

On the role of electromagnetic boundary conditions in single molecule fluorescence lifetime studies of dyes embedded in thin films

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Abstract

Single molecule fluorescence lifetime studies are generally performed in thin polymer films, where the influence of the interface on the behaviour of fluorescing molecules is not negligible. In order to describe this influence, we investigate annealed films of different thickness. We show that the distribution of fluorescence lifetimes of the embedded dyes is shifted to lower values as the thickness of the film increases. We explain this shift by simple electromagnetic arguments related to the boundary conditions at the interfaces of the polymer film with air and glass, respectively. The conclusion is that extreme care must be taken in order to interpret single molecule data with respect to the true chemical nature of the phenomena. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

Thin supported polymer films have received much attention because of their use in the microelectronics industry [1], in membrane technology [2], in coatings [3] and in nanotechnology [4]. In polymer technology, the processes of wetting, adsorption and adhesion have been studied in detail [5].

In order to better understand the physics of glassy polymers, we investigate thin films of atactic polystyrene by optical means, looking at the photodynamics of embedded dyes that act as ‘nanoreporters’ of their local environment. Most detailed information is obtained by detecting at the single molecule level, which rules out the averaging effect of bulk experiments, and hence allows one to determine full distributions of particular properties of dyes embedded in solutions [6], gels [7], polymer matrix [8,9], etc., at room temperature. In this study, we focus specifically on the fluorescence lifetime, which proved to be a very sensitive parameter to provide new insights into the photo-

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physics and photochemistry of dyes. Furthermore, the fluorescence lifetime is affected by the presence of the polymeric nanoenvironment, as shown by both near-field [10] and far-field optical microscopy [11,12], using time-correlated single-photon counting (TCSPC). However, in single molecule studies of individual dyes embedded in a thin polymer film, the fluorescence lifetime is not only determined by local photochemistry, but also by the geometry of the surrounding dielectric media [11]. Especially, molecular orientation and position with respect to thin film boundaries strongly influence the fluorescence decay. This so-called electromagnetic boundary conditions (EBC) effect can dominate the underlying photochemistry. Therefore, in this Letter, we present a thorough investigation of the role of these EBC effects on the behaviour of individual dyes embedded in thin amorphous polymer films.

2. Experimental

Thin films of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine (DiD) fluorescence dye (Molecular Probes) in polystyrene (Polymer Standard Service) with a number average molar mass of 89 300 g/mol and a polydispersity index of 1.06 were prepared (Fig. 1).

The dye was chosen as it possesses a high fluorescence quantum yield (close to one), an absorption cross-section of $7.456 \text{ \AA}^2/\text{molecule}$, and is highly photostable when embedded in a polymer matrix [11,13]. Films of different thicknesses (10, 30, and 70 nm) were prepared by spin coating of polymer-dye solutions. First different amounts of polystyrene (0.25, 0.50, and 1.00 wt%, respectively) were dissolved in toluene (Aldrich, 99.8%) [14]. DiD probe molecules were subsequently dissolved in the polymer solution at a low concentration (4, 2, and 1 nM respectively), prior to spin coating at typically 3000 rpm for 45 s.

Finally, the thin film samples obtained were annealed in vacuum following a two-stage procedure (first 4 h at 60 °C, subsequently 2 h at 105 °C), to remove remaining solvent and to relax the stresses induced by the deposition procedure. The annealed films were investigated by AFM, to ac-

