FLIMfit software: brief list of main GUI features

Abbreviations

FLIM	Fluorescence Lifetime Imaging Microscopy
TCSPC	Time Correlated Single Photon Counting
IRF	Instrument Response Function
TVB	Time Varying Background
FOV	Field of View (synonymous to "image")

1. Introduction: fluorescence decay models and fitting expressions

IRF

The concept of the **instrument response function (IRF)** is central to the correct analysis of FLIM measurements. The IRF describes the spread of photon arrival times due to purely instrumental effects. The IRF can sometimes be measured directly in the imaging system used. In some cases, it is instead convenient to measure a fluorophore with a monoexponential decay, which is referred to as a **reference IRF** $g_R(t)$ to obtain information about the IRF. The Reference IRF is typically a high-quality experimental acquisition of a mono-exponential fluorescence decay with known **fluorescence lifetime** τ_R obtained from a suitable sample in the spectral window of interest.

The IRF can be pre-processed by baseline-subtraction and it is then normalized to have unit area in order to represent the instruments' **photon arrival time probability density**.

When using a reference decay that sufficiently short that it can be considered to be practically instantaneous (τ_R =0), the reference IRF becomes an impulse response function, or **delta-function IRF** $g_\delta(t)$.

Artefacts present in FLIM data such as non-delayed (scattered, "fast") light and the background intensity, will be mentioned further on.

Fitting expressions

Consider the fluorescence decay caused by the arrival of fast laser pulses at times $t = -nT$ (n=0,1,2,… ∞), and recorded in the time window [0,T], where T is the **pulse repetition period**. One could derive the following model expression to fit the measured decay intensity to the N_{exp} exponential components, basing on the delta-function IRF $g_\delta(t)$:

$$\tilde{D}_\delta(t) = I_{tot}\left(\sum_{k=1}^{N_{\exp}} \frac{f_k}{\tau_k} \frac{1}{1-e^{-T/\tau_k}} e^{-t/\tau_k}\right) \otimes g_\delta(t) \qquad (1)$$

where I_{tot} is the **total number of photons** in the decay ("total intensity"), τ_k are fluorescence lifetimes, and f_k are **fractional contributions** of components to the total decay (f_k are summing to 1). Formula (1) takes into account fluorescence intensities originating from the current laser pulse at t=0, and from

all previous pulses. The term in brackets is the photon arrival time probability density for the model decay, as it integrates to 1 in the range [0,*T*]. The convolution of this term with the IRF then gives the joint probability density of the photon arrival at time $t \in [0,T]$ due to independent delays of photons in the sample and in the instrument. The tilde is used to indicate that a decay model has been convolved with the IRF.

In the case when reference IRF $g_R(t)$ is used instead of $g_\delta(t)$, the decay model is modified to compensate for the additional convolution of the IRF with the reference decay. The model becomes

$$\tilde{D}_R(t) = I_{tot}\left(\tau_R \sum_{k=1}^{N_{\exp}} \frac{f_k}{\tau_k} \left\{ \delta(t) + \left(\frac{1}{\tau_R} - \frac{1}{\tau_k} \right) \frac{1}{1-e^{-T/\tau_k}} e^{-t/\tau_k} \right\} \right) \otimes g_R(t) \qquad (2)$$

where $\delta(t)$ is Dirac delta. As expected, the expression (2) reduces to (1) in the limit $\tau_R \to 0$, i.e. when $g_R(t) \to g_\delta(t)$.

For fluorescence decays, the fractional contributions of the decay components f_k are proportional to their lifetime τ_k and to the **molecule fraction β_k** of the corresponding fluorescent emitters, i.e. $f_k \propto \tau_k \cdot \beta_k$. Substituting this into the models (1) and (2) and introducing the **intensities of the scatter light *S* and the background *B***, one can rewrite these expressions in the form convenient for fitting purposes, as

$$\tilde{D}_\delta(t) = S \cdot g_\delta(t) + \sum_{k=1}^{N_{\exp}} A_k \left\{ \frac{1}{1-e^{-T/\tau_k}} e^{-t/\tau_k} \otimes g_\delta(t) \right\} + B \qquad (3)$$

$$\tilde{D}_R(t) = S \cdot g_\delta(t) + \sum_{k=1}^{N_{\exp}} A_k \left\{ g_R(t) + \left(\frac{1}{\tau_R} - \frac{1}{\tau_k} \right) \frac{1}{1-e^{-T/\tau_k}} e^{-t/\tau_k} \otimes g_R(t) \right\} + B \qquad (4)$$

The advantage of using intensity amplitudes A_k in the fitting model is that they are directly proportional to the above fractions β_k of fluorescent molecules participating in corresponding decays (not to numbers of photons they emit). Molecule fractions are then calculated as $\beta_k = A_k \Big/ \sum_{k=1}^{N_{\exp}} A_k$, and reported by the software.

