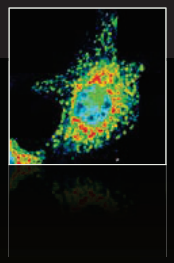
Applications

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

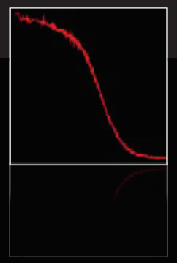
Expanding the capabilities of an LSM with one of PicoQuants Upgrade Kits gives the user access to a large variety of innovative time-resolved applications.

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

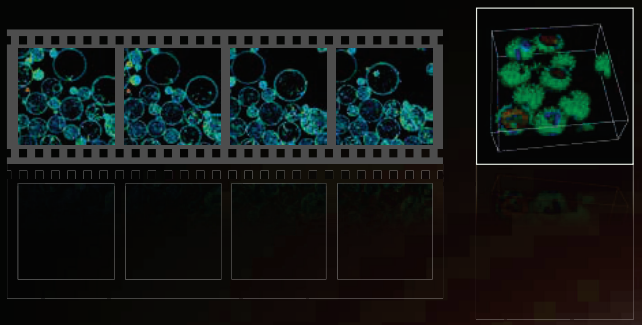


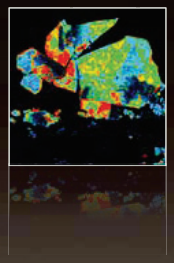
FCS

- Concentration measurements in cells
- Diffusion behavior of molecules

rapid FLIM^{HiRes}

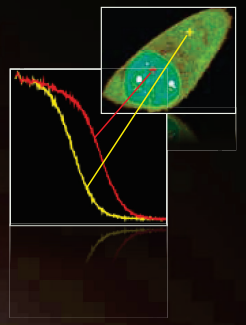
- In vivo FLIM
- FRET in rapidly moving objects
- Fast imaging of environmental parameters





PLIM

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

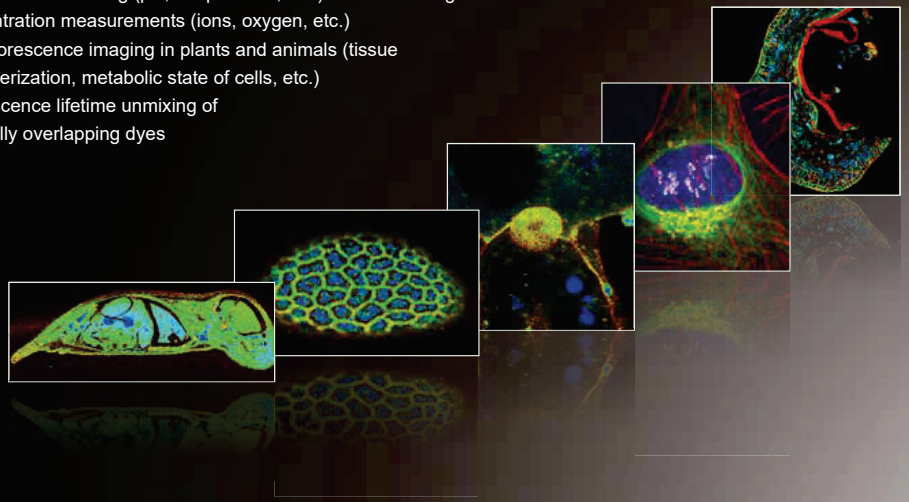


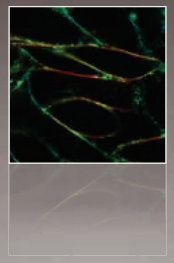
FLCS

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

FLIM

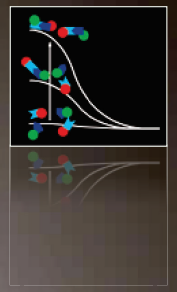
- Environmental sensing (pH, temperature, etc.) in cells and organs
- Concentration measurements (ions, oxygen, etc.)
- Autofluorescence imaging in plants and animals (tissue characterization, metabolic state of cells, etc.)
- Fluorescence lifetime unmixing of spectrally overlapping dyes





Anisotropy

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



FLCCS

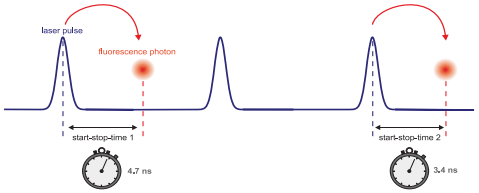
- Cross-talk free detection of molecular interactions
- Complex formation, stoichiometry, and binding kinetics

FLIM

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

Principle

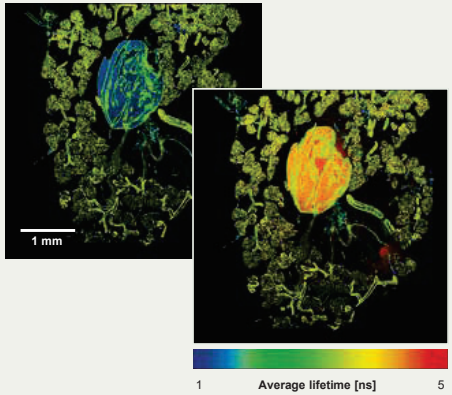
FLIM produces an image by recording for every pixel differences in the excited state decay rate from a fluorescent sample, which are typically in the range of picoseconds to nanoseconds. In a FLIM image, contrast is based on the fluorescence lifetime, which is defined as the average time a molecule remains 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



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^{HiRes}

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

Principle

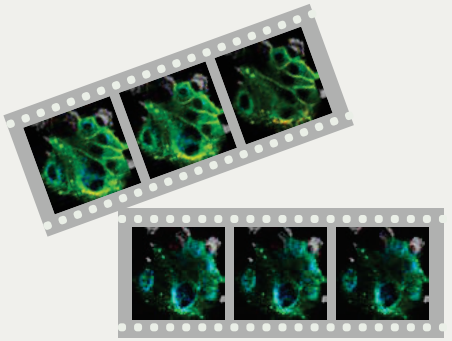
rapidFLIM^{HiRes} is a novel approach for acquiring FLIM images in a very fast manner by exploiting optimized hardware components such as TCSPC boards and detectors with ultra short dead times. The optimized hardware eliminates the measurement artifacts that would otherwise occur in conventional FLIM 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.

