Criteria for model evaluation in the case of deconvolution calculations

M. Ameloot and H. Hendrickx

Citation: The Journal of Chemical Physics **76**, 4419 (1982); doi: 10.1063/1.443557
View online: http://dx.doi.org/10.1063/1.443557
View Table of Contents: http://scitation.aip.org/content/aip/journal/jcp/76/9?ver=pdfcov
Published by the AIP Publishing

Criteria for model evaluation in the case of deconvolution calculations

M. Ameloot and H. Hendrickx

Division of Biophysics, Department W.N.F., Limburgs Universitair Centrum, Universitaire Campus, B-3610 Diepenbeek, Belgium
(Received 9 June 1981; accepted 10 December 1981)

The time-resolved fluorescence relaxation obtained by a pulse method can be described by a convolution product of the measured excitation profile and the fluorescence response of the sample. In this paper, the behavior of this convolution model, with respect to the parameters, is examined. The model happens to be nearly linear in the neighborhood of the least-squares estimates, according to an adapted nonlinearity measure. As a consequence, the lack of fit-test and the extra sum of squares-test from the theory of linear models can be applied to decide how many components should be included in the fluorescence response function. The proposed method can be easily extended to other areas involving exponential relaxation.

I. INTRODUCTION

The most important step in the study of experimental data sets is the problem of model evaluation. In the case of the measurements of fluorescence decay data, this problem reduces more specifically to the number of exponentials that have to be considered in the model. Since fluorescence decay measurements can be done very accurately, the problem is worthwhile dealing with. Moreover, the popular single photon counting method[1] (SPC) makes the problem suited for a statistical treatment because the error distribution of the experimental data is well known. A reconvolution method with the aid of an iterative nonlinear least squares technique has been found to be convenient to obtain the pre-exponentials and the lifetimes of the decay components.[2,3]

For an experimental set of fluorescence decay data, the specific problems, with regard to the evaluation of the model and of the parameters, are of a double nature: first, the nonorthogonality of the exponential functions involved, and second, the nonlinearity of the model function, i.e., the partial derivative with respect to a parameter depends on that parameter.

The evaluation criteria for a linear model are well formulated.[4–6] However, when the model is nonlinear they no longer apply. For instance, the usual F tests for regression and "lack of fit" are not strictly valid in the general nonlinear case, because the distributions of the various statistics are not known, even when the distribution of the experimental errors is well established.[4]

In this paper, we try to solve this evaluation problem by investigating whether the methods used for linear models can be adapted to nonlinear models.

In this context, measures of nonlinearity can be used in deciding whether linearized results provide acceptable approximations. The nonlinearity measures suggested in the statistical literature[7–9] hold only for observations which follow a normal distribution with common variance. As in the measurement of exponential decays, the data range covers several decades; the variances can be expected to be very different. Consequently, adapted nonlinearity measures applicable to this experimental environment have to be derived. Therefore, the nature of the fluorescence decay data is described in the first part of this paper. In the subsequent sections, the original nonlinearity measures of Beale[7] and Linssen[9] are introduced and corresponding modified measures suggested. According to these adapted measures, the convolution model has been found to have a low intrinsic nonlinearity. This means that, for the case of fluorescence decay measurements, model evaluation can be done, relying on the methods of linear regression. As a consequence, the model building procedures by a χ^2 test (lack of fit) and by a F test (extra sum of squares) are recommended to solve the problem of the number of exponentials in the model. Their convenience is then demonstrated on some simulated and real data sets.

II. NATURE OF THE FLUORESCENCE DECAY DATA

Before the investigation of the nonlinearity of the fluorescence decay models, the experimental procedure of the SPC technique and the subsequent data analysis are briefly reviewed and discussed, because the geometry of the sample space is influenced by the nature of the experimental data.

The SPC experiments, for fluorescence lifetime determinations down to the nanosecond region, are performed by repetitive excitation of the sample. The time interval distribution between the excitation and the first detected photon is recorded with a combination of a time-to-amplitude converter and a multichannel analyzer (MCA). As has been pointed out by Harris and Selinger,[10] the zeroth pulse interval density is recorded. In this work, it will be assumed that the ratio of the mean detection rate to excitation rate is sufficiently low, so that the histogram of the number of counts in a channel against channel number in the MCA is a direct analog of the decay of the sample.

In an ideal single photon counting experiment, the observed time-resolved fluorescence $g(t)$ has to be considered as the convolution of the measured excitation profile $l(t)$ and the fluorescence delta-response function $f(t, \theta)$, i.e.,

$$g(t, \theta) = \int_0^t l(u) f(t-u, \theta)\, du \,. \tag{1}$$

In most cases, the fluorescence reponse function can

 0021-9606/82/094419-14$02.10

be described by

$$f(t,\ \theta) = \sum_{i=1}^{p} \theta_i \exp(-t/\theta_{p+i}) ,$$

where $\theta = (\theta_1, \ldots, \theta_p, \theta_{p+1}, \ldots, \theta_{2p})$ denotes the unknown parameter vector. This model function is linear in the subset $a = (\theta_1, \ldots, \theta_p)$ and nonlinear in the subset $\tau = (\theta_{p+1}, \ldots, \theta_{2p})$.

In order to determine the delta-response function $f(t, \theta)$, one measures $g(t)$ and $l(t)$ at a set of values of t, the independent variable, which is assumed to be errorless.

Knight and Selinger[11] suggested that the reconvolution method would be the best way to estimate $f(t, \theta)$. In this method, the parameters of a postulated model function are determined according to some fitting criteria. This idea has been worked out by several authors (e.g., Grinvald and Steinberg[2]) and reviewed and discussed by McKinnon *et al.*[9] and O'Connor *et al.*[12]

It should stressed that, in order to get a reliable data analysis, the error distribution of the obtained data must be determined. Under the mentioned assumptions, concerning the experimental conditions, the relaxation of the excited sample will behaves as a time-dependent source described by expression (1). The total number of collected counts within the finite time window $[0, T]$ of the MCA follows a generalized Poisson distribution[13] given by

$$P_x(T) = \frac{\mu^x(T) \exp[-\mu(T)]}{x!} ,$$

with

$$\mu(T) = \int_0^T g(t)\, dt .$$

The n observations of the fluorescence response $g(t)$ will be denoted by $(y_1, \ldots, y_n)$. For a fixed total number of collected counts, the channel contents y_i of the MCA follow a multinomial distribution because successive detections of emitted photons are believed to be independent. It should be stressed that under these conditions the y_i are mutually independent and Poisson distributed.[14]

Therefore, the maximum likelihood estimators for the parameter vectors a and τ can be obtained by minimizing

$$S(\theta) = \sum_{i=1}^{n} w_i [\, y_i - g_i(\theta)]^2 ,$$

where $\{g_i(\theta)\}$ are the theoretical values corresponding to $\{y_i\}$.[15–17] The weighting factors $\{w_i\}$ are given by the inverse of the expected values, i.e., $w_i = [\, g_i(\theta)]^{-1}$. For the nonlinear models, this weighting results in an iteratively reweighted least squares method.[17,18] It should be mentioned that this weighting implies that the equality

$$\sum_{i=1}^{n} y_i = \sum_{i=1}^{n} g_i(\theta)$$

is fulfilled automatically.

III. MEASURES OF NONLINEARITY–OVERVIEW

Before suggesting nonlinearity measures adapted to the nature of the data, the original measures from the literature are briefly reviewed.

Consider the general model η described by the particular model function g

$$\eta = g(\theta,\ \xi) ,$$

where θ is a $(p \times 1)$ vector of parameters and ξ is a vector of independent variables. It will be supposed that n independent observations $(y_1, \ldots, y_u, \ldots, y_n)$ of the response η have been made, where the uth observation y_u was obtained when the independent variables ξ were set at ξ_u.

The experimental errors are assumed to be independent and normally distributed with a common variance σ^2.

Some idea of the nonlinearity of the model involved can be obtained by the study of the hyperellipsoidal confidence region, obtained under the assumption that the linearized form of the model is valid in the neighborhood of $\hat{\theta}$, the least squares estimate of θ, as suggested elsewhere.[4] This $100(1-\alpha)\%$ confidence hyperellipsoid is given by

$$(\theta - \hat{\theta})' \hat{X}' \hat{X} (\theta - \hat{\theta}) \leq p s^2 F_{p,n-p;\alpha} , \qquad (2)$$

with

$$X_{uj} = \left. \frac{\partial g(\theta,\ \xi_u)}{\partial \theta_j} \right|_{\hat{\theta}} , \quad u = 1, \ldots, n , \quad j = 1, \ldots, p .$$

s^2: estimate of σ^2 with $(n-p)$ degrees of freedom; $F_{p,n-p;\alpha}$: Upper $100\alpha\%$ point of the F distribution with $(p,\ n-p)$ degrees of freedom; and $(')$: notation for the transposed form.

