BRAIN SLICE IMAGING AND PHOTO-STIMULATION AT HIGH SPATIO-TEMPORAL RESOLUTION WITH LATTICE LIGHT-SHEET MICROSCOPY

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Lattice light-sheet microscopy[1] (LLSM) is a recent technique based on selective plane illumination microscopy[2] (SPIM) that has several key advantages: intrinsic optical sectioning, very fast imaging, low photo-toxicity, and, due to the non-diffractive lattice beam shaping, a very high and uniform spatial resolution. The great benefits of LLSM for fast 3D cellular imaging are now well established in various fields of biology.
In neuroscience, local control of neuronal activity is critical for many studies. Unfortunately, LLSM can not be combined with electrophysiology due to the limited space above the sample, and the brain slice can only be stimulated globally with pharmacological agents. To circumvent this limitation we built a LLSM in which we added a photo-stimulation module (Fig. 1) that allows illumination of any user-defined region of interest with millisecond temporal precision and sub-micrometric spatial resolution.

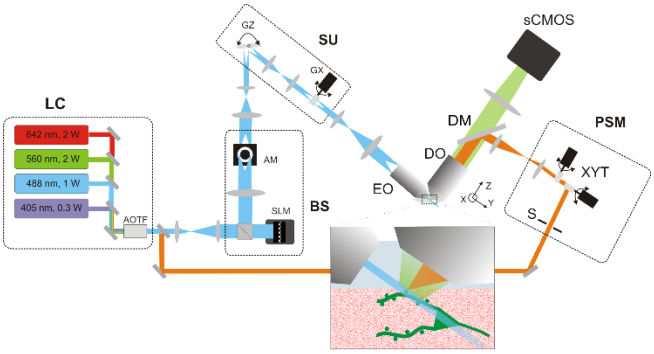

Figure 1: Lattice light-sheet microscope equipped with photostimulation module (PSM) to target any user defined ROI at high spatiotemporal resolution. Inset: Schematic zoom at the sample with light sheet (blue), detected fluorescence (green), and photostimulation beam (orange).

We performed targeted uncaging or FRAP while imaging neurons or glia inside live rodent brain slices. We demonstrated the performances of LLSM and photo-stimulation in several ongoing studies: measurement of AMPA receptor diffusion at single spines, vesicular transport in dendrites, spontaneous and stimulated local calcium activity in neurons and astrocytes. Sub-micrometric neuronal compartments such as spines could be imaged and targeted down to ~20 µm below the surface.
Overall, the addition of targeted photo-stimulation module opens the way to all-optical neurophysiology studies and significantly expends the potential of LLSM in neuroscience.

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[2] J. Huisken, et al., "Optical sectioning deep inside live embryos by selective plane illumination microscopy.," *Science*, 305, 5686, pp. 1007–1009, 2004.