



www.fluorolog.com



Modular Design

HORIBA's Fluorolog-3 is manufactured with our own gratings, CCDs, InGaAs array detectors, and fast PMTs, offering you everything you need to build the perfect system. Our quality components are housed in solidly constructed compartments with all-reflective optics to provide the highest light throughput and sensitivity on the market. Below are four classic examples of Fluorolog configurations. DeltaTime can easily be added to any system to bring the fastest TCSPC collection to your laboratory.

Fluorolog 3-11

The basic configuration consists of single-grating monochromators in excitation and emission positions, and a red-sensitive photomultiplier. Add any accessory now, or expand your capabilities later.

The FL-3-11 provides outstanding sensitivity and performance for most experimental requirements.



Fluorolog 3-21

One of our most popular configurations, the Fluorolog 3-21 configuration is an L-Format fluorometer with a double excitation monochromator on the excitation channel, and a single emission monochromator on the right hand side of the sample compartment. This configuration offers excellent stray light rejection and versatility for most UV-Vis applications, and is ideal for highly scattering samples.

Fluorolog 3-221

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 for the same resolution you would get in a single-grating monochromator. Standard intermediate slits let you reduce stray-light even further. This system also adds a second emission monochromator, so-called T-format, for simultaneous dual emission detection.

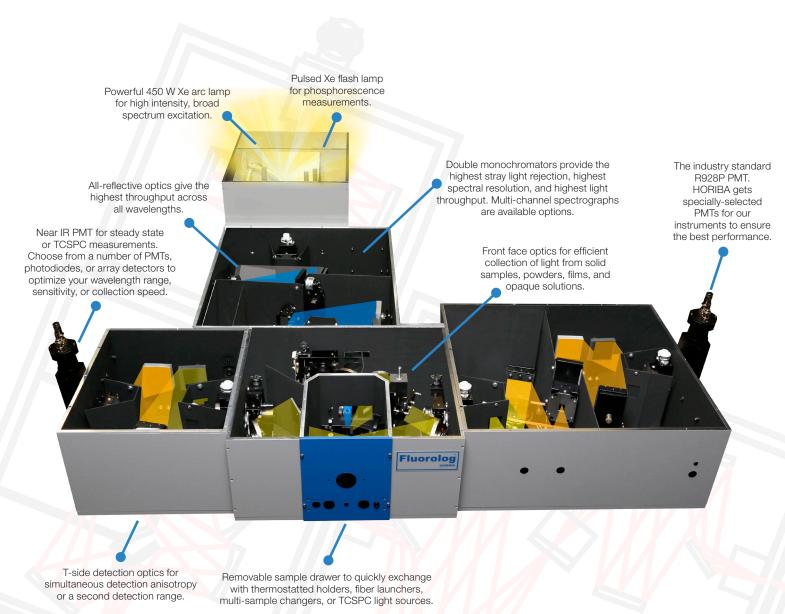
Nanolog®

Dedicated SWCNT and nanomaterials fluorometer.

Alternate between the best in scanning resolution and stray light rejection to the instantaneous acquisition and spatial resolution of an imaging spectrometer with a NIR InGaAs array and UV-Vis CCD. Our Nanolog is the prime example of this configuration, specially optimized for analyzing single-walled carbon nanotubes (SWCNT), quantum dots, and other nanomaterials.



A look under the hood



Considering Options

Single monochromator vs. double monochromator

A double monochromator is recommended for solid (powders, films, devices, etc.) and turbid (cell suspensions, colloids, aggregates, etc.) samples, where scattered light can significantly interfere with the signal.

"Stray light" is unintended light from the sample or optical path that reaches the detector. It contributes to the background signal, and in extreme cases overwhelms the desired signal. The best strategy for removing stray light is the use of a second monochromator in line. HORIBA uses a true double monochromator with an adjustable center slit for the best stray light rejection available.

L- vs. T-format

T-format is best for multiple detectors and real-time anisotropy usage.

The Fluorolog is modular and accessible, allowing the user to trade one detector for another. If your research will routinely switch between multiple detectors, a great deal of time will be saved with a dedicated second emission monochromator for your other detector. This is particularly important if you want to optimize one detection channel for Vis/UV, and the other for NIR.

In addition, if you plan to do many anisotropy measurements or anisotropy kinetics, T-format allows you to efficiently collect two polarization angles simultaneously. This decreases measurement time, and increases kinetics time resolution.

Array vs. PMT

Array detectors are faster and will give greater signal-tonoise (S/N) for a given collection time. PMTs are more sensitive for extremely weak samples, and necessary for any time-domain measurements. CCDs or InGaAs arrays collect a wide spectral range in a fraction of a second. If you have many samples to collect, want to collect excitation-emission matrices (EEMs), want to minimize photobleaching/photo-damage, or simply want to save time, you should consider an array detector. If, on the other hand, you expect to do phosphorescence or TCSPC measurements, a PMT is essential. The very weakest of samples will have a slight advantage with a PMT, and if this is the focus of your research, a PMT is your better choice.

