FelixGX Data Analysis

Contents

A Quick Tour of FelixGX Data Analysis

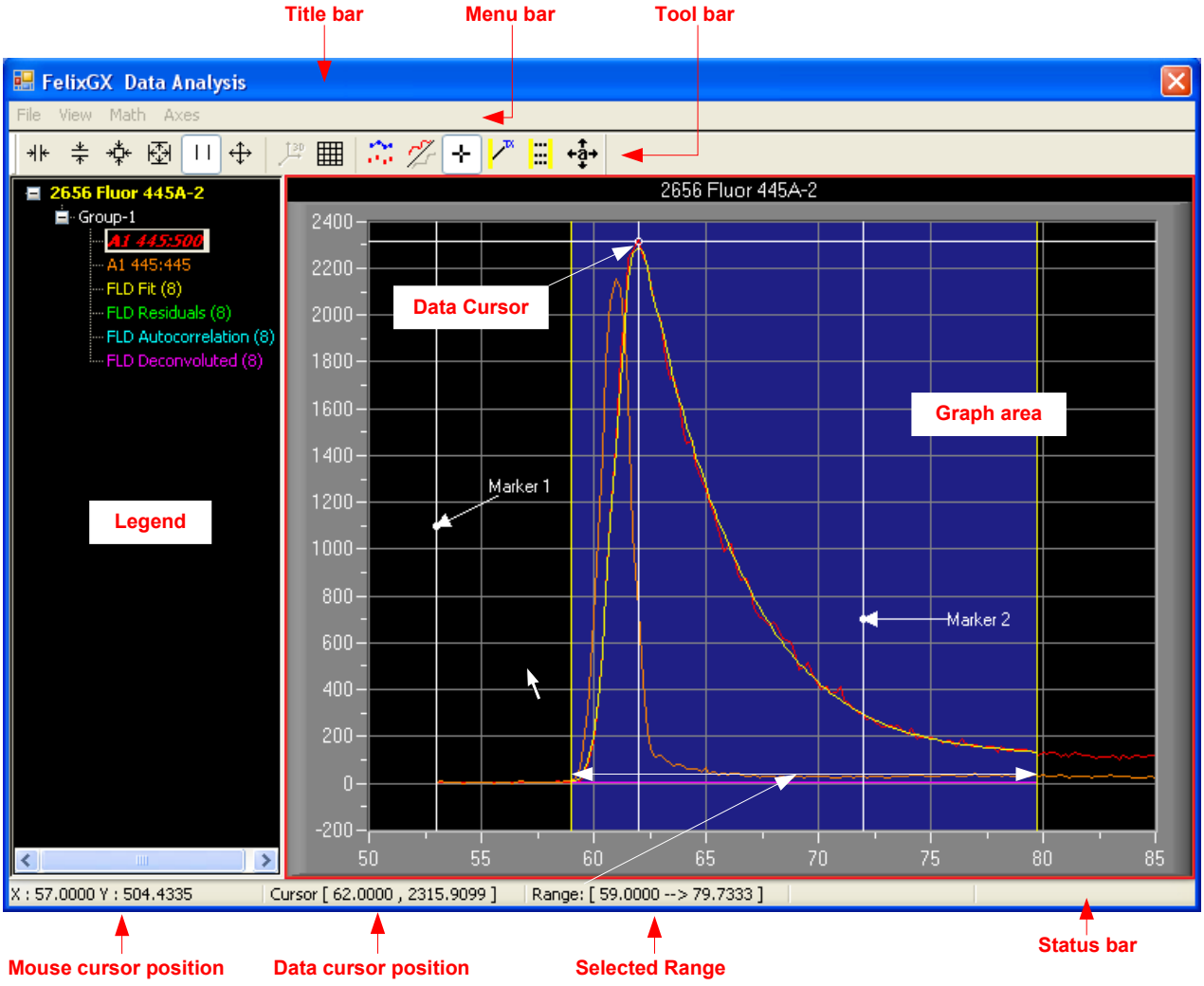
Launching the FelixGX Data Analysis window

To launch the Data Analysis window, in FelixGX select a single group name in the legend, then click on the menu items **File | Send to Data Analysis**.

Note: in this manual the terms *curve* and *trace* are used interchangeably.

NOTE: the FelixGX Data Analysis program window sits "On Top" of most other program windows and several of its own dialogs. If a dialog or other program window does not appear, move the FelixGX Data Analysis window to one side and the dialog or other window may be there.

The principal parts of the FelixGX Data Analysis screen are shown here and described below.



Across the top of the window runs the Title Bar. To move a window occupying less than the full monitor screen, click and drag on the title bar. To resize this window click and drag on an edge or corner of the window. By right-clicking on the Title Bar, you can see the version information about PTIGraphicX (PGX).

Beneath the Title Bar is the Menu Bar. Each heading in the Menu Bar contains a group of related commands. Below the Menu Bar is the Toolbar. The icons on the Toolbar provide instant access to a number of the most frequently used commands.

Beneath the Menu Bar and Toolbar is the space where data is displayed.

The Legend along the left side lists the names of the records, groups and traces. Click on a record, group, or trace name in the legend to make it active. Depending on the visibility of groups and traces in a record, all visible traces will be shown in the graph area. Right-clicking on a record, group or trace name in the Legend will show different command menus (see Legend submenus below). Double-clicking on a record or group name in the Legend will contract or expand the list below it. You can also click on a + sign in front of an record or group name to expand it, or click on a - sign in front of an record or group name to minimize it.

The dividing bar between the legend and graph area can be moved by clicking and dragging it with the mouse cursor. The right side of this window has an area where traces are plotted in Graph Mode, or displayed in spreadsheet form in Grid Mode. Right-clicking on the graph or grid area will show a menu of relevant commands (see Graph submenus below). If FelixGX Data Analysis is launched by clicking on a group name in the FelixGX legend and using the command **File | Send to Data Analysis**, the record name in FelixGX Data Analysis will be TempDataAnalysis. This name will also be shown as the graph title. If FelixGX Data Analysis uses the command **File | Open** to open a data file, then the file name will be used as the record name and graph title.

Across the bottom of the Workspace is the Status Bar that gives information about cursor positions and trace names. From left to right are fields for the current mouse cursor position (or most recent position if the mouse cursor has left the graph area), the data cursor position, the selected range, and the name of the trace that the mouse cursor is pointing to (if the mouse cursor is not on a trace, this field will be blank).

Toolbar

Note: whether the graph is at Full Autoscale or displaying an expanded region, the arrow keys on the keyboard can shift the graph in any direction as long as the Data Cursor is not active. When the Data Cursor is active, then the arrow keys move the Data Cursor around and Shift+arrow keys move the graph around. When the FelixGX Data Analysis is first opened, a new graph must be rescaled in any way before it can be moved around.

Zoom X

Zoom X causes the selected region to fill the entire X-Axis in the window. The toolbar shortcut is the only location to use this function. Select the icon, click and drag the mouse left or right in the graph area. Releasing the mouse button will expand the desired region.

The X-Axis is scaled in six modes. The other modes are:
Full Autoscale on the Axes menu and toolbar 
2x X-Zoom In on the Axes menu
2x X-Zoom Out on the Axes menu
Fixed X-Min & Max on the Axes menu
Zoom Rect on the toolbar 

 **Zoom Y**

Zoom Y causes a selected region to fill the entire Y-Axis in the window. The toolbar shortcut is the only location to use this function. Select the icon, click and drag the mouse up or down in the graph area. Releasing the mouse button will expand the desired region.

The Y-Axis is scaled in one of six modes. A symbol will appear in the Axes menu next to Full Autoscale, Autoscale from 0, Fixed Y-Min & Max, and Logarithmic Y-scale depending on the mode in use. The other modes are:
Full Autoscale on the Axes menu and toolbar 
Autoscale from 0 on the Axes menu
Fixed Y-Min & Max on the Axes menu
Logarithmic Y-Scale on the Axes menu
Zoom Rect on the toolbar 

 **Zoom Rect**

Zoom Rect causes a selected rectangular region to fill the entire window. The toolbar shortcut is the only location to use this function. Select the icon, click and drag the mouse in the graph area. Releasing the mouse button will expand the desired region.

 **Full Autoscale**

Displays the full range of data. This mode is also available on the Axes menu.

 **Range Toggle**

Some math functions are performed on a selected region of a curve (a subset of the X values). To select this region, first choose the target curve by clicking on its name in the legend. Then select the **Range Toggle** icon () from the graph toolbar and use the mouse to click and drag within the graph display over the desired region of the curve. For more precise control, you can then enter *Low X* and *High X* values into the text boxes provided. The selected region will be highlighted, and the desired math value will be displayed. The math function dialog box can be left open while different regions are selected, and math values, when displayed, will change dynamically.

 **Pan Graph**

Select this icon then click and drag on the graph and the graph will move with the cursor. Releasing the mouse button will turn this function off. If the Data Cursor is off, then the arrow keys will pan the graph in steps. If the data Cursor is on, then use Shift+arrow keys to pan the graph.

 **3D**

This icon is initially inactive. If a group has more than one trace, then clicking on the session, group, or trace name will activate the 3D icon. Clicking on this icon opens the PTIGraphicX 3D dialog (see 3D Display for a description).

 **Grid View**

Toggles the display between Grid mode and Graph mode. In grid mode curve(s) will be presented numerically on a grid in spreadsheet fashion.

 **Change Plot Mode**

This icon can be pressed 3 times to get different plot modes: dot, dot+line, line.

 **Toggle Trace Visibility**

Toggles the visibility OFF/ON of the selected traces.

 **Data Cursor Toggle**

Toggles the Data Cursor ON/OFF. The Data Cursor highlights a data point with a small circle at the intersection of a horizontal and a vertical line. When the Data Cursor is enabled the trace in the legend which it is assigned to will become italic. Use the left or right keys to move the Data Cursor along a trace, and the up or down keys to switch between traces. You can also click and drag the vertical bar to move the Data Cursor along a trace. The Data Cursor can also be enabled/disabled by double-clicking on a trace in the Legend. Each record can have its own Data Cursor.

Note: if you click outside the graph area then the graph area is no longer the active window and the Data Cursor will not move in response to arrow keys. You must click on the graph area again to make it and the Data Cursor active.

 **Add Event Marker**

Opens the “Add Event Marker” dialog, where events can be manually created.

 **Edit Event Markers**

Opens the “Events Window” dialog where events can be edited, deleted or hidden.

 **Move Annotation Captions**

Allows you to move the caption of an event marker. NOTE: to reposition the arrow move the caption to a Y value where you’d like the arrow and double-click the caption. This will create a horizontal arrow using the new position of the caption. You can activate **Move Annotation Captions** by double-clicking on a caption.

Legend submenus

Record:
Rename: rename the record
Close: closes a record that has been imported into the FelixGX Data Analysis window.
New Group: creates a new group
Import Group: import a PTIGraphicX Group file (*.pxg) as a new group
Export Record: same as **File/Save Record As...**
Import Events: import events from a file
Export Events: export events to a file using one of the formats shown at **File/Import** (active only if there are events)
Unmatch events: this will unmatch the events from a trace and removes the caption merging
Axis Properties: this makes adding/deleting X,Y,Z axes and editing the axes titles possible either for a record or a group. Note: the Z-axis is not available at this time.

Group:
Toggle Visibility: toggles the visibility of traces inside the selected group(s)
Hide All/Show All: hide/show all the traces in the selected group(s)
Plot Mode: specifies the plot mode of the traces inside the group
Rename: rename the group
Delete: remove the selected group(s) and all traces inside
Export Group: export the group to a file using the PTIGraphicX Group file (*.pxg), Felix Text File (*.txt) or Aligned Text File (*.atf) format.
Axis Properties: this makes adding/deleting X,Y,Z axes and editing the axes titles possible either for a record or a group. Note: the Z-axis is not available at this time.
Open 3D Display: opens the PTIGraphicX 3D dialog. See 3D Display for a description.

You can copy one or more groups by selecting them and then clicking and dragging the selected groups away from the group names.

Trace:
Toggle Visibility: changes the visibility of the selected trace(s)
Color: change the color of the trace using the Windows color dialog
Plot Mode: change the plot mode for the selected traces
Rename: rename the trace
Delete: remove the selected trace(s)
Trace Math: traces can be arithmetically combined and data can be fitted, smoothed, averaged, integrated, normalized, differentiated, etc. See the description of the Math Commands
Align Events: this will use the points from the trace to match them to the events that were added
Properties: shows trace properties. Not fully implemented at this time

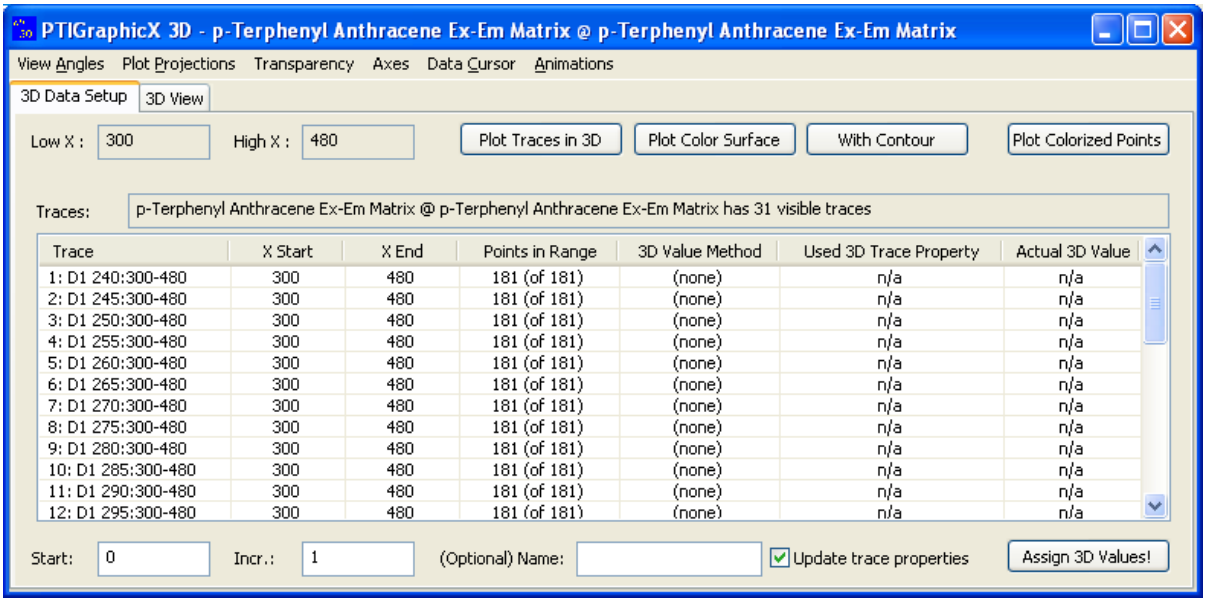
You can copy one or more traces by selecting them and then clicking and dragging the selected groups away from the trace names.
NOTE: some Group/Trace commands are available when selecting multiple items.

Graph submenus

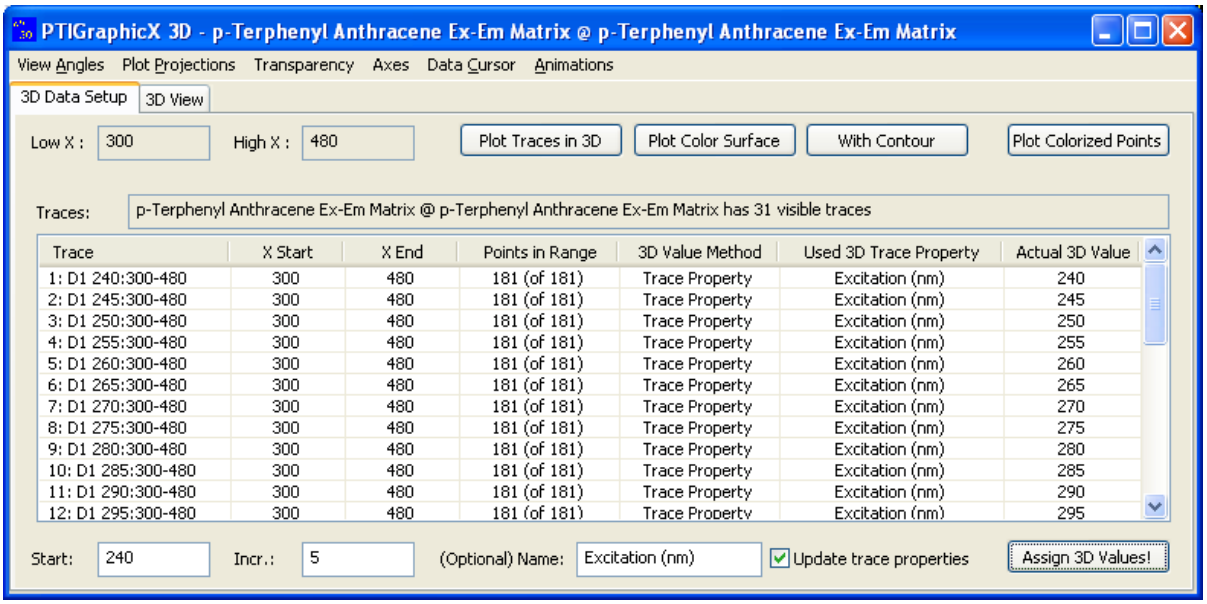
Activated by right-clicking on the graph area.
File: shows the File menu
    Open Record
    Save Record As...
    File Format Options...
    Import from Felix32
    Print Options
    Print...
View: shows the **View** menu
    Toolbar: toggles the Toolbar display Off/On
    Statusbar: toggles the Statusbar display Off/On
    Legend: toggles the Legend display Off/On
    Menu: toggles the Menu display Off/On
Graph: only appears if there are traces in the legend. Shows the **Axes** and **Math** menus, and
    Event Markers: **Add** and **List**.