The above description is suitable for TCSPC and widefield time-gated FLIM modalities.

Background compensation

In some cases, the background intensity *B* might be present in the measured FLIM data, i.e. a background decay that is due to the sample preparation or instrument, and therefore should be included as an additional component in the fitting model. The expression for *B* becomes

$$B = Vb(t) + Z \qquad (5)$$

where $b(t)$ is a spatially invariant time-varying background (TVB) function. This TVB can be determined by measuring a control sample without fluorescent objects of interest. The contribution *V*

of this TVB can be fitted but it is preferred if it can be measured experimentally where possible. The term *Z* represents a constant offset to the data, e.g. due to detector dark/thermal current, CCD readout noise or a background of stray room light.

In the case of reference reconvolution, i.e. using equation (4), but when it is necessary to fit a scattered contribution, the true IRF $g_\delta(t)$ can be estimated from the reference IRF $g_R(t)$ through the use of a Fourier deconvolution method.

Integrated Intensity

The integrated intensity for pixel *s* is calculated by summing the data over time bins or gates, accounting for any provided background and scatter and therefore represents an estimation of the total signal of interest. Using (5), one can write:

$$I_{s,tot}^{\ decay} = \sum_{i=1}^{N_{bins}} [y_s(t_i) - V_s b(t_i) - S \cdot g_{\delta,s}(t_i)] - Z_{SV,s} - Z_{const} \qquad (6)$$

where t_i is the photon arrival time assigned to the *i*-th bin or gate, $y_s(t_i)$ is the measured photon count of the pixel *s* in the *i*-th time bin (gate). The time-independent contribution to background is split into the spatial varying ($Z_{SV,s}$) and constant (Z_{const}) components for convenience. Note that, depending on software settings, any of the parameters except $y_s(t_i)$, $b(t_i)$ and $g_{\delta,s}(t_i)$ might be fitted parameters and therefore the integrated intensity may change when the data is refitted using a different model.

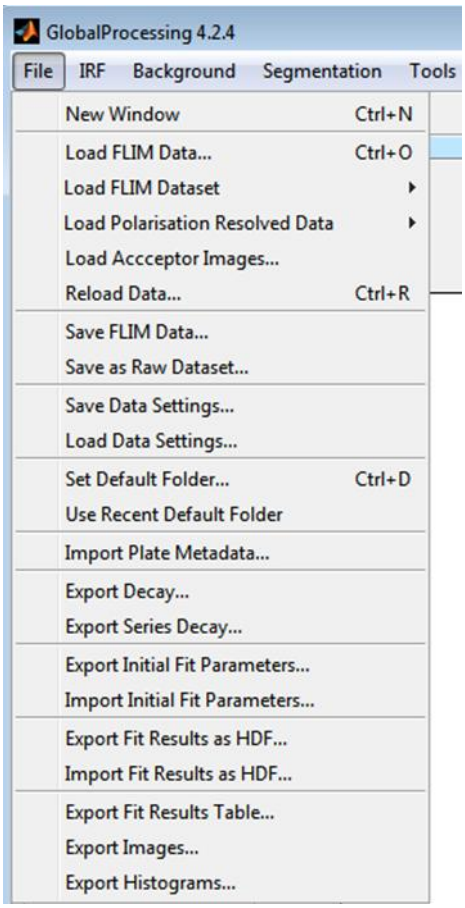
Fitting modes

In the software, the parameters of the expressions (3), (4) and (5) might be **fitted locally** (i.e. separately for every pixel in the FLIM image), **globally** (when their values are the same for groups of fitted pixels such as an image or a group of images), or **fixed** (set up by the user to a constant, not fitted). As the molecule fractions are of primary interest, they are often fitted locally.

The parameters N_{exp} and *T* are always fixed. The IRFs and TVB functions are also considered fixed. However, the IRFs (as well as scatter light terms) might be time-shifted, or different for different pixels in the image, or both. These shifts are not shown in the formulas in order to keep them observable.

