Photobleaching of Fluorescent Dyes in Polymer Films

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Abstract

Photobleaching is measured on millisecond time scales in Rhodamine 6G in SAN 25. The data is found to obey a double exponential decay on time scales of tens to hundreds of milliseconds. Photobleaching is studied for different intensities and temperatures. If is found that the photobleaching decay rate increases until saturation at incident excitation intensities of about 100 kW/cm². In the region of lower intensities, increasing ambient temperature increases the rate of photobleaching.

Introduction

Current optical data storage (ODS) technology utilizes reflection as a means to read data. Figure 1 contains microscope images of CD, DVD, and Blu-Ray (BR) discs. There are pits in the surface of the disc which are used to store data. When in operation, the disc is spinning, and as a laser is scanned across the disc, the pits will scatter the light. The variation of reflected light intensity translates into bits. The storage capacity is limited by the size of the pits and the separation between the layers. BR players increase storage capacity by decreasing the wavelength of the laser and decreasing the spot size of the focused beam. However, since light scattering is the method of read out, bits are limited to the surface of the disc, or at best, to only a few layers.

Co-extruded polymer films have the potential to be a useful media for ODS. The advantage of a polymer film is the ability to store on multiple layers, thus, increasing storage capacity. A multitude of layers can also be made in large areas at very low cost, compared to commercial techniques, which require several different deposition processes. If fluorescence (FL) is the method of readout, the beam will penetrate further into the sample and storage capacity can be enhanced by storing data in three dimensions. Successful writing of data has been demonstrated in a coextruded polymer film of PETG containing the dye C18-RG². Figure 2 (left) shows confocal microscope images of separate layers in the film. The images are written with a BluRay laser by bleaching the dye. Each layer can be separately resolved clearly with limited cross talk between each layer.

Figure 2 (right) is a diagram of the co-extrusion process used to make the films. Two polymers are melted and stacked on top of each other. Then, they are pushed through layer multipliers, which cut
the stack in half and flow the two resulting stacks on top of each other. Each time the stack goes through a multiplier, the number of layers is doubled. The process is repeated until the desired number of layers is reached and the result is an A-B sequence of polymer layers. One polymer is optically active, and will be used to store data while the other provides a barrier between optically active layers to allow for the layers to be resolved in a confocal microscope. At the end of the process two protective layers are added to the surface of the film.

Although writing and reading of data has been achieved, the physical mechanisms of photobleaching are not yet understood. For ODS, a material with a nonlinear response during writing and little response during reading is required. Photobleaching does not normally exhibit these properties, but it has not been studied for C18-RG. Such a response might be obtained due to the localized laser-induced heating of film during writing. The goal is to study the effects of temperature on the bleaching to determine where the rates begin to be change. This will not only help in determining if the laser heating can reasonably affect the bleaching during normal writing conditions, but possibly also help to identify the possible mechanisms that are leading to bleaching.

Figure 1: Microscope images of CD, DVD, and Blu-ray discs with the associated focused laser beam.
Figure 2: (left) Confocal microscope images of pictures drawn in a co-extruded film through photobleaching. Each square is a separate layer in the film. (right) Diagram of the co-extrusion process.

Theory

Dye Levels and Transitions

Figure 3 is a general model of dye levels and transitions taken from a paper by Eggling et al.\textsuperscript{3}. The molecule starts in the ground singlet state represented by $s_0$. The absorption of photons transition the molecule from the ground singlet to higher singlet states represented by $s_n$. With sufficient energy, vibrational states, $s_1^\prime$, can be excited. The $t_n$ states represent triplet states, which the molecule can transition to after being excited into higher singlet states. The rate between the first excited singlet and the triplet, $k_{isc}$, is called the intersystem crossing time. It is proposed that many bleaching reactions occur from the first excited triplet state. B represents the bleached state, a permanent non-fluorescing state due to some change in the molecules.
In a paper by Talhavini and Atvars, photobleaching was studied at different temperatures in fluorescein and Rose Bengal dissolved in poly (vinyl alcohol) (PVA). In this study they found an increase in the rate of photobleaching with increased temperature. The increase in rate was attributed to an increase in a dye-dye chemical reaction involving electron transfer between two dye molecules initially in the triplet state. The result of the chemical reaction are two dye molecules, an oxidized molecule and a protonated semireduced molecule. There can be further reactions involving these molecules and other triplet molecules as well.

Talhavini and Atvars worked in a cryostat without oxygen. Therefore, any expected reactions involving oxygen were neglected in their study. In a series of papers by Zondervan, the effects of oxygen as well as reactions involving the host matrix studied. The study involved Rhodamine 6G in PVA. It was speculated from this study that oxygen had two effects on photobleaching, that it reacted with metastable states and increased photobleaching but also reduced photobleaching by reducing the lifetime of those states. As for the PVA host matrix, it is suspected that an electron transfer occurs from PVA to the Rhodamine 6G molecule to form a radical anion which doesn’t fluoresce.

