FargoFit Help

Welcome to FargoFit help system.

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FargoFit is a software for global analysis of fluorescence decays measured using direct digitizing method with instrument designed at the University of Minnesota. Program design was inspired by the software **Globals Unlimited** and a lot of ideas was adopted from it. Large number of processes (quenching, energy transfer, anisotropy, chemical reactions) can be combined in fitting model to fit observed multidimensional data surface. The program implements an iterative reconvolution of the experimental data with nonlinear least-squares minimization using either Marquardt-Levenberg or Simplex algorithm. Additionally any fitting parameters can be manually adjusted. Because the program is focused on analysis of data obtained by direct digitizing, uniform weighting scheme is implemented by default, but weighting by signal standard deviation can also be used. The entire workspace can be saved in a single file for future use; fitting results can be printed or exported in text format.

The Microsoft© .NET Framework 4.0 or newer is required for successful work of **FargoFit** program

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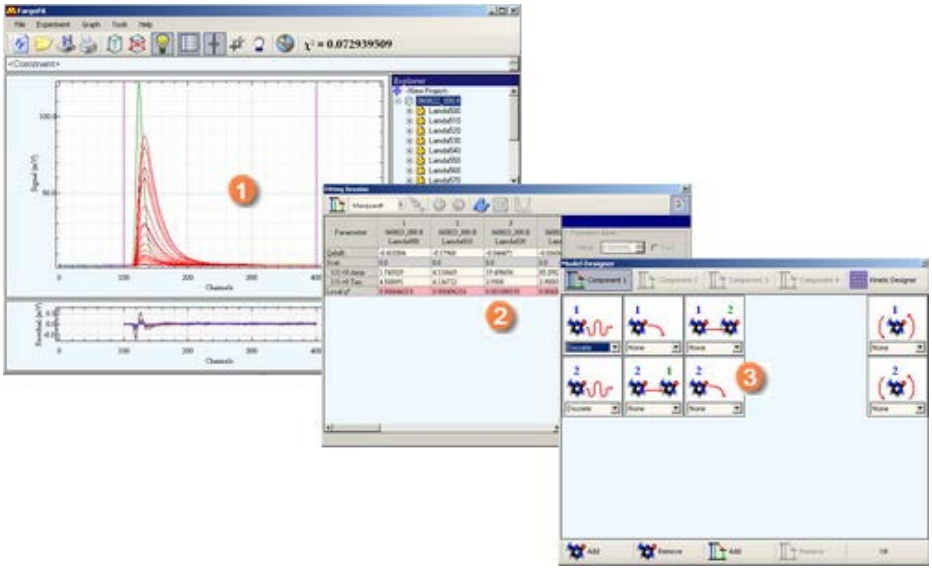
FargoFit Help

Introduction

The FargoFit user interface provides easy access to the various tools and features you can use to browse and analyze your data. FargoFit consists of three main parts: the Signal Browser, the Fitting Dialog, and Model Designer.



1 Browser

The FargoFit Signal Browser is the main browsing and managing component of the user interface, and that is what user sees when he/she starts FargoFit. In the Signal Browser, a user can load and view data, fitting results, set physical parameters of experiments and fitting region, access data simulation and manipulation tools.

The Signal Browser window has a menu, toolbar strips, and a **Comment** panel. The rest of the window is divided in two panels. Right panel contains **Project Explorer** and **Property** sub-windows. Left panel has up to three graph pannels (signal, fitting residual and signal standard deviation), that show data for the selected item in **Project Explorer**. All windows can be resized at the expense of the others. To resize a window, click the frame and drag.

2 Fitting Dialog

The Fitting Dialog shows a list of parameters for the current model. Parameter properties panel on the right side of the window displays properties of the current parameter. Using this panel user can change value of this parameter, link/unlink other parameters to the current parameter, and fix/unfix the current parameter. Program keeps track of parameter changes. As long as model isn't changed, previous value of parameters can be recalled.

3 Model Designer

The Model Designer allows user to combine different processes, like quenching, energy transfer, anisotropy, chemical reactions in one fitting model. Program supports up to four chemical reaction components and up to four fluorecsence decay components.

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Main window menu

File

New - creates new FargoFit project
Open - open existing FargoFit project
Save - save current project
Copy Graph - copies entire graph area of program window as image to clipgoard
Print Preview - organize and preview printed report
Print - print report
Exit - closes the program

Experiment

Add - add data from experiment file to the project
Remove - remove experiment file from the project
Open IRF - use IRF from another experiment file
Recalculate IRF - recalculate IRF using a different value for a reference lifetime
View Data - View data from the selected experiment in a table format
Save as *.frg - save the selected waveform in old data format *.frg (Could be used to export data in text format)
Save as *.fl - save selected waveform or experiment in new data format *.fl

Graph

X axis - toggles X axis between **Time** and **Channels** units
Y axis - toggles Y axis of signal graph between **Lin** and **Log** format
Grid - switch grid of graph between **Fine**, **Coarse**, and **None**
Mouse action - Switches action of left mouse button in **Signal** graph area between **Move Cursor**, **Zoom**, and **Move Graph**
Show IRF - show IRF associated with experiment
Show cursors - show cursors that determine fitting region
Remove background - subtract background for each waveform before plotting
Animation - controls **Zoom** animation on the signal graph
Show standard deviation - toggles display the signal standard deviation graph

Tools

Global analysis - open **Fitting Session** dialog
Analysis wizard - Perform data analysis using preexisting script file
Support plane error analysis - open **Support Plane Error Analysis** dialog
Waveform batch setting - open **Waveform Properties** dialog that allows user to change properties of waveforms
Baseline correction - open **Baseline Correction** dialog
Simulation - open **Data Simulation** dialog
Options - open **Options** dialog

Help

Content - open this help document
About - information about the program

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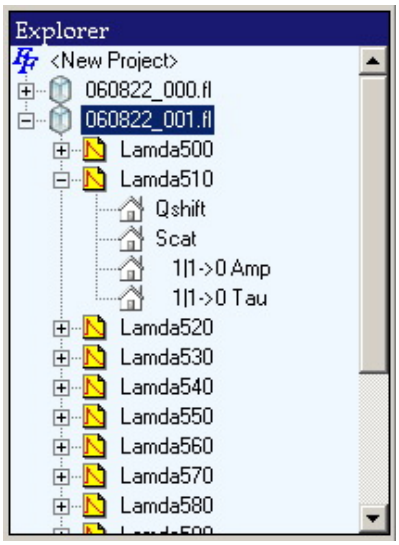
Project structure

Each FargoFit project contains all information about data in the project and a fitting model. The data hierarchy has four levels - **Project, Experiment, Waveform,** and **Fitting Parameter**. The program shows a structure of the current project in the *Project Explorer*. By selecting different items of the project in the *Project Explorer* user can access properties of different elements of the project. Properties of the selected element are shown in the *Properties Panel*. Some of them are *"Read Only"* others can be changed. Data associated with the selected item are plotted in the *Graph Panel*.

Fitting model.

All FargoFit projects have an associated fitting model. Even a new project has a default model. FargoFit program doesn't provide list of available models. Instead, user can combine different processes in fitting model using Model Designer. The program then analyses the model and generates a list of fitting parameters that is available in the Fitting Dialog. For each **Waveform** program uses values of fitting parameter associated with it to calculate a fit.



Project

The **Project** is a root element of FargoFit's data structure. It contains list of experimental files opened in the program for global analysis. The text of **Project** element is a project's file name. If project hasn't been saved, the *<New Project>* is used. All waveforms in the **Project** with property *Show = True* are plotted in the **Graph Panel** along with fits calculated for parameter values associated with this waveform and residuals in the **Residual panel**.

Experiment

The **Experiment** element represents data from single data file and may contain several waveforms, measured during one experiment, and an associated IRF function. The text of **Experiment** element is a name of data file. All waveforms of experiment with property **Show** = ***True*** are plotted in **Graph Panel** along with fits calculated for parameter values associated with this waveform and residuals in the **Residual panel**.

Waveform

The **Waveform** is a signal trace included in a data file. In **FargoFit** there are several physical parameters associated with each waveform. Some of them come with data file, others user has to set manually. Not all physical parameters have to be defined. Some of them don't have a meaning for a particular experiment. For example, parameter **Wavelength** has no meaning for an experiment with filter-based instrument. The text of the **Waveform** element represents arbitrary name of the waveform. Waveform data and IRF function are plotted in the **Graph Panel** along with the fit calculated for parameter valuess associated with this waveform and residual in **Residual panel**. All waveforms in a project have similar sets of fitting parameters associated with them. If user changes a model, program regenerates parameter sets for all waveforms according to the new model and calculates fit curves using these parameters on the fly.


