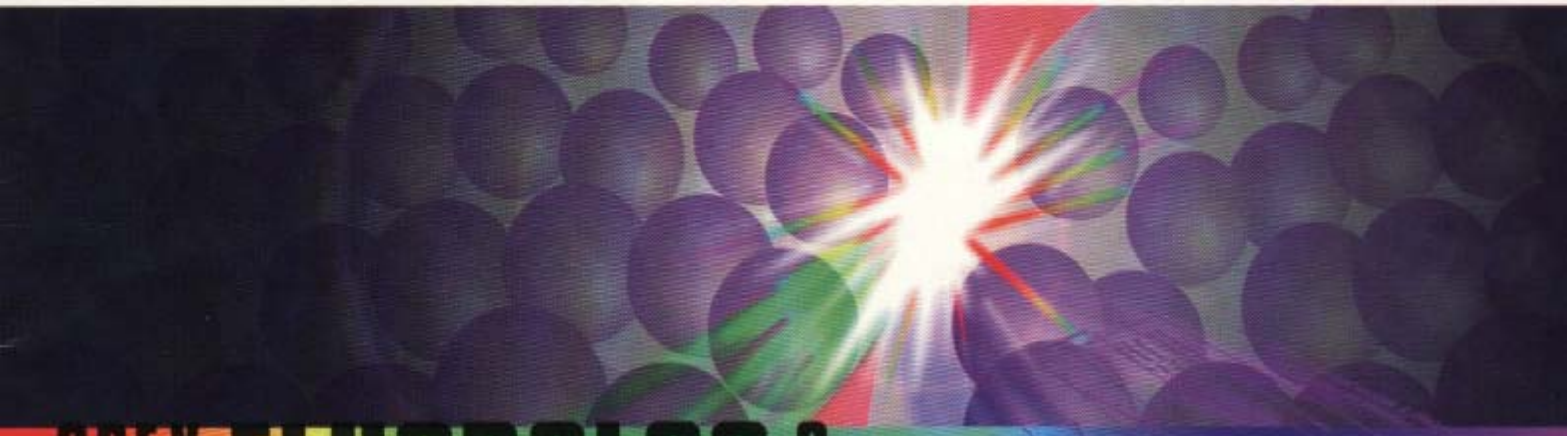
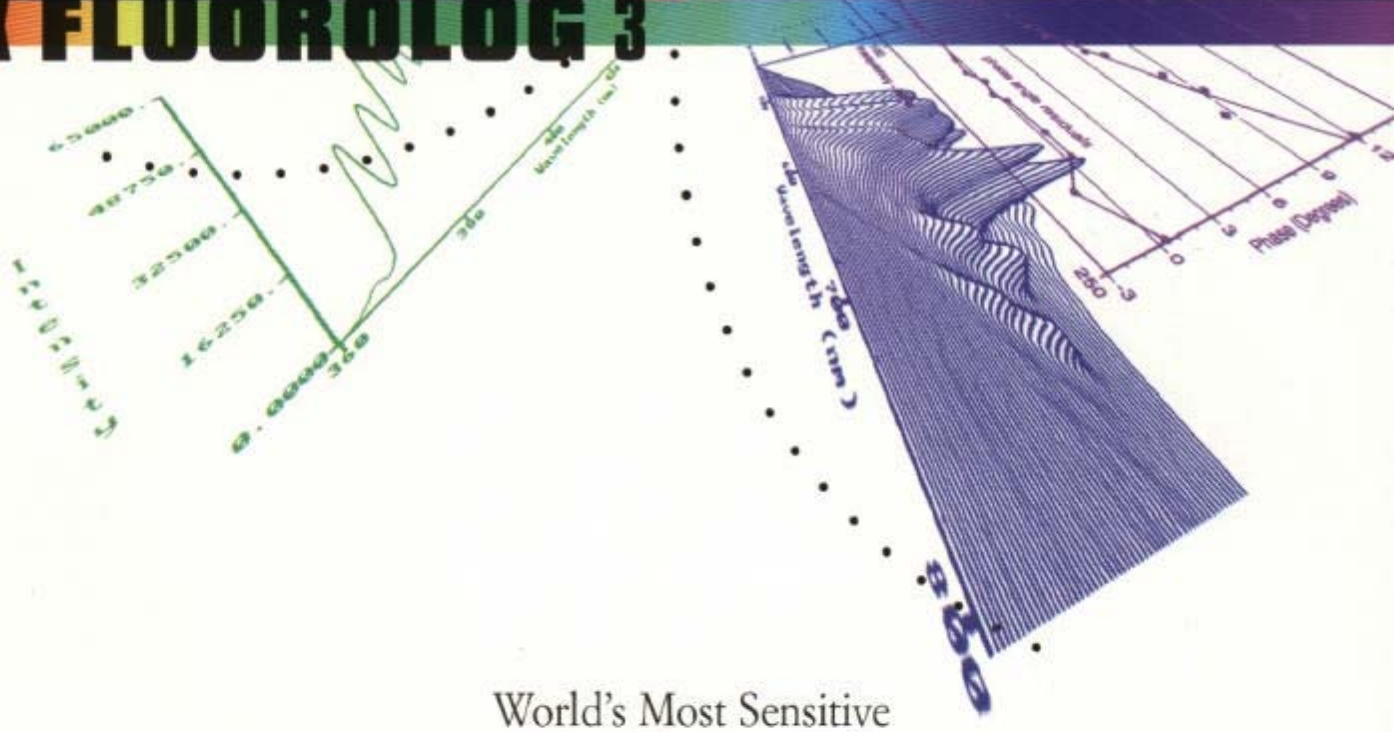
How to Build a Spectrofluorometer



SPEX FLUOROLOG 3



World's Most Sensitive
Modular Spectrofluorometer
Dynamic or Steady State

ISA JOBIN YVON · SPEX
HORIBA Group

How Fluorolog-3 Adapts to YOUR Sample

Fluorolog-3 is the latest 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're working in steady state or molecular dynamics, your selections will deliver the perfect balance of these crucial benefits:

SENSITIVITY

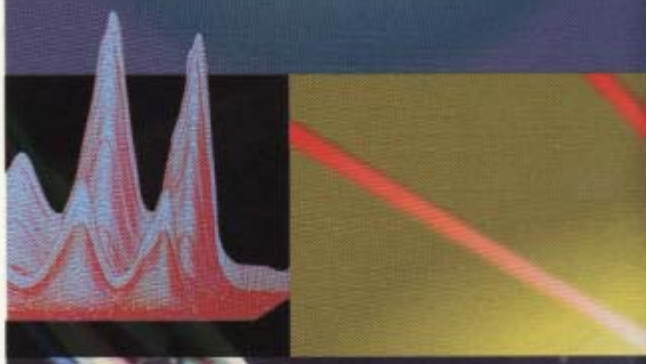
SPEED

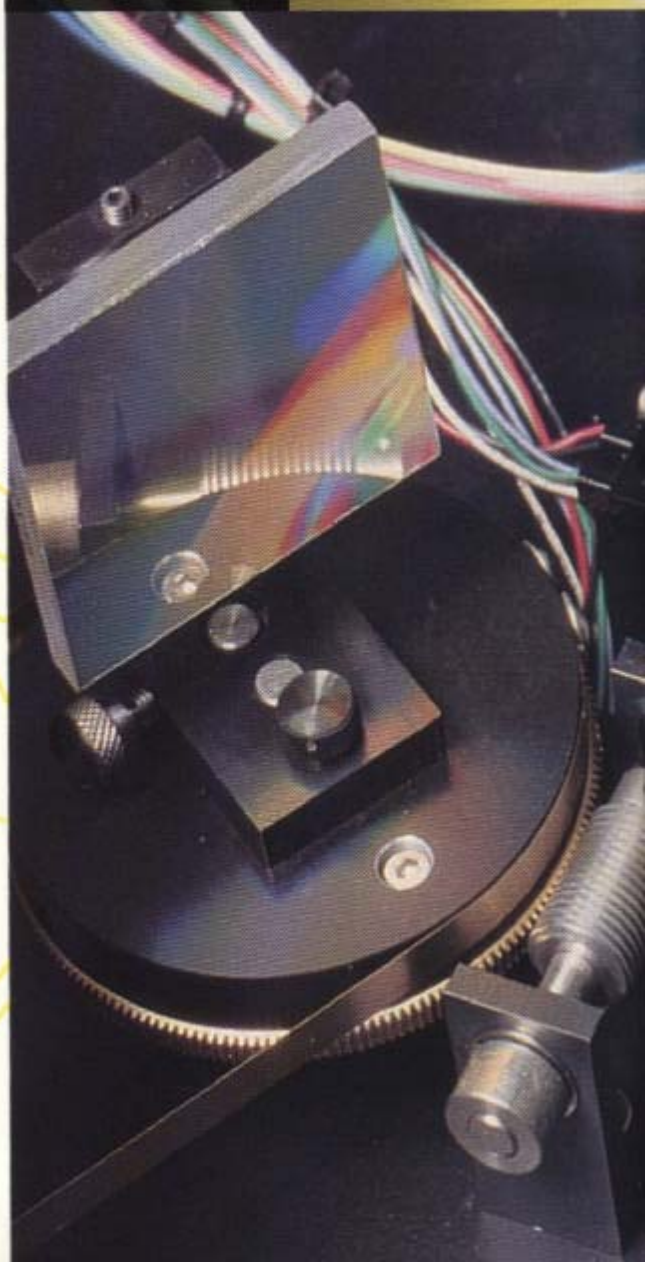
MODULARITY

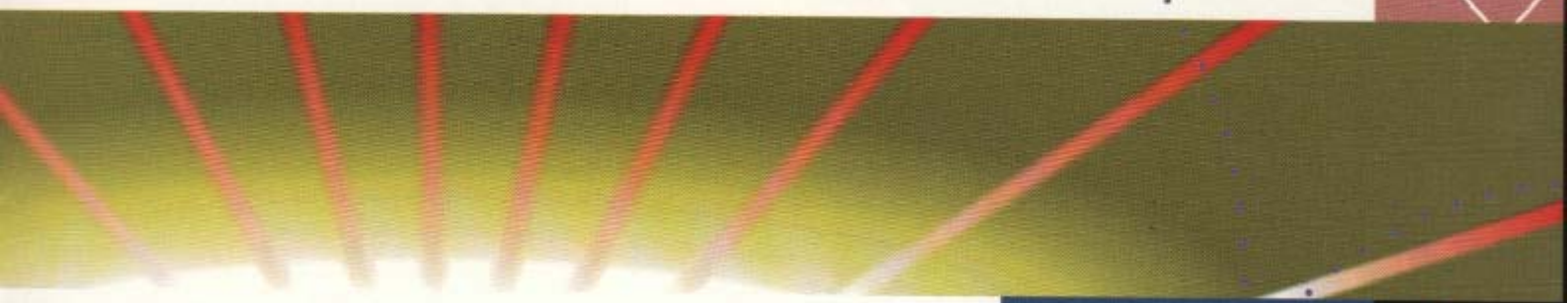
AUTOMATION

VERSATILITY

REAL WORLD PERFORMANCE







Sensitivity

A Fluorolog-3 delivers the ultimate in sensitivity. This not only means that you 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 by time, photobleaching, or other means that can invalidate your data. Fluorolog-3 is the fastest scanning modular instrument made.

Modularity

No one system can provide the answers to all problems. That's why Fluorolog-3 is modular. Choose a source, spectrometer, sample compartment, detector and accessories that match the wavelength range, time domain, or physical characteristics and parameters of your sample, such as temperature, solid, liquids, even remote sensing through fibers. And when you need to probe the mechanisms of molecular dynamics, the Tau-3 Frequency Domain Lifetime Upgrade delivers pico-second 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 in slit and wavelength settings from memory. Automated sampling accessories include polarizers, sample changers, plate readers, grating turrets and more.

Versatility

Fluorolog-3 has an accessory for virtually any sample—and if we don't have what you need, we can always make a special device for you

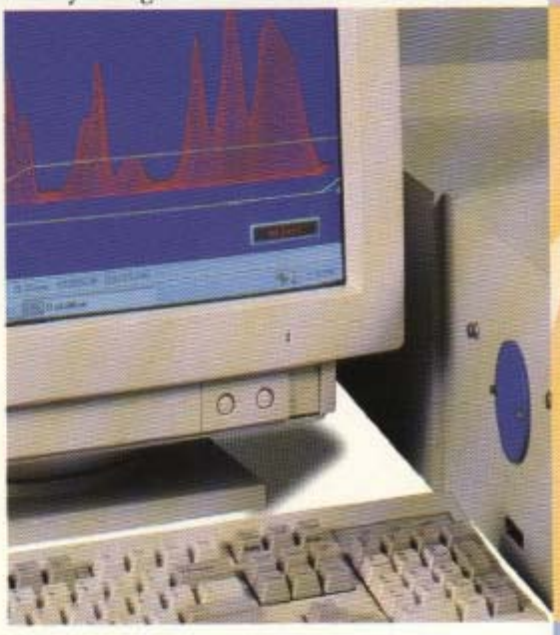
Real World Performance

IR probes to energy transfer, dynamic polarization to CCD detection—it's all in the Fluorolog-3 spectrofluorometer.

Sensitivity

How We Achieve the Best Sensitivity:

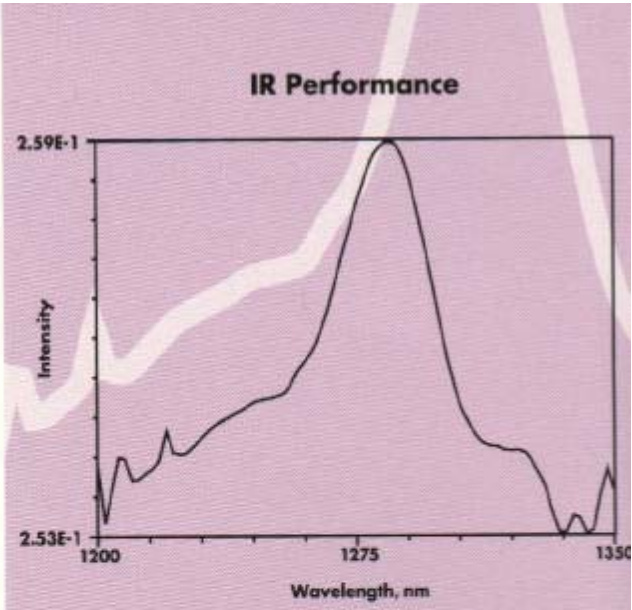
1. Our excitation lamps are mounted vertically to image the arc on the slit for more throughput and longer life as a bonus.
2. All-reflective optics keep the light in focus at all wavelengths, unlike lenses.
3. Plane, kinematic gratings also remain focused at all wavelengths and are easily changed to maximize any wavelength range. Ruled gratings eliminate the polarization anomalies of holographic gratings.
4. Photon-counting detection strips noise from weak signals.
5. Windows-based software supplies data-extraction routines.



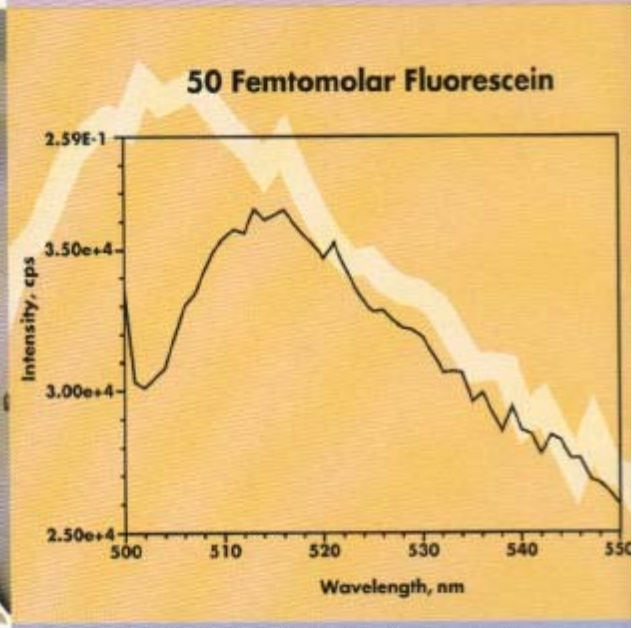
What Sensitivity Means to Your Data:

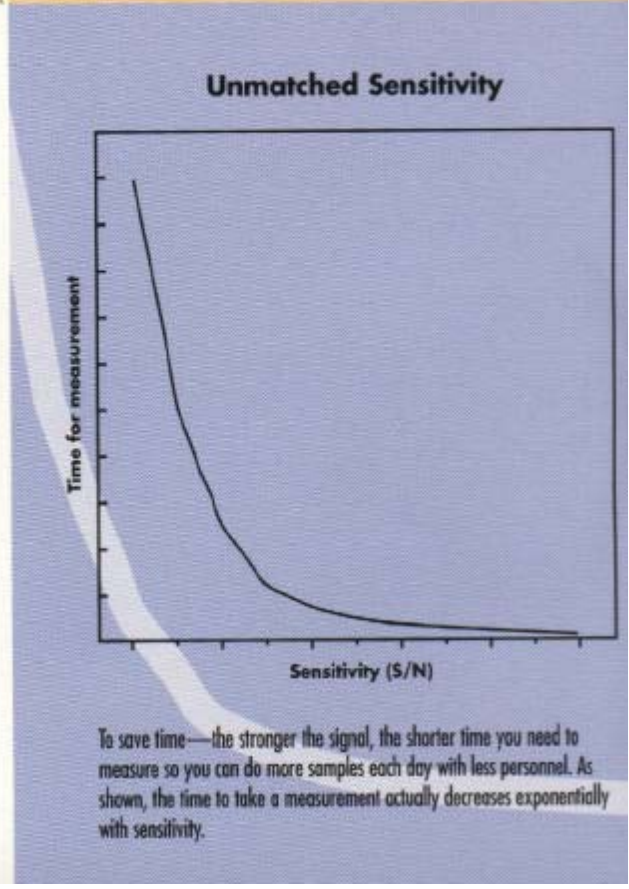
1. You can analyze samples at lower concentrations, obtaining data unavailable with other instruments.
2. Save time—the stronger the signal, the more samples you can measure in a given time.
3. More accurate data. The stronger the signal, the better the statistics, the lower the noise, the better the accuracy.

Save Time



Luminescence spectrum of singlet oxygen emission taken with a Ge detector. The sample was Rose Bengal. FL3 can change gratings and detectors to meet any sample conditions.



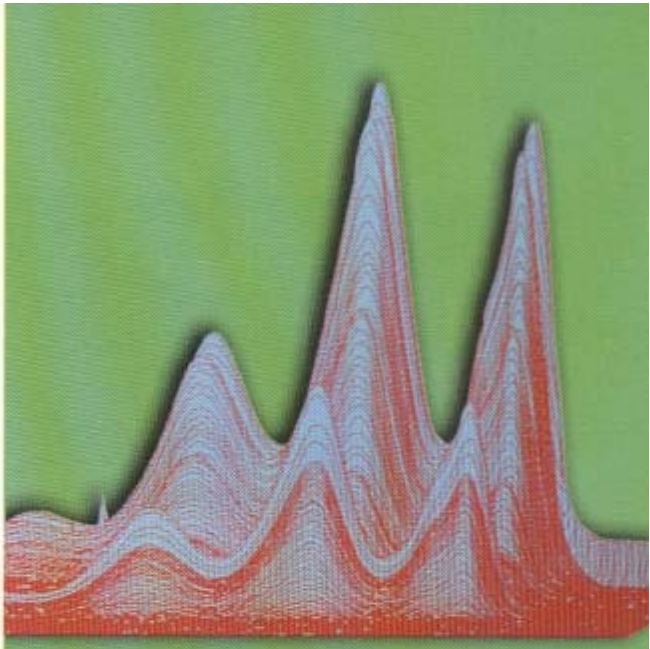


To save time—the stronger the signal, the shorter time you need to measure so you can do more samples each day with less personnel. As shown, the time to take a measurement actually decreases exponentially with sensitivity.

Speed

Matrix Scanning

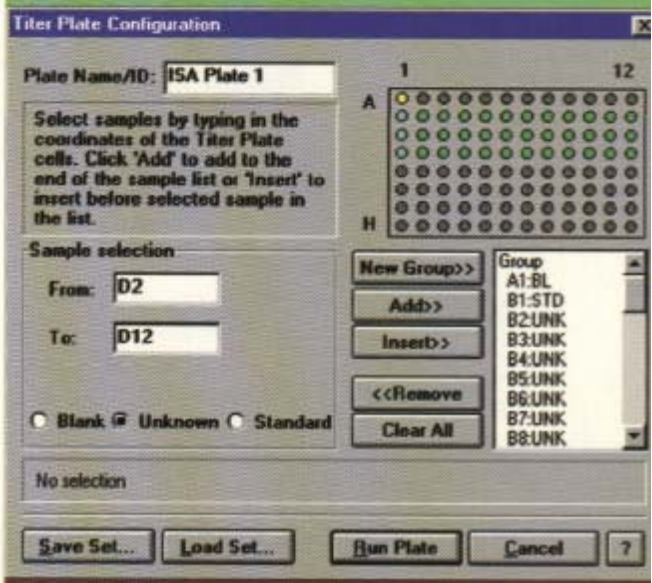
Not only does Fluorolog-3 software include routines for automatic scanning of emission spectra for a defined set of excitation spectra, to produce an excitation/emission matrix that fully characterizes the samples fluorescence, the spectrometer unique design supplies fast scanning (150 nm/sec), that makes these scans practical. Your samples can be totally characterized in a matter of minutes, as shown by the matrix at right.



Anthracene excitation/emission matrix fully characterizes the samples.

Multiwavelength Data

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

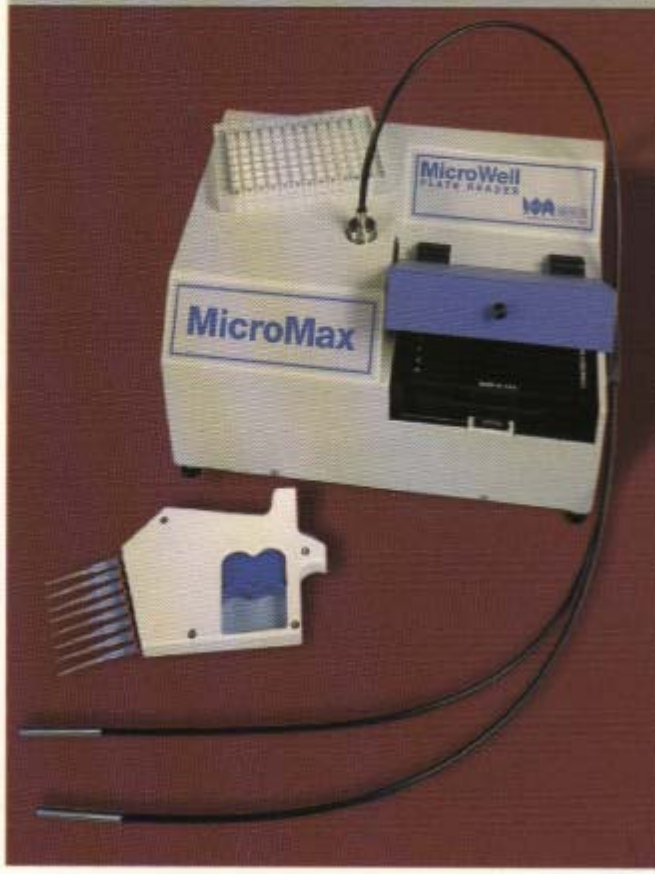


Tau-3 Picosecond Lifetime Unit

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 Tau-3 Lifetime System will turn your Fluorolog-3 into a picosecond time machine. And you can upgrade any Fluorolog already in use, should your demands change in the future.

