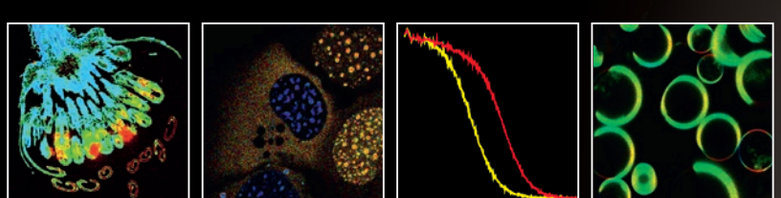
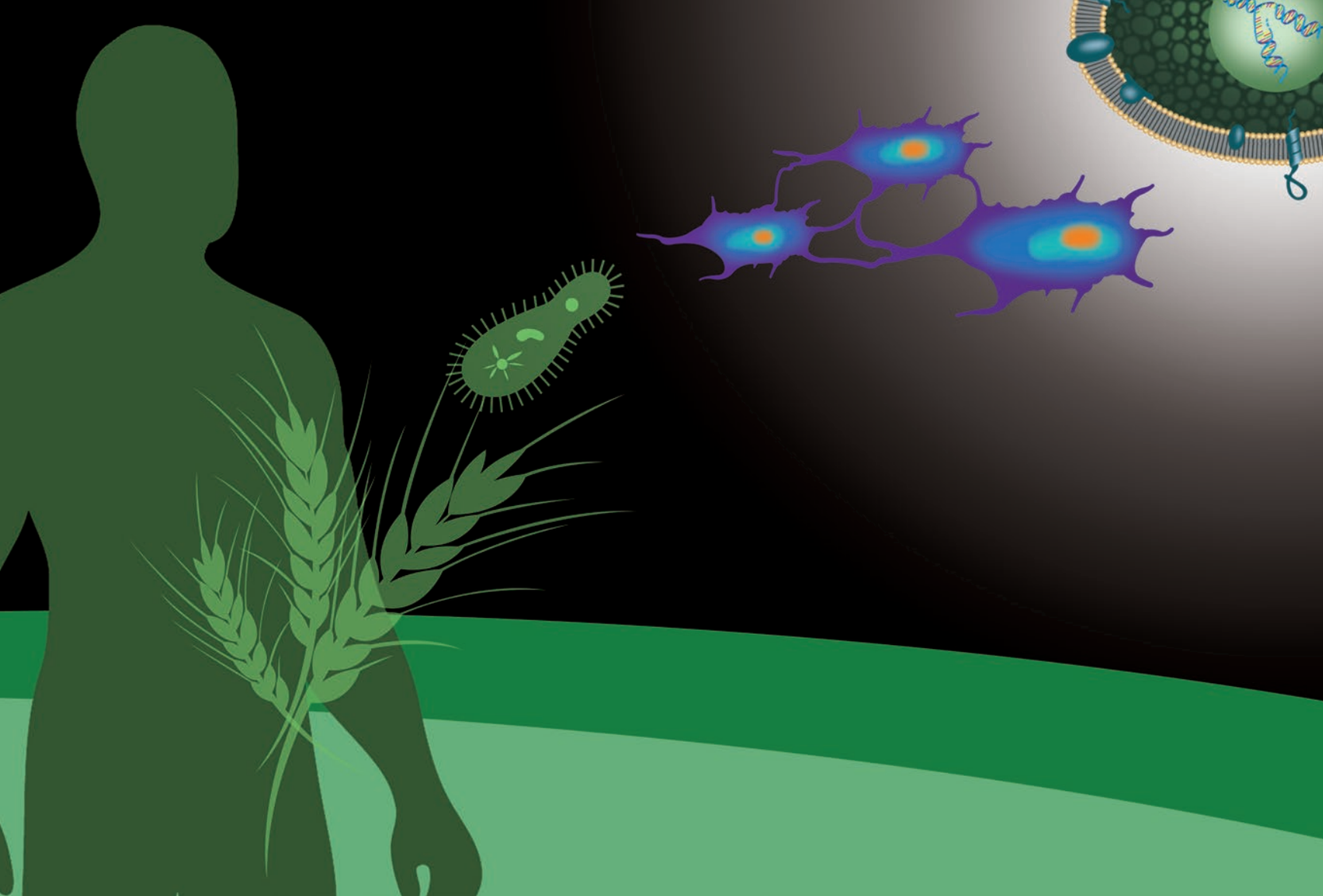
PicoQuant

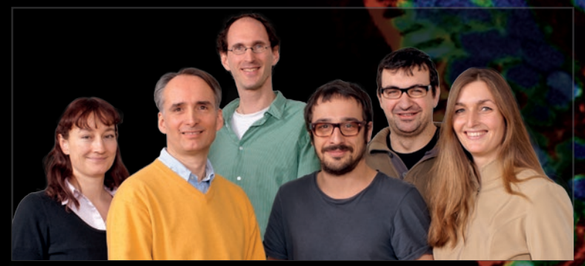
LSM Upgrade Kit

Compact FLIM and FCS Upgrade Kit for Laser Scanning Microscopes





Foreword



Dear Researcher,

PicoQuant has a successful history of more than 20 years in developing and manufacturing instrumentation for both time-resolved spectroscopy and single molecule detection. Since their introduction in 2008, our easy to use and modular Laser Scanning Microscope (LSM) Upgrade Kits are used in leading research institutes, imaging facilities, and industry laboratories all over the world.

