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Quenching of Fluorescence by Light: A New Method to Control the Excited-State Lifetimes and Orientations of Fluorophores[1]

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We report steady-state and time-resolved studies of quenching of fluorescence by light i.e., "light quenching." The dyes rhodamine B (RhB) and 4-dicyanomethylene-2-methyl-6-(*p*-dimethamino)-4H-pyrane (DCM) were excited in the anti-Stokes region from 560 to 615 nm. At a high illumination power the intensities of DCM and RhB were sublinear with incident power, an effect we believe is due to stimulated emission, and *not* ground-state depopulation. The extent of light quenching was proportional to the amplitude of the emission spectrum at the incident wavelength, as expected for light-stimulated decay from the excited state. Control measurements at a decreased average illumination power, and in solvents of various viscosities, indicated that the effect was not due to undesired photochemical processes. Importantly, the frequency-domain intensity decays remained single exponentials, and the lifetimes were unchanged with light quenching, which suggests that the effect was not due to heating or other photochemical effects. These results are consistent with a quenching process which occurs within the quenching pulse. Importantly, as expected for light quenching with a single pulsed laser beam, the time 0 anisotropies of RhB and DCM were decreased due to orientation-dependent quenching of the excited-state population. In closing we discuss some possible future applications of light quenching to studies of dynamic processes.

KEY WORDS: Light quenching; excited-state lifetime; fluorophores.

INTRODUCTION

The phenomenon of the quenching of fluorescence by light, or "light quenching," is the diminishing of the excited-state population by stimulated emission of atoms or molecules by external illumination. Early experiments carried out by Russian spectroscopists showed that light quenching required overlap of the quenching wavelength with the emission spectrum [3] and displayed the same photoselection rules as does excitation [4]. Mazurenko *et al.* [5] observed depolarization at high illumination intensities, apparently due to selective stimulated decay of the vertically polarized component.

To date essentially all studies of light quenching have been performed using the intense giant pulses from Q-switched lasers and using long quenching pulses which are comparable to continuous, nonpulsed illumination. We now show that significant light quenching can occur with the lower energy pulses from a cavity-dumped picosecond dye laser. Observation of light quenching with

[1] This report is partially based on the experimental data published previously [1,2].

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this commonly available laser source offers the opportunity to control the lifetime and orientation of the excited-state populations, which can have numerous applications of time-resolved fluorescence. We believe that the use of light quenching will enable a new class of fluorescence experiments, in which the sample is prepared by one or more quenching pulses prior to the time-resolved or steady-state observations.

THEORY

We consider a dilute solution of a fluorophore in the optically inactive solvent and assume that the absorption and emission oscillators of the fluorophore are colinear. We also assume that the viscosity of the solvent is high enough that significant rotational diffusion does not occur during the lifetime of the fluorophore's excited state. To describe the time evolution of the excited-state population during and after the excitation, we use a spherical coordinate system in which the fluorophore transition dipole makes an angle θ from the vertical axis and ϕ from the direction of illumination (Scheme I). We suppose that the angular distribution of the transition dipoles before excitation is uniform.

Assuming that the changes in the ground-state population are negligible, the excited-state population $n(\theta,t)$ is governed by

$$\frac{dn(\theta, t)}{dt} = NP(t)\,\sigma_a \cos^2\theta - n(\theta, t)\left[\frac{1}{\tau} + P(t)\,\sigma_{1q}\cos^2\theta\right] \quad (1)$$

