

New SPCM Version 9.78 Comes With New Software Functions

Abstract: This application note describes new functions implemented in Version 9.78 of the bh SPCM software [1]. Version 9.78 especially addresses the needs of bh's Fast-Acquisition FLIM system [3]. FLIM data from up to four TCSPC modules can be combined into a single FLIM data set, and displayed as gated intensity images or as lifetime images. Moreover, decay curves can be selected from ROIs or POIs within the images, and displayed online in a separate decay curve window.

Combination of Data from Several SPC Modules

Many bh TCSPC and FLIM systems use several parallel TCSPC modules to record data in several wavelength channels, different polarisation channels, or from different source positions at an object of large spatial extension [1]. Multi-module systems are also used to increase the throughput rate of a TCSPC system. In this case, a single optical signal is either spread on several detectors the signals of which are recorded in parallel TCSPC channels [1, 5], or the photon pulses of a single detector are distributed into four parallel TCSPC FLIM channels [3, 4, 5]. SPCM Version 9.78 has a function to combine the data of these channels and to display intensity or lifetime images of the combined data. To activate the channel-combination function select the 'FIFO Imaging Mode' and open the '3D Trace Parameters'. Under 'Module' select M1-4. Leave the other definitions as they are. Data Type is 'PS', Display Mode is 'F(x,y)', see Fig. 1.

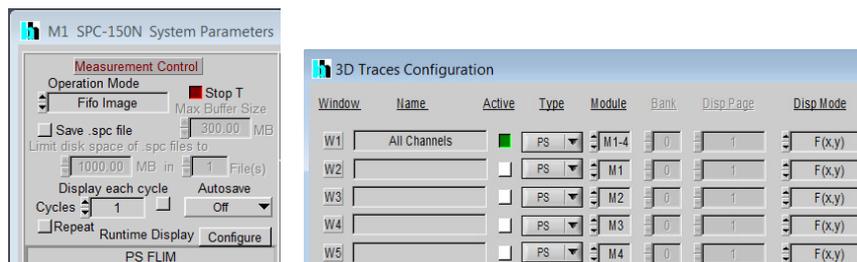


Fig. 1: Combination of TCSPC channels. Left: Measurement control section of the SPCM System Parameters. Right: 3D Display Parameters

Selecting a channel combination does not discard the data of the individual channels - it acts only on the display of the data and on how SPCM interprets the data. A 'Save' operation will save the original channel data, independently of what is defined in the 3D trace parameters.

Intensity Images from Combined Channels

SPCM is able to display (time-gated) intensity images or colour-coded lifetime images [1]. The result of the settings shown above is a main panel as shown in Fig. 2, with an image showing a (time-gated) intensity image of the combined data of SPC modules 1 to 4. Several such images can be displayed for different time gates, please see [1], [6] or [7] for details. In addition to the combined data, it is possible to display also the data of the individual channels. To do so, activate the 'active' buttons for the individual TCSPC modules, M1, M2, M3, M4, in the 3D Trace definitions. Please see Fig. 5 and Fig. 6.

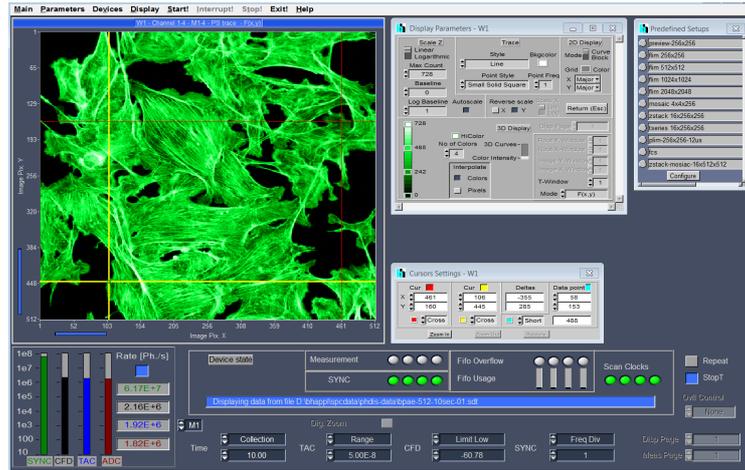


Fig. 2: Main panel, display of the combined data of four TCSPC FLIM Channels

Lifetime Images from Combined Channels

The channel-combination function works also in combination with online lifetime-image display [1, 2]. The trace parameters are shown in Fig. 3. Data Type is 'LIFET', the data of TCSPC Modules M1-4 are combined into one image.

