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Multidimensional Luminescence Measurements

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Luminescence spectroscopy has long been recognized as a tool for the quantitative and qualitative identification of a wide range of molecular systems. However, the resolving power of these techniques is often limited for similar compounds because absorption and emission peaks are typically broad banded and leads to overlapping spectra. To increase the resolving power of luminescence studies, recent studies have used multidimensional luminescence measurements (MLM) (use of two or more parameters of luminescence) to increase selectivity.

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Multidimensional Luminescence Measurements

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Introduction

Analytical measurement techniques that use more than one parameter for a single measurement may be termed "multi-parameter techniques." Thus, the measurement of luminescence is inherently a multiparameter technique since the simplest luminescence measurements will involve the exploitation of more than one parameter. For example, the measurement of the luminescence (phosphorescence or fluorescence) from a sample will involve the simultaneous use of an absorption wavelength and a luminescence wavelength since a molecule must absorb energy at wavelength, λ_{ex}, and be promoted to an excited state before luminescing at wavelength, λ_{em}. The measured luminescence intensity, I_L , may then be represented as a function of λ_{ex} and λ_{em}, i.e.,

$$I_L = f(\lambda_{ex}, \lambda_{em}). \tag{1}$$

Selective measurement of individual luminophores in simple [1,2] or even complex mixtures [3,4] may be achieved by varying these two parameters; a number of examples were detailed as early as 1969 in a review article by Sawicki[4].

Another parameter often used in luminescence measurement is the lifetime of the luminophore. The luminescence decay after initial excitation will generally follow first order kinetics. The time for the decay to reach 1/e of its initial value is a characteristic parameter of the molecule. Thus, the use of this parameter in combination with equation (1) provides increased selectivity. A number of representative examples where the decay time of luminescing species is used to achieve additional selectivity may be cited[5-7].

In recent years, novel advances in instrumentation[8] and data reduction strategies[9] have encouraged the simultaneous exploitation of

multiple parameters to achieve selective measurements. Consequently, the selectivity of luminescence measurements may be enhanced through both instrumental and mathematical approaches.

Figure 1 is a diagrammatic representation of several parameters of luminescence which may be used for selective luminescence measurements. Clearly, many parameters of luminescence may be simultaneously exploited to achieve a high degree of selective measurement and ultimately specific measurement. One obvious approach to additional selectivity is to obtain I_L in terms of three parameters, i.e.,
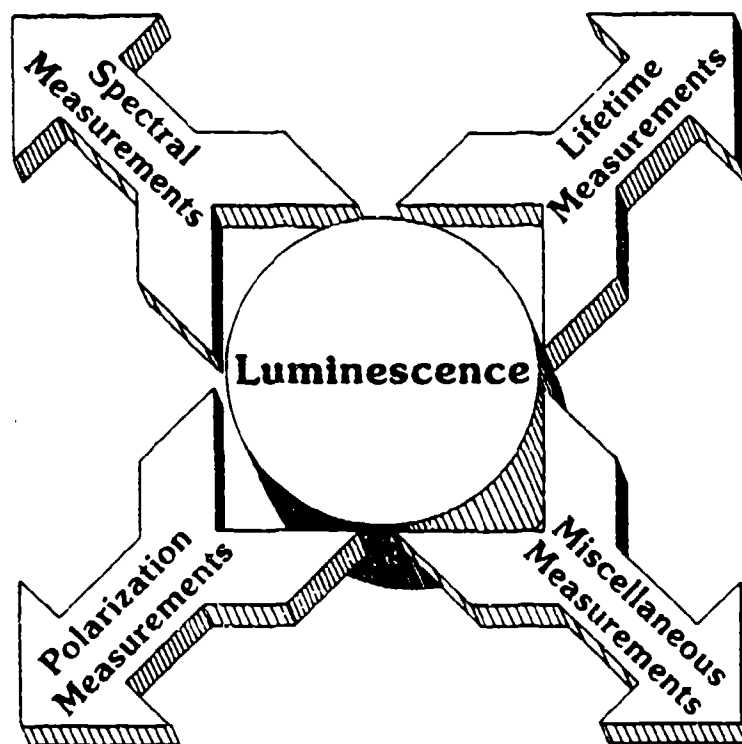
$$I_L = f(\lambda_{ex}, \lambda_{em}, P) \qquad (2)$$

where P is an arbitrary parameter selected from among the many listed in Figure 1. Furthermore, more than three variables could be used in equation (2) to increase the selectivity of the measurement. A number of other parameters could also be included[10] in Figure 1, particularly under the miscellaneous category. However, we have necessarily limited our diagram to include those parameters which are widely used and those which are increasingly being used for selective measurement. It should also be noted that some of the parameters listed in the miscellaneous category are really not characteristic parameters of the molecule in the excited state. However, these characteristic properties of the molecule may be combined with excited state information to increase selectivity.

This manuscript discusses multidimensional luminescence measurement (MLM) and its applications in analytical chemistry. The MLM approach involves the simultaneous use of two or more parameters of luminescence for selective measurement. We term this approach multidimensional rather than multimetric since, in this article, we will primarily emphasize data

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- Excitation spectrum
- Emission spectra
    (fluorescence and/or phosphorescence)
- Synchronous spectrum
- Low temperature spectra
    (matrix isolation, Shpol'skii, etc.)

- Fluorescence
- Phosphorescence

Time Resolved and Phase Resolved

Spectral Measurements

Lifetime Measurements

Luminescence

Polarization Measurements

Miscellaneous Measurements

- Depolarization
- Circularly polarized luminescence
- Fluorescence detected circular dichroism

- Quenchometry
- Surfactant enhanced luminescence
    (micelles, etc.)
- Sensitized measurements
- Chemical derivatization
- Chromatographic detector

Figure 1

acquisition, display, and processing in a multidimensional format. There are a number of advantages for reduction of data using a multidimensional format.[9] An example of such an approach was presented in a recent "A page" article by McGown et al.[11] where phase resolved fluorescence intensity is derived as a function of synchronously scanned wavelengths and of detector phase angle. Our discussion in this manuscript will be divided into the four major classifications of 1) spectral measurements, 2) lifetime measurements, 3) polarization measurements and 4) miscellaneous approaches, as depicted in Figure 1. The utility of MLM will be demonstrated using one or two examples in each classification.

