Time Resolved Imaging with Stimulated Emission in Pump-Probe Microscopy

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Pump-probe microscopy has become a common platform for imaging based on nonlinerar optical processes, such as stimulated emission (SE), ground state depletion (GSD), and excited state depletion (ESD). The capacity includes molecular specificity, improved resolution, and enhanced penetration deapth [1]. In the past years, stimulated emission based pump-probe microscopy has demostrated dark chromophore detetion [2] and fluorescence lifetime imaging [3]

In this work, a pump-probe microscope is configured for the simultaneous detection of stimulated gain and spontaneous loss. We have used a pulsed diode laser, λ_{pu} = 635 nm as the pump (excitation) beam and a mode-locked Ti-sapphire laser, λ_{pr} = 780 nm, as the probe (stimulation) beam. The time delay (τ) between the pump and probe pulses are precisely controlled by adjusting the length of the triggering cables and setting the delay generator. The probe beam pulses are passed through two 15-cm long dispersive glass rods (SF-6) for extending into a pulse width of 1.5 ps. Both beams are coupled into the scanner and focused on the sample by an objective lens (10X, NA=0.3).
For stimulated gain, the pump beam is modulated at a frequency, f_1, and the probe beam is demodulated accordingly to extract the signal in the transmission direction with a photodiode as the detector (PDA 36A, Thorlabs). For spontaneous loss, the probe beam is modulated at frequecny, f_2, the spontaneous loss signal is than demodulated from the fluorescence detected in the reflection mode by a PMT. In all cases, a high performance lock-in amplifier (HF2LI, Zurich Instruments) is used. The output signal of the lock-in amplifier is then fed to the A/D channel of the scanning unit for image reconstruction. The scan rate is set at a frequency 500 Hz, to match the time constant (1.99 ms) of the lock-in amplifier.

By demodulating fluorescence signal, the fluorescence lifetime and optical section images can be obtained with greatly reduced background, in which shot noise is attributed. Additionally, this technique improves signal-to-noise ratio and enhances penetration depth like multiphoton microscopy, without expansive femtosecond lasers.

[1] Martin C. Fischer, Jesse W. Wilson, Francisco E. Robles, and Warren S. Warren, “Invited Review Article: Pump-probe microscopy,” Rev. Sci. Instrum.87, 031101 (2016).

[2] W. Min, S. Lu, S. Chong, R. Roy, G. R. Holtom, and X. S. Xie, "Imaging chromophores with undetectable fluorescence by stimulated emission microscopy," Nature 461, 1105-1109 (2009).

[3] P.-Y. Lin, S.-S. Lee, C.-S. Chang, and F.-J. Kao, "Long working distance fluorescence lifetime imaging with stimulated emission and electronic time delay," Optics express 20, 11445-11450 (2012).