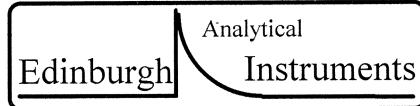


B. Hess

Operating Instructions

TCC900 Computer Module for Time Correlated Single Photon Counting

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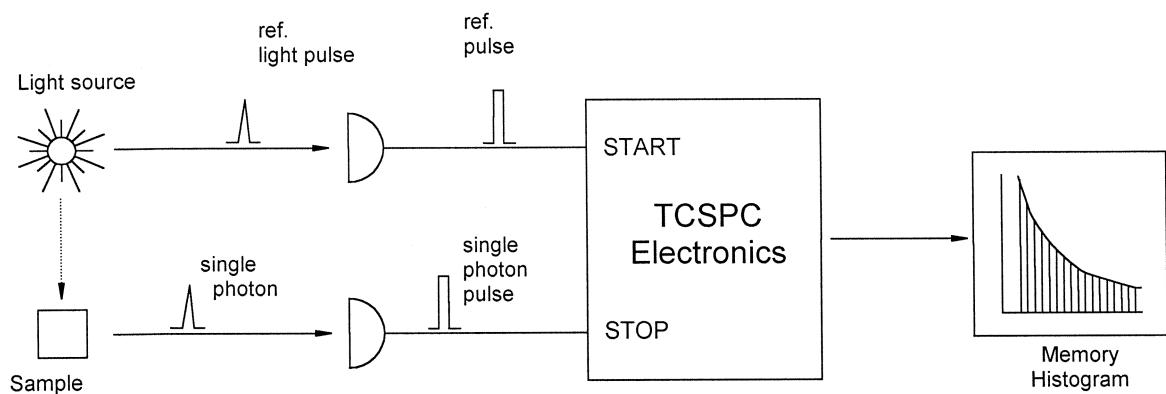


1. Introduction

1.1. Time Correlated Single Photon Counting

Time Correlated Single Photon Counting (TCSPC) is a well established and the most common technique for fluorescence lifetime measurements, but is also becoming increasingly important for measurements of photon migration, optical time domain reflectometry and time of flight studies.

The principle of TCSPC is the detection of single photons and the measurement of their arrival times in respect to a reference signal, usually from the excitation light pulse. TCSPC is a statistical method and a highly repetitive light source is needed to accumulate a sufficient number of photon events for a required statistical data precision.



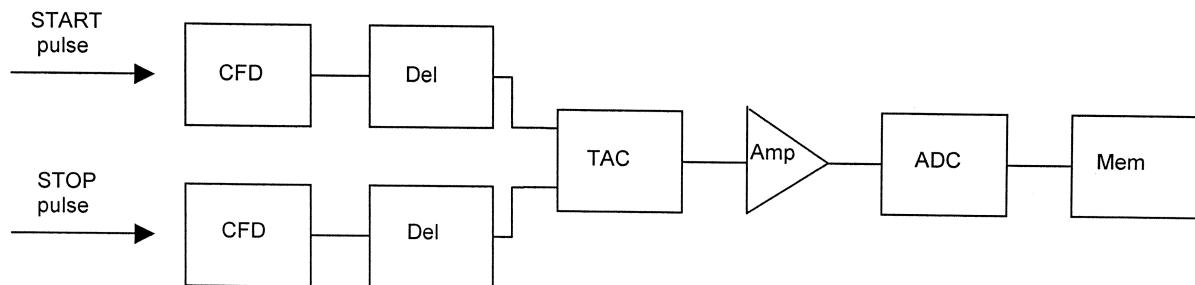
The TCSPC electronics can be compared to a fast stopwatch with two inputs. The clock is started by the START signal pulse and stopped by the STOP signal pulse. The time measured for one START – STOP sequence will be represented by the addition of one more count to a histogram in which the channels on the x-axis represent the time. With a highly repetitive light source millions of START – STOP sequences can be measured in a short time. The resulting probability histogram of photon counts versus channels represents the shape of the fluorescence decay.

Generally, one of the pulses to the TCSPC electronics (either START or STOP) will be generated by a single emission photon. Single photons can be detected with photo-detectors having an intrinsic high gain. These are usually photomultipliers or micro-channel plate photomultipliers, but also single photon avalanche photodiodes are also sometimes used. For statistical reasons it is important to ensure that no more than one single photon event per light flash is detected. Multi-photon events will affect the histogram statistics and will yield to erroneous measurement results. (This is known in the scientific literature as the “pulse pile-up problem”.) In order to ensure that only one photon per light flash is detected the photon rate is kept low (usually 5% or lower) in comparison to the repetition rate of the exciting lamp.

The fact of two substantially different rates at the two input channels of the TCSPC electronics results in two different modes of operation, known as the “forward” and the “reverse” mode.

1.2. TCSPC Electronics

The main components for signal processing in TCSPC are constant fraction discriminators (CFD), electrical delays (Del), the Time-to-Amplitude Converter (TAC), Amplifier (Amp), Analogue to Digital Converter (ADC) and digital memory (Mem).



At the input of the electronics, incoming pulses are evaluated in respect to the pulse height. Only pulses higher than a given **Threshold** will be accepted for further signal processing. In this way small amplitude noise pulses are readily eliminated.

The constant fraction discriminators on both the START and the STOP input then analyse the pulse shape of the individual pulses. The portion of the steepest slope of the initial edge on the incoming pulses (generally negative pulses) is taken as a criteria for the temporal position. Which portion of the slope is taken will depend on the **Fraction**, the **Constant Fraction Delay** (or shaping delay), and the **Zero Crossing Level**. Threshold, fraction, constant fraction delay, and zero crossing level will depend on the type of detector used and need to be matched to the individual detector.

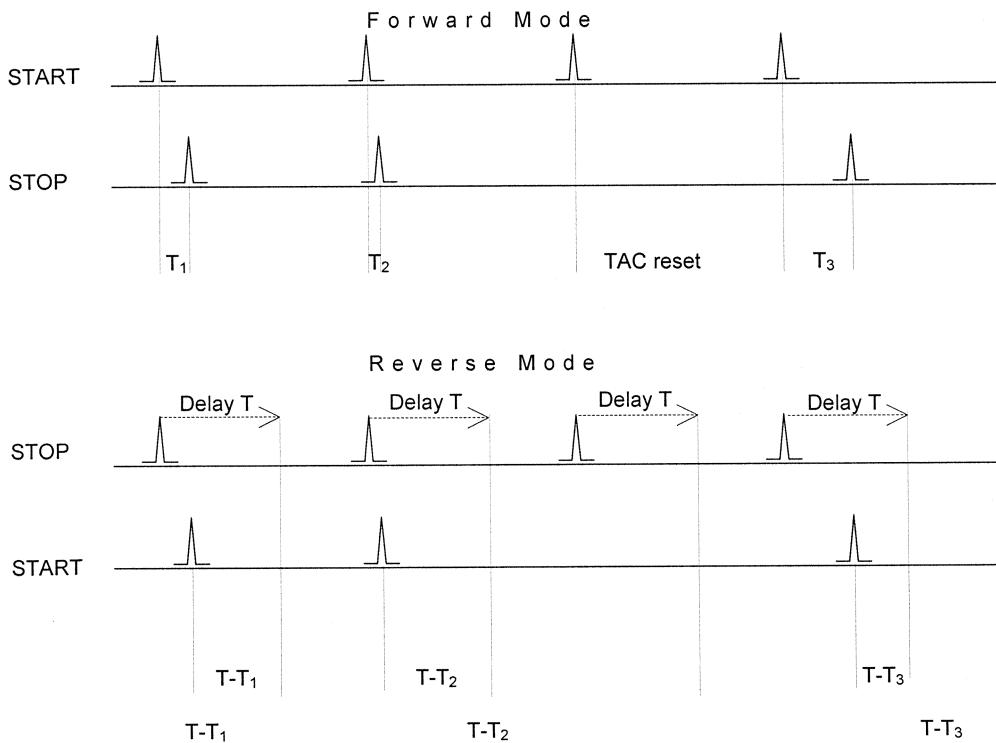
At the output of the CFD pulses are re-shaped to a standard height and shape. They can then be delayed by an electronic **Shifting Delay**. The implementation of a delay will result in a left or right shift of the entire measurement on the time axis.