Reverse Saturable Absorption
It is important, for an ODS dye, that it be a reverse saturable absorber. Saturable and reverse saturable absorption is explained, for example, in a paper by Srinivas et. al. At low intensities, if the lifetime of the first excited singlet state is short enough, any excited molecule will fluoresce and go back to the ground state. However, if a buildup of molecules in the singlet state occurs, photons can excite molecules into higher singlet states. If the absorption of the higher energy transitions is low, the dye will become transparent at higher intensities. Such a dye is called a saturable absorber. A reverse saturable absorber will absorb more photons at high intensities. A reverse saturable absorber would aid in the ODS process because it reduces destructive reading. A dye can be read at low intensities, and if there is no bleaching, it can be read as many times as necessary without losing the data. Assuming bleaching occurs from higher singlet states only, bleaching can be enhanced in a reverse saturable absorbing dye if the absorbance of those higher singlet states is high.

**Experimental Methods**

The sample is a film (thickness about 2 μm) of the polymer SAN 25 doped with Rhodamine 6G. The concentration of the dye is 0.12 wt. %. Essentially, the apparatus is a home built confocal microscope which measures the fluorescence of the sample as it is exposed to incident laser light. A diagram of the setup is given in figure 4. The source light is a 405nm BD laser diode (cw). A pinhole is used to eliminate higher order diffraction from the grating inside the apparatus of the diode itself. The beam travels through a beam splitter into a microscope objective. The resulting fluorescence from the sample is collected through the same objective and the beam splitter reflects the fluorescence away from the initial path. A filter is located after the beamsplitter to suppress residual excitation light and an additional lens focuses the fluorescence to an avalanche photodetector.
The fluorescence is monitored in time after the incidence of the cw excitation source. The laser is unblocked by hand (about 1 ms rise time) and photobleaching is measured by the decay in the fluorescence after the sample is exposed to light. The sample is mounted on a glass slide coated with Indium Tin Oxide (ITO) connected to a variac in order to heat the sample. Measurements are performed at temperatures from room temperature and up. Temperatures of about 75 °C can be achieved in this setup before the ITO coated slide begins to crack.

**Results and Discussion**

Figure 5 (left) shows a typical photobleaching curve. Due to the complicated processes involved in the photobleaching, fitting to an exact theory is difficult. Phenomenologically, the behavior of the curve is captured well by a double exponential fit. The quality of the double exponential fit is strongly dependent on the time scale fitted. Here, the time scale being investigated is on the order of tens to hundreds of milliseconds. Any bleaching on longer time scales appear as a constant. Fits incorporating longer times result in rates that are clearly to slow and don’t fit the first milliseconds of the curve well.
For different powers and at 25 °C and 50 °C, the fast rate constant from the fits are shown in figure 5 (right). Error bars are estimated from the standard deviation of multiple trials (typically about 8). At room temperature, the bleaching rate increases with power until saturation. This likely happens because all the states reach equilibrium on these time scales. Figure 6 shows the first 50 milliseconds of high and low temperature bleaching curves at that 50 kW/cm². The high temperature curve clearly has a faster rate for the first few milliseconds. As the power increases further, though, the rate levels off and actually bleaches slower than at 25 °C, which could be due to a reduction in the dye concentration during the heating process. At larger powers however the errors bars become very large since the decay is very fast. The two temperatures at the low powers, however, show a clear discrepancy.
Figure 6: Photobleaching curves at 50 kW/cm² for three different temperatures

The fast rate of the 75 °C curve is very similar to the 50 °C curve, though there is a large drop in amplitude at 75 °C. The slower decay constant also appears a bit smaller. Both of these latter effects are probably due to the evaporation of material during heating. Unfortunately, higher temperatures could not be obtained because 75 °C is the maximum temperature achievable before the ITO slide begins to crack.

Some mechanisms that can be affected at low temperatures, below the glass transition, level off quickly. As discussed by Zondervan, the intensity dependence of photobleaching can yield insight into the rates of higher energy singlet and triplet states. Since the higher energy levels are very reactive, the fact that photobleaching levels off at large intensities is an indication that either the populations have saturated or that the rate has increased beyond the resolution of the system, which is about a 1-ms based on the turn on time of the laser. In the previous studies, there is no leveling off of the rate even though similar intensities are used³, so to study the higher intensities relevant to ODS it is likely a better setup is required.

