MULTIMODAL AND LABEL-FREE SUPER-RESOLUTION IMAGING

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In the last decade, super-resolution optical microscopy has become an appealing tool for breaking the diffraction limit in biological imaging that typically has been developed based on fluorescent labelling. Nevertheless, deep tissue and label-free imaging remain challenging, especially for tick and highly scattering biological specimens [1]. The advent of ultrashort pulse laser sources has led non-linear optical (NLO) light-matter interaction to have a central role in 3D label-free imaging due to efficient signal generation in the non-linear process [2]. The advantages of NLO approaches include the reduction of scattering due to the use of near-infrared radiation, which enables high penetration depth and reduces the aberrations introduced by the specimen [1]. Non-linear interaction comprises the generation of sum frequencies, high-harmonic generation (SHG/ THG), Raman scattering, two-photon fluorescence, and others [3]. Transient absorption, also known as pump-probe microscopy, is a non-linear optical imaging technique that can probe the dynamics of the excited states. Two types of non-linear interaction, including transient absorption and stimulated Raman scattering (SRS), can be exploited with a pump-probe approach to provide novel contrast mechanisms in weakly or non-fluorescent samples [1]. Besides, utilizing the combination of excited state absorption with ground-state depletion and saturation can help in reaching high- and super-resolution label-free microscopy. Studying complex biological systems typically demands high speed, sensitivity, spectral and multi-contrast capability, and spatial and temporal resolution [1]. In this context, we aim to develop a multimodal microscope by combining conceptions of non-linear processes and multiphoton techniques to obtain multi-contrast imaging. In this work, we perform super-resolution label-free imaging using our custom-built near-infrared pump-probe microscope. Three femtosecond pulsed laser beams, generated by an OPO pumped by a Ti:sapphire laser (Chameleon Ultra II and compact OPO Coherent), are coupled into a commercial laser scanning confocal microscope (Nikon A1 MP). We explore the saturation of transient absorption using a doughnut-shaped beam in the STED-like approach to saturate the absorption [3] and SHG, SFG, and Raman scattering processes to achieve label-free imaging at an improved spatial resolution.

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