The values of the sum of the squared residuals $S(\theta)$, calculated from the actual model function at several points of this ellipsoid, can be compared with each other. In the linear theory, these values have to be equal. Secondly, one can compare these values with the value S_α predicted from the linear theory,[19] and given by

$$S_\alpha = S(\hat{\theta}) \left(1 + \frac{p}{n-p} F_{p,n-p;\alpha} \right) . \qquad (3)$$

Although the correct distribution properties are not known in the nonlinear case, one can calculate confidence contours with an approximate confidence level by using expression (3), which can then be used as approximate $100\ (1-\alpha)\%$ confidence contours. The comparison of these approximate confidence regions with the linearized forms given by Eq. (2), can give an idea of the nonlinearity involved. Finally, one can compare the actual value of the sum of squares for points on these approximate contours with the values obtained for the same points using the linearized model.

However, these studies do not provide a formal criterion and give only a qualitative impression of the nonlinearity. A more elaborate numerical measure of nonlinearity has been described by Beale[7,8,19] and is

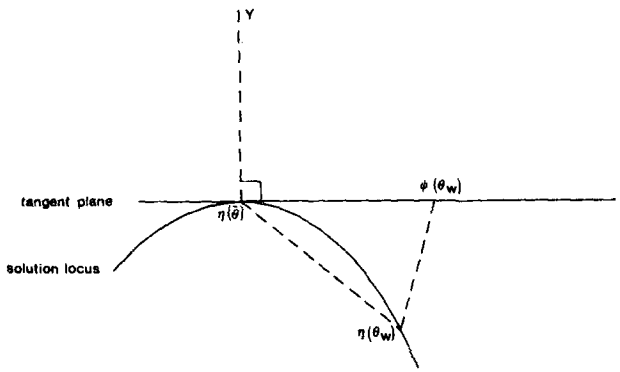

FIG. 1. Schematic representation of the solution locus and the tangent plane approximation at $\eta(\hat{\theta})$ in the sample space, including the data vector Y used in the estimation procedure of $\hat{\theta}$.

adapted in this work for SPC data.

It is common to consider a sample space of n dimensions in which each coordinate n_u represents a possible observation for $\xi = \xi_u$. The solution locus is defined as the surface in the sample space generated by the points $\eta(\theta)$ with components $\eta_u = g(\theta, \xi_u)$, $u = 1, \ldots, n$, and regarding θ as a variable. Each point θ_0 in the parameter space maps to a point $\eta(\theta_0)$ on the solution locus. Lines in the parameter space through the point θ_0 map to curves on the solution locus through $\eta(\theta_0)$. The tangent plane approximation to the solution locus in the neighborhood of $\hat{\theta}$ is given by

$$\psi_u(\theta) = \eta_u(\hat{\theta}) + \sum_{i=1}^{p} (\theta_i - \hat{\theta}_i) \left. \frac{\partial g(\theta, \xi_u)}{\partial \theta_i} \right|_{\hat{\theta}}, \quad u = 1, \ldots, n,$$

or in matrix notation

$$\psi(\theta) = \eta(\hat{\theta}) + X(\theta - \hat{\theta}) .$$

This approximation forms a linear subspace of the sample space, which has a uniform system of coordinates defined by the derivatives of the model function in $\hat{\theta}$.

Consider the points θ_w (w: $1, \ldots, m$) in the neighborhood of $\hat{\theta}$ (see Fig. 1). An empirical measure of nonlinearity is provided by the quantity

$$\hat{N}_\theta = ps^2 \frac{\sum_{w=1}^{m} \sum_{u=1}^{n} \{\eta_u(\theta_w) - \eta_u(\hat{\theta}) - \sum_{i=1}^{p} (\theta_{iw} - \hat{\theta}_i)[\partial g(\theta, \xi_u)/\partial \theta_i]|_{\hat{\theta}}\}^2}{\sum_{w=1}^{m} \{\sum_{u=1}^{n} [\eta_u(\theta_w) - \eta_u(\hat{\theta})]^2\}^2} ,$$

or

$$\hat{N}_\theta = ps^2 \frac{\sum_{w=1}^{m} \| \eta(\theta_w) - \psi(\theta_w) \|^2}{\sum_{w=1}^{m} \| \eta(\theta_w) - \eta(\hat{\theta}) \|^4} . \tag{4}$$

The dimensionless quantity $\hat{N}_\theta$ is a quadratic average of the distances between corresponding points on the solution locus and the tangent plane approximation, the normalization being provided by the square of the distances from the points on the solution locus to $\eta(\hat{\theta})$. Beale states that the value of $\hat{N}_\theta$ may depend on the configuration of the points $\eta(\theta_w)$ around $\eta(\hat{\theta})$, but should not depend significantly on the number of points used or their distances from $\eta(\hat{\theta})$, if these distances are not too large. Corresponding to the empirical measure given by Eq. (4), a theoretical measure N_θ can be defined as the limit of $\hat{N}_\theta$, as the number of test points θ_w goes to infinity and the points θ_w have a particular configuration. Guttman and Meeter[19] have checked the conditions under which $\hat{N}_\theta$ is a valuable estimate of N_θ.

Beale states that the model could be regarded as being obviously nonlinear if

$$\hat{N}_\theta > 1/F_{p,n-p;\alpha} ,$$

and that the linear approximation is valid if

$$\hat{N}_\theta < 0.01/F_{p,n-p;\alpha} .$$

If $\hat{N}_\theta$ falls between the given levels, Beale suggests a transformation of the parameters, because $\hat{N}_\theta$ measures both the model nonlinearity and the nonlinearity due to the parametrization in the particular model function. A nonlinear transformation $\psi = \psi(\theta)$ of the parameters θ_i may provide a more uniform coordinate system on the solution locus, thereby reducing the nonlinearity of the model function. The minimum nonlinearity will be attained when $\psi(\theta_w)$ is always at the foot of the perpendicular from $\eta(\theta_w)$ on the tangent plane at $\eta(\hat{\theta})$ to the solution locus. This minimum value, resulting under the particular transformation $\phi = \phi(\theta)$, will be denoted by $\hat{N}_\phi$. The limiting value of $\hat{N}_\phi$ for an infinity of points around $\eta(\hat{\theta})$ is denoted by N_ϕ, called the intrinsic nonlinearity of the model. N_ϕ can be regarded as the curvature of the solution locus at $\eta(\theta)$,[8] which depends only on the model and the experimental design.

According to the transformation $\phi = \phi(\theta)$, $\hat{N}_\phi$ will be given by[19]

$$\hat{N}_\phi = ps^2 \frac{Q_\phi}{\sum_{w=1}^{m} \| \eta(\theta_w) - \eta(\hat{\theta}) \|^4} ,$$

where

$$Q_\phi = \sum_{w=1}^{m} \sum_{u=1}^{n} \left[\eta_u(\theta_w) - \eta_u(\hat{\theta}) - \sum_{j=1}^{p} \phi_{wj} \left. \frac{\partial g(\theta, \xi_n)}{\partial \theta_j} \right|_{\hat{\theta}} \right]^2 .$$

The values ϕ_{wj} are obtained from the normal equations $X'[\eta(\theta_w) - \eta(\hat{\theta}) - X\phi_w] = 0$; $w = 1, \ldots, m$ which define m sets of p simultaneous linear equations.

The measure N_ϕ can be used in the following way. If N_ϕ is not too large, a confidence region with associated probability greater than or equal to $(1 - \alpha)$ is given by

$$S(\theta) - S(\hat{\theta}) \leq ps^2 k_1 F_{p,n-p;\alpha} ,$$

where

$$k_1 = 1 + N_\phi , \qquad (p = 1) ,$$

$$k_1 = 1 + (p + 2/p) N_\phi , \quad (p \geq 2) ,$$

if s^2 is an independent estimator of σ^2.

If s^2 is estimated from the sample, k_1 has to be modified as follows:

$$k_1 = 1 + \frac{n}{n-1} N_\phi \ , \quad (p=1) \ ,$$

$$k_1 = 1 + \frac{n(p+2)}{(n-p)p} N_\phi \ , \quad (p \geq 2) \ .$$

The lack of fit test becomes

$$S(\hat{\theta}) \leq (n-p)\, s^2 [1 + (p - 2/n - p) N_\phi] F_{p,n-p;\alpha} \ . \tag{5}$$

However, the nonlinearity may be underestimated if the points $\eta(\theta_w)$ on the solution locus, used in the calculation of $\hat{N}_\theta$ or $\hat{N}_\phi$, are taken too far away from $\eta(\hat{\theta})$.[4] This risk can be minimized by the use of a modified measure that has been suggested by Linssen.[6] In this new measure, the denominator is determined by $\eta(\hat{\theta})$ and $\psi(\theta_w)$

$$\hat{M}_\theta = \frac{s p^{1/2} [\sum_{w=1}^{m} \| \eta(\theta_w) - \psi(\theta_w) \|^2]^{1/2}}{\sum_{w=1}^{m} \| \eta(\hat{\theta}) - \psi(\theta_w) \|^2} \ .$$

Following a similar line of thought as Beale did, the corresponding low-nonlinearity criterion is supplied by

$$\hat{M}_\theta < \frac{0.1}{(F_{p,n-p;\alpha})^{1/2}} \ .$$

Accordingly, the intrinsic nonlinearity measure $\hat{M}_\phi$ can be defined. In the case of a small nonlinearity, one should note that

$$\hat{M}_\theta^2 \approx \hat{N}_\theta \ , \quad \hat{M}_\phi^2 \approx \hat{N}_\phi \ .$$

IV. NONLINEARITY MEASURES FOR FLUORESCENCE DECAY MODELS

In the described nonlinearity estimators, distances measured in the sample space have to be compared. This means that a metric has been defined. It should be emphasized that the nature of the error distribution of the observations determines the geometry to be applied.[8] In the preceding section, the data were supposed to be independent and normally distributed with common variance, so that the usual Euclidian metric was used.