Spectral Measurements

Theory. The discussion provided here is necessarily brief, and one of two useful monographs[12,13] may be consulted for detailed discussions on the general theory of luminescence. Luminescence measurements have received widespread applications in the analytical studies of large, unsaturated, highly conjugated, organic molecules such as polynuclear aromatic compounds (PNAs). Organic molecules generally have an even number of electrons. Therefore, the absorption and fluorescence transitions will occur between singlet states. Phosphorescence will generally involve an intersystem crossing to a t.iplet state with subsequent radiative decay to the ground singlet state. Since this transition is quantum mechanically forbidden (long-lived), and therefore more subject to non-radiative decay processes in fluid solutions, phosphorescence is usually observed under cyrogenic conditions. Some recent studies have provided useful approaches to room temperature phosphorescence measurements.[14]

Two useful properties of most luminescing molecules provide the selectivity of equation (1) described earlier. The first property is that for most pure luminophores, the observed emission spectrum is independent

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of the excitation wavelength. The second property is that the excitation spectrum is independent of the monitored emission wavelength. Thus, variations in the emission profile with variations in the excitation wavelength would suggest that more than one luminescing component is present in a sample. A similar conclusion could be drawn from variations in the excitation spectrum with monitored emission wavelengths. Many early analytical applications of luminescence measurements varied the excitation and monitored luminescence wavelengths to achieve selectivity.[2,4] However, for complex molecules with broad band absorption and emission spectra, the selectivity of this approach decreases rapidly as the number of components increase. Optimum selectivity is likely to occur with narrow band excitation and with an emission resolution better than the bandwidth of the monitored components. In addition, the number of excitation and/or emission wavelengths employed should equal or exceed the number of emitting components.[15]

To illustrate the preceding point, Figure 2 is a contour plot of a typical one component fluorescence/excitation matrix. Note that, within the defined contour levels, this pure compound adheres to the two properties of excitation and emission previously discussed. A more complex, six component, mixture is shown in Figure 3. It is visually obvious that this is a multicomponent system since the emission profile changes several times with variations in the excitation wavelength. A similar conclusion may be drawn about variations in the excitation spectra with monitored emission wavelength. In fact, the number of changes noted in the emission or excitation profile is an indication of the minimum number of luminescing species (rank) contributing to the matrix.
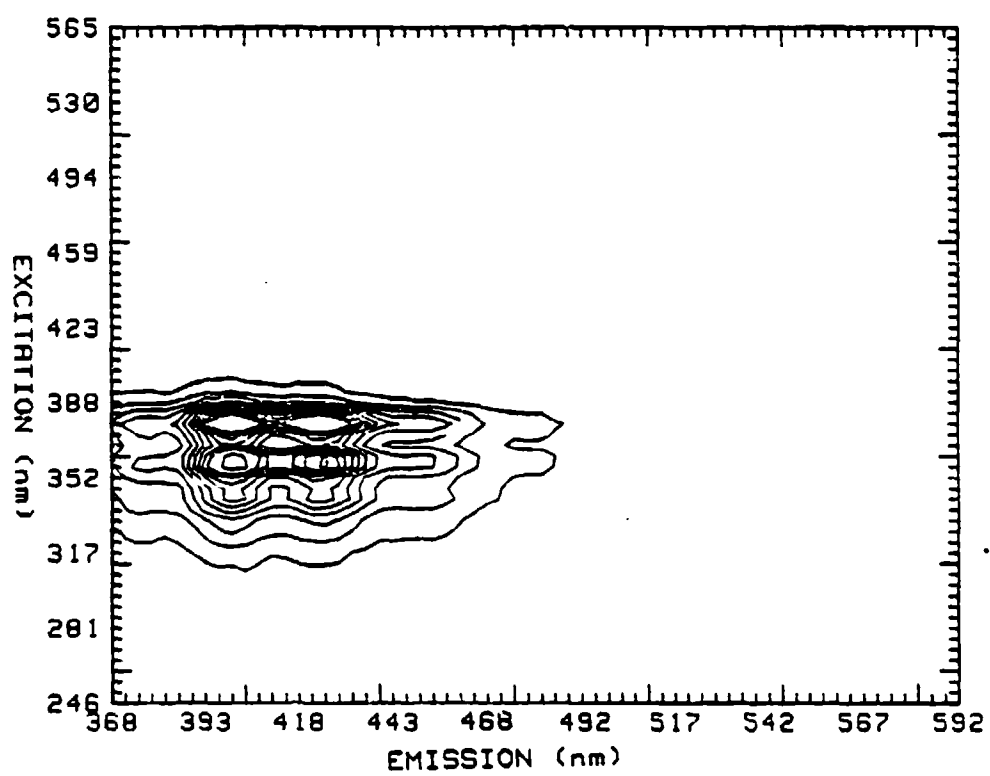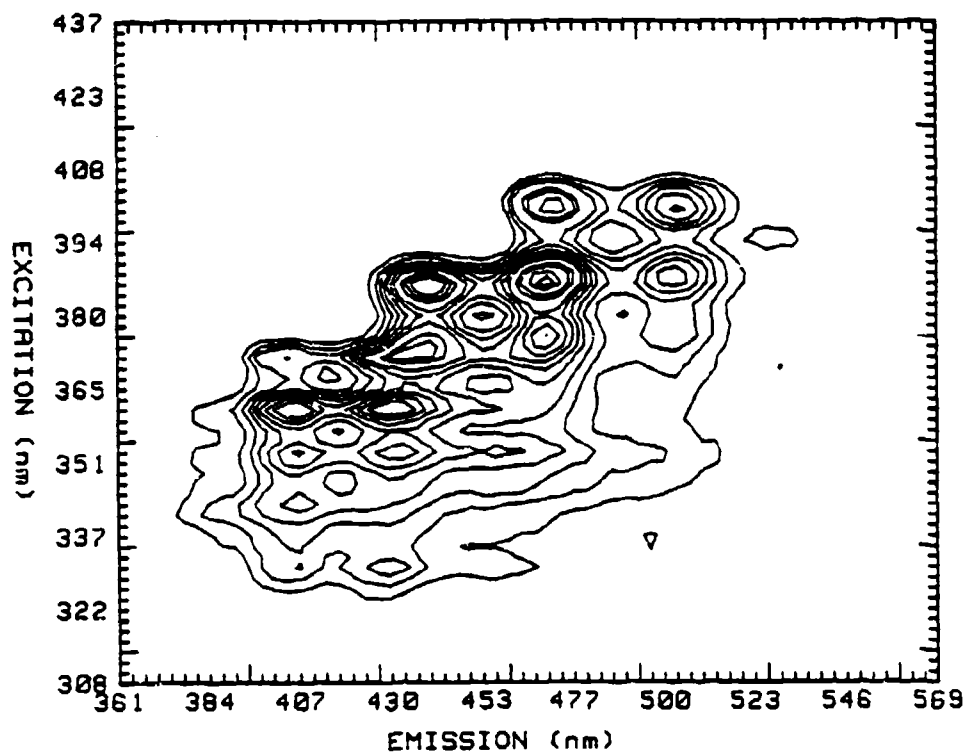
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Figure 2

