





LSM Upgrade Kit



PICOQUANT

Compact Lifetime and FCS Upgrade Kit for Laser Scanning Microscopes

- Picosecond time resolution
- Turn-key diode lasers
- Time-Correlated Single Photon Counting (TCSPC)
- Advanced data analysis software
- Supported LSMs:

 TCS SP5, SP2,  C1, C1si, A1,  LSM510, LSM710, LSM780
 FluoView FV300, FluoView FV1000, Fluoview FV1000MPE



1) Sample courtesy of Sandra Orthaus, former member of Fritz Lipmann Institute, Leipzig, Germany
2) Sample courtesy of Philippe Bastiaens, Max Planck Institute of Molecular Physiology, Dortmund, Germany

Applications

- Time-resolved microscopy in biology and chemistry
- Fluorescence Lifetime Imaging (FLIM)
- Förster Resonance Energy Transfer (FRET)
- Fluorescence Correlation Spectroscopy (FCS, FLCS, FCCS)
- Single molecule spectroscopy
- Mapping of cell parameters (pH, protein binding, ion concentration, etc.)

Components

The complete kit to upgrade Laser Scanning Microscopes (LSMs) towards time-resolved measurements consists of a laser excitation system, a photon counting detector, a data acquisition unit and dedicated software to analyze the measurement results. Optical fibers are used to couple the excitation light into the microscope and to guide the fluorescence emission to the detector. The synchronization with the LSM is done via the corresponding signals provided by the LSM controller.

Excitation

The excitation subsystem consists of a pulsed diode laser driver and different laser heads with pulses in the picosecond time regime (additional, cw mode is available as an option). The available wavelengths range from 375 nm to 900 nm. The laser heads are integrated along with multiple optical components in one Laser Combining Unit (LCU) for easier handling and coupling into an optical fiber.



TCSPC data acquisition

The photon counting module PicoHarp 300 contains the complete timing electronics for Time-Correlated Single Photon Counting (TCSPC) with picosecond resolution. The versatile Time-Tagged Time-Resolved mode (TTTR) is used to study fluorescence dynamics and allows to synchronize the measurement with external events through special marker signals. These markers allow later the reconstruction of 2D or 3D images. If more than one detector is required, a router solution allows to connect and record the signals of up to 4 detectors simultaneously.



Detector

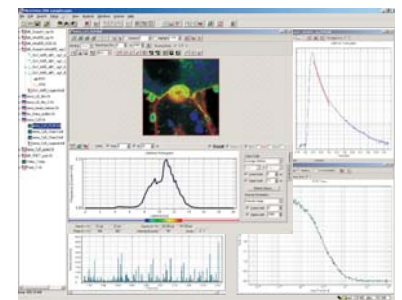
The standard detectors for the upgrade kit are Single Photon Avalanche Diodes (SPADs) which feature a high temporal resolution and a very high detection efficiency, necessary e.g. for single molecule studies or Fluorescence Correlation Spectroscopy (FCS). As an alternative, Photomultiplier tubes (PMTs) can be used. As they have a lower detection efficiency than SPADs, they are not suited for FCS, but can still be used for FLIM. In case of the Olympus FluoView FV1000MPE system it is also possible to use the internal NDD PMT detectors for FLIM measurements.

One or two detector channel set-ups are available with integrated filter holders that allow quick change of emission filters in order to adapt to different experimental conditions. The detector(s) are usually connected to the LSM via a suited optical fiber to an appropriate fiber exit port of the microscope (except NDD set-ups).

