

SymPhoTime 64 Analysis Tips and Tricks

~~TOC~~

Summary

This tutorial explains a few features of SymPhoTime64 that can help to make working with the software more comfortable. In detail, this tutorial covers:

- Different file-types used or generated by SymPhoTime64
- Image Display
- ROI-handling
- Export options

This tutorial assumes SymPhoTime V2.0

General UI Philosophy

• right clicking on graphs/images gives additional options and tools otherwise hidden

File-Type Overview

Workspace: may contain these Filetypes	Content of the file	How to get such a file in the workspace	How to open/process
.ptu	Raw data in a unified TTTR- format	 Generated by a measurement Import of older TTTR files from PicoQuant boards (.pt3,.pt2,.thr,.t3r,.t2r) By copying a .ptu file into the workspace folder before opening the workspace 	 Double click: opens a comment window in which the info entered in the acquisition field is displayed Highlight and run an analysis script you want to apply to the raw data.

Workspace: may contain these Filetypes	Content of the file	How to get such a file in the workspace	How to open/process
.pqres	Result file	 Generated from the online analysis selected during the measurement (the Countrate- Display is not saved as a result file) By highlighting a ptu-file, applying a script and saving the result By double click on a result file to modify the analysis and then saving again the new result (the old result is never overwritten) 	1. Double click. This opens the script with the stored result 2. For online FCS-results, the calculated molecular brightness can be displayed by highlighting and going to the main menu onto File \rightarrow Show Comment
.pck	internal file	 is generated, when a post-acquisition analysis is performed speeds up the processing for additional analysis. This file type was introduced with version 2.5 	this file type is for software internal purposes only, so the user does not need to use it.
.pco	Comment file, contains manually entered text (e.g. information about the experiment)	Menu: File → Create Comment	Double Click
.bmp	Contains a camera image	Save a camera image generated by the fluorescence or back reflection camera (only MT200 - users)	Double Click

Working with the different file types

Raw data (".ptu") files

• double click on any .ptu (raw data) file opens a file viewer, in which you find information about the file type and acquisition settings

SymPhoTime 64 - [Fast Preview of TS-	Bead_immo_xy-scan_D	ual Focus.ptu]		
🙀 File Edit View Settings Scripts Ana	alysis Window Help			
	Analysis			
Cy5_diff_IRF+FLCS-pattern.p	General Information		Comment Header	
BJL Atto655_diff_FLCS-pattern.pt	Name	TS-Bead immo xy-scan Dual	Created on 2/21/2007 12:49:11 PM	*
i →	Туре	TTTRFile	Z-Scan at:	
Atto488_diff_cw_total_correl	Date	2012-08-29 10:55:38	0	
Cy5_immo_FLIM+Pol-Imaging.;	Acquisition time	00.01-24	0	
ig	TCSPC Mode	73		
DaisyPollen_cells_FLM.ptu	Heasurament mode	13		
GFP_RFP_cells_FLIM-FRET.pt	Measurement mode	mage		
TS-Bead immo xy-scan Dua	Number of Pixels	200 x 200		
C TS-Bead_immo_xy-scan_	Scan direction	х-у		
Left_Focus.pqres	StartPoint (x, y)	83.15 µm, 68.95 µm		
TS Read immo x7-acan atu	Image Size	3.00 µm x 3.00 µm		
i BA488+IBA547_unlinked_mix	Dwell Time	3.0 ms		
BA488+547_crosslinked.ptu	Scanning pattern	Monodirectional		
non-FRET sample.ptu	Stop after	00:01:24		
Atto488 485nm pulsed.otu	Stop Reason	Time over		
Status Messages:				
Workspace: Successfully opened.				

