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A Two-Color Scanned Light-Sheet Microscope for Expanded Mouse Brain Sections

In Expansion Microscopy (ExM) a sample with fluorophores linked to a swellable gel is expanded homogeneously by a factor of approx. 4 [1]. This leads to a virtual optical resolution of up to 60 nm laterally and 250 nm axially. Applied to mouse brain samples this allows for resolution of neuronal network details on length scales of 100 nm, which are normally below the diffraction limit of optical microscopes. Combining ExM and Light-Sheet Fluorescence Microscopy (LSFM) results in an imaging technique, which features low phototoxicity, high frame rates and super resolution. Compared to super resolution point-scanning confocal microscopes the data acquisition time can be reduced by a factor of 20 [2].
Here, we constructed a scanned LSFM specifically for Expansion LSFM. The instrument features a water-dipping, high numerical aperture, long working distance objective lens, simultaneous two-color detection and a confocal data acquisition mode [3]. The instrument was devised to investigate expanded gel samples with dimensions of up to 20x20x2.5 mm^3. The fragile gels require a gentle sample handling, which was considered for the design of the sample holder. For fast image acquisition in two colors a simultaneous detection with the GEMINI-2C from Hamamatsu was installed. Combined with two sCMOS cameras in rolling shutter mode, a confocal image acquisition is feasible. An automated image acquisition with frame rates up to 40 Hz is implemented, which will reduce the imaging duration for a 1 mm^3 sample (before expansion) from currently 110 to only 5 hours.
The instrument will be used to analyze the 3D structure of extended, dense and sparsely labeled neuronal networks in subregions of mouse brains in super-resolution.

[1] Chozinsky et al., “Expansion microscopy with conventional antibodies and fluorescent proteins”, Nat. Methods 13(6), 2016
[2] Bürgers et al., submitted
[3] Baumgart et al., “Scanned light sheet microscope with confocal slit detection”, Opt. Expr. 20(18), 2012

Affiliation

University of Bonn

Terms and Conditions

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Primary author(s) : Ms BÜRGERS, Jana (Institute of Physical and Theoretical Chemistry, University of Bonn, 53115 Bonn, Germany)

Co-author(s) : Mr FELDHOFF, Dennis (Institute of Physical and Theoretical Chemistry, University of Bonn, 53115 Bonn, Germany); Dr SCHWARZ, Martin K. (Institute for Experimental Epileptology and Cognition Research, Functional Neuroconnectomics Group, University of Bonn, 53127 Bonn, Germany); Prof. KUBITSCHECK, Ulrich (Institute of Physical and Theoretical Chemistry, University of Bonn, 53115 Bonn, Germany)

Presenter(s) : Ms BÜRGERS, Jana (Institute of Physical and Theoretical Chemistry, University of Bonn, 53115 Bonn, Germany)

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