The TAC is the fast clock, started by the START and stopped by the STOP pulse. The START pulse initiates the growth of a ramp signal. Depending on the arrival time of the STOP pulse the ramp will have a lower or higher height. Once the growth of the ramp has been stopped, the level will remain constant for a defined period. The TAC output pulse can then be amplified, thus effectively stretching the time axis. Minimum and maximum available (amplified) TAC amplitude determine the **Time Range**.

The amplified TAC output pulse is effectively an analogue pulse of a height corresponding to the measured time difference of a single START – STOP sequence. For further processing the pulse height will be measured by a digital pulse height measurement device, the ADC. The ADC resolution determines, how many discrete time values are possible. All possible measured TAC pulse amplitudes will therefore be put into different time bins. The width of the time bin is the ratio of the full time range to the resolution of the ADC in channels. This is the time resolution, usually given in picoseconds / channel or nanoseconds / channel.

As described in paragraph 1.1. there are two different operating modes in TCSPC applications: In **Forward Mode** the pulse rate from the light source is connected to the START input. This rate (with generally equally spaced pulses in time) is substantially higher than the (more or less random) pulses from the detector connected to the STOP input. The advantage of the forward mode is that no shifting delay is required or that it is relatively small and that photon events with long retardation times are represented at the longer time scales, so that an additional inversion of the time scale is not required.

t high rates of the light source, however, the forward mode has a clear disadvantage. Because the vast majority of TAC circles will be started by the START pulse, but never stopped by a STOP signal, it needs to be reset at overflow. The electronics is kept busy more than 20 times than that actually required. For electronic circuits with an upper limit of operating circles per second (given by the dead time of the overall system) this means the highest count rate processed will be reduced by a factor more than 20. To avoid this (and to utilise the full capability of signal processing) TCSPC electronics can be operated in the **Reverse Mode**, where the signal cable carrying the high count rate from the light source is connected to the STOP input and the low rate is connected to START. The drawback is that the reference pulses from the light source need to be shifted by a usually long shifting delay, so that they arrive at the input of the TAC later than the START pulses from the detector. A rule of thumb is that this delay is slightly longer than the time scale chosen for the measurement. In reverse mode the time axis of the memory histogram is internally reversed in order to see photon events with long retardation times demonstrated at the right part of the time axis.

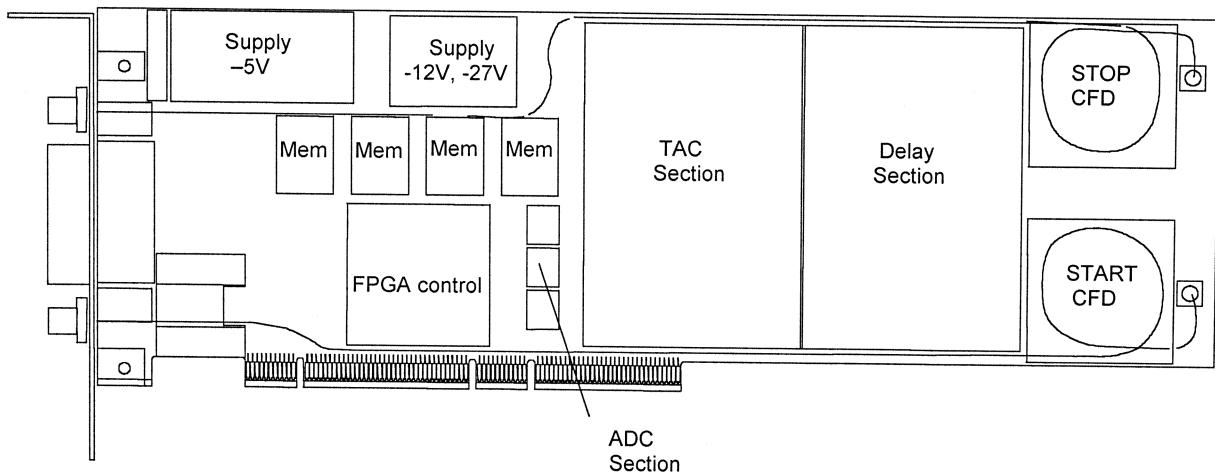


1.3. The TCC900 Module

Whereas in the past equipment for time correlated single photon counting was build up of a number of individual electronic modules (e.g. NIM modules), the TCC900 board comprises all electronics required. It is a computer plug-in board and all what is needed is to connect the synchrope output of the light source and the pulse output from the detector to the computer card.

The important parameters highlighted in the previous paragraph are all software controlled, with the exception of the constant fraction delay which is an exchangeable plug. A number of useful other parameters are software controlled or monitored by the software, such as START and STOP pulse rate, data acquisition stop conditions, etc.

The general layout of the TCC900 module is shown below.



There is no setup (such as jumper positioning, microswitches, etc.) to be made by the user. The only exception is the exchange of the constant fraction delay plugs for both the START and the STOP, if required. The delay plugs are located under the shielding can of each of the CFDs. They can be exchanged after removal of the can lid. The standard delay fitted at manufacture is 200ps, which is appropriate for most modern photomultiplier types.

2. Transit and Packing

All hard- and software are supplied in a robust ABS carrier case with foam packaging. The electronic board is protected by an anti-static bag.

If at the time of the delivery the package has signs of damage, then please contact the vendor immediately.
Open the carrier case carefully.

The carrier case includes the following items:

- the electronic board,
- the T900 software, supplied on three floppy disks,
- the software protection devise,
- 6 additional delay plugs
- the manual.

3. Hardware Installation

3.1. Computer Requirements

A Pentium PC or at least a ATX 486 computer is needed for the operation of the TCC900 module. The computers operating system must be WINDOWS 95 or higher and it should have a mouse or trackball for convenience.

The computer must have at least one free full size PCI slot, capable for operation at 33MHz. As the TCC900 is heavily populated with electronic components no computer components such as heat sinks, fans, cables, etc. should obscure the space needed for a full size card.

3.2. Installation of the TCC900 module

The TCC900 has been designed for automatic detection by the host computer. Direct memory access and interrupt functions will be set automatically. Therefore the only action is to insert the TCC900 into the computers free PCI slot and secure it appropriately.

Warning

When handling the TCC-900 avoid electrostatic shock. It is recommended to use anti-static equipment suitable for handling delicate electrical devices.

After the card has been physically fitted to the computer and the computer is switched on, WINDOWS will realise that a new hardware device has been installed. The WINDOWS program will try to load a device driver for this new hardware device. No separate device driver should be loaded. The driver loading routine should be processed with "Next" and "Finish". Do not apply "Cancel" during this process. This will ensure the computer will not "look" for a driver again next time it is switched on.

Should there be any malfunction, the reason may be an insufficient power supply in the computer or insufficient computer memory.

3.3. Connection of External Cables

In order to perform measurements at least both the START and the STOP inputs need to be connected to external devices like trigger outputs of lamps or lasers and detectors. The control / routing input can be left unconnected. If there is no external routing signal supplied to the router input, then all measurements will be routed to the first curve container of the memory.

Warning

Although the inputs are protected against high voltage, it is good practice to ensure that the signal cables are not charged up before connection is made to the TCC-900 module. If the detector output is not charged down by a bleeding resistor, then the high voltage of a photomultiplier tube must be switched off and the cable discharged before making a connection.

Signal pulses should be provided by 50Ω cables. The input connectors accept plugs of the SMA series. The pulses must have negative amplitudes. Electrical parameters must never exceed the maximum ratings (see paragraph 6, Technical Specification).

Note: The T900 software does not support signal routing.

If signal routing is required, than the routing signal must be supplied to the correct pins of the routing input. Depending on the binary signal supplied to the routing input, photon pulses will be routed to the curve container corresponding to the binary code of the signal. The plug for the routing input is of the JAE Electronics TX20A series, model TX20A-26PH1-D2P1-D1.

