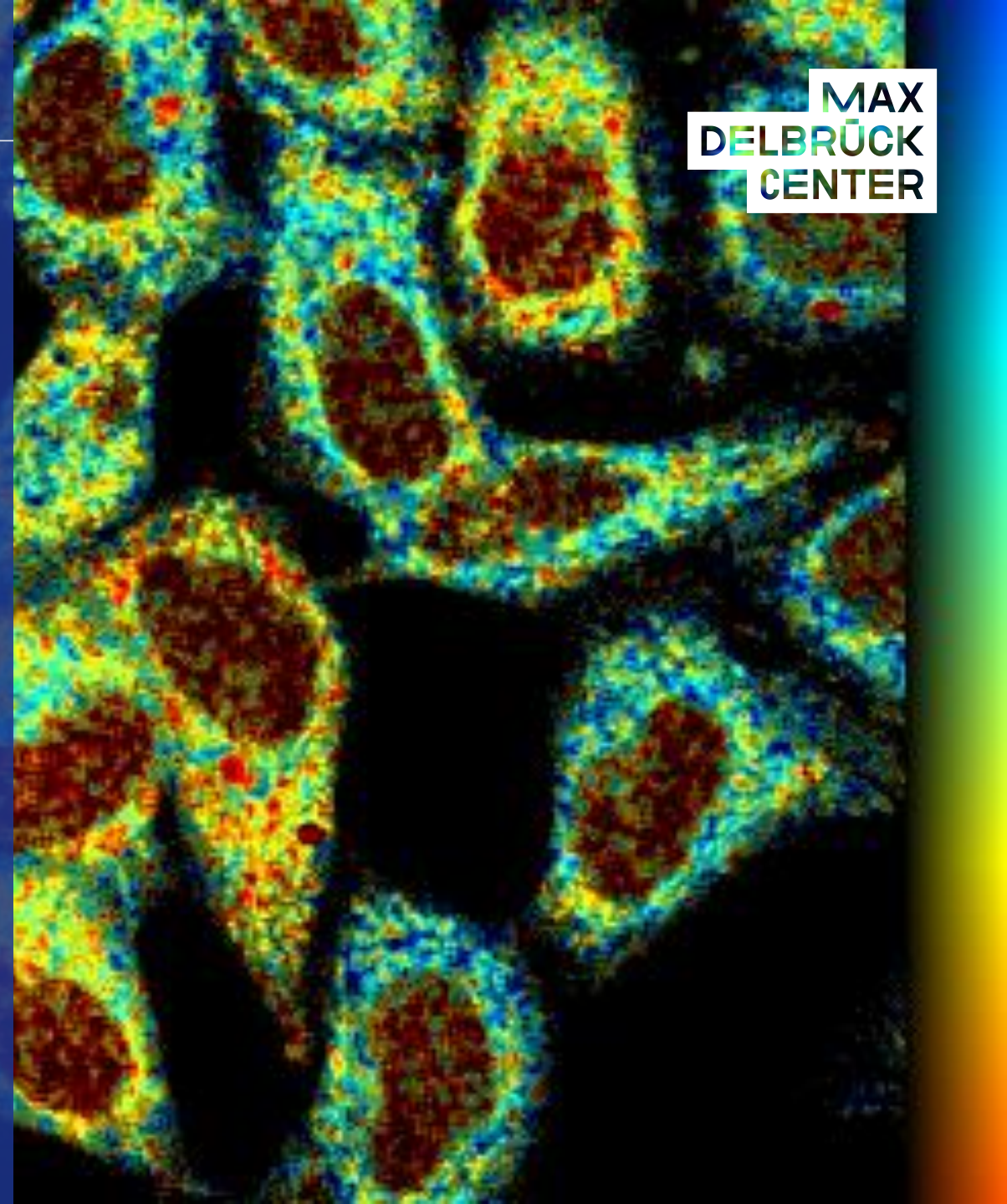


ANALYSING DATA WITH *FLIMfit*

Anca Margineanu
Advanced Light Microscopy

MAX
DELBRÜCK
CENTER



- **Publication:** Warren et al. (2013), *Rapid Global Fitting of Large Fluorescence Lifetime Imaging Microscopy Datasets*, PLoS ONE 8(8): e70687. doi:10.1371/journal.pone.0070687
- **Website:** <https://flimfit.org/>
- Developed in **Matlab**, offered as a **compiled GUI** that runs independently (only the **MCR** necessary) to analyse **time-domain FLIM** data
- Several versions are implemented in **Omero**

- **Global fitting algorithm for multiexponential decays** applied on **hundreds of images**

$$y(t) = \beta_1 e^{-t/\tau_1} + \beta_2 e^{-t/\tau_2}$$

- * **Global analysis:** lifetimes are considered invariant across all images or regions of interest and are fitted simultaneously by minimising a global χ^2

tau_1	1.8588e+03	1.8588e+03	1.8588e+03	1.8588e+03	1.8588e+03	1.8588e+03	1.8588e+03	1.8588e+03	1.8588e+03	1.8588e+03
tau_2	166.7117	166.7117	166.7117	166.7117	166.7117	166.7117	166.7117	166.7117	166.7117	166.7117
offset	0.0563	0.0446	0.0438	0.0456	0.0497	0.0393	0.0484	0.0499	0.0438	0.0449
beta_1	0.2558	0.2465	0.2308	0.2296	0.2218	0.2082	0.2155	0.2040	0.2007	0.2089
beta_2	0.7442	0.7535	0.7692	0.7704	0.7782	0.7918	0.7845	0.7960	0.7993	0.7911

