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FOCUS ON FLUORESCENCE

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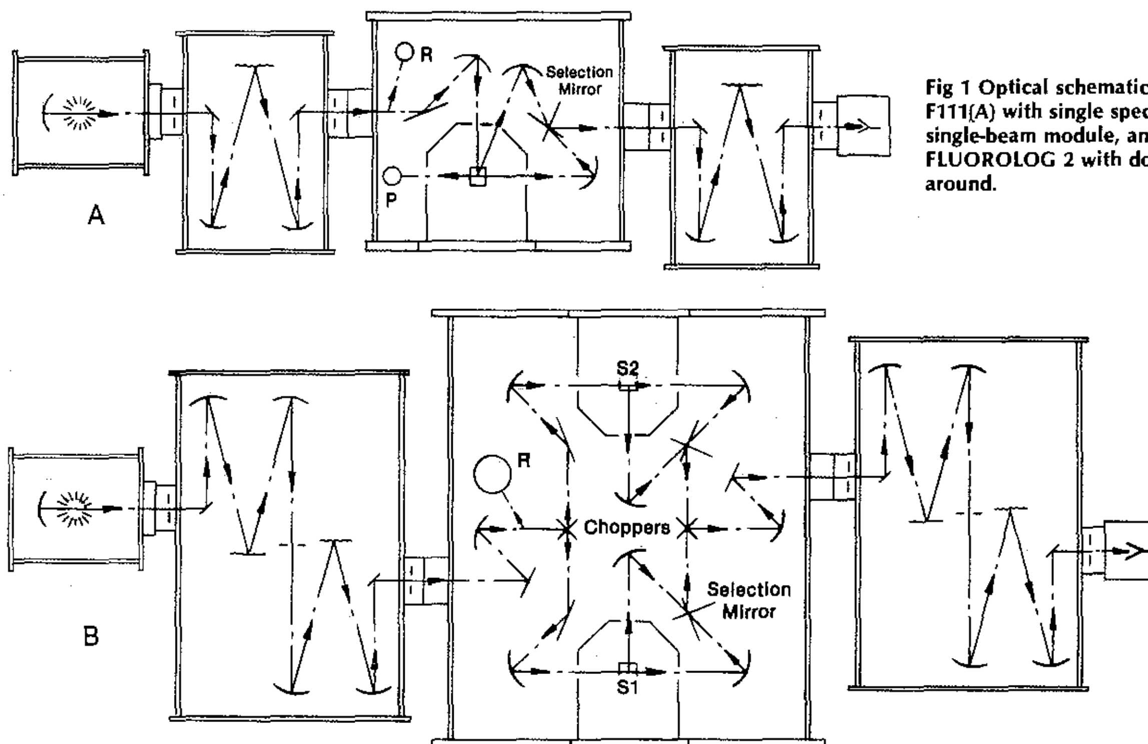

Fig 1 Optical schematics for the F111(A) with single spectrometers and a single-beam module, and the F222(B) FLUOROLOG 2 with doubles all around.

When biologists and chemists first tried to exploit the phenomenon of fluorescence, its temperamental personality must have been a nuisance. Some substances fluoresced only weakly or only in special solvents, while others stubbornly refused to yield any light at all, no matter what tricks were played on them. Fortunately, as time passed and experience accumulated, this acute environmental dependence, or specificity as it is often known, was recognized for what it is: a powerful, responsive probe into the microscopic world inhabited by molecules and cells, a beacon that signals the subtlest changes in its surroundings.

Examples are easy to find. When a fluorophore latches on to a protein, a sudden surge in its fluorescence may mark the event. An alteration of the proton gradient across a cell boundary can tilt the balance of peak heights in an excitation spectrum. Polarization, temperature, lifetimes, and quantum yields are all variables in the fluorescence equation. Each of these parameters claims its own distinctive perspective. And it takes a special breed of spectrofluorometer to translate so many different views into a coherent picture.

The Right Questions

At times, the possibilities and permutations inherent in the fluorescence technique seem almost as numerous as the researchers in the field. So many questions are asked that no single instrument can provide all the answers. To that end, the FLUOROLOG 2 Series of spectrofluorometers was devised. The FLUOROLOG 2 is not one, two, or even three spectrofluorometers designed in a compromise effort to please most of the people most of the time. Instead the basic concept of the FLUOROLOG 2 is modular; individual instrument systems are custom-assembled for a particular lab. You get to choose among sources, spectrometers, sample compartments, detectors and accessories that will work best for you.

Consider the simplest and most sophisticated optical layouts in Fig 1. The most economical version — with the highest throughput — is Model F111: a single-grating spectrometer for excitation radiation, a single-beam module for right-angle or front-face sampling, and a single spectrometer in the emission beam. The quintessential Model F222, in a higher price bracket, delivers the ultimate in resolution and stray-light rejection with two double spectrometers, and a dual-beam module for comparing two samples in real time. Nested intermediate between these are the F211, the F121, etc. Different as they are, all share such convenient features as a gap-bed sample compartment for accessories and unique sampling schemes; and all interact with the Spex DATAMATE, for overall spectrometer and detector control, data manipulation, bulk spectral storage, and conveniently labeled hard copies.

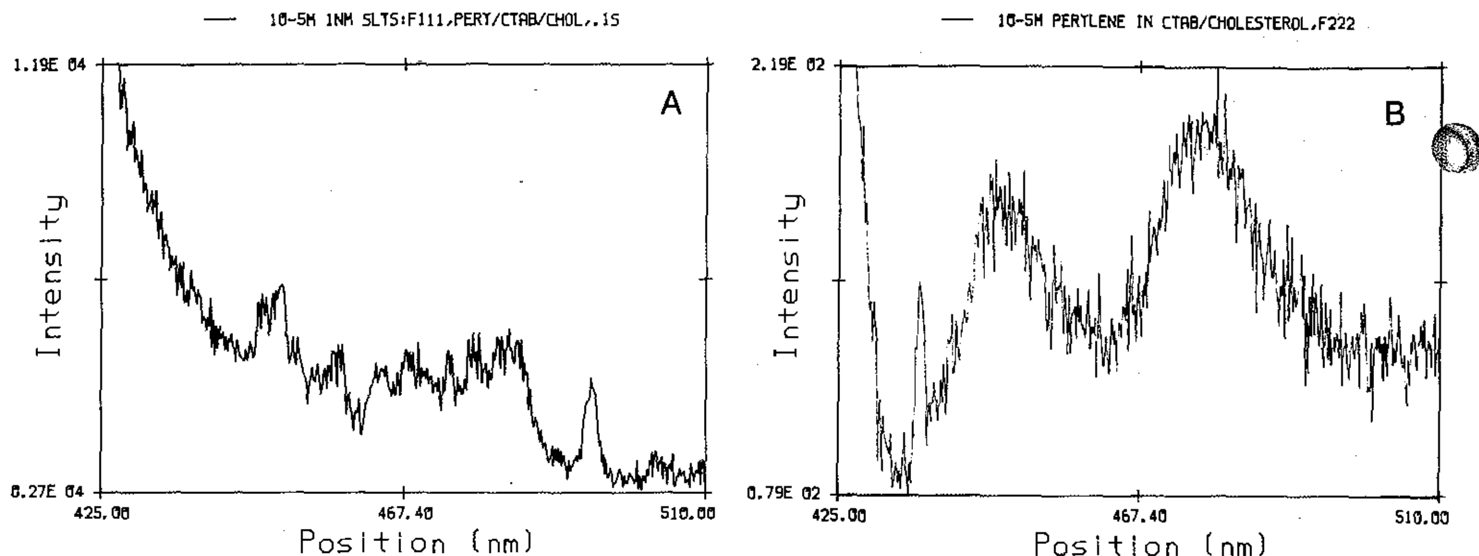

Fig 2 Highly scattering micellar solutions of CTAB and cholesterol introduce a level of stray light that masks perylene fluorescence with single spectrometers (A); the same sample yields clearly definable peaks with double spectrometers (B).

One Grating — or Two?