Routing signals can be supplied stationary or quasi-stationary, or – for synchronous TCSPC - they are correlated to each individual photon event. While synchronous TCSPC requires an external router, stationary and quasi-stationary signals can be supplied by simply supplying a TTL signal to the corresponding pin number.

The control / routing connector pin assignment is given in the list below:

Pin	Assignment	Input	Output	Description
1	STROBE	•	•	Latching signal, e.g. from router, TTL, active high
2	COINCID	•		Coincidence signal, e.g. from router, TTL, active high
3	EXTACQ	•		External acquisition enable signal, TTL, active low
4	EXTINCDEC	•		Ext. increm./decrem., TTLhigh-add data, TTLlow-subtract data
5	ROUTER 0	•	•	
6	ROUTER 1	•	•	
7	ROUTER 2	•	•	
8	ROUTER 3	•	•	
9	ROUTER 4	•	•	
10	ROUTER 5	•	•	12 bit, encoding 4096 data curves, TTL, active high
11	ROUTER 6	•	•	
12	ROUTER 7	•	•	
13	ROUTER 8	•	•	
14	ROUTER 9	•	•	
15	ROUTER 10	•	•	
16	ROUTER 11	•	•	
17	SPI - Clock		•	SPI clock signal
18	SPI - Data		•	SPI data, 32 bit word
19	SPI - Chip Select		•	SPI chip select line
20	GRD	•	•	
21	+ 5V		•	ext. voltage supply, max. load 200mA
22	+ 5V		•	
23	+ 12V		•	ext. voltage supply, max. load 100mA
24	- 12V		•	ext. voltage supply, max load 100mA
25	GRD	•	•	
26	GRD	•	•	
27	N/C			
28	N/C			

4. Software Installation

The TCC900 is operated by the T900 software. This T900 software package requires WINDOWS 95 or higher as computer operating system. The software T900 is protected by means of a software protection device to be fitted to the parallel port of the computer. The software protection device is programmable and has been loaded so that features of the T900 software package can be operated in accordance with the specific requirement.

The T900 software comes on 3 floppy disks. Insert disk 1 into the floppy drive of your computer and run setup.exe (by use of either the WINDOWS explorer or the RUN button).

The installation program will guide you through the installation.

At the end of the installation an icon will be arranged at the desktop of your computer. Double click on the icon will start up the program and will enable you to operate the TCC900 module.

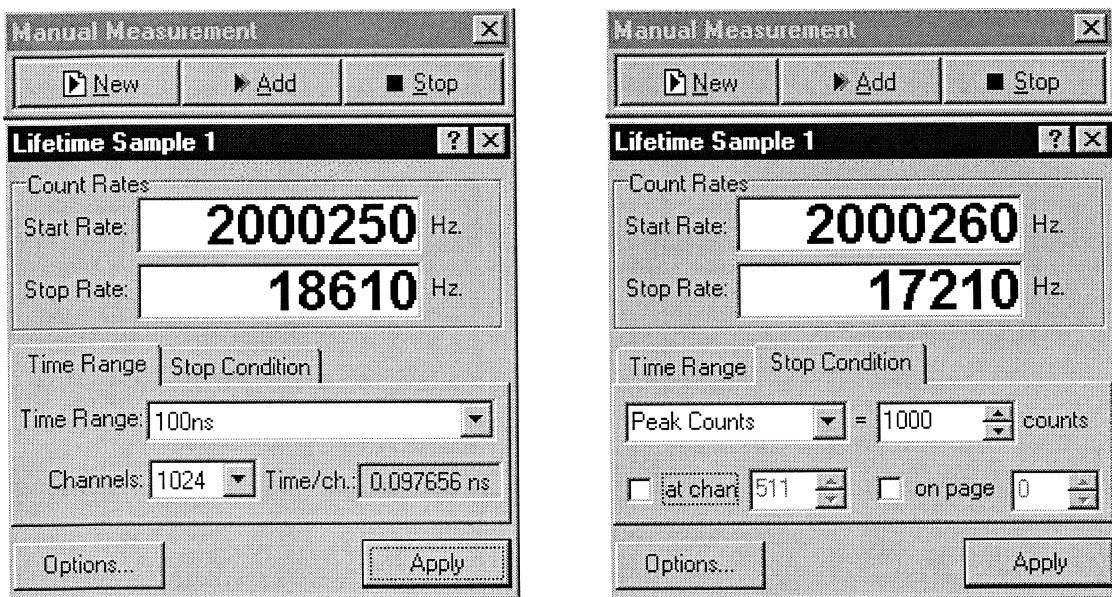
Once the hard- and software installations are completed, the TCC900 will be operated via the dedicated T900 software. Double click on the T900 icon at the desktop of the WINDOWS interface will start up the T900 program. The user will then see the T900 main frame, from which lifetime data acquisition, data fitting, data manipulation, data presentation, as well as hardcopy and file outputs can be made.

5. Acquiring Data

Fluorescence lifetime data acquisitions can be made using the data acquisition dialogue box. This box can be accessed either via **File / New Measurement / ...** or by clicking the τ button of tool bar on the top left side of the desktop. The acquisition dialogue box allows to set-up all functions for routine measurements.

On the top of this box both the Start and the Stop signal rates are demonstrated with a resolution of either Hz or kHz (to be set up in the Options menu – see later). Start and Stop rates depend on the threshold settings of the constant fraction discriminators. These settings can be modified under **Options**.

The acquisition dialogue box further contains two tabbed dialogue boxes, **Time Range** and **Stop Conditions**.



5.1. Time Range

The **Time Range** box allows to select the full time range for the measurement. Values between 2.5ns and 50 μ s with 3 steps per decade are pre-set as default values.

Channels is the total number of data bins (channels) of the measurement. Thus this value determines the resolution of the x-axis (time axis). Values of 512, 1024, 2048, and 4096 channels can be selected. If one of the two values, Time Range or Channels, has been changed, then the time calibration (**Time/ch.**) will be updated as soon as the **Apply** button is pressed.

5.2. Stop Conditions

Stop Conditions defines the conditions at which the measurement will be automatically stopped. The stop conditions selectable are:

- Stop at a pre-defined number of counts in either the channel with the maximum number of photon counts, or in a pre-selected channel;
- Stop after a pre-defined time has elapsed;
- Stop manually;
- Stop with an external interrupt signal.

For **Peak Count** stop conditions any value for the maximum number of counts can be selected. If the **at channel** box has not been ticked on, then the measurement will stop at the entered maximum counts of the channel with the maximum counts (the peak of the curve). If the "at channel box" is ticked and a value in the range of the total number of channels of the curve has been entered, than the measurement will stop as soon as the pre-defined number of counts at that channel has been reached.

For stop at elapsed **Time** conditions any time in seconds can be entered. **Manual** stops can be made at any time, even when Peak Counts, Time or External has been selected.

Measurements can be externally stopped (**External**) by a TTL signal supplied to the control port (refer to paragraph 3.2).

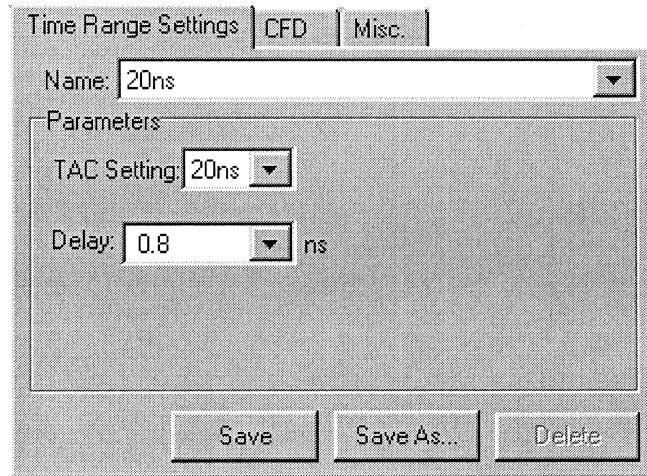
5.3. Options...

