

Instantaneous Fluorescence Quantum Yield—A New Quantity with Characteristic “Fingerprints” for Excited-State Processes

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The instantaneous fluorescence quantum yield ϕ_{ins} —a new quantity for fluorescence studies defined as the ratio of the fluorescence intensity to the optical density, both measured at the moment of the maximum of the exciting pulse—proves to be a very sensitive function for excited-state processes. Dependent on the excitation intensity ϕ_{ins} exhibits characteristic features (maxima/minima) indicating, for example, excited-state absorptions and annihilation processes. ϕ_{ins} is therefore more informative as the intensity dependence of the usually utilized fluorescence yield, the information content of which is restricted because this function is hardly structured. In the paper the influences of specific molecular parameters (excited-state absorption cross section, annihilation constant) on ϕ_{ins} are given, problems of the experimental accessibility of ϕ_{ins} are discussed, and an experimental setup for determination of this new quantity is presented. The application of the method is demonstrated for identification of excited-state absorptions of organic molecules in solution.

KEY WORDS: Fluorescence quantum yield; fluorescence yield; excited-state processes; exciton annihilation.

INTRODUCTION

Generally the intensity dependence of pulse-excited time-integrated fluorescence yield is utilized for the characterization of deactivation channels and bimolecular interactions (e.g., singlet–singlet annihilation processes) in systems of fluorescent organic molecules. The content of information, however, is restricted because this function is hardly structured. Especially in the case of excited-state absorption the differentiation of the distinct processes is problematic [1]. To overcome these difficulties a few years ago the so-called instantaneous fluorescence

quantum yield ϕ_{ins} was introduced on the basis of analytical investigations [2]. It has been originally defined for a three-level energy system at the end of an rectangular exciting pulse of the intensity I [cf. Equ. (1)].

$$\phi_{\text{ins}} \propto \frac{n_2}{I[\sigma_{12}(n_1 - n_2) + \sigma_{23}(n_2 - n_3)]} \quad (1)$$

where n_i are the normalized population densities; n_2 , the population density of the fluorescing level; and σ_{12} and σ_{23} , the cross sections of ground- and excited-state absorption.

As shown theoretically [2] ϕ_{ins} in dependence on excitation intensity proves to be a very sensitive function for excited-state processes, with characteristic features (maxima/minima) indicating excited-state absorptions and annihilation processes.

In the following, problems of the experimental accessibility are discussed and an experimental setup for

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determination of ϕ_{ins} is presented. The application of the method is demonstrated for identification of excited-state absorptions of organic molecules in solution.

MATERIALS AND METHODS

Chemicals. The compound tetra-(4-t-butyl)-phthalocyanatomagnesium ($t_4\text{-PcMg}$) was synthesized by a modification of a literature procedure [3]. Ethanol was UVA-SOL grade.

Fluorescence and Absorption. Absorption spectra were recorded on a Lamda-19 spectrometer (Perkin Elmer). Fluorescence spectra were measured with a Fluorolog FL-112 (Instruments S.A.), and emission cross sections calculated according to Peterson *et al.* [4].

EXPERIMENTAL ACCESSIBILITY OF ϕ_{ins}

By means of numerical calculations it was shown that the information content of ϕ_{ins} is conserved for more complicated energy level models (number of levels >3) and even if ϕ_{ins} is related to the maximum of a Gaussian pulse [5]. This improves considerably the experimental accessibility of ϕ_{ins} . Figure 1 shows schematically the experimental situation: A strong, intensity-variable laser pulse $I_1^0 = I_{\text{max}} i_0(t)$ with $i_0(t_0) = 1$ (t_0 is the time of the

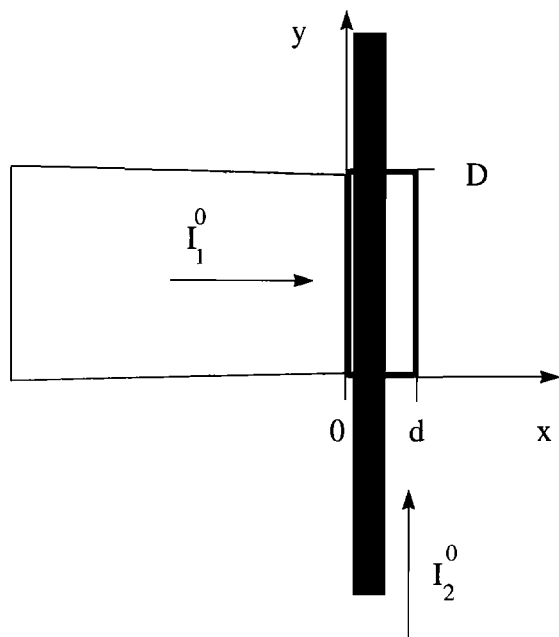


Fig. 1. Geometrical arrangement for the experimental accessibility of ϕ_{ins} (top view).

