

SymPhoTime Lifetime Fitting

Summary

This tutorial shows step-by-step, how to fit the lifetime of a measured sample. As an example, a single exponential reconvolution fit is used to determine the lifetime of ATTO655 diluted in water.

Step-by-Step Tutorial

Select a file and start the script

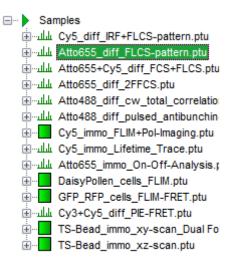
- Start SymPhoTime 64 software.
- Open the "Samples" workspace via "File\open Workspace" from the main menu.

Note: The "Samples" workspace is delivered with the SymPhoTime 64 and on the CD-ROM and contains example data to show the function of the SymPhoTime data analysis. If you haven't installed it on your computer, copy it from the DVD onto a local drive before going through this tutorial.

Response: The files of the sample workspace are displayed in the workspace panel on the left side of the main window.

SymPhoTime 64			
File Edit View Settings Scripts Analys			
	Analysis		
E Samples	Imaging		
in Cy5_diff_IRF+FLCS-pattern.ptu فاسلس Atto655_diff_FLCS-pattern.ptu			
int_recs-pattern.ptu int_recs-pattern.ptu	Time Trace		
tto655_diff_2FFCS.ptu	TCSPC		
Atto488_diff_cw_total_correlatio سلاس	_		
itto488_diff_cw_antibunching.pt ملاس	FCS		
ie Cy5_immo_FLIM+PoI-Imaging.ptu ناسلس Cy5_immo_Lifetime_Trace.ptu	 Grouped Analysis 		
iiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiii			
DaisyPollen_cells_FLIM.ptu	 Alignment 		
GFP_RFP_cells_FLIM-FRET.ptu	 User Defined Script 		
in Cy3+Cy5_diff_PIE-FRET.ptu			
TS-Bead_immo_xy-scan_Dual Fo			

• Highlight the file ATT0655_diff_FLCS-pattern.ptu by a single mouse click.



• Select the "Analysis" tab and in there, open the drop down menu "TCSPC".

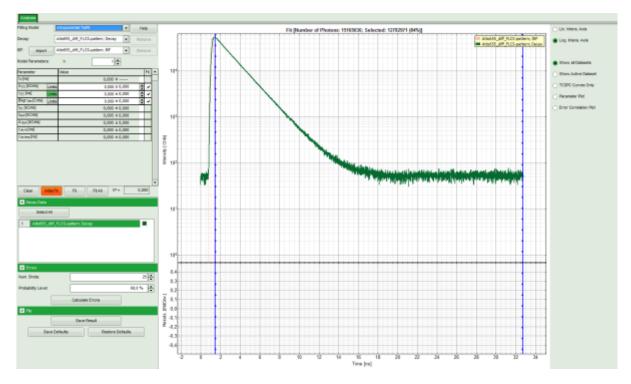
· · ·	Analysis		
E Samples Samples الله Cy5_diff_IRF+FLCS-pattern.ptu الله Atto655_diff_FLCS-pattern.ptu الله Atto655+Cy5_diff_FCS+FLCS.ptu الله Atto655_diff_2FFCS.ptu الله Atto655_diff_Cw_total_correlatio	TCSPC		
	TC SPC Decay Calculates a TCSPC decay and an artificial IRF from a TTTR file. Select a TTTR file and press 'Start'. Help Start FCS	TCSPC Fitting Least squares fitting of TCSPC decay curves. Select one or several TTTR files and press 'Start'. Help Start	
⊞∎ TS-Bead_immo_xy-scan_Dual Fo ∎ TS-Bead_immo_xz-scan.ptu	 Grouped Analysis Alignment User Defined Scripts 		

Note: The drop down menu can be opened and closed by clicking on the grey button on the left side of the header of the drop down menu:

• Start the "TCSPC Fitting" script by clicking on "Start".