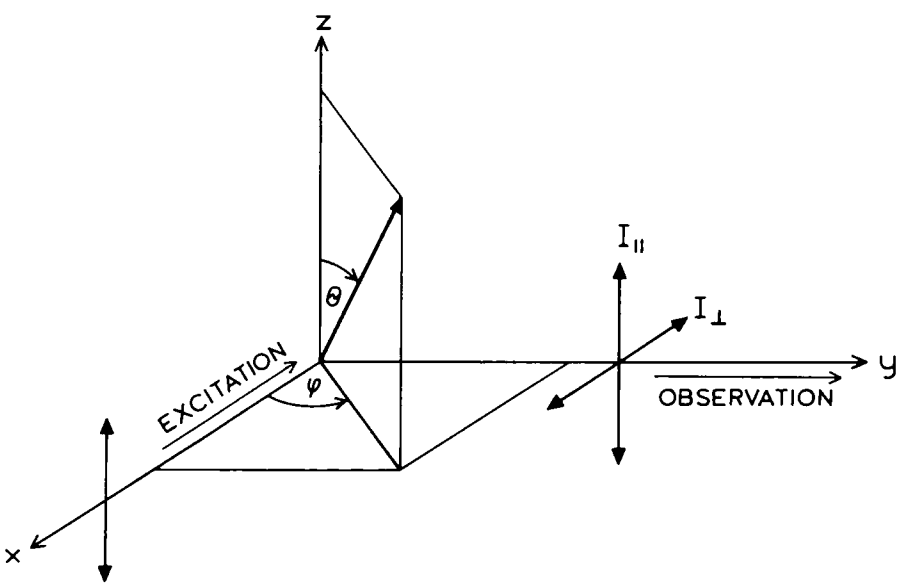
where N is the ground-state population, σ_a and σ_{1q} the absorption and light quenching cross sections, respectively, $P(t)$ the power density [photons/(cm^2/s)] of the incident light, and τ the unquenched lifetime. For short pulses Eq. (1) yields

$$n(\theta,t) = N\frac{\sigma_a}{\sigma_{1q}}\left(1 - e^{-W_p\sigma_{1q}\cos^2\theta}\right)e^{-t/\tau} \quad (2)$$

where

$$W_p = \int_0^\infty P(t)\,dt \quad (3)$$

is the number of photons passing the unit area of the sample during the single pulse. W_p is proportional to the laser power. Importantly, it follows from Eq. (2) that for short pulses $n(\theta,t)$ does not depend on the shape of the incident pulse. The polar plots of the function $n(\theta,t)$ in the absence and presence of light quenching are shown in Fig. 1. These plots show that the extent of orientation along the z axis (right) is decreased by light quenching.



Scheme I. Coordinate system for a fluorophore. The transition moment makes an angle θ with the Z axis and ϕ with the X axis.

