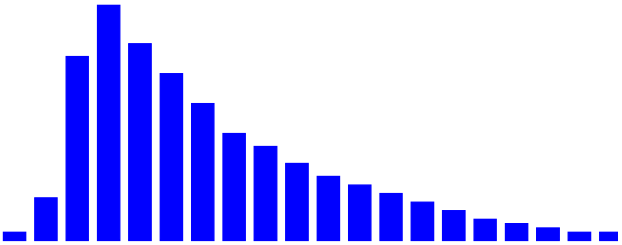

# Becker & Hickl GmbH

## The bh FLIM Technique -

## More than

## Fluorescence Lifetime Imaging

2024

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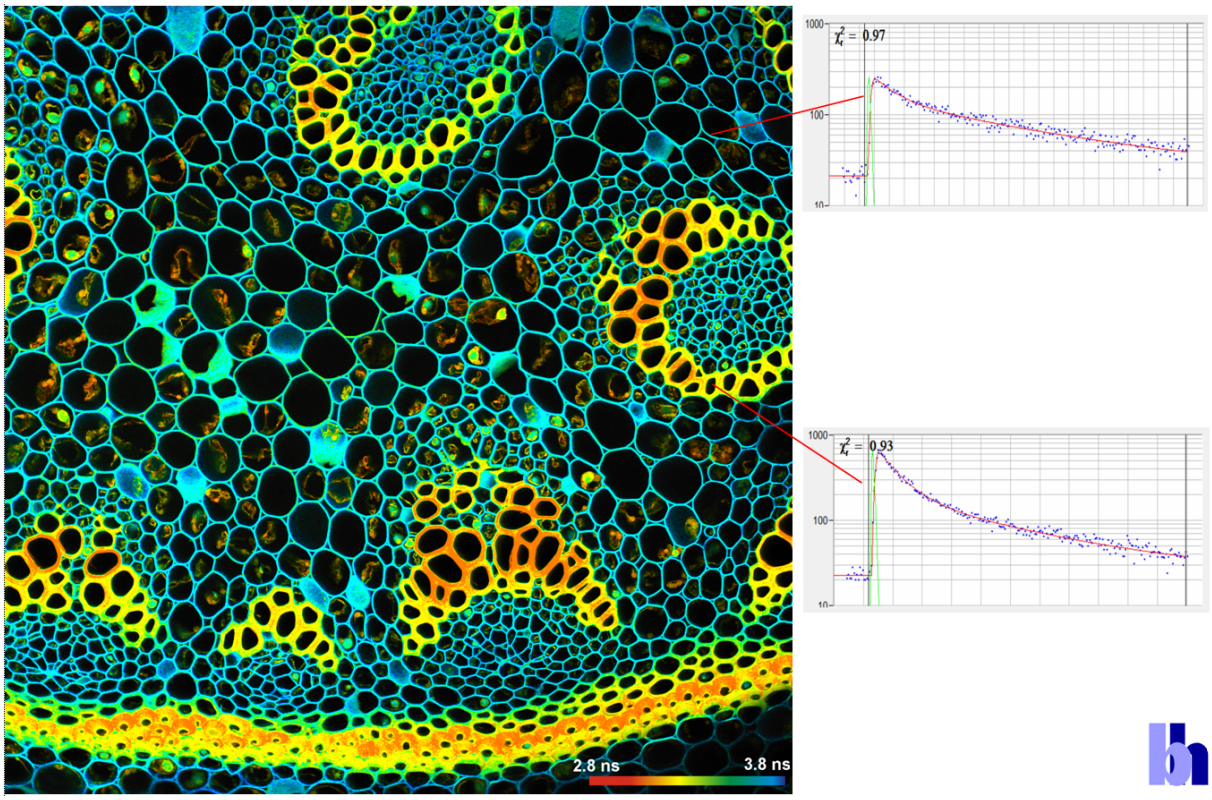
# bh FLIM: More than Fluorescence-Lifetime Imaging

## From Basic FLIM to High-End Molecular Imaging

bh FLIM systems record FLIM images of unprecedented temporal and spatial resolution at an accuracy level close to the theoretical limit given by photon statistics [1, 23]. But bh FLIM systems do more that that: The bh FLIM technique is based on a new understanding of FLIM in general [2]. FLIM is not just considered a way to add additional contrast to microscopy images. It is considered and designed as a molecular imaging technique. bh FLIM exploits the fact that the fluorescence decay function of a fluorophore is an indicator of its molecular environment, and that multi-exponential decay analysis delivers molecular information, such as the metabolic state of live cells and tissues, protein conformation and protein interaction, reaction of cells to drugs and molecular environment, or mechanisms of cancer development and cancer progression. To reach this target, bh FLIM systems have features not available by other systems: Compatibility with live-cell imaging, extraordinarily high time resolution and photon efficiency, capability to split decay functions into several components, excitation-wavelength multiplexing in combination with parallel-channel detection, recording of dynamic lifetime effects caused by fast physiological effects, and simultaneous FLIM/PLIM [2]. The most important ones of these features will be described in this brochure.

## Precision Megapixel FLIM Images

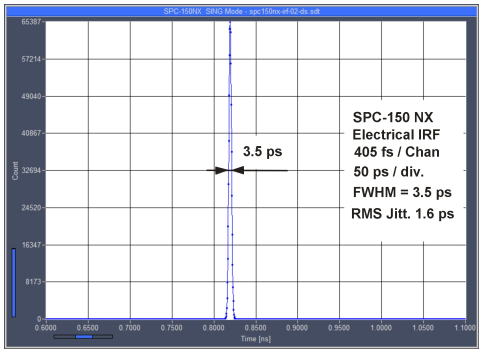
bh FLIM is characterised by spatial resolution in the megapixel range and temporal resolution in the 10-ps range. An example is shown below.

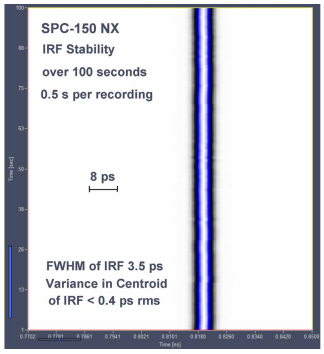


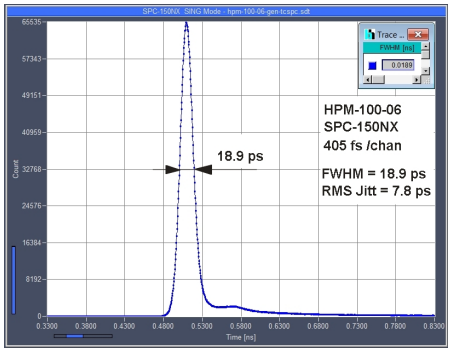
# TCSPC FLIM

## The Ultimate in FLIM Time Resolution and Timing Stability

The electrical time resolution of the bh FLIM modules is 3.5ps fwhm, or about 1.5 ps rms [2]. Timing stability is better than 0.4ps rms. The system IRF of multiphoton systems is <19 ps fwhm, or 8.3 ps rms, including detector and laser. No need to record an IRF for a system this fast!