Figure 3

Instrumentation. The utility of obtaining luminescence spectra as a function of multiple excitation and multiple emission wavelengths encouraged some researchers to digitize and generate such information by manual manipulation of data from luminescence spectrometers.[16] Such studies demonstrated the need for an automated luminescence spectrometer. An early automated luminescence spectrometer was developed and described by Wampler and DeSa in 1971.[17] The data were digitized and could be output in a suitable form for postprocessing. An automated instrument was also described by O'Haver et al. in 1973.[18] However, this instrument acquired data in analog form for plotting on an x-y recorder. Consequently, the data were not in a suitable form for postprocessing. In the same year, Holland et al.[19] described a completely automated system capable of simultaneous absorption and fluorescence measurements. This system was interfaced to a dedicated digital computer for data processing. Perhaps the most completely automated luminescence spectrometer was described by Haugen et al. in 1975.[20] This spectrometer was interfaced to a general purpose computer and operated in a time-share mode. Six modes of operation were possible for data acquisition, and the data could be stored on magnetic tape for further processing. Commercially available instrumentation capable of similar operations has been reported.

Despite the high degree of automation in the instrumentation described in the preceding paragraph, the acquisition of luminescence data as a function of multiple excitation and multiple emission wavelengths was a time consuming process. Table I provides a chronology of the improvements in the data acquisition time of luminescence data. Note that a significant decrease in data acquisition time was not realized until around 1974 and in 1975, when a video fluorometer was first described by workers at the University of Washington.[23] The decrease in data acquisition time by

the video fluorometer was due to a novel concept of polychromatic illumination coupled with the rapid two dimensional data acquisition capabilities of an intensified vidicon detector.[24] As is often the case with such developments, some tradeoffs had to be made. In this case, the video fluorometer is less sensitive than conventional luminescence instrumentation. However, the sensitivity of the instrumentation could be increased by using the integrating capabilities of the vidicon detector with a concomitant sacrifice in rapid data acquisition. A video fluorometer similar to the one developed at the University of Washington has also been described.[25,26] The incorporation of shutters and cryogenic equipment for low temperature operation has also allowed this instrumentation to be used for phosphorescence studies.[28,29]

Lifetime Measurements

Theory. As mentioned earlier, the luminescence lifetime is another parameter that can be used for multiparametric evaluation of the luminescence information. This can be an especially important property of the luminescing molecules if there is a significant overlap in the excitation and emission bands. In such cases, the lifetime of the luminophore may be a distinguishing factor. The luminescence lifetime is the time required for the luminescence intensity to decay to $1/e$ of its initial value.[12] Hence, the luminescence intensity can be obtained as a function of monitored wavelength of emission and the observation time after termination of the excitation pulse, t_i, i.e.,

$$I_L = f(\lambda_{em}, t_i) \tag{3}$$

A complete mathematical description of the luminescence intensity would require an equation in the form of equation (2), i.e.,

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$$I_L = f(\lambda_{ex}, \lambda_{em}, t_1) \ . \qquad\qquad (4)$$

Practical considerations determine the approach to acquiring data in the form of equations (3) and (4). For instance, two parameters may be fixed and the third one varied.[30] Obviously, the specific instrumentation will determine the necessary acquisition time and data format. Since several methods of determining the luminescence lifetime may be used, it is useful at this point to briefly discuss the theory of the two commonly used techniques.[12] These methods are based on the use of two types of instrumentation: (1) time resolved and (2) phase resolved. In the time resolved method, the sample is excited with a short duration pulse of light, and the time dependent decay of the luminescence intensity may be used to calculate the lifetime. In the phase resolved method, the sample is excited with sinusoidally modulated light. The phase shift and demodulation of the emission, relative to the incident light, are parameters that may be used for calculation of the lifetime. Both methods require some calculations to obtain the luminescence lifetime of the luminophore. This is especially true for method (2) if the sample contains more than one luminophore.

In general, the excitation of a luminophore with an infinitely short pulse of light (δ function), provides the best approach to explaining the luminescence decay. The luminescence intensity as a function of time after the excitation pulse is assumed to be an exponential decay curve since the luminescence decay process usually obeys first rate kinetics, i.e.

$$\frac{I_L(t)}{I_L(0)} = e^{-kt} \qquad\qquad (5)$$

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where $I_L(t)$ and $I_L(0)$ are the intensities of the luminescence at time t and time 0, respectively. The parameter k is the first order rate constant for the decay process. Equation (5) indicates that a time/luminescence decay curve may be obtained if an ideal delta pulse excitation is used. Unfortunately, any practical instrument is non-ideal and can provide only an excitation pulse of finite duration. In addition, the associated electronics will have a response function of finite width. Consequently, the use of a non-ideal lifetime instrument will produce data which are a convolution of several response characteristics. A mathematical process is therefore needed to deconvolute the time decay curve.

With an appropriate modulation frequency for the excitation beam, the luminescence will also have an associated modulation depending on the time delay. This property is used in phase resolved luminescence measurements. The phase resolved method uses a continuous sinusoidally modulated excitation beam. Knowing the modulation frequency (f) and degree of modulation of the excitation source, a simple derivation[12] of the phase difference, ϕ, between the excitation source and the fluorescence gives the lifetime, τ, as:

$$\tau = \frac{1}{2 \pi f} \ (\tan \phi). \tag{6}$$

Similarly, the observation of the demodulation (M) is also suitable for calculation of the lifetime since

$$\tau = \frac{1}{2 \pi f} \sqrt{\frac{1}{M^2} - 1}. \tag{7}$$

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Equations (6) and (7) are valid if the emission of the luminophore is spectrally homogeneous. If the system is more complex, the calculation of the lifetimes is more complicated.

