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Maximum Entropy Analysis of Analytically Simulated Complex Fluorescence Decays

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Abstract We tested a Maximum Entropy Method developed for oversampled data (SVD-MEM) on complex analytically simulated exponential decay data consisting of both noisy and noiseless multi-exponential fluorescence decay curves. We observed recovery of simulated parameters for three sets of data: a decay containing three exponential functions in both intensity and anisotropy curves, a set of intensity decays composed of 4, 5 and 6 exponential functions, and a decay characterized by a Gaussian lifetime distribution. The SVD-MEM fitting of the noiseless data returned the simulated parameters with the high accuracy. Noise added to the data affected recovery of the parameters in dependence on a data complexity. At selected realistic noise levels we obtained a good recovery of simulated parameters for all tested data sets. Decay parameters recovered from decays containing discrete lifetime components were almost independent of the value of the entropy scaling parameter γ used in the maximization procedure when it changed across the main peak of its posterior probability. A correct recovery of the Gaussian shaped lifetime distribution required selection of the γ-factor which was by several orders of magnitude larger than its most probable value to avoid a band splitting.

Keywords SVD-MEM · Oversampled data · Fluorescence lifetime distributions · Fluorescence anisotropy distributions · Synthetic data · Influence of noise

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Introduction

An analysis of multi-exponential fluorescence decays belongs to difficult tasks in data analysis. For many years such analyses were done almost exclusively by so called parametric fitting where an explicit parametric model decay was taken to fit the experimental data by nonlinear least squares methods [1]. Besides a difficulty to find the global overall minimum of the χ^2 hyper-surface by the parametric minimization procedure, χ^2 statistical criteria very rarely allowed to fit the analyzed decay curve by more than three exponential functions.

About 30 years ago a new strategy of the data analysis called Maximum Entropy Method appeared and found its applications in time-resolved fluorescence spectroscopy. Tests of so called classical Maximum Entropy Method (MEM) [2, 3] on the synthetic fluorescence decay data were done in [4, 5]. Compared to the parametric approach these tests proved that the new method is able to decode more complicated decays, e.g. those containing lifetime distributions, without assumption of any preferred decay model. In this article we explored another solution of the Maximum Entropy problem developed for oversampled data [6] and we tested its resolving power on analytically simulated multi-exponential fluorescence decay and anisotropy data.

If amount of independent information in measured data is much lower than the number of data points N, the maximization of the quantity Q=γS-L, where S is an entropy of the parameter map, L=$1/2\chi^2$ and γ is a positive entropy scaling factor, can be solved in the singular space of the transform between the searched parameter map and the data. This procedure leads to a considerable reduction of variables and gives a fast and accurate maximization algorithm. Compared to the previous classical implementations, this strategy gives a set of maps depending on the entropy scaling factor γ instead of an unique γ-value calculated on the criterion of χ^2=N that generally tends to underfit the data [6].

To test capability of the letter Maximum Entropy algorithm, which we abbreviate SVD-MEM, three sets of model multi-exponential decays were generated: the decay containing three exponential functions in both intensity and anisotropy decay curves, a set of decays containing 4, 5 and 6 exponential functions, and a decay characterized by a Gaussian lifetime distribution.

Materials and methods

Time-domain exponential fluorescence decays f(t) were analytically simulated according to equations:

$$f(t) = g(t)*i(t) = \int_0^t g(x)i(t-x)\,dx \tag{1}$$

$$g(t) = ct^2[\exp(-k_2t) - \exp(-k_1t)] \tag{2}$$

$$i(t) = \sum_{i=1}^{n} \alpha_i \exp(-t/\tau_i) \tag{3}$$

where i(t) is a δ-pulse response of the chromophore, g(t) an apparatus response function, and f(t) the resulting intensity decay. Functions g(t) and f(t) were then integrated across the channel time-width to obtain sets of measurable quantities G_i and F_i for i=1,2,….,N [7]. The response function was simulated with parameters of k_1=15.0 ns^{-1} and k_2=14.5 ns^{-1} that resulted in the response function half-width of about 290 ps.

Simulated fluorescence anisotropy decay components were calculated similarly as described above using equations:

$$i_{||}(t) = \frac{1}{3} i(t)[1 + 2\,r(t)] \tag{4}$$

$$i_{\perp}(t) = \frac{1}{3} i(t)[1 - r(t)] \tag{5}$$

$$r(t) = \sum_{i=1}^{n} \beta_i \exp(-t/\Phi_i) \tag{6}$$

where $i_{||}(t)$ and $i_{\perp}(t)$ is the parallel and perpendicular decay component, respectively, and r(t) is an anisotropy decay.

In all tests we analyzed both the noisy and noiseless decay curves. Calculated channel intensities of the noiseless curves were rounded to integers, noisy curves were obtained by subsequent implementation of the Poisson noise.

All simulated data were analyzed by the SVD-MEM using PC routines coded according to Bryan [6]. The SVD-MEM routines as well as routines developed for the Marquardt parametric analysis use advanced higher polynomial approximations of the apparatus response function in calculations of the convolution integral [8]. All program codes used for both the data generation and data analyses were developed by (Vecer J., *FluoDecay* software pack).

Results and discussion

Triple exponential intensity and anisotropy decay

To show capability of SVD-MEM to analyze fluorescence and anisotropy decays often found for proteins in aqueous solutions we generated data containing both triple exponential intensity and anisotropy decays. Such decay data lay on the border of complexity that can be handled by parametric methods.

