PicoQuant

SymPhoTime 64

A software package for cutting edge fluorescence applications

Vision

A one-stop solution for cutting edge applications

The SymPhoTime 64 software package is an integrated solution for data acquisition and analysis using PicoQuant's time-resolved confocal microscopes, LSM Upgrade Kits or TCSPC electronics. The clearly structured layout and powerful analysis routines allow the user to focus on the results rather than on data processing.

Time-resolved fluorescence spectroscopy has evolved to become a fundamental method for a wide field of research topics ranging from biological to materials research. These multiple applications put high demands on the capabilities of a software that should address the needs of different users working in time-resolved fluorescence spectroscopy. The conformance to the various requirements were the fundamental design goal of SymPhoTime 64, a software package that aims at facilitating data acquisition as well as data analysis by providing a clearly structured layout and a wide range of customizable analysis procedures. With SymPhoTime 64 PicoQuant answers the demands of the ever-evolving area of time-resolved fluorescence spectroscopy.

Intuitive user interface

Data workspace, user-defined settings, different themes, ...

Extensive online visualization

FLIM, FCS, time traces or TCSPC histograms

Image analysis

FLIM, FRET, anisotropy, multiple ROIs, ...

Correlation analysis

FCS, FCCS, FLCS, antibunching, total correlation, ...

Time trace analysis

MCS, (PIE-)FRET, on/off histograms, anisotropy, ...

Integrated scripting language

User-defined analysis procedures, GUIs, additional fitting models, ...

Intuitive data acquisition

For MicroTime Series and LSM Upgrade Kits

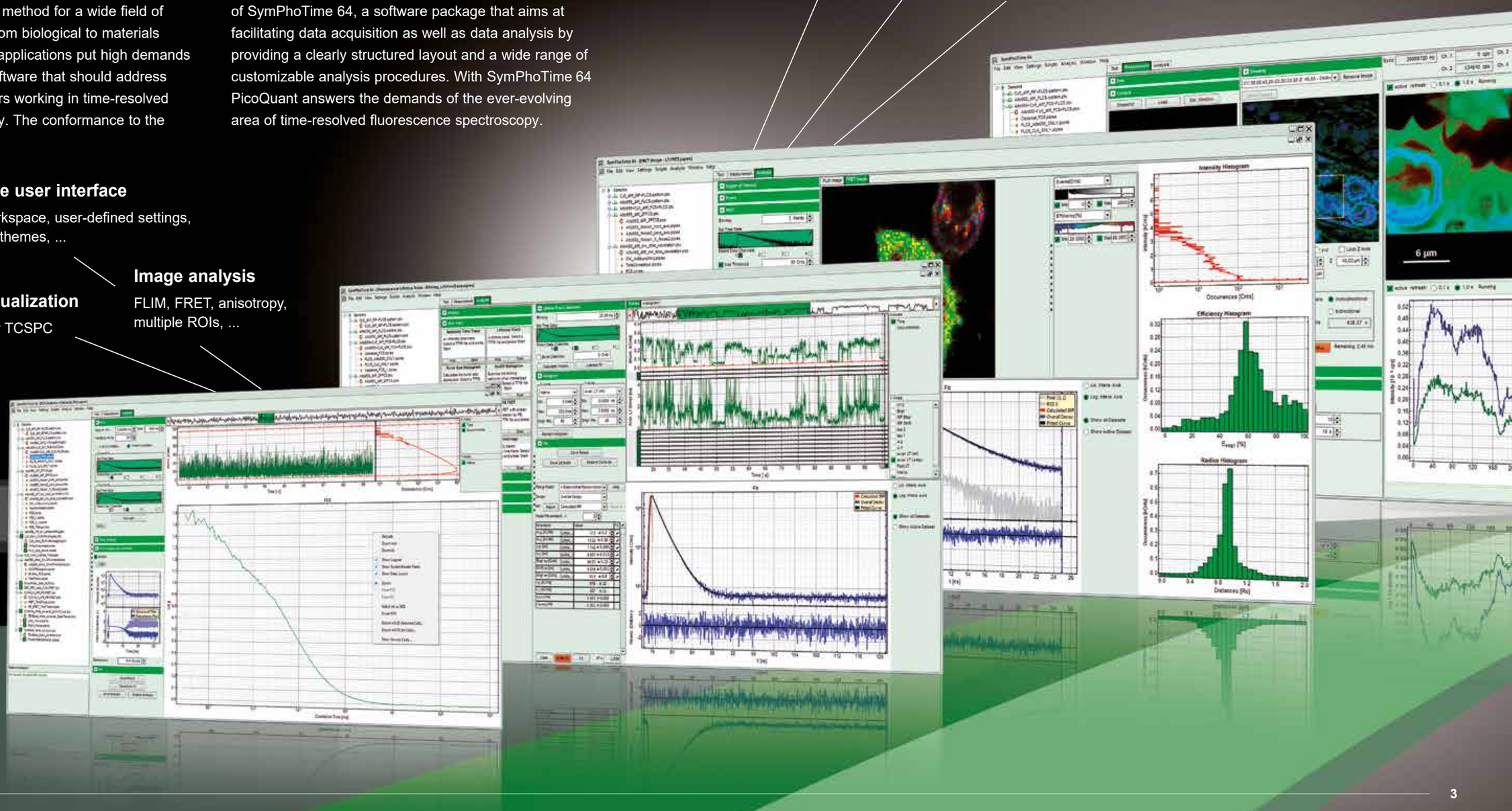
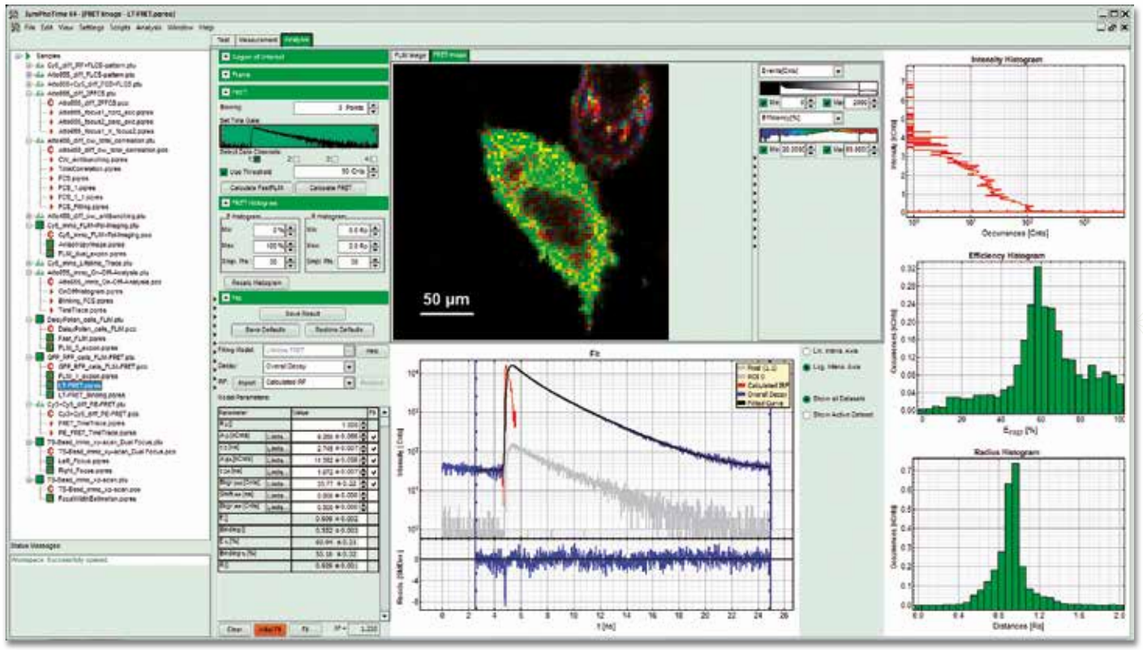