It should be apparent that each lifetime method has certain advantages and disadvantages. The selection of the proper method will depend on several variables including the nature of samples, deviation of decay from ideality, and lifetime range desired.[12] For this discussion, namely, use in multidimensional luminescence measurements, the format of the data must first be examined. Several choices in multiparametric representation of the luminescence data are available depending on the complexity of the data set. If one desires a two-dimensional format, the data can be acquired using either the emission spectra or the excitation spectra as a function of luminescence decay time while fixing the other parameter. In both cases, a two-dimensional data matrix is formed.[30,31] The intensity data may be represented as an (m x n) matrix where m is the number of wavelengths observed in each excitation or emission spectrum, and n is the number of time intervals at which spectra are obtained. The time difference between the two excitation or emission measurements greatly depends on the lifetime of the luminophore in the mixture. Typically, this value may range from ∿ 0.1 ns (fluorescence) to the order of ms or s (phosphorescence). Obviously, the multidimensional representation can also be extended according to equation (4). Here, the intensity data is represented as an (m x n x o) matrix where m is the number of excitation wavelengths, n is the number of emission wavelengths, and o is the number of time intervals at which spectra are obtained. The advantages of this approach are clear since it is very unlikely that two components will have the same lifetime as well as the same excitation and emission spectra.

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Instrumentation. Due to the short lifetime of most fluorophores, phosphoresence lifetime techniques were developed much earlier than fluorescence lifetime techniques. A pulse source phosphorescence lifetime technique was described in 1974.[32] A complete two-dimensional time-resolved technique, according to equation (3), was reported as early as 1975.[33] The method was fairly tedious and complicated due to mechanical control of the emission monochromator. However, this instrument was able to acquire data in an emission/time matrix (ETM) format. A more sophisticated time resolved phosphoresence spectrometer was developed by Goeringer and Pardue in 1979.[31] A silicon intensified target (SIT) vidicon was used to collect the data in the ETM format (Figure 4). However, this instrumentation did not exploit the excitation information since the sample was irradiated with undispersed light. A complete time resolved technique was reported in 1982.[29] Since this instrumentation incorporates a polychromator to disperse the excitation source, a complete phosphorescence/excitation/time matrix (PETM) is obtained. Although the representation of such data in a four-dimensional format is difficult due to the number of variables (Fig. 5), it should be clear that this format of the data provides the greatest information. The development of comparable fluorescence instrumentation would require much more sophisticated techniques due to the much shorter time intervals (nanoseconds). Although a great deal of work has been done in that field,[34] the unavailability of complete multidimensional fast scanning instrumentation similar to that reported by Ho and Warner[29] is understandable due to the lack of superfast (response time in the order of ns) SIT vidicons. However, there are several methods available that are able to obtain the data in a fluorescence/excitation/time matrix (FETM).[33,35] It is interesting to note that the ultrafast, picosecond
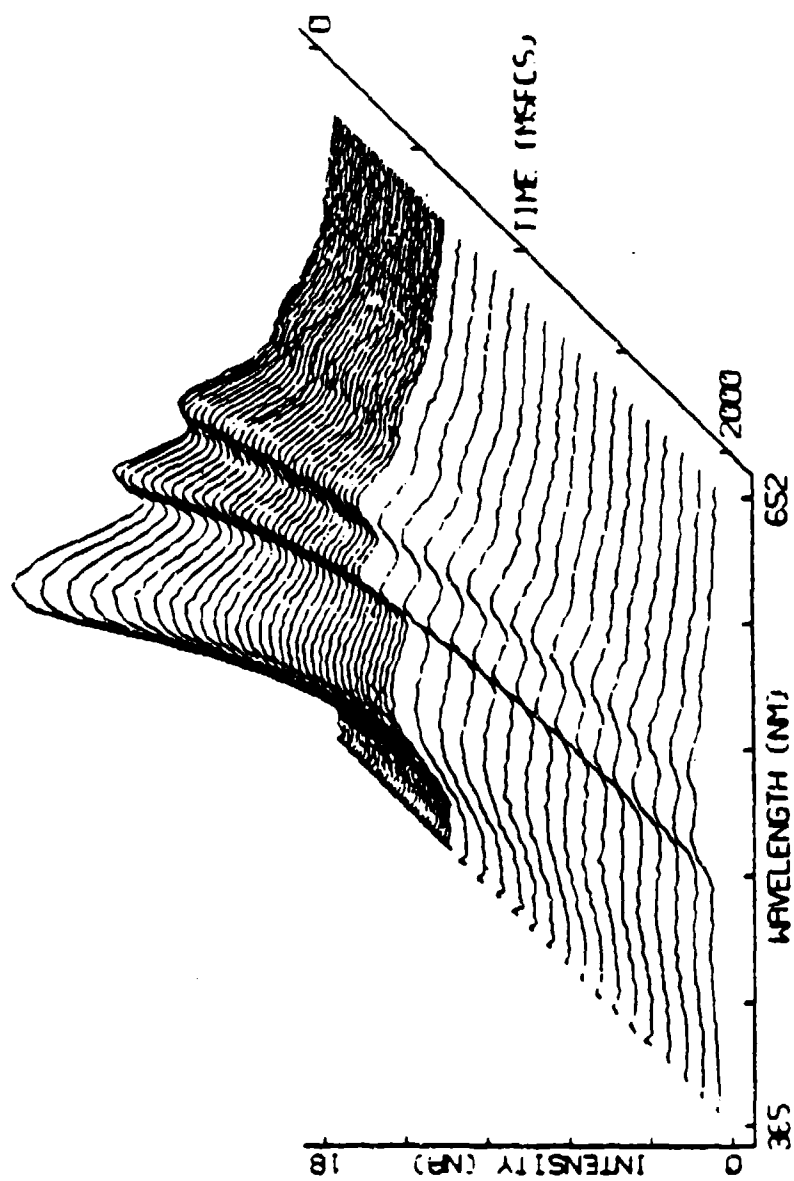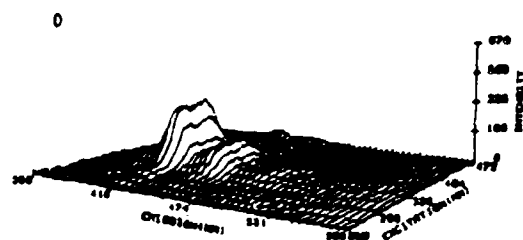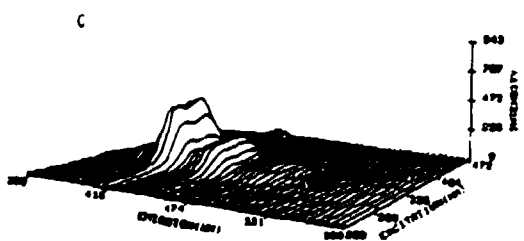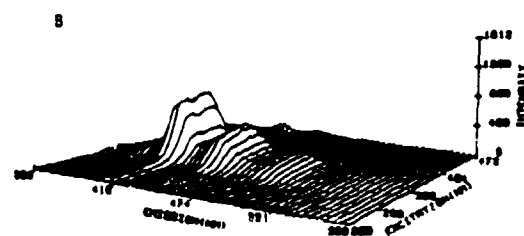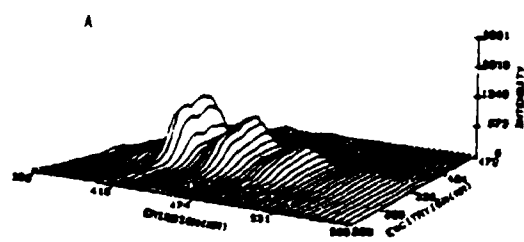
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Figure 4

Figure 5

spectroscopic techniques belong to the general field of multiparametric measurement. Although these methods have been used for studies of ultrafast physical and chemical processes, the use of streak cameras[36] should also be applicable to ultrafast multidimensional fluorescence measurements.