3D Display

Open this dialog by clicking on the **3D icon**  , or by clicking on the **Open 3D Display** command on the Legend Group command.



Initially, the **3D Value Method** column shows (none), and the **Used 3D Trace Property** and **Actual 3D Value** columns show n/a. Enter a start value for the third axis parameter in the **Start** text box, an increment value in the **Incr.** text box, and optionally a name for the third axis in the **Name** text box, and then click **Assign 3D Values!** to update the table.



If the **Update trace properties** check box has been checked (default = yes), then the trace properties will also be updated with the 3D values.

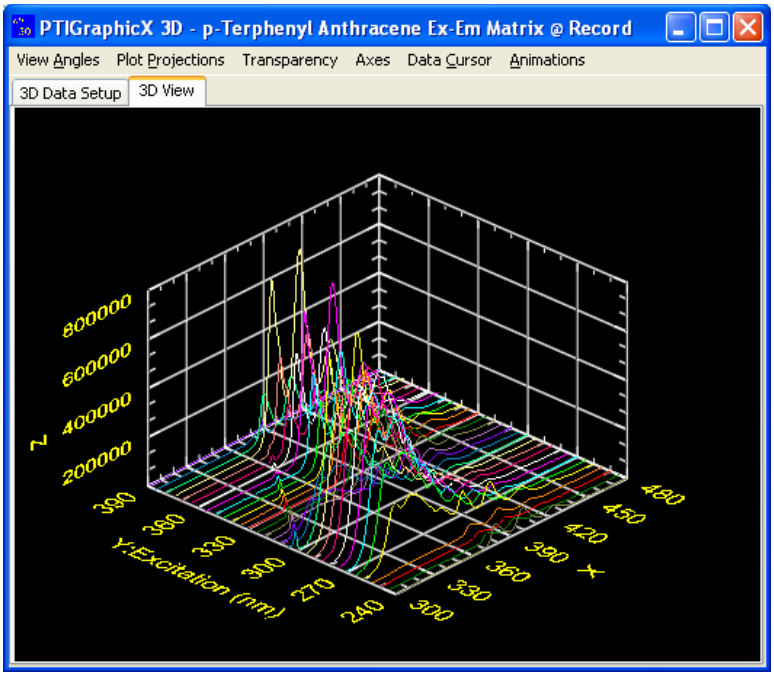
If the record is saved or exported as a PTIGraphicX Record File (*.pxa), or the group is exported as a PTIGraphicX Group file (*.pxg), then the 3D values will be saved with the file. Saving or exporting the record or group as a Felix text file (*.txt) or Aligned Text Format file (*.atf) does not save the 3D values with the file.

All of the traces in the group are displayed in the 3D graph, whether or not the visibility of some traces is turned off in 2D display. If an X-range of the data is defined by using the Range tool , then this range is shown in the **3D Data Setup** by the **Low X** and **High X** values and only that X-range will be displayed in the 3D graph. If the Range tool is not used, then all of the X-range will be displayed. If a 3D graph is currently displayed, and the Range tool is used to change the desired X-range on the 2D graph area, then you must click on the **3D Data Setup** button to refresh the 3D data table and then click on one of the **Plot** buttons to refresh the 3D graph with the new X-range.

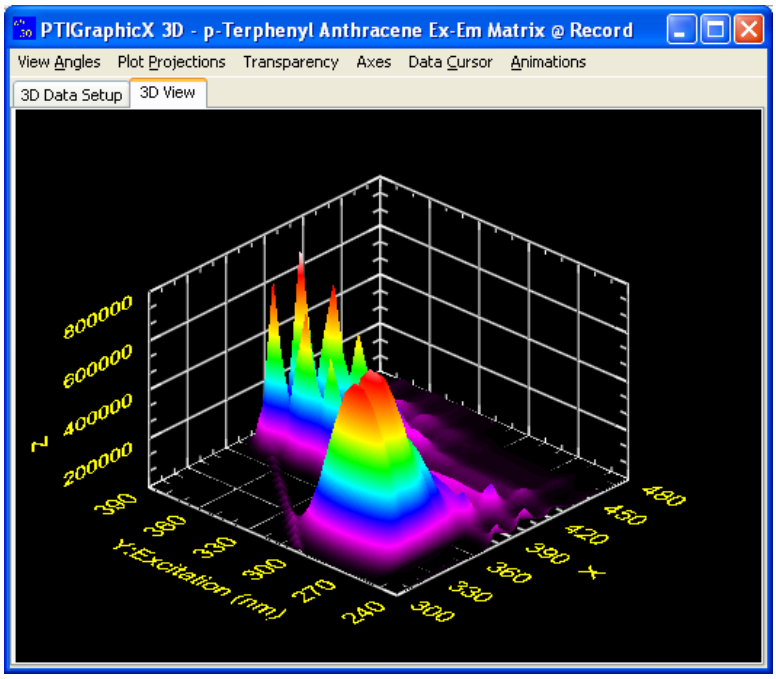
Plot buttons

These buttons show different ways of viewing the data in 3-D. In any 3D graph, clicking and dragging horizontally on the graph area rotates the graph about a Z- (Intensity-) axis (i.e., perpendicular to the XY plane) through the center of the screen. Clicking and dragging vertically on the graph area rotates (tilts) the image about a horizontal axis across the center of the screen. Pressing a Shift key and dragging on the graph area pans the image. Pressing an Alt key and dragging up or down on the graph area or using the mouse wheel zooms the image. A 3D graph is initially shown as an isometric view. Subsequent views of the same 3D data preserve the orientation of the last view. Grid lines, axes titles and grid labels are shown in all 3D views.

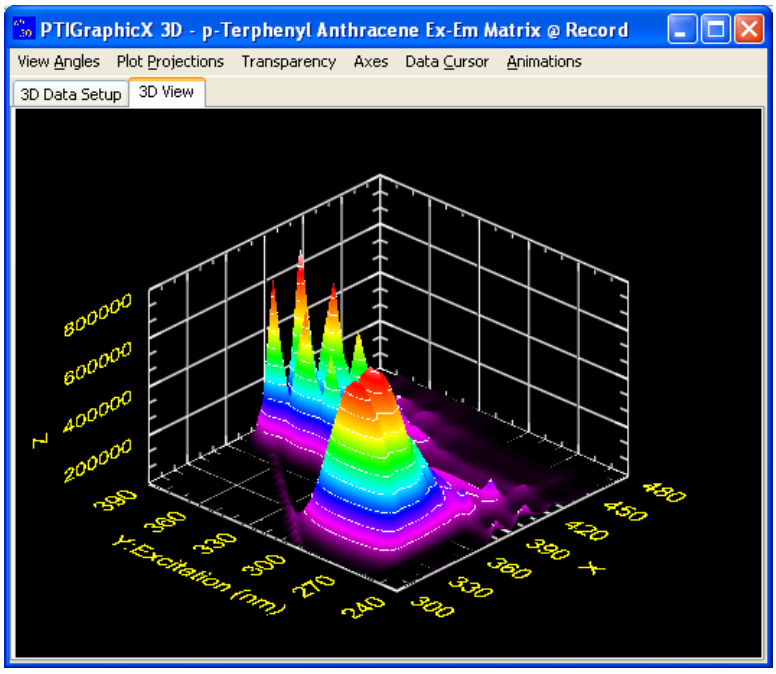
Plot Traces in 3D: shows the traces as separate lines in 3D using the same trace colors as in the legend and 2D graph.



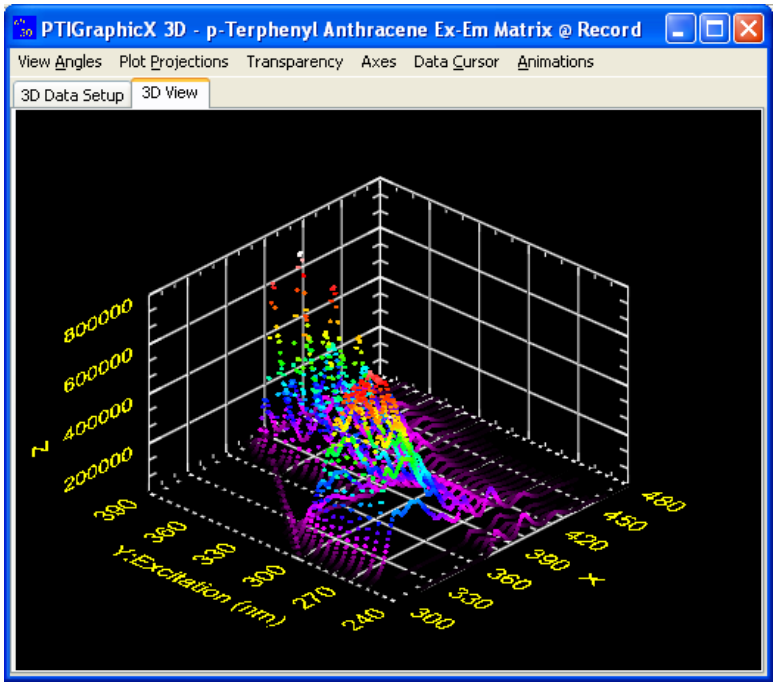
Plot Color Surface: shows a pseudo colored smooth surface with colors ranging from black for the minimum Z-value, then through violet to red, and then white for the maximum Z-value.



With Contour: shows the same as **Plot Color Surface** plus white constant Z-value contour lines.



Plot Colorized Points: shows only the data points with the same colors as in **Plot Color** Surface.



3D View: if this button is clicked before any of the Plot buttons have been clicked, then the 3D View will simply show an empty 3D graph with the axes and grid lines only. Once a Plot button has been used, then 3D View will show the last used 3D plot View.

View Angles

Towards X-Y Plane: Rotates the 3D View to show only the X-Y plane.

Towards Y-Z Plane: Rotates the 3D View to show only the Y-Z plane.

Towards X-Z Plane: Rotates the 3D View to show only the X-Z plane.

Reset 3D View: Changes the 3D graph to an isometric view.

Clicking and dragging on any of these views rotates the 3D view as described under Plot buttons.

Plot Projections

Show X-Y Projection: Shows a projection of the 3D plot onto the X-Y grid plane.

Show Y-Z Projection: Shows a projection of the 3D plot onto the Y-Z grid plane.

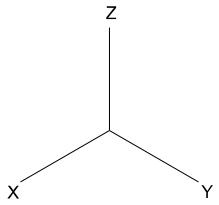
Show X-Z Projection: Shows a projection of the 3D plot onto the X-Z grid plane.

Show only Projections: Hides the 3D view and shows only the projections on the grid planes.

Transparency: Select the transparency of the 3D graph data – all views and projections.

Axes: Change how the data is displayed along each axis.

Inverted: Toggles inversion of the order of data along each axes ON/OFF. The default 3D view is isometric as shown, with the origin at the intersection of the axes, and increasing X, Y, or Z values along the axes towards the respective axes names. However, the default display of the data for X and Y values is to have smaller values at the bottom of the picture (in the center) and increasing towards the top of the picture. This data orientation is called inverted. The Z-axis is not inverted in the default view. The data orientation is preserved as the 3D view is rotated.



Logarithmic: Toggles the logarithmic scaling of data along an axis ON/OFF. When OFF, the data is displayed linearly along an axis from minimum to maximum values. When ON, the data is is displayed on a logarithmic scale using full decades.

Data Cursor

Show Cursor: Toggles the display of the 3D data cursor ON/OFF. When displayed, the (X, Y, Z) values are displayed at the center of dashed lines.

The following planes are only visible if the 3D cursor is visible.

Show X-Y Plane: Toggles ON/OFF a transparent gray plane parallel to the X and Y axes, at the Z-value of the data point.

Show X-Z Plane: Toggles ON/OFF a transparent gray plane parallel to the X and Z axes, at the Y-value of the data point.

Show Y-Z Plane: Toggles ON/OFF a transparent gray plane parallel to the Y and Z axes, at the X-value of the data point.

Animations

Rotate: Toggles ON/OFF the rotation of the 3D graph about the Z- (Intensity-) axis (i.e., perpendicular to the XY plane) through the center of the 3D graph window. Rotation is in one direction only. Clicking any of the View Angle commands or switching to the 3D Data Setup stops the rotation.

Shuffle: Toggles ON/OFF shuffling the above rotation between clockwise and counterclockwise directions.

Increment Speed (Num+): Press and hold the Num Lock and + keys on the numerical keypad to increase the rotation/shuffle speed. Clicking this command on the menu only increments the speed once per click.

Decrement Speed (Num-): Press and hold the Num Lock and - keys on the numerical keypad to decrease the rotation/shuffle speed. Clicking this command on the menu only decrements the speed once per click.

Menus

File Menu

Open Record

Use the **Open Record** command to open one or more data files stored on disk or on the network. The **Open** dialog box is a standard Windows Open dialog and shows the file name, and by clicking on the View Menu icon,  , and choosing Details, you can also show the time and date the file was last saved, and the file size.

To choose a different file location, click on the **Look in** text box and browse the file structure to find the location you want.

Note: FelixGX Data Analysis allows you to import other data files up to a maximum of 16 data records to be open at one time.

File name
Type a file name in the text box, or select the file name from the list.

Files of type
Depending on the settings selected in **File | File Format Options**, you can choose to show PTIGraphicX Record Files (*.pxa), Felix Text files (*.txt), Aligned Text Format files (*.atf), FeliX32 Analysis Dataset files.(*.ana), FeliX32 Analysis Group files(*.ang), TimeMaster 1.X files (*.tma), FeliX 1.X files (*.flx), GRAMS files (*.spc), or Andor Text Format files (*.asc). PTIGraphicX Record Files (*.pxa) are saved in a binary format and preserve groups and group names, events, and axes names. FeliX32 Analysis Dataset files.(*.ana), FeliX32 Analysis Group files(*.ang), TimeMaster 1.X files (*.tma), FeliX 1.X files (*.flx), GRAMS files (*.spc), or Andor Text Format files (*.asc) are other binary file formats. Felix Text Files (*.txt), and Aligned Text Files (*.atf) are tab delimited text (ASCII) and remove group name and axes name information – when reopened, all traces appear in one group named *"Group-1"* with unlabeled axes. Felix Text Files (*.txt), and Aligned Text Files (*.atf) must have the following format.

LINE 0: Aligned Text Files (*.atf) have "ALIGNED_FORMAT" inserted before the first line of the following.
LINE 1: number of curves, followed by carriage return/line feed
LINE 2: number of data pairs for first curve, two tab characters, number of data pairs for second curve… This line must end with one tab character and carriage return/line feed after the number of data points for the last curve.
LINE 3: first curve label, two tab characters, second curve label… This line must end with one tab character and carriage return/line feed after the curve label for the last curve.
LINE 4: first curve X-Axis label, tab character, first curve Y-Axis label, tab character, second curve X-Axis label, tab character, second curve Y-Axis label… ending with carriage return/line feed after the Y-axis label for the last curve.

LINES 5-N: first curve X value, tab character, first curve Y value, tab character, second curve X value, tab character, second curve Y value… ending with carriage return/line feed.

Note: All unused data fields must be padded with tab characters. For example, if the first curve has fewer data pairs than the second, tab pairs must be used prior to the remaining second

curve values. However, if latter curves have fewer data points than preceding curves, do not pad these data fields.

Note: In *.txt format files all curves start data in line 5. In *.atf (***a****ligned* ***t****ext* ***f****ormat)* files, the X-Y data pairs for each curve are placed so same X-data are placed on the same row starting with the smallest X-data in row 5.

Maximum number of characters per field is 32.
Maximum number of curves (or data pairs) is 30.
Maximum number of data pairs for any curve is unlimited.

For *.txt files the file name will be displayed as the record name. The information on line 4 (axes labels) will not be displayed on the screen. To label the axes, select the individual curves from the legend and use the Axes/Edit Axis Properties dialog.

Example of a *.txt format data file:
The following ASCII file contains data for three curves. The first is called TEST1 and has 3 data pairs representing Time versus Counts. The second is called TEST2 and has 5 data pairs representing Time versus Intensity. The third is called TEST3 and has 4 data pairs representing Wavelength versus Intensity.

```
3 \R \N
3 \T \T 5 \T \T 4 \T \R \N
TEST1 \T \T TEST2 \T \T TEST3 \T \R \N
Time \T Counts \T Time \T Intensity \T Wavelength \T Intensity \R \N
10 \T 100 \T 1 \T 160 \T 340 \T 1655 \R \N
20 \T 110 \T 2 \T 165 \T 345 \T 1650 \R \N
30 \T 120 \T 3 \T 170 \T 350 \T 1630 \R \N
\T \T 4 \T 175 \T 355 \T 1570 \R \N
\T \T 5 \T 180 \R \N
```

\R is a carriage return, ASCII code 10.
\N is a line feed, ASCII code 13.
\T is a tab character, ASCII code 9.
Note: Spaces are used in this example for clarity. No spaces would be used in the ASCII file. Tab characters are the only acceptable field delimiters.
If the file contents of the sample file above is viewed in a text editor (such as Windows Notepad), it would appear as follows:

```
3
3       5       4
TEST1       TEST2       TEST3
Time  Counts Time  Intensity  Wavelength  Intensity
10  100     1   160     340         1655
20  110     2   165     345         1650
30  120     3   170     350         1630
```

4 175 355 1570
5 180

Save Record As...

