# The relationship between near-surface chlorophyll and solar-stimulated fluorescence: biological effects

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## ABSTRACT

The fluorescence of chlorophyll *a* (Chl) near 683 nm can be detected in water leaving radiance and related quantitatively to the concentration of Chl. Solar-induced fluorescence has also been related to photosynthesis in deeper waters. However, little is known about the relationships between Chl, fluorescence, photosynthesis, and irradiance near the sea surface. Quantum yields of fluorescence and photosynthesis, as well as the ratio of fluorescence to photosynthesis, change during exposures to bright light. Several physiological processes are at play. Consequently, it is difficult to construct models of near-surface quantum yields. Experimentation and comprehensive sampling in the field are required for critical information. Some approaches are presented here. Radiometer buoys that measure downwelling irradiance at 490 nm, Ed(490), and upwelling spectral radiance, Lu($\lambda$) are good tools for measuring solar-stimulated fluorescence during studies of near-surface biology. Results can be compared with experimental measurements using a fluorometer with a very weak measuring beam that does not perturb the balance between fluorescence and photosynthesis. Comparisons indicate that relationships between near-surface Chl, fluorescence, photosynthesis and irradiance can vary widely for reasons that are not yet well resolved. Still, Lu(683), corrected for backscatter and normalized to Ed(490), is a useful measure of near-surface Chl in many environments.

Key words: fluorescence, chlorophyll, photosynthesis, reflectance, upwelling radiance, quenching.

## 1. INTRODUCTION

Solar-stimulated fluorescence of chlorophyll *a* can be detected in spectra of upwelling radiance or irradiance[1-3] and related quantitatively to the concentration of chlorophyll (Chl) in surface waters[4] and deeper in the water column.[5,6] Consequently, the fluorescence of Chl can be detected with passive instruments on moorings, profilers, drifters, or remote platforms. These measurements of fluorescence represent an important complement to estimates of Chl concentration from observations of ocean color.[7,8] To make good use of the data, it is critical to understand the relationship between Chl and solar-stimulated fluorescence as influenced by environmental, physiological and taxonomic factors.

The problem of estimating fluorescence emission (photons $m^{-3} s^{-1}$) from radiance reflectance has been addressed (ref 6 and references therein). However, the biological processes that influence the relationships between Chl, light absorption, photosynthesis, and solar-induced fluorescence are not well studied for high and variable irradiance characteristic of the sea-surface.[9] It is thus difficult to validate or improve models that describe the relationships between Chl or photosynthesis and the fluorescence signal in upwelling radiance.[10-12] Experimentation, as well as comprehensive sampling in the field under different regimes of solar irradiance and vertical mixing, are required. Some approaches are presented here.

## 2. METHODS

During several deployments in coastal waters, a tethered spectral radiometer buoy (TSRB) measured downwelling irradiance (490 nm) above the surface [Ed(490)] and near-surface upwelling radiance, [$Lu_{TSRB}(\lambda)$] in six wavebands corresponding to the SeaWiFS sensor, plus 683 nm to detect fluorescence of Chl.[13,14] Meantime, discrete samples were obtained. Only fluorometric determinations of Chl are reported here. More recently, we used an instrument that measures 14 wavebands of Lu and Ed in the UV and visible, with the radiance sensors about 10 cm below the surface vs 45 cm for the TSRB; otherwise, the measurements reported here are essentially the same for both instruments. Data collected at 1 $s^{-1}$ were reduced to medians for 60-s bins. The fluorescence signal corrected for backscatter, $Lu(683)_{corr}$, was calculated with linear

baseline correction to $Lu_{TSRB}(683)$, interpolating $Lu_{TSRB}$ at 670 and 700 nm. For the analyses discussed here, no attempt was made to calculate fluorescence emission in photons $m^{-3}$ $s^{-1}$. [2,6,15]

