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Microscopy for Global Challenges

touching atoms, molecules, nanostructures and cells by multidimensional microscopy

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IT-3-O-1457 Imaging of cleared biological samples with the Ultramicroscope

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In the last years we have developed a special Ultramicroscope (light-sheet microscope) for visualizing neuronal networks in whole brains. In the Ultramicroscope whole cleared brains are illuminated with a sheet of light and the optical sections are used for 3D reconstructions. This approach allows one to employ also low power, wide field objectives for imaging of large samples.

By clearing neuronal tissue with organic solvents (BABB) after dehydration, we could visulalize GFP-labelled neuronal networks in the whole brain [1.[Improving our clearing technology by using tetrahydrofuran for dehydration and dibenzylether (THF/DBE) for clearing we were able to image GFP-labelled axons even in heavily myelinated spinal cord [2,3]. Also nervous and muscle structures in drosophila melanogaster can be imaged [4]. Our and other clearing solutions have non standard refractive indices. Due to a heavy refractive index mismatch imaging in these solutions with e.g. air or water immersion objectives gives therefore suboptimal results. We thus developed special objective devices that allow refractive index matched imaging. We show that high resolution imaging through 10 mm clearing medium is possible (Fig.1).

Furthermore we substantially increased the axial resolution of our light-sheet microscope by developing completely new optics for light sheet generation. These optics create an extremely thin light sheet by the use of a Powell- and several aspheric lenses. As light sheet thickness determines the axial resolution it is of pivotal importance for the performance of the light-sheet microscope. Our light sheet is static and will thus in future allow combination with other microscopic techniques which need constant nonscanned illumination. Examples for the application of the ultramicroscope as imaging of mouse brain, spinal cord and whole drosophila are given.

References

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