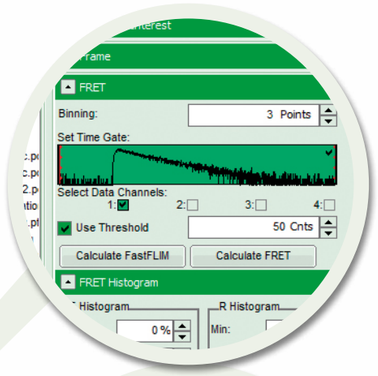
Image analysis

With SymPhoTime 64, analysis of (time-resolved) imaging measurements will be easier and faster than ever before. The software provides specially adapted interfaces for many standard analysis procedures ranging from Fluorescence Lifetime Imaging (FLIM) to Förster Resonance Energy Transfer (FRET) and anisotropy. Each interface provides only those procedures that are directly required for the special analysis. This ensures that users can fully concentrate on analyzing their data.



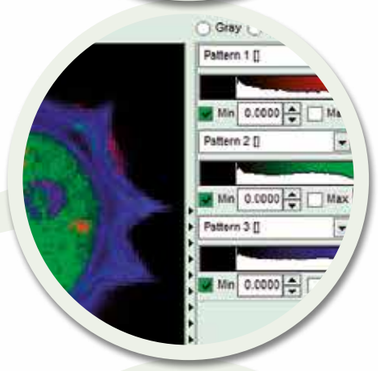
Fluorescence Lifetime Imaging (FLIM)

SymPhoTime 64 supports analysis of images with dimensions up to 4096 x 4096 pixels. As a first analysis step, a special “fast FLIM” procedure can be applied that yields immediate results and is therefore very useful for a quick preview, assessment of the image quality or the selection of regions-of-interest (ROIs) for more detailed analysis. A detailed FLIM analysis is then based on fitting an exponential decay function to the acquired fluorescence decay in each image pixel. In that way, the SymPhoTime 64 software permits to extract up to five different lifetimes and related amplitudes from the measured data. Even the finite temporal resolution of the system (Instrument Response Function, IRF) can be corrected using a dedicated numerical reconvolution algorithm with either measured data or individually calculated correction profiles.



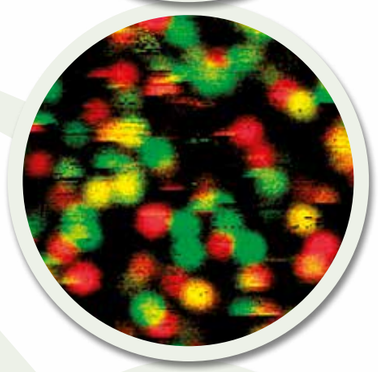
Time gating, binning, multichannel support

Time gating, binning and up to eight detection channels are supported for all measurements. Arbitrary time gates permit to use only selected parts of the raw data, which can be used to e.g., remove scattered light. Binning combines several adjacent image pixels and can lead to an improved signal-to-noise ratio in the final image.



