LaserStrobe

Time-Resolved Spectrofluorometer

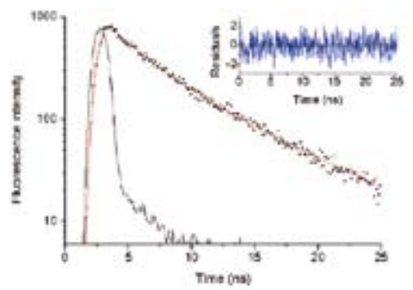
The LaserStrobe™ spectrofluorometer is an L-format system for time-resolved fluorescence measurements from less than 100 picoseconds up to the microsecond range. PTI's lifetime instruments are the only instruments on the market today that employ the patented Stroboscopic Lifetime Technique: the newest, most advanced, less expensive, and easy to use technology compared to alternative methods. The LaserStrobe™ was designed to provide the most important features (missing in many commercial instruments) to the user: nearly continuous wavelength coverage from UV to NIR, excellent lifetime resolving power attained by the inclusion of nonlinear timescales, such as the arithmetic progression and logarithmic timescales, and robust electronics that require no user adjustments. The LaserStrobe™ spectrofluorometer features the same Open Architecture design as other PTI instruments, allowing for easy upgrades to steady state, phosphorescence, or NIR capabilities, and can be also easily converted to lifetime microscopy.

Intensity And Lifetime Measurements Are Complementary In Luminescence

It is often necessary to combine results from steady state and lifetime studies in order to obtain the most complete information about the studied object. In the simplest example, both measurements are necessary to fully characterize the excited state of an organic molecule - to find out what are the rate constants for the emission and for the non radiative deactivation. This information is readily available by combining the lifetime from the time-resolved measurement with the quantum yield from the steady state. Another example would be to characterize a molecule under study and its interactions with the surrounding environment. In the steady state measurement alone, which can provide a fluorescence spectrum, fluorescence quantum yield or anisotropy value, most of this information is scrambled, as the measured parameters are time averages and the information about specific processes is lost. This lost information becomes especially important when fluorescent molecules are used as probes to study complex systems such as proteins, nucleic acids, membranes, polymers, surfactants (micelles), etc. These systems frequently exhibit multiple structural domains and conformations. The fluorescence decay will reveal this information by exhibiting multiple lifetimes, while on the other hand this information will be totally obscured in the steady state measurement alone.

Consider a simple case of a protein containing one Trp residue such as human serum albumin HSA. Carry out a steady state measurement and you'll get a typical Trp spectrum reflecting no particular information about the protein, except that it contains Trp. However, if you measure its fluorescence decay, you'll find that this single Trp residue has 3 different lifetimes! You know immediately that the protein exists in at least 3 different conformational states.

Complex fluorescence decay of human serum albumin (HSA) containing a single tryptophan residue. A 3-exponential model was required to obtain a satisfactory fit: τ1=0.45 ns (18%), τ2=3.10 ns (25%), τ3=6.51 ns (15%).

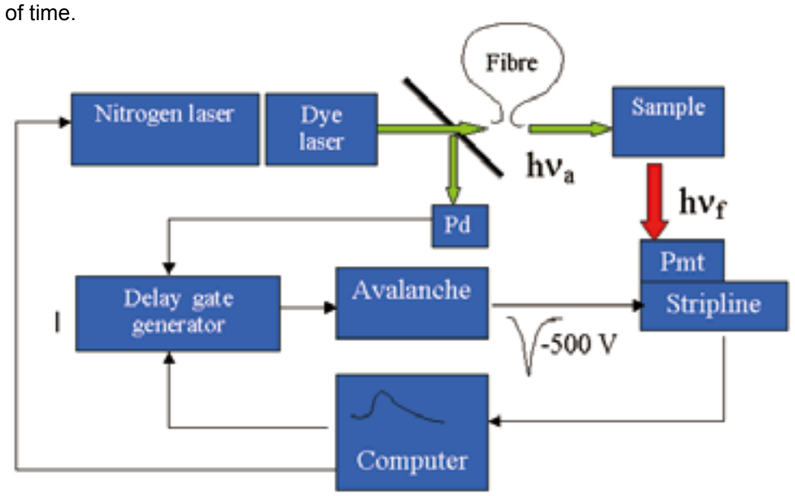


How much information are you missing by only measuring intensity?
Applications using time-resolved luminescence have been growing very rapidly during the last decade. They encompass very diverse disciplines, ranging from such traditional areas as photochemistry, photophysics and photobiology to medicine, numerous applications in industry and even agriculture. Now you don't have to have an engineering degree to operate the lifetime equipment... utilizing the LaserStrobe™ makes time-resolved measurements fast and easy.

The Stroboscopic Technique

The Strobe technique is a time-domain technique. It measures fluorescence decay curves (i.e. fluorescence intensity as a function of time) directly and the researcher has full advantage of seeing the physical mechanism in the course of the experiment. Frequently, a qualitative judgement about a particular mechanism can be made by examining raw decay data and a proper fitting function can be selected.

The Strobe technique is the most recent, and electronically, the simplest technique. It utilizes a pulsed light source (a laser or an LED) and measures fluorescence intensity at different time delays after the pulse. As a result, a fluorescence decay curve is collected. The principle of operation of the LaserStrobe™ system, based on PTI nitrogen and dye lasers, is outlined in the diagram below. The laser is triggered by the software/interface with the repetition rate up to 20 Hz controlled by the user. The optical pulse from the dye laser (or optional frequency doubler) is fed by a single optical fiber to the sample compartment and excites the sample. In order to eliminate any potential jitter (i.e. the uncertainty between the time the laser is triggered and the time it actually fires), a photodiode (Pd) is placed in front of the laser and the pulse from the Pd is routed to a digital delay gate generator (DGG) unit, which outputs a delayed TTL pulse. The DGG is under computer control and the value of the TTL pulse delay is determined in the acquisition software. The delayed pulse triggers an avalanche circuit, which provides a narrow high voltage pulse (ca. -500V) for the detection circuitry. This pulse creates the gain and the temporal discrimination gate for the photomultiplier. Scanning the gate (time delay) across the fluorescence decay allows the acquisition of fluorescence intensity as a function of time.



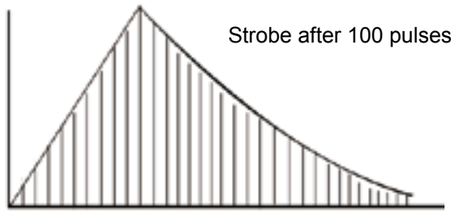
One of the advantages of the Stroboscopic technique is the ability to utilize low repetition, inexpensive lasers, such as PTI's nitrogen/dye laser, which can provide virtually continuous excitation wavelengths from 360 to 990 nm or from 235 to 990 nm with the optional frequency doubler. There is simply no other system on the market with this capability!

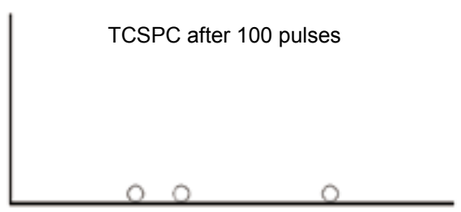
The Advantages Of The Stroboscopic Technique

- Time domain: direct measurement of fluorescence decay curves
- Sensitivity: picomolar concentrations of fluorophores measured in minutes
- Works with low rep rate N_2/dye lasers: unsurpassed coverage from 235 to 990 nm
- Speed: fast measurements achieved by measuring fluorescence intensity directly and unlike photon counting methods, is not limited by photon counting statistics
- Superior lifetime resolvability: the only technique that uses logarithmic and arithmetic progression timescales in addition to a conventional linear timescale
- Random acquisition: eliminates bias when measuring samples that are inherently unstable

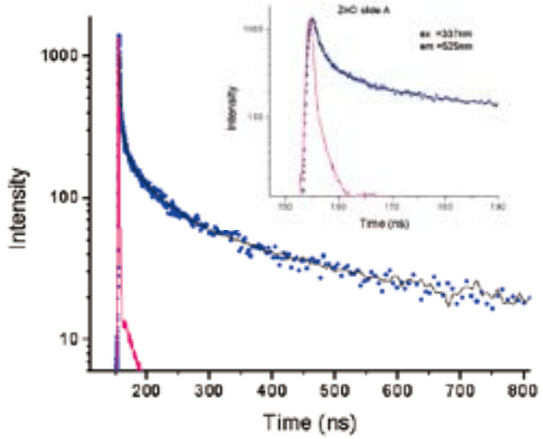
Data Acquisition – Fast And Flexible

The Stroboscopic technique has been designed with performance and efficiency in mind. Unlike the conventional single photon counting, which is plagued by its infamous pulse pile-up problem and can detect only 2-3 photons per 100 light pulses, the LaserStrobe™ measures emission intensity directly after each excitation pulse.

