

PULSE5QT

users' manual

**Fluorescence Anisotropy Decay Analysis
by
Quantified Maximum Entropy Method**

Jean-Claude Brochon

Copyright Spring 1998

Table of Contents

Read me first	2
Theoretical background	3
Getting started	6
Entry file	6
How to run the program	10
Output files	15
Figures and Demo results	16
Example of analysis report	22

1. Read me first

The following are quick notes to enable you to set up and run the PULSE5QT program as supplied to you. It is essential that this document is read first.

The files are supplied on a PC diskette in DOS standard format (see listing on page 3). The files on the disk contain the following :

Executable codes

FLAME5Q.exe : Auto-decompressed executable code of PULSE5QT.EXE program

Demo files :

test-2qt.go : Input File used to run the fluorescence anisotropy decay analysis example as a demo. It contains all the necessary responses to the questions asked by the program PULSE5QT which requires the companion test-2st.go file (see below).

test-pol.prm : Parameter file with all options for the instrument configuration and the experimental method.

memsys.prm : Parameter file contains all MemSys control variables.

test-2st.go : Input File used to run the total fluorescence decay analysis example as a demo. It contains all the necessary responses to the questions asked by the program PULSE5 and the required data filenames (see PULSE5 users' manual).

test-2.vv, test2.vh : Files containing data used in our demo example . Each file contains also the excitation profile. There is no blank data file in use.

test-2qt.log: Output result file of the current demo data analysis

test-2qt.dis: Output file containing θ and $\rho(\theta)$ distribution for further use and plot.

test-2qt.crv: Output file containing data , fitting values, residuals and autocorrelations.

test-2qt.tab: Output file containing a table of the parameters of the $\rho(\theta)$ distribution: peak position, peak surface, peak width with their associated error bars.

Warning: Running the module PULSE5QT is the second step of the analysis of the polarised fluorescence decays $I_{vv}(t)$ and $I_{vh}(t)$. The total fluorescence decay $T(t)$,see below, should be

analysed first and then the anisotropy analysis can take place. The program needs all the parameters files in use for T(t) analysis: test-2qt.go, test-pol.prm and memsys.prm

2. Brief theoretical background

A detailed introduction to the Quantified Maximum Entropy Method for time-resolve fluorescence data analysis is given in Methods in Enzymology (1994) Vol. 240 chapter 13.

With an exciting flash of vertically polarised light having a finite experimental width $E(t)$ the measured parallel I_{VV} and perpendicular I_{Vh} components of the emitted fluorescence are:

$$I_{VV} = \frac{1}{3} E(t) \otimes \left\{ \int_0^\infty \int_0^\infty \int_{-0.2}^{0.4} \mathbf{g}(\mathbf{t}, \mathbf{q}, A) e^{-t/\tau} \left(1 + 2A \frac{-t/\theta}{e} \right) dt d\mathbf{q} dA \right\} \quad (1)$$

$$I_{Vh} = \frac{1}{3} E(t) \otimes \left\{ \int_0^\infty \int_0^\infty \int_{-0.2}^{0.4} \mathbf{g}(\mathbf{t}, \mathbf{q}, A) e^{-t/\tau} \left(1 - A \frac{-t/\theta}{e} \right) dt d\mathbf{q} dA \right\} \quad (2)$$

where $\mathbf{g}(\mathbf{t}, \mathbf{q}, A)$ are the number of fluorophore with fluorescence decay τ , rotational correlation time θ and initial anisotropy A which is related to the angle between absorption and emission dipole moments. Symbol \otimes denotes the convolution with time.

Formally the problem is reduced to a determination of the lower 2-dimensional projected distribution of $\mathbf{g}(\mathbf{t}, \mathbf{q}, A)$ on the $\mathbf{b}(\mathbf{t}, \mathbf{q})$ plane :

$$\beta(\tau, \theta) = \int_{-0.2}^{0.4} \gamma(\tau, \theta, A) dA \quad (3)$$

In practice, the initial anisotropy A is determined separately by a steady-state fluorescence polarisation measurement.

Total fluorescence decay

The fluorescence decay is obtained by summing the two polarised components:

$$T(t) = I_{VV}(t) + 2g I_{Vh}(t) = E_1(t) \otimes \int_0^\infty h(\mathbf{t}) e^{-t/\tau} dt \quad (4)$$

where $h(\mathbf{t})$ is the distribution of fluorescence lifetimes given by:

$$h(\mathbf{t}) = \int_0^\infty \int_{-0.2}^{0.4} A \mathbf{g}(\mathbf{t}, \mathbf{q}, A) d\mathbf{q} dA \quad (5)$$

and g is a correction factor for the response of the optics and the detection of the vertically and horizontally polarised emission (if any). If we are only interested in the fluorescence decay, $T(t)$ is usually measured in one data curve by setting the emission side polariser at the "magic" angle of 54.75° .

Current time-resolved anisotropy analysis

In analysing the polarised time-resolved fluorescence data many experimenters use the sum $T(t)$ and the "quotient" $Q(t)$ defined as:

$$Q(t) = \frac{I_{vv}(t) - g I_{vh}(t)}{I_{vv}(t) + 2g I_{vh}(t)} \quad (6)$$

and taking into account definitions in formula 1 and 2

$$Q(t) = \frac{E_1(t) \otimes \left\{ \int_0^\infty \int_0^\infty \int_{-0.2}^{0.4} A \mathbf{g}(\mathbf{t}, \mathbf{q}, A) e^{-t/\tau} e^{-t/q} dt d\mathbf{q} dA \right\}}{E_1(t) \otimes \int_0^\infty h(t) e^{-t/\tau} dt} \quad (7)$$

First the τ values are extracted from $T(t)$ and then the θ values, assuming that all lifetimes have exactly the same rotational dynamics. In this model, the distribution $\mathbf{g}(\mathbf{t}, \mathbf{q}, A)$ can be reduced to the product of two distributions $\mathbf{b}(\mathbf{a}, \mathbf{q}) = h(\mathbf{t}) \mathbf{r}(\mathbf{q})$ and $Q(t)$ is rewritten as

$$Q(t) = \int_0^\infty \mathbf{r}(\mathbf{q}) e^{-t/q} d\mathbf{q} \quad (8)$$

$$\mathbf{r}(\mathbf{q}) \text{ is normalised and } \int_0^\infty \mathbf{r}(\mathbf{q}) d\mathbf{q} = A \quad (9)$$

and the polarised components can be rewritten as:

$$I_{vv} = \frac{1}{3} E_1(t) \otimes \left\{ \int_0^\infty h(t) e^{-t/\tau} dt \left(1 + 2 \int_0^\infty \mathbf{r}(\mathbf{q}) e^{-t/q} d\mathbf{q} \right) \right\} \quad (10)$$

$$I_{vh} = \frac{1}{3} E_1(t) \otimes \left\{ \int_0^\infty h(t) e^{-t/\tau} dt \left(1 - \int_0^\infty \mathbf{r}(\mathbf{q}) e^{-t/q} d\mathbf{q} \right) \right\} \quad (11)$$

Maximum Entropy

Without any prior information we should assume that the probability for a given molecule having a given rotational correlation time θ is independent of its fluorescence lifetime τ . The general formulation of the entropy is then:

$$S = \int_0^\infty \int_0^\infty \int_{-0.2}^{0.4} \mathbf{g}(\mathbf{t}, \mathbf{q}, A) - m(\mathbf{t}, \mathbf{q}, A) - \mathbf{g}(\mathbf{t}, \mathbf{q}, A) \log \frac{\mathbf{g}(\mathbf{t}, \mathbf{q}, A)}{m(\mathbf{t}, \mathbf{q}, A)} dt d\mathbf{q} dA \quad (12)$$

where $m(\mathbf{t}, \mathbf{q}, A)$ is the prior probability for the distribution $\mathbf{g}(\mathbf{t}, \mathbf{q}, A)$.

