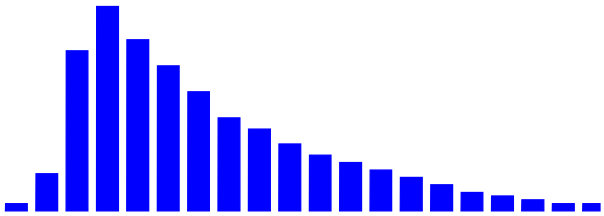

Becker & Hickl GmbH

FLIM Systems for Zeiss LSM 980 Laser Scanning Microscopes

Addendum to

Handbook for Modular FLIM Systems for Zeiss LSM 710 / 780 / 880 Laser Scanning Microscopes

7th Edition

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FLIM Systems for Zeiss LSM 980

Overview

This brochure is an addendum to the handbook ‘Modular FLIM Systems for Zeiss LSM 710 / 780 / 880 Family Laser scanning Microscopes’ [1]. It describes the FLIM systems for the new Zeiss LSM 980 microscopes, explains the differences compared to the LSM 710 / 780 / 880 systems, and shows technical innovations which have become available since the previous handbook has been published.

There are no changes in the general signal recording principles. The systems are still using bh’s multi-dimensional TCSPC process, yielding near-ideal photon efficiency, extremely high time resolution, and unprecedented timing stability [2, 3]. The LSM 980 are using the new SPC-150NX modules, reaching an IRF width of <20 ps.

As for the LSM 710 / 780 / 880, the standard detector is the bh HPM-100-40 GaAsP hybrid detector. However, the ultra-fast HPM-100-06 detector has been added as an option. The opto-mechanical detector interface has been updated to match the requirements of the laser-safety loop of the LSM 980.

Significant progress has been made on the excitation side. Four ps diode lasers of different wavelength have been combined in a four-wavelength laser module. Inside the laser module, the beams of the lasers are combined into one single-mode fibre. The fibre is connected to the LSM 980 scan head via the normal LASOS precision fibre connector. The lasers are software-controlled from the FLIM system. Moreover, they can be multiplexed synchronously with the pixels, lines, or frames of the scan. In combination with the multidimensional features of the recording process, new operation modes became possible. Example are simultaneous dual-excitation and dual-emission wavelength recording, or simultaneous FLIM / PLIM recording with visible excitation wavelength.

New functions have been added also to the SPCM data acquisition software [2]. For example, SPCM is now able to display lifetime images and decay curves from regions of interest online during the measurement. SPCM also got extended multi-threading features. As a result, the systems can record at high sustained count rate even in combination with the new online-display functions.

The data analysis software has been put on an entirely new basis. New Generation SPCImage NG [2, 4] is a combination of time-domain analysis and phasor analysis. The combination provides image segmentation functions to extract precision decay data even from noisy images or from images of moving objects. The new analysis software uses maximum-likelihood estimation (MLE) to fit the time-domain data. The MLE algorithm is not only faster than the previously used weighted least-square (WLS) fit, it also delivers unbiased decay parameters for FLIM data with low photon number. Most importantly, SPCImage NG runs the data analysis on a graphics processing unit (GPU). The GPU runs massive parallel processing, reducing processing times from previously minutes to a few seconds.

System Architecture

Principle of TCSPC FLIM

The general principle of the FLIM data recording has not changed. The bh FLIM systems use a combination of bh's multidimensional time-correlated single-photon counting process with confocal or multiphoton laser scanning. The sample is repetitively scanned by a high-repetition rate pulsed laser beam, single photons of the fluorescence signal are detected, and each photon is characterised by its time in the laser pulse period and the coordinates of the laser spot in the scanning area in the moment its detection. The recording process builds up a photon distribution over these parameters, see Fig. 1. The photon distribution can be interpreted as an array of pixels, each containing a full fluorescence decay curve in a large number of time channels.

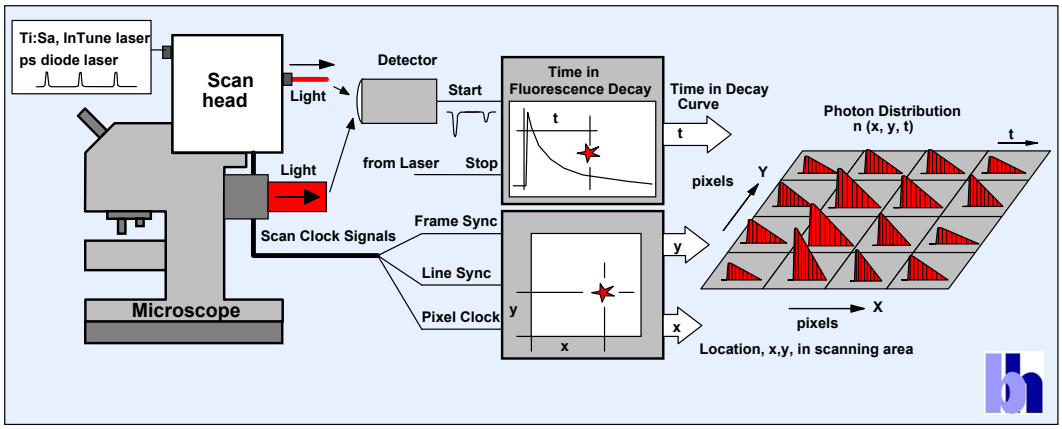

Fig. 1: Principle of TCSPC FLIM

The main application of FLIM is the investigation of biological systems. These systems are characterised by a multitude of parameters. Moreover, the live systems are dynamic. The TCSPC FLIM process shown in Fig. 1 can be extended by including additional parameters in the photon distribution. These can be the depth of the focus in the sample, the wavelength of the photons, the time after a stimulation of the sample, or the time within the period of an additional modulation of the laser. These techniques are used to record Z stacks or lateral mosaics of FLIM images, multi-wavelength excitation and emission FLIM images, images of physiological effects occurring in the sample, or to record simultaneously fluorescence and phosphorescence lifetime images. The options for using these advanced features have greatly improved with the extended control functions of the new LHB-104 four-laser assembly [6]. Please see page 7.

Standard FLIM systems have two parallel channels of the architecture shown in Fig. 1. The systems can be upgraded for up to four parallel detection and recording channels. Different than older systems, the LSM 980 FLIM system use the new SPC-150N or SPC-150NX TCSPC modules. The new modules yield higher time resolution with ultra-fast detectors [8] and have improved timing stability [2]. The modules reach < 5 ps FWHM electrical IRF width and < 1 ps low-frequency timing wobble [2].