The phase resolved approach differs significantly in implementation. Since the data are not easily represented in an ETM or FETM format, a different approach to multidimensional luminescence measurement is necessary. A good example of this is the utilization of synchronously scanned wavelengths as a function of phase angle.[11]

Polarization Measurements

Theory. When a luminophore is excited with linearly polarized light, those molecules with their absorption dipoles aligned with the electric vector of the polarized light have the highest probability of excitation. The rules of photoselection dictate that the luminescence which results from this absorption is also polarized. Since these molecules remain in the excited state for a finite time before they luminesce, and because they are free to rotate during this time, some depolarization will occur. The amount of depolarization due to rotational diffusion is dependent on the size and shape of the molecule as well as solvent viscosity and is often used in biochemistry to investigate many important processes such as ligand binding, denaturation and aggregation.[12] In some cases energy transfer is a major contributor to depolarization, and luminescence polarization experiments can yield information on the excited states of the luminescing molecule.[12]

In luminescence polarization measurements, spectra are obtained by scanning wavelengths selected by an excitation monochromator and measuring

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the luminescence intensity parallel ($\parallel$) and perpendicular ($\perp$) to the polarization of the exciting light. The degree of polarization is then given by

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} \tag{8}$$

where $I_{\parallel}$ and $I_{\perp}$ are, respectively, the intensities of the components of the emission parallel and perpendicular to the polarization plane of the excitation beam.

In much of the luminescence literature, polarization data are reported in terms of anisotropy, which eases the theoretical derivation of model systems. The anisotropy is related to the polarization and is defined as

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} \; . \tag{9}$$

Circularly polarized light may also be used for molecular excitation. When light is passed through a doubly refracting crystal that has been oriented so that the light traverses the crystal in a direction perpendicular to the optical axis, the ordinary and extraordinary rays are not separated but travel at different speeds. When the rays exit the crystal, they are out of phase and the resulting beam of light is said to be elliptically polarized. The ellipticity is then given by the angle, θ , whose tangent is equal to the ratio of the minor axis to the major axis of the optical ellipse. If the crystal is of a thickness such that the rays exit with a phase difference of $\Pi/2$ for a given frequency, the light is circularly polarized.[37] In circular polarization, the electric vector does not oscillate in one plane but actually is constant in magnitude and describes a circular motion about the axis of propagation either clockwise (righthanded) or counterclockwise (lefthanded).

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In 1895, A.M. Cotton[38] discovered that chiral molecules differentially absorb left and right circularly polarized light. This discovery laid the foundation for the field of circular dichroism (CD). Experimentally, the CD spectrum of a sample is obtained by measuring the difference in the intensities of transmitted left and right circularly polarized light. Today, CD is an accepted technique for the study of chiral molecules and has been used most often in determining structural interactions between biomolecules.[39]

A new technique has been developed within the last ten years that uses the Cotton Effect to analyze chiral fluorescent molecules. Fluorescence detected circular dichroism (FDCD) is related to the classical CD method much as the fluorescence excitation spectrum is related to the absorption spectrum. Douglas Turner et al.[40] first presented the concepts and preliminary experimental results of FDCD in 1974. This method is based on the assumption that the experimentally corrected excitation spectrum of a luminophore has the same profile as its absorption spectrum. This assumption is generally valid in the absence of effects such as energy transfer. In FDCD, the differential absorption of left and right circularly polarized light is detected by the relative intensity differences of the fluorescence light. Thus, the FDCD spectrum is related to the CD spectrum since the intensity of fluorescence is proportional to the intensity of absorbed light at low absorbance. The major advantages gained by FDCD are greater sensitivity and selectivity. Selectivity is gained because not every chromophore in a chiral mixture will fluoresce. This technique allows the potential of isolating a single chiral fluorophore in a mixture of chiral chromophores for determination of the structure at that particular location.

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A theoretical discussion of the FDCD technique has already been published.[41] Early publications cited some problems in the theoretical and experimental techniques[42-44], but these were resolved in a later manuscript.[45] The first application of FDCD demonstrated the validity of the technique and showed the usefulness of this method as a bioanalytical tool.[46]

Instrumentation. Conventional spectrometers can be used to measure luminescence polarization by adding linear polarizers in the excitation and emission beams of light. Several commercial instruments are available with polarization attachments as options. Polarization spectra must be corrected to account for a wavelength-dependent polarization response inherent in the detection system (monochromator, optics, detector). There are several methods to calculate the instrumental correction constant which is termed the G factor. Techniques for determining the G factor and greater details on the theory of luminescence polarization and depolarization are given elsewhere.[12]