By virtue of their superior stray-light rejection, double spectrometers are the obvious choice for turbid, highly scattering samples. What this means to your research can be seen in Fig 2 where perylene was dissolved in a particularly murky solution of 10^{-3}M cetyltrimethylammonium bromide (CTAB)/cholesterol micelles. The peaks of the fluorophore are utterly swamped by stray light leaking through single spectrometers (A), yet all are clearly defined by doubles (B)

If a double performs so well, why consider a single at all? Well, first of all a single spectrometer, with fewer optical surfaces, is more efficient. Its higher throughput may be needed to analyze weakly fluorescing samples or others at rarified concentrations. Economy is another reason; a single spectrometer is simply less expensive. And when Stokes shifts are routinely large, and spectral features fairly broad, the full potential of double spectrometers may not be significant.

How Many Beams?

Is single or double-beam better? That depends . . .

A single beam module (as in Fig 1) keeps the number of optical surfaces to a minimum, increasing the throughput and decreasing the price. Note also that a swingaway mirror collects sample luminescence either in a normal right-angle direction, or from the front face of the sample (22.5° from the excitation angle). This oblique position is especially valuable for solid, turbid, or highly absorbing samples such as the one that provided the tryptophan spectra in Fig 3. Trace A was acquired in the right-angle position where very little fluorescence escaped the optically dense solution. But with the flip of a knob, trace B was produced — now with so intense a signal that the DATAMATE versatility was tested; we switched from pc to dc detection. An ideal application for a single beam instrument.

Some problems, however, do not lend themselves to single beam treatment. Should your sample be temperature or light sensitive, or undergo a kinetic reaction that might sabotage reproducibility between two runs, then the value of double-beam simultaneous operation becomes evident. Note the 2-naphthol (solid line) and triethylamine (NEt_3) in acetonitrile spectra in Fig 4. When these two solutions are combined and excited, they form excited state complexes (or exciplexes) which may yield luminescent

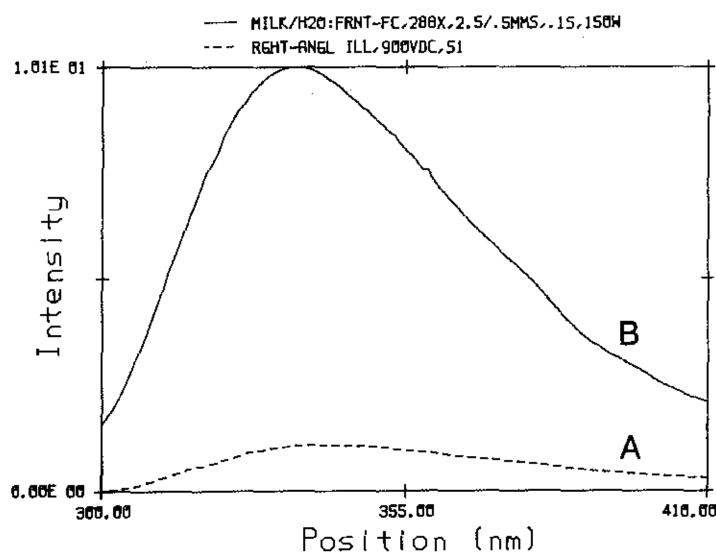

Fig 3 When milk is viewed at right angles to the exciting beam (A), its fluorescence is trapped inside. Viewed front face on the single-beam module, however, the sample displays the characteristic spectrum of tryptophan (B).

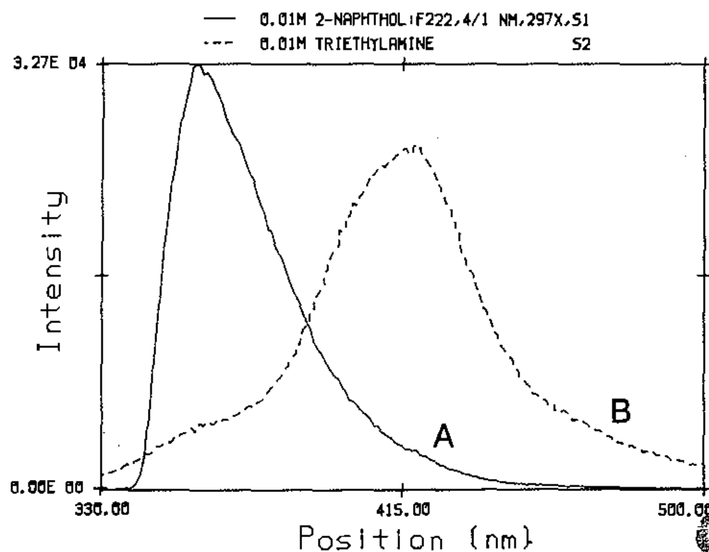

Fig 4 Spectra of separate solutions of 10^{-2}M 2-naphthol (A) and triethylamine (B).

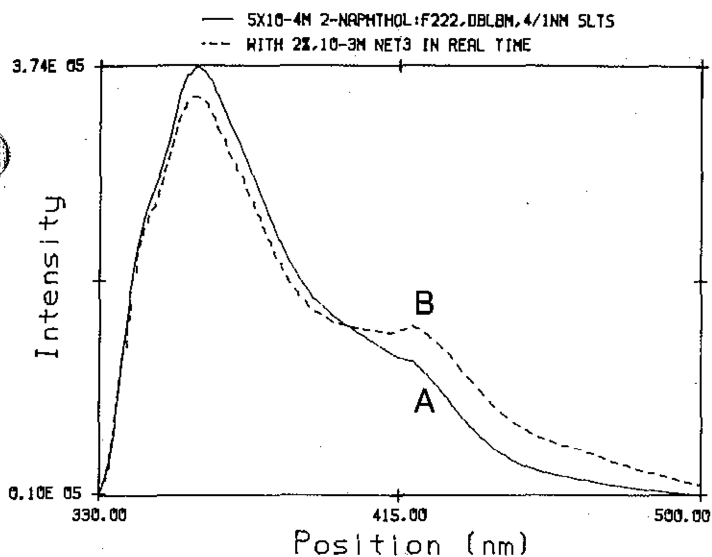

Fig 5 When 2-naphthol (A) is mixed with 10^{-3}M triethylamine (NEt_3), exciplex formation results in quenching and the appearance of a bulge above 415 nm (B).

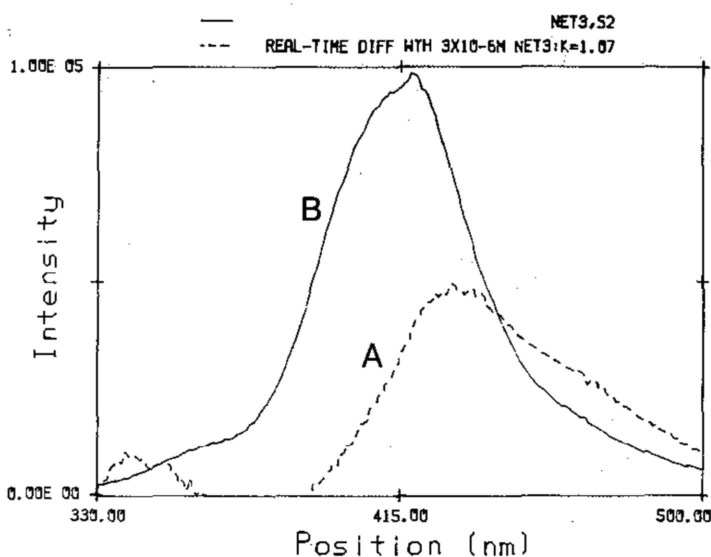

Fig 6 Real-time subtraction (A) with the FLUOROLOG series dual-beam sample module demonstrates that the new fluorescent bulge in Fig 5 is not simply NEt_3 (B) but a characteristic emission from exciplex formation.

spectra significantly different from either substance alone. These exciplexes have been identified as intermediaries in many photochemical reactions [1]. Fig 5 compares the spectra of 2-naphthol before and after the addition of 10^{-4}M NEt_3. Quenching of 2-naphthol fluorescence is immediately evidenced by the drop in signal intensity of the main peak (B). The increase at about 415nm, however, may mistakenly be ascribed to NEt_3 itself. Fig 6 denies this assumption. With 2-naphthol in one beam, and the mixture in the other compartment of the dual-beam module, DATAMATE portrayed their real-time difference spectrum (6A). Even a cursory comparison with the NEt_3 spectrum (6B) shows that the difference spectrum is actually a new fluorescence band characteristic of the exciplex itself.