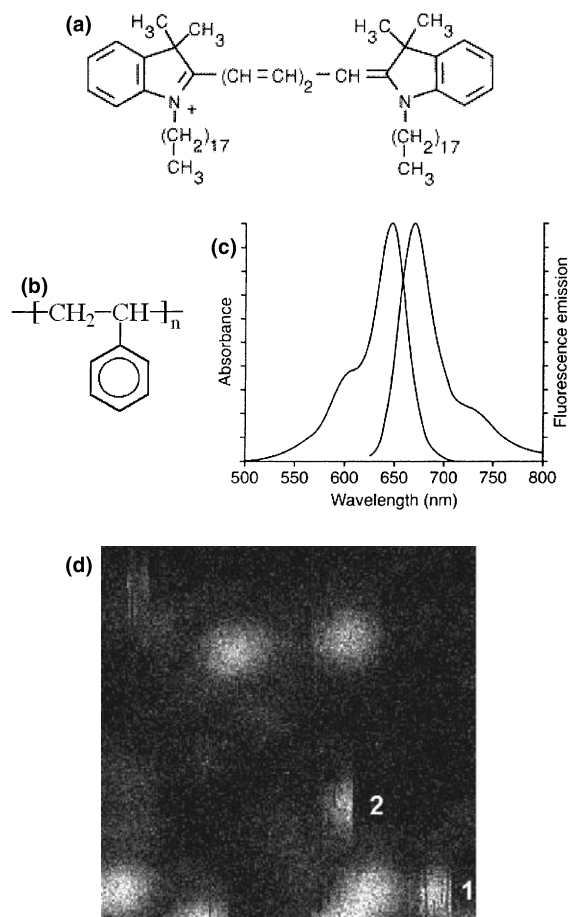


Fig. 1. Schematic views of (a) the 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine (DiD) dye, (b) the polystyrene repeating unit, (c) absorption and emission spectra of the dye, (d) $5 \times 5 \mu\text{m}^2$ area of DiD dyes in a 10 nm thick sample of polystyrene, obtained with a step size of 40 nm/pixel at a pixel rate of 2 kHz. Molecule 1 and 2 exemplify discrete processes of blinking and photobleaching, respectively.

curately determine the film roughness and thickness, using the so-called 'scratch method' [15,16].

The DiD molecules were excited by 130 fs pulses at a wavelength of 647 nm, generated by a frequency doubled optical parametric amplifier (Spectra Physics, OPAL 1100–1300 nm, 500 mW) pumped by a Ti:Sa laser (Spectra Physics, Tsunami model 3960, 775 nm, 2.5 W). Bandpass excitation (640DF20) and IR blocking filters were inserted at the entrance of a confocal scanning fluorescence microscope (CFM, Zeiss inverted

microscope), before focusing the circularly polarized light on the sample. A light beam of $3 \mu\text{W}$ was focused on the sample using an oil immersion objective lens (Olympus NA1.4, $100\times$), resulting in an excitation power of $3 \text{ kW}/\text{cm}^2$, well below the saturation intensity. The fluorescence light emitted by the dye was collected through the same objective, separated from the excitation light by use of a dichroic mirror (650DRLP) and a long pass filter (RG695), and directed to the small detection area ($180 \mu\text{m}$ diameter) of an avalanche photodetector (SPCM-AQ-14, EG&G Electro Optics). This APD determines the arrival time of a photon with an accuracy of 300 ps. Finally, an SPC 500 time-correlated single-photon counting card (Becker & Hickl) was used to measure the time lag between excitation pulse and fluorescence photon with a timing resolution of 13 ps, the accuracy being actually limited by the APD.

The whole setup was computer controlled by custom made LabView software.

To localize the molecules, fluorescence lifetime images were acquired by scanning the sample over areas of $10 \times 10 \mu\text{m}^2$, at a pixel frequency of 2 kHz with a step size of 40 nm/pixel. Fluctuations in fluorescence intensity and lifetime were then recorded on specific molecules that were centred, in consecutive experiments, at the laser spot for on-line TCSPC. The experimental determination of fluorescence lifetime was performed by using a fitting procedure of the exponential decay obtained by sampling photon arrival times during 100 ms. During this period, the detector counted more than 2000 events. The procedure was repeated 250 times to get a full transient.

In general, TCSPC data satisfied the assumptions of least-squares (LS) analysis [17], and since it was shown to be the most general and reliable method for analysis of time-resolved data [18], we used its implementation. In order to take into account the background fluorescence, an offset was introduced in the decay analysis. It should be noted that, although the use of an LS analysis is quite reliable for 2000 events [19], for fewer events (below 1000) the maximum likelihood estimator (MLE) must be used [20].

