

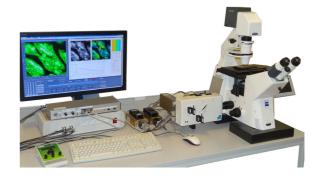
DCS-120 Confocal Scanning FLIM Systems

Based on bh's Multidimensional Megapixel FLIM Technology

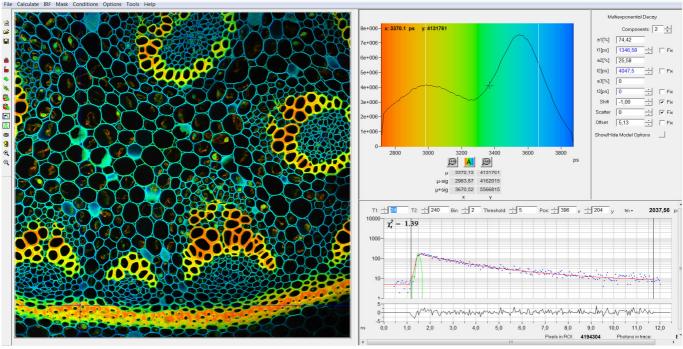
Complete Laser Scanning FLIM Microscopes FLIM Upgrades for Existing Conventional Microscopes

FLIM with up to 2048 x 2048 pixels Decay curves recorded with up to 4096 time channels Time-channel width down to 813 femtoseconds One-photon and multiphoton versions Scanning by fast galvanometer mirrors Two fully parallel detection channels One or two BDL-SMN or BDS-MM picosecond diode lasers Laser wavelengths 375, 405, 440, 473, 488, 510, 640, 685, 785 nm Two confocal detection channels, fully parallel acquisition or Two non-descanned detection channels (multiphoton version) 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 Ultra-high-speed hybrid detectors 16-channel multi-wavelength GaAsP detector module Fully integrated scan, laser, and detector control Z-stack FLIM acquisition with Zeiss Axio Observer Z1 Spatial Mosaic FLIM via motorised sample stage (optional) Online lifetime calculation, online display of lifetime images Simultaneous FLIM and PLIM Fluorescence lifetime-transient scanning (FLITS) Ultrafast time-series recording by temporal mosaic FLIM

Software for Windows 7 and Windows 10, 64 bit









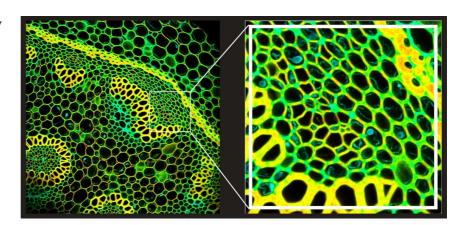
Nunsdorfer Ring 7-9 12277 Berlin, Germany Tel. +49 / 30 / 212 800 20 Fax. +49 / 30 / 212 800 213 email: info@becker-hickl.com

Recker & Hickl GmhH

Confocal Scanning FLIM Systems

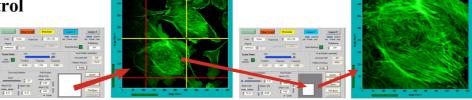
Megapixel FLIM Technology

Extremely large images
Image size up to 2048 x 2048 pixels
Record a large number of cells under
identical conditions
Select cells of interest from recorded



Fast Preview Function Interactive Scanner Control

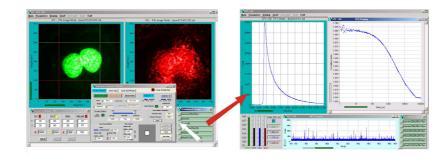
Easy focusing and sample positioning
Easy scan area selection
No stop of scanner required



Easy Change of Instrument Configuration

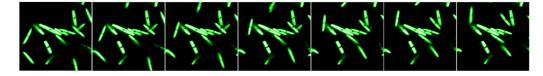
Select system configuration from list of 'Predefined Setups'

Change instrument configuration on a single mouse click



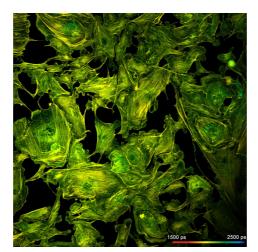
Fast Scanning 0.5 s snapshots every 1 s

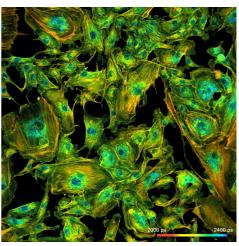
Bacteria catched in flight



Dual-Channel FLIM

Separate filters and pinholes Fully parallel TCSPC channels High data throughput Short acquisition times No amplitude or lifetime crosstalk