Through our deep understanding of scientific applications and excellent expertise in time-resolved techniques, we engineered a series of turn-key Upgrade Kits integrated into LSMs from all major microscopy companies. Our constant development keeps these kits at today's cutting-edge of research and provide a simple way to enhance your research with time-resolved methods such as FLIM, PLIM, FRET, anisotropy, or FCS.

If you are interested in such an outstanding Upgrade Kit for your LSM, please contact us – we are always happy to discuss your individual requirements in detail to enrich your research.

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Vision

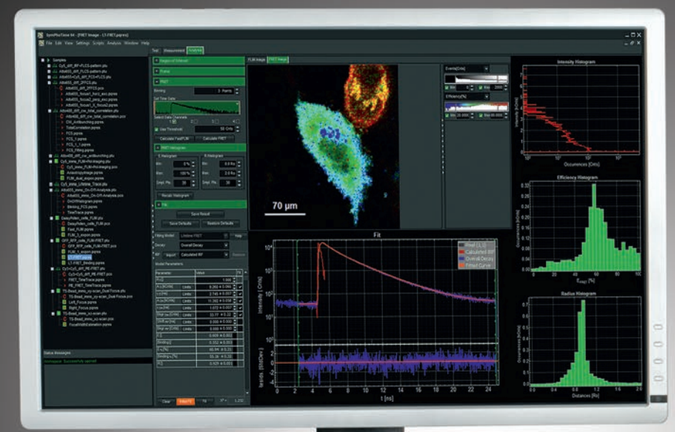
The confocal Laser Scanning Microscope (LSM) is a powerful and widely used tool in life science. It combines high optical resolution with excellent sensitivity, enabling scientists to visualize small structures by recording the spatial location of fluorescent labels in all three dimensions. The capabilities 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 all 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!

Advanced time-resolved applications in a turn-key setup

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

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.



Study various samples with highest sensitivity

Investigate all kinds of samples and labels like in classic confocal microscopy 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.

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 TCSPC wiki.

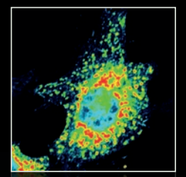
Powerful software integration

The Upgrade Kit features excellent integration with the software from the LSM manufacturer, enabling you to perform time-resolved experiments with ease. Spend your time analyzing your data, not working the software!

Applications

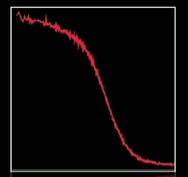
Bring your LSM into the time dimension and gain access to new information on your samples.

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

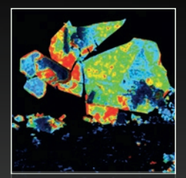


FCS

- Concentration measurements in cells
- Diffusion behavior of molecules

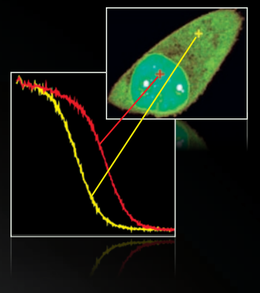
rapid FLIM

- 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

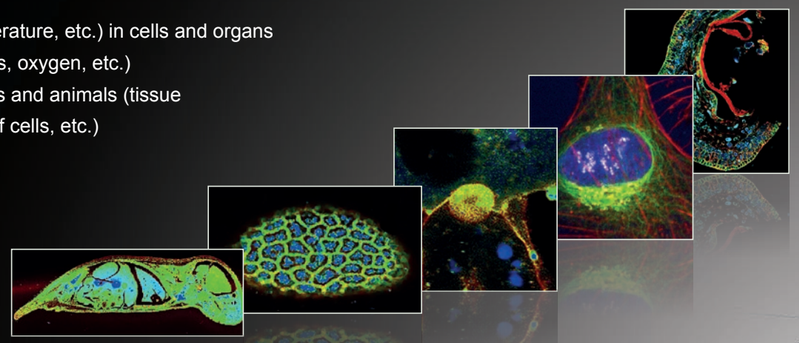


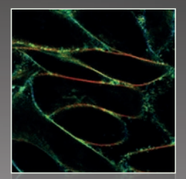
FLCS

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

FLIM

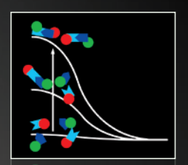
- Environmental sensing (pH, temperature, etc.) in cells and organs
- Concentration measurements (ions, oxygen, etc.)
- Autofluorescence imaging in plants and animals (tissue characterization, metabolic state of cells, etc.)
- Fluorescence lifetime unmixing of spectrally overlapping dyes





