



## Make Life Easier by Using Modern Features of the SPCM Software

*Abstract.* Over a long period of time the SPCM operating software of the Becker & Hickl TCSPC systems has continuously been upgraded with new measurement control functions and user interface features. Despite of a tremendous growth in functionality, the general user interface and the data and setup file structure has been kept compatible. The fact that compatibility has been maintained over many software versions has led to the situation that many users do not know and do not exploit the full range of functions of their TCSPC systems. This application note describes how modern features can help users work with their TCSPC or TCSPC-FLIM systems more efficiently.

### Development of the SPCM Software

The first version of SPCM operating software has been released with the introduction of the SPC-630 and SPC-730 TCSPC modules in 1996. Since then, the SPCM software has been continuously upgraded to keep pace with new hardware developments, such as continuous-flow recording and imaging, FIFO imaging, or parallel operation of TCSPC channels. Other SPCM upgrades have added functionality to existing operation modes of SPC modules. Typical examples are page stepping functions, on-line FCS calculation, and online FCS fit. Other features have been introduced to make the use of the software more comfortable. There are functions that allow the user to easily change between different instrument configurations, better see the progress of the measurement, or save measurement data automatically. At the same time, Becker & Hickl have kept the general structure of the software and the structure of the data and setup files compatible. As a result, you can still load setup data of a 10-years old experiment, start the acquisition, and reproduce the results of the earlier measurement. The fact that compatibility has been maintained over so many years has led to a paradox situation: Many TCSPC users have upgraded their systems with the latest SPCM software versions, but do not know and, consequently, do not use the full range of available functions, work with old and inefficient user interface configurations, or run their experiments blind, without using any of the online display features of the SPCM software. It also happens that users start into time-consuming DLL programming of recording sequences although the required control functions are available in the SPCM standard software. This application note therefore addresses a number of SPCM features which, if only enabled by the user, make working with TCSPC and FLIM easier and more efficient.

### Configure the SPCM Main Panel to your Requirements

The internal signal processing hardware of the bh TCSPC modules can be changed by a software command. Depending on the SPC module type, up to 12 different operation modes are available. The data of the photons can be used to build up photon distributions in the SPC hardware, or be transferred in the computer photon by photon. Within the individual operating mode, single- and multi-wavelength operation, single or sequential recording, programming of measurement cycles, triggered accumulation, and imaging is possible. The bh TCSPC modules can thus be used for a

wide variety of applications [1, 2]. The SPCM software therefore allows the user to configure the user interface to fit the requirements of different TCSPC experiments.

Four typical instrument configurations are shown in Fig. 1. The upper row, left, shows a configuration for conventional fluorescence lifetime experiment. A combined fluorescence decay / FCS experiment is shown right. The lower row shows typical main panel configurations for fluorescence lifetime imaging applications. A single-wavelength experiment with the bh DCS-120 confocal scanning system [6] is shown left. An online image is displayed on the left of the main panel. The scanner control panel is kept open on the right. A detector control window has been placed at the lower right. A multi-spectral FLIM configuration [7] is shown in the lower row, right. Images in eight wavelength intervals are displayed, and the scanner and detector control panels are kept open at the lower right. For details of opening, resizing and positioning the individual windows, please see [2].