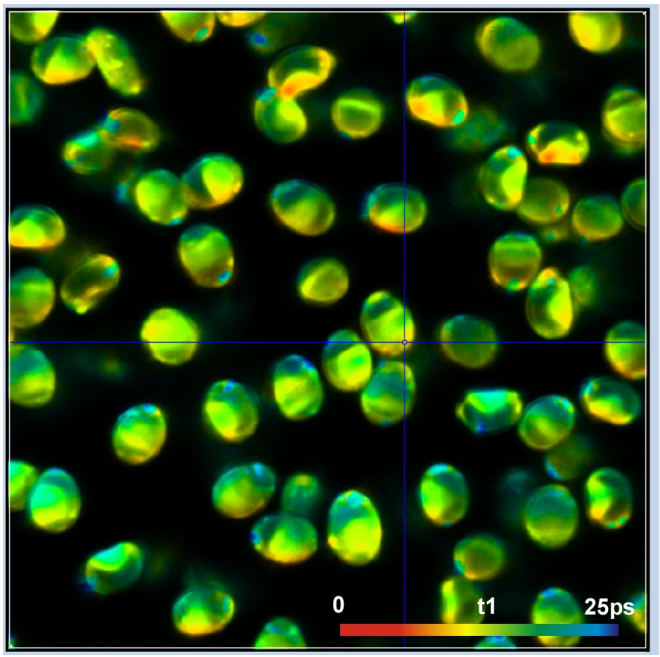


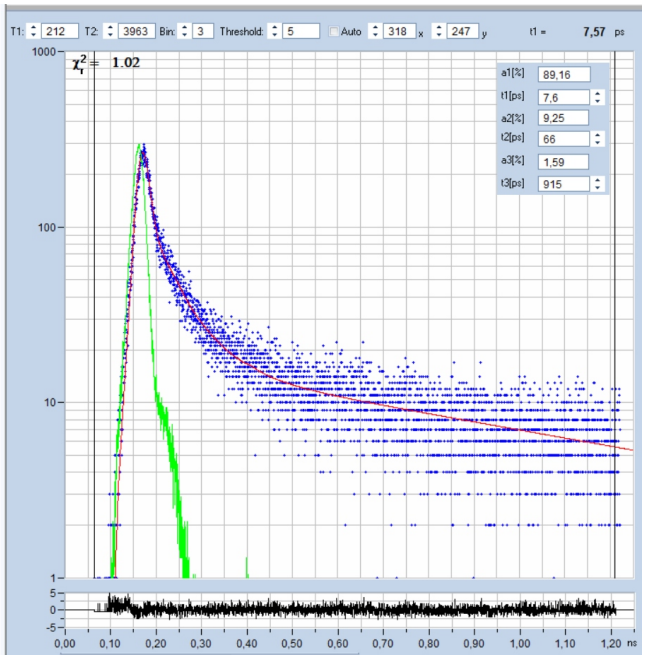




## Ultra-High Resolution FLIM

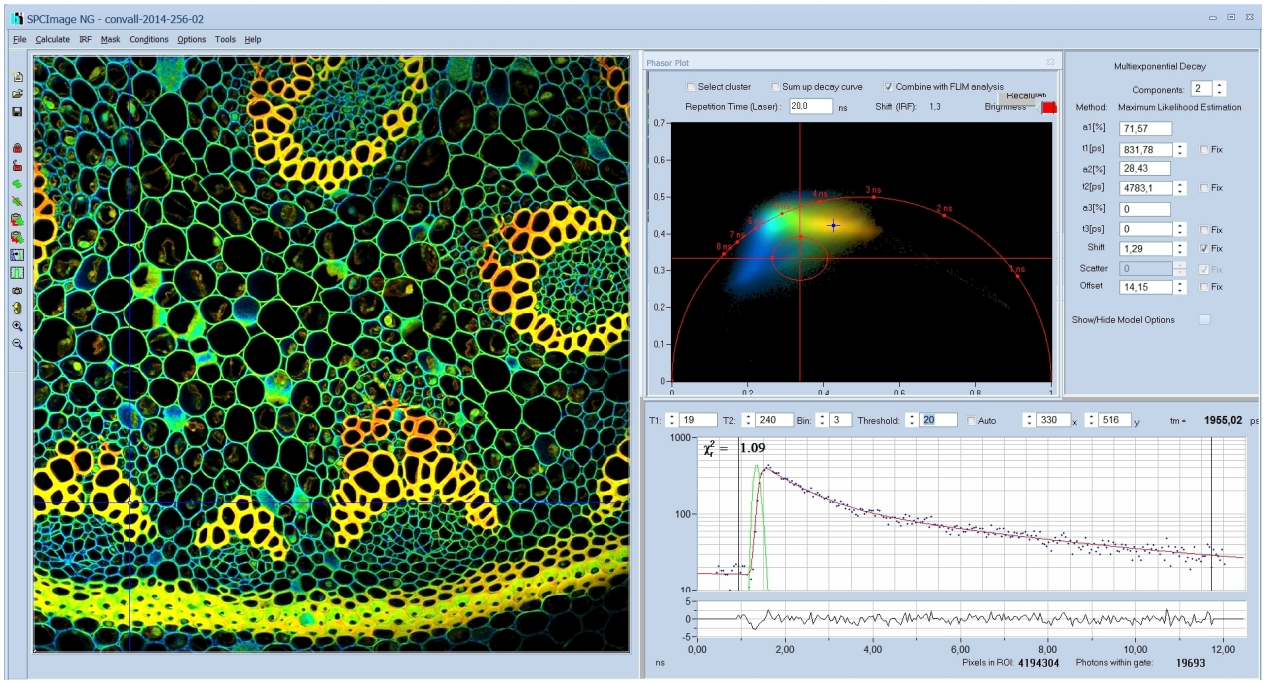
Ultra-short decay times in biological systems are more frequent than commonly believed. They are often considered difficult or impossible to measure. However, lifetimes in the 10-ps range are no problem for bh FLIM systems. The data below were recorded at a time-channel width of 300 femtoseconds, and with an IRF width of 19 ps [7]. The dominating decay component has a lifetime of 7.6 picoseconds.



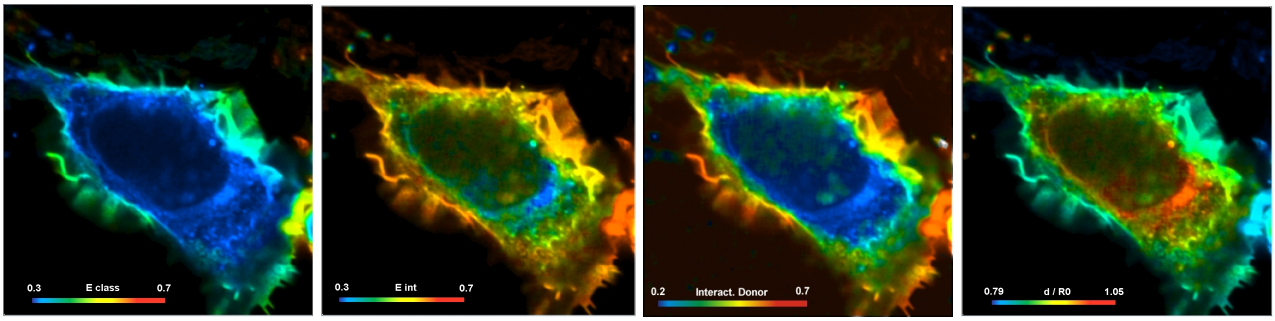
## FLIM Data Analysis by SPCImage NG

Data analysis is an integral part of the bh FLIM systems [2, 24]. GPU processing, MLE fit, multi-exponential analysis, combination with phasor plot, automatic IRF synthesis - these are the main features. Precision multi-exponential decay analysis occurs within seconds, MLE yields high fit stability, no reference measurement is needed, and the combination with phasor analysis allows the user to obtain precision lifetimes for low-intensity data by image segmentation. Biologically relevant parameters, such or FRET intensities and FRET distances, ion concentrations, membrane potentials, and metabolic ratios are directly available from the decay data.



