Research paper

Fluorescence lifetime, dipole orientation and bilayer polymer films

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ARTICLE INFO

ABSTRACT

Bilayer films consisting of the optically transparent polymers, polystyrene (PS) and poly(methyl methacrylate) (PMMA) were spin-cast on glass substrates. The upper 13.5 nm layer (PS) was lightly doped with Rhodamine-6 G (RH6G) or MEH-PPV. While the fluorescence of MEH-PPV was independent of PMMA thickness, the lifetime of RH6G increased 3-fold as the underlying PMMA thickness increased from 0 to 500 nm while the collected flux decreased suggesting a reorientation of the smaller molecule's dipole with respect to the air-polymer interface with PMMA thickness. This suggests lifetime may find application for nondestructive thickness measurements of transparent films with sub-micron lateral resolution and large range.

1. Introduction

Fluorescence or photo-luminescence of single molecules is not only fundamental interest but also of much practical interest as probes in illuminating processes. In particular fluorescence lifetime imaging, due to the strong dependence of lifetime on the local environment, is finding increasing application throughout the physical and biological sciences [1] since the lifetime does not depend on initial perturbation conditions (e.g. wavelength of excitation, duration or method of light exposure), is not affected by photobleaching, and is relatively independent of the fluorescence intensity and fluorophore concentration at low concentrations. Lifetime is; however, sensitive to a great variety of internal factors (i.e. fluorophore structure) and external factors including temperature, polarity, refractive index, the presence of fluorescence quenchers, and even geometry. As summarized by Berezin and Achilefu [1], 'this combination of environmental sensitivity and parametric independence renders fluorescence lifetime a separate yet complementary method to traditional fluorescence intensity measurements.'

Concerning geometry, back in the 1970s, Drexhage [2] calculated that the lifetime of fluorophores within ∼20 nm of an interface is dependent on their orientation relative to the interface. While molecules whose dipoles are oriented parallel to the interface are unaffected by the interface, the fluorescence lifetime of molecules whose dipoles are oriented perpendicular are lengthened by a factor related to the cube of the ratio of the refractive indexes. For a typical polymer-air interface ($n_{polymer}/n_{air} \sim 1.5$) this can result in a threefold lengthening of the fluorescence lifetime.

Some 25 years later, with the advent of single molecule spectroscopy, a renewed interest in the effect of an interface on fluorescent lifetime of single emitters was prompted by the observation of substantial variations in lifetimes for spin-coated films of fluorescent DiI (1,1′-didodecyl-3,3,3′,3′-teramethylindocarbocyanine) molecules embedded in an inert matrix. Macklin attributed this to effects of the polymer-air interface [3]. In similar system, Vallee observed a lengthening in fluorescence lifetime as the films became thinner. Vallee's group attributed this to the effect of the orientation on the fluorescence of individual fluorophores near the air/polymer boundary [4]. The advent of the annular illumination technique [5] allowed Wild's group [6] to measure the orientation of individual DiI molecules within the overall isotropic distribution and correlate this with fluorescence lifetime. This allowed for the first direct comparison with theory and thus verified the strong influence of the orientation of a single molecule's transition dipole moment relative to the air-dielectric interface on florescence lifetime for molecules within 20 nm of the interface.

In the work presented here we embedded, at low concentration, fluorophores in a 13.5 nm thick polymer matrix which was spin cast onto an underlying layer of a second optically inactive polymer of similar index of refraction. The thickness of the upper layer was chosen to ensure that all the embedded fluorophores were close to the interface so that orientation effects on lifetime could be observed while the thickness of the lower layer was varied. We note that similar bilayer films have recently been deposited on graphene coated substrates to studying the distance dependence of energy transfer [7–9] between fluorophores and graphene. By varying the

thickness of the buffer layer, energy transfer was shown to be non-radiative (Foster-like) as evidenced by the concurrent dropping of both fluorescence lifetime and intensity as buffer layer thickness decreased. Two of these papers also noticed, but did not comment on, a secondary effect that observed lifetimes of the fluorophores also varied in the absence of graphene [8,9].