For the total fluorescence decay, the entropy is only dependent of $h(\tau)$ and

$$S = \int_0^{\infty} h(\mathbf{t}) - m(\mathbf{t}) - h(\mathbf{t}) \log \frac{h(\mathbf{t})}{m(\mathbf{t})} d\mathbf{t} \quad (13)$$

and for the fully correlated anisotropy analysis, an entropy function is defined as independent of τ and A :

$$S = \int_0^{\infty} \mathbf{r}(\mathbf{q}) - m(\mathbf{q}) - \mathbf{r}(\mathbf{q}) \log \frac{\mathbf{r}(\mathbf{q})}{m(\mathbf{q})} d\mathbf{q} \quad (14)$$

Maximum Entropy is an optimal criterion for reconstructing a positive distribution $\rho(\theta)$ from imperfect data. In this application, the maximum entropy method is used to reconstruct the two polarised decays I_{vv} and I_{vh} from noisy pulse-fluorescence data.

In this expression, m is a measure, usually taken to be flat in logarithmic space (see references for the reason why), which quantifies the relative importance of the various channels. S measures the deviation of the distribution $\rho(\theta)$ from this measure, attaining its global maximum of zero when $\rho(\theta)$ is equal to $m(\theta)$. Because S is maximised by distributions which are as close as possible to the uniform and featureless (in log. space) measure m , maximum entropy uniquely gives the most probable reconstruction: there must be evidence in the data for any structure seen in a maximum entropy reconstruction. Suitably normalised, S is also minus the information content of $\rho(\theta)$, so that maximum entropy affords a uniquely comprehensible reconstruction, having only that minimum of information which is required to fit the data.

3. Getting started

You can now run the program interactively and reply to the questions asked by the program. The explanations and responses to the questions are given in the chapter 4. The output should be similar to the example outputs enclosed (from a PC running under DOS). However different machine may produces slightly different results. These differences may accumulate as the iterates proceed but will not strongly affect the final result as the program will iterate to the unique maximum of entropy solution. It may take a few more or less iterates to converge however.

Finally you can edit the parameter file containing the answers to all the questions and you re-run the program in batch mode. Characters after the `./.` label in the lines of data are ignored by the operating system (the program does not know they are there). They are thus an useful way of reminding the user of the content of the appropriate question. The label `/x/` contains the line number `x` of the current question and it refers to the corresponding explanation in the users' notes.

4. Entry files

The program asks a number of questions, so that it can set up the options you require. These can be typed in response to the questions if running in interactive mode or they can be edited in as parameter files if running in batch mode. As we expect that users will mainly run in batch mode once they have become familiar with the system, we have set up the questions with this in mind. Thus the program always asks the same number of questions in the same order even if as a result of a previous question the result is meaningless.

Finally you edit only one parameter file containing all answers to question and you re-run the program in batch mode. An example is given in `test-2qt.go`, *in page 9*. This file contains respectively the parameters for anisotropy analysis from both polarised decays, through the instrument set-up, taking into account the lifetime distribution already recovered from PULSE5 program. Characters after the `...n/` label in the lines of data are ignored by the operating system (the program does not know they are there). They are thus an useful way of reminding the user of the content of the appropriate question. The label `...n/` contains the line number `n` of the current question and it refers to the corresponding explanations in "*How to run the program PULSE5QT*" in page 10.

Total Fluorescence Decay Analysis

First the fluorescence decay was analysed in using the program PULSE5. The following input files has to be created (see PULSE5 users' manual).

Companion parameter files

File:test-2st.go

test-pol.prm / 1/ Filename for instrument set up and methods
test-2ST.log / 2/ Filename of the analysis listing
test-2.vv / 3/ Filename of the I-Magic or I_Vertical decay curve
test-2.vh / 4/ Filename of the I_Horizontal decay curve
excit.fl / 5/ Filename of the FIRST Flash or Reference decay curve
bid.dat / 6/ Filename of the SECOND flash or Reference decay curve
bid.dat / 7/ Filename of the BLANK_Magic or BLANK_Vertical decay curve
bid.dat / 8/ Filename of the BLANK_Horizontal decay curve
18 Feb. 1998 DEMO test: PULSE5/PULSE5QT.EXE Mock data convolved with excit.fl
Two exponentials; taus: 0.3 2.8ns / Alpha's: 2.80E04 and 1.2E04
(TWO Correlation times: 0.5ns and 10ns) R0=0.25
0.05 10. 100 /12a/ To_Minimum, To_Maximum, Number of lifetimes
0. 0. 0 /../ To_Minimum, To_Maximum, Number of lifetimes
0 /13/ Addition of Scattered light : 0= No , 1= Yes
0 /14/ Addition of a Background channel : 0= No , 1= Yes
0. 0. 0. 0. 0. /15/ Extra 5 single exponentials (outside of the To domains)
1, 1945, 1961, 2020 /16/ Four channel # for Fluo. decay and Backgrd domains
1937 /17/ Fluo. decay chl number from where the CHI-SQUARE starts
0.0 1 /18/ Excitation shift in channel unit, Flag for convolution type
1 /19/ First analysis (with a flat distribution):1=Yes, 0:Rerun
0. /20/ Level of flat map: neg. or =0 ==> Calculated / >0.= Given
0 /21/ Flag for the PRIOR MODEL of the distribution
1 1 /22/ Graphics, Intermediate results after N: <N= No , >N= Yes
-0.5 -0.15 -0.02 /23/ 1st_Rate 2d_Rate Third Rate
1 /24/ Automatic peak-finding? : 1 = Yes, 0 = No
0 0 /25/ Upper and lower peak marker numbers (manual peak analysis)

The second companion parameter file contains all options for the instrument configuration and for the experimental methods:

File: test-pol.prm

02-10-95 / exp-1/ date of the experiments: DD-MM-YY
0.004 / exp-2/ Nanosecond/channel value in your MultiChannel Analyser
1 1 A / exp-3/ Flag to reverse curves: 0=No 1=Yes; Compression factor
1 0.82 / exp-4/ Flag Excitation polariser; G_factor:I_ver./I_hor. response
0.0 / exp-5/ Coefficient for BLANK_Magic or BLANK_Vertical curve
0.0 / exp-6/ Coefficient for BLANK_Horizontal curve
0. / exp-7/ Lifetime of the reference (in ns)
0 / exp-8/ Flag for average of 2 excitations
4.0 / exp-9/ Excitation pulse frequency in Mhz
-2. 0. /exp-10/ Background and its error (I_Magic or I_Vertical decay curve)
-2. 0. /exp-11/ Background and its error (I_Horizontal decay curve)
-2. 0. /exp-12/ Background and its error (FIRST Flash or Reference curve)
-2. 0. /exp-13/ Background and its error (SECOND Flash or Ref._Horizontal)
-2. 0. / exp-14/ Background and its error (BLANK_Magic or BLANK_Vert. curve)
-2. 0. /exp-15/ Background and its error (BLANK_Horizontal decay curve)
0 /exp-16/ Flag for entry of data 1./variance: 0=calculated, 1=given

