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THE METHOD OF CELL ROTATION FOR COMPUTER BASED CORRECTION OF FLUORESCENCE MEASUREMENTS FOR ATTENUATIONS DUE TO PRIMARY AND SECONDARY ABSORPTION

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THE METHOD OF CELL ROTATION FOR COMPUTER BASED CORRECTION OF FLUORESCENCE MEASUREMENTS FOR ATTENUATIONS DUE TO PRIMARY AND SECONDARY ABSORPTION

By

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ABSTRACT

THE METHOD OF CELL ROTATION FOR COMPUTER BASED CORRECTION OF FLUORESCENCE MEASUREMENTS FOR ATTENUATIONS DUE TO PRIMARY AND SECONDARY ABSORPTION

By

Karlis Adamsons

An improved computerized right-angle spectrofluorometer capable of automatically implementing primary and secondary absorption corrections was constructed. The instrument incorporates a unique approach utilizing off center cell rotation to allow regulation of the thickness of the sample solutions through which the excitation and emission beams penetrate. Knowledge of the transmittance as a function of pathlength along both the excitation and emission optical axes permits determination of fluorescence signal attenuation caused by sample absorption. Equations have been determined and programs written to allow calculation of the correction factors to be done in real time. Once the factors are calculated, they are automatically instituted to generate fluorescence data corrected for these absorption effects.

Successful primary absorption corrections were obtained for quinine sulfate solutions in the concentration range $1x10^{-8}$ to $1x10^{-5}$ M with 1^{o} absorbances $7x10^{-5}$ to $7x10^{-2}$. Successful secondary absorption corrections were obtained for solutions containing quinine sulfate, $1x10^{-5}$ M, and fluoroscein over the concentration range $1x10^{-6}$ to $1x10^{-4}$ M with 2^{o} absorbances $6x10^{-3}$ to $2.3x10^{-1}$.

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Dedication

The most beautiful thing we can experience is in the unveiling of yet another artistic masterpiece from nature's museum. The success of this work is dedicated to all of those who provided me the freedom and the means for such an experience in scientific discovery. Witnesses were we to the birth of a new means for looking at the finer details of nature's composition and brush strokes. With new vision the extent of the unveiling we are about to encounter is unknown...

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Acknowledgements

Credit must be primarily extended to man's development of the scientific method and, thereby, the means of separating our fantasies from nature's truths. The method is the key that will unlock these truths, but only to an open mind. As long as one is only biased it does not make any difference, because if one's bias is wrong a perpetual accumulation of experiments will perpetually annoy one until they cannot be disregarded any longer. They can only be disregarded if one is absolutely sure ahead of time of some precondition that science has to have. In fact it is necessary for the very existence of truth in science that open minds exist which do not allow that nature must satisfy some preconceived conditions.

Credit is due also to the open-minded individuals who helped me in discovering the key for myself: Andrew Timnick, Jack Holland and John Sell. The relentless programming of Mike Reid and the words of insight from Bill Franz are gratefully acknowledged. The closeness of the association between us all has been the catalyst to the successes achieved and those only now dreamed of.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Fig

Fig

Fig

Fig

Figu

Figu

Figu

Figu

Figu

Figu

Figu

Figu

Figu

Figure

Figure

LIST OF SYMBOLS

E	Herein refers to quantized energy equal to the product of Planck's constant and the frequency of the radiation.
h	is Planck's constant.
ν	Refers to the frequency of radiation.
Φ	Known as fluorescence quantum efficiency and is defined as a ratio of the total energy emitted to the total energy absorbed as a function of time and independent of excitation wavelength.
C_f	is the fluorophore concentration
ϕ_f	is the fluorescence radiant flux
ϕ_o	is the incident (excitation beam) radiant flux
ϵ	is the molar absorptivity.
b	is the cell pathlength in centimeters.
K	is a product of three constants: ϵ, Φ and b.
$f(\theta)$	is the geometrical factor dependent on the effective solid angle viewed by the detector.
$g(\lambda)$	is the response characteristic of the detector varying with wavelength.
K'	is a product of five contants, $f(\theta)$, $g(\lambda)$, ϵ, Φ, and b, for a particular set of experimental conditions.
C_{max}	is the concentration level of a fluorophore above which nonlinearity is observed in a plot of ϕ_f with C_f.
ϵ_λ	is the molar absorptivity at a specific wavelength of excitation.
Ex	is the excitation radiation or source of said radiation.
Em	is the emission radiation, fluorescence radiation in particular.
X_α, X_β	are distances of the fluorescence detector axis from the inner surface of the excitation cuvette face; X_α is the shorter penetration distance, X_β is the longer penetration distance.
Y_α, Y_β	are distances of the excitation beam axis from the emission port cuvette face; Y_α is the shorter penetration distance, Y_β is the longer penetration distance.
Δd	is the distance between the focal points of fields of detection along either the excitation or emission optical axes; refers to distance between foci of positions 1 & 4, 2 & 3, or 1 & 2 and 3 & 4.
(0)	is an ideal (non-existing) cell position which is attained by correcting for primary and secondary absorption attenuations.

(1) is a position refered to in the method of cell rotation possessing the least primary absorption and the least secondary absorption with regard to other attainable cell positions.

(2) is a position refered to in the method of cell rotation possessing the least primary absorption and most secondary absorption with regard to other attainable cell positions.

(3) is a position refered to in the method of cell rotation possessing the most primary absorption and the most secondary absorption with regard to other attainable cell psotions.

(4) is a position refered to in the method of cell rotation possessing the most primary absorption and the least secondary absorption with regard to other attainable cell positions.

λ_1 is the wavelength of excitation radiation.

λ_2 is the wavelength of emission (or fluorescence) radiation.

X_i refers in general to the distance of the detection axis from the inner source excitation face of the sample cell.

ϕ_i refers to the component of radiant flux being detected in a slice of solution volume perpendicular to the propagation axis of excitation.

n is the number of component slices of detected radiant flux across the emission window.

ϕ is an idealized component slice of detected radiant flux across the emission window; here the radiant flux is assumed identical for each slice regardless of penetration distance.

T_λ is the sample transmittance at a specified wavelength.

ω_α is equal to the ratio x_α/b, a dimensionless emission window parameter.

ω_β is equal to the ratio x_β/b, a dimensionless emission window parameter.

Θ_α is equal to the ratio Y_α/b, a dimensionless excitation window parameter.

Θ_β is equal to the ratio Y_β/b, a dimensionless excitation window parameter.

Θ_i is equal to the ratio Yi/b, a nonspecific dimensionless excitation window parameter.

f is the fraction radiant flux in each slice of the detected emission beam; it describes the ratio ϕ/ϕ_o.

$\Delta\omega$ refers to the difference in magnitude between W_α and W_β, $W_\beta - W_\alpha$.

$\Delta\Theta$ refers to the difference in magnitude between Θ_α and Θ_β; $\Theta_\beta - \Theta_\alpha$.

ϕ_{ω_α} is the fluorescence radiant flux detected at ω_α

ϕ_{ω_β} is the fluorescence radiant flux detected at ω_β

A_1 refers to the absorption of excitation radiant energy as a function of penetration distance along the detected excitation propagation axis.

GF is the geometric factor required to extrapolate the absorption of excitation radiation from the inner cell surface to the focus of position one.

$\phi_{f(1)}$ is the detected fluorescence radiant flux at position one.

ϕ_{cf1} is the detected fluorescence radiant flux at position one corrected for primary absorption.

$\phi_{e\alpha}$ is the fluorescence radiant flux detected at θ_{α}.

$\phi_{e\beta}$ is the fluorescence radiant flux detected at θ_{β}.

A_2^o refers to the absorption of emission radiant energy as a function of penetration distance along the detected emission propagation axis.

GF' is the geometric factor required to extrapolate the absorption of fluorescence radiation from the inner cell surface to the focus of position one.

ϕ_o' is the effective field generated emission beam radiant flux prior to penetration of solution toward the emission port.

Cc is the concentration of the secondary absorber, either the fluorophore itself or another chromophore.

ϕ_{cf2} is the detected fluorescence radiant flux at position one corrected for secondary absorption.

f_1 is the correction factor derived from primary absorption measurements; $(GF)\Delta A_1^o$

f_2 is the correction factor derived from secondary absorption measurements; $(GF)\Delta A_2^o$

$\phi_{cf1\ ^o2^o}$ is the detected fluorescence radiant flux at position one corrected for both primary and secondary absorption.

ϕ_{f2} is the detected fluorescence radiant flux at position two.

ϕ_{f4} is the detected fluorescence radiant flux at position four.

QS refers to quinine sulfate

S/N is the ratio of detected fluorescence signal to the background noise.

CHAPTER I

INTRODUCTION

When a beam of electromagnetic radiation passes through a material or sequence of different materials (i.e., the sample and its containing vessel), its energy may be channeled into a variety of processes (Figure 1). Part of the radiation will be absorbed and transduced into whatever system dependent deactivation processes (Table 1) are in effect, part will be reflected, part will be transmitted, and a part will be scattered in various ways (i.e., Tyndall, Rayleigh and Raman scattering). Absorption takes place in discrete units of quanta, the energies of which are equal to the product $h\nu$, where ν is the frequency of the radiation and h is Planck's constant. Quanta of visible or ultraviolet radiation are of sufficient energy to raise a molecule to an excited electronic state, from which this energy may be converted into rotational, vibrational or kinetic energy (i.e., heat) or into chemical energy (i.e., photochemical reactions, aggregation phenomena) or part of the energy may be re-emitted as quanta of lower energy (i.e., as fluorescence or phosphorescence).

For analytical applications, fluorescence has been the most utilized of the molecular deactivation processes due to its facility for characterizing excitable chemical systems and its remarkable

Figure 1. Schematic Diagram of Activation and Deactivation Processes For Molecules

Key: (1) Activation step, excitation of singlet ground state, So, to the first excited singlet state, S_1^*.
(2) Radiationless deactivation of S*
(3) Radiational deactivation of S* (fluorescence)
(4) Intersystem crossing S* to the ground triplet state, To
(5) Intersystem crossing To $\longrightarrow$ S*
(6) Radiationless deactivation of To
(7) Radiational deactivation of To (phosphorescence)

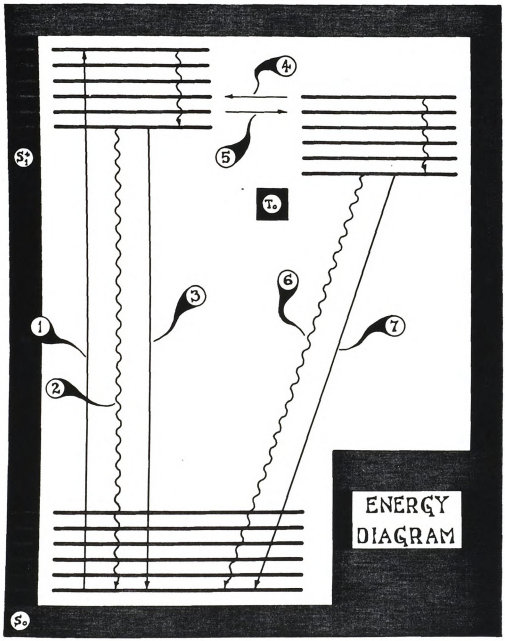


FIGURE 1

Table 1. Activation and Deactivation Processes[1]

Reactants	Rate Constant	Products	Description	Type (Figure 1)
$S_0 + h\nu_a$	$\xrightarrow{k_a}$	S*	Excitation Step	1
$S^* + S_0$	$\xrightarrow{k_{sq}}$	2So	Quenching due to Collision with So	2
S* + Y	$\xrightarrow{k_{sy}}$	So + Y	Quenching due to Collision with Y	2
S*	$\xrightarrow{k_{sp}}$	Products	Photochemical Reaction	2
S* + So	$\xrightarrow{k_{sd}}$	$(So)_2$	Dimerization	2
S* + Y	$\xrightarrow{k_{sf}}$	So + Y*	Forster-type intermolecular energy transfer	2
S*	$\xrightarrow{k_f}$	$So + h\nu_f$	Fluorescence	3
S*	$\xrightarrow{k_{si}}$	To	Intersystem crossing (spin forbidden)	4
To	$\xrightarrow{k_{ti}}$	S*	Intersystem crossing (spin forbidden)	5
To + So	$\xrightarrow{k_{tq}}$	2So	Quenching due to collision with S	6
To + Y	$\xrightarrow{k_{ty}}$	So + Y	Quenching due to Collision with Y	6
To	$\xrightarrow{k_{tp}}$	Products	Photochemical reaction	6
To + So	$\xrightarrow{k_{td}}$	$(So)_2$	Dimerization	6
To + Y	$\xrightarrow{k_{tf}}$	So + Y	Forster-type intramolecular	6
To	$\xrightarrow{k_p}$	$So + h\nu_p$	Energy Transfer Phosphorescence	7

Symbols defined: So = ground singlet state of molecule; S* = excited singlet state of molecule; To = lowest triplet state of molecule; h = planck's constant; ν_a = frequency of absorbed radiation; ν_f = frequency of fluorescence radiation; ν_p = frequency of phosphorescence radiation; Y = solvent or impurity molecule; Y* = excited nonluminescing solvent or impurity molecule; $(So)_2$ = non-luminescing dimer.

sensitivity. The initial excitation or absorption of radiation by a molecule in solution is dependent on the energy difference between its electronic states. In general, the molecules will absorb a narrow band of radiation the wavelengths of which are equivalent to the energy level separation between two electronic energy states and associated vibrational and rotational energy levels. Since for each electronic state there are many vibrational and rotational levels, an absorption band is observed instead of absorption at a single wavelength.

Scientific literature over recent years spanning a wide variety of areas such as trace metal determination, analyses for traces of organic materials, and particularly for determining trace constituents of biological systems, attests to the ever increasing interest in and importance of fluorescence.

Instrumentation for the measurement of fluorescence has evolved over the last several decades to where it now routinely provides a means for obtaining accurate qualitative and quantitative information about these molecules.

In brief, fluorometry, no matter how simple or complicated the instrumentation, consists of three basic components: a source of radiant energy with which to irradiate (excite) the sample; a sample holder; and a detector to observe the fluorescence emitted by the sample. The complexity of the instrument depends on the degree to which the frequency and radiant flux of the exciting radiation need be controlled, the degree to which specific frequencies of fluorescence can be selected and the sensitivity and precision with which the selected fluorescence radiant flux can be measured.

Table 2a. Instrumental Variables

A. Source Stability
B. Source Spectral Distribution
C. Excitation Monochromator
    1. Intensity Variation
    2. Bandwidth Control
    3. Stray Excitation Radiation
D. Cell Geometry
E. Emission Monochromator
    1. Intensity Variation
    2. Bandwidth Control
    3. Stray Excitation Radiation
F. Detector
    1. Sensitivity
    2. Linearity
    3. Stability
    4. Spectral Response
G. Amplifier
    1. Stability
    2. Linearity
H. Read Out
    1. Stability
    2. Linearity

Table 2b. Photophysical Variables

A. Quantum Efficiency
    1. Radiationless Collisions
    2. Internal Conversion
    3. Phosphorescence
B. Absorption
    1. Primary
    2. Secondary
C. Reflections and Refractive Index
D. Light Scattering
E. Re-Emission

In filter fluorometers, selection of the frequency is made by inserting appropriate filters in the beams of the exciting and fluorescence radiation. If a monochromator is used to select the frequency of either beam, the instrument by definition becomes a spectrofluorometer. It can then be used to measure the spectral distribution of the fluorescence radiation (i.e., "fluorescence emission spectrum"), or the variation of fluorescence radiant flux with frequency of the exciting radiation (i.e., "Fluorescence excitation spectrum").

Spectrofluorometry has the advantages over spectrophotometry of being potentially far more analytically sensitive and providing two spectra as criteria for identification and characterization instead of one. It has the obvious limitation that not every absorbing substance fluoresces.

Molecules that fluoresce do so with a characteristic quantum efficiency, Φ, defined as a ratio of the total quanta emitted to the total quanta absorbed per unit time and is independent of the excitation wavelength.

$$\Phi = \frac{\text{Number of quanta fluoresced}}{\text{Number of quanta absorbed}}$$

Where both the quantity in the numerator and denominator are measured in a given time frame for a specific emission and excitation transition, respectively. The relationship between fluorescence measured and the fluorophore concentration, C_f, is given as:

$$\phi_f = \Phi \cdot \phi_o \quad (1 - e^{-\epsilon bc_f}) \qquad (1)$$

and expanded,

$$= \Phi \cdot \phi_o \cdot (1-1 + \epsilon bc_f - \frac{(\epsilon bc_f)^2}{2!} + \frac{(\epsilon bc_f)^3}{3!} - \ldots) \quad (2)$$

Where : ϕ_f = fluorescence radiant flux; ϕ_o = incident radiant flux; ϵ = molar absorptivity; b = pathlength of cell; C_f = molar concentration of fluorophore.

For dilute solutions in which only a small fraction of the exciting radiation is absorbed (ϵbc_f is small and only the first term in Equation 2 is significant):

$$\phi_f = k \cdot \phi_o \cdot C_f \quad (3)$$

Where k represents the product of the constants Φ, ϵ and b. A plot of ϕ_f vs C_f should yield a straight line. The linearity, however, breaks down at higher concentrations when $\epsilon bc_f > 0.01$ and higher order terms in Equation 2 become significant. Table 2 provides a listing of instrumental and photophysical factors which can account for observed deviations from linearity.

The above equation demonstrates ϕ_f is directly related to ϕ_o. To obtain higher analytical sensitivity, ϕ_o can be increased. With modern photomultipliers and amplifiers the detection of very low concentrations of fluorescent substances is feasible. A fundamental distinction of spectrofluorometry from spectrophotometry becomes apparent in that for the latter the detection limit is set by the minimum detectable difference in radiant flux between the incident and transmitted radiation and extremely precise measurements of radiant flux are required to attain as high a sensitivity. Furthermore, in spectrophotometry, a ratio ϕ/ϕ_o is evaluated. Increasing ϕ_o will increase ϕ in a proportionate amount so that the ratio is constant for a particular concentration.

Outside of instrumental factors quenching and inner filter effects (i.e., primary and secondary absorptions) are the major influences on the accuracy of quantitative fluorometric measurements. Lack of appreciation of the elementary principles governing these two effects, or confusion between the two, has in the past led to the use of unsatisfactory equipment or procedures and to the incorrect interpretation of fluorescence measurements. Quenching includes all those processes that result in lowering the quantum efficiency from that of the isolated ideal state by diverting radiant energy absorbed by the molecule into channels other than the fluorescence emission process. Primary absorption involves absorption of the exciting radiation by the fluorophore or other chromophores in the background matrix. Secondary absorption involves absorption of the fluoresced radiation either by self-absorption due to partial overlap of the absorption and emission bands or by absorption by other chromophores. The combined effects of the absorption processes have been called the innter filter effects in the period prior to clear definition of causation. This term is still used by some authors.

Over recent years, investigators have evaluated these inner filter effects and have, to varying degrees, succeeded in compensating for the associated attenuations. Historical aspects leading up to the current state-of-the-art instrumentation, optical geometries and data correction procedures follow.

The goal of this present work was to design an improved computerized instrument to make accurate and correct corrections in fluorescence measurements for primary and secondary absorption effects and to

evaluate its performance. To this end, a novel method employing cell rotation was developed which allows regulation of the thickness of sample through which the excitation and emission beams pass.

CHAPTER II

HISTORICAL

A. Problems

A lucid and exhaustively detailed account of the problems caused by excessive sample absorption, the conventional methods applied in dealing with excessive sample absorption, and the mathematical expressions used to correct spectra attenuated by primary and secondary absorptions is compiled in Christmann's Ph.D. dissertation[2].

Inner filter effects diminish the quality, and therefore, the usefulness of the fluorescence data obtained from a particular chemical system. Both quantitative fluorescence measurements and qualitative spectral determinations are adversely affected. The discussion will focus individually on these aspects of spectrofluorometry.

1. Quantitative Fluorescence Measurements.

The analytical versatility of fluorescence spectroscopy is due not only to its inherently high sensitivity, but also to the relationship between observed fluorescence and fluorophore concentration in ideal situations. This point is made in many current analytical textbooks that include even rudimentary descriptions of the processes of photoluminescence. This relationship can be represented as follows:

$$\phi_f = f(\theta).g(\lambda).\phi_o.\Phi.\ (1-e^{-\epsilon bc})$$

$$= f(\theta).g(\lambda).\phi_o.\Phi.x$$

$$\left[1-(1-2.3\ \epsilon bc + \frac{(2.3\,\epsilon bc)^2}{2!} - \frac{(2.3\,\epsilon bc)^3}{3!} + \ldots + \frac{(2.3\,\epsilon bc)^n}{n!}\ )\ \right]$$

$$= f(\theta).g(\lambda).\phi_o.\Phi.x$$

$$\left[\ (2.3\,\epsilon bc - \frac{(2.3\,\epsilon bc)^2}{2!} + \frac{(2.3\,\epsilon bc)^3}{3!} + \ldots + \frac{(2.3\,\epsilon bc)^n}{n!}\ \right] \quad (4)$$

Where $f(\theta)$ is the geometry depending on the effective solid angle viewed by the detector, $g(\lambda)$ is the response characteristic of the detector varying with wavelength, and ϵ, b and c have their usual Beer's law connotations.

When the fluorophore is very dilute (i.e., when the primary absorbance of the solution is less than 0.01[1], only the first term of the power series is significant. The expression reduces to the linear form:

$$\phi_f = f(\theta).g(\lambda).\phi_o.\Phi.\epsilon.b.c. \quad (5)$$

$$= K'.\phi_o.c. \quad (6)$$

Fluorescence radiant flux is a linear function of concentration and of incident radiant flux. The term K' for a particular set of experimental conditions is equal to a collection of constants, $f(\theta).g(\lambda).\Phi.\epsilon.b$.

At higher fluorophore concentrations it becomes necessary to include additional terms from the power series. Examination of the expanded equation shows that the terms alternate in sign. As more terms are evaluated (for higher concentrations), those which are negative begin to influence the overall value significantly.

The concentration level above which non-linearity becomes apparent can be determined by:

$$C_{maximum} = \frac{0.01}{\epsilon_{\lambda} b} \quad (7)$$

Where ϵ_{λ} is the molar absorptivity at the wavelength of excitation and b is the sample pathlength along the axis of excitation. For establishing a standard curve it is important to determine the fluorophore concentration at which the ϕ_f vs. C_f plot becomes significantly non-linear for the accuracy desired.

At sufficiently high concentrations the fluorescence radiant flux reaches a peak and then decreases rapidly. This observation[1] demonstrates that fluorescence radiant flux is a function of the penetration distance of the exciting radiation and increasing fluorophore concentration (Figure 2). Recently investigators have described this phenomenon as an apparent shifting of the most intensely fluorescing region of the sample solution toward the excitation face of the cell as the penetration depth of the exciting radiation is reduced by increased absorption (Figure 3). It has been shown that the position of the geometrical detection window is directly related to the curvature of the fluorescence response[10]. Departure from linearity is mainly due to primary absorption (Figure 4). Other key processes involved are self-quenching and secondary absorptions (Figure 5).

The primary absorption process is responsible for supplying energy that is subsequently reemitted as fluorescence radiation (Table 1). Its molecular basis had been first identified in the early thirties. Later studies[11-13] further described these forms of energy transition, both

Figure 2. Fluorescence Radiant Flux as a Function of the Penetration Distance of UV-VIS Radiation Over a Hypothetical Series of Increasing Fluorophore Concentrations

Key: (A) very dilute, no detectable ϕ_f attenuation across emission window; (H) very concentrated, extreme ϕ_f attenuation; (B) through (G) successive intermediate concentrations.

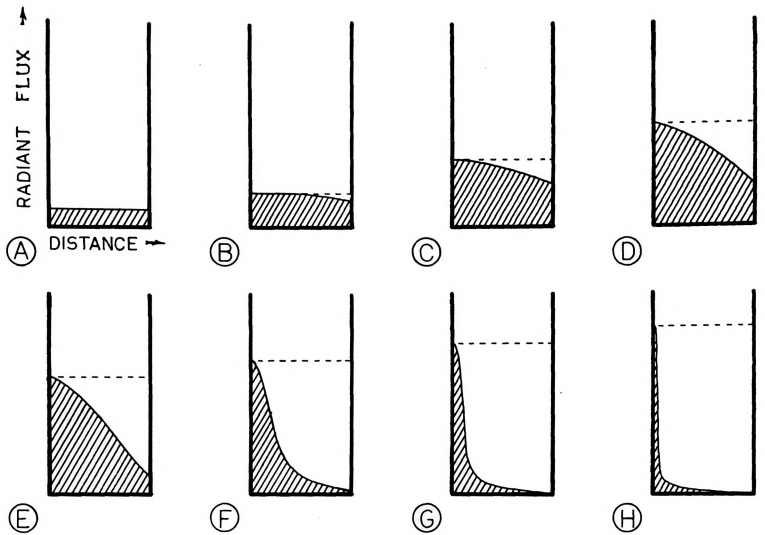


FIGURE 2

Figure 3. Apparent Fluorescence Emitting Region As a Function of Fluorophore Concentration

Key: EX = Excitation Radiation
EM = Detected Fluorescence Radiation
CONC = Fluorophore Concentration
O = Emission Port Detector

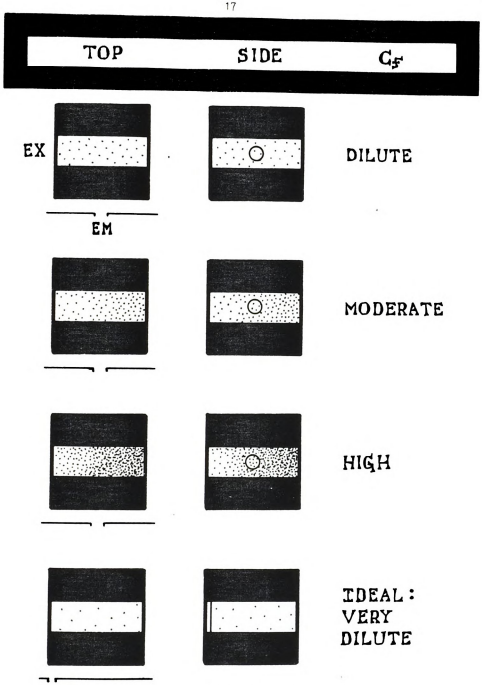


FIGURE 3

Figure 4. Effect of Primary Absorption Along Excitation Axis With Respect to Apparent Luminosity

Top = Reference medium (negligible primary absorption)

Bottom = Sample medium (detectable primary absorption)

Key: X_1, X_2 = distances of fluorescence detector axis from the excitation cuvette face
EX = Excitation Source Radiation
EM = Emission Radiation

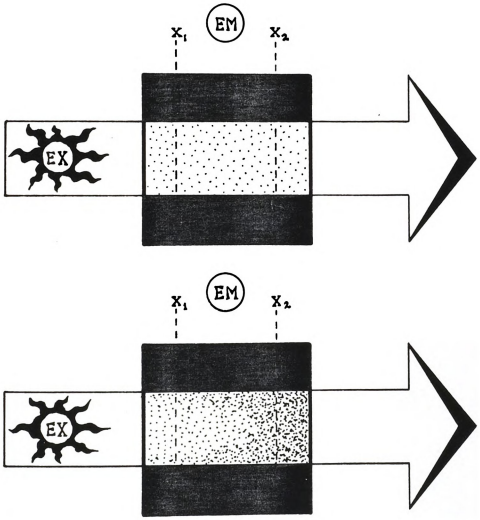


FIGURE 4

Figure 5. Effect of Secondary Absorption Along Emission Axis With Respect to Apparent Luminosity

Top = Reference medium (negligible secondary absorption)

Bottom = Sample medium (detectable secondary absorption)

Key: Y_1, Y_2 = distances of excitation beam axis from the emission port cuvette face
EX = Excitation Source Radiation
EM = Emission Radiation

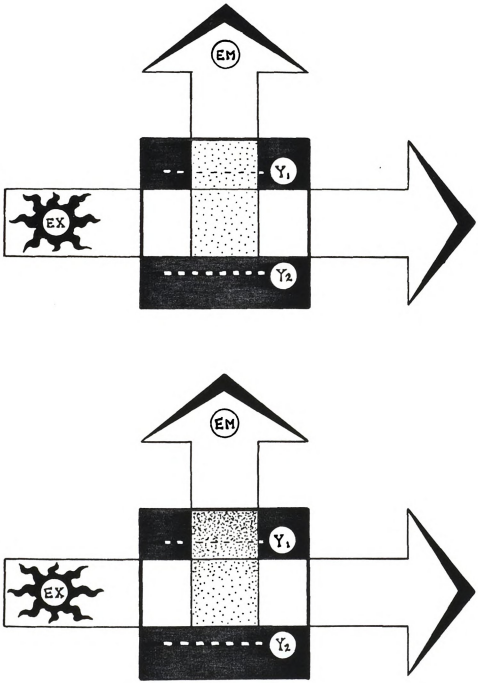


FIGURE 5

theoretically and experimentally. It was not until the seventies[9,11] that an instrument was developed to correct fluorescence measurements conveniently for this attenuation. Prior to the advent of correction procedures, accurate analysis could only be attained by working with dilute solutions where the attenuation caused by primary absorption was negligible. However, the limitation of measuring only dilute solutions is not a universal remedy. This point will be further discussed in the section under conventional methods.

The secondary absorption processes include self-absorption and absorption of fluorescence by other chromophores in the matrix. Both processes will cause attenuation of the ϕ_f vs. C_f analytical curve. Self-absorption is a result of overlap between a molecule's absorption and emission bands. The effect is noticeable when the fluorescence is measured on the short wavelength side of an emission band so that the fluorophore itself may absorb its own fluorescence radiation. This attenuation is observed as an exponential decrease of the relative portion of radiation transmitted by the solution at the emission wavelength with increasing concentration of the fluorophore[14]. Usually fluorescence is measured outside of this region of the spectrum unless it is necessary to use this region to avoid more severe interferences from other absorbing species. Fluorescence absorption by other solution components depends on their respective concentrations and overall characteristics of the absorption spectra. When possible the background matrix should be prepared without inclusion of such component species. If they cannot be eliminated and these effects are significant, then a correction for the attenuation must be applied.

Performance of routine fluorescence analyses requires that the calibration (standard) curve is prepared with a set of standards possessing an identical background matrix. If this practice is not observed, there is no compensation made for the different degrees of primary and secondary absorption encountered[15,16].

2. Qualitative Fluorescence Measurements

Fluorescence and ultraviolet-visible spectrometry provide similar information regarding qualitative analyses. The excitation spectrum of a fluorescent molecule theoretically is similar to its uv-vis absorption spectrum[17,18]. This signifies that fluorophores may be characterized by direct comparison to universally available uv-vis standard spectra reference files such as those published by Sadtler Research[19] and API[20].

In theory, the emission spectrum should be a mirror image of the excitation spectrum. However, since an excitation spectrum is normalized with respect to energy along the wavelength or wavenumber axis and that the emission spectrum is not, severe distortions from the expected may result[17,18]. Also when complex transitions are involved, as in the case of quinine sulfate, emission spectra will not be a mirror image of excitation spectra.

Instrumental factors such as the variation of the source energy with wavelength and variations in the spectral sensitivity of the detection system distort the fluorescence excitation and emission spectra[12,13,21-26]. Several commercially available spectrofluorometers correct for these effects.

Photophysical factors such as primary and secondary absorption can result in severe spectral distortions[12]. In dilute solutions of primary absorbance of less than 0.01 these factors are usually negligible. An exception would be a system containing a strong secondary radiation absorbing species which is transparent to the excitation radiation. As primary absorption begins to exceed 0.01 a decrease in the fluorescence excitation spectrum relative to the uv-vis absorption spectrum is observed[27]. As was noted in making quantitative measurements, even higher increases in absorption will reduce the effective penetration distance of uv-vis radiation through the media (Figure 2). This will cause the ϕ_f vs. C_f plot to pass through a maximum and proceed to bow down toward the concentration axis. This may grossly distort the excitation spectrum further departing from it's expected "true" characteristics.

Attenuations caused by the absorption of emission radiant energy will incorporate a negative error in relative quantum yield determinations employing area integration techniques[26].

Self-absorption can be differentiated from other chromophores since the loss of radiant flux will be greater on the short wavelength side of the fluorescence emission spectrum.

Secondary absorption by the background matrix will reduce the observed fluorescence emission. The matrix components will affect the emission spectrum only in the spectral regions where they themselves absorb. In this case quantum yields will also be low.

3. The Effects of Primary and Secondary Absorption on Other Forms of Information Derived From Fluorescence Measurements

Thermodynamic quantities and associated chemical equilibrium constants have been extracted from fluorescence data[28]. Unfortunately, these data, unless corrected for both instrumental and photophysical errors, will give erroneous values for such quantities. Calculations of this nature assume linearity in the ϕ_f vs. C_f interrelationship throughout the concentration range under study. The most highly regarded determinations of these quantities[2] involved preparation of calibration curves to encompass the concentration range. In all cases, background matrices were matched to that of the sample.

If there are absorption changes, the fluorescence data collected over time as a reaction proceeds must be continually corrected. In a monitored reaction one must be aware of the potential for a changing background matrix. These changes must be accounted for prior to evaluation of rate constants and activation parameters used in establishing system specific rate laws. In recent years correction factors for primary and secondary absorption have been applied to static systems[2,9,29,30]. An automatic and rapid absorption correcting instrument adaptable to repetitive scanning of dynamic chemical systems has not yet been reported. Until the advent of such instrumentation, the meaningfulness of these quantities should be viewed with suspicion.

B. Conventional Methods

Various approaches, both in experimental procedure and in instrumentation, have been devised in overcoming or correcting for primary and secondary absorption. The following methods will be discussed: sample dilution; optimum choice of excitation and emission wavelengths; detection geometries (i.e., transmission, front-surface, right-angle (Figure 6); non-typical designs of sample cells; and two-photon excitation. Limitations encountered in each method will be emphasized.

1. Sample Dilution

A common "textbook" approach for minimizing excessive sample absorption was to dilute the sample until the linear portion of the ϕ_f with C_f curve was attained[17,31]. For many chemical systems, this was the easiest method for reducing the attenuations due to these absorbances. This remedy was not found universally applicable. Chemical systems containing a fluorophore at very low concentrations and a highly absorbing background matrix are an example. Further dilution of such a system must encounter the trade-off of decreasing C_f below detection sensitivity of the instrument and never attaining the linear region of the calibration curve. Also, since a wide variety of chemical species are concentration dependent (i.e., possible conformational changes, alterations in intermolecular or intramolecular bonding, formation of different solvent structures) unidentified chemical events can reduce the concentration of the fluorescing species or may cause other significant changes in fluorescence. As a result, errors of unknown magnitude are introduced into the measurements.

As simple as dilution appears, extreme care must be exercized to avoid contamination of the sample by the dilution procedure. Purity of all media used in dilution must be ascertained and an identical background matrix should be included. These precautions will give more confidence to the resulting measurements.

2. Optimum Choice of Excitation and Emission Wavelengths

The use of filters and monochromators has made possible the selection of narrow bandwidths of either the excitation or emission radiation. The chosen excitation or emission wavelengths are those at which primary and secondary absorption interferences are minimal. This approach is convenient with commerically available spectrofluorometers since they have both an excitation and an emission monochromator.

Problems with this approach arise when the sample complexity precludes the existence of such wavelengths where it may be impossible to find wavelengths where primary and secondary absorption effects are negligible. Where one chromophore does not absorb, another may. The absorption and emission characteristics of other fluorophores may cause spectral interferences.

A fundamental limitation is in the reduction of sensitivity by selection of wavelengths shorter or longer than the excitation or emission maxima.

Wavelengths should be chosen to minimize Rayleigh scattering. The magnitude of the scatter peak depends upon the absorption characteristics of the solvent and sample, the size and number of particles suspended, and the wavelength of the exciting radiation.

Also wavelengths that encompass intense Raman scattering should be avoided. Raman scatter is the result of interaction of photons with the vibrational energy levels of solvent or solute molecules which results in inelastic scattering of photons of lower or higher energy. A blank is used to determine the radiant flux of the Raman bands which can be subtracted from the sample fluorescence radiant flux. Often it is possible to minimize Raman interferences by exciting the sample not at the peak of the excitation band, but at a shorter wavelength on the shoulder of the excitation band. This reduces sensitivity to some extent, but in the process moves the Raman band toward shorter wavelengths, thus reducing its interference with the emission band of interest.

Many commercial double beam spectrofluorometers subtract fluorescence radiation from a reference solution containing only the background matrix from the sample solution. This will eliminate the scatter bands and the effect of matrix chromophores and fluorophores which do not interact significantly with the fluorophore of interest.

3. Detection Geometries

Cell geometry describes the cell orientation as it relates to the excitation beam and emission optics. The evolution of geometries applied to account for the absorption artifacts will be briefly presented here and with regard to applied correction factors in the following section.

Figure 6. Detection Geometries

A. Transmission
B. Front Surface
C. Right Angle

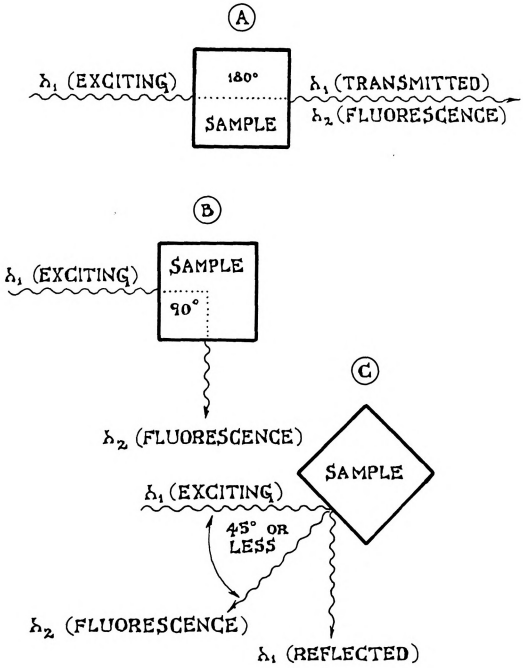

A
λ1 (EXCITING)
180°
SAMPLE
λ1 (TRANSMITTED)
λ2 (FLUORESCENCE)
B
λ1 (EXCITING)
SAMPLE
90°
C
λ2 (FLUORESCENCE)
SAMPLE
λ1 (EXCITING)
45° OR LESS
λ2 (FLUORESCENCE)
λ1 (REFLECTED)

FIGURE 6

a. Transmission Geometry

The arrangement in transmission geometry is shown in Figure 6a. Fluorescence is viewed along the same axis that the excitation radiation propagates. Generally an emission monochromator is required to isolate only fluorescence wavelengths.

The advantage, aside from the simplified instrumental set-up, is that attenuation of of measured fluorescence due to primary absorption is much less pronounced than in instruments using a right-angle geometry[32]. The actual primary absorption over a constant distance, Δd, is unchanged between geometries, but as this attenuation increases and, subsequently, the maximal (apparent) fluorescing region compresses toward the source of exciting radiation a dramatic difference between the two geometries becomes apparent. In the right-angle mode of detection the region of maximal fluorescence on increasing primary absorption will be at the entrance cell wall for the excitation beam from where it is virtually impossible to isolate emission radiation. With the transmission arrangement all emitted radiation within the detection window is monitored. Negative deviations from the ideal linear ϕ_f and C_f plot are thus reduced in the ideal case, dependent only on the inverse square loss of the emitted radiation as its origins become more remote from the viewing detector.

The major drawback to transmission geometry remains, however, the quality of the emission optical systems needed for viable separation of stray light from the desired fluoresced radiation.

A disadvantage relative to right angle detection is the apparent increase in the effect of secondary absorption[32]. This is caused by an

increase in the average pathlength traversed by the fluorescence radiation in the transmission geometry. This average is further increased if the primary absorption is high[33]. The reason, discussed in the problems section, for this observation is depicted in Figures 2 and 3.

b. Front Surface Geometry

The arrangement in front-surface geometry is shown in Figure 6c. Fluorescence is viewed at a 45° (or smaller) angle of reflection from the axis of propagation for the excitation radiation.

Frontal illumination has the advantage of permitting the measurement of the emission spectrum of a solution containing a high concentration of a fluorescent substance. Obtaining such a spectrum with right-angle or transmission geometries may be impossible since in very concentrated solutions a large portion of the exciting light is absorbed close to the region of entry into the cell. For concentrated systems, this geometry is the only feasible way of keeping the fluorescing region in the detection window. This greatly reduces the primary and secondary absorptions that are encountered by further penetration of the solution. However, this approach only reduces the magnitude of these absorptions, they are not eliminated. Various investigators have demonstrated that, indeed, secondary absorption can be quite high with some systems[33,35]. Later, suggestions were made for exciting only at absorption maxima and thereby reducing the high concentration of fluorophore required[17,35].

