How to Build the World's Most Sensitive Spectrofluorometer

The source
Starting with a xenon source that supplies prime UV performance, we mount the bulb vertically, because horizontal mounting leads to sagging, instability, and shorter arc-life. The xenon source is focused onto the entrance-slit of the excitation monochromator with an elliptical mirror. Besides ensuring efficient collection, the reflective surface keeps all wavelengths focused on the slit, unlike lenses (with chromatic aberrations that make them optimally efficient only at one wavelength).

The slits
The slits are bilateral, continuously adjustable by the software in units of bandpass (wavelength) or millimeters. This preserves maximum resolution and instant reproducibility.

The excitation monochromator
The excitation monochromator is an aspheric design which ensures that the image of the light diffracted by the grating fits through the slit. The gratings themselves are blazed and planar, avoiding the two major disadvantages of the more common concave holographic gratings: poor polarization performance and inadequate imaging during scans that throws away light. The unique wavelength-drive scans the grating at speeds as high as 80 nm/s. The grating's grooves are blazed to provide maximum light in the UV and visible region.

The reference detector
Before the excitation light reaches the sample, a photodiode reference detector monitors the intensity as a function of time and wavelength to correct for any change in output due age or wavelength. The photodiode detector is traceable to NIST standards out to 1000 nm, and requires no maintenance.

The sample chamber
A spacious sample chamber is provided to allow the use of a wide variety of accessories for special samples, and encourage the user to experiment with many sample schemes.

The emission monochromator
All the outstanding features of the excitation monochromator are also incorporated into the emission monochromator. Gratings are blazed to provide maximum efficiency in the visible. Correction-factor files traceable to NIST lamps remove optical artifacts from the optical path through the monochromator.

The detector
Emission-detector electronics employ photon-counting for the ultimate in low-light-level detection. Photon-counting concentrates on signals that originate from fluorescence photons, ignoring the smaller pulses originating in photomultiplier-tube electronics. Lower-performance fluorometers with analog detection—in contrast—simply add noise and signal together, hiding low signals within the noise. The emission-detector housing also contains an integral high-voltage supply which is factory-set to provide the maximum count-rate, while eliminating most of the dark noise.

Computer-control
The entire control of the FluoroMax®-4 originates in your PC, from our most powerful software, FluorEssence™. On start-up, the system automatically calibrates and presents itself for new experiments, or stored routines instantly called from memory. Professional, publication-ready plots and data-analysis are based on world-renowned Origin®.

Accessories
A wide variety of accessories are applications-oriented and detailed in previous pages of this brochure.

Specifications
Below are our guaranteed specifications for the FluoroMax®-4 spectrofluorometer. Compare them with other instruments, and you'll see why FluoroMax®-4 is uniquely suited to your application.

Optics	All-reflective for focusing at all wavelengths and precise imaging for microsamples
Source	150 watt Ozone-free xenon lamp eliminates venting
Spectrometers	Plane-grating Czery-Turner design maintains focus at all wavelengths
Excitation	200–950 nm, optimized in the UV
Emission	200–950 nm, optimized in the visible
Bandpass	0–30 nm, continuously adjustable from computer
Wavelength Accuracy	± 0.5 nm
Scan Speed	80 nm/s
Integration Time	1 ms to 160 s
Emission Detector	Photomultiplier, range 200–850 nm
Reference Detector	Photodiode selected for stability
Water-Raman Signal	400,000 counts/second minimum at 350 nm excitation, 397 nm emission, 5 nm bandpass, 1 s integration time
Signal-to-Noise Ratio	3000:1 (steady-state mode)
Nanosecond Lifetime Option	Lifetime range 200 ps–0.1 ms (100-200 ps - 0.1 ms) Minimum resolution < 7 ps/channel Excitation with interchangeable NanoLEDs: 265–785 nm TCSPC detection
Phosphorescence Lifetime Option (in FluoroMax®-4P)	Lifetimes down to 10 µs Delay variable 50 µs–10 s Sampling time variable 50 µs–10 s Excitation with broadband pulsed UV xenon lamp Flash rate 0.05–25 Hz Flash duration 3 µs FWHM; low-intensity tail > 30 µs Flashes per data point 1–999
System Control	PC, with FluorEssence™ software
Dimensions (w x h x d)	32.5" x 11" x 19"; 82.6 cm x 28 cm x 48 cm
Sample Compartment (w x h x d)	5.5" x 7" x 7"; 14 cm x 18 cm x 18 cm
Power Requirements	5 A, 120 V; 2.5 A, 240 V; 50 or 60 Hz, single-phase
Weight	75 lbs; 34 kg

