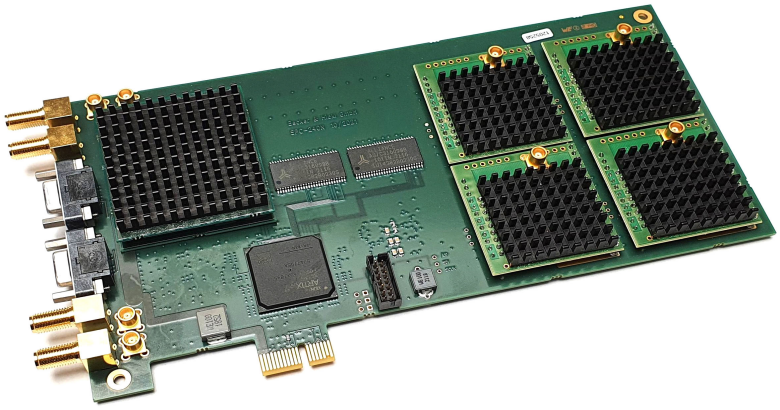
# Becker & Hickl GmbH

## SPC-QC-104

## 3-Channel TCSPC / FLIM Module

## User Manual



**3 Parallel TCSPC / FLIM Channels**
**1 Timing Reference Channel**

**or**

**4 Parallel Absolute-Timing Channels**

**2022**

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August 2022

# Contents

# Overview

The SPC-QC-104 TCSPC / FLIM module has three parallel TCSPC / FLIM channels or four absolute timing channels on a single PCI-express board. The module features high temporal and spatial resolution, high peak count rate, and extraordinarily high timing stability. The electrical IRF width is less than 50 ps FWHM; the timing jitter is about 20 ps RMS. The timing stability over 10 minutes is better than 5 ps RMS, including a bh BDS-SM ps diode laser and a bh HPM-100-06 detector. The peak count rate is on the order of 80 MHz per channel. The module is operated by bh's SPCM data acquisition and control software [1], running under Windows 10 and Windows 11. By using bh's multi-dimensional TCSPC process [1, 2, 3], a multitude of different operation modes is available. There are modes for standard recording of temporal waveforms of optical signals, sequential recording, time- and parameter-tag recording, FLIM, spatial and temporal mosaic FLIM, triggered accumulation of fast time series of curves and images, excitation-wavelength multiplexing, multi-wavelength imaging, and simultaneous FLIM / PLIM [1, 3, 12]. Pixel numbers in the FLIM mode can be as high as 4096 x 4096, pixel rates can be in the MHz range. Data analysis is performed by bh's SPCImage NG software [1, 16]. Examples of TCSPC and FLIM recordings in different operation modes of the SPC-QC-104 are shown in the figures below.

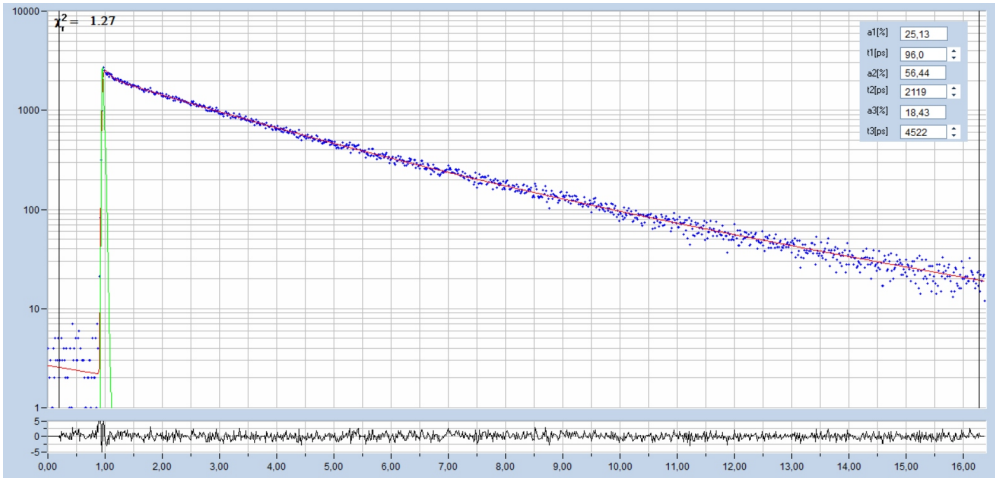



Fig. 1: Fluorescence-decay curve of FAD, 2p excitation at 780 nm, 4096 time channels, 4 ps per channel. Data analysis by SPCImage NG.

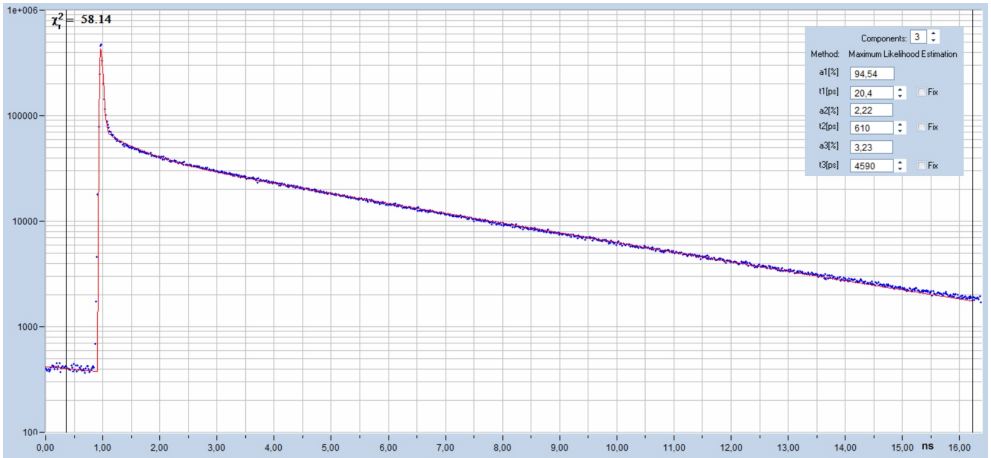



Fig. 2: Fluorescence decay with an ultra-fast component. Analysis by SPCImage NG delivers a decay time of 20.4 ps for the fast component.

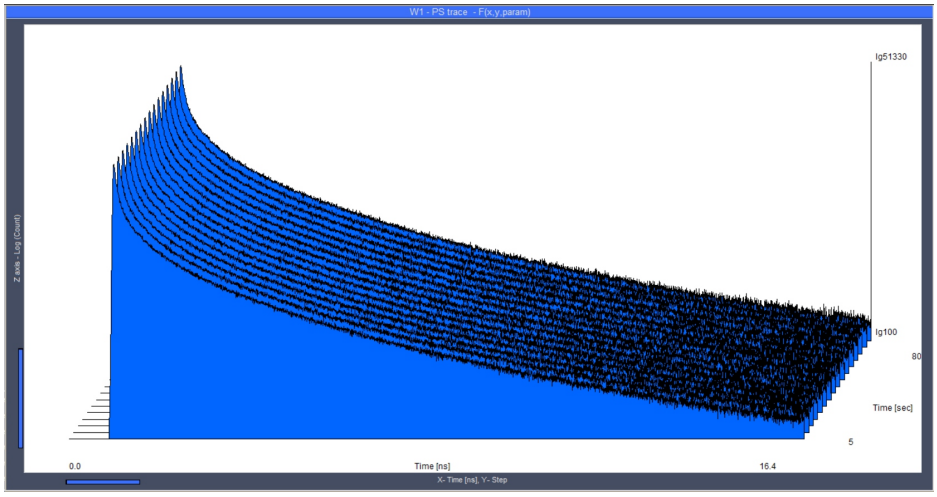



Fig. 3: Sequential recording of decay curves, 16 curves, Acquisition time 5 seconds per curve, 4096 time channels, 4 ps per channel. 3D Display function of SPCM.

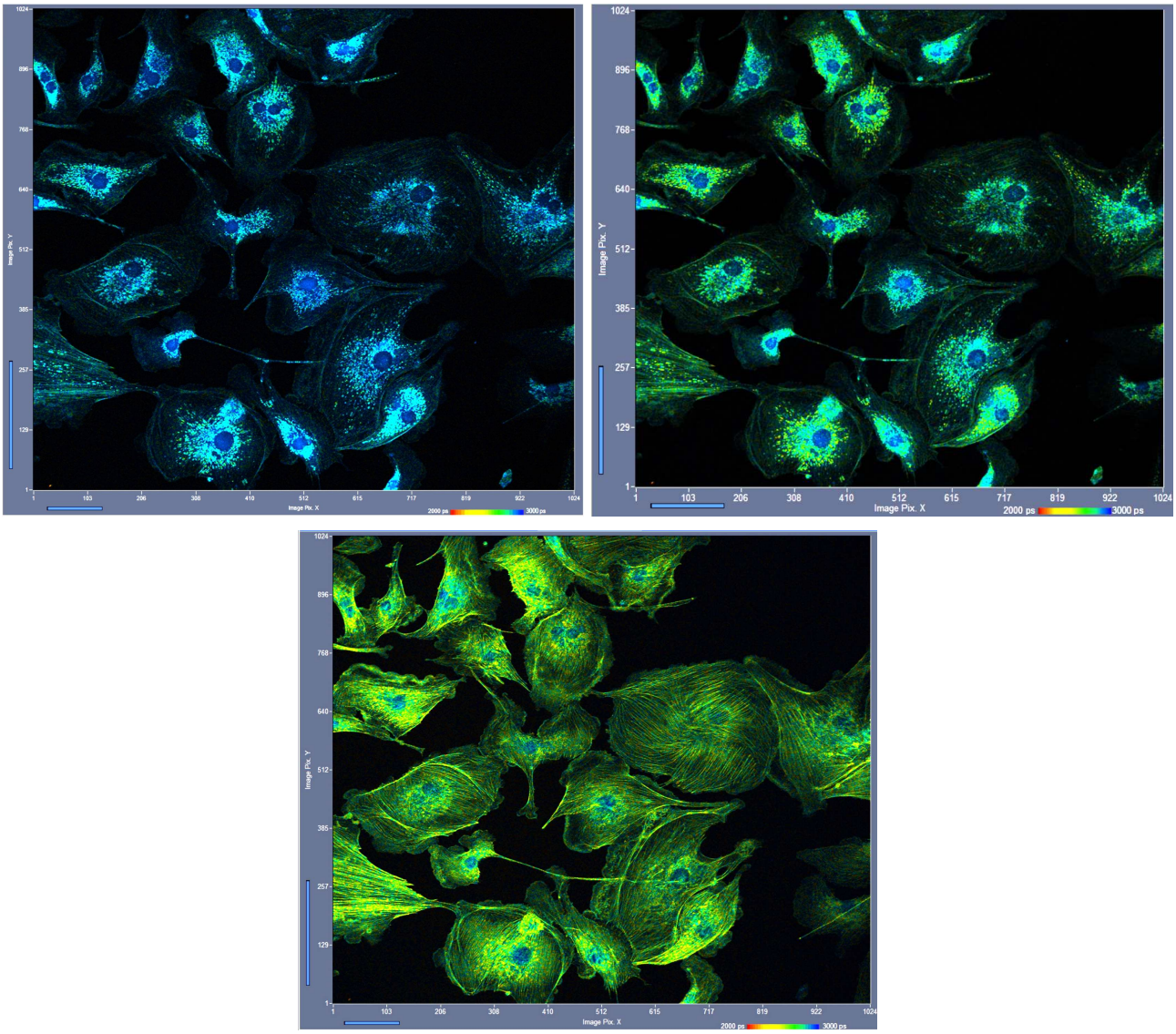



Fig. 4: SPC-QC-104, 3-channel FLIM of BPAE cells, bh DCS-120 confocal FLIM system, 1024 x 1024 pixels.

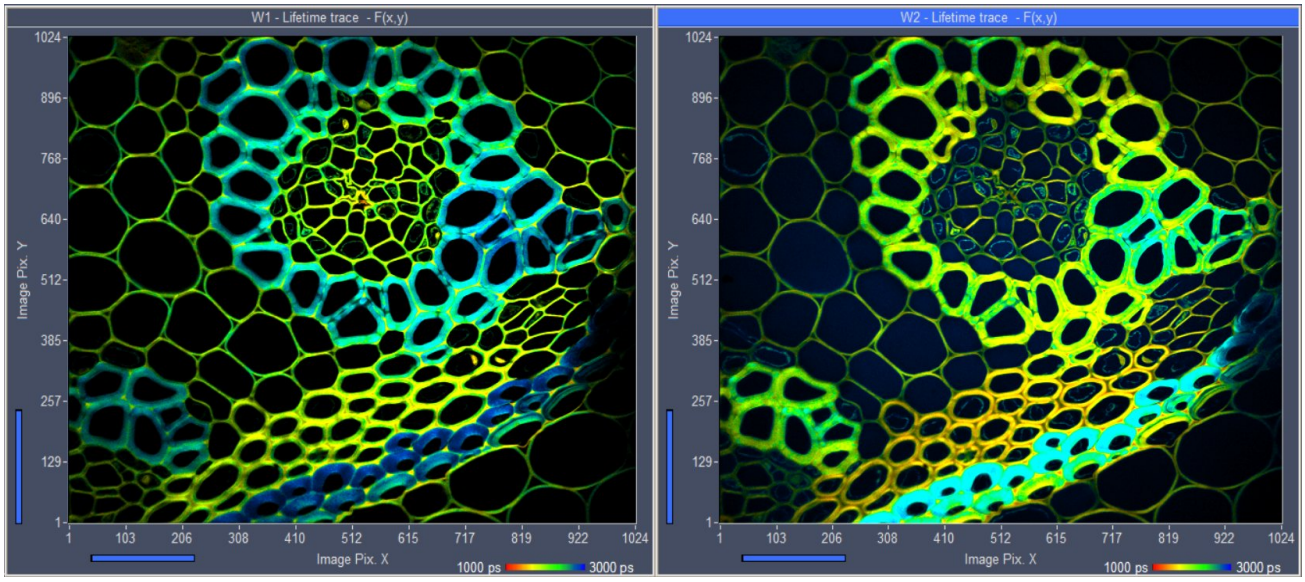



Fig. 5: Convallaria sample, 2p excitation, 2 spectral channels, 1024x1024 pixels, 1024 time channels

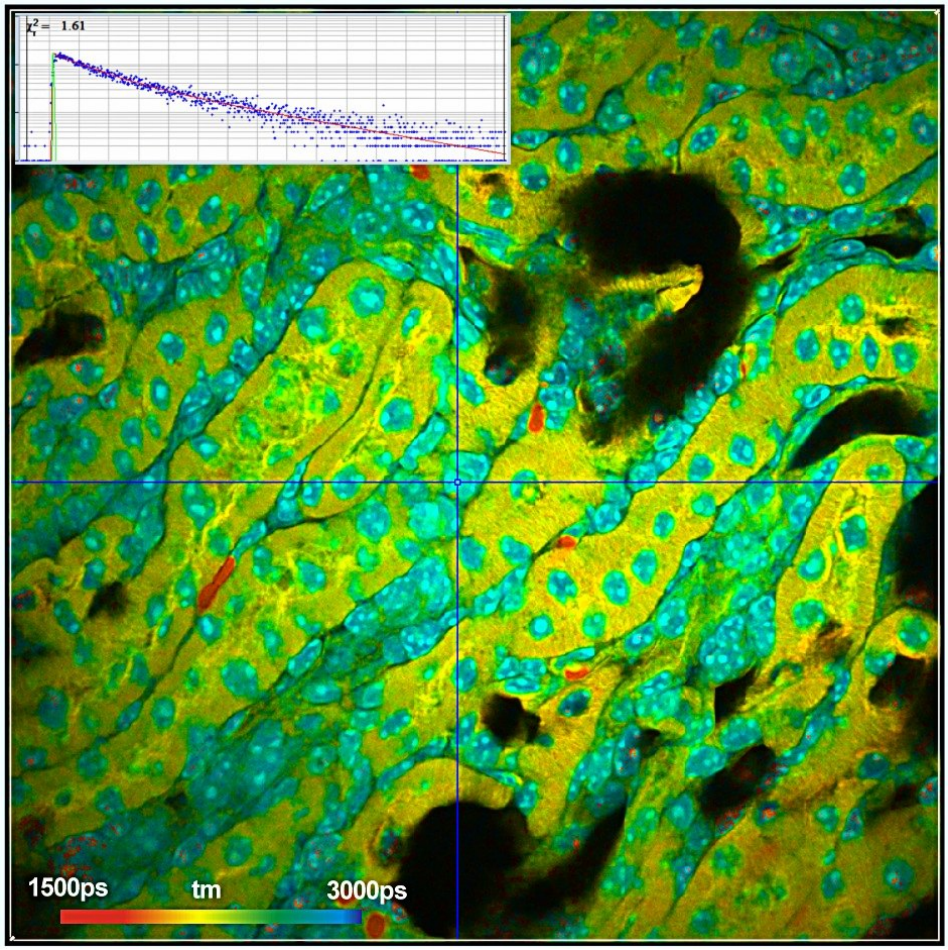



Fig. 6: High-resolution multiphoton FLIM, mouse-kidney sample, 1024x1024 pixels, 1024 time channels. Recorded with DCS-120 MP system, 3-exponential decay analysis by SPCImage NG. Insert shows decay curve at cursor position.

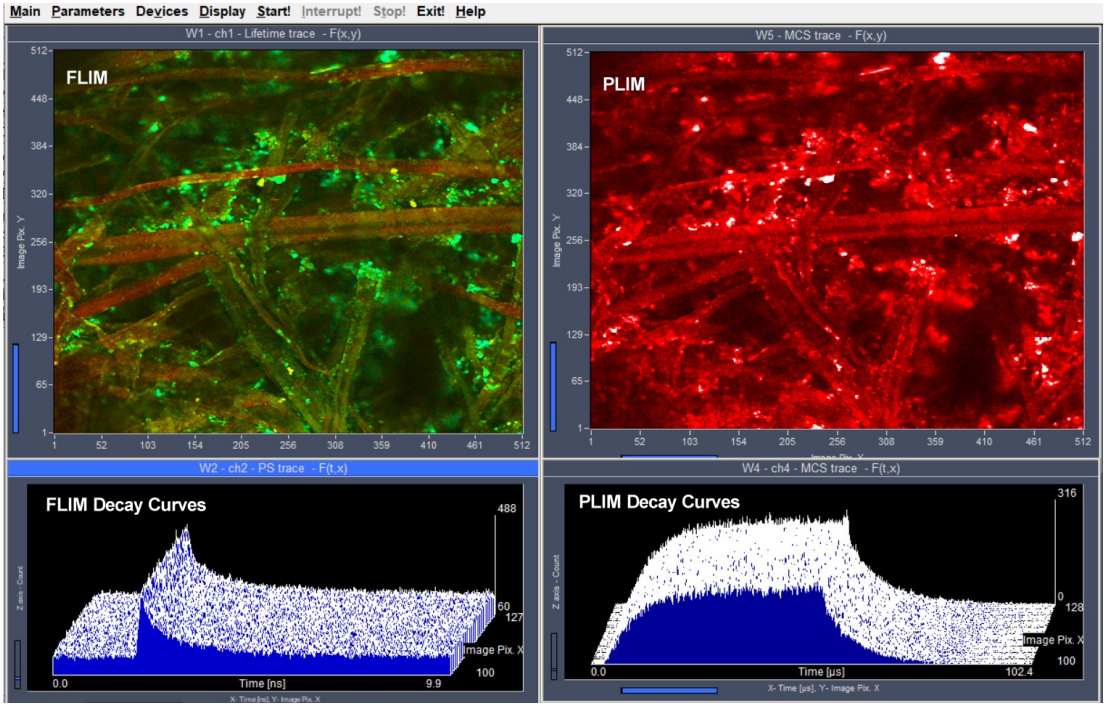



Fig. 7: Simultaneous FLIM / PLIM. DCS-120 confocal FLIM system with SPC-QC-104, Pixel time 8.8 µs, laser-on time 4.4 µs. Image and curve display of SPCM.

## Module Architecture

The architecture of the SPC-QC 104 is shown in Fig. 8. At the input are four identical constant-fraction discriminators, CFD1 through CFD4. The CFDs provide defined trigger pulses the temporal position of which is independent of the amplitude of the input pulses. The CFDs are identical with those of the SPC-150N and -180N series. For principle of the CFDs and parameter setup please see section 'Discriminators' of this manual or bh TCSPC Handbook [1], section 'Constant-Fraction Discriminators' and 'System Optimisation'. CFD1, CFD2, and CFD3 are intended to receive detector pulses. CFD4 can either receive a fourth detector signal or a reference pulse from the excitation source.

The CFD output pulses are fed into four TDCs (Time-to-Digital Converters), please see section 'Time-Conversion Principle' in this manual. The TDCs determine absolute times of the pulses with reference to the system clock sequence. Because all TDCs are operating on the same system clock and are implemented in the same FPGA they deliver photon times which are exactly comparable. The TDC results are fed into the signal-processing block of the FPGA. In addition, the signal-processing block receives routing signals, a 'Count Enable' signal, and an 'Experiment Trigger' signal. Moreover, there are three markers, M1...M3 to include external events in the data stream. These can be pixel, line, and frame clock pulses from a scanner or a reference pulse from an external stimulation of the sample.

Depending on the operation mode, the signal-processing logics performs the following operations:

- In the internal-histogram modes (Single, Oscilloscope, f(t,T) it calculates the differences between the times delivered by TDC 1, 2 and 3 against the times delivered by TDC 4. The resulting values are the times of the photons after the previous laser pulse. The processing logics builds up the photon distributions over these times in the on-board memory. In addition to the photon times the procedure can use the state of the routing signals to create separate photon distributions for different states of the routing signals, please see Fig. 13.

- In the FIFO (Parameter-Tag) and FIFO Imaging modes it calculates the differences between the times delivered by TDC 1, 2 and 3 against the times delivered by TDC 4. Photon by photon, it sends the results to the bus interface. From there, the values are read by the SPCM software, which then builds up the photon distributions. The on-board memory is used as a FIFO (first-in-first-out) buffer to bridge time intervals in which the software is not able to read the data. In addition to the photon times the processing logics sends the state of the routing signals for the individual photons. Using these data, SPCM routes the photons into separate photon distributions for different routing vectors, please see Fig. 13. Moreover, external events, such as scan clocks from a scanning device, or other reference pulses from the experiment setup are included in the data stream. The software builds up lifetime images, multi-wavelength lifetime images, or simultaneous FLIM/PLIM images from such data. In addition to building up photon distributions, SPCM can save the parameter-tagged data into a file. For every photon, the parameter-tag data contain the time after the laser pulse, the absolute time from the start of the experiment, and the state of the routing signals. The data also contain entries for the marker events. Please see SPCM description in the bh TCSPC Handbook [1].
- In the absolute timing mode the TDC times of all four channels are send to the bus interface. The on-board memory is used as a FIFO (first-in-first-out) buffer. SPCM runs correlation calculations on the data and stores the raw data in a file. Also here, the routing information is included in the data of the individual photons, and marker events are included in the data stream.

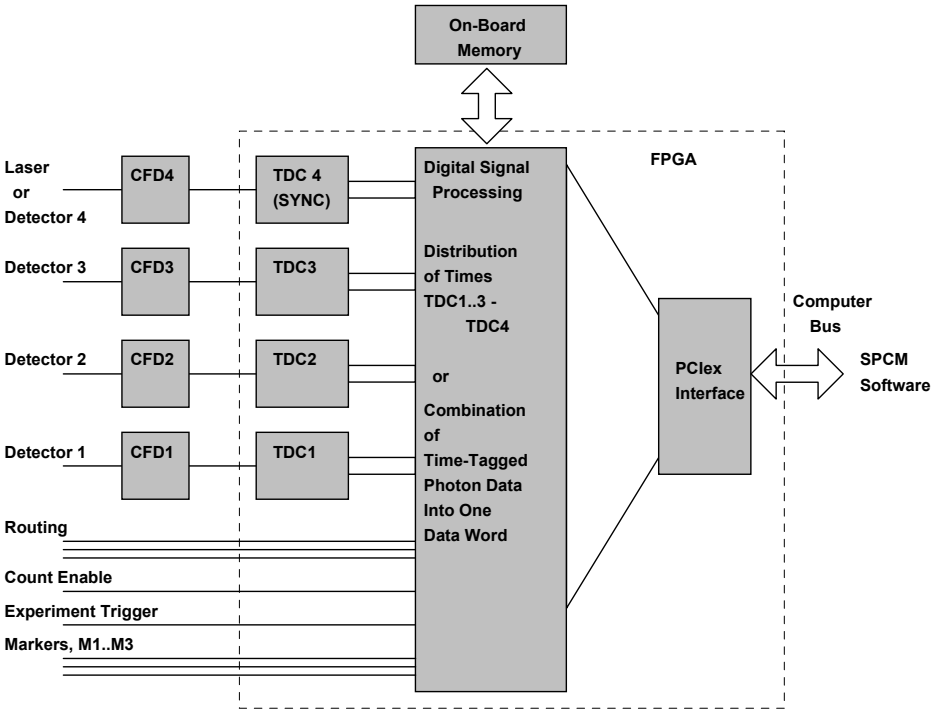



Fig. 8: Architecture of the SPC-QC 104 module

## Discriminators

Single-photon pulses from a PMT have a considerable amplitude jitter. If the pulses were sent through a normal discriminator the amplitude jitter would cause a timing jitter, Δt, on the order of the duration of the leading edge the photon pulses, see Fig. 9, right. For normal (high-speed) PMTs this would be about 1 ns - too much for the standards of TCSPC.

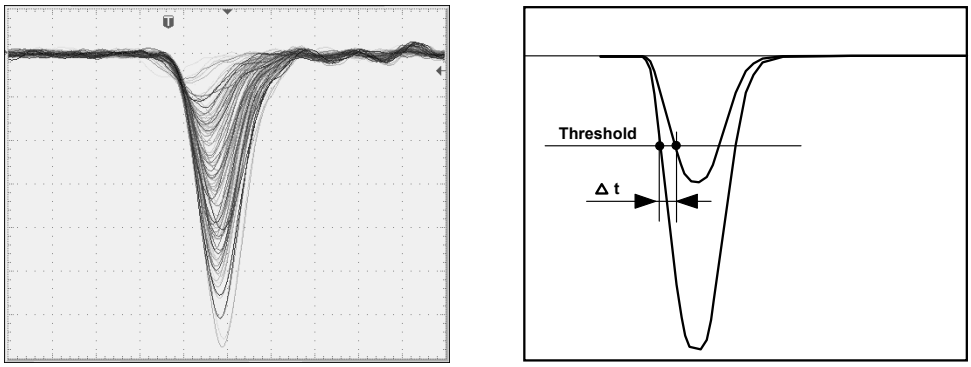



Fig. 9: Left: Single-photon pulses from a normal PMT. Right: When the pulses are received by a normal discriminator the amplitude jitter would induce a timing jitter, Δt.

Obviously, a TCSPC device needs a discriminator which triggers at a constant fraction of the pulse amplitude, see Fig. 10, left. If the discriminator threshold is varied in a way that it is a constant fraction of the pulse amplitude the timing variation, Δt, vanishes. Please see Fig. 10, right.

The principle of a CFD is usually described as shown in Fig. 10, left. However, the principle has a flaw: The electronical implementation is impossible. When the leading edge of the pulse crosses the threshold the final amplitude of the pulse and thus the correct threshold are not known yet. The practical implementation is therefore different. The detector pulse is re-shaped by an electrical network (usually a system of short delay lines, see [1]) so that a bipolar pulse is obtained, see Fig. 10, right. The temporal position of the zero-cross point is independent of the amplitude. Therefore a discriminator picks of the baseline transition of the bipolar pulse. In practice, the optimum 'zero-cross level' may be not exactly at the zero line but slightly above or below. The zero-cross level is therefore adjustable to optimise the timing performance for a given detector. Moreover, there is a second discriminator which looks at the original detector pulse. Only if the pulse exceeds the threshold of this discriminator the zero-cross discriminator is enabled. The complete CFD therefore has two control voltages - a threshold voltage and a zero-cross voltage. The timing performance is optimised be the Zero-Cross-Level, the amplitude range by the Threshold voltage.

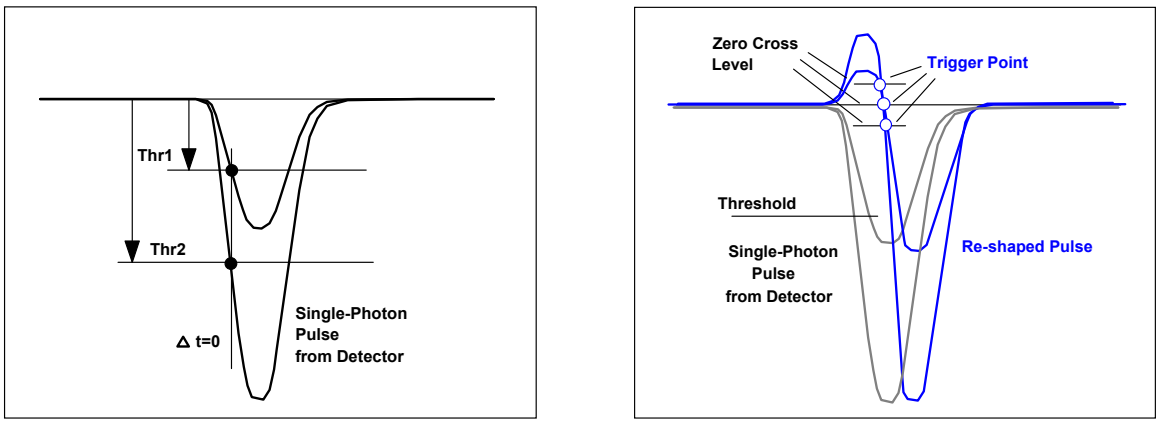



Fig. 10: Commonly described Principle of a CFD (left) and practical implementation (right)

## Time-Conversion Principle

TCSPC is based on the detection of single photons, the determination of their detection times, and the buildup of photon distributions or correlations of the times of the photons. The core of any TCSPC

device is therefore a time-measurement circuit which converts the photon times - either absolute or relative ones - into digital data words.

There are two generally different time-conversion principles. The bh SPC-130, -150, -160 and -180 TCSPC modules use a TAC/ADC principle; the SPC-QC-104 uses direct time-to-digital (TDC) conversion.

The two principles are illustrated in the figure below. The TAC-ADC principle is shown left. It uses a linear voltage ramp between a start pulse (usually the photon) and a stop pulse (usually a reference from the laser). The voltage is converted in a digital data word which expresses the time of the photon in the laser pulse sequence. The advantage of the TAC/ADC principle is the extremely high time resolution. The IRF width of the SPC-150NXX and -180 NXX modules is 2.8 ps fwhm (full width at half maximum), the effective timing jitter is about 1.5 ps (rms) [1, 3]. A resolution this high is high is not reached by any other TCSPC device. It is more than 17 times higher than for the TDC principle.