In order to avoid extra difficulties, the use of the Euclidian metric in the sample space, generated by the fluorescence decay measurements, should be advised. This requires in the SPC technique a sufficient number of counts in the channels of the MCA, in order that the normal approximation to the Poisson distribution would be valid. The observations y_i can then be transformed into a data set with common variance by multiplying each observation with the appropriate weighting factor[4] $w_i^{1/2} = y_i^{-1/2}$. However, this weighting can be interpreted as a transformation of coordinates in the sample space, so that the shape and the curvature of the solution locus are changed accordingly.

Taking this effect into account, the estimators $\hat{N}_\theta$ and $\hat{N}_\phi$ have to be modified into a kind of "weighted" nonlinearity measures $\hat{N}'_\theta$ and $\hat{N}'_\phi$

$$\hat{N}'_\theta = p s^2 \frac{\sum_{w=1}^{m} \sum_{u=1}^{n} w_u [\eta_u(\theta_w) - \eta_u(\hat{\theta}) - \sum_{j=1}^{p} (\theta_{jw} - \hat{\theta}_j)(\partial g / \partial \theta_j)|_{\hat{\theta}}]^2}{\sum_{w=1}^{m} \{\sum_{u=1}^{n} w_u [\eta_u(\theta_w) - \eta_u(\hat{\theta})]^2\}^2} \ , \tag{6}$$

and

$$\hat{N}'_\phi = p s^2 \frac{\sum_{w=1}^{m} \sum_{u=1}^{n} w_u [\eta_u(\theta_w) - \eta_u(\hat{\theta}) - \sum_{j=1}^{p} \phi_{wj} (\partial g / \partial \theta_j)|_{\hat{\theta}}]^2}{\sum_{w=1}^{m} \{\sum_{u=1}^{n} w_u [\eta_u(\theta_w) - \eta_u(\hat{\theta})]^2\}^2} \ , \tag{7}$$

where $w_u = y_u^{-1}$.

Some criticism can be given on these expressions because, therein, more weight is given to observations that are below their expected value than to observations that are above their expected value. This remark can be anticipated however by replacing the weights by the inverse of the expected values $g_i(\hat{\theta})^{-1}$. The use of these weights suggests a kind of Poissonian weight. The effect of this transformation is not entirely clear. We prefer the expressions (6) and (7). Besides, if the data values are large, the relative deviations from y_i^{-1}, with respect to $g_i(\hat{\theta})^{-1}$, will be small.

The estimators $\hat{M}_\theta$ and $\hat{M}_\phi$ have to be adapted accordingly into the new measures $\hat{M}'_\theta$ and $\hat{M}'_\phi$.

It will be shown on real and simulated data sets that, according to the modified nonlinearity measures, the convolution model for fluoresence decay data has a low intrinsic nonlinearity. For convenience, the original and the adapted nonlinearity measures, together with their interrelations, are summarized in Fig. 2.

V. MODEL EVALUATION OF THE FLUORESCENCE DECAY DATA

In this section, a procedure is suggested for the model evaluation of fluorescence decay data. Because the con-

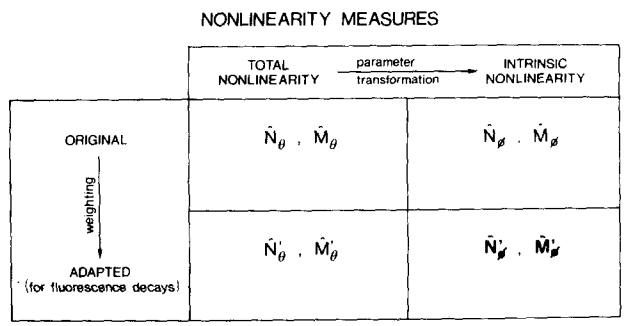

FIG. 2. Overview of the original and adapted nonlinearity measures with their interrelations.

volution model will demonstrate a low intrinsic nonlinearity, statistics used in the evaluation of linear models can be proposed.

The parameters of the fluorescence response function will be estimated by using the deconvolution method. In this procedure, a model has to be proposed *a priori* and evaluated with appropriate tests.

The χ^2 test for "goodness of fit" is suggested in the literature for that purpose. Because the distributions of the statistics in the estimation of nonlinear models are not exactly known, the use of this χ^2 test may be not correct, even for low intrinsic nonlinearity. Indeed, the χ^2 test can be regarded as coming from the "lack of fit" test for linear models under the assumption of normally distributed errors

$$S(\hat{\theta}) \leq (n-p)\, s^2 F_{n-p,\nu;\alpha} ,$$

where s^2 is an independent estimate of the common variance σ^2 with ν degrees of freedom. When the normal approximation to Poisson distributed data is used and the weights are chosen accordingly, the variance σ^2 should be one. In the limit $\nu \to \infty$, s^2 should converge to σ^2. Taking into account the correction for the nonlinearity of the model, as expressed by Eq. (5), the χ^2 test has to be written as

$$S(\hat{\theta}) \leq \chi^2_{n-p}\left(1 + \frac{p-2}{n-p}\, N'_\phi\right) .$$

However, the correction factor in the right-hand side of this inequality is nearly one because the value of $\hat{N}'_\phi$, the estimate of N'_ϕ, will be small even in the case of a moderate intrinsic nonlinearity and because the number of observations n is usually greater than 200. So, the lack of fit test can be used without correction for fluorescence decay rate.

If there is lack of fit, considering another model is imperative. When $S(\hat{\theta})$ is well below the critical value, it should be common practice to fit the data to another model because the only admitted conclusion is that the model is plausible and not been found inadequate by the data.[4] This does not guarantee that the proposed model is the best description of the data.

So, the question arises of whether it is worthwhile to include an extra exponential term. This problem can be investigated by examining the sum of squares values of the competing models in the same way as one does for linear models.

Consider two models without lack of fit: model M_1 with p parameters and $S_1(\hat{\theta}_1)$, where $\theta_1 = (\theta_{11}, \ldots, \theta_{1p})$; and model M_2 with q parameters and $S_2(\hat{\theta}_2)$, $q < p$

$$\theta_2 = (\theta_{21}, \ldots, \theta_{2q}) .$$

The errors are supposed to be normally distributed. The objective is to see if the inclusion of the $(p-q)$ extra parameters in the model M_1 gives a significant reduction of $S_1(\hat{\theta}_1)$, as compared to $S_2(\hat{\theta}_2)$.

The F statistic given by

$$F = \frac{[S_2(\hat{\theta}_2) - S_1(\hat{\theta}_1)]/p-q}{S_1(\hat{\theta}_1)/n-p}$$

has to be compared with $F_{p-q,n-p;\alpha}$ to test the hypothesis $H_0: \theta_{q+1} = \theta_{q+2} = \cdots = \theta_p = 0$.

If the test is significant, the extra parameters have to be included; otherwise, the extra terms are rejected. This method has been called "the extra sum of squares principle".[4] As for the χ^2 test, the examination of the nonlinearity of convoluted fluorescence decay data justifies the use of this F test.

VI. MATERIALS AND METHODS

A. Instrumentation

The fluorescence decays were measured by means of a conventional SPC apparatus. The optical part and the detector (Mullard 56 DUVP/03) were purchased from Applied Photophysics Ltd. (London, U.K.). The NIM electronics were delivered by Canberra (Meriden, Conn.) and Ortec (Oak Ridge, Tenn.). The MCA was directly connected to a PDP 11/34 (Digital Equipment Corp., Marlboro, Mass.).