JOBIN YVON Technology

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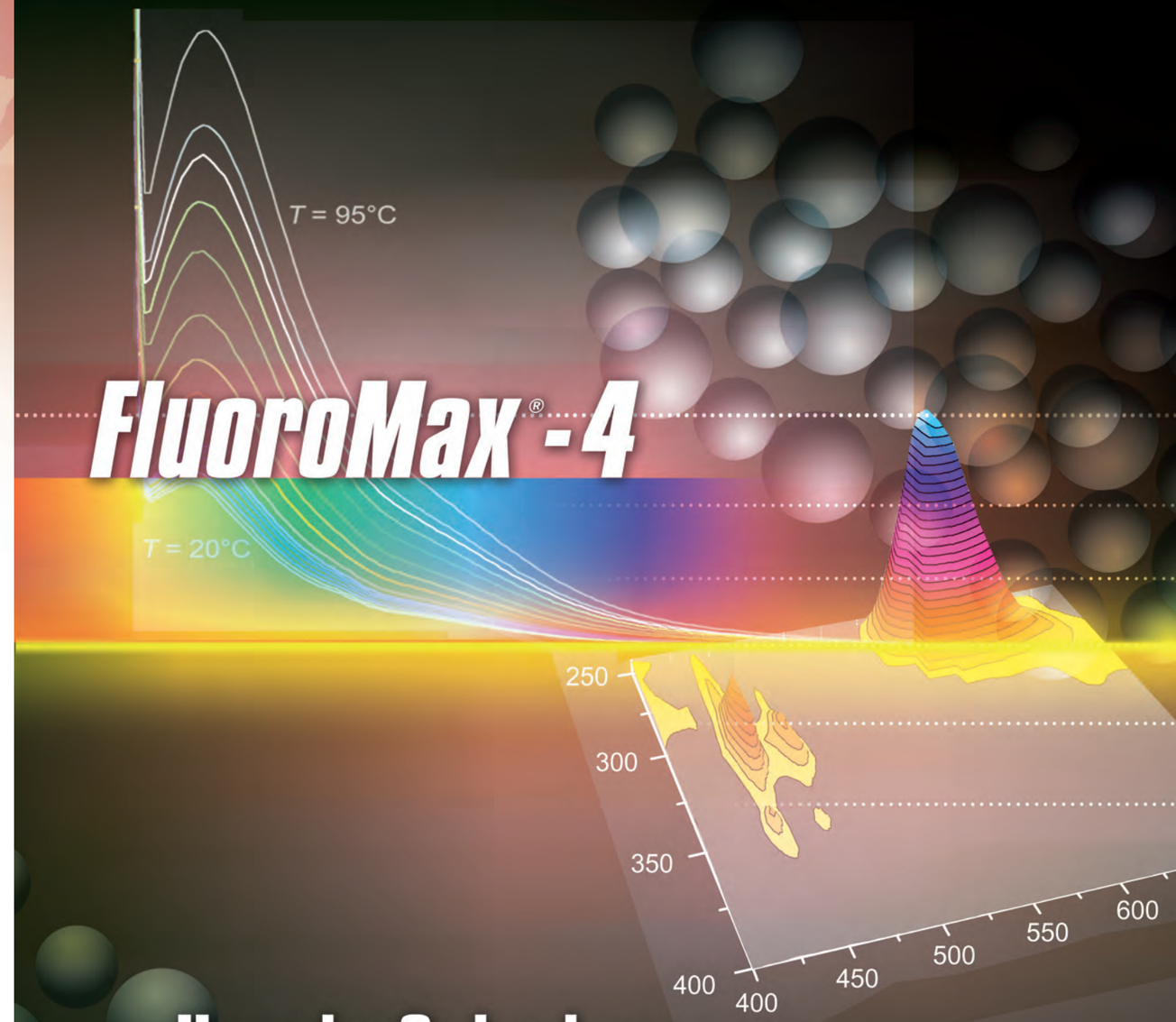
HORIBA Scientific



FluoroMax®-4

How to Select a Spectrofluorometer

JOBIN YVON Technology

Explore the future
Automotive Test Systems | Process & Environmental | Medical | Semiconductor | Scientific
HORIBA

The World's Most Sensitive Spectrofluorometer

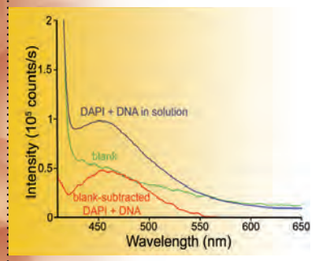
New phosphorescence and nanosecond TCSPC measurements!