The TDC principle is shown on the right. The photon pulses from the detector(s) and the reference pulses from the laser are sent through chains of delay elements. The timing logics looks at the data in the delay chains, identifies start-stop pairs of photons and laser pulses, and this way determines the temporal positions of the photons in the laser pulse sequence. The time resolution of the TDC principle is significantly lower than that of the TAC/ADC principle. The IRF width of the SPC-QC-104 is about 48 ps (fwhm), the effective timing jitter about 20 ps (rms). The advantage of the TDC principle is that works up to extremely high photon rates, and that several timing channels can be implemented on a single PC board.

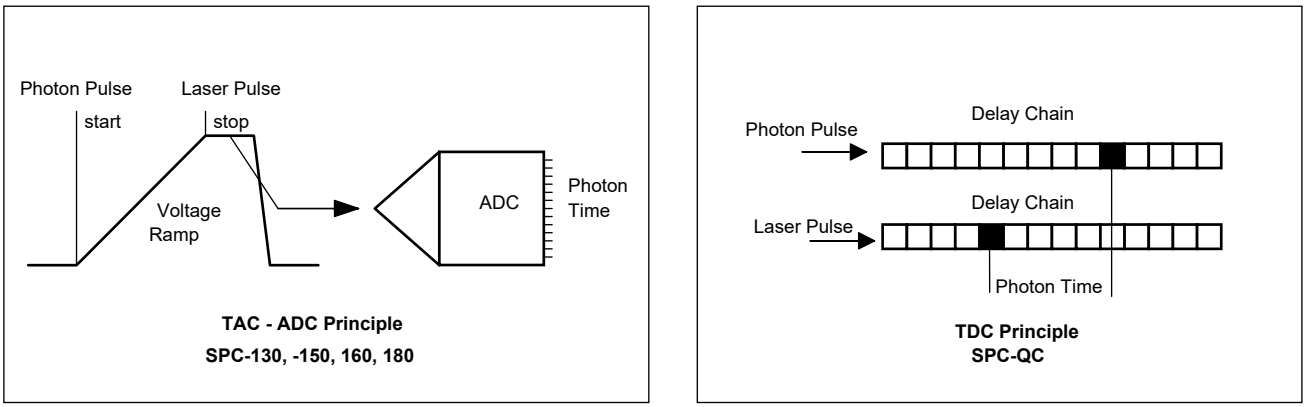



Fig. 11: Time-conversion principles. Left: TAC/ADC principle. Right: TDC principle

## Buildup of Fluorescence-Decay Data

As all bh TCSPC devices, the SPC-QC records time-resolved optical data by detecting single photons of a repetitive light signal and building up photon distributions over the times of the photons after a timing-reference pulse. In the simplest case the photon distribution can just be built up over these times, in other cases it includes also other parameters, such as the wavelength, the time from a stimulation of the sample, or the position of the excitation beam within a scanning area. The buildup of the photon distribution does not differ substantially from the procedure used in the bh SPC modules [1]. The only difference is in the method of the time measurement and in the fact that the SPC-QC measures the time from the laser pulse to the photon, whereas the SPC modules measure it from the photon to the next laser pulse [1, 2, 3].

## Classic TCSPC Mode

Classic TCSPC builds up a photon distribution over the times of the photons in the excitation pulse period. The principle is illustrated in Fig. 12.

When a photon is detected, the arrival time of the corresponding detector pulse in the signal period is measured. The detection events are collected in a memory by adding a '1' at an address proportional to the detection time. After many signal periods a large number of photons has been detected, and the distribution of the photons over the time in the signal period has been built up. The result represents the waveform of the optical pulse.

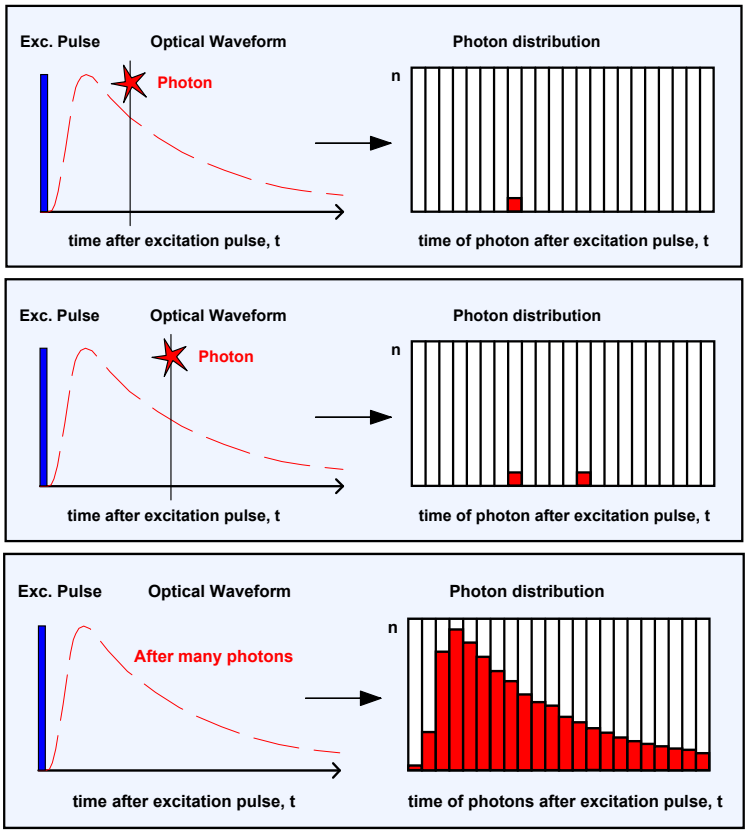



Fig. 12: Classic TCSPC records a distribution over the times of the photons after the excitation pulses.

## Multi-Wavelength TCSPC

Multi-wavelength TCSPC is based on splitting the light spectrally into a number of detector channels (or channels of a multi-anode PMT), and using the number of the channel in which the photon arrived as a second coordinate of the photon distribution. The principle is shown in Fig. 13. For each photon, the detector delivers a single-photon pulse which indicates the detection time, and a 'Channel' signal which indicates in which spectral channel the photon arrived. The TCSPC module builds up a photon distribution over the photon time and the channel number. The result is identical with a set of decay curves for (in this case 16) different wavelengths.

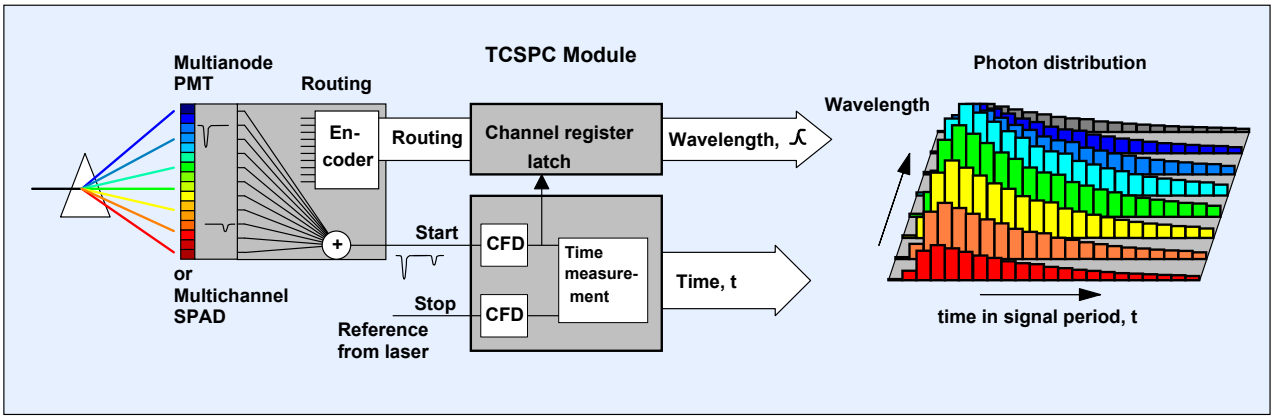



Fig. 13: Principle of multi-wavelength TCSPC

Please note that multi-wavelength TCSPC does not imply any wavelength scanning, detector switching, or multiplexing. Every photon is put into a place in the photon distribution according to its detection time and its wavelength. Compared to scanning the spectrum with a monochromator and recording individual decay curves, the efficiency is much higher. Multi-wavelength detection, especially in combination with FLIM, has therefore become a commonly used technique of spectroscopy of biological samples [9, 10, 11].

## FLIM

FLIM by multi-dimensional TCSPC is based on scanning a sample by the focused beam of a high-repetition rate laser and detecting single photons of the fluorescence signal. The times of the photons are determined by the timing (TDC) electronics, the position of the laser beam in the moment of the photon detection by a scanning interface. The scanning interface counts pixel clock, line clock, and frame clock pulses from the scanner and this way obtains the position of the beam in the scan area. The recording process builds up a photon distribution over these parameters [1, 2, 4, 5, 7]. The principle is illustrated in Fig. 14.

The result is an array of pixels, each containing a full fluorescence decay curve with a (typically large) number of time channels. The process works at any scan rate, and delivers near-ideal photon efficiency and extremely high time resolution [1, 3].

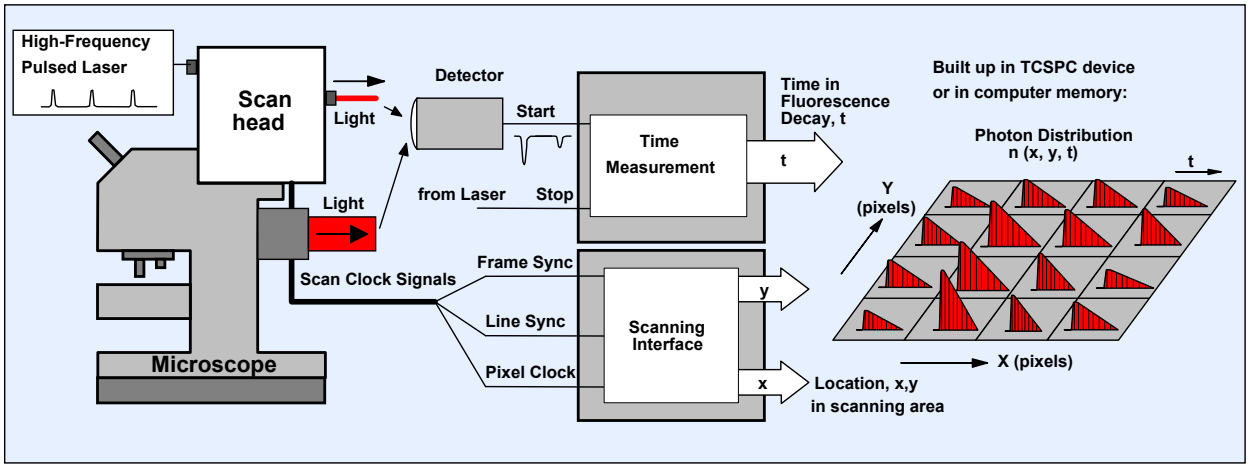



Fig. 14: Fluorescence lifetime imaging

## Multi-Wavelength FLIM

Multi-wavelength (or 'multi-spectral') FLIM uses a combination of the FLIM architecture shown in Fig. 14 with multi-wavelength detection principle described in Fig. 13. In addition to the times of the photons and the positions, x, and y, of the scanner, the TCSPC module determines the (spectral) detector channel that detected the photon. These pieces of information are used to build up a photon distribution over the times of the photons in the fluorescence decay, the wavelength, and the coordinates of the image [1, 2, 6, 7, 8]. The result is an image that contains several decay curves for different wavelength in each pixel. The principle of multi-wavelength FLIM imaging is shown in Fig. 15.

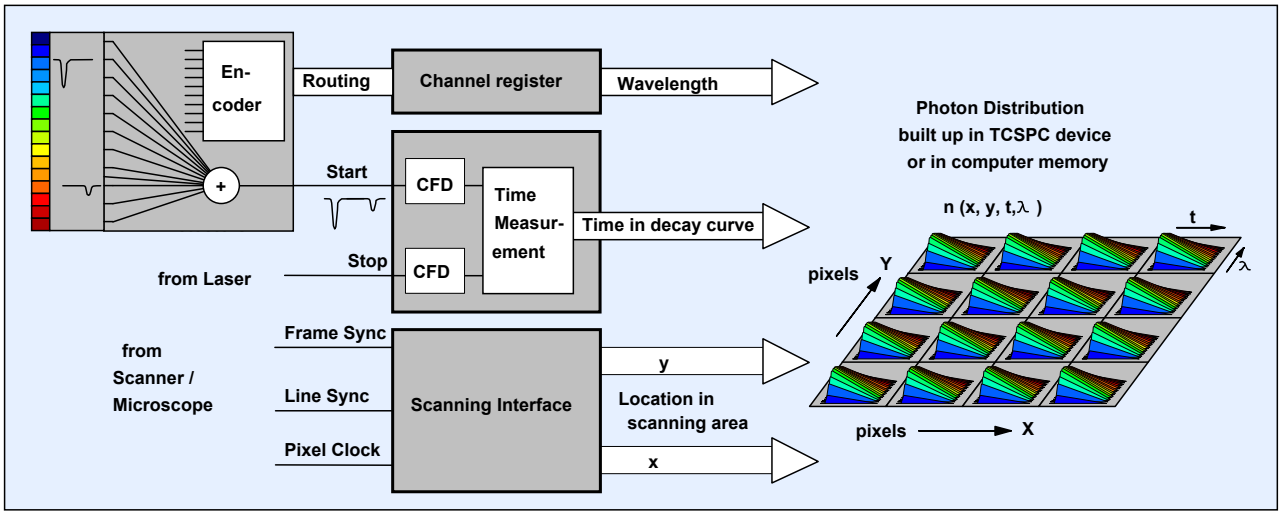



Fig. 15: Multi-wavelength FLIM. The recording process builds up a photon distribution over x,y,t, and λ.

## Simultaneous FLIM/PLIM

A bh TCSPC module can, in principle, record an optical waveform simultaneously on two different time scales. One time scale comes from the TDC times, the other from the system clock. By tagging the photons with these two times, simultaneously photon distributions on the picosecond and on the microsecond time scale can be build up.

The technique is used to record fluorescence and phosphorescence decay data simultaneously [1, 12, 13, 14, 15]. The principle is shown in Fig. 16. A high-frequency pulsed laser is on-off modulated with a period in the microsecond or millisecond range. The TCSPC module determines photon times, t, within the laser pulse period, and photon times, T, within the laser modulation period. Fluorescence decay curves are obtained by building up a photon distribution over t, phosphorescence decay curves by building up a the photon distribution over T. The technique solves a number of problems of phosphorescence decay measurement. The most significant one is that for phosphorescence decay measurement the duration and the period of the excitation must be long. This conflicts with the capabilities of the available lasers, and with the requirement that for fluorescence decay measurement the pulses and the pulse period must be short. With the principle shown in Fig. 16, the effective excitation pulse for the phosphorescence measurement is the laser-on period, but for fluorescence it is the laser pulse period.

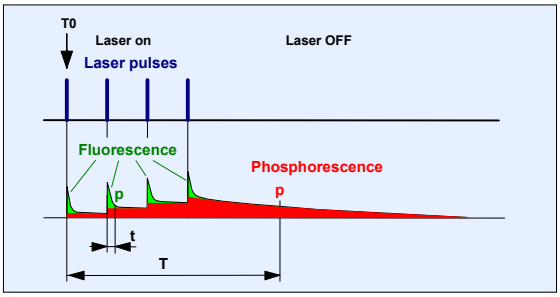



Fig. 16: Build-up of phosphorescence within a burst of laser pulses

To combine the technique shown in Fig. 16 with imaging the scan coordinates must be included in the parameters of the photon distributions. A phosphorescence lifetime image (PLIM) is obtained by recording the photons versus the times, T, in the laser on-off period and the scan coordinates, a fluorescence lifetime image (FLIM) by recording the photons versus the TDC times, t, and the scan coordinates. Both processes can be run simultaneously. The TCSPC system architecture for simultaneous FLIM / PLIM is shown in Fig. 17. Technical details and applications of simultaneous FLIM / PLIM are described in the bh TCSPC Handbook [1].

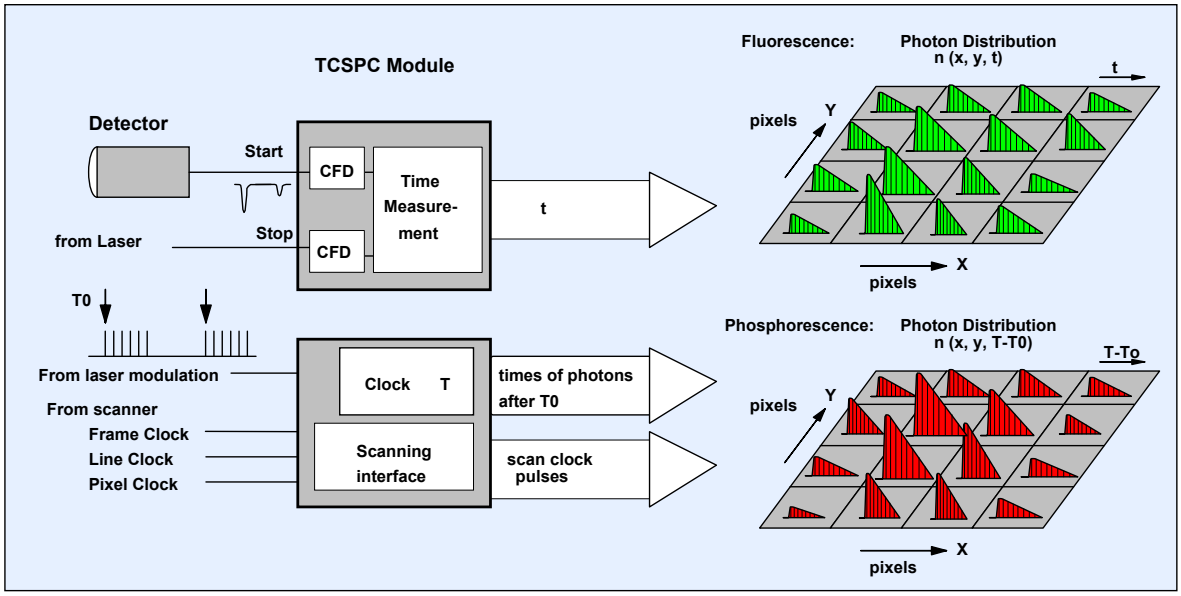



Fig. 17: Simultaneous fluorescence and phosphorescence lifetime imaging in the parameter-tag mode

The procedure can be refined by using the laser on/off information as a routing signal to better separate the fluorescence in laser-on phases from the phosphorescence in the laser-off phases. Please see bh TCSPC Handbook [1].

## Comparison with bh SPC-130 to SPC-180 series modules

The advantage of the TDC principle is that the timing electronics can be implemented in an FPGA (Field-Programmable Gate Array). Therefore several recording channels can be implemented on one TCSPC board. Another feature where the TDC is superior to the TAC is that the TDC principle works up to extremely high count rates. In practice, the count rate is limited by pile-up, dead time in the detector-discriminator combination, degradation of the detector timing performance at high count rate, and, of course, sample degradation.

On the downside, the time resolution is much lower than for the TAC ADC principle. A comparison of the electrical IRF of an SPC-180NXX and an SPC-QC-104 is given in the figure below. The IRF width for the SPC-180NXX (left) is 2.8 ps FWHM, for the SPC-QC-104 (right) it is 48 ps FWHM. Although 48 ps FWHM is an excellent value for a TDC the SPC-QC-104 does not exploit the full time resolution of ultra-fast detectors, such as SSPDs, MCP-PMTs and ultra-fast hybrid detectors.

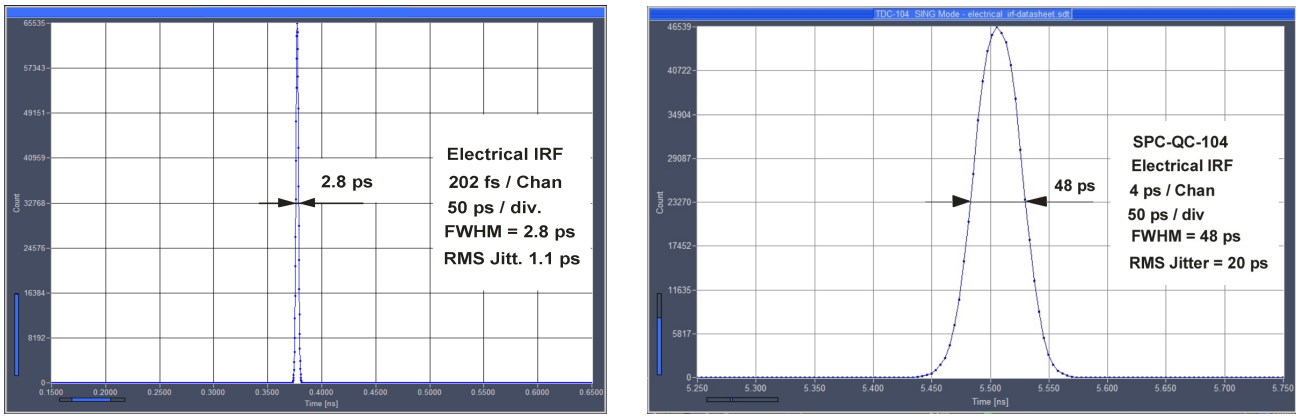



Fig. 18: IRF for SPC-180NXX (left) and SPC-QC-104. Same time scale, 50 ps /division

Another critical feature is timing stability. For may years stability was a problem for the TDC. In the SPC-QC-104 the stability problem has largely been overcome by a new TDC-logics structure. A comparison of the timing stability of an SPC-180 NXX and an SPC-QC-104 is shown in the figure below. For the SPC-180 NXX the stability of the first moment of the IRF is better than 0.4 ps RMS, for the SPC-QC-104 it is better than 5 ps RMS (note different time scales). Although the SPC-QC does not reach the stability of the SPC-180NXX possible timing drift remains far below the IRF width and thus is rarely a problem in practical application.


Fig. 19: Timing stability of an SPC-180NXX (left) and of an SPC-QC-104 (right). Time-series of measurements, display in Colour-Intensity mode. Note different time scale.

# System Setup

## Software Installation

We recommend to install the software from the https://www.becker-hickl.com. You are then sure that you get the latest software version. Please open www.becker-hickl.com (Fig. 20, left). Click on the ‘Products’ button. In the Products panel, click on ‘Software’ (Fig. 20, right).

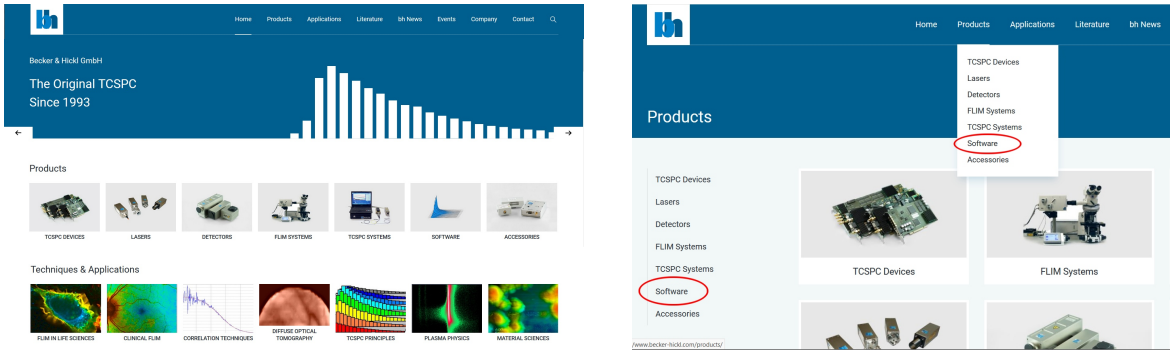



Fig. 20: For TCSPC software installation open www.becker-hickl.com, and click the ‘Products’ button’. Then select ‘Software’

This opens the panel shown in Fig. 21, left. Select ‘tcspc-setup_64.exe’ or (for older 32-bit computers) ‘tcspc-setup_32.exe’. You can start the installation right away or first save tcspc_setup.exe on the hard disc and run it from there. The start of the setup program opens the usual Windows installer, see Fig. 21, right.

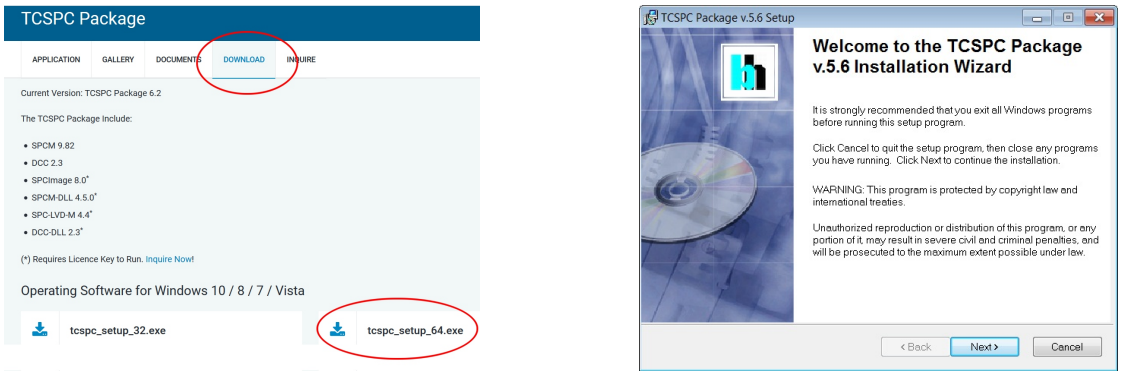



Fig. 21: Selection of the TCSPC software (left) starts the Windows installer (right)

In the next step you can select what exactly you want to install, see Fig. 22, left. Non-imaging applications may need only the SPCM and the DCC application. The installation of these components is free. For fluorescence-decay analysis and FLIM data analysis you need SPCImage NG. For installation you have to purchase a license number.

The installation procedure lets you also install DLLs for the bh TCSPC systems. The DLLs are only needed if you plan to develop your own instrument software. SPCImage and the DLLs require a license number - please type in as shown in Fig. 22, right. If you purchased SPCImage the number has been delivered with your system. If you don’t find it please request one from bh.

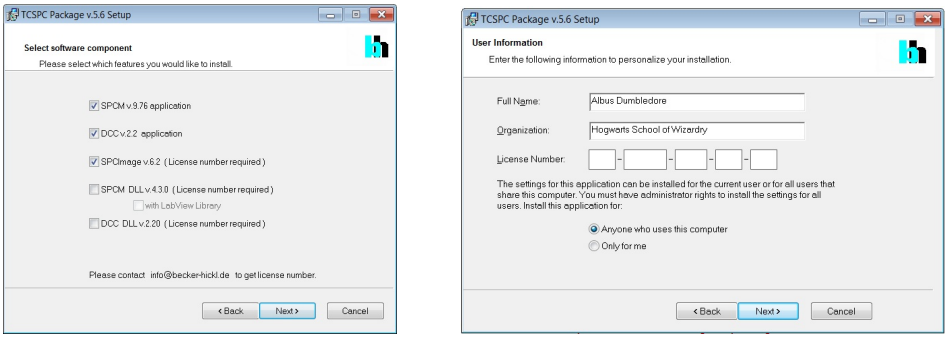



Fig. 22: Selection of software components (left) and license number input for SPCImage and DLLs

After that, please follow the instructions of Windows and finish the installation. Problems can occur if Windows on your computer has not been updated for a longer period of time. The TCSPC package may then not be compatible with the Windows installer. Therefore, please run pending Windows updates before you start the installation.

## Hardware Installation

The SPC-QC-104 is a PCI-express module for installation in a standard PC. To exploit the full capability of the SPC-QC-104 the computer should have 32 GB of memory.

The SPC-QC-104 module occupies a single PCI-ex slot. Both slots with short PCIex connectors and with long PCIex connectors can be used. To install the module, turn off the computer, remove the blind plate of a free slot, insert the module, and fix its front plate to the computer frame it with the screw that previously held the blind plate. Please see Fig. 23. Some computers have other mechanisms to hold the module in place. In any case it is important that the module is securely fixed. If it is not, you almost certainly get contact problems and EMC (electromagnetic compatibility) problems. If additional experiment-control modules, such as a DCC-100, a GVD-104 or a GVD-240 are to be used, slots have to be provided also for these. PCIex versions of these modules may have additional inputs for power supply. Please connect one of the internal power supply lines to the corresponding connector on the board. Please see bh TCSPC Handbook [1], chapter 'Installation of the bh TCSPC Modules'.

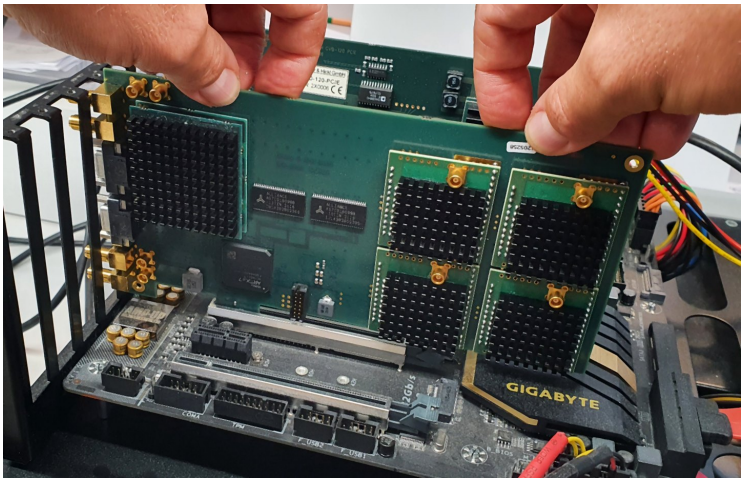



Fig. 23: Insertion of a SPC-QC-104 in a PC. The module can be inserted both in long and in short PCIex connectors.

## Software Start

When the module has been inserted in the computer switch on, wait until Windows has started, and start the SPCM Software. If SPCM has not been installed yet, install it now. After starting SPCM the initialisation panel shown in Fig. 24, left should appear. The installed modules are marked as ‘In use’. The modules are shown with their serial number, PCI address and slot number.

If the SPC module is not found at this stage for whatever reasons, the software starts in an emulation mode (see below, ‘Starting the SPC software without an SPC module). What you see in this mode is generated by the software or loaded from a file - it is not the data recorded by the SPC module.