Many early experiments in the field of FDCD were done on conventional CD instruments which were modified by placing a photomultiplier tube at right angles to the excitation beam. Recently a new multidimensional instrument has been described which acquires ellipticity as a function of multiple excitation and emission wavelengths through the use of an intensified diode array combined with a polychromatic dispersion system.[47] A block diagram of this instrument is given in Figure 6. The instrument acquires complete fluorescence spectra at an excitation wavelength by illuminating the sample with left circularly polarized light and then with right circularly polarized light. The FDCD in terms of ellipticity spectra are acquired as a difference in these two measurements.
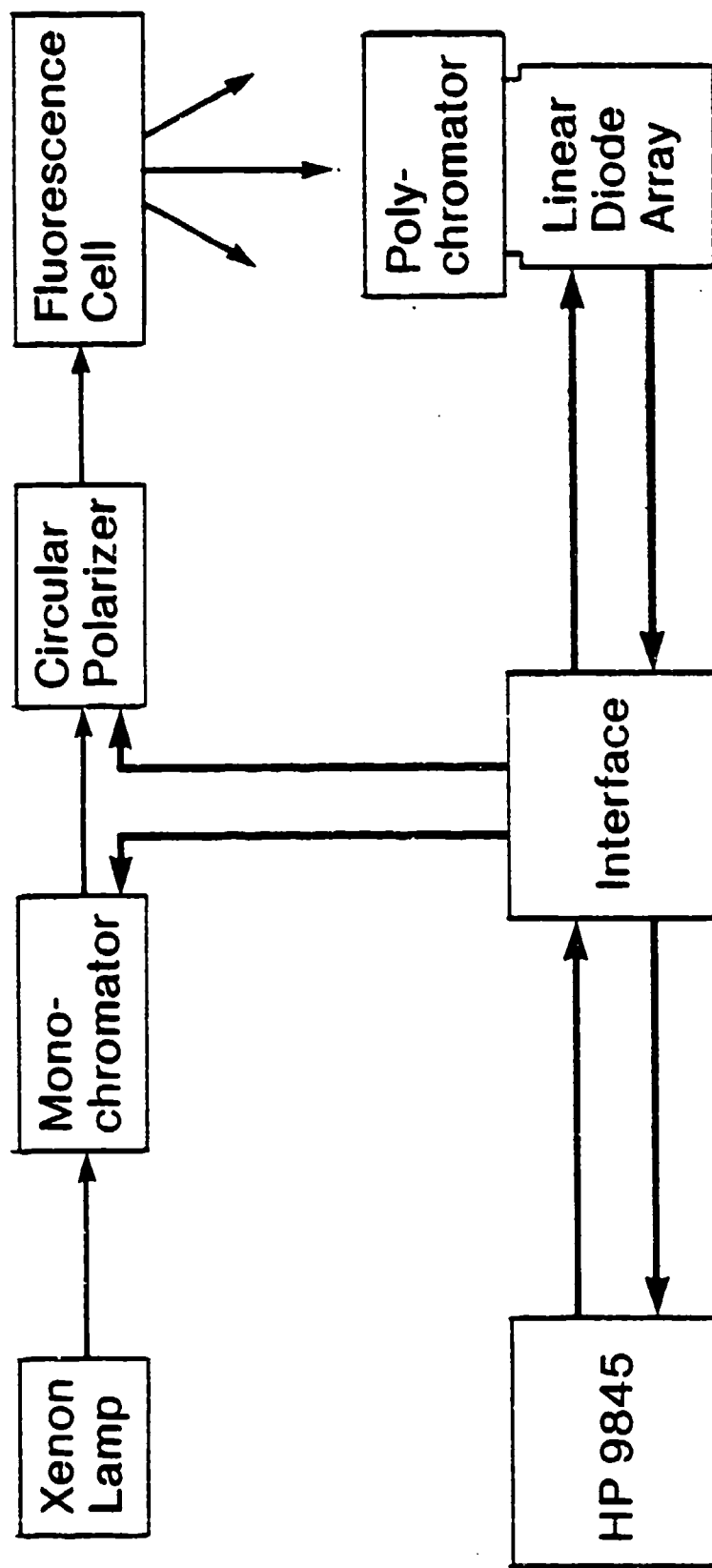
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Figure 6

The data are temporarily stored in the memory of a microprocessor-based interface which also controls the excitation monochromator, polarizers and detection interfaces allowing for total instrumental control. Several FDCD spectra may be obtained as the excitation monochromator is scanned through a series of wavelengths.

This new instrument allows acquisition of fluorescenc⸱ ⸱ form of a matrix of ellipticity as a function of multi⸱ ⸱ and emission wavelengths. Figure 7 is an example of such data⸱ ⸱ is evident that a number of possibilities exist for more rapid analysis of multicomponent chiral fluorophores. This technique should minimize the necessity of time consuming preseparation procedures for complex mixtures of optically active luminophores. This is particularly important since preseparation often introduces changes in the chemistry of the system under investigation.

Miscellaneous Measurement

Theory. Note in Figure 1 that we listed five possible parameters that could be used to increase selectivity in this category. This list is obviously abbreviated since other parameters such as luminescence induced by chemical reactions (chemiluminescence) could also be included.[48] The important point to recognize is that while these parameters are often not properties of the luminescence measurement, they are properties of the molecule that can be coupled with the luminescence measurement. For example, fluorescence has long been recognized as a selective chromatographic detector for high performance liquid chromatography (HPLC).[49] Thus, the selectivity of the fluorescence measurement coupled with the great resolving power of modern HPLC techniques provides an immensely selective measuring technique.[50] Other examples in this
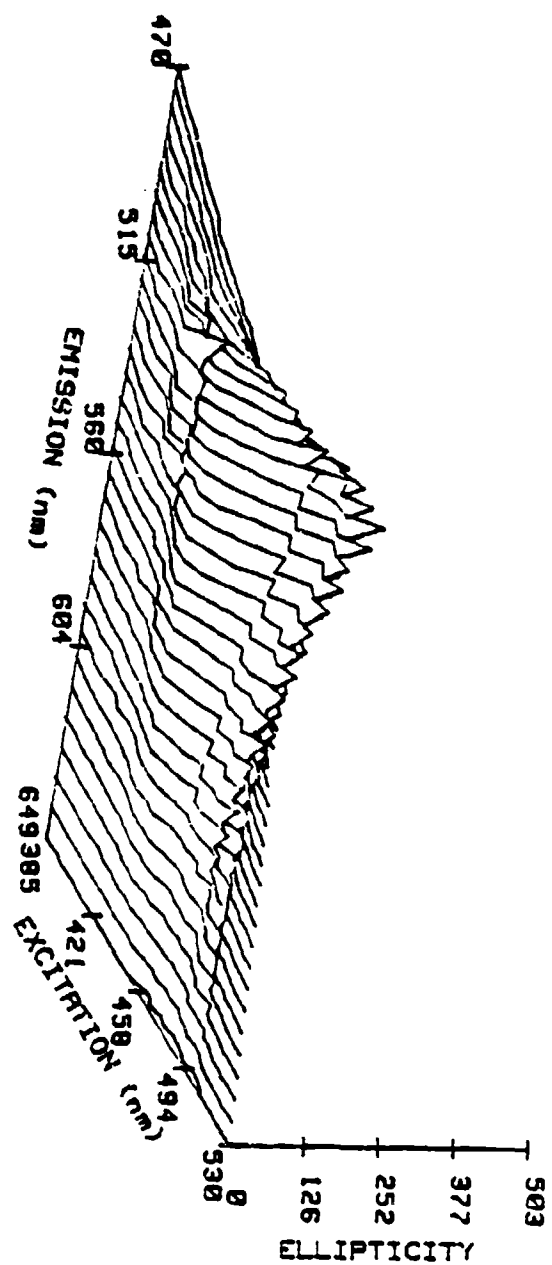
Figure 7

category use selective fluorescence quenching,[51] micelle enhanced luminescence[14,52,53] and chemical derivatization[54] to increase the selectivity of the measurement.