B. Data analysis

The data were analyzed with a nonlinear least squares algorithm according to Marquardt.[20] The program has been implemented in Fortran and uses double precision arithmetic (17 decimals). In most cases, starting values for the iterative procedure could be obtained from a preceding Laplace deconvolution,[21] which has been modified with respect to the cutoff correction. The weights during the least squares search could be chosen to be Gaussian or Poissonian. The program has the possibility of evaluating a sum of three exponentials in the presence of a correction for lampscatter and zero-time shift.[22] After the estimation of the parameters, the cross sections of the confidence hyperellipsoid and the nonlinearity measures ($\hat{N}'_\theta$, $\hat{N}'_\phi$, $\hat{M}'_\theta$, $\hat{M}'_\phi$) are calculated.

C. Chemicals

The decay measurements were performed on a degassed solution of 1,3-di(β-naftoxy)-propane in isooctane at a concentration of 2×10^{-5} M, purified by HPLC on silica (10 μ), using a mixture of n-hexane and chloroform (80/20) as eluens, and recrystallized from absolute ethanol. The quantum yields of monomer and excimer fluorescence were, respectively, 0.25 and 0.03. The sample was excited at 280 nm and the fluorescence of the monomer was measured at 335 nm.

D. Real decay data

From a previous temperature study,[23] it could be concluded that the monomer flourescence decay of 1,3-di(β-naftoxy)-propane is described by a sum of three exponentials with well-separated decay constants below 20 °C. A description of this data with two exponentials causes a pronounced lack of fit. When the temperature is increased, the separations between the decay constants decrease, so that a decay measured at room temperature provides a model building test case (dataset RT2073). A satisfiable fit could only be obtained with three relaxation times with values well

TABLE I. Estimates for the nonlinearity measures of the convolution model, together with their respective low nonlinearity criteria. The complete description of the respective model functions are given in Tables II–VI. Simulations are labeled with SY, the real data with RT.

Data set	Number decays	$\hat{N}_\vartheta'$	$\hat{N}_\phi'$	$\frac{0.01}{F}$	$\hat{M}_\vartheta'$	$\hat{M}_\phi'$	$\frac{0.1}{\sqrt{F}}$
SYL038	2	6×10^{-3}	4×10^{-8}	2×10^{-2}	2.0	2×10^{-4}	2×10^{-1}
SYL039	2	1×10^{-3}	1×10^{-7}	2×10^{-2}	6.0	4×10^{-4}	2×10^{-1}
SYL047	2	2×10^{-4}	6×10^{-7}	4×10^{-3}	16.0	8×10^{-4}	6×10^{-2}
SYL055	2	1×10^{-2}	2×10^{-6}	4×10^{-3}	2.0	1×10^{-3}	6×10^{-2}
SYL042	3	3×10^{-5}	1×10^{-7}	5×10^{-3}	35.0	4×10^{-4}	7×10^{-2}
SYL016	3	9×10^{-4}	3×10^{-8}	5×10^{-3}	6.0	2×10^{-4}	7×10^{-2}
TR2073	3	7×10^{-3}	3×10^{-6}	5×10^{-3}	2.0	2×10^{-3}	7×10^{-2}
SYL015	3	6×10^{-2}	4×10^{-4}	5×10^{-3}	0.7	2×10^{-2}	7×10^{-2}
RT2047	3	8×10^{-2}	7×10^{-4}	5×10^{-3}	0.4	3×10^{-2}	7×10^{-2}
SYL053	3	8×10^{-2}	7×10^{-4}	5×10^{-3}	0.5	3×10^{-2}	7×10^{-2}
RT2049	3	4×10^{-4}	8×10^{-9}	5×10^{-3}	7.0	9×10^{-5}	7×10^{-2}
SYL072	3	1×10^{-4}	5×10^{-10}	5×10^{-3}	14.0	2×10^{-5}	7×10^{-2}

within the line of the results of the previous study. The experiment was repeated with a somewhat maladjusted lamp, which resulted in an excitation profile with a larger full width at half-maximum (FWHM) (data sets RT2047, RT2049) in order to increase the problem of data analysis. In this manner, real data sets are available for which the kinetic scheme of the corresponding fluorescence relaxation is known from the analysis of RT2072. The peak channel of the excitation profile contains at least 20 000 counts.

E. Simulations

Simulated decay data sets were generated by convoluting a nonsmoothed measured excitation profile with a sum of exponential decays. Counting noise was added according to the Gaussian asymptotic approximation of the Poisson distribution. The function RAN of the Fortran package supplied by Digital was used at the start of the noise generating sequence to give uniformly distributed random numbers on $]0, 1[$. Gaussian distributed random numbers were then obtained with an algorithm, according to Moshman.[24] Instrumental distortions such as scatter and zero-time shift error can be built in, if desired. All simulated data sets in the text are labeled with SY.

VII. RESULTS

A. Nonlinearity of the convolution model

The application of the modified nonlinearity measures proposed in Sec. IV on the simulated and real data sets will be described next.

In an earlier version of the computer program, the calculations of the nonlinearity were made by using points θ_w, taken from cross sections through $\hat{\theta}$ of the confidence hyperellipsoid of the linearized model. These intersections nearly coincide with the sections calculated with the actual model using the value S_α (see also Appendix A). This confirms the nearly ellipsoidal contours in the (τ_i, τ_j) plane at the 95% confidence level calculated by Selinger for a biexponential decay without convolution, simulating well-designed experiments.[18]

This may underestimate the total nonlinearity. Therefore, the nonlinearity is calculated at end points of the major axes of the confidence hyperellipsoid of the linearized model. These points can be found by taking the eigenvectors of the unique symmetric matrix, appearing in the quadratic expression (2), as the new basis in the parameter space. By choosing these end points as θ_w and using the quadratic averaging formula as suggested by Beale, a more reliable empirical estimate of the nonlinearity can be obtained.[9] The results of the determination of the nonlinearity of the convolution model can be found in Table I.

From the table, the following can be concluded: The measure $\hat{M}_\vartheta'$ always exceeds its limit value; $\hat{N}_\vartheta'$ in most cases, but not always. This suggests that $\hat{N}_\vartheta'$ sometimes underrates the total nonlinearity, which is in agreement with earlier results.[9,19] However, the measures of intrinsic nonlinearity $\hat{N}_\phi'$ and $\hat{M}_\phi'$ are always below the criteria which implies a low intrinsic nonlinearity of the convolution model. Analogous results are obtained by using the inverse of the function values as the weighting factors. It may be concluded that there exists a parametrization for which the convolution model has a negligible nonlinearity. Because the convolution product leaves a linear model completely linear, the mentioned result could be expected from the findings of Bates and Watts,[8] who state that the nonlinearity of the most models is mainly due to the parametrization.

The large values of $\hat{M}_\vartheta'$ were mainly due to the nonlinearity on only a few principal axes of the linearized confidence hyperellipsoid. Because the actual $S(\theta)$ values for the end points on these axes are larger than the value predicted from linear theory, the linearized confidence region includes the corresponding region of the actual model. A conservative confidence region can then be obtained by circumscribing the confidence hy-

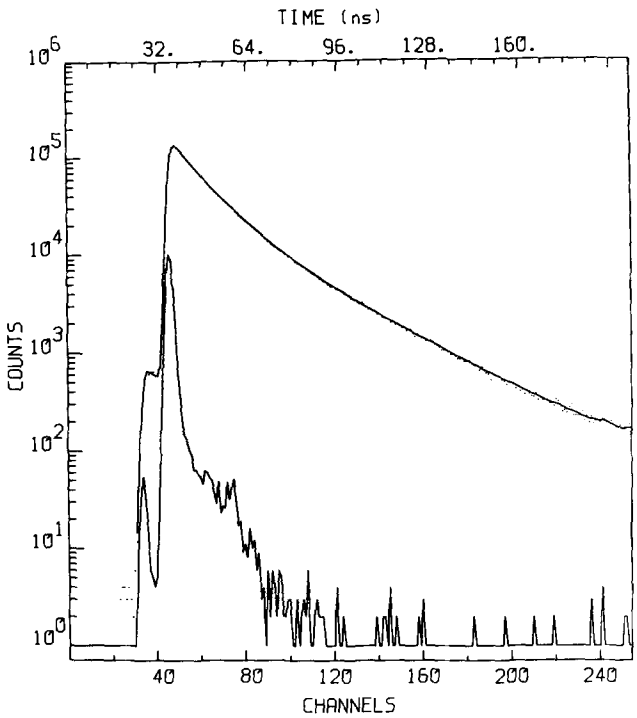

FIG. 3. Data representation of simulation SYL016. The excitation profile and the convolution containing three components are represented with a solid line. The dots indicate the simulated decay.

perellipsoid for the linear model by tangent hyperplanes, each pair of planes being parallel to one of the coordinate axes in the parameter space. This enables the determination of the support plane intervals.[20,22]

The linear behavior of the convolution model, with respect to the parameters, could be suspected from a remark given by Grinvald and Steinberg.[2] They found comparable confidence intervals by applying the support plane method to the linear approximation and the rigorous method of Hamilton,[6] in which the maximum interval of a parameter is investigated by allowing the other parameters to maximize that inverval without exceeding the value S_α.

