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Title

Experimental evaluation of diffusion constant in a thin polymer film by triplet lifetime analysis of single molecules

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Abstract

We propose a method for dynamically sensing oxygen concentration in a local nanoscale domain of a thin polymer film using histograms of photon interdetection times of single molecules. By temporally analyzing the histograms, a dynamical change in the triplet lifetime of single molecules in thin polymer films with different thicknesses can be measured with respect to the oxygen concentration in a gas cell. From the time lags for observing the triplet lifetime change in the histograms due to differences in the oxygen diffusion times in the films, we estimate the diffusion constants in a local nanoscale domain of films, which approximately corresponds to the reported values in the bulk film. Thus, we conclude that this method is applicable to in situ nanoscale environmental sensors with high spatial resolution in heterogeneous materials, such as polymer blends, biological cells, and thin-film devices.

1. Introduction

Since single molecules/nanocrystals are widely used in ultrasmall and thin devices, such as DNA optical-mapping devices [1], single-photon sources [2], pH sensors [3], light-emitting devices [4], and transistors [5], single-molecule spectroscopy is being extensively employed for evaluating and improving the performance of these devices in various fields ranging from biomedicine to photonics. Because the performances of these small devices strongly depend on the nanoscale environmental and morphological conditions, it is essential to obtain local environmental information of these small devices with high spatial resolution. In particular, oxygen molecules play important roles in various optical devices and biological samples via the effects of oxygen quenching on emission processes and of oxidative photobleaching or radical oxygen species on biological tissues. Therefore, the information of the oxygen concentration in local nanoscale domains is beneficial.

For sensing the surrounding environmental information, the triplet state of single molecules has been useful, because the lifetime of the nonemissive triplet state is typically sensitive to the surrounding environmental conditions, such as collisions of oxygen molecules [6], the density of host polymer films [7], and electron transfer in host polymer films [8]. To determine the nonemissive triplet lifetime, several methods have been reported, *e.g.*, methods using histograms of off-time durations of triplet blinking [6,9,10] and time-autocorrelation function of fluorescence intensities [10,11-13]. Among these methods, we have employed a method that uses histograms of photon interdetection times (PIT) under the low-excitation condition [14-17]. This method can dynamically

and accurately determine the triplet lifetime, even for cases of low-photon count rates and short triplet lifetimes under the low-excitation condition, in comparison with previous methods. In our previous study, we successfully used this method to dynamically determine the triplet lifetime as a function of the change in the surrounding oxygen concentration [16]. Furthermore, we also confirmed that the change in the trap state due to a substrate on which quantum dots were dispersed strongly affected the emission dynamics of single quantum dots [17]. From these results, we expect that this method can be applied to dynamical local environmental sensors, because the triplet/trap state lifetime of single nanoemitters is sensitive to changes in local environmental conditions.

In this study, we performed PIT measurements to determine the dynamical oxygen diffusion in local nanoscale domains of a thin polymer film. The dynamical change in the triplet state lifetime of a single molecule in a thin polymer film was observed as a function of the oxygen concentration in a gas cell. Furthermore, from the time lags for initiating the change in the triplet lifetimes of single molecules in thin polymer films with different thicknesses in the order of μm, we obtained the diffusion constant of oxygen molecules, which approximately corresponded to the reported value in bulk film. These results suggest that we can monitor the oxygen diffusion in a thin polymer film, which has been difficult to evaluate with conventional methods. Therefore, we think that single-molecule spectroscopy combined with the PIT method can facilitate high spatial resolution observations of local nanoscale environments in heterogeneous materials such as polymer blends, biological samples, and small optical devices, which should provide important insight into industrial applications.

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2. Experiment

1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) molecules dispersed in a thin polymethylmethacrylate (PMMA) film were used in the experiments [16]. DiI molecules (10^{-9} mol/l, Lambda) and PMMA powder ($M_n = 48,300$, 1.0 wt%, Aldrich) were dissolved in toluene. Twenty microliters of the solution was spin-coated on a silica cover slip (22×22 mm^2) and then dried at room temperature in air (film thickness ~100 nm). We confirmed that approximately 10 DiI molecules were dispersed in a 5×5 μm^2 area of the prepared sample by observations with a confocal microscope. In addition, a water solution of polyvinyl alcohol (PVA, $M_w = 89,000–98,000$, Aldrich) was overcoated on the film and then dried in air at room temperature. We prepared 5- and 20-μm-thick PVA overcoated films; the film thicknesses were controlled by the concentration of the PVA water solution and determined by scanning-electron-microscopic observations after the measurements.