Waveform is included in global analysis


Waveform is flagged as overflowed


Waveform is excluded from global analysis

Fitting parameter

The **Fitting Parameter** element represents data what program uses to calculate a fit curve. The physical meaning of each parameter depends on the model used. Main purpose of this element is to contain a parameter value. It also keeps additional information such as parameter's bounds, and error estimation results.


This icon in the **Fitting Parameter** element means that the parameter is **Local**- it is used just in one place


This icon in the **Fitting Parameter** element means that the parameter is **Global** - it is used in more than one place

 If icon in the **Fitting Parameter** element is grey, the support plane error analysis has not been performed for this parameter

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Project properties

ConfLevel

Level of confidence used for support plane error analysis.

Count

Number of experiments.

FileName

Name of the project file.

Weighting

Specify if standard deviation of the signal is used as weighting factor for χ^2 calculation.

χ^2

Global χ^2 of project.

Print

Boolean, property that shows if project report is included in print queue.

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Experiment properties

χ^2

Global χ^2 of experiment.

Left

Left cursor position

Right

Right cursor position

Comment

Experiment comment what come with data file. Could be changed at any time.

Count

Number of waveforms in the data file.

Date

Time stamp of the experiment.

Dwell

Time between data points (ns).

File name

Name of the data file.

User

Name of user that created the experimental file.

Version

Version # of Fargoland program that was used to perform the experiment.

IRF ampl

Amplitude of the IRF signal.

IRF aver

Number of averages that was made to measure IRF function.

IRF back

Background of the IRF function.

IRF color

Color of the IRF trace.

IRF lamda

Wavelength at which IRF function was measured.

IRF overflow

Shows if signal reached the instrument limit during IRF measurement.

IRF ref

Lifetime of reference sample that was used to calculate IRF function.

Print

Boolean property, shows if the experiment report is included in the print queue.

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Waveform properties

Fit

Boolean property, shows if the waveform is included in global analysis.

Show

Boolean property, shows if the waveform is shown in the graph panel when project is selected in the **Project Explorer**.

χ^2

Local χ^2 of the particular waveform.

Concentration

Concentration of component. Physical parameter used in some fitting models.

Delay

Delay between start of the experiment and the time when waveform was measured. Physical parameter used in some fitting models. It usually comes with data file.

Wavelength

Wavelength at which the waveform was measured. Physical parameter used in some fitting models. It usually comes with data file.

Polarizer

Polarizer position. Physical parameter used in some fitting models. It usually comes with the data file.

Temperature

Physical parameter used in some fitting models. It usually comes with the data file.

Average

Number of averages that was made to measure the waveform.

Color

Color of the waveform trace.

IsOverflow

Boolean property, shows if signal reached maximum level during waveform measurement.

Name

Arbitrary name of waveform.

Amplitude

Waveform amplitude.

Background

Waveform background.

Print

Boolean property, shows if project report is included in the print queue.

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Fitting parameter properties

Asymptotic

Result of asymptotic parameter error estimation performed automatically at the end of each fitting session.

LConfBound

Lower Confident Bound found during support plane error analysis.

UConfBound

Upper Confident Bound found during support plane error analysis.

Category

Physical meaning of the parameter.

IsLinked

Read only Boolean property, shows if the parameter is used in more than one place.

Value

Value of the parameter.

Vary

Read only property, shows if the parameter is varied during fitting session.

LBound

Max possible value of the parameter.

UBound

Min possible value of the parameter.

Print

Boolean property, shows if project report is included in the print queue.

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New project

To open new FargoFit project, do one of the following:

- Select **New** from the Main Window **File** menu.
- Press **Ctrl+N**.
- In the Main Window toolbar click the **New Project**  button from the toolbar.

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Open project

To open saved FargoFit project, do one of the following:

- Select **Open** from the the Main Window **File** menu.
- Press **Ctrl+O**.
- Click the **Open Project**  button in Main Window toolbar.

Pick a file with **.ff** extension using **Open File** dialog.

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Save project

To save FargoFit project, do one of the following:

- Select **Save** from the Main Window **File** menu.
- Press **Ctrl+S**.
- Click the **Save Project**  button in the Main Window toolbar.

Project is saved as a binary file with **.ff** extension.

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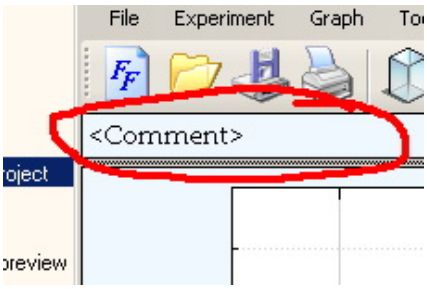
Project comment

User can make comments for project in Comment panel.



Height of this panel can be extended and more than one line of text can be used

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How to load data

To load experiment to FargoFit project, do one of the following:

- Drag and Drop files from Windows Explorer to FargoFit Explorer panel
- Select **Add** from the **Experiment** menu and pick a file using an extended **Open File** dialog.
- Click the **Add Experiment**  button from the main window toolbar and pick a file using an extended **Open File** dialog. Several data files can be selected and added to the project simultaneously.

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How to unload data

To unload experiment to FargoFit project, do one of the following:

- Select proper experiment or one of its waveform in the **Project Explorer**. Click **Remove** from the **Experiment** menu.
- Select proper experiment or one of its waveform in the **Project Explorer**. Click the **Remove Experiment**  button in the Main Window toolbar.

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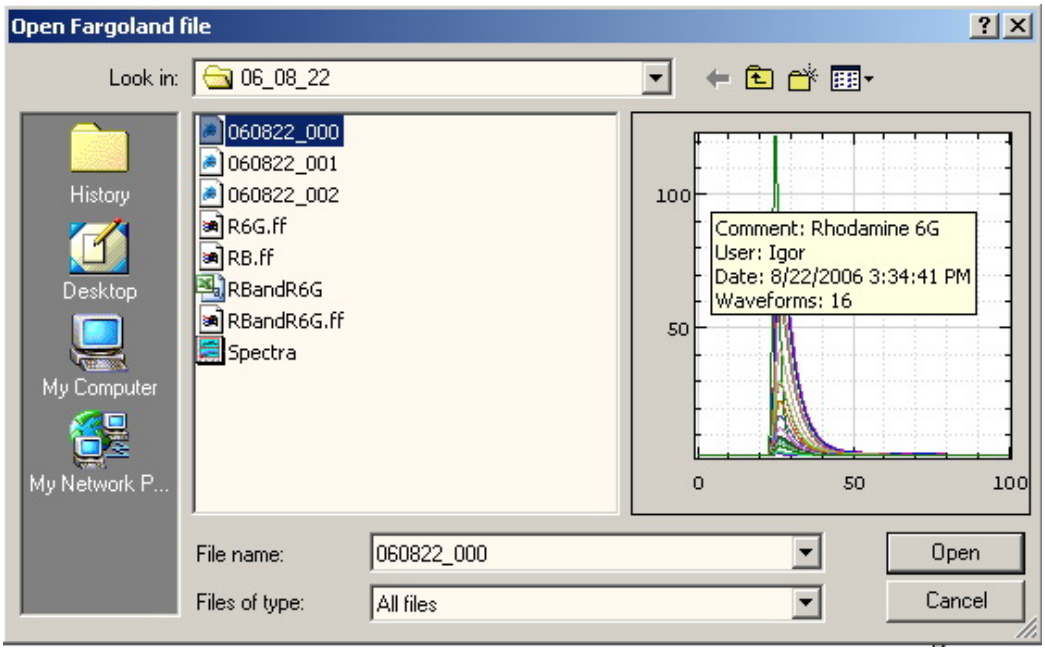
Open File Dialog

FargoFit uses this modified **Open File** dialog to add data files to a project.



On the right side of dialog user can see a data in the **Preview Panel**. A tool tip shows some additional information about the data file if mouse pointer is on graph area. This information includes:

- Comment
- Instrument operator
- Time stamp
- Number of waveforms

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Supported file formats

The **FargoFit** supports two kinds of data files:

*.fl files

*.fl files are <XML> files generated by the current version of data acquisition program **Fargoland**.