The "Options..." box in the data acquisition menu does not need to be accessed in routine measurements. Access to the options is only required for

- Setting up the CFD parameters
- Changing the shifting delay
- Change between Forward to Reverse mode of Operation
- Change the displayed resolution of the frequency / pulse count rate

5.3.1. Time Range Settings

The main function of this dialogue box is to enter a new value of the shifting delay. A shifting delay other than "zero" is required to shift the entire measurement (decay and/or IRF measurement) either left or right on the measurement window.



Generally for large time ranges (above 100ns) a shifting delay is required in order to shift the measurement to the right to cover the full lifetime measurement, including the rising edge. For short time ranges a small shifting delay may be required in order to compensate for the different cable lengths in the start and stop channels. By "placing" the measurement right full use of the x-axis resolution can be made.

A large shifting delay is further required when working in the "Reverse Mode" (see paragraph 5.1.3.3.). In this mode the exciting light pulse (usually a laser pulse) needs to be shifted in time to arrive at the TCC900 card later than latest expected decay photon, i.e. the shifting delay must be slightly longer than the selected time range. (see also paragraph 1.2.)

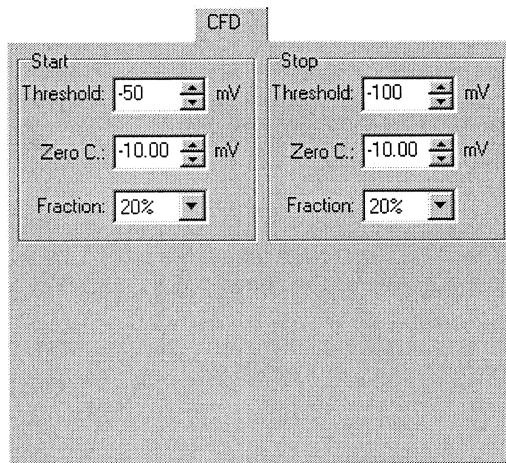
By clicking the arrow of the "Delay" box a list opens up to show the delay values available. The list is a combination of individual delay lines on the card. Select the required delay value by clicking on it.

Once the "Time Range Settings" box has been closed the selected delay value will always be active for the particular time range. Other time ranges maintain the old delay values.

5.3.2. CFD Parameter Options

The CFD set-up is important in detector / electronics optimisation works, in particular when the TCSPC module has been recently installed or when a new detector has been installed.

Both START and STOP input of the TCC900 card have a CFD to time optimise and time shape the incoming pulses. Both CFDs can be set up independently. The majority of the CFD parameters can be optimised by software, i.e. Threshold, Zero Crossing Level, and Fraction. Only one CFD parameter, the CFD Delay, must be exchanged by hand.



The editable ranges are:

- Threshold: -2000 mV ... 0 mV
- Z/C: -100 mV ... 100mV
- Fraction: 20%, 30%, 35%

As a general guide line the initial set-up should be made as follows:

Threshold: select a value about 10% of the average pulse height of the input pulses. (Trigger pulses from a trigger diode will not have a large pulse height fluctuation, pulses from a photomultiplier will fluctuate largely.)
 Z/C: Start with a small negative value, e.g. -5mV
 Fraction: Start with 20%

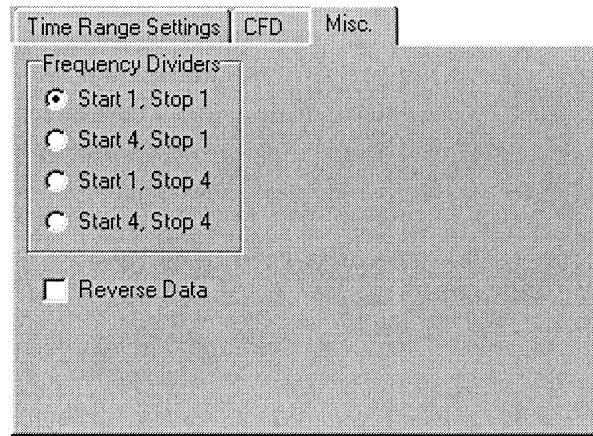
All the above parameters need to be optimised in order to achieve good measurement results, e.g. in respect to the pulse width of the instrument response, to the ratio of the number of dark counts to the number of signal counts, to the elimination of RF and interference problems, etc.

The CFD delay also needs to be optimised for the best measurement result. As a general rule the CFD delay should be about 70% of the pulse rise time.

Different delay plugs are supplied with the TCC900 card. The standard CFD delay plugs fitted to both the START and the STOP channel are 0.8ns. If this value is not appropriate for the particular detector, than it can easily be exchanged. For this the card needs to be removed from the computer and the lid of the small shielding can of the relevant CFD (see graph in paragraph 1.3) to be removed. (A small screw driver might be of help when removing this lid.) The brown delay plug can be easily located when the lid is removed. To exchange simply unplug the original plug and insert the new plug instead. Refit the shielding can afterwards and re-install the TCC900 card in the computer.

5.3.3. Miscellaneous

This dialogue box allows to change between Forward and Reverse mode of operation and it enables the customer to change the resolution of the pulse frequency / count rate displayed in the main measurement dialogue box.



The **Frequency Divider** settings can be chosen as required. For count rates up to about 10Mcounts/s no division needs to be applied. For light sources and detectors exceeding approximately 10Mcounts/s division by 4 should be applied.

Reverse Data should be ticked when TCSPC is performed in Reverse Mode (i.e. the light source reference signal is connected to the STOP input, the detector signals are connected to the START input). This will ensure that the data demonstrated on the screen will decay towards the positive end of the time scale.

6. Analysis of Lifetime Data

6.1. Introduction

6.1.1. 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:

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

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.

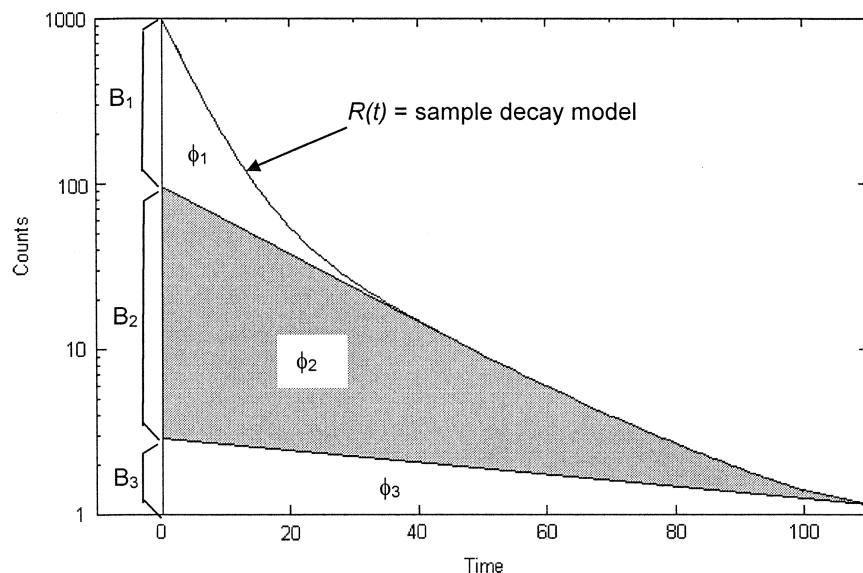
The characteristic lifetimes τ_i are the most important lifetime parameters. They are specific for different decay (or growth) processes and express the time it takes to decay from the beginning of the decay to a level of about 37% of the original value.

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 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.