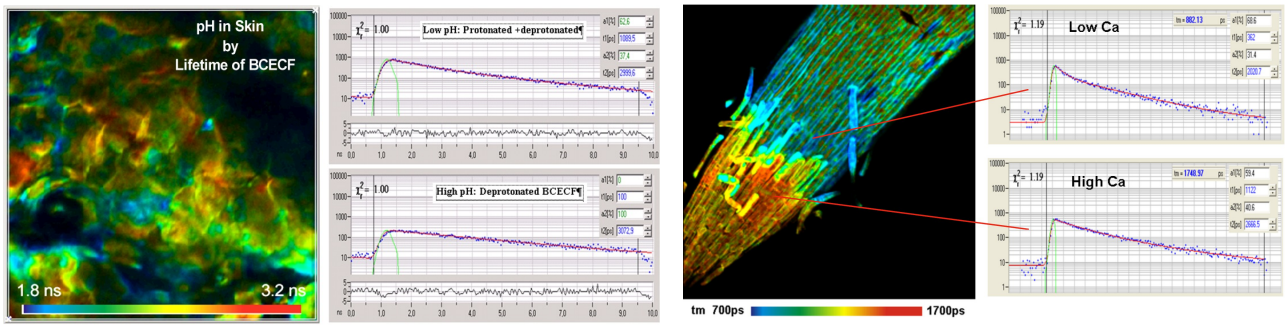

## Protein Interaction - Quantitative FRET Results

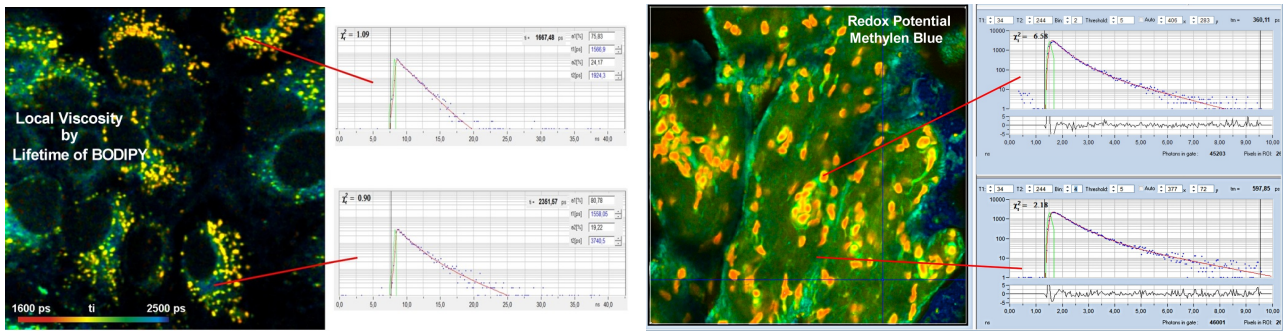
Precision FLIM-FRET is performed by double-exponential FRET analysis. In contrast to single-exponential techniques, the method delivers correct FRET efficiencies and FRET distances [8] even for incomplete donor-acceptor linking, and without reference measurement of a donor-only sample [9]. The classic FRET efficiency, the FRET efficiency of the interacting donor, the amount of interacting donor, and the donor-acceptor distance are displayed directly by SPCImage NG [2].



## Molecular Parameters - Derived from Fluorescence-Decay Data

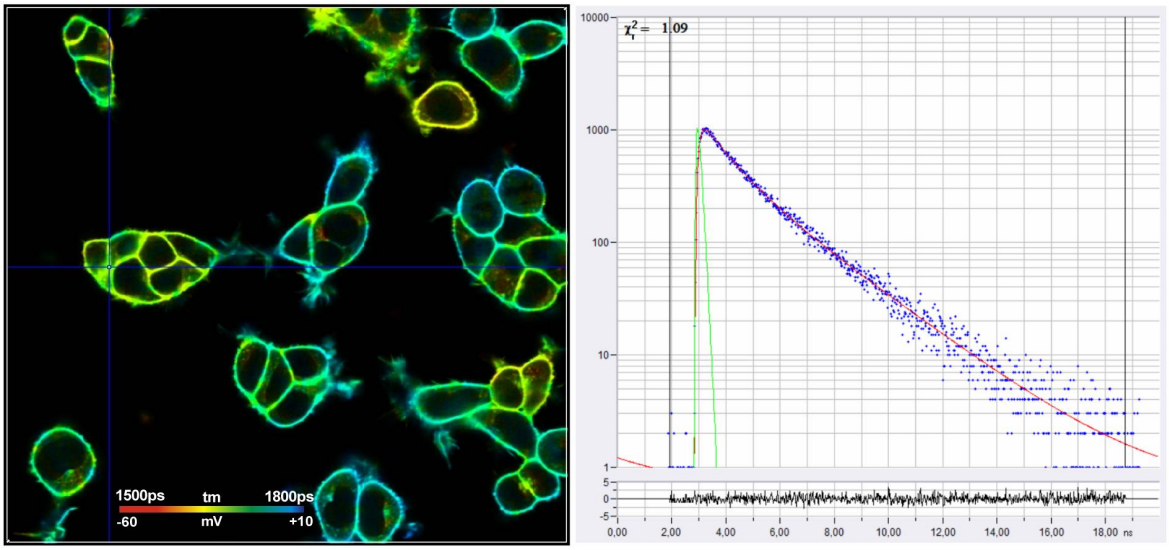
Molecular parameters, such as local pH, ion concentrations, local viscosity or redox potential are available through precision decay analysis [2]. The results are quantitative, i.e. independent of the laser power, the fluorophore concentration, and the parameters of the optical-system. Examples are shown below.



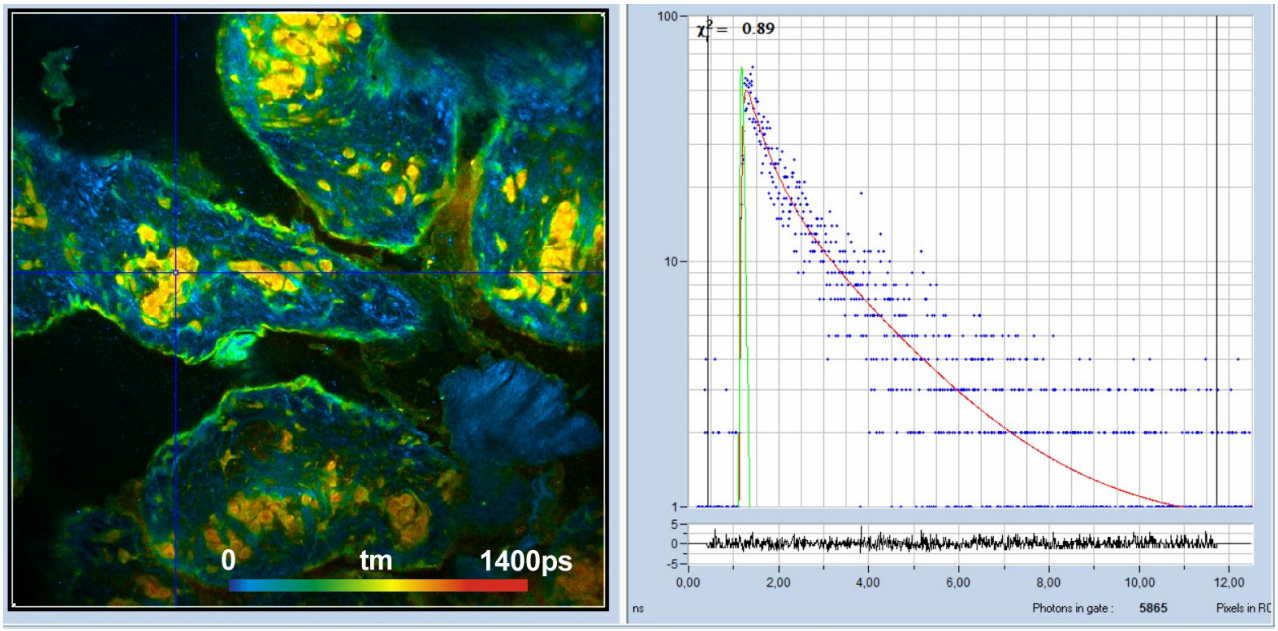

## Membrane Potential

Membrane potentials can be measured by FLIM of voltage sensitive dyes. The lifetime change over the physiological range of membrane potentials is not very large but can well be resolved by bh's TCSPC FLIM systems [10].



