

SymPhoTime Lifetime Fitting

Summary

This tutorial shows step-by-step, how to fit a selected model to a decay curve in order to get 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.

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File Edit View Settings Scripts Analys	sis Window Help
	Analysis
E··· ▶ Samples	▼ Imaging
اللس Atto655_diff_FLCS-pattern.ptu	Time Trace
in mini Atto655_diff_2FFCS.ptu	TCSPC
Atto488_diff_cw_antibunching.pt	FCS
ty5_immo_FLIM+PoFImaging.ptu الملد Ey5_immo_Lifetime_Trace.ptu	Grouped Analysis
السلسية: Atto655_immo_On-Off-Analysis. بسية: DaisyPollen_cells_FLIM.ptu	Alignment
GFP_RFP_cells_FLIM-FRET.ptu السطيل Cy3+Cy5_diff_PIE-FRET.ptu	User Defined Scripts
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		
Samples السنيليل Cy5_diff_IRF+FLCS-pattern.ptu السنيليل Atto655_diff_FLCS-pattern.ptu السنيليل Atto655+Cy5_diff_FCS+FLCS.ptu السنيليل Atto655_diff_2FFCS.ptu السنيليل Atto655_diff_2FFCS.ptu			
الله Atto488_diff_pulsed_antibunchin Cy5_immo_FLIM+PoI-Imaging.ptu Cy5_immo_Lifetime_Trace.ptu Atto655_immo_On-Off-Analysis.; DaisyPollen_cells_FLIM.ptu GFP_RFP_cells_FLIM-FRET.ptu Cy3+Cy5_diff_PIE-FRET.ptu	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 use a n-exponential tailfit or a n-exponential reconvolution model fit. A tailfit can be used when the expected lifetimes are significantly longer than the FWHM of instrument response function. Still a reconvolution fit is usually preferable, because the complete decay, including its rising edge is analyzed, 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 fitted model I 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 using the smallest amount of adjustable parameters is selected. \Rightarrow In this example, the fit is already sufficient.

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

File		
	Save Result	
Save Default	ts Restore Defaults	

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

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- 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".

Analysis					
Fitting Model: n		-Exponential Reconvolution		Help	
Decay:	[Atto655_diff_FLCS-pattern; Decay	Ren	ove	
IRF: Impor	t	Atto655_diff_FLCS-pattern; RF	Remove		
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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.

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time[rs] Intensity[Cnts] 0.7.83999996867293 0 0.7.913999968633320 129 0.7.91399996833320 129 0.7.91399996833320 237 0.7.91399996833320 129 0.7.91399996833320 237 0.7.91399996833320 237 0.807390906731364 632 0.813999996673444 651 0.81399999667328 1572 0.81399999667328 1572 0.813999996653265 2210 0.853909096537652 2244 0.8539090965318662 3751 0.873909096513662 3751 0.87999999445137 5906 0.879999994419763 6990	Atto655_diff_ELCS-pa Time[rs] Intensity[Cn 0 34 0 0,007999999680136 52 0,0159999996801365 0,0339999996041008 48 0,031999999840168 42 0,0479999998840168 42 0,0479999998740232 52 0,053999997424588 56 0,0719999998742688 56 0,0719999996742345 53 0,0719999996483656 49 0,0859999996144032 48 0,10395999996184437 45 0,11199999955247 53	<pre>ttern: Decay ts] Time[ns] Intens: 0,0079999999680336 0,01999999960672 0,021999999960672 0,021999999960672 0,0319999998721344 0,039999998742688 0,0479999998082016 0,051999997442688 0,07199999987442688 0,07999999680336 0,079999999680336 0,079999999680336 0,079999999684437 0,013999999584477 0,11199999995247</pre>	Fitted curve [ty[Cnts] 51.8027040100098 51.8027040100098 51.8027040100098 51.8027040100098 51.8027040100098 51.8027040100098 51.8027040100098 51.8027040100098 51.8027040100098 51.8027040100098 51.8027040100098	
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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 Tailfit	
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Model Parameters:	n 1 🔺	
Parameter	Value Fit	*
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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	
τ Av Amp [ns]	0,947 ± 0,001	
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Clear	Fil Fil Filai 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.

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• 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.

fitting values - Editor		-	- Annual Contraction of the International Contractional	
Datei Bearbeiten Format Ansich	ht <u>?</u>			
A[1] tau[1] Dataset Number[] A 1 54,81758984375 1 2 72,099546875 2	8kor_Dec 1[1] [1][kcnts] Dataset Number[] 1,83132298214872 1 0,947262934758441 2	T_SUB tau[1][n3] Dataset 52,9291534423828 41,6631622314453	A_Sum Tau_AV_Int Number[] Bkgr_Dec[Cnt 1 12548,5890634994 2 8537,15358005857	Tau_Av_Amp 5] Dataset Number[] 1 12548,589C 2 8537,15358

• 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).



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