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Photobleaching of disodium fluorescein in water

J. R. Saylor

Abstract Photobleaching of disodium fluorescein dissolved in water is experimentally investigated using laser induced fluorescence (LIF). It is demonstrated that significant photobleaching occurs on the millisecond time scale, resulting in a large decrease in the fluorescence signal emanating from a constant concentration sample. The importance of avoiding photobleaching when using LIF with disodium fluorescein for concentration measurements in water flow experiments is demonstrated. A half-life for photobleaching is introduced and measured for disodium fluorescein and is shown to be a more appropriate measure than the traditional 'bleaching quantum efficiency'. It is demonstrated that the photobleaching of disodium fluorescein is at least partially reversible.

1 Introduction

Photobleaching is the decrease in fluorescence intensity of a substance as it is exposed to light. When LIF is used to measure dye concentration in fluid mechanics experiments this decrease in fluorescence intensity is undesirable since it can erroneously be interpreted as a reduction in concentration. Measurements of the fluorescence emitted from samples of disodium fluorescein, subjected to laser irradiation, were performed to determine the magnitude and time scale of photobleaching.

2 Experimental method

The dye used was disodium fluorescein (Fisher Scientific Co., A-833) mixed to a concentration of 10^{-6} M in deionized water. Figure 1 shows a schematic representation of the experimental setup. The laser beam was focussed to a 33 µm measurement volume inside a 3.5 ml quartz cuvette. The beam was not attenuated by anything other than lenses as it traveled to the cuvette, and so the power delivered to the measurement volume is assumed to be equal to the 1 Watt laser output. The fluorescent radiation emitted by the disodium fluorescein in the measurement volume was focused onto two identical photomultiplier tubes (PMTs) (Hamamatsu, R928). One of these PMTs was used as a trigger signal to initiate data collection from the 'signal' PMT with a delay of 1 µs. Data from the signal PMT was digitized and recorded at 10^6 points per second. A yellow glass filter and a green short-wave-pass interference filter were placed in front of the signal PMT to ensure that light corresponding only to dye fluorescence, and not scattered laser light, was passed through to the PMT.

Experiments were run with the optical chopper (pulsed mode) and without the optical chopper (continuous mode). During the pulsed mode experiments, the pulse frequency was 1100 Hz and the pulse duration was 0.45 ms. The variation in laser intensity was less than 2% and the variation in the duration of the pulses was less than 1% for all runs. Error due to diffusion of dye into or out of the measurement volume during an experiment was less than 3%.

3 Results

The result of a sample continuous mode photobleaching experiment is presented in Fig. 2a. This plot is the fluorescence response of disodium fluorescein to a step function in laser light. The data are scaled to the maximum value of the run. The decrease in fluorescence intensity is rapid and nonlinear.

Previous researchers have quantified photobleaching using the bleaching quantum efficiency, B, which is defined as

$$B = \frac{N_b}{N_a}, \tag{1}$$

where N_b is the number of molecules bleached and N_a is the number of incident photons absorbed. This relation assumes that photobleaching proceeds linearly in time, which Fig. 2a discounts. This explains the huge discrepancies observed in the values of B measured by previous researchers (Ippen et al. 1971, Beer and Weber 1972, Britt and Moniz 1972, Weber 1973, Sahar and Treves 1977) who did their measurements over widely varying time intervals, and obtained values for B varying from 4.0×10^{-6} to 1.9×10^{-2}. Clearly B is an inappropriate measure of photobleaching, and a half-life, $\tau_{1/2}$, corresponding to the time interval between the peak response and one-half of the peak response, was used instead. The average value of $\tau_{1/2}$ was 2.9 ms for the continuous mode experiments.

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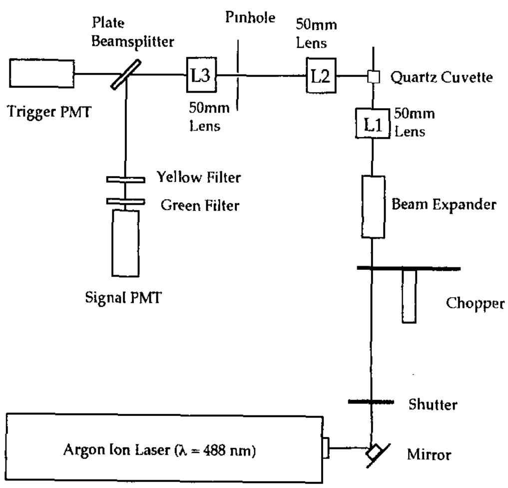