Use the **Save Record As** command to name and save the data of the active record. The **Save** dialog box is a standard Windows Save dialog and shows the file name, and by clicking on the View Menu icon,  , and choosing Details, you can also show the time and date and file size of existing files. Select **Save,** or select **Cancel** whereby the dialog box will close and nothing will be saved.

To choose a different file location, click on the **Look in** text box and browse the file structure to find the location you want.

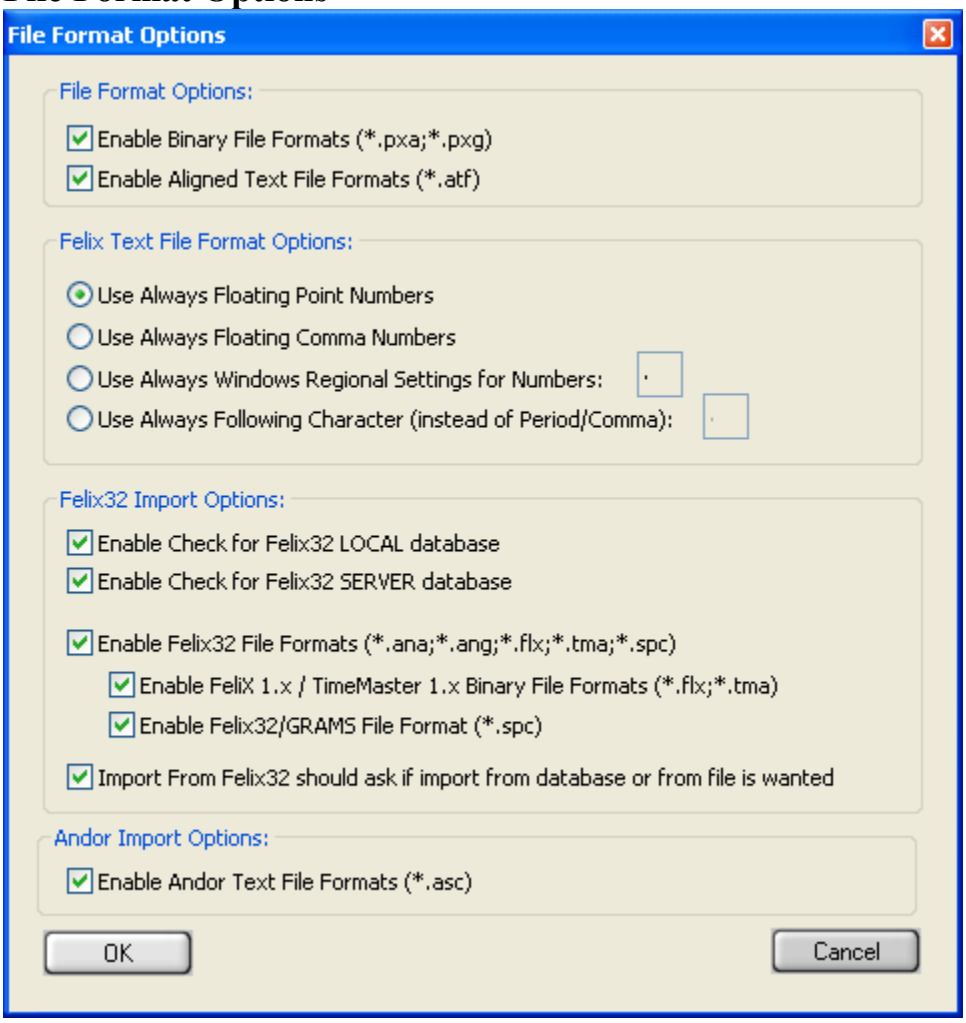
File name
Type a file name in the text box, or select the file name from the list. If you try to save as an existing file name, you will get a message that the file already exists. You must answer **Yes** to this question to save the data under an existing file name. Otherwise, choose **No** and enter a new file name.

Save as type
Depending on the settings selected in **File | File Format Options**, you can choose to save the active record as a PTIGraphicX Record File (*.pxa), Felix Text File (*.txt), or Aligned Text File (*.atf). PTIGraphicX Record Files (*.pxa) are saved in a binary format and preserve groups and group names, events, and axes names. Felix Text File (*.txt) and Aligned Text File (*.atf) are tab delimited text (ASCII) format and remove group name and axes name information – when reopened, all traces appear in one group named *"Group-1"* with unlabeled axes. The Aligned Text File (*.atf) type is shown only if the selected traces have different X-value distributions.

Save: saves the file.

Cancel: the dialog box will close and nothing will be saved.

File Format Options



This dialog enables you to choose the display of file types in the **File | Open Record**, **File | Save Record As**, and **File | Import from FeliX32** menu commands, or the Legend commands for importing or exporting records or groups.

File Format Options

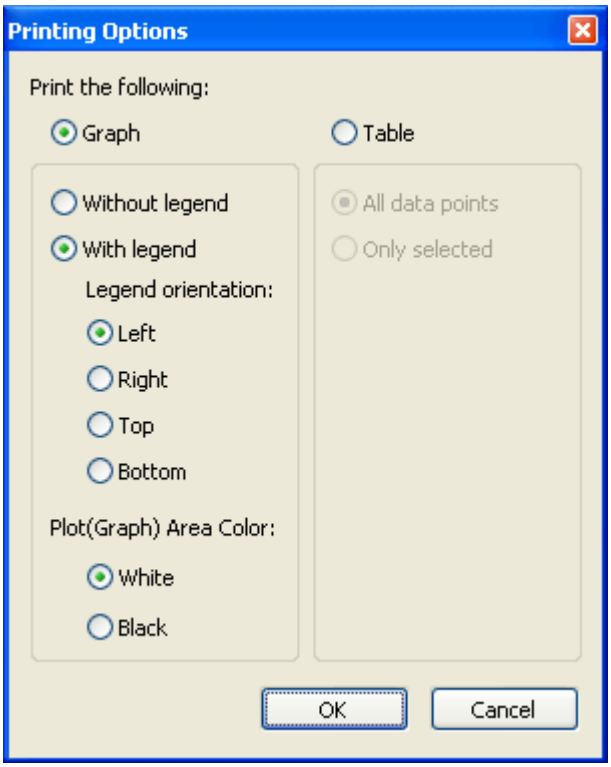
Enable Binary File Formats (*.pxa, .pxg): toggles the display of PTIGraphicX Record File (*.pxa) and PTIGraphicX Group File (*.pxg) file types.

Enable Aligned Text File Formats (*.atf): toggles the display of Aligned Text File Formats (*.atf).

Import from Felix32

Use the **Import from Felix32** command to log in to a Felix32 database and select a dataset to import into FelixGX Data Analysis. You can only select one dataset at a time from the database.

Print Options...



This dialog sets the options for printing a graph or table. Only those active traces in the active record will be printed in a graph or a table. If the Plot Area Color is made white, then a white trace will print as a black line.

Print

Use this command to print the contents of the active workspace. This command opens a dialog box where you can specify the range of pages to be printed, the number of copies, the destination printer, and other printer options. Refer to Windows documentation and online help for details on using this dialog box.

View Menu

Math/FitOutput

When doing any kind of Lifetime Data Analysis a text window named **Math/Fit Output** pops up containing identification information, fitted parameters and various statistics associated with the fit. Since this is a text window, the text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window.

This command reopens the **Math/Fit Output** text window.

File menu commands

Open: Opens a text file (for example result file from previous performed analysis).

Save: Saves text in the **Math/Fit Output** window as a text file (default extension .txt).

Edit menu commands

Cut: Cuts selected text area out of the text.

Copy: Copies selected text area.

Paste: Pastes from clipboard (for example text area which was cut / copied).

Select All: Selects all the text in the text window.

Clear: Deletes all text in the text window.

Identification Information

Analysis Function: Type of analysis.

Curves: Names of the curves the analysis is based on.

Time Range: Characterized by Start Time and End Time.

Start Parameters: Fixed or floating start values of the used parameters.

Statistic Results

Fitted Curve: Name of the curve generated by the fitting procedure.

Residuals: Name of the curve displaying the weighted difference between the calculated fit and the real data.

Autocorrelation: Name of the Autocorrelation curve.

Deconvoluted: Name of the Deconvoluted curve.

Chi2: Chi Square Statistic for testing correlation.

Durbin Watson: Durbin-Watson parameter for testing correlation.

Z: Parameter expressing the result of a Runs Test.

Pre-exponential: Defined as a_i in the equation $I(t) = \Sigma [(a_i)\exp(-t/\tau_i)]$, where t is time and τ is the lifetime.

Lifetime: Defined as τ in the equation $I(t) = \Sigma [(a_i)\exp(-t/\tau_i)]$, where t is time and a_i is the pre-exponential factor.

F1: Relative integrated intensities defined as $F_i = a_i\tau_i / \Sigma a_i\tau_i$, where a_i and τ_i are the pre-exponential factors and lifetimes, respectively.

Tau-av1: Steady state average lifetime defined as $\text{Tau-av1} = \Sigma a_i\tau_i^2 / \Sigma a_i\tau_i$, where a_i and τ_i are the pre-exponential factors and lifetimes, respectively.

Tau-av2: Amplitude average lifetime defined as $\text{Tau-av2} = \Sigma a_i\tau_i / \Sigma a_i$, where a_i and τ_i are the pre-exponential factors and lifetimes, respectively.

Fitted Parameters: Values and Deviations of the curve parameters resulting from the fit.

The following View menu commands are also available by right-clicking in the Graph area.

Toolbar
Toggles the Toolbar display Off/On.

Statusbar
Toggles the Statusbar display Off/On.

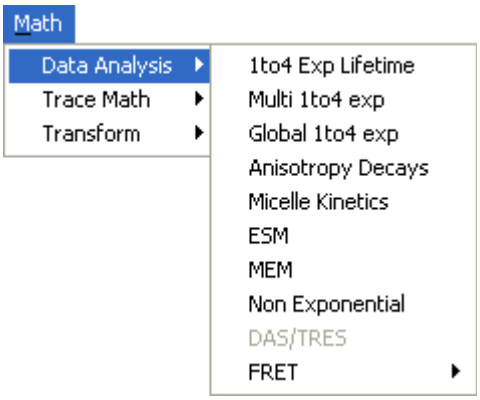
Legend
Toggles the Legend display Off/On.

Menu
Toggles the Menu display Off/On.

Math

Acquired or imported data can be processed mathematically through the commands in the Math menus. The Math menus are also available as Graph\Math by right-clicking on the graph area. **Data Analysis:** lifetime decay analysis functions which include but are not limited to 1-4 exponential fit, global analysis, MEM and ESM, and anisotropy decays. **Trace Math:** traces can be arithmetically combined and data can be fitted, smoothed, averaged, integrated, normalized, differentiated, etc. **Transform** allows you to convert spectra from energy to/from quanta, and wavelength to/from wavenumber.

Data Analysis



The various methods of data analysis are found under Math Analysis. They are:

1. 1 To 4 Exp. Lifetime
2. Multi 1 To 4 Exp.
3. Global 1 To 4 Exp.
4. Anisotropy Decays
5. Micelle Kinetics
6. ESM, MEM
7. Non Exponential
8. DAS/TRES – not available
9. FRET

These methods are covered in separate sections that are independent of each other. Thus, only the section of interest needs to be read. However, it is recommended that the **General Introduction** is read first as most of the concepts and topics used in the other sections are introduced there.

General Introduction

Fitting function

Every method of lifetime analysis depends on a model or fitting function for the decay of luminescence intensity. This may be as simple as a single exponential decay or as complicated as schemes for micelle kinetics including quenchers and distributions of micelle sizes etc. The various methods of analysis presented here differ mostly in the model they employ. The fitting function (explicitly dependent on time) is denoted in what follows as D(t) and can be thought of as the time dependent luminescence excited by a delta function (infinitely short) excitation pulse.

Convolution

In any pulsed excitation fluorescence lifetime instrument the finite width of the excitation pulse will distort the free decay of fluorescence as described by D(t). This distortion is known as convolution and the mathematical description is given by *Equation 1*:

$$I(t) = \int_0^t L(t-s)D(s)ds \qquad \text{Eq. 1}$$

where L(t) is the instrument response function (IRF) also known as the excitation pulse curve, and I(t) is the experimentally determined decay intensity at time t. The meaning of Equation 1 is that the intensity of the decay at time t is determined by both the continuous re-pumping of the fluorescence excitation during the emission from the nanosecond flash lamp or laser and the decay of fluorescence emission that has occurred up to time t.

The convolution distortion cannot be removed experimentally. Instead, the IRF L(t) is measured by using a scattering solution in a companion measurement prior to determining the fluorescence decay of the sample. This experimental L(t) is then used to actually determine I(t) from Equation 1 by the procedure known as iterative reconvolution.

Curve Fitting Procedure

The fitting procedure uses an iterative fitting procedure based on the Marquardt algorithm (Bevington, 1969) where the experimental data are compared to a model decay based on Equation 1. Deviations from the best fit are characterized by the reduced chi-square statistic, χ^2, as shown in *Equation 2*:

$$\chi^2 = \frac{1}{N-n-1} \sum_1^N \frac{(I(i)_{calc} - I(i)_{exp})^2}{s(i)^2} \qquad \text{Eq. 2}$$

where N is the number of data points (or channels), n is the number of fitting parameters, and s is the standard deviation (see below). The best fit is determined when chi-square is minimized. If the standard deviations are estimated correctly, a perfect fit to the data will produce a chi-square close to 1.0. Good results typically produce χ^2's of 0.9 to 1.2.

It is necessary to incorporate an estimate of the data precision when using a statistical fitting procedure. For the case of the stroboscopic optical boxcar the standard deviation is determined from within the decay by actually measuring the "noise" at time t and applying a special procedure developed for this type of experimental data. (James *et al*, 1992)

Artifacts

There are several well-known artifacts due to the intrinsic nature of photomultiplier tubes, etc… which must be accounted for during the analysis procedure.

***Color Shift Artifact Correction*:** Photomultiplier tubes do not respond identically at all wavelengths of incident light. This is due to the fact that the photoelectrons ejected from the photocathode will have excess kinetic energy when the incident photon is more energetic, i.e., bluer. This effect manifests itself primarily, although not exactly, as a zero-time shift in the excitation position relative to the decay. This time shift can be approximated by a single parameter δ as shown in *Equation 3*:

$$I(t) = a \exp\left(\frac{-(t+\delta)}{\tau}\right) \qquad \text{Eq. 3}$$

This parameter may be either determined experimentally or incorporated in the fitting procedure as a variable parameter. Both options are available in this program. The fitting procedure for using the variable time shift parameter is based on that from *Time-Correlated Single Photon Counting* by O'Connor and Phillips.

Analog Baseline Offset: Since the stroboscopic technique is based on an analog measurement, there will always be a small difference between the measured baseline for the IRF and that for the fluorescence decay, typically on the parts per thousand level. This offset can cause inaccuracies to the determination of fluorescence lifetimes since it will be treated as due to a true convolution effect. The effects of this offset can be removed (James *et al*, 1992) by determining the offset during the data analysis by assigning a non-convolved constant, c, to the decay intensity as shown in *Equation 4*:

$$I(t)_{corrected} = I(t)_{uncorrected} + c \qquad \text{Eq. 4}$$

Useful Statistical Parameters

A variety of statistical parameters have been developed to assist in determining the quality of the analysis. First among these is the reduced χ^2 parameter as discussed above. Others are:

Randomness of the Residual Pattern: The residual R_i is the difference between the calculated fit and the real data at time t_i. Weighted residuals r_i are the ratio of R_i to s_I, i.e., $r_i = R_i/s_i$ and should range from about -3.3 to 3.3.

A plot of these residuals should produce a flat pattern randomly distributed about zero with no features. Periodic oscillations or other deviations indicate a poor fit. This is a simple and reliable test for the goodness of the fit.

Autocorrelation Function of the Weighted Residuals: This function is calculated from *Equation 5* (Grinvald and Steinberg, 1974):

$$Cr_j = \frac{\frac{1}{m}\sum_{i=n_1}^{n_1+m-1} r_i r_{i+j}}{\frac{1}{n_3}\sum_{i=n_1}^{n_2} [r_i]^2} \qquad \text{Eq. 5}$$

where $n_3=n_2-n_1+1$, n_1 and n_2 are the first and last channels chosen to do the calculation over. An upper limit is set at $j = n_3/2$ to allow for maximal testing of a finite data set.

By definition $Cr(0)=1$. For the remaining points Cr_j should form a flat band of high frequency low amplitude noise about zero. Any pattern indicates a lack of fit. The autocorrelation function is very sensitive to any radio-frequency (RF) noise.

Durbin-Watson Parameter: This parameter was introduced by Durbin and Watson (Biometrika, 1950, 1951) to test for correlations. The parameter DW is defined in *Equation 6* as:

$$DW = \frac{\sum_{i=n_1+1}^{n_2} [r_i - r_{i-1}]^2}{\sum_{i=n_1}^{n_2} [r_i]^2} \qquad \text{Eq. 6}$$

where the other parameters are defined above.

This parameter may be interpreted as follows; the fit is likely satisfactory if the value of DW is greater than 1.7, 1.75 and 1.8 for single, double and triple exponential fits respectively (O'Connor and Phillips, 1984).

Runs Test: The runs test determines the number of positive and negative groups or runs of the residuals as defined in *Equation 7*:

$$z = \frac{zn}{\sqrt{(zd)}} \qquad \text{Eq. 7}$$

where

$$zn = (nn + np) - \frac{(2 \times nvn \times nvp)}{(nvn + nvp)} + 1$$

$$zd = \frac{2 \times nvn \times nvp \times (2 \times nvn \times nvp - nvn - nvp)}{(nvn - nvp)^2 \times (nvn + nvp - 1)}$$

and np is the number of positive transitions, nn is the number of negative transitions, nvn is the number of negative residuals and nvp is the number of positive residuals.