Fluorescence yield under experimentally controlled irradiance was measured in two ways.[16] For samples from perennially ice-covered Antarctic lakes, fluorescence emission was detected with a Biospherical Instruments PNF-300 natural fluorometer [Lu(683) detector] mounted looking downward from the mouth of a clear container illuminated by a projector lamp filtered through two blue-green (Corning 4-97) filters and neutral density screen. Temperature was near 0°C and samples were dark-adapted for 30 min. Actinic irradiance (PAR) was measured with a submersible scalar irradiance sensor (Biospherical Instruments QSL-100), and steady-state fluorescence as a function of PAR was recorded for individual samples after exposure for about 5 min, when transients had subsided. Relative fluorescence yield was estimated as Lu(683)/PAR. Laboratory measurements on a culture of the diatom *Thalassiosira pseudonana* (clone 3H) were made with a PAM fluorometer (Walz, Effeltrich, Germany).[17] Steady-state fluorescence yield under ambient irradiance ($F_S$) is that measured during stimulation by a very weak, rapidly pulsed, non-actinic measuring beam. Relative fluorescence emission is calculated as $F_S$ times irradiance, as measured with a QSL-100.

## 3. RESULTS AND DISCUSSION

Although more sophisticated calculations can be made, near surface Chl can be estimated by normalizing the upwelling radiance (or irradiance) signal to downwelling irradiance.[4] A plot of the fluorescence signal from our radiometer buoys, [$Lu(683)_{corr}$ / Ed(490)] vs surface Chl (Fig. 1A) shows a relationship comparable to what has been observed from aircraft.[4] The apparent strength of the relationship between Chl and the fluorescence signal comes from the large range of Chl sampled. When the efficiency of fluorescence is approximated with a ratio [$Lu(683)_{corr}$/(Ed(490) · Chl)] and plotted as a function of irradiance (Fig. 1B),[18] variability is much more prominent (see refs 15,19). In turbid waters, apparent fluorescence yield can vary due to changes in the attenuation of exciting and emitted photons;[2,6] otherwise, variability in $Lu(683)_{corr}$/(Ed(490) · Chl) is the consequence of differences in absorption coefficient of phytoplankton ($m^2$ mg $Chl^{-1}$) and the quantum yield of fluorescence (mol photons emitted · mol photons absorbed$^{-1}$). Variability in absorption coefficient is associated with pigment packaging (a function of cell size and intracellular pigment concentration), and accessory pigmentation, which are linked to differences in species composition, nutrition and photoacclimation.[15,20-24] Also, there can be changes in the proportion of