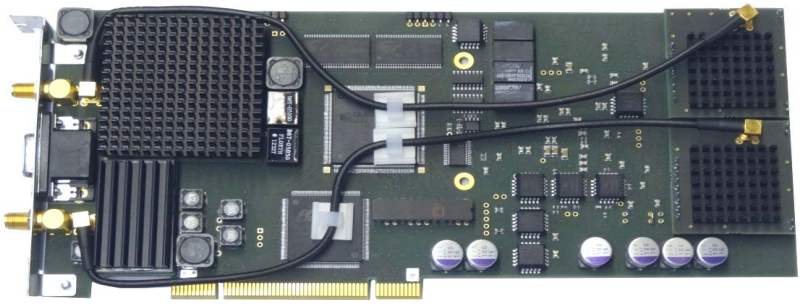

Fig. 2: SPC-150NX module. Compared to the older SPC-150 the SPC-150NX yields higher time resolution and higher timing stability.

Detectors

The standard FLIM detectors are still the bh HPM-100-40 GaAsP hybrid detectors [2]. The detectors have extremely high sensitivity and deliver an IRF width on the order of 100 to 120 ps FWHM. The combination of high sensitivity and high speed is almost ideal for the majority of FLIM applications. For applications which require ultra-high time resolution, e.g. for precision double-exponential analysis of fast decay components, the HPM-100-06 is available. With the SPC-150NX the detector delivers 19 ps FWHM IRF width in multiphoton systems, and 40 to 60 ps FWHM IRF width in diode-laser based systems [8]. The MW FLIM GaAsP multi-wavelength detectors [2] of the LSM 710 / 780 / 880 systems can be used with the LSM 980 as well.

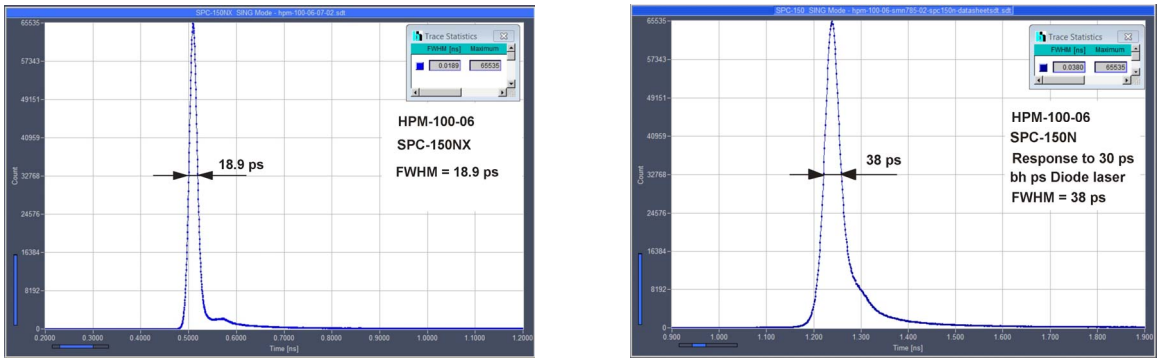

Fig. 3: System IRF (Instrument-Response Function) with HPM-100-06. Left: Ti:Sa laser. Right: ps diode laser.

The use of the Zeiss BIG-2 detectors for FLIM [1, 9] is supported also for the LSM 980. However, the TCSPC time resolution of the BIG-2 is inferior to that of the HPMs. The BIG 2 can be used when the task is just to determine fluorescence lifetimes within the pixels of a FLIM image. For extracting decay components of multi-exponential decay profiles, as it is required for FRET experiments and metabolic FLIM, the BIG 2 is not recommended.

Detector Interface

The optical principle of the detector interface has remained unchanged. Please see [1], pages 3 to 4 and pages 43 to 46. The laser-safety connector of the detectors has been adapted to the new double-safety loop of the LSM 980, see Fig. 4, left. The bh beamsplitter module has the same safety connector, see Fig. 4, middle. Also the cover of the new beamsplitter module is

included in the safety loop, see Fig. 4, right. The safety pins are connected in series. A safety violation is thus reported to the LSM if either a detector is removed from the beamsplitter module, the beamsplitter module is de-attached from the microscope, or the cover of the beamsplitter module is opened.

Fig. 4: Safety connector pins at the detector (left), at the beamsplitter module, and between the beamsplitter module case and the cover of the beamsplitter cube compartment (right)

The detectors and detector assemblies work both for the confocal output of one-photon systems and for the NDD output of multiphoton (LSM 980 NLO) systems.

Lasers

Multiphoton Systems

Multiphoton systems use a Ti:Sa laser for excitation. There is no change compared to the LSM 710 / 780 / 880 systems. For using LSM 980 Multiphoton FLIM systems, please follow the instructions in [1].

One-Photon (Confocal) Systems

In terms of one-photon FLIM excitation the LSM 980 differs considerably from LSM 710 / 780 / 880 systems. The LSM 710 / 780 / 880 were available with integrated ps diode lasers. Standard LSM 980 systems are not. In the standard version, they even have no free laser port to which external lasers could be connected. However, the LSM 980 can be purchased with (or modified for) an additional laser input. The bh FLIM systems for the LSM 980 is available with a four-laser assembly (the LHB-104 ‘Laser Hub’) that can be connected to this input. The LHB-104 ‘Laser Hub’ contains up to four bh BDS-SM picosecond-diode lasers [7]. The optical outputs of the individual lasers are combined into one single-mode fibre. The fibre output connector is compatible with the laser port of the LSM 980. The LHB-104 contains wavelength-multiplexing electronics, inputs for laser-intensity and laser-modulation signals, and outputs for the synchronisation signals to bh SPC modules [6]. The LHB-104 is shown in Fig. 5.

Fig. 5: LHB-104 ‘Laser Hub’. Fibre output via LASOS Precision Connector, compatible with LSM 980 laser input port.

The lasers of the LHB-104 are controlled by software via a DCU-400 or DCU-800 detector-laser controller. A DCU-400 detector/laser controller is shown in Fig. 6.

Fig. 6: DCU-400 detector/laser controller

By controlling the LHB-104 box from the FLIM computer, lasers of different wavelengths can be selected freely. Moreover, different laser wavelengths can be multiplexed to record FLIM images for different excitation wavelength quasi simultaneously [2]. The LHB-104 also supports simultaneous FLIM / PLIM, a technique introduced by bh in 2013 [2, 5]. Other applications include diffuse optical tomography by NIRS techniques, fibre-based lifetime spectroscopy of biological systems, and fluorescence-lifetime spectrometers.