Microwell Plate Reader

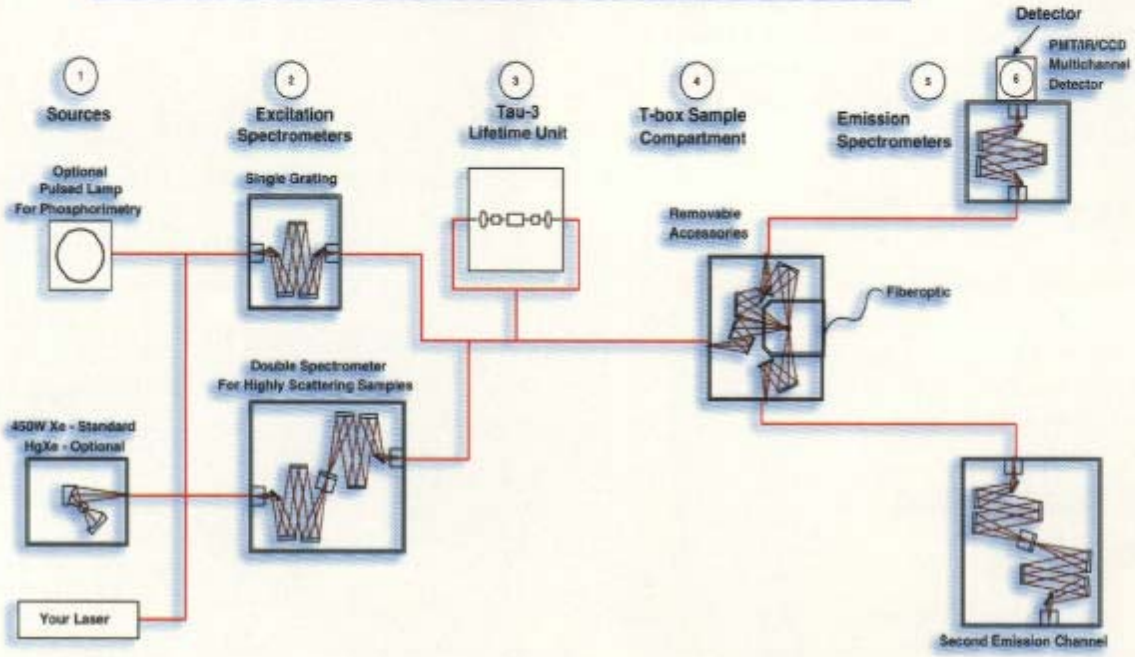
When you have a large number of samples to run, the plate reader is ideal. Coupled to the instrument through angled optical fibers, fluorescence data is quickly acquired at a speed of about 100 samples per minute. Routines are also included for auto background subtraction, standard calibration curves,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



SAVE TIME AND IMPROVE YOUR DATA

Modularity

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



MODULARITY MEANS YOU DON'T COMPROMISE YOUR DATA

① Sources

450W xenon cw lamp is standard. Options include a Hg/Xe for more uv intensity, pulsed Xe for phosphorimetry, or put your laser on any of our convenient laser ports.

② Excitation Spectrometer

Choose a single grating unit with kinematic gratings to customize your wavelength range (a three grating turret is available if you need to automatically switch between several ranges) 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.

③ Tau-2 Lifetime System

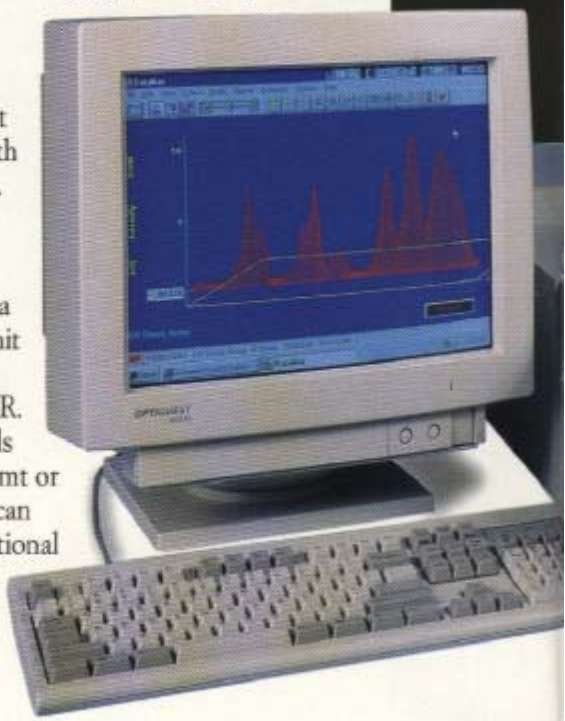
Add picosecond lifetime capability, now or later, with our frequency domain lifetime unit. Switch between cw or lifetime in a matter of seconds.

④ T-Sample Compartment

All reflective optics in the sample compartment mean the sample is always in focus, no matter what the size, and 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 page 9.

⑤ Emission Spectrometer

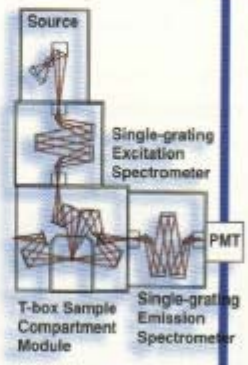
You have the same choices as with excitation with the additional option of an imaging spectrograph that lets you mount a CCD detector for instant spectra. The spectrograph can also mount a second detector with automated switching.



Detectors

⑥ The standard pmt is a room temperature unit that covers the range from the uv to near IR. A TE-cooled unit aids sensitivity, or other pmt or solid state detectors can be mounted for additional wavelengths in the IR region.

Recommended Modular Configurations

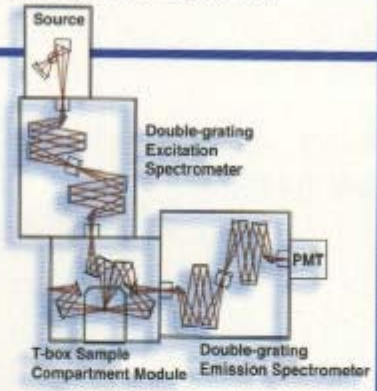


FL3-11

The basic Fluorolog-3 configuration is formed from single-grating spectrometers in excitation and emission positions, a T-sample compartment, and a red-sensitive photomultiplier. Add any accessory now, or expand your capabilities later. The FL3-11 provides outstanding sensitivity and performance at the lowest price.

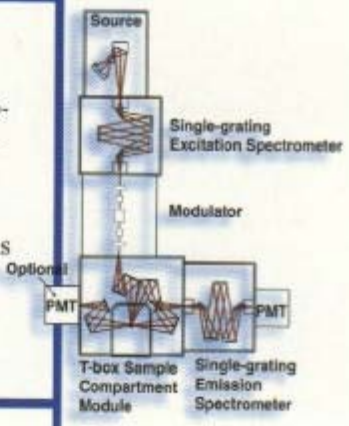
FL3-22

The ultimate in stray-light rejection, the double grating spectrometers 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 resolution as well as sensitivity. Optional automated center slits let you push the stray-light envelope even further back.



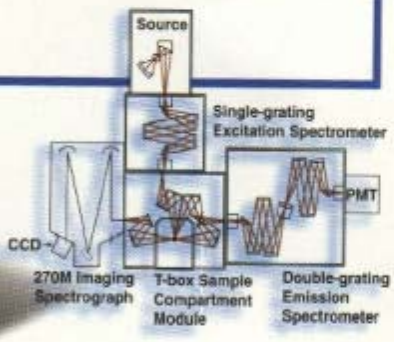
FL3-11-Tau3

Switch from steady-state measurement to picosecond lifetimes with the optional Tau-3 automated system as easily as clicking on a mouse —without any realignment. Tau-3 is the most sophisticated system for molecular dynamics as you probe the microworld of energy transfer, dynamic depolarization, or an endless list of other time-dependent applications.



FL3-12-270M-CCD

Alternate between the best in scanning resolution and stray-light rejection to the instantaneous acquisition and spatial resolution of an imaging spectrometer with a Charge Coupled Device (CCD).



AND MORE!

Multiple, automated ports on the 270M spectrograph, IR Detectors, grating turrets—ask any Spex Fluorolog-3 applications engineer today to help you assemble your most versatile spectrofluorometer.

FL3-11 waiting to run your samples.

Automation
Means it's Easy to Use!

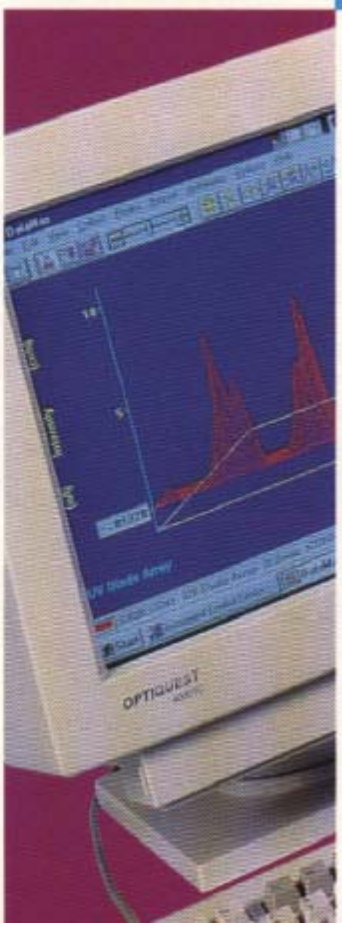
Hardware

Fluorolog-3 is self calibrating, which means you begin taking data once the unit is turned on. Wavelength scanning, slit settings (for bandpass control or resolution) are all automatic, as are sample changers, temperature control, plate readers, polarizers, and more. You get to concentrate on your samples and data and not have to worry about twisting knobs, sliding slits, or other forgettable, neglectable items—and remember, because the settings are electronic, they're much more reproducible.

Software

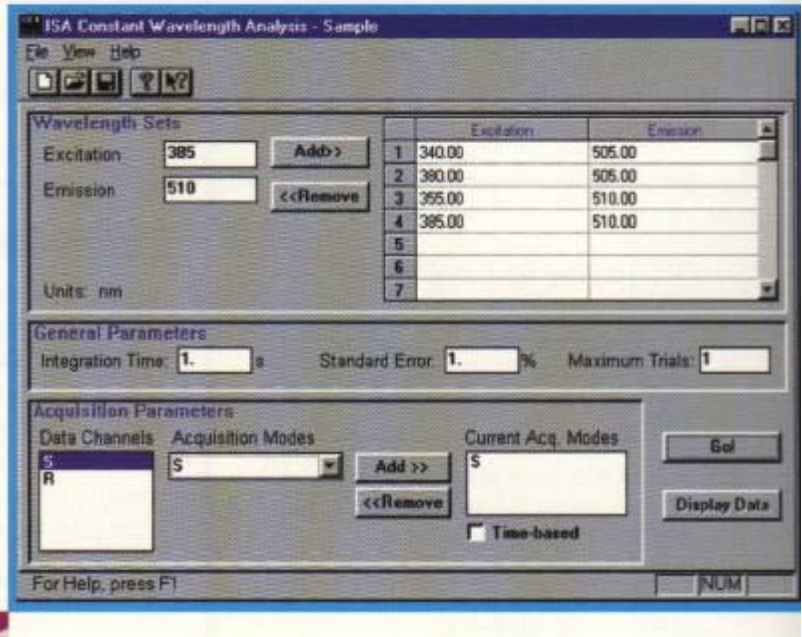
For advanced operations in a Windows™ operating environment, DataMax-STD software has expanded features that revolutionize the way you operate your spectrofluorometer. All it takes is a glance at the familiar button tool bars and context-sensitive help menus, then you're instantly taking data. DataMax produces a comfortable environment that never forgets 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. You can even set up different prompt sophistication to suit your level of experience; Novice, Intermediate, or Expert Levels are available.

- Simplified pull-down menus for all operations and accessories
- Detector Algebra to customize the way you acquire data
- Matrix scanning to produce three-dimensional and total luminescence data
- Optional developmental tools like Array Basic™ and Visual Basic™ encourage customized operations
- Real-time display lets you optimize parameters
- AND the full power of GRAMS/386™ is part of every DataMax package.



Some of the Data display and processing routines include:

- Zooming and scaling
- Contour Maps
- Integrate
- Curve Fit
- Excitation/Emission Correction
- Standard Arithmetic
- 3D Perspective
- Smoothing
- Deconvolve
- Derivative
- Single-point Analysis in Spreadsheet format



SPEX Automated Temperature Control Accessories

The fluorescence emission of a sample is influenced by temperature. Intensity will be reduced as temperature is raised. A series of measurements of intensity as a function of the temperature allows the calculation of various sample parameters, such as an Arrhenius plot, from which the activation energy of a process may be determined. For protein studies, information about the thermal stability of a protein may be obtained, by performing a series of scans as a function of temperature.

Automated temperature control may be easily added to any of the SPEX fluorescence instruments, including the Fluorolog-3 Tau-3 Lifetime system, operating under DataMax-STD software. The complete accessory is available as part number TEMP1, which consists of a microprocessor controlled circulator, a remote temperature sensing probe, an interface card and all necessary cables. This accessory adds a new dimension to all experiments supported by DataMax, in a completely automatic fashion.

Versatility

Fluorolog-3 Accessories

Fiber Optic Platform
1950, 1950-1M

This accessory is used for remote sensing in the region between 250nm and 850nm for samples that cannot be positioned in the sample chamber.

Liquid Nitrogen Dewar Assembly
FL-1013

For phosphorescence or delayed fluorescence measurements, samples are often frozen at liquid nitrogen temperatures to preserve the fragile triplet state. A Dewar is used to freeze and maintain the temperature of the sample. The Dewar is placed on a pedestal within the sampling module of the spectrofluorometer, and the sample is placed in a quartz cell and slowly immersed in the liquid nitrogen-filled Dewar.

Automated Polarization Accessories

The Model FL-1018 automated L-format Polarization Accessory permits complete control of your polarization experiments from the computer keyboard and lets you automate all the necessary polarizer rotation for the measurement of VV, VH, HH, and HV components. An optional T-format configuration is also available.

Automated Four-Position Thermostatted Cell Holder
FL-1011

The Model Automated Four-Position Thermostatted Cell Holder keeps a sample at a constant temperature from -10°C to 80°C.

The temperature of the sample is maintained by a liquid mixture pumped through from an external circulating temperature bath (not included).

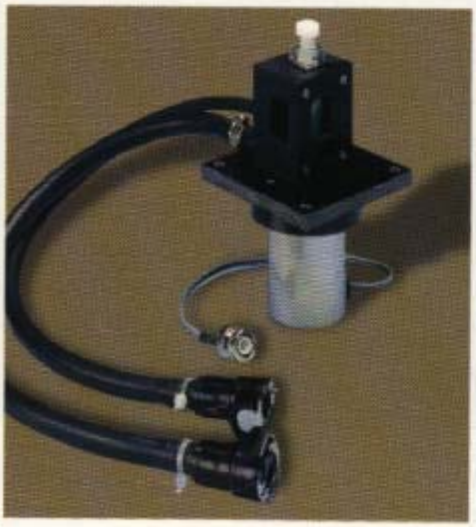
The holder also includes a magnetic stirrer to mix a turbid or a viscous sample while it is positioned in the light beam.

Solid Sample Holder
1933

The Model 1933 Solid Sample Holder is designed for samples such as thin films, powders, pellets, microscope slides, and fibers. The holder consists of a base upon which a bracket, spring clip, and sample block rest.

Single-Position Thermostatted Cell Holder with Magnetic Stirrer
1931B

The Single-Position Thermostatted Cell Holder keeps a sample at a constant temperature from −15°C to 100°C. The temperature is maintained by an ethylene glycol-water mixture pumped through from an external circulating temperature bath (not included). The holder also includes a magnetic stirrer to mix turbid or viscous samples.

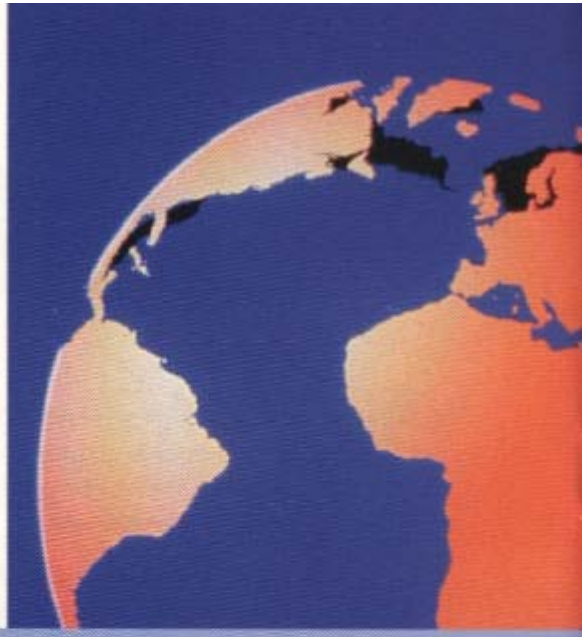


YOU HAVE THE SAMPLE– FL3 HAS THE ACCESSORY

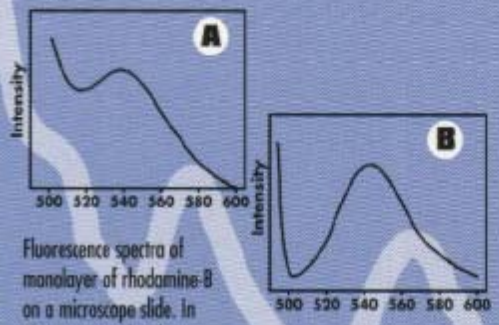
Model	Item
FL-1014	SHUTTER, Emission
1920	CUVETTE, 4ml, Quartz Capped
1923	CELL, MICRO, 50µl, Cylindrical, Quartz (Requires Model 1923A Adapter)
1923A	ADAPTER for 50µl Micro Cell
1924	CELL, MICRO, 250µl, Cylindrical, Quartz (Requires Model 1924A Adapter)
1925	CUVETTE, 4ml, Quartz, Stoppered
1933	HOLDER, Solid Sample
1939	FILTER, SET, CUT-ON
1950-1M	FIBER OPTIC BUNDLE, 1 Meter, Bifurcated, Randomized (For Use with Model 1950)
1950-2M	FIBER OPTIC BUNDLE, 2 Meters, Bifurcated, Randomized (For Use with Model 1950)
1950-5M	FIBER OPTIC BUNDLE, 5 Meters, Bifurcated, Randomized (For Use with Model 1950)
1950	FIBER OPTIC PLATFORM (Requires Fiber Optic Bundle)

Model	Item
1955	CELL, HPLC Flow, 20µl
1931B	HOLDER, Thermostatted Single-Cell with Magnetic Stirrer
FL-1020	POLARIZATION ACCESSORY, Manual, L-format
FL-1015	INJECTION PORT
FL-1013	DEWAR, Liquid Nitrogen Assembly
FL-1018	POLARIZATION ACCESSORY, Automated, L-format
1930U	AUTOMATED if purchased with 1937A
FL-1012	HOLDER, Two-Position, Automated. Thermostatted with Magnetic Stirrer
FL-1011	HOLDER, Four-Position, Automated. Thermostatted with Magnetic Stirrer
CM-MH	HOLDER, Coverslip
QC-SX	CELL, REDUCED-VOLUME, 1 ml, 5mmx5mm. (Includes Adapter and Magnetic Stirrer)
TRIG-15/25	ACCESSORY, TRIGGER.

Real World Performance

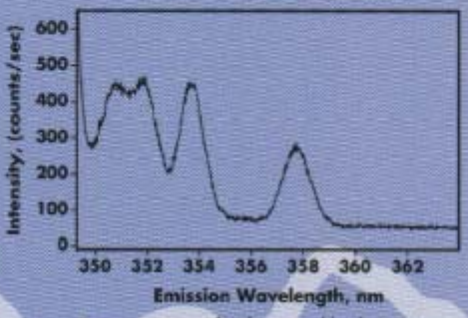


Whether you're working in Biochemistry or material science, measuring calcium migration, intermolecular distances, or laser crystals, the sensitivity and flexibility of a Fluorolog-3 Spectrofluorometer will help you gather more information on more samples in a smaller amount of time. And when your research focus changes, so does FL3, modularly adapting to the demands of your work with upgrades and innovation. Here are just a few examples.



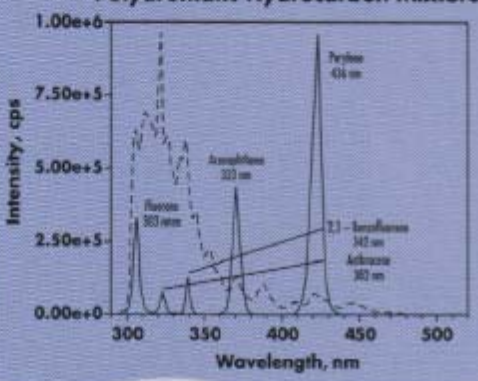
Fluorescence spectra of monolayer of rhodamine-B on a microscope slide. In Plot A, acquired with a single-grating spectrometer system, the fluorescence tends to be masked by the excitation. Scanning with a double-grating system yields a clearly defined peak at 540 nm as shown in plot B.

Carbon Tetrachloride Raman Scan (excitation at 348.0 nm)



Raman spectrum of carbon tetrachloride (CCl_4) acquired double-grating FL3.

Synchronous Scan of a Polyaromatic Hydrocarbon Mixture



Synchronous scan (broken line) and normal emission scan (solid line) of a mixture of polynuclear aromatic hydrocarbons.

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 spectrometer on the emission side drastically improves stray-light rejection.

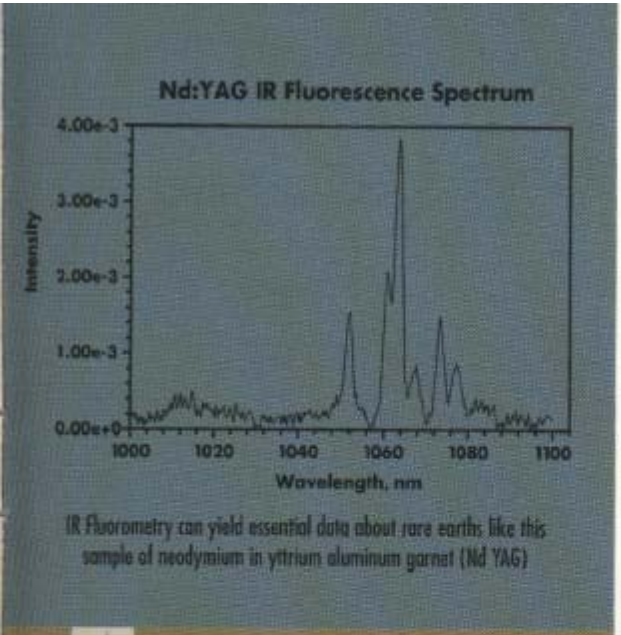
The Rhodamine-B data above compares the performance of a single-grating and a double-grating system on the same highly scattering sample: a thin monolayer of rhodamine-B on a microscope slide. The sample was scanned in the front-face fluorescence detection mode with our best single-grating spectrofluorometer system, and then with the model which has double-grating spectrometers for both excitation and emission. In Plot A, stray-light from the sample masks the rhodamine-B fluorescence. Plot B, measured by the double-grating, shows a well defined fluorescence peak at 540 nm.

Carbon Tetrechloride data illustrates the unmatched stray-light rejection capability of the Fluorolog-3 by showing all four Raman bands that occur at 350.7, 351.8, 353.6 and 357.7 nm for carbon tetrachloride (CCl_4). The excitation wavelength was 348 nm and the band-pass settings on the excitation and emission spectrometers were 0.5 and 0.7 nm respectively. Narrow slit widths and the capability to step the spectrometer in small increments are critical in resolving the 350.7 and 351.8 bands.

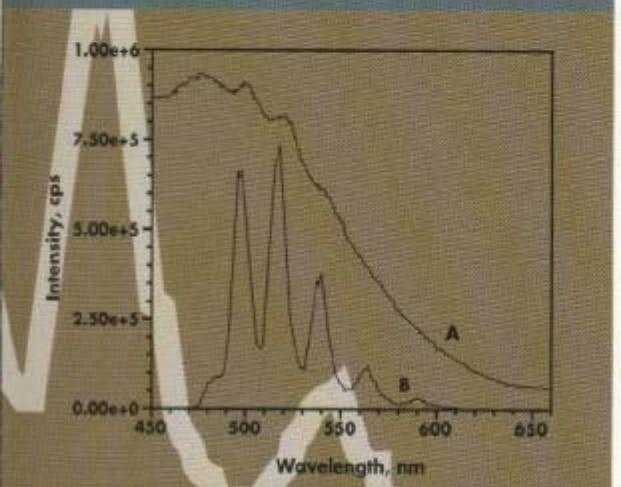
Synchronous Scanning for Characterizing Complex Mixtures

The observed fluorescence spectrum of a complex mixture often contains overlapping spectral features. Synchronous scanning offers a solution to this problem by simultaneous scanning the excitation and emission spectrometers with a constant offset between them (specified in wavelength or cm^{-1}).

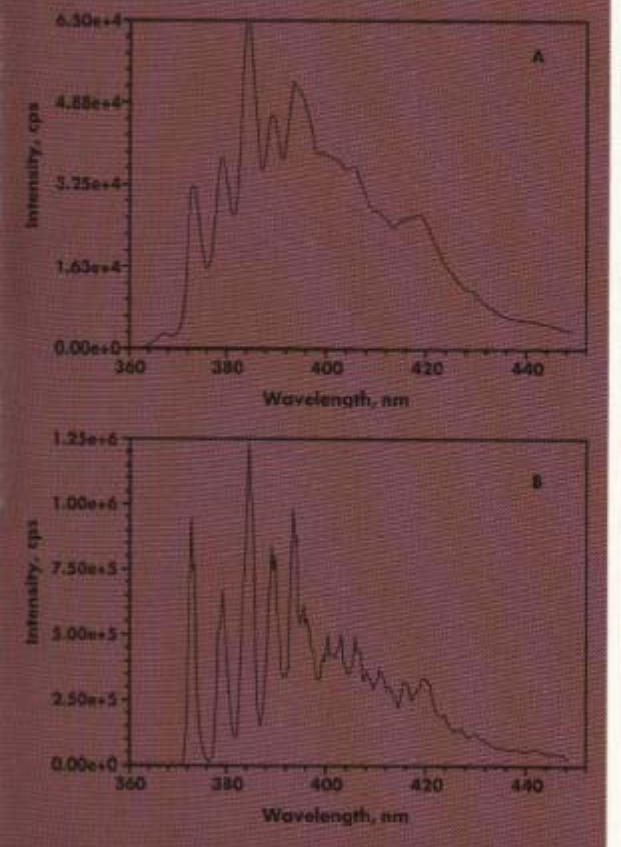
The polynuclear aromatic hydrocarbons figure compares a synchronous spectrum and a conventional emission spectrum for a mixture of five polynuclear aromatic hydrocarbons. The solid line is the emission spectrum acquired on a Fluorolog-3 system with constant-wavelength excitation. When the sample is scanned synchronously, five individual components are resolved into unique sharp peaks, as shown by the broken line.



IR fluorometry can yield essential data about rare earths like this sample of neodymium in yttrium aluminum garnet (Nd YAG)



Organic fluorescence in natural groundwater masks uranyl phosphorescence emission under continuous excitation (A). Signal-gating ignores fluorescence, uncovers the phosphorescence (B, expanded for comparison)



Emission spectra for pyrene, acquired at room temperature (A) and at 77°K (B)

Working with Fluorescence in the IR

The Fluorolog-3 series can be equipped for the IR region, opening up totally new areas of applications for fluorescence spectroscopy. For example, manufacturers of pharmaceuticals can employ IR fluorescence to identify toxic agents. In materials science, as illustrated by Yttrium aluminum garnet figure, IR fluorometry can help to study the sites occupied by rare-earth ions incorporated into single-crystal lattices. Also, probes in the red avoid interference from native fluorescence in the blue. An IR spectrofluorometer must be equipped with a red-sensitive photomultiplier or a solid state detector whose response is effective far into the IR region. With a PMT sensitive to 1 micron, only minimal system modification is necessary. For fluorescence detection above 1 micron, a variety of solid-state detectors covering different wavelength ranges, are available. Only a SPEX Fluorolog-3 IR system includes such components as integrated features.

Fluorolog-3 IR systems also have interchangeable gratings and optional grating turrets to enhance efficiency in the IR region giving Fluorolog-3 spectrofluorometers IR capabilities unmatched by any other instrumentation.

Phosphorescence for Time-Resolved Data

Fluorescence from the singlet state 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, looking at environmental effects on a sample or following changes in the hydrodynamic characteristics of macromolecular systems.

In phosphorescence experiments the Fluorolog-3 and the 1934D Phosphorimeter, excited by a pulsed light source synchronized with user-specified delay and sampling times, produces time-resolved spectral data.

A delay permits acquisition of a phosphorescence spectrum without fluorescence interference, as shown graphically in the organic fluorescence figure at left. This selectivity is particularly important for samples in which the phosphorescence of the analyte can be overwhelmed by strong fluorescence from extraneous materials.

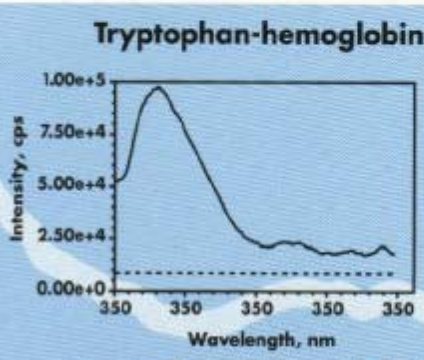
Time resolved data acquisition also makes it possible to acquire phosphorescence decay curves and compute lifetimes.

Low-Temperature Scans for Enhanced Fluorescence

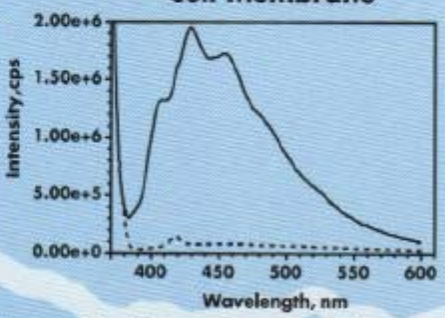
One way to protect a sample from molecular collisions that quench luminescence is by isolating the sample in a rigid matrix. Thus, cooling with liquid nitrogen enhances the phenomenon of fluorescence, even for otherwise dormant sample. The Pyrene data at left compares the fluorescence

spectra of a pyrene sample acquired at room temperature (A) and at liquid-nitrogen temperature (B). The Fluorolog-3 Liquid Nitrogen Dewar accessory was used for the low-temperature scan.

As dramatically demonstrated in Plot B, the low-temperature technique intensifies fluorescence emission for the pyrene sample and sharpens peaks to reveal greater structural detail. The superior resolution of a Fluorolog-3 double-grating spectrometer system optimizes measurements under these conditions.



Comparison of results of right-angled (broken line) and front-face (solid line) viewing of the β37 tryptophan in the 2αβ region of hemoglobin.



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

Front-Face Detection for Absorbent or Solid Samples

Fluorescence is typically collected at 90 degrees 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 degrees because of interference from reflected light. In highly absorbent samples like hemoglobin or milk, most of the emitted light is reabsorbed internally before the fluorescence can be measured.

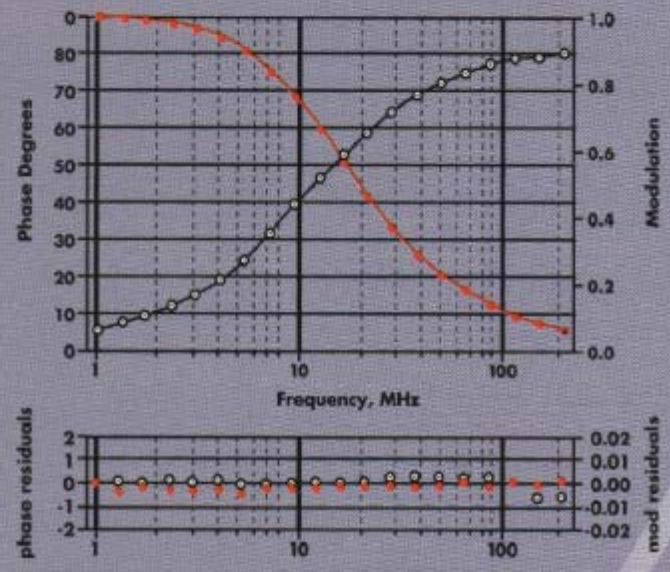
A significant optional feature of Fluorolog-3 spectrofluorometers is a choice between conventional right-angle or front-face fluorescence detection, ideal for solid, turbid or highly absorbent samples such as pellets, powders and monolayers on microscope slides. In front-face viewing, the fluorescence is collected from the sample's surface.

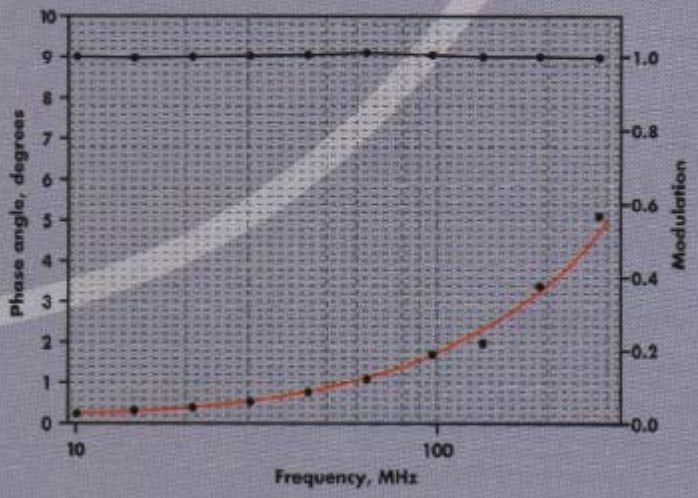
The tryptophan figure compares the spectra for right-angle and front-face viewing of a hemoglobin sample. For many years, hemoglobin was thought to be non-fluorescent because the fluorescence could not detected at the conventional 90 degree angle. With front-face viewing one can easily obtain the fluorescence spectrum for the β37 tryptophan in the αβ region of hemoglobin.

Detecting Trace Quantities of Biological Probes with Fluorescence Polarization.

Used in conjunction with the large number of fluorescent dyes suitable for biological research, fluorescence spectroscopy has greatly expanded our understanding of metabolic processes on the molecular level. SPEX Fluorolog-3 design offers unparalleled sensitivity for such work. The Cell Membrane figure illustrates the clear spectral differentiation between unlabeled cell membranes and membranes labeled with 1μM of DPH, a widely used probe for polarization and anisotropy measurements.

Fluorolog-3 And Molecular Dynamics





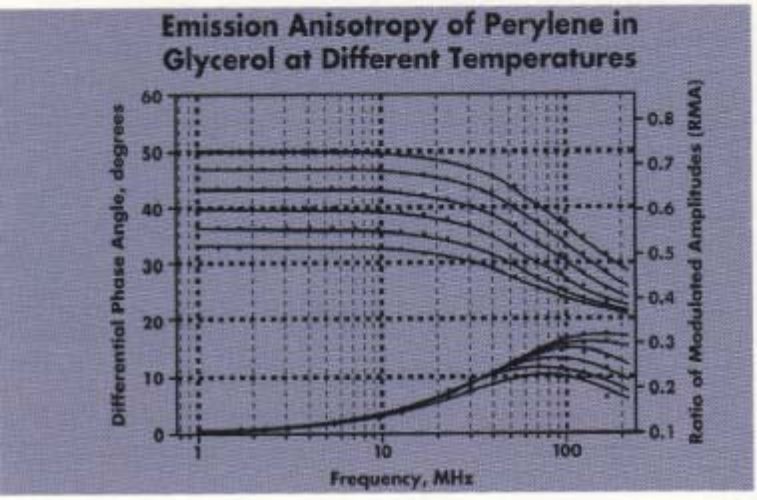
Precise measurement of the 47ps lifetime was critical in the understanding of the quenching effect of the iodide counterion to BIC (a benzimidazolocarbocyanine dye) in methanol. From this, and other time-resolved results, it was possible to determine that twisting around the polymethine bridge controlled the photophysics of BIC in solution. Data provided by Serdar Ozcelik, Daniel Akins and Ardie Walser of the City College of the City University of New York..

Time-resolved fluorescence measurements reveal significantly more information about the kinetics of molecular processes than steady-state emission spectroscopy . Now, lifetime techniques are applied to diverse fields of study including 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 two decades. Instruments, S.A. offers the Fluorolog-Tau3 for time- resolved fluorescence measurements in the frequency-domain. The Tau3 option provides picosecond lifetimes, anisotropy decay, time-resolved spectra and lifetime resolved spectra, while retaining the high performance found in the steady-state, photon-counting Fluorolog-3 models. Our choice of a frequency-domain design has its basis in the proven advantages of this approach. The Tau3 exploits the features of high sensitivity, precision and ease of use to give you an advantage in your research.

Real world biological samples often present complex fluorescence lifetime decays. The wide frequency range of the SPEX Fluorolog-Tau3 allows the resolution of complex fluorescence lifetimes with high precision and accuracy. Hematoporphyrin, a molecule of biological interest, decays as a triple exponential with widely spaced lifetimes. The Fluorolog-Tau3 easily resolves the fluorescence lifetimes of this molecule, which range from 840 picoseconds to 15 nanoseconds. The upper plot shows the frequency response of the sample, in which the phase shift and modulation of the sample are measured as a function of frequency. The lower plot shows the residuals of the fitted data, in which the difference between the data and the fitted decay function are plotted as a function of frequency. The reduced chi-square, a measure of the statistical goodness of fit, was 1.008 for this data set.

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 drug/protein interactions may be measured as well. Steady-state measurements

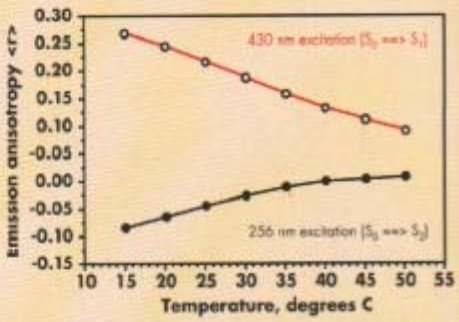


yield time-averaged values. Resolution of the decay of anisotropy provides much more detail about molecular motions on the fluorescence timescale. Adding temperature as a variable allows the study of complex phenomena such as phase transitions in membranes and the photophysics of molecules in solution.

Perylene is a small disk-like fluorophore that rotates anisotropically in solvent. The rotational rates of perylene in glycerol vary with the viscosity. As the temperature increases, the solvent viscosity decreases, and the perylene molecules rotate more freely. The T-format SPEX Fluorolog-Tau3, with optional Autopolarizer and TEMP1 temperature controller, measures these rotational motions as a function of temperature automatically. The upper curves show the RMA (Ratio of Modulated Amplitudes) as the temperature increased from 20°C to 45°C in 5 degree steps. The lower curves show the differential phase angle (V-H). As the molecule rotates faster, the RMA value 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.

Steady state emission anisotropy of perylene in glycerol

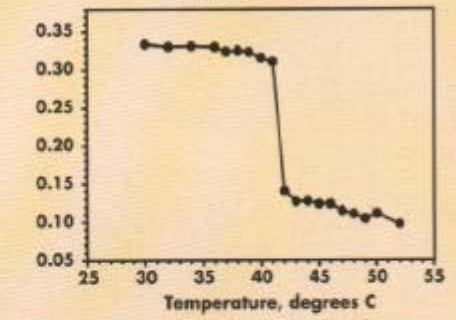
Temperature-based fluorescence provides information about the environment of the fluorophore, such as the viscosity. An example of this is shown below. Perylene has two absorption dipoles in the molecular plane. One of these is collinear with the emission dipole (S0 ==> S1) and the other orthogonal (S0 ==> S2). Excitation at 430 nm results in positive anisotropy, and excitation at 256 nm results in negative anisotropy, due to the orthogonality of the absorption and emission dipoles. The steady-state anisotropy of perylene in glycerol, acquired at both 430 and 256 nm excitation, as a function of temperature, was collected automatically with the Autopolarizer and TEMP1 accessories. As the temperature is increased, the bulk viscosity of the glycerol decreases, and the perylene molecules rotate faster. These faster motions depolarize the fluorescence emission. From this, the viscosity of the solvent as well as the size, shape and hydrodynamic volume of a fluorophore may be evaluated.



Steady State emission anisotropy of perylene in glycerol. Excitation at two wavelengths results in different anisotropies (see text). The emission anisotropy decreases with temperature, reflecting viscosity changes in the solvent.

DPH labeled DPPC MLVs (1:500)

Phase transition curve



Phase transition curve of DPPC, a phospholipid model of biological cell membranes.

Phase transition of Phospholipids

The addition of small proteins or the effects of lipid composition on cell membranes may be determined using anisotropy versus temperature. Above, such an experiment uses a model membrane of a phospholipid multilamellar vesicle. The phospholipid (DPPC) was labeled with DPH, a rodlike probe which partitions itself in the hydrophobic region of the vesicle. The anisotropy of DPH embedded in DPPC shows a sharp reduction at the phase transition of the phospholipid.