PMT vs. Photodiodes

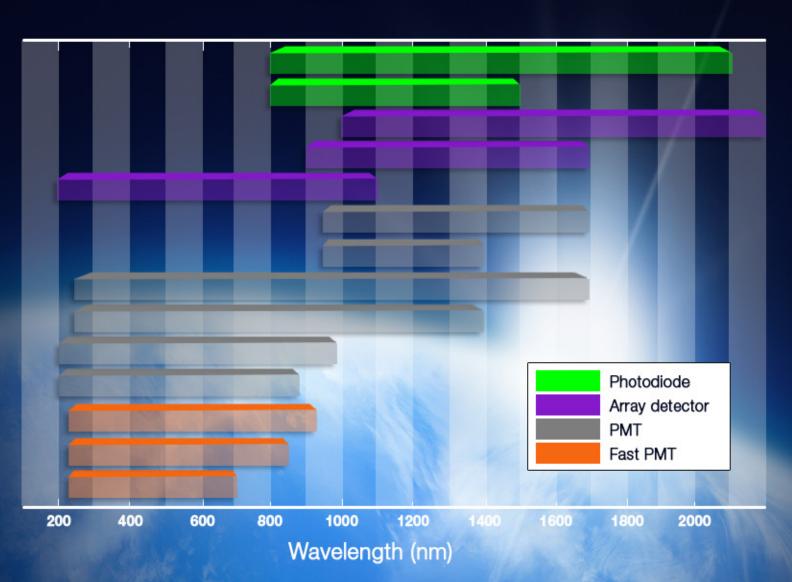
Photomultiplier tubes provide better sensitivity and better repeatability. Photodiodes can cover very large ranges well into the NIR, and can be more affordable.

We use our PMTs in digital, single-photon counting mode, which means each collected photon is registered as a single count. The noise from this kind of measurement is simple and easily predicted, making single-photon counting the high-mark of fluorescence measurement for many years. In the near IR, we offer NIR PMT detectors and analog photodiodes. If you need detection beyond 1700 nm, photodiodes are the only option, and we can support measurements extending beyond 20 µm.

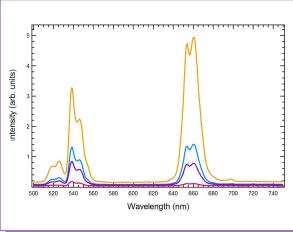
Steady State vs. Lifetime

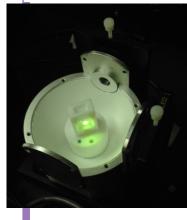
Frequently, the information of a steady state spectrum is sufficient, but the additional information of time-domain spectroscopy can clarify ambiguous data. Most fluorescence spectra are broad and often overlap each other, making quantification difficult. In biomolecules, there may be several Tryptophans fluorescing from different regions of a protein with very similar spectral properties. In these cases, it is helpful to look in the time domain, as fluorophores with similar spectral properties may have drastically different lifetimes. Furthermore, lifetime spectroscopy avoids photobleaching artifacts that confound steady state measurements.

Detector Options



Optimize for the Near IR





Upconverters excited with HORIBA's 980 laser, miniature integrating sphere

Upconverters can be excited with HORIBA's 980 nm laser, or your own custom source. Emission spectra (left) were collected from an integrating sphere accessory (right).

- Single channel photodiodes
- Options from 0.8-40 µm

HORIBA offers a number of photodiode detectors that cover the NIR-IR from 800 nm to 40 μ m, allowing you to use our industry-leading instrument on advanced semiconductors and nanomaterials.



Range

- Steady state or TCSPC PMT
- 950-1700 nm
- LN- or TE-cooling for ultra low dark counts

We use TE-cooled Hamamatsu® near IR PMTs to offer the sensitivity of single photon counting for weakly emitting samples, as well as the versatility of ultrafast lifetime measurements.



- 1024 channel InGaAs Array
- 800-1700 nm (optional 800-2200 nm)
- LN- or TE-cooling for ultra low dark counts

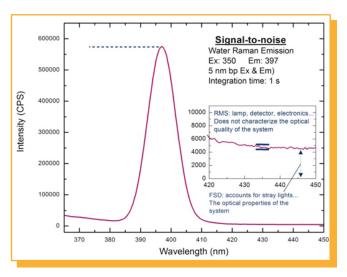
HORIBA makes its own TE- and LN-cooled CCDs and InGaAs array detectors. Two detectors can cover from 200–2300 nm, and collect complete spectra in a millisecond. High-throughput or excitation-emission matrix (EEM) measurements should utilize this technology.



