Operating Instructions

F900

Analysis of Lifetime Data

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EDINBURGH INSTRUMENTS

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1. Introduction

1.1. Exponential Sample Decay Model

Raw lifetime data require generally a numerical analysis procedure for recovering the intrinsic lifetime parameters, either growth or decay parameters. In the majority of cases the growth or decay processes are either of single or multi-exponential nature, or they can be simulated by a sum of exponentials.

An exponential growth or decay process is expressed in mathematical terms as follows:

$$R(t) = A + \sum_{i=1}^{4} B_i \; e^{-\frac{t}{\tau_i}} \quad ,$$

with pre-exponential factors B_i, the characteristic lifetimes τ_i, and an additional background A. *R(t)* is often called the sample decay model. It is a theoretical expression for the response of the sample to an infinitely short excitation. *R(t)* must to be distinguished from the more complex sample response function *X(t)* described in the next paragraph and from the raw data *F(t).*

The expression above contains four exponential terms. Many measurements contain only one or two terms. On the other hand samples can theoretically contain many more lifetimes and can even be so complex that a lifetime distribution analysis would be justified. However, practically all real lifetime measurements can be approximated with no more than four exponential terms. And often the number of exponential terms can be reduced by careful experimental planning and clean practice.

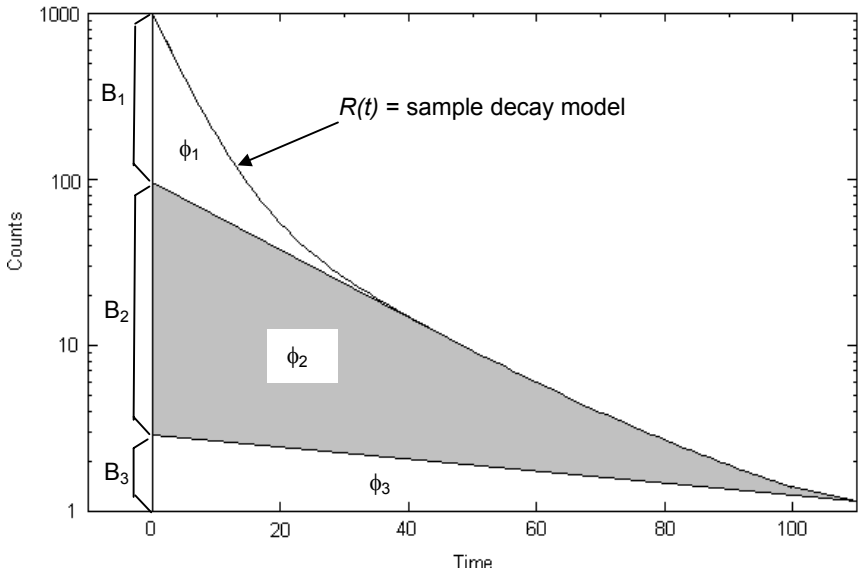
The characteristic lifetimes τ_i are the most important lifetime parameters. They are specific for different decay (or growth) processes and express the time it takes to decay from the beginning of the decay to a level of about 37% of the original value.

The pre-exponential factors B_i are values which include technical (instrumental) parameters and sample parameters. Used in relative terms, they are still valuable sample parameters. In a multi-component system, for instance, the concentration ratio of the individual components can be determined (see table below). In absolute terms the B_i-values are also affected by instrumental parameters like efficiency of the system, geometrical conditions of the sample, intensity of the excitation source, etc. These instrumental parameters can increase or a decrease the measured sample signal, which effectively will result in an (simultaneous) increase or decrease of the B_i values.

The pre-exponential factors can be either positive or negative. A positive B_i value represents a decay process, while negative B_i values are characteristic for growth processes.

The following table summarises sample parameters which are often used by scientists to describe specific sample features, parameters which are derived from the original B_i and τ_i values. For better illustration a 3-component system (with a sum of three exponential terms) was chosen as an example.

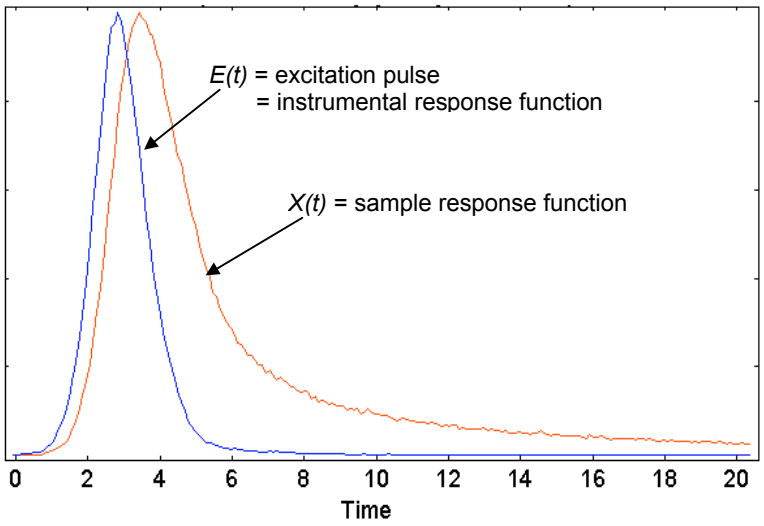
$c_2 = \frac{B_2}{B_1 + B_2 + B_3}$	relative concentration of the second component
$\phi_2 = \frac{B_2 \tau_2}{B_1 \tau_2 + B_2 \tau_2 + B_3 \tau_3} \cdot 100\%$	relative fluorescence intensity of the second component, as a percentage
$\langle \tau \rangle = \frac{B_1 \tau_1^2 + B_2 \tau_2^2 + B_3 \tau_3^2}{B_1 \tau_1 + B_2 \tau_2 + B_3 \tau_3}$	average lifetime of the entire decay process



