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TECHNICAL NOTE

THE USE OF SHORT LIVED FLUORESCENT DYES TO CORRECT FOR ARTIFACTS IN THE MEASUREMENTS OF FLUORESCENCE LIFETIMES

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Abstract—The ultrafast emission for fluorophores can be used as the effective excitation pulse for prompt response measurements by photo detectors in the same spectral region as that of unknown samples. This method corrects for such artifacts as wavelength and spatial dependence of the response function of the photodetector. It is shown that the emission from triphenylmethane dyes is an excellent effective pulse with relaxation time $\lesssim 2$ ps in the red region of the spectrum. A microchannel plate photomultiplier has only a 35 ps increase in response or lag time between excitation at 420 nm and emission at 680 nm.

INTRODUCTION

The availability of fast photon measuring devices and picosecond laser light pulses has opened a wide field of research on the photophysics of various molecules. Probably the simplest, yet highly useful, measurements are those of fluorescence lifetimes. This method allows a direct study of the properties of the excited singlet states of molecules. As the measurements have been refined, several sources of systematic errors have been found (see e.g. Demas, 1983). One of the most difficult to measure and correct is the wavelength dependence of the response function of some photomultipliers (see Demas, 1983, p. 174; Lewis *et al.*, 1973 and Rayner *et al.*, 1977). The situation is aggravated in the study of photosynthetic systems where excitation in the blue or near ultraviolet is followed by fluorescence in the far red. James *et al.* (1983) have suggested the use of a short lived fluorophore to mimic the excitation pulse shape at the desired emission wavelength. By exchanging the sample and fluorophore the desired fluorescence and the effective excitation pulse are measured under identical conditions. This technique has been applied only to the near ultraviolet and blue regions and to relatively long lived fluorophores with lifetimes in 1–5 ns regions. However, the fluorescence lifetimes in photosynthetic systems vary from 50 ps to 2 ns (Breton and Geacintov, 1980). Thus to be useful the fluorophore must have a very short lifetime and the emission must span a wavelength region including the far red. In addition, the compounds should be readily available and easy to purify. These rather esoteric requirements are met by the triphenylmethane dyes. The fluorescence lifetimes of these dyes had previously been investigated because of their strong dependence on viscosity of the medium (Yu *et al.*, 1977; Sundstrom *et al.*, 1982). In this note, it is shown that triphenylmethane dyes are excellent pulse fluorophores for emission in the red region of the spectrum for prompt temporal response measurements of photo-detection systems such as streak photon counting and pulse time resolved fluorimetry.

MATERIALS AND METHODS

To determine the fluorescence kinetic profile a CPM† ring dye laser and amplifier system (see Fork *et al.*, 1981) combined with a streak camera system were used. The laser pulse of 120 femtoseconds (fs) from the 114 MHz laser oscillator output had about 200 pJ pulse energy at 625 nm wavelength. This laser pulse train was sent into a four-stage dye amplifier pumped with a frequency doubled Nd:YAG laser at 1–10 Hz. Each amplified fs laser pulse has about 0.6 ps pulse duration and 1 mJ pulse energy. The streak camera system consists of a Hamamatsu model HTVC 1370-1 streak camera, a silicon intensified target vidicon camera, a temporal analyzer, and a video monitor. The performance and operating characteristics of the streak camera have been described elsewhere (see Tsuchiya, 1983; Inuzuka *et al.*, 1982; Ho *et al.*, 1984). The wavelength selection of the dye fluorescence signal was obtained by inserting color filters and Ditric narrow band filters in front of the input slit of the streak camera. The total system time resolution is about 2.5 ps.

The steady state time integrated fluorescence spectra of the samples were measured by using an Ar laser pump source, a SPEX ½ meter double spectrometer, and a PAR lock-in amplifier. The output power of the Ar laser was about 100 mW at 488 nm wavelength and the input slit width of the spectrometer was set to be 1 mm.

In the pulse-lifetime measurement technique, a pulse of light at 420 nm was generated from a 300 ps, 30 μW pulse of 337 nm light from an atmospheric pressure N_2 laser (Photochemical Research Associates LN100) incident on a

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†*Abbreviations*: CPM, colliding pulse mode locked; PPO, 2,5-diphenyloxazole.

simple dye laser containing PPO in dioxan. The fluorescence of the sample in a 1 mm cuvette was incident through filters on a microchannel plate photomultiplier, Hamamatsu R12940-01, operated at −2kV. The photosignal was measured by a Tektronix 7912AD digitizer. The sweep of the digitizer was triggered by a fraction of the excitation pulse incident on a fast photodiode, Lasermetrics 3711PD. Groups of pulses internally summed in the digitizer were transferred to a computer, HP9825, for further summation and baseline subtraction. Fit of the data was by re-iterative convolution (Mauzerall, 1984, see Demas, 1983). This measurement system was very stable. The random error of time resolution in this experiment is estimated to be about ± 20 ps.