Fig. 1: ".ptu" file information and comment

The general file information contains for example:

- the number of pixels
- the image size (if the image has been acquired with a scanner controlled by the SymPhoTime software or the size info has been entered before the measurement, either manually in the **"Acquisition"** tab or automatically for remotely controlled FLIM-imaging
- the imaging mode (mono- or bidirectional)
- whether it's a t2 or t3 type file
- Info that was entered into the text field during the acquisition (you cannot change this information after the acquisition)

More detailed information can be found in the **"Header"** - tab:

Test Measurement	Test Measurement Analysis						
 General Information 		Comment Header					
Name	Lunge_unstained8.ptu	1. File_GUID	{22A85BDE-4355-4256-B1A7-ABDAD8E55002}				
Туре	TTTRFile	2. File_CreatingTime	2015-01-12 2:17:35 PM				
Date	2015-01-12 2:17:35 PM	3. Measurement_SubMode	3				
Acquisition time	10:00:00	4. File_Comment					
TCSPC Mode	Т3	5. TTResult_StopReason	0				
Measurement mode	Image	6. Fast_Load_End	<empty></empty>				
Number of Pixels	400 x 400	7. CreatorSW_Name	SymPhoTime 64				
Scan direction	х-у	8. CreatorSW_Version	2.0				
StartPoint (x, y)	-972.90 µm, -1393.40 µm	9. CreatorSW_SVNBuild	3660				
Image Size	401.80 µm x 401.80 µm	10. CreatorSW_Modules	0				
Dwell Time	2.0 ms	11. UsrOverviewGUID	{107AC57D-7514-4E38-B8F7-EBEC9F98CFEE}				
Scanning pattern	Monodirectional	12. ImgHdr_Dimensions	3				
Laser Power Diode	325.3 a.u.	13. ImgHdr_Ident	5				
Stop after	00:11:26	14. ImgHdr_BiDirect	FALSE				
Stop Reason	Time over	15. ImgHdr_TimePerPixel	2				
L		16. lmgHdr_X0	-972.9				
		17. ImgHdr_Y0	-1393.4				
		18. ImgHdr_PixX	400				
		19. lmgHdr_PixY	400				
		. . .					

Comment (".pco") files

• To read or edit a comment-file (.pco), just double click it.

Contrary to the comment entered prior the start of the measurement, the comment file can be edited at any time. The comment entered before the measurement is stored directly inside the .ptu file and cannot be modified after the measurement (see figure 1).

Result ("pqres") files

• Double clicking a .pqres (result) file opens the result of a particular analysis script.

Result files also contain a comment which can be displayed via the file menu: **File → Show Comment**.



Typically the result-file opens with a preview display (Image, FCS-traces, TCSPC-curve or Time Trace - depending on the type of analysis). Additionally the **"Comment"** - tab of an online-analysis will contain relevant information about the measurement. To check the values calculated during an online analysis, select the "**Comment**"-tab.

The kind and amount of the information of course depends on the type of online analysis:

Image



- Max. Photons (in the brightest pixel for the selected data channel)
- Max. Countrate (calculated taking into account the pixel recording times)
- Avg. Photons (in a pixel)
- Avg. Countrate (calculated taking into account the pixel recording times)
- Frames (especially important for LSM upgrade kits where in general several frames are recorded, while for an image acquired with the MicroTime, the image consists of a single frame)

FCS (for each of the max.2 data channels)



- Max. Countrate
- Avg. Countrate
- G(0)
- Num. Molecules (calculated from the average countrate and G(0))
- mol. Brightness (in kcps/s/molec.)
- if 2 data channels are active, also G(0) of the crosscorrelation is calculated.

Time-Trace

- Max. Counts
- Av. Counts

TCSPC

• Max. Counts (number of photons in the peak TCSPC-channel)

You can also add additional information and save this using the **"Save Comment"** - button. The modified comment is stored in the same (.pqres) file.

Image Display

• Images can be drawn in 3 ways (for this, open the file FLIM_3_expon.pqres, associated to the raw data

file DaisyPollen_cells_FLIM.ptu)

 toggling between the different display options can be done by selecting the desired display option above the scale.

Grey Scale	Rainbow Scale	RGB Scale
Electricity and the second sec	DaayProse_Galle, TAM (Size	Daty/Poletr_cols_(C.M.plo Daty Ranker Daty V(0)0.4.1 Annee V(0)0.4.1 Annee V(0)0.4.1 V(0)0.4.1 Annee V(0)0.4.1 V(0)0.4.1 V(0)0.4.1 Annee V(0)0.4.1 V(0)0.4.1 V(0)0.4.1 V(0)0.4.1 Annee
1 selectable parameter encoded: 1. brightness	2 selectable parameters encoded as: 1. brightness 2. pseudocolor	3 selectable parameters encoded as: 1. Red 2. Green 3. Blue

- some parameters are only available after performing a FLIM-Fit (i.e. the different lifetime components and amplitudes
- to adjust the scale, you can either
 - $\circ\,$ type the min and max-value
 - $\circ~$ place the cursor above the scale, right click and select "Scale (ALL)".