The third companion parameter file contains all MemSys control variables and are rarely changed:

File: memsys.prm

500 /memsys-1/ Maximum number of Iterates
30 /memsys-2/ Number of iterates between plots on screen
1 1 /memsys-3/ Method options
10 /memsys-4/ LEVEL of diagnostic output
1.0 /memsys-5/ Aim
0.10 /memsys-6/ Tolerance
0 /memsys-7/ Number of samples for movies
4321 /memsys-8/ Iseed
1 /memsys-9/ Nrand
0 /memsys-10/ VESA-defined mode supported
1 /memsys-11/ Flag for graphics on screen, 0=No 1=Yes

Fluorescence Anisotropy Decay Analysis

The following file is an example of responses requested by the program for fluorescence anisotropy data analysis and supplied to the user for demonstration. The program knows through the test-2qt.go file all needed values and parameters.

In other words the files test-2qt.go , test-pol.prm and test-2qt.go are fully linked in order to avoid the anisotropy decay analysis running with a wrong lifetime distribution and bad parameters.

File:test-2qt.go

test-2qt.log	/1/ Filename of the analysis listing
test-2st.go	/2/ Filename of the parameters for total fluo decay analysis
0.1 100. 100	/3a/ To_Minimum, To_Maximum, Number of lifetimes
0. 0. 0	/3/ To_Minimum, To_Maximum, Number of lifetimes
0.	/ 4/ Addition of a Infinite Theta channel : 0=NO , 1=YES
0. 0. 0. 0. 0.	/ 5/ Extra 5 single exponentials (outside of the To domains
1 1920	/ 6/ First and last channels of the decay fitting.
1	/ 7/ First analysis (withe a flat map distribution)
0.	/ 8/ Level of flat map
0	/ 9/ Flag for the PRIORMODEL of the distribution
1 1	/10/ Graphics flags : distr. , chi2
-0.40 -0.10 -0.03	/11/ Rate1 , rate2, rate3
1	/12/ Automatic search of peaks
0 0	/13/ Upper and lower peak marker numbers

6. How to run the program PULSE5QT

Load your program as usual.

Notational Convention: *Italics* indicate questions asked by the program

The first question asks the name of the first parameter file containing the answers to the questions for the selected decay analysis:

Please enter the FILENAME of analysis PARAMETERS to run the program in "batch" mode

OR enter TWO blank characters for running the program interactively

====> If you wish to run the program automatically, please enter the filename, up to 12 characters in the format *.xxx (no more than three characters after the point).

For demo supplied: **test-2qt.go**

====> If you wish to run the program interactively, please enter two blank or dash characters.

Then the program asks the following questions. These are printed below with an explanation of the response and suggested values from the test-2qt.go file supplied with the package:

Q: / 1/ *Enter the Filename of the analysis listing*

R: The length of the filename is maximum of 12 characters. The first eight characters will be automatically imposed as the root of the filenames *.dis and *.crv , *.tab for final outputs (see below).

For demo supplied: **test-2qt.log**

Q: / 2/ *Enter the Filename used previously to start total fluorescence decay analysis*

R: The length of the filename is maximum of 12 characters. (see below).

Then all data filenames and lifetime distribution file will be automatically assigned as well as flag for scattered light, excitation shift (if any), and all experimental conditions

For demo supplied: **test-2qt.go**

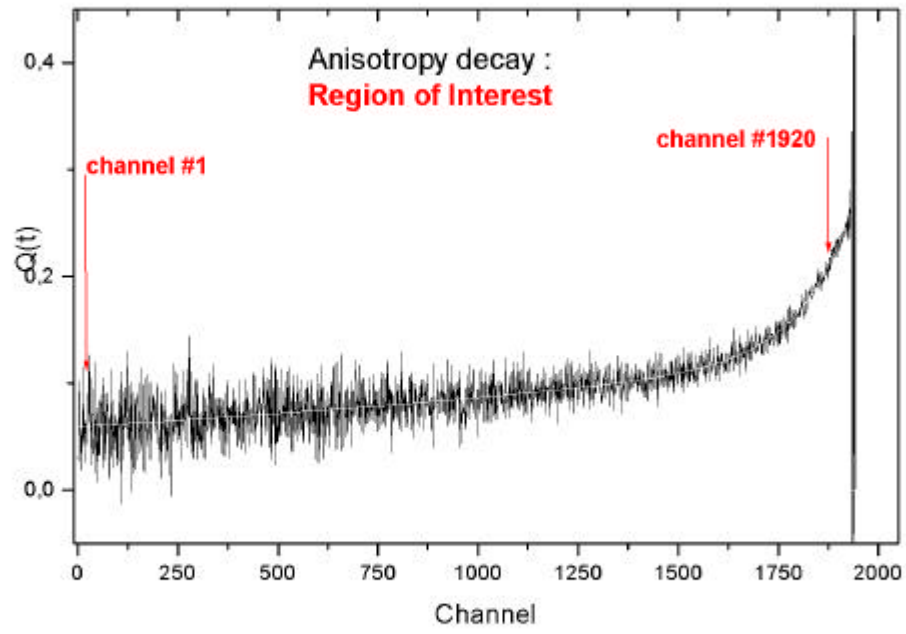
Q: / 3./ *Enter Theta_Minimum, Theta_Maximum, Number of correlation times*

-if the number of correlation time is set positive then LOG scaling

-if it is set negative then scaling is LINEAR

R: "Theta" means θ . These are the lower and upper limits of correlation time domain (in nanosecond) in use to delimit the range of rotational correlation times to be considered by the

program. The correlation time values will be spaced logarithmically or linearly between these limits. An entry of a negative value is a flag to select linear scaling (the absolute value is used in the program).



Once the number of correlation times is sufficiently large to capture all the structure in the data, then increasing it will not affect the shape of the result (and will merely increase the computing time approximately linearly). Thus it is recommended to have the number of points large enough for the digital resolution required.

Values for demo supplied: **0.1 100.0 100**

Q: /3./ *Enter Theta_Minimum, Theta_Maximum, Number of rotational correlation times*
-if the number of correlation time is set positive then LOG scaling
-if it is set negative then scaling is LINEAR

R: Five correlation time domains can be defined as far as the total number of correlation times does not exceed the limit of 200 set in the program.

Values for demo supplied: **0.0 0.0 0**

Q: /4/ *Do you add an infinite correlation time?*
Enter: 0 = No
1 = Yes

R: In case of large rotational correlation time it could be better to set a infinite θ . The user should take into account the ratio of the average lifetime and the largest correlation accessible to its measurement. This ratio depends on the data counting of both decays I_{vv} and I_{vh} . It is rather reasonable to consider a maximum ratio θ/τ of 30-40. For above θ values the distribution will become broad or even flat telling us that all those large values of θ have the same capability to fit the data as well as θ_{∞} .