Fig. 1. Experimental setup

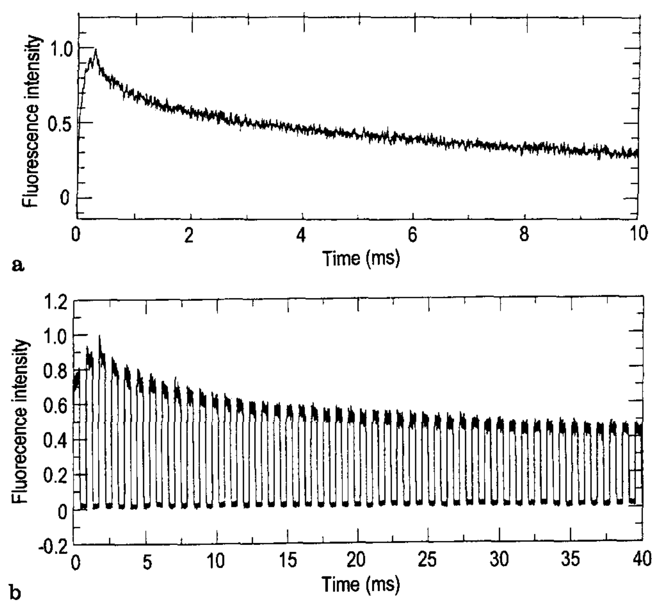

Fig. 2. **a** Sample plot of fluorescence response to continuous laser irradiation, **b** Sample plot of fluorescence response to pulsed laser irradiation

A sample pulsed mode photobleaching experiment is presented in Fig. 2b. The purpose of the pulsed mode runs was to see if $\tau_{1/2}$ would change when the laser was periodically blocked to allow time for photobleaching recovery. To compare the pulsed mode runs with the continuous mode runs, $\tau_{1/2}$ was computed after removing points corresponding to times when the laser was blocked. The average half-life for the pulsed mode runs was $\tau_{1/2}=12.3$ ms, about four times larger than for the continuous mode runs, indicating that a significant amount of photobleaching recovery occurred during the pulsed mode runs.

4
Discussion of results

The experiments presented above indicate that photobleaching of disodium fluorescein can result in a significant decrease in the measured fluorescence intensity after very short periods of laser irradiation (O(1 ms)). Photobleaching of this magnitude would have negative effects on LIF experiments where the measured fluorescence is assumed to be directly related to the dye concentration.

Various authors have demonstrated that photobleaching is influenced by irradiation intensity and dye concentration (e.g., Sahar and Treves 1977; Goldman 1960; Benson et al. 1985). Consequently, the photobleaching half-life data presented here are specific to the concentration and laser intensity used during the experiments. While the dye concentration used here is typical of that used in fluid mechanics experiments, the laser intensity used varies from study to study. The intensity is determined by the laser power and the laser beam waist. While the laser power used by many fluid mechanics researchers employing LIF tends to fall within the 1–10 Watt range (e.g. Dahm and Dimotakis 1987, Prasad and Sreenivasan 1990), the diameter of the beam waists can vary significantly. For example, Dahm and Dimotakis (1987) used a 1000 μm diameter beam waist, Long et al. (1992) used a 200 μm diameter beam waist, and Prasad and Sreenivasan (1990) used a 5 μm diameter beam waist. The beam waist for the work presented here was 33 μm, and researchers using beam waists of this size or smaller with a 1 Watt or greater laser power level should take steps to prevent contamination of their data due to photobleaching. The degree of photobleaching at lower intensities (i.e. smaller laser power and/or larger beam waists) is uncertain, and further work is necessary to quantify the effect of laser intensity on photobleaching.

A simple method for avoiding photobleaching at any non-zero fluid velocity is to pulse the laser beam for a period of time much shorter than the measured value of $\tau_{1/2}$. By separating the laser pulses by the residence time τ_r (the beam diameter divided by the local fluid velocity), photobleaching can be avoided without compromising the temporal resolution of the dye structures present in the flow. This technique is further described in Saylor (1993).

The half-life obtained in the continuous mode experiments was shorter than that obtained in the pulsed mode experiments, indicating that the photobleaching of disodium fluorescein is at least partially reversible. The fluorescence intensity at the beginning of a pulse was always greater than at the end of the preceding pulse. This increase in fluorescence intensity from the trailing edge of one pulse to the leading edge of the next was 20% shortly after the experiment was initiated ($t<10$ ms), and 3% after long times ($t>20$ ms), indicating that photobleaching recovery occurs, but diminishes as time progresses. After 20 ms, a dynamic balance between photobleaching and photobleaching recovery is approached, and the fluorescence signal approaches a constant value.

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