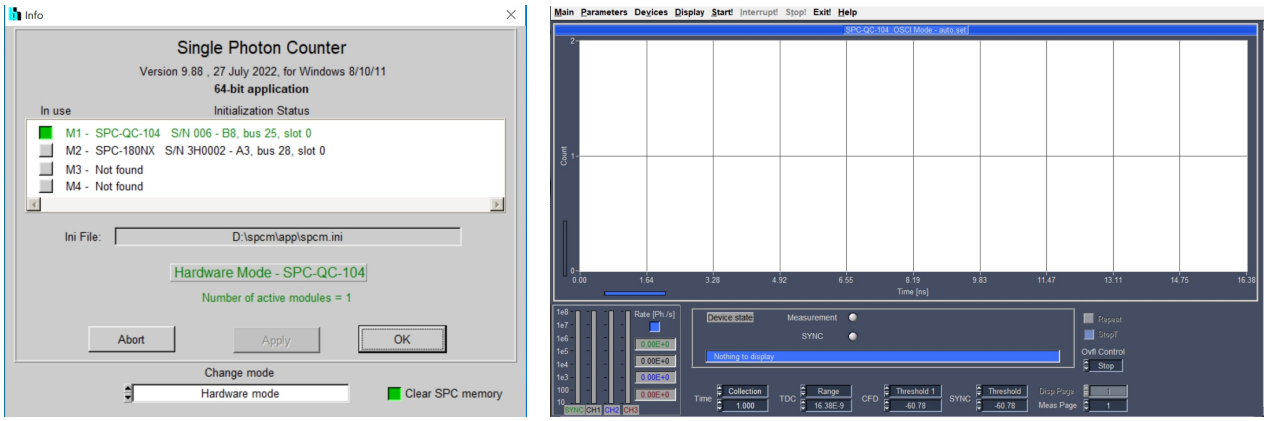



Fig. 24: Starting the SPCM software. Initialisation panel (left) and main panel of SPCM (right).

The software runs a hardware test when it initialises the modules. If an error is found, a message ‘Hardware Errors Found’ is given and the corresponding module is marked red. In case of non-fatal hardware errors you can start the SPCM main window by selecting ‘Hardware Mode’ in the ‘Change Mode’ panel. Please note that this feature is intended for trouble shooting and repair rather than for normal use.

When the initialisation window appears, click on ‘OK’ to open the main window of the SPCM Software (Fig. 24, right). At the first start the software comes up with default parameters which may not by appropriate for your measurement problem. Therefore, changes may be required for your particular application. Please see section 'SPCM Software'.

## Starting the SPCM Software without an SPC Module

You can use the SPCM Software without a SPC-QC module. In its start window the software will display a warning that the module is not present, see Fig. 25.

To start the software for the desired module type, click into the ‘Change Mode’ field and select the desired module type from the list. Then click on ‘Apply’, ‘OK’. The software will start in a special mode and emulate the SPC-QC device memory in the computer memory. You can set the TCSPC system parameters, load, save, process, convert and display data, i.e. do everything except for a real measurement.

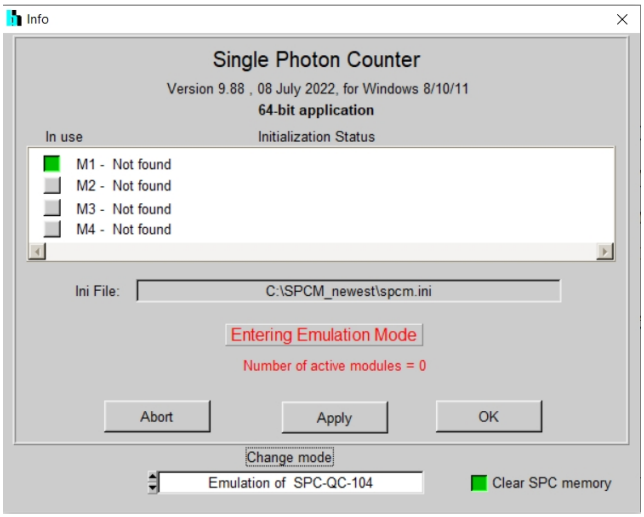



Fig. 25: Startup panel in the emulation mode (without a TCSPC module)

## First Light

This section describes the general procedure for the initial setup of a measurement system using the SPC-QC-104. For simplicity, the procedure is described at the example of a simple fluorescence-decay experiment. Complex systems, such as FLIM systems with scanners, dual or triple channel systems, systems with multi-wavelength detection, or systems with laser multiplexing are based on the same general principles, and the initial setup is done in a similar way.

A basic system is outlined in Fig. 26. A pulsed light source - usually a ps diode laser - is directed into a fluorescent sample, and the fluorescence light is detected by a single-photon detector. A filter or a package of filters may by used in front of the detector to make the setup daylight-compatible and to suppress scattered excitation light. Additional optical elements are not required for basic system setup, although they certainly exist in more complex experiments.

The synchronisation signal from the laser is connected to the SYNC input of the SPC-QC module, the detector signal to one of the CFD inputs. Systems with PMCS-150 detectors, id-100 detectors or other SPADs work as indicated in Fig. 26, left. For HPM-100 detectors or PMC-150 detectors a DCC-100 detector controller is necessary, see Fig. 26, right.

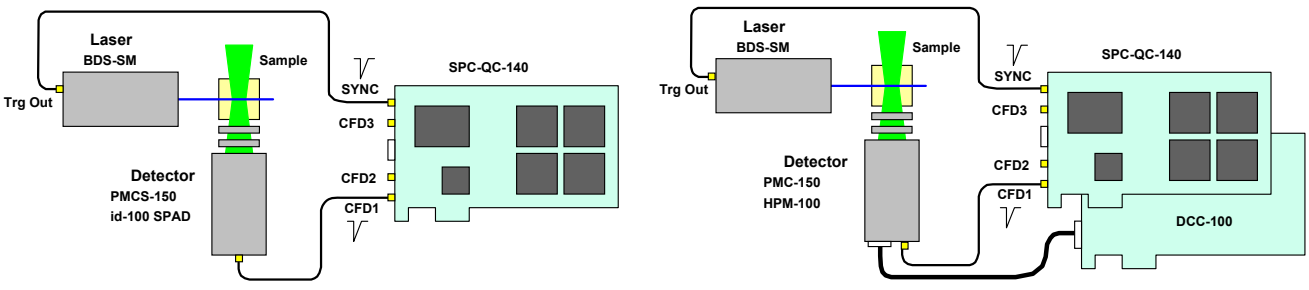



Fig. 26: Basic fluorescence-decay measurement system

When all system connections are in place turn on the system computer, and start SPCM. For the initial setup, we recommend to use system parameters as shown in Fig. 27.

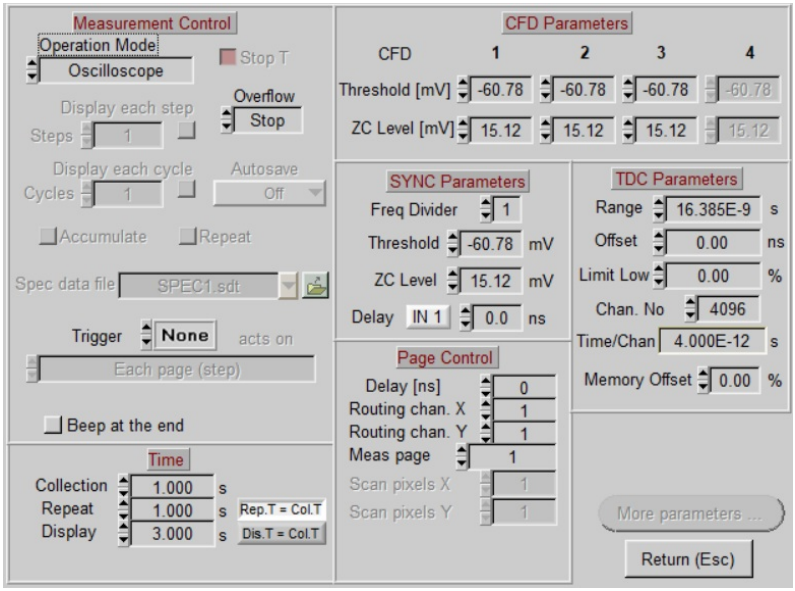



Fig. 27: System parameters for initial system setup

Operation mode is 'Oscilloscope', collection time is 1 second. Trigger is 'None'. The thresholds of the detector and Sync discriminators are -60 mV, the zero-cross levels are +15 mV. The TDC parameters are: Range = 16 ns, Offset = 0, Limit Low = 0, Channel Number 4096. This gives a time-Channel width of 4 ps. Memory Offset is zero. The page control parameters are: Delay = 0 ns, Routing X and Y = 1, Measurement Page =1. With these settings, the TDC-QC should record at least some photons for all conceivable detectors and under all conceivable circumstances.

The display of curves in SPCM is controlled by the Trace Parameters in combination with the Display Parameters. To display the desired data these parameters must be appropriately set. The recommended Trace Parameter setup is shown in Fig. 28.

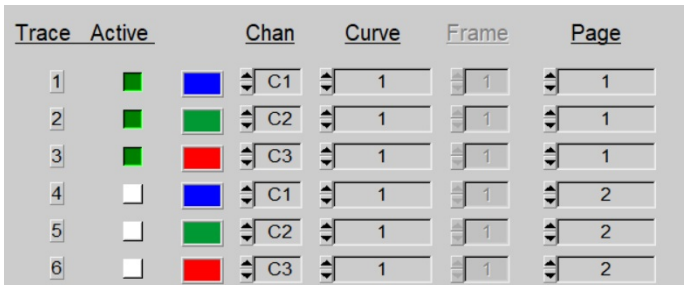



Fig. 28: Trace Parameters for system setup

With the parameters shown, SPCM displays three individual curves built up from the photons in the specified channels. The colours for curve 1, 2, and 3 are blue, green, and red, respectively. With the detector connected to CFD 1 (see Fig. 26) a blue curve should show up. Traces (4 to 6) for a second memory page are reserved but not turned on.

Curve style, vertical range, and background / grid colours are defined by the 'Display Parameters'. For system setup we recommend the settings shown in Fig. 29.

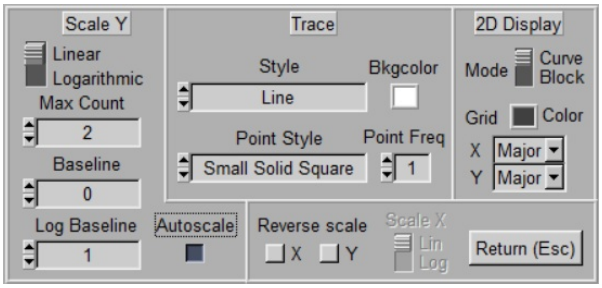



Fig. 29: Display Parameters for system setup

With the settings shown above the user interface should be as shown in Fig. 30. The curve window is resizable - we recommend to pull it up to the full size as shown in the figure.

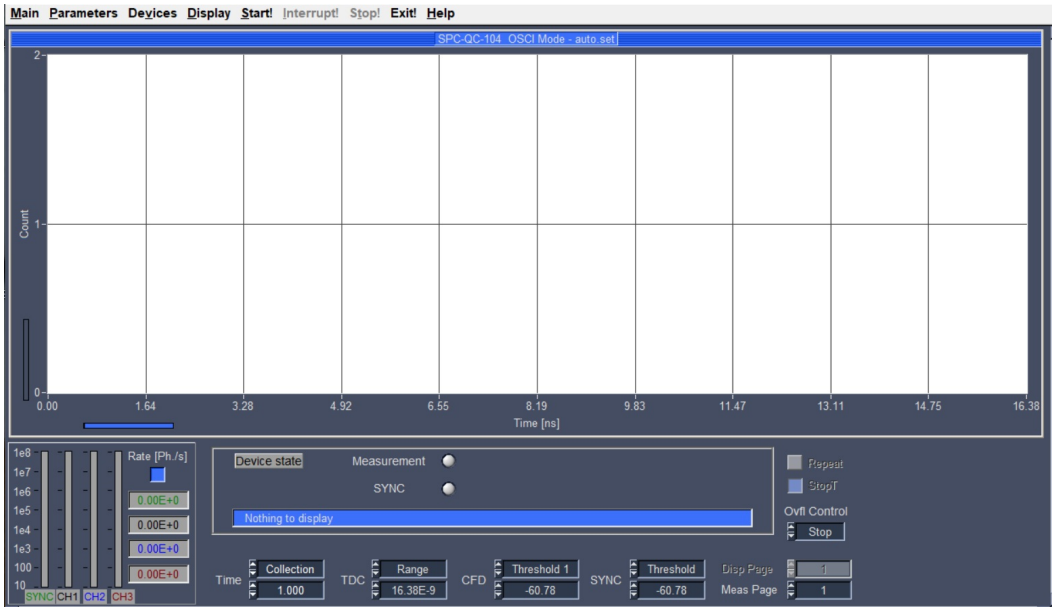



Fig. 30: SPCM user interface for system setup

When the main panel is as in Fig. 30, turn on the laser and the detector. If the laser has selectable repetition rate, use 40 MHz to 80 MHz. The 'SYNC' rate bar in the lower left should show the laser repetition rate. The rate bar for Channel 1 should show a photon rate. For detectors which are controlled via a DCC card, enable the DCC output to which the detector is connected, and increase the 'Gain' until you see a count rate in Channel 1. Details of detector setup are described in the bh TCSPC Handbook [1], chapter 'System Optimisation'.

When you click the Start button a decay curve should show up in the curve window. If you are lucky it looks as shown in Fig. 31, page 23. The decay curve is perfectly placed in the observation-time interval and it has been recorded at a reasonable time scale. No further refinements are needed in this case.

In most cases, however, the decay curve will be badly shifted, or there will be no decay curve visible at all. If no decay curve shows up the reason is almost certainly a mistake in the optical setup or in the detector setup. Make sure that the detector is turned on, that the detector has not been driven in an overload shutdown, and that it receives a reasonable amount of fluorescence light. If necessary, try with more or less filters. If the detector is operated via a DCC card make sure a reasonable detector gain is set. Please refer to the instructions in the bh TCSPC Handbook [1], chapter 'System Optimisation'.

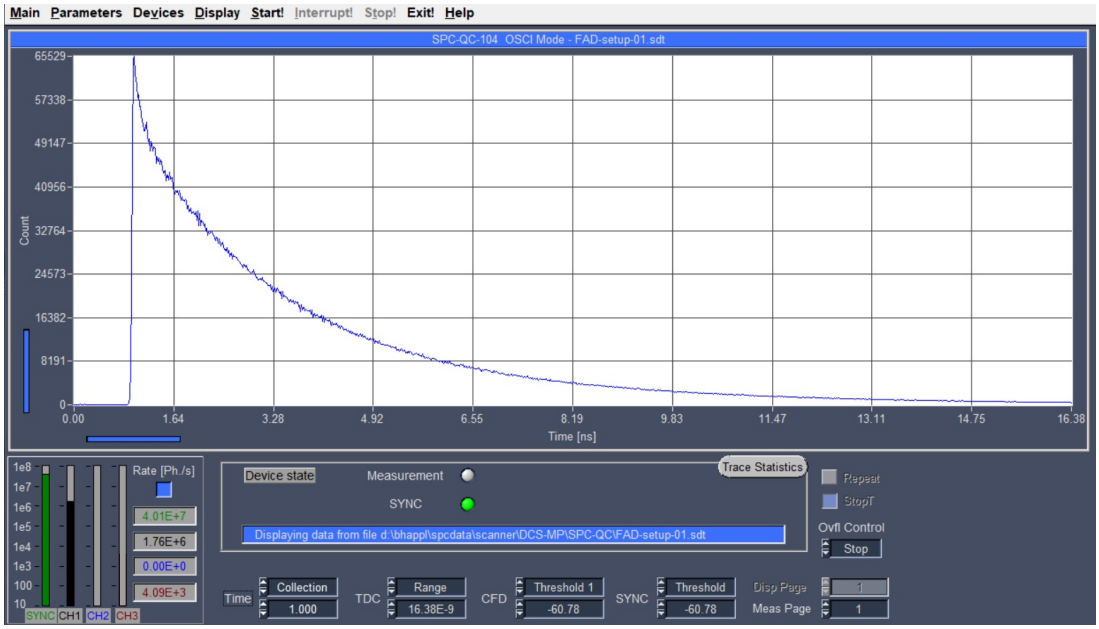



Fig. 31: Laser running, detector receiving photons, Measurement started, decay curve displayed in curve window

A shift in the curve results from different optical and electrical signal transit time in the Sync and detection channel. To correct it, change the 'Offset' in the TDC timing parameters, see System Parameters, Fig. 27. To conveniently adjust the offset during the measurement, the parameter is also accessible directly from the main panel, see Fig. 32. Increasing the offset shifts the curve to the right, decreasing the offset shifts it to the left, see Fig. 33.

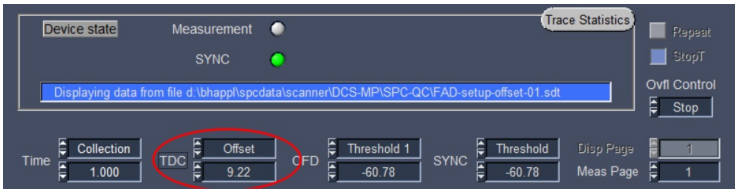



Fig. 32: Changing the TDC Offset from the main panel

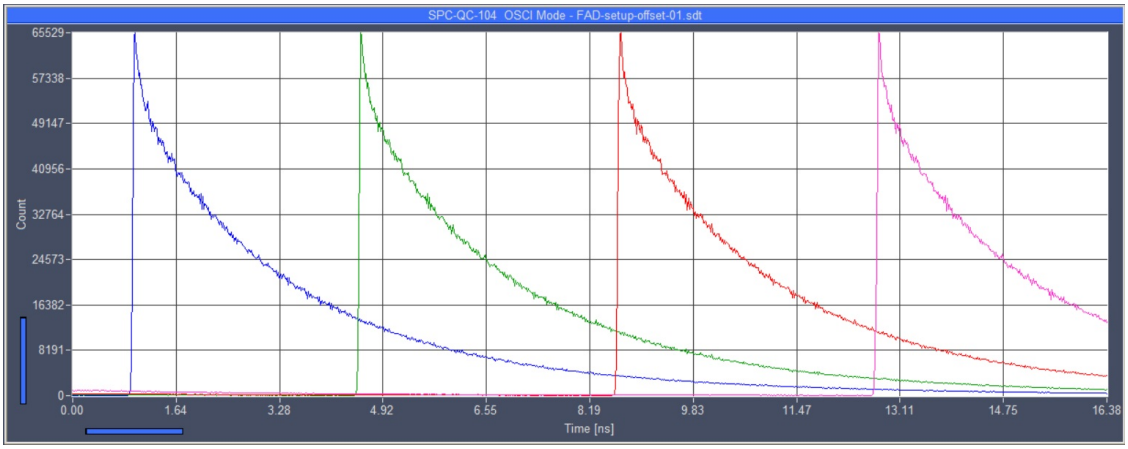



Fig. 33: Shifting the curve by the TDC Offset parameter. From left to right, the curves were recorded with increasing Offset.

The parameters used above are appropriate for fluorescence decay times on the order of a few nanoseconds. For longer decay times a different recording time interval may be desirable. The interval is defined by the TDC Range parameter, see system parameters (fig. ???). Recordings with different

TDC ranges are shown in Fig. 34. With decreasing range the curves are stretched to the right, as can be seen in Fig. 34. Please note that very long recording time intervals may require lower laser repetition rate.

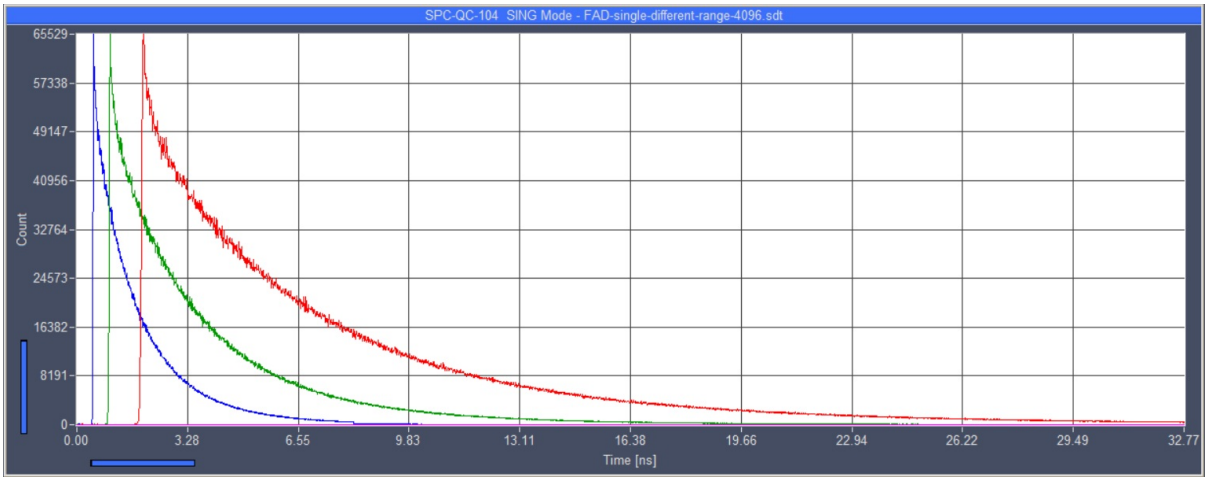



Fig. 34: Fluorescence decay recorded with different TDC Range

# SPCM Software

## Overview

All bh TCSPC modules come with bh's SPCM data acquisition software. SPCM runs the data acquisition in the various operation modes of the SPC modules while controlling peripheral devices, such as detectors, lasers, scanners, or motor stages [1]. Operation modes are available for almost any conceivable TCSPC application. There are modes for fluorescence and phosphorescence decay recording, multi-wavelength decay recording, laser-wavelength multiplexing, recording of time series, FCS and photon counting histograms, and there are modes for FLIM, multi-wavelength FLIM, Mosaic FLIM, time-series FLIM, Z stack FLIM, and simultaneous FLIM/PLIM. Since July 2019 SPCM comes with extended multi-threading capabilities, greatly improving the throughput rate even in case of complex online data and display operations.

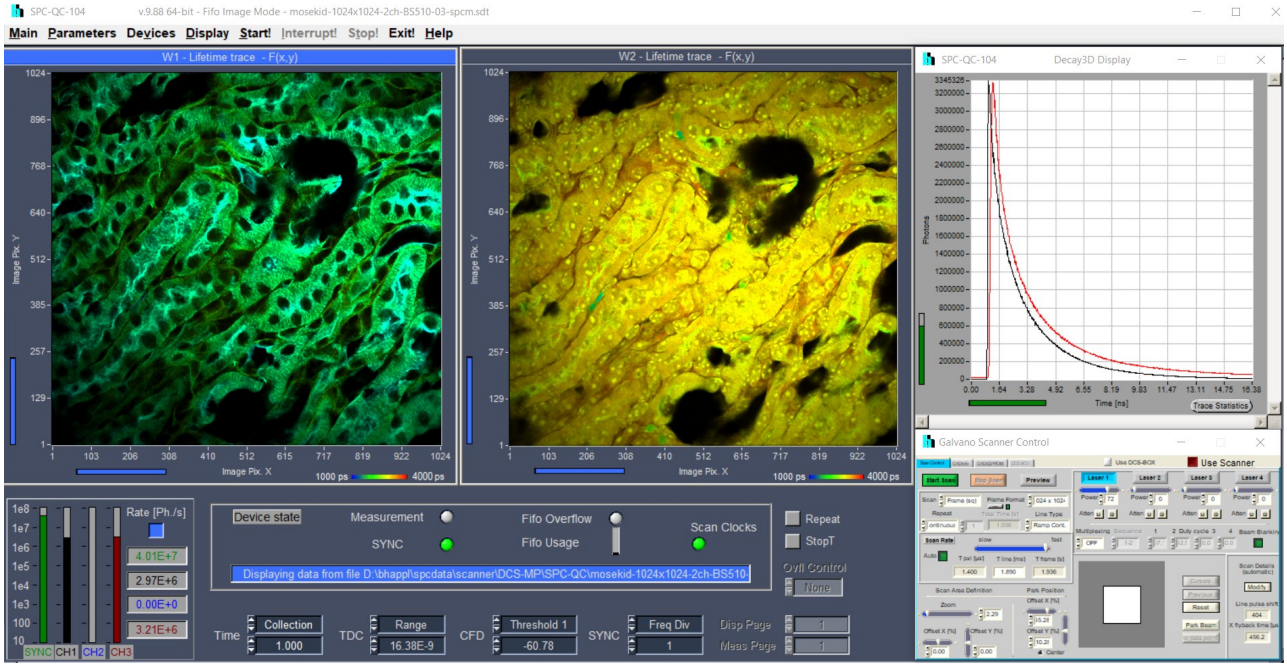



Fig. 35: Example of SPCM Main panel. Dual-channel FLIM, DCS-120 scanner, online-lifetime display, decay curves in ROIs of images

SPCM includes the management of measurement and control parameters, user-interface configuration parameters, and parameters for online data visualisation. Load and save functions are provided to handle measurement and setup data. A large part of SPCM provides display functions for multi-dimensional data. There are display functions for decay curves, series and arrays of decay curves, intensity and lifetime images, and arrays of images. Different projections can be used to create images from selected planes within a multi-dimensional data array. Display functions also contain online calculation and display of decay curves from ROIs within lifetime images. A direct link is provided for communication with SPCImage NG FLIM analysis software.

A 150-page description of the SPCM software is available in the bh TCSPC Handbook [1], chapter 'SPCM Software'. Most of the SPCM functions can be used for the SPC-QC modules as described there. The following sections describe features which are either essential to the function of all TCSPC modules or special for the SPC-QC. For functions not described, please refer to the bh TCSPC Handbook.

## System Parameters

### Overview

The System Parameters define the internal functions of the SPC or SPC-QC module hardware and the data transfer between the hardware and the software. The parameters include the operation mode, control parameters for sequential measurements, page stepping, repeat, accumulation and autosave functions. Furthermore, the system parameters control the settings of the CFD, SYNC, and TDC parameters as well as the routing and scanning parameters. Because not all parameters may be applicable to all operation modes and SPC modules some details of the system parameter panel change with the operation mode and module type. A typical system parameter panel for an SPC-QC-104 system is shown in Fig. 36.

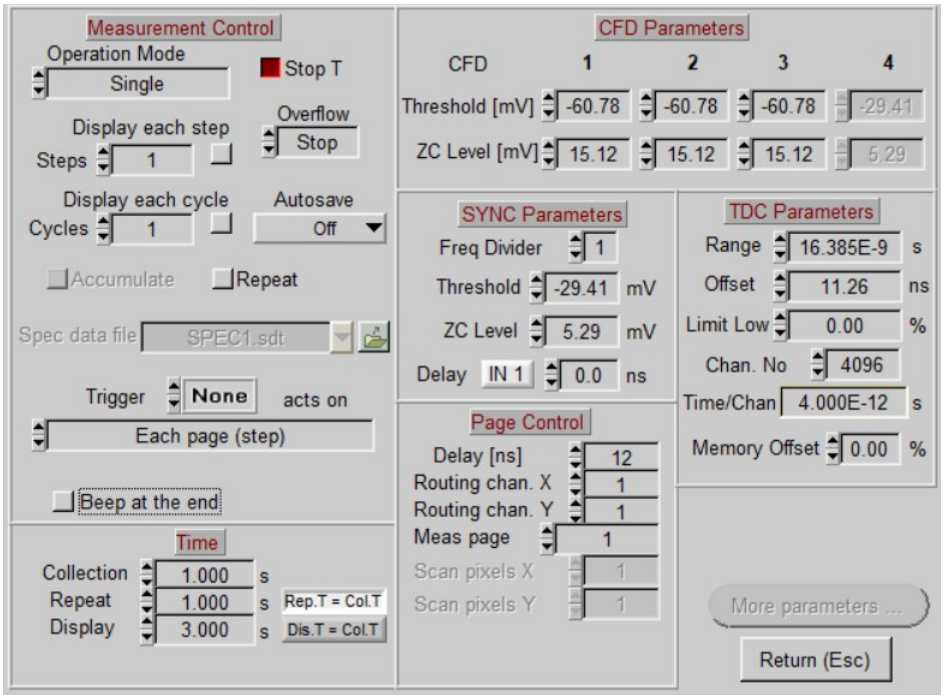



Fig. 36: SPCM system parameter panel, SPC-QC-104, 'Single' mode

### Measurement-Control Parameters

The Measurement-Control part allows the user to define the operation mode, to configure the measurement procedure via the page stepping, cycle, accumulate and autosave functions, and to define the action of the experiment trigger. Collection time, repeat time, and display time are defined in the 'Time' section at the bottom of the measurement control parameters. The measurement control part of the panel can change slightly changes with the SPC module type and with the operation mode. However, most of the measurement control parameters are the same for the SPC-QC and the other SPC modules. The differences are mainly in the available operation modes, see below.

### Operation Mode

Available operation modes of the SPC-QC-104 are Single, Oscilloscope, F(txy), F(t,T), F(t,EXT), FIFO, or FIFO Imaging, see Fig. 37. The general function of these modes is the same as for the SPC-series modules. We will therefore just give an overview here. However, there are some subtle differences which we will point out below.

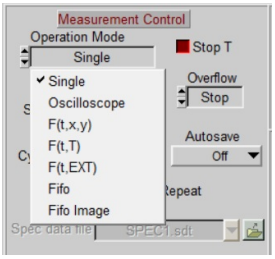



Fig. 37: Operation modes of the SPC-QC-104

### Single Mode

'Single' performs a classic TCSPC measurement. In the simplest case it records a fluorescence decay curve or another waveform of an optical signal. An example is shown in Fig. 38. The recording can be run over a predefined collection time ('Stop T'), continued until 65.535 photons are recorded in the maximum of a curve ('Stop Overflow') or continued until the operator manually stops the measurement. The difference to the SPC series modules is that the SPC-QC module have three recording channel per board. Therefore three such measurements can be performed simultaneously. Another difference to the SPC modules is that the SPC-QC can record into a larger number of time channels. The SPC-QC module can work with up to 65536 time channels, the SPC modules only with 4096 channels. The routing function works the same way for the SPC-QC and the SPC modules. Also the Trigger and the Page Stepping and Cycle functions are the same as for the SPC modules. Please see [1].

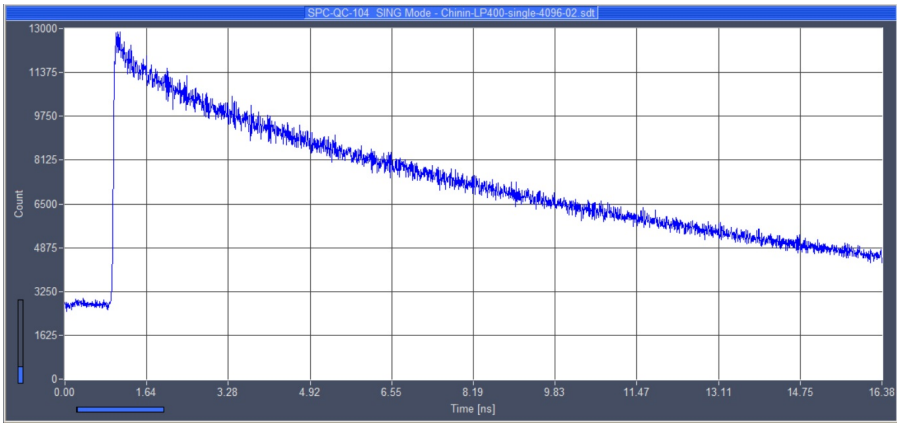



Fig. 38: Fluorescence decay curve of quinine sulphate, recorded in the 'Single' mode

### Oscilloscope Mode

In the 'Oscilloscope' mode the SPC-QC performs a repeated measurement in intervals of the selected 'Collection Time'. Parameters and control features are the same as for the Single mode.