TCSPC Fitting				
Least squares fitting of TCSPC decay curves. Select one or several TTTR files and press 'Start'.				
Help Start				

Response: The TCSPC Fitting script is applied to the file ATT0655_diff_FLCS-pattern.ptu. Thereby, a new Window opens:

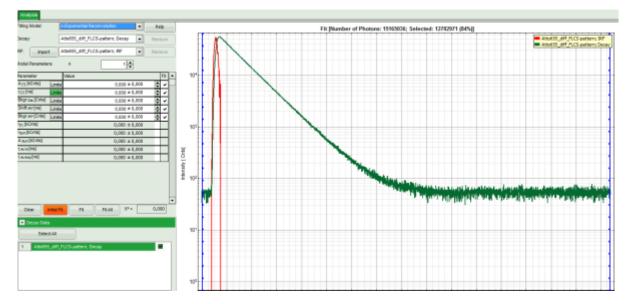


Note: The window contains two different regions:

- 1. Left: Fitting and analysis options.
- 2. Center/right: TCPSC fitting graph showing the TCSPC histogram of the measured sample.
- In the decay form on the left, select n-exponential reconvolution as fitting model.

Response:

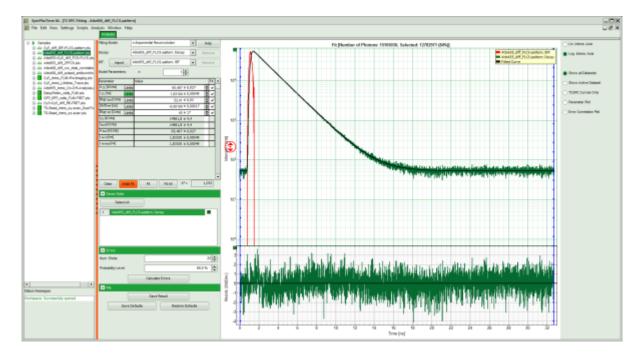
- In the TCSPC window, the IRF is displayed in bright red and the data fitting limits are moved to the border of the TCSPC window.
- The new fitting parameters "Shift IRF" and "Bkgr IRF" (=background IRF) appear in the fitting parameter table.



Note: The software offers the possibility to fit the data using a n-exponential tailfit or a n-exponential reconvolution fit. A tailfit can be used when the fitted lifetimes are significantly longer than the instrument response function. Still a reconvolution fit is usually preferable, because the complete decay is fitted, while for a tailfit, the start of the fitting range is usually a bit arbitrary.

For explanation about the fitting model and the used equations, click on the "Help" button next to the selected model. This opens a help window containing the fitting equation and the explanation of the different parameters.

• Click: "Initial Fit" (marked in orange).



Response:

- In the TCSPC window, the fit is displayed as a black line. Below, the residuals (= raw data fit values) are displayed.
- Fit values appear in the fitting table.

Note: Usually, a decent fit is characterized by the following criteria:

The fitted curve overlays well with the decay curve. In the residual window, the values spread randomly around 0.

The χ^2 -value approaches 1.

The calculated fitting values are reasonable.

Usually the fitting model with least parameters is selected.

 \Rightarrow In this example, the fit is already sufficient.

• Store the result file by selecting: "Save Results".

File				
		Save Resu	t -	
	Save Defaults		Restore Defaults	

Response: A result file (TCSPC_Fitting.pqres) is stored under the raw data file (ATT0655_diff_FLCS-pattern.ptu).

--- Samples Ey5_diff_IRF+FLCS-pattern.ptu لللد Atto655 diff FLCS-pattern.ptu الملد Atto655_diff_FLCS-pattern.pt FCS.pqres TCSPC_Fitting.pqres Atto655+Cy5_diff_FCS+FLCS.ptu it Atto655_diff_2FFCS.ptu الملد⊷ Atto488_diff_cw_total_correlatio اللله Atto488 diff pulsed antibunchin لللد Cy5_immo_Lifetime_Trace.ptu الملد Atto655_immo_On-Off-Analysis. DaisyPollen_cells_FLIM.ptu GFP_RFP_cells_FLIM-FRET.ptu ⊡سلس Cy3+Cy5_diff_PIE-FRET.ptu TS-Bead_immo_xy-scan_Dual Fo TS-Bead_immo_xz-scan.ptu

- Later, clicking on the result file reopens the file in the same way as it was stored.
- Now all necessary steps are done. There are several possibilities how to continue:

Export the Fitting Values

- To transfer the fitting data into another program, place your mouse cursor over the fitting table into a grey region, use a right mouse click to open the context menu and select "Copy" or simple use "<CRTL>" + "C" to copy the fitting table into the clipboard.
- Open Notepad or Excel or any other program you want to copy the data into, and past the data there
 using "<CRTL>" + "V".