While spin-casting of single layer films is well established, bilayer polymer films provide many more challenges due to the need for selective solvents. We will focus on the ubiquitous poly (methyl methacrylate) (PMMA) and polystyrene (PS) system. PMMA and PS, both transparent in the optical region, is a widely used model system for investigations including interfacial width measurements, block copolymer morphology, antireflection coatings, physical blending, dewetting dynamics, isotope driven segregation, and reactive compatibilization [10]. The system also illustrates one of the challenges of bilayer films: while spin-casting of PS on PMMA, although challenging, is possible, the reverse process, spin-casting PMMA on PS is not currently possible due to solvent compatibility problems [10]. By embedding Rhodamine 6G (RH6G) at low concentrations in the PS layer, we show that the fluorescence lifetime and spectra can be correlated to the thickness of the underlying layer of PMMA. While previous work has shown that when individual chromophores are aligned perpendicular to an air/polymer interface there is a drastic increase in fluorescence lifetime, we present the first evidence of a macro-level increase in fluorescence lifetime of an ensemble of small molecules suggesting alignment on the ensemble level. We further demonstrate that the degree of alignment can be controlled by the insertion of a thin spacer layer.

2. Materials & methods

2.1. Sample preparation

PMMA (Aldrich, $M_w \sim 996kD$) was dissolved in toluene and mixed for 7 days at 300 K. The solution was then spin coated onto cleaned (acetone then DI water) glass microscope slides (FEA or fused silica) and dried under vacuum conditions for 2 days at room temperature to remove remaining solvent. The thickness of the film (from 0 to 2000 nm) was controlled by varying the concentration of PMMA in toluene [11]. The active layer was processed as follows. The fluorophore (Rhodamine 6G (Rh6G, Mw = 479 g/mol) or Poly [2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV, M_n = 150–250kD)) was first mixed with polystyrene (PS, Fluka, M_w = 184kD M_n = 178kD) at a ratio of 0.1 wt% and dissolved in 1-Chloropentane (2 mg/ml). The low concentration was chosen to ensure the fluorophores were sufficiently separated so that the probability of aggregate or dimer formation was negligible. 1-Chloropentane (Acros) was chosen as the solvent since (1) its low vapor pressure allows very uniform and smooth thin films to be spin-coated; and (2) it is an orthogonal solvent to the toluene based PMMA layer ensuring that the underlying PMMA spacer layer remains intact and that fluorescent molecules do not leach into the it [10]. After mixing for 7 days at 300 K and filtering (0.45 μm) to remove aggregates, the solution was spin coated onto the PMMA coated glass slide to form a t = 13.5 ± 1.5 nm (as measured by AFM) thick fluorescent film. Finally the films were dried under vacuum conditions for 2 days at room temperature to remove remaining solvent. The basic structure of the spin cast device is shown in Fig. 1(inset).

2.2. Observation and analysis

The resulting bilayer films were observed using our home built vacuum confocal microscope [12]. 5 ps (FWHM) λ_{excite} = 470 nm
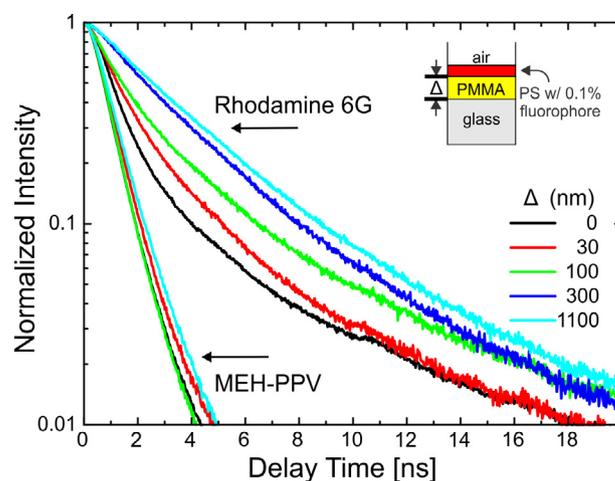