- * **Global binning:** the photons from all the pixels in a region of interest are combined to create a single histogram with more photons

- **Loading multidimensional file formats:**

- * B&H, Picoquant, ome-tiff LaVision Biotec, Leica .pt3, .tiff
- * Single images
- * Series of images: z stacks, time lapses, concentration
- * Multiple channels (time-resolved anisotropy)

- **Instrument response function (IRF) (image/ascii):**

- * **Fluorescent reference**
- * **Scatter** (multiphoton)

- **Background:**

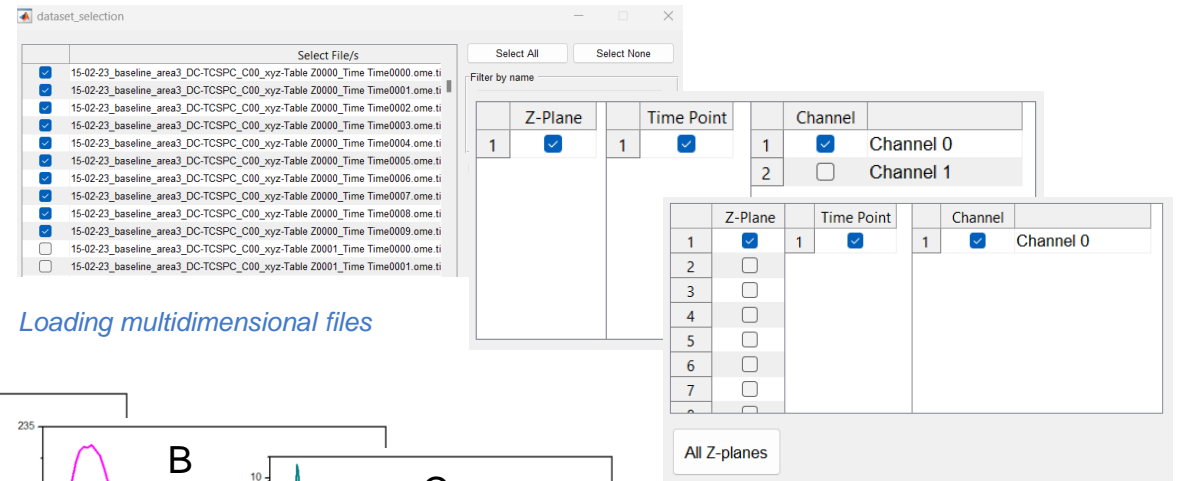
- * **Constant**
- * **Time-varying**

- **Scattered light**

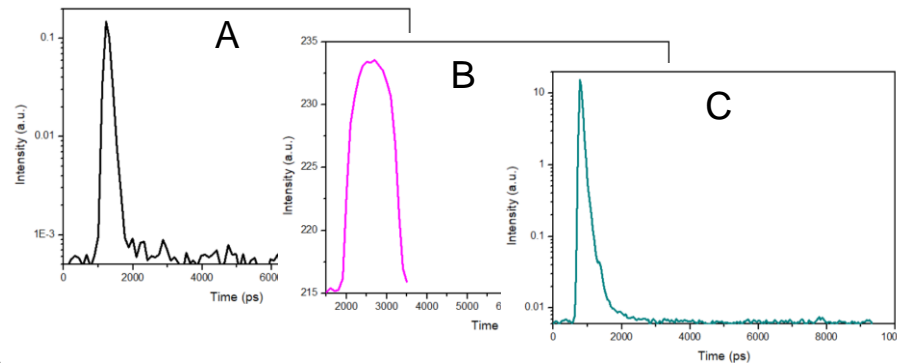
- **Pulse train correction** (i.e. incomplete decays fitting)

- **FRET model** for donors with complex fluorescence decay

- **Time-resolved anisotropy decays analysis**

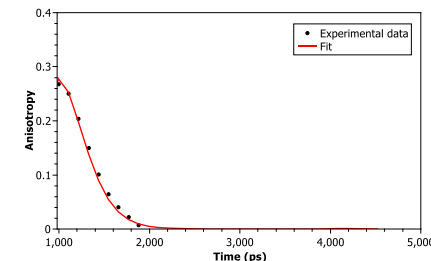


Loading multidimensional files



Examples of IRFs of different instruments:
A- scatter (multiphoton TCSPC)
B- erythrosin B in water (time-gated)
C- erythrosin B in water (TCSPC)

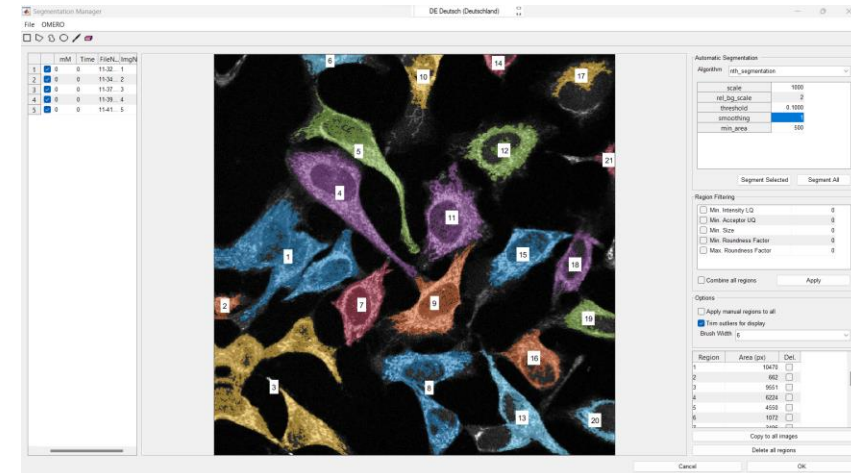
Time resolved anisotropy decay of rhodamine 6G in water



