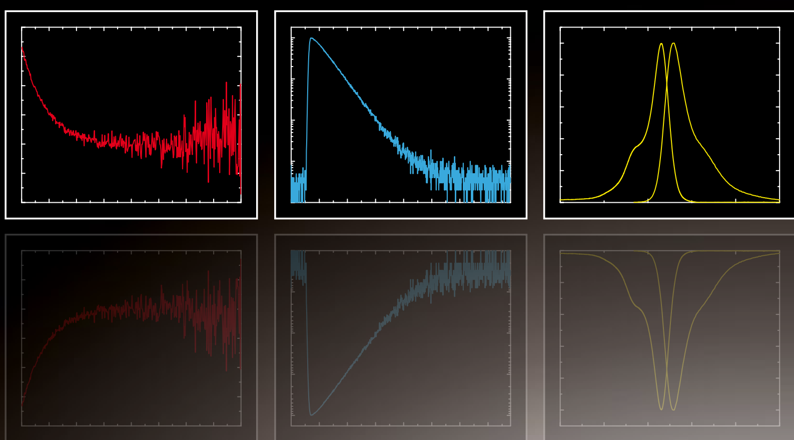
FluoTime 300 “EasyTau”

PicoQuant

A fluorescence spectrometer
for beginners and experts



Foreword

Dear Researcher,

PicoQuant has a long and successful history in instrumentation for time-resolved spectroscopy and microscopy. Our picosecond diode lasers and photon counting electronics can be found in more than a thousand working systems around the world. Their compactness and ease-of-use enable scientists to perform previously complex measurements on a daily basis. Nonetheless, we considered that time-resolved fluorescence measurements could be made even easier than ever before and therefore developed a fully automated fluorescence lifetime and steady-state spectrometer, the FluoTime 300. This system is in constant development. It is continually evolving to match the requirements of today's research, and to make the acquisition of fluorescence spectra easier than before.

If you are interested in this outstanding instrument please contact us. We are always happy to discuss your individual requirements in detail, because your needs drive our development.

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Vision

A fluorescence spectrometer for beginners and experts

The FluoTime 300 is an advanced research-grade fluorescence lifetime spectrometer with steady-state option. Its intuitive system software EasyTau, featuring application targeted wizards, allows researchers to focus on their samples without concerning themselves finding the best instrument settings for their experiments.

Fluorescence spectroscopy has been established as one of the fundamental spectroscopic laboratory methods. It is used for routine quality control as well as sophisticated measurements in research labs. An ideal instrument combines the needs of both worlds and makes the method usable for highly trained specialists as well as for occasional users. This ambition was the fundamental design principle of the FluoTime 300.

Designed by scientists for scientists, it surely sets a new standard for fluorescence spectrometers.

- **Fully automated system**
  All adjustable parts controlled by system software
- **Turn-key excitation sources**
  Picosecond pulsed diode lasers, LEDs, Xenon lamps, or other excitation sources
- **Ultra high sensitivity**
  Single photon counting for ultimate sensitivity with better than 26 000:1 signal-to-noise ratio
- **Large sample chamber with specialized sample holder**
  Accommodates various easy and on-the-fly switchable sample holders
- **Wide lifetime range**
  Fluorescence lifetimes < 10 ps and up to seconds
- **Wide spectral range**
  Fluorescence detection from UV to NIR with sub-nm resolution
- **Comprehensive system software**
  Intuitive user interface with application targeted wizards

System Layout

Fully automated system with high quality components

The FluoTime 300 is designed for maximum light throughput and highest sensitivity. This is achieved by single photon counting data acquisition methods and by careful design and selection of all optical components. The heart of the FluoTime 300 is a large multifunctional sample chamber with fully automated optical elements to control intensity and polarization of the excitation beam and fluorescence signal.

Fully automated optical components

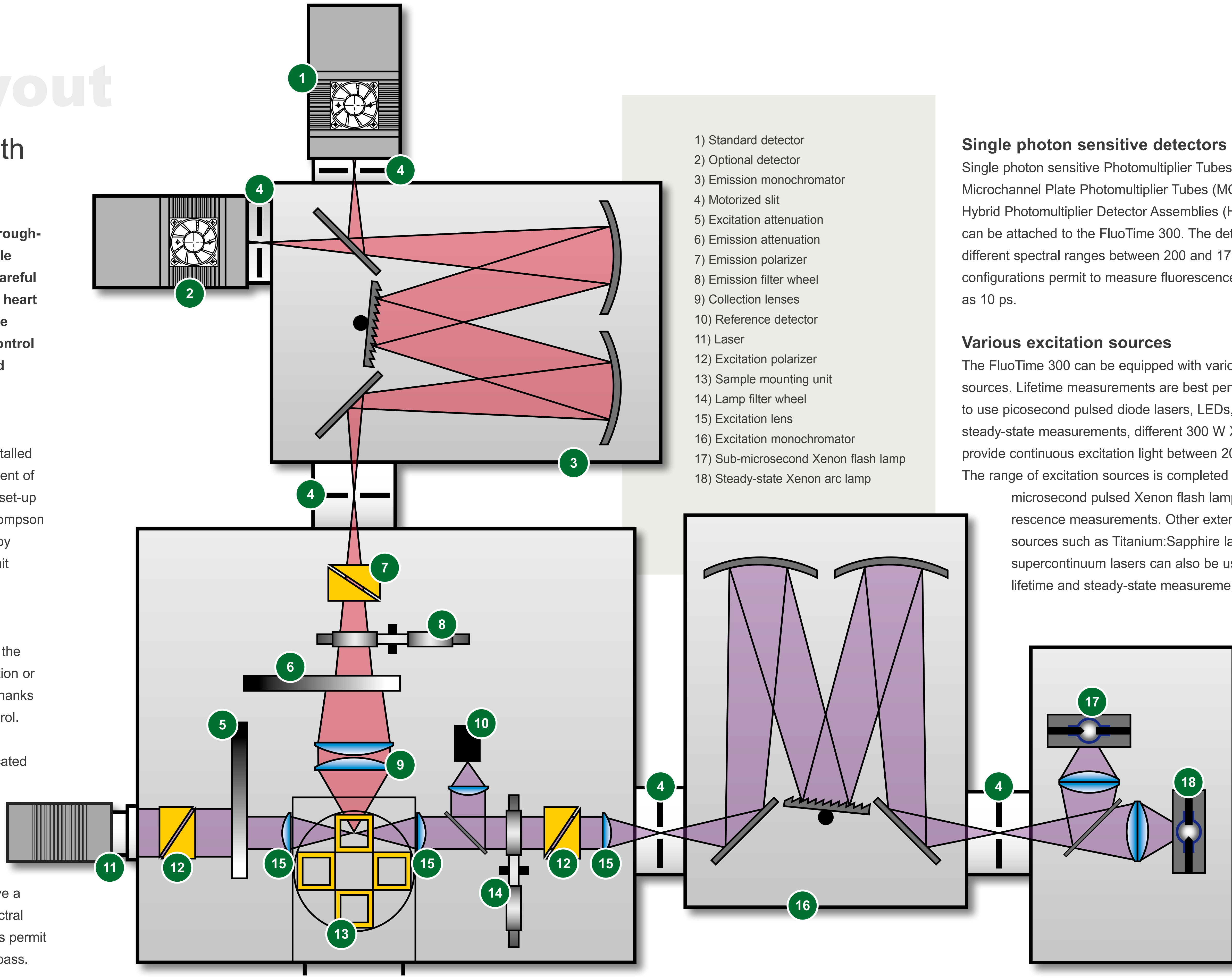
Fully automated polarizers and signal attenuators are installed in all optical beam paths. The attenuators permit adjustment of signal intensity over a wide range and thus adapting the set-up to the corresponding sample requirements. The Glan-Thompson polarizers can be freely rotated in 0.1° steps for anisotropy studies. They feature a 15 mm clear aperture and transmit throughout the wavelength range from 200 to 2000 nm.

Multifunctional sample holders

A single cuvette holder is the standard sample holder for the FluoTime 300. It can be supplied with a liquid cooling option or a cryostat. Multiple sample measurements are possible thanks to a 4-position sample changer with full temperature control. Magnetic stirrers with variable speed are available for all holders. Solid samples can also be studied using a dedicated front face sample holder.

Efficient monochromators

The FluoTime 300 uses Czerny-Turner monochromators that are equipped with one or two high quality diffraction gratings. These monochromators achieve a high spectral resolution down to 0.1 nm over a broad spectral wavelength range from 200 up to 1700 nm. Motorized slits permit easy adjustment of each monochromator's spectral bandpass.



1) Standard detector
2) Optional detector
3) Emission monochromator
4) Motorized slit
5) Excitation attenuation
6) Emission attenuation
7) Emission polarizer
8) Emission filter wheel
9) Collection lenses
10) Reference detector
11) Laser
12) Excitation polarizer
13) Sample mounting unit
14) Lamp filter wheel
15) Excitation lens
16) Excitation monochromator
17) Sub-microsecond Xenon flash lamp
18) Steady-state Xenon arc lamp

Single photon sensitive detectors

Single photon sensitive Photomultiplier Tubes (PMTs), Microchannel Plate Photomultiplier Tubes (MCP-PMTs), or Hybrid Photomultiplier Detector Assemblies (Hybrid-PMTs) can be attached to the FluoTime 300. The detectors cover different spectral ranges between 200 and 1700 nm. Selected configurations permit to measure fluorescence lifetimes as short as 10 ps.

Various excitation sources

The FluoTime 300 can be equipped with various excitation sources. Lifetime measurements are best performed with easy to use picosecond pulsed diode lasers, LEDs, or Solea. For steady-state measurements, different 300 W Xenon arc lamps provide continuous excitation light between 200 and 1000 nm. The range of excitation sources is completed by a sub-microsecond pulsed Xenon flash lamp for phosphorescence measurements. Other external excitation sources such as Titanium:Sapphire lasers or supercontinuum lasers can also be used for both lifetime and steady-state measurements.

System Software EasyTau

Acquiring fluorescence spectra has never been easier

Acquiring fluorescence anisotropy spectra and especially time-resolved fluorescence measurements are very often considered to be tasks for experts. With the operation and analysis software EasyTau, this myth can be put to rest. The software features application wizards that guide the inexperienced user through all necessary steps to acquire high quality fluorescence data. An alternative “customized measurement” mode with full control over all system parameters and a versatile scripting language make the software also attractive to experts.

System status at a glance

The system status of the FluoTime 300 is permanently monitored and displayed. The user is always informed about all important parameters such as excitation and detection wavelength, polarizer position, signal intensity, temporal resolution, pile-up rate, and sample temperature.

