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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. To achieve this, it is necessary to exactly define volumes of interest (VOI) in the data of the first microscope. Further, the precise relocation of the identical VOI in the second microscope is essential as well as the registration of the 3D object in all spatial directions. However, the correlation of 3-dimensional data from different microscopes (e.g. laser scanning microscopes and focussed ion beam scanning electron microscopes) is feasible due to cross correlation methods but this workflow is not fully-automated up to now [1,2].

The 3D workflow can be simplified by reducing the scale of the object in one dimension. One popular approach is slicing the object into serial sections (correlative array tomography) [3]. Thus, the segmentation in one dimension is done mechanically and only 2-dimensional microscope images have to be correlated as shown in Fig. 1.

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

After detection of characteristic features [4], the patterns can be described using a variety of different algorithms [5, 6] A comparison of the features in the single sections followed by an alignment of the features results in an accurate alignment of the single sections (Fig. 2). Finally, the full volume can be reconstructed by a similar slice-to-slice stack alignment.

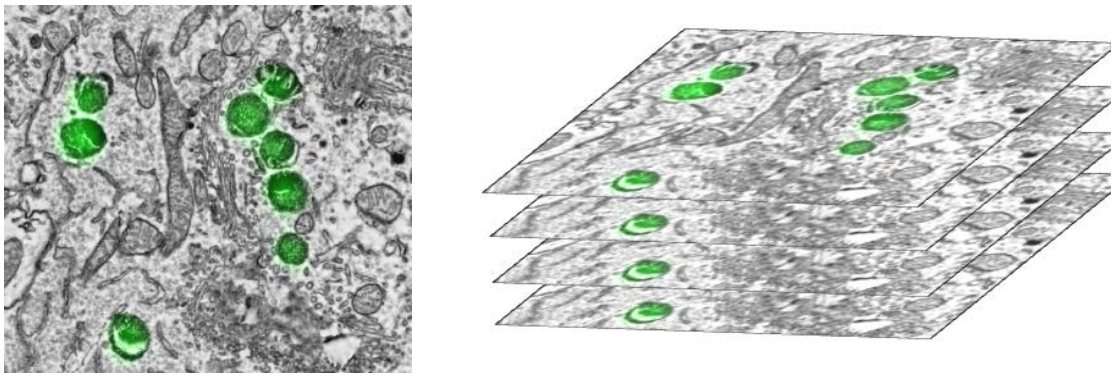

Figure 1. Ultrathin brain sections. (A) Overlay of a fluorescence image and an image taken with a scanning electron microscope. (B) Stack of overlaid 2D-images.

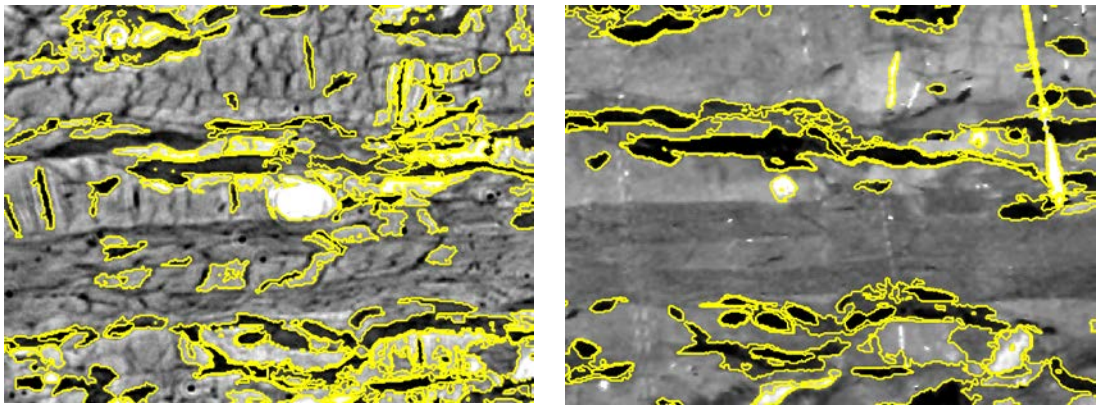

Figure 2. Determination of characteristic features in the same region of interest of a skin section. A phase contrast image is shown in (A), (B) depicts an electron microscope image detected with a secondary electron detector.

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