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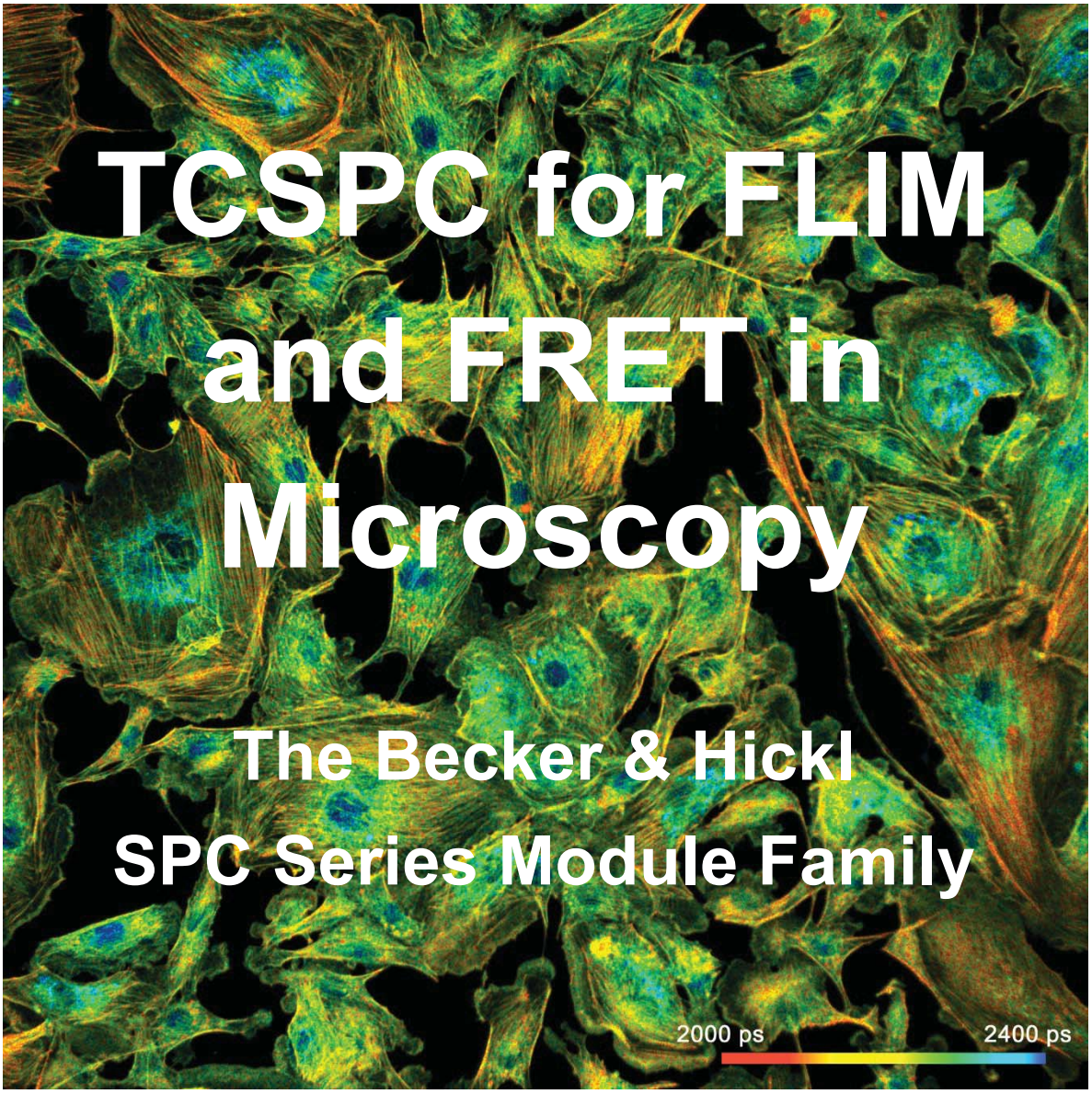

TCSPC for FLIM and FRET in Microscopy

The Becker & Hickl SPC Series Module Family

PC Based Systems

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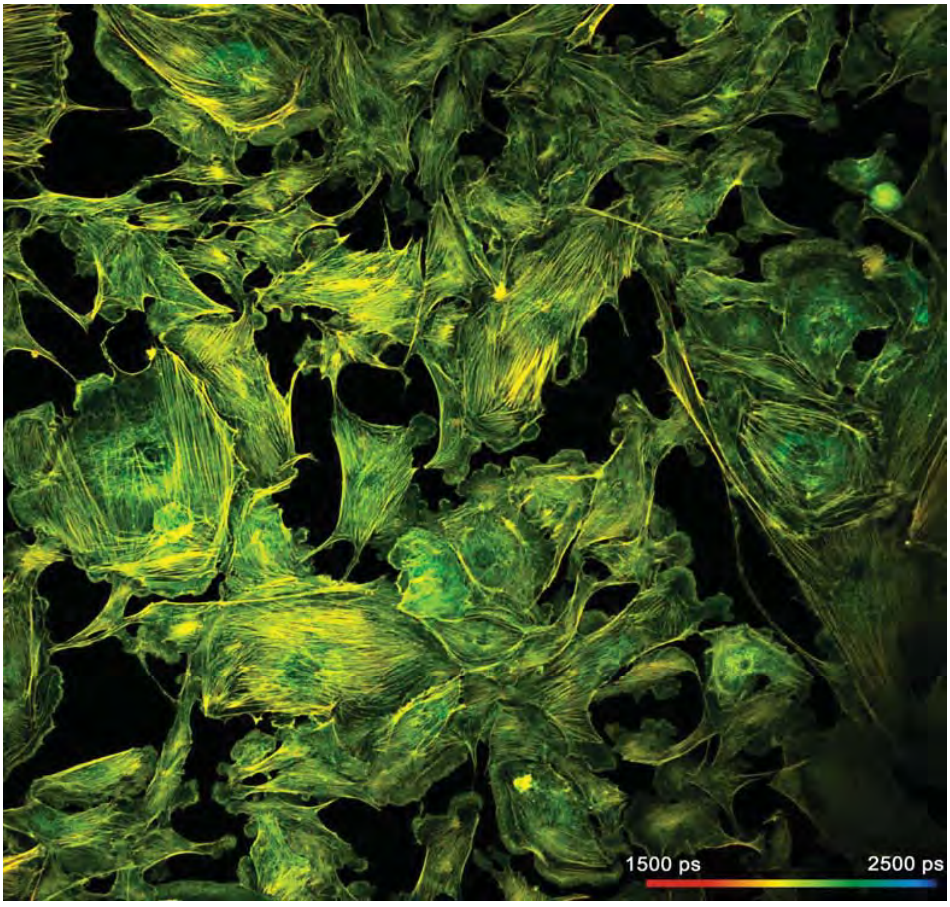
FLIM Systems for
Laser Scanning Microscopes



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FLIM Systems for Laser Scanning Microscopes

General Features

Principle

The FLIM systems are based on bh's multi-dimensional time-correlated single photon counting (TCSPC) process in combination with confocal or multiphoton scanning by a high-frequency pulsed laser beam. 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 of its detection. The recording process builds up a photon distribution over these parameters. The result is an array of pixels, each containing a full fluorescence decay curve in a large number of time channels [9, 15, 16].

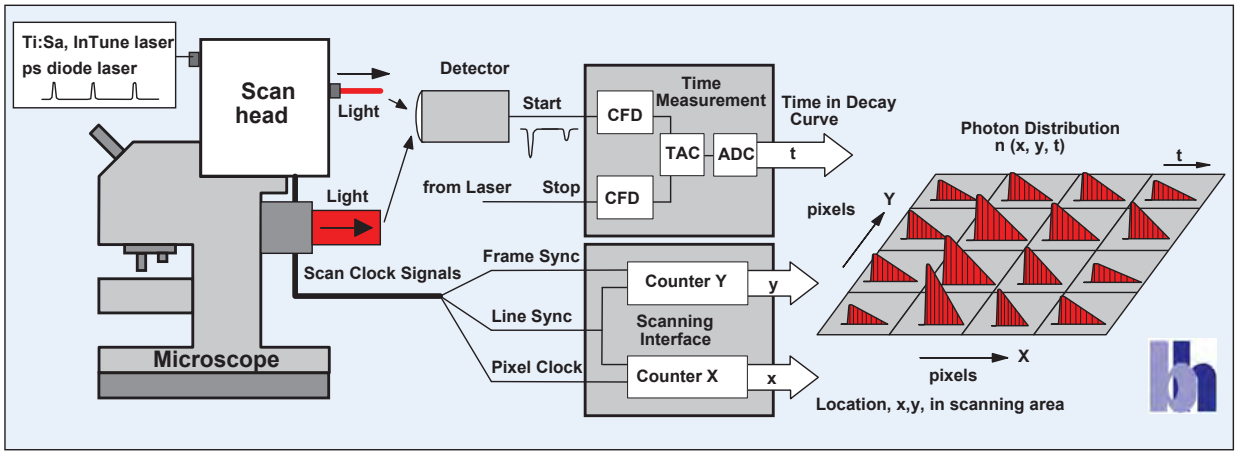


Fig. 1: bh's multi-dimensional TCSPC FLIM process probes the sample by randomly emitted photons

The principle shown in Fig. 1 can be extended to simultaneously detect in 16 wavelength channels. The optical spectrum of the fluorescence light is spread over an array of 16 detector channels. The TCSPC system and determines the detection times, the channel numbers in the detector array, and the position, x, and y, of the laser spot for the individual photons. These pieces of information are used to build up a photon distribution over the time of the photons in the fluorescence decay, the wavelength, and the coordinates of the image. The principle of multi-wavelength FLIM is shown in Fig. 2.

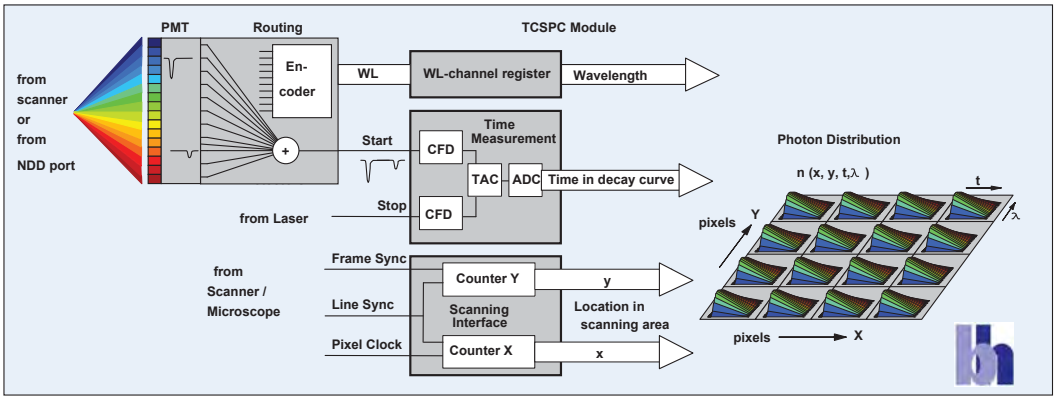


Fig. 2: Principle of Multi-Wavelength TCSPC FLIM

As for single-wavelength FLIM, the result of the recording process is an array of pixels. However, the pixels now contain several decay curves for different wavelength. Each decay curve contains a large number of time channels; the time channels contain photon numbers for consecutive times after the excitation pulse.

The technique works at even the fastest scan rates available in laser scanning microscopes, combines near-ideal photon efficiency, excellent time resolution, excellent timing stability, fast recording speed, multi-wavelength capability, and resolution of multi-excellent decay functions into their components with optical sectioning capability and suppression of lateral scattering [12, 15]. The systems are live-cell and live-tissue compatible, able to record fast physiological effects in the sample, record spatial mosaics and Z stacks of FLIM images, and to simultaneously record fluorescence and phosphorescence lifetime images.

The bh FLIM systems are using 64-bit data acquisition software [16]. As a result, images with extremely high spatial and temporal resolution can be recorded. Images can be large as 2048 x 2048 pixels with 256 time channels per pixel, or 1024 x 1024 pixels with 1024 time channels. Such images cover the full field of view of even the best microscope lenses at diffraction-limited resolution. Multiwavelength FLIM is possible with 16 wavelength intervals and up to 512 x 512 pixels and 256 time channels.

Data Acquisition Hardware

The bh FLIM system contain one or several (usually two) TCSPC FLIM modules, a detector controller, and, if the bh DCS-120 scan head is used, a scan controller module. The modules can be operated inside a PC, or in an extension box connected to a laptop computer, see Fig. 3.

Fig. 3: Left: PC-based FLIM system, shown with DCS-120 scan head, BDL-SMC picosecond diode laser, and HPM-100 hybrid detectors. Middle: Simple-Tau 152 dual-channel FLIM system, containing tw0 SPC-150 TCSPC FLIM cards and a DCC-100 detector controller. Right: Simple-Tau II system, containing two SPC-160pcie TCSPC FLIM cards and a DCC-100pcie detector controller.

bh FLIM systems are compatible with almost any high-frequency pulsed excitation source. These can be Ti:Sa lasers of multiphoton microscopes, super-continuum lasers, or pulsed fibre lasers. For FLIM with scanning microscopes that have no pulsed excitation source bh deliver a wide range of picosecond diode lasers.

