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**ANALYSIS OF LASER FLUOROSENSOR
SYSTEMS FOR REMOTE ALGAE
DETECTION AND QUANTIFICATION**

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ANALYSIS OF LASER FLUOROSENSOR SYSTEMS FOR REMOTE ALGAE

DETECTION AND QUANTIFICATION

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SUMMARY

The development and performance of single- and multiple-wavelength laser fluorosensor systems for use in the remote detection and quantification of algae are discussed in this paper. The appropriate equation for the fluorescence power received by a laser fluorosensor system is derived in detail, and the result is found to differ by as much as a factor of 10 from those previously reported in the literature. Experimental development of a single-wavelength system and a four-wavelength system, which selectively excites the algae contained in the four primary algal color groups (green, golden-brown, red, and blue-green), is reviewed, and test results are shown. A comprehensive error analysis is reported which evaluates the uncertainty in the remote determination of the chlorophyll a concentration contained in algae by single- and multiple-wavelength laser fluorosensor systems. Environmental parameters which can greatly affect the fluorescence cross section of algae include long- and short-term light history and nutrient and age effects. Results of the error analysis indicate that the remote quantification of chlorophyll a by a laser fluorosensor system requires optimum excitation wavelength(s), remote measurement of marine attenuation coefficients, and supplemental instrumentation to reduce uncertainties in the algal fluorescence cross sections. Without these additional measurements, both the single- and multiple-wavelength laser fluorosensor systems can only provide qualitative information about chlorophyll a concentration contained in the algae, and the single-wavelength system yields results which are comparable to a multiple-wavelength system which has optimum excitation wavelengths.

INTRODUCTION

An algae laser fluorosensor system consists of a laser, which is used to directly or indirectly excite the chlorophyll a pigment contained in algae, and a collocated telescope receiver system, which detects the emitted fluorescence from chlorophyll a at 685 nm. The quantification of chlorophyll a in vivo (chlorophyll a contained in living algal cells) represents a measure of the concentration and distribution of algae. These measurements are of interest because they are indicative of primary productivity and chemical, thermal, and mechanical conditions in the marine environment.

Passive techniques have been developed to remotely evaluate chlorophyll a in vivo concentration from aircraft and satellites (refs. 1 to 5). These techniques rely on the spectral characteristics of reflected sunlight for determination of the chlorophyll a concentration contained in the algae and, thus, are

limited to day observations and relatively clear sky conditions. Active remote sensing systems do not have these limitations. A laser fluorosensor system which is operated from a low-altitude aircraft can have day/night operation and can be used when there is high-altitude cloud cover. Because of the rapid surveillance capability of an active system, it can be used for "ground truth" measurements for high-altitude passive instruments and for localized investigations which may require greater spatial resolution than is usually available with passive techniques. Also, a four-wavelength laser fluorosensor system can provide information about the distribution of algae contained in the four algal color groups, whereas current passive techniques do not differentiate between different types of algae.

This paper discusses the importance of algae and algal measurements, the spectral characteristics of algae, and the development of single- and multiple-wavelength laser fluorosensor systems. Emphasis is given to a detailed derivation of the equation for the fluorescence power received by a laser fluorosensor system and to the error analysis for determination of chlorophyll a in vivo concentration by single- and multiple-wavelength laser fluorosensor systems.

SYMBOLS

A	matrix of elements a_{ij}
A_r	area of receiving telescope, m^2
A_z	area illuminated by laser at depth z , m^2
a	water absorption coefficient, m^{-1}
a_{ij}	constants
B	diagonal matrix of elements b_{jj}
b_{jj}	elements of diagonal matrix such that $\sigma_{ij} = a_{ij}b_{jj}$
b	water scattering coefficient, m^{-1}
D	telescope diameter, m
$F(\lambda_f)$	laser-induced fluorescence emitted from chlorophyll <u>a</u> in vivo at 685 nm, W/nm
H_z	laser irradiance per unit laser wavelength bandwidth, $W/m^2\text{-nm}$
k, k_i	$(\gamma_l + \gamma_f)$ and $(\gamma_f + \gamma_i)$, respectively
m	index of refraction for water
N	matrix of elements n_j

n	concentration of chlorophyll <u>a</u> contained in algae, mole/m ³
P_o	laser output power, W
P_r	power received by detector, W
R	distance from laser fluorosensor system to surface of water, m
X	matrix of elements x_i
x_i	defined in equation (10)
z	water depth of differential volume under examination, m
α	single-scattering attenuation coefficient, m ⁻¹
β	atmospheric extinction coefficient, m ⁻¹
γ	effective attenuation coefficient of water, m ⁻¹
$\Delta\lambda$	wavelength bandwidth, nm
ϵ_{ij}	matrix elements of Σ^{-1}
λ	wavelength, nm
ξ	optical efficiency of receiver system
ρ	water surface reflectivity
Σ	matrix of elements σ_{ij}
σ	cross section for fluorescence at 685 nm per chlorophyll <u>a</u> molecule contained in algae when excited by laser wavelength λ_l , m ² /mole
σ_{ij}	$= \sigma_j(\lambda_i)$

Subscripts:

d	detector
f	fluorescence, or at fluorescence wavelength, 685 nm
i	laser excitation wavelength corresponding to four algal color groups
j	four algal color groups
l	laser, or at laser excitation wavelength

Abbreviations:

ALOPE Airborne Lidar Oceanographic Probing Experiment

PMT photomultiplier tube

A δ before a symbol denotes the standard deviation of the parameter.

IMPORTANCE OF ALGAE AND ALGAL MEASUREMENTS

The types and concentration of algae in various bodies of water are important indicators of primary productivity and marine environmental conditions. In the ocean, algae are practically the only producer of organic matter. The photochemical conversion of inorganic matter to organic matter via photosynthesis is known as productivity. Also, algae are an important part of the lowest level on the food chain and are thus important to the survival of all higher forms of marine life.

Marine environmental conditions - such as chemical, thermal, and mechanical factors - can have major influence on algal populations in any body of water. Man-created conditions often upset the natural ecological balance, and this leads to growth in some algal species and death in others. The presence of nondegradable heavy metals, for example, mercury and lead, or slowly degradable chemicals such as DDT, has a dramatic effect on the survivability of different algal species. Likewise, the thermal and mechanical properties of the water which are influenced by electrical power plants, industrial plants, and waste disposal systems, to name only a few, also alter the makeup of algal populations. However, it can be difficult to establish direct effects of a pollution source on algal populations because of changes which are seasonal or locally transient in nature; for example, changes in algal populations can be altered by a sudden weather change.

Several algal species are toxic to fish and man, and when their concentrations reach bloom proportions (typically greater than 10^6 cells/liter), fish are killed. During this condition the shellfish which are affected concentrate the toxin. People who have eaten contaminated shellfish have been known to die from paralytic shellfish poisoning. Gymnodinium breve, the Red Tide of Florida; Gonyaulax tamarensis, the Red Tide of New England; and Gonyaulax catenella, which is found along the California coast, are examples of toxic algae.

Research is necessary to learn more about the parameters which affect the marine ecosystem. Since the type, concentration, and distribution of algae are affected by the chemical, thermal, and mechanical properties of the marine environment, remote detection of algae can be used to infer important information about marine conditions.

SPECTRAL CHARACTERISTICS OF ALGAE

All optical methods of algae detection employ the spectral properties of the primary light-absorbing pigments, which are present in different algal

species in different proportions. Most algal species fall into one of four basic color groups - green, golden-brown, red, and blue-green. The color group is determined by the apparent color of the pure algal culture. The spectral characteristics of the primary pigments contained in algae are shown in figure 1 (ref. 6). Algal pigments have the following natural colors: chlorophyll a and chlorophyll b are green, carotenoids are orange, phycoerythrin is red, and phycocyanin is blue. All algae contain chlorophyll a; in addition, green algae contain chlorophyll b, golden-brown algae contain carotenoids and fucoxanthin (not shown in fig. 1), red algae primarily contain phycoerythrin, and blue-green algae primarily contain phycocyanin. There are similarities between the spectral absorption characteristics of the primary pigments and the absorption spectra of algae, as can be seen in figure 2 (ref. 7). As would be expected, the absorption features are not as distinct as those of the individual extracted pigments. This limits the use of specific pigment absorption features in identification of algal species. The primary spectral difference between the green and golden-brown algae is the presence of fucoxanthin in the golden-brown color group. This pigment allows spectral differentiation between these two color groups. Blue-green algae, which are not represented in figure 2, have an absorption spectrum indicative of their primary pigment, phycocyanin.

The exchange of energy between pigments in algae is presented in an elementary form in figure 3. All pigments can absorb appropriate wavelengths of light directly, and the energy is then transferred toward chlorophyll a. Since this process is less than 100-percent efficient for all but chlorophyll b, some of the energy which is not transferred to the next pigment is lost by nonradiative processes (usually conversion to heat), and the remainder is dissipated as fluorescence by the pigment. The energy reaching chlorophyll a excites it above the first singlet state of the molecule. Energy stored in the chlorophyll a is then used in photosynthesis with any remaining energy dissipated as fluorescence. Since all algae contain chlorophyll a, its fluorescence properties are important for use in active remote sensing techniques. A typical fluorescence spectrum for green and golden-brown algae is shown in figure 4. The primary fluorescence peak at 685 nm results from deexcitation of the first singlet state of chlorophyll a to the ground state. The secondary peak at 735 nm is generally lower than the primary peak, and it is a result of a secondary energy exchange mechanism associated with photosynthesis. Red and blue-green algae also have their primary fluorescence peak at 685 nm. Thus, active remote sensing techniques are based upon detection of the laser-induced fluorescence of chlorophyll a in vivo at 685 nm.

Normalized excitation spectra for representative species of algae in the four primary algal color groups (golden-brown, green, red, and blue-green) are shown in figure 5. In this figure, the fluorescence of chlorophyll a in vivo was monitored at 685 nm while the wavelength of the excitation light was scanned from 360 nm to 680 nm by a Perkin-Elmer fluorescence spectrophotometer. The peaks of the excitation spectra were normalized to a value of 10 for comparison between color groups. Spectrophotometer studies have indicated that these spectra are qualitatively representative of other algal species contained in the same color groups (ref. 8).

DERIVATION OF POWER RECEIVED BY LASER FLUOROSENSOR SYSTEM

Unexplained assumptions by many authors (refs. 8 to 14) in deriving the governing equation for the power received by a laser fluorosensor system have resulted in various relationships which differ by as much as a factor of 2π . A thorough derivation of this equation with justified assumptions is needed to establish the appropriate form of the equation.

