

# Single dye molecules in an oxygen-depleted environment as photostable organic triggered single-photon sources

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We demonstrate, that under nitrogen atmosphere, 20% of single DiIC<sub>18</sub>(3) (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) molecules in poly(methylmethacrylate) show an extremely low photobleaching quantum yield of  $(4.66 \pm 0.07) \times 10^{-8}$  together with a reasonably short triplet lifetime. We exploit these properties to demonstrate that the system can be used to produce a triggered single-photon source based exclusively on organic materials.

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Ensemble and single-molecule fluorescence experiments with organic dyes are limited by photobleaching. Photobleaching irreversibly terminates the emission of a single emitter in a single step after a period of fluorescence emission. Typical photobleaching quantum efficiencies for organic dye molecules range between  $10^{-5}$  for molecules in solvents,<sup>1</sup> over  $10^{-6}$  in polymeric hosts,<sup>2-4</sup> and the extraordinarily small value of  $10^{-8}$  for molecules in a molecular crystal.<sup>5</sup> Much effort has been devoted to the understanding of photobleaching and finding ways to decrease the photobleaching quantum efficiency. This would strongly benefit all types of single-molecule experiments ranging from biophysical<sup>6</sup> to quantum optics and communication experiments.<sup>7</sup> The current understanding, though far from being complete, suggests an important role of singlet oxygen that is created in the immediate vicinity of the molecule by self sensitization involving triplet-triplet annihilation<sup>8-10</sup> This view is supported by the fact that the triplet-state lifetime is strongly reduced in the presence of oxygen.<sup>10,11</sup> Moreover, the photobleaching quantum yield of rhodamine 6G in oxygen-depleted polymer films was found to be dependent on the excitation intensity. Here, the triplet state is thought to be a precursor for the photobleaching reaction.<sup>12,13</sup>

In this letter, we report that for a subensemble of organic dye molecules embedded in a  $\sim 10$  nm polymer film in a nitrogen atmosphere, a 60 times decreased photobleaching quantum yield, even at elevated excitation intensities is observed. At the same time, the triplet lifetime is low enough to allow considerable fluorescence emission rates. We demonstrate that for single emitters, count rates of 40 000 photons/s can be maintained for as long as 500 s. Start-stop experiments<sup>14</sup> performed with ps-pulsed excitation of single dye molecules in the polymer matrix show clear antibunching characteristics and demonstrate a single-photon source based on organic materials.

Samples are prepared from a cosolution of poly(methylmethacrylate) [(PMMA) 10 mg/ml] and DiI [1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiIC<sub>18</sub>(3)),  $10^{-9}$  M, obtained from Molecular Probes,

Leiden, The Netherlands (D-282)] in toluene by spin coating onto cleaned microscope cover slips. The resulting films have a thickness of 10 nm as measured by atomic force microscopy. Prior to spin coating, the cover slips are baked at 500 °C in an ambient atmosphere for 6 h in order to remove fluorescent contaminants. Importantly, blank samples consisting of polymer films without DiI were prepared for each measurement and investigated in parallel to ensure the absence of contaminations.

The molecules were excited at an average power of 4 kW cm<sup>-2</sup> at a wavelength of 532 nm by a ps-pulsed frequency-doubled Nd:Van laser (GE-100, Time-bandwidth Products, Zurich, Switzerland, pulse width 8 ps, 80 MHz repetition rate) and focused to a diffraction limited spot by an oil immersion microscope objective with a numerical aperture of 1.3. The small active area of a single-photon counting module [(SPCM) AQR-13, Perkin Elmer, Fremont, CA] served as the confocal pinhole in the detection path. Fluorescence images were recorded by raster scanning the sample through the focus and counting the photons for each pixel within a selectable integration time.

To remove ambient oxygen from the sample, a tube of about 1 cm inner diameter was fixed closely ( $< 1$  mm) above the sample and connected to a nitrogen gas bottle. A laminar flow of nitrogen gas was established to remove the ambient oxygen. The removal of oxygen is a very fast process due to the fast diffusion of gases in PMMA.<sup>10</sup> To re-establish ambient oxygen, the tube was removed from the sample and the nitrogen supply was turned off.