Fig. 1. Fluorescence decay time for the bilayer films with different thicknesses of PMMA underlayer. The active layer is 13.5 nm thick polystyrene (PS) doped with 0.1%wt of either Rhodamine 6G or MEH-PPV. [inset] Schematic of the typical device structure used.

pulses (PicoQuant, PDL 800-B) were focused onto the sample plane using a 60x, NA = 0.85 objective lens (Nikon) except where noted. (Excitation power on sample was ~600 W/cm²). The fluorescence light emitted by the dye molecules was collected through the same objective lens (epifluorescence mode), and after appropriate filters, focused by a f = 200 mm doublet lens into a φ = 100 μm optical fiber and directed alternatively to a spectrometer (BWTEK BRC111A) or an SPCM avalanche photodiode (Perkin-Elmer) connected to a time-correlated single-photon counting card (SPC 630, Becker & Hickl) (to obtain fluorescence lifetime data). During all measurements, a peizo-electric stage (Physic Instruments) was used to scan the sample over an area of 30 × 30 μm², at a pixel frequency of 100 Hz and step size of 1 μm/pixel to avoid photobleaching and average over an ensemble of fluorophores.

The fluorescent lifetime decay curves were fit using two exponential decay:

$$I = A_{short}e^{\left(\frac{-t}{\tau_{short}}\right)} + A_{long}e^{\left(\frac{-t}{\tau_{long}}\right)} \tag{1}$$

In all cases, the shorter time constant dominated the decay curve, i.e. $A_{short} > A_{long}$, with purely single-exponential decay seen occasionally.

As the shape of the spectra was relatively constant during the experiments, following Liang [13] we defined a spectral coefficient (S) to characterize the changes in the spectra:

$$S = \frac{\int_{555}^{\infty} I(\lambda)d\lambda - \int_{0}^{555} I(\lambda)d\lambda}{\int_{0}^{\infty} I(\lambda)d\lambda} \tag{2}$$

where $I(\lambda)$ is the flux at the wavelength λ. The advantage of this is that it allows changes in spectra to be specified by a single number rather than a complete spectrum when the shape is constant.

Finally the dependence of the lifetime and spectral shift on the thickness (Δ) of the underlying PMMA layer was fit to an exponential curve:

$$X(\Delta) = A + B \times \exp\left(\frac{\Delta}{\Delta_o}\right) \tag{3}$$

where X represents either S or τ, A and B are constants and Δ_o is the characteristic decay distance.

3. Results and discussion

3.1. Lifetime and spectra

Fig. 1 presents typical results for the time decay of fluorescence following excitation with the pulsed layer for the two molecules. For the large fluorophore (MEH-PPV, cf Fig. 2a inset) the curves for fluorescence intensity as a function of time delay are very similar. The lifetime first lengthens then shortens then lengthens again – all within experimental error. In contrast, for the small molecule (Rh6G) data, as the film thickens, the fluorescence drops much more slowly with time. In short, while there is no obvious correlation between PMMA thickness and rate of fluorescence decay for the large molecule, there is a strong correlation for the small molecule.

