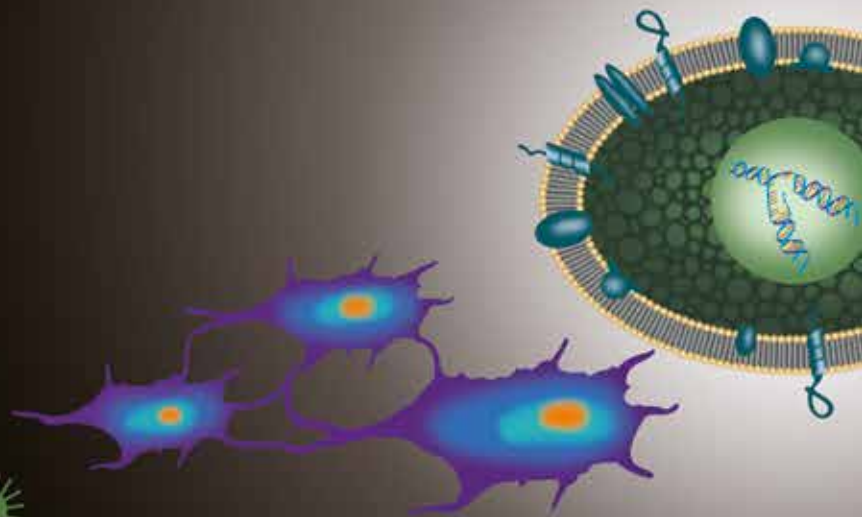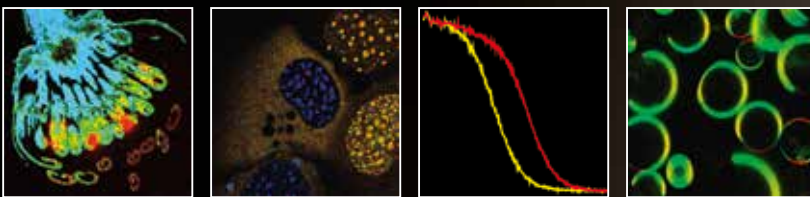# LSM Upgrade Kit

Compact FLIM and FCS Upgrade Kit for Laser
Scanning Microscopes

PicoQuant

Your Benefits

A new dimension for your laser scanning microscope

The confocal Laser Scanning Microscope (LSM) is a powerful tool commonly used in many areas of life sciences that lets you see what happens within your samples. Expand the versatility of your LSM by adding one of our Upgrade Kits to gain access to a whole new dimension of information: the time domain. Gain deeper insights into the systems you are studying as well as into their dynamics thanks this additional contrast source.

- **Maintenance free turn-key system**

    Comfortable performance without time-consuming adjustments

- **Highest sensitivity with broad lifetime range**

    Detect lifetimes ranging from < 100 ps to µs with up to 4 detection channels

- **rapidFLIM^{HiRes}: new high speed data acquisition**

    Record fluorescence lifetime images (FLIM) at rates of up to several frames per second

- **Highly customizable**

    Adapted to most major LSMs with various configuration for ultimate flexibility

- **Advanced data analysis software**

    Supports Fluorescence Lifetime Imaging (FLIM), Förster Resonance Energy Transfer (FRET) and Fluorescence Correlation Spectroscopy (FCS)

Vision

The confocal Laser Scanning Microscope (LSM) is a powerful and widely used tool in life science. It combines high optical resolution with excellent sensitivity, enabling scientists to visualize small structures by recording the spatial location of fluorescent labels in all three dimensions. The capabilities of an LSM can be further enhanced by implementing time-resolved techniques, giving access to a whole new dimension of information. Upgrade Kits from PicoQuant are the highly flexible,

turn-key solution to expand LSMs from major manufacturers with single photon counting techniques.
Each kit encompasses all of the innovative hardware and software required to grant you access to a wide range of exciting applications such as quantification of FRET experiments, studying environmental parameters, and measuring concentrations or molecular mobility for various kinds of samples.
It is time to bring your LSM into a new dimension!

Study various samples with highest sensitivity

Investigate as in classic confocal microscopy all kinds of samples and labels exhibiting luminescence lifetimes ranging from < 100 ps up to µs, such as live or fixed cells and tissues (human, animal, and plant), membranes, semiconductors, quantum dots, and even single molecules.

Get an additional image contrast

See more properties of your sample, identify sites of molecular interaction, quantify your measurements, image more fluorescent labels in multicolor experiments via lifetime unmixing, differentiate your sample based on autofluorescence, and improve the fluorescence signal by removing unwanted artifacts.

Advanced time-resolved applications in a turn-key setup

Get access to innovative techniques such as Fluorescence or Phosphorescence Lifetime Imaging (FLIM, PLIM), rapidFLIM[HiRes], quantitative Förster Resonance Energy Transfer (FRET) measurements, Fluorescence Correlation Spectroscopy (FCS/FLCS, FCCS), polarization anisotropy studies, and autofluorescence characterization.

Excellent support

We provide on-site customer training for both

device and software during installation as well as online support through a team of highly qualified specialists. PicoQuant also organizes yearly workshops,
software training days, and hosts a Time-Correlated Single Photon Counting (TCSPC) wiki.

Maintenance-free and easy to use

After on-site integration of the Upgrade Kit, no further time-consuming adjustments are needed. Enjoy the ease of use, versatility, system integration, and comfortable performance of your newly time-resolved LSM.
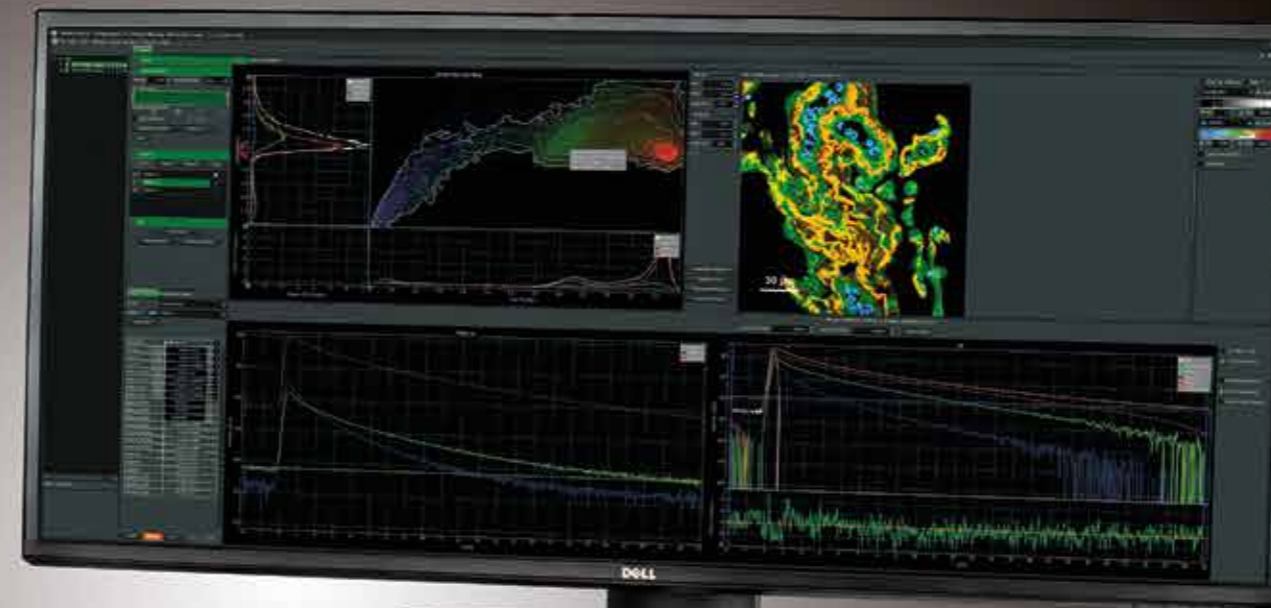
Powerful software integration