The function *R(t)* is a mathematical expression for a fluorescence or phosphorescence decay, or for other temporal changes of the sample intensity (e.g. caused by chemical reaction, degradation, etc.). However, *R(t)* expresses the decay or growth process only in certain approximations. Two important features of raw lifetime data are not included in the expression *R(t)*. This is the statistical noise of the data and the process of sample excitation, which is often accomplished by an optical pulse of a light source of short – but not infinitely short – duration.

1.2. Reconvolution

In practice, many lifetime measurements do not only decay starting right after a prompt, infinitely short, signal rise, but they have a finite rising edge often caused by the exciting light pulse. This initial part of the raw data contains valuable information. For example, short lifetimes can often only be precisely recovered if the initial part of the fluorescence decay is included in the analysis.



In this case the sample response function can not be described with the simple decay model *R(t).* In the initial part (the formation part and the initial decay part) the sample response is determined primarily by the instrumental response function (which includes the optical pulse widths as well as possible electrical effects). The mathematical relationship between sample response function (*X(t)*), the instrumental response function (*E(t)*) and the sample decay model (*R (t)*)is the convolution integral:

$$X(t) = \int_0^t E(t')\ R(t-t')\ dt'$$

Apart from the noise *X(t)* fully describes the measured data, i.e. the rise of the signal, the initial part of the decay, and the tail of the decay. In order to calculate *X(t)* one needs to know both the theoretical model for the decay (*R(t)*) and the (separately measured) instrumental response function (*(E(t)*). Only in the case of an infinitely short sample excitation is the sample response function identical to the model function.

The F900 software offers two fitting routines. What the two procedures have in common is that they try to find the best set of parameters B_i and τ_i to match the theoretical sample response *X(t)* to the raw data *F(t).* The two routines are different in respect to the theoretical sample response function used. In the "**Tail Fit**" routine *X(t)* is identical to *R(t).* This routine is only applicable for data which are fitted in a region with no further sample signal generation, either by the exciting light pulse or by sample formation (e.g. excimer generation). The "**Reconvolution Fit**" routine is more universal. It fits the (convoluted) sample response *X(t)* to the data. This procedure allows one to fit over the rising edge of the data. In other words: The Tail Fit procedure eliminates the statistical noise from the raw data, but can not handle the region in which sample excitation takes place. The Reconvolution Fit procedure eliminates both the noise and the effects of the exciting light pulse.

1.3. Numerical Fit

The F900 offers two numerical routines to extract the decay parameters B_i and τ_i from the raw data. The routines use either the pure decay model "exponential process" (rise or decay) as it is expressed in the form *R(t)* or the Convolution Integral in the form *X(t)* and fits this model to the raw experimental data *F(t)* by modifying the B_i and τ_i.

The numerical procedure behind the search for the best B_i and τ_i is the Marquardt-Levenberg algorithm. This is an iteration procedure which searches for the best B_i and τ_i by a controlled and directed minimisation of the "goodness of fit", χ_g^2, which is defined as.

$$\chi_g^2 = \sum_k w_k^2\ (X_k - F_k)^2$$

(*k* is the index for the individual data points to be fitted, the sum expands over all these data points.) The w_k are the weighting factors for the individual data points. Using the correct weighting factors for a specific set of raw data is important. The correct type of weighting factors is determined by the type of noise specific to the data and hence is inherited from the method which was used to collect the data. For example, lifetime data acquired by TCSPC or gated single photon counting (MCS) obey Poissonian noise statistics with the well defined weighting factor for each data point (F_k) of $w_k = 1/\sqrt{F_k}$. Data acquired by an oscilloscope obey Gaussian noise statistics with the $w_k = const$.

Following Marquardt-Levenberg the partial derivatives of χ_g^2 with respect to the B_i and also with respect to the τ_i are both set to zero. This results in a set of equations which can be solved simultaneously by linearisation. The solution of the set of equations provides the B_i and τ_i which fit the raw data best. A distinct feature of the Marquardt-Levenberg algorithm is also a so called acceleration parameter, which is an artificial number that is added to the main diagonal of the matrix formed by set of equations before each new iteration step. The acceleration parameter increases the speed of the fitting process dramatically without scarifying the robustness and stability of the fitting routine.