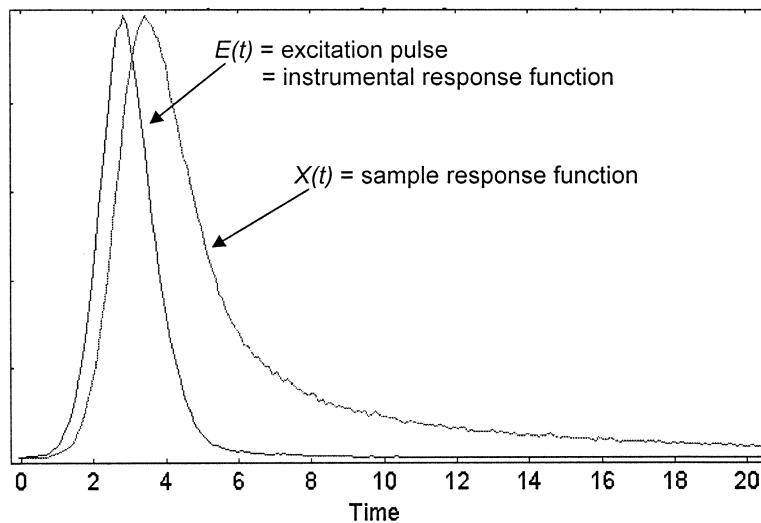
$c_2 = \frac{B_2}{B_1 + B_2 + B_3}$	relative concentration of the second component
$\phi_2 = \frac{B_2 \tau_2}{B_1 \tau_1 + B_2 \tau_2 + B_3 \tau_3} \cdot 100\%$	relative fluorescence intensity of the second component, as a percentage
$\langle \tau \rangle = \frac{B_1 \tau_1^2 + B_2 \tau_2^2 + B_3 \tau_3^2}{B_1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

6.1.2. 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 T900 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.

6.1.3. Numerical Fit

The T900 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.)

6.1.4. 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 11.1.3. 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 its 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 11.3), 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

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

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 T900 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.

6.1.5. Data Types and Weighting Factors

The two lifetime data analysis routines available from the T900, "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:

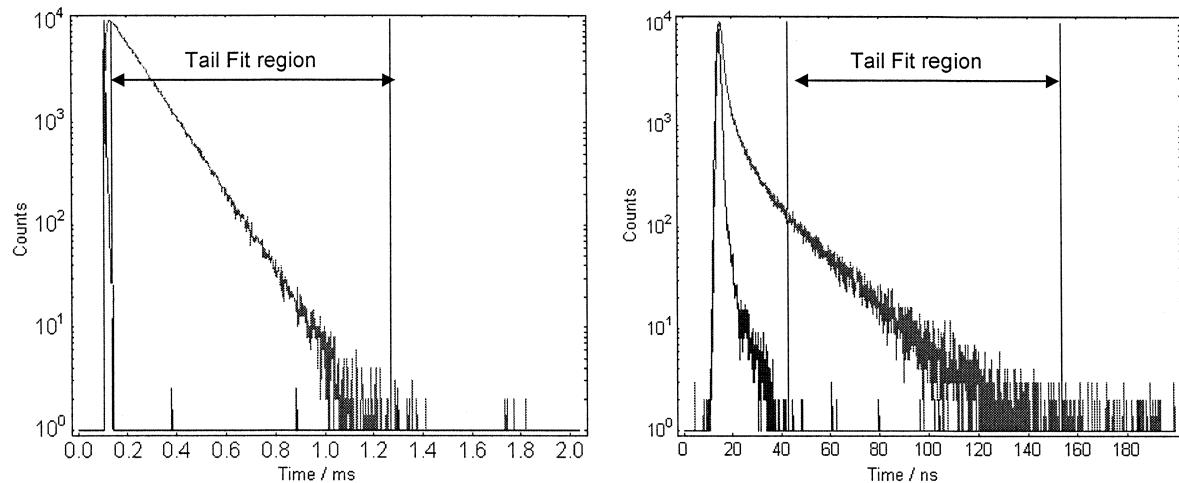
Data Type	Data Acquisition Technique	Weighting Factors (w_k)
Lifetime Data	TCSPC / MCS	$w_k = 1 / \sqrt{F_k}$
Kinetic Data	SPC	$w_k = 1 / \sqrt{F_k}$
Transient Data	Oscilloscope	$w_k = \text{const.}$
Time Resolved Anisotropy Data	TCSPC / MCS	error propagation from original data

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

- Fit Data,
- Residual Data
- Autocorrelation Data.

6.2. 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.

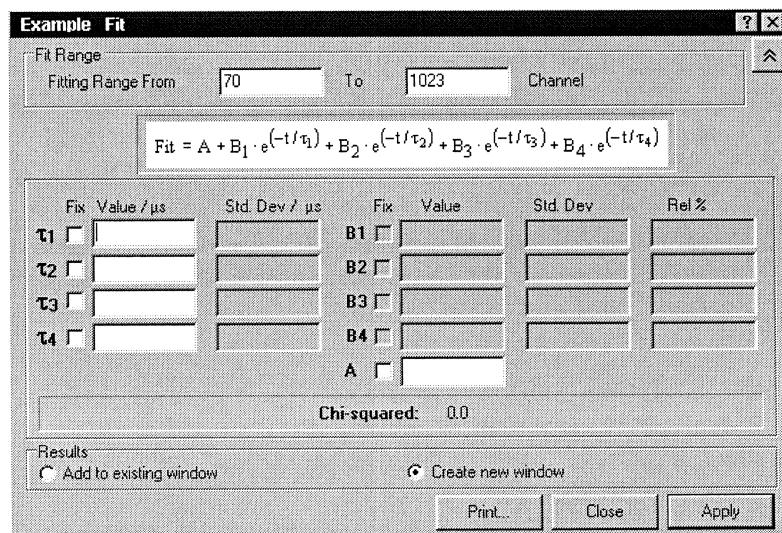
Tail Fits (as well as Reconvolution Fits) can only be performed at one decay data file at a time. Therefore the active data window should only contain one decay measurement. (Instrumental response functions as well as fitted curves can be present in the active data window.) If more than one decay measurement 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 6.1.1. "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.

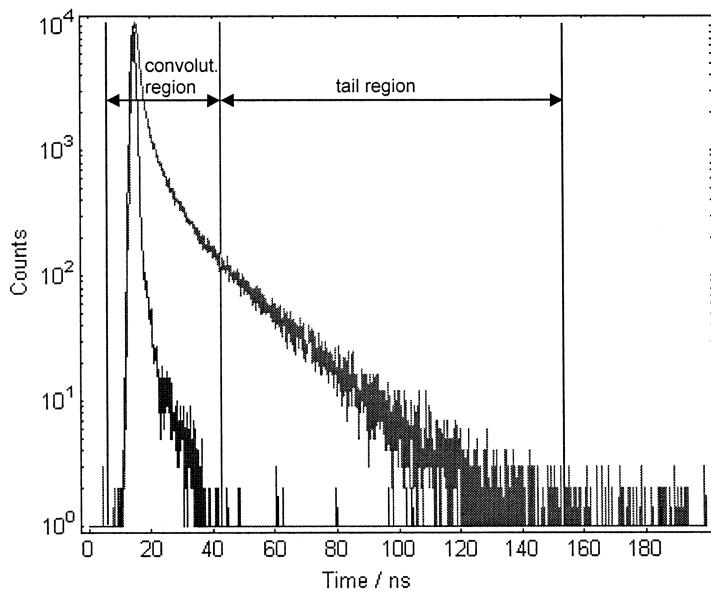
The third section contains a table which is used to enter and to view both initial fit parameters and fit results, respectively. Before attempting a fit (**Apply**) at least one initial lifetime (usually τ_1) needs to be entered. Whether the lifetimes must be entered in unit of channels or in unit of 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, μ s, 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 11.4. "Fit Evaluation and Re-Fitting".

6.3. 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.

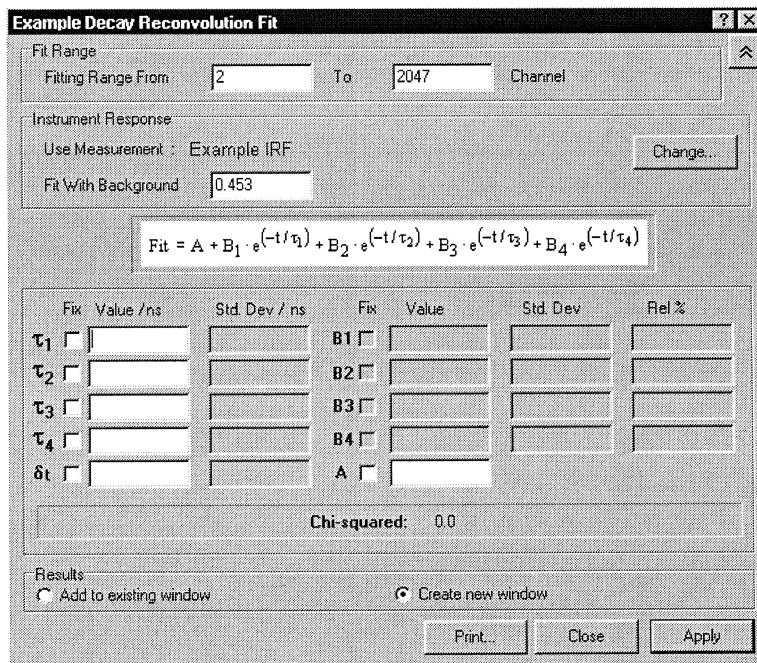


Reconvolution Fits can only be performed at one decay data file at a time. The active data window must contain at least the decay measurement and the instrumental response. If more than one decay measurement is present in the data window then a single decay measurement and the instrumental response function 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 5 "Acquiring Data") 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 6.1.1. "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.

