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FLUOR - A PROGRAM TO ANALYSE FLUORESCENCE DATA

by

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FLUOR - a program to analyse fluorescence data

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1. Introduction.

The program FLUOR is used to analyse fluorescence data from the Synchrotron Radiation Source (SRS) at Daresbury Laboratory to obtain fluorescence and anisotropy decay times. It grew out of two earlier codes, FLUORFIT and FLUOROT, the former was used to analyse lifetime and the latter anisotropy data.

The purpose of this document is twofold; to describe the numerical models used in the data analysis and to describe certain aspects of the computer code to enable the user to understand the usage of the code and to assist in program maintainance.

The structure of this memorandum is as follows. In section 2 we give a general overview of the task of analysing fluorescence data. This is followed by a discussion in section 3 of the reasons behind the decision to rewrite the earlier codes. The details of the models used to analyse the data are given in section 4 and in section 5 we describe the code menus and how to drive the code. In the appendices are given a sample output file and graphs, and a diagram of the subroutine structure.

2. The task of analysing fluorescence data.

The data to be analysed are of two main types; either the total fluorescence is measured or the signals with polarisation parallel and perpendicular to the polarisation axis of the incident radiation are measured. Both types of data can be analysed to obtain the lifetime(s) of the total fluorescence from the sample.

Anisotropy (defined below) is a measure of the difference in the fluorescence which is polarised parallel and perpendicular to the polarisation of the incident photon beam. For example, linear molecules preferentially absorb light polarised along the axis of the molecule but they may rotate before fluorescing and hence emit light not necessarily polarised in the same direction as the incident beam. In this case, the time dependence of the anisotropy depends on the rotational time scales of the molecules. FLUOR can be used to analyse fluorescence emitted parallel and perpendicular to the initial photon polarisation direction to obtain the lifetime(s) for the anisotropy.

The measured fluorescence signal is not a straight forward decay but is a convolution of the fluorescence decay with a 'prompt', plus a background. The prompt is the average time profile of the single bunch in the storage ring convoluted with the detector electronics and is usually measured for each experiment. Where appropriate, the background may be measured from the fluorescence of the buffer solution only, or else has to be estimated from the data.

A model is chosen to represent the time behaviour of the fluorescence or the anisotropy; normally this will be a sum of exponentials but the code has been written so that it is easy to add different models for the decays. The model function can then be convolved with the prompt and fitted to the experimental data. This is done by minimising the weighted sum of the squares of the residues (the differences between the experimental and model data) to obtain the model parameters, including the fluorescence lifetimes or the anisotropy lifetimes. If the decay times are very much longer than the duration of the prompt, it is possible to just use a model function without the convolution. More details of the models used in the fitting are given in section 4.

The output from the minimisation consists of the values of the model parameters, the fitting function, the residues and the autocorrelation of the residues. Also given are

the results of various statistical parameters; the variance, the Durban-Watson parameter, Skewness and Kurtosis. The user can display the graphs of the experimental data, the fits, the residues and their correlation and may save the plots to file for sending to a hard copy device. A log file with a record of the different models tried and the results of the fit (ie fitting parameters and parameters indicating goodness of fit) is also produced.

3. Rewriting the codes.

The earlier fluorescence codes had been used extensively to analyse lifetime and anisotropy data, see eg Behan et al 1992. It was decided to redesign and recode the programs for two main reasons, namely to make the codes more 'user friendly' and to increase the flexibility of the code. The specification given was that the new code had to include all the functionality of the earlier codes, plus it had to be menu driven to make it easier to use, and it had to be easy to add new features, eg different minimisation techniques or model functions as mentioned in the previous section. Combining the lifetime and anisotropy analysis into one program means that there is no longer the problem of one code being modified and getting out of phase with the other, and also saves duplication of code.

A design study for the new program was carried out, looking in particular at the flow of data through the code and the organisation of the processes within the program. It was judged better to consider the design from scratch rather than 'reverse engineering' the old programs. In order to make the code easy to maintain and upgrade, the code has to be modular, both in the way information is stored in the program and in the way it is processed. For example, it is desirable to store separately those parameters describing the data which are determined by the experiment and those which can be varied and are model dependent, to enable ease of changing the model of the time dependence of the fluorescence. The part of the code which carries out the minimisation has now been separated out and interfaced through a routine which passes an array of the values of the variables in the model and the name of the subroutine to calculate the model dependent function to be minimised, hence it is easy to change or add new minimisation techniques or new models to the code.

Having a menu driven code means that the user now has more control over the order of processes within the code, eg, after data have been read in it is now possible to plot them before fitting whereas before processes were performed in a given sequence with plotting only occuring after the data had been fitted. The starting values for the model parameters in the fits are also input using a menu type display and are saved so that the user does not have to retype all the parameters for each fit.

Two new features to aid ease of use were added. A log file is produced which keeps track of which fits have been tried. The name of the data-set being analysed plus the description of the fit are stored in a 'startup file' so that the user can pick up the problem from where he/she left off when rerunning the code.

4. Numerical Methods.

In this section the details of the analysis of the raw data are given; in particular how the fitting functions are constructed. The data from a multi-channel analyser are stored in standard SRS data sets in 'MCA' format, ie each experimental signal is given by a list of

1024 numbers which are the counts in each of the MCA's channels. The first two channels contain the elapsed time and live time of the experiment respectively.

