LIGHT SHEET FLUORESCENCE MICROSCOPY OF EXPANDED SAMPLES

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Critical details of neuronal connectivity (e.g. synapses) occur on 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 µm 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 exceeding those of point-scanning instruments by a factor of ~20.

Large sample blocks of mouse brains are isotropically expanded. The heavy adsorption of water during the expansion process renders the sample transparent allowing imaging by confocal light sheet fluorescence microscopy [4]. This yields a virtual lateral and axial optical resolution of 75 and 250 nm, respectively, with depths up to effectively 2 mm. Exploiting state-of-the art genetic and rabies virus-based labeling approaches to selectively label specific subsets of synaptically connected neurons we can generate a structural connectivity map of subregions of a mouse brain in super resolution.

Notably, the method is applicable to all types of tissue, we will display examples such as microtubuli and nuclear pores in mammalian cells and transcription site topology in *Chironomus tentans* salivary gland cells.

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