The general configuration used in this derivation is given in figure 6. A common assumption in all derivations is that the algae and all scattering material are uniform with depth in the water. The laser irradiance in $\text{W/m}^2\text{-nm}$ which reaches a depth of z in the water is given by

$$H_z = \frac{P_o e^{-\beta_l R} (1 - \rho_l) e^{-\gamma_l z}}{\Delta\lambda_l A_z} \quad (1)$$

where

P_o	laser output power, W
β_l	atmospheric extinction coefficient at laser wavelength, m^{-1}
R	distance from laser fluorescence system to water, m
ρ_l	water surface reflectivity at laser wavelength
γ_l	effective attenuation coefficient of water at laser wavelength, m^{-1}
z	water depth of differential volume under examination, m
$\Delta\lambda_l$	wavelength bandwidth of laser energy, nm
A_z	area illuminated by laser at a depth of z , m^2

The effective water attenuation coefficient may vary with depth (ref. 15) because of changes in the angular distribution of laser light as it is multiply scattered and deviated from collimated light at the surface to relatively diffuse light several attenuation lengths underwater (ref. 16). The single-scattering attenuation coefficient for a collimated beam is

$$\alpha = a + b$$

where a is the absorption coefficient and b is the scattering coefficient. It is assumed in the derivation of α that if a photon is scattered it is lost to the beam, and the energy in the unscattered beam is exponentially reduced by the amount $e^{-\alpha r}$ where r is the distance traveled in the water (ref. 16). Since the laser excitation wavelengths for algae are between 400 and 650 nm,

the scattering coefficient is, in general, many times larger than the absorption coefficient (ref. 17). The irradiance at depth z includes unscattered and scattered light. The effective attenuation coefficient is then less than α because the scattered light is mostly forward scattered (ref. 18), and thus it can also become part of the irradiance at depth z . The diffuse attenuation coefficient determines the exponential attenuation of diffuse light with depth. Duntley (ref. 19) empirically determined that the diffuse attenuation coefficient k is

$$k = a + \frac{b}{6}$$

Because of the large contribution of scattered light to the irradiance of the laser light, the effective attenuation coefficient for laser penetration γ_l is better approximated by the diffuse attenuation coefficient k_l at the laser wavelength than by the single-scattering attenuation coefficient. The limiting value of γ_l is a_l for very large collection cone angles, that is, greater than 40° (ref. 20); however, this extreme value for the effective attenuation coefficient is not felt to be generally applicable for most laser irradiance cases. In this analysis it is assumed that γ is constant with depth. It should be noted that Koeppen and Walker (ref. 15) have reported that γ may change with depth; however, no generalized relationship was given for this variation.

The laser-induced fluorescence, in W/nm, emitted from the chlorophyll a in vivo at 685 nm contained in the differential volume $A_z dz$ at a depth of z is

$$dF(\lambda_f) \Delta\lambda_f = H_z \Delta\lambda_l \sigma n A_z dz \quad (2)$$

where

$\Delta\lambda_f$ fluorescence bandwidth at 685 nm, nm

σ cross section for fluorescence at 685 nm per chlorophyll a molecule contained in the algae when excited by laser wavelength λ_l , $m^2/\text{molecule}$

n concentration of chlorophyll a contained in the algae, molecule/ m^3

The fluorescence cross section per chlorophyll a molecule is equal to the fluorescence energy emitted per chlorophyll a molecule at 685 nm divided by the incident excitation energy per unit area.

The fluorescence which is emitted from the volume $A_z dz$ in the direction of the laser fluorosensor system is refracted at the water surface. Thus, the criterion for fluorescence to be collected by the telescope receiver system is

$$z \tan \theta + R \tan \theta' \leq \frac{D}{2}$$

where θ is the angle of the fluorescence emitted from the volume $A_z dz$ measured from the vertical, θ' is the angle of the fluorescence after refraction at the water surface, and D , the telescope diameter, is equal to $\sqrt{4A_r/\pi}$ where A_r is the area of the receiving telescope. By using Snell's Law and assuming that θ and θ' are small angles, the previous criterion becomes

$$\theta \leq \frac{D}{2(z + mR)}$$

where m is the index of refraction for water. The fluorescence which is emitted from the volume $A_z dz$ into the solid angle $\pi(z\theta)^2/z^2$ is collected by the telescope receiver system. Based on the limiting values for θ , the maximum value of the collection solid angle is $A_r/(z + mR)^2$. Since the fluorescence from the volume $A_z dz$ is emitted into 4π steradians, the fractional proportion of emitted fluorescence to be collected by the telescope is $A_r/4\pi(z + mR)^2$. Thus, the differential power received at the detector in the laser fluorosensor system with the field of view of the receiver large enough to accept the fluorescence emitted from the algae contained in the volume $A_z dz$ is

$$dP_r = dF(\lambda_f) \frac{A_r}{4\pi(z + mR)^2} e^{-\gamma_f z} (1 - \rho_f) e^{-\beta_f R} \xi \Delta\lambda_d \quad (3)$$

where

γ_f	effective attenuation coefficient of water at 685 nm, m^{-1}
ρ_f	water surface reflectivity at 685 nm
β_f	atmospheric extinction coefficient at 685 nm, m^{-1}
ξ	optical efficiency of receiver system
$\Delta\lambda_d$	receiver wavelength bandwidth, nm

Since the absorption coefficient a is in general larger than the scattering coefficient b at 685 nm for a wide range of water turbidity (ref. 17), the effective attenuation coefficient for the fluorescence γ_f is dominated by a . The form of γ_f is

$$\gamma_f = a_f + \frac{b_f}{c}$$

where c can range from 1 to 6 depending upon the ratio a/b . For example, if $a/b > 2$, c may be in the range $1 < c < 3$, and if $a/b < 1/3$, c may range from $3 < c < 6$. With most of the fluorescence power reaching the receiver being emitted within two attenuation lengths of the surface and with $a > b$ for most cases, the optical depth due to scattering is less than 1. From data presented in reference 18 on the propagation of light from a spherical lamp, it can be reasoned that for scattering optical depths less than 1 the value of c falls in the range $1 < c < 2$. Since the effective attenuation coefficient varies with depth and turbidity, its form can only be roughly approximated by

$$\gamma_f \approx a_f + \frac{b_f}{1.5}$$

Combining equations (1), (2), and (3) yields

$$dP_r = K \frac{e^{-(\gamma_l + \gamma_f)z}}{(z + mR)^2} dz \quad (4)$$

where

$$K = \frac{P_o \xi A_r \Delta \lambda_d (1 - \rho_l)(1 - \rho_f) e^{-(\beta_l + \beta_f)R} \sigma_n}{4\pi \Delta \lambda_f}$$

The total power received by the telescope is obtained by integration of dP_r over all values of z . Thus,

$$P_r = K \int_0^{\infty} \frac{e^{-(\gamma_l + \gamma_f)z}}{(z + mR)^2} dz$$

Making the transformation $w = \frac{mR + z}{mR}$ yields

$$P_r = \frac{K}{mR} e^{(\gamma_l + \gamma_f)mR} \int_1^{\infty} \frac{e^{-(\gamma_l + \gamma_f)mRw}}{w^2} dw$$

By using the definition for a second-order exponential integral,

$$E_2(x) = \int_1^{\infty} \frac{e^{-xy}}{y^2} dy$$

(eq. 5.1.4 of ref. 21) and the recurrence relationship,

$$E_2(x) = e^{-x} - xE_1(x)$$

(eq. 5.1.14 of ref. 21), the power received equation can be written as

$$P_r = \frac{K}{mR} \left\{ 1 - (\gamma_l + \gamma_f)mR e^{(\gamma_l + \gamma_f)mR} E_1[(\gamma_l + \gamma_f)mR] \right\} \quad (5)$$

Since the smallest values for the effective attenuation coefficients are the respective absorption coefficients, $a_f = 0.45 \text{ m}^{-1}$ at 685 nm and $a_l > 0.05 \text{ m}^{-1}$ between $400 \text{ nm} \leq \lambda_l \leq 650 \text{ nm}$ (ref. 17). Thus, the value of $\gamma_l + \gamma_f$ must be greater than 0.50 m^{-1} , and since $m \approx 1.33$ and $R \geq 100 \text{ m}$ for most cases, $(\gamma_l + \gamma_f)mR \geq 66$. The functional representation for $E_1(x)$ can be shown to be approximated with less than 0.1-percent error by the first two terms in its asymptotic expansion,

$$E_1(x) \approx \frac{e^{-x}}{x} \left(1 - \frac{1}{x} \right)$$

when $x \geq 66$ (eq. 5.1.51 and table 5.2 of ref. 21). Substitution of this approximation into equation (5) yields the following general equation for the fluorescence power detected by the laser fluorosensor system:

$$P_r = \frac{P_o \xi A_r \Delta \lambda_d (1 - \rho_l)(1 - \rho_f) e^{-(\beta_l + \beta_f)R} \sigma_n}{4\pi \Delta \lambda_f (\gamma_l + \gamma_f) m^2 R^2}$$

The uncertainty discussed previously in specifying the values of γ_l and γ_f makes the small correction factors for surface reflectivity and atmospheric attenuation for ranges less than 1 km negligible. Thus, the simplified form of the power received is

$$P_r = \frac{P_o \xi A_r \Delta \lambda_d \sigma n}{4\pi \Delta \lambda_f (\gamma_\ell + \gamma_f) m^2 R^2} \quad (6)$$

where the approximations for γ_ℓ and γ_f are $\gamma_\ell \approx a_\ell + b_\ell/6$ and $\gamma_f \approx a_f + b_f/1.5$. With laser excitation wavelength in the range $400 \text{ nm} \leq \lambda_\ell \leq 650 \text{ nm}$ and with $\lambda_f = 685 \text{ nm}$, the value of γ_f is more than 2 times the value of γ_ℓ determined from the data taken from Vineyard Sound (ref. 22) and Chesapeake Bay (ref. 23). The equations given in references 8 to 14 for the power received by a laser fluorosensor system range from a factor of $2\pi m^2$ larger to a factor of γ_ℓ less than equation (6). Equation (6) is felt to be the correct representation of the power received, and the concentration of chlorophyll a in vivo can be obtained by solving for n in equation (6). Thus,

$$n = \frac{P_r 4\pi \Delta \lambda_f (\gamma_\ell + \gamma_f) m^2 R^2}{P_o \xi A_r \Delta \lambda_d \sigma} \quad (7)$$

This is the basic relationship used with all single- and multiple-wavelength laser fluorosensor systems for determination of chlorophyll a in vivo concentrations.

SINGLE-WAVELENGTH LASER FLUOROSENSOR SYSTEMS

The first laboratory tests involving laser-induced fluorescence of algae were conducted by Hickman and Moore (ref. 9) in 1970 using a nitrogen laser operating at 337 nm and two algal species, Anacystis nidulans (a blue-green algae) and Chlorella pyrenoidosa (a green algae). The fluorescence of chlorophyll a contained in the algae was detected at 680 nm. Laboratory experiments revealed that laser-induced fluorescence from a chlorophyll a concentration of 6 $\mu\text{g/liter}$ which was contained in Anacystis nidulans was remotely detectable from less than 1 m. Projections of these measurements suggested that an airborne laser/receiver system could detect these same concentrations of chlorophyll a in vivo from a distance of 100 m. Friedman and Hickman (ref. 10) were the first to state that various algal species could be identified by detection of their fluorescence signatures using a tunable laser as the excitation source. They concluded that if only chlorophyll a in vivo is of interest, the optimum excitation wavelength is $600 \pm 50 \text{ nm}$ and the optimum detection wavelength is 685 nm. Measurement of concentrations as low as 1 $\mu\text{g/liter}$ for chlorophyll a in vivo from a distance of 500 m using a 100-kW peak power laser was calculated to be feasible.