This adjusts the minimum to the minimum value of this

parameter in the image. This may also mean that if there a a few pixels with extreme values, e.g. very long lifetimes of some background pixels, that the contrast within the structures of the image is not as clearly visible anymore. On the other hand, as the min and max values are then display as scale limits, you can get an easy idea of the parameter range in the image.

- place the cursor above the scale, right click and select **"Clip Outliners"**. This feature ignores outliners of the parameter (i.e. the 5% lowest and highest values).
- when looking at the Fast Lifetime parameter, in the unbinned image, which calculates the mean of the photon arrival times, you will notice that the lifetime values have partially negative values:



- this happens, as the rising edge of the decay is chosen as 0. Background pixels with just a single photon which might be before this pixel can therefore have negative Fast Lifetime values.
- in fitted images, constraints can be set (e.g. 0 as minimum lifetime) in the TCSPC fitting panel
- the fast lifetime in an ideal system should equal the intensity weighted average lifetime [τ av. int.].
 Usually, fitted lifetimes tend to be slightly shorter, as the background photons are excluded from the

calculation.

• In images obtained with PIE-excitation, the fast lifetimes are usually meaningless, as in this case the photons from 2 decays are taken into account.

Color Smoothing

- In a "Rainbow Scale Image", a color smoothing can be introduced. When checking **"Smooting"** below the intensity axis, a width defined can be defined for a Gaussian distribution which is overlaid to the image and used to improve the optical appearance of the image. As in many cases the pixel size in the image is smaller than the optical resolution, taking into account the neighbouring pixels is also a valid approach to de-noise the image.
- The smoothing is only applied to the parameter encoding the color scale. Take a look at the effect in the following example from the Fast_FLIM.pqres file which is associated to the file DaisyPollen_cells_FLIM.ptu



Fig. 6: No Smoothing Fig. 7: Smoothing = 100nm





Fig. 9: Smoothing = 400nm

- As can be seen for this example, moderate smoothing can despeckle the image slightly, while high smoothing can completely mask certain features and therefore also introduce artifacts. Therefore please handle the tool with care
- NOTE: As the smoothing is just a display function that does not affect the raw data, smoothed images can be exported as .bmp, but not as ASCII or .tif, and also the smoothing does not affect the lifetime histogram
- Take care that you record the data with the correct physical dimensions. While in images taken with the MT200 this is done automatically, in LSM upgrade systems, which are not remotely controlled by the LSM software via a handshake, the image size is not known unless typed in before the acquisition (see online tutorials about FLIM and FLIM-FRET imaging)

Working with ROIs

Selecting ROIs

In Images, ROIs can be selected using the context menu (Right Click):



- you can mark several areas with the same or different selection tools by keeping the <Shift> key pressed while drawing the different ROIs
- if you want to remove some of the pixels from the ROI selection, just keep the <CTRL> key pressed when using a ROI selection tool to unselect unneeded pixels from the selection.

- If you want to start from scretch again, select "Select all as ROI"
- you can also invert the selection using "Invert ROI"

The following ROI-selection tools are available:

Paint ROI



Marks the pixels the mouse passes over. The faster the mouse pointer moves, the broader is the marked line of pixels

Free ROI



Rectangle ROI



Ellipse ROI



Magic Wand ROI



marks areas with a similar fast lifetime

Undo ROI selection

Using <CTRL>+<Z> you can undo a selection (you can also redo by pressing <CTRL>+<Shift>+<Z>), as shown in this example:

1. Start image, in this case the daisy-pollen image from the demo workspace, we choose the "FLIM_3_expon.pqres". The task is to define a ROI from the blue area below the pollen for this illustration example.