Intelligent data storage

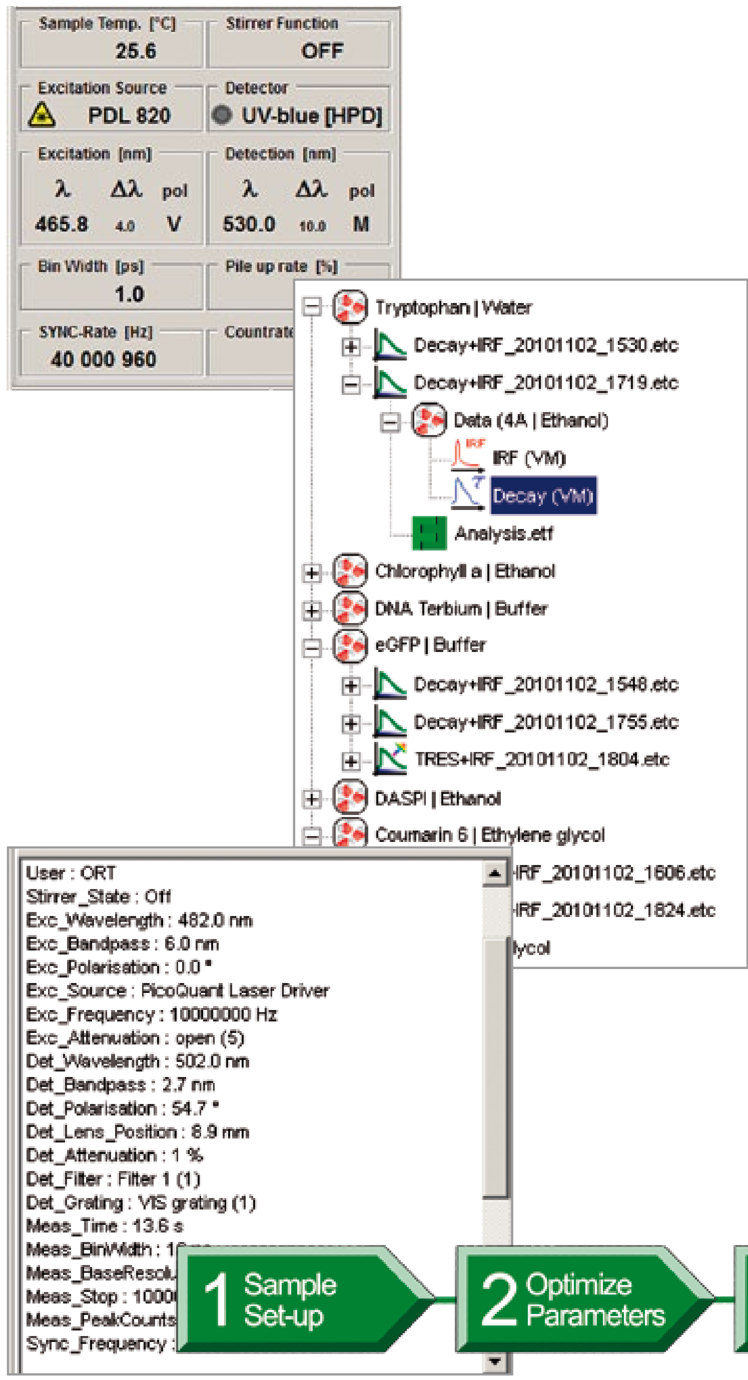
The EasyTau software features a well structured data storage architecture. All measurement data files and related analysis results are stored in a well-arranged workspace. Measurement data is sorted according to the studied sample, which permits a quick assessment of sample properties.

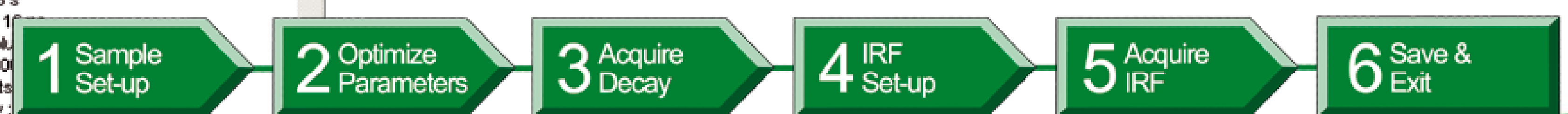
Complete logging of parameters

The complete system and acquisition settings for each measurement are logged and stored with each data file, and can thus be recapped at any time. As a feature for multiuser facilities, the software even permits to store the current user name along with the measurement data.

Powerful application wizards

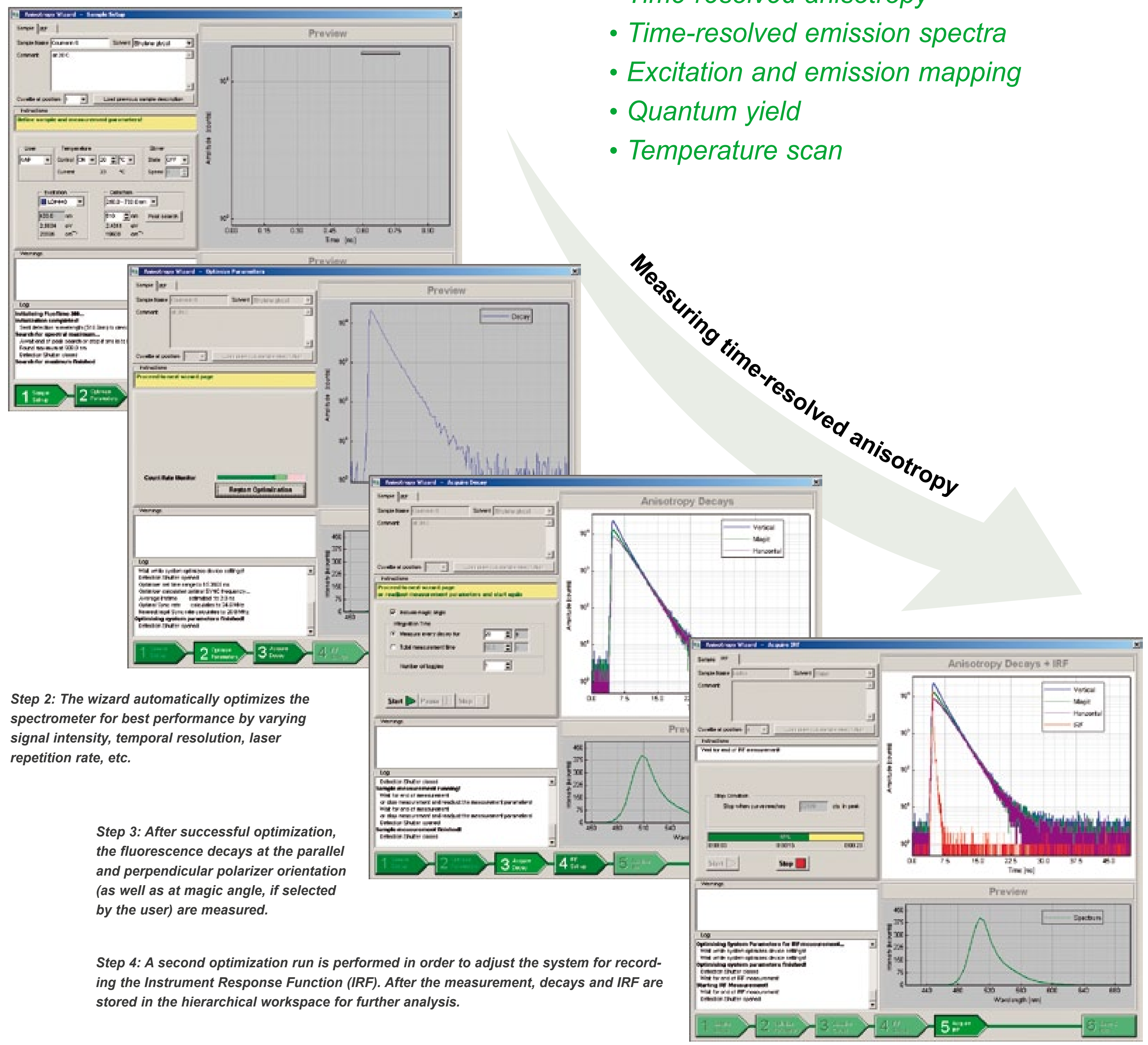
The EasyTau application wizards enable even inexperienced users to acquire high quality fluorescence data. They are built on a powerful yet simple to use scripting language and on more than 20 years of fluorescence expertise gathered by PicoQuant. Every wizard optimizes the spectrometer settings according to the sample characteristics. The provided wizards can be edited by the end-user and saved separately in their workspace, allowing customization for the individual application.





WIZARDS INCLUDED

- *Excitation and emission spectra*
- *Excitation and emission anisotropy*
- *Intensity and lifetime kinetics*
- *Fluorescence decays*
- *Time-resolved anisotropy*
- *Time-resolved emission spectra*
- *Excitation and emission mapping*
- *Quantum yield*
- *Temperature scan*



Step 1: The user is asked to enter information about the sample and to define relevant measurement parameters such as solvent, excitation, and detection wavelength.

Step 2: The wizard automatically optimizes the spectrometer for best performance by varying signal intensity, temporal resolution, laser repetition rate, etc.

Step 3: After successful optimization, the fluorescence decays at the parallel and perpendicular polarizer orientation (as well as at magic angle, if selected by the user) are measured.

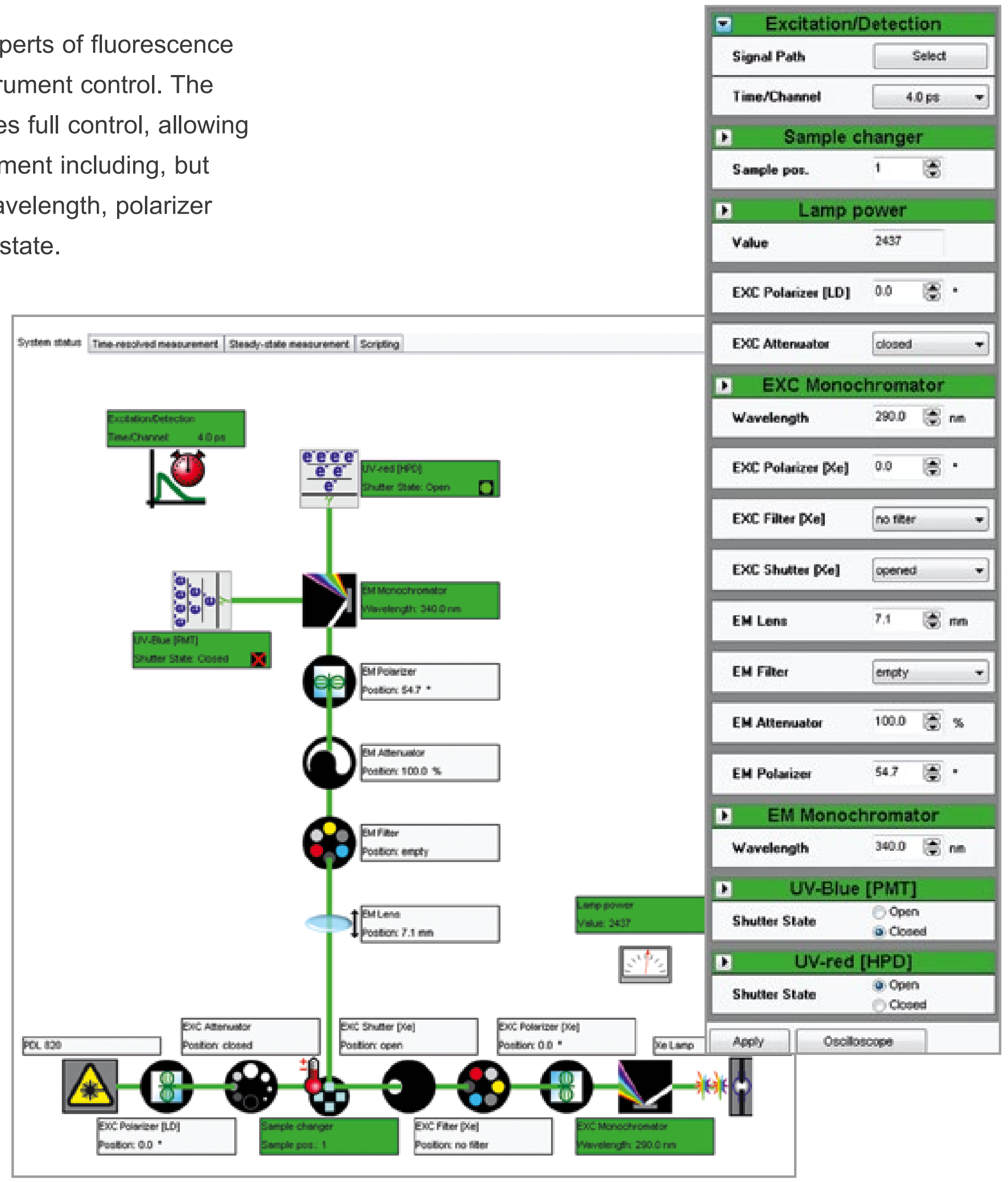
Step 4: A second optimization run is performed in order to adjust the system for recording the Instrument Response Function (IRF). After the measurement, decays and IRF are stored in the hierarchical workspace for further analysis.