Values for demo supplied: **0**

Q: /5/ *Addition of up to five SINGLE EXPONENTIAL,*
(outside of the already set correlation time domains).
Please enter FIVE values which are, either:
positive for the selected correlation time values (ns)
or null if not in use

R : Where the sample contains compound having single correlation times and if they are already precisely known, then the program needs these values as a prior information in order to supply the best reconstruction of correlation time distribution allowed by the data. For example that is recommended in studying a mixture of monomer-dimer-aggregate protein in solution if the monomer rotational correlation time is already known.

Values for demo supplied: **0.0 0.0 0.0 0.0 0.0**

Q: /6/ *Give the channel numbers which delimit the ANISOTROPY decay time domain to fit*

R: These are the markers (channel number) which delimit the useful anisotropy decay.

The background markers and convolution markers have been already set in **test-2qt.go** file during the fluorescence decay analysis.

Values for demo supplied: **1** **1920**

Q: / 7/ *Is the analysis starting from the beginning or continuing from a previous run ?*

Enter 1 for starting from the beginning

Enter 0 for continuing with a distribution saved from a previous run

R: If a run has stopped after the imposed number of iterations (see memsys.prm file) without termination to the most probable solution (which is reached for an OMEGA value close to 1.0), the calculation can be continued from the previous result for additional iterates, preventing the need to repeat initial calculations. That often happens if the rates (see question 23 below) are too small and slow down the progress on the entropy trajectory. The user must reply 0. Then the files *.are and *.sav allocated to the previous partial result are automatically read in.

Value for demo supplied: **0**

Q: / 8/ *Setting up the default value of the initial flat distribution.*

Enter a value,

positive : the default level is imposed at this value

null : the level is calculated from the decays Ivv and Ivh and flash integrals

*negative X: the calculated default level is then multiplied by 10^{**X}*

If continuing an analysis, please enter any value.

R: A standard default level for the initial pre-exponential distribution can be automatically calculated from the polarized fluorescence decays.

If you assume that the peaks should be more sharply defined you may enter a negative value (X) then the calculated default level is multiplied by 10^{**X} . This option can be used in order to force a better resolution of close peaks or to enhance sharpness of a flat distribution. The validity of this option should be check in looking at the auto-correlation graph which should be centred on zero axes.

Value for demo supplied: **0.0**

Q: / 9/ *If starting from the beginning,*

do you want to use a PRIOR MODEL of distribution ?

Enter: 0= No , 1= Yes

- If yes, the filename of the model distribution

should be model.dis (see PULSE5QT users" manual)

- If no , the model is assumed to be flat and set

at the same level as the starting flat distribution

R: The MaxEnt formalism allows the user to test whether there is evidence in the data for a deviation from some expected (model) distribution of correlation times. (This model might be, for example, either expected from theory or measured at some different physical conditions).

Usually there is no model and the expectation is set to be flat over the expected range of solution at the default level and reply 0.

Otherwise the user should respond 1 and the file model.dis containing the expected distribution of correlation time is automatically read in from the file model.dis The scaling in θ should identical to those given in question 3, see above).

Value for demo supplied: **0**

Q: /10/ *Enter two flags for graphics updates
of the progress of the calculation : 1=yes, 0=no
First flag for the distribution $r(\theta)$
Second flag for chisq and omega progress*

R: This enables two graphics pages to be displayed: first the intermediate $\rho(\theta)$ distributions and second the chisquare and Omega progress. That may be of a particular interest if the selected rates for driving the convergence are not appropriate i.e. too low or much too high.

Value for demo supplied: **1 1**

Q: /11/ *Enter Rate1, Rate2 and Rate3
[if the entry is 0. 0. 0. then the default values
are set to -0.5 -0.15 -0.05]*

R: Rate controls the change in the distribution which is allowed in each iterate. Small values ensure that the MaxEnt trajectory is closely followed but the computation time can be long. Using higher values allows the MaxEnt algorithm more freedom and although the computation may be dramatically faster but there is an increased risk of the program diverging from the MaxEnt trajectory ,not converging or losing its way. The symptoms are that the program goes to the maximum of allowed iterates with Test, Entropy, Omega, Chisq diagnostics bouncing and failing to converge or in extreme cases losing its way and getting very high values.

Similarly, setting rate negative provides further freedom and shorter computation times but does not enhance the risk of convergence not being reached.

Although the chance of the program not converging is small, the setting of rate id dependent on the quality of data presented to MaxEnt. Therefore, where time is important, the user should explore how much the rate setting may be "abused" for the types of data being processed.

In general, the large rate Rate1 will enable the program to initially converge rapidly during few tens of iterates and the rate is lowered as OMEGA reaches 0.1. Then the rate can be lowered at the Rate2 value till OMEGA reaches value of 0.75 and at this stage of the trajectory the rate is automatically set to the Rate3 value.

Value for demo supplied: **-0.40 - 0.10 -0.03**

Q: /12/ *Automatic peak-finding?, 0 = No , 1 = Yes*

R: In entering a zero value, the user can impose the lower and upper limits of peaks to analyse in the recovered distribution. If this option for the imposed selection of peaks (0 value) is given, then the program asks for the lower and upper limits of each selected peak. For example, this option may be useful in case of a soft shoulder in a broad peak which cannot be detected by the automatic search peak.

Value for demo supplied : **1**

Q: /13/ *Please enter lower and upper peak markers.*

0,0 ==> exit of peak analysis routine.

R: The two markers of a region of interest (ROI) are given in each of the next lines.

Please enter 0 0 if there is no more ROI to consider. The number of ROI in the distribution is arbitrarily limited to 20.

Recommended value for demo supplied : **0 0**

7. Output Files:

The program produces a number of results and diagnostic on the analysis.

The corresponding files have a common root name with the "*.log" filename which is given by the user (for the test supplied **test-2qt.log**) but they have specific extension names.

test-2qt.log : This file contains information on the data : integral, peak position, average correlation time etc... It recall the correlation time domain parameters and the MaxEnt options in use. It also indicates the maximum number of data points and the maximum length of the distribution allowed by the current version of the delivered program. It provides diagnostics on the progress of the maximum entropy trajectory. The final distribution and the corresponding residuals are recorded along with a peak search results. Finally the error bars on the peak parameters are also given.