pulse maximum) is focused on the sample by means of a cylindrical lens. Simultaneously a weak pulse I_2^0 probes the transmission perpendicular to the strong laser pulse in the excitation region near $x = 0$ at the edge of the sample cell. Both excitation and probe pulses have to be exactly synchronized in time.

The instantaneous fluorescence quantum yield ϕ_{ins} for $x = 0$ is defined as follows:

$$\phi_{\text{ins}}(I_{\text{max}}) = \frac{n_i(t_0; I_{\text{max}})}{|(\partial/\partial x) I_1(t_0; I_{\text{max}})|} \quad (2)$$

where $n_i(t_0; I_{\text{max}})$ is the population density of the fluorescing energy level at $x = 0$, which is independent along the optical pathway of the probe beam I_2 ($0 < y \leq D$).

The excitation pulse I_1^0 generates a population density $n_i(t_0; I_{\text{max}})$ in the sample. The nominator of Eq. (2) represents the instantaneous fluorescence intensity $I_{\text{fl}}^{\text{ins}}(t_0; I_{\text{max}})$ at the time of the pulse maximum of the excitation and for the denominator one yields

$$\frac{\partial}{\partial x} I_1(t_0; I_{\text{max}}) = -I_{\text{max}} \cdot g(t_0; I_{\text{max}}) \quad (3)$$

with

$$g(t_0; I_{\text{max}}) = \sigma_{12} n_1(t_0; I_{\text{max}}) - \sigma_{21} n_2(t_0; I_{\text{max}}) \pm \quad (4)$$

This quantity is probed by means of the weak probe beam I_2^0 in the y -direction (transmission T_2):

$$T_2(t_0; I_{\text{max}}) = \exp(-D \cdot g(t_0; I_{\text{max}})) \quad (5)$$

that is,

$$|g(t_0; I_{\text{max}})| \propto |\ln T_2(t_0; I_{\text{max}})| \quad (6)$$

Thus for the experiment ϕ_{ins} yields from the ratio of the instantaneous fluorescence yield ϕ_{ins} and the optical density, both measured at the moment of the maximum of the exciting pulse:

$$\phi_{\text{ins}} \propto \frac{I_{\text{fl}}^{\text{ins}}(t_0; I_{\text{max}})}{I_{\text{max}} \cdot |\ln T_2(t_0; I_{\text{max}})|} = \frac{\phi_{\text{ins}}(t_0; I_{\text{max}})}{|\ln T_2(t_0; I_{\text{max}})|} \quad (7)$$

INFLUENCE OF EXCITED-STATE PARAMETERS ON ϕ_{ins}

To show how excited-state processes, especially excited-state absorption and singlet-singlet annihilation, influence the intensity dependence of ϕ_{ins} , numerical simulations for a standard three-level energy scheme (cf. Fig.

2) were carried out utilizing a 100-ps excitation pulse. For that, the rate equation system in Fig. 2 was solved for definite parameter sets and theoretical curves for the intensity dependence of ϕ_{ins} were calculated.

The upper part of Fig. 3 demonstrates the influence of the excited-state absorption (absorption cross section σ_{23}) by different values for the ratio of excited-state to ground-state absorption ($q = \sigma_{23}/\sigma_{12}$). As can be seen ϕ_{ins} is a monotonously decreasing curve for strong σ_{23} ($q > 0.2$), but under the condition $q < 0.2$ the intensity dependence of ϕ_{ins} offers a distinct maximum which is the higher the lower the value of σ_{23} is. This maximum is a direct “fingerprint” for the strength of the absorption in the excited state. In the lower part of Fig. 3 the singlet–singlet-annihilation constant γ was changed. With increasing annihilation the beginning of nonlinearity is shifted to lower excitation intensities, and besides the normal monotonous decrease in ϕ_{ins} in dependence on excitation intensity, annihilation is indicated by a minimum for $\gamma > 10^{11} \text{ s}^{-1}$.