Whether a double or single-beam module is the final choice, 1-to-1 focusing of the spectrometer slit image onto the sample permits precise alignment of microsamples. In Fig 7, for instance, the spectrum of fluorescein in NaOH was recorded from a 10 μl capillary. Extrapolation from this data indicates that the FLUOROLOG 222 could see fluorescence from a speck of fluorescein as minute as 0.04 picograms.

The variety of FLUOROLOG 2 optical layouts is flexible enough to follow almost any twist in the course of your research. For those recalcitrant samples, those eccentric solutions that demand individual attention, we prescribe one or more of the following fluorescence options and sample-pampering accessories.

An Exciting Technique

Before a substance radiates, it must be excited. Thus, one of the fundamental tasks of a spectrofluorometer is to determine which excitation wavelengths contribute the most to fluorescence. This excitation scan is acquired by positioning the emission spectrometer on a known fluorescence peak while scanning the excitation spectrometer and recording intensity as a function of excitation wavelength. The results resemble an absorption spectrum. But they do differ. As a rule, every substance with a fluorescence spectrum generates a corresponding absorption spectrum. The converse need not be true; absorbed energy can be released by processes other than the emission of light (heat, for example).

For a dilute solution, a relationship

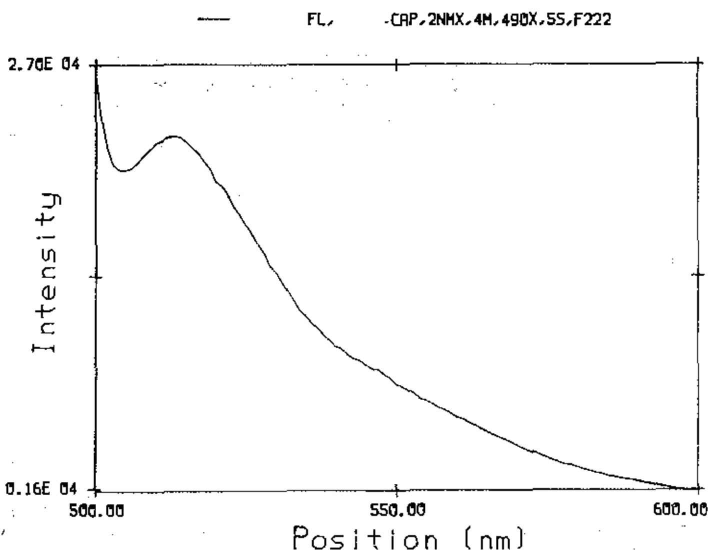

Fig 7 For this spectrum, fluorescein in NaOH was injected into a 10μl capillary and aligned in the gap-bed of an F222. One-to-one focusing of the excitation and emission slits onto the sample allows detection of sub-picogram quantities of the material.

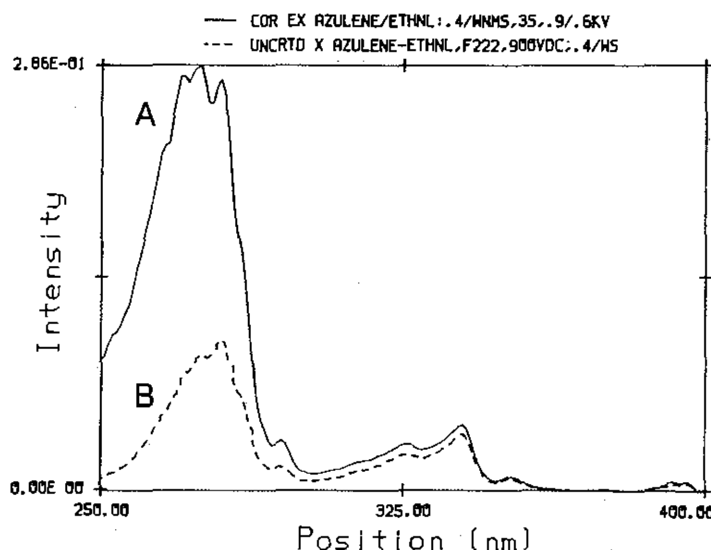

Fig 8 The true excitation spectrum of azulene in ethanol is drastically different from one uncorrected by the FLUOROLOG's reference counter (B).

can be drawn between excitation and absorption spectra by the equation:

$$I_m[\lambda] = kI_x[\lambda]Q\,\varepsilon\,[\lambda]$$

Here I_x equals the intensity of the light source, Q the quantum efficiency of the sample (amount of radiation emitted per radiation absorbed), ε the absorption coefficient, k a constant of proportionality, and I_m the intensity of fluorescence emission. Note that both ε and I_x depend on wavelength. To measure ε with a spectrofluorometer, therefore, the variation of excitation intensity with wavelength must be considered. All models of the FLUOROLOG accomplish this with a quantum counter in the sample module (R, Fig 1). A fused-silica beam splitter directs a small portion of the exciting light to a quantum counter which reports the resulting signal to the DATAMATE. As data accumulates in this S1/R1 mode (Emission detector signal scaled by reference quantum counter signal), any change in the intensity of the exciting light is automatically compensated and

$$\varepsilon\,[\lambda] = I_m/(I_x kQ) = S1/R1 \times Q$$

Fig 8 demonstrates the radical difference this excitation correction produced. The dotted line traces the uncorrected spectrum. An absorption spectrum of the same sample (also run on the FLUOROLOG) appears in Fig 9. Dissimilarities between absorption and excitation spectra arise when the quantum efficiency (Q) is a function of wavelength. This may originate from impurities, from the formation of excited complexes, or from energy transfer between molecules. Excitation spectra inherently contain more information than absorption spectra. By comparing the two, astute scientists often discover a new molecular handle at their disposal. In fact, excitation presents several advantages over absorption:

1. Selectivity — In a mixture of substances, each component will contribute its own absorption spectrum and therefore peaks can overlap, destroying identifying signatures. However, if one or more of the components do not fluoresce (Q = O), they will contribute nothing to the excitation spectrum and the interference will be eliminated. Also, fluorescence provides two independently variable wavelength parameters (excitation and emission) while absorption offers only one.
2. Detectivity — Revealing excitation spectra can be obtained from samples at concentrations that are orders of magnitude below the limits of detection of the absorption technique.
3. Versatility — Obviously, absorption spectra cannot be obtained from an opaque sample. On the other hand, the excitation spectrum will not only characterize the substance, but will also demonstrate the best wavelength at which to excite the sample during an emission scan.

The real question is not which of the two techniques is superior; rather which applies best to a given situation or sample. Together, excitation and absorption spectra by the FLUOROLOG 2 allow an added degree of freedom to suit the technique to the sample — a much more rational approach than trying to fit the sample to the technique.

The Correct Way

To fully exploit the potential of a fluorescence probe in biological systems, the quantum efficiency of the fluorophore must be obtained [3]. Such information can frequently be extracted from a comparison of the compound's spectrum with some standard of known quantum efficiency [4]. In this case, accuracy mandates that the inevitable distortions due to wavelength dependence of detector response, grating efficiencies and the like, be removed from the data; a radiometric correction must convert the raw, recorded intensity values into true spectra.

One of the most consistently reliable methods for correcting emission spectra is based upon the output of a standard tungsten lamp [5]. The SPEX correction option relies upon an NBS calibrated lamp with irradiance values certified from 250 to 1000 nm. After a scan of the emission spectrometer over the lamp spectrum, the certified values, divided by the measured intensity values, yield appropriate correction factors. These factors may then be stored in the EAROM, (non-volatile portion of DATAMATE's memory) to be applied on demand, in real time or at your leisure, to fluorescence emission spectra. All manipulations and required interpolations are automatic. The effectiveness of such radiometric correction is evidenced in Fig 10, with the apparent (B) and true (A) spectra of quinine sulfate.

Polarization

Assuming a molecule remains motionless between the time it absorbs radiation and the instant of emission, the original polarization of the exciting light will be preserved. If, however, a molecule rotates far enough during this interval (a period shorter than the lifetime of the excited state), it disrupts this orientation and the emission is depolarized. Within these two extremes lies the province of fluorescence polarization.

