

Single-snapshot and intensity-resolved two-photon fluorescence measurements

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We present a single-snapshot (SSS) method for obtaining intensity-resolved two-photon fluorescence (TPF). This simple method uses a digital camera to image the TPF spot on a liquid dye jet. By making a comparison between the local laser and TPF intensities, TPF probabilities are reconstructed. We compare our intensity-resolved TPF results with those obtained by the more common intensity scanning (IS) and z -scan methods. The dependence of the TPF probability on intensity obtained by the SSS method for coumarin-30 exhibits a clear maximum around $I \sim 4 \times 10^{12}$ W/cm² and a postsaturation decrease, while no such effects were found in the data obtained by the other methods. Additionally, theoretical models are presented to extract the *overall* probability from within the volume integral. To our knowledge, we present the first reported measurements of such intensity-resolved TPF. © 2009 Optical Society of America

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Two-photon processes play a prominent role in several modern applications, such as two-photon fluorescence (TPF) microscopy [1,2], visualization of nonlinear beam propagation in liquids [3,4], and characterization of ultrashort pulses [5]. Many TPF experiments have been designed to operate in the strictly quadratic regime (low intensities). However, for higher intensities, deviations from the expected $S \propto I^2$ scaling are known to occur [6]. Following common practice, one can integrate fluorescence from the entire focal region of the laser beam. However, this results in a fluorescence signal S that has been averaged over a broad range of intensities. This effect is known in the literature as *volumetric weighting* or *spatial averaging* [6–8]. Spatial averaging can mask subtle features appearing in presaturation signals, and it can completely conceal physically relevant information that may arise in postsaturation signals.

Because we plan to apply coherent control of TPF in biological systems and over a broad range of intensities, accurate knowledge of intensity-dependent TPF is desirable. The distribution of the laser intensity over the cross section of the laser beam as well as diffraction effects make it necessary to develop a procedure for extracting physical parameters from the measured signals. Furthermore, evolutionary algorithms are commonly used in coherent control experiments and may require significant processing time; therefore fast feedback is advantageous.

In this Letter, we present an approach to overcome spatial averaging and thus obtain intensity-resolved TPF. A digital camera was used to take a *single-snapshot* (SSS) image of laser induced TPF from a dye jet. The local TPF and laser intensities were then used to reconstruct TPF probabilities. To show how our results differ from those obtained by more common methods, we compared our data with that of intensity scanning (IS) [6] and intensity-selective scanning (ISS) [9,10]. Additionally, theoretical models were developed to extract intensity-dependent fluorescence probabilities and to describe the TPF signal obtained from z -scan experiments.

A commonly employed technique used to measure TPF involves placing a glass cuvette containing a dye solution directly into the path of a laser beam [11]. This method works well at lower intensities required for the observation of TPF in the quadratic regime [6]. However, for higher intensities and shorter pulse durations, nonlinear effects such as self-focusing, self-phase modulation, and white-light generation can compromise the results of the experiment [3]. To avoid the influence of glass in our experiments, we used a liquid dye jet from a dye laser.

A schematic of our experimental setup is shown in Fig. 1. Femtosecond laser pulses from a multipass amplifier (35 fs duration, 5 kHz repetition rate, 0.8 mJ per pulse) were sent through a liquid dye jet delivering a 1 mM solution of coumarin-30 dissolved in ethylene glycol. Typical flow speed was ~ 10 m/s, and the dimensions of the liquid jet were $500 \mu\text{m}$ along the propagation direction of the beam and ~ 4 mm transverse to the beam. The displacement z between the focus and the jet was manually varied using a translational stage with a resolution of $10 \mu\text{m}$. The input laser power was adjusted using a variable neutral density filter. All recorded TPF sig-

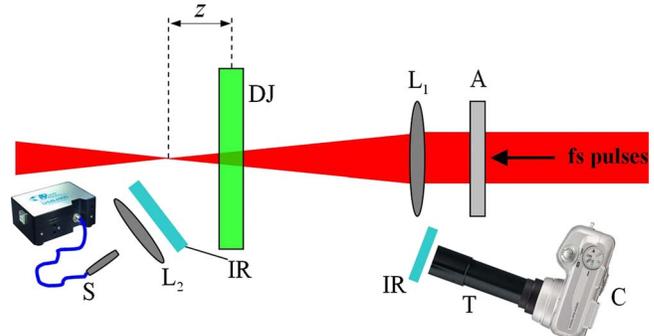


Fig. 1. (Color online) Experimental setup as viewed from above. A, variable attenuator; L_1 , 32 cm focusing lens; DJ, dye jet; IR, BG-39 IR filter; L_2 , collection lens; S, spectrometer; T, telescope; C, camera; z , relative distance between the focus and jet.

nals were optically filtered using BG-39 IR filters. The filtered signals were then recorded using either a spectrometer (Ocean Optics, SD2000) or a digital camera (Nikon Coolpix 4300).