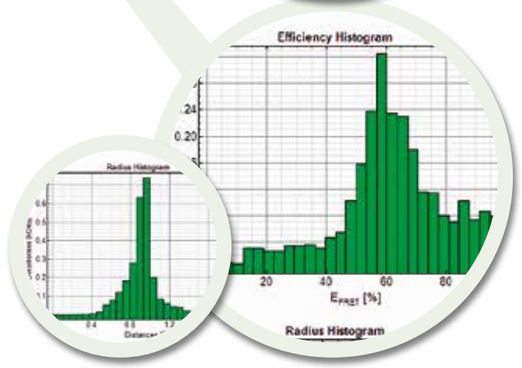
Pattern matching for advanced analysis

SymPhoTime 64 includes a unique image analysis procedure based on a pattern matching algorithm that enables decomposition of an image into the contributions from individual subcomponents based on overall decay shapes. This procedure thus generates individual images for the separate subcomponents, e.g., autofluorescence contribution, FRET and non-FRET species or different fluorescence dyes contained in the sample.



Fluorescence anisotropy imaging

With the SymPhoTime 64 fluorescence anisotropy measurements based on the fluorescence intensity can be evaluated. Separate images are generated for both parallel and perpendicular fluorescence orientation, and a histogram of the anisotropy values in each image pixel is calculated. The different detection efficiencies of both detection channels can also be taken into account (“G-Factor”).

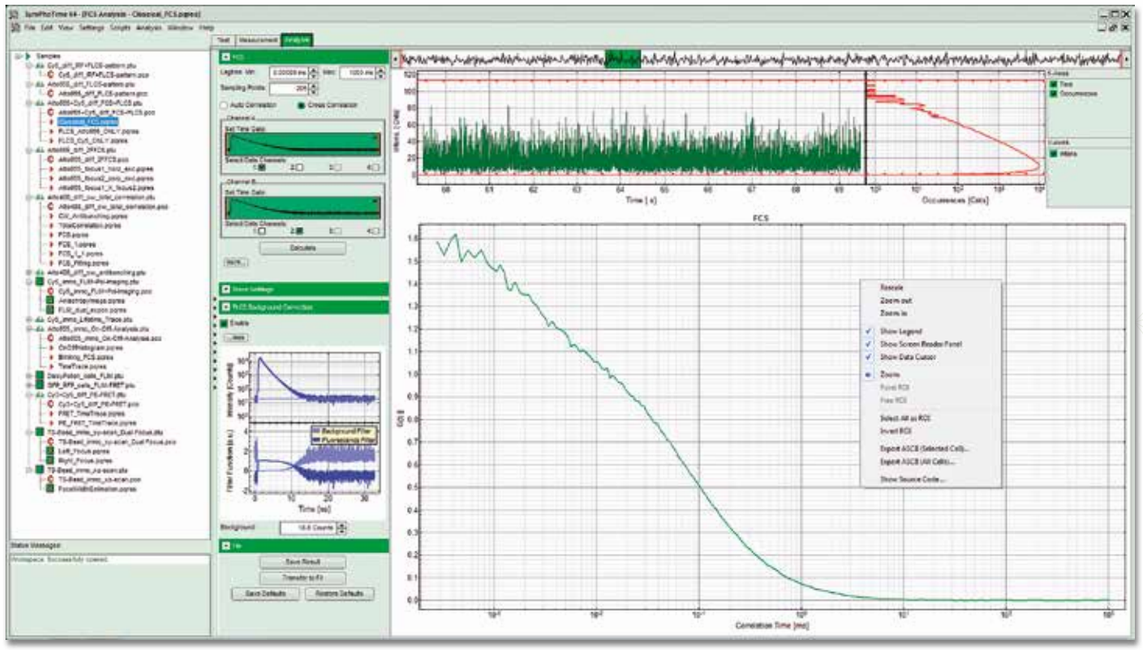


Förster Resonance Energy Transfer (FRET)

FLIM-FRET measurements can be evaluated using either the fluorescence intensity or lifetime as parameter. With the latter, a dedicated fitting function is applied. FLIM-FRET analysis results can be directly visualized in a false color representation. The FRET efficiency histogram and the histogram of the donor-acceptor distances in units of the Förster distance are always calculated.

