

Fit Software Module for the Confocor 2

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1. Model

In general case, mathematical model for the correlation function, used in the fit module, is given by:

$$G(t) = \frac{I + \frac{T}{I-T} e^{-t/t_T}}{N} \left(\sum_{i=1}^M \frac{F_i}{(I+t/t_i) \sqrt{I+t/(S^2 t_i)}} \right) + I, \quad (1)$$

where M is the number of fluorescent components, N is the average number of fluorescent molecules in the detection volume, T and t_T are, respectively, the fractional population and decay time of the triplet state, F_i and t_i are, respectively, the contribution and translational diffusion time of the i -th fluorescent component and S is the “structural” parameter of the instrumental setup: $S = \frac{\mathbf{v}_2}{\mathbf{v}_1}$, where \mathbf{v}_2 and \mathbf{v}_1 are the distances from the center of the laser beam focus in the axial and radial directions, respectively, at which the collected fluorescence intensity has dropped by a factor of e^2 compared to its peak value for Gaussian beam profile. The contributions F_i are subject to the normalization condition:

$$\sum_{i=1}^M F_i = 1. \quad (2)$$

For many applications simpler models which are special cases of the model (1) can be used. These are the models without triplet fraction ($T=0$) and different reductions to a particular numbers of components (normally no more than 3: $M \leq 3$). In the case of cross correlation measurements the “triplet state” model can be used as well.

The model (1), given Eq. (2), is totally identifiable, i.e. all parameters of the models can be uniquely determined on the basis of only one measured correlation function.

2. Fit

Fit is based on the approximation of the experimental correlation curve by the theoretical function (1) with the condition (2). Theoretical model is characterized by a final set of parameters $(N, S, T, \mathbf{t}_T, F_1, \dots, F_M, \mathbf{t}_1, \dots, \mathbf{t}_M)$, which have to be estimated, provided that the number of components M is known. The estimators provide the optimal value of a priori chosen criterion. The criterion, that should be optimized, is a functional with respect to the experimental and theoretical curves. As theoretical function is completely determined by a set of unknown parameters, the criterion optimal value corresponds to the required combination of the parameters values.

We use \mathbf{c}^2 criterion, which is a sum of squared weighted differences of an experimental $G^E(t)$ and theoretical $G^T(t, \mathbf{a})$ correlation curves in a number of the detected time points t_i , $i=1, \dots, K$:

$$\mathbf{c}^2(\mathbf{a}) = \sum_{i=1}^K w(t_i) \{G^E(t_i) - G^T(t_i, \mathbf{a})\}^2, \quad (3)$$

where

$$\mathbf{a} = \{N, S, T, \mathbf{t}_T, F_1, \dots, F_M, \mathbf{t}_1, \dots, \mathbf{t}_M\} \quad (4)$$

is the vector of unknown parameters, K is the number of time points, $w(t_i)$ is the weighting factor. In general case we will assume that vector \mathbf{a} is consisted of a number of elements $(\mathbf{a} = \{a_1, \dots, a_m\})$, which are correspondent to the elements of the vector \mathbf{a} from Eq. (4).

There are many various searching algorithms, developed for the minimization of the criterion function \mathbf{c}^2 [1,2]. These algorithms are usually based on the iterative process of search, where, starting from a priori chosen initial guesses, a new set of parameters is generated after the comparison of the criterion on the current and previous iterations. Search stops when either value of criterion or values of parameters do not change greater than a priori chosen threshold, or number of iteration overflows some critical value. The implementations of the algorithms differ in a way of the generation of a new set of parameters.

3. Marquardt algorithm

One of the most wide-spread and rigorous iterative algorithms is Marquardt non-linear least-squares algorithm [3,4]. The idea consists of the linearization of the model (1) in a truncated Taylor series in order to make use of linear least-squares analysis, and attain the desired minimum value of \mathbf{c}^2 criterion by an iterative sequence of calculations.