The Upgrade Kit features excellent integration with

the software from the LSM manufacturer, enabling you to perform multidimensional time-resolved experiments with ease. Spend your time analyzing your data, not working the software!
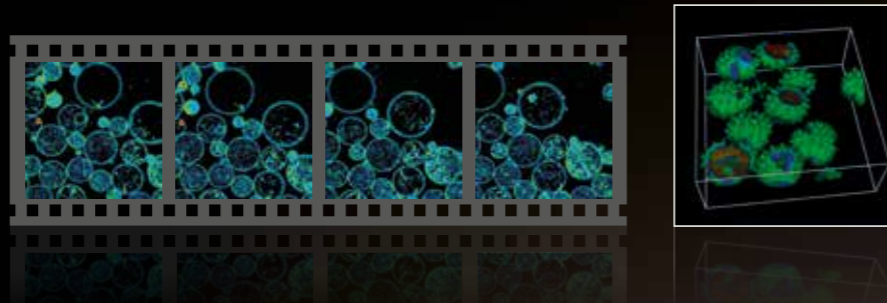
Applications

Bring your LSM into the time dimension and gain access to new information on your samples.

Expanding the capabilities of an LSM with one of PicoQuants Upgrade Kits gives the user access to a large variety of innovative time-resolved applications.

These techniques provide a new layer of information for all kinds of luminescent materials or labels, offering deeper insights into structure, function, and behavior.
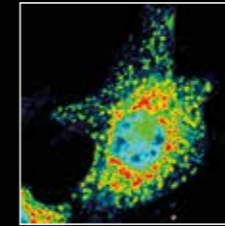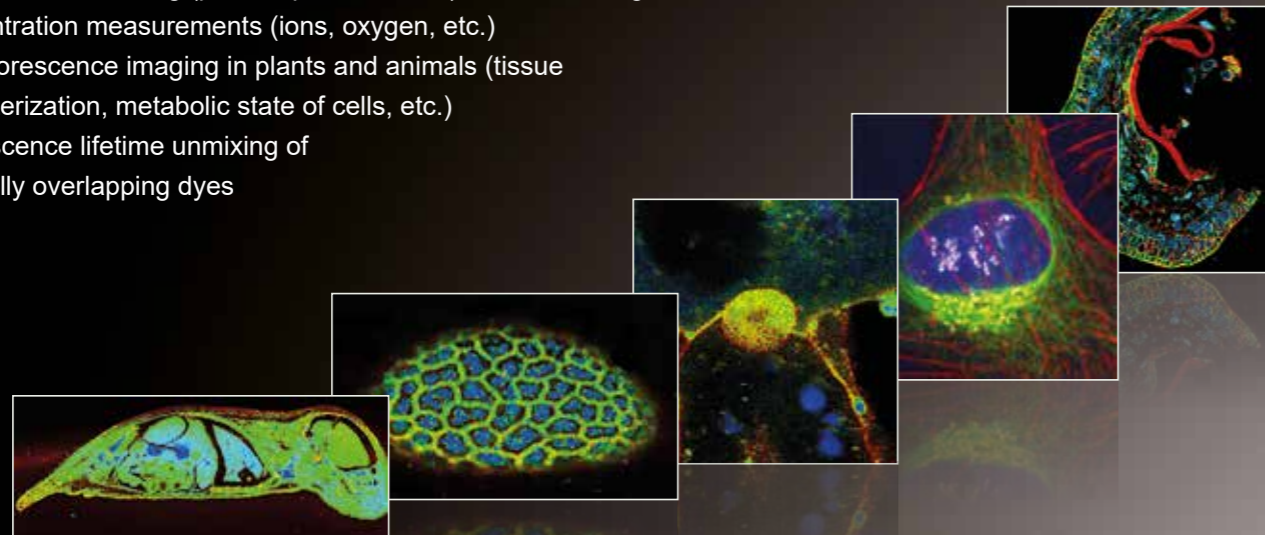
FRET

- Intra- and intermolecular interactions
- Enzyme activity
- Protein folding

FCS

- Concentration measurements in cells
- Diffusion behavior of molecules

rapid FLIM^HiRes

- In vivo FLIM
- FRET in rapidly moving objects
- Fast imaging of environmental parameters

PLIM

- Intracellular oxygen measurements
- Semiconductor quality control
- Characterization of charge carrier lifetimes
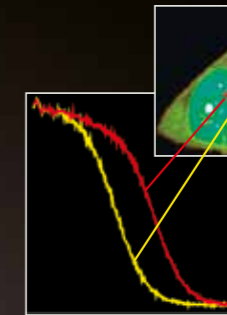
FLCS

- Determination of absolute molecular concentrations
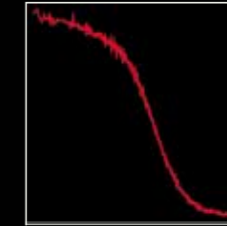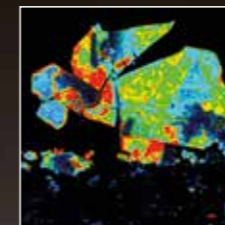- Artifact-free analysis of protein mobility and lipid dynamics

FLIM

- Environmental sensing (pH, temperature, etc.) in cells and organs
- Concentration measurements (ions, oxygen, etc.)
- Autofluorescence imaging in plants and animals (tissue characterization, metabolic state of cells, etc.)
- Fluorescence lifetime unmixing of spectrally overlapping dyes
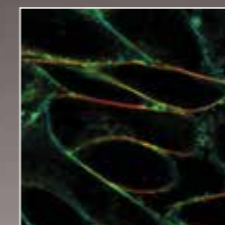
Anisotropy

- Protein interactions (Homo-FRET)
- Membrane structure and composition
- Probe orientation and sample viscosity
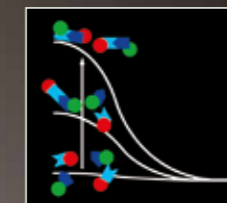
FLCCS

- Cross-talk free detection of molecular interactions
- Complex formation, stoichiometry, and binding kinetics

FLIM

Use Fluorescence Lifetime Imaging (FLIM) to gain new insights into what's going on in your samples by exploiting the time dimension.

Principle

FLIM produces an image by recording for every pixel differences in the excited state decay rate from a fluorescent sample, which are typically in the range of picoseconds to nanoseconds. In a FLIM image, contrast is based on the fluorescence lifetime, which is defined as the average time a molecule remains in an excited state prior to returning to its ground state by emitting a photon. Fluorescence lifetimes can be precisely measured by recording the time difference between an ultra short laser excitation pulse and the arrival of the first photon at
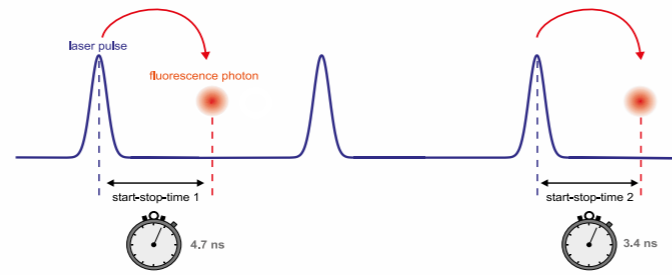
the detector. Fluorescence lifetime is an intrinsic feature of each fluorophore. However, it can be influenced by a broad range of environmental conditions, such as pH, ion concentration, molecular binding, or the proximity of energy acceptors in FRET.