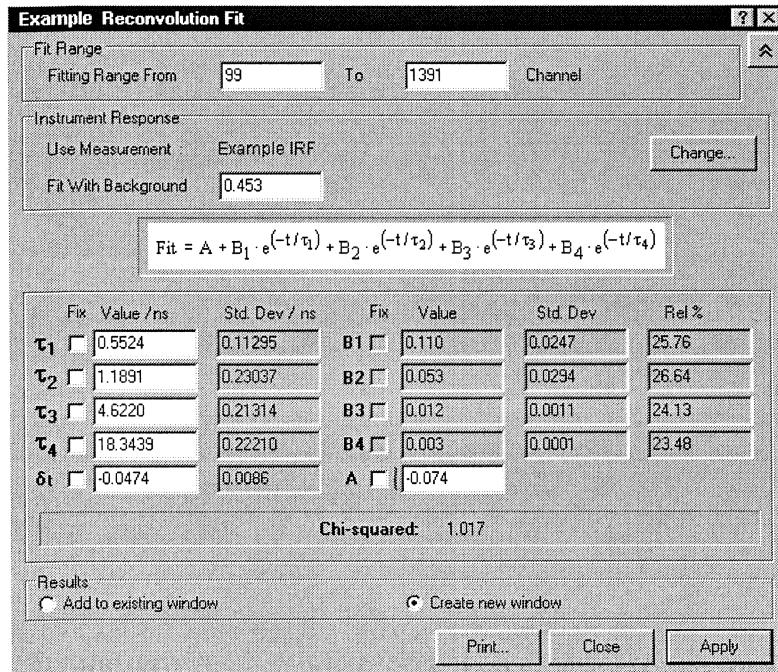
The fourth section of this dialogue box contains a table which is used to enter and to view both initial fit parameters and fit results, respectively. Before attempting a fit (**Apply**) at least one initial lifetime (usually τ_1) needs to be entered. Whether the lifetimes must be entered in unit of 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, μ s, 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.

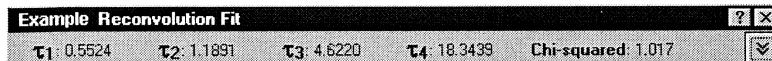
The fitting procedure is started using the **Apply** button. A successful fit will result in the new lifetime parameters, an update of the χ^2 value displayed beneath the table of lifetime parameters, in a fitted decay curve, and in a residual curve. The user can choose prior to the fit whether the resulting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

6.4. 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 "▲" button on the top right side or by just pressing the return button. Repeating these actions for the second time will result in re-maximisation.

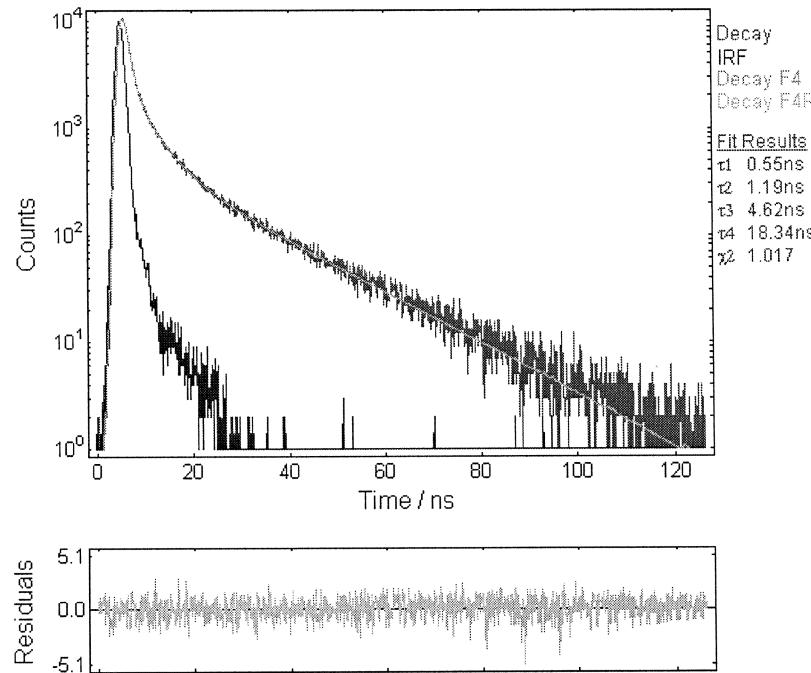


The data window will be updated automatically with the fitted sample response function ($X(t)$) and the residual curve ($Y(t)$). The residual curve will be shown in a separate bar underneath the original data.

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.

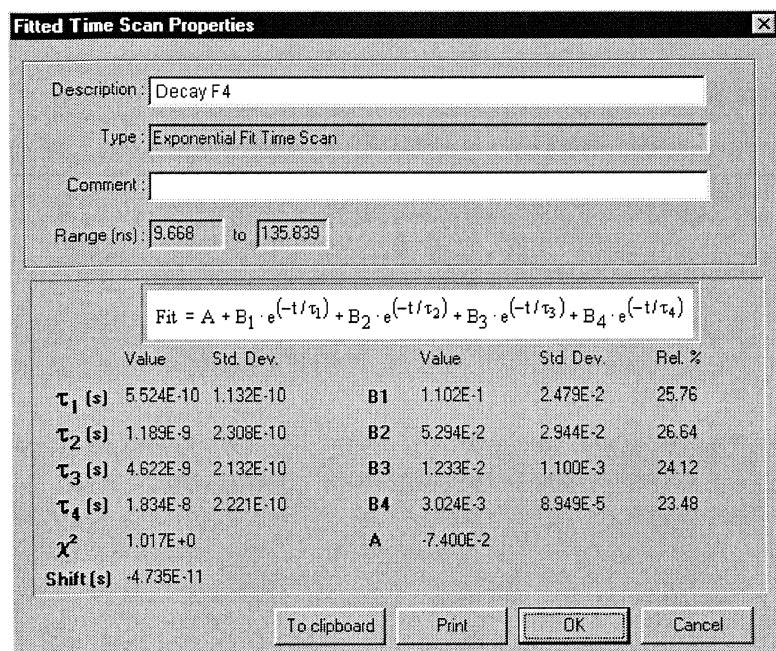
If the initial lifetime (or one of the initial lifetimes) chosen was too small, then there is a likelihood that during the fitting process the lifetime will "run" towards Zero. A τ -value of Zero is physically meaningless and can not be dealt with mathematically during the fit. An error message will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



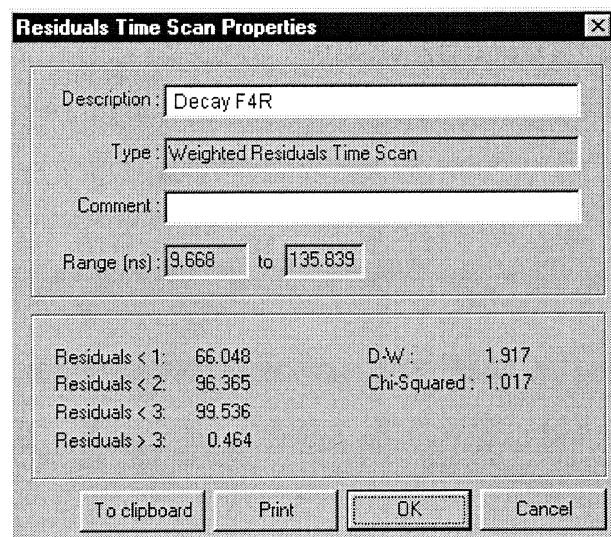
If the initial lifetime (or one of the initial lifetimes) chosen was too small, the fit procedure might not “run” into a singularity but will try to increase the τ -value. However, the fit routine limits final τ -values to 10 times the original (start) value. After 50 iteration steps a result will be 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.