Anything that inhibits a molecule's freedom of movement alters the polarization of its fluorescence. For instance, when a small probe binds to a massive protein, the observed polarization takes

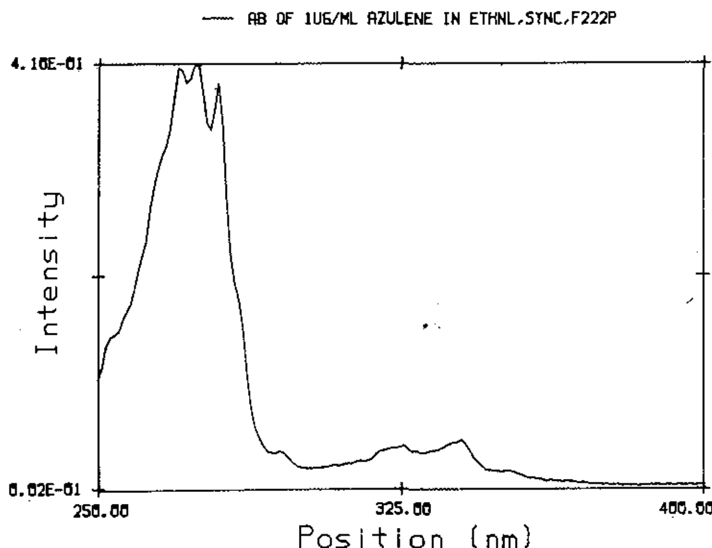

Fig 9 The absorption spectrum of azulene taken on the Model F222 is almost identical, in form, to its excitation spectrum.

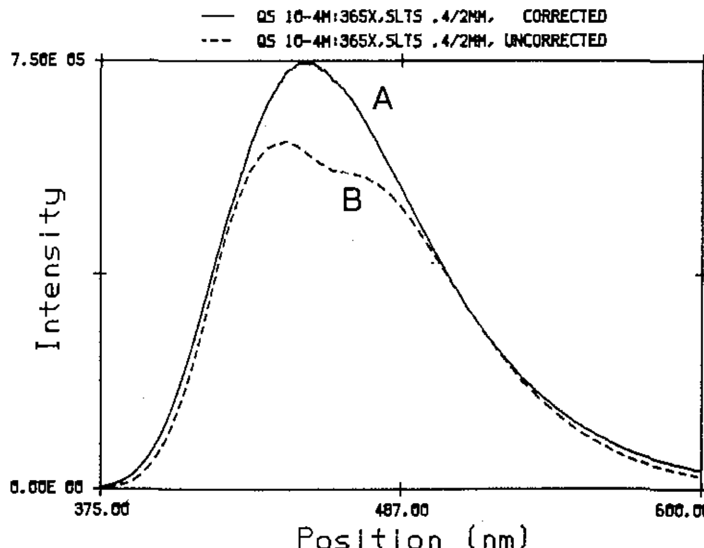

Fig 10 Uncorrected (B) emission spectrum of quinine sulfate and the same spectrum radiometrically corrected by DATAMATE.

a jump, the magnitude of this enhancement depending on the size and conformation of the protein, the tightness of binding, etc. For unbound probes, on the other hand, the more viscous the medium in which the fluorescing molecule finds itself, the slower its rotation. In general, the depolarization characteristics of a probe imbedded in a sample are an accurate measure of the host's microviscosity as well as the degree of its anisotropy. From these data, parameters such as the volume of micellar aggregates can be calculated. FLUOROLOGs accept two different accesories for the measurement of polarization. They differ by their arrangement of the Glan Thompson polarization analyzers around the sample. (Fig 11A). In the simplest configuration, a polarizer before the sample converts unpolarized exciting light to a chosen orientation. Similarly, the analyzer mounted at right-angles to the sample allows only that radiation with the selected orientation to pass on to the emission detector where intensity can be recorded. If we call the polarization component in the plane of the drawing vertical (v) and the one perpendicular horizontal (h) four fluorescent intensities can be measured (I_{vv}, I_{hh}, I_{vh}, I_{hv}). From them we calculate a polarization ratio.

$$P_r = (I_{vv} \times I_{hh})/(I_{vh} \times I_{hv})$$

which relates to the actual degree of polarization by $P = (P_r-1)/(P_r+1)$. Relationships such as the fundamental Perrin equation [6] tie the degree of polarization to parameters like viscosity, temperature, lifetime, and the effective volume of the fluorescent sphere. Another scheme for measuring polarization, which also cancels out any specious effects of lamp fluctuations during measurements, is the T-configuration [7] shown in Fig 11B. Here emission from the sample is viewed in two directions simultaneously, through analyzers oriented in opposite directions. By recording the ratios of these signals, only two measurements (at each position of the excitation polarizer) rather than four, yield the degree of polarization. In fact, with DATAMATE user programmability, keyboard commands can be strung together into a complete routine that will scan the polarization/excitation spectrum in one polarizer orientation, pause for a flip of the polarizer, then continue with the second scan. Once both spectra are safely stored in memory, DATAMATE will calculate and display the result automatically.

Among the most suitable probes for the study of micellar systems are polynuclear, aromatic hydrocarbons. Charged with small dipole moments, they are unlikely to perturb the environment they explore, and these probes easily penetrate the surfactant aggregates. In particular, perylene has been shown to be suitable [8] since it absorbs strongly in the visible, has a high quantum yield, and possesses a lifetime compatible with the range of microviscosities measurable with the polarization technique.

Fig 12 shows the polarization spectra (P) calculated from the excitation scans of perylene immersed in a 30/70 ethanol/glycerol solution at three different temperatures. Note how the degree of polarization steadily rises as the temperature of the 1931 Sample Heater/Cooler decreases thereby increasing the viscosity of the sample.

Out in the Cold

In solutions, mobile fluorophores fall prey to interactions which, at the very least, smudge the spectral features; at worst, fluorescence is completely quenched. At room temperature, the main culprit responsible for broadening of spectral features is the inhomogeneous nature of the solvent [9]. In effect, the environment of each fluorophore perturbs the natural line width of its spectral features to a given degree and/or direction. When these individual contributions are summed in the fluorescence spectrum, the peaks inflate as well as overlap. However, the closer the matrix in which the fluorophore finds itself approximates a rigid regular crystal, the smaller will be this inhomogeneous broadening. Freezing the sample is an effective way to accomplish this.

A direct outgrowth of this enhanced resolution at low temperatures is an increase in selectivity. A highly resolved spectrum not only is easier to identify, especially in the company of competing fluorescence, but the increased separation between absorption and emission spectra reduces the possibility that the sample will reabsorb its own emission and bleed away signal intensity.

Though FLUOROLOG's variable-temperature accessory works well with fluorophores suspended in viscous media, many solvents remain fluid far below this accessory's range (-70C). Accordingly, SPEX designed a cryogenic sample chamber around a non-fluorescing, fused-silica Dewar flask that suspends sample tubes in liquid nitrogen during analyses. At 77K, most solvents freeze, either into crystals, glasses, or snows. These rigid media imprison fluorescing molecules, keeping them in protective custody, out of reach of quenching molecules. Fig 13 illustrates the degree of resolution enhancement attainable from typical samples in liquid nitrogen. Pyrene fluorescence, whose vibrational fine-structure pattern has probed into the hydrophobic regions of membranes and

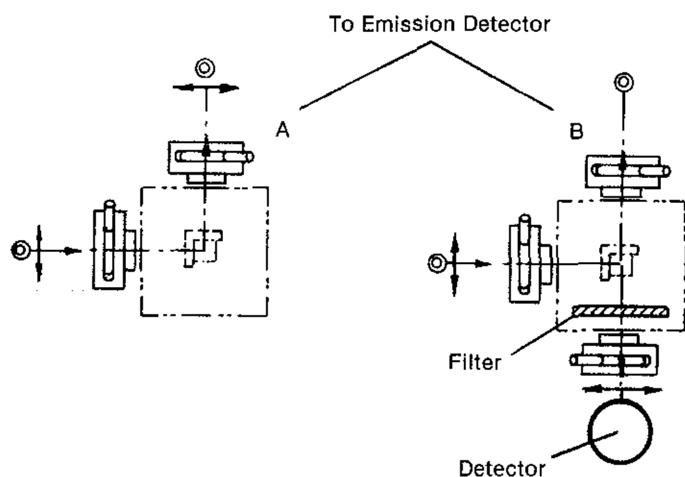

Fig 11 The normal polarizer scheme (A) requires four measurements while the T-configuration (B), which views two analyzers simultaneously, only requires two measurements.