When dilute solutions of a fluorophore are measured, the observed fluorescence is proportional to concentration and an excitation spectrum unattenuated by primary absorption is recorded. At higher concentrations where primary absorption begins to attenuate the ϕ_f with C_f

plot, distortions appear in the excitation spectrum.

The major disadvantages inherent in this mode of detection are high secondary absorption effects, an increase in the probability of quenching because of the high concentrations, significant distortions of the emission spectra due to re-emission of absorbed radiation[32,34] and reduction of fluorescence if analyte association occurs. In addition, non-orthagonality between excitation and emission beams and their respective cell faces can induce severe polarization artifacts and multiple internal reflections.

c. Right-Angle Geometry

The arrangement in right-angle geometry is shown in Figure 6b. Fluorescence is viewed at an angle of 90° to the propagation axis of the excitation radiation.

The major advantage of right-angle detection, where the detector monitors only the illuminated liquid and not the illuminated cell face, is that interference by stray excitation radiation arising from either reflections at the cuvette faces or any fluorescence of the cuvette itself is minimized.

A limitation is the fact that the excitation radiation has to pass through a depth of solution, Δd, before reaching the region viewed by the detector where its effective radiant flux may be reduced by primary absorption. This arrangement is suitable for moderately absorbing solutions, which generally refers to dilute solutions of the fluorophore. Considerably larger concentrations of a transparent solute in the background matrix can be tolerated with no adverse effect.

4. Designs of Sample Cells

The normal cell employed with commercial spectrofluorometers has dimensions of 1 cm x 1 cm x 4.5 cm height. Occasionally instruments using cells of different dimensions have been reported.

Absorption interferences can be reduced by taking advantage of shorter pathlengths since primary absorption is proportional to distance of solution traversed. In many cases, the analysis can be confined to the linear portion of the ϕ_f with C_f curve[36]. In addition to absorption, deviation from linearity may be caused by light scattering and fluorescence of the cell walls when right-angle geometry is applied. Care must be taken that the detector monitors only a region within the solution, otherwise large distortions may result.

Fiber optics have been used as channeling devices for both excitation and emission radiation. The fiber optic bundles are attached directly to the faces of the cuvette. This approach has been observed to account for a three-fold decrease in primary absorption artifacts[37].

5. Two-Photon Excitation

A novel method has been developed to eliminate virtually all interference of primary absorption[38]. In principle, photons possessing half of the energy required for absorption are passed through the media containing the fluorophore. When two such photons impact on a fluorophore molecule simultaneously, they are absorbed and the system successfully reaches its first excited singlet state. The investigators have found that very concentrated solutions could be monitored without excess absorption artifacts.

Disadvantages[2] include considerably more expensive and complex instrumentation, continued presence of secondary absorption effects, and the requirement for a laser source to increase the probability of simultaneous impact of low energy photons. The method provides an interesting, although impractical for routine analyses, approach to the problem of primary absorption.

C. Mathematical Corrections

The objective of the conventional methods described in the last section was to eliminate artifacts due to excessive absorption. None of the instrumental arrangements was found to be a universal remedy. A direct means of eliminating these attenuations may prove to be elusive, but an indirect means has already been introduced. If either the primary or secondary absorption can be described by a mathematical expression, then a correction factor can be derived and applied to the fluorescence measurements.

1. Absorption Corrections for Transmission Geometry

Crude attempts to describe primary absorption were made as early as 1930[39]. Little success was achieved. The expressions used were later found to be simplistic and incomplete. Soon after in 1938[40,41], more elaborate expressions were derived in terms of primary and secondary absorbances. It was later deduced that these were based on an incorrect assumption. All of the excitation radiation absorbed does not, as the expressions indicated, contribute to the emitted fluorescence. Background matrix components sometimes absorb and may not fluoresce. In 1944[42] similar equations were derived. This time agreement was satisfactory in the comparison of calculated and measured ϕ_f values.

However, the systems were composed of a single fluorophore in a non-absorbing solvent. In 1973[43] a strictly theoretical work appeared accounting for these absorptions, but that an accurate compensation for the factors would require homogeneous, monochromatic and collimated excitation radiation and monochromatic and collimated fluorescence radiation.

2. Absorption Corrections for Front-Surface Geometry

For front-surface geometry the first expressions to account for both primary and secondary absorptions appeared in 1938[40,41]. Similar descriptions of these effects appeared later in 1951[44] and again in 1973[43]. None provided experimental evidence. From 1956 to 1959[45-48] a series of papers presented very elaborate corrections and experimental verification. Effects such as multiple reabsorptions and re-emissions were considered and incorporated into the correction schemes. In 1961[49] these expressions were adapted to account for the apparent receding of the maximal fluorescing region from the front face on dilution. Limitations at the detectable extremes of fluorophore concentration were discussed.

Other work reported on corrective expressions for this geometry was concerned with correcting fluorescence quantum yields[32,50-53].

3. Absorption Corrections for Right-Angle Geometry

The first theoretically derived expressions to account for primary and secondary absorption appeared in 1938[40]. They were later found to be too simplistic. They failed to include the possibility of absorbing species other than the single fluorophore. Also, the geometrical models used failed to describe accurately the actual experimental arrangement.

During the early fifties renewed interest in this area was awakened. One theoretical paper[44] was presented that based all of the fluorescence as originating from a point source. Experimental evidence was lacking. Two more papers quickly followed[55,56] and offered more elaborate expressions. Both ignored the excitation and emission window dimensions, critical in avoiding attenuations by the cell walls.

The late fifties offered a theoretical description of primary absorption based only on the solution (sample) absorption of excitation radiation and dimensions of the emission window[27]. Both the fluorophore and it's background matrix were considered.

In the early sixties[59] a more extensive treatment of derivations based on this measurement geometry was published. Both calculated and analytically measured curves of ϕ_f with C_f from several chemical systems were in agreement below a primary absorbance of 0.5. Large deviations were found to occur at higher concentrations. Inaccuracy in defining the applied detection geometry and failure to incorporate secondary absorption effects were limitations in extending plot linearity into higher concentrations.

Further documented attempts at experimental verification were not to follow until the early[57] and mid seventies[11,58]. Finally an accurate explanation of the validity of this approach had surfaced. Holland[9] found primary absorption to be independent of the nature of the absorbing species and the excitation and emission wavelengths. A computer centered spectrofluorometer (capable of making simultaneous absorption and fluorescence measurements) was developed for testing a newly developed mathematical model, more detailed than the ones previously proposed, that was derived to account for this absorption. This provided

the basis for calculating the required correction factor. Holland, Kelly and co-workers[11,29] found that this model resulted in primary-absorption-corrected fluorescence that was linear with the fluorophore concentration in solutions with total absorbances as high as 2.0. However, a limitation of this approach was not including a correction factor for the effects of secondary absorption.

In the mid sixties[60] a theory that developed expressions for primary and secondary absorption as a function of observed signal to noise ratio in photoluminescence spectrometry was formulated. Investigators[2] later questioned the validity of the expressions since they were partly derived from another theory describing self-absorption in flame photometry.

A comprehensive theoretical work[43] in the early seventies appeared to account completely for the geometry, all components responsible for both primary and secondary absorptions, and reflections within the sample cuvette. Soon afterwards, experimental verification was forthcoming[61]. The above theoretical expressions were adapted to procedures requiring fluorescence measurements to be made at three cell positions. Here the sample cell was manually shifted with respect to the excitation and emission optical axes. The positions are illustrated in Figure 8. A correction factor was calculated for the attenuation of the fluorescence signal as a function of distance along either axis. This procedure was called the "cell shift method"[2,61]. Practical use of this method was severely limited by problems in the manual re-positioning of the sample cell[66].

Over the last two years, a series of publications appeared by Christmann and co-workers[30,62,63] offering a detailed critical

Figure 7. Instrumental Components and Their Arrangement for the Moving Mirror Method

Cell compartment including:

A. Reference Cell
B. Sample Cell
C. Emission Collimation Baffles
D. Quantum Counter
E. Image Rotation Device

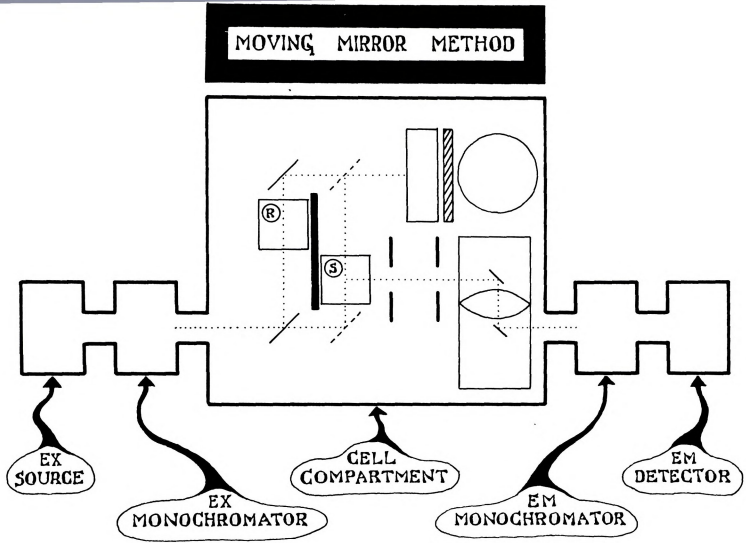

MOVING MIRROR METHOD
R
S
EX SOURCE
EX MONOCHROMATOR
CELL COMPARTMENT
EM MONOCHROMATOR
EM DETECTOR

FIGURE 7

Figure 8. Instrumental Components and Their Arrangement for the Cell Shift Method

Cell Compartment including:

A. Chopper
B. Quantum Counter
C. Beam Splitter
D. Cell, Sample
E. Emission Collimator

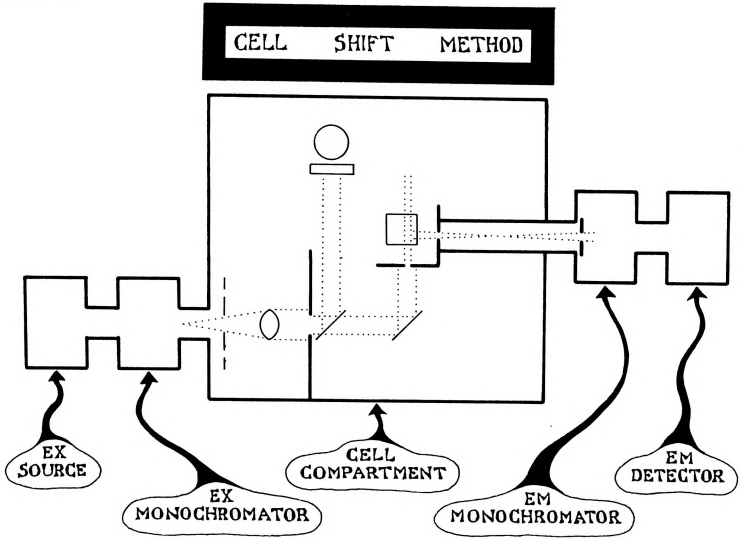

CELL SHIFT METHOD
EX SOURCE
EX MONOCHROMATOR
CELL COMPARTMENT
EM MONOCHROMATOR
EM DETECTOR

FIGURE 8

evaluation of the cell shift method. An automated computer controlled instrument (Figure 16) was constructed to compare conveniently the calculated with experimental results. A cell positioner was built that was computer controlled and highly accurate in cell repositionment. The method was shown accurate to two percent or better up to a sample absorbance of 2.7. Limitations to application of this method are noted when re-emission, reflection, and scattering phenomena are significant. A correction factor was determined to account for internal reflections.

CHAPTER III

CORRECTIONS FOR RIGHT-ANGLE GEOMETRY

A DETAILED EXAMINATION

A. Introduction

Correction factors for primary absorption have been derived and tested by investigators during recent years[2,33,27,58,62]. Accurate corrections for the interference have been demonstrated for sample absorbances at the excitation wavelength as high as 2.. Instrumentation in all of the above cases involved right-angle geometry for fluorescence measurement. As long as secondary absorption is negligible these corrections are adequate. When the sample solution is no longer transparent to the emitted fluorescence radiation, correction of primary absorption alone is not enough. To account for components that absorb fluorescence radiation a second correction factor was applied. Both corrections are essential to preserve precision and accuracy of the measurements.

A variety of attempts to describe and correct for secondary absorption have been made[14,61,64]. In some cases no experimental verification is provided. In other cases the accuracy of the model is not clearly demonstrated. The cell shift method[2,61] provided both clear

experimental evidence and accuracy in employing a correction for secondary absorption.

The major drawback of the cell shift method was, however, its slowness in data acquisition and processing. The object of this investigation has been to develop a novel and rapid technique to correct for primary and secondary absorption effects. This approach involves cell rotation instead of cell shifting. The geometry used is identical to that of the cell shift method; however, movement between positions is very fast, thus this technique useful with not only static chemical systems, but also dynamic systems. The design and performance evaluations of this instrument and technique will be described in subsequent chapter(s).

1. Need for Both Absorbance and Fluorescence

The problem of absorption by the sample system requires that absorption corrections are made at each wavelength. Since absorption is wavelength dependent, knowledge of the absorption as well as the fluorescence at each data point is necessary. To avoid errors introduced when measuring absorbance and fluorescence by two different instruments, it is essential that simultaneous absorption and fluorescence measurements are made with one instrument system. By use of such a simultaneous measurement technique, as introduced by Holland and coworkers[11], high confidence could be placed in the interrelationship between absorption and emission, since both measurements are made with the same solution, at the same time, and with the same optical system. The complexity of the instrumentation and the multiplicity of corrections applied to account for both instrumental and photophysical

factors made it essential to integrate the spectrofluorometer with a computer into one system that could record the fluorescence and absorption measurements and calculate and apply the various corrections.

B. Theory

1. The Moving (Vibrating) Mirror Method

a. Geometry

The optical configuration employed in this method is shown in Figure 9. A focusing lens is positioned to give a 1:1 image ratio at the entrance slit and a field lens which is compatible with the optical speed of the emission monochromator. The edges of the mask at X_1 and X_2 confine the observation window to a fixed fraction of the total fluorescence viewing area.

b. The Basic Assumptions:[11]

(1) The measured quantities (R, S, and F) are expressed in terms directly proportional to the absolute number of quanta involved (N). As shown below, the proportionality constants for the reference and sample beams are equal but may be different for the fluorescence beam.

$$R = kN_r$$

$$S = kN_s$$

$$F = k'N_f$$

(2) The absorption processes within the cell exponentially attenuate the excitation beam.

$$\phi_{(x)} = \phi_o e^{-kx}$$

Figure 9. Geometrical and Optical Configuration for the Collection of Fluorescence Radiation by the Moving Mirror Method

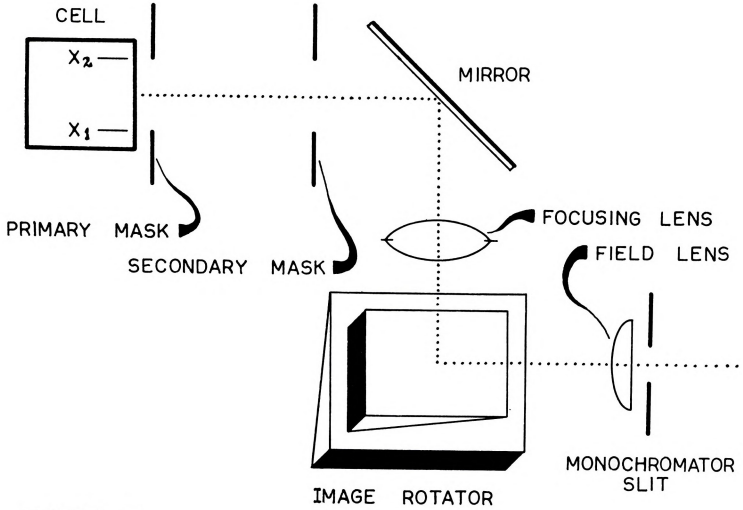

CELL
X2
X1
MIRROR
PRIMARY MASK
SECONDARY MASK
FOCUSING LENS
FIELD LENS
IMAGE ROTATOR
MONOCHROMATOR SLIT

FIGURE 9

(3) For the duration of any observation period, the quanta fluoresced by any absorbing fluorophore species are linearly related to the quanta absorbed by that species

$$F = k'' \Phi (\phi_o - \phi)$$

Where Φ = quantum efficiency and k" = geometric and instrumental constant.

(4) The ratio of the fluorophore absorbance to the total absorbance in the cell is equal to the ratio of the quanta, Q_f, absorbed by the fluorophore to the total quanta absorbed, Q_T.

$$\frac{A_f}{A_T} = \frac{Q_F}{Q_T}$$

(5) A fixed fraction of the fluorescence radiation generated within the observation window is viewed by a detector with uniform sensitivity.

$$F \text{ measured} = k''' \cdot F \text{ window}$$

(6) Only the fluorescence of a single fluorophore is measured and any absorbance of this fluorescence is negligible.

(7) The effects of scattered light, refractive indices, and anisotropic characteristics are assumed to be negligible.

c. The Beam Intensity as a Function of Measured Quantities

Assuming that the excitation beam is attenuated exponentially, the radiant flux at any point on the beam axis in the cell may be represented as a function of the distance from the point of entry.

$$d\phi = -\phi\epsilon \, c \, x \, dx$$

In measuring absorption, the beam usually passes through the entire cell, and thus x is equal to the pathlength b. If a partial penetration of the cell is considered, the following expression can be derived[11]:

$$\phi_x = Re^{(\frac{x}{b}\ln T)}$$

$$= RT^{(x/b)}$$

Substitution of x/b by ω, the fractional distance across the cell, produces:

$$\phi_\omega = RT^\omega$$

representing the radiant flux of the beam at any fraction of the cell length, ω, from the plane of entry.

d. Emission Radiation (Fluorescence) in Terms of Measured Quantities and the Observation Window.

Since F is assumed equal to the product of a constant and the quanta absorbed within the observation window, and that the fraction of the total quanta absorbed by the fluorophore is $A_f/(A_f + A_c)$, the derived expression for fluorescence becomes:

$$F = \frac{2.3 \; K \; A_f \; R \; (T^{\omega_2} - T^{\omega_1})}{\ln T}$$

e. Dependence of Corrected Fluorescence on Absorption. (Two Approaches)

(1) Absorption-corrected fluorescence may be represented as the sum of segments $\Delta\omega$ wide across the window from ω_1 to ω_2. Here it is assumed that the source-corrected fluorescence ratio F/ϕ_o

actually describes the limiting condition as sample absorption approaches zero. The following expressions were presented:

$$F_{co} = \frac{F_{\omega 1}}{\phi_{\omega 1}} + \frac{F_{(\omega 1 + \Delta\omega)}}{\phi_{(\omega 1 + \Delta\omega)}} + \ldots + \frac{F_{\omega 2}}{\phi_{\omega 2}}$$

$$= \sum_{\omega 1}^{\omega 2} \frac{F_{\omega}}{\phi_{\omega}}$$

$$= 2.3 \; K \; A_f \cdot \sum_{\omega_1}^{\omega_2} \Delta\omega$$

$$= 2.3 \; K \; A_f \; (\omega_1 - \omega_2)$$

(2) Absorption-corrected fluorescence may also be defined as the observed fluorescence divided by the average radiant flux of the excitation beam across the window from $_1$ to $_2$. The following expressions were reported:

$$F_{co} = \frac{F}{\int_{\omega_1}^{\omega_2} \frac{\phi_{\omega} \, d\omega}{\omega_2 - \omega_1}}$$

$$= \frac{F \; \ln T \; (\omega_2 - \omega_1)}{R \; (T^{\omega 2} - T^{\omega 1})}$$

$$= 2.3 \; K \; A_f \; (\omega_2 - \omega_1)$$

(3) Both approaches led to the same solution. The $\Delta\omega$ value will be a constant for any system where the observation and detection geometry are not changed, therefore:

$$A_{co} \propto A_f$$

The important implication here is that fluorescence values corrected in the above manner will be linear with the absorbance of the fluorophore even if one or more chromophores are present.

f. The Primary Absorption Correction Factor

The absorption correction factor is defined as:

$$f_a = \frac{\ln T\,(\omega_2' - \omega_1')}{(T^{\omega_2} - T^{\omega_1})}$$

and the corrected fluorescence becomes simply:

$$F_{co} = \left(\frac{F}{R}\right) \times f_a$$

Note that a similar correction factor had been introduced earlier[27].

g. Limitations

Use of the determined correction factor, f_a, was considered valid only if the assumptions used in its derivation are reflected in the chemical and experimental measurement conditions. Assumption (1) is found valid from consideration of their instrumental arrangement. Assumptions (2), (3), (4), (6) and (7) could be satisfied by the correct choice of fluorophores and chromophores. The remaining assumption, (5), required further consideration.

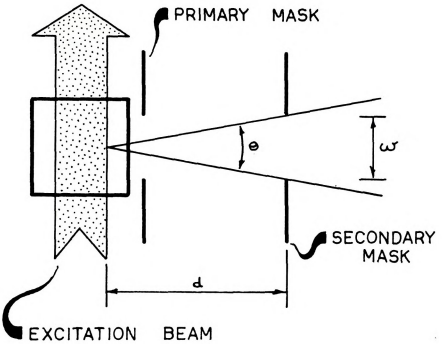
Ideally, all lateral information along the excitation beam axis between X_1 and X_2 should reach the detector. However, such a goal cannot be reached without geometric modifications due to emission monochromator slit orientation. The approach used was suited to the case where the slit height of the monochromator is larger than the horizontal dimension of the fluorescence window. Here, this end was

accomplished by a 90^{o} rotation of the fluorescence cell image through the use of front surface mirrors. As mentioned in introducing the applied geometry, a 1:1 image ratio was attained at the entrance slit to the monochromator.

The second requirement assumed that the fluorescence must be observed at a fixed observational angle at any point across the fluorescence window. This goal was closely approached by placement of a second mask, with the same window dimensions, ω, as the window in the primary mask, at a distance, d, from the source of fluorescence as shown in Figure 10. The θ and ϕ angles are represented as the limiting values for the observation angles across the fluorescence window. The differences between θ and ϕ for various d values were expressed as relative percent error. Ideally, it would be desired to collect only radiation which is parallel to the optical axis. However, the large secondary mask distance would be prohibitive and greatly reduce efficiency in radiation collection. A compromise was reached allowing a maximum error of 1%. This resulted in a secondary mask distance of 4.0 cm. The error in observation angles across the cell was small while permitting the passage of a reasonable amount of fluorescence to the detector.

The last requirement inherent in assumption (5) dealt with the uniformity of the detector sensitivity. Once this was ascertained as satisfactory, all of the requirements of this assumption were fulfilled. With use of the proper fluorophores in conjunction with the instrumental configuration outlined, correction of raw fluorescence data could be found to give an extended linear relationship between fluorescence and the fluorophore absorbance. Evidence presented demonstrated that the absorption-corrected fluorescence is linear with

Figure 10. Limiting Conditions for the Fluorescence Observation of Fluorescence Radiation by the Moving Mirror Method


PRIMARY MASK
θ
ω
SECONDARY MASK
d
EXCITATION BEAM

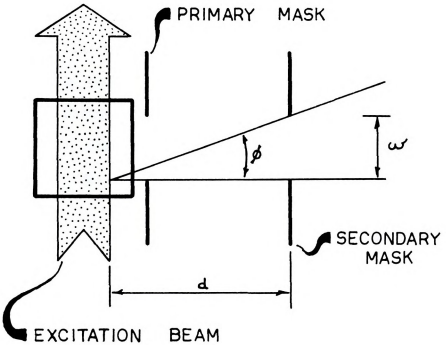

PRIMARY MASK
ϕ
ω
SECONDARY MASK
d
EXCITATION BEAM

FIGURE 10

fluorophore absorbance, up to a solution absorbance of 2.

3. The Cell Shift Method

a. Geometry

The geometry used in this method is illustrated in Figure 11. A square cell of internal dimensions b x b cm was employed. The minimum and maximum pathlengths (cm) for absorption of exciting radiation at λ are X_{α} and X_{β}, respectively. The minimum and maximum pathlengths (cm) for absorption of fluorescence radiation at λ' and y_{α} and y_{β}, respectively.

b. The Basic Assumptions[30]

In addition to the cell geometry, it was intially convenient to make the following assumptions.

(1) The excitation beam is homogeneous, collimated, and monochromatic with a wavelength λ.

(2) The emission beam is collimated and monochromatic with wavelength λ'.

(3) Fluorescence photons which are absorbed in the cell are not re-emitted by the sample.

(4) Scattered light, refractive index effects, and reflections within the cell are negligible.

(5) The sample is homogeneous and contains a single fluorophore although other chromophores may be present.

(6) Both primary and secondary absorption processes obey Beer's Law.

c. The Attentuation by Primary Absorption

A derivative form of the correction factor determined by Holland and coworkers[11] for primary absorption was applied. However, the usefulness of such corrected measurements was limited to where the absorbance at the excitation wavelength is not higher than 2.0 A. The problem deduced was that the primary absorption-corrected fluorescence is directly proportional to the fluorophore concentration only if the sample is completely transparent to the emitted fluorescence. Because many real samples contain components which absorb the fluorescence of the analyte, consideration was given to the attenuation caused by secondary absorption.

d. The Attenuation by Secondary Absorption

The fluorescing volume of solution, which is viewed by the detector was represented as a collection of n parallel and equally spaced plane sources of light, each at a different distance y_i (cm) from the emission face of the cell. Each of these planes contributes a component of radiant flux to the emission beam such that beam flux at the cell face can be given as:

$$\phi = \sum_{i=1}^{n} \phi_i$$

In a case where secondary absorption is negligible and if assumptions (1), (2) and (5) are valid, each plane contributes an equal component to the emission beam. But, if secondary absorption occurs, the power contributed from each plane is attenuated in accordance with the Beer-Lambert law.

The steps in the derivation of attenuation by secondary absorption

are detailed in the 1980 paper by Christmann and coworkers[30]. The result obtained was:

$$\lim_{n\to\infty} f = \frac{T^{\Theta_\beta} - T^{\Theta_\alpha}}{\Delta e \ln T}$$

This indicated that the fraction of radiant flux in the emission beam transmitted to the cell wall is an explicit function of sample transmittance at the emission wavelength λ' and excitation window parameters e_β and e_α.

e. Absorption Effects on the Measured Fluorescence Signal

It was found that actual measurements required that conditions of assumptions (1) and (2) must be relaxed. In instrumental setup the excitation and emission beams are nearly collimated over small distances, but never truly monochromatic.

f. The Correction Factors

When the spectral bandpasses for excitation and emission are made sufficiently narrow, the sample transmittances at the respective wavelengths λ and λ' do not vary significantly over the particular bandpasses. The expression for the fluorescence signal current was given as:

$$i_{fpa} = 2.303\ K\ A_{f\lambda}\ \frac{(T_\lambda)^{\omega_\beta} - (T_\lambda)^{\omega_\alpha}}{\ln T_\lambda}\ x$$

$$\frac{(T_{\lambda'})^{\Theta_\beta} - (T_{\lambda'})^{\Theta_\alpha}}{(\Theta_\beta - \Theta_\alpha)\ln T_{\lambda'}} \cdot \int\int \cdots$$

All wavelength dependent factors are included within the integral. In the case where T_λ and $T_{\lambda'}$ go to unity, the fluorescence signal becomes totally absorption free:

$$i_o = 2.303 \; K \; A_{f\lambda} \; (\omega_\beta - \omega_\alpha) \cdot \int\cdot\int \circ \circ \circ$$

The relationship between i_{fpa} and i_o then becomes:

$$i_o = \left[\frac{(\omega_\beta - \omega_\alpha) \ln T_\lambda}{(T_\lambda)^{\omega_\beta} - (T_\lambda)^{\omega_\alpha}}\right]\left[\frac{(\theta_\beta - \theta_\alpha) \ln T_{\lambda'}}{(T_{\lambda'})^{\theta_\beta} - (T_{\lambda'})^{\theta_\alpha}}\right] \cdot i_{fpa}$$

$$= f_{a1} \cdot f_{a2} \cdot i_{fpa}$$

Where f_{a1} is the primary absorption correction factor and f_{a2} is the secondary absorption correction factor.

g. A Limitation of the Secondary Absorption Correction

When the absorbed fluorescence is re-emitted by the sample, this type of correction breaks down in a violation of assumption (3). This was considered to be a potentially serious limitation of this secondary absorption correction because many fluorophores have highly overlapping absorption and emission spectra, and thus absorb and re-emit their own fluorescence.

Solutions in which the re-emission of absorbed fluorescence radiation is negligible permit correction of right-angle fluorescence measurements for secondary absorption interference. When both primary and secondary absorption correction factors are applied, totally absorption-free fluorescence information is generated.

4. Method of Cell Rotation

a. Geometry

A diagram of right-angle geometry is shown in Figure 11. The fields of detection are shown for both the emission axis (top) and excitation axis (bottom) of Figure 12. For the following mathematical treatment it is assumed that the sample cell wall is transparent to the radiation, and of negligible thickness.

b. Basic Assumptions

(1) The excitation radiation is homogeneous in wavelength, λ_1, and is collimated.

(2) The emission radiation is observed as a collimated monochromatic beam of fixed dimensions with wavelength, λ_2. The width of the window of emission observation is much less than the total cell dimension (width) such that distinct slices with varying cell positions are obtained.

(3) Photons of emission radiation which are absorbed by the sample solution will not be re-emitted.

(4) Effects due to scattering of excitation radiation, changes in refractive index, and internal reflections are all negligible.

(5) Samples are composed of a single fluorophore, but may contain other chromophores.

(6) The cuvette walls are matched in transmission characteristics for both excitation and emission radiation, and the walls parallel to the viewing optics do not affect the observed fluorescence.

Figure 11. Geometry for Right-Angle Fluorometric with a Square Cell of Internal Dimensions b x b cm.

Key: X_α = pathlength (cm) for absorption of exciting radiation at nm, minimum detected in window.

X_β = pathlength (cm) for absorption of exciting radiation at nm, maximum detected in window.

Y_α = pathlength (cm) for absorption of fluorescence radiation at nm, maximum detected in window.

Y_β = pathlength (cm) for absorption of fluorescence radiation at nm, minimum detected in window.

Def: $w_\alpha = X_\alpha / b$

$w_\beta = X_\beta / b$

$e_\alpha = Y_\alpha / b$

$e_\beta = Y_\beta / b$

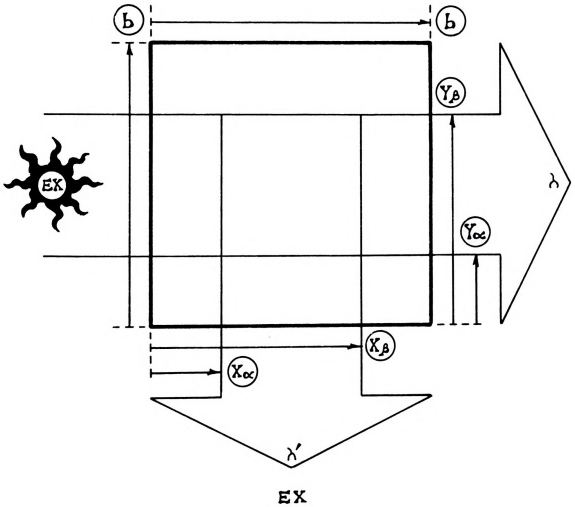


FIGURE 11

Figure 12. Regions of Observation for the Method of Cell Rotation

A. Along emission axis.

B. Along excitation axis.

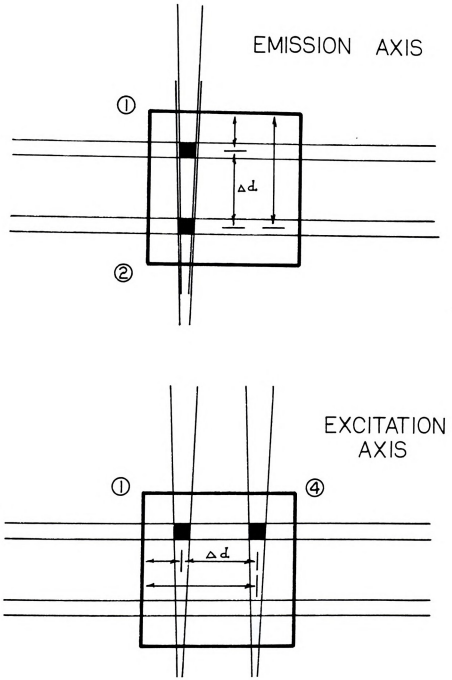


FIGURE 12

(7) Both primary and secondary absorption processes obey Beer's Law.

c. Primary Correction Factor

The top half of Figure 13 shows a typical case where primary absorption is manifested. Two illuminated slices are detected, one at ω_α and one at ω_β. The change in absorbance between ω_α and ω_β is defined as follows:

$$\Delta A_2 = \ln\left(\frac{\phi_o}{\phi_{\omega_\beta}}\right) - \ln\left(\frac{\phi_o}{\phi_{\omega_\alpha}}\right)$$

$$= \ln(\phi_{\omega_\alpha}) - \ln(\phi_{\omega_\beta})$$

$$= \ln\left(\frac{\phi_{\omega_\alpha}}{\phi_{\omega_\beta}}\right)$$

The calculated change in absorbance between the entry plane and ω_α is obtained as follows:

$$\Delta A_1 = \Delta A_2 \left(\frac{\omega_\alpha}{\omega_\beta - \omega_\alpha}\right)$$

$$= \ln\left(\frac{\phi_o}{\phi_{\omega_\alpha}}\right)$$

In this manner it is possible to calculate ϕ_o. Since the fluorescence radiant flux is directly proportional to the incident radiant flux of the excitation radiation, according to $\phi_f = k\, c\, \phi_o$, then the following relationships can be given:

$$\left(\frac{\phi_{\omega_\alpha}}{\phi_o}\right) \propto \left(\frac{\phi_{f,\omega_\alpha}}{\phi_o}\right)$$

$$\left(\frac{\phi_{\omega_\beta}}{\phi_o}\right) \propto \left(\frac{\phi_{f,\omega_\beta}}{\phi_o}\right)$$

This allows correction for primary absorption according to the following expression:

$$\phi_{cf1^o} = \phi_{f(1)} \cdot e^{\left(\frac{\omega_\alpha}{\omega_\beta - \omega_\alpha}\right) \cdot \Delta A_2}$$

Where ϕ_{cf1^o} is primary absorption corrected fluorescence and $\phi_{f(1)}$ is the uncorrected fluorescence reading at position (1). The above correction factor is determined while holding secondary absorption constant. This situation occurs between positions 1 and 4 of Figure 12.

d. Secondary Correction Factor

The bottom half of Figure 13 shows a typical case where secondary absorption is manifested. Two illuminated slices of volume are detected, at fractional distances of e_α and e_β. The change in absorbance between these planes, e_α and e_β, may be defined as follows:

$$\Delta A_2' = \ln\left(\frac{\phi_o'}{\phi_{e_\alpha}}\right) - \ln\left(\frac{\phi_o'}{\phi_{e_\beta}}\right)$$

$$= \ln(\phi_{e_\beta}) - \ln(\phi_{e_\alpha})$$

$$= \ln\left(\frac{\phi_{e_\beta}}{\phi_{e_\alpha}}\right)$$

The calculated change in absorbance between the exit plane (since $Y_\alpha = (b - Y_\beta)$) and e_β is obtained as follows:

$$\Delta A_1' = \Delta A_2' \left(\frac{e_\beta}{e_\alpha - e_\beta}\right)$$

$$= \ln\left(\frac{\phi_o'}{\phi_{e_\beta}}\right)$$

Figure 13. Attenuation of Radiation

A. Along excitation axis

B. Along emission axis

ENTRY PLANE OF EXCITATION RADIATION
ΔA1
ΔA2
A
T
EMERGENCE OF TRANSMITTED EXCITATION RADIATION
ωα
ωβ
EXCITATION
EXTRAPOLATED ENTRY PLANE OF EMISSION RADIATION
ΔA1'
ΔA2'
A
T
EMERGENCE OF TRANSMITTED EMISSION RADIATION
θβ
θα
EMISSION

FIGURE 13

In this manner, it is possible to determine ϕ_o'. Since the attenuation of fluorescence caused by secondary absorption is measured directly, the following terms are equal:

$$\phi_{e_\alpha} = \phi_{fe_\alpha}$$

$$\phi_{e_\beta} = \phi_{fe_\beta}$$

The secondary absorption is corrected for according to the following expression:

$$\phi_{cf_2^o} = \phi_{f(1)} \cdot e^{\left(\frac{e_\beta}{e_\alpha - e_\beta}\right) \cdot \Delta A_2'}$$

Where $\phi_{cf_2^o}$ is secondary absorption corrected fluorescence. The above correction factor is determined while maintaining primary absorption constant. This occurs between positions 1 and 2 of Figure 12.

e. Combined Correction Factor

As in the case of many chemical systems both primary and secondary absorption attenuations are observed. The following expression accounts for both:

$$\phi_{cf_1^o 2^o} = \phi_{f(1)} \cdot e^{\left(\frac{\omega_\alpha}{\omega_\beta - \omega_\alpha}\right) \cdot \Delta A_2} \cdot e^{\left(\frac{e_\beta}{e_\alpha - e_\beta}\right) \cdot \Delta A_2'}$$

$$= \phi_{f(1)} \cdot f_1^o \cdot f_2^o$$

Where f_1^o is determined under conditions where secondary absorption is constant and f_2^o is determined under conditions where primary absorption is constant.

f. Satisfying the Assumptions

Assumption (1) is closely approximated with use of an excitation monochromator. A very narrow band of wavelengths are passed and the beam is essentially collimated over the short distances spanned within the cell.

Assumption (2) has been somewhat relaxed in a compromise between having the detected emission beam collimated and maintaining a practical distance prior to entry into the emission monochromator. Masking studies have found the detection fields approximately rectangular as viewed from the top in either case represented in Figure 12. The width of the window of emission observation is considerably less than the total cell dimension. Monochromatic emission radiation is obtained with a high quality emission monochromator.

Assumption (3) is valid when chemical systems are chosen that do not possess overlapping absorption and emission spectra, which would cause re-emission of absorbed fluorescence. The limitation resulting from re-emission is, of course, dependant on the degree of overlap between the absorption and emission spectra.

Assumption (4) is valid only with use of proper sample cell materials and cell orientation in the instrument. Of the cited problems, internal reflections are thought to be the most significant. Corrections for this factor have been derived and implemented in recent work upon the cell shift method[2].

Assumption (5) involves proper choice of chemical systems. This would be no problem with prepared samples, but either biologically or environmentally derived samples may incorporate other fluorophores of unknown concentration.

Assumption (6) has been found valid by rotating through all the cell facets at each position. No significant differences were observed. The cell walls parallel to the viewing optics were shielded by the design of the cell holder to prevent their interference with the observed fluorescence.

CHAPTER IV

AN AUTOMATED INSTRUMENT TO CORRECT FLUORESCENCE MEASUREMENTS FOR PRIMARY AND SECONDARY ABSORPTION EFFECTS VIA THE METHOD OF CELL ROTATION

A. Introduction

Earlier it was emphasized that a variety of factors exist which can affect the linearity of the measured fluorescence with fluorophore concentration relationship. Investigators have demonstrated experimentally that such factors can be mathematically described and that fluorescence measurements can be corrected. Multiplication of the sample signal by the appropriate correction factors produces extended linearity in the above fluorescence with concentration plot. Linearity up to sample absorbances of slightly higher than 2.7 has been achieved[2].

The "cell shift" approach as introduced by Novak[61] involved manual cell positioning which was found to be very time consuming and prone to significant errors. Development of an automated cell positioning device by Christmann[2] reduced these problems and allowed a detailed examination of the cell shift technique[62,63]. This device did have limitations, however. The 12V permanent magnet DC motors used in driving perpendicular slides through two 0.5 mm pitch lead screws were susceptible to overheating. The dovetail tracks supporting the slides

required constant inspection of the state of lubrication. Build-up of dust or metal (aluminum) oxide, or thinning of the lubricating grease, could cause seizure of the slide against the track. Any increase in friction or a seizure results in greatly elevated current input to the motor. If the current is substantially above the motor tolerance, the heat generated over even short periods of time can burn out the motor coils. Such problems make a system like this impractical for prolonged use in repetitive routine measurements.

A more efficient, high speed, and very reproducible means of cell positioning has been developed in this current work. The positioning device can be used repeatedly over long periods of time with no detriment. The relatively small spacial requirements of this device allow ready conversion of commercially available spectrofluorometers to instruments that can automatically correct for both primary and secondary absorption attenuations. Instrument sensitivity can be easily optimized with fine-tuning adjustments in three-dimensional space. The device can be made adaptable over a wide range of temperatures or atmospheres with negligible decrease in performance. The device is computer interfaced to conveniently initiate and control the cell positioning, data acquisition and correction, overlay graphing capabilities and print out, and data storage. In this portion of the thesis, the basic design and operation of this integrated computer/cell-positioner/spectrofluorometer system are described.