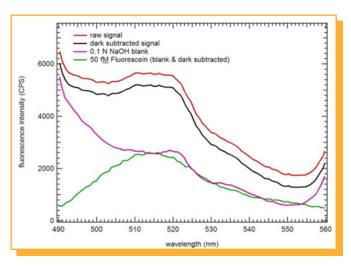
Maximizing signal, Minimizing noise

It can be confusing to try and compare the sensitivity of one instrument to another with the multiple specifications and differing definitions that exist.

Sensitivity of a spectrofluorometer is generally reported as a minimum measurable concentration of a fluorophore (commonly Fluorescein), or the signal-to-noise ratio (S/N) of the Raman scattering peak of water. Most companies opt for the second, as it is a more readily verified number, especially in the field. Unfortunately, this specification can be confusing since two calculation methods prevail in the industry: First Standard Deviation (FSD) and Root Mean Square (RMS).



The Raman band of ultra-pure water was measured in a standard 1 cm quartz cuvette. HORIBA qualifies its instruments with two measures of signal-to-noise: Root Mean Square (RMS) and First Standard Deviation (FSD) of light from its standard Xenon lamp source.



50 fM Fluorescein in 0.01 N NaOH. Subtracting the dark counts and the signal from the solvent alone reveals the clean Fluorescein spectrum at ultra-low concentration.

To avoid confusion, HORIBA Scientific quotes both specifications. And no matter how you look at it, the Fluorolog series has always been the most sensitive modular research fluorometers available. This is the result of increased signal due the optimized design of the all reflective optics, the photon counting signal collection method, and the reduction of stray light by the choice of gratings and baffling.

Measure smaller samples, detect smaller changes

Big discoveries come from small changes

Don't let your research get lost in the noise. Using the most sensitive fluorometer means having confidence in small changes in your data. These are the same small changes that drive curiosity and promote new discoveries.

Dilute and small volume samples

Higher sensitivity means you can also measure more dilute samples, or need less sample to begin with. Given the cost and effort often required for sample preparation, this means you save both time and money.

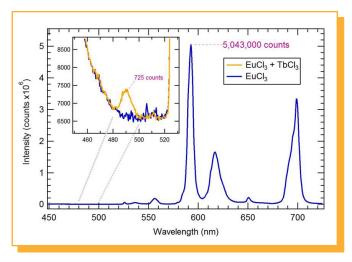
Sensitivity means speed

The real limit on how rapidly you can acquire data is not how fast you can scan the grating, but how long you have to measure at each wavelength to get an acceptable signal-to-noise. The higher sensitivity of the Fluorolog series means that not only can you measure weaker samples, but also that you can actually acquire good quality spectra faster.

Dynamic Range

Measure more than 6 orders of magnitude of signal levels in the same spectrum

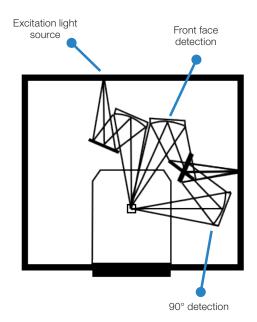
Fortunately not all samples are weak. In fact, some samples are quite strong. But some are both, which creates a challenge to measure the strong and weak peaks in the same scan. With an intra-spectral range of over 6 orders of magnitude, even wildly varying multi-component spectra are no problem to measure in one scan. Not only does this save time, but it is essential for kinetics studies when one cannot afford to repeat a measurement at different integration times to keep all peaks within range.

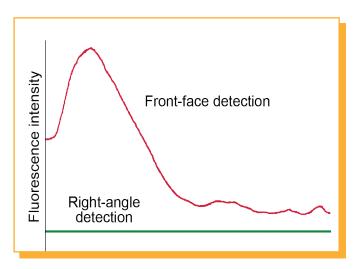


Spectrum of a mixture of Terbium Chloride and Europium Chloride in ultrapure water. The inset is a zoomed view of the spectrum showing the very weak Europium Chloride peak measured in the same scan as the extremely strong Terbium Chloride peaks recorded in the same measurement. The spectra of pure Terbium Chloride and pure Erbium Chloride are also shown in the zoomed in view for reference.

Optional Front Face Collection Optics

Solid and highly turbid liquid samples are more efficiently collected at an angle that is very close to the incident illumination beam, rather than at 90°. This unique detection scheme provides optimum fluorescence signal from samples that are very challenging for other instruments that do not have a front face detection capability.





The plot of Tryptophan in hemoglobin compares 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 $\beta 37$ Tryptophan in the $\alpha \beta$ region of hemoglobin can be easily obtained.

CCD and InGaAs Array Detectors

HORIBA can offer the speed and performance of high-end array detectors at an affordable price because we design and manufacture our own! These multi-channel detectors are ideal for high throughput, EEMs and microscopy mapping.

