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"Multiparameter Fluorescence Methods
for Analysis in the Marine Environment"



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FINAL TECHNICAL REPORT

N00014-83-K0026

Summary of All Work Accomplished

(1) Technical Report #1, Oldham, P., Patonay, G. and Warner, I.M., "A Portable, Microprocessor Controlled, Multichannel Fluorometer for Marine Analysis," Analytica Chimica Acta, 158, 277-285 (1984).

The design of a portable, multichannel fluorometer which provides enhanced sensitivity and rapid data acquisition is described. The advantages of multidimensional fluorescence detection are discussed with special reference to the continuous monitoring in vivo chlorophyll fluorescence in the marine environment. Sensitivity, detection limit, linearity of detection, and other parameters are used to evaluate the instrument design. Preliminary experiments are presented in regard to chlorophyll determinations in the open ocean.

(2) Technical Report #2, Ho, C.-N., Rollie, M. and Warner, I.M., "Multiparametric Detection in Modern Luminescence Spectrometry," Optical Engineering, 22, 571-575 (1983). (Other support - NSF)

Due to its sensitivity and selectivity, luminescence detection is often the choice in a wide variety of analytical applications. However, until recently, only one parameter of luminescence was being monitored in a single experiment. For example, emission spectrum scanned at a fixed single excitation wavelength, and decay of luminescence observed at a single wavelength of excitation and emission. In this paper we will present some of the recent developments to provide multiparametric detection of luminescence phenomena. For example, a complete intensity map of phosphorescence, simultaneously, at multiple wavelengths of excitation and emission over time. Another area involves the use of polarized radiation in effecting luminescence detection. We will discuss the instrumentation and data analysis for these kinds of experiments.

(3) Technical Report #3, Warner, I.M., Oldham, P.B., Zillioux, E., and Patonay, G., "Marine Analysis Using a Rapid Scanning Multichannel Fluorometer," NATO Advanced Study Institute on Advanced Techniques on Marine Pollution Studies, Beaulieu-Sur-Mer, France, October 4-14, 1984.

The use of a rapid scanning multichannel fluorometer capable of ship-board operation is described. The advantages of multichannel detection with an intensified linear photodiode array are discussed in reference to the continuous monitoring of oceanic phytoplankton populations by their in vivo fluorescence. The utility of the excitation-emission matrix (EEM) for

fingerprinting marine algae is presented along with preliminary data acquired from unialgal cultures. Experimental data acquired at San Diego Bay, California in December '83 is presented to illustrate the utility of the instrumentation.

(4) Technical Report #4, Warner, I.M., Patonay, G. and Thomas, M., "Multidimensional Luminescence Measurements," Analytical Chemistry, 57, 463A-474A (1985). (Other support - NSF)

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. Complex mixtures may be analyzed by rapidly acquiring fluorescence data as a function of multiple excitation and emission wavelengths. Advances in instrumentation and data reduction strategies allow the discrimination of mixtures in overlapping spectra by capitalizing on differences in fluorescence lifetime or fluorescence depolarization measurements. Fluorescence detected circular dichroism (FDCD) has been used to resolve optically active fluorescent compounds. Additionally, luminescence measurements may be combined with a range of measurements such as quenchometry and chromatography to yield very selective determinations.

(5) Technical Report #5, Oldham, P.B., Patonay, G. and Warner, I.M., "Optical Output Stabilization Method for Direct Current Arc Lamps," Review of Scientific Instruments, 56, 297-302 (1985).

A simple, effective technique for stabilizing the optical output of direct current (dc) arc lamps is described. The large output fluctuation due to arc wander in a commercially available lamp and power supply is minimized by the introduction of an alternating current (ac) waveform superimposed on the dc source voltage in conjunction with detector averaging. Arc stability is monitored indirectly by the detection of arc excited fluorescence from a standard sample. The monitored lamp output is typically maintained to within 1% relative standard deviation (RSD) by this method. Data are presented supporting the theory that arc wander is significantly reduced by the addition of an ac component to the dc lamp power. Various methods of ac introduction are discussed along with the design of a controllable oscillator circuit. The effects of variations in ac voltage and frequency on optical output stability are examined.