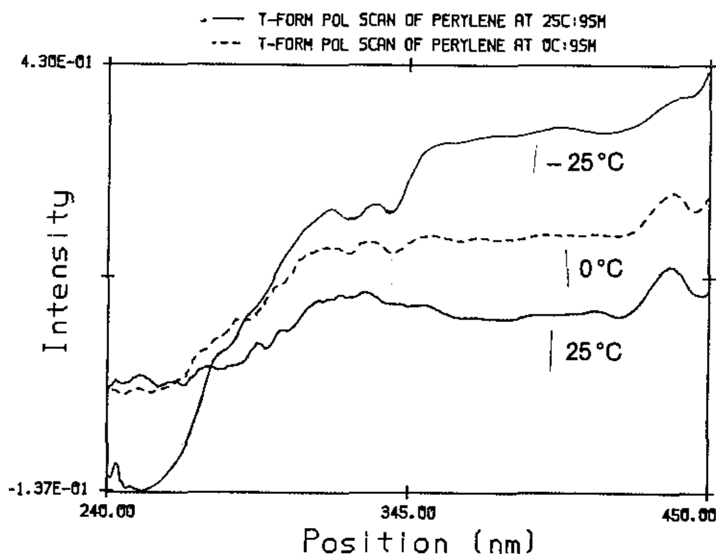

Fig 12 Excitation/polarization scan of perylene in 30/70 ethanol/glycerol at three different temperatures demonstrate the increasing viscosity of the probe's environment.

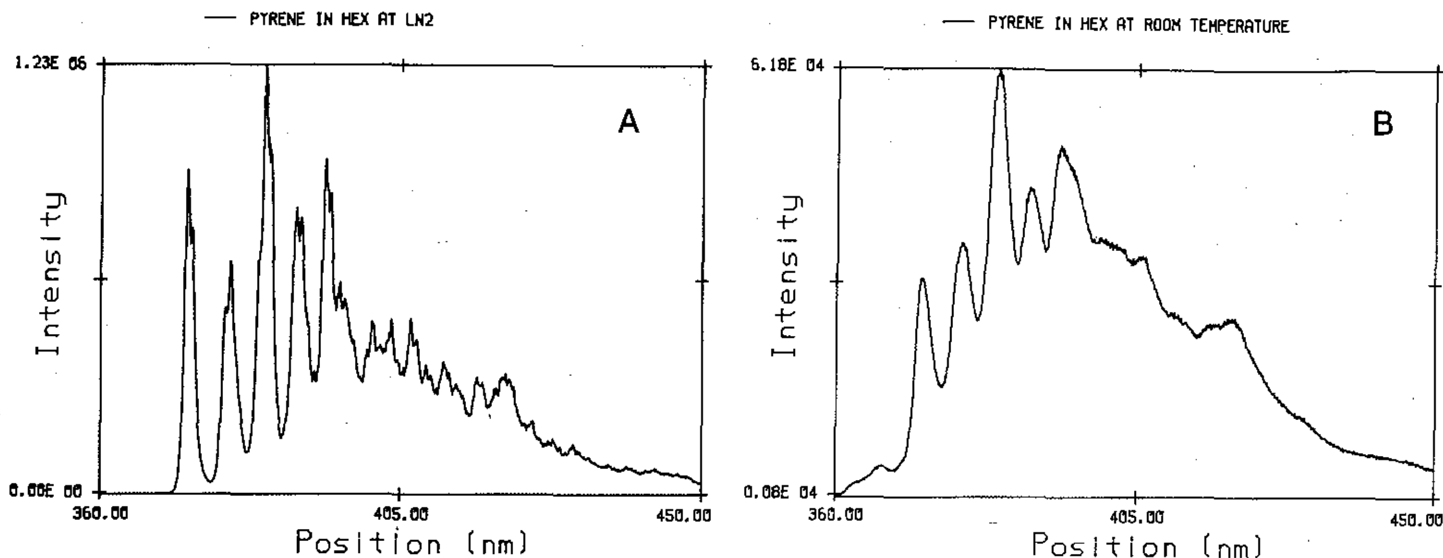

Fig 13 At 77K (A), pyrene fluorescence separates out into its fine-structure contributions, though at room temperature they merge (B).

proteins, is shown at both room temperature (B) and 77K. In its frozen state over 100 individual transitions can actually be distinguished in hydrocarbon solvents [10,11].

Of course, to profit from this detail, a spectrofluorometer must be able to resolve the peaks. FLUOROLOG has no trouble separating the two lines (Fig 14), a scant half nanometer apart.

Note also the expanded upper portions of the traces in Fig 15. At 77K, the triplet excited state now lives long enough to phosphoresce above 500nm (A). In contrast, at room temperature (B) every trace of this long-wavelength luminescence is extinguished.

MSRTP

The ability of the cryogenic accessory to coax normally dormant phosphorescence out into the open demonstrates why phosphorimetry, at least until the last few years, has been shackled to the liquid nitrogen Dewar flask. Only in the strictly ordered environment of a frozen sample, it seemed, could the triplet state survive to release its delayed glow. Unfortunately, not all samples lend themselves to freezing. Biological systems cannot be examined *in vivo*, and ice crystals may puncture delicate cell walls. That, and the nuisances of special handling, impose a rather severe limit on the applicability of the technique.

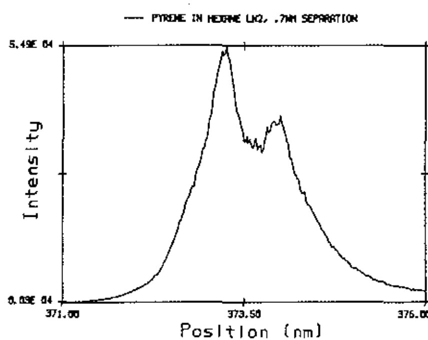

Fig 14 The F222 easily resolved these two closely spaced peaks out of the fluorescence pack.

One method for inducing phosphorescence at room temperature is simply to adsorb the sample compound on some solid surface, say filter paper [12]. This rigid matrix isolates molecules from quenching by moisture or oxygen. Because of its sensitivity and selectivity, this method of observing room-temperature phosphorescence (RTP) has been successfully applied to trace analyses of organic compounds [13]. However, the approach has also been criticized as cumbersome, time consuming, and plagued by background luminescence from the substrate. Responding to these complaints, micelle-stabilized room temperature phosphorescence (MSRTP) has been proposed to view delayed emission in aqueous solutions while also speeding analyses and limiting background [14]. Here, phosphorescing molecules become sequestered inside aggregates of amphiphilic molecules (micelles) which protect them from destructive encounters with quenching species. The results can be seen in Fig

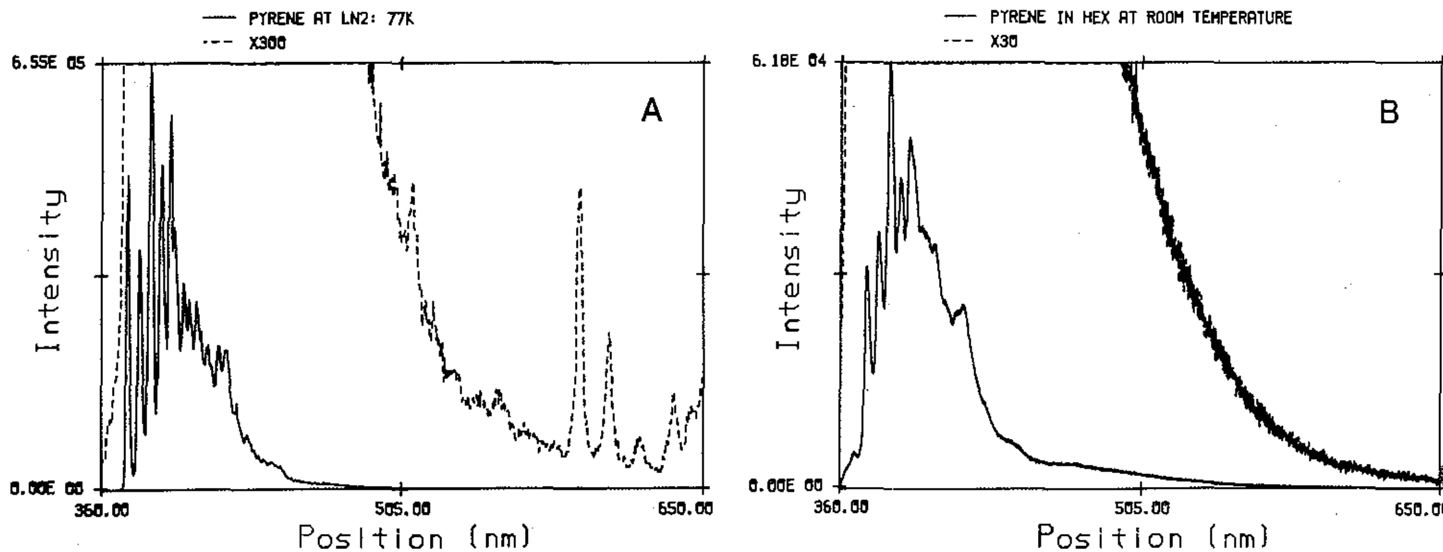

Fig 15 Liquid nitrogen temperatures also allow the triplet state to live long enough to emit radiation as phosphorescence (A). At room temperature these triplets are easily annihilated (B) so no peaks appear in the expansion.