FLIM is a more robust imaging method than intensity based ones as the lifetime does not depend on intensity fluctuations, fluorophore concentration, sample thickness, or system settings. This allows for direct comparison of results amongst different samples, like in cell measurements with varying labeling density or expression levels without the need of additional control experiments.

These properties make FLIM the technique of choice for most kinds of functional imaging.

Applications
• Sensing the local environment (pH, ion concentration, polarity, temperature, etc.)
• Monitoring molecular interactions over space and time
• Studying conformal changes in proteins and nucleic acids
• Detecting enzyme activity (proteolysis, phosphorylation, etc.)
• Enhanced multi-color, artefact-free imaging via lifetime unmixing
• Characterizing samples by their autofluorescence (differentiating anatomical structures, identifying cancerous alterations, etc.)

Example

1 mm

1   **Average lifetime [ns]**   5
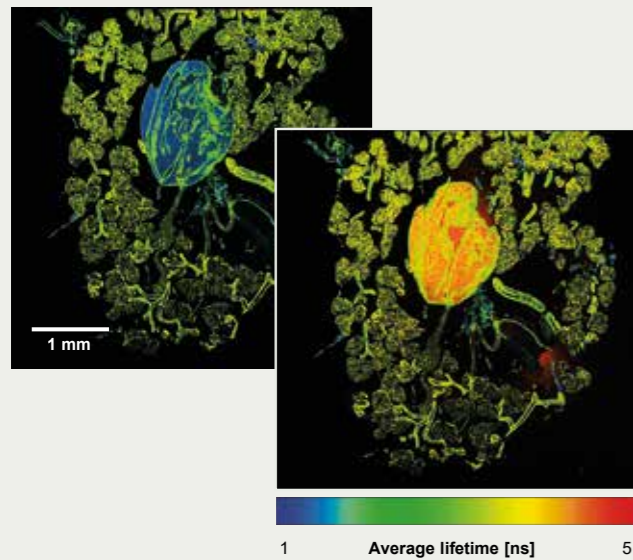
Monitoring chloride ion (Cl-) concentration in insect organs by exploiting fluorophore lifetime dependence on the local environment. Cockroach salivary glands allow studying epithelial ion transport by staining the organ with the Cl- sensitive dye MQAE. Recording FLIM images with physiological (174 mM, left) and reduced (2 mM, right) Cl- concentrations enabled mapping of the Cl- concentration throughout the whole organ by detecting changes in fluorescence lifetime. The central reservoir displayed a significant change in Cl- concentration (changing from blue to orange), while the lifetime difference in the surrounding salivary glands was much less pronounced (green).

In collaboration with C. Dosche and C. Hille, University of Potsdam, Germany.

laser pulse
fluorescence photon
start-stop-time 1   4,7 ns
start-stop-time 2   3,4 ns

rapidFLIM^HiRes

A novel approach to make visible fast processes with fluorescence lifetime imaging.

Principle

rapidFLIM^HiRes is a novel approach for acquiring FLIM images in a very fast manner by exploiting optimized hardware components such as TCSPC boards and detectors with ultra short dead times. The optimized hardware eliminates the measurement artifacts that would otherwise occur in conventional FLIM measurements at high count rates. Significantly higher detection count rates can now be used, thus greatly reducing the required measurement time for FLIM images, with only a slight sacrifice in temporal resolution.

The rapidFLIM^HiRes approach preserves the high optical resolution of confocal microscopy and the intuitive operation of time-domain FLIM. Depending on sample brightness and image size, more than 15 frames can now be captured per second.

Applications
• Following dynamic lifetime processes (changing interaction states, chemical reaction, highly mobile species, in vivo measurements, etc.)
• Dynamic lifetime changes
• Fast imaging of environmental parameters (pH, ion, or oxygen concentration, etc.)
• Investigating the FRET dynamics of transient molecular interactions in cells or mobile structures (mobility of cell organelles, vesicle trafficking, particle movement, or cell migration)
• Performing live cell observations (fast acquisition of FLIM z-stacks, time series, etc.)
• Using the characteristic autofluorescence of tissue types for studying dynamics of cell metabolism
• High throughput FLIM screening
• FRET in rapidly moving objects and mobile samples

"rapidFLIM opens the door to investigate interaction during dynamic processes, our goal is a FRET movie."

Dr. Stefanie Weidtkamp-Peters,
Center for Advanced Imaging (CAI), University of Düsseldorf, Germany

Example
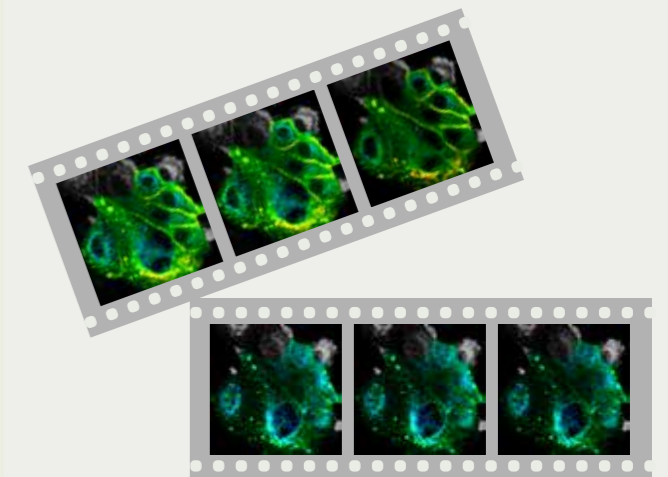
This example shows how the fluorescent probe FliptR (Fluorescent lipid tensor Reporter) can be used to imaging lipid composition and membrane tension in both live cell and artificial membranes. The probe displays a significant change in fluorescence lifetime when embedded in a confining environment. If lateral pressure is applied, FliptR undergos a planarization which leads to a change in fluorescence lifetime. Here the membranes of MDCK cells were stained with FliptR and subjected to an hyperosmotic shock, where the membrane tension rapidly decreases resulting in a shorter lifetime.

Sample (and data) courtesy of Dr. Thomas Korte, Humboldt University Berlin, Germany.

PLIM

Phosphorescence Lifetime Imaging (PLIM) allows characterizing samples with long emission lieftimes and is ideally suited for life as well as materials science.

Principle

PLIM is similar to FLIM, but images longer emission lifetimes of up to several microseconds. The contrast in a PLIM image is based on the phosphorescence lifetime of individual fluorophores, which indicates the average time that a molecule remains in an excited triplet state prior to returning to the ground state by emitting a photon. In life sciences, typical phosphorescent probes include metal ions (e.g., Ru, Ir, Pd, Pt) complexed with organic ligands, which are used as sensors to image specific environmental properties, like oxygen concentrations in tissues and to analyze their effects on cell metabolism, or to study aggregation of species related to Alzheimer's disease. Complexes based on lanthanide ions, mainly Tb^{3+} or Eu^{3+}, are also used as donors in Luminescence Resonance Energy Transfer (LRET) measurements, which is especially usefull for analyzing mixtures. Furthermore, nanoparticles and quantum dots can also exhibit long fluorescence lifetime components and be thus imaged by PLIM.