Conclusion

Most of the rate change with temperature occurs up to around 100 kW/cm², and the rate is similar for both 50 °C and 75 °C. The rate increases by at most a factor of 2 for intermediate intensities. Higher temperatures may give more insight into the physical mechanism because the properties of the polymer host would change drastically around the glass transition temperature. There are many possible physical
mechanisms that may be causing photobleaching, and could be activated in different temperature regimes. The fact that the temperatures studied here are below the polymer glass transition is an indication of the nature of one of these mechanisms. This could be the dye-dye interaction or an accelerated S₁-T₁ or T₁-S₀ transition, which can increase with temperature independently of the polymer. Studying the effects of oxygen as well as going below room temperature and trying lower concentrations might help determine the actual mechanism.

Since it is proposed that a chemical reaction between oxygen and triplet dye molecules causes bleaching, it is important to investigate how the sample bleaches without oxygen present. In order to perform such an experiment a more sophisticated experimental setup must be used, such as a cryostat. A proper cryostat would also provide a means to heat to higher temperatures.

It is important to understand what happens at higher intensities to allow for the practical application of reading and writing in ODS. However, for this sample, the interesting physics is at lower intensities. This setup is not sensitive enough to detect fluorescence at much lower powers. A more sensitive detector is required. It would be difficult to detect fluorescence in samples with lower concentrations. This is important because the temperature dependence suggests that a dye-dye chemical reaction might be taking place. The dye-dye interaction can be eliminated completely if the molecules are not in close enough proximity to react which may be achieved with lower concentrations.

We have shown that the photobleaching increases at most by a factor of 2 from 25 °C to 75 °C at intensities below 100 kW/cm². In addition to determining the physical mechanisms involved in photobleaching, the temperature dependence is relevant to any practical application involving such high intensities where laser heating may be significant.

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Appendix: Design of Optical Setup

The problem of the experiment is to construct a setup that will measure photobleaching.

Constraints

- Must collect fluorescence while the sample is exposed with cw laser light
- Must be able to alter temperature and atmosphere

Approaches:

The first approach was to put the sample in a Leica Confocal Microscope. Such a microscope was available and eliminated the need to build anything. The problem with using the microscope, however, was that the laser scanned. It would be impossible to hold the laser in one place at a time. Such measurements would complicate the data too much. The next approach was to build a home-built microscope on an optical table. It would have to be a simpler setup but the laser would stay still.

Analysis:

The advantage of using the Leica, however, was that it was already built and could do a lot already. The setup wouldn’t have to be realigned constantly. It would be easy to attach an incubation system which could regulate the temperature and flow inert gas through it. Furthermore, measurements with different wavelength lasers could be performed at ease. Data analysis is the main complication of the Leica. Because of the complication of the data analysis, the home-built design was chosen. The Leica did prove to be useful as a means to easily perform preliminary measurements on different samples. Such measurements did not need a complicated data analysis procedure to understand generally what the experiment was going to be like.

The goal of the project was to measure photobleaching under different atmospheric conditions. Changing atmospheric conditions was the main complication of the home-built setup. The first approach to this problem was simply to attach an incubation system designed for a microscope to a stage on the optical table. However, since mounting the system became complicated, the temperature and atmosphere regulation was inefficient. The incubation system was replaced with a glass slide coated with ITO which could heat the sample sufficiently, but the measurements would have to be done in air.
Iterations:

Optimization of the setup involved testing different detectors, samples, and measurement techniques. The detector would need sufficient speed and sensitivity to detect the signal. Of what was available, an avalanche photodetector was the best option. It could detect speeds up to 100 MHz and the sensitivity was higher than any other detector. Different samples were tested to find which could yield the best measurements. The characteristics of the samples were the type of dye, the thickness, and the concentration. Some dyes fluoresce less than others and low concentration samples would fluoresce less than high concentration ones. For very low concentrations, the detector wouldn’t have the necessary sensitivity to detect the signal. Thin dyes were used to avoid the possibility of the detector collecting fluorescence from different depths in the sample, which would be exposed to different intensities if the Rayleigh length of the sample was much smaller than the thickness. After measuring the Rayleigh length, the necessary thickness could be determined. It turned out to be possible to make samples close to the Rayleigh length. If this were not possible, a pinhole would have to be added to the setup before the detector.

Standards:

The measurement was automated with LabView code. A VI was written which would sample from the signal outputted from the detector. The VI could control the sampling rate and could average multiple readings together. Both properties of the VI controlled the time resolution of the experiment. Testing had to be done to find a time resolution that matched the time scales of the experiment. Noise could be significantly reduced by averaging multiple scans, but if the resolution of the experiment was milliseconds and it was found that bleaching occurred on time scales of hundreds of microseconds, the number of averages would have to be adjusted. Different trials were run to maximize the signal to noise ratio while making sure the resolution was fast enough.
References


