Correlative Light and Electron Microscopy
– on the way from 2D towards 3D

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Correlative microscopy bridges the gap between light and electron microscopy. The previously introduced “Shuttle & Find” interface is the first easy to use solution for imaging one and the same sample regions in different microscope systems. It allows the straightforward relocation of an area of interest which was investigated before in a different microscope system. The next step will be to address correlative 3D applications. Two approaches for the generation of 3D correlative data sets are of major interest: Correlative array tomography and Correlative 3D LM-FIB.

Correlative array tomography allows the detection of fluorescent labels as well as ultrastructural investigations on ultrathin serial sections. [1]. Regions of interest can be marked and automatically imaged within all the individual sections building up long ribbons. The challenge of this approach is on one hand the alignment of the consecutive 2D images taken on light microscope and electron microscope and on the other hand their subsequent registration to a correlative 3-dimensional data set.

While correlative array tomography is sample preserving it can only be performed on fixed and sliced specimen. For the correlation of life cell imaging with highly resolved ultrastructure the use of optical sectioning methods and FIB is the combination of choice. This allows to analyse large volume dynamics and correlate these data with the ultrastructural information semi-automatically. The combination of 2-Photon microscopy and FIB-SEM avoids the typical problems which are normally encountered with serial tomography. Crossbeam systems provide 3D datasets of comparatively large volumes that can be analysed with maximum precision and high spatial resolution. Time consuming alignment procedures generally used in tomography are not needed, simple cross correlation methods are sufficient to achieve precise alignment of data obtained [2].

References