2. GUI Features

1. File (menu)



1.1 Use the **New Window** menu option to open a new instance of FLIMfit in a new window.

1.2 Use the **Load FLIM Data** menu option to load the raw FLIM data acquired from a single FOV. The program can load the following FLIM file formats: B&H .sdt, LaVision TCSPC OME-tiffs, ASCII .txt files, a set of wide-field time-gated .tif images in a single directory and FLIMfit .raw files.

1.3 Use the **Load FLIM Dataset** menu option to load the raw FLIM data from multiple FOVs or multiple time or Z (depth) points. These FOVs can then be analysed together as a single dataset.

1.4 Use the **Load Polarization Resolved Data** menu option to do just that. Polarization analysis needs 2 images acquired at different polarizer angles, so the data must have at least 2 FLIM channels (this is currently only implemented for for B&H TCSPC .sdt images).

1.5 The **Load Acceptor Images** menu option can be used when analysing FLIM-FRET data. It allows you to load a set of acceptor images that correspond to a set of FLIM images of the donor that you have already loaded. The acceptor images may be used to segment the data into ROIs based on both the donor and acceptor channel in inter-molecular FRET experiments and to plot the acceptor intensity. The file names of acceptor images must contain the names of the corresponding FLIM files to be recognised.

1.6 Use the **Reload Data** menu option to attempt to reload the currently loaded dataset. This can be useful when processing a dataset during acquisition when not all of the images had been acquired when the data was first loaded. This option requires that the data folders have been created in advance of the acquisition.

1.7 **Save FLIM Data:** currently under development

1.8 Use the **Save as Raw Dataset** menu option to save the currently loaded data as a .raw file. The .raw file is useful as it can be rapidly reloaded using the **Load Raw Dataset** option, which can save time when dealing with large datasets.

1.9 You can save the settings of the **Data** tab into an XML file by using **the Save Data Settings** menu option. You can then load the settings back into FLIMfit when necessary by using **Load Data Settings** menu option.

1.10 Use **Set Default Folder** and **Use Recent Default Folder** menu options to set default folder where data are loaded from. This is useful as it saves repeatedly navigating to the folder that your data is stored in when opening e.g. IRF files.

1.11 Use the **Import Plate Metadata** menu option to load the description of multi-well plate reader data. This description is stored in special Excel format.

1.12 Use the **Export Decay** menu option to save the current pixel decay and the IRF displayed in the **Decay** tab, in text format. This option asks the user to select a directory and a file name, and the program then saves two .txt files called filename.txt and filename_irf.txt.

1.13 You can save all the settings shown in the fitting parameters tab into an XML file by using the **Export Initial Fit Parameters** menu option. These settings can then be loaded back in to FLIMfit when necessary by using **Import Initial Fit Parameters** menu options.

1.14 You can save the fitted decay parameters into a HDF format by using the **Export Fit Results as HDF** menu option. You can then load the results back into FLIMfit by using the **Import Fit Results as HDF** menu option. The HDF ("hierarchical data format") is suitable for storing large amounts of data with complex structure.

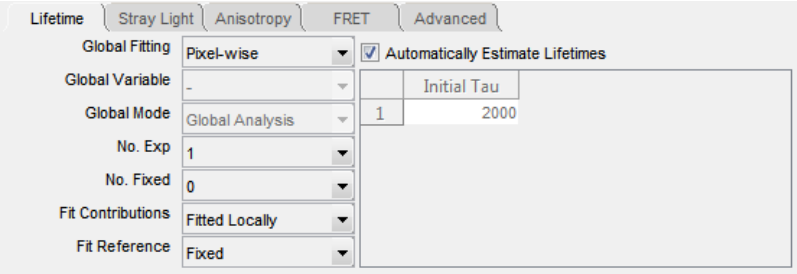
1.15 Use the **Export Fit Results Table** menu option to save the mean and global fitted variables shown in the **Parameters tab** to a text file (comma separated values, CSV).

1.16 Use the **Export Images** menu option to export the fit parameter images selected in the **Plot** tab as both colormapped images (i.e. including colour bar and scale) and floating point grayscale parameter image files (tiff images).

1.17 Use the **Export Histograms** menu option to save the current data in the **Histogram** tab as a text file (CSV).

Fitting setups

2. Lifetime (tab)



2.1. Use the Global Fitting combo-box to specify the scope of the global variables. The following choices are available:

- **Pixel-wise:** decays are fitted on a pixel-by-pixel basis
- **Image-wise:** global fitting is performed across each image individually, i.e. lifetimes are constant across each image
- **Global:** global fitting is performed across the entire dataset, i.e. lifetimes are constant across all images.

