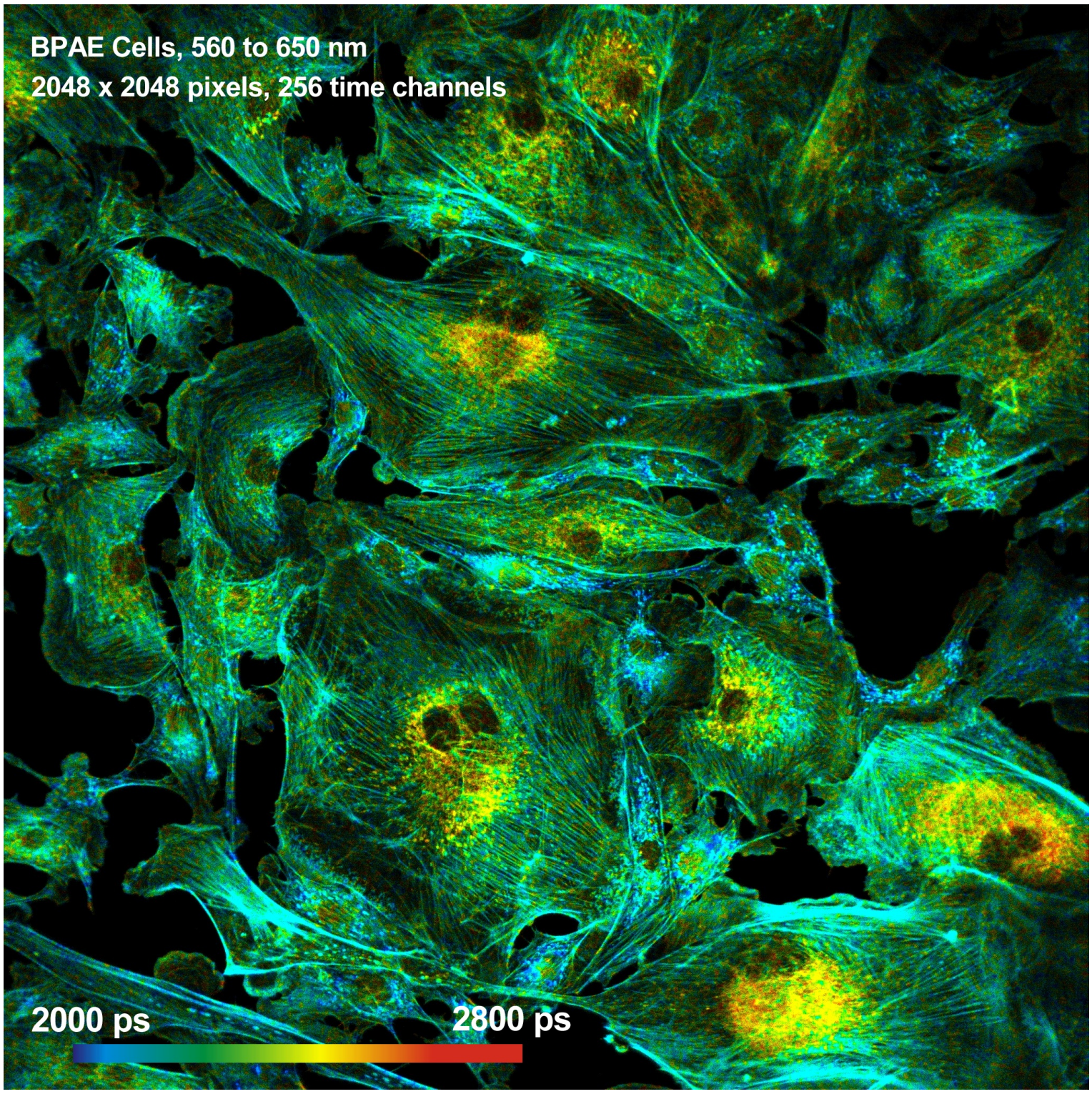
Becker & Hickl GmbH

Beautiful FLIM



Multi-Dimensional TCSPC

In 1993 Becker & Hickl introduced a new multi-dimensional TCSPC technique. Different than classic devices which recorded a photon distribution over only one parameter - the time of the photons in a fluorescence decay - the new devices recorded photon distributions also over the wavelength of the photons. Multi-dimensional TCSPC was born. Within a few years, more dimensions were added. Fast sequential recording was introduced with the SPC-430 in 1995, fluorescence-lifetime imaging in combination with fast scanning with the SPC-535 in 1996. These developments provided the basis for TCSPC FLIM. The first application of the bh TCSPC FLIM technique appeared in laser scanning ophthalmology in 1996. Applications to laser scanning microscopy appeared in 1998 with the SPC-730 module. Since then, the FLIM images constantly became bigger and better. The first images had 128 x 128 pixels and 256 time channels, then the data format increased to typically 256 x 256 pixels. In 2014, Becker & Hickl were the first to pass to the 64-bit world of Windows. Due to the large data space available in the 64-bit environment images could now be recorded with up to 4096 pixels, and with 1024 time channels. More importantly, additional dimensions were be added to the recording process. The corresponding photon distributions can be four- or five-dimensional, the data representing multi-spectral FLIM, FLIM Z stacks and lateral FLIM mosaics, time-series FLIM, simultaneous FLIM-PLIM, or even combinations of these. Possible applications are the investigation of fast physiological effects in live systems, such as Ca^{++} transients or chlorophyll transients, dynamics of protein interaction, and metabolic imaging in combination with pO_2 measurement. There may be more which have not even been considered yet.

FLIM images recorded by these advanced techniques are not only scientifically meaningful, they are also aesthetically appealing. This brochure presents a selection of the most beautiful images recorded with bh FLIM systems.

Megapixel FLIM Technology

BPAE Cells, High-Resolution Lifetime Image, Green Wavelength Channel

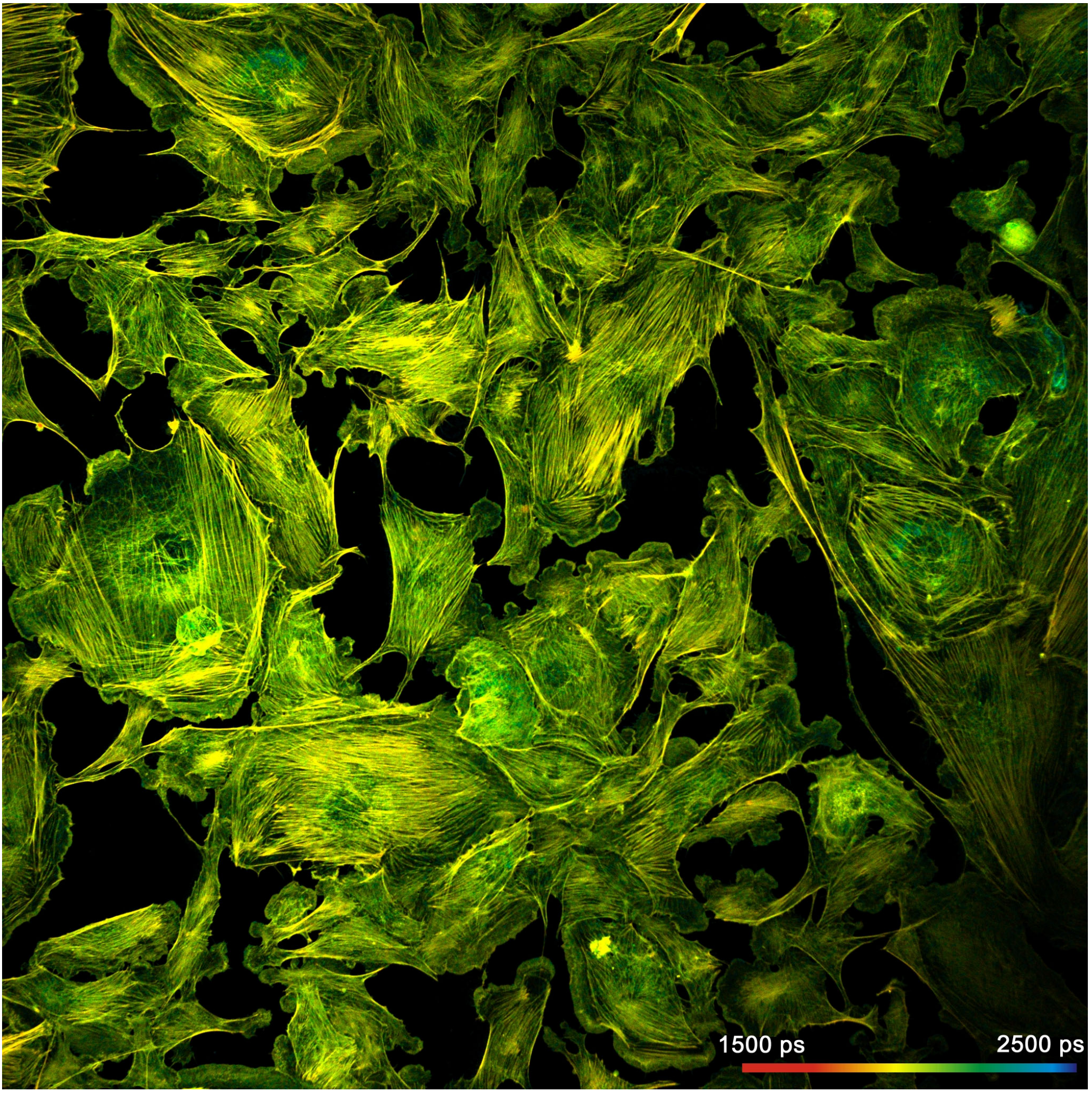


Image page 3:

FLIM of BPAE sample (Invitrogen)

Imaging Parameters: Excitation wavelength 470 nm, Detection wavelength 495 to 560 nm, 2048 x 2048 pixels, 256 time channels per pixel.

Instrument: DCS-120 Confocal Scanning FLIM System with two SPC-150 TCSPC FLIM modules and HPM-100-40 GaAsP hybrid detectors.

Principle of TCSPC FLIM

FLIM by multi-dimensional TCSPC is based on scanning the sample by a high-repetition rate pulsed laser beam and detecting single photons of the fluorescence signal returned from the sample. 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. The result can be interpreted as an array of pixels, each containing a full fluorescence decay curve in a large number of time channels.

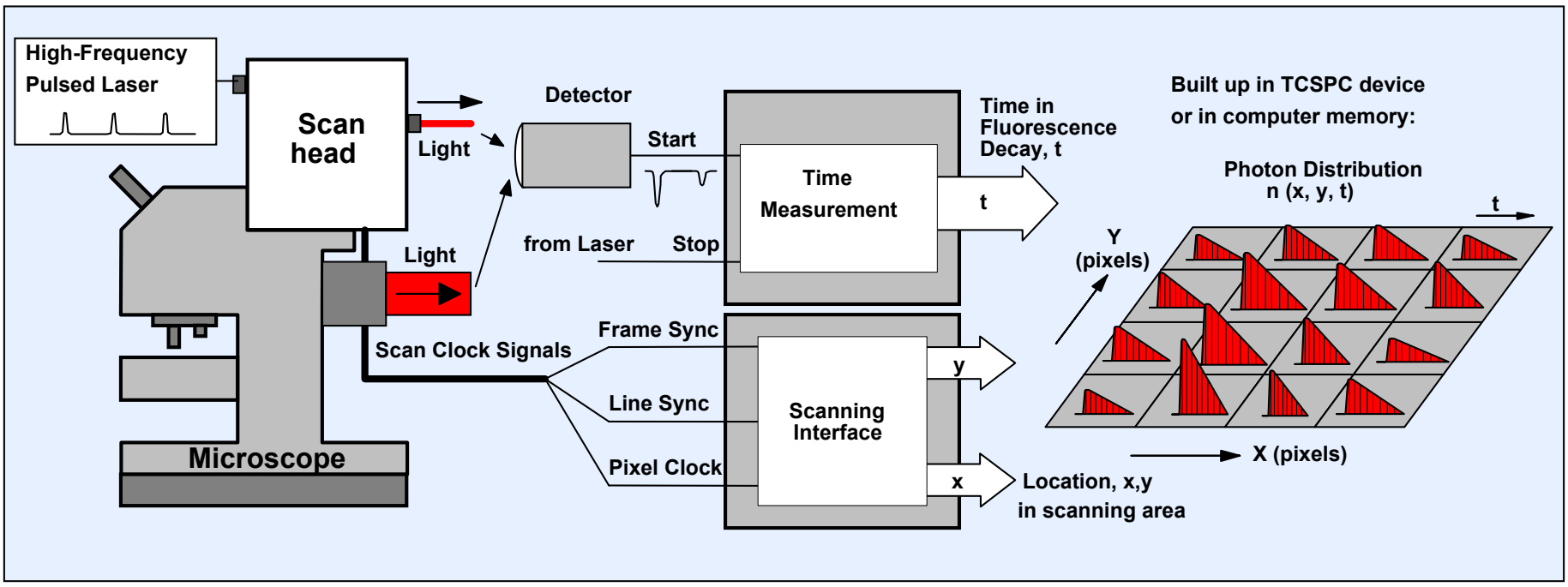


Fig. 1: Principle of TCSPC FLIM

Megapixel FLIM Technology

BPAE Cells, High-Resolution Lifetime Image, Red Wavelength Channel

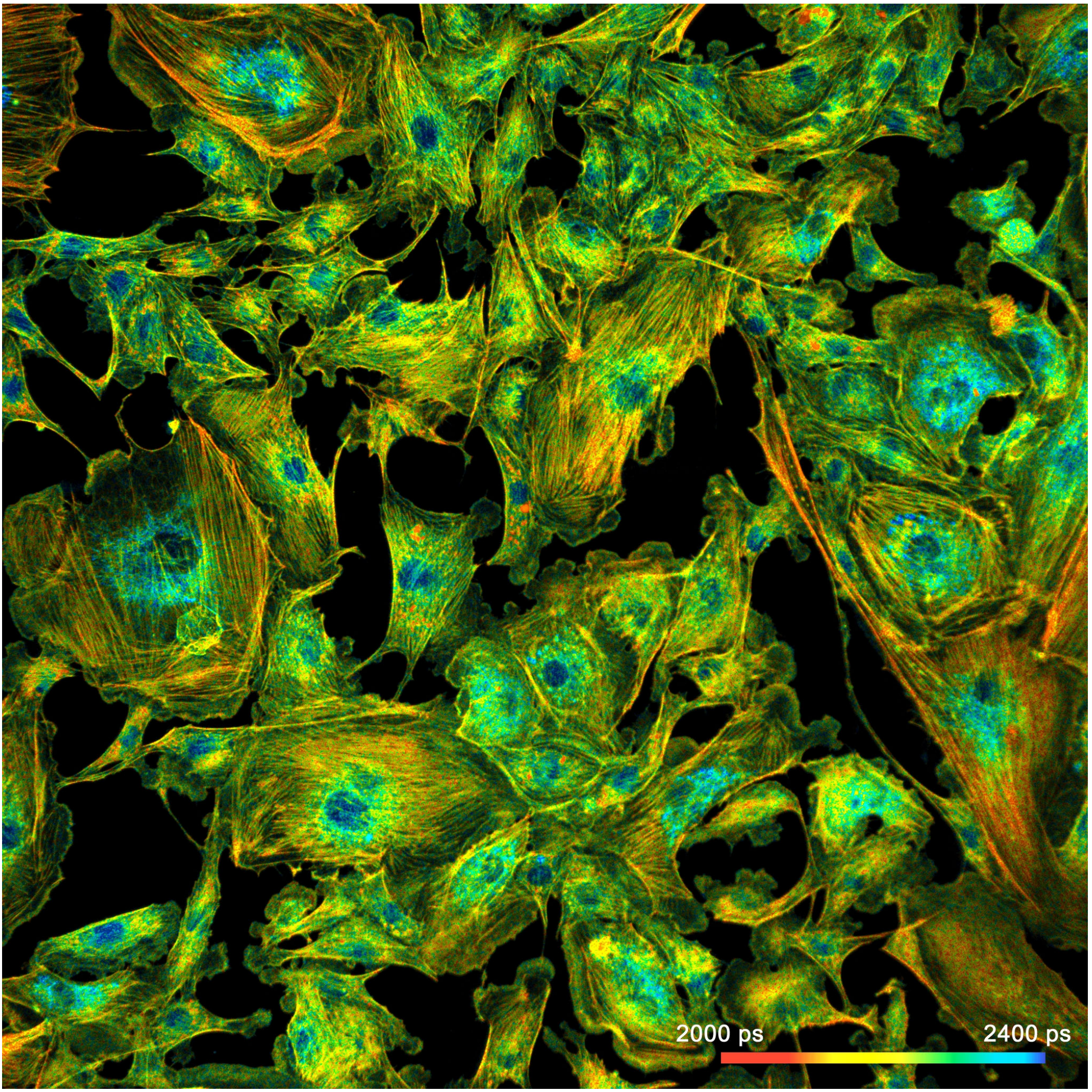


Image page 5:

FLIM of BPAE sample (Invitrogen).

Imaging Parameters: Excitation wavelength 470 nm, detection wavelength 560 to 650 nm, 2048 x 2048 pixels, 256 time channels per pixel.

Instrument: DCS-120 Confocal Scanning FLIM System with two SPC-150 TCSPC FLIM modules and HPM-100-40 hybrid detectors.

Dual-Channel Confocal Scanning

The principle of the DCS-120 confocal scanner is shown in the figure below. Two laser beams are coupled into the scanner. They are combined by a beam combiner, pass the main beamsplitter, and are deflected by the scan mirrors. The scan lens sends the beam down the microscope beam path in a way that the scan mirror axis is projected into the back aperture of the microscope lens. The motion of the scan mirrors causes a variable tilt of the beam in the plane of the microscope lens. The laser is thus scanning an image area in the focal plane of the microscope lens. The scanning can be very fast - the line time can be as short as a millisecond, an entire frame can be scanned in less than a second.

The fluorescence light is collected back through the microscope lens, passes the scan lens, and is again reflected at the scan mirrors. The reflected beam is stationary, independently of the motion of the scan mirrors. It is separated into two spectral or polarisation components, and projected into confocal pinholes. Only light from the excited spot in the focal plane of the microscope can pass the pinholes and reach the detectors. The result is a clean image from a defined depth inside the sample, without contamination by out-of-focus blur and lateral scattering.