The experimental setup is shown in Fig. 1 [16]. The prepared sample was set on a piezo stage on a confocal microscope stage (IX-70, Olympus). A CW Ar$^+$ laser ($\lambda = 488$ nm, Showa Optronics) was used as the excitation light source with circular polarization; it was introduced into the microscope and focused onto the sample by an oil-immersion microscope objective ($100\times$, N.A. = 1.4, focal spot size ~ 430 nm). Typically, the excitation power was 60 W/cm^2. Emission from the sample was collected by the same objective and imaged onto a 100-μm-diameter pinhole (1 μm at the sample plane). Then, this emission was passed through a notch filter to eliminate the excitation

light, after which the emission was detected by an avalanche photodiode (APD, SPCM-AQR-14, EG&G). The time intervals between two adjacent photon detection pulses from the APD were recorded by a time interval analyzer (PCI-6602, National Instruments; time resolution 12.5 ns). The sample was covered by a gas cell, and nitrogen gas with constant flow (1.6 l/s) was injected into the gas cell to control the temporal oxygen concentration. The oxygen concentration in the gas cell was measured by an oxygen monitor (Taiei Denki), which was placed at a distance of 15 cm from the sample. Using this setup, emission images were measured by scanning the sample two dimensionally, and the locations of single DiI molecules were specified. The center position of the bright spot corresponding to each single DiI molecule was able to be estimated with an averaged deviation of about 5 nm using a two-dimensional Gaussian function fitting [18]. After selecting a specific single molecule, the time intervals were recorded. Finally, PIT histograms and time traces of the fluorescence intensity were constructed with the desired time resolutions on a computer.

We can determine the triplet lifetimes by fitting the PIT histogram data with a double-exponential function. From refs. 14 and 16, if the molecule can be treated as a three-level system and the experiment is conducted under the weak-excitation condition (that is, the excitation rate is considerably smaller than the decay rate of the excited state), the histogram of interdetection times is theoretically given by a double-exponential function as

$$d(t) = A_1 \exp\left[-\left(I_{on} + I_b\right)t\right] + A_2 \exp\left[-\left(k_t + I_b\right)t\right], \tag{1}$$

with

$$I_{on} = \Phi \varphi_f k_{ex},$$ (2)

where $d(t)$ is the histogram of interdetection times, A_1 and A_2 are arbitrary constants, k_t is the relaxation rate from the triplet state, k_{ex} is the excitation rate, I_b is the background count, φ_f is the fluorescence quantum yield, and Φ is the detection efficiency. The first decay rate I_{on} denotes the photon count rate when the molecule is not in the triplet state, while the second decay rate k_t directly corresponds to the decay rate of the triplet state. Therefore, the triplet lifetime can be directly determined from the second decay rate by fitting the histogram.

3. Results and discussion

Figure 2 shows the typical time traces of fluorescence intensities from single DiI molecules in films overcoated with PVA of different thicknesses: (a) 0 μm, (b) 5 μm, and (c) 20 μm. Initially, the gas cell was filled with air (O_2 concentration ~ 21.0%). Then, nitrogen gas was injected after a lapse of 120 s. In air (0–120 s), the intensity fluctuated around 30–40 counts / 10 ms for all samples, and the intensity fluctuations did not reach the background level (1–2 counts / 10 ms), because oxygen quenching at this high oxygen concentration caused the triplet lifetime of the molecule to be a few hundred microseconds [19], which was less than the binning time of 10 ms. After nitrogen gas was injected, the intensity fluctuations reached the background level after 180 s for the sample without the PVA film coating (Fig. 2(a)) and after 360 s for the sample with the 5-μm-thick PVA film coating (Fig. 2(b)). This behavior inferred that the decreased oxygen concentration in the film resulted in a

longer lifetime of the nonradiative triplet state. However, no change was observed in the time trace for the sample with the 20-μm-thick PVA film coating (Fig. 2(c)) and the intensity fluctuations did not reach the background level even after 600 s, which is the longest time our system could measure. These results suggest that the temporal behavior of the intensity fluctuations strongly depends on the thickness of the overcoated PVA film.