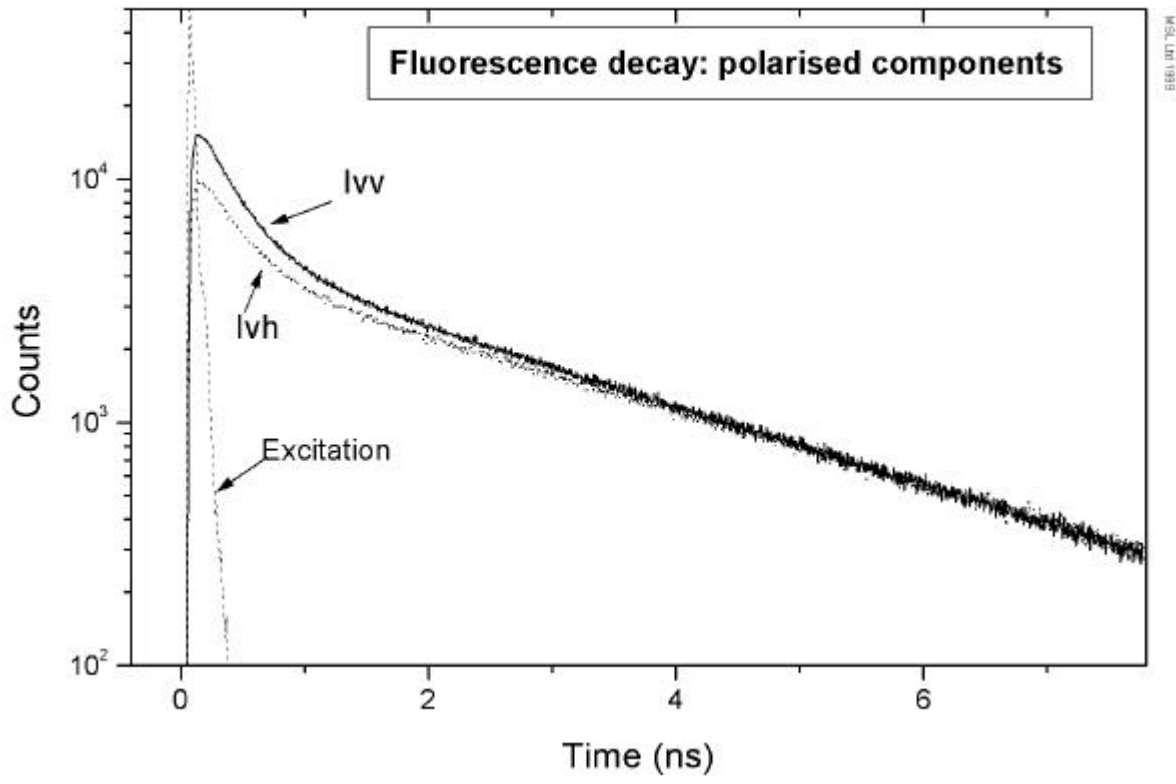
test-2qt.dis : This file contains four columns corresponding respectively to the "pixel" value, the correlation time in nanosecond, the pre-exponential factors $\beta(\theta)$ and the peak label of the recovered distribution. In case of scattered light option, the two first pre-exponential factors does not correspond to any correlation. As the initial anisotropy could be biased the two first pixel contains values corresponding respectively to scattered light in $I_{vv}(t)$ and $I_{vh}(t)$. The corresponding correlation time are artificially set to one tenth and two tenth of the MCA channel value in nanosecond. These two first value may be skipped before plotting.

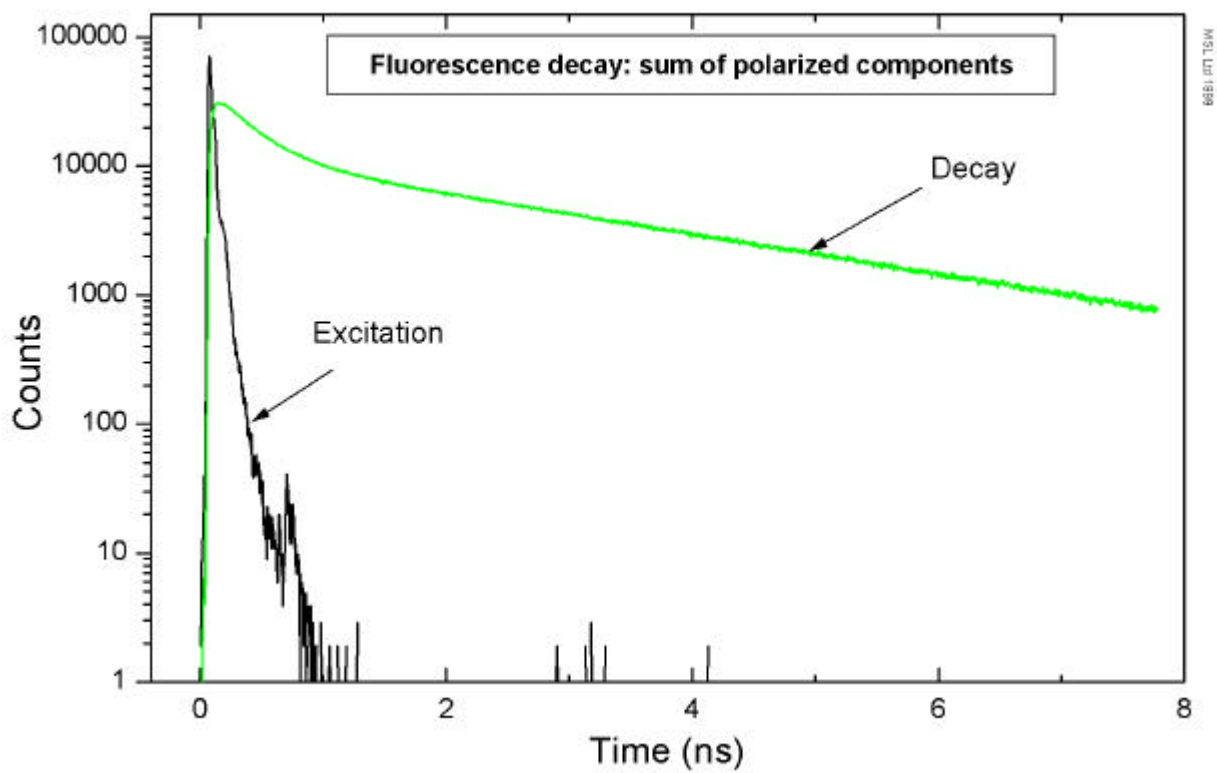
test-2qt.tab : This file is a table which summarise the peak search in the distribution. The associated error bars are also given. This file may be sent directly to the printer.

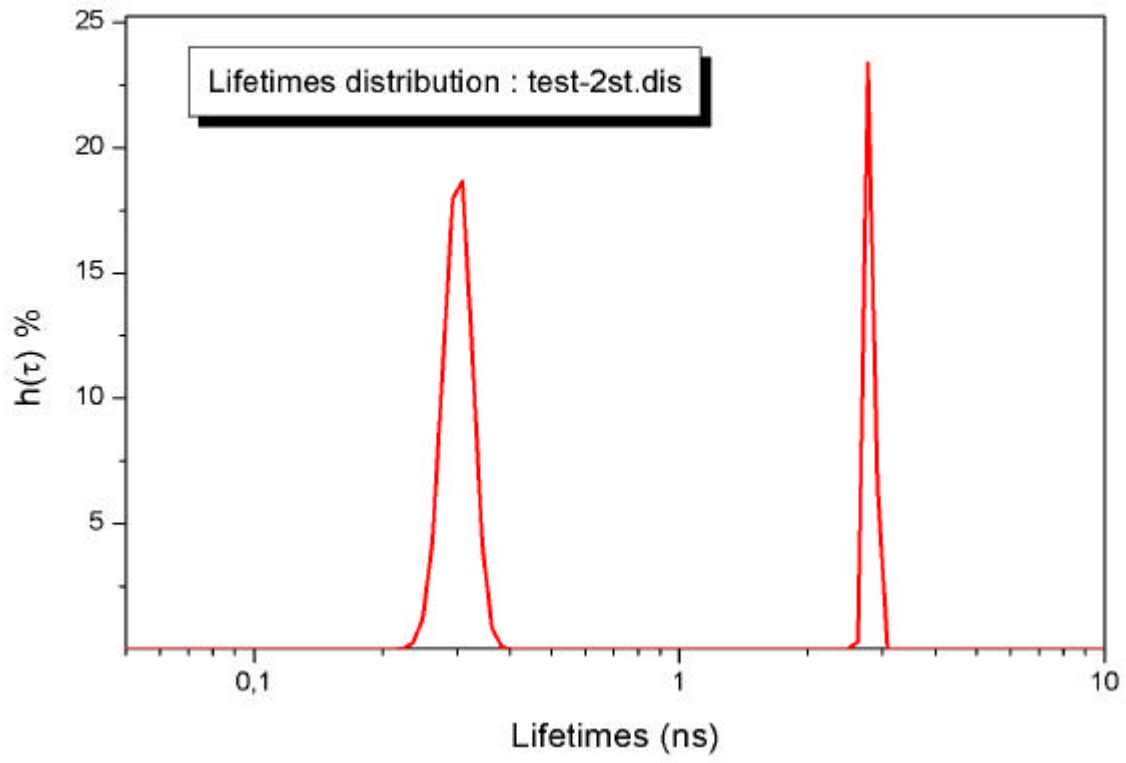
test-2qt.crv : This file consists of height columns corresponding respectively to:

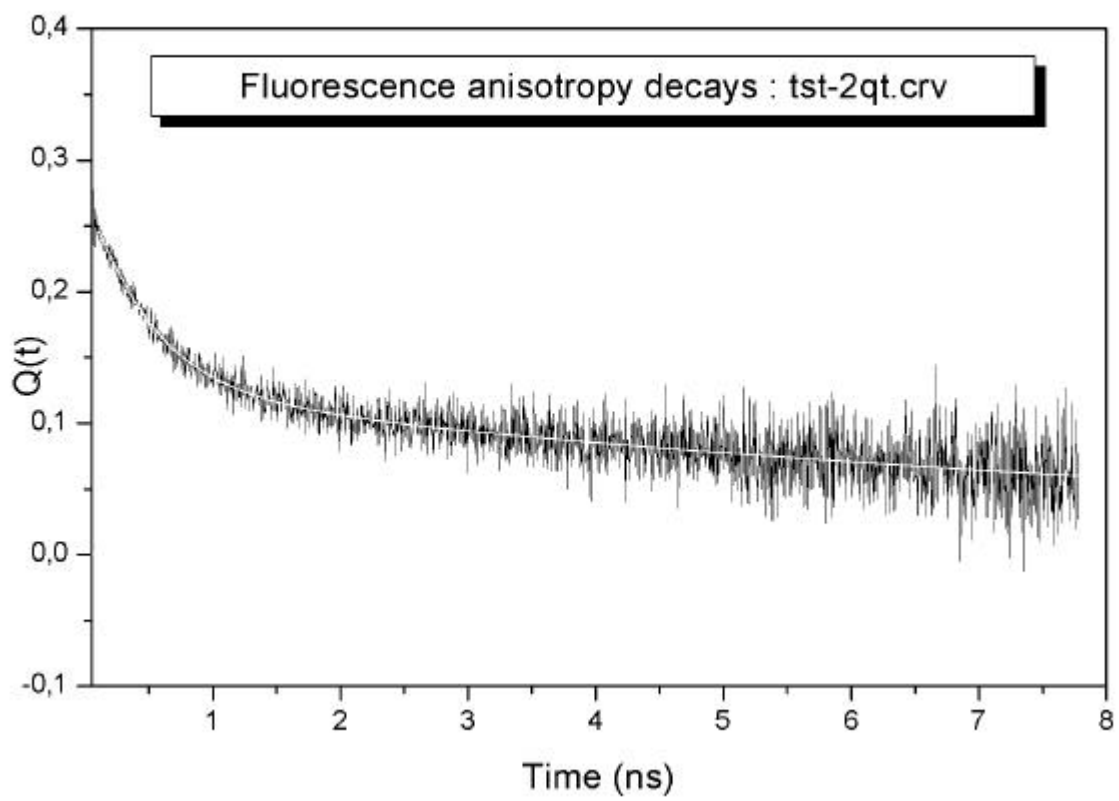
- 1) the channel number
- 2) the flash intensity profile $E(t)$ or the reference fluorescence decay
- 3) the experimental $Q(t)$ (from data I_{vv} and I_{vh}) after background subtractions
- 4) the calculated $Q(t)$ anisotropy decay
- 5) the I_{vv} residuals
- 6) the I_{vh} residuals
- 7) the autocorrelations values corresponding to I_{vv}
- 8) the autocorrelations values corresponding to I_{vh}

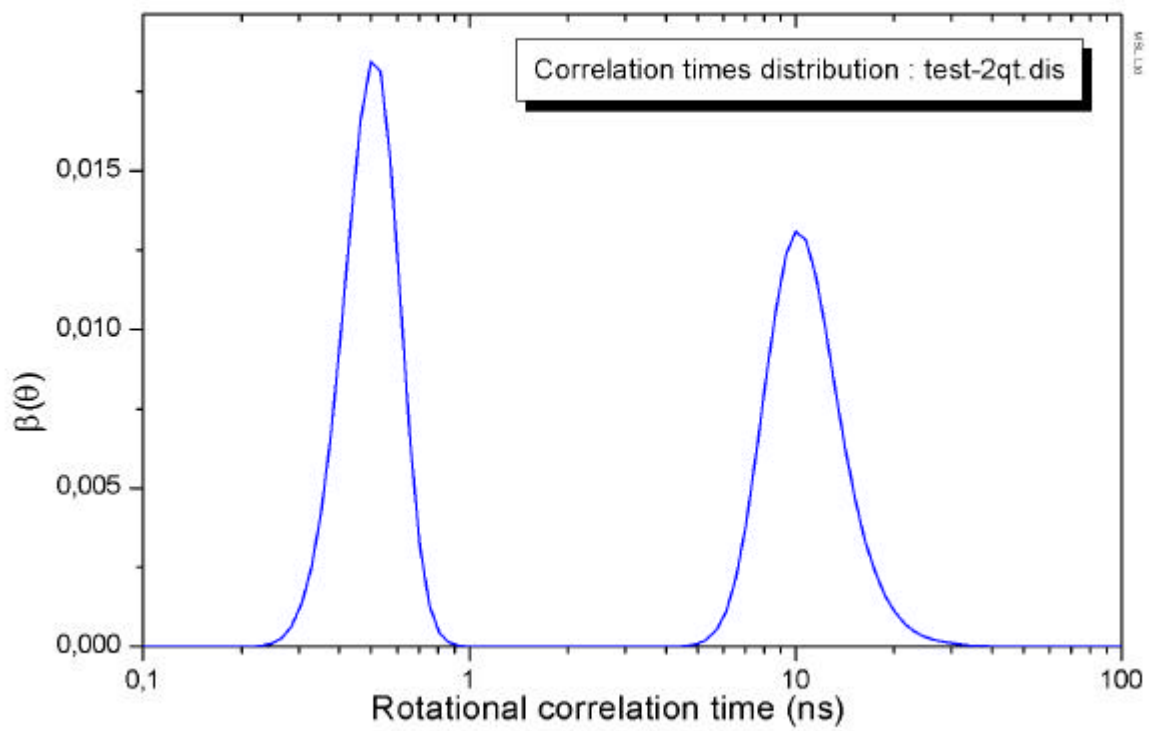
test-2qt.are and **test-2qt.sav** are the files which contain all the areas and internal parameters saved within the computer after the last iteration.

