Title
A new approach to the measurement of the multiphoton excitation cross-section of a fluorophore

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Abstract

Multiphoton excitation of fluorophores has recently found widespread use across the range of fluorescence measurement techniques. Measurement of the multiphoton excitation cross section as a function of wavelength is an important parameter in the characterization of existing fluorophores and development of new indicators for these applications. We describe a technique that addresses some typically encountered problems when making this measurement. The desire for efficient excitation naturally suggests the use of a short pulse laser system (e.g., 100 femtosecond pulse duration) however the. chromatic dispersion of the laser pulse in the experimental system may lead to significant uncertainties in the temporal properties of the excitation pulse. We find that the use of a picosecond Ti:Sapphire laser, with its smaller spectral bandwidth can reduce this potential source of error. A second factor to be considered is the separation of different order effects e.g., two photon excitation and three photon excitation. In a "frequency domain" approach, we slowly modulate (~100Hz) the intensity of input laser pulse train and analyze the emission signal in terms of the amplitude and phase of the harmonics of this modulation. Thus for example we expect a signal in the third harmonic if three photon excitation is present. The system is implemented in a microscope using a photomultiplier and analogue to digital converter in the detection system. The picosecond pulse train is modulated using a Pockels cell. Results on commonly used fluorophores for two and three photon excitation will be presented.