

Two-photon fluorescence excitation in fluorecein

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Abstract

We report the implementation of the two-photon fluorescence excitation technique to study the two-photon absorption cross-section in organic materials. This method was applied to a fluorecein sample, which is a calibration material for this kind of experiment.

Introduction

Two-photon process has been found to be a very powerful research tool to be used in many fields of materials characterization and devices. Two-photon excitation is created when, by focussing an intense light source, the density of photons per unit of volume and per unit time become high enough for two photons to be absorbed simultaneously into same chromophore. In this case, the absorbed energy is the sum of energy of the two photons. This process is thus a quadratic process with respect to illumination intensity. For instance, the two-photon absorption (2PA) has been used for dense three dimension optical data storage, localized photodynamic therapy, two photon sensitization and optical limiting [1,2]. Also the two-photon process can be observed in two-photon fluorescence excitation (2PFE) [3] that has been used mainly to study the electronic structure of excited state in molecules and more recently for multi-photon (two-photon) fluorescence microscopy for enhancing the sensitivity and resolution. In all these application, it is the magnitude of the two-photon absorption cross-section, σ_2' , which is of crucial importance. It is a great challenge to design highly efficient two-photon (2P) chromophore, which fulfils the above requirements. Recently, a design strategy based on the concept that symmetric charge transfer from the ends of a conjugated symmetric system to the middle, or vice versa, is correlated to enhance value of δ . This concept was tested in a series of bis (styryl) benzene derivatives with donor-conjugation-donor (D- π -D), donor-acceptor-donor (D-A-D) and acceptor-donor-acceptor (A-D-A) structural motifs with exceptionally large value of σ_2' and theoretical prediction agreements [1].

Nowadays, femtosecond laser system have become available and enabled the study of two-photon processes, once it can be easily observed. In this work we report the implementation of a technique to study a 2PFE process using femtosecond pulses. A fluorecein solution was used as a testing material, since it has well known two-photon cross section. The employment of this technique will allow further investigation of 2PFE in new organic materials with potentially high 2P cross section. Also, this 2PFE can be used with Z-scan technique as complementary technique to improve the understanding of 2P processes [4,5].

Experimental

The fluorecein/methanol solution was prepared and placed in a quartz cuvette (optical path: 1 cm). The linear absorption was measured in a Cary 17-A UV-Vis spectrophotometer. The experimental set-up for measuring the two-photon fluorescence employed a femtosecond Ti:sapphire laser ($\lambda=775$ nm, 190 fs pulse duration and 1 kHz repetition rate) as the excitation beam. The fluorescence signal spectrum was measured in a conventional fiber optic spectrophotometer (Ocean Optics PC2000). While the excitation beam passes through the fluorecein solution, the input of the spectrometer optical fiber collects the fluorescent light from cuvette side. The pump beam intensity was varied with a set of calibrated neutral filter in order to study the dependence of emitted light on the excitation irradiance.

Results and Discussions

The sample used in this work present an absorption peak around 490 nm, being completely transparent at 775 nm, the wavelength used in the two-photon experiment, as shown Fig.1. Figure 2 shows the fluorescence spectrum for the fluorecein solution when two-photon excitation was used (excitation at 775 nm), obtained with a pump irradiance of 5 GW/cm². As expected, this spectrum is identical to the one found in the literature for excitation at 388 nm.

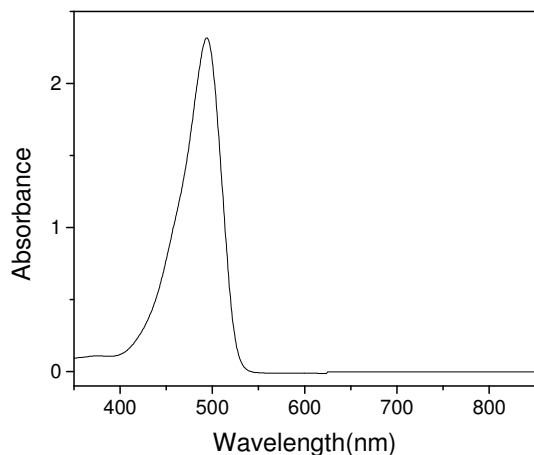


Figure 1– Absorbance spectrum of fluorescein in the methanol solution.