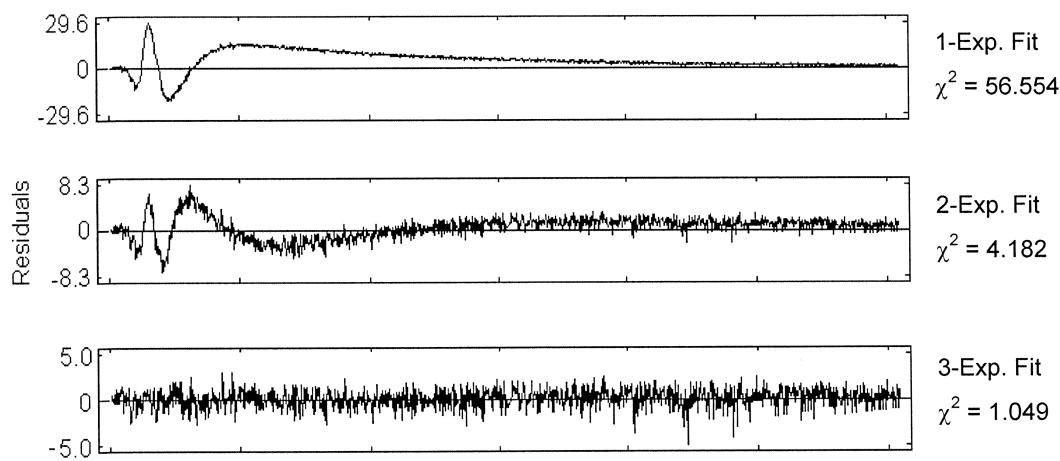
The table of lifetimes may be printed (**Print**). If the Fit dialogue box has been closed (**Close**) the table of lifetime parameters will no longer be available in the current form. However, it is available in a slightly different form via the data properties facility: **File / Properties ...**, or **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.



7. Technical Specification

Maximum Ratings

Current into the inputs	100 mA (DC) 500 mA (pulse, <1us)
Voltage at control inputs	-0.5V ... +5.5 V
Currents at the	
+5V output	200 mA
5V output	200 mA
+12V output	100 mA
12V output	100 mA
Supply Voltage at PCI Connector	
Vcc (+5V)	-0.5 ... +5.5 V
Vpp (+12V)	-0.5 ... +13 V
Signal Voltages at PCI connector	-0.5 ... +5.5 V
Ambient temperature	0°C ... +55°C

Signal Inputs

Principle	Constant Fraction Discrimination (CFD)
Inputs	Two identical inputs for START and STOP
Impedance:	50 Ω
Input Pulse Amplitude Range	-10 mV to -1 V
Electrical Time Jitter (FWHM)	5 ps
Time Walk	30 ps
Threshold Range	-10 mV to -1V
Zero Cross Adjust	-100 mV to + 100 mV
CFD Fraction	20% / 30% / 35% adjustable
Integrated delay range	256ns on both channels independently

Time-to-Amplitude Converter (TAC)

Principle	Ramp Generator
TAC Ramp Range	10 ns to 50 μs
TAC Offset	0 to 50% of TAC Range

Analogue to Digital Convector (ADC)

Principle	Flash ADC with error correction
Resolution	4096 channels (12 bits)
Biased Amplifier Gain	1 or 4

Dynamic Characteristics

Time Range	2.5 ns to 50 μs
Min. Time / Channel	610 fs
Differential Nonlinearity	< 2% RMS (from 5% to 95%) of full time range

Data Acquisition

Dead Time	112.5 ns
Max. number of Curves	4096, 512 data points per curve
	2048, 1024 data points per curve
	1024, 2048 data points per curve
	512, 4096 data points per curve
	up to 4096
Max. number of Detector Channels	4 10^9 counts (32 bits)
Max. number of Counts / Channel	at maximum, at cursor position, after time elapsed,
Stop Conditions:	
after external interrupt, manual	
Memory Control	per software control / per external routing signal
Signal Rate Monitor	for START and STOP input

Operation Environment

Computer System	Pentium PC, ATX 486
PC bus interface	PCI 32 bit, 33MHz
Power Consumption	20 W at +5V, 2.0 W at +12V
Dimensions	337 mm x 120 mm x 35 mm

7. Warranty

- 1 a) The Company guarantees the equipment forming the subject of the contract/quotation against defective materials and workmanship for a period of one year from the date of delivery to the Purchaser.
 - b) In the case of sub-assemblies of equipment not manufactured by the Company, but incorporated in the equipment ordered, the Purchaser will be entitled only to the benefit and/or limitations of any guarantee given by the makers of such assemblies.
 - c) In no event shall the Company be liable for any consequential loss or damage arising from failure of the equipment under warranty.
 - d) At the end of the one year period referred to herein, all claims upon all liability of the Company shall be absolutely at an end.
 - 2 a) The Company also warrants that the equipment conforms to specifications contained in current brochures or to extra specifications confirmed in writing at the time of order acknowledgement.
 - b) No warranty is made or implied as to the suitability of any equipment for the Purchaser's intended use beyond such performance specifications as form part of the contract.
3. The purchaser warrants:
- a) That he will carefully examine and list all parts of the equipment supplied by the Company and notify the Company in writing of any shortage, defect or failure to comply with the contract, which is or ought to be apparent upon such examination and test, within 48 hours of the equipment being delivered to or collected by the Purchaser.
 - b) The equipment will be operated in accordance with the instructions and advice detailed in the appropriate operating instructions manual, or any other instructions which may be provided by the Company. The Company shall not be held responsible for any defect arising from the Purchaser's failure to comply with these recommendations and instructions or from damage arising from negligence or exposure to adverse environmental conditions.
4. The warranty is effective when:
- a) Any defects in the equipment supplied are notified immediately by the Purchaser to the Company.
 - b) The equipment is returned to the Company at its Edinburgh premises, transportation and insurance prepaid, and undamaged by the failure to provide sufficient packaging.
 - c) The Purchaser has made payment in full for the contract in accordance with the Company's normal trading terms, ie. 30 days from date of invoice.
5. The warranty covers:
- a) Engineer's time costs during inspection and repair.
 - b) Any materials or components, which require to be replaced.
 - c) Return carriage costs to the Purchaser
6. However, if the Purchaser requests a service engineer to carry out the necessary inspection and repair of the equipment covered by the warranty on site, the Purchaser will be liable, at the Company's discretion, for:
- a) Engineer's travelling time costs.
 - b) Engineer's travelling and accommodation expenses.

The timing of the inspection and repair of the equipment will be determined entirely at the discretion of the Company.