The data to be analysed are either total fluorescence decay signals or 'anisotropy' data, ie fluorescence signals with polarisation parallel (F_1) or perpendicular (F_2) to the incident beam polarisation during the anisotropy experiment. An equivalent notation common in the literature is to use the terms 'vertical' and 'horizontal' to describe the fluorescence polarisations, instead of 'parallel' and 'perpendicular' respectively. For a general reference to the analysis of fluorescence data, see for example O'Conner and Phillips 1984.

This section is split into obtaining fluorescence lifetimes and anisotropy parameters.

4.1 Lifetime analysis.

The experimental data F_{exp} to be fitted are either obtained from measuring the total fluorescence decay signal from a sample or can be synthesised from 'anisotropy data'. In the latter case,

$$F_{exp} = gfac * trat * F_1 + 2 * F_2$$

where gfac is the ratio of the efficiency of the analyser when perpendicular and parallel, trat is the ratio of the live-time that the analyser spends perpendicular to parallel. The weighting factors used in the minimisation of the squares of the residues are given by $1/F_{exp}$ for the decay data and by $1/((gfac * trat)^2 * F_1 + 4F_2)$ for the anisotropy data. For data values less than 1, the weight is set to unity. For the lifetime data only, a second minimisation is carried out using $1/F_{fit}$ for the weights; this usually results in a better fit to the background.

If there are measurements from buffer solutions, then a background can be obtained from

$$background = tfac * buffer$$

for decay data and

$$background = gfac * tfac * buffer_1 + 2 * tfac * buffer_2$$

for the anisotropy data, where tfac is the ratio of the live-times of the perpendicular decay and buffer measurements, $buffer_1$ and $buffer_2$ are the fluorescence signals from the buffer with polarisation parallel and perpendicular to the incident polarisation.

Let us assume we have exponential decays only. The model as a function of time t is then of the form

$$F_{model}(t) = \Sigma_{i=1}^{n} \alpha_i e^{-t/\tau_i} + background$$

where the τ are the fluorescence lifetimes. If there is no buffer measurement, the background is taken to be a constant, which can be varied in the minimisation. The experimental data can be fitted to this function or to the model convolved with the prompt P. In the latter case the fitting function is given by

$$F_{fit}(t) = \int_0^t P(t_1 + \Delta t) F_{model}(t - t_1) dt_1 + background + P(t) * elas$$

where Δt allows for a possible time shift between the prompt and the decay signal, *elas* gives the proportion of the incident light which is scattered elastically. It is also possible to

subtract a constant background from the prompt signal. Note that it may not always be appropriate to include the time shift, elastic scattering etc. Any of the model parameters can be fixed or varied in the minimisation and the range over which the function is to be fitted can also be chosen.

The integral in the convolution is carried out using the trapezoidal rule. The weighted sum of the squares of the differences between F_{fit} and F_{exp} is then minimised. At present the minimisation is carried out using an implementation of the Levenberg-Marquardt algorithm from the IMSL Mathematical Library (see Dennis and Schnabel 1983) but the code has been designed with a view to adding different minimisation techniques. The derivatives of the function with respect to the model variables required for the minimisation are carried out numerically (within the IMSL routines) to allow flexibility in the definition of the model.

4.2 Anisotropy analysis.

Anisotropy r is defined by

$$r = (f_1 - f_2)/(f_1 + 2f_2) \qquad (1)$$

where the fluorescence with polarisation parallel and perpendicular to the incident polarisation direction are denoted by f_1, f_2. The problem of analysing anisotropy data is that the convolution of r with the prompt is not equal to the RHS of equation (1) with f_1 and f_2 replaced by the measured signals F_1 and F_2 (which are convolved with the prompt), hence we cannot fit directly to an expression for the anisotropy.

Two methods of analysing the anisotropy data, named for the purpose of the program 'reconvolution' and 'response function' have been implemented. In the discussion below, an exponential model for the anisotropy has been assumed, given by

$$r_{model}(t) = r_\infty + \Sigma_{j=1}^{m} \beta_j e^{-t/\phi_j} \qquad (2)$$

where the ϕ 's give the anisotropy lifetimes.

4.2.1 Reconvolution method.

An 'experimental anisotropy' is defined from the measured signals F_1, F_2 by

$$r_{exp} = (gfac \times s_1 - s_2)/(gfac \times s_1 + 2s_2))$$

s_1 and s_2 are the parallel and perpendicular signals without the background, ie

$$s_1 = trat \times F_1 - backgrd_1; \qquad s_2 = F_2 - backgrd_2$$

where the backgrounds can either be fixed constants or obtained from buffer measurements $\times tfac$. The data resulting from this expression are fitted in two stages.

The denominator in the expression for the anisotropy is just the total fluorescence signal and can be fitted as in the lifetime analysis. In this case, as we do not need to know the physical decay lifetimes, the model is taken to be a sum of 8 exponentials with fixed exponents. This model can then be convolved with the prompt as before and fitted to the lifetime experimental data.

The numerator in the expression for the anisotropy, $f_1 - f_2$, is r multiplied by the total fluorescence decay. Hence we can write a model for the numerator as the product of the exponential sums for the anisotropy and the decay.

$$r_{numer}(t) = \Sigma_{i=1,8} \Sigma_{j=1}^{m} \alpha_i \beta_j e^{-t/\tau_i} e^{-t/\phi_j}$$

The fitting function for the 'experimental anisotropy' is then defined as follows

$$r_{fit}(t) = r_\infty + [\int_0^t P(t_1 + \Delta t) r_{numer}(t - t_1) dt_1]/F_{fit}(t)$$

where F_{fit} contains the values of the fitting function to the lifetime minus the background. The values of the βs, ϕs and r_∞ are found from the fit. The value of Δt, if required, is taken from the lifetime fit.