System Software EasyTau

Full instrument control

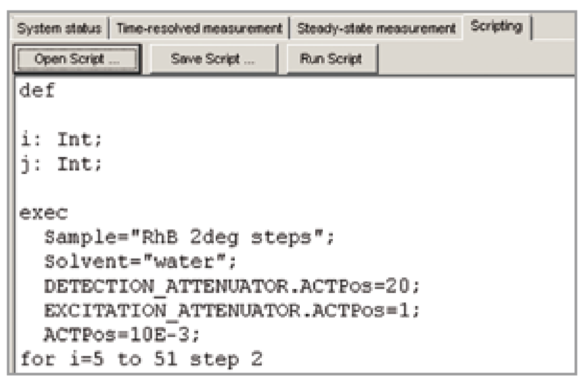
The EasyTau software is attractive for experts of fluorescence spectroscopy who prefer to have full instrument control. The “customized measurement” mode provides full control, allowing the adjustment of every part of the instrument including, but not limited to, excitation and detection wavelength, polarizer position, attenuator settings, and shutter state.

The “customized measurement” mode supports time-resolved as well as steady-state measurements. In steady-state mode, intensity can be recorded as a function of various parameters, including polarizer angle, time, and collection lens position. The polarizers can be moved in or out of the beam path on the fly, without having to restart the EasyTau software, as the software is aware of their actual position.



Scripted data acquisition

Besides full instrument control, the “customized measurement” mode also includes a unique feature: a scripting language that allows development of user-defined measurement protocols. The scripting language can access and control every single component of the FluoTime 300. It supports time-resolved as well as steady-state measurements. Even novices in programming can create a script by clicking on the component and setting the desired value. Through the use of control structures, loops, and mathematical functions, the scripting language allows the experienced user to develop even the most complex measurement protocols. Measurement protocols that have to follow predefined procedures will greatly benefit from scripted data acquisition.



“The FluoTime 300 makes time-resolved measurements accessible to all laboratories.”

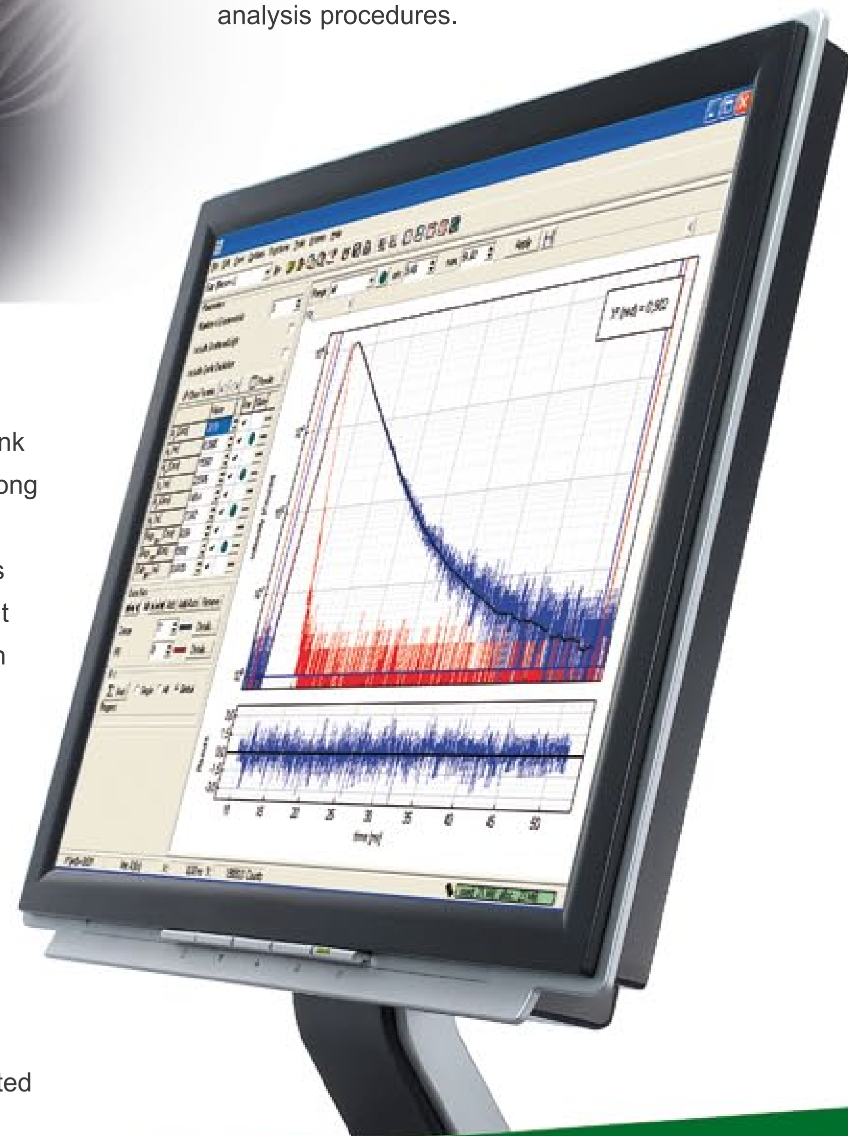
Joseph R. Lakowicz, Center for Fluorescence Spectroscopy, University of Maryland, USA

Multi-exponential decay analysis

For analysis of time-resolved measurements, a direct data link to the established FluoFit software is provided. FluoFit is a long standing and powerful global data analysis software for fluorescence decay and anisotropy measurements. Tail fitting as well as numerical reconvolution to account for the Instrument Response Function (IRF) can be performed. The association of the experimental data curves to the decay and IRF or to different polarization orientations for anisotropy measurements is automatically performed. Exponential decay models up to 4th order or, alternatively, different lifetime distribution models can be fitted to the decay data. Initial values for the fitted parameters are automatically estimated. Reduced chi-square, weighted residuals, and autocorrelation traces can be used to assess the goodness of fit. Advanced error analysis using support plane or bootstrap analysis assigns realistic confidence intervals to the fitted parameters.

Complex data arithmetic

The EasyTau software allows direct processing and analysis of the experimental data through a powerful mathematical function processor. Basic operations such as subtractions or multiplications, and even advanced operations such as smoothing, derivatives, integrations, or trigonometric functions can be applied on either experimental data results or previous calculations. Data can be normalized and displayed in linear or logarithmic plots. Plots can be freely zoomed, and all measurement or analysis results can be exported as presentation-ready images or as multicolumn ASCII data for further analysis. The experimental data files are never modified in any analysis step, ensuring data authenticity for further analysis procedures.



Steady-state Spectroscopy

Steady-state Spectroscopy

Steady-state measurements are classical tasks performed with a fluorescence spectrometer. They refer to a measurement condition in which the fluorescence is not temporally resolved, but integrated over time. Typical examples are excitation and emission spectra as well as steady-state anisotropy.

World's most sensitive single monochromator spectrometer

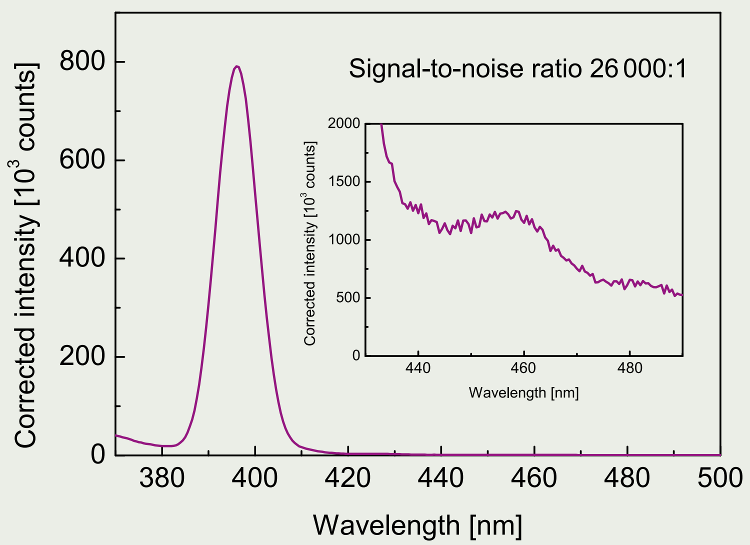
The FluoTime 300 is based on single photon counting, which is the most suited method for measuring weak fluorescence with the highest dynamic range. The signal-to-noise ratio achievable with this method is therefore higher than for any other detection method. This allows measurements of fluorescence spectra of extremely diluted dye solutions even down to picomolar concentration ranges. The sensitivity can be demonstrated by the water Raman band, which is traditionally used to compare the performance of different spectrometers. With the FluoTime 300, a signal-to-noise ratio of > 26 000:1 can be routinely obtained using a standard blue sensitive PMT detector.

Spectral correction with a mouse click

Spectral properties of the excitation source, the monochromator, and the detector affect the recorded excitation and emission spectra. For quantitative measurements, all these influences have to be taken into account. With the FluoTime 300, excitation spectra are easily corrected using the automatically recorded signal from a built-in reference detector which monitors a fraction of the excitation light.

For correction of emission spectra, instrument specific calibration files are delivered with each spectrometer. The correction routines are included in the EasyTau software and can be applied by a simple mouse click.

Examples



Water Raman spectrum: For this measurement, an emission spectrum of HPLC grade water in a 1 cm path length quartz cuvette was recorded from 370 to 500 nm with a 5 nm detection bandwidth, 1 nm step size, and 1 s integration time per wavelength. The excitation source was a 300 W coaxial Xenon arc lamp with the excitation monochromator set to 350 nm with a 5 nm bandwidth. The signal was detected with a standard blue sensitive photomultiplier. The resulting signal-to-noise ratio is > 26 000:1 based on the measured peak counts at 397 nm (800 000 cps) and the background signal at 450 nm (1 000 cps).

