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LIGHT SHEET FLUORESCENCE MICROSCOPY FOR HIGH RESOLUTION WHOLE-BRAIN 3D MAPPING

Rabies virus-based retrograde tracing is a powerful approach for visualizing synaptically connected neurons. Combined with a refined tissue clearing technology [1], this approach enables e.g. the visualization of transplanted neurons and synaptically connected host cells in whole-mouse brain preparations [2]. In order to visualize and 3D reconstruct such a transplant connectome we optimized a light sheet fluorescence microscope (LSFM) for high resolution imaging of complete cleared mouse brains.

The instrument features two long working distance, refractive index corrected objective lenses suitable for organic clearing solutions such as BABB. The objectives have magnifications of 4.44 and 12, and numerical apertures of 0.3 and 0.53, respectively. Due to the two-sided Gaussian beam illumination of the sample the image quality in both brain hemispheres is identical. A pivoting of the scanned light sheets significantly reduces shadow artefacts. An autofocus routine based on Vollath's F4-function is used to optimize illumination and detection planes. We used submicrometer fluorescent beads embedded in epoxy resin to characterize the experimental point spread function and compared the achievable optical resolution to that realized in mouse brain samples. Finally, we compared the effect of Bessel and Gaussian beams for imaging in strongly scattering samples. Bessel beam illumination yielded a clearly superior contrast in such samples.

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

[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, 19 January 2017

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