The weighting factors for the squares of the residues are given by

$$weight = (gfac \times s_1 + 2 \times s_2)^4/[9 \times gfac^2(\delta s_1^2 \times s_2^2 + \delta s_2^2 \times s_1^2)]$$

where $\delta s_1, \delta s_2$ are the uncertainties in s_1 and s_2.

4.2.2 Response function method.

In the response function method, the individual measured decays, F_1, F_2, are fitted using a general function; again an 8 exponential sum with fixed exponents, convolved with the prompt, has been used. This allows the determination of the decays deconvolved from the prompt, which should be a good representation of f_1 and f_2. The anisotropy can then be calculated from equation (1) and fitted to a function of the form given in equation (2). Note that in this method, the anisotropy fit is for a model curve, not to experimental data, and hence the statistical tests used in the other fits cannot be applied as they are appropriate for data with Gaussian statistics. Also the curve which is being fitted is not convolved with the prompt and hence the range in decay time for which the curve can be generated is from t=0 to t' where t' is the range of time over which F_1, F_2 are fitted.

4.3 Goodness of fit.

The program calculates several statistical parameters which give guidance to how well the data are fitted. The information below is mainly taken from O'Conner and Phillips (1984).

The first parameter is the χ^2 defined by

$$\chi^2 = \Sigma_i \sigma_i^2 [y_i - y(t_i)]^2 = \Sigma_i r_i^2$$

where σ_i is the weight at each point, y_i is the value of the model at time t_i, $y(t_i)$ is the data point and r_i is the weighted residual at point i. The reduced χ^2 is defined by

$$\chi_\mu^2 = \chi^2/(n - p)$$

where n is the number of data points to be fitted and p is the number of parameters determined by the fitting. For data with a Poisson distribution, as in counting experiments,

χ^2_μ should be approximately unity for a good fit; values in the range 0.8 - 1.2 are usually considered acceptable.

The residuals should be randomly distributed about zero. Any correlations among the residuals may show up in systematic deviations from zero in the autocorrelation plot; non-correlated values should show small high-frequency deviations from zero. The Durban Watson parameter, defined by

$$DW = \Sigma_i(r_i - r_{i-1})^2/(\Sigma_i r_i^2)$$

gives a measure of the correlation of the residues between neighbouring points. There is a lower limit to this value which is acceptable; this limit depends on the number of data points and the number of variables. For example, for fits to 256 or 512 data points, DW should be greater than 1.7, 1.75 and 1.8 for single, double or triple exponential fits respectively.

A measure of the skewness of the fit is given by

$$SK = \{n/[\Sigma_i(r_i - r_{mean})]^3\}^{1/2} \times \Sigma_i(r_i - r_{mean})^3$$

Results for a normal distribution of residues would have a mean of zero and a standard deviation of $(6/n)^{1/2}$, hence an absolute value of SK greater than $(6/n)^{1/2}$ is an indication that the fit is skewed.

The last parameter is the Kurtosis, defined by

$$K = n[\Sigma_i(r_i - r_{mean})]^4/[\Sigma_i(r_i - r_{mean})]^2$$

For a normal distribution of residues, this would have a value of 3 and for samples of more than 1000 data points, the standard deviation would be $(24/n)^{1/2}$. Values greater than 3 indicate there are too many residuals near the mean value and far from the mean. Values less than 3 indicate that the distribution of residuals is flatter than for a normal distribution.

5. Running the code.

5.1 The Program Menus.

The menu layout consists of a main menu, with four sub-menus, all of which are accessed via the main menu. The sub-menus are for changing the type of analysis which is to be carried out (lifetime or anisotropy), reading in the data, editing the fitting parameters and plotting out the data and fits. The plot sub-menu also has a sub-menu for altering the display parameters of the plot. All of the menus consist of three parts - the top and middle parts are used to display information/options and the bottom part is used to input the option chosen. There are three different types of menu used in this program. These are described below.

The main, change analysis and read menus are of the following form. The top part of the screen is used to display information, eg analysis type chosen, type of data, datasets read in. The middle part of the menu is the list of options for that menu. To execute an option type the first letter(s) of the chosen option and hit return. These menus offer

default options, which should be the most commonly used path through the program, to minimise the amount of key presses needed to run the program. You can also type ahead, without waiting for the menus to come up. To exit from any of these menu you simply type 'q'. All of these menus will accept upper or lower case commands.

Another type of menu is used by the edit and plot parameter menus which need to input values to the parameters. All of the parameters have a reference number. The top part of the menu displays all the parameters which have only two possible settings - on or off. To change these parameters simply type the parameter number and hit return. The parameters displayed in the bottom half of the menu require their new value to be typed in. To change these values type the parameter number, a space, then the new value. If any further information is required you will be prompted for it, eg whether the variable is allowed to vary or not. You cannot type ahead in this menu. To exit you have to type '0'.

The third type of menu is the plot selection menu. The top part of the screen displays information about which data sets have been read in. In the middle part a numbered list of all the curves which can be plotted is given. Up to six curves may be overlaid on one plot; to execute a plot type the numbers of the individual curves required separated by a space. Type '13' to enter the menu which changes the plot axes etc., and type '0' to exit from the menu.