The first field test of a single-wavelength laser system for the detection of chlorophyll a contained in algae was conducted in December of 1971 by Mumola and Kim (ref. 11) from a fixed platform on the Thimble Shoals Channel Pier of the Chesapeake Bay Bridge Tunnel. A coaxial, flash-lamp-pumped, rhodamine 6G dye laser was used as the excitation source, and a 15-cm-diameter telescope,

with a narrow band-pass filter which was centered at 685 nm, was used as the receiver. Results from this field trip are shown in figure 7. Chlorophyll a in vivo concentrations were determined at 1-hour intervals over a 24-hour period. In situ data (data obtained by analyzing samples taken from the water) were supplied by the Environmental Protection Agency (EPA). The fluorescence signals were normalized to EPA findings at 2000 EST on December 29, 1971. The results of this experiment were encouraging, and subsequent helicopter flight experiments were conducted near Wallops Island, Virginia. However, these flight experiments resulted in only limited success.

Using the same basic laser system which was used in field tests over the Chesapeake Bay, Kim (ref. 12) flew the instrument in a helicopter over Lake Ontario at the request of EPA. A transect was made of Lake Ontario from west of Rochester, New York, to the Canadian shore at an altitude of 30 m. The remotely sensed chlorophyll a in vivo concentrations were found to be 50 percent higher along the United States side than in midlake or on the Canadian side. The results of this flight are presented in figure 8. No sea truth measurements were reported for this experiment. One of the primary drawbacks of the single-wavelength laser system was its inability to account for differences in absorption by various pigments contained in different algal color groups. Thus, the laser system would not be able to accurately determine chlorophyll a in vivo concentrations if the mixture of algal types changed over the flight path. Also, any change in the light scattering properties of the water during the flight would also produce errors in the calculated values for chlorophyll a in vivo concentrations.

MULTIPLE-WAVELENGTH LASER FLUOROSENSOR SYSTEM

A four-color airborne fluorosensor (ALOPE - Airborne Lidar Oceanographic Probing Experiment) was developed at NASA Langley Research Center (refs. 8, 13, and 14) for determining the distribution and chlorophyll a in vivo concentrations of the four primary algal color groups. The laser excitation wavelengths used for the golden-brown, green, red, and blue-green color groups were 454, 539, 598, and 618 nm, respectively. These were the most optimized laser wavelengths which could be obtained with the low-gain laser design used in the ALOPE system. A single linear flash lamp is used to simultaneously pump four dye cells symmetrically spaced about the flash lamp, and a rotating aperture permitted lasing from only one dye laser cavity at a time. The ALOPE system is shown schematically in figure 9. A system of bending mirrors directs the laser light coaxial with the 25.4-cm-diameter telescope receiving system. The fluorescence of the chlorophyll a in vivo is collected by the telescope system, which passes only light at 685 nm.

By assuming uniform vertical distribution of turbidity and chlorophyll a in vivo concentration in the water, a set of four coupled equations results from summing the fluorescence contribution to the power received by the laser fluorosensor system from each algal color group for each laser excitation wavelength. The pertinent equations, derived from equation (6), are

$$P_r(\lambda_i) = \frac{P_o(\lambda_i) \xi_{A_r} \Delta\lambda_d}{4\pi(\gamma_f + \gamma_i)m^2R^2 \Delta\lambda_f} \sum_{j=1}^4 \sigma_j(\lambda_i) n_j \quad (i = 1,2,3,4) \quad (8)$$

where subscript i denotes the laser excitation wavelength, and subscript j denotes the algal color group. It should be noted that these equations are different from the equations derived in references 8, 13, and 14 (which are all by the same authors) for a multiple-wavelength laser fluorosensor system. The matrix elements for equation (8) can be written as

$$x_i = \sum_{j=1}^4 \sigma_{ij} n_j \quad (i = 1,2,3,4) \quad (9)$$

where

$$x_i = \frac{P_r(\lambda_i)}{P_o(\lambda_i)} \frac{4\pi(\gamma_f + \gamma_i)m^2R^2 \Delta\lambda_f}{\xi_{A_r} \Delta\lambda_d} \quad (10)$$

and

$$\sigma_{ij} = \sigma_j(\lambda_i)$$

Thus the matrix form of equation (8) is

$$X = \Sigma N$$

The concentration matrix N can then be determined from

$$N = \Sigma^{-1} X \quad (11)$$

where Σ^{-1} is the inverse matrix of Σ . The matrix derivation of equation (11) is similar to that given in references 8, 13, and 14. In order for the concentrations of chlorophyll a for each algal color group to be determined from equation (11), the fluorescence cross sections of the algae $\sigma_j(\lambda_i)$ must be known for each measurement, and the parameters $P_r(\lambda_i)$, $P_o(\lambda_i)$, γ_f , and γ_i must be determined for each laser firing. In the initial experiments which were conducted with the ALOPE system, only the power received by the detection system $P_r(\lambda_i)$ was recorded for each laser shot. It was assumed that the power output of each laser was predictable and that the attenuation coefficients γ_f and γ_i would be determined by in situ measurement techniques.

The first field test of the ALOPE system was conducted from the George P. Coleman Bridge 30 m above the surface of the York River at Yorktown, Virginia.

Ground truth data for chlorophyll a in vivo concentrations, salinity, and algal species identification were supplied by the Virginia Institute of Marine Sciences (VIMS), and water temperature and attenuation coefficients were measured for each test. The results from that test are shown in figure 10 (ref. 8). Because of ambiguous data obtained at the laser wavelengths of 598 nm and 618 nm, only results obtained at the laser wavelengths of 454 nm and 539 nm, which corresponded to optimum excitation of green and golden-brown algae, were used to determine the total chlorophyll a concentration given in the figure. The calculation was made according to a matrix equation (ref. 13) which was similar to equation (11). However, the constant in the power-received equation in reference 13 is a factor of 2 larger than equation (8), and reference 13 defines γ_f and γ_i as the single-scattering attenuation coefficients α_f and α_i . Since γ_f and γ_i , as defined previously, are always less than α_f and α_i , the value of $P_r(\lambda_i)$ would be smaller because of the single-scattering attenuation coefficient assumption. Thus the errors in the analysis of ALOPE data were somewhat offsetting, and the results which were obtained were encouraging enough so that the instrument was subsequently flown over the lower end of the James River in Virginia. Figure 11 shows the flight path of the helicopter on the 138-km flight. The distance from the laser system to the water was approximately 100 m, and, as a result of a 2-second interval between each of the four laser firings, one complete data set was obtained over each 270 meters. The averages of the remotely sensed chlorophyll a concentrations, calculated from the power-received equation in references 8 and 14 were obtained for each leg of the flight and are presented only for information in figures 11 and 12. (The error bars represent differences between outgoing and incoming legs of the flight.) Note the $2\pi m^2$ difference between equation (8) and the power-received equation in references 8 and 14 and also note attenuation coefficient definition differences. In addition, it should be mentioned that the data obtained with the rhodamine 6G laser, which operates at 598 nm, were not used because of filter blocking problems and because ground truth measurements indicated that there were no red algae present in the water. The fluorescence cross sections given in reference 14 were used in the data reduction, and attenuation coefficients were obtained from in situ measurements over the flight path of the helicopter. No comparison of these results with in situ measurements of chlorophyll a concentrations was reported. The calculated concentrations of chlorophyll a in each algal color group can only be expected to predict the actual concentrations when the proper relationship, as given in equation (8), is used to relate the fluorescence power received by the laser fluorosensor system to the chlorophyll a concentration in the water.

ERROR ANALYSIS OF LASER FLUOROSENSOR SYSTEMS

Single-Wavelength Systems

The chlorophyll a in vivo concentration is determined for a single-wavelength laser fluorosensor system by equation (7). To find the statistical variance of the chlorophyll a in vivo concentration, it is first assumed that P_o , P_r , $(\gamma_l + \gamma_f)$, and σ are independent random variables and the other parameters in the equation are constants. The general variance equation, which is based upon propagation of errors by least squares (ref. 24), is given by

$$(\delta f)^2 = \sum_{i=1}^n \left(\frac{\partial f}{\partial u_i} \bigg|_{u_1, u_2, u_3, u_4} \right)^2 (\delta u_i)^2 \quad (12)$$

where δf is the standard deviation and $(\delta f)^2$ is the variance of the function f which has n independent variables u_i ; δu_i is the standard deviation and $(\delta u_i)^2$ is the variance of u_i . It can be readily shown that from equation (7) the following variance relationship exists:

$$\left(\frac{\delta n}{n} \right)^2 = \left(\frac{\delta P_r}{P_r} \right)^2 + \left(\frac{\delta P_o}{P_o} \right)^2 + \left(\frac{\delta \sigma}{\sigma} \right)^2 + \left(\frac{\delta k}{k} \right)^2 \quad (13)$$

where $k = \gamma_l + \gamma_f$. The normalized variance of each of the variables thus contributes equally to the normalized variance of the chlorophyll a in vivo concentration. Table 1 contains example solutions of equation (13). It can be seen that the variable with the largest normalized standard deviation dominates the normalized standard deviation for the chlorophyll a concentration. Since power measurements can be made to an accuracy of 2.5 percent, the concentration error is due mostly to uncertainties in fluorescence cross section and effective attenuation coefficient. The expected magnitude of these uncertainties is discussed in a subsequent section.

Multiple-Wavelength Systems

Two different approaches to the error analysis for a multiple-wavelength system are used. In the first approach, all of the fluorescence cross sections $\sigma_j(\lambda_i)$ in equation (8) are considered independent variables. The second approach assumes that the fluorescence cross sections for each algal color group always have the same relative magnitude and vary only in absolute magnitude.

