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SPECTROFLUOROMETRY MOVES FORWARD WITH THE FLUOROLOG

In myriad areas of analytical chemistry, the impact of fluorescence techniques has been so great that dozens of models of commercial instruments are now manufactured, and thousands are in operation. They range from hand-held, inexpensive filter black-boxes dedicated to a specific analytical task all the way to sophisticated, state-of-the-art systems geared to the diverse and complex problems associated with basic research. The Spex FLUOROLOG and its computerized counterpart, the FLUOROCOMP, are new research systems designed to nudge the fluorescence frontiers still further. How this has been accomplished is the theme of this paper.

Regarded simply if somewhat inexactly, fluorescence refers to the simultaneous emission of electromagnetic radiation by a substance upon its exposure to radiation of a higher frequency. Phosphorescence arises in a similar manner, but the emitted light persists for some time after removal of the excitation source. Actually, both fluorescence and phosphorescence are family members of a more general effect, luminescence. Luminescence encompasses the entire gamut of emission phenomena where the frequency of the emitted light is independent of the exciting energy. This energy can take many forms. Light produced by chemical action (chemiluminescence) and that emitted as the result of altering or breaking a crystal (triboluminescence) are two lesser known relatives.

Any type of luminescence can be measured by the FLUOROLOG. One of its strongest attributes is its modularity, encouraging researchers to change the source, the detector, the sample compartment, the electronics, and the optics. So modified, the system can handle almost any luminescence measurement, at any temperature, in any atmosphere, in a magnetic field, and under experimental conditions dictated by the effect sought.

While luminescence is produced in many different ways, fluorescence and phosphorescence have become particularly important as analytical tools. Not only do they serve to single out a specific substance in a complex chemical system, but by virtue of their known lifetimes they help sort out the mechanisms of complex reactions.

Although fluorescence often refers to any emission with a lifetime between 10^{-9} and 10^{-7} sec, the term is defined rigorously as emission from an excited singlet state after absorption of electromagnetic energy. Wher a molecule in the ground state absorbs light it is raised to an upper vibrational level of an excited singlet state in about 10^{-15} sec (the Franck-Condon principle). Then in 10^{-13} to 10^{-11} sec the molecule loses vibrational energy and drops to the lowest vibrational level of the first excited singlet state. The lifetime of this state is 10^{-9} to 10^{-7} sec. Should the molecule finally return to the ground state through emission of a photon, the process is fluorescence.

In a similar manner, phosphorescence often refers to emission with a lifetime greater than 10^{-5} or 10^{-4} sec. Actually, a transition to the lowest triplet state can occur while the molecule is in the lowest singlet state. Rates for this intersystem crossing are comparable to those of most fluorescence emission, 10^{7} to 10^{8} per sec. Once in the metastable triplet state the molecule loses further vibrational energy until it returns to the lowest vibrational level of the triplet state with a characteristic lifetime of 10^{-4} to 10 sec or more. The return of such a molecule to the ground state by emission of a photon is phosphorescence. Thus, the theoretical and pragmatic differentiations between fluorescence and phosphorescence agree.

Knowledge of luminescent properties, particularly of proteins, predates modern science. Luminescence of an extract of the wood **Lignum nephriticum** was reported by Monardes as long ago as 1565 (1). In 1746 Becconi observed phosphorescence of his hands after vigorous washing and exposure to strong sunlight (2). And in about 1852 G. G. Stokes conclusively associated fluorescence or phosphorescence with proteins and proteinaceous materials (3). In 1913 Lehmann succeeded in photographing luminescence of cells viewed through a microscope (4). Although some of these early interpretations are suspect because of probable contaminants, fluorescence was unquestionably observed.

Until about 1920 the choice of excitation sources was restricted to arcs between carbon or metal electrodes. Modern fluorometry came of age with the introduction of a stable, intense, and long-lasting source, the gas discharge lamp. On the heels of this advance came comparable milestones in monochromators, in photomultipliers, and in high-performance amplifiers. Framed in terms of results, general quantitative determinations of luminescent materials below ppb levels, concentrations not detectable by most other analytical methods, became a reality.

Many of the accomplishments of the late 40s and early 50s were byproducts of investigations of the readily available heme proteins and the porphyrin molecule aromatic ring structure which is the basis for all heme groups. Since then fluorescence has broken out of its limited biological and biochemical shell to become a versatile quantitative tool in

many other areas. Development and refinement of instrumentation and techniques have enhanced the technology on all levels.

Its inherent advantages over both other types of spectroscopy and physical analytical methods have earned fluorometry widespread acceptance. Being an emission effect, it is a measure of a weak signal against a totally empty background. By contrast, at low concentrations, absorption spectroscopy depends on measurement of the difference between an intense signal and an intense reference. It is like weighing the difference between an elephant with a penny on its back and an elephant. As a consequence, detection in fluorescence is limited by noise arising from the electronics, especially the photomultiplier; detection in absorption is limited by the stability of the instrument. Given the best equipment in both, the detectivity in fluorescence plunges several orders below that of absorption.

