MAXIMUM LIKELIHOOD ESTIMATION FOR SUPERIMPOSED EXPONENTIALLY DECAYING FLUORESCENCE PROCESSES

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ABSTRACT

In this paper, we address the problem of estimating the components of superimposed exponentially decaying signals. Usual estimation techniques, e.g. least squares or eigenvalue and eigenvector based methods, are not adequate for exponentionally decaying fluorescence processes due to their rather simple signal modelling. Therefore, we introduce a better suited parametric model by exploiting the statistical properties of the exponentially decaying emission of fluorescence photons (time dependent Poisson statistics). Using this model maximum likelihood estimates for the fluorescence intensity spectrum and the decay parameters are derived. The performance of the maximum likelihood estimates is compared with the least squares estimates by means of simulations and real data experiments. The results indicate the superiority of the maximum likelihood estimates.

INTRODUCTION

Fluorescence spectroscopy is a powerful tool in analytical chemistry. Large molecules, however, like polycyclic aromatic hydrocarbaons (PAH) for example, have broad fluorescence spectra and the spectra of different PAH may overlap. In this case a second parameter is needed for identification.

We perform time-resolved fluorescence measurements to achieve a higher selectivity with respect to the different PAH. In this case the fluorescence intensity spectrum and the decay parameters of the fluorescence process are the characteristic parameters for classifying PAH. Therefore, the initial step in a classification procedure should be the accurate estimation of these parameters for each component.

We propose adequate parameter models for the time-resolved spectrum taking into account a time-dependent Poisson statistics for the emission of the fluorescence photons. Maximum likelihood estimates (MLE) are developed on this basis for the fluorescence intensity spectrum and the decay parameters.

By means of simulations the performance of the MLE is compared to usual least squares estimates (LSE) which are only using deterministic decay curves embedded in (white gaussian) noise for modelling the measurements. Finally, the MLE is applied to measured time-resolved fluorescence data.

DATA MODEL

For a certain wavelength of interest a fluorescence process describes the emission of photons over time. In analogy to the radioactive decay law the times at which photons are detected can be interpreted as realizations of exponentially distributed random variables.

Now, let $R \gg r$ denote the number of possible and emitted photons, respectively. After partitioning the observation intervall $[0, T)$ in the sub-intervalls $[n\Delta, (n+1)\Delta)$ $(n = 0, \ldots, N-1)$ the probability for observing a photon in the nth intervall is

$$p_n = \frac{r}{R} \int_{n\Delta}^{(n+1)\Delta} \frac{1}{\tau} \exp\left(-\frac{t}{\tau}\right) dt = \frac{r}{R}\, a \exp\left(-\frac{n\Delta}{\tau}\right)$$

with

$$a = 1 - \exp\left(\frac{\Delta}{\tau}\right).$$

Thus, the probability for counting $x_1, \ldots, x_{N-1}$ photons in the intervalls $[n\Delta, (n+1)\Delta)$ $(n = 0, \ldots, N-1)$ is given by

$$P(X_0 = x_0, \ldots, X_N = x_N) = \frac{R!}{x_0! \cdots x_N!}\; p_0^{x_0} \cdots p_N^{x_N},$$

where

$$x_N = R - \sum_{n=0}^{N-1} x_n$$

and

$$p_N = 1 - \sum_{n=0}^{N-1} p_n.$$

Approximating the multinomial distribution by a Poisson distribution, cf. Poissons theorem [4], we obtain

$$P(X_0 = x_0, \ldots X_{N-1} = x_{N-1}, X_N = x_N) \approx$$

$$\prod_{n=0}^{N-1} \frac{\mu_n^{x_n}}{x_n!} \exp(-\mu_n),$$

where

$$\mu_n = R\,p_n = b\,\exp\left(-\frac{n\Delta}{\tau}\right)$$

with

$$b = r\,a.$$

In practice usually several fairly independent fluorescence processes are simultaneously excited. Supposing the number M of superimposed processes to be known, the number of photons observed in the nth time intervall is

$$y_n = \sum_{m=1}^{M} x_{n,m},$$

where $x_{n,m}$ represents the counts of the mth process in the nth intervall. Since the sum of independent Poisson distributed random variables is also Poisson distributed, we obtain

$$P(Y_0 = y_0, \ldots, Y_N = y_N) \approx \prod_{n=0}^{N-1} \frac{\nu_n^{y_n}}{y_n!} \exp(-\nu_n)$$

with

$$\nu_n = \sum_{m=1}^{M} b_m \exp\left(-\frac{n\Delta}{\tau_m}\right).$$

Furthermore, if the time spread of the detector is significant the parameters ν_n $(n = 0, \ldots, N-1)$ should be rather modeled by

$$\nu_n = \int_0^\infty h(n\Delta - t) \sum_{m=1}^{M} b_m \exp\left(-\frac{t}{\tau_m}\right) dt.$$

Herein $h(t)$ denotes the expected impulse response of the detector. Empirical investigations have shown, that the impulse response of a typical detector can be satisfactorily described by

$$h(t) = \beta\, t^2\, \exp(-\alpha t) \qquad t \geq 0,$$

where α is supposed to be known or estimated in advance. Thus, after some cumbersome calculations the parameters ν_n $(n = 0, \ldots, N-1)$ can be reformulated to

$$\nu_n = \sum_{m=1}^{M} c_m \left(\gamma_m^3 \exp\left(-\frac{t}{\tau_m}\right) - \left(\frac{\gamma_m}{2} t^2 + \gamma_m^2 t + \gamma_m^3\right) \exp(-\alpha t)\right),$$

with

$$c_m = \beta\, b_m$$

and

$$\gamma_m = \left(\alpha - \frac{1}{\tau_m}\right)^{-1}.$$

MAXIMUM LIKELIHOOD ESTIMATES

Now, the MLE can be constructed as follows. Taking the logarithm of the counting density

$$f_y(y_0, \ldots, y_N) = P(Y_0 = y_0, \ldots, Y_N = y_N) = \prod_{n=0}^{N-1} \frac{\nu_n^{y_n}}{y_n!} \exp(-\nu_n),$$

we obtain the log likelihood function

$$L(\underline{a}, \underline{\tau}) = \sum_{n=0}^{N-1} \left(y_n \log(\nu_n) - \nu_n - \sum_{i=1}^{y_n} \log(i) \right).$$

After ignoring the constant term, changing the sign and replacing ν_n by $c_M\, \eta_n$, where

$$\eta_n = \left(\exp\left(-\frac{n\Delta}{\tau_M}\right) + \sum_{m=1}^{M-1} d_m \exp\left(-\frac{n\Delta}{\tau_m}\right) \right)$$

with

$$\underline{d} = \left(d_1, \ldots, d_{M-1}\right)' = \left(c_1, \ldots, c_{M-1}\right)' / c_M,$$

we get the minimization criterion

$$q(c_M, \underline{d}, \underline{\tau}) = \sum_{n=0}^{N-1} \left(c_M \eta_n - y_n \log(\eta_n) - y_n \log(c_M) \right).$$

Minimization of $q(c_M, \underline{d}, \underline{\tau})$ over c_M without restrictions yields the explicit solution

$$\hat{c}_M = \frac{\sum_{n=0}^{N-1} y_n}{\sum_{n=0}^{N-1} \eta_n}.$$

Substituting the estimate $\hat{c}_M$ for c_M and ignoring again a constant term, we can determine the maximum likelihood estimates of the decay parameter vector $\underline{\tau}$ and the remaining vector of the amplitude ratios $\underline{d}$ by minimizing

$$Q(\underline{d}, \underline{\tau}) = \sum_{n=0}^{N-1} y_n \log\left(\frac{\sum_{n=0}^{N-1} \eta_n}{\eta_n} \right).$$

EXPERIMENTAL RESULTS

The minimization of the criterion $Q(\underline{d}, \underline{\tau})$ requires a global search and a local optimization technique. Therefore, the convergence behavior and the robustness of several globale and locale optimization methods have been investigated empirically.

The performance of the MLE constructed above and the LSE decribed in [2] and applied in [1] and [3] is compared by means of monte carlo simulations. Fig.1 and Fig.2 show the analysis results for simulated data supposing two equally strong fluorescent components with decay parameters of 10ns and 20ns, respectively.

The superiority of the MLE can be clearly observed as the two clusters are not separated using the LSE algorithm. In Fig.3 and Fig.4 the MLE for successively measured time-resolved fluorescence data of a mixture of two PAH are plotted. Furthermore, Fig.4 indicates that about 9000 snapshots (laser exitation pulses) are required to form three satisfactory contracted clusters. The clusters are due to monomere and excimere fluorescence of the two components.

CONLUDING REMARKS

For the estimation of time-resolved fluorescence spectra a physically well motivated parametric model has been developed and the maximum likelihood principle has been applied. The MLE are obtained by an iterative optimization procedure. Various global and local optimization techniques have been empirically considered. The most convincing results are provided by combining the genetic algorithm for a row global search with a local optimization procedure, e.g. simplex algorithm or gradient based methods. Numerical experiments with simulated and real data indicate that the proposed maximum likelihood estimator has a higher resolving power than the LSE or the eigenvalue/eigenvector methods.

In completing the PAH classification procedure parametric and nonparametric cluster analysis algorithm in conjunction with decision or evidence theoretic approaches are currently under research.

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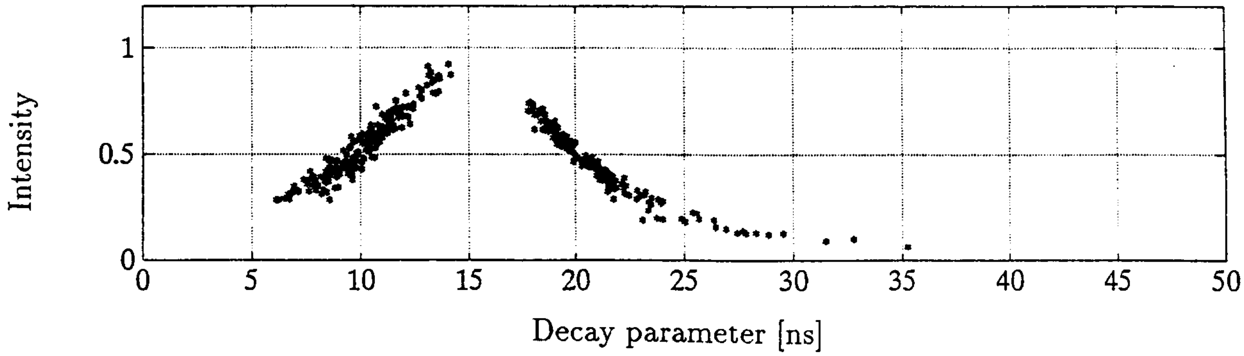

Figure 1. Maximum likelihood intensity and decay parameter estimates for 200 monte-carlo simulations.

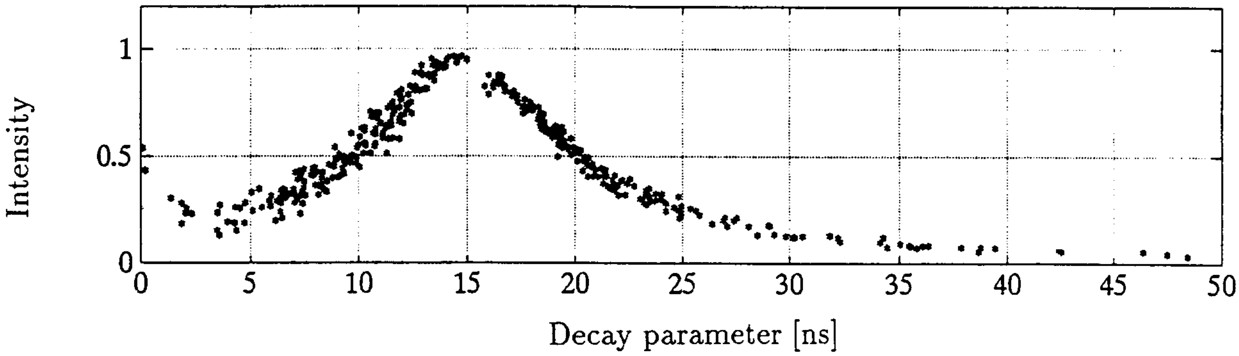

Figure 2. Least squares intensity and decay parameter estimates for 200 monte-carlo simulations.

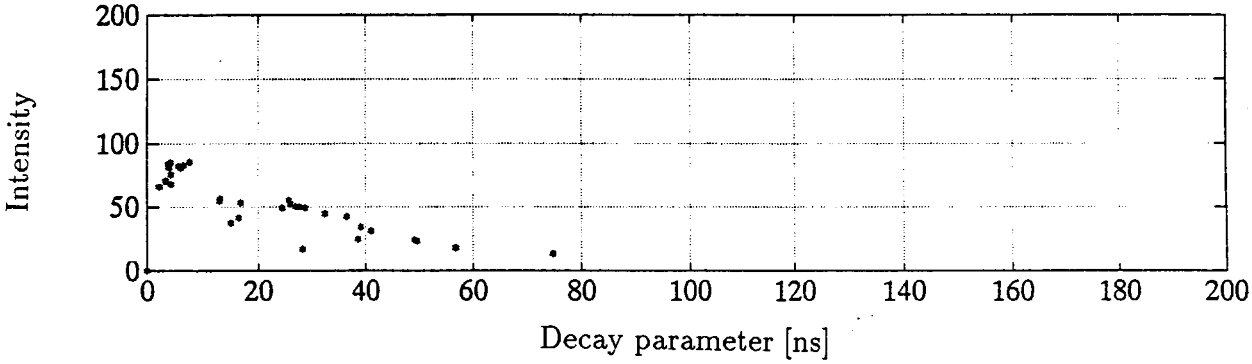

Figure 3. MLE of the intensity and decay parameters for real data (mixture of two PAH, 3000 snapshots).

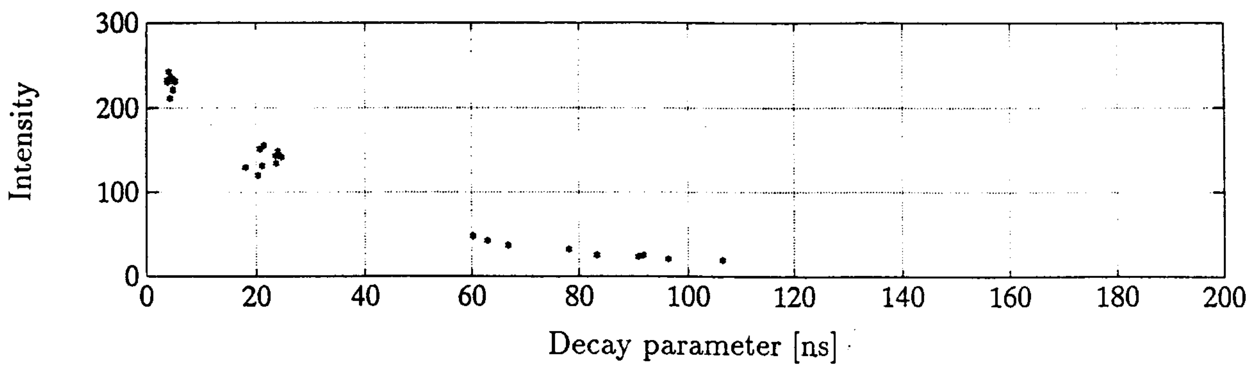

Figure 4. MLE of the intensity and decay parameters for real data (mixture of two PAH, 9000 snapshots).