



## Picosecond Lifetime Performance of the TimeMaster™ Fluorescence Lifetime Spectrometer

### Introduction

Fluorescence lifetime measurements have found numerous applications over the last two decades. A knowledge of the fluorescence lifetime is invaluable to the photochemist and photophysicist in their quest to unravel light-induced reaction mechanisms and electronic structure of the excited states of organic and inorganic molecules. Fluorescence lifetimes are widely used by biomedical researchers who employ endogenous and exogenous fluorescent probes to study properties of biomembranes, enzymes, photosynthetic systems, secondary structure and interactions in nucleic acids, and even malignancy of tissues. The technique has also found its way into industry, where it is used for studying semiconductors, laser dyes, polymers and other materials. It is becoming recognized by environmentalists in their effort to identify pollutants and characterize their properties.

The most direct way to determine the fluorescence lifetime is to excite the sample with a short pulse of light and measure the fluorescence response as a function of time. In the simplest case, the excited molecules will return to the ground state following a first order kinetic law. The fluorescence intensity,  $F$ , will exhibit an exponential dependence on time:

$$F(t) = F_0 \exp\left(\frac{-t}{\tau}\right)$$

*Eq. 1*

where  $\tau$  is the fluorescence lifetime and  $F_0$  is the intensity at time zero. Equation (1) represents an ideal case where the excitation pulse is infinitely narrow. If the pulse has a finite temporal width, the exponential fluorescence decay (1) will be convoluted with the pulse profile function  $L(t)$ , resulting in the more general expression

$$F(t) = F_0 \int_0^t L(t-t') \exp\left(\frac{-t'}{\tau}\right) dt'$$

*Eq. 2*

When the width of  $L(t)$  becomes zero, equation (2) transforms into (1). In a typical experiment, both  $L(t)$  and  $F(t)$  are measured, and the lifetime ( $\tau$ ) is recovered numerically, usually by an iterative least squares algorithm.

If the functions  $L(t)$  and  $F(t)$  were measured with infinite precision, there would be no restrictions on the measurement of short lifetimes with a given pulsed source. In a real experiment, however, the noise component will eventually cause  $L(t)$  and  $F(t)$  to become indistinguishable from one another when the lifetime becomes significantly shorter than the excitation pulse width.

It is difficult to specify an unqualified lower limit on the fluorescence lifetime that can be measured by an instrument. Factors such as quantum yield, fluorophore concentration, and decay kinetics can affect the measurement. In order to demonstrate performance, one must use a well-characterized standard or propose a special, convincing protocol. We do both.

The object of this paper is to establish and demonstrate a reliable limit of short lifetime determination for the instrument. This issue is important as performance claims from various instrument suppliers are largely unsubstantiated and very misleading.

## Instrumentation

The instrument tested was a Model C-71 *TimeMaster* Fluorescence Lifetime Spectrometer from Photon Technology International. The instrument employed a nanosecond flash lamp generating pulses of 1.6 ns (FWHM) when filled with hydrogen gas and operating at a frequency of 25kHz. The Instrument uses a patented stroboscopic detection system described elsewhere [1]. The optical system consisted of excitation and emission monochromators equipped with flipping mirrors which allow the use of a 75 watt xenon lamp continuous light source and a photon counting detector for steady state fluorescence measurements. This configuration makes it easy to measure fluorescence lifetimes or steady state spectra by convenient selection from a software menu. The monochromators were coupled to a sample compartment containing a motorized, thermostatable four-position cuvette holder. The system operates completely under computer control. The fluorescence decay curves were analyzed with the standard multiexponential fitting software from PTI.

## Results

To assess the subnanosecond lifetime performance of the instrument, *p*-terphenyl fluorescence decays were measured in the presence of varying concentration of potassium iodide (KI) in a water-ethanol mixture (1:1) [2]. The heavy I<sup>-</sup> ions act as fluorescence quenchers by enhancing the non-radiative intersystem crossing between the excited singlet and triplet states of *p*-terphenyl. When quenching is governed by a collisional mechanism, the lifetime dependence on quencher concentration is given by the Stern-Volmer equation [3]

$$\frac{\tau_0}{\tau} = 1 + k_q \tau_0 [Q]$$

*Eq. 3*

where [Q] is the quencher concentration,  $\tau_0$  and  $\tau$  are the fluorescence lifetimes in the absence and presence of quencher, respectively, and  $k_q$  is the bimolecular quenching rate constant. By increasing the quencher concentration, a sequence of gradually quenched fluorescence decays can be recorded and analyzed numerically for lifetimes. The  $\tau_0/\tau$  vs. [Q] dependence can be plotted and tested for linearity according to equation (3).