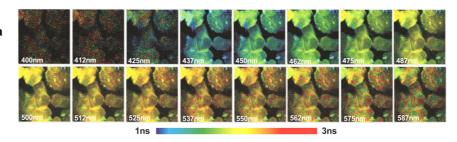
Confocal Scanning FLIM Systems

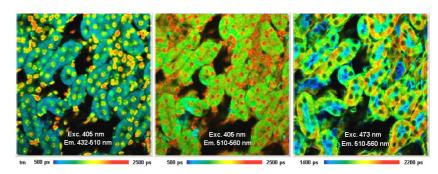
Multi-Wavelength FLIM

Simultaneous detection in 16 wavelength channels

GaAsP multi-wavelength detector No wavelength scanning, no time gating Near-ideal recording efficiency Maximum SNR at minimum sample exposure

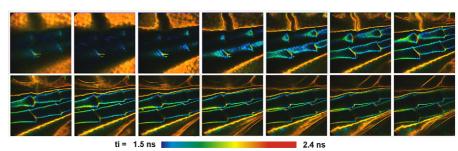
Laser Wavelength Multiplexing Pixel, line, or frame multiplexing Separation of fluorophores by excitation and emission wavelength





Z Stack FLIM

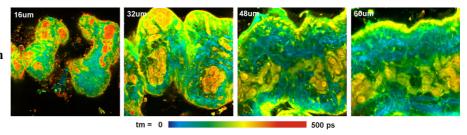
Automatic FLIM acquisition in consecutive Z planes



Multiphoton FLIM

Ultra-high sensitivity combined with deep-tissue imaging capability Simultaneous, fully parallel detection in two wavelength channels Simultaneous FLIM and SHG imaging

Extraction of SHG component from FLIM data

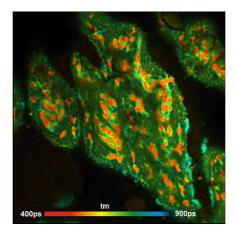


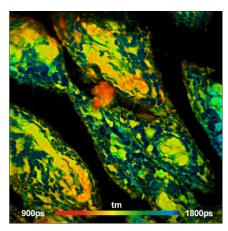
Near-Infrared FLIM

Confocal FLIM with NIR fluorophores

Excitation by ps diode lasers, 640 to 785nm, by supercontinuum laser, or by Ti:Sa laser

Large penetration depth
No autofluorescence background









Confocal Scanning FLIM Systems

Multi-Exponential FRET Analysis

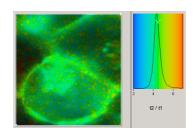
FRET, separation of interacting and non-interaction donor Self-calibrating FRET Correct conventional FRET efficiencies

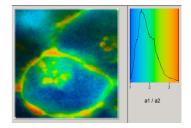
Time-Series FLIM

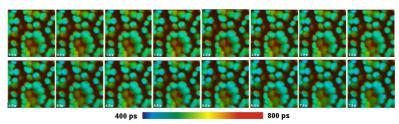
Time-series as fast as 2 images per second
Physiological effects
Chlorophyll transients

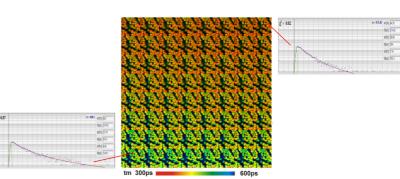


Time series at rates down to 40 ms per image Record Ca²⁺ transients by FLIM Record chlorophyll transients



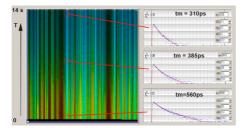


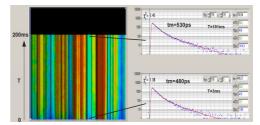




FLITS

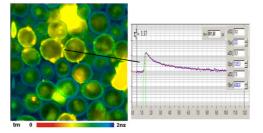
Resolve transient lifetime effects down to 1 millisecond by line scanning Record photochemical chlorophyll transients Record Ca²⁺ transients in live neurons

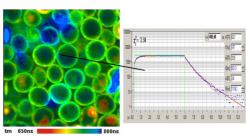




PLIM

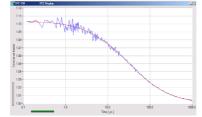
Simultaneous recording of phosphorescence and fluorescence lifetime images Record metabolic effects by FLIM and track O₂ concentration by PLIM

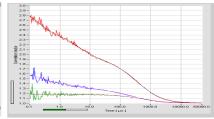




FCS

High-efficiency by GaAsP hybrid detectors No afterpulsing peak Online calculation, online fit Time-gating for suppression of Raman light