A value of $-1.96 < Z$ indicates a satisfactory fit at the 95% confidence level (Hamburg, 1985).

The general structure of each analysis program is the same, so there is considerable similarity in running the programs. For each method the data to be analyzed and time range over which to analyze must be selected. Then the initial model parameters must be selected, perhaps holding some of them at pre-selected values. Finally, the fit is run and the results interpreted.

1 To 4 Exponential Lifetime

Theory

This is the simplest and arguably the most generally useful of the fitting procedures. It is suitable for the analysis of fluorescence decays consisting of up to 4 exponentials and associated pre-exponentials.

Fitting Function

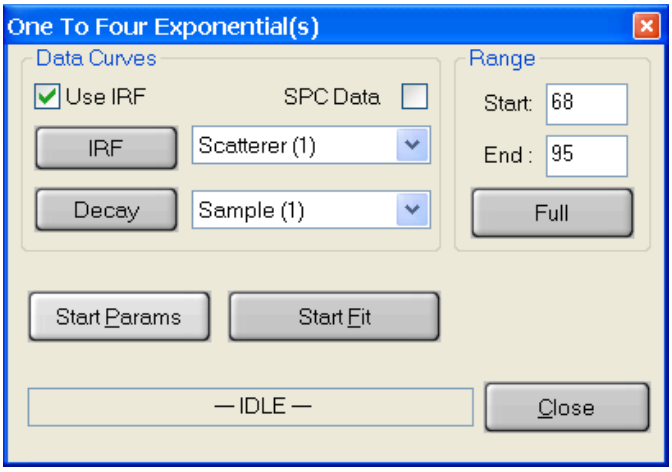
This analysis program can fit up to a 4 exponential decay which follows the fitting law:

$$D(t) = \sum a_i \exp\left\{\frac{-t}{\tau_i}\right\} \qquad \text{Eq. 1}$$

where D(t) is the delta function generated decay at time t. This fitting function allows for negative a_i's so that risetimes can also be determined with this program.

Using the Program

The initial **One To Four Exponential(s)** dialog box is shown below.



Data Curves

The **Use IRF** check box selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** check box is used only when single photon counting data has been imported.

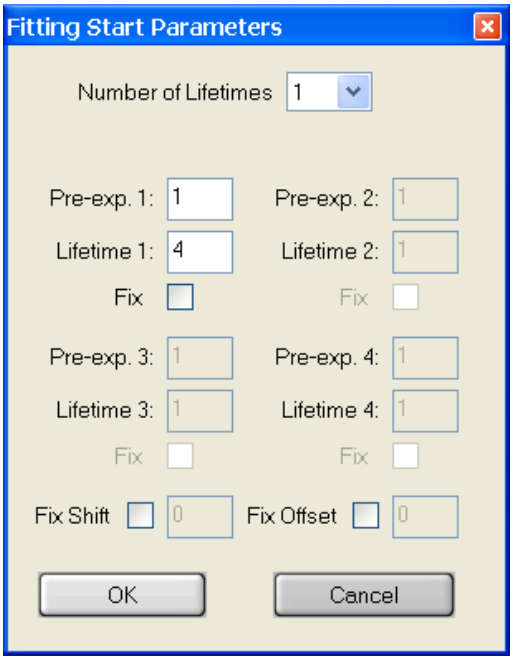
The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will appear in the box beside the button.

The **Decay** button selects the curve to be analyzed. Select a curve by clicking on its name in the legend then click on the **Decay** button. The name of the selected curve will appear in the box beside the button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box that allows these values to be entered.

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.



The **Start Parameters** dialog box for the **1 To 4 Exp**. **Lifetime** method is shown at left.

The **Number of Lifetimes** text box selects the number of different lifetimes used to analyze the decay curve. Select a number between 1 and 4. Normally, for the first fit of a new sample, the number one is chosen.

Pre-exp., Lifetime, Fix: For each of the lifetimes to be used in the fit an initial guess for the lifetime and the pre-exponential factor (*Pre-exp*) must be given. Only the relative values of the pre-exponential factors are relevant here so that for a single exponential fit the value 1 is normally used. Each of the lifetimes chosen for the analysis may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are very poor. If this occurs, try changing the starting values.

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Fix Offset: Because of difficulties in establishing a noise free baseline there may be a small intensity offset for a decay curve. This offset may be included as a parameter in the analysis. The offset parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the offset is allowed to float, a value of 0.0 is used as the initial guess.

Study of the portion of the sample and scatterer curves before the laser pulse may indicate whether an offset is required. However, it is often better to adjust the sample and scatterer curves to average zero before the pulse (using the math functions provided in the graphics module) than to trust the fit. Once all parameters have been set, click the **OK** button to return to the previous dialog box and then **Start Fit** to start the analysis.

Results

The results of the analysis are displayed in two forms.

1. The fitted curve, the weighted residuals, the autocorrelation function and the deconvoluted decay (i.e. D(t)) appear in the workspace.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.

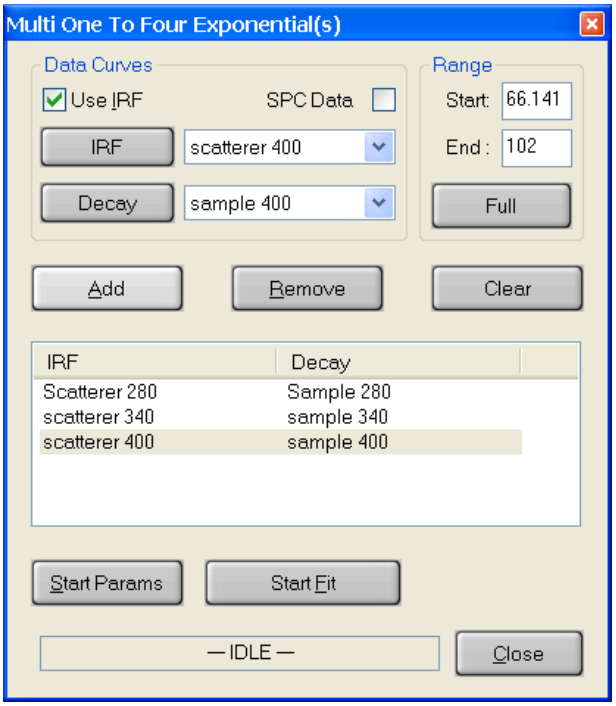
Multi 1 To 4 Exponential

The multiple file one to four exponential lifetime method, as its name implies, allows the analysis of multiple scatterer/sample pairs at the same time. Each pair will be separately analyzed over the same range with the same number of exponentials and the same options. The analysis results in a set of parameters (lifetimes and pre-exponential factors) for each data pair. The theory for this method is exactly the same as that for the **1 To 4 Exp. Lifetime** method.

This type of analysis is useful when a series of otherwise identical decay curves has been collected as a function of some parameter (temperature, composition or wavelength for example). Trends in the values of the lifetime parameters may then be recognized rather easily.

Using the Program

The initial dialog box for **Multi One To Four Exponential(s)** is shown below.



Data Curves

The **Use IRF** check box selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** check box is used only when single photon counting data has been imported.

Multiple scatterer/sample pairs are selected by first choosing a single pair.

The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will appear in the box beside the button.

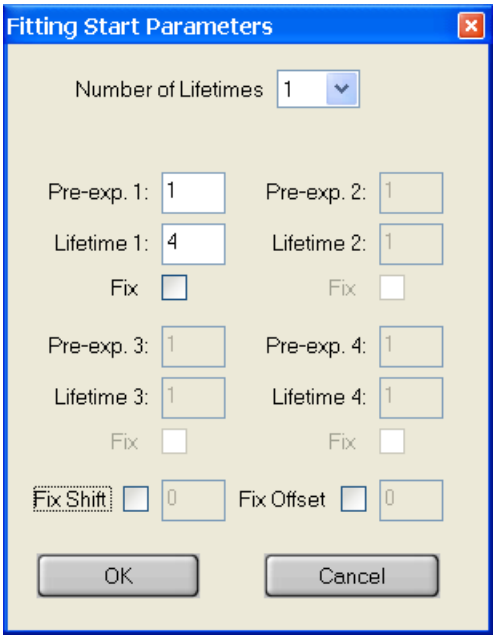
The **Decay** button selects the curve to be analyzed. Select a curve by clicking on its name in the legend then click on the **Decay** button. The name of the selected curve will appear in the box beside the button.

Enter this data pair into the analysis by clicking the **Add** button at which point their names appear in the text window. Data pairs may be deleted by clicking on the appropriate line in the text window to highlight the line then clicking on the **Remove** button. All sample pairs may be removed by clicking on the **Clear** button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box that allows these values to be entered.

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.



The **Start Parameters** dialog box for the **Multi 1 To 4 Exp**. method is shown at left and is identical to the dialog for **1 to 4 Exp. Lifetime**.

The **Number of Lifetimes** text box selects the number of different lifetimes used to analyze the decay curves. Select a number between 1 and 4. Normally, for the first fit of a new sample, the number one is chosen.

Pre-exp., Lifetime, Fix: For each of the lifetimes to be used in the fit an initial guess for the lifetime and the pre-exponential factor (*Pre-exp*) must be given. Only the relative values of the pre-exponential factors are relevant here so that for a single exponential fit the value 1 is normally used. Each of the lifetimes chosen for the analysis may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are very poor. If this occurs, try changing the starting values.

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see the **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Fix Offset: Because of difficulties in establishing a noise free baseline there may be a small intensity offset for a decay curve. This offset may be included as a parameter in the analysis. The offset parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the offset is allowed to float, a value of 0.0 is used as the initial guess.

Study of the portion of the sample and scatterer curves before the laser pulse may indicate whether an offset is required. However, it is often better to adjust the sample and scatterer curves to average zero before the pulse (using the math functions provided in the graphics module) than to trust the fit.

Once all parameters have been set, click the **OK** button to return to the previous dialog box and then **Start Fit** to start the Analysis.

Results

The results of the analysis are displayed in two forms.

1. The fitted curve, the weighted residuals, the autocorrelation function and the deconvoluted decay (i.e. D(t)) appear in the workspace.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.

Global 1 To 4 Exponential

Theory

This analysis program provides for the analysis of up to 4 exponential decays for a number of data files simultaneously. The global analysis assumes that the lifetimes are linked among the data files but that the associated pre-exponentials are free to vary.

Fitting Function

The analysis program can fit up to a 4 exponential decay, which follows the fitting law:

$$D(t) = \sum a_i \exp\left\{\frac{-t}{\tau_i}\right\} \qquad \text{Eq. 1}$$

where D(t) is the delta function generated decay at time t. This fitting function allows for negative a_i's so that risetimes can also be determined with this program.

Global Analysis

Global analysis is a procedure whereby several data sets, which have parameters in common may be analyzed simultaneously (Knutson, Beechem and Brand, 1983). This program assumes that the lifetimes are linked among the data files, ie., the lifetimes are the same for all decays. This is accomplished by using a matrix mapping of the fitting parameters whereby the pre-exponentials are unique for each decay curve while the lifetimes are mapped to the same value for each decay.

For example, two linked lifetimes with 2 unique pre-exponentials each and 4 decay curves map as:

$$\begin{vmatrix} a(1,1) & a(1,2) & a(1,3) & a(1,4) \\ \tau(1,1) & \tau(1,1) & \tau(1,1) & \tau(1,1) \\ a(2,1) & a(2,2) & a(2,3) & a(2,4) \\ \tau(2,1) & \tau(2,1) & \tau(2,1) & \tau(2,1) \end{vmatrix} \qquad \text{Eq. 2}$$

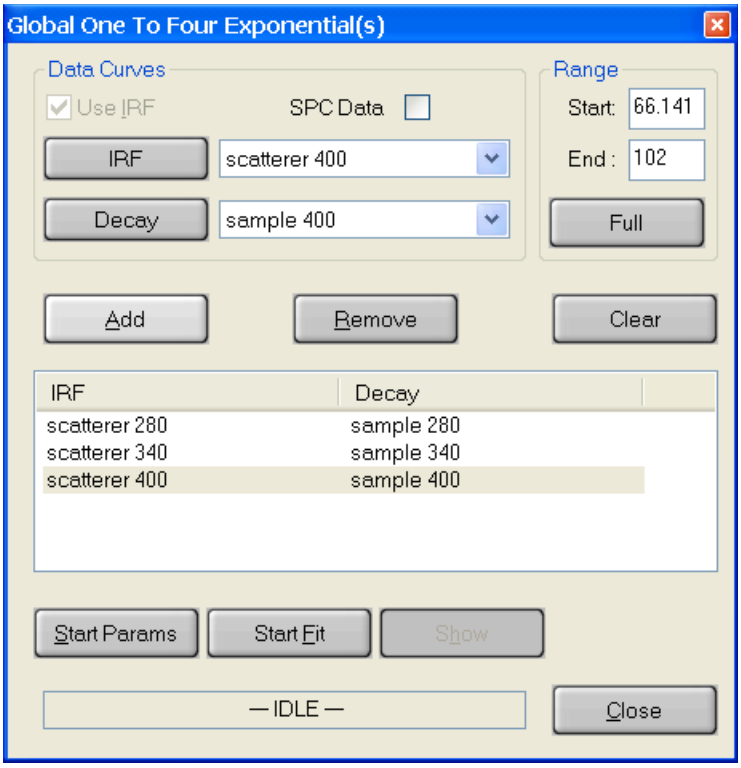
Least squares data analysis using the Marquardt algorithm is done on all data files simultaneously using the map to substitute parameters appropriately while minimizing the global χ^2_g:

$$\chi^2_g = \sum_j \chi^2_i \qquad \text{Eq. 3}$$

where χ^2_i is given by Equation 2 of the **General Introduction**. Refer to the **General Introduction** for a discussion of fitting procedures and statistical parameters. This type of analysis is useful when a series of otherwise identical decay curves has been collected as a function of some parameter, which alters the relative amounts of two or more fluorophores without altering their lifetimes. For example, this form of analysis could be used for various mixtures of non-interacting fluorescent compounds.

Using The Program

The initial dialog box for **Global One To Four Exponential(s)** is shown below.



Data Curves

Note: Global 1-4 Exponential analysis requires that all the data curves to have the same time data (X-axis data).

The **Use IRF** check box selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** check box is used only when single photon counting data has been imported.

Multiple scatterer/sample pairs are selected by first choosing a single pair.

The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will appear in the box beside the button.

The **Decay** button selects the curve to be analyzed. Select a curve by clicking on its name in the legend then click on the **Decay** button. The name of the selected curve will appear in the box beside the button.

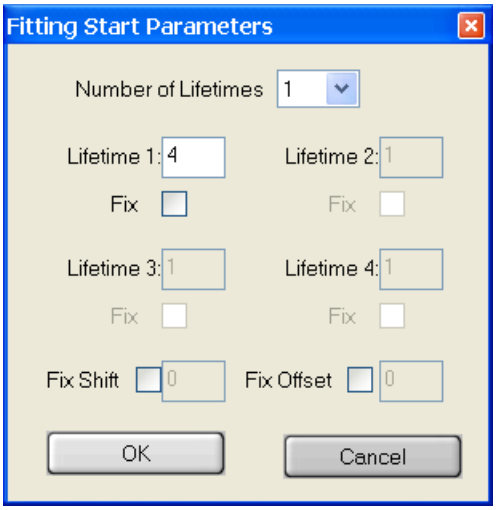
Enter this data pair into the analysis by clicking the **Add** button at which point their names appear in the text window. Data pairs may be deleted by clicking on the appropriate line in the text window to highlight the line then clicking on the **Remove** button. All sample pairs may be removed by clicking on the **Clear** button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box, which allows these values to be entered.

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.

Show: In order to avoid screen congestion, only selected analysis curves will be displayed. Data pairs are selected by clicking on the appropriate line in the text window to highlight the line. Clicking on the **Show** button will then display the fitted curve, the residuals, autocorrelation, and deconvoluted curves associated with this data pair.



The **Start Parameters** dialog box for the **Global 1 To 4 Exp**. method is shown at left.

The **Number of Lifetimes** text box selects the number of different lifetimes used to analyze the decay curves. Select a number between 1 and 4. Normally, for the first fit of a new sample, the number one is chosen.

Lifetime, Fix: For each of the lifetimes to be used in the fit an initial guess for the lifetime must be given. Each of the lifetimes chosen for the analysis may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are very poor. If this occurs, try changing the starting values.

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see the **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Fix Offset: Because of difficulties in establishing a noise free baseline there may be a small intensity offset for a decay curve. This offset may be included as a parameter in the analysis. The offset parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the offset is allowed to float, a value of 0.0 is used as the initial guess.

Study of the portion of the sample and scatterer curves before the laser pulse may indicate whether an offset is required. However, it is often better to adjust the sample and scatterer curves to average zero before the pulse (using the math functions provided in the graphics module) than to trust the fit.

Once all parameters have been set, click the **OK** button to return to the previous dialog box and then **Start Fit** to start the Analysis.

Results

The results of the analysis are displayed in two forms.

1. The results may be displayed in graphical form. However, in order to avoid screen congestion, only selected analysis curves will be displayed. Data pairs are selected by clicking on the appropriate line in the text window of the **Global One To Four Exponential(s)** dialog box to highlight the line. Clicking on the **Show** button will then display the fitted curve, the weighted residuals, autocorrelation and deconvoluted curves associated with this data pair.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.

Anisotropy Decays

Theory

This program allows for the calculation of up to four rotational correlation times plus a residual anisotropy term. The program first allows the user to calculate the fluorescence lifetime(s) from the parallel and perpendicularly polarized emission intensities. The user can then calculate the rotational correlation time(s).