The options on some of the menus vary according to what data is present in memory and also what analysis method has been chosen.

5.2 Typical Path Through the Program.

The program is located on the Molecular Science compute server, mscsv1. Versions with X and Tektronics graphics are available, type fluorx for the former, fluor for the latter. If using the X version, remember to direct X output to your terminal, eg to send X output to xterm6, you would type 'setenv DISPLAY xterm6:0.0'. The main menu is shown in fig. 1. Below is a description of a session running fluor, using the default path through the program:-

1. Read in data.
2. Edit the fit parameters.
3. Fit the data.
4. Plot out the data.
5. goto 2 or Quit.

It is also possible to save the experimental data, final fit and residuals to a user specified file using the 'Save' option from the main menu.

5.2.1 Inputting the data.

To read in a dataset hit return to bring up the read menu (see fig. 2). Before any reading is done the data type must be set correctly. Assuming that this is correct ie lifetime or anisotropy, hit return. If it is not correct select "Change" to set it to the correct data type. There are also options for whether channel numbers are present or not, and for reversed or forward data. Datasets can contain data which is collected with time increasing as you move down the dataset ie forward data, or with time decreasing as you move down the dataset ie reversed data. The default values for the options are set for SRS datasets.

Selecting the read option brings up the prompts for the file names and blocks to be read in. The first option is the prompt. Type in the full name of the dataset, including the directory, followed by the block number eg. "/srsdata/ha12/r4400.dat 1". The next prompt is for the decay data. The program automatically uses the same file as the prompt, with block 2 for lifetime data, and blocks 2 and 3 for the anisotropy parallel and perpendicular data. If these are correct hit return, otherwise type in dataset details as for the prompt. The next question is for the buffer file, if there is one. Unless you have read in a previous buffer file this filename will be blank. You can either type in buffer file name, or type 'n' if you do not wish to read a buffer file in. The data is then read in and the menu rewritten showing the data read in in the top part of the screen.

The program stores the last file name it read in for each option and uses them as the default for the next input. When you have read in the data type 'q' to return to the main menu.

5.2.2 Fitting the data.

The type of analysis that can be done depends on the type of data that you have. Only lifetime analysis can be carried out on lifetime data. Both lifetime and anisotropy analysis can be carried out on anisotropy data. If the default option is not the analysis required then "Change" brings up a sub-menu of the available data analysis methods.

The parameters to be used to fit the data can now be set by hitting return to bring up the edit menu. The edit menu varies according to whether you are doing lifetime or anisotropy analysis. It also varies according to the method you choose to do the anisotropy. Examples of the various kinds of edit menu are shown in fig. 3. For information on how to change the variables see the section 5.1. When all the variables have been set type '0' to exit the edit menu.

To carry out a fit on the data hit return. The values of the parameters obtained by fitting are printed up on the screen, together with the statistical parameters described in section 4.3.

The following information is stored in a log file which can be printed later:

1. Names, type and block numbers of datasets read in.
2. Fit parameters set by the user.
3. Final fit parameters, with fit statistics.

This file can be viewed at any time using the "Display" option on the main menu.

5.2.3 Plotting the results.

To plot the data hit return to bring up the plotting menu. The list of possible plots is displayed on the screen. The options displayed depend on the type of data and on the type of fit (if any) that has been performed. A typical plot menu, after an anisotropy fit has been done, is shown in fig 4. For information on how to use this menu see section 5.1.

The plots are not automatically saved in the grid file for sending to a plotter later. To save them type 'y' when prompted if you wish to save the plot which is displayed on the screen. When the program has been exited it will ask if you wish to send the grid file to the printer. This menu also allows you to view the final fit parameters and the fit statistics again by typing '12'.

The plots can also be altered to show different areas, use different scales, etc. Typing

'13' takes you to the plot parameter menu, shown in fig 5. The default parameters are taken from the last plot drawn. The options are as follows: the y-axis can be logarithmic or linear; the y-axis can be scaled automatically; the x-axis can be labelled with time or channel numbers; the printing of the final parameters in the top right hand side of the fitted data plots can be disabled. This latter is useful if the fitted curve is a multi-exponential and the parameters overlap part of the plot.

Numerical limits for the x-axis and, if not-autoscaled, for the y-axis can be input. Typing '0' exits from the menu.

References.

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Dennis J.E. Jr. and Schnabel R.B., 1983, Numerical Methods for Unconstrained Optimisation and Non-linear Equations, Prentice-Hall, Engelwood Cliffs, New Jersey.