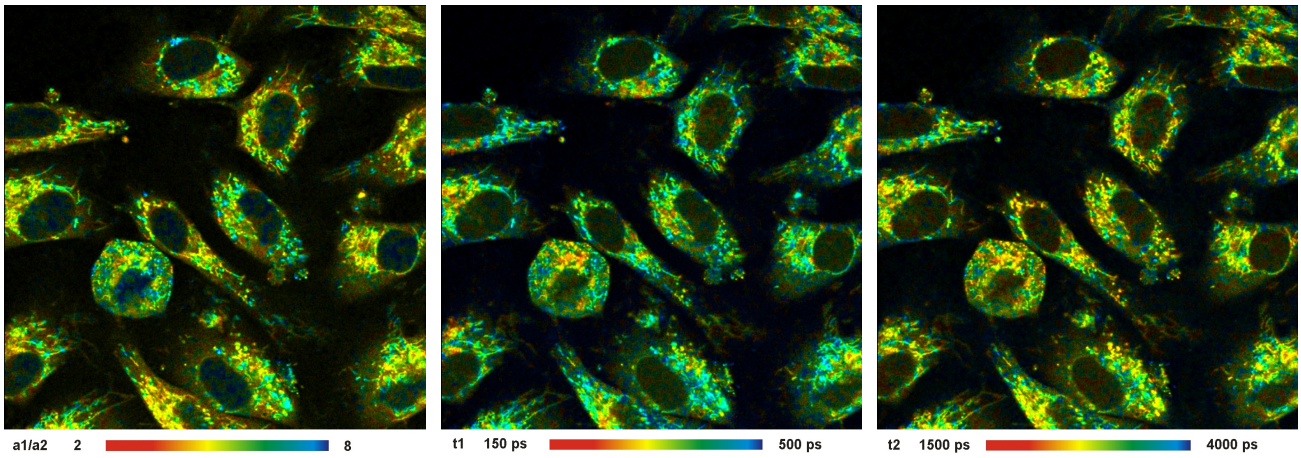
## Label-Free Multiphoton Imaging of Cells and Tissues

Use high-resolution multiphoton FLIM to record label-free FLIM from deep layers of biological tissue. Benefit from high penetration depth, high image contrast and from the metabolic information contained in the data [2].



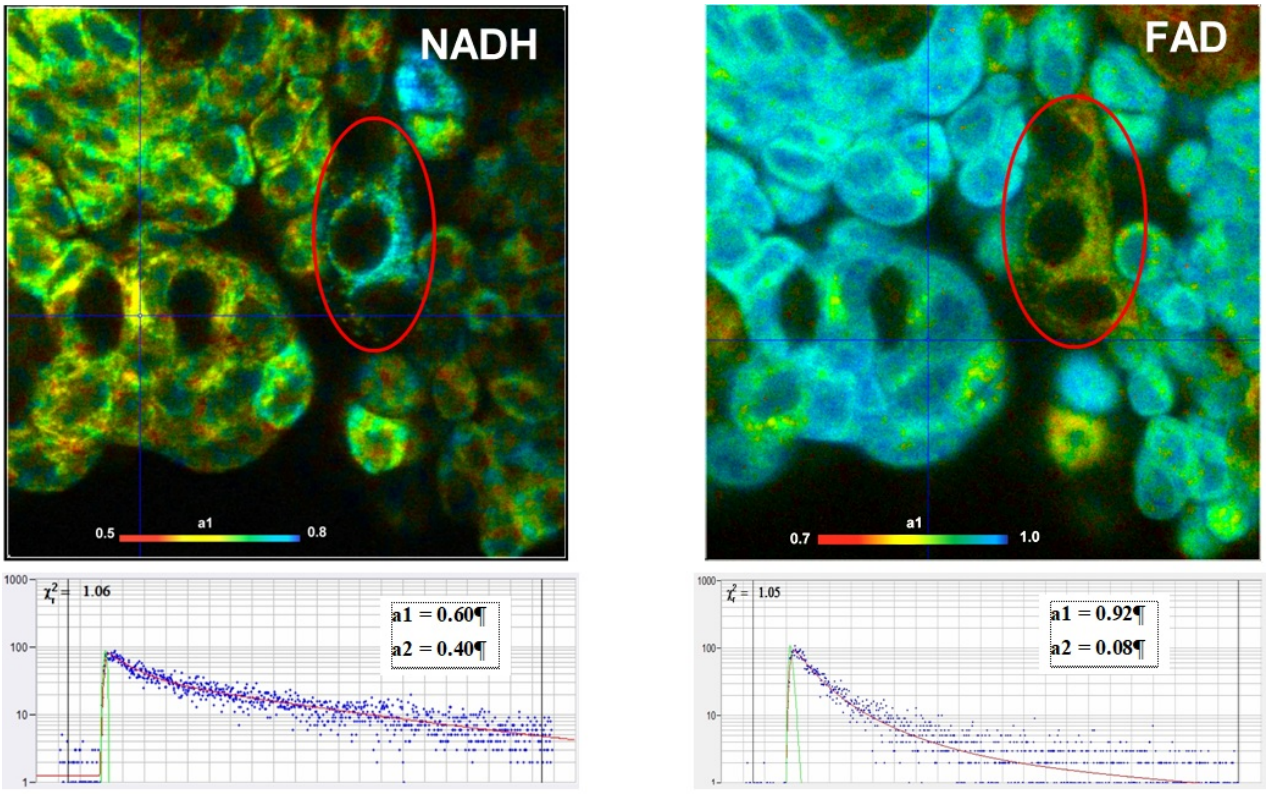

## Metabolic Imaging by FLIM of NADH

Record multi-exponential decay parameters of unbound and bound NADH. Component lifetimes and component amplitudes bear information on the metabolic state of cells and tissues [2].



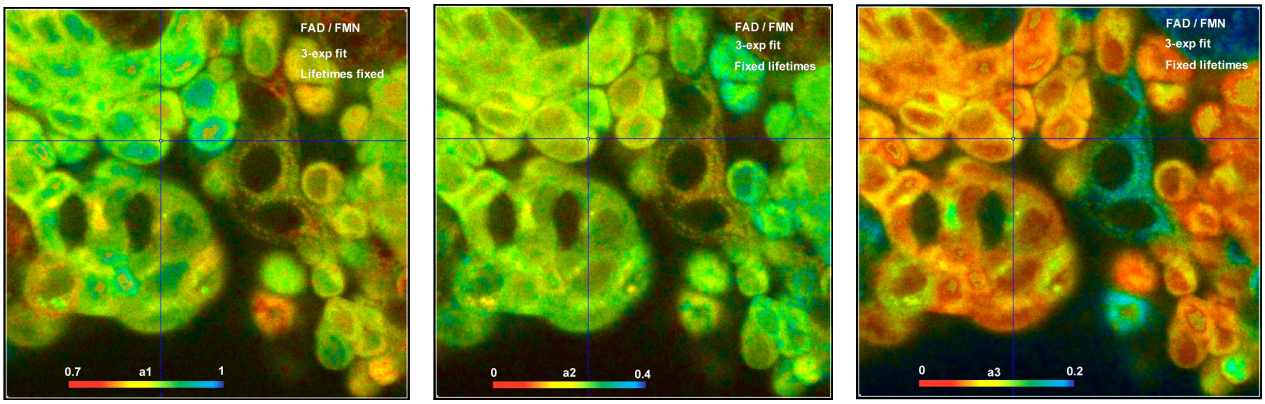
## Metabolic FLIM of NADH and FAD by Laser Multiplexing - Increased Reliability of Tumor Detection

Record Metabolic FLIM by excitation-wavelength multiplexing and simultaneous imaging of NADH and FAD. Benefit from perfect separation of NADH and FAD. Discriminate tumor cells from good cells via the amplitudes of decay components [12, 13]. Below: Human bladder cells, amplitude image, tumor cells marked.