*.dat files

*.dat files are simple ASCII files. They contain 3 comma or tab separated columns with time, IRF intensity and luminescence decay intensity values. Optional 4th column can contain standard deviation of intensity. For proper weighting of TCSPC data 4th column should be calculated as square root of intensity.

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Data visualization overview

The graph area of main program window shows the data for currently selected item in project explorer. The view of graph area depends on a sort of this selected item.

Project

All waveforms with property **Show** set to ***True*** are plotted in the **Signal** area. Fitting results are also plotted in the **Signal** area if **Fit** property is set to ***True***. Residuals are plotted for the same waveforms in the **Residual** area. If **Standard Deviation** area is visible then standard deviation data are plotted there if they exists in data file.

Experiment

All waveforms in the selected experiment are plotted in the **Signal** area. Fitting results for waveforms with **Fit** property set to ***True*** are plotted in **Signal** area. Residuals are plotted for the same waveforms in the **Residual** area. If **Standard Deviation** area is visible then standard deviation data are plotted there if they exists in data file. Left and right cursors show the part fo waveform that is used for fitting

Waveform

Selected waveform is plotted in the Signal area. Fitting result is plotted in the **Signal** area if **Fit** property of selected waveform is set to ***True***. Residual is plotted in **Residual** area. If **Standard Deviation** area is visible then standard deviation data are plotted there if they exists in data file.

Parameter.

Result of **Support Plane Error Analysis** is plotted if analysis has been performed.

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Graph axis.

Vertical axis

The vertical axis of the **Signal** sub-window can be changed between **Linear** and **Logarithmic** using **Y axis** item from the **Graph** menu.

Horizontal axis

The horizontal axis of **Signal, Residual, Standard deviation** sub-windows can be changed between **channels** and **nanoseconds** using **X axis** item from the **Graph** menu.

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Graph grid

Grid in the graph sub-windows can be changed between **None, Coarse, Fine** using **Grid** item from the **Graph** menu.

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Mouse actions

Signal and **Support plane error** sub-windows have left mouse button click functions. Some of them are general, another are specific. User can change mouse function using **Mouse action** section of **Graph** menu or toolbar buttons. The meaning of each function is listed below.

Cursor

If Experiment is selected in project explorer and **Show cursors** option is ***On*** there are two vertical lines on the Signal sub-window that represent fitting region. With this function activated user can change this region by clicking on a cursror and dragging it to a new position. This action will be followed by new fitting model calculation.

Zoom

The user can zoom into a part of full graph in Signal sub-window using this option. User should press left mouse button and select area of interest by dragging rectangle cursor over it. In order to return back to full graph right mouse button should be clicked with cursor is somewhere in graph area.

Move

Once zoomed in, this option will enable user to move the zoomed area through entire graph.

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Additional visualization features

Additional options available from **Graph** menu.

Show IRF

If this item is checked IRF associated with the experiment is shown.

Show cursors

This option controls cursors visibility.

Remove background

If this item is checked, background is subtracted from each waveform before plotting.

Animation

If this item is checked the zoom operation go through 10 intermediate stages that makes an effect of smooth movement. This option can slow the program down especially for a big number of plotted waveforms.

Show Standard Deviation

If this item is checked, the graph for signal's standard deviations is shown at the bottom of the graph area.

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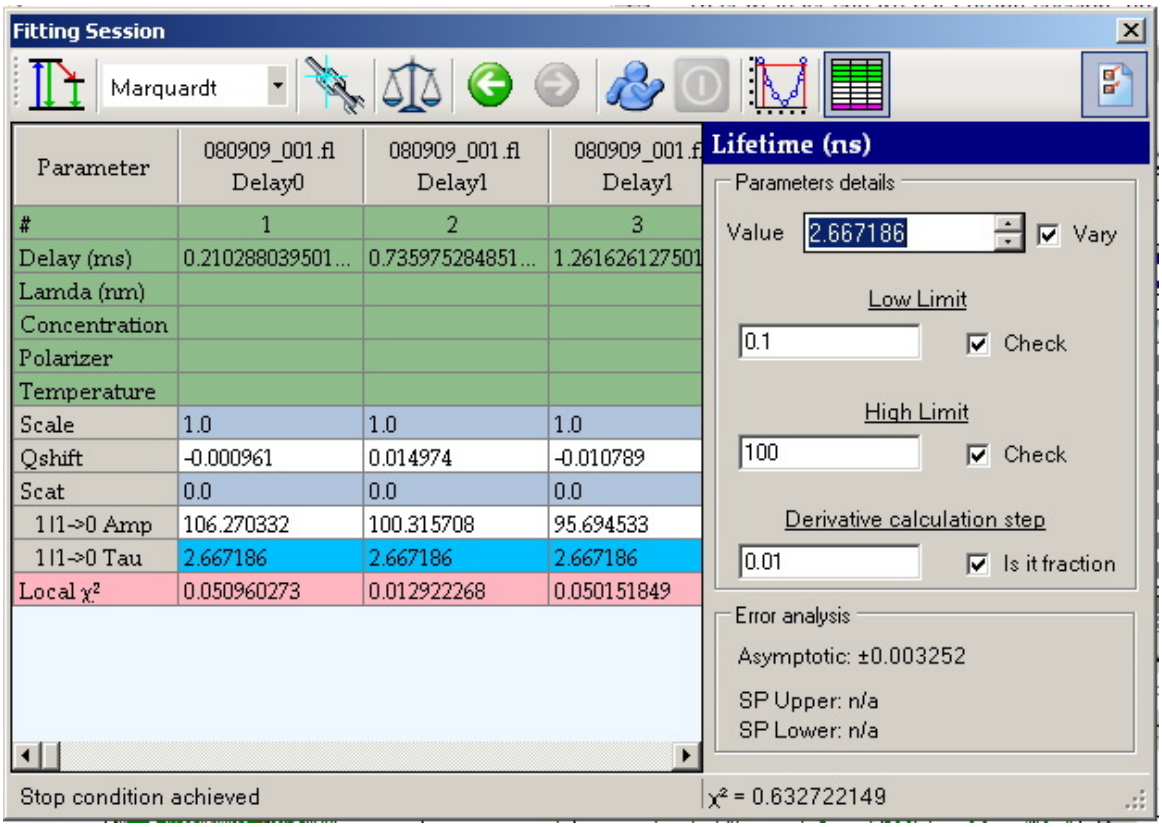
Introduction to Fitting Session dialog

To start fitting session, do one of the following:

- Select **Global Analysis** from the **Tools** menu.
- Click the **Global Analysis**  button from the Main Window toolbar.

The **Fitting Session** dialog has a toolbar, a table of parameters, a current parameter setting dialog, and a status bar.



Toolbar

 Opens **Model Designer**

 This button switches between Marquardt and Simplex fitting algorithms.

 This button enables **Link parameters mode** if checked.

 If this button is checked the residual is weighted by signal standard deviation.

 Undo/Redo parameter values

 Starts/Stops fitting

 Performs **Support Pane** error analysis

 Shows/Hides **Waveform properties**

 Shows/Hides **Current Parameter properties** control

Parameter table

The parameter table shows list of fitting parameter values for all waveforms included in a project. The first column lists the name of parameters used in current fitting model. Every project has a fitting model associated with is. New project starts with default single exponent model. Each waveform included in a project has a column with parameter values associated with it. The header of each column contains file name and waveform name. If **Show Waveform Properties** option is checked, the table also shows experiment conditions associated with each waveform: delay, emission wavelength, concentration, emission polarizer orientation, and temperature. Values of these experimental conditions can't be changed from this table, user must use **Properties** control in the main window instead. Last row

of the table shows χ value for each waveform.

For user's convenience cell background color represents cell's status:

green	Experimental condition
white	Variable parameter
gray	Fixed parameter
blue	Current parameter
pink	χ^2 value

Current Parameter Properties panel

The name of the current parameter is displayed at the top of the panel.

Value of the parameter and its lower and upper limits are displayed below. These values are changed by either typing in proper text box, or by pressing the Up/Down buttons on right of the value text box. By checking the **Vary** box, the parameter is varied during fitting, otherwise the parameter will be fixed. he parameter value will be constrained by upper or lower limits if the **Check** box is checked for corresponding limit. The **Derivative calculation step** text box determines the step size that is used to calculate χ^2 derivative for the current parameter in Marquardt algorithm. If **Is it fraction** check box is checked, the value in text box is treated by the program as a fraction of the parameter value, otherwise it is an absolute value of step size.