The Multiplex Advantage

In a side-by-side comparison, we took a 45 second measurement of Rhodamine B with both detectors under equal conditions. Even with 1/2 the wavelength resolution (PMT: 1 nm steps, CCD: 0.5 nm steps), the CCD shows 5x to 15x greater signal-to-noise. This is due to the better quantum efficiency of CCDs and the number of measurements that can be made with them in a short amount of time.

Signal-to-noise

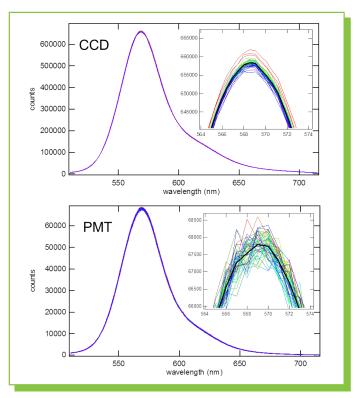
Simultaneous acquisition of the entire luminescence spectrum means more signal can be collected for a given period of time.

Low noise

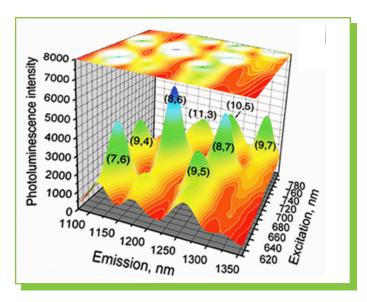
Our CCDs and InGaAs arrays are thermo-electrically or liquid nitrogen cooled, virtually eliminating their thermal noise. This helps the signal from your weakest samples stand out even more.

Speed of acquisition

Array detectors let you collect the entire spectrum in a fraction of a second. This facilitates possibilities of fast Excitation Emission Matrices (EEMs), full spectrum kinetics, or high throughput measurements of your samples.



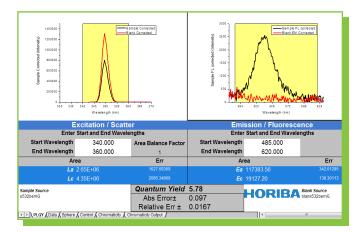
50 repeated, 45 s emission scans of Rhodamine B were subtracted from the averaged scan to measure deviations. Measurements were adjusted for sample photobleaching. In the time given, the CCD (top) shows 5 to 15 times the signal-to-noise of the PMT (bottom).



3D EEM of SWCNT acquired with an InGaAs array. Acquisition time for this experiment was <10 minutes. The same experiment can be performed with a single channel InGaAs detector, but the acquisition time would be up to 2 hours for comparable data quality.

Absolute Quantum Yield Measurements

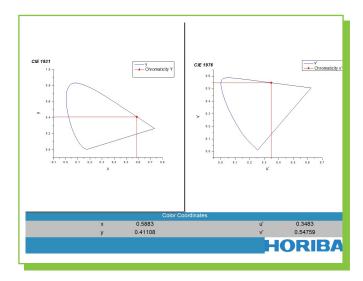
Measure absolute quantum yields (QY) with the only accessory optimized for liquids, powders, films, and solids. The Quanta- ϕ is a large 15 cm integrating sphere made of Spectralon® for the highest reflectivity over the broadest spectral range for the most accurate and reproducible QY values. The solid sample port can also accept wired sources for electroluminescence measurements. The ww- ϕ can be equipped with cuvette or solid sample holders.



High precision absolute quantum yield characterization of a quantum dot sample enabled by the Quanta- ϕ integrating sphere accessory.

Color Coordinate Calculator

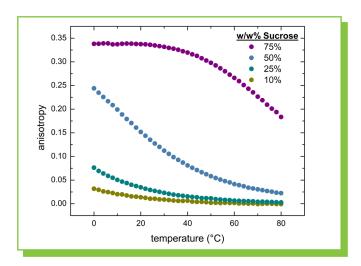
In many applications, such as phosphors for screen displays, multi-color LEDs, fluorescent additives to consumer products, etc., there is a need to quantify a visual perception of color. HORIBA provides a Color Coordinate Calculator based on two widely accepted standards introduced by the International Commission on Illumination, CIE 1931 and CIE 1976. The CIE 1931 uses x, y chromaticity coordinates where each x, y pair corresponds to a unique color within the colored shape. The CIE 1976 uses a system with more uniform perceptual chromaticity to define the color space using u, v coordinates. Upon highlighting a spectral trace and clicking on CIE 1931 and CIE 1976 Color Coordinates, HORIBA will display both CIE pairs.



Fluorolog-3 software also automatically generates CIE 1931 and 1976 values for your sample.