### F(t,x,y) mode

F(t,x,y) is a one- or two-dimensional routing mode. Typically, signals are recorded by a multi-anode detector. The channels are arranged in one dimension along an x coordinate or in two dimensions along an x coordinate and a y coordinate. The recording procedure builds up a photon distribution over t (the photon time) and x (the distance along x) or a photon distribution over t and the distances along x and y. The main application is multi-wavelength decay recording by the process described in Fig. 13. With its three channels, the SPC-QC can, in principle, build up three multi-wavelength photon distributions simultaneously. A typical result is shown in Fig. 39.

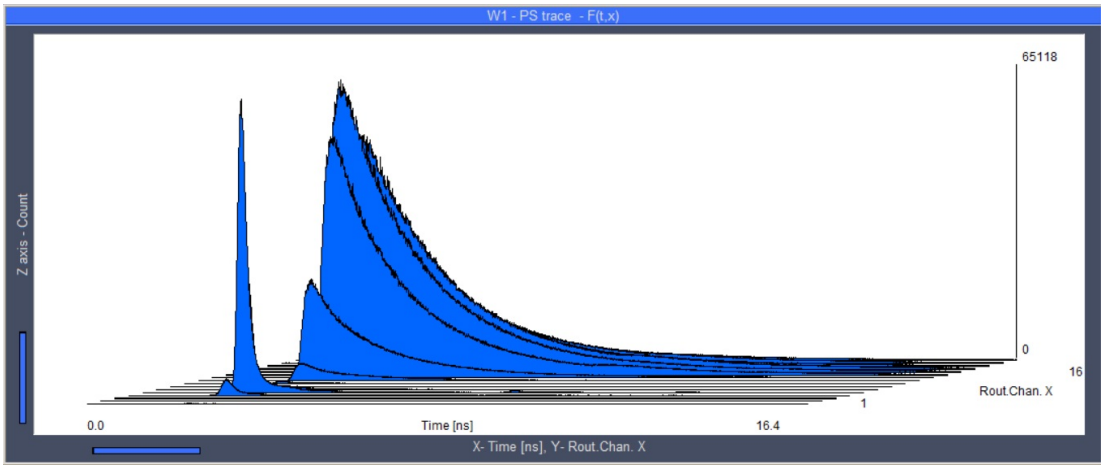



Fig. 39: F(t,x,y) mode. Multi-wavelength fluorescence-decay measurement

### F(t,T), F(t,EXT) modes

F(t,T) and F(t,EXT) are sequential recording modes. Sequences of fluorescence decay curves or other optical waveforms are recorded as a function of time or as a function of an external parameter. Three such sequences can be recorded by the thee channels of the SPC-QC, the individual channels can be further extended by routing. The measurement-control parameters are the same as for the SPC modules. Please see [1].

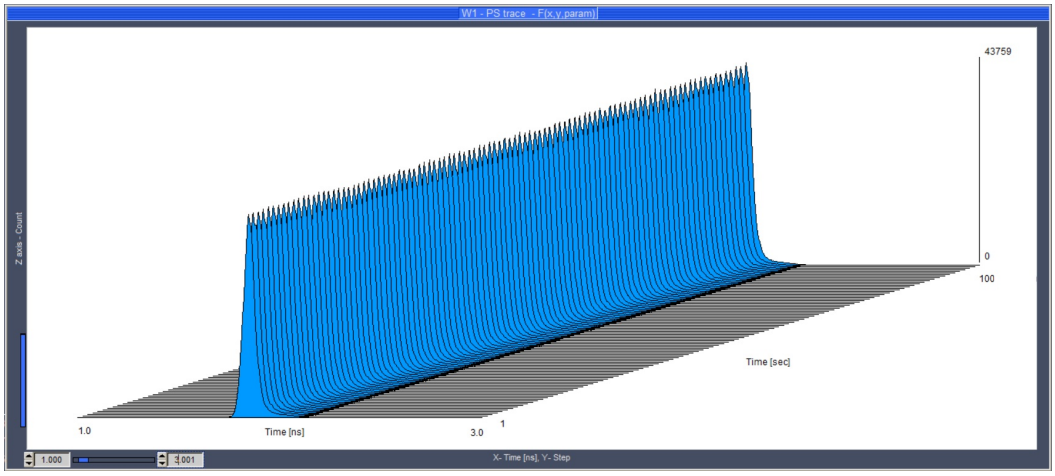



Fig. 40: F(t,T) mode. Sequential recording of an optical signal.

### FIFO Mode

In the FIFO mode the SPC-QC creates a data stream of time- and parameter-tagged single photon data and sends them to SPCM. SPCM stores these data for further analysis, and/or immediately calculates decay curves, auto- and cross-correlation functions, photon counting histograms, or time traces (or Multichannel Scaler, MCS curves) from them. A special feature is triggered MCS recording, which is used for simultaneous fluorescence and phosphorescence decay recording. Three channels can be recorded simultaneously, each channel can be extended by routing. Markers can be included in the data stream to tag the times of external events.

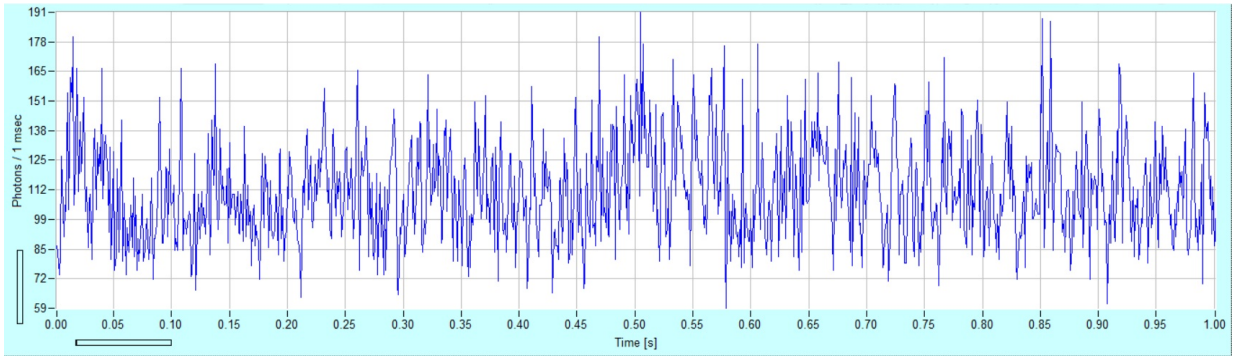



Fig. 41: FIFO Mode, Intensity (MCS) trace, diffusion of fluorescent molecules through laser focus

## FIFO Imaging

The FIFO Imaging mode builds up FLIM data from a stream of time- and parameter-tagged single-photon and pixel, line, and frame marker data. Each pixel marker indicates the transition to the next pixel, each line marker the transition to the next line, and each frame marker the transition to the next frame. SPCM analyses these data, determines the times of the individual photons and the x-y position of the scanner in the moment of their detection, and builds up a photon distribution over these parameters. The recording process is illustrated in Fig. 14, page 13. The result is an array of pixels, each containing a fully resolved decay curve in a large number of time channels. Typical FLIM data formats are 512x512 to 2048x2048 pixels, with 256 to 1024 time channels per pixel. An example is shown in Fig. 42.

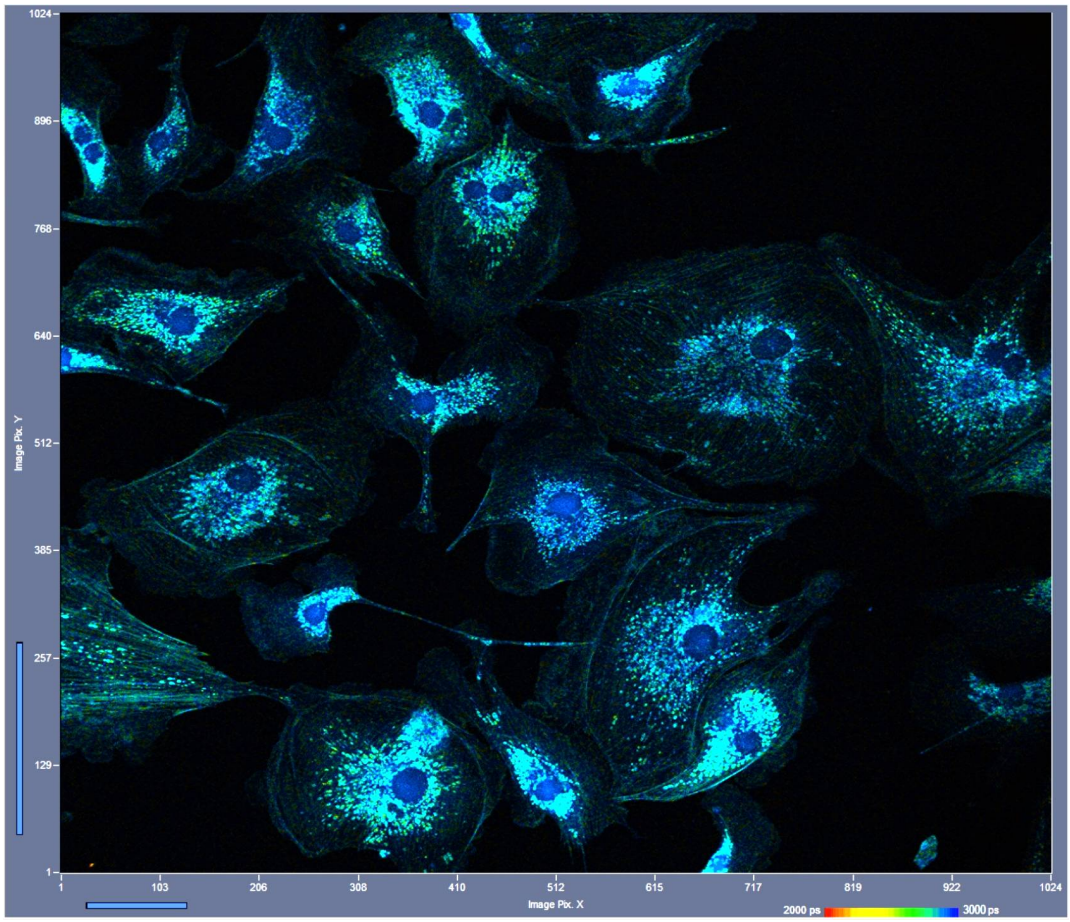



Fig. 42: FIFO Imaging Mode. FLIM image with 1024x1024 pixels and 1024 time channels per pixel

With the SPC-QC module three such FLIM images can be built up simultaneously. Each channel can be further extended by routing, allowing for multi-wavelength detection and laser wavelength multiplexing.

Another extension is 'Mosaic FLIM'. In combination with a motorised sample stage, SPCM records FLIM images of large sample areas without restriction by the limited field of view of the microscope lens. Moreover, Mosaic FLIM can be used to record extremely fast time series of FLIM images. By using periodic stimulation of a transient process in the sample and synchronisation of the mosaic recording with the stimulation, it is possible to accumulate such 'temporal mosaic' data over many stimulation periods. The speed of the mosaic series is then no longer limited by decrease in the photon number. Details are described in the bh TCSPC Handbook [1], chapter 'SPCM Software'.

The FIFO Imaging mode is also able to build up lifetime images from MCS data. It can do so even simultaneously with the buildup of FLIM data. The result is simultaneous FLIM / PLIM, a method which has found wide application in live sciences since its introduction by bh in 2015. Please see bh TCSPC Handbook [1], chapter ???.

## CFD Parameters

The CFD parameters control the threshold and the zero-cross levels of the constant-fraction discriminators, see Fig. 10, page 10. The settings in the System Parameters are shown in Fig. 43. The panel shows the settings for CFDs which are used to receive detector signals. In configurations which use channel 4 for synchronisation with a pulsed light source these are CFDs 1 to 3; the CFD4 settings are shown under 'SYNC Parameters'.

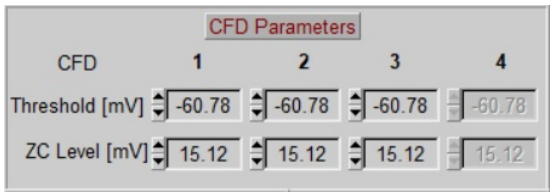



Fig. 43: CFD Parameters

## SYNC Parameters

The SYNC parameter section of the System Parameters is shown in Fig. 44. It contains the Threshold and the Zero Cross level of the discriminator, a Frequency Divider ratio, and the setting for a USB-controlled delay unit (see page 60) which may be connected in the SYNC signal path. With frequency-divider ratios larger than one several signal periods of the optical signals are recorded. The SYNC delay is used to balance the signal transit time in the SYNC channel with that of the detector (CFD) channels. It can be used if, for whatever reasons, the TDC offset (see below) is not sufficient to place the recorded part of an optical signal correctly in the recording-time interval.

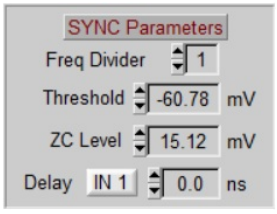



Fig. 44: SYNC parameters

## TDC Parameters

The TDC-parameter section controls the parameters for time measurement in the TDCs. 'Range' is the time-conversion range, 'Offset' the position of a recorded waveform inside the observation time interval, and 'Range' is the length of the observation time interval. Examples are shown in Fig. 33 and Fig. 34, page 23. 'Chan. No'. is the number of time channels, and 'Time/Chan' the time-channel width resulting from the selected combination of 'Range' and 'Chan. No'. Please note that the minimum time-channel width is 4 ps. Therefore not all combinations of parameters are possible. SPCM therefore corrects the user inputs and suggests parameter combinations which are within the capabilities of the hardware. Examples are shown in Fig. 45 and Fig. 46.

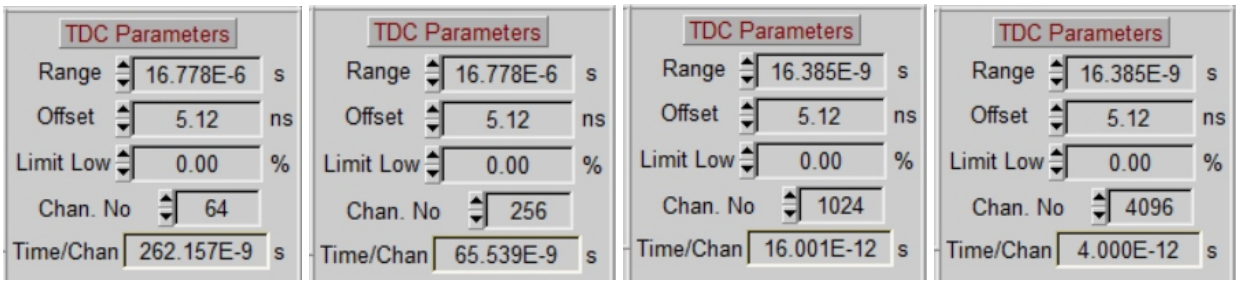



Fig. 45: TDC settings, different Channel Number with same Range. The combinations yield different Time/Channel.

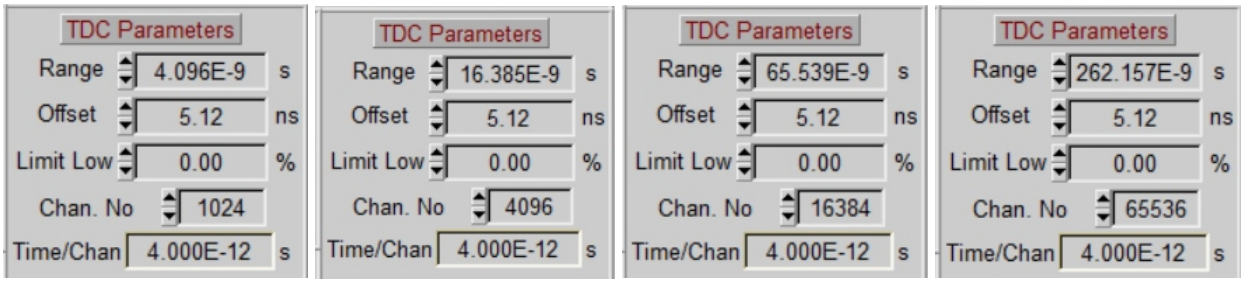



Fig. 46: TDC settings, different Range with same Time/Channel, different Channel Number.

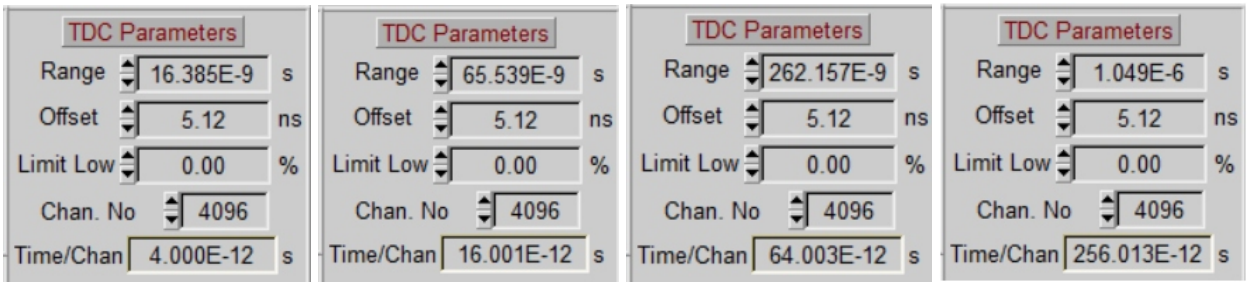



Fig. 47: TDC settings, different Range with same Channel Number, different Time/Channel

Not all combinations are available for all operation modes. For examples, the maximum Channel Number for FIFO Imaging is 4096, and for the FIFO mode Channel Number is always 4096.

## Page Control

Page Control contains parameters related to the data structure of the results and the associated system configuration. 'Delay' is the time after the detection of a photon when the routing signals are read. 'Routing chan. X' and 'Routing chan. Y' are the numbers of routing channels in X and Y direction. Image pixels X and Y is the pixel format for lifetime images. Channel Correction opens a table by which the delay non-uniformity of the channels of a multi-wavelength detector can be corrected. Mosaic Imaging opens the menu that defines the format of image mosaics. 'Max Image Size' opens a

menu to define the memory space claimed by SPCM. The Page control section is different for different operation modes. Examples are shown in Fig. 48.

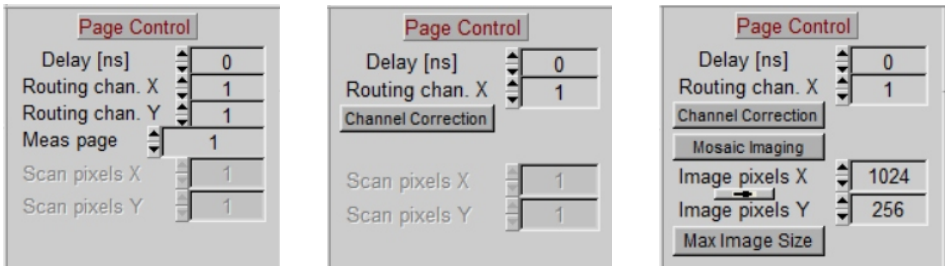



Fig. 48: Page Control section of SPCM system parameters. Left to right: Single Mode, FIFO Mode, FIFO Imaging Mode.

The Page Control parameters are the same as for other bh SPC modules. Please refer to [1] for details.

## More Parameters

'More Parameters' opens a menu showing parameters which are specific to the selected operation mode. For example, in the FIFO Imaging mode the 'More Parameters' define the synchronisation of the recording with the frame, line, and pixel clock pulses of the scanner, the pixel clock source and the type of the scan (unidirectional or bidirectional). The corresponding parameter panel is shown in Fig. 49.

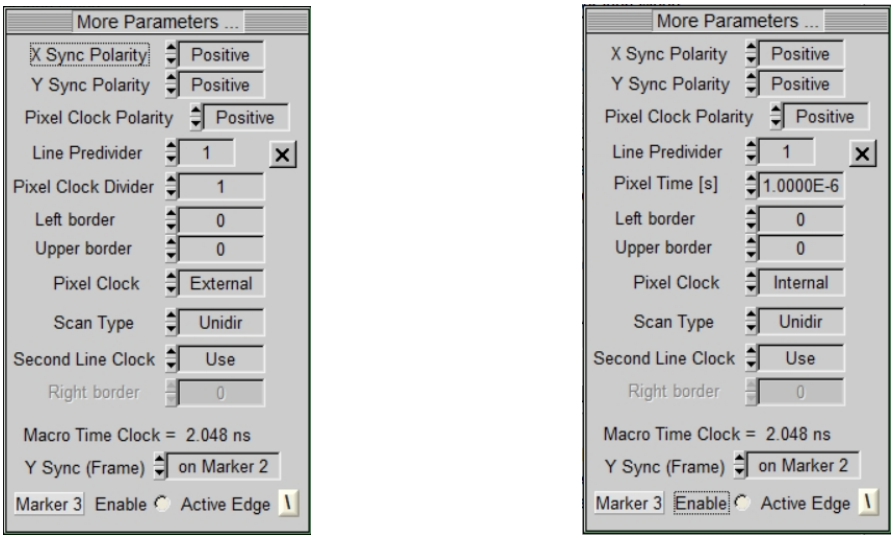



Fig. 49: 'More Parameters' panel for the FIFO Imaging mode. The parameters define the interaction of the recording process with the scanner. Left: External pixels clock. Right: Internal pixel clock.

For function of the parameters and their influence on the recording process please see bh TCSPC Handbook [1].

## Display Functions

The bh TCSPC modules record multi-dimensional data. In the simplest case, the result of a measurement may just be a single fluorescence decay curve or a single waveform of another optical signal. However, a measurement can also produce several curves simultaneously. In case of the SPC-QC, the curves can represent data recorded by different channels of the module, data recorded by several detectors connected to a single channel via a router, data recorded by several channels of a bh multi-wavelength detector, data recorded with several multiplexed lasers, or data from subsequent steps of a time-series recording procedure. Several such data sets can by recorded in different memory pages. Moreover, the curves may not represent waveforms of light signals at all but FCS curves, photon counting histograms, or time-resolved spectra. A few examples of the display of TCSPC curves are shown in Fig. 50.

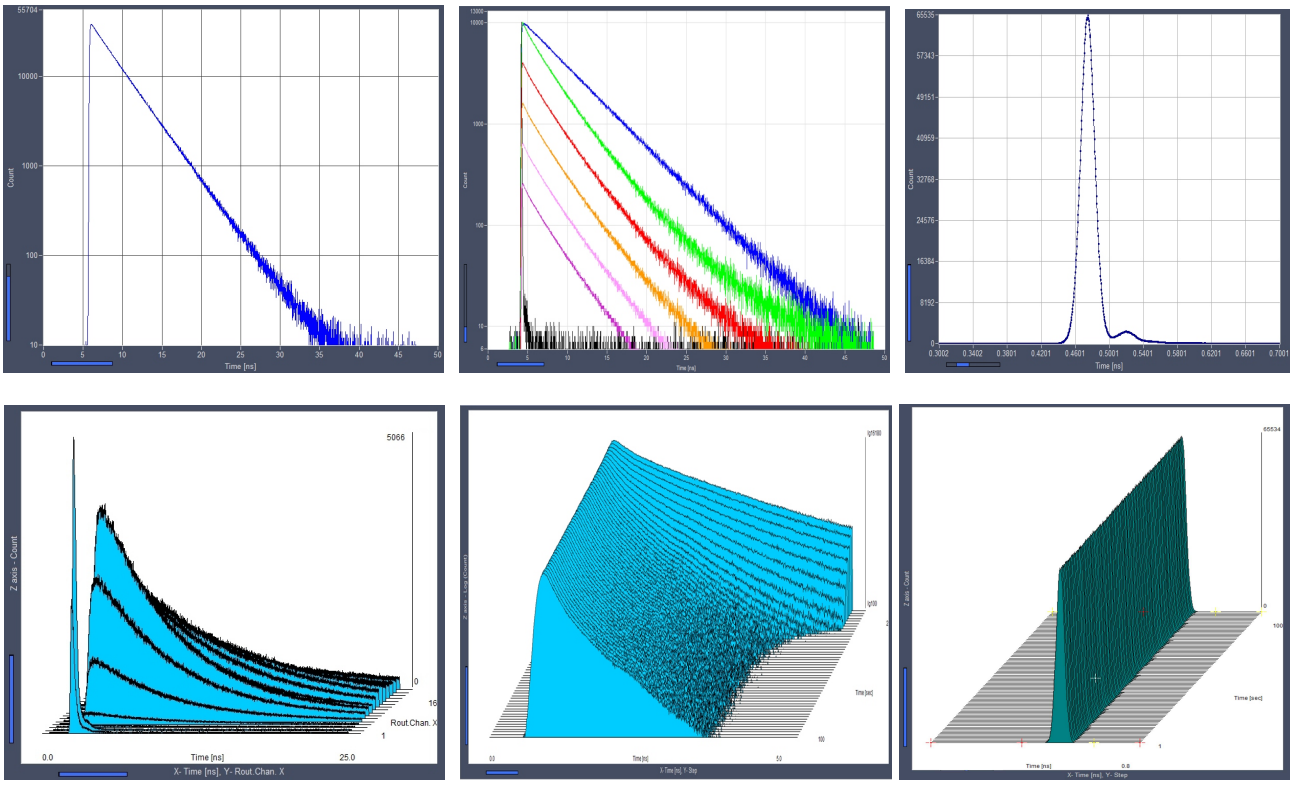


Fig. 50: Curve display in SPCM. Upper row: 2D curve display. Lower row: 3D curve display.

The structure of TCSPC data becomes even be more complex when an SPC module is used in the imaging mode. A single TCSPC image is an array of pixels, with each pixel containing a decay curve in form of photon numbers in consecutive time channels. Several such data arrays may exist if a multi-wavelength detector is used, or if the measurement system is using routing or laser multiplexing. Even more, mosaic imaging delivers a two-dimensional array of TCSPC images. Data with more than two dimensions cannot easily be displayed by a two-dimensional display device or printer. The data must be projected in a single plane, and the way this projection is performed must be suitably selected. Examples are shown in Fig. 51.

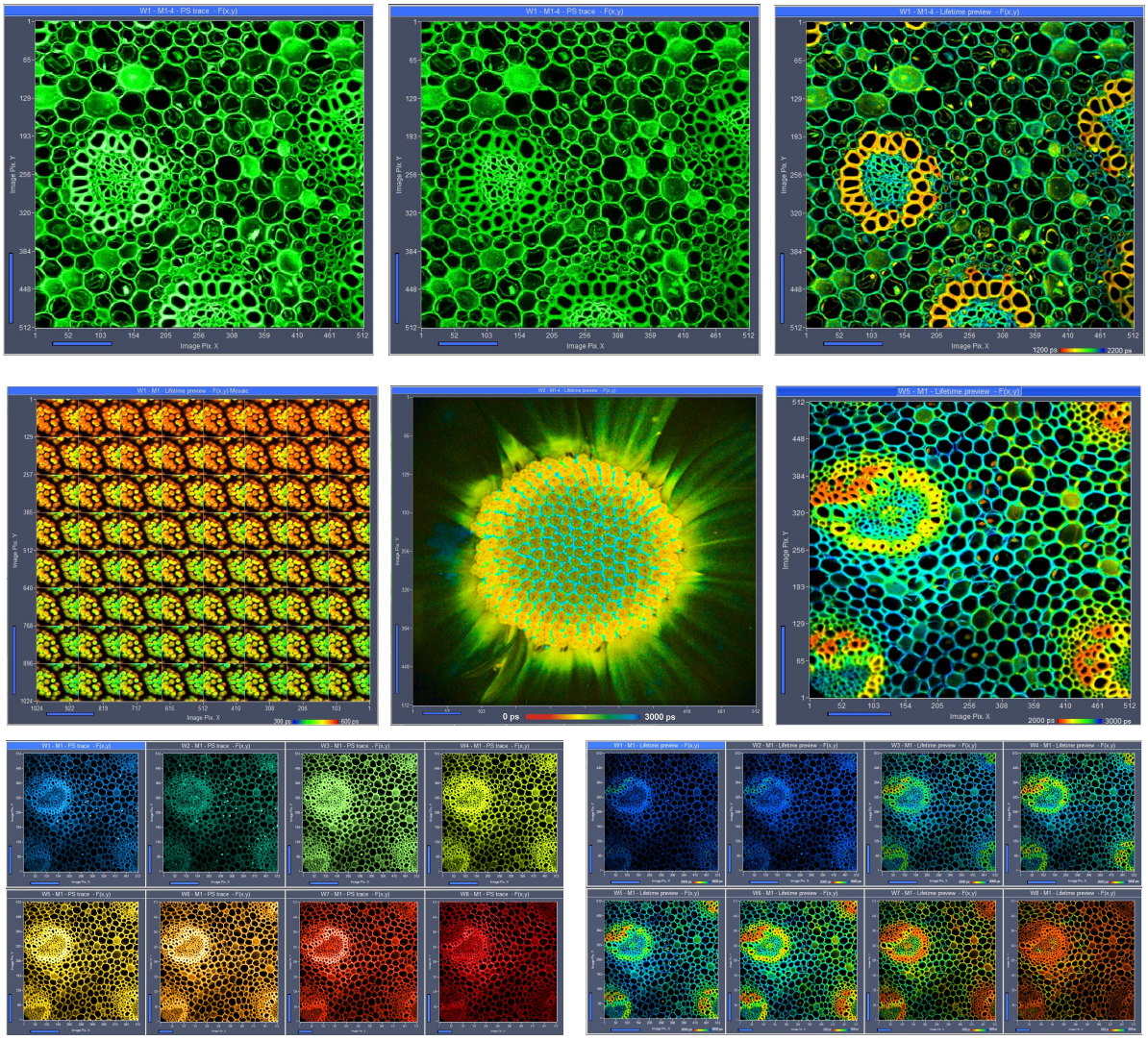


Fig. 51: Examples of SPCM image display. Upper row: Intensity image, gated intensity image (of late photons), lifetime image. Middle row: Mosaic FLIM image, image from several combined channels of a fast FLIM system, single image from multi-wavelength FLIM data. Lower row: Multi-wavelength intensity image in 8 routing windows selecting 2 adjacent channels each, colours adjusted to real emission wavelengths, multi-wavelength image, colour-coded lifetime.