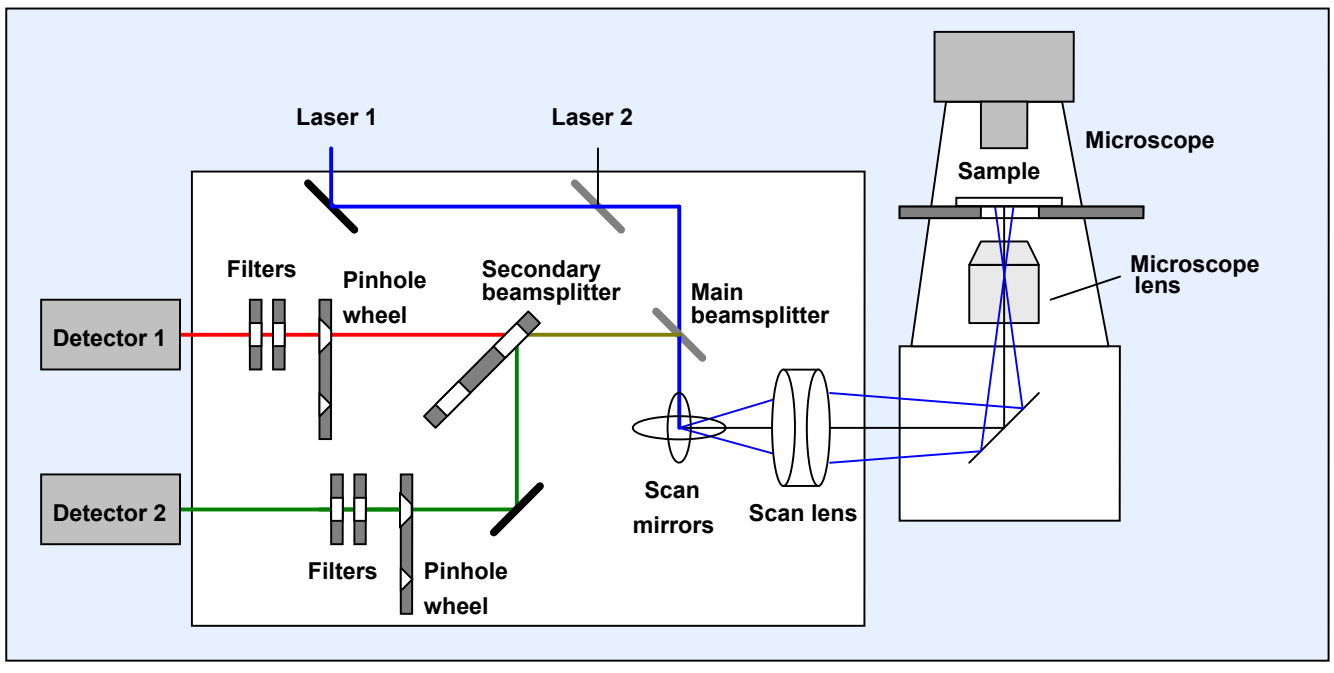


Fig. 2: Principle of dual-channel confocal scanner

Megapixel FLIM Technology

High Resolution Multiphoton Lifetime Image of Convallaria Sample

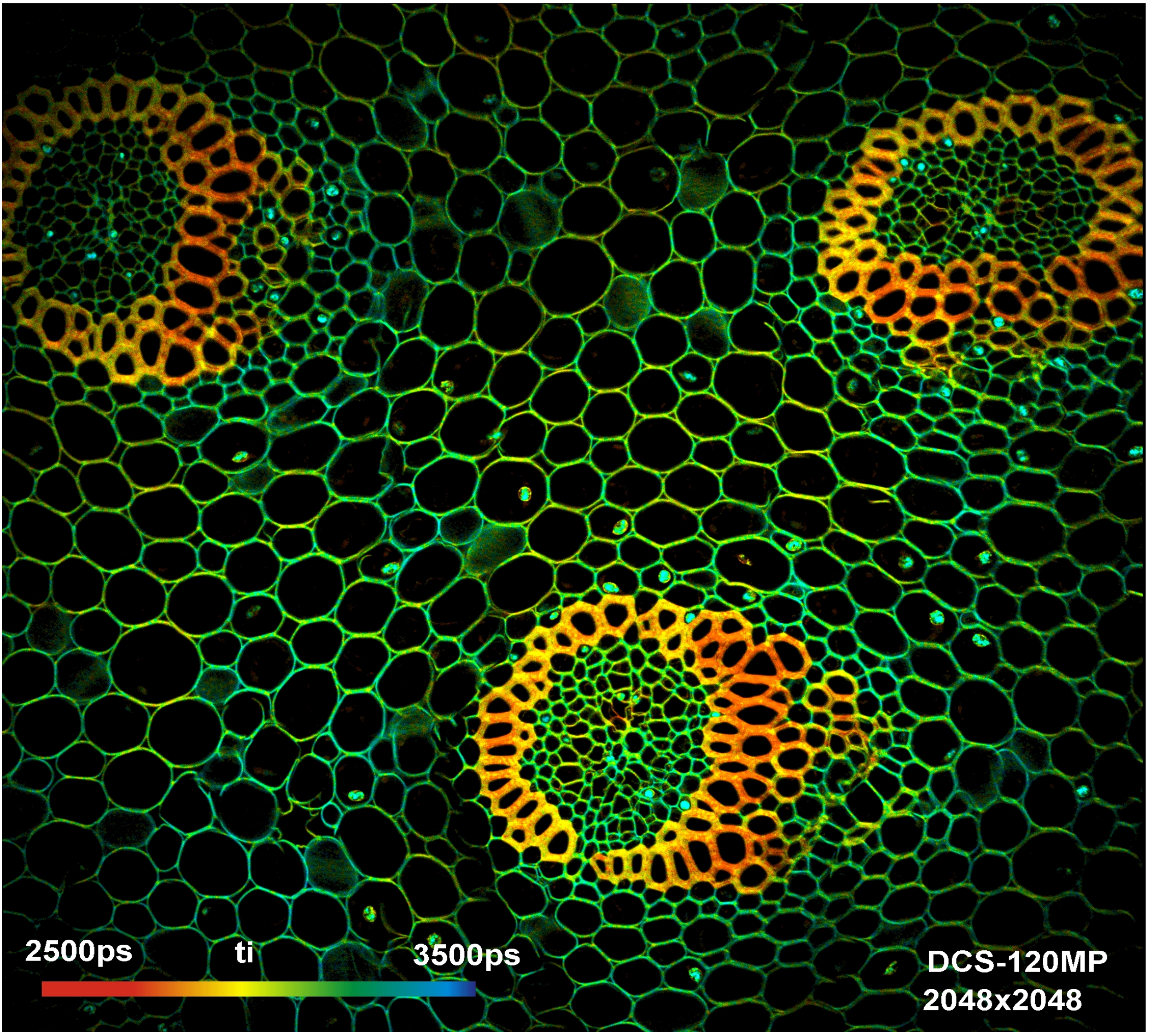


Image page 7

Convallaria sample stained with Acridin Orange

Imaging Parameters: Two-photon excitation at 820 nm, 2048 x 2048 Pixels, 256 time channels per pixel.

Instrument: DCS-120 Multiphoton FLIM System, with two SPC-160 TCSPC FLIM Modules, HPM-100-40 detectors, non-descanned detection.

Two-Photon Excitation

Two-Photon (or 'Multiphoton') excitation uses several (usually two) photons of the excitation light to generate one fluorescence photon. To make two-photon excitation efficient a femtosecond titanium-sapphire laser and focusing through a high-NA microscope lens is required. Due to the nonlinear nature of the two-photon process, excitation occurs only in a confined layer around the focal plane of the microscope lens. Two-photon excitation has several advantages over one-photon excitation: First, the laser wavelength is in the NIR, where absorption and scattering coefficients are low. Consequently, deep layers of the sample can be reached. Second, fluorophores with excitation wavelengths in the UV can be reached without the need of UV optics. Third, since excitation occurs only in the focal plane, photochemical effects in the sample are reduced. A fourth advantage is that light scattered on the way out of the sample can efficiently be recorded without impairing the image quality.

Non-Descanned detection

With two-photon excitation, no pinhole is needed to suppress the detection of out-of focus fluorescence. Consequently, there is no need to send the fluorescence light all the way back through the scanner. Instead, the fluorescence is split from the excitation directly behind the microscope lens, and directly sent to the detectors. The result is that even photons scattered on the way out of the sample have a chance to reach the detectors. The fact that scattered photons are detected does not impair the image quality - the data acquisition system automatically assigns them to the x-y position of the laser beam, not to the position where they left the sample. The result is high image quality and high detection efficiency from deep sample layers. The principle is shown in the figure below.

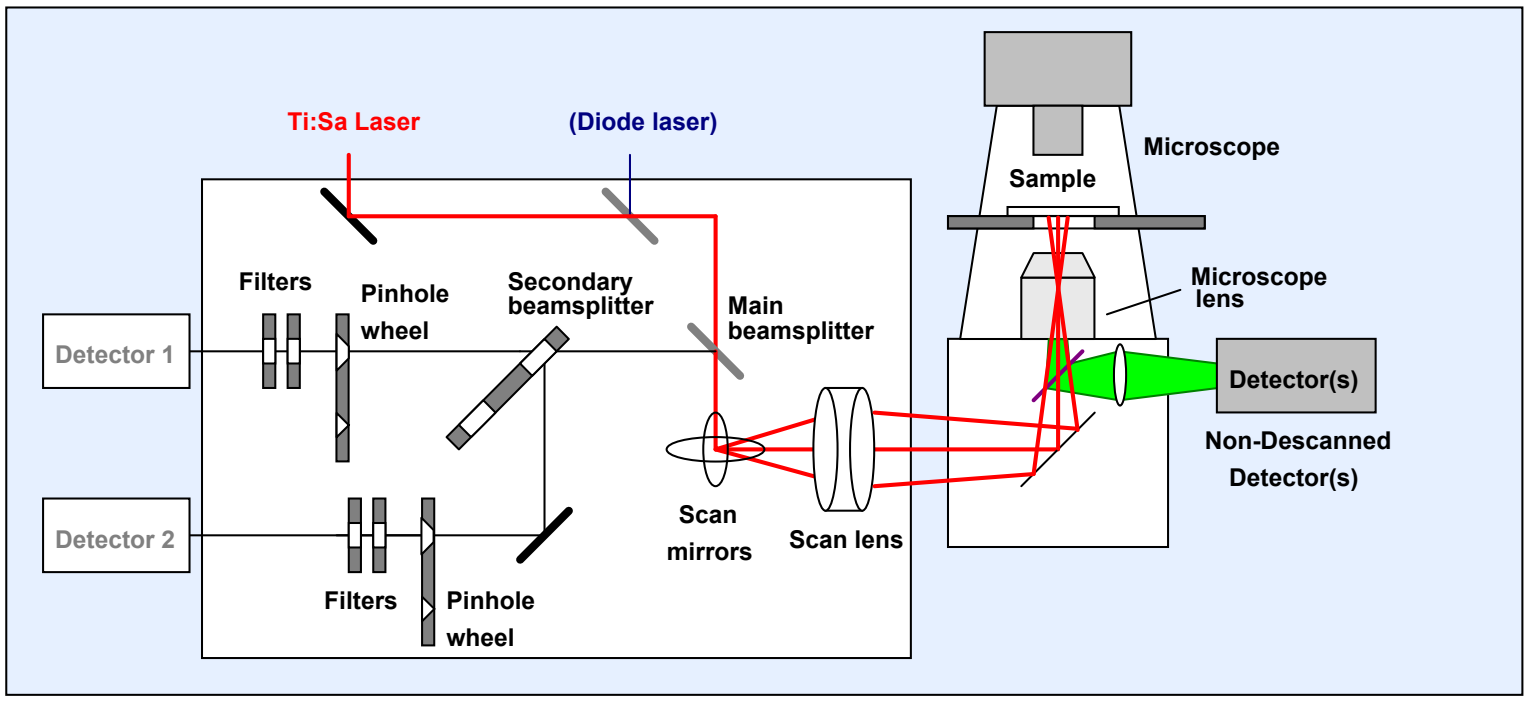
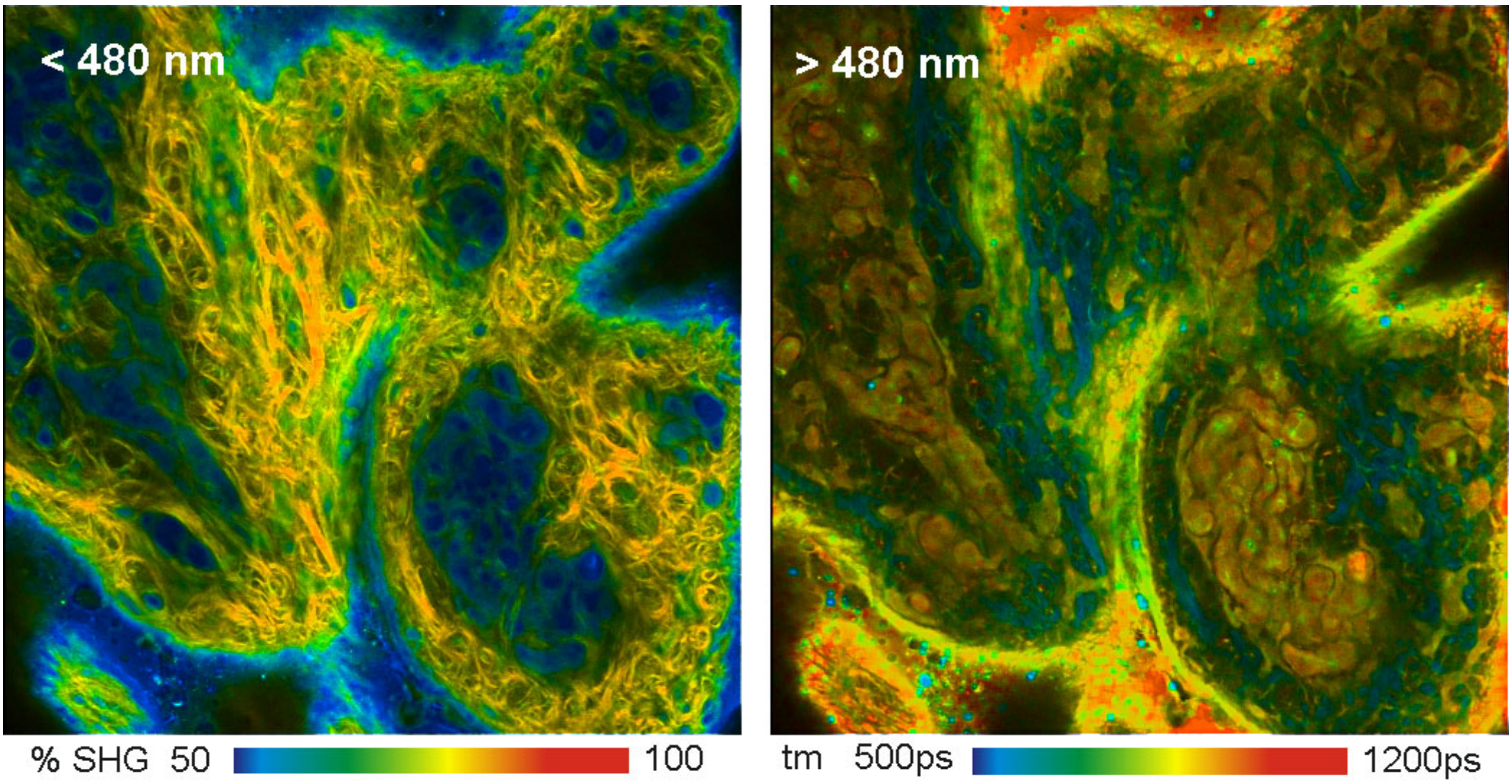


Fig. 3: Two-photon scanning with non-descanned detection

Megapixel FLIM Technology

Deep-Tissue FLIM with Two-Photon Excitation and Non-Descanned Detection



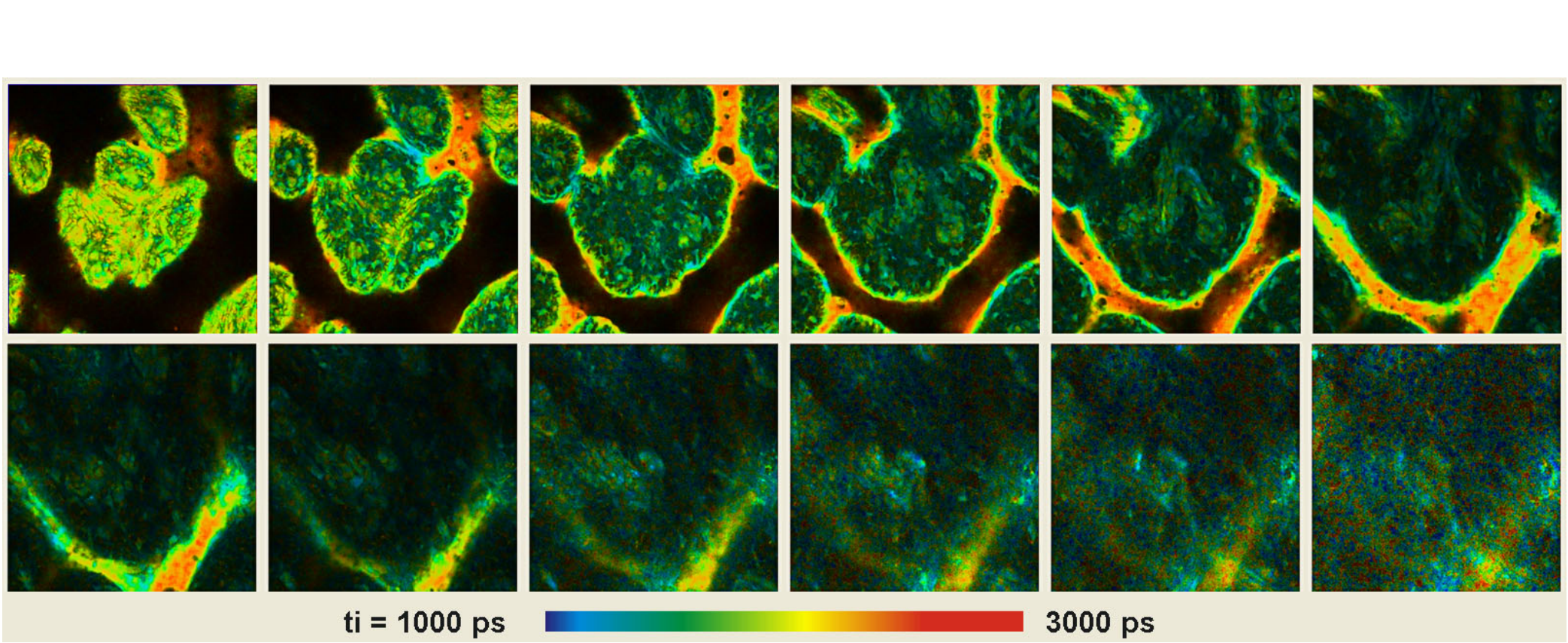


Image page 9

Autofluorescence and SHG images of pig skin

Imaging Conditions: Top: Two Photon excitation at 800 nm, non-descanned detection, parallel recording in two wavelength intervals, 440 to 480 nm and 480 to 600 nm. 512x512 pixels, 256 time channels. The image from the 440 to 480 nm channel was processed to show the percentage of SHG in the signal. The image from the 480 to 600 nm shows the amplitude-weighted lifetime obtained from a double-exponential fit of the decay functions.