The Marquardt-Levenberg algorithm does not only produce the best lifetime parameters, but also the standard deviation for each of the fitted parameters. (They can be taken from the main diagonal of the so-called error matrix.)

1.4. Fit Quality Parameters

The quality of the fit result can be evaluated in several ways. If the fit result is entirely wrong, then a simple visual comparison between the raw data and the fitted curve might be sufficient to find the reason for the mis-fit. In most cases, however, the visual comparison between these two data is not sufficient. Other parameters need to be calculated to allow a much more precise fit evaluation.

The most common parameters are the following:

The Reduced Chi-Square

Using the expression of χ_g^2 outlined in paragraph 1.3. and dividing it by the number of free parameters *n* (which is approximately the number of fitted data points subtracted by the number of lifetime parameters used in the fit) will result in

$$\chi^2 = \sum_k w_k^2 \frac{[X_k - F_k]^2}{n}$$

χ^2 is called the "reduced chi-square"; it is the scaled "goodness of fit". The reduced chi-square has a distinct advantage over the goodness of fit in that it's value is independent of the number of data points and the number of fitting parameters. This allows one to compare different fits.

For Poisson distributed data ($w_k = 1/\sqrt{F_k}$) the reduced chi-square has the theoretical limit 1.0. *χ^2-values* above Unity indicate a bad fit result, although values of about 1.1, 1.2 or even 1.3 are acceptable under certain conditions. If the fitting range has been inappropriately chosen (see paragraph 3), χ^2 can be slightly less than 1.0.

[Principally one always needs to distinguish between the chi-square (χ_g^2) and the reduced chi-square (χ^2). As for fit evaluation only the reduced chi-square is used, the word "reduced" is omitted in this manual.]

The Residual Data

Using the fit result data (X_k), the measured raw lifetime data (F_k), and the appropriate weighting factors (w_k - see next paragraph) the residual data can be calculated as follows:

$$Y_k = w_k (X_k - F_k)$$

The residual data are the difference between the fitted curve and the original data to be fitted, weighted by the standard deviation of each data point. A good fit should give a residual curve that only contains random noise distributed around Zero. Any deviation from the randomness would give an indication for a mis-fit, either because the appropriate exponential model contains more exponential terms than were used in the fit or because of instrumental artefacts.

The Autocorrelation Data

The autocorrelation function of the residuals is defined as

$$Z_k = \frac{\frac{1}{n_H - k - n_L} \sum_{i=nL}^{nH-k} X_i X_{i+k}}{\frac{1}{n_H - n_L} \sum_{i=nL}^{nH} X_i^2},$$

with n_L and n_H being the lower and upper limit of the fitting range, respectively. The residual autocorrelation data (Z_k) show more clearly than the residual data themselves (Y_k) whether the residuals are fully randomly distributed, or whether there is a repetitive pattern in the residuals. As each residual data point is correlated with itself the first autocorrelation data point is always 1.0. All other autocorrelation data points should be randomly distributed around Zero if the residuals are "clean".

The Durbin-Watson Parameter

This parameter is used by some scientists to evaluate the quality of the fit. It is defined as

$$DW = \frac{\sum_{i=nL+1}^{nH} (X_i - X_{i-1})^2}{\sum_{i=nL}^{nH} X_i^2},$$

with n_L and n_H being the lower and upper limit of the fitting range, respectively. The Durbin Watson parameter can only be considered in absolute terms for a defined number of exponentials in the tried model. DW-values of less than 1.7, 1.75 and 1.8 are indicative for poor fits to single, double, and triple exponential decay models.

The F900 fitting routines provide all four fit quality data and parameters, the reduced χ^2-value, the residual data, the residual autocorrelation data and the Durbin-Watson Parameter. The most commonly used parameters among many scientists today are the former two.

1.5. Data Types and Weighting Factors

The two lifetime data analysis routines available from the F900, “Tail Fit” and “Reconvolution Fit”, require the following data types:

- Lifetime Data
- Kinetic Data
- Transient Data
- Time Resolved Anisotropy Data.

Different data types will be treated differently by the data analysis routines due to their different weighting factors:

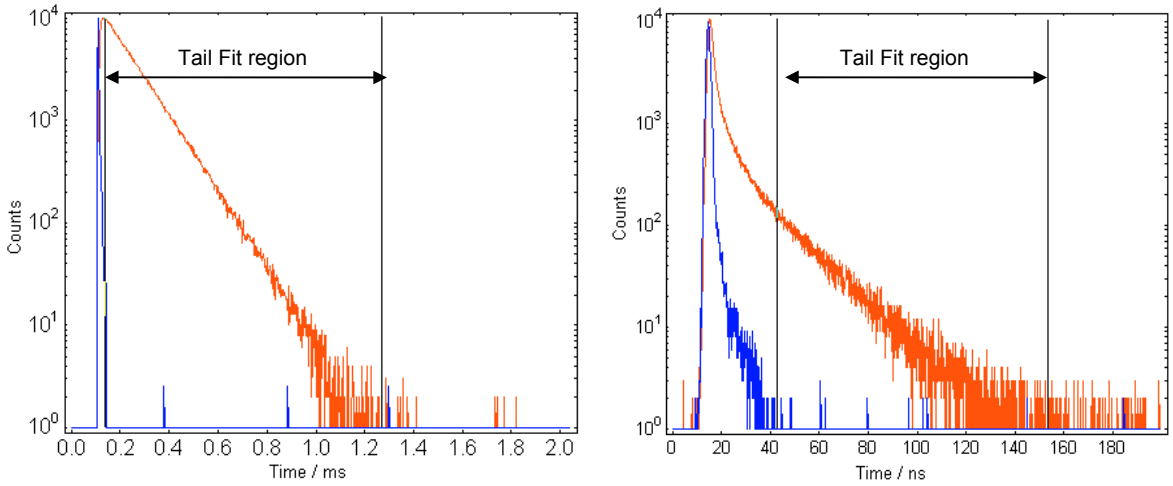
Data Type	Data Acquisition Technique	Weighting Factors (w_k)
Lifetime Data	TCSPC / MCS	$w_k = 1/\sqrt{F_k}$
Kinetic Data	SPC	$w_k = 1/\sqrt{F_k}$
Transient Data	Oscilloscope	$w_k = const.$
Time Resolved Anisotropy Data	TCSPC / MCS	error propagation from original data

The following data types are produced at the end of a numerical fit and after autocorrelation analysis:

- Fit Data,
- Residual Data
- Autocorrelation Data.

2. Tail Fit Analysis

The two pictures below show typical measurement examples. Also indicated are the data regions which can be analysed with Tail Fits. As stated before Tail Fits should only be performed in a region with no further sample excitation, i.e. in a region where the exciting light pulse has disappeared. Consequently the Tail Fit routine will be used to analyse those samples with long decay times. The pictures not only show the decay data but also the instrumental response function. This is only to demonstrate the fitting range. For a Tail Fit the instrumental response function is not needed, and as far as the beginning of the fitting range is known the instrumental response function will in general not even be measured.



While the Tail Fit applied to the measurement in the left picture will Yield to all lifetime information intrinsic in the decay measurement, a Tail Fit applied to the measurement in the right picture will obviously only result in lifetime parameters which have a dominating effect in the longer time region. The lifetime parameters in the shorter region (out with the indicated fitting range) can not be recovered by using the Tail Tit routine. For these data it is far more appropriate to use the Reconvolution Fit routine described in the next paragraph.

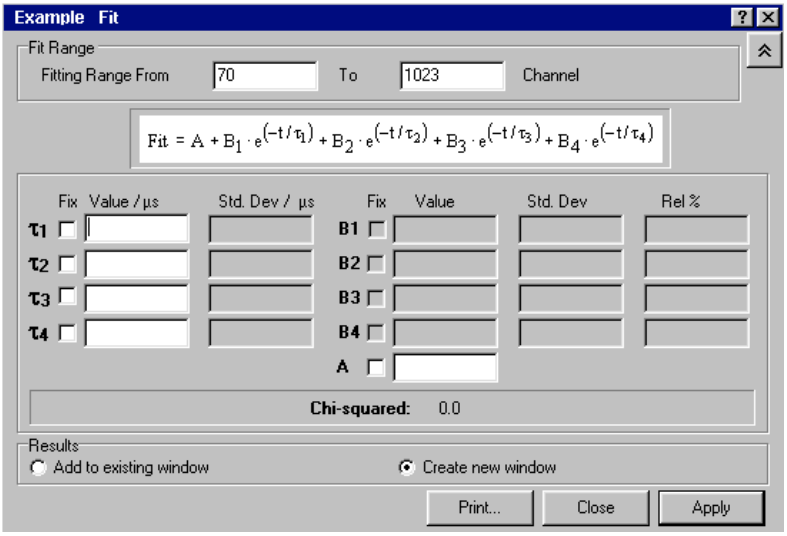
Tail Fits (as well as Reconvolution Fits) can only be performed at one decay data file at a time. Therefore the active data window should only contain one decay measurement. (Instrumental response functions as well as fitted curves can be present in the active data window.) If more than one decay measurement is present in the data window then a single decay measurement must be extracted ***(Window / Extract…***, or ***right mouse click on data container / Extract…*** *).*

After the decay measurement has been chosen the Tail Fit routine can be started. The Tail Fit dialogue box is accessed as follows: ***Data / Exponential Fit / Tail Fit …*** .

The top section of the Tail Fit dialogue box contains the fitting range, given in data channels. For Tail Fits the default setting of the lower end of the fitting range is determined by the data channel containing the biggest value. The default setting of the upper end of the fitting range is the maximum channel number of the measurement data. Alternatively, if the zoom facility is used to select a zoomed range of the data container, then this selected range will be given as the default fitting range. The lower limit defined by the channel with the maximum data still applies.

The fitting range can be changed. Careful and educated selection of the fitting range is essential for successful fitting. At the lower end the fitting range must start at least one channel above the channel containing the maximum signal. If a smaller value is edited, then an error message will appear when attempting to fit.

