Orientation dependence of fluorescence lifetimes near an interface

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The fluorescence lifetime of single DiI-dye molecules in a 20 nm polymer film on glass is measured as a function of the orientation of the absorption dipole moment. A strong dependence of the lifetime on the orientation of the dye molecules relative to the polymer/air interface is found. Molecules with a dipole moment perpendicular to the interface exhibit a lifetime which is by a factor of 2.1 ± 0.1 longer than the lifetime of molecules with parallel dipole moments. The general trend of the results is in good agreement with theoretical predictions. However there are significant deviations which are attributed to varying molecular environments. © *2002 American Institute of Physics.*
[DOI: 10.1063/1.1515732]

Observables of fluorescent molecules like absorption and emission spectra, fluorescence lifetime, anisotropy, quantum yield, and others are influenced by their local environment. Therefore, fluorophores can be used as truly nanoscopic sensors in biology[1,2] and material science.[3,4] Furthermore, they give detailed insight in fundamental optics.[5,6] The interpretation of any data obtained in such applications requires a detailed understanding of the interactions between a molecule and its environment. In such interactions different length scales can be of relevance, which are due to different physical origins. Theoretical understanding of the behavior of single molecules is usually based on considering a molecule as classical radiating dipole in a homogeneous environment with a piecewise constant complex dielectric constant. In this picture, the fluorescence lifetime, for example, is strongly influenced by the number of decay channels, i.e., the density of electromagnetic modes available to the photons emitted by molecule. This effect can be important over length scales that are large compared to molecular dimensions due to the long range of the electromagnetic interaction. In contrast to this, it is also assumed, that variations in the excited state lifetimes between identical guest molecules in a solid matrix can occur due to variations in the local dielectric constant around a molecule, i.e., the exact chemical nature of molecular nanoenvironments. Here, we are focusing on the investigation of long-ranged electromagnetic effects in a simple geometry. We consider molecules within a distance of 0–20 nm to a planar interface between two lossless dielectrics. The behavior of fluorescent molecules at interfaces has been investigated in the past. Drexhage *et al.*[7] studied lifetime variations as a function of the distance from a metallic interface, using fatty acid multilayers as tunable spacers between the interface and the fluorophores. A reduction of the lifetime by orders of magnitude was observed when the distance of the

dye from the metal is in the nm range. These studies were extended to purely dielectric interfaces[8] where a pronounced but less drastic effect was found.

A major drawback of the early experiments of Drexhage is that the theoretically predicted distinct dependence of the effect on the out-of-plane orientation of molecular dipoles could not be directly verified since this parameter could not be determined for the system under investigation. Macklin *et al.*[5] experimentally investigated the variation in lifetimes of single molecules near a plane polymer-air interface. They attributed variations in the lifetime to different out-of-plane orientation of the molecular dipoles with respect to the interface which they deduced from shifts in the emission spectra.

Vallee *et al.*[9] found strong lifetime variations of dy molecules in the vicinity of a dielectric interface which they attributed to different orientations of the dye molecules.

Xie *et al.*[10] estimated the dipole moment orientation of a dye molecule using a doughnut-shaped laser mode. Two molecules with differing fluorescence lifetimes were consistently found to have different orientations.

In this article we disentangle long-ranged electromagnetic effects from the influence of short-ranged inhomogeneity on the fluorescence lifetimes of single dye molecules in the vicinity of a dielectric interface. We precisely determine the out of plane component of the molecular dipoles by evaluating the patterns generated in annular illumination.[6] This allows us to systematically and quantitatively study the influence of different out-of-plane orientations on the lifetime of single fluorescent molecules near a dielectric/air interface. The investigation of a large number of molecules yields sufficient statistical evidence to allow for a quantitative comparison of our results to the predictions of classical electromagnetic analysis of molecular fluorescence in planar layered structures.[11–18] We find that the lifetimes follow the general trend (longer lifetime for large out-of-plane angles) predicted by the theory. However, we also find significant deviations which are indicative of inhomogeneous nanoenvironments sensed by the molecules. Our results open the way to truly quantitative studies of effects due to varying molecular nanoenvironments on the level of single molecules.