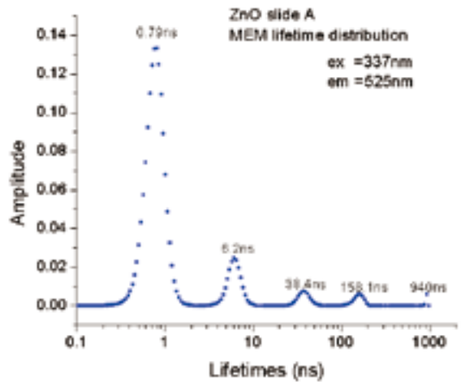




The key feature of the Stroboscopic technique is a pulsed photomultiplier as part of the detection system. The timing is very precise, with steps as small as 25 ps. As a direct consequence of software control over the timing, the Stroboscopic technique has a unique ability to acquire data with a nonlinear time base and in a random sequence. For example, with the use of our Logarithmic Timebase Acquisition protocol, it is possible to measure lifetimes differing by four orders of magnitude in one single experiment! The Random Acquisition mode, on the same hand, eliminates bias when measuring samples that are inherently unstable.



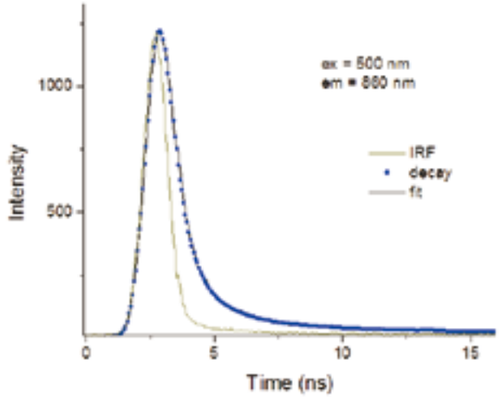
Fluorescence decay of ZnO sample measured at 525 nm band with the LaserStrobe™. The decay is very complex, with lifetimes ranging from hundreds of ps to hundreds of ns. In order to enhance temporal resolution, a logarithmic timescale was used, which provides higher density of data points at short times. See expanded decay (insert).



MEM lifetime distribution analysis of ZnO decay. The lifetime distribution function shows 5 distinct lifetime peaks. The lifetime values shown are the averages over respective distribution peaks. Such complex analysis is impossible with a discrete multiexponential fitting function – the MEM, aided by the use of logarithmic timescale, is the only feasible alternative.

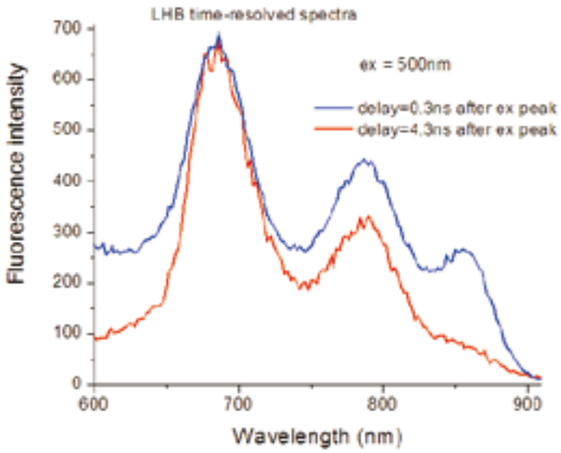
Data Acquisition – Continued

Time-resolved spectra measurements are just as easy to perform on the LaserStrobe™ as with a steady state system. The emission monochromator scans the desired wavelength range based on the time delay after the excitation pulse that is entered into the software - it's as simple as that!



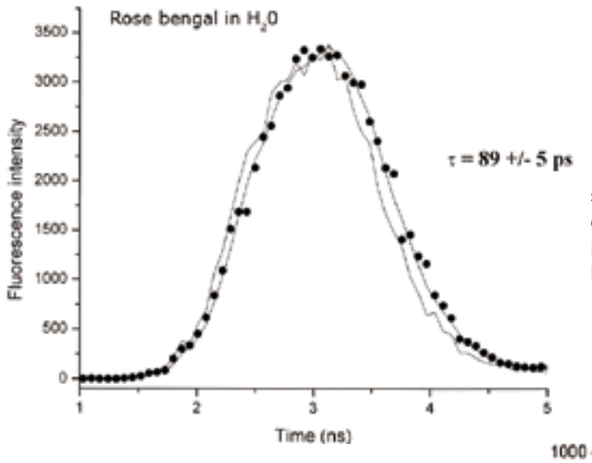
Fluorescence decay of light harvesting chlorophyll complex LHB measured with the LaserStrobe™. The recovered lifetimes are: 0.43 ns (85%), 1.17 ns (13%) and 7.6 ns (2%). (Sample courtesy of Dr. Robert Blankenship, Washington Univ, St. Louis.)

Fluorescence time-resolved spectra (TRES) of LHB scanned directly at two different delay times after the excitation pulse reveal spectral differences between short and long lifetime components.



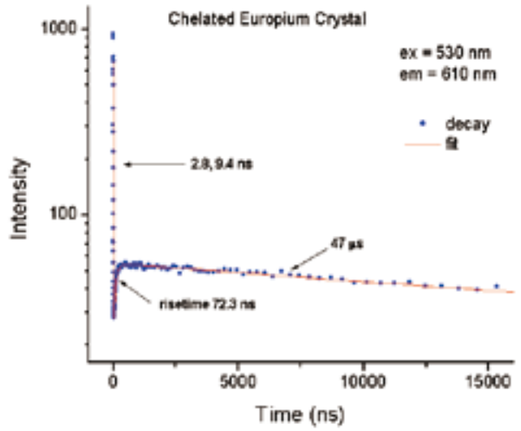
Lifetime Range

The shortest lifetime that can be measured with a lifetime instrument depends on the temporal pulse width, pulse stability and electronic response. The optical pulse of the N2 laser and the timing electronics of the LaserStrobe™ are extremely stable, which greatly contributes to its excellent temporal resolution. By utilizing an iterative reconvolution algorithm, the LaserStrobe™ is capable of determining lifetimes ranging from under 100 ps to approximately 50 µs.



Due to the LaserStrobe's excellent pulse stability, lifetimes as short as 100 ps and lower can be deconvoluted from decay curves. Here is an example of Rose Bengal in water whose literature reported lifetime is 91 ps.

Due to our unique Stroboscopic technique, the LaserStrobe™ can easily capture multiple lifetimes and risetimes ranging from single nanoseconds to tens of microseconds in a single decay sweep. Note the logarithmic time scale employed, which makes this experiment possible. (Sample courtesy of Dr. Mary Berry, Univ. of South Dakota).



Wavelength Range

Sample excitation is afforded by PTI's own GL-3300 nitrogen laser coupled to the high-resolution GL-302 dye laser. This excitation source provides a clean optical pulse of approximately 800 picosecond duration with an extremely low spectral bandwidth of 0.04 nm. When pumped by a nitrogen laser, the dye laser is tunable over a wide spectral range from 360 to 990 nm. The laser dyes are placed in the dye laser cavity in stoppered 1x1 cm cuvettes, so changing from one dye to another takes seconds. The optional frequency doubler allows for powerful UV output down to 235 nm.

The basic LaserStrobe™ system is equipped with an automated emission monochromator with a standard PMT that can detect from 200 to 680 nm. An optional red sensitive PMT is available, which will extend the detection range to approximately 930 nm.

Software

FeliX32™ Software Package

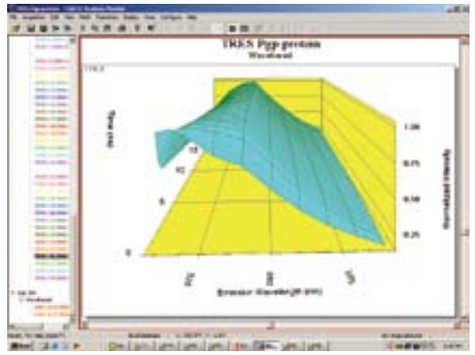
PTI's FeliX32™ is the most comprehensive software package on the market. It's easy to use Windows™ based interface offers one software solution for all your fluorescence measurements. FeliX32™ uses full 32-bit implementation graphics capabilities, including sophisticated 3-dimensional plotting and full motion rotation. All major data handling packages are included: multi-exponential fits, global analysis, non-exponential analysis, anisotropy decay as well as maximum entropy methods. FeliX32™ also uses script controlled data acquisition so that specialized experimental routines can be easily created by the end user via FeliX32™ macro commands. This allows for unsurpassed flexibility in acquisition, calculation,

Time Resolved Fluorescence with FeliX32™

- **Fluorescence & phosphorescence decays**
  Measure lifetimes from less than 100 ps to seconds
- **Time-Resolved Spectra (TRES)**
  Characterize components in a mixture or follow processes in the excited state
- **Gated spectra**
  Separate phosphorescence from fluorescence or eliminate scattered light and impurity fluorescence for your lanthanide-based assays
- **Random acquisition**
  Remove measurement bias for photo unstable samples
- **Nonlinear timescales**
  Choose logarithmic or arithmetic progression timescales to improve resolvability of multiple lifetimes
- **Powerful lifetime analysis modules**
  Select from 1-to-4 exponential, global, stretched exponential, micelle quenching, anisotropy decay, TRES and DAS, FRET Calculator or the advanced MEM and ESM lifetime distribution analysis.