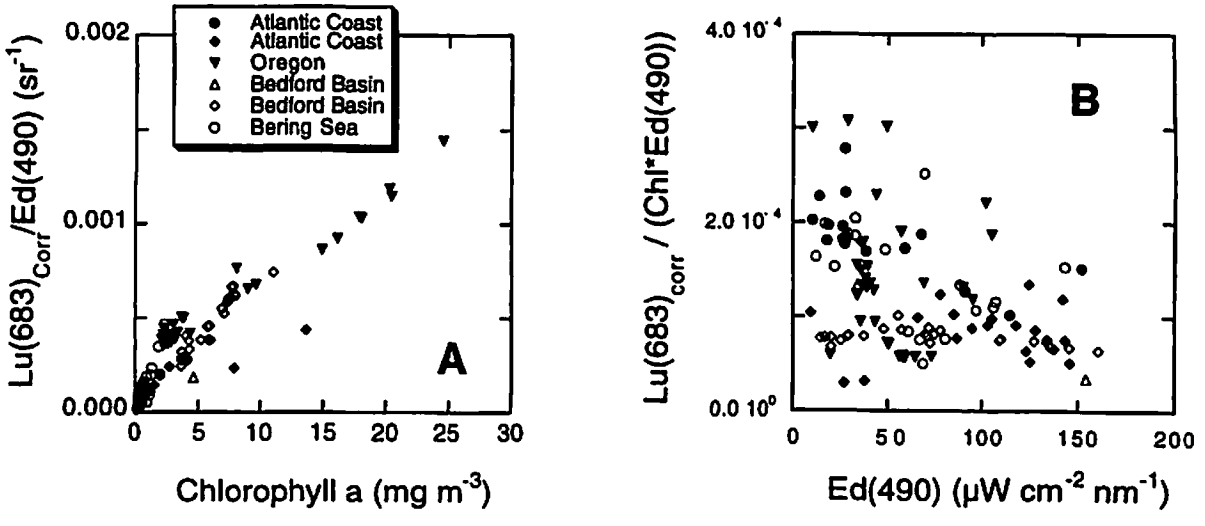


Fig. 1. Relationships between solar-stimulated fluorescence, as measured with radiometer buoys, and surface Chl, extracted and measured fluorometrically. A. The fluorescence signal is $Lu_{TSRB}(683)$, corrected with a linear baseline, normalized to Ed(490) measured above the surface. Samples were collected during two cruises off the Atlantic coast of the US in the summer of 1996, off the Oregon coast in Sept. 1994 (including offshore waters and a diatom bloom in the plume of the Columbia River[14]), in the Bering Sea during the spring of 1996, and during August, 1996 in Bedford Basin, Nova Scotia. B. Ignoring for now the attenuation of light that could reduce the fluorescence signal in turbid waters,[6] we plot a measure related to fluorescence yield, $Lu(683)_{corr}$ / [Chl · Ed(490)], units: $sr^{-1} \cdot (mg\ m^{-3})^{-1}$.

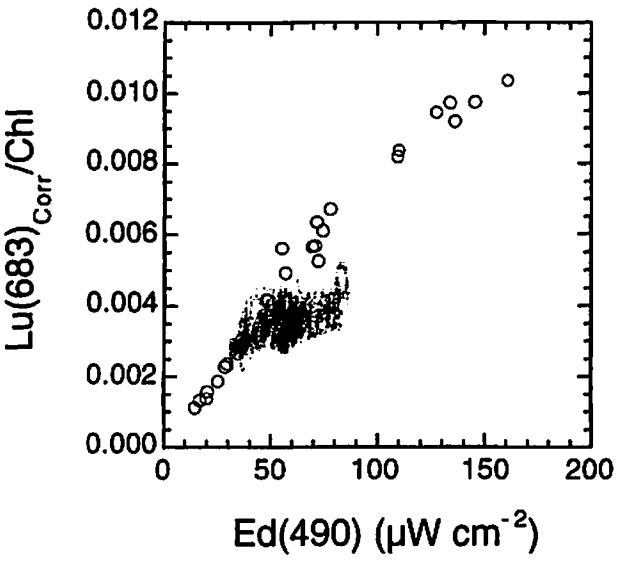


Fig. 2. Relationships between fluorescence per unit Chl [(μW $cm^{-2}$ $nm^{-1}$ $sr^{-1}$ (mg $m^{-3})^{-1}$] and Ed(490 during deployments of radiometer buoys. Open circles: measurements from Bedford Basin, Nova Scotia on 21 and 22 August, 1996. Chlorophyll was measured directly on surface samples. Small points are from a diatom bloom in the plume of the Columbia River off Oregon, Sept. 20, 1994.[14] Each point is a median for 60 records, collected 1 $s^{-1}$. Chlorophyll was calculated from a power fit to records of $Lu_{TSRB}(490)/Lu_{TSRB}(555)$ vs Chl during the day (N = 8, $R^2$ = 0.97): Chl = 9.57*($Lu_{TSRB}(490)$ / $Lu_{TSRB}(555))^{-1.96}$. The record from Oregon is consistent with strong quenching of fluorescence at relatively low irradiance.

light absorbed by photosynthetically active pigments.[25,26] In nature, these properties change over time scales of several hours to many days. The quantum yield of fluorescence can change more rapidly, in response to variable irradiance.[27-29] Under some circumstances, changes in fluorescence yield can be related to changes in the quantum yield of photosynthesis.[27,30,31]

In the context of the variability in Fig. 1B and related sets of data,[15,18,19] we focus here on short-term (seconds - minutes) light-induced changes in fluorescence yield that bear directly on the relationships between fluorescence, photosynthesis and Chl in surface waters.[28,31] The relevant processes are photochemical quenching and nonphotochemical quenching of fluorescence.