Example: marking several spots with a similar lifetime:

1. Click on the file FAST_FLIM.pqres, which is associated to the file DaisyPollen_cells_FLIM.ptu	7 um	Events(Cnts) Lifetime[ns] 0
2. first mark one structure with the magic wand tool. All other pixels turn grey, therefore it is not possible anymore to know which pixels to select.	-7 µт	Events[Cnts] Lifetime[ns] 0 1.8 0 0
3. Therefore invert the ROI	y ym	Fast Events[Cnts] 0 1 0 0 8
4. then keep the <crtl>-button pressed and click on the other areas with a long lifetime (it is also possible to change the ROI-selection tool).</crtl>	7 µm	2000 Events[Cnts] 0 1.8 Uffetime[ns] 0.8
5. now invert the ROI again, and you have marked all similar areas within the Pollen.	7 µm	2000 Events[Cnts] 0 1.8 Uiretime[ns] 0.8

How to use the magic wand tool

The magic wand tool marks region with a similar lifetime range. "Similar" in this sense is an arbitrary definition, but the sensitivity "Magic Wand Thres." of the magic wand tool can be adjusted in a box below the intensity and color scaling options (see in the screenshots below). Adjusting the Magic Wand Threshold can help to easier mark objects in the image as shown in the example:



The magic wand tool is based on the displayed image, therefore the sensitivity of the selection is also dependent on the scaling of the color-encoded component (in general, the lifetime). Changing the color scale can also change the sensitivity of the tool as shown in this example:



How to get values of a parameter along a line

In SymPhoTime 64 there is the option to get the Intensity profile along a line from the GUI element **"slice direction"** in the following GUIs



- FLIM
- Pattern Matching
- Fast Pattern Matching
- Focal Width
- Gated STED Image
- Confocal + STED Image

In Analysis GUIs "FLIM" and "Pattern Matching" the feature is made available upon clicking on the "Resolution

Decay Fitting	Resolution Estimation	
Fitting Model:	n-Exponential Reconvolution	Help
	- 14 - 14 - 14 - 14 - 14 - 14 - 14 - 14	

estimation" tab.

or

Pattern Matching	Resolution Estimation	
Decay:	Overall Decay	
Pattern:		

Let us take as an example the "FLIM Analysis" GUI



Now use the drop-down list to select the parameter used for the "gray-scale" and select "FastLT" (or any other parameter of your choice)



Then you get the corresponding gray level image according to "FastLT" and by clicking on the selected line the

graph in the lower panel is updated and the now the profile of the the "FastLT" along the line is displayed.



Note: The title and units of the Y axis of the graph will not be updated. Rescaling of the lower graph might be necessary

As a final step you can right click on the graph and export the line profile data.

Export Options

Export of graphs and images can usually be done via the context menu via a right mouse click with the mouse being over the image or graph. Some scripts also have defined export buttons in the control panel on the left.

Images

For **images**, there are the following export options (right clicking on any displayed image will show the export dialogue:



Formats

Bitmap



- The image is exported as shown on the screen.
- The color scale is not exported
- If the image size on the screen is increased (e.g. by iding the script-tab), the scale bar may adapt.
- The scale bar can be hidden by unchecking "show scale bar" in the context menu

Bitmap with Color Scale



- The image is exported as shown on the screen.
- The intensity and/or color scale is exported
- If the image size on the screen is increased (e.g. by iding the script-tab), the scale bar may adapt.
- The scale bar can be hidden by unchecking "show scale bar" in the context menu

1 to 1 Bitmap



- Every pixel in the image is exported as one single pixel in the bitmap
- The color scale is not exported
- changes in the intensity scale are adapted to the image

