

Polarized Stimulated Emission Depletion in Enhanced Green Fluorescent Protein (EGFP)

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Stimulated emission depletion (STED) population and polarisation dynamics are used together with time-resolved fluorescence anisotropy and absorption measurements to determine the full alignment of EGFP molecules following near infra red two-photon excitation. Conventional fluorescence techniques do not provide this information and as molecular alignment plays a significant role in biophysically important processes such as resonance energy transfer, the determination of 'hidden' degrees of molecular order created by multiphoton excitation is highly desirable. Time-resolved linear and circularly polarised two-photon fluorescence anisotropy measurements are used to determine the structure of the transition tensor in the neutral and anionic states of EGFP; these are combined with STED intensity and anisotropy measurements to yield the hitherto 'hidden' degrees of molecular order created by two-photon excitation. The efficiency of STED in molecular systems is governed by the interplay between the STED cross-section, molecular alignment and the rate of vibrational relaxation in the ground state. Vibrational relaxation dynamics in the ground electronic state of EGFP are investigated using shaped picosecond and femtosecond depletion pulses. EGFP is seen to possess a significant cross section (ca. 10^{-15}cm^2), an extremely high degree of initial molecular alignment and efficient vibrational relaxation (t_{vib} ca. 250fs).