## Display of 2D Data

Decay curves recorded in a single channel or in several channels of an SPC-QC module, data recorded by several detectors, by several channels of a bh multi-wavelength detector, by several multiplexed lasers, or data from the steps of a time-series recording procedure can be displayed by the 2D display functions of the SPCM software. The 2D function puts the curves in a single t-y diagram, as shown in Fig. 50. The display of 2D data is controlled by the 2D Trace Parameters in combination with the Display Parameters. The 2D Trace Parameters determine which data are displayed, the Display Parameters determine how they are displayed. The parameters act both on the display of data in the SPCM main window and on the '2D Curve' routine under 'Display' in the top task bar of SPCM.

## Selection of the Data to be Displayed

The data to be displayed are selected in the 'Trace Parameter' panel. Up to 16 Traces (curves) can be defined. The traces can be curves from different memory pages into which the data have been recorded (Fig. 52, left), curves from different routing channels of an SPC or SPC-QC module (Fig. 52, middle), or curves from different channel of an SPC-QC module (Fig. 52, right).

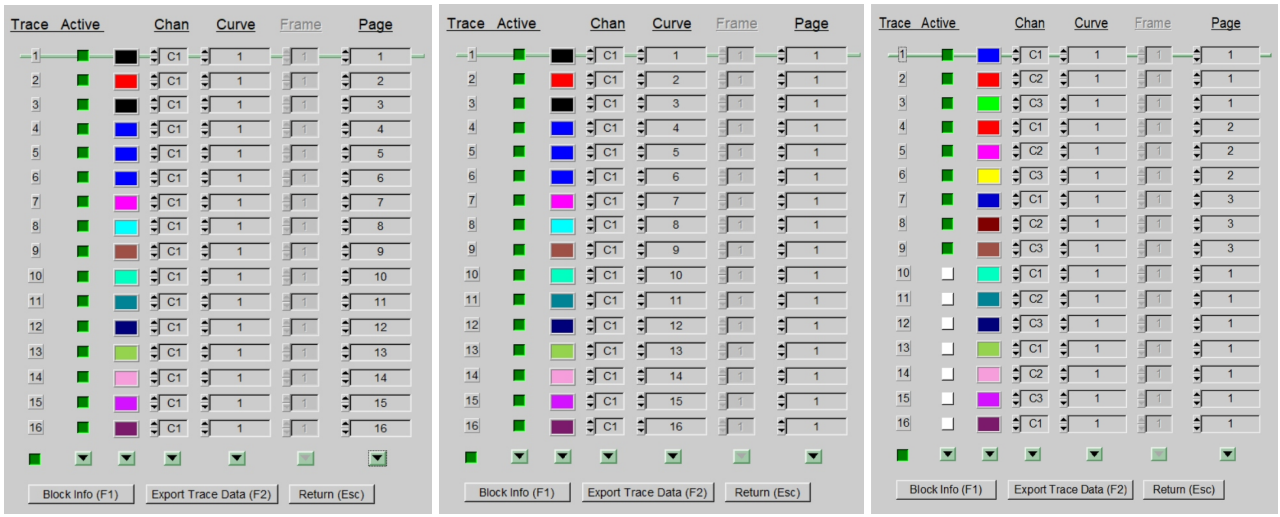



Fig. 52: Examples of trace parameter definitions. Subsequent measurement pages (left), curves from subsequent routing channels (middle), curves from different channels of an SPC-QC module in three different measurement pages (right).

## Display Range and Curve Style

The display range, linear or logarithmic scale, and the style of the curves is defined in the 'Display Parameters'. Examples are shown in the figures below. The display parameters also allow the user to define a grid for the display. The autoscale button automatically adjusts the display range to the maximum of the largest curve in the display.

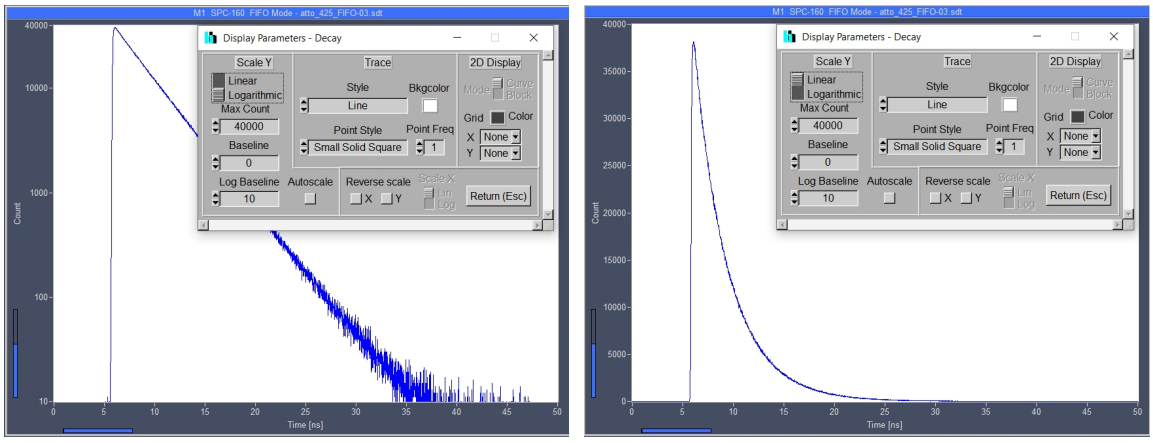



Fig. 53: Curve display for various display-parameter settings. Linear and logarithmic scale.

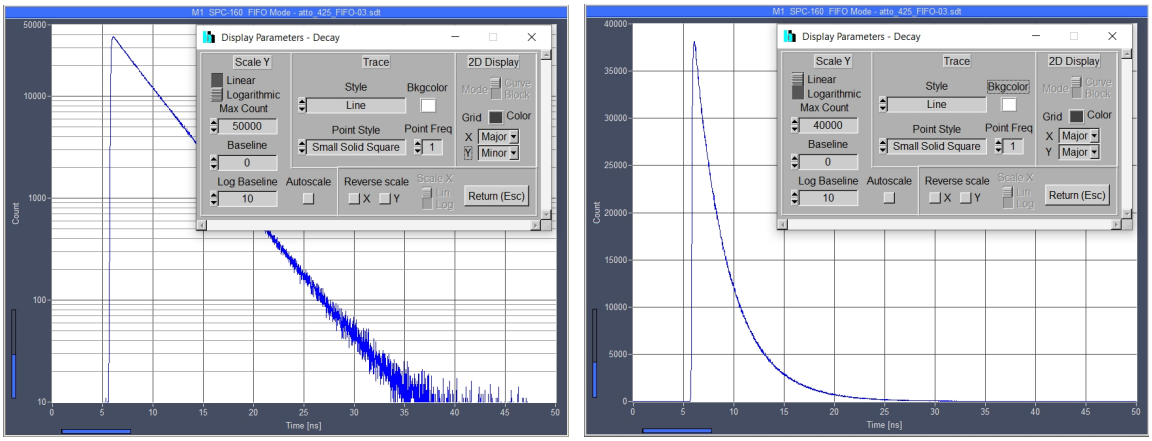



Fig. 54: Curve display for various display-parameter settings. Linear and logarithmic grid.

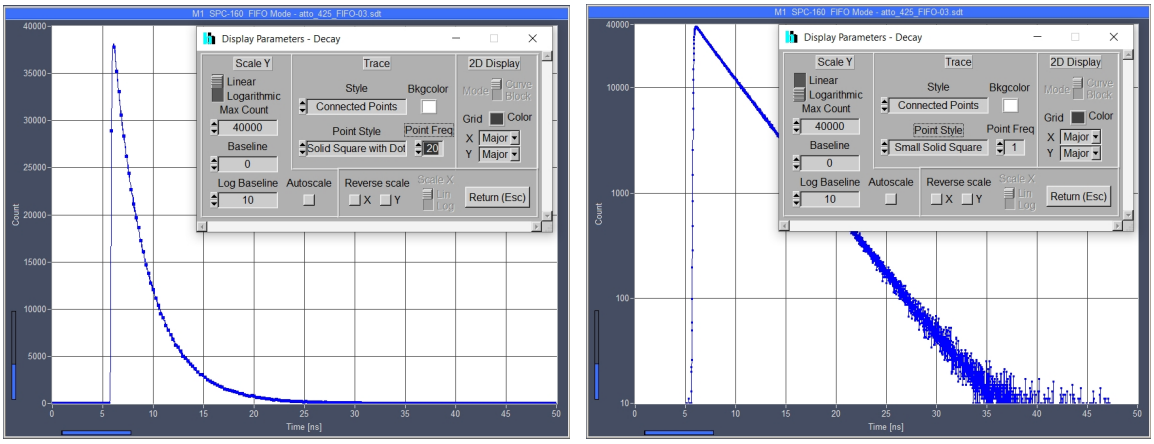



Fig. 55: Curve display for various display-parameter settings. Curve style '^Connected Points', different point frequency.

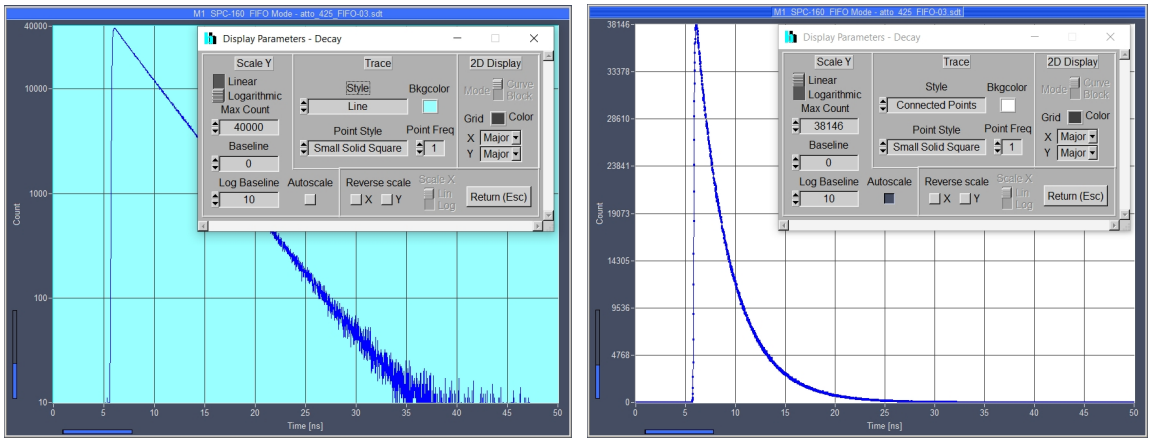



Fig. 56: 2D display for different display-parameters. Different background colours.

## Display of 3D Data

The 3D display function is used to display data from sequential measurements, multi-detector measurements, multi-wavelength measurements, or from combinations of these. The corresponding photon distributions may be built up versus several variables, such as an externally varied parameter, the time in a fluorescence decay, the time in a sequence of decay curves, or the wavelength.

## Display Modes

Examples of the display of 3D data are shown in Fig. 57 and Fig. 59. Fig. 57 shows a sequence of decay curves recorded by scanning the wavelength of a monochromator, Fig. 59 shows 3D data recorded with a 16-channel PML-GaAsP multi-wavelength detector. There are two different modes for 3D display. The data can be displayed as an array of curves (curve mode, shown left) and as a pseudo image in which the brightness and the colour represent the photon numbers (colour-intensity mode, shown right).

## Display Parameters

The display mode and display style are controlled by the Display Parameters. The parameters corresponding to the display configurations of Fig. 57 and Fig. 59 are shown in Fig. 58 and Fig. 60. Display mode is 3D Curves (left) and Colour Intensity (right). Offset X and Offset Y are the offsets of the subsequent curves in the 3D Curve mode. The colour bar associates the colours of the colour-intensity mode to the count numbers in the time channels. The number of colours can be changed by 'No. of Colours', the colours can be selected from a colour palette. Click into the colour bar to do this. An additional 'Mode' parameter defines the third coordinate of the display. It is 'f(x,y,param)' for the monochromator scan, and f(t,x) for the multi-wavelength recording. Please see [1] for further details.

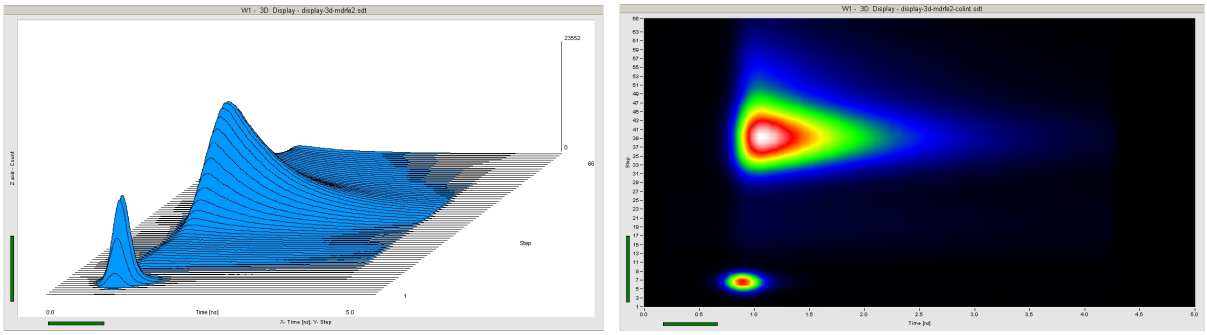


Fig. 57: Result of f(t,ext) measurement, wavelength scan by monochromator. Left curve mode, right colour intensity mode

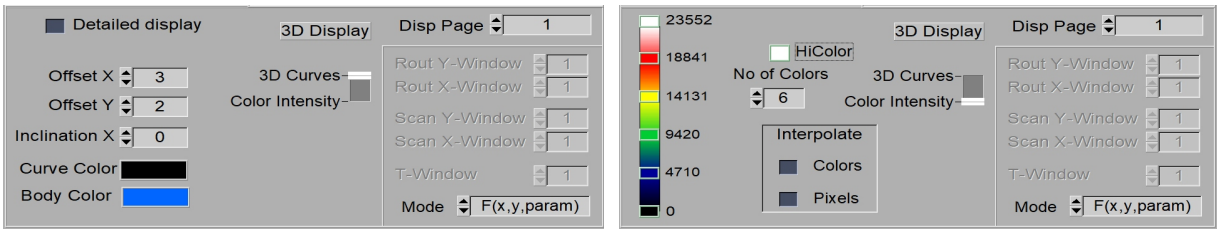



Fig. 58: Display parameters for Fig. 57. Left: Curve mode. Right: Colour-intensity mode.

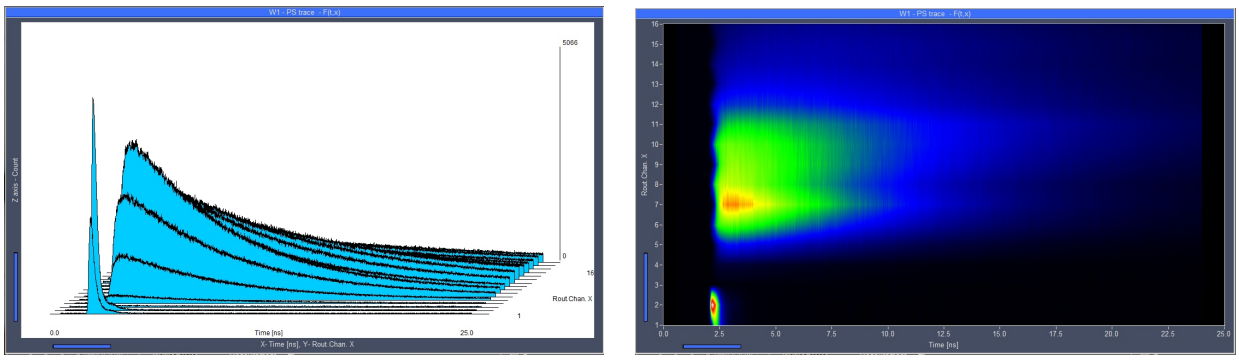


Fig. 59: Result of f(t,x) multi-wavelength measurement with the PML-16 GaAsP detector. Left Curve Mode, right colour intensity mode

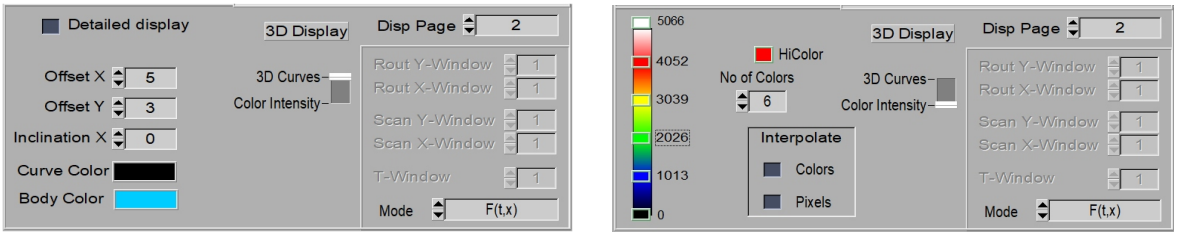



Fig. 60: Display parameters for the data in Fig. 59. Left: Curve mode. Right: Colour-intensity mode.

## Display of FLIM Data

The results of a FLIM measurement are multi-dimensional data arrays. In the simplest case, the dimensions are the two coordinates of the scanning area, X and Y, and the time in a fluorescence of phosphorescence decay, t. However, FLIM data can be more complex. There may be additional coordinates, such as the detector-channel number in multi-wavelength FLIM data, the time step within a series of recordings, or other parameters which were assigned to the photons in the recording process. It may be necessary to display the data in different parameter spaces, e.g. as pure intensity images, as time-gated images, or as colour-coded fluorescence lifetime images. It may even be desired to display the data not as images but as sequences of decay curves integrated over selected spatial windows within the images. The SPCM software allows the user to define up to eight display windows for data of different source, different data type or different projection within the data parameter space. The display of the data is controlled by the '3D Trace Parameters', the 'Window Parameters' and the 'Display Parameters'. This section explains the parameters setup for the display of FLIM data for typical FIFO Imaging measurements and demonstrates it at typical examples.

### Intensity Images from FLIM Data

#### One Image from One Channel of an SPC-QC Module

Fig. 61 shows the parameters for displaying an intensity image from the combined photon numbers in all time channels of a single FLIM image. In the 3D Trace parameters, only one display window (W1) is enabled. The data type to be displayed is ps FLIM, the data come from TCSPC module M1, and the display mode is F(x,y). The Window parameters (bottom left) define a single Time Window, from time channel 1 to 256, and two spatial windows, both from pixel 1 to pixel 1024. In other words, the windows incorporate the entire FLIM data array.

The Display Parameters, Fig. 62, define a colour-intensity image with linear intensity scale. The intensity is coded by colour, the colour scale goes from black over red to white. The image is displayed for Time Window 1 - this is the only time windows defined (see Window Intervals, Fig. 61). It contains the photons of all time channels (from 1 to 256). The SPC Main panel with an image defined by these parameters is shown in Fig. 63.

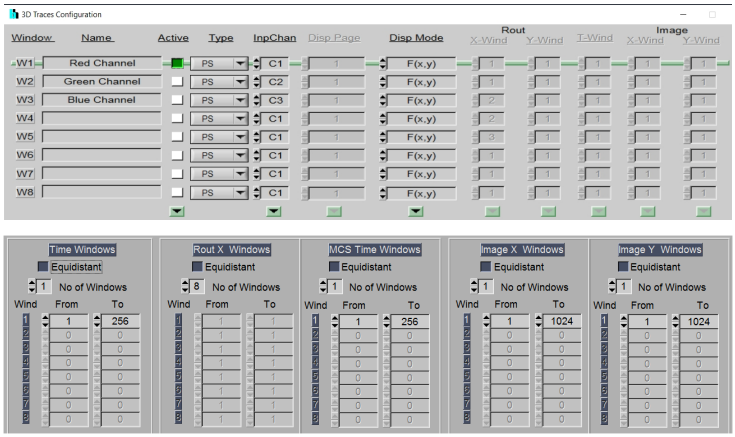



Fig. 61: 3D Trace parameters and Window Parameters for the display of a single image

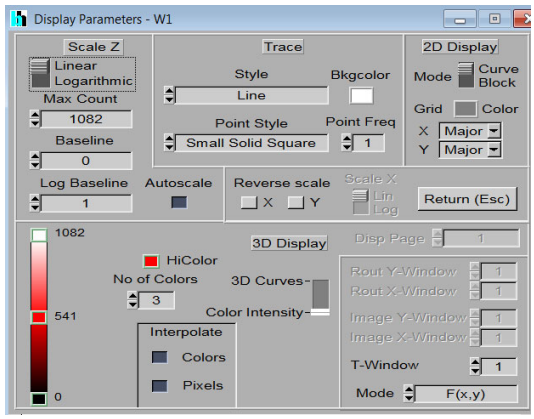



Fig. 62: Display parameters

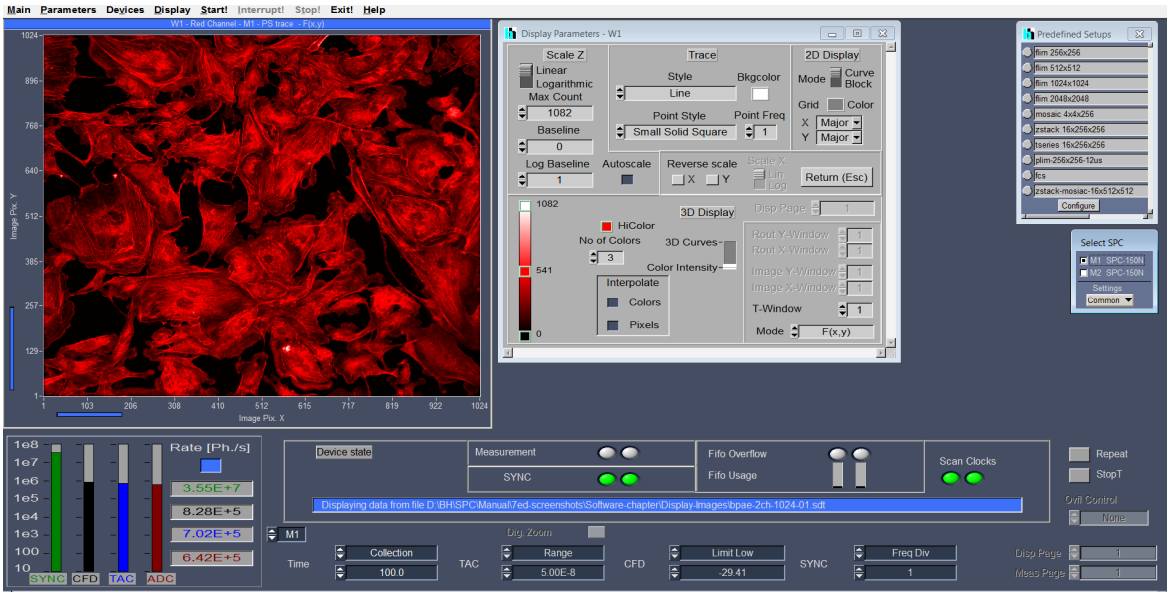



Fig. 63: SPCM Main panel with the image defined by the parameters shown above

### Images from Two Channels of an SPC-QC Module

The parameter setup for the display of two images from different channels is shown in Fig. 64. The Trace Parameters define two display windows. They contain data from two SPC modules, M1, and

M2. The window parameters are the same as for a single image - there is only one time window, containing the photons of all TCSPC time channels from 1 to 256.

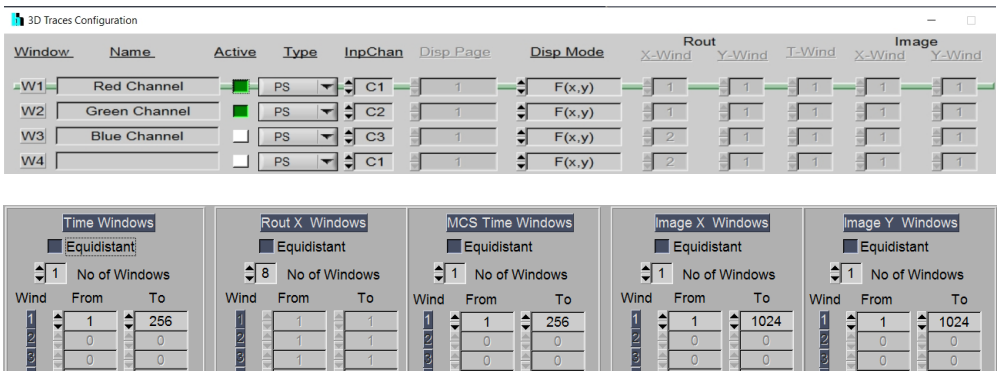



Fig. 64: 3D Trace parameters and Window parameters for the display of the images from two SPC modules, M1 and M2

The two images are displayed with separate display parameters, see Fig. 65. The SPCM main panel with the two images is shown in Fig. 66.

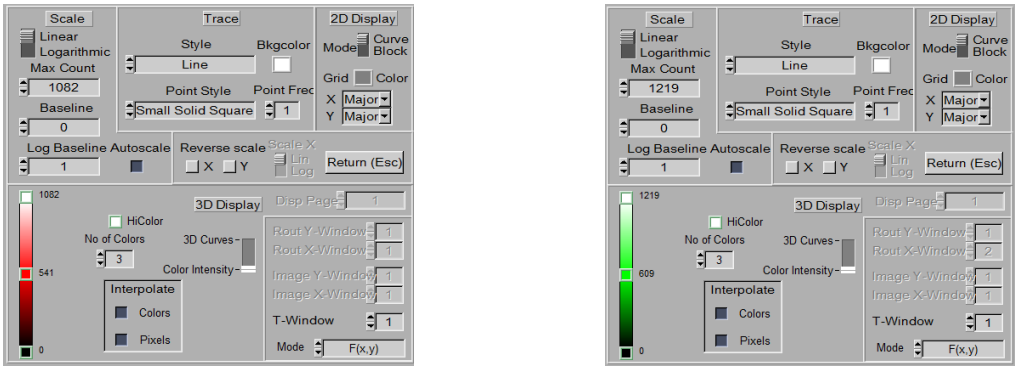



Fig. 65: Display parameters for the two images defined by the parameters in Fig. 64

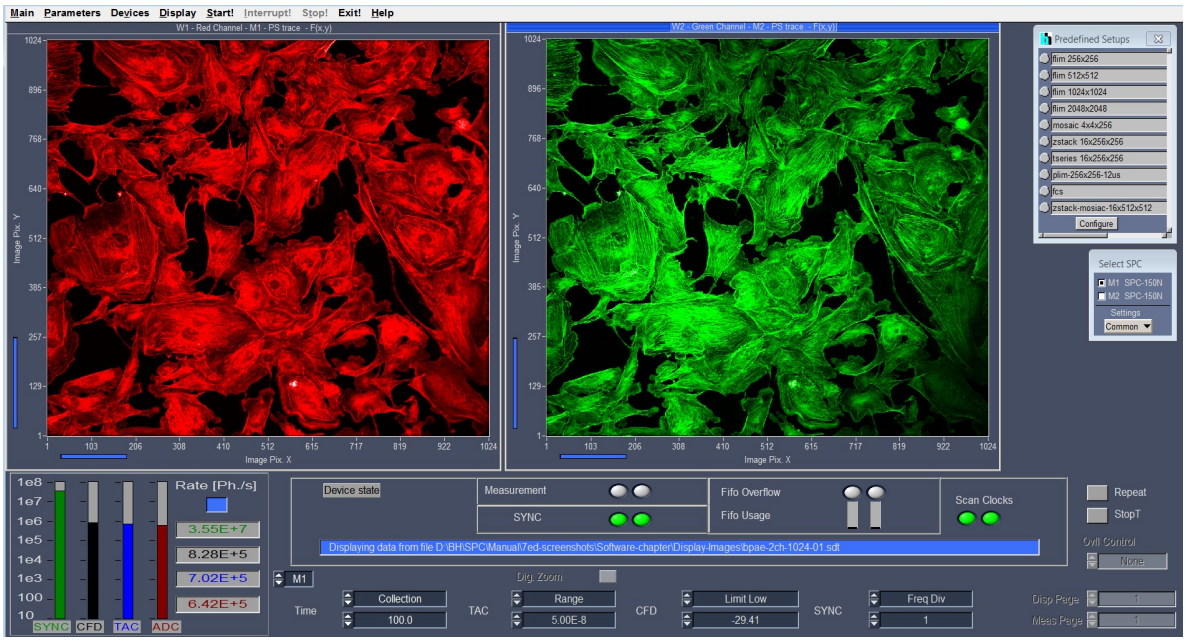



Fig. 66 SPCM Main panel. Two images for separate SPC-QC channels.

**Intensity Images from Routing or Laser-Multiplexing Channels**

Images from several routing channels are displayed as shown in Fig. 67. The 3D Trace Parameters define several display windows for different routing channels. The data in these channels come from two detectors connected to channel 1 and channel 2 of the SPC-QC (Em 560 and Em 405), the routing bits come from the multiplexing of two lasers (Ex 488 nm and Ex 405 nm). The 3D trace parameters define the number of display windows used, and the information to be displayed in the windows. The Window parameters define which routing channels or groups of routing channels are displayed in the windows. The 3D Trace parameters and Window parameters and the images created by this setup are shown in Fig. 67.