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

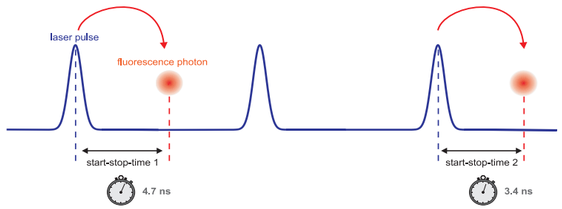
FLIM

Fluorescence Lifetime Imaging (FLIM) provides lifetime as an additional image contrast and allows characterizing the sample based on new parameters

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.

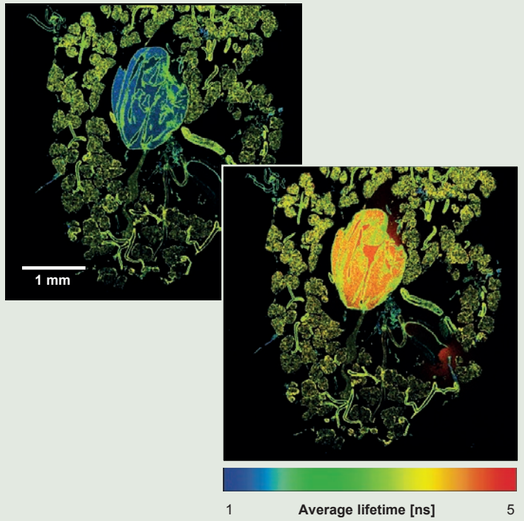


Fluorescence lifetime is an intrinsic feature of each fluorophore. However, it can be influenced by a broad range of environmental conditions, such as pH, ion concentration, molecular binding, or the proximity of energy acceptors in FRET.
FLIM is a more robust imaging method than intensity based ones 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



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.

rapidFLIM

Fast acquisition technique for dynamic FLIM imaging with several frames per second.

Principle

rapidFLIM 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 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.

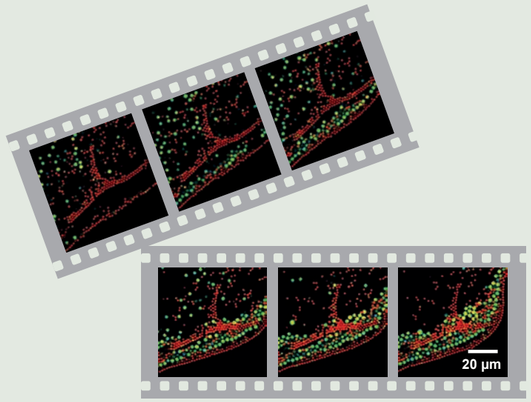
Applications

- 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



The pictures above show the diffusion behavior of two kinds of dye-labeled beads, monitored via rapidFLIM. The large beads with a size of 3.4 µm were labeled with Nile Red showing a fluorescence lifetime of 3.3 ns. The smaller beads (diameter of 2.7 µm) contained the dye Dragon Green, featuring a fluorescence lifetime of 4.0 ns. The two species were distinguished based on their lifetime difference of about 700 ps. The beads formed a random structure at the edge of a water droplet, whose assembly was followed over time. The movie was acquired with 8 µs pixel dwell time, corresponding to about 3 frames per second. FLIM image analysis was performed using the pattern matching FLIM approach.

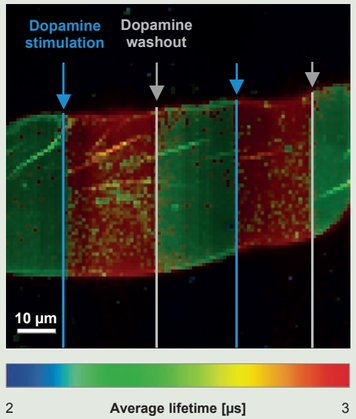
PLIM

The imaging method for characterizing samples with long emission lifetimes, ideally suited for life and materials science.

Principle

Phosphorescence Lifetime Imaging (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.

Example



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.