EXPERIMENTAL SETUP

The apparatus for the experimental studies is depicted in Fig. 4. As the first step nanosecond fluorescence techniques should be applied. Therefore excitation was performed with a frequency-doubled Nd:YAG laser (Type Brilliant; Quantel) as the main light source, which pumped a dye laser DL (Type UDL 201; LTB Berlin) amplifier system. This setup provided single pulses of 60–160 μJ with a pulse duration (FWHM) of about 3 ns

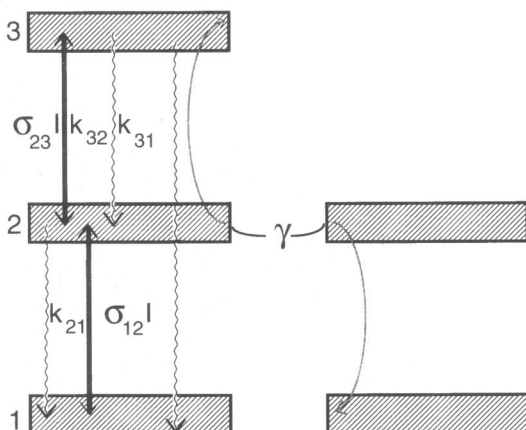


Fig. 2. Standard three-level energy scheme for the numerical simulation presented in Fig. 3. Cross sections of ground- and excited-state absorption: $\sigma_{12} = \sigma_{23} = 10^{-16} \text{ cm}^2$. Relaxations: $k_{21} = 10^9 \text{ s}^{-1}$, $k_{32} = 10^{11} \text{ s}^{-1}$, $k_{31} = 0 \text{ s}^{-1}$. Annihilation constant: $\gamma = 10^9 \text{ s}^{-1}$.

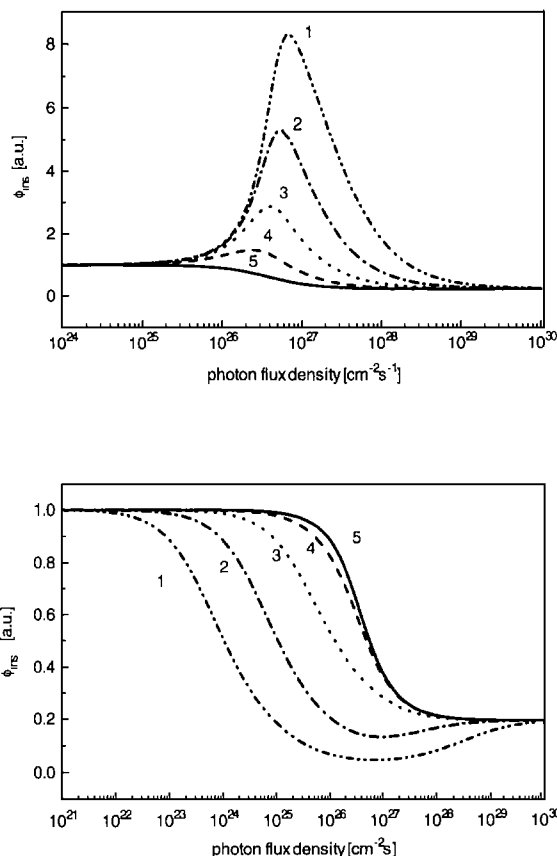


Fig. 3. Numerical simulation of the influence of excited state parameter on ϕ_{ins} by means of the energy-level model in Fig. 2 (excitation pulse duration: 100 ps). Above: Variation of the excited-state absorption σ_{23} ($q = \sigma_{23}/\sigma_{12}$): (1) $q = 0.01$, (2) $q = 0.032$, (3) $q = 0.10$, (4) $q = 0.32$, and (5) $q = 1$. Below: Variation of the singlet annihilation γ : (1) $\gamma = 10^{13} \text{ s}^{-1}$, (2) $\gamma = 10^{12} \text{ s}^{-1}$, (3) $\gamma = 10^{11} \text{ s}^{-1}$, (4) $\gamma = 10^{10} \text{ s}^{-1}$, and (5) $\gamma = 10^9 \text{ s}^{-1}$.

tunable in the range of 620 to 690 nm (with DCM in dimethyl sulfoxid).

The main part of the laser beam (I_1^0 ; cf. Fig. 1) passed through an attenuator ATT that could be tuned by a step motor SM over six orders of magnitude without any displacement or angle distortion of the laser beam. This strong excitation beam, whose intensity was monitored by the photodiode PD3, was focused on sample S by a cylindrical lens L3. Its energy in the sample region amounted to 40 μJ at 676 nm; the focus line was 100 $\mu\text{m} \times 3 \text{ mm}$.