Samples were prepared by spin-casting a solution of

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poly-methyl-methalcrylate (PMMA) in toluene containing the chromophore 1,1-dioctadecyl-3,3,3′,3′- tetramethylindo-carbocyanine (DiI, Molecular Probes) in a concentration of 10^{-10} mol l^{-1} onto a clean microscope cover glass. This yields 20 nm thick polymer films on glass with an areal density of ≈ 0.5 dye molecules per μm^2. Experiments were performed with a fluorescence scanning confocal optical microscope.[6] A pulsed, frequency doubled NdYag laser (Antares, Coherent, $\lambda = 532$ nm, pulse length 150 ps) was used as excitation light source. The beam passes a $\lambda/2$ plate before it is coupled into a single mode optical fiber for spatial filtering. The polarization change in the fiber is compensated by applying stress to the fiber (Polarite polarization controller, L.O.T.), thus allowing for switching between two orthogonal linear polarizations at the output of the fiber by appropriate turning of the $\lambda/2$ plate. After the fiber, the light is collimated by a lens. A circular disk with a diameter of 3 mm then blocks the inner part of the beam and implements annular illumination geometry.[6] A dichroic mirror directs the light into a microscope objective where it is focused onto the interface. The fluorescence light is collected by the same objective, passes the dichroic mirror, and is detected by a single photon counting avalanche photodiode. For each photon, the time delay relative to the next laser pulse is recorded as well as the absolute photon arrival time during the recording of the image using a TCSPC computer card (SPC 402, Becker and Hickl). This allows for both the construction of a fluorescence image and the local analysis of the fluorescence lifetime at each pixel. The absorption dipole moment of a large number of single molecules was determined by evaluating the intensity patterns that arise from excitation by annular illumination.[6] In brief, annular illumination leads to an electric field distribution in the focus in which all three fundamental cartesian field components (defined by optical axis and the direction of polarization) are of comparable strength but exhibit very distinct patterns in the geometrical focal plane which are shown in Fig. 1 (upper part). An arbitrarily oriented molecule will exhibit an image pattern which is a superposition of the three fundamental patterns. In order to enhance the orientational sensitivity of the method for small out of plane angles, for each molecule the image patterns were measured for two orthogonal linear polarizations of the exciting laser beam. This allows for a precise assignment of an absorption dipole orientation by simultaneous least-square fitting of the two measured to the respective theoretical patterns, finally yielding the polar angle θ relative to the surface normal.

It should be noted that the fitting procedure never yields polar angles of the transition dipole moment very close to the perfectly parallel or perpendicular case. This behavior is intrinsic to our method of orientation determination as was verified by investigating synthetic data with a statistical error, generated by a Monte Carlo algorithm. The resulting patterns were evaluated by the same least-square routine as the measured data.

From these simulations, we found the most probable true value for θ is slightly different from the value as obtained from the fit, additionally, the error in θ could be estimated.
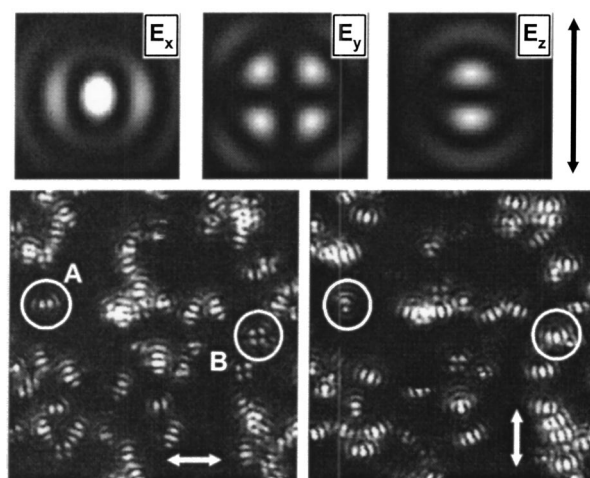

FIG. 1. Upper part: calculated intensity of the electrical field components for annular illumination. Each image corresponds to a 1 μ wide square area at the interface which is coinciding with the focal plane. The double arrow indicates the E field of the incident beam (x axis). Lower part: Experimental data. The two images were recorded with linearly polarized light, the double arrows indicate the electrical field vector. The two molecules A and B are examples for which fluorescence lifetime analysis is presented

This most probable θ with the corresponding error was used for further evaluation of the data.

The excited molecule is described as a radiating point dipole with a transition dipole moment, $\mathbf{p}_0$, located at the position $\mathbf{r}_0$ at a distance d from a plane interface. The molecular dipole is located in medium 1 with dielectric constant $\epsilon_1 = 2.25$ (PMMA film on glass), the second medium is air with $\epsilon_2 = 1$. θ is the angle between the surface normal and $\mathbf{p}_0$. For further theoretical analysis it is convenient to consider decay rates $P = \tau^{-1}$ instead of lifetimes τ because rates are additive. The decay rate P_{tot} of an excited fluorophore can be divided into a nonradiative contribution P_{nr} and the emission of photons (electromagnetic processes) P_{em}. Thus

$$\frac{1}{\tau} = P_{\mathrm{tot}} = P_{\mathrm{nr}} + P_{\mathrm{em}}. \tag{1}$$