To verify that this setup yields results consistent with earlier work using fluorescence dyes [6], the TPF signal was measured as a function of peak laser intensity I_0 by holding z constant and varying the input power (IS method). The measured TPF data are shown in Fig. 2 and are plotted in a log-log representation. For lower intensities, the slope of the curve is consistent with that of a two-photon process as expected from lowest-order perturbation theory [12]. A deviation in the signal from $S \propto I^2$ to $S \sim I^{1/2}$ can be seen around an intensity of $I_S \sim 4 \times 10^{12}$ W/cm². This deviation indicates the onset of saturation. For intensities exceeding I_S , it can be shown that the measured signal (for our geometry) has an intensity dependence of $S(I_0) \propto \ln|I_0/I_S|$ [10]. This increase in yield is known to be a geometric artifact of the experimental configuration, and any decrease that may have been present in the TPF probability will be hidden by spatial averaging [13].

A known method used to overcome spatial averaging is ISS [10]. ISS has been successfully used in time-of-flight experiments to obtain ionization probabilities [10,14]. This method is adopted here to obtain intensity resolved TPF. The measuring technique used in ISS is similar to the common z -scan method used in TPF experiments [9]. Typically a thin sample is translated along the beam direction, and as a result the sample is subjected to different local on-axis peak intensities $I_{0L}(z)$. To extract overall TPF probabilities, raw z -scan data must be deconvolved [10]. Because the thickness of our dye jet (500 μ m) is smaller than twice the Rayleigh range (1.3 mm), integration over the z direction can be neglected. The reduced dimensionality of our experimental configuration allows us to deconvolve raw z -scan data $S(z)$. The TPF probability $P(I)$ can then be obtained from the volume integral [10]

$$S(I_0) \propto \int_0^{I_0} P(I) \frac{\partial V}{\partial I} dI. \quad (1)$$

An exact theoretical description for the interaction of intense ultrashort pulses with dye solutions is complex and beyond the scope of this Letter. For this reason, only several contributing factors governing the overall probability $P(I)$ are mentioned: laser-dye interactions (excitation, fluorescence, and bleaching) [8], laser-solvent interactions (thermal effects) [6,9], and experimental configurations (detection scheme) [8]. The volumetric weighting factor in Eq. (1) is inversely proportional to the product of local on-axis intensity and intensity $\partial V/\partial I \propto 1/I_{L0}(z)I$. Multiplying Eq. (1) by $I_{0L}(z)$, differentiating with respect to z and applying Leibniz's integral rule, the probability $P(I)$ can be extracted from within the integral

$$P(I_{0L}) \propto \left(\frac{I_{0L}(z)}{dI_{0L}(z)/dz} \right) \frac{d}{dz} [I_{0L}(z)S(z)]. \quad (2)$$

Our measured z -scan data $S(z)$ and its deconvolution [Eq. (2)] are shown by the circles in Figs. 3(a) and 3(b). The solid curve in Fig. 3(a) is a theoretically determined signal $S(z) \propto w^2 \ln|1 + (I_0 w_0^2 / I_S w^2)^2|$ (here $w = w_0 \sqrt{1 + z^2/z_0^2}$ is the beam size, w_0 is the beam waist, and I_S is the saturation intensity) calculated by integrating a model probability $P(I) \propto I^2 / (1 + I^2/I_S^2)$ in Eq. (1). The deconvolution of the theoretically determined TPF signal $S(z)$ is shown by the solid curve in Fig. 3(b). This model probability proved useful in determining the experimental configuration and is in good agreement with the measured data. The experimentally found ISS probability curve [Fig. 3(b)] was found to have a dependence of $S \propto I^2$ for lower intensities. For intensities exceeding $I_S \sim 4 \times 10^{12}$ W/cm², the ISS data exhibit an intensity dependence having a smaller slope than the postsaturation yield found in the IS data.

To obtain TPF probabilities using the SSS method, local TPF and laser intensities at the position of the jet were compared. A digital camera was used to image the TPF on the jet, and knife-edge measurements were used to determine the beam size at the position of the jet. To determine the probabilities, all one must do is integrate the fluorescence signal around a suffi-

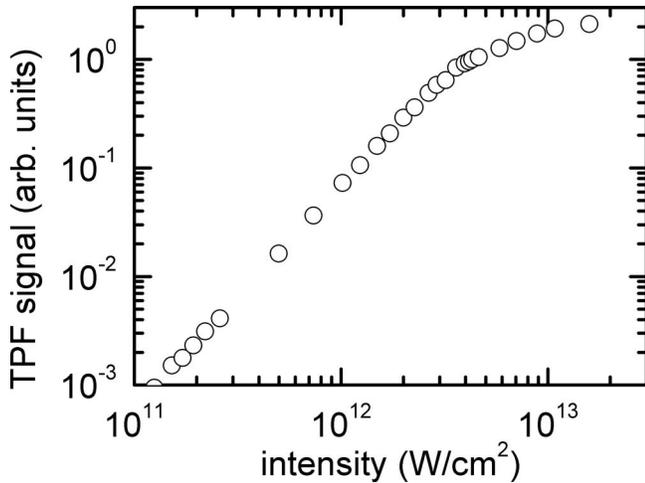


Fig. 2. Spatially averaged TPF signal obtained using the IS method.