This model data set comprises of the parallel and perpendicular decay curves $F_{||}$ and $F_{\perp}$, the apparatus response function G, and the intensity decay curve F_m calculated as $F_m = F_{||} + 2\,F_{\perp}$ for both noiseless and noisy data. Parameters α_i and τ_i selected for the simulation of the triple exponential intensity decay are displayed in Table 1, parameters β_i and Φ_i used for the simulation of the triple anisotropy decay are shown in Table 2.

The SVD-MEM analysis of the decay curve F_m resulted in a lifetime distribution showing dependence of pre-exponential factors α_i on lifetimes τ_i equidistantly distributed on the logarithmic time scale. Figure 1 shows such distribution found by the SVD-MEM analysis of the noisy data. The mean positions of peaks $\langle\tau\rangle$ found in the lifetime distribution were calculated as $\langle\tau\rangle = \sum f_i\tau_i / \sum f_i$ using fractional intensities $f_i = \alpha_i\tau_i$, for the index i running over the selected peak. The mean peak pre-exponential factors $\langle\alpha\rangle$ were found as corresponding peak areas $\langle\alpha\rangle = \sum \alpha_i$. The calculated mean peak values of $\langle\alpha\rangle$ and $\langle\tau\rangle$ for both noisy and noiseless data are compared with those used in simulations in Table 1. We characterize the accuracy of the SVD-MEM fit by the reduced value of the chi-square $\chi^2_N = \chi^{2/N}$ where N is a number of analyzed data points, because the SVD-MEM analysis starts from a flat distribution of lifetimes whose number does not change during the whole fitting process. This is a difference compared to the parametric analysis where the number of model parameters changes during the fitting process and number of degrees of freedom ν instead of N is used. The χ^2_N value of the noiseless data fit refers mainly to the convolution error. Assuming that the simulated noiseless curve is a "correct

Table 1 Comparison of the fluorescence decay parameters recovered by the SVD-MEM analysis with those taken in the triple exponential intensity decay simulation: α, τ—simulated decay parameters with the "correct" value of χ_N^2; ⟨α⟩, ⟨τ⟩—mean values of the recovered decay parameters with the χ_N^2 value of the fit

Simulated Decay	χ_N^2	α_1	τ_1 (ns)	α_2	τ_2 (ns)	α_3	τ_3 (ns)
3 exp	1.0108	3.000	0.500	3.000	2.000	3.000	10.000
Analysis	χ_N^2	$\langle\alpha\rangle_1$	$\langle\tau\rangle_1$ (ns)	$\langle\alpha\rangle_2$	$\langle\tau\rangle_2$ (ns)	$\langle\alpha\rangle_3$	$\langle\tau\rangle_3$ (ns)
Noiseless	0.00037	2.997	0.500	3.002	1.997	3.003	9.999
Noisy	1.0005	2.968	0.500	3.031	2.002	3.001	10.008

Table 2 Comparison of the anisotropy decay parameters recovered by SVD-MEM analysis with those taken in the triple exponential anisotropy decay simulation: β, Φ—simulated anisotropy parameters with the "correct" value of χ_N^2; ⟨β⟩, ⟨Φ⟩—mean values of the recovered anisotropy parameters with the χ_N^2 value of the fit

Simulated anisotropy	χ_N^2	β_1	ϕ_1 (ns)	β_2	ϕ_2 (ns)	β_3	ϕ_3 (ns)
3 exp	1.0252	0.100	1.000	0.100	4.000	0.200	15.000
Analysis	χ_N^2	$\langle\beta\rangle_1$	$\langle\phi\rangle_1$ (ns)	$\langle\beta\rangle_2$	$\langle\phi\rangle_2$ (ns)	$\langle\beta\rangle_3$	$\langle\phi\rangle_3$ (ns)
Noiseless	0.00014	0.100	1.000	0.100	4.004	0.200	15.008
Noisy	1.0139	0.099	0.953	0.111	4.138	0.193	15.371

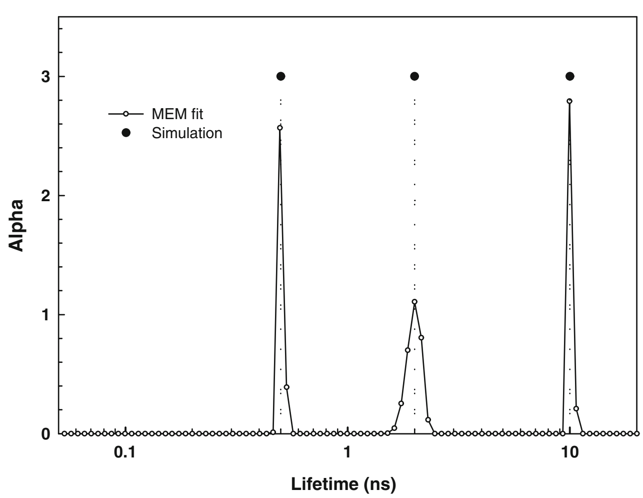


Fig. 1 Lifetime distribution recovered by the SVD-MEM analysis of the noisy triple exponential intensity decay for 100 lifetimes logarithmically distributed on the time scale from 0.02 to 20 ns. Simulated parameters are marked by *full circles*

fit" of the simulated noisy curve, we calculated also the "correct χ_N^2" of the simulated data in the row of Table 1 where simulated parameters are shown. In Fig. 2 we present also residual and autocorrelation functions of the decay curve fit [9] as an important accuracy test complementary to the χ_N^2 value. Both functions are randomly distributed around the zero level which confirms correct fitting of the decay curve. The mean peak values shown in Table 1 were obtained by the SVD-MEM analysis of data for the most probable value of the entropy scaling parameter γ [6]. Nevertheless, we verified that they practically do not change when γ changes across the main peak of its posterior probability (data not shown).

