FAST CONFOCAL FLUORESCENCE IMAGING IN FREELY-BEHAVING MICE

Clara Dussaux[1], Jozsua Fodor[1], Vivien Szabo[2], Yan Chastagnier[2], Jean-François Léger[1], Laurent Bourdieu[1], Julie Perroy[2] and Cathie Ventalon[1]
(1) IBENS, CNRS UMR 8197, INSERM U1024, Ecole Normale Supérieure, 46 rue d'Ulm, 75005 Paris, France
(2) Institut de Génomique Fonctionnelle, 141, rue de la Cardonille, 34094 Montpellier Cedex 05, France
E-mail : dussaux@biologie.ens.fr

KEY WORDS: *In vivo* brain imaging, Freely-moving mice, Multipoint confocal imaging, Fluorescence microscopy, Fiberscope, Spinning disk microscopy

We developed a new fiberscope that allows fluorescence imaging with optical sectioning at high speed (up to 200Hz) deep in the brain (up to 150µm) of freely-behaving mice. The system relies on a home-made confocal microscope coupled to the animal's brain with a 2-meter long optical fiber bundle attached to an imaging micro-objective. The head-mounted part weighs less than 2g, which allows experiments in freely-behaving mice. The fiberscope is inspired from a system previously developed [1], but imaging speed and signal to noise ratio have been significantly improved thanks to the use of multipoint confocal imaging.
Multipoint confocal imaging is a variation on conventional confocal microscopy that consists in illuminating the sample with an array of light points created with a matrix of pinholes, and detecting fluorescence through the same matrix. The matrix is scanned rapidly to illuminate the full field-of-view during acquisition of a single image with a camera. This system allows a significant improvement of imaging speed compared to conventional confocal microscopy (reaching rates >100Hz) while keeping similar sectioning capacity and resolution. In our case, as in [2], the matrix of pinholes is created using a Digital Micromirror Device (DMD). The fast refreshing rate of DMDs (up to 22kHz) allows displacing the pinhole matrix at high speed, with no-moving part. The key advantage of this implementation is that the size and density of pinholes can be easily adapted to the sample under investigation (whereas in traditional implementations, such as in spinning disk confocal imaging, the matrix is fixed).

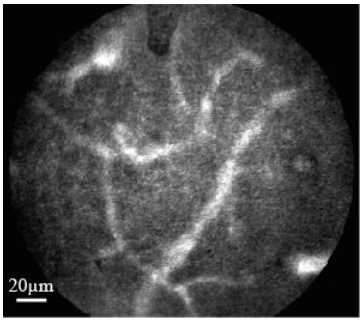

Figure 1: Imaging of blood vessels in the cortex of an anesthetized mouse

RESULTS

Our system allows recording optical sections of thickness down to 13µm (limited by the aberrations of the grin lens based micro-objective), with a resolution compatible with cellular imaging and a field-of-view of 230µm. We imaged microvasculature in the cortex in anesthetized and freely-behaving mice following intravenous injection of a red fluorescent dextran protein (Figure 1). Images could be recorded at high speed (up to 200Hz), and at depths up to 150 µm. Comparison with conventional epifluorescence imaging showed enhanced contrast and background rejection.

[1] V. Szabo, C. Ventalon, V. De Sars, J. Bradley, and V.Emiliani, "Spatially Selective Holographic Photoactivation and Functional Fluorescence Imaging in Freely Behaving Mice with a Fiberscope", *Neuron*, **84,** 1157-1169 (2014).
[2] F. P. Martial, and N. A. Hartell, "Programmable Illumination and High-Speed, Multi-Wavelength, Confocal Microscopy Using a Digital Micromirror," Plos One **7,** e43942 (2012).