Improvements in two-photon fluorescence microscopy

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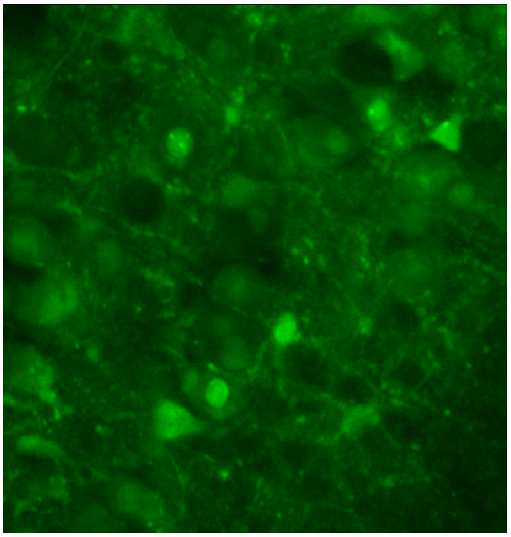
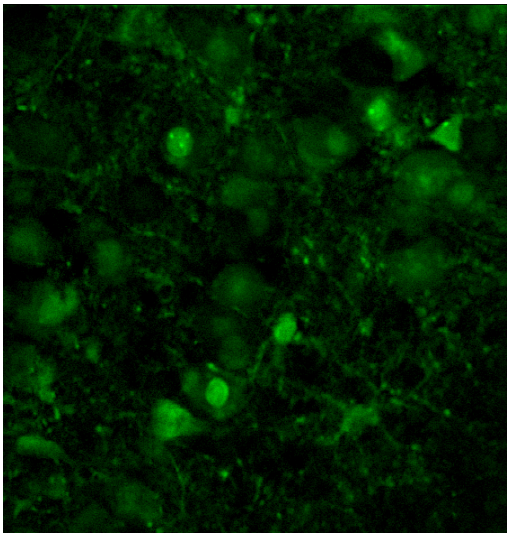
ABSTRACT

Two-photon excitation fluorescence (TPEF) microscopy is a powerful technique to image deep biological living tissue [1]. Given that TPEF is mostly generated by ballistic (unscattered) light, this technique provides high resolution images even within scattering media. Recently, we have shown that the rejection of out-of-focus TPEF background in scattering tissue can be significantly enhanced with a differential aberration technique [2,3]. This technique is based on an intermittent introduction of extraneous aberrations to control the phase profile of the near-infrared femtosecond laser beam in the back aperture of the focusing objective. The subtraction of an aberrated image from a standard image then leads to enhanced background rejection.



Standard TPEF image of sulforhodamine-labeled mouse olfactory bulb slice



DAI-TPEF image of sulforhodamine-labeled mouse olfactory bulb slice

We report an improvement on this technique based on the use of a novel, fast, cost-effective deformable mirror, allowing us to perform differential aberration at a rate of 400kHz. In particular, the problem signal-to-noise ratio is discussed, and techniques to maximize signal-to-noise ratio are evaluated. We present a heuristic description of our technique which we corroborate with experimental results. Images of labeled mitral cells in a mouse olfactory bulb are compared with standard TPEF microscopy images,

demonstrating significant out of focus TPEF background rejection and better image quality with our technique.

Moreover, we describe a simple TPEF imaging strategy, called targeted path scanning (TPS), to monitor the dynamics of spatially extended neuronal networks with higher spatiotemporal resolution than possible with standard raster scanning [4]. Our strategy combines the advantages of mirror-based scanning, minimized dead time, ease of implementation, and compatibility with high-resolution low-magnification objectives.

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KEYWORDS
Adaptive optics, TPEF microscopy, thick tissue imaging.