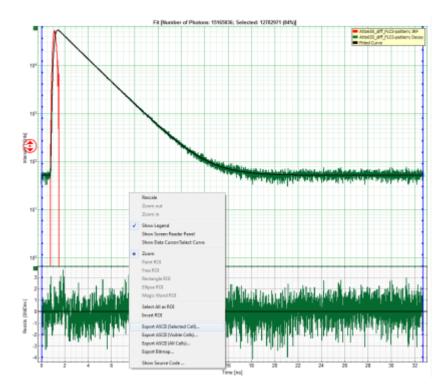
Fitting Model:	1	-Exponential Reconvolution		lp	
Decay:		Atto655_diff_FLCS-pattern; Decay	Remove		
RF: Impo	rt /	tto655_diff_FLCS-pattern; RF		Remove	
Model Paramete	ira:	n 1 🗮			
Parameter		Value		Fit	
A [i] [kCnts]	Lints	65,467 ± 0,027		~	
t [i] [ns]	Linits	1,83100 ± 0,00049		~	
Bkgr Det [Crits]	Linits	52,41 ± 0,52	÷	~	
Shift er [ns]	Linits	-0,00100 ± 0,00017	1	~	
Bkgr 🔫 (Cnts)	Linits	45 ± 17	÷	~	
Ipj [kCnts]		14981,8 ± 4,4			
Isun [kCnts]		14981,8 ± 4,4			
A _{Bum} [kCnts]		Copy Ctrl+C 5,467 ± 0,027			
tavm[ns]		3100 ± 0,00049)		
TAVANU [15]		1,83100 ± 0,00049)		

↓

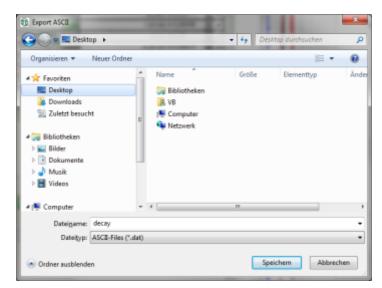


Export the Complete Decay

• If the complete decay should be exported to plot the graph in another program, place the cursor over the decay graph, use the right mouse click to open the context menu and select one of the ASCII export options, in this case "selected cell".



Response: A window opens and asks for a file name to store the exported result file. Select e.g. the name decay.dat.



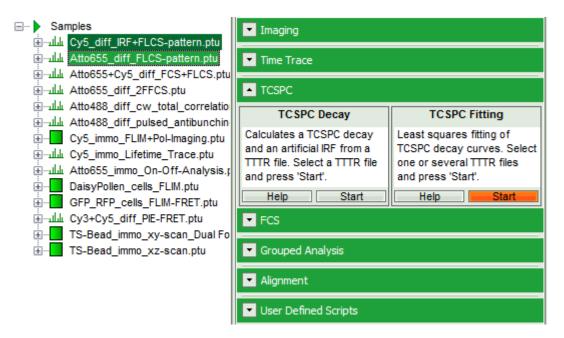
Note: The .dat file contains the TCSPC curve, the estimated instrument response function (IRF) and the fitted

curve.

decay - Editor	1.00	International Property lies of the local division of the local div	
Datei Bearbeiten Format Ansicht ?			
Atto655_diff_FLCS-pattern; IRF time(ns] Intensity[cnts] 0,78399996867293 0 0,79199996867293 0 0,7919999683326 129 0,80799999673042 237 0,807999996730427 347 0,815999966739427 347 0,831999996673944 651 0,83599999661328 1572 0,84599999661326 2210 0,85599999653562 2255 0,8799999643266 5284 0,88799999643173 5906 0,88799999643173 5906 0,88799999643173 5906	Atto655_diff_FLCS_patte Time[rs] Intensity[Cnt3] 0,00799999998080336 52 0,013999999808072 58 0,023999999808721344 60 0,03199099987412468 42 0,05399999980802016 45 0,0539999998742688 56 0,071999999742688 56 0,071999999742688 55 0,079999996164032 48 0,039999996164032 48 0,03999999552447 53	rn: Decay Fitted Curve Time[ns] Intensity[Cnts] 0,0079999999680336 0,01599999998060336 0,02399999998071344 51,8027040100098 0,03199999980521344 51,8027040100098 0,0479999998062016 51,8027040100098 0,0479999998062016 51,8027040100098 0,053999999742685 51,8027040100098 0,079999999742685 51,8027040100098 0,0799999996164032 51,8027040100098 0,055999996164032 51,8027040100098 0,0559999996164032 51,8027040100098 0,055999995614032 51,8027040100098 0,01399999955247 51,8027040100098	
<i>i</i>			*