3. Results and discussion

Samples of each film thickness were scanned by CFM to localize the molecules. Fig. 1 shows a typical scan over an area of $5 \times 5 \mu\text{m}^2$. Close inspection of this figure reveals the photodynamics involved in such a single molecule experiment with discrete on–off blinking (molecule 1) and photobleaching (molecule 2). Once localized, we positioned the molecule in the focus of the laser spot in order to record the fluorescence lifetime in time, leading to a transient for each individual molecule. Fig. 2 shows transients for two selected molecules. Fig. 2a shows a continuous transient presenting a stable fluorescence intensity as well as fluorescence lifetime τ . One-step photobleaching is observed

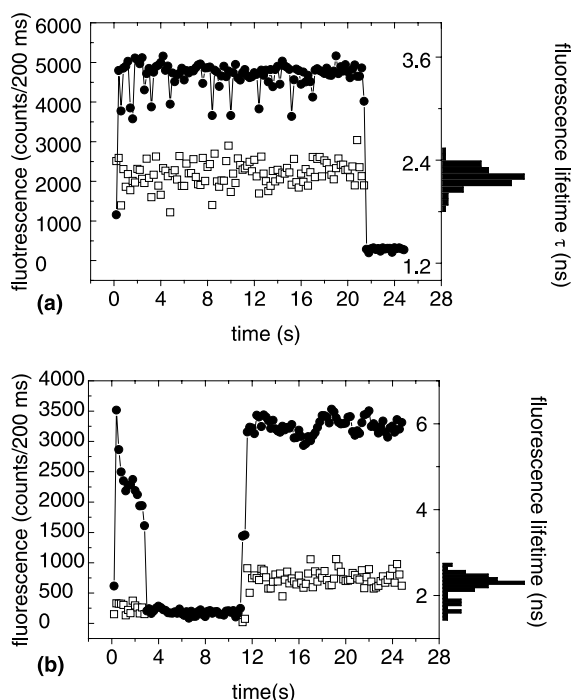


Fig. 2. Fluorescence transients of two particular DiD molecules embedded in a 30 nm thick polystyrene film at an excitation power of $3 \text{ kW}/\text{cm}^2$. Data were binned in 100 ms time intervals. Molecule (a) shows a highly stable fluorescence lifetime and intensity prior to photodissociation after 21 s. Molecule (b) exhibits variations in the fluorescence intensity and stops to fluoresce for 8 s during the acquisition procedure. On the right side of this graph are plotted the distributions of fluorescence lifetimes corresponding to the transients shown.

after 21 s. In contrast Fig. 2b shows the emission of a molecule that exhibits variations in intensity and lifetime. In this particular case, the variations in both parameters are correlated, possibly due to the opening of an extra decay channel in the polymer matrix. Notice that between 3 and 11 s, the molecule is in a ‘dark’ state and does not emit photons. From each transient, we extract a fluorescence lifetime distribution, as plotted on the right margins of Fig. 2a and b, respectively. By collecting the transients for about 200 molecules, a fluorescence lifetime distribution was built up for each sample.

Fig. 3 shows these full distributions for each sample with different thickness values. The key observation on this graph is the shift to lower lifetime when the thickness of the film increases, peaking at 2.22, 2.10 and 1.88 ns for the considered 10, 30, and 70 nm thick films, respectively. Also of great importance is the width of the lifetime distributions. As can be observed, the width ($w_\tau = 1.54$ ns) of the distribution is largest for the thinnest film (10 nm). If the film’s thickness is increased (70 nm), the width of the distribution decreases ($w_\tau = 1.07$ ns). In order to explain the observed dependence of the fluorescence lifetime