In many cases it is not suitable to use the default values. For example, if one would fit over the default fitting range offered by the Tail Fit routine dialogue box for the right of the two measurements above, then erroneous fit results would be guaranteed as the channel with the maximum signal is well before the suggested start of the fitting range.



At the upper end of the fitting range one must pay attention not to allow it to expand over a range where there is no information in the original raw data, i.e. the data are Zero. The consequences of involving that range are not as severe as choosing an inappropriate value at the shorter end of the fitting range, but the goodness of fit (χ^2) would be below the theoretical limit (= 1.0 for Poisson distributed data).

The second section of Tail Fit dialogue box contains the formula of the mathematical model to be used (= *R(t),* see paragraph 1.1. “Exponential Sample Decay Model”). Four exponential terms and a constant background (*A*) are shown, although this dialogue box is also used for single, double, and triple exponential decay analysis.

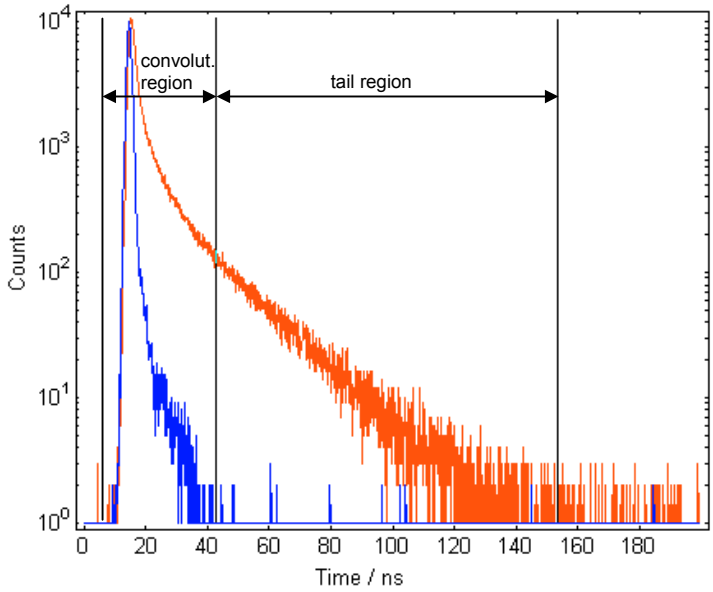
The third section contains a table which is used to enter and to view both initial fit parameters and fit results, respectively. Before attempting a fit (***Apply***) at least one initial lifetime (usually τ_1) needs to be entered. Whether the lifetimes must be entered in unit of channels or in unit of times will depend on what the x-axis of the data container has been set to (***Options / Plot Defaults / 2D / Lifetime,*** or ***right mouse click on data container / Plot Options / Lifetime***). If the lifetimes are given (or requested) in units of time, then the software will automatically scale the τ-values to ps, ns, µs, ms, or seconds. The time unit used is displayed next to the title “value / ” on top of the τ-value column.

The fitting procedure is started using the ***Apply*** button. A successful fit will result in the new lifetime parameters, an update of the χ^2 value displayed beneath the table of lifetime parameters, in a fitted decay curve, and in a residual curve. The user can choose prior to the fit whether the resulting curves should be added to the original data window or if a new data window is to be created.

For evaluation of the fit and for re-fitting proceed with paragraph 4. “Fit Evaluation and Re-Fitting”.

3. Reconvolution Fit Analysis

If the raw data *F(t)* are not only superimposed by noise but are also affected by the effects of sample excitation and signal generation the numerical procedure requires the use of the convolution integral *X(t)* to extract the lifetime parameters B_i and τ_i .The fit range expands over the convolution region and the tail region.



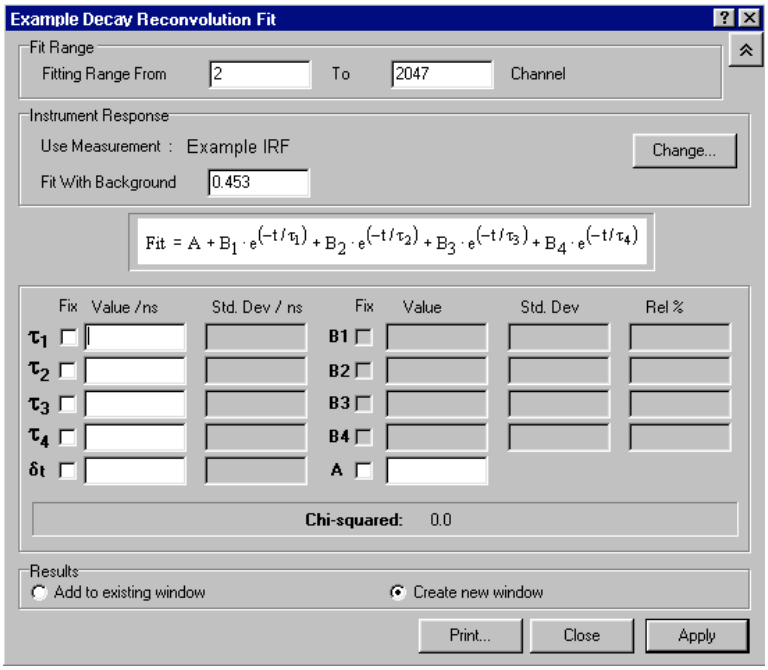
Reconvolution Fits can only be performed at one decay data file at a time. The active data window must contain at least the decay measurement and the instrumental response. If more than one decay measurement is present in the data window then a single decay measurement and the instrumental response function must be extracted ***(Window / Extract…***, or ***right mouse click on data container / Extract…*** *)*.