2.2. Use the **Global Variable** combo-box to confine the scope of the global fit to a particular multi-well plate metadata parameter (e.g. 'Row' or 'Column'). If a parameter is selected all images with the same parameter value will be globally analysed together.

2.3. Use **Global Mode** combo-box to set up the method of global analysis. The following choices are available:

- **Global binning:** All pixels in the global fit are binned into a single decay. This decay is then fitted to determine the global parameters. Each pixel is then fitted individually with the global parameters fixed (using the method of linear least square minimisation) to determine the local variables.
- **Global analysis:** All the pixels in the global fit are simultaneously analysed using Variable Projection algorithm (see in **Advanced tab**)

2.4. Use the **No. Exp** combo-box to set up the number of exponential decay components N_{exp}

2.5. Use the **No. Fixed** combo-box to set up the number of fixed fluorescence lifetimes τ_k. The first **No. Fixed** values in the **Initial Tau** list are fixed.

2.6. Use the **Fit contributions** combo-box to set up the fitting mode for the amplitudes A_k (formula (3) and (4)). The following choices are available:

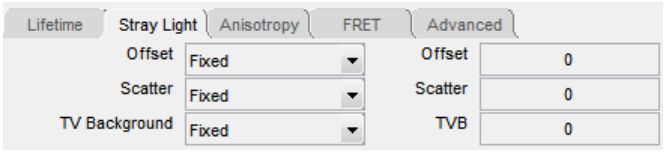
- **Fixed:** the fractions β_k are fixed for corresponding amplitudes (set up by the textboxes appearing to the right)
- **Fit locally:** amplitudes are fitted locally, i.e. on a pixel-by-pixel basis
- **Fit globally:** - amplitudes are fitted globally, i.e. they are invariant across the image. Use this option when fitting a complex donor FRET model or polarisation resolved data.

- **Fit globally (Grouped):** use this option to fit a model where we have a mixture of several fluorophores with complex decays. The lifetimes and contributions are specified in groups, indexed from zero. Within each group the lifetime and fractional contributions are invariant across the image but the fractional population of each group varies locally. The groups are specified using the **Initial tau** list.

2.7. Use the **Fit Reference** combo-box to set up the fitting mode for the variable τ_R (formula (4)), as **Fixed** or **Fitted**. The reference lifetime is fitted globally.

2.8. Use the **Initial Tau** combo-box to choose the initial values of lifetimes. These are estimated automatically unless 'Automatically estimate initial guesses' is deselected.

3. Stray Light (tab)

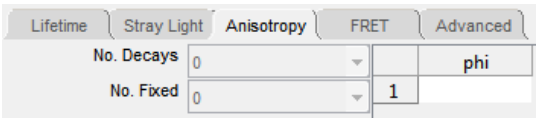


You can select the fitting scope of the "stray light" variables entering the model expressions (3-5), by choosing either **Fixed**, or **Fitted Locally**, or **Fitted Globally.** The scope is set for the following variables:

- **Offset**: A constant background value, *Z* in the formula (5)
- **Scatter**: A fast-light scatter contribution, *S* in the formulas (3) and (4)
- **TV Background**: A time-varying background contribution that is scaled by the coefficient *V*, see formula (5). This option can only be used if a time varying background has been loaded. The *V* coefficient can be fitted globally or fixed by setting the TVB Background to 'Fixed' and TVB to 1.

4. Anisotropy (tab)

These options are used to set up an anisotropy decay model if polarisation resolved data has been loaded.



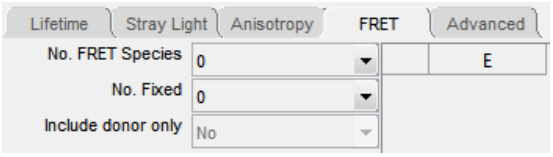
4.1. Use the **No Decays** combo-box to specify the number of rotational correlation times to fit.

4.2. Use the **No Fixed** combo-box to specify the number of rotational correlation times that are fixed. The first **No Fixed** are fixed to the value specified in the **Phi** list.

4.3. Use **Phi** textbox to specify the initial guesses and fixed values for the anisotropy correlation times.

5. **FRET (tab)**

These options are used to fit a complex-donor FRET decay model. In this model we assume that the donor fluorophore has two or more conformations with associated lifetimes whose FRET efficiencies are linked by their relative quantum yields. To use this mode **Fit Contributions** should be set to **Fixed** or **Globally** in the **Lifetime tab**, as we assume that the fractional contribution of the various donor species are invariant across the image.