PLIM, or generally the analysis of phosphorescent compounds, is of great importance in the field of materials science, especially in the characterization of Organic Light Emitting Diode (OLED) materials, or for determining charge carrier mobility in semiconductors.
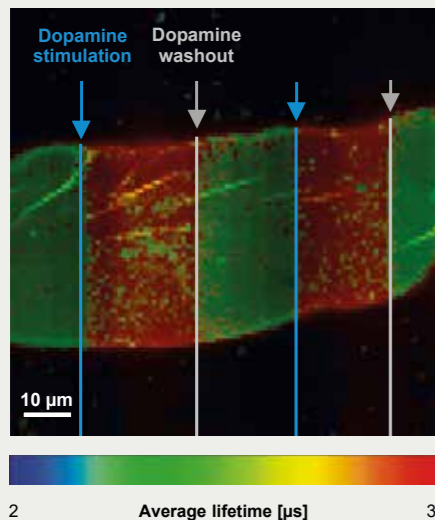
Applications

• Sensing environmental conditions (e.g., oxygen concentrations) in cells and living tissues with the help of metal organic complexes
• Monitoring singlet oxygen emission
• Chemical sensing applications
• Characterizing charge carrier mobility in semiconductors
• Detecting defect sites in semiconductor wafers and solar cells

Example

Dopamine stimulation   Dopamine washout

10 µm

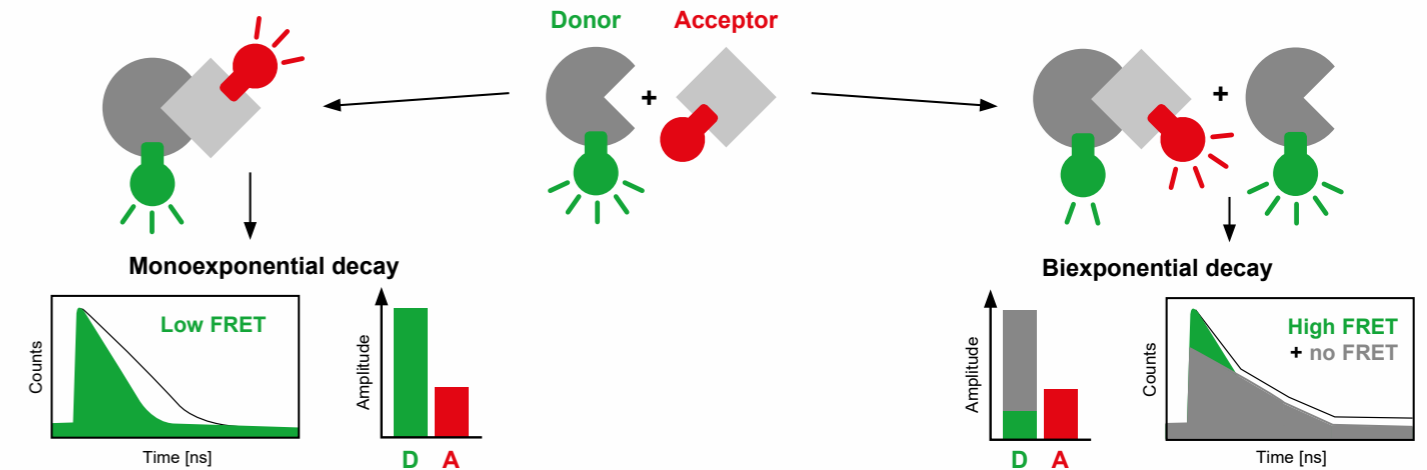2   **Average lifetime [µs]**   3

Oxygen was imaged in male cockroach salivary glands by using KR341, a phosphorescent probe based on a Ru complex with a lifetime in the microsecond (µs) range that is quenched by molecular oxygen.
The sample was slowly scanned and during the image scan, dopamine was added or removed by washing with Ringer buffer. Dopamine triggers the metabolism which increases oxygen consumption. Thus, stimulation of cellular metabolism by dopamine exposure decreased the oxygen concentration within the salivary glands, which was measured by an increase in the phosphorescence lifetime of KR341. This effect was reversible.
After washing out the dopamine, the oxygen consumption decreased resulting in a higher oxygen concentration and stronger lifetime quenching of the oxygen sensor KR341.

Data courtesy of K. Jahn, C. Hille, University of Potsdam, Germany.

FRET

Lifetime-based Förster Resonance Energy Transfer (FRET) enables distance measurements in the nanometer range as well as observation and quantification of molecular interactions.
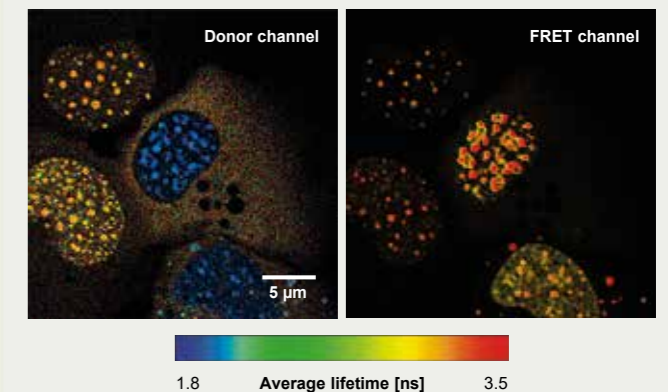

Monoexponential decay   **Biexponential decay**

Donor   Acceptor

Low FRET   High FRET + no FRET

Counts   Time [ns]   Amplitude   D A
Amplitude   D A   Counts   Time [ns]

Principle

FRET is commonly used in life science as a "molecular ruler" to measure distances between pairs of matched fluorescent molecules. FRET is a non-radiative process whereby energy from an excited fluorescent molecule (donor) is transferred to a second, non-excited fluorophore (acceptor) in its direct vicinity, resulting in quenching of the donor and a shorter donor fluorescence lifetime. As an outstanding benefit, FLIM-FRET allows distinguishing between molecules that do or do not show FRET in each image pixel, and thus quantifying the extent of energy transfer and binding.

Applications

• Imaging and quantification of molecular interactions
• Studying distribution and assembly of protein complexes in space and time
• Receptor/ligand interactions
• Monitoring enzyme activity (protease)
• Analyzing intramolecular interactions to follow conformational changes in nucleic acids and protein folding
• Nucleic acid hybridization and primer-extension assays for detecting mutations
• FRET sensors to monitor ion flux

"This innovative LSM Upgrade Kit enables simultaneous FLIM and PLIM measurements, which significantly contributes towards unraveling complex cellular functions."

Dr. Carsten Hille, University of Potsdam, Germany

Example

Donor channel   FRET channel

5 µm

1.8   **Average lifetime [ns]**   3.5

Using FLIM-FRET to investigate the role of small GTPase during the embryonic development of zebrafishes. Quantitative FLIM-FRET analysis allows determining the spatial and temporal activity of two or more interacting molecules. The GTPase activity can be monitored via the FRET efficiency from one chromophore to another. By measuring the donor fluorescence lifetime, relevant information can be collected. Here the small GTPase Rac protein was fused to variants of CFP (donor) and YFP (acceptor). When the acceptor fluorophore is bleached (Fig.1; lower region of the image), the lifetime of the donor increases (Fig. 2). However, FRET efficiency is not affected by the acceptor bleaching.