First approach.— The variance equation which is based upon the first approach can be derived from equation (9) by using the inverse matrix Σ^{-1} defined in equation (11) and the general variance equation defined in equation (12). The derivation of the variance equation for chlorophyll a concentration (private communication from R. T. Thompson, Jr., Old Dominion University, Norfolk, Virginia) results in the following relationship:

$$(\delta n_j)^2 = \sum_{m=1}^4 \left[(\epsilon_{jm})^2 (\delta x_m)^2 + \sum_{i=1}^4 (\epsilon_{jm} n_i)^2 (\delta \sigma_{mi})^2 \right]$$

where ϵ_{jm} are the elements Σ^{-1} and the other parameters are the same as those previously defined in deriving equation (11). The normalized form of this equation is

$$\left(\frac{\delta n_j}{n_j}\right)^2 = \frac{1}{n_j^2} \sum_{m=1}^4 (\epsilon_{jm} x_m)^2 \left(\frac{\delta x_m}{x_m}\right)^2 + \frac{1}{n_j^2} \sum_{m=1}^4 \sum_{i=1}^4 (\epsilon_{jm} \sigma_{mi} n_i)^2 \left(\frac{\delta \sigma_{mi}}{\sigma_{mi}}\right)^2 \quad (14)$$

The definition of x_i (eq. (10)) and the general variance equation (12) can be readily used to show

$$\left(\frac{\delta x_i}{x_i}\right)^2 = \left[\frac{\delta P_r(\lambda_i)}{P_r(\lambda_i)}\right]^2 + \left[\frac{\delta P_o(\lambda_i)}{P_o(\lambda_i)}\right]^2 + \left(\frac{\delta k_i}{k_i}\right)^2 \quad (15)$$

where $k_i = \gamma_f + \gamma_i$. For the present discussions the subscripts are dropped from the normalized variances (e.g., $\delta \sigma_{mi} / \sigma_{mi} = \delta \sigma / \sigma$ for $m = 1, 2, 3, 4$ and $i = 1, 2, 3, 4$). Equations (14) and (15) then become

$$\left(\frac{\delta n_j}{n_j}\right)^2 = \left[\frac{1}{n_j^2} \sum_{m=1}^4 (\epsilon_{jm} x_m)^2 \right] \left(\frac{\delta x}{x}\right)^2 + \left[\frac{1}{n_j^2} \sum_{m=1}^4 \sum_{i=1}^4 (\epsilon_{jm} \sigma_{mi} n_i)^2 \right] \left(\frac{\delta \sigma}{\sigma}\right)^2 \quad (16)$$

and

$$\left(\frac{\delta x}{x}\right)^2 = \left(\frac{\delta P_r}{P_r}\right)^2 + \left(\frac{\delta P_o}{P_o}\right)^2 + \left(\frac{\delta k}{k}\right)^2 \quad (17)$$

These equations were evaluated for two cases. In case I, the calculated normalized variance for the chlorophyll a concentration was based upon the laser excitation wavelengths and fluorescence cross sections which are used in the ALOPE system (ref. 14) and are shown in table 2. In case II, a set of optimized laser wavelengths were visually chosen from figure 5 so that the difference between each algal color group and the other three would be maximized. The fluorescence cross sections for each algal color group at each laser wavelength were obtained from the data presented for representative algal species in figure 5 of reference 14. Table 3 lists these parameters. No consideration was given to the practicality of using lasers which operate at these wavelengths; however, all wavelengths throughout the visible spectrum can be obtained with high gain dye lasers. For both cases I and II it was assumed that there was a mean concentration of 20 $\mu\text{g/liter}$ of chlorophyll a in vivo in all algal color groups present in the water, and the power received for each excitation wavelength was calculated from equation (8) using the following laser fluorosensor system parameters from reference 14:

$$\xi = 0.25$$

$$A_r = 0.05 \text{ m}^2$$

$$R = 100 \text{ m}$$

$$\Delta\lambda_d = 5 \text{ nm}$$

$$\Delta\lambda_f = 20 \text{ nm}$$

$$P_o(\lambda_i) = 5000 \text{ W} \quad (\text{peak power at each laser excitation wavelength } \lambda_i)$$

The normalized standard deviation for the chlorophyll a concentration for cases I and II was determined from equations (16) and (17) for various values of the normalized standard deviations of P_r , P_o , k , and σ . These results are shown in table 4.

Second approach.— In the second approach to the error analysis of the chlorophyll a concentration, it is assumed that the ratios between the fluorescence cross sections at the four excitation wavelengths for each algal color group are constant and only the absolute magnitude of the fluorescence cross sections can change. With these assumptions, equation (9) can be reduced to

$$x_i = \sum_{j=1}^4 a_{ij} b_{jj} n_j$$

where $\sigma_{ij} = a_{ij} b_{jj}$ and a_{ij} are constants. In matrix form this may be written

$$X = A B N$$

where B is a diagonal matrix and A is a matrix of constants. Taking a derivative of both sides and using the chain rule results in the following equation:

$$X' = A B' N + A B N'$$

By using matrix algebra, N' can be determined from

$$N' = B^{-1} A^{-1} X' - B^{-1} B' N \quad (18)$$

where A^{-1} and B^{-1} are the inverse matrices for A and B . It can also be shown that $B^{-1} A^{-1} = \Sigma^{-1}$ where Σ^{-1} was previously defined. In terms of matrix elements equation (18) can be written

$$dn_j = \sum_{m=1}^4 \epsilon_{jm} dx_m - \frac{1}{b_{jj}} db_{jj} n_j$$

where ϵ_{jm} are the matrix elements of Σ^{-1} , and since B is a diagonal matrix, B^{-1} is a diagonal matrix and has as its matrix elements $1/b_{jj}$. From this equation the following partial derivatives can be obtained:

$$\frac{\partial n_j}{\partial x_m} = \epsilon_{jm}$$

and

$$\frac{\partial n_j}{\partial b_{jj}} = - \frac{n_j}{b_{jj}}$$

Substitution of these relationships into the general variance equation (12) yields

$$(\delta n_j)^2 = \sum_{m=1}^4 (\epsilon_{jm})^2 (\delta x_m)^2 + \left(\frac{n_j}{b_{jj}} \right)^2 (\delta b_{jj})^2$$

The normalized form of this equation becomes

$$\left(\frac{\delta n_j}{n_j} \right)^2 = \frac{1}{n_j^2} \sum_{m=1}^4 (\epsilon_{jm} x_m)^2 \left(\frac{\delta x_m}{x_m} \right)^2 + \left(\frac{\delta \sigma_{ij}}{\sigma_{ij}} \right)^2 \quad (19)$$

where

$$\left(\frac{\delta \sigma_{ij}}{\sigma_{ij}} \right)^2 = \left(\frac{\delta b_{jj}}{b_{jj}} \right)^2$$

and

$$\left(\frac{\delta x_m}{x_m} \right)^2 = \left(\frac{\delta P_o}{P_o} \right)^2 + \left(\frac{\delta P_r}{P_r} \right)^2 + \left(\frac{\delta k}{k} \right)^2$$

The first term on the right side of equation (19) is the same as that in equation (14). This would be expected because the only difference in the two approaches was in the statistics dealing with the fluorescence cross sections and not the x_i parameters. The variance of the normalized chlorophyll a concentration contained in an algal color group is dependent upon the variance in the normalized fluorescence cross section at any excitation wavelength of the same algal color group. Results of calculations of $\delta n_j/n_j$ from equation (19) for cases I and II are given in table 5. It is assumed that the normalized variance for fluorescence cross sections is the same for all algal color groups.

Interpretation of the data presented in tables 4 and 5 can only be made after consideration is given to the magnitude and source of the uncertainties which can lead to errors in the determination of the chlorophyll a concentration contained in each algal color group.

Parameters Which Can Alter Fluorescence of Chlorophyll a In Vivo

Some of the factors which can alter the fluorescence cross section of algae include long- and short-term light history, nutrient and age effects, and water temperature (refs. 25 to 30). With the exception of the work of Campillo, Kollman, and Shapiro, who used a laser excitation source at 530 nm, all the data presented in the following sections are from experiments where a broadband excitation source in the blue spectral region was used to excite broadband fluorescence at 685 nm. The reported data are in terms of the ratio of algal fluorescence to chlorophyll a in vivo concentration. This ratio is proportional to the fluorescence cross section discussed previously.

Long-term light history.- Changes in fluorescence levels of chlorophyll a in vivo result from irradiation of the algae by different amounts of solar radiation. Munday and Govindjee (ref. 25) explain this phenomenon as a "membrane" reaction where different reaction centers in the cell "move" with respect to each other and thus alter the fluorescence properties of the cell. Kiefer's experiments in the Gulf of California and North Central Pacific (ref. 26) established that algal fluorescence is inversely related to solar irradiation and that this effect was limited to algae near the surface of the water. Laboratory tests were performed by Blasco (ref. 27) with the marine diatom Skeletonema costatum to determine the day/night effect on algal fluorescence. Figure 13 shows chlorophyll a concentration versus fluorescence for day and night conditions. It can be seen that the ratio of fluorescence to chlorophyll a concentration is 80 percent larger at night than during the day. Blasco concluded that prediction of chlorophyll a concentrations from fluorescence measurements is greatly influenced by the background irradiation intensity.

Short-term light history.- The transient change in fluorescence yield of chlorophyll a in vivo during the initial few seconds of illumination has been studied by Munday and Govindjee (refs. 25 and 28). They conducted experiments into the nature of the short-term, less than 2 sec, fluorescence transient associated with the algae Chlorella. A representative fluorescence curve is given in figure 14. The algae were exposed to a 2-sec pulse of low-intensity

light. The fluorescence yield rises instantaneously to the initial level O. It then rises to level I in 20 to 50 msec, and remains constant or decreases slightly for a brief period before rising to the peak P after 0.25 to 1 sec. Within 1 to 2 sec, it decreases to the steady-state level S. The fluorescence at O is the only part of the curve which was found to be directly proportional to the intensity of the excitation light. Unlike the other parts of the curve, it is not affected by preillumination of the algae. An additional result found by Munday and Govindjee (refs. 25 and 28) was that the time to peak P is inversely proportional to the intensity of the excitation light. This has important implications for laser light excitation of algae because with high-intensity illumination the fluorescence curve could go directly to P without establishing O (private communication from Govindjee, University of Illinois, Urbana, Illinois). The qualitative shape of the curve shown in figure 14 was confirmed by E. V. Browell, O. Jarrett, Jr., F. Farmer, and C. A. Brown, Jr., using Dunaliella euchlora. A Perkin-Elmer fluorescence spectrophotometer with a flowing sample cell was used to permit exposure times greater than 2 msec for the algae. Current research into fluorescence emission of algae using picosecond laser excitation pulses has revealed that there is a decrease in the fluorescence cross section with increasing pulse intensity (ref. 30). Also, there is a change in this cross section if a series of picosecond excitation pulses separated by several nanoseconds are used because of changes which occur in the algae after each pulse. Thus, data obtained with excitation times shorter than a nanosecond or greater than several milliseconds are not directly applicable to flash-lamp-pumped laser excitation pulses of 300 to 500 nsec. The determination of the fluorescence nature of chlorophyll a in vivo in the submicrosecond range is very important in determining the relationship between chlorophyll a in vivo concentration and fluorescence yield for flash-lamp-pumped laser excitation applications.