Most bh FLIM systems are using the bh HPM-100 hybrid detectors [11]. However, the system also work with single-photon avalanche diodes (SPADs), with InGaAs SPADs [3], with conventional PMTs [4], with MCP PMTs [8], and even with superconducting NbN detectors [6]. bh TCSPC FLIM hardware delivers unsurpassed time resolution and timing stability. Time-channel widths down to 405 fs are feasible, and the electronic jitter is less than 3 ps rms. With ultra-fast detectors fluorescence lifetimes in the 20 ps range can be resolved. Please see [16] for details.

Data Acquisition Software

The bh FLIM systems use bh SPCM data acquisition software [16]. Since 2013 the SPCM software is available in a 64-bit version. SPCM 64 bit exploits the full capability of Windows 64 bit, resulting in faster data processing, capability of recording images of extremely large pixel numbers, and availability of additional multi-dimensional FLIM modes.

The main panel of the SPCM data acquisition software is configurable by the user. Four configurations for FLIM systems are shown in Fig. 4. During the acquisition the SPCM software displays intermediate results in predefined intervals, usually every few seconds. The acquisition can be stopped after a defined acquisition time or by a user commend when the desired signal-to-noise ratio has been reached. Frequently used operation modes and user interface configurations are selected from a panel of predefined setups.

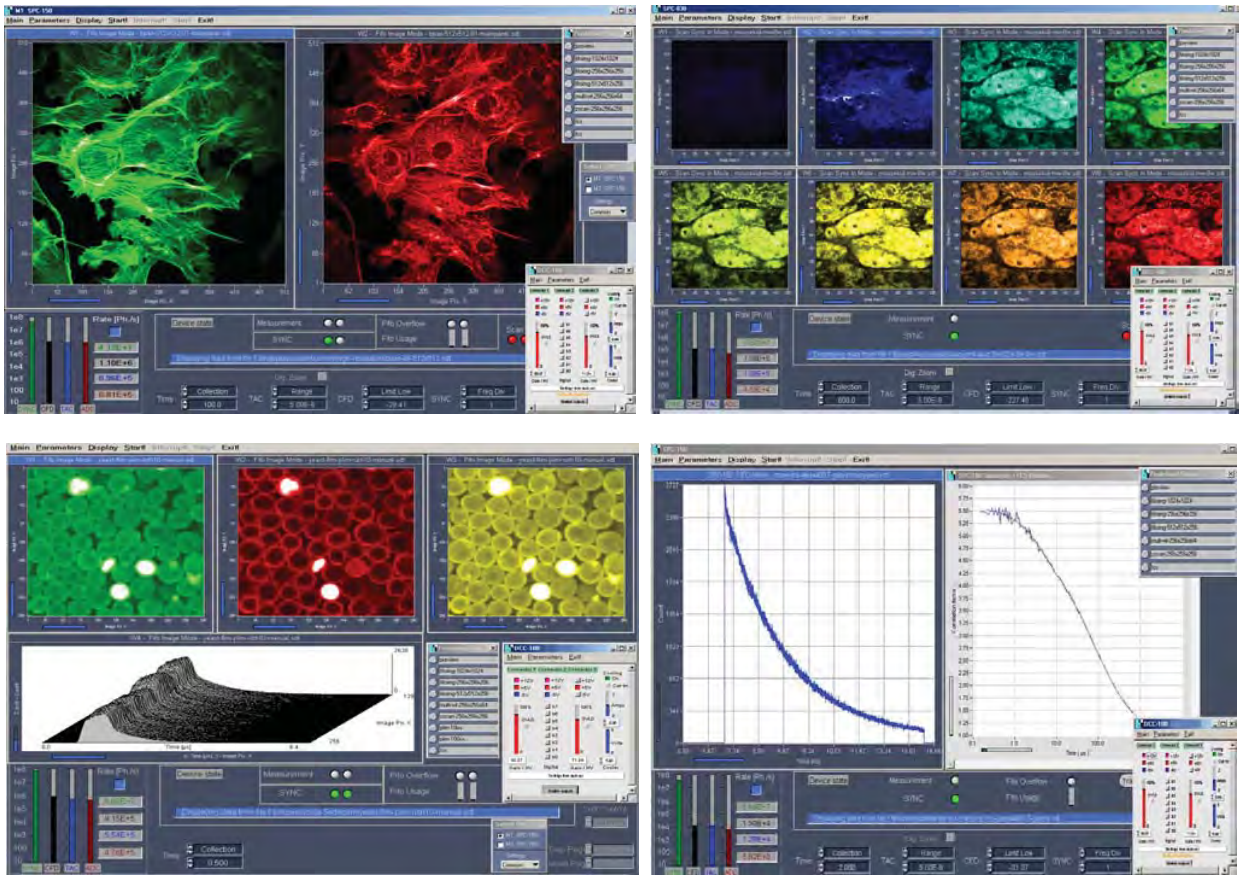


Fig. 4: SPCM software panel. Top left to bottom right: FLIM with two detector channels, multi-spectral FLIM, combined fluorescence / phosphorescence lifetime imaging (FLIM/PLIM), fluorescence correlation (FCS).

FLIM Data Analysis

All bh FLIM systems use bh SPCImage data analysis software. SPCImage runs a de-convolution on the decay data in the pixels of FLIM data. It uses single, double, or triple-exponential decay analysis to produce pseudo-colour images of lifetimes, amplitudes, or intensities of decay components, or of ratios of these parameters. An ‘incomplete decay’ model is available to determine long fluorescence lifetimes within the short pulse period of the Ti:Sa laser of a multiphoton system. Moreover, SPCImage avoids troublesome recording of an instrument response function (IRF) by extracting the

IRF from the FLIM data themselves. Please see data analysis chapters in [1, 2] or [16] for further details.

The main panel of the SPCImage data analysis is shown in Fig. 5. It shows an intensity image (upper left), a lifetime image (upper middle), a lifetime distribution over the pixels of a region of interest (upper right), and the fluorescence decay curve in a selected spot of the image (lower left). The basic model parameters (one, two or three exponential components) are selected in the lower right.

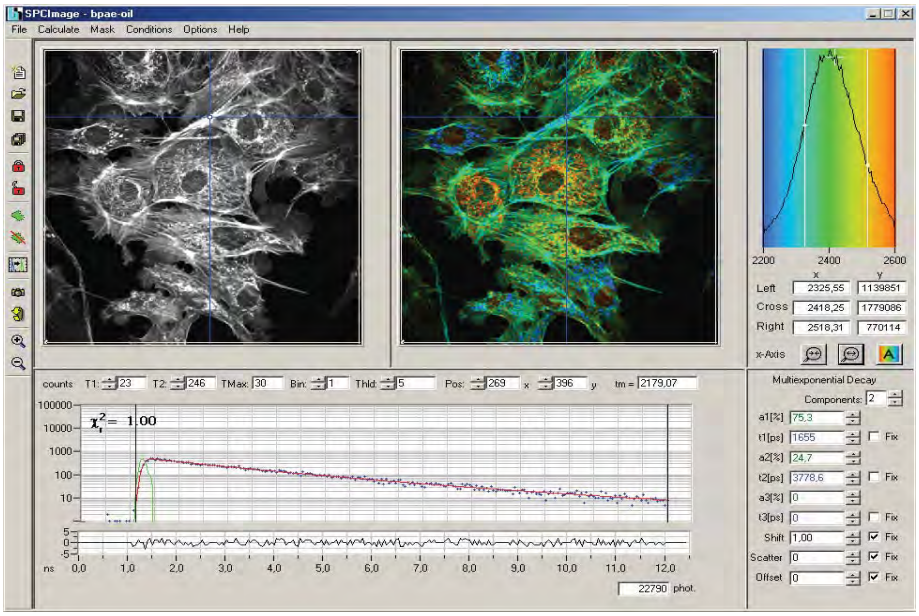


Fig. 5: Main panel of the SPCImage data analysis

FLIM Functions in Brief

Easy Change Between Instrument Configurations

Frequently used instrument configurations are stored in a ‘Predefined Setup’ panel. Changing between the different configurations and user interfaces is just a matter of a single mouse click, see Fig. 6.

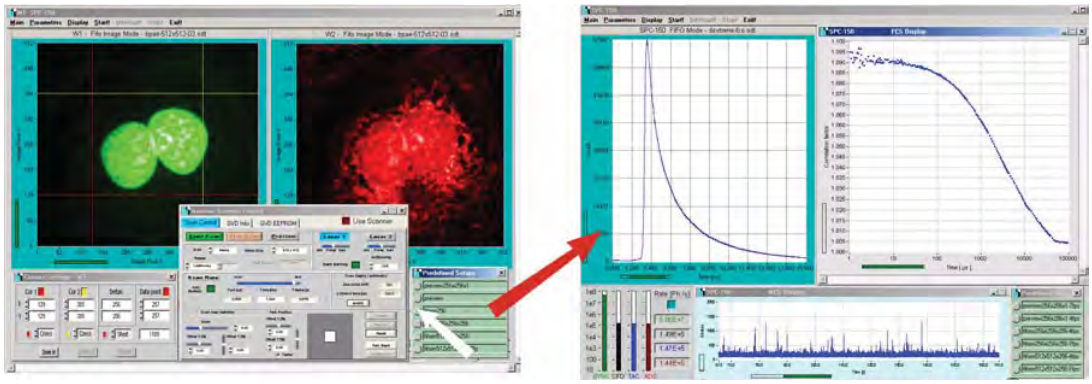


Fig. 6: Changing between different instrument configurations: The software switches from a FLIM configuration into an FCS configuration by a simple mouse click

Interactive Scan Control

Any change in the scan area of the microscope immediately becomes effective in the recorded images.

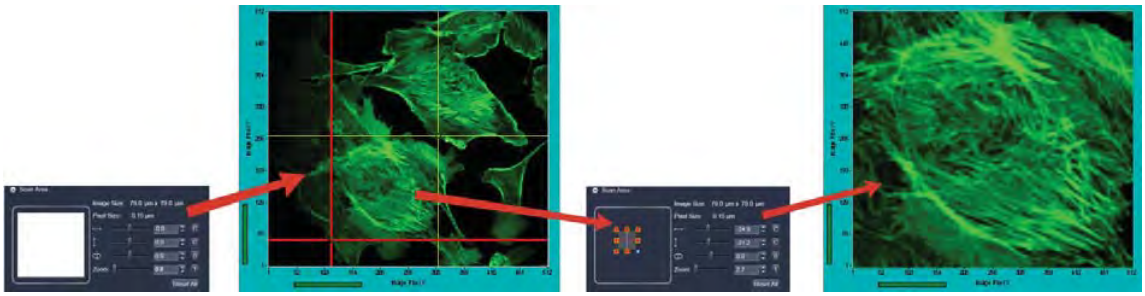


Fig. 7: Interactive scanner control for external microscope software. Example for Zeiss LSM 780/880.

For systems using the GVD-120 (such as the bh DCS 120 system) the control of the scanner is integrated in the SPCM data acquisition software. The zoom factor and the position of the scan area can be adjusted via the scanner control panel or via the cursors of the display window. Changes in the scan parameters are executed online, without stopping the scan.

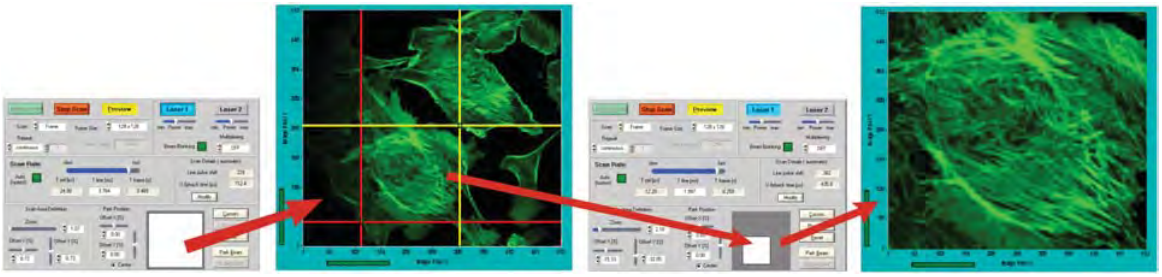


Fig. 8: Interactive scanner control for systems using the bh GVD-120 scan controller module

Fast preview function

When FLIM is applied to live samples the time and the sample exposure needed for positioning, focusing, laser power adjustment, and selection of the scan region has to minimised. Therefore, the bh FLIM systems have a fast preview function. The preview function displays images in intervals of 1 second and faster. Both intensity and lifetime images can be displayed.

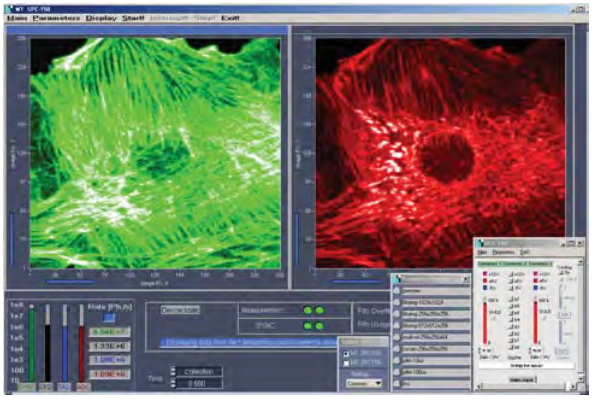


Fig. 9: SPCM software in fast preview mode. 1 image per second, two parallel FLIM channels recording in separate wavelength intervals.

