A TWO-PHOTON MICROSCOPE EMPLOYING AN ACOUSTO-OPTIC LIGHT MODULATOR FOR FAST 3D VOLUME SCAN

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Animals are able to evaluate the benefits and risks associated with different behaviors in rapid response to perceived changes in the environment. In mammals, the underlying calculations involve neuronal circuits in cortex, the outer sheet of the mammalian brain. Decoding of these and other brain functions from electrical activity in cortical circuits requires the data of cellular activity from large numbers of individual neurons at millisecond resolution in behaving animals. While two-photon scanning microscopy (TPM) of activity indicators, like genetically-encoded calcium indicators (GECIs), may yield such data, common pivoting mirror-based laser scanners usually permit only moderately fast sampling (~10-100 Hz) from neurons if these are located in the plane of the optical section, and significantly slower rates (~1-10 Hz) when neurons in different sections are included. New scanning methods are needed for a more comprehensive and fast sampling of neurons in 3D cortical networks.

We developed a new type of two-photon microscope employing a dispersion-compensated acousto-optic spatial light modulator (AO-SLM) [1] to realize lateral and axial displacements of the laser focus at equal speed. By time-locking the output pulses of a regenerative and parametric laser amplifier (800–1250 nm) to the 40 kHz refresh cycle of the AO-SLM, individual laser pulses are independently modulated with tilt and defocus, allowing consecutive pulses to be directed to arbitrary positions within the accessible 3D scan volume, irrespective of their distance. In our configuration, the scan volume corresponded to a cube of 400 μm edge length. The resulting point-spread function (PSF) was diffraction-limited in the center of the scan volume while expanding by less than a factor two in lateral (axial) size at focus positions close to the lateral (axial) volume border. The observed aberrations are in agreement with the expected effect of residual chromatic wave front dispersion created in the AO crystals and of spherical aberration of wave front curvature created in the objective, respectively. We implemented several scan modes, including a saltatory mode for increased sampling speed where the laser visits neuron cell bodies without scanning the space in-between. Further gain of speed is achieved in a holographic mode where the laser beam is additionally modulated such that a single focus is replaced by an array of multiple foci at close enough distance (< 5 μm) as to target the same cell body and thus boosting the single pulse photon yield. Using recordings from eGFP and GCaMP6-expressing neurons we demonstrate scan speeds of up to 1 KHz from individual neurons in sensory cortex of mice under anesthesia.

[1] W. Akemann, J.F. Léger, C. Ventalon, B. Mathieu, S. Dieudonné, L. Bourdieu, "Fast spatial beam shaping by acousto-optic diffraction for 3D non-linear microscopy", *Optics Express,* **23(22),** 28191-205 (2015).