Sample and data courtesy of M. Gonzalez-Gaitan, Biochemistry Department, University of Geneva, Switzerland.
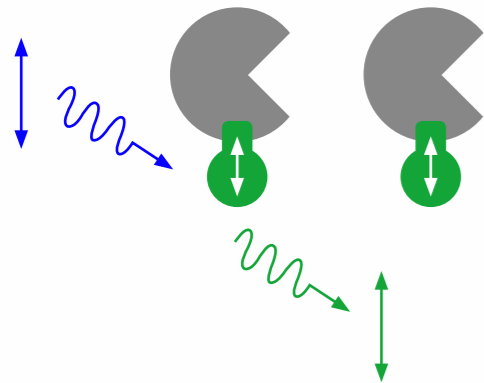
Fluorescence Anisotropy

Fluorescence anisotropy indicates the decree of polarization, which can be used to study the orientation, mobility, and interaction of molecules.
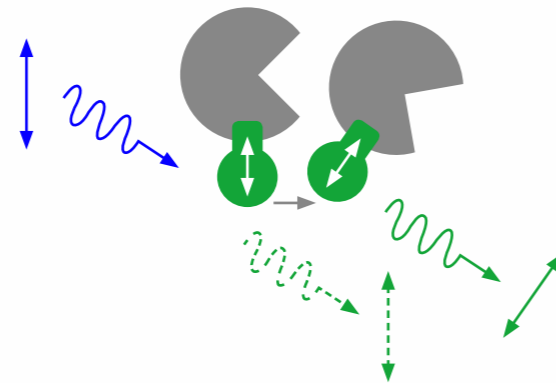
No FRET
Polarization vector is parallel to excitation

Homo-FRET
Polarization vector has changed due to Homo-FRET

Principle

After excitation with polarized light the fluorescence emitted by a molecule is also polarized. The extent of fluorescence polarization is described as anisotropy. The emission can become depolarized by a number of processes, including rotational diffusion, which depends on the viscosity of the solvent, as well as molecular size and shape. Thus, following changes in fluorescence ani-

sotropy over time provide fascinating insights into molecular mobility as well as into the processes that affect it. Another important application lies in studying Homo-FRET, where energy is transferred between two fluorophores of the same type. Such measurements are typically used to detect dimerization or oligomerization of proteins of the same species.

The Homo-FRET process causes fluorescence depolarization and leads to a decease in anisotropy that can be measured in order to identify and quantify the Homo-FRET process. Other factors that can influence fluorescence anisotropy, and hence be studied by it, include random molecular motion (Brownian rotation) or conformational flexibility within molecules.

Applications

- Probing the local microviscosity in cytoplasm, liquids, and polymers
- Investigations of biomembrane fluidity and rigidity
- Determining the orientation of molecular probes or other molecules in various matrices
- Quantifying protein denaturation
- Monitoring FRET between identical molecules (Homo-FRET) as in protein-protein interactions (crowding, oligomerization)
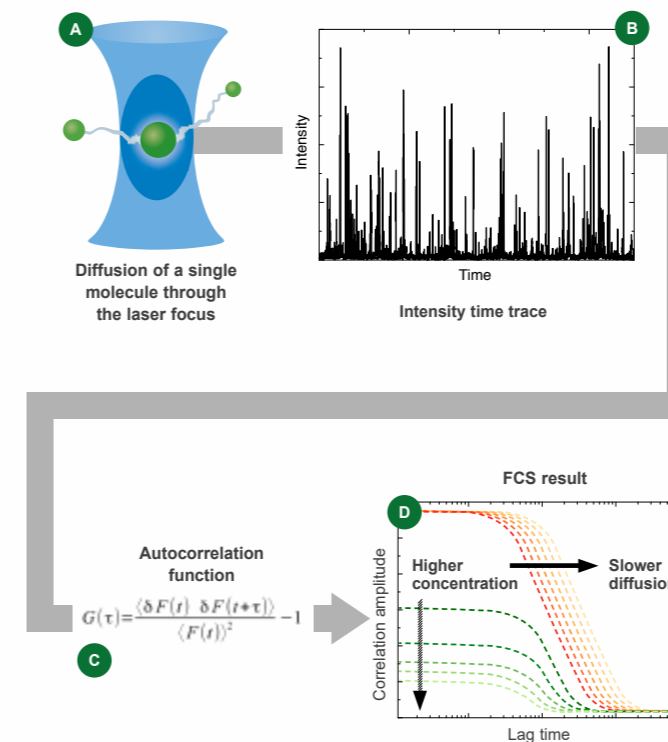
Example

Anisotropy to study protein oligomerization of viral membrane proteins. A specific viral membrane protein (Gp41) which is part of HIV, is responsible for membrane fusion of the HI virus with the T-cell membrane. After infection and synthesis, it is known to be transported to the plasma membrane via intracellular transport vesicles, Golgi apparatus, and endoplasmatic reticulum (ER). Living Chinese Hamster Ovary (CHO) cells transfected with Gp41-mYFP showed a lower anisotropy value at the outer cell membrane compared to the intracellular protein localization. This decrease was caused by clustering of the viral membrane protein at the cell surface leading to Homo-FRET.
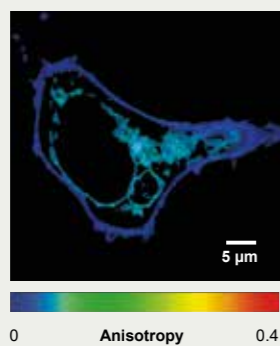
Data courtesy of R. Schwarzer, Humbold University Berlin, Germany.

5 µm

0   **Anisotropy**   0.4

FCS

Fluorescence Correlation Spectroscopy (FCS) analyzes fluorescence intensity fluctuations to determine mobility, concentration, and interactions of diffusing molecules in cells or membranes.

Diffusion of a single molecule through the laser focus

Intensity time trace

Time

Autocorrelation function

$$G(\tau) = \frac{\langle \delta F(t)\, \delta F(t+\tau)\rangle}{\langle F(t)\rangle^2} - 1$$

FCS result

Higher concentration   Slower diffusion

Lag time

Principle

FCS uses a statistical analysis of fluctuations in fluorescence intensity to extract information on processes such as molecular diffusion and concentration. In an FCS measurement, one records a time-trace (B) of changes in emission intensity caused by single fluorophores passing through the small detection volume (A).

These intensity changes can be quantified in their strength and duration by temporal autocorrelation of the recorded intensity signal (C), leading to the average number of fluorescent particles in the detection volume as well as their average diffusion time through the focus (D). Eventually, important biochemical parameters such as concentration and size or shape of the particle (molecule) or viscosity of the environment can be determined.

Applications

FCS
- Concentration measurements in cells and solutions
- Diffusion behavior of molecules in space and time
- Conformational dynamics inside polypeptides and nucleic acids
- Polymerization, surface adsorption

FLCS
- Absolute determination of molecular concentrations
- Artifact-free determination of protein mobility and lipid dynamics

FLCCS
- Cross-talk free detection of molecular interactions
- Complex formation including stoichiometry and binding kinetics

Example

1 µm

$D_{(Cytoplasm)} = $ ~5.4 µm^2/s

$D_{(Nucleus)} = $ ~13.7 µm^2/s

Cytoplasm
Nucleus

Lag time
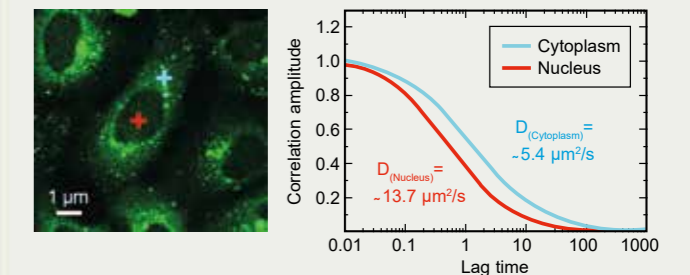
Resolving Different Complexes in Cellular Subcompartments. Ago2, an argonaut protein, is part of the RNA-Induced Silencing Complex (RISC). Understanding the localization and assembly mechanism of RISC helps to unveil the regulation of gene expression. The mobility of GFP-Ago2 was measured in ER293 cells in the nucleus (red cross) and the cytoplasm (blue cross).