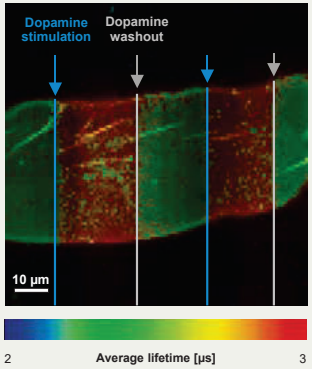
Principle

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



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.

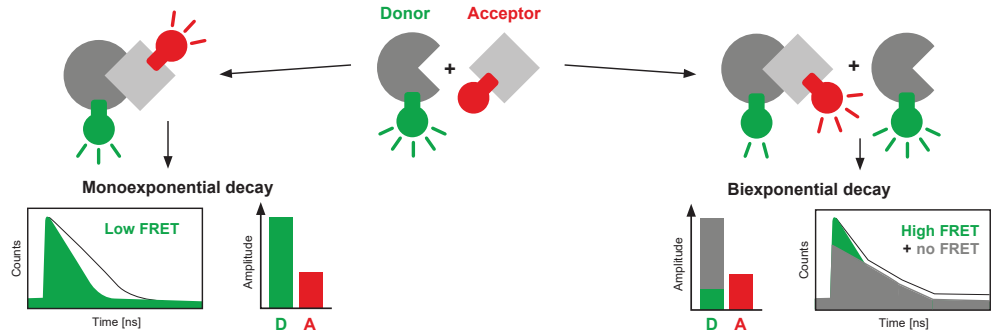
Data courtesy of K. Jahn, C. Hille, University of Potsdam, Germany.

"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 (FRET) enables distance measurements in the nanometer range as well as observation and quantification of molecular interactions.



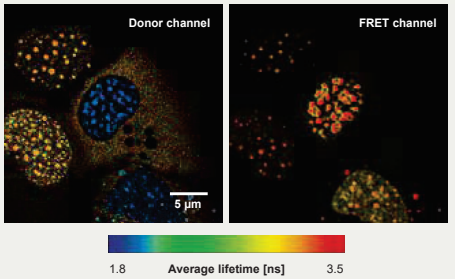
Principle

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

Example



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.

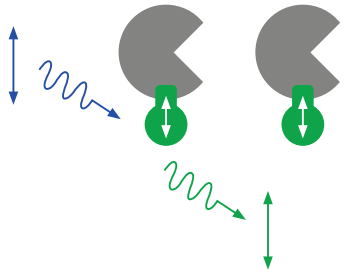
Sample and data courtesy of M. Gonzalez-Gaitan, Biochemistry Department, University of Geneva, Switzerland.

Fluorescence Anisotropy

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

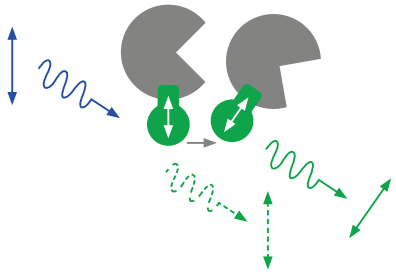
No FRET

Polarization vector is parallel to excitation



Homo-FRET

Polarization vector has changed due to Homo-FRET



Principle

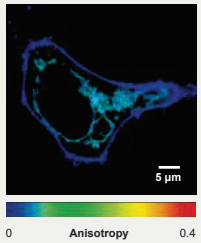
After excitation with polarized light the fluorescence emitted by a molecule is also polarized. The extent of fluorescence polarization is described as anisotropy. The emission can become depolarized by a number of processes, including rotational diffusion, which depends on the viscosity of the solvent,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

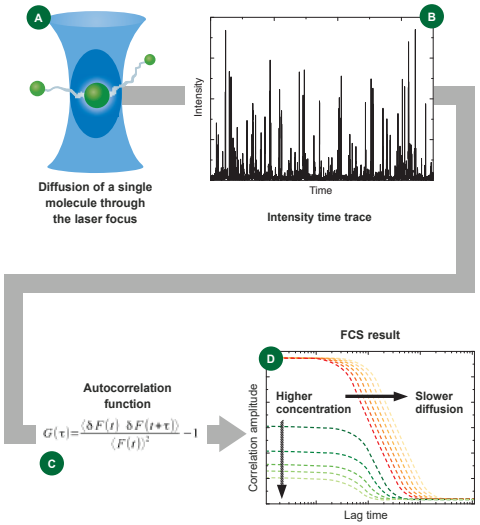


Anisotropy to study protein oligomerization of viral membrane proteins. A specific viral membrane protein (Gp41) which is part of HIV, is responsible for membrane fusion of the HI virus with the T-cell membrane. After infection and synthesis, it is known to be transported to the plasma membrane via intracellular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

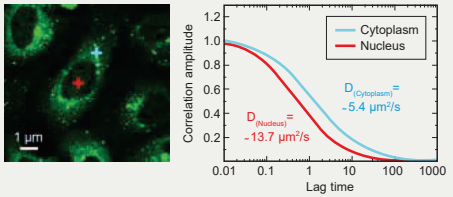
FLCS

- 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.