Laser Wavelength Multiplexing

The LHB-104 is able to multiplex two of the available lasers synchronously with the pixels, the lines, or the frames of the scan. Simultaneously with switching the lasers it sends a routing signal to the TCSPC modules. The TCSPC module uses this signal to send photons excited by different lasers into separate images [2].

To multiplex lasers, turn the multiplexing switch of the LHB-104 module to either Frame, Line, or PXL, see Fig. 7, left. FR means frame multiplexing, L1 means multiplexing line by line, L2 means multiplexing each second line, X means pixel multiplexing. In the OFF position multiplexing is disabled, both lasers are on. Connect the outputs of the MPM to the ‘Laser ON’ inputs of the two lasers to be multiplexed (Fig. 7, middle). In the DCC or DCU panel, turn on the lasers which are to be multiplexed, see Fig. 7, right. To multiplex the lasers the multiplexing module of the LHB needs the scan clocks from the laser scanning microscope.

Therefore, make sure that the scan clocks from the microscope to the TCSPC system are fed through the MPM, as shown in Fig. 8.

Fig. 7: Left: MPM mode switch. Middle: Cables from MPM to Laser ON inputs, lasers 3 and 4 are multiplexed by MPM outputs 1 and 2. Right: DCU panel with lasers 1 and 2 turned on

Fig. 8: Connection of the Scan clock signals through the MPM module. 'SCAN In' is the input from the scan controller, 'To SPC is the output to the TCSCP module(s)

TCSPC Process with Laser Multiplexing

The TCSPC FLIM process with laser multiplexing is illustrated in Fig. 9. In addition to the time and the x,y position of the photons the TCSPC modules receive a bit that identifies the laser by which the current photon was excited. The TCSPC module uses this bit to route the photon into the image for the corresponding laser. Each TCSPC module thus records two separate FLIM images for different excitation wavelength. Two TCSPC modules are running in parallel, with their detectors detecting in different emission wavelength intervals. Thus, the FLIM system records four images for the four combinations of excitation and emission wavelengths.

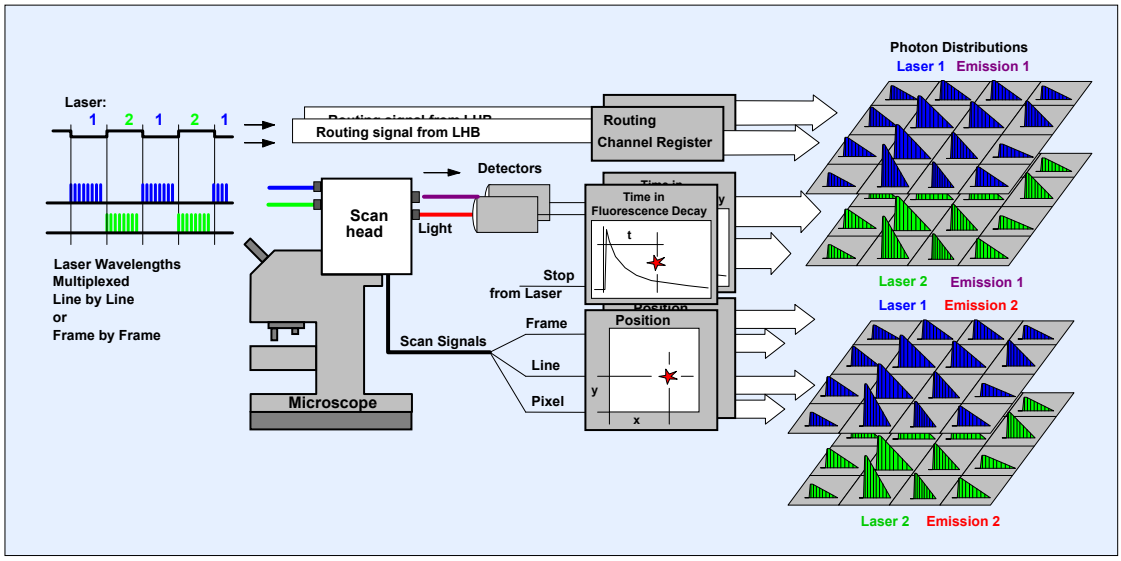

Fig. 9: TCSPC FLIM with laser multiplexing

Data Acquisition Software

SPCM Software

SPCM runs the data acquisition in the various operation modes of the SPC modules while controlling peripheral devices, such as detectors, lasers, scanners, motor stages, or other experiment control devices. 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, there are modes for FLIM, multi-wavelength FLIM, Mosaic FLIM, time-series FLIM, Z stack FLIM, and simultaneous FLIM / PLIM. Please see [1, 2] for details. 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.

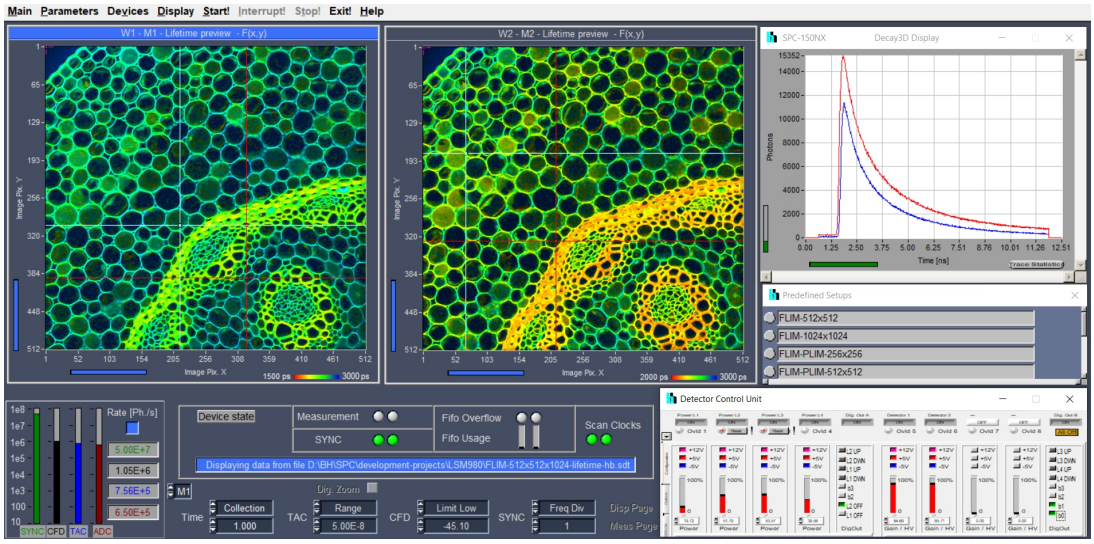

Fig. 10: Example of SPCM Main panel

SPCM includes the management of the measurement and measurement 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, images, and arrays of images. Different projections can be used to visualise data from selected planes within a multi-dimensional data array. Display functions also contain online calculation and display of pseudo-colour fluorescence-lifetime images and display of decay curves from ROIs within lifetime images. A direct link is provided for communication with SPCImage NG FLIM analysis software.

