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Simulations of Measurements of Fluorescence Lifetimes Using Noise-Modulated Light

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At the Pattern Recognition group at the Delft University of Technology, we are working on new ways to measure fluorescence lifetimes. There are two well-known ways to measure lifetimes; the phase method and the pulse method. In the phase method fluorescent material is stimulated by sinusoidally modulated light. The emitted fluorescent light will have the same modulation frequency, but there will be a phase shift between the excitation and the emission light. Measuring this phase shift will, after some simple calculation, give the lifetime of the fluorescent material. The second method is the pulse method. Short pulses of light are used to excite the material. The emitted light is detected, and from these measurements the decay curve of fluorescent light is determined. In our research we want to use a new method that may allow us to measure a mixture of lifetimes. We want to use excitation light that is modulated by a white noise signal. We are currently building an experimental setup for these measurements. We have been working on numerical and electrical simulations to investigate the properties of noise signals. Some results of these simulations are presented in this paper.

KEY WORDS: Fluorescence lifetime measurement; ARMAX estimation; white noise; filters.

INTRODUCTION

We want to measure the lifetimes of a mixture of fluorophores. Measurement of lifetimes has been done using sinusoidal modulated light and using short pulses of light. There have been reports[5–9] of experiments where a mixture of sinusoids was used to measure multiple lifetimes, although when one sinusoid is used, only one average lifetime can be measured. It is also possible to measure multiple lifetimes using the pulse method.[10,11] For accurate measurements the measurement time can be quite long and a difficult deconvolution algorithm has to be applied to the data to get the lifetimes. We are working on a new way to measure multiple fluorescence lifetimes. The main difference from the other two methods is that we use a light source that is modulated by a white noise signal.

We consider a mixture of fluorophores[1] as a system with multiple poles. The transfer function of this system is a sum of exponentials. We want to get the parameters of these exponentials by exciting the system with a white noise signal. Such a signal has a flat frequency spectrum. By measuring the input and output signal we can get an estimation of the transfer function of the system. The system transfer function is

$$N(t) = \sum_{k=1}^{P} N_k e^{-t/\tau_k}\, u(t) \tag{1}$$

where $u(t)$ is the unit step function, τ_k are the lifetimes, N_k are the relative concentrations of the component, and P is the number of components.

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In the Laplace domain this is

$$H(s) = \sum_{k=1}^{P} \frac{N_k \tau_k}{1 + s\tau_k} \tag{2}$$

Since we sample input and output signal, we are working in the discrete domain. The transfer function in the discrete domain is

$$\begin{aligned} N_D(t) &= \sum_{k=1}^{P} N_k e^{-nT/\tau_k}\, u(n) \\ &= \sum_{k=1}^{P} N_k (e^{-T/\tau_k})^n\, u(n) \end{aligned} \tag{3}$$

and

$$\begin{aligned} H_D(z) &= \sum_{k=1}^{P} \frac{N_k}{1 - e^{(-T/\tau_k)}\, z^{-1}} \\ &= \sum_{k=1}^{P} \frac{N_k}{1 - a_k z^{-1}} \end{aligned} \tag{4a}$$

with $a_k = e^{-T/\tau_k}$, T is the sampling interval, and τ_k are the lifetimes.

If we have a two-lifetime system, the transfer function is

$$\begin{aligned} H_D(z) &= \frac{N_1 + N_2 - a_2 N_1 z^{-1} - a_1 N_2 z^{-1}}{(-1 + a_1 z^{-1})(-1 + a_2 z^{-1})} \\ &= \frac{z^{-1}(-a_2 N_1 - a_1 N_2) + N_1 + N_2}{a_1 a_2 z^{-2} - (a_1 + a_2) z^{-1} + 1} \\ &= \frac{q_0 + q_1 z^{-1}}{p_0 + p_1 z^{-1} + p_2 z^{-2}} \end{aligned} \tag{4b}$$

When such a system is used in combination with a white noise input signal, we have made an autoregressive moving average system. The equation for such a system is

$$\begin{aligned} p_0 y(n) + p_1 y(n-1) + p_2 y(n-2) \\ = q_0 u(n) + q_1 u(n-1) + e(n) \end{aligned} \tag{5}$$

where $u(n)$ is white noise and $e(n)$ additive noise. After measurement of the input and output signal we use an ARMAX estimator[1,2,4] to get an estimation of the system parameters p_n and q_n. From these parameters the lifetimes can be calculated. The ARMAX estimator we used is in the System Identification toolbox, which is an extension to Matlab.[4]

RESULTS

We performed numerical and electrical simulations of ARMAX systems. We first give the results of the numerical simulations, then those of the electrical simulations.