The Reconvolution Fit Analysis menu will not be active if the software does not recognise exactly one sample decay measurement and one instrumental response function. The instrumental response function (IRF) must be assigned as such. If the assignment has not been made prior to the measurement (see paragraph 7 “Lifetime Data Acquisition”) then the flag needs to be set in the file properties: ***(File / Properties…***, or ***right mouse click on data container / Properties…)***. Once the relevant file has been selected in the properties dialogue box the IRF assignment is made by ticking the box on the bottom left of the property dialogue box: ***“Is instrument Response”***.

The Reconvolution Fit dialogue box is accessed as follows: ***Data / Exponential Fit / Reconvolution Fit …*** .

The top section of the Reconvolution Fit dialogue box contains the fitting range, given in data channels. If no Zoom was applied to the data window the default setting for the fitting range is identical to the number of data points of the raw measurement. If Zoom was applied before accessing the Reconvolution dialogue box, then the selected range will be given as the default fitting range.

With Reconvolution Fits the fitting range should generally expand over the entire measurement, starting from about Zero, ranging over the rising edge, the decay, down to Zero (see picture above). The user must pay attention not to allow the fitting range to expand over a range where there is no information in the original raw data. The consequences of involving that range are that the goodness of fit (χ^2) would be below the theoretical limit (= 1.0 for Poisson distributed data).



The second section of the Reconvolution Fit dialogue box contains information of the instrumental response function. It shows the name of the IRF and the background level of the instrumental response function detected by the software. Generally this value does not need to be changed. However, it can be edited if required. An alternative IRF can be loaded by using the button on the right side: ***Change***.

The third section contains the formula of the mathematical model to be used (= *R(t),* see paragraph 1.1. "Exponential Sample Decay Model"). Four exponential terms and a constant background (*A)* are shown, although this dialogue box is also used for single, double, and triple exponential decay analysis.

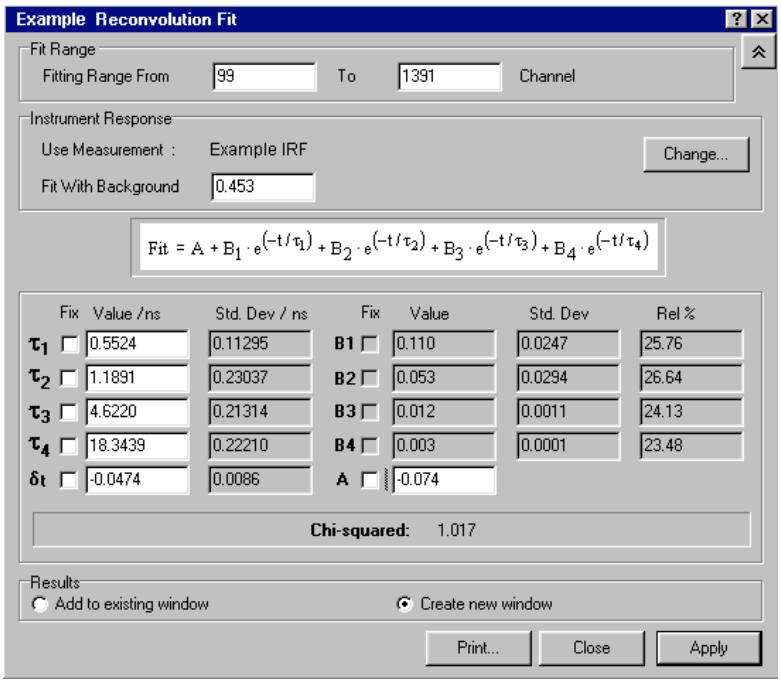
The fourth section of this dialogue box contains a table which is used to enter and to view both initial fit parameters and fit results, respectively. Before attempting a fit (***Apply***) at least one initial lifetime (usually τ_1) needs to be entered. Whether the lifetimes must be entered in unit of channels or in unit of times will depend on what the x-axis of the data container has been set to (***Options / Plot Defaults / 2D / Lifetime,*** or ***right mouse click on data container / Plot Options / Lifetime***). If the lifetimes are given (or requested) in units of time, then the software will automatically scale the τ-values to ps, ns, µs, ms, or seconds. The time unit used is displayed next to the title "value / " on top of the τ-value column.

In addition to the τ_i, the B_i, and the background A, one more parameter δt is listed in the table of lifetime parameters. δt is the shift between the instrumental response function and the lifetime measurement. The shift parameter is iterated in the reconvolution fit in a similar way as the B_i and τ_i are. Both background A and shift δt do not require an initial parameter to be entered.