Photochemical quenching reflects a trade-off in the fate of photons absorbed by photosynthetic pigments associated with photosystem II (PSII). Absorbed photons can be directed to photosynthesis, fluorescence, or radiationless decay (heat).[30] When photon flux is very low, photosynthetic reaction centers are mostly open (*i.e.*, available for photosynthesis) and photosynthetic quantum yield, hence quenching due to photosynthesis, is relatively high; in turn, fluorescence yield is relatively low. As irradiance increases, photons cannot be processed as rapidly as they are absorbed, and a greater proportion of reaction centers are closed: photosynthetic quantum yield declines, photochemical quenching decreases in importance, and fluorescence yield increases. The inverse relationship between photosynthetic quantum yield and fluorescence quantum yield as a function of irradiance is the basis for an algorithm relating photosynthesis to solar-stimulated fluorescence.[11] The authors did not intend their algorithm for use near the surface, where other physiological processes are at play and where backscattered solar irradiance contributes to Lu(683) and confounds measurements of fluorescence with a single passive sensor.[6]

Excess irradiance can damage photosynthetic systems, particularly sites associated with the reaction center of PSII.[32] It is thus adaptive for plants to reduce excessive "excitation pressure"[33] on PSII. This is accomplished by photoacclimation[34] on time scales of hours to days,[35] whereby the balance between the rate of light absorption and the rate of utilization is restored.[36] On shorter time scales under variable irradiance, responses involve nonphotochemical quenching of absorbed photons, *i.e.*, dissipation of absorbed irradiance as heat.[29,30] Nonphotochemical quenching reduces the quantum yields of both photosynthesis and fluorescence, complicating the relatively straightforward relationships between photosynthesis, fluorescence and irradiance that hold when only photochemical quenching influences fluorescence yield.[31] Presently, there is very little information on which to base models of solar-stimulated fluorescence and photosynthesis near the sea-surface (ref 9 , but see ref. 28). Where can we look for more information?

One approach is to describe variations in solar-stimulated fluorescence as a function of irradiance in nature (see ref. 18). Solar-stimulated fluorescence yield [$Lu(683)_{corr}$/(Ed(490) · Chl), units: $sr^{-1}$ (mg $m^{-3})^{-1}$] is determined during deployments of a radiometer buoy. If solar irradiance varies substantially while the phytoplankton assemblage changes little, fluorescence vs. irradiance (Fl vs *E*) relationships can be described (see also Fig. 4 in ref. 9). Chlorophyll can be measured directly, or estimated from reflectance ratios.[18] Our records from deployments in coastal waters indicate big differences in Fl vs *E* (Fig. 2). It is very likely that nonphotochemical quenching plays an important role in these relationships.

Clearly, there is a need to quantify and understand nonphotochemical quenching under bright and variable irradiance characteristic of surface layers. There are a great many experimental studies that examine changes of fluorescence during

exposures to variable irradiance,[27,29,37] but it is not always easy to relate yields for stimulated fluorescence to fluorescence emission under natural conditions (ref. 38, but see refs. 28,39). A problem with many instruments is the need to remove samples from the ambient environment to make measurements. Fluorometers with remote probes, LIDAR systems and *in situ* fluorometers don't have that problem, but many of them utilize measuring beams that can induce photochemistry and thereby alter *fluorescence* yield during the course of the measurement.[38] At least two experimental approaches are suitable for studying Fl vs *E* relevant to solar-stimulated fluorescence: direct measurement of Lu(683) during exposures to blue-green light,[16] and use of non-actinic (i.e., very weak and very short) measurement beams during active fluorometry (the "probe" during "pump and probe"[28,40] or the non-actinic measuring beam in pulse-amplitude-modulated (PAM) fluorometry[17]).