B. Model evaluation procedures

Although the values of $\hat{N}'_\theta$, $\hat{M}'_\theta$ and $\hat{N}'_\phi$, $\hat{M}'_\phi$ suggest that one has to perform a parameter transformation to establish the low intrinsic nonlinearity of the convolution model, one can use the original model function in the evaluation of the χ^2 test and the F test because the sum of squares values do not depend on the parametrization. Indeed, a small value of $\hat{N}'_\phi$ or $\hat{M}'_\phi$ is necessary and sufficient to apply these tests.

In many cases, the χ^2 test will exclude the alternative model because of the high $S(\hat{\theta})$ value. It is a good practice to fit various models to the same set of experimental data to see if some decay component was not partially masked by a bad experimental design. If one finds two models with a sufficiently low $S(\hat{\theta})$ value, the final decision should be made by using the F test. The adequacy of a model can also be checked by investigating the weighted residuals because the latter should confirm the assumptions concerning the errors when the model is correct. It should be mentioned that most tests on the residuals are exact for nonlinear models and may be applicated without modification. So, the randomness of the weighted residuals may be investigated by the run test (see Appendix B) and the autocorrelation function,[2] while their distribution can be compared with the standard normal distribution. In order to illustrate the use of these tests and to discuss their respective merits, a few simulated and experimental data sets were selected.

1. Simulations

In Tables II and III, simulated data are given for which the competing models are, respectively, a mono- and a bi-exponential decay and a two and a three exponential decay. The analysis of these data sets always started from the first channel for which the normal approximation was valid.

It can be deduced from Tables II and III that there is a pronounced effect of the total collected counts on the model building procedure to be followed. When the decay data are sufficiently large, all tests favor the same alternative, except the check for the standard normal distribution which gives no real information concerning the problem. In the case that the total count is relatively small, the two competing models can be fitted without lack of fit. It can be concluded from Figs. 4

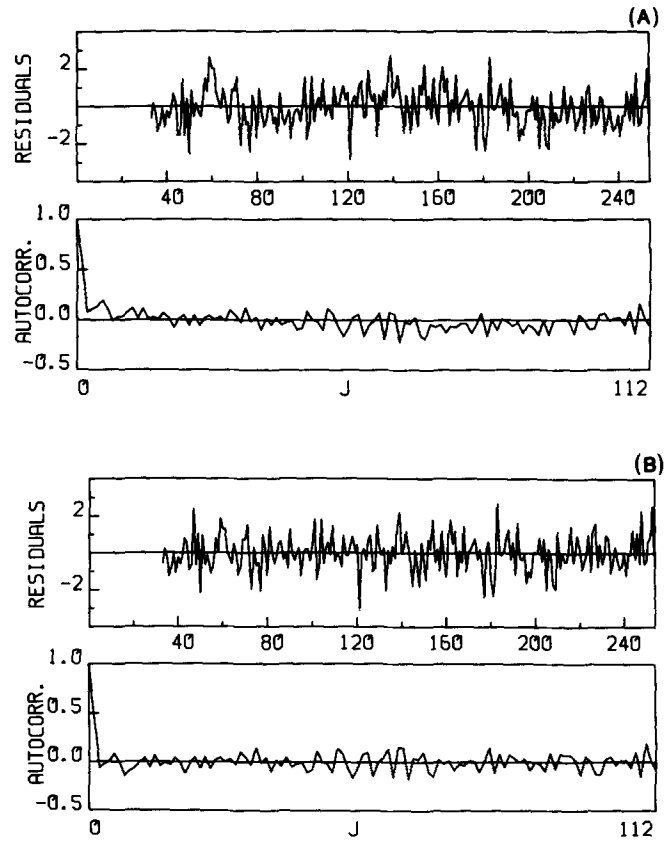

FIG. 4. Weighted residuals vs channel number and their autocorrelation for SY8042 analyzed for two components (A) and three components (B).

TABLE II. Simulated data sets for which the correct model is a two component decay. Column I contains the parameters used in the generation sequence. Columns II and III contain the estimates from a two and one component analysis. The amplitudes are expressed in ns^{-1} and the lifetimes in ns.

Data set	SYL038			SYL039			SYL047			SY8055		
Number of channels	183			183			233			243		
Channel width (ns)	0.196			0.196			0.451			0.451		
FWHM-lamp (ns)	5			5			3.5			3.5		
Total counts decay	670 524			1 005 469			373 390			2 490 187		
Peak value decay	13 463			20 133			6846			45 600		
	I	II	III	I	II	III	I	II	III	I	II	III
a_1	0.02	0.013	0.2178	0.03	0.03	0.3264	0.15	0.15	0.1759	1	1.02	1.174
σ_{a_1}	...	0.004	0.0003	...	0.01	0.0005	...	0.03	0.0004	...	0.03	0.002
a_2	0.2	0.209	...	0.3	0.31	...	0.03	0.03	...	0.2	1.84	...
σ_{a_2}	...	0.004	...	...	0.01	...	...	0.03	...	...	0.03	...
τ_1	3	2.2	4.873	3	2.7	4.875	20	20.0	19.07	20	19.9	19.06
σ_{τ_1}	...	0.7	0.006	...	0.6	0.005	...	0.6	0.04	...	0.1	0.02
τ_2	5	4.95	...	5	4.98	...	12	13	...	12	11.6	...
σ_{τ_2}	...	0.03	...	...	0.04	...	...	3	...	...	0.8	...
$S(\hat{\theta})$		178.4	208.5		179.3	224.3		219.8	263.8		208.7	668.3
(χ^2 crit)[a]		(211.0)	(213.2)		(211.0)	(213.2)		(265.1)	(267.3)		(275.9)	(278.0)
Z[b]		1.48	−1.12		1.48	−1.10		0.92	−0.29		−0.13	−7.73
autocorrelation[c]		46	39		46	31		54	43		79	3
$N(0,1)$[d]		y[d]	y		y	y		y	y		y	y
F test			15.1			L.O.F.[f]			22.9			L.O.F.[f]
(F crit)[e]			(3.0)						(3.0)			

[a]Upper 95% point of the χ^2 distribution with the appropriate degrees of freedom.
[b]Z is the statistic of the run test (see Appendix B).
[c]The number of sign changes of the autocorrelation function of the weighted residuals.
[d]$N(0,1)$ denotes the test for a standard normal distribution of the weighted residuals. y is the positive result.
[e]Upper 95% point of the F distribution with appropriate degrees of freedom.
[f]L.O.F. denotes that the F test is not applied becasue the χ^2 test is significant.

TABLE III. Simulated data sets for which the correct model is a sum of three decays. The alternative contains two components. Column I contains the parameters used in the simulation. Columns II and III contain the estimates from a three and a two component analysis. The explanation of the symbols is given in the footnotes of Table II. The data of simulation SYL016 are represented in Fig. 3.

Data set	SY8042			SYL016		
Number of channels	222			222		
channel width (ns)	0.784			0.784		
FWHM lamp (ns)	2.5			2.5		
Total counts decay	791 580			2 949 170		
Peak value decay	36 000			135 182		
	I	II	III	I	II	III
a_1	1	1.0	1.345	4	4.1	5.08
σ_{a_1}	...	0.2	0.006	...	0.2	0.01
a_2	0.4	0.4	0.342	1.6	1.6	1.24
σ_{a_2}	...	0.2	0.006	...	0.2	0.01
a_3	0.2	0.24	...	0.8	0.7	...
σ_{a_3}	...	0.05	...	...	0.1	...
τ_1	8	8.2	9.27	8	8.0	9.10
σ_{τ_1}	...	0.6	0.06	...	0.2	0.04
τ_2	15	14	26.3	15	16	27.0
σ_{τ_2}	...	3	0.3	...	1	0.1
τ_3	30	28	...	30	30.7	...
σ_{τ_3}	...	1	...	...	0.8	...
$S(\hat{\theta})$	...	218.7	249.5		216.2	445.4
(χ^2 crit)[a]		(251.1)	(253.4)		(251.1)	(253.4)
Z[b]		1.60	−0.46		2.24	−4.72
autocorrelation[c]		76	60		59	9
$N(0,1)$[d]		y[d]	y		y	y
F test			15.2			L.O.F.[f]
(F crit)[e]			(3.0)			

and 5 and Tables II and III that, in this case, a correct choice can be made only by using the F test.

Similar conclusions concerning the autocorrelation function have already been drawn.[2,22] The power of the autocorrelation function, with respect to the randomness of the residuals, becomes more pronounced when the noise level on the decay data is lower.

2. *Real experiments and corresponding simulations*

In order to illustrate the similar behavior of the various tests, the model building procedures are applicated on the real data RT2073, RT2047, RT2049, and on simulations generated by using the estimates from the experiments. Thereby, the estimation procedure and the subsequent model evaluation have been applicated on data sets with and without truncating of the raising part of the fluorescence response. We remind that several authors apply this truncation to reduce the effect of instrumental artifacts.