O'Conner D.V. and Phillips D., 1984, Time-correlated Single Photon Counting, Academic Press, London.

```
SRSLIB PROGRAM FLUORFIT.                VERSION 2 (Last Update 06/02/91)
Analysis type: ANISOTROPY
Data_type    : ANISOTROPY
Prompt file: r4400.dat                  block  1    0 points
Decay  file: r4400.dat                  block  2    0 points PARALLEL
 TOP MENU
Change analysis
Read experimental data
Edit fit parameters
Fit data
Plot data
Save files
Display log file
Quit program
================================================================================


>>> Select command [READ]: 
```

Fig.1

```
 DEFAULT SETUPS
Data_type    : ANISOTROPY      channel numbers: YES      mode: REVERSE
Prompt file: r4400.dat                        block  1    0 points
Decay  file: r4400.dat                        block  2    0 points PARALLEL
 READ MENU
Read Files
Data type [Lifetime / Anisotropy ... ]
CHannel numbers (ON/OFF)
COllection mode (REVERSE/FORWARD)
QUIT
================================================================================


>>> Select option [READ]: 
```

Fig. 2

```
LIFETIME        SETUP OPTIONS
( 1) Use_prompt          : YES          ( 2) Use_Buffer          : NO
( 3) Use_Time_shift      : NO           ( 4) No of Exponentials:  2
( 5) Gfac (ratio effic):  1.00          ( 6) Trat (ratio times):  1.00
CALCULATION PARAMETERS
( 7) first chan          1              ( 8) last chan         1024
(10) elast.scat      0.000  VAR         (11) Scale            0.039
(12) backgrd         0.000  FIX
(13) lamp_bckgr      0.000  FIX
(15) alpha  1        0.020  VAR         (16) tau    1         1.000  VAR
(17) alpha  2        0.020  VAR         (18) tau    2         5.000  VAR
( 0) EXIT
```

```
>>> Enter parameter INDEX and new VALUE:
```

```
ANISOTROPY      SETUP OPTIONS
ANALYSIS METHOD RECONVOLUTION
(-1) change analysis method
( 1) Use_prompt          : YES          ( 2) Use_Buffer          : NO
( 3) Use_time_shift      : NO
( 4) No of Exponentials:  2             ( 5) Scale        0.03891
( 6) GFAC (effic perp/parall    1.000   ( 7) TRATIO (times perp/parall    1.000
( 8) PFAC (rescales anisotropy  1.000
CALCULATION PARAMETERS
(10) first chan          100            (11) last chan         800
(12) lamp_bckgr      0.000
(13) parall back     0.000              (14) perp back        0.000
(15) r infinity      0.000  FIX
(17)  beta  1        0.200  VAR         (18) phi    1         1.000  VAR
(19)  beta  2        0.200  VAR         (20) phi    2         5.000  VAR
( 0) EXIT
```

```
>>> Enter parameter INDEX and new VALUE:
```

Fig. 3

```
DATA IN MEMORY
Prompt file: r4400.dat                  block  1 1024 points
Decay  file: r4400.dat                  block  2 1024 points PARALLEL
                                        block  3 1024 points PERPENDICULAR
PLOT MENU (VERSION 1)
 (1) Prompt
 (2) Decay parallel
 (3) Decay Perpendicular
 (6) Experimental Decay data
 (8) Experimental Anisotropy data
 (9) Anisotropy fit
(10) Residuals
(11) Autocorrelation
(12) Output final parameters
(13) Change plotting parameters
 (0) QUIT
```

```
>>> Enter number(s) to be plotted, separated by spaces (n1 n2 n3...n6) :
```

Fig. 4

Appendix A

Output from FLUOR.

Below are given a printout of the log file and sample graphs from a double exponential fit to anisotropy data read from file r4400.dat. Figure A1 gives the experimental data, ie prompt, parallel and perpendicular decay curves; figure A2 the fit to the anisotropy; figure A3 shows plots of the residuals and autocorrelation function.

```
 PLOT OPTIONS
(1) Y-axis scale : LINEAR              (2) X-axis scale : CHANNEL NO
(3) Print final parameters : YES       (4) Automatic Y-axis range : NO
 PLOT PARAMETERS
X-axis     (5) start :   100           (6) finish :   800
Y-axis     (7) start :     -0.50       (8) finish :      1.00
(0) QUIT
================================================================================




>>> Enter parameter INDEX:
```

Fig.5

```
*** READ :-
DATA_TYPE = ANISOTROPY
PROMPT r4400.dat                block  1
DECAY r4400.dat                blocks  2 3
```

```
@@@ INITIAL ANISOTROPY PARAMETERS
 ANISOTROPY                          ANALYSIS METHOD RECONVOLUTION
 Use_prompt         : YES             Use_Buffer         : NO
 Use_time_shift     : NO
 No of Exponentials:  2               Scale      0.03891
 GFAC (effic perp/parall    1.000     TRATIO (times perp/parall    1.000
 PFAC (rescales anisotropy   1.000
 first chan         100               last chan         800
 lamp_bckgr       0.000
 parall back      0.000               perp back         0.000
 r infinity       0.000  FIX
 beta  1          0.200  VAR         phi    1          0.001  FIX
 beta  2          0.200  VAR         phi    2          5.000  VAR
```