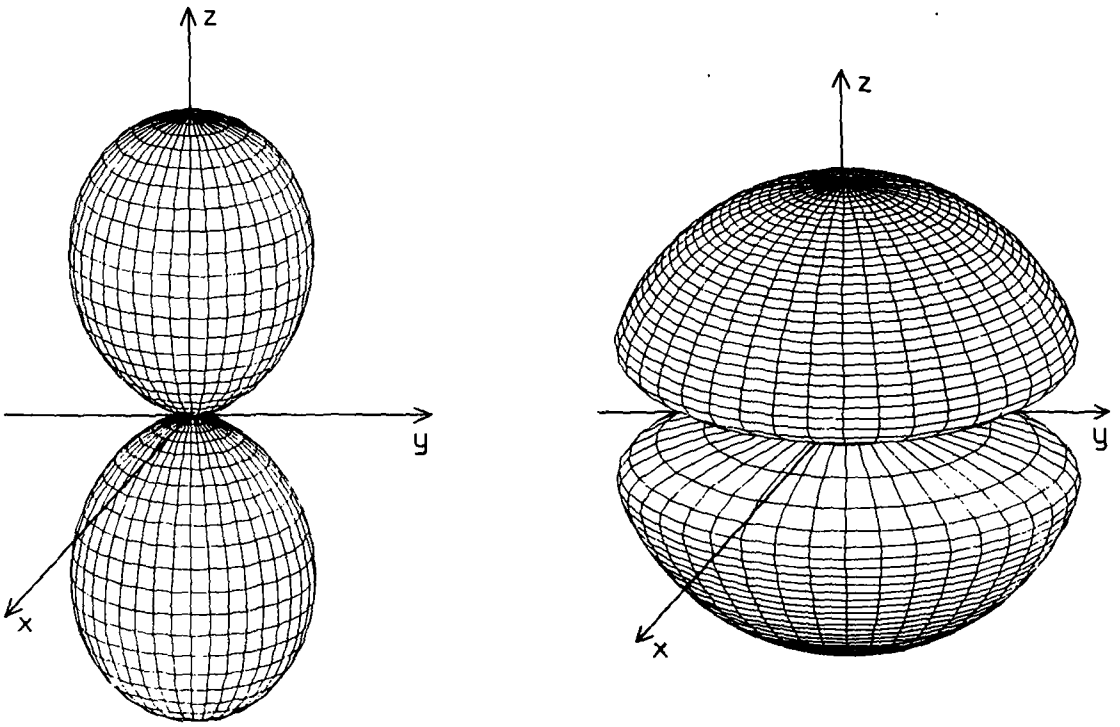
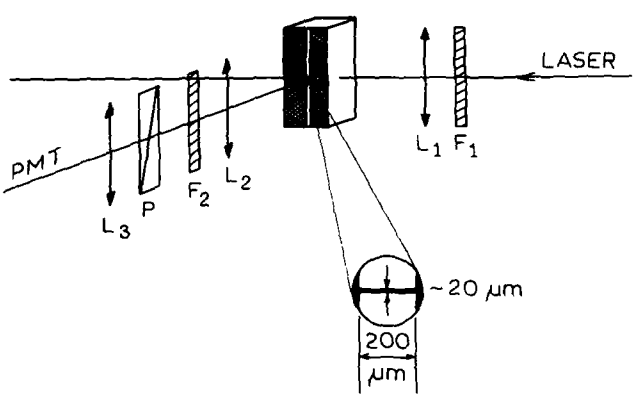


Fig. 1. Spatial distribution $n(\theta,t)$ [Eq. (2)] of the excited fluorophore transition dipoles immediately after excitation ($t = 0$) for $W_p\sigma_{1q} = 0$ (left) and for $W_p\sigma_{1q} = 50$ (right).



Scheme II. Sample focusing configuration used for light quenching studies of RhB and DCM.

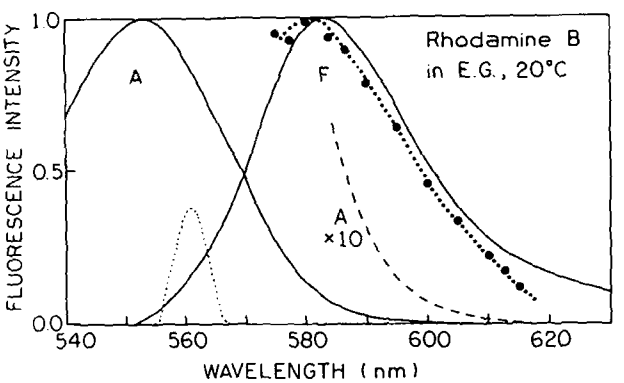


Fig. 2. Absorption and emission spectra of RhB in ethylene glycol. The filled circles on the emission spectrum show the relative light quenching cross sections $(Q-1)/W_p$ [Eq. (8)]. The emission of RhB was isolated using a 560-nm interference filter (------).

The time-dependent intensities of the polarized components are given by

$$I_{\parallel}(t) = c \int_0^{2\pi} \int_0^{\pi/2} n(\theta, t) \cos^2\theta \sin\theta \, d\theta \, d\phi \qquad (4)$$

$$I_{\perp}(t) = c \int_0^{2\pi} \int_0^{\pi/2} n(\theta, t) (1 - \cos^2\theta)\cos^2\phi \sin\theta \, d\theta \, d\phi \qquad (5)$$