Fast Online FLIM

The bh TCSPC/FLIM systems record and display fluorescence lifetime images at a rate of up to 10 images per second. The function is used to select interesting cells within a larger sample for subsequent high-accuracy FLIM acquisition. In FLIM experiments with longer acquisition time it helps the user evaluate the signal-to-noise ratio of the data and decide whether enough photons have been recorded to reveal the expected lifetime effects in the sample.

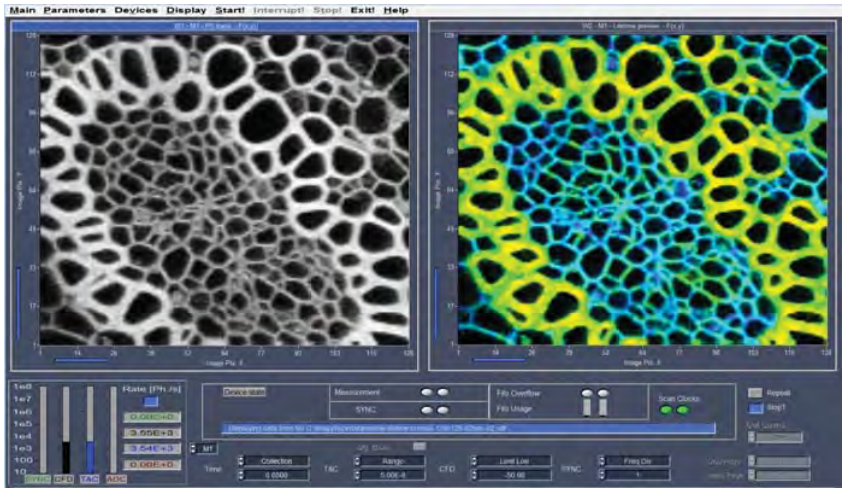


Fig. 10: Fast online FLIM. Intensity image (left) and lifetime image (right). Images 128 x 128 pixels, recorded at a speed of 5 images per second.

Two fully parallel TCSPC FLIM Channels

Standard bh FLIM systems record in two wavelength intervals simultaneously. The signals are detected by separate detectors and processed by separate TCSPC modules [16]. There is no intensity or lifetime crosstalk. Even if one channel overloads the other channel is still able to produce correct data.

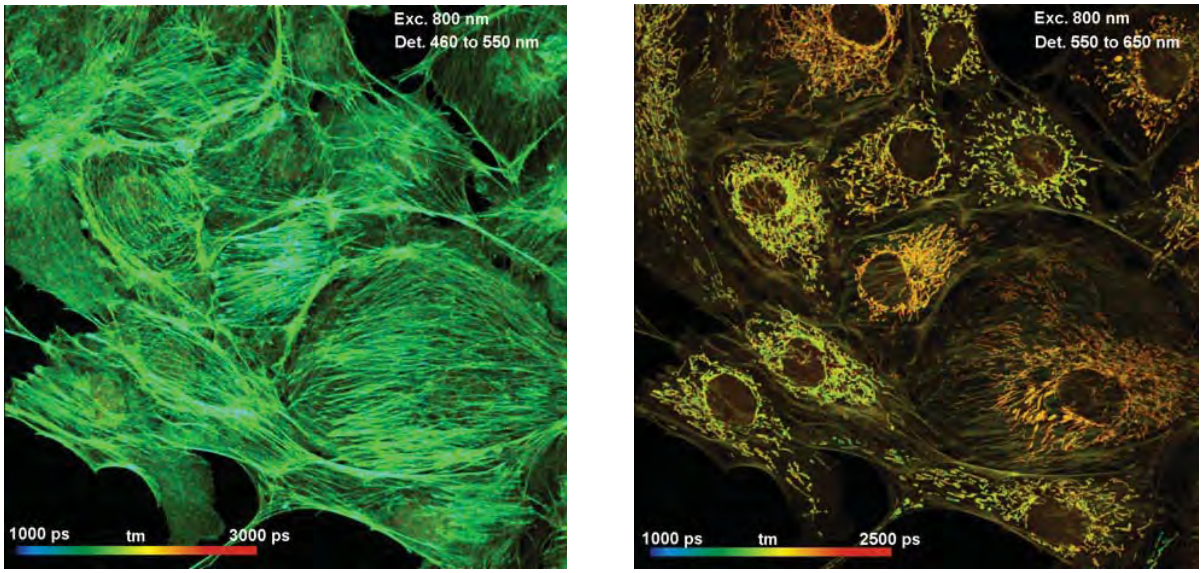


Fig. 11: Dual-channel detection. BPAE cells stained with Alexa 488 phalloidin and Mito Tracker Red. Left: 460 nm to 550 nm. Right: 550 nm to 650 nm.

Megapixel FLIM Images

With 64 bit SPCM software pixel numbers can be increased to 2048 x 2048 pixels, with a temporal resolution of 256 time channels. Two such images can be recorded simultaneously in different wavelength channels.

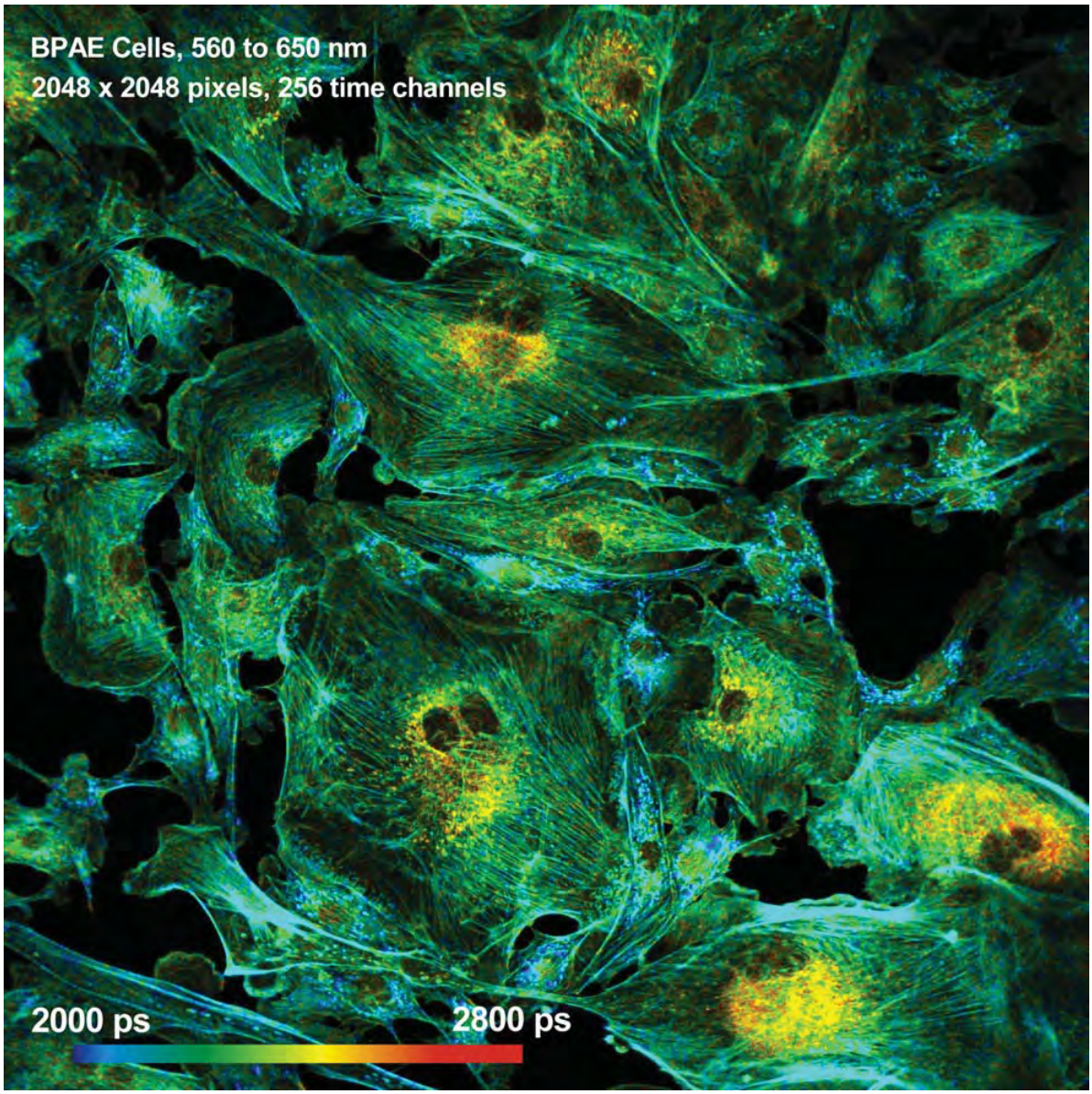


Fig. 12: BPAE cells, recorded with a spatial resolution of 2048 x 2048 pixels. 256 time channels per pixel.

Multiphoton NDD FLIM: Clear Images from Deep Tissue Layers

bh FLIM systems for multiphoton microscopes are compatible with non-descanned detection (NDD). With non-descanned detection, fluorescence photons scattered on the way out of the sample are detected efficiently and assigned to the correct pixels of the image. The result is that bright and clear images are obtained from deep tissue layers. An example is shown in Fig. 13.

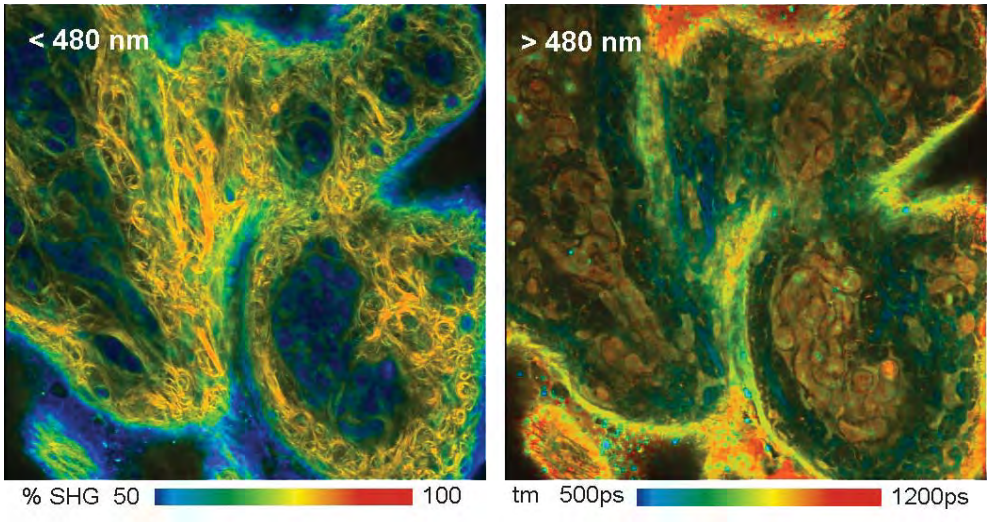


Fig. 13: Two-photon FLIM of pig skin. LSM 710 NLO, HPM-100-40, NDD. Left: Wavelength channel <480nm, colour shows percentage of SHG. Right: Wavelength channel >480nm, colour shows amplitude-weighted mean lifetime.

Multi-Spectral FLIM

bh FLIM systems are able to record simultaneously in 16 wavelength channels. The images are recorded by an extended multi-dimensional TCSPC process which uses the wavelength of the photons as a coordinate of the photon distribution [10, 16]. An example is shown in Fig. 14.

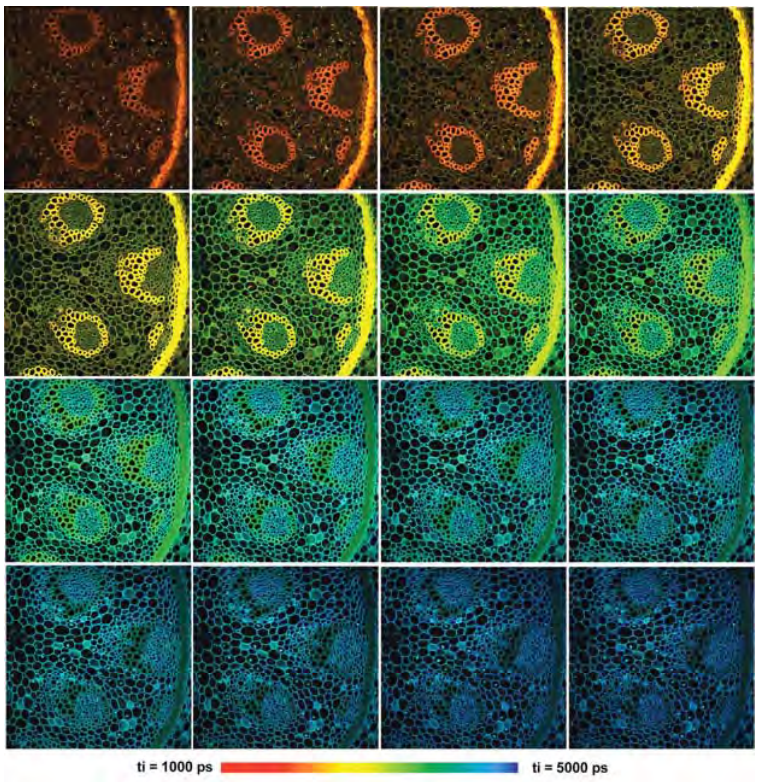


Fig. 14: Multi-wavelength FLIM, 16 images with 512 x 512 pixels and 256 time channels were recorded simultaneously. bh DCS-120 confocal scanner, bh MW-FLIM GaAsP 16-channel detector, Zeiss Axio Observer microscope.