Bottom: FLIM Z stack, images from the surface of the skin of down to 100 µm depth.

Instrument: Zeiss LSM 710 NLO Multiphoton Microscope, Simple-Tau 152 dual-channel FLIM system, HPM-100-40 GaAsP hybrid detectors.

Multiphoton FLIM with the Zeiss LSM 710/780/880 MP Microscopes

The scan head of the microscope scans the sample with the focused beam of a Ti:Sa laser. The fluorescence light is separated from the excitation light by a dichroic mirror in one of the filter cubes in the microscope filter carousel. The fluorescence beam is leaving the microscope at one of its side ports. A ‘Zeiss T Adapter’ splits the light into two wavelength channels, the signals of which are delivered to two Zeiss-type output ports. Two bh HPM-100-40 hybrid detectors are attached to the ports of the T Adapter. The photon pulses from the detectors are processed by the two parallel TCSPC channels of a bh Simple-Tau 152 system.

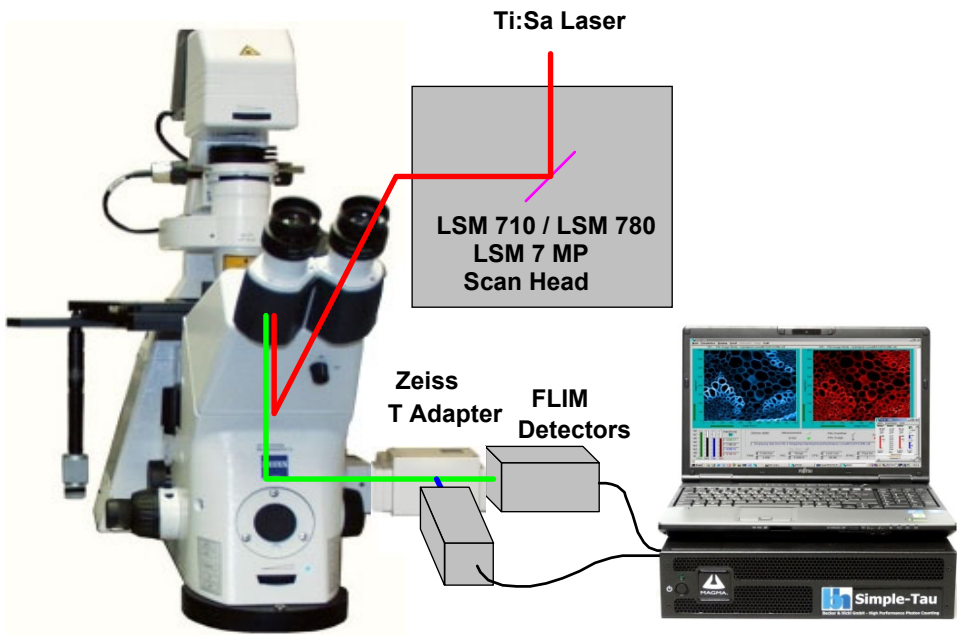


Fig. 4: Non-descanned FLIM detection with the Zeiss LSM 710/780/880 NLO microscopes

Z Stack FLIM

Multiphoton FLIM with non-descanned detection can favourably be combined with Z stack recording. The microscope scans planes of consecutive depth in the sample, the FLIM system records the corresponding FLIM images and stores them in consecutive data files or in consecutive element of a FLIM Mosaic. Photobleaching and photodamage is less a problem than for one-photon excitation, because the scanning of one plane does not cause degradation effects in another.

Megapixel FLIM Technology

Two-Photon Autofluorescence FLIM

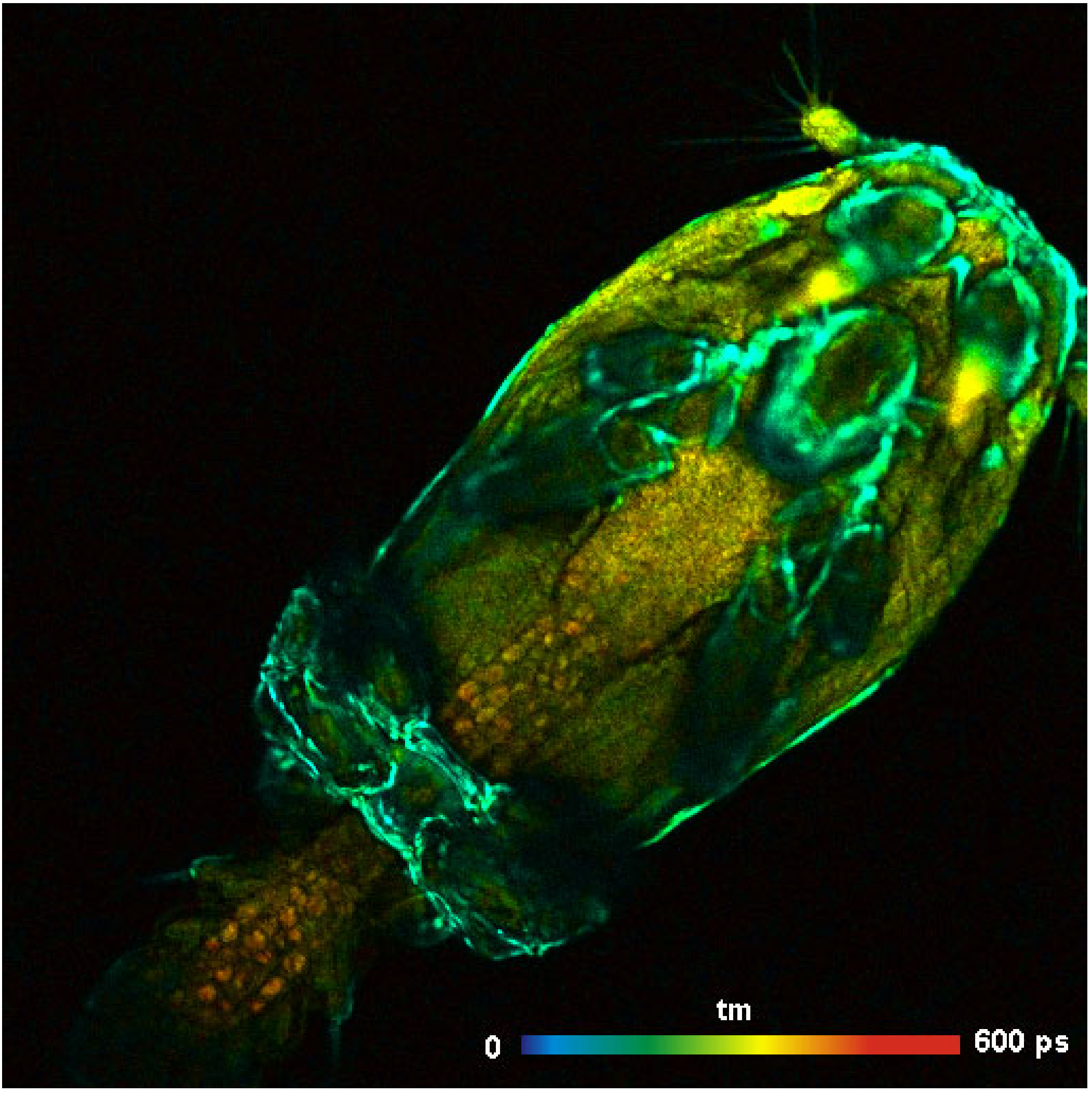


Image page 11

Autofluorescence FLIM of a live salmon louse (*sepeophtheirus salmonis*)

Imaging Parameters: Two-photon excitation at 780 nm, 512 x 512 pixels, 256 time channels, detection from 450 to 600 nm.

Instrument: SPC-150 with Sutter MOM Moveable Objective Multiphoton Microscope

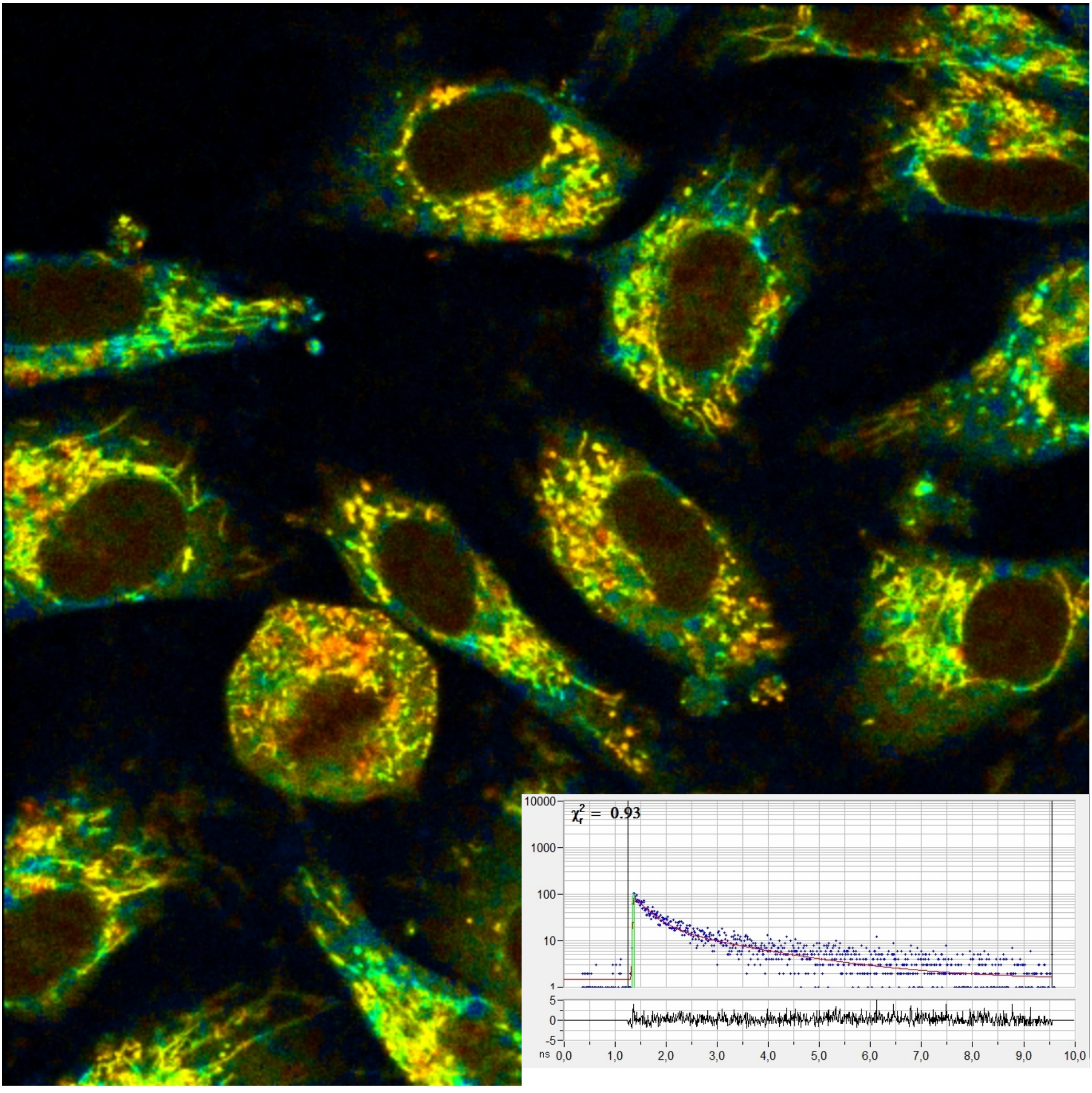
Autofluorescence FLIM

Biological tissue contains a wide variety of endogenous fluorophores. For many years, autofluorescence measurements of cells and tissue have mainly been performed by spectrally resolved techniques. The problem of purely spectrally resolved techniques is that the emission and excitation spectra of the endogenous fluorophores are broad and poorly defined. Many of the fluorophores are mixtures of closely related compounds. The shape of the spectra can therefore vary. Moreover, absorbers present in the tissue can change the apparent fluorescence spectra. It is therefore difficult to disentangle the fluorescence components by their emission spectra alone. The most serious drawback of purely spectral techniques is, however, that different binding states of fluorophores cannot be distinguished. FLIM not only helps distinguish different fluorophores but also shows differences in the binding states and in the composition and conformation of the fluorophores.

Autofluorescence FLIM is often performed in deep layers of biological tissue. Moreover, the excitation wavelengths for most endogenous fluorophores are in the ultraviolet region, which poses problems to standard microscope optics. The imaging method of choice is therefore multiphoton excitation with non-descanned detection: Multiphoton excitation shifts the excitation wavelength into the NIR, and non-descanned detection efficiently collects photons from deep sample layers.

Megapixel FLIM Technology

NAD(P)H FLIM with Ultra-Fast Detectors



tm 500 ps 1200 ps

Image page 13

Autofluorescence (NAD(P)H) FLIM of live Ea.hy926 cells.

Imaging Parameters: Two-photon excitation at 750 nm, detection from 430 to 470 nm. FLIM data format 512x512 pixels, 1024 time channels. Time-channel width 10ps.

Instrument: SPC-150N with Zeiss LSM 880 Multiphoton Microscope, ultra-fast HPM-100-06 hybrid detector, IRF width 20 ps FWHM.

NADH FLIM

Of special interest in autofluorescence FLIM is NAD(P)H (nicotimamide adanine (pyridine) dicucleotide). NAD(P)H is involved in the metabolism of the cell. It exists in a bound and an unbound state, with distinctly different fluorescence lifetimes. The relative amount of bound and unbound NADH depends on the type of metabolism. Phosphorylation is related to a higher, glycolysis to a lower bound/unbound ratio. FLIM can therefore be used to determine the state of the metabolism. Metabolic information can, in principle, be obtained from the mean (amplitude-weighted) lifetime of the decay functions in the FLIM pixels. More accurate information is contained in the pre-exponential factors, a_1 and a_2, of the decay functions. In typical FLIM images with about 120 ps IRF width it is difficult to determine a_1 and a_2 at good signal-to noise ratio. The situation improves significantly with decreasing IRF width. With the new bh HPM-100-06 detectors an IRF width of less than 20 ps is achieved. This results in an almost perfect separation of the decay components, accurate a_1, a_2, τ_1 and τ_1 values, and thus in reliable metabolic information.

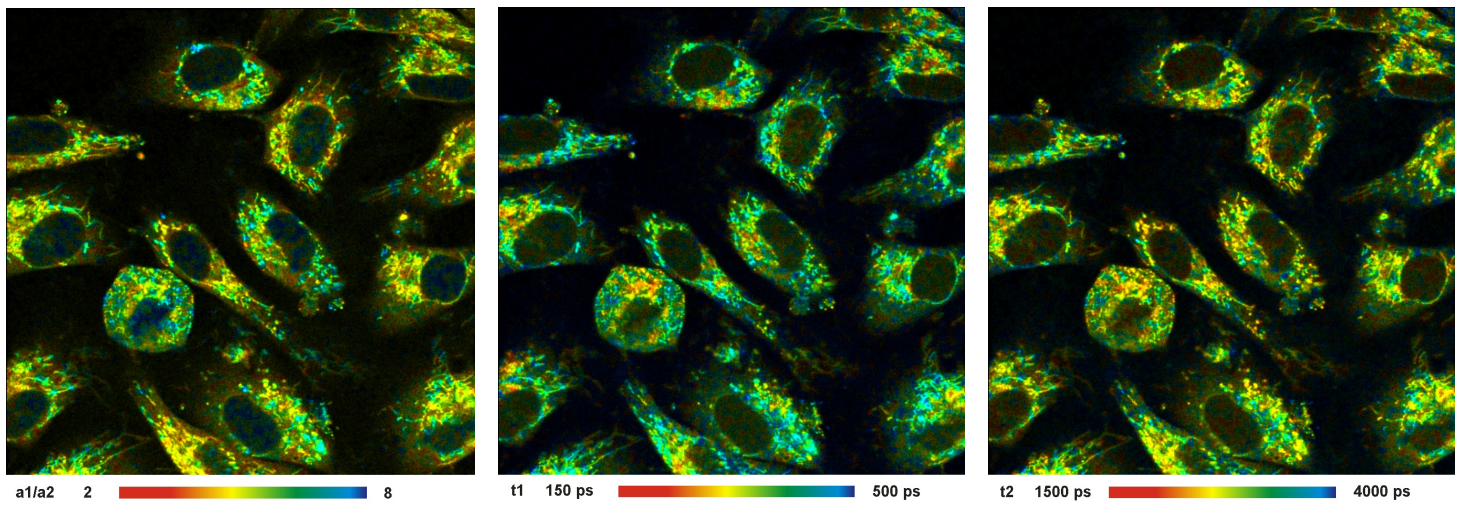


Fig. 5: Left to right: Images of the amplitude ratio, a1/a2 (unbound/bound ratio), and of the fast (t1, unbound NADH) and the slow decay component (t2, bound NADH). Extracted from the data shown at page 13.