Instrumentation. The basic luminescence instrumentation described in the previous sections is also applicable here. Certain modifications such as miniaturization of the sample cell may be required for specific applications such as HPLC detection.[49] Many of these requirements are adequately covered in the monographs[12,13] or references previously listed[52,53] and will not be discussed here.

One example of multidimensional HPLC detection involves the use of a video fluorometer as a detector for HPLC.[55] In this example, a video fluorometer is interfaced to a high performance liquid chromatograph (HPLC) using a laminar flow cell to minimize dead volume and scattered light. The characteristic polychromatic illumination method of the video fluorometer was used to acquire real-time fluorescence/excitation matrices as a function of chromatographic retention time. Figure 8 provides an example of data from this study. In this example, the top EEM was acquired at the retention time of benzo(a)pyrene (BaP) using a BaP standard mixture while the bottom EEM was acquired at the same retention time for a shale oil sample. Another video fluorometer has been used with a conventional flow cell[56] to characterize the fluorescent components from a sample of burned crude oil.

Another excellent example of multidimensional instrumentation in this category is a second-generation laser video fluorometer-HPLC system recently developed by Skoropinski et al.[57] at the University of Washington. This instrument features a tunable dye laser as excitation source and a microchannel plate intensified diode array detector for fluorescence characterization of the HPLC eluate (Figure 9). The dye laser
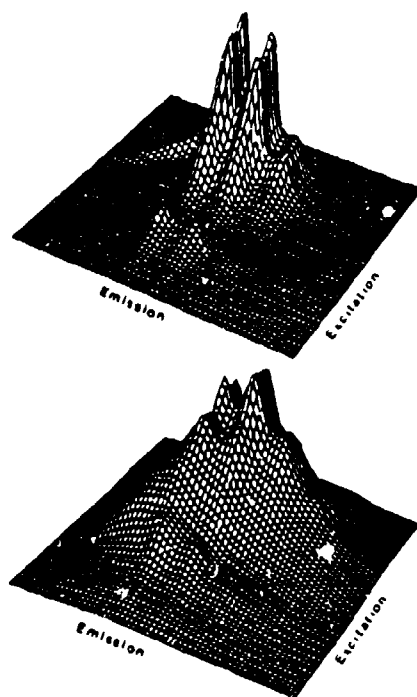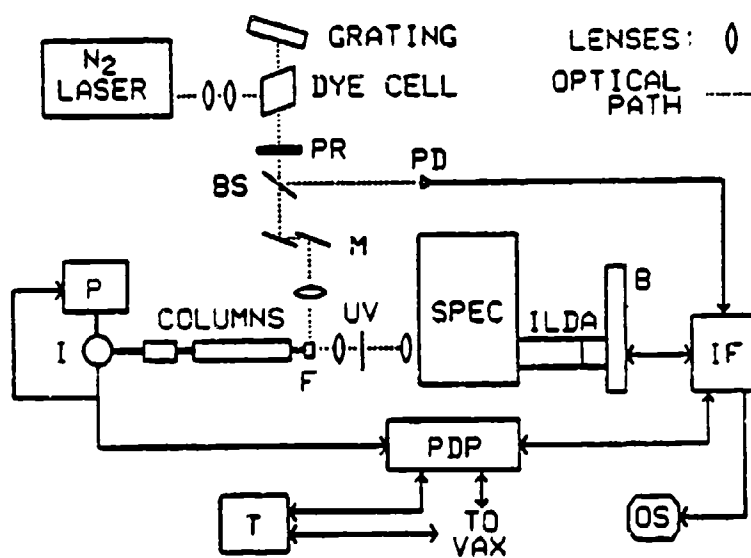
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Figure 8

Figure 9

grating is mounted on a galvanometer scanner, permitting the laser to be tuned to a specific wavelength on the millisecond time scale. The computer controlled system can obtain and store up to 10 fluorescence spectra per second as a chromatographic peak elutes.

A portion of a two-dimensional chromatogram of a soil extract obtained with the laser video fluorometer HPLC system, described in the preceding paragraph is shown in Figure 10A. These data were analyzed using standard chromatograms of the compounds benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(a)pyrene, known to elute in this region. These chromatograms were fit to the observed matrix by least squares. The residual matrix, however, shown in Figure 10B, clearly revealed the presence of a "hidden" fourth component. Comparison with the spectra of other PNAs of comparable composition led to the identification and quantitation of the compound as 2 ng of perylene.

A final example in this category is the coupling of fluorescence lifetime with fluorescence detection in HPLC.[58] In this example, data are presented in a three-dimensional display of fluorescence intensity versus chromatographic retention time and luminescence decay time. The utility of this approach was demonstrated using structurally similar PNAs.