(6) Technical Report #6, Oldham, P.B., Zillioux, E.J. and Warner, I.M., "Spectral 'Fingerprinting' of Phytoplankton Populations by Two-Dimensional Fluorescence and Fourier-Transform-Based Pattern Recognition," Journal of Marine Research, 43, 893-906 (1985).

The selectivity of fluorescence spectroscopy is exploited for the characterization of marine algae. Two-dimensional, digital images of in vivo fluorescence intensity versus excitation and emission wavelengths, called excitation-emission matrices (EEMs), are used as spectral "fingerprints" for marine phytoplankton populations. Fourier-transform-based pattern recognition is described along with its inherent strengths and weaknesses for the analysis of natural populations. The EEMs of unknown algae are compared to a library of standard EEMs representing 23 algal species and 6 classes with better than 80% accuracy. The EEMs acquired under different physiological conditions are used in determining pattern recognition reliability. The potential for fingerprinting mixed populations and oceanographic regions is also discussed.

(7) Technical Report #7, Ho, C.-N., Patonay, G. and Warner, I.M., "Bioanalytical Applications of Fluorescence Quenching," Trends in Analytical Chemistry, 5(2), 37-43 (1986). (Other support - PRF, NSF)

Bioanalytical applications of fluorescence quenching are described. Both static and dynamic quenching processes, as applied to biochemistry, are reviewed. New developments and future trends of research in the area are discussed with regard to improved measurements.

(8) Technical Report #8, Vicsek, M., Neal, S.L. and Warner, I.M., "Time Domain Filtering of Two-Dimensional Fluorescence Data," Applied Spectroscopy, 40(4), 542-548 (1986). (Other support - NSF)

Four time domain filtering methods are applied to simulated and experimental two dimensional fluorescence data in order to evaluate their performance. The methods that were evaluated are 1) moving average, 2) Savitsky-Golay polynomial smoothing, 3) Chebyshev filtering, and 4) Bicubic spline filtering. The methods are compared using mean square error analysis and the difference in the amplitudes of the filtered noisy and ideal data. The two dimensional version of the Savitsky-Golay filtering and the spline method produced the best overall results.

(9) Technical Report #9, Neal, S.L., Patonay, G., Thomas, M.P. and Warner, I.M., "Data Analysis for Multidimensional Luminescence," Spectroscopy, 1(3), 22-28 (1986). (Other support - NSF)

The application of digital analysis and reduction methods to multidimensional luminescence measurements is reviewed. The applicability and performance of linear, frequency domain and optimization methods is discussed. The range of problems being solved by these methods is also reported. Linear methods have been developed to the greatest extent. Frequency domain and

optimization techniques are being used more often as the need for digital analysis methods increases.

(10) Technical Report #10, Nelson, G., Patonay, G. and Warner, I.M., "An Inexpensive Intelligent Interface for Coupling a PRA Fluorescence Lifetime Instrument to a Microvax II," Analytical Instrumentation, 15(3), 215-225 91986). (Other support - NSF)

An inexpensive interface which can perform serial and parallel I/O operations necessary for instrument control and data transfer operations using time-correlated single photon counting instrumentation is described. Development of a compatible stepper motor system for controlling peripherals is also provided. This interface was implemented using an inexpensive Z80A based microcomputer.

(11) Technical Report #11, Oldham, P.B., Patonay, G. and Warner, I.M., "Evaluation of AC Stabilization of a DC Arc Lamp for Spectroscopic Applications," Analytical Instrumentation, 16(2), 263-274 (1987). (Other support - NSF)

Spectroscopic applications of the recently described method of arc lamp stabilization by the addition of a small (<2V) alternating current (AC) to a direct current (DC) arc lamp are discussed. A possible explanation for the improved arc stability is presented. Evaluation of arc formation and spatial behavior is monitored by a series of still photographs of the arc image. Observation of the electrode surfaces in similar photographs provide insight into the distribution of heat in the interelectrode region. A photodiode array imaging device is used to accurately detect lateral movement of the arc image over prolonged time periods. Advantages of AC stabilized lamps in spectroscopic applications are also presented.

