# SYNCHRONOUSLY PUMPED DYE LASERS IN FLUORESCENCE DECAY MEASUREMENTS OF MOLECULAR MOTION*

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## Summary

The advent of stable and reliable synchronously pumped dye laser systems has greatly improved the measurement of fluorescence decay times in both the picosecond and the nanosecond time domains. When used in conjunction with time-correlated single-photon counting techniques, the enhanced signal-to-noise ratio permits analysis of multicomponent fluorescence such as is obtained in many heterogeneous systems. Examples of this type of analysis are given with particular reference to macromolecular excimer-forming systems in comparison with the analogous free molecules in solution. For picosecond measurements, the nonlinear phenomenon of sum frequency generation in anisotropic crystals offers several advantages over single-photon counting techniques. These include improved time resolution, the ability to detect fluorescence in the far red and IR without resort to special red-sensitive photodetectors and the measurement of fluorescence anisotropy. Examples are given of rotational diffusion measurements on the dyes cresyl violet and oxazine.

## 1. Introduction

The advent of synchronously mode-locked dye laser systems using ion laser pumping enabled advances to be made in the analysis both of fluorescence decay times in the picosecond time scale and of complex multicomponent decays in the nanosecond region. We describe below methods used and applications.

## 2. Analysis of multicomponent decay

The synchronously pumped dye lasers used in these experiments in conjunction with single-photon counting detection have been described adequately elsewhere [1, 2]. What is of importance is that the stability of the laser pulses and the high repetition rates allow meaningful analysis of experimental fluores-

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cence decay curves in terms of multiple components, containing for example up to three weighted exponentials:

$$i(t) = \sum_{i=1}^{n} Ai \exp\left(-\frac{t}{\tau_i}\right) \tag{1}$$

The curve-fitting routines and acceptance criteria have been documented elsewhere [2, 3]. It should be stressed that stringent criteria (which will be described) are applied to test the reliability of the modelling procedures. The ability to resolve decay curves into several components with confidence is of particular value in heterogeneous systems such as are encountered routinely in biology. We describe below, however, an application in the fluorescence of synthetic polymers bearing aromatic moieties pendant to the polymer backbone.

*2.1. Application to excimer formation in polymers*

Conventional excimer formation in most free aromatic molecules in solution is completely and adequately modelled by the kinetic scheme in Fig. 1 which predicts that the decay of the monomer fluorescence is the sum of two exponential terms, that of the excimer fluorescence being represented by the difference in these two exponentials:

$$i_M(t) = A_1 \exp(-\lambda_1 t) + A_2 \exp(-\lambda_2 t) \tag{2}$$

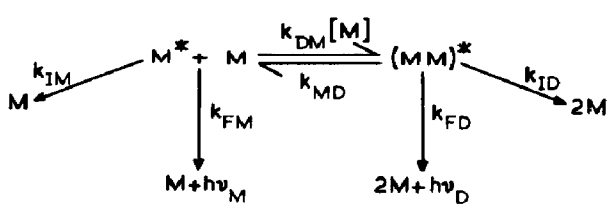
$$i_D(t) = A_3 \exp(-\lambda_1 t) - \exp(-\lambda_2 t) \tag{3}$$

where

$$\lambda_1 + \lambda_2 = \tau_1^{-1} + \tau_2^{-1}$$

$$= k_{FM} + k_{IM} + k_{DM}[M] + k_{FD} + k_{ID} + k_{MD}$$

Although early work has suggested that the same scheme could be applied to synthetic polymers of the general type $\text{-(CH}_2\text{-CHX)-}_n$ where X is an aromatic moiety in dilute solution, we found this to be rarely the case. In general the fluorescence decay of the monomer requires a minimum of three exponential terms for adequate fitting, and the results require a kinetic scheme at least as complex as the type shown in Fig. 2. In this scheme two types of monomer units can be distinguished kinetically (but not spectrally) depending on the ability through molecular motion to form excimers. Thus some monomer units $M_1$ in close prox-



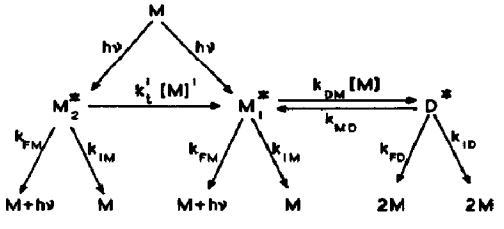




Fig. 1. A kinetic scheme for excimer formation in solution (after Birks).