HORIBA Scientific delivers the FluoroMax®-4: analytical speed, easy to use, and MAXimum sensitivity

The FluoroMax®-4 is a compact spectrofluorometer from Spex®, yet it offers the ultimate sensitivity in fluorescence investigations as well as features not found in most table-top fluorescence-detection systems.

Applications for the FluoroMax®-4

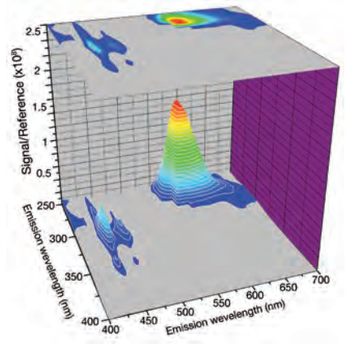
Pharmaceuticals and Medicine



The high sensitivity of fluorescence offers the unique ability to study the molecular environment of biologically significant compounds. This figure demonstrates the unsurpassed sensitivity of the FluoroMax®-4's photon-counting detection: 10 fg/mL of DAPI bound to DNA.

The inherent sensitivity of fluorescence makes this technique ideal for advanced research, routine analysis, and quality control in pharmaceutical and medical fields. Fluorescence provides information on the dynamics, rigidity, and structure of DNA, proteins, and viruses. Fluorescence-immunoassay methods, in pharmaceuticals, can specifically identify a limitless number of analytes in concentrations as low as picomolar levels.

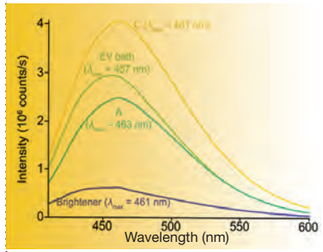
Environmental Science



3-D matrix scan over a range of excitation wavelengths of a sample of petroleum. The signal is divided by the reference detector to remove temporal and spectral variations of the excitation light. Variations in scans from sample to sample can be used for quality-control and analysis of impurities.

Fluorescence can monitor trace quantities of organic, inorganic, toxic, mutagenic, or carcinogenic substances in air, water, and soil. In complex, real-world samples, high sensitivity and selectivity are required to measure these trace constituents because of multiple sources of interference and high background signals. 3-D matrix scanning and contour-mapping (shown here, also called a "total luminescence spectrum") provide a unique fingerprint that qualitatively identifies a compound. A highly publicized application for 3-D matrix scans involves tracing the geological source of different oil samples.

Analytical Chemistry



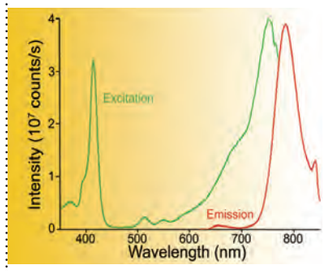
Spectra at different concentrations characterize an electrogalvanizing bath.

Analytical chemists probe the molecular environment and study luminescence spectra and quantum efficiencies of fluorescent species. Once the basic characteristics such as excitation, emission, and quantum yields are determined for a fluorescent probe, routine assays and methods can be developed for testing laboratories.

Analytical fluorescence properties for chemical probes include:

- General solvent effects
- Quantum yields and lifetimes
- Excited-state dipole-moments
- Heavy-atom and temperature effects
- Room- and low-temperature effects
- Reactions on various surfaces

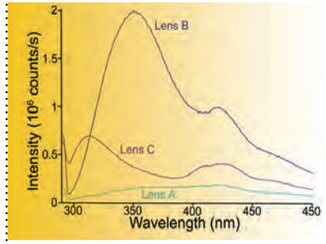
Food Science and Agriculture



Measuring the ratio-corrected excitation of chlorophyll in the near-IR region supplies information on leaf senescence. The ratio-corrected excitation and emission spectra of chlorophyll from most green vegetation are shown.