RESULTS AND DISCUSSION

It has now been verified, using a sub-picosecond light pulse of 625 nm and ultrafast streak camera that both crystal violet and malachite green in water at room temperature have lifetimes of less than 2 ps (see Fig. 1). The emission spectrum of malachite green (Fig. 2) and crystal violet (Fig. 3) show that the wavelength range 600 to 740 nm can be covered by these two dyes. Measurement of the fluorescence of crystal violet on excitation with a dye laser pulse of 300 ps width at 420 nm using a micro-channel plate photomultiplier and a fast digitizer shows that the wavelength effect of this kind of photomultiplier is rather small: 35 ± 10 ps for the wavelength difference between 420 and 680 nm (Fig. 4). Although the data were best fit (least squares) by a combination of lag or time base shift (11 ps) a single lifetime (26 ps), the exact separation of these responses is not possible. Similarly, measurement with the 337 nm pulse directly from the atmospheric pressure nitrogen laser showed a response lag and/or lifetime of 60 ± 10 ps. A complication is a long-lived (2–3 ns) fluorescence (due to impurities?) excited by this short wavelength light which is poorly absorbed by these dyes. This long lived component is readily separated from the desired component by numerical analysis. The measurement of effects less than ½0th the halfwidth of the response peak is possible because of the excellent stability of the system.

CONCLUSIONS

The availability of such a short-lived fluorophore with ≤ 2 ps lifetimes allows its use as an "effective" excitation pulse in determining short and mixed

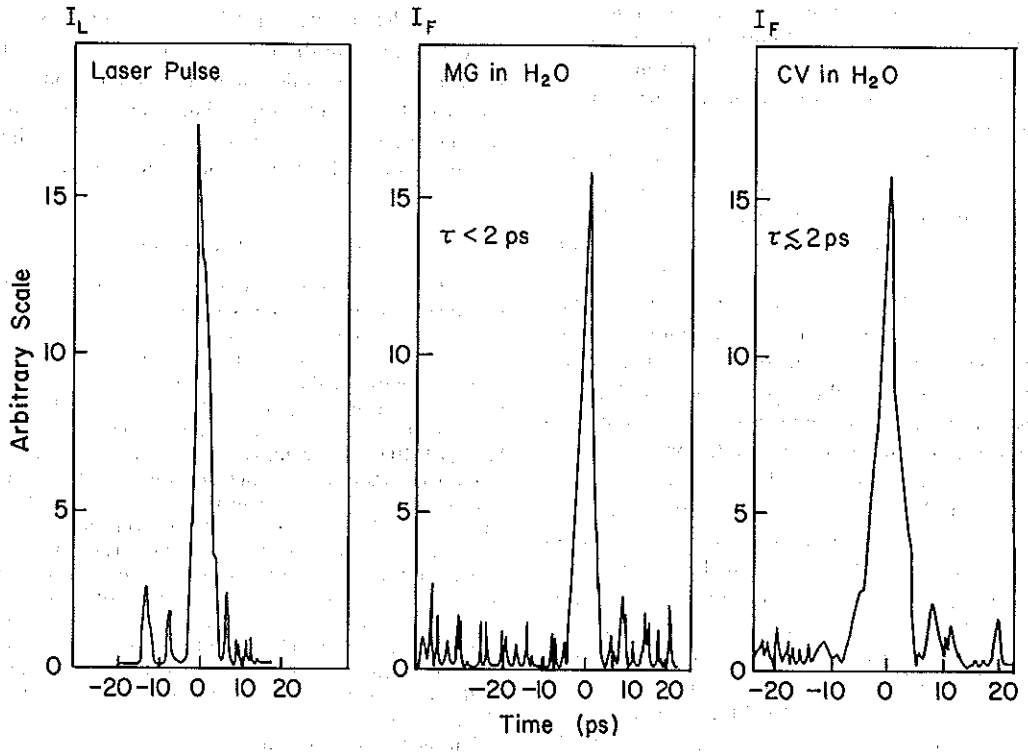

Figure 1. (a) A 0.6 ps laser pulse at 625 nm wavelength scattered from an aluminium plate measured by a Hamamatsu model HTV C1370-01 streak camera. The FWHM of this prompt curve is about 2.5 ps, (b) Fluorescence intensity decay profile of malachite green in water solution measured by a 2.5 ps (FWHM) time resolution streak camera. (c) Fluorescence intensity decay profile of crystal violet in water solution measured by a 2 ps (FWHM) resolution streak camera. The exciting laser pulse duration is about 0.6 ps and wavelength at 625 nm. The optical density of this sample at 625 nm is about 1.8 and the crystal violet concentration was estimated to be about 3×10^{-3} *M*. Both fluorescence decay profiles in (b) and (c) are not resolved with this time measurement system and the entire emission band was measured.