Megapixel FLIM Technology

Multi-Wavelength FLIM

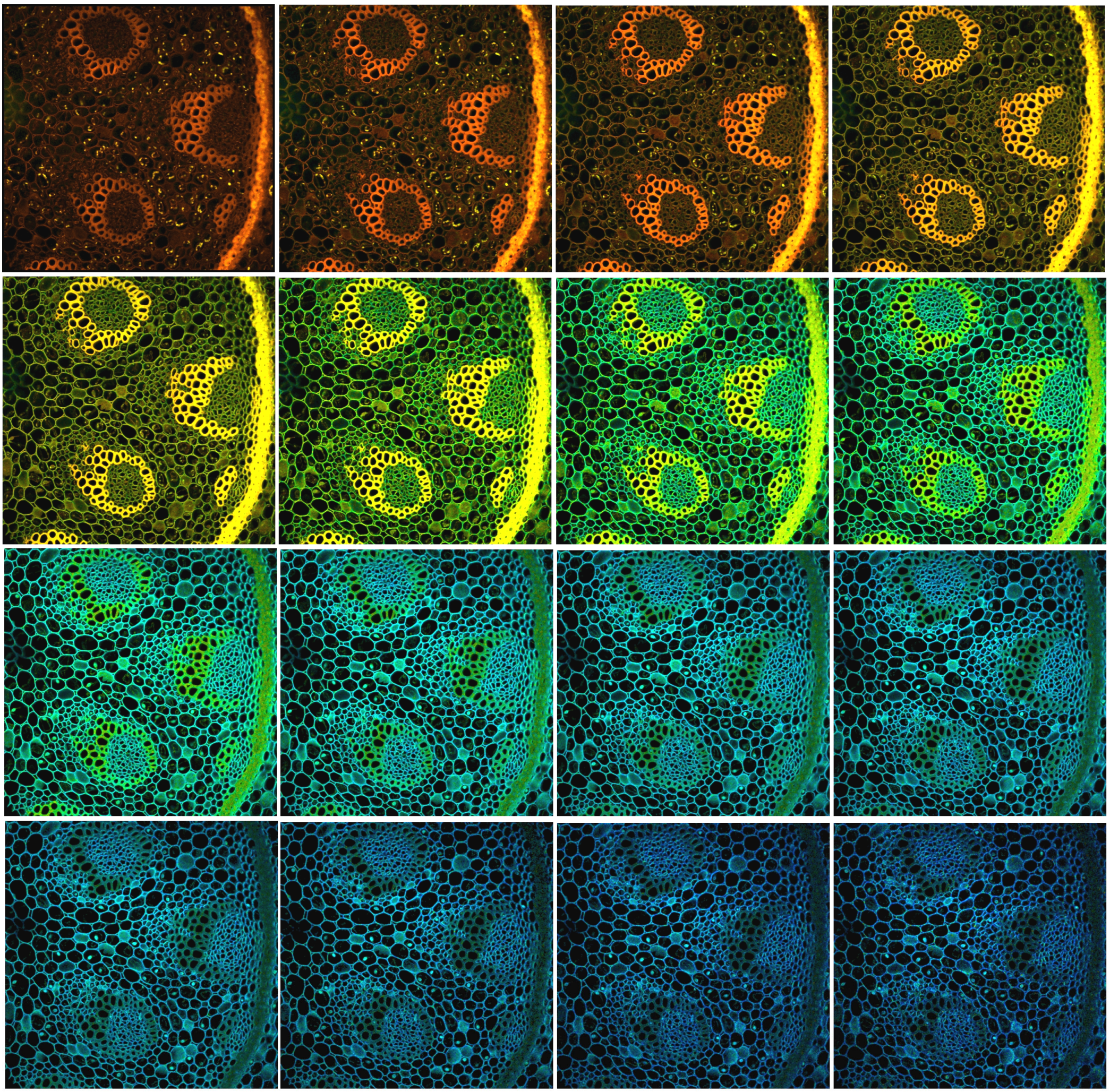


Image page 15

Convallaria sample stained with Acridin Orange,

Imaging Parameters: One-photon excitation at 470 nm, 512 x 512 Pixels, recorded into 16 wavelength channels and 256 time channels per pixel.

Instrument: DCS-120 Multiphoton FLIM System, with two SPC-160 TCSPC FLIM Modules, PML-16 GaAsP Multi-Wavelength detector, confocal detection.

Multi-Wavelength FLIM

The principle of TCSPC FLIM 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 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. 6.

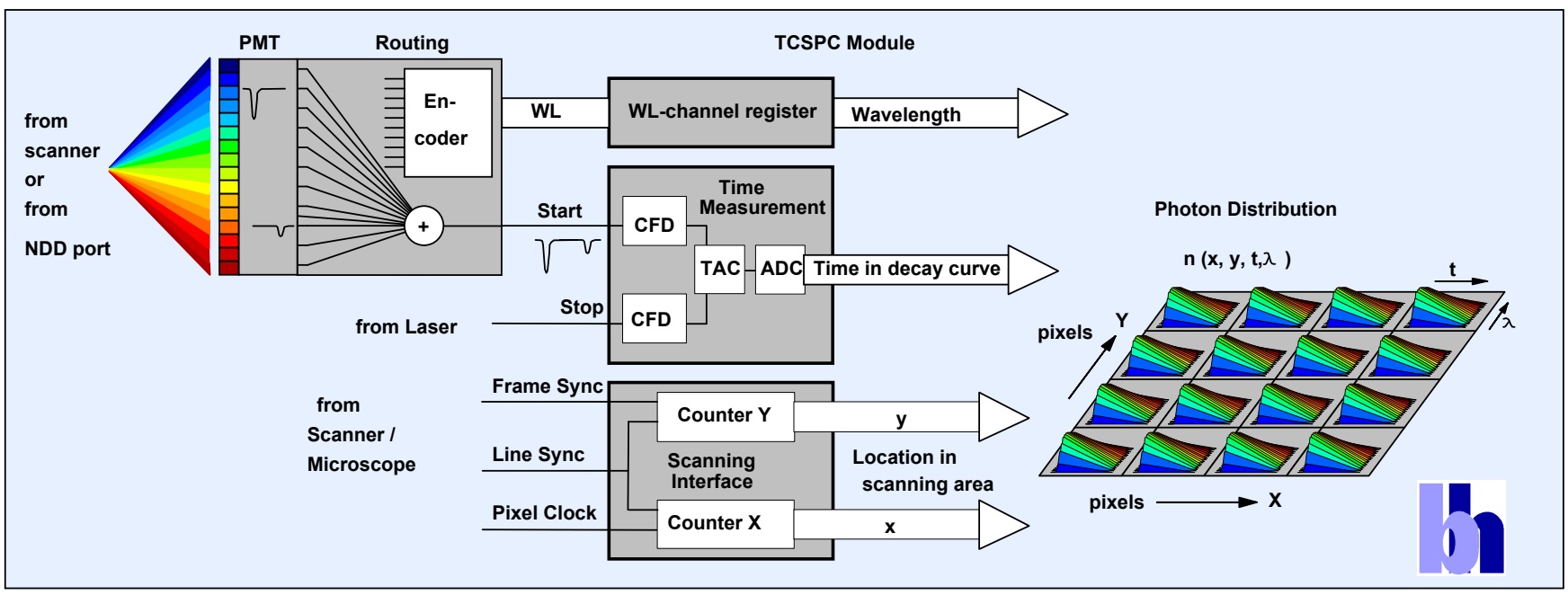


Fig. 6: Principle of Multi-Wavelength TCSPC FLIM

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.

Megapixel FLIM Technology

Two-Photon High-Resolution Lifetime Mosaic Image

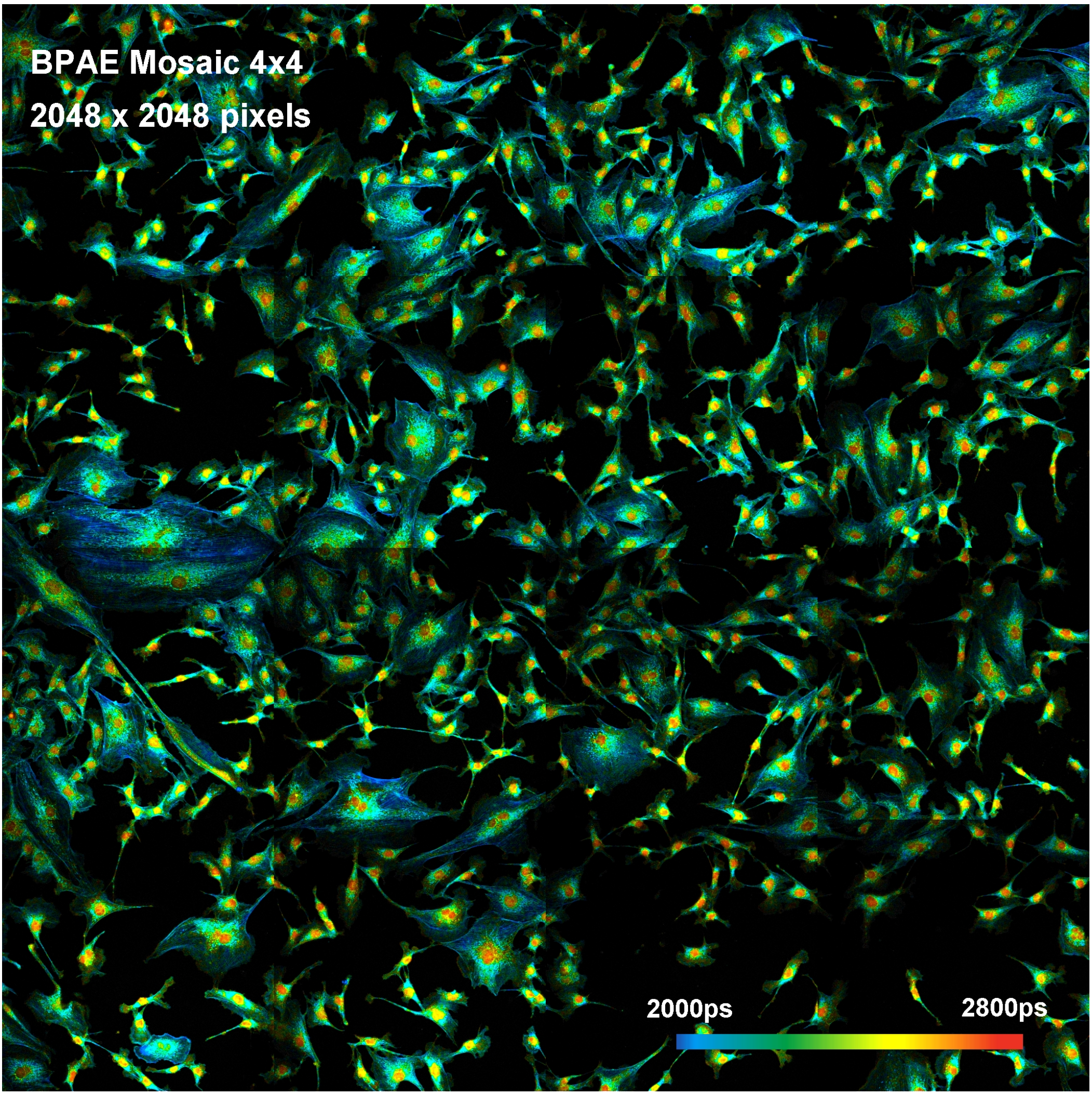


Image page 17

Mosaic FLIM of BPAE sample stained with Alexa 488 and Mito Tracker Red (Invitrogen)

Imaging Parameters: Two-photon excitation at 820 nm, mosaic with 4x4 elements, each element scanned with 512 x 512 Pixels, 256 time channels per pixel. The entire image has 2048 x 2048 pixels.

Instrument: DCS-120 MP Multiphoton FLIM System, with two SPC-160 TCSPC FLIM Modules, HPM-100-40 GaAsP hybrid detectors, non-descanned detection.

Spatial Mosaic FLIM

Spatial Mosaic FLIM records an array of lifetime images into a single, large photon distribution. A lifetime image is recorded into the first element of the mosaic-data array, then the sample is shifted by the diameter of the scan area, and a new image is recorded into the next element. The process is repeated until the desired sample area has been imaged. Compared with an image taken through a low-magnification (low-NA) lens the spatial resolution is better, and a higher photon collection efficiency is obtained. In the case of two-photon excitation also a higher excitation efficiency is obtained because the high-NA yields a high local energy density in the focus.

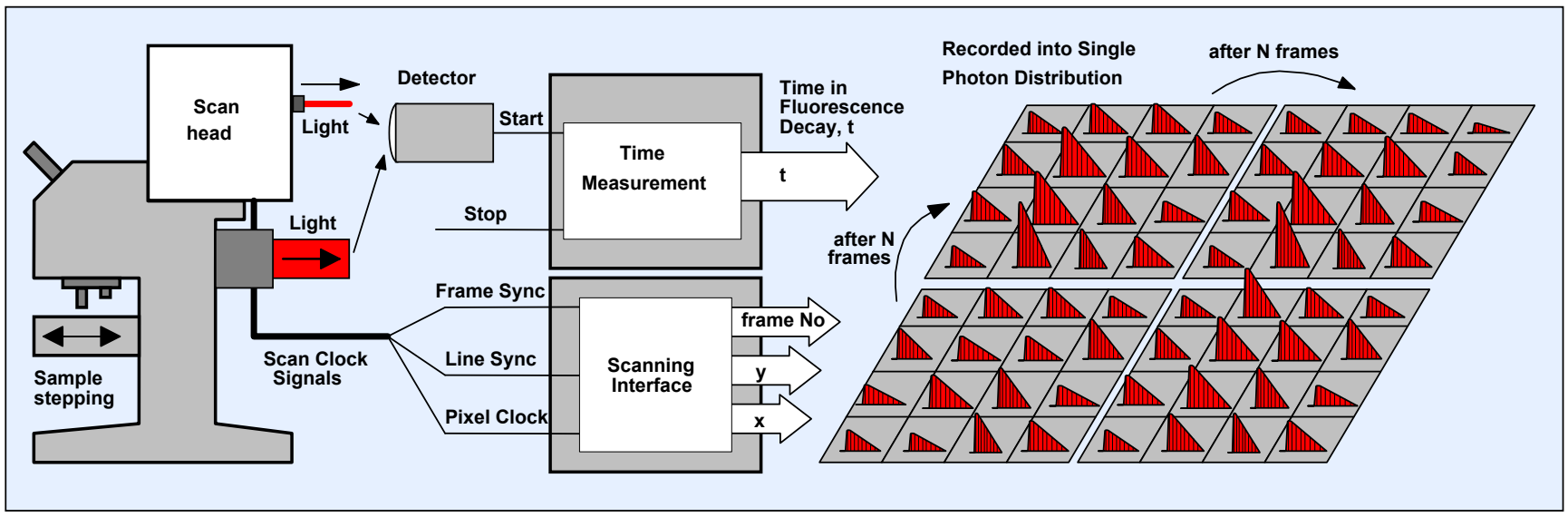
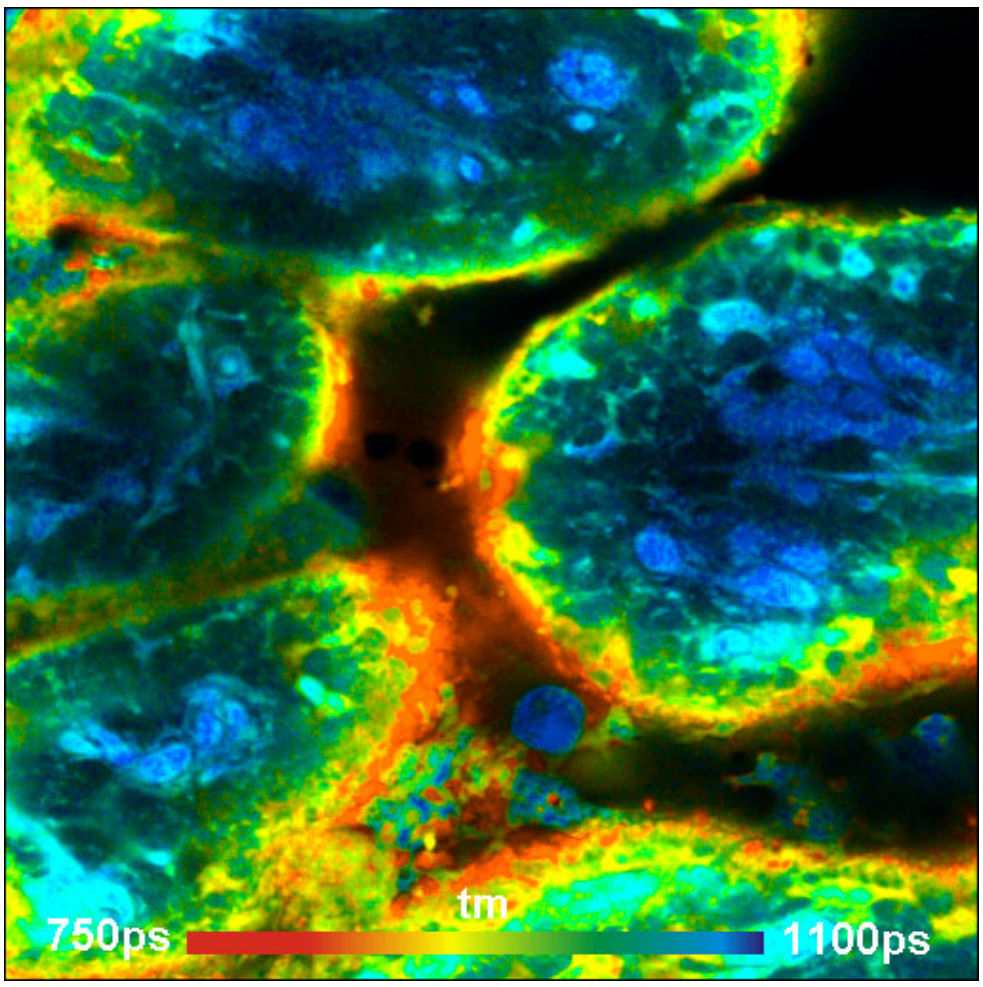
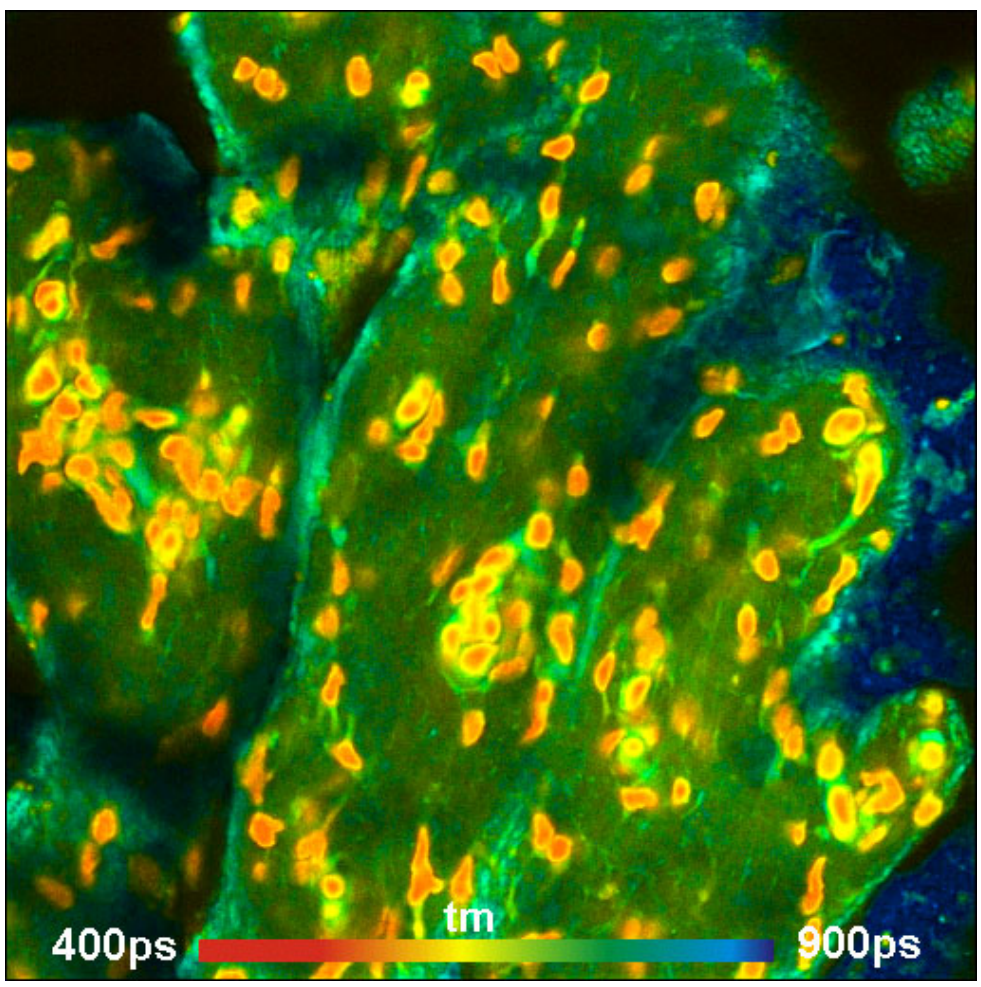