Fitting Function for Fluorescence Lifetimes from Polarized Emissions

The analysis program can fit up to a 4 exponential decay, which follows the decay law:

$$D(t) = \sum a_i \exp\left\{\frac{-t}{\tau_i}\right\} \qquad \text{Eq. 1}$$

where D(t) is the delta pulse excited decay function at time t. This fitting function allows for negative a_i's so that risetimes can also be determined with this program.

For polarized light, D(t) may be calculated from the raw data:

$$D(t) = I(t)_{par} + 2 \times G \times I(t)_{per} \qquad \text{Eq. 2}$$

where $I(t)_{par}$ is the intensity of light detected with a vertical excitation polarizer and a vertical emission polarizer (*i.e.*, the polarizers are parallel to each other), $I(t)_{per}$ is the intensity of light detected with a vertical excitation polarizer and a horizontal emission polarizer (*i.e.*, the polarizers are perpendicular to each other), and G is the correction term for the relative throughput of each polarization through the emission optics (see below).

Convolution

Refer to the **General Introduction** for information on convolution.

Decay of Anisotropy

Anisotropy, r(t), is defined as:

$$r(t) = \frac{I(par) - G \times I(per)}{I(par) + 2 \times G \times I(per)} \qquad \text{Eq. 3}$$

where I(par) and I(per) are as defined above and the time dependence is assumed. This function is known to decay with a multi-exponential decay law (Phillips *et al*, 1985):

$$r(t) = \sum_{i=1}^{5} b_i \exp\left(\frac{-t}{\phi_i}\right) + b_\infty \qquad \text{Eq. 4}$$

Although the sum can run to 5 terms for completely anisotropic rotational motion, at lower precision levels and with relatively symmetric rotors, equation 4 will only yield in practice 1 or 2 terms. The b_∞ term refers to residual anisotropy remaining after all the transient terms have decayed and is commonly interpreted to imply restricted motion of the rotor.

G-Factor

The instrumental G-Factor is measured in a separate experiment that can employ the same sample for which the polarization is being measured. The experimental procedure is to set the excitation polarizer to the horizontal orientation and then measure the intensity of emission for both the vertical and horizontal orientations of the emission polarizer. The G-Factor is then defined as:

$$G = \frac{I_{HV}}{I_{HH}} \qquad \text{Eq. 5}$$

Since the excitation polarization is horizontal, and thus perpendicular to both the vertical and horizontal orientations of emission, any movement of the emitting molecules will make equal contributions to both orientations of emission. Thus the intensity of both vertically and horizontally polarized light reaching the emission monochromator will be equal and will have identical time evolution. Any differences in the observed intensity must then be due to polarization artifacts of the detection channel (diffraction gratings are well known to have different efficiencies for light polarized parallel and perpendicular to the rulings). Equation 5 determines the ratio of such artifacts and provides a correction factor.

For luminescence decays the G factor may be determined in either of two ways.

The decay may be recorded with both orientations of the analysing polarizer and the G factor determined from the ratio of the integrated intensities.

$$G = \frac{\int I(t)_{HV}\,dt}{\int I(t)_{HH}\,dt} \qquad \text{Eq. 6}$$

Since the time evolution of both decays is identical, the G factor may be determined from the ratio of signals recorded with both orientations of the analyzing polarizer at *any* time. It is convenient to determine this ratio using Fluorescence Timebased measurements with the delay set just after the peak of the luminescence decay. This method is likely to be faster than the first method.

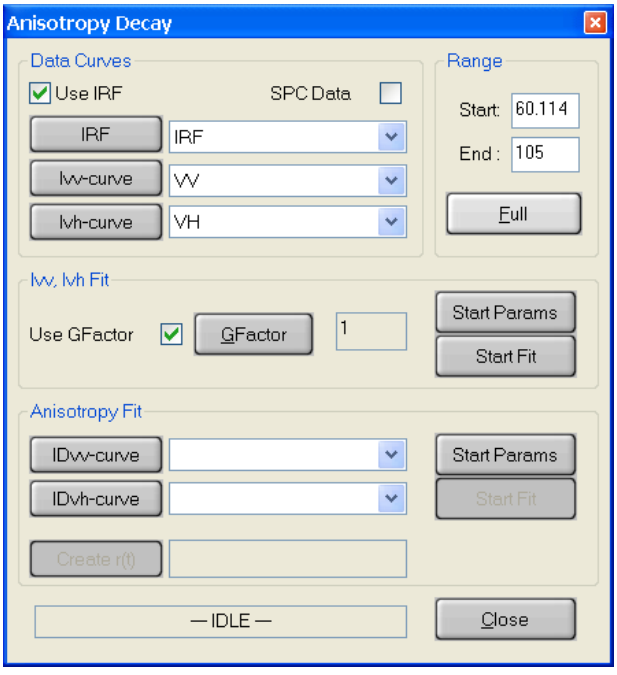
Curve Fitting Procedure

Refer to the General Introduction for information on fitting procedures and fitting statistics. Numerical analysis of anisotropy decay data is non-trivial. For this software, we chose to perform fits on the raw data files, I_{vv} and I_{vh}, without manipulating the curves prior to analysis. In the first step, the I_{vv} and I_{vh} curves are analyzed simultaneously by the global multi-exponential program. The fitted deconvoluted curves, ID_{vv} and ID_{vh} are then used to create the anisotropy function r(t) according to Equation 3. The so-constructed r(t) is free of any convolution effects and can be directly fitted to Equation 4. It must be remembered that r(t), being constructed from fitted curves, contains no experimental noise and therefore typical fit criteria like the χ^2 value, randomness of weighted residuals, D-W parameters etc… do not apply. Instead, the guiding criterion should be the minimum value of the sum of the least squares obtained with different kinetic models, *i.e.*, different numbers of parameters.

The interpretation of the b_j's is that b_j at t=0 is the initial polarization of the molecule (for single exponential decays, often known as r(0) and b_∞ is the residual polarization, often known as r_∞.

Using the Program

The initial **Anisotropy Decay** dialog box is shown below.



Data Curves

The **Use IRF** checkbox selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** checkbox is used only when single photon counting data has been imported.

The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will appear in the box beside the button.

The **Ivv-curve** and **Ivh-curve** buttons select the two curves to be analyzed. Select a curve by clicking on its name in the legend then click on the **Ivv-curve** or **Ivh-curve** button. The name of the selected curve will appear in the box beside the button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Ivv, Ivh Fit

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box, which allows these values to be entered (see below).

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.

The user has the option of using a G factor in the analysis or not. Toggle this option on or off by clicking on **Use GFactor**. Clicking on the **GFactor** button opens a dialog box that allows the G factor to be entered in several different ways (see below).

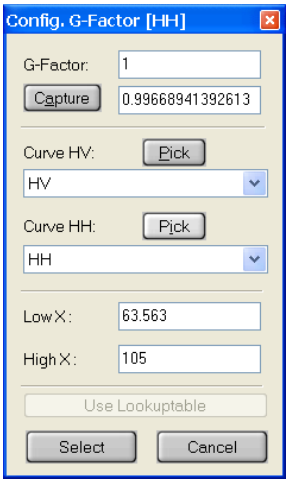
Anisotropy Fit

The **IDvv-curve** and **IDvh-curve** buttons select the two deconvoluted curves to be analyzed. Select a curve by clicking on its name in the legend then click on the **IDvv-curve** or **IDvh-curve** button. The name of the selected curve will appear in the box beside the button.

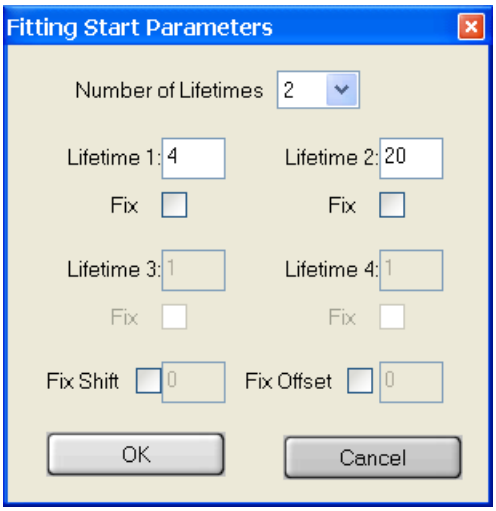
The **Create r(t)** button calculates r(t) from the ID_{vv} and ID_{vh} curves.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box, which allows these values to be entered (see below).

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.



The **Configure G-Factor** dialog box is shown at left. The G-factor may be entered directly into the **G-factor** text box or captured from HV and HH decays. To capture the G-factor select the HV curve in the left legend and click on the **Curve HV Pick** button. Select the HH curve in the left legend and click on the **Curve HH Pick** button. Select the region of the curves to be used in calculating the G-factor in the normal manner (usually this is the whole decay curve). The ratio of the integrals under the HV and HH curves is displayed in the **Capture** text box. Click on **Capture** to accept this value for the G-factor, then click on the **Select** button to copy this value to the Anisotropy Decay dialog and close the Configure G-Factor dialog. It will be displayed in the **G-Factor** text box. **Use Lookuptable** is not available at this time.



The **Start Parameters** dialog box for the **Ivv, Ivh Fit** is shown at left.

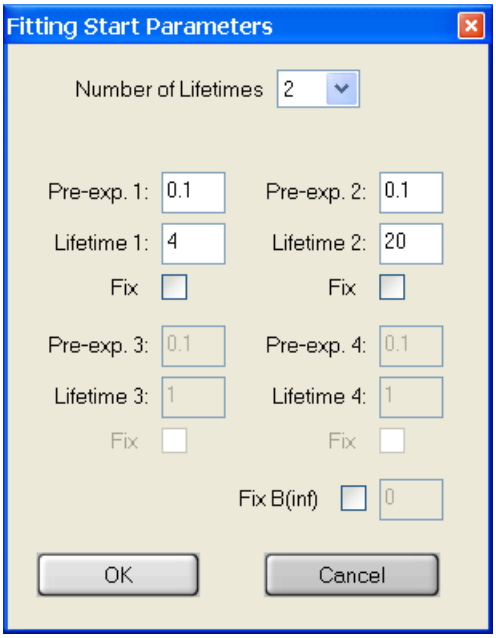
The **Number of Lifetimes** text box selects the number of different lifetimes used to analyze the decay curve. Select a number between 1 and 4.

Lifetime, Fix: For each of the lifetimes to be used in the fit an initial guess for the lifetime must be given. Each of the lifetimes chosen for the analysis may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are very poor. If this occurs, try changing the starting values.

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see the **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Fix Offset: Because of difficulties in establishing a noise free baseline there may be a small intensity offset for a decay curve. This offset may be included as a parameter in the analysis. The offset parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the offset is allowed to float, a value of 0.0 is used as the initial guess.

Study of the portion of the sample and scatterer curves before the laser pulse may indicate whether an offset is required. However, it is often better to adjust the sample and scatterer curves to average zero before the pulse (using the math functions provided in the graphics module) than to trust the fit. Once all parameters have been set, click the **OK** button to return to the previous dialog box and then **Start Fit** to start the Analysis.



The **Anisotropy Fit** start parameters dialog box is shown at left.

The **Number of Lifetimes** text box selects the number of different lifetimes used to analyze the r(t) curve. Select a number between 1 and 4.

Lifetime, Fix: For each of the lifetimes to be used in the fit an initial guess for the lifetime must be given. Each of the lifetimes chosen for the analysis may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are very poor. If this occurs, try changing the starting values.

Fix B(inf): B_∞ is the long time residual polarization and may be included as a parameter in the analysis. The parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the offset is allowed to float, a value of 0.0 is used as the initial guess.

Once all parameters have been set, click the **OK** button to return to the previous dialog box and then **Start Fit** to start the Analysis of r(t).

Results

The results of the analysis are displayed in two forms.

1. The fitted curve, the weighted residuals, the autocorrelation function, the deconvoluted decay curves (i.e. D(t) or IDvv for example), the anisotropy, etc… appear in the workspace.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.

Micelle Kinetics

Theory

This program allows for the analysis of quenching processes in micelles.

Fitting Function

The analysis program uses the "stretched exponential" fitting function (Rogers *et al*, 1978). This function can be used to describe the quenching in micelles when quencher molecules are Poisson distributed among the micelles. The fitting function is:

$$D(t) = a_1 \exp\{-a_2 t - a_3[1 - \exp(-a_4 t)]\} \qquad \text{Eq. 1}$$

For the case of quenching in micelles these parameters can be interpreted as:

a_1 = scale factor for the fitting function
$a_2 = 1/\tau$, the reciprocal of the unquenched fluorophore lifetime
a_3 = aggregation number
$a_4 = 1/k_q$, the reciprocal of the quenching rate constant

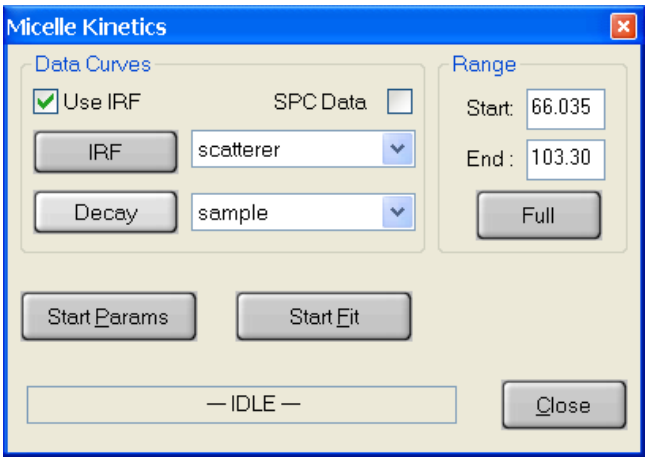
providing that the quenching process is fast relative to exchange of species.

Equation 1 implies that the fluorescence decay can be represented by a set of exponential decays with Poisson distributed amplitudes and discretely spaced lifetimes, which means that this type of data can also be analyzed with the PTI Maximum Entropy Method program (Siemiarczuk and Ware, 1990).

Please see the **General Introduction** for a discussion of the fitting procedures and statistics.

Using The Program

The initial dialog box for **Micelle Kinetics** is shown below.



Data Curves

The **Use IRF** checkbox selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** check box is used only when single photon counting data has been imported.

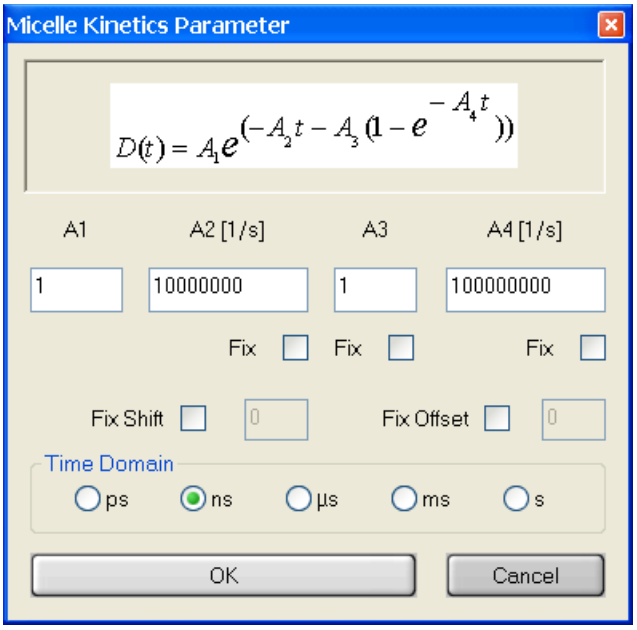
The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will appear in the box beside the button.

The **Decay** button selects the curve to be analyzed. Select a curve by clicking on its name in the legend then click on the **Decay** button. The name of the selected curve will appear in the box beside the button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box that allows these values to be entered.

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.



The **Parameters** dialog box for the **Micelle Kinetics** method is shown at left.

The fitting function is shown in a text box as a reminder of what the various parameters are.

A1-A4: For each of the parameters to be used in the fit an initial guess must be given. Each of the parameters chosen for the analysis may be fixed at the input value (except **A1**) or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are very poor. If this occurs, try changing the starting values.

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see the **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Fix Offset: Because of difficulties in establishing a noise free baseline there may be a small intensity offset for a decay curve. This offset may be included as a parameter in the analysis. The offset parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the offset is allowed to float, a value of 0.0 is used as the initial guess.

Time Domain: This method can be used to analyze fluorescence or phosphorescence data and imported data. The units used on the time axis may be different for each of these cases. For PTI instruments the units are nanoseconds for fluorescence and microseconds for phosphorescence.

Results

The results of the analysis are displayed in two forms.

1. The fitted curve, the weighted residuals, the autocorrelation function and the deconvoluted decay (i.e. D(t)) appear in the workspace.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.

Non-Exponential Decay

Theory

This program allows for the analysis of data by a general fitting function consisting of two exponentials multiplied together each with variable exponents of time. The exponents can be either varied or fixed which provides a powerful general function for models such as Förster energy transfer and time-dependent quenching.

Fitting Function

The fitting function is:

$$D(t) = a_1\exp(-a_2t^n)\exp(-a_3t^m) \qquad \text{Eq. 1}$$

The parameters are:

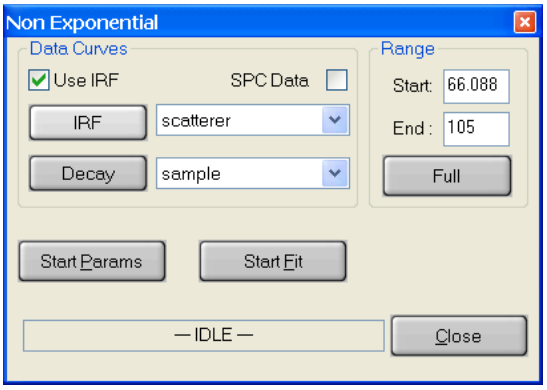
a_1 = scale factor for the fitting function
$a_2 = 1/\tau$, the reciprocal of the "slow" decay component
$a_3 = 1/\tau$, the reciprocal of the "fast" decay component
n = exponent of the "fast" component
m = exponent of the "slow" component if n>m.