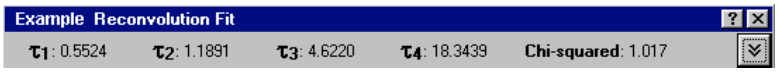
The fitting procedure is started using the ***Apply*** button. A successful fit will result in the new lifetime parameters, an update of the χ^2 value displayed beneath the table of lifetime parameters, in a fitted decay curve, and in a residual curve. The user can choose prior to the fit whether the resulting curves should be added to the original data window or if a new data window is to be created.

4. Fit Evaluation and Re-Fitting

The numerical fit will usually take a fraction of a second. A successful fit will result in an update of the data window, an update of the lifetime parameters, and a new χ^2 value.



In many cases the Fit dialogue box containing the fit results is demonstrated on top of the data container containing the new fitted curve and the residual curve. To make fit evaluation easier the Tail Fit dialogue box can be minimised by using the “ ‘ ıtton on the top right side or by just pressing the return button. Repeating these actions for the second time will result in re-maximisation.

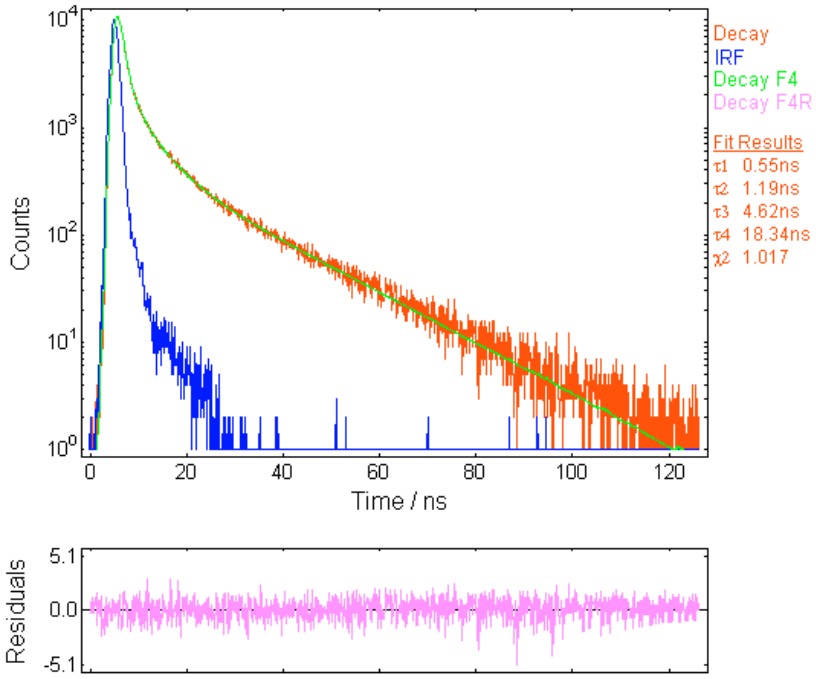


The data window will be updated automatically with the fitted sample response function (*X(t)*) and the residual curve (*Y(t)*). The residual curve will be shown in a separate bar underneath the original data.

The two additional curves will carry the name of the original measured decay data with the extensions Fx and FxR for the fitted sample response and the residual data, respectively (x standing for the number of exponentials fitted).

A fit might not be successful. If no stable minimum for the χ^2 was found after 50 iteration steps, then the fit will automatically be stopped and a message “***A suitable fit was not found within 50 iterations.”*** will appear on the screen. New start parameters should be tried or the fitting range needs to be re-checked.

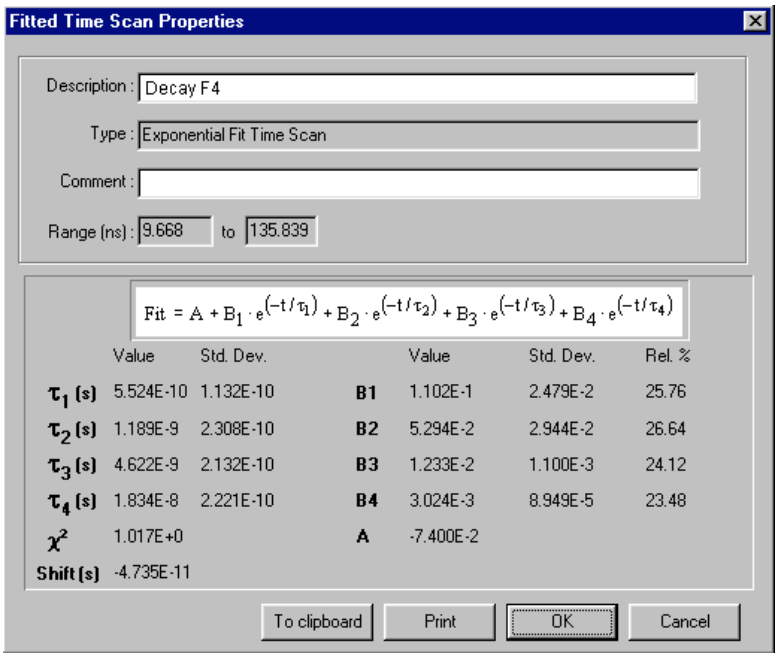
If the initial lifetime (or one of the initial lifetimes) chosen was too small, then there is a likelihood that during the fitting process the lifetime will “run” towards Zero. A τ-value of Zero is physically meaningless and can not be dealt with mathematically during the fit. An error message will appear on the screen: ***“A matrix singularity occurred during fitting.”*** New initial lifetimes should be chosen, which are bigger than the ones used before.



