; GATAN 3View; FEI Quanta 200F). This technique allows the visualization of large volume at the ultrastructure level. At the same time, by matching coordinates systems between microscopes and by using landmarks, it is possible to link the fluorescence information obtained by light microscopy in vivo or after fixation with the three dimensional ultrastructure. In order to extract the components to be analyzed, different image processing tools have been developed. The microtome SBFSEM allows the reconstruction of entire neurites arborescence. In complement, the FIB technique allows extracting more high resolution information from a smaller volume, highlighting the cytoplasmic structures. These two methods are highly complementary techniques for studying neural ultrastructure.