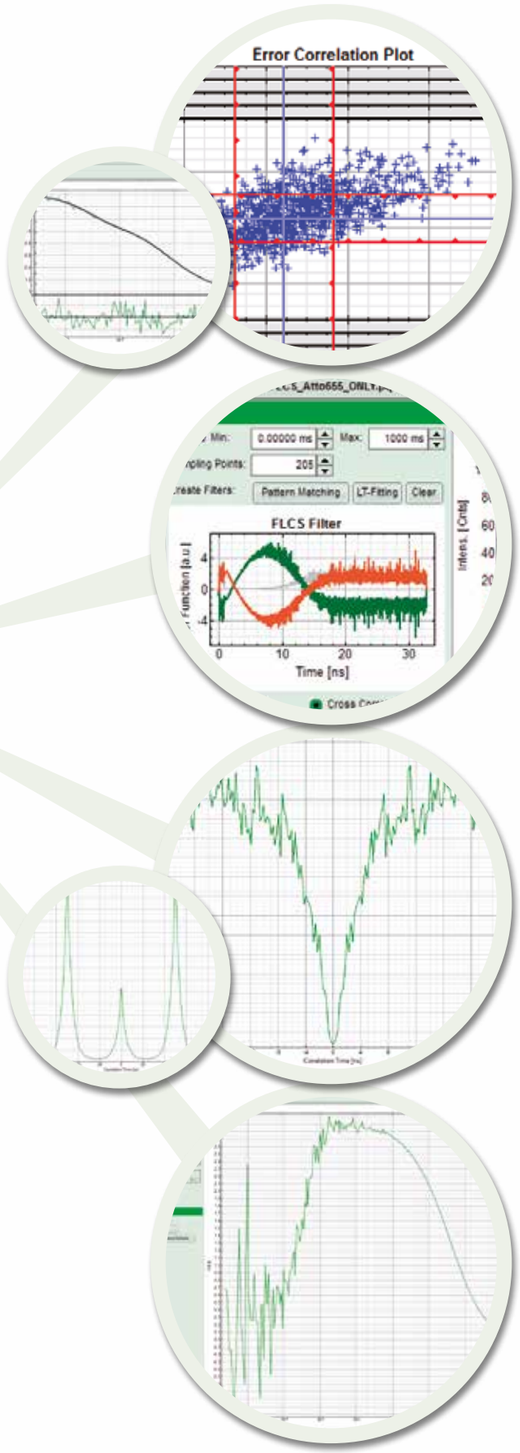
Correlation spectroscopy

SymPhoTime 64 sets a new standard for analysis of Fluorescence Correlation Spectroscopy (FCS) measurements. The software provides a wide range of specially adapted correlation analysis procedures, which range from classic auto-correlation and cross-correlation (FCCS) to lifetime based correlation analysis (FLCS) and total correlation. Even coincidence-correlation analysis is possible based on the unique time-tagging modes of PicoQuant's Time-Correlated Single Photon Counting (TCSPC) modules. By exploiting the full power of a multi-core computer system, SymPhoTime 64 is one of the fastest software correlators on the market.



Auto- and cross-correlation

SymPhoTime 64 includes one of the fastest software correlators for the calculation of auto- and cross-correlation functions. This is made possible by the unique time-tagging mode of PicoQuant's TCSPC modules that save the arrival time for each detected photon on each detection channel. Up to eight individual detection channels or the sum of selected channels can be included in the correlation analysis. The maximum and minimum time range of the correlation time as well as the number of data points can be defined for an individually optimized correlation result. Freely adjustable intensity thresholds and time gates for each detection channel enable the analysis of subsets of the measurements. Even correction of signal background is possible using Fluorescence Lifetime Correlation Spectroscopy (FLCS) without performing any extra measurements. The resulting correlation curves can be further analyzed by fitting dedicated models to the result or exported to standard formats for custom data processing.



Advanced fitting options

Several standard fitting models are included in SymPhoTime 64, which allows determining seveal important parameters, such as diffusion coefficients or molecular concentrations of one or more labeled species. All fitting procedures are automatically followed by a dedicated error analysis. As a result, not only confidence intervals are established for each fitting parameter, but even correlations between parameters become visible at a glance.

Lifetime based correlation

A special feature of the Symphotime 64 are FLCS experiments, where measured fluorescence lifetimes are included to discriminated labeled species. FLCS can also be used for the correction of background signal and detector afterpulsing removal. Even the separation of species with the same diffusion constant but different fluorescence lifetimes becomes possible.

Antibunching

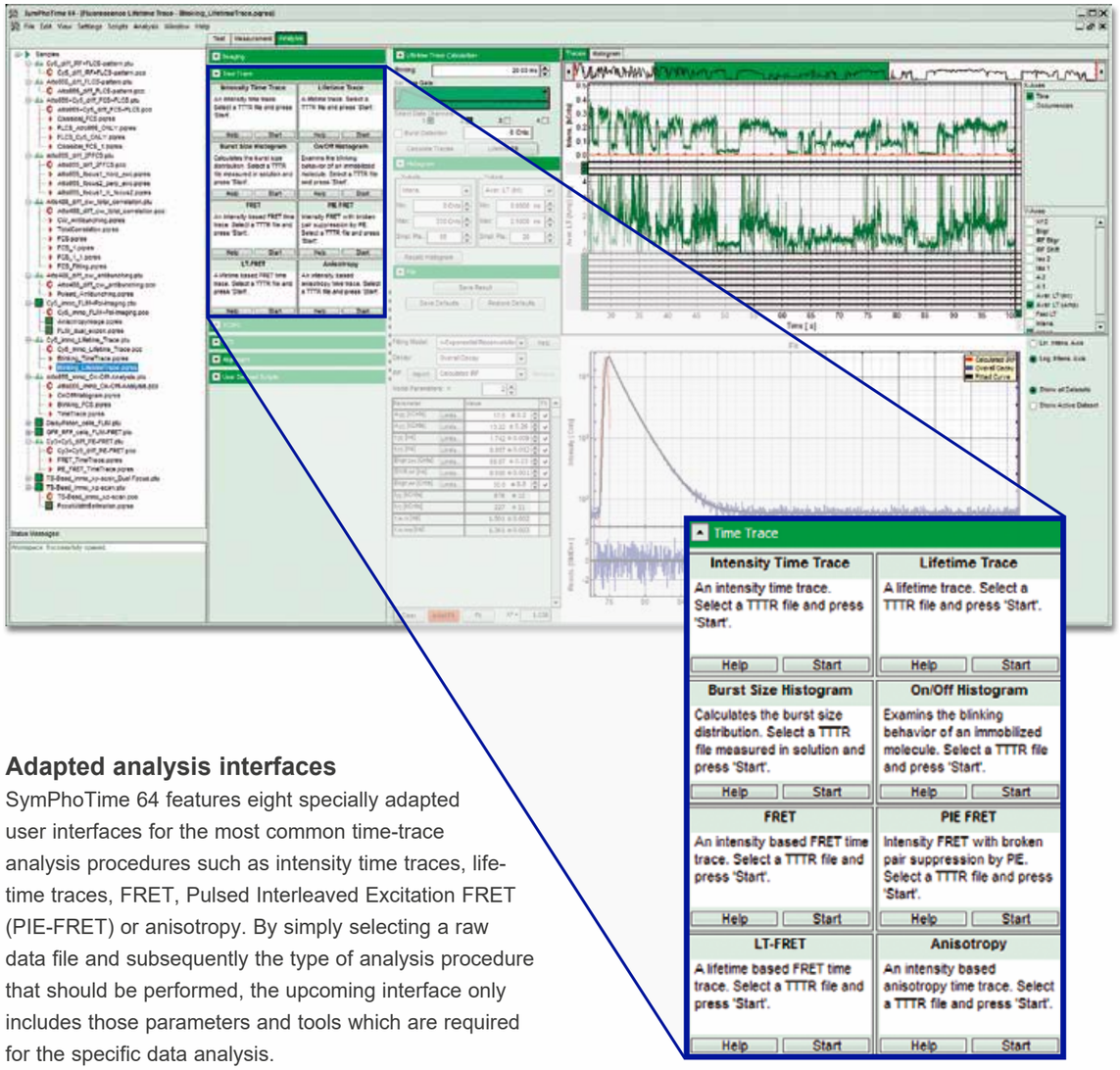
SymPhoTime 64 also permits to study correlations on the picosecond to nanosecond time scales. These calculations are made possible by the special "T2" time-tagging mode of PicoQuant's TCSPC electronics. With SymPhoTime64, antibunching correlations can be directly calculated, taking possible temporal shifts between the two detection channels into account. The resulting correlation curve can then be exported for further data processing.