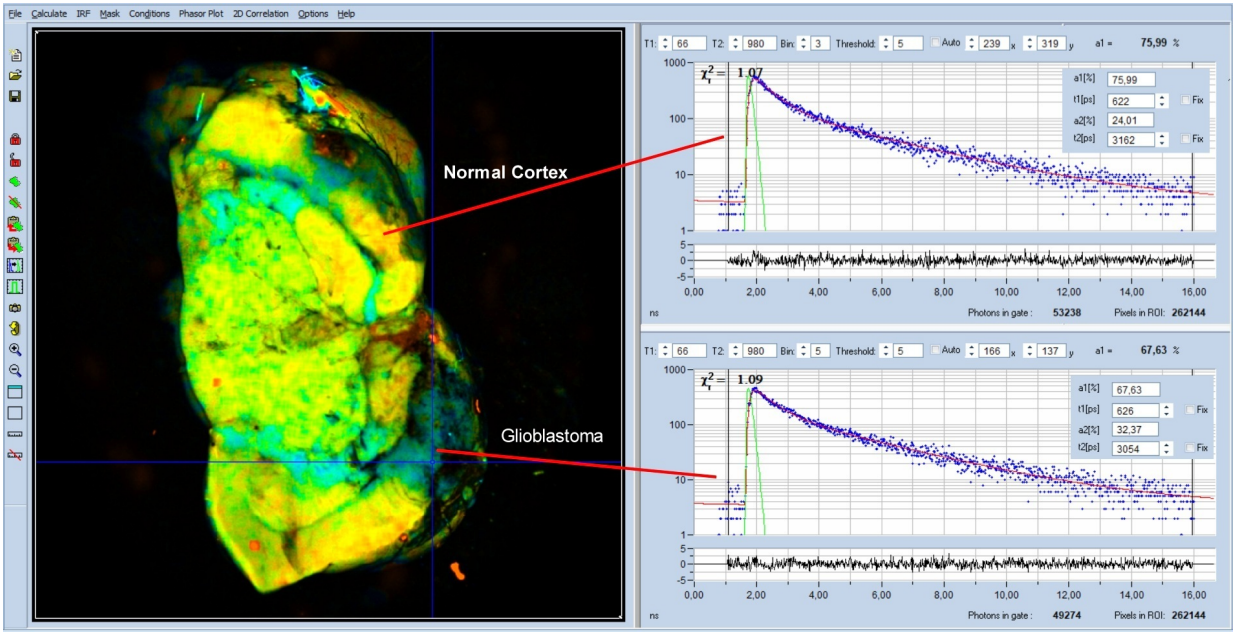

## FMN in Cells

Distinguish FMN from FAD by triple-exponential decay analysis [14]. Below: Relative concentration of bound FAD, free FAD, and FMN in human bladder cells.



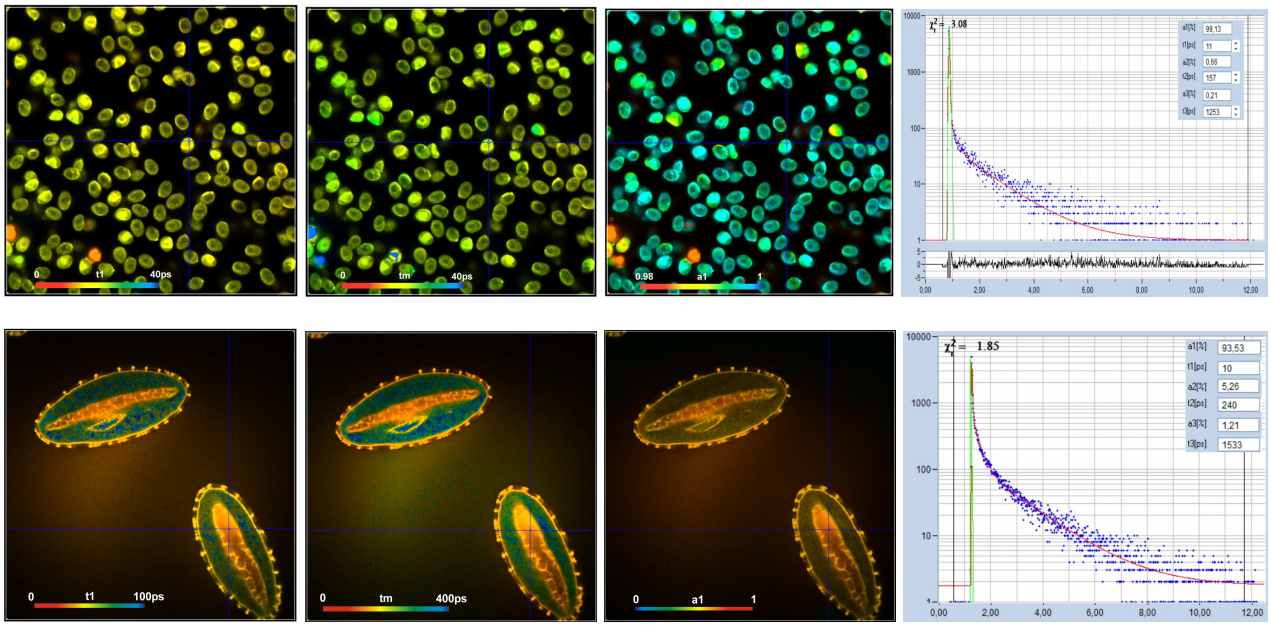
## Metabolic FLIM of Macroscopic Objects

Record metabolic FLIM of macroscopic objects [15]. Below: FLIM image of a whole rat brain. Colour parameter is amplitude of fast decay component, a1, characterising the metabolic state of the tissue [16].