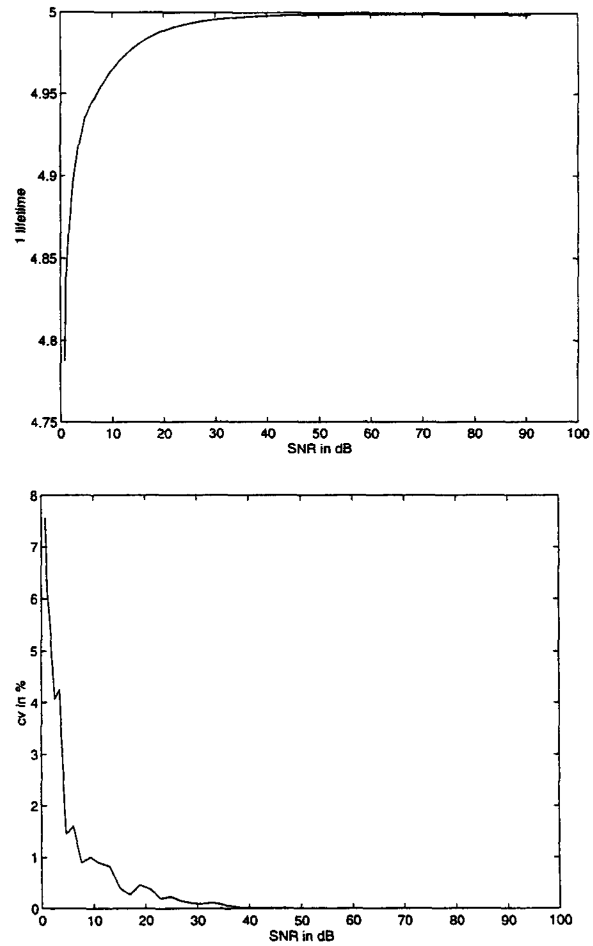

Fig. 1. Estimated lifetime and CV of a one-component system as a function of SNR.

Numerical Simulations

We investigated the sensitivity of the ARMAX estimation process to additive noise. When no additive noise is present the ARMAX estimator gives the exact value of the parameters since it is equivalent to solving a linear system. In Eq. (5) we have varied the noise term $e(n)$ and calculated the estimation of the parameters p_n and q_n. From these parameters the lifetime is calculated. We did the experiment as follows. We took a lifetime (e.g., 5 ns) and calculated the system parameters p_n and q_n that would give us a system with a lifetime of 5 ns. We then took a random noise signal $u(n)$. With the parameters p_n and q_n we calculated signal $y(n)$. We added a small random noise signal $e(n)$. This resulting signal was put into the ARMAX estimator, which gave