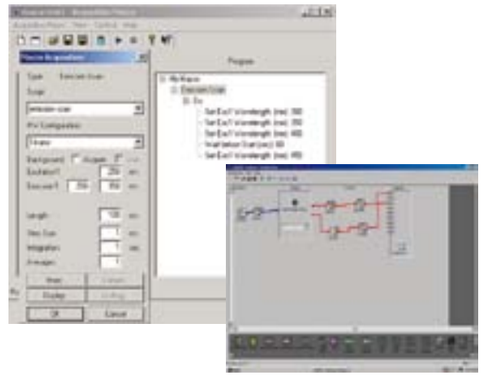
SteadyState Fluorescence with FeliX32™

- **Excitation & emission ratios**
  Determine ion concentrations using shifted probes
- **Excitation, emission, & synchronous scans**
  Determine spectra or purity of samples
- **Multidye analysis**
  Fura-2 for calcium and BCECF for pH
- **Time-based polarization**
  Measure antibody-antigen binding and follow structural transitions in proteins and nucleic acids
- **Automated excitation and emission spectra correction**
  Real-time excitation correction
- **Automated routine builder**
  Create and save automated protocols
- **Contour maps and 3D plots**
  Generate rotating three-dimensional plots
- **Extensive mathematical analysis tools**
  Linear fits, averages, derivative, integrations, smoothing, and much more!



The most comprehensive software package!

**Create and save automated protocols-
Set it up and walk away!**



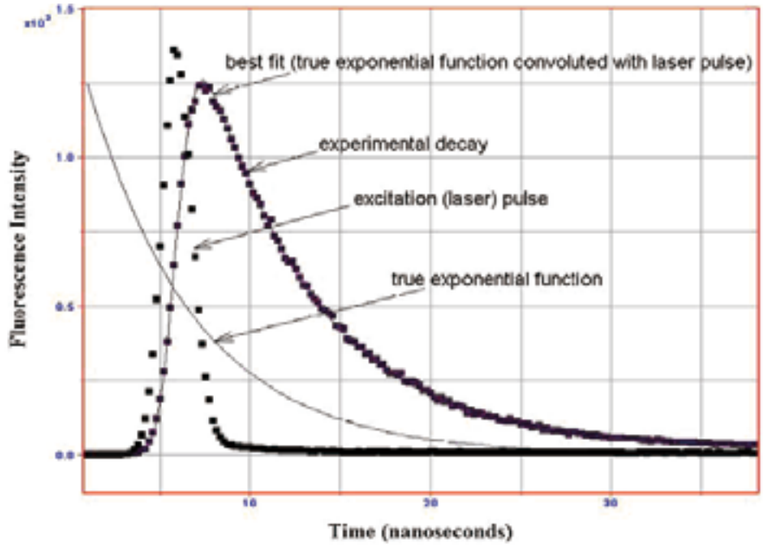
**One easy-to-use software
for all measurement capabilities**

Applications

Time-Resolved Data Analysis

Fitting Function

Every method of lifetime analysis depends on a model or fitting function for the decay of luminescence intensity. This may be as simple as a single exponential decay or as complicated as schemes for micelle kinetics including quenchers or distributions of lifetimes in a heterogeneous environment. The various methods of analysis offered with the LaserStrobe™ differ mostly in the model they employ. The fitting function (explicitly dependent on time) is denoted as D(t) and can be thought of as the time dependent luminescence excited by a delta function (infinitely short) excitation pulse. In the simplest case D(t) is represented by a single-exponential function $D(t)=A*\exp(-t/\tau)$.



Convolution

In any pulsed excitation fluorescence lifetime instrument the finite width of the excitation pulse will distort the free decay of fluorescence as described by D(t). This distortion is known as convolution, which can be expressed as:

$$I(t) = \int_0^t L(t-s)D(s)ds$$

where L(t) is the instrument response function (IRF – the measured shape of the laser pulse) and I(t) is the experimentally determined decay intensity at time t. In order to remove the convolution distortion, the IRF L(t) is measured by using a scattering sample. This experimental L(t) is then used to determine the parameters (e.g. the lifetime) of the fitting function D(t) using a procedure known as iterative reconvolution.

Time-Resolved Data Analysis Continued

Curve Fitting Procedure

The fitting procedure is based on the Marquardt algorithm where the experimental data are compared to a model decay convoluted with the IRF. Deviations from the best fit are characterized by the reduced chi-square (c2): where N is the number of data channels, n is the number of fitting parameters, and s is the standard deviation (see below). The best fit is determined when chi-square is minimized. If the standard deviations are estimated correctly, a perfect fit to the data will produce a chi-square close to 1.0. Good results typically produce c2's of 0.9 to 1.2.

$$\chi^2 = \frac{1}{N-n-1}\sum_1^N \frac{(I(i)_{calc} - I(i)_{exp})^2}{s(i)^2}$$

Goodness of Fit

In addition to the chi-square value, a number of statistical parameters is calculated to assess the quality of the fit, such as:

- Weighed residuals
- Autocorrelation function
- Durbin-Watson
- Runs test parameter

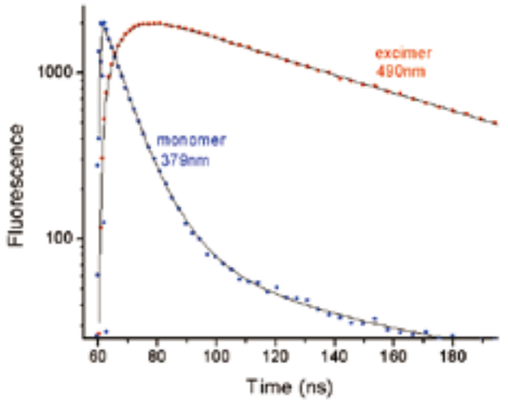
These criteria enable the user to decide whether the fitting model adequately describes the experimental data or a different fitting function is required. The decay fitting functions offered with the FeliX32™ package, including powerful distribution analysis MEM and ESM modules, will be more than sufficient for any kinetic scenario.

LaserStrobe Applications

The LaserStrobe™ is the most versatile lifetime fluorometer on the market. Due to its unprecedented excitation wavelength tunability from UV to NIR, this instrument can be used for virtually any application that requires fluorescence lifetimes. It is used by researchers from all imaginable fields around the world: biologists, biochemists, chemists, physicists, materials scientists, industrial R&D labs, environmental researchers and more. The following are the most typical applications for the LaserStrobe™:

Properties of excited states

A very basic research area, where a fluorescing molecule is a research object on its own rather than a means of studying something else. Fluorescence lifetimes are measured in order to gain insight about the nature of electronic transition, determine radiative and non radiative rate constants, follow excited state relaxation processes, intrinsic changes in molecular geometry, electron and energy transfer, interactions with solvent, etc.

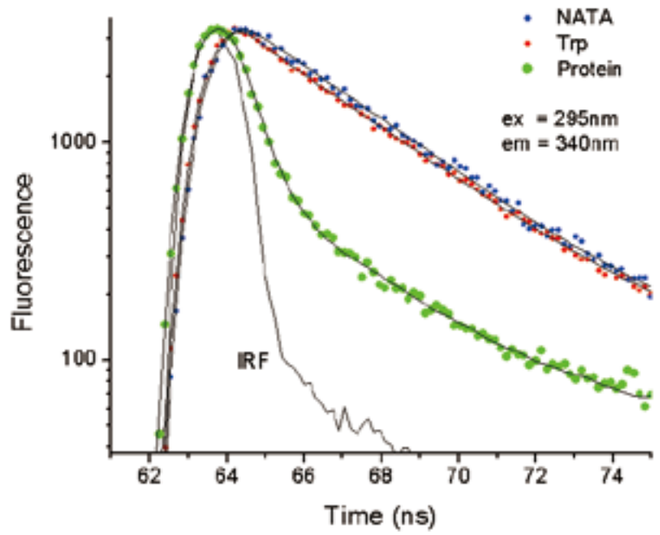


The monomer decay and intramolecular excimer formation in di-pyrenyl-propane are followed simultaneously at 2 emission wavelengths with the dual channel (T-format) LaserStrobe™. Global analysis recovered 3 decay constants common to both emissions: 7.2, 15.7 and 78.7 ns.

More Applications

Protein Structure and Dynamics

- A distance between two protein sites labeled with an energy donor and acceptor can be measured via FRET (fluorescence resonance energy transfer). Energy transfer efficiency is obtained from a decrease of the donor lifetime.
- Segmental and global motion of proteins can be studied by anisotropy decays of intrinsic (tryptophan, tyrosine) or artificial fluorescence probes.
- Localization of certain groups in proteins (e.g. accessibility to water) can be studied by the effect of external quenchers on the lifetime of the probe.
- Protein folding/unfolding can be monitored by changes in the probe lifetime and/or rotational correlation time.