Fluorescence decays of *p*-terphenyl were measured for six different concentrations of KI ranging from 0 to 0.32 M. Since there was some noticeable precipitation at room temperature, the experiments were carried out at T = 332 deg. K. The decay curves and the numerical fits are shown in Figure 1. The lifetimes obtained from the exponential analysis are shown in Table 1. The values range from 1.21 ns for the sample with no quencher to 170 ps for [KI] = 0.32M.

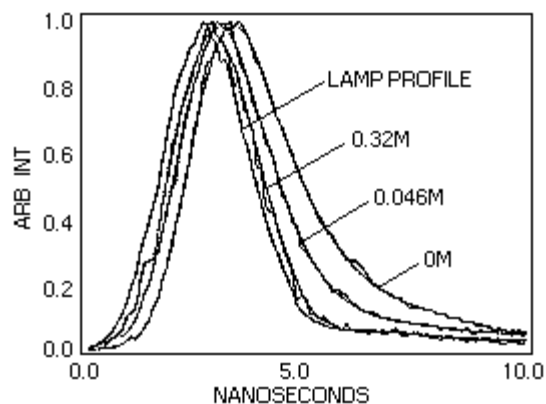


Figure 1. Fluorescence decays of *p*-terphenyl at different concentrations of quencher, KI.

[KI] / mole L <sup>-1</sup>	$\tau$ /ns
0.0	1.21
0.045	0.65
0.102	0.42
0.152	0.33
0.231	0.22
0.318	0.17

Table 1. Fluorescence lifetimes of *p*-Terphenyl at varying concentrations of quencher, KI.

To ascertain that the recovered short lifetime values were meaningful, a Stern-Volmer dependence was plotted according to equation (3). This dependence, as shown in Figure 2, is almost perfectly linear. The value of  $k_q = 1.58 \times 10^{10} \text{ s}^{-1} \text{ mole}^{-1} \text{ L}$  was determined from the slope of the plot. This value of the quenching rate constant is in excellent agreement with the diffusion-controlled bimolecular rate constant,  $1.55 \times 10^{10} \text{ s}^{-1} \text{ mole}^{-1} \text{ L}$ , reported for water at  $T = 332 \text{ deg. K}$  from the Smoluchowski and Stokes-Einstein equations [4]. The average deviation of the measured lifetimes from those calculated from the theoretical straight line in the Stern-Volmer plot (Fig. 2) is only 14 ps. These results lend confidence to the ability of the instrument to measure lifetimes to less than 200 ps with good precision.

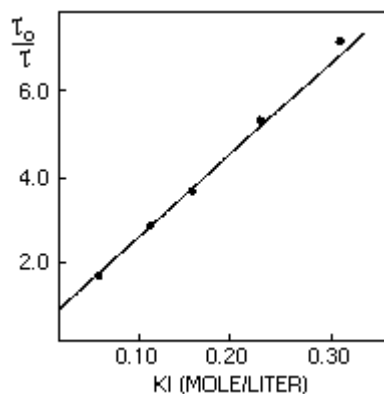
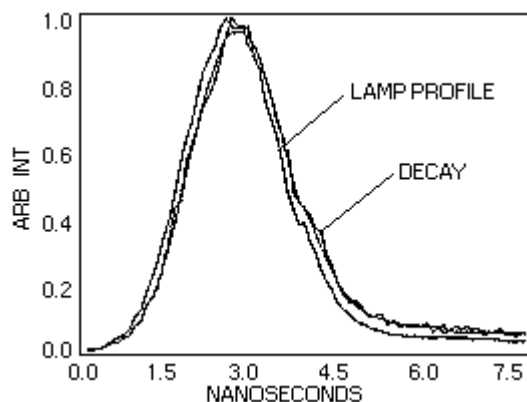


Figure 2. Stern-Volmer plot for fluorescence quenching of *p*-terphenyl by KI.

To test the performance of the instrument in the 100 ps range, the lifetime of rose bengal was measured in water. Its lifetime is known to be 91 ps as measured with a picosecond laser with timecorrelated single-photon counting detection [5].



*Figure 3. Fluorescence decay of rose bengal in water.*

A decay curve obtained with the instrument for rose bengal in water is presented in Figure 3. The measurement was repeated three times and resulted in an average lifetime of  $99 \pm 9$  ps. This result is in very good agreement with the literature value cited above. It proves that the TimeMaster can measure lifetimes as short as 100 ps with the relative error of less than 10%.

## Conclusion

The results presented prove that a well-designed analog system, such as the TimeMaster from PTI, equipped with a conventional flash lamp, is capable of measuring fluorescence lifetimes down to the 100 picosecond range with high precision. The lifetime uncertainty at the lowest limit does not exceed 10%.

The instrument's short lifetime performance equals or exceeds that of time-correlated single-photon counting. When combined with its reliable and relatively simple electronic design, simplicity of use, and low price, it is the instrument of choice for most routine fluorescence lifetime applications.

## References

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