The results are summarized in Tables IV, V, and VI. The real and simulated data could be fitted with a convolution sum of three decays by taking the raising part into account (see Fig. 7).

The possibility that the shortest decay component may be due to an artifact has been ruled out by the previous study[23] and the fact that RT2073 could not be fitted with-

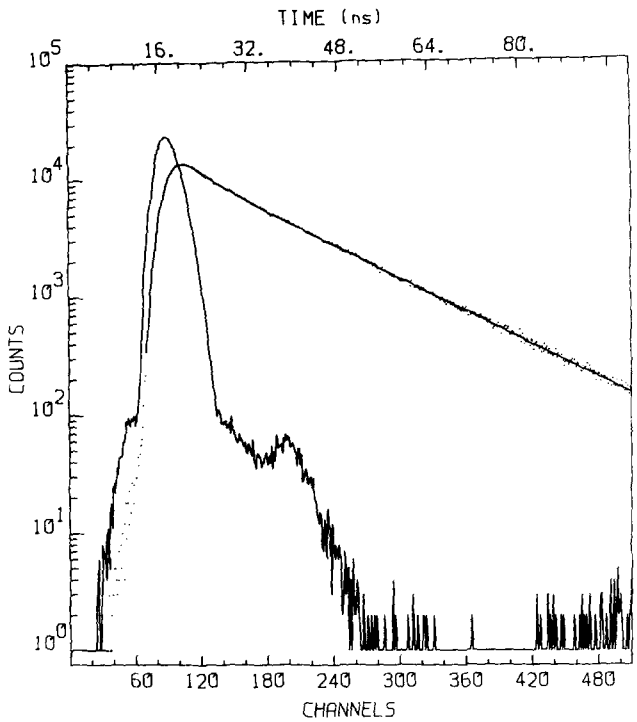

FIG. 6. Data representation of experiment RT2047. The excitation profile and the convolution containing three components are represented with a solid line. The dots indicate the observed decay.

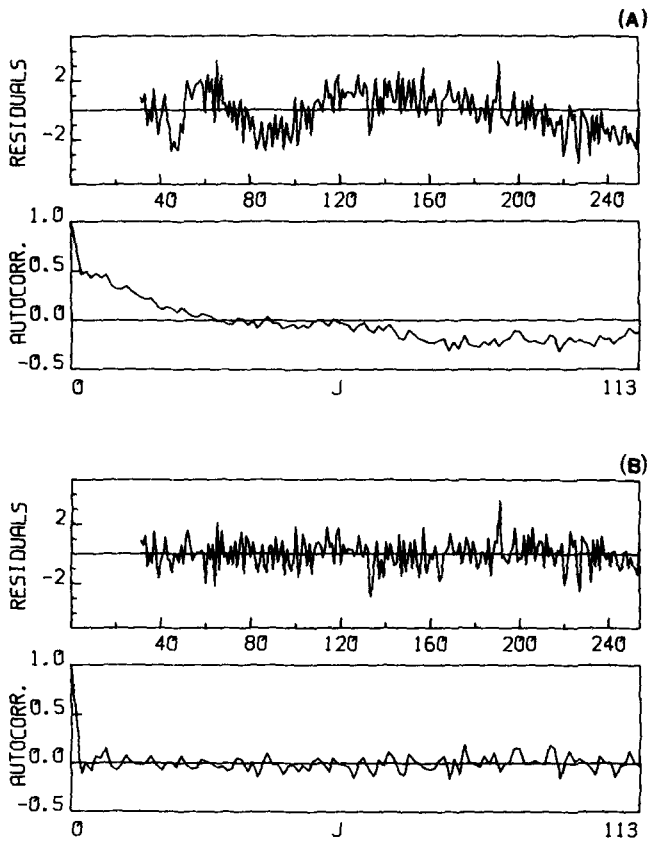

FIG. 5. Weighted residuals vs channel number and their autocorrelation for SYL016-analyzed for two components (A) and three components (B). SYLO16 contains a greater total count than the corresponding simulation SY8042 (see Fig. 4).

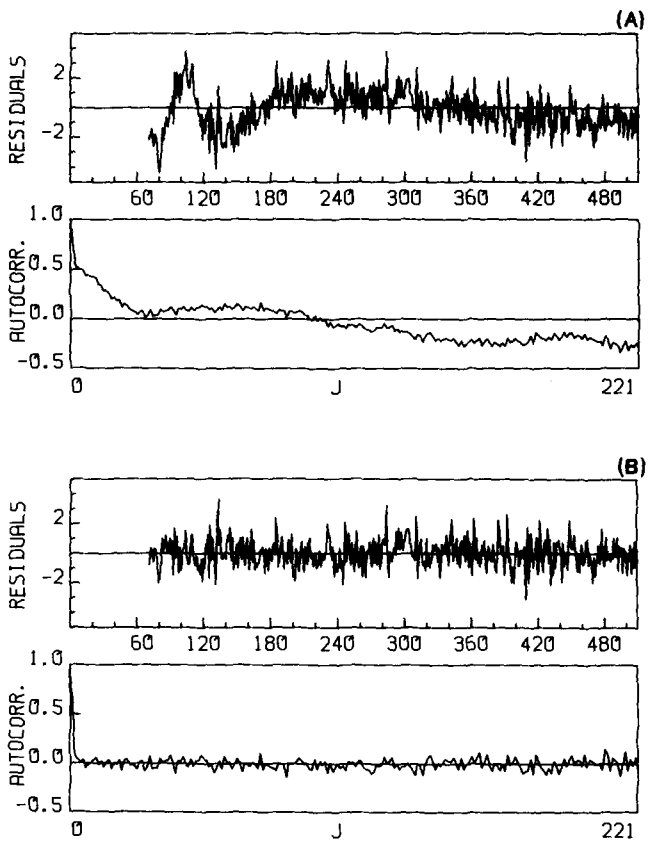

FIG. 7. Weighted residuals vs channel number and their autocorrelation for experiment RT2047, analyzed for two exponentials (A) and for three exponentials (B) by taking the raising part of the measured decay into account.

TABLE IV. Analysis of experiment RT2073 and of simulation SYL015 using different models. The evaluation started before and after the maximum decay value. The simulation SYL015 was generated by using the excitation profile of RT2073 and the six estimates from the analysis of RT2073 starting at channel 71. The explanation of the symbols is given in the footnotes of Table II.

Data set	RT2073				SYL015			
Channel width (ns)	0.165				0.165			
FWHM lamp (ns)	3.5				3.5			
Total counts decay	1 194 919				1 195 585			
Peak value decay	10 142				20 285			
start ch.	71		111		71		111	
Total No. ch.	439		399		439		399	
	I	II	I	II	I	II	I	II
a_1	0.062	0.0719	0.06	0.055	0.061	0.0734	0.049	0.055
σ_{a_1}	0.002	0.0009	0.01	0.001	0.002	0.0009	0.008	0.001
a_2	0.060	0.1270	0.047	0.119	0.046	0.1270	0.047	0.119
σ_{a_2}	0.009	0.0005	0.004	0.001	0.002	0.0005	0.003	0.001
a_3	0.08	...	0.103	...	0.106	...	0.105	...
σ_{a_3}	0.01	...	0.007	...	0.004	...	0.006	...
τ_1	1.8	3.21	1.6	5.2	1.5	3.16	1.9	5.3
σ_{τ_1}	0.1	0.07	0.3	0.2	0.1	0.07	0.5	0.2
τ_2	10	18.21	9	18.71	8.4	18.22	9	18.73
σ_{τ_2}	1	0.05	1	0.07	0.7	0.04	1	0.07
τ_3	20.4	...	19.5	...	19.4	...	19.5	...
σ_{τ_3}	0.6	...	0.4	...	0.2	...	0.3	...
$S(\hat{\theta})$	448.2	672.6	394.6	446.6	411.8	631.3	374.4	411.9
$(\chi^2_{crit})^a$	(482.4)	(484.5)	(440.1)	(442.2)	(482.4)	(484.5)	(440.1)	(442.2)
Z^b	−2.82	−6.05	−1.95	−3.83	1.91	−4.38	1.51	−1.10
autocorrelation[c]	70	19	75	47	104	15	95	93
$N(0,1)^d$	y^d	y	y	y	y	y	y	y
F test		L.O.F[f]		L.O.F		L.O.F.		
(F crit)[e]							19.7 (3.0)	

out lack of fit, by a sum of two exponentials and introducing a correction for scatter and/or time-shift. The analysis of the corresponding simulations suggest that the recovery of the six parameters is reasonable and is better for the smaller excitation profile. The two component alternative was excluded by the χ^2 test and the tests for the randomness of the residuals excepted for the real data set with the largest lamp profile where the F test was decisive.