Nutrient and age effects.- Since algae go from an exponential growth phase to a stationary growth phase as a result of exhaustion of one of the nutrients in the medium, it is primarily the limiting nutrient which causes changes in fluorescence properties of the algae. Blasco's analysis of the data obtained from investigations performed in northwestern Africa (ref. 27) showed that when a culture reaches the stationary growth phase, the ratio of fluorescence to chlorophyll a concentration increases. These results are shown in figure 15. Nitrates are directly linked to pigment formation within cells, and as a result, the ratio of fluorescence to chlorophyll a concentration increases immediately after the cells have exhausted the nitrates in the medium. Phosphate-limited cultures which were tested by Blasco did not exhibit an immediate change in fluorescence until 6 days after most of the phosphate had been depleted by the algae. In both cases the exhaustion of nutrients affects the growth of pigments and, in turn, reduces the photosynthetic activity which allows more energy to be dissipated by fluorescence. The change in the ratio of fluorescence to chlorophyll a concentration can be over 100 percent for both nitrate- and phosphate-exhausted algal cultures.

Water temperature effect.- There are conflicting reports in the literature of the influence of temperature on the fluorescence characteristics of algae. Lorenzen (ref. 29) reported a large decrease in fluorescence when the temperature of the algal medium was increased from 12° C to 35° C in a short period of time. Blasco (ref. 27) argues that the sudden change in temperature caused

a stressed condition in the algae which caused this change in fluorescence. Laboratory results which were reported by Blasco (ref. 27) are shown in figure 16. These data were taken over a 20-minute period of time, and they exhibit a positive change in fluorescence with temperature. In contrast to these two sets of data, Kiefer (ref. 26) found, while conducting experiments in the Gulf of California, that temperature had no effect on the fluorescence of chlorophyll a in vivo. It is assumed that within naturally occurring limits, temperature is a relatively unimportant parameter in the determination of chlorophyll a in vivo fluorescence.

Additional Uncertainties in the Remote Quantification of

Chlorophyll a In Vivo

It has been assumed in the preceding derivation of the equation for the power received by a laser fluorosensor system that measurements at different excitation wavelengths are made for the same water sampling volume. Under this condition, it would be difficult to predict the effective attenuation coefficients to greater than 30-percent accuracy even if the values for the absorption and scattering coefficients were known precisely. This is because the form of the equation for the effective attenuation coefficient is only an approximation and because the attenuation coefficients vary depending upon the scattering characteristics, and the amount and vertical distribution of turbidity. If no knowledge of the scattering coefficient can be remotely obtained for the sampling volume, the uncertainty in γ_l and γ_f would be greater than 50 percent. Further, the effective attenuation coefficient at each excitation wavelength has at least a 5- to 10-percent uncertainty relative to other excitation wavelengths. If the measurements for all excitation wavelengths cannot be made for the same sampling volume, the uncertainty in the attenuation coefficients for each laser excitation becomes very large, unless the body of water is uniform in turbidity over the distance traveled during one measurement cycle.

For a field measurement of laser output power and received fluorescence power, it is assumed that a measurement accuracy of 5 percent is typical with 2.5 percent attainable.

A greater than 40-percent variation in the fluorescence cross section for various species within the same algal color group (ref. 14) can create large uncertainties in determination of the chlorophyll a in vivo concentration by the laser fluorosensor system. Some of this intragroup variation may only be in absolute magnitude, and the relative spectral characteristics may be the same. For example, an error in the measurement of chlorophyll a contained in the algae can arise because of difficulty in complete extraction of all chlorophyll a from the algal cells. Part of the uncertainty in fluorescence cross section can result from changes in the relative excitation spectra of various species within a color group. The source and nature of this cross section variation is not known; however, it is realistic to assume a 5- to 10-percent uncertainty in the relative fluorescence cross section at each excitation wavelength.

The vertical distribution of algae can change dramatically in the first 5 m and the ratio of fluorescence to chlorophyll a concentration changes with depth because of different amounts of solar irradiance reaching different depths (ref. 26). In terms of the present analysis, only average values of chlorophyll a concentrations and fluorescence cross sections can be used. The errors involved in these assumptions are difficult to quantify in terms of their impact on the accuracy of chlorophyll a in vivo concentration because each measurement condition is different. The horizontal variation of algae can be very large over only a few meters, especially during bloom conditions; and with large changes in algal concentrations, large changes in the attenuation coefficients occur. For these reasons it is imperative that all measurements be made over the same sampling volume.

Discussion of Error Analysis Results

For single-wavelength laser fluorosensor systems, it can be seen from table 1 that increased measurement accuracy of output and received power from 5 to 2.5 percent does not significantly decrease the error in the chlorophyll a concentration when the error in the effective attenuation coefficient is greater than 10 percent. Without remote measurement capability for the attenuation coefficient, the error in its estimation can be large, greater than 50 percent. If the standard deviation of the effective attenuation coefficient is small, the uncertainty in the chlorophyll a concentration is nearly the same as it is for fluorescence cross section. Thus, the resulting standard deviation for chlorophyll a concentration depends primarily upon both $\delta k/k$ and $\delta \sigma/\sigma$.

From the fluorescence cross section data presented in reference 14, an excitation wavelength can be selected for which the fluorescence cross sections for all four algal color groups are nearly equal. The approximate wavelength is 618 nm. The average fluorescence cross section information at this wavelength for the color groups is given in table 2. If an average fluorescence cross section of $0.15 \times 10^{-21} \text{ m}^2$ is assumed for any algal composition encountered with the single-wavelength laser fluorosensor system, the maximum error in the fluorescence cross section is 40 percent, and thus the standard deviation $\delta n/n$ would be less than 40 percent. Uncertainties in effective attenuation coefficients and fluorescence cross section due to factors listed in previous sections also influence the error in the chlorophyll a concentration, as shown in table 1. With a supplemental remote measurement of attenuation coefficients, it is felt that the concentration of chlorophyll a can be determined within 100-percent accuracy, which is sufficient for many applications.

For the multiple-wavelength laser fluorosensor to be able to determine the chlorophyll a concentration in each algal color group, the uncertainties of the fluorescence cross sections must be reduced so that the normalized standard deviation for the chlorophyll a concentration in all color groups is less than 1.0. Under these conditions, matrix equation (11) can be solved without difficulty for the concentration matrix. If $\delta n/n$ is greater than 1.0 for any color group due to an uncertainty in σ , Σ is an ill-conditioned matrix, which can produce completely unrealistic values for the chlorophyll a concentrations (e.g., negative concentrations). The error analysis for multiple-wavelength

systems was separated into two parts as a result of different statistics applied to the fluorescence cross sections. The variance relationship between P_0 , P_r , k , and n is the same in both cases. In table 4 the results are presented for the normalized standard deviation of the chlorophyll a concentration when the fluorescence cross section is considered independent of excitation wavelength. Even if the same sample volume is used for all excitation wavelengths, at least a 10-percent uncertainty in the relative excitation spectra for each of the color groups is anticipated. If different sample volumes are used during a measurement cycle, the uncertainty in the fluorescence cross section increases because of the possibility of changing algal concentrations and composition. In table 4 it can be seen that even for $\delta\sigma/\sigma = 0.1$, case I had a value of $\delta n/n > 1.0$. Not until $\delta\sigma/\sigma$ was greater than 0.3 did case II produce $\delta n/n > 1.0$. It was explained previously that case I represented the ALOPE parameters (table 2) and case II represented the set of optimized excitation wavelengths given in table 3. Optimistic power measurement accuracies (2.5 percent), knowledge of relative effective attenuation coefficients at all excitation wavelengths to 5 percent, and a modest 10-percent standard deviation of fluorescence cross section produce 177-percent uncertainty in the concentration of chlorophyll a contained in the golden-brown algae when ALOPE parameters are used and only 53 percent when the optimized excitation wavelengths of case II are used. (Measurements for golden-brown algae have the most uncertainty in $\delta n/n$.) In general, use of the optimized excitation wavelengths reduce the uncertainty in chlorophyll a concentration by a factor of 3 from the ALOPE parameters.

When the spectral variation in the fluorescence cross sections for a color group is assumed to be in a constant ratio, even though the absolute magnitude may change due to light, age, and chemical factors, the chlorophyll a concentration standard deviations given in table 5 result. As was discussed previously, the magnitude of the fluorescence cross section can vary over 100 percent by chemical stress. The ALOPE system parameters, which were used in case I, produce unacceptably high uncertainties for all the input parameters shown in the table. The optimized excitation wavelengths used in case II yield values for $\delta n/n$ which are less than 100 percent even for large uncertainties in the fluorescence cross sections.

The data presented in tables 4 and 5 are a result of different statistical approaches which attempt to evaluate extreme values for the uncertainties in chlorophyll a concentrations. In both approaches, the ALOPE system parameters produced standard deviations in n greater than 3 times those produced in case II, which assumed more optimized excitation wavelengths.

CONCLUDING REMARKS

Accuracy in the remote determination of chlorophyll a in vivo concentration depends upon the use of the correct equation for the power received by a laser fluorosensor system. The appropriate form of this relationship is derived in this paper (eq. (6)). Similar equations have been reported in the literature; however, they differ from the present equation by as much as a factor of 10.

Low-intensity long-exposure excitation of algae, which is used in fluorescence spectrophotometer studies, must be correlated with high-intensity short-pulse laser excitation of algae before spectrophotometer measurements of algal fluorescence cross sections can be used in laser fluorosensor system investigations. Also, additional research needs to be conducted on the variation of fluorescence yield which occurs in algae because of varying ambient light levels and nutrient concentrations. The uncertainties associated with these factors directly affect the accuracy of the remote chlorophyll a in vivo concentration measurements.

The use of optimized excitation wavelengths in a four-wavelength laser fluorosensor system can reduce uncertainties in the chlorophyll a concentration measurement by more than a factor of 3 from the present ALOPE (Airborne Lidar Oceanographic Probing Experiment) system. Assuming optimistic estimates of power measurement accuracies and of uncertainties in attenuation coefficients and algal fluorescence cross sections, a laser fluorosensor system with optimized excitation wavelengths would produce chlorophyll a concentration uncertainties in all four color groups of less than 53 percent.

The use of a "ground-truth" point to calibrate the laser fluorosensor system can only be used in large bodies of water where the water conditions change slowly over long distances. However, under those circumstances the variation in algal types would not be expected to change significantly, and a single-wavelength system could be used for the chlorophyll a measurement.

In water where marine conditions vary widely over short distances, supplemental remote measurements of marine attenuation coefficients and environmental factors which affect the fluorescence cross section of algae (e.g., nutrient concentration and ambient light intensity) are necessary at each sample point of the laser fluorosensor system. Without this additional measurement capability, both the single- and multiple-wavelength laser fluorosensor systems can only provide qualitative information about the chlorophyll a contained in the algae, and the single-wavelength system yields results which are comparable to a multiple-wavelength system with optimum excitation wavelengths.

