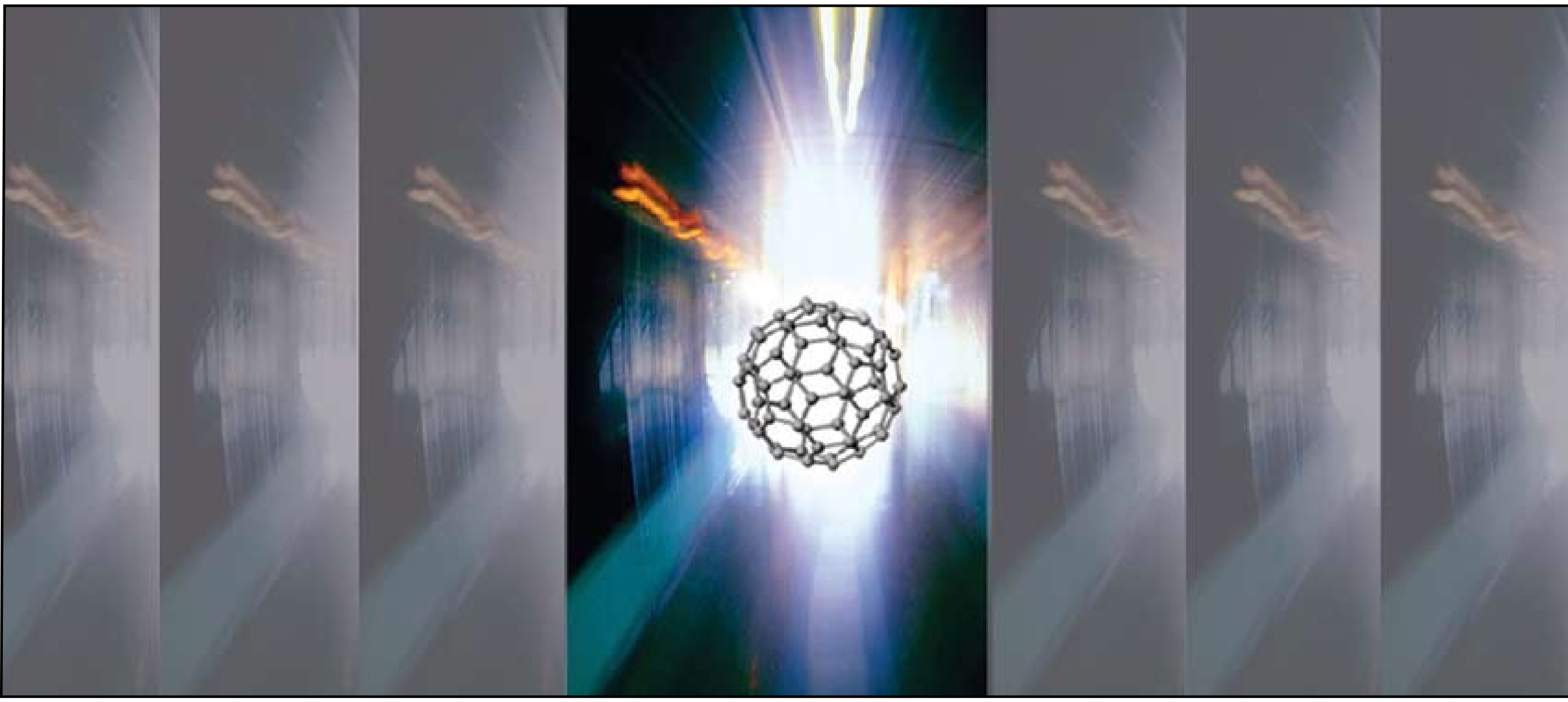
HORIBA JOBIN YVON



How to Build the World's Fastest Spectrofluorometer

MF^2 Multi-Frequency Fluorometer

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Time is always on your side with a HORIBA Jobin Yvon MF² (Multi-Frequency Fluorometer) lifetime spectrofluorometer. The MF2 is a revolution in fluorescence dynamics, because it can obtain ALL frequency-domain data in one measurement...as fast as 1 millisecond! Now you can plot the course of reactions that were inaccessible with other instruments, or simply take more data with the time available, even when there are multiple components in the sample. You can do it with a system as simple as a filter box, or one that incorporates multiple spectrometers and detectors, polarizers, and more for a complete fluorescence laboratory encouraging steady state as well as lifetime measurements with our World's Most Sensitive spectrofluorometer. We've put together solutions that fit any research need at a fraction of the cost of comparable systems, without compromising the reliability and repeatability you require, while including RAPID and TRUE picosecond performance.

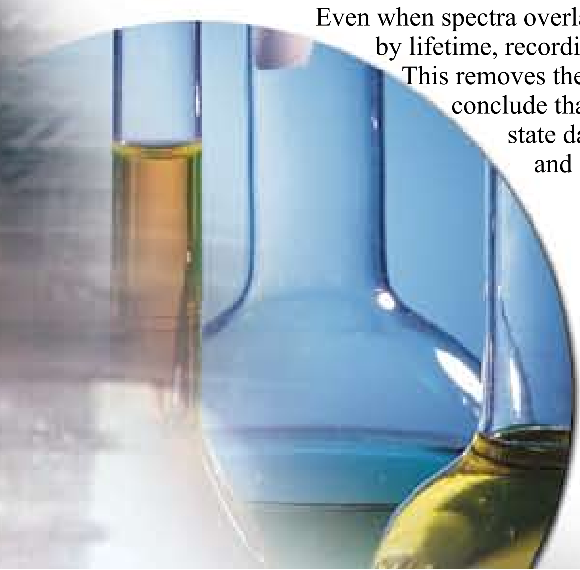
Why Lifetimes?

Because of the unique sensitivity, selectivity, and non-destructive nature of fluorescence spectroscopy, the technique has become ever more popular, especially in biochemistry, pharmaceuticals, and materials science. It is an essential tool for DNA-sequencing, cell-identification, *in-vitro* and *in-vivo* studies of biological events, clinical chemistry, and even in the frontiers of nanomaterials.

Steady-state measurement of fluorescence produces an averaged picture of a substance: its absorption and resultant emission of light in the UV, visible, and IR region of the spectrum. By introducing time-discrimination, much more information is revealed. The motion, size, environment, intermolecular distances, and many other intermolecular parameters can be deduced from the behavior of a material's fluorescence as a function of time.

For example, you can record similar fluorescence spectra from two molecular species. These two spectra, however, can be the product of very distinct—and different—mechanisms. The differences between the lifetimes recorded for each spectrum, however, could reveal that the molecule with the shorter lifetime is subject to collisional quenching by interacting with other molecules in the surrounding medium. On the other hand, the species with the longer lifetime could be trapped inside a protein, leaving it immune to external collisions.

Even when spectra overlap, it may be possible to separate temporally contributions by lifetime, recording independent emission spectra at different lifetimes. This removes the interference between the two spectra. You might conclude that the difference between recording lifetimedataand steady-state data is like the difference between a motion picture and a snapshot.



Why Frequency Domain?

In the frequency domain, the excitation source has its intensity modulated at high frequency. As a result, the sample's emission is also modulated, but out of phase with the excitation. Both the phase-difference and the modulation of the emission are directly linked to the lifetime. This gives you twice as many parameters to determine the lifetime.

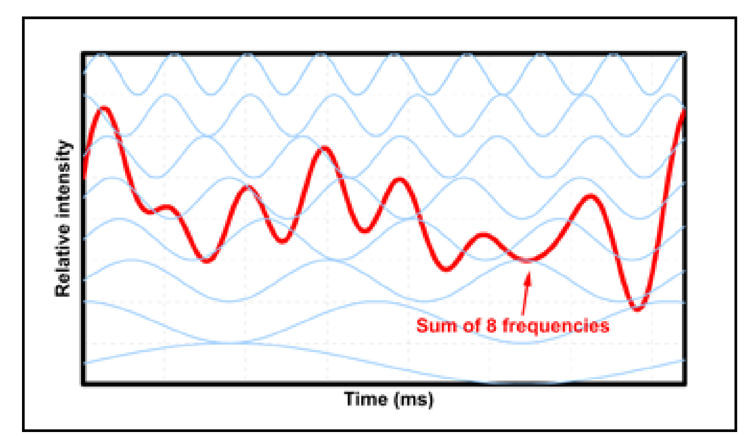
Frequency-domain measurements often are faster and convenient, especially with typical liquid samples in cuvettes emitting at a higher quantum-efficiency. Frequency-domain measurements also produce two observables—phase and modulation—from which lifetimes can be calculated. Not only does this overdetermine the lifetime, it also supplies additional precision at either longer or shorter lifetimes.

The real advantage of multi-frequency fluorometry

Though one frequency would be sufficient to measure a single-exponential decay, real-world samples rarely contain only a single component, and the individual components probably have their lifetimes distributed over some range. The more frequencies used, the better is the ability to discriminate between components and characterize their distributions.

Typical frequency-domain instruments excite the sample with various frequencies of light in a sequence, meaning that completing the experiment requires waiting for all frequencies to be used. Such an experiment might take hours to record.

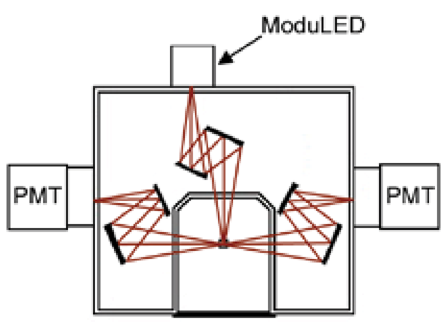
We at HORIBA Jobin Yvon used recent and patented advances in frequency-synthesis to develop wholly new technologies to bypass conventional limitations. By grouping multiple frequencies components into one high-frequency waveform and greatly expanding the available frequency range, we decreased the time it takes to measure a fluorescence lifetime from minutes to milliseconds, a 10,000-fold change. We call this revolution in instrumentation the MF², which delivers previously unavailable experimental speed and performance. Many complete lifetime experiments can be performed in only millliseconds. Not only that, but one instrument and one experiment can determine lifetimes over a huge range, from picoseconds to milliseconds and beyond.

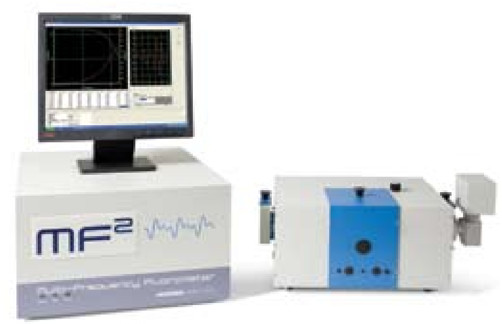


A schematic of eight separate frequencies (light blue) and their sum, applied simultaneously in the MF² method.

Why MF²?

HORIBA Jobin Yvon carries on the tradition of high-performance instrumentation of Spex®, IBH, and SLM-Aminco. HORIBA Jobin Yvon's instruments are fully automated, making experiments easy and reducing chances of operator error. The technique of multi-frequency fluorometry offers reduced price, bringing the world of lifetime spectrometry into the realm of cost-conscious budgets, without sacrificing the quality and service you expect. ONLY HORIBA Jobin Yvon can supply you with this patented, unique, exceedingly fast lifetime instrument.





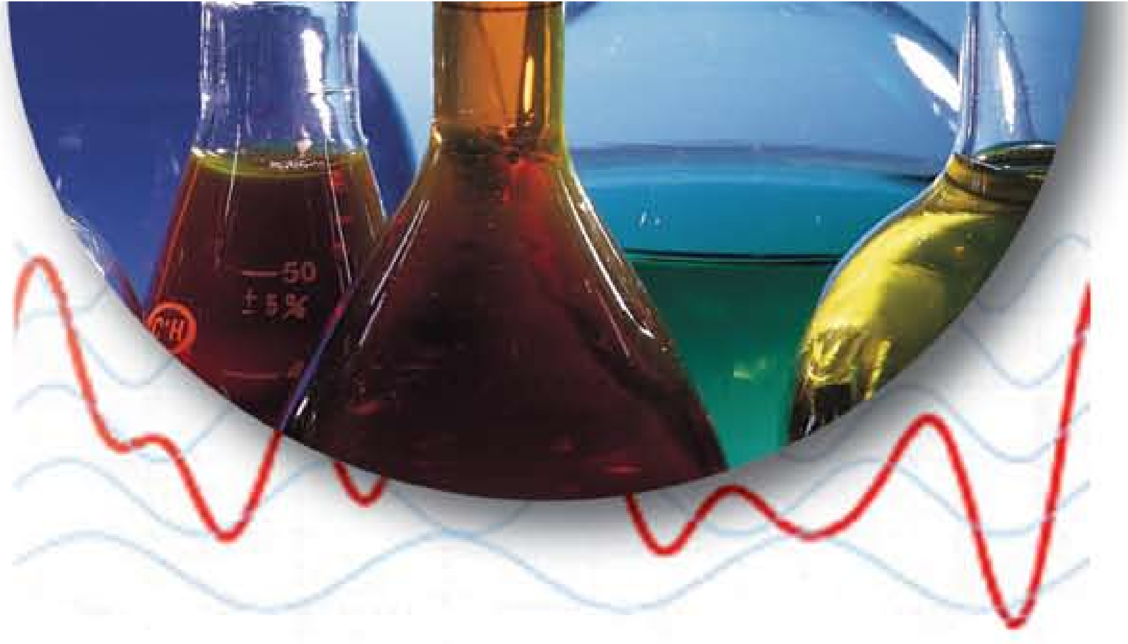
Simple MF² with ModuLED source, T-format sample chamber, and two photomultiplier tubes (PMT) for detectors. In the photograph, the MF² control unit supports the monitor.

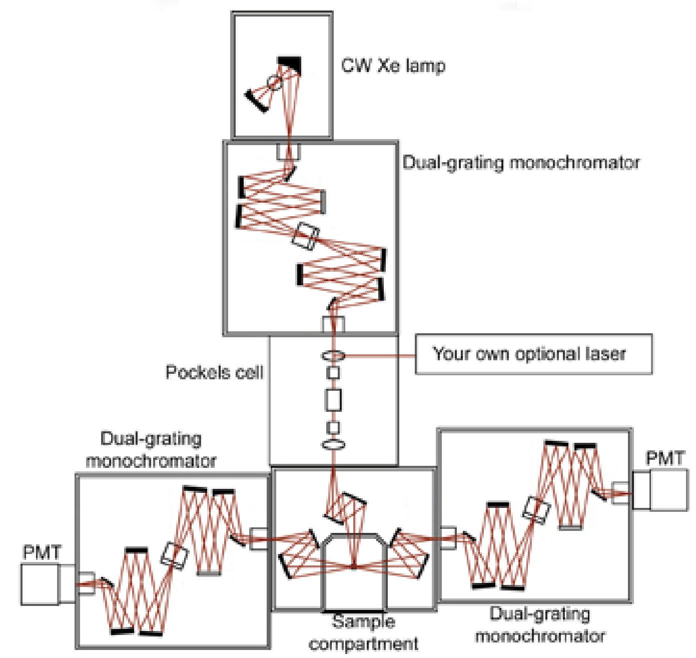
A Total Fluorescence Laboratory in One Complete System—Small or Big!

You can start with a simple filter-based system, excited by our ModuLED or laser sources. Single or dual detectors are possible, as are all the accessories for the sample compartment. You can even do dynamic anisotropy. Upgrade to a full spectrofluorometer at any time!

Whenever you want, you can upgrade your simple filter-based unit to a full spectrofluorometer laboratory. You can add up to three monochromators, multiple detectors, even CCDs, to do steady-state investigations with a 450 W xenon lamp. Add a Pockels cell to run MF² with YOUR OWN high-powered CW laser, to get the fastest, most accurate data ever. With the Pockels cell coupled to the xenon lamp, on the other hand, you can take lifetime data anywhere in the spectrum that your photomultiplier tube can see.

No matter which configuration you choose, you get the patented frequency-synthesis and mixing instrumentation, plus the interchangeable, solid-state ModuLED sources. You also get the proven performance of lifetime detection only from HORIBA Jobin Yvon. The MF² offers wide, flat frequency-response over an unequaled range—from 500 Hz to 310 MHz. Analysis of an unknown requires fitting the data to a model by the well-established non-linear least-squares method. With the MF², you have the power and computing speed to implement and test multiple models as you go. And all of this runs with Windows®-compatible software exclusively from HORIBA Jobin Yvon.





Upgraded MF² with dual-grating excitation and emission monochromators, Pockels cell, CW xenon-lamp source, and your own laser, plus two PMTs. The photograph below shows the instrument with a filter-based PMT on the left, and a PMT attached to a monochromator on the right.

Data You Can Expect from Your MF²

Analyze single and multi-component fluorescence decays

Figure x is an example of a simple lifetime-analysis in the frequency domain. The experiment took only xxx ms. The datapoints are fit to a single-exponential model. Two parameters are measured: The modulation and the phase-shift of the fluorescence from the sample. A least-squares simultaneous fit reveals a fluorescence lifetime of xxxx ns.

Many samples contain several species, or their decay mechanism involves several different fluorescent lifetimes. A multi-exponential lifetime analysis is shown in Figure x. A fit of the decay reveals three separate lifetimes, at x ns, y ns, and z ns.

Resolve picosecond lifetimes

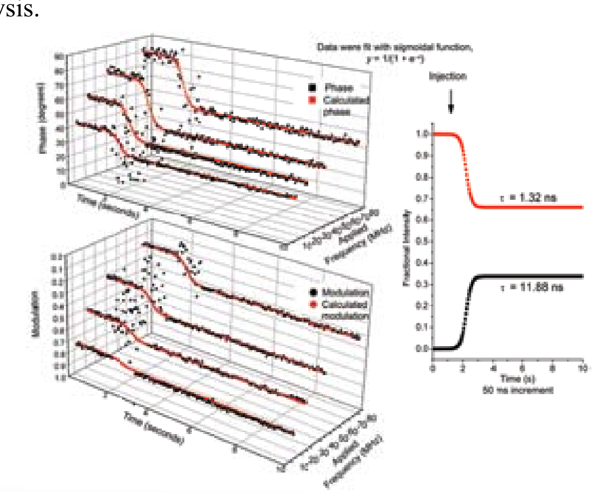
Another superior characteristic of HORIBA Jobin Yvon's MF² is its ability to measure ultra-short lifetimes in the picosecond range. Figure x shows data from xxxx, revealing a lifetime of x ps. The fit is confirmed by the small χ^2 and low residuals.

Deconvolve multiple spectra

A lifetime-resolved acquisition, or phase-resolved spectrum, can separate overlapping spectra with different lifetimes. Here, in Figure x, the spectra of xxxx and xxxx are deconvoluted within a methanol mixture using this technique.

Watch the time-evolution of spectra

Molecules are dynamic systems, so their spectra are continuously evolving. HORIBA Jobin Yvon's MF² easily defines the evolution of spectra with time. Figure x shows a time-resolved spectrum over the course of xxx ns, in a 3-D view, of xxxx. Different emission characteristics appear when excited by different wavelengths. Individual decay-curves and spectra can be extracted for further analysis.



Four applied frequencies simultaneously monitor a mixing experiment, and the change in intensity of the lifetimes of two components in the mixture. On the left, the black points are the data, and red lines are the calculated fit to a sigmoidal function. On the right is the calculated change in intensities of the two lifetimes.

Run kinetic experiments

A crucial part of biochemistry and chemistry is understanding how systems evolve when compounds react. The MF² is able to resolve changes in chemical composition down to the millisecond timescale.

Detect the denaturing of proteins

The rapid nature of the MF^2 technique allows you to capture small changes in lifetime while the experiment is in progress, such as when a protein unfolds as the temperature rises.

Record time-resolved polarization spectra

With optional automated poalrizers, our lifetime spectrofluorometers run time-resolved emission experiments to determine:

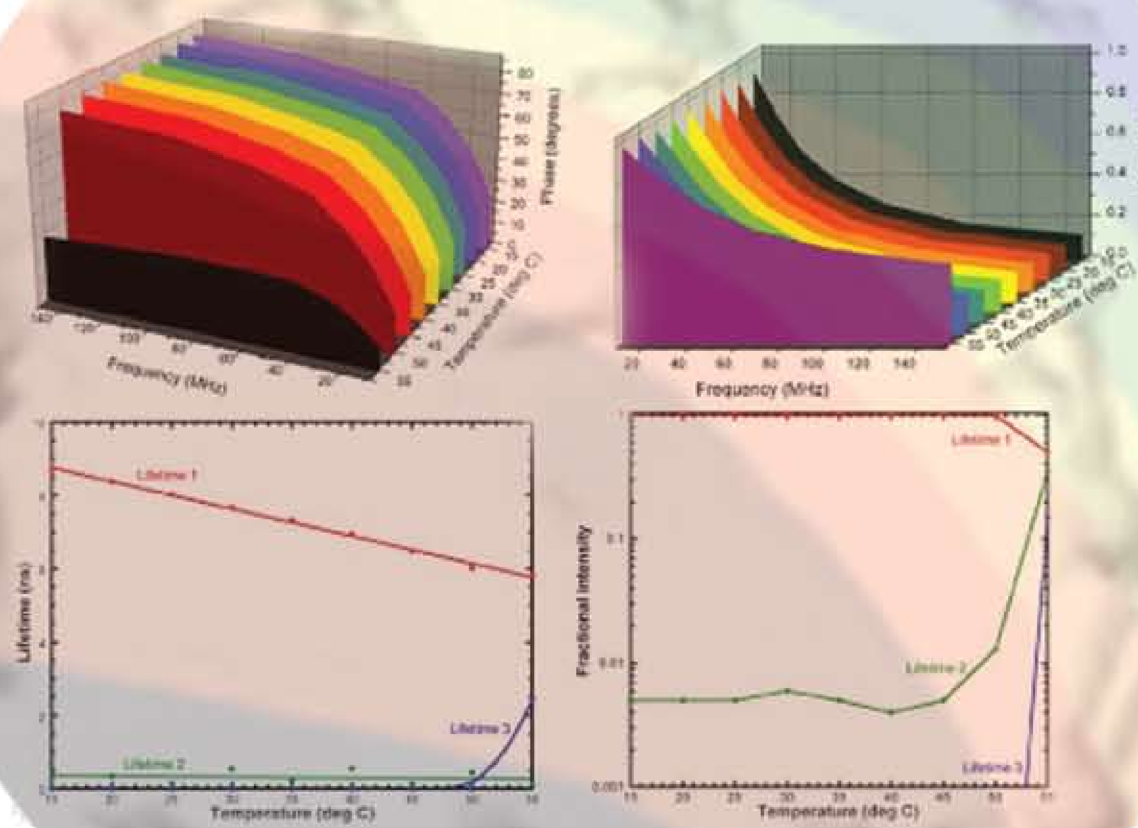
- Complex rotational behavior of molecules
- Molecular size and shape
- Protein structure and dynamics
- Phuscal properties of polymers, liquid crystals, and membranes

Determine phosphorescence lifetimes

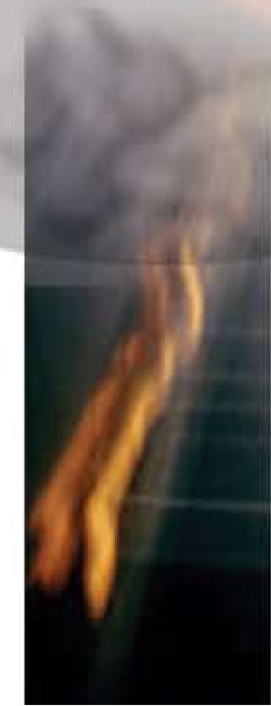
Phosphorescence occurs over a much longer timespan than fluorescence. The MF^2 can examine luminescence lifetimes extending out to the millisecond—or longer—ranges.

Measure infrared luminescence

Near-infrared luminescence is widely studied in the fields of materials science and nanotechnology. With optional near-IR detectors, the MF^2 can detect and analyze emissions from carbon nanotubes and other near-IR emitters.



Here the phase-change (upper left) and modulation (upper right) vary with temperature as a protein is heated. The change in calculated lifetime (and respective fractional intensity) with temperature is shown in the bottom plots. Note how lifetime 1 falls steadily with temperature, lifetime 2 is unaffected, but lifetime 3 appears only at the highest temperature. (A bi-exponential fit, not shown for 55°C, gives an unacceptably large χ^2.) The entire experiment took only 60 s, with a lifetime resolution of 0.1 to 10 ns.



Specifications

An MF^2 is a complete, stand-alone lifetime spectrofluorometer with both lifetime and steady-state modes. All HORIBA Jobin Yvon spectrofluorometers operate on user-friendly Windows® XP-compatible software, offering instrument control, data-acquisition, and data-processing, with non-linear least-squares lifetime modeling.

Major components of the MF^2

- Pulsed solid-state excitation source
- Optional Pockels cell with CW source
- Sampling module compatible with L- or T-format detection
- Optional single- or double-grating excitation and emission monochromators
- Signal detector
- MF^2 chassis, the system controller
- FluorEssence™ data-acquisition and modeling software
- All cables and connectors

Modes of operation

- Steady-state
- Frequency-domain, lifetime mode: 10 picoseconds to 1 millisecond (for a 2° phase-shift at 250 MHz, and 0.1 modulation at 1 MHz)
- Frequency-domain, time-resolved mode: 1 ms

Frequency range

500 Hz to 310 MHz, up to 8 simultaneous frequencies
Resolution of 0.1° at 10 MHz, 1° at 300 MHz, with reduced $\chi2 < 1.0$

Source

- Choice of fixed-wavelength, interchangeable ModuLED solid-state pulsed excitation source (wavelengths available from 250 nm in the UV to 1310 nm in the near-IR). Standard optical-pulse durations are < 200 ps (< 100 ps typical) for laser-diodes, < 1.5 ns for LEDs. High repetition-rate.
- User-supplied external laser with optional Pockels cell.

Signal-to-noise ratio

4000:1, measured at 450 nm in steady-state mode, with the Raman band of water at 397 nm, at 5 nm bandpass, excitation at 350 nm, integration time of 1 s, using 1st standard-deviation in the signal-to-background noise.

Optional monochromators

All-reflective *f*/3.6 Czerny-Turner, with kinematic, interchangeable gratings from UV to IR. Range is 0–1100 nm (up to 4000 nm with optional gratings), minimum step size is 0.0625 nm, accuracy is ±0.5 nm (with a 1200 grooves/nm grating); resolution is 0.2 nm; speed is 80 nm/s. Determination of lifetimes is limited to 300-850 nm.

Sample holder

Automated, motorized 4-position, thermostatted cuvette holder with magnetic stirrer. Temperature range = –10°C to +80°C (with optional water-bath).

Detector

R928P photomultiplier tube is standard for 240–850 nm response. Other optional detectors to 1100 nm.

Accessories

Compatible with all Fluorolog® accessories.

Why HORIBA Jobin Yvon?

Local support

Who else can give you the service and applications support you need, in order to achieve the total potential from your instrument? HORIBA Jobin Yvon has fill applications laboratories staffed by fluorescence experts in the USA, Europe, and Asia. HORIBA Jobin Yvon's affiliates and sister companies are in the UK, Germany, France, Italy, China, Korea, and Japan. Add to this a global network of representatives, and you can rest assured that you will have the support you expect only from HORIBA Jobin Yvon. We are part of HORIBA, a $1 billion company with almost 5000 employees world-wide.