Fig. 2 presents the spectra for the two molecules for different thicknesses of PMMA. The spectra for MEH-PPV (Fig. 2a) is the same at 40 nm as it is at 700 nm – clearly independent of the thickness of the underlying PMMA layer. For polystyrene film doped with Rh-6G (Fig. 2(b)), while the shape of the spectrum is unchanged with PMMA spacer layer thickness, there is a gradual blue shift as the PMMA thickness increases from 0 to 100 nm. For thicknesses greater than 100 nm, the spectrum remains
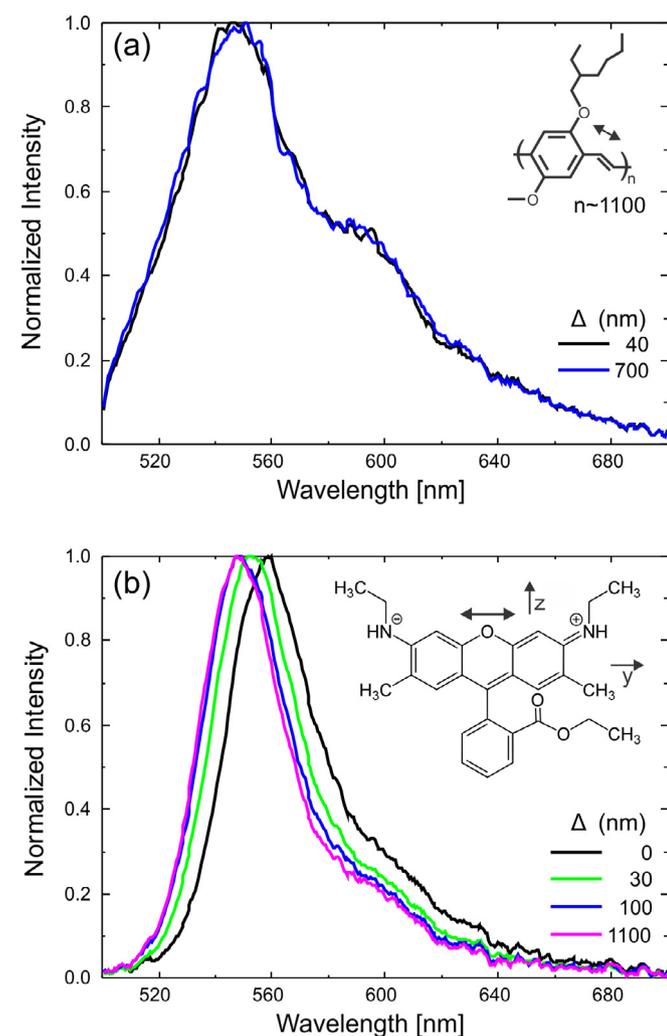
unchanged. Since there is no change in the shape of the spectra, in the following discussion, we will make use of a single number, spectral coefficient (as defined in Eq. (2)) to describe the spectra.

Table 1 summarizes the fluorescence results for the limits of thin (<15 nm) and a thick (>700 nm) under-layer of PMMA. The PS layer in which the chromophores are embedded is 13.5 nm thick and the majority of results recorded using a 60x NA = 0.85 objective lens. The fluorescent lifetime was obtained by curve fitting the decay curves using Eq. (1). As the decay curves were well described by a single exponential decay, the fluorescent lifetime is presented as a single number. The spectral coefficient was obtained using Eq. (2). The error bars were obtained by repeated measurements on different samples and substrates of different types of glass. As can be seen from the error bars, the type of glass (fused silica or FEA glass) had only a small effect on these fluorescence – either lifetime or spectral coefficient. For the large fluorophore (MEH-PPV) the presence or absence of the underlying layer of PMMA has very little effect on fluorescence. Under all conditions the changes in fluorescence lifetime, spectral coefficient and flux are within the limits of experimental error. In contrast, when the small fluorophore (Rh6G) was embedded in the polystyrene matrix, fluorescence was strongly affected by the underlying PMMA layer. The existence of a thick PMMA under-layer resulted in a threefold increase in the fluorescence lifetime of Rh6G relative to that measured in its absence (from ∼1 ns to ∼3 ns). Concurrently the spectral coefficient was reduced from 0.5 to 0.05 reflecting a blue shift of the spectra. In order to check if there might be an index of refraction effect, the glass substrate (n = 1.5) was replaced with a sapphire substrate (n = 1.8). While the spectral coefficient (S) was affected by the change in substrate, the change resulted only in a small increase in fluorescence lifetime (within experimental error). In summary, while fluorescence – in terms of lifetime, flux and spectrum –from the large fluorophore (MEH-PPV) is robust, independent of the PMMA underlayer, that from the small fluorophore is clearly impacted by the 600 nm thick PMMA underlayer.