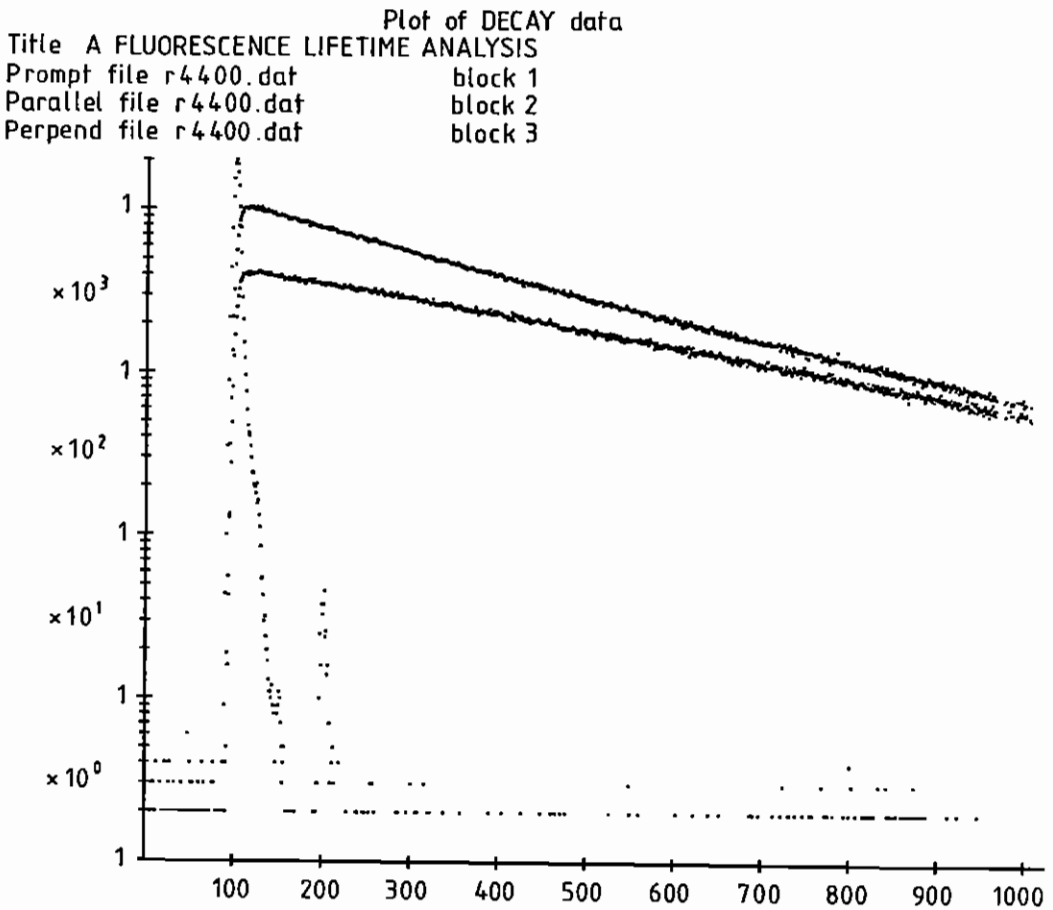
```
### FINAL ANISOTROPY PARAMETERS
 FUNCTION PARAMETERS FOR EXPONENTIAL FIT TO ANISOTROPY
                   *** REDUCED CHI2 =      0.9940 ***

   beta  1          0.1316  VAR         phi    1          0.0010  FIX
   beta  2          0.3422  VAR         phi    2         20.7018  VAR

  r infinity       0.0000  FIX
  first chan         100               last chan         800

 8 EXPONENTIAL FIT FOR DECAY
 ***chi2 for decay     1.4607***
     exponential      coeff
          1.0000        -0.0007
          5.0000        -0.0104
         10.0000         0.0141
         20.0000        -0.0195
         50.0000         0.0441
        100.0000        -0.0797
        200.0000         0.1142
        600.0000         0.0463

 ****values of statistical parameters****
 reduced chi2                 0.9940     should be in range 0.8 to 1.2
 Durban Watson parameter      0.5327
 Skewness factor             -0.1544     abs value should be less than      0.9252E-01
 Kurtosis factor              3.031      should be of the order of 3
```