B. Theory

The positions achieved by the method of "cell rotation" are shown in Figure 14. One full rotation permits measurements to be obtained in any of the positions, one through four. Position zero is obtained only by correcting for both primary and secondary absorptions. This position is not a physical location of the cell at any time, but a calculated ideal. At each of the four internal positions, a fixed volume of solution is excited by the radiation source and observed by the fluorescence emission detector. Varying the thickness of solution through which the exciting radiation must pass, from position one to four or from position two to three, provides information on the primary absorption. Varying the thickness of solution through which the emission radiation must pass, from position one to two or from position four to three, provides information on secondary absorption.

Derivations of expressions to account for sample absorption were presented in the previous chapter. The following expression was used to correct simultaneously for the primary and secondary components of absorption:

$$\phi_{cf12} = \phi_{f1} \cdot e^{(GF)\cdot \Delta A_T}$$

Where ΔA_T is ΔA_1 and $\Delta A_1'$ combined, and GF is the normalized geometrical factor. Recall that:

$$\Delta A_2 = \ln\left(\frac{\phi_{f1}}{\phi_o}\right) - \ln\left(\frac{\phi_{f4}}{\phi_o}\right)$$

Figure 14. Cell Positions Available Using the Cell Rotation Method.

The normal measurement sequence is counterclockwise beginning with position one and finishing with position one. The cell compartment walls are broken at the excitation and emission ports. The small square within the sample cuvette indicates the region under observation by the detector. Position zero is obtained by correcting for primary and secondary absorption by application of the correction factors.

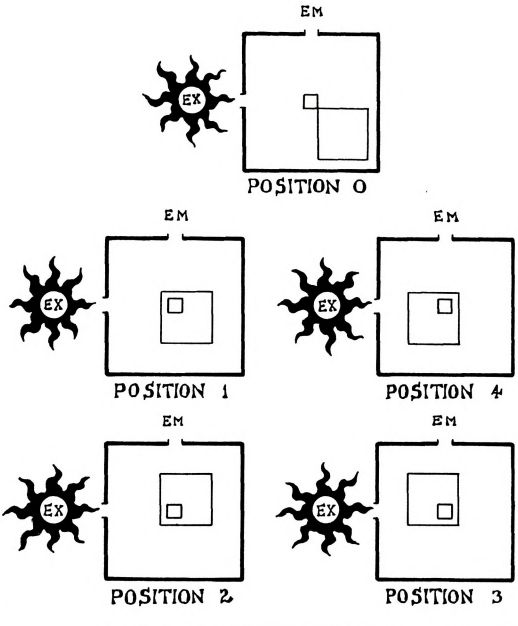

EM
EX
POSITION 0
EM
EX
POSITION 1
EM
EX
POSITION 4
EM
EX
POSITION 2
EM
EX
POSITION 3
CELL ROTATION METHOD

FIGURE 14

$$\Delta A_2' = \ln\left(\frac{\phi_{f1}}{\phi_o}\right) - \ln\left(\frac{\phi_{f2}}{\phi_o}\right)$$

$$\Delta A_T = \ln \frac{(\phi_{f1})^2}{(\phi_{f2})(\phi_{f4})} = \frac{2 \ln(\phi_{f(1)})}{(\phi_{f(2)})(\phi_{f(4)})}$$

Where ΔA_2 and $\Delta A_2'$ are functions of the emission and excitation window parameters. Assumptions inherent in this expression are that the excitation and emission beams are collimated and of narrow spectral bandwidth relative to the sample absorption bands, and that stray excitation radiation, scattering phenomena, internal reflections, and re-emission of absorbed fluorescence are negligible in isolated viewing situations.

C. Instrument Design

1. Overview

The specifications for the Perkin-Elmer Model 512 double beam spectrofluorometer are listed in the Appendix (Table 7). The basic components of the absorption-corrected spectrofluorometer and their arrangement are shown in Figure 15. A flowchart of all physical devices employed in the cell rotation system is presented in Figure 16. Associated interfaces are detailed in the appendix in Figures 41 through 45. The system designed for removing ozone from two simultaneously operating spectrofluorometers is detailed and shown in the appendix in Figure 46. Design details of the cell rotation device are given later in this chapter in Figures 17-19. The stepper motor and power supply specifications are listed in Table 6 of the Appendix. A standard PDP 8/e computer has been dedicated to system control and output is available

Figure 15. Block Diagram of Instrumentation Used in the Cell Rotation Method

Cell Compartment Contents

1. Chopper mirror
2. Reference cell or scatter plate
3. Quantum counter or scatter plate
4. Filter - Red Pass
5. Photomultiplier Tube
6. Sample cell - showing off - center rotation (stepper motor & post).
7. Lattice mirror.

CELL ROTATION METHOD

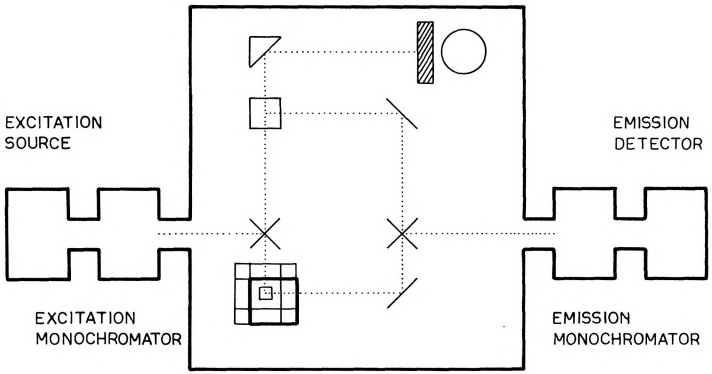


FIGURE 15

Figure 16. Flowchart of Physical Devices Employed in the Cell Rotation Method

Detailed specifications of the following components are included in the appendix:

1. Stepper motor
2. Spectrofluorometer output amplifier
3. Multiplexer.
4. Analog to digital converter.
5. Pulse controller for stepper motor
6. Ozone exhaust system.

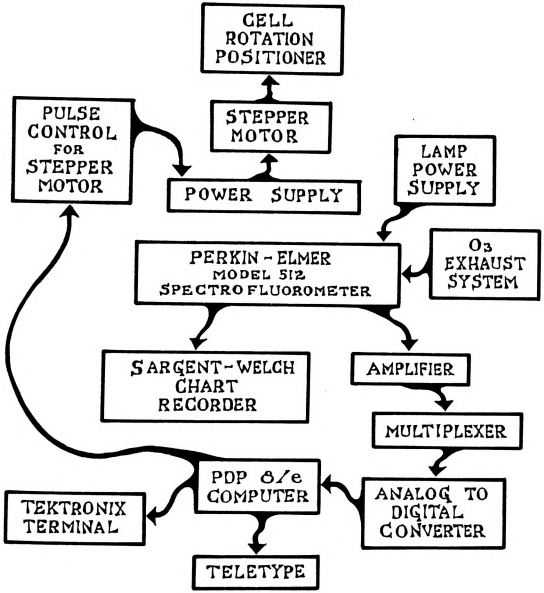

CELL ROTATION POSITIONER
STEPPER MOTOR
PULSE CONTROL FOR STEPPER MOTOR
POWER SUPPLY
LAMP POWER SUPPLY
PERKIN - ELMER MODEL 512 SPECTROFLUOROMETER
O3 EXHAUST SYSTEM
SARGENT-WELCH CHART RECORDER
AMPLIFIER
MULTIPLEXER
PDP 8/e COMPUTER
ANALOG TO DIGITAL CONVERTER
TEKTRONIX TERMINAL
TELETYPE

FIGURE 16

Figure 17. Stepper Motor and Lock-In Platform

The motor is held by a series of eight 8-32 brass screws. The lower plate is ½ inch aluminum stock, employed for stability. Stepper motor specifications are available in the appendix.

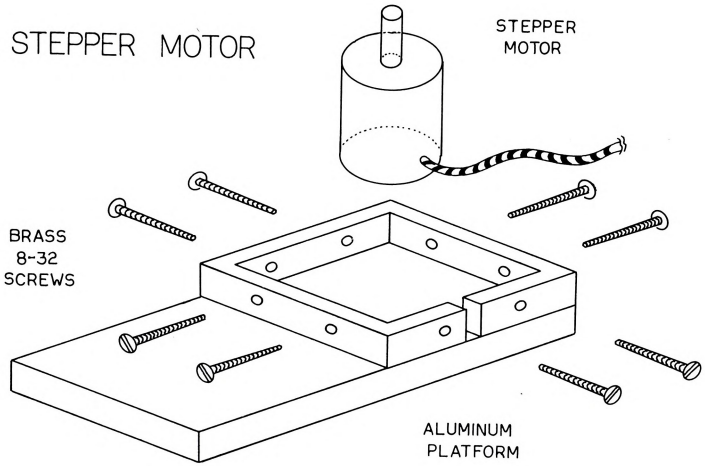

STEPPER MOTOR
STEPPER
MOTOR
BRASS
8-32
SCREWS
ALUMINUM
PLATFORM

FIGURE 17

Figure 18. Post and Cell Holder Platform

The ½ inch aluminum post is secured to the stepper motor rod with two rings of three 8-32 screws each. See text for z axis truing. The cell holder platform, is held to the top of the post with a single 8-32 screw. The cell holder is locked into the desired position with a series of eight 8-32 screws. The exact position determines the profile and dimensions of rotation, circular or elliptical.

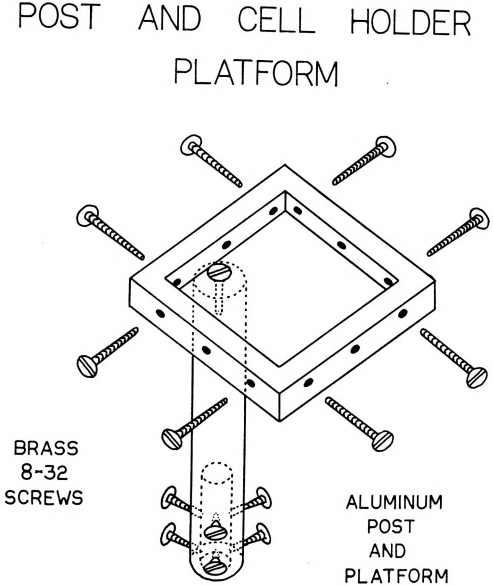

POST AND CELL HOLDER
PLATFORM
BRASS
8-32
SCREWS
ALUMINUM
POST
AND
PLATFORM

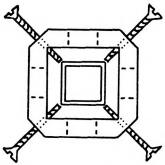
FIGURE 18

Figure 19. The Cell Holder

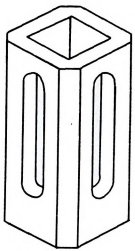
The holder is symmetrical. The walls are of $\frac{1}{4}$ inch aluminum. The slots are 1/64 inch in from either cell wall as shown in the top view. The holder is painted ultra flat black to minimize reflections. The cell is supported by a series of eight nylon 10-42 screws.

are of ¼ inch
from either cell
older is painted
ns. The cell is
-42 screws.

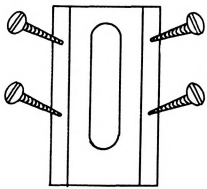
THE CELL HOLDER



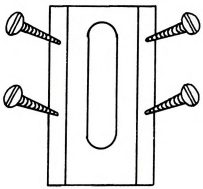
TOP VIEW



AN ISOMETRIC VIEW



FRONT VIEW



SIDE VIEW

FIGURE 19

via a Sargent-Welch chart recorder, a teletype, or a Tektronix video terminal.

2. Data Treatment

Fluorescence is detected by a photomultiplier (PE R-446F). The photocurrents generated are converted to voltages. The instrument output, without modification, is connected to a Sargent-Welch chart recorder (Model SR). The analog output is also connected through a follower with gain of x100 (amplifier interface) to a four channel CMOS multiplexer (switching interface for dual instrument access - Datel Systems MX series MSD-409) and subsequently into an analog to digital converter (Analogic - 0-10 V high speed 12 bit MP 2112) for processing by the computer (PDP 8/e). The software used is shown in a flowchart in Figure 20. The software incorporates two functions: (1) data display, correction routines, storage and output; (2) control of cell positioner, setting of scan and plot parameters, initiation of scan, and collection of scan data.

The correction routine of the program automatically cycles through scans at positions (1), (2), and (4) as shown in Figure 21. Each position can be independently examined and output. Position scans can be overlapped for visual comparisons. Corrected spectra can be independently examined, overlapped with any uncorrected position scans, and output. Long term storage of position and corrected spectra is accomplished with a floppy disk drive system (Data Systems, Inc.). Generally all data runs are stored on floppy disks in a data library for future reference.

Figure 20. Flowchart of System Program

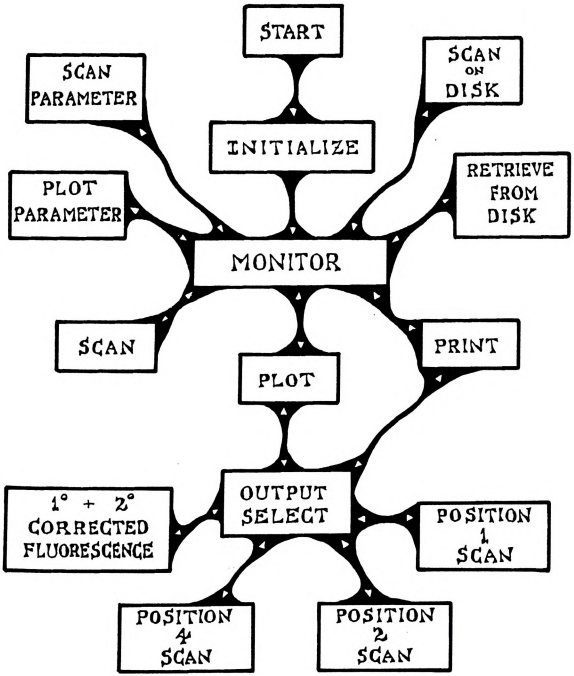

START
SCAN PARAMETER
SCAN ON DISK
INITIALIZE
PLOT PARAMETER
RETRIEVE FROM DISK
MONITOR
SCAN
PRINT
PLOT
1° + 2° CORRECTED FLUORESCENCE
OUTPUT SELECT
POSITION 1 SCAN
POSITION 4 SCAN
POSITION 2 SCAN

FIGURE 20

Figure 21. Positions of Emission Detection Fields.

Key: 1. Least Primary Absorption, least secondary absorption.
2. Least Primary Absorption, most secondary absorption.
3. Most primary absorption, most secondary absorption.
4. Most primary absorption, least secondary absorption.
0. Corrected for attenuations by primary and secondary absorptions.

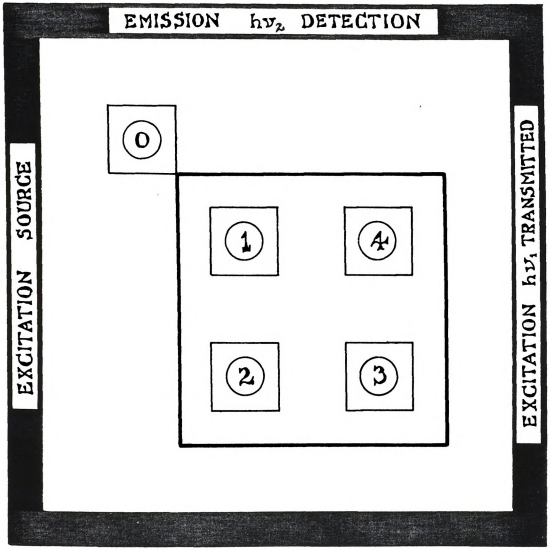

EMISSION $h\nu_2$ DETECTION
EXCITATION SOURCE
EXCITATION $h\nu_1$ TRANSMITTED
0
1
2
3
4

FIGURE 21

3. The Cell Positioner

This device, detailed in Figures 17-19, and photographed in Figure 22, was designed to provide a very rapid and highly reproducible means of automatically correcting for both primary and secondary absorption effects. The durable construction and the choice of stepper motor was to allow continual service over long periods of time without the need for re-alignments.

The positioner has a range for Δd between 0.2 cm to 1.0 cm along either of two perpendicular axes. This permits a large choice in the rotation patterns, both in magnitude and in ellipticity. While holding the chosen major axis constant for Δd and decreasing Δd along the minor axis one may readily obtain rotation patterns as shown in Figure 23. The cell displacement along either axis is a function of the respective lead screw rotations, and is found to be highly reproducible. This is shown in Figure 24.

This figure demonstrates the linearity and accuracy of the positioner along either of the optical axes. Vernier calipers were employed to ascertain cell displacements in X and Y dimensions from the stationary outer walls of the cell holder platform. These displacements were plotted against lead screw revolution. Linear regression gives a correlation coefficient (essentially) equal to unity in both cases.

The precision of the positioner was determined by measuring A/D converter output after each of five successive 360° rotations and averaging to obtain the error distribution. Possible shifting of the average was determined by several sets of five successive readings taken consecutively. The error bars between sets did not alter in magnitude and differences between the averages were negligible.

Figure 22. Cell Positioner as Employed by the Cell Rotation Method

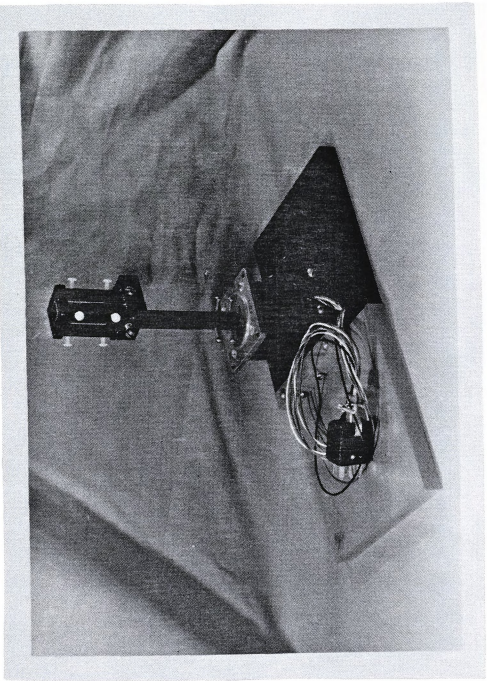


FIGURE 22

Figure 23. Elliptical Cell Rotation

All measurements reported in this thesis were conducted with larger or smaller circular rotation patterns. Other possible rotation patterns include ellipses with major axes along either the X- or Y- direction. Extremes of ellipticity would allow study of either the primary absorption or secondary absorption alone.

ELLIPTICAL CELL ROTATION

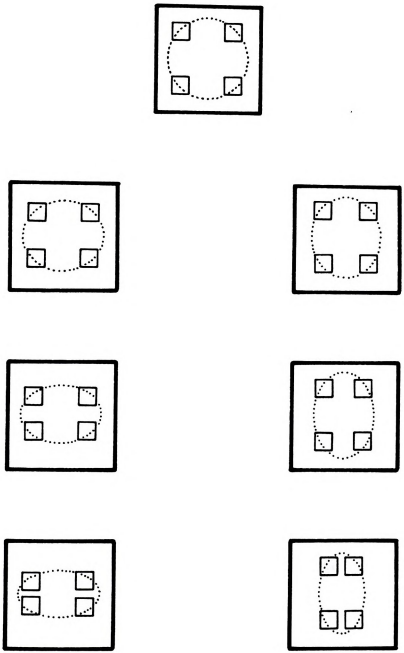


FIGURE 23

Figure 24. Cell Displacement in X- and Y-Dimension as a Function of Lead Screw Rotation

Off center positioning and securing of the cell holder on the post platform is accomplished by perpendicular sets of 8-32 brass lead screws. Circular rotation patterns possessing Δ d values anywhere from 2 mm to 10 mm can be set by matching lead screw revolutions. Note that in a circular pattern Δd primary is equal to Δd secondary.

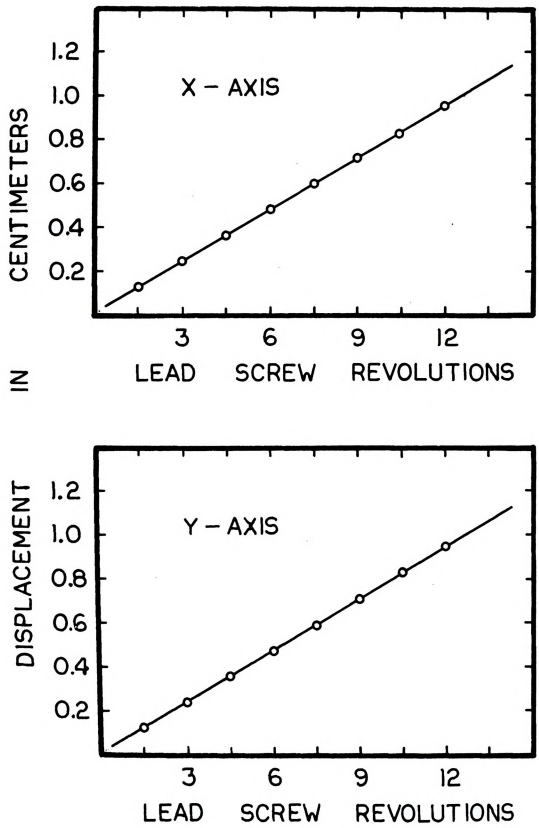

DISPLACEMENT IN CENTIMETERS
1.2
1.0
0.8
0.6
0.4
0.2
X – AXIS
3
6
9
12
LEAD SCREW REVOLUTIONS
DISPLACEMENT
1.2
1.0
0.8
0.6
0.4
0.2
Y – AXIS
3
6
9
12
LEAD SCREW REVOLUTIONS

FIGURE 24

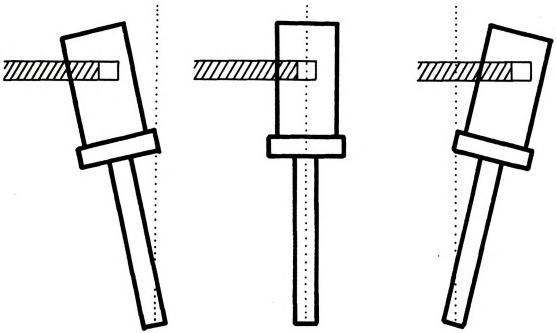
The sample cell is held by eight nylon screws against the four beveled edges as shown in Figure 19. The cell is suspended vertically with the four slots of the holder centered. The walls of the slots are 1/64 inch in from the inner faces of the cell. Several thin coats of ultra flat black paint were used to minimize reflections. The rugged design of the holder was intended to prevent transferred distortions to the cell edges while being locked in position.

The post cell holder platform is tightly held on the post by one brass 8-32 screw. The post is countersunk into the base of the platform to aid in locking the two components together. The lower end of the post contains a cylindrical opening, the inside diameter of which is 0.003 inch larger than the outside diameter of the stepper motor drive rod. The six lock screws are arranged in two sets of three as diagrammed in Figure 18. They are used both to align vertically the post, and subsequently the sample cell itself, and to secure the post on the rod. The lack of vertical truing can lead to a 2 to 3 fold decrease in the detected fluorescence signal, as shown in Figure 25.

The stepper motor is tightly locked on the heavy aluminum platform by a series of eight 8-32 screws as shown in Figure 17. The platform possesses an extension that permits use of a clamp device to attach it to the instrument table. An alignment tool has been designed to aid in rapid initial positioning of the entire stepper motor platform. This tool is shown in Figure 26. Both the stepper motor and platform, readily fit into the lower half of the Perkin-Elmer Model 512 spectrofluorometer cell compartment.

Figure 25. Z Axis Truing

The cell holder post is designed to allow fine tuning along the vertical axis. In a rotating system, this is critical to aid in preventing a forward and backward wobble of the cell surfaces perpendicular to the excitation and emission optical axes.
The post can be raised or lowered to accomodate different volumes of solution. A shift of $\frac{1}{4}$ inch is possible with maintenance of all six lock-in screws.


FORWARD
VERTICAL
BACKWARD

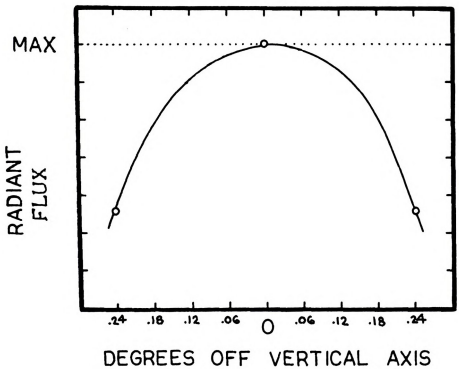

MAX
RADIANT FLUX
.24
.18
.12
.06
0
.06
.12
.18
.24
DEGREES OFF VERTICAL AXIS

FIGURE 25

Figure 26. Stepper Motor Platform Alignment Tool

This device permits rapid alignment (coarse) of the field of detection within the cell. Once the approximate position is reached the stepper motor platform is locked onto the bench. In the secured state any vibrations encountered by the cell following a rotation are minimal.

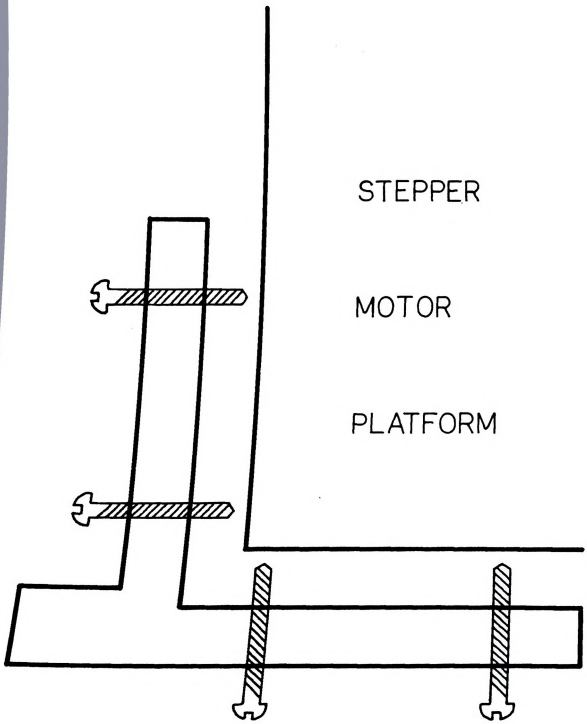


FIGURE 26

A shield to prevent access of stray external radiation divides the upper and lower halves of the cell compartment. A slot in the shield facilitates the post positioning in alignment of the cell with the optical axes. A black sliding disk is secured to the post over the slot to keep unwanted radiation from penetrating to the top half of the cell compartment.

The specifications for the stepper motor are provided in Table 6 (Appendix). The rotation rate is 2.5 msec per 1.8^{o} or 125.0 msec to shift between adjacent cell positions (90^{o} apart). The stepper motor is rotated counterclockwise by a preset sequence of fifty pulses for each 90^{o} turn. These pulses proceed through an interface that increases pulse width (Figure 45, Appendix) for compatibility with the power translator (Superior Electric Company, Slo-Syn Translator Model STM-1800).

4. Detection Field Characteristics

Study of the narrowness of the field of detection was completed. Both primary and secondary masks were used at a separation of 3 cm. The thickness of the primary masks was 1/32 inch. The thickness of the secondary mask was 1/8 inch.

Primary masks were attached 3/8 inch from the outside surfaces of the cell walls flush with the outer walls of the cell holder as shown in Figure 27. They were secured in place with rubber cement. Each mask covered $\frac{1}{2}$ of the surface area and had been painted ultra flat black to absorb most of the incident radiation.

A single secondary mask was placed in the emission port filter clip s shown in Figure 28. This mask possessed a milled $\frac{1}{4}$ inch vertical

Figure 27. Study of Detection Field Using Primary Masks

The masks were 3/8 inch from the outer faces of the sample cuvette and they extended halfway across the cell as shown in each of the four positions. They were painted ultra flat black to absorb most of the incident radiation.

The differences between measurements made with the masks and without them were negligible. This supports the belief that the field of detection is quite narrow.

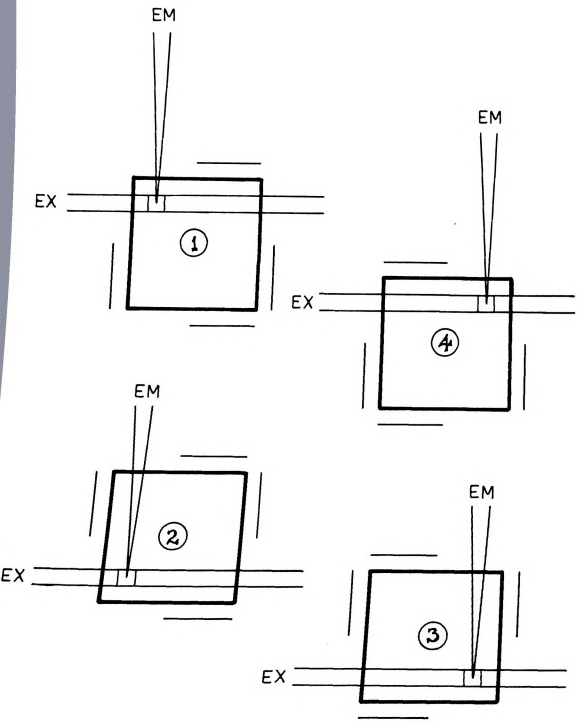


FIGURE 27

Figure 28. Study of Detection Field Using Both a Primary and Secondary Mask

The primary mask was situated 3/8 inch from the outer face of the sample cuvette while in position one. The secondary mask was placed in the emission port filter clip. The primary mask extended across half of the cell face in both cases. The secondary mask was centered on the detection "hot spot" of the parabolic mirror. A verticle slot of ¼ inch width was not found to appreciably attenuate the measured signal. This further supports the contension that the field of detection is very narrow.

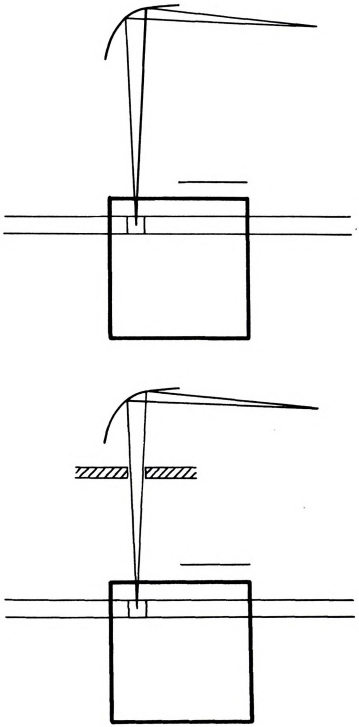


FIGURE 28

slit which was centered on the detection "hot spot" of the recessed parabolic mirror. This mask was also painted ultra flat black.

Table 3a illustrates the effect of the primary masks. A comparison of measurements made with and without the masks indicate negligible differences. This demonstrates that no attenuation is evident and that the masked region of the solution has no emitted radiation reaching the fluorescence detector.

Table 3b illustrates the effect of the secondary mask. Providing that the verticle slot is $\frac{1}{4}$ inch (or wider) and is centered on the "hot spot", the differences observed with and without the mask are negligible. Inclusion of both primary and secondary masks verifies that the field of detection is quite narrow. This provides a basis for the initial assumption that the emission radiation is observed as a very narrow bandwidth and collimated beam with a fixed limited angle of admittance.

5. Detection Window Calibrations

The emission window was calibrated to determine the maximum allowable Δd between positions one and four, two and three. The chosen positions must be such that no significant attenuation is encountered by having the field of detection interact with either cell wall. Measurement of changes in fluorescence radiant power across the cell interior were made in increments of 0.5 mm. The sample studied was 1×10^{-6} M quinine sulfate in a background matrix of 0.1 N H_2SO_4 prepared with house distilled water. This sample was chosen because very little attenuation of the excitation beam occurs.

The solution was excited at 250 nm and the emission was monitored

Table 3a. Effect of Primary Masking

Conc. Q.S.	Mask	Cell Positions (1)	(2)	(3)	(4)
1×10^{-6} M	No	365	368	308	310
1×10^{-6} M	Yes, 1^{o}	362	367	310	310
2×10^{-6} M	No	718	713	559	562
2×10^{-6} M	Yes, 1^{o}	720	717	560	564

Table 3b. Effect of Secondary Masking

Conc. Q.S.	Mask	Cell Positions (1)	(2)	(3)	(4)
1×10^{-6} M	Yes, 1^{o}	360	365	306	307
1×10^{-6} M	Yes, $1^{o}+2^{o}$	360	362	306	309
2×10^{-6} M	Yes, 1^{o}	720	718	565	562
2×10^{-6} M	Yes, $1^{o}+2^{o}$	716	716	560	557

Note: Quinine Sulfate in 0.1 N H_2SO_4
Sensitivity 0.1x
Slits EX/EM 10/10 nm
Ref. Attenuation Control 600V

(Adjusts reference radiant power to use full range of A/D converter output for largest analyte peak, 0000 to 4096).

at 460 nm. The fluorescence profile along the emission window is shown in Figure 29. To avoid attenuations positions were chosen 3mm removed from either cell wall. This led to the routine use of a 4 mm cell centered Δd value along this axis.

The excitation window was calibrated to determine the maximum allowable Δd between positions one and two, three and four. In this case, the chosen positions must be such that negligible attenuations are encountered by having the excitation beam interact with either the front or back cell surfaces. The fluorescence radiant flux was measured at increments of 0.5 mm across this window. At each of these increments the emission window axis was also calibrated from wall to wall. On completion a full matrix of values for the excitation/emission window mapping were obtained. The fluorescence profile along the excitation window is shown in Figure 30. It became apparent that a 4 mm cell centered Δd_o value was also the maximum along this axis.

The above mapping was generated with the cell originally in position one. Rotations of the cell within the cell holder were made to study the quality of the matching between the four vertical cell surfaces. Minor differences within 2 mm of the cell walls were detected. Otherwise all four cell surfaces were matched. If significant differences were to exist then the correction procedures used would have to account for the transmission differential for two adjacent surfaces after each cell rotation. Use of a circular rotation pattern resulting in d's of 4 mm avoids the need for such two surface matching corrections.

Figure 29. Emission Window Sample Cell

The plot represents the detected fluorescence radiant flux as a function of distance from the inner cell walls. The quartz cell has dimensions of 1 cm by 1 cm by 4.5 cm. Attenuations close to the cell walls led to the routine choice of 0.4 cm centered for Δd along this axis.

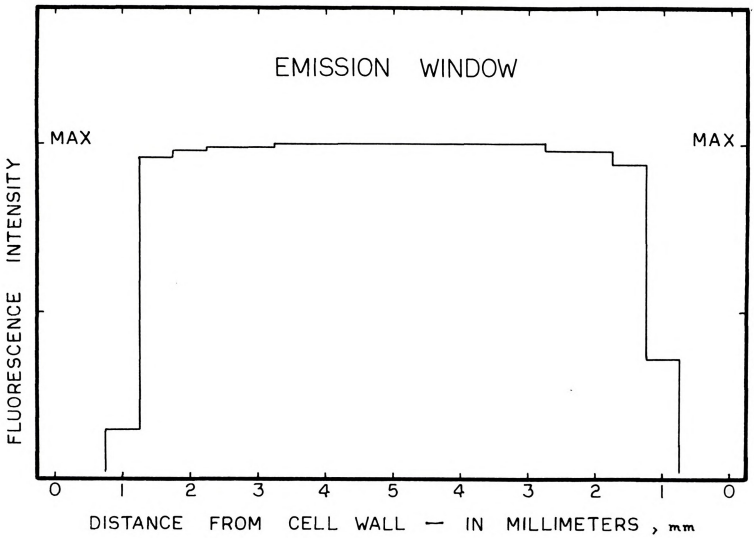

EMISSION WINDOW
MAX
MAX
FLUORESCENCE INTENSITY
0 1 2 3 4 5 4 3 2 1 0
DISTANCE FROM CELL WALL — IN MILLIMETERS , mm

FIGURE 29

Figure 30. Excitation Window Sample Cell

The plot represents the detected fluorescence radiant flux as a function of distance from the inner cell walls. The quartz cell has dimensions of 1 cm by 1 cm by 4.5 cm. Attenuations close to the cell walls led to the routine choice of 0.4 cm centered for Δ d along this axis.

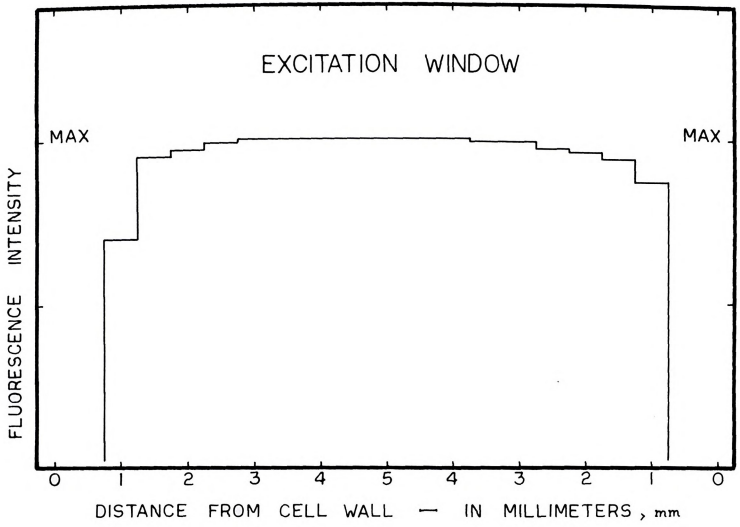

EXCITATION WINDOW
MAX
MAX
FLUORESCENCE INTENSITY
0 1 2 3 4 5 4 3 2 1 0
DISTANCE FROM CELL WALL — IN MILLIMETERS, mm

FIGURE 30

6. Error Analysis

There will always be a certain amount of error or irreproducibility in making instrumental measurements. Understanding of the various causative factors is necessary to develop means of eliminating, reducing or accounting for them. The following is a summary of commonly encountered sources of noise and their significance in the present system.

Fluctuations in temperature of the instrumental components and sample solution can cause variations in ϕ_f. The instrument warms up to a constant running temperature in twenty minutes. The source lamp temperature is maintained constant by a fan and by a specially designed ozone exhaust pumping system. The cell positioner is well shielded from the lamp chamber. The stepper motor maintains a constant temperature whether "stepping" or holding position. Thus this source of error is expected to be minimal.

Stray excitation radiation which reaches the emission detector would increase ϕ_f values. This problem is virtually eliminated by the use of an emission monochromator employing a small bandpass. Specifications for the Model 512 Perkin-Elmer Spectrofluorometer indicate less than 2% stray light penetration exists at a wavelength of 350 nm. This is a systematic error.

Internal reflections of excitation or emission radiation from sample cuvette surfaces can result in ϕ_f signal attentuation. Although the assumption of negligible reflections has been thus far made in calculating correction factors for primary and secondary absorption, this is not always true. Reflections, when significant, are a source of

inaccuracy. Typical glass sample cuvettes reflect about 4% of the radiation transmitted by the sample back into the sample by the air-glass interface of the cell wall. The reflected excitation radiation would enhance the ϕ_f signal. Also a portion of the emission radiation initially emitted away from the emission detector will be reflected back and added to the ϕ_f signal. The inaccuracy in the measured ϕ_f due to these effects is a function of the transmittance at each of these wavelengths. Recently investigators have reported[2,11,62,63] application of mathematical models and subsequently derived correction factors to account for reflectance phenomena. The present sample cuvette walls are of quartz to avoid absorption of ultraviolet radiation. Glass normally employed in cuvette walls will absorb ultraviolet radiation. A problem at this time, however, is that the reflection characteristics of the quartz-air interface are unknown. This can be calculated if the refractive index of the type of quartz is known.

Random error is caused by variations in source radiation output, flicker noise if measurement of ϕ_f is conducted in the energy mode. This problem is essentially eliminated by taking readings in the ratio mode that is available with the Model 512 Perkin-Elmer Spectrofluorometer. This mode automatically compensates for light source intensity variations. The manner in which the ratio mode operates is detailed in the Model 512 instruction manual. All measurements throughout the current work have been conducted in the ratio mode.

Baseline or 0% noise includes three types of noise associated with transducers and amplifiers. They are all involved with uncertainty in making the zero adjustment. They include dark current shot noise, dark

current excess noise and amplifier excess noise. In modern spectrofluorometers, 0% noise is seldom significant except at the wavelength extremes of the instrument and at the limits of detection for fluorescence[4]. This noise is independent of the baseline setting.

Johnson noise is due to Brownian motion of electrons in resistive components of spectrofluorometers. This noise in photomultiplier tubes and amplifiers is so small in magnitude relative to other sources of noise that for all practical purposes it can be neglected. It is not dependent on the magnitude of the current and may occur in the absence of a current.