Total correlation

Thanks to the "T2" time-tagging mode, SymPhoTime 64 can also perform total correlations. The total correlation does not only include the results from antibunching and classic FCS, but also gives access to dynamics in the nanoseconds time scale, which allows e.g., the study of rotational dynamics of molecules and triplet effects.

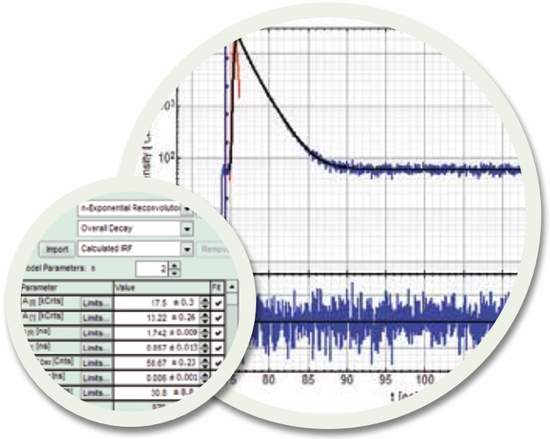
Time-trace analysis

The analysis of fluorescence intensity time traces is another core feature of SymPhoTime 64. Fluorescence intensity time traces display the measured fluorescence dynamics and can be analyzed in a variety of ways. Prominent examples are on/off histograms, burst size histograms, and fluorescence lifetime traces, but also FRET or anisotropy analysis belong to this category.



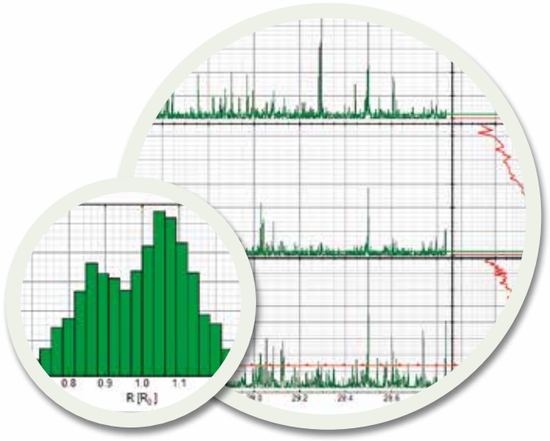
Adapted analysis interfaces

SymPhoTime 64 features eight specially adapted user interfaces for the most common time-trace analysis procedures such as intensity time traces, lifetime traces, FRET, Pulsed Interleaved Excitation FRET (PIE-FRET) or anisotropy. By simply selecting a raw data file and subsequently the type of analysis procedure that should be performed, the upcoming interface only includes those parameters and tools which are required for the specific data analysis.



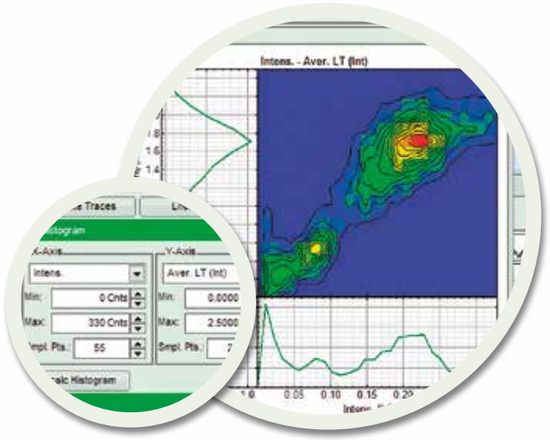
Efficient decay analysis

SymPhoTime 64 includes an efficient decay fitting algorithm, which provides the determination of up to five different fluorescence lifetimes from the measurement data. The software supports tail as well as reconvolution fitting using either measured or calculated Instrument Response Functions (IRF). All fits are automatically followed by a sophisticated error analysis based on the bootstrap method. In that way, confidence intervals are established for each fitting parameter and correlations between parameters become visible at a glance.



FRET, PIE-FRET, Lifetime-FRET

FRET is one of the core time-trace analysis methods supported by SymPhoTime 64. It can be calculated either by using the intensity of the donor and acceptor or by using the measured fluorescence lifetime of the donor molecule. In both cases, FRET distance and efficiency histograms are calculated and displayed. Adjustable thresholds allow to select subsets of the whole measurement file as well as to define background and burst levels for FRET calculation. SymPhoTime 64 also supports PIE-FRET analysis, a technique that corrects the results for incomplete FRET pairs in the sample.