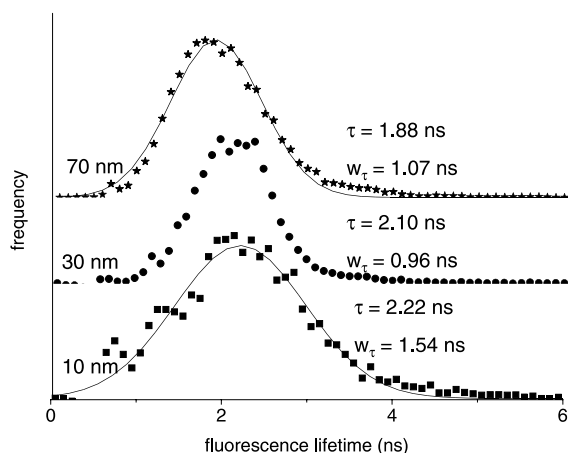


Fig. 3. Histograms and corresponding Gaussian fits of determined fluorescence lifetimes for DiD molecules embedded in different polystyrene films of thicknesses 10, 30, and 70 nm (200 molecules were investigated for each sample). The fluorescence lifetime distributions shift to lower values when the thickness of the film increases.

on the film’s thickness, we have to invoke either photochemical or photophysical effects affecting the dyes in the matrix. Since early experiments by Drexhage et al. [21], it is known that the lifetime of an excited molecule fluorescing near an interface between two media can be altered substantially owing to reflection and absorption at the interface. Therefore, if we want to address polymer dynamics, we must first isolate the photophysical effects related to interfaces. To this end we must consider the EBCs at the interfaces of the polymer with air on the one side, and glass on the other side.

Let us now consider the model used to study the effect of EBC on the behaviour of the dyes. Chance et al. [22] and Lukosz and Kunz [23] have described this interaction, showing that the field of the dipole is perturbed in the presence of a second medium. In a simple approximation a dye molecule can be considered as an oscillating dipole, when driven by the alternating electric field of the incoming light wave. The dipole acts as a source of a secondary wave. When surrounded by an isotropic medium, this secondary wave does not influence the oscillator. The well-known butterfly pattern for the total power W radiated by the molecule can be described by the Larmor formula

$$W = \frac{\mu\omega^4|\vec{p}|^2}{12\pi c}, \quad (1)$$

with \vec{p} the dipole moment, μ the permeability of the considered medium, ω the dipole emission frequency and c the speed of light.

If a fluorescing dye molecule is placed close to an interface, interference between the direct (ray 1) and reflected (ray 2) parts of the emitted light wave will occur (Fig. 4a). The amplitude of the resultant wave is determined by superposition of these two parts. The result depends on the path difference between the two rays and on the optical properties (phase shift, reflectivity) of the interface. The reflection of electromagnetic waves at a plane interface can be treated using the classical Fresnel formulas. This dependence on the reflection coefficients illustrates that the total radiated power is strongly dependent on the distance d separating the dipole from the interfaces, as well as on the orientation α of the dipole with respect to the interface. In the underlying problem the dipole is so