(12) Technical Report #12, Rollie, M.E., Patonay, G. and Warner, I.M., "Automated Sample Deoxygenation for Improved Luminescence Measurements," Analytical Chemistry, 59, 180-184 (1987). (Other support - NSF)

Quenching of luminescence by molecular oxygen is an inherent limitation in fluorescence and phosphorescence analysis. Consequently, greater analytical sensitivity, as well as lower detection limits, can be achieved by removal of oxygen from the sample prior to luminescence analysis. An automated system for rapid deoxygenation is described in this paper. This apparatus combines a multiple sampling valve and a membrane barrier to generate a concentration gradient for sample deoxygenation. This automatic, multisample, on-line deoxygenation technique reduces sample handling to a minimum because deoxygenation and luminescence analysis occur within a closed system. The usefulness of this procedure is discussed in terms of automation, reproducibility, detection limit, deoxygenation efficiency, and applications to a variety of compounds. In addition, potential application of this procedure to other analytical subdisciplines

is also discussed.

(13) Technical Report #13, Rollie, M.E., Patonay, G. and Warner, I.M., "Deoxygenation of Solutions and Its Analytical Applications," Industrial and Engineering Chemistry, 26(1), 1-6 (1987). (Other support - PRF and NSF)

A representative survey of current methods of solution deoxygenation is presented. Some of the novel, non-conventional methods of degassing are also highlighted. The deoxygenation methodology is examined with special emphasis on luminescence spectroscopy, reductive electrochemical analysis, high-performance liquid chromatography and liquid chromatography with reductive electrochemical detection. Each of these areas of analytical chemistry is examined with regard to (1) the adverse effects that are caused by the presence of dissolved oxygen in samples and solutions and (2) how the current deoxygenation methodology is applicable to each analytical technique.

(14) Technical Report #14, Oldham, P.B. and Warner, I.M., "Analysis of Natural Phytoplankton Populations by Pattern Recognition of Two-Dimensional Fluorescence Spectra," Spectroscopy Letters, 20(5), 391-413 (1987). (Other support - NSF)

Two-dimensional Fourier transform based pattern recognition is used to characterize natural populations of marine algae by their two-dimensional, in vivo fluorescence spectra. The two-dimensional fluorescence spectrum is called an excitation-emission matrix (EEM) and is acquired by a portable, multichannel fluorescence spectrophotometer (PMFS). Natural populations in the Gulf of Mexico south of Louisiana and the coastal area near Savannah, Georgia are characterized by their in-situ fluorescence. Characterization of unknown populations is achieved by comparing the unknown EEMs to a collection of standard EEMs acquired from 23 species (6 classes) of marine algae. Pattern recognition results from the data collected along the Georgia coast was confirmed by microscopic examination of selected samples.

(15) Technical Report #15, Nelson, G., Patonay, G. and Warner, I.M., "Fluorescence Lifetime Study of Cyclodextrin Complexes of Substituted Naphthalenes," Applied Spectroscopy, 41(7), 1235-1238 (1987). (Other support - NSF)

The interactions of α , β , and γ -cyclodextrins and selected naphthalene derivatives as observed through fluorescence lifetime measurements are discussed in detail. These systems can be quickly characterized using the parameters obtained from experimental fluorescence decay curves. The formation of inclusion complexes can be followed with the appearance of a long-lived fluorophore which contributes to the total fluorescence according to the cyclodextrin concentration. This fluorophore is determined to be an inclusion complex between a

naphthalene and cyclodextrin.

(16) Technical Report #16, Patonay, G., Nelson, G. and Warner, I.M., "Recent Advances in Multidimensional Fluorescence Spectrometry," Progress in Analytical Spectroscopy, 10, 561-571 (1987). (Other support - NSF and NIH)

This review article discusses some of the latest developments and applications in the field of multidimensional fluorescence techniques. Numerous important advances have been made in the past few years, mostly due to improvements in automated instrumentation. In addition, the increased use of multiple parameters has provided recognized advantages. The multiparametric term has been used most often in systems only where the fluorescence intensity is observed as a function of two variables (e.g. excitation and emission). We review the utility of other parameters, including circular dichroism, fluorescence lifetime and chromatographic retention time, in enhancing the fluorescence measurement. In addition, we discuss powerful algorithms which have been developed to extract meaningful information from various types of multidimensional fluorescence data.