Figures 1(a) and 1(b) show subsequent scans recorded at 10 ms pixel integration time of the same area illustrating the characteristic difference in the emission of single DiI molecules in PMMA in the presence and absence of oxygen. The images are scanned from the bottom to top and the lower half of Fig. 1(a) was recorded in the presence of ambient oxygen. The molecules fluoresce brightly with peak count rates exceeding 100 counts/ms. Triplet blinking and photobleaching, evident from occasionally visible dark pixels and half-moon-shaped spots, respectively, demonstrates the observation of individual molecules. As the nitrogen flow is turned on after half way through the scan (arrow), a strong increase in the triplet lifetime is observed. This is obvious from the hardly

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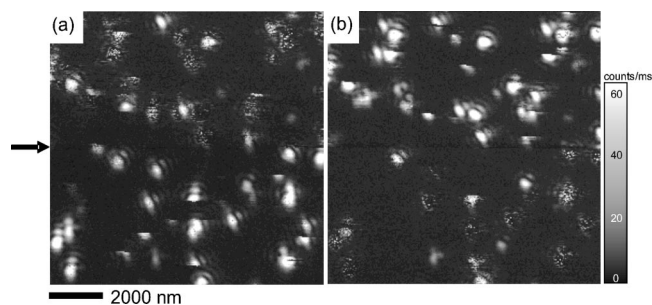


FIG. 1. Scanning confocal optical microscopy of single DiI molecules in PMMA. Scan direction bottom to top,  $256 \times 256$  pixels, integration time 10 ms/pixel. (a) and (b) show subsequent scans of the same area. Lower half in (a) and upper half in (b) recorded without supplying  $N_2$  (ambient oxygen present). In the presence of nitrogen, for most of the molecules, the triplet lifetime increases instantaneously; obvious from the hardly visible fluorescence patterns. Note the existence of a subensemble of molecules (bright spots) that are hardly affected by the absence of oxygen.

visible fluorescence patterns that appear to be interrupted by long and frequent dark periods. The duration of the triplet dark states estimated from the duration of dark periods is about 100–120 ms, in agreement with previous findings.<sup>2,3,9,10</sup> In Fig. 1(b), the same area is scanned, however, with the  $N_2$  supply in the lower half and ambient oxygen in the upper half showing the reverse behavior as in Fig. 1(a). This demonstrates the reversibility of the observed triplet quenching effect.

Surprisingly, even in the absence of oxygen, roughly 20% of all molecules still appear as bright undisrupted patterns in the image in Fig. 1. The obviously short triplet lifetime, in spite of the absence of the triplet quencher oxygen, indicates that for such molecules alternative pathways for reverse intersystem crossings do exist.<sup>15</sup> The peak emission rate with decreased triplet decay rate is slightly reduced as expected within the steady-state description of a three-level system. Accordingly, we find that the maximum pixel count rate detected on average from 21 molecules in the presence of oxygen is about  $140 \times 10^3$  counts/s, and  $120 \times 10^3$  counts/s under nitrogen flow. The fluorescence emission behavior of this group of molecules was thus hardly distinguishable from those of molecules in the presence of oxygen and is investigated further in the following. It is important to note that here we take full advantage of the fact that in a single-molecule experiment, one can select only those molecules for further analysis which show a desired behavior. This is a unique feature of single-molecule experiments and strongly improves the accuracy and reliability of results.

We have selectively investigated the photobleaching quantum yield of the species with the apparently short triplet state lifetime under nitrogen and compare the results to the case of molecules in the presence of oxygen. Toward this end, we performed repetitive line scans ( $1 \mu\text{m}$ , 256 pixels, 5 ms/pixel) over single fluorescence molecules until the molecule photobleached (see Fig. 2). Data with and without oxygen were taken from the same sample. The resulting images were stored on a personal computer and processed with Igor Pro (WaveMetrics, Inc., Oregon). To determine the total numbers of emitted photons  $N$ , for each molecule, the most frequent pixel count rate at background level was subtracted for each line scan and the remaining counts were integrated. In the presence of oxygen, molecules were selected ran-