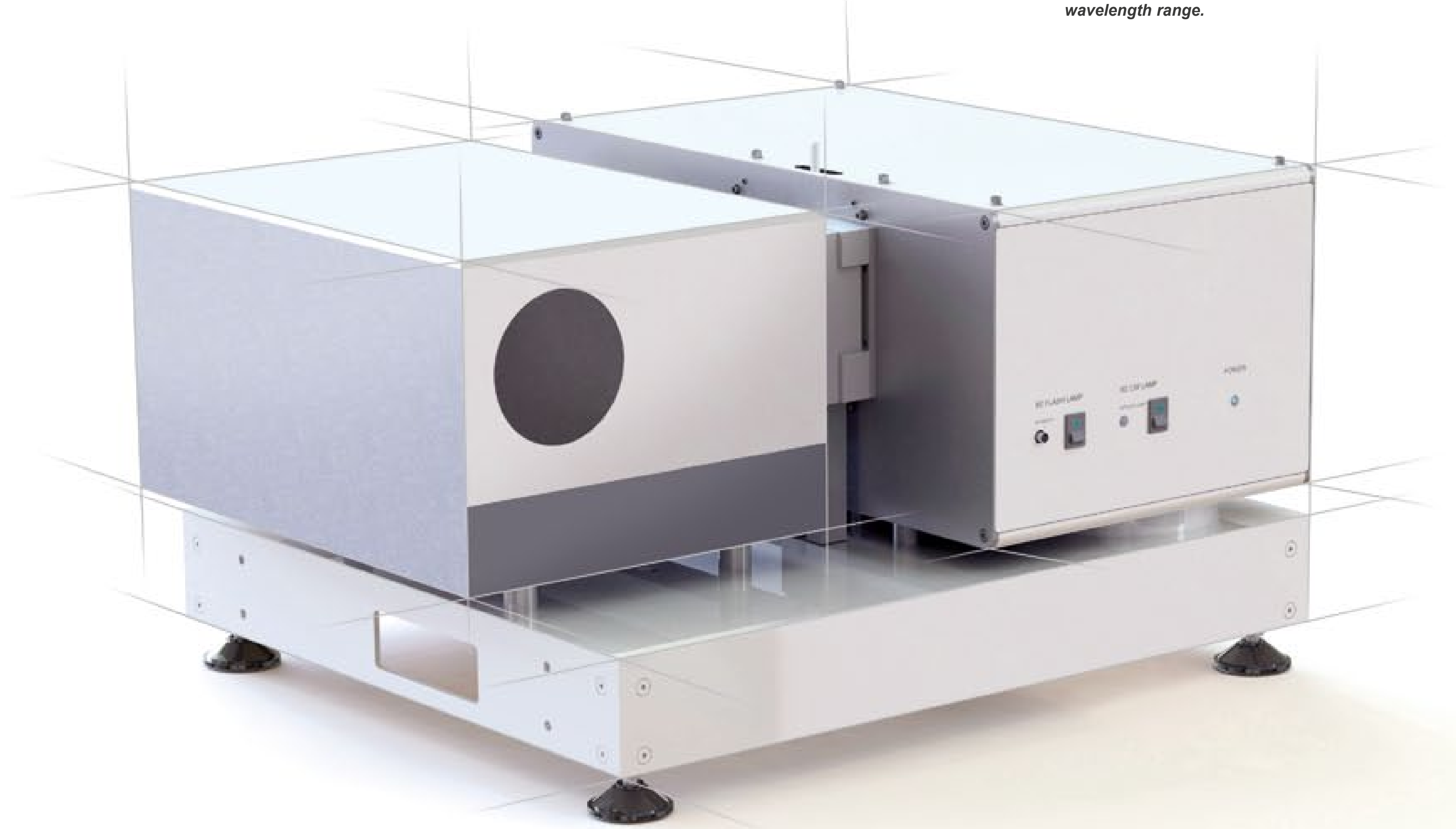
Selected applications

Steady-state fluorescence spectroscopy can be used in many research areas:

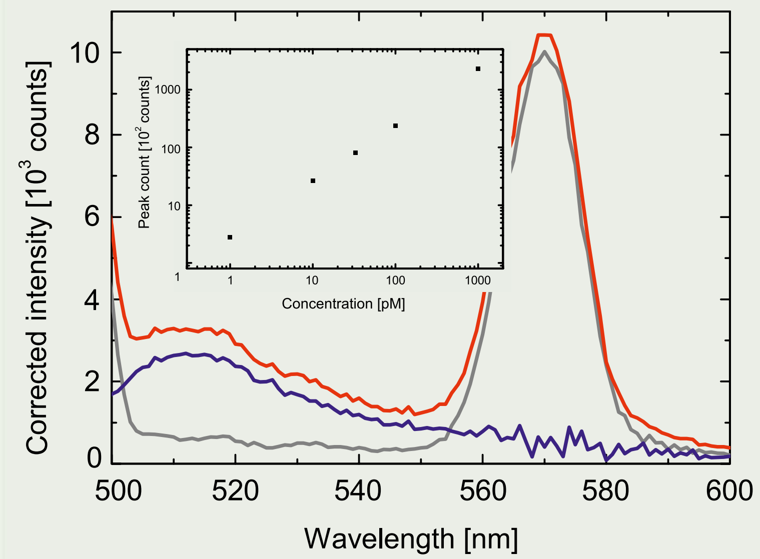
- analytical chemistry – identify species by their excitation or emission spectra and determine their concentration
- photochemistry – characterize photochromes or follow reaction kinetics
- industrial quality control – monitor the quality of polymers, analyze proteins or DNA, efficiencies of solar cells
- environmental science – monitor traces of organic or inorganic substances in solvents or solid samples
- food science – study growth or aging phenomena of fruits and plants

Highest sensitivity
> 26 000:1

Modular housing for white light excitation sources that can hold up to two Xenon arc lamps (operating in either CW or sub-millisecond flash mode). The housing also comes with an excitation monochromator, allowing to select or scan over any excitation wavelength range.



Examples



Sensitivity: Maintaining high sensitivity in the spectral range between 500 and 600 nm requires special detectors and balanced performance of the other spectrometer components.

The result shows a benchmark test using a popular fluorescent dye, Fluorescein, dissolved in carbonate buffer (pH = 10) with a concentration of 10 pM. The sample was excited at 478 nm using a 300 W coaxial Xenon arc lamp with 4 nm excitation and emission slit.

The fluorescence was detected at magic angle conditions at 1 s accumulation time per wavelength step using a standard blue sensitive PMT. The signal intensity (inset) at peak wavelength shows excellent linear dependence on sample concentration down to 1 pM. At this concentration, the emission intensity is equal to the Raman scattering intensity of water.

By subtracting the corresponding blank spectrum (grey) from the measured intensity (red), it is still possible to recover the correct Fluorescein emission spectrum (blue).

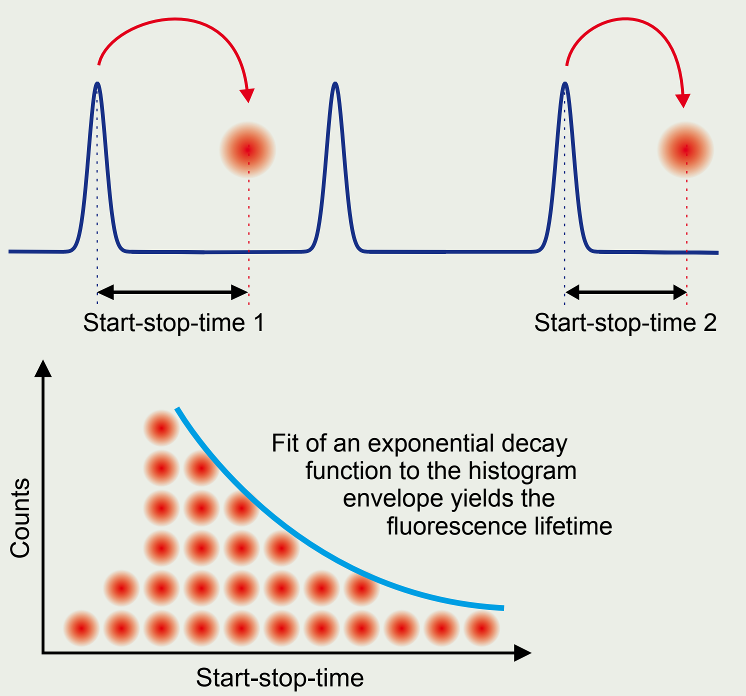
Time-resolved Spectroscopy

In time-resolved measurements, the detected fluorescence photons are registered with respect to the excitation pulse. This allows the study of phenomena ranging from the picosecond to the millisecond time scale. Typical examples are measurements of the fluorescence and phosphorescence lifetime as well as time-resolved anisotropy.

Measurement results within seconds

The time-resolved data acquisition of the FluoTime 300 is based on Time-Correlated Single Photon Counting (TCSPC), which is the most sensitive and precise method to measure fluorescence lifetimes. The raw measurement data directly represents the temporal change in fluorescence intensity and is thus very intuitive. Plotting the data in logarithmic scale, it is easy to check whether decays exhibit only a single lifetime or multiple lifetimes – even polarization effects can be instantly observed. With TCSPC, fluorescence decays can be measured in seconds or even fractions of a second.

Principle of TCSPC



Time-Correlated Single Photon Counting (TCSPC) is based on the precise measurement of the time difference between the moment of excitation and the arrival of the first fluorescence photon at the detector. The measurement of the time difference is repeated many times to account for the statistical nature of fluorescence emission and all measured time differences are sorted into a histogram. This histogram can then be analyzed to extract the fluorescence lifetime and signal amplitude.

Dynamics from ps to s

With a fast Microchannel Plate Photomultiplier Tube (MCP-PMT) or a Hybrid-PMT detector together with excitation sources that have short pulse widths and high repetition rates, lifetimes down to a few picoseconds can be resolved. TCSPC can also be used to measure lifetimes up to several hundred nanoseconds by changing the repetition rate of the excitation sources. Even phosphorescence studies with lifetimes up to a few milliseconds can be performed if the FluoTime 300 is equipped with a dedicated Multichannel Scaling board.

Lifetimes shorter than the instrument response

Fluorescence decays usually show an exponential dependence on time. Extracting the fluorescence lifetime is therefore possible by fitting a suited exponential decay function to the experimental data, taking the characteristics of the instrument into account. These characteristics which include, e.g., the finite pulse width of the excitation source, are commonly described by the Instrument Response Function (IRF), which can be precisely measured with the FluoTime 300. Elaborate data analysis corrects for the finite width of the IRF and thus allows resolving fluorescence lifetimes much shorter than the IRF itself.

"The FluoTime 300 is a versatile, state-of-the-art and exceptionally user friendly fluorescence spectrometer."