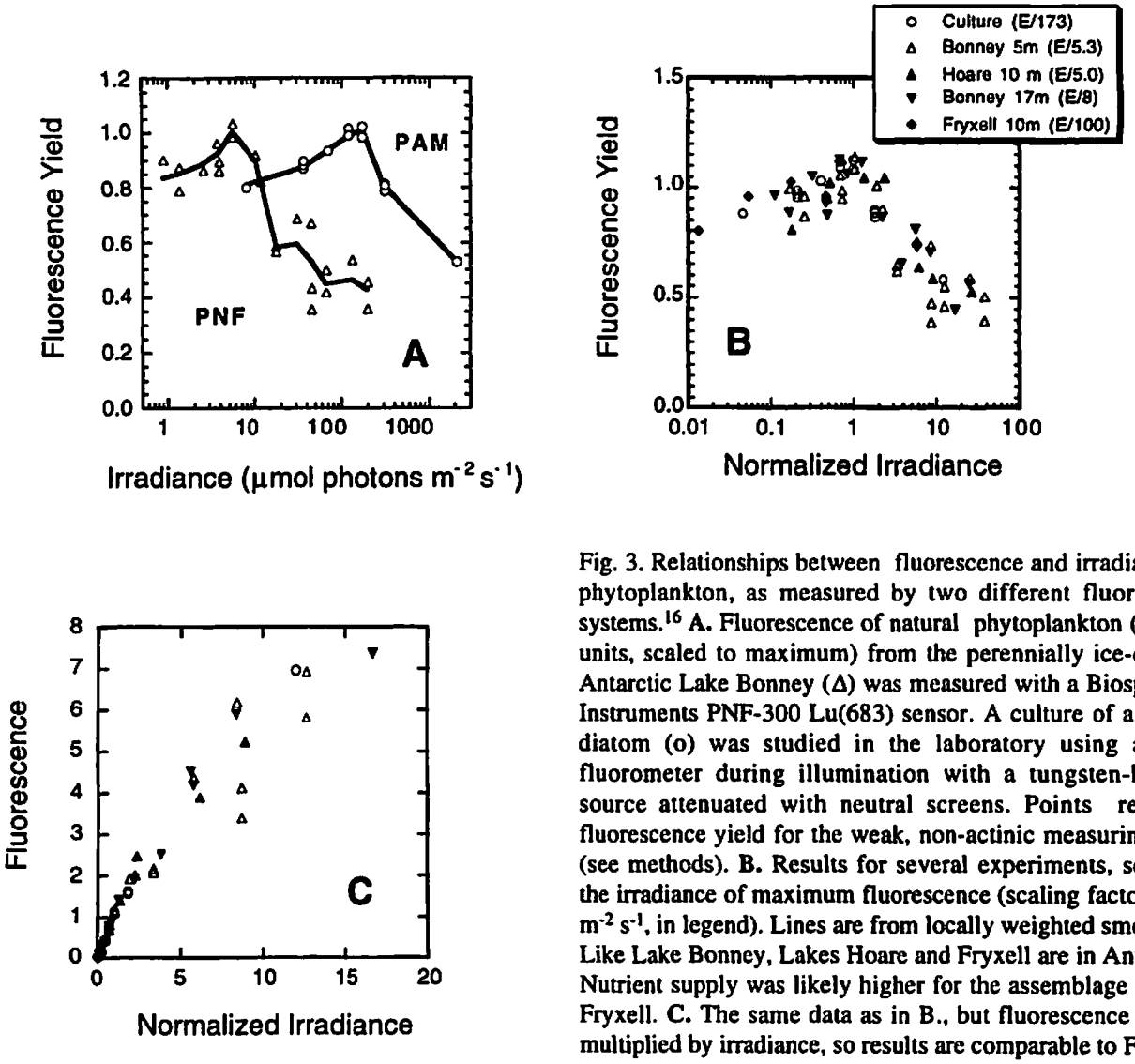


Fig. 3. Relationships between fluorescence and irradiance for phytoplankton, as measured by two different fluorometric systems.[16] **A.** Fluorescence of natural phytoplankton (relative units, scaled to maximum) from the perennially ice-covered Antarctic Lake Bonney (Δ) was measured with a Biospherical Instruments PNF-300 Lu(683) sensor. A culture of a marine diatom (o) was studied in the laboratory using a PAM fluorometer during illumination with a tungsten-halogen source attenuated with neutral screens. Points represent fluorescence yield for the weak, non-actinic measuring beam (see methods). **B.** Results for several experiments, scaled to the irradiance of maximum fluorescence (scaling factor, µmol $m^{-2}$ $s^{-1}$, in legend). Lines are from locally weighted smoothing. Like Lake Bonney, Lakes Hoare and Fryxell are in Antarctica. Nutrient supply was likely higher for the assemblage from L. Fryxell. **C.** The same data as in B., but fluorescence yield is multiplied by irradiance, so results are comparable to Fig. 2.