The last group of controls shows the confidence interval for the current parameter if it is available. The **Asymptotic** confidence interval is calculated at the end of each successive fitting process using information about curvature matrix at the minimum of χ^2 hypersurface. **SP** (Support Plane) confidence limits are calculated by reconstructing real shape of χ^2 hypersurface projection.

Status bar

The status bar contains two panels. First panel shows number of fitting iteration being calculated. If the Marquardt algorithm is used, this panel also shows number of unsuccessful fitting loops that leads to increase of Marquardt dumping factor and this factor value. When fitting is terminated the reason for termination is shown here. The second panel shows the current value of χ^2.

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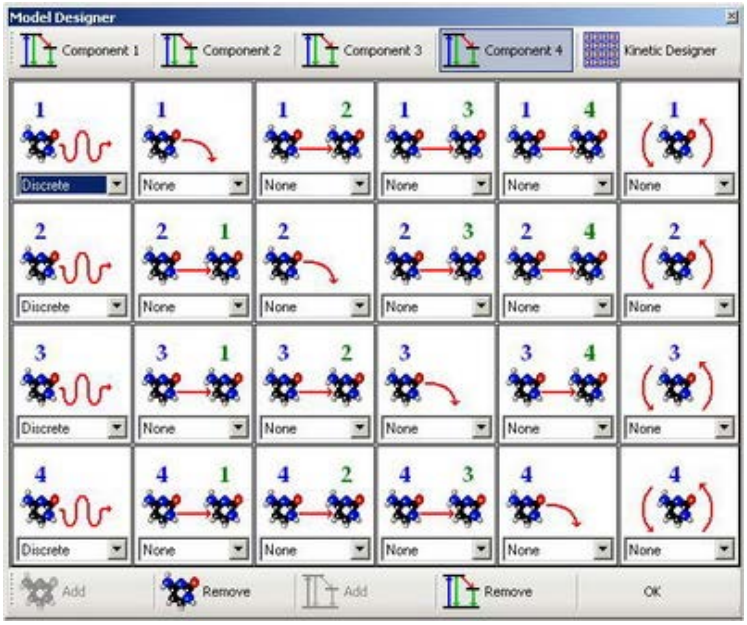
Model designer

Designer window

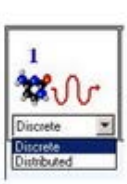
Model designer lets a user to create data a fitting model as a combination of components and processes. Click on **Model designer** button in toolbar of **Fitting session** window to bring up the following dialog:



Luminescent components

The simplest element of the model is a luminescent component . Every component is represented by row in **Model designer** table. Model can have up to 4 luminescent components. Final observed luminescence intensity decay is calculated as a sum of the component decays.To change number of components buttons **Add component** and **Remove component** at the bottom of **Model designer** window should be used.

Every component has a process of luminescence associated with it. This process could have single lifetime or single Gaussian distribution of lifetimes. This could be specified by using combo box in first column of **Model designer** table.



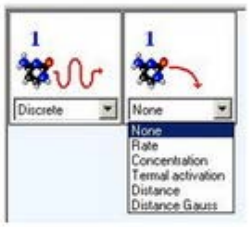
$$I(t) = \begin{cases} Ae^{-\frac{t}{\tau}} \\ \frac{A}{\sigma\sqrt{2\pi}} \int_{\tau\,\min}^{\tau\,\max} e^{-\frac{1}{2}\left(\frac{\tau-\tau_0}{\sigma}\right)^2} e^{-\frac{t}{\tau}} d\tau \end{cases}$$

In case non-radiative de-excitation process presents in a system, like collision quenching or energy transfer to non-luminescent acceptor, the rate constant of this process can be included in a model:

$$I(t) = Ae^{-\left(\frac{1}{\tau}+k\right)t}$$

Using combo box in a cell for quenching process  the user can map this rate constant of this process to one of these functions:

- None
- Rate constant
- Stern-Volmer equation
- Thermal activation
- Resonance Energy Transfer with discrete distance
- Resonance Energy Transfer with distributed distance



$$k = \begin{cases} 0 \\ k \\ \frac{1}{\tau} k_{SV}[Q] \\ qe^{-\frac{E_{act}}{R_c(T+273)}} \\ \frac{1}{\tau}\left(\frac{R_0}{R}\right)^6 \\ \frac{1}{\tau}\left(\frac{R_0}{R}\right)^6, R\ distributed\ \frac{e^{-\frac{1}{2}\left(\frac{R_c-R}{\sigma}\right)^2}}{\sigma\sqrt{2\pi}} \end{cases}$$

Anisotropy decay what is associated with component can be specified using combo box in last column of **Model designer** window. Available options of anisotropy model are:

- None
- Single exponent
- Double exponent
- Triple exponent

$$r(t) = \begin{cases} 0 \\ r_0((1-A_\infty)e^{-\frac{t}{\phi_1}} + A_\infty) \\ r_0(A_1 e^{-\frac{t}{\phi_1}} + (1-A_1-A_\infty)e^{-\frac{t}{\phi_2}} + A_\infty) \\ r_0(A_1 e^{-\frac{t}{\phi_1}} + A_2 e^{-\frac{t}{\phi_2}} + (1-A_1-A_2-A_\infty)e^{-\frac{t}{\phi_3}} + A_\infty) \end{cases}$$

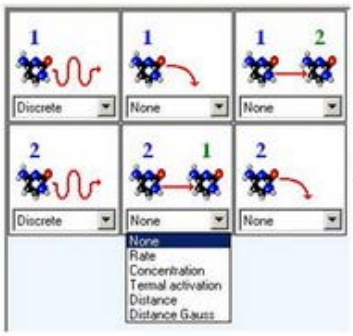
Observed intensity decay for this component is then calculated by:

$$I_{ob}(t) = \frac{I(t)\left(1 + r(t)\left(3\cos^2(\theta) - 1\right)\right)}{\sqrt{1 - \left(1 - G^2\right)\sin^2\theta}}$$

where:
I(t) - intensity decay of component
r(t) - anisotropy decay of component
G - g-factor of instrument
θ - orientation of emission polarizer

Fast kinetics

The processes of exchange between luminescent components in excited state can be included in a model. These must be first order processes like A*+B→A+B*. These processes are called **Fast kinetic** because they occur on a timescale, that is limited by lifetime of excited luminescence component. These processes can be described by system of coupled linear differential equations, where rate constants are mapped to function of exchange process. To assign exchange process between components A and B, use the combo box in cell (A,B) of **Model designer** window.



$$k = \begin{cases} 0 \\ k \\ \frac{1}{\tau} k_{SV}[Q] \\ qe^{-\frac{E_{act}}{R_c(T+273)}} \\ \frac{1}{\tau}\left(\frac{R_0}{R}\right)^6 \\ \frac{1}{\tau}\left(\frac{R_0}{R}\right)^6, R\ distributed\ \frac{e^{-\frac{1}{2}\left(\frac{R_c-R}{\sigma}\right)^2}}{\sigma\sqrt{2\pi}} \end{cases}$$

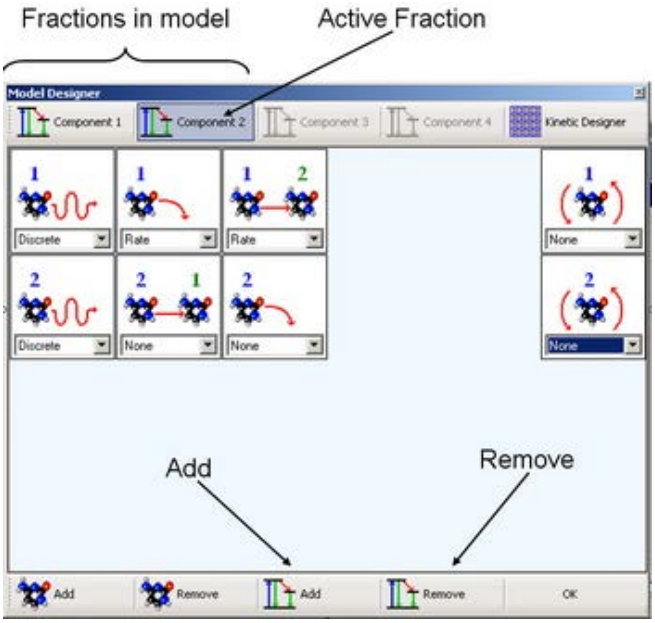
Available exchange functions are the same as for non-radiative de-excitation processes.