Again, because fluorescence entails the emission of light, the effect is a linear function of concentration over a wide dynamic range. That for absorption, an exponentional function, is restricted to about three decades. Even with a sample as small as 200 μl, the linearity of fluorescence analysis stretches from ng/ml to mg/ml.

Thus far, we have dwelt on fluorescence emission spectra: plots of the changing wavelength of the emitted light while the wavelength of the exciting light remains fixed. Conversely, fluorescence measurements can also be made in an excitation mode: a plot of the changing wavelength of excitation while the wavelength of emission remains fixed. Normalized to the number of quanta incident on the sample, an excitation spectrum and an absorption spectrum ordinarily turn out to be identical. Since the quantum yield of a typical fluorescent molecule remains constant over its absorption spectrum irrespective of the exciting (absorbing) wavelength, the number of quanta emitted is a constant fraction of the number absorbed.

In practice an excitation and an absorption spectrum may differ, but almost invariably the excitation spectrum provides sounder information. At low concentrations, instabilities obscure the true absorption spectrum; at high concentrations, absorption may be essentially complete, again departing from the true. Further, whereas absorption measurements of obstinate samples such as solids, plastic films, or turbid liquids are often impossible, such materials behave normally in excitation fluorescence.

Fluorescence is also more specific than absorption in the uv and visible regions. Although all fluorescent molecules absorb light, only about 5% of all absorbers fluoresce. Potential interference is diminished proportionately. And finally, since there are two different properties to consider (excitation, or absorbance, and emission), the results may be combined in various fashions to yield much more information than is available from the absorbance alone.

Fluorescent molecules (fluorophores) can be classified as extrinsic or intrinsic. A fluorophore is intrinsic if it is present in the original sample. If it is the only such molecule in the system, so much the better, for then it can be characterized with a minimum of uncertainty: only perturbations equal to the energy added by the exciting light can possibly interfere.

Frequently, however, no suitable intrinsic fluorophore is present. For example, the fluorescence lifetime of tryptophan in a protein ($\sim$4 ns) is too short to record the rotational relaxation times ($\geq$100 ns) for large proteins. Or there is the situation at low concentrations where the poor quantum yield of an intrinsic fluorophore limits detection.

In recent years, extrinsic fluorophores have become a powerful means for overcoming such limitations. Commonly termed "label," "probe," or "tag," extrinsic fluorophores are added to mark transformations not otherwise traceable. Labels may be covalently bound within a compound or more loosely associated through hydrophobic affinity, hydrogen bonding, ionic interactions, or some such weaker force. Choosing a specific label which can be covalently bound to the analyte makes it easy to track. Another method, which has the advantage of minimal interference, is to choose a fluorescent chemical analog of the analyte or a compound which interacts with the analyte.

Fluorophores normally contribute information about a system by their preferential interactions, reaction kinetics, or effects on energetics of the system. A fluorophore with an affinity for a particular solvent provides information about the immediate environment of the label. In a classic example 1-anilinonaphthalene-8-sulfonate (ANS) reacts preferentially with hydrophobic species resulting in a fifty-fold increase in quantum yield and a blue shift in the emission maximum by as much as 40 nm. The time scale for fluorescence measurements (10^{-12} to 10^{-8} sec) is suitable for detecting events ranging from submolecular motions to rotations and translations of large macromolecules; that span is handy for characterizing many biochemical mechanisms. By comparison, spectral absorption is limited to changes that occur in the 10^{-15} sec dictated by the Franck-Condon principle, during which interval little kinetic information can be gained. In fluorescence, kinetics can be observed directly with very fast electronics, as in the measurement of a time-dependent spectral shift in emission with solvent reorientation (5, 6), or indirectly through a continuously measured parameter such as polarization. The degree of depolarization is a function of a molecule's structure and rate of rotation between the times it absorbs and the times it emits; this rate can be determined (7) from the emission lifetime. Recently, the energetics of interactions within a system have been related to changes in lifetime and to degree of binding of the label (8).

As an analytical technique, fluorescence has yet another advantage: most procedures, chemicals, and ancillary equipment are interchangeable with those for colorimetry and uv absorbance. And because samples can be viewed front-face, fluorescence demands no complicated sample preparation or handling.