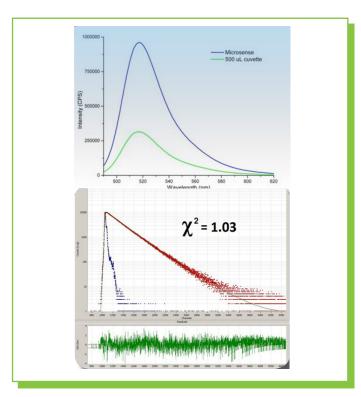
Polarization

Adding optional polarizers to a Fluorolog-3 allows anisotropy measurements and hence, the changes in molecular rotation in the sample. This indirect measure of the local viscosity gives information on sample aggregation, structural changes, molecular binding, and other mechanisms.



Fluorescein dissolved in four aqueous solutions of sucrose. With increased solution temperature, viscosity decreases, yielding faster rotation times and correspondingly lower anisotropies. Similarly, anisotropy is an excellent tool for understanding changes in macromolecule shape, as well as molecular binding.

Microvolumes



Using the Microsense, 5 μ L of AlexaFluor 488 labeled IgG is enough to get steady state or TCSPC data.

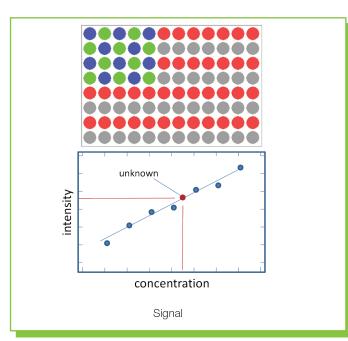
Measure ultra-low sample volumes with easy sample recovery

HORIBA's Microsense, the most advanced microliter accessory on the market, is designed to get cuvette-quality steady state or lifetime data from a 1-5 μ L of sample.

Avoiding dilution and nearly total recovery minimizes the need for sample, while preserving measurement sensitivity. Based on all quartz optics, Microsense is compatible with UV to NIR measurements.



Fluorescence Well Plate Accessory



Maximize your throughput

Search for "hits" or automate measurements of a large number of samples.

The MicroMax 384 accessory lets you automate your measurements for high-throughput data collection. Based on standard microtiter well plates, MicroMax saves you time and money by automating spectral, kinetics, single point, and time-resolved measurements on anywhere from 6 to 384 samples at a time. Fluorolog-3 software includes calibration curve routines for simple quantification of your results.

Full Spectral Microscopy and FLIM

All instruments in the Fluorolog family can be fiber-coupled to virtually any upright or inverted microscope. Our confocal microscope coupling allows you to go beyond simple filter-based fluorescence microscopy, to full spectral analysis of spatially varying or extremely small volume samples.

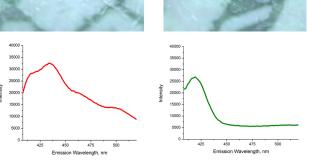
Couple a microscope to a Fluorolog-3 and:

- Measure a complete spectrum of a sample as small as 1 µm.
- Get spectra from as little as a few molecules of sample.
- Perform localized FRET measurements.

Add an automated stage and camera and:

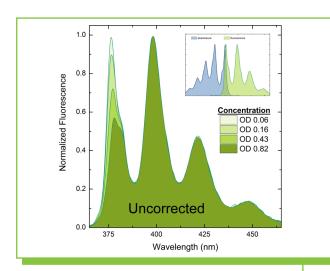
- Create complete spatial/spectral maps.
- Perform repetitive QC characterization of structured samples like photovoltaics.





Data from a real and forged US 5 dollar bill. Fluorescence imaging shows no obvious differences. However it is easy to spectrally distinguish the real from a fake hill

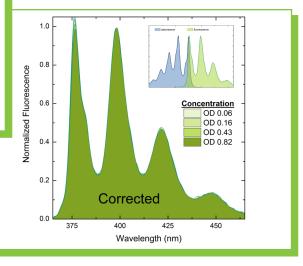
Absorbance/Transmittance Accessory



Reabsorption of fluorescence photons can occur even at moderate concentrations, and lead to increasing distortion of spectra with increasing concentration (left). The Fluorolog-3 software can correct this phenomenon, (known as the inner-fliter effect), using the sample absorbance measured by the absorbance accessory (right).

Add absorbance to your fluorescence measurement.

Correct your fluorescence spectra for innerfilter effects in concentrated samples without dilution.



Peltier-based Heated/Cooled Cuvette Holders

Rapid temperature control in single or multiple cuvette models with built-in magnetic stirring.

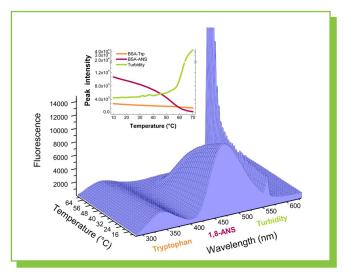
Precise temperature control for precise data on your protein folding, micellization, solubility, conformation, phase, and rotational transitions.