Signal shot noise is due to two factors: 1) random rate of arrival of photons at the photocathode; and 2) random emission of photoelectrons down the dynode chain. This noise increases in magnitude with increases in the size of the quantity being measured. Although the significance of this noise for the Model 512 has not been ascertained, it is probably the major source of noise.

Cell positioning uncertainty has been determined to be a probable limiting source of random error in high quality spectrophotometers[66]. Due to their similarity, spectrofluorometers are prone to suffer from this type of error. However, in spectrofluorometers the resultant error is much less significant. The specifications of the stepper motor (which controls the cell position through rotation) indicate that the reposition accuracy after one complete rotation (scanning in positions one, two, and four) is 3% of each last step before a halt. Since each step is 1.80° and three halts are involved the maximum position inaccuracy would be $\pm\ 0.16^{\circ}$. Several hundred such cycles of measurement

resulted in negligible misalignments. However, even if such potential misalignments were to become significant in such a cummulative fashion, full scans are run at each of the three positions virtually eliminating this type of error. Figure 31 shows the cumulative effects of rotation misalignments.

Software for system data collection normally involves taking forty readings, calculation of the average value and error bar (difference between high and low values in set of forty) for each data point stored. If spectrofluorometer outputs are held constant at various levels one observes a regular senusoidal oscillation with time as shown in Figure 32. The oscillation is at 60 cycles/second and remains constant as shown also in a plot of A/D converter output with voltage in Figure 34. The fluorescence signal to noise ratio with voltage is shown in Figure 33.

It becomes apparent from the preceding discussion and from the data that the limiting factor of system error is in the 60 Hz line noise. Due to S/N ratios obtained it is preferable to monitor data at voltage levels above 2.0 V. The software has an available smoothing routine to average data over a full cycle of the 60 Hz noise and thus eliminate this problem. (Another means of eliminating this noise is with a 60 Hz notch filter).

D. Results of Correction Factor Analysis

1. Study of Primary Absorption

The range of linearity in the ϕ_f vs C_f plot was found to extend to approximately 1×10^{-5} M quinine sulfate in a background matrix of 0.1 N H_2SO_4. Utilizing a Fluke 8020 A digital multimeter detection was

Figure 31. Potential Misalignment Problems Encountered During Cell Rotation Method Tune-Up

The top diagram shows expected alignment of cell walls with the propagation axes of the excitation radiation and detected emission radiation. The middle diagram shows skewing of the sample cell. A few degrees off from the perpendicular can severely attenuate the detected fluorescence. The bottom diagram shows a problem with Z axis truing. Here the cell faces are not vertical and significant attenuations of the fluorescence signal may result. The process of eliminating these potential problems is described in Chapter III.

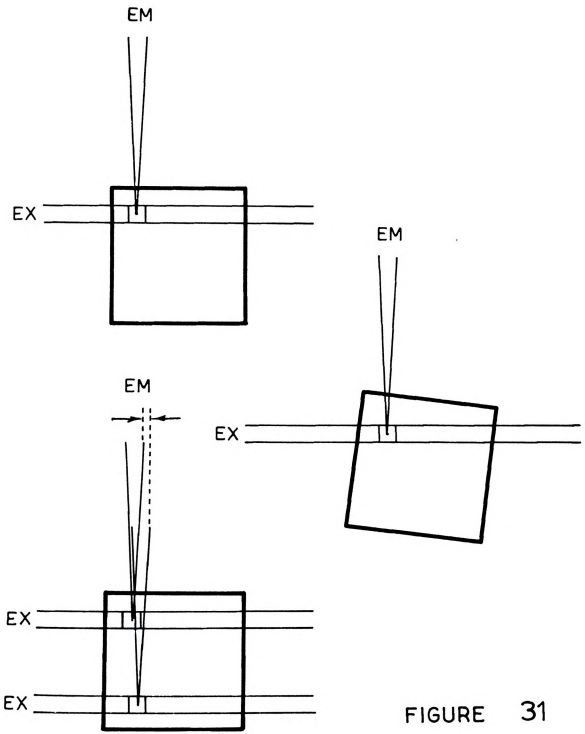


FIGURE 31

Figure 32. Exploded View of Noise Monitored at Various Output Voltage Levels

60 Hz line noise is the predominant source of error at the selected output voltage levels. The magnitude of the error bar due to this noise is constant regardless of analog to digital converter output. *

*The magnitude of 60 Hz oscillation is approximately 30/4096 relative to the maximum A/D converter output at 10 volts.

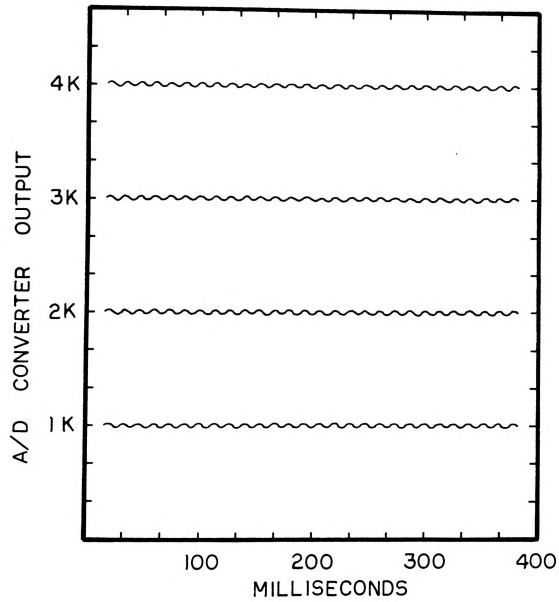


FIGURE 32

Figure 33. Signal to Noise Ratio for Full Range of A/D Converter Output

These ratios are independent of sample solution measured; H_2O, ethanol, 0.1 N H_2SO_4, 1.0 N H_2SO_4, quinine sulfate in acidic media were monitored for S/N characteristics. The correlation coefficient of the 0.1 N H_2SO_4 data is 0.9933 indicating a linear rise in S/N with voltage.

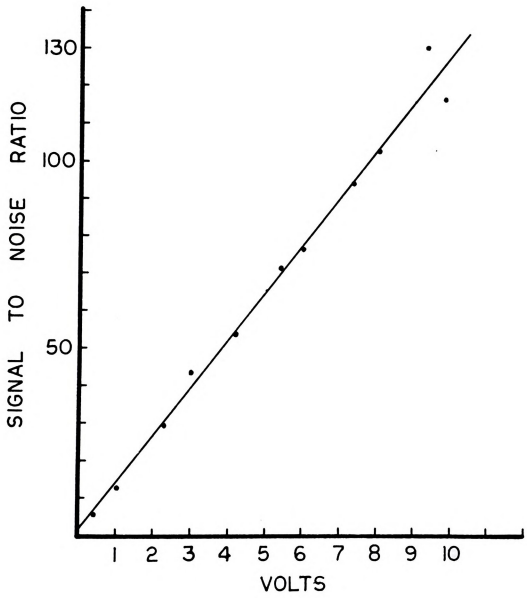


FIGURE 33

Figure 34. Reproducibility of Cell Position One with Multiple Rotations.

The full range of A/D converter output was investigated. The readings taken after each of five successive 360° rotations were averaged to determine the magnitude of error inherent in realignment. Error bars were calculated as the difference between the high and low values registered in any of the five rotations.

The difference between readings of subsequent rotations was negligible. The measurements vary over a constant sinusoidal component of 60 cycle per second line noise irregardless of voltage input to the A/D converter. A solution of 1×10^{-6} M quinine sulfate in 0.1 N H_2SO_4 was used, output level voltage was varied by the high voltage reference attenuation adjust.

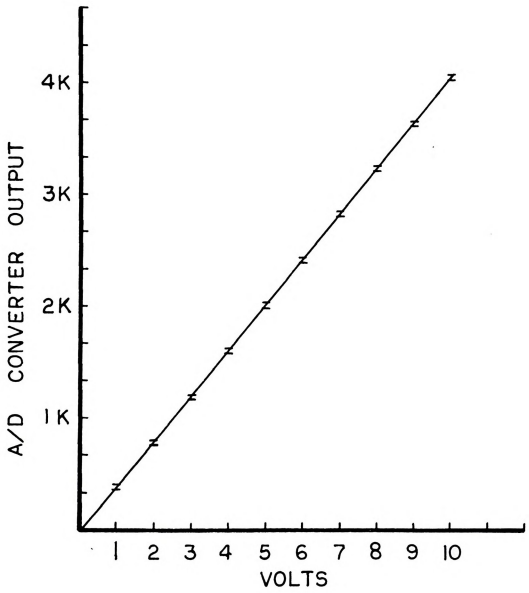


FIGURE 34

was possible down to $1x10^{-9}$ M. Figure 35 shows in plots A through E the extent of the above detectable linear range.

Primary absorption differences for quinine sulfate over a range of concentrations is shown in Figure 36. Significant primary absorptions are encountered at $1x10^{-7}$ M and above. Only two curves are apparent even though measurements were conducted at all four cell positions. This is the case since excitation at 250 nm is absorbed by the sample, but the emission at 460 nm is not absorbed. As was expected there is no secondary absorption thus matching position 1 and 2 readings and position 3 and 4 readings.

A plot of the logarithmic difference between the window edge positions along the excitation optical axis with concentration is shown in Figure 37. The plot is observed to be linear over the range of concentrations measured. Since this expression, $(\ln(\phi_f/\phi_o)_1 = \ln(\phi_f/\phi_o)_4$, appears to describe adequately the attenuation of incident excitation radiation with distance, a correction factor can be determined.

An emission scan of $1x10^{-6}$ M quinine sulfate in 0.1 N H_2SO_4 in four cell positions is shown in Figure 38. Scans in position 1 and 2 are well matched, as are scans in positions 3 and 4. Application of the correction routine for primary and secondary absorptions resulted in curve 0.

2. Study of Secondary Absorption

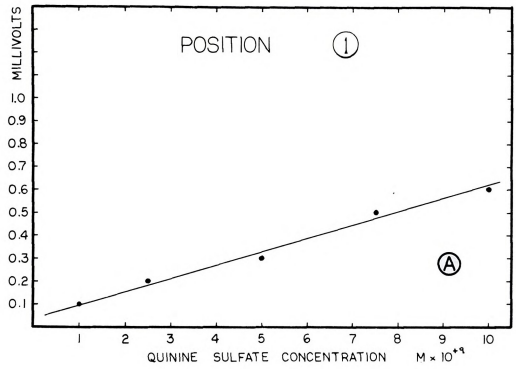
A plot of the logarithmic difference between the window edge positions along the emission detection axis with concentration is shown in Figure 39. The plot is linear over the range of concentrations

Figure 35. Instrumental Output with Concentration for Quinine Sulfate

A. 1×10^{-9} to 1×10^{-8} M
B. 1×10^{-8} to 1×10^{-7} M
C. 1×10^{-7} to 1×10^{-6} M
D. 1×10^{-6} to 1×10^{-5} M
E. 1×10^{-5} to 1×10^{-4} M

Specifications:

Solvent 0.1 N H_2SO_4
Excitation Wavelength 350 nm
Emission Wavelength 450 nm
Reference Attenuation 600 V
Sensitivity 0.1x
Ratio Mode
Slits 20/20 nm


POSITION (1)
MILLIVOLTS
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
1 2 3 4 5 6 7 8 9 10
QUININE SULFATE CONCENTRATION M × 10+9
(A)

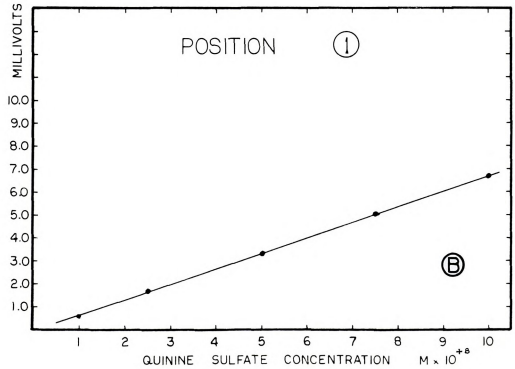
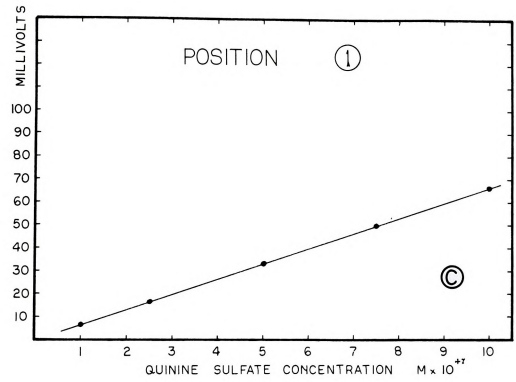

POSITION (1)
MILLIVOLTS
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
1 2 3 4 5 6 7 8 9 10
QUININE SULFATE CONCENTRATION M × 10+8
(B)

FIGURE 35



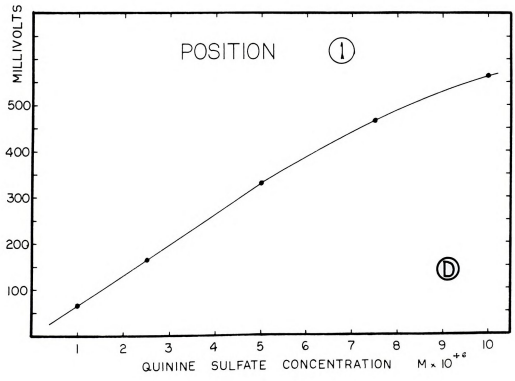


FIGURE 35
CONTINUED

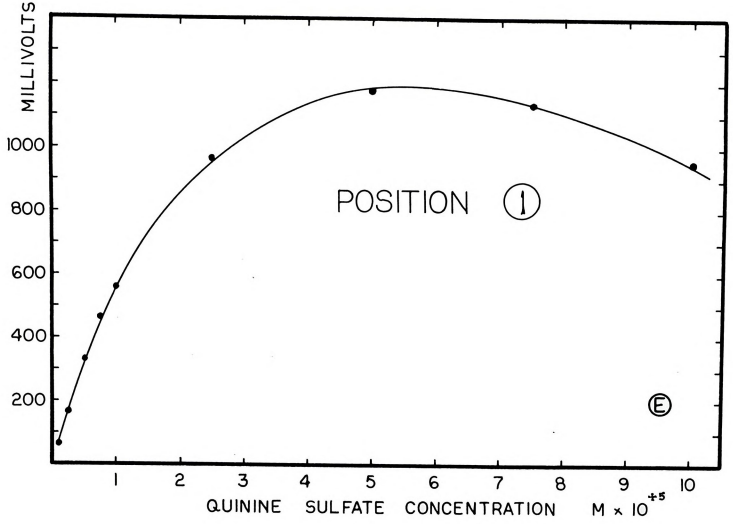


FIGURE 35
CONTINUED

Figure 36. Quinine Sulfate - Fluorescence Emission Scan as Detected in Four Cell Positions and Absorption Corrected.

Specifications:

Concentration Quinine Sulfate 1×10^{-6} M
Solvent 0.1 N H_2SO_4
Excitation Wavelength 250 nm
Emission Wavelength 460 nm
Reference Attenuation 650 V
Sensitivity 0.1x
Ratio Mode
Slits 10/10 nm

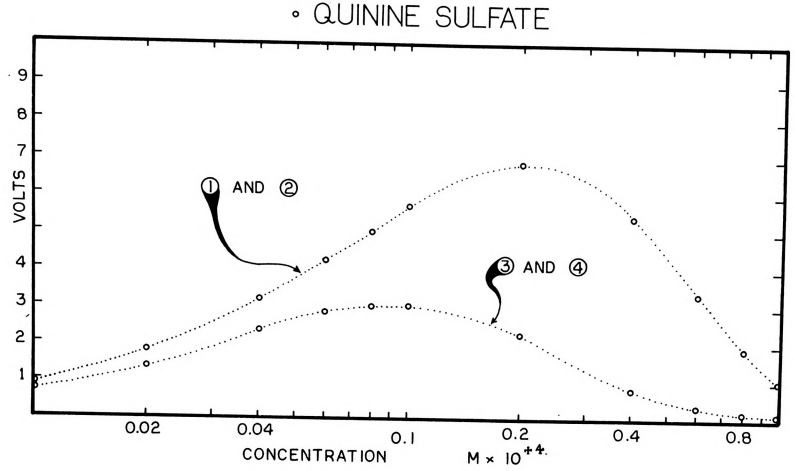

° QUININE SULFATE
VOLTS
9
8
7
6
5
4
3
2
1
① AND ②
③ AND ④
0.02
0.04
0.1
0.2
0.4
0.8
CONCENTRATION
M × 10⁺⁴

FIGURE 36

Figure 37. Study in Linearity of Primary Absorption with Increasing Quinine Sulfate Concentration

The plot represents the change in $\ln(F/R)_1$ $\ln(F/R)_4$ with concentration. The quinine sulfate concentration range extends from 1×10^{-7} M to 1×10^{-5} M. The background matrix consists of 0.1 N H_2SO_4 in house distilled water.

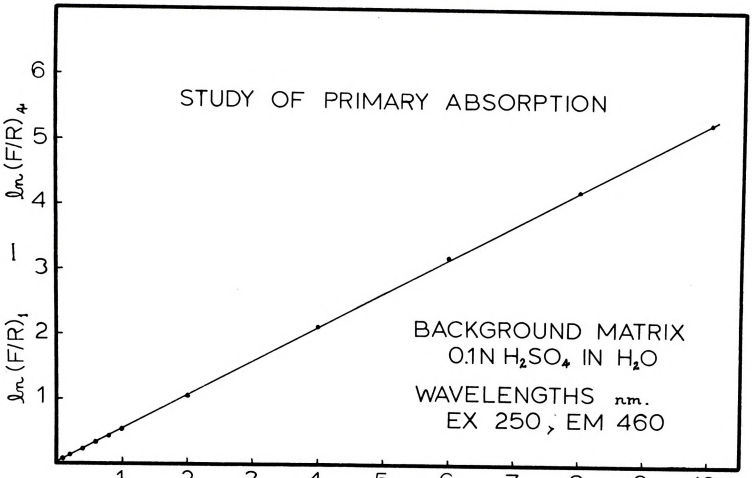

STUDY OF PRIMARY ABSORPTION
BACKGROUND MATRIX
0.1N H_2SO_4 IN H_2O
WAVELENGTHS nm.
EX 250, EM 460
$\ln (F/R)_4$
I
$\ln (F/R)_1$
1
2
3
4
5
6
7
8
9
10
M x 10^{+6} QUININE SULFATE

FIGURE 37

Figure 38. Quinine Sulfate - Fluorescence Emission Scan as Detected in Four Cell Positions and Absorption Corrected

Specifications:

Concentration Quinine Sulfate 1×10^{-6} M
Solvent 0.1 N H_2SO_4
Excitation Wavelength 250 nm
Emission Wavelength 460 nm
Reference Attenuation 650 V
Sensitivity 0.1x
Ratio Mode
Slits 10/10 nm

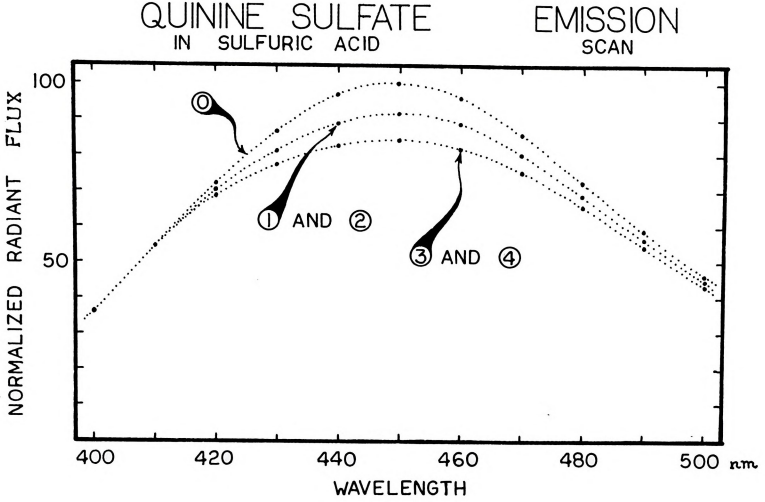

QUININE SULFATE
IN SULFURIC ACID
EMISSION
SCAN
NORMALIZED RADIANT FLUX
100
50
0
1 AND 2
3 AND 4
400
420
440
460
480
500 nm
WAVELENGTH

FIGURE 38

Figure 39. Study in Linearity of Secondary Absorption with Increasing Fluorescein Concentration

The plot represents the change in $\ln(F/R)_1 - \ln(F/R)_2$ with concentration. The fluorescein (sodium salt) concentration range extends from 1×10^{-6} M to 1×10^{-4} M. The background matrix consists of 1×10^{-5} M quinine sulfate and 0.1 N H_2SO_4 in house distilled water.

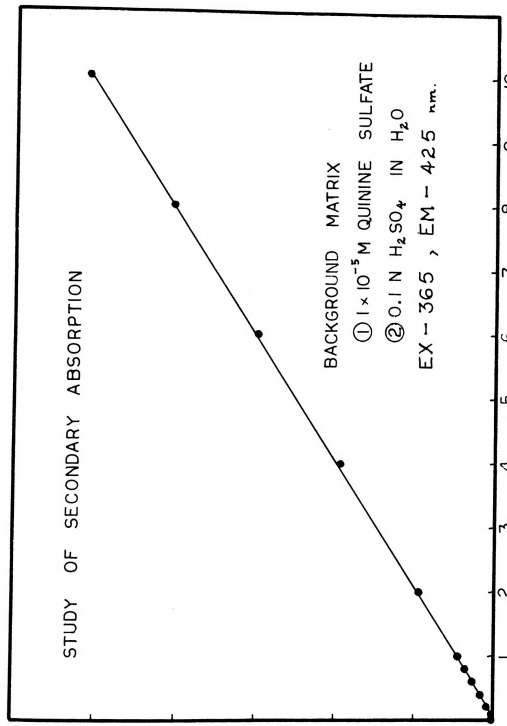

STUDY OF SECONDARY ABSORPTION
BACKGROUND MATRIX
① 1 × 10⁻⁵ M QUININE SULFATE
② 0.1 N H₂SO₄ IN H₂O
EX – 365 , EM – 425 nm.
2.5
2.0
1.5
1.0
0.5
1
2
3
4
5
6
7
8
9
10
M × 10⁺⁵ FLUORESCEIN

FIGURE 39

measured. The expression, $\ln(\phi_f/\phi_o)_1 - \ln(\phi_f/\phi_o)_2$, describes the attenuation of radiation at the emission wavelength with distance. From this a correction for secondary absorption can be determined.

A series of scans were conducted for mixtures of quinine sulfate and fluorescein as shown in Figures 40A, B, and C. In each case, the correction routine for primary and secondary absorption was applied to generate curve 0.

Figure 40A-C. Fluorescence Emission Scan for Mixtures of Quinine Sulfate and Fluorescein

A. Quinine Sulfate 1×10^{-5} M
   Fluorescein 2×10^{-5} M
B. Quinine Sulfate 1×10^{-6} M
   Fluorescein 6×10^{-5} M
C. Quinine Sulfate 1×10^{-6} M
   Fluorescein 2×10^{-5} M

Specifications:

Solvent 0.1 N H_2SO_4
Excitation Wavelength 365 nm
Emission Scan 400-500 nm
Reference Attenuation 600 V
Sensitivity 0.1 x
Ratio Mode
Slits 10/10 nm

AN EMISSION SCAN

QUININE SULFATE AND FLUORESCEIN

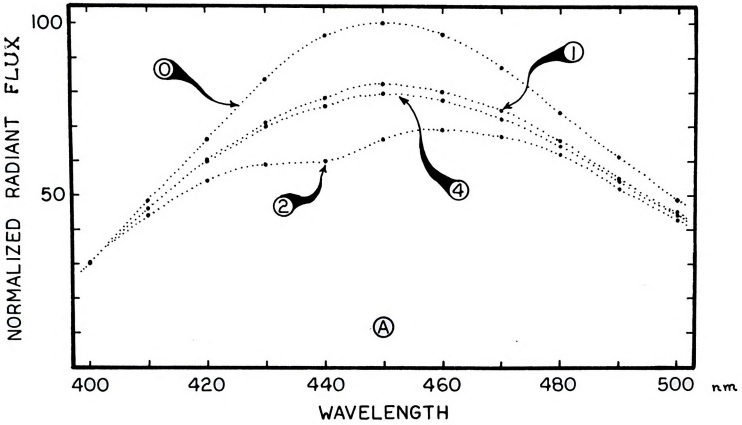


FIGURE 40

AN EMISSION SCAN

QUININE SULFATE AND FLUORESCEIN

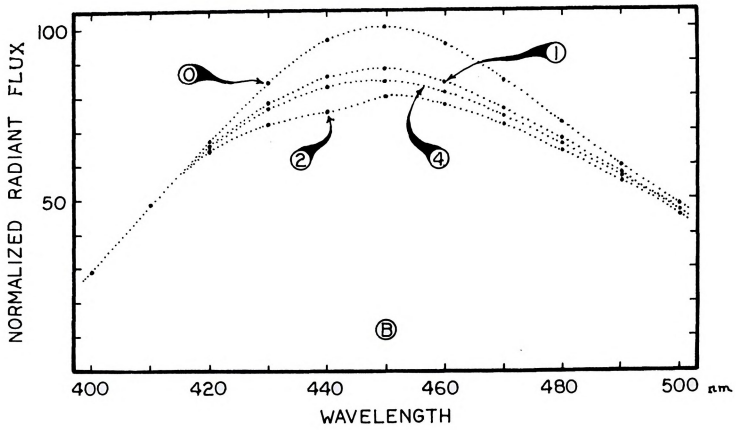


FIGURE 40
CONTINUED

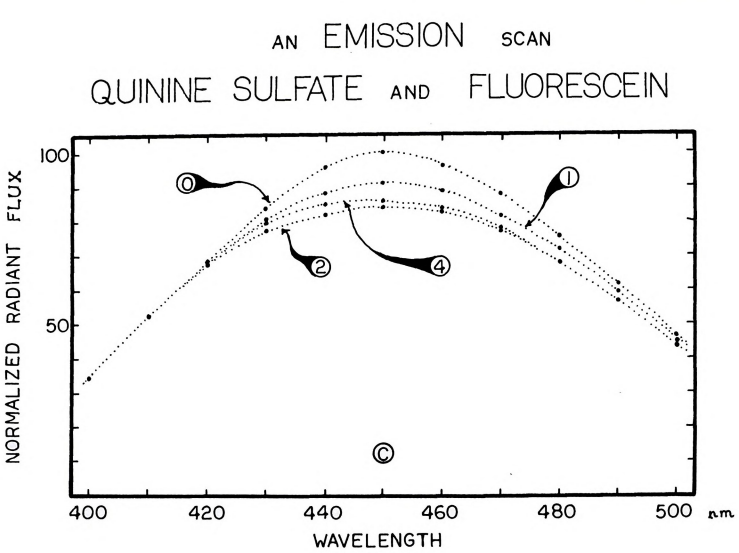


FIGURE 40
CONTINUED

CHAPTER V

CONCLUDING STATEMENTS

A. Summary

The original goal of this research has been realized. An instrument interactive with the laboratory PDP 8/e computer has been constructed to correct automatically for attenuation of the detected fluorescence signal caused by the sample's characteristic primary and secondary absorption. The instrument's ease of operation and overall performance have been evaluated.

At the outset of this project, emphasis was placed on ease of operation, reliability, precision, accuracy, rapid data acquisition, rapid spectral correction, and the adaptability for conversion of commercially available spectrofluorometers into absorption correcting units without the need for costly reconstruction.

Design of an integral system was implemented. The flowchart of all physical devices employed in the computer controlled absorption correcting spectrofluorometer is shown in Figure 16. Most of the devices involved are readily available in typical, modern analytical laboratories. Components such as the stepper motor and its power supply are commercially available. The circuitry required to adapt the computer's stepper motor command pulses to the power drive of the motor is straight

forward and inexpensive as shown in Figure 45 (appendix). The heart of the correction system, the cell positioner, can easily be constructed from diagrams detailing the three sections shown in Figures 17-19.

A spectrofluorometer incorporating right-angle geometry was selected as most appropriate since sample transmission at both the excitation and emission wavelengths can be accurately computed from fluorescence monitored at the three cell positions thus obviating the need for a second instrument.

The cell positioner was designed to meet the requirements for the necessary data acquisition in computation of and correction for primary and secondary absorbance. The present arrangement has a preset range for Δd between 0.2 to 1.0 cm along either the excitation or emission optical axis. The practical maximum limit to the usable Δd values with normal 1 cm by 1 cm cells has been determined from window profiling to be within a 0.4 cm by 0.4 cm volume element centered within the cell.

The stepper motor requires only 125 msec to fully shift and halt in a position 90° removed in a counterclockwise direction. Although the shift is moderately rapid between positions there is no apparent problem with introducing air bubbles at this rate.

The simple design and massive construction of the cell positioner were essential for durability over prolonged periods of measurement, for maintenance of a high degree of precision and accuracy, and to minimize vibration caused misalignments.

The relatively small size of the cell holder of the positioner assembly will potentially allow its incorporation into the sample cell compartments of other commercially available right-angle spectrofluorometers. The pedestal design should be amenable to controlled

environments covering a wide range of solution temperatures or noncorrosive atmospheres (i.e., nitrogen, helium, argon).

Accommodation of smaller samples is possible by vertical adjustments of the post on the motor drive rod and a series of shim plates, each 1/8 inch thick and 5 by 10 inches in surface area. The maximum attainable height within the current cell compartment is 2 cm, at which position the sample volume requirements are cut by a factor of two.

The detection aperture for fluorescence must be quite narrow for use of normal 1 cm by 1 cm cells allowing Δd values sufficiently large to permit accurate calculation of the primary and secondary absorption correction factors.

The collimation of the excitation and emission radiation required for correction by the method of cell rotation is a potential limiting factor in this technique. However, narrowness of the detection field in the Perkin-Elmer Model 512 is sufficient to allow accurate calculations. Other units may not be so fortunate. In the cell shift method[2,61], this factor was suspected by the authors as being the method's greatest practical limitation due to the resultant reduction in measurement precision and sensitivity.

The precision of the absorption corrected fluorescence is obviously dependent on the inate measurement precision of the spectrofluorometer. In this case the Perkin-Elmer Model 512 again was more than adequate. Fortunately, the measurement precision characteristic of modern spectrofluorometers is excellent. This, therefore, will not be a limit to other applicatons of this technique.

B. Future Work

1. Chemical Systems

a. Repetitive scans for generating an absorption corrected fluorescence spectrum can be collected over a period between 5 and 15 minutes depending on the wavelength range scanned with current instrumentation. This permits study of dynamic chemical systems that undergo changes in such a timescale. Examples would be systems that directly alter a fluorophore concentration through reaction, cases where the environment (background matrix) interacting with the fluorophore is undergoing change, or where association/dissociation phenomena are observed involving the fluorophore molecules.

b. The determination of a diagnostic, the partial quantum efficiency, is feasible with minor alteration of the current setup. A quantum counter would have to be aligned to monitor the rotating cell's transmittance at each cell position. Since the counter responds linearly to the number of photons which enter the counter, the difference between the quantities $\phi\omega_1$ and $\phi\omega_2$ is proportional to the quanta absorbed within the window $(\omega_1 - \omega_2)$. After comparison of the quantum counter and emission PMT outputs for identical radiant flux and wavelength settings, the emission PMT could be converted to a signal which was proportional to the number of quanta emitted at a particular wavelength. The partial quantum efficiency can be calculated by dividing the number of photons emitted by the number of photons absorbed within the window. Such information would provide insight on an environment's influence upon a fluorophore (i.e., changes in pH or ionic strength, aggregation or dissociation phenomena over a range of concentrations) as well as rapid identification as to whether or not primary

and/or secondary absorption is occurring.

c. Increasing temperature of a sample solution usually decreases fluorescence radiant flux because the increased random motion of molecules increases the probability of collisional deactivation of an excited molecule. Controlled cooling of the cell compartment, and subsequently the sample itself, may provide greater insight into the effect of temperature on both quantitative and qualitative fluorescence analyses. It is anticipated that lower temperatures may significantly increase the fluorescent emission, and thus the detectability of lower fluorophore concentrations.

d. A novel technique available with the current instrumental arrangement for measurement of particle concentrations is absorption corrected nephelometry. This technique is based on the scattering of incident radiation by particles suspended in the solution. The beam of radiation is monitored at a 90^{o} angle with the right-angle geometry. Because of the angle of measurement this technique is most suited for determining very low concentrations. For optimum scattering efficiency using ultraviolet and visible regions of the spectrum the optimum particle size is in the range of about 10 to 10,000 $\mathring{A}$, or colloidal size[5].

e. Clinically it is very important to determine accurately the concentrations of various drugs present in pharmaceutical preparations. Many drugs exhibit fluorescence after reaction with organic species that will form fluorophore products. When fluorescence is used as the analytical technique absorption corrections are necessary to prevent output signal attenuations. Due to this problem, the apparent

drug concentrations appear to be substantially lower than the actual concentrations. An instrument with absorption correcting capability should significantly improve the safety margin in the packaging and dispensing of such drug preparations.

f. In general, inorganic ions do not exhibit fluorescence. Fortunately, many of these same ions are capable of forming with aromatic organic molecules chelate complexes which may be highly fluorescent, even if the organic species itself does not exhibit fluorescence. This approach has in the past provided the basis for very sensitive analyses of many elements including most of the transition elements. Absorption corrected measurements would significantly increase the accuracy of such determinations, particularly in concentrations ranging to 1×10^{-6} M and higher for the chelate complexes.

g. Bioluminescence, the emission of visible light by living systems, is one of the most fascinating of natural phenomena and can readily be studied by the absorption correcting instrument. The mechanistic aspects of the chemistry involved in luminescent bacteria, glowing toadstools, protozoa that can light up ocean waves, luminous clams, fantastically illuminated worms, and the common firefly have all been the objects of curiosity. The chemical problem is an interesting one. The firefly's emitted radiation with a wavelength of 560 nm has an energy of 214 kJ/einstein. A natural question is then, "What kind of chemical reaction can lead to an energy yield that high?". It is far too much energy to be provided by the splitting of ATP. Even the oxidation of NADH by oxygen would barely provide the necessary energy.

A clue comes from the fact that chemiluminescence is very common when O_2 is used as an oxidant in non-enzymatic processes. The slow oxidation of alcohols, aldehydes, and many nitrogen compounds is accompanied by emission of radiation visible to the eye. Chemiluminescence is especially pronounced in those reactions that are thought to occur by free radical mechanisms. The recombination of free radicals provides enough energy to permit the release of visible radiation. Any loss of this emitted radiation through absorption can automatically be corrected for.

h. The spectra of certain types of enzyme solutions differ radically depending on the presence or absence of molecular oxygen (O_2). Pea seedling diamine oxidase and hog kidney diamine oxidase are two such enzymes. Anaerobic studies can be accomplished by degassing the solution and maintaining an oxygen free environment in the cell compartment. A positive (in excess of 1 ATM) atmosphere of N_2 or Ar within the cell chamber will create such an environment. Aerobic studies require only that the inert atmosphere be removed and that oxygen be permitted to diffuse into the solvent matrix. The kinetics of the enzyme/substrate interactions could be better understood with controlled oxygen (one of the substrates required) diffusion over time. Here absorption corrected data are essential.

2. Instrumental Improvements.

a. The cell rotation method must be further characterized and compared to the cell shift method[2]. Of particular interest would be knowledge of the concentration ranges where the various correction factors are valid. This would include corrections as applied to primary

and secondary absorption, internal reflections, and re-emission. By comparison with results obtained using the cell shift method one could determine the advantages and the disadvantages to correction by either approach.

b. A sample cell with dimensions of 2.0 cm by 2.0 cm would permit much larger values of Δ d along each of the optical axes. Current Δ d limitation is 0.4 cm, centered, in either dimension. The expected increase would be to 1.4 cm for both axes. The larger observation windows would allow significantly more accurate determinations of both primary and secondary absorption. Previously the largest routinely used window was achieved by Christmann with the cell shift method[2]. An 0.8 cm window had been attained.

c. An X·Y positioner analogous to that employed by Christmann in the cell shift method has been constructed. Instead of employing DC motors which are prone to positional errors and are not practical for repetitive scanning, two stepper motors were used. In half-step mode each motor takes 8,000 steps per cm along each optical axis. Such an arrangement would allow 64,000,000 potential setting positions across the surface of a 1 cm by 1 cm cell. Thus full and highly accurate maps of window calibrations could be routinely made. Also realignments for the various cell positions would be very reproducible.

d. A unique system has been designed possessing a stationary sample cell. This system would employ a pulsed tunable laser as the excitation source. Fiber optics (UV-VIS) would be used to guide emitted fluorescence from three cell positions to a series of three scanning

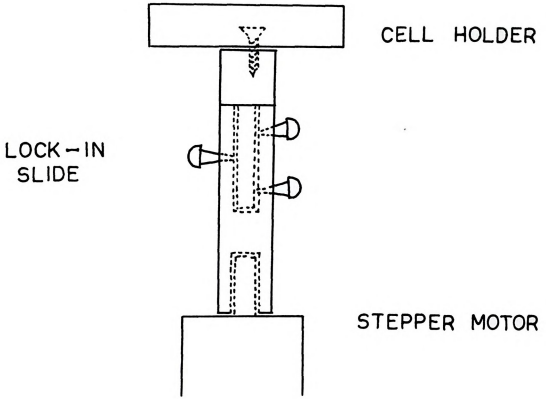
monochromators (matched). The outputs of the monochromators would go to a set of three PMT's (matched) from which outputs would go to a multiplexer to allow high speed switching and essentially simultaneous scanning of the three cell positions required for implementing corrections for primary and secondary absorption. Such a system would be characterized by high speed in achieving absorption corrected spectra, by ideal positional alignment since the cell does not move to allow emission readings from different positions, and by very high sensitivity due to the pulsed laser source.

e. The sensitivity of the current instrumental arrangement could be increased several orders of magnitude by application of a pulsed tunable laser as the source. A pulsed laser would used to eliminate the problems of solution heating and saturation of excited energy levels encountered with use of continuous wave lasers. Minor modification of the cell compartment would be required in adapting the laser source.

f. With the aid of a pulsed laser source, fluorescence lifetimes could also be measured. This would enable the instrument to resolve multi-component mixtures in a sample by time resolved fluorescence spectrometry.

g. Use of a solid state array detector in place of the photomultiplier tube could reduce the time required to obtain a fluorescence spectrum. The intrinsically low levels of emission radiant flux characteristic of the instrument would indicate that a long integration time would be necessary with this kind of detector, but that data might be obtained more quickly than with a scanning monochromator system[2].

h. Small sample volumes may preclude using current cell positioner design. A modification of the cell holder post should allow use of volumes as low as 1.5 ml. The modification is illustrated below:



Normally available (1 cm x 1 cm x 4 cm) cells can still be used, thus avoiding the expense of having specially designed cells made by the manufacturer.

APPENDIX A

Figure 41. Modified M1709 Omnibus Interface Foundation Module

This module is a general interface card that allows the user to interface a variety of equipment to the PDP-8/e computer. The custom designed M1709 is detailed. The module provides all necessary omnibus interface logic (i.e., bus drivers/receivers, device selectors, interrupt/skip circuitry).

M1709 OMNIBUS INTERFACE FOUNDATION MODULE AS MODIFIED

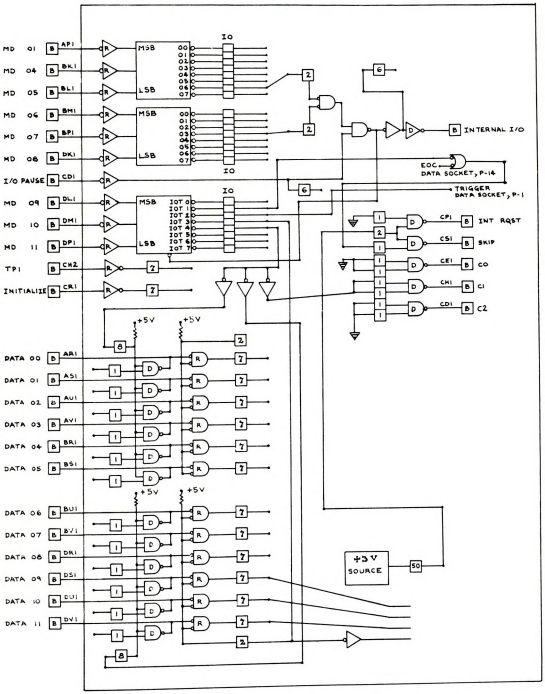


FIGURE 41

Figure 42. Analog to Digital Conversion Circuitry

The Analogic MP 2112 is high-speed and low cost. Full 12-bit conversions are completed in less than 7μs, which provide throughput speed up to 140,000 conversions per second. Allowing up to 3μs for the multiplexer and the sample-and-hold amplifier to settle in conjunction with the 7μs, the resulting throughput rate is 100 kHz. Other characteristics include a differential linearity of ½ LSB, a ± 0.012% relative accuracy, less than 7 ppm/°C gain temperature coefficient, and both parallel and serial outputs.

