Chen-Yuan Dong, Christof Buehler, Peter T C So, and Enrico Gratton.

Frequency-domain pump-probe stimulated emission spectroscopy using a fluorescence microscope.

41st Annual Meeting of the Biophysical Society, New Orleans, Louisiana, 1997.
Biophys J. 1997; 72(2 Pt 2), Tu-Pos422.

Abstract

We developed a pump-probe stimulated emission system for spectroscopic studies inside a microscope. Our pump-probe technique involves focusing two pulsed laser beams onto a fluorescent sample. One laser, the pump, is used to excite the fluorophores. The other laser, the probe, is used to induce stimulated emission from the molecules in the excited state. The repetition frequencies of the two lasers are offset by an amount small compared to the base laser repetition frequency; this results in a signal at the difference frequency between the two lasers and corresponding harmonics. There are several advantages of this technique. First, the high frequency content of pulsed lasers can be analyzed at a very low frequency. As a result, fast optical detectors are not needed to investigate ultrafast molecular phenomena. Second, optical overlapping of two laser beams is efficient inside the microscope. With relatively high numerical aperture objectives, tight focusing and effective signal collection can be achieved. Consequently, low laser power is necessary to observe the pump-probe stimulated emission effect. Furthermore, multiple harmonics can be acquired simultaneously resulting in reduced data acquisition time and improved signal-to-noise ratio. The instrument's temporal resolution is determined by the laser pulse width and relative jitter between the two lasers. We present time-resolved spectroscopic data acquired with this instrument including lifetime and polarization. (This work was supported by NIH RRO3 155.)