LaserStrobe™ equipped with the optional frequency doubler is ideal for measuring tryptophan lifetimes, regardless of the complexity of Trp decay. The recovered lifetime of NATA is 3.18 ns, while Trp and protein show 2-exponential decays: 0.41 ns (27%) and 3.26 ns (73%) for Trp and 0.47 ns (95%) and 3.7 ns (5%) for the protein.

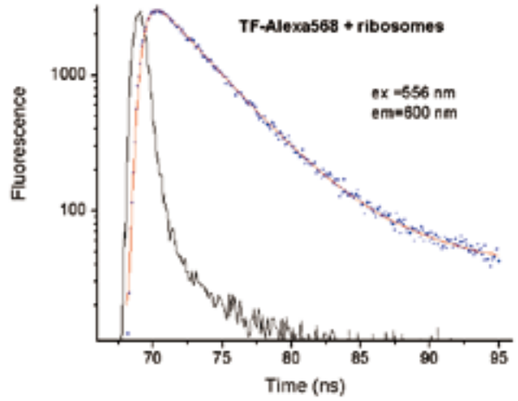
Membrane Fluidity

One of the classical biological applications of time-resolved fluorescence spectroscopy. Typically, an elongated hydrophobic (i.e. water insoluble) molecule is used as a probe (e.g. DPH) and the anisotropy decay is measured. Due to topology of the membrane, the probe rotations are limited to the space within a cone. The rotational correlation time and the cone angle are obtained from the anisotropy data.

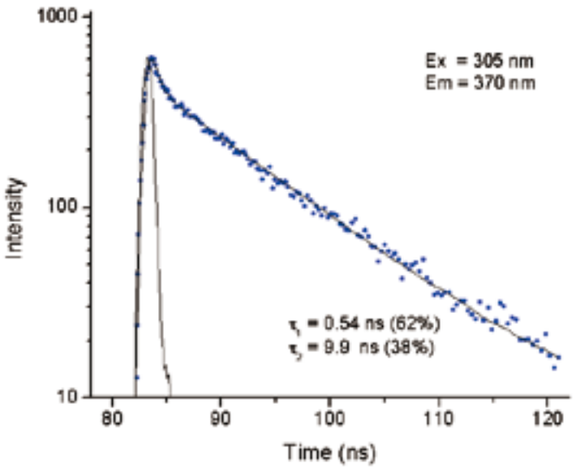
More Applications

Nucleic Acids Studies

- Fluorescence lifetimes and time-resolved anisotropy are powerful tools for studying segmental mobility and hybridization of DNA. The technique is also commonly used to study trigger factors and peptide binding to ribosomes.



Fluorescence decays of Alexa 568-labeled trigger factor bound to ribosomes. The recovered lifetime is 3.54 ns. The decay also exhibits a risetime of 0.56 ns, which is indicative of an excited state structural relaxation.



Fluorescence decay of 50 nM aminopurine bound to RNA.

More Applications

Cyclodextrins (nanocavities)

Cyclodextrins (CDs) are cyclic sugar molecules that possess internal cavities capable of complexing hydrophobic organic and organometallic molecules in aqueous solution. The CDs are shaped like truncated cones with three different cavity diameters: 6.5 A (a-CD), 7.5 A (b-CD) and 9.0 A (g-CD). Since the CDs are water soluble, but have a hydrophobic interior, they can be used to deliver hydrophobic drugs. Interactions and inclusion of drugs, steroids and other molecules with the CDs have been extensively studied with the time-resolved fluorescence. The CDs are also used to study the effects of restricted geometries on the photochemistry and dynamic behavior of guest molecules.

Excited state intramolecular charge transfer (ICT)

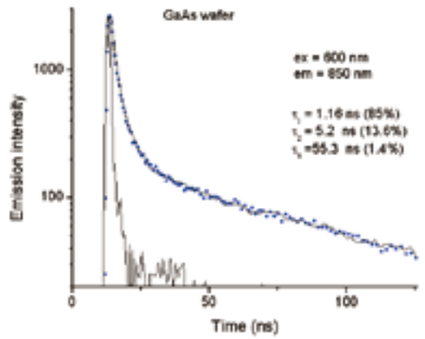
Many molecules exhibit substantial degree of charge redistribution upon excitation. Some may even undergo a full charge separation where an electron jumps from one end of the molecule to the other. In many cases changes in molecular geometry accompany the electron transfer (e.g. TICT: twisted intramolecular charge transfer states). Such molecules are often used as fluorescent probes, since the highly polar excited states make them very sensitive to the environment. The ICT phenomenon also happens naturally, e.g. as one of primary processes in photosynthesis. Fluorescence lifetime is a common tool to study the ICT; it gives the rate constant for the charge transfer directly. Fluorescence decay kinetics, combined with time resolved fluorescence spectra, could elucidate the kinetic mechanism that leads to the ICT state as well as the mechanism of subsequent relaxation.

Asphaltenes

Asphaltenes are components of crude oils and strongly affect the properties of oils. They are large aromatic hydrocarbons composed of one or two fused ring systems. Their structure and molecular weight are not very well defined and has been a subject of long-lasting controversy. The time-resolved Fluorescence Depolarization spectroscopy (FD) is used to measure rotational correlation time (τc) of asphaltenes extracted from oil and dissolved in a solvent. The rotational correlation time is directly proportional to the volume of the molecule under study. Knowing the volume we can calculate the molecular radius and the molecular mass of asphaltenes. The measurement is simple and involves recording the fluorescence decay curves of asphaltenes with two different orientations of the emission polarizer.

Materials Research

The flexibility of the excitation wavelength selection of the LaserStrobe™ and its choice of linear and nonlinear timescales make the instrument very suitable for material research, which includes lifetime measurements from semiconductors, powders, wafers, coatings, etc. The extremely narrow spectral band of the dye laser helps a lot in reducing stray light artifacts from these strongly scattering samples.



Emission decay from GaAs wafer measured with LaserStrobe™ equipped with a red-sensitive PMT.

Specifications

Range of Lifetimes From less than 100 picoseconds to 50 microseconds

Sensitivity Capable of measuring decay of 7 pM fluorescein

Excitation Source

Type	Pulsed nitrogen laser pumping dye laser
Spectral Range	337,360 to 990 nm (depending on dye used) Optional 235 to 990 nm with frequency doubler
Bandwidth	0.04 nm
Peak Power	275 KW at 5 Hz
Pulse Width	800 picoseconds
Pulse Energy	200 microjoules per pulse at 500 nm, 5 Hz
Adjustment	XYZ, focusing, rear mirror

Emission Monochromator

Type	Czerny-Turner
Focal Length	200 nm
Stray Light Rejection	10^{-4} (10^{-8} for double monochromators)
F #	4
Bandpass	0 to 25 nm
Accuracy	+/- 1 nm
Minimum Step Size	0.25 nm

Emission Grating

Type	Ruled
Standard	1,200 l/mm
Blazed	400 nm
Resolution	0.5 nm

Detector

Standard 1527 PMT	185 to 680 nm
Optional 928 PMT	185-680 nm

Sample Compartment

PTI's universal QuadraCentric™ sample compartment comes standard with a 10x10 mm thermostatable cuvette holder equipped with a variable speed stirrer, high efficiency quartz optics, filter holders, active excitation correction, lid activated emission shutter, and one quartz cuvette. The Open Architecture modular design allows for numerous options such as polarizers, solid or powdered sample holder, cryostats, polarizers, titrators, stop flows, and many other options for limitless application solutions.