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TABLE 1.- NORMALIZED STANDARD DEVIATION OF PARAMETERS IN
SINGLE-WAVELENGTH LASER FLUOROSENSOR ERROR ANALYSIS

$\delta P_o/P_o$	$\delta P_r/P_r$	$\delta k/k$	$\delta \sigma/\sigma$	^a $\delta n/n$
0.05	0.05	0.1	0	0.12
.025	.025	.1	0	.11
.025	.025	.1	.3	.32
.025	.025	.1	.7	.71
.025	.025	.1	1.0	1.01
.025	.025	.3	0	.30
.025	.025	.3	.3	.43
.025	.025	.3	.7	.76
.025	.025	.3	1.0	1.04
.025	.025	.5	0	.50
.025	.025	.5	.3	.58
.025	.025	.5	.7	.86
.025	.025	.5	1.0	1.12

^aCalculated from equation (13).

TABLE 2.- ALGAL FLUORESCENCE CROSS SECTIONS FOR ALOPE SYSTEM^a

Algal color group	Fluorescence cross section, m ² , at excitation wavelength, nm, of -			
	454.4	539.0	598.7	617.8
Green	1.152×10^{-21}	0.139×10^{-21}	0.164×10^{-21}	0.232×10^{-21}
Golden-brown	.653	.151	.081	.110
Red	.133	.450	.447	.255
Blue-green	.071	.050	.152	.239

^aFrom reference 14.

TABLE 3.- OPTIMIZED FLUORESCENCE CROSS SECTIONS FOR FOUR-WAVELENGTH LASER FLUOROSENSOR SYSTEM

Algal color group	Fluorescence cross section, m ² , at excitation wavelength, nm, of -			
	482	520	562	640
Green	1.13×10^{-21}	0.28×10^{-21}	0.16×10^{-21}	0.39×10^{-21}
Golden-brown	.42	.28	.14	.10
Red	.09	.22	.64	.13
Blue-green	.03	.03	.06	.60

TABLE 4.- NORMALIZED STANDARD DEVIATION OF CHLOROPHYLL a CONCENTRATION FOR
INDEPENDENT VARIATION OF FLUORESCENCE CROSS SECTIONS

$\delta P_O/P_O$	$\delta P_R/P_R$	$\delta k/k$	$\delta \sigma/\sigma$	^a $\delta n/n$							
				^b Case I				^c Case II			
				Green	Golden-brown	Red	Blue-green	Green	Golden-brown	Red	Blue-green
0.05	0.05	0	0	0.85	1.46	0.26	0.58	0.22	0.44	0.15	0.18
.05	.05	.05	0	1.05	1.79	.31	.71	.27	.54	.18	.22
.05	.05	.1	0	1.48	2.54	.44	1.00	.38	.76	.25	.31
.025	.025	0	0	.43	.73	.13	.29	.11	.22	.07	.09
.025	.025	.05	0	.74	1.27	.22	.50	.19	.38	.13	.15
.025	.025	.1	0	1.28	2.20	.38	.87	.32	.66	.22	.26
.025	.025	.2	0	2.46	4.20	.73	1.66	.63	1.26	.42	.51
0	0	0	.1	.73	1.24	.21	.46	.21	.37	.14	.16
0	0	0	.2	1.45	2.48	.42	.91	.41	.74	.27	.32
0	0	0	.3	2.18	3.71	.63	1.37	.62	1.11	.41	.47
.05	.05	.05	.1	1.28	2.18	.37	.85	.34	.65	.23	.27
.05	.05	.05	.2	1.79	3.06	.52	1.15	.49	.92	.32	.39
.025	.025	.1	.1	1.47	2.52	.43	.98	.38	.76	.26	.31
.025	.025	.1	.2	1.93	3.32	.57	1.26	.52	.99	.35	.41
.025	.025	.05	.1	1.04	1.77	.30	.68	.28	.53	.19	.22
.025	.025	.05	.2	1.63	2.79	.47	1.04	.45	.83	.30	.35

^aCalculated from equations (16) and (17).

^bExcitation wavelengths and fluorescence cross sections from table 2.

^cExcitation wavelengths and fluorescence cross sections from table 3.

TABLE 5.- NORMALIZED STANDARD DEVIATION OF CHLOROPHYLL a CONCENTRATION FOR DEPENDENT
VARIATION OF FLUORESCENCE CROSS SECTIONS WITHIN ALGAL COLOR GROUPS

$\delta P_o/P_o$	$\delta P_r/P_r$	$\delta k/k$	$\delta \sigma/\sigma$	^a $\delta n/n$							
				^b Case I				^c Case II			
				Green	Golden-brown	Red	Blue-green	Green	Golden-brown	Red	Blue-green
0.05	0.05	0.05	0	1.05	1.79	0.31	0.71	0.27	0.54	0.18	0.22
.05	.05	.05	.2	1.07	1.80	.37	.74	.34	.58	.27	.30
.05	.05	.05	.4	1.12	1.83	.51	.81	.48	.67	.44	.46
.05	.05	.05	.7	1.26	1.92	.77	1.00	.75	.88	.72	.73
.025	.025	.1	0	1.28	2.20	.38	.87	.32	.66	.22	.26
.025	.025	.1	.2	1.30	2.21	.43	.89	.38	.69	.30	.33
.025	.025	.1	.4	1.34	2.23	.55	.96	.51	.77	.46	.48
.025	.025	.1	.7	1.46	2.31	.63	1.12	.77	.96	.73	.75
.025	.025	.05	0	.74	1.27	.22	.50	.19	.38	.13	.15
.025	.025	.05	.2	.77	1.28	.29	.54	.28	.43	.24	.25
.025	.025	.05	.4	.84	1.33	.46	.64	.44	.55	.42	.43
.025	.025	.05	.7	1.02	1.45	.74	.86	.73	.80	.71	.72

^aCalculated from equation (19).

^bExcitation wavelengths and fluorescence cross sections from table 2.

^cExcitation wavelengths and fluorescence cross sections from table 3.

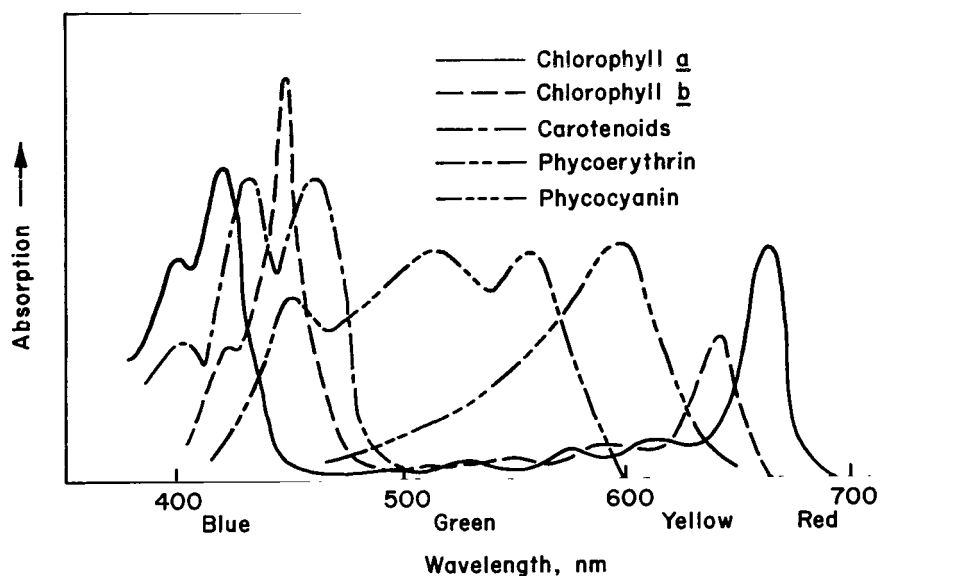


Figure 1.- Absorption spectra of pigments found in living algae.
(Adapted from *The Absorption of Light in Photosynthesis* by Govindjee and Rajni Govindjee, Copyright © 1974 by Scientific American, Inc. All rights reserved.)

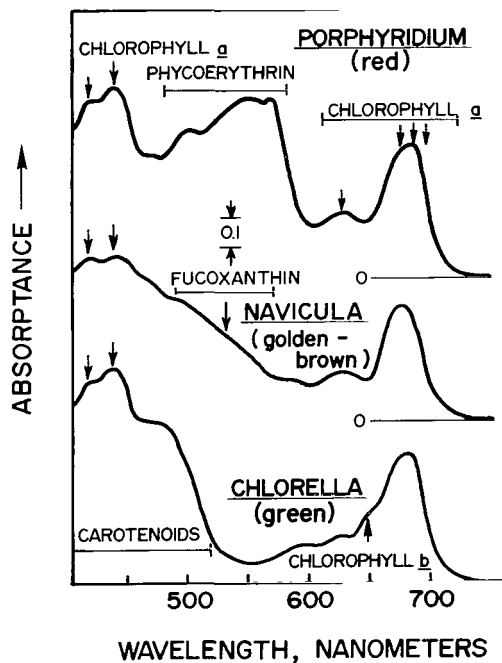


Figure 2.- Absorbance spectra of three algal species which are representative of different color groups. Algal pigments which dominate light absorption are indicated. (From *Molecular Approaches to Plant Physiology* by C. A. Price, c.1970, McGraw-Hill Book Co., Inc. with permission of publisher.)

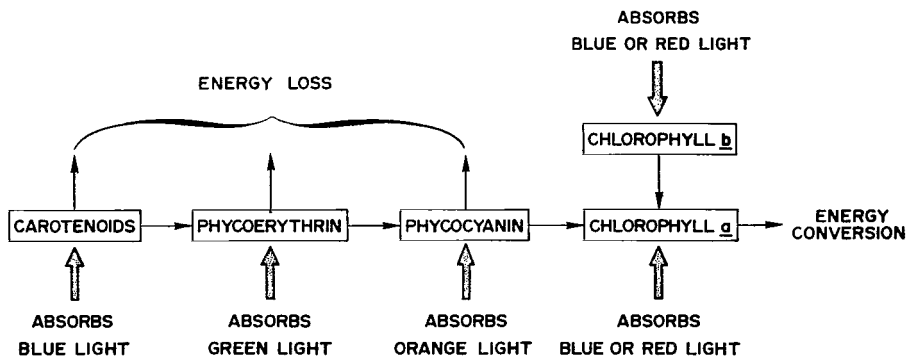


Figure 3.- Light absorption by pigments contained in living algae and path of energy transfer within algal cell. (Adapted from The Absorption of Light in Photosynthesis by Govindjee and Rajni Govindjee, Copyright © 1974 by Scientific American, Inc. All rights reserved.)