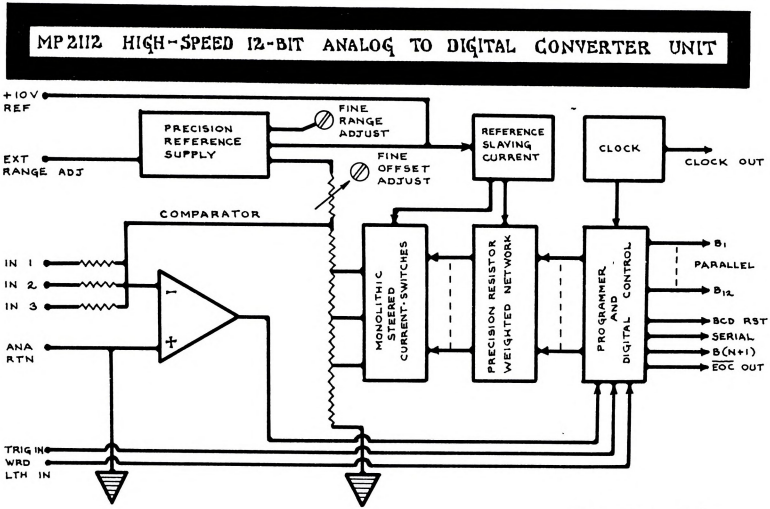

MP2112 HIGH-SPEED 12-BIT ANALOG TO DIGITAL CONVERTER UNIT
+10V REF
EXT RANGE ADJ
PRECISION REFERENCE SUPPLY
FINE RANGE ADJUST
FINE OFFSET ADJUST
REFERENCE SLAVING CURRENT
CLOCK
CLOCK OUT
COMPARATOR
IN 1
IN 2
IN 3
ANA RTN
MONOLITHIC STEERED CURRENT-SWITCHES
PRECISION RESISTOR WEIGHTED NETWORK
PROGRAMMER AND DIGITAL CONTROL
B1
PARALLEL
B12
BCD RST
SERIAL
B(N+1)
EOC OUT
TRIG IN
WRD LTH IN

FIGURE 42

Figure 43. Four Channel CMOS Multiplexer

The MXD-409 analog multiplexer (Datel Systems, Inc.) is a 4 channel monolithic device manufactured with a dielectrically isolated complementary MOS process. The circuits incorporate analog and digital input protection which protects the units from both overvoltage and loss of power. The digital inputs are DTL/TTL/CMOS compatible and address the proper channel by means of a 2 bit binary code. Another important feature of these multiplexers is the use of break-before-make switching to insure that no two channels are ever momentarily shorted together.

Transfer accuracies of 0.01% can be achieved at channel sampling rates up to 200 kHz and over ± 10V signal ranges. Power consumption is only 7.5 mW at standby and 15 mW at 100 kHz switching rate. Power supply range is ± 5V to ±20V.

4 CHANNEL CMOS MULTIPLEXER MXD-409 CIRCUIT

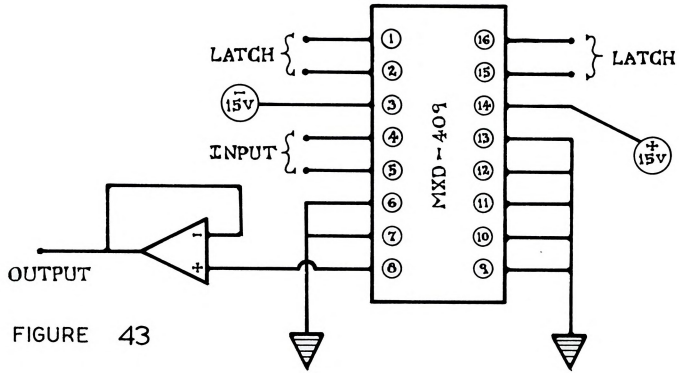


FIGURE 43

Figure 44. Amplification Circuit

To boost Perkin-Elmer model 512 spectrofluorometer output to required voltage levels for analog to digital conversion circuitry.

Key:
- 1 KΩ = one kilo-ohm resistor, ½ watt power rating.
- 200 KΩ = two hundred thousand ohm maximum variable resistor, ½ watt power rating.
- 1 MΩ = one meg. ohm resistor, ¼ watt power rating.
- 741 = operational amplifier.

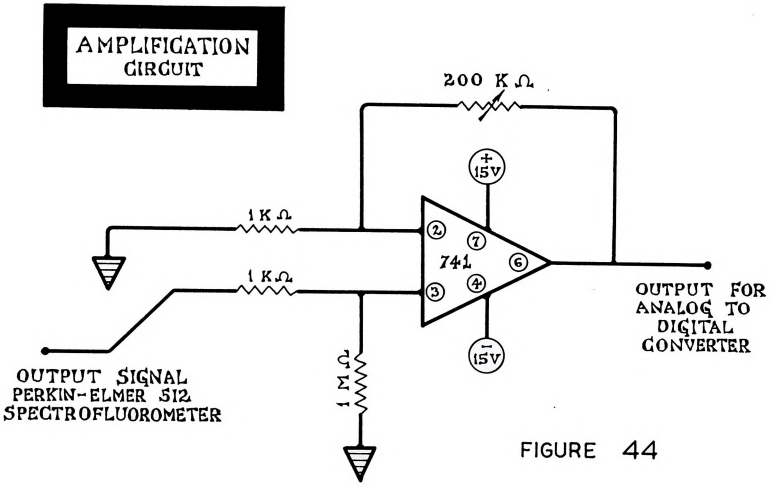

AMPLIFICATION CIRCUIT
200 KΩ
+15V
1 KΩ
741
1 KΩ
OUTPUT FOR ANALOG TO DIGITAL CONVERTER
-15V
OUTPUT SIGNAL PERKIN-ELMER 512 SPECTROFLUOROMETER
1 MΩ

FIGURE 44

Figure 45. Pulse Fitting Circuit

This circuit is interfaced between the I/O port of the PDP 8/e computer and the power supply of the stepper motor. The timing (RC) circuit is set for a value of 70 s for each pulse.

Key: 74-121 = one shot.
SK-3025 = PNP transistor with 1 amp capacity.
All resistors are of $\frac{1}{4}$ watt capacity.

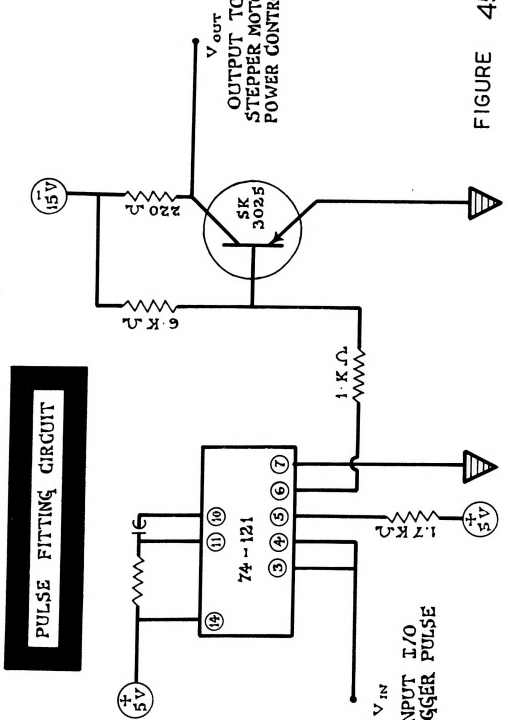

PULSE FITTING CIRCUIT
+5V
14
11
10
74 - 121
3
4
5
6
7
V IN
INPUT I/O
TRIGGER PULSE
1.7 K Ω
+5V
1 K Ω
6.8 K Ω
-15V
220 Ω
V OUT
OUTPUT TO
STEPPER MOTOR
POWER CONTROL
SK
3025

FIGURE 45

Figure 46. Ozone Exhaust System

This system was designed to eliminate the buildup of ozone in our laboratory when two spectrofluorometers are operating concurrently. The ozone is pumped beneath the floor into a drainage line. Since the half-life of ozone is about 17s, this method effectively reduces any potential hazards.

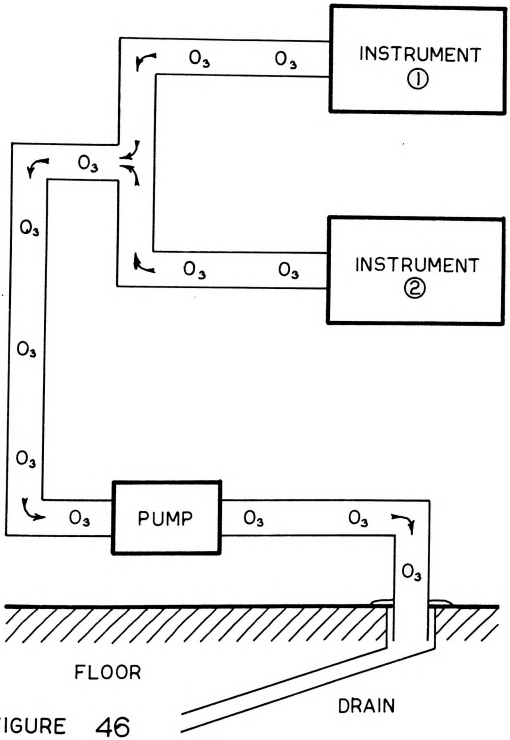


FIGURE 46

APPENDIX B

Table 7. Model 512 Specifications

The Model 512 Fluorescence Spectrophotometer Specifications

I. General Specifications

a. Main Unit

1) Monochromators... Excitation and emission
   Optical system : Czerny-Turner mount, aperture value F3.5
   Diffraction grating: 600 lines/mm, blazed at 300 nm
2) Spectral bandpass : 3, 10, 20 nm (changeable in 3 steps)
3) Wavelength range
   Emission measurement 200 to 900 nm and zero order
   Absorption measurement 250 to 640 nm
4) Wavelength accuracy: ±1 nm, zero order: ±3 nm
5) Wavelength reproducibility: Within 0.2 nm
6) Wavelength backlash: Wavelength backlash, when turning from long wavelength to short wavelength: within 0.5 nm
7) Scan speed : 60, 120, 240, 480 nm/min
8) Light source : Xenon lamp 150 W
9) Detector : Photomultiplier R-446F
10) Photometric system : Direct (ENERGY)
    Ratio recording
    Subtraction mode %T mode
    Absorption mode
11) Sensitivity : 0.1, 0.3, 1, 3, 10, 30, 100 (selectable in 7 steps)
12) Response : Fast, medium slow (Changeable in 3 steps)
13) Dimensions : 67 cm W x 39 cm D x 27 cm H
14) Weight : 54 kg
15) Signal Output : 10 mv for potentiometer recorder
    1 volt for digital voltmeter

b. Xenon Lamp Power Suply

1) Constant-current (7.5 A) pulse ignition type
2) Dimensions : 25 cm W x 29 cm H x 35 cm D
3) Weight : 22 kg

II. Fluorescence Mode Specifications

1) Stray light : Excitation monochromator = 1% at λ = 250 nm
   Emission monochromator = 2% at λ' = 350 nm
2) Sensitivity : The Raman band of H_2O will show a 25% pen deflection under the following conditions:
   High voltage = 700-800 V
   Sensitivity = X100
   Energy mode
   Excitation wavelength = 350 nm
   Slit = 10 nm
   Emission Slit = 10 nm

Table 7 (cont'd)

	Response = medium Noise level = within 5% or less of full scale (Make measurement at wavelength at Raman Peak)
3) Stability in Ratio Mode:	(Power must be on for at least 30 minutes) Drift is 1%/hour under the following conditions. 1. Diffuser plates should be in both sample and reference beams. 2. Wavelength of excitation should be 400 nm, slit = 10 nm. 3. Wavelength emission = 400 nm, slit = 10 nm

III. Subtract Mode Specifications

1. Flatness of zero baseline	1% under the following conditions: 1. 1 ppm quinine solution placed in both beams. 2. excitation = 350 nm, slit = 10 nm. 3. emission = 380-580 nm scan, slit = 10 nm
2. Subtract Dial Magnification:	More than 5 times

IV. Transmission Mode Specifications

1) 100% Line flatness	:	+2% from 250 to 640 nm
2) Stability	:	1% drift/hour at 100 T; 0.1% drift/hour at 0%T (Measurement should be done at 30 minutes after power turn-on).
3) Photometric Accuracy:	:	±0.77%T ABSORBANCE: ±0.01 from 0 to 0.5A, ±0.03 from 0.5 to 1.0A.

Table 6. Stepper Motor (Rapid-Syn) Specifications

Model Number	23D - 6102
Accuracy	3% noncummulative
Resistance/ Ø	5.1 ohms
Rated Voltage	5.1 V
Current per phase	1.0 amp
Inductance per phase	10.0 milli henries
Time for single step	2.5 milli sec
Holding Torque	53 oz in
Maximum Running Torque	35 oz in
Detent Torque	5 oz in
Maximum Thrust Load	25 lbs
Maximum Overhang Load	15 lbs
Rotor Inertia	87 gm cm^2
Weight	20 oz
Length	2.0 in
Degrees per step	1.8° (full step mode)
Source voltage	28 V

APPENDIX C

Table 4. Quartz Sample Cuvette Cleaning Proceudre

I. Aqueous Solutions
   A. Rinse cell thoroughly with lukewarm tap water.
   B. Give final rinse with chemically pure distilled water.
   C. Occasionally it may be necessary to cleanse first with detergent solutions or similar liquids and 3-5% aqueous hydrochloric acid.

II. Aqueous Protein Solutions
   A. Soak cell in solution of 0.5% hydrochloric acid (pH 1-2) and pepsin (1g/100 ml). Soak overnight if necessary.
   B. Follow procedure for aqueous solutions.

III. Organic Solutions
   A. Rinse cell with suitable water soluble orgfanic solvent such as acetone, dioxane or alcohol, possibly mixed with lye or acid.
   B. Follow procedure for aqueous solutions.

IV. Heavy Metal Traces
   A. Wash cell with tap water.
   B. Dip cell into clean lukewarm concentrated nitric acid (aqua regia if appropriate).
   C. Give final rinse with chemically pure, distilled water.

V. Preservation of Cuvette Windows
   A. Follow the appropriate procedure above for the type of sample solution run in the cell.
   B. Remove all traces of water by using a suction pump (aspirator).
   C. Dip the cell into chromosulfuric acid (a saturated solution of potassium bichromate in pure concentrated sulfuric acid) at a temperature of 35°C. Let soak if necessary, for no more than one hour.
   D. Remove cell from acid and allow residual acid to drip from cell, or remove it by suction. Leave cell in open air to allow humidity in air to dilute any residual acid in cell corners.
   E. Rinse cell thoroughly with lukewarm tap water.
   F. Give final rinse with chemically pure distilled water.
   G. Allow to dry in dust free air.
   H. Polish outside of cell windows wiht optical cleaning tissue free of chlorine or acid traces.
   I. Store cell in dust free location until next use.

Table 9. Reagents List

Sulfuric Acid - Mallinckrodt. Lot KLMK
Ethanol - Anhydrous from MSU Chem. Stores
Fluorescein (sodium salt also known as uranine) - Fisher Scientific Company; laboratory grade
Quinine Sulfate Dihydrate - Aldrich, high purity lot LD
Distilled H_2O - house

Table 5. Proper Selection of Slit Widths

Purpose	Excitation Slit	Emission Slit	Comments
Excitation Spectra	Narrow	Wide	Both of the slit widths can be wide for measuring samples possessing broad peaks
Emission Spectra	Wide	Narrow	
Quantitative Analyses	Wide	Wide	For obtaining high S/N ratio the slit widths should be as wide as possible.

Table 8. List of I/O Instructions

Information for the use of FLROT (11-30-81)

Start-Up

1. The core image file is on the disk marked "SYS:" in pencil. This disk goes in the left slot of the floppy disk drive.
2. With the disk in the drive, boot the system by loading 7600 octal in the switch register, press the clear button on the front panel (this should cause the drive to click), and then press the continue button on the panel.
3. The computer should cause the terminal to respond with A "." which indicates that OS-8 is active.

Under OS-8

1. To load the core image file of FLROT.PA from OS-8, type:
   R FLROT
   The computer should respond with a <bell> "*". When this happens the FLROT program is in control of the computer.

FLROT

1. Whenever the computer responds with a "<bell> *", it means that the program monitor is in control. With the program monitor in control, the user has the following commands available:

   Table 1

   EM: Sets the scan parameters for an emission scan
   EX: Sets the scan parameters for an excitation scan
   SC: Initiates the scan sequence
   XA: Changes the number of units per inch of the x-axis
   PR: Prints the results of the scan
   DI: Displays the results of the scan
   SA: Saves the current table on dsk:
   GE: Gets a named table from DSK:
   TO: Returns to OS-8

   Each of these commands consists of a two letter beginning which is given above and a terminator which is either a ":" or a <CR>. The first two letters of a command must match one of the command above or the computer will respond with a "?".

2. Below is a more detailed description of each of the commands

   EM

   This command allows the user to select scan parameters for an emission scan. The computer asks for the starting wavelength and the excitation wavelength (excitation=).

Table 8 (cont'd)

Each of these values is a decimal integer terminated by a space.

EX

This command allows the user to select the scan parameters for an excitation scan. The computer asks for the same information as in the emission scan except it substitutes "emission=" for "excitation=". The parameters are entered in the same manner.

SC

The scan command (SC:) initiates the scanning sequence. The delimitter to end the command must be depressed at the same time the scan is started. Position 1 is scanned first and when this is finished, the cell is rotated and the computer types "reset monochromator". The user must reset the monochromators to the starting wavelength and depress the space bar at the same time the next scan is started. The computer then scans position two. The same sequence is followed after this position is scanned to scan position 4. When position four has been scanned, the computer types "finished" and automatically smooths all three scans. When the smoothing is finished, the computer returns to the monitor.

XA

The XA command allows the user to change the number of scan units per inch. There are 10 inches across the screen.

PR

The print command allows the user to print out the values of a particular variable from the scan. The list of variables is in Table 2.

Table 2

RR:	P1
SS:	P2
FF:	P4
A1:	P0

Where:

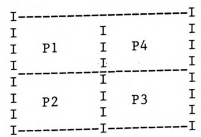


Table 8 (cont'd)

The variable is selected by typing the two letter symbol from the table above and following it with a ";" or <CR>.

DI

The display routine draws a graph of any of the variables from Table 2 from an entire scan.