When, however, the raising part of the decay is neglected, the short component is not well represented in the data so that in various cases a satisfiable fit could also be obtained with a two component model (see Fig. 8). The truncation of the data results in an insufficient discriminative power of the χ^2 test, the run test, and the autocorrelation function, and necessitates the use of the F test. Although the uncertainties of the estimates for the two exponential model are smaller than

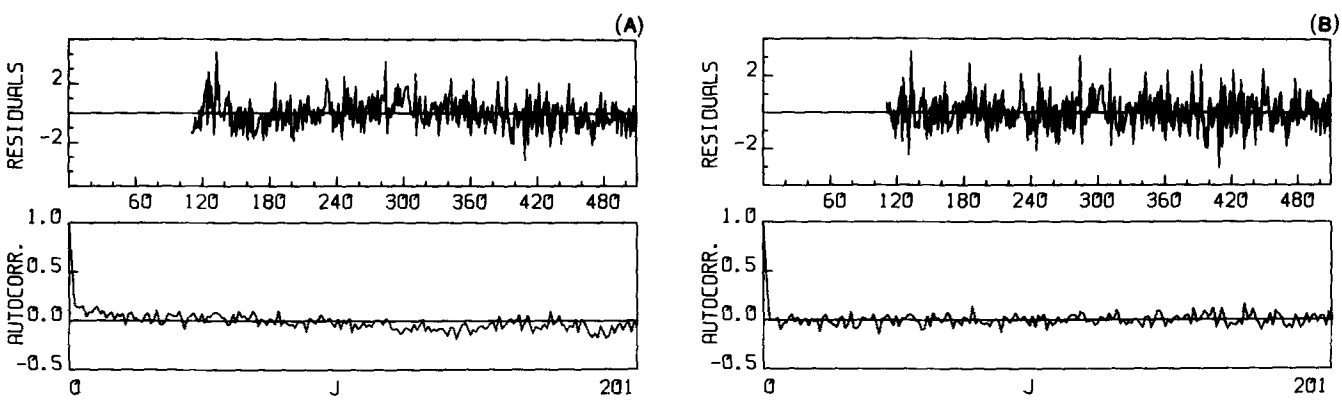

FIG. 8. Weighted residuals vs channel number and their autocorrelation for experiment RT2047, analyzed for two exponentials (A) and for three exponentials (B) by neglecting the raising part of the measured decay. Compare with Fig. 7.

TABLE V. Analysis of experiment RT2047 and of simulation SYL053 using different models. The evaluation started before and after the maximum decay value. The simulation was generated by using the excitation profile of RT2047 and the six estimates from the analysis of RT2047 starting at channel 71. The explanation of the symbols is given in the footnotes of Table II. The data of RT2047 are represented in Fig. 6.

Data set	RT2047				SYL053			
Channel width (ns)	0.196				0.196			
FWHM lamp (ns)	4.7				4.7			
Total counts decay	1 412 870				1 470 258			
Peak value decay	13 695				14 230			
Start ch.	71		111		71		111	
Total No. ch.	439		399		439		399	
	I	II	I	II	I	II	I	II
a_1	0.128	0.116	0.09	0.065	0.133	0.119	0.05	0.058
σ_{a_1}	0.004	0.002	0.01	0.002	0.004	0.002	0.01	0.001
a_2	0.041	0.1144	0.030	0.1089	0.041	0.1193	0.035	0.1123
σ_{a_2}	0.002	0.0004	0.002	0.0005	0.002	0.0004	0.009	0.0005
a_3	0.1050	...	0.099	...	0.1100	...	0.109	...
σ_{a_3}	0.0009	...	0.003	...	0.0009	...	0.002	...
τ_1	0.86	2.03	1.8	3.8	0.85	1.95	1.8	4.2
σ_{τ_1}	0.06	0.04	0.3	0.1	0.06	0.04	0.8	0.1
τ_2	5.6	17.66	8.0	18.03	5.5	17.73	6	18.18
σ_{τ_2}	0.3	0.03	1.0	0.04	0.3	0.03	1	0.04
τ_3	18.26	...	18.6	...	18.30	...	18.3	...
σ_{τ_3}	0.06	...	0.2	...	0.06	...	0.08	...
$S(\hat{\theta})$	413.5	830	368.8	428.6	417.2	863.4	382.9	393.0
$(\chi^2_{crit})^a$	(482.4)	(484.5)	(440.1)	(442.2)	(482.4)	(484.5)	(440.1)	(442.2)
Z^b	−1.36	−6.66	0.22	−1.16	1.70	−7.06	0.69	1.05
autocorrelation[c]	112	5	104	62	112	19	99	101
$N(0,1)^d$	y[d]	y	y	y	y	y	y	y
F test	L.O.F.[f]		31.9		L.O.F.		5.2	
(F crit)[e]			(3.0)				(3.0)	

for the sum of three exponentials, the F tests in the various cases indicate a significant reduction of $S(\hat{\theta})$ when a third component has been included. It should be emphasized that the raising part of the convoluted decay contains information which should not be wasted, not only because of the number of data points but especially because of the different contribution of the respective relaxation times to the various channel contents. This has been verified by utilizing the same number of data points and starting the analysis at different channels. When the raising part of the convoluted decay is not considered, the correlations between the estimates tend to increase and therefore the estimates themselves depend more on the starting values used in the iterative search procedure.

Even in the presence of artifacts, a more reliable model can be obtained by taking the raising part into account and by correcting for these artifacts. Further investigations concerning the instrumental artifacts and corresponding data analysis are in progress.

VIII. DISCUSSION

The question of testing the model adequacy has been discussed critically in several papers.

McKinnon *et al.*[3] pointed out that criteria based on the sum of the weighted squared residuals should not be used by themselves because they may not detect significant correlations among the residuals. As the order of the residuals are not taken into account, a large number of small deviations of the same sign may be not detected. The nonparametric Kolmogorov–Smirnov test should then be more suitable,[25] but the results of the calculations on the presented data sets are along the same line with the χ^2 test. Also, the conclusions drawn from the tests on the randomness of the residuals do not contradict the corresponding χ^2 value. Nevertheless, it should indeed be advised that the sequence of the residuals should be examined when the χ^2 value is below the criterion.

Another point to be stressed is that when the adequacy of a model is being tested, the performance of the whole instrumental setup and its ability to conform to the convolution relation are unavoidably taken into account.[3] In this context, a subjective criterion for model evaluation has been formulated[22]: an acceptable fit for an unknown decay function is the one which yields that characteristic shape of the autocorrelation function and of the residual distribution, observed for suited decay standard under the same experimental conditions, rather than the best fit in the statistical sense.

In our opinion, it is possible to detect and correct for

TABLE VI. Analysis of the experiment RT2049 and of the simulation SYL072 using different models. The evaluation started before and after the maximum decay value. The simulation was generated by using the excitation profile of F RT2049 and the six estimates from the analysis of RT2049 starting at channel 91. The explanation of the symbols is given in the footnotes of Table II.

Data set	RT2049				SYL072			
Channel width (ns)	0.165				0.165			
FWHM lamp (ns)	5.0				5.0			
Total counts decay	1 434 023				1 412 738			
Peak value decay	11 658				11 361			
Start Ch.	91		115		91		115	
Total No. ch.	419		395		419		395	
	I	II	I	II	I	II	I	II
a_1	0.050	0.0585	0.051	0.054	0.050	0.0576	0.047	0.052
σ_{a_1}	0.005	0.0006	0.007	0.001	0.004	0.0006	0.005	0.001
a_2	0.022	0.0963	0.030	0.0960	0.028	0.0949	0.023	0.0936
σ_{a_2}	0.004	0.0004	0.006	0.0005	0.009	0.0004	0.003	0.0005
a_3	0.086	...	0.091	...	0.08	...	0.085	...
σ_{a_3}	0.009	...	0.002	...	0.01	...	0.008	...
τ_1	2.9	3.77	1.9	4.1	2.8	3.7	2.8	4.2
σ_{τ_1}	0.3	0.08	0.6	0.1	0.3	0.08	0.6	0.1
τ_2	9	17.99	7	18.05	10	17.99	9	18.10
σ_{τ_2}	3	0.04	1	0.05	3	0.04	3	0.05
τ_3	18.6	...	18.4	...	19.0	...	18.6	...
σ_{τ_0}	0.5	...	0.2	...	0.7	...	0.4	...
$S(\hat{\theta})$	420.8	452.8	389.3	417.1	429.4	470.2	412.4	432.1
$(\chi^2_{crit})^a$	(461.3)	(467.6)	(435.9)	(438.0)	(461.3)	(467.6)	(435.9)	(438.0)
Z^b	−0.14	−1.33	0.65	−1.42	1.12	−0.90	0.86	0.27
autocorrelation[c]	99	65	90	68	111	85	112	106
$N(0,1)^d$	y^d	y	y	y	y	y	y	y
F test	15.7		13.9			L.O.F.[f]	9.3	
(F crit)[e]	(3.0)		(3.0)				(3.0)	

instrumental artifacts by a rigorous data analysis so that objective tests can be used.