The simulated parallel $F_{||}$ and perpendicular $F_{\perp}$ polarized decay curves were analyzed globally using results of the previous intensity decay analysis. The analysis resulted in a distribution of rotational correlation times Φ_i showing dependence of β_i-coefficients on rotational correlation times Φ_i equidistantly distributed on the logarithmic time scale.

Fig. 2 Fit accuracy of the noisy triple exponential decay curve visualized by the residual and autocorrelation functions. Decay curve parameters: 46.1×10^{6} counts per decay, 1,024 of data points, calibration 0.05 ns/channel

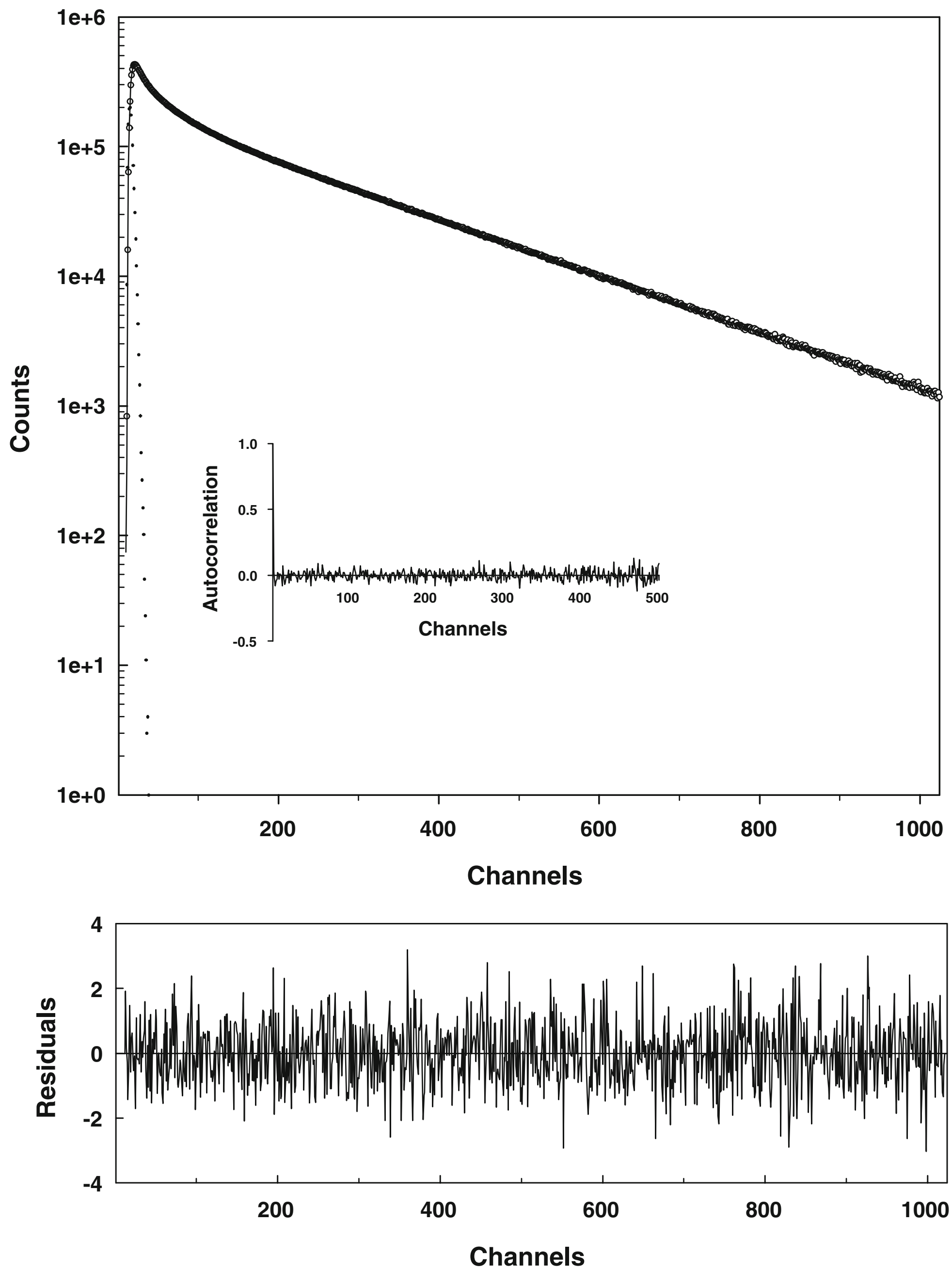


Figure 3 shows the distribution obtained by the SVD-MEM analysis of the noisy data. The mean positions of the Φ-peaks were calculated from the distributions of the rotational correlation times as $\langle\Phi\rangle=\sum\beta_i\phi_i/\sum\beta_i$ for the index i running over the selected peak and the corresponding mean values of coefficients $\langle\beta\rangle$ as the peak areas, $\langle\beta\rangle=\sum\beta_i$. The resulting mean peak values of $\langle\beta\rangle$ and $\langle\Phi\rangle$ are compared with those used in simulations in Table 2. Similarly to the intensity decay fit, the residual and autocorrelation functions of both parallel and perpendicular noisy decay curves do not exhibit any systematic deviations, Fig. 4. Accuracy of the anisotropy decay ft is displayed in Fig. 5.