There is no time gating, no wavelength scanning and, consequently, no loss of photons in this process. The system thus reaches near-ideal recording efficiency. Moreover, dynamic effects in the sample or photobleaching do not cause distortions in the spectra or decay functions. The individual images in the 16 wavelength channels are recorded at a resolution of up to 512x512 pixels and 256 time channels.

Fig. 15 and Fig. 16 demonstrate the true resolution of the data. Images from two wavelength channels, 502 nm and 565 nm, were selected form the data shown Fig. 14, and displayed at larger scale and with individually adjusted lifetime ranges. With 512x512 pixels and 256 time channels, the spatial and temporal resolution of the individual images is comparable with that normally used for single-wavelength FLIM.

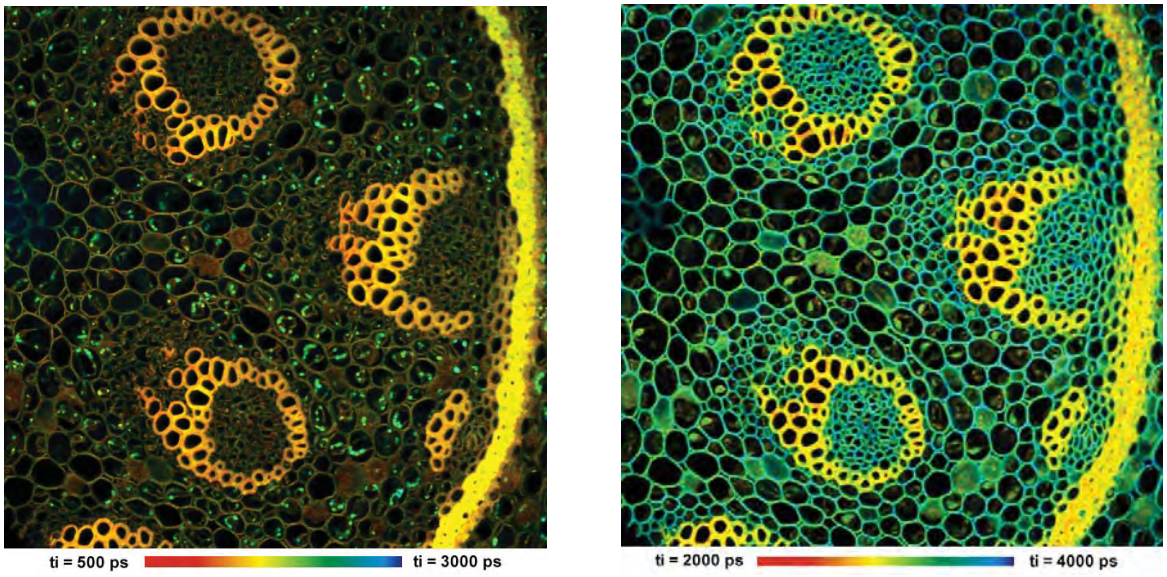


Fig. 15: Two images from the array shown in Fig. 14, displayed in larger scale and with individually adjusted lifetime range. The images have 512 x 512 pixels and 256 time channels.

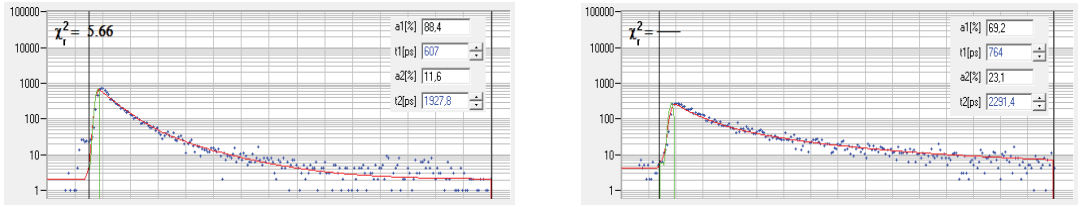


Fig. 16: Decay curves at selected pixel position in the images shown above. Blue dots: Photon numbers in the time channels. Red curve: Fit with a double-exponential model.

Multiphoton Multispectral NDD FLIM

bh's MW FLIM is the world's first simultaneously detecting multiphoton multispectral NDD FLIM system [10]. It uses a special optical interface that connects the NDD ports of multiphoton microscopes to the input slit of the detector [1, 2, 16]. A typical result is shown in Fig. 17.

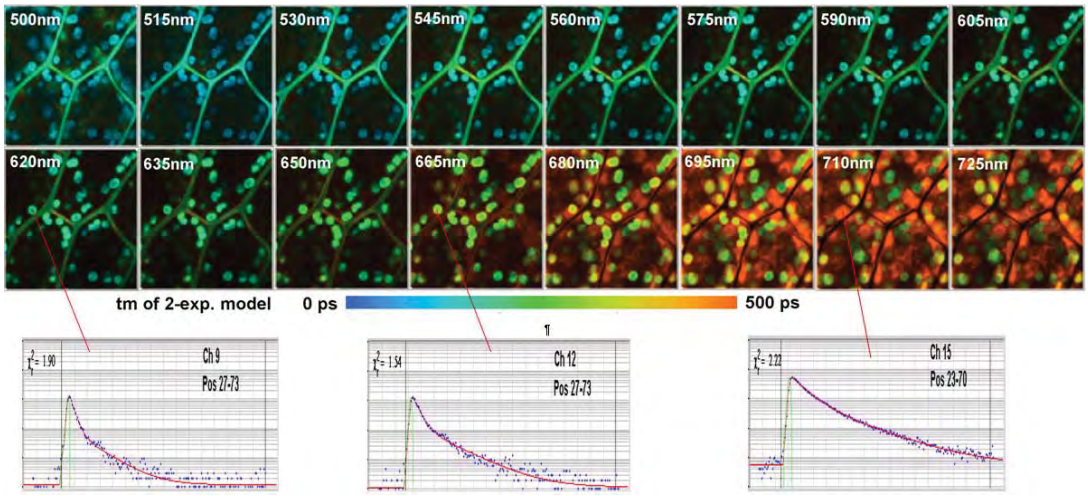


Fig. 17: Multiphoton Multispectral NDD FLIM. Lifetime images and decay curves in selected pixels and wavelength channels. LSM 710 NLO, bh MW FLIM detector

Time-Series FLIM by Record-and-Save Procedure

Time-series FLIM is available for all system versions, and all detectors [1, 2, 16]. Time series as fast as 2 images per second can be obtained. A time series taken at a moss leaf is shown in Fig. 18. Time-series FLIM at higher speed can be performed by temporal mosaic FLIM, see Fig. 22 and Fig. 23.

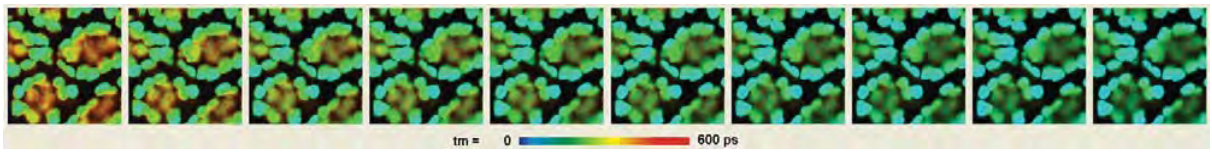


Fig. 18: Time-series FLIM, 1 image per second. Chloroplasts in a leaf, the fluorescence lifetime of the chlorophyll decreases with the time of exposure.

Z Stack Recording by Record-and-Save Procedure

For each Z plane, a FLIM image is scanned and acquired for a specific ‘collection time’. Then the data are saved in a file, the microscope steps to the next plane, and the next image is acquired. The procedure continues for a specified number of Z planes. A Z stack of autofluorescence images taken at a water flee is shown in Fig. 19. Z stack FLIM can be performed also by mosaic FLIM, please see Fig. 21.

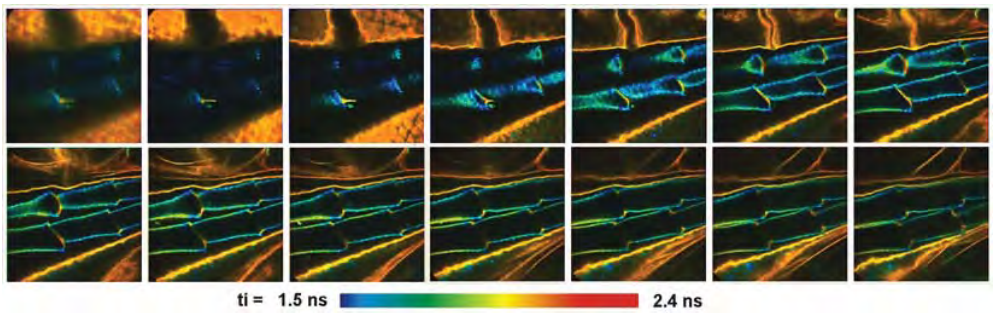


Fig. 19: Z stack recording, part of a water flee, autofluorescence. Images 256x256 pixels, 256 time channels.

Mosaic FLIM

Mosaic FLIM is based on bh's 'Megapixel FLIM' technology introduced in 2014. Mosaic FLIM records a large number of images into a single FLIM data array [16]. The individual images within this array can be for different displacement of the sample (spatial mosaic), different depth within the sample (z-stack mosaic), of for different times after a stimulation of the sample (temporal mosaic). Spatial mosaic FLIM combines favourably with the Tile Imaging capability of the Zeiss LSM 710/780/880 and similar procedures in other microscopes. An example is shown in Fig. 20. The complete data array has 2048 x 2048 pixels, and 256 time channels per pixel. Compared to a similar image taken through a low-magnification lens the advantage of mosaic FLIM is that a lens of higher numerical aperture can be used, resulting in higher detection efficiency and higher spatial resolution.

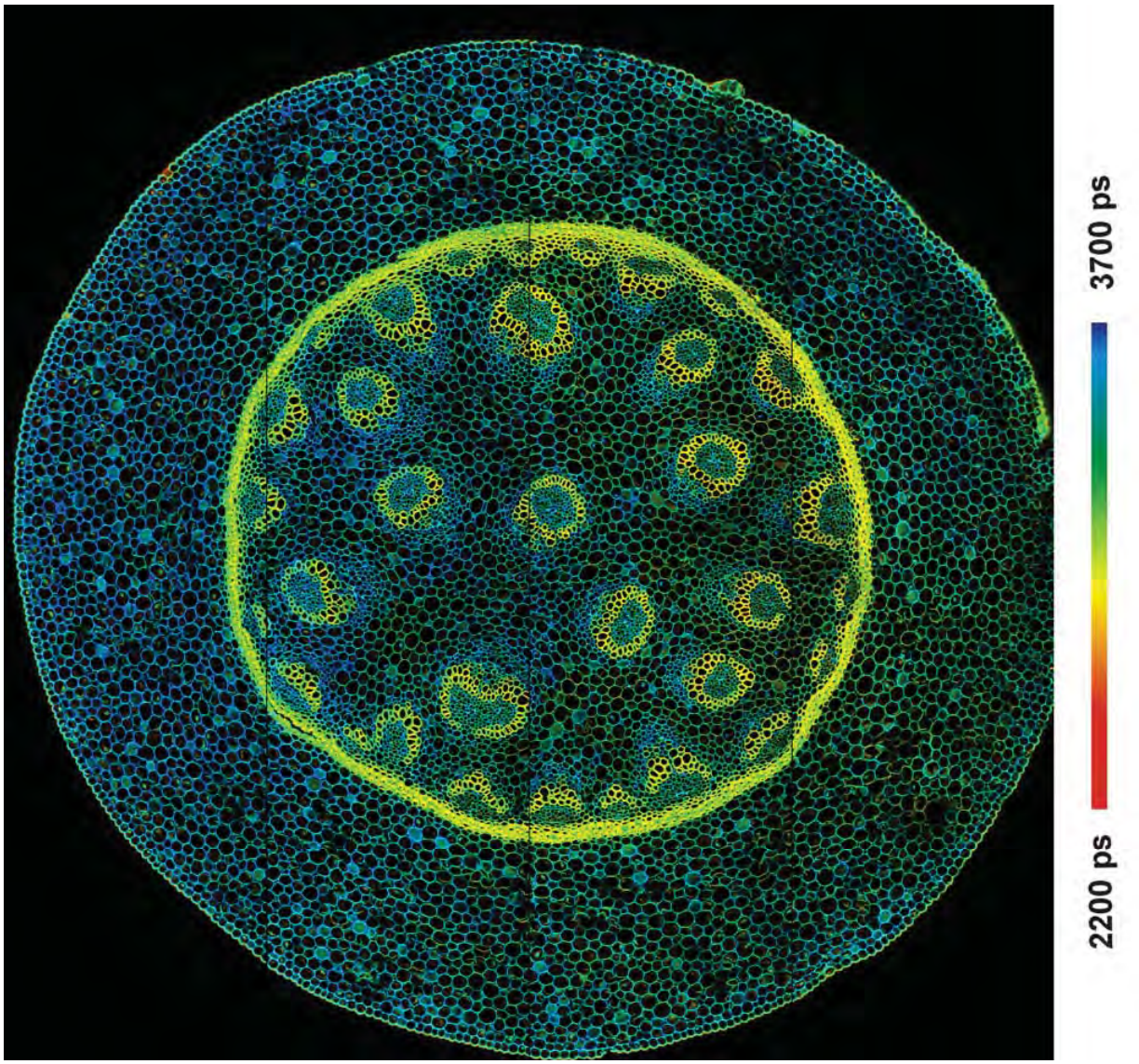


Fig. 20: Mosaic FLIM of a Convallaria sample. The mosaic has 4x4 elements, each element has 512x512 pixels with 256 time channels. The complete mosaic has 2048 x 2048 pixels, each pixel holding 256 time channels. Zeiss LSM 710 with bh Simple-Tau 150 FLIM system. Total sample size covered by the mosaic 2.5 x 2.5 mm.