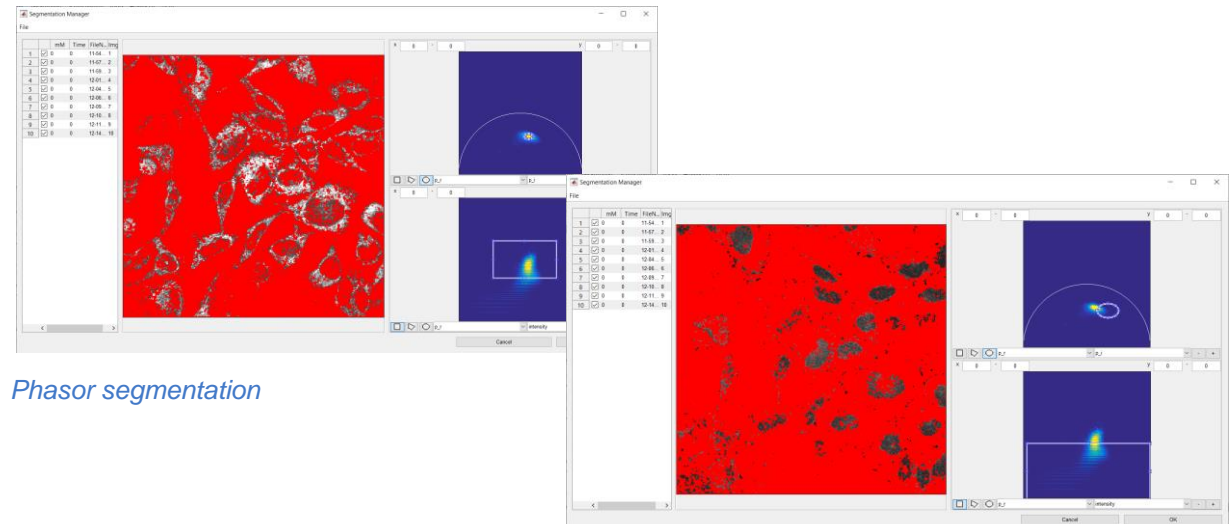
FLIMfit IMPLEMENTATIONS

- **Segmentation:**

- * **Manual**
- * **Automated:** Watershed algorithm + options from Cell Profiler
- * **Acceptor images** (for FRET experiments)
- * **Phasor segmentation** (sine vs. cosine transforms of the decay, or intensity vs. sine/cosine transforms)
- * **Save and load** multiple segmentation images using logical operators



Automated watershed segmentation



Phasor segmentation

FLIMfit: DATA VISUALISATION

Time resolved fluorescence decay and fit

C:\Users\amargine\Data\GerBio FLIM\2018-08015 HeLa cells lifetime\all\ (5.0.3)

File OMERO IRF Background Segmentation Tools Advanced Help

Dataset Parameters Images Gallery Histogram Correlation Plotter Plate

Decay

Plot	Dis...	Me...	Min	Max	Auto
tau_1	<input type="checkbox"/>	<input type="checkbox"/>	1620	1660	<input checked="" type="checkbox"/>
tau_2	<input type="checkbox"/>	<input type="checkbox"/>	106	109	<input checked="" type="checkbox"/>
offset	<input type="checkbox"/>	<input type="checkbox"/>	6.9000e...	0.1240	<input checked="" type="checkbox"/>
beta_1	<input type="checkbox"/>	<input type="checkbox"/>	0.1190	0.3550	<input checked="" type="checkbox"/>
beta_2	<input type="checkbox"/>	<input type="checkbox"/>	0.6450	0.8810	<input checked="" type="checkbox"/>
l0	<input type="checkbox"/>	<input type="checkbox"/>	1.6100	27.2000	<input checked="" type="checkbox"/>
l	<input type="checkbox"/>	<input type="checkbox"/>	10	132	<input checked="" type="checkbox"/>
mean_tau	<input type="checkbox"/>	<input type="checkbox"/>	291	650	<input checked="" type="checkbox"/>
w_mean_t...	<input type="checkbox"/>	<input type="checkbox"/>	1130	1480	<input checked="" type="checkbox"/>
chi2	<input type="checkbox"/>	<input type="checkbox"/>	0.2330	4.4400	<input checked="" type="checkbox"/>

Param	Type	Value
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	

Invert Colorscale? No
Display Colormap? Yes
Display Limits? Yes

Intensity Decay

Time (ps)

Norm Residual

Progress

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
im_group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
region	1	1	1	1	1	1	1	1	1	1	1	1	1	1
success %	0	0	0	0	0	0	0	0	0	0	0	0	0	0
iterations	12	12	12	12	12	12	12	12	12	12	12	12	12	12

Fit Selected Decay

Fit Dataset

FLIMfit: DATA VISUALISATION

Table of fitted parameters (results)

The screenshot shows the FLIMfit software interface. The 'Parameters' tab is selected and highlighted with a pink circle. The main window displays a table of fitted parameters for 8 image groups. The parameters include im_group, region, success %, iterations, pixels, tau_1, tau_2, offset, beta_1, beta_2, I0, I, mean_tau, w_mean_tau, and chi2. The values are provided for each of the 8 image groups.