To analyze this phenomenon more quantitatively, from the data in Fig. 2, we measured histograms of the interdetection times for samples with different PVA film thicknesses ((a) 0 μm, (b) 5 μm, and (c) 20 μm), as shown in Fig. 3 [16]. In the figure, a histogram was obtained for each 60-s period in Fig. 2, and each dot in the histogram was binned in 125-μs intervals. The histograms were measured at 30-s intervals, and we plotted the histograms at 60-s intervals from bottom (0–60 s) to top (540–600 s) in Fig. 3. Solid lines indicate the single- or double-exponential fitting function. Initially (0–120 s), the histograms exhibited single-exponential decays, and the second decay component was not observed for all samples. Because these decay rates are equivalent to I_{on} in the time traces in Fig. 2, oxygen quenching would cause the relaxation rate from the triplet state (k_t) to be less than I_{on} [19]. Note that the triplet lifetime could be determined even in air, because I_{on} can increase if the excitation power increases. After sometime, the second decay component (k_t) appeared at different times for different PVA thicknesses (Fig. 3(a) and (b)) because of the decrease in oxygen concentrations. However, in the case of the sample with the 20-μm PVA film coating, no change was observed in the histograms even after 600 s, as seen in Fig. 3(c). This result suggests that it took more than 600 s for oxygen molecules to diffuse within the 20-μm-thick PVA film, and oxygen molecules could not be evacuated from around single

DiI molecules in that time. Note that the fast decays did not change for all samples over the entire 600-s measurement period, which means that the excitation conditions did not change during this time.

To further discuss the influence of film thickness, Fig. 4 shows temporal changes in the triplet lifetimes for samples with different PVA film thicknesses. In this figure, circles, triangles, and squares represent the triplet lifetimes of samples with overcoated PVA film thicknesses of 0 μm, 5 μm, and 20 μm, respectively. Note that when the histograms appeared as single-exponential functions, the inverse of the exponent (I_{on}^{-1}) was plotted instead of the triplet lifetime. The solid line indicates the oxygen concentration in the gas cell, which started decreasing when the nitrogen gas injection began at 2 min, and it decreased to 1.7% by the end of the measurement. Before the nitrogen gas injection, the triplet lifetimes for all samples with different PVA thicknesses almost corresponded to each other. After nitrogen gas was injected, for the sample without the PVA film, the change in the triplet lifetime almost followed the change in the oxygen concentration, and the lifetime became approximately 5 ms. Note that even when the thickness of the PVA film was 0 μm, there was a time lag from the starting time of the nitrogen gas injection. This time lag represents the time for gas replacement in the gas cell and PMMA thin film. When the sample was overcoated with the 5-μm-thick PVA film, there was a further time lag for observing the change in the triplet lifetime. On the other hand, when the sample was overcoated with the 20-μm-thick PVA film, no change was observed in the triplet lifetime during the measurement time, because it took more than 600 s to evacuate oxygen molecules around single DiI molecules. Thus, because the

time lag for observing the change in the triplet lifetime depends on the thickness of the overcoated PVA film, the differences among the samples with different PVA thicknesses can be attributed to differences in the oxygen diffusion time through the thin PVA film.

From these experimental data, we estimated the diffusion constant from the simple relation $D \sim d^2/t$, where D, d, and t are the diffusion constant, PVA film thickness, and diffusion time in the PVA film, respectively. Figure 5 shows the PIT histograms of different single DiI molecules in the center part of the same film overcoated with 5-μm PVA film. The time lags for the start of the change in the triplet lifetime have a certain distribution ((a) 260 s, (b) 380 s, (c) 140 s). Thus, we measured 10 different single molecules for the sample without and with a 5-μm-thick PVA film, and the averaged time lags were 140 ± 90 s (without PVA) and 230 ± 110 s (5-μm-thick PVA film). The time lag for the sample without PVA film coating represents the time for gas replacement in the gas cell and diffusion in the DiI-dispersed PMMA thin film. From the difference between the time lags for the samples without and with a 5-μm-thick PVA film, the diffusion time of oxygen molecules in the 5-μm-thick PVA film was approximately estimated as ~90 s (diffusion constant $\sim 2.6 \times 10^{-9}$ cm^2/s). For a bulk PVA film, the diffusion constant was reported to be on the order of 10^{-12}–10^{-9} cm^2/s [20, 21], which strongly depended on the relative humidity and temperature. Although values of the diffusion constant will fluctuate in nanoscale domains because of local heterogeneity, the estimated diffusion constant approximately agreed with the reported value in the bulk film. Considering this, we thought that the oxygen diffusion in a local nanoscale domain of the film was measured accurately. Furthermore, for the 20-μm-thick PVA film, because the diffusion

time was estimated to be 1500 s using the estimated diffusion constant, and this time scale exceeded our measurement time, we could not observe any change in the triplet lifetime. Thus, we concluded that single DiI molecules analyzed by the PIT method accurately probed the local oxygen concentration and readily evaluated the diffusion constant in a local nanoscale domain of the film. Note that we measured different single DiI molecules at different locations in the film and observed the deviations in time lags. We considered that these deviations would be due to film inhomogeneity, which suggests that the present method could probe differences in local environments of thin films.