In front of the attenuator ATT a weak probe beam was split from the main beam by a beamsplitter (BS3) and fed through the excitation region in the sample with a cross section of about 60 μm . The transmission T_2 of this beam was measured by means of the photodiodes PD1 and PD2. Probe and excitation beams were exactly

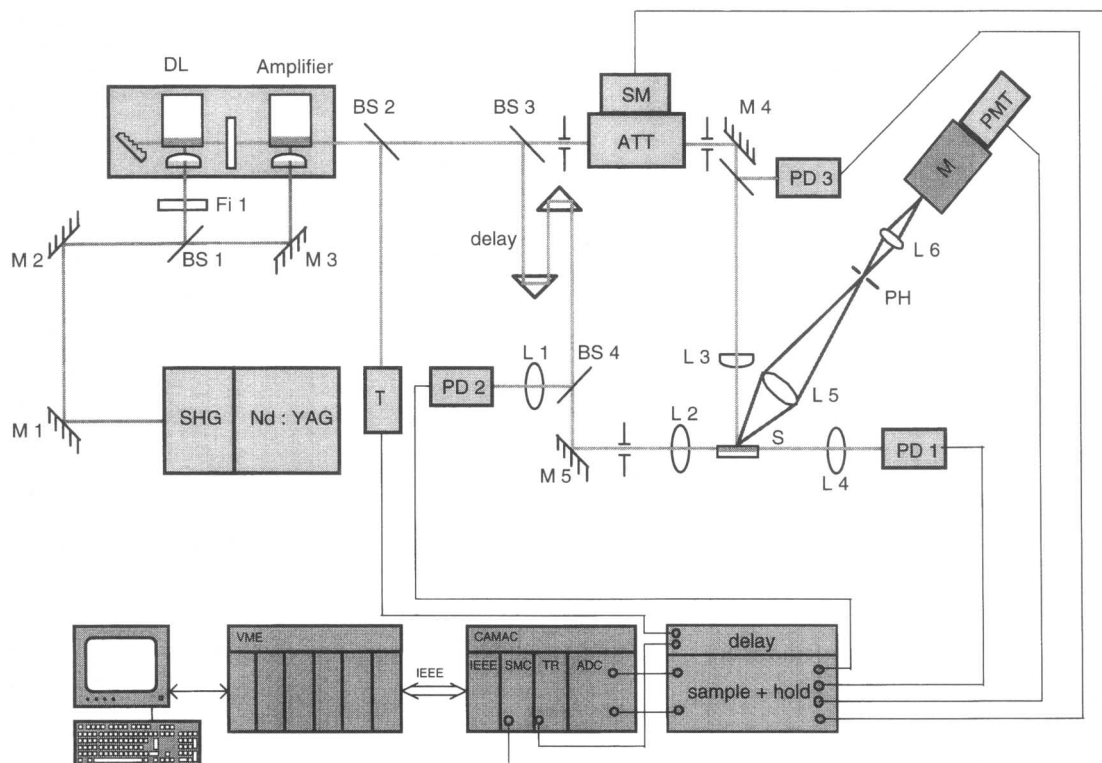


Fig. 4. Experimental setup (for explanation see text).

synchronized with an optical delay line. Homogeneous illumination of the sample required an appropriate sample configuration, with the optical pathway (i) in the x -direction, that is, the direction of the excitation beam, $d = 200 \mu\text{m}$ with a sample transmission of $>80\%$ in this direction, and (ii) in the y -direction, that is the direction of the probe beam, $D = 3 \text{ mm}$ (transmission for low excitation intensities $>10\%$).

For the fluorescence detection a method according to Schödel *et al.* [6] was used: with a lens L3 (150-mm focal length) the emitting area of the sample was displayed through a pinhole PH. The magnification factor was about 3. A pinhole of $200 \mu\text{m}$ permitted the selection of the fluorescence only from the region of the highest intensity and almost-constant photon density. The emission was then spectrally selected by a monochromator and detected by a fast microchannel photomultiplier PMT.

The signals from the photodiodes (PD1 and PD2 for determination of transmission T_2) and the photomultiplier all measured at time t_0 of the maximum of the excitation pulse were transferred to a boxcar integrator which was operating in the gate mode (gate width: 150 ps) and was connected to a computer. For dataprocessing procedures were used, which are contained in the CALE software package [7].