The electromagnetic contribution P_{em} of the total decay rate is determined by integrating the Poynting vector over a suitable surface enclosing the dipole. Using Poyntings theorem this integration can be reduced to the calculation of the back-reacted field at the dipole position[14,17]

$$\frac{P_{\mathrm{em}}}{P_0} = 1 + \frac{6\pi\epsilon_0\epsilon_1}{p_0^2 k_1^3} \mathrm{Im}[\mathbf{p}_0^* \cdot \mathbf{E}_{\mathrm{br}}(\mathbf{r}_0)], \tag{2}$$

where ω and k_1 are the frequency of the emitted radiation and the wave vector in medium 1, respectively. $\mathbf{E}_{\mathrm{br}}$ is the back-reacted electrical field which is defined as the difference between the field in the presence of the interface and the field of the same dipole in a homogenous dielectric medium. A detailed account of how to calculate $\mathbf{E}_{\mathrm{br}}$ for a system of stratified layers is found in Refs. 17 and 18. In the case of a plane dielectric interface, for symmetry reasons, the parallel (perpendicular) component of the back reacted field generated by a perpendicular (parallel) dipole vanishes. Therefore, the decay rate for a dipole with arbitrary polar angle
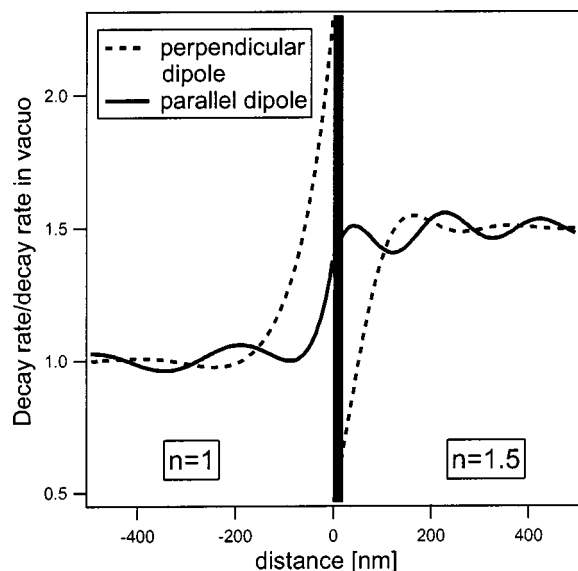
FIG. 2. Fluorescence decay rates of a radiating dipole at an interface between two dielectrics with refractive indices $n = 1$ and $n = 1.5$.

FIG. 3. Histograms for the time delay between excitation and fluorescence emission for the molecules A and B in Fig. 1. The exponential fit is shown as a straight line, the resulting fitting parameters are indicated in the graph.

$P_{em}(\theta)$ is simply the linear superposition of the parallel ($\|, \theta = 90°$) and the perpendicular ($\perp, \theta = 0°$) case according to

$$P_{em}(\theta, d) = \sin^2(\theta) P_{em\|}(d) + \cos^2(\theta) P_{em\perp}(d). \quad (3)$$

Figure 2 shows the variation in P_{em} as a function of the distance from a dielectric-dielectric interface for the two fundamental polarizations.[17,18] In close proximity to the interface, a distinct difference between the two investigated orientations is observed. While $P_{em}(d)$ is continuous for the dipole oriented parallel to the interface, for the perpendicular case a discontinuity is observed with

$$\frac{\lim_{d\to 0^+} P_{em,\perp}(d)}{\lim_{d\to 0^-} P_{em,\perp}(d)} = \left(\frac{\epsilon_1}{\epsilon_2}\right)^2. \quad (4)$$

At a distance of several wavelengths away from the interface the respective bulk values are recovered. Since the measurements reported in this paper were performed using fluorophores with arbitrary orientation, located in a 20 nm thick polymer layer on top of a glass slide, this regime, exhibiting a strong variation of decay rates with orientation is marked in Fig. 2. It is clearly seen that a large difference in P_{em} of the different orientations is observed while the variations in the lifetime of equally oriented chromophores due to different distances from the interface is almost negligible.

In the lower part of Fig. 1 a typical pair of fluorescence images obtained with our setup is shown. The characteristic image patterns of single molecules are due to the interaction of the absorption dipole moment of the DiI molecules with the electric field distribution in the focus created by annular illumination. Some molecules are aligned almost parallel to one of the main axes of the system, leading to patterns similar to the theoretical patterns depicted in Fig. 1 (upper part). The determination of the orientation of the absorption dipoles is based on the least-squares fitting routine described above.
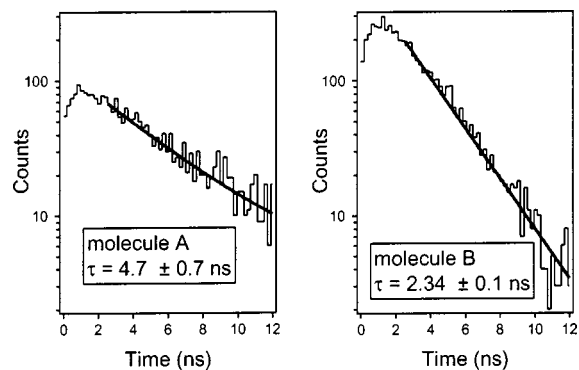
It should be noted that Sund *et al.*[19] report for DiI instead of single absorption- and emission-dipole moments two pairs, both being symmetric with respect to a common axis. For the measurements reported here, this leads to effects which are not distinguishable from both absorption and emission dipole moment being collinear to this axis.