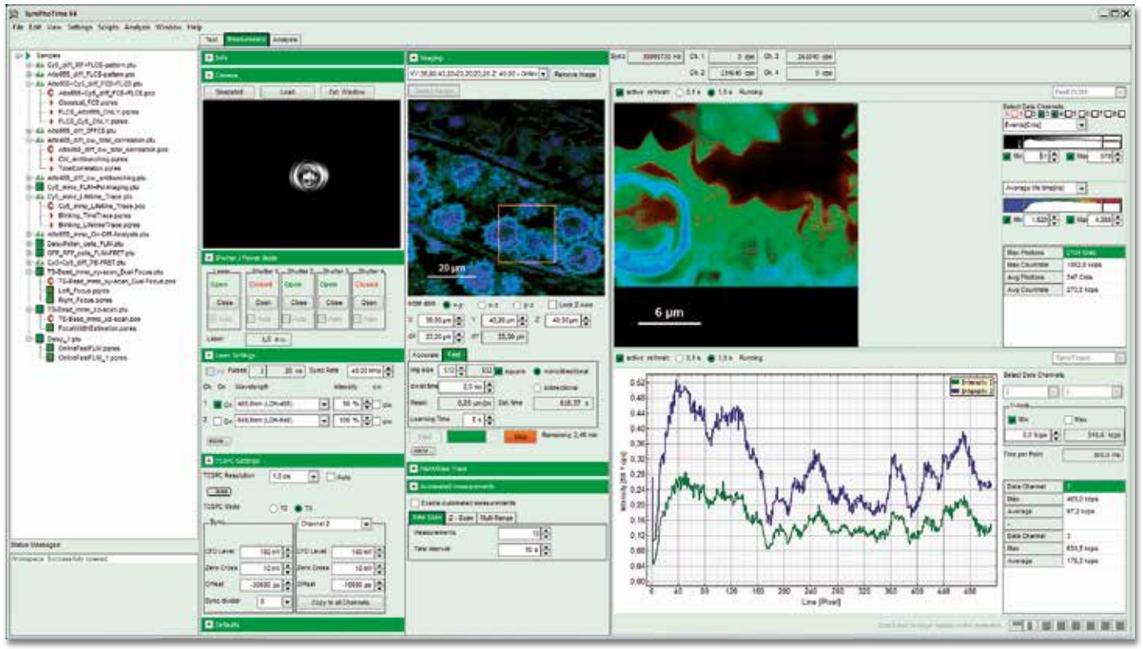


Classic single molecule methods

Several classic single molecule methods are supported within the time-trace analysis interface. This includes on/off histograms to study the blinking behaviour of an immobilized molecule or burst size histogramming used in, e.g., fluorescence biomedical assays. The results from a fluorescence lifetime analysis of the time-trace can be visualized through correlograms displaying various parameter dependencies.

Data acquisition

SymPhoTime 64 is the dedicated data acquisition software for PicoQuant's time-resolved confocal microscopes and upgrade kits for laser scanning microscopes. It can also be used with custom set-ups based on PicoQuant's TCSPC electronics. A clear and structured layout makes data acquisition and hardware control easier than ever before.



Time-tagged data acquisition

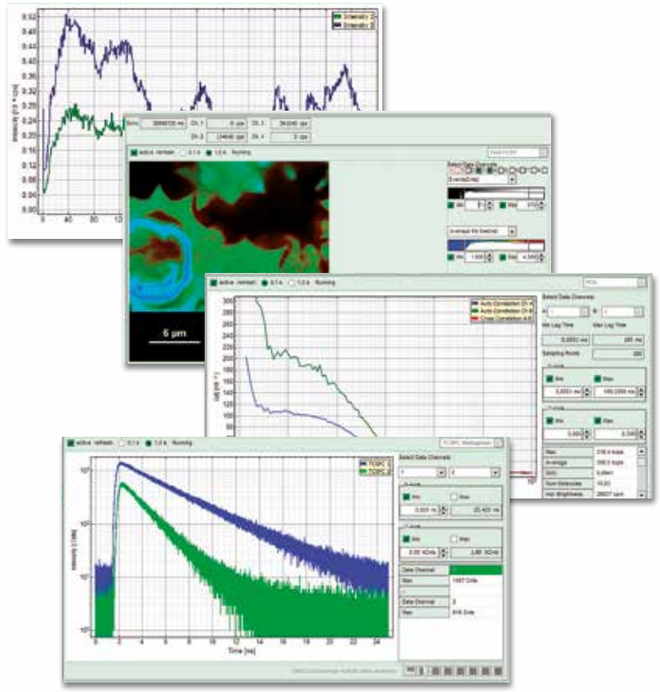
All data acquisition and analysis features of SymPhoTime 64 are based on the unique time-tagging modes of PicoQuant's TCSPC modules. Hereby, photons in each detection channel are tagged with the time difference to the last laser pulse or, in certain cases, with the absolute arrival time since the beginning of the measurement. Synchronization signals from a scanning system or other external devices can also be time tagged and included in the data file. This scheme preserves all photon timing information and allows a large variety of data interpretations ranging from simple TCSPC histograms to complex imaging and correlation analyses.