3.2. Origin of the lifetime changes

At this point we would like investigate the origin of this threefold lengthening of the lifetime of Rh6G by first ruling out three effects. While fluorescence lifetime is known to depend on the effective refractive index of the host via the n^2 dependence of the radiative rate constant, this is ruled out by (1) the fact that the layers involved all have similar refractive indexes and (2) any refractive index change should also affect the larger molecule. A second possibility is the Purcell effect wherein a resonant cavity enhances the spontaneous emission rate thus reducing lifetime. This explanation is excluded as such an effect would also impact the MEH-PPV lifetime – which it does not, and by the absence of any significant differences in refractive index between the layers in the system. A third possibility is energy transfer. Fluorescence lifetime is used as a sensitive probe for non-radiative (Foster-like) energy transfer. This is ruled out since in the case of energy transfer, reduced lifetime is accompanied by reduced flux – the opposite of what is observed in this work.

This leaves dipole reorientation. According to Drexhage's [2] calculations, the presence of the air-dielectric interface above the PS layer results in a threefold lengthening of the fluorescence lifetime for those dipoles both (1) close to and (2) orientated perpendicular to the interface relative to other dipoles. On an ensemble level, this results in slight lengthening of the lifetime of chromophores in a thin film relative to the bulk due since in an isotropic distribution, only a few chromophores satisfy both conditions [4]. The large change in lifetime seen in this experiment suggests that not only a few molecules are oriented perpendicular to the

Fig. 2. Effect of PMMA thickness on the bilayer film spectra. The active layer is 13.5 nm thick PS with 0.1 %wt doping of the fluorophore. (a) MEH-PPV doped PS (b) Rhodamine 6G doped PS [insets] Schematics of the (a) Rhodamine 6G (after Ref. [19]) and (b) MEH-PPV. The direction of the dipole moment for the two molecules is denoted by double arrows (↔).

Table 1
Comparison of fluorescence lifetime (t), spectral shift (S) and Flux for fluorophores (1% wt concentration) embedded in a 13.5 nm thick polystyrene (PS) matrix spin-cast for thin and thick underlayers of PMMA.

fluorophore	Buffer Layer Thickness	NA	τ	S	Flux
MEH-PPV	<15 nm PMMA	0.85	0.84 ± 0.1	0.3 ± 0.05	1.0 ± 0.05
MEH-PPV	3500 nm PMMA	0.85	1.0 ± 0.1	0.24 ± 0.03	1.0 ± 0.05
Rh6G	<15 nm PMMA	0.85	1.1 ± 0.2	0.5 ± 0.1	1.2 ± 0.05
Rh6G	>500 nm PMMA	0.85	3.0 ± 0.2	0.05 ± 0.03	0.4 ± 0.1
Rh6G	<15 nm PMMA	0.3	1.04 ± 0.2	–	–
Rh6G	>500 nm PMMA	0.3	2.2 ± 0.2	–	–

interface but that the whole distribution of dipole moments shifts from predominately isotropic to predominately perpendicular to the interface for the smaller molecule on an *ensemble* level. The sharp drop in the collected flux (by a factor of 3) as thickness increases strongly supports this interpretation. Since the excitation light transmitted through the objective lens is preferentially polarized in the parallel to the substrate, dipoles perpendicular to the substrate are excited with lower efficiency than those oriented in the plane of the film. Once excited, more light emitted by dipoles oriented perpendicular to the film is trapped within the film relative to those in the plane of the film [14–16]. Reorientation of dipoles distribution from isotropic to preferentially perpendicular thus results in less intensity due to less efficient excitation of the dipoles and less efficient collection of light by the objective lens.