TSC
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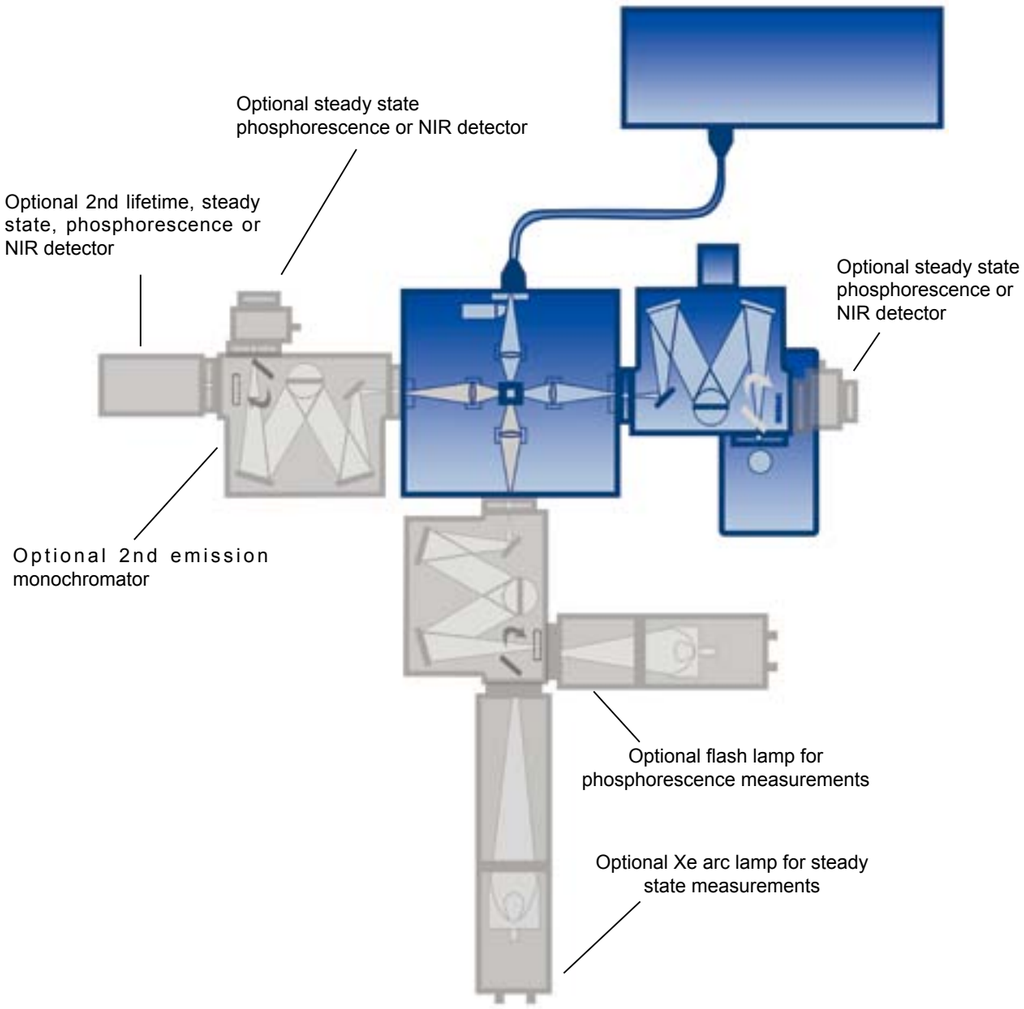


EasyLife V™

EasyLife V™ Complements your fluorescence intensity measurements

EasyLife V™ Offers Easy Solutions For Your Applications

The EasyLife V™ is an integrated solution that provides answers that you have been unable to obtain until now! Using our patented lifetime fluorescence technique, the EasyLife V™ obtains the maximum information about any molecule, something you simply cannot get with conventional steady state techniques.
Whether you are involved in biology, chemistry, pharmaceutical science, food technology, or materials science your work will be greatly enriched by utilizing the EasyLife V™.

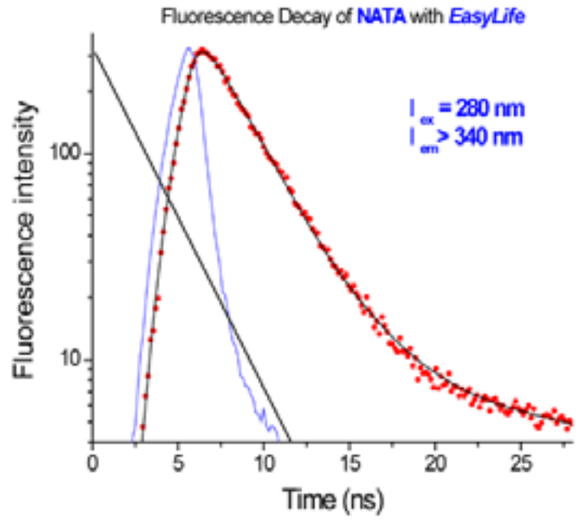
Take A Close Look At EasyLife V ™ – OBB's New, Amazingly Simple, Powerful, Yet Affordable Solution Machine.

- Picomolar sensitivity
- Lifetimes from less than 100 ps
- Powerful software with FRET Calculator
- Large selection of state-of-the-art pulsed LEDs
- Small footprint (a coffee table will suffice!)
- Fully portable
- Turn-key operation
- Maintenance free
- Priced below steady state

Why Measure Time Resolved Fluorescence?

An important advantage of acquiring lifetime measurements is that they are an "intrinsic" molecular parameter. As a result, the lifetime value is independent of fluorescence intensity that can suffer losses due to light scattering and depends on local probe concentration. Therefore, the lifetime measurement is much more informative and reliable when studying highly scattering and solid samples.

In the steady state measurement alone, measured parameters such as spectra, intensity, and polarization are time averaged and the information about dynamic processes is lost. This missing information becomes especially important when fluorescent molecules are used as probes to study complex systems. These systems, including proteins, nucleic acids, membranes, polymers, and micelles frequently exhibit multiple structural domains and conformations. The use of time resolved fluorescence will reveal this information by detecting multiple lifetimes, which reflect structural diversity and interactions.



Principles of Lifetime Determinations

Applications

Ideal for use with biological fluorescent probes to study:

Protein structure dynamics
Protein-Protein interactions
Protein ligand binding
Enzymatic assays
Biomembranes
Nucleic acid conformation
Nucleic acid interactions
Photosynthesis
Liposomes and lipids

And more.....

EasyLife V™ is also an excellent choice for:

Molecular sensors
TR FRET
Material quality control
Quantum dot research
Laser dyes characterization
Development of MLC probes
Photosensitizers research

And more....

Why do you need the EasyLife V™?

The EasyLife V™ adds a new dimension to many research areas by utilizing time resolved fluorescence techniques, which have never been so affordable or easy to execute.

The superior performance of the EasyLife V™ allows measurements on the picosecond and nanosecond timescales, unravelling processes unavailable from conventional fluorescence measurements.

The Stroboscopic Technique (Strobe)

This is the most recent and electronically the simplest technique. While the technique is the newest, it is already more than 10 years old and well established and validated. It utilizes a pulsed light source (an LED, a laser diode or a nitrogen/ dye laser) and measures fluorescence intensity at different time delays after the pulse. As a result, a fluorescence decay curve is collected. The diagram below shows the basic elements of a strobe instrument that utilizes a pulsed LED.

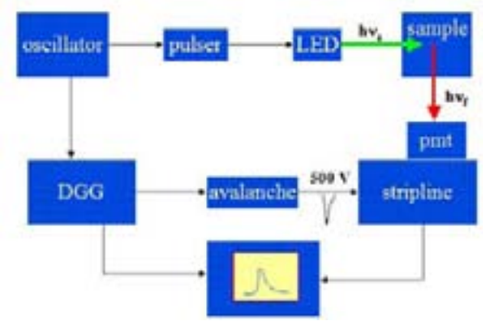


Fig. 1 Block diagram of an LED-based EasyLife stroboscopic system

A master clock (oscillator) generates pulses at a fixed 25 kHz frequency. The pulses are routed simultaneously to the LED pulser and a digital delay gate generator (DGG) unit.

The pulser triggers the LED; the LED flashes and excites the sample, which subsequently emits fluorescence. At the same time the pulse synchronized with the LED pulse triggers the DGG, which outputs a delayed TTL pulse.

The DDG is under computer control and the value of the TTL pulse delay is determined in the acquisition software. The delayed pulse triggers an avalanche circuit, which provides a high voltage pulse (ca. 500 V) for the detection circuitry. This pulse creates the gain and the temporal discrimination gate for the photomultiplier.

An important feature is that the strobe technique does not use a conventional voltage divider network for providing interdynode voltages in the photomultiplier (PMT). Instead, the PMT dynodes are interconnected by a stripline circuit. The pulse from the avalanche is injected in the stripline at the time delay specified by the DGG. The pulse travels along the dynode chain amplifying the primary photoelectrons generated at the specific time delay. This way high amplification and time gating are simultaneously achieved in the PMT strobe circuit. The measured analog signal is fed to a 12-bit A/D converter. Scanning the gate (time delay) across the fluorescence decay allows the acquisition of fluorescence intensity as a function of time.

One of the advantages of the stroboscopic technique is the ability to utilize relatively inexpensive pulsed LEDs.

The strobe technique can also be very fast; this is because it measures fluorescence intensity directly and, unlike photon counting techniques, is not limited by photon counting statistic and can therefore take advantage of high intensity fluorescence.

A unique feature of the strobe is the ability to measure decays with the use of non-linear timescale. This is possible because the software controls the delayed output of the DGG. The stroboscopic instruments employ arithmetic progression and logarithmic timescale acquisition protocols in addition to the conventional linear timescale. These nonlinear timescale protocols enhance the lifetime resolving power and allow for the acquisition of complex decays with underlying lifetimes differing by orders of magnitude using fewer data points than would be required with the linear timescale.