(17) Technical Report #17, Thomas, M., Nelson, G., Patonay, G. and Warner, I.M., "A Versatile Interface for Instrument Control," Laboratory Microcomputer, 6(4), 164-167 (1987). (Other support - NSF)

An inexpensive interface which uses Zilog Z-80 microprocessor compatible interface chips and allows instrumental control and data acquisition using a Radio Shack TRS-80 Model 4 microcomputer is described. This interface provides parallel and serial data handling capabilities useful to a wide variety of instrumentation. A block diagram of the TRS-80 compatible interface is presented and the TTL signals which are used for chip selection, control and input/output functions are discussed. Examples of the application of this interface to a commercial Photochemical Research Associates fluorescence lifetime instrument and an in-house, multidimensional fluorescence detected circular dichroism spectrometer are provided.

(18) Technical Report #18, Warner, I.M., Patonay, G., Rollie, M., Thomas, M. and Nelson, G., "Optimization of Fluorescence Measurements," Progress in Analytical Luminescence, ASTM, STP 1009, D. Eastwood and L.J. Cline Love, Eds., American Society for Testing and Materials, Philadelphia, 1988, pp. 1-11. (Other support - NSF)

Various components of a conventional fluorometer are discussed. Several approaches to optimization of some of these components are discussed by citing specific research examples. The four areas of research that are described include: stabilization of a xenon arc lamp, improvements in sample chemistry, increased detector selectivity, and development of data reduction

strategies for complex data. Improvements in the fluorescence measurement process are cited with each example.

(19) Technical Report #19, Nelson, G., Patonay, G. and Warner, I.M., "The Effects of Selected Alcohols on Cyclodextrin Inclusion Complexes of Pyrene Using Fluorescence Lifetime Measurements," Analytical Chemistry, 60(3), 274-279 (1988). (Other support - NSF)

This study focuses on the inclusion complexes of γ -cyclodextrin with pyrene as indicated by fluorescence lifetime measurements and the effects of alcohols on these systems. The pyrene complex has a longer lifetime than free pyrene, yet quenching is observed in the presence of γ -cyclodextrin. This apparent conflict is discussed. Also, short chain alcohols participate in the pyrene/ γ -cyclodextrin inclusion complexes producing a longer lifetime of the pyrene complex. This participation is described through evaluation of the lifetime data. The changes in fluorescence lifetime with increasing cyclodextrin concentration are observed to follow the changes in equilibrium fractions of the pyrene/cyclodextrin system. The study of these systems using fluorescence lifetime measurements yields a method of evaluating this equilibrium and estimating the formation constants for pyrene/cyclodextrin complexes.

(20) Technical Report #20, Tarr, M., Nelson, G., Patonay, G. and Warner, I.M., "The Influence of Mobile Phase Alcohol Modifiers on HPLC of Polycyclic Aromatics Using Bonded Phase Cyclodextrin Columns," Analytical Letters, 21(5), 843-856 (1988). (Other support - NSF)

Recent studies have shown that formation constants for cyclodextrin inclusion complexes are increased in the presence of certain alcohols. This paper describes a preliminary investigation of the effects of mobile phase alcohol modifiers on HPLC separation of polynuclear aromatics using cyclodextrin bonded phases. Dramatic changes in the chromatography are reported in the presence of alcohol. Some discussion is provided as to how these results may be used to increase the selectivity of HPLC separations using cyclodextrin bonded phases.

(21) Technical Report #21, Patonay, G., Fowler, K., Nelson, G. and Warner, I.M., "Investigation of Ternary Cyclodextrin Complexes Using Fluorescence Spectrometry," Analytica Chimica Acta, 207, 251-258 (1988). (Other support - NSF)

Ternary complexes of cyclodextrin are apparently formed in the presence of saturated alcohols, glycols and acids. The role of third components in these complexes is investigated. Pyrene is used as a probe to determine the degree of compartmentalization, the change in the hydrophobicity of the microenvironment, and the formation constant.