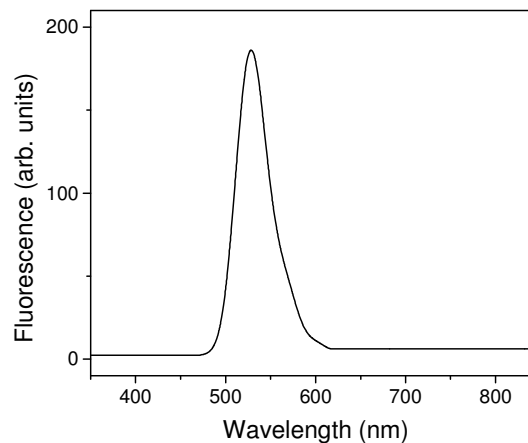


Figure 2– Two-photon fluorescence spectrum in the fluorescein methanol solution, for excitation at 775 nm.

Two-photon fluorescence excitation measurements, similar to the one showed in Fig. 2, were carried out for different pump beam irradiances, in order to study the emitted light dependence on the excitation intensity. Figure 3 shows that the fluorescence signal exhibit a quadratic dependence on the irradiance, as expected for pure 2PFE processes. The solid line in this figure represents the theoretical fitting obtained with a quadratic polynomial function. These results reveal the reliability of our experimental systems, allowing its use in future two-photon studies in organic compounds, once fluorescein is a standard material for this kind of experiments.

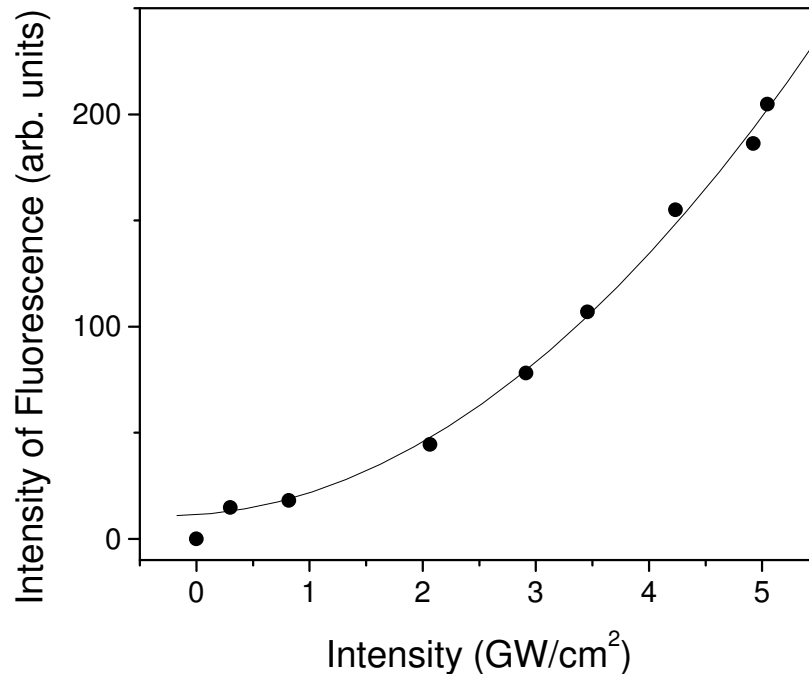


Figure 3– Two-photon fluorescence spectrum, for excitation at 775 nm, in the fluorescein methanol solution.

Conclusions

We have implemented the 2PFE experimental setup to investigate the two-photon absorption cross-section in organic materials. This technique was applied to a fluorescein methanol solution, that is a standard material for this type of experiment. The obtained results are in agreement with those presented in the literature, showing the reliability of this method for future works.

Acknowledgements

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