As seen from Tables 1 and 2 the SVD-MEM analysis of the noiseless data accurately returns all simulated decay parameters. In the limit of the zero noise all parameters are found with deviations smaller than about 0.15%. The fit of the noisy data resulted in peak parameters that deviated not more than 1% for both α-values and lifetimes, 11% for β-values and 3.5% for rotational correlation times. Better recovery of the intensity decay parameters compared to those of the anisotropy decay is not surprising since the decay curve F_m is composed of only 3 exponential functions. Due to a higher intensity it also contains lower noise compared to $F_{||}$ and $F_{\perp}$ components ($F_m=F_{||}+2F_{\perp}$). Moreover, $F_{||}$ and $F_{\perp}$ are much more

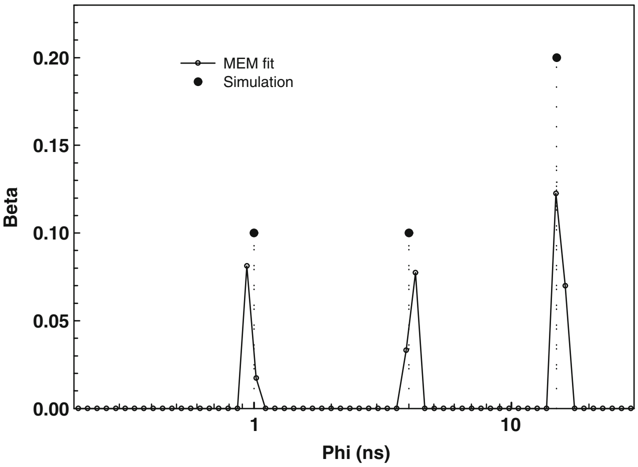


Fig. 3 Distribution of the rotational correlation times found by the SVD-MEM analysis of the noisy components $F_{||}$ and $F_{\perp}$ containing three exponential functions in both intensity and anisotropy decays for 100 ϕ-values distributed logarithmically on the time scale from 0.05 to 200 ns. Simulated parameters are marked by *full circles*

complex as they are both composed of 12 different exponential functions, see Eqs. 4–6. Substantial influence of noise on results of the data analysis is evident also from lower χ^2_N values of the noisy data fits compared to their "correct χ^2_N" values. This shows that after the noise addition the simulated noiseless curves are no more the best fits of noisy data. The good accuracy of all noisy data fits is confirmed by the symmetric distribution of both the residual and autocorrelation functions around the zero level, Figs. 4 and 5.

Multi-exponential fluorescence decays

In order to evaluate capability of the SVD-MEM to resolve highly complex fluorescence decays we simulated a set of three decay curves F_m composed of 4, 5 and 6 exponential functions. Both noisy and noiseless data were analyzed. Lifetime distributions obtained by analysis of realistic noisy data are displayed in Fig. 6. Mean peak values of the decay parameters $\langle\alpha\rangle$ and $\langle\tau\rangle$ calculated from the distributions as described above are compared with those used in simulations in Table 3.

It can be seen from Table 3 that the SVD-MEM analysis of the noiseless data accurately returned decay parameters used in simulations. The deviations of the parameters are smaller than 0.5%, 0.8% and 2.0% for 4, 5 and 6 exponentials, respectively. Figure 6 shows that up to 6 exponential decay components can be resolved in the noisy data by the SVD-MEM without any prior knowledge about the analyzed decays. With increasing number of exponentials the decay parameters are recovered with less accuracy. At selected noise levels the largest deviations from the simulated values reach 3.0%, 4.5% and 17.3% for 4, 5 and 6 exponentials, respectively. Similarly to the previous example, the mean values of the recovered decay parameters are almost independent of the entropy scaling factor γ changed across the main band of its posterior probability (data not shown). Mean lifetimes τ_{mean} as integral characteristics of the decays are recovered with high accuracy. This accuracy is better than 0.3% for the most complex noisy decay composed of six exponentials.

