Synchronized subharmonic modulation in stimulated emission microscopy

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In this work, we have further improved the stimulated emission (SE)-based pump-probe microscopy [1] with subharmonic fast gate synchronization, which allows over an order of magnitude improvement in signal-to-noise ratio. Critically, the alternative way of modulation is implemented with the highest possible frequency that follows the lasers' repetition rate. Its working is based on a homemade frequency divider that divides the repetition frequency (76 MHz) of the Ti:sapphire (probe) laser to half of the repetition frequency, 38 MHz, which is used to synchronously drive the pump laser and to provide the reference signal for the ensuing lock-in detection. In this way, SE can be detected with sensitivity reaching the theoretical (shot noise) limits, with a much lower time constant (0.1 ms) for faster image acquisition [2, 3]. Note that raising the modulation frequency in the lock-in detection (ideally >1 MHz) serves two purposes: (1) The image acquisition time is greatly reduced by shortening the pixel dwell time (2) High modulation frequency also effectively minimizes the ubiquitous 1/f noise.

Experimentally, we used a pulsed diode laser, λ_{pm} = 635 nm, as the pump (excitation) beam and a mode-locked Ti-sapphire laser, λ_{pb} = 780 nm, as the probe (stimulation) beam. We performed our measurements on ATTO647N fluorescent dye.

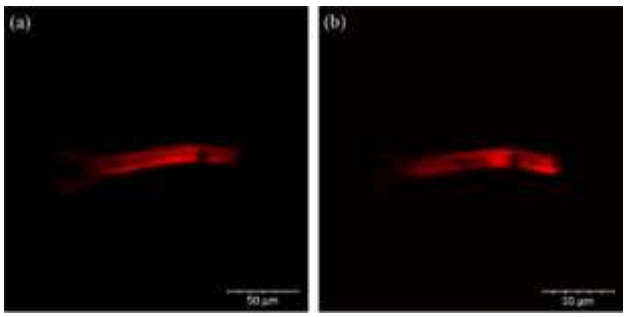

Figure 1. (a) Confocal image versus (b) subharmonic SE image of a blood vessel. The SE image is recorded at 512×512 pixels with a scale bar of 50 µm. The time constant of the lock-in amplifier, matching the pixel dwell time, is set at 0.1 ms.

The time delay (τ) between the pump and probe pulses is precisely controlled with an auto-correlator setup, to allow the extraction of fluorescence lifetime. We have also demonstrated very high temporal resolution (~4 ps) for fluorescence lifetime measurement that is based on stimulation emission (SE) in pump-probe configuration, compared with the temporal resolution (~ 30 ps) of the conventional time correlated single photon counting (TCSPC).

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[3] S. Das, *et al.*, *Opt. Express* 27, 27159-27167 (2019).