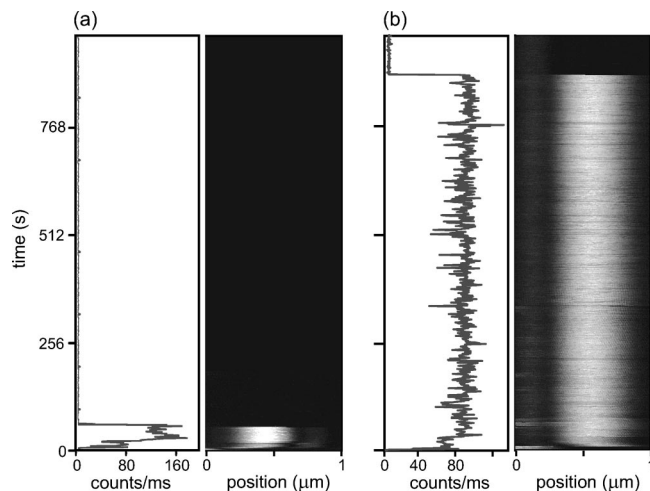


FIG. 2. Successive line scans over single DiIC<sub>18</sub> ( $1 \mu\text{m}$ , 256 pixels, 5 ms/pixel) with an excitation flux of about  $4 \text{ kW cm}^{-2}$  until photobleaching occurred. (a) Sample exposed to oxygen, (b) sample under laminar nitrogen flow.

domly. In the oxygen-depleted polymer under the nitrogen flow, we selected the species of DiIC<sub>18</sub> which exhibited a triplet lifetime short enough so that they yielded an appreciable count rate above background also in the absence of oxygen at a pixel integration time of 5 ms.

Figure 2 compares typical traces of repetitive line scans of single DiI in [Fig. 2(a)] the presence of and [Fig. 2(b)] absence of oxygen. The molecule in contact with ambient oxygen in Fig. 2(a) maintained about  $100 \times 10^3$  counts/s peak pixel intensity for about 40 s. The molecule shown in Fig. 2(b) in an oxygen-depleted environment showed a peak pixel intensity of approximately  $80 \times 10^3$  counts/s for roughly 800 s. Note again the decrease of about 20% in the pixel count rate. Following the analysis described in a previous study,<sup>5</sup> we have quantitatively determined the photobleaching quantum efficiency  $\phi_b = (\eta\phi_f)/N$  from the total number  $N$  of photons detected from each molecule. The detection efficiency of our setup is  $\eta = 0.065$ . For the fluorescence quantum yield, we chose  $\phi_f = 1$  which probably underestimates the resulting photobleaching quantum yields.

Figure 3 shows the distributions of  $\phi_b$  resulting from about 250 (oxygen) and 120 (nitrogen) molecules. Visual inspection immediately shows that the photobleaching quantum yield is strongly increased in the presence of oxygen. For a quantitative assessment of the data, we fitted the distributions in Fig. 3 using the distribution function  $P(\phi_b) = c \exp(-\phi_0/\phi_b)/\phi_b^2$ . Here,  $c$  is a normalization constant and  $\phi_0/2$  is the most probable photobleaching quantum efficiency. This distribution function is derived by assuming that the survival probability of a molecule decreases exponentially with time and that the number of detected photons is proportional to this survival time. In the presence of oxygen, we obtain  $\phi_0^{\text{oxygen}} = (2.9 \pm 0.2) \times 10^{-6}$ , whereas in the presence of nitrogen the fit yields  $\phi_0^{\text{nitrogen}} = (4.96 \pm 0.1) \times 10^{-8}$ . This means that the photobleaching is reduced under a nitrogen atmosphere compared to ambient conditions by a factor of  $\phi_0^{\text{oxygen}}/\phi_0^{\text{nitrogen}} = 60$ .

Since the photostability of the observed subensemble of DiI molecules in PMMA under a nitrogen atmosphere reaches high values for organic systems,<sup>5</sup> our results are of