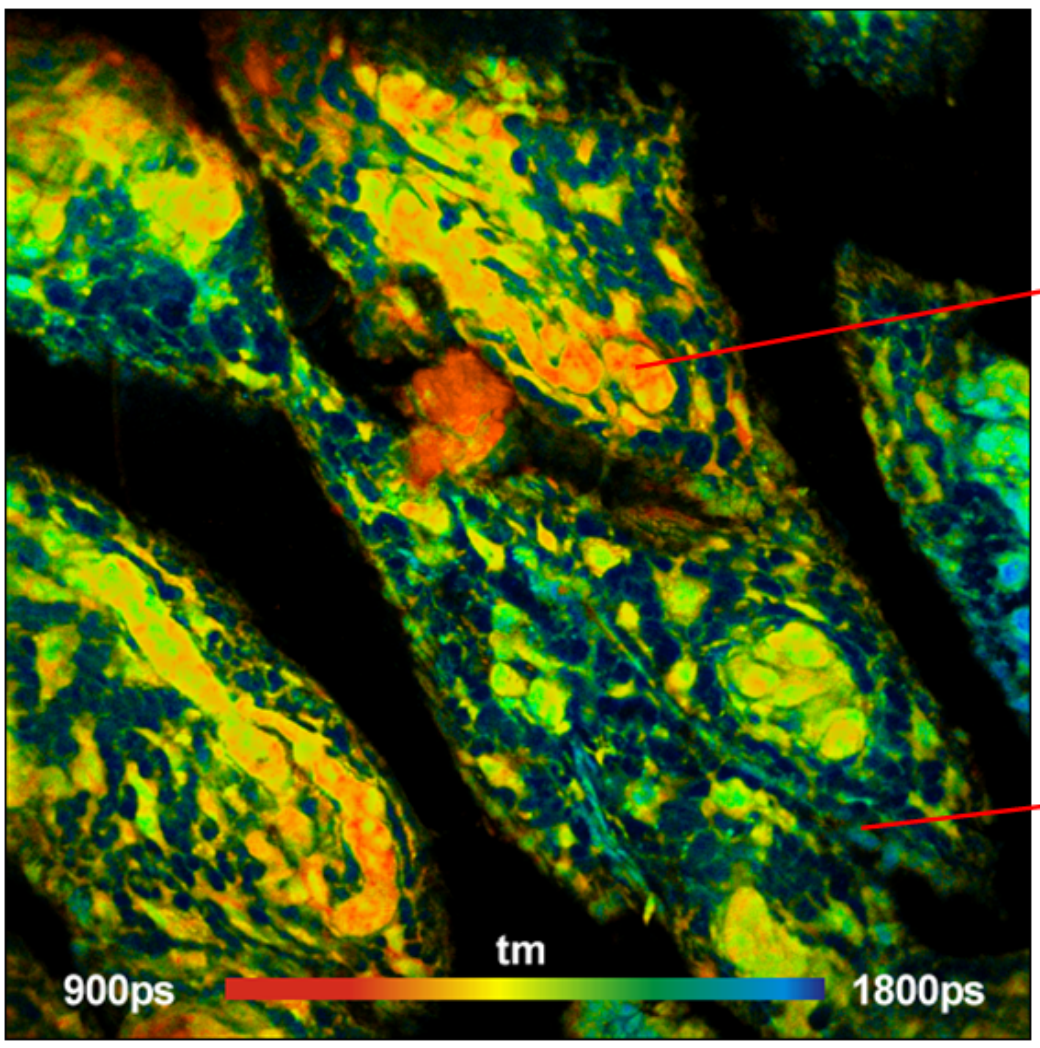
Fig. 7: Mosaic FLIM. The TCSPC system records a spatial mosaic of lifetime images in to a single, large photon distribution.

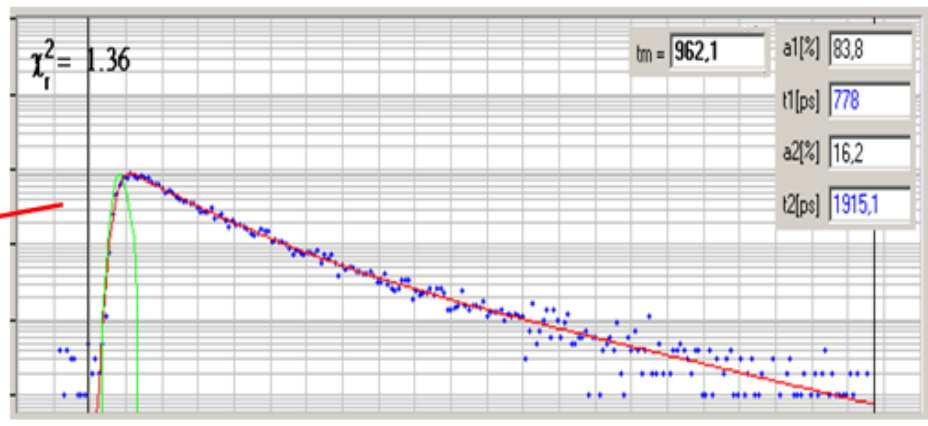
Megapixel FLIM Technology

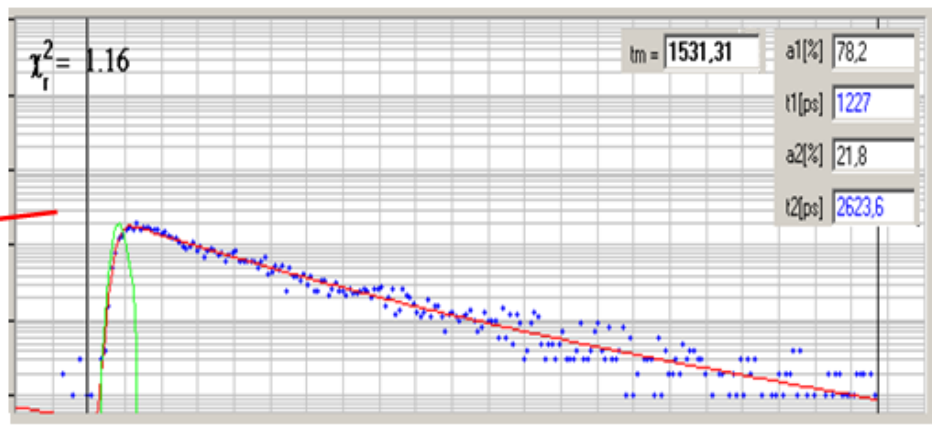
Near Infrared FLIM - Deep-Tissue Imaging at Affordable Price











Images page 19

Pig skin, stained with DTTCC (upper left and lower left) and with methylene blue (upper right)

Imaging conditions: Scanning with 512 x 512 pixels, recorded into 256 time channel per pixel, confocal detection. Excitation and detection wavelengths 780 nm and 800 to 850 nm (upper left), 645 nm and 660 to 900 nm (upper right), 650 nm and 680 to 900 nm (lower left)

Instruments: Upper left: Zeiss LSM 710 NLO with Simple-Tau-152 FLIM system and HPM-100-50 detector, Ti:Sa laser used as one-photon excitation source. Upper right: LSM 710 Intune with Simple-Tau-152 FLIM system and HPM-100-50 detector, Lower left: DCS-120 WB system with wideband beamsplitter BDL-SMN-650 nm ps diode laser and HPM-100-50 detector.

Near-Infrared FLIM

Near-infrared (NIR) dyes show surprisingly large variation in their fluorescence lifetimes depending on the local molecular environment. They are therefore potential markers for biologically relevant parameters. Near-infrared FLIM requires excitation sources in the red and near-infrared region, suitable detectors, and scanner optics adapted for the wavelength range of interest. On the excitation side the problem can be solved by using the Ti:Sa lasers of multiphoton microscopes as one-photon excitation sources, or by using ps diode lasers with wavelengths in the range from 650 to 785 nm. On the detection side, wideband beamsplitters and HPM-100-50 hybrid detectors can be used.

In tissue FLIM applications NIR FLIM is a cost efficient alternative to multiphoton FLIM. Because the scattering coefficient of the tissue at NIR wavelengths is small the excitation reaches deep tissue layers, and the emission can leave the tissue without substantial loss. The recorded images are therefore of high quality. The short lifetime of most NIR dyes - often considered a disadvantage - is no problem for the bh TCSPC FLIM devices.

Megapixel FLIM Technology

FLIM of Macroscopic Objects

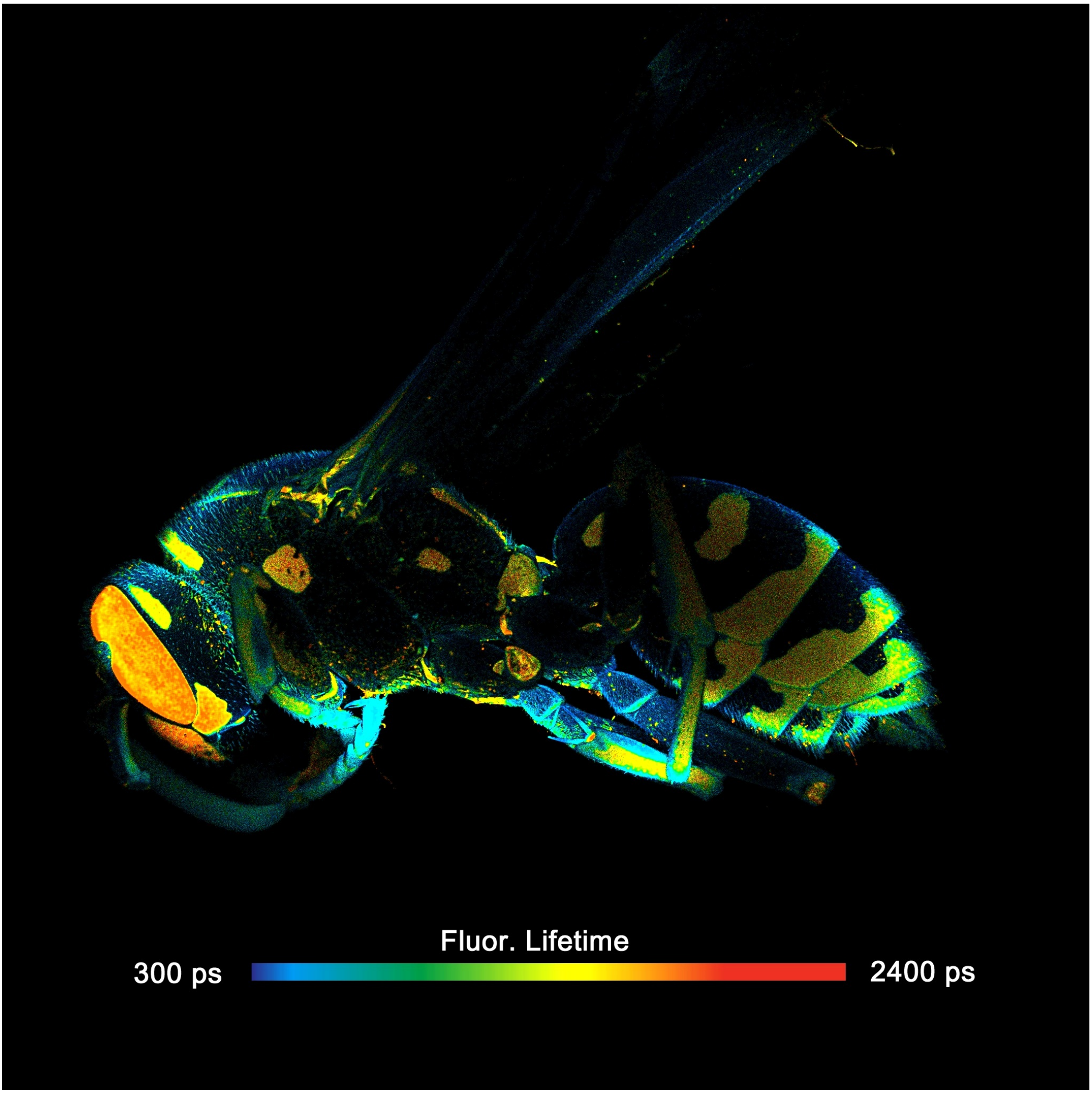


Image page 21

FLIM of a wasp (*vespula germanica*)

Imaging Conditions: Scanning and recording with 2048 x 2048 pixels, 256 time channels per pixel. Excitation wavelength 470 nm, detection wavelength 495 to 700 nm.

Instrument: DCS-120 MACRO FLIM System

FLIM of Macroscopic Objects

Samples larger than typically used in microscopy can be scanned by placing the sample directly in the primary focal plane of a confocal scanner. A complete instrument for FLIM of macroscopic objects is the DCS-120 MACRO system, available from Becker & Hickl.

The system is based on the scanner of the DCS-120 confocal FLIM system. The laser beam is scanned by two fast-moving galvanometer mirrors. The scan lens focuses the laser beam into an image plane located closely in front of the scanner. The image plane of the scan lens is brought in coincidence with the sample surface. As the galvanometer mirrors change the beam angle the laser focus scans across the sample. Fluorescence light excited in the sample is collected and collimated by the scan lens, de-scanned by the galvanometer mirrors, and separated from the excitation light by the main dichroic beamsplitter. The fluorescence beam is further split into two spectral or polarisation components, and focused into pinholes. Light passing the pinholes is sent to the detectors. The systems scans an image area of approximately 15x15 mm. For imaging of objects larger than the scan area the system can be extended with a motorised sample stage.

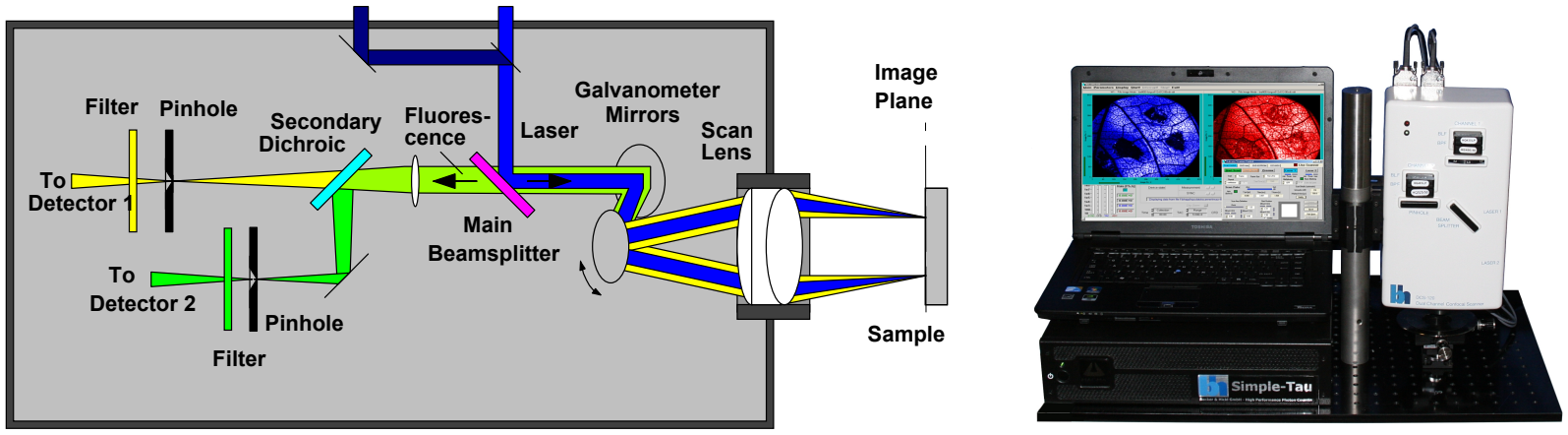


Fig. 8: Left: Scanning of a macroscopic sample in the primary image plane of the DCS-120 scanner. Right: bh DCS-120 MACO system.