Z Stack Mosaic FLIM

The Mosaic FLIM function can be used to record Z Stacks of FLIM images. As the microscope scans consecutive images planes in the sample the FLIM system records the data into consecutive elements of a FLIM mosaic. The advantage over the traditional record-and-save procedure is that no time has to be reserved for save operations, and that the entire array can be analysed in a single analysis run.

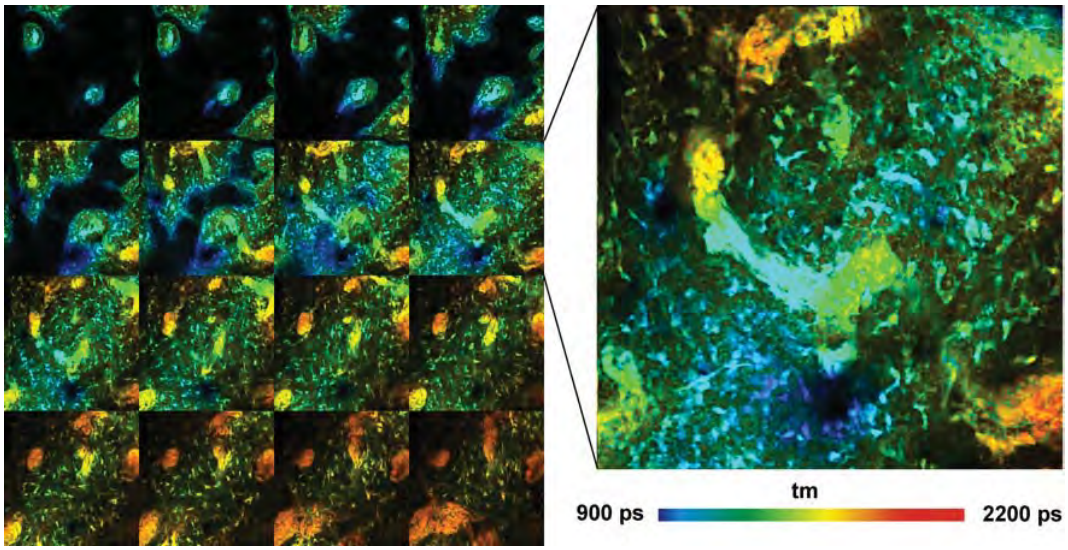


Fig. 21: FLIM Z-stack, recorded by Mosaic FLIM. Pig skin stained with DTTC. 16 planes, 0 to 60 um from top of tissue. Each element of the FLIM mosaic has 512x512 pixels and 256 time channels per pixel. Plane 8 is shown magnified on the right. LSM 7 OPO system, HPM-100-50 GaAs hybrid detector.

Temporal Mosaic FLIM

Mosaic FLIM can be used to record FLIM time series. An example is shown in Fig. 22.

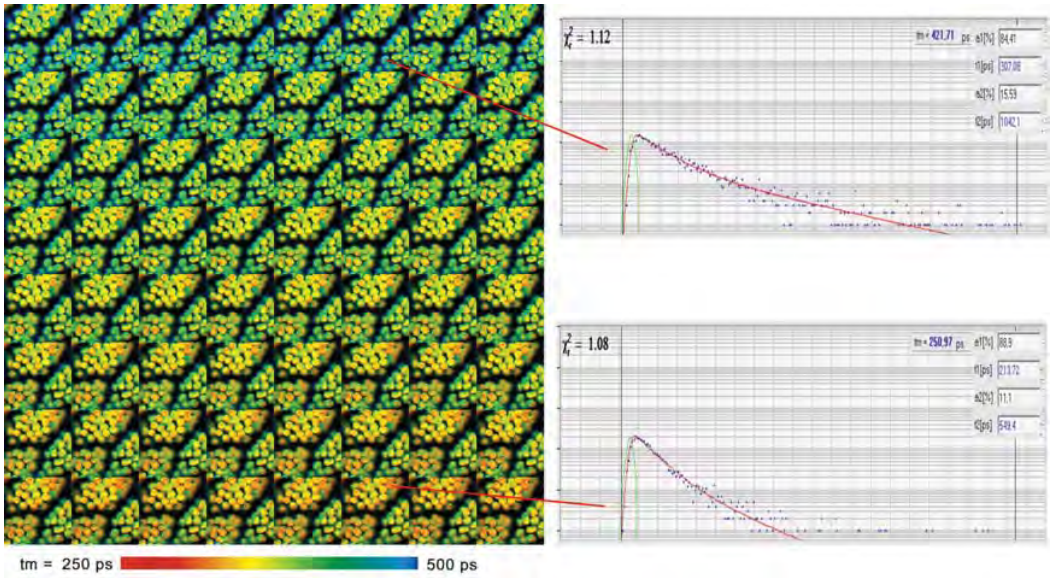


Fig. 22: Time series acquired by mosaic FLIM. Recorded at a speed of 1 mosaic element per second. 64 elements, each element 128 x 128 pixels, 256 time channels, double-exponential fit of decay data. Sequence starts at upper left. Moss leaf, lifetime changes by non-photochemical chlorophyll transient.

Also here, the advantage is that no time has to be reserved for save operations during the recording sequence. A Mosaic-FLIM time series can therefore be made very fast. The most important advantage is, however, that temporal Mosaic FLIM data can be accumulated. A lifetime change in the sample is stimulated periodically, and a mosaic recording sequence started for each stimulation. Because the entire photon distribution is kept in the memory the photons from the subsequent runs are automatically accumulated. The result is that the signal-to-noise ratio no longer depends on the speed of the series. The only speed limitation is the minimum frame time of the scanner. For many laser scanning microscopes frame times of less than 50 milliseconds can be achieved. This brings the transient-time resolution down to the range where physiological effects in live samples occur. One example is the recording of Ca^{2+} transients in neurons. An example is shown in Fig. 23.

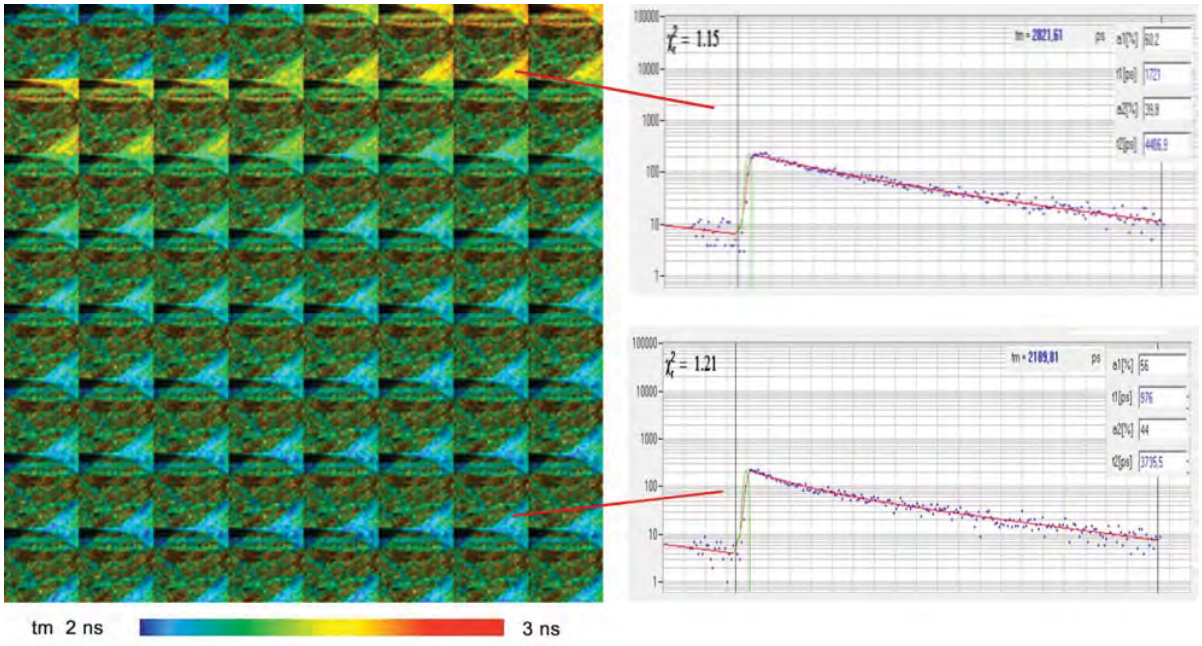


Fig. 23: Temporal mosaic FLIM of the Ca^{2+} transient in cultured neurons after stimulation with an electrical signal. The time per mosaic element is 38 milliseconds, the entire mosaic covers 2.43 seconds. Experiment time runs from upper left to lower right. Photons were accumulated over 100 stimulation periods. Zeiss LSM 7 MP multiphoton microscope and bh SPC-150 TCSPC module. Data courtesy of Inna Slutsky and Samuel Frere, Tel Aviv University, Sackler Faculty of Medicine.

FLITS: Fluorescence Lifetime-Transient Scanning

FLITS records transient effects in the fluorescence lifetime of a sample along a one-dimensional scan. The technique is based on building up a photon distribution over the distance along the scan, the arrival times of the photons after the excitation pulses, and the experiment time after a stimulation of the sample. The maximum resolution at which lifetime changes can be recorded is given by the line scan time. With repetitive stimulation and triggered accumulation transient lifetime effects can be resolved at a resolution of about one millisecond [13].

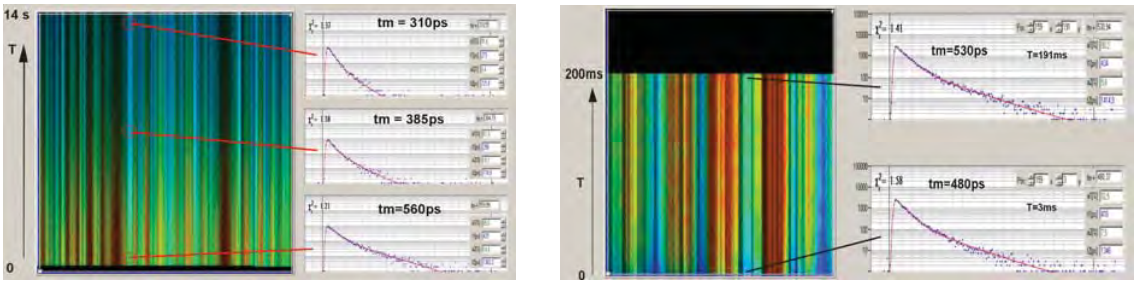


Fig. 24: FLITS of chloroplasts in a grass blade, change of fluorescence lifetime after start of illumination. Left: Non-photochemical transient, transient resolution 60 ms. Right: Photochemical transient. Triggered accumulation, transient resolution 1 ms.

Excitation Wavelength Multiplexing

By multiplexing several ps diode lasers images can be obtained quasi-simultaneously for different excitation wavelength. With the two detection channels of the bh systems, images for three or four combinations of excitation and emission wavelength are obtained. An example is shown in Fig. 25.

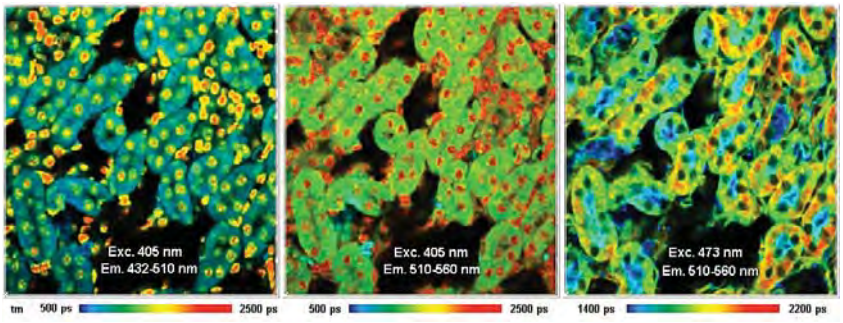


Fig. 25: Excitation wavelength multiplexing, 405 nm and 473 nm. Detection wavelength 432 nm to 510 nm and 510 nm to 550 nm. Mouse kidney section, stained with Alexa 488 WGA, Alexa 568 phalloidin, and DAPI.

Near-Infrared FLIM

Near-infrared FLIM can be performed by one-photon excitation with ps diode lasers, by one-photon excitation with Ti:Sapphire lasers, or two-photon excitation with OPOs [5, 14].

