HORIBA Scientific

Fluorolog®-3

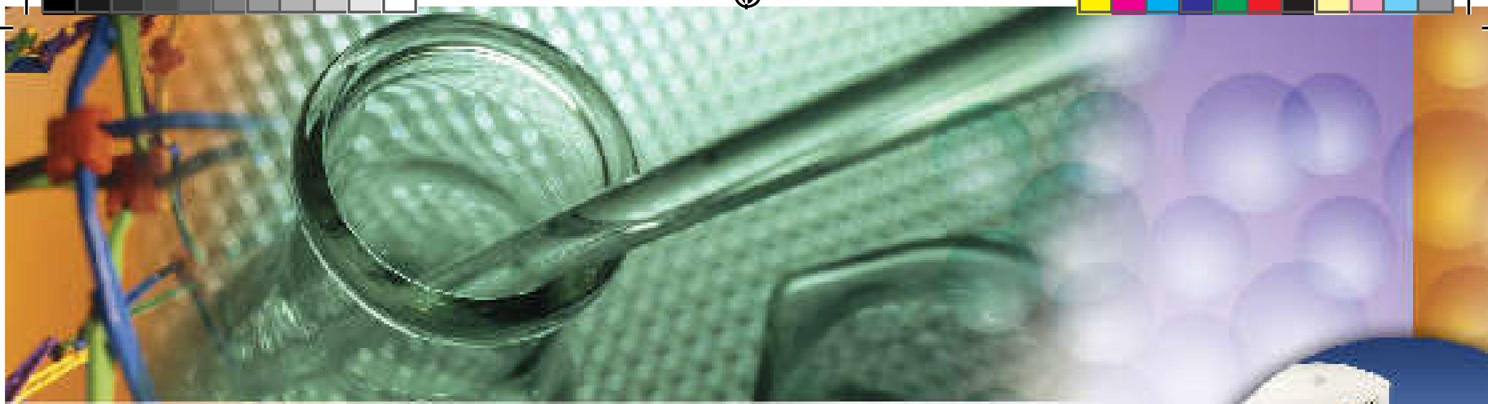
How to Build a Spectrofluorometer

How the Fluorolog® adapts to YOUR sample

The Fluorolog® is the final concept in fluorescence engineering, an instrument that encourages you to custom-tailor a spectrofluorometer's performance to the work you need to accomplish. Whether you use steady-state or molecular dynamics, your selections will deliver the perfect balance of these crucial benefits:

- Sensitivity
- Speed
- Modularity
- Automation
- Versatility
- Exclusivity
- Real-world performance

Sensitivity	The Fluorolog® delivers the ultimate in sensitivity. This means not only that you can see lower concentrations, but you also take data faster, which means more work done, with more accuracy.
Speed	Not only does fast scanning produce more data, it also limits degradation of samples over time, by photobleaching, or other means that can invalidate your data. The Fluorolog® is the fastest scanning modular instrument made.
Modularity	No one system can provide the answers to all problems. That's why the Fluorolog® is modular. Choose a source, monochromator, sample compartment, detector, and accessories that match the wavelength-range, time-domain, or physical characteristics and parameters of your sample, such as temperature, physical state (solid or liquid), and even remote sensing through fiber-optics. When you need to probe the mechanisms of molecular dynamics, the frequency-domain or TCSPC upgrades deliver picosecond time-discrimination at the twist of a knob.
Automation	Turn the power on and you're ready to take data. The instrument calibrates itself, and you can load slit and wavelength settings from memory. Automated sampling accessories include polarizers, sample-changers, microwell-plate readers, automatic titrators, temperature baths, stopped-flow systems, and more.
Versatility	The Fluorolog® has an accessory for virtually any sample—and if we don't currently have what you need, we can always make a special device for you.
Exclusivity	HORIBA Scientific is the only company that offers both types of dynamic experiments, both phase and pulsed upgrades for ALL your applications.
Real-world performance	From nanotechnology, to biotechnology, energy transfer, and dynamic polarization to CCD or multi-channel detection from the UV to the IR, it's all in the Fluorolog® spectrofluorometer.



Sensitivity

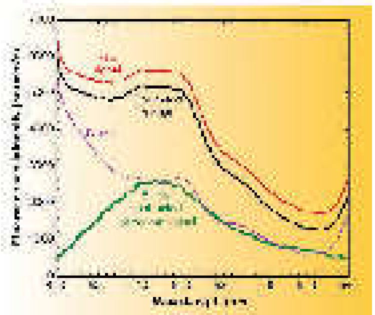
How we achieve the best sensitivity:

1. Our CW xenon excitation lamps are mounted vertically to image the arc on the slit for more throughput—with longer lamp-life as a bonus.
2. All-reflective optics keep the light in focus at all wavelengths, unlike lenses.
3. Kinematic plane-gratings also remain focused at all wavelengths, and are easily changed to maximize any spectral range. Ruled gratings eliminate the polarization anomalies of holographic gratings and deliver more photons to your sample and detector.
4. Photon-counting detection strips noise away from weak signals.
5. FluorEssence™ software based on the familiar Windows® operating system runs data-analysis and post-processing routines.

FluorEssence™, our most powerful fluorescence software ever.

What sensitivity means to your data:

1. You can analyze samples at lower concentrations, obtaining data unavailable with other instruments.
2. Save time-the stronger the signal, the more samples you can measure in a given time with the same accuracy.
3. More-accurate data. The stronger the signal, the better the statistics, the lower the noise, the better the accuracy.
4. Time-correlated single-photon counting (TCSPC) for molecular dynamics is the ultimate in sensitivity. With TCSPC you get true single-photon counting, instead of a DC background that is unavoidable with analog systems.



The super-sensitive Fluorolog® can detect 50 fM (femto!) fluorescein in 0.1 M NaOH.

Speed

Matrix scanning

Not only does the Fluorolog® software include routines for automatic scanning of emission spectra for a defined set of excitation spectra, to produce an excitation-emission matrix that fully characterizes the sample's fluorescence, the monochromator's unique design supplies fast scanning (150 nm/s) to make these scans practical. Your samples can be totally characterized in a matter of minutes, as shown by the matrix at right. If you want extra speed, choose a multi-channel detector, such as a CCD or InGaAs diode-array to obtain your spectra without scanning at all.

Multiwavelength data

For probes with multiple excitation or emission wavelengths, our software delivers routines to slew quickly between specified wavelengths while acquiring data. This lets you handle probes for calcium, pH, magnesium, and many others automatically.

MF² and TCSPC lifetime units

Whether you need to do Fluorescence Resonance Energy Transfer (FRET), molecular dynamics, anisotropy-decay, or simply need to resolve spectra on the basis of lifetime, the MF² (phase) and TCSPC (pulsed) systems will turn your Fluorolog®-3 into a picosecond time machine. You can upgrade any Fluorolog® in use, should your demands change in the future.

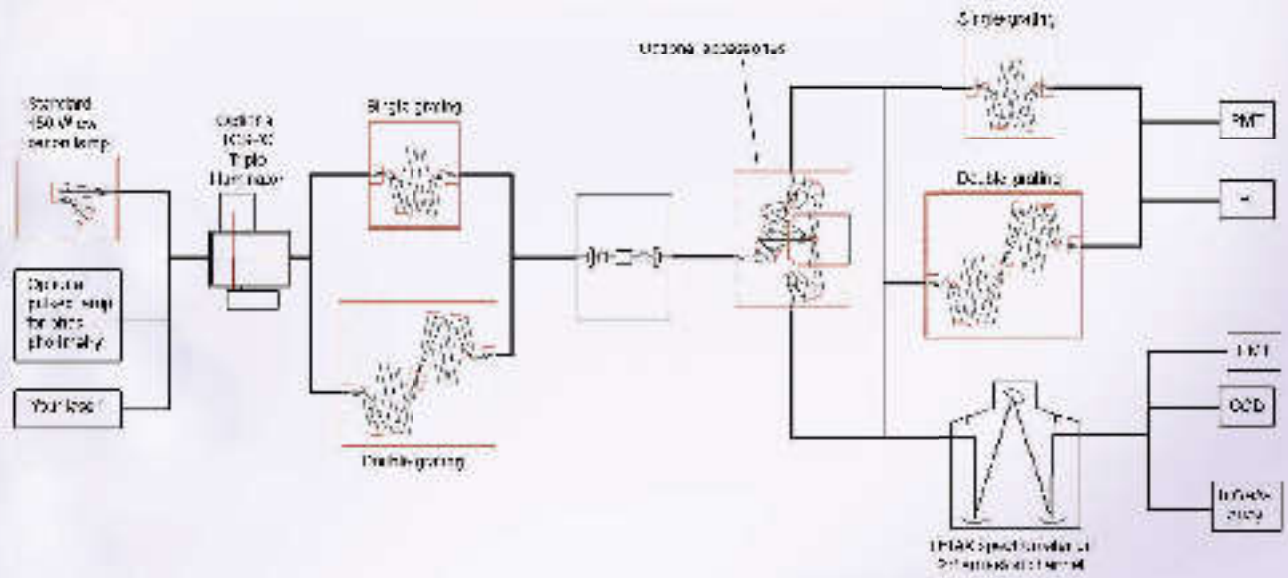
Microwell-plate reader

When you have a large number of samples to run, the microwell-plate reader is ideal. Coupled to the instrument through fiber-optics, fluorescence data is quickly acquired at a speed of about 100 samples per minute. Routines are also included for automatic background subtraction, standard calibration curves, kinetics, and computation of results in concentration or user-specified units.

Modularity

Choose the components you need to maximize the sensitivity, speed, wavelength, timing, sample-handling, or other important parameters.

1. Sources	2. Excitation Monochromator	3. Optional MF² (Phase) Lifetime Unit	4. Sample Compartment	5. Emission Monochromator(s)	6. Detectors



Sources

450 W xenon CW lamp is standard. Options include a pulsed xenon lamp for phosphorimetry, a laser port for your own laser source, NanoLED solid-state pulsed sources, a triple-illuminator option to mount nanosecond or microsecond flash-lamps, and more.

Excitation monochromator

Choose a single-grating unit with kinematic gratings to customize your spectral range, or a double-grating unit for highly scattering samples. Slits and calibration are automated, and therefore reproducible for even the most inexperienced users, and scanning is the fastest.

Lifetime systems

Add picosecond lifetime capability, now or later, with our frequency-domain MF² unit, or time-domain TCSPC upgrade. With the TCSPC Triple-Illuminator accessory you even get the option of multiple sources, including spark lamps and solid-state pulsed NanoLEDs.

T-sample compartment

All-reflective optics in the sample compartment means the sample is always in focus, no matter what the size or spectral range. Facilities are available for a second emission-channel for dual-wavelength probes or T-format polarization studies. A gap-bed sample compartment accepts custom sampling accessories or any listed on pages 6 and 7.

Emission monochromator

You have the same choices as with excitation, with the additional option of an imaging spectrograph that lets you mount a CCD or InGaAs-array detector (infrared) for instant spectra. The spectrograph even accepts a second detector for automated switching.

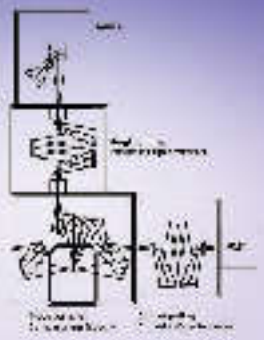
Detectors

The standard detector is a photomultiplier tube (PMT) that covers the full range from UV to near-IR. A thermoelectrically cooled unit aids sensitivity, or other PMTs and solid-state detectors can be mounted for additional wavelengths in the IR, plus multi-channel arrays that we manufacture for perfect integration into the Fluorolog®.

Recommended Modular Configurations

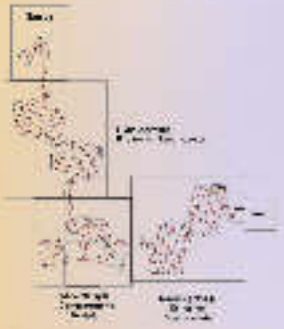
FL3-11

The basic "T-geometry" configuration is formed from single-grating monochromators for excitation and emission positions and a 1-watt cooled red-sensitive photomultiplier. Add any accessory now or easily in the future. The FL3-11 provides a great value in sensitivity and performance at the lowest price.



FL3-22

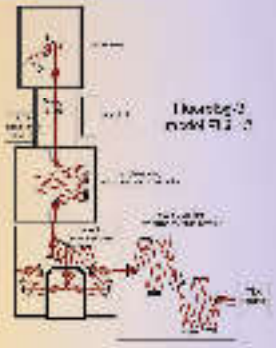
The ultimate in stray-light rejection, the double-grating monochromators in excitation and emission positions are perfect for highly scattering biological samples like lipids and proteins, or solids like powders, semiconductors, or phosphors. You also get a bonus in sensitivity. The additive grating design allows you to open the slits twice as wide as for the same resolution you would get in a single-grating monochromator. Standard center third slits let you push the stray-light envelope even further.



FL3-11-MF²

Nanolog®

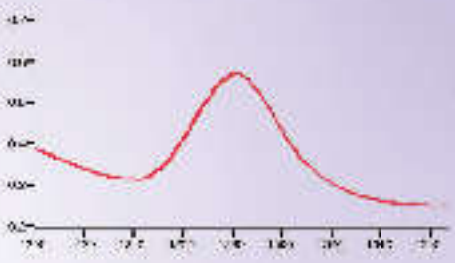
FL3-TCSPC



...and more—such as the Fluorolog®-IR, optimized for detection above 1 µm!

Multiple, automated ports on the spectrograph, IR detectors, grating turrets—ask any Spex® Fluorolog® applications engineer today to help you assemble your most versatile spectrofluorometers.

NanoLog® spectrofluorometer, your best choice for analyzing nanomaterials.



Near-IR emission spectrum of singlet O_2 generated from $[Ru(bpy)_3]Cl_2$ in D_2O.

Versatility
Fluorolog® Accessories

Fiber-optic platform F-3000

Use this accessory for remote-sensing from 250–850 nm for samples that cannot be placed in the sample chamber.

Liquid-nitrogen Dewar FL-1013

To measure phosphorescence or delayed fluorescence, samples are often frozen at liquid-nitrogen temperature to preserve the fragile triplet state. A Dewar flask is used to freeze and maintain the temperature of the sample. The sample is placed in a quartz cell, and slowly immersed in the liquid-nitrogen-filled Dewar. The Dewar is on a pedestal within the Fluorolog®'s sample compartment.

Automated polarizers

The FL-1044 automated L-format polarization accessory permits complete control and calibration of your polarization experiments from the computer keyboard. You can automatically rotate the polarizers to determine VV, VH, HH, and HV components. An optional T-format configuration (FL-1045) is also available.

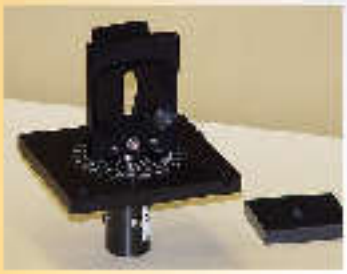
Automated single-position thermostated cuvette-holder FL-1027

This cuvette-holder keeps a sample at a constant temperature from –10°C to +80°C. The temperature is maintained by a liquid mixture pumped through from an external circulating temperature bath (F-1000/F-1001, not included). The holder also includes a magnetic stirrer to mix a turbid or viscous sample while positioned in the optical path.

Automated four-position thermostated cuvette-holder FL-1011

This cuvette-holder keeps up to four samples at a constant temperature from –10°C to +80°C. The temperature is maintained by a liquid mixture pumped through from an external circulating temperature bath (F-1000/F-1001, not included). The holder also includes a magnetic stirrer to mix a turbid or viscous sample while positioned in the optical path. A dual-position thermostated sample-holder (FL-1012) is also available.

Solid-sample holder 1933



The solid-sample holder is designed for solids including thin films, powders, pellets, microscope slides, and fibers. The holder consists of a base upon which a bracket, spring clip, and sample-block rest.

MicroMax 384 Microwell-plate reader

The MicroMax 384 can rapidly scan hundreds of tiny samples within minutes automatically. Useful for pharmaceuticals and nanomaterials, you can determine the fluorescence characteristics fast using microwell plates with up to 384 wells.

Microscope interface

For recording fluorescence experiments under a microscope, this accessory consists of a fiber-optic adapter plus excitation and emission fiber-optic bundles that carry the light-source to the microscope optics, and fluorescence emission from the sample back to the Fluorolog®.

Chopper with Lock-in Amplifier FL-1069L

For improved data-acquisition in extended-IR applications, try our chopper and lock-in amplifier accessory.

Thermoelectric heater/ cooler F-3004

For heating and cooling samples without external circulating baths. You can rapidly heat and cool your fluorescent material through a wide range of temperatures using the Peltier effect. A magnetic stirrer is included.

Stopped-flow Accessory

The stopped-flow accessory adds the dimension of kinetics research to your instrument, perfect for analyzing fluorescence reactions on the millisecond time-scale.

More accessories for the Fluorolog®:

Model	Item
F-3026	Standard-lamp correction factor kit
1914F	Thermoelectrically cooled R928P photomultiplier tube
1920	4 mL quartz cuvette with cap
F-3011	250 µL cylindrical quartz microcell adapter
F-3012	250 µL cylindrical quartz microcell (requires F-3011 adapter)
1925	4 mL quartz cuvette with stopper
1938	Set of 5 cut-on optical filters, 1" x 2"
1939	Set of 5 cut-on optical filters, 2" x 2"
1955	20 µL HPLC flow cell
F-1000/1001	External circulating temperature bath
F-3029	Integrating sphere for quantum yields
F-3023	Cryostat
FL-1001	Front-face viewing option
FL-1014	Sample-compartment electronics
FL-1030	Thermoelectrically cooled near-IR photomultiplier tube
QC-SK	Reduced-volume 1 mL cell, 5 mm x 5 mm, with adapter and magnetic stirrer
TRIG-15/25	Trigger accessory

Contact your Fluorescence Representative for an up-to-date list of new accessories to enhance your experiments.

Exclusivity

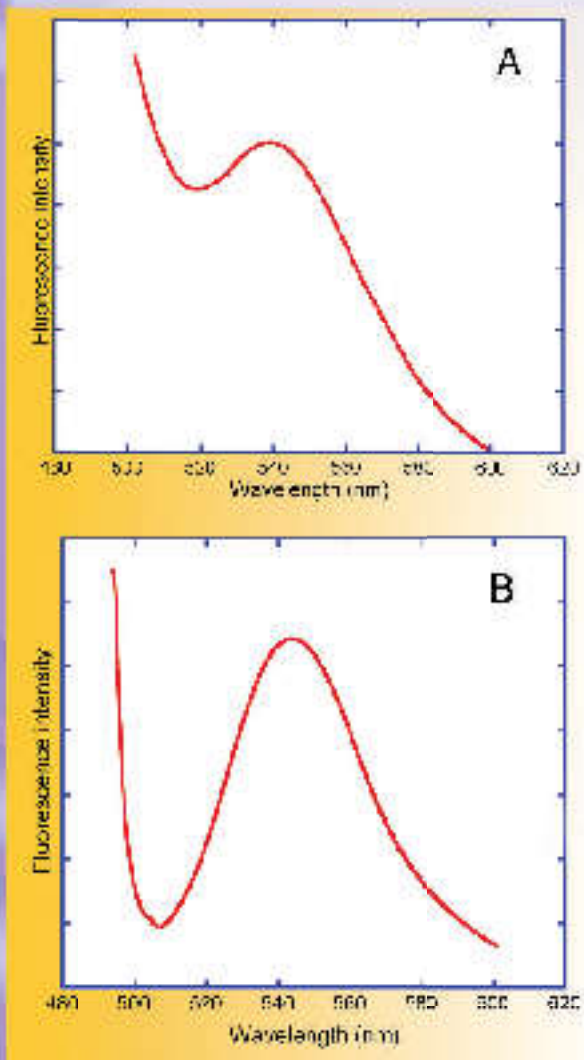
No other company offers you the choice of time-domain or frequency-domain upgrades. Who else can supply the applications support and service to get the full potential from your instrument? HORIBA Scientific has full applications laboratories in the USA, Europe, and Asia, plus affiliates and representatives the world over. You can rest assured that you have the support you expect only from HORIBA Scientific.

Real-World Performance

Whether you're working in biochemistry or nanomaterials, measuring calcium-migration, intermolecular distances, or laser crystals, the sensitivity and flexibility of a Fluorolog® spectrofluorometers will help you gather more information on more samples in a smaller amount of time. When the focus of your research changes, so can the Fluorolog®, adapting modularly to the demands of your work with upgrades and innovations. Here are just a few examples.

Detecting fluorescence in highly scattering samples

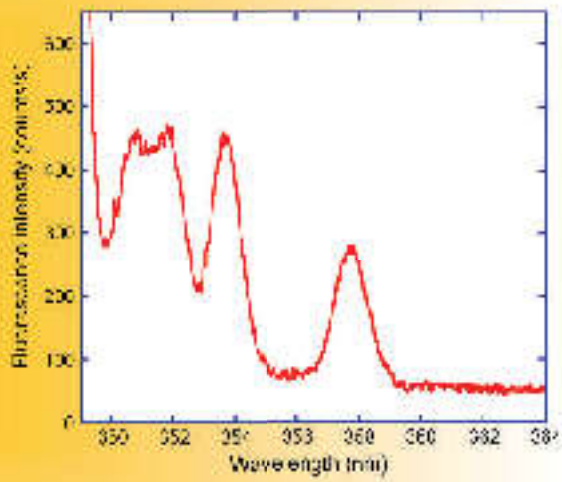
With highly scattering samples, fluorescence signals may be overwhelmed by stray or scattered light from the sample, making quantitative and qualitative analytical determinations impossible. However, a double-grating monochromator on the emission side drastically improves stray-light rejection.



Emission scan in front-face mode of a monolayer of rhodamine-B fluorescence with a (A) single-grating monochromator and (B) double-grating monochromator. Note the improved resolution of the peak near 540 nm when the double-grating monochromator rejects scatter from the sample.

The procedure for detecting fluorescence in highly scattering samples is illustrated by comparing the performance of a single-grating and a double-grating system on the same highly scattering sample. A monolayer of procaine-HCl on a microscope slide was scanned in front-face fluorescence detection mode with our best single-grating system and then with a model with double-grating monochromators on both excitation and emission. In plot A, stray light from the sample masks the procaine-HCl fluorescence. Plot B—measured using the double-grating system—shows a well-defined fluorescence peak at 540 nm.

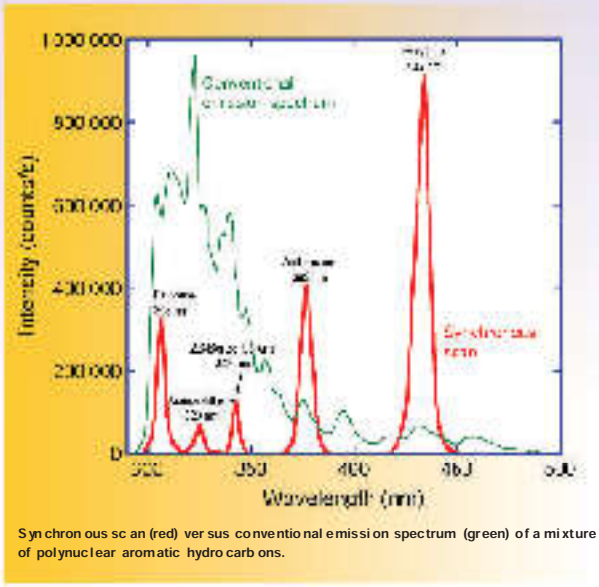
Carbon-tetrachloride data illustrate the unmatched stray-light rejection of the Fluorolog® by revealing all four Raman bands, at 350.7, 351.8, 353.6, and 357.7 nm for CCl_4. The excitation wavelength was 348 nm, and the bandpass settings on the excitation and emission monochromators were 0.5 and 0.7 nm, respectively. Narrow slit-widths and the ability to step the monochromator in small increments are critical in resolving the 350.7 and 351.8 peaks.



The four peaks of the Raman spectrum of CCl4 are easily resolved with double-grating monochromators in a Fluorolog®.

Synchronous scanning for characterizing complex mixtures

The observed fluorescence spectrum of a complex mixture often contains overlapping spectral features. Synchronous scanning offers a solution to this problem by simultaneously scanning the excitation and emission monochromators with a constant offset between them (in units of wavelength or wavenumbers).



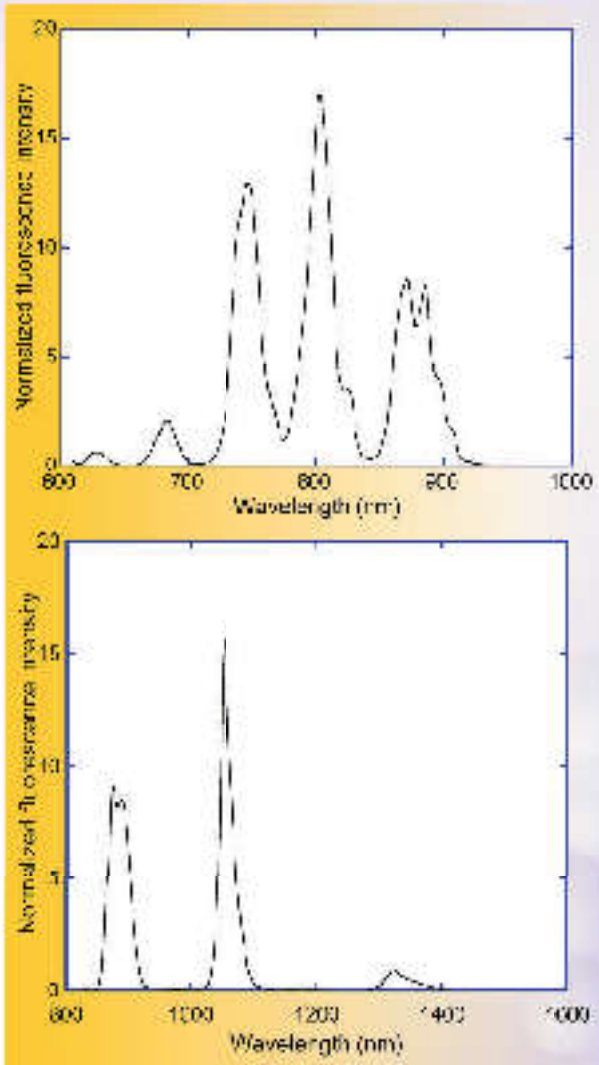
Synchronous scan (red) versus conventional emission spectrum (green) of a mixture of polynuclear aromatic hydrocarbons.

The scan of a mixture of polynuclear aromatic hydrocarbons (PAHs) compares a synchronous spectrum and a conventional emission spectrum for a mixture of five PAHs. The green line is the emission spectrum acquired on a Fluorolog® system with constant-wavelength excitation. When the sample is scanned synchronously (red line), five individual components are resolved into unique sharp peaks.

Infrared fluorescence (CW and lifetime)

Our Fluorolog® systems can be equipped to detect IR fluorescence, opening up totally new applications for fluorescence spectroscopy. For example, manufacturers of pharmaceuticals can employ IR fluorescence to identify toxic agents. In the world of nanomaterials, IR fluorometry can determine the composition of mixtures of single-wall carbon nanotubes. Also, probes in the red avoid interference from native fluorescence in the blue. An IR spectrofluorometer must be equipped with a red-sensitive photomultiplier tube (PMT) or solid-state detector whose response is effective far into the IR region. For fluorescence detection at wavelengths longer than 850 nm, there are two possible paths: the simplest is to mount either a PMT or InGaAs array that takes you as far 2.2 µm, depending on your choice; the alternative provided by a variety of solid-state detectors, covering different spectral regions, is available, as are choppers and lock-in amplifiers for enhanced sensitivity. Only a Spex® Fluorolog® IR system includes these components as integrated features.

Fluorolog® IR systems also have interchangeable gratings and optional grating-turrets to enhance efficiency in the IR region, giving Fluorolog® spectrofluorometers IR capabilities unmatched by any other instruments.



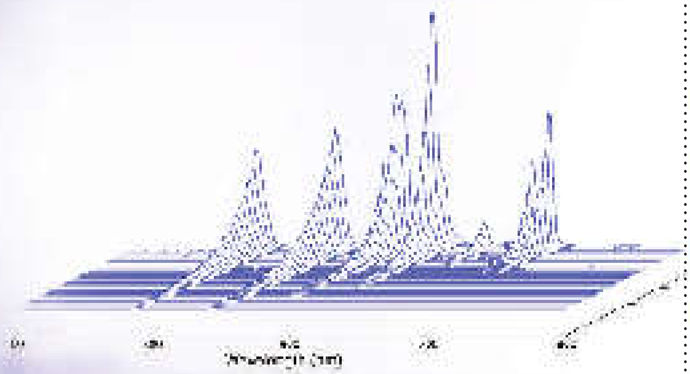
Normalized excitation (above) and emission (below) spectra from Nd-doped phosphate laser-glass in the red to near-IR.

Real-World Performance, continued

Fluorescence from singlet states usually occurs within a few nanoseconds after excitation. Because triplet transitions are more inhibited, the average phosphorescence-decay times are longer, ranging from microseconds to seconds, offering a longer observation period for monitoring reactions, viewing effects of the local molecular environment on a sample, or following changes in the hydrodynamic characteristics of macromolecular systems.

In phosphorescence experiments, the FluoroLog® with the FL-1040 dual-lamp housing—which includes a pulsed light source—can excite your sample with synchronized, user-specified delay and sampling windows, and can record time-resolved spectral data.

A delay permits acquisition of a phosphorescence spectrum without fluorescence interference. This selectivity is particularly important for samples in which the analyte can be overwhelmed by strong fluorescence from extraneous materials. In the matrix scan of an aqueous mixture of terbium-ligand and europium-ligand below (ligand = benzophenone-amine-tetra chromophore), the Eu-ligand luminescence (especially at 580 and 700 nm) decays faster (0.5 ms) than the Tb-ligand luminescence (1.1 ms).

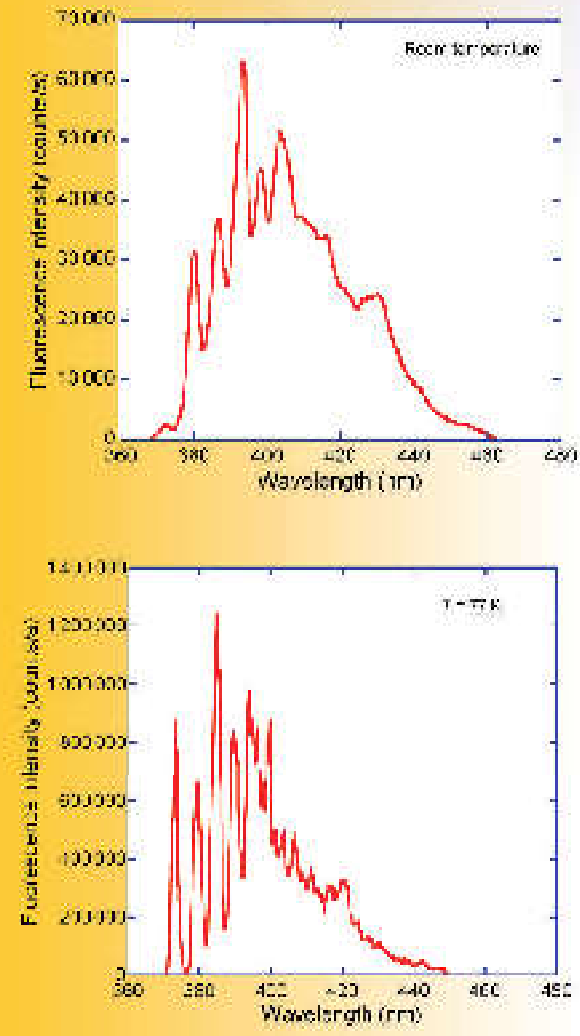


Time-gated matrix scan of an aqueous mixture of Tb-ligand and Eu-ligand.

Time-resolved data-acquisition also allows you to acquire phosphorescence-decay curves and compute phosphorescence lifetimes.

Low-temperature scans for enhanced fluorescence

One way to protect a sample from molecular collisions that quench luminescence is to isolate the sample in a rigid matrix. This cooling with liquid nitrogen enhances the phenomenon of fluorescence, even for an otherwise dormant sample. The graph below compares the fluorescence spectra of pyrene acquired at room temperature (upper) and at liquid-nitrogen temperature (lower). The FL-1013 Dewar accessory was used to chill the sample. As dramatically demonstrated in the overlaid plot, the low-temperature technique intensifies fluorescence emission from the pyrene and sharpens peaks to reveal greater structural detail. The superior resolution of a FluoroLog® double-grating system optimizes measurements under these conditions.



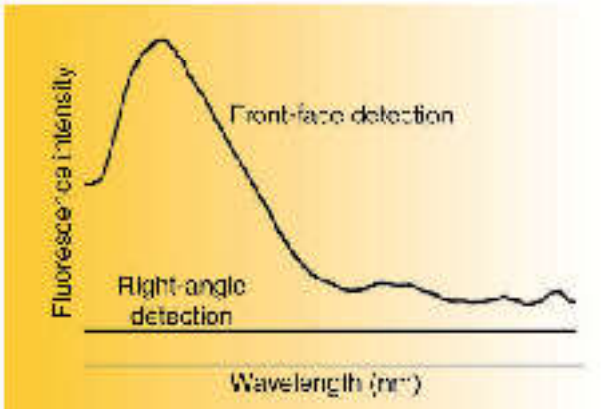
Emission spectra for pyrene acquired at (top) room temperature, and (bottom) at 77 K.

Front-face detection for absorbent or solid sample

Fluorescence is typically collected at 90° to the excitation beam to minimize interference from scattered light. Yet right-angle viewing is not feasible with some samples. Imprint paper, for example, should not be viewed at 90° because of interference with reflected light. In highly absorbent samples like hemoglobin or milk, most of the emitted light is reabsorbed internally before the fluorescence can be measured.

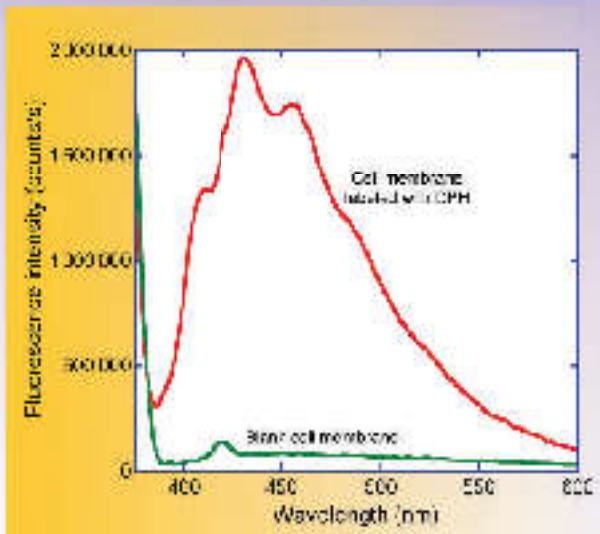
A significant optional feature of the Fluorolog® is a choice between conventional right-angle or front-face fluorescence detection. Ideal for solid or highly absorbent samples such as powders, powders, and microlayers on microscope slides. In front-face viewing, the fluorescence is collected from the sample's surface.

The color of tryptophan in hemoglobin contributes the spectra for right-angle and front-face viewing of a hemoglobin sample. For many years, hemoglobin was thought to be non-fluorescent because the fluorescence could not be detected at the conventional 90° angle. With front-face viewing, the fluorescence spectrum for the β-37 tryptophan in the α2β2 region of hemoglobin can be easily collected.



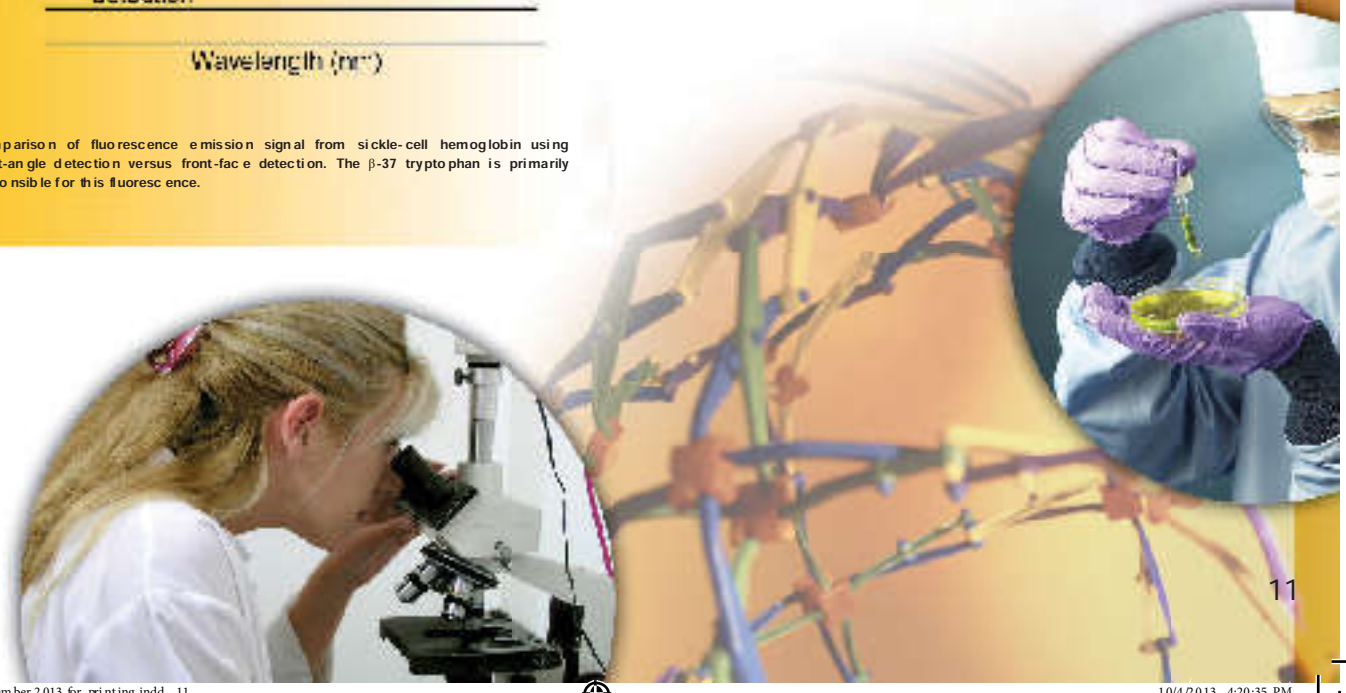
Comparison of fluorescence emission signal from sickle-cell hemoglobin using right-angle detection versus front-face detection. The β-37 tryptophan is primarily responsible for this fluorescence.

Detecting trace quantities of biological probes with fluorescence polarization



These fluorescence spectra clearly differentiate between a blank cell membrane and a membrane tagged with the biological probe DPH.

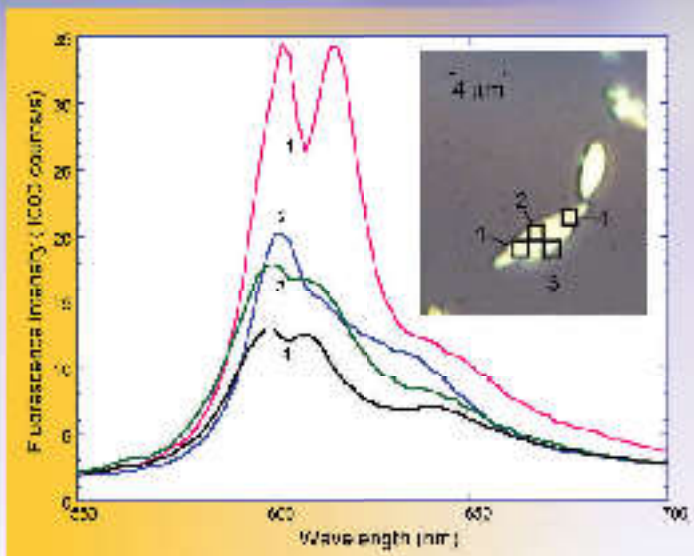
Used in conjunction with the variety of fluorescent dyes suitable for biological research, fluorescence spectroscopy has greatly expanded our knowledge of the molecular processes of the living cell. The Fluorolog® - 3 design offers unparalleled sensitivity for this work. The figure above illustrates the clear spectral differentiation between fluorescence in membranes and membranes labeled with DPH, a widely used probe for polarization and anisotropy measurements.



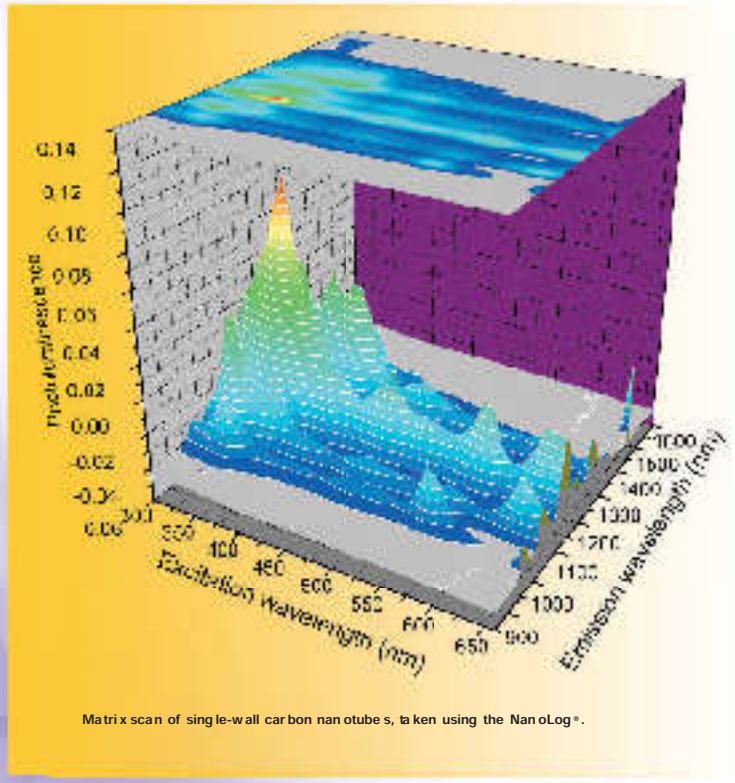
Real-World Performance, continued

Fluorescence mapping of single molecules and cells

The optional FluoroMap upgrade includes a microscope, fiber-optic bundles to bring excitation to and emission from the microscope, and even a digital camera for screen captures of your samples' luminescence. Mapping the microscopic variability in fluorescence of your biological samples or nanomaterials was never so easy. The graph to the right shows differences in fluorescent spectra from a single tiny crystal of PIC, a fluorescent dye and photographic sensitizer, resting on a microscope slide. The inset shows a microphotograph of the crystal and different areas on it from which spectra were taken.



PIC fluorescent spectra, taken with the FluoroMap from 4 different areas on a single microscopic crystal.



Matrix scan of single-wall carbon nanotubes, taken using the NanoLog®.

Scan and analyze mixtures of carbon nanotubes

The world of nanomaterials, including quantum dots and nanotubes, is open to you with the NanoLog®, a Fluorolog® instrument specially optimized for characterizing materials that fluoresce in the near-IR. The NanoLog® is fitted with a iHR320 imaging spectrometer and InGaAs-array detector for super-fast recording of spectra. Coupled with our exclusive Nanosizer™ software, you can even automatically and completely determine the composition (chirality and diameter) of mixtures of nanotubes. Here is an emission-excitation spectra of a mixture of single-wall carbon nanotubes recorded with the NanoLog®.

Automation
Means it's easy to use!

Hardware

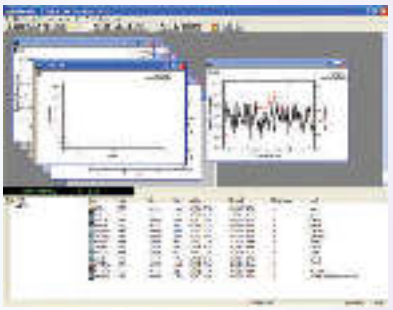
The Fluorolog® is self-calibrating, which means you begin taking data once the unit is turned on. Wavelength-scanning and slit-settings for bandpass control or resolution are all automatic, as are sample-changers, temperature control, microwell-plate readers, polarizers, and more. You can concentrate on your samples and data, and not worry about twisting knobs, sliding slits, or other forgettable items. Remember, because the settings are electronic, they are much more reproducible.

Temperature-control

The fluorescence emission of a sample is influenced by temperature: intensity falls as the temperature rises. Measuring intensity as a function of the temperature allows you to calculate various parameters, including activation energy from an Arrhenius plot, or thermal stability of proteins. Automated temperature-control includes a microprocessor-controlled circulator, remote temperature-probe, interface card, and all cables.

Software

For advanced operations in a Windows® operating system, the NEW FluorEssence™ software package has expanded features that revolutionize the way you operate your spectrofluorometer. Only a glance at the familiar toolbars and context-sensitive help-menus, and you're instantly recording data. FluorEssence™ is a comfortable environment, never forgetting that fluorescence is the reason you're there. Click to select the type of scan, your accessories, or bring back a complete experiment you run routinely. FluorEssence™ even comes with video tutorials that get you going as soon as you sit down at the keyboard.



- Simplified drop-down menus for operations
- Detector algebra to customize data-acquisition
- Matrix-scanning to produce 3-D and total-luminescence data
- Real-time control lets you see instantly the effect of changing hardware settings
- All the power of Origin® 8 Pro

Some of the display and processing routines include:

- Zooming and scaling
- Integrate
- Excitation and emission correction
- 3-D perspective plots
- Deconvolution
- Single-point analysis
- Contour maps
- Curve-fit
- Standard arithmetic
- Smoothing
- Derivative

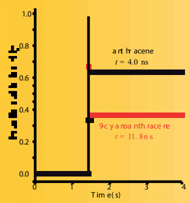
Fluorolog® and Molecular Dynamics

Time-resolved fluorescence measurements reveal significantly more information about the kinetics of molecular processes than steady-state spectroscopy. Now, lifetime techniques are applied to diverse fields such as photochemistry, biology, molecular biophysics, polymers, and semiconductors. The increased value of fluorescence lifetimes and anisotropy-decay coincides with the great strides in both time-resolved instrumentation and on-line data-analysis that have taken place within the last 30 years. HORIBA Scientific is the first firm that offers both phase- (with the MF²) and time-domain (with TCSPC) upgrades for time-resolved fluorescence measurements. These provide picosecond lifetimes, anisotropy-decay, time-resolved spectra, and lifetime-resolved spectra, while retaining the high performance found in the steady-state photon-counting Fluorolog®. Choose the technique that's best for you.

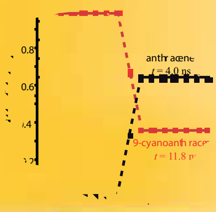
World's fastest frequency domain with MF²

Real-world samples often present complex fluorescence-lifetime decays, which can be analyzed precisely and accurately using the wide frequency range MF² upgrade. The mixing of two fluorescent organic species, anthracene and 9-cyanoanthracene (see plot), can be captured using our optional stopped-flow accessory. The Fluorolog®-MF², applying four simultaneous frequencies, easily resolves the mixing process within milliseconds and determines the lifetime of each species. The top graph shows the frequency response of the sample, in which the phase-shift of the sample is measured over time (interval between points = 20 ms) as a function of frequency. The middle graph shows the fitted data using our exclusive Universalizer fitting package, showing the relative intensities and the fitted lifetime values for each species. The bottom graph is an expanded view of the middle plot, showing how the MF² resolves the mixing process.

Raw phase data of stopped-flow mixing of anthracene and 9-cyanoanthracene, using four simultaneous applied frequencies. Each data set corresponds to a 20 ms time-interval.



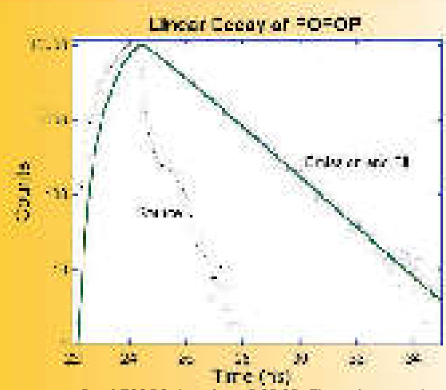
Lifetimes of anthracene (4 ns) and 9-cyanoanthracene (11.8 ns) extracted from raw data using the Universalizer fitting routine. Initially the cuvette contained only 9-cyanoanthracene (fractional intensity = 1 at time = 0). Time interval between points = 20 ms.



Expanded view of previous graph, showing individual data points separated by 20 ms. The MF² captures and resolves the mixing point (t = 1.48 s) during the experiment.

Time domain with TCSPC

The TCSPC (Time-Correlated Single-Photon Counting) upgrade merges the world-renowned Fluorolog® with the speed and ultimate sensitivity for lifetimes of the Fluorocube, the flexibility of pulsed excitation sources, whether solid-state LEDs from UV through near-IR, spark lamps for wideband power or even your own Ti:sapphire laser. This is the hallmark of TCSPC on the Fluorolog®. Time-Correlated Single-Photon Counting gives the best precision and sensitivity, combined with easy use with solid samples, temperature-dependent materials, and infrared fluorescence. Below is a lifetime-decay of the fluorophore POPOP, which exhibits a classic single-exponential fluorescence decay. HORIBA Scientific software deconvolves the pulse-profile of the NanoLED solid-state source from the emission to give an excellent fit (reduced χ^2 of 0.951) of 1.32 ns for POPOP's fluorescence lifetime.

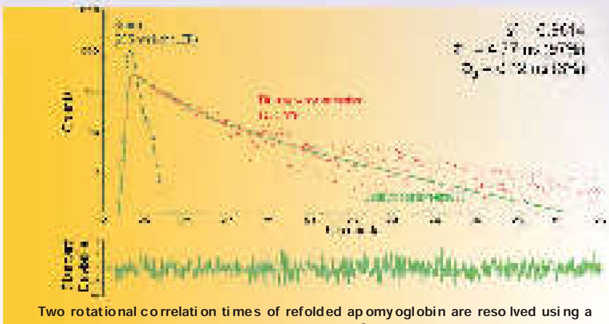


Least-squares fit of TCSPC data from POPOP. The points are the data; the green line is the fit, giving a lifetime of 1.32 ns with a reduced χ^2 of only 0.951.

For more details on lifetime measurements, see our Phase or Pulse: How to Select the Best Lifetime Spectrofluorometer brochure.

Emission Anisotropy

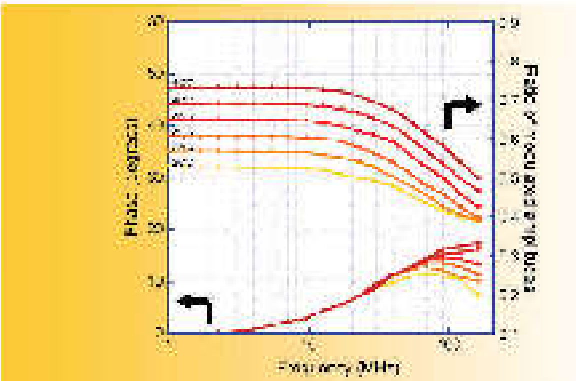
Emission anisotropy, or polarization, gives information about the size and shape of molecules, and also the environment of the fluorophore. The hydrodynamic volume of molecules, viscosity of solvents, microviscosity of local membrane environments, and long-range interactions may be measured as well. Steady-state measurements yield time-averaged values. Resolution of the decay of this anisotropy provides much more detail about molecular motions on the fluorescence timescale. Adding temperature as a variable allows the study of dynamic processes such as phase-transitions in membranes and the photophysics of molecules in solution. One example of a biological application is studying the rotational motion of proteins in solution, using a TCSPC accessory with polarizers on the Fluorolog® and optional temperature bath. Refolded apomyoglobin in solution was scanned at 20°C using a 280 nm pulsed NanoLED source. Emission was collected at 335 nm. A difference fit with excellent reduced χ^2 (0.9614) using DAS6 Decay Analysis Software gave two rotational correlations: a longer lifetime (4.77 ns) probably caused by overall molecular rotation, and a shorter lifetime (1.40 ns) possibly from internal molecular motion.



Two rotational correlation times of refolded apomyoglobin are resolved using a UV pulsed source and TSCPC on the Fluorolog®.

Temperature-based emission anisotropy

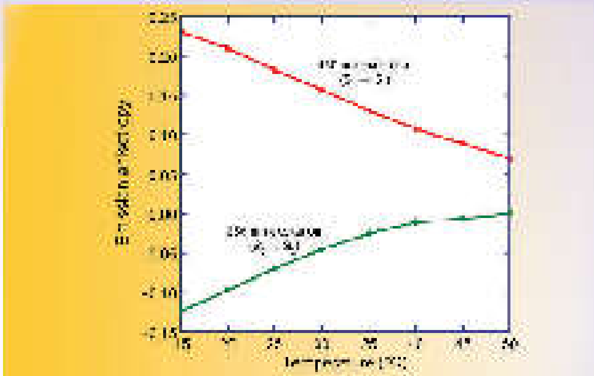
Perylene is a small disk-like fluorophore that rotates anisotropically in a solvent. The rotational rate of perylene in glycerol varies with the viscosity. As the temperature increases, glycerol's viscosity drops, and perylene rotates more freely. The Fluorolog®-MF2 with optional autopolarizer and temperature controller measures these rotations as a function of temperature automatically. The upper curves show the ratio of modulated amplitudes (ΛAC) as the temperature rose from 20°C to 75°C in 5° steps. The lower curves show the differential phase-angle (Δφ). As the molecule rotates faster, the ΛAC increases and the differential phase-angle shifts to higher frequencies. These data may be analyzed in terms of rotational correlation times as functions of temperature.



Emission anisotropy of perylene in glycol at various temperatures.

Steady-state emission anisotropy

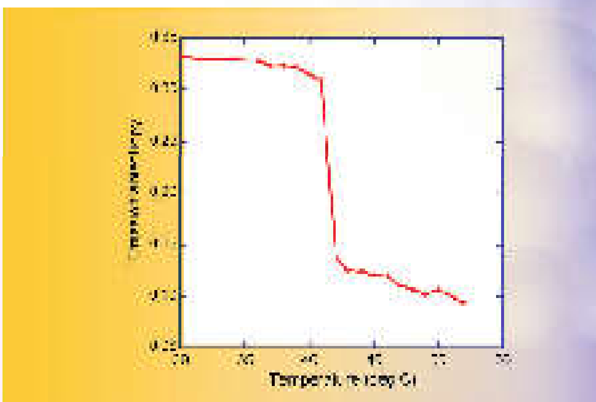
Temperature-based fluorescence provides information about the environment of the fluorophore, such as the viscosity. An example of this is shown below. Perylene has two absorption dipoles in the molecular plane. One of these is collinear with the emission dipole ($S_0 \rightarrow S_1$) and the other orthogonal ($S_0 \rightarrow S_2$). Excitation at 430 nm results in positive anisotropy, while excitation at 258 nm gives negative anisotropy (the absorption and emission dipoles are orthogonal). The steady-state anisotropy of perylene in glycerol, acquired at 430 nm and 258 nm excitation as a function of temperature, was collected automatically with the optional autopolarizer and temperature bath. As the temperature rises, the bulk viscosity of the glycerol falls and the perylene rotates faster, depolarizing the fluorescence emission. From this, the viscosity of the solvent and the size, shape, and hydrodynamic volume of the fluorophore may be evaluated.



Steady-state anisotropy of perylene in glycol. Excitation at two different wavelengths results in different anisotropies that are reduced as the temperature rises.

Phase-transition

The addition of small probes into cell membranes, or the effects of lipid composition on cell membranes, may be determined using anisotropy as a function of temperature. Here a model membrane of a phospholipid multilamellar vesicle was used. The phospholipid DPPC was labeled with DPH, a rod-like probe which partitions itself into the hydrophobic region of the vesicle. The anisotropy of DPH embedded in DPPC shows a sharp reduction at the phase-transition (42°C) of the phospholipid.



Phase-transition curve of DPPC multilamellar vesicles (labeled with DPH, 1:500), a phospholipid model of cell membranes.

Specifications

If it fluoresces, you'll see it in a Fluorolog®

The basic Fluorolog®-3 spectrofluorometer system consists of the following components:

1. A 450 W xenon lamp and its power supply, inside a housing
2. A single-grating excitation monochromator
3. A T-format sample compartment with excitation reference detector
4. A single-grating emission monochromator
5. An emission photomultiplier tube with photon-counting detection
6. All necessary electronics and software to attach to the serial port of your compatible PC.

Any of these components can be replaced or augmented by additional components that will alter stray-light (spectral purity) characteristics, sensitivity, wavelength-range, or any of the other parameters that dictate the success of your research.

Excitation source

450 W xenon short-arc mounted vertically in an air-cooled housing. Light collection and focusing by off-axis mirror for maximum efficiency at all wavelengths. Optional pulsed lamp for phosphorescence measurements, and spark sources and diodes for pulsed lifetime-acquisitions.

Monochromators

Czerny-Turner design with kinematic gratings and all-reflective optics. Optional double-grating units available for highest stray-light rejection and sensitivity. (Specifications based on 1200 grooves/mm grating, but many other gratings are available)

- **Accuracy** 0.5 nm
- **Speed** 150 nm/s
- **Range** 0–1300 nm mechanical range (longer ranges for different gratings); throughput based on grating's blaze
- **Gratings** 330 nm blaze for excitation (200–700 nm range); 500 nm blaze for emission (300–1000 nm range); other gratings available for different ranges.
- **Bandpass** Set automatically (0–30 nm single-grating, 0–15 nm double-grating) with auto-calibration on start-up.
- **Imaging** Imaging spectrometer option for multi-channel acquisition or multi-port applications.
- **Resolution** As good as 0.04 nm with iHR320 spectrograph option and 1800 grooves/mm (better with iHR550).
  Three-grating turret supplied.

Sample compartment

T-box design to allow second emission-detection channel. Gap-bed removable for sampling-accessory replacement. Optional front-face detection.

Detectors

Photodiode for excitation correction from 240–1000 nm. Standard emission detector is R928P photomultiplier tube for high sensitivity in photon-counting mode (240–850 nm). Other PMTs to 1700 nm, with thermoelectrically cooled option. Solid-state detectors for higher-wavelength emissions. CCD or diode-array multi-channel detectors for rapid emission spectra and sample spatial information.

Software

Windows® based FluorEssence™ supplies all scanning, time-based, and accessory data-acquisition as well as complete control of all hardware.

Lifetime upgrades

MF^2 (frequency-domain) has a lifetime range of 20 ps to 10 ms and frequency range of 1 kHz to 300 MHz; with 1 to 8 frequencies simultaneously (maximum 100 frequencies with sequential operation), depending on excitation source and sample. TCSPC (time-domain) has a lifetime range from 50 ps to 1 second, depending on detector source, and electronics. Consult your representative for your requirements.

Sensitivity

S/N can be MUCH better than 30 000:1 by exchanging PMT, grating, calculation method, etc. Standard PMT (R928) and gratings (1200 grooves/mm) produce better than 12 000:1 (30 000:1 RMS) with excitation at 350 nm, 5 nm bandpass, and the 1st standard deviation of background noise at 450 nm. (When comparing S/N, be sure that all settings and hardware are the same. For a full explanation on comparison of S/N, please see our Application Note FL-13, "Sensitivity of the Fluorolog® and FluoroMax® Spectrofluorometers".

info-sci.fr@horiba.com
www.horiba.com/scientific

JOBIN YVON Technology

HORIBA Scientific

USA: +1 732 494 8660 **France:** +33 (0)1 69 74 72 00 **Germany:** +49 (0)89 4623 17-0
UK: +44 (0)20 8204 8142 **Italy:** +39 0 2 5760 3050 **Japan:** +81 (0)3 38618231
Spain: +34 91 490 23 34 **China:** +86 (0)10 8567 9966 **Brazil :** +55 11 5545 1540
Other Countries: +33 (0)1 69 74 72 00