Improving nutritional quality, shelf-life, and packaging of food-products are critical to the food-science industry. Bacterial growth is particularly destructive and dangerous, as evidenced by lawsuits involving contamination, illness, and even death. To ensure a reliable product, food-providers need to identify contaminants and vulnerability to infectious growth, micro-organisms, molds, and even pesticides normally used to prevent their spread. Packaging is equally important, both as a protective membrane against oxidation and a possible source of trace plasticizers and polymers. Researchers want to know how crop-yields and quality can be improved through the correct application of fertilizers.

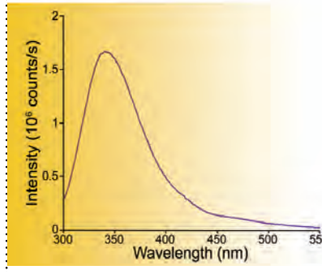
Manufacturing and Industry



This is a comparison of proteins adhering to the surface of contact lenses. Manufacturers of enzymatic cleaning solutions can thus evaluate the product's effectiveness to improve quality.

Manufacturers use fluorescence to monitor the quality of paints, plastics, polymers, optical brighteners, and phosphor coatings. Biotechnology researchers analyze drugs, hormones, proteins, vitamins, and DNA with fluorescence. Medical and clinical instrument-manufacturers investigate fiber-optic-based invasive systems that can be snaked into arteries or other orifices. Cosmetic and health-care companies evaluate the effectiveness of new products for the consumer marketplace such as broad-spectrum sunscreens, lipid-based emollients to improve skin-quality, and anti-aging creams.

Photochemistry

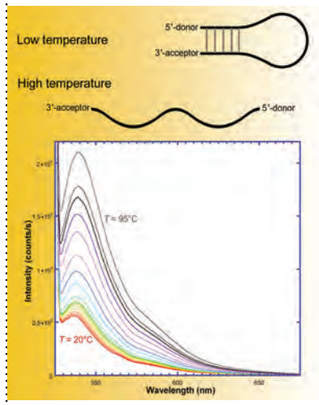


An emission scan of tryptophan in human skin, using the remote fiber-optic accessory with the FluoroMax®. In vivo fluorescence offers insights into skin remittance, cellular turnover, and effectiveness of sunscreens, cosmetics, and pharmaceuticals.

Photochemical research unravels the complexities of molecular microenvironments. The mechanisms of light-absorption and the photophysical properties of a substance determine its function in chemical and biological processes. Photochemical applications of fluorescence include:

- Molecular mechanisms of transmembrane protein-transfer in bacteriorhodopsin
- Photodynamic therapy, a technique for tumor location, identification and control
- Biological energy-conversion in green-plant photosynthesis
- Use of quantum dots as biological probes in cancer diagnosis and tissue studies
- Characterization of flavins, carotenoids, and other photoreceptors

Cell Biology



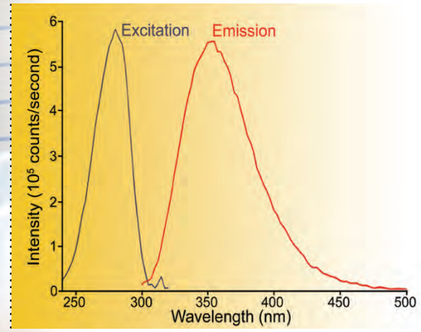
TET, a donor and fluorescent dye, was attached to the 5' end of a DNA fragment, and QSY, an acceptor or quencher, was attached to the 3' end. The DNA formed a loop, with a stem keeping the donor and acceptor together at low temperatures. When heated, the loop opened, removing the acceptor from the donor's proximity, and the fluorescence increased. Excitation was at 521 nm.

A wide variety of fluorescent tracers assist in the study of basic biological processes. These substances can be characterized by their fluorescence excitation and emission spectra. Using a ratio technique in which the fluorescence intensities at several wavelengths are monitored offers advantages over measuring the absolute intensity at a single wavelength.

The World's Most Sensitive Spectrofluorometer

How FluoroMax®-4's speed AND sensitivity produce maximum performance

The FluoroMax®-4 scans at up to 80 nm/second—but speed is useless without sensitivity!



