SIMULTANEOUS DUAL-PLANE IMAGING WITH A MULTI-SITE MESOSCOPE

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High-throughput imaging of large volumes is needed to analyze the collaborative behavior of large neuronal populations which is regarded fundamental to understand brain function.

To achieve this goal, new developments in multi-photon microscopy include objective lenses with both large field-of-view (FoV) and high numerical aperture [1]. Such combination of optical properties can only be achieved by constructing objective lenses of large physical size and subsequent high inertia. Therefore, traditional fast focusing techniques, such as positioning the objective lens with piezo-actuators, cannot be utilized. Recently, remote focusing [2] has been introduced to overcome this hurdle. In principle, this method keeps the primary objective lens stationary, using a secondary lens to generate an aberration-free real image to be viewed with rapidly adjusting focus. The recently introduced MesoScope [3] implements this approach, exceeding sequential imaging rates of 40 volumes-of-interest (VoI) per second.

To further increase the imaging throughput, we have extended this microscope scheme to support simultaneous dual-plane imaging. This extension adds a second remote focusing unit to the MesoScope, utilizing an orthogonal beam polarization not employed in the original design. Focal plane illumination is alternated at twice the laser pulse rate, i.e. 160 MHz, resulting in true simultaneous dual-plane imaging. Cross-talk between planes depends on the fluorescence lifetime of the employed molecules. The laser power delivered to both planes can be independently controlled to optimize the depth-dependent multi-photon excitation. The system extension also includes fast de-multiplexing electronics. The upgrade to the system is an add-on to the original design that does not affect its original functioning and is handled by a custom modification of the ScanImage-based control- and data-acquisition software. As intended, the modified MesoScope performs at twice the imaging throughput of the original system, i.e. 80 VoI/s [4].

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