Fig. 2. The minimal scheme capable of explaining data on vinyl aromatic polymers in solution.

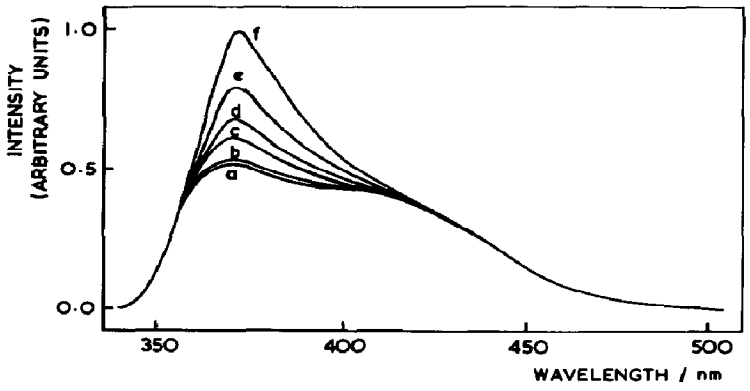



Fig. 3. Fluorescence from poly(*N*-vinyl carbazole) as a function of temperature: curve a, 295 K; curve b, 275 K; curve c, 260 K; curve d, 240 K; curve e, 220 K; curve f, 200 K.

imity can on excitation undergo reversible excimer formation by rotation of aromatic moieties or segmental motion. A second type $M_2$ behave essentially as isolated chromophores, and excimers are formed from these only very weakly as a result of long-range electronic energy transfer to other sites. The detailed arguments leading to these conclusions are presented in this paper and other papers at this conference and in some cases have been reported elsewhere [3, 4].

In the specific case where X is the carbazole chromophore, it is of interest to note that no monomer fluorescence is observable, only two excimer sites being distinguishable spectrally (Fig. 3). The analysis of the high energy excimer again requires three exponential terms for adequate modelling, from which it can be deduced that there are two types of high energy excimer which are indistinguishable spectrally. We speculate that these are (i) high energy excimers formed by *partial* overlap of carbazolyl units in a nearest-neighbour configuration which are able reversibly through chain motions to achieve complete overlap, thus forming the deep excimer trap which fluoresces at 420 nm (these interactions can only occur in isotactic sequences in the polymer) and (ii) excimers formed in syndiotactic regions of the polymer where nearest-neighbour overlap is structurally impossible (the high energy excimer thus results from weaker next-to-nearest-neighbour interactions where nearest-neighbour overlap is structurally impossible).

It is clear that the ability to recognize multiple components in fluorescence in these systems is of great value for gaining an understanding of the molecular interactions giving rise to excimer formation, and this in turn permits use of the knowledge to study polymer mobility.

## 3. Sum frequency generation

The non-linear phenomenon of sum frequency generation in anisotropic crystals used to measure fluorescence decays has several advantages over time-

correlated single-photon counting, such as much improved time resolution and the measurement of fluorescence in the far red and IR regions without resort to red-sensitive photomultipliers. A straightforward measurement of fluorescence anisotropy is also possible. Thus decays as short as 6 ps have been measured and, for example, fluorescence at 900 nm when mixed with 600 nm laser light generates a sum frequency at 360 nm which is easily detected. The apparatus has been described fully elsewhere [5] and is shown in Fig. 4. The instantaneous power of the sum frequency in a crystal of length $L$ is given by [6]

$$P_w(L) \approx k\, L^2 P_1 P_2 \frac{w_3}{w_1} \tag{4}$$

where the subscripts 1, 2 and 3 refer to the gating pulse, the fluorescence and the sum frequency (given by $w_3 = w_2 \pm w_1$) respectively. By appropriate phase matching the difference frequency can be generated which can be used to measure fluorescence in the UV.