Gaussian lifetime distribution

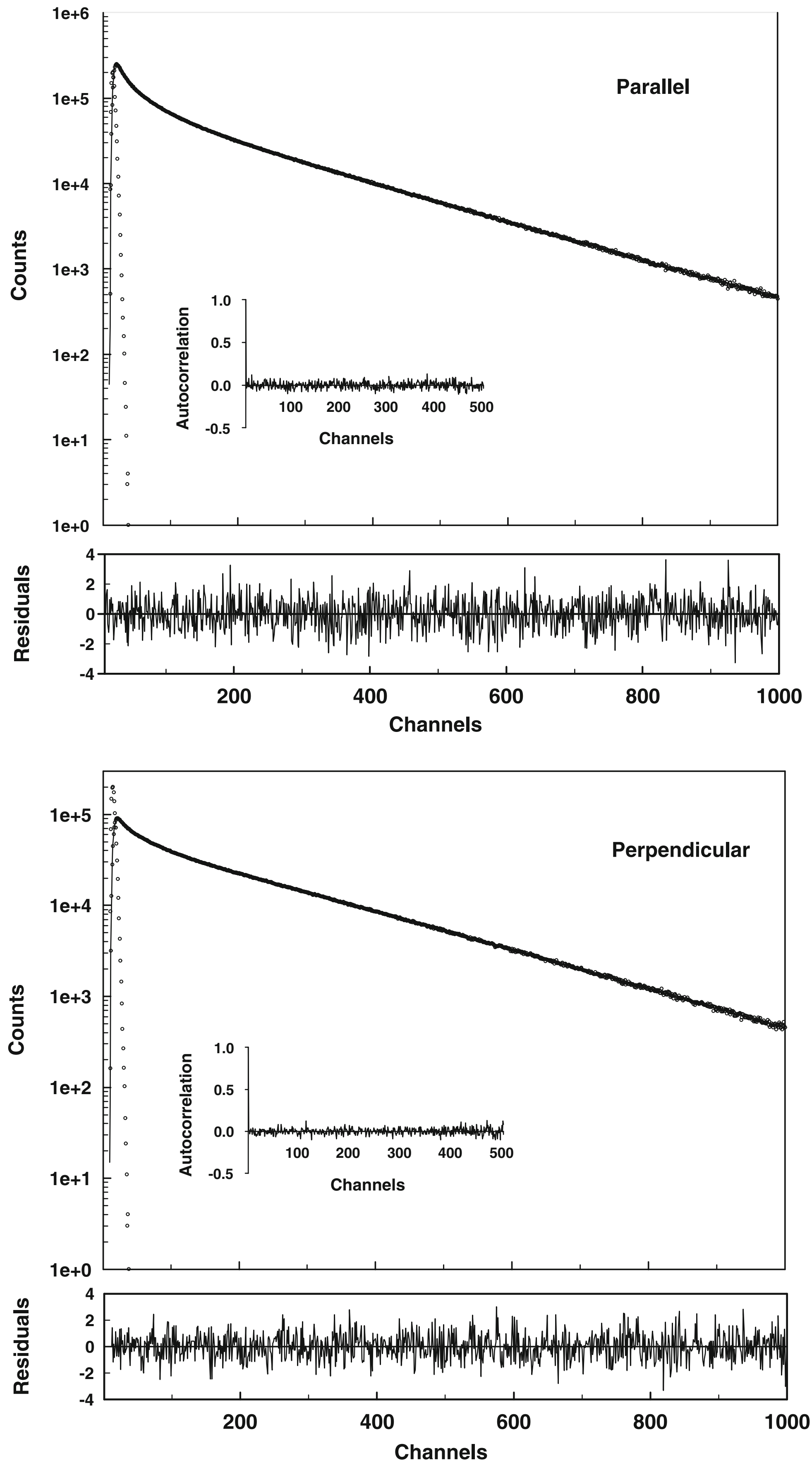
Recognition and precise quantification of lifetime distributions hidden in fluorescence decays is supposed to be a difficult task. Such decays can be often parametrically fitted by several exponential functions with sufficient statistical precision. On the other hand, lifetime distributions can be found by the MEM analysis of decays containing discrete lifetime components. This can mainly happen if the decays are poorly determined, e.g. the data are too noisy and/or only a part of the decay is analyzed.

We tested capability of the SVD-MEM in the analysis of simulated decays composed of exponential components with a Gaussian distribution of the lifetime amplitudes. The distribution was centered at τ=10 ns with a half-width of Δτ=5 ns. We transformed the distribution shape to the logarithmic time scale with the same scale division as it was used later in the SVD-MEM analysis. In particular, the distribution was represented by 30 exponential functions. Finally, the distribution was normalized to 1 at its maximum. Synthetic intensity decay curves F_m were generated in 1,024 data points with calibration constant of 0.05 ns/channel, and the total number of 1.9×10^7 counts per decay. The "correct" χ^2_N of the noisy data was found to be 1.0138.

The lifetime distributions recovered from both the noiseless and noisy data are shown in Figs. 7 and 8. For comparison we analyzed the same curves by the Marquardt parametric method. Sufficient statistical accuracy of the fit was obtained with a discrete bi-exponential model. Residuals of the SVD-MEM fits are compared with those of the parametric analysis in insets of Figs. 7 and 8.

In Fig. 7 the lifetime distributions found by the SVD-MEM in the noiseless data are compared with the original Gaussian distribution that gave rise to the data. The distributions recovered by the SVD-MEM fit perfectly the simulated shape for the values of the entropy scaling factors γ that changes over 4 orders of the magnitude. It can be seen that the same decay was also satisfactorily fitted by 2 exponentials using the Marquardt parametric method. Despite only a small difference of about 0.02 in χ^2_N, the residual function of the parametric fit exhibits severe systematic deviations indicating usage of an inadequate model.

Fig. 4 Fit accuracy of the noisy decay components $F_{||}$ (21.1×10^6 counts per decay) and $F_{\perp}$ (12.5×10^6 counts per decay) visualized by residual and the autocorrelation functions: 1,024 of data points, calibration 0.05 ns/channel



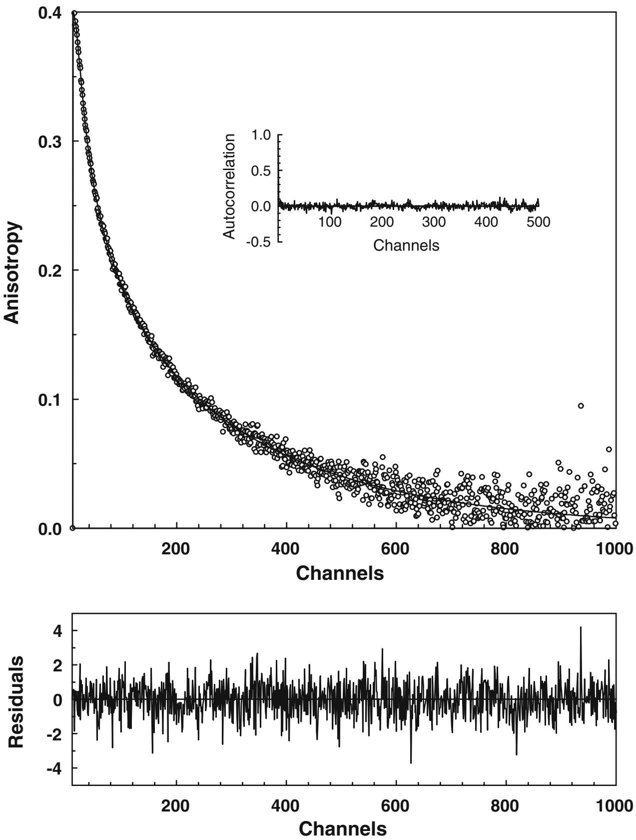


Fig. 5 Fit of the noisy triple exponential anisotropy decay curve calculated from the corresponding fits of the noisy components $F_{||}$ and $F_{\perp}$. The fit accuracy is visualized by the residual and autocorrelation functions

