Fitting software for fluorescence correlation spectroscopy

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Introduction

The renaissance of fluorescence correlation spectroscopy is accompanied by the design of appropriate models, which are used to fit the measured correlation functions. A consistent approach to the data analysis requires the development of user-friendly software suitable for managing and modeling of experimental data.

Software tools have been developed to analyze correlation functions obtained by fluorescence correlation spectroscopy. The analysis is performed on the basis of the global approach, where several autocorrelation traces measured under different conditions are combined and simultaneously fitted. Standard manipulations with the parameters such as parameter fixing and putting constraints are implemented in the program. The quality of the fit can be judged by the χ^2 criterion and by visual inspection of the residuals. The confidence intervals for each parameter can be obtained by the exhaustive search method. The built-in experimental database allows for easy storage and management of experimental data. The software has been developed to run under Windows 95/NT 4.0 on the basis of a modular object-oriented architecture and includes the possibility of two- and three-dimensional graphical representation of the measured and recovered correlation functions. At this moment the model library contains two types of models: the "Triplet-State" model and the "Conformational" model (for details see [1]). The "Triplet-State" model is:

$$G(t) = 1 + \frac{(1 - F_{trip} + F_{trip} e^{-t/T_{trip}})}{N} \left(\sum_i \frac{F_i}{\left(1 + \frac{t}{T_i}\right)\sqrt{1 + \frac{t}{a^2 T_i}}} \right),$$

in which N is the average number of fluorescent molecules in the detection volume, F_{trip} and T_{trip} 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 a is the "structural" parameter of the instrumental setup.

The modular object-oriented architecture allows for easy extension of the model library.

Results and Discussion

To illustrate the performance of the developed global program, three data sets have been analyzed simultaneously in terms of the "Triplet-State" model: Rhodamine Green (RG, 0.7 kDa), Rhodamine Green Dextran (RGD, 10 kDa) and a mixture of both compounds (MRGD), all dissolved in water. The experimental data were obtained with a Zeiss-Evotec ConfoCor® instrument [2]. The triplet-state parameters were linked over all three data sets. The diffusion times were fitted according to the following scheme. The RG- and RGD-data sets were approximated by a one-component diffusion model and the MRGD-data set by a double-component diffusion model. The diffusion time of the RG-data set was linked to the shorter diffusion time of the MRGD-data set (T_1), and the diffusion time of the RGD-data set was linked to the longer diffusion time of the MRGD-data set (T_2). The confidence intervals have been determined at the 67% confidence level. A typical result of a fit is shown in fig. 1, which presents a single autocorrelation trace of a mixture (MRGD). The following values of the diffusion times were obtained: T_1 = 0.132 [0.131;0.133] ms and T_2 = 0.325 [0.324; 0.327] ms.

References

1. Widengren, J. and Rigler, R., Cell. Mol. Biol. 44 (1998) 857.
2. Hink, M. and Visser, A.J.W.G., In Rettig, W., Strehmel, B., Schrader, S. and Seifert, H. (Eds.) Applied Fluorescence in Chemistry, Biology and Medicine, Springer-Verlag, Berlin, 1998, p. 101.

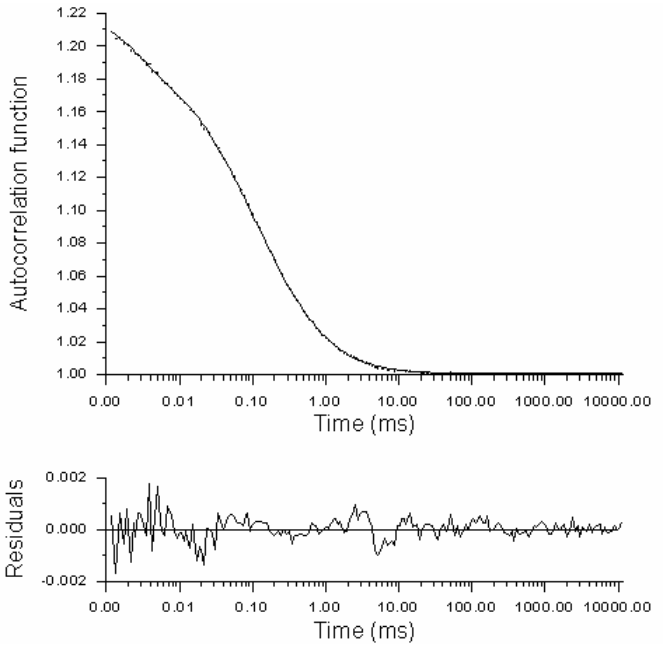

Fig. 1. *Example of experimental and fitted autocorrelation traces (top) and residuals (bottom)*