Plot of ANISOTROPY data

title A FLUORESCENCE LIFETIME ANALYSIS

Prompt file r4400.dat block 1

Parallel file r4400.dat block 2

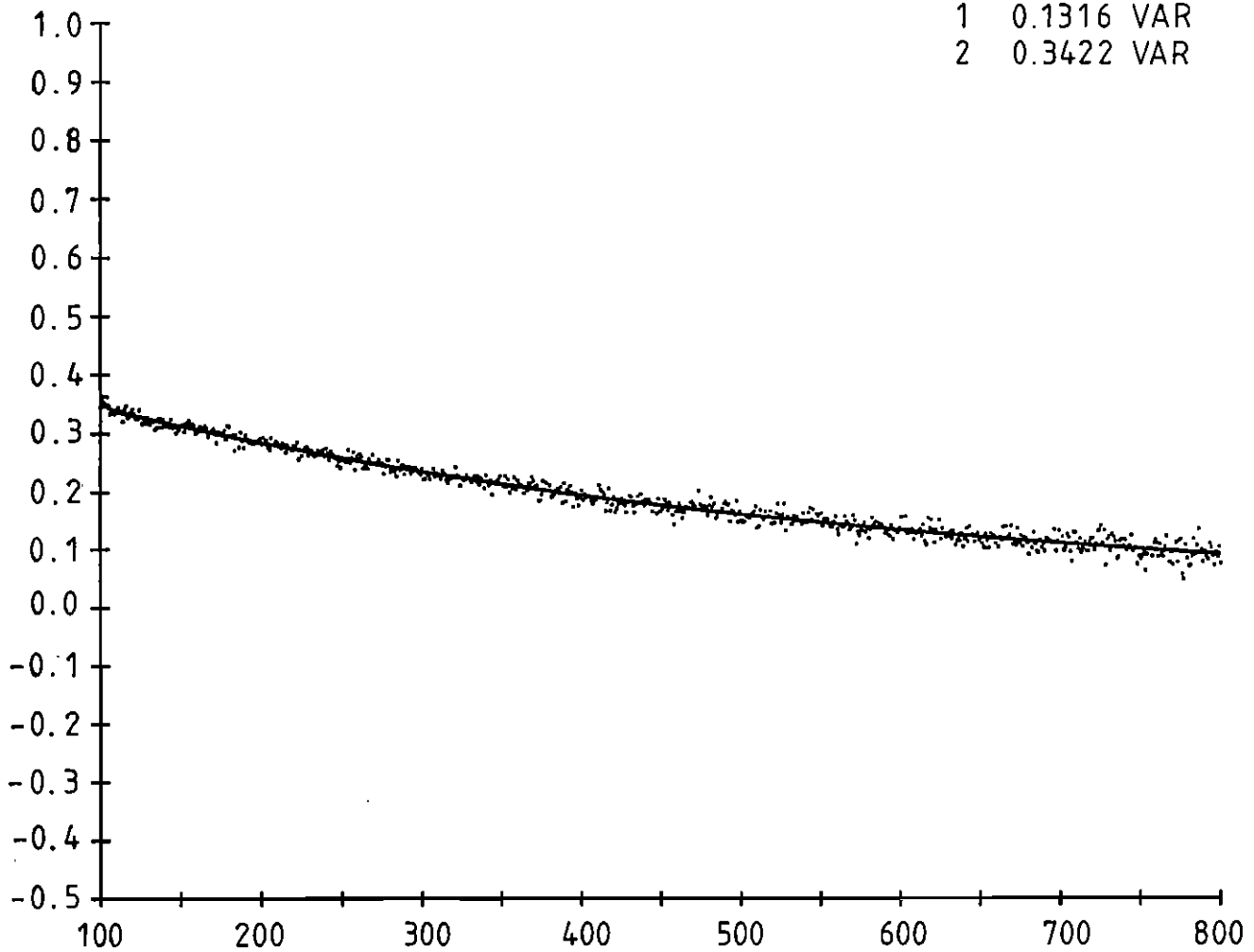
Perpend. file r4400.dat block 3

Fitting between channels 100 and 800

Chisqr 0.9940 R infinity 0.0000 FIX

Para.bkgrd 0.0000 FIX Perp.bkgrd 0.0000 FIX

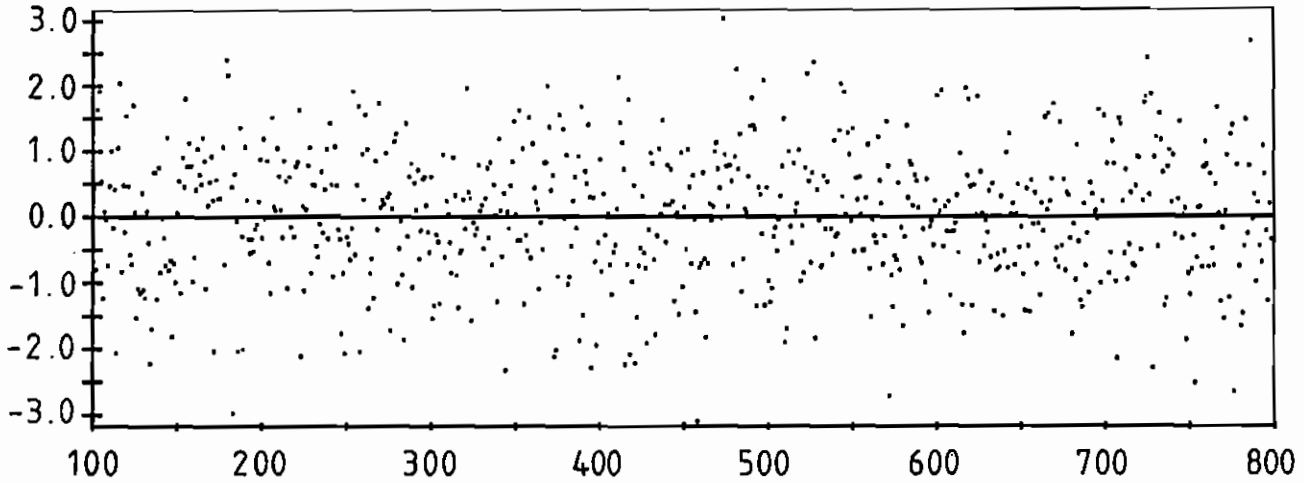
BETA		PHI	
1	0.1316 VAR	1	0.0010 FIX
2	0.3422 VAR	2	20.7018 VAR