For some applications, photo bleaching of the single molecules may be a problem. In this experiment, 12% of single DiI molecules survived for the whole measurement time (10 min.). This problem can be solved by reducing the pump laser power with the cost of the signal to noise ratio. Optimization of the excitation wavelength [22], choice of a more robust molecule, and an intermittent observation will be also useful to overcome this problem.

4. Conclusion

We proposed a method that uses histograms of photon interdetection times from single molecules to dynamically sense local environments (oxygen concentrations) in a thin polymer film. Using this method, we observed that following the decrease in the oxygen concentration in the gas cell, the triplet lifetimes of single DiI molecules increased with time lags depending on the thickness of the overcoated PVA film. From these time lags, we estimated the oxygen diffusion constant, which approximately

corresponded to the reported diffusion constant of oxygen molecules in bulk PVA film. The results suggest that oxygen diffusion in a local nanoscale domain of a thin PVA film could be dynamically monitored using single DiI molecules by the PIT method. Thus, we expect that this method can be applied to in situ nanoscale environmental sensors with high spatial resolution in heterogeneous materials such as polymer blends, biological cells, and thin-film devices.

Figure captions

Figure 1. Experimental setup.

Figure 2. Time traces of fluorescence intensity fluctuations of a single DiI molecule in a PMMA film overcoated by a PVA film of the following thicknesses: (a) 0 μm, (b) 5 μm, and (c) 20 μm. Nitrogen purging started at 2 min to decrease the oxygen concentration.

Figure 3. Histograms of photon interdetection times of a single DiI molecule in a PMMA film overcoated by a PVA film of the following thicknesses: (a) 0 μm, (b) 5 μm, and (c) 20 μm. These histograms were obtained from the results in Fig. 2 for each 60-s period (dots) from 0 s to 600 s. Solid lines represent the single- or double-exponential fitting function. The histograms were measured at 30-s intervals, and only the histograms at intervals of 60-s intervals from 0 s are presented from bottom to top.

Figure 4. Temporal change in triplet lifetimes of a single DiI molecule in a PMMA film overcoated by a PVA film, as determined by the fittings shown in Fig. 3. Circles, triangles, and squares indicate samples with PVA film thicknesses of 0 μm, 5 μm, and 20 μm, respectively. The solid line indicates the oxygen concentration in a gas cell measured by an oxygen monitor.

Figure 5. PIT histograms of different single DiI molecules in a same film (PVA film

thicknesses 5 μm).   Nitrogen purging started at 2 min to decrease the oxygen

concentration. Arrows indicate the starting point of the change in the triplet lifetime.