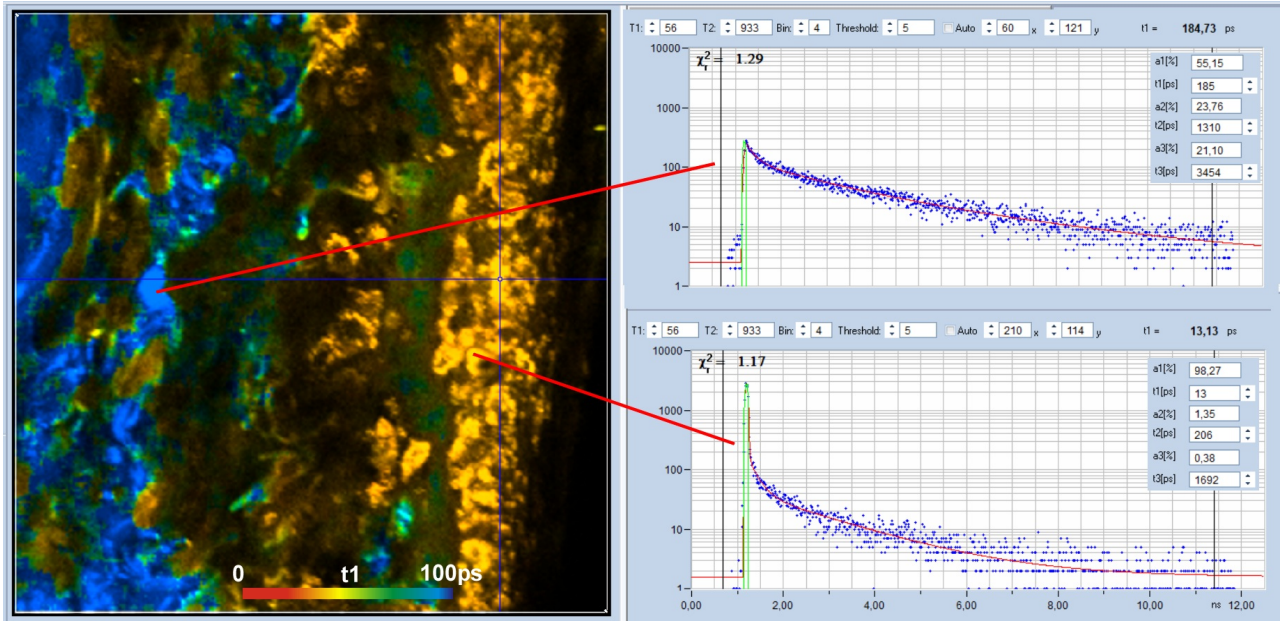

## Ultra-Fast Decay Processes in Biological Material

Explore fluorescence-decay processes which have never been seen before [17, 18, 19]. Below: Mushroom spores and pollen grains, fast decay component of 10 to 11 ps.



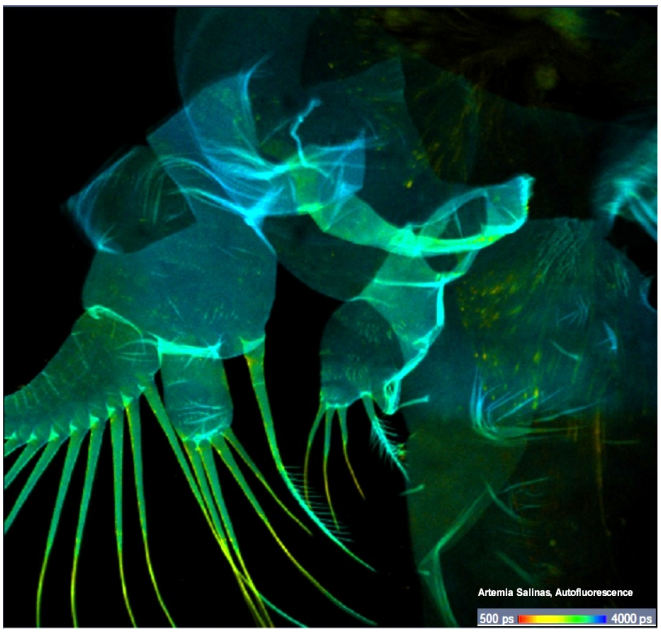
## Ultra-Fast Fluorescence Decay in Malignant Melanoma

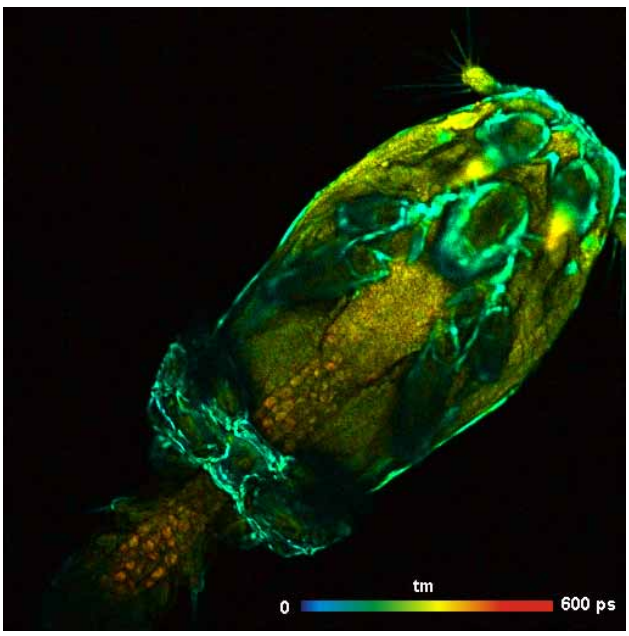
Use ultra-fast FLIM for melanoma detection. Below: Melanoma sample, decay curves of healthy tissue and tumor tissue. The tumor has a fast decay component of 13 ps [20].




## Autofluorescence Imaging of Small Organisms

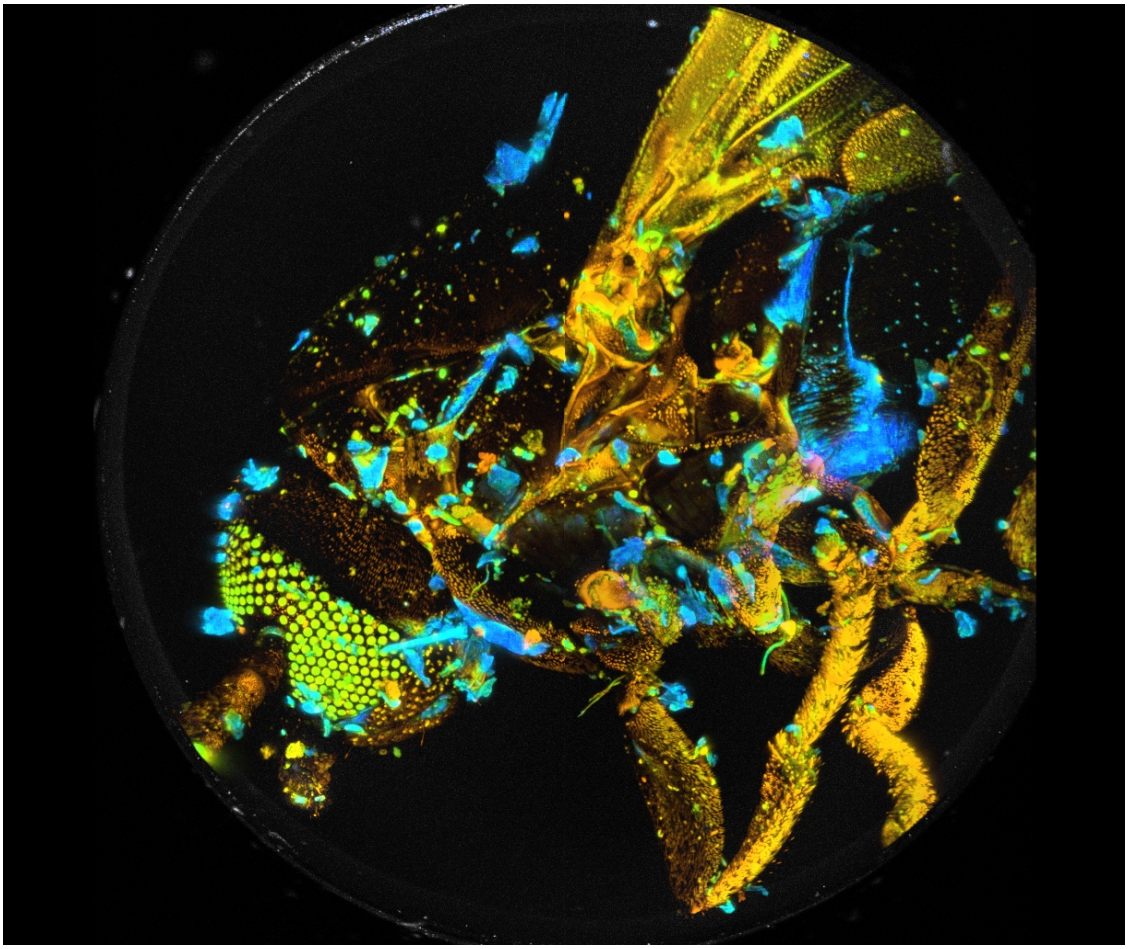
Study environment effects on small organisms by recording autofluorescence. Benefit from the fact that FLIM parameters are sensitive to the metabolic state.