The exponents can be held constant or found as parameters of the fit. For example, by setting n=1 and m=0.5, this fitting function is suitable for Förster energy transfer kinetics (Förster, 1949, Birks, 1948, Steinberg *et al*, 1983) or time-dependent quenching (Ware and Andre, 1983). Any other decay law, which can be modeled by two exponentials multiplied together, can be analyzed by this program.

Refer to the **General Introduction** for a discussion of the fitting procedures and statistical parameters.

Using the Program

The initial dialog box for **Non-exponential Decay** is shown below.



Data Curves

The **Use IRF** check box selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** check box is used only when single photon counting data has been imported.

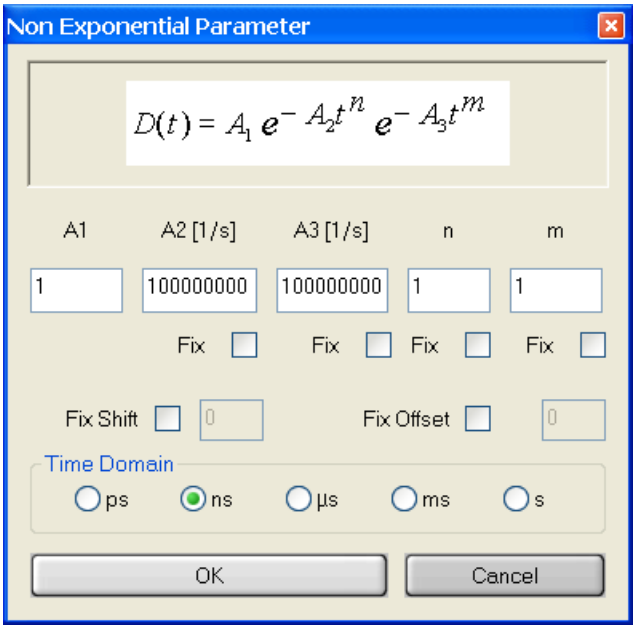
The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will appear in the box beside the button.

The **Decay** button selects the curve to be analyzed. Select a curve by clicking on its name in the legend then click on the **Decay** button. The name of the selected curve will appear in the box beside the button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box that allows these values to be entered.

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.



The **Parameters** dialog box for the **Non-exponential Decay** method is shown at left.

The fitting function is shown in a text box as a reminder of what the various parameters are.

A1-A3, m, n: For each of the parameters to be used in the fit an initial guess must be given. Each of the parameters chosen for the analysis may be fixed at the input value (except **A1**) or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are very poor. If this occurs, try changing the starting values.

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see the **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Fix Offset: Because of difficulties in establishing a noise free baseline there may be a small intensity offset for a decay curve. This offset may be included as a parameter in the analysis. The offset parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the offset is allowed to float, a value of 0.0 is used as the initial guess.

Time Domain: This method can be used to analyze fluorescence or phosphorescence data and imported data. The units used on the time axis may be different for each of these cases. For PTI instruments the units are nanoseconds for fluorescence and microseconds for phosphorescence.

Results

The results of the analysis are displayed in two forms.

1. The fitted curve, the weighted residuals, the autocorrelation function and the deconvoluted decay (i.e. D(t)) appear in the workspace.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.

ESM – Exponential Series Method

Theory

Fluorescence lifetime measurements often result in complex decays requiring a more sophisticated approach than a single- or double-exponential fitting function (James and Ware, 1986, Siemiarczuk *et al*., 1990). This applies especially to the emission originating in such intrinsically complex systems as:

- bichromophoric molecules exhibiting distributions of conformers in the excited state
- fluorophores adsorbed on surfaces
- fluorophores attached to polymers
- fluorescent probes in micelles and liposomes
- fluorescent probes in biomembranes and other biological systems
- fluorophores in monolayers
- intrinsic fluorescence from proteins
- systems undergoing Förster-type energy transfer
- and many others…

Even intuitive considerations would lead one to expect distributions of lifetimes in these systems. Quite often, however, especially for low precision data, a good fit can be obtained with a double- or triple-exponential function for a system, which in fact represents a continuous distribution of lifetimes. In general, however, the parameters recovered from such a fit have no physical meaning.

The Exponential Series Method (ESM) is designed to recover lifetime distributions without any *a priori* assumptions about their shapes. This method uses a series of exponentials (up to 200 terms) as a probe function with fixed, logarithmically-spaced lifetimes and variable pre-exponentials. This allows covering a lifetime range of several orders of magnitude. In many situations the ESM is capable of differentiating between continuous distributions and discrete, multi-exponentials decays.

Fitting Procedure

The fluorescence decay is approximated by the exponential series:

$$F(t) = \sum_{i=1}^{N} a_i \exp\left(\frac{-t}{\tau_i}\right) \qquad \text{Eq. 1}$$

where a_i are the variable amplitudes, τ_i are the lifetimes which are fixed and logarithmically-spaced, and N is the number of terms. Initially all a_i are set equal. In order to recover amplitudes a_i the ESM uses an iterative procedure to minimize the chi-square function that is defined as follows:

$$C = \left(\frac{1}{n}\right) \sum_{k=1}^{n} \frac{\left(Y_k - \sum_{i=1}^{N} D_{ki}\, a_i\right)^2}{\sigma_k^2} \approx 1.0 \qquad \text{Eq. 2}$$

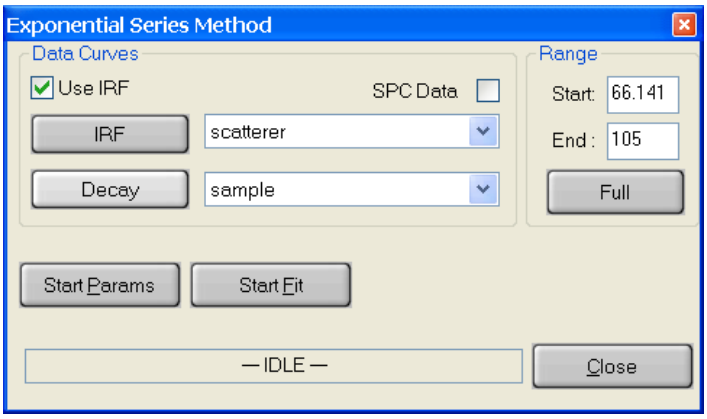
where Y_k represents the fluorescence intensity (e.g. number of photons) in the kth channel, σ_k is the standard deviation in the k^{th} channel, n is the number of data points (or channels), D_{ki} is the convolution matrix:

$$D_{ki} = \int_0^{t_k} L(t_k - t) \exp\left(\frac{-t}{\tau_i}\right) dt \qquad \text{Eq. 3}$$

where L(t) comprises the excitation pulse profile and the instrument response function.

Using the Program

The initial **Exponential Series Method** dialog box is shown below.



Data Curves

The **Use IRF** checkbox selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** checkbox is used only when single photon counting data has been imported.

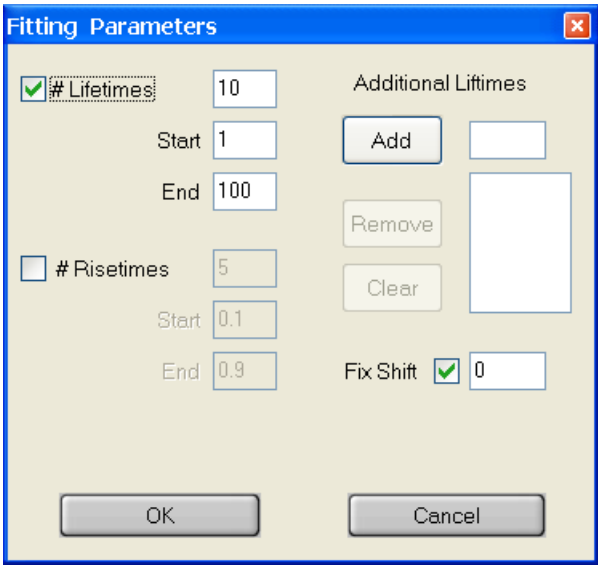
The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will appear in the box beside the button.

The **Decay** button selects the curve to be analyzed. Select a curve by clicking on its name in the legend then click on the **Decay** button. The name of the selected curve will appear in the box beside the button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box that allows these values to be entered.

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.



The **Fitting Parameters** dialog box for the **Exponential Series** method is shown at left.

The **# Lifetimes** check box and text box select the number of different lifetimes used in the analysis of the decay curve. These are distributed in a logarithmic manner between the **Start** lifetime and the **End** lifetime.

The **# Risetimes** check box and text box select the number of different risetimes used in the analysis of the decay curve. These are distributed in a logarithmic manner between the **Start** lifetime and the **End** lifetime.

Additional Lifetimes

Additional fixed lifetimes may be entered one at a time in the text box. Clicking on the **Add** button enters this value on the lower text window. Lifetimes may be deleted by clicking on the appropriate line in the text window to highlight the line then clicking on the **Remove** button. All lifetimes may be removed by clicking on the **Clear** button. This option is useful when there are some lifetimes lying far outside the range of the distribution. Extending the range of the lifetime distribution to include these would be very wasteful since most of the lifetimes would lie in regions with zero amplitude.

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see the **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Once all parameters have been set, click the **OK** button to return to the previous dialog box and then **Start Fit** to start the Analysis.

Results

The results of the analyses are displayed in several forms.

1. The fitted curve, lifetime distribution, the weighted residuals, the autocorrelation function and the deconvoluted decay (i.e. D(t)) appear in the workspace. Initially, only the fitted curve and the lifetime distribution are displayed the others being hidden to avoid clutter. The lifetime distribution curve contains most of the information from this analysis. Commonly, all other files must be hidden to see this curve since the Y scale is much smaller than most data curves. The numerical values associated with the distribution are not included in the Math/Fit Output text window since they are, typically, very numerous. The numerical values can be viewed by hiding all other curves except the distribution curve and using the **Grid View** button to display a spreadsheet of the results.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.
3. While the fit is executing a **Fit Status** window displays the current lifetime distribution and residuals on a logarithmic time scale. Once this window is closed it cannot be recovered. Should the user wish to capture this window, this can be done by making **Fit Status** the active window (click on title line), saving the active window to the clipboard (**Alt+ Print Scrn**), opening a graphics program e.g. Paint and pasting the clipboard into the program (**Ctrl+V**).

MEM – Maximum Entropy Method

Theory

Fluorescence lifetime measurements often result in complex decays requiring a more sophisticated approach than a single- or double-exponential fitting function (James and Ware, 1986, Siemiarczuk *et al*, 1990). This applies especially to the emission originating in such intrinsically complex systems as:

- bichromophoric molecules exhibiting distributions of conformers in the excited state
- fluorophores adsorbed on surfaces
- fluorophores attached to polymers
- fluorescent probes in micelles and liposomes
- fluorescent probes in biomembranes and other biological systems
- fluorophores in monolayers
- intrinsic fluorescence from proteins
- systems undergoing Förster-type energy transfer
- and many others…

Even intuitive considerations would lead one to expect distributions of lifetimes in these systems. Quite often, however, especially for low precision data, a good fit can be obtained with a double- or triple-exponential function for a system, which in fact represents a continuous distribution of lifetimes. In general, however, the parameters recovered from such a fit have no physical meaning.

The Maximum Entropy Method (MEM) is designed to recover lifetime distributions without any *a priori* assumptions about their shapes (Skilling and Bryan 1989, Smith and Grady, 1985). This method uses a series of exponentials (up to 200 terms) as a probe function with fixed, logarithmically-spaced lifetimes and variable pre-exponentials. This allows covering a lifetime range of several orders of magnitude. In many situations the MEM is capable of differentiating between continuous distributions and discrete, multi-exponentials decays.

Fitting Procedure

The fluorescence decay is approximated by the exponential series:

$$F(t) = \sum_{i=1}^{N} a_i \exp\left(\frac{-t}{\tau_i}\right) \qquad \text{Eq. 1}$$

where a_i are the variable amplitudes, τ_i are the lifetimes which are fixed and logarithmically-spaced, and N is the number of terms. Initially all a_i are set equal. The MEM theory utilizes the Shannon-Jaynes entropy function:

$$S = -\sum_{i=1}^{N} a_i \log\left(\frac{a_i}{\sum_{i=1}^{N} a_i}\right) \qquad \text{Eq. 2}$$

which has to be maximized in order to recover the least biased set of amplitudes $\{a_i\}$ out of all feasible solutions. On the other hand, to ensure that the recovered solution is in agreement with the experimental decay, the following constraint based on the chi-square statistics is implemented:

$$C = \left(\frac{1}{n}\right) \sum_{k=1}^{n} \frac{\left(Y_k - \sum_{i=1}^{N} D_{ki}\, a_i\right)^2}{\sigma_k^2} \approx 1.0 \qquad \text{Eq. 3}$$

where Y_k represents the fluorescence intensity (e.g. number of photons) in the kth channel, σ_k is the standard deviation in the kth channel, n is the number of channels, D_{ki} is the convolution matrix:

$$D_{ki} = \int_0^{t_k} L(t_k - t) \exp\left(\frac{-t}{\tau_i}\right) dt \qquad \text{Eq. 4}$$

where L(t) comprises the excitation pulse profile and the instrument response function. Conditions (2) and (3) can be combined in one function:

$$Q = \alpha S - C \qquad \text{Eq. 5}$$

where α is a Lagrange multiplier. Q is then maximized by an iterative procedure thus ensuring simultaneous maximization of S and minimization of C until constraint (3) is satisfied. After a target value of chi-square is reached, the program keeps maximizing S with C kept constant until the entropy test parameter:

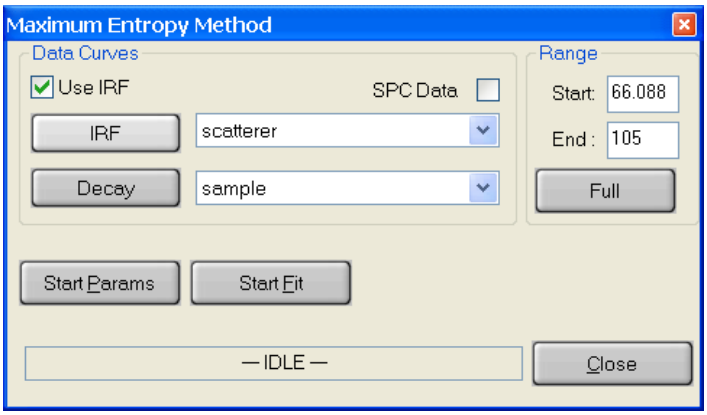
$$0.5\left[\left(\frac{\text{gradC}}{|\text{gradC}|}\right) - \left(\frac{\text{gradS}}{|\text{gradS}|}\right)\right] < 0.1 \qquad \text{Eq. 6}$$

This condition ensures that the global maximum of Q has been reached.

Using the Program

The initial **Maximum Entry** method dialog box is shown below.

Data Curves



The **Use IRF** checkbox selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** checkbox is used only when single photon counting data has been imported.

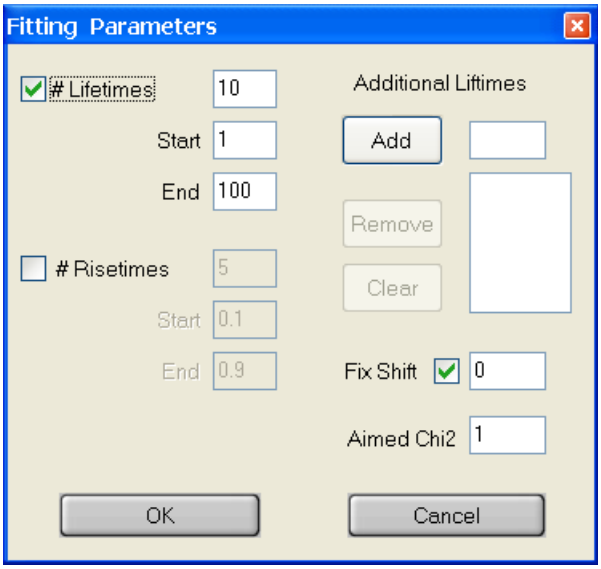
The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will appear in the box beside the button.

The **Decay** button selects the curve to be analyzed. Select a curve by clicking on its name in the legend then click on the **Decay** button. The name of the selected curve will appear in the box beside the button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box that allows these values to be entered.

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.



The **Fitting Parameters** dialog box for the **Maximum Entropy Method** is shown at left.

The **# Lifetimes** check box and text box select the number of different lifetimes used in the analysis of the decay curve. These are distributed in a logarithmic manner between the **Start** lifetime and the **End** lifetime.

The **# Risetimes** check box and text box select the number of different risetimes used in the analysis of the decay curve. These are distributed in a logarithmic manner between the **Start** lifetime and the **End** lifetime.

Additional Lifetimes

Additional fixed lifetimes may be entered one at a time in the text box. Clicking on the **Add** button enters this value on the lower text window. Lifetimes may be deleted by clicking on the appropriate line in the text window to highlight the line then clicking on the **Remove** button. All lifetimes may be removed by clicking on the **Clear** button. This option is useful when there are some lifetimes lying far outside the range of the distribution. Extending the range of the lifetime distribution to include these would be very wasteful since most of the lifetimes would lie in regions with zero amplitude.