Applications

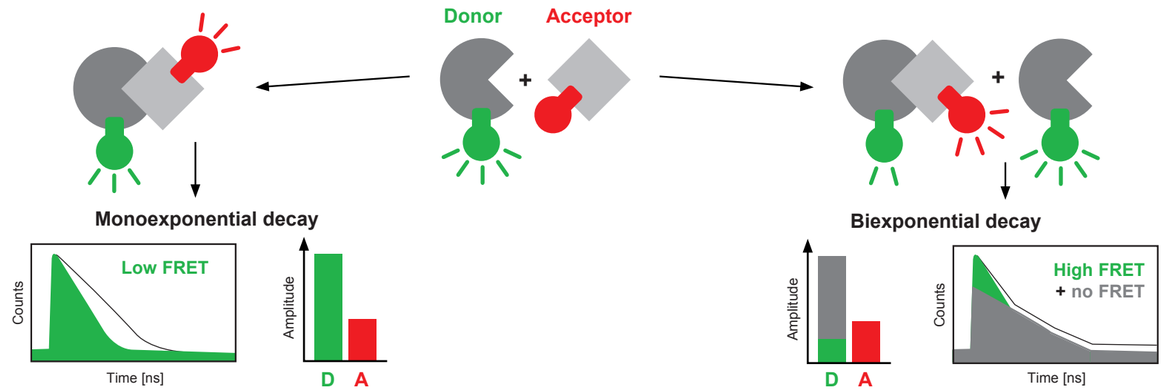
- Sensing environmental conditions (e.g., oxygen concentrations) in cells and living tissues with the help of metal organic complexes
- Monitoring singlet oxygen emission
- Chemical sensing applications
- Characterizing charge carrier mobility in semiconductors
- Detecting defect sites in semiconductor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

"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

FRET

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



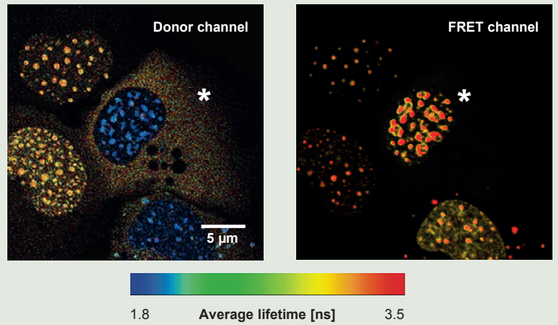
Principle

Förster Resonance Energy Transfer (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
- Distribution and transport of lipids

Example



FLIM images of human U2OS cells expressing the donor CENP-B-Cerulean and additionally the acceptor EYFP-CENP-A. Both kinetochore proteins have been labeled either at their C- or N-terminus and display nuclear localization at the centromeres.
Donor and acceptor fluorescence were monitored with two separate detectors upon selective donor excitation at 485 nm. Due to FRET, the central cell (asterisk) exhibited a quenched donor lifetime (blue color in donor channel) and strong acceptor fluorescence (in the FRET channel) due to the interaction between the proteins CENP-A and CENP-B. Thus demonstrating that both N-terminus of CENP-A and the C-terminus of CENP-B were in direct vicinity.

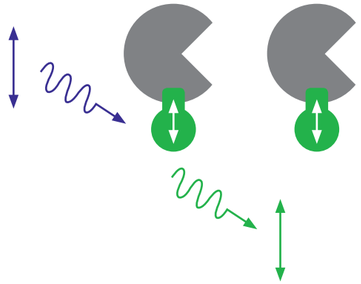
Sample courtesy of S. Orthaus, former member of Leibniz Institute for Age Research, Fritz Lipmann Institute (FLI), Jena, Germany.

Fluorescence Anisotropy

Fluorescence anisotropy indicates the decree of polarization, which can be used to study the orientation, mobility, and interaction of molecules

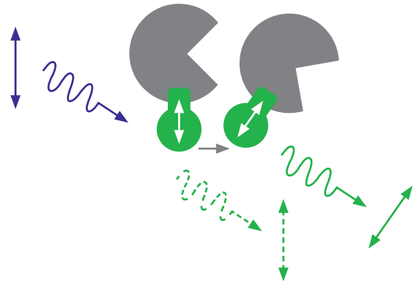
No FRET

Polarization vector is parallel to excitation



Homo-FRET

Polarization vector has changed due to Homo-FRET



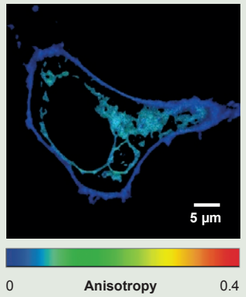
Principle

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 anisotropy 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

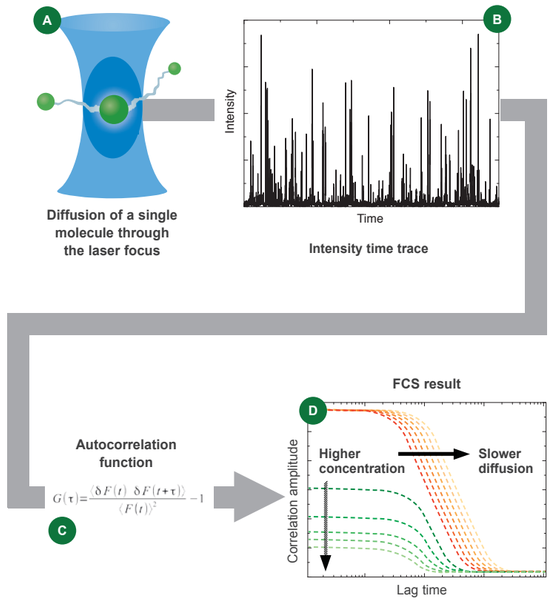


Anisotropy to study protein oligomerization of viral membrane proteins.
A specific viral membrane protein (Gp41) which is part of HIV, is responsible for membrane fusion of the HI virus with the T-cell membrane. After infection and synthesis, it is known to be transported to the plasma membrane via intracellular 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.