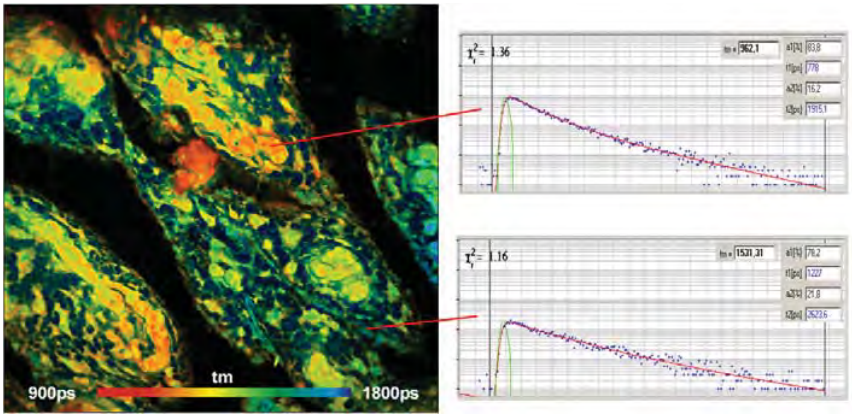


Fig. 26: Near-Infrared FLIM with ps diode laser, bh DCS-120 system. Pig skin sample stained with 3,3'-diethylthiatricarbocyanine, detection wavelength from 780 nm to 900 nm.

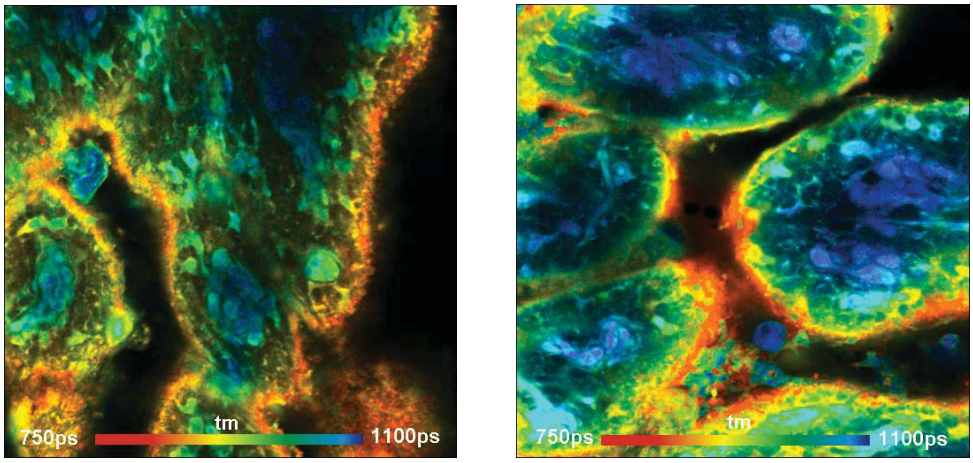


Fig. 27: Pig skin samples stained with 3,3'-diethylthiatricarbocyanine. Zeiss LSM 780 NLO system, one-photon excitation with Ti:Sa laser at 780nm, confocal detection at 800nm to 900nm

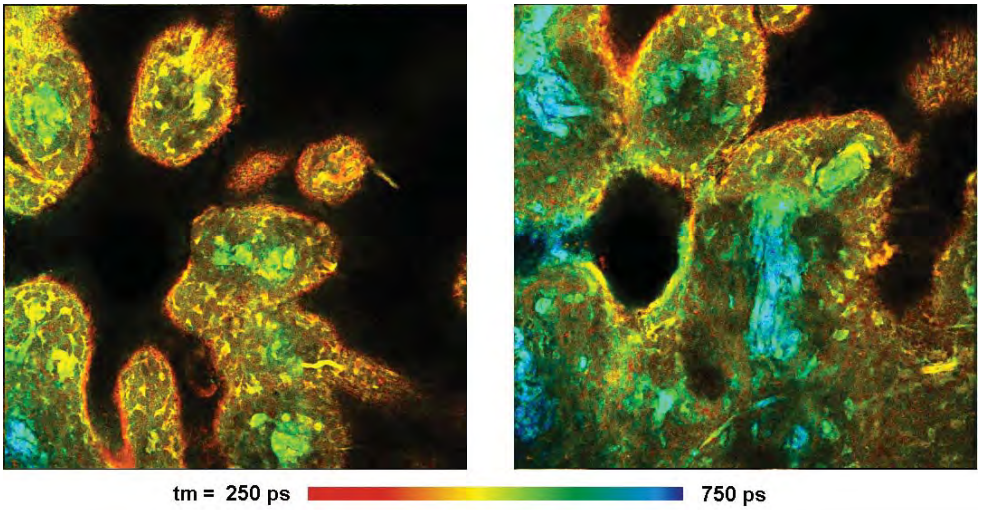


Fig. 28: Pig skin stained with Indocyanin Green. Zeiss LSM 780 OPO system, two-photon excitation at 1200 nm, non-descanned detection, 780 to 850 nm. Depth from top of tissue 10 µm (left) and 40 µm (right).

FLIM / PLIM: Simultaneous Fluorescence and Phosphorescence Lifetime Imaging

Phosphorescence and fluorescence lifetime images are recorded simultaneously by bh's proprietary FLIM/PLIM technique. The technique is based on modulating a ps diode laser synchronously with the pixel clock of the scanner. FLIM is recorded during the 'On' time, PLIM during the 'Off' time of the laser [7, 16]. The SPCM software delivers separate images for the fluorescence and the phosphorescence which are then analysed with SPCImage FLIM/PLIM analysis software.

Currently, there is increasing interest in PLIM for background-free recording and for oxygen sensing. In these applications, the bh technique delivers a far better sensitivity than PLIM techniques based on single-pulse excitation. The real advantage of the bh FLIM/PLIM technique is, however, that FLIM and PLIM are obtained *simultaneously*. It is thus possible to record metabolic information via FLIM of the NADH and FAD fluorescence, and simultaneously map the oxygen concentration via PLIM [17]. An example is shown in Fig. 29.

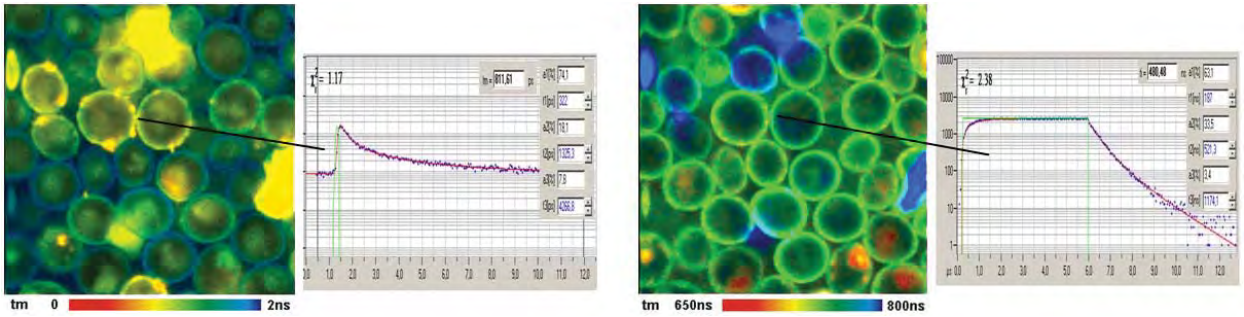


Fig. 29: Yeast cells stained with (2,2'-bipyridyl) dichlororuthenium (II) hexahydrate. FLIM and PLIM image, decay curves in selected spots.

FLIM of Macroscopic Objects

With the bh DCS-120 MACRO version objects as large as 15 mm can be scanned [2]. An image obtained with the DCS-120 MACRO is shown in Fig. 30.

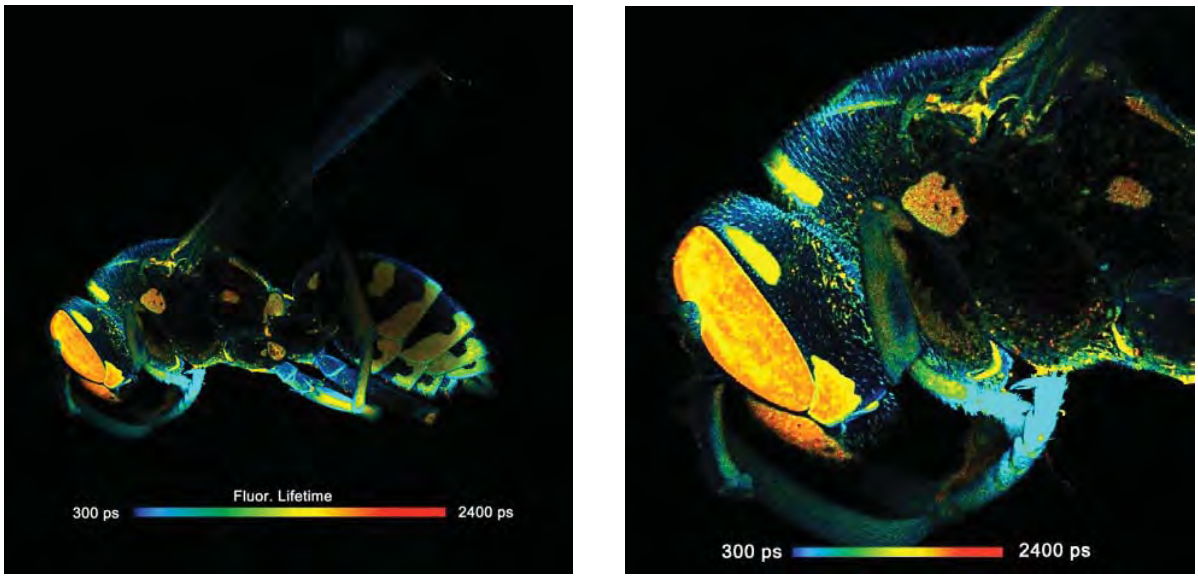


Fig. 30: FLIM of a macroscopic object. Resolution 2048 x2048 pixels, 256 time channels. Left: Original image. Right: digital zoon into recorded FLIM image, showing the excellent resolution of the data.

Clinical FLIM

Clinical FLIM applications use the fact that pathological processes induce changes in the molecular environment or in the conformation of endogenous fluorophores. These, in turn, cause detectable changes in the fluorescence decay profiles. Please see [15] or [16] for an overview. bh FLIM has been introduced into clinical instruments for opthalmology and dermatology, please see [15, 16] technical details. The first clinical instruments are on the market. FLIM images recorded with the FLIO ophthalmic lifetime imaging system of Heidelberg Engineering and with the MPT Flex multiphoton skin tomography system are shown in Fig. 31.

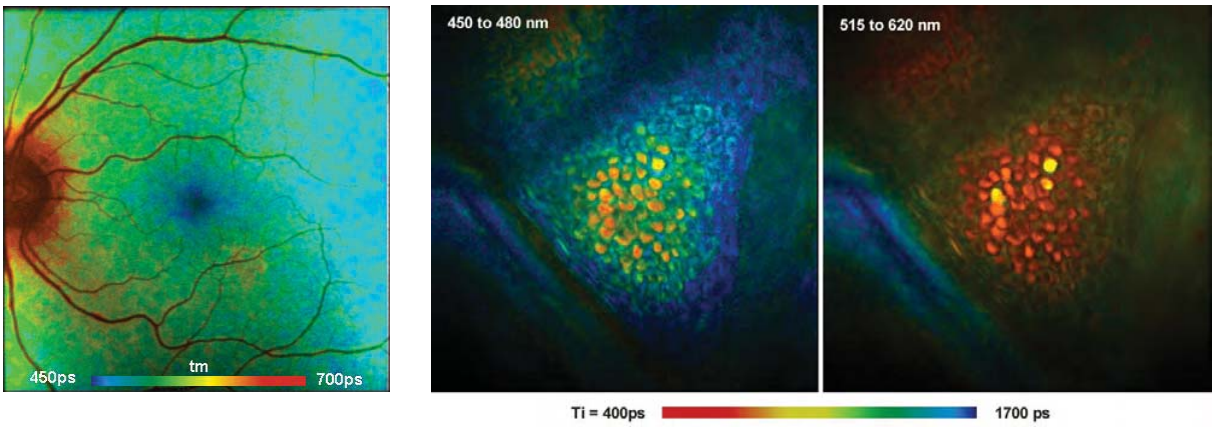


Fig. 31: Left: FLIM of a human retina, recorded in vivo with Heidelberg Engineering FLIO ophthalmoscope. Right: Multiphoton FLIM of human skin, reocrded in vivo with Jenlab MPT FLEX multiphoton tomography system.

STED FLIM

TCSPC FLIM can be combined with STED [16]. The combination of a STED microscope of Abberior Instruments (Göttingen, Germany) with the bh Simple-Tau 150/154 TCSPC FLIM system records FLIM data at a spatial resolution of better than 40 nm. The image format can be as large as 2048 x 2048 pixels, with 256 time channels per pixel. An image area of 40 x 40 micrometers can thus be covered with 20 nm pixel size, fully satisfying the Nyquist criterion. With smaller numbers of time channels even larger pixel numbers are possible. The system especially benefits from Windows 64 bit technology used both in the Abberior and in the bh data acquisition software, from the combined processing power of two parallel system computers, and the high data throughput of up to four parallel TCSPC FLIM channels. The system achieves peak count rates in excess of 5 MHz per FLIM channel, resulting in unprecedented signal-to-noise ratio and short acquisition time.