Megapixel FLIM Technology

FLIM of Macroscopic Objects

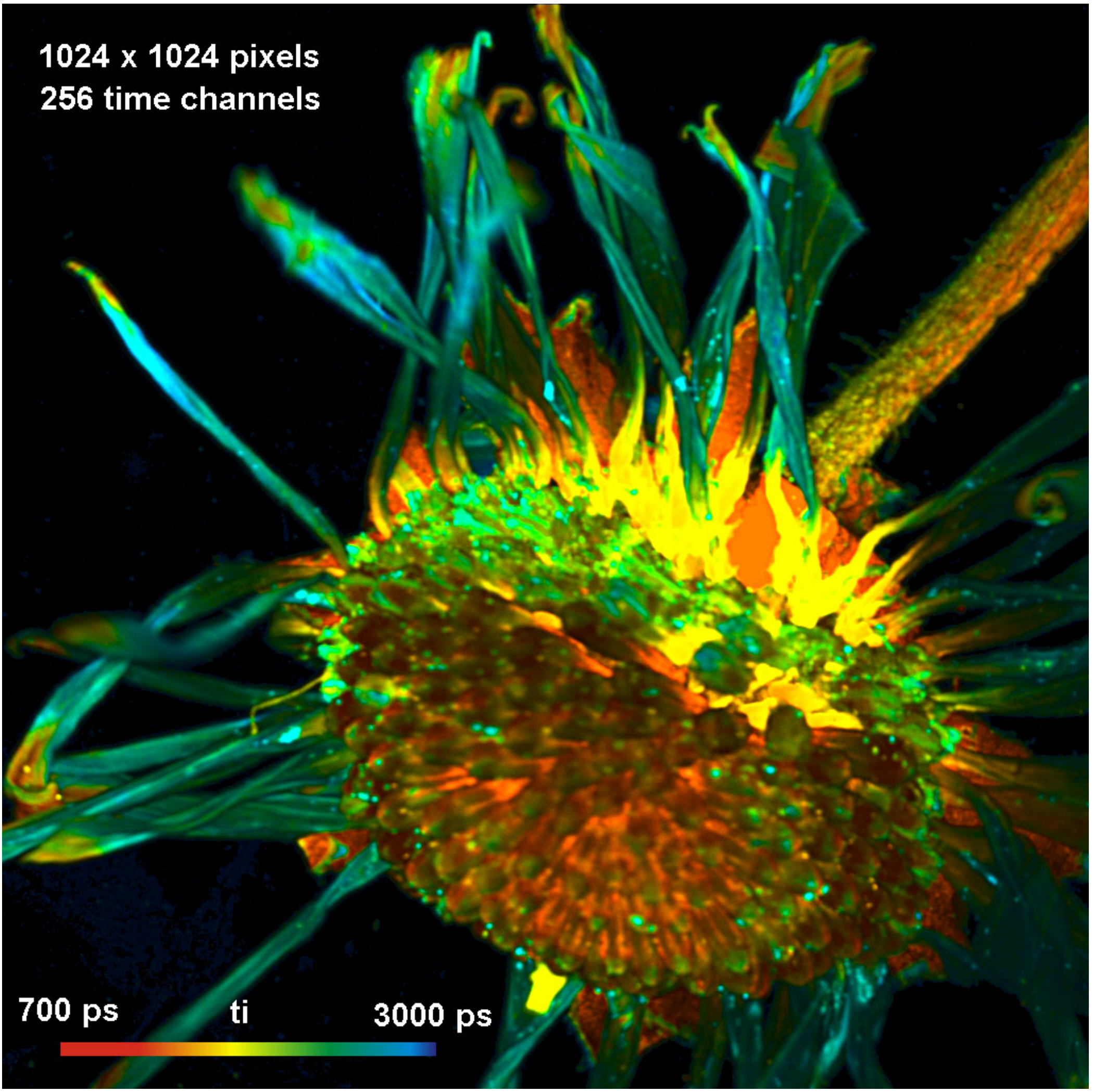


Image page 23

FLIM image of Daisy Flower (*Bellis perennis*)

Imaging Conditions: Scanning and recording with 1024 x 1024 pixels, 256 time channels per pixel. Excitation wavelength 470 nm, detection wavelength 495 to 700 nm.

Instrument: DCS-120 MACRO FLIM System

FLIM of Plants

Plants are the first organisms to respond to enivironmental damage or to climat change. Macro FLIM helps reveal changes in the plant meatabolism, turn up early stages of fungus infection, or display side effects of herbecides and fertilisers used in agriculture.

Megapixel FLIM Technology

STED FLIM at 20 nm Resolution

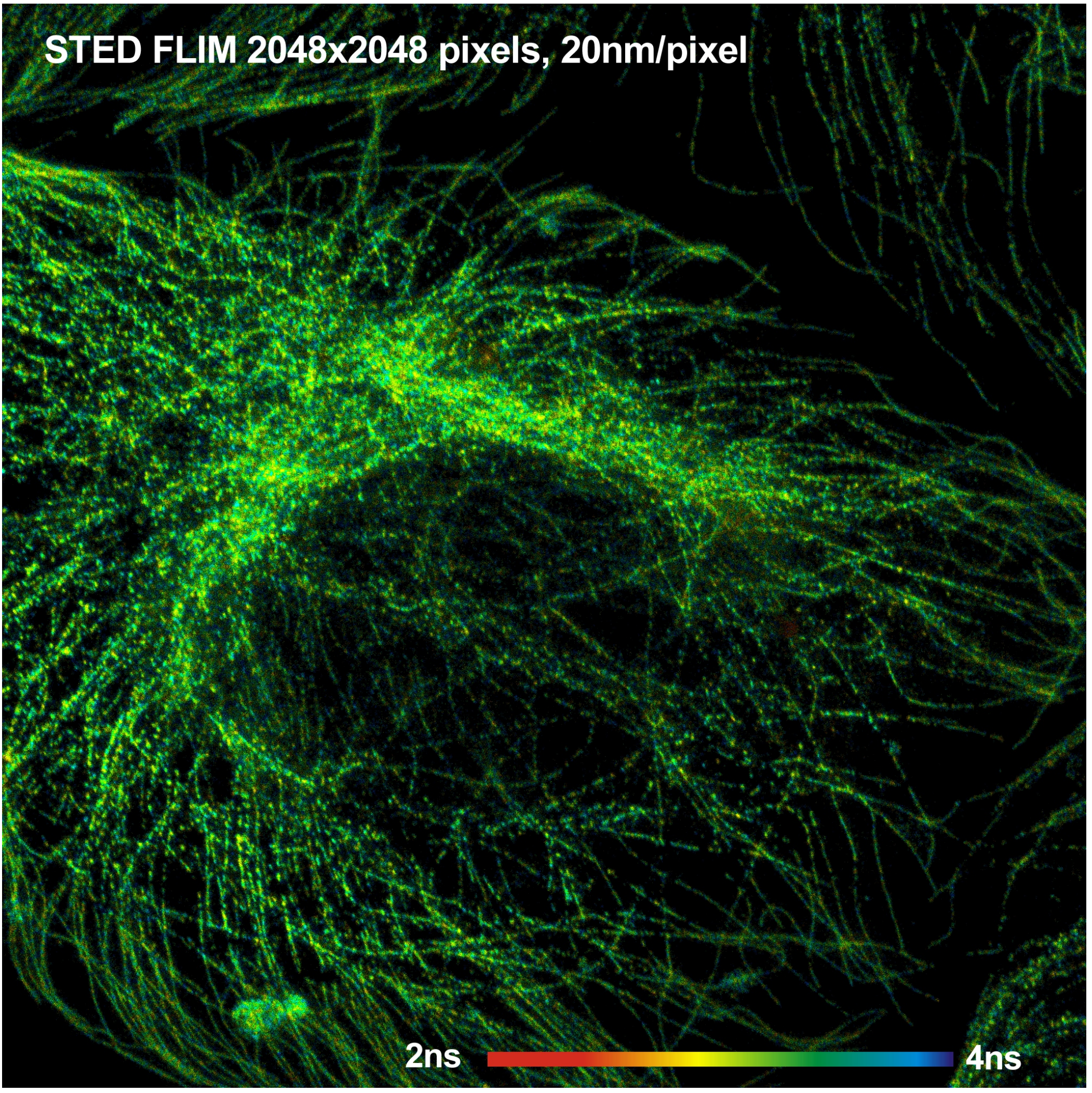


Image page 25

SETD FLIM of BPAE Cell, tubulin fibres stained with Alexa 488.

Imaging Parameters: 2048 x 2048 pixels, 256 time channels per pixel. Optical resolution 20 nm per pixel.

Instrument: Abberior STED Microscope with SPC-150 TCSPC FLIM module

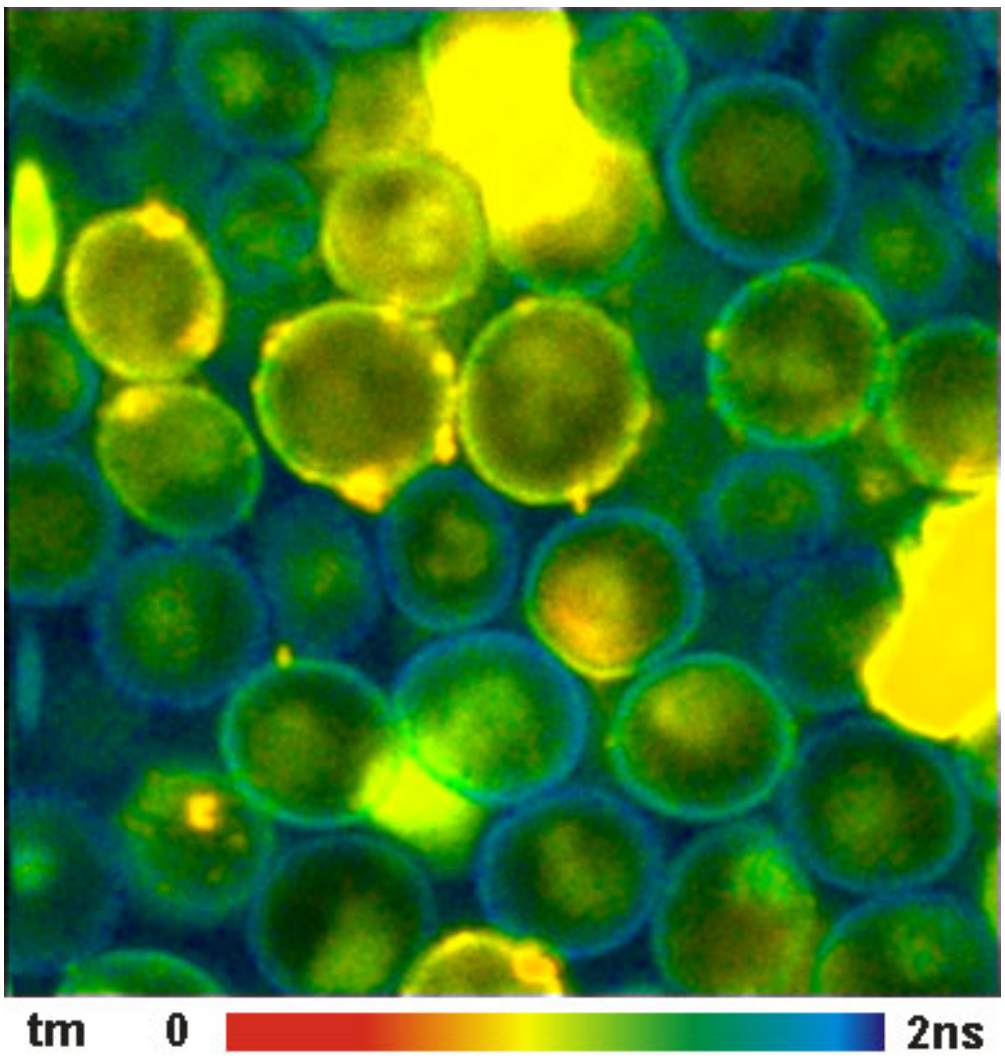
STED FLIM

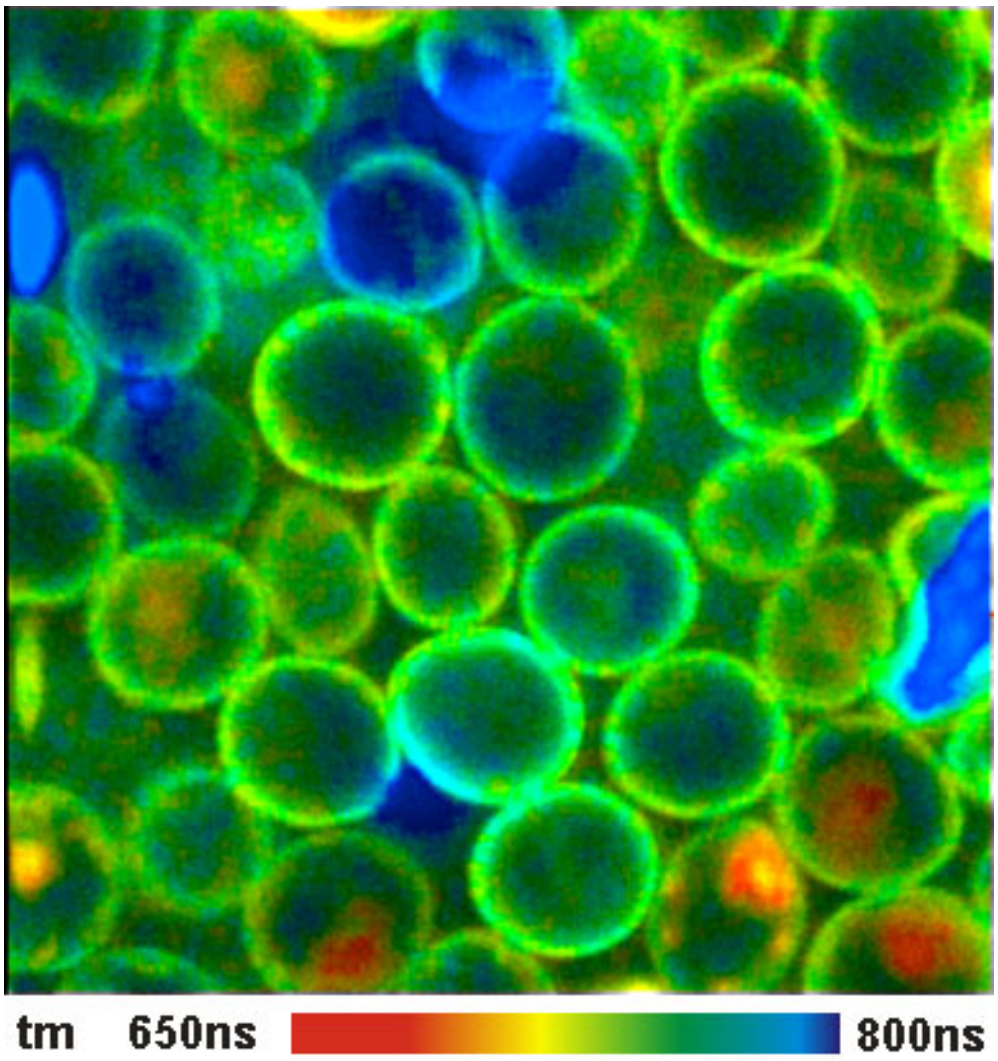
Stimulated Emission Depletion (STED) microscopy exploits the nonlinearity of stimulated emission to obtain optical super-resolution. The fluorescence excited by the excitation beam in a scanning microscope is depleted by stimulated emission induced by a second (STED) laser beam. The wavefront of the STED beam is manipulated to obtain a diffraction pattern that either has doughnut shape laying in the x-y plane or a dumbbell shape oriented along the z axis. Fluorescence remains un-depleted in the centre part of the doughnut or the dumbbell. Because stimulated emission is highly nonlinear the un-depleted volume can be made considerably smaller than the point-spread function of the excitation beam. By scanning both beams together images with optical super-resolution are obtained.

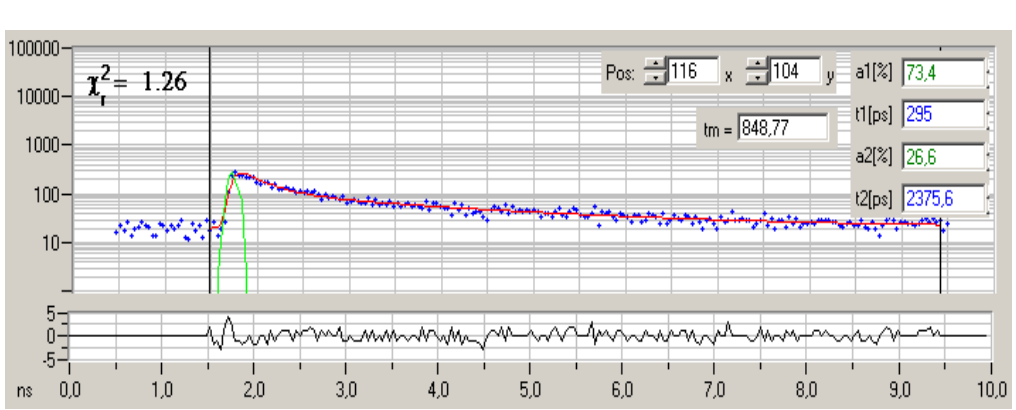
Because STED imaging is a scanning process it can be combined with TCSPC FLIM. The combination of the Abberior STED system with the bh Simple-Tau 150/154 TCSPC FLIM system records FLIM data at a spatial resolution of better than 40 nm. The image format can be as large as 2048 x 2048 pixels with 256 time 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. The system especially benefits from Windows 64 bit technology used both in the Abberior and in the bh data acquisition software, and from the high data throughput of up to four parallel TCSPC FLIM channels.

