Laboratoire d'optique et biosciences
Ecole Polytechnique – CNRS – INSERM – IP Paris

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Ecole Polytechnique
Amphithéâtre Curie

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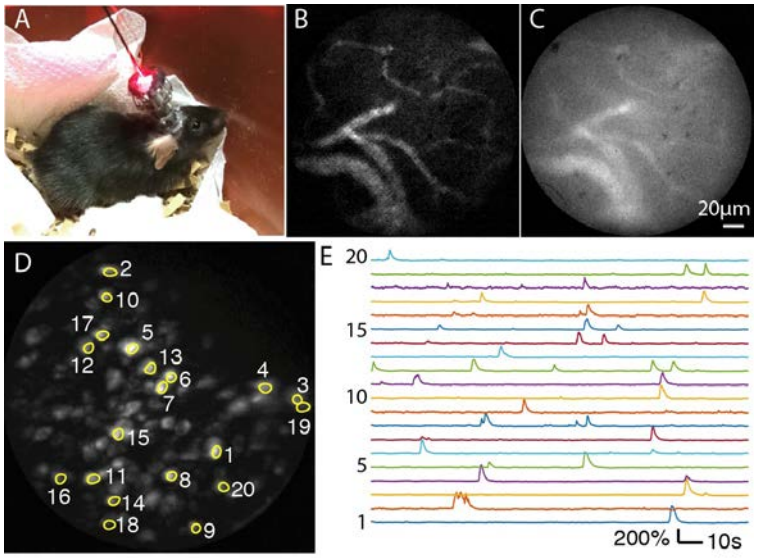
Fast confocal fluorescence imaging and photoactivation in freely behaving mice

A longstanding goal in Neuroscience is to unravel the neural basis of perception, memory formation and behaviors. To address this goal, it can be useful to manipulate and record neuronal activity with cellular precision while the animal (here, a rodent) is performing specific behavioral tasks. Such experiments can be performed with optical methods, using photostimulation of optogenetic actuators and fluorescence imaging of calcium reporters. To apply optical methods to freely-behaving mice, two approaches have been followed: a microscope can be fully miniaturized and placed on the rodent head, or an image guide can be used as a relay between a regular-size custom-made microscope and the animal, which allows partly overcoming miniaturization constraints.

Using this second strategy, we have recently developed two fiberscopes. The first system allows for photoactivation with near-cellular resolution in freely-behaving mice [1]. This is achieved by shaping the illumination beam for photoactivation using phase modulation with a liquid-crystal display. The second system allows for fast fluorescence imaging with optical sectioning, using multipoint- and line-scanning confocal imaging [2]. For both imaging modalities, the illumination beam is shaped by intensity modulation with a digital micro-mirror device, providing high adaptability to the sample and imaging conditions. Using this device, we demonstrated fast (>100 Hz) fluorescence imaging of blood flow and neuronal activity in the brain of freely-behaving mice, with reduced out-of-focus background compared with widefield imaging (see figure).

[1] Szabo et al, Neuron 84, 1157–1169 (2014)
[2] Dussaux et al, Scientific Reports 8, 16262 (2018).



Fast confocal imaging in freely behaving mice. A. Picture of mouse with the fiberscope probe fixed on the skull. **B-C.** Multipoint-scanning confocal (B) and widefield (C) images of microvasculature in the cortex. Acquisition rate: 150Hz. **D-E.** Neuronal activity recording in the hippocampus using line-scanning confocal imaging of the calcium indicator GCaMP6. **D.** Maximum ΔF image computed over an acquisition of 25 minutes, with 20 neurons outlined. **E.** Neuronal activity (calcium traces, $\Delta F/F$) of the 20 neurons outlined in D and plotted as a function of time. Acquisition rate: 100 Hz.