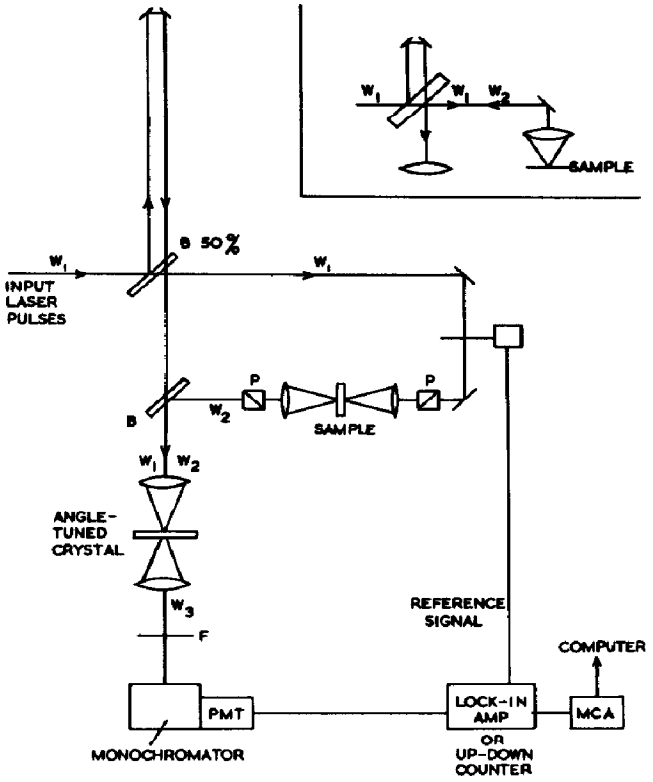



Fig. 4. Experimental arrangements for time-resolved fluorescence up-conversion: F, filters; P, polarizers; C, a sectored disc chopper connected to a lock-in amplifier or photon counter; $W_1$, laser beam; $W_2$, fluorescence. The crystal is $LiIO_3$ (path length, 1 nm). The sample is contained in a glass cell of path length 1 nm mounted perpendicularly to the exciting beam and spun about an axis parallel to the beam, and the fluorescence is collected from the front face of the cell along the exciting beam axis. In an alternative method (inset) the sample is flowed through a cell or pumped through a nozzle to form a jet, and fluorescence is collected at 180° to the exciting beam.

The fluorescence intensity $I_s$ generated by the exciting pulse has a time profile given by

$$I_s(t) = \int_{-\infty}^{t} I_f(t')L(t-t')\,dt' \tag{5}$$

where $t'$ is the time delay, $L(t)$ is the laser pulse shape and $I_f(t)$ is the molecular fluorescence intensity at time $t$. The signal $S(t)$ after the crystal is given by

$$S(t) = \int_{-\infty}^{\infty} I_s(t')L(t-t')\,dt' \tag{6}$$

which combined with eqn. (5) gives

$$S(t) = \int_{-\infty}^{t} I_f(t')\,A\mathrm{T}(t-t')\,dt' \tag{7}$$

where $A(t)$ is the measured autocorrelation function of the laser pulse. As the autocorrelation function is readily measured, the signal can be fitted to the desired decay function at the shortest times with no *a priori* knowledge of the laser pulse shape. This offers a significant advantage over transient absorption methods [7, 8] where it is necessary to make assumptions about the laser pulse shape to facilitate curve fitting.

*3.1. Application to rotational relaxation of dyes*

From measurements of fluorescence decays collected parallel and perpendicular to the polarization of the exciting light, the fluorescence anisotropy $r(t)$ can be calculated if we know the normalization factor $G$ [4]:

$$r(t) = \frac{I_{11}(t)G - I_1(t)}{I_{11}(t)G + 2I_1(t)} \tag{8}$$

Here

$$I_{11} = \{1 + 2r(t)\}\exp\left(-\frac{t}{\tau_f}\right)$$

$$I_1 = \{1 - r(t)\}\exp\left(-\frac{t}{\tau_f}\right)$$

Figure 5 shows an anisotropy measured in this way, and results on a variety of compounds are summarized in Table 1.