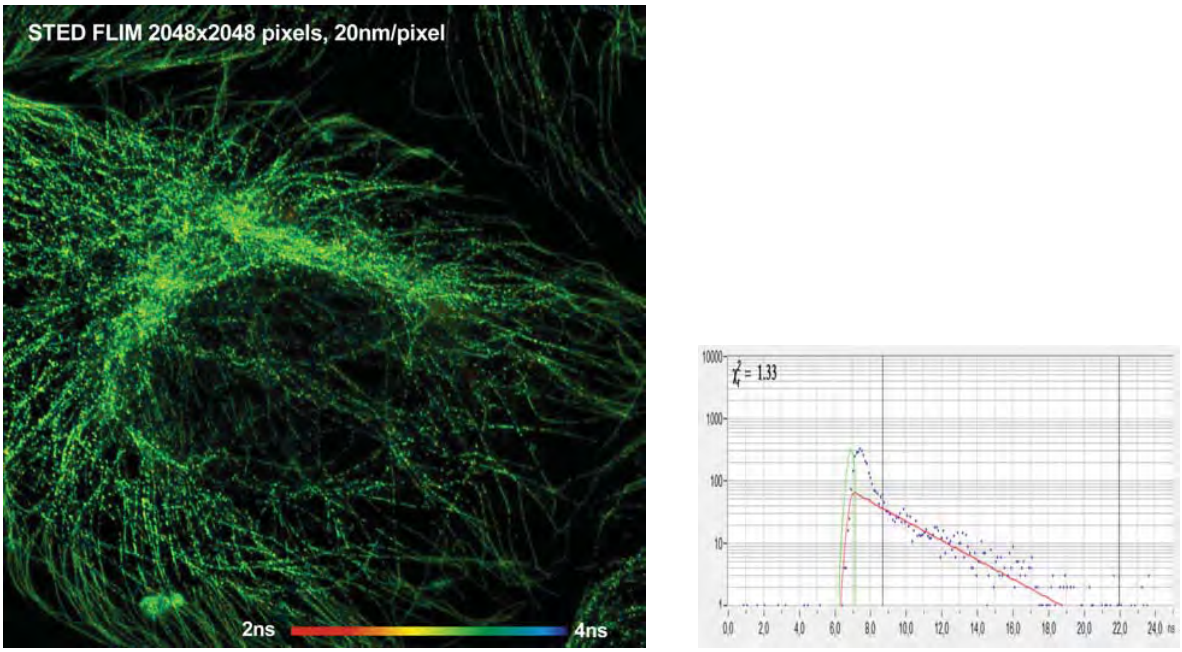


Fig. 32: STED FLIM with Abberior Intruments STED microscope. 2048 x 2048 pixels. Single cell, stained with tubulin-binding dye, recorded at a resolution of 20 nm per pixel. Decay curve in selected pixel shown on the right. The initial peak is undepleted fluorescence. It is gated off in the intensity data of image shown on the left.

FCS

The bh GaAsP hybrid detectors of the bh FLIM systems deliver highly efficient FCS [1, 11, 16]. Because the detectors are free of afterpulsing there is no afterpulsing peak in the autocorrelation data. Thus, accurate diffusion times and molecule parameters are obtained from a single detector. Compared to cross-correlation of split signals, correlation of single-detector signals yields a four-fold increase in correlation efficiency. The result is a substantial improvement in the SNR of FCS recordings [11, 16]. FCS is be obtained both with confocal systems and with multiphoton NDD systems. Gated FCS is obtained by hardware gating the photon times within the TCSPC modules, FCCS by cross-correlating the signals of two TCSPC channels.

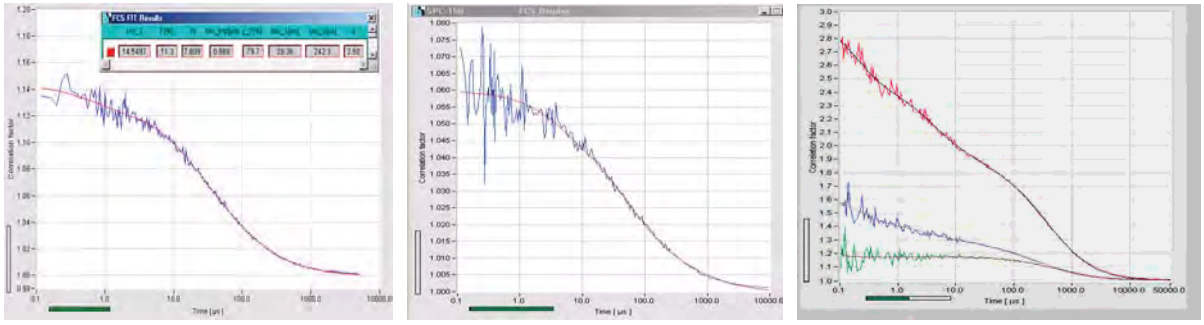
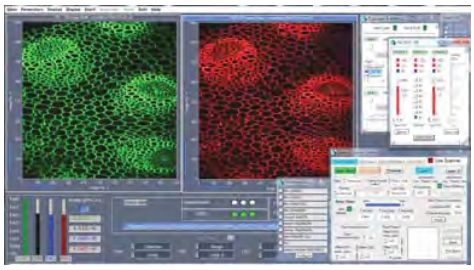


Fig. 33: FCS with bh TCSPC FLIM systems, GaAsP hybrid detectors. Left to right: Confocal FCS with ps diode laser, two-photon NDD FCS, cross correlation of photons recorded in different detection channels.

bh FLIM Systems for Various Microscopes

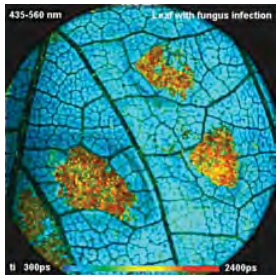
DCS-120 Confocal Scanning FLIM Systems

FLIM with up to 2048 x 2048 pixels
Complete Confocal Laser Scanning FLIM microscopes
FLIM upgrade for existing conventional microscopes
Includes scanner with fast galvanometer mirrors
Two fully confocal detection channels
One or two BDL-SMC or BDL-SMN picosecond diode lasers
Laser wavelengths 375, 405, 440, 473, 488, 510, 640, 685, 785 nm
Tuneable excitation by super-continuum laser with AOTF
One or two confocal detection channels, parallel acquisition
Channel separation by dichroic or polarising beamsplitters
Individually selectable pinholes, individually selectable filters
GaAsP hybrid detectors for visible range, GaAs hybrid detectors for NIR range
16-channel multi-wavelength GaAsP detector module
Z-stack FLIM acquisition with Zeiss Axio Observer Z1
Simultaneous fluorescence and phosphorescence lifetime imaging (PLIM)
Fluorescence lifetime-transient scanning (FLITS)
Ultrafast time-series recording by temporal mosaic FLIM function
Wideband (WB) version, compatible with tuneable lasers
Electronic pinhole alignment



DCS-120 MP Multiphoton FLIM Systems

Multiphoton version of DCS-120 scanning system
Excitation by Ti:Sa laser
Laser control integrated in SPCM data acquisition software
Laser intensity control and PLIM laser modulation by AOM
One or two non-descanned detection channels
One or two bh HPM-100-40 hybrid detectors
Clear Images from deep tissue layers
Excellent spatial and temporal resolution
Images up to 2048 x 2048 pixels, 256 time channels
Full field of view of microscope lens scanned
Optional 16-channel multi-wavelength GaAsP detector module
Z-stack FLIM acquisition with Zeiss Axio Observer Z1
Simultaneous fluorescence and phosphorescence lifetime imaging (PLIM)
Fluorescence lifetime-transient scanning (FLITS)
Ultrafast time-series recording by temporal mosaic FLIM function
Please see [2] for details



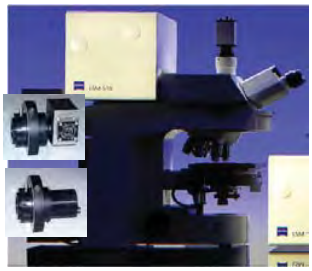
DCS-120 Macro System

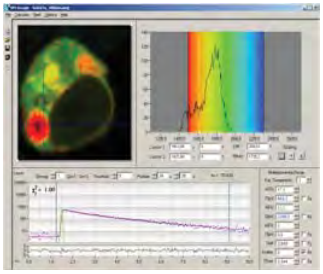
FLIM of macroscopic objects
Scan field up to 15 mm diameter
FLIM with up to 2048 x 2048 pixels
Scanning by fast galvano mirrors
Two fully confocal detection channels
One or two BDL-SMC or BDL-SMN picosecond diode lasers
Laser wavelengths 375, 405, 440, 473, 488, 510, 640, 685, 785 nm
Tuneable excitation by super-continuum laser with AOTF
One or two confocal detection channels, parallel acquisition
Channel separation by dichroic or polarising beamsplitters
Individually selectable pinholes, individually selectable filters
GaAsP hybrid detectors for visible range, GaAs hybrid detectors for NIR range
16-channel multi-wavelength GaAsP detector module
Simultaneous fluorescence and phosphorescence lifetime imaging (PLIM)
Fluorescence lifetime-transient scanning (FLITS)
Ultrafast time-series recording by temporal mosaic FLIM function
Wideband (WB) version, compatible with tuneable lasers
Electronic pinhole alignment
Please see [2] for details

FLIM Systems for Zeiss LSM 710 / 780 / 880 Microscopes

LSM 710 / 780 / 880 NLO, LSM 7MP Multiphoton Microscopes
LSM 710, LSM 780, LSM 880 Confocal Microscopes
LSM 710, LSM 780, LSM 880 In Tune systems
FLIM with up to 2048 x 2048 pixels
Multiphoton FLIM, PLIM, multispectral FLIM, FCS
Confocal FLIM, PLIM, multispectral FLIM, FCS
FLIM with bh HPM hybrid detectors or Zeiss BIG-2 detectors
Fast preview mode, both for intensity and lifetime
Mosaic FLIM
Z Stack FLIM
Fast Time-series FLIM
Acquisition by 1, 2, 3 or 4 parallel TCSPC FLIM channels
Detection by bh HPM-100 hybrid detectors or Zeiss BIG 2 detector
Simultaneous fluorescence and phosphorescence lifetime imaging (PLIM)
Fluorescence lifetime-transient scanning (FLITS) fully integrated
Ultrafast time-series recording by temporal mosaic FLIM function
Confocal NIR FLIM up to 900 nm detection wavelength
Two-Photon OPO FLIM up to 900nm detection wavelength

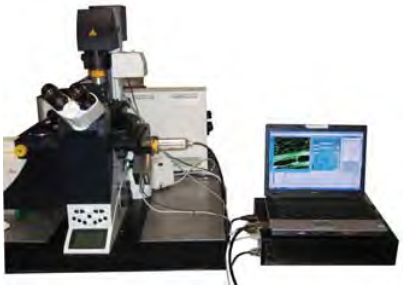
Please see [1] for details

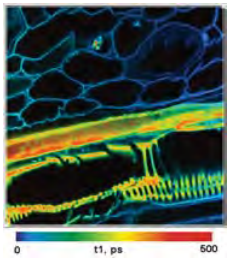




Still available: FLIM Systems for Zeiss LSM 510 NLO Multiphoton Microscopes

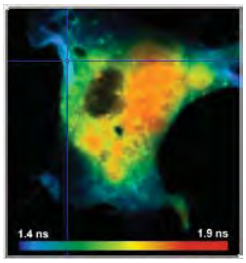
FLIM with up to 2048 x 2048 pixels
Multiphoton excitation with non-descanned detection
Detectors connected to Zeiss NDD switch box
Single-wavelength NDD FLIM
Dual-wavelength NDD FLIM
Multi-spectral NDD FLIM
Fast preview mode
Mosaic FLIM
Z Stack FLIM
Fast time-series FLIM
HPM-100-40 hybrid detectors
One or two parallel SPC-150 TCSPC channels
PC-based systems or Simple-Tau TCSPC systems
Portable to LSM 710, 780, 880 microscopes





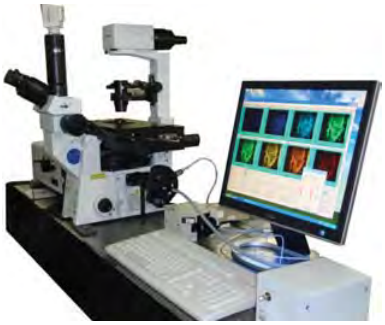
Non-Descanned FLIM Systems for Leica SP5 MP, SP8 MP Microscopes

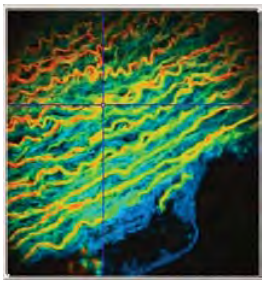
Non-descanned detection via Leica RLD port
1 detector coupled directly to RLD port
2 detectors via external beamsplitter
Simple-Tau 150 or 152 TCSPC systems
Acquisition in 1 or 2 parallel TCSPC FLIM channels
bh HPM-100-40 hybrid detectors or Leica HYD detectors
Multi-spectral FLIM with 16-channel GaAsP detector
Works at any scan rate of SP microscope
No nonlinearity by Leica sinusoidal scan
Fast acquisition, fast preview mode
Megapixel FLIM, 2048 x 2048 pixels
Fluorescence lifetime-transient scanning (FLITS)
Ultra-fast time series by temporal mosaic FLIM
Simultaneous FLIM / PLIM



FLIM Systems for Olympus FV1000 and FV300 Confocals

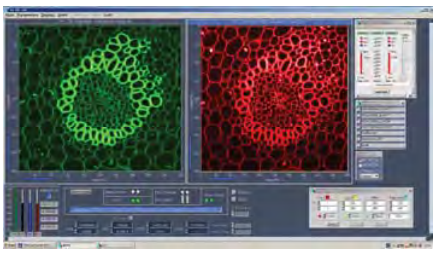
Excitation by bh BDL-405 SMC or BDL-473 SMC picosecond diode laser
High efficiency by direct coupling of detectors
Single-wavelength detection: PMT, MCP-PMT, or Hybrid PMT
Multi-wavelength detection: bh PML-SPEC detector
Full overload protection of detectors
ROI and Zoom functions of FV1000 or FV300 available
Works at any scan rate
FCS capability
Fluorescence lifetime-transient scanning (FLITS)