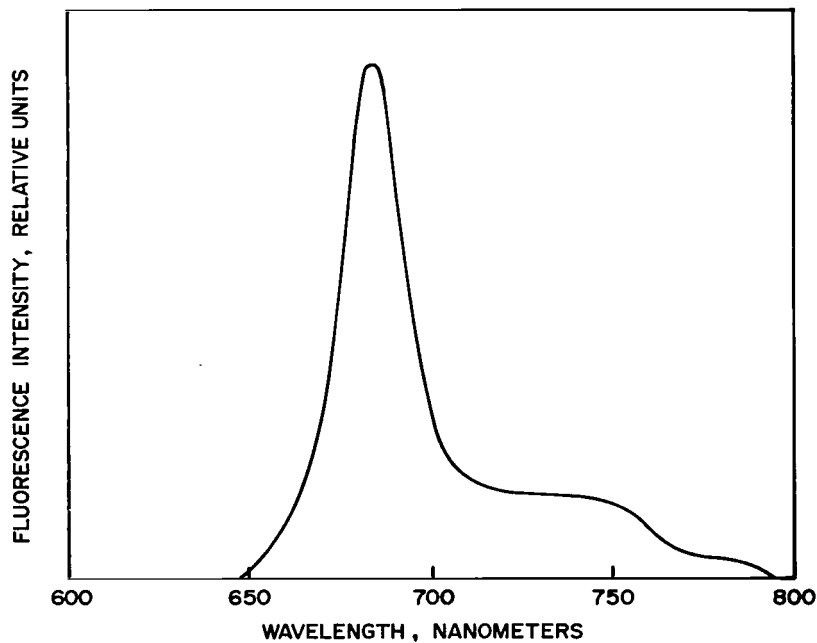


Figure 4.- Typical fluorescence spectrum for green and golden-brown algae.

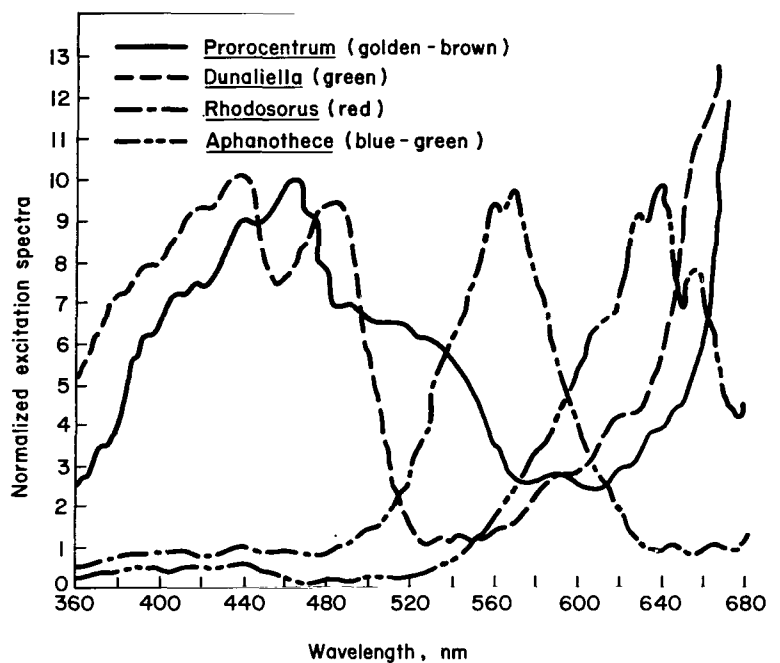


Figure 5.- Normalized excitation spectra for representative algal species from four algal color groups. Fluorescence emission at 685 nm was detected while excitation wavelength was changed. (Adapted from ref. 8.)

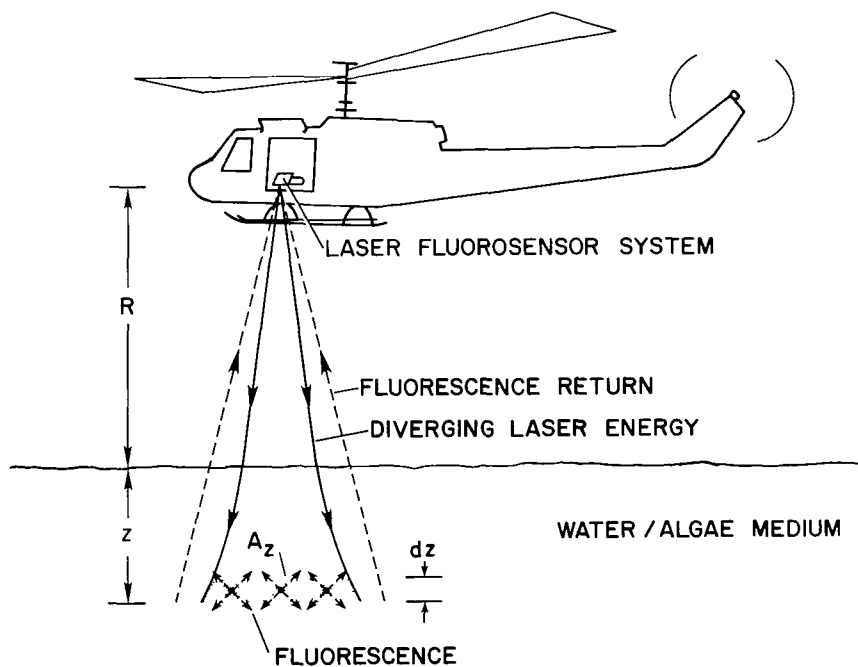


Figure 6.- Diagram used in derivation of equation for power received by a laser fluorosensor system.

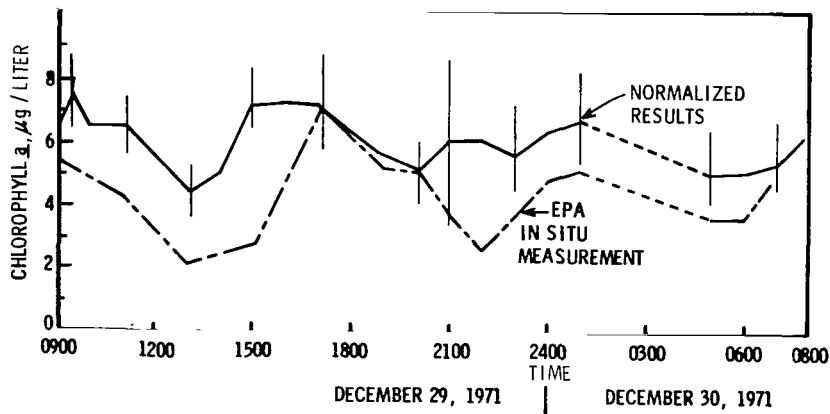


Figure 7.- Chlorophyll a in vivo concentrations determined by single-wavelength laser system from Thimble Shoals Channel Pier of Chesapeake Bay Bridge Tunnel and normalized to in situ measurements at 2000 EST on December 29, 1971. (From ref. 11.)

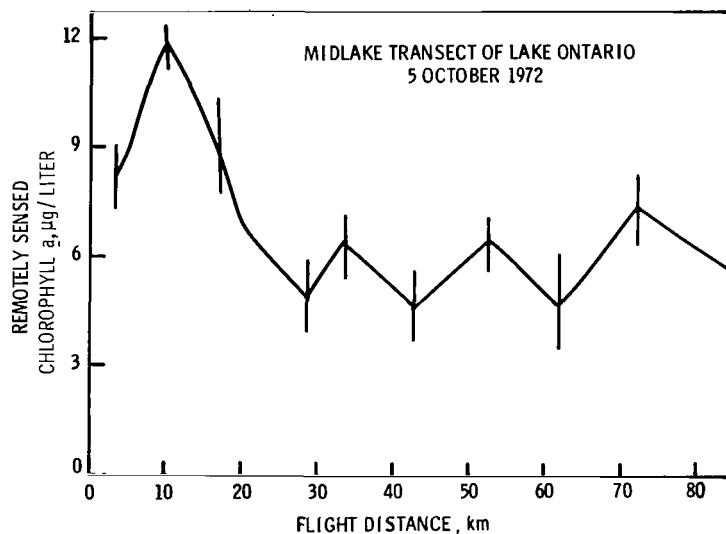


Figure 8.- Chlorophyll a in vivo concentrations measured by airborne laser fluorosensor system which was flown from west of Rochester, New York, to Canadian shore. (Adapted from ref. 12.)

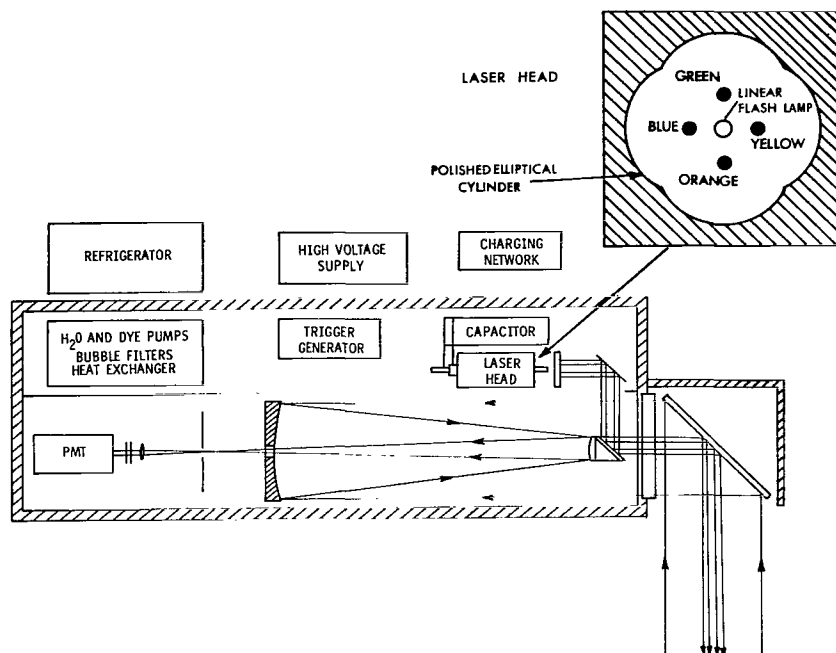


Figure 9.- ALOPE system with four-wavelength laser head (only one laser wavelength is emitted at a time), 25.4-cm-diameter receiving telescope, and associated equipment. (Adapted from ref. 13.)

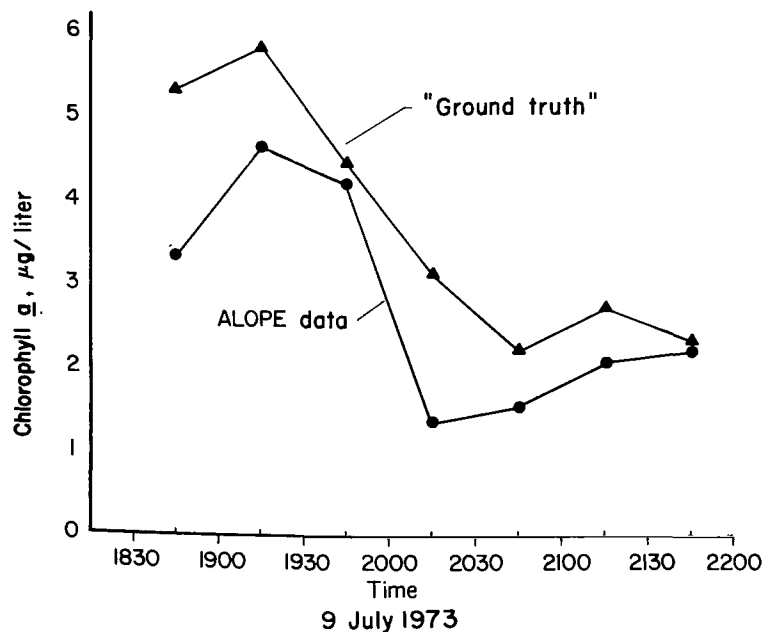


Figure 10.- Chlorophyll a in vivo concentrations obtained by ALOPE system from George P. Coleman Bridge, which is 30 m above York River. Blue (454 nm) and green (539 nm) wavelengths only were used in data reduction. (From ref. 8.)