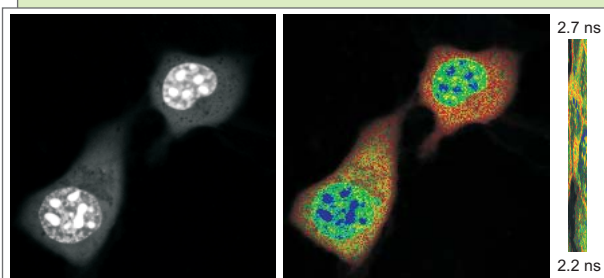


Software

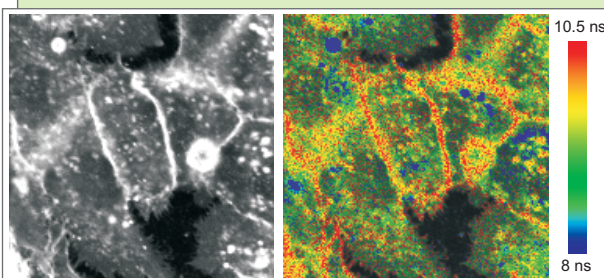
The SymPhoTime software is based on the powerful but generic TTTR data collection. Users can perform an unlimited number of analysis steps without losing track of the interdependence and origin of their measurement and analysis data. All derived data is maintained in the hierarchic workspace, including a log file, keeping track of all measurement and analysis steps. The analysis possibilities include intensity time traces, burst analysis, lifetime histogramming, Fluorescence Correlation Spectroscopy (FCS), Förster Resonance Energy Transfer (FRET) and Fluorescence Lifetime Imaging (FLIM), to name only a few. The lifetime image as well as FCS auto- and cross correlation curves are already displayed during the data acquisition. Up to four lifetimes can be fitted for each pixel or selected regions of the image. Mixtures of different dye molecules can thus be analysed. Reconvolution of the TCSPC histogram can be performed with a measured or fitted Instrument Response Function (IRF) in order to yield high accuracy even for very short lifetimes.



Measurement Examples



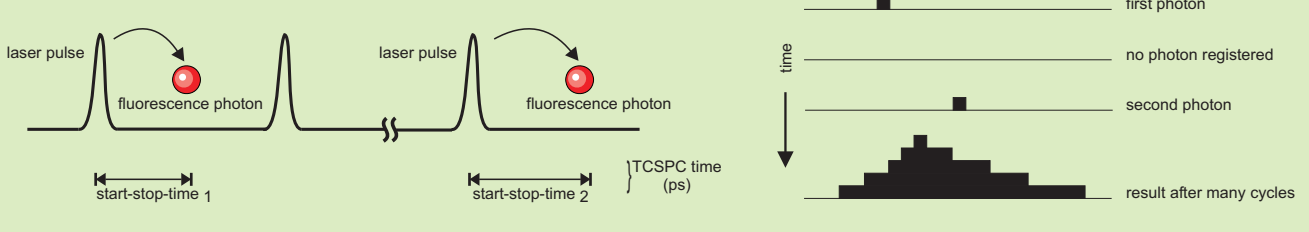
Interactions of protein partners in their natural environment inside living cells can be studied with time-resolved FRET microscopy. The technique was used to characterize intra-nuclear dimer formation for the transcription factor C/EBP α in living pituitary GHFT1-5 cells of mice. Members of the C/EBP family of transcription factors are critical determinants of cell differentiation. Dimerization of CFP-YFP-C/EBP Δ 154 protein molecules in the cell nucleus could be detected with FRET. Excitation with 440 nm, detection around 470 nm. (Sample courtesy of Ye Chen and Ammasi Periasamy, FRET Workshop, Keck Center for Cellular Imaging, University of Virginia)



For this lifetime image a cancer cell line of liver cells was cultivated. The cells were stained with phospholipids labeled with NBD, a dye whose lifetime is depending on the hydrophobicity of its surrounding environment. The lifetime allows to gain information about the molecular structure of cellular compartments. The fluorescence was excited with a wavelength of 470 nm and detected using a 500 nm longpass filter. Left image: intensity image; right image: calculated fluorescence lifetime image from the time-resolved measurement in false coloring. (Sample courtesy of Astrid Tannert and Thomas Korte, Humboldt University Berlin, Molecular Biophysics, Germany)