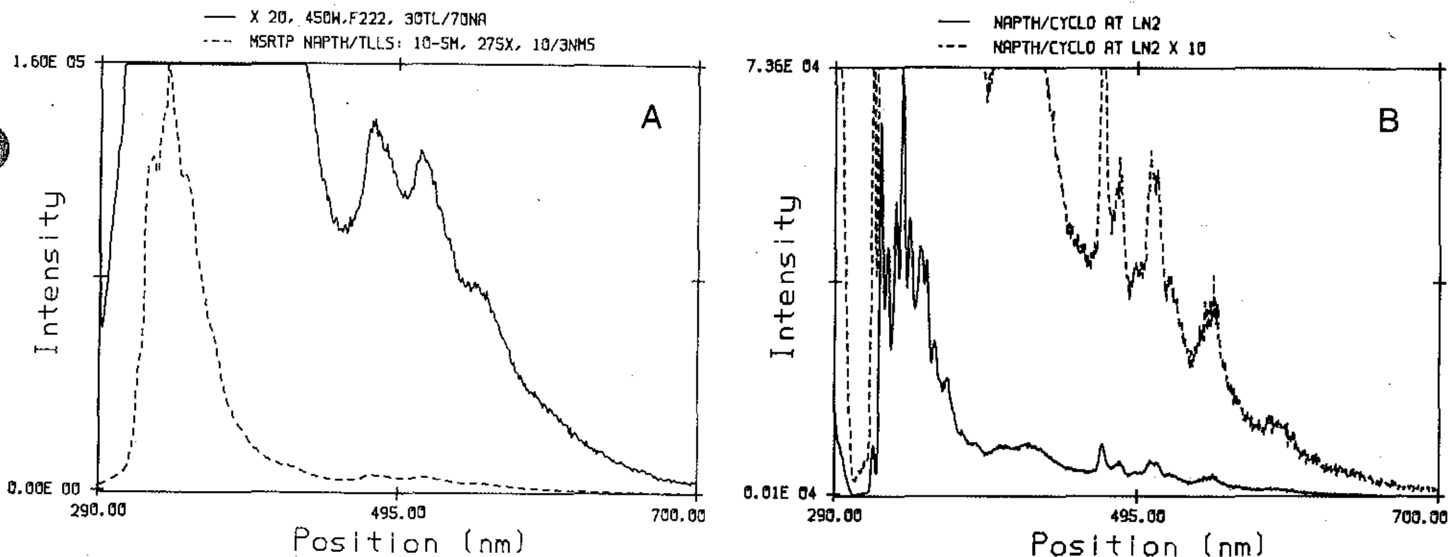

Fig 16 MSRTP spectrum of naphthalene in thallium lauryl sulfate exhibits phosphorescence in the expanded view (A). That compares to the phosphorescence of naphthalene at 77K shown in (B).

16, where naphthalene has been introduced to an aqueous solution of thallium lauryl sulfate (TlLS) micelles.

These particular micelles are doubly effective. Not only do they shield the fragile triplet excited state, but the presence of thallium introduces a perturbation (heavy-atom effect) that facilitates the transition to the ground state singlet through the phosphorescence pathway. Saved from extinction, the phosphorescence clearly emerges in the region above about 500 nm (expanded trace). Compare this to naphthalene at 77K, shown in Fig 16B, which exhibits the same phosphorescence structure, albeit better resolved.

Delaying Tactics

Even if phosphorescence is the most familiar manifestation, it cannot claim to be the sole mechanism responsible for persistent luminescence. Delayed fluorescence, an interesting variant on long-lived emission, can also occur.

Normal fluorescence results when a molecule in an excited singlet state (S1) releases energy in the form of radiation which rapidly decays back to the ground state singlet (S0). The process takes only a few nanoseconds to complete. Delayed fluorescence, on the other hand, may be of two types, depending on the mechanisms responsible for the lag between excitation and emission [15]. In E-type delayed fluorescence, the molecule detours through the excited triplet state responsible for phosphorescence, only to be knocked back into the singlet state by thermal agitation, from where it can then fluoresce. P-type involves the transfer of energy between two such triplets, one of which is promoted back into the excited singlet, the other of which is deactivated radiationless to the ground-state singlet. All three mechanisms (normal, E-type or P-type delayed fluorescence) involve identical emitting transistions (S1→S0).

As a consequence, their spectra are virtually indistinguishable with a typical spectrofluorometer. What better candidate to demonstrate the time-discrimination power of the 1934 Pulsed-lamp Phosphorimeter accessory to the FLUOROLOG 2?

Delayed fluorescence appears to be a general feature of chloroplasts in the leaves of green plants and may also be an integral part of the primary photosynthetic reaction [16]. Fig 17 presents three spectra obtained *in vivo* from a rhododendron leaf excited by the SPEX phosphorimeter. The resulting spectra were sampled after delays of 20, 30 and 40 µsec respectively. The decay of delayed fluorescence did not follow an exponential relationship and was further complicated by a mixture of E-type and P-type fluorescence. But signal-gating circuitry eliminated any possible contributions from normal short-lived fluorescence.

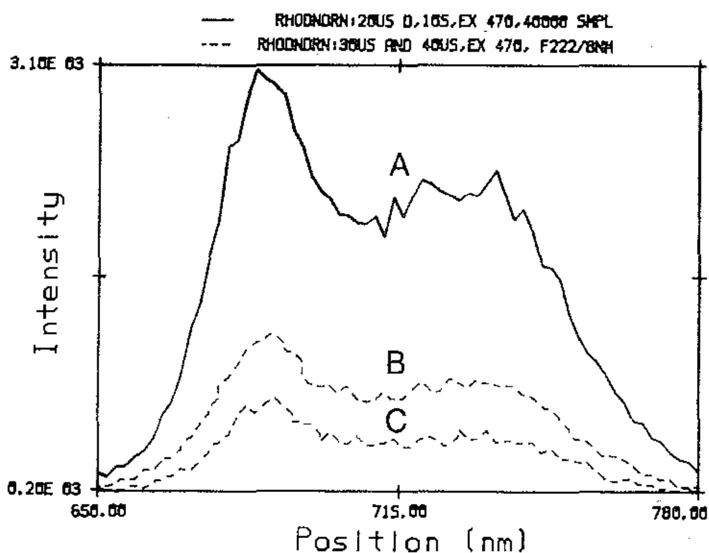

Fig 17 Delayed fluorescence from a rhododendron leaf after a 20 (A), 30 (B), and 40 (C) µsec delay has been introduced between excitation and sampling.

