Modular Spectrofluorometer The Fluorolog®-3

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Abstract

The Fluorolog®-3 modular spectrofluorometer, developed by Jobin Yvon, lets researchers choose the source, spectrometers, detectors, and accessories for their custom needs. The Fluorolog®-3 delivers the ultimate in sensitivity, speed, and automation for all types of steady-state fluorescence research in quantum chemistry, materials science, biology, analysis, and quality control. The Fluorolog®-3 is completely computer-controlled, and delivers high-quality fluorescence data throughout the ultraviolet, visible, and near-IR regions of the electromagnetic spec-trum.

1 Introduction

Luminescence can be divided into two realms: fluorescence and phosphorescence. Fluorescence is light emitted from a sample, induced during excitation of the sample, whereas phosphorescence is light that persists (greater than ~ 10^{-6} s) from a sample, after the excitation is removed (Fig. 1). Both of these types of luminescence are used to answer scientific questions about molecular shape, size, and activity. Popularly, they are often lumped together under "fluorescence spectroscopy". Fluorescence spectroscopy has certain advantages over other optical spectroscopies, namely sensitivity (< 10^{-12} mol) and specificity. Fluorescence spectroscopy is sensitive to the micro-environment of a molecule (up to ~ 10 nm away), and depends on molecular motion. For example, the following dynamic processes can affect the fluorescence spectrum of a sample: rotational diffusion of proteins and membrane-bound molecules; collision of molecules with quenchers; translational diffusion; formation of complexes; and changes in an excited state.

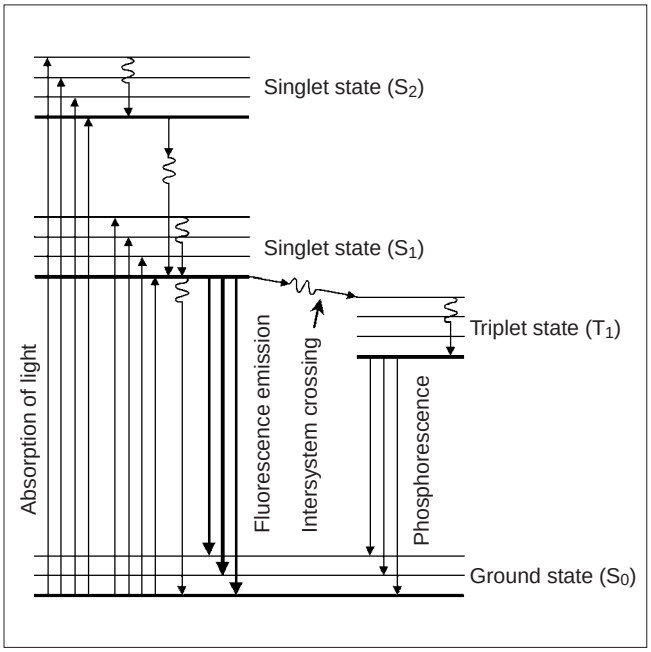

Fig. 1 Jabłonski Diagram of Absorption and Emission of Light in a Molecule

Phosphorescence occurs when an electron flips its spin (intersystem crossing), a "forbidden transition", which occurs more slowly than "allowed" transitions.

Fluorescence spectroscopy has found use in the following applications: protein conformation and transport; trace levels of biologically active compounds and carcinogens; quality-assurance of medications; monitoring of drug-delivery and interactions; properties of macromolecules and nanoparticles; photoreactivity of organic compounds; detection of chemical reactions; structure-property relations; monitoring pollutants in air,

water, and soil; photoluminescence and photoluminescence excitation; and manufacturing quality-control. Jobin Yvon (JY)'s Fluorolog®-3 (Fig. 2) offers outstanding performance in all of these fields, measuring both fluorescence and phosphorescence.

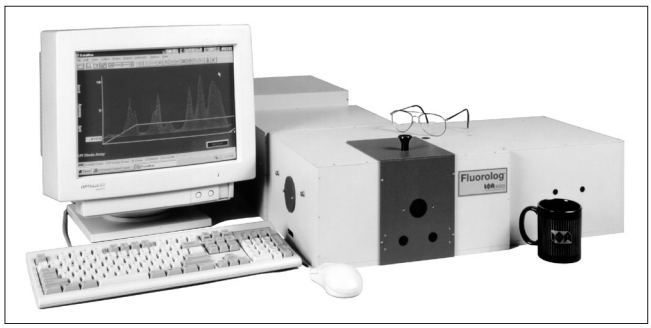

Fig. 2 Basic Fluorolog®-3 Spectrofluorometer

2 Measurement Principles

In general terms, a spectrofluorometer is constructed from a light source, a method of choosing the excitation wavelength, a sample holder, an emission monochromator, and a detector. The Fluorolog®-3 usually contains a 450 W continous-wave xenon arc lamp as the excitation source, a Spex® monochromator with ruled plane gratings as the excitation wavelength selector, an interchangeable T-format sample-holder in which a sample cuvette may be placed, another Spex® monochromator to select the emitted luminescence from the sample, and an R928P photomultiplier tube in photon-counting mode as the emission detector. Accessories for specialized measurements are available, and described later in this article.