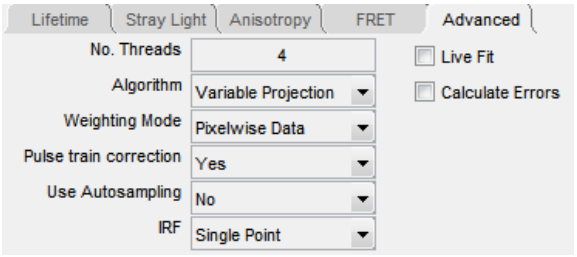


5.1. Use the **No. FRET Species** combo-box to specify the number of different FRET conformations present. For example, for an intra-molecular FRET probe where there are likely to be two different conformations with different (but non-zero) FRET efficiencies, set this to two and set **Include Donor Only** to **No**. For an inter-molecular FRET probe where the unbound state will have a zero FRET efficiency, set this to one and set **Include Donor Only** to **Yes**.

5.2. Use **No. Fixed** combo-box to set the number of FRET conformations which have fixed efficiencies. The first **No. Fixed** FRET efficiencies in the **E** list will be fixed.

5.3. Use **Include Donor Only** combo-box to specify whether a donor-only contribution should be included, that is a contribution from a species which is not FRETing.

6. **Advanced (tab)**



6.1. Use the **No. Threads** textbox to set up the number of threads used in multi-threading processing. This is automatically set to the number of cores present on the local machine which in general is optimal.

6.2. Use the **Algorithm** combo-box to choose the method and the type of error function minimization. The following methods are available:

- **Variable Projection:** Use separable fitting to minimise the mean-square error function.
- **Maximum Likelihood:** Use the maximum likelihood fitting metric, only applicable to pixel-wise fitting. Use this option for fitting data with low photon count numbers, particularly TCSPC data.

7. Use **Weighting Mode** combo-box to select the method of weighting the residuals entering the mean-square error function when using Variable Projection

$$\textit{Error function} = \sum_{i}^{N_{bins}} \frac{(D_{\text{model}}(t_i) - y(t_i))^2}{\sigma(t_i)}$$

where t_i are the bin (delay) times, $\sigma(t_i)$ is weighting coefficient, and $D_{\text{model}}(t_i)$ and $y(t_i)$ are the model and measured intensity values, respectively.

- **Average Data**: Weight according to the average measured decay across the global scope. This is the default, and advised option.
- **Pixelwise Data:** Weight according the measured decay in each pixel ($\sigma^2(t_i)=y(t_i)$). Can display significant bias at low photon counts.
- **Model**: Weight according to the model decay ($\sigma^2(t_i)=D_{\text{model}}(t_i)$). May lead to problems with convergence if initial estimates are significantly different from the true values.

7.1. Use the **Pulse Train Correction** combo-box to specify whether incomplete decays should be included in the fit. Unless you have a very low repetition rate laser source or are fitting very fast decays, this option should only be disabled for testing and when fitting data simulated without incomplete decays.

7.2. Use **Auto Resampling** combo-box to dynamically combine bins to ensure that there are at least 10 counts in each bin. This option is superseded by Maximum Likelihood fitting.

7.3. **Use IRF combo-box** to set up the type of IRF. The following choices are available:

- **Single Point**: Use the same IRF for all pixels
- **Use SV IRF**: Use a spatially varying IRF that has been loaded from the IRF menu. Each pixel is fitted using the IRF from the corresponding pixel in the spatially varying IRF.
- **Use IRF shift map**: Use an IRF shift map that has been loaded from the IRF menu. The same IRF is used in each pixel, time shifted according to the shift map loaded.

7.4. You can check the **Live Fit** checkbox to dynamically refit the currently selected decay as fitted parameters are changed or as the selected region is changed. This option does not automatically fit the whole dataset.

7.5. You can check **Calculate Errors** checkbox to estimate confidence intervals on the global parameters based on the F statistic. This option should currently be considered experimental and significantly increases the fitting time.

Instrument and Preprocessing Setups

1. Data (tab)

The settings in the tab relate to transformations applied to the data prior to fitting and can be used to specify data acquisition parameters that are not stored in within the loaded data file format.

1.1 Use the **Smoothing** combo-box to set up the kernel size for smoothing. The data will be smoothed by XY convolution of the data with a uniform square kernel of the size specified.