LED's and Laser Diodes

OBB engineers have developed a broad range of proprietary pulsed light emitting diodes (LEDs) to be used as excitation sources with the EasyLife systems. These small but robust light sources are available in a broad range of wavelengths, from UV to NIR

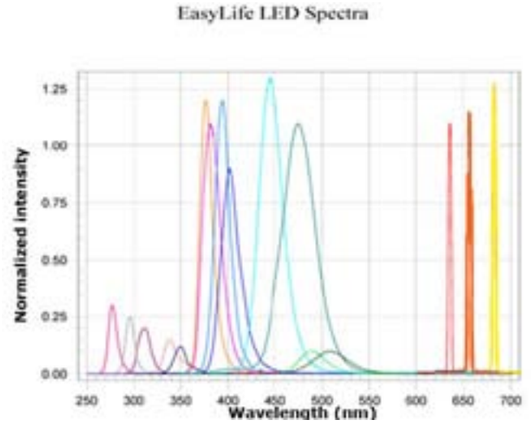
LED Sources

Central Wavelength / Type

- 280 nm LED
- 295 nm LED
- 310 nm LED
- 340 nm LED
- 365 nm LED
- 370 nm LED
- 380 nm LED
- 393 nm LED
- 405 nm LED
- 410 nm LED
- 435 nm LED
- 445 nm LED
- 450 nm LED
- 460 nm LED
- 505 nm LED
- 525 nm LED
- 635 nm Laser Diode
- 650 nm Laser Diode
- 670 nm Laser Diode

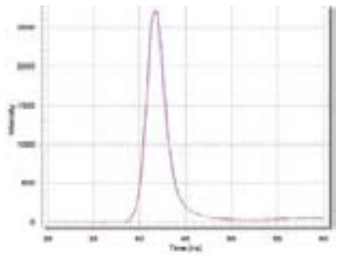
Other LED's and laser diodes are available on request.

To exchange sources is real easy, just snap off and then snap on and it is done – no alignment required.

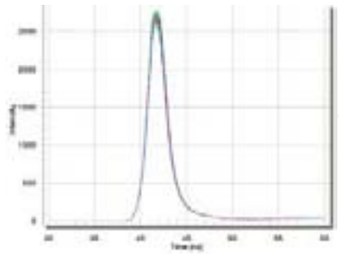


Reproduciblity

6 Hours Repeat Acquisition



Repeat After 4 Days



Optional Accessories

- Magnetic stirrer
- Manual sheet polarizers
- Bandpass filters
- Liquid nitrogen dewar
- Solid sample holder
- Long-pass filters
- Neutral density filters
- Microcuvette with adapter

Compact Sample Compartment

Standard
Thermostatable
Sample Holder

Standard
Lid Actavated
Shutter

Standard
Focusing
Optics

Standard
Mounting Hardware For Filters and/or Polarizers

The sample compartment is compact but it has lenty of room to accommodate what you need to make a host of various sample measurements

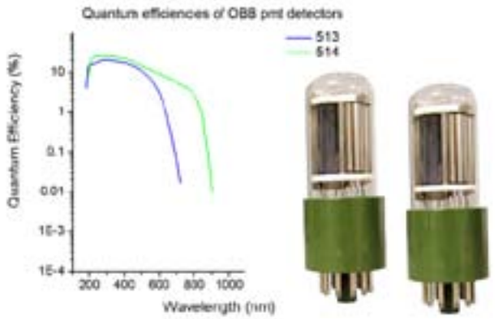
PMT Options

- Extended IR version

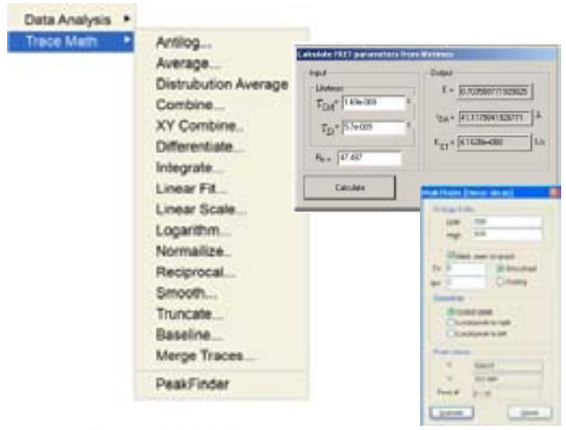
The standard system uses a specially selected PMT that allows for the measurement of lifetimes from 100 picoseconds to 3 microseconds. The wavelength range of detection is from 185 nm to 680 nm.

At the time of order you may select, as an option, an extended wavelength range PMT tube that can detect from 185 nm to 900 nm

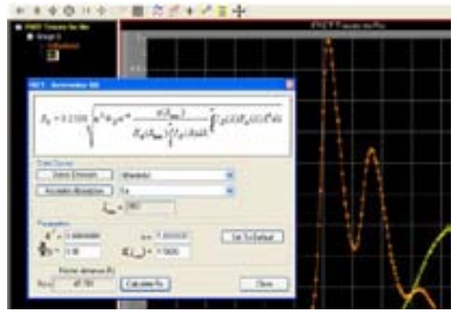
Detector Options



Math Features



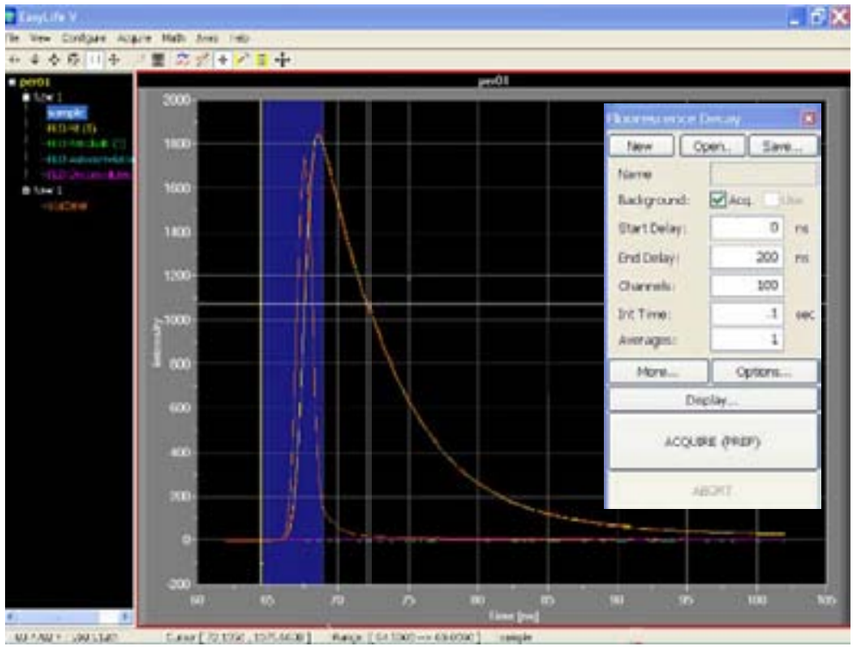
Built In FRET Calculator



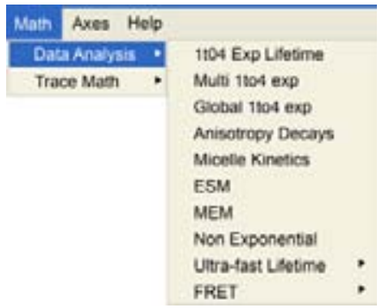
Global
Analysys

Anisotropy

EasyLife V™ Softwaredesigned for ease of Use

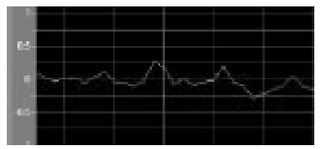


Data Analysis



Micelle Kinetics

Output



Chi2
Random Residuals

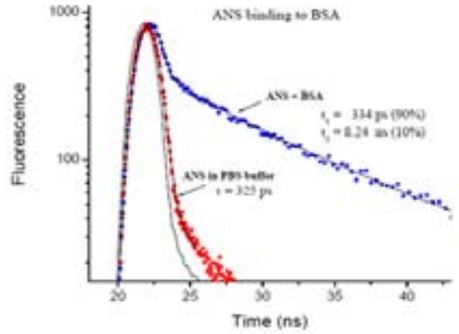
And so much more.....

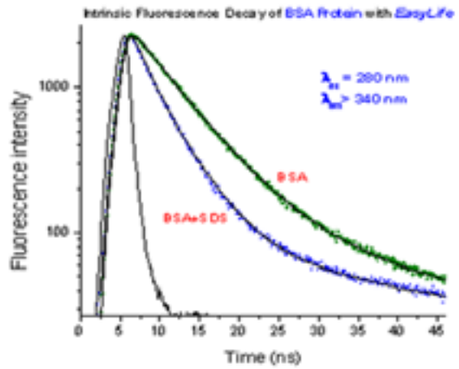
Applications

Proteins

Most proteins fluoresce due to the presence of any or all three fluorescent amino acids: tryptophan, tyrosine, and phenylalanine. Intrinsic time-resolved fluorescence of tryptophan is commonly used to study the structure and dynamics of proteins. These experiments require pulsed light sources emitting in the UV, between 270 and 295 nm. The EasyLife V™, equipped with the 280 or 295 nm pulsed LED source, is a very robust yet fast instrument perfectly suited for use with tryptophan and tyrosine fluorophores.

If you happen to use external fluorophores, there is a large selection of pulsed LEDs available for any wavelength in the UV-VIS range. A polarity sensitive, hydrophobic probe such as ANS is a good illustration of binding of an extrinsic probe to a protein. ANS binding to bovine serum albumin was monitored with the EasyLife V™ equipped with the 370 nm LED. The lifetime of ANS in the buffer is very short, 325 ps, and increases to 8 ns upon binding to BSA. The ratio of free ANS to BSA bound ANS (9:1) can be easily determined from the double exponential fit to the fluorescence decay.