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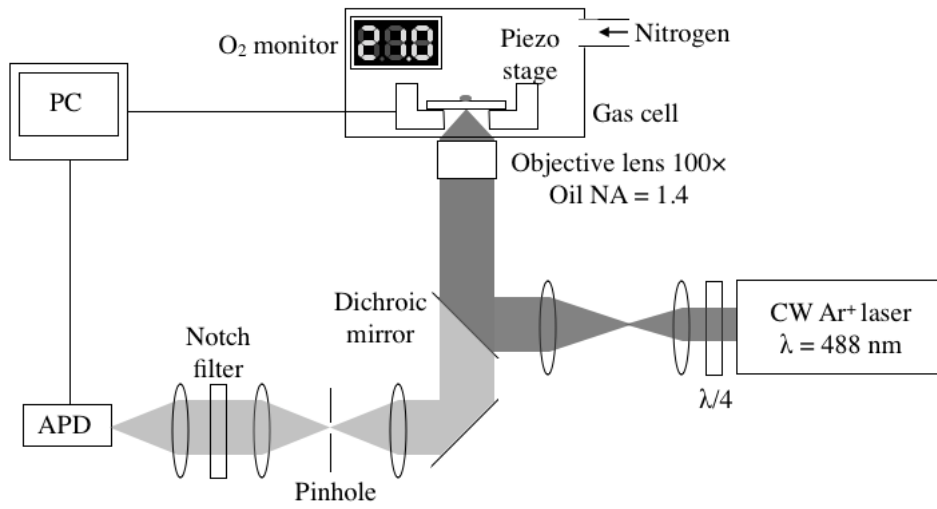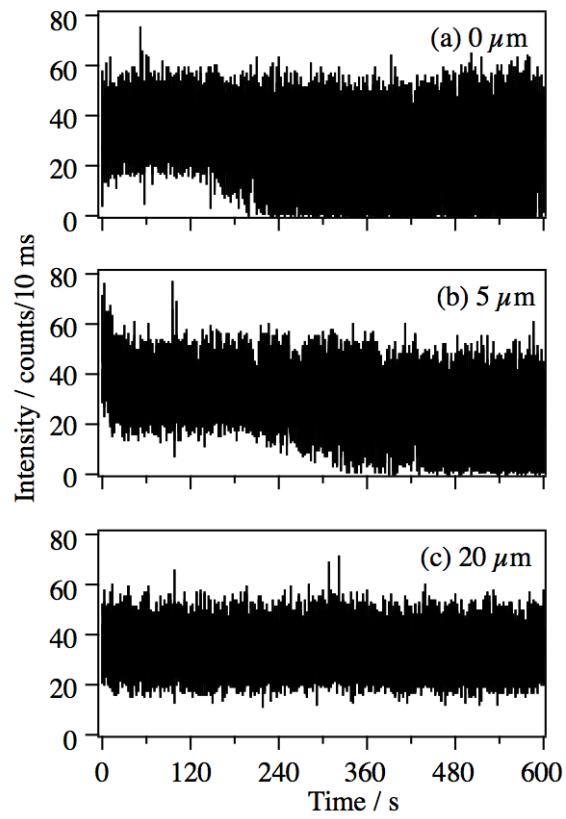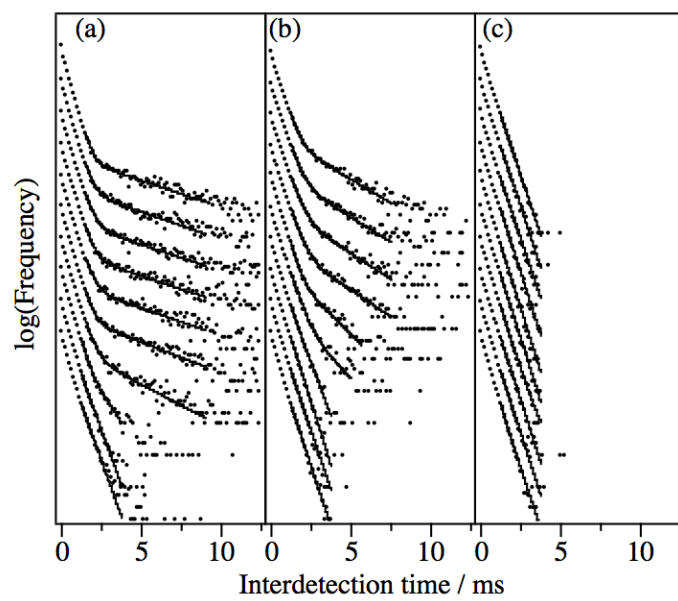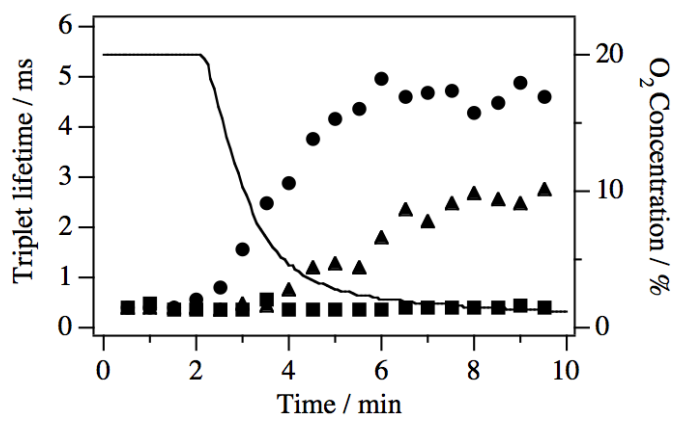
Figures

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