FCS

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



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

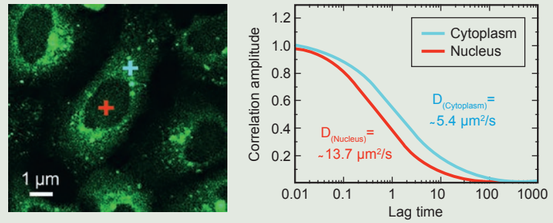
FLCS

- 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



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.

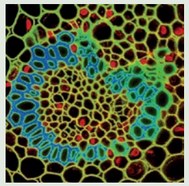
Courtesy of M. Gärtner, P. Schwille, Technical University Dresden, Germany.

TTTR Data Acquisition

The unique Time-Tagged Time-Resolved (TTTR) data acquisition mode is the basis for various applications and records four pieces of information for each photon:

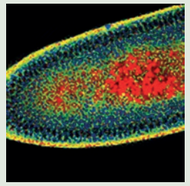
- Start-stop (TCSPC) time **t** needed for FLIM
- Time tag (elapsed time since measurement start) **T** for FCS/FCCS
- Marker signals from the scanner (spatial information) for imaging **M**
- Channel information (polarization or wavelength information) **CH**

Through flexible combination and analysis of the information stored in the TTTR data file, a broad range of analytical methods can be carried out with a single data set.