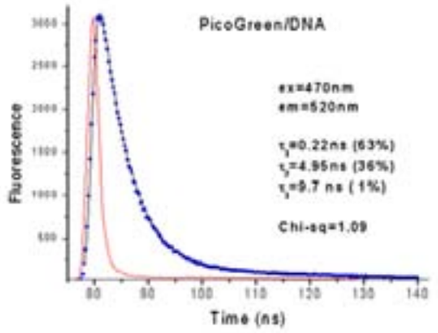
Fluorescence decays of bovine serum albumin (BSA) in PBS buffer were measured with the EasyLife V™. The native protein shows a nearly single-exponential decay with an average lifetime of 6.31 ns. After being treated with SDS detergent, BSA undergoes a structural transition and its fluorescence decay exhibits two shorter lifetimes, 1.47 ns (37%) and 4.43 ns (63%).

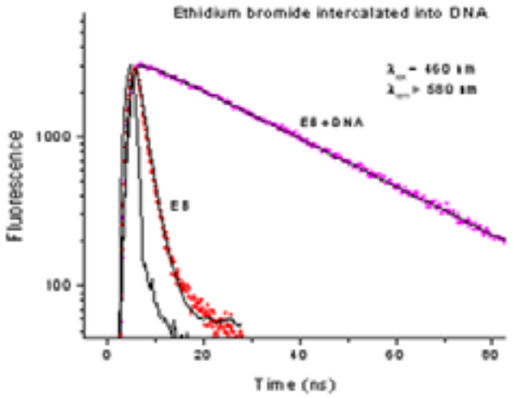
Applications

Nucleic Acids

If you study conformational features or hybridization of DNA, the EasyLife V™ is the right system for you. A probe molecule in a buffer will show very little or no anisotropy. Attach it to a protein, DNA, or membrane, however, and the anisotropy is increased. This is all that the steady state experiment can tell you: the probe is attached to a much bigger entity. However, if you measure the lifetime of the probe, you can estimate the rate of rotational diffusion in addition to the size of the macromolecule that is attached to the probe.

Ethidium Bromide (EB) is a commonly used DNA probe, which readily intercalates between the DNA bases. EB is weakly fluorescent in aqueous media, but becomes strongly fluorescent after intercalation into DNA. The lifetime of EB in buffer is 1.71 ns and increases dramatically to 22.7 ns after binding to calf thymus DNA.





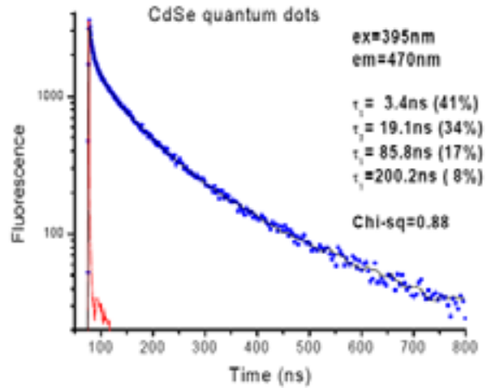
Fluorescence decay of PicoGreen/DNA measured with an EasyLife V™ lifetime system. Conformational diversity may result in multiple lifetimes of the probe bound to DNA. The EasyLife V™ is fully capable of measuring and analyzing such complex decays. The decay of PicoGreen, a common probe for doublestranded DNA, exhibits a clearly multi-exponential behavior, resulting in three lifetimes that range from 220 ps to 9.7 ns.

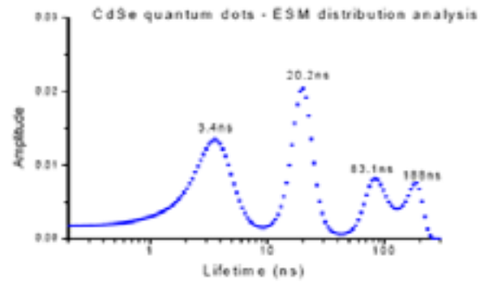
Applications

Nucleic Acids

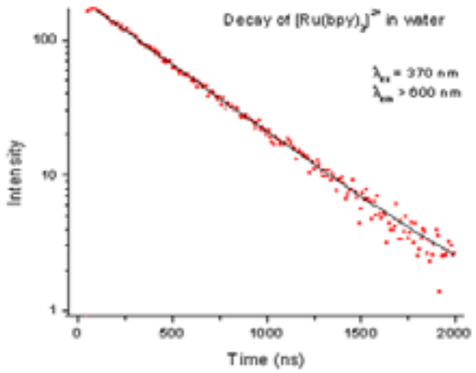
Nanotechnology has become one of the fastest growing disciplines. Quantum dots, semiconductor crystals on the nanometer scale, have become a new type of fluorescent probe, often replacing more troublesome and photo-unstable organic fluorophores. Optical properties of quantum dots, such as absorption, emission spectra, and lifetimes are determined by their size and shape.

The fluorescence decay of CdSe quantum dots measured with the EasyLife V™ indicates a highly heterogeneous nature of the sample. A unique feature of the EasyLife V™, the ability to acquire data using logarithmic or arithmetic progression timescales, facilitates greatly in analysis of multi-exponential decays with an underlying broad range of lifetimes. Here, a 4-exponential decay function was needed to adequately describe the experimental decay, acquired with the arithmetic timescale. This result was alidated by the ESM lifetime distribution analysis, another powerful analytical tool in the EasyLife V™ software, which confirmed the lifetime values from the discrete 4-exponential analysis.





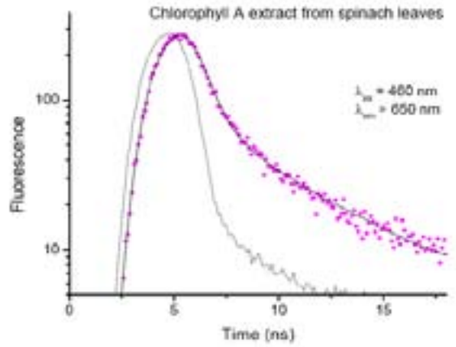
Metal-ligand complexes (MLC) have become very popular probes due to their relatively long lifetimes. They are particularly suitable to studying large macromolecular systems such as nucleic acids and proteins. The figure shows a decay of one of the most common types of LC, tris(2,2'-bipyridyl) ruthenium (V). The probe has a rather low quantum yield (about 4%). Not a problem for the EasyLife V™! The recovered lifetime is 429 ns.



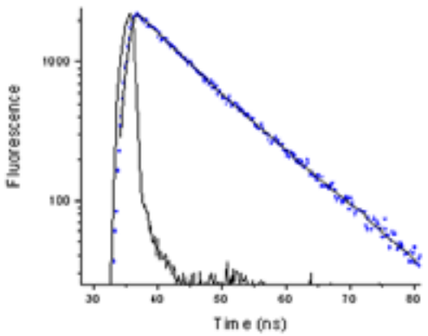
Applications

Porphyrins and Chlorophylls

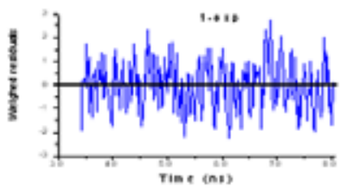
If you study conformational features or hybridization of DNA, the EasyLife V™ is the right system for you. A probe molecule in a buffer will show very little or no anisotropy. Attach it to a protein, DNA, or membrane, however, and the anisotropy is increased. This is all that the steady state experiment can tell you: the probe is attached to a much bigger entity. However, if you measure the lifetime of the probe, you can estimate the rate of rotational diffusion in addition to the size of the macromolecule that is attached to the probe.



Fluorescence decay of Chl A extracted from spinach leaves and suspended in buffer. The decay is double exponential due to the presence of Chl A aggregates. The recovered lifetimes are 570 ps (93%) and 4.2 ns (7%).



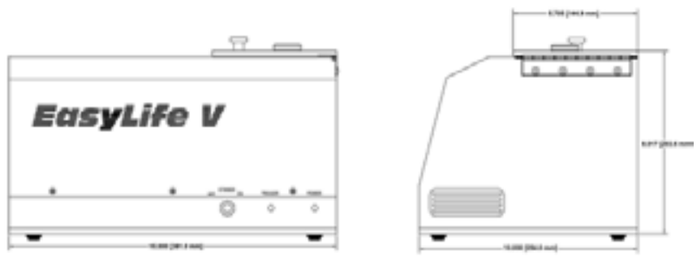
Fluorescence decay of meso-tetraphenylporphyrin (m-TPP) in chloroform measured with the EasyLife V™ equipped with a 370 nm LED source. Fitted with a single exponential function, the recovered lifetime is 9.16 ns. The chi-square value of 1.07 and the random residual function indicate that the single exponential model adequately describes the decay of m-TPP.