The user interface of SPCM is configurable by the user. Different configurations can thus be created for different applications and measurement tasks. The configurations can be stored in a Predefined Setup panel and recalled on demand by a single mouse click.

SPCM runs under Windows 10. SPCM is a true 64-bit application. Provided the computer has enough memory there is virtually no limitation in the data size of TCSPC / FLIM results. SPCM thus records 'Megapixel' FLIM data with unprecedented pixel numbers. Moreover, SPCM records data of high complexity, such as multi-emission wavelength or multi-excitation wavelength FLIM data, dynamically variable FLIM data, or spatial FLIM mosaics.

Laser and Detector Control

Detectors and lasers are controlled via a DCU-400 or a DCU-800 device [6]. The DCU provides gain regulation, ON/OFF control, cooling and overload shutdown for detectors and power control and intensity and ON/OFF control for the lasers. It operates also the optical attenuators of the lasers.

Operation of the DCU is integrated in the SPCM data acquisition software. The software panel for controlling four lasers and two detectors is shown in Fig. 11. The electrical power of the four lasers is controlled by the four sliders on the left, the gain of the detectors by the two sliders in the middle. Two outputs are left unused. They can be used for additional detectors or other control tasks within the FLIM system. The laser ON/OFF buttons and the attenuator buttons are located in the middle (Laser 1, 2) and on the right (Laser 3, 4). Please see [6] for further details.

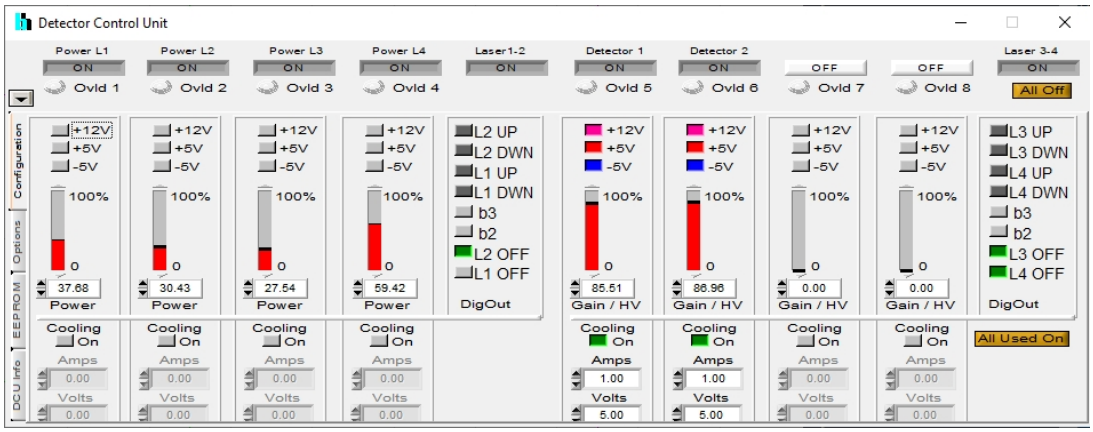

Fig. 11: DCU-800 software pane, control of four lasers and two detectors

System Parameter Setup

The TCSPC system parameters for the basic FLIM modes are described in [1], see 'System Parameters for Basic FLIM Experiments'. The parameters for time-series recording, Z-stack FLIM, spatial and temporal mosaic FLIM, FLITS, PLIM, and FCS are described under 'Advanced FLIM Techniques and Procedures'. There is no need to change these setups for the LSM 980. However, new setups have to be generated for FLIM with laser multiplexing.

Laser Wavelength Multiplexing

Frame Multiplexing

System Parameters

The TCSPC system parameters for Frame Multiplexing are shown in Fig. 12. The system parameters are essentially the same as for normal single-channel and dual-channel FLIM. The only difference is that 'Routing Channels' is 2 instead of 1. This allows the TCSPC modules to receive the multiplexing information, i.e. the identifier of the laser that excited the current photon (see Fig. 9). The TCSPC modules thus build up two separate images for the two la-

sers. The scan parameters are the same as for normal FLIM with a 512 x 512 pixel LSM scan and 512 x 512 pixel FLIM recording, see ‘More Parameters’ on the right.

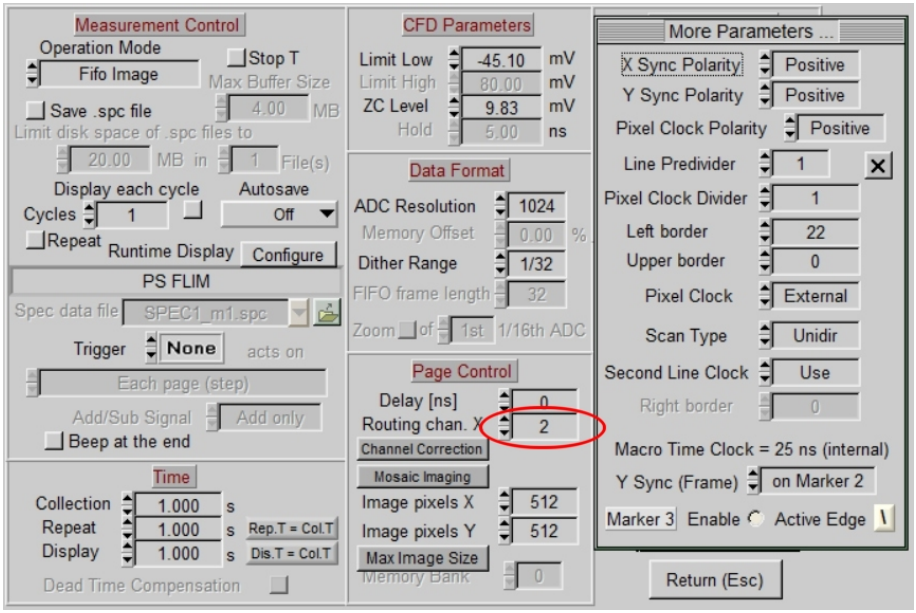

Fig. 12: System parameters for Frame Multiplexing

3D Trace Parameters and Window Intervals

The 3D Trace Parameters are shown in Fig. 13. The parameters define which of the available images are displayed in the SPCM main panel. With the settings shown, all four combinations of detection channels and lasers are displayed. Display windows 1 to 4 show the images for TCSPC Module 1 / Laser 1, TCSPC Module 1 / Laser 2, TCSPC Module 2 / Laser 1, and TCSPC Module 2 / Laser 2, respectively. Lifetime display by the SPCM online function has been selected. You can also select (gated) intensity images, see examples in [1]. Please note that not all display windows may contain useful photons. This is especially the case when a detection wavelength interval is on the short-wavelength side of one of the laser wavelengths. In that case, you can turn off the display of these data by de-activating the ‘Active’ button.