In order to determine the fluorescence lifetime, all photons that contribute to a certain pattern are selected by a threshold criterium and a histogram of the number of photons with a certain time delay relative to the exciting laser pulse is generated. For the two molecules indicated in Fig. 1 by "A" and "B," the respective histograms are presented in Fig. 3. The fluorescence lifetime τ is determined by an exponential fit to the data, weighted with the appropriate statistical error.

Figure 4 shows the fluorescence decay rate as a function of $\sin^2(\theta)$. This way of data representation is chosen because of the resulting linear functional dependence [see Eq. (3)]. The standard deviation in $\sin^2(\theta)$ is determined from the Monte Carlo simulations. Two errors of the measured fluorescence lifetimes are taken into account quantitatively and therefore contribute to the standard deviation in τ. The uncertainty of the exponential fit (see Fig. 3) and the error introduced by the varying distance from the interface. The decay rate of parallel dipoles is considerably enhanced rela-
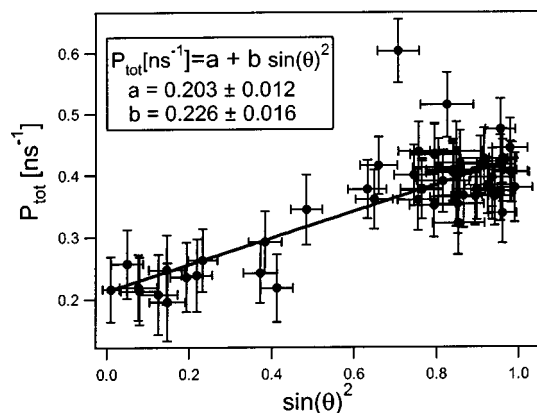

FIG. 4. Fluorescence decay rate of DiI molecules in as a function of $\sin^2(\theta)$. The straight line is a linear fit to the data, the fitting parameters are indicated in the graph.

tive to the ones oriented perpendicular to the interface. A linear fit to the data yields an enhanced rate of a perfectly parallel dipole relative to a perpendicular one of

$$\frac{P_{\text{tot}\parallel}}{P_{\text{tot}\perp}} = \frac{a+b}{a} = 2.11 \pm 0.1 \tag{5}$$

with the parameters a and b of the linear fit as indicated in the inset of Fig. 4. It should be noted, though, that already inspection by eye suggests that there is a stronger deviation of the data from the fit as it would be expected from the errors. Indeed, our set of experimental data and standard deviations yields a $\chi^2 = 215$. Assuming only normally distributed errors the probability Q for obtaining a χ^2 larger than this value amounts to 10^{-21}.[20]

Classical electromagnetism predicts a ratio of the radiative decay rates of

$$\frac{P_{\parallel,\text{rad}}}{P_{\perp,\text{rad}}} = 2.54. \tag{6}$$

This value is slightly larger than the experimental result for the total decay rates, indicating a nonvanishing contribution from nonradiative decay processes. Agreement between experiment and theory can be established when P_{nr} is set to 0.056 ns^{-1}.

The observed scatter of the measured data around the ideal behavior can be assigned to differences in the molecular environment, induced by the polymer matrix. These differences in the nanoenvironment lead to an additional, intrinsic contribution P_{int} to the fluorescence decay rate. The observation that the two most prominent outliers have a significantly enhanced decay rate, suggests an asymmetric probability distribution for P_{int}.

An estimate for the significance of these variations may be obtained assuming a normal distribution around 0 with a standard deviation $\sigma(P_{\text{int}})$. In this case a $\sigma(P_{\text{int}}) = 0.044 \text{ ns}^{-1}$ would yield the value for χ^2 with the highest probability ($\chi^2 = 53$). It should be kept in mind, though, that the probability distribution for P_{int} appears to be more complicated than a normal distribution. Its precise shape is governed by the interactions of the dye molecules with the sur-

rounding matrix. Therefore these deviations deserve focused attention in future studies since here effects on molecular dimensions can be measured by purely optical means.

In conclusion, it has been shown that the major contribution to variations in the fluorescence lifetime of DiI in the direct vicinity to a plane dielectric interface can be assigned to orientational effects that lead to variations in the electromagnetic emission probability. Nevertheless, some other contributions play a role as well to obtain a complete picture of fluorescence in nontrivial dielectric surroundings. It can be expected that future research on single molecules will give a detailed insight in these mechanisms.

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