with c being a constant. After integration over time and

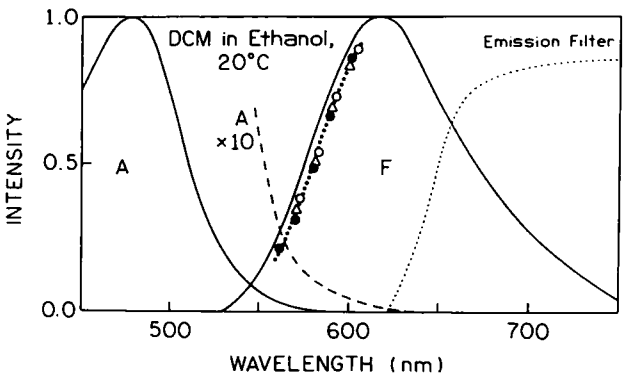


Fig. 3. Absorption and emission spectra of DCM in ethanol. The symbols on the emission spectrum show the relative cross section of light quenching $(Q-1)/W_p$ [Eq. (8)], at the indicated wavelength, in propylene glycol (•), isobutanol (○), and ethanol (△). The right, short-dashed line shows the transmission spectrum of the filter used to isolate the emission.

using the substitution $\cos\theta = x$, one obtains the following equations valid for the case of steady-state observation:

$$I_{\parallel} = 2c\pi\tau N\frac{\sigma_a}{\sigma_{1q}}\int_0^1 (1 - e^{-W_p\sigma_{1q}x^2})\,x^2\,dx \qquad (6)$$

$$I_{\perp} = c\pi\tau N\frac{\sigma_a}{\sigma_{1q}}\int_0^1 (1 - e^{-W_p\sigma_{1q}x^2})\,(1 - x^2)\,dx \qquad (7)$$

The total intensity may be calculated as $I = I_{\parallel} + 2I\perp$. The amount of quenching is characterized by the parameter $Q = I_0/I$, where $I_0 = I(\sigma_{1q}=0) = (2/3)c\pi\tau NW_p\sigma_a$.

Based on Eqs. (6) and (7) one can show that for small values of $W_p\sigma_{1q}$,

$$Q \approx 1 + \frac{3}{10}W_p\,\sigma_{1q} \qquad (8)$$

Equation (8) constitutes the Stern–Volmer relation in light quenching. Equations (6) and (7) can be also used for calculation of the steady-state anisotropy,

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} \qquad (9)$$

Due to the light quenching the steady-state anisotropy decreases when the laser power increases. In the absence of rotational depolarization the time-dependent anisotropy $r(t)$, which can be calculated from Eq. (9) using Eqs. (4) and (5) instead of Eqs. (6) and (7), does not depend on time and is equal to the steady-state anisotropy. The effect of rotational diffusion can be added by assuming the anisotropy decays, with a single correlation time Θ,

$$r(t) \sim r_{0q}e^{-t/\Theta} \qquad (10)$$

where r_{0q} is the time 0 anisotropy in the presence of light quenching.

MATERIALS AND METHODS

The light source was the cavity-dumped output of a rhodamine 6G dye laser, which was pumped by the 514 output of a mode-locked argon ion laser. The excitation was polarized vertically, as occurs from the output of our argon ion and dye lasers. The pulse width of the dye laser was near 5 ps and was focused to a spot