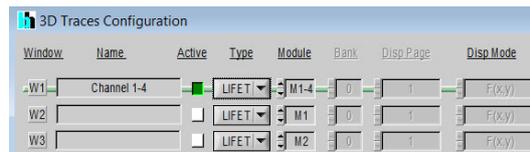


Fig. 3: 3D Trace Parameters for lifetime image display of combined channels

The corresponding main panel is shown in Fig. 4. The Display Parameter panel is open on the right. In its lower part it contains the definitions for the lifetime range, the reference lifetime, binning, and brightness and contrast settings, please see [1] for details.

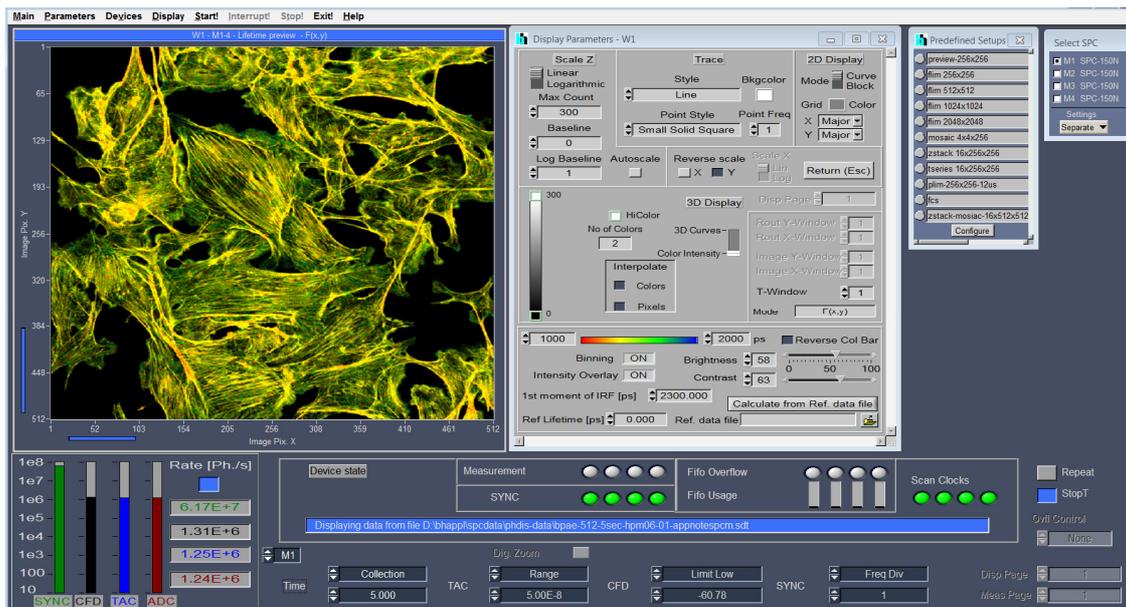


Fig. 4: SPCM Main panel with lifetime display of combined channels

SPCM is able to display up to eight images of different data type and/or from different TCSPC modules simultaneously. With the trace parameters shown in Fig. 5, a lifetime image of four combined channels is displayed together with the lifetime images of the individual channels. An SPCM main panel for this configuration is shown in Fig. 6.

Window	Name	Active	Type	Module	Bank	Disp Page	Disp Mode
W1	Channel 1-4	<input checked="" type="checkbox"/>	LIFET	M1-4	0	1	F(X,Y)
W2	Channel 1	<input checked="" type="checkbox"/>	LIFET	M1	0	1	F(X,Y)
W3	Channel 2	<input checked="" type="checkbox"/>	LIFET	M2	0	1	F(X,Y)
W4	Channel 3	<input checked="" type="checkbox"/>	LIFET	M3	0	1	F(X,Y)
W5	Channel 4	<input checked="" type="checkbox"/>	LIFET	M4	0	1	F(X,Y)

Fig. 5: 3D trace parameters for display of combined channels together with images of individual modules