ASCII

🔄 Ascii-Image.dat - Editor 🗕 🗖 🗙
Datei Bearbeiten Format Ansicht ?
A[θ][a.u.] ^
(x0 y0) = (33.742[µm] 81.126[µm])
(x1 y1) = (56.942[µm] 104.326[µm])
48.1130367256701 38.1194343566895 27.8771915435791 20.
883706510067 1.57704854011536 2.0063445661217 3.8899574643001 2.0
43.3114891052246 28.7642789743841 18.0941982269287 24.
2.3033666610718 5.2004656791687 -0.280249253381044 1.50240385532379
36.4441757202148 23.1448974609375 22.1630558108445 23.
5878 7.36094188690186 2.16265559196472 2.09216284751892
27.4307747607527 29.2554246552754 20.195990100503 15.44089698
5 14.0675230026245 4.43429946899414 2.53525349497795
22.6835460662842 21.4575099945068 18.9643862247467 15.
19.5395488739014 5.82253885269165 5.17786169052124 4.2
23.7337322235107 21.5069664157927 20.9220823198557 14.
9.840238571167 9.26663017272949 4.43016910552978 -0.07618889
19.3119793236256 19.3550316691399 20.7237081900239 17.
290496826 11.3244619369507 7.43768787384033 6.237090066
۱. «

- the file is exported as a text file
- Every plotted parameter is saved as an array in the text file, before every array, there's a small header that indicates dimensions and the shown parameter.

Thus depending on the plot type, the file contain several arrays:

- greyscale images : 1 array, i.e. intensity
- rainbow images: 2 arrays, i.e. intensity + fast lifetime
- RGB images: 3 arrays, i.e. amplitude 1-3
- for importing into processing programs as ImageJ, best export each parameter as greyscale image, delete the header lines and import as text image into ImageJ. Make sure that the decimals number have the same country settings in both programs to avoid import problems.

TIFF



- Every pixel in the image is exported as one single pixel in the tiff
- the tiff format for the number is float, therefore the real values can be displayed.
- Each parameter is stored as different greyscale-frame, therefore export of an RGB-image results in a tifimage stack with 3 layers
- As absolute values are exported, the displayed intensity scale is not relevant
- The image size is exported as well, however the current ImageJ always shows cm.

You can manually set it to μm via: Image Properties \rightarrow Unit of Length $\rightarrow \mu m$.

Clipboard

• This feature exports the image as in the bitmap-option, but into the clipboard. The image can than be pasted into a graph or text program (e.g. word document) via <CTRL>+<V>

Binary

- This export option is not found in the context menu, but in the FLIM-script and a few others as a separate button.
- It exports the image as a .bin-file, which can be imported e.g. into phasor analysis software and is also supported by the open microscopy platform.
- TCSPC-binning is applied to the export of these images.
- see Structure of the pre-histogrammed Image Data File

Graphs

• for **graphs**, e.g. the TCSPC-Decay plot in the FLIM-script, can be exported in an ASCII-format as well as in a bitmap format. If the graphs consists on different cells, e.g. the decay and the residuals as shown, it is possible to select, which cells should be exported.



Export ASCII selected cell



- the active cell is the cell below the mouse pointer, the color of the lines in the active cell change to green.
- The active cell in this example is the TCSPC decay window, exporting the active cell just exports the decays in the upper window without the residuals
- the exported file is a text file that can be loaded into Excel or other programs.

Export ASCII visible cells



• all open cells are exported in this option, i.e. if the graph is plotted as shown above, the decays and the

residuals are exported.

- If the residual cell is closed by clicking on the small green "-" on the upper left corner of each cell (see below), the active cell equals the visible cell, and the export result is the same as exporting just the active frame
- the exported file is a text file that can be loaded into Excel or other programs.

Export ASCII all cells

• all cells are exported. In our example, TCSPC-decays and residuals are both exported, independent from whether the all cells are open or not.

Export bitmap



• the graph is exported as plotted bitmap.

Copy to clipboard

- the graph is exported as an image to the clipboard as plotted and can be pasted into a graphic program or e.g. a word document via <CTRL>+<V>
- In contrast to the bitmap-export, a highlighted cell in the the clipboard-copy remains highlighted. If this is not wanted, just move the mouse slightly out of the cell before exporting to clipboard

Fitting Results

• For *Fitting Results*, e.g. Lifetime-Fits, the values can be exported as an ASCII-table either into a file or the clipboard, from where it can be directly pasted into e.g. Excel or a similar program. To export, you can use either the context menu, as with graphs and images, or the icons above the fitting panel.