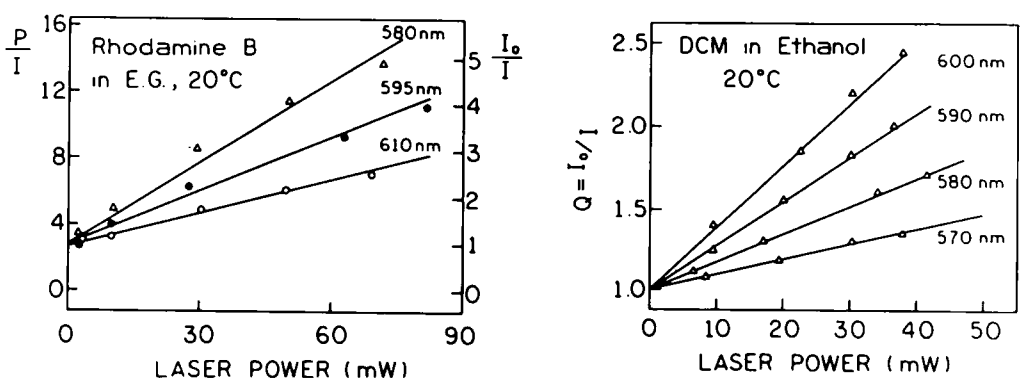
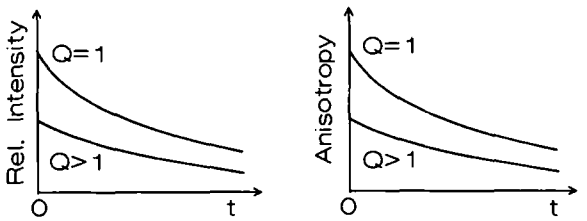


Fig. 4. Wavelength-dependent quenching of RhB in ethylene glycol (left) and DCM in ethanol (right).



Scheme III. Schematic effects of intense pulsed excitation on the intensity and anisotropy decay of a fluorophore. The excitation and quenching pulse arrives at time $t = 0$. The parameter Q describes the amount of light quenching and is defined as $Q = I_0/I$, where I_0 and I are the photoluminescence intensity without and with the presence of light quenching, respectively.

size about 20 μm in diameter. Hence, an incident power of 50 mW corresponds to a maximum intensity of 1.0 $\times$ 10^9 W/cm^2. Without focusing, the spot size was about 0.2 cm in diameter.

The intensity, anisotropy, and frequency–domain data were obtained using the instrumentation described previously [6–8]. The detector was a red-sensitive R928 PMT from Hamamatsu. The average and peak power of the incident light were varied by insertion of neutral density filter (F_1) into the excitation beam (Scheme II). To determine if sample heating altered the data, the total power was decreased without changing the instantaneous power.

RESULTS

Stationary Measurements of Light Quenching

Absorption and emission spectra of RhB in ethylene glycol and DCM in ethanol, together with their wavelength-dependent cross sections for light quenching, are shown in Figs. 2 and 3. The relative cross section for quenching (σ_{1q}) can be calculated from a plot of W_p/I versus laser power (W_p) [Eq. (8)]. The fact that the cross sections follow the emission spectrum strongly suggests that we are observing light quenching rather than some other photochemical effect.

The Stern–Volmer plots for RhB and DCM are shown in Fig. 4. For small amounts of quenching [Eq. (8)] the relative cross section for quenching (σ_{1q}) is proportional to the slope of the plot. The relative values of σ_{1q} are seen to decrease as the incident wavelength increases.

Frequency-Domain Intensity and Anisotropy Decays

The DCM and RhB frequency-domain intensity decays remain a single exponential in the presence of light quenching. For both species in the presence of light quenching there is a small increase in mean lifetime (3.8% and 4.7%, respectively), which is not presently understood. These data suggest that there are no significant photochemical effects.