Rapidly vary sample temperature over a range of -25 to 105°C (-40 to 150°C optional). Fluorolog-3 software also simplifies automating temperature dependence measurements, including complex ramps and profiles.

Optional LN₂ and He Cryostats

For greater temperature range and sample type flexibility, the Fluorolog-3 supports various cryostats offering temperature control down to 4 K.





Thermal unfolding of Bovine Serum Albumin in PBS and 1,8-ANS followed using three observables: the quenching of intrinsic Tryptophan fluorescence by water, the quenching of the 1,8-ANS, and the increased 2nd order scattered light which reports turbidity from protein aggregation.

Temperature

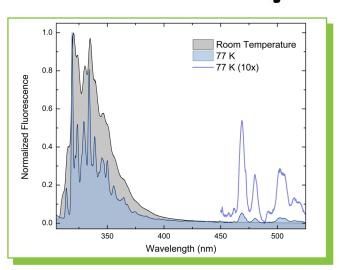
Bath

An alternative to Peltier-based units. Better precision (0.01°C), range (-25° to 100°C) and long term stability, but with much slower temperature ramping.



Economic LN
Temperature
Measurement
Dewar
Accessory

Cryogenic temperatures enable measurements of fine structure, enhanced phosphorescence, and rare conformations/states often not possible at room temperature. This low cost accessory readily permits measurements of samples at 77 K.



Temperature can add thermal broadening to fluorescence spectra and increase phosphorescence quenching. Spectra of Naphthalene dissolved in Methanol measured at room temperature (298 K) and in a liquid nitrogen dewar accessory (77 K). The low temperature spectrum reveals rich vibrational structure and longer wavelength phosphorescence. Phosphorescence peaks are also shown magnified 10x for clarity.

Temperature Contro

Other Accessories

The Fluorolog-3 series includes the most comprehensive line of accessories that enable researchers to extend the utility of their instrument to as many experiments as possible. The following is a partial list of accessories available, in addition to those previously discussed.

- Auto-titrator (injector) dual syringe, dual valve
- Stopped flow rapid kinetics accessory
- Solid sample holder designed for viewing front-face fluorescence of thin films, powders, pellets, paper, fibers, or microscopic slides. Variable alignment angle.
- 2-position sample holder with magnetic stirring bar
- 4-position sample holder with magnetic stirring bar
- 250 µl reduced volume cell
- 500 µl cuvette 5x5 mm
- 20 µl HPLC flowcell
- Fiber optic probe, bifurcated, randomized. Ideal for samples which cannot fit inside a standard sample chamber. Requires fiber adapter.
- Sealed water standard in scratch-proof housing for water Raman S/N verification
- Emission correction factor kit
- Excitation correction factor kit
- Purge port, quartz windows for sample compartment for use with nitrogen purging





Specifications: Fluorolog-3

Optics	All reflective optics for high sensitivity at all wavelengths, and for microsamples
Source	450 W CW Ozone-free Xenon arc lamp (250 to 2500 nm)
Monochromators	Czerny-Turner design with plane gratings for optimized focus at all wavelengths and minimum stray light
Excitation grating	1200 groove/mm blazed at 330 nm
Emission grating	1200 groove/mm blazed at 500 nm
Bandpass	0 to 30 nm (single mono, 1200 gr/mm grating), continuously adjustable 0 to 15 nm (double mono, 1200 gr/mm grating), continuously adjustable
Wavelength Accuracy	± 0.5 nm
Integration Time	1 ms to 160 s
Base detector	Photomultiplier R928P, spectral coverage 200 to 870 nm
Reference Detector	UV enhanced silicon photodiode
Water Raman S/N	> 30,000:1 RMS (> 15,000:1 FSD)
Dimensions (FL3-11)	77.8 cm (w) x 34.9 cm (h) x 102.2 cm (d)*
TCSPC Lifetime Options	
Lifetime range with standard detector	< 150 ps to 1 s
Lifetime range with optional PPD detector	< 25 ps to 1 s
Lifetime range with suitable laser and detector	< 5 ps to 1 s
Phosphorescence Option	< 10 µs to >10 s

Take your measurements to the next dimension: Time

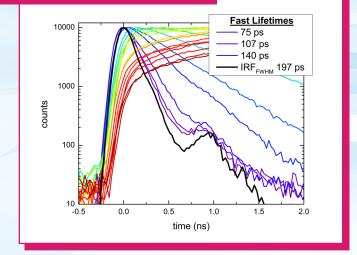
TCSPC

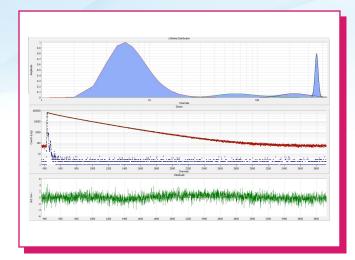
(Time Correlated Single Photon Counting)

- 40 years of experience in TCSPC innovation
- Industry-leading true 100 MHz system operation allows for millisecond acquisition time
- Robust data, independent of concentration and photobleaching.
- Lifetimes from 5 ps to seconds.
- Unique SpectraLEDs for highest efficiency phosphorescence measurements.
- TCSPC lifetimes, anisotropy, TRES, and kinetics.