Recent experimental results illustrate the influence of photochemical and nonphotochemical quenching on steady-state fluorescence yield (Fig. 3): at low irradiance, fluorescence yield increases with irradiance (photochemical quenching diminishes as photosynthetic quantum yield declines), and at higher irradiance, fluorescence yield decreases because of nonphotochemical quenching. Thresholds for the transition (scaling irradiances in Fig. 3B) are strongly dependent on growth conditions (see also ref. 28). Although it was possible here to find a fairly strong pattern that was largely a function of appropriately scaled irradiance (Fig. 3B), it should be remembered that there are several quenching processes in phytoplankton and that they are time-dependent,[41] a function of preconditioning,[42] and somewhat species-dependent.[29] Much more experimentation is necessary before robust generalizations can be developed. It should be recognized, however, that

even fairly strong fluorescence quenching causes noticeable, but not extreme, bending in plots of fluorescence (*i.e.*, $Lu(683)_{corr}$ / Chl) vs irradiance (compare Fig. 2 with Fig. 3C); even stronger quenching would be required to saturate the Fl vs $E$ relationship, as suggested in data from the equatorial Pacific.[9] Regardless, the slope of fluorescence/Chl vs irradiance (see Fig. 2 and ref 18) can probably be related to quenching under restricted circumstances.

## 4. CONCLUSIONS

We have shown that several tools can be used to explore the relationships between fluorescence, Chl and irradiance in surface waters. Changes of fluorescence as a function of irradiance, observed experimentally and in the field, indicate that nonphotochemical quenching can be important in surface waters. The significance to photosynthesis is unresolved, however. Direct measurements of short-term carbon assimilation [43] or estimates of photosynthesis from special fluorometric methods[40] could help to improve our extremely sketchy understanding of the relationships between solar-stimulated fluorescence and near-surface photosynthesis.[9] Indications are that through careful consideration of the expanding literature on fluorescence, along with prudent design of experiments, rapid progress can be made. Meantime, it is encouraging that solar-stimulated fluorescence appears to be a readily detectable and reasonably robust indicator of near-surface chlorophyll.

