

CONFOCAL TWO-PHOTON LIGHT SHEET MICROSCOPY FOR IN VIVO IMAGING OF ZEBRAFISH LARVAE

**Alexander Harder, Bhuvaneshwari Nagarajan*, Eugen Baumgart,
Peter Königshoven, Jana Bürgers, Benjamin Odermatt*, Ulrich Kubitscheck**
Institute of Physical and Theoretical Chemistry, *Institute of Anatomy,
Rheinische Friedrich-Wilhelms-Universität Bonn
Wegelerstraße 12, 53115 Bonn, Germany
E-mail: harder@pc.uni-bonn.de

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Light Sheet Fluorescence Microscopy (LSFM) has become a popular method for in vivo imaging of biological samples. It allows 3D imaging at subcellular resolution while featuring minimal fluorescence bleaching and photo toxicity as well as high image acquisition rates. This makes the method feasible for live imaging of either long-term developmental or fast biological processes.

We present a confocal LSFM setup that uses scanned light sheet illumination [1] in combination with confocal detection by synchronizing laser scanning and rolling shutter readout of a scientific CMOS camera. This has proven to reduce background by scattered light, improving overall image contrast [2]. We combine this detection method with two-photon excitation in order to further enhance image contrast and to avoid stimulation of the visual system of the specimen by visible light. Since two-photon excitation is proportional to the squared laser power this reduces the effective field of view to an area around the focal spot. However, this field of view is sufficient for small samples like a zebrafish spinal cord. The microscope is also capable of illuminating the sample from two opposing directions. This can either be used to reduce stripe artifacts [3] or to increase the effective field of view. The light sheet for both illumination sides is independently generated by two scanning mirrors. Each of these comprises two axes, allowing to record 3D-stacks without moving the sample. Instead, the objective is moved by a piezo stage to keep the light sheet in focus. While the speed of two-photon imaging is more restricted by excitation power than its single-photon counterpart, the microscope enables live imaging of neuronal activity at the cellular level. When only a few cells within a volume are of interest, the image planes of these cells can be addressed individually. This significantly improves acquisition time compared to full volumetric imaging.

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[3] Misha B. Ahrens, Michael B. Orger, Drew N. Robson, Jennifer M. Li, Philipp J. Keller, "Whole-brain functional imaging at cellular resolution using light-sheet microscopy", *Nature Methods* **10**, 413–420 (2013)