	1	2	3	4	5	6	7	8
im_group	1	2	3	4	5	6	7	8
region	1	1	1	1	1	1	1	1
success %	0	0	0	0	0	0	0	0
iterations	19	19	19	19	19	19	19	19
pixels	109770	68077	83683	104095	123717	85441	123538	105489
tau_1	1.7326e+03	1.7326e+03	1.7326e+03	1.7326e+03	1.7326e+03	1.7326e+03	1.7326e+03	1.7326e+03
tau_2	130.3074	130.3074	130.3074	130.3074	130.3074	130.3074	130.3074	130.3074
offset	0.0541	0.0488	0.0471	0.0450	0.0442	0.0420	0.0441	0.0471
beta_1	0.2519	0.2430	0.2274	0.2247	0.2189	0.2065	0.2124	0.2021
beta_2	0.7481	0.7570	0.7726	0.7753	0.7811	0.7935	0.7876	0.7979
I0	6.6015	5.8728	6.9358	6.3692	6.4521	7.1412	7.4274	7.9184
I	38.1444	33.4537	36.8116	33.7980	33.3744	34.9984	36.8810	38.3937
mean_tau	533.9747	519.6494	494.7354	490.3133	481.0867	461.2153	470.5798	454.0565
w_mean_tau	1.4348e+03	1.4216e+03	1.4013e+03	1.3961e+03	1.3872e+03	1.3672e+03	1.3776e+03	1.3596e+03
chi2	1.1871	1.0157	1.1266	1.0163	0.9921	1.0483	1.1162	1.1555

Below the main table, there is a 'Progress' table showing the number of iterations for each image group across 14 channels:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
im_group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
region	1	1	1	1	1	1	1	1	1	1	1	1	1	1
success %	0	0	0	0	0	0	0	0	0	0	0	0	0	0
iterations	12	12	12	12	12	12	12	12	12	12	12	12	12	12

FLIMfit: DATA VISUALISATION

Image gallery of selected fitted parameters

The screenshot shows the FLIMfit software interface. The 'Gallery' tab is highlighted with a pink circle. The main window displays a grid of images for various parameters. The 'Dataset' panel on the left shows a list of frames (41-54) with columns for 'mM' and 'Time'. The 'Parameters' panel shows settings for 'Background', 'IRF', and 'Testing'. The 'Lifetime' panel shows 'Global Fitting' and 'Global Variable' settings. The 'Progress' table at the bottom shows the status of the fit for each region.

Param	Type	Value
tau_1		1620
tau_2		106
offset		6.9000e...
beta_1		0.1190
beta_2		0.6450
l0		1.6100
l		10
mean_tau		291
w_mean_t...		1130
chi2		0.2330

im_group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
region	1	1	1	1	1	1	1	1	1	1	1	1	1	1
success %	0	0	0	0	0	0	0	0	0	0	0	0	0	0
iterations	12	12	12	12	12	12	12	12	12	12	12	12	12	12

FLIMfit: DATA VISUALISATION

Histograms of selected fitted parameters

The screenshot displays the FLIMfit software interface. The 'Histogram' tab is highlighted with a pink circle. The histogram shows the frequency distribution of the weighted mean tau parameter, with a peak around 1450. The interface includes various control panels for data, fitting, and visualization.

Dataset Table:

	mM	Time
41	10	0
42	10	0
43	10	0
44	10	0
45	10	0
46	10	0
47	10	0
48	10	0
49	10	0
50	10	0
51	10	0
52	10	0
53	10	0
54	10	0

Parameters Table:

Plot	Dis...	Me...	Min	Max	Auto
tau_1	<input type="checkbox"/>	<input type="checkbox"/>	1620	1660	<input checked="" type="checkbox"/>
tau_2	<input type="checkbox"/>	<input type="checkbox"/>	106	109	<input checked="" type="checkbox"/>
offset	<input type="checkbox"/>	<input type="checkbox"/>	6.9000e...	0.1240	<input checked="" type="checkbox"/>
beta_1	<input type="checkbox"/>	<input type="checkbox"/>	0.1190	0.3550	<input checked="" type="checkbox"/>
beta_2	<input type="checkbox"/>	<input type="checkbox"/>	0.6450	0.8810	<input checked="" type="checkbox"/>
l0	<input type="checkbox"/>	<input type="checkbox"/>	1.6100	27.2000	<input checked="" type="checkbox"/>
l	<input type="checkbox"/>	<input type="checkbox"/>	10	132	<input checked="" type="checkbox"/>
mean_tau	<input type="checkbox"/>	<input type="checkbox"/>	291	650	<input checked="" type="checkbox"/>
w_mean_t...	<input type="checkbox"/>	<input type="checkbox"/>	1130	1480	<input checked="" type="checkbox"/>
chi2	<input type="checkbox"/>	<input type="checkbox"/>	0.2330	4.4400	<input checked="" type="checkbox"/>

Progress Table:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
im_group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
region	1	1	1	1	1	1	1	1	1	1	1	1	1	1
success %	0	0	0	0	0	0	0	0	0	0	0	0	0	0
iterations	12	12	12	12	12	12	12	12	12	12	12	12	12	12

FLIMfit: DATA VISUALISATION

Correlation plots of selected fitted parameters

The screenshot shows the FLIMfit software interface. The 'Correlation' tab is highlighted in pink. The main window displays a heatmap showing the correlation between two parameters: 'weighted mean tau' (x-axis, 1150-1500) and 'per pixel/per object' (y-axis, 20-120). The plot shows a strong positive correlation, with a bright yellow/red region indicating high correlation. The interface includes a dataset list on the left, a main image window, and various control panels for data processing and fitting.