- None
- Rate constant
- Stern-Volmer equation
- Thermal activation

- Resonance Energy Transfer with discrete distance
- Resonance Energy Transfer with distributed distance

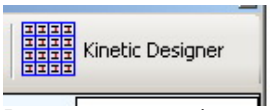
Fractions

If investigated system is a mixture of physical species with luminescence decays more complicated than single exponent every species can be represented in a model as a **Fraction**. Every **Fraction** may contain up to 4 luminescent components what may have inner conversion and interaction terms. The observed luminescence decay is calculated as a sum of decays of individual **Fractions** multiplied by weighting factors of corresponded **Fractions**. The weighting factor is a relative concentration of corresponded **Fraction**. Weighting factor of last **Fraction** is assumed to be 1 minus the sum of weighting factors of another fractions. Fractions are represented in **Model designer** window by picture  . Number of fractions can be changed from 1 to 4 using **Add** and **Remove** fraction buttons at the bottom of **Model designer** window. All fractions have same number of luminescent components but additional inner conversion and interaction processes can be different. In order to change these processes for particular **Fraction** this fraction should be selected at the top of **Model designer** window.



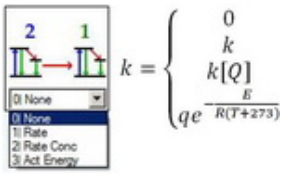
Fraction kinetics

Kinetic of fraction transformation can be included in a fitting model in form of first order process like **Fraction1→Fraction2** or pseudo-first order process like **Fraction1+Fraction2→Fraction3** in case of surplus concentration of **Fraction1** or **Fraction2**. Process of fraction dissipation or process of interaction with non-fluorescent component like **Fraction1+A→Fraction2** in case of surplus concentration of non-fluorescent component is also valid. These processes are described by system of coupled linear differential equations with time-dependent fraction weighting factors. Rate constants of these equations can be included in a model using **Kinetic designer** tab what can be shown using button on top of **Model designer** window.



Rate constant can be specified using the combo box in corresponding cell of **Kinetic designer**. Available options are:

- None
- First-order rate constant
- Second-order rate constant multiplied by concentration of the surplus component (pseudo-first order condition).
- Thermal activation



$$k = \begin{cases} 0 \\ k \\ k[Q] \\ qe^{\frac{E}{R(T+273)}} \end{cases}$$

During model evaluation, the program solves a system of coupled linear differential equations and calculates fraction weighting factors at delay specified for waveform as experiment conditions using values of **Fraction** parameters as initial values at **0** delay. Please note what this analysis is available only for waveforms with presented value of **Delay** experimental condition, i.e. stopped-flow and kinetic experiments.

FargoFit Help

Descriptor of parameter

Descriptor of parameter

Parameter descriptor is a string what appears in Parameter column. Besides parameter name it can include one or more indexes.Parameters that only have a name in the descriptors are general parameters for a waveform. Parameters that belong to specific fraction have its number in descriptor followed by symbol "**|**", Eg. **1|1->0Amp**. If a parameter is a rate constant determined from fraction kinetics it will have two indexes that specify fractions involved in the kinetic process, Eg. **1->2|SlowRate**. If second index is "**0**" this is a kinetic of dissipation of fraction specified by first index. The numbers after symbol "**|**" specify indexes of exponential components involved in a process described by parameter. If second index isn't "**0**" this is energy transfer process between two exponential components, otherwise this is luminescence or inner conversion process to ground state.

List of parameters

Scale	scale factor, is used to compensate difference of concentration, excitation intensity, etc. for different waveforms in global analysis
Qshift	color shift
Scat	amplitude of scattered (zero-lifetime) component
Bound	boundary conditions (time-zero relative absorbance of each species)
Amp	amplitude of exponential component
Tau	lifetime of exponential component
TauFWHM	half-width of lifetime Gauss distribution
ForstDist	Forster distance
Rate	rate constant of first-order process
SternVolmer	Stern-Volmer quenching constant
Freq	Arrhenius frequency factor
ActEnerg	Arrhenius activation energy
FRET_Dist	distance of Forster Energy Transfer
FRET_FWHM	half-width of Forster energy transfer distance Gauss distribution
AnisoMax	time-zero initial anisotropy term
AnisoFract	pre-exponential fraction associated with anisotropy correlation time
CorrTime	rotational correlation time
AnisoFractInf	fraction associated with constant anisotropy term
Gfact	g-factor of instrument
Fraction	relative concentration of **Fraction** component (relative concentration at zero-time for double-kinetic experiments)
SlowRate	rate constant of first order slow-kinetic process
SlowRateConc	rate constant of pseudo-first order slow-kinetic process
SlowFreq	Arrhenius frequency factor for slow-kinetic process
SlowActEnerg	Arrhenius activation energy for slow-kinetic process
DeadTime	dead-time of stopped-flow instrument

FargoFit Help

Link parameters

Parameters linking means to force different parameters to have same value during fitting. There are two ways to perform parameter linking.

Link across all.

User can force equivalent parameters in different experiments to be the same. Since different waveforms correspond to different columns in the parameters table this means making all parameters in a row the same. In order to perform this linking right click on a cell in the row. This will make the parameter in this cell a **Current parameter** and will open a pop-up menu. Click **Link across all** item. This will link parameters in all cells of the current row to the **Current parameter**. Unlinking is done in the same way.

Link across experiment.

It works in exactly the same way as **Link Across All** but only for waveforms within a single experiment. Pick the parameter that you want other parameters to be linked to and right click on its cell. Click on **Link across experiment** in the pop-up menu. This will link parameters in all cells of the current row for current experiment to the **Current parameter**.

Individual linking.

To perform more complicate linking user can switch program to the **Link mode** by checking the  button in a toolbar or using hot-key combination CTRL+SHIFT. In this mode all parameters with the same physical meaning can be linked to the **Current parameter**. Moving cursor over cells with these parameters will make cursor looks like a padlock. If the padlock is unlocked, the parameter isn't linked to the **Current parameter** and can be linked by clicking on its cell. If padlock is locked, parameter is already linked and could be unlinked by clicking on the cell. When linking is done program should be switched to the **Fitting mode** by unchecking  button.

FargoFit Help

Copy values of fitting parameters to clipboard

User can copy values of fitting parameters to clipboard in order to transfer them to another program using pop-up menu of **Parameters** table. If **Waveform properties**  button is checked the property values can be copied as well. Several options for copying values are available:

Copy Cell

It copies values of **Current Parameter**

Copy Row

It copies values of all parameter in same row with **Current Parameter**

Copy column

It copies values of all parameter in same column with **Current Parameter**

Copy table

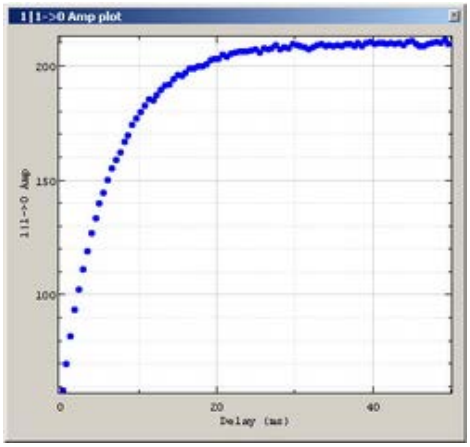
It copies entire table

FargoFit Help

Plot parameter values vs experiment conditions

Plot parameter values vs experiment conditions

Performing global fit the fitting parameters on same row of **Parameters** table can be plotted as a function of several arguments such as number of the corresponding waveform, delay, concentration, polarizer position, temperature, emission wavelength. Select **Plot vs -> <*argiment*>** from pop-up menu to bring up the following window:



Using pop-up menu user can:

- copy graph as a picture to clipboard
- save graph as a picture in file
- copy graph data in ASCII format to clipboard
- save graph data in ASCII format in file

Asymptotic Error Analysis

Asymptotic Error Analysis

Asymptotic Error Analysis method is a simplest way to measure the reliability of parameter values calculated by fitting. This method is based on examination of the diagonal elements of χ^2 curvature matrix for the local area of parameters set. This analysis yields a value of of standard deviation of hypothetical Gaussian distribution centered at the parameter value and is called Asymptotic Standard Error (ASE). Program automatically calculates and lists ASE for all non-fixed parameters after a successful fitting procedure.