(22) Technical Report #22, Blyshak, L., Rossi, T., Patonay, G. and Warner, I.M., "Cyclodextrin-Modified Solvent Extraction for Polynuclear Aromatic Hydrocarbons," Analytical Chemistry, 60(19), 2127-2131 (1988). (Other support - NSF)

The extraction efficiencies of several polynuclear aromatic hydrocarbons (PAHs) between isopropyl ether/water and between isopropyl ether:1-butanol (1:4)/water are measured in the presence of an aqueous γ -cyclodextrin (CDx) modifier at room temperature. The distribution of certain PAHs into the aqueous phase is increased by the presence of 10^{-2} M γ -CDx. For compounds such as perylene and coronene which show the most marked effects, the extraction efficiencies into aqueous phase from pure isopropyl ether are 95.1% and 93.7%, respectively, when the CDx modifier is used. In the mixed solvent system with 1-butanol, these values are 63.5% and 98.1%, respectively. In both systems, the increased distribution into water is based in part on the size relationship between the PAH and the CDx cavity. In the case of relatively small molecules like anthracene, little or no extraction is observed in the presence of the CDx modifier. This type of extraction system may be useful for selective extraction of large PAHs from mixtures. Extraction results for a variety of PAHs are presented and discussed.

(23) Technical Report #23, Zung, J., Nelson, G. and Warner, I.M., "An Effective Stepper Motor Controller for Updating a Perkin-Elmer 650-10S Fluorometer," accepted by Laboratory Microcomputer. (Other support - NSF)

A stepper motor controller circuit for effectively updating all mechanical operations of a Perkin-Elmer 650-10S fluorometer by use of an Apple II+ computer is described. This controller/interface provides complete control of the fluorometer including positioning, stepping and homing of the monochromators. The controller described, can also be used for controlling devices which require accurate and reproducible positioning. The design and implementation of an optoelectronic device for accurately homing both monochromators is also described.

(24) Technical Report #24, Woodlee, R.L., Fuh, M.-R., Patonay, G. and Warner, I.M., "Enhanced DC Arc Lamp Performance for Spectroscopic Applications," submitted to Review of Scientific Instruments. (Other support - NSF)

The performance of a high pressure xenon arc lamp is enhanced by application of a combination of two techniques which (1) reduces noise due to arc wander and (2) increases the lamp intensity. Fluctuations in lamp intensity due to arc wander is minimized by the superimposition on an alternating current (AC) on the direct current (DC) source voltage in conjunction with detector averaging. Lamp intensity is increased by the application of a static magnetic field to the arc plasma. Arc stability and intensity are monitored indirectly by measuring arc excited Rhodamine B fluorescence. Data illustrating the effects of each

technique individually and in combination are presented. Lamp output is typically maintained to less than 1% relative standard deviation (RSD) while intensity is increased by as much as 127%. Possible explanations for the enhanced intensity and increased stability is discussed.

(25) Technical Report #25, Woodlee, R.L., Fuh, M.-R. and Warner, I.M., "Effects of Select Pollutants on the Multidimensional Fluorescence of Marine Phytoplankton," submitted to Environmental Science and Technology. (Other support - NSF)

The ability of multidimensional fluorescence to detect the effects of select pollutants on algal fluorescence and production is demonstrated. By monitoring a range of excitation and emission wavelengths, multidimensional fluorescence is ideally suited to rapidly measure algal fluorescence generated by both chlorophyll a and accessory pigments, as well as any changes induced by pollutants. Laboratory cultured and natural algae samples from classes Chlorophyceae, Bacillariophyceae, and Cyanophyceae were exposed to substituted nitroaromatics and fluorescence spectra of the algae recorded. Notable spectroscopic changes and fluorescence quenching were observed.