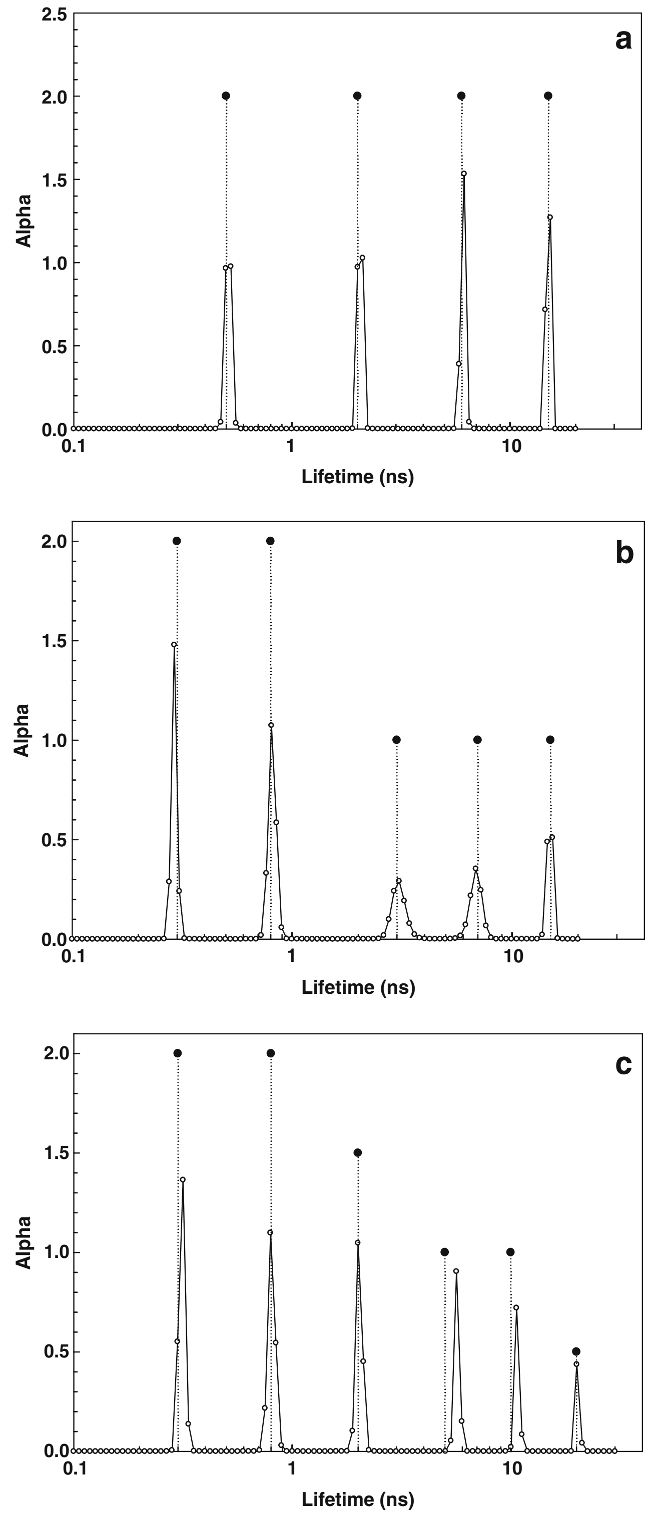


Fig. 6 Lifetime distributions obtained by SVD-MEM analysis of simulated noisy multi-exponential decays: 1,024 data points, 100 lifetimes logarithmically distributed on the time scale. Other details: 4 exponentials: time scale from 0.1 to 20 ns, calibration 0.05 ns/channel, total number of counts per decay $N=5.7\times10^7$; 5 exponentials: 0.1–20 ns, 0.1 ns/channel, $N=1.8\times10^7$; 6 exponentials: 0.1–30 ns, 0.1 ns/channel, $N=2.0\times10^7$. Simulated parameters are marked by *full circles*

The situation substantially changes after addition of noise to the data. As evident from Fig. 8., the lifetime distributions recovered by the SVD-MEM exhibit a band splitting for $\gamma<0.01$. This is caused by too low entropy contribution in the maximized quantity $Q=\gamma S\text{-}L$ that allows noise to scramble the information. For $\gamma>1$ the recovered distributions start to closely resemble the simulated band shape. Fortunately, there are at least three indications that the correct result of the SVD-MEM analysis is a single band and not a bimodal distribution. First, the smaller peak at 6.9 ns found for $\gamma=0.01$ is not significant as a standard deviation σ of its area is larger than its peak area $\langle\alpha\rangle$. Second, an increase of χ^2_N takes only 0.0004 when γ changes from 0.01 to 1, and third, ones the correct shape of the distribution is found for γ close to 1 it does not depend any more on its further increase over a broad interval of γ-values. The existence of the lifetime band can be by no means predicted by the parametric Marquardt analysis because the reduced chi-square value of the bi-exponential noisy data fit $\chi^2_\nu=1.024$ is statistically acceptable. For $\nu=1{,}000$ degrees of freedom the standard deviation σ of the reduced chi-square distribution is about 0.045, therefore