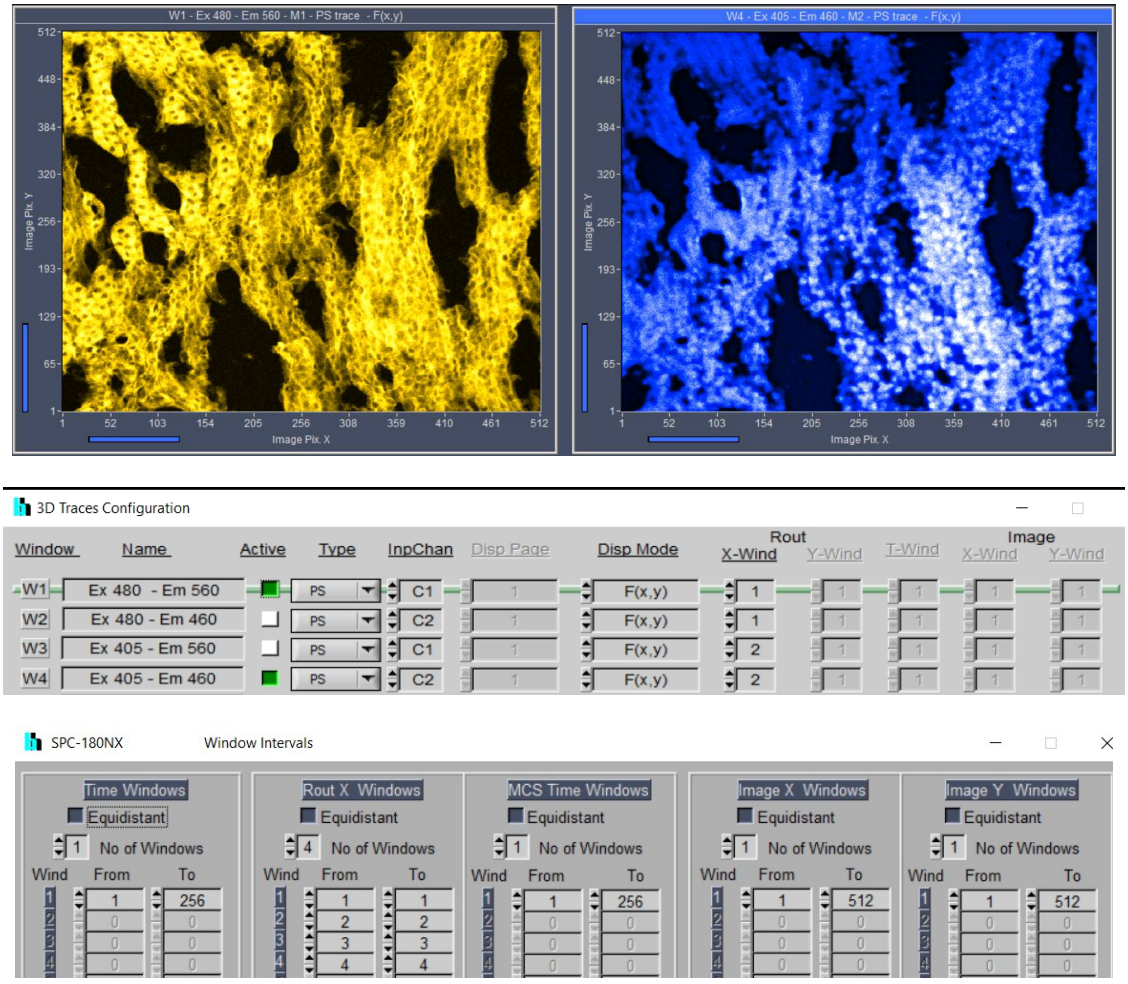



Fig. 67: Two images from a dual-channel wavelength-multiplexing system, intensity display. 3D trace parameters and Window parameters shown at the bottom. Four combination of excitation and emission wavelength exist, two of them are switched active in the 3D trace parameters and displayed in two image windows of SPCM.

**Multi-Wavelength Intensity Images**

The display of multi-wavelength FLIM data from a 16-channel detector is shown in Fig. 68 trough Fig. 70. The trace parameters define eight display windows. Data type is ps FLIM. The individual images in the display windows are derived from subsequent ‘Routing Windows’. Each routing window contains the data of two subsequent routing channels, see Window Parameters. This way, data of every two wavelength channels are combined in one image.

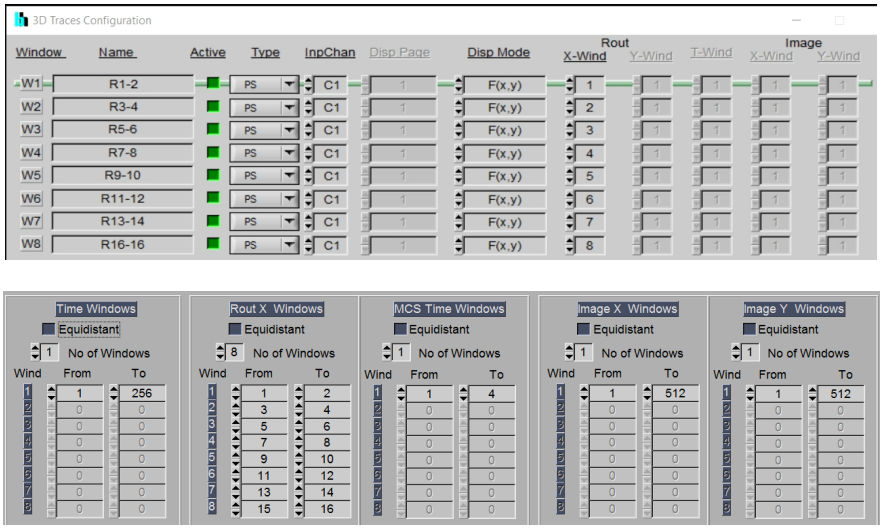



Fig. 68: 3D Trace Parameters and Window Parameters for multi-wavelength FLIM

The individual images have separate display parameters. The display parameters for the first three images are shown in Fig. 69. They contain the colour definition for the individual images, and the routing window. Since the intensities in different wavelength channels can vary over a wide range Autoscale is turned on for all images. The main panel of SPCM with the eight images is shown in Fig. 70.

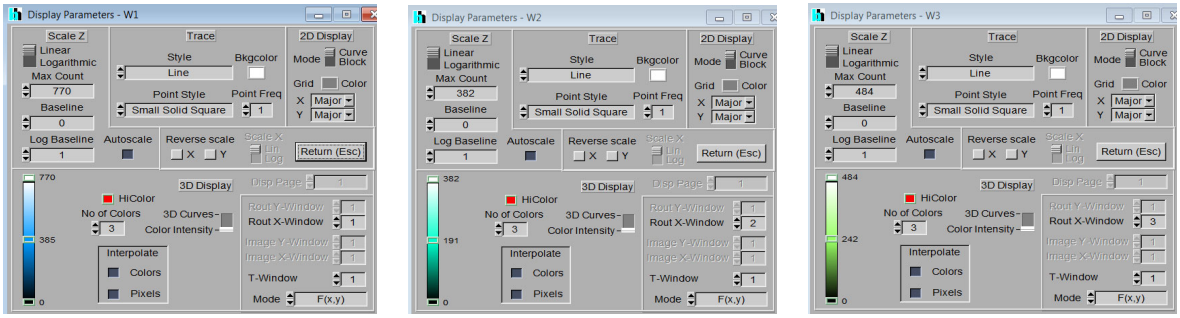



Fig. 69: Display parameters in the first three display channels of a multi-wavelength measurement

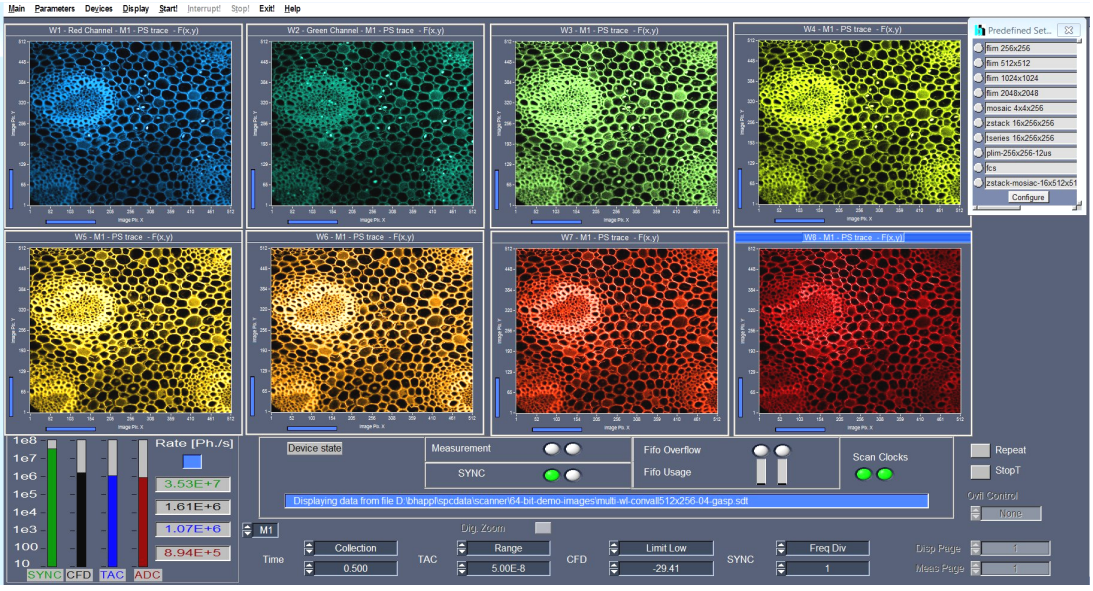



Fig. 70: SPCM main panel for multi-wavelength FLIM

## Colour-Coded Lifetime Images

Fig. 71 shows the definition of the parameters for run-time lifetime display for a two-channel SPC-QC system. For online-lifetime display of colour-coded lifetime images, select 'Type' = 'LIFET' in the 3D trace parameters.

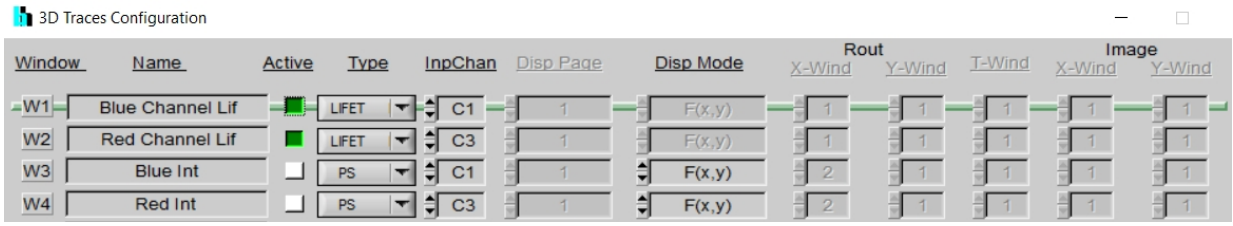



Fig. 71: 3D trace parameters for run-time display of lifetime images. Two SPC-QC channels.

The 'Type' definition need not be identical in all channels. For example, you can display a lifetime in one channel together with an intensity image in the same channel or in another channel, and switch the images 'Active' or inactive on demand.

The display of lifetime-image display requires more parameters than the display of intensity images. The online-lifetime function calculates the first moment of the decay data in the pixels, and subtracts the first moment (the centroid) of the IRF from it [1, 18]. Therefore the temporal location of the IRF must be known. Moreover, the display range for the lifetime, the direction of the colour scale must be known. These parameters are defined in the Display Parameters, see Fig. 72. As usual, there is a separate set of display parameters for each image. In the lower part of the panels the lifetime range, the direction of the colour bar, the brightness and the contrast, and the reference moment for the IRF is defined. The reference moment can be determined with SPCImage or calculated from a reference FLIM file of a sample with known fluorescence lifetime. For principle of run-time lifetime calculation please see [18] or section 'Fast Online FLIM' in the bh TCSPC Handbook. The main panel of the SPCM software with the lifetime images of two SPC channels is shown in Fig. 73.

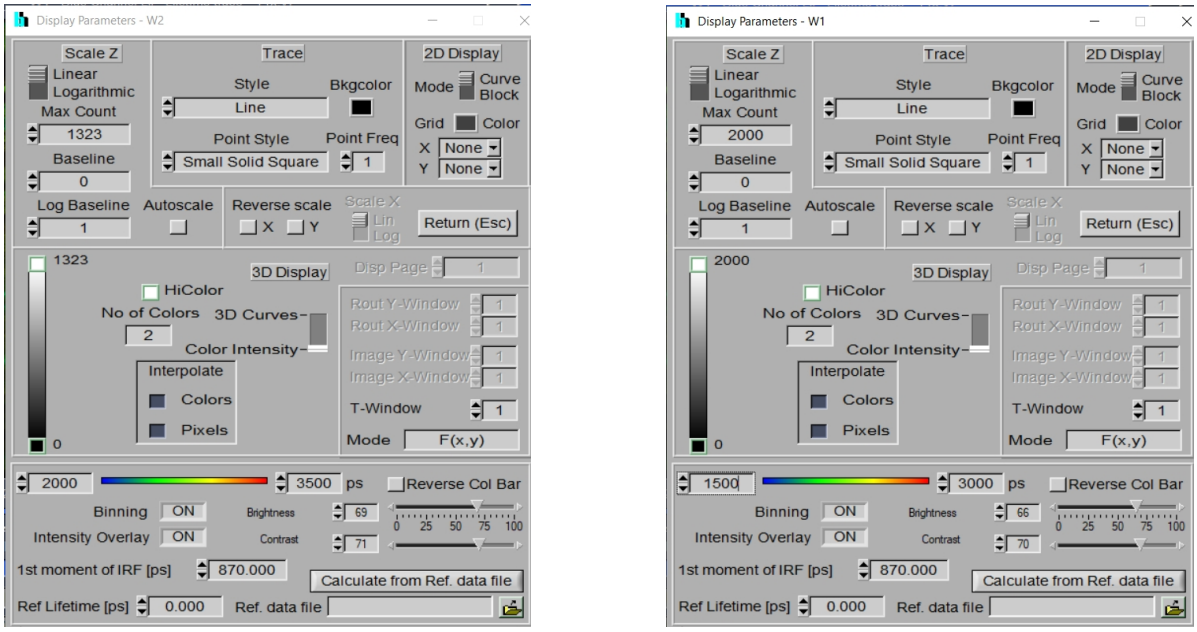



Fig. 72: Display parameters for run-time lifetime display. Two channels with different lifetime range.

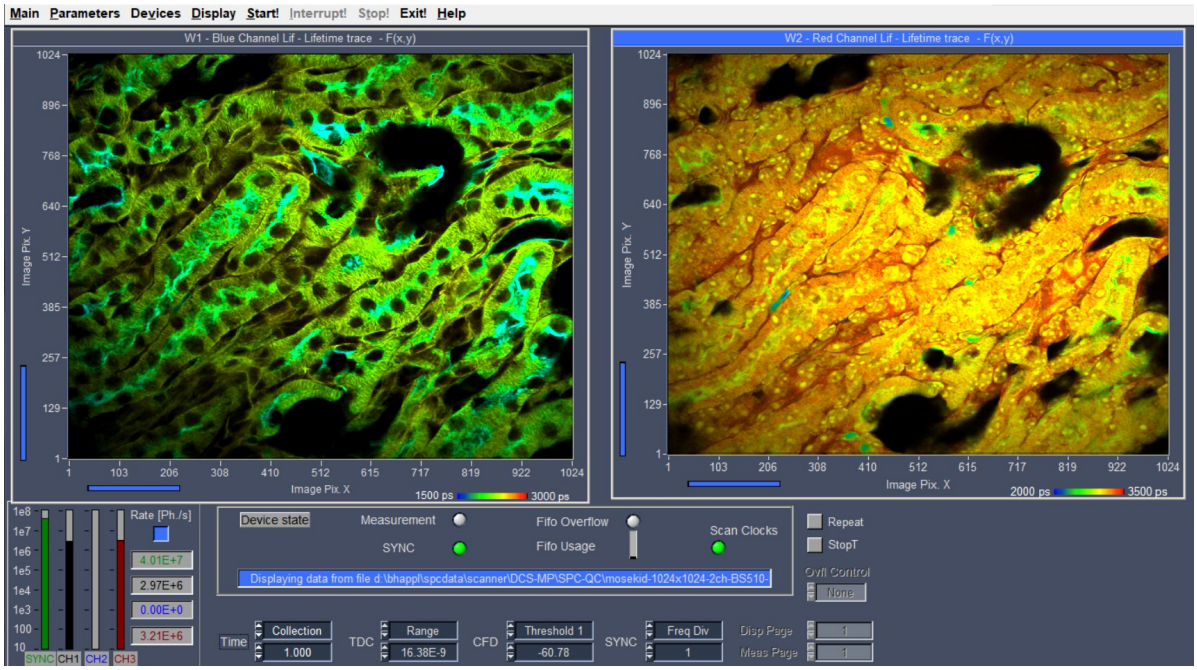



Fig. 73: SPCM main panel with run-time lifetime calculation. SPC-QC, channels 1 and 3.

## Display of Decay Data in Point or Region of Interest

A right-mouse click into one of the image in the SPCM main panel opens a small menu 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. 74, right.

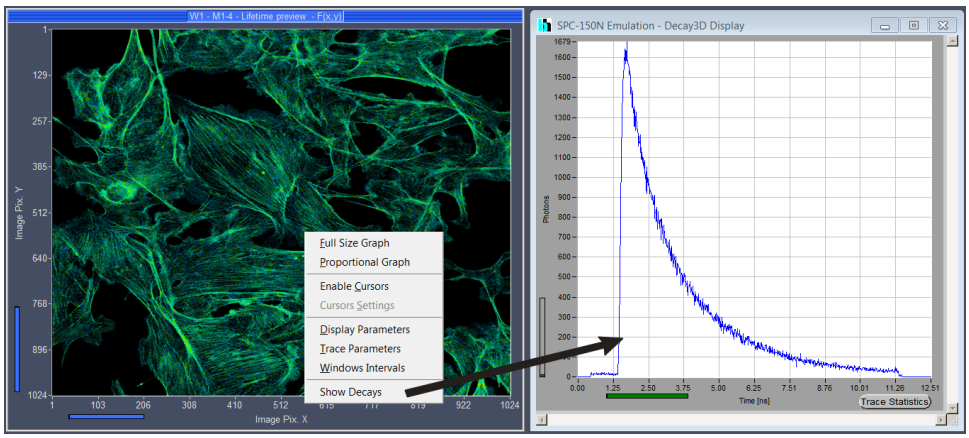



Fig. 74: A right mouse click into an image and a click on 'Show Decays' opens the decay curve panel shown on the right

The SPCM main panel with the decay curve window is shown in Fig. 75. 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. 75, 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. 75 displays a lifetime image 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.

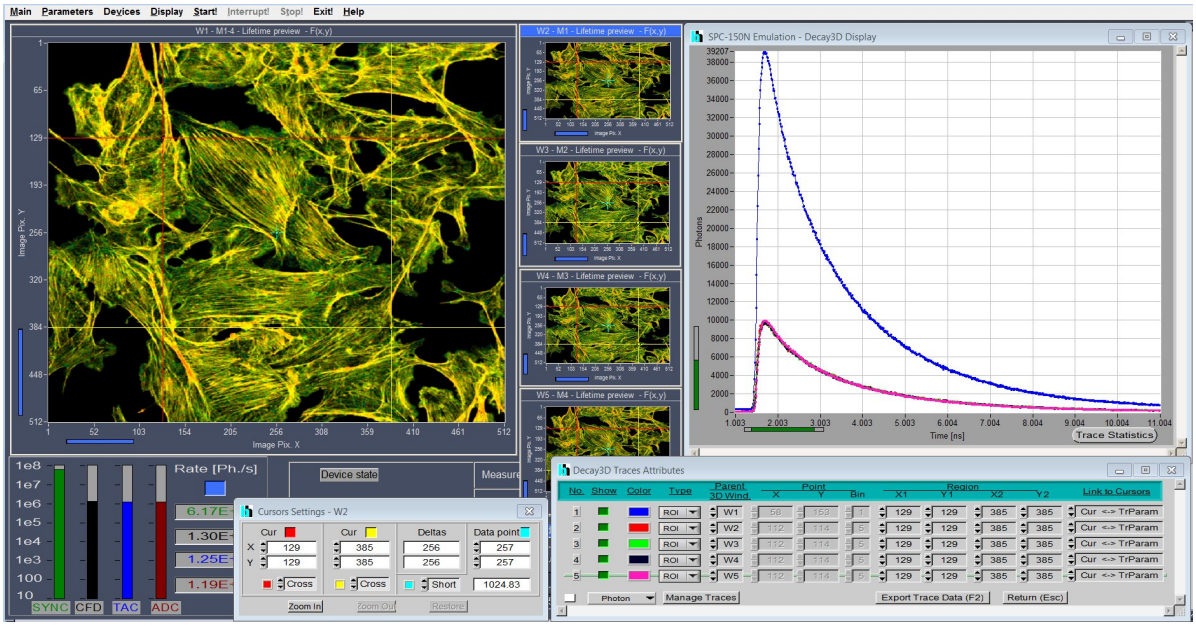



Fig. 75: SPCM Main Panel with lifetime images and decay curve window

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 SPCM-QC channels, or images from different routing channels.

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 'Display of 2D Data'.

## Save and Load Functions

Save and Load functions are the same as for other modules of the bh SPC series. You can save and load measurement data together with the setup parameters or only setup parameters. In the first case, SPCM creates .sdt files, in the second case .set files. The save and load panels are shown in ???. Please see bh TCSPC Handbook for details.

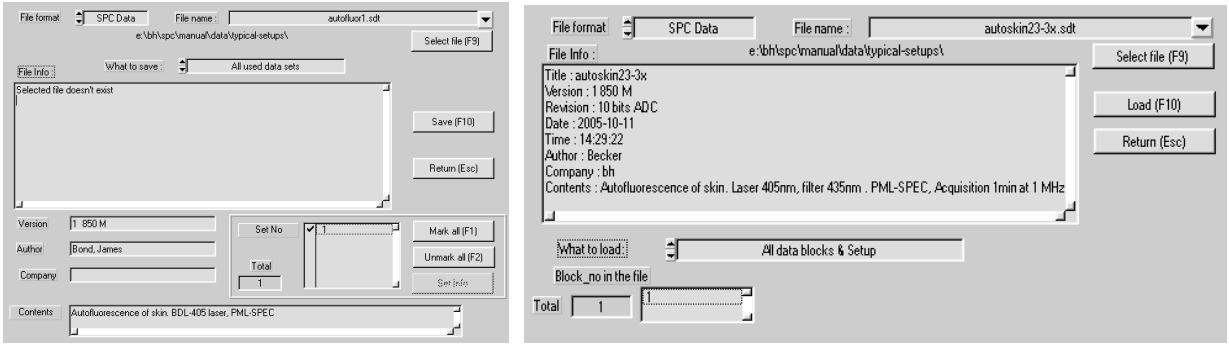



Fig. 76: SPCM functions for loading and saving data and setup parameters

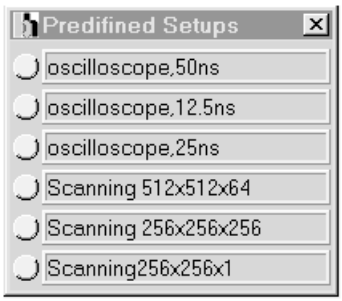
## Autosave at SPCM Exit

When you are exiting SPCM the setup data are automatically saved into an 'auto.set' file. When you later open SPCM the data are automatically loaded so that the system starts with the parameters of the previous SPCM session.

## Predefined Setups

Setups of frequently used system configurations can be added to a list of ‘Predefined Setups’. Changing between these setups then requires only a mouse click.

To use the predefined setup function, click on ‘Main’, ‘Load Predefined Setups’. This opens the panel shown right. A setup is loaded by clicking on the button left of the name of the setup.




To add or delete setups to or from the list, or to change the names of the setups, click into one of the name fields with the right mouse key. This opens the panel shown in Fig. 77.

To add a setup, click on the disk symbol right of the ‘File Name’ field. Select the files you want to put into the list of predefined setups, and click on the ‘Add’ button. Every setup has a user-defined ‘nickname’. The default nickname is the file name of the .set file. To change the nickname, click into the nickname filed and edit the name. Then click on ‘Replace’.

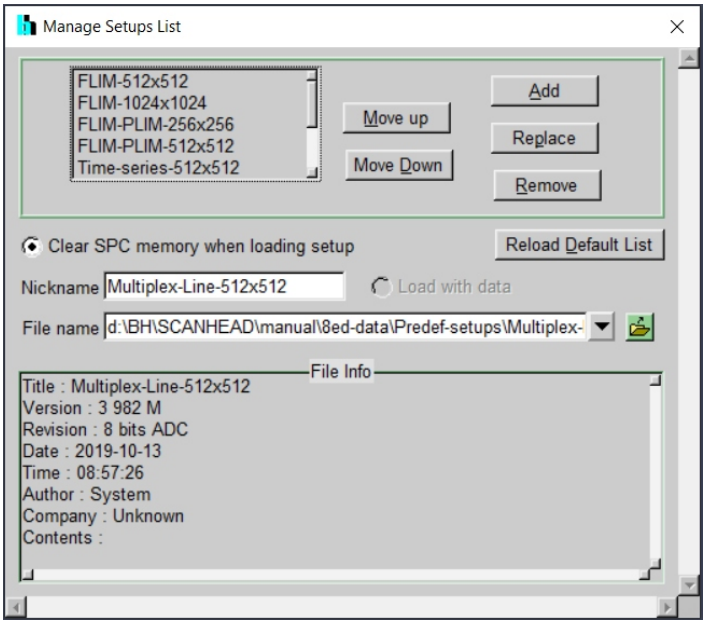



Fig. 77: Editing the list of predefined setups

# SPCImage NG Data Analysis Software

## General Functions

SPCImage NG is a new generation of bh's TCSPC-FLIM data analysis software. It combines time-domain and frequency-domain analysis, uses a maximum-likelihood algorithm to calculate the parameters of the decay functions in the individual pixels, and accelerates the analysis procedure by GPU processing. In addition to FLIM data, SPCImage NG processes single-curve decay data, multi-wavelength data, excitation-multiplexed data, PLIM data, mosaic FLIM data, and other multi-dimensional TCSPC data sets. SPCImage NG provides decay models with one, two, or three exponential components, incomplete-decay models, and a shifted-component model. Another important feature is advanced IRF modelling, making it unnecessary to record IRFs for the individual FLIM data sets. 1D and 2D parameter histograms are available to display the distribution of the decay parameters over the pixels of the image or over selectable ROIs. Image segmentation can be performed via the phasor plot or the 2D parameter histograms. Pixels with similar phasor or 2D parameter signature can be combined for high-accuracy time-domain analysis, resulting in photon numbers known only from cuvette-based lifetime experiments. The following section gives a brief overview on SPCImage NG. For a comprehensive description please see chapter 'SPCImage NG Data Analysis Software' in the bh TCSPC Handbook [1]. Please see also [16] and [17].

The main panel of SPCImage in its basic configuration is shown in Fig. 78.

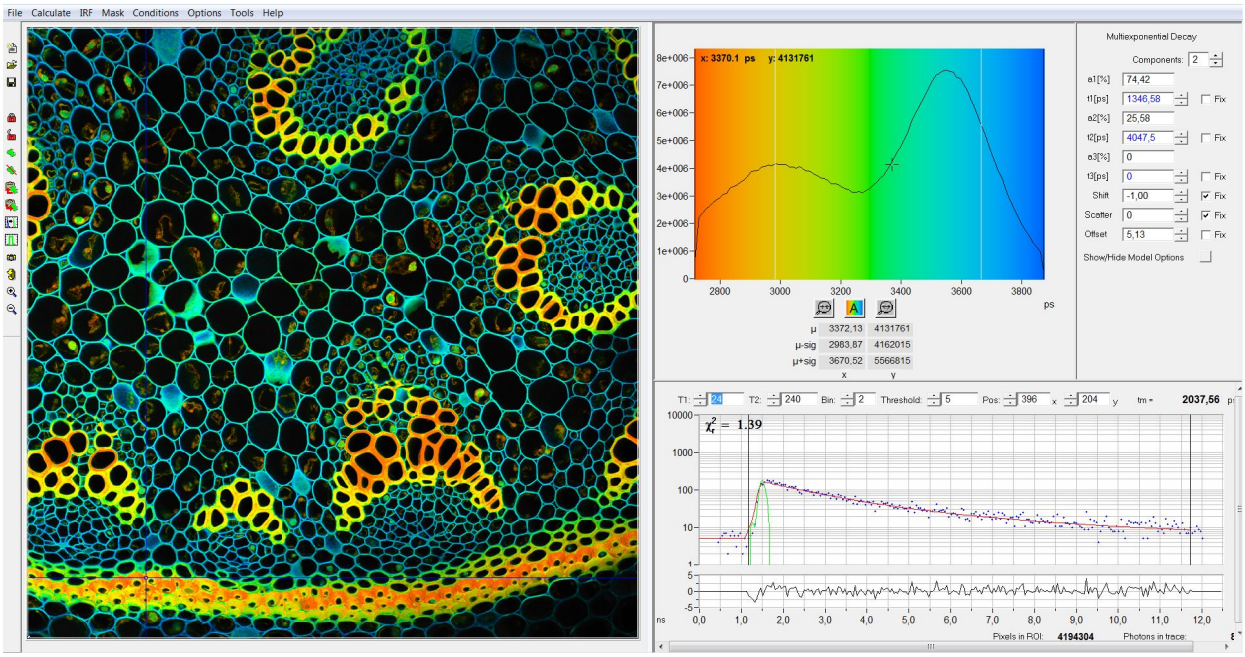



Fig. 78: Main panel of SPCImage NG

## Combination with Phasor Plot

SPCImage FLIM analysis software combines time-domain multi-exponential decay analysis with phasor analysis. Phasor analysis expresses the decay data in the individual pixels as phase and amplitude values in a polar diagram, the 'Phasor Plot'. Pixels with similar decay signature form distinct clusters in the phasor plot. Clusters of interest can be selected and back-annotated in the lifetime image for further processing or for combination of pixel data. An example is shown in Fig. 79.

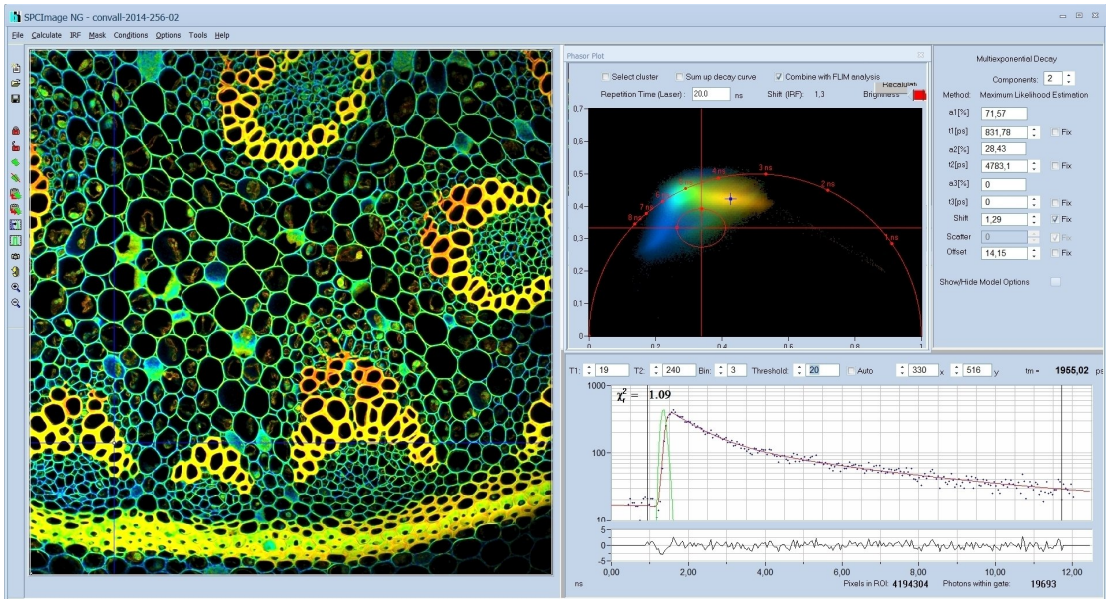



Fig. 79: Combination of time-domain analysis (left and lower right) and phasor plot (upper right)

## Images of Decay Parameters

SPCImage NG produces images of fluorescence lifetimes and other fluorescence decay parameters from TCSPC FLIM data. It runs an iterative fit and de-convolution procedure on the decay data of the individual pixels of the FLIM images. In the simplest case, the result is the lifetime of the decay functions in the individual pixels. For complex decay functions the fit procedure delivers the lifetimes and amplitudes of the decay components. SPCImage then creates colour-coded images of the amplitude- or intensity-weighted lifetimes in the pixels, images of the lifetimes or amplitudes of the decay components, images of lifetime or amplitude ratios, and images of other combinations of decay parameters, such as FRET intensities, FRET distances, bound-unbound ratios, or the fluorescence-lifetime redox ratio, FLIRR. A few examples are shown in Fig. 80 through Fig. 83.