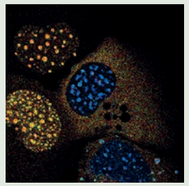
FLIM imaging

T + **t** + **M**



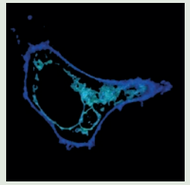
Spectral FLIM imaging

T + **t** + **CH** + **M**



FRET

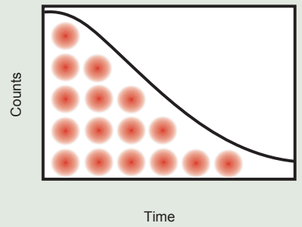
T + **t** + **CH** + **M**



Anisotropy imaging

T + **t** + **CH** + **M**

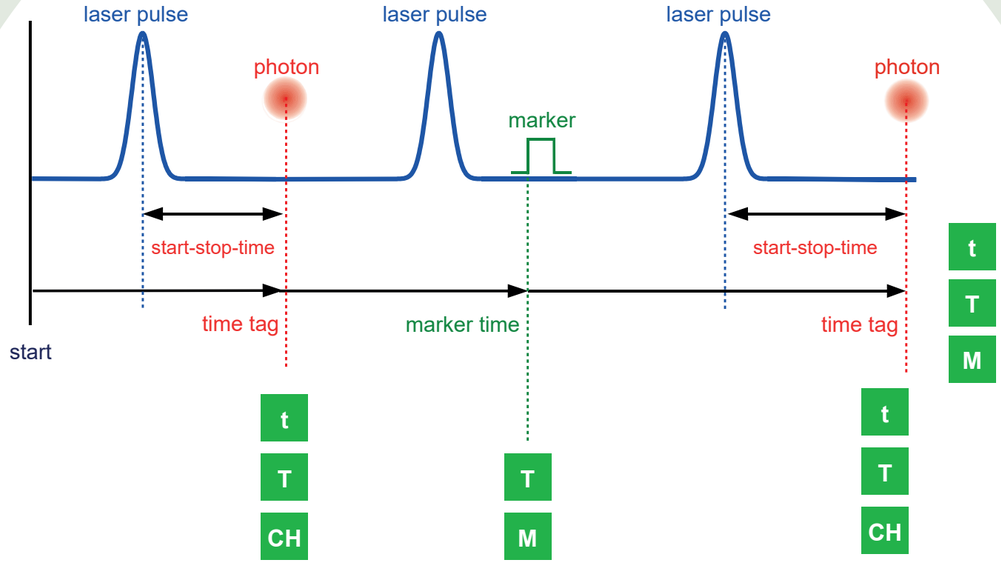
Lifetime measurements **t**

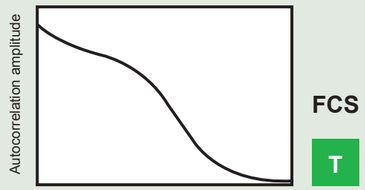


Photon filtering

Photon correlation

Scanner integration



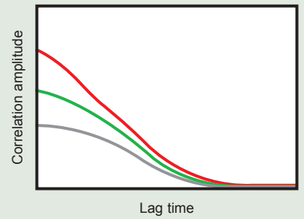


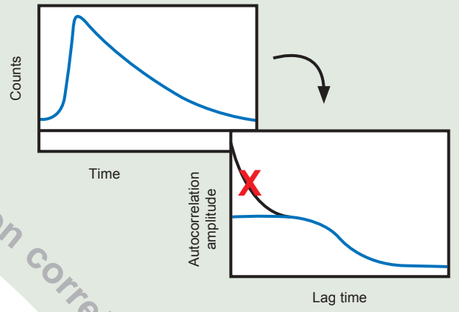
FCS

T

FCCS

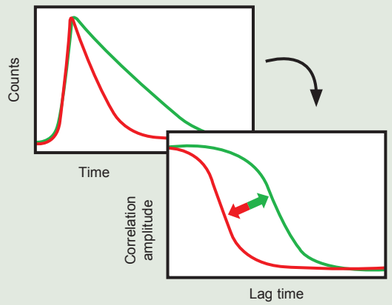
T + **CH**





FLCS

t + **T**



FLCCS

t + **T** + **CH**

System Layout

The PicoQuant LSM Upgrade Kit is an external add-on for laser scanning microscopes granting easy to use, 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

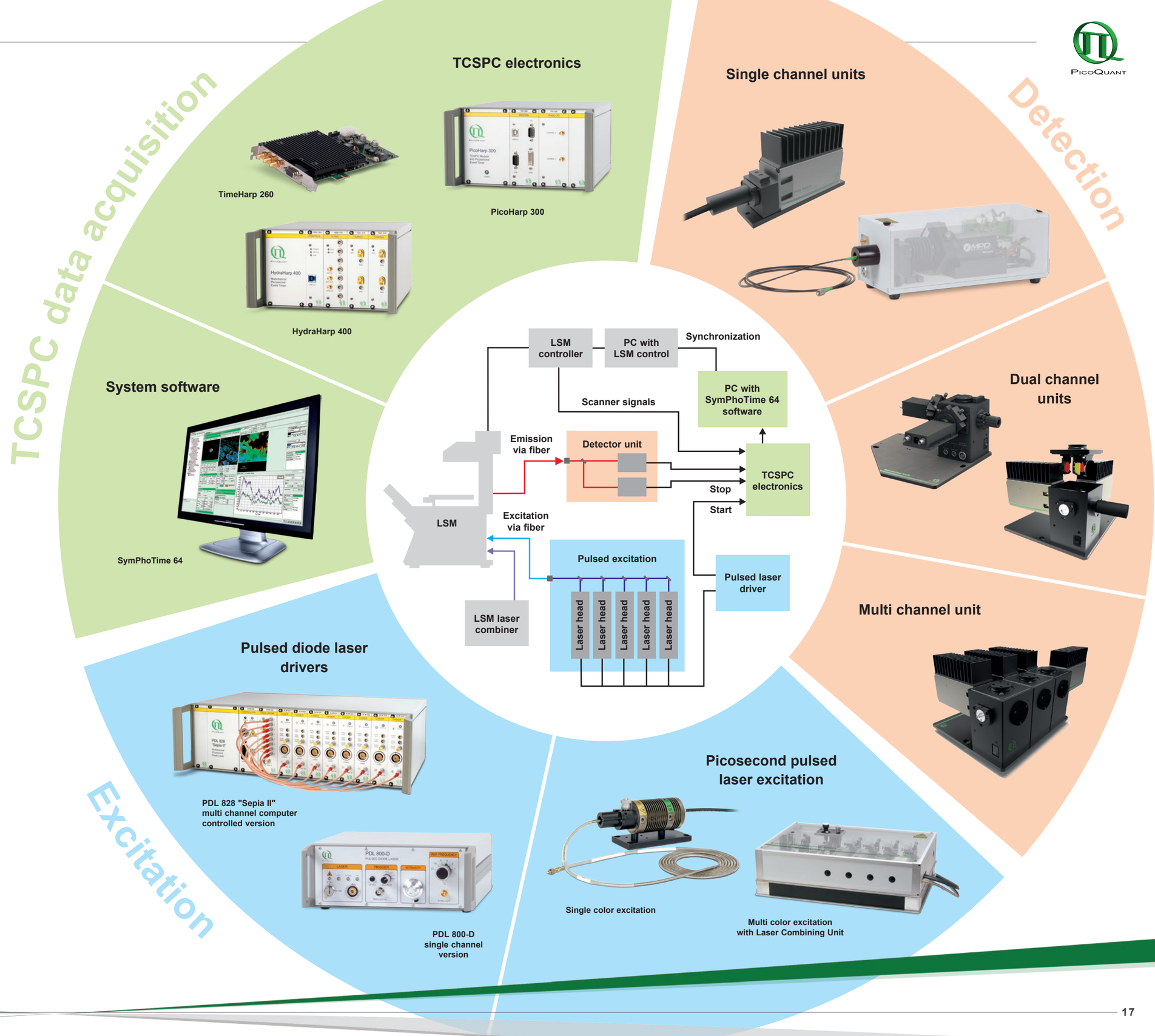
The compact and maintenance-free kit is available for many LSM types from the major microscopy companies Leica, Nikon, Olympus, and Zeiss.