## 5. ACKNOWLEDGMENTS

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## 6. REFERENCES

1. R. A. Neville and J. F. R. Gower, "Passive remote sensing of phytoplankton via chlorophyll fluorescence," *J. Geophys. Res.*, **82**, 3487-3493 (1977).
2. H. Gordon, "Diffuse reflectance of the ocean: the theory of its augmentation by chlorophyll *a* fluorescence," *Appl. Opt.*, **21**, 2489-2492 (1979).
3. A. Morel and L. Prieur, "Analysis of variations in ocean color," *Limnol. Oceanogr.*, **22**, 709-722 (1977).
4. J. F. R. Gower and G. Borstad, "Use of the *in-vivo* fluorescence line at 685 nm for remote sensing surveys of surface chlorophyll *a*," *Oceanography from Space*, J.F.R. Gower, (ed.), pp. 281-294, Plenum Press, New York, 1981.
5. M. S. Kishino, S. Sugihara and N. Okami, "Influence of fluorescence of chlorophyll *a* on underwater upward irradiance spectrum," *La Mer*, **22**, 224-232 (1984).
6. D. A. Kiefer, W. S. Chamberlin and C. R. Booth, "Natural fluorescence of chlorophyll *a*: relationship to photosynthesis and chlorophyll concentration in the western South Pacific gyre," *Limnol. Oceanogr.*, **34**, 868-881 (1989).
7. H. R. Gordon, O. B. Brown, R. H. Evans, J. W. Brown, R. C. Smith, K. S. Baker and D. K. Clark, "A semianalytic radiance model of ocean color," *J. Geophys. Res.*, **93**, 10,909-10,924 (1988).
8. K. L. Carder, S. K. Hawes, K. A. Baker, R. C. Smith, R. G. Steward and B. G. Mitchell, "Reflectance model for quantifying chlorophyll *a* in the presence of productivity degradation products," *J. Geophys. Res.*, **96**(C11), 20,599-20,611 (1991).
9. J. J. Cullen and M. R. Lewis, "Biological processes and optical measurements near the sea-surface: some issues relevant to remote sensing," *J. Geophys. Res.*, **100** (C7), 13,255-13,266 (1995).
10. B. J. Topliss and T. Platt, "Passive fluorescence and photosynthesis in the ocean: Implications for remote sensing," *Deep-Sea Res.*, **33**, 849-864 (1986).
11. W. S. Chamberlin, C. R. Booth, D. A. Kiefer, J. R. Morrow and R. C. Murphy, "Evidence for a simple relationship between natural fluorescence, photosynthesis and chlorophyll in the sea," *Deep-Sea Res.*, **37**, 951-973 (1990).
12. P. M. Stegmann, M. R. Lewis, C. O. Davis and J. J. Cullen, "Primary production estimates from recordings of solar-stimulated fluorescence in the equatorial Pacific at 150° W," *J. Geophys. Res.*, **97**(C1), 627-638 (1992).
13. J. J. Cullen, A. M. Ciotti and M. R. Lewis, "Observing biologically induced optical variability in coastal waters," *SPIE Ocean Optics XII*, **2258**, 105-115 (1994).
14. J. J. Cullen, Á. M. Ciotti, R. F. Davis and M. R. Lewis, "Optical detection and assessment of algal blooms," *Limnol. Oceanogr.*, in press (1996).
15. M. S. Kishino, S. Sugihara and N. Okami, "Estimation of quantum yield of chlorophyll *a* fluorescence from the upward irradiance spectrum in the sea," *La Mer*, **22**, 233-240 (1984).
16. P. J. Neale and J. C. Priscu, "Fluorescence quenching in phytoplankton of the McMurdo Dry Valley Lakes (Antarctica): Implications for the function of the photosynthetic apparatus," *Ant. Res. Ser.* submitted, 1996.