APPENDIX D

PROGRAM LISTING

On the following pages, a listing of the source files of the system are presented.

```
XLIST                                          PAL8-V10A NO DATE   PAGE 1

                        /FLOURESCENCE PROGRAM              11/19/81
                    /PREFLOURO
                0010 *10
00010           0000 FROM,      0
00011           0000 TO,        0
                0020 *20
00020           0200 USR,       200
00021           6223 MONPTR,    CDFCIF CODFLD
00022           5423            JMP I .+1
00023           0200            200
00024           0605 STRGIN,    PACKER  /ROUTINE TO INPUT FILENAME
00025           7402 ERR,       HLT     /ERROR RETURN
00026           0000 ENTRY,     0       /ENTRY POINT FOR DEVICE HANDLER
```

```
/FLOURESCENCE PROGRAM          11/19/81  PAL8-V10A NO DATE   PAGE 2

                    / INITIALIZATION CODE FOR FLOURESCENCE PROGRAM
                    / 1.)CALLS USR IN
                    / 2.)FETCHES DEVICE HANDLER
                    / 3.)JUMPS TO THE MONITOR IN FIELD 2
                0200 *200
00200           6201 BEGIN,     CDF 0
00201           6212            CIF 10
00202           4623            JMS I USRIN
00203           0010            10
00204           7200            CLA
00205           6201            CDF 0
00206           6212            CIF 10
00207           4420            JMS I USR
00210           0001            1
00211           0423 DEVNUM,    DEVICE DSK
00212           1300
00213           1001 NTRE,      1001
00214           5025            JMP ERR
00215           7200            CLA
00216           1213            TAD NTRE
00217           3026            DCA ENTRY
00220           6223            CDFCIF CODFLD
00221           5622            JMP I .+1
00222           0200            200
00223           7700 USRIN,     7700
```

```
/FLOURESCENCE PROGRAM            11/19/81  PALB-V10A NO DATE   PAGE 4

                0400  *400
                      /TABLE STORAGE ROUTINE
                      /
                4030          WFCW=4030
                0030          RFCW=0030
                      /
                      /     CODE TO SAVE A RUN
                      /     ******************
                      /
00400  1371   SAVRUN, TAD P20
00401  3010          DCA FROM
00402  1371          TAD P20
00403  3370          DCA FROM1
00404  1366          TAD P3000
00405  3011          DCA TO
00406  1367          TAD P30
00407  3336          DCA TO1
00410  1373          TAD PPP12
00411  4330          JMS MOVEBK
00412  1256          TAD FNLNTH
00413  4424          JMS I STRGIN
00414  0600          FILNAM
00415  1261          TAD FNAME
00416  3226          DCA SBLCK
00417  3227          DCA SBLCK+1
00420  1662          TAD I DEV
00421  0260          AND FILINF
00422  6201          CDF 0
00423  6212          CIF 10
00424  4420          JMS I USR
00425  0003          3
00426  0600   SBLCK,  FILNAM
00427  0000          0
00430  5025          JMP ERR
00431  7200          CLA
00432  1226          TAD SBLCK
00433  3240          DCA BLKNUM
00434  6203          CDFCIF 0
00435  4426          JMS I ENTRY
00436  4030   FCW,    WFCW
00437  0000   BUFPTR, 0
00440  0000   BLKNUM, 0
00441  5025          JMP ERR
00442  1261          TAD FNAME
00443  3252          DCA FN
00444  1662          TAD I DEV
00445  0260          AND FILINF
00446  6201          CDF 0
00447  6212          CIF 10
00450  4420          JMS I USR
00451  0004          4
00452  0600   FN,     FILNAM
00453  0020          20
00454  5025          JMP ERR
00455  5021          JMP MONPTR
```

```
/FLOURESCENCE PROGRAM                    11/19/81  PALB-V10A NO DATE   PAGE 4-1

  95   00456  0004  FNLNTH,  4
  96   00457  0600  GFLNAM,  FILNAM
  97   00460  0017  FILINF,  17
  98   00461  0600  FNAME,   FILNAM
  99   00462  0212  DEV,     DEVNUM+1
 100                /
 101                /CODE TO RETRIEVE A TABLE FROM DISK
 102                /
 103   00463  7200  GETRUN,  CLA
 104   00464  1256           TAD FNLNTH
 105   00465  4424           JMS I STRGIN
 106   00466  0600           FILNAM
 107   00467  7200           CLA
 108   00470  1261           TAD FNAME
 109   00471  3301           DCA SBLOCK
 110   00472  3302           DCA SBLOCK+1
 111   00473  1662           TAD I DEV
 112   00474  0260           AND FILINF
 113   00475  6201           CDF 0
 114   00476  6212           CIF 10
 115   00477  4420           JMS I USR
 116   00500  0002           2
 117   00501  0600  SBLOCK,  FILNAM
 118   00502  0000           0
 119   00503  7200           CLA
 120   00504  1301           TAD SBLOCK
 121   00505  3312           DCA BLOCNM
 122   00506  6203           CDFCIF 0
 123   00507  4426           JMS I ENTRY
 124   00510  0030  RRFCW,   RFCW
 125   00511  0000  BUFFPT,  0
 126   00512  0000  BLOCNM,  0
 127   00513  5025           JMP ERR
 128   00514  7200           CLA
 129   00515  1366           TAD P3000
 130   00516  3010           DCA FROM
 131   00517  1367           TAD P30
 132   00520  3370           DCA FROM1
 133   00521  1371           TAD P20
 134   00522  3011           DCA TO
 135   00523  1371           TAD P20
 136   00524  3336           DCA TO1
 137   00525  1373           TAD PPP12
 138   00526  4330           JMS MOVEBK
 139   00527  5021           JMP MONPTR
 140   00530  0000  MOVEBK,  0
 141   00531  7041           CIA
 142   00532  3372           DCA BKLNTH
 143   00533  1370           TAD FROM1
 144   00534  4342           JMS TRIN
 145   00535  4351           JMS TROUT
 146   00536  0000  TO1,     0
 147   00537  2372           ISZ BKLNTH
 148   00540  5333           JMP .-5
 149   00541  5730           JMP I MOVEBK
```

```
/FLOURESCENCE PROGRAM          11/19/81  PAL8-V10A NO DATE   PAGE 4-2

 150   00542   0000  TRIN,     0
 151   00543   1364            TAD CDFCDF
 152   00544   3345            DCA CFLD
 153   00545   0000  CFLD,     0
 154   00546   1410            TAD I FROM
 155   00547   6201            CDF 0
 156   00550   5742            JMP I TRIN
 157   00551   0000  TROUT,    0
 158   00552   3365            DCA SLOC
 159   00553   1751            TAD I TROUT
 160   00554   1364            TAD CDFCDF
 161   00555   3360            DCA .+3
 162   00556   2351            ISZ TROUT
 163   00557   1365            TAD SLOC
 164   00560   0000            0
 165   00561   3411            DCA I TO
 166   00562   6201            CDF 0
 167   00563   5751            JMP I TROUT
 168   00564   6201  CDFCDF,   CDF 0
 169   00565   0000  SLOC,     0
 170   00566   3000  P3000,    3000
 171   00567   0030  P30,      30
 172   00570   0000  FROM1,    0
 173   00571   0020  P20,      20
 174   00572   0000  BKLNTH,   0
 175   00573   0012  PPP12,    12
```

```
/FLOURESCENCE PROGRAM            11/19/81  PAL8-V10A NO DATE   PAGE 5

 176            0600  *600
 177   00600    0000  FILNAM, ZBLOCK 5
 178                  /ROUTINE TO PACK CHARACTERS FOR OUTPUT BY TYPIST
 179                  /CALLING SEQUENCE:
 180                  /       JMS PACKER
 181                  /NOTES:
 182                  /               C(AC)=TWICE THE NUMBER OF CHARACTERS TO BE
INPUTTED
 183                  /               RETURN ADRESS EQUALS A POINTER TO THE STRI
NG LOCATION
 184                  /
 185   00605    0000  PACKER, 0
 186   00606    7041    CIA
 187   00607    3305    DCA NODCHR      /MINUS THE NUMBER OF STORAGE WORDS
 188   00610    1605    TAD I PACKER    /GET STRING ADDRESS
 189   00611    3304    DCA STARTI
 190   00612    2205    ISZ PACKER      /BUMP RETURN ADDRESS
 191   00613    7200    CLA
 192   00614    4710  READIN, JMS I INCHAR /GET NEXT CHARACTER
 193   00615    3300    DCA TEMCHR      /SAVE AT TEMCHR
 194   00616    1300    TAD TEMCHR
 195   00617    1302    TAD CLF
 196   00620    7440    SZA             /IS IT A <LF>?
 197   00621    1303     TAD NEG3
 198   00622    7640    SZA CLA         /IS IT A <CR>?
 199   00623    7410     SKP
 200   00624    5255    JMP SPCCHR      /TREAT SPECIALLY
 201   00625    4710  READ2N, JMS I INCHAR /GET SECOND CHARACTER
 202   00626    3301    DCA TMCHR2      /SAVE
 203   00627    1301    TAD TMCHR2
 204   00630    1302    TAD CLF
 205   00631    7440    SZA             /IS IT A LF?
 206   00632    1303     TAD NEG3
 207   00633    7640    SZA CLA         /IS IT A CR?
 208   00634    7410     SKP
 209   00635    5261    JMP SPCR2       /TREAT SPECIALLY
 210   00636    1301    TAD TMCHR2      /GET SECOND CHARACTER
 211   00637    7417  SHIFTR, LSR       /SHIFT SIGNIFICANT BITS TO MQL
 212   00640    0005    5
 213   00641    7200    CLA
 214   00642    1300    TAD TEMCHR      /GET FIRST CHARACTER
 215   00643    0306    AND KK77         /MASK 6 BITS OUT
 216   00644    7413    SHL             /SHIFT BACK TO AC
 217   00645    0005    5
 218   00646    3704    DCA I STARTI    /STORE IN STRING
 219   00647    2304    ISZ STARTI      /BUMP STRING POINTER
 220   00650    2305    ISZ NODCHR      /DECREMENT #OF WORDS LEFT
 221   00651    5214     JMP READIN     /IF ROOM READ NEXT CHARACTER
 222   00652    7201  ENDSTR, CLA IAC   /STRING FULL
 223   00653    3704    DCA I STARTI    /PUT A ONE AT THE END OF STRING
 224   00654    5605    JMP I PACKER    /RETURN
 225   00655    1300  SPCCHR, TAD TEMCHR /GET CHAR
 226   00656    3301    DCA TMCHR2      /STORE IN SECOND CHAR
 227   00657    3300    DCA TEMCHR      /CLEAR FIRST CHAR
 228   00660    5236    JMP SHIFTR-1    /STORE IN STRING
 229   00661    7300  SPCR2, CLA CLL    /SPEC CHAR LOWER HALF
 230   00662    1300    TAD TEMCHR      /GET UPPER HALF
```

```
/FLOURESCENCE PROGRAM            11/19/81  PALB-V10A NO DATE   PAGE 5-1

 231   00663  0306    AND KK77          /MASK OUT SIGNIFICANT DIGITS
 232   00664  7006    RTL
 233   00665  7006    RTL
 234   00666  7006    RTL
 235   00667  3704    DCA I STARTI      /STORE IN STRING
 236   00670  2304    ISZ STARTI        /BUMP STRING POINTER
 237   00671  1301    TAD TMCHR2        /GET SECOND CHAR
 238   00672  3300    DCA TEMCHR        /PUT IN FIRST CHAR
 239   00673  2305    ISZ NODCHR        /DECREMENT #OF WORDS LEFT
 240   00674  5225     JMP READ2N       /IF ROOM READ NEXT CHAR
 241   00675  1307    TAD CC100         /APPEND STRING ENDER
 242   00676  3704    DCA I STARTI      /STORE IN STRING
 243   00677  5605     JMP I PACKER     /RETURN
 244   00700  0000  TEMCHR, 0
 245   00701  0000  TMCHR2, 0
 246   00702  7766  CLF, -12
 247   00703  7775  NEG3, -3
 248   00704  0000  STARTI, 0
 249   00705  0000  NODCHR, 0
 250   00706  0077  KK77,   77
 251   00707  0100  CC100,  100
 252   00710  0711  INCHAR, CHINPT
 253                /
 254                /CHARACTER INPUT ROUTINE
 255                /
 256   00711  0000  CHINPT, 0
 257   00712  6031         KSF
 258   00713  5312         JMP .-1
 259   00714  6036         KRB
 260   00715  6046         TLS
 261   00716  5711         JMP I CHINPT
```

```
/FLOURESCENCE PROGRAM          11/19/81  PALB-V10A NO DATE   PAGE 6

 262                    /FL11  FLUORO       7/19/73  (AUTO SMOOTHING ADDED)
 263            0002    FIELD 2
 264                    / CODE FIELD DECLARATION:
 265            0020                CODFLD=20
 266            0005    *5
 267   20005    7400    FLIN, 7400  /FLOATING IN
 268   20006    7200    FOUT, 7200  /FLOATING OUT
 269            0007    *7  /THIS MUST BE 7
 270   20007    5600    FLTPT, 5600   /START OF FLOATING POINT INTERPRETTER
 271            0020    *20
 272   20020    0000    EMFLG, 0  /ON EM THIS IS 1
 273   20021    0000    EMFLG2, 0     /SAME AFTER SCAN
 274   20022    0000    START, 0    /STARTING MONOCHROMETER PARAM
 275   20023    0000    START2, 0
 276   20024    0000    END, 0   /ENDING WAVE VALUE
 277   20025    0000    END2, 0
 278   20026    0000    MORX, 0     /FIXED MONOCHROM WAVE VALUE
 279   20027    0000    MORX2, 0
 280   20030    0000    WAVE,   0
 281   20031    0000    NOVAL,  0        /# OF DATA POINTS
 282   20032    0000    WLCH, 0  /TEMP WAVE CHANNEL HOLDER
 283   20033    7773    N5, -5
 284   20034    0000    TEMP1, 0
 285   20035    0000    TEMP2, 0
 286   20036    0000    TEMP3, 0
 287   20037    1453    SPARSE, SPASE  /TYPE TWO SPACES
 288                    *62   /LEAVE HOLE FOR FLOATING POINT
 289   20062    0000    TEMP4, 0
 290   20063    0000    TEMP5, 0
 291   20064    0000    TEMP6, 0
 292   20065    0042    EXCH,   42       /EXCITATION CHANNEL
 293   20066    0043    EMCH,   43       /EMISSION MONOCHROM CHANNEL
 294   20067    0540    ROUND, OFF    /ROUND OFF ROUTINE
 295   20070    1050    A2D, ADAVER   /A-D ROUTINE
 296   20071    4000    P4000, 4000
 297   20072    4722    INPUT, ENPUT    /GENERAL CHAR INPUT
 298   20073    0200    MONIT, MONITR   /POINTER TO MONITOR  (MAY BE CHANGED
BY ABORTS)
 299   20074    0200    MONSAV, MONITR    /PERMANENT POINTER TO MONIT
 300   20075    0004    INTVAL, 4   /WAVE LENGTH OR WAVENUMBER INTERVAL
 301   20076    0004    INTVL2, 4
 302   20077    4000    NUMOUT, DECPRT    /SINGLE PREC DIG OUT.
 303   20100    3340    NUMIN, SINGLE   /SINGLE PRECISION INPUT
 304   20101    4063    TYPIST, TYPSTG    /PACKED CHAR STRING PRINTER
 305   20102    0317    PRINT1, TTOUT     /GENERAL CHAR PRINTER
 306   20103    0342    IITYCR, TYCR    /CR, LF
 307   20104    0347    YORN, YESNO
 308   20105    0600    NULE1, NULE    /WAVE NUMBER TO LAMBDA
 309   20106    0271    TTOINT, KBRK    /INTERRUPT ON ^C
 310   20107    0000    R1, 0
 311   20110    0000    F1, 0
 312   20111    0000    S1, 0
 313   20112    0000    R2,0
 314   20113    0000    F2, 0
 315   20114    0000    S2, 0
 316   20115    7766    N12, -12
```

```
/FLOURESCENCE PROGRAM          11/19/81  PALB-V10A ND DATE   PAGE 6-1

 317   20116  0747  GEXSET, EXSETQ   /"EXCITATION="
 318   20117  1310  GEMSET, EMSETQ
 319   20120  0740  GSTART, STARTQ  /"START="
 320   20121  0744  GEND, ENDQ
 321   20122  1470  SETUP, UPSET    /SETUP FOR OUTPUT
 322   20123  1601  GOTCHA, GOTCH1    /GET RSF FOR OUTPUT
 323   20124  4447  OUTSTQ, STIGMA    /STRING OF OUTPUT OPTIONS
 324   20125  3363  RFLOT, RFL
 325   20126  3360  SFLOT, SFL
 326   20127  3366  FFLOT, FFL
 327   20130  3560  BUF1, BUFF1
 328   20131  0756  ONEFLT, FLUTE1    /FLOATING POINT 1
 329   20132  3566  BUF3, BUFF3
 330   20133  0004  P4, 4
 331   20134  0213  ENTURP, INTPRT   /INTERPRETTER FOR COMMAND OPTIONS
 332          0057  NLAT=57     /LAST CHAR ON FLOATING NUMERICAL INPUT
 333   20135  7526  NASTY, -252
 334   20136  0012  P12, 12
 335   20137  3522  YUNORM, UNORM   /UNNORMALIZE
 336   20140  3322  NORM, NORMAL    /NORMALIZE
 337   20141  5200  DRPLOT, PLOTX    /PLOTTERDRIVER
 338   20142  4330  NEGA1, NEG11   /ROUTINE TO CIA FOR NU AND NOP FOR LE
  (WAVELENGTH=LE)
 339   20143  4333  LINCOM, LINKER  /" TO CML FOR NU AND NOP FOR LE (WAV
ENUMBER=NU)
 340   20144  0334  TYOM, GMOM  /TYPE ?
 341   20145  3563  BUF2, BUFF2
 342   20146  0210  P310, 310   /200D
 343   20147  4460  DIAG, DINOQ    /PRINT MESSAGES CONCERNING BACKGROUND,
 ART, PM, ETC.
 344   20150  0207  BELL, 207
 345   20151  1317  USEREG, REGUSE   /ROUTINE TO OUTPUT A CONTROL US TO
RETURN TO TEXT MODE.
 346   20152  3627  XOFF1, XOFF   /OFFSET BUFFER
 347   20153  2407  LOGT, LOGTT  /LOG(R/S)
 348   20154  0001  SMFLG, 1   /AUTO SMOOTHING FLAG
 349   20155  5042  SMITCH, SMUST2
 350   20156  0000  FTEMP0, 0; 0; 0
 351   20157  0000
 352   20160  0000
 353   20161  0000  FTEMP1, 0; 0; 0
 354   20162  0000
 355   20163  0000
```

```
/FLOURESCENCE PROGRAM                    11/19/81  PAL8-V10A NO DATE   PAGE 7

  356             0200  *200
  357    20200    4551  MONITR, JMS I USEREQ
  358    20201    4342    JMS TYCR    /PRINT CR, LF
  359    20202    1150    TAD BELL
  360    20203    4317    JMS TTOUT
  361    20204    1311    TAD ASTR
  362    20205    4317    JMS TTOUT   /PRINT *
  363    20206    1212    TAD CMNDS   /POINTER TO LIST OF COMMAND OPTIONS
  364    20207    4213    JMS INTPRT  /GO TO THE INTERPRETTER
  365    20210    0000    0   /THIS LOC WILL HAVE THE COMMAND FOUND BY THE I
NTERPRETTER
  366    20211    5610    JMP I .-1
  367    20212    4400  CMNDS, CMDSTQ     /POINTER TO LIST OF OPTIONS (THIS M
UST BE SET BY USER)
  368                    /
  369    20213    0000  INTPRT, 0      /INTERPRETTER, INPUT AND PROCESS A COM
MAND
  370    20214    3304    DCA LOCLOC    /ETER WITH POINTER TO OPTION STRING
IN AC
  371    20215    3307    DCA CHRPR   /CLEAR COMMAND WORD FOR PREMATURE DELI
MITER
  372    20216    4257    JMS CHARIN    /GET THE FIRST COMMAND CHAR
  373    20217    0310    AND P77
  374    20220    7106    CLL RTL     /MOVE TO LEFT HALF
  375    20221    7006    RTL
  376    20222    7006    RTL
  377    20223    3307    DCA CHRPR
  378    20224    4257    JMS CHARIN    /GET SECOND CHAR
  379    20225    0310    AND P77
  380    20226    1307    TAD CHRPR
  381    20227    3307    DCA CHRPR
  382    20230    4257    JMS CHARIN
  383    20231    5230    JMP .-1     /KEEP READING AND ECHOING UNTIL DELIMIT
ER.   CR OR :
  384    20232    4437    JMS I SPARSE  /TYPE A FEW SPACES AFTER *
  385    20233    1304  DELIMR, TAD LOCLOC   /A DELIMITER WAS FOUND
  386    20234    3305    DCA TEMPRM
  387    20235    1705  CHECKI, TAD I TEMPRM
  388    20236    7440    SZA       /GET A COMMAND WORD FROM THE OPTION STRIN
G
  389    20237    5243      JMP .+4
  390    20240    4334  ERRERR, JMS GMGM    /END OF COMMAND OPTION STRING SI
GNALED BY A 0
  391    20241    4342    JMS TYCR    /PRINT ? CR LF
  392    20242    5215    JMP INTPRT+2    /AND TRY AGAIN
  393    20243    7041     CIA      /MAKE COMMAND NEGATIVE
  394    20244    1307    TAD CHRPR     /AND SEE IF IT EQUALS ENTERED COMMAND

  395    20245    7650    SNA CLA
  396    20246    5252     JMP .+4
  397    20247    2305    ISZ TEMPRM  /THESE COMMANDS DON'T MATCH SO MOVE TO
NEXT COMMAND
  398    20250    2305    ISZ TEMPRM
  399    20251    5235    JMP CHECKI
  400    20252    2305    ISZ TEMPRM    /FOUND CORRECT COMMAND WORD
  401    20253    1705    TAD I TEMPRM    /GET ASSOCIATED COMMAND
  402    20254    3613    DCA I INTPRT  /PLACE COMMAND AT RETURN ADDRESS
  403    20255    2213    ISZ INTPRT    /INCREMENT RETURN ADDRESS
  404    20256    5613    JMP I INTPRT    /RETURN
  405    20257    0000  CHARIN, 0   /READ AND ECHO CHAR, LOOK FOR CR OR : AS
DELIMITERS
  406    20260    4777'   JMS ENPUT     /INPUT A CHAR AND TYPE IT
  407    20261    1314    TAD N272    /IS IT A COLON
  408    20262    7450    SNA
  409    20263    5232    JMP DELIMR-1    /YES
  410    20264    1315    TAD P55   /NO, IS IT A CR
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/FLOURESCENCE PROGRAM          11/19/81  PAL8-V10A NO DATE   PAGE 7-1

  411    20265  7650     SNA CLA
  412    20266  5233      JMP DELIMR
  413    20267  1306     TAD FCHAR    /NOT DELIMITER, RETURN WITH CHAR JUST
INPUT
  414    20270  5657      JMP I CHARIN
  415    20271  0000     KBRK, 0    /INPUT AND INTERRUPT SERVICER
  416    20272  6031      KSF
  417    20273  5671      JMP I KBRK    /NO INPUT
  418    20274  6036      KRB
  419    20275  3306      DCA FCHAR
  420    20276  1306      TAD FCHAR
  421    20277  1316      TAD CNTRLC   /-CODE FOR ^C
  422    20300  7650      SNA CLA
  423    20301  5473      JMP I MONIT
  424    20302  1306      TAD FCHAR    /GET THE CHAR AND RETURN
  425    20303  5671      JMP I KBRK
  426    20304  0000     LOCLOC, 0
  427    20305  0000     TEMPRM, 0
  428    20306  0000     FCHAR, 0
  429    20307  0000     CHRPR, 0
  430    20310  0077      P77,77
  431    20311  0252     ASTR, 252  /*
  432    20312  0215     CR215, 215  /CR
  433    20313  0212     LF212, 212  /LF
  434    20314  7506     N272, -272  /-COLON
  435    20315  0055     P55, 55  /COLON-CR
  436    20316  7575     CNTRLC, -203    /-CODE FOR ^C
  437    20317  0000     TTOUT, 0  /SUBROUTINTE TO PRINT A CHAR
  438    20320  6046      TLS
  439    20321  6041      TSF
  440    20322  5321      JMP .-1
  441    20323  7041      CIA  /CHECK FOR CR
  442    20324  1312      TAD CR215
  443    20325  7640      SZA CLA
  444    20326  5331      JMP .+3
  445    20327  1313      TAD LF212
  446    20330  5320      JMP TTOUT+1
  447    20331  4271      JMS KBRK  /LOOK FOR ABORT
  448    20332  7200      CLA
  449    20333  5717      JMP I TTOUT
  450    20334  0000     GMGM, 0
  451    20335  4342      JMS TYCR
  452    20336  1341      TAD QM    /PRINT A ?
  453    20337  4317      JMS TTOUT
  454    20340  5734      JMP I GMGM
  455    20341  0277     QM, 277
  456    20342  0000     TYCR, 0
  457    20343  7200      CLA    /TYPE CR, LF
  458    20344  1312      TAD CR215
  459    20345  4317      JMS TTOUT
  460    20346  5742      JMP I TYCR
  461    20347  0000     YESNO, 0   /YES OR NO ROUTINE:  NO=RETURN, YES=RETUR
N+1
  462    20350  7201      CLA IAC
  463    20351  1347      TAD YESNO
  464    20352  3363      DCA YUP
  465    20353  1347      TAD YESNO



/FLOURESCENCE PROGRAM          11/19/81  PAL8-V10A NO DATE   PAGE 7-2

  466    20354  3365      DCA NOPE
  467    20355  1361      TAD YESTRG
  468    20356  4213      JMS INTPRT
  469    20357  0000      0
  470    20360  5757      JMP I .-1
  471    20361  0362     YESTRG, .+1
  472    20362  3105       3105  /YES
  473    20363  0000     YUP, 0
  474    20364  1617       1617  /NO
  475    20365  0000     NOPE, 0
  476    20366  0000      0   /STRING ENDER
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/FLOURESCENCE PROGRAM                11/19/81  PALB-V10A NO DATE   PAGE 8

 477   20377  4722
 478          0400  *400
 479   20400  1264  CALIB, TAD Q200     /SET UP MONOCHROMETER CALIBRATION

 480   20401  4501    JMS I TYPIST    /PRINT "SET TO 200"
 481   20402  4472    JMS I INPUT
 482   20403  7300    CLA CLL         /WAIT FOR TTO RESPONSE AND THEN DISC
ARD INPUT
 483   20404  1065    TAD EXCH    /EXCITATION CHANNEL
 484   20405  4234    JMS CAD     /INPUT D1 RELATING TO LE1  (200NM)
 485   20406  3266    DCA EXD1
 486   20407  1066    TAD EMCH  /EMISSION CHANNEL
 487   20410  4234    JMS CAD
 488   20411  3270    DCA EMD1
 489   20412  4503    JMS I IITYCR
 490   20413  1265    TAD Q650   /SET TO 650
 491   20414  4501    JMS I TYPIST    /PRINT
 492   20415  4472    JMS I INPUT
 493   20416  7300    CLA CLL
 494   20417  1065    TAD EXCH
 495   20420  4234    JMS CAD    /GET D2 FOR LE2
 496   20421  7041    CIA
 497   20422  1266    TAD EXD1   /D2-D1
 498   20423  7041     CIA
 499   20424  3267    DCA EXD2D1
 500   20425  1066    TAD EMCH
 501   20426  4234    JMS CAD
 502   20427  7041    CIA
 503   20430  1270    TAD EMD1
 504   20431  7041     CIA
 505   20432  3271    DCA EMD2D1
 506   20433  5473    JMP I MONIT    /DONE
 507   20434  0000  CAD, 0   /A-D INPUT AND AVERAGE
 508   20435  7000    NOP /*****  ADSC  /SET CHANNEL FROM AC
 509   20436  7300    CLA CLL
 510   20437  3034    DCA TEMP1
 511   20440  3035    DCA TEMP2   /CLEAR LOW AND HIGH SUMMATION REGISTER
S
 512   20441  1033    TAD N5
 513   20442  3036    DCA TEMP3
 514   20443  7000    NOP /*****  ADSF   /WAIT FOR CHANNEL CHANGE
 515   20444  7000    NOP /*****  JMP  -1
 516   20445  4470    JMS I A2D   /GET A TIME AVERAGED A-D VALUE
 517   20446  7100    CLL
 518   20447  1035    TAD TEMP2   /LEAST SIG
 519   20450  3035    DCA TEMP2
 520   20451  7430    SZL
 521   20452  2034    ISZ TEMP1   /CARRY TO MOST SIG
 522   20453  2036    ISZ TEMP3  /COUT
 523   20454  5245    JMP  -7
 524   20455  1035    TAD TEMP2  /GET ANSWER
 525   20456  7421    MQL
 526   20457  1034    TAD TEMP1
 527   20460  7407    DVI
 528   20461  0005    5
 529   20462  4467    JMS I ROUND    /ROUND AND GET ANSWER
 530   20463  5634    JMP I CAD
 531   20464  0596  Q200, Q0200
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/FLOURESCENCE PROGRAM            11/19/81  PAL8-V10A NO DATE   PAGE 8-1

  532   20465  0564  G650, GG650
  533   20466  1742  EXD1, 1742
  534   20467  3616  EXD2D1, 3616      /CALIBRATION CONSTANTS
  535   20470  2553  EMD1, 2553
  536   20471  3731  EMD2D1, 3731
  537   20472  0000  LE2DIG, 0   /CHANGE WAVELENGTH TO CORRESPONDING DIGI
TAL NUMBER FOR
  538   20473  7425    MQL MUY   /COMPARISON WITH A-D MONOCHROMETER CHANN
EL
  539   20474  0000  D2D1, 0     /SET BY EM OR EX ROUTINES
  540   20475  7407    DVI
  541   20476  7020    7020    /(650-200)*8
  542   20477  4467  JMS I ROUND
  543   20500  1302  TAD D1
  544   20501  5672    JMP I LE2DIG      /RETURN WITH ANSWER AFTER ADDING
INTECEPT
  545   20502  0000  D1, 0   /A-D VALUE CORRESPONDING TO 200NM WHICH IS 0
PSEUDO WAVELENGTH
  546   20503  7201  EM, CLA IAC  /SET FOR EMISSION
  547   20504  3020    DCA EMFLG   /SET EMSISSION FLAT
  548   20505  1271    TAD EMD2D1    /SET UP CALIBRATION CONSTANTS
  549   20506  3274    DCA D2D1
  550   20507  1270    TAD EMD1
  551   20510  3302    DCA D1
  552   20511  1116    TAD GEXSET    /"EXCITATION="
  553   20512  3064  EITHER, DCA TEMP6
  554   20513  1120    TAD GSTART    /START=
  555   20514  4501    JMS I TYPIST
  556   20515  4737    JMS I PARM1   /NU OR LE INPUT ROUTINE
  557   20516  3022    DCA START     /SET STARTING WAVE PARAMETER
  558   20517  1121    TAD GEND    /ASK   "END="
  559   20520  4501    JMS I TYPIST
  560   20521  4737    JMS I PARM1
  561   20522  3024    DCA END
  562   20523  1064    TAD TEMP6
  563   20524  4501    JMS I TYPIST    /ASK FOR WAVE VALUE OF FIXED MONO
  564   20525  4737    JMS I PARM1
  565   20526  3026    DCA MORX
  566   20527  5473    JMP I MONIT   /DONE
  567   20530  3020  EX, DCA EMFLG   /CLEAR EM FLAG TO SET FOR EMISSION
  568   20531  1267    TAD EXD2D1    /SET UP MONCHROM CALIBRATION CONSTANT
S
  569   20532  3274    DCA D2D1
  570   20533  1266    TAD EXD1
  571   20534  3302    DCA D1
  572   20535  1117    TAD GEMSET  /ASK "EMISSION="
  573   20536  5312    JMP EITHER   /OR NUIN   WAVE LENGTH OR NUMBER INPUT
ROUTINE
  574   20537  0624  PARM1, LEIN   /ROUND OFF DIVISION ROUTINE  (JMS RIGHT AFT
ER DIVISOR)
  575   20540  0000  OFF, 0   /ROUND OFF DIVISION ROUTINE
  576   20541  3355    DCA REMAIN  /SAVE REMAINDER
  577   20542  7344    CLA CLL CMA RAL   /-2
  578   20543  1340    TAD OFF
  579   20544  3372    DCA REMAN2    /POINTS TO DIVISOR
  580   20545  1772    TAD I REMAN2
  581   20546  7110    CLL RAR   /DIVISOR BY 2
  582   20547  7141    CIA CLL
  583   20550  1355    TAD REMAIN
  584   20551  7701    CLA MQA
  585   20552  7430    SZL
  586   20553  7001    IAC   /ROUND OFF
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587   20554  5740    JMP I OFF
588   20555  0000  REMAIN, 0
589   20556  2305  GG200, TEXT "SET TO 200"
590   20557  2440
591   20560  2417
592   20561  4062
593   20562  6060
594   20563  0000
595          0563    * -1
596   20563  0001    1  /STRING ENDER
597   20564  2305  GG650, TEXT "SET TO 650"
598   20565  2440
599   20566  2417
600   20567  4066
601   20570  6560
602   20571  0000
603          0571    * -1
604   20571  0001    1
605   20572  0000  REMAN2, 0
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/FLOURESCENCE PROGRAM                    11/19/81  PAL8-V10A NO DATE   PAGE 9

 606                0600  *600
 607   20600  0000  NULE, 0   /CHANGE WAVENUBER IN AC TO WAVELENGTH
 608   20601  3220    DCA NULE2
 609   20602  1220    TAD NULE2
 610   20603  7425    MQL MUY
 611   20604  3100    3100   /1600 IN OCTAL (8*200)
 612   20605  3034    DCA TEMP1      /WAVELENGTH IS 8*(LE-200)   WITH LE
IN NANOMETERS
 613   20606  7501    MQA   /WAVE NUMBER IS 8*NU WITH NU IN 100 INVERSE
CM
 614   20607  7141    CIA CLL     /DOUBLE PRECISION SUBTRACT
 615   20610  1071    TAD P4000   /LEAST SIG PART OF 64*10E5
 616   20611  7421    MQL   /FORMALA IS 64*10E5*NUMBER-8*200*NUMBER  ALL
DIVIDED BY NUMBER
 617   20612  1034    TAD TEMP1
 618   20613  7040    CMA
 619   20614  7430    SZL
 620   20615  7001    IAC
 621   20616  1223    TAD P3032   / MOST SIG PART OF 64E5
 622   20617  7407    DVI
 623   20620  0000  NULE2, 0
 624   20621  4467    JMS I ROUND   /ROUND OFF AND GET ANSWER
 625   20622  5600    JMP I NULE
 626   20623  3032  P3032, 3032
 627   20624  0000  LEIN, 0  /INPUT WAVELENGTH AND SEE IF INBOUNDS
 628   20625  4500    JMS I NUMIN   /INPUT AN INTEGER WAVELENGTH IN NM
 629   20626  1265    TAD N310   /SUBTRACT 200  /LOWER BOUNDS
 630   20627  7510    SPA
 631   20630  5242    JMP LERR  /ERROR
 632   20631  3244    DCA NUIN
 633   20632  1244    TAD NUIN
 634   20633  1266    TAD N702    /-450 TO MAKE UPPER LIMINT 650
 635   20634  7740    SMA CLA SZA
 636   20635  5242    JMP LERR
 637   20636  1244    TAD NUIN  /OK
 638   20637  7106    CLL RTL  /SO MULT BY 8
 639   20640  7004    RAL
 640   20641  5624    JMP I LEIN
 641   20642  4544  LERR, JMS I TYGM  /ERROR
 642   20643  5225    JMP LEIN+1   /TYRY AGAIN
 643   20644  0000  NUIN, 0  /INPUT WAVE NUMBER
 644   20645  4500    JMS I NUMIN
 645   20646  3224    DCA LEIN
 646   20647  1224    TAD LEIN
 647   20650  1267    TAD N232  /-154 LOWER LIMIT
 648   20651  7710    SPA CLA
 649   20652  5263    JMP NERR  /TOO SMALL
 650   20653  1224    TAD LEIN
 651   20654  1270    TAD N764   /-500 UPPER LIMIT
 652   20655  7740    SMA SZA CLA
 653   20656  5263    JMP NERR
 654   20657  1224    TAD LEIN  /OK
 655   20660  7106    CLL RTL
 656   20661  7004    RAL  /SCALE UP BY 8
 657   20662  5644    JMP I NUIN
 658   20663  4544  NERR, JMS I TYGM  /ERROR
 659   20664  5245    JMP NUIN+1 /TRY AGAIN
 660   20665  7470  N310, -310  /200
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/FLOURESCENCE PROGRAM              11/19/81  PAL8-V10A NO DATE   PAGE 9-1

 661   20666  7076  N702, -702   /-450
 662   20667  7546  N232, -232  /-154
 663   20670  7014  N764, -764   /500
 664   20671  1120  OUTRNG, TAD GSTART   /SET NEW OUTPUT LIMITS
 665   20672  4501    JMS I TYPIST  /ASK "START="
 666   20673  4737    JMS I PARM2  /LEIN OR NUIN
 667   20674  3034    DCA TEMP1
 668   20675  1034    TAD TEMP1
 669   20676  7141    CIA CLL
 670   20677  1022    TAD START
 671   20700  7450    SNA
 672   20701  5305  ZO1, JMP OK11   /SNL CLA FOR NU
 673   20702  4543    JMS I LINCOM
 674   20703  7630  ZO2, SZL CLA /SNL CLA FOR NU
 675   20704  5271    JMP OUTRNG  /ERROR   ASK AGAIN
 676   20705  1034  OK11, TAD TEMP1
 677   20706  3023    DCA START2
 678   20707  1121    TAD GEND    /"END="
 679   20710  4501    JMS I TYPIST
 680   20711  4737    JMS I PARM2
 681   20712  3034    DCA TEMP1
 682   20713  1034    TAD TEMP1
 683   20714  7141    CIA CLL
 684   20715  1024    TAD END
 685   20716  7450    SNA
 686   20717  5323    JMP OK12
 687   20720  4543    JMS I LINCOM
 688   20721  7620  ZO3, SNL CLA  /OF SZL CLA FOR NU
 689   20722  5307    JMP OK11+2   /ERROR  ASK AGAIN
 690   20723  1034  OK12, TAD TEMP1 /OK
 691   20724  3025    DCA END2
 692   20725  1021    TAD EMFLG2
 693   20726  7650    SNA CLA
 694   20727  5332    JMP .+3
 695   20730  1116    TAD GEXSET   /TYPE "EXCITATION="
 696   20731  7410    SKP
 697   20732  1117    TAD GEMSET
 698   20733  4501    JMS I TYPIST   /ASK FOR NEW VALUE OF FIXED MONOCHR
OM
 699   20734  4737     JMS I PARM2
 700   20735  3027    DCA MORX2
 701   20736  5473    JMP I MONIT
 702   20737  0624  PARM2, LEIN  /OR NUIN
 703   20740  2324  STARTG, TEXT "START="
 704   20741  0122
 705   20742  2475
 706   20743  0000
 707          0743    *.-1
 708   20743  0001    1
 709   20744  0516  ENDG, TEXT "END="
 710   20745  0475
 711   20746  0000
 712          0746    *.-1
 713   20746  0001    1
 714   20747  0530  EXSETG, TEXT "EXCITATION= "
 715   20750  0311
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/FLOURESCENCE PROGRAM              11/19/81  PAL8-V10A NO DATE   PAGE 9-2

 716   20751  2401
 717   20752  2411
 718   20753  1716
 719   20754  7540
 720   20755  0000
 721          0755    *.-1
 722   20755  0001    1
 723   20756  0001  FLUTE1, 1   /FLOATING POINT:    1
 724   20757  2000      2000
 725   20760  0000    0
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/FLOURESCENCE PROGRAM                11/19/81  PAL8-V10A ND DATE   PAGE 10

 726                    1000  *1000
 727   21000  7240  SCAN,  CLA CMA  /SCAN AND COLLECT DATA
 728   21001  3010         DCA 10
 729   21002  3031         DCA NDVAL  /DATA POINT COUNTER
 730   21003  1022         TAD START  /MOVE SCAN PARAMETERS TO PERMANENT STOR
AGE
 731   21004  3023         DCA START2
 732   21005  1024         TAD END
 733   21006  3025         DCA END2
 734   21007  1075         TAD INTVAL
 735   21010  3076         DCA INTVL2    /MOVE ALL PARAMETERS TO STORAGE SO L
ATER CHANGES WILL
 736   21011  1026         TAD MORX    /NOT FOUL UP OUTPUT
 737   21012  3027         DCA MORX2
 738   21013  1020         TAD EMFLG
 739   21014  3021         DCA EMFLG2
 740   21015  1022         TAD START
 741   21016  3030         DCA WAVE
 742   21017  1155         TAD SMITCH    /SET RETURN ADDRES FOR AUTO SMOOTH
 743   21020  3073         DCA MONIT
 744   21021  1020         TAD EMFLG
 745   21022  7650         SNA CLA
 746   21023  5226         JMP  +3
 747   21024  1066         TAD EMCH  /EMISSION CHANNEL
 748   21025  7410         SKP
 749   21026  1065         TAD EXCH
 750   21027  3032         DCA WLCH  /SET MONCHROM CHANNEL
 751   21030  4720  SCAN2, JMS I DATAIN  /BIG ENOUGH, TAD DATA
 752   21031  1030         TAD WAVE
 753   21032  7141         CIA CLL
 754   21033  1024         TAD END
 755   21034  7450         SNA
 756   21035  5646         JMP I FNSCN  /FINISHED
 757   21036  4543         JMS I LINCOM
 758   21037  7620  Z04, SNL CLA  /OR SZL CLA
 759   21040  5646         JMP I FNSCN  /DONE
 760   21041  1075         TAD INTVAL
 761   21042  4542         JMS I NEGA1    /EITHE NOP FOR LE OR CIA FOR NU
 762   21043  1030         TAD WAVE
 763   21044  3030         DCA WAVE
 764   21045  5230         JMP SCAN2   /REPEAT
 765   21046  1124  FNSCN,  DONEO
 766   21047  1200  GFIN,  GFING   /FINISHED
 767   21050  0000  ADAVER, 0   /A TO D ROUTINE
 768   21051  7300         CLA CLL
 769   21052  1303         TAD PP4
 770   21053  7041         CIA
 771   21054  3017         DCA 17
 772   21055  3062         DCA TEMP4
 773   21056  3063         DCA TEMP5
 774   21057  1321  KLOCK,  TAD DELTIM   /SET CLOCK FOR TIME INTERVAL
 775   21060  3177         DCA 177
 776   21061  6532         ADCV
 777   21062  6531         ADSF
 778   21063  5262         JMP .-1
 779   21064  7200         CLA
 780   21065  6534         ADRB
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/FLOURESCENCE PROGRAM 11/19/81 PAL8-V10A NO DATE PAGE 10-1

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 781  21066  7100          CLL
 782  21067  1062          TAD TEMP4
 783  21070  3062          DCA TEMP4
 784  21071  7430          SZL
 785  21072  2063          ISZ TEMP5  /CARRY TO HIGH PRECISION
 786  21073  2177          ISZ 177      /TIMING LOOP
 787  21074  5273          JMP .-1  /WAIT ON CLOCK
 788  21075  2017          ISZ 17
 789  21076  5257          JMP KLOCK
 790  21077  1062          TAD TEMP4
 791  21100  7421          MQL
 792  21101  1063          TAD TEMP5
 793  21102  7407          DVI   /AVERAGE
 794  21103  0004  PP4, 4
 795  21104  4467          JMS I ROUND
 796  21105  7001          IAC
 797  21106  7440          SZA
 798  21107  5315          JMP .+6    /NOT SATURATED
 799  21110  1150          TAD BELL   /UH OH, SATURATED, SOUND ALARM
 800  21111  6041          TSF
 801  21112  7410          SKP
 802  21113  6046          TLS
 803  21114  7200          CLA
 804  21115  7041          CIA   /RESUBTRACT 1
 805  21116  7140          CMA CLL
 806  21117  5650          JMP I ADAVER
 807  21120  1230  DATAIN, INDATA
 808  21121  4647  DELTIM, 4647    /2NM/SEC ON THE PERKIN ELMER
 809  21122  0472  LEDIQ1, LE2D1Q
 810  21123  1124          DONE0
 811  21124  7201  DONE0,  CLA IAC
 812  21125  4777'         JMS TURN
 813  21126  4503          JMS I IITYCR
 814  21127  1371          TAD GRESET
 815  21130  4501          JMS I TYPIST
 816  21131  4472          JMS I INPUT
 817  21132  7200          CLA
 818  21133  3010          DCA IO
 819  21134  3031          DCA NOVAL
 820  21135  1022          TAD START
 821  21136  3030          DCA WAVE
 822  21137  1342          TAD DONE1-1
 823  21140  3246          DCA FNSCN
 824  21141  5230          JMP SCAN2
 825  21142  1143          DONE1
 826  21143  7325  DONE1,  CLA CLL IAC CML RAL
 827  21144  4777'         JMS TURN
 828  21145  4503          JMS I IITYCR
 829  21146  1371          TAD GRESET
 830  21147  4501          JMS I TYPIST
 831  21150  4472          JMS I INPUT
 832  21151  7201          CLA IAC
 833  21152  3010          DCA IO
 834  21153  3031          DCA NOVAL
 835  21154  1022          TAD START
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/FLOURESCENCE PROGRAM 11/19/81 PAL8-V10A NO DATE PAGE 10-2