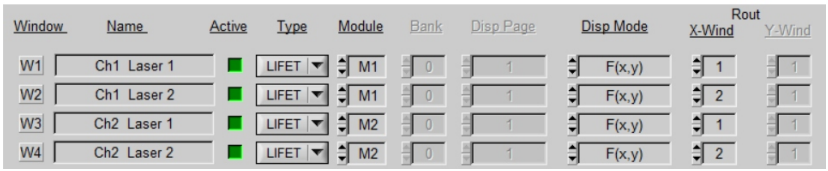

Fig. 13: 3D trace parameters for laser multiplexing and two parallel TCSPC channels

The ‘Routing Windows’ selected in the 3D Trace parameters can be combinations of several TCSPC routing channels. For laser wavelength multiplexing each routing window contains just one routing channel. The corresponding definitions are made in the ‘Window Intervals, see Fig. 14.

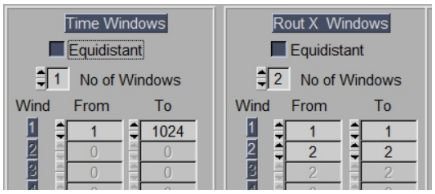

Fig. 14: Window parameters for laser multiplexing

SPCM Main Panel

The SPCM Main panel obtained with the definitions above is shown in Fig. 15. It has display windows for the four combinations of lasers and TCSPC detection channels. The control panel for the lasers and detectors is in the lower right.

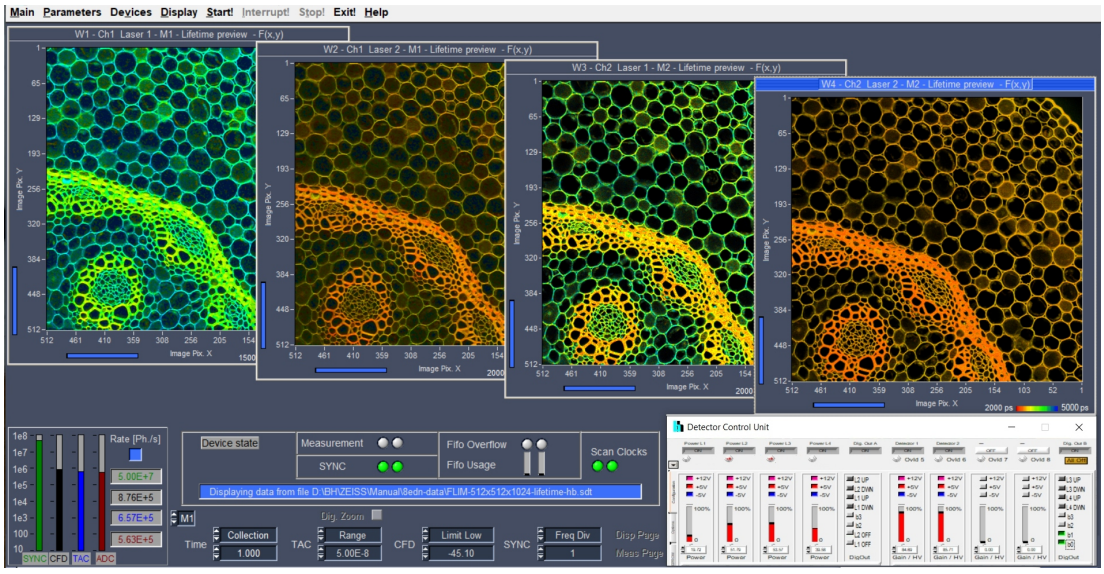

Fig. 15: SPCM main panel for laser-wavelength multiplexing. Two parallel TCSPC channels, two lasers. Images of all combinations of lasers and TCSPC channels displayed.

Line and Pixel Multiplexing

The system parameters for line and pixel multiplexing are shown in Fig. 16. The parameters are mostly the same as for frame multiplexing. Differences are in the scan parameters. With line or pixel multiplexing, the laser wavelength changes for each second line or each second pixel. Controlled by the routing signal, the TCSPC module sends the photons of even lines or even pixels into one image, the photons of uneven lines or uneven pixels into a second one. The resulting images thus have only half the number of lines or half the number of pixels of the LSM scan. To obtain the correct image size a ‘Line Divider’ or ‘Pixel Clock Divider’ of 2 has to be used for the image buildup. We recommend to use dividers of 2 both for the lines and the pixels, see Fig. 16, right. The LSM scan can then be performed just with twice the pixel number of the FLIM images. Note that with the settings of Fig. 16 the FLIM images are recorded with 512 x 512 pixels. The LSM therefore has to run a 1024 x 1024 pixel scan or, if available, a 512 x 512 scan with line and pixel averaging. The 3D trace parameters, the Window Parameters, and the SPCM main panel are the same as for frame multiplexing.