Aimed Chi2: Enter the target value of χ^2.

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see the **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Once all parameters have been set, click the **OK** button to return to the previous dialog box and then **Start Fit** to start the Analysis.

Results

The results of the analyses are displayed in several forms.

1. The fitted curve, lifetime distribution, the weighted residuals, the autocorrelation function and the deconvoluted decay (i.e. D(t)) appear in the workspace. Initially, only the fitted curve and the lifetime distribution are displayed the others being hidden to avoid clutter. The lifetime distribution curve contains most of the information from this analysis. Commonly, all other files must be hidden to see this curve since the Y scale is much smaller than most data curves. The numerical values associated with the distribution are not included in the Math/Fit Output text window since they are, typically, very numerous. The numerical values can be viewed by hiding all other curves except the distribution curve and using the **Grid View** button to display a spreadsheet of the results.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.
3. While the fit is executing a **Fit Status** window displays the current lifetime distribution and residuals on a logarithmic time scale. Once this window is closed it cannot be recovered. Should the user wish to capture this window, this can be done by making **Fit Status** the active window (click on title line), saving the active window to the clipboard (**Alt+ Print Scrn**), opening a graphics program, e.g., Paint and pasting the clipboard into the program (**Ctrl+V**).

Fluorescence Resonance Energy Transfer (FRET)

Theory

The Fluorescence Resonance Energy Transfer (FRET) takes place between an excited donor molecule (D) and the ground-state acceptor molecule (A) over a range of distances, typically 10-100 Å. FRET is a non-radiative process (i.e. there is no photon emitted or absorbed during the energy exchange). The efficiency of FRET is strongly dependent on the D-A distance and is characterized by the Förster critical radius R_o, a unique parameter for each D-A pair. When the D-A distance is R_o, the efficiency of energy transfer is 50%. Once R_o is known, the D-A pair can be used as a molecular ruler to determine the distance between sites labeled by D and A.

There are two basic methods to determine the efficiency of FRET: a) by measuring a decrease of fluorescence intensity of D in the presence of A, and b) by measuring the fluorescence lifetime of D, which becomes shortened as a result of FRET. In some cases, one can also monitor an enhancement of the acceptor fluorescence or the acceptor lifetime, if it is much shorter than the donor lifetime. PTI provides specialized systems for both steady state and time-resolved FRET techniques, which can be used for a variety of FRET applications.

If any quantitative information is expected from a FRET experiment, it is imperative that the R_o value is known. The R_o value can be calculated as follows:

$$R_o = 0.2108\ (\kappa^2 \Phi_D n^{-4} J_{DA})^{1/6} \qquad \text{Eq. 1}$$

where κ^2 is the orientation factor, Φ_D is the quantum yield of D in the absence of A, n is the refraction index of the medium, and J_{DA} is the spectral overlap integral between the excitation spectrum of A and the emission spectrum of D. The overlap integral J_{DA} can be calculated if the absorption (excitation) spectrum of A and the fluorescence emission spectrum of D are known, i.e.

$$J_{DA} = C\int_0^\infty I_D(\lambda) E_A(\lambda) \lambda^4 d\lambda \qquad \text{Eq. 2}$$

where I_D is the emission spectrum of D, E_A is the absorption (excitation) spectrum of A and C is the normalization factor defined as:

$$C = \frac{\varepsilon(\lambda_{max})}{E_A(\lambda_{max}) \int_0^\infty I_D(\lambda) d\lambda} \qquad \text{Eq. 3}$$

where $\varepsilon(\lambda_{max})$ is the molar extinction coefficient of A at the absorption (excitation) maximum.

The value of κ^2 depends on a relative orientation of D and A transition moments. If the transition moments have fixed orientations, κ^2 will vary from 0 (transition moments perpendicular) to 4 (transition moments collinear). For parallel transition moments $\kappa^2 = 1$. When A molecules are randomly distributed about D in a rigid medium, $\kappa^2 = 0.476$. If D and A undergo a rotational motion, which is faster than the decay time of D, $\kappa^2 = 2/3$.

The energy transfer efficiency E can be calculated from either fluorescence intensity or lifetime measurements for D alone and D in the presence of A.

$$E = 1 - \frac{I_{DA}}{I_D} \qquad \text{Eq. 4}$$

where I_D and I_{DA} are fluorescence intensities of D in the absence and presence of A, respectively, or

$$E = 1 - \frac{\tau_{DA}}{\tau_D} \qquad \text{Eq. 5}$$

where τ_D and τ_{DA} are fluorescence lifetimes of D in the absence and presence of A, respectively.

Once R_o and E are know, the distance r between D and A can be calculated:

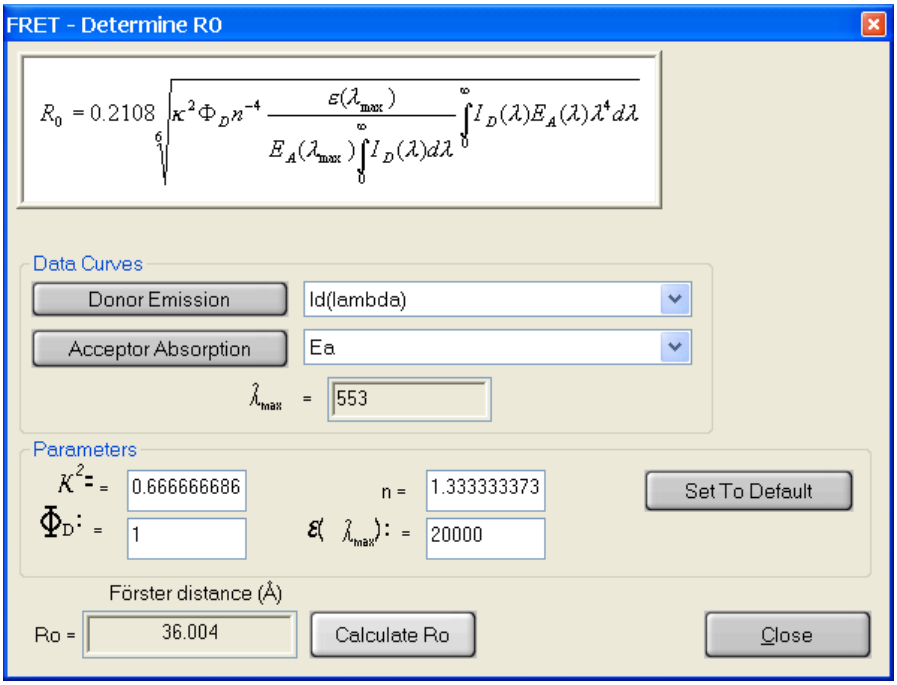
$$r = \left(\frac{1}{E} - 1 \right)^{1/6} R_o \qquad \text{Eq. 6}$$

If the lifetime of D is known, the FRET rate constant can also be calculated:

$$k_{ET} = \frac{1}{\tau_D} \left[\frac{R_o}{r} \right]^6 \qquad \text{Eq. 7}$$

Using the FRET Calculator
Clicking on **Math**, **Data** Analysis, and then on **FRET** can access the FRET Calculator. The FRET drop down menu gives three choices: **Determine Ro**, **Calculate FRET Parameters (steady- state)** and **Calculate FRET Parameters (lifetimes)**.

Determine R_o



Donor Emission: This button selects the curve to be used as donor emission spectrum. Select a curve by clicking on its name in the legend and then click on the **Donor Emission** button. The name of the selected curve will appear on the box beside the button.

Acceptor Absorption: This button selects the curve to be used as acceptor absorption (excitation) spectrum. Select a curve by clicking on its name in the legend and then click on the **Acceptor Absorption** button. The name of the selected curve will appear on the box beside the button.

Once the curves have been defined, the acceptor absorption (excitation) maximum wavelength will be displayed in the **λ_{max}** box.

κ^2: Enter the value for the orientation factor in the box or leave the default value of 2/3 for the fast rotation limit.

Φ_D: Enter the value for the donor emission quantum yield in the box (the default value is 1).

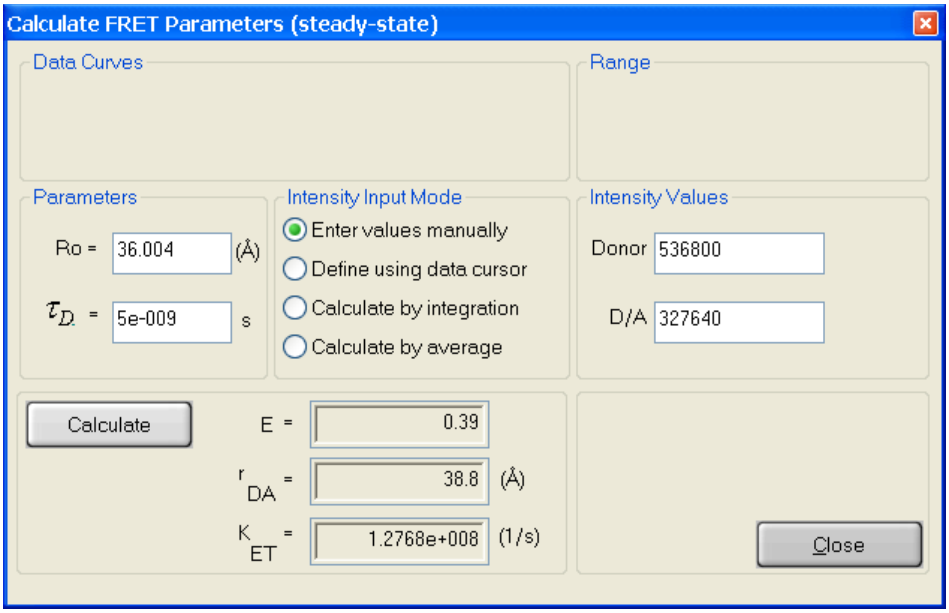
n: Enter the value for the index of refraction in the box (the default value is 1.33333 for water).

ε (λ_{max}): Enter the value for the molar extinction coefficient for the acceptor at the absorption (excitation) maximum in the box (the default value is 20000).

Calculate R_o: Click on this button to show the Förster distance R_o in the **R_o** box.

Set To Default: Click on this button to reset all the parameters to default values.

Calculate FRET Parameters (steady-state)

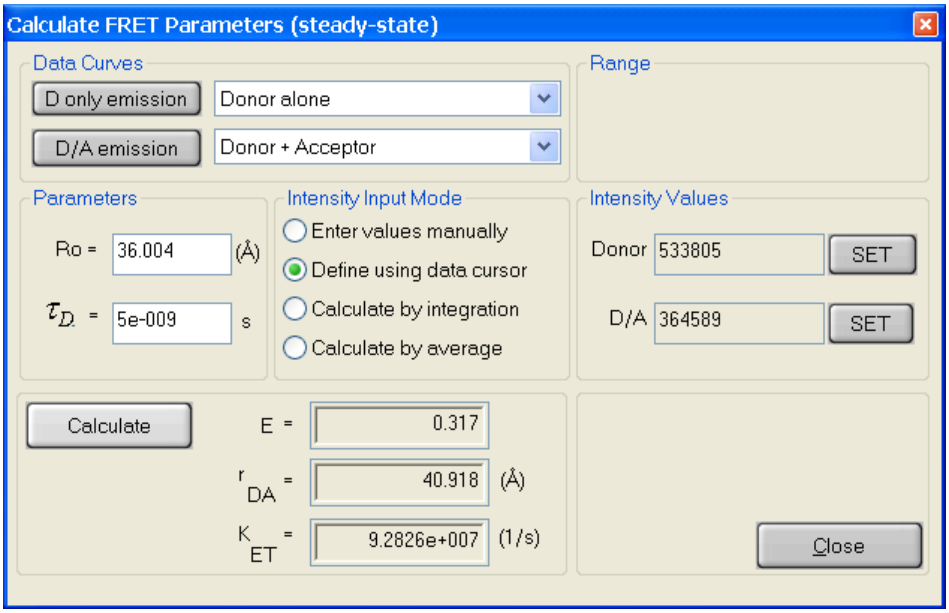


R_o: In the **Parameters** box, either enter the value of the Förster distance R_o or retain the value calculated in the **Determine R_o** option.

τ_D: In the **Parameters** box, enter the donor lifetime if you want the FRET rate constant to be calculated.

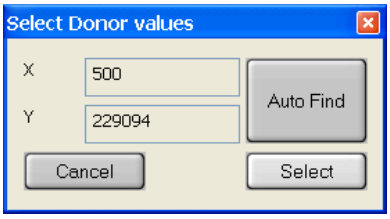
- To enter intensity values manually, click on the **Enter values manually** radio button in the **Intensity Input Mode** box. Then type in intensity values for donor alone in the **Donor** box and for donor in the presence of acceptor in the **D/A** box. Click on **Calculate** and the FRET efficiency **E**, donor-acceptor distance (r_{DA}) and FRET rate constant k_{ET} will be displayed. The k_{ET} value will only have any meaning if the correct τ_D has been entered, otherwise it should be ignored.

- To enter donor intensities using the data cursor, click on the **Define using data cursor** radio button. The **Data Curves** box becomes available.



The **D only emission** button selects the donor emission curve. Select a curve by clicking on its name in the legend, then click on the **D only emission** button.

The **D/A emission** button selects the donor emission curve measured in the presence of acceptor. Select a curve by clicking on its name in the legend, then click on the **D/A emission** button.

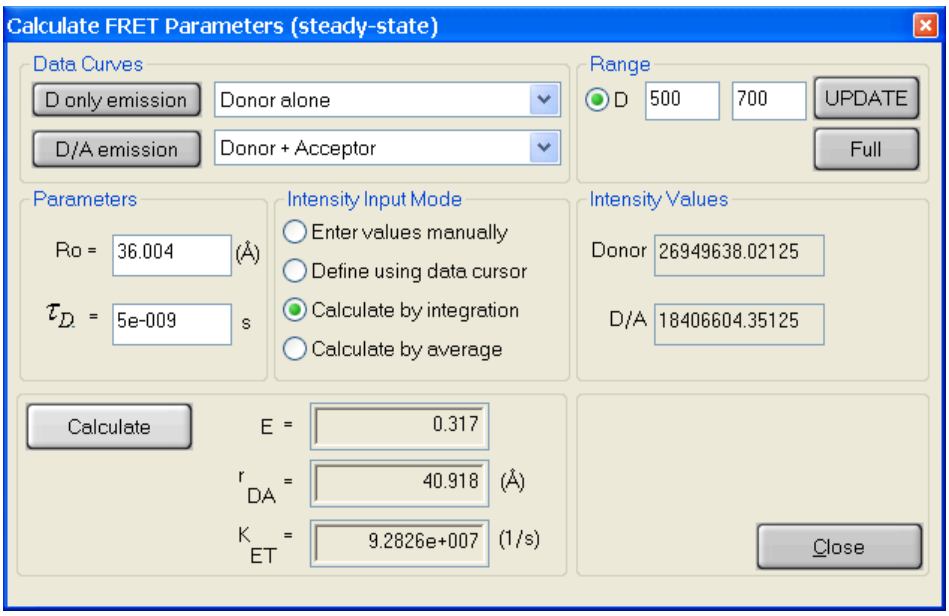


In the **Intensity Values** box click on the **SET** button next to the **Donor** box, which opens the **Select Donor values** box. The data cursor initially is at the left edge of the selected trace. Using the mouse or the left and right arrow keys move the cursor to the desired position for the intensity readout. Click **Auto Find** to automatically position the cursor at the maximum of the donor curve. Click on **Select** to capture the intensity of the Donor and close the dialog.

Similarly, in the **Intensity Values** box click on the **SET** button next to the **D/A** box, which opens the **Select D/A values** box. The data cursor initially is at the left edge of the selected trace. Using the mouse or the left and right arrow keys move the cursor to the desired position for the intensity readout. Click **Auto Find** to automatically position the cursor at the maximum of the D/A curve. Click on **Select** to capture the intensity of the Donor in the presence of the acceptor and close the dialog.

Click on the **Calculate** button and the FRET efficiency **E**, donor-acceptor distance ($\mathbf{r_{DA}}$) and FRET rate constant $\mathbf{k_{ET}}$ will be displayed. The $\mathbf{k_{ET}}$ value will only have any meaning if the correct $\boldsymbol{\tau_D}$ has been entered, otherwise it should be ignored.

- To calculate donor and D/A intensities by integration, click on the **Calculate by integration** radio button. The **Data Curves** box becomes available and the **Selected Range** function is activated.



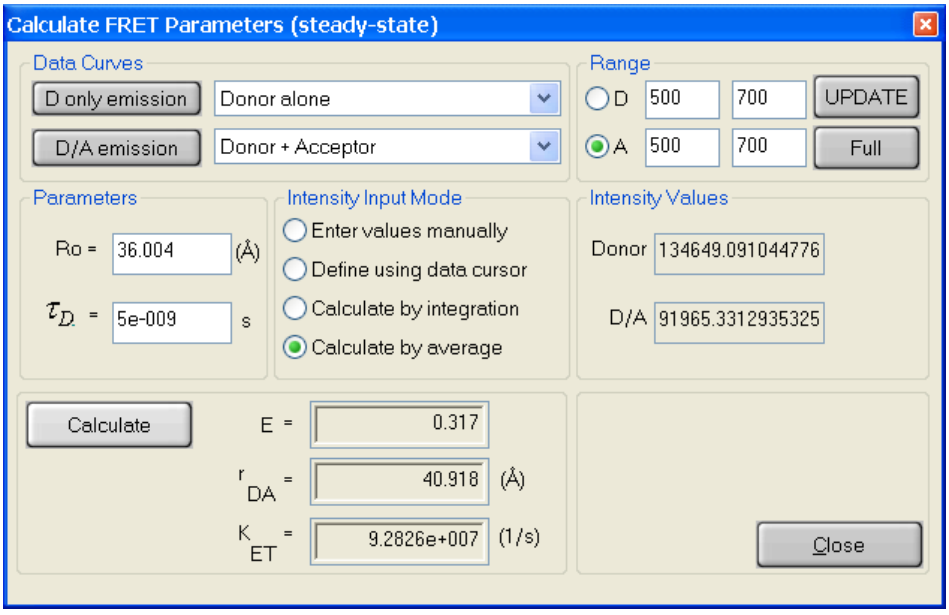
The **D only emission** button selects the donor emission curve. Select a curve by clicking on its name in the legend, then click on the **D only emission** button.