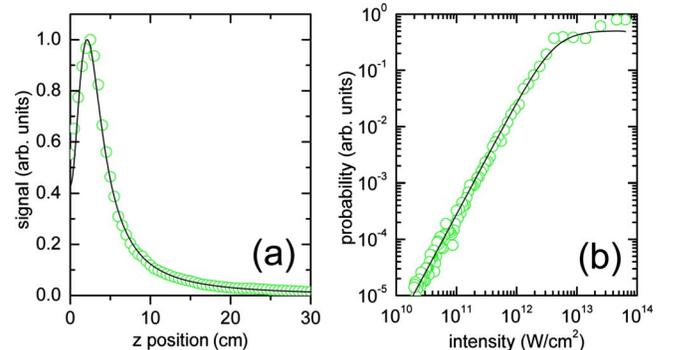


Fig. 3. (Color online) (a) TPF measured during z scan. (b) Reconstructed probability obtained by deconvolving the data in (a).

ciently thin ring of radius ξ , divide by the area of that ring, and plot the result against the corresponding local average laser intensity. In this experiment it was verified that the beam could be accurately described by a Gaussian function. In the case of a nonidealized beam, isointensity contours of the beam profile can be used. This makes the SSS method independent of the beam intensity profile unlike that of ISS, which requires a Gaussian beam.

To show how $P(I) \propto S(I)$ for the SSS method, consider a thin cylindrical shell of dye molecules in a laser beam and having a density defined by $\rho(r) = \sigma \delta[f(I)]$. Here $f(I) = r(I) - \xi$, σ is the surface density, and ξ is the radius of the shell. The intensity-resolved TPF probabilities can then be found from the volume integral [Eq. (1)]

$$S(I_R) = 2\pi\sigma\Delta z \int_0^{I_0} \delta(r(I) - \xi) P(I) r \frac{dr}{dI} dI. \quad (3)$$

Differentiation of the radial coordinate is for a Gaussian beam, and the Dirac delta function can be transformed using $\delta(f(I)) = \delta(I - I_R) / |df(I)/dI|_{I_R}$ [where $I_R(\xi)$ is the root of $f(I)$]. After some calculations, the probability is found to be proportional to the TPF signal $P(I_R) = S(I_R)/N$ (here N is the number of dye molecules). Measured results using the SSS [ISS] method depend on how well the local radial intensity $I(\xi)$ [on-axis peak intensity $I_{0L}(z)$] can be experimentally determined or prepared.

In our SSS experiment, the displacement z was held fixed at $z \approx 1$ cm, and the beam was observed to focus behind the jet indicating that the surface of the liquid jet was of optical quality. Figure 4 shows the recorded TPF spot on the dye jet (inset) as taken with the digital camera. A hole can be seen at the center of this TPF profile; analysis of the digital image files assured that the camera's CCD was not saturated. By applying the reconstruction algorithm to raw SSS data, the observed hole in the TPF signal gave rise to a clear decrease in the TPF probabilities following saturation. This decrease in probability with increasing intensity is consistent with the presence of higher-order nonlinear processes that limits TPF.

In conclusion, we present a simple single-snapshot method for obtaining intensity-resolved TPF. This method is general and can be used to investigate other phenomena affecting TPF, in particular for higher intensities. In the case of the ISS method, the reconstruction algorithm was found to be sensitive to raw z -scan data [Fig. 3(a)] taken near the focus (post-saturation regime), and for this reason we found ISS to be less reliable than the SSS method. Finally, we produce what we believe to be the first reported intensity-resolved TPF probabilities.

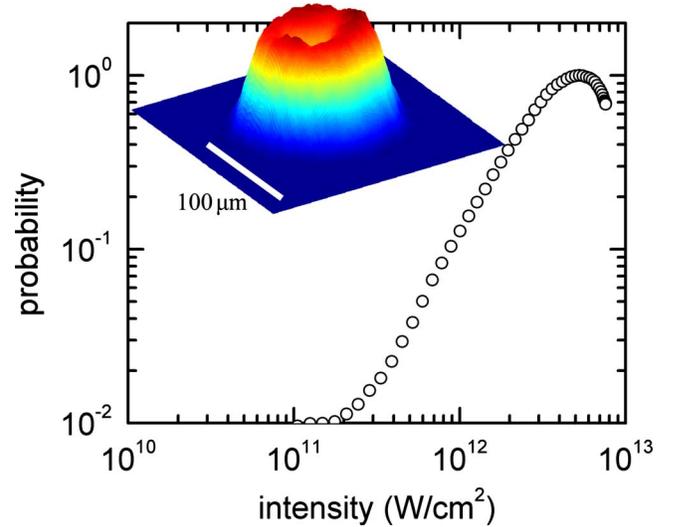


Fig. 4. (Color online) Reconstructed intensity-resolved TPF probability using the SSS method. Inset, digital image of the TPF spot on the dye jet.

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