Table 3 Results of the SVD-MEM analysis obtained for the set of simulated multi-exponential fluorescence intensity decays containing 4, 5 and 6 exponential functions: α, τ—simulated decay parameters with the "correct" value of χ^2_N; ⟨α⟩, ⟨τ⟩—mean values of the recovered decay parameters with the χ^2_N value of the fit; τ_{mean} is the mean lifetime of the whole decay

Simulation	χ^2_N	τ_{mean} (ns)	α_1	τ_1 (ns)	α_2	τ_2 (ns)	α_3	τ_3 (ns)	α_4	τ_4 (ns)				
4 exp	0.9872	11.287	2.000	0.500	2.000	2.000	2.000	6.000	2.000	15.000				
Analysis	χ^2_N	τ_{mean} (ns)	$\langle\alpha\rangle_1$	$\langle\tau\rangle_1$ (ns)	$\langle\alpha\rangle_2$	$\langle\tau\rangle_2$ (ns)	$\langle\alpha\rangle_3$	$\langle\tau\rangle_3$ (ns)	$\langle\alpha\rangle_4$	$\langle\tau\rangle_4$ (ns)				
Noiseless	0.00014	11.287	2.000	0.500	2.000	2.001	2.001	6.002	2.000	15.001				
Noisy	0.9814	11.293	2.017	0.512	2.005	2.061	1.964	6.108	1.987	15.027				
Simulation	χ^2_N	τ_{mean} (ns)	α_1	τ_1 (ns)	α_2	τ_2 (ns)	α_3	τ_3 (ns)	α_4	τ_4 (ns)	α_5	τ_5 (ns)		
5 exp	1.0063	10.458	2.000	0.300	2.000	0.800	1.000	3.000	1.000	7.000	1.000	15.000		
Analysis	χ^2_N	τ_{mean} (ns)	$\langle\alpha\rangle_1$	$\langle\tau\rangle_1$ (ns)	$\langle\alpha\rangle_2$	$\langle\tau\rangle_2$ (ns)	$\langle\alpha\rangle_3$	$\langle\tau\rangle_3$ (ns)	$\langle\alpha\rangle_4$	$\langle\tau\rangle_4$ (ns)	$\langle\alpha\rangle_5$	$\langle\tau\rangle_5$ (ns)		
Noiseless	0.00003	10.461	1.990	0.302	1.994	0.799	0.993	2.980	1.004	6.943	1.010	14.969		
Noisy	0.9904	10.447	2.012	0.292	2.067	0.815	0.955	3.089	0.985	6.886	1.024	14.905		
Simulation	χ^2_N	τ_{mean} (ns)	α_1	τ_1 (ns)	α_2	τ_2 (ns)	α_3	τ_3 (ns)	α_4	τ_4 (ns)	α_5	τ_5 (ns)	α_6	τ_6 (ns)
6 exp	1.0338	11.009	2.000	0.300	2.000	0.800	1.500	2.000	1.000	5.000	1.000	10.000	0.500	20.000
Analysis	χ^2_N	τ_{mean} (ns)	$\langle\alpha\rangle_1$	$\langle\tau\rangle_1$ (ns)	$\langle\alpha\rangle_2$	$\langle\tau\rangle_2$ (ns)	$\langle\alpha\rangle_3$	$\langle\tau\rangle_3$ (ns)	$\langle\alpha\rangle_4$	$\langle\tau\rangle_4$ (ns)	$\langle\alpha\rangle_5$	$\langle\tau\rangle_5$ (ns)	$\langle\alpha\rangle_6$	$\langle\tau\rangle_6$ (ns)
Noiseless	0.00002	11.013	2.011	0.306	1.983	0.812	1.487	2.017	0.996	5.020	0.998	10.006	0.501	19.997
Noisy	1.0295	11.032	2.059	0.313	1.896	0.807	1.609	2.029	1.109	5.677	0.827	10.688	0.479	20.200

the unacceptable χ^2_v value would be larger than 1.045. Neither, the residual function of the noisy decay fit shows evident systematic distortions (see Fig. 8). However, with increasing number of data points N the standard deviation of the reduced chi-square distribution σ decreases according to the equation $\sigma = \sqrt{2/N}$ [10] to the value of 0.016 for 8,000 data points. From this point of view, an increase of data points could help to recognize lifetime distributions hidden in the decay data by classical parametric methods.

Conclusions

The SVD-MEM analysis of the noiseless data accurately returned simulated decay parameters of all studied decays. It confirms not only the correct functionality of the SVD-MEM software but it also sets limits for analyses of corresponding noisy data. All presented results on the noiseless data were obtained by choosing the most probable value of the entropy scaling parameter γ [6], however, there