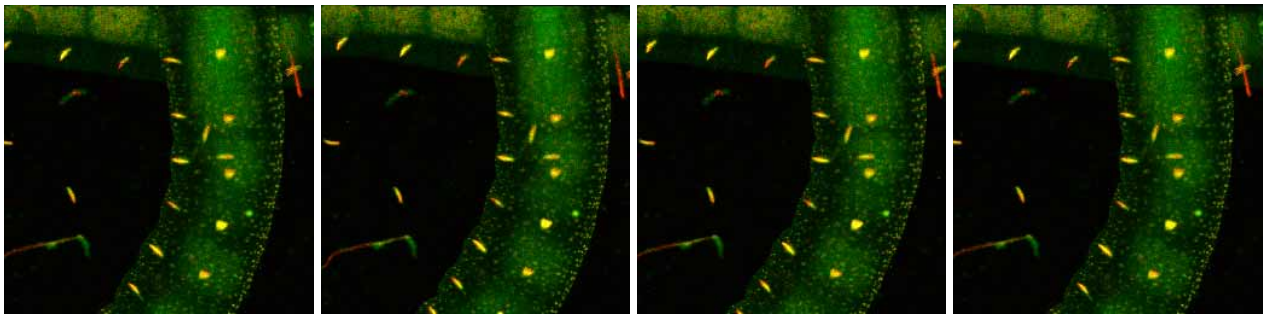
## High-Resolution Z Stacks

Resolve fluorescence dynamics through the entire depth of small organisms. Benefit from high spatial and temporal resolution. Please see [21] for details.



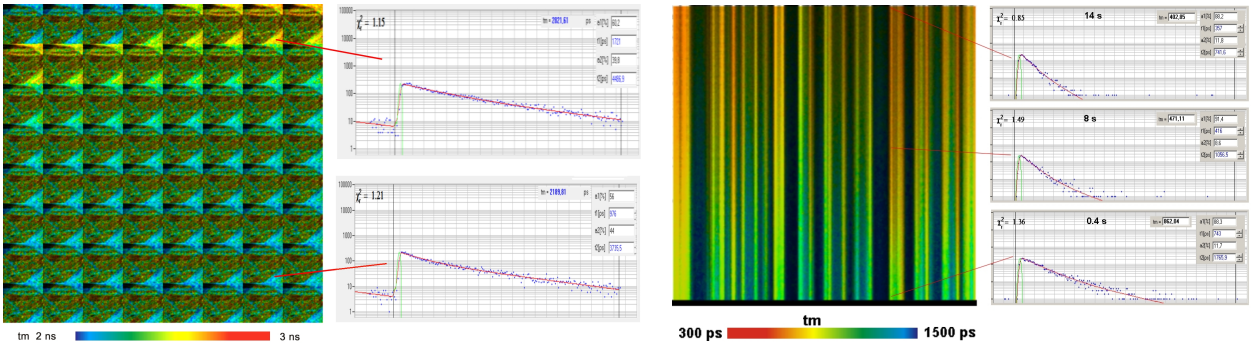
## Express FLIM: Video Sequences from Dynamic Objects

Record video sequences from dynamically changing objects. Below: FLIM sequence from Enchytraeus albidus, 5 frames per second.



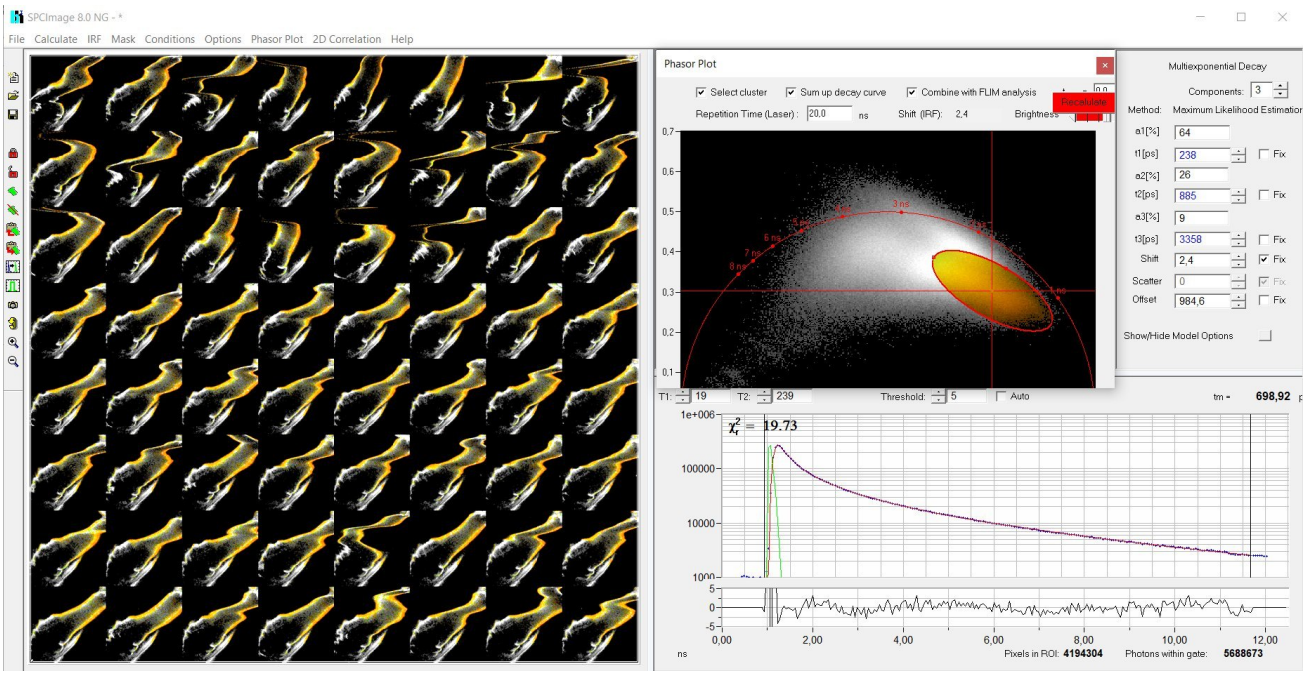
## Triggered Accumulation of Time Series - Recording of Fast Physiological Effects

Record FLIM data of fast physiological effects as fast as the scanner can run [2, 22]. Below, left: Calcium transient in cultured neurons, temporal mosaic imaging, 40 ms per image. Right: Chlorophyll transient, line scanning, 0.5 s per line.