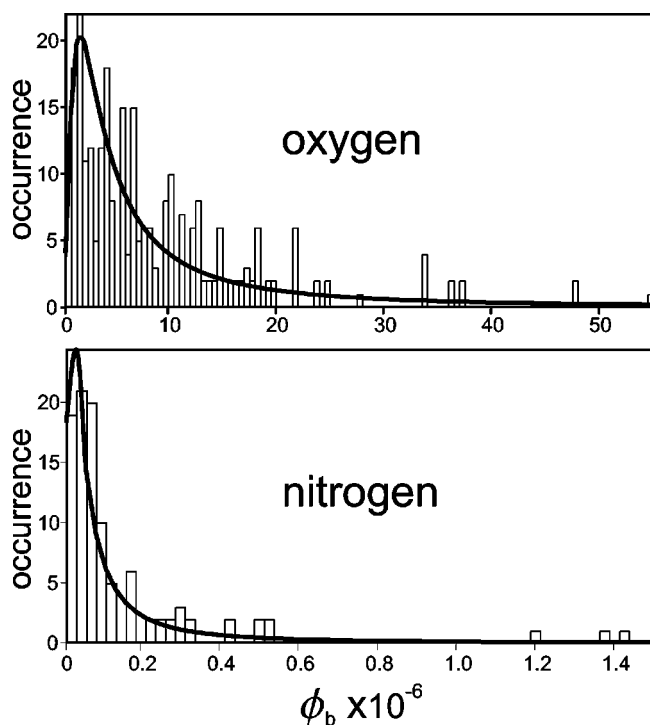


FIG. 3. Histogram of photodestruction quantum efficiency  $\phi_b$  of 250 (oxygen) and 120 (nitrogen) individual photobleached molecules. The smooth curves are fits to the data using the distribution function  $P(\phi_b) = c \exp(-\phi_0/\phi_b)/\phi_b^2$ .

potential interest with respect to quantum optical experiments with single molecular quantum systems. To demonstrate this, we employ a single DiI molecule as a triggered source of single photons.<sup>7</sup> Toward this end, we split the fluorescence light of a single molecule by a nonpolarizing beam-splitter in two equal-intensity beams and focus them to two separate SPCMs to realize a Hanbury–Brown and Twiss detection scheme.<sup>14</sup> The output pulses of the SPCMs, corresponding to single-photon detection events, are fed into a time-correlated single-photon counting computer card (Timeharp 200, Picoquant, Berlin, Germany) where they serve as start and stop triggers, respectively, for the measurement of interphoton times. Figure 4 shows a typical histogram of interphoton times obtained from a single DiI molecule in PMMA under a nitrogen atmosphere. The distance of the peaks corresponds well with the expected time difference between subsequent laser pulses of 12.5 ns. The width of the peaks is given by the excited-state lifetime of DiI and a contribution due to the instrument response (see dotted curve in Fig. 4). The missing peak at zero time delay indicates a negligible probability for the emission of more than one fluorescence photon per excitation laser pulse. This clearly demonstrates a triggered source of single photons based on the organic chromophore/matrix system DiI in PMMA.<sup>7</sup>

We have found a sizeable subensemble of DiIC<sub>18</sub> molecules in PMMA (20%) which under a nitrogen atmosphere on average emit about  $10^6$ – $10^8$  photons before photobleaching while still showing a reasonably short triplet lifetime. We

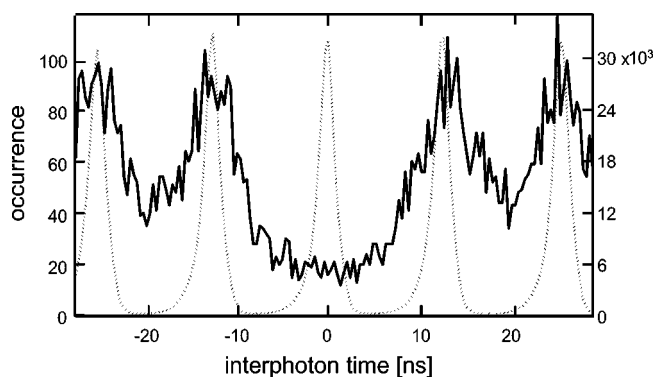


FIG. 4. Histograms of interphoton times. Solid curve (left-hand side scale) recorded with a single DiI molecule in PMMA under nitrogen atmosphere within 20 min. The dotted curve (right-hand side scale) is the instrument response function recorded with scattered laser light only (10 min integration time). Binwidth for both curves is 350 ps.

speculate that specific conformations of DiIC<sub>18</sub> molecules in PMMA related to *cis/trans* isomerization lead to this behavior.<sup>3,15–17</sup> The immense photostability of the system opens possibilities for applications in photonics, biology, and quantum communication.

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