FargoFit Help

Support Plane Error Analysis

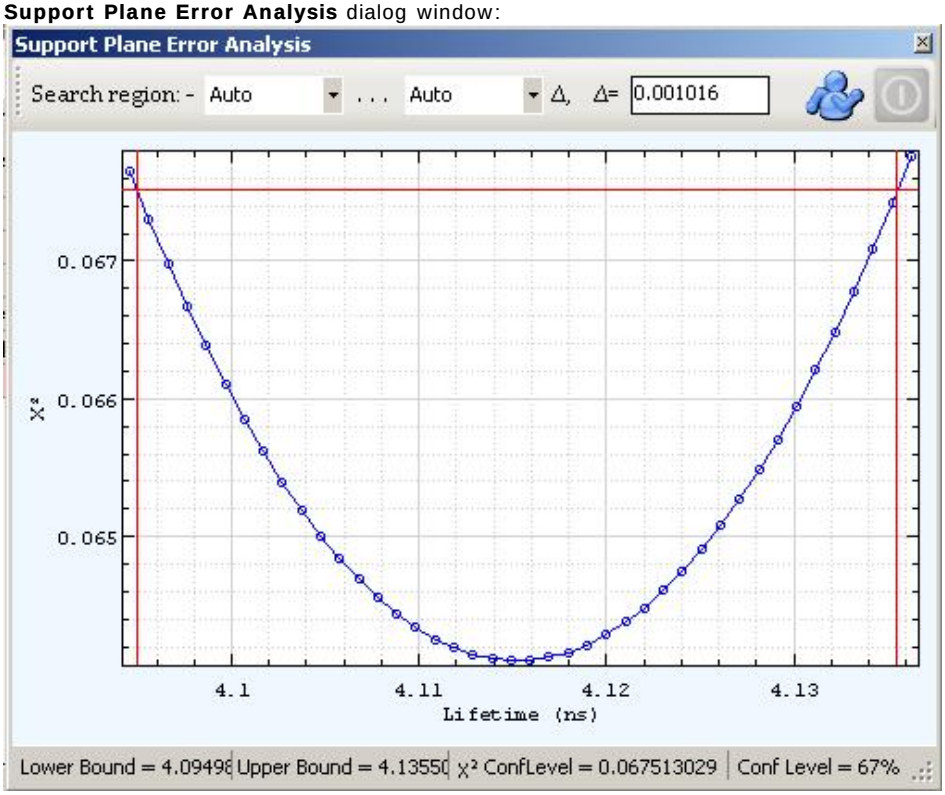
Support Plane Error Analysis

The goal of support plane error analysis is to calculate the projection of χ^2 hyper surface to χ^2 - Investigated Parameter plane. The parameter under investigation is fixed at the trial value near the best fit value, but all other parameters are allowed to vary to minimize χ^2, and fitting procedure is performed. In this way, a set of minimum χ^2 values, which are calculated as a function of the particular parameter, is built. The F-test statistical criteria is then used to determine tolerance level of the best-fit χ^2. Confidence level for F-statistic is a project property and by default its value is 67%. The intersections of this level with χ^2 plot represent upper and lower confidence limits of the investigated parameter.

To open Support Plane Error Analysis dialog do one of the following:

- Select parameter in **Explorer** and click on item **Support Plane Error Analysis** of **Tools** menu.
- Select parameter in **Explorer** and click on **Globe**  button in the main window toolbar.
- Using **Fitting Dialog** window select parameter and click **Support Plane Error Analysis**  button in toolbar

Support Plane Error Analysis dialog window:



Using text fields in the toolbar user could specify explored region in term of steps that program will make to increase and decrease the parameter value and size of this step. If user specifies **Auto** as a number of steps the program will make steps until it reaches the level calculated using F-statistic. Program will stop to change parameter value if it reaches limit specified for this parameter. Default step size is 10% of asymptotic standard error but it could be changed.

Buttons **Run** and **Stop**  are used to start and stop the analysis process. Result of analysis is shown on graph in the middle of dialog window. It can be exported in a text format using **Data View** item of the **Experiment** menu of the main window.

Status bar has four fields:

- Low limit of confident interval.
- High limit of confident interval.
- χ^2 tolerance calculated using F-statistic.
- Confidence level.

FargoFit Help

Use different IRF

Every Fargoland data file has information about **I**nstrument **R**esponse **F**unction (**IRF**) but IRF from another data file can be associated with particular experiment.

To use IRF from another data file with current experiment:

Select **Open IRF** from the **Experiment** in the Main Window menu

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FargoFit Help

Recalculate IRF

Every Fargoland data file has information about **I**nstrument **R**esponse **F**unction (**IRF**). IRF is measured using scattered sample or sample with single exponent luminescence decay. Lifetime of this decay is used to calculate IRF and is saved in data file as reference time. In case of scattered sample **0** reference time is used. User can recalculate IRF using another reference time.

To recalculate IRF using another reference time:

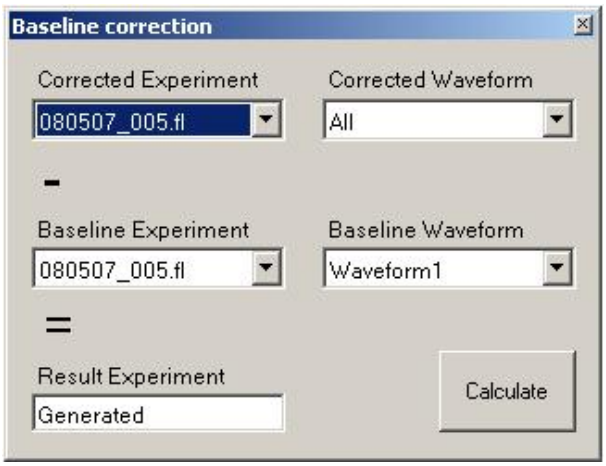
Select **Recalculate IRF** from the Main Window **Experiment** menu. Enter the reference time in dialog box.

FargoFit Help

Baseline correction

Baseline correction

In some cases it can be necessary to subtract one waveform from another, usually in order to correct data for background luminescence or scattered light. Click on **Baseline Correction** item of **Tools** menu to bring up the following dialog:



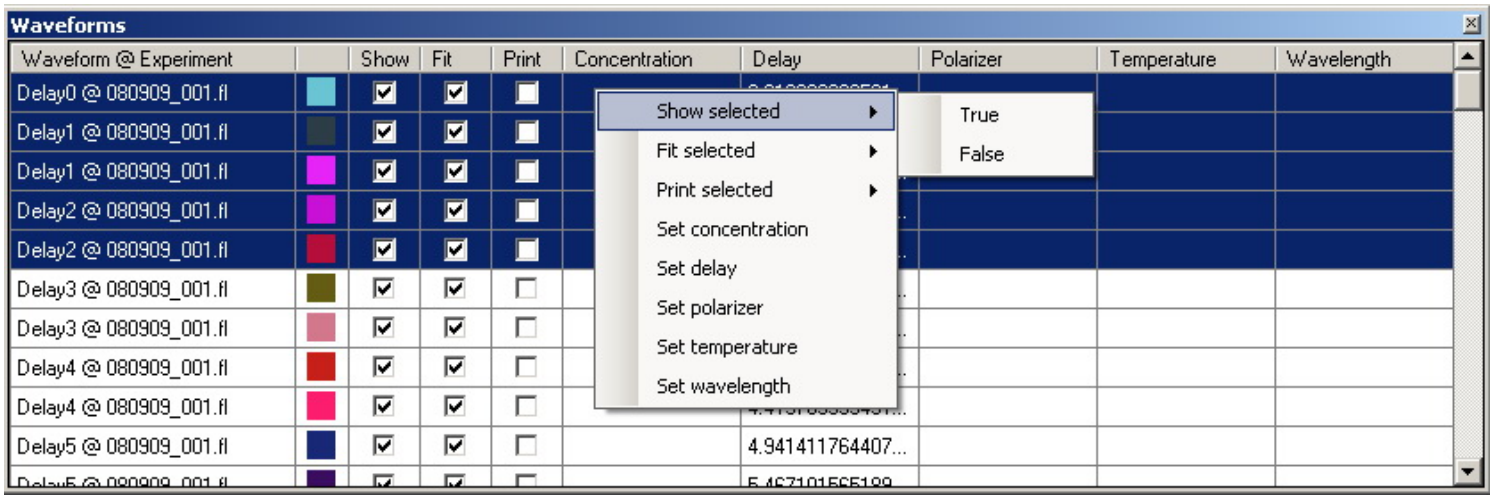
Pick waveform what need to be corrected using combo boxes **Corrected Experiment** and **Corrected Waveform**. If item **All** is selected in **Corrected Waveform** the correction will be applied to all waveforms of experiment selected in **Corrected Experiment**, otherwise only selected waveform will be corrected. Pick waveform what will be subtracted using combo boxes **Baseline Experiment** and **Baseline Waveform**. Click on **Calculate** button to calculate the result. Experiment with name specified in field **Result Experiment** will be created and result will be placed in it. Please note that if standard deviation data exist for both operands the standard deviation of result is calculated as square root of sum of squares of standard deviations.