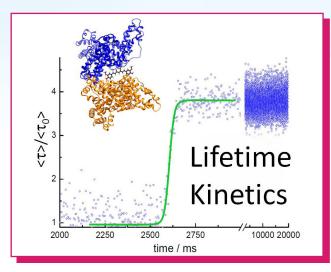
Lifetimes of Rhodamine 6 G in Methanol measured using optional ultrafast PPD detector. At high concentrations, self-quenching results from homodimers and trimers formation. Lifetimes as short as 75 ps are seen, as well as homo-FRET at lower concentrations.

Add lifetime measurement to any Fluorolog with the DeltaTime™ TCSPC accessory. Working in the time domain removes the confounding influences of concentration and photobleaching. DeltaTime has the same footprint as a mouse pad, but is powerful enough to deliver 12 decades of lifetimes. With its industry-leading true 100 MHz system operation, DeltaTime offers TCSPC acquisition rates, with all decays being acquired in mere milliseconds, allowing for TCSPC lifetime kinetics of fast reactions. Its crystal-locked timing circuits never require recalibration. Select from our current catalog of over 70 compact pulsed light sources, with more being added all the time. And once you get your data, powerful DAS6 analysis software lets you choose among 9 fitting models.





Non-extensive distribution fits: 1,8-ANS exists in free solution and partitions into several disparate environments in Bovine Serum Albumin. Within each environment, a distribution of states exists with a corresponding distribution of lifetimes. Fluorolog-3 software not only offers standard discrete exponential fittin, but also several energy transfer and distribution models, including the proprietary non-extensive distribution shown here.



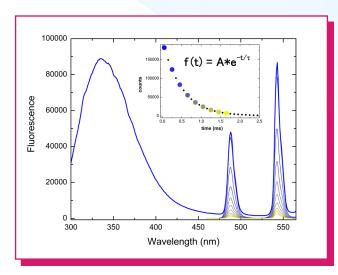
Sample photobleaching corrupts kinetic data by adding an exponential term. Lifetimes are more robust than steady-state intensities. To support rapid kinetics, the Fluorolog-3 is capable of measuring a complete lifetime decay in as little as every 10 ms. Example shown is the binding of Curcumin to Serum Albumin.



Phosphorescence

Measure phosphorescence spectra, lifetimes from microseconds to seconds.

The Fluorolog-3 can be upgraded with an optional pulsed Xenon flash lamp, enabling lifetime measurements down to 10 µs with no additional electronics or detectors. Ideal for measuring Lanthanide tagged samples or rare earth phosphors used in lighting applications.



Complex solutions like this mixture of Bovine Serum Albumin (BSA) and Terbium Chloride (Tb³+) can be challenging to interpret. Using the pulsed light source of the phosphorescence option allows you to temporally "gate out" the BSA fluorescence, leaving only the Tb³+ phosphorescence. Inset shows the single wavelength phosphorescence decay of Tb³+ in this mixture; colored circles correspond to the gated spectra in the main figure.

FluorEssence™ Software

Simple enough for the occasional user; Powerful enough for the most elaborate experiments.

Fluorescence software that works like you do

- Efficiently develop your experimental method and then save it for future use.
- Data collection, analysis and report generation are easily streamlined.
- Full software control of accessories.
- Automate repetitive experiments with a built-in batch mode.
- All instrument calibration parameters are automatically applied per method.
- Of course, all experimental parameters are always saved, along with the data file for comparison with previously collected data.

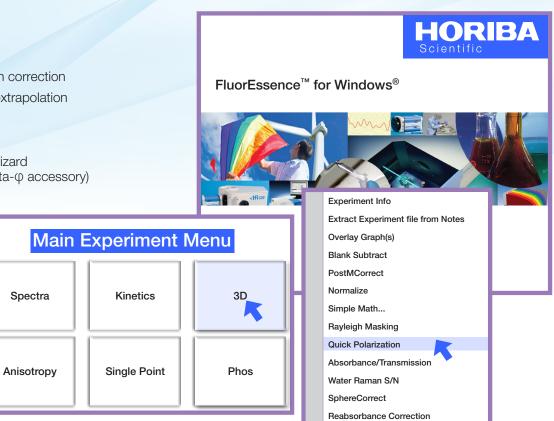
Convert data to answers

Powerful processing and data-management tools of OriginPro™ include a complete suite of data reduction tools.