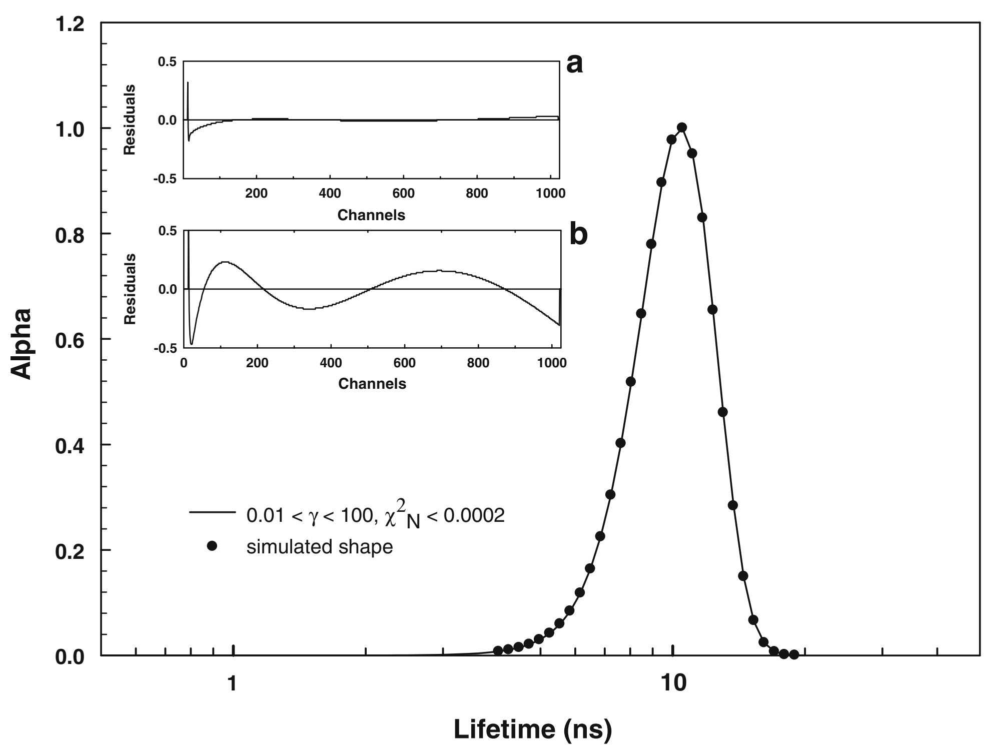


Fig. 7 Comparison of the lifetime distribution recovered by the SVD-MEM analysis of noiseless decay data (*solid line*) with the simulated Gaussian lifetime distribution (*full circles*). *Insets* show residuals of the decay fits: *A*) SVD-MEM fit, χ^2_N=0.0002; *B*) double exponential fit by the Marquardt parametric method, χ^2_v=0.0209

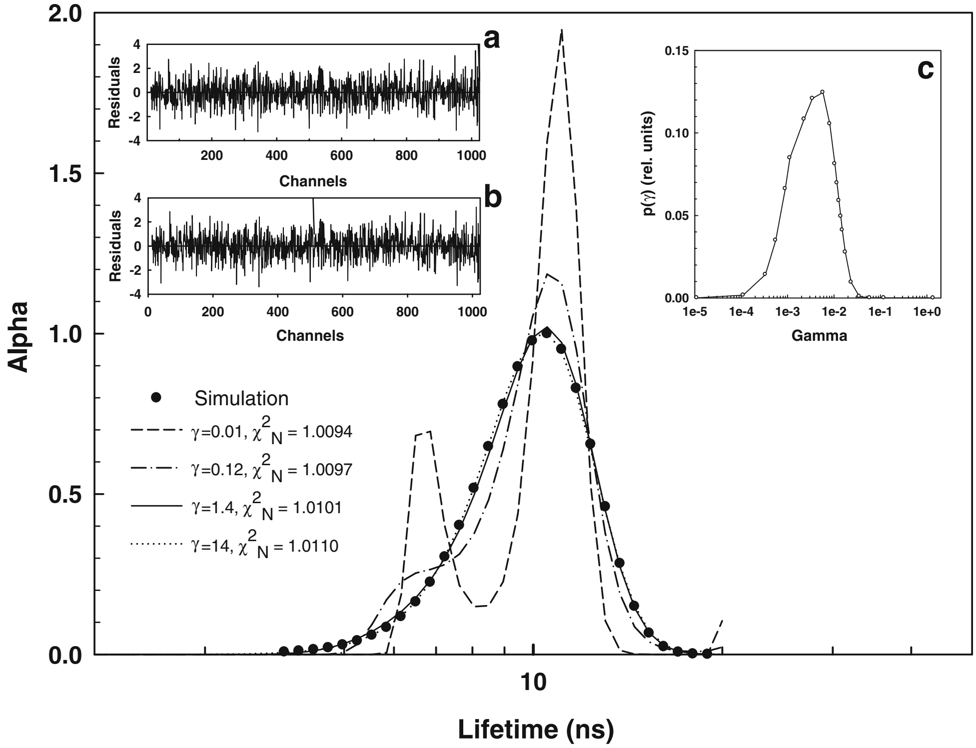


Fig. 8 Comparison of the lifetime distributions obtained by SVD-MEM analysis of the noisy decay data (*lines*) with the simulated Gaussian lifetime distribution (*full circles*). *Insets A* and *B* show residuals of the decay fits: *A*) SVD-MEM fit, χ^2_N=1.0110; *B*) double exponential fit by the Marquardt parametric method, χ^2_ν=1.0242. *Inset C*: Posterior probability of the entropy scaling factor γ [6]

were found very little changes in recovery of the simulated parameters when γ changed across the main peak of its posterior probability p(γ).

The SVD-MEM analysis of the noisy data returned decay parameters that were affected by the noise in dependence on complexity of the studied decays. At the selected noise levels we obtained good recovery of simulated parameters for all tested decays. In contrary to the noiseless data, the results of the SVD-MEM analysis depended more on the value of the γ-factor. In general, the lower the entropy scaling factor the narrower peaks of the parameter distributions were found for decay curves containing discrete lifetime components. Nevertheless, the calculated mean peak parameter values were practically independent of γ changing across the main peak of its posterior probability p(γ). The different situation was found for the Gaussian decay distribution where the low values of γ led to the band splitting in the recovered distribution. The correct band-shape was found for γ-values exceeding more than 100 times the most probable γ-value expected from the theory, inset C of Fig. 8.

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