(26) Technical Report #26, Neal, S.L., Sell, D., Thomas, M.P., Monnier, V.M. and Warner, I.M., "Videofluorometric Analysis of Aging Human Collagen," submitted to Analytical Biochemistry. (Other support - NSF)

The development of fluorescent pigments in aging human collagen has been observed, but neither the source of these compounds nor their nature has been described. Recently two distinct fluorophores were isolated from aging insoluble human collagen rich tissue following a sequence of proteolytic digestions and chromatographic separations. Using the videofluorometer, which monitors the fluorescence intensity of a sample as a function of several excitation and emission wavelengths, the fluorescence of the collagen rich tissue at various stages of the separation process were analyzed to determine the number of fluorescent components in each of the samples and estimate their component spectra. The analysis indicated that the isolated fluorophores were indeed single-component samples and that the insoluble collagen-rich fraction contains two major fluorophores whose spectra are consistent with the spectra of the isolated compounds.

(27) Technical Report #27, Zung, J.B., Fuh, M.-R. and Warner, I.M., "Design and Characterization of a Fiber Optic Based Multidimensional Fluorometer," submitted to Analytica Chimica Acta. (Other support - NSF)

The design and characterization of a novel fiber optic based multidimensional fluorometer is reported. This fluorometer uses a white light source and an intensified photodiode array detector. A bifurcated sensing configuration is used to transmit

excitation and emission radiation to and from the solution under study. Detection limits in the nanomolar range were obtained for several test compounds.

(28) Technical Report #28, Neal, S.L., and Warner, I.M., "Survey of Sequential Target Factor Analysis Algorithms," submitted to the Journal of Chemometrics. (Other support - NSF, NOBCChe, P&G)

The performance of three target factor analysis spectral resolution algorithms on two dimensional fluorescence data is described and evaluated. The effects of random noise and spectral overlap were evaluated by systematically varying the spectral properties of synthetic data matrices. The results indicate that the S/N should be at least five in order for the method to generate verifiable estimations of the component spectra and that the convention used to choose the first estimate to the component spectra is significant.

Index of All Publications

- 1) Oldham, P., Patonay, G. and Warner, I.M., "A Portable, Microprocessor Controlled, Multichannel Fluorometer for Marine Analysis," Analytica Chimica Acta, 158, 277-285 (1984).
- 2) Ho, C.-N., Rollie, M. and Warner, I.M., "Multiparametric Detection in Modern Luminescence Spectrometry," Optical Engineering, 22, 571-575 (1983).
- 3) Warner, I.M., Oldham, P.B., Zillioux, E., and Patonay, G., "Marine Analysis Using a Rapid Scanning Multichannel Fluorometer," NATO Advanced Study Institute on Advanced Techniques on Marine Pollution Studies, Beaulieu-Sur-Mer, France, October 4-14, 1984.
- 4) Warner, I.M., Patonay, G. and Thomas, M., "Multidimensional Luminescence Measurements," Analytical Chemistry, 57, 463A-474A (1985).
- 5) Oldham, P.B., Patonay, G. and Warner, I.M., "Optical Output Stabilization Method for Direct Current Arc Lamps," Review of Scientific Instruments, 56, 297-302 (1985).
- 6) Oldham, P.B., Zillioux, E.J. and Warner, I.M., "Spectral 'Fingerprinting' of Phytoplankton Populations by Two-Dimensional Fluorescence and Fourier-Transform-Based Pattern Recognition," Journal of Marine Research, 43, 893-906 (1985).
- 7) Ho, C.-N., Patonay, G. and Warner, I.M., "Bioanalytical Applications of Fluorescence Quenching," Trends in Analytical Chemistry, 5(2), 37-43 (1986).
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- 10) Nelson, G., Patonay, G. and Warner, I.M., "An Inexpensive Intelligent Interface for Coupling a PRA Fluorescence Lifetime Instrument to a Microvax II," Analytical Instrumentation, 15(3), 215-225 (1986).
- 11) Oldham, P.B., Patonay, G. and Warner, I.M., "Evaluation of AC Stabilization of a DC Arc Lamp for Spectroscopic Applications," Analytical Instrumentation, 16(2), 263-274 (1987).
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- 13) Rollie, M.E., Patonay, G. and Warner, I.M., "Deoxygenation of Solutions and Its Analytical Applications," Industrial and Engineering Chemistry, 26(1), 1-6 (1987).
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