The corrsponding FCS curves are shown in the graph. Measuring the diffusion coefficient inside the nucleus (about 13.7 µm/cm2) and in the cytosol (approx. 5.4 µm/cm2) revealed that RISCs complexes differ in their diffusion behaviour, which was confirmed to be due to size differences of nuclear and cytosolic RISCs.

Courtesy of M. Gärtner, P. Schwille, Technical University Dresden, Germany.

System Layout

The PicoQuant LSM Upgrade Kit is an external add-on for laser scanning microscopes granting versatile and comfortable performance without time-consuming adjustments. It enhances the capabilities of confocal laser scanning microscopes by providing access to a wide range of time-resolved techniques for all kinds of samples and labels as used in confocal laser scanning microscopy.

A turn-key LSM Upgrade Kit encompasses three major parts:
• Picosecond pulsed excitation sources
• Single photon counting detectors
• TCSPC acquisition unit along with system software

The compact and maintenance-free kit is available for many LSM types from the major microscopy companies Nikon, Olympus, Scientifica and Zeiss. Numerous options allow tailoring each Upgrade Kit to the sample and experimental requirements of the user. Later extensions and modifications are very straightforward thanks to the modular design of the set-up. PicoQuants Upgrade Kits feature extensive integration into the system software from most of major LSM manufacturers and allow carrying out sophisticated time-resolved experiments in a simple, comfortable, and well documented way. The LSM Upgrade Kit also comes with the powerful, yet easy to use software package SymPhoTime 64, which enables users to focus on analyzing their samples and results thanks to its clearly structured layout and versatile analysis routines.

"The modular and turn-key nature of the LSM Upgrade Kit as well as support provided by PicoQuant make it easy to integrate FLIM into a Core Facility."

Dr. Anna Lladó, Dr. Julien Colombelli, IRB, Barcelona Institute of Science and Technology, Barcelona, Spain

TCSPC data acquisition

TCSPC electronics

TimeHarp 260

MultiHarp 150

HydraHarp 400

System software

SymPhoTime 64

Detection

Single channel units

Dual channel units
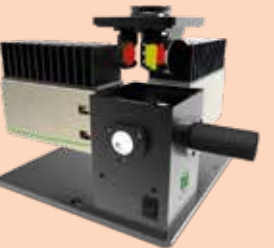
Multi channel unit
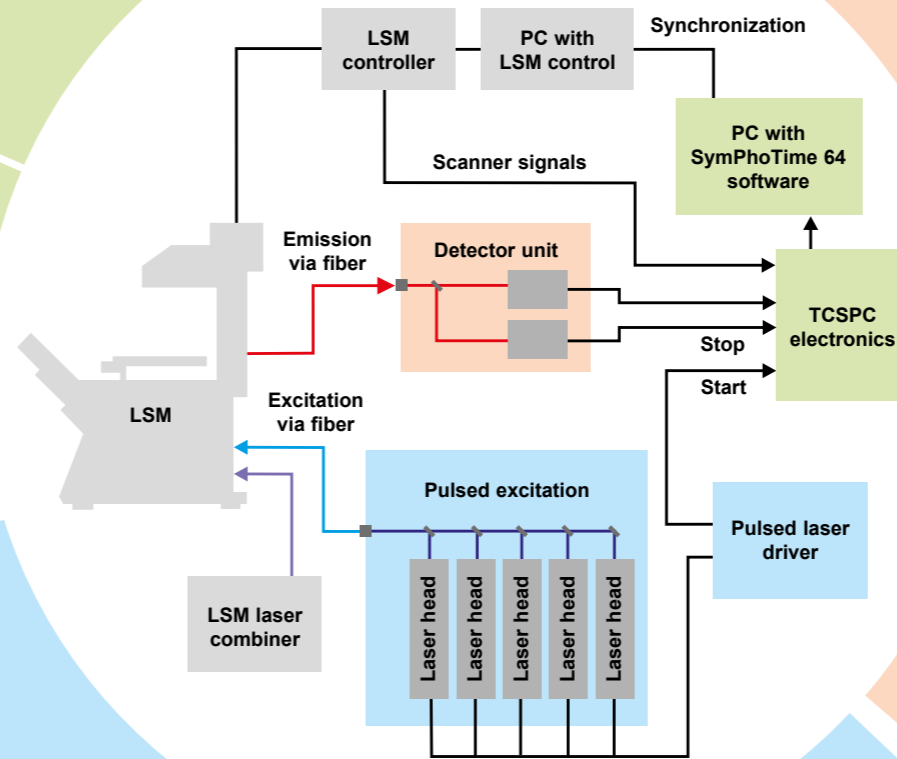
Excitation

Pulsed diode laser drivers

PDL 828 "Sepia II" multi channel computer controlled version

PDL 800-D single channel version

Picosecond pulsed laser excitation

Single color excitation

Multi color excitation with Laser Combining Unit

Diagram labels

LSM controller — PC with LSM control — Synchronization

PC with SymPhoTime 64 software

Scanner signals

Emission via fiber

Detector unit

TCSPC electronics

Stop

Start

LSM

Excitation via fiber

Pulsed excitation

Pulsed laser driver

LSM laser combiner

Laser head, Laser head, Laser head, Laser head, Laser head

System Components

Best data quality for time-resolved measurements

Time-Correlated Single Photon Counting (TCSPC) is the most sensitive and accurate method to determine fluorescence lifetimes occurring in the picosecond to microsecond time regime. TCSPC works by using the laser excitation pulse as the measurements start point and stops after the detector registers the first arriving photon. The time difference between these two points is then stored and the sample fluorescence decay profile is constructed by collecting single photons from a large number of excitation/emission cycles.

PicoQuant developed a unique data acquisition mode called Time-Tagged Time-Resolved (TTTR) for recording many other experimentally relevant parameters for each photon. Beside the start-stop times (TCSPC time), the elapsed time since the start of the data acquisition is also recorded (time tag) allowing for FCS/FCCS and, in conjunction with synchronization signals from the scanner, for the reconstruction of intensity and lifetime images. The channel information is also monitored for each photon, which determines either their wavelength or polarization, depending on the setup. This data format preserves complete information for every single photon, thereby providing a universal basis for a large variety of analysis methods.

Multi-color pulsed excitation

The excitation subsystem can be configured with multiple laser heads integrated in a Laser Combining Unit (LCU) with each providing a single

Wavelength [nm]

350	400	450	500	550	600	650	700

375   405   440   470   485   510   530   560   590   640
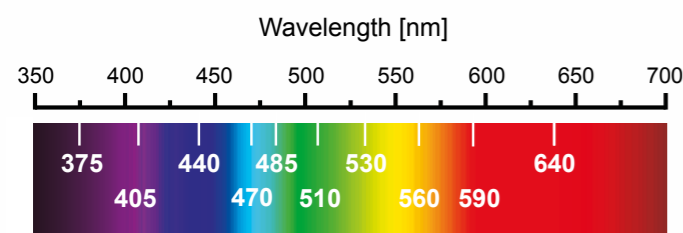
excitation wavelength ranging from 375 to 640 nm (depending on the LSM type). Output power and pulse repetition rate are controlled by a laser driver from the PDL Series. For automated systems (motorized LCUs available for Nikon and Zeiss LSMs) and the realization of multi color excitation schemes, the PDL 828 "Sepia II" laser driver is required. This computer-controlled version allows for advanced excitation schemes, such as those used in PIE (Pulsed Interleaved Excitation), cw operation of laser heads, and the generation of pulse trains to measure very long lifetimes via PLIM.