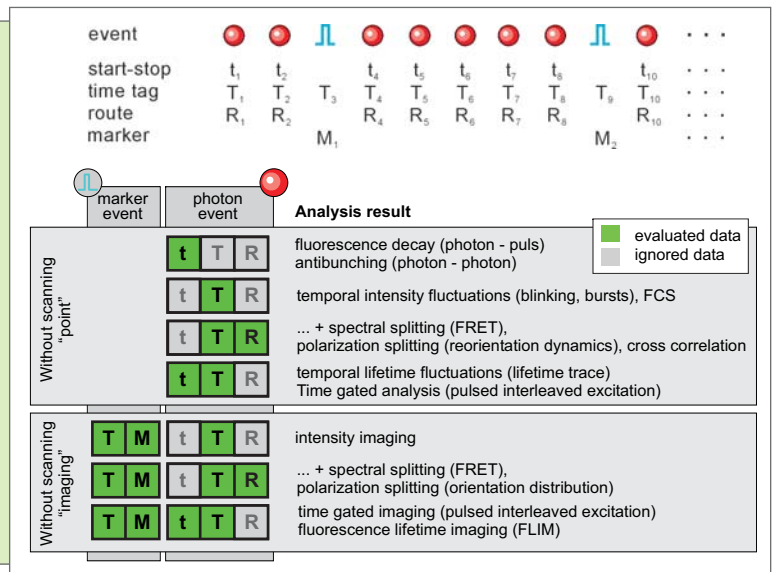
What is TCSPC ?

Time-Correlated Single Photon Counting (TCSPC) is the most powerful method to measure fluorescence lifetimes, especially for very short lifetimes, e.g. in the picosecond to nanosecond regime and low fluorescence intensities, e.g. in single molecule studies. The method is based on the repetitive precise measurement of the time difference between the excitation (e.g. the laser pulse) and the subsequent emission of a fluorescence photon. As only a single photon is registered, the measurement is repeated very often and the measured time differences are sorted into a histogram. This histogram of photon arrival times can then be analyzed to extract the fluorescence lifetime.



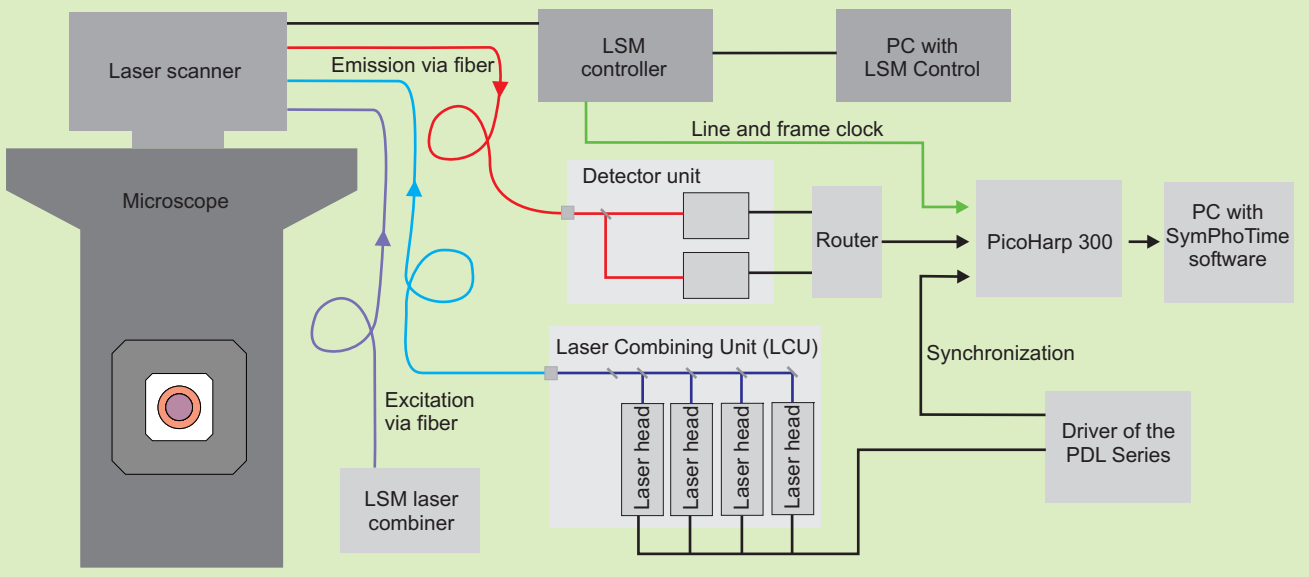
What is TTTR ?