Plot	Dis...	Me...	Min	Max	Auto
tau_1	<input type="checkbox"/>	<input type="checkbox"/>	1620	1660	<input checked="" type="checkbox"/>
tau_2	<input type="checkbox"/>	<input type="checkbox"/>	106	109	<input checked="" type="checkbox"/>
offset	<input type="checkbox"/>	<input type="checkbox"/>	6.9000e...	0.1240	<input checked="" type="checkbox"/>
beta_1	<input type="checkbox"/>	<input type="checkbox"/>	0.1190	0.3550	<input checked="" type="checkbox"/>
beta_2	<input type="checkbox"/>	<input type="checkbox"/>	0.6450	0.8810	<input checked="" type="checkbox"/>
l0	<input type="checkbox"/>	<input type="checkbox"/>	1.6100	27.2000	<input checked="" type="checkbox"/>
l	<input type="checkbox"/>	<input type="checkbox"/>	10	132	<input checked="" type="checkbox"/>
mean_tau	<input type="checkbox"/>	<input type="checkbox"/>	291	650	<input checked="" type="checkbox"/>
w_mean_t...	<input type="checkbox"/>	<input type="checkbox"/>	1130	1480	<input checked="" type="checkbox"/>
chi2	<input type="checkbox"/>	<input type="checkbox"/>	0.2330	4.4400	<input checked="" type="checkbox"/>

Param	Type	Value
▼	▼	
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▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	

Progress	1	2	3	4	5	6	7	8	9	10	11	12	13	14
im_group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
region	1	1	1	1	1	1	1	1	1	1	1	1	1	1
success %	0	0	0	0	0	0	0	0	0	0	0	0	0	0
iterations	12	12	12	12	12	12	12	12	12	12	12	12	12	12

FLIMfit: DATA VISUALISATION

Plots of selected fitted parameters across all images

The screenshot displays the FLIMfit software interface. The 'Plotter' tab is highlighted in pink. The main plot shows 'weighted mean tau' on the y-axis (ranging from 1150 to 1500) versus 'ImqNum' on the x-axis (ranging from 0 to 55). The plot shows a series of data points with vertical error bars, indicating the weighted mean tau for each image. The values fluctuate between approximately 1250 and 1450.

The left panel shows a list of images (41 to 54) with columns for 'mM' and 'Time'. A preview of a cell image is shown in the center. The bottom left panel shows various fitting parameters and options, including 'Smoothing', 'Integrated Min.', 'Time Min.', 'Counts/Photon', 'Global Fitting', and 'Global Variable'.

The right panel shows a table of fitted parameters and their values:

Plot	Dis...	Me...	Min	Max	Auto
tau_1	<input type="checkbox"/>	<input type="checkbox"/>	1620	1660	<input checked="" type="checkbox"/>
tau_2	<input type="checkbox"/>	<input type="checkbox"/>	106	109	<input checked="" type="checkbox"/>
offset	<input type="checkbox"/>	<input type="checkbox"/>	6.9000e...	0.1240	<input checked="" type="checkbox"/>
beta_1	<input type="checkbox"/>	<input type="checkbox"/>	0.1190	0.3550	<input checked="" type="checkbox"/>
beta_2	<input type="checkbox"/>	<input type="checkbox"/>	0.6450	0.8810	<input checked="" type="checkbox"/>
I0	<input type="checkbox"/>	<input type="checkbox"/>	1.6100	27.2000	<input checked="" type="checkbox"/>
I	<input type="checkbox"/>	<input type="checkbox"/>	10	132	<input checked="" type="checkbox"/>
mean_tau	<input type="checkbox"/>	<input type="checkbox"/>	291	650	<input checked="" type="checkbox"/>
w_mean_t...	<input type="checkbox"/>	<input type="checkbox"/>	1130	1480	<input checked="" type="checkbox"/>
chi2	<input type="checkbox"/>	<input type="checkbox"/>	0.2330	4.4400	<input checked="" type="checkbox"/>

Below the parameter table, there are options for 'Invert Colorscale?' (No), 'Display Colormap?' (Yes), and 'Display Limits?' (Yes).

The bottom panel shows a 'Progress' table with columns for 'im_group' (1-14) and rows for 'region', 'success %', and 'iterations'.

FLIMfit: DATA VISUALISATION

Average values of selected fitted parameters in a 96-well plate

The screenshot displays the FLIMfit software interface. The central focus is a 96-well plate heatmap where each cell's color represents the average value of a selected fitted parameter. The color scale ranges from dark blue (low values) to red (high values). The values 2700 and 2200 are labeled on the heatmap. The 'Plate' tab is highlighted with a pink circle. To the left, a 'Dataset' panel shows a list of wells (41-54) with checkboxes and columns for 'mM' and 'Time'. Below this is a 'Data' panel with settings for 'Smoothing', 'Integrated Min.', 'Time Min.', 'Counts/Photon', 'Rep. Rate', 'Gate Max.', and 'Time Max.'. The 'Lifetime' panel includes 'Global Fitting', 'Global Variable', 'Global Mode', 'No. Exp.', 'No. Fixed', 'Fit Contributions', 'Fit Reference', and 'Fit IRF Shift'. A 'Progress' table at the bottom shows the status of the fit for each well.