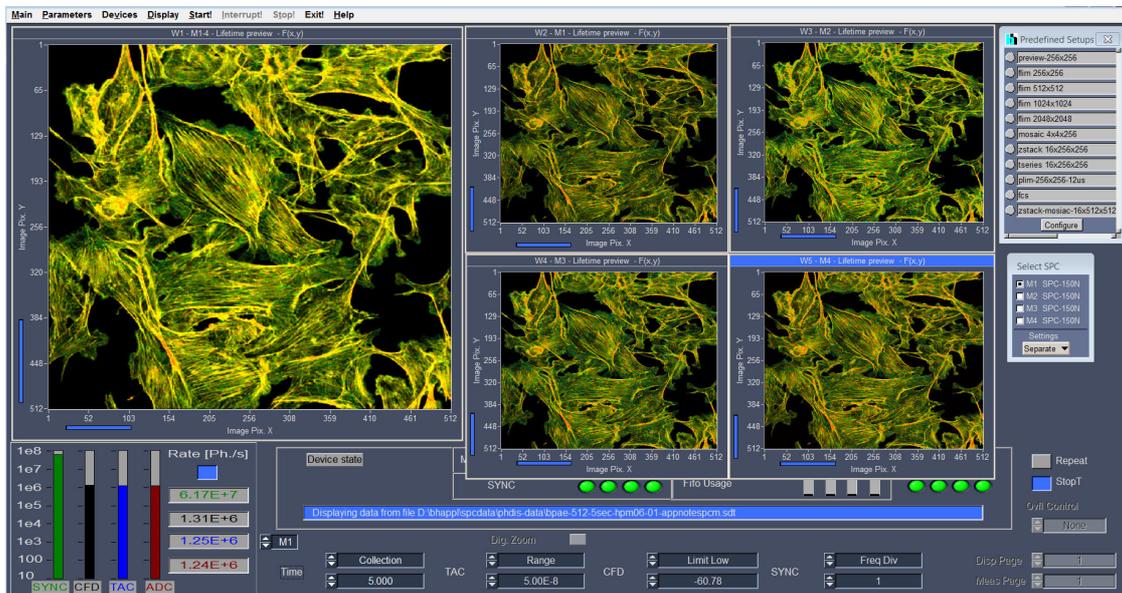


Fig. 6: Main panel with combined images of Channel 1-4 and images of individual channels

Display of Decay Data in Point or Region of Interest

A right-mouse click into one of the image windows of SPCM opens a small panel from where you can access the image cursors, the display parameters, trace parameters, window parameters, etc. A click into ‘Show Decays’ opens a decay-curve window as shown in Fig. 7, right.

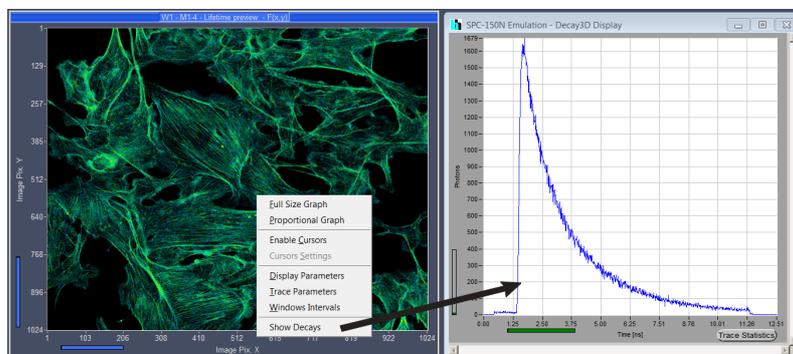


Fig. 7: A right mouse click into an image and a click on ‘Show Decays’ opens the decay curve panel shown on the right

The SPCM main panel with the decay curve window is shown in Fig. 8. The curves can be displayed for all active image windows, and for individual regions or points of interest (ROIs or POIs). The ROIs are defined by the image cursors, the POI by the ‘Data Point’. A right mouse click into the decay window opens a panel with the Trace Parameters for the individual curves. The panel can be seen in Fig. 8, lower right. You can add or remove curves, activate or de-activate curves, define colours for the curves, define whether a curve is from an ROI or a POI, and from which image display window the curve comes. The coordinates of the POI and of the ROI cursors are displayed on the right. As an example, Fig. 8 displays a lifetime images of the combined channels of a four-channel-fast acquisition system (large image), the lifetime images of the individual channels (small images), and the decay data in an ROI of the combined channels and the individual channels.