FargoFit Help

Batch waveform properties change

To change properties **Show, Fit, Print, Concentration, Delay, Polarizer, Temperature, Wavelength** of batch of waveforms do following:

Select **Waveform batch setting** from the **Tools** menu. Following dialog will appear.



This dialog shows a list of all waveforms in a project. User can change **Show, Fit, Print** properties of an individual waveform using corresponded check boxes or highlight several waveforms and change properties **Show, Fit, Print** at once using pop-up menu. Value of **Concentration, Delay, Polarizer, Temperature, Wavelength** can be changed using pop-up menu for one or several selected waveforms as well.

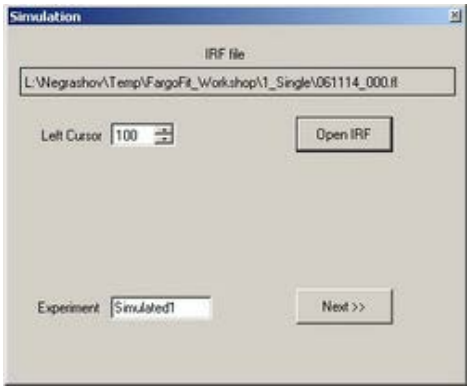
FargoFit Help

Data simulation

Data simulation

To simulate experimental data click on **Simulate** item of **Tools** menu.

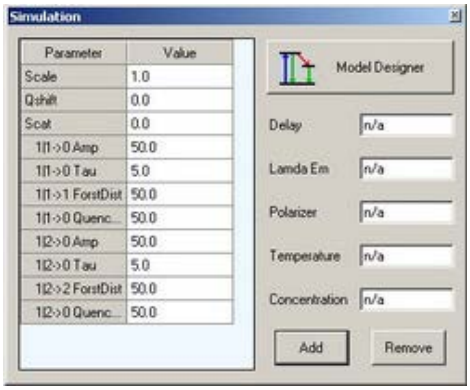
Data simulation is a two stage process. At first stage user specify source file for IRF data and left cursor position what limits data range used to calculate IRF background value. User also specify the name of new experiment what will contain simulated data.



At second stage user can create fitting model using **Model Designer** dialog opened by clicking **Model Designer** button. At second stage user can create fitting model using **Model Designer** dialog opened by clicking **Model Designer** button. Then user can change parameters values using

Parameters

table. If fitting model requires data for delay, concentration, polarizer orientation, or temperature they must be entered in corresponded fields. New simulated waveform is generated by clicking **Add** button. Last generated button can be deleted by clicking **Remove** button.



Numerical values of simulated data can be seen using **View Data** item of **Experiment** menu. Whole simulated experiment can be saved using **Save as *.fl** item of **Experiment** menu.

Copy graph to clipboard

Copy graph to clipboard

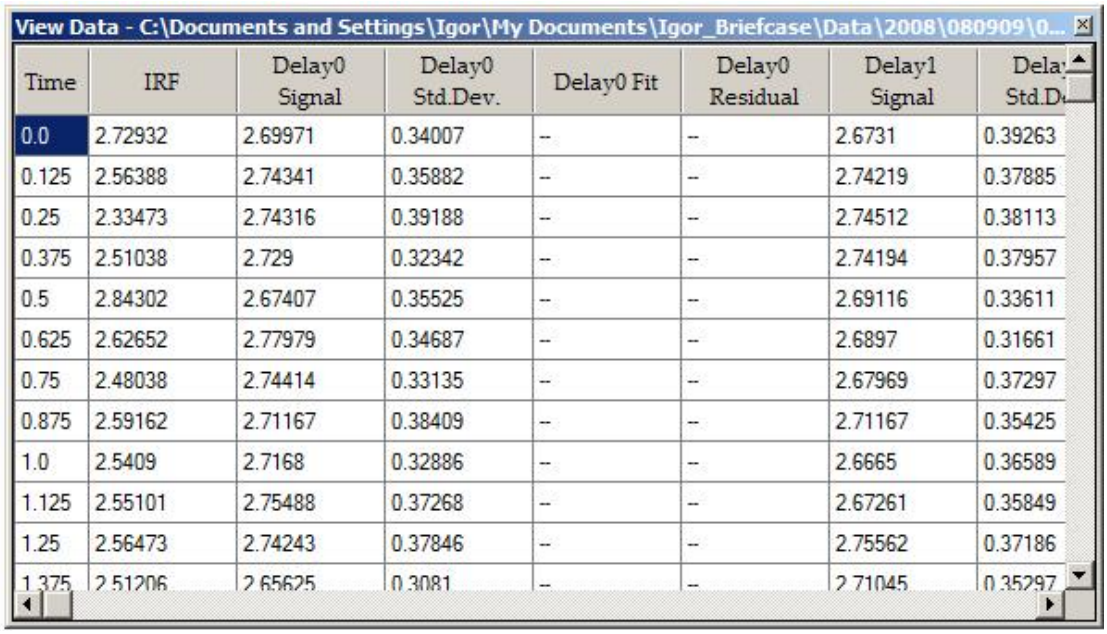
To copy entire graph area of program window as image to clipboard click on **Copy Graph** item in the **File** menu. Graph area is copied as it is seen including aspect ratio with signal graph, residual graph, and optionally signal standard deviation graph.

FargoFit Help

Export data as table

Export data as table

Experimental data and result of analysis can be viewed in the form of a table by clicking **View Data** item in the **Experiment** menu.



Time	IRF	Delay0 Signal	Delay0 Std.Dev.	Delay0 Fit	Delay0 Residual	Delay1 Signal	Dela Std.D
0.0	2.72932	2.69971	0.34007	--	--	2.6731	0.39263
0.125	2.56388	2.74341	0.35882	--	--	2.74219	0.37885
0.25	2.33473	2.74316	0.39188	--	--	2.74512	0.38113
0.375	2.51038	2.729	0.32342	--	--	2.74194	0.37957
0.5	2.84302	2.67407	0.35525	--	--	2.69116	0.33611
0.625	2.62652	2.77979	0.34687	--	--	2.6897	0.31661
0.75	2.48038	2.74414	0.33135	--	--	2.67969	0.37297
0.875	2.59162	2.71167	0.38409	--	--	2.71167	0.35425
1.0	2.5409	2.7168	0.32886	--	--	2.6665	0.36589
1.125	2.55101	2.75488	0.37268	--	--	2.67261	0.35849
1.25	2.56473	2.74243	0.37846	--	--	2.75562	0.37186
1.375	2.51206	2.65625	0.3081	--	--	2.71045	0.35297

Table content depends on item currently selected in **Explorer**

- **Project** - Option is not available.
- **Experiment** - Table contains columns with time, instrument response function, and data for all waveforms in the experiment file.
- **Waveform** - Table contains column with time, instrument response function, and data for particular waveform.
- **Fitting parameter** - Table contains result of support plane error analysis: column with parameter values and column with correspondent χ^2 values. If support plane error analysis was not performed for this parameter no table rows will be displayed.

Waveform data contains four columns: signal, signal standard deviation, fit, and residual. Some columns may be hidden/shown using table pop-up menu.

Data in the cells can be highlighted using left mouse click. Alternatively, left click on a column header highlights the whole column. Highlighted data can be copied to clipboard using table pop-up menu.

FargoFit Help

Print result of analysis

Print result of analysis

Printed report can be created for every element of data hierarchy such as **Project**, **Experiment**, **Waveform**, and **Fitting Parameter**. Every element has a Boolean property **Print** what controls if report for this element is included in print queue. This property can be changed using:

- **Property** table when this element is selected in **Explorer**.
- **Waveform Batch Setting** item of **Tools** menu for waveforms.
- **Print Preview** dialog.