17. U. Schreiber, U. Schliwa and B. Bilger, "Continuous recording of photochemical and nonphotochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer," *Photosyn. Res.*, **10**, 51-62 (1986).
18. M. R. Abbott and R. M. Letelier, "Bio-optical drifters — scales of variability of chlorophyll and fluorescence," SPIE Ocean Optics XIII (1996).
19. C. Roesler and M. J. Perry, "In situ phytoplankton absorption, fluorescence emission, and particulate backscattering spectra determined from reflectance," *J. Geophys. Res.*, **100**(C7), 13,279-13,294 (1995).
20. W. S. Chamberlin and J. Marra, "Estimation of photosynthetic rate from measurements of natural fluorescence: analysis of the effects of light and temperature," *Deep-Sea Res.*, **39**, 1695-1706 (1992).
21. D. A. Kiefer, "Fluorescence properties of natural phytoplankton assemblages," *Mar. Biol.*, **22**, 263-269 (1973).
22. D. A. Kiefer, "Chlorophyll *a* fluorescence in marine diatoms: responses of chloroplasts to light and nutrient stress," *Mar. Biol.*, **23**, 39-46 (1973).
23. J. J. Cullen, "The deep chlorophyll maximum: comparing vertical profiles of chlorophyll a," *Can. J. Fish. Aquat. Sci.*, **39**, 791-803 (1982).
24. A. Morel and A. Bricaud, "Inherent properties of algal cells including picoplankton: Theoretical and experimental results.," *Photosynthetic Picoplankton*, T. Platt and W.K.W. Li, (eds.), pp. 521-559, 1986.
25. H. M. Sosik and B. G. Mitchell, "Light absorption by phytoplankton, photosynthetic pigments and detritus in the California Current System," *Deep-Sea Res. I*, **42**(10), 1717-1748 (1995).
26. G. Johnsen, E. Sakshaug and M. Vernet, "Pigment composition, spectral characterization and photosynthetic parameters in *Chrysochromulina polylepis*," *Mar. Ecol. Prog. Ser.*, **83**, 241-249 (1992).
27. P. G. Falkowski and Z. Kolber, "Variations in chlorophyll fluorescence yields in phytoplankton in the world oceans," *Aust. J. Plant Physiol.*, **22**, 341-355 (1995).
28. A. M. Chekalyuk and M. Y. Gorbunov, "Diel variability of in vivo chlorophyll fluorescence in near-surface water layer," *SPIE Ocean Optics XII*, **2258**, 140-151 (1994).
29. B. W. Ibelings, B. M. Kroon and L. R. Mur, "Acclimation of photosystem II in a cyanobacterium and a eukaryotic green alga to high and fluctuating photosynthetic photon flux densities, simulating light regimes induced by mixing in lakes," *New Phytol.*, **128**, 407-424 (1994).
30. G. H. Krause and E. Weis, "Chlorophyll fluorescence and photosynthesis: the basics," *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **42**, 313-349 (1991).
31. D. A. Kiefer and R. A. Reynolds, "Advances in understanding phytoplankton fluorescence and photosynthesis," *Primary Productivity and Biogeochemical Cycles in the Sea*, P.G. Falkowski and A. Woodhead, (eds.), pp. 155-174, Plenum, 1992.
32. P. J. Neale, "Algal photoinhibition and photosynthesis in the aquatic environment," *Photoinhibition*, D.J. Kyle, C.B. Osmond, and C.J. Arntzen, (eds.), pp. 35- 65, Elsevier, Amsterdam, 1987.
33. D. P. Maxwell, S. Falk and N. P. A. Huner, "Photosystem II excitation pressure and development of resistance to photoinhibition," *Pl. Physiol.*, **107**, 687-694 (1995).
34. P. G. Falkowski and J. LaRoche, "Acclimation to spectral irradiance in algae," *J. Phycol.*, **27**, 8-14 (1991).
35. J. J. Cullen and M. R. Lewis, "The kinetics of algal photoadaptation in the context of vertical mixing," *J. Plankton Res.*, **10**, 1039-1063 (1988).
36. R. J. Geider, H. L. MacIntyre and T. M. Kana, "A dynamic model of photoadaptation in phytoplankton," *Limnol. Oceanogr.*, **41**(1), 1-15 (1996).
37. J. J. Cullen, C. S. Yentsch, T. L. Cucci and H. L. MacIntyre, "Autofluorescence and other optical properties as tools in biological oceanography.," *Proc. SPIE Int. Soc. Opt. Eng.*, **925**, 149-156 (1988).
38. P. J. Neale, J. J. Cullen and C. M. Yentsch, "Bio-optical inferences from chlorophyll a fluorescence: What kind of fluorescence is measured in flow cytometry?," *Limnol. Oceanogr.*, **34**, 1739-1748 (1989).
39. M. Estrada, C. Marrasé and J. Salat, "*In vivo* fluorescence/chlorophyll *a* ratio as an ecological indicator in oceanography," *Sci. Mar.*, (1996).
40. Z. Kolber and P. G. Falkowski, "Use of active fluorescence to estimate phytoplankton photosynthesis in situ," *Limnol. Oceanogr.*, **38**, 1646-1665 (1993).
41. C. S. Ting and T. G. Owens, "The effects of excess irradiance on photosynthesis in the marine diatom *Phaeodactylum tricornutum*," *Pl. Physiol.*, **106**, 763-770 (1994).
42. W. Arsalane, B. Rousseau and J. C. Duval, "Influence of the pool size of the xanthophyll cycle on the effects of light stress in a diatom - competition between photoprotection and photoinhibition," *Photochem. Photobiol.*, **60**, 237-243 (1994).
43. J. J. Cullen, M. R. Lewis, C. O. Davis and R. T. Barber, "Photosynthetic characteristics and estimated growth rates indicate grazing is the proximate control of primary production in the equatorial Pacific," *J. Geophys. Res.*, **97**(C1), 639-654 (1992).