We confirmed this interpretation by interrogating the angular dependence of the lifetime of dipoles in the film. While this can be done using the methodology of Ref. [21] for the film as a whole, the same information with a higher signal to noise ratio can be obtained by simply varying the Numerical Aperture (NA) of the objective lens. (The maximum half-angle (θ_{max}) of the cone of light that can exit (or be collected by) the lens is related to the numerical aperture (NA) of the lens by $\theta_{max} = \sin^{-1}(NA/n)$. For an isotropic distribution of dipole moments, NA should have little effect on the observed lifetime as the majority of the dipoles which lie in the lateral plane. However, if dipoles oriented perpendicular to the interface are making a significant contribution to the observed ensemble lifetime, the observed lifetime will drop with decreasing NA as the subset of dipoles oriented perpendicular would be excited (and light collected) less efficiently relative to those oriented in the parallel to the interface. In Table 1, the effect of reducing objective lens NA from NA = 0.85 ($\theta_{max} = 58°$) to NA = 0.3 ($\theta_{max} = 17°$) is presented. For the film on glass (or with a thin buffer layer), reducing NA resulted in a slight decrease in lifetime – as would be expected for an isotropic distribution of dipoles in which there are few dipoles oriented perpendicular to the substrate. In contrast, with a thick underlying buffer layer, the observed lifetime dropped by ~30% as the NA was decreased. This provides clear evidence that dipoles perpendicular are making a strong contribution to the observed lifetime.

A final supporting argument in favor of a change in dipole orientation for RH6G is the lack of any change in fluorescence for the larger molecule (MEH-PPV). Previous work has shown that the forces involved in spin-casting force the dipole moments of this large chain polymer to lie in the substrate plane [17].

3.3. Thickness dependence and its implications

This leads to three questions. 'Over what thickness range of PMMA do changes in fluorescence occur?' and, 'Are changes in lifetime related to the shift in spectrum?', and thirdly, 'Over what range can we estimate the thickness of the transparent PMMA layer in the bilayer PS/PMMA film by monitoring the change in fluorescence of the fluorophores embedded in the PS layer?' Fig. 3 presents the effect of the thickness of the PMMA layer on the lifetime, spectral coefficient and flux observed through the 60x objec-

tive lens for the fluorophores embedded in the 13.5 nm thick PS films. For MEH-PPV fluorescence – lifetime, spectral and flux, is nearly independent of the thickness of PMMA except for a slight variation for buffer layer thicknesses <30 nm.

In contrast, the fluorescence lifetime of the Rh-6G doped PS increases continuously as the underlying PMMA thickness increases and saturates for thicknesses greater than 500 nm (Fig. 3(a)) while the spectrum (Fig. 3(b)), shifts to the blue as the thickness of the PMMA spacer layer increases and saturates for thicknesses greater than 100 nm. The decrease in flux (Fig. 3(c)) occurs over thickness range similar to the change in lifetime suggesting that these effects are correlated.

In order to better quantify the relationship between thickness and fluorescence the spectral coefficient (S) and fluorescence lifetime (τ) data for Rh-6G doped films were fit by an exponential functions (Eq. (3)) to obtain a characteristic thickness over which the change occurs. Results are summarized in Table 2. Based on the above fitting, it can be seen that the spectral coefficient changes on a length scale of ($\Delta_o = 44$ nm) thickness while the fluorescent lifetime varies on a much longer length scale ($\Delta_o = 221$ nm). The different length scales involved with the suggest that the changes in lifetime are connected with the changes in flux but not related to the changes in spectral coefficient.

We believe that the shift in spectrum (the Huang-Rhys factor does not change) is unrelated to changes in flux and lifetime as (1) the length scale in question is different (44 nm (spectral coefficient) versus 221 nm for lifetime/flux) and (2) changes in spectrum are also seen in the PL of MEH-PPV in the range for PMMA thicknesses of 0 to 30 nm. The lack of change in spectral shape suggests that this is similar to the solvochromatic effect described by the Lippert-Mataga equation and is due to the slight difference in the substrate index of refraction (1.5) and that of PMMA (1.6) affecting the effective index of refraction for the fluorophores in the thin layer of PS. As discussed by Sebastion (in [23] and previous work) the distance dependence between the substrate and the embedded Rh6G (or MEH-PPV) molecules is governed by a d^{-4} relation (rather than d^{-6} as for point sources) allowing the effects of the substrate to have an influence in emission out to ~30 nm.