PicoQuant lasers, consisting of laser heads and driver unit are coupled to the LSM via an optical fibre. Alternatively, external systems such as Titanium:Sapphire lasers can be used, enabling Multi Photon Excitation (MPE) for deep tissue FLIM imaging or spectral excitation.

Highest sensitivity with up to 4 detection channels

TCSPC measurements require detectors with single photon sensitivity and high time resolution. The Upgrade Kit can accommodate up to 4 detection channels that can be each equipped with a choice of different detector types best matched to the sample and fluorophore:
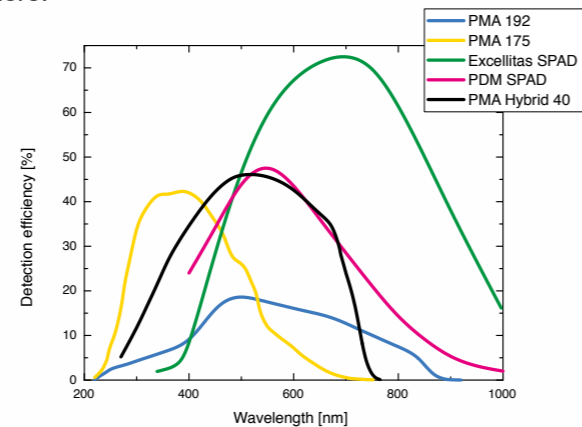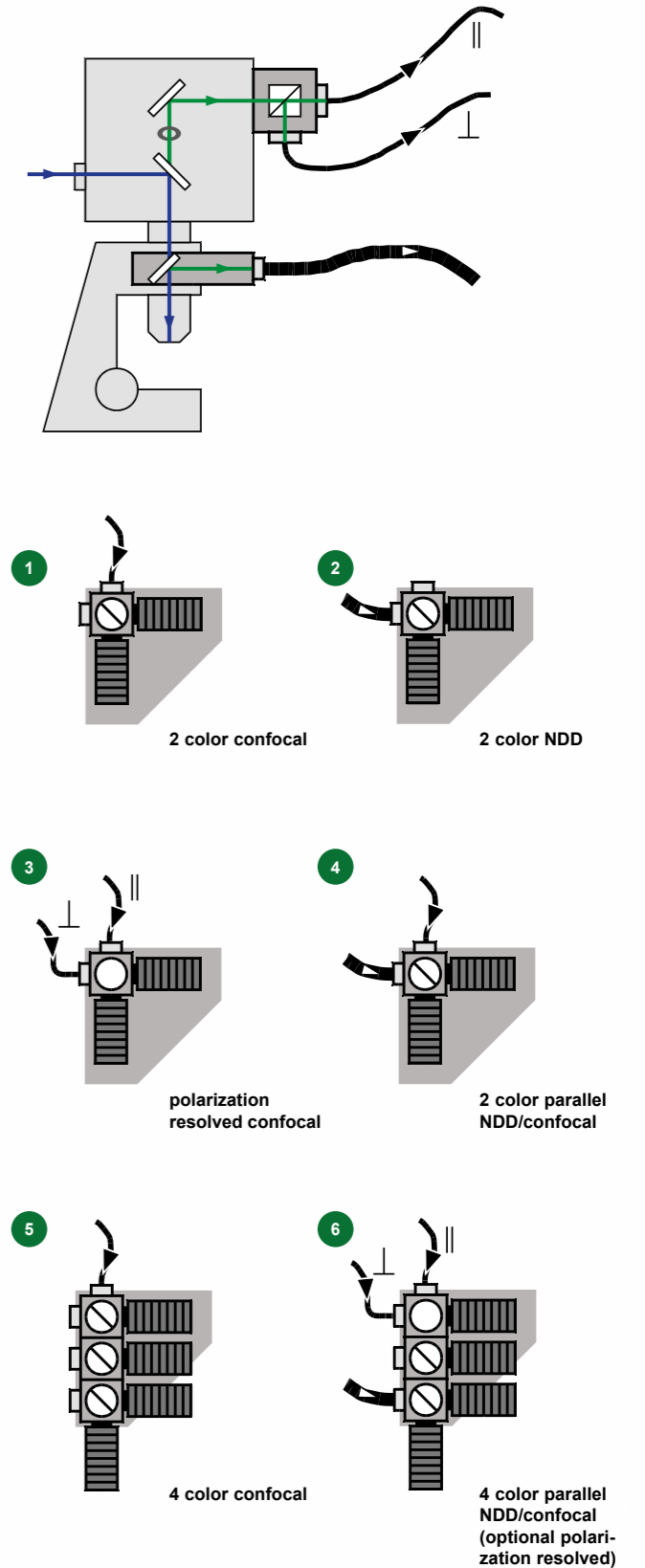

Hybrid photomultiplier tubes from the PMA Hybrid Series (best all-rounder for FLIM, FCS, and deep tissue imaging), single photon avalanche diodes from the PDM

series (fastest detectors for FLIM and FCS), red-sensitive SPADs (highest sensitivity for FCS), and photomultiplier tubes (economic variant for FLIM and deep tissue imaging). The detectors can be attached either in confocal mode using a pinhole or in non-descanned detection (NDD) for multi photon excitation.

To offer greatest flexibility and multiple applications in one system, the multi-channel detection unit can be configured for parallel confocal, polarization, and NDD measurements for multi color FLIM, FRET, deep tissue FLIM imaging, auto- and crosscorrelation (FCS, FLCS, FCCS, FLCCS) as well as anisotropy studies. Several detection configurations are available:

1. Dual-channel confocal detection for FLIM, FRET, auto- and cross correlation (FCS, FLCS, FCCS, FLCCS)

2. Dual channel NDD set-up for deep tissue FLIM and FRET

3. Dual channel polarization resolved confocal detection for FLIM, FRET, anisotropy imaging, auto- and cross-correlation (FCS, FLCS, FCCS, FLCCS)

4. Parallel dual channel set-up enabling confocal and non-descanned detection for dual color FLIM, FRET, deep tissue FLIM imaging, auto- and cross correlation (FCS, FLCS, FCCS, FLCCS)

5. Four-channel confocal detection for FLIM, FRET, auto- and cross correlation (FCS, FLCS, FCCS, FLCCS)

6. Parallel four channel set-up enabling confocal as well as non-descanned detection and polarization measurements for multi color FLIM, FRET, deep tissue FLIM imaging, auto- and crosscorrelation (FCS, FLCS, FCCS, FLCCS), and anisotropy.


1 2 color confocal   2 2 color NDD

3 polarization resolved confocal   4 2 color parallel NDD/confocal

5 4 color confocal   6 4 color parallel NDD/confocal (optional polarization resolved)

SymPhoTime 64

Advanced and user-friendly software package for cutting edge time-resolved fluorescence applications with multiple analysis tools.

The data acquisition and analysis software "SymPhoTime 64" uses the special TTTR data format and features a clearly structured graphical user interface (GUI), guiding the user through all necessary steps of an individual measurement or analysis process. The software is working on a 64 bit operating system to speed up analysis and enable the handling of large data sets as often obtained from FLIM z-stacks and time series. It includes a wide range of analysis methods and automated analysis routines.

