BRIEF COMMUNICATION

A NOMOGRAM FOR DECONVOLUTION OF SINGLE EXPONENTIAL FLUORESCENCE DECAYS

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ABSTRACT An extremely rapid technique for deconvolving single exponential luminescence decay data is described that involves essentially no mathematical manipulation of the experimental data. The method permits "real time" measurement of deconvolved luminescence lifetimes with conventional pulsed, lifetime-fluorometers and phosphorimeters. The method assumes that the true luminescence decay of the chromophore is accurately represented by a single exponential decay function.

INTRODUCTION

Fluorescence decay times, when measured with pulsed lifetime fluorimeters, must be extracted or "deconvolved" from the observed data. The measured data include a convolution of the instrumental response function and the temporal profile of the excitation source with the sample fluroescence decay function. For systems with decay profiles described by a sum of two exponentials, this process of deconvolution is best handled in a mathematical fashion using "iterative reconvolution" (1–3). When the sample decay function is described by a sum of three exponentials with different coefficients and exponents, the mathematical problem is essentially an ill-posed problem and reliable algorithms which do not require data of extraordinary accuracy have yet to be developed. For single exponential decays there are many methods for mathematically manipulating the observed data to obtain reliable estimates of the sample lifetime (3). Probably the most rapid and accurate method is that described by Demas and Crosby (4) and Demas and Adamson (5). However, even this method involves mathematical processing of the data.

It is the purpose of this paper to demonstrate an extremely rapid technique for finding single exponential sample decay times with an accuracy comparable to instrumental limitations. The method accounts for the distortion introduced by the excitation source but involves essentially no manipulation of the data.

EXPERIMENTAL

For many time-dependent measurements, the molecular response function is a simple exponential. Provided that this is verified for any system being studied, the deconvolution of the response function

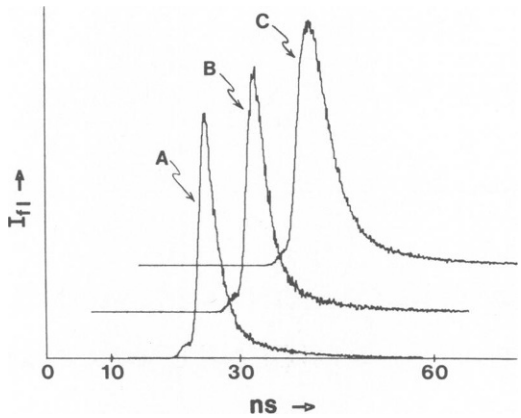

FIGURE 1 Excitation lamp profiles used. Curves *B* and *C* were synthesized from curve *A* and random noise was added. Curve *A* is the profile of an 8 psi N_2 nanosecond flashlamp. FWHM values of these curves are *A*, FWHM = 2.8 ns; *B*, FWHM = 3.4 ns; *C*, FWHM = 5.7 ns.

from the observed luminescence decay can be obtained numerically by a number of techniques, most yielding fluorescence decay times accurate to ±2% or 50 ps, whichever is greater. These methods involve processing of the data by some linear or nonlinear least-squares procedure.

To assess the possibility of doing away with this requirement, at least for experiments where decay time accuracy of ±0.15 ns (150 ps) is sufficient, ~100 "observed" fluorescence decay functions were synthesized from three different exciting flashes using a computer and the recursion relationships outlined by Ware et al. (6).

The exciting flash profiles used are shown in Fig. 1. Curve *A* is the flash observed from a free-running 8 psi N_2-flashlamp. The decay fluorometer used to measure curve *A* was built with an Ortec 473A constant fraction discriminator (Ortec Inc., Oak Ridge, Tenn.), a LeCroy model 3001 TAC-multichannel analyzer (LeCroy Research Systems Corp., Spring Valley, N.Y.), and an RCA 4084 photomultiplier tube (RCA Solid State, Somerville, N.J.). Curve *B* is a simulated excitation flash generated by convolving curve *A* with an exponential response function, $\exp(-t/0.5\text{ ns})$, to enable the calculation of data observed with an excitation flash of profile quite different from the standard N_2-lamp profile. Curve *C* has been generated by taking curve *A* and convolving it with the exponential response function, $\exp(-t/2\text{ ns})$. Random Gaussian noise was added to obtain curves *B* and *C*.