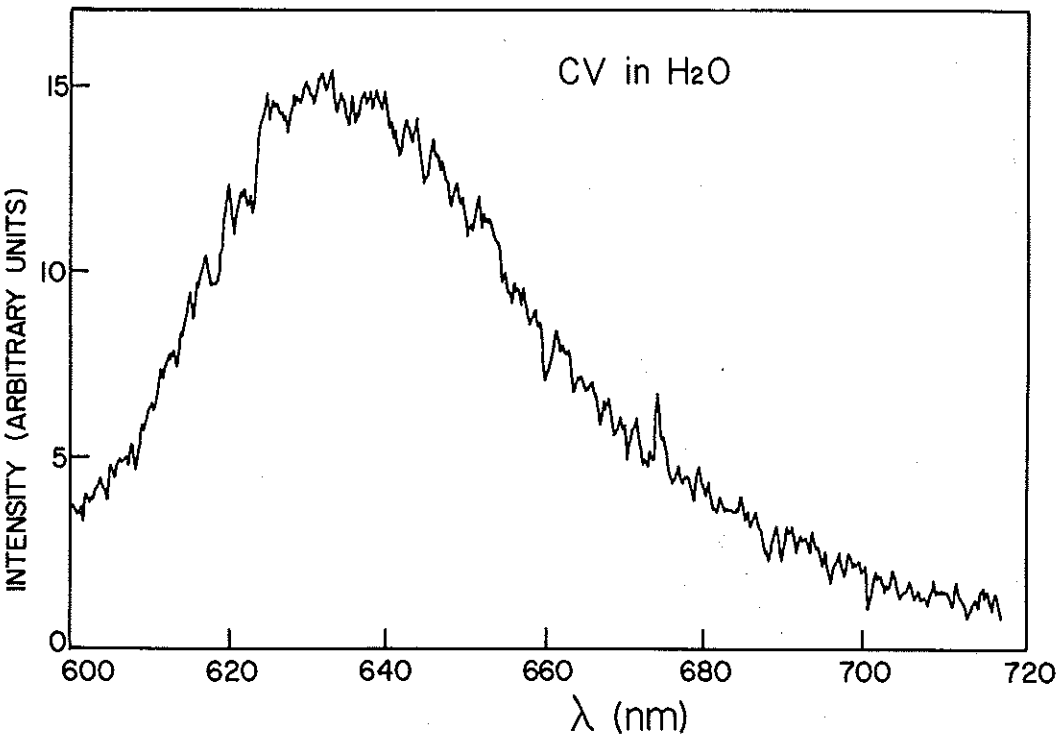

Figure 2. Fluorescence spectrum of malachite green in water excited by an argon laser at 488 nm wavelength.

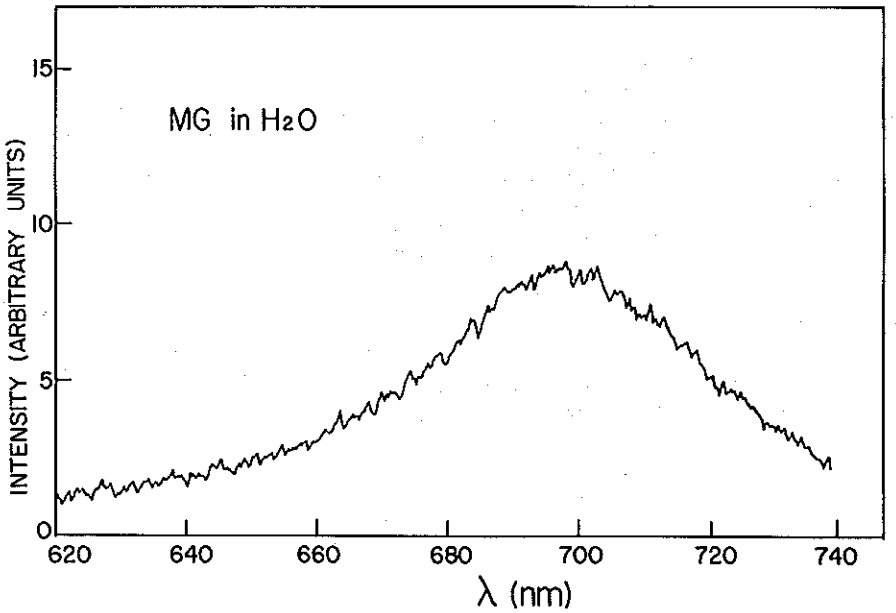

Figure 3. Fluorescence spectrum of crystal violet in water solution excited by an Ar laser at 488 nm wavelength.