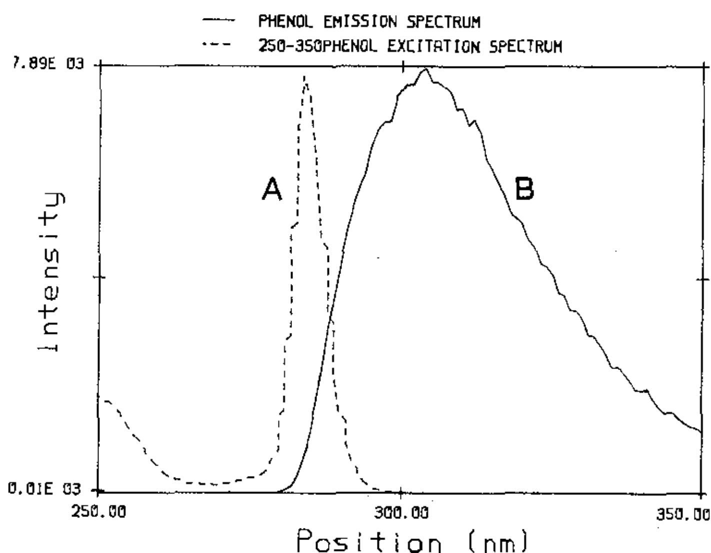

Fig 18 Excitation (A) and emission (B) spectra of phenol display significant overlap between 280 and 290 nm.

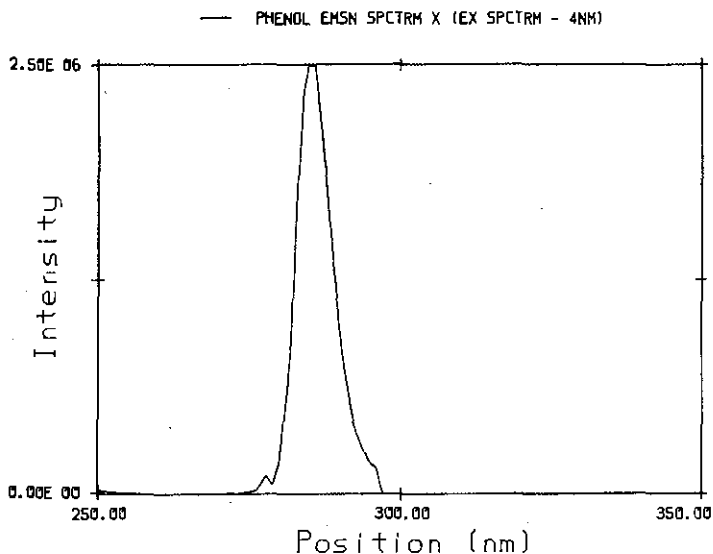

Fig 19 Synchronous spectrum of phenol with 4-nm offset between spectrometers enhances this overlap while ignoring the rest of the spectrum.

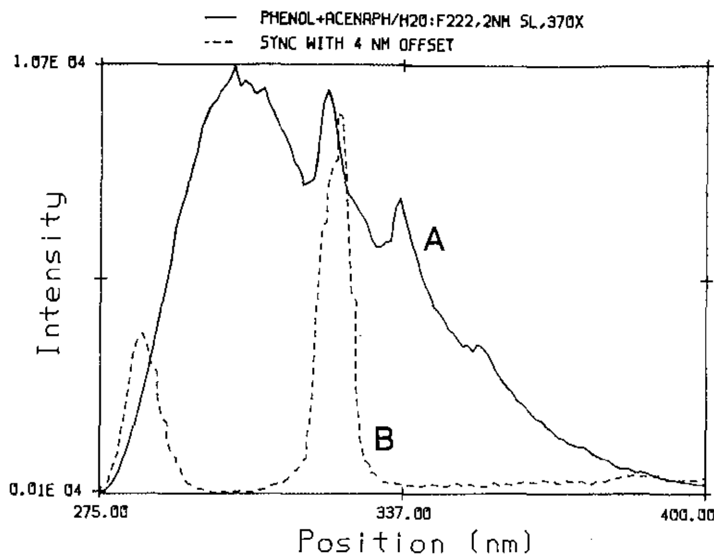

Fig 20 Emission scan of a mixture of phenol and acenaphthalene (A) shows severe interference between components. With synchronous scanning, the components are clearly separated.

Synchronized Scanning

This particular application has earned the distinction of being the most misunderstood of all spectrofluorometer techniques. Where most of us are accustomed to scanning either the excitation or the emission spectrometer while the other is held stationary on a given fluorescence or absorption maximum, synchronous spectrometery has us scan both spectrometers simultaneously, usually with a specific wavelength offset. The result is a convolution of the emission and absorption (more precisely, the excitation) spectra [17]. For example, if the emission spectrum of the substance is described by the function E (λ), while the excitation is represented by X(λ), the synchronous spectrum will be

$$S(\lambda) = E(\lambda + \Delta\lambda) * X(\lambda)$$

where $\Delta\lambda$ is the difference in wavelength between the two spectrometers during the scan.

The primary application of synchronous spectrometry to date has been in the realm of pollutants. Crude oils, for instance, usually reveal more spectral structure with the spectrometers locked together. Fluorescence peaks then separate in an approximation of the number of rings in the aromatic system [18]. A similar partitioning is observed with complex environmental samples where synchronous scanning separates mixtures of fluorescing substances into their constituents.

Take for example the spectra of phenol in Fig 18. The dotted curve (A) is the excitation spectrum, X(λ); the solid line (B) is the emission, E(λ). Fig 19 is the synchronous spectrum for $\Delta\lambda = 4$nm.

In Fig 20 phenol has been introduced into a solution of acenaphthalene which severely overlaps in the emission spectrum. Applying the synchronous technique results in a clean chromatogram-like separation of the components, placing phenol at 280 nm, acenaphthalene at 325 nm (B).

TO SCAN - OR NOT TO SCAN?

All models of the FLUOROLOG 2 include photon-counting detection for the ultimate in detectivity; DATAMATE's internal data circuitry accepts from one to over five million counts without a blink of its CRT. For stronger signals, DATAMATE converts to dc with a few keystroke commands and substitution of a dc preamp for the pc. We haven't neglected the trend toward multichannel detection either.

The allure of grafting an Optical Multichannel Analyzer (OMA) onto a FLUOROLOG translates into speed. By monitoring 500 or 1000 detection elements simultaneously, an entire spectrum can be viewed in real time as it evolves. These instant pictures can trace ephemeral events such as the formation of excited states, free radicals, or reaction intermediates. Just as easily, a large number of spectra can be captured in rapid succession to, for one example, monitor a liquid-chromatograph effluent.

Any FLUOROLOG that includes a single-grating emission spectrometer may be fitted with a longer focal length mirror (Fig 21) that projects a flat image onto the sensitive area of an OMA. In our lab, we mounted an intensified diode array (PARC model 1454 with 1450 controller) onto an F211 FLUOROLOG. The 512 channels of this detector sprawl over 12.5 mm. With a 300 gr/mm grating, this captures 165 nm at any instant, the perfect instrument for monitoring a reaction in progress.

Researchers in kinetics are noted for brandishing exotic pulsed lasers at their samples, but a single pulse of the SPEX phosphorimeter produced the spectrum shown in Fig 22. Although the low-powered, non-destructive excitation lasted only 3 μs, the main peaks of this $Eu(C_4H_7NO)_3(NO_3)_3$ sample are clearly visible.

For automated work and multi-spectra acquisition, a flow cell would be ideal,

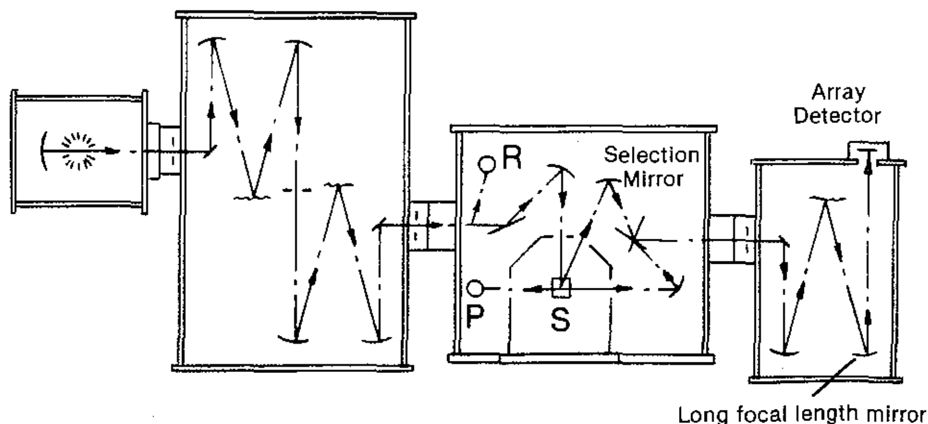

Fig 21 FLUOROLOG 211 fitted with Array Detector and long-focal-length focusing mirror in the emission spectrometer.