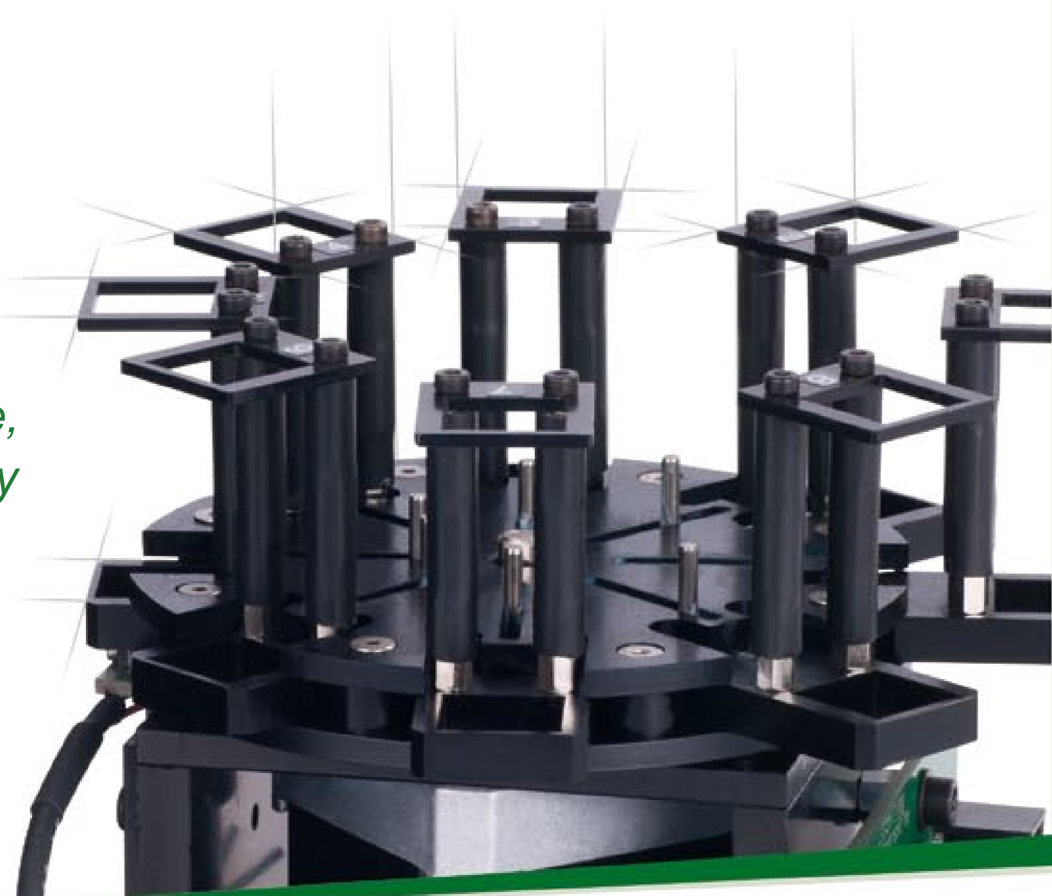
Tebello Nyokong, Rhodes University, South Africa

A probe for the molecular environment

The fluorescence lifetime is characteristic for each fluorophore and can be used to characterize the sample. It is, however, also influenced by the chemical composition of its environment. Additional processes like Förster Resonance Energy Transfer (FRET), quenching, charge transfer, solvation dynamics, or molecular rotation also effect the decay kinetics. Lifetime changes can therefore be used to gain information about the local chemical environment, to follow reaction mechanisms, or as a tool for quality control in industrial applications.

"Acquiring fluorescence spectra has never been easier."

Zygmunt Gryczynski, Texas Christian University, USA

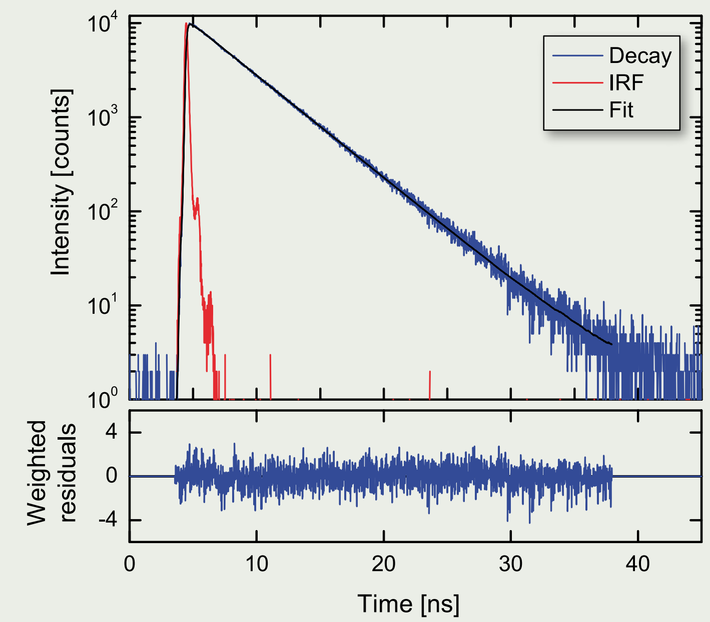


Selected applications

Time-resolved fluorescence spectroscopy can be used in many research areas:

- analytical chemistry - identify or separate species by their fluorescence lifetime, monitor changes in the environment
- biochemistry – study protein folding or signaling pathways
- photobiology – detect singlet oxygen for photodynamic therapy
- biophysics – study membrane rigidity or enzyme/substrate interactions
- industrial quality control – monitor the quality of wafers, semiconductors, or solar cells

Examples

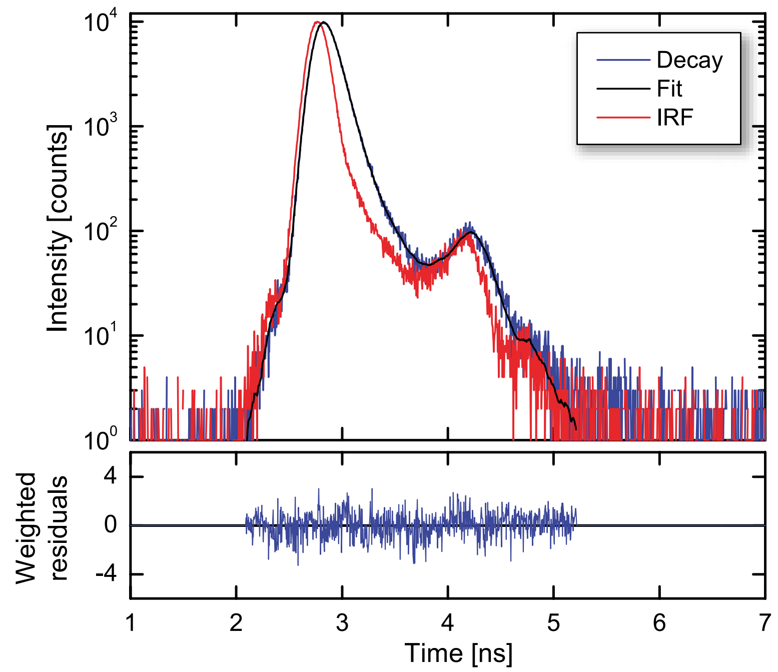


Fluorescence lifetime analysis of a 1 µM Fluorescein solution in pH 10 phosphate buffer. The plot shows the acquired TCSPC histogram (blue) along with the additionally measured Instrument Response Function, IRF (red). During the analysis process, the finite width of the IRF is taken into account by a numerical reconvolution process. The weighted residual trace (bottom panel) clearly shows an excellent agreement between fit and experimental histogram. The recovered lifetime is 3.99 ± 0.01 ns. The measurement was completed in less than 10 seconds.

Measurement Examples

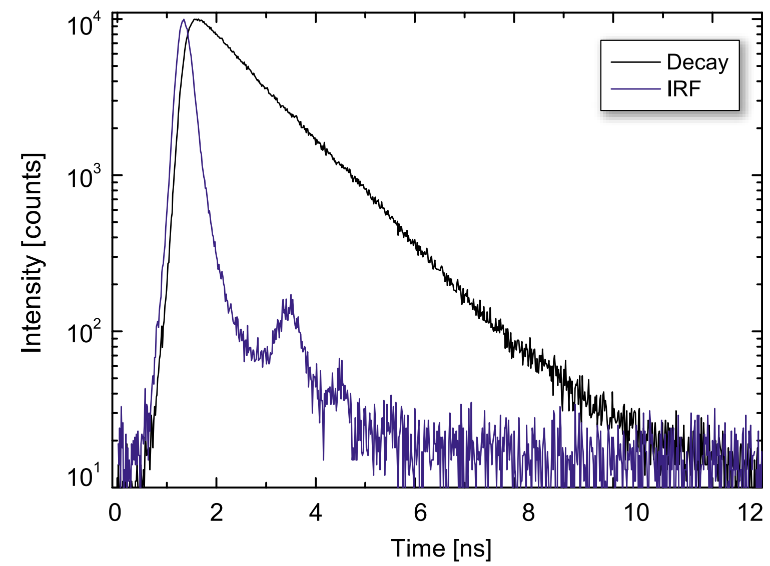
Determination of lifetimes shorter than the instrument response

The excellent stability of PicoQuant lasers, the good temporal resolution of the compact PMT detectors, and the low jitter of the timing electronics make it possible to routinely resolve decay times on the order of tens of picoseconds, even without a MCP-PMT detector. The figure shows the fluorescence decay of a 100 nM solution of Erythrosin B in distilled water. The sample was excited with 531 nm laser light (pulse width 72 ps, FWHM), and the fluorescence was detected at 550 nm with 3 nm bandpass through a polarizer set at magic angle conditions. The IRF was determined to be 200 ps (FWHM). Data analysis using numerical reconvolution resulted in a single lifetime of 89 ps ± 4 ps ($\chi^2 = 1.08$), which is in excellent agreement with the published literature value of 89 ps ± 3 ps.



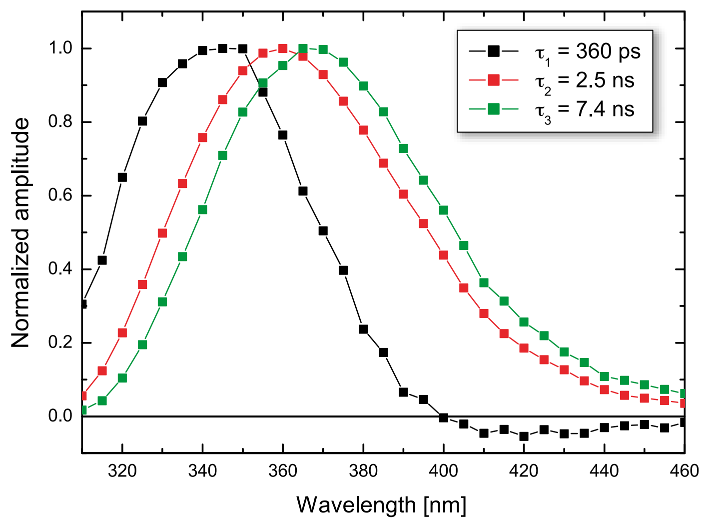
Near infrared fluorescent dyes

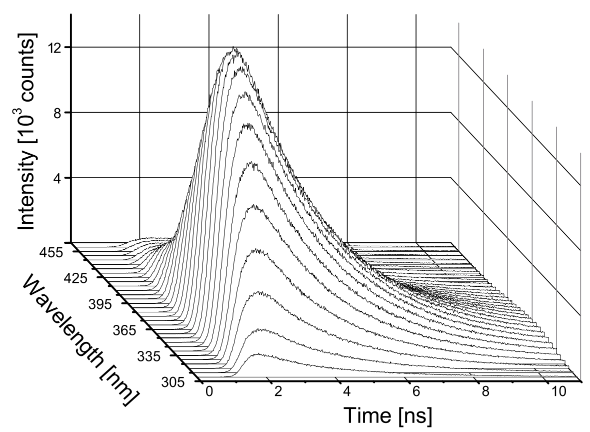
Near infrared (NIR) fluorescent dyes are becoming more and more important as probes in biological systems as they are less affected by light absorption and scattering from biological tissue compared to classical UV and visible fluorophores. The number of new water soluble NIR fluorophores that can be used in biological systems has therefore grown substantially in recent years. Studying these fluorophores requires NIR sensitive detectors with low dark counts and a good temporal resolution to resolve the fluorescence dynamics of these dyes. The example shows the time-resolved fluorescence of the common dye IR-140 in acetone, acquired using a dedicated IR sensitive PMT along with a high quality grating blazed in the NIR. The IRF was determined to be 600 ps (FWHM). Data analysis using numerical reconvolution resulted in a liftetime of 1.16 ± 0.01 ns ($\chi^2 = 1.17$).



