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ABSTRACTS

Visualization of neuronal networks in the mouse brain, spinal cord and mouse embryos by ultramicroscopy

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It would be very helpful for the analysis of neuronal networks of the brain, if one could visualize these networks in 3 dimensions. Up to now this was only possible with limited resolution by sequential slicing and reconstruction of the brain. This time consuming attempt is easily hampered by artifacts as shrinkage and distortion induced by standard histological procedures.

To overcome these problems we used a microscopy based on extreme darkfield illumination with a light sheet, once called ultramicroscopy. This microscopy allows optical sectioning of whole mouse brains and was combined with an approach to clear fixed neuronal tissue: Mouse brains were made completely transparent by immersion in oil of the same refractive index as protein. By illuminating the brains with blue light (λ = 488 nm), neurons labeled with GFP were visualized by fluorescence. This way we could detect single neurons in hippocampi inside whole brains.

By surface rendering the shape and position of hippocampi relative to the brain surface could be depicted. In complete excised hippocampi subcellular resolution was obtained by 3D reconstruction from several hundred optical sections. The dendritic trees of CA1 hippocampal neurons with dendrites and dendritic spines could be visualized.

Many proteins can be labelled in transgenic mice with genetically encoded fluorescent markers. Using these markers our approach will represent a high-throughput screening method for protein expression in 3 D. This expression can be monitored with µm resolution and should allow the elucidation of complex neuronal networks in the brain and spinal cord.

We show that ultramicroscopy allows also optical sectioning and detailed 3D reconstruction of whole mouse embryos by imaging autofluorescent structures. Especially the circulatory system in the body and brain became apparent as blood remaining in the preparation showed strong fluorescence. Also other applications like e.g. visualization of nerve bundles in whole embryos and visualization of plaques in brains of mice with Alzheimers disease will be shown. In general the method is well suited for high-throughput phenotype screening of transgenic mice and thus will benefit the investigation of disease models.

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