Note: in order to ensure that thresholding and background subtraction of the raw data does not affect the choice of background or threshold values, data is averaged prior to fitting rather than summed. This does not affect the weighting of the data during either variable projection fitting or maximum likelihood fitting. A correction factor is applied to the χ^2 value to adjust for the change in photon statistics due to the smoothing level applied:

$$\chi^2 = \chi_{fit}{}^2 [Smoothing]^2$$

The equivalent *binning* setting in Becker and Hickl software is provided for reference.

1.2 Use the **Integrated Min.** textbox to set the integrated intensity threshold. Pixels which have an integrated intensity of less than **Integrated Min** will not be included in the fit. The integrated intensity is calculated after accounting for any supplied background values (see section XX) and is not smoothed (see formula (6)).

1.3 Use the **Time Min** and **Time Max** textboxes to define the time limits of the FLIM signal. Time bins or gates with times less than **Time Min.** or greater than **Time Max.** will be excluded from the fit. This does not affect the integrated intensity image.

1.4 Use **Counts/Photon** textbox to specify the average number of counts recorded per detected photon event on the GOI. This is used to correct the χ^2 value to account for the scaled Poissonian statistics for time-gated FLIM only, as follows:

$$\chi^2 = \chi_{fit}{}^2 [Counts / Photon]^{-2}$$

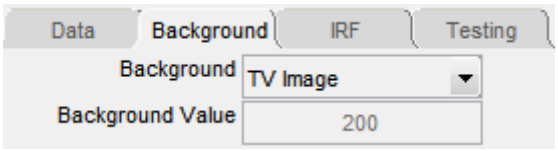
1.5 Use the **Rep. Rate** textbox to set up the repetition rate f_{rep} of the laser used to acquire the data, in MHz. The pulse repetition period is then defined as $T=1/f_{rep}$. This is used to correct for incomplete decays. Can cause significant artefacts if set incorrectly!

1.6 Use **Gate Max.** textbox to define the saturation limit of the data. Pixels which have a time bin or gate with **Gate Max.** or more counts will be excluded from the fit. This may be used to exclude saturated pixels - for 16-bit TCSPC a value of 65,536 should be used, for

12-bit time gated data a value of 4,096 should be used. The **Gate Max.** threshold is applied prior to any background subtraction and/or smoothing.

2. Background (tab)

The settings in this tab are used to set the background subtraction. These options subtract a background from the data before fitting. A background due to detector dark noise or stray room light should be added to the model (see section XXX) rather than being subtracted, otherwise the fit will be weighted incorrectly. However, it is currently not possible to add a spatially and time varying background to the model, and so this option must be used if you wish to account for a spatially and temporally varying background.



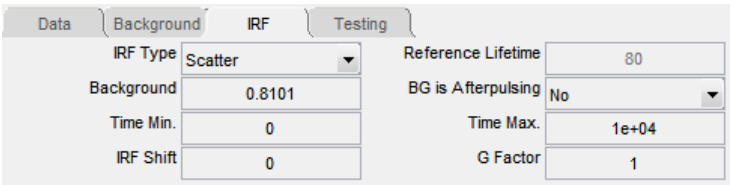
2.1 Use the **Background** combo-box to select the type of subtracted background. The following choices are available:

- **None**: no background is subtracted from the data.
- **Single Value**: the value specified by **Background value** is subtracted uniformly from all the data. This is most commonly used to subtract a digital camera offset value in time gated data.
- **Image:** A background image, loaded via the **Background** menu, is subtracted uniformly from every time bin or gate. This can be used to subtract a background that is not time dependent but varies across the image, e.g. from room light or non-uniformity in the camera. If no background is loaded, this option has no effect.
- **TV Image**: A time varying background (TVB) image is subtracted (the background varies both in XY and in *t*.

2.2 **Background Value** The background value subtracted if **Single Value** is selected in **Background** combo-box.

3. IRF (tab)

The settings in this tab relate to the Instrument Response Function (IRF) and how it is processed.



3.1 Use the **IRF Type** combo-box to specify the type of IRF loaded. The following choices are available:

- **Scatter:** true IRF $g_\delta(t)$, recorded using e.g. a scattering sample or Raman scatter.
- **Reference:** monoexponential decay recorded with a reference dye $g_R(t)$.
- **Reference using deconvolution**: the reference IRF $g_R(t)$ is deconvolved from the mono-exponential τ_R decay using Richardson-Lucy deconvolution, and the result is used as delta-function IRF $g_\delta(t)$.

3.2 Use the **Reference Lifetime** textbox to set the lifetime τ_R of the reference decay loaded. This value will only be used if **IRF type** is set to **Reference**.