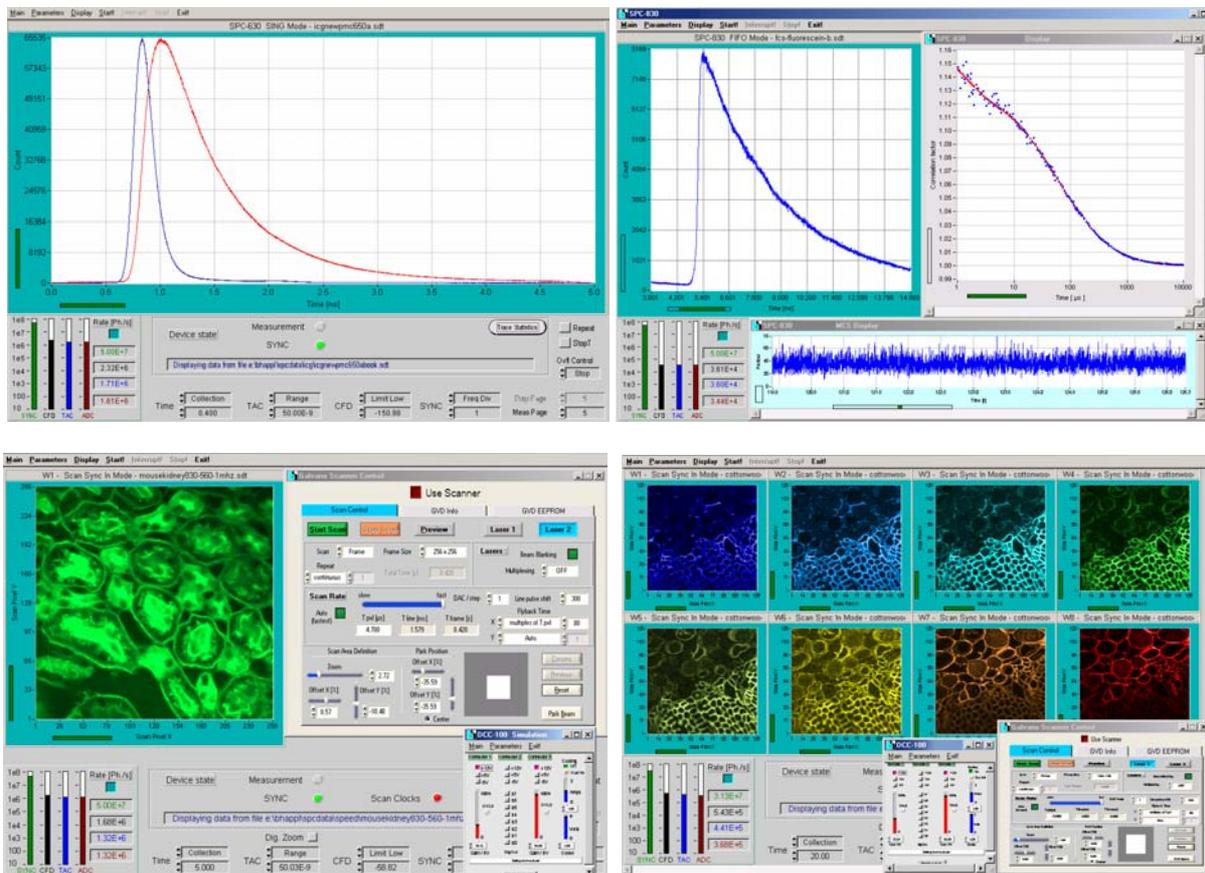


Fig. 1: Main panel configuration for typical TCSPC experiments. Upper row: Conventional fluorescence decay experiment (left), combined fluorescence decay / correlation experiment (right). Lower row: Single-detector FLIM experiment (left), multi-spectral FLIM experiment (right), both with bh DCS-120 scanner.

The instrument configuration is saved in the TCSPC data or TCSPC setup files. Thus, loading a data file not only restores the measurement system parameters but also the appearance of the SPCM user interface. It is thus possible to change the configuration of the TCSPC instrument by only loading a different setup or data file.

## Change Between Different Instrument Configurations on a Mouse Click

To facilitate the change between different instrument configurations you may put frequently used software configurations into a list of 'predefined setups'. By using the panel shown in Fig. 2 you can change between different instrument configurations by a single mouse click. The panel is opened by clicking into 'Main', 'Predefined Setups' (Fig. 2, left).

For editing the list of predefined setups, put the mouse cursor into the panel, and click on the right mouse key. This opens the panel shown in Fig. 2, right. Any setup (.set) or setup/data file (.sdt) can be selected and added to the predefined setup panel. For data files you can select whether only the setup part or the complete setup and data file is to be loaded. Moreover, you can assign names to the setups by using the 'nickname' field. Please see [2] for details.



Fig. 2: Left: Opening the Predefined-Setup panel. Middle: Predefined-Setup panel, opened. You can change between different instrument configurations by a single mouse click. Right: Editing the predefined setup panel. Any setup or data file of the SPCM software can be selected and put into the predefined setup panel.