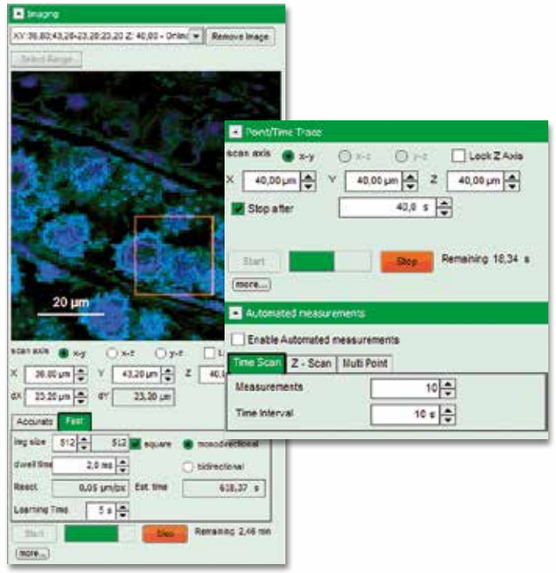
Integrated device control

SymPhoTime 64 controls all relevant hardware of the time-resolved confocal microscopes MicroTime 100 and MicroTime 200, such as the available piezo scanners, video camera, power photodiode and all installed shutters. For data acquisition, SymPhoTime64 supports all current TCSPC units from PicoQuant (PicoHarp 300, HydraHarp 400, TimeHarp 260, and MultiHarp 150). The relevant settings, such as the temporal bin width or the discriminator settings of the input channels can be adjusted within the software. An interface for PicoQuant's PDL 828 "Sepia II" laser driver is also included to adjust laser power and repetition rates of the connected laser heads.

Extensive online visualization

SymPhoTime 64 can display up to four parallel and independent measurement previews during data acquisition. This includes "fast FLIM" images with adjustable intensity and color scale as well as the calculation of auto- and cross-correlation FCS traces for selected detection channels. In addition, SymPhoTime 64 permits to calculate and display TCSPC histograms and intensity time traces in real-time for individual detection channels or the sum of all detection channels. The number and type of displayed measurement previews can be widely defined by the user. This special feature allows quick judgement of data quality or changes of the sample properties already during the measurement. All calculated previews are saved along with the raw data file in the workspace for later analysis.





Imaging and point measurements

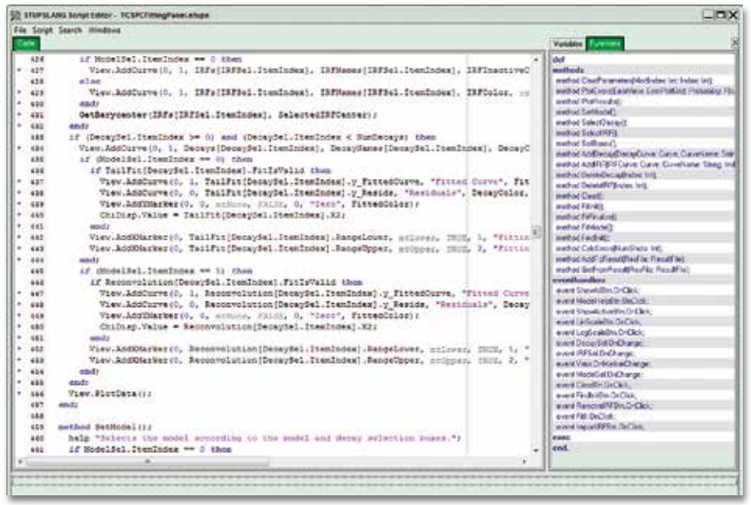
SymPhoTime 64 can directly control the scanning devices used in the MicroTime 200 system. Single point, multi-point, and line scans for scanning FCS as well as 2D imaging measurements are supported. Even image stacks for 3D measurements are possible if the system is equipped with a suited z-scanning device.
Data acquisition with other scanning devices such as LSMs and AFMs is also possible. In that case, the scanning itself must, however, be controlled by an additional software such as the operation software of the LSM. SymPhoTime 64 is then used in a remote (slave) mode and images are calculated based on suitable synchronization signals from the scanning controller that are stored in the time-tagged data file.
SymPhoTime 64 also features a dedicated pre-measurement mode ("oscilloscope mode" or "test mode"), which permits to fine tune system parameters and data acquisition settings without actually creating any measurement data.

Customizable software

SymPhoTime 64 is designed to guide the user through all necessary steps for an individual analysis or measurement process. This is achieved by a clearly structured graphical user interface (GUI) with different themes and specially adapted analysis procedures. SymPhoTime 64 can be further customized using the integrated scripting language that enables the user to add, e.g., additional fit models, GUI components or analysis procedures.

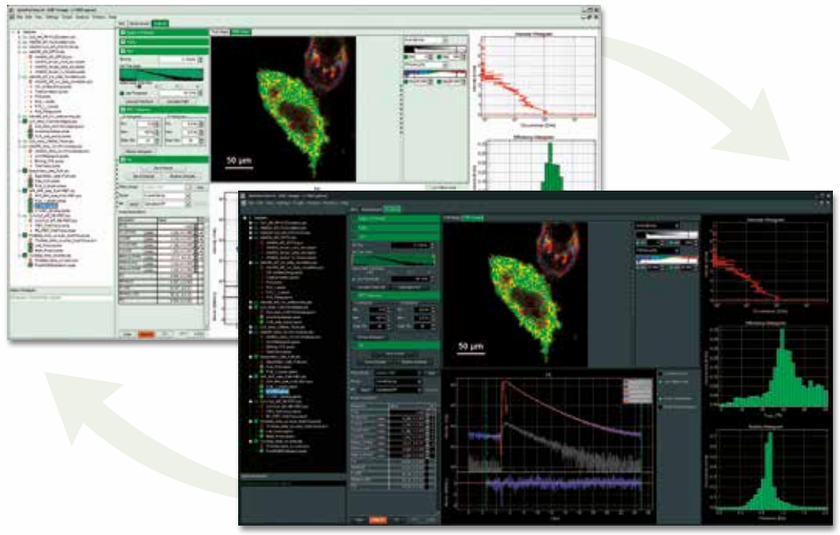
Selectable themes

SymPhoTime 64 features different themes, i.e. different color schemes of the user interface.
While the standard theme uses classic Windows colors, a special dark theme has been designed for light sensitive measurements, e.g., NDD deep tissue imaging.