DCS-120 Confocal Scanning FLIM Systems

SPCImage FLIM and PLIM Data Analysis

Single, double, and triple-exponential decay analysis

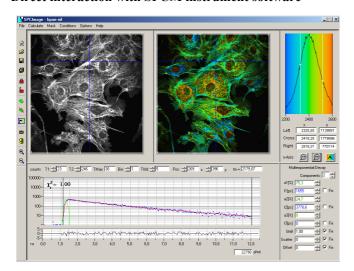
No IFR measurement required

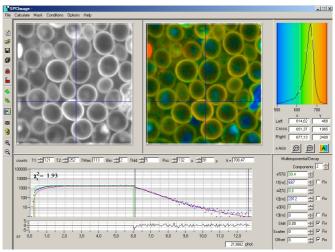
Display of lifetimes, amplitudes, intensities or ratios of decay parameters

Histograms of lifetimes, amplitudes, intensities or ratios of parameters in regions of interest

Phasor plot

Direct interaction with SPCM instrument software





Burst Analyzer Single-Molecule Data Analysis

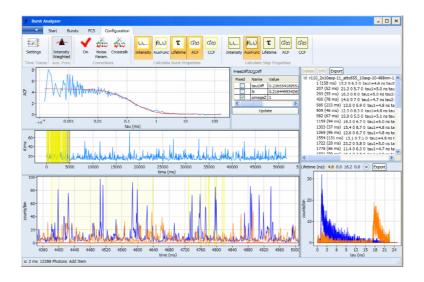
Identification of photon bursts from single molecules in parameter-tag data

Calculation of intensity and lifetime histograms

Calculation of single-molecule FRET intensities

Advanced FCS calculation

Time-gated FCS, selection of time windows in PIE data







DCS-120 Confocal Scanning FLIM Systems

System Components

Lasers



Available Wavelengths Repetition rates Pulse width

Intensity control, electronic Beam correction optics

Fibre coupling

Power delivered into fibre:

Laser Multiplexing Beam blanking

375nm, 405 nm, 445 nm, 473 nm, 488 nm, 515nm, 640nm, 685nm, 785nm

20 MHz, 50 MHz, 80 MHz, CW

375nm to 445nm: typ. 40 ps 473nm to 785nm: typ. 60 ps

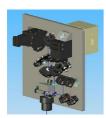
Beam-profile and astigmatism correction

Single-mode, into Qioptiq fibre

405 nm: 1 mW, 440 nm: 0.4 mW, 473 nm: 0.4 mW, 488 nm: 0.4 mW

Within each pixel, line by line, or frame by frame, PIE During x and y flyback, via bh GVD-120 scan controller

Scanner



Laser inputs Laser power regulation, optical

Laser beam combiner

Main beamsplitter

Secondary beamsplitter

Pinholes Filters

Point-Source Kineflex push-and click fibre manipulator

Attenuators, 1:1 to 1:50

Dichroic mirror

Close-coupled galvanometer mirrors

Dichroic 420 nm, 450 nm, 490 nm, 405/473 nm

Beamsplitter wheel. Dichroics 510 nm, 560 nm, polarising beamsplitter,

100% channel 1, 100% channel 2. Other dichroics on request

From approx. 0.5 AU to 10 AU $^{1)}$

Filter sliders, standard filters LP 435, LP 485, BP 480/40, BP535/30, BP 620/60

Detectors





GaAsP hybrid detectors Standard PMTs

High-speed MCP-PMTs Multi-wavelength detector

Detector control

HPM-100-40 hybrid PMT modules. IRF width 130 ps²⁾ PMC-100-1 or -20 cooled PMT modules. IRF width 180 ps²⁾

R3809U-50 MCP PMTs, IRF width 70 ps2)

bh MW FLIM GaAsP detector. Please see individual data sheet. Gain, cooling, overload shutdown, via bh DCC-100 detector controller

TCSPC Modules



1 SPC-150 TCSPC module

2 SPC-150 TCSPC modules

Single-channel FLIM systems with one HPM-100-40, one PMC-100, or one

multispectral FLIM detector

Dual-channel FLIM systems with two HPM-100-40, two PMC-100, or two

multispectral FLIM detectors High-speed FLIM systems

Scanner Control





Scan controller Generation of scan signals

Image size

Max. scan rate, time/frame Zoom factor

Beam blanking Laser multiplexing

Beam park function Scan control software Galvo driver amplifier bh GVD-120 (single-slot PCI module) Hardware, digital signal synthesis

16 x 16 to 2048 x 2048

128x128: 0.32s, 256x256: 0.6s, 512x512: 1.5s 3)

1:1 to 1:10⁴⁾

During flyback, on / off selectable

Pixel by pixel, line by line, or frame by frame

any location within scan area

Integrated in standard SPCM TCSPC software

bh GVP-120

Electronics box and system computer



Lap-top PC with

PCI-extension box

Extension box contains: 1 or 2 SPC-150 TCSPC modules 1 GVD-120 scan controller 1 DCC-100 detector controller