Using the predefined setup panel is especially recommended to FLIM users [3, 4, 5, 6]. Often FLIM data are acquired with different pixel numbers, or different numbers of time channels. There may also be different scan formats of the microscope, which require different binning in the TCSPC hardware. The ability to quickly change between different setups is especially useful for Leica SP2 FLIM users. The SP2 has no pixel clock, so that different setups are required for different scan rates [4]. Moreover, scanning microscopes may also be used for FCS experiments. By putting an FCS setup in the predefined-setup panel, changing between FLIM and FCS is a matter of a single mouse click.

## Online Display in the Imaging Modes

FLIM experiments on samples of low photostability often have to be performed at low count rates. Consequently, long acquisition times can be required to obtain the desired signal-to-noise ratio. Under these conditions, online display allows the user to watch the buildup of the images, check whether the sample still delivers reasonable signals, or to stop the measurement when the desired signal-to-noise ratio has been obtained. The online display works in a different way in the Scan Sync In mode and in the FIFO Imaging mode.

The Scan Sync In mode builds up the images exclusively in the hardware. Displaying intermediate data during the measurement requires the acquisition to be stopped for data readout. In order to avoid the recording of incomplete frames the readout is only possible when the current frame is completed [2]. This synchronisation with the scan is achieved by defining a large number of measurement 'cycles' and activating 'accumulate' and 'display each cycles' in the system



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parameters of the SPCM software, see Fig. 3, left. The display interval is defined by ‘Collection Time’, see Fig. 3, right.



Fig. 3: System control parameters for on-line display in the Scan Sync In mode

The setting shown runs 100 cycles of the specified ‘collection time’, accumulates the data, and displays the accumulated data after each cycle. In other words, the settings shown in Fig. 3 are equivalent to a single measurement cycle of 500 seconds. Please note that you do not necessarily have to run the measurement over the specified number of cycles. The online display allows you to estimate the quality of the data, and, when you are happy with the signal-to-noise ratio, stop the measurement.

In the FIFO Imaging mode the photon data are transferred into the computer photon by photon, and the images are built up by software [2]. The data stream is buffered by the SPC device memory, which is used as a FIFO (first-in-first-out) buffer. A stop of the acquisition for data readout is therefore not required. The display action is just started in specified intervals controlled by a timer on the SPC module. The display interval is set by ‘Display Time’ in the measurement control section of the SPC system parameters, see Fig. 4. The ‘Cycle’ function of the SPCM software is disabled by setting ‘Cycles’ to 1, see Fig. 4, right.

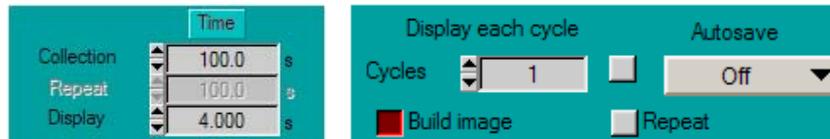


Fig. 4: Online-display time for the FIFO Imaging mode

The display of the data itself is controlled by the ‘Display Parameters’ and ‘Window Parameters’, see [2]. We recommend to activate the ‘Autoscale’ function in the Display Parameters. The corresponding part of the Display Parameters is shown in Fig. 5.

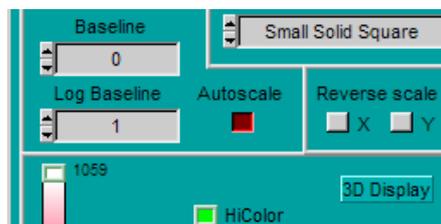


Fig. 5: Activation of the autoscale function (part of the display parameter panel)

## Recording a Series of Measurements: The Page Stepping Function

In many cases the data of a single measurement occupy only a small fraction of the device memory of the SPC module. The memory is then partitioned in a large number of ‘pages’, each of which holds the data of one measurement. The SPCM software contains a number of functions for recording a series of measurements into subsequent memory pages[2, 6].

The simplest way of using the page function is running several related measurements by manually controlling of the ‘page’ function. Each measurement of the series is recorded in a different part of the SPC memory. Eventually, the data of all the individual measurements are saved into one common data file.

An example is shown in Fig. 6. An instrument response function and three decay curves (in this case for different polarisation) were recorded one after another into different pages of the SPC memory. The ‘Trace Parameters’, were defined to display the first four pages of the SPC memory. The destination page for the data of the subsequent measurements is controlled by ‘Meas Page’ in the lower right corner of the main panel, see Fig. 6, right.