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 the 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



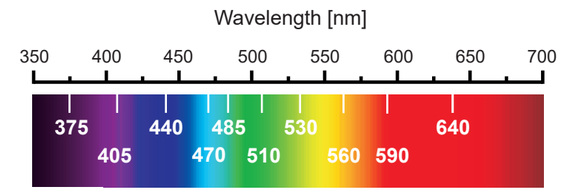
System Components

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.

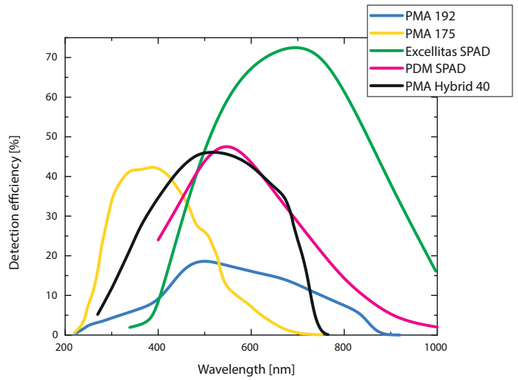
Multi-color pulsed excitation

The excitation subsystem can be configured with multiple laser heads integrated in a Laser Combining Unit (LCU) with each providing a single 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 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 or white light lasers from the Solea family* 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 cross-correlation (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)

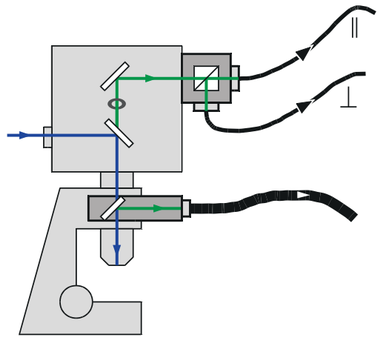
2. Dual channel NDD set-up for deep tissue FLIM and FRET

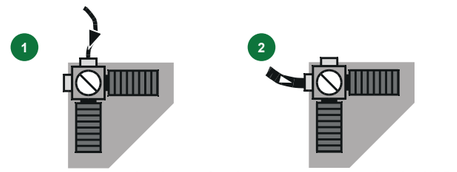
3. Dual channel polarization resolved confocal detection for FLIM, FRET, anisotropy imaging, auto- and crosscorrelation (FCS, FLCS, FCCS, FLCCS)

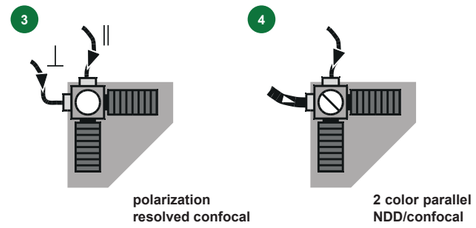
4. Parallel dual channel set-up enabling confocal and non-descanned detection for dual color FLIM, FRET, deep tissue FLIM imaging, auto- and cross correlation (FCS, FLCS, FCCS, FLCCS)

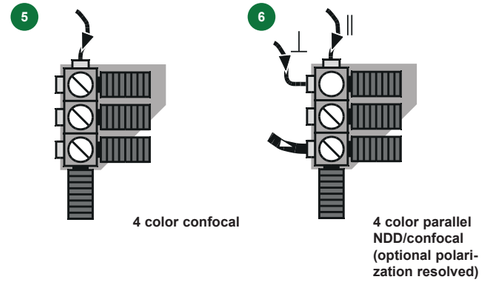
5. Four-channel confocal detection for FLIM, FRET, auto- and cross correlation (FCS, FLCS, FCCS, FLCCS)

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.