The nonlinear theoretical model function $G^T(t_i, \mathbf{a})$ is linearized by the expansion in a truncated Taylor series near the vector of initial guesses \mathbf{a}^0 :

$$G^T(t_i, \mathbf{a}) = G^T(t_i, \mathbf{a}^0) + \sum_{j=1}^m \left(\frac{\mathcal{J}G^T(t_i, \mathbf{a}^0)}{\mathcal{J}a_j} \right) \mathbf{d}a_j, \quad (5)$$

where unknown coefficients $\mathbf{d}a_1, \dots, \mathbf{d}a_m$ are corrections to the parameters a_1^0, \dots, a_m^0 , and are assumed to be small enough to expand $G^T(t_i, \mathbf{a})$ in a Taylor series and to truncate it after the first-order terms. Eq. (5) is the equation of linear regression with respect to the coefficients $\mathbf{d}a_1, \dots, \mathbf{d}a_m$. These coefficients can be found by the linear least squares method, applied directly to Eq. (5), as the solution of a set of linear algebraic equations

$$\left(\frac{\mathcal{J}\mathbf{c}^2}{\mathcal{J}\mathbf{d}a_j} \right) = 0, \quad j = 1, \dots, m. \quad (6)$$

After substituting Eq. (3) into Eq. (6), one obtains:

$$\sum_{i=1}^n w(t_i) \left[G^E(t_i) - G^T(t_i, \mathbf{a}^0) - \sum_{j=1}^m \left(\frac{\mathcal{J}G^T(t_i, \mathbf{a}^0)}{\mathcal{J}a_j} \right) \mathbf{d}a_j \right] \left(\frac{\mathcal{J}G^T(t_i, \mathbf{a}^0)}{\mathcal{J}a_j} \right) = 0, \quad j = 1, \dots, m. \quad (7)$$

Introducing weighted residuals $E(t_i) = w(t_i)(G^E(t_i) - G^T(t_i, \mathbf{a}^0))$, set (7) can be rewritten as:

$$\sum_{k=1}^m \sum_{i=1}^n w(t_i) \left(\frac{\mathcal{J}G^T(t_i, \mathbf{a}^0)}{\mathcal{J}a_k} \right) \left(\frac{\mathcal{J}G^T(t_i, \mathbf{a}^0)}{\mathcal{J}a_j} \right) \mathbf{d}a_k = \sum_{i=1}^n w(t_i) E(t_i) \left(\frac{\mathcal{J}G^T(t_i, \mathbf{a}^0)}{\mathcal{J}a_j} \right), \quad j = 1, \dots, m. \quad (8)$$

Once the vector of coefficients $\mathbf{da}_1, \dots, \mathbf{da}_m$ is obtained from the set (8), a new realization of the vector \mathbf{a} can be calculated:

$$a_j = a_j^0 + \mathbf{da}_j, j = 1, \dots, m. \quad (9)$$

The improved estimate of a_j replaces a_j^0 in Eq. (5) and iteration starts again. Marquardt [3,4,5] had developed a method that exhibited a gradient like search direction when far from the minimum and then moved smoothly into the analytical method near the minimum. The method improves the conditioning of the matrix of partial derivatives

$$\mathbf{B} = \sum_{i=1}^n w(t_i) \begin{pmatrix} \mathbb{J}G^T(t_i, \mathbf{a}^0) \\ \mathbb{J}a_k \end{pmatrix} \begin{pmatrix} \mathbb{J}G^T(t_i, \mathbf{a}^0) \\ \mathbb{J}a_j \end{pmatrix}, j, k = 1, \dots, m. \quad (10)$$

The off diagonal elements of Eq. (10) are left unchanged but the diagonal elements are redefined as follows:

$$b_{ii} = (1 + \mathbf{I})b_{ii}, i = 1, \dots, m. \quad (11)$$

If $\mathbf{I} = 0$, the analytical solution is provided by Eq. (10). If \mathbf{I} is large, the off diagonal elements $b_{ij} (i \neq j)$ become insignificant compared to the diagonal elements. The search direction is then along the path of steepest descent or the gradient method [4].

The Marquardt method adjusts \mathbf{I} to ensure that after each iteration \mathbf{c}^2 decreases; \mathbf{I} is reduced at each iteration as long as \mathbf{c}^2 decreases. If the solution causes \mathbf{c}^2 to increase, however, \mathbf{I} is increased. In this manner, failure of the analytical-like solution causes \mathbf{I} to increase, which makes the solution more steepest-descent-like until \mathbf{c}^2 is reduced. As the minimum is approached, however, the analytical solution usually becomes more accurate and \mathbf{I} approaches zero.

The covariance matrix \mathbf{C} of the fit parameters is given by

$$\mathbf{C} = \mathbf{B}^{-1}, \quad (12)$$

when $\mathbf{I} = 0$.

4. Initial Guesses

Iterative methods, granting high quality of fitting, are relatively slow and need the initial guesses for the parameters to be known. When the initial guesses are far from the true values, the convergence may be very slow and time consuming. The common way of the experimental data analysis may be composed of non-iterative routine, generating initial guesses, and iterative procedure, starting search with those initials.

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