```
 836  21155  3030          DCA WAVE
 837  21156  1361          TAD DONE3-1
 838  21157  3246          DCA FNSCN
 839  21160  5230          JMP SCAN2
 840  21161  1162          DONE3
 841  21162  7200  DONE3,  CLA
 842  21163  4777'         JMS TURN
 843  21164  1323          TAD DONE0-1
 844  21165  3246          DCA FNSCN
 845  21166  1247          TAD QFIN
 846  21167  4501          JMS I TYPIST
 847  21170  5473          JMP I MONIT
 848  21171  1207  GRESET, GGRSET
```

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/FLOURESCENCE PROGRAM            11/19/81  PALB-V10A NO DATE   PAGE 11

 849   21177  1222
 850          1200  *1200
 851   21200  0015  GFING,   15
 852   21201  0012          12
 853   21202  0611          TEXT "FINISHED"
 854   21203  1611
 855   21204  2310
 856   21205  0504
 857   21206  0000
 858          1206          *.-1
 859   21206  0001          1
 860   21207  2205  GQRSET,  TEXT "RESET MONOCHROMATORS"
 861   21210  2305
 862   21211  2440
 863   21212  1517
 864   21213  1617
 865   21214  0310
 866   21215  2217
 867   21216  1501
 868   21217  2417
 869   21220  2223
 870   21221  0000
 871          1221          *.-1
 872   21221  0001          1
 873   21222  0000  TURN,    0
 874   21223  6213          CDFCIF 10
 875   21224  4627          JMS I ROCEL
 876   21225  7200          CLA
 877   21226  5622          JMP I TURN
 878   21227  3067  ROCEL,   ROCELL
 879   21230  0000  INDATA,  0  /DAT COLLECTION SUBROUTINE
 880   21231  7326    CLA CLL CML RTL        /SET MULTIPLEXER CHANNEL
 881   21232  6533    ADSC
 882   21233  7200    CLA
 883   21234  1115    TAD N12   /AVERAGE 10 OF THEM
 884   21235  3013    DCA I3
 885   21236  3111    DCA S1  /CLEAR SUMMATION REG
 886   21237  3114    DCA S2
 887   21240  4470  SMPWT,  JMS I A2D
 888   21241  7100    CLL   /SUM UP
 889   21242  1111    TAD S1
 890   21243  3111    DCA S1
 891   21244  7430    SZL
 892   21245  2114    ISZ S2
 893   21246  2013    ISZ I3  /COUNT DATA POINTS
 894   21247  5240    JMP SMPWT
 895   21250  1111    TAD S1
 896   21251  7421    MQL   /NOW AVERAGE
 897   21252  1114    TAD S2
 898   21253  4270    JMS SAVDR
 899   21254  2010    ISZ 10
 900   21255  2010    ISZ 10
 901   21256  2031    ISZ NOVAL  /COUNT NUMBER OF COMPOSITE POINTS
 902   21257  1010    TAD 10
 903   21260  1266    TAD N2775   /LIMIT OF DATA STORAGE
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/FLOURESCENCE PROGRAM              11/19/81  PAL8-V10A NO DATE   PAGE 11-1

 904   21261  7710    SPA CLA
 905   21262  5630    JMP I INDATA
 906   21263  1267    TAD GFULL   /DATA TABLE FULL
 907   21264  4501    JMS I TYPIST
 908   21265  5473    JMP I MONIT
 909   21266  5003  N2775, -2775
 910   21267  1300  GFULL, GGFUL
 911   21270  0000  SAVGR, 0
 912   21271  7407    DVI
 913   21272  0012    12
 914   21273  4467    JMS I ROUND
 915   21274  6231    CDF 30   /AVERAGE AND STORE
 916   21275  3410    DCA I 10
 917   21276  6221    CDF CDDFLD
 918   21277  5670    JMP I SAVOR
 919   21300  0015  GGFUL, 15
 920   21301  0012  12
 921   21302  2401    TEXT "TABLE FULL"
 922   21303  0214
 923   21304  0540
 924   21305  0625
 925   21306  1414
 926   21307  0000
 927          1307    *.-1
 928   21307  0001    1
 929   21310  0515  EMSETQ, TEXT "EMISSION= "
 930   21311  1123
 931   21312  2311
 932   21313  1716
 933   21314  7540
 934   21315  0000
 935          1315    *.-1
 936   21315  0001    1
 937   21316  0237    237  /CODE FOR CONTROL US
 938   21317  0000    REGUSE, 0
 939   21320  7200    CLA
 940   21321  1316    TAD .-3
 941   21322  4502    JMS I PRINT1
 942   21323  5717    JMP I REGUSE
 943          0055  *55
 944   20055  0000  0     /SET SO NO CR ON OUTPUT
 945   20056  0000  0
 946          7345  *7345
 947   27345  4502  JMS I PRINT1  /CHANGE FLOATING POINT OUTPUT
 948   27346  7000    NOP
 949   27347  7000    NOP
 950          7170  *7170
 951   27170  7441  -337   /TO CHANGE RUBOUT TO BACKARROW FOR CONSISTENC
Y
 952   27171  0122  337-215
 953          7144  *7144
 954   27144  4472    JMS I INPUT
 955   27145  7000    NOP
 956   27146  7000    NOP   /OVERLAY OF FLOATING INPUT
 957          7150  *7150
 958   27150  7200    CLA



/FLOURESCENCE PROGRAM              11/19/81  PAL8-V10A NO DATE   PAGE 11-2

 959   27151  7000    NOP   /INPUT ALREADY HAS BEEN ECHOED
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/FLOURESCENCE PROGRAM            11/19/81  PALB-V10A NO DATE   PAGE 12

 960          1400  *1400
 961    21400 4263  PRINT, JMS GOODIE   /GET THE OUTPUT PARAM
 962    21401 4503  PRINTU, JMS I IITYCR
 963    21402 4547    JMS I DIAG
 964    21403 5232    JMP PRIN3-3   /JUMP PAST THIS ISLAND.
 965    21404 0000  ADVIS, 0
 966    21405 1120    TAD GSTART   /PRINT ASSOCIATED DATA
 967    21406 4501    JMS I TYPIST
 968    21407 1023    TAD START2
 969    21410 4322    JMS UNDO
 970          0077  PRTDEC=NUMOUT
 971    21411 4253    JMS SPASE  /TYPE SOME SPACES
 972    21412 1121    TAD GEND
 973    21413 4501    JMS I TYPIST
 974    21414 1025    TAD END2
 975    21415 4322    JMS UNDO
 976    21416 4253    JMS SPASE
 977    21417 1021    TAD EMFLG2
 978    21420 7650    SNA CLA
 979    21421 5224    JMP .+3
 980    21422 1116    TAD GEXSET  /EM SCAN
 981    21423 7410    SKP
 982    21424 1117    TAD GEMSET  /EX SCAN
 983    21425 4501    JMS I TYPIST
 984    21426 1027    TAD MORX2
 985    21427 4322    JMS UNDO
 986    21430 3010    DCA 10  /CLEAR LINE COUNTER
 987    21431 5604    JMP I ADVIS
 988    21432 4522    JMS I SETUP    /SETUP R,S,F POINTERS
 989    21433 4523    JMS I GOTCHA    /GET FIRST RSF VALUES
 990    21434 5240    JMP .+4
 991    21435 4523  PRIN3, JMS I GOTCHA
 992    21436 2016    ISZ 16
 993    21437 5247    JMP .+10
 994    21440 4503    JMS I IITYCR  /ONLY 4 PER LINE SO GIVE CR, LF
 995    21441 2010    ISZ 10
 996    21442 1010    TAD 10
 997    21443 4477    JMS I PRTDEC  /PRINT LINE NUMBER
 998    21444 4253    JMS SPASE
 999    21445 1257    TAD N4
1000    21446 3016    DCA 16
1001    21447 4666    JMS I PRUNE   /DO THE CONVERSION AND OUTPUT IT
1002    21450 4406    JMS I FOUT
1003    21451 4253    JMS SPASE
1004    21452 5235    JMP PRIN3
1005    21453 0000  SPASE, 0   /SPACE PRINTER
1006    21454 1260    TAD SPASQ
1007    21455 4501    JMS I TYPIST
1008    21456 5653    JMP I SPASE
1009    21457 7774  N4,-4
1010    21460 1461  SPASQ, .+1
1011    21461 4040    4040
1012    21462 0001    1
1013    21463 0000  GOODIE, 0  /GET CONVERSION TYPE
1014    21464 1124    TAD OUTSTG  /STRING LOCATIN FOR OUTPUT OPTIONS
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/FLOURESCENCE PROGRAM          11/19/81  PAL8-V10A NO DATE   PAGE 12-1

1015   21465  4534    JMS I ENTURP  /INTERPRETTER
1016   21466  0005    PRINT, 5
1017   21467  5663    JMP I GOODIE
1018   21470  0000  UPSET, 0  /ROUTINE TO SETUP RSF POINTERS
1019   21471  7240    CLA CMA
1020   21472  3015    DCA 15  /DATA PICKUP
1021   21473  1031    TAD NOVAL
1022   21474  7040    CMA
1023   21475  3063    DCA TEMP5  /-# OF POINTS-1
1024   21476  1022    TAD START
1025   21477  3030  SETUP3, DCA WAVE
1026   21500  1023    TAD START2
1027   21501  7141    CIA CLL
1028   21502  1030    TAD WAVE
1029   21503  7450    SNA
1030   21504  5670    JMP I UPSET  /RETURN
1031   21505  4543    JMS I LINCOM
1032   21506  7630    SZL CLA
1033   21507  5670    JMP I UPSET
1034   21510  7325    CLA STL RAL IAC
1035   21511  1015    TAD 15
1036   21512  3015    DCA 15
1037   21513  2063    ISZ TEMP5
1038   21514  7410    SKP
1039   21515  5473    JMP I MONIT  /ALREADY OUT OF DATA
1040   21516  1076    TAD INTVL2
1041   21517  4542    JMS I NEGA1  /NOP OR CIA
1042   21520  1030    TAD WAVE /UPDATE WAVE
1043   21521  5277    JMP SETUP3
1044   21522  0000  UNDO, 0    /CHANGE PSEUDO WAVE PARAMETER INTO REAL P
ARAMETER
1045   21523  7417    LSR
1046   21524  0002    2
1047   21525  1146  Z77, TAD P310   /ADD IN 200 IF WAVELENGTH
1048   21526  4477    JMS I PRTDEC
1049   21527  5722    JMP I UNDO
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/FLOURESCENCE PROGRAM            11/19/81  PAL8-V10A NO DATE   PAGE 13

1050            1600  *1600
1051    21600   4000  PMCP, 4000  /START OF PM TABLE
1052    21601   0000  GOTCH1, 0  /GET REF VALUES, FLOAT
1053    21602   2063    ISZ TEMP5   /OUT OF DATA?
1054    21603   7410    SKP
1055    21604   5473    JMP I MONIT  /YES
1056    21605   1030    TAD WAVE
1057    21606   7141    CIA CLL
1058    21607   1025    TAD END2
1059    21610   7450    SNA
1060    21611   5215    JMP GUD2  /OK, LAST POINT
1061    21612   4543    JMS I LINCOM
1062    21613   7620    SNL CLA
1063    21614   5473    JMP I MONIT  /PAST END, DONE
1064    21615   6231  GUD2, CDF 30   /MOVE IN DATA
1065    21616   1415    TAD I 15
1066    21617   3107    DCA R1
1067    21620   1415    TAD I 15
1068    21621   3111    DCA S1
1069    21622   1415    TAD I 15
1070    21623   3110    DCA F1
1071    21624   6221    CDF CODFLD
1072    21625   5226          JMP GOTU        /*****  TAD EMFLG2   /CHECK
FOR EM OR EX
1073    21626   1107  GOTU, TAD R1
1074    21627   4540    JMS I NORM  /FLOAT ANS SAVE R
1075    21630   3363    RFL
1076    21631   1111    TAD S1
1077    21632   4540    JMS I NORM
1078    21633   3360    SFL
1079    21634   1110    TAD F1
1080    21635   4540    JMS I NORM
1081    21636   3366    FFL
1082    21637   1076          TAD INTVL2
1083    21640   4542          JMS I NEGA1
1084    21641   1030          TAD WAVE
1085    21642   3030          DCA WAVE
1086    21643   5401          JMP I GOTCH1
1087    21644   4040  GGFAC, TEXT "  SCALE FACTOR= "
1088    21645   2303
1089    21646   0114
1090    21647   0540
1091    21650   0601
1092    21651   0324
1093    21652   1722
1094    21653   7540
1095    21654   0000
1096            1654  *.-1
1097    21654   0001  1
1098    21655   4040  GGMARX, TEXT "  MAX="
1099    21656   1501
1100    21657   3075
1101    21660   0000        '
1102            1660  *.-1
1103    21660   0001  1
1104    21661   0521  GEQ2, TEXT "EQUIVALENCE WAVELENGTH (WAVENUMBER)="
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/FLOURESCENCE PROGRAM            11/19/81  PALB-V10A NO DATE   PAGE 13-1


1105    21662   2511
1106    21663   2601
1107    21664   1405
1108    21665   1603
1109    21666   0540
1110    21667   2701
1111    21670   2605
1112    21671   1405
1113    21672   1607
1114    21673   2410
1115    21674   4050
1116    21675   2701
1117    21676   2605
1118    21677   1625
1119    21700   1502
1120    21701   0522
1121    21702   5175
1122    21703   0000
1123            1703    *.-1
1124    21703   0001    1
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/FLOURESCENCE PROGRAM                11/19/81  PAL8-V10A NO DATE   PAGE 14

1125            2200  *2200
1126   22200    0000  RR, 0  /OUTPUT RAW R
1127   22201    4407    JMS I FLTPT
1128   22202    5525    FGET I RFLOT
1129   22203    0000    FEXT
1130   22204    5600    JMP I RR
1131   22205    0000  SS, 0  /OUTPUT S
1132   22206    4407    JMS I FLTPT
1133   22207    5526  FGET I SFLOT
1134   22210    0000    FEXT
1135   22211    5605    JMP I SS
1136   22212    0000  FF, 0  /FLUOR
1137   22213    4407    JMS I FLTPT
1138   22214    5527    FGET I FFLOT
1139   22215    0000    FEXT
1140   22216    5612    JMP I FF
1141   22217    0000  CO, 0  /CORRECTED FLUOR  F/R
1142   22220    0000  AB9, 0   /ABSOBENCE, -LOG(S/R)=LOG(R/S
1143   22221    4407    JMS I FLTPT
1144   22222    5525    FGET I RFLOT
1145   22223    6156            FPUT FTEMPO
1146   22224    3525            FMPY I RFLOT
1147   22225    6525            FPUT I RFLOT
1148   22226    5527            FGET I FFLOT
1149   22227    6161            FPUT FTEMP1
1150   22230    3526            FMPY I SFLOT
1151   22231    6527            FPUT I FFLOT
1152   22232    5525            FGET I RFLOT
1153   22233    4527    FDIV I FFLOT
1154   22234    0000    FEXT
1155   22235    7240    CLA CMA
1156   22236    1044    TAD 44
1157   22237    7500    SMA
1158   22240    5247    JMP .+7
1159   22241    7200    CLA   /AB=0 R/S<=1
1160   22242    4540    JMS I NORM
1161   22243    0044    44
1162   22244    4540    JMS I NORM
1163   22245    2357    LOGAB
1164   22246    5620    JMP I AB9
1165   22247    4540  JMS I NORM   /N LOG2     /FOR ALGORITHM SEE FLOATING
 POINT MANUAL
1166   22250    0044    44
1167   22251    4407    JMS I FLTPT
1168   22252    3321    FMPY LOG2
1169   22253    6530    FPUT I BUF1
1170   22254    5525    FGET I RFLOT
1171   22255    4527    FDIV I FFLOT
1172   22256    0000    FEXT
1173   22257    7201    CLA IAC   /MAKE 1<=X<2
1174   22260    3044    DCA 44
1175   22261    4407    JMS I FLTPT
1176   22262    2331    FSUB I ONEFLT  /SUTRACT 1 TO GET "Y" FOR SERIES (S
EE MANUAL)
1177   22263    6532    FPUT I BUF3
1178   22264    3354    FMPY LB
1179   22265    1351  FADD L7
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/FLOURESCENCE PROGRAM          11/19/81  PAL8-V10A NO DATE   PAGE 14-1

1180   22266  3532     FMPY I BUF3
1181   22267  1346     FADD L6
1182   22270  3532     FMPY I BUF3
1183   22271  1343     FADD L5
1184   22272  3532     FMPY I BUF3
1185   22273  1340     FADD L4
1186   22274  3532     FMPY I BUF3
1187   22275  1335     FADD L3
1188   22276  3532     FMPY I BUF3
1189   22277  1332     FADD L2
1190   22300  3532     FMPY I BUF3
1191   22301  1327     FADD L1
1192   22302  3532     FMPY I BUF3
1193   22303  1530     FADD I BUF1  /THIS IS THE NATURAL LOG
1194   22304  6357     FPUT LOGAB          /SAVE FOR USE WITH AB
1195   22305  5156           FGET FTEMP0
1196   22306  6525           FPUT I RFLOT
1197   22307  5161           FGET FTEMP1
1198   22310  6527           FPUT I FFLOT
1199   22311  5357           FGET LOGAB
1200   22312  3362           FMPY GEOFAC      /MULTIPLY BY A GEOMETRIC FAC
TOR
1201   22313  0000     FEXT
1202   22314  4765           JMS I EXPNT
1203   22315  4407           JMS I FLTPT
1204   22316  3525           FMPY I RFLOT
1205   22317  0000           FEXT
1206   22320  5620     JMP I AB9
1207   22321  0000   LOG2, 0  /NATURAL LOG OF 2
1208   22322  2613     2613
1209   22323  4414     4414
1210   22324  0002   COMMON, 2  /LN 10  TO MAKE COMMON LOGS
1211   22325  2232     2232
1212   22326  7307     7307
1213   22327  0000   L1, 0
1214   22330  3777     3777
1215   22331  7742   7742
1216   22332  7777     L2, 7777
1217   22333  4000     4000
1218   22334  4100     4100
1219   22335  7777     L3, 7777
1220   22336  2517     2517
1221   22337  0307     0307
1222   22340  7776     L4, 7776
1223   22341  4113     4113
1224   22342  7211     7211
1225   22343  7776     L5, 7776
1226   22344  2535     2535
1227   22345  3301     3301
1228   22346  7775     L6, 7775
1229   22347  4746     4746
1230   22350  0771     0771
1231   22351  7774   L7, 7774
1232   22352  2236     2236
1233   22353  4304     4304
1234   22354  7771   L8, 7771


/FLOURESCENCE PROGRAM          11/19/81  PAL8-V10A NO DATE   PAGE 14-2

1235   22355  4544   4544
1236   22356  1735   1735
1237   22357  0000   LOGAB, 0
1238   22360  0000    0
1239   22361  0000   0
1240   22362  0000   GEOFAC, 0000; 3000; 0000
1241   22363  3000
1242   22364  0000
1243   22365  2415   EXPNT,  FEXP
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/FLOURESCENCE PROGRAM                11/19/81  PAL8-V10A NO DATE   PAGE 15

1244            2400  *2400
1245   22400    0000  GCBUFF, 0
1246   22401    0000  0
1247   22402    0000  0
1248   22403    0000  FBF, 0
1249   22404    0000  0
1250   22405    0000  0
1251   22406    2357  LOGAB1, LOGAB
1252   22407    0000  LOGTT, 0
1253   22410    0000  0
1254   22411    0000  0
1255   22412    0000  FAKB, 0
1256   22413    0000  0
1257   22414    0000  0
1258   22415    0000  FEXP,   0
1259   22416    4407          JMS I FLTPT
1260   22417    3277          FMPY LG2E
1261   22420    6156          FPUT FTEMP0
1262   22421    0000          FEXT
1263   22422    4537          JMS I YUNORM
1264   22423    3045          DCA 45
1265   22424    1045          TAD 45
1266   22425    3310          DCA FLAG2
1267   22426    4540          JMS I NORM
1268   22427    0045          45
1269   22430    4407          JMS I FLTPT
1270   22431    6161          FPUT FTEMP1
1271   22432    5156          FGET FTEMP0
1272   22433    2161          FSUB FTEMP1
1273   22434    6156          FPUT FTEMP0
1274   22435    3156          FMPY FTEMP0
1275   22436    6161          FPUT FTEMP1
1276   22437    1274          FADD D
1277   22440    6311          FPUT FTEMP2
1278   22441    5271          FGET C
1279   22442    4311          FDIV FTEMP2
1280   22443    2156          FSUB FTEMP0
1281   22444    1263          FADD A
1282   22445    6311          FPUT FTEMP2
1283   22446    5266          FGET B
1284   22447    3161          FMPY FTEMP1
1285   22450    1311          FADD FTEMP2
1286   22451    6311          FPUT FTEMP2
1287   22452    5156          FGET FTEMP0
1288   22453    4311          FDIV FTEMP2
1289   22454    3305          FMPY FLOT2
1290   22455    1302          FADD FLOT1
1291   22456    0000          FEXT
1292   22457    1310          TAD FLAG2
1293   22460    1044          TAD 44
1294   22461    3044          DCA 44
1295   22462    5615          JMP I FEXP
1296   22463    0004  A,      0004; 2372; 1402
1297   22464    2372
1298   22465    1402
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/FLOURESCENCE PROGRAM            11/19/81  PAL8-V10A NO DATE   PAGE 15-1


1299    22466   7774  B,        7774; 2157; 5157
1300    22467   2157
1301    22470   5157
1302    22471   0012  C,        0012; 5454; 0343
1303    22472   5454
1304    22473   0343
1305    22474   0007  D,        0007; 2566; 5341
1306    22475   2566
1307    22476   5341
1308    22477   0001  LQ2E,     0001; 2705; 2435
1309    22500   2705
1310    22501   2435
1311    22502   0001  FLOT1,    0001; 2000; 0000
1312    22503   2000
1313    22504   0000
1314    22505   0002  FLOT2,    0002; 2000; 0000
1315    22506   2000
1316    22507   0000
1317    22510   0000  FLAG2,    0
1318    22511   0000  FTEMP2,   0; 0; 0
1319    22512   0000
1320    22513   0000
1321    22514   0007  FLT100,   7; 3100; 0
1322    22515   3100
1323    22516   0000
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/FLOURESCENCE PROGRAM          11/19/81  PALB-V10A NO DATE   PAGE 16

1324                 3000  *3000
1325          23000  7000  N1000, -1000
1326          23001  0000  FCNT, 0
1327          23002  0000  RCNT, 0
1328          23003  3344  GNEW, GGNEW
1329          23004  1124  PLOT, TAD OUTSTG  /PLOT THE REQUESTED INFO
1330          23005  4534    JMS I ENTURP  /GET REQUEST
1331          23006  0000  PLOPT, 0
1332          23007  4547    JMS I DIAG   /PRINT DIAGNOSTICS
1333          23010  1344    TAD PLTRET  /SETUP RETURN ADDRESS RETPLT
1334          23011  3073    DCA MONIT
1335          23012  4522    JMS I SETUP
1336          23013  4540    JMS I NORM
1337          23014  3161    YCAL  /CLEAR YMAX BUFFER
1338          23015  4540    JMS I NORM
1339          23016  3627    XOFF   /CLEAR OFFSET BUFFER
1340          23017  4523  GORSH, JMS I GOTCHA  /GET RSF
1341          23020  4606    JMS I PLOPT  /CALCUALTE Y
1342          23021  4407    JMS I FLTPT
1343                 0010    FABS=10      /COMMAND TO TAKE THE ABSOLUTE VALUE OF
 THE FAC
1344          23022  0010    FABS
1345          23023  2361    FSUB YCAL
1346          23024  0000    FEXT
1347          23025  1045    TAD 45
1348          23026  7710    SPA CLA
1349          23027  5217    JMP GORSH
1350          23030  4407    JMS I FLTPT
1351          23031  1361    FADD YCAL  /BIGGER
1352          23032  6361    FPUT YCAL
1353          23033  0000    FEXT
1354          23034  4506    JMS I TTOINT
1355          23035  7200    CLA
1356          23036  5217    JMP GORSH
1357          23037  1074  RETPLT, TAD MONSAV  /COME HERE AFTER FINDING MAX
1358          23040  3073    DCA MONIT  /RESET ABORT
1359          23041  1371    TAD GPLTMX  /MAX=
1360          23042  4501    JMS I TYPIST
1361          23043  4407    JMS I FLTPT
1362          23044  5361    FGET YCAL
1363          23045  0000    FEXT
1364          23046  4406    JMS I FOUT  /PRINT LARGEST Y VAL
1365          23047  1372    TAD GFAC  /ASK FOR "  SCALE FACTOR="
1366          23050  4501    JMS I TYPIST
1367          23051  4405    JMS I FLIN
1368          23052  4407    JMS I FLTPT
1369          23053  6361    FPUT YCAL
1370          23054  0000    FEXT
1371          23055  1057    TAD NLAT  /GET LAST CHAR
1372          23056  1135    TAD NASTY  /WAS ASTERISK?
1373          23057  7640    SZA CLA
1374          23060  5266    JMP .+6
1375          23061  7001    IAC  /SET PEN UP FOR OVERPLOT
1376          23062  4541    JMS I DRPLOT  /MOVE PEN TO ORIGIN
1377          23063  0000    0
1378          23064  0000    0
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/FLOURESCENCE PROGRAM            11/19/81  PAL8-V10A NO DATE   PAGE 16-1

1379   23065  5277    JMP PLTARE
1380   23066  1366    TAD P117
1381   23067  7200  Z06, CLA    /NOP
1382   23070  1023    TAD START2
1383   23071  7427    MQL DVI
1384   23072  0120    120
1385   23073  7605    CLA MUY
1386   23074  0120    120
1387   23075  7701    CLA MQA
1388   23076  3367    DCA PLTEM     /START2 ROUNDED TO THE NEXT LOWER 10
1389   23077  7240  PLTARE, CLA CMA
1390   23100  4541    JMS I DRPLOT  /INITIALIZE
1391   23101  4522    JMS I SETUP
1392   23102  7201    CLA IAC
1393   23103  3010    DCA 10   /SET PEN STATUS
1394   23104  1370    TAD RET2  /SET RETURN
1395   23105  3073    DCA MONIT
1396   23106  4523  GDRSH2, JMS I GOTCHA
1397   23107  4606    JMS I PLOPT   /GET Y VALUE
1398   23110  4407    JMS I FLTPT
1399   23111  3361    FMPY YCAL
1400   23112  1552    FADD I XOFFI    /ADD IN OFFSET  (0 OR 500)
1401   23113  0000    FEXT
1402   23114  4537    JMS I YUNORM
1403   23115  3353    DCA YREG
1404   23116  1353    TAD YREG
1405   23117  7141    CIA CLL
1406   23120  1365    TAD PP1750    /CHECK FOR OFF SCALE
1407   23121  7630    SZL CLA
1408   23122  5327    JMP PLOKE
1409   23123  1365    TAD PP1750  /TOO BIG
1410   23124  3353    DCA YREG
1411   23125  1150    TAD BELL
1412   23126  4502    JMS I PRINT1  /SOUND ALARM
1413   23127  1353  PLOKE, TAD YREG
1414   23130  1136    TAD P12   /OFFSET TO ALLOW FOR TICS ON BASELINE
1415   23131  3353    DCA YREG
1416   23132  1030    TAD WAVE
1417   23133  7041    CIA
1418   23134  1367    TAD PLTEM
1419   23135  4542    JMS I NEGA1
1420   23136  1076    TAD INTVL2
1421   23137  7041    CIA
1422   23140  4540    JMS I NORM  /X COORDINATE
1423   23141  0044    44
1424   23142  4407    JMS I FLTPT
1425   23143  4760    FDIV I XSCL3
1426   23144  0000    FEXT
1427   23145  4537    JMS I YUNORM
1428   23146  1136    TAD P12   /ALLOW FOR TICS
1429   23147  3352    DCA XREG
1430   23150  1010    TAD 10   /PEN STATUS
1431   23151  4541    JMS I DRPLOT
1432   23152  0000  XREG, 0
1433   23153  0000    YREG, 0
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/FLOURESCENCE PROGRAM          11/19/81  PAL8-V10A NO DATE   PAGE 16-2

1434   23154  3010     DCA 10
1435   23155  4506     JMS I TTOINT  /LOOK FOR INTERRUPT
1436   23156  7200     CLA /
1437   23157  5306     JMP GORSH2
1438   23160  3613  XSCL3, XSCAL     /PLOT BUFFER  XAXIS SCALE FACTOR.
1439   23161  0000  YCAL, 0
1440   23162  0000  0
1441   23163  0000  0
1442   23164  3037  PLTRET, RETPLT
1443   23165  1750  PP1750, 1750
1444   23166  0117  P117, 117
1445   23167  0000  PLTEM, 0
1446   23170  3200  RET2, RET2I
1447   23171  1655  GPLTMX, GGMARX
1448   23172  1644  GFAC, GGFAC
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/FLOURESCENCE PROGRAM            11/19/81  PAL8-V10A NO DATE   PAGE 17

1449                    3200  *3200
1450    23200   1057    RET2I, TAD NLAT  /RETURN HERE AFTER PLOT
1451    23201   1135      TAD NASTY   /IF * ON SCALE FACTOR, AVOID DRAWING B
ASELINE
1452    23202   7650      SNA CLA
1453    23203   5204      JMP LASPLT  /WAS OVERPLOT SO DON'T BOX
1454    23204   1313      TAD PP117
1455    23205   7000    ZP3, NOP  /CLA
1456    23206   1025      TAD END2
1457    23207   7427      MQL DVI
1458    23210   0120      120
1459    23211   7605      CLA MUY
1460    23212   0120      120
1461    23213   7701      CLA MQA
1462    23214   3240      DCA PLTEM2   /ROUNDOFF END TO NEAREST TEN
1463    23215   1314      TAD RET3  /SET NEW ABORT ADDRESS
1464    23216   3073      DCA MONIT
1465    23217   1240      TAD PLTEM2
1466    23220   4276      JMS XXPL  /CALCULATE PLOT POSITION OF END
1467    23221   3224      DCA PLTEND
1468    23222   7001      IAC
1469    23223   4541      JMS I DRPLOT   /MOVE TO END OF PLOT
1470    23224   0000    PLTEND, 0
1471    23225   0012      12
1472    23226   1224      TAD PLTEND
1473    23227   3231      DCA PLUT10
1474    23230   4715      JMS I SIDE  /PLOT SIDE (10 TICS)
1475    23231   0000    PLUT10, 0   /X COORD OF SIDELINE
1476    23232   0012      12   /YCOORD OF SIDELINE
1477    23233   0012      12   /DIRECTION AND LENGTH OF TIC
1478    23234   0144      144
1479    23235   1716      TAD I PLTEMI  /NOW PLOT TOP
1480    23236   3244      DCA PLTEM2+4
1481    23237   4717      JMS I BASTOP
1482    23240   0000    PLTEM2, 0   /X STARTING POINT
1483    23241   7660      -120
1484    23242   1762      1762  /Y COORD
1485    23243   1774      1774   /Y COORD OF TIC
1486    23244   0000      0   /X ENDING POINT
1487    23245   4715      JMS I SIDE
1488    23246   0012      12   /X COORD OF SIDELINE
1489    23247   1762      1762  /Y COORD OF SIDELINE
1490    23250   7766      -12  /DIRECTION AND LENGTH OF TIC
1491    23251   7634      -144  /DIRECTION AND DISTANCE BETWEEN TICS
1492    23252   1240      TAD PLTEM2
1493    23253   3263      DCA MKKR
1494    23254   1244      TAD PLTEM2+4
1495    23255   3257      DCA .+2
1496    23256   4717      JMS I BASTOP
1497    23257   0000      0
1498    23260   0120      120
1499    23261   0012      12
1500    23262   0000      0
1501    23263   0000    MKKR, 0  /ENDING POINT OF PLOT
1502    23264   1074    LASPLT, TAD MONSAV
1503    23265   3073      DCA MONIT  /RESET ABORT POINTER
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/FLOURESCENCE PROGRAM            11/19/81  PALB-V10A NO DATE   PAGE 17-1


1504   23266  1224    TAD PLTEND
1505   23267  1320    TAD P62  /50=ONE HALF INCH
1506   23270  3273    DCA .+3
1507   23271  7001    IAC
1508   23272  4541    JMS I DRPLOT
1509   23273  0000  NORM7, 0
1510   23274  0000    0
1511   23275  5473    JMP I MONIT
1512   23276  0000  XXPL, 0  /CALCULATE X COORD OF PLOT
1513   23277  7041    CIA
1514   23300  1716    TAD I PLTEM1
1515   23301  7041    CIA
1516   23302  4542    JMS I NEGA1  /NOP OR CIA
1517   23303  4540    JMS I NORM
1518   23304  0044    44
1519   23305  4407    JMS I FLTPT
1520   23306  4721    FDIV I XSCALI  /SCALE
1521   23307  0000    FEXT
1522   23310  4537    JMS I YUNORM
1523   23311  1136    TAD P12
1524   23312  5676    JMP I XXPL
1525   23313  0117  PP117, 117
1526   23314  3264  RET3, LASPLT
1527   23315  3400  SIDE, PLTSID
1528   23316  3167  PLTEM1, PLTEM
1529   23317  3447  BASTOP, PLTBAS
1530   23320  0062  P62, 62
1531   23321  3613  XSCALI, XSCAL
1532   23322  0000  NORMAL, 0  /NORMALIZE NUMBER IN AC
1533   23323  3046    DCA 46
1534   23324  3045    DCA 45
1535   23325  1337    TAD PP27
1536   23326  3044    DCA 44  /EXPONENT
1537   23327  1722    TAD I NORMAL
1538   23330  3340    DCA SINGLE
1539   23331  2322    ISZ NORMAL
1540   23332  4407    JMS I FLTPT
1541   23333  7000    FNOR
1542   23334  6740    FPUT I SINGLE
1543   23335  0000    FEXT
1544   23336          / / SINGLE
1545   23337  0027  PP27, 27
1546   23340  0000  SINGLE, 0  /INTEGER INPUT
1547   23341  4405    JMS I FLIN
1548   23342  4537    JMS I YUNORM
1549   23343  5740    JMP I SINGLE
1550   23344  1605  GGNEW, TEXT "NEW?"
1551   23345  2777
1552   23346  0000
1553          3346  * -1
1554   23346  0001  1
1555   23347  0015  GGPER, 15
1556   23350  0012    12
1557   23351  2516    TEXT "UNITS/INCH? "
1558   23352  1124
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/FLOURESCENCE PROGRAM            11/19/81  PALB-V10A NO DATE   PAGE 17-2


1559   23353  2357
1560   23354  1116
1561   23355  0310
1562   23356  7740
1563   23357  0000
1564          3357  *.-1
1565   23357  0001  1
1566   23360  0000  SFL, 0
1567   23361  0000  0
1568   23362  0000  0
1569   23363  0000  RFL, 0
1570   23364  0000  0
1571   23365  0000  0
1572   23366  0000  FFL, 0
1573   23367  0000  0
1574   23370  0000  0
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/FLOURESCENCE PROGRAM          11/19/81  PAL8-V10A NO DATE   PAGE 18

1575          3400  *3400   /LAST OF PLOT GARBAGE (ROUTINES TO PLOT BASE
LINES AND SIDES)
1576    23400 0000  PLTSID, 0  /PLOT SIDELINE
1577    23401 1136    TAD P12
1578    23402 7040    CMA
1579    23403 3034    DCA TEMP1  /10 TICS
1580    23404 4243    JMS GOTP
1581    23405 3223    DCA PLUT  /X COORD
1582    23406 4243    JMS GOTP
1583    23407 3224    DCA PLUT+1  /Y COORD
1584    23410 4243    JMS GOTP
1585    23411 3017    DCA 17  /DIRECTION OF TIC
1586    23412 4243    JMS GOTP
1587    23413 3016    DCA 16  /DIRECTION OF PLOT
1588    23414 5220    JMP .+4  /ENTER IN MIDDLE OF LOOP TO PLOT FIRST TI
C
1589    23415 1224  PLUT4, TAD PLUT+1
1590    23416 1016    TAD 16
1591    23417 3224    DCA PLUT+1
1592    23420 7240    CLA CMA
1593    23421 3010    DCA 10
1594    23422 4541    JMS I DRPLOT
1595    23423 0000  PLUT, 0
1596    23424 0000    0
1597    23425 2010    ISZ 10
1598    23426 5240    JMP PLUT2
1599    23427 1223    TAD PLUT  /PLOT TIC
1600    23430 1017    TAD 17
1601    23431 3235    DCA PLUT3
1602    23432 1224    TAD PLUT+1
1603    23433 3236    DCA PLUT3+1
1604    23434 4541    JMS I DRPLOT
1605    23435 0000  PLUT3, 0
1606    23436 0000    0
1607    23437 5222    JMP PLUT-1  /MOVE PEN BACK
1608    23440 2034  PLUT2, ISZ TEMP1  /PLOT AGAIN/?
1609    23441 5215    JMP PLUT4  /YES
1610    23442 5600    JMP I PLTSID  /NYET, RETURN
1611    23443 0000  GOTP, 0
1612    23444 1600    TAD I PLTSID
1613    23445 2200    ISZ PLTSID
1614    23446 5643    JMP I GOTP
1615    23447 0000  PLTBAS, 0  /PLOT BASELINE OR TOP
1616    23450 4315    JMS GOTB
1617    23451 3035    DCA TEMP2
1618    23452 4315    JMS GOTB
1619    23453 4542    JMS I NEGA1
1620    23454 3017    DCA 17
1621    23455 4315    JMS GOTB
1622    23456 3276    DCA PLOO1+1  /YCOORD
1623    23457 4315    JMS GOTB
1624    23460 3305    DCA PLOO5  /Y COORD TIC
1625    23461 4315    JMS GOTB
1626    23462 3016    DCA 16  /END POINT
1627    23463 5267    JMP .+4    /ENTER LOOP TO PLOT FIRST TIC
1628    23464 1035    PLTBS1, TAD TEMP2
1629    23465 1017    TAD 17  /SET NEW X
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1630   23466  3035    DCA TEMP2
1631   23467  1035    TAD TEMP2
1632   23470  4721    JMS I XXPLI
1633   23471  3275    DCA PL001  /X COORD
1634   23472  7240    CLA CMA
1635   23473  3010    DCA 10
1636   23474  4541    JMS I DRPLOT
1637   23475  0000  PL001, 0
1638   23476  0000    0
1639   23477  2010    ISZ 10
1640   23500  5307    JMP PL002
1641   23501  1275    TAD PL001  /PLOT TIC
1642   23502  3304    DCA .+2
1643   23503  4541    JMS I DRPLOT
1644   23504  0000    0
1645   23505  0000  PL005, 0
1646   23506  5274    JMP PL001-1  /RESET PEN POSITION
1647   23507  1035  PL002, TAD TEMP2  /DONE?
1648   23510  7041    CIA
1649   23511  1016    TAD 16
1650   23512  7640    SZA CLA
1651   23513  5264    JMP PLTBS1  /AGAIN
1652   23514  5647    JMP I PLTBAS  /DONE
1653   23515  0000  GOTB, 0
1654   23516  1647    TAD I PLTBAS
1655   23517  2247    ISZ PLTBAS
1656   23520  5715    JMP I GOTB
1657   23521  3276  XXPLI, XXPL
1658   23522  0000  UNORM, 0  /UNNORMALIZE NUMBER IN FAC
1659   23523  1045    TAD 45  /RETURN WITH # IN AC
1660   23524  7750    SPA SNA CLA
1661   23525  5722    JMP I UNORM  /NUMBER <=0
1662   23526  4407    JMS I FLTPT
1663   23527  1355    FADD AHALF  /ROUND OFF
1664   23530  0000    FEXT
1665   23531  1044    TAD 44
1666   23532  7750    SPA SNA CLA
1667   23533  5722    JMP I UNORM   /LESS THAN 1
1668   23534  1044    TAD 44
1669   23535  7041    CIA
1670   23536  1354    TAD P14
1671   23537  7500    SMA
1672   23540  5343    JMP .+3
1673   23541  7240    CLA CMA  /OVERFLOW
1674   23542  5722    JMP I UNORM
1675   23543  3350    DCA .+5
1676   23544  1046    TAD 46
1677   23545  7421    MQL
1678   23546  1045    TAD 45
1679   23547  7415     ASR
1680   23550  0000    0
1681   23551  7413    SHL
1682   23552  0001    1
1683   23553  5722    JMP I UNORM
1684   23554  0014  P14, 14
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/FLOURESCENCE PROGRAM                11/19/81  PAL8-V10A NO DATE   PAGE 18-2

1685   23555  0000  AHALF, 0
1686   23556  2000    2000
1687   23557  0000    0
1688   23560  0000  BUFF1, 0
1689   23561  0000  0
1690   23562  0000  0
1691   23563  0000  BUFF2, 0
1692   23564  0000  0
1693   23565  0000  0
1694   23566  0000  BUFF3, 0
1695   23567  0000  0
1696   23570  0000  0
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/FLOURESCENCE PROGRAM                11/19/81  PAL8-V10A NO DATE   PAGE 19

1697          6554  *6554       /IN FLOATING POINT INTERPRETTER
1698   26554  3620  FAB   /ABSOLUTE VALUE
1699   26555  6000  6000    /NEGATE FAC
1700          3600  *3600
1701   23600  1216  XAX, TAD GPER   /AK "UNIT/INCH?"
1702   23601  4501    JMS I TYPIST
1703   23602  4405    JMS I FLIN
1704   23603  4407    JMS I FLTPT
1705   23604  4617    FDIV I A100
1706   23605  6213    FPUT XSCAL
1707   23606  0000    FEXT
1708   23607  7325    CLA STL IAC RAL  /3
1709   23610  1213    TAD XSCAL
1710   23611  3213    DCA XSCAL  /MULT BY 8
1711   23612  5473    JMP I MONIT
1712   23613  0001  XSCAL, 1   /SCALE FACTOR DEFAULT WITH 20 UNITS PER I
NCH
1713   23614  3146    3146
1714   23615  3146    3146
1715   23616  3347  GPER, GGPER
1716   23617  2514  A100, FLT100
1717   23620  0000  FAB, 0   /ROUTINE TO TAKE ABSOLUTE VALUE OF FAC
1718   23621  3047    DCA 47    /CLEAR OUT 3RD WORD OF PREC.
1719   23622  1045    TAD 45
1720   23623  7710    SPA CLA
1721   23624  4626    JMS I .+2  /NEGATE
1722   23625  5620    JMP I FAB
1723   23626  6000  6000    /LOCATION IN FLOATING POINT OF ROUTINE NEGAT
ER
1724   23627  0000  XOFF, 0
1725   23630  0000  0
1726   23631  0000  0
1727   23632  0000  OFFSET, 0
1728   23633  1237    TAD PDD500
1729   23634  4540    JMS I NORM  /SET OFFSET TO 500 (HALF SCALE)
1730   23635  3627    XOFF
1731   23636  5632    JMP I OFFSET
1732   23637  0764  PDD500, 764
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/FLOURESCENCE PROGRAM            11/19/81  PAL8-V10A NO DATE   PAGE 20

1733            4200  *4200
1734    24200   4540  AVERAGE, JMS I NORM  /CLEAR SUMMATIN BUFFER
1735    24201   4237    AVBUFF
1736    24202   1124    TAD OUTSTG   /CHOOSE OUTPUT OPTION
1737    24203   4534    JMS I ENTURP
1738    24204   0000  AVPARM, 0
1739    24205   4547    JMS I DIAG   /PRINT DIAGNOSTIC MESSAGES
1740    24206   3544    DCA I TYGM
1741    24207   4522    JMS I SETUP
1742    24210   1236    TAD AVRET
1743    24211   3073    DCA MONIT   /SET ABORT RETURN
1744    24212   4523    JMS I GOTCHA
1745    24213   4604    JMS I AVFARM
1746    24214   4407    JMS I FLTPT
1747    24215   1237    FADD AVBUFF
1748    24216   6237    FPUT AVBUFF
1749    24217   0000    FEXT
1750    24220   2544    ISZ I TYGM
1751    24221   5212    JMP .-7
1752    24222   1074  RETAV, TAD MONSAV
1753    24223   3073    DCA MONIT
1754    24224   1544    TAD I TYGM
1755    24225   4540    JMS I NORM
1756    24226   3560    BUFF1
1757    24227   4407    JMS I FLTPT
1758    24230   5237    FGET AVBUFF
1759    24231   4530    FDIV I BUF1
1760    24232   0000    FEXT
1761    24233   4503    JMS I IITYCR
1762    24234   4406    JMS I FOUT
1763    24235   5473    JMP I MONIT
1764    24236   4222  AVRET, RETAV
1765    24237   0000  AVBUFF, 0
1766    24240   0000    0
1767    24241   0000    0
1768    24242   3062  LE, DCA TEMP4
1769    24243   1267    TAD INTG  /ASK FOR SIZE OF INTVAL
1770    24244   4501    JMS I TYPIST
1771    24245   4500    JMS I NUMIN
1772    24246   3075    DCA INTVAL
1773    24247   1300    TAD SWOOP   /START OF STRING OF LE, NU CHANGES
1774    24250   3034    DCA TEMP1
1775    24251   1434  LE3,  TAD I TEMP1     /GET AN ADDRESS
1776    24252   7450    SNA   /END WITH 0
1777    24253   5473    JMP I MONIT
1778    24254   3035    DCA TEMP2
1779    24255   1034    TAD TEMP1
1780    24256   7001    IAC
1781    24257   1062    TAD TEMP4
1782    24260   3036    DCA TEMP3
1783    24261   1436    TAD I TEMP3
1784    24262   3435    DCA I TEMP2
1785    24263   2034    ISZ TEMP1
1786    24264   2034    ISZ TEMP1
1787    24265   2034    ISZ TEMP1
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/FLOURESCENCE PROGRAM            11/19/81  PALB-V10A NO DATE   PAGE 20-1


1788   24266  5251    JMP LE3
1789   24267  4270  INTG,  +1
1790   24270  1116  TEXT "INTERVAL= "
1791   24271  2405
1792   24272  2226
1793   24273  0114
1794   24274  7540
1795   24275  0000
1796          4275    *.-1
1797   24275  0001  1
1798   24276  7201  NU, CLA IAC
1799   24277  5242    JMP LE
1800   24300  4301  SWOOP,.+1
1801   24301  0537  PARM1/ADDRESS
1802   24302  0624  LEIN/LENGTHCOMMAND
1803   24303  0644  NUIN/NUMBER COMMAND
1804   24304  0737    PARM2
1805   24305  0624    LEIN
1806   24306  0644    NUIN
1807   24307  1031    SCAN2+1
1808   24310  3067  Z06
1809   24311  7200    CLA
1810   24312  7000    NOP
1811   24313  4331  NEG11+1
1812   24314  7000  NOP
1813   24315  7041  CIA
1814   24316  4334  LINKER+1
1815   24317  7000  NOP
1816   24320  7020  CML
1817   24321  3205    ZP3
1818   24322  7000    NOP
1819   24323  7200    CLA
1820   24324  1525  Z77
1821   24325  1146    TAD P310
1822   24326  7000    NOP
1823   24327  0000  0
1824   24330  0000  NEG11, 0    /AC NEGATER DUE TO BASIC DIFF BETWEEN LAM
BDA AND WAVENUMBER
1825   24331  7000    NOP  /CIA
1826   24332  5730    JMP I NEG11
1827   24333  0000  LINKER, 0    /LINK COMPLEMENTER FOR SAME REASON
1828   24334  7000    NOP
1829   24335  5733    JMP I LINKER    /NOP MAY BE CML
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/FLOURESCENCE PROGRAM            11/19/81  PAL8-V10A NO DATE   PAGE 21

 1830           4400  *4400    /STRINGS OF COMMAND OPTIONS
 1831    24400  0301  CMDSTG,  0301  /CA
 1832    24401  0400           CALIB
 1833    24402  0515           0515
 1834    24403  0502           EM
 1835    24404  0530           0530
 1836    24405  0530           EX
 1837    24406  1725           1725
 1838    24407  0671           OUTRNG
 1839    24410  2303           2303
 1840    24411  1000           SCAN
 1841    24412  2022           2022
 1842    24413  1400           PRINT
 1843    24414  2417           2417
 1844    24415  5106           OSB     /  OSB
 1845    24416  2315           2315   /SM   FOR AUTO SMOOTH
 1846    24417  5025           SMUST
 1847    24420  0411           0411
 1848    24421  5003           DISPLA    /PLOT ON SCOPE
 1849    24422  2014           2014
 1850    24423  5000           PLOTG
 1851    24424  0126           0126
 1852    24425  4200           AVERAGE
 1853    24426  1405           1405
 1854    24427  4242           LE
 1855    24430  1625           1625
 1856    24431  4276           NU
 1857    24432  3001           3001   /XA
 1858    24433  3600           XAX   /SET X-AXIS SCALE
 1859    24434  6161           6161   /11
 1860    24435  5010           SM11
 1861    24436  6167           6167   /17
 1862    24437  5014           SM17
 1863    24440  2217           2217    /RO
 1864    24441  4512           ROTAT
 1865    24442  2301           2301
 1866    24443  5100           SAVE    /SAVE A RUN
 1867    24444  0705           0705    /GE
 1868    24445  5103           GET     /GET TABLE FROM DISK
 1869    24446  0000           0
 1870    24447  2222  STIGMA,  2222    /STRINGS OF OUTPUT OPTIONS FOR PLOT,
 PRINT, PUNCH,
 1871    24450  2200   RR      /AND AVERAGE
 1872    24451  2323   2323
 1873    24452  2205    SS
 1874    24453  0606   /FF
 1875    24454  2212   FF
 1876    24455  0161           0161    /A1
 1877    24456  2220           AB9     /PRIMARY CORRECTED ABSORBANCE
 1878    24457  0000   0
 1879    24460  0000  DINOG, 0   /ROUTINE TO PRINT BRIEF DIAGNOSTICS TO MI
NIMIZE USER ERROR
 1880    24461  4715   JMS I VISAD     /PRINT SOME ADVICE
 1881    24462  4503   JMS I IITYCR
 1882    24463  1270   TAD GDIG  /PRINT "SMOOTH?"
 1883    24464  4501   JMS I TYPIST
 1884    24465  1154   TAD SMFLG
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/FLOURESCENCE PROGRAM            11/19/81  PALB-V10A NO DATE   PAGE 21-1

1885   24466  4277    JMS NOGIN   /TELL IF AUTO SMOOTHED
1886   24467  5660    JMP I DINOG
1887   24470  4471  GDIG, .+1
1888   24471  2315    TEXT "SMOOTH?:    "
1889   24472  1717
1890   24473  2410
1891   24474  7772
1892   24475  4040
1893   24476  4000
1894          4476    *.-1
1895   24476  0001    1
1896   24477  0000  NOGIN, 0
1897   24500  7650    SNA CLA      /PRINT Y IF AC IS 1
1898   24501  7305    CLL CLA IAC RAL   /SET TO 2
1899   24502  1305      TAD GGY
1900   24503  4501    JMS I TYPIST
1901   24504  5677    JMP I NOGIN
1902   24505  4506  GGY,  +1
1903   24506  3140    3140  /Y
1904   24507  0001    1
1905   24510  1640    1640  /N
1906   24511  0001    1
1907   24512  6213  ROTAT,  CDFCIF 10
1908   24513  5714          JMP I .+1
1909   24514  3000          3000
1910   24515  1404  VISAD,  ADVIS
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/FLOURESCENCE PROGRAM            11/19/81  PAL8-V10A NO DATE   PAGE 22

1911            5000  *5000
1912   25000    1205  PLOTQ,  TAD XPLOT  /PLOT ON PLOTTER
1913   25001    3141    DCA DRPLOT
1914   25002    5607    JMP I LPLOT
1915   25003    1206  DISPLA,  TAD SCPLOT  /PLOT ON SCOPE
1916   25004    5201    JMP .-3
1917   25005    5200  XPLOT,  PLOTX
1918   25006    4600  SCPLOT,  PLOTSC
1919   25007    3004  LPLOT,  PLOT
1920   25010    1223  SM11,  TAD P13   /SET UP FOR 11 POINT CURVE SMOOTH
1921   25011    3112    DCA R2
1922   25012    1220    TAD WPON
1923   25013    5622    JMP I SMOR
1924   25014    1224  SM17,  TAD P21
1925   25015    3112    DCA R2
1926   25016    1221    TAD WPON+1
1927   25017    5622    JMP I SMOR
1928   25020    5551  WPON,  W1
1929   25021    6663    C1
1930   25022    5400  SMOR,  SMOOTH
1931   25023    0013  P13,13
1932   25024    0021  P21,21
1933   25025    1234  SMUST,  TAD GSMUST   /ASK " SMOOTH? "
1934   25026    4501    JMS I TYPIST
1935   25027    4504    JMS I YORN
1936   25030    7410    SKP   /NO
1937   25031    7201    CLA IAC
1938   25032    3154    DCA SMFLG    /SET AUTO SMOOTHING FLAG ACCORDINGLY
1939   25033    5473    JMP I MONIT
1940   25034    5035  GSMUST,  +1
1941   25035    2315    TEXT "SMOOTH?  "
1942   25036    1717
1943   25037    2410
1944   25040    7740
1945   25041    4000
1946            5041    *.-1
1947   25041    0001    1
1948   25042    1377  SMUST2,  TAD (SMUST3)   /COME HERE AT END OF ALL SCAN
S
1949   25043    3073    DCA MONIT   /RESET MONITR POINTER FOR LOOPING
1950   25044    1154    TAD SMFLG   /SHOULD WE SMOOTH?
1951   25045    7650    SNA CLA
1952   25046    5274    JMP SMUST7    /NO
1953   25047    3267    DCA SMUST4   /SETUP R,S,F POINTER (0=R)
1954   25050    7346    CLA CMA CLL RTL   /-3 FOR R,S,F
1955   25051    3270    DCA SMUST5
1956   25052    1224  SMUST8,  TAD P21     /SETUP FOR 17 POINT SMOOTH
1957   25053    3112    DCA R2
1958   25054    1221    TAD WPON+1
1959   25055    3114    DCA S2
1960   25056    1031    TAD NOVAL
1961   25057    7040    CMA
1962   25060    1112    TAD R2
1963   25061    7500    SMA
1964   25062    5274    JMP SMUST7     /NO POINTS
1965   25063    3034    DCA TEMP1
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/FLOURESCENCE PROGRAM            11/19/81  PAL8-V10A NO DATE   PAGE 22-1

1966   25064  7240     CLA CMA
1967   25065  1267     TAD SMUST4    /0=R  1=S  2=F
1968   25066  5776'    JMP SMUST6    /HOP INTO SMOOTHING ROUTINE
1969   25067  0000  SMUST4, 0
1970   25070' 0000  SMUST5, 0
1971   25071  2267  SMUST3, ISZ SMUST4     /COME HERE WHEN DONE WITH A PA
RT OF DATA
1972   25072  2270     ISZ SMUST5      /DONE ALL 3?
1973   25073  5252     JMP SMUST8       /NO   GO DO NEXT SET
1974   25074  7200  SMUST7, CLA     /ALL DONE
1975   25075  1074     TAD MONSAV
1976   25076  3073     DCA MONIT     /RESET MONITOR POINTER
1977   25077  5473     JMP I MONIT     /AND QUIT
1978   25100  6203  SAVE,    CDFCIF 0
1979   25101  5702           JMP I .+1
1980   25102  0400           SAVRUN
1981   25103  6203  GET,     CDFCIF 0
1982   25104  5705           JMP I .+1
1983   25105  0463           GETRUN
1984   25106  6203  DSB,     CDFCIF 0
1985   25107  5710           JMP I .+1
1986   25110  7600           7600
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/FLOURESCENCE PROGRAM            11/19/81  PALB-V10A NO DATE   PAGE 23

1987   25176  5421
1988   25177  5071
1989          5400  *5400
1990   25400  3114  SMOOTH, DCA S2
1991   25401  1031    TAD NOVAL   /SMOOTH FIVE POINTS ASSUMING CUBIC FIT
 PS_OSSIBLE
1992   25402  7040    CMA      /SEE "METHODS IN NUMERICAL ANALYSIS" BY NI
ELSEN
1993   25403  1112    TAD R2  /# OF POINTS IN SMOOTHING INTERVAL
1994   25404  3034    DCA TEMP1
1995   25405  1034    TAD TEMP1
1996   25406  7700    SMA CLA
1997   25407  5216    JMP SMOO2  /NOT ENOUGH POINTS
1998   25410  1330    TAD SMOOCH
1999   25411  4501    JMS I TYPIST  /"INPUT 0,1,2 FOR R,S,F"
2000   25412  4500    JMS I NUMIN
2001   25413  1033    TAD N5
2002   25414  7510    SPA
2003   25415  5220    JMP .+3
2004   25416  4544  SMOO2, JMS I TYQM
2005   25417  5473    JMP I MONIT  /INCORRECT ENTRY
2006   25420  1133    TAD P4
2007   25421  3010  SMUST6,  DCA 10  /PICKUP POINTER
2008   25422  1112    TAD R2
2009   25423  7110    CLL RAR
2010   25424  3113    DCA F2   /CREATE TRUNCATED N/2
2011   25425  1113    TAD F2
2012   25426  7104    CLL RAL
2013   25427  1113    TAD F2   /3*IN/2]
2014   25430  1010    TAD 10
2015   25431  3011    DCA 11  /PUT-BACK POINTER
2016   25432  1112    TAD R2
2017   25433  7041    CIA
2018   25434  3035    DCA TEMP2
2019   25435  4252    JMS SMIFT  /NOW GET IN FIRST 5 POINTS
2020   25436  2035    ISZ TEMP2
2021   25437  5235    JMP .-2
2022   25440  4252  SMOO3, JMS SMIFT   /NOW GO THRU SMOOTHING LOOP
2023   25441  4537    JMS I YUNORM   /UNORMALIZE NEW MIDDLE VALUE
2024   25442  6231    CDF 30
2025   25443  3411    DCA I 11
2026   25444  6221    CDF CODFLD
2027   25445  2011    ISZ 11      /STORE IN DATA TABLE
2028   25446  2011    ISZ 11
2029   25447  2034    ISZ TEMP1
2030   25450  5240    JMP SMOO3
2031   25451  5473    JMP I MONIT
2032   25452  0000  SMIFT, 0  /SHIFT IN NEW VALUES AND FORM SMOOTHED POI
NT WHICH IS
2033   25453  1114    TAD S2   /ESSENTIALLY A WEIGHTED AVERAGE OF THE 5
NEARBY POINTS
2034   25454  3036    DCA TEMP3   /POINTER TO WEIGHT FACTORS
2035   25455  7325    CLA IAC STL RAL   /3
2036   25456  3016    DCA 16     /FOR SPACING UP POINTER TO WEIGHT FACTOR
S
2037   25457  1113    TAD F2
2038   25460  7041    CIA
2039   25461  3015    DCA 15   /SPACE UP 5, THEN SPACE DOWN FIVE
2040   25462  3107    DCA R1
2041   25463  3110    DCA F1  /CLEAR SUMMATION BUFFER
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/FLOURESCENCE PROGRAM                11/19/81  PAL8-V10A NO DATE   PAGE 23-1

2042   25464  3111     DCA S1
2043   25465  1327     TAD PUSHER    /PUSH-DOWN STORAGE BUFFER
2044   25466  3062     DCA TEMP4
2045   25467  1062     TAD TEMP4
2046   25470  3017     DCA 17  /PICKUP POINTER
2047   25471  1112     TAD R2
2048   25472  7041     CIA
2049   25473  3063     DCA TEMP5
2050   25474  1417   SMU, TAD I 17   /GET A POINT
2051   25475  3462     DCA I TEMP4   /PUSH IT DOWN
2052   25476  1462     TAD I TEMP4
2053   25477  2062     ISZ TEMP4   /GET BACK POINT FOR AVERAGE
2054   25500  4540     JMS I NORM
2055   25501  0044      44
2056   25502  4407     JMS I FLTPT
2057   25503  3436     FMPY I TEMP3   /MULT BY WEIGHT
2058   25504  1107     FADD R1
2059   25505  6107     FPUT R1
2060   25506  0000   FEXT
2061   25507  1036     TAD TEMP3
2062   25510  1016     TAD 16   /MOVE UP OR BACK
2063   25511  3036     DCA TEMP3
2064   25512  2015     ISZ 15
2065   25513  5316     JMP .+3
2066   25514  7346     CLL CMA CLA RTL   /-3    FOR SPACING BACK DOWN TAB
LE OF FACTORS
2067   25515  3016     DCA 16    /(LAST 5 FACTORS SAME AS FIRST FIVE)
2068   25516  2063     ISZ TEMP5
2069   25517  5274     JMP SMU
2070   25520  6231     CDF 30
2071   25521  1410     TAD I 10
2072   25522  2010     ISZ 10        /MOVE IN NEW POINT INTO PUSHDOWN BUFF
ER
2073   25523  2010     ISZ 10
2074   25524  6221     CDF CODFLD
2075   25525  3462     DCA I TEMP4
2076   25526  5652     JMP I SMIFT
2077   25527  6717   PUSHER, PUSHY
2078   25530  5531   SMODCH, .+1
2079   25531  0516     TEXT "ENTER 0,1, OR 2 FOR R,S, OR F:"
2080   25532  2405
2081   25533  2240
2082   25534  6054
2083   25535  6154
2084   25536  4017
2085   25537  2240
2086   25540  6240
2087   25541  0617
2088   25542  2240
2089   25543  2254
2090   25544  2354
2091   25545  4017
2092   25546  2240
2093   25547  0672
2094   25550  0000
2095           5550   *.-1
2096   25550  0001   1
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/FLOURESCENCE PROGRAM                11/19/81  PAL8-V10A NO DATE   PAGE 23-2

2097   25551  7775  W1,7775     /-36/429        ALL SUCCEEDING FACTORS ARE
 DIVIDED BY 429
2098   25552  5241    5241
2099   25553  0755    0755
2100   25554  7773  W2, 7773    /9
2101   25555  2536    2536
2102   25556  7023    7023
2103   25557  7775  W3, 7775   /44
2104   25560  3220    3220
2105   25561  3210    3210
2106   25562  7776  W4, 7776    /  69
2107   25563  2445    2445
2108   25564  4576    4576
2109   25565  7776  W5, 7776    /84
2110   25566  3104    3104   /
2111   25567  0154    0154
2112   25570  7776  W6, 7776   /89
2113   25571  3243    3243
2114   25572  4020    4020
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/FLOURESCENCE PROGRAM                11/19/81  PAL8-V10A NO DATE   PAGE 24

2115           6663  *6663
2116   26663   7775  C1, 7775   /THESE ARE WEIGHT FACTORS FOR 17 POINT SMO
OTH
2117   26664   5726    5726
2118   26665   6233    6233
2119   26666   7773  C2,7773
2120   26667   5476    5476
2121   26670   4735    4735
2122   26671   7773    7773
2123   26672   2614    2614
2124   26673   2210    2210
2125   26674   7774    7774
2126   26675   3442    3442
2127   26676   0503    0503
2128   26677   7775    7775
2129   26700   2531    2531
2130   26701   4365    4365
2131   26702   7775    7775
2132   26703   3274    3274
2133   26704   5022    5022
2134   26705   7775    7775
2135   26706   3672    3672
2136   26707   2020    2020
2137   26710   7776    7776
2138   26711   2051    2051
2139   26712   1513    1513
2140   26713   7776  C9, 7776
2141   26714   2102    2102
2142   26715   4430    4430
2143   26716   0000    0
2144   26717   0000    PUSHY, 0
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/FLOURESCENCE PROGRAM          11/19/81  PAL8-V10A NO DATE   PAGE 25

2145                    /FL33  FLUORD
2146              4600  *4600   /NEW SCOPE VERSION OF PLOTSC
2147   24600      0000  PLOTSC, 0  /PLOT ON SCOPE
2148   24601      7500    SMA
2149   24602      5221    JMP SC1
2150   24603      7200    CLA  /INITIALIZE /BUT DON'T MOVE UNTIL FIRST ACTUA
L PLOT
2151   24604      3244    DCA SCSAVX
2152   24605      3245    DCA SCSAVY
2153   24606      5600    JMP I PLOTSC
2154   24607      0000  PLOLD, 0     /VECTOR PLOT POINTS
2155   24610      1245    TAD SCSAVY  /OLD Y COORD
2156   24611      7421    MQL
2157   24612      7325    STL CLA IAC RAL  /3
2158   24613      4276    JMS PLPT  /OUTPUT Y COORD
2159   24614      1244    TAD SCSAVX
2160   24615      7421    MQL
2161   24616      7305    CLA CLL IAC RAL    /2
2162   24617      4276    JMS PLPT   /OUTPUT X COORD  (THE 3 AND THE 2 ARE O
UTPUT CODES)
2163   24620      5607    JMP I PLOLD
2164   24621      3246  SC1, DCA SCBRYT   /1 MEANS UNWRITTEN PLOT
2165   24622      1242    TAD GS1
2166   24623      4250    JMS PRINT3   /SET FOR VECTOR PLOT
2167   24624      4207    JMS PLOLD    /PLOT TO OLD COORD.
2168   24625      4256    JMS SCSCAL   /GET X TIMES .76
2169   24626      3244    DCA SCSAVX
2170   24627      4256    JMS SCSCAL   /SAME FOR Y
2171   24630      3245    DCA SCSAVY
2172   24631      1246    TAD SCBRYT
2173   24632      7650    SNA CLA
2174   24633      5236    JMP .+3    /IS BRIGHT PLOT
2175   24634      1242    TAD GS1
2176   24635      4250    JMS PRINT3   /IS DARK
2177   24636      4207    JMS PLOLD    /OUTPUT NEW COORD.
2178   24637      1243    TAD US1
2179   24640      4250    JMS PRINT3  /SET BACK TO NORML PRINT MODE
2180   24641      5600    JMP I PLOTSC  /DONE, RETURN
2181   24642      0235  GS1, 235  /VECTOR PLOT
2182   24643      0237  US1, 237  /NORMAL TEXT
2183   24644      0000  SCSAVX, 0
2184   24645      0000  SCSAVY, 0    /X AND Y COORD.
2185   24646      0000  SCBRYT, 0    /IS ONE FOR DARK, 0 FOR BRIGHT
2186   24647      0000  SCXN, 0   /SCRATCH
2187   24650      0000  PRINT3, 0
2188   24651      6046    TLS
2189   24652      7200    CLA
2190   24653      6041    TSF
2191   24654      5253    JMP .-1
2192   24655      5650    JMP I PRINT3
2193   24656      0000  SCSCAL, 0  /GET COORD TIMES .76
2194   24657      1600    TAD I PLOTSC
2195   24660      2200    ISZ PLOTSC
2196   24661      7425    MQL MUY
2197   24662      1370    1370    /760
2198   24663      7407    DVI
2199   24664      1750  PD1750, 1750  /1000
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/FLOURESCENCE PROGRAM                11/19/81  PAL8-V10A NO DATE   PAGE 25-1

2200   24665  7701     CLA MQA
2201   24666  7141     CIA CLL
2202   24667  1264     TAD PD1750   /CHECK TO SEE IF IT IS OVER 1000D
2203   24670  7620     SNL CLA
2204   24671  5274     JMP .+3
2205   24672  7701     CLA MQA   /LEGITIMATE
2206   24673  5636     JMP I SCSCAL
2207   24674  1264     TAD PD1750
2208   24675  5636     JMP I SCSCAL  /TOO BIG
2209   24676  0000   PLPT, 0  /OUTPUT VECTOR COORDINATES
2210   24677  3247     DCA SCXN  /CODE
2211   24700  7413     SHL
2212   24701  0006     6  /GET TOP 5 BITS
2213   24702  1311     TAD PD240  /MORE CODES
2214   24703  4250     JMS PRINT3
2215   24704  1247     TAD SCXN
2216   24705  7413     SHL
2217   24706  0004     4
2218   24707  4250     JMS PRINT3
2219   24710  5676     JMP I PLPT
2220   24711  0240   PD240, 240
2221   24712  0000   SCHOME, 0
2222   24713  1320     TAD PD33
2223   24714  4250     JMS PRINT3    /CLEAR SCREEN AND HOME
2224   24715  1321     TAD PD14
2225   24716  4250     JMS PRINT3
2226   24717  5712     JMP I SCHOME
2227   24720  0033   PD33, 33
2228   24721  0014   PD14, 14
2229   24722  0000   ENPUT, 0  /INPUT AND ECHO A CHAR
2230   24723  7200     CLA
2231   24724  4777'    JMS KBRK     /REST OF MONITOR IS AT 200
2232   24725  7450     SNA
2233   24726  5324     JMP .-2
2234   24727  4776'    JMS TTOUT    /ECHO IT
2235   24730  1375     TAD (-212
2236   24731  1774'    TAD FCHAR    /GET BACK CHAR AND TEST FOR LINE FEED
2237   24732  7650     SNA CLA
2238   24733  5323     JMP ENPUT+1   /IGNORE LINE FEEDS COMPLETELY
2239   24734  1774'    TAD FCHAR   /
2240   24735  5722     JMP I ENPUT
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/FLOURESCENCE PROGRAM            11/19/81  PAL8-V10A NO DATE   PAGE 26

2241   24774   0306
2242   24775   7566
2243   24776   0317
2244   24777   0271
2245           5200   *5200
2246
2247                  /DIGITAL 8-12-U
2248                  /PLOT SUBROUTINE
2249                  /CALLING SEQUENCE
2250
2251                  /   C(AC)=-1;  INITIALIZE
2252                  /   C(AC)= 0;  PLOT WITH PEN DOWN
2253                  /   C(AC)= 1;  PLOT WITH PEN UP
2254                  /      JMS PLOTX
2255                  /      X CO-ORDINATE (IN STEPS) (RETURN IF AC=-1)
2256                  /      Y CO-ORDINATE (IN STEPS)
2257
2258   25200   0000   PLOTX,  0
2259   25201   7510           SPA               /MOVE THE PEN?
2260   25202   5220           JMP PLOTA         /NO: CONTINUE
2261   25203   1361           TAD PLOTPN        /ADD PEN STATUS
2262   25204   7112           CLL RTR
2263   25205   7710           SPA CLA                   /ANY CHANGE?
2264   25206   5227           JMP PLOT1         /NO: CONTINUE
2265   25207   7620           SNL CLA
2266   25210   5214           JMP .+4                   /LOWER THE PEN
2267   25211   3361           DCA PLOTPN        /RAISE THE PEN
2268   25212   6504           PLPU
2269   25213   5216           JMP .+3
2270   25214   2361           ISZ PLOTPN        /LOWER THE PEN
2271   25215   6524           PLPD
2272   25216   4370           JMS PLOTWT        /WAIT FOR FLAG
2273   25217   5227           JMP PLOT1         /CONTINUE
2274   25220   7200   PLOTA,  CLA
2275   25221   6504           PLPU              /RAISE THE PEN
2276   25222   3361           DCA PLOTPN
2277   25223   3362           DCA PLOTNX        /0 TO X CO-ORDINATE
2278   25224   3363           DCA PLOTNY        /0 TO Y CO-ORDINATE
2279   25225   4370           JMS PLOTWT
2280   25226   5600           JMP I PLOTX
2281
2282
2283                  /DIGITAL 8-12-U
2284                  /PAGE 2
2285                  /PICK UP ARGUMENTS
2286
2287   25227   1362   PLOT1,  TAD PLOTNX        /FETCH PREVIOUS X CO-ORDINAT
E
2288   25230   7141           CIA CLL
2289   25231   1600           TAD I PLOTX       /FORM NX-NPX
2290   25232   7420           SNL               /L=0: NX<NPX
2291   25233   7041           CIA
2292   25234   3364           DCA PLOTDX        /ABSOLUTE VALUE OF DIFFERENC
E
2293   25235   7004           RAL
2294   25236   3367           DCA PLOTMV        /SAVE SIGN BIT
2295   25237   1600           TAD I PLOTX       /SET NEW
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/FLOURESCENCE PROGRAM                11/19/81  PAL8-V10A NO DATE   PAGE 26-1

2296   25240   3362                  DCA PLOTNX      /PREVIOUS X
2297   25241   2200                  ISZ PLOTX       /INCREMENT POINTER
2298   25242   1363                  TAD PLOTNY      /FETCH PREVIOUS Y CO-ORDINAT
E
2299   25243   7141                  CIA CLL
2300   25244   1600                  TAD I PLOTX     /FORM NY-NPY
2301   25245   7420                  SNL             /C=0: NPY<NY
2302   25246   7041                  CIA
2303   25247   3365                  DCA PLOTDY      /ABSOLUTE VALUE OF DIFFERENC
E
2304   25250   1367                  TAD PLOTMV      /SAVE SIGN BIT
2305   25251   7004                  RAL             /BIT 10(1)= DRUM-DOWN(POSITI
VE)
2306   25252   3367                  DCA PLOTMV      /BIT 11(1)=PEN-LEFT (POSITIV
E)
2307   25253   1600                  TAD I PLOTX     /SET NEW
2308   25254   3363                  DCA PLOTNY      /PREVIOUS Y
2309   25255   2200                  ISZ PLOTX       /INCREMENT POINTER
2310   25256   1364                  TAD PLOTDX
2311   25257   7141                  CIA CLL
2312   25260   1365                  TAD PLOTDY
2313   25261   7620                  SNL CLA                 /L=0: DELTA Y < DELT
A X
2314   25262   5275                  JMP PLOT2
2315   25263   1364                  TAD PLOTDX      /REVERSE NUMBERS
2316   25264   3366                  DCA PLOTNA
2317   25265   1365                  TAD PLOTDY
2318   25266   3364                  DCA PLOTDX
2319   25267   1366                  TAD PLOTNA
2320   25270   3365                  DCA PLOTDY
2321   25271   7001                  IAC             /SET MAJOR MOTION
2322   25272   0367                  AND PLOTMV      /INSTRUCTION
2323   25273   1342                  TAD PLOTT1
2324   25274   5300                  JMP .+4
2325
2326
2327                 /DIGITAL 8-12-U
2328                 /PAGE 3
2329
2330   25275   1367  PLOT2,          TAD PLOTMV
2331   25276   7110                  CLL RAR
2332   25277   1345                  TAD PLOTT2
2333   25300   3366                  DCA PLOTNA
2334   25301   1766                  TAD I PLOTNA
2335   25302   3340                  DCA PLOT4
2336   25303   1367                  TAD PLOTMV      /SET COMBINED MOTION
2337   25304   1350                  TAD PLOTT3
2338   25305   3367                  DCA PLOTMV
2339   25306   1767                  TAD I PLOTMV
2340   25307   3331                  DCA PLOTDB
2341   25310   1364                  TAD PLOTDX
2342   25311   7110                  CLL RAR
2343   25312   3366                  DCA PLOTNA
2344   25313   1364                  TAD PLOTDX
2345   25314   7040                  CMA
2346   25315   3367                  DCA PLOTMV
2347   25316   2367  PLOT3,          ISZ PLOTMV
2348   25317   7410                  SKP
2349   25320   5600                  JMP I PLOTX     /ALL DONE
2350   25321   1366                  TAD PLOTNA
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2351   25322   1365           TAD PLOTDY
2352   25323   3366           DCA PLOTNA
2353   25324   1366           TAD PLOTNA
2354   25325   7140           CMA CLL
2355   25326   1364           TAD PLOTDX
2356   25327   7630           SZL CLA
2357   25330   5340           JMP PLOT4        /SINGLE MOTION
2358   25331   0000  PLOTDB,  0                /COMBINED MOTION
2359   25332   1364           TAD PLOTDX
2360   25333   7041           CIA
2361   25334   1366           TAD PLOTNA
2362   25335   3366           DCA PLOTNA
2363   25336   4370           JMS PLOTWT
2364   25337   5316           JMP PLOT3
2365
2366   25340   0000  PLOT4,   0
2367   25341   5336           JMP .-3
2368
2369   25342   5343  PLOTT1,  .+1
2370   25343   6511           PLPR             /PEN-RIGHT
2371   25344   6521           PLPL             /PEN-LEFT
2372   25345   5346  PLOTT2,  .+1
2373   25346   6512           PLDU             /DRUM-UP
2374   25347   6514           PLDD             /DRUM-DOWN
2375   25350   5351  PLOTT3,  .+1
2376   25351   6513           PLDU PLPR        /UP-RIGHT
2377   25352   6523           PLUD PLPL        /UP-LEFT
2378   25353   6515           PLDD PLPR        /DOWN-RIGHT
2379   25354   4355           JMS .+1                  /DOWN-LEFT
2380   25355   0000           0
2381   25356   6514           PLDD
2382   25357   6521           PLPL
2383   25360   5755           JMP I .-3
2384
2385
2386                  /DIGITAL 8-12-U
2387                  /PAGE 4
2388
2389   25361   0000  PLOTPN,  0
2390   25362   0000  PLOTNX,  0
2391   25363   0000  PLOTNY,  0
2392   25364   0000  PLOTDX,  0
2393   25365   0000  PLOTDY,  0
2394   25366   0000  PLOTNA,  0
2395   25367   0000  PLOTMV,  0
2396
2397   25370   0000  PLOTWT,  0
2398   25371   6501           PLSF             /WAIT FOR DONE FLAG
2399   25372   5371           JMP .-1                  /NOT YET
2400   25373   6502           PLCF             /CLEAR FLAG
2401   25374   5770           JMP I PLOTWT     /EXIT
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/FLOURESCENCE PROGRAM                11/19/81  PAL8-V10A NO DATE   PAGE 27

2402                    4000  *4000
2403                          /THIS ROUTINE DOES WHAT DIGITAL'S 8-22-U UNSIGNED DE
CIMAL PRINT
2404                          /SHOULD HAVE.  IT IS NOT DEPENDENT ON 8-19-U AND NEE
DS NO
2405                          /CHANGES TO RUN.  IT ALSO TYPES BLANKS FOR LEADING Z
EROES.
2406                          /CHANGE SZA AT ARROW+7 TO NOP TO GET LEADING ZEROES.

2407                          /CALL WITH JMS DECPRT--NUMBER TO BE PRINTED IN AC.
2408
2409   24000  0000  DECPRT,   0
2410   24001  3260            DCA VALUE             /SAVE INPUT
2411   24002  3261            DCA DIGIT             /CLEAR
2412   24003  3255            DCA FLAGE
2413   24004  1250            TAD CNTRZA
2414   24005  3262            DCA CNTRZB            /SET COUNTER TO FOUR
2415   24006  1247            TAD ADDRZA
2416   24007  3214            DCA ARROW             /SET TABLE POINTER
2417   24010  7410            SKP
2418   24011  3260            DCA VALUE             /SAVE
2419   24012  7100            CLL
2420   24013  1260            TAD VALUE
2421   24014  1251  ARROW,    TAD TENPWR            /SUBTRACT POWER OF TEN

2422   24015  7430            SZL
2423   24016  2261            ISZ DIGIT             /DEVELOP BCD DIGIT
2424   24017  7430            SZL
2425   24020  5211            JMP ARROW-3           /LOOP
2426   24021  7200            CLA                   /HAVE BCD DIGIT
2427   24022  1261            TAD DIGIT             /GET DIGIT
2428   24023  7440            SZA                   /BLANK LEADING ZEROES
2429   24024  5236            JMP .+12
2430   24025  1255            TAD FLAGE
2431   24026  7640            SZA CLA
2432   24027  5237            JMP .+10
2433   24030  1262            TAD CNTRZB
2434   24031  7001            IAC
2435   24032  7650            SNA CLA
2436   24033  5237            JMP .+4
2437   24034  1256            TAD K20
2438   24035  7410            SKP
2439   24036  2255            ISZ FLAGE
2440   24037  1257            TAD K260              /MAKE IT ASCII
2441   24040  4502      JMS I PRINT1
2442   24041  7200            CLA
2443   24042  3261            DCA DIGIT             /CLEAR
2444   24043  2214            ISZ ARROW             /UPDATE POINTER
2445   24044  2262            ISZ CNTRZB            /DONE ALL FOUR?
2446   24045  5212            JMP ARROW-2           /NO:  CONTINUE
2447   24046  5600            JMP I DECPRT          /YES:  EXIT
2448   24047  1251  ADDRZA,   TAD TENPWR
2449   24050  7774  CNTRZA,   -4
2450   24051  6030  TENPWR,   -1750                 /ONE THOUSAND
2451   24052  7634            -0144                 /ONE HUNDRED
2452   24053  7766            -0012                 /TEN
2453   24054  7777            -0001                 /ONE
2454   24055  0000  FLAGE,    0
2455   24056  7760  K20,      -20
2456   24057  0260  K260,     260


/FLOURESCENCE PROGRAM                11/19/81  PAL8-V10A NO DATE   PAGE 27-1

2457   24060  0000  VALUE,    0
2458   24061  0000  DIGIT,    0
2459   24062  0000  CNTRZB,   0
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2460
2461                    /DIGITAL 8-20-U
2462                    /CHARACTER STRING TYPE-OUT
2463                    /CALL WITH STRING ADDRESS IN
2464                    /C(AC): ALL CODES MAY BE DEVELOPED
2465                    /RETURN FOLLOWING THE JMS
2466
2467  24063  0000  TYPSTG,  0
2468  24064  3351           DCA TEMG              /STORE INITIAL ADDRESS

2469  24065  3353           DCA FLAG              /CLEAR FLAG
2470  24066  1751  TSCC1,   TAD I TEMG           /PICK UP DATA
2471  24067  7012           RTR                   /ROTATE 6 BITS RIGHT
2472  24070  7012           RTR
2473  24071  7012           RTR
2474  24072  4277           JMS TSCC2             /TYPE FIRST CHARACTER
2475  24073  1751           TAD I TEMG            /PICK UP DATA
2476  24074  4277           JMS TSCC2             /TYPE SECOND CHARACTER

2477  24075  2351           ISZ TEMG              /INCREMENT STORAGE ADD
RESS
2478  24076  5266           JMP TSCC1             /GO BACK FOR MORE
2479
2480  24077  0000  TSCC2,   0
2481  24100  0354           AND K77               /MASK OFF 6 BITS
2482  24101  3352           DCA TEMR              /SAVE CHARACTER
2483  24102  1353           TAD FLAG              /TEST "SPECIAL" FLAG
2484  24103  7640           SZA CLA
2485  24104  5314           JMP TYPSP             /SET: TYPE SPECIAL
2486  24105  1352           TAD TEMR              /NO: REGULAR CHARACTER

2487  24106  7450           SNA                   /IS IT ZERO?
2488  24107  5312           JMP  +3               /YES: SET FLAG
2489  24110  4333  TYPAT,   JMS PRINT6            /NO: PRINT IT
2490  24111  5677           JMP I TSCC2           /RETURN
2491  24112  2353           ISZ FLAG              /SET "SPECIAL" FLAG
2492  24113  5677           JMP I TSCC2           /EXIT
2493
2494  24114  3353  TYPSP,   DCA FLAG             /CLEAR "SPECIAL" FLAG
2495  24115  1352           TAD TEMR              /TEST FOR "0"
2496  24116  7041           CIA
2497  24117  7450           SNA
2498  24120  5310           JMP TYPAT             /0: TYPE "@"
2499
2500
2501
2502                         IAC                   /TEST FOR 01
2503  24121  7001           SNA CLA
2504  24122  7650           JMP I TYPSTG          /YES: EXIT CODE
2505  24123  5663           TAD SKIPMA            /ALTER INSTRUCTION
2506  24124  1360           DCA SWITCH            /TO BE "SMA"
2507  24125  3335           TAD TEMR              /TYPE CHARACTER
2508  24126  1352           JMS PRINT6
2509  24127  4333           TAD SKIPPA            /ALTER INSTRUCTION
2510  24130  1361           DCA SWITCH            /TO BE "SPA"
2511  24131  3335           JMP I TSCC2           /RETURN
2512  24132  5677
2513
2514  24133  0000  PRINT6,   0
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/FLOURESCENCE PROGRAM            11/19/81  PAL8-V10A NO DATE   PAGE 28-1

2515   24134  1355            TAD M40                 /COMPARE WITH 40
2516   24135  7510  SWITCH,   SPA                     /OR SMA FOR SPECIAL CO
DES
2517   24136  1356            TAD C100
2518   24137  1357            TAD C240
2519   24140  4502     JMS I PRINT1
2520   24141  1747     TAD I FCHARI
2521   24142  1350     TAD CNTRLP
2522   24143  7640     SZA CLA
2523   24144  5733            JMP I PRINT6
2524   24145  3747     DCA I FCHARI
2525   24146  5663     JMP I TYPSTG
2526
2527                   /CONSTANTS AND TEMPORARY REGISTERS
2528   24147  0306      FCHARI, FCHAR
2529   24150  7560     CNTRLP, -220    /ABORT TYPE OUT ONLY ON ^P
2530   24151  0000     TEMQ,    0                      /CONTAINS STRING ADDRE
SS
2531   24152  0000     TEMR,    0                      /CONTAINS 6 BIT CHARAC
TER
2532   24153  0000     FLAG,    0                      /"SPECIAL" FLAG
2533   24154  0077     K77,     77
2534   24155  7740     M40,     -40
2535   24156  0100     C100,    100
2536   24157  0240     C240,    240
2537   24160  7500     SKIPMA,  SMA
2538   24161  7510     SKIPPA,  SPA
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/FLOURESCENCE PROGRAM          11/19/81  PAL8-V10A NO DATE   PAGE 29

2539            0001  FIELD 1
2540            3000  *3000
2541                  /
2542                  /CODE TO ROTATE THE CELL
2543                  /
2544    13000   4235  ROTATE, JMS NEWNUM
2545    13001   0225          AND MASK3
2546    13002   4205          JMS ROTIT
2547    13003   6223          CDFCIF CODFLD
2548    13004   5646          JMP I MONPT
2549    13005   0000  ROTIT,  0
2550    13006   3244          DCA CURTEM
2551    13007   1243          TAD CURENT
2552    13010   7041          CIA
2553    13011   1244          TAD CURTEM
2554    13012   1245          TAD FOUR
2555    13013   0225          AND MASK3
2556    13014   7040          CMA
2557    13015   3226          DCA ROTX
2558    13016   2226          ISZ ROTX
2559    13017   5223           JMP .+4
2560    13020   1244          TAD CURTEM
2561    13021   3243          DCA CURENT
2562    13022   5605          JMP I ROTIT
2563    13023   4247          JMS TURNIT
2564    13024   5216          JMP .-6
2565    13025   0003  MASK3,  3
2566    13026   0000  ROTX,   0
2567    13027   7775  SRDLAY, 7775
2568    13030   7716  FOURTH, 7716
2569    13031   0000  FIFTY,  0
2570    13032   0000  COUNT,  0
2571    13033   7577  MUSEC,  7577
2572    13034   0000  DELMU,  0
2573    13035   0000  NEWNUM, 0
2574    13036   6031          KSF
2575    13037   5236          JMP .-1
2576    13040   6036          KRB
2577    13041   6046          TLS
2578    13042   5635          JMP I NEWNUM
2579    13043   0000  CURENT, 0
2580    13044   0000  CURTEM, 0
2581    13045   0004  FOUR,   4
2582    13046   0200  MONPT,  200
2583    13047   0000  TURNIT, 0
2584    13050   7200          CLA
2585    13051   1230          TAD FOURTH      /FIFTY STEP
2586    13052   3231          DCA FIFTY       /COUNTER
2587    13053   1227  READSR, TAD SRDLAY      /GET TIME DELAY
2588    13054   3232          DCA COUNT       /SET UP TIME DELAY COUNTER
2589    13055   1233  TLOOP,  TAD MUSEC
2590    13056   3234          DCA DELMU       /MICRO LOOP
2591    13057   2234          ISZ DELMU
2592    13060   5257           JMP .-1
2593    13061   2232          ISZ COUNT       /INCREMENT TIME DELAY COUNTE
R
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/FLOURESCENCE PROGRAM          11/19/81  PAL8-V10A NO DATE   PAGE 29-1

2594    13062   5255           JMP TLOOP
2595    13063   6530          PULS
2596    13064   2231          ISZ FIFTY       /INCREMENT FIFTY STEP COUNTE
R
2597    13065   5253           JMP READSR
2598    13066   5647          JMP I TURNIT
2599    13067   0000  ROCELL, 0
2600    13070   4205          JMS ROTIT
US
2601    13071   0000          CDCIF CODFLD
2602    13072   5667          JMP I ROCELL
2603                  PAUSE
```