A number of possible arrangements of the Fluorolog®-3's modules are shown in Fig. 3. Choice of the arrangement of components is dictated by the user's requirements for sensitivity and selectivity, as well as type of sample to be measured, for example, polarized scans, front-face measurements, high-speed acquisitions, etc.

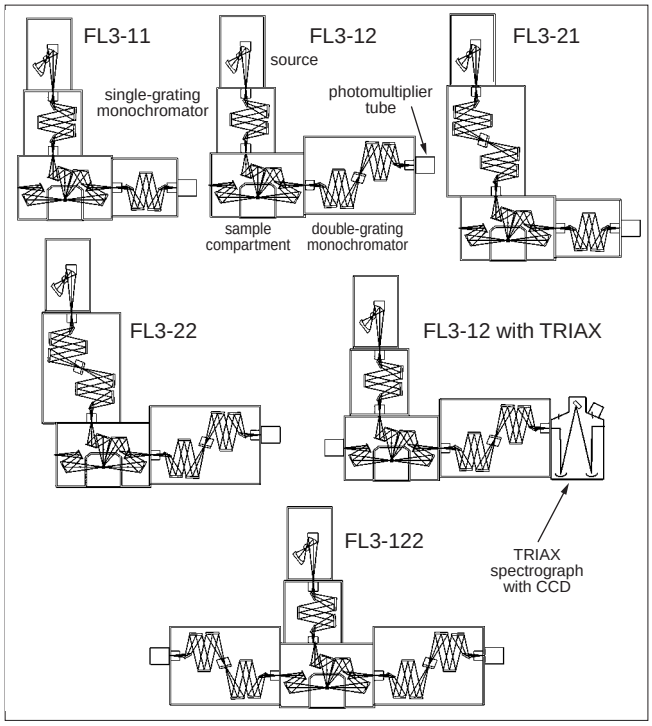

Fig. 3 Some of the Configurations Possible with the Fluorolog®-3 Modular Spectrofluorometer

3 Features of the Fluorolog®-3

3.1 Advantages

A xenon arc lamp, the start of the optical train, and standard on the Fluorolog®-3, is vertically mounted, in order to image the arc on the entrance slit, and also results in longer lamp life. Within the monochromators, the gratings used to disperse the light are ruled plane-gratings, kinematically mounted. These gratings remain focused at all wavelengths (unlike lenses, with a variable refractive index). Ruled gratings eliminate polarization anomalies of holographic gratings. Throughout the Fluorolog®-3, all the optics are reflective, removing the distortions induced by lenses. At the end of the optical train, JY relies on photon-counting detectors to strip noise away from weaker signals. Finally, JY's own custom software, based on Windows™, controls the entire system for collecting all data and performing data-analysis. Not shown in Fig. 3 is a SpectrAcq controller, which handles low-level commands to and from the spectrofluorometer, and the host computer, in which resides the high-level controlling software for the SpectrAcq. The Fluorolog®-3 is self-calibrating; all control of slits, wavelengths, integration times, temperature controls can be accessed through the software. Data from the Fluorolog®-3 can be exported to popular spreadsheet programs for further refinements in presentation.

3.2 Measurement Types

The Fluorolog®-3 offers the researcher versatility. Many different scan types are available as shown in Table 1.

Table 1 Various Measurement Types using Fluorolog®-3

Emission scan	The excitation wavelength is fixed, while the emission monochromator scans the sample.
Excitation scan	The emission wavelength is fixed, while the excitation monochromator changes the incident light.
Synchronous scan	Both excitation and emission monochromators are scanned, with a constant wavelength distance (the offset) between them.
Time-based scan	Takes data with respect to time to study scan photodecay and kinetics. Both ex-citation and emission wavelengths are fixed.
Discover scan	Surveys samples in a preliminary scan with unknown characteristics, in which both excitation and emission wavelengths are varied.
Batch scan	Runs a series of pre-defined experiments on one or several samples.
Matrix scan	Scans both excitation and emission wavelengths, to create a three-dimensional matrix of the excitation and emission spectra.
Temperature scan	Runs an experiment while monitoring scan and varying the temperature of the sample.
Polarization scan	Records spectra while varying scan polarization components of the excitation and emission.
Microplate scan	Records spectra from up to 384 different wells in a microwell plate, using the MicroMax microwell plate reader.

3.3 Specifications

The specifications of the basic Fluorolog®-3 are given in Table 2.