Decay Associated Spectra (DAS) of Tryptophan

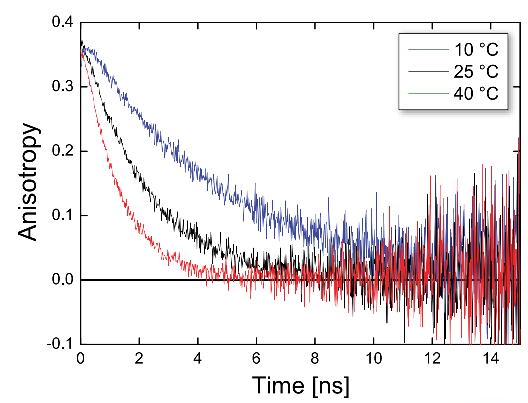
The outstanding sensitivity of the FluoTime 300 together with the high performance pulsed light sources and the analysis software from PicoQuant allows the user to routinely perform studies that previously required special purpose set-ups and custom software development. Examples are Time-Resolved Emission Spectra (TRES) and Decay Associated Spectra (DAS) of Tryptophan. A 17 µM solution of Tryptophan in a pH 7.4 phosphate buffered saline was excited with a pulsed LED at 290 nm, and the fluorescence was detected from 310 to 460 nm. An IRF and 31 decay curves were automatically collected within less than 12 minutes. Global analysis reveals that all 31 decay curves can be well described by three global lifetimes: 360 ps, 2.5 ns, and 7.4 ns. By plotting the wavelength dependent relative amplitudes of these three components, one gets the DAS of Tryptophan, which reveals a relatively slow spectral relaxation. The 360 ps decay component depopulating the blue edge of the emission spectrum contributes to the long wavelength side as a rising (negative amplitude) component.

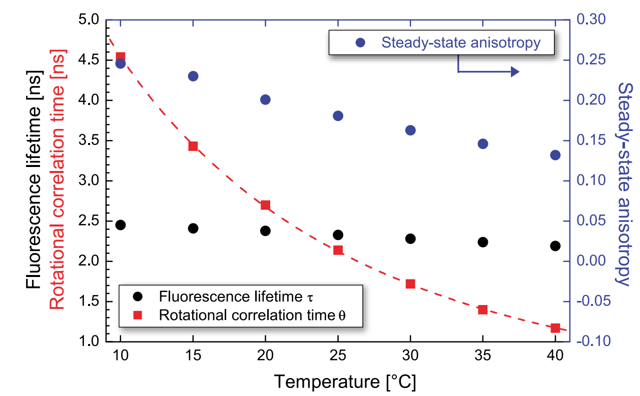




Dynamic anisotropy

Motorized, large aperture, UV-transparent Glan-Thompson polarizers, programmable sample temperature control, and EasyTau software scripting are the key components of the complex measurement sequence that lead to the results presented below. Sample: Coumarin 6 in ethylene glycol excited at 440 nm, emission detected at 510 nm with 3 nm bandpass. The system automatically set the sample temperature, and at each temperature step four measurements were automatically performed: IRF, VV (parallel), VH (perpendicular), and VM (magic angle) polarized decay measurements. A quick analysis of VV and VH decays clearly shows temperature dependent behavior of the emission anisotropy. For detailed quantitative results, global reconvolution anisotropy analysis of the data set was performed with the FluoFit software. A model of a single, spherical, rotating particle with a single exponential fluorescence lifetime describes the system very well, as expected for a small, highly polar particle in polar solvent. The result reveals the slightly temperature dependent single exponential fluorescence lifetime of Coumarin 6. The temperature dependence of the viscosity could be fitted to the experimentally obtained change in the rotation times. Even a precise calculation of the steady-state anisotropy values was possible, which were found to be in perfect agreement with the anisotropy values estimated by the Perrin equation.

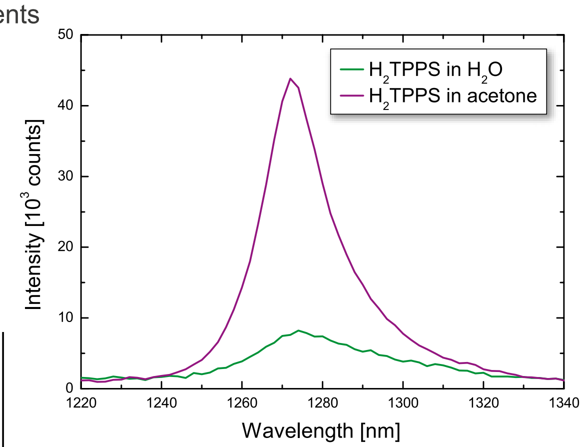


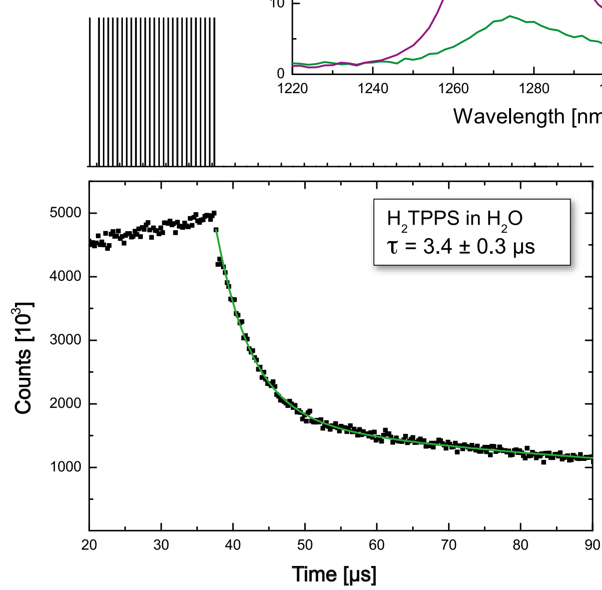


Measurement Examples

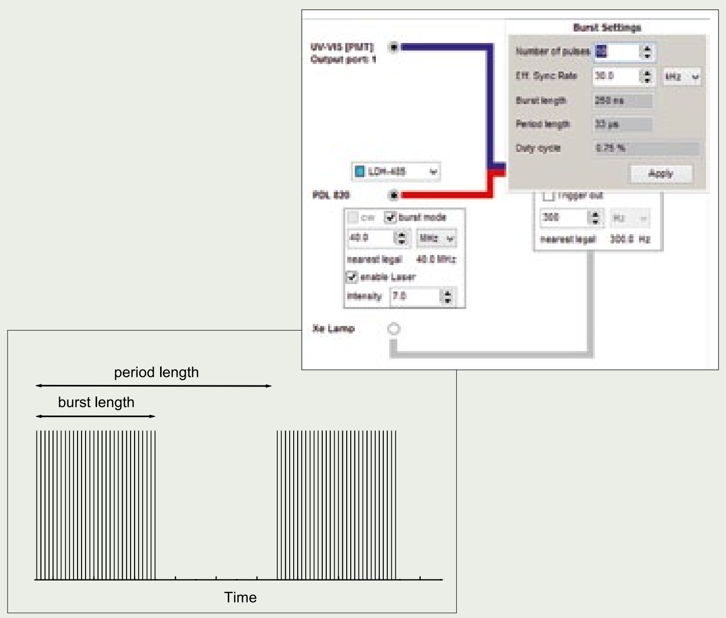
Singlet oxygen phosphorescence of photosensitizer for photodynamic therapy

Singlet oxygen is the common name of an electronically excited state of molecular oxygen which is less stable than molecular oxygen in the electronic ground state. It is typically generated via energy transfer from the excited state of a photosensitizer to the oxygen molecule. The reactive properties of singlet oxygen are, for example, used to destroy cancer cells in photodynamic therapy. In order to optimize such therapies, current research tries to design photosensitizer molecules that optimize the generation of singlet oxygen. Other studies focus on the emission lifetime of singlet oxygen which is solvent dependent, and can therefore be used to gain information about the environment of the emitting oxygen molecules. Singlet oxygen studies are usually performed by steady-state and time-resolved phosphorescence measurements with emission detection around 1270 nm. Such measurements are usually challenging, because the singlet oxygen emission is very weak compared to, e.g., the fluorescence signal of the photosensitizer. The example shows the steady-state spectrum of the singlet oxygen emission produced by H_2TTPS in acetone and even in H_2O, which is especially challenging due to the spectral overlap of the OH vibration of water and singlet oxygen emission. The second graph additionally shows the time-resolved singlet oxygen measurement using the burst mode feature of the FluoTime 300. A tail fit yields a lifetime of 3.4 ± 0.3 µs, which is in excellent agreement with the published literature value. The high sensitivity of the FluoTime 300 even in the NIR region thus make additional set-ups to perform time-resolved and steady-state measurements of singlet oxygen even in H_2O obsolete.





Burst mode



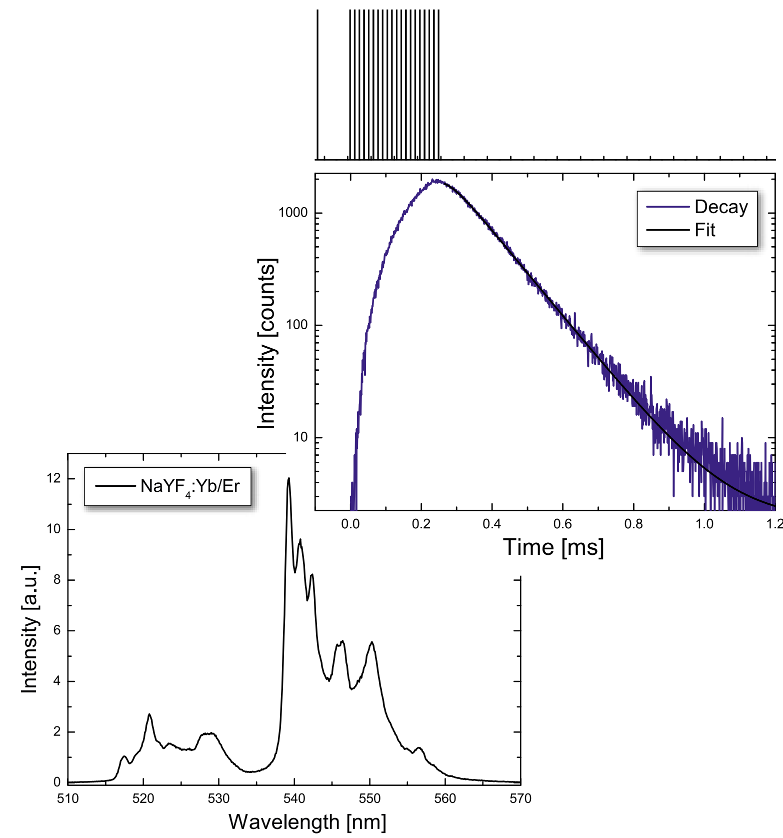
PicoQuant's pulsed diode laser heads are optimized for repetition rates in the MHz range, which allows a fast measurement of fluorescence lifetimes in the ps to ns range. Measuring longer lifetimes up to the ms range requires corresponding low repetition rates of the excitation source. As diode laser are often not efficient excitation sources at low repetition rates, due to their limited pulse energy, such measurements are usually performed using pulsed Xenon flash lamps. This does, however, require an additional excitation path with a suited excitation monochromator.