The theory of light quenching predicts a decrease

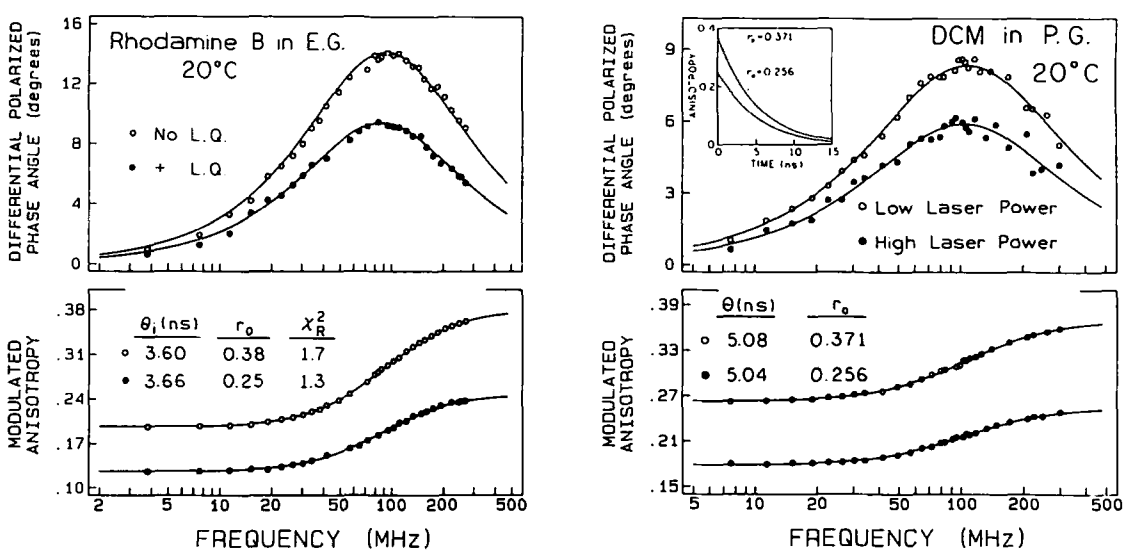


Fig. 5. Frequency-domain anisotropy decay data for RhB in ethylene glycol and DCM in propylene glycol. In the presence of light quenching, the time 0 anisotropy is reduced from 0.38 to 0.25 and from 0.37 to 0.26 for RhB and DCM, respectively. The correlation time is unchanged.

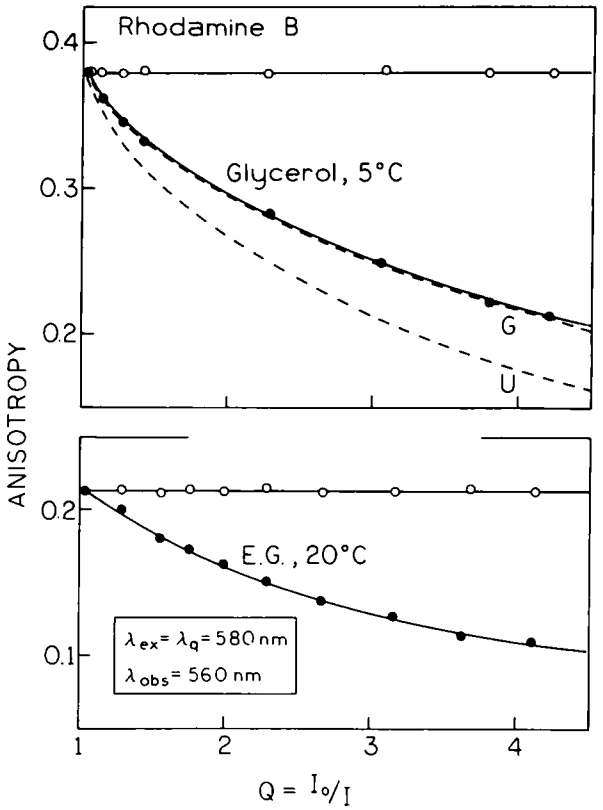


Fig. 6. Steady-state anisotropies of RhB in glycerol (top) and ethylene glycol (bottom) observed with focused illumination. The open circles show the anisotropies measured with unfocused laser illumination. The dashed lines show the theoretical prediction for a uniform (U) or a Gaussian (G) beam profile.

in time 0 anisotropy, with no change in the decay time or correlation time (Scheme III). No change in decay time or correlation time is expected because the light quenching occurs within the laser pulse, and the measurement occurs after the pulse. Additionally, lifetime and decay time measurements are usually normalized and are not dependent on the total signal level. To test this prediction, we measured the frequency-domain (FD) anisotropy decay of RhB and DCM in the absence (○) and presence (●) of light quenching (Fig. 5). The FD anisotropy data show a uniform decrease in the differential phase angles and modulated anisotropies, which is expected for an apparent decrease in the fundamental (time 0) anisotropy.