APPLICATIONS

The many advantages of fluorescence were first widely recognized and applied in the fields of biochemistry and biology, primarily as a research tool. Conformation of proteins and changes in conformation as a function of parameters such as pH, ionic strength, or temperature have been studied. Binding constants for fluorescent substrates of enzymes are directly measurable by changes in fluorescence quantum efficiency. For example, the binding energy of reduced nicotinamide adenine dinucleotide (NADH) to various

dehydrogenases is determined by the increase in NADH fluorescence emission at 420 nm during binding. Kinetics of individual steps in enzyme mechanisms are isolated by stopped-flow fluorescence; assays for specific enzymes are based on the rate of appearance or disappearance of a fluorescent substrate; and orientations of small molecules bound to some macromolecules are definable. Energies of interaction between small molecules bound to proteins are calculated from effects on the binding constant of a fluorescent substrate. Biologists add fluorescent labels to systems as complex as muscle (9) and nerve tissue (10) and observe changes due to external stimuli. Special microscopes make fluorescence within individual cells visible to human eyes.

In the biology-related field of clinical chemistry, fluorescence has long been a widespread quantitative and semiquantitative tool. Examples abound. The aromatic amino acids tyrosine, tryptophan, and phenylalanine are routinely assayed on the basis of their characteristic emission spectra. Some antibodies are quantified by the changes in polarization of fluorescent antigens (11). As the small antigen is bound to a large antibody, its rotational relaxation time and its polarization rise markedly. Udenfriend (12) is recognized for developing and publishing many early clinical procedures based on fluorophores; now numerous assays are continually being published.

Fluorescence is at home in the quality control of pharmaceuticals; penicillin, thiazine, hormones, and vitamins are among the host of products regularly assayed in production laboratories. In forensic science fluorescence (along with chromatography) is essential for the detection of exceedingly minute concentrations of barbiturates, alkaloids, salicylates, and hallucinogens that tend to evade other analytical techniques.

Although biological challenges spurred development of fluorescence instrumentation, many industries have hopped on the bandwagon. One of the more exciting new applications is in petroleum and petrochemistry. Both emission and excitation fluorescence spectra of oil are distinctive for different source oil fields. This is true whether the oil is refined or still crude. The Coast Guard is developing methods to identify the sources of oil spills in harbors from characteristic fluorescence spectra. Similarly, since the oil's spectra change as a function of aging or oxidation, it may be possible to predict the remaining life of lubricating oils from their fluorescence.

In the detergent industry fluorescence has become an aid as well as an analytical tool. Madison Avenue decrees that a detergent must do much more than merely lift dirt from laundry. One essential attribute of a salable detergent is its ability to mask the yellowing of cloth with age. To achieve this, brighteners—materials that fluoresce in the blue when energized by violet or ultraviolet light—are added. Spectrofluorometers easily evaluate the brighteners' effectiveness as to both degree and persistence after repeated rinsing.

In inorganic chemistry, too, fluorescence analyses have found many applications. Because of the ease of sample preparation and the extremely low limit of detection, fluorometry is widely favored.

The growing worldwide concern for air and water quality has given impetus to development of new techniques for detecting and identifying pollutants. Fortunately, many harmful contaminants in air and water are aromatic organic molecules which fluoresce. Often influenced by such readily controlled parameters as pH, fluorescence helps identify the molecule sought even in the presence of others. Thanks to its ppb detection limits fluorometry frequently eliminates tedious extractions.

A relatively recent entrant into polymer chemistry, fluorometry takes on tasks difficult or impossible to handle by absorption: front-surface geometry is the key. Plastic films can be analyzed quantitatively without regard for thickness variations. Solids can be examined directly to detect and quantify antioxidants, ultraviolet screening agents, plasticizers, and impurities in films, powders, pellets, or finished products. Since different polymers have different emission characteristics, the morphology of samples, as well as molecular weights (13) and orientations within polymers (14), can be obtained through fluorescence.

SPEX FLUOROLOG: DESIGN FEATURES

Right from the source, careful consideration went into the design of the FLUOROLOG. Either a 150 or 450W xenon arc can be plugged in. Normally operated cw, the lamp can be pulsed with a special phosphorimetry attachment; then the pulse rate can be varied up to 60 Hz and the sampling time from 1 μs to 10 ms. To maintain focus on a sample regardless of the excitation wavelength, and to assure maximum collection of light from the lamp, a large, off-axis ellipsoidal mirror was selected.