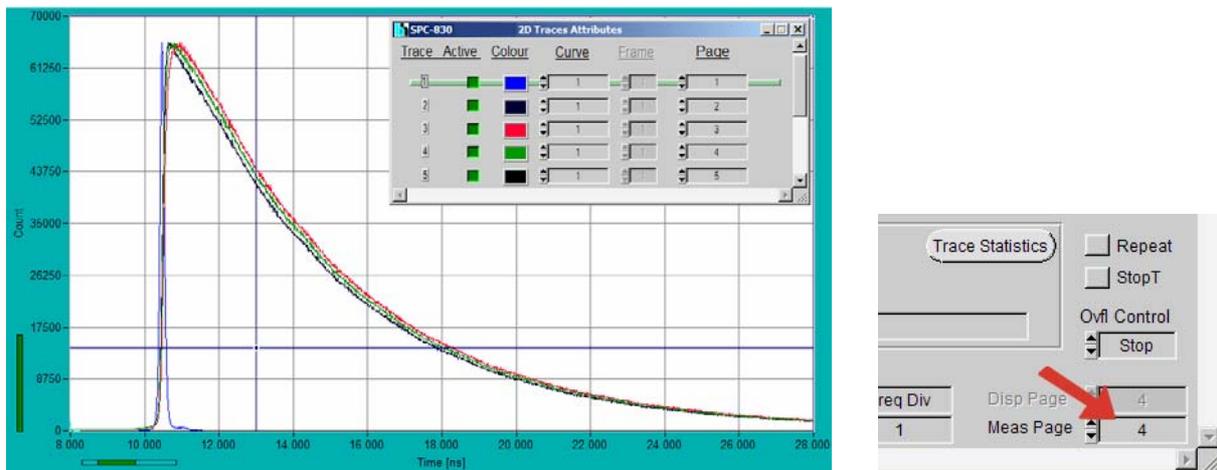


Fig. 6: Recording into different pages of the SPC device memory (left). The memory page is controlled manually (right)

A page stepping sequence can be performed also automatically. An appropriate setup for a time-controlled sequence in the ‘Single’ mode is shown in Fig. 7. A series of 50 subsequent 2-second recordings is taken in steps of 10 seconds.

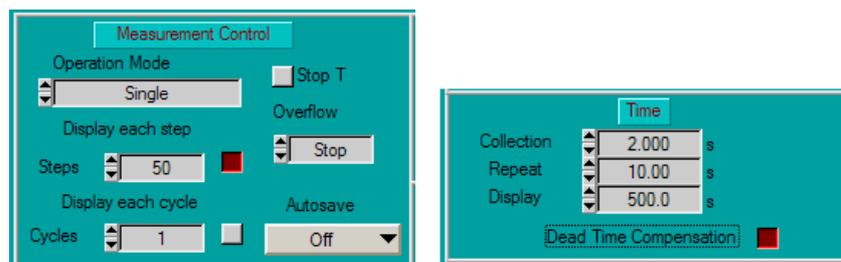


Fig. 7: Automatic page stepping. A series of recordings is taken into 50 subsequent memory pages.

Please note that page stepping can be combined with multi-detector operation. In this case, each step contains a number of curves corresponding to the number of detector channels. Moreover, page stepping can be triggered by an external TTL signal, please see [2] for details.

## Measurement Cycles: The Cycle and Autosave Function

Another way of recording a sequence of measurements is via the ‘Cycle’ function. The measurement is repeated for a number of ‘cycles’. The data of the subsequent cycles are stored into individual data files. The ‘cycle’ function is normally used for measurements that deliver data

blocks too big to be stored in subsequent memory pages. The typical applications are time-series of TCSPC images taken in the Scan Sync In or FIFO imaging mode, or complex measurements that require to repeat a page stepping sequence for a number of cycles. Typical setups for a series of Scan Sync In and FIFO imaging measurements are shown in Fig. 8.