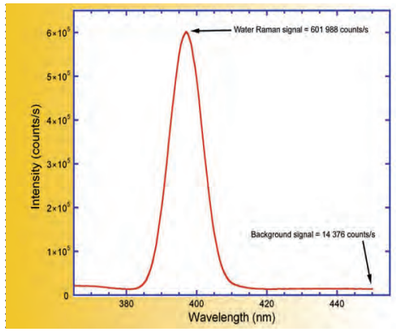
Excitation and emission spectra of 10^{-6} M tryptophan. The data were automatically acquired, blank-subtracted, and displayed in 20 s.

The water-Raman test of sensitivity

Only when you combine speed AND sensitivity do you achieve true time-saving, which means you can run more samples. Hence the cost to run each sample decreases, for one instrument with one operator can do the work of two or more. That's real progress! Because it is independent of sample preparation, the water-Raman test of the signal-to-noise ratio is a good measure of relative sensitivity between different instruments, provided the experimental conditions used to compare the systems are the same. Unfortunately, there are a number of different ways of handling the data, all of which are valid but which will give quite different numbers. Therefore, it is important not only to know how the water-Raman signal-to-noise ratio (S/N) is measured, but also how the data were treated.

What HORIBA Jobin Yvon does

There are various methods to measure S/N, but we do it this way. The water-Raman S/N test method combines a value for system sensitivity (with a signal) with a value for system noise (no signal present) to show the overall performance of the instrument. At HORIBA Jobin Yvon, we define the S/N as the difference of peak and background signal, divided by the square root of the background signal. This method is derived from an assumption of random noise and Gaussian statistics, so that the first standard deviation equals the square root of the measured number—in our case, the background signal. The peak signal is measured at the water-Raman peak (397 nm for 350 nm excitation). The noise is measured in a region where no Raman signal is present (450 nm). An "ideal" system would give a noise value of zero. In both measurements, our bandpass is set to 5 nm on all slits on the entrance and excitation monochromators.



Water-Raman spectrum on a typical FluoroMax® instrument. Excitation was at 350 nm, integration time was 0.5 s, 5 nm bandpass, with data recorded every 0.5 nm from 365–450 nm. Signal (at 397 nm) and background (at 450 nm) are indicated in the plot.

Actual data (a typical FluoroMax® instrument) serve to show our method. The experimental conditions were as follows:

- Excitation 350 nm with 5 nm bandpass
- Emission 365–450 nm with 5 nm bandpass
- Interval 0.5 nm
- Integration time 0.5 s
- No smoothing of data points
- Standard room-temperature red-sensitive detector
  Note: Make sure the test is carried out with the actual detector you use. All HORIBA Jobin Yvon systems are specified with a R928P photomultiplier tube at room temperature).

The measurements provided the following data:

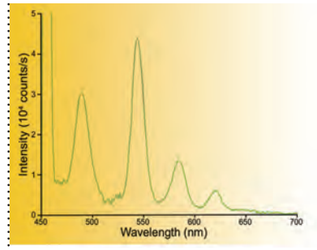
- Peak signal (at 397 nm) = 601 988 cps
- Background signal (at 450 nm) = 14 376 cps

Therefore, our method gives a water-Raman *S/N* of:
$(601\,988 - 14\,376) / (14\,376)^{1/2} = 4901$

Why our method is more accurate

We assert that our method better characterizes the instrument, although it gives a lower value. Other methods only take into account the detector noise and the shot noise of the electronics. On the other hand, by using the background total intensity as a measure of noise, our method is more representative of a real "live" experiment where noise is also influenced by factors such as the quality of the optics and scattered light in the system. These additional factors influence the ability to measure a very low signal from a sample and ought not be ignored.

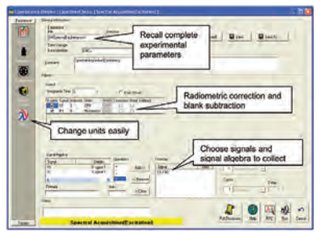
Photon-counting means sensitivity



$TbCl_3$ at the extremely low concentration of 10^{-5} M, used as a probe.

Only the FluoroMax®-4 offers the ultimate sensitivity of photon-counting. FluoroMax®-4 is the only instrument in its range to deliver photon-counting as standard. With photon-counting you measure only the signal that originates from sample photons—noise from the detector is rejected. That means your weakest signals aren't swamped by electronic background, and you can analyze concentrations undetectable with other instruments.