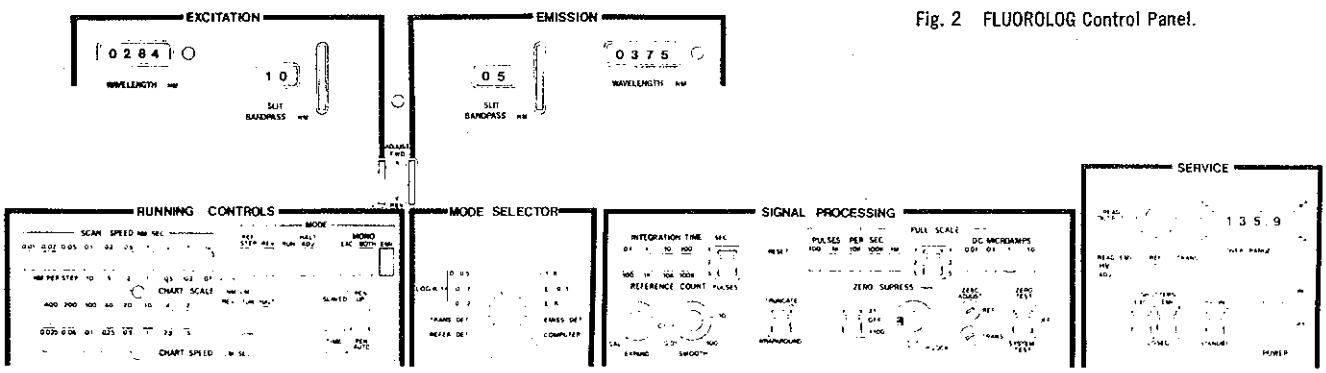

Fig. 2 FLUOROLOG Control Panel.

In monochromators, low scattered light and high optical throughput are the two paramount criteria for discerning the weak signals typical of fluorescence. Almost the entire amount of scattered light in any monochromator originates from imperfect gratings. Irrespective of their quality, gratings scatter some of the incident light instead of diffracting all of it: overall background fog is generated. In a double monochromator the scattering level can be reduced by the square of that in a single monochromator, while the loss in signal from the additional optics is only about 50%. Assuming a scattering reduction from 10^{-5} to 10^{-10}* going from a single to a double monochromator, S/N is thus improved nmensely.

Taking advantage of our extensive experience in Raman spectroscopy, we designed two identical DOUBLEMATE monochromators into the FLUOROLOG. Their optical configuration results in so-called subtractive dispersion: by teaming the gratings so they rotate in unison but in opposite directions, the light impinging on the sample (excitation) or on the photomultiplier (emission) is homogenized. That means that instead of being spectrally dispersed it is rendered uniform across the slit. Think of this as light produced by an ideal (but non-existent), variable, square-wave, band-pass filter. Homogeneous light avoids errors that might occur if adjacent regions of a sample or of the photomultiplier received different wavelengths of light.

High luminosity is achieved by incorporating into the monochromators large gratings (50 mm square), blazed for greatest efficiency at 250 nm for excitation and 450 nm for emission. Mounting these gratings in a modified Czerny-Turner configuration of 0.22m focal length results in a high aperture ratio of f/4.

The Spex modification of the Czerny-Turner layout consists of separating the two mirrors to skirt a source of interfering radiation that is often confused with scattered light. Properly referred to as rediffracted light, this results when long-wavelength diffracted light from the grating finds a reflection path back to the grating and contributes to the background. Spatial separation of the mirrors in the Spex design is a factor in achieving such low detection limits.

A digitized detection system of photon counting is among the most boastworthy FLUOROLOG features. Numerous advantages accrue from counting the actual photons impinging on the PMT cathode instead of measuring the resulting direct current:

1) signal-to-noise is about 3 times better (15);

2) digital outputs for display, logging, and computer-entry are not subject to analog-to-digital converter errors;

3) "box-car" integration accumulates all the signal with much greater accuracy than RC exponential-decay integration;

4) the emission signal is independent of fluctuating high voltage;

5) the FLUOROLOG's nine-digit counter provides superior accuracy and precision;

6) digital signals mate with auxiliary instrumentation, some, yet to be developed.

The signal path starts with the emission detector, a high-gain PMT operated at a fixed (plateau) voltage. Already, there is one advantage: voltage regulation is less than critical since within the plateau a voltage change of as much as 1% does not affect the photon counting rate. Pulses from the PMT are processed in a preampdiscriminator adjacent to the PMT to minimize cable capacitance and provide higher counting rates. The discriminator is essentially an electronic threshold, passing only those pulses large enough to be true photon signals.

Signal pulses then enter a nine-decade counter accumulating up to 999,999,999 counts, read and reset to zero at intervals set by the mode and method of operation. In a ratio mode, resetting is done when the reference counter is filled; for "box-car" integration, resetting occurs after a fixed time.

In the FLUOROCOMP, the counters are read directly into the computer. In the FLUOROLOG, they are read into a dedicated calculator which is also connected to full scale and integration time switches. Correcting for the integrating time the calculator multiplies the total count by appropriate factors to generate a number equal to the actual percent of the selected full scale value.

This number, the intensity value sought by the analyst, is dispatched along various electronic paths. It is presented digitally on a panel meter that reads from 0 to 10 times the recorder full-scale value; through a D→A converter it is sent to the x-y recorder; for data logging it is sent to a digital interface. The display meter has an overrange capability permitting it to process signals 10 times as high as anticipated. Analog presentation on the recorder displays the same degree of overranging through a wrap-around feature: upon reaching the top of the scale, the pen slews back to zero and continues to trace.