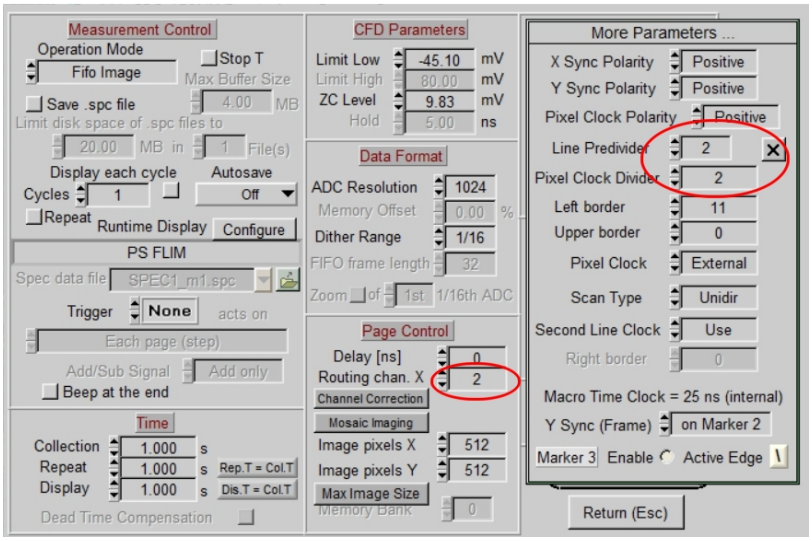

Fig. 16: System parameters for pixel or line multiplexing

Data Analysis

bh FLIM systems for the Zeiss LSM 980 run Next-Generation SPCImage NG data analysis software [2, 4]. SPCImage NG is a combination of time-domain and phasor analysis, see Fig. 17. Phasor clusters can be defined in the phasor plot and back-annotated in the time-domain images. The corresponding pixels be combined for high-accuracy multi-exponential decay analysis. Moreover, histograms of decay parameters, such as amplitude or intensity weighted lifetimes, component lifetimes, component amplitudes, or ratios of these parameters can be created within the selected phasor range. Vice versa, ROIs in the time-domain images can be defined, and a phasor plot be displayed for the selected area. Other innovative features are a Maximum-Likelihood Estimation algorithm, GPU Processing, and new decay models and new IRF modelling functions. Please see [2], chapter ‘FLIM data analysis’ for details.

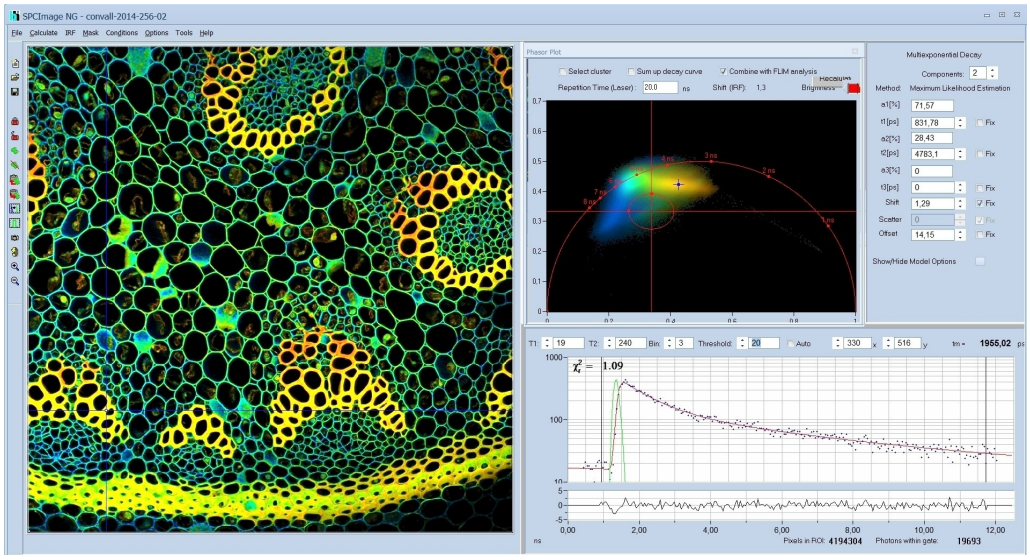

Fig. 17: SPCImage NG main panel, with time domain fit and phasor plot

General Analysis Functions

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. 18 through Fig. 21.

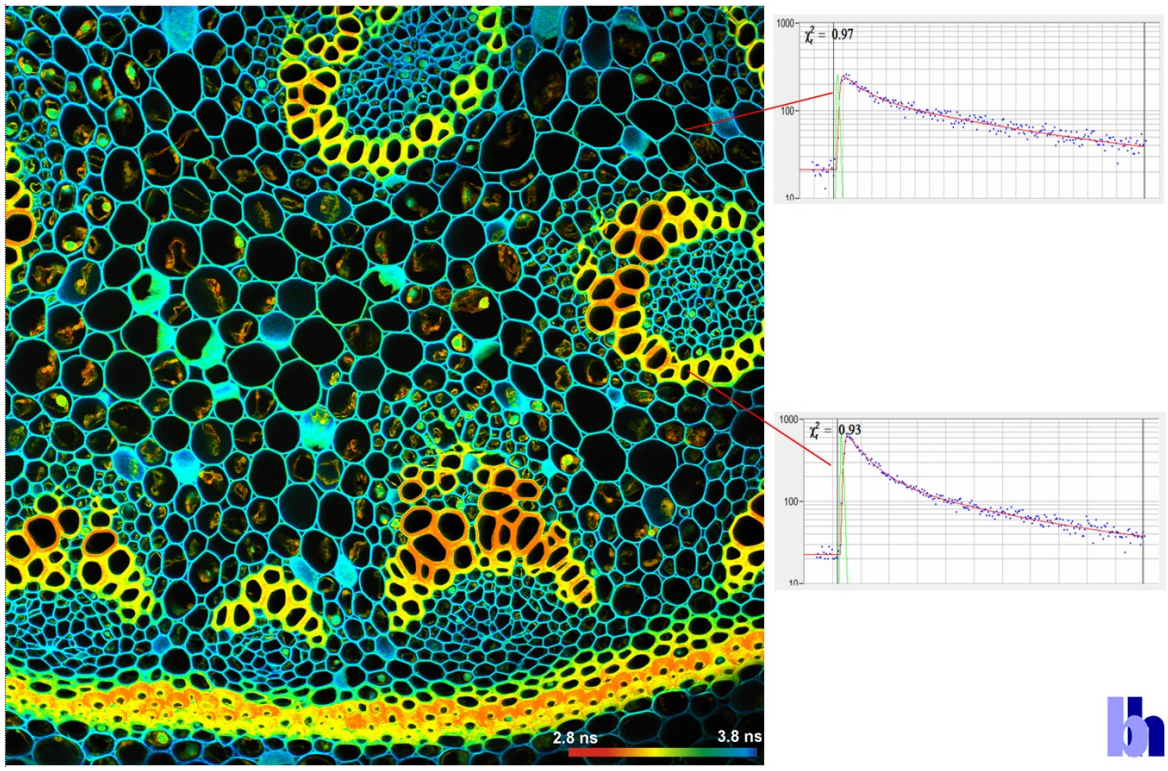

Fig. 18: Image of the amplitude-weighted lifetime, tm, of a double-exponential decay. Right: Fluorescence decay curves in selected pixels.