* not available in the US

SymPhoTime 64

Advanced and user-friendly software package for cutting edge 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 Leica, 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 (Leica, Nikon, Zeiss)
- Multi-color Fluorescence lifetime imaging (FLIM)
- rapidFLIM 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, 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
Wavelengths	(375)/405-640 nm, Solea supercontinuum laser
Repetition rate	up to 40 MHz (optional 80 MHz)
Pulse width	down to 70 ps (FWHM)

TCSPC Data Acquisition

Type	TimeHarp 260 PICO + long range mode	TimeHarp 260 NANO	HydraHarp 400	PicoHarp 300
Number of detection channels	2	2	up to 8	1
Time resolution (bin width)	25 ps / 2.5 ns	250 ps	1 ps	4 ps
Dead time	< 25 ns / < 2.5 ns	< 2 ns	< 80 ns	< 95 ns
Sustained data throughput	up to 40 million cps	up to 40 million cps	up to 40 million cps	up to 5 million cps

Detectors

Type[1]	Hybrid PMT	SPAD (SPCM-AQRH)	SPAD (PDM Series)	PMT (PMA Series)
Spectral range	300-720 nm	400-1040 nm	400-1000 nm	230-920 nm
Dark counts (at 20°, typ. value)	300-100 cps	< 100 cps	< 250 cps	< 900 cps
Instrument Response Function (IRF at 650 nm)	< 120 ps	< 350 ps	< 100 ps[6]	< 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 (antibunching)
Fluorescence Lifetime Imaging (FLIM)	(Time-gated) Fluorescence Intensity Imaging; Fluorescence Lifetime Imaging (FLIM); 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]

Leica	TCS SP8, TCS SP5
Nikon	A1, C2, C1si
Olympus	FluoView FV 3000, FVMPE-RS, FV 1200 (MPE), FV 1000 (MPE)
Zeiss	LSM 880 (NLO), LSM 780, LSM 710

1) Other laser lines or detector types available upon request.
2) Existing multiphoton (Ti:Sapphire) 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

PicoQuant for Scientists

The annual workshop on Single Molecule Spectroscopy and Super-resolution Microscopy in the Life Sciences brings together top researchers from this field.

Application lab

We at PicoQuant are committed to provide innovative products for time-resolve spectroscopy and microscopy applications to our customers. Our team of experts is at your disposal for discussing your scientific challenges and offer solutions tailored to your requirements. Of course, all of our products, including the single molecule sensitive MicroTime microscopy platform and time-resolved FluoTime fluorescence spectrometers, are available for testing and evaluation.

PicoQuant also hosts a number of Application and Technical Notes, tutorial videos and extensive bibliography on www.picoquant.com/scientific as well as a wiki focusing on TCSPC and its many applications (www.tcspc.com).

Courses on time-resolved microscopy

To improve the understanding and promote the use of time-resolved fluorescence spectroscopy and microscopy, PicoQuant established the “European Short Course on Time-resolved Microscopy and Correlation Spectroscopy ” as an annual event in 2009. This course, along with a similar one on the “Principles and Applications of Time-resolved Fluorescence Spectroscopy", is intended for individuals seeking an in-depth introduction to the principles of fluorescence microscopy and spectroscopy as well as their applications in many scientific fields. The courses consist of lectures by renowned scientists followed by practical sessions on the instrumentation and its software.

Workshop on single molecule spectroscopy

Since 1994, scientists from PicoQuant organize the annual workshop on “Single Molecule Spectroscopy and Super-resolution Microscopy in the Life Sciences” that brings together top researchers from this field. With this event, we continue to encourage the exchange of knowledge and new ideas between experts in single molecule spectroscopy and super-resolution microscopy, interested scientists from other fields, and potential users from the life science industry.

PicoQuant GmbH

PicoQuant was founded in 1996 to develop robust, compact, and easy to use time-resolved instrumentation and systems. Since April 2008 sales and support in North America is handled by PicoQuant Photonics North America Inc.

Today, PicoQuant is known as a company leading in the field of pulsed diode lasers, time-resolved data acquisition, single photon counting, and fluorescence instrumentation. Our instruments are present all over the world. They are used in the laboratories of Nobel Laureates to help generating data for papers in high-ranking journals as well as for carrying out routine quality control in production processes of global industrial players.

Starting from traditional time-resolved fluorescence detection in bioanalytics, the range of applications is continuously increasing and includes semiconductor quality control, diffuse optical imaging and materials research, quantum information processing, optical detector testing, and telecommunications. Due to the ease of use of our products, researchers can now focus on their scientific questions in biology, medicine, environmental science, quantum optics, or chemistry without needing a large background in physics, electronics, and optics.

We offer state-of-the-art technology

Our goal is to offer state-of-the-art technology that has been co-developed and tested by renowned researchers, at an affordable price for both scientific groups and price conscious industry.

We have successfully teamed up with all major confocal microscopy companies to develop dedicated equipment that permits time-resolved fluorescence studies on their laser scanning microscopes. Following this philosophy, we are always looking for new challenges. PicoQuant especially encourages OEM inquiries for its products, notably for applications where implementing time-resolved techniques were considered too expensive and cumbersome in the past.

More than 20 years of R & D work

The combination of more than 20 years of R & D work, several thousand units sold, and cooperation with international experts for special applications forms the basis for new outstanding developments which are always driven by our customers’ needs and inspirations. We invite you to visit our website or contact our product and application specialists directly to discuss your specific needs. And, of course, you are always welcome to visit our application labs during your travels to Germany.

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