Well a detailed explanation for dipole re-orientation is beyond the scope of this paper, we would like to propose a possible mechanism based on the recent work of Torkelson's group [22]. In their work, stiffness in PS films supported on silica was found decrease as the film thickness increased to 250 nm after which it remained constant (this critical thickness was temperature dependent). An additional stiffness gradient was found at the air/PS interface extending approximately 20–50 nm into the PS film. While correlation does not indicate causality, the similar length scales involved suggest stiffness gradients may be the mechanism for reorientation of the Rh6G molecules. Such a gradient would not be expected to have an effect on the more bulky polymer.

3.4. Implications and applications

Finally, we would like to consider some possible applications of this effect. While the PS/PMMA system is of great interest in and of
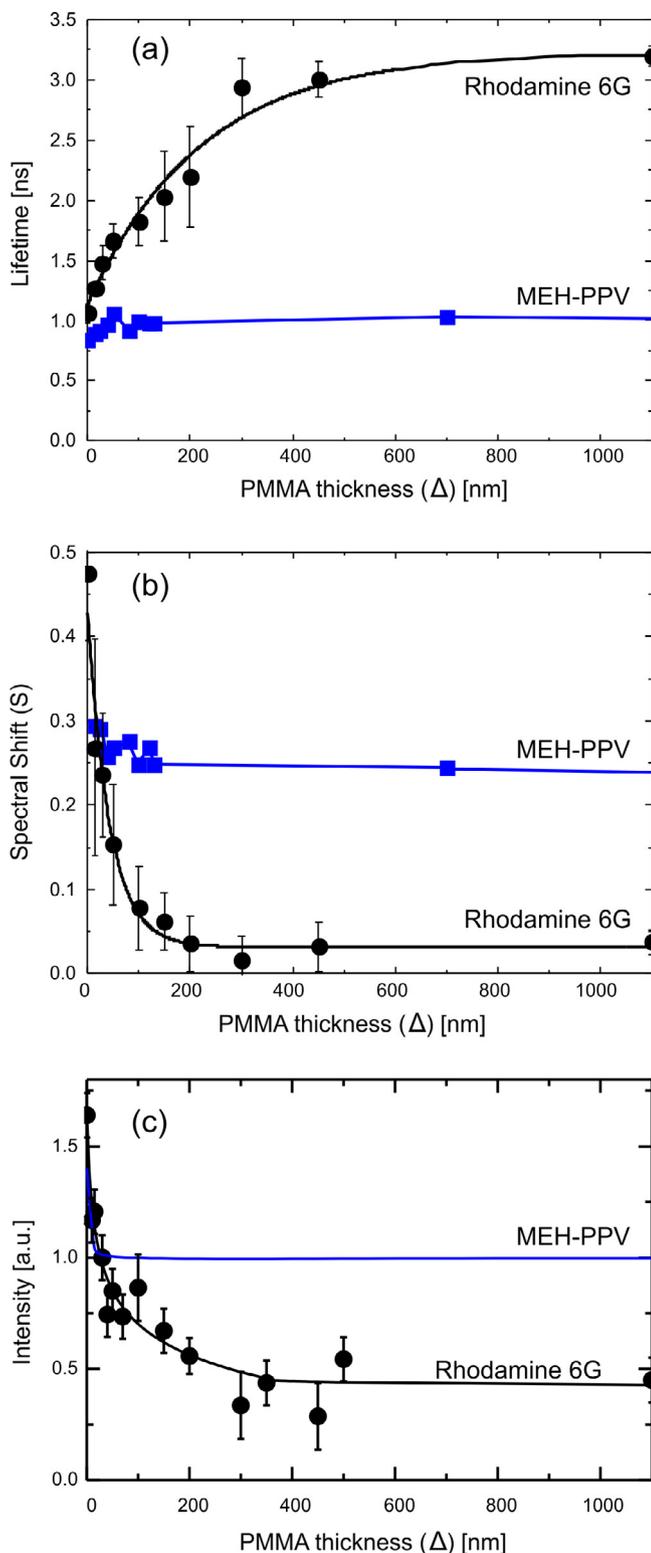
Fig. 3. Emission properties of Rhodamine 6G (black circles and lines) and MEH-PPV (blue squares and lines) doped Polystyrene (PS) thin films on a glass substrate as a function of PMMA thickness (Δ). (a) Fluorescence Lifetime (τ). (b) Spectral Shift (S). (c) Flux. The lines for the Rhodamine 6G data represent exponential curve fits (with characteristic distances of 44 and 221 nm for the spectral coefficient and fluorescence lifetime respectively) while those for MEH-PPV are to direct the eyes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Fitting parameters used to describe the variation of spectral coefficient (S) and fluorescence lifetime of Rh6G embedded in a 13.5 nm thick polystyrene (PS) matrix as a function of PMMA thickness.