Reference and transmission detectors are normally exposed to a level of light far greater than that measurable by counting individual photons. Accordingly, dc currents are measured and signal-to-noise is, of course, not a problem. The PMTs are matched pairs, energized by matched rhodamine B quantum converters whose response is flat over the range 200-600 nm. This, coupled with identical optical paths to both detectors, assures accurate comparison regardless of wavelength, polarization, and intensity.

Output of the detectors is first balanced then converted to digital pulses through V→F techniques. All subsequent signal handling is the same as in the emission channel. This includes division of transmission by reference, scaling, division by integration time to yield count rate instead of total count, and output to the interface and display. Only one compromise has been made in our obsession for digitizing: in deference to the bulk of scientists conditioned to interpret data in units of hills and dales, an analog chart emerges on the x-y recorder. This can be presented either as straight in-

* Too often, measurement of instrumental scatter has deteriorated to a numbers game. To avoid ambiguity, our figures are based on the intensity of a spectral line originating from a monochromatic laser line. From a continuous xenon source, scattering, though difficult to quantify, is usually stipulated at about 10^{-3} for a single monochromator.

tensity or as the negative log of intensity for absorbance readings. Note, however, that the x-axis of the recorder is run by a stepper motor to preserve the digitizing. Its speed can be varied for expanding wavelength presentation.

The sample chamber of the FLUOROLOG is a box about 18 x 18 x 20 cm attached to the basic instrument so that a gap is opened on the side and bottom of the instrument when it is removed. Hence, borrowing from lathe terminology, the term "gap bed." As in a lathe, the gap bed in the FLUOROLOG permits the experimenter a wide latitude in the construction of sampling devices. Liquid nitrogen and liquid helium cryostatic sample holders will fit, as will a fair-sized magnet. Of equal importance, the gap bed 1) makes the beam from the excitation monochromator accessible for processing before it impinges on the sample, and 2) permits excitation by a laser which, by virtue of its being monochromatic, need not be passed through the excitation monochromator.

MODES OF OPERATION

The FLUOROLOG can operate in many modes. The optical paths are combined in Fig. 4 where it may be seen that the path from the source to the beam splitter is common to all modes. Light from the xenon arc source is dispersed by the excitation monochromator and may be polarized before it reaches the beam splitter—a coarse (8 grooves/mm) reflection grating with opposing facets ruled at equal angles to the incident light. This and subsequent optics assure identical treatment of the two light paths, a prerequisite for accurate optical comparison.

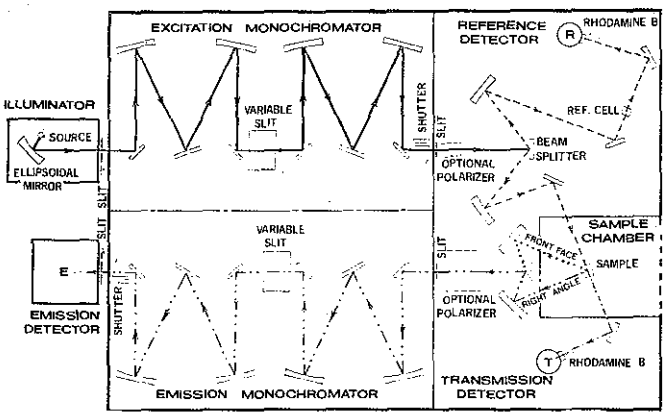

Fig. 4 FLUOROLOG Light paths. Light traverses all paths shown whenever the source is lit and shutters open, but choice of mode determines operational detectors.

In the simplest mode, a reference spectrum of the source is recorded. The light path is represented in Fig. 4 by the solid line and the dashed line from the beam splitter to the reference detector. In the transmission mode the intensity of light transmitted by the sample is recorded, uncorrected for incident light. This is the path shown as the solid line, the dashed line to the sample, and the dash-dot line to the transmission detector. The identical electronics of the two modes are diagrammed in Fig. 5.

Then, as a dual-beam spectrophotometer, the FLUOROLOG can record either transmittance (T/R) or absorbance (log (R/T)) of a sample and automatically include the blank correction. Half of the light from the beam splitter is passed

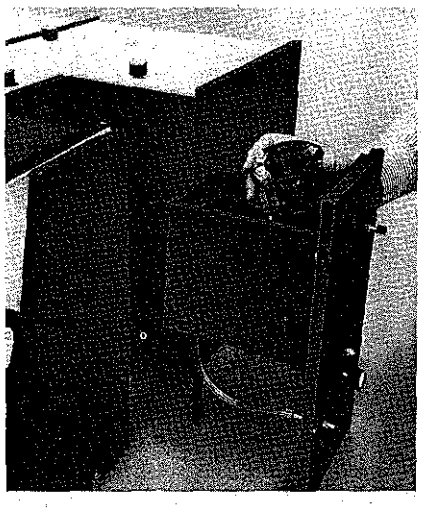

Fig. 3 Rotary Sample Holder, its Sample Compartment opened to demonstrate the ease of handling.