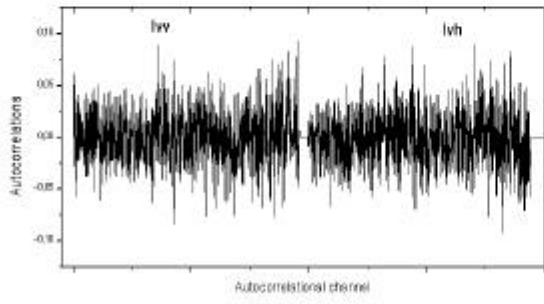
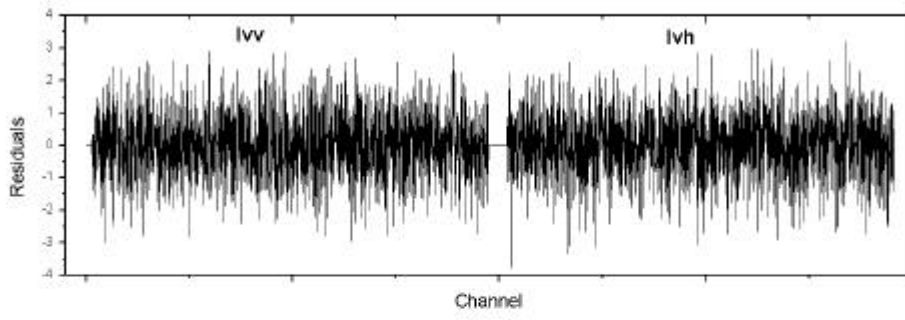












Filename: test-2qt.log

18/ 2/1998 at 13h 2

```

*****
*
*
* PPPP  U  U  L      SSS  EEEEE  55555      *
* P  P  U  U  L      S    E    5      QQQ  TTTTT *
* PPPP  U  U  L      SS   EEEE  5555  Q   Q   T   *
* P      U  U  L      S    E      5      QQ   T   *
* P      UUU  LLLLL  SSS   EEEEE  555      Q   T   *
*
*
*
*
*          Pulsed Fluorescence Analysis      *
*                    by                      *
*          Quantified Maximum Entropy      *
*
*
*
*
* J-C Brochon & MSL                        *
* Version 1.21, Winter 1998                *
* Copyright (C) Logitas J.C. Brochon      *
*****

```

< Unauthorized access to this program is forbidden >

```

.....
.....
18 Feb. 1998 DEMO test: PULSE5/PULSE5QT.EXE Mock data convolved with
excit.fl fi
Two exponentials; taus: 0.3 2.8ns / Alpha's: 2.80E04 and 1.2E04
(TWO Correlation times: 0.5ns and 10ns) R0=0.25
.....
.....

```

```

Number of parameters in the Total Fluorescence analysis: 100
- Flags: Scattered light = 0      Background = 0
- The 100 lifetimes are:
0.5000000000000000D-001  0.5270000000000000D-001  0.5560000000000000D-001
0.5870000000000000D-001  0.6190000000000000D-001  0.6530000000000000D-001
0.6890000000000000D-001  0.7270000000000000D-001  0.7670000000000000D-001
0.8090000000000000D-001  0.8540000000000000D-001  0.9010000000000000D-001
0.9500000000000000D-001  0.1003000000000000      0.1058000000000000
0.1116000000000000      0.1177000000000000      0.1242000000000000
0.1310000000000000      0.1382000000000000      0.1458000000000000
0.1538000000000000      0.1623000000000000      0.1712000000000000
0.1806000000000000      0.1906000000000000      0.2010000000000000
0.2121000000000000      0.2238000000000000      0.2361000000000000
0.2490000000000000      0.2627000000000000      0.2772000000000000
0.2924000000000000      0.3085000000000000      0.3254000000000000
0.3433000000000000      0.3622000000000000      0.3821000000000000
0.4031000000000000      0.4253000000000000      0.4487000000000000
0.4733000000000000      0.4994000000000000      0.5268000000000000
0.5558000000000000      0.5863000000000000      0.6186000000000000

```

0.6526000000000000	0.6884000000000000	0.7263000000000000
0.7662000000000000	0.8083000000000000	0.8528000000000000
0.8997000000000000	0.9491000000000000	1.0013000000000000
1.0563000000000000	1.1144000000000000	1.1757000000000000
1.2403000000000000	1.3085000000000000	1.3804000000000000
1.4563000000000000	1.5364000000000000	1.6209000000000000
1.7100000000000000	1.8040000000000000	1.9032000000000000
2.0078000000000000	2.1182000000000000	2.2346000000000000
2.3575000000000000	2.4871000000000000	2.6238000000000000
2.7681000000000000	2.9202000000000000	3.0808000000000000
3.2501000000000000	3.4288000000000000	3.6173000000000000
3.8162000000000000	4.0260000000000000	4.2473000000000000
4.4808000000000000	4.7272000000000000	4.9871000000000000
5.2612000000000000	5.5505000000000000	5.8556000000000000
6.1775000000000000	6.5172000000000000	6.8754000000000000
7.2534000000000000	7.6522000000000000	8.0729000000000000
8.5167000000000000	8.9849000000000000	9.4789000000000000
10.0000000000000000		

The pre-exponential coefficients of the total fluorescence decay are:

0.0000000000000000	0.0000000000000000	0.0000000000000000
0.0000000000000000	0.0000000000000000	0.0000000000000000
0.0000000000000000	0.0000000000000000	0.0000000000000000
0.0000000000000000	0.0000000000000000	0.0000000000000000
0.0000000000000000	0.0000000000000000	0.0000000000000000
0.0000000000000000	0.0000000000000000	0.1186700000000000D-018
0.6912300000000000D-016	0.2530200000000000D-013	0.5775900000000000D-011
0.8259400000000000D-009	0.7507500000000000D-007	0.4435400000000000D-005
0.1749300000000000D-003	0.4736000000000000D-002	0.9032900000000000D-001
1.2380000000000000	12.3380000000000000	89.5340000000000000
468.29000000000000	1727.10000000000000	4350.30000000000000
7191.60000000000000	7465.50000000000000	4653.50000000000000
1671.80000000000000	334.5600000000000000	36.4010000000000000
2.1233000000000000	0.6617900000000000D-001	0.1108200000000000D-002
0.1008600000000000D-004	0.5055000000000000D-007	0.1412500000000000D-009
0.2220800000000000D-012	0.1972300000000000D-015	0.9923000000000000D-019
0.0000000000000000	0.0000000000000000	0.0000000000000000
0.0000000000000000	0.0000000000000000	0.0000000000000000
0.0000000000000000	0.0000000000000000	0.0000000000000000
0.0000000000000000	0.0000000000000000	0.0000000000000000
0.0000000000000000	0.0000000000000000	0.0000000000000000
0.0000000000000000	0.0000000000000000	0.0000000000000000
0.0000000000000000	0.0000000000000000	0.0000000000000000
0.0000000000000000	0.0000000000000000	0.4941100000000000D-014
0.2899200000000000D-007	0.1157700000000000D-001	111.6700000000000000
9350.30000000000000	2533.00000000000000	0.882780000000000000
0.1716200000000000D-006	0.8938800000000000D-017	0.000000000000000000
0.0000000000000000	0.0000000000000000	0.000000000000000000
0.0000000000000000	0.0000000000000000	0.000000000000000000
0.0000000000000000	0.0000000000000000	0.000000000000000000
0.0000000000000000	0.0000000000000000	0.000000000000000000
0.0000000000000000	0.0000000000000000	0.000000000000000000
0.0000000000000000	0.0000000000000000	0.000000000000000000
0.0000000000000000	0.0000000000000000	0.000000000000000000
0.0000000000000000	0.0000000000000000	0.000000000000000000
0.0000000000000000	0.0000000000000000	0.000000000000000000
0.0000000000000000	0.0000000000000000	0.000000000000000000