The **D/A emission** button selects the donor emission curve measured in the presence of acceptor. Select a curve by clicking on its name in the legend, then click on the **D/A emission** button.

Range: To select the integration range, use the mouse to drag the left and right edges of the **Selected Range**. Alternatively, type in the start and end values for the range and click on the **UPDATE** button. The integrated intensity values will be displayed in the **Intensity Values** box. If the integration is to be carried out over the entire range, just click on the **FULL** button and the intensities will be captured and displayed.

Click on the **Calculate** button and the FRET efficiency **E**, donor-acceptor distance ($\mathbf{r_{DA}}$) and FRET rate constant $\mathbf{k_{ET}}$ will be displayed. The $\mathbf{k_{ET}}$ value will only have any meaning if the correct $\boldsymbol{\tau_D}$ has been entered, otherwise it should be ignored.

➢ To calculate donor and D/A intensities by average, click on the **Calculate by average** box. The **Data Curves** box becomes available and the **Selected Range** function is activated.



The **D only emission** button selects the donor emission curve. Select a curve by clicking on its name in the legend, then click on the **D only emission** button.

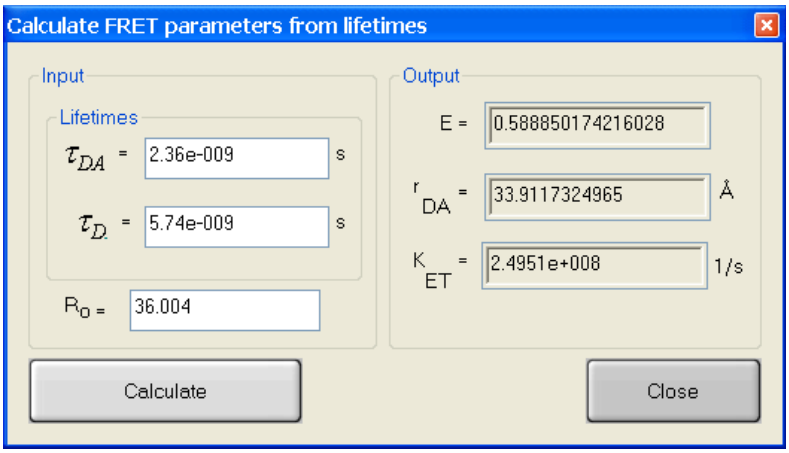
The **D/A emission** button selects the donor emission curve measured in the presence of acceptor. Select a curve by clicking on its name in the legend, then click on the **D/A emission** button.

Range for D: To select the averaging range for the donor alone, click on the **D** radio button in the **Range** box, and use the mouse to drag the left and right edges of the **Selected Range**, or type in the start and end values for the range and click on the **UPDATE** button. The average intensity value for **D** will be displayed in the **Intensity Values** box. If the averaging is to be carried out over the entire range, just click on the **FULL** button and the intensity will be captured and displayed.

Range for D/A: To select the averaging range for the donor in the presence of acceptor, click on the **A** radio button in the **Range** box, and use the mouse to drag the left and right edges of the **Selected Range**, or type in the start and end values for the range and click on the **UPDATE** button. The average intensity value for **D/A** will be displayed in the **Intensity Values** box. If the averaging is to be carried out over the entire range, just click on the **FULL** button and the intensity will be captured and displayed.

Click on the **Calculate** button and the FRET efficiency **E**, donor-acceptor distance ($\mathbf{r_{DA}}$) and FRET rate constant $\mathbf{k_{ET}}$ will be displayed. The $\mathbf{k_{ET}}$ value will only have any meaning if the correct $\boldsymbol{\tau_D}$ has been entered, otherwise it should be ignored.

Calculate FRET Parameters (lifetimes)



In the **Input** box, enter the lifetime value of donor in the presence of acceptor (τ_{DA}) and the donor alone (τ_D). At the bottom of the **Input** box, either enter the value of R_o or retain the value calculated in the **Determine R_o** option.

Click on the **Calculate** button and the FRET efficiency **E**, donor-acceptor distance (r_{DA}) and FRET rate constant k_{ET} will be displayed.

Trace Math Commands

The commands in the Trace Math menu allow specific mathematical functions to be carried out on single curves or selected regions of a curve. Many of the math dialog boxes can be left open so that multiple operations can be performed.

Settings and controls that are common to all dialog boxes are presented under the heading Common Math Controls. The descriptions for the configuration dialog boxes that follow provide details on the specific math function as well as settings and controls that are unique to them.

Note. Some math functions are performed on a selected region of a curve (a subset of the X values). To select this region, first choose the target curve by clicking on its name in the legend. Then select the **Range Toggle** icon (| |) from the graphing toolbar and use the mouse to click and drag within the graph display over the desired region of the curve. For more precise control, you can then enter **Low X** and **High X** values into the text boxes provided. The selected region will be highlighted, and the desired math value will be displayed. The math function dialog box can be left open while different regions are selected, and math values, when displayed, will change dynamically.

Common Math Controls

The trace name operand is shown in the dialog title bar after the trace math command name.

Create New Data
If checked, a new curve will be created. The original (source) data will be preserved.

Replace Old Data
If checked, the original curve will be permanently lost, as it will be replaced by the new data.

Label
Type the name of the new curve in the text box. If no label is specified, the new curve will be listed in the legend with a name comprised of a generic math function descriptor (e.g., Smooth, or Logarithm) added to the source curve's original name.

Execute
Carries out the operation. If you type in new values to select an X-axis region, **Execute** is required to perform the new calculation.

Lock to trace
The values in the dialog are locked to that curve, even if you then select another curve.

Close
Closes the math function dialog box.

Antilog

Calculates the antilogarithm of the selected curve.

Average

Calculates the average value of the Y-axis parameter on a selected region of a curve. The average value is the sum of the values divided by the number of points.

The standard deviation is also determined using the equation:

$$\sigma = \sqrt{\frac{\sum_i y_i^2 - \frac{1}{n}(\sum_i y_i)^2}{n-1}}$$

Where y_i is the Y-value of a data point and n is the total number of data points in the portion of the data trace being averaged.

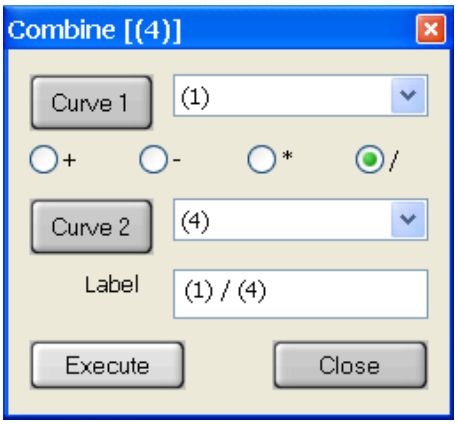
Distribution Average

Calculates the **Integrated Amplitudes** and **Distribution Average** of the selected region of a trace. The integrated amplitudes is the sum of the Y-values. The distribution average is determined using the equation:

$$\frac{\sum_i x_i y_i}{\sum_i y_i}$$

Combine

The combine command allows you to add one curve to another, subtract a curve from another, multiply a curve by another, or divide a curve by another. The math is performed in a point-by-point fashion. Only the portions of the curves that overlap are combined.



Curve 1, Curve 2
Select curves for the operation by clicking on their names in the drop-down list boxes. Alternatively, select a curve from the legend and click on the Curve 1 or Curve 2 button.

Operation
Check an operator to add (+), subtract (-), multiply (x), or divide (/) Curve 1 by Curve 2.

XY Combine

This feature allows the user to construct a new data trace, using the X values of one trace, and the Y values of another trace. In this way, complex data, such as time-dependent temperature ramps and correlated data, can be converted into new traces that have compatible X axes to simplify the display and treatment of the data.

Source trace with X data
Use the drop-down menu to choose the trace from which to create the X data. Alternatively, select a curve from the legend and click on the **Pick** icon beside the **Source trace with X data** header.

Source trace with Y data
Use the drop-down menu to choose the trace from which to create the Y data. Alternatively, select a curve from the legend and click on the **Pick** icon beside the **Source trace with Y data** header.

Note: If the X-data of the two operands are different, then the resulting Y-values will be linearly interpolated or extrapolated from the two nearest Y-values of the second operand.

Differentiate

Differentiate takes the derivative of the selected curve. Subsequent application of the differentiate command results in the second derivative, etc. Differentiation is done using the 5 point Savitzky-Golay algorithm, which provides a smoothed derivative.

Integrate

This function integrates within the range of the selected region of a curve. The Total Area is the integral of the data above the absolute X-axis. The Peak Area is used to integrate a peak within a curve.

Total Area
Displays the total integrated area within the selected range. If there is negative data, then the total integrated area may also be negative.

Peak Area
Displays the integral of the peak above the background. The graph module projects an imaginary line between the points where the boundaries of the range intersect the curve. Peak Area is the integrated area above that line. If most of the curve data lies below this line, then the Peak Area will be a negative number.

Lock to trace
The values in the Integrate dialog are locked to that curve, even if you then select another curve.

Linear Fit

Calculates and overlays a linear fit to the selected region of a curve. The slope, intercept, and correlation coefficient are displayed.

Linear Scale

The Linear Scale is used to shift a curve or a selected region of a curve on either the X- or the Y-axis. The curve can be shifted on the Y-axis by a multiplier, divisor, or an addend. The curve can be shifted on the X-axis by an addend only.

Y and X Value
Multiplier: Multiplies all Y values in the curve by the specified multiplier.

Divisor: Divides all Y values in the curve by the specified divisor.

Offset: Adds the specified value to the X or Y value for each point in the curve.

Select Range
Applies the transformation only within the region selected by the user. The remainder of the trace is also copied but without transformation. The range is selected dragging the edges of the **Selected Range**.

Logarithm

Calculates the logarithm of the selected curve.

Normalize

Normalizes a curve to a set value. The normalization function reference may be either a peak or a specified point.

Reference
Select **Peak** or **Specified Point**. Enter the X value of the specified point in the text boxes.

Normalize to:
Enter the value to which the curve will be normalized.

Reciprocal

Calculates the reciprocal (1/Y) of the Y-axis data in the selected curve.

Smooth

This function performs a Savitzky-Golay smoothing of the selected curve.

Buffer Size
Select a 7, 15, 21, or 33-point buffer. A higher buffer results in greater smoothing.

Truncate

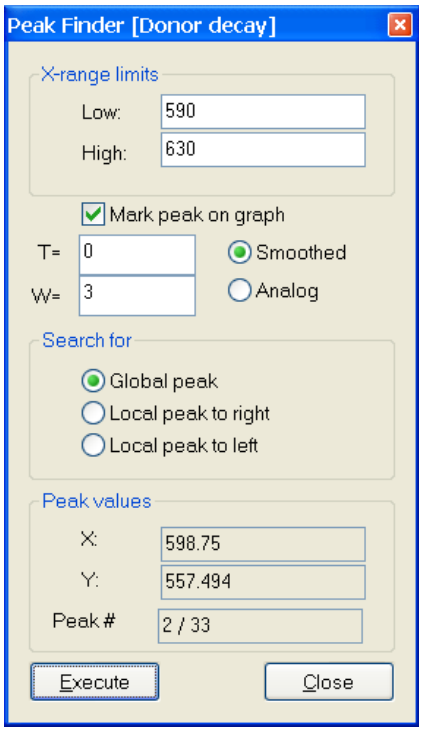
Truncate is used to reduce the X-axis range on the selected curve. The selected region of the curve is preserved and all X values above and below this region are permanently deleted. The region may be selected by entering the endpoints of the region, or by clicking and dragging the **Selected Range** edges.

Baseline

Baseline suppression causes a **Selected Range** of a curve to be set to a constant Y value (commonly zero). The range is selected as described in the introduction to this chapter. The chosen Y-value is entered into the text box and the function is performed by pressing the **Execute** button. This function is useful when noise in the baseline of the scatterer affects the lifetime of the sample. This happens because the IRF (scatterer) is convoluted with the sample lifetimes to give the observed decay. Thus noise in the scatterer is also convoluted and becomes a major problem for long-lived samples when observations are recorded out to many sample lifetimes. The convoluted noise has the same effect as small light pulses long after the real light pulse has ended. The derived lifetimes therefore appear to be smaller than they really are. See the **Analysis** chapter for further details.

Peak Finder

This function uses two algorithms. **Smoothed** fits a quadratic polynomial to sequential groups of data points to find peaks within the range of the **Selected Range** of a curve. Because of the polynomial fitting, the apparent X and Y **Peak values** and may be shifted slightly. **Analog** finds the global peak as the highest Y-value and local peaks as being higher than immediate left and right neighboring points.



X-range limits: Displays the low and high limits set by the **Selected Range**. These limits may be moved by using the cursor to grab and move the vertical edge lines.

Mark peak on graph: Shows a crosshair at the peak position on the graph.

Smoothed: Uses polynomial fitting to find the peaks.

T= Displays the threshold value. Only peaks above this threshold will be found.

W = Specifies the number of consecutive data points to use in the quadratic least squares fit. This value should not exceed approximately half of the half-width of the peak. It can be much smaller for noise-free data. The best choice for well-defined peaks is 3.

Analog: Does not smooth the data before finding peaks.

Search for:

Global peak: The peak within the selected range with the highest Y-axis value.

Local peak to right/left: Click on **Execute** to find the next peak to the right or left.

Peak values:

X: the X value of the specified peak.

Y: the Y value of the specified peak.

Peak #: Currently shown peak / Total peaks found.

Transform Commands

Note: These commands should only operate on spectral data and give faulty results if applied to timebased data.

Energy => Quantum

Converts the selected spectrum from energy units to quantum units proportional to the number of photons per second.

Quantum => to Energy

Converts the selected spectrum from quantum units, expressed as the number of photons detected at a given wavelength (or wavenumber), to energy units proportional to the number of photons detected at a given wavelength (or wavenumber) multiplied by the photon energy.

Wavelength => Wavenumber

Converts the selected trace from units of wavelength (nm) to wavenumber (1/cm). This command will also convert the trace to wavelength from wavenumber. Selection of which direction to convert is performed automatically.

Axes Menu

This option applies to the currently visible acquisition. The commands in the axes menu allow expansion or contraction of the axes for viewing and analyzing specific regions of a trace. The labels applied to the axes may also be altered. Some of these commands will appear as buttons on the Toolbar and as the submenu Graph\Axes when right-clicking on the graph area.

Full Autoscale

Scales the X and Y-axis to provide maximum space for the displayed curves in the graph window. A check mark will appear next to this command when this scaling mode is in effect.
Shortcut:  Use the toolbar icon to re-scale the axes after zoom features are used.

Autoscale From 0

Scales the Y-axis to provide maximum space, starting at 0, for the displayed curves in the graph window. A checkmark will appear next to this command when this scaling mode is in effect.

Fixed Y-Min. & Max…

Assigns a minimum and a maximum value to the Y-axis. The Y-scale will remain fixed within this range even when the X-axis is zoomed in or out. A checkmark will appear next to this command in the menu when this scaling mode is in effect.

Logarithmic Y-Scale

Makes the Y-axis logarithmic. The default log scale is automatic decade selection. If there are zero or negative values in the displayed curve(s) the automatic log scale may not be optimized. You can change the number of decades to display the data over using the **Visible log Decades** command in the *Axes* menu. A check mark will appear next to this command in the menu when this scaling mode is in effect.

Visible log Decades

Use this menu to define the number of log decades to be displayed on the screen. The default will automatically scale the Y-axis using an appropriate number of decades. If there are zero or negative values in the displayed curve(s), automatic selection of log decades may not be optimal for the display. The user can select from two to eight log decades to display the data over. To view the displayed curves in log scale select **Logarithmic Y-Scale** from the *Axes* menu.

2x X-Zoom In

Expands the X-axis by a factor of 2, beginning at the center of the display.

2x X-Zoom Out

Contracts the X-axis by a factor of 2, beginning at the center of the display.

Fixed X-Min. & Max…

Assigns a minimum and a maximum value to the X-axis. The X-scale will remain fixed within this range even when the Y-axis is zoomed in or out. A check mark will appear next to this command in the menu when this scaling mode is in effect.

Edit Axis Properties

Here you can modify the Axis settings for the record or the groups (depending on which radio button at the bottom you select). If you click on an Enabled button it will add a new axis with the caption and units specified. Clicking again on the button will remove the axis. You can only add/remove group axes. Record axes titles can only be edited.

If you click on a Fixed button you can set the minimum and maximum values to that axis.

Selecting a unit from the Unit drop down list will put that title in the Caption box where it can then be edited. If there are units in the parentheses these will show on the axis title. Note: changing a unit (e.g., from ms to s) changes the axis label only, it does not change the data point values.

NOTE: The graph module supports multiple X axes (each group can have one), although the graph only allows one to be displayed at once, so you have to click on the traces in the groups to make that axis become visible.

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