An integrated scripting language ("STUPSLANG") enables creating new analysis procedures or customizing existing ones. The data acquisition is integrated into the LSM software (for Nikon, and Zeiss) allowing for performing advanced acquisition schemes, such as multi point FCS, time-series FLIM, and FLIM z-stacks in a comfortable and easy way.
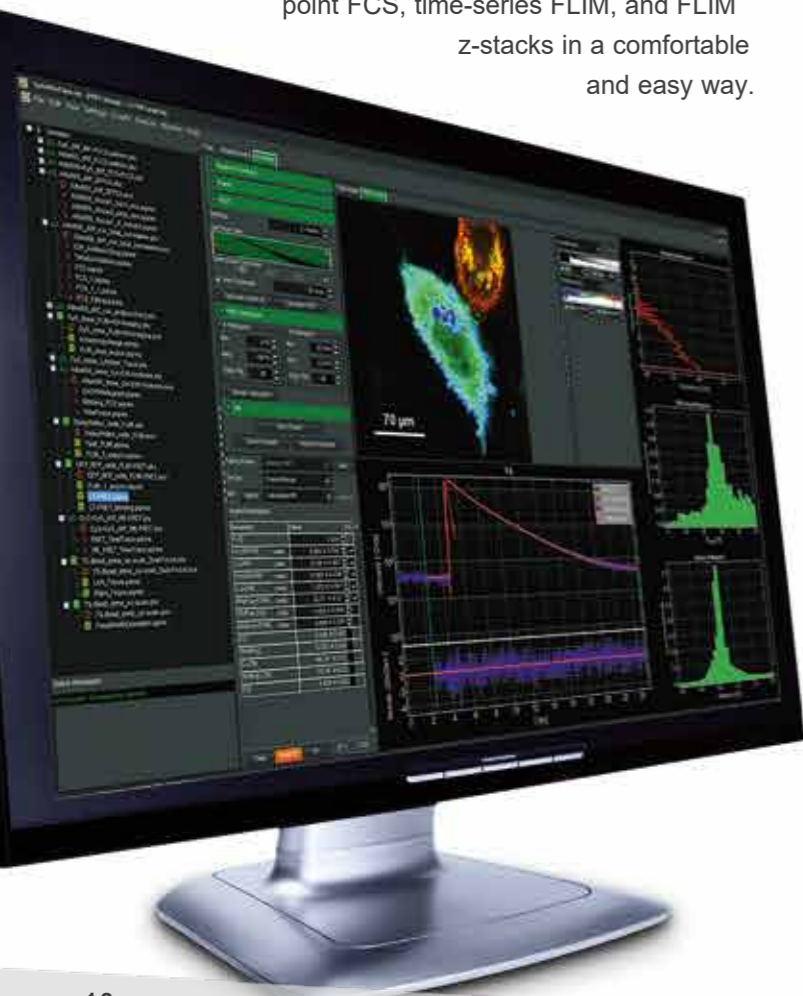
Features

- Integration of FLIM and FCS data acquisition into LSM system software (Nikon, Zeiss)
- Multi-color Fluorescence lifetime imaging (FLIM)
- rapidFLIM[HiRes] for fast FLIM acquisition with up to several frames/sec
- Phosphorescence Lifetime Imaging (PLIM)
- Combined FLIM and PLIM measurements
- Deep tissue FLIM imaging
- Fluorescence (Cross-) Correlation Spectroscopy (F(C)CS) with a time range from picoseconds to seconds
- Fluorescence Lifetime Correlation Spectroscopy (FLCS)
- Lifetime-based Foerster resonance energy transfer (FRET) for calculating FRET efficiency and radius as well as the fraction of complete FRET molecules in respect to donor-only pairs for the whole image
- Fluorescence anisotropy (polarization) measurements (Homo-FRET)
- Real time parallel calculation and display of up to 4 different previews (FastFLIM, TCSPC histogram, FCS, FCCS, or time-trace) during data acquisition
- Image analysis: (time-gated) fluorescence intensity imaging; multi-color FLIM and PLIM; rapidFLIM[HiRes], FLIM-FRET analysis calculating FRET efficiency, FRET radius, amplitude ratio etc. in each image pixel; intensity FRET; bleed-through correction; pattern matching analysis, Fluorescence lifetime histogram; time-gated analysis; TCSPC histogram for region of interest; steady-state anisotropy
- Point measurement analysis: MCS trace, FCS, FCCS, FLCS, PIE-FCS, TCSPC histogramming and fitting with advanced error treatment, on/off-state histogram (blinking), burst size analysis, BIFL (Burst Integrated Fluorecence) analysis, FRET histogram incl. accounting for Pulsed Interleaved Excitation (PIE), particle brightness analysis, lifetime histogram, total correlation and coincidence correlation histograms (antibunching)
- Grouped analysis of multiple measurements

Specifications

Excitation source[1,2]

Light source[3]	Picosecond laser diode heads, motorized laser attenuation upon request
Wavelengths	(375)/405-640 nm
Repetition rate	up to 40 MHz (optional 80 MHz)
Pulse width	down to 70 ps (FWHM)

INVISIBLE OR VISIBLE LASER RADIATION
AVOID DIRECT EXPOSURE TO BEAM
CLASS 3B LASER PRODUCT
IEC / EN 60825-1

TCSPC data acquisition

Type	TimeHarp 260 PICO + long range mode	MultiHarp 150	HydraHarp 400	PicoHarp 300
Number of detection channels	2	4 or 8	up to 8	1
Time resolution (bin width)	25 ps / 2.5 ns	10 ps	1 ps	4 ps
Dead time	< 25 ns / < 2.5 ns	650 ps	< 80 ns	< 95 ns
Sustained data throughput	up to 40 million cps	up to 80 million cps	up to 40 million cps	up to 5 million cps

Detectors

Type[1]	Hybrid PMT	SPAD (SPCM-AQRH)	PMT (PMA Series)
Spectral range	300-720 nm	400-1040 nm	230-920 nm
Dark counts (at 20°, typ. value)	300-100 cps	< 100 cps	< 900 cps
Instrument Response Function (IRF at 650 nm)	< 120 ps	< 350 ps	< 180 ps

Power requirements

110/230 V, 50/60 Hz

Software features[4]

General concept	64 bit operation system; versatile TTTR file format for data acquisition and analysis; time gating for all methods; Graphical User Interface (GUI) for automated analysis and measurement processes; automated routines for many measurements and standard analysis procedures
Point measurements	MCS trace; FCS; FCCS; FLCS; PIE-FCS calculation and fitting; TCSPC histogram; on/off-state histogram; burst size analysis; BIFL (Burst Integrated Analysis); (PIE-)FRET histogram; particle brightness analysis; lifetime histogram; total correlation and coincidence correlation histograms (anti-bunching)
Fluorescence Lifetime Imaging	(Time-gated) Fluorescence Intensity Imaging; Fluorescence Lifetime Imaging (FLIM); rapidFLIM[HiRes], fluorescence lifetime histogram; time-gated analysis; TCSPC histogram for region of interest; FLIM-FRET image analysis incl. FRET efficiency, FRET radius and amplitude ratio; Intensity FRET; bleed-through correction; pattern matching; steady-state anisotropy

Supported LSMs[5]

Nikon	A1, C2, C1si
Olympus	FluoView FV 3000, FVMPE-RS, FV 1200 (MPE), FV 1000 (MPE)
Zeiss	LSM 980 (NLO), LSM 880 (NLO), LSM 780, LSM 710
Scientifica	HyperScope, VivoScope

1) Other laser lines or detector types available upon request.
2) Existing multiphoton (Ti:Sa) lasers can be implemented.
3) Class 3B lasers – they will increase the classification of your LSM accordingly.
4) For details, please see our SymPhoTime 64 brochure.
5) Depending on configuration, upgrades for other LSM types are possible, please contact us for details.
6) Increases in blue spectral range