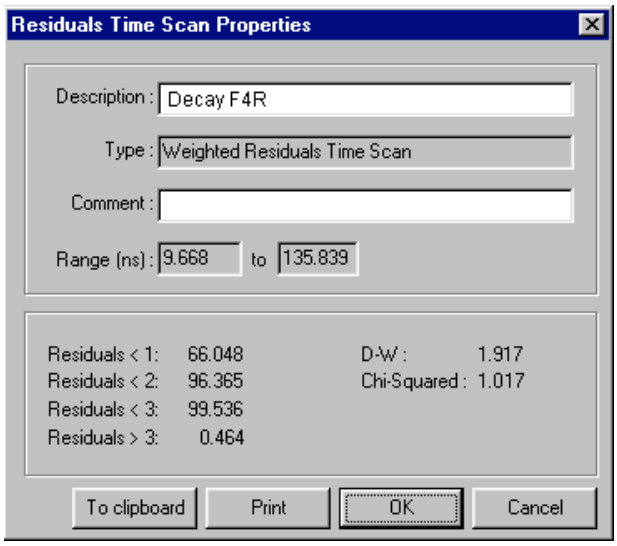
If the initial lifetime (or one of the initial lifetimes) chosen was too small, the fit procedure might not "run" into a singularity but will try to increase the τ-value. However, the fit routine limits final τ-values to 10 times the original (start) value. After 50 iteration steps a result will be displayed and the (or one of the) fitted τ-values will be exactly 10 times the original value. The result would not be a good fit. In this case the fit simply needs to be re-started (***Apply***).

The table of lifetime parameters also shows the standard deviation for the fitted parameters. Lifetimes as well as the shift parameter and the constant background can be fixed during the fit by ticking the relevant box in the table of lifetime parameters The cell for the standard deviation will remain empty for fixed parameters. If one or more negative pre-exponential factor have been obtained, then the column of relative fluorescence intensity will remain empty.

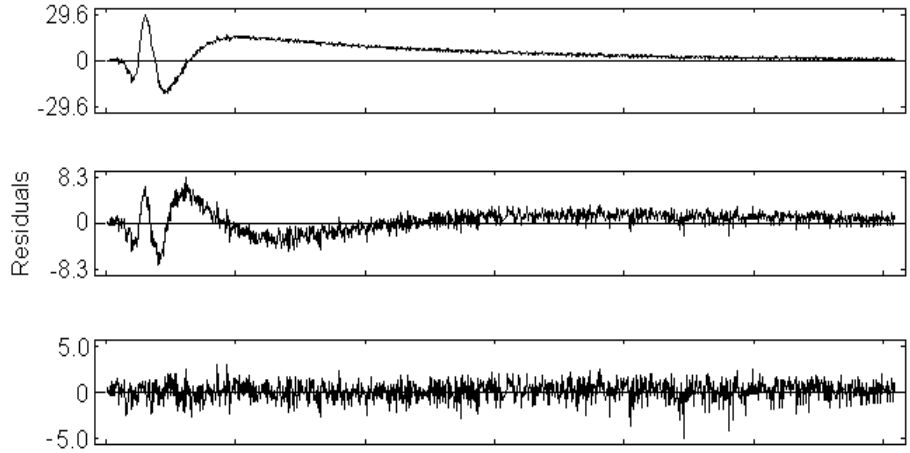
The table of lifetimes may be printed (***Print***). If the Fit dialogue box has been closed (***Close***) the table of lifetime parameters will no longer be available in the current form. However, it is available in a slightly different form via the data properties facility: ***File / Properties …,*** or ***right mouse click on the data container / Properties***. – load fit data. There the data are given in scientific format which improves the resolution, particularly for the *B*-values.



The properties of the residual data contain additional information: This property box does not only show the χ^2-value, but also the Durbin-Watson-Parameter (***D-W***:) and the number of residual data points within one, two, or three times the standard deviation.



It is advisable to start any lifetime data analysis with a single exponential sample decay model, i.e. only one lifetime should be entered before the fit is started with ***Apply***. If the fit is not satisfactory a model with more exponentials can be tried. Most scientists evaluate the fit result using both the χ^2-value and the residual data. The picture overleaf shows an example for the development of χ^2 and the residuals when changing the model from single to 3-exponential. For this example a 3-exponential fit would be appropriate to fit the data.


29.6
0
-29.6
1-Exp. Fit
χ^2 = 56.554
Residuals
8.3
0
-8.3
2-Exp. Fit
χ^2 = 4.182
5.0
0
-5.0
3-Exp. Fit
χ^2 = 1.049