The important features of the results are as follows. In oxazine and cresyl violet the rotational relaxation measurements do not correlate well with spectral shifts observed in these compounds in various solvents. Thus the compounds behave similarly with respect to solvent shifts in that the spectra of both are shifted in 1,4-dioxan (hydrogen bonding and polar) but not in acetonitrile (not hydrogen bonding but polar); excited states of both compounds are quenched in water. However, Table 1 shows that the rotational relaxation behaviour in the two compounds is different, cresyl violet exhibiting a much longer relaxation than oxazine which approximates "stick" behaviour. It is unlikely that this is due to the change in shape between the molecules, since related compounds which are more prolate than oxazine is also deviate from normal stick behaviour in relaxation. The anomaly can be explained if specific hydrogen bonding to the amino

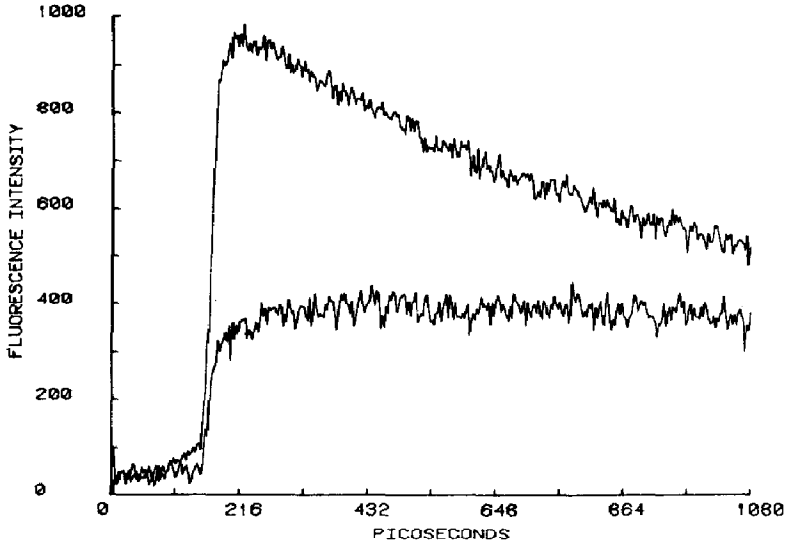



Fig. 5. Fluorescence anisotropy of cresyl violet obtained from measurement of the polarized components of fluorescence.

group occurs in cresyl violet, which is not possible in oxazine. This will have the effect of slowing the molecular rotation particularly since the bonding occurs at the end of the long axis of the ellipsoid of revolution. Solvent attachment on the central ring containing oxygen and nitrogen will be nearer to the symmetry axis of the molecule and will impede rotation to a lesser extent. It is thus concluded that a qualitative description of the properties of oxazine and cresyl violet requires that there are two sites of solvent–solute interaction: (i) the ring containing oxygen and nitrogen which affects spectral and excited state relaxation properties but not rotational relaxation and (ii) the amino substituent which does not markedly affect electronic properties but greatly slows down rotational relaxation.

TABLE 1

Experimental excited state properties of cresyl violet and oxazine

| *Solvent* | *Viscosity* (cP) | *Cresyl violet* | | *Oxazine*[a] | |
|---|---|---|---|---|---|
| | | $\tau_R$ (ps) | $\tau_f$ (ps) | $\tau_R$ (ps) | $\tau_f$ (ps) |
| Acetone | 0.32 | 78 ± 4 | 3670 | 57 | 1122 |
| Methanol | 0.55 | 134 ± 4 | 3650 | 84 | 813 |
| Water | 1.03 | 130 ± 5 | 2390 | 141 | 552 |
| Ethanol | 1.2 | 350 ± 14 | 3510 | 136 | 1024 |
| Propanol | 2.2 | 696 ± 15 | 3870 | 237 | 1122 |

Calculated "stick" lines for (a) cresyl violet (volume, 288 Å$^3$), 84 ps cP$^{-1}$, and (b) oxazine (volume 317 Å$^3$), 120 ps cP$^{-1}$.

[a] Data from Fleming *et al.* [9].

## 4. Conclusions

The ability to make accurate fluorescence decay measurements in both heterogeneous systems and on a short time scale by use of synchronously pumped dye lasers allows a much more detailed probe of molecular motion than has hitherto been possible.

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