As an alternative to pulsed Xenon flash lamps, the FluoTime 300 features a special "burst mode" which does not require any additional hardware and still makes lifetimes measurements up to ms possible. In burst mode, the diode laser first emits a variable number of laser pulses at repetition rates in the MHz range and is then switched off to detect the emission of the sample. The individual laser pulses act like one single pulse with a much higher pulse energy. In that way, a large number of molecules can be excited and the resulting fluorescence can easily be detected by the FluoTime 300. Measurements of phosphorescence and excited states with long lifetimes are possible without the use of further excitation sources.

Fluorescence upconversion of nanoparticles

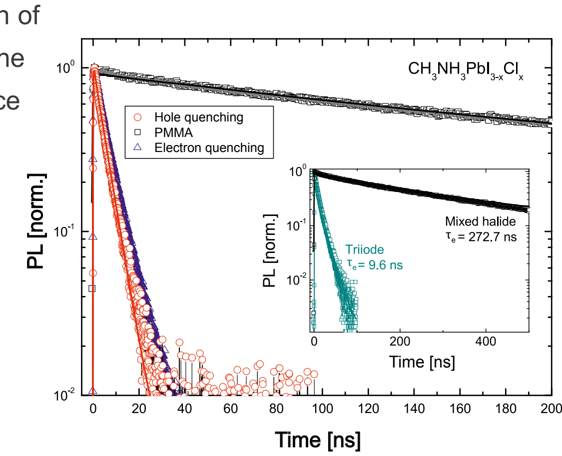
Fluorescence upconversion particles absorb light in the near infrared (NIR) and emit light in the visible range. These particles are currently in the focus of research because of their prospective application for, e.g., in vivo optical imaging, as they allow excitation in the NIR spectral range where light absorption and scattering from biological tissue is minimized. Another possible application are dye sensitized solar cells. A standard solar cell does not absorb in the near infrared spectral range, as the energy of the NIR light is too low for injecting electrons into the conducting band of the solar cell material. A possible option to overcome this limitation and thus create solar cells with a higher light conversion efficiency are dye sensitized solar cells in which upconversion particles are used to convert the NIR light into visible light which then has enough energy to leap the band gap of the semiconducting material.

The example shows the steady-state upconversion spectrum of $NaYF_4$ nanoparticles co-doped with erbium and ytterbium in cylcohexane in the visible range under laser excitation at 980 nm. In order to measure a time-resolved spectrum, the burst mode feature of the FluoTime 300 was used. A tail fit of the data reveals two fluorescence lifetimes of 100 µs and 30 µs. Although fluorescence upconversion is a rather small effect and therefore normally difficult to observe, the high sensitivity of the FluoTime 300 together with the NIR laser of the LDH Series renders these experiments feasible.



Determining electron-hole diffusion lengths in perovskite solar cells

A critical parameter in understanding the photophysics of semiconductor solar cells is the diffusion length of the photo-excited electrons and holes in the material. Time-resolved photoluminescence quenching experiments are a valuable tool for determining diffusion lengths. The example shows data obtained from mixed halide and triiodide organometal perovskite layers in presence of either an electron (blue) or hole (red) quenching layer, or a PMMA coating (black). The decay curves were recorded at 780 nm, corresponding to the peak emission of both materials. The measured decay dynamics can be fitted to a diffusion model, allowing to derive diffusion lengths. Here, the diffusion length of the electrons and holes in the mixed halide perovskite was 1 µm while the triiodide material featured a much shorter length of 100 nm, correlating well with performance of these materials as solar cells.



S. D. Stranks et al., Science, 342 (2013), p.341

System Components

System Components

Compact in size, ultimate in performance

The FluoTime 300 has exceptional sensitivity in combination with unprecedented ease-of-use. The system builds on key technologies like picosecond pulsed diode lasers and Time-Correlated Single Photon Counting (TCSPC) electronics developed by PicoQuant and complemented by high-end optomechanics, detectors, and accessories.

High quality optical components

The FluoTime 300 includes various high quality optical elements such as lenses, polarizers, attenuators, and filter wheels.
All components are motorized and their position is either automatically optimized by the EasyTau software wizards or manually adjustable in the “customized measurements” mode. Even the entrance and exit slits of the monochromators are under complete computer control, leaving the placement of the sample to be the only necessary manual step for measuring a fluorescence spectrum.

Efficient monochromators

The Czerny-Turner monochromators have a focal length of either 150 or 300 mm. Both can be equipped with various high quality diffraction gratings in order to achieve spectral resolution down to 0.1 nm over a broad wavelength range. Wavelength tuning is computer controlled with a minimum step size of 0.01 nm. The spectral bandpass of the monochromators can be easily adjusted via the system software.

The heart of the FluoTime 300 is a large multifunctional sample chamber with fully automated optical elements and support for different sample holders including cryostats.

TCSPC and MCS data acquisition

Two outstanding data acquisition units are available for time-resolved as well as steady-state fluorescence measurements: the TimeHarp 260 and the PicoHarp 300.

The TimeHarp 260 is a state-of-the-art, compact, and easy to use PCIe board that offers an adjustable temporal resolution from 25 ps to 5.2 ms and is therefore the perfect choice for measuring steady-state and time-resolved luminescence decays with lifetimes ranging from a few ten picoseconds up to several seconds. The TimeHarp 260 is a perfect match for systems equipped with photomultiplier tubes or hybrid photomultiplier tube detectors. For measuring ultrafast dynamics, the stand-alone unit PicoHarp 300 offers an even better temporal resolution down to 4 ps and is therefore recommended for FluoTime 300 systems equipped with MCP-PMT or blue sensitive hybrid photomultiplier tube detectors.

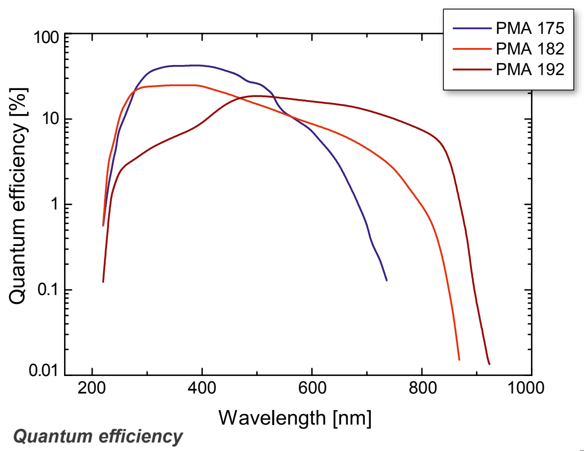
The two data acquisition units PicoHarp 300 and TimeHarp 260 offer Time-Correlated Single Photon Counting (TCSPC), Multi-Channel Scaling (MCS), coincidence correlations, or event timing with temporal resolutions between picoseconds and milliseconds. The units work in histogramming mode and offer two time tagging modes.

System Components

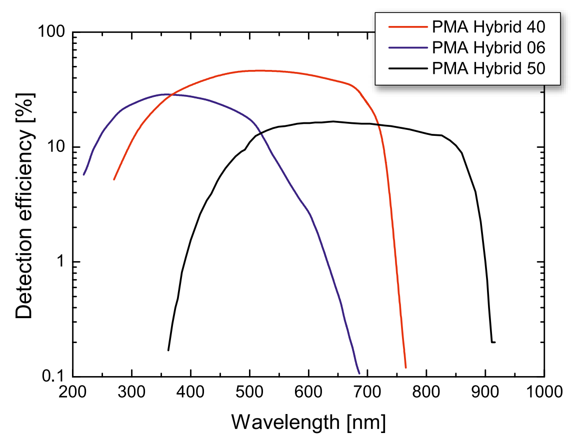
Photon counting detectors

The FluoTime 300 can be configured with one or two single photon counting detectors. Each detector includes an electro-mechanical shutter, optional cooling, and an overload protection that can be operated from the system software. The available detectors are either PMTs, MCP-PMTs, or Hybrid-PMT modules.
PMTs of the PMA Series cover the spectral range from 200 to 920 nm. Selected and configured for optimum detection efficiency and temporal resolution, the PMTs of the PMA Series are the best choice for resolving lifetimes down to 50 ps. For applications that require even higher temporal resolution, MCP-PMTs are the detectors of choice. When operated along with an appropriate short pulsed laser system, their very fast response time permits resolving lifetimes even down to 10 ps. All MCP-PMTs can be equipped with a thermoelectric cooler to reduce dark counts. A relatively new but excellent alternative to MCP-PMTs and PMTs is the Hybrid-PMT. Hybrid-PMTs of the PMA Hybrid Series feature a high detection efficiency up to 45 % along with a very good timing response, which is even comparable to a MCP-PMT for certain models. Hybrid-PMT detectors are available in a spectral range between 220 and 900 nm. Infrared optimized PMTs cover the spectral range between 950 and 1700 nm. All NIR-PMTs have an internal thermoelectric cooler (coding system), eliminating the need for liquid nitrogen and/or cooling water. The response time of these detectors is fast enough to resolve lifetimes down to 100 ps.

Typical detectors for the FluoTime 300: NIR-PMT, PMT, and Hybrid-PMT.



Quantum efficiency as a function of wavelength of available PMT detectors.



Detection efficiency as a function of wavelength of available Hybrid-PMT detectors.

Excitation subsystem

The excitation subsystem consists of compact turn-key picosecond pulsed diode lasers (LDH Series) or LEDs (PLS Series) covering the wavelength range from 245 nm to the infrared. The lasers emit pulses as short as 40 ps, and even the LEDs reach pulse widths well below 1 ns. Repetition rate and output power of all lasers can be adjusted via software, making them ideal excitation sources for fluorescence lifetime measurements.

Since all LEDs and laser heads work with the same driver unit, switching wavelengths is as easy as changing an electric bulb on a desk lamp. For excitation and emission spectra, different steady-state and pulsed Xenon arc lamps provide continuous excitation light between 200 and 1000 nm.

Other external lasers such as Titanium:Sapphire lasers or PicoQuants supercontinuum laser family Solea can also be used.

The Solea combines short pulse widths with wavelength tunability from 480 to 900 nm, allowing to perform steady-state emission and excitation scans as well as time-resolved measurements with the same light source. Controls for the Solea laser series are fully integrated into the EasyTau software. Another option for the excitation subsystem is the versatile pulsed laser module VisUV offering picosecond pulses at up to three wavelengths (266, 355, and 532 nm). Combined with flexible repetition rates from single shot to 80 MHz and pulse widths < 70 ps, the VisUV is an excellent choice for time-resolved fluorescence spectroscopy applications, especially in the ultraviolet for, e.g., the excitation of amino acids in order to investigate intrinsic protein fluorescence.