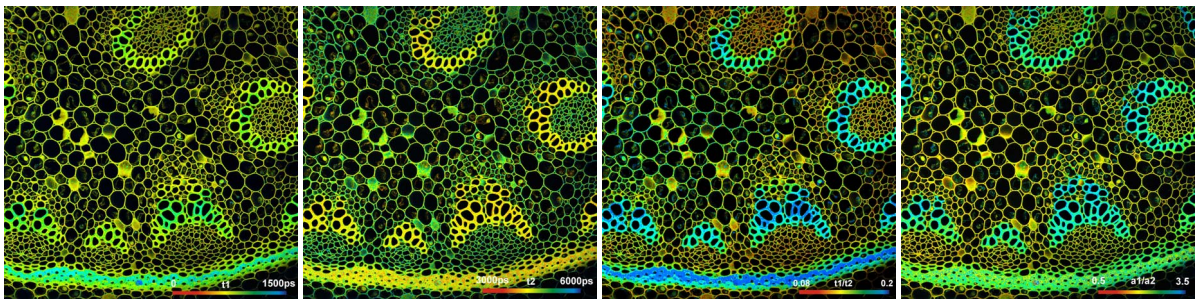

Fig. 19: Left to right: Colour-coded images of the lifetimes of the fast component, t1, and the slow component, t2, of a double-exponential decay, Images of the amplitude ratio, a1/a2, and the lifetime ratio, t1/t2, of the fast and the slow decay component.

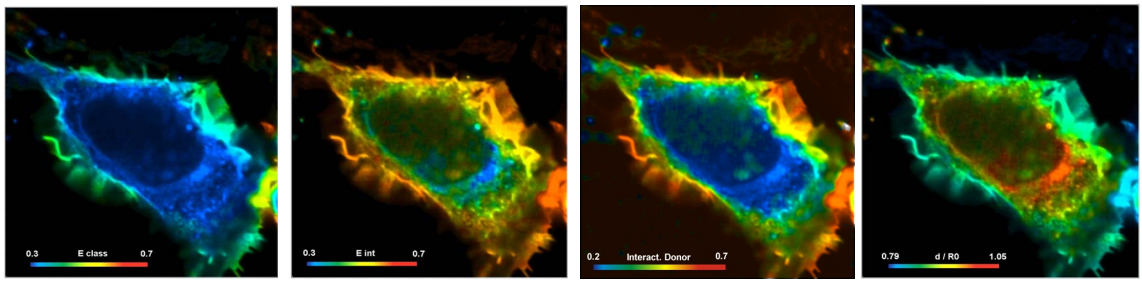

Fig. 20: Cell with interacting proteins, labelled with a FRET donor and a FRET acceptor. Left to right: Classic FRET efficiency, FRET efficiency of interacting donor fraction, amount of interacting donor, FRET distance.

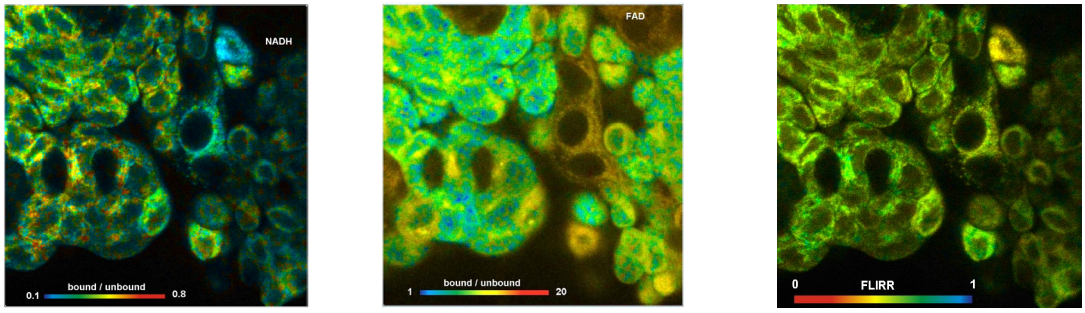

Fig. 21: Metabolic FLIM. Bound-unbound ratio of NADH, Bound/unbound ratio of FAD, Fluorescence-Lifetime Redox Ratio, FLIRR.

Maximum-Likelihood Algorithm

In addition to the traditional weighted least-square (WLS) fit SPCImage NG has a Maximum-Likelihood Estimation (MLE) implemented. The MLE algorithm is not only faster than the WLS fit, it also delivers superior lifetime accuracy for low photon numbers. A comparison is given in [2].

GPU Processing

Data produced by state-of-the art 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-100-06 detectors [8]. In terms of size, such data sets are equivalent to a stack of 1024 one-megapixel images. Processing such amounts data by the CPU of even a fast computer can take tens of minutes. SPCImage NG therefore uses GPU (Graphics Processor Unit) processing. 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. GPU processing is running on NVIDIA cards and a number of other NVIDIA-compatible display devices. When SPCImage NG finds a suitable device in the computer it automatically runs the data analysis on it.

Fig. 22: Left: Progress panel during lifetime calculation, showing that GPU processing is used. Right: Part of 'Preferences' panel, indicating that a Quadro K1100M CUDA 7.0 device was found.

Fig. 23 shows an image recorded with 1024 x 1024 pixels and 1024 time channels per pixel. Processing times for triple-exponential analysis were:

Traditional WLS fit, CPU, multicore processing:	16 minutes
MLE fit, CPU, multicore processing:	5 minutes
MLE fit, NVIDIA GeForce MX230 on Lenovo laptop	40 seconds
MLE fit, NVIDIA GeForce GTX 1050 TI	8 seconds
MLE fit, NVIDIA GeForce RTX 2080 Ti 7.0	2 seconds