Item	A	B	Δ_o (nm)
Spectral Coefficient (S)	0.03	+0.4	44
Lifetime (τ)	3.2	−2	221

itself (see Ref. [10] and the numerous papers it cites), we believe that this dependence of the fluorescence of Rh6G embedded in PS on the thickness of the underlying PMMA layer in PS/PMMA bilayer films as well as being a caution also has potential application beyond this immediate system. As a caution, when this bilayer structure is used as a means to characterize energy transfer as in Refs. [7–9], one must either employ a capping layer above the chromophore layer (as done in [8]), utilize a large molecule, like MEH-PPV, whose lifetime is not affected by buffer layer thickness, or ensure that the lifetime changes are small compared to the effects one is trying to observe (as in [9]). As an application, by using orthogonal solvents, the top layer can be cleanly removed. This opens the possibility of using fluorescence as a means to monitor film thickness as a complementary technique to ellipsometry and Atomic Force Microscopy (AFM). Concerning ellipsometry, while it is a powerful method for determining film thicknesses, it is an indirect method where a model analysis must first be preformed and the thickness obtained by interactive fitting of the data. Reliable results are difficult to obtain when either (1) there are variations in layer thickness on the sub-millimeter level as focused laser spot sizes are of the order of 100 by 40 μm due to the need to interact with the samples at an oblique angle [18], or (2) there is little contrast provided by refractive index differences. Concerning AFM, high lateral resolution allows variations of thickness to be mapped with nanometer lateral resolution in a small area of the film. Fluorescence mapping (lifetime and spectral shift) which supports a lateral resolution of 0.3 μm in the visible spectral region along with the ability to quickly observe large areas, is useful when lateral resolution higher than ellipsometry is required over a range greater than is convenient with AFM. Fluorescence may also prove useful as an independent measurement of thickness for films with little refractive index contrast. One caveat to this is that one must be able to find a solvent orthogonal to the layer whose thickness is to be determined. For example, in the investigated system, while embedding RH6G in PS allows the PMMA thickness to be evaluated, the reverse process, dissolving RH6G in PMMA to monitor PS thickness is not possible since all known solvents capable of dissolving PMMA also dissolve polystyrene (PS). Finally, in terms of application, we note that the ability to influence the orientation of emission dipoles is of considerable concern in the construction of light emitting diodes (OLED) due to the trapping of up to 80% of emitted light inside the thin film structure. While improving outcoupling technologies is one way of reducing loss, controlling the direction of light emission through dipole orientation is an alternative strategy to address this problem intrinsically and is currently being pursued by groups such as that of Rene Janssen [20] and Mark Thompson [21]

4. Conclusions

In conclusion, the fluorescence lifetime of Rh6G embedded ~13.5 nm of polystyrene in bilayer PS/PMMA films was found to

increase drastically as the thickness of the underlying PMMA layer increased from 0 to 500 nm. Coupled with the decrease in flux with increased lifetime, this suggests a reorientation of dipoles perpendicular to the substrate as the underlying layer increases in thickness. As well as shedding insight into the important PS/PMMA system, this work suggests that fluorescence – lifetime and spectra – microscopy can provide a complementary method to ellipsometry and atomic force microscopy for the nondestructive measurement of the thickness of transparent films.

Acknowledgement

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