3.3 Use the **Background** textbox to set the background signal level that is subtracted from the raw IRF signal before normalization. The background signal level may be estimated automatically, see **Estimate IRF Background** option in the **IRF** menu.

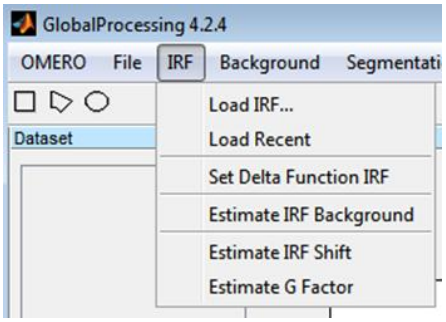
3.4 Use the **BG is Afterpulsing** combo box to specify if the background on the IRF is due to afterpulsing. If so, timebins from the IRF which are excluded from the fit using **Time Min** and **Time Max** are set to the background value and no background is subtracted from the IRF. This correctly compensates for the presence of afterpulsing, which is common in TCSPC data acquired using a photomultiplier tube.

3.5 Use the **Time Min.** and **Time Max.** combo-boxes to set the time limits of the IRF signal. Time bins or gates in the IRF with times less than **Time Min.** or greater than **Time Max.** will be set to zero if **Afterpulsing Correction** is off or to the value specified by **Background** if **Afterpulsing Correction** is on.

3.6 Use the **IRF Shift** textbox to shift the IRF in time by the value specified. This shift is applied using cubic interpolation so sub-time bin values may be specified. This value may be estimated by using the **Estimate IRF Shift** menu option (see below).

3.7 Use the **G Factor** textbox to set up the G factor, i.e. the relative sensitivities of the parallel and perpendicular detector channels. This value only applies when analysing Polarisation resolved data.

IRF (menu)



1. Use the **Load IRF** menu option to load an IRF from file. If image data is selected, all the pixels will be averaged into a single decay.

2. The **Load Recent** menu option loads the most recently loaded IRF.

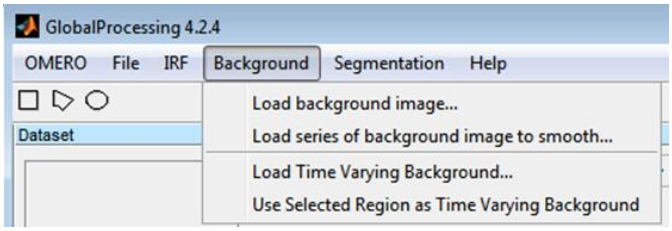
3. Use the **Set Delta Function IRF** menu option to set the IRF to a delta-function, i.e. the model functions will be used without convolution.

4. Use the **Estimate IRF Background** menu option to estimate and subtract the background from the IRF. The background is estimated by fitting a Gaussian with a constant background to the IRF. The fitted constant value is then applied automatically to the background cell/value in the IRF tab and replaces any previous value.

5. Use the **Estimate IRF shift** menu option to estimate any time shift present in the loaded IRF relative to the true IRF due to, for example, colour effects in the detector. The shift is determined by finding the IRF shift that minimizes the χ^2 value (the goodness of fit measure) obtained for a fit to the region currently selected by the user.

6. Use the **Estimate G factor** menu option to estimate the G factor using a dye sample with a short rotational correlation time. The G factor is estimated using the knowledge that the anisotropy tends to zero towards the end of the decay. This procedure is used only when analysing Polarization resolved measurements.

Background (menu)



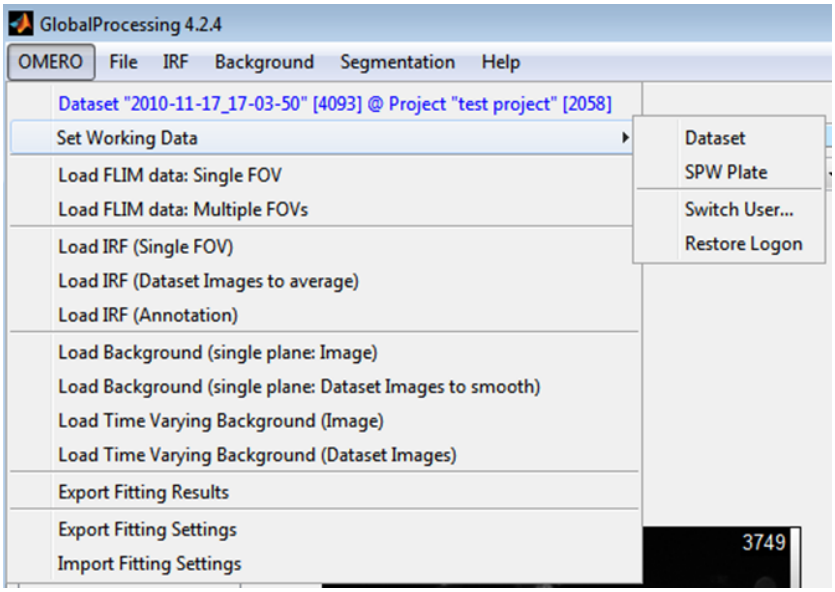
1. Use the **Load Background image** menu option to load a spatially varying background from an image file on disk that contains a single plane image matching the XY size of the loaded FLIM data. This sets the values of spatial-varying background Z_{SV} (see above).