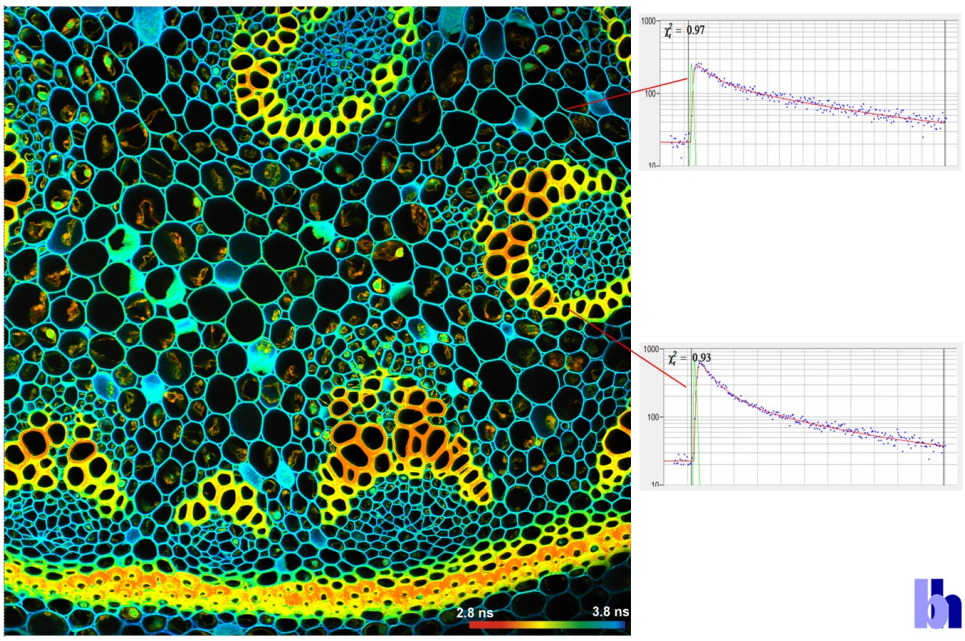



Fig. 80: Image of the amplitude-weighted lifetime, $t_m$, of a double-exponential decay. Right: Fluorescence decay curves in selected pixels.

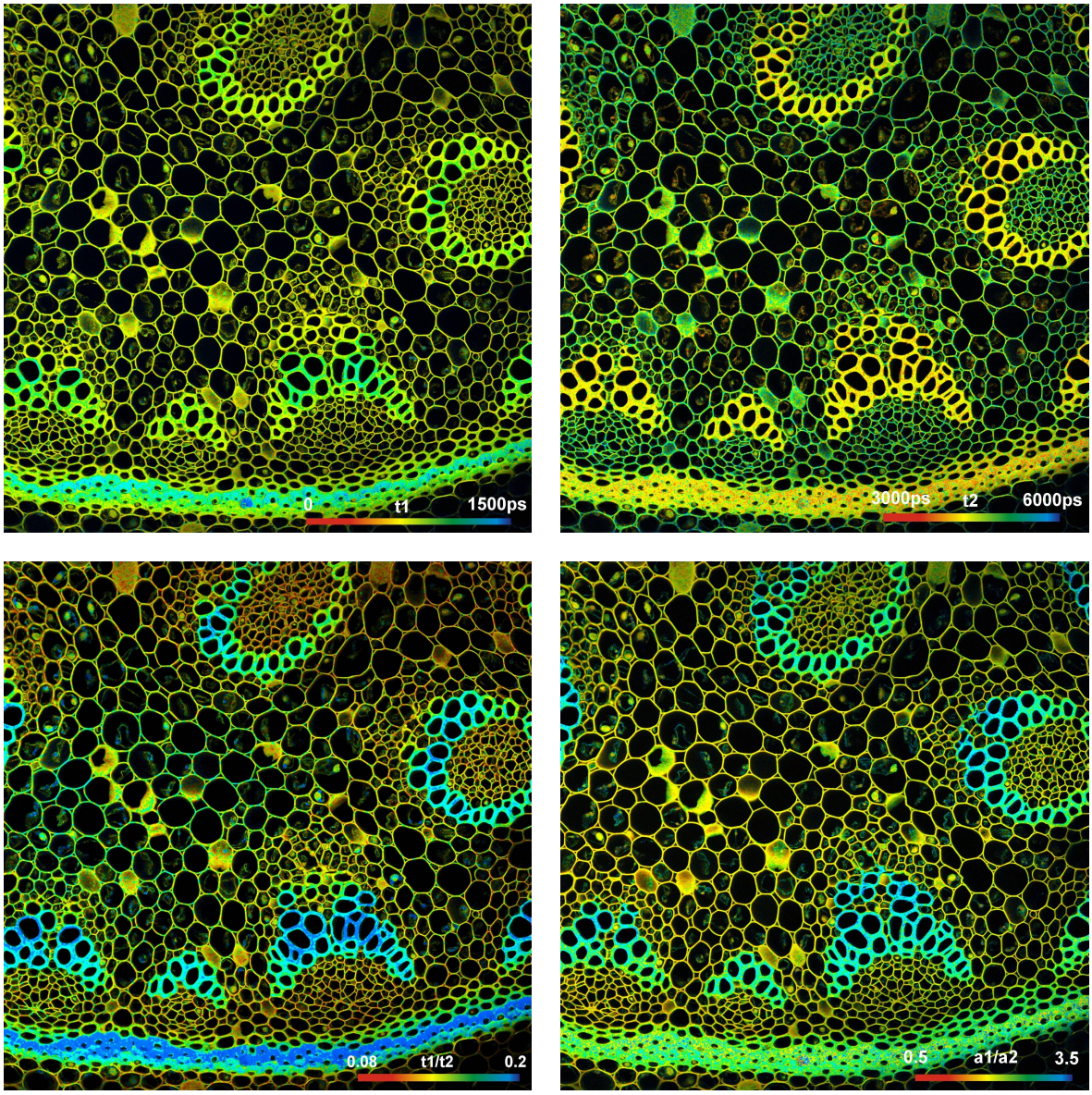



Fig. 81: Upper row: Images of the lifetimes of the fast component, $t_1$, and the slow component, $t_2$, of a double-exponential decay. Lower Row: Images of the amplitude ratio, $a_1/a_2$, and the lifetime ratio, $t_1/t_2$, of the fast and the slow decay component.

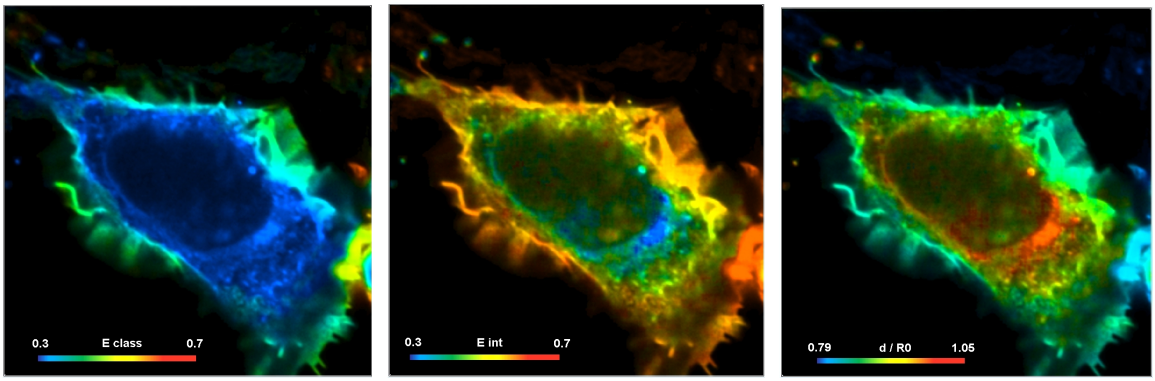



Fig. 82: Cell with interacting proteins, labelled with a FRET donor and a FRET acceptor. Left to right: Classic FRET efficiency, FRET efficiency of interacting donor fraction, FRET distance

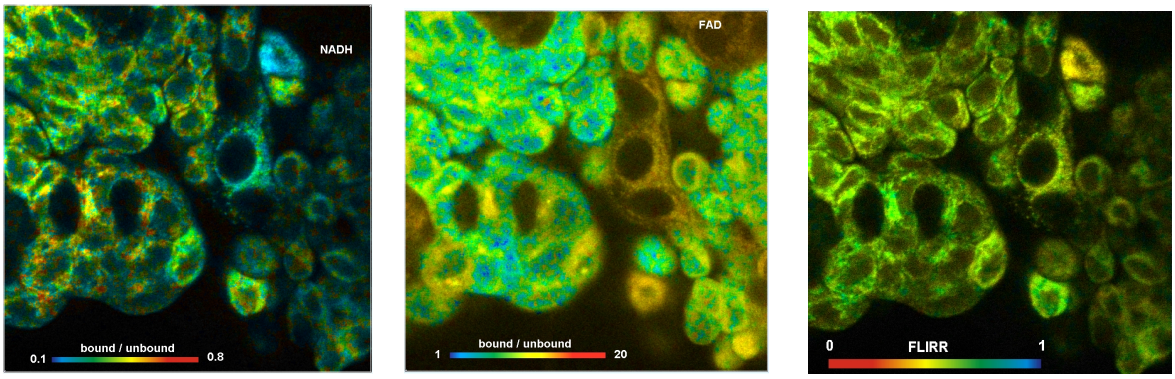



Fig. 83: Metabolic FLIM. Bound-unbound ratio of NADH, Bound/unbound ratio of FAD, Fluorescence-Lifetime Redox Ratio, FLIRR.

In addition to the functions described above SPCImage NG analyses single fluorescence decay curves, data from excitation-multiplexed FLIM. multi-wavelength FLIM, spatial and temporal mosaic FLIM, and simultaneously recorded FLIM/PLIM. It can extract phosphorescence intensities from normal FLIM data, extract SHG signals, and distinguish regions with single-exponential decay from regions where the decay is multi-exponential. Moreover, SPCImage is able to display time-gated images, e.g. to enhance SHG signals in FLIM images, or to reject un-depleted fluorescence in STED-FLIM images [Fig. 1]. A batch-processing function and a batch export function are available for analysing a large number of FLIM data sets automatically and to convert them into bmp or tiff images.

## Maximum-Likelihood Algorithm

Different than previous versions, SPCImage NG uses a maximum-likelihood estimation (MLE) process to determine the decay parameters in the pixels. In contrast to the frequently-used weighted least-square (WLS) fit, MLE is based on calculating the probability that the values of the model function correctly represent the data points of the decay function. Compared to the least-square method, the fit accuracy is improved especially for low photon numbers, and there is no bias toward shorter lifetime as it is unavoidable for the least-square fit. Please see [1], chapter 'SPCImage NG Data Analysis Software'.

## Modelling of the Instrument-Response Function

Recording the 'Instrument Response Function' (IRF) is a permanent problem of time-resolved fluorescence spectroscopy. Recording the IRF in a FLIM system is difficult, and often impossible. As a result, there is rarely an IRF that was recorded in a FLIM system and represents the temporal behaviour of the system correctly. Therefore, SPCImage NG does away with IRF recording altogether. Instead, the IRF is extracted from the FLIM data themselves. Earlier SPCImage versions had an 'Auto IRF', which was derived from the rising edge of the fluorescence decay function. The Auto IRF has been used successfully for more than 20 years. It works well for decay functions which are not too far from a single-exponential function but has deficiencies if very fast decay components are present. A new approach introduced by SPCImage NG is the 'Synthetic IRF'. It is created by modelling the IRF with a generic function. The exact parameters of this function are determined by fitting it to the FLIM data together with the selected decay model. The results of this procedure are so good that an accurate IRF is obtained even for decay functions containing ultra-fast components.

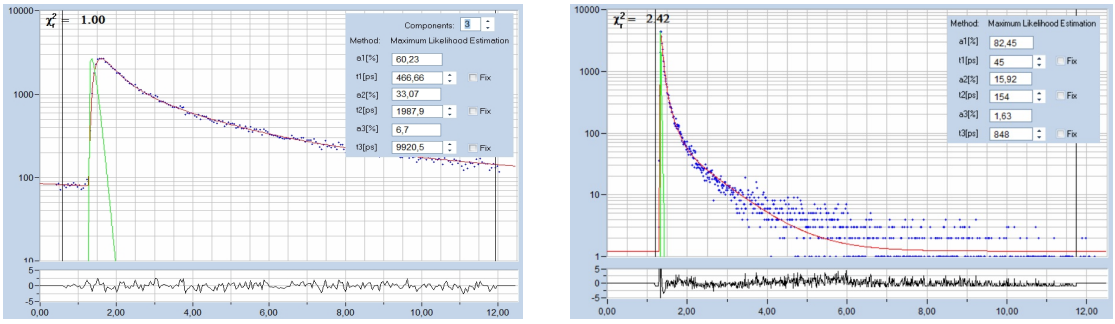



Fig. 84: Analysis with synthetic IRF. Left: Fluorescence excited by diode laser. Right: Ti:Sa laser, sample with extremely fast decay component. Green curve IRF, blue dots data points, red curve fit with triple-exponential decay model.

## Decay Models

SPCImage NG provides single-, double-, and triple-exponential decay models. An 'Incomplete Decay' option is available to determine long fluorescence lifetimes within the short pulse period of the Ti:Sa laser of a multiphoton system. SPCImage NG provides also a 'Shifted-Component' model. In this model, the decay components of a multi-exponential model functions can be shifted in time by predefined values. The model is used for ophthalmic FLIM, where different decay components come from different depth within the eye. For details please see [1].

## GPU Processing

Data recorded with bh FLIM systems can contain an enormous number of pixels and time channels. Images with 1024 x 1024 or even 2048 x 2048 pixels are not uncommon, and time-channel numbers of 1024 are routinely used in combination with fast HPM detectors [1]. An example is shown in Fig. 85.

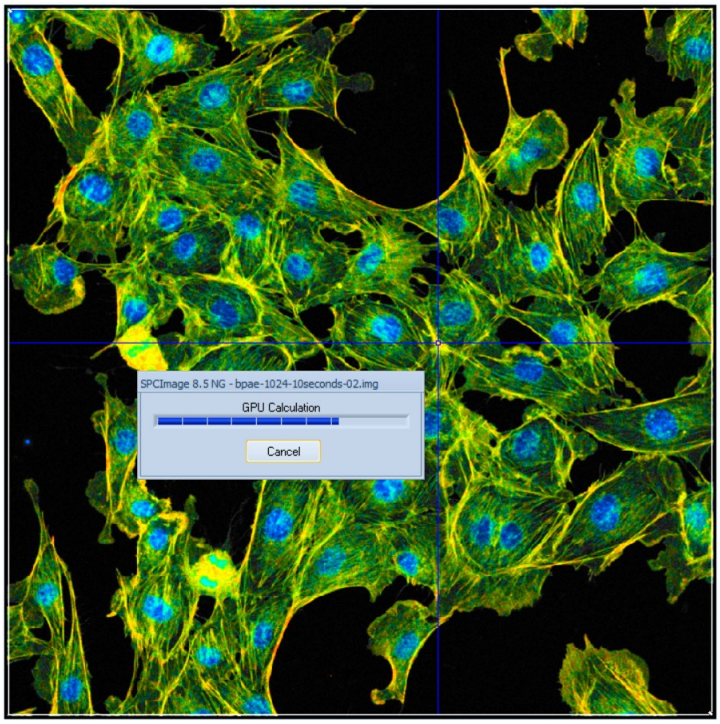



Fig. 85: A lifetime image with 1024 x 1024 pixels and 1024 time channel per pixel. The image was calculated on an NVIDIA GPU in 5 seconds.

Processing such amounts of data by the CPU of even a fast computer takes tens of minutes. SPCImage NG therefore runs the data analysis on a GPU (Graphics Processor Unit). The image data are transferred into the GPU, which then runs the de-convolution and fit procedure for a large number of

pixels in parallel. Data processing times are thus massively reduced. The image shown in Fig. 85 was calculated on a medium-speed NVIDIA GPU within five seconds. Conventional data analysis of this image takes about 10 minutes.

## Parameter Histograms

SPCImage has histogram functions for the decay parameters. The histogram shows how often pixels of a given parameter value occur in the lifetime image. The histogram refers either to a selected region of interest or, if no ROI was defined, to the entire lifetime image. Together with the various options to select decay parameters and combinations of decay parameters a wide variety parameter histograms can be obtained. Two examples are shown in Fig. 86.

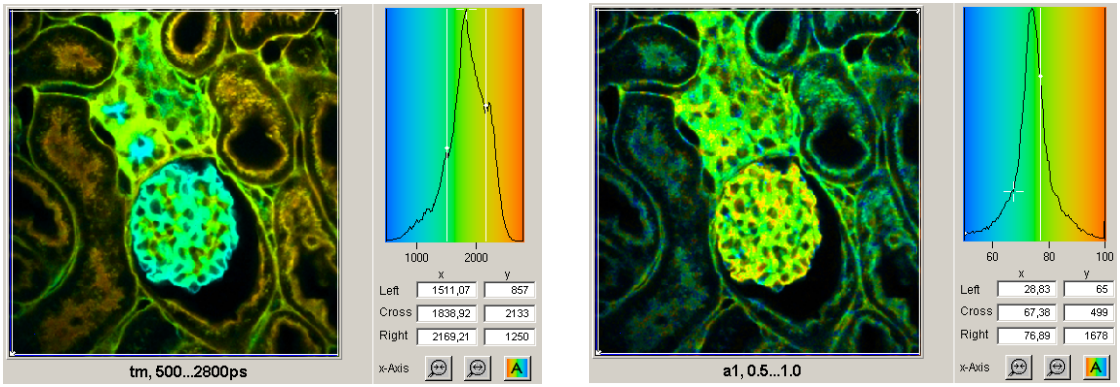



Fig. 86: Histograms of the mean (amplitude weighted) lifetime of double-exponential fit (left) and of the amplitude of the fast decay component, $a_1$ (right)

## 2-D Histograms

2D histograms present density plots of the pixels over two selectable decay parameters. The decay parameters can be lifetimes, $t_1$, $t_2$, $t_3$, or amplitudes, $a_1$, $a_2$, $a_3$, of decay components, amplitude or intensity-weighted lifetimes, $t_m$ or $t_i$, or arithmetic conjunctions of these parameters. An example is shown in Fig. 87. A histogram of the amplitude, $a_1$, of the fast decay component versus the amplitude-weighted lifetime, $t_m$, has been created. Cursors in the histogram are available to select special parameter combinations and back-annotate the corresponding pixels in the lifetime image.

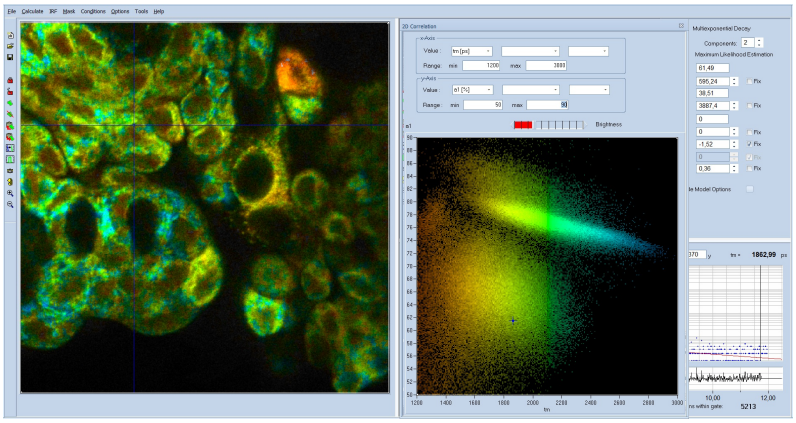


Fig. 87: 2-D histogram showing density plot of pixels over amplitude-weighted lifetime, $t_m$, and amplitude of fast component, $a_1$.

## ROIs

SPCImage NG allows the user to define ROIs in the images. Both rectangular and polygonal ROIs can be defined. Parameter histograms are displayed for the selected ROI, see Fig. 88.

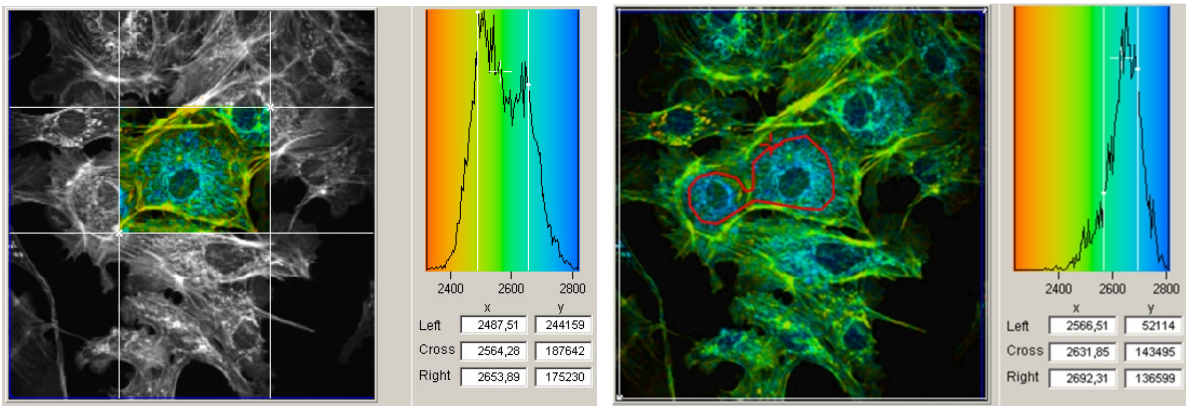



Fig. 88: ROI Definition. Left: Rectangular ROI. Right: Polygonal ROI

Several polygonal ROIs can be defined, and the corresponding parameter histograms be selected via the buttons on top of the histogram window. Please see Fig. 89.

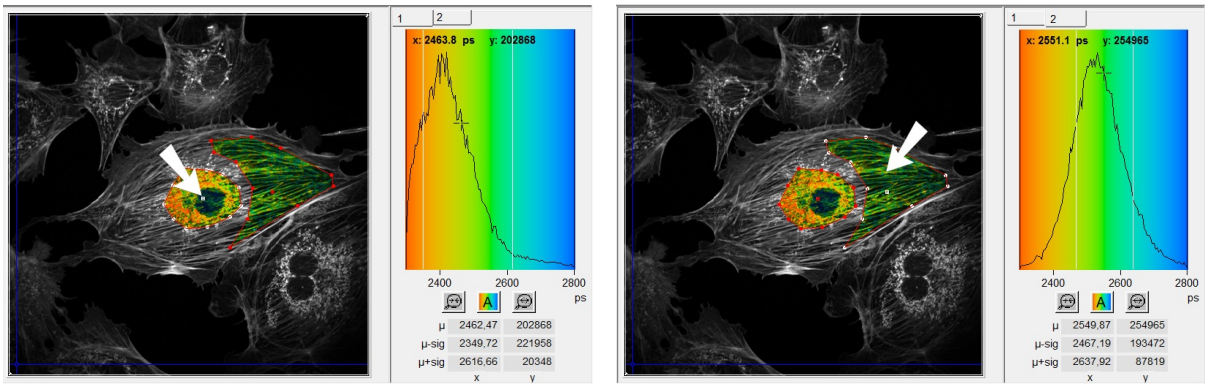



Fig. 89: Multiple ROIs, with selection of parameter histogram.

## Image Segmentation

Images taken at high pixel numbers and Mosaic FLIM images can contain a large number of cells. In these cases, it is time-consuming, if not impossible, to manually select regions of interest for each of the cells in the image. SPCImage NG therefore provides automatic image segmentation functions via the phasor plot and the 2D histograms. Areas with different decay signature form separate clusters in these presentations. Interesting clusters can be selected and back-annotated in the images. An example is shown in Fig. 90. The image area contains a large number of cells. A phasor plot of the image was calculated, and the phasor range of the cell nuclei selected. The decay data of the corresponding pixels are combined. The result is single decay curve, containing an enormous number of photons. This curve can be analysed at high precision with double- and triple-exponential decay models, see Fig. 90, bottom right. Please see [1] for further details.

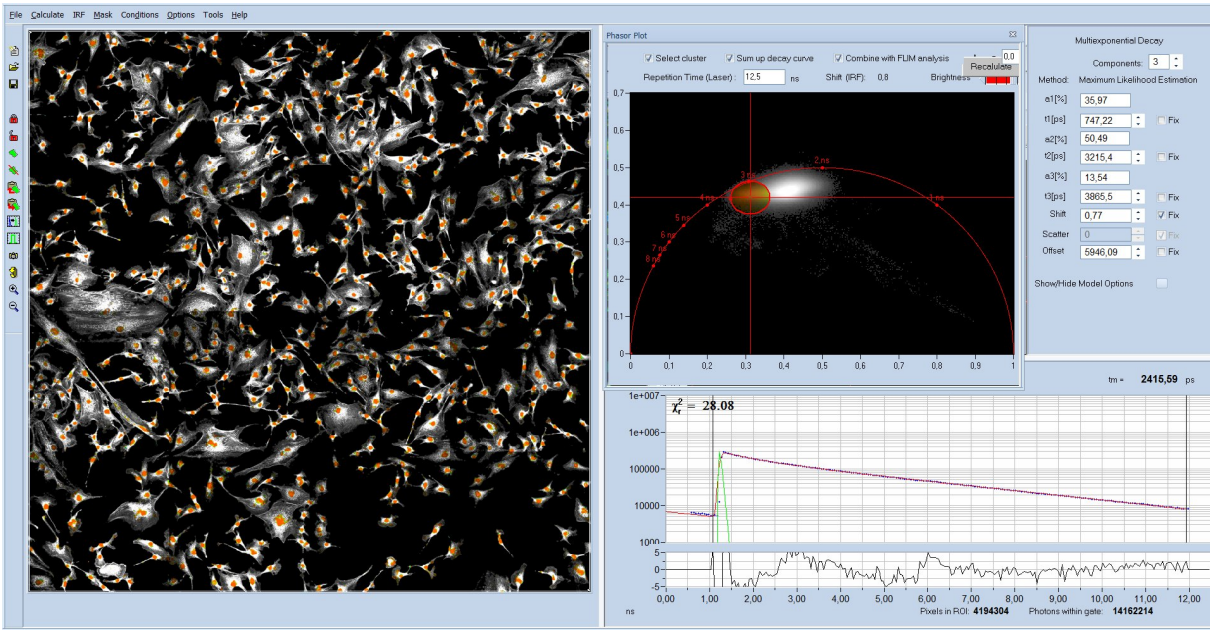



Fig. 90: The phasor range of the nuclei of the cells has been selected by the 'Select Cluster' Function. The decay-parameter histogram (shown right) refers to the selected pixels. A combined decay curve for the selected pixels is displayed by the 'Sum up decay curves' function.

# Peripheral Components

Every measurement system needs peripheral components, such as lasers, detectors, and experiment control devices. A variety of such devices is available from bh. All are compatible with the SPC and SPC-QC modules. Frequently used peripheral devices are described below. For details please see TCSPC Handbook and individual data sheets and manuals.

## BDS-SM Series Lasers

The BDS-SM series picosecond diode lasers [22] are OEM Modules with a size of only 40 mm x 40 mm x 110 mm. The lasers contain the entire driver electronics. They are operated from a simple +12 V power supply and can be controlled via a bh DCC-100 card, a GVD-120 or GVD-104 card, or via an independent manual-control box. The lasers are available both with free-beam and single-mode fibre output. The pulse width is on the order of 40 to 90 ps, the pulse repetition rate can be switched between 80 MHz, 50 MHz, 20 MHz, and CW. All the typical diode laser wavelengths from 375 nm to 785 nm are available. The output power is stabilised by an internal regulation loop, and fast on-off switching is implemented. The lasers have a synchronisation output to the bh TCSPC modules and a trigger input for synchronisation with other pulsed lasers.

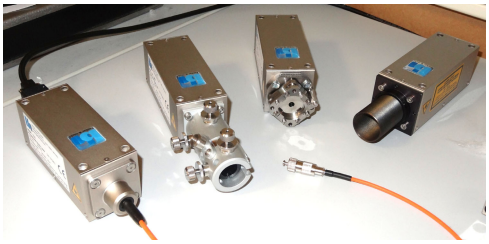


Fig. 91: BDS-SM series laser with pig-tail single-mode fibre, Qioptiq Kineflex adapter, Lasos Precision Connector, and free-beam output through a C-Mount adapter

## BDU-SM USB-Controlled Picosecond/CW Diode Lasers

The BDU-SM family picosecond / CW diode lasers are fully controlled and powered via a USB interface. Available wavelengths range from 375 nm to 785 nm. The lasers are available with elliptical or circular free-beam output, and with single-mode fibre output. As the BDS lasers, the BDU lasers feature extraordinarily high timing stability and intensity stability. Repetition rates are 20, 40, 80 MHz, or CW. Optical power at 80 MHz is typically 3 to 5 mW. Pulse width is 40 ps to 300 ps, depending on the wavelength version and selected power. The laser, a typical pulse shape, and the control panel are shown in Fig. 92

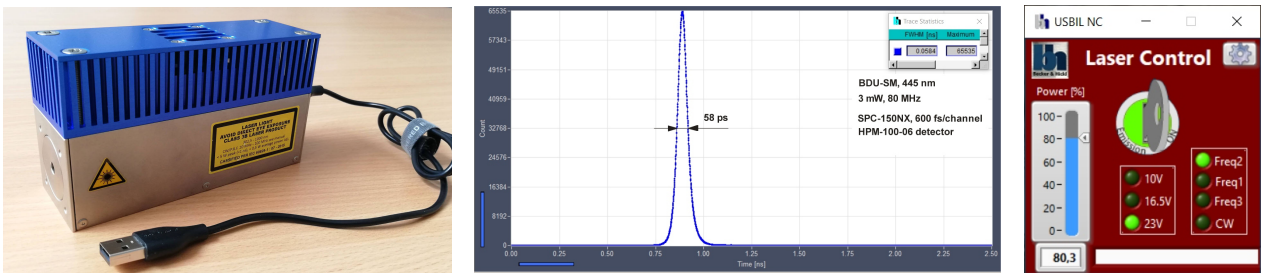



Fig. 92: BDU-SM series picosecond diode laser

## Detectors

bh TCSPC devices work with almost any single-photon detector. For some detectors an electrical attenuator or a pulse inverter may be required. An overview on detectors, their working principles and their performance with TCSPC can be found in the bh TCSPC Handbook [1], chapter 'Detectors for TCSPC'. An overview on the most frequently used detectors is given below.