Also available:

SPC, GVD, and DCC modules installed in standard PC

- depends on microscope lens used
 Includes pulse width of ps diode laser
- maximum scan rate depends on zoom factor
 useful zoom range depends on microscope





Confocal Scanning FLIM Systems

SPCImage FLIM Data analysis The gold standard in FLIM analysis

Analysis of FLIM and PLIM data

Analysis by iterative convolution and fit procedure

Analysis by first moment of photon distribution

Combination of time-domain analysis and phasor plot

Automatic IRF determination

Multi-tread calculation procedure

Single, double, and triple-exponential decay models

Incomplete decay models

Baseline correction

Free or fixed lifetimes of decay components

Pseudo-global analysis

Synthetic, measured or manually defined IRF

Extraction of IRF from SHG components in FLIM image

Region-of-interest selection

Single and double-exponential FRET

Display of lifetime images

Display of FRET images

Display of lifetimes, amplitudes, intensities

Display of ratios of parameters

Calculation of FRET efficiencies

Histograms of lifetimes, amplitudes, or parameter ratios

Multiple region of interest definition

Two-dimensional histograms of decay parameters

Phasor plot

Export of lifetime data

Export of images

Direct interaction with SPCM software

Automatic transfer of SPCM data to SPCImage

Transfer of data of selected channels or of all channels

Processing of Megapixel FLIM data

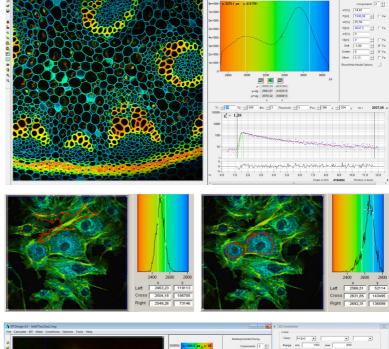
Processing of Mosaic FLIM data

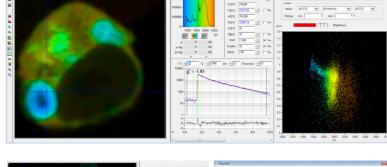
(2017), www.becker-hickl.com

Batch processing of multiple FLIM files

Batch processing of multi-wavelength FLIM data

Batch export of FLIM images and decay data





For detailed description please see: FLIM Systems for Zeiss LSM 710 / 780 / 880 family laser scanning microscopes, user handbook. 7th edition





DCS-120 Confocal Scanning FLIM Systems

TCSPC Technique

	Single-Detector Systems	Dual-Detector Systems	Multi-Spectral Systems
TCSPC system	Simple-Tau 150	Simple-Tau 152	Simple-Tau 150
Components	Lap-top computer PCI extension box SPC-150 TCSPC module DCC-100 detector controller	Lap-top computer PCI extension box Two SPC-150 TCSPC modules DCC-100 detector controller	Lap-top computer PCI extension box SPC-150 TCSPC module DCC-100 detector controller
Saturated count rate Sustained count rate	10 MHz	20 MHz	10 MHz
Scan Sync In mode	10 MHz	20 MHz	10 MHz
FIFO Imaging Mode	4 MHz	4 MHz	4 MHz
Scan rate	any	any	any
Scan synchronisation	via pixel clock, line clock and frame clock in all imaging modes		
Zoom	automatic zoom with zoom in microscope		
Online display	in programmable intervals, 1 second or larger		
Max. image size, 64 bit SPCM software			
$pxl_x x pxl_y x \Delta t$	2048x2048x256	2x 2048x2048x256	16x 512x512x64
(examples)	1024x1024x1024	2x 1024x1024x1024	16x 512x512x256
	512x512x4096	2x 512x512x4096	16x 256x256x1024
	256x256x4096	256x256x4096	

Requirements to Microscope

Confocal FLIM Side port of microscope available MP FLIM with NDD Side port and Lamp port or NDD port available

More specifications: Please see [1] or [2]

Other FLIM configurations: Please note that the bh FLIM systems are highly modular. Therefore a large number of different

FLIM system configurations are possible. Please see [1] for details or contact bh.

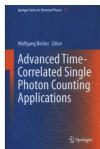
Literature:

- [1] DCS-120 confocal scanning FLIM systems, user handbook, 7th ed., www.becker-hickl.com
- [2] DCS-120 confocal scanning FLIM systems. Overview brochure. www.becker-hickl.com
- [3] The bh TCSPC Handbook, 7th ed., www.becker-hickl.com
- [4] W. Becker, Advanced Time-correlated single photon counting techniques. Springer 2005
- [5] W. Becker, ed., Advanced time-correlated single photon counting applications. Springer 2015

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