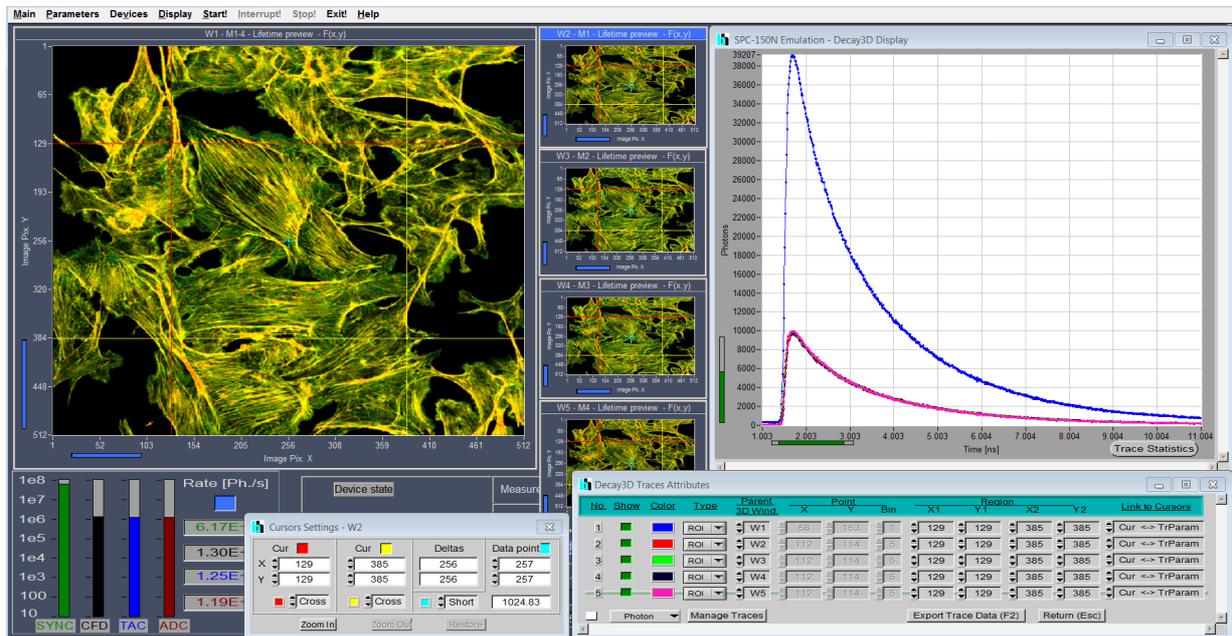


Fig. 8: SPCM Main Panel with lifetime images and decay curve window

The decay-curve display works for all images that contain decay data in their pixels. These can be intensity images, time-gated intensity images, lifetime images, combined images from several TCSPC modules, or images from different routing channels of a TCSPC module.

The display of the decay curves itself is controlled by the ‘Display Parameters’ the same way as for decay curves recorded in the ‘Single’, ‘Oscilloscope’, or ‘FIFO’ mode. The display scale can be linear or logarithmic, an autoscale function is available, and the curves can be displayed as individual data points, lines, or data points connected by lines. Please see software description in the bh TCSPC Handbook [1] or in the handbooks of the DCS-120 FLIM system [6] or of the FLIM systems for the Zeiss LSM 710/780/880 family microscopes [7].

Other New SPCM Features

There are other new SPCM features which have already been added in previous software versions. For example, SPCM is able to control Ti:Sa lasers and the bh AOM module [1]. Ti:Sa and AOM control is used in the DCS-120 MP multiphoton FLIM microscopes, please see [6]. The features can, however, also be used for user-built two-photon microscopes and for other Ti:Sa applications. Control of a motorised sample stage has been implemented in SPCM version 9.76. In combination



with the bh DCS-120 confocal and multiphoton scanning FLIM systems, the stage is used to record mosaics of FLIM images and to control the microscope remotely. Please see [1] and [6].

References

1. W. Becker, The bh TCSPC handbook. Becker & Hickl GmbH, 7th ed. (2017). Available on www.becker-hickl.com
2. Becker & Hickl GmbH, SPCM Software Runs Online-FLIM at 10 Images per Second. Application note, available on www.becker-hickl.com
3. Becker & Hickl GmbH, Fast-Acquisition TCSPC FLIM System with sub-25 ps IRF Width. Application note, available on www.becker-hickl.com
4. Becker & Hickl GmbH, Fast-Acquisition Multiphoton FLIM with the Zeiss LSM 880 NLO. Application note, available on www.becker-hickl.com
5. Becker & Hickl GmbH, Fast-Acquisition TCSPC FLIM: What are the Options? Application note, available on www.becker-hickl.com
6. Becker & Hickl GmbH, DCS-120 confocal and multiphoton FLIM systems. User Handbook, available on www.becker-hickl.com
7. Becker & Hickl GmbH, FLIM systems for Zeiss LSM 710/780/880 family microscopes. User Handbook, available on www.becker-hickl.com