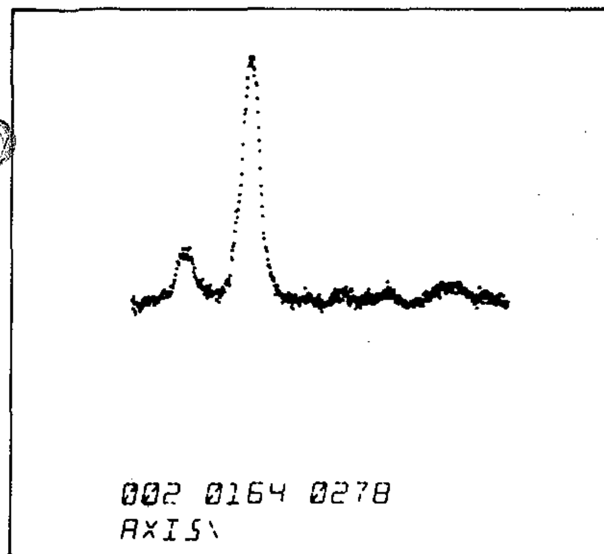

Fig 22 Emission spectrum of solid $Eu(C_4H_7NO)_3(NO_3)_3$ obtained from a single flash of the phosphorimeter lamp. Excitation = 385 nm, excitation bandpass = 5 nm, emission = 640 nm, exposure = 0.05s, accumulations = 1.

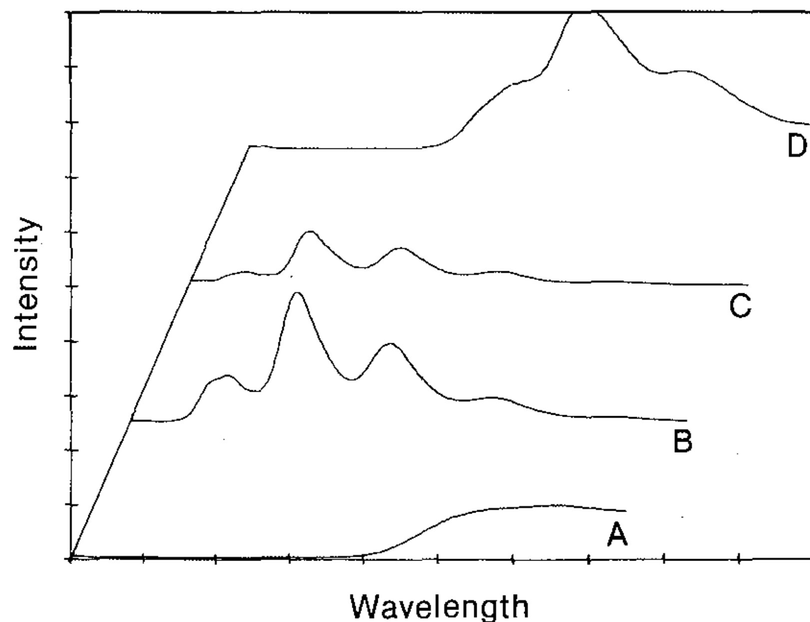

Fig 23 Emission spectra of: A—0.5mg/5ml of 3-aminofluranthene; B—3mg/5ml of 9-methylanthracene; C—4mg/5ml of anthracene; D—5mg/5ml of 9-aminoacridine mono HCl. Excitation = 360nm, bandpass = 15 nm, emission = 440 nm, exposure = 0.05s, accumulations = 1, delay between accumulations = 10s.

yet even by manually positioning samples into the excitation beam, we produced the four completely different spectra of Fig 23 in less than one minute. In this case, however, a cw 450W lamp supplied the excitation.

Yes, multichannel performance can be impressive. But it can equally be limiting, especially when samples of low concentration are the main concern. As a rule, at least an order of magnitude better sensitivity is achieved with a PMT scanned system as compared to a cooled, intensified diode array.

EPILOGUE

Having described results with samples that have recently run through our applications lab, we suspect you may now be contemplating a few puzzles of your own. Perhaps there's a baffling sample whose fluorescence has been overwhelmed by scatter and a double spectrometer may clear the haze. Or is there some troublesome noise that DATAMATE's processing may silence? Whatever the problem or question we'll gladly place our experience at your disposal.

Next year's questions? Next year's problems? Wouldn't you really feel more secure with a modular FLUOROLOG 2 spectrofluorometer insuring your lab's updateability?

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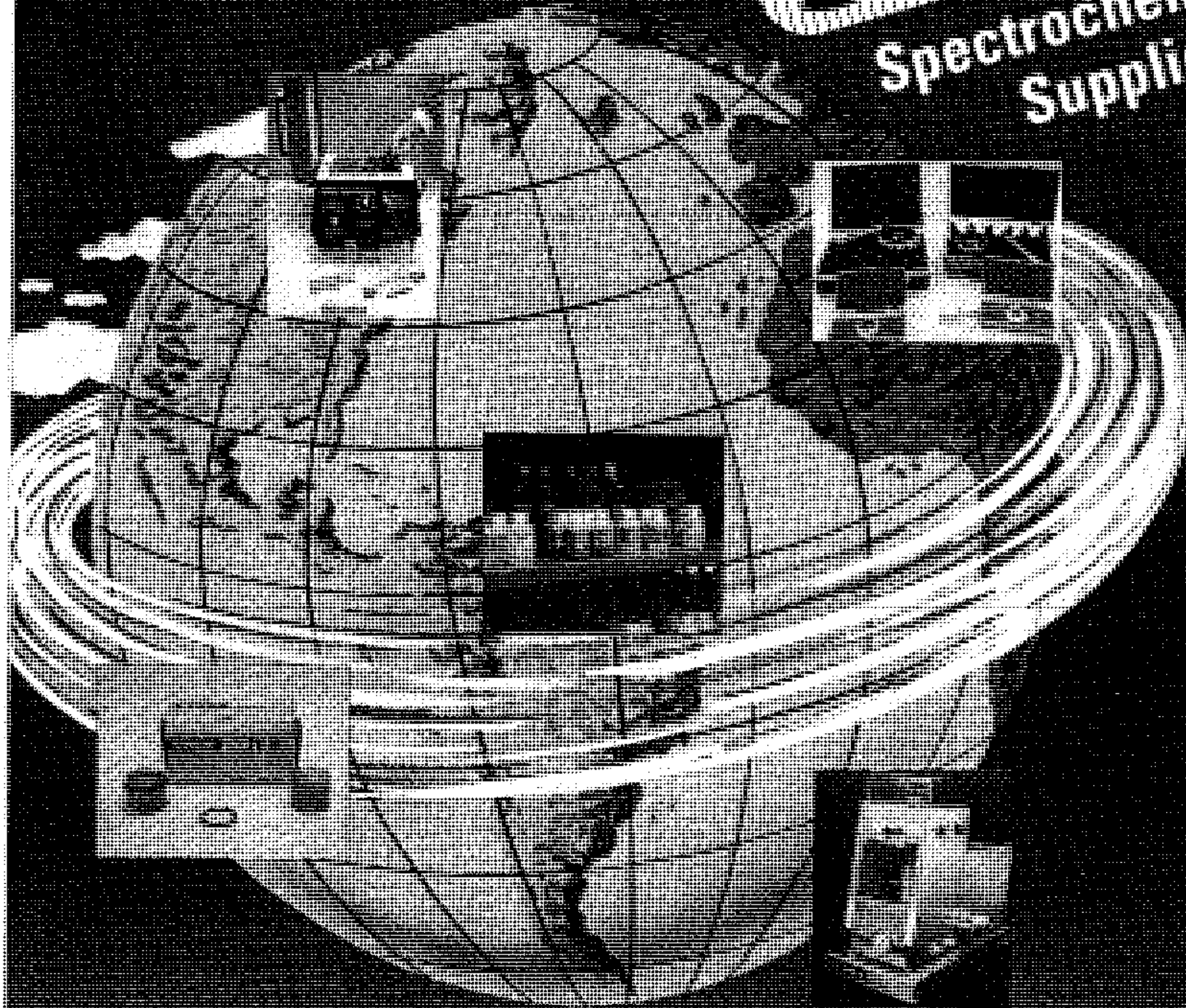

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