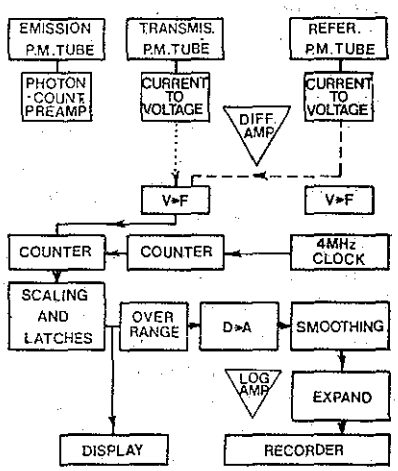

Fig. 5 Reference and Transmission Modes. Dashed line represents logic for Reference mode, dotted line that of Transmission mode, and solid line that common to both.

through the blank in the reference cell, the other half through the sample. In these modes counts of transmission signal are accumulated for a selected number of reference pulses, after which the FLUOROLOG automatically scales the ratio of accumulated transmission to reference signals and transmittance is digitally displayed at the same time that either the absorbance or transmittance spectrum is recorded. The electronics schematic is Fig. 6.

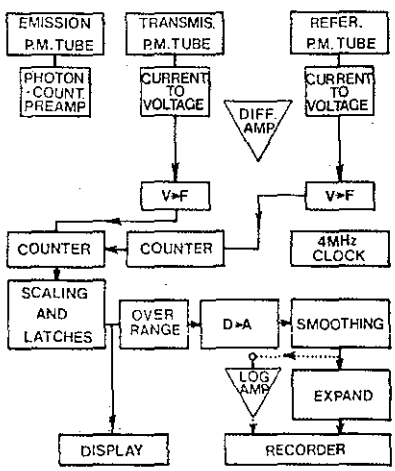

Fig. 6. Transmittance and Absorbance Modes. The dotted lines represent the extra step to record Absorbance; solid lines denote logic flow common to both modes.

Performing its ultimate function of producing emission and excitation spectra, the FLUOROLOG puts both its monochromators to work. When the excitation monochromator is scanned while the emission is held constant, an uncorrected excitation spectrum is produced; when the emission monochromator is scanned while the excitation is held constant, the result is an uncorrected emission spectrum. These emission and excitation spectra are obtained in the emission mode, but they can also be recorded in another mode—emittance (or E/R). The emission path is the solid line, the dashed line to the sample, either the dotted line or the dash-dot line to the switchable mirror, and the dash-dot line to the emission detector. For emittance this beam is referenced to that impinging directly on the reference quantum counter (Fig. 7). Since the reference response is proportional to the number of incident quanta, and the emission response is proportional to emitted quanta, the ratio as a function of exciting wavelength is a corrected excitation spectrum.

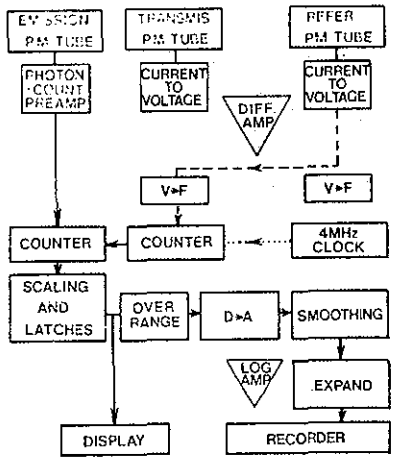

Fig. 7 Emission and Emittance Modes. The solid lines denote the sequences in both modes. The dashed lines represent the reference input for Emittance, and the dotted line the configuration for uncorrected Emission.

Attaining truly corrected emission spectra is no easy task. Responses of both emission monochromator and photomultiplier vary with wavelength and polarization of the light. Geometry of the collection optics, linearity of the detector amplifier, and stability of the light source also must be considered. In addition to instrumental factors, such sample characteristics as absorption, quantum efficiency, quenching, refractive index, scattering, and anisotropism must be corrected for. Whether to correct an emission to units of quanta with a constant wavelength bandpass or to units of energy with a constant energy bandpass, as is required for quantum yield measurements, adds another dimension to the problem. Usually good photomultiplier and monochromator choices obviate the need for corrections. In those few cases where a corrected emission spectrum is truly needed, it is better to make all corrections at one time than to make a simple wavelength response correction first and then have to adjust for other effects. To accommodate these instances, the FLUOROLOG is designed to interface directly to a computer (as in the Spex FLUOROCOMP) or other calculating device capable of performing the complex arithmetic.