Plot	Dis...	Me...	Min	Max	Auto
tau_1	<input type="checkbox"/>	<input type="checkbox"/>	1620	1660	<input checked="" type="checkbox"/>
tau_2	<input type="checkbox"/>	<input type="checkbox"/>	106	109	<input checked="" type="checkbox"/>
offset	<input type="checkbox"/>	<input type="checkbox"/>	6.9000e...	0.1240	<input checked="" type="checkbox"/>
beta_1	<input type="checkbox"/>	<input type="checkbox"/>	0.1190	0.3550	<input checked="" type="checkbox"/>
beta_2	<input type="checkbox"/>	<input type="checkbox"/>	0.6450	0.8810	<input checked="" type="checkbox"/>
l0	<input type="checkbox"/>	<input type="checkbox"/>	1.6100	27.2000	<input checked="" type="checkbox"/>
l	<input type="checkbox"/>	<input type="checkbox"/>	10	132	<input checked="" type="checkbox"/>
mean_tau	<input type="checkbox"/>	<input type="checkbox"/>	291	650	<input checked="" type="checkbox"/>
w_mean_t...	<input type="checkbox"/>	<input type="checkbox"/>	1130	1480	<input checked="" type="checkbox"/>
chi2	<input type="checkbox"/>	<input type="checkbox"/>	0.2330	4.4400	<input checked="" type="checkbox"/>

Param	Type	Value
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	
▼	▼	

Progress														
im_group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
region	1	1	1	1	1	1	1	1	1	1	1	1	1	1
success %	0	0	0	0	0	0	0	0	0	0	0	0	0	0
iterations	12	12	12	12	12	12	12	12	12	12	12	12	12	12

FLIMfit: EXPORTING RESULTS

- **Export images:**
 - * FLIM values (.tif, raw 32-bit)
 - * FLIM (.tif, RGB)
 - * Intensity-weighted FLIM (.tif, RGB)
 - * Intensity (.tif, 16-bit)
- **Export results table** (.csv)
- **Export histograms and plots** (.csv)
- **Export to Power Point:**
 - * Decay + IRF
 - * Galerie
 - * Histogram
 - * Correlation plots
 - * Plots

The screenshot shows the FLIMfit software interface with the 'Parameters' tab selected. A table on the right lists parameters for export, with checkboxes for 'Dis...' and 'Me...'. A blue arrow points from the text 'Select parameters to be displayed as image' to the 'Dis...' column. Below the table is a section for 'Set parameter limits' with a table for 'Param', 'Type', and 'Value'. At the bottom, there are three dropdown menus for 'Invert Colorscale?', 'Display Colormap?', and 'Display Limits?'.

Plot	Dis...	Me...	Min	Max	Auto
tau_1	<input type="checkbox"/>	<input type="checkbox"/>	2070	2120	<input checked="" type="checkbox"/>
tau_2	<input type="checkbox"/>	<input type="checkbox"/>	244	250	<input checked="" type="checkbox"/>
offset	<input type="checkbox"/>	<input type="checkbox"/>	0.0088	0.0832	<input checked="" type="checkbox"/>
beta_1	<input type="checkbox"/>	<input type="checkbox"/>	0.1680	0.3500	<input checked="" type="checkbox"/>
beta_2	<input type="checkbox"/>	<input type="checkbox"/>	0.6500	0.8320	<input checked="" type="checkbox"/>
I0	<input type="checkbox"/>	<input type="checkbox"/>	1.2300	13.4000	<input checked="" type="checkbox"/>
I	<input type="checkbox"/>	<input type="checkbox"/>	11	99	<input checked="" type="checkbox"/>
mean_tau	<input type="checkbox"/>	<input type="checkbox"/>	559	894	<input checked="" type="checkbox"/>
w_mean_t...	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	1410	1770	<input checked="" type="checkbox"/>
chi2	<input type="checkbox"/>	<input type="checkbox"/>	0.2930	2.7400	<input checked="" type="checkbox"/>

Param	Type	Value

Invert Colorscale? No
Display Colormap? Yes
Display Limits? Yes

Select and adjust the parameters to be displayed as image

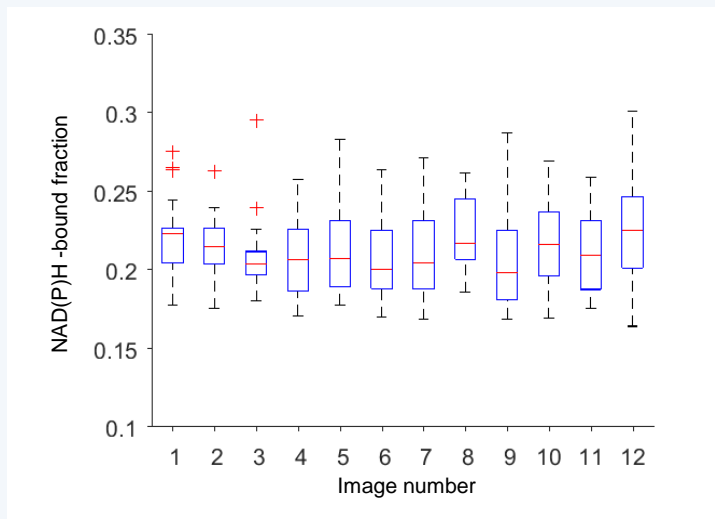
BIOLOGICAL APPLICATIONS OF FLIM GLOBAL ANALYSIS

1. Estimate the free and bound NAD(P)H fractions in live cells

1. Global analysis with double exponential decay

- Set the global analysis for all the images acquired in a given condition (i.e. tissue type, cell type, substrate, inhibitors concentrations etc.)
- The short NAD(P)H lifetime corresponds to the free form
- The long NAD(P)H lifetime corresponds lifetime to the bound form
- Segment the images using the watershed segmentation available in FLIMfit to get statistics per cell