EXPERIMENTAL IDENTIFICATION OF EXCITED-STATE ABSORPTIONS

For testing the apparatus mentioned above the compound t_4 -PcMg dissolved in ethanol was used. With respect to the suitability of this compound as a potential sensitizer of a new start mechanism for photodynamic tumor therapy (PDT) from a stepwise excited higher singlet state, the spectroscopic properties of t_4 -PcMg were investigated some years ago [8]. T_4 -PcMg in ethanol exhibits a high fluorescence quantum yield ($\phi_F > 0.9$) with a fluorescence lifetime of 5.7 ns and a very low intersystem crossing yield. Further, we found that an excited state can be effectively populated by two stepwise absorption transitions at about 675 nm. Therefore t_4 -PcMg should be a good candidate to demonstrate the specific "fingerprint" of ϕ_{ins} with respect to excited-state absorptions.

For determination of ϕ_{ins} the compound t_4 -PcMg was excited at 676 nm (maximum of the longwavelength absorption band). The emission was detected at 730 nm. Figure 5 shows the result: as can be seen ϕ_{ins} clearly exhibits a maximum whose peak indicates an excited-state absorption. Numerical curve fitting utilizing the four-level energy scheme in Fig. 6 (known parameters

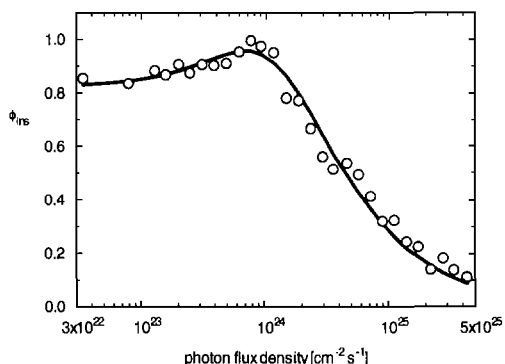


Fig. 5. ϕ_{ins} as a “fingerprint” for the excited-state absorption in t_4 -PcMg in ethanol. Excitation wavelength, 676 nm; emission wavelength, 730 nm. (○) Data points from the experiment [ϕ_{ins} was calculated from φ_{ins} and $\ln T_2$ according to Eq. (7)] and (—) after numerical curve fitting [optimal fit for σ_{exc} (676 nm) = $2.7 \times 10^{-16} \text{ cm}^2$].

are given in the legend) yields an optimal fit of experimental and calculated data for the variable excited-state absorption cross section $\sigma_{\text{exc}} = 2.7 \times 10^{-16} \text{ cm}^2$. This value is in good agreement with data recently obtained by nonlinear absorption studies [9]. Because of the strong spectral overlap of absorption and fluorescence, addition-

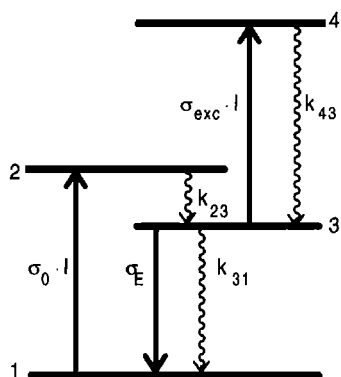


Fig. 6. Energy-level scheme for data evaluation of Fig. 5: σ_0 (676 nm) = $8 \times 10^{-16} \text{ cm}^2$, σ_{exc} (676 nm) = variable; σ_E (676 nm) = $6 \times 10^{-16} \text{ cm}^2$, $k_{23} = 1 \times 10^{12} \text{ s}^{-1}$, $k_{31} = 1.8 \times 10^9 \text{ s}^{-1}$, and $k_{43} = 1 \times 10^{12} \text{ s}^{-1}$.

ally the stimulated emission (cross section σ_E in Fig. 6) had to be considered for the numerical curve fitting. This process acts as direct counterpart to the excited-state absorption.

CONCLUSIONS

The instantaneous fluorescence quantum yield ϕ_{ins} is a new quantity with characteristic “fingerprints” for excited-state processes. Besides the identification of excited-state absorptions in molecular systems, the method should be especially useful for the characterization of (i) exciton annihilation in aggregate systems and (ii) energy transfer processes. Furthermore, shorter excitation pulses, in the picosecond range, and corresponding fluorescence detection (e.g., streak systems) should be applied. This is currently in progress.

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