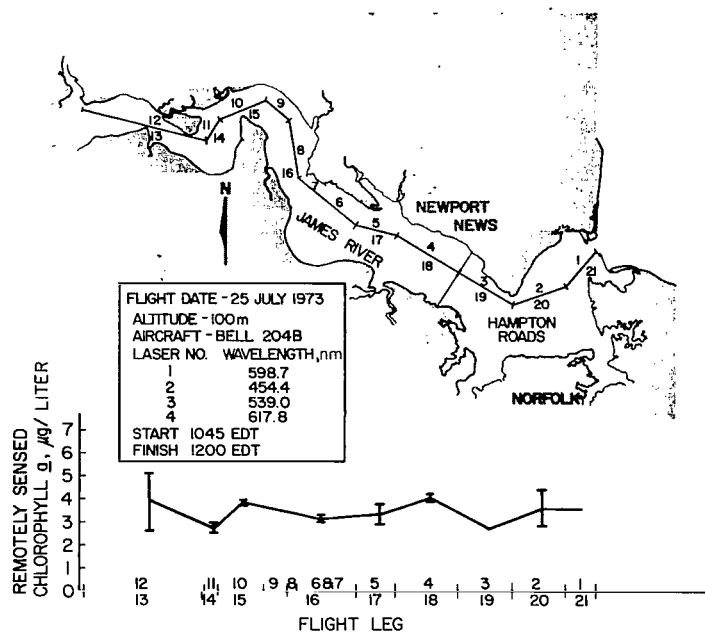


Figure 11.- Total chlorophyll a in vivo concentrations measured by ALOPE system on flight over James River. Red (598 nm) wavelength was not used in data reduction. (Adapted from ref. 8.)

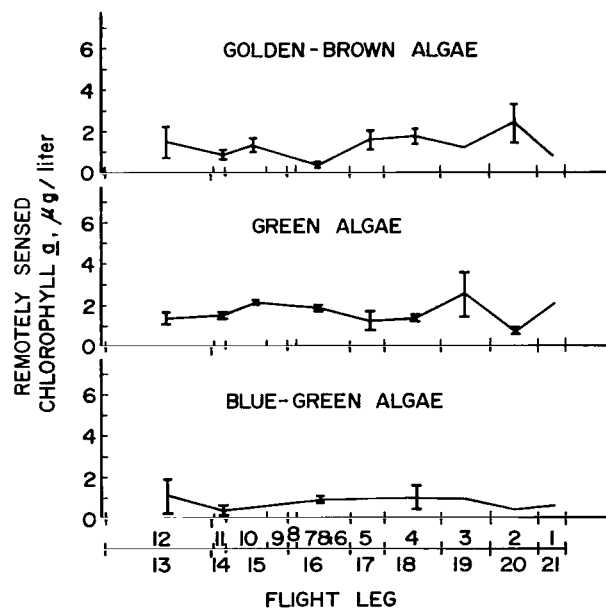


Figure 12.- Chlorophyll a contained in algal color groups as measured by ALOPE system on flight over James River. Concentrations of red algae were not determined. (Adapted from ref. 8.)

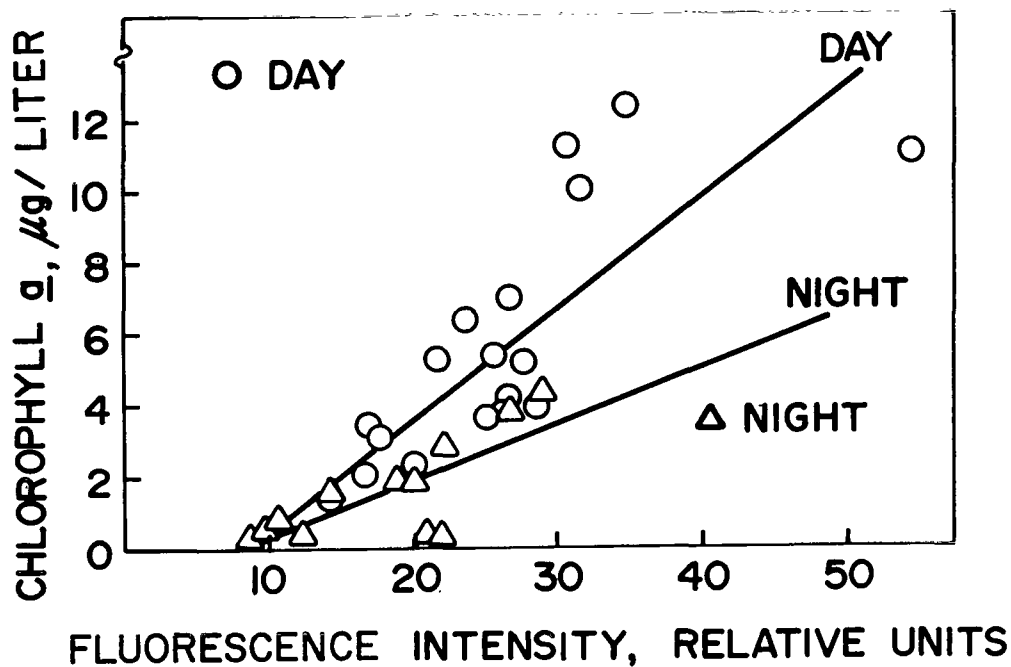


Figure 13.- Relationship between chlorophyll a concentration and fluorescence intensity for algae tested during day and night conditions. (Adapted from ref. 27.)

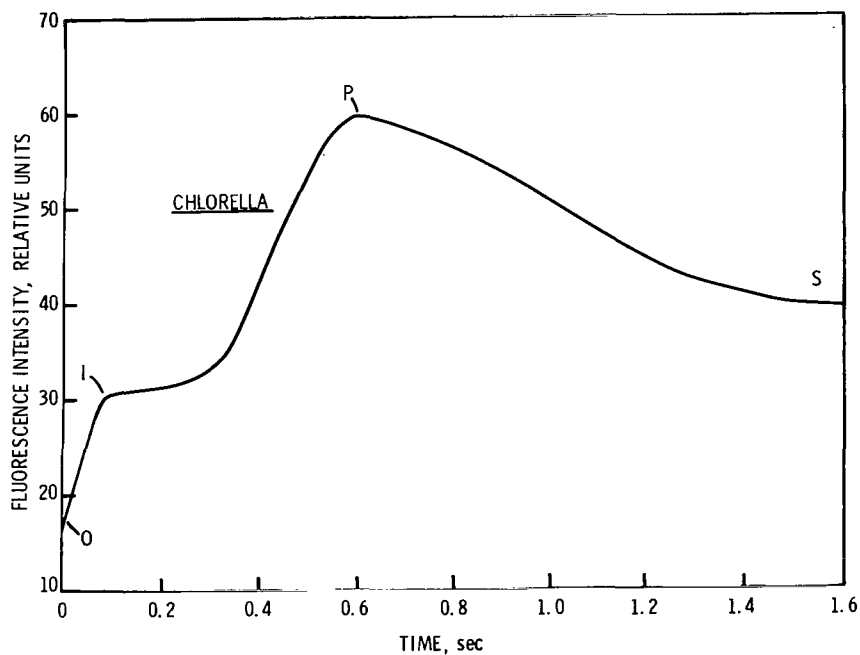


Figure 14.- Transient fluorescence curve for Chlorella when it is excited by 2-sec pulse of low-intensity light. (Adapted from ref. 25.)

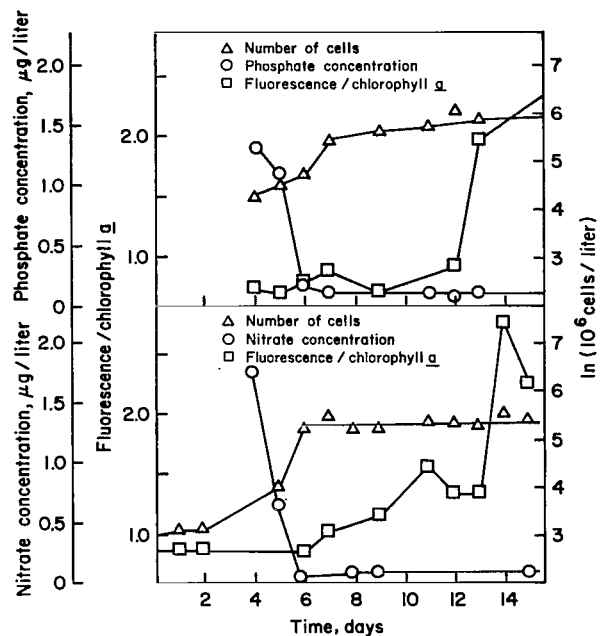


Figure 15.- Algal cell concentrations and fluorescence/chlorophyll *a* ratio as a function of phosphate and nitrate levels in algal growth medium. (Adapted from ref. 27.)

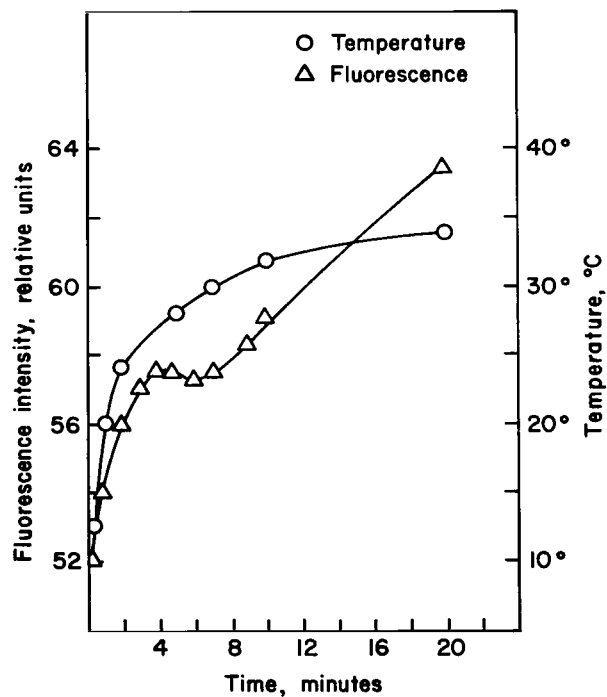


Figure 16.- Relationship between temperature and algal fluorescence. (Adapted from ref. 27.)



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