Scripting for complete customization

SymPhoTime 64 includes the dedicated scripting language “STUPSLANG” that can be used to extend the software even further. The scripting language is a powerful tool to develop, e.g., new analysis procedures, which are not part of the standard functionality. Herewith, new fitting functions can be added and even new user interfaces can be designed. On top of that, external hardware such as scanners are accessible via a dedicated interface. This special feature makes the SymPhoTime 64 one of the most versatile software packages on the market.



Software features

Data acquisition features

Supported TCSPC modules	PicoHarp 300, HydraHarp 400, TimeHarp 260, MultiHarp 150
Supported configurations	MicroTime 200 with 100 × 100 (× 100) µm or 10 x 7.5 cm piezo scanner or FLIMbee galvo scanner Laser Scanning Microscopes (LSMs) from Nikon, Olympus, Scientifica or Zeiss Stand-alone TCSPC modules Remote control via TCP/IP interface (software handshake with ZEN and NIS Elements) MicroTime 100 with 80 x 80 (x 100) µm or 10 x 7.5 cm piezo scanner
Number of detection channels	1 to 8 detectors
Measurement modes	Single point, multi-point, x line scan (with FLIMbee), 2D imaging (XY, XZ, YZ), 3D imaging (XYZ), time lapse (XYT), oscilloscope mode for alignment purposes
Measurement previews	FLIM, FCS and FCCS, time traces, TCSPC histogram Parallel calculation and display of up to 4 different previews
Supported laser driver	PDL 828 “Sepia II”

Data analysis features

General features	Time gating, binning, GUI themes TCSPC fitting (multi-exponential decay (1 to 5 exponentials), least squares fitting, MLE fitting, IRF reconvolution, tailfit, bootstrap error analysis)
Fluorescence intensity traces	Blinking (on/off histogramming), count rate histogram (PCH), burst size histogram, intensity gated TCSPC, fluorescence lifetime traces, lifetime histogram, BIFL (Burst Integrated Analysis)
Correlation	FCS, FCCS, FLCS, PIE-FCS, scanning FCS, FCS calibration, STED-FCS, STED-FLCS FCS fitting (models: diffusion constants, triplet state, conformational, protonation, gaussian PSF, user defined models via scripting, bootstrap error analysis) Antibunching/coincidence correlation, total correlation
Imaging	FLIM, FLIM-FRET, intensity FRET, anisotropy imaging, (time gated) fluorescence intensity imaging, adjustable color scale, region of interest (ROI), simultaneous confocal and STED, gated STED, pattern matching for multicolor STED
FRET	FLIM-FRET, intensity FRET, PIE-FRET (Pulsed Interleaved Excitation) with bleedthrough correction
Steady state anisotropy	Objective correction factors included
Export data formats	BMP, ASCII, TIFF, BIN
User scripting (STUPSLANG)	User defined analysis procedures, fitting functions, multiparameter filtering Control of external hardware via suitable interface

Operating Environment

Required PC	2.2 GHz (or better) quad-core CPU, minimum 4 GB RAM (suggested 16/32 GB), Windows 10 x64 with Full HD Display, USB slot for protection module

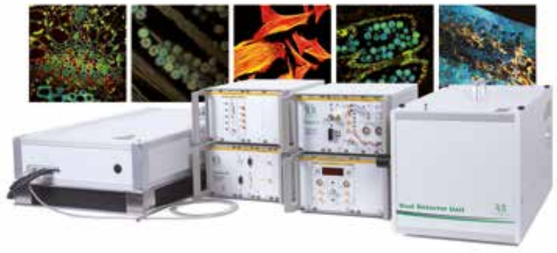
Specifications are subject to changes without prior notification.

Systems and components

SymPhoTime 64 is the dedicated data acquisition software for PicoQuant's time-resolved confocal microscopes and LSM upgrade kits. It can also be used with custom set-ups based on PicoQuant TCSPC electronics. PicoQuant offers several TCSPC modules and complete systems that are individually matched to the requirements of the user.

Photon counting instrumentation

High accuracy timing and fast photon counting is one key area of PicoQuant's leading technological competence. Notably, the HydraHarp 400 and MultiHarp 150 Time-Correlated Single Photon Counting (TCSPC) modules have become acknowledged brands worldwide. These versatile instruments for event timing and TCSPC readily support sophisticated techniques in single molecule spectroscopy, correlation spectroscopy, quantum optics and scanning applications. The product range is completed by high speed photon counting detectors and various other accessories.



Fluorescence lifetime systems

PicoQuant offers turn-key time-resolved upgrade kits for Laser Scanning Microscopes (LSM) as well as complete time-resolved confocal fluorescence microscopes. All systems are highly modular and can be adapted to meet the requiements of even the most demanding applications such as single molecule detection.

PicoQuant

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

Today, PicoQuant is known as a leading company 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 as well as for carrying out routine quality control in production processes of global industry players. Starting from traditional time-resolved fluorescence detection, the range of covered applications continuously grew to include semiconductor quality control, diffuse optical imaging, materials research, quantum information processing, optical detector testing, and telecommunications. Due to the ease of use of our products, researchers can focus on their scientific questions in biology, medicine, environmental science, quantum optics, or chemistry without needing a large background in physics, electronics, or 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 scientists and price conscious industry.

We have successfully teamed up with major confocal microscopy companies to develop dedicated equipment that permits carrying out 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 or cumbersome.

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 forms the basis for new outstanding developments which are always driven by our customers' needs and inspirations. Visit our website or contact our product and application specialists directly to discuss your needs. Of course, you are always welcome to visit our application labs during your travels to Germany.

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