Light sheet fluorescence expansion microscopy of spacious mouse brain sections

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Critical details of neuronal connectivity such as synapses show length scales of about 100 nm. Structures small like this can optically be resolved using super resolution light microscopy. This is not feasible for the reconstruction of extended neuronal networks, because all available super resolution approaches are restricted to thin samples of about 20 m in depth. However, synaptically connected neurons can be spatially separated from each other by hundreds of micrometers.

A recently introduced tissue expansion method allows to isotropically increase the sample size and thus the virtual optical resolution by a factor of four [1]. Macromolecules that are as close as ~60 nm before expansion can be resolved with no detectable distortion of the relative protein position. This increase in effective resolution enables imaging of critical neuronal details using conventional light microscopes thus bypassing the depth-limit of super resolution techniques. We combine tissue expansion with light sheet fluorescence microscopy to allow fast and gentle imaging of $(2\ mm)^3$ samples (after expansion) *[2, 3]*. These two methods are an ideal match to obtain super-resolved images of extended neuronal circuits at imaging rates largely exceeding those of point-scanning instruments.

Large sample blocks of mouse brains were isotropically expanded. The heavy adsorption of water during the expansion process rendered the sample transparent allowing imaging by confocal light sheet fluorescence microscopy [4]. Theoretically, this yields a virtual lateral and axial optical resolution of 75 and 250 nm, respectively, with imaging depths up to effectively 2 mm, using an 25x objective lens featuring a numerical aperture of 1.1. We demonstrated the capabilities of light sheet fluorescence expansion microscopy by performing fast volumetric super resolution imaging of mouse dentate gyrus molecular-, granule cell- and polymorphic layers using a custom-built instrument. Our method enabled an exact evaluation of granule cell morphology within the context of large cell ensembles spanning several orders of magnitude in resolution. Subsequent deconvolution allowed to reduce the virtual optical resolution almost to the theoretically expected values.

[1] Chen, F., Tillberg, P.W., and Boyden, E.S. (2015). Optical imaging. Expansion microscopy. Science 347, 543 - 548.

[2] J. Doerr, M. K. Schwarz, D. Wiedermann, A. Leinhaas, A. Jakobs, F. Schloen, I. Schwarz, M. Diedenhofen, N. C. Braun, P. Koch, D. A. Peterson, U. Kubitscheck, M. Hoehn, and O. Brüstle (2017). Whole-brain 3D mapping of human neural transplant innervation. Nat. Comm. 8: 14162. doi: 10.1038/ncomms14162

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[3] Schwarz, M. K., Scherbarth, A., Sprengel, R., Engelhardt, J., Theer, P., Giese, G. (2015) Fluorescent-Protein Stabilization and High-Resolution Imaging of Cleared, Intact Mouse Brains. PlosOne, DOI:10.1371.

[4] Baumgart, E., and U. Kubitscheck. 2012. Scanned light sheet microscopy with confocal slit detection. Opt. Expr. 20: 21805 - 21814

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