### Hybrid Detectors

The bh HPM-series hybrid detectors feature high sensitivity, high time resolution, low dark count rate, and almost complete absence of afterpulsing [24]. With their introduction in 2009 they quickly replaced PMT detectors in almost all high-end TCSPC applications. More than 900 pieces were manufactured since 2009. With the introduction of GaAs cathode versions the application was extended into NIRS spectroscopy [25]. Finally, with the ultra-fast bialkali and multialkali versions, the HPM-100 modules joined the exclusive club of sub-20-ps detectors, which previously was entirely governed by MCPs and SSPDs [23]. Photos of the HPM-detector are shown in Fig. 93.

Fig. 93: Left: bh HPM-100 hybrid PMT module. Left to right: Versions with C-Mount adapter, version with Zeiss BIG adapter, fibre-version, version with thermoelectric cooler

The HPM-100 modules come in different cathode versions. Detectors with different cathodes have different IRF width. Typical values are listed in the table below. The IRF width was measured with an SPC-150NX module, i.e. with an electrical IRF width of 3.5 ps. With the 48 ps electrical IRF width of

the SPC-QC the effective IRF width becomes, of course, longer. Typical values are shown in the last column of the table.

| | Cathode Type | Wavelength Range | IRF Width, SPC-150NX | IRF Width, SPC-QC |
|---|---|---|---|---|
| HPM-100-06 | Bialkali | 300 - 600 nm | 18 ps | 52 ps |
| HPM-100-07 | Multialkali | 250 - 800 nm | 18 ps | 52 ps |
| HPM-100-40 | GaAsP | 300 - 720 nm | 90 - 130 ps | 100 - 104 ps |
| HPM-100-41 | GaAsP | 300 - 800 nm | 100 - 130 ps | 110 - 104 ps |
| HPM-100-50 | GaAs | 400 - 900 nm | 130 - 200 ps | 104 - 200 ps |

## PMC-150 and PMCS-150 Cooled PMT Modules

The PMC-150 and the PMCS-150 are cooled PMT modules. They are based on Hamamatsu photosensor modules. These modules contain a miniature PMT together with a high-voltage generator. IRF width is about 120 ps. The modules come in different cathode version, see individual data sheets. The detectors are shown in Fig. 94.


Fig. 94: Left: PMC 150 detector. Right: PMCS-150 detector.

The PMC-150 is operated via a DCC-100 detector controller. The controller provides for power supply, gain setting, and overload shutdown. The PMCS-150 has fixed gain and internal overload shutdown. It can be used both with and without a DCC-100. IRF profiles are shown in Fig. 95.

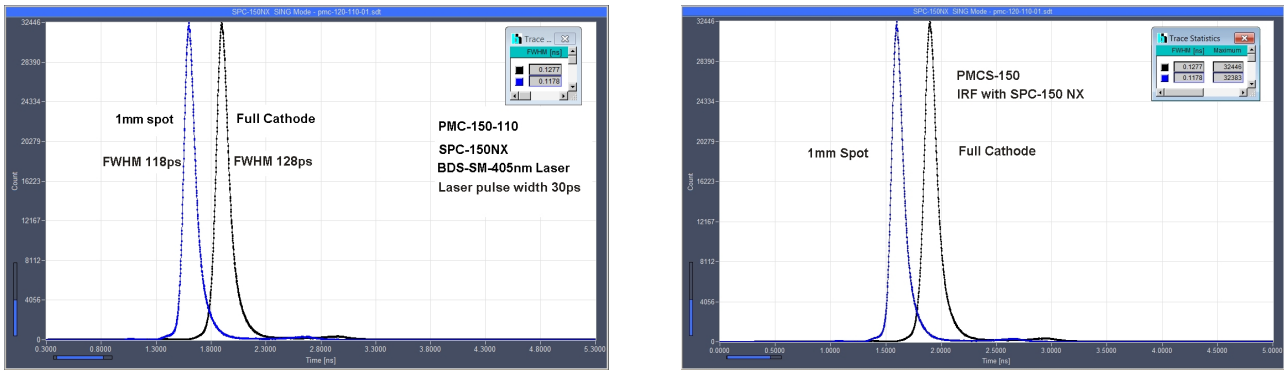



Fig. 95: IRFs of PMC-150 and PMCS-150

## Multi-Wavelength Detectors

The bh PML-16C and PML-16 GaAsP detectors provide 16 simultaneously recording channels for TCSPC. The detectors are shown in Fig. 96, left and second left. Multi-wavelength detection assemblies with the PML-16 detectors are shown in Fig. 96, second right and right.

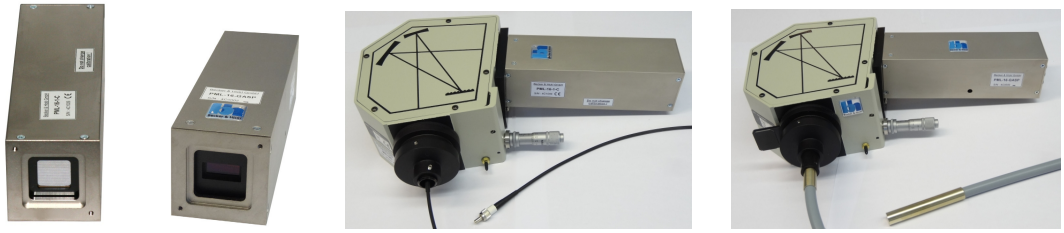


Fig. 96: Left to right: PML-16C, PML-16 GaAsP, PML-SPEC assembly with fibre input, MW-FLIM assembly with fibre-bundle input.

The detectors are operated via a DCC-100 detector controller card. They are directly compatible with the bh SPC and SPC-QC modules. IRFs for the PML-16-C and the PML-16 GaAsP are shown in Fig. 97. The IRF width is 150 ps FWHM for the PML-16-C, and 203 ps for the PML-16 GaAsP. For further details please see bh TCSPC Handbook.

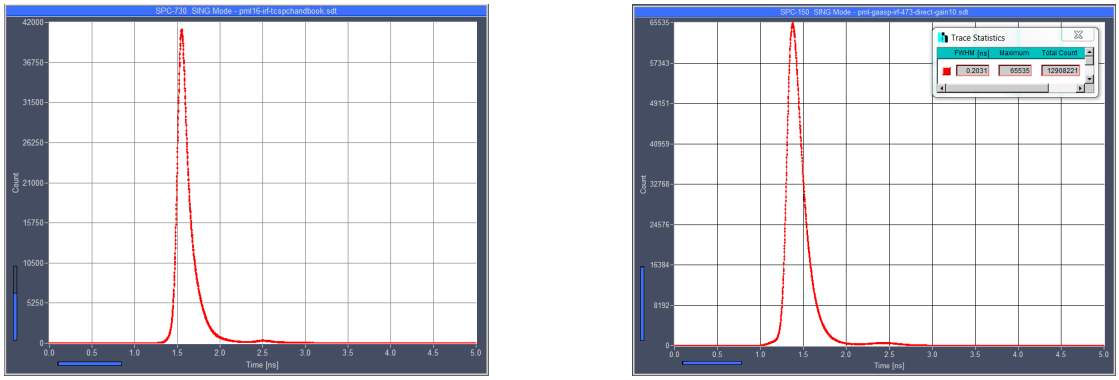


Fig. 97: IRF of PML detectors, one channel of 16. Left: PML-16C, FWHM = 150 ps. Right: PML-16 GaAsP, FWHM = 203 ps

## SPADs

SPADs (Single-Photon Avalanche Photodiodes) connect to the SPC and SPC-QC cards without problems. For most of them a pulse inverter and an attenuator is required. These parts are available from bh. SPADs with high NIR sensitivity tend to produce slower IRFs. Practically achieved IRFs range from about 25 ps to a few 100 ps FWHM. Measured IRFs for a fast and a slow SPAD are shown in Fig. 98.

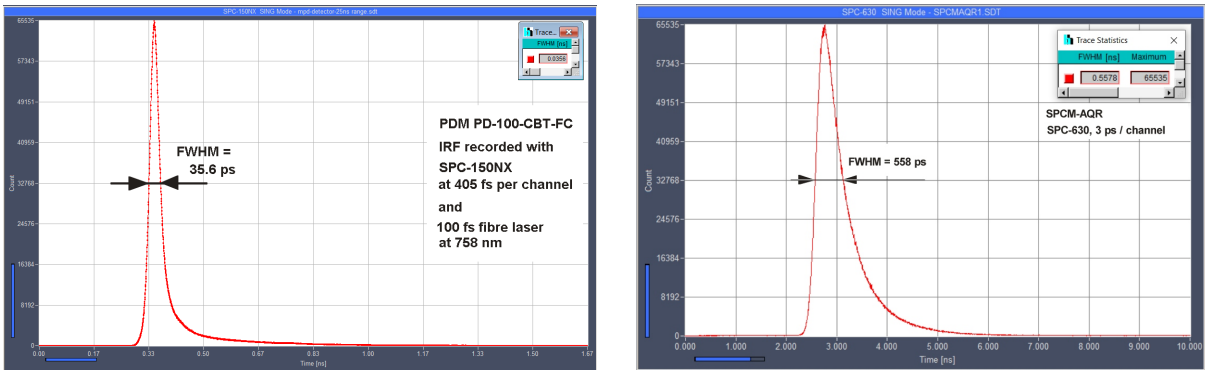



Fig. 98: IRF of a fast SPAD (MPD PDM PD-100-CBT-FC) and of a slower one (Perkin Elmer SPCM-AQR series, for low dark counts). The FWHM is 35.5 ps versus 270 ps. Note different time scale.

The FWHM values show that the full resolution of fast SPADs can only be exploited by the SPC series modules. For SPADs with larger than 100 ps IRF width there is no significant difference between the SPC and the SPC-QC modules.

## SSPDs

SSPDs (Superconducting Single Photon Detectors) have extremely fast IRFs. Two examples are shown in Fig. 99. One is from a SCONTEL TCORPS-UF-10 detector [26], the other from a single-nanowire SSPD of JPL, Pasadena [27].


Fig. 99: IRF of a SCONTEL TCORPS-UF-10 SSPD (left) and IRF of a single-nanowire SSPD of Jet Propulsion Laboratory (JPL). Both recorded with SPC-150NXX module. Note different time scale.

It is obvious that the time resolutions of detectors this fast cannot be exploited by an SPC-QC or any other TDC-based timing instrument. Only the SPC-150NXX and the SPC-180NXX come close to the timing resolution required for SSPDs. Unless other detector parameters than the time resolution are in focus one of these SPC modules should be used instead of the SPC-QC-104.

## DCC-100 Detector Controller

The DCC-100 card is used to control the detectors of bh TCSPC systems. It is also able to control the BDL-SMC, BDL-SMN, and BDS-SM ps diode lasers, and to control electro-mechanical shutters. Please see bh TCSPC Handbook for technical details. The software panel of the DCC-100 is shown in Fig. 100.

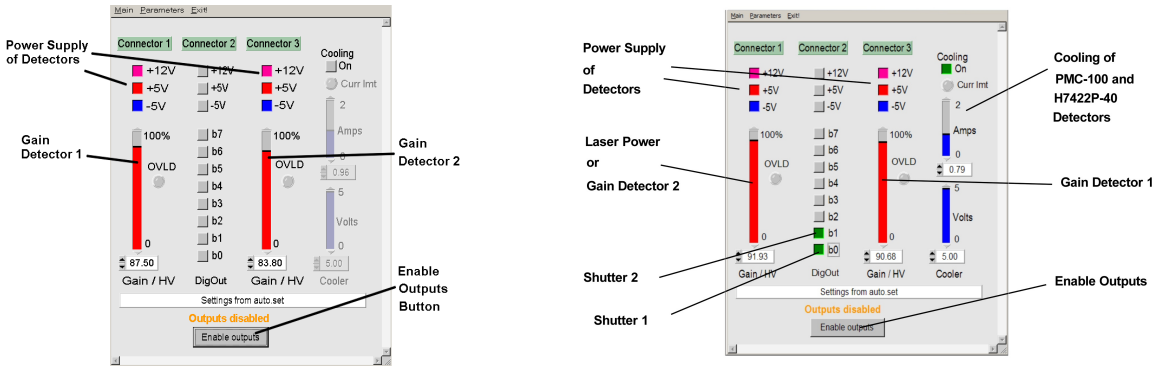



Fig. 100: Detector control panel. Left: Control of two detectors. Right: Control of one laser and one detector.

## GVD-120 and GVD-104 Scan Controllers

The GVD-120 and GVD-104 san controller modules are used to control fast galvanometer scanners or piezo scan stages with analog input. The devices also control the scanner of the bh DCS-120 Confocal Scanning FLIM System [20]. In addition to driving the scanners the GVD devices control bh BDS and

BDL picosecond diode lasers. The GVD-120 controls two, the GVD-104 four lasers. Laser control includes beam blanking during the flyback of the scanner, intensity control, laser on-off switching, and laser multiplexing. With the GVD cards, the operation of the scanner and the lasers is fully integrated in the SPCM software. The user interfaces are shown in Fig. 101. For technical details please see [1] and [20].

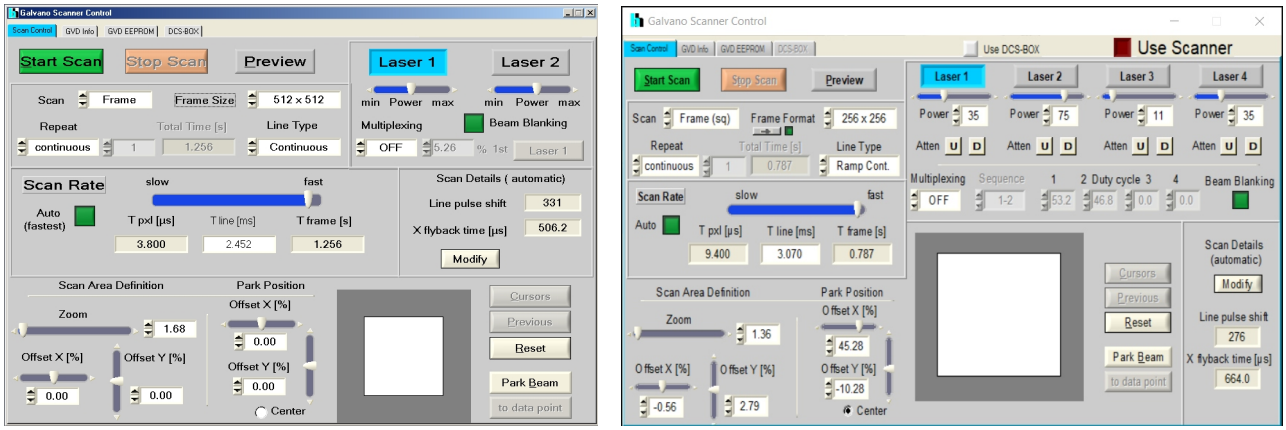



Fig. 101: GVD control panel. Left: GVD-120. Right: GVD-104

## DCS-120 Scan Head

The DCS-120 scan head is used to scan objects with focused laser beams and return fluorescence signals from the object to two detectors. The scan head has two optical inputs for bh BDL-SMC or BDS-SM lasers, two confocal detection paths, internal beamsplitters and filters, individually selectable pinholes, and two optical outputs to detectors. The detectors are attached to the back of the scan head. Scanning is performed by fast galvanometer mirrors. The scan head is controlled via a GVD-120 or GVD-104 scan controller card. The user interface is fully integrated in SPCM, see above, Fig. 101. A photo of the scan head is shown in Fig. 102.

Fig. 102: DCS-120 scan head

Normally, the scanner is part of the bh DCS-120 Confocal and Multiphoton FLIM System, see [20]. However, it can be used also independently of this system. Its use is not only attractive to solve user-specific FLIM problems but also to upgrade conventional microscopes with FLIM. The DCS-120 scan head can also be a real alternative to - often desperate - attempts to attach FLIM systems to scanning microscopes which are not designed for this kind of operation. In these cases, a DCS-based system may be not only easier to set up but also perform better. Moreover, the DCS design results in a fully integrated system, operated from one piece of software, and from one computer. For details please see TCSPC Handbook [1] and handbook of the DCS-120 system [20].

## DB-32 SYNC Delay Box

The DB-32 is a USB-controlled passive delay box. It is used to adapt the signal transit time in the SYNC path or in a detector path of a TCSPC system to different optical configurations. Moreover, it can switch between to different SYNC sources or two different detectors. The SYNC-Delay Box is shown in Fig. 103.


Fig. 103: Sync Delay Box

The delay and the signal source are selected via a 'Delay' parameter in the SYNC parameter part of the System Parameters, or directly via the SYNC field in the main panel, see Fig. 104.

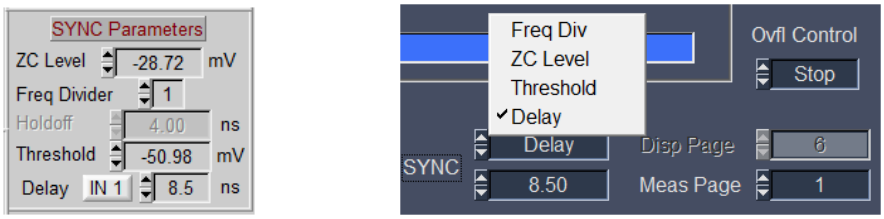



Fig. 104: Delay setting in the system parameters (left) and in the SYNC field of the main panel (right)

## SIS 2x4 USB-Controlled Signal Switch

The SIS 2x4 module contains two 4:1 or 1:4 USB-controlled signal switches. The device is used to select between different detectors and different SYNC sources. Different hardware configurations of a TCSPC system can thus be realised and selected by loading the corresponding setup data via the Load function or the Predefined Setup panel. The SIS 2x4 is shown in Fig. 105, left, the control panel in Fig. 105, right.

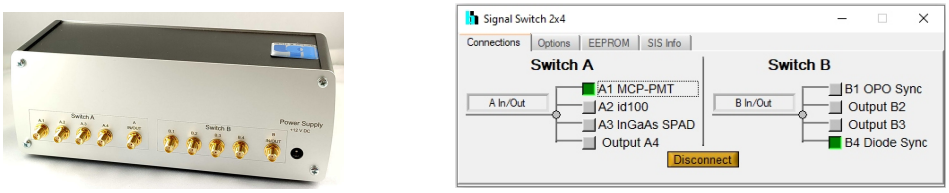



Fig. 105: SIS 2x4 USB-controlled signal switch

## DDG-210 Pulse Generator Card

The DDG-210 pulse generator card is used in systems which do not contain a GVD card to on-off modulate lasers for phosphorescence decay measurements and phosphorescence lifetime imaging. The DDG-210 panel is shown Fig. 106. The card generates pulses to the laser or to an AOM of a laser, and a routing signal to the SPC module. The pulses can be generated in a free-running mode, or be

triggered by the pixel clock of a scanner. The DDG-210 is part of the bh FLIM systems for the Zeiss LSM 710 family microscopes [19]. Please see [19] and bh TCSPC Handbook [1] for further details.

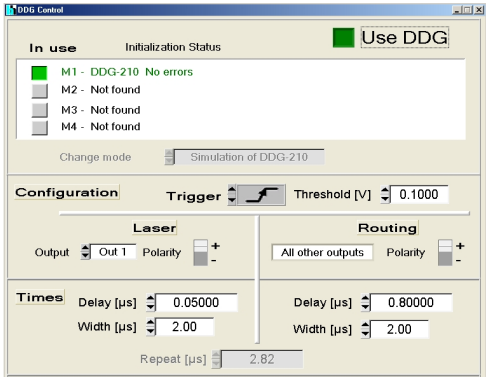



Fig. 106: Left: Starting the DDG-210 control. Right: DDG-210 control panel

## Ti:Sa Laser and AOM Control

The SPCM software is able to control a Ti:Sa laser and an AOM. The control panel opens by a click into ‘Devices’, 'Ti:Sa laser & AOM Control’. The control panel is shown in Fig. 107

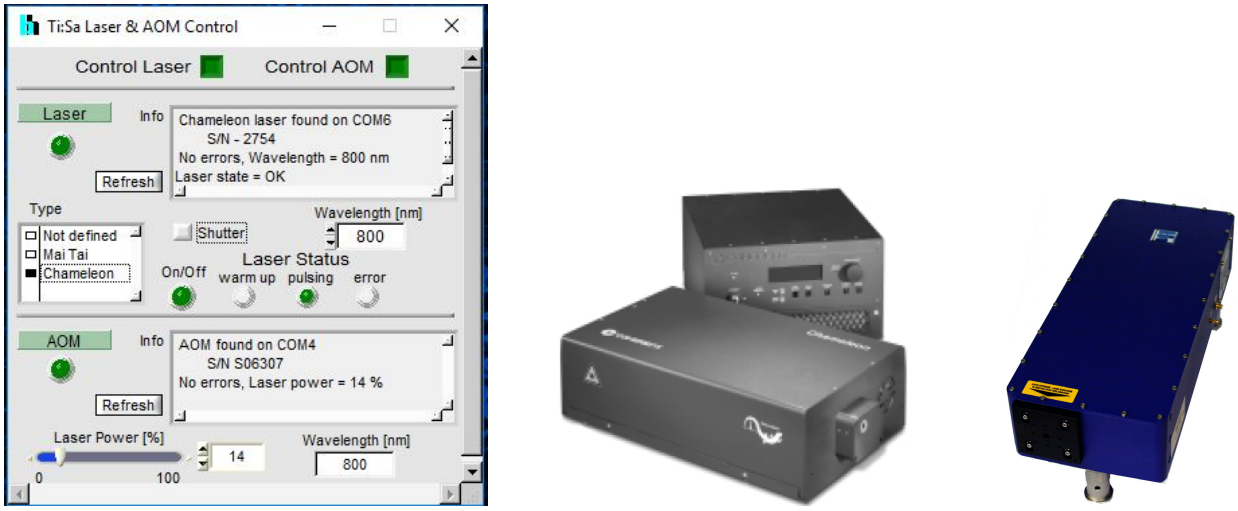



Fig. 107: Left: Ti:Sa laser and AOM control panel. Right: Coherent Chameleon laser and bh AOM

The laser is controlled in the upper part of the panel. The type of Ti:Sa laser must be selected under ‘Laser’, ‘Type’. The system computer connects to the laser via a USB interface. The laser panel controls the shutter of the laser and the wavelength, and displays the status of the laser.

The lower part of the panel controls the bh AOM. Since an AOM is wavelength-dependent the AOM controller has to know the wavelength selected for the laser. If both the laser and the AOM are controlled from SPCM the wavelengths for the laser and the AOM are automatically coupled.

## Motor Stage

SPCM is able to control a motorised sample stage. The stage can be used to control the position of a sample manually or to record mosaics of images in cooperation with the GVD-120 or GVD-104 scan controller of the bh DCS-120 system.

The motor stage control panel is shown in Fig. 108. The panel shown left lets the user manually control the sample position. When a DCS scan is started the system records a normal FLIM image in the selected place of the sample. The panel on the right has Tile Imaging enabled. The motor stage interacts with the scanner, and, in cooperation with the DCS scanner, records a spatial mosaic of images.

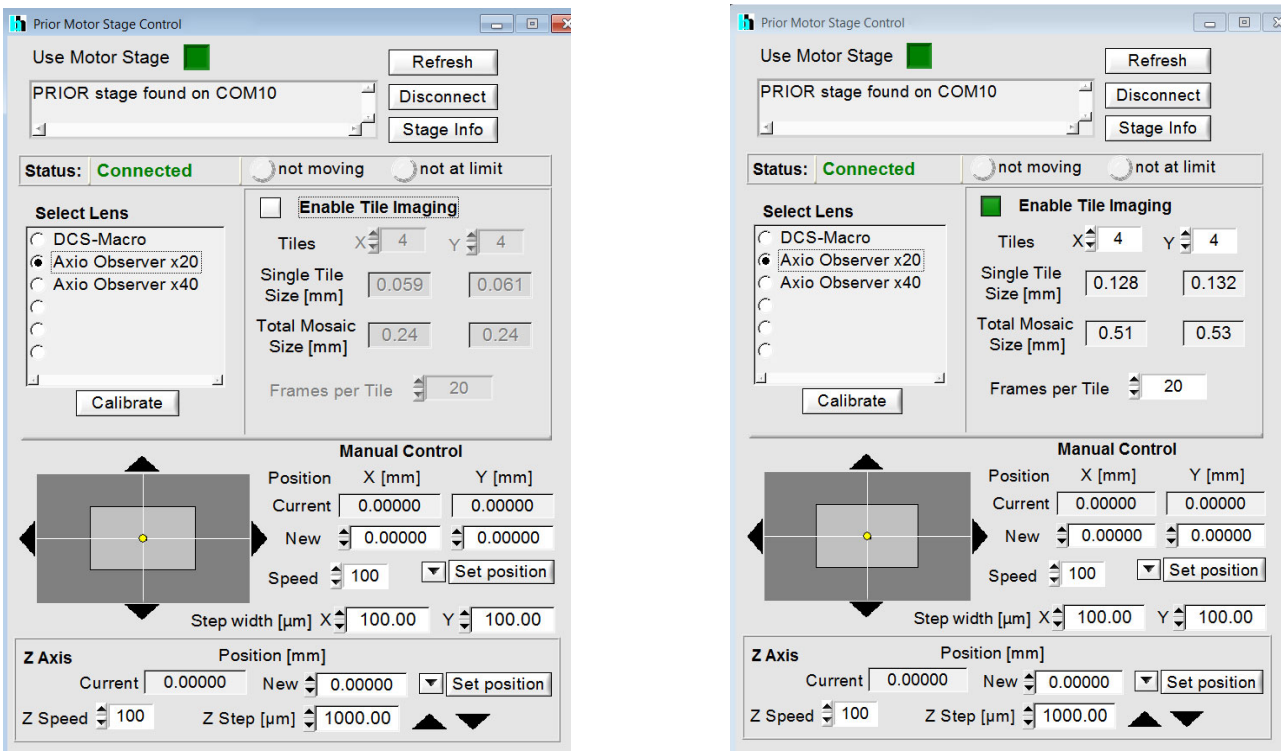



Fig. 108: Control panel for motor stage. Left: Normal scanning, manual control of motor stage. Tile imaging disabled. Right: Tile imaging enabled. Spatial mosaic scanning and manual control of stage.

Fig. 109 shows how the optical scanner interacts with the motor stage. When Tile Imaging is enabled the step width of the motor stage automatically adjusts to the scan area (Zoom factor) selected in the DCS scanner panel. When the measurement is started the SPC system records a mosaic of FLIM images the elements of which have the same x and y size as the step width of the motor stage. The result is a large image consisting of individual scans offset by the motor-stage step width. The data of the individual elements of the mosaic (or tiles) can be accumulated over a selectable number of frames (20 in Fig. 109). The number of frames per tile can be selected both in the motor stage panel and in the scanner panel. It is automatically adjusted to the same value.

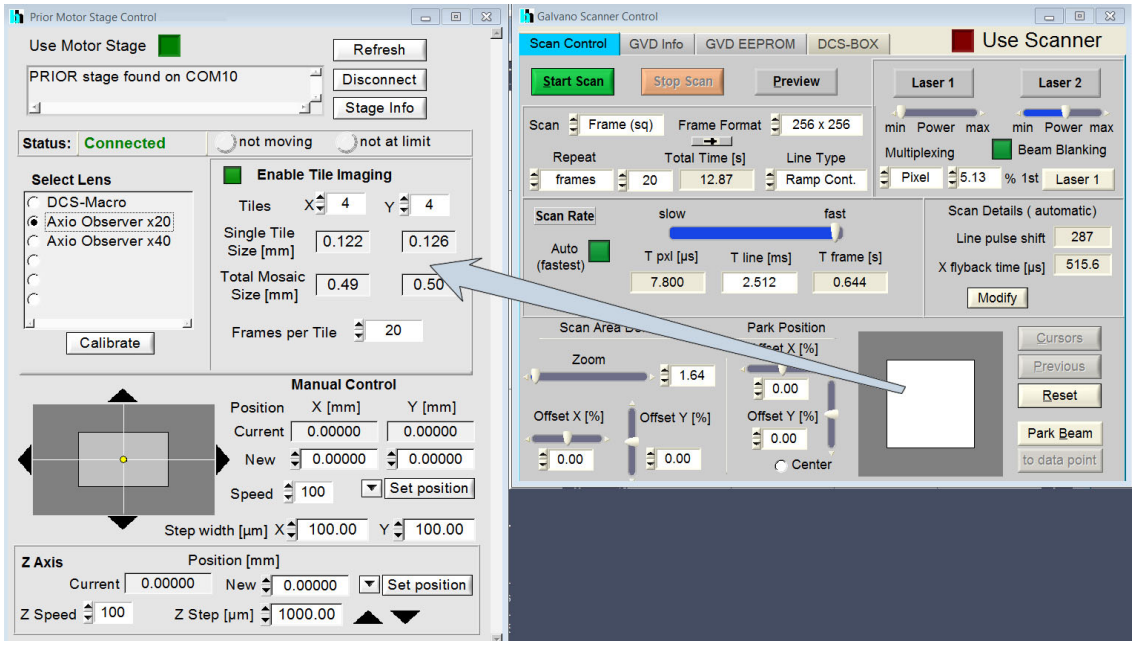



Fig. 109: Interaction of the motor stage with the DCS-120 scanner

## Support

We are happy to give you any possible support to the use of your TCSPC devices. Support starts with help for finding the most promising experimental strategy to solve a given scientific problem, choosing the right system components to implement this strategy, and choosing data recording and data analysis procedures that yield a maximum of information from the experiment. Best case, support should start even before you buy the system components. Once you have the wrong laser and the wrong detectors the best technical support cannot correct the mistakes. Therefore contact us before it is too late.

An important component of support is the bh TCSPC Handbook. It presents solid information on any level: Detection of fast low-level light signals, TCSPC principles, noise sources and their minimisation, detectors and detector principles, setup of experiments, multi-dimensional features of advanced TCSPC and TCSPC FLIM, applications, parameter setup, and data analysis. Moreover, the book contains more than 1200 references related to the bh TCSPC and FLIM devices and their application. Please take a look into this book - if you want a printed copy please request one from bh.

If you have problems with your TCSPC module or with the experiment in which it is used, please contact us under info@becker-hickl.com. Please do not waste your and our time preparing screen shots or lengthy power-point documents to describe the problem. What we need is a data file. Only from the data file we can conclude what is wrong in your system. We can even reproduce a possible problem here.

If you can't save the data (.sdt) send us a setup file (.set). Screen shots are only appropriate if nothing else is working.

In most cases, however, the source of a problem is not directly in the TCSPC module but in the experimental setup. Therefore add a short description of the problem, the setup, and the application for which it is used. If necessary, we can remotely troubleshoot your system via 'Team Viewer' software. Please contact us - we will come back to you with the details.

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