Specifications

- Lifetime range: 100 ps to 3 µs
- Sensitivity: 400 picomolar fluorescein
- Excitation: OBB proprietary nanosecond LEDs
- Optical pulse width: 1.5 ns (typical)
- Excitation range available: 280-670 nm
- Emission range: 185-680 nm, Optional to 900 nm
- Wavelength selection: 2” square or 1” round filters
- Detection: Patented lifetime detector
- Typical acquisition time: 20 s (sample dependent)
- Timescale (menu selectable): Linear, arithmetic and logarithmic
- Acquisition mode: Sequential or random
- Sample holder: Single 1 x 1 cm cuvette
- Software: FeliX GX
- Lifetime analysis: Complete package:
  1-4 exponential, global, non-xponential, micelle kinetics, lifetime distribution (ESM and MEM), anisotropy, FRET calculator
- QuickStart DVD: Included

* All specifications subject to change without notice



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EasyLife™ L

Luminescence Lifetime LRET System

EasyLife™ L—An Exceptionally Fast, Sensitive and Accurate Luminescence Spectrometer for LRET Assays and General Luminescence

Examples of Applications

- LRET based kinase activity assays
- Conformational changes in ligand-binding glutamate receptor
- Sigma factor binding to RNA polymerase
- K+ channel voltage sensor movement in cell membranes
- Detection of salicylic acid in blood by sensitized Tb luminescence
- Assays for determining antibiotics (norfloxacin, garenoxacin, grepafloxacin) in urine and serum
- DNA-lanthanide assays for multi-drug resistance of TB strains

Features and Benefits

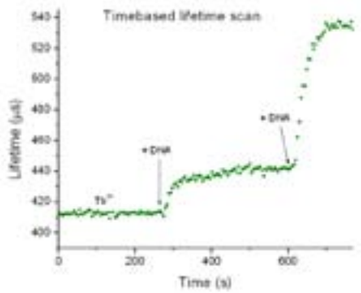
- Single-shot operation
- Ideal for LRET assays and phosphorescence
- Ultra-high sensitivity and acquisition speed
- Very high precision
- High stability and reproducibility
- Instant lifetime determination
- Timebased lifetime scan
- Timebased intensity scan
- Lifetime temperature ramping
- Intensity temperature ramping
- Reduced bleaching
- Low maintenance

Optional Accessories

- Manual sheet polarizers
- Liquid nitrogen dewar
- Rotatable solid sample holder
- Rotatable powder sample holder
- Bandpass filters
- Long-pass filters
- Neutral density filters
- Microcuvette with adapter

EasyLife™ L Offers Special Features

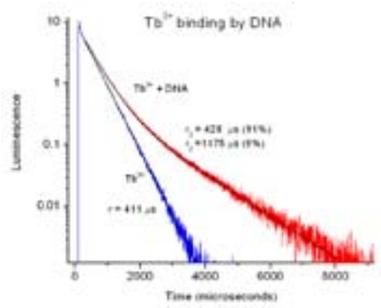
Due to its unique 'single-shot' detection technique, the EasyLife™ L is lightening fast. It can measure up to 500 complete decays in a second. The decays are instantly analyzed in real time enabling the user to follow reaction kinetics by plotting the lanthanide lifetime rather than the intensity as a function of time. This is the basis for a new, unique feature that only EasyLife™ L can offer: the Timebased Lifetime Scan. This makes the experiment immune to artifacts that usually affect intensity, such as light scattering, concentration fluctuations, precipitation etc. The lifetime-based technique is ideal for sensing applications, where binding of a substrate changes the lanthanide lifetime.



The binding kinetics of Tb3+ to DNA can be followed free of intensity artifacts by using the unique Timebased Lifetime Scan. Here the EasyLife™ L performs continuous rapid measurements of the average terbium lifetime in real time.

Advantages of Lanthanide-Based Probes

Chelated lanthanide probes offer some distinct advantages over conventional organic fluorophores. Lanthanides exhibit luminescence lifetimes ranging from hundreds of microseconds to milliseconds, which are orders of magnitudes higher than typical fluorescence lifetimes. By utilizing a pulsed excitation source combined with time-resolved detection and selecting only the long-lived emission from the lanthanide ion, one can easily eliminate the background signal due to native or impurity fluorescence and scattered light, thus greatly enhancing the sensitivity and accuracy of an assay. This has led to development of numerous assays based on Luminescence Resonance Energy Transfer (LRET), which utilize chelated lanthanide donors rather than organic fluorophores.



DNA-lanthanide assays are used in a number of diagnostic tests, for example DNA-Tb3+ in testing for antibiotic resistance of tuberculosis strains. Terbium luminescence is enhanced upon binding to DNA, which results in 3 fold increase of Tb3+ lifetime.

Specifications

Lifetime Range	200 ns to 300 ms
Multiple Automatic Lifetime Fitting	1 to 4 exponential
Excitation	High powered pulsed xenon lamp
Repetition rate	1 to 500 Hz under software control
Optical Pulse Width	1 µs (FWHM)
Excitation Range	200 to 2000 nm
Emission Range	185 to 680 nm, optional to 900 nm
Wavelength Selection	1 inch round bandpass and longpass filters
Sample Temperature Control	Air-cooled peltier
Temperature Range	20 to 50 ° C
Sample Stirrer	Built in, variable speed
System Control	FluoroScan software
Dimensions	16.9 x 11 x 7.7 inches
Weight	12 lbs

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EasyLife™ TCSPC

Lifetime Fluorometer

Take A Close Look At EasyLife™ TCSPC — OBB's New, Amazingly Simple, Powerful, Yet Affordable Solution Machine.

The EasyLife™ TCSPC is an integrated solution that provides answers that you have been unable to obtain until now! Using the Time Correlated Single Photon Counting technique, the EasyLife™ TCSPC obtains the maximum information about any molecular system, something you simply cannot get with conventional steady state techniques. Whether you are involved in biology, chemistry, pharmaceutical science, food technology, or materials science your work will be greatly enriched by utilizing the EasyLife™ TCSPC.

Features and Benefits

- Lifetimes from approximately 20 ps (LED dependent)
- Femtomolar sensitivity
- Powerful analysis software
- Large selection of state-of-the-art pulsed LEDs
- Stable, snap-in pulsed LEDs provide great reproducibility
- Small footprint
- Portable
- Turn-key operation
- Maintenance free
- Great for multi-user lab

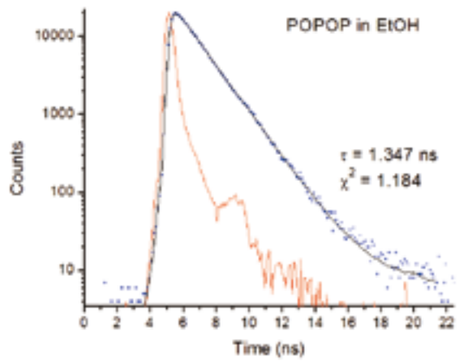
Applications

Ideal for use with biological fluorescent probes to study:

- Protein structure and dynamics
- Protein interactions
- Biomembranes
- Liposomes and lipids
- Nucleic acid conformation
- FRET experiments
- Photosynthesis

EasyLife™ TCSPC is also an excellent choice for:

- Very short lifetimes
- Molecular sensors
- FRET validation
- Material quality control
- Quantum dot research
- Laser dyes characterization
- Development of MLC probes
- Photosensitizers research



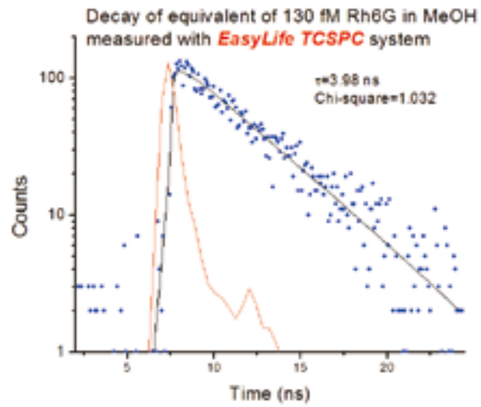
The TCSPC technique provides a benefit of a high dynamic range, typically 4-5 orders of magnitude of photon count levels, which ensures excellent precision of lifetime determination. Since photon counting is governed by Poisson statistics, the signal-to-noise is highly predictable and data analysis is very robust due to well-defined standard deviations.

Specifications

Lifetime Range	From about 20 ps to 3 us (LED dependent)
Sensitivity	100 fM rhodamine
Excitation	OBB proprietary nanosecond LEDs
Excitation Range Available	260 to 670 nm
Emission range	185 to 820 nm
Wavelength Selection	2 inch square or 1 inch round filters
Detection	Fast photon counting pmt
Channel Resolution	< 1 ps
Sample holder	Single, thermostatable 1 x 1 cm cuvette
System Control	TCSPC PCI board
Dimensions	16.9 x 11 x 7.7 inches
Weight	12 lbs

Optional Accessories

- Magnetic stirrer
- Manual sheet polarizers
- Liquid nitrogen dewar
- Solid sample holder
- Microcuvette with adapter
- Bandpass filters
- Long-pass filters
- Neutral density filters



For very low concentrations of fluorophore the EasyLife™ TCSPC is a clear winner: fluorescence lifetimes at femtomolar levels can be easily determined.