Steady-State Anisotropies and Comparison with Theory

We compared the extent of depolarization caused by light quenching with that predicted by our theory [Eqs. (6), (7), and (9)]. For this comparison we used the anisotropies of RhB in glycerol at 5°C and in ethylene glycol at 20°C (Fig. 6). The anisotropy (●) decreased progressively with the incident power in both solvents. No change in anisotropy was observed if the laser beam was unfocused. (○). To obtain agreement of the theoretical and experimental data, it was necessary to account for the spatial distribution of the incident light in the sample [2]. We assumed that the laser beam intensity was distributed as a Gaussian.

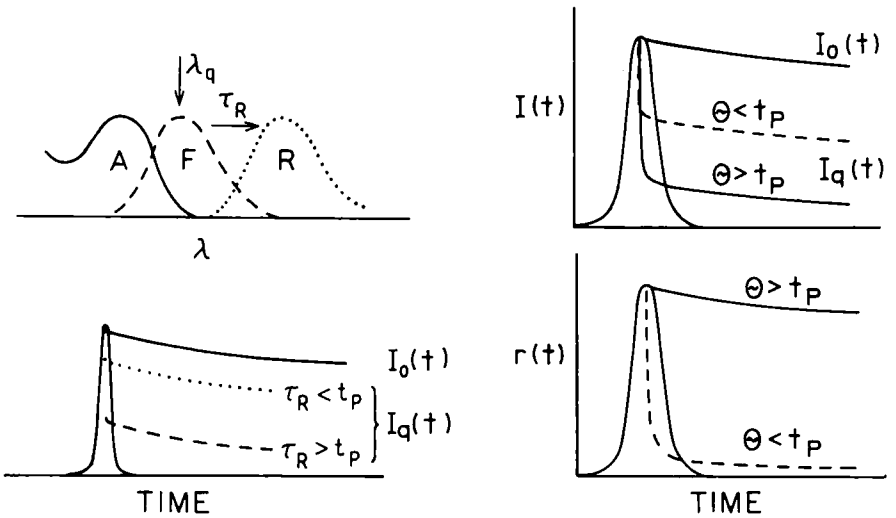
CONCLUSIONS

In our experiments with focused laser excitation we observed that the emitted fluorescence displayed spectral properties which are typical for the light quenching phenomenon. The light quenching cross section clearly followed the RhB or DCM emission spectra in all investigated solvents. At low and moderate intensities of the incident light, the amount of quenching fulfilled the Stern–Volmer relation. Light quenching decreased the steady-state intensity and anisotropy but did not change the decay time or rotational correlation time. We also found that the extent of depolarization caused by light quenching was in agreement with that predicted by our theory, but it was necessary to account for the spatial distribution of the incident light in the sample.

Applications of Light Quenching

Light quenching of fluorescence provides a new method to control the excited-state population and orientation of flourophores. For instance, the extent of light quenching can be expected to depend on the solvent relaxation time (τ_R), the rotational correlation time (Θ), and the extent to which these processes occur within the width of the quenching pulse. For instance, for excitation and quenching by a single pulse of width t_p (see Scheme IV), more light quenching is expected for $\tau_R > t_p$ because the emission spectrum has not yet relaxed from overlap with the quenching wavelength (left). Similarly, rotational diffusion away from the electronic vector of the incident light, during the laser pulse, is expected to decrease the amount of light quenching (right). This indicates that the single-beam light quenching experiments can reveal the rates of fluorophore motion, or more rapid rotations of the electronic moments [9–11], from the extent of light quenching.

Light quenching provides a new method for study of the picosecond and femtosecond dynamics of fluorophores and macromolecules, as revealed by their flu-



Scheme IV. Schematic effects of intense pulsed excitation in the presence of solvent relaxation or rotational diffusion.

orescence emissions. The comparison of the experiments and theory described above demonstrates that light quenching can be significant using practical laser sources and without photochemical damage. It has numerous potential applications for studies of the dynamics of fluorescent substances.

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