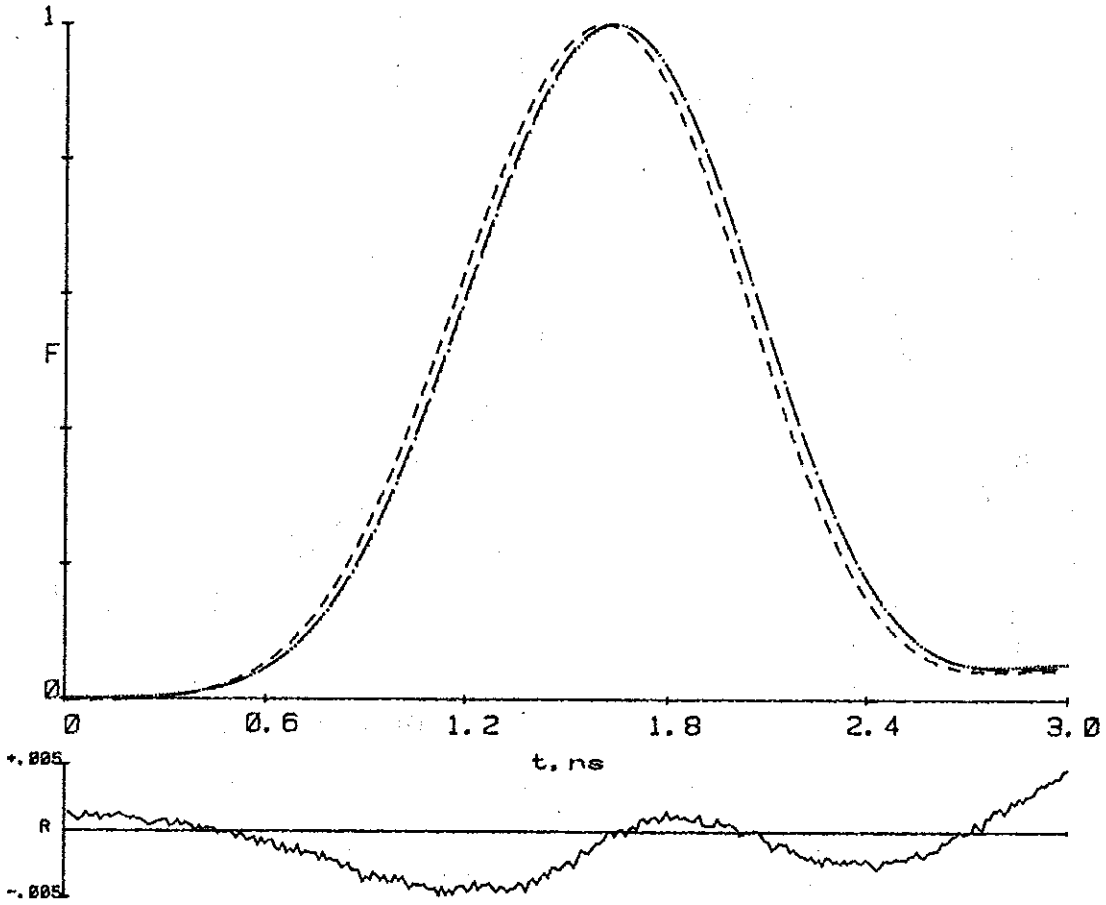

Figure 4. The fluorescence of crystal violet excited by a 420 nm dye laser pulse and measured at 680 nm with a microchannel plate photomultiplier. The short dashed line is the excitation pulse measured at 420 nm. The dots are the fluorescence of the crystal violet measured at 680 nm and the long dashes are the convolution of the excitation pulse by a lifetime of 26 ps together with a one channel shift (10.8 ps) of this pulse to longer times. The residuals (fluorescence minus the calculated values, normalized to the peak height) are plotted below. The measured values are the sum of 128 sweeps.

fluorescence lifetimes by various computational methods. This allows one to bypass corrections to the instrument response by making use of the associative property of convolutions. The response function cancels out of the calculation if, and only if, the excitation and response are measured under identical conditions. The use of an ultra-short-lived fluorophore with lifetimes $\lesssim$ 2 ps allows wavelength and spatial effects to be easily made very similar and thus to come closer to the ideal conditions. The triphenyl-methane dyes are excellent fluorophores for measurements on photosynthetic systems, porphyrins and other long wavelength emitters.

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