In a typical TCSPC measurement, the measured time differences between the excitation pulse and the fluorescence photon are sorted into a histogram, which represents the fluorescence decay. This method is, however, not applicable to study dynamic processes, e.g. molecules diffusing through the detection volume or the movement of a laser scanner. For this purpose, the start-stop events are not sorted into a histogram, but stored directly along with an additional time information (time tag) that represents the arrival time of the photon with respect to the beginning of the experiment. This is called Time-Tagged Time-Resolved (TTTR) measurement mode. An additional feature of this mode is that external marker signals or routing information can also be incorporated, leading to a great flexibility in measurement and analysis modes.





Synchronization

In order to perform imaging, the spatial origin of the photons must be recovered, which requires a synchronization information from the LSM controller. Typically an implementation with synchronization signals at the beginning of each line and frame is used. The TTTR mode allows to record such synchronization signals with a ns time tag. These signal markers will appear as special bits in the TTTR data stream generated during the measurement. This makes it possible to reconstruct the 2D image from the stream of TTTR records, since the relevant XY position of the laser scanner can be determined during the data analysis. Each individual frame remains available for analysis.



Specifications

Excitation Source ¹⁾				
Light source ⁴⁾	Picosecond Laser Diode Heads			
Wavelengths	405 - 510 nm, 530 nm, 635 - 900 nm			
	375 nm on request			
Repetition rate	up to 40 MHz (optional 80 MHz)			
Pulse width	down to 70 ps (FWHM)			
Detectors				
Type ¹⁾	PMT (PMA Series)	SPAD (PDM Series)	SPAD (τ-SPAD)	
Spectral range	185 - 650 nm	185 - 820 nm	400 - 1000 nm	400 - 1000 nm
Dark counts (at 20°C)	< 50 cps	< 900 cps	< 250 cps	< 100 cps
Instrument Response Function (IRF λ=650 nm)	typ. 200 ps	typ. 50 ps	typ. 50 ps	typ. 400 ps
TCSPC Data Acquisition				
Type	PicoHarp 300			
Time resolution (bin width)	4 ps			
Dead time	< 95 ns			
Time ranges	260 ns to 2 μs			
Time tag resolution (TTTR mode)	equals excitation period			
Sustained data throughput in TTTR mode	up to 5 million counts/second			
Maximum collection time	virtually unlimited, restricted only by computer memory/harddisk capacity			
Software Features ²⁾				
General concept	use of versatile TTTR file format for data acquisition, data archiving in workspace, time gating for all methods, separation of up to four detector signals			
Point measurements	data conversion to: MCS trace, FCS calculation and fitting, TCSPC histogram, on/off-state histogram, burst size analysis, (PIE-)FRET histogram, photon counting histogram, lifetime histogram, FCCS, FLCS			
Fluorescence Lifetime Imaging (FLIM)	data conversion to: fluorescence intensity images (max. image size 512 × 512 pixel), fluorescence lifetime images, time gated analysis, TCSPC histogram for region of interest			
Operational and Electrical				
Operating environment	PC Pentium/Athlon class, 3 GHz, dual core, 4 GB memory with Windows™ 7			
Power requirements	110/230 V, 50/60 Hz			
Supported LSMs ³⁾				
Leica	TCS SP5, SP2			
Nikon	C1, C1si, A1			
Olympus	FluoView FV300, FluoView FV1000, FluoView FV1000MPE			
Zeiss	LSM 510, LSM 710, LSM 780			
<p>1) Lasers, other detectors and cooling available upon request.</p> <p>2) For details please see our SymPhoTime data sheet.</p> <p>3) Upgrades of other LSM types are also possible, please contact us for details.</p> <p>4) Class 3B lasers - will increase the classification of your LSM accordingly.</p>				

Other PicoQuant Systems

FluoTime 300 "EasyTau"
Automated fluorescence lifetime spectrometer



FluoTime 200
High performance fluorescence lifetime system



MicroTime 200
Inverse time-resolved fluorescence microscope



MicroTime 100
Upright time-resolved fluorescence microscope



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