The Scattering "intensity" =0.000000E+00

**** CORRELATION TIME DOMAIN 1 ****

Smallest correlation time 0.10 ns
 Largest correlation time 100.00 ns
 Number of correlation times 100
 Log. scaling
 No Scattered light channels in use
 No positive constant anisotropy
 Total number of anisotropy parameters 100

**** DATA MEASUREMENT PARAMETERS ****

Analysed assuming 0.0040 ns/channel
 The following M.C.A. channels were used to
 delimit fluorescence and background data :
 N1 Fluorescence channel 1
 N2 Fluorescence channel 1945
 N-CHI2 limited at channel 1920
 Number of data points set "unmeasured" in
 the fluorescence leading edge was 25

 N1 Background channel 1961
 N2 Background channel 2020
 Excitation was vertically polarised.
 Polarised fluorescence decay analysed.

Solution "blanks" were NOT measured :

**** EXCITATION PARAMETERS ****

Frequency of the excitation 4.000 MHz
 Convolution calculated with "n" excitation curves.
 Convolution calculated with true excitation data
 and a polynomial approximation of degree 1
 Flash (Reference) curve is NOT shifted.

**** DATA ****

I VERT. or I_magic fluorescence test-2.vv
 Peak channel in M.C.A. was 1911
 The corresponding peak value was 15296.
 The total counts between markers was 4238399.
 The background level was automatically calculated:
 This background was 0.00
 The corresponding error was 0.00

I HOR. fluorescence test-2.vh
 Peak channel in M.C.A. was 1910
 The corresponding peak value was 9718.
 The total counts between markers was 3490394.
 The background level was automatically calculated:

This background was 0.00
 The corresponding error was 0.00

FLASH 1 or Reference decay 1 excit.fl
 Peak channel in M.C.A. was 1927
 The corresponding peak value was 71251.
 The total counts between markers was 759092.
 The background level was automatically calculated:
 This background was 0.07
 The corresponding error was 0.03

CURVE INTEGRALS:

Excitation 0.7589623E+06
 Fluorescence 0.9962645E+07
 Mean lifetime 1.855 ns
 Averaged anisotropy 0.1381

The estimated default level..... 0.138144E-02
 The total switched out data points are 25
 Started from a flat map:
 The estimated default level was 0.0014
 Constant default level used as a model

MEM parameters were:

Method === 1 1
 Level === 10
 Aim === 1.0000E+00
 Default === 1.3814E-03
 Rate1 === -0.40 up to Omega=0.1
 Rate2 === -0.10 up to Omega=0.75
 Rate3 === -0.03

Maximum vector sizes allowed by this program:

Experimental data4000
 Correlation times + Scattered lights + r_infinite .. 210

++++
 The number of "pixels" in the distribution is: 100
 The rotational correlation times are:
 0.10 0.11 0.11 0.12 0.13 0.14 0.15 0.16 0.17 0.19
 0.20 0.22 0.23 0.25 0.27 0.28 0.31 0.33 0.35 0.38
 0.40 0.43 0.46 0.50 0.53 0.57 0.61 0.66 0.71 0.76
 0.81 0.87 0.93 1.00 1.07 1.15 1.23 1.32 1.42 1.52
 1.63 1.75 1.87 2.01 2.15 2.31 2.48 2.66 2.85 3.05
 3.27 3.51 3.76 4.04 4.33 4.64 4.98 5.34 5.72 6.14
 6.58 7.05 7.56 8.11 8.70 9.33 10.00 10.72 11.50 12.33
 13.22 14.17 15.20 16.30 17.48 18.74 20.09 21.54 23.10 24.77
 26.56 28.48 30.54 32.75 35.11 37.65 40.37 43.29 46.42 49.77
 53.37 57.22 61.36 65.79 70.55 75.65 81.11 86.97 93.26100.00

++++

Sum of contents of spectrum 0.2533965E+00
 Iterations executed 30 / 500

```

Current ENTROPY ,ISTAT ..... -0.220977E+00  001010
Current CHI2, OMEGA ..... 0.964499E+00  0.143131E+00

Sum of contents of spectrum ..... 0.2529033E+00
Iterations executed ..... 60 / 500
Current ENTROPY ,ISTAT ..... -0.323230E+00  001010
Current CHI2, OMEGA ..... 0.964085E+00  0.531204E+00

Sum of contents of spectrum ..... 0.2528213E+00
Iterations executed ..... 86 / 500
ENTROPY, ISTAT..... -0.366907E+00  000000
CHI2, OMEGA ..... 0.96404E+00  0.99446E+00

```

MEM DISTRIBUTION OF EXPONENTIALS:

```

0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000
0.0000 0.0000 0.0000 0.0001 0.0003 0.0006 0.0013 0.0025 0.0042 0.0067
0.0098 0.0133 0.0165 0.0185 0.0181 0.0153 0.0109 0.0065 0.0032 0.0013
0.0004 0.0001 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000
0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000
0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0001 0.0002 0.0005 0.0011
0.0022 0.0038 0.0060 0.0084 0.0107 0.0123 0.0131 0.0128 0.0117 0.0101
0.0082 0.0063 0.0047 0.0034 0.0024 0.0016 0.0011 0.0007 0.0005 0.0003
0.0002 0.0001 0.0001 0.0001 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000
0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000

```

Maximum value = 0.18454E-01

Level of discrimination for peak search= 0.18454E-05

```

Sum of Rho(Theta)=.252821E+00   +or- 0.138E-02   ==>   0.55 %
Mean of Theta"s= 5.7032E+00     +or- 1.341E+00   ==>  23.515 %

```

```

-----
*****
*   PEAK POSITIONS   *
*****

```

```

Left marker  12           Right marker 34           Top position 24
Left marker  54           Right marker 94           Top position 67

```

*** PEAK NUMBER= 1 ***

```

Total flux = 0.1297E+00   +or- 4.415E-03   ==>   3.40 %
Centroid   = 5.0225E-01   +or- 3.125E-02   ==>   6.22 %
Dispersion = 9.7422E-02   +or- 9.810E-02   ==>  100.69 %

```

*** PEAK NUMBER= 2 ***

```

Total flux = 0.1231E+00   +or- 4.242E-03   ==>   3.45 %
Centroid   = 1.1182E+01   +or- 2.495E+00   ==>  22.31 %
Dispersion = 3.4863E+00   +or- 1.400E+01   ==>  401.65 %
-----

```

=== Elapsed time: 1mn 9sec ===