Method files: Recall complete experiments instantly



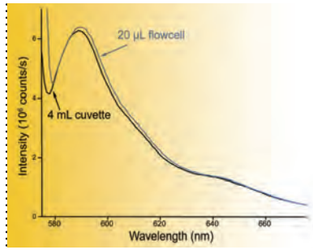
FluorEssence™'s tabbed windows with bold icons are dedicated to fluorescence experiments.

When you need to rerun an experiment or sample (or just verify which conditions you used to collect a particular data file), simply recall all the parameters from memory—including bandpass settings—with a single command. No guess-work. No leafing through the pages of your laboratory notebook. And you can't make a mistake!

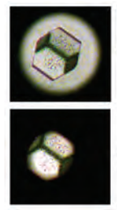
Fluorescence microscopy

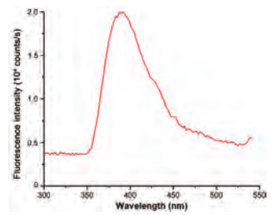
Small-volume samples, too

FluoroMax®-4's precise imaging is perfect for HPLC cells or small-sample volumes such as 20 µL. But when samples are simply too small for cuvettes, you need a mapping microscope. This is a simple option with our various fiber-optic bundles that deliver excitation light down into the deep UV for biological samples or nanomaterials and even collect the fluorescence to return it for characterization through the FluoroMax®-4's emission monochromator.



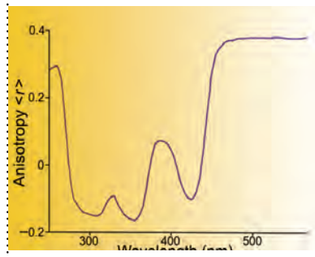
Sometimes small volumes resist conventional measurements. This plot compares fluorescence spectra of 20 n*M* resorufin in a standard 4-mL cuvette, and our 1955 20 µL flowcell. High sensitivity is achieved regardless of sample volume.





On the left are digital images of a single glucose isomerase crystal, only 70 µm across, using a mapping microscope with different pinholes in the emission path, a 10x objective, and our Microscope Adapter. On the right is an emission spectrum of the crystal, with an excitation wavelength of 280 nm.

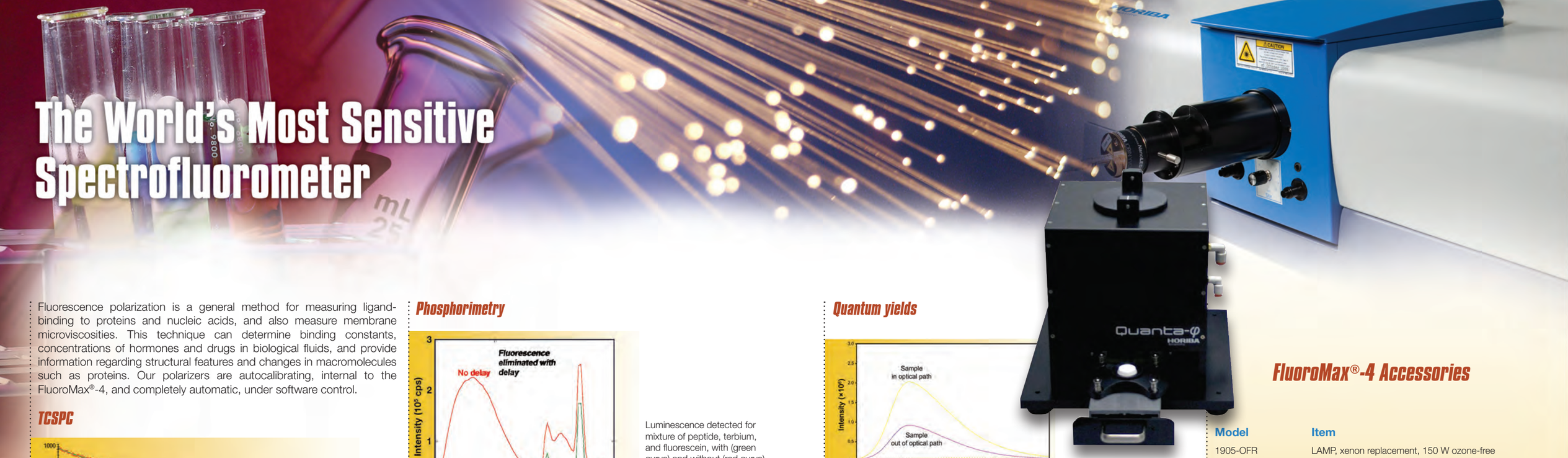
Polarization and dynamic anisotropy



Polarization excitation spectrum of rhodamine B demonstrates wavelength-sensitivity of polarization.