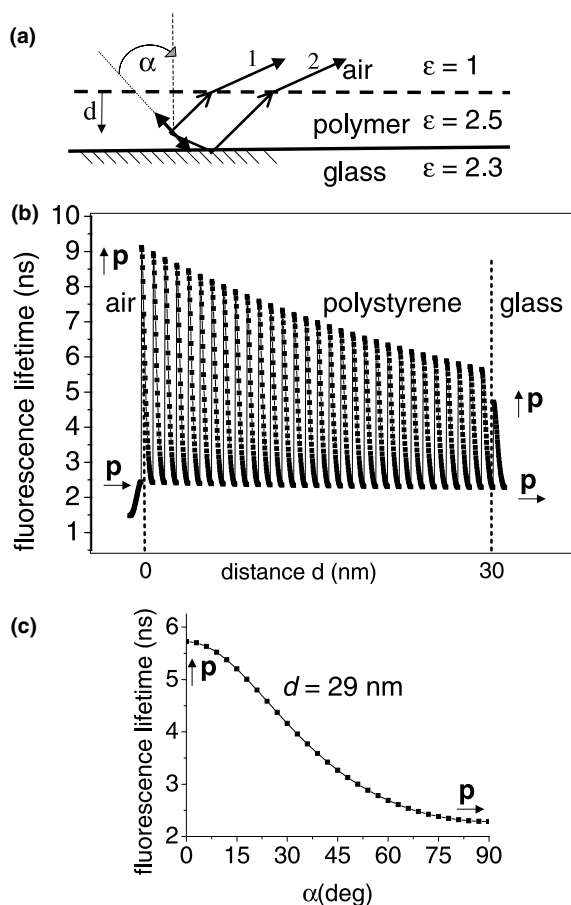


Fig. 4. (a) Schematic of the model used in this study: fluorescing molecules are represented by their dipole moments which are oriented with an angle α relative to the normal of the air–polymer interface, and placed at a distance d from this interface. Molecules are assumed to have random orientation and are placed in all depths within the polymer layer. (b) Calculated dependence of the fluorescence lifetime on the distance and orientation of the dye with respect to the air–polymer interface. (c) Fluorescence lifetime for a particular molecule situated 29 nm from the left interface as a function of the angle α between the normal to the surface and the dipole moment orientation, ranging from completely out of plane to in plane.

close to the interface between the different media that the exponentially decaying part of the fields plays an important role and should be included. This is performed by taking the complex Fresnel coefficients as a function of the dielectric constant rather than depending on the angles employed in a more familiar geometric optical picture. Lukosz

and Kunz [23] calculated the following expression for the total power radiated by an arbitrarily oriented dipole in the presence of a second medium:

$$\frac{W(d)}{W} = \cos^2 \alpha \left[\frac{W(d)}{W} \right]_{\perp} + \sin^2 \alpha \left[\frac{W(d)}{W} \right]_{\parallel}, \quad (2)$$

where the radiated power emitted by the dipole in a homogeneous medium (Eq. (1)) normalizes the result. The ratio of observed fluorescence lifetimes in two different optical environments is then given by the inverse ratio of the total power radiated by the fluorescent molecule.

$$\frac{\tau(d)}{\tau} = \left[\frac{W(d)}{W} \right]^{-1}, \quad (3)$$

where τ is the fluorescence lifetime in vacuum. The determination of the fluorescence lifetime is thus equivalent to measuring the power radiated in the whole space surrounding the dipole.

How does this scheme apply in our case and is it able to explain changes observed in the lifetime distributions shown in Fig. 3?

First of all, as the fluorescence lifetime depends on the angle and on the distance separating the dipole from the interfaces, we consider a particular disposition of all molecules in the polymer matrix. The most reasonable assumption is to consider a random arrangement of the molecules placed in all depths within the polymer, each with an isotropic distribution of orientations. We thus model the thin film as a three-layer system consisting of the polystyrene with the DiD dyes embedded in the matrix, sandwiched between air and the glass substrate. Knowing the dielectric constants of the three media, $\epsilon = 1, 2.5, 2.3$, respectively, for air, PS and glass, we determined numerically the fluorescence lifetimes of all the molecules embedded in the polymer. In our calculations, for each molecule situated a distance $d = 1, 2, 3, \dots$ nm away from the air interface, we considered the angle α ranging from 0° to 90° in steps of 3° .

Results for a 30 nm thick film are shown in Fig. 4b. This is a plot of fluorescence lifetime versus depth of the molecule in the matrix, with $d = 0$ at the air–polymer interface, for the various angles (α) of the dipoles with respect to the normal. The key observation on this graph is the decrease in the

fluorescence lifetime when the molecule is far away from the air interface, especially for a fully out-of-plane oriented dipole, while the fluorescence lifetime of an in-plane dipole remains almost unchanged (see also Fig. 4c). The graph also nicely represents boundary conditions originating from Maxwell's equations, with continuity (discontinuity) of the in-plane (out-of-plane) component of the electric field through the interfaces. Furthermore, performing such calculations for 10–70 nm thick films shows an overall shift to lower values of the fluorescence lifetimes with increasing film thickness. Fig. 5, illustrating the simulated distributions of fluorescence lifetimes for films of three different thicknesses, exemplifies this result. Note that in this graph only dipoles with an angle larger than 30° have been included, since out-of-plane molecules with a smaller angle are hardly excited by the CFM. Direct comparison with the experimental distributions in Fig. 3 leads to the following observations.

