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Two-photon excitation of 2,5-diphenyloxazole using a low power green solid state laser

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Abstract

This Letter concerns two-photon excitation of 2,5-Diphenyloxazole (PPO) upon illumination from a pulsed 532 nm solid state laser, with an average power of 30 mW, and a repetition rate of 20 MHz. A very agreeable emission spectrum position and shape has been achieved for PPO receiving one- and two-photon excitation, which suggests that the same excited state is involved for both excitation modes. Also, a perfect quadratic dependence of laser power in the emission intensity function has been recorded. We tested the application of a small solid state green laser to two-photon induced time-resolved fluorescence, revealing the emission anisotropy of PPO to be considerably higher for two-photon than for one-photon excitation.

1. Introduction

Rapid progress in the development of ultrafast excitation sources, in particular femtosecond Ti:sapphire lasers, made it possible to observe nonlinear optical effects of different types of chemical and biological samples [1–4]. Two-photon excitation has been applied to two-photon fluorescence microscopy for imaging, as well as two-photon fluorescence correlation spectroscopy [5,6]. The advantages of the two-photon method include better temporal and spatial resolution, and the possibility of deeper penetration of samples characterized by high scattering features, even including tissue samples [7–9]. Moreover, multi-photon excitation restricts photobleaching to only the focal volume. Although for different types of measurements the two-photon mode carries different advantages, the most prominent seems to be possibility of molecule excitation in the transparent wavelength region where ultraviolet absorption and fluorescence studies can be performed with laser pulses from visible/near infra red regions.

Development and facilitation in nonlinear spectroscopy depend strongly on laser light sources. Presently most ultrafast lasers can deliver a large excess of power in one ultrashort pulse duration (commonly from the Ti:Sapphire). A more commercially cost effective choice, due to their availability and reliability, is the use of solid state lasers such as neodymium:yttrium vanade, neodymium-doped lithium yttrium fluoride, or lasers based on Cr:fosterite crystal [10].

Here we present a study on two-photon absorption and subsequent emission of PPO at a fixed excitation wavelength with a solid state 532 nm laser. This excitation provides a smaller photon flux in the pulse with a high stability, which makes this light source ideal in time-resolved fluorescence. In addition, the 20 MHz repetition rate fits much better for nanosecond decays than the repetition rate from most of Ti:sapphire lasers (about 80 MHz).

2. Experimental

2.1. Chemicals

2,5-Diphenyloxazole (PPO) was provided by Fisher Scientific, IL and used without further purification. Different concentrations of PPO solutions were prepared in ethanol (EtOH) depending on type of experiment. EtOH HPLC/Spectrophotometric grade was purchased from Sigma–Aldrich (Lot. I.D. 07F08).

2.2. Steady state measurements

The electronic absorption spectrum of PPO diluted in EtOH was recorded with a single-beam UV–Vis spectrophotometer model Cary 50 Bio® (Varian Inc. Australia). The emission spectrum of PPO following single photon excitation (at 265 nm) was recorded with a Cary Eclipse (Varian Inc., Australia) spectro-fluorometer. Spectral analysis was performed with **GRAPHER 6** software from **GOLDEN** Software. The steady state two photon fluorescence spectra were constructed directly from time-resolved measurements recorded at different wavelengths with a 5 nm step.

Two-photon time-resolved emission spectra and fluorescence intensity decays were performed using a FluoroTime200 system (PicoQuant GmbH, Germany), used in time-correlated single photon counting (TCSPC) mode. The measurement set up was equipped with an excitation source neodymium-doped 532 nm pulsed solid state laser (Time–Bandwidth, Switzerland), Hamamatsu multi channel photomultiplier (MCP) for detection, and Time-Harp300 TCSPC board (PicoQuant) for data processing. The laser operated at a fixed 20 MHz repetition rate, providing pulses of about 10 ps duration. Excitation light was expanded and focused again inside the cuvette using 5× objective (0.12 numerical aperture). The emission light was collected through the monochromator supported with a 532 nm notch filter (Shamrock). For lifetime measurements the monochromator was set to a position of 360 nm. We used a Glan–Tylor polarizer at the magic angle position for lifetime data and in appropriate positions for anisotropy measurements. The response function of the instrument was recorded using a colloidal silica scatterer (Ludox). The fluorescence intensity decay curves ($I(t)$) were analyzed using the FluoFit software (PicoQuant) based on a multi-exponential model which involved an iterative deconvolution process. A sum of exponentials, where α_i and τ_i are the pre-exponential factor and fluorescence lifetime, respectively, was used to describe the data:

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i) \quad (1)$$

For two-photon anisotropy study of PPO solution, two fluorescence intensity decay components were measured: $I_{\parallel}(t)$ and $I_{\perp}(t)$, the symbols representing fluorescence signal measured with analyzing polarizer aligned vertically and horizontally relative to the vertical polarization of the excitation light, respectively. From these decays, the anisotropy as a function of time was determined:

$$r(t) = \frac{I_{\parallel}(t) - GI_{\perp}(t)}{I_{\parallel}(t) + 2GI_{\perp}(t)} \quad (2)$$

where G is a correction factor (G -factor) compensating the instrument sensitivity difference for vertically and horizontally polarized light. The anisotropy value is usually higher in the two-photon mode. Theoretically, for one-photon and two-photon, the maximal values of anisotropy are 0.4 and 0.57, respectively. This is a consequence of the photoselection (9, 11). The anisotropy decay data were fitted to the single-exponential function:

$$r(t) = r_o e^{-\frac{t}{\theta}} \quad (3)$$

where r_o is an initial anisotropy contribution component in the fitting range channel and θ is a rotational correlation time.

3. Results and discussion

Figure 1 presents the absorption and emission spectra of PPO in ethanol. The absorption spectrum contains a weakly structured band characteristic of PPO, centered at 305 nm. The fluorescence spectra recorded for one- (continuous line) and two-photon (dots) modes are presented on the same figure. The emission spectra were recorded with 266 and 532 nm for one- and two-photon excitation, respectively. One can see that emission bands recorded for both excitation modes are similar, and that they are very close to the emission band reported previously for PPO in methanol [11,12]. Present study shows that in EtOH, a fine structure of the PPO's emission band can be resolved even at room temperature. The concentration of PPO solution was 7×10^{-3} M.

Figure 2 shows the dependence of the emission signal on average incident laser power. Because the laser produced relatively little power (<30 mW) we used additional focusing (5× objective) to focus the light beam inside the cuvette. The plot has a slope of two (1.96) indicating two-photon excitation.

The temporal behavior of two-photon induced fluorescence of the PPO in EtOH was also probed based on the time-correlated single photon counting (TCSPC) technique. The fluorescence decay profile of the studied PPO solution is shown on Figure 3 together with instrument response function (IRF). The reconvolution of the decay curve and IRF according to a single exponential model resulted in a 1.58 ns lifetime. This result is in agreement with measurements gathered from one-, two- and three-photon excitation of PPO using frequency-domain technique [11,12]. It was revealed that single-exponential dependence is identical for one-, two-, and three photon induced fluorescence lifetimes. In our experiment we also found that this phenomenon was independent of the excitation process.

Next, we examined the anisotropy decay of the PPO with two-photon excitation. Figure 4 shows a decay fit to a time-dependent anisotropy decay model (Eq. (3)). For the analysis of the anisotropy decay curve, one rotational correlation time (0.073 ns) was sufficient. The two-photon initial anisotropy (r_o) obtained from the same fit equals 0.51. This is the result of higher photoselection induced by two-photon absorption over one-photon with a maximum value of 0.4. Anisotropy decay analysis was performed additionally with two components of the correlation time and simultaneous observation of the residual changes. Second component application did not improve the quality of the fit. This is a typical result recorded

in the cases of solutions characterized with very low viscosity and a short correlation time. The data were tail fitted without instrument response function deconvolution and this is the reason for less than perfect χ^2_R value.

4. Conclusions

In this manuscript we explored the possibility of using a relatively low power, green solid state laser for two-photon measurements. It was demonstrated that with using PPO solution as an example, two-photon induced fluorescence measurements are entirely possible. The recovered intensity and anisotropy decays revealed accurate parameters and an excellent signal to noise ratio. Observation of two-photon induced fluorescence with less than 30 mW laser power can have implications for the use it in spectroscopy/microscopy fields.

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Figure 1. Normalized absorption (dashed) and emission (continuous) spectra of PPO in ethanol gathered in one-photon excitation mode. The fluorescence spectrum was received at 265 nm excitation. (dots) fluorescence spectrum taken for two-photon mode of excitation. The insert presents the chemical structure of PPO (2,5-Diphenyloxazol).

Figure 2. Logarithmic dependence of the fluorescence intensity (I) in function of laser power (P).

Figure 3. Time-domain intensity decay of PPO in ethanol received in two-photon excitation mode using green solid state laser.

Figure 4. Anisotropy decay of PPO in ethanol for two-photon excitation at 532 nm. The observation was at 360 nm.