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

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Point-scanning two-photon microscopy is used routinely for in vivo, volumetric biological imaging, especially in deep tissues. Despite the excellent penetration depth, a conventional point-scanning two-photon microscopy is slow due to the need for raster scanning and imaging time scales linearly with increasing volume, hampering studies of fast biological dynamics. An attractive alternative to point-scanning geometries is wide-field two-photon microscopy, typically called temporal focusing microscopy (TFM) since optical sectioning is achieved by focusing a beam temporally while maintaining wide-field illumination. However, TFM suffers from scattering in tissue resulting in limited imaging depth.

We introduce a novel computational de-scattering technique called De-scattering by Excitation Patterns in TFM or 'DEEP-TFM'. We use wide-field temporal focusing patterned excitation and the signal is measured with a wide-field detector, such as a camera. Briefly, we built a modified temporal focusing microscope that projects arbitrary excitation patterns onto the focal plane using a digital mirror device (DMD). Emission light from the modulated excitation is then detected by a camera. Due to their NIR wavelengths, the excitation patterns maintain their fidelity despite travelling through scattering medium. However, the emission photons are scattered by tissues and the strength of scattering is strongly depth dependent. This assumption holds for most biological tissue. In practice, TFM images are minimally affected by scattering at or near the surface; as the imaging depth increases, scattering gradually degrades high-frequency information in the images. However, low frequencies in the images are retained for most depths even with wide-field detection. Single pixel detection approaches discard this low frequency information, and hence require a large number of excitation patterns. We then combine the information about the excitation patterns with the acquired images, to computationally reconstructed a de-scattered image. Experimentally, to de-scatter a single FOV, multiple pattered excitations (and images) are needed; the number depends on the loss of high-frequency information due to scattering, and hence on the imaging depth.

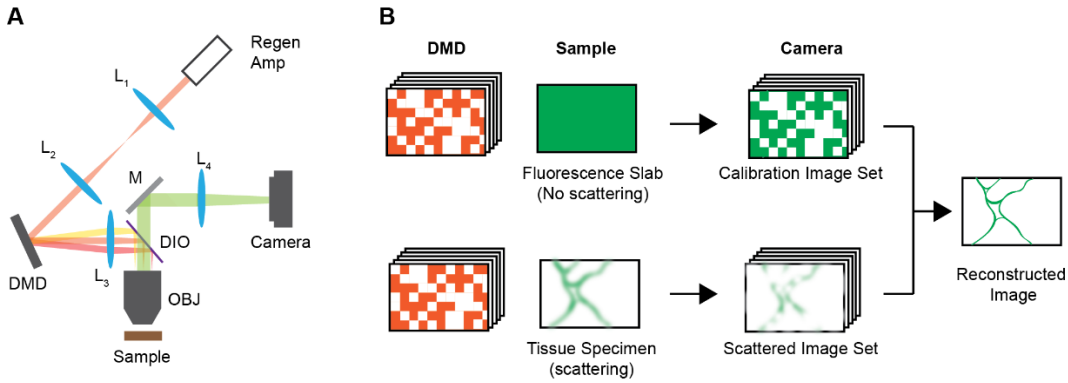

Figure 1. (A) Optical schematic of the imaging system: L1, L2, L3, L4 – lenses; DMD- Digital Mirror Device; DIO – Dichroic mirror; OBJ – Microscope objective. (B) Proposed computational imaging strategy. First a set of patterns are projected on a calibration specimen (homogeneous thin fluorescent layer) to record the calibration image set at the absence of any scattering. Then the same patterns are projected to record the encoded images through a scattering medium. Then the de-scattered images are reconstructed.