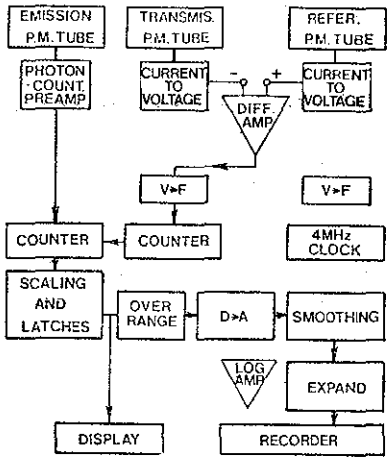

Fig. 8 Relative Fluorescence Efficiency Mode. This diagrams the electronics which assemble data according to the formula E/(R-T).

A mode of operation unique to the FLUOROLOG among commercial instruments is "relative fluorescence efficiency" (Fig. 8). Defined as E/(R-T), it is the number of quanta emitted per quantum absorbed. It has been shown that if the emission spectrum is invariant with excitation wavelength, then the relative fluorescence efficiency measured at one wavelength is linearly related to the total quantum efficiency (18, 19). The relative fluorescence efficiency is constant across an absorption band for an ideal compound; deviations from the ideal indicate the presence of impurities or anisotropism.

Computer Control

The FLUOROLOG has been designed so that it can operate with a computer controlling both wavelength scanning and data gathering. A system incorporating the necessary computer and software will be offered as the FLUOROCOMP. This system not only acts as a data collection device, but also makes decisions in real time to change such parameters as integration time, scaling, and signal-to-noise (total number of photon counts). Subroutines already developed or being written include: repeat scanning of emission or excitation spectra; storage and retrieval of data in a floppy disc mass storage system; manipulation of stored data (such as arithmetic, smoothing, peak detection, and changing individual values); data entry via remote keyboard; printing, plotting, or punching output; automatic calculation of "white body" emission correction curves; provision for additional programs (Fortran or Basic) to manipulate stored data.

In operation, all three detectors are sensed by the computer. Data from all channels are transferred to the computer in a BCD (binary-coded decimal) bit-parallel character-serial format. Monochromator and recorder movements are directed by pulses from the computer. Calculated results are either displayed on the instrument's standard x-y plotter or on any peripheral which is directly connected to the computer. In short, the fluorometer functions as a slave to the computer.

ACKNOWLEDGMENTS

A continued and intensive effort, development of the Spex FLUOROLOG involved almost everyone associated with the company during the past four years. Rather than single out a few of the major contributors and thereby hint at a lack of appreciation for the others, we take this opportunity of thanking the more than a score of dedicated, conscientious people whose ideas and labors have culminated in a product of which we are all deservedly proud.

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FLUOROLOG SPECIFICATIONS

OPTICAL

Source: Choice of: 150 watt xenon arc lamp, focusing through an off-axis ellipsoidal mirror; **OR** 450 watt xenon arc lamp

Beam Splitter: 8 gr/mm, equal-angle reflection grating

Emission Optics: Front-face or right-angle viewing selected by flat mirror controlled externally

Polarization Kit (optional): Optics consist of a single Glan-Thompson polarizer in the excitation beam, and a uv Polacoat film polarizer and a uv scrambler in the emission beam

Emission PMT: Choice of: bi-alkali, low dark count, quartz window, uncooled; range 190-650 nm; **OR** GaAs, thermoelectrically cooled with less than 30 pps dark count; range 190-860 nm; **OR** trialkali, liquid nitrogen cooled, range 650-1000 nm

Reference and Transmission PMTs: Side window, S-20 response, matched

MONOCHROMATORS

Type: f/4, double-grating 0.22m Czerny-Turner with subtractive dispersion

Dispersion: 4 nm/mm

Range: 190-1000 nm (At wavelengths below 200 nm, nitrogen purging is required)

Readout: Direct in nm on two 4-digit counters, readable to ±0.1 nm

Accuracy: ±1 nm

Precision: ±0.2 nm

Entrance and exit slits: 5 removable slits; widths nominally 0.25, 0.5, 1.25, 2.5, and 5 mm

Intermediate slit: continuously variable from 0 to 65 nm bandpass, readable to ±0.1 nm

Gratings: 1200 gr/mm, kinematically mounted for easy replacement: excitation gratings blazed at 250 nm, emission gratings blazed at 450 nm

ELECTRICAL

Lamp Power Supply: 150 watt, low-ripple, power regulated; **OR** 450 watt, low-ripple, current regulated

High Voltage Supplies:

Emission: 400-2000 V, front panel adjusted

Reference and Transmission: 400-1000 V, front panel adjusted

Photon Counting (Emission):
Pulse Pair Separation: 30 nsec, for a maximum synchronous rate of 33 MHz; preamplifier bandwidth is 100 MHz