The "observed" fluorescence decay curves were synthesized with the addition of Gaussian noise to simulate the Poisson-type noise observed in photon-counting experiments. The variance of the noise equaled the square root of the intensity of the data in each channel. This noise was calculated using the IBM-Scientific Subroutine Package algorithm. The "observed" decay curves had maximum intensities of 10–30,000 counts. All synthetic data were calculated on an Apple-II microcomputer (Apple Corporation, Cupertino, Calif.) using a Basic program.[1]

RESULTS

When the synthetic curves for single exponential decays were analyzed, it was realized that the time at which an observed fluorescence intensity had decayed to some arbitrary fraction of its maximum value was a smooth function of the deconvolved molecular decay time. Because these functions were smooth, they were seen to have value for predicting the molecular decay time.

[1]A program in Basic for calculation of this curve from any given excitation profile is available upon request from the author.

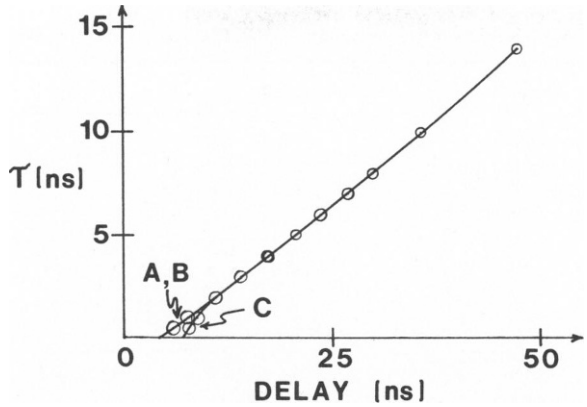

FIGURE 2 Calibration curves for lamp profiles *A*, *B*, and *C*. The ordinate represents the τ-value in nanoseconds. The abscissa corresponds to the delay between the $1/e$ point of the excitation flash and the $0.075 * I_{max}$ point of the observed fluorescence decay curve.

Fig. 2 clarifies the procedure. "Calibration" nomograms are presented for lamp profiles *A*, *B*, and *C*, where the 0 delay position on the abscissa is the fluorometer channel number or delay time corresponding to the $1/e$ point of the excitation flash. The delay coordinate for a given decay lifetime is the time between the $1/e$ time of the excitation flash and the time at which the observed fluorescence decay drops to 7.5% of its maximum intensity. The nomograms were obtained from synthetic data with no random noise added. Because the three nomograms show considerable overlap, the coordinates of the points used to generate Fig. 2 are tabulated in Table I. The use of the 7.5% I_{max} point is a matter of choice, but it was felt that this particular number represented a good compromise between the reliability with which the delay time could be ascertained and the steepness of the associated calibration nomogram.

TABLE I
NOMOGRAM DATA FOR
THREE DIFFERENT EXCITATION PROFILES

	Delay Time		
	Lamp A	Lamp B	Lamp C
		(ns)	
1.0	7.7	7.4	9.0
2.0	11.0	10.9	11.3
3.0	14.3	14.3	14.6
4.0	17.5	17.4	17.5
5.0	20.6	20.5	20.5
6.0	23.8	23.5	23.5
7.0	26.8	26.5	26.5
8.0	29.8	29.5	29.4
9.0	—	32.3	32.2
10.0	35.6	35.1	35.1
12.0	—	40.7	40.7
14.0	47.1	46.4	46.5
16.0	—	52.0	52.1

TABLE II
COMPARISON OF MEASURED WITH REAL LIFETIMES

τ used	τ measured	Average
(ns)		
1.7	1.5	1.5 ± 0.1
1.7	1.5	
1.7	1.6	
4.3	4.3	4.2 ± 0.1
4.3	4.1	
4.3	4.2	
8.1	8.0	8.0 ± 0.1
8.1	8.1	
8.1	7.9	
12.6	12.6	12.4 ± 0.2
12.6	12.4	
12.6	12.2	