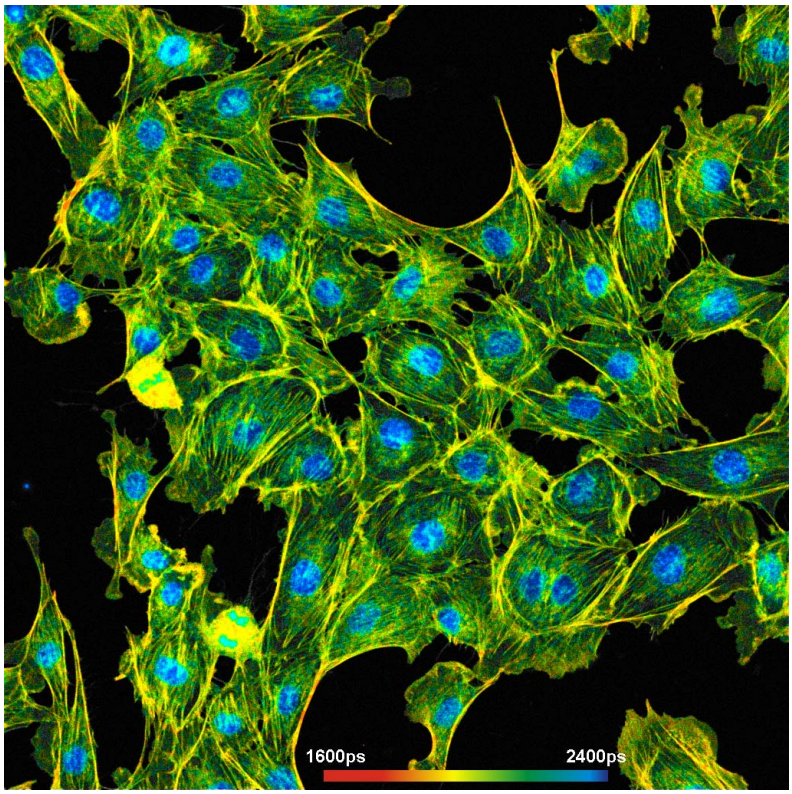

Fig. 23: Lifetime image with 1024 x 1024 pixels and 1024 time channel per pixel, recorded by bh FASTAC FLIM system on a Zeiss LSM 880 NLO. Processing time is reduced from 16 minutes on CPU to 2 seconds on NVIDIA GeForce RTX 2080 Ti 7.0 GPU.

IRF Modelling

In a laser scanning system it is difficult, if not impossible, to measure an IRF. The excitation wavelength is usually blocked by filters, and a fluorophore with sufficiently short lifetime does not exist. In multiphoton microscopes recording of an SHG signal is an option but also this is not free of pitfalls [2]. From the beginning, SPCImage therefore offered ways to estimate an IRF from the recorded data.

Previous SPCImage versions offered an ‘Auto IRF’ which was derived from the rising edge of the fluorescence signal in a selectable area of the FLIM image. The IRF obtained this way is reasonably correct when the recorded signal is fluorescence with a lifetime several times longer than the width of the IRF. Systematic deviations may occur when SHG or an extremely fast fluorescence decay component are present, or when the rising edge is distorted by laser leakage. Nevertheless, the 'Auto IRF' works well [2], and has been used successfully since the introduction of SPCImage in 2000.

SPCImage provides a fully synthetic IRF. The calculation is based on fitting an IRF-model function together with the selected decay model to the FLIM data. The IRF model is a function of the type $x \cdot e^{-x}$ which closely resembles the IRF of hybrid detectors with GaAsP cathodes. It also fits reasonably well to the response of other detectors. For lasers with pulse width above 1 ps the detector IRF can be convoluted with a laser pulse of selectable width. The definitions are shown in Fig. 24, two examples for IRFs of the $x \cdot e^{-x}$ type are shown in Fig. 25. In both cases, the synthetic IRF yields a virtually perfect fit of the decay model function to the photon data.

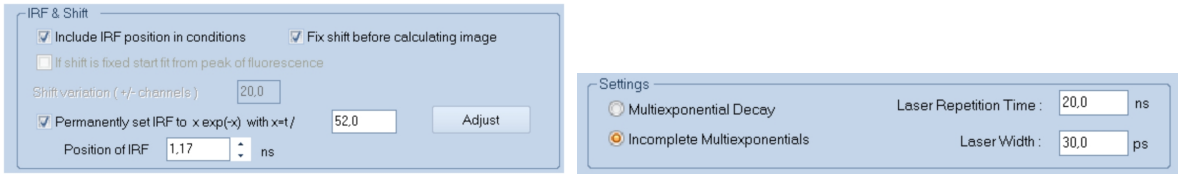

Fig. 24: Definitions for IRF of the type $x \cdot e^{-x}$

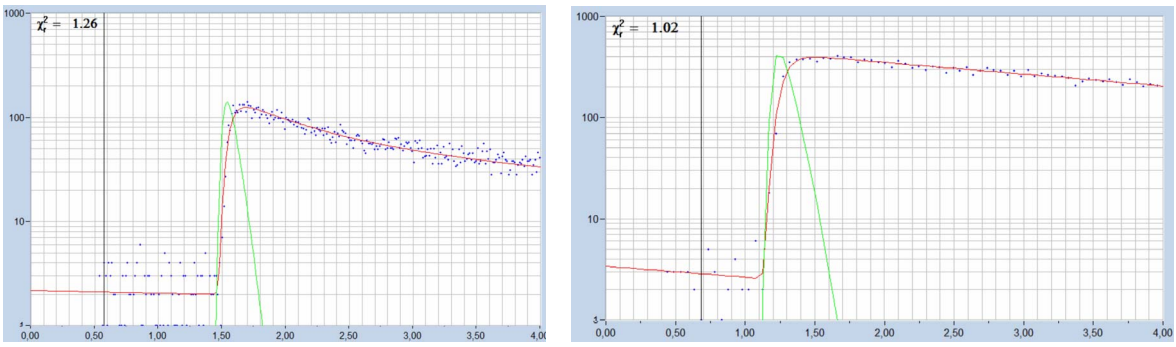

Fig. 25: IRF of the type xe^{-x}. Left: Modelled for ps diode laser plus HPM-100-06 hybrid detector. Right: IRF modelled for HPM-100-40 hybrid detector and ps diode laser.

References

1. Becker & Hickl GmbH, Modular FLIM systems for Zeiss LSM 710 / 780 / 880 family laser scanning microscopes. User handbook, 7th ed. (2017). Available on www.becker-hickl.com
2. W. Becker, The bh TCSPC handbook. 8th edition, Becker & Hickl GmbH (2019), available on www.becker-hickl.com
3. Becker & Hickl GmbH, The bh TCSPC Technique. Principles and Applications. Available on www.becker-hickl.com.
4. SPCImage NG Next Generation FLIM data analysis software. Overview brochure, 20 pages, available on www.becker-hickl.com.
5. Becker & Hickl GmbH, Simultaneous Phosphorescence and Fluorescence Lifetime Imaging by Multi-Dimensional TCSPC and Multi-Pulse Excitation. Application note, available on www.becker-hickl.com
6. Becker & Hickl GmbH, LHB-104 Laser Hub. User Manual. Available on www.becker-hickl.com.
7. BDS-SM Family Picosecond Diode Lasers. Extended data sheet, www.becker-hickl.com
8. Becker & Hickl GmbH, Sub-20ps IRF width from hybrid detectors and MCP-PMTs. Application note, available on www.becker-hickl.com
9. Becker & Hickl GmbH, Zeiss BiG-2 GaAsP detector is compatible with bh FLIM Systems. Application note, available on www.becker-hickl.com

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