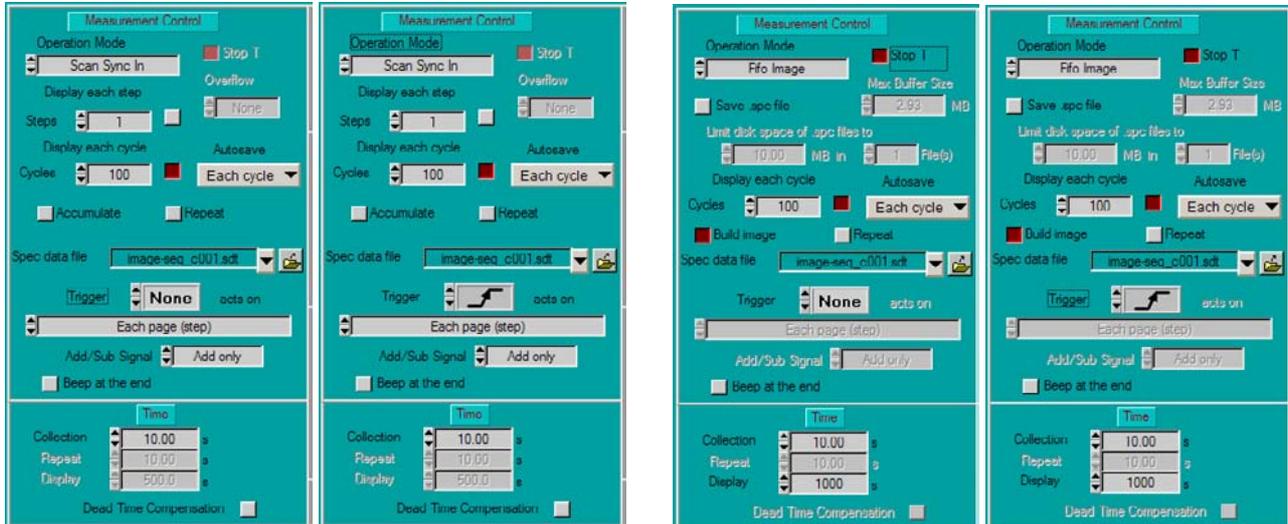


Fig. 8: Defining series of measurements in the imaging modes. Left to right: Scan Sync In without trigger, Scan Sync In with trigger, FIFO imaging without trigger, FIFO imaging with trigger.

The two setups shown left are for the Scan Sync In imaging mode. Both setups define a measurement of 100 cycles. Each cycle acquires for 10 seconds. The images are saved into subsequent data files. In the setup shown second left the experiment trigger function has been enabled. That means the acquisition of each cycle is started by an experiment trigger pulse [2].

Two similar setups for the FIFO Imaging mode are shown on the right. The lifetime images are built up online from the stream of incoming photon data. ‘Save .spc data’ has been disabled, i.e. the single-photon (time-tag) data are discarded.

## Summary

The SPCM operating software of the bh SPC modules has a number of advanced functions that allow the user to adapt their TCSPC instrument to different experiments. By using the predefined-setup function, the user can change between different instrument configurations by a single mouse click. Online display is available in all operation modes. Complex recording tasks can be performed by using the page stepping, cycling, and autosave functions. Users who want to make better use of their SPC modules are encouraged to contact bh for help with their system setup.

## References

1. W. Becker, Advanced time-correlated single-photon counting techniques. Springer, Berlin, Heidelberg, New York, 2005
2. W. Becker, The bh TCSPC handbook. Becker & Hickl GmbH (2005), [www.becker-hickl.com](http://www.becker-hickl.com)
3. Becker & Hickl GmbH, Modular FLIM systems for Zeiss LSM 510 and LSM 710 laser scanning microscopes. User handbook. Available on [www.becker-hickl.com](http://www.becker-hickl.com)



4. Becker & Hickl GmbH and Leica Microsystems, Leica MP-FLIM and D-FLIM Fluorescence Lifetime Microscopy Systems. User handbook. Available on [www.becker-hickl.com](http://www.becker-hickl.com)
5. Becker & Hickl GmbH, Modular FLIM systems for Olympus laser scanning microscope FV1000. User handbook. Available on [www.becker-hickl.com](http://www.becker-hickl.com)
6. Becker & Hickl GmbH, DCS-120 Confocal Scanning FLIM Systems, user handbook. [www.becker-hickl.com](http://www.becker-hickl.com)
7. Becker & Hickl GmbH, PML-16-C 16 channel detector head for time-correlated single photon counting, user handbook. Available on [www.becker-hickl.com](http://www.becker-hickl.com)