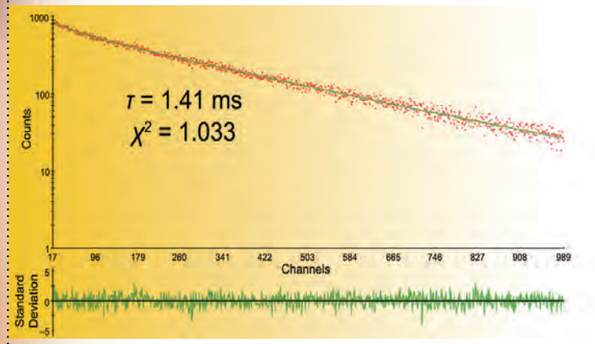
Fluorescence polarization can demonstrate a change in the rotational Brownian motion of a small molecule upon binding to a larger one. The small molecule thus assumes the slower motion of the larger molecule. The alteration in mobility of a small fluorescent ligand also can be detected with high sensitivity from the depolarization of the emission following excitation with polarized light.

The World's Most Sensitive Spectrofluorometer



Fluorescence polarization is a general method for measuring ligand-binding to proteins and nucleic acids, and also measure membrane microviscosities. This technique can determine binding constants, concentrations of hormones and drugs in biological fluids, and provide information regarding structural features and changes in macromolecules such as proteins. Our polarizers are autocalibrating, internal to the FluoroMax®-4, and completely automatic, under software control.

TCSPC



Förster Resonance Energy Transfer TCSPC decay of lanthanide tied to fluorescein, with a donor-acceptor distance of 5.44 nm. On the upper graph, red dots are data, and the green line is the fit, giving a lifetime of 1.41 ms.

Time-correlated single-photon counting (TCSPC) is an option for your FluoroMax®-4 to determine fluorescence lifetimes into the nanosecond range quickly and accurately. Our pulsed light-sources are the advanced, interchangeable solid-state NanoLEDs. Advantages of TCSPC include:

- Variations of the excitation beam's intensity are irrelevant
- Detects individual photons
- No pulse-smearing from boxcar gates
- High speed data-acquisitions
- Digital precision, and no analog offset required for fitting results
- The entire decay curve is examined at once

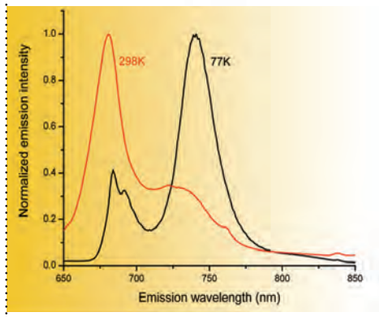
The systematic errors associated with stroboscopic techniques used by other manufacturers are absent with TCSPC. For example, flashlamps generate stray RF which the stroboscopic detection electronics can detect. TCSPC avoids this by rejecting low-level noise and accepting only high-level signals. Poisson statistics provide robust estimates of the standard deviation in each channel via TCSPC, but there is no method to determine stroboscopic uncertainties.

Phosphorimetry

Luminescence detected for mixture of peptide, terbium, and fluorescein, with (green curve) and without (red curve) a 50-µs phosphorimeter delay. Note how the delay removes unwanted fluorescence, leaving only long-lived phosphorescence.

Triplet transitions occur more slowly, from microseconds to seconds, than singlet transitions. With our phosphorimeter option, a built-in xenon flashlamp emits broadband excitation, and a synchronized variable delay rejects any fast fluorescence interference. A FluoroMax®-4P (FluoroMax®-4 outfitted with the phosphorimeter) contains both pulsed lamp and CW lamp for phosphorescence and fluorescence detection. Switching from fluorescence to phosphorescence detection is done entirely by a computer-controlled mirror!

