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Spatial Resolution Enhancement using Non-Linear Temporal Cross-Correlation Techniques in Pump-Probe Confocal Microscopy.

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Abstract

Pump-probe microscopy uses multiple illumination wavelengths to excite and de-excite a fluorescent sample. A pump laser beam, with a wavelength in the absorbance band of the fluorophore, excites the fluorophore into the electronic excited state as in conventional fluorescence microscopy. Unlike conventional fluorescence microscopy, a second laser beam, the probe, also illuminates the sample. The wavelength of the probe beam lies in the red edge of the fluorescence emission band and nearly instantaneously brings the excited fluorophore into the ground state via stimulated emission. In this manner, the detected fluorescence emission can be modulated spatially and temporally by the probe.
Various schemes have been developed to apply pump-probe illumination to microscopy. Using modulated or pulsed lasers, pump-probe microscopy has been used to measure ultra-fast fluorescent lifetimes and molecular rotations. Linear, temporal cross-correlation of pump and probe illumination has been found to increase the spatial resolution of the microscope, even when the pump and probe beams completely overlap spatially. More recently, non-linear STimulated Emission Depletion (STED) techniques have achieved substantial resolution enhancements using partially spatially overlapped beams in the steady-state.
In this poster, we propose using non-linear STED effects to increase the resolution enhancement by temporal cross-correlation of spatially overlapped pump and probe beams. In this approach, spatial zones of different intensities within the point spread function are modulated at different harmonics due to mixing under the non-linear STED conditions. We are using Super Continuum Generation (SCG) from a single mode-locked Ti:Sapphire laser coupled with photonic crystal fiber to generate synchronized, ultra-fast pulsed illumination ... [truncated at 250 words]