2. The **Load series of background image to smooth** menu option loads a set of single plane images and automatically calculates an average background image from them. The user is required to select a folder containing suitable image files.

3. Use the **Load Time Varying Background** menu option to load a time varying background. This is not spatially varying background - a single trace is loaded from a .txt file that was saved previously using the **Export Decay** menu option.

4. The **Use Selected Region as Time Varying Background** menu option defines the time varying background signal that is not XY dependent. The time varying background is set to the average of the region that is currently selected by the user.

OMERO (menu)



1. Use the **Set Working Data** menu option to select the current OMERO storage containing FLIM images – either **Dataset** or **SWP Plate**. The logon options include possibility to enter as different user (**Switch User**), and restore server connection (**Restore Logon**).

2. Use the **Load FLIM data: Single FOV** menu option to load a single FLIM image from the pre-set OMERO storage.

3. Use the **Load FLIM data: Multiple FOVs** menu option to load multiple FLIM images from the pre-set OMERO storage. These images should have the same XYZCT dimensions.

4. Use the **Load IRF (Single FOV)** menu option to load an IRF from a multi-plane image. **A** multi-plane OMERO image with the same XY dimensions and FLIM delays as the loaded FLIM data is expected. The data in this image are averaged over XY to produce 1-dimensional IRF signal.

5. Use the **Load IRF (Dataset Images to average)** menu option to load the IRF from an OMERO dataset containing a set of single plane images with the values of FLIM delays (expressed in picoseconds) encoded in image names. This command is equivalent to loading the IRF from a folder on disk using the **File**, **Load IRF** menu option.

6. Use the **Load IRF (Annotation)** menu option to load an IRF from an OMERO Annotation file within the current working data (Dataset or Plate). The files, including single-pixel B&H decays are loaded by the same procedure as when loading from the disk.

7. Use the **Load Background (single plane: Image)** menu option to load a background image. A single-plane image is expected. This is analogous to the **Load Background Image** option from the **Background** menu.

8. Use the **Load Background (single plane: Dataset Images to smooth)** menu option to calculate an averaged background image from a selected Dataset containing a set of single-plane images. This is analogous to the **Load series of background images to smooth** option in the **Background** menu.

9. Use the **Load Time Varying Background (Image)** menu option to load a TVB image. This is analogous to the **Load Time Varying Background** option in the **Background** menu.

10. Use the **Load Time Varying Background (Dataset Images)** menu option to load a TVB image from an OMERO Dataset. This is analogous to the **Load IRF (Dataset Images)**. The 1-dimensional time-dependent TVB signal is calculated by averaging this data over the XY dimensions.

11. Use the **Export Fitting Results** menu option to store the current values displayed in the fitting results tab into OMERO. Internally within FLIMfit, the results of a fit are stored as double precision XY parameter images (i.e. several for each FOV, depending on the number of fit parameters). When exporting the fit parameter data into OMERO, these parameter images are placed in a multi-plane "double" OMERO image along the "channels" dimension. In other words, the OME dimension of each exported FOV results in an image with XY dimensions equal to the FOV, Z=1, T=1 and C = N_{fit_params}, where N_{fit_params} is the number fitted parameters. Fitted data are exported as a new OMERO container of the same storage type as working data (i.e. either a new Dataset or a new Plate) with the name derived from the name of the analysed working data. The name of the new OMERO container also contains the global time when the fit was performed.

12. Use the **Export Fitting Settings** menu option to attach an XML file containing the current fitting settings of the program as a File Annotation to the current working data in OMERO (Dataset or Plate). This allows the fit settings to be reloaded back into FLIMfit from OMERO by using the **Import Fitting Settings** menu item.