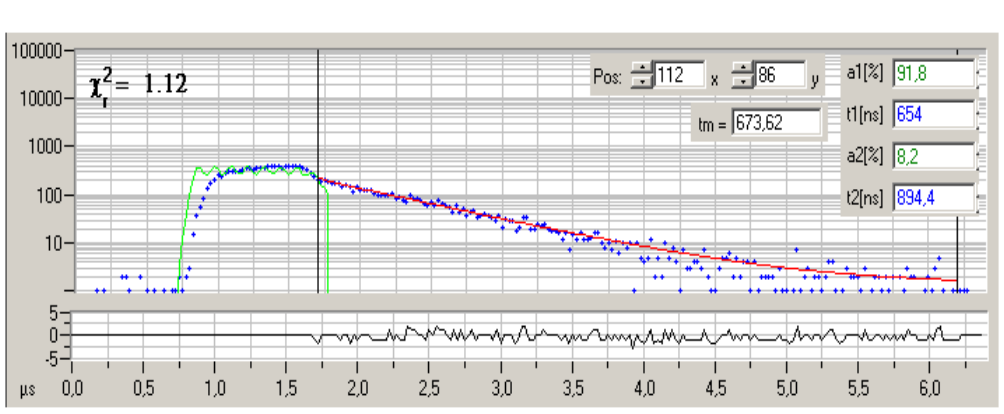
Megapixel FLIM Technology

Simultaneous FLIM - PLIM









Images page 27

Yeast cells stained with a Ruthenium dye, autofluorescence FLIM and PLIM image

Imaging Conditions: Excitation with 405 nm ps diode laser, pulse period 20 ns, modulation period 20 µs, FLIM recorded at 440 to 480 nm, PLIM recorded at 600 to 700 m.

Instrument: DCS-120 Confocal FLIM System

Phosphorescence Lifetime Imaging

There is a number of radiative relaxation mechanisms which occur on a much longer time scale than fluorescence. The commonly known one is phosphorescence, i.e. emission from the triplet state of organic dyes. Phosphorescence is usually weak at room temperature. However, strong phosphorescence emission is obtained for organic complexes of ruthenium, platinum, terbium, and palladium. Of special interest for live-cell imaging is that phosphorescence is strongly quenched by oxygen. The phosphorescence lifetime is therefore used as a sensing function for the local oxygen concentration, or oxygen partial pressure, pO2.

Oxygen measurement is especially important for metabolic imaging. The metabolic state of cancer cells and normal cells is different, but it also depends on the availability of oxygen. pO2 measurement therefore has to be performed simultaneously with NAD(P)H FLIM.

The task is solved by modulating the excitation laser of a FLIM system at a period in the microsecond of millisecond range. A FLIM image is obtained from the photon times in the laser pulse period, a FLIM image from the times in the modulation period.

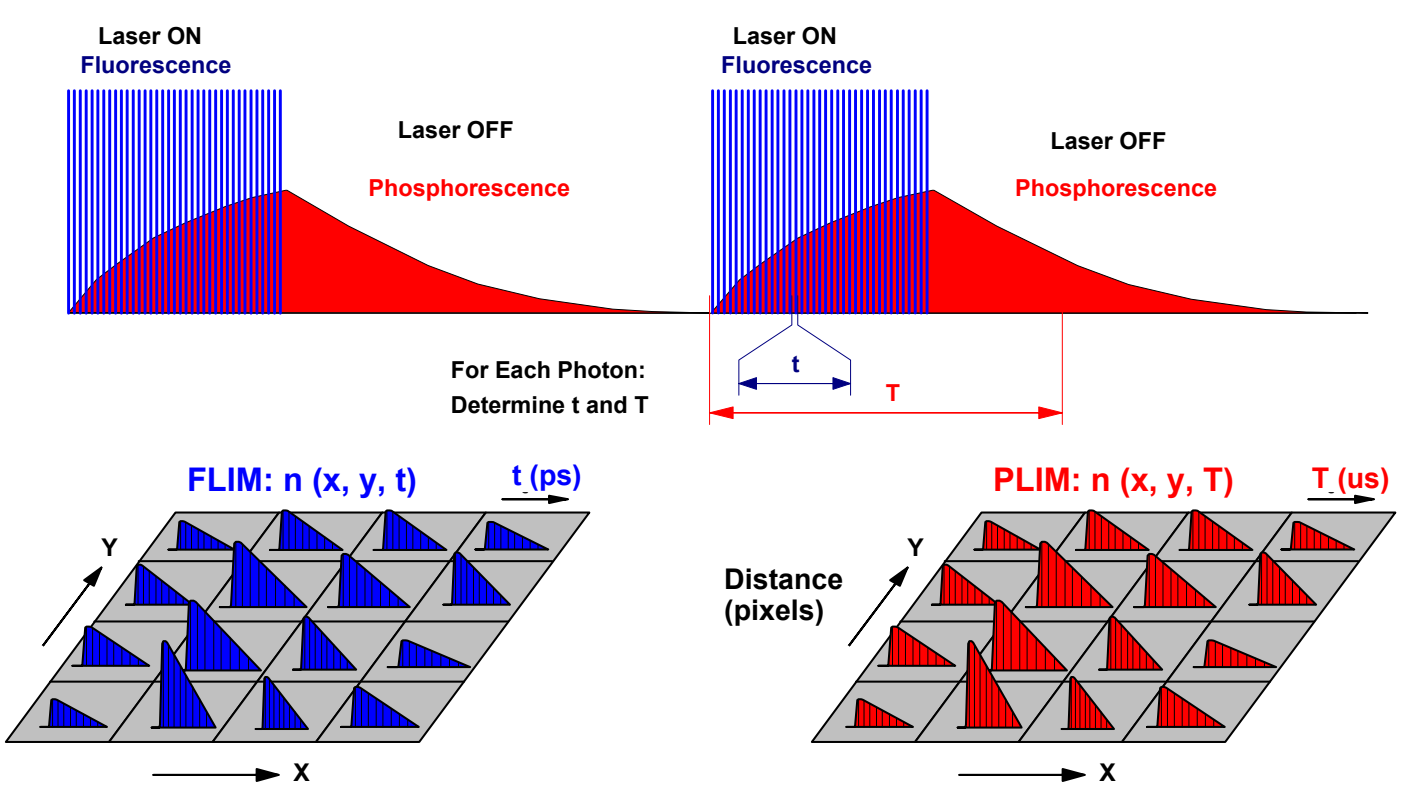


Fig. 9: Simultaneous FLIM and PLIM

Megapixel FLIM Technology

Temporal Mosaic FLIM - Recording of Chlorophyll Transients

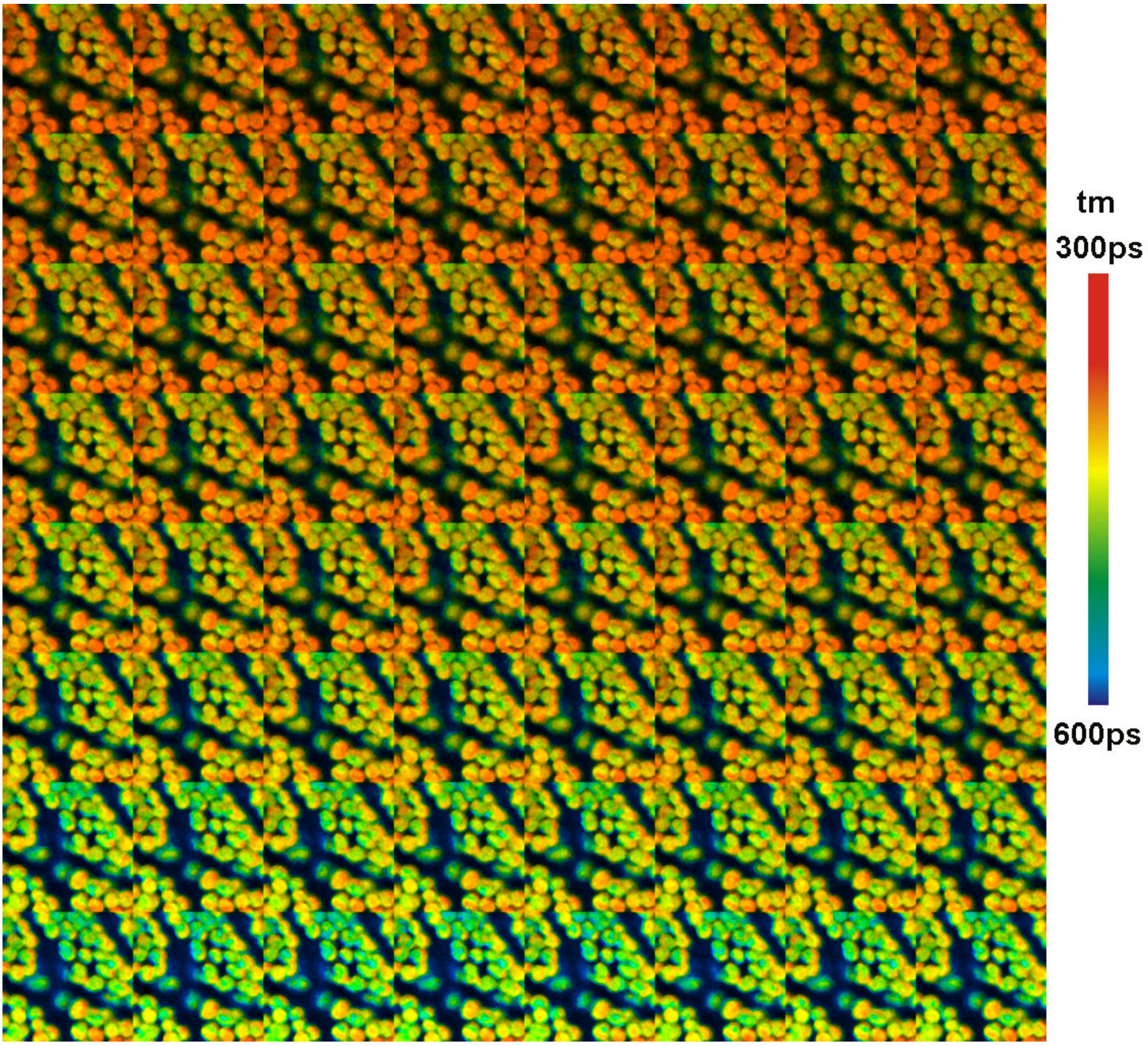


Image page 29

Moss leaf, decrease in fluorescence lifetime of chlorophyll with time of exposure.

Imaging Conditions: Excitation 470 nm, Mosaic FLIM with 64 elements, acquisition time per element 1 second, 128 x 128 pixels per mosaic element, 256 time channel.

Instrument: DCS-120 System Confocal FLIM System

Temporal Mosaic FLIM

Temporal Mosaic FLIM records a series of FLIM images into a single, large data array. Subsequent images are recorded in consecutive elements of the FLIM mosaic. The result is a time series, the time step of which is a multiple of the frame time. The entire data set is recorded into a single, large photon distribution. The principle is shown in Fig. 10.

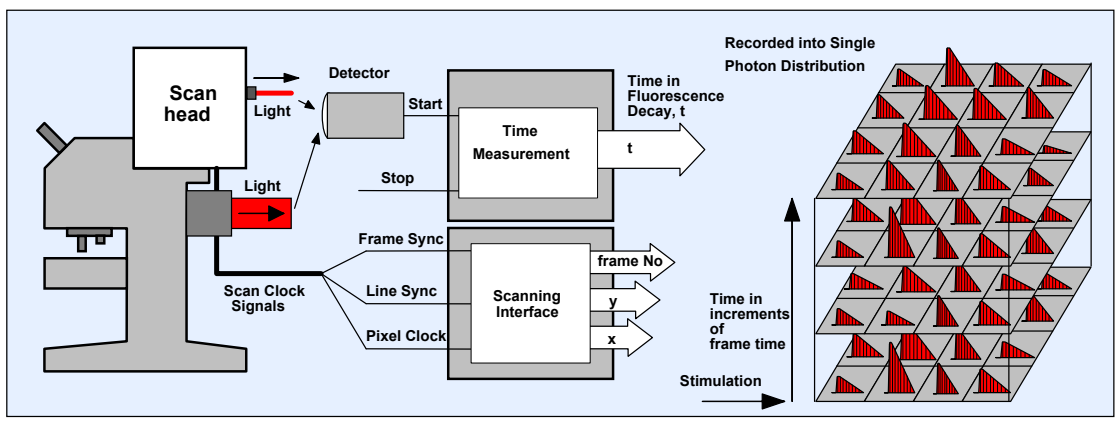


Fig. 10: Temporal Mosaic FLIM

Compared to the conventional record-and-save procedure of time-series recording Mosaic FLIM has several advantages: No time has to be reserved for the save operations, and the data can be better analysed with global-parameter fitting.

The biggest advantage is, however, that mosaic time series data can be accumulated: A sample would be repeatedly stimulated by an external event, and the start of the mosaic recording be triggered by the stimulation. With every new stimulation the recording procedure runs through all elements of the mosaic, and accumulates the photons. Accumulation allows data to be recorded without the need of trading photon number and lifetime accuracy against the speed of the time series. Consequently, the time per step (or mosaic element) is only limited by the minimum frame time of the scanner.

Megapixel FLIM Technology

Recording of Ca^{++} Transient in Live Neurons

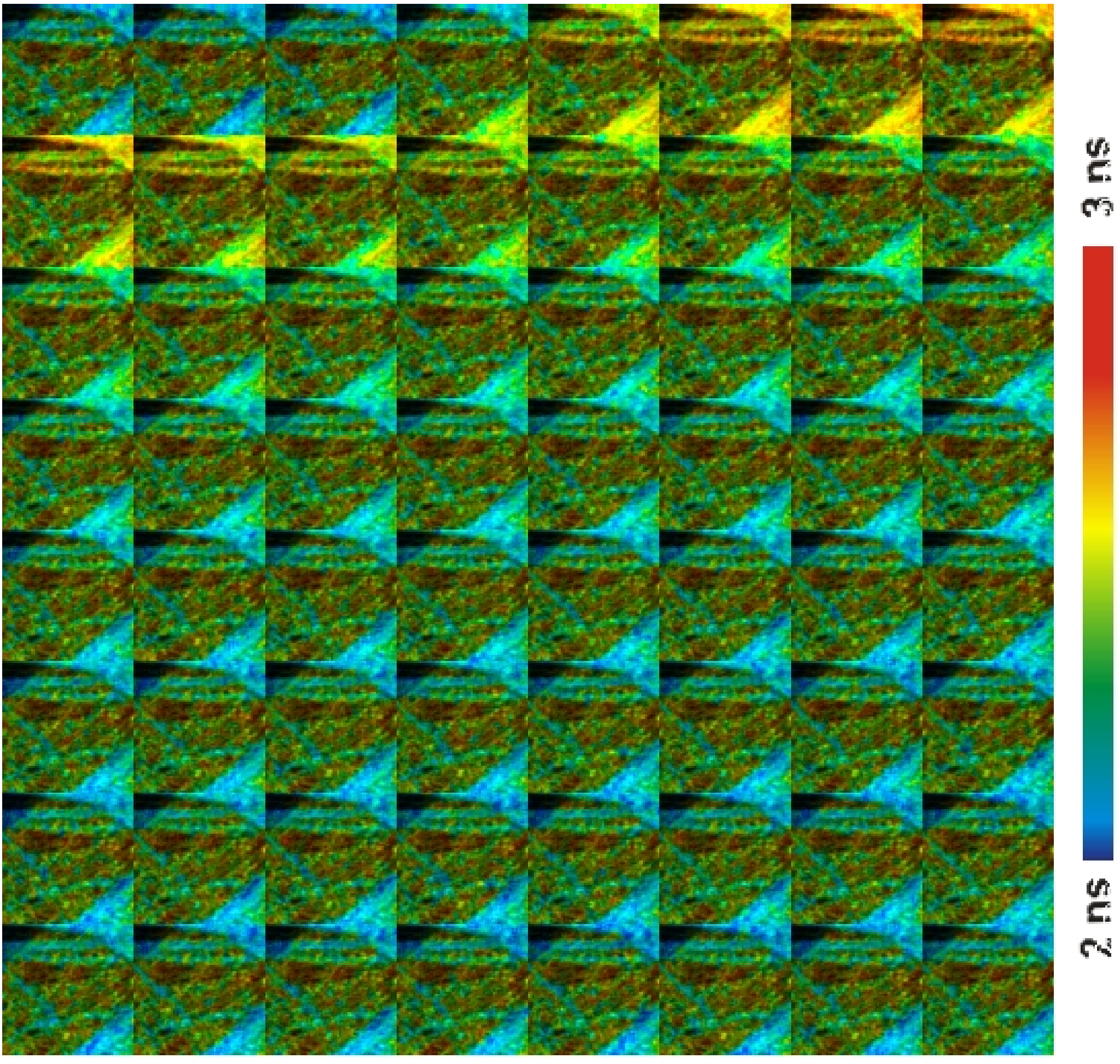


Image page 31

Live neurons stained with Calcium Green Bapta. Change in Ca^{++} concentration after electrical stimulation.

Imaging Conditions: Mosaic FLIM with triggered accumulation, two-photon excitation, 128 x 128 pixels per mosaic element, 256 time channels. Scan time per mosaic element 38 milliseconds.

Instrument: SPC-150 TCSPC FLIM Module, Zeiss LSM 7 MP Multiphoton microscope, Simple-Tau 152 TCSPC system, HPM-100-40 GaAsP hybrid detectors, non-descanned detection.