The shift to lower values of fluorescence lifetimes for dyes embedded in an annealed polymer matrix when the thickness of the film increases can be explained by simply taking into account the role of electromagnetic boundary conditions on the fluorescence rate of the dyes. It must be noted that

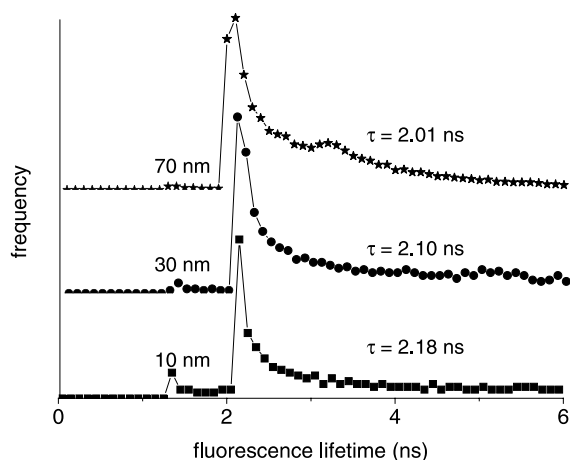


Fig. 5. Histograms and corresponding Lorentzian fits of calculated fluorescence lifetimes for DiD molecules embedded in different polystyrene films of thicknesses 10, 30, and 70 nm.

this clear dependence of the fluorescence lifetime on the thickness of the films does not hold for non-annealed samples. In this case, the photophysical properties of the dyes are probably dominated by inhomogeneities of the films, such as solvent residues and/or frozen stresses induced by the spin coating.

A close examination of Figs. 3 and 5 also indicates broader experimental distributions when compared to simulated ones taking only the EBC effect in consideration. This and the fact that the standard deviations are larger than the statistically expected values (± 47 ps) [19] were attributed to the strong influence of the inhomogeneous nanoenvironment on the photophysical behaviour of the probe molecule. Furthermore, for the 10 nm thick sample, the width of the experimental distribution is larger than the one of the 30 and 70 nm thick films. This indicates that possibly a more heterogeneous set of configurations is probed by the dyes in the thinnest film.

Finally, photoinduced chemical alterations of the dyes can also contribute to the width of the experimental distributions: Fig. 2a for example could be the behaviour of a dye photooxidized during the time of measurement. In analogy to a scheme suggested recently by Basché [24], the change in the fluorescence lifetime observed after the 8 s off period could be explained by considering a change in the length of the conjugation path of the molecule due to photooxidation. Indeed, as one could expect from Eqs. (1) and (3), the increase in the fluorescence lifetime observed after the long dark period would result in a lower radiated power of the dipole, causing a red shift of about 50 nm in the emission spectrum. Also Lu and Xie [25] attributed spectral fluctuations of such amplitude and timescale to a photoinduced mechanism, in which radiationless relaxations take place, leading to variations of the nuclear coordinates. Of course, serious investigations must be performed in order to prove that this long off period shown in Fig. 2a and the subsequent change of lifetime are really due to photooxidation. Oxygenated and de-oxygenated experiments would probably vary the relative occurring proportion of such events and thus permit conclusion on this point.

4. Conclusions

In this Letter, we have shown that electromagnetic boundary conditions can play an important role on the fluorescence rate of dyes embedded in a polymer. By modeling thin films as three-layer systems consisting of a polymer embedding dye layer sandwiched between air and a glass substrate, we demonstrated that EBC effects cause a shift to lower values of the full distributions of fluorescence lifetimes, with increasing thickness of annealed films. The widths of the distributions, on the other hand, indicate that other effects are going to play a role, like nanoheterogeneous properties of the polymer film, photooxidation of the dyes, thermal inhomogeneous broadening, etc.

When interpreting the data and trying to get information about a polymer thin film, extreme care must be taken in order to attribute the behaviour of the dye correctly to either photophysical or photochemical effects occurring inside the matrix.

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