

A LABEL-FREE MULTIMODAL NONLINEAR MICROSCOPE FOR BIOLOGICAL APPLICATIONS

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INTRODUCTION: We developed a home-made, innovative and label-free transmission inverted multimodal nonlinear microscope with off-the-shelf components able to perform two-photon excitation fluorescence (TPEF), second harmonic generation (SHG), coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) microscopy [1]. This set-up is highly versatile, can be easily reconfigured and allows the observation of vital, complex and thick biological samples without invasive laser sources and staining. In this work, we performed nonlinear imaging of different biological samples, from single cells to highly heterogeneous tissues to validate our acquisition system.

METHODS: The microscope is fed by a multi-branch Erbium-doped amplified fiber laser. In the CARS/SRS modalities, pump (at 780nm wavelength) and tunable Stokes pulses (in the 940-1200nm range) with picosecond duration are employed, thus covering the CH-stretch region ($2500\text{-}3200\text{cm}^{-1}$). TPEF/SHG signals are excited by a third branch delivering 100fs pulses at 780nm. The sample is mounted on an x-y translational stage, enabling us to image large sample areas.

RESULTS: Figure 1 shows results on a murine coccygeal vertebra section. Distribution of collagen is detected in the CARS modality (a), while autofluorescence of the sample in the TPEF is shown in (b). We employed this microscope also to investigate unknown components of vital breast cancer cells [2], thus enhancing the potentiality of nonlinear microscopy respect to conventional imaging technique. This technique represents a turning point towards clinical applications [3].

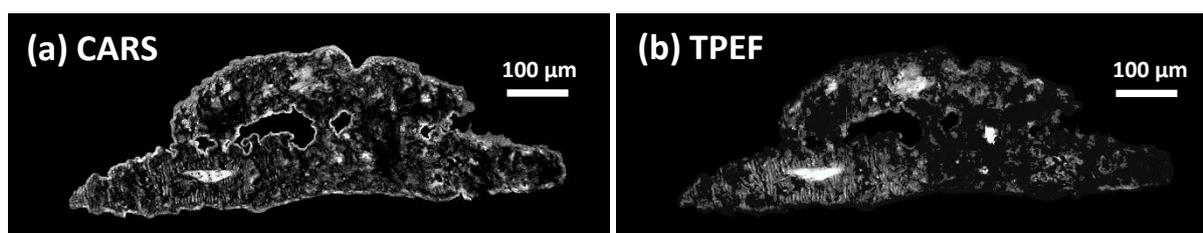


Figure 1: Multimodal image of murine coccygeal vertebra section. (a) CARS signal at 2897 cm^{-1} Raman shift, (b) TPEF signal. Furthermore, each channel was acquired with a total dimension of $1000\mu\text{m} \times 400\mu\text{m}$, 1000×400 pixels, and a pixel dwell time of 5ms.

References:

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