To send queue of print reports to printer, do one of the following:

- Select **Print** from the Main Window **File** menu.
- Press **Ctrl+P**.
- Click on **Print**  button from the Main Window toolbar.

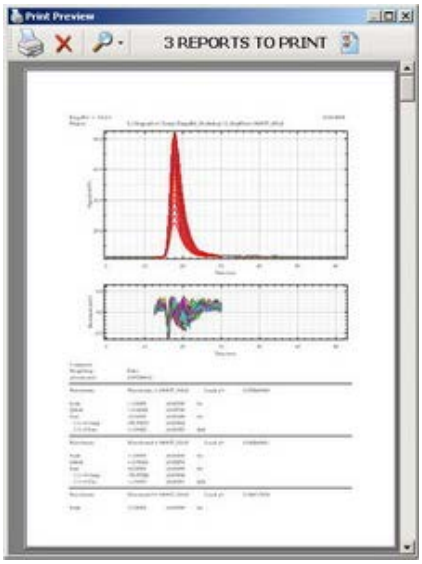
Standard Windows **Print** dialog will appear.

FargoFit Help

Print preview

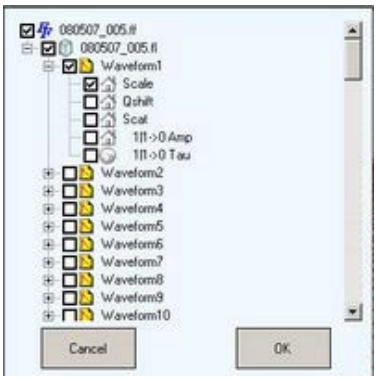
Print preview

Printed report can be created for every element of data hierarchy such as **Project**, **Experiment**, **Waveform**, and **Fitting Parameter**. Every element has a Boolean property **Print** what controls if report for this element is included in print queue. To see this queue, click on **Print Preview** item of **File** menu. The dialog similar to shown on picture will be opened.



Number of reports in queue is shown on the top of dialog. In order to change queue content, click on **Show Explorer**  button. The window with project tree structure similar to

Project Explorer will appear.



Using check boxes at project components user can include/exclude report for particular element from print queue. Use button **OK** to see result or button **Cancel** to discard changes.

To print the queue, click on **Print**  button on toolbar. Standard Windows **Print** dialog will appear.

FargoFit Help

Analysis wizard

Parameters wizard

Parameters wizard is designed to automate creation of fitting model. It has a script language what performs model creation by adding required fractions, luminescence and quenching components, rotational components, and component interactions. Once created, script can be saved in a file for future reuse. To open Parameters wizard one needs to click on the **Wizard**  button in the Fitting Session window toolbar. The Parameters Wizard window has 4 buttons on a top - Open, Insert, Save, and Run - and a text area where script is actually entered. The easiest way to make a script is to click on Insert button and pick proper command from the list. In order to make a comment semicolon should be placed in front of it.

FargoFit Help

How to make a script file for analysis wizard

Script file for Parameters Wizard.

script example

Parameters Wizard script is a text file with list of commands, using one command per line. Semicolon character is used to mark line as a comment.

General commands.

- **Default** - Creates default fitting model what is a model with one exponent and one fraction.

Model creation commands.

- **AddFract** - Increase number of fractions
- **RemoveFract** - decrease number of fractions
- **AddExp** - Increase number of exponential components
- **RemoveExp** - decrease number of exponential components
- **Exp F I S** - switch exponential component **I** of fraction **F** between discrete (**S="0"**) and distributed (**S = "1"**) states
- **Inter F I1 I2 S** - specify a model for a process of energy conversion from exponential component **I1** to exponential component **I2** of fraction **F** as:
  S = "0" - none
  S = "1" - simple rate constant
  S = "2" - Stern-Volmer eqation
  S = "3" - energy of activation
  S = "4" - FRET
  S = "5" - FRET with distance distribution
  If **I1** = **I2** this is a process of internal energy conversion from exponential component **I1** to ground state.
- **Aniso F I S** - set anisotropy decay function associated with exponential component **I** of fraction **F** as:
  S = "0" - none
  S = "1" - single exponent
  S = "2" - double exponent
  S = "3" - triple exponent
- **Kinetic F1 F2 S** - set rate constant of conversion of fraction **F1** to fraction **F2** as:
  S = "0" - none
  S = "1" - first order rate constant
  S = "2" - product of second order rate constant and concentration
  S = "3" - energy of activation

Parameter Commands

- **Value Col Row Val** - set value of parameter in column **Col** and row **Row** equal **Val**
- **Vary Col Row Bool** - set parameter in column **Col** and row **Row** fixed if **Bool = "True"** and variable if **Bool = "False"**
- **Link Col1 Row1 Col2 Row2** - link parameter in column **Col2** and row **Row2** to parameter in column **Col1** and row **Row1**
- **LinkAcross Col Row** - link all parameters in **Row** to parameter in column **Col**
- **LinkAcrossExp Col Row** - link all parameters in **Row** to parameter in column **Col** for experiment which **Col** belongs to
- **Unlink Col Row** - unlink parameter in column **Col** and row **Row**
- **UnlinkAcross Row** - unlink all parameters in row **Row**

FargoFit Help

Program options

General parameters not associated with particular project can be set using **Options** dialog. To see this dialog click on item **Options** of **Tools** menu.

χ^2 tolerance

This is criterion for stop of fitting process - smallest relative tolerance of χ^2 value between two iterations.

Maximum Marquardt iterations

Maximum number of iteration for Marquardt algorithm

Maximum Marquardt factor increasing

Maximum number of unsuccessful increases of Marquardt factor

Maximum Simplex iterations

Maximum number of iteration for Simplex algorithm

Marquardt factor initial value

Initial value of Marquardt factor

Threshold of the smallest SVD values

FargoFit program uses SVD (Singular Value Decomposition) to calculate matrix inversion for Marquardt algorithm. Parameters that are undetermined or ill-determined by model and have corresponding singular values smaller than **Threshold of Smallest SVD Values** are excluded from analysis by setting them fixed.

Minimum of logarithm plot scale

Minimum value of vertical axes of signal graph in logarithm scale

Gauss integral limits

The integration procedures for distributed parameters are performed not from 0 to infinity but from mean of distribution minus specified number of FWHM to mean plus the same number of FWHM. If lower limit of distribution becomes negative it automatically corrected by the program to be 0.

FargoFit Help

Literature

Optical spectroscopy.

J. R. Lakowicz: *Principles of Fluorescence Spectroscopy.* Second Edition. Kluwer Academic/Plenum Publishers, New York, 1999.

Bernard Valeur: *Molecular fluorescence. Principles and applications.* Wiley-VCH Verlag GmbH, 2002.

Data analysis.

P. R. Bevington and D. K. Robinson: *Data Reduction and Error Analysis for the Physical Sciences.* McGraw-Hill, 1992

W. H. Press, S. A. Teukolsky, W. T. Vetterling and B. R. Flannary: *Numerical Recipes in C.* Cambridge University Press, 1992

Joseph M. Beechem, Marcel Ameloot, and Ludwig Brand: *Global analysis of fluorescence decay surfaces - excited-state reactions.* Chemical Physics Letters, Vol. 120, p. 466-472, 1985

Joseph M. Beechem: *Picosecond fluorescence decay curves collected on millisecond time scale: Direct measurement of hydrodynamic radii, local/global mobility, and intramolecular distances during protein-folding reactions.* Methods in enzymology, Vol. 278, p. 24-49, 1997

```
;FRET for 2-exponent decay
;first file is D, second - DA
Default
AddFract
AddExp

Inter 1 1 1 4
Inter 1 2 2 4

Vary 2 1 True
Value 1 4 0.0
Vary 2 4 True

Value 1 5 80.0
Link 1 5 2 5
Link 1 5 1 13
Link 1 5 2 13

Value 1 6 18.0
Link 1 6 2 6
Link 2 6 1 14
Link 2 6 2 14

Link 1 7 2 7
Link 1 7 1 11
Link 1 7 2 11

Link 1 8 2 8
Link 1 8 1 12
Link 1 8 2 12

Value 1 9 10.0
Link 1 9 2 9
Link 1 9 1 15
Link 1 9 2 15

Value 1 10 3.0
Link 1 10 2 10
Link 1 10 1 16
Link 1 10 2 16
```