A	2463	C2	6666	FFLOT	0127	LE	4242
AB9	2220	C240	4157	FIFTY	3031	LEDIG1	1122
ADAVER	1050	C9	6713	FILINF	0460	LEIN	0624
ADDRZA	4047	D	2474	FILNAM	0600	LERR	0642
ADVIS	1404	DATAIN	1120	FLAG	4153	LEZDIG	0472
AHALF	3555	DECPRT	4000	FLAGE	4055	LE3	4251
ARROW	4014	DELIMR	0233	FLAG2	2510	LF212	0313
ASTR	0311	DELMU	3034	FLIN	0005	LG2E	2477
AVBUFF	4237	DELTIM	1121	FLOT1	2502	LINCOM	0143
AVERAG	4200	DEV	0462	FLOT2	2505	LINKER	4333
AVPARM	4204	DEVNUM	0211	FLTPT	0007	LOCLOC	0304
AVRET	4236	DIAG	0147	FLT100	2514	LOGAB	2357
A100	3617	DIGIT	4061	FLUTE1	0756	LOGAB1	2406
A2D	0070	DINOG	4460	FN	0452	LOGT	0153
B	2466	DISPLA	5003	FNAME	0461	LOGTT	2407
BASTOP	3317	DONE0	1124	FNLNTH	0456	LOG2	2321
BEGIN	0200	DONE1	1143	FNSCN	1046	LPLOT	5007
BELL	0150	DONE3	1162	FOUR	3045	L1	2327
BKLNTH	0572	DRPLOT	0141	FOURTH	3030	L2	2332
BLKNUM	0440	D1	0502	FOUT	0006	L3	2335
BLOCNM	0512	D2D1	0474	FROM	0010	L4	2340
BUFFPT	0511	EITHER	0512	FROM1	0570	L5	2343
BUFF1	3560	EM	0503	FTEMP0	0156	L6	2346
BUFF2	3562	EMCH	0066	FTEMP1	0161	L7	2351
BUFF3	3566	EMD1	0470	FTEMP2	2511	L8	2354
BUFPTR	0437	EMD2D1	0471	F1	0110	MASK3	3025
BUF1	0130	EMFLG	0020	F2	0113	MKKR	3263
BUF2	0145	EMFLG2	0021	GEOFAC	2362	MONIT	0073
BUF3	0132	EMSETG	1310	GET	5103	MONITR	0200
C	2471	END	0024	GETRUN	0463	MONPT	3046
CAD	0434	ENDG	0744	GODDIE	1463	MONPTR	0021
CALIB	0400	ENDSTR	0652	GORSH	3017	MONSAV	0074
CC100	0707	END2	0025	GORSH2	3106	MORX	0026
CDFCDF	0564	ENPUT	4722	GOTB	3515	MORX2	0027
CFLD	0545	ENTRY	0026	GOTCHA	0123	MOVEBK	0530
CHARIN	0257	ENTURP	0134	GOTCH1	1601	MUSEC	3033
CHECKI	0235	ERR	0025	GOTP	3443	M40	4155
CHINPT	0711	ERRERR	0240	GOTU	1626	NASTY	0135
CHRPR	0307	EX	0530	GS1	4642	NEGA1	0142
CLF	0702	EXCH	0065	GUD2	1615	NEG11	4330
CMDSTG	4400	EXD1	0466	IITYCR	0103	NEG3	0703
CMNDS	0212	EXD2D1	0467	INCHAR	0710	NERR	0663
CNTRLC	0316	EXPNT	2365	INDATA	1230	NEWNUM	3035
CNTRLP	4150	EXSETG	0747	INPUT	0072	NLAT	0057
CNTRZA	4050	FAB	3620	INTPRT	0213	NOGIN	4477
CNTRZB	4062	FABS	0010	INTG	4267	NOOCHR	0705
CO	2217	FAKB	2412	INTVAL	0075	NOPE	0365
CODFLD	0020	FBF	2403	INTVL2	0076	NORM	0140
COMMON	2324	FCHAR	0306	KBRK	0271	NORMAL	3322
COUNT	3032	FCHAR1	4147	KK77	0706	NORM7	3273
CR215	0312	FCNT	3001	KLOCK	1057	NOVAL	0031
CURENT	3043	FCW	0436	K20	4056	NTRE	0213
CURTEM	3044	FEXP	2415	K260	4057	NU	4276
C1	6663	FF	2212	K77	4154	NU1N	0644
C100	4156	FFL	3366	LASPLT	3264	NULE	0600

NULE1	0105	PLOT3	5316	GFIN	1047	SCAN2	1030
NULE2	0620	PLOT4	5340	GFING	1200	SCBRYT	4646
NUMIN	0100	PLPT	4676	GFLNAM	0457	SCHOME	4712
NUMOUT	0077	PLTARE	3077	GFULL	1267	SCPLOT	5006
N1000	3000	PLTBAS	3447	GM	0341	SCSAVX	4644
N12	0115	PLTBS1	3464	GMGM	0334	SCSAVY	4645
N232	0667	PLTEM	3167	GNEW	3003	SCSCAL	4656
N272	0314	PLTEM1	3216	GPER	3616	SCXN	4647
N2775	1266	PLTEM2	3240	GPLTMX	3171	SC1	4621
N310	0665	PLTEND	3224	GQFAC	1644	SETUP	0122
N4	1457	PLTRET	3164	GQFUL	1300	SETUP3	1477
N5	0033	PLTSID	3400	GQMARX	1655	SFL	3360
N702	0666	PLUT	3423	GQNEW	3344	SFLOT	0126
N764	0670	PLUT10	3231	GQPER	3347	SHIFTR	0637
OFF	0540	PLUT2	3440	GQRSET	1207	SIDE	3315
OFFSET	3632	PLUT3	3435	GQV	4505	SINGLE	3340
OK11	0705	PLUT4	3415	GQ200	0556	SKIPMA	4160
OK12	0723	PMCP	1600	GQ650	0564	SKIPPA	4161
ONEFLT	0131	PPP12	0573	GRESET	1171	SLOC	0565
OSB	5106	PP117	3313	GSMUST	5034	SMFLG	0154
OUTRNG	0671	PP1750	3165	GSTART	0120	SMIFT	5452
OUTSTG	0124	PP27	3337	Q200	0464	SMITCH	0155
PACKER	0605	PP4	1103	Q650	0465	SMOOCH	5530
PARM1	0537	PRINT	1400	RCNT	3002	SMOOTH	5400
PARM2	0737	PRINTU	1401	READIN	0614	SMOO2	5416
PDD500	3637	PRINT1	0102	READSR	3053	SMOO3	5440
PD14	4721	PRINT3	4650	READ2N	0625	SMOR	5022
PD1750	4664	PRINT4	4133	REQUSE	1317	SMPNT	1240
PD240	4711	PRIN3	1435	REMAIN	0555	SMU	5474
PD33	4720	PRTDEC	0077	REMAN2	0572	SMUST	5025
PLOKE	3127	PRUNE	1466	RETAV	4222	SMUST2	5042
PLOLD	4607	PUSHER	5527	RETPLT	3037	SMUST3	5071
PLOO1	3475	PUSHY	6717	RET2	3170	SMUST4	5067
PLOO2	3507	P117	3166	RET21	3200	SMUST5	5070
PLOO5	3505	P12	0136	RET3	3314	SMUST6	5421
PLOPT	3006	P13	5023	RFCW	0030	SMUST7	5074
PLOT	3004	P14	3554	RFL	3363	SMUST8	5052
PLOTA	5220	P20	0571	RFLOT	0125	SM11	5010
PLOTDB	5331	P21	5024	ROCEL	1227	SM17	5014
PLOTDX	5364	P30	0567	ROCELL	3067	SPARSE	0037
PLOTDY	5365	P3000	0566	ROTAT	4512	SPASE	1453
PLOTMV	5367	P3032	0623	ROTATE	3000	SPASG	1460
PLOTNM	0556	P310	0146	ROTIT	3005	SPCCHR	0655
PLOTNX	5362	P4	0133	ROTX	3026	SPCR2	0661
PLOTNY	5363	P4000	0071	ROUND	0067	SRDLAY	3027
PLOTPN	5361	P55	0315	RR	2200	SS	2205
PLOTQ	5000	P62	3320	RRFCW	0510	START	0022
PLOTSC	4600	P77	0310	R1	0107	START1	0704
PLOTT1	5342	QCBUFF	2400	R2	0112	STARTQ	0740
PLOTT2	5345	QDIQ	4470	SAVE	5100	START2	0023
PLOTT3	5350	QEMSET	0117	SAVOR	1270	STIGMA	4447
PLOTWT	5370	QEND	0121	SAVRUN	0400	STRGIN	0024
PLOTX	5200	QEQ2	1661	SBLOK	0426	SWITCH	4135
PLOT1	5227	QEXSET	0116	SBLOCK	0501	SWOOP	4300
PLOT2	5275	QFAC	3172	SCAN	1000	S1	0111

S2	0114	XSCALI	3321
TEMCHR	0700	XSCL3	3160
TEMPRM	0305	XXPL	3276
TEMP1	0034	XXPLI	3521
TEMP2	0035	YCAL	3161
TEMP3	0036	YESNO	0347
TEMP4	0062	YESTR0	0361
TEMP5	0063	YORN	0104
TEMP6	0064	YREG	3153
TEMQ	4151	YUNORM	0137
TEMR	4152	YUP	0362
TENPWR	4051	ZP3	3205
TLOOP	3055	Z01	0701
TMCHR2	0701	Z02	0703
TO	0011	Z03	0721
TO1	0536	Z04	1037
TRIN	0542	Z06	3067
TROUT	0551	Z77	1525
TSCC1	4066		
TSCC2	4077		
TTOINT	0106		
TTOUT	0317		
TURN	1222		
TURNIT	3047		
TYCR	0342		
TYPAT	4110		
TYPIST	0101		
TYPSP	4114		
TYPSTG	4063		
TYGM	0144		
UNDO	1522		
UNORM	3522		
UPSET	1470		
USEREG	0151		
USR	0020		
USRIN	0223		
US1	4643		
VALUE	4060		
VISAD	4515		
WAVE	0030		
WFCW	4030		
WLCH	0032		
WPON	5020		
W1	5551		
W2	5554		
W3	5557		
W4	5562		
W5	5565		
W6	5570		
XAX	3600		
XOFF	3627		
XOFFI	0152		
XPLOT	5005		
XREG	3152		
XSCAL	3613		

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