High definition stimulated emission microscopy with synchronized subharmonic modulation

SUBIR DAS,[1] YI-CHIH LIANG,[1] SHUNJI TANAKA,[2] YASUYUKI OZEKI[2], AND FU-JEN KAO[1,*]

[1]*Institute of Biophotonics, National Yang-Ming University, Taipei, 11221, Taiwan*
[2]*Department of Electrical Engineering and Information Systems, University of Tokyo, Tokyo 113-8656, Japan*
[1]**fjkao@ym.edu.tw*

In the past years, stimulated emission based pump-probe technique [1] has demonstrated the novelty of dark chromophore detection [2], fluorescence lifetime imaging [3], long working distance detection, and the detection of fluorophores under ambient light. In this presentation, we will elucidate subharmonic synchronized modulation in improving the signal-to-noise ratio for the detection of the stimulated gain and the spontaneous loss.

Critically, a high frequency divider circuit which divides the repetition frequency (76 MHz) of the probe laser (Ti:sapphire) to the half repetition frequency (38 MHz), which drives the pump laser (pulsed gain-switched diode laser) synchronously and provides the reference signal for lock-in detection. In this way, the highest possible modulation frequency can be achieved for lock-in detection with shot noise limited sensitivity. The greatly shorten time constant (< 0.1 ms) further improved imaging speed [4].

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. The time delay (τ) between the pump and probe pulses is precisely controlled, to allow the extraction of fluorescence lifetime. We performed our measurements on ATTO647N fluorescent dye.

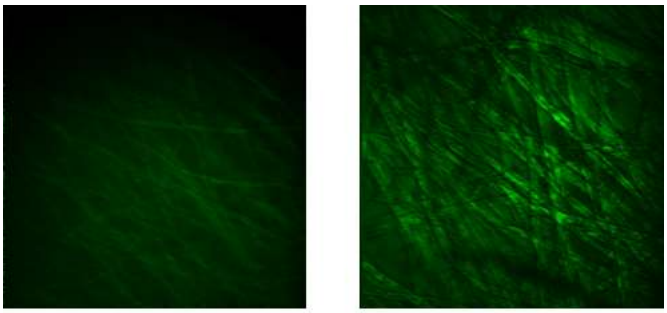

Figure 1. A comparison of imaging between regular low frequency (100KHz) modulation and subharmonic modulation (38MHz).

The modalities of detection includes stimulated gain and spontaneous loss. For stimulated gain, the pump beam is modulated at a frequency, f_1, and the probe beam is demodulated accordingly. For spontaneous loss detection, the probe beam is then modulated at frequency, f_2, and the spontaneous emission is then demodulated from the fluorescence detected in the reflection mode, allowing the removal of large background that usually exists in pump-probe detection.

[1] M. C. Fischer, *et al.*, *Rev. Sci. Instrum.*87, 031101 (2016).
[2] W. Min, *et al.*, *Nature* 461, 1105-1109 (2009).
[3] P.-Y. Lin, *et al.*, *Opt. Express* 20, 11445-11450 (2012).
[4] Y. Ozeki, *et al.*, *Opt. Express* 18, 13708-13719 (2010).