Conclusion

The discussion provided here demonstrates the tremendous selectivity of luminescence measurements. The selectivity is even applicable to structurally similar compounds. This is in direct contrast to the usual notion that absorption and luminescence measurements are spectrally too broad banded to be useful for analytical measurement of similar compounds. It is apparent from the above discussion that a combination of multidimensional luminescence measurement techniques provides the greatest
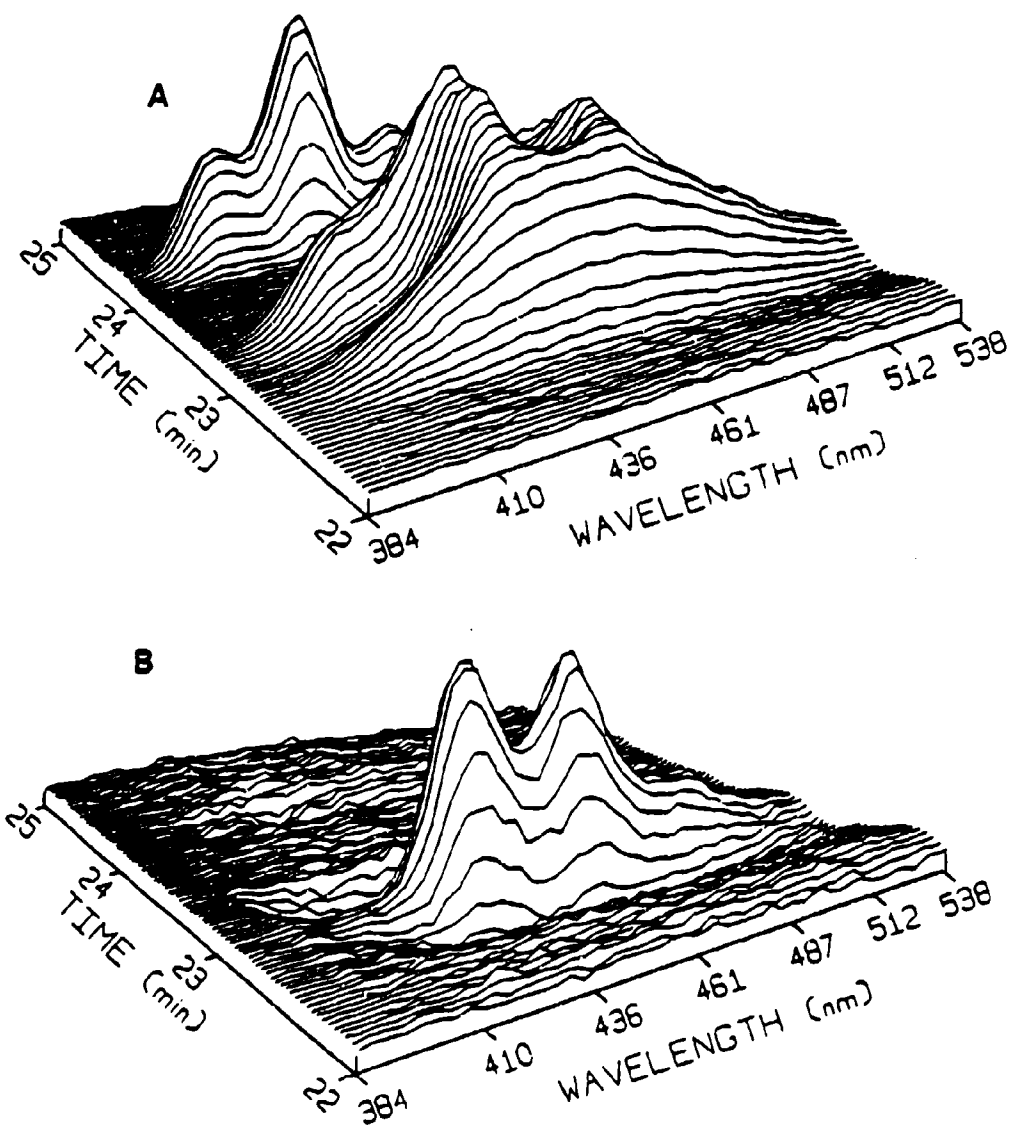
Figure 10

selectivity. It should be noted that no single instrument has ever been developed for measurement of all of the various luminescence parameters that we have discussed. It is doubtful that such an instrument will ever be developed. However, one can easily conceive of a complete fluorescence laboratory with an array of luminescence instrumentation capable of measuring many of the parameters provided in Figure 1. Thus, the desired selectivity may be achieved through the use of a combination of luminescence instrumentation. The cost of such instrumentation will likely decrease along with increased instrumental sophistication due to improvements in electronics, detectors, optics and computers for data acquisition and control.

Finally, we have primarily emphasized the theory and instrumentation of MLM techniques. The development of such techniques necessarily require the development of novel computer algorithms for reduction and interpretation of such large data sets. This parallel development has provided novel algorithms for the quantitative and qualitative analysis of these data.[9,30,59-61]

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Table I.   Chronology of Developments in Luminescence Spectrometers

Instrument (Year noted in Literature)[a]	# λ_{ex} by # λ_{em}	Acquisition Time[b]	Reference
Turner Fluorometer (1964)	100 X 100	several hours	21
Microfluorometer (1974)	100 X 500	3.2 sec.	22
Computerized Fluorometer (1975)	200 X 134	hours	20
Video Fluorometer (1975)	256 X 256	17 msec.	23
Commercial Fluorometer (1979)	200 X 200	1.5 hours	27

(a)   These are only selected examples from the literature.   Other examples were published around the same time.

(b) Most of these times are estimates from the information presented in the referenced articles.   In general, these times do not include digitization, storage and processing time.

Figure Captions

Figure 1:   Examples of many parameters of luminescence which can be exploited to achieve selective measurement.

Figure 2:   The contour plot of an emission/excitation matrix of 8.3 x 10^{-5}M anthracene in cyclohexane. Data were acquired in 0.6 s on a video fluorometer[29] in a 64 x 64 format.

Figure 3:   The contour plot of an EEM of an approximately equifluorescent six component mixture of the PNAs benzo(k)fluoranthrene, 2,3-benzanthracene, fluoranthrene, perylene, 2-ethylanthracene and pyrene in cyclohexane. Data were acquired in 0.6 s on a video fluorometer[29] in a 64 x 64 format.

Figure 4:   Typical time resolved ETM of 2-naphthoic acid and p-aminobenzoic acid. Reprinted from reference 61.

Figure 5:   Time resolved PETM of a binary mixture of phenanthrene and triphenylene. (A) t = 0 s; (B) t =5.5 s; (C) t = 12.5 s; (D) t = 17.5 s. Reprinted from reference 38.

Figure 6:   Block diagram of fluorescence detected circular dichroism spectrometer.

Figure 7:   Spectrum of cyclodextrin induced chirality in acridine orange acquired on FDCD instrument in 32 x 32 format.

Figure 8.   Three-dimensional projection of EEMs acquired at retention time of Benzo(a)pyrene (BaP) from (A) BaP standard chromatogram and (B) shale oil chromatogram. Reprinted from reference 54.

Figure 9.   The laser video fluorometer HPLC system: PR, partial reflector in the dye laser cavity; BS, beam splitter; PD, photodiode; M, beam steering mirrors; P, HPLC solvent

programmer, dual solvent pumps, and solvent mixer; I, HPLC injector; F, HPLC fluorescence flow cell; UV, ultraviolet cutoff filter; SPEC, spectrograph; ILDA, microchannel-plate-intensified, cooled, linear photodiode array; B, Reticon diode array evaluation board; IF, computer interface; T, CRT terminal; PDP, Digital Equip. Corp. PDP-1104 mini-computer; OS, oscilloscope.

Figure 10.   Partial fluorescence chromatogram of an extract of soil that was found to be contaminated with polynuclear aromatic compounds.   (A) A segment of the chromatogram in which benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(a)pyrene (BaP) are known to elute.   BaP is the compound with the longest retention time. (B) The same segment as in (A), but after subtraction of the appropriate amount of the known standard matrices.

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Acknowledgment

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