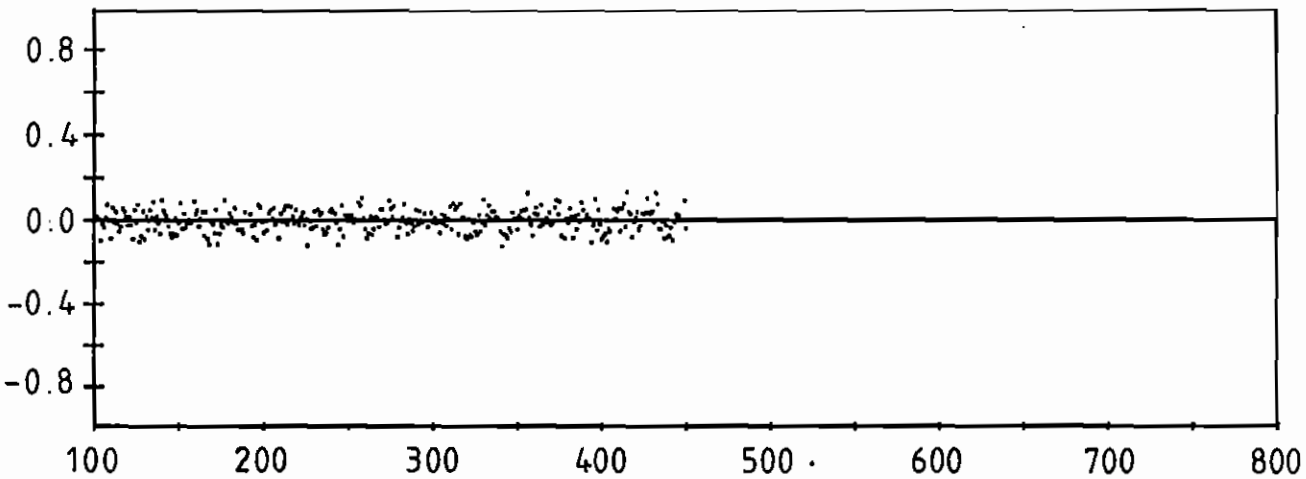


Plot of RESIDUALS and AUTOCORRELATION data

Prompt file r4400.dat block 1 Analysis : ANISOTROPY Red CHISQR : 0.9940

Decay file r4400.dat block 2 title A FLUORESCENCE LIFETIME ANALYSIS

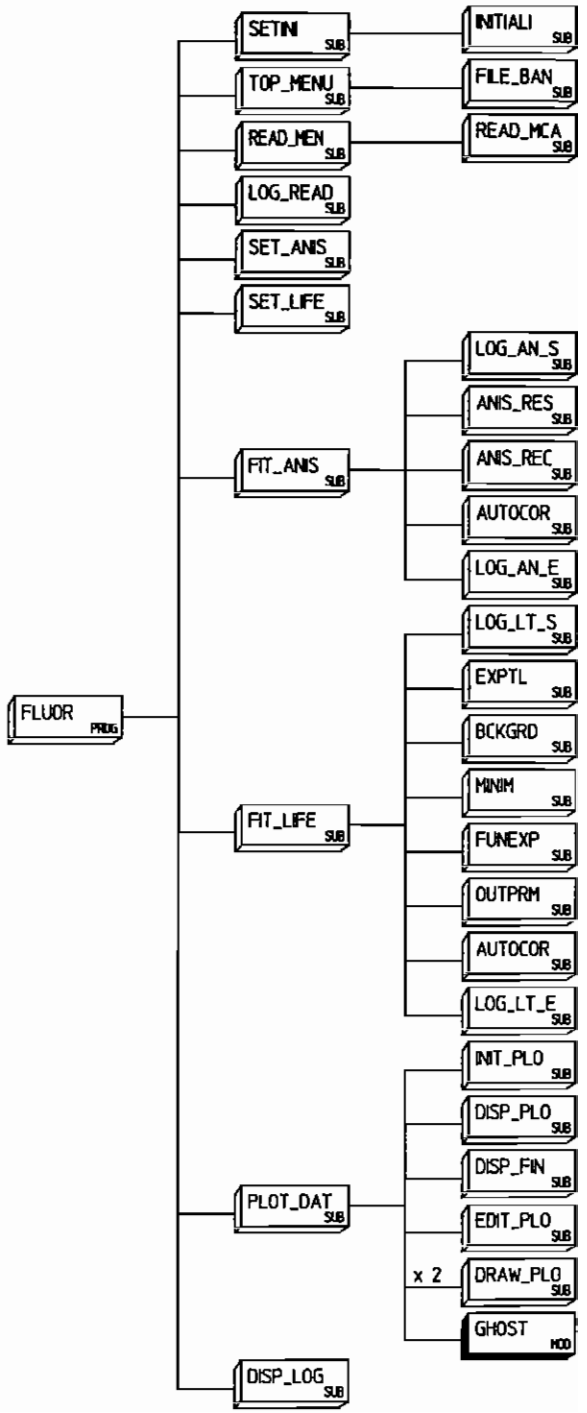




Appendix B

The first module chart of FLUOR (figure B1) shows the overall strucure of the code, the branches to the initialisation of the code, displaying the menu, reading in data, setting up the log file, editing the model parameters for anisotropy or lifetime analysis, fitting the data (anisotropy or lifetime) and plotting data and results. The boxes with heavier outline denote libraries, in this figure the ghost graphics library. Routines to carry out screen handling, selecting values from the terminal etc have been omitted.

Figure B2 gives more detail on the anisotropy fitting, the two main branches being the different ways to analyse anisotropy data. The fitting routines, DUNLSF and routines called from there, are from the IMSL Math Library and are interfaced through a single routine MINIM with auxilliary routines PACK and UNPACK which organise the variable model parameters into an array to be passed to DUNLSF. DUNLSF also requires a user supplied routine to calculate the model functions. FUNC (not shown) performs this task and calls the required routines, eg the FUNEXP routines which evaluate the exponential type functions given in this paper for life-times and anisotropy. The SETPRM routines select the required parameters for the model. The experimental data to be fitted are selected in EXPTL and BCKGRD and for the response function method, ANIS evaluates the anisotropy from the individual perpendicular and parallel decays which is subsequently fitted.



MODULE CHART OF FIT_ANIS