Typical excitation sources are compact pulsed diode lasers with picosecond pulses, LEDs with sub-nanosecond pulses, supercontinuum lasers like the Solea series, or versatile multi-wavelength laser modules like the VisUV.

System Components

Sample mounting units

- **Integrating sphere with sample holder for liquid and solid samples**
  Determination of absolute quantum yields with high precision
- **Adjustable front face sample holder with slide clamp**
  Tunable sample position and light incidence angle
- **Peltier-cooled 4-position sample holder**
  Fully motorized and software-controlled temperature settings
- **8-position sample holder**
  Ideal for high throughput measurements
- **Adjustable front face sample holder with inclined inset**
  Excellent for the investigation of powder samples
- **Peltier cooled sample holder for single cuvette**
  Programmable temperature controls
- **Temperature stabilized sample holder**
  Temperature range of -20 °C to 135 °C depending on external bath

System Components

Specialized sample holders
The large multifunctional sample chamber of the FluoTime 300 can accommodate a wide variety of specialized sample holders. Their modular design allows for easy and on-the-fly switching between mounting units, offering unprecedented flexibility in measuring liquid, solid, or powder samples under a range of conditions.

Sample holder with fiber coupler
This unit features a fiber coupler instead of a sample holder, allowing to interface a fiber coupled optical microscope with the FluoTime 300. In this way, the FluoTime 300 can be used to measure either steady-state or time-resolved luminescence spectra from a sample in the microscopes focal spot. The full range of optical elements of the FluoTime 300, such as automated polarizer, filter wheel, attenuator, and monochromator can be used along with the analytical tools of the EasyTau and FluoFit software package to investigate light emitted from the microscope focal volume.

Adjustable front face sample holder
Characterizing the luminescent properties of solids, powders, films, coatings, or wafers requires a front face sample holder. This mounting unit allows to manually adjust the sample position and its angle with respect to both incident and detection beam paths with great accuracy. Tuning this angle permits not only to compensate for light scattering from the sample but also to optimize the sample position. The holder is designed for samples with sizes of up to 2 inches and comes either with a slide clamp or an inclined insert.

Sample mounting unit with temperature stabilized holder for 1 cm x 1 cm cuvettes
This mounting unit features a temperature stabilized sample holder for standard 1 cm x 1 cm cuvettes. The sample holder is connected to an external circulating thermal bath via the cable glands on the front panel of the unit. Additionally, an inlet for dry purging gas is provided on the unit front panel to help prevent humidity condensing on the cuvette.

Sample mounting unit with 8-position holder
This sample mounting unit features slots for up to 8 standard optical cuvettes (1 cm x 1 cm). The sample holder is fully motorized and controlled by the EasyTau operating software, allowing to seamlessly switch from one sample to another without manual intervention. This holder assembly is neither temperature stabilized nor cooled by a Peltier element. It is ideal for high throughput fluorescence measurements at ambient temperatures, where many different samples (e.g., a concentration series) should be measured in a single experiment.

Peltier cooled sample holder for 1 cm x 1 cm cuvettes
The sample mounting unit features a Peltier cooled sample holder for standard 1 cm x 1 cm optical cuvettes. The Peltier element allows setting and maintaining the sample temperature in the range of -15 °C to 110 °C and is fully controlled by the EasyTau operating software, allowing not only to set a fixed value but also to map out the temperature dependence of the fluorescence properties. The unit is also equipped with an inlet for purging with dry gas to prevent water condensation from occurring on the cuvette walls.

Peltier cooled sample mounting unit with 4-position holder
A revolver head featuring sample holders for up to four 1 cm x 1 cm cuvettes is provided in this mounting unit. The entire holder head is cooled by a Peltier element, allowing operation in the temperature range from -15 °C to 110 °C. The holder is connected to an external circulating thermal bath via cable glands on the unit front panel for thermal stabilization. An inlet for dry purging gas is available to prevent humidity from condensing on the cuvette. The 4 slot revolver head of the sample holder is fully motorized and controlled by the EasyTau operating software.

Sample mounting unit for liquid nitrogen Dewar
This sample mounting unit is an inexpensive solution for luminescence measurements at 77 K. The FluoTime 300 can be equipped with a quartz Dewar in a specially designed support, allowing clear access to the sample from all four directions. The liquid or powder sample is filled into a quartz tube that is then submerged in liquid nitrogen. The simple design of the Dewar and sample tubes allows to quickly swap out samples while minimizing down times waiting for thermal stabilization. This sample mounting unit comes with a specially designed top hat lid for closing the sample chamber.

Integrating sphere for quantum yield measurements
Luminescence quantum yield measurements are typically done by comparing the emission intensity of a compound with a standard of know quantum yield under identical measurement conditions. This method is well established and precise, but also time consuming. In addition, target compound and reference have to have similar absorption and emission wavelength ranges. In cases where a suitable standard is not available or, generally speaking, for all scattering samples, the use of an integrating sphere to determine the absolute luminescence quantum yield is mandatory.
The FluoTime 300 can be equipped with an integrating sphere for measuring solutions as well as solid samples. It has been thoroughly designed, calibrated, and is able to reproduce published literature data of selected quantum yield standards such as Rhodamine 6G, Cumarin 153, and Ru(byp)3 with high precision. The complete work flow for quantum yield determination is included in the EasyTau software as a dedicated wizard. Precise quantum yield measurements can be routinely performed in a few minutes even by untrained users.
All relevant fluorescence characteristics can therefore be acquired with only one instrument, streamlining the work flow and keeping all calibration schemes simple.

System Components

Rapid kinetic (stopped-flow) accessory

A rapid kinetic or stopped-flow accessory (SFA-20 series from TgK Scientific Ltd) monitors fast reactions in solutions on a millisecond time scale. The accessory has an empirical dead time < 8 ms, which makes it possible to monitor reactions like enzyme kinetics, quenching, association/dissociation, and so on. As an option, a microvolume version, pneumatic drive, anaerobic kit, variable ratio mixing, multimixing version, and advanced analysis software (Kinetic studio) can be provided along with the FluoTime 300.

Cryogenic temperatures

For applications that require cryogenic sample conditions, the FluoTime 300 features a large multifunctional sample chamber that can be equipped with a liquid nitrogen cryostat from Oxford Instruments (Optistat Series) or a closed-cycle helium cryostat from ARS (DE-202 Series).

The Oxford OptistatDN is a nitrogen bath cryostat providing cryogenic environments ideal for optical measurements between 77 and 500 K. Changing the sample is simple and does not require breaking the insulating vacuum or warming up the cryostat. The control of the cryostat is fully integrated into the EasyTau software, which allows to record even time intensive temperature scan maps fully automatically.

For measurements at even lower temperatures, a closed-cycle helium cryostat DE-202 from ARS can be supplied along with the FluoTime 300.

This cryostat is a compact, axially symmetrical, closed cycle cryocooler which is ideal for small heat loads and enables low temperature photoluminescence measurements from 4 to 500 K.

Specifications

Optical configuration
- L-Geometry

Mode of operation
- Time-Correlated Single Photon Counting (TCSPC)
- Multichannel Scaling (MCS)

Sensitivity
- Signal-to-noise ratio typically better than 26 000:1, as measured from a water Raman spectrum, excitation wavelength 350 nm, spectral bandwidth 5 nm, integration time 1 s

Lifetime range
- 50 ps to s with PMT detector and TCSPC electronics
- < 20 ps to s with Hybrid-PMT detector and TCSPC electronics
- < 10 ps to s with MCP-PMT detector, TCSPC electronics, and suitable laser
- Up to several s with PMT, Hybrid-PMT or MCP-PMT, and MCS electronics

Excitation sources
- Picosecond pulsed diode lasers or LEDs with repetition rates up to 80 MHz, common driver unit
- Solea: broadband tunable laser with repetition rates up to 40 MHz
- Sub-microsecond pulsed Xenon flash lamp
- 300 W Xenon arc lamp
- External lasers such as Titanium:Sapphire lasers

Monochromators
- Czerny-Turner type
- Focal length: 150 or 300 mm, single or dual exit slits
- Grating with 1200 g/mm, blazed at 500 nm or 600 g/mm blazed at 1250 nm (other gratings on request)
- Slit width adjustable between 10 µm and 4 mm (continuously adjustable, full computer control)
- Stray light rejection typically $1:10^{-5}$ with suitable longpass filter $1:10^{-10}$

Detectors
- Cooled or uncooled detectors
- Photomultiplier Tubes (PMTs) with different spectral ranges between 200 and 920 nm
- Microchannel Plate Photomultiplier Tubes (MCP-PMTs) with various spectral ranges between 185 and 910 nm
- Near infrared sensitive Photomultiplier Tubes (PMTs) with different spectral ranges between 950 and 1700 nm
- Hybrid-PMTs with spectral range between 300 and 890 nm

Software
- Easy to use and comprehensive Windows based system and analysis software
- Data archiving in workspace, data export features, data arithmetic
- Application wizards for several typical measurement tasks
- Customized measurement mode with full instrument control and optional measurement parameter optimizer
- Scripting language for user-defined data acquisition and measurement sequences
- Lifetime analysis based on numerical reconvolution procedure, up to 4th exponential decay functions, lifetime distributions, anisotropy, global analysis, rigorous error analysis

Specifications are subject to changes.

PicoQuant for Scientists

The annual workshop on Single Molecule Spectroscopy brings together the top researchers in this field.

Application lab

We always welcome scientists to visit our PicoQuant application labs and see the FluoTime 300 working, or do test measurements with their own samples. We perform a quick and qualified investigation of your scientific problems. We discuss your needs and try to offer a solution tailored to your requirements. Of course, all of our other products, including the single molecule sensitive MicroTime 200 microscope system, are also available for testing and evaluation.

Courses on time-resolved fluorescence

To improve the understanding and promote the use of time-resolved fluorescence spectroscopy and microscopy, PicoQuant established the "European Short Course on Principles and Applications of Time-resolved Fluorescence Spectroscopy" as an annual event in 2003. In 2009, an additional event was introduced focusing on time-resolved microscopy. Both courses are intended for individuals seeking an in-depth introduction to the principles of fluorescence spectroscopy and microscopy and their applications to the life sciences. They consist of lectures by renowned scientists as well as comprehensive instrumentation and software hands-on training.

Workshop on single molecule spectroscopy

Since 1994, the scientists from PicoQuant organize the annual workshop on "Single Molecule Spectroscopy and Super-resolution Microscopy in the Life Sciences" that brings together the top researchers in this field. With this event, we continue to encourage the exchange of knowledge and new ideas between the experts in single molecule spectroscopy, 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 used all over the world. They are used in the laboratories of Nobel Laureates and help to prepare papers in high-ranking journals as well as carrying out routine quality control and 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 tomography, quantum information processing, optical detector testing, and telecommunications. Due to our easy to use products, researchers can now focus on their problems 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 intention is to offer state-of-the-art technology which has been co-developed and tested by renowned researchers, at a price affordable to 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 provides a stable basis for new outstanding developments 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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