FLUOROMETRY FOR BIOLOGICAL SENSING

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Introduction

Fluorescence is the optical process whereby light of short wavelength (high energy per photon) is absorbed by molecules (organic or inorganic) in their ground state, exciting the absorbing compound to a higher state. The decay to the ground state is usually a two-stage process: first to an intermediate state (usually metastable, i.e., longer-lived) with a fractional loss of energy (sometimes radiationless); and in a second stage to the ground state, with the emission of radiation of less energy per photon than that originally absorbed, i.e. of longer wavelength. There are many fluorescence phenomena in the marine environment, some of which have biological or biogeochemical significance. Most notable of these is the fluorescence of the ubiquitous phytoplankton pigment chlorophyll-*a* (red light emitted, ~ 683 nm *in vivo* and ~ 670 nm *in vitro*) when excited by blue light over a broad spectrum in the 'Soret' band (400–470 nm). Chlorophyll-*a* (Chl*a*) fluorescence is the archetypal phytoplankton phenomena of marine biogeochemical significance. Through the use of simple filter fluorometers, the measurement of Chl*a* in the marine environment has been exploited widely, over the whole of the Earth's oceans, for over three decades. Chlorophyll-*a* is universally accepted as the key variable in the simplest models of phytoplankton biomass and productivity. All plants contain Chl*a*, and Chl*a* is a crucial component of phytoplankton photosynthetic apparatus. Other chlorophylls (-*b* and -*c*) and their degradation products (pheophytins and pheoborides) also fluoresce when excited by blue light, with emissions close to Chl*a* fluorescence. Phycobiliproteins (phycoerythrin and phycocyanin), which are specific to cyanobacteria and cryptomonads, fluoresce at yellow (560–580 nm) and orange (620–640 nm) wavelengths when excited by green-yellow light (530–580 nm). Cyanobacteria form a major fraction of the biomass in tropical and sub-tropical oceans. Their fluorescences are covered briefly in this article. The remaining accessory pigments (carotenoids, both photosynthetic and photoprotective) do not fluoresce. Dissolved organic compounds/materials (DOC/DOM), which may be autochthonous, that is, produced *in situ* from the degradation of biogenic materials, fluoresce across the UV and visible spectrum when excited by UV and blue light. In coastal environments, additional allochtonous DOM of terrestrial, riverine origin (also termed gelbstoff or yellow substances) may coexist but not covary with DOM of biogenic origin. DOM are important in marine biogeochemical cycles and are included in this article. Petrochemicals, polycyclic and polynuclear aromatic hydrocarbons (PAH), which have fluorescence signatures similar to DOM, may originate in marine environments (seeps) but have little oceanographic relevance. Of course they may occur from anthropogenic activities – discharges (pollution) in coastal zones – and can be mapped and tracked by ship-borne and airborne fluorometers. Of minor biogeochemical significances and excluded from this article are the fluorescences from inorganic ions (e.g., NO_3^- has weak fluorescence in the UV).

This article concentrates on the fluorescence of chlorophyll-*a*, used widely for estimating phytoplankton occurrence and abundance in the marine environment, both *in vitro* (extracted into solution) and *in vivo* (living plants). Recently active fluorescence methods have emerged for investigating photosynthetic processes and determining photosynthetic rates and parameter values. These include fluorescence induction, pulse amplitude modulation (PAM) fluorometry, pump and probe fluorometry (PPF), and fast repetition rate fluorometry (FRRF). Discussion of these techniques forms a major section of this article. Brief sections address passive solar stimulated fluorescence (PSSF), its detection *in situ* and from aircraft and satellites, laser-stimulated phytoplankton fluorescences from ships or aircraft, laser stimulation of photosynthetic quantum efficiency, and automatic flow cytometry (AFC).

Chlorophyll-*a* and Phytoplankton Pigment Fluorescences

The red fluorescence of chlorophyll was first observed in the eighteenth century from green leaves photosynthesizing under direct sunlight. Since then, it has been used as a chemical assay technique for quantifying Chl*a* and as a mechanism for studying photosynthesis in plants. A simplified excitation scheme and the energy levels of 'bulk' chlorophyll are illustrated in **Figure 1**. Strictly this scheme