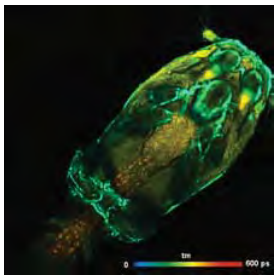
Non-descanned FLIM Systems for Olympus FV1000 and FV300 Multiphoton Microscopes

Multiphoton FV1000 and FV300 systems with inverted microscopes
High efficiency by non-descanned FLIM detection
Deep-tissue imaging capability
Single-wavelength detection: PMT, MCP-PMT, or Hybrid PMT
Multi-spectral FLIM with 16-channel GaAsP detector
Full overload protection of FLIM detectors
ROI and Zoom functions of FV1000 or FV300 available
Works at any scan rate
Fluorescence lifetime-transient scanning (FLITS)



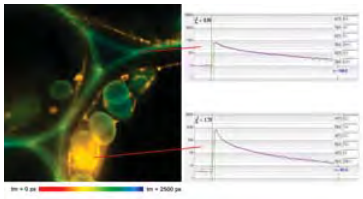
Non-descanned FLIM Systems for Nikon A1 MP Multiphoton Microscopes

64-bit megapixel FLIM technology
One FLIM channel or two parallel FLIM channels
High-efficiency PMH-100 hybrid detectors
Non-descanned detection for deep-tissue imaging
Multi-spectral FLIM with 16-channel GaAsP detector
ROI and Zoom functions of A1 available
Works at any scan rate
Megapixel FLIM
Fluorescence lifetime-transient scanning (FLITS)
Ultra-fast time series by temporal mosaic FLIM



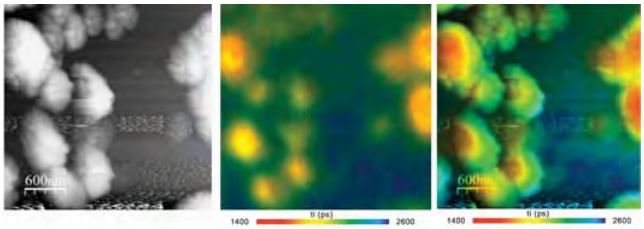
FLIM Systems for Sutter Instrument MOM Microscopes

Up to four parallel FLIM channels
Multiphoton excitation by Ti:Sa laser
Non-descanned detection for deep-tissue imaging
Overload protection of FLIM detectors
Up to 1024 x 1024 pixels, 1024 time channels
High efficiency
Fast acquisition
SPCM Online FLIM function available
Simultaneous FLIM / PLIM



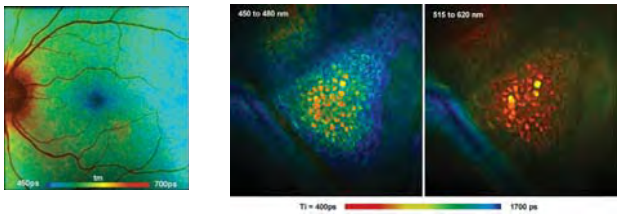
PZ-FLIM-110 Stage-Scanning FLIM System

Sample scanning by piezo scan stage
Excitation by BDL or BDS series ps diode lasers
Confocal detection
HPM-100 hybrid detector
Optional PML-SPEC GaAsP multi-spectral detector
Excellent contrast and resolution
Fully controlled by bh SPCM TCSPC/FLIM data acquisition software
Compact electronics, integrated in bh Simple Tau system
Megapixel FLIM technology - images up to 2048 x 2048 pixels
Lateral (x-y) an vertical (z) scanning
Simultaneous FLIM / PLIM



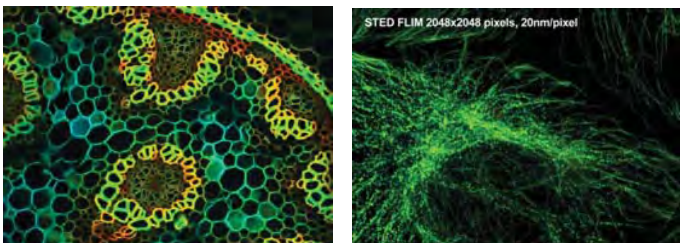
FLIM for NSOM Systems

For NSOM systems of Nanonics and NT-MDT
Combines atomic-force and fluorescence lifetime information
High sensitivity by HPM-100 hybrid detectors
Fluorescence and phosphorescence lifetime imaging
Single-point transient-lifetime recording
Please see bh TCSPC Handbook [16]or contact bh.



FLIM Systems for Clinical Imaging

FLIM systems for ophthalmology
FLIM systems for dermatology
FLIM systems for tissue imaging
FLIM through endoscopes
Time-resolved NIRS and fNIRS Imaging
Online FLIM at rates of up to 10 images per second
Please see bh TCSPC Handbook [16] or contact bh



FLIM for other Scanning Systems

bh FLIM systems can be configured for almost any conceivable laser scanning system. They work with galvanometer scanners, polygon scanners, resonance scanners, and motor-driven and piezo-driven scan stages.

Left: FLIM recorded with Lucid Vivascope, ultra-fast polygon scanner

Right: STED FLIM recorded with STED microscope of Abberior Systems, Goettingen

Please see bh TCSPC Handbook [16] or contact bh.

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Specifications

General Principle

Lifetime measurement	time-domain
Excitation	high-frequency pulsed lasers
Buildup of lifetime images	Single-photon detection by multi-dimensional TCSPC [**16**] Builds up distribution of photons over photon arrival time after laser pulses, scan coordinates, time from laser modulation, time from start of experiment.
Multi-wavelength FLIM	uses wavelength of photons as additional coordinate of photon distribution
Excitation wavelength multiplexing	uses laser number as additional coordinate of photon distribution
Scan rate	works at any scan rate
Buildup of fluorescence correlation data	correlation of absolute photon times [**16**]
General operation modes	FLIM, two spectral or polarisation channels Multi-wavelength FLIM Time-series FLIM, microscope-controlled time series Z-Stack FLIM Mosaic FLIM, x,y, z, temporal Excitation-wavelength multiplexed FLIM FLITS (fluorescence lifetime-transient scanning) PLIM (phosphorescence lifetime imaging) simultaneous with FLIM FCS, cross FCS, gated FCS, PCH Single-point fluorescence decay recording

Data recording hardware, please see [16] for details

TCSPC System	bh Simple Tau 152 TCSPC system, extension box coupled to laptop PC
Coupling to PC	bus extension cable or thunderbolt interface
Number of parallel TCSPC / FLIM channels	typ. 2, min. 1, max 4
Number of detector (routing) channels in FLIM modes	16 for each FLIM channel
Principle	Advanced TAC/ADC principle
Electrical time resolution	2.3 ps rms / 6.8 ps fwhm
Minimum time channel width	813 fs
Timing stability over 30 minutes	typ. better than 5ps
Dead time	100 ns
Saturated count rate	10 MHz per channel
Dual-time-base operation	via micro times from TAC and via macro time clock
Source of macro time clock	internal 40MHz clock or from laser
Input from detector	constant-fraction discriminator
Reference (SYNC) input	constant-fraction discriminator
Synchronisation with scanning	via frame clock, line clock and pixel clock pulses
Scan rate	any scan rate
Synchronisation with laser multiplexing	via routing function
Recording of multi-wavelength data	simultaneous in 16 channels, via routing function
Experiment trigger function	TTL, used for Z stack FLIM and microscope-controlled time series
Basic acquisition principles	on-board-buildup of photon distributions buildup of photon distributions in computer memory generation of parameter-tagged single-photon data online auto or cross correlation and PCH
Operation modes	f(t), oscilloscope, f(txy), f(t,T), f(t) continuous flow FIFO (correlation / FCS / MCS) mode Scan Sync In imaging, Scan Sync In with continuous flow FIFO imaging, with MCS imaging, mosaic imaging, time-series imaging Multi-detector operation, laser multiplexing operation cycle and repeat function, autosave function

Max. Image size, pixels (SPCM 64 bit software)	4096x4096	2048x2048	512x512	256x256
No of time channels, see [16]	64	256	1024	4096

Data Acquisition Software, please see [16] for details

Operating system	Windows 7, Windows 10, 64 bit
Loading of system configuration	single click in predefined setup panel
Start / stop of measurement	by operator or by timer, starts with start of scan, stops with end of frame
Online calculation and display, FLIM, PLIM	in intervals of Display Time, min. 1 second

Online calculation and display, FCS, PCH	in intervals of Display Time, min. 1 second
Number of images diplayed simultaneously	max 8
Number of curves (Decay, FCS, PCH, Multiscaler)	8 in one curve window
Cycle, repeat, autosave functions	user-defined, used for for time-series recording, Z stack FLIM, microscope-controlled time series
Saving of measurement data	User command or autosave function Optional saving of parameter-tagged single-photon data
Link to SPCImage data analysis	automatically after end of measurement or by user command

Data Analysis: bh SPCImage, integrated in bh TCSPC package, see [1, 2] or [16]

Data types processed	FLIM, PLIM, MW FLIM, time-series, Z stacks, single curves
Procedure	iterative convolution or first-moment calculation
IRF	synthethic IRF or measured IRF
Model functions	single, double, triple exponential decay single, double, triple exponential incomplete decay models
Parameters displayed	amplitude- or intensity-weighted average of component lifetimes ratios of lifetimes or amplitudes, FRET efficiency fractional intensities of components or ratios of fractional intensities parameter distributions
Parameter histograms, one-dimensional	Pixel frequency over any decay parameter or ratio of decay parameters
Parameter histograms, two-dimensional	Pixel frequency over two decay parameters, Phasor plot

Excitation Sources, One-Photon Excitation, please see [1] for details

Picosecond Diode Lasers	Modified bh BDL-SMC lasers
Number of lasers simultaneously operated	2
Wavelengths	405nm, 445nm, 473nm, 488nm, 515nm, 640nm, 685nm, 785nm
Mode of operation	picosecond pulses or CW
Pulse width, typical	40 to 100 ps
Pulse frequency	selectable, 20MHz, 50MHz, 80MHz
Power in picosecond mode	0.4mW to 1mW injected into fibre. Depends on wavelength version.
Power in CW mode	20 to 40mW injected into fibre. Depends on wavelength version.
Tuneable Lasers in visible range	Zeiss Intune Laser
Wavelength	480 to 640 nm
Repetition rate	40 MHz
Other Vis-Range Lasers	
Visible and UV range	any ps pulsed laser of 20 to 80 MHz repetition rate
Coupling requirements	Point Source-Kineflex compatible fibre adapter
Wavelength	any wavelength from 400nm to 800nm
Synchronisation / Modulation of lasers	
Laser Multiplexing	Diode lasers, pixel by pixel, line by line, frame by frame requires bh DDG-210 card and multiplexing indimo
Interleaved excitation	sync of diode laser to diode laser or diode laser to intune laser requires bh laser sync option
Laser Modulation for PLIM	Diode lasers, requires bh DDG-210 card and PLIM indimo

Excitation Sources, Multi-Photon Excitation, please see [1] for details

Femtosecond NIR Lasers	any femtosecond Ti:Sa laser or Ti:Sa pumped OPO
Wavelength	650 to 1000
Repetition rate	typ. 80 MHz
Laser Modulation for PLIM	requires bh DDG-210 card and PLIM indimo

Detectors

Interface to LSM 710 family microscopes	NDD / BIG adapter DC port adapter (older LSMs only, not recommended)
Beamsplitter, NDD port	Zeiss NDD T Adapter
Beamsplitter, confocal port	bh beamsplitter assembly with Zeiss-type filter cubes detectors are portable between NDD and (confocal) BIG port

Hybrid Detectors (standard)	bh HPM-100-40 hybrid detector
Spectral Range	300 to 710nm
Peak quantum efficiency	40 to 50%
IRF width with bh diode laser	120 to 130 ps
Detector area	3mm
Background count rate, thermal	300 to 2000 counts per second
Background from afterpulsing	not detectable
Afterpulsing peak in FCS	not detectable
Power supply and overload shutdown	via DCC-100 controller of TCSPC system

Hybrid Detectors for NIR (optional)	bh HPM-100-50 hybrid detector
Spectral Range	400 to 900nm
Peak quantum efficiency	12 to 15%
IRF width with bh diode laser	120 to 130 ps
Detector area	3mm
Background count rate, thermal	1000 to 8000 counts per second
Background from afterpulsing	not detectable
Power supply and overload shutdown	via DCC-100 controller of TCSPC system

Multi-Wavelength FLIM Detector (optional)	bh MW FLIM assembly
Spectral range	380 to 630nm or 380 to 750nm
Number of wavelength channels	16
Spectral width of wavelength channels	12.5 nm
IRF width with bh diode laser	250 ps
Power supply and overload shutdown	via DCC-100 controller of TCSPC system

Detectors (optional)	PMZ-100 PMT modules	id100-50 SPAD modules	R3809U MCP PMTs
IRF width (fwhm)	200ps	60ps	28ps
Other specifications		please see [**16**]	

Return by fax or postal mail or scanned email to the address below

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❒ **TCSPC for Microscopy**
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