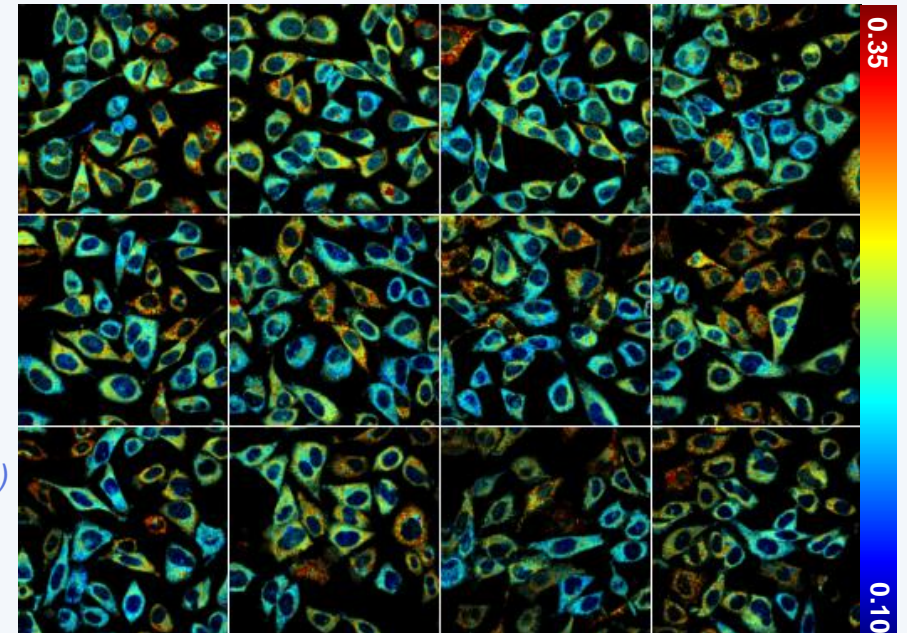
2. Display the results (e.g. images, statistical graphs)



*The bound NAD(P)H fraction
in HeLa cells metabolising lactate:*

- *Box plot of bound fraction/cell/image (left)*
- *Intensity merged images
of the bound fraction (right)*

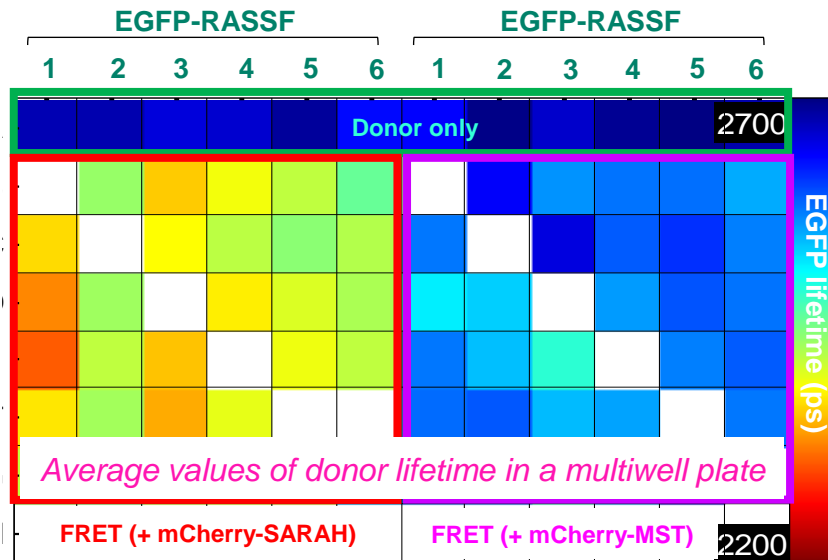
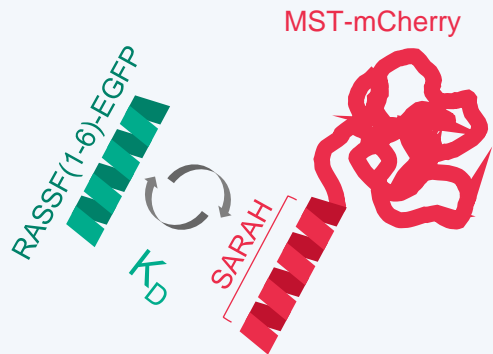
(A. Margineanu, Max Delbrück Centrum Berlin)



BIOLOGICAL APPLICATIONS OF FLIM GLOBAL ANALYSIS

2. Estimate dissociation constants (K_D) using intermolecular FLIM-FRET

The biological interactions explored by FRET



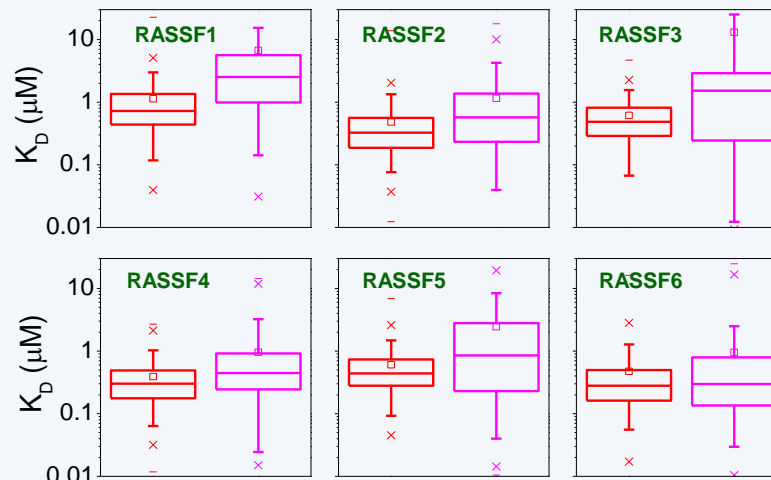
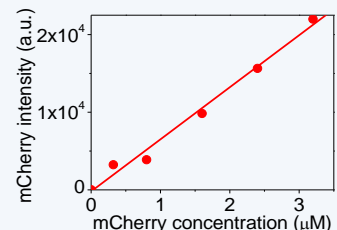
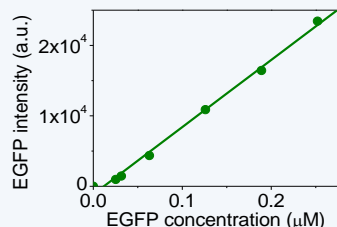
Summary of the K_D calculations