Decay Fitting Resolution Estimation							
Fitting Model:	n-Expo	nen	tial Reconvolutior Help				
Decay:	Overal	De	cay	-			
IRF: Import	Calcula	ted	IRF		Re	mov	/e
Model Paramete	Model Parameters: n 3						
Show Al	Export	t to (Clipboard	Export	ns A	SC	D
Parameter		俥	Overair Deca	7		~	-
A [I] [kCnts]	Limits		1490.8 ±	7.5	÷	~	
A [2] [kCnts]	Limits		332.5 ±	1.5	÷	~	
A [3] [kCnts]	Limits		20.300 ±	0.093	÷	~	
τ(η (ns)	Limits		0.480 ±	0.000	÷		
τμ] [ns]	Limits		1.446 ±	0.000	÷		
τ[8] [ns]	Limits		3.939 ±	0.000	÷		
Bkgr pec [Cnts]	Limits		235.94 ±	0.53	÷	~	
Shift (RF [ns]	Limits		0.01775 ±	0.00081	÷	~	
Bkgr rer [Cnts]	Limits		211 ±	104	÷	~	
l[1] (kCnts)			18978 ±	95			
I ₍₂₎ [kCnts]			12752 ±	58			
I [2] [kCnts]			2120.6 ±	9.7			
Isum[kCnts]			0.000 ±	0.000			
A sun [kCnts]			0.000 ±	0.000			
•						Þ	Ť
Clear Initial	FR 📃	Fit	FitAl	X2 =	6.4	156	

• The feature can not only export the fitted values of one curve, but also be applied for the fits of several curves simultneously. To illustrate this, mark several ROIs in the image, as illustrated in the image below:



- click on "initial fit" to get resonable staritng parameters, then "fit all"
- When data are exported now, the fitting values of each fitted data set are stored in an additional line

		Unbenannt - Editor					×
Datei Bearbeiten Format Ansicht	2						
Parameter A[1][kCnts	s] ±Err A[2][kC	nts] ±Err	A[3][kCnts]	tErr	tau[1][ns]	tErr	\sim
Overall Decay 333.100 0.	.000 1489.200	0.000 20.549	0.000 1.443	0.000	0.480 0.000	3.926	
Pixel (1,1) 333.100 0.	.000 1489.200	0.000 20.549	0.000 1.443	0.000	0.480 0.000	3.926	
ROI 0 333.100 0.000 14	489.200 0.000	20.549 0.000	1.443 0.000	0.480	0.000 3.926	0.000	
ROI 1 333.100 0.000 14	489.200 0.000	20.549 0.000	1.443 0.000	0.480	0.000 3.926	0.000	
ROI 2 333.100 0.000 14	489.200 0.000	20.549 0.000	1.443 0.000	0.480	0.000 3.926	0.000	
ROI 3 333.100 0.000 14	489.200 0.000	20.549 0.000	1.443 0.000	0.480	0.000 3.926	0.000	
							× 1
<						;	1

• If you want to view all the fitting parameters in one window, you can just click on "Show all" on the upper

fere, and a window opens, which displays an nearly parameters side by sid	left ,	and a	a window	opens,	which	displays	all fitting	parameters	side by side	de
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All Export to Clipboard Export as ASCI