Calcium Imaging by Temporal Mosaic FLIM

Neuronal activity is accompanied by a change in the concentration of free Calcium ions. The changes occur on a time scale on the order of 50 milliseconds. The Ca^{++} concentration can be monitored by a Ca^{++}-sensitive dye. Activity in the neurons is stimulated periodically by electrical pulses. For every stimulation, a series of recordings into a temporal FLIM Mosaic is started. The time per mosaic element must be on the order of 50 ms or faster. To obtain a sufficient singal-to-noise ratio for a sequence this fast the data are accumulated for a large number of stimulation periods. With fast galvanometer scanners the a resolution on the transient-time scale of 40 ms or faster can be reached.

Megapixel FLIM Technology

Clinical FLIM of the Human Eye

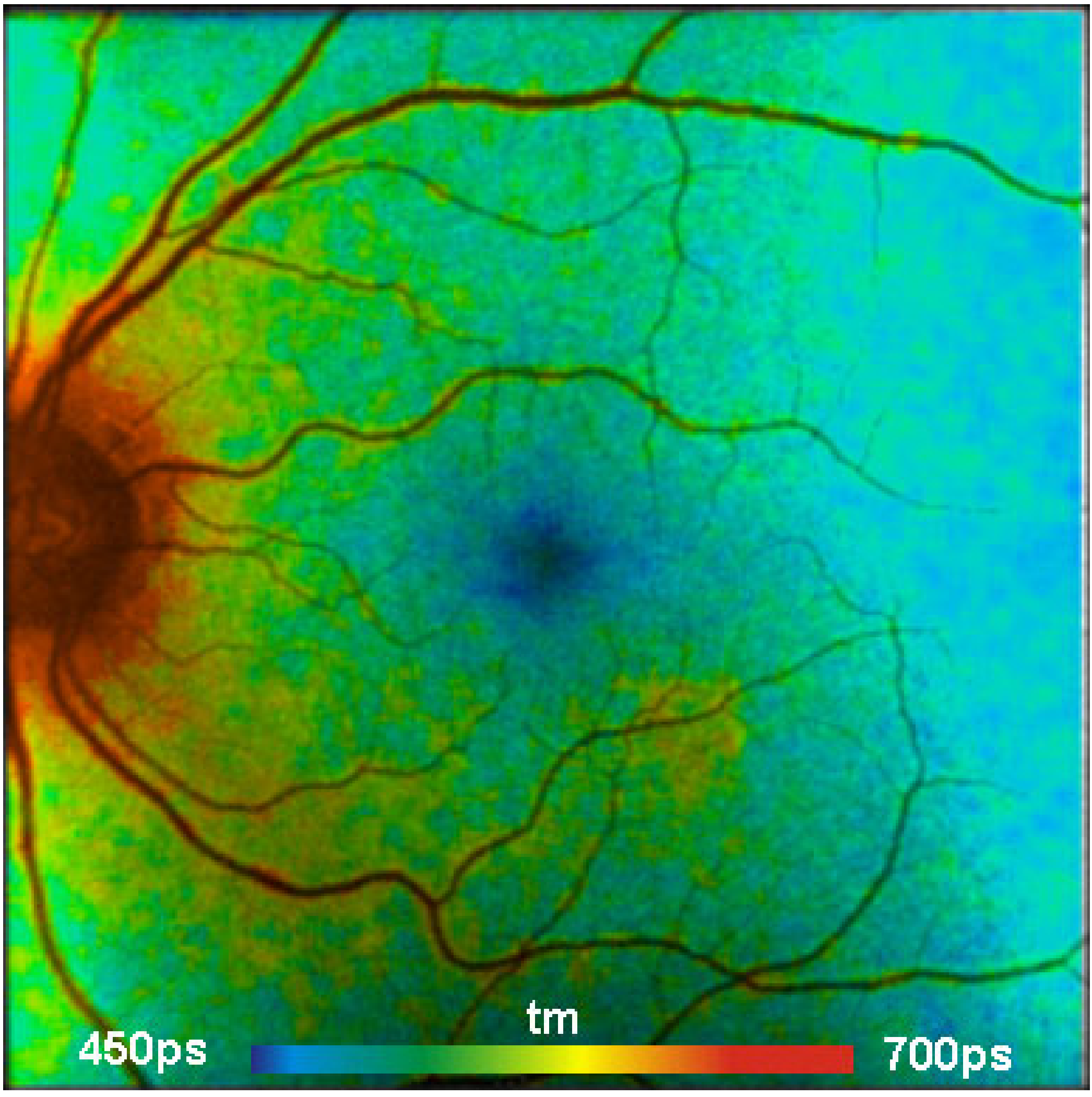


Image page 33

Fluorescence lifetime image of the fundus of a human eye, recorded in vivo.

Imaging Conditions: Excitation 473 nm, detection 560 to 700 nm. 512 x 512 pixels, 1024 time channels.

Instrument: Heidelberg Engineering FLIO Scanner with BDL-SMC ps diode laser and two SPC-150 TCSPC FLIM modules.

Ophthalmic FLIM

TCSPC FLIM is so sensitive that it is able to record lifetime images of the fundus (background) of the human eye. Ophthalmic FLIM uses fast beam scanning in combination with excitation by a picosecond diode laser. The scanner of the Heidelberg Engineering FLIO system uses resonance scanning in x and galvanometer scanning in y. The scan rate is on the order of 16 frames per second. The laser beam is projected into the pupil of the eye through the front lens of the scan head. The fluorescence light returning fro the eye background is descanned, projected into a pinhole, split into two wavelength channels, and detected by two HPM-100-40 GaAsP hybrid detectors. The photon pulses from the detectors are processed in two parallel SPC-150 TCSPC FLIM modules. In parallel with FLIM imaging, infrared reflection images are recorded in short intervals. These images are used to compensate eye motion in the FLIM recording. The principle of the instrument is shown in Fig. 11.

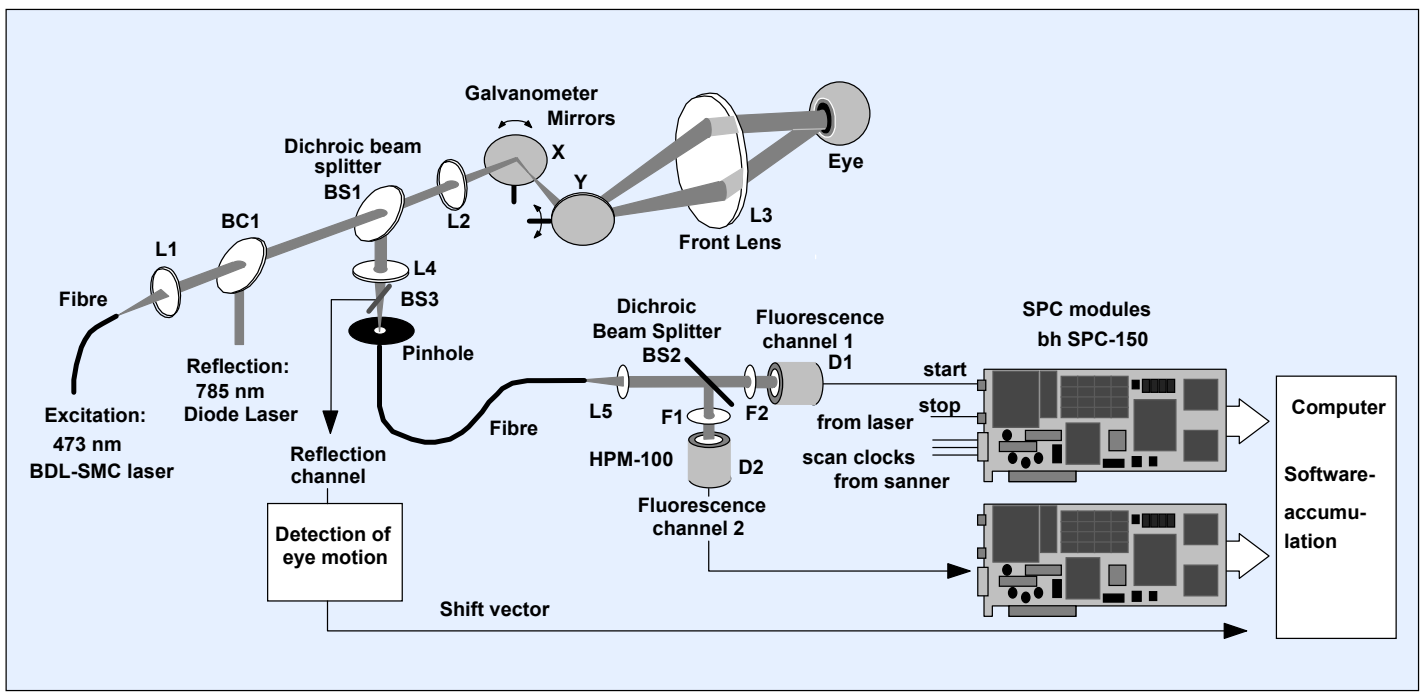
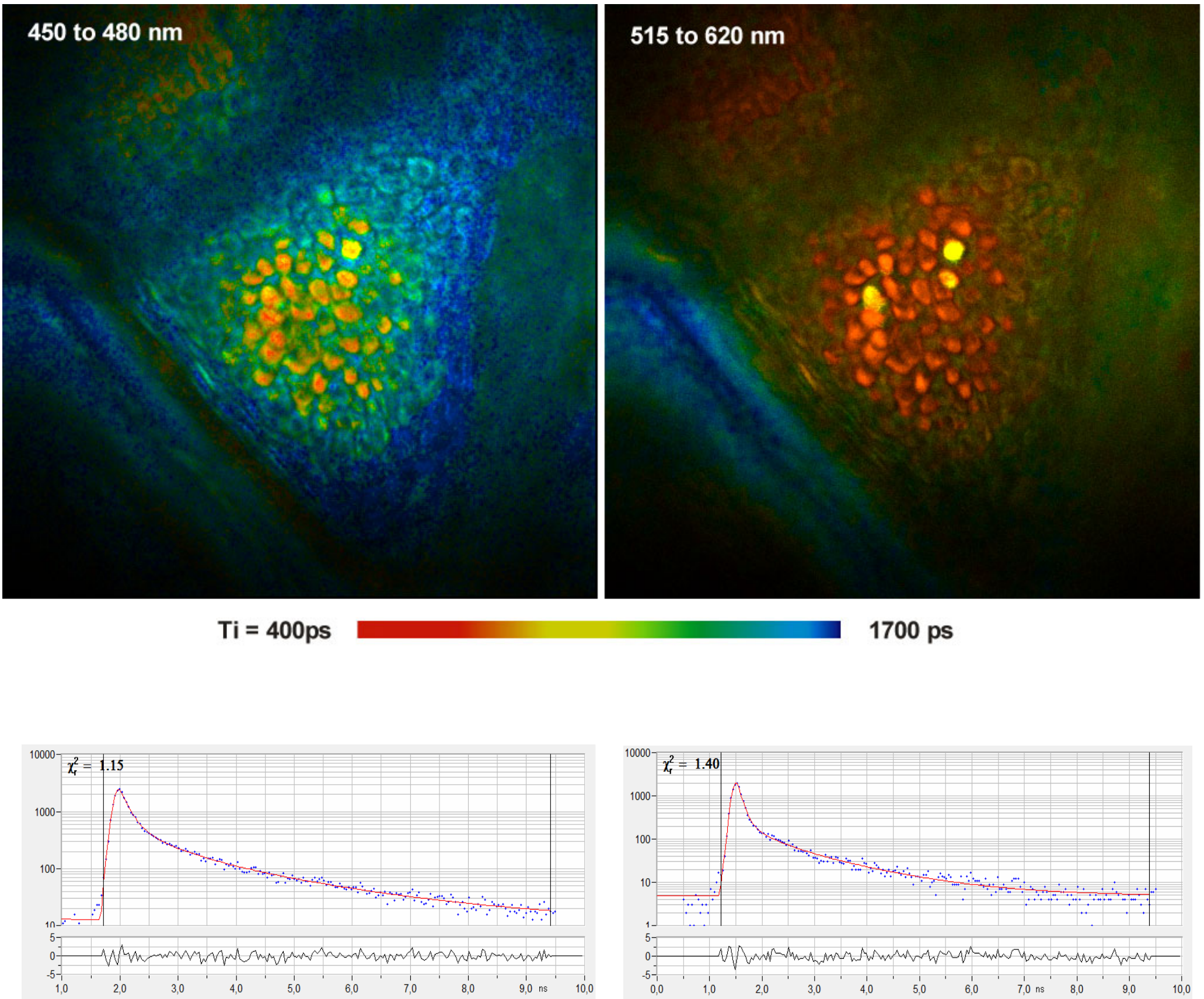


Fig. 11: Principle of the Heidelberg Engineering FLIO system

Early stages of eye diseases are often accompanied by metabolic changes in the fundus of the eye. These, in turn, cause changes in the fluorescence decay parameters of endogenous fluorophores. Ophthalmic FLIM is currently at the sage of clinical tests. The results show that the technique is able to show early stages of eye diseases before these are detectable by conventional methods, and, importantly, before they have caused permanent damage to the retina.

Megapixel FLIM Technology

Multiphoton-FLIM Tomography of Human Skin



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Top: Human Skin at the forearm of a volunteer. Bottom: Decay curves in 5x5 pixel spot of interest.

Imaging Conditions: Two-photon excitation at 750 nm, FLIM data format 512 x 512 pixels, 256 time channels, parallel detection in two wavelength channels.

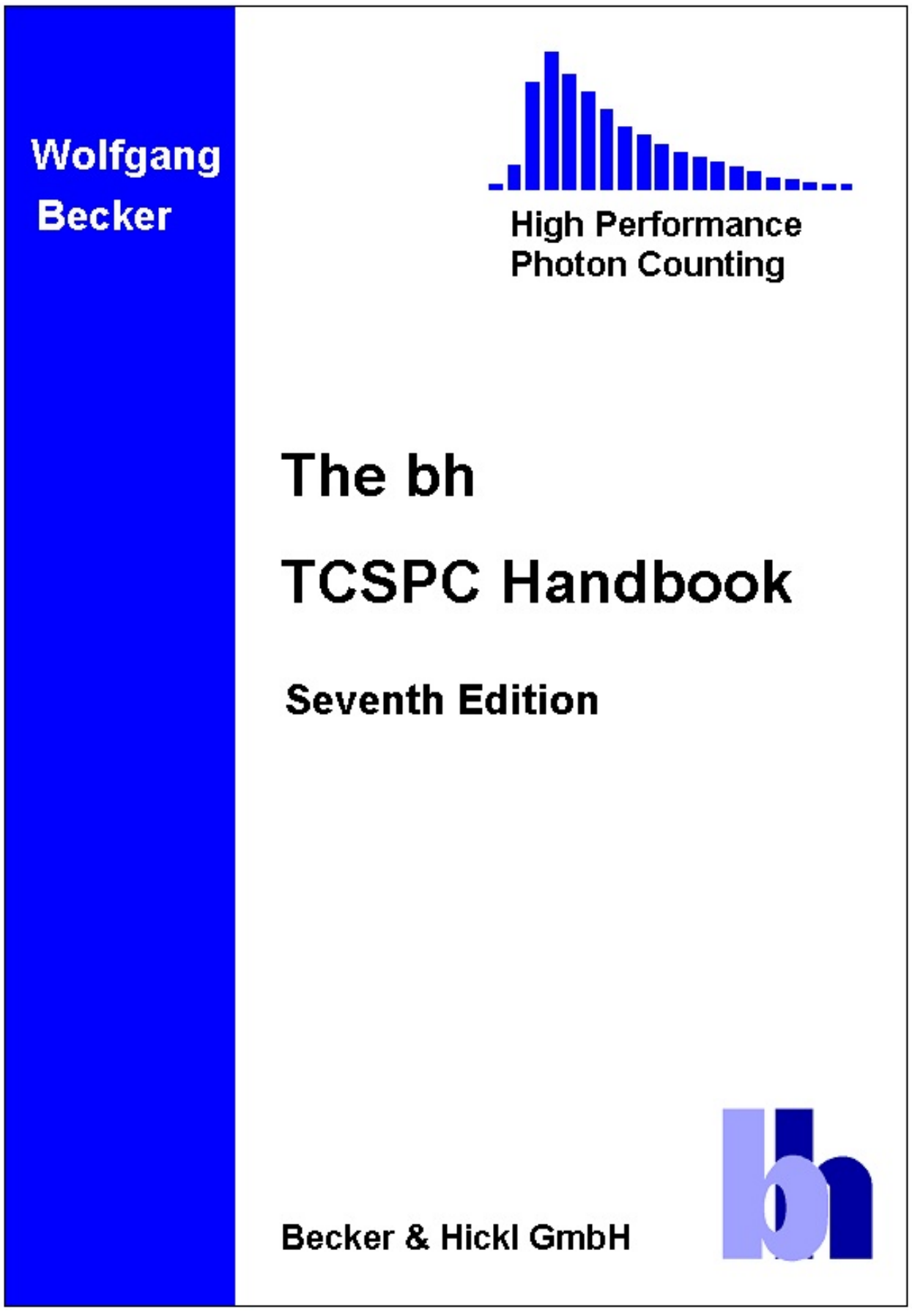
Instrument: Jenlab Dermainspect with three bh SPC-150 TCSPC FLIM modules.

Multiphoton Tomography of Human Skin

Multiphoton tomography of human skin exploits the fact that by two-photon excitation in combination with non-descanned detection delivers optically sectioned images of tissue layers as deep as 100 µm. The tree-dimensional tissue structure can be reconstructed from the data at sub-cellular resolution. The technique goes back to the work of Gratton, König, Masters, So and Tromberg who showed that in-vivo two-photon imaging of cells and, especially, human skin is possible without impairing the viability of the skin.

Because the technique is based on scanning it can be combined with TCSPC FLIM. FLIM not only helps identify different fluorophores, it also delivers information on the metabolic state of the tissue. It therefore helps to discriminate between different skin lesions, inflammation, and, especially, between healthy, precancerous, and cancerous tissue.

Megapixel FLIM Technology



891 pages

1155 References

The Standard Book of TCSPC.

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