8. CE Declaration of Conformity

CE

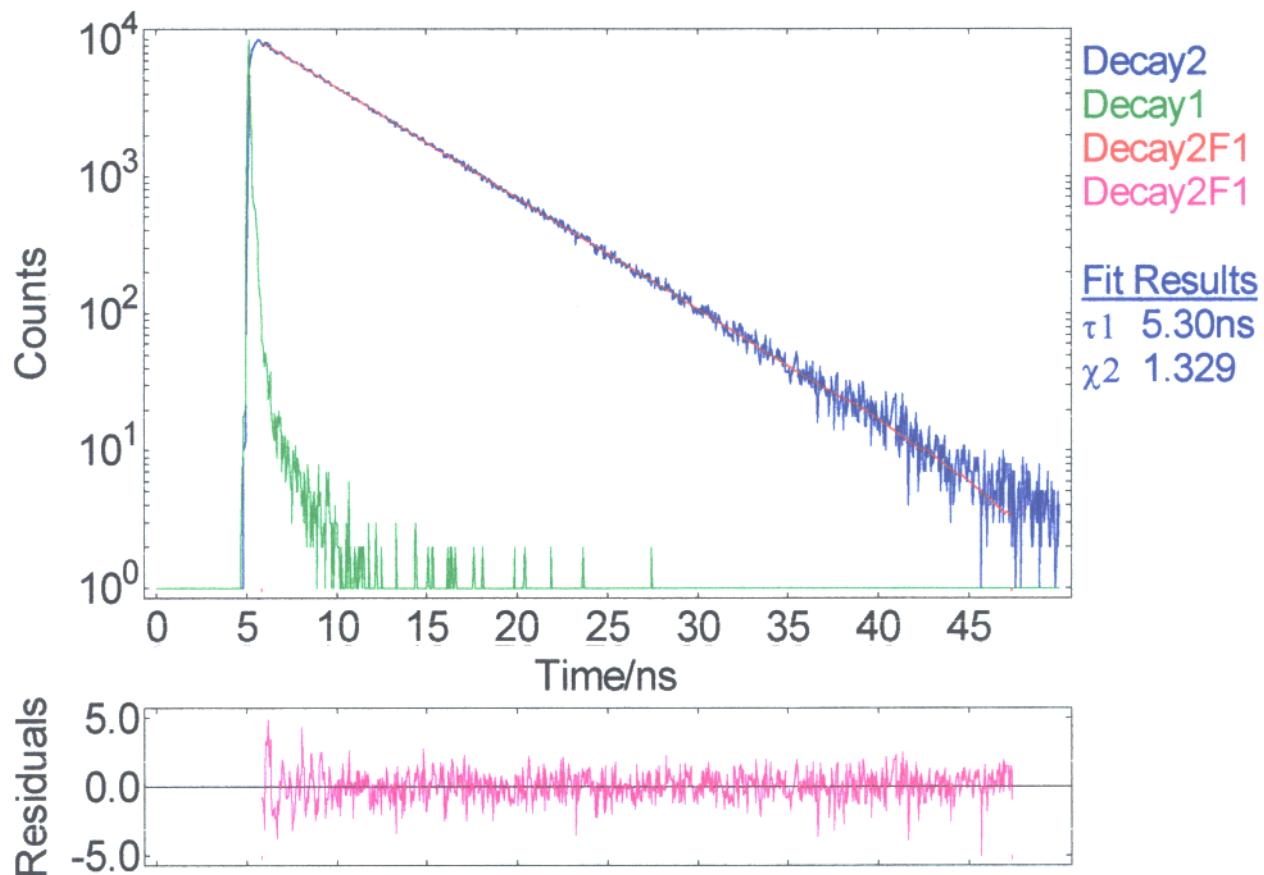
Manufactured by:

Edinburgh Instruments Ltd.
2 Bain Square
Livingston
EH54 7DQ
Tel.: + 44 1506 425 300
Fax.: + 44 1506 425 320

Applicable Standards:

Generic Immunity	EN 50082-1 : 1992
Generic Emission	EN 50081-1 : 1992
Electrical Safety Standards	EN 61010-1 : 1993

Edinburgh Instruments Ltd. certify that this equipment conforms with the protection requirements of the above Directives.

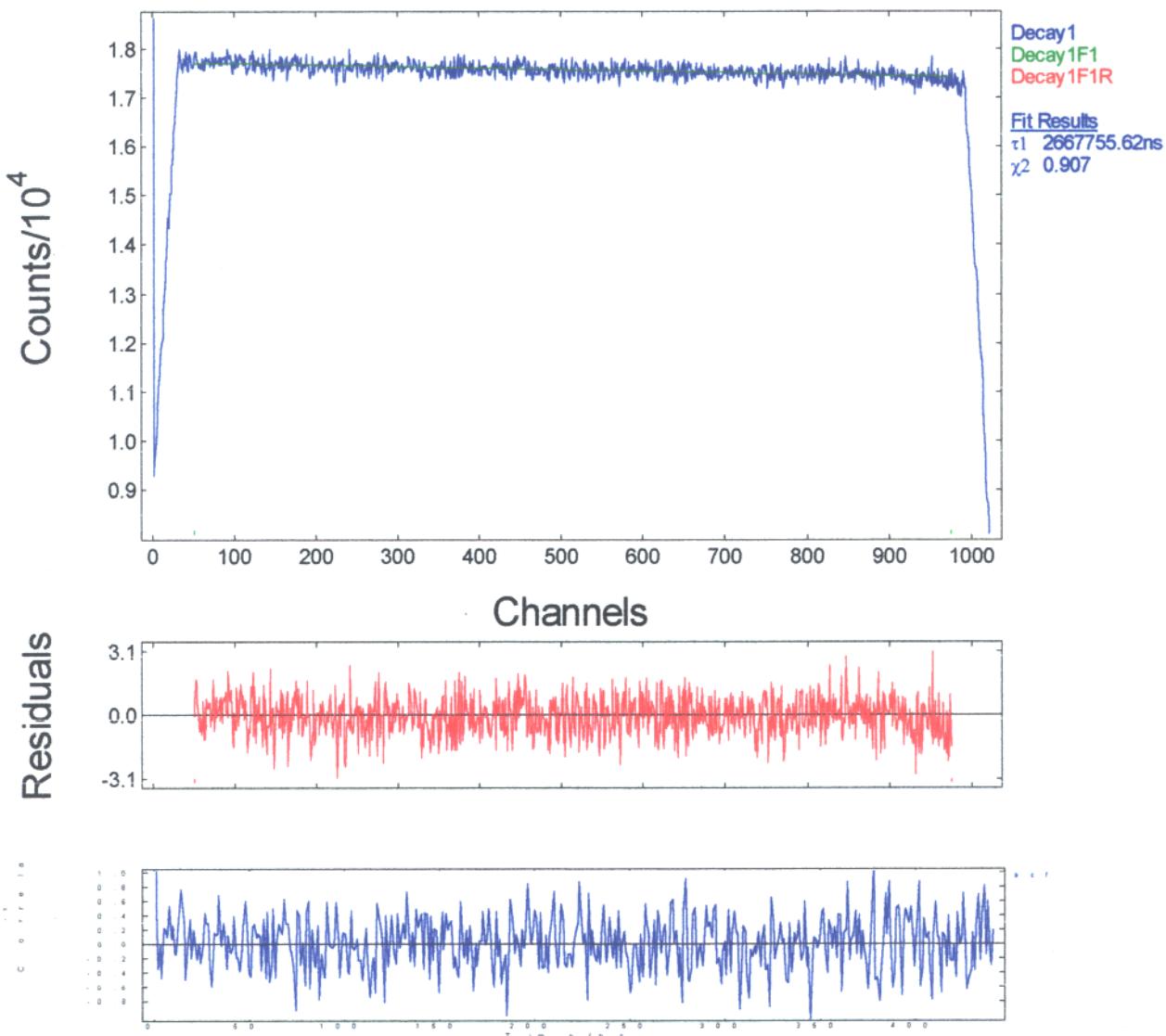


	START	STOP
Source:	Diode Laser	MCP-PMT + Amp.
CFD Delay:	1.0ns	0.2ns
Rates:	1MHz	20000cps
Threshold:	-100mV	-10mV
Z/C:	-50mV	-10mV
Fraction:	20%	20%

Mode: Forward
 Shifting Delay: 0
 No. of Channels: 1024
 Fit range: 120-970, Reconvolution

Sample: Coumarin 307 in methanol
 Excitation: 400nm
 Emission: 500nm

IRF: 125ps



	START	STOP
Source:	Diode Laser	MCP-PMT + Amp.
CFD Delay:	1.0ns	0.2ns
Rates:	1MHz	10000cps
Threshold:	-100mV	-10mV
Z/C:	-50mV	-10mV
Fraction:	20%	20%

Mode: Forward
Shifting Delay: 0
No. of Channels: 1024
Fit range: 50-970, Tail Fit

Sample: Random Light
Note: Slope due to pulse pile up
 Second bar = autocorrelation