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Parameter		0	Overall Decay			~	-															
Anj [kCnts]	Limits		1489 ± 13		¢	¥																
A [z] [kCnts]	Limits		333.1 ± 3.5		÷	~																
A [3] [kCnts]	Linits		20.55 ± 0.38		٥	~																
ជ្យ [ns]	Limits		0.4800 ± 0.002	27	÷.	~																
t [2] [ns]	Linits		1.443 ± 0.006	5	¢	¥																
τpj [ns]	Limits		3.926 ± 0.016	5	÷.	v																
Bkgr Dec [Cnts]	Limits		234.9		÷	¥																
Shift pr [ns]	Limits		0.01772 ± 0.000	166	÷.	~																
Bkgr 🔫 [Cnts]	Limits		219.0 ± 99.5		÷	¥																
Ing [kCnts]			18956 ± 127		Ĩ																	
Ipp [kCnts]			12746 ± 71	_	Η																	
Ipj [kCnts]			2140 ± 31		Π																	
Isum [kCnts]			33842 ± 69		Π																	
A sum [kCnts]			1843 ± 12																			
41						F	•															
•																						
Clear Initial	Ft .	Fit	FRAI X ² -		6.4	180																
Clear Initial	Ft	Fit	F&AI X*-		6.4	180																_
Clear Initial	Ft	Fit	FitAl X ^a -		6.4	180										Exp	ort t	o Clipboard	Expo	rt as	AS	CII
Clear Initial Close Parameter	Ft	Fit Ov	F&AI X ^a =	-	6.4 Pix	180 el (1	,1)		~	R010		~	RDI 1	~	R012	Exp	ort t	o Clipboard ROI 3	Expo	rt as	ASI	Cill
Clear Initial Close Parameter A _[1] [kCmts]	F4	Fit €Ov	Fit All X ² =	~ ~	6.4 Pbo	880 el (1	,1) 88 ± 0.00	55	> >	R010 82.69 ± 0.26	÷	- -	R0I 1 26.94 ± 0.18	v v	R012 2.973 ± 0.049	Exp	ort t	o Clipboard ROI 3 6.86 #	Expo 0.11	it as	A50	CIII
Clear Initial Close Rarameter A (r) [RCnts] L A (r) [RCnts] L	Fat inits	Fit €	F#All X* =	> > >	6.4	el (1 0.01	,1) 88 ± 0.00 01 ± 0.00	55	> > >	ROI 0 82.69 ± 0.26 11.839 ± 0.042	- -		RDI 1 26.94 ± 0.18 11.337 ± 0.048	2 2 2	RDI 2 2.973 ± 0.049 2.310 ± 0.029	Exp	ort t	0 Clipboard ROI3 6.86 # 3.448 #	Expo 0.11 0.019	rt as	A50	CI
Clear Initial Close Parameter Arg (kCnts) L Apg (kCnts) L	Fat imits imits imits	Fit ● Ov	F#All X* =	* * * *	6.4	180 el (1 -0.0	,1) 88 ± 0.00 01 ± 0.00 71 ± 0.00	55 2 041	x x x x	R010 82.69 ± 0.25 11.839 ± 0.042 0.3309 ± 0.0056	4 4 4	x x x x	RDI 1 26.94 ± 0.18 11.337 ± 0.048 0.783 ± 0.011	2 2 2 2	RDI 2 2.973 ± 0.049 2.310 ± 0.029 1.0817 ± 0.0098	Exp	ort to	Clipboard ROI3 8.88 ± 3.448 ± 0.3916 ±	Expo 0.11 0.019 0.0059	it as	A50 > > > > >	CI
Clear Initial Close Parameter A _[1] (kCnts) L A _[2] (kCnts) L A _[2] (kCnts) L A _[2] (kCnts) L A _[2] (kCnts) L	Ft imits imits imits	Fit Ov	F# All X* = erall Decay 1489 ± 13 1489 ± 13 20.55 ± 0.38 0.4800 ± 0.0027 20	* * * * *	6.4	el (1 0.01 -0.0 0.4	.1) 88 ± 0.00 01 ± 0.00 71 ± 0.00 80 ± 0.00	55 🛊 2 🛊)41 🛊	x x x x x	R010 82.89 ± 0.25 11.839 ± 0.042 0.3309 ± 0.0056 0.480 ± 0.000	- - -	v v v v	RDI 1 26.94 ± 0.18 11.337 ± 0.048 0.783 ± 0.011 0.480 ± 0.000	1 1 1 1 1	ROI 2 2.973 ± 0.049 2.310 ± 0.029 1.0817 ± 0.0098 0.480 ± 0.000	Exp	ort to	Clipboard ROI 3 6.86 ± 3.448 ± 0.3916 ± 0.480 ±	Expo 0.11 0.019 0.0059 0.000	11 as	AS > > > > > > >	CII