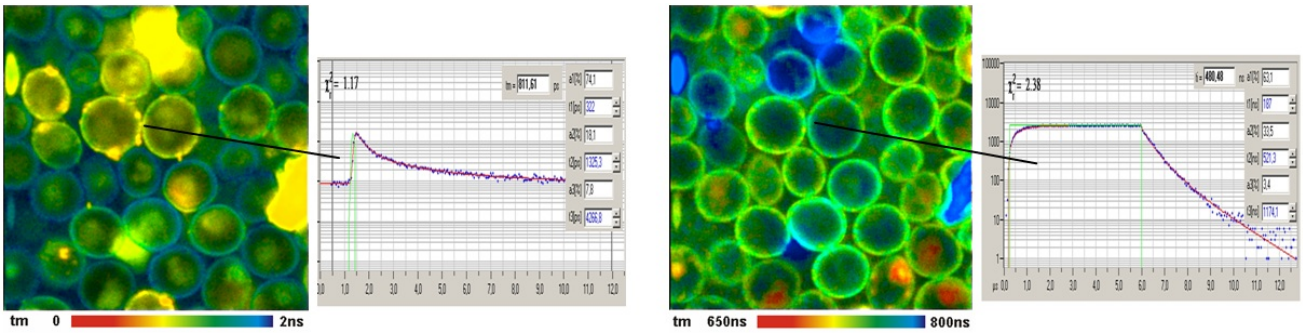

## Temporal Mosaic FLIM: Precision Lifetime Analysis of Moving Objects

Record precision decay data from moving objects. Below: Metabolic FLIM on the moving leg of a water flee. bh Temporal Mosaic FLIM with subsequent image segmentation [2, 24]. Precision decay curve shown lower right.



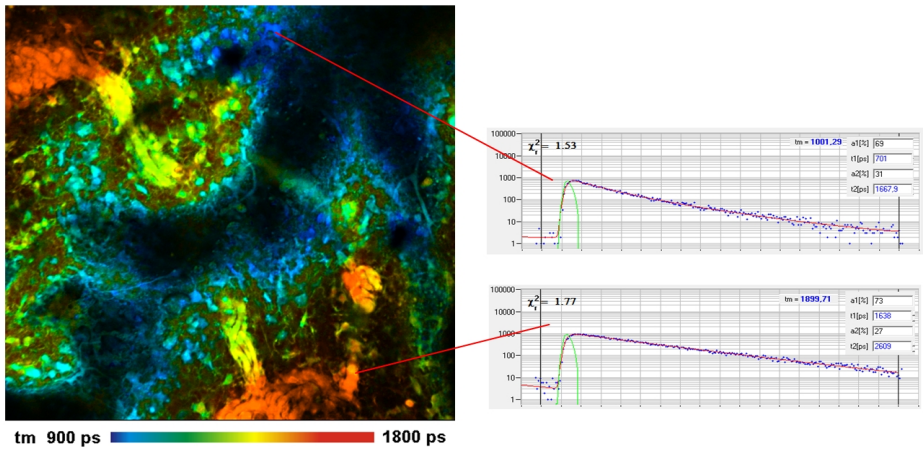
## Simultaneous FLIM / PLIM

bh FLIM systems are able to record fluorescence and phosphorescence simultaneously. Use simultaneous FLIM and PLIM to record the metabolic state of cells in dependence of the oxygen concentration [25, 26].



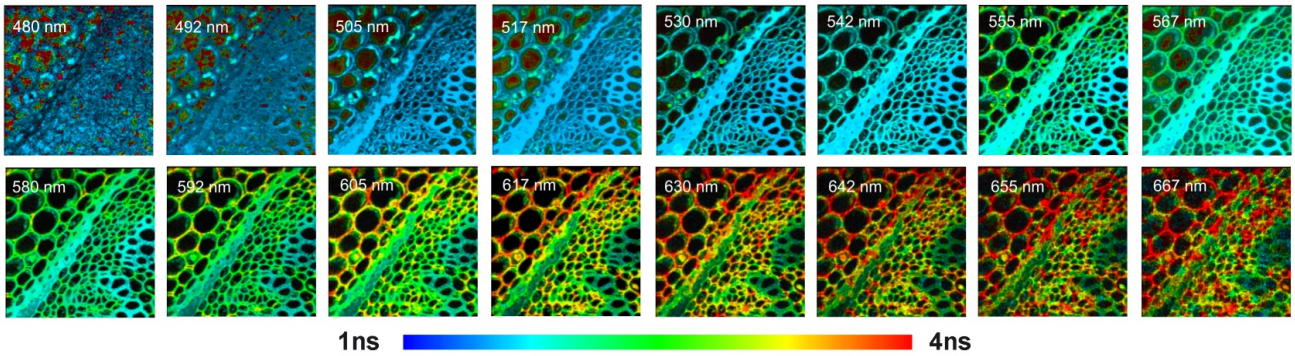

## FLIM with NIR Dyes

NIR dyes often show large variation in their fluorescence decay with the molecular environment [2]. Explore the use of NIR fluorophores as molecular sensors! Below: Pig skin stained with DTTCC.




## Multi-Wavelength Detection

Explore the unexplored: Simultaneous Detection in 16 wavelength channels. Please see [1, 2] for details.



# bh Lifetime Imaging Systems

**DCS-120 Confocal**

Confocal scanning by bh DCS scanner, two ps diode lasers, hybrid detectors, two parallel SPC-180 TCSPC / FLIM channels. Controlled by SPCM software, data analysis by SPCImage NG. Expandable with more lasers and detectors [3].

**DCS-120 Multiphoton**

Multiphoton scanning by bh DCS scanner, excitation by Ti:Sa laser, non-descanned detection, hybrid detectors, two parallel SPC-180 TCSPC / FLIM channels. Controlled by SPCM software, data analysis by SPCImage NG [3].

**DCS-120 Multiphoton Fibre**

Multiphoton scanning by bh DCS scanner, excitation by femtosecond fibre laser, non-descanned detection, hybrid detectors, two parallel SPC-180 TCSPC / FLIM channels. Controlled by SPCM software, data analysis by SPCImage NG [3].

**DCS-120 MACRO**

Confocal scanning in the image plane of a bh DCS scanner. No microscope needed. Image size up to 15 mm, resolution 15 µm. Two ps diode lasers, two hybrid detectors, two parallel SPC-180 TCSPC / FLIM channels. Controlled by SPCM software, data analysis by SPCImage NG [3].

**FLIM System for Zeiss LSM 980 confocal**

Confocal scanning, up to four bh ps diode lasers, two hybrid detectors, two SPC-180 TCSPC FLIM channels. Controlled by Zeiss ZEN software and bh SPCM software. SPCM integrated in ZEN via TCP interface. Data analysis by SPCImage NG [4, 5].

**FLIM System for Zeiss LSM 980 NLO Multiphoton**

Multiphoton scanning by Ti:Sa laser, non-descanned detection, hybrid detectors, two SPC-180 TCSPC FLIM channels. Controlled by Zeiss ZEN software and bh SPCM software. SPCM integrated in ZEN via TCP interface. Data analysis by SPCImage NG [4, 5].

**FLIM System for Zeiss LSM 980 NLO Multiphoton with BIG detector**

Multiphoton scanning by Ti:Sa laser, non-descanned detection by Zeiss BIG detector, recording by two channels of one SPC-QC-104 TCSPC FLIM module. Controlled by Zeiss ZEN software and bh SPCM software. SPCM integrated in ZEN via TCP interface. Data analysis by SPCImage NG [4, 5].

**FLIM System for Leica SP 5 / SP 8 multiphoton with Hyd detectors**

Multiphoton scanning by Ti:Sa laser, non descanned detection by Leica Hyd detectors. Two SPC-180 TCSPC FLIM channels, data analysis by SPCImage NG [2, 6].

**FLIM Systems for other laser scanning microscopes**

Please see The bh TCSPC Handbook [1], available on www.becker-hickl.com.

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