- Find the bound fraction β from the global double exponential fit of the FRET decays

$$I_{(t)} = I_0 \left[(1 - \beta)e^{-\frac{t}{\tau_D}} + \beta e^{-\frac{t}{\tau_{DA}}} \right]$$

- Calibrate the EGFP and mCherry intensity vs. concentrations

⇒ Determine the **dissociation constants K_D** : $K_D = \frac{[D_{free}][A_{free}]}{[DA]}$



Estimated values of the intracellular K_D

(statistics per cell):

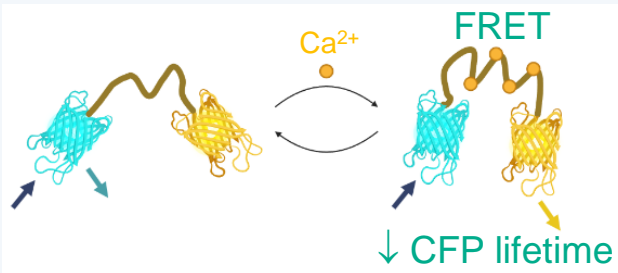
- RASSF(1-6) with the SARAH domain (red box plots)
- RASSF(1-6) with the full length MST (magenta box plots)

Margineanu et al. (2016), Sci Rep. 6:28186, doi: 10.1038/srep28186

BIOLOGICAL APPLICATIONS OF FLIM GLOBAL ANALYSIS

3. Estimate the free and bound fractions of a Ca^{2+} biosensor labeled with a multiexponential donor using the FRET model

CerTn-L15 Ca^{2+} biosensor (Cerulean-Citrine)



1. Multiexponential donor (Cerulean)

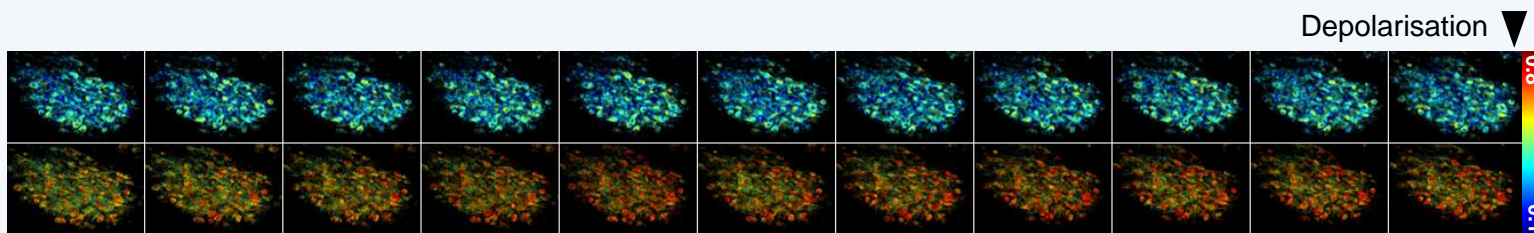
Get the values of the lifetimes and contributions of the donor not interacting with the acceptor and fix them

Lifetime	Stray Light	Anisotropy	FRET	Advanced	
Global Fitting	Global	<input type="checkbox"/>	Automatically Estimate Lifetimes		
Global Variable	-		Initial Tau	Beta	
Global Mode	Global Analysis		1	3700	0.6000
No. Exp	2		2	1500	0.4000
No. Fixed	2				
Fit Contributions	Fixed				
Fit Reference	Fixed				
Fit IRF Shift	Fixed				

2. Set 2 FRET species with low (Ca^{2+} - free) and high (Ca^{2+} -bound) FRET efficiencies

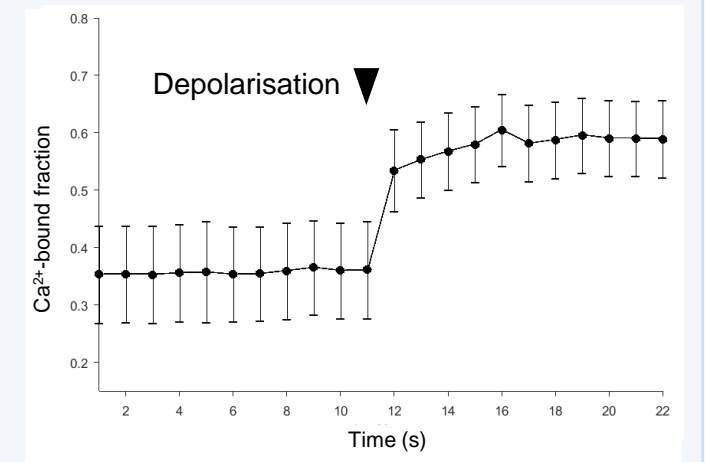
Lifetime	Stray Light	Anisotropy	FRET
No. FRET Species	2		E
No. Fixed	0	1	0.1000
Include donor only	No	2	0.4000

3. Display and plot the fraction of the component with high FRET efficiency (Ca^{2+} -bound)



→Time (s)

Time series of increased Ca^{2+} signalling in live mouse neurons after KCl depolarisation (V. Siffrin, A. Margineanu, Max Delbrück Centrum Berlin)



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