To test the predictive value of these "calibration" nomograms, a variety of decay curves with maximum intensity of 10,000 counts were synthesized in which random noise was added as described earlier. The time at which the observed decay had dropped in intensity to 7.5% of the maximum was then used with the appropriate calibration nomogram to infer the true molecular decay time. The results obtained using the excitation flash described by curve *A* in Fig. 1 are given in Table II. The results show an SD of 0.1 ns with the average τ-value measured typically ~0.1 ns short of the true value. The maximum total error in these measurements is therefore $\lesssim$ 0.2 ns. The systematic error of 0.1 ns in the measured value arises from the method for determining the time at which the intensity has fallen to 7.5% of I_{max}. The data in Table II were obtained using a delay corresponding to the first channel with intensity <7.5% of I_{max}. Because of the random noise on the data, this channel actually represented the first point on the observed decay to fall to <7.5% of I_{max} within the amplitude of the noise. The noise-free delay time will be a little longer than this measured delay. It is recommended that the delay be measured to the point where the average intensity of the observed decay passes through the 7.5% I_{max} point.

The principal errors associated with this method come from errors in the measurement of the $1/e$ time of the excitation flash and the 7.5% I_{max} time of the observed decay curve. These errors may arise from noise in the data or from drifts in the delay between the system starting trigger pulse and the $1/e$ time of the excitation flash profile. To minimize the results of such errors, the 7.5% I_{max} time of the observed decay curve was chosen for measurement instead of the $1/e$ point or the 50% I_{max} point. Using the 7.5% value, a fairly linear nomogram is obtained with a shallow slope of ~0.33. Thus, an error in combined delay time measurements ($1/e$ of the flash and 7.5% I_{max} of the observed decay) of 1 ns, for example, results in a 0.33-ns error in the inferred decay lifetime. If a point of greater intensity than 7.5% I_{max} in the observed decay profile is used, a nomogram with a correspondingly larger slope is obtained. In such cases, the error in the inferred τ_F value due to an error in the measured delay times is also greater.

Although the accuracy of the data presented in Table II is limited to ±0.2 ns, the

considerable ease with which decay times can be determined would seem to indicate that this method will find wide use by experimenters whose experimental results are not greatly affected by the reduced accuracy. An additional advantage is that the method yields data that are relatively insensitive to small drifts in lamp profile, as shown by the similarity of the three calibration curves in Fig. 2 obtained for three different excitation flash profiles. However, the technique is valid for use only in those cases where a single exponential decay correctly describes the luminescence decay of the chromophore. Furthermore, it is best used to analyze data obtained with a storage oscilloscope or a photoncounting apparatus. With such units, the error introduced in measuring the delay times will be small. Researchers using oscilloscope data recording will find that the 7.5% I_{max} point is difficult to measure accurately and may prefer to use the $1/e$ point of the observed decay, instead. A nomogram using the $1/e$ point can then be constructed, as presented in Fig. 2. This nomogram will not, in general, be linear, as suggested by Munro and Ramsay (7). When using oscilloscope data recording, it is essential that the oscilloscope be externally triggered by the flashlamp. Only then will the delay corresponding to the ordinate in the calculated nomogram be related to the delay measured from the oscilloscope trace.

While the results listed in Table II show that τ_F values much less than the full width at half maximum (FWHM) of the excitation lamp can be measured reliably, the final lower limit will be set by the precision with which the $1/e$ point of the excitation flash and the 7.5% I_{max} point of the observed decay curve can be measured.

CONCLUSION

In summary, it has been shown that the tedium of mathematical deconvolution of single exponential decay times from observed fluorescence decay curves can be avoided by the use of calibration nomograms. Curve *B* in Fig. 2 is generally applicable to all N_2-flashlamp systems without further manipulation, provided the profile of these lamps is described reasonably well by Fig. 1, Curve *a*. For other lamps, a calibration curve can be readily calculated (footnote 1).

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