Clear Initial Close Parameter A _[1] (kCnts] L A _[2] (kCnts] L A _[2] (kCnts] L A _[2] (kCnts] L T _[2] (kCnts] L T _[2] (na) T	Ft ints ints ints ints	Fit Ov	Ft All X ^x = 1469 ± 13 333.1 ± 3.5 20.55 ± 0.38 0.4800 ± 0.0027 1.443 ± 0.006 \$\$\$	> > > > > >	6.4	el (1 -0.0 0.4 1.4	(1) 88 ± 0.00 01 ± 0.00 71 ± 0.00 80 ± 0.00 46 ± 0.00	55 4 2 4 0 41 4 0 4	* * * * * *	ROI 0 82.89 ± 0.25 11.839 ± 0.042 0.3309 ± 0.0056 0.480 ± 0.000 1.445 ± 0.000		× × × × ×	RD(1 26.94 ± 0.18 11.337 ± 0.048 0.783 ± 0.011 0.480 ± 0.000 1.446 ± 0.000		RDI 2 2.973 ± 0.049 2.310 ± 0.029 1.0817 ± 0.0098 0.480 ± 0.000 1.446 ± 0.000		v v v v v	Clipboard ROI 3 6.86 ± 0.3916 ± 0.480 ± 1.446 ±	Expo 0.11 0.019 0.0059 0.000 0.000	11 as	A < < < < < < < < < < < < < < < < < < <	CI
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Clear Initial Close Parameter Arg (NCnts) L Arg (NCnts) L Arg (NCnts) L Top (na) L Top (na) L Shift ser (na) L Ing (NCnts) L Shift ser (na) L Ing (NCnts) L	Fit mits mits mits mits mits mits mits mi		F£ All X ^x = erall Decay 1489 ± 13 1489 ± 13 1 333.1 ± 3.5 2 20.55 ± 0.38 1 0.4800 ± 0.0027 1 1.443 ± 0.006 2 23.9 ± 1.8 1 0.1772 ± 0.00066 1 219.0 ± 99.5 1 18956 ± 127 1 12746 ± 71 1 33842 ± 69 1 1843 ± 12 1.0606 ± 0.0024		6.4	el (1 0.01 -0.0 0.4 1.4 3.9 -0.0 0.0 0.115 0.2 -0.0 0.0 0.0 0.0 0.115 0.2 -0.0 0.0 1.15 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.	(1) 88 ± 0.00 01 ± 0.00 171 ± 0.00 80 ± 0.00 46 ± 0.00 05 ± 0.00 18 ± 0.07 74 ± 0.08 45 ± 0.07 74 ± 0.04 68 ± 0.08 33 ± 0.00 27 ± 0.22	55 4 2 44 0 44 0 44 0 44 0 44 0 44 0 44 0		R010 82.89 ± 0.26 11.839 ± 0.042 0.3309 ± 0.0056 0.480 ± 0.000 1.445 ± 0.000 9.9 ± 0.2 -0.0030 ± 0.0007 417 ± 115 1052,7 ± 3.4 454.0 ± 1.6 34.56 ± 0.58 1541.3 ± 4.4 94.86 ± 0.29 0.8421 ± 0.0011			R01 1 26.94 ± 0.18 0.783 ± 0.048 0.783 ± 0.011 0.480 ± 0.000 1.446 ± 0.000 3.997 ± 0.027 0.0158 ± 0.0011 194 ± 195 342.9 ± 2.3 434.8 ± 1.9 81.8 ± 1.1 859.5 ± 1.5 3.906 ± 0.051 1.2980 ± 0.0018		RDI 2 2 973 ± 0.049 2 310 ± 0.029 1 .0817 ± 0.0098 0.480 ± 0.000 1.445 ± 0.000 1.445 ± 0.000 3.939 ± 0.000 0.2245 ± 0.029 -1147 ± 1709 37.85 ± 0.62 88.6 ± 1.1 113 ± 1 239.45 ± 0.85 6.355 ± 0.032 2.4698 ± 0.0067			Clipboard RDI3 6.86 ± 3.448 ± 0.3916 ± 0.480 ± 1.446 ± 3.939 ± 1.267 ± 0.0051 ± -249 ± 87.3 ± 132.22 ± 40.91 ± 260.44 ± 10.698 ± 1.5138 ±	Expo 0.11 0.019 0.0039 0.000 0.000 0.000 0.0013 308 1.4 0.72 0.61 0.88 0.096 0.0065			CI

- Exporting from this window exports also all fitting values.
- In an analogous fashion, also FCS-fitting data can be exported.

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