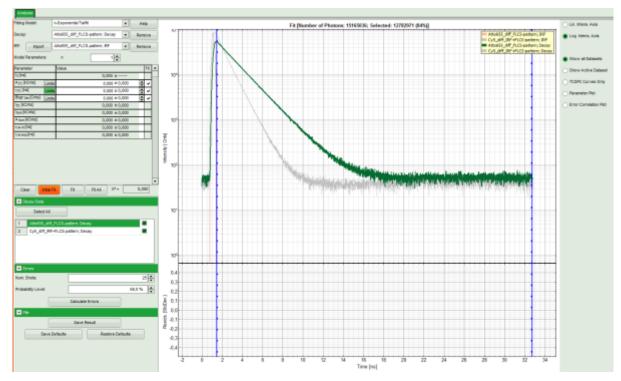
Process Several Files and Calculate Averages

 If several raw data files are marked in the workspace and the TCSPC fitting script is then applied, the decays of all loaded files are displayed in the script. For illustration, mark the two files Cy5_diff_IRF+FLCS-pattern.ptu and ATT0655_diff_FLCS-pattern.ptu with one mouse click each and start the TCSPC fitting script.

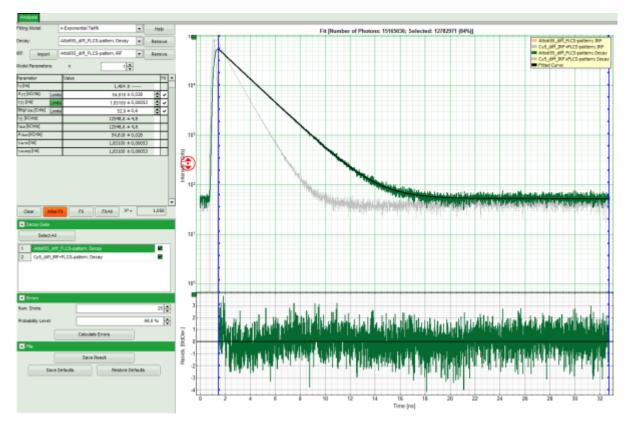


Response: The TCSPC fitting window is opened and the TCSPC histograms of both files are loaded. The TCSPC histogram from the file "ATTO655_diff_FLCS-pattern.ptu is marked in green, indicating that it is the active file. Under decay data all files are listed, the active file is always highlighted in green.

Note: The file Cy5_diff_IRF+FLCS-pattern.ptu contains a lifetime measurement of the dye Cy5 in water. In the TCSPC histogram it can be clearly seen that the lifetime of this dye is significantly shorter than the lifetime of ATTO655.



• Click on "Initial Fit" to perform a monoexponential tailfit of the ATTO655 dataset.



• If both dyes can be fitted with the same model, click "Fit All".

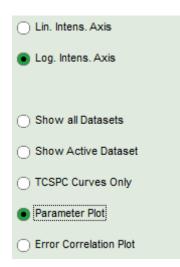
Response: Both data sets are fitted with a single exponential tailfit model. The values of the last dataset are displayed in the fitting table.

Analysis	
Fitting Model:	n-Exponential Tailft Help
Decay:	Cy5_diff_IRF+FLCS-pattern; Decay Remove Remove
RF: Import	Cy5_diff_RF+FLCS-pattern; IRF Remove
Model Parameters:	n 1 📥
Parameter	Value Fit .
to[ns]	1,464 ±
A [1] [kCnts] Limi	s 72,10 ± 0,16 🚔 🗸
τ[1] [08] Limi	8 0,947 ± 0,001 🔮 ✔
Bkgr pec [Cnts] [Limi	
I[1] [kCnts]	8537 ± 12
I sum [kCnts]	8537 ± 12
A sum [kCnts]	72,10 ± 0,16
t Av int [NS]	0,947 ± 0,001
t Av Amp [ns]	0,947 ± 0,001
	-
Clear	Fit Fit FitAl X ² = 3,714

• To toggle between the fitted values of the different data sets and check the fits, click onto the different datasets to activate them.

