A FOUR-COLOR SCANNED LIGHT-SHEET MICROSCOPE FOR IMAGING LARGE, EXPANDED SAMPLES

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The physical expansion of microscopic samples is a simple and elegant way to visualize structures with nano-scale dimensions by standard light microscopy [1]. However, expanding large samples creates especially extended specimen. The microscopic analysis of such specimen without compromises requires an instrument that allows to image fast and with high resolution and that features a long working distance and low phototoxicity. We constructed such a microscope, which was designed specifically for imaging expanded mouse brain sections.

In Expansion Microscopy (ExM) a sample with fluorophores linked to a swellable gel is expanded homogeneously and isotropically by a factor of 4 to 20. This allows to resolve neuronal network details on length scales of, e.g., synapses, which are normally below the diffraction limit of optical microscopes. Combining ExM and Light-Sheet Fluorescence Microscopy (LSFM) results in an imaging technique, which fulfills the above named requirements. Compared to a super resolution point-scanning confocal microscope the data acquisition time can be reduced by a factor of at least 20 [2].

Our instrument features a water-dipping 40x NA 1.0 objective lens with a working distance of 2.5 mm and four solid state lasers for fluorescence excitation. The fragile expanded specimen require a gentle sample handling. This was considered for the design of a dedicated sample holder, which takes up samples with dimensions of up to 20x20x2.5 mm^3. Image acquisition of two fluorescence channels occurs simultaneously by two sCMOS cameras in full frame or rolling shutter mode. The latter allows acquisition of confocal images [3]. Acquisition of diffraction limited image stacks occurs automatically in a tiled fashion yielding and reaches 8.5 hours for a 1 mm^3 sample (before expansion) with effective resolutions of 100 nm laterally and 325 nm axially, respectively, for green fluorescence.

We present experimental data from expanded mouse dentate gyrus molecular-, granule cell- and polymorphic layers that were obtained with such a custom designed LSFM [2]. The achieved super resolution in dual colors is demonstrated by spatial analysis of the pre- and post-synaptic proteins bassoon and shank2, respectively.

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