Drift: 0.01% plus 0.3% of any zero suppression

Accuracy: 0.1% plus 0.3% of any zero suppression

Zero Suppression: 0-500 and 0-50,000 counts/sec

Full-Scale Ranges: 15 from 100 counts/sec to 5 million counts/sec, in 5 decades, with a 1, 2, 5 sequence

Direct Current (Reference and Transmission):
Linearity: 0.5%

Drift: 0.1% after initial warm-up, plus 0.3% of any zero suppression

Accuracy: 1%

Zero Suppression: 0-0.5μA and 0-50 μA

Full-Scale Ranges: 12 from 10 to 50 μA, in 4 decades, with a 1, 2, 5 sequence

Absorbance Scales: 0-0.5, 0-1, and 0-2
Drift: 2%

Accuracy: 2%

Analog Output: 0 to 100 mV for the chart recorder

Digital Output: TTL-compatible accumulated counts to computer on 4 lines: BCD, bit-parallel, character-serial **AND** TTL-compatible count rate, on 16 lines: BCD parallel

Digital Panel Meter: 4-digit counter, switchable to display high voltage or output

Monochromator Drives: Reversible stepping motors; 50 steps/nm; maximum stepping rate 500 steps/sec; continuous or burst scanning

Speed Ranges: 0.01 to 10 nm/sec in 4 decades with a 1, 2, 5 sequence (continuous mode)

Data Point Spacing: 0.1 to 10 nm/data point in 3 decades with a 1, 2, 5 sequence; dwell time per data point 0.1 to 500 sec **OR** dwell time dependent on 100 to 5 million reference counts (burst modes)

Reverse Slew: 10 nm/sec

External Pulses: Accepts TTL pulses up to 500 Hz (650 Hz if ramped); pulse rate of 50 Hz corresponds to 1 nm/sec

Recorder: 100 mV full scale, potentiometric flat-bed; x-y type for 28×43 cm

Interfacing: Facilitates addition of digital peripherals and/or computer. All lines are buffered, TTL-compatible. Intensity channels are readable in BCD digit-serial form; four parallel BCD digits of processed data are provided for data logging. Important gate outputs and control lines, including those to the stepping motors in the monochromators and recorders, are also accessible.

SAMPLE COMPARTMENT: "Gap-Bed" for ready replacement or modification
Dimensions: 18×18×20 cm

Standard Holder: For single sample cell, 10-mm path, of non-fluorescing fused silica, Teflon stopper

Rotary Holder (optional): For sequential positioning of six 10-mm cells

SERVICE REQUIREMENTS: 115V, 50/60 Hz

DIMENSIONS: 122×75×52 cm

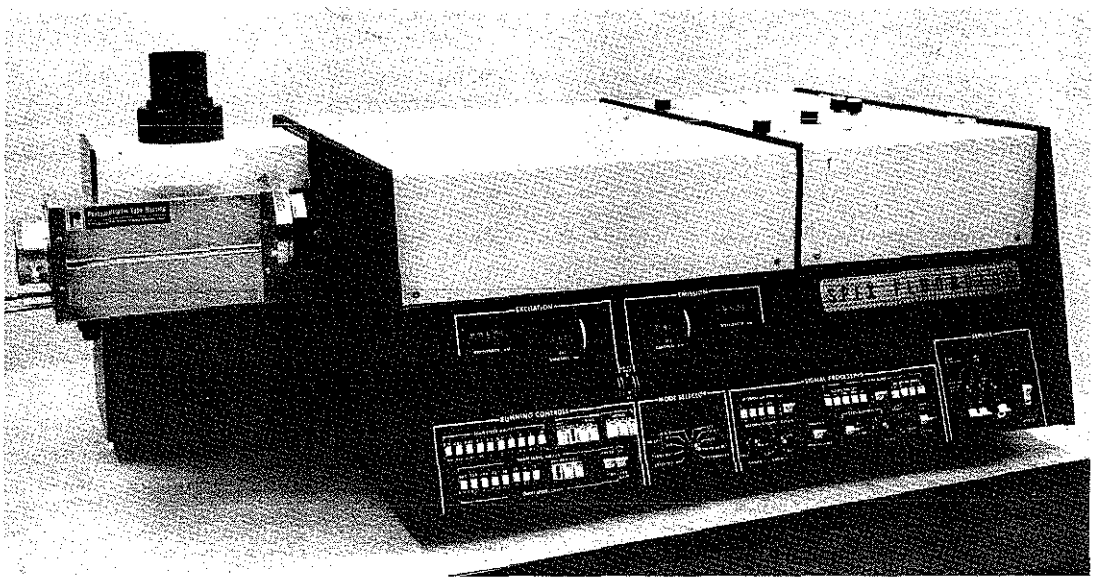

Fig. 1 Spex FLUOROLOG spectrofluorometer. The #1911 emission photomultiplier is shown in place (at left, in front of illuminator housing).

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