DE-SCATTERING WITH EXCITATION PATTERNING IN TEMPORALLY-FOCUSED MICROSCOPY (DEEP-TFM)

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Multiphoton excitation is the method of choice for deep, in vivo microscopic imaging [1]. Functional imaging of neurons down to nearly 1.5 mm has been achieved with two-photon excitation [2]. For deep imaging, most multiphoton microscopes are configured as point scanning systems requiring slow, sequential raster scanning of a diffraction limited focus over the region of interest. In contrast, parallelized approaches, such as temporal-focused-two-photon-microscopy [3], provide wide-field optically-sectioned excitations. However, the emission photons are of much shorter wavelengths. They scatter significantly before reaching the camera. Thus, the advantages of wide-field excitation are hardly utilized. To overcome this limitation, here we introduce a new computational imaging scheme called, "de-scattering with excitation patterning in temporally-focused microscopy" or DEEP-TFM, based on spatially modulated temporally focused excitation followed by computational demodulation.

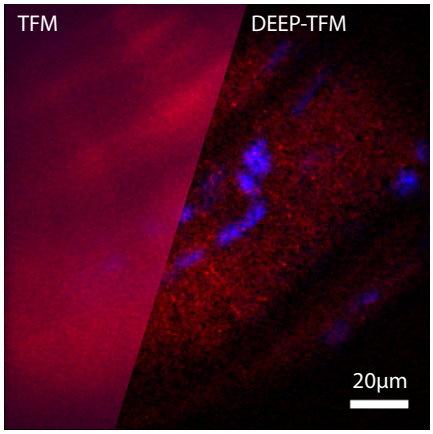

Figure 1. TFM, and DEEP-TFM images of a mouse muscle specimen at a $190\mu m$ deep imaging plane. The blue and red channels are respectively: nucleus (stained with Hoechst 33342) and F-actin (stained with Alexa Fluor 568 Phalloidin).

Firstly, we project a set of random excitation patterns onto the sample being imaged; the excitation patterns maintain their fidelity through the scattering medium due to two-photon excitation wavelengths. Secondly, we image the emission light through the scattering medium onto a camera for each excitation pattern. Although the scattering of emission light distorts the imaged features, we theoretically demonstrate that noise-limited reconstruction is possible through the use of multiple patterns. We, then, demonstrate the proposed imaging scheme in silico in simulations. Finally, we show experimental validation of DEEP-TFM by imaging biological specimen through scattering media (Figure 1).

We believe that DEEP-TFM can speed up in-vivo two photon imaging by an order to two orders of magnitude compared to the point scanning two-photon microscopy without sacrificing image quality.

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