Decay Data				
	Select All			
1	Atto655_diff_FLCS-pattern; Decay			
2	Cy5_diff_IRF+FLCS-pattern; Decay			

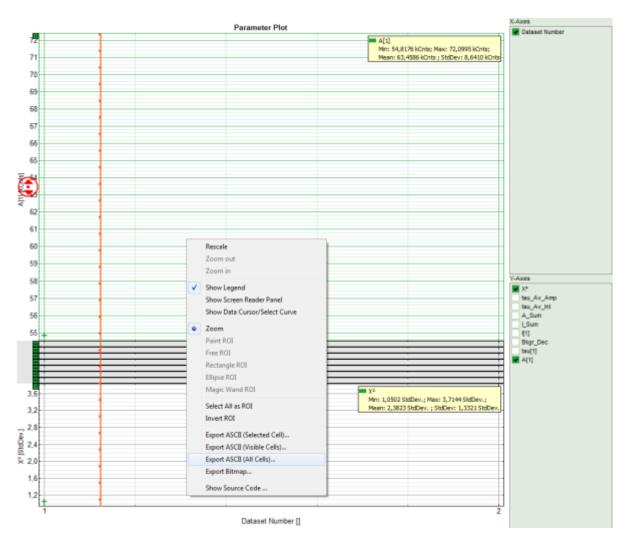
• To export all fitting values for all fits, activate the "Paramater Plot".



Response: The parameter plot is shown. The parameters to be displayed are plotted as points in a graph, with the first point on the left belonging to the first data set, etc.

Note: The parameter plot is in this case not very illuminating, as only 2 datasets are present. It's full potential can be generated, if the same dye is measured several times, because in this case it graphically shows the deviation of the fitting values. It also calculates an average for each fitting value over the data sets. In our example, it is of course meaningless, as two different dyes were fitted.

• Place the mouse over the graph, activate the context menu with a right mouse click and select the "Export ASCII (All cells)" option. This allows to store the fitting values of all fitted data sets at once e.g. to load them into Excel or a similar program for further processing.



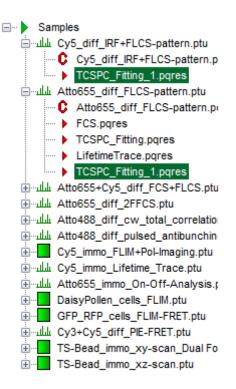
Response: A window opens and asks for a file name and a folder to store the data, e.g. as fitting values.dat.

	CANCE AND	×
🕞 🕞 – 📃 Deskto	cop 🕨 🔫 😽 Desktop durchsuchen	٩
Organisieren 🔻	Neuer Ordner	0
 ★ Favoriten ■ Desktop ▶ Downloads ™ Zuletzt besuch ■ Bibliotheken ■ Bilder ■ Dokumente ▶ Musik ■ Videos 	ht Rome Größe Elementtyp	Änder
🖳 Computer	▼ (- F
Datei <u>n</u> ame:	fitting values	•
Datei <u>t</u> yp:	ASCII-Files (*.dat)	-
Ordner ausblende	en Speichern Abbrech	ien

• Store the file. You can open the data e.g. with the notepad function of the PC to have a look at the data structure. Every data set is stored as a line and the fitting values are arranged as columns. The order of the data sets corresponds to the order of the data sets in the SymPhoTime table.

📑 fittin	g values - Editor			-	and the second s		
Datei	Bearbeiten Format An	sicht ?					
A[1] Datase 1 2	tau[1] et Number[] 54,81758984375 72,099546875	Bkgr_Dec A[1][kcnts] Data 1 1,8313229821 2 0,9472629347	I[1] set Number[] 4872 1 58441 2	z_Sum tau[1][rs] Data 52,9291534423828 41,6631622314453	A_Sum set Number[] 1 12548, 2 8537,1	tau_Av_Int Bkgr_Dec[Cnts] 589053494 5358005857	Tau_AV_Amp _ Dataset Number[] 1 12548,589C 2 8537,15358
×		II.					*

• Don't forget to save the data in SymPhoTime by clicking on "Save results". This generates an analysis result file (.pqres), which in this case is storedalong with both corresponding raw data files (.ptu).



Copyright of this document belongs to PicoQuant GmbH. No parts of it may be reproduced, translated or transferred to third parties without written permission of PicoQuant GmbH. All information given here is reliable to our best knowledge. However, no responsibility is assumed for possible inaccuraciesor omissions. Specifi cations and external appearances are subject to change without notice.



PicoQuant GmbH Rudower Chaussee 29 (IGZ) 12489 Berlin Germany P +49-(0)30-1208820-89 F +49-(0)30-1208820-90 info@picoquant.com www.picoquant.com