A complete library of video tutorials to get you started

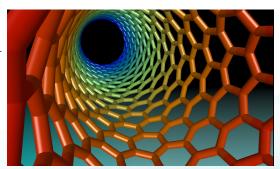
Features

- · Data views in workbook formats, keeping graphs, tables and notes together for each experiment
- Integrate, differentiate, or fit fluorescence data to Gaussian, Lorentzian, and custom curves
- Zooming and scaling
- · Contour maps and profiles from 3D plots
- Peak finding
- Standard arithmetic
- 3D perspective
- Smoothing
- Deconvolution
- · Excitation/emission correction
- Interpolation and extrapolation
- Blank subtraction
- Normalization
- PLQY calculator wizard (for use with Quanta-φ accessory)



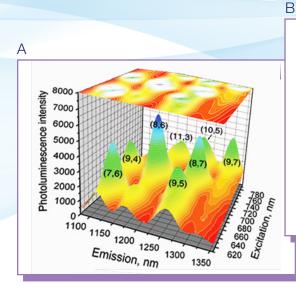
Nanosizer® Software

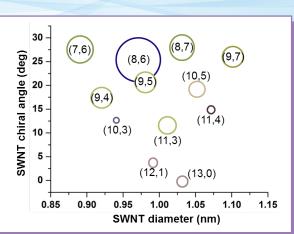
Single-walled carbon nanotubes (SWCNTs) come in a large variety of structures and diameters. With a sample of these nanotubes, the Nanolog configurations can run an excitation-emission matrix scan. With the finished scan, you transfer these parameters to our exclusive Nanosizer software, which automatically determines the chirality and diameter from the spectral landscape. Below is an actual scan and complete analysis run on a NanoLog:



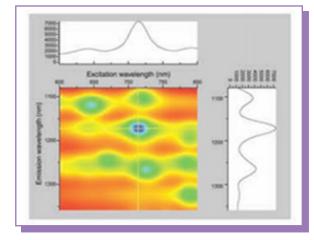
Benefits

- 3-D spectral surface simulation
- Simultaneous analytical simulation of spectral surfaces
- Rapid preliminary scanning to recognize peaks and their shapes for easy model-fitting
- Complete, easy-to-edit model-parameter table for nanotube mixtures
- Nanotube species-recognition with editable library
- Nanotube species-recognition with user's analytical simulations
- Complete reports and charts in common spreadsheet format
- Optional "enhanced" fitting-engines for statistically robust simulations

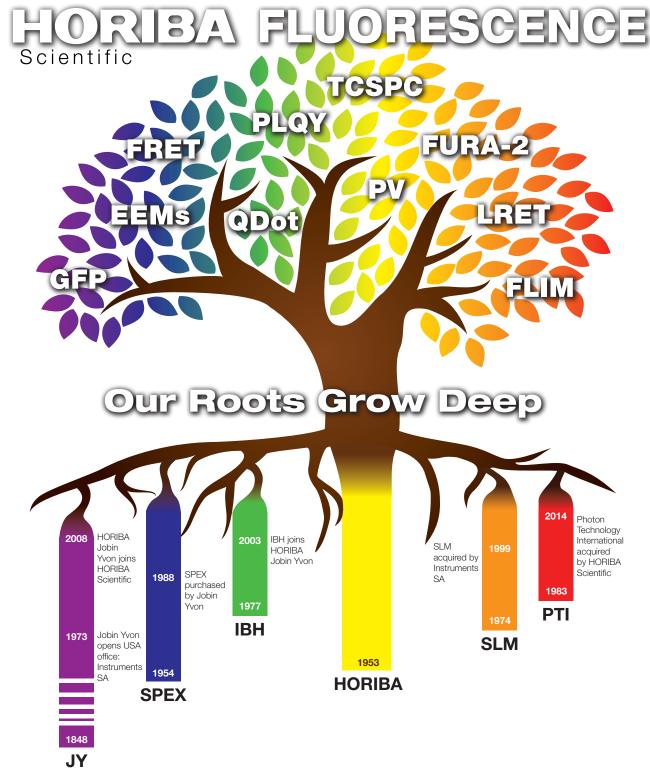




Emission-excitation scan of a mixture of single-walled carbon nanotubes. Plot A is the spectral landscape recorded by the Nanolog; plot B is the structural assignment determined by the Nanosizer software. Diameters and colors of circles in plot B are related to peak-intensities in plot A.



Screen-capture from FluorEssence data acquisition and analysis software showing selected excitation and emission spectra centered on the intense peak emitted from the (8,6) species of carbon nanotube determined in Plots A and B.



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