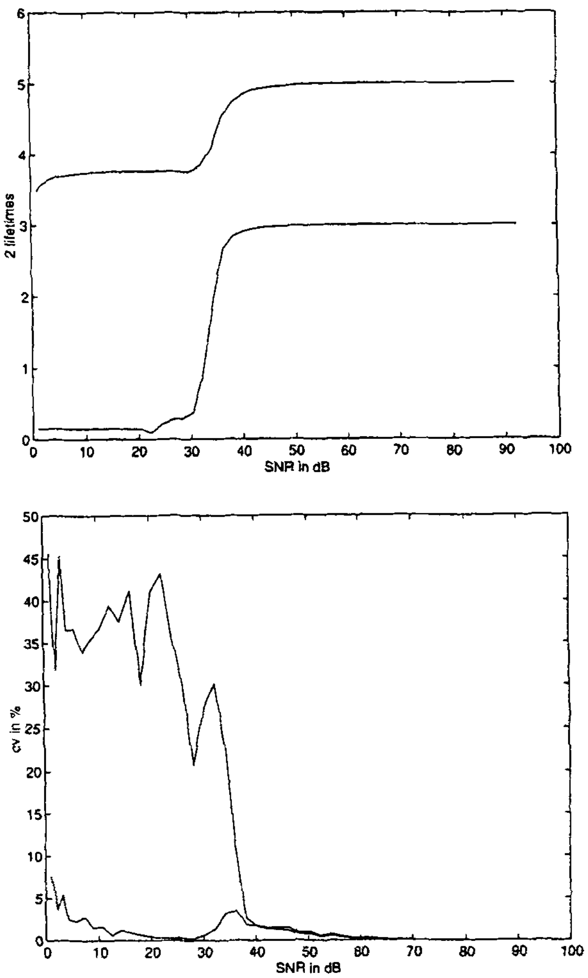

Fig. 2. Estimated lifetimes and CV of a two-component system as a function of SNR.

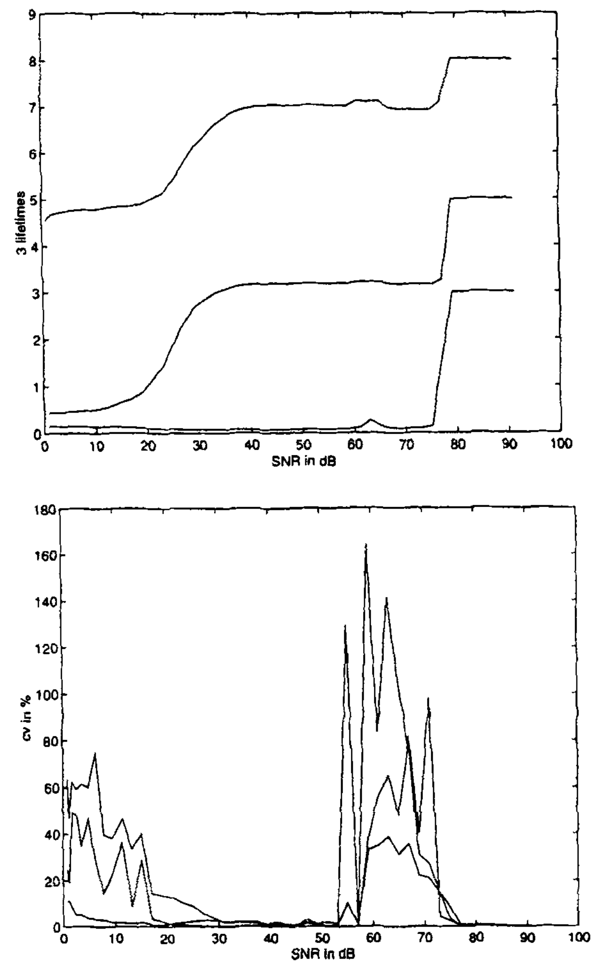

Fig. 3. Estimated lifetimes and CV of a three-component system as a function of SNR.

us an estimation of p_n and q_n. From these estimates the lifetimes are calculated. We have repeated this experiment for different noise signals $e(n)$. We show the results in Figs. 1–3. The signal-to-noise ratio (SNR) value on the horizontal axis is defined as follows:

$$\mathrm{SNR} = 10 * \log\left(\frac{\mathrm{var}(y(n) + e(n))}{\mathrm{var}(e(n))}\right) dB \qquad (6)$$

where dB stands for decibels. On the vertical axis the estimated lifetime is plotted. In Fig. 1 the lifetime to be found was 5 ns. In Fig. 2 two lifetimes, of 3 and 5 ns, have to be estimated. Figure 3 shows the result of a three-lifetime system with values of 3, 5, and 8 ns. In the figures the straight lines on the right give the values of the lifetimes to be found. The number of lines equals the number of lifetimes. In all experiments the sample time was 1 ns. We can see from Fig. 1 that we need a minimum SNR of 20 dB to estimate the parameter of a first-order system. Above 20 dB the value of 5 ns is estimated correctly. When the SNR is below 20 dB the estimation results become unacceptable. The bias then gets too high and the coefficient of variation (CV) is too large, so that the results are unpredictable. For a second-order system we see from Fig. 2 that we need a SNR value of 40 dB. For a third-order system we need a value of 80 dB. This is plotted in Fig. 3. These high SNR values are needed to distinguish between different components of a multiexponential model. The flat area in the CV plot in Fig. 3 between 30 and 50 dB does not mean that the estimation procedure was successful. The bias is far too large. What happens here is the mapping of complex values on a real axis, ignoring the imaginary part. For multiexponential systems the SNR of the measured signals must be rather high to get good estimation results with this procedure. Single-exponential systems are easy to analyze. A SNR value of 20 dB is usually not a problem in a real measurement system.

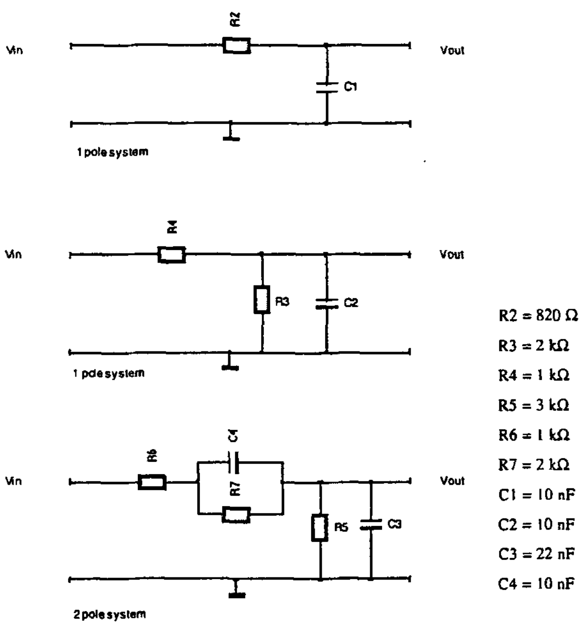

Fig. 4. Layout of electrical components.

Table I. Results of Electrical Simulations

Pole	N	Real (kHz)	Value	Estim 1	CV 1 (%)	Bias 1 (%)	Estim 2	CV 2 (%)	Bias 2 (%)
1	5000	19.4		19.6	8.5	1			
1	5000	10		9.5	8.1	−5			
1	5000	23.8		24.5	21.2	3			
2	5000	3.8	29.6	3.6	15.3	−5	24.8	13.5	−16

Electrical Simulations

After these numerical simulations we performed electrical simulations. We built an electrical filter that has the same transfer function as a multiexponential system. We chose the values of the resistors and capacitors, so we know the location of the −3-dB points of the system. We want to recover these points using our ARMAX estimator. We have put a white noise signal from a Wavetek signal generator at the input of our filter and sampled the input and output signal using a LeCroy digitizing oscilloscope. These data sets are fed into the estimator. From the estimated parameters the −3-dB points are calculated. The electrical filters used are shown in Fig. 4. The results are listed in Table I.

In the column "real value" the value of the −3-dB point of the filter is printed. This value is determined by the values of the components. "Estim" is the estimated value of this −3-dB point, and the CV is determined by repeating the experiment 10 times. The number of samples used in these experiments was 5000. We also tested a three-lifetime system. The results were not acceptable. This is because the SNR value is not high enough. It can be shown that sampling with an 8-bit quantizer gives a SNR value of 59 dB. In Fig. 3 we show that this is not high enough for estimating the parameters of a three-lifetime system.

CONCLUSIONS

We conclude that the ARMAX estimator is capable of estimating the parameters of multiexponential systems in numerical simulations. When real data are used, the estimator can give an accurate estimation of single- and double-exponential systems. Higher-order exponential systems cannot be handled at the moment. The electrical simulations can be compared to the measurements we are going to do on fluorescent material. Although the frequencies we used in the electrical simulations (20 kHz) are much lower than those we shall use in fluorescence measurements (200 MHz), the estimation procedure will be the same. These simulations indicate that we will be able to measure the lifetimes of two-component fluorescent material.

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