Table 2 Fluorolog®-3 Specifications (basic instrument)

Component	Description
Excitation source	450-W xenon short-arc lamp, mounted vertically in an air-cooled housing. Pulsed lamp for phosphorescence measurements is optional.
Spectrometers	Single-grating, Czerny-Turner with all-reflective optics. Resolution = 0.2 nm Accuracy = ± 0.5 nm Speed = 150 nm/s Range = 0–1300 nm Double-grating monochromators are optional.
Gratings	Kinematically mounted, classically-ruled Excitation blaze = 330 nm (200–700 nm range) Emission blaze = 500 nm (300–1000 nm range) Other optional gratings are available.
Sample compartment	T-format, removable gap-bed for optional compartment sample accessories.
Reference detector	Calibrated photodiode for excitation correction from 240–1000 nm
Sample detector	R928P photomultiplier tube in photon-counting mode, from 240–850 nm. Optional detectors are available with sensitivity into the infrared.
Sensitivity	4000:1 signal-to-noise ratio, using double-distilled water, in a Raman scan, using the 397 nm peak, 5 nm bandpass, 1 s integration time, and background noise first standard-deviation measured at 450 nm.
Software	JY custom software for Windows™. Controls all experimental parameters, accessories, data-collection, and analysis.

4 Accessories

A large number of accessories to enhance the performance of the Fluorolog®-3 are available from JY. Among them are as follows.

(1) Sources

In order to measure long-lived phosphorescence (> 1 μs), the optional phosphorimeter can be ordered from JY. This accessory consists of a xenon flash-lamp in a housing, and associated electronics.

(2) Monochromators

These modules disperse light into its component wavelengths. Both double-grating monochromators, with greater dispersive ability, and single-grating monochromators are available from JY. TRIAX spectrographs, with multiple gratings are also able to be mounted on the Fluorolog®-3 for custom research and increased speed with CCD-array detectors.

(3) Optics

In many cases, fluorescence measurements involve removing interference, such as second-order peaks or Rayleigh bands. The use of cut-on and cut-off optical filters removes these distortions of the measurements. Such optional filters and filter-holders can be purchased from JY. In addition, measurements of polarized light is possible with optional automated polarizers, whose rotation is controlled via software. To optimize sensitivity over a broad range of wavelengths, interchangeable ruled gratings are available for the monochromators.

(4) Sample-holders

The Model 1933 sample-holder for supporting crystals, thin films, pellets, powders, fibers, and cells, is useful for certain types of fluorescence research. For viewing fluorescence from the front face of a sample, rather than through the sample, the FL-1001 front-face accessory is necessary. Holders for two or four cuvettes simultaneously, with integral thermostats, help keep a liquid's temperature constant.

(5) Temperature Control

Kinetics and molecular motion – and thus luminescence – are temperature-dependent. Therefore, JY offers a full range of accessories to control a sample's temperature. A circulating water-bath and a Peltier-thermocouple drive have wide flexibility, while liquid-nitrogen dewar flasks operate at cryogenic temperature (77 K) for "freezing" a sample's molecular motions.

(6) Time-based Measurements

The autotitration injector provides automated, controlled repeated injection of aliquots into a sample, with precision to 0.1 % of the total syringe volume, all controlled via the software. For individual injections, the injector port plus the trigger box is available. The MicroFlow, a stopped-flow accessory for kinetics measurements, offers automated control of sample mixing.

(7) Multiple Samples

The MicroMax microwell titer-plate reader allows multiple samples and multiple wavelengths to be scanned in one experiment. The MicroMax is controlled via software; excitation to the MicroMax and fluorescence from the samples is transmitted to the Fluorolog®-3 with a fiber-optic probe. Plates containing up to 384 wells are compatible.

(8) Detectors

A number of photomultiplier tubes can be used in the Fluorolog®-3 to expand the range of detectable wavelengths into the near-infrared (longer than 800 nm). Various CCD-arrays, with scanning spectrographs, increase the speed of data-acquisition, for they can record a full spectrum at once.

(9) Lifetime Measurements

To measure the decay of fluorescence lifetimes, the Fluorolog®-3 can be upgraded to a Fluorolog®-Tau-3. This involves addition of a Pockels-cell modulator box and extra controlling electronics, to measure lifetimes using the frequency-domain. The lifetime capability allows measurements down to the picosecond (10^{-12} s) range, for extra information on intra- and intermolecular motions, protein dynamics, solvent-relaxation, binding, bilayer viscosities, etc. A laser-port is built in, for an external laser excitation source.

5 Conclusion

The Fluorolog®-3 offers wide flexibility, encouraging both the novice and seasoned researcher to customize the spectrofluorometer to the desired use. The Fluorolog®-3's speed produces more data, limits the degradation of photosensitive samples, and avoids photobleaching. Modularity, sensitivity, and reliability all contribute to the Fluorolog®-3's reputation as the workhorse for fluorescence research world-wide.

Note

Fluorolog® is registered in the USA. Spex® is registered in the USA and European Union.

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