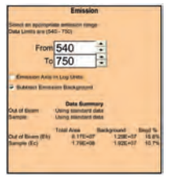
Cryogenic measurements

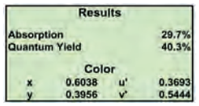


Normalized comparison of *Festuca* spp. chlorophyll fluorescence at room temperature and liquid-nitrogen temperature, using a Dewar accessory in a FluoroMax®, excited at 440 nm. Note how the 681 nm peak from Photosystem II sharpens into a doublet (CP43 and CP47 complexes) upon chilling, while the Photosystem I peak at 740 nm broadens and increases strongly in intensity at low temperature, from the Lhca1 and Lhca4 complexes.

When you want to freeze molecular motions to sharpen up spectral bands, or preserve the fragile triplet state, our liquid-nitrogen-cooled Dewar is the perfect accessory to chill the sample. The Dewar is placed on a pedestal within the sample compartment. The sample, within a quartz cell, is slowly immersed into the liquid-nitrogen-filled Dewar.

Quantum yields

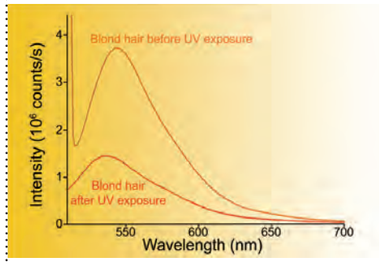




In the Integrating Sphere accessory, a sample of dye-impregnated plastic was scanned. Data and screenshots of results are shown. The color of the sample can be plotted both in CIE 1931 *xy* and CIE 1976 *u'v'* coordinates. All calculations are automatically performed by our Quantum Yield and Color Calculator software.

Accurate and reliable photoluminescence quantum yields for fluorescent samples are now within your grasp with the Quantum Yield Accessory, perfect for research on OLEDs, DNA sequencing and detection, immunology, nanocrystals, green fluorescent protein, quantum dots, and phosphors. Specially designed to slide into the sample compartment of the FluoroMax®-4, the Quantum Yield Accessory includes a 4" (10 cm) integrating sphere, sample holders for liquids and thin solid films, and special, exclusive software for automatically determining quantum yields.

Fiber optics—for samples even we haven't thought of



Comparison of blond hair before and after UV exposure, using a remote fiber-optic accessory. Hair-care products can be improved to protect hair from the sun's UV damage.

Sometimes the sample just doesn't fit—even in the spacious sample compartment of the FluoroMax®-4. That's when you need bifurcated fiber-optic probe that directs exciting light to the sample, and also collects resulting fluorescence. This is perfect for *in vivo* UV-A evaluation of cosmetics, hair, or sunscreens, photodynamic therapy, skin-remittance studies, living creatures in aqueous environments.... Use your imagination!

FluoroMax®-4 Accessories

Model	Item
1905-OFR	LAMP, xenon replacement, 150 W ozone-free
J1920	CUVETTE, 4 mL, quartz, capped
J1925	CUVETTE, 4 mL, quartz, stoppered
J1933	HOLDER for solid samples
J1938	FILTERS, 1"x 2" (2.5 cm x 5 cm), cut-on, set
J1939	FILTERS, 2"x 2" (5 cm x 5 cm), cut-on, set
J1955	CELL, HPLC flow
F-1000/1	TEMPERATURE BATH
F-3004	PELTIER DRIVE, sample heater/cooler
F-3005	INJECTOR, autotitration
F-3029	QUANTUM-YIELD accessory
F-3012	250 µL reduced volume cell
F-3011	Adapter for F-3012
F-3023	Cryostat
FM4-3000	Fiber optic adapter
FL-1010	HOLDERS (2) for filters
FL-1011	CELL-HOLDER, automated four-position thermostatted, with magnetic stirrer
FL4-1012	CELL-HOLDER, automated dual-position thermostatted, with magnetic stirrer
FL-1013	LIQUID-NITROGEN DEWAR assembly,
FL4-1015	PORT, injector
FL4-1027	CELL-HOLDER, single-position thermostatted, with magnetic stirrer
FM4-2000	POLARIZER, automated L-format
FM-2005	PHOSPHORIMETER upgrade
FM-2007	WINDOWS for the FluoroMax®-4 sample compartment
FM-2008	Filter holder, set of 2
FM4-2015	TCSPC upgrade
MicroMax 384	PLATE-READER, Microwell
J650518	50 µL cuvette
J650519	Adapter for 50 µL cuvette
F-3025	STOPPED-FLOW accessory
J400981	TRIGGER accessory, external