By using the method of moments, a component incrementation test has been proposed for the determination of the number of exponentials.[26] Although we have no direct experience with this method, we do have with the Laplace deconvolution technique,[21] which is also a transformation method. In both methods, the decay constants and the pre-exponential factors are the roots of a polynom. The incrementation test rejects the extra exponential term when a very small amplitude or a negative decay constant is found. From our experience with the Laplace deconvolution, we can conclude that it was not always possible to resolve multicomponent decays. Although these transformation methods have their own merits, the incrementation test may be not reliable.

Due to the nonorthogonality of exponential functions, the correlations between the estimates tend to be relatively large, even when the lifetimes are well separated. By considering an extra exponential term in the decay, the correlations may become very large. Bacon[27] has suggested that, when this is the case, the simpler model may be appropriate. However, this decision has to be taken very carefully when the F test is significant and another experimental design should be considered.

It should be remarked that the data of the excitation profile are only approximative due to the counting noise. The effect of these uncertainties can be interpreted as the introduction of an extra error term, added to the counting noise of the decay data. Although the observed decay data are independent (see Sec. II), correlations between the channel contents may now occur and the weighting in the estimation procedure may be not correct.[28] The consequences of the noise on the lamp data have been investigated on the experiments discussed in this paper and were found to be small. Indeed, the variance of the extra error term is about 1% of the variance of the corresponding decay data point.

IX. CONCLUSIONS

In this work, a modified nonlinearity measure has been suggested for which the data points may have different variances. This is an extension of earlier described measures. According to this new measure, it has been found that when the fluorescence response is given by a sum of exponential decays, the convolution model behaves like a linear one in the neighborhood of the least squares estimates. Hence, the lack of fit test and the "extra sum of squares" test from the theory of linear models can be applied to decide how many com-

ponents have to be included in the response function. It has been shown that other tests on the residuals such as the run test, and the check for their distribution and their autocorrelation, give no real information for model discrimination when two models can be fitted to the data without lack of fit. Only the extra sum of squares principle gives a firm basis to draw appropriate conclusions. The effect of an insufficient number of counts in the data channels and the truncation of the raising part of the fluorescence response have been investigated. Both factors tend to reduce the discriminative power of the χ^2 test. The final decision can only be made with the F test.

ACKNOWLEDGMENTS

The authors are grateful to Dr. R. Todesco for supplying the fluorochrome used in this work. They are indebted to Ir. N. Linssen (T. H. Eindhoven, The Netherlands), Dr. E. M. L. Beale (U.K.), and to Dr. H. Callaert and Dr. N. Veraverbeke of their department for helpful comments and discussions. The authors also like to express their appreciation to Miss L. Verboven and Miss R. Heymans for their accurate type work.

APPENDIX A: THE CALCULATION OF THE CONFIDENCE REGIONS

After the estimation of $\hat{\theta}$, the confidence region corresponding to the linearized model can be calculated by using expression (2). Therein the differentiation between the parameter vector $a \equiv (\theta_1 \cdots \theta_p)$ and the nonlinearity in the parameter vector $\tau \equiv (\theta_{p+1} \cdots \theta_{2p})$ of the model function disappears. Cross sections of the confidence hyperellipsoid, with B_j and B_k as variables, can be obtained by solving for each value of B_k, the quadratic expression in θ_j, keeping $\theta_i (i \neq j,\ i \neq k)$ fixed.

One can also calculate the approximate confidence regions for the nonlinear models defined in Sec. III by using the value

$$S_\alpha = S(\hat{\theta}) \left(1 + \frac{2p}{n-2p}\ F_{2p,n-2p;\alpha}\right) ,$$

where $(2p)$ denotes the dimension of the parameter space. When more than two parameters are involved, cross sections of the confidence hyperellipsoid can be obtained in two ways. The first method is to select a grid of points in the parameter space and to evaluate the sum of squares function at every point of the grid.[18] The second possibility for getting sectional drawings uses an analytical expression obtained by separating the linear parameter from the model.[4] The sum of squares function can then be written as

$$S(a,\ \tau) = \sum_{i=1}^{n} \frac{1}{y_i} \left(y_i - \sum_{j=1}^{p} a_j g'_{ji}\right)^2 , \qquad \text{(A1)}$$

where $g'_{ji} = \partial g(t_i)/\partial a_j$. By equating Eq. (A1) to the value S_α, an approximate confidence region becomes defined as

$$\sum_{i=1}^{n} \frac{1}{y_i} \left(y_i - \sum_{j=1}^{p} a_j g'_{ji}\right)^2 = S_\alpha . \qquad \text{(A2)}$$

A confidence contour which relates a_j and τ_k can be obtained by solving the quadratic equation (A2) in a_j for each value of τ_k, by fixed values of the other parameters.

Cross sections showing the relation between the nonlinear parameters τ_i and τ_j can be obtained in a similar way. For each value of τ_i, the two corresponding values of τ_j are calculated, while keeping the other parameters fixed. This can be done with the Newton–Raphson algorithm.

APPENDIX B: THE RUN TEST

In the run test, the randomness of the signs of the residuals versus channel number is investigated.[4] The number of subsequent groups of residuals with the same sign (= runs) will be denoted by u.

Define n_1 and n_2 as the number of residuals of each sign. Let

$$\mu = \frac{2n_1 n_2}{n_1 + n_2} + 1 ,$$

$$\sigma^2 = \frac{2n_1 n_2 (2n_1 n_2 - n_1 - n_2)}{(n_1 + n_2)^2 (n_1 + n_2 - 1)} .$$

If $n_1 > 10$ and $n_2 > 10$, then

$$Z = \frac{(u - \mu + 1/2)}{\sigma}$$

is a unit normal deviate.

The sequence of the signs of the residuals will be considered to be random at the 95% confidence level if $|Z| < 1.96$.

[1] W. R. Ware, in *Creation and Detection of the Excited State*, edited by A. Lamolla (Dekker, New York, 1971).
[2] A. Grinvald and I. Z. Steinberg, Anal. Biochem. **59**, 583 (1974).
[3] A. G. McKinnon, A. G. Szabo, and D. R. Miller, J. Phys. Chem. **81**, 1564 (1977).
[4] N. R. Draper and H. Smith, *Applied Regression Analysis* (Wiley, New York, 1966).
[5] J. V. Beck and K. J. Arnold, *Parameter Estimation in Engineering and Science* (Wiley, New York, 1977).
[6] W. C. Hamilton, *Statistics in Physical Science* (Ronald, New York, 1964).
[7] E. M. L. Beale, J. R. Stat. Soc. Ser. B **22**, 41 (1960).
[8] D. M. Bates and D. G. Watts, J. R. Stat. Soc. Ser. B **42**, 1 (1980).
[9] H. N. Linssen, Stat. Neerlandica **29**, 93 (1975).
[10] C. M. Harris and B. K. Selinger, Aust. J. Chem. **32**, 2111 (1979).
[11] A. G. Knight and B. K. Selinger, Spectrochim. Acta Part A **27**, 1223 (1979).
[12] D. V. O'Connor, W. A. Ware, and J. C. Andre, J. Phys. Chem. **83**, 1333 (1979).
[13] A. Ruark and L. Devol, Phys. Rev. **49**, 355 (1936).
[14] H. U. Gerber, Am. Stat. **33**, 85 (1979).
[15] T. Awaya, Nucl. Instrum. Methods **165**, 317 (1979).
[16] T. Awaya, Nucl. Instrum. Methods **174**, 237 (1980).
[17] R. I. Jennich and M. L. Ralston, Annu. Rev. Biophys. Bioeng. **8**, 195 (1979).
[18] B. K. Selinger, NATO ASI *Time Resolved Fluorescence Spectroscopy in Biochemistry and Biology* (Plenum, New York, 1980).

[19]I. Guttman and D. A. Meeter, Technometrics 7, 623 (1965).
[20]D. W. Marquardt, J. Soc. Ind. Appl. Math. 11, 431 (1963).
[21]A. Gafni, R. L. Modlin, and L. Brand, Biophys. J. 263, 15 (1975).
[22]A. Grinwald, Anal. Biochem. 75, 260 (1976).
[23]R. Todesco, Ph. thesis, Leuven, Belgium, 1981.
[24]J. Moshman, in *Mathematical Methods for Digital Computers*, edited by A. Ralston and H. S. Wilf (Wiley, New York, 1967).
[25]J. H. Pollard, *Numerical and Statistical Techniques* (Cambridge University, Cambridge, 1977).
[26]I. Isenberg, R. D. Dyson, and R. Henson, Biophys. J. 13, 1090 (1973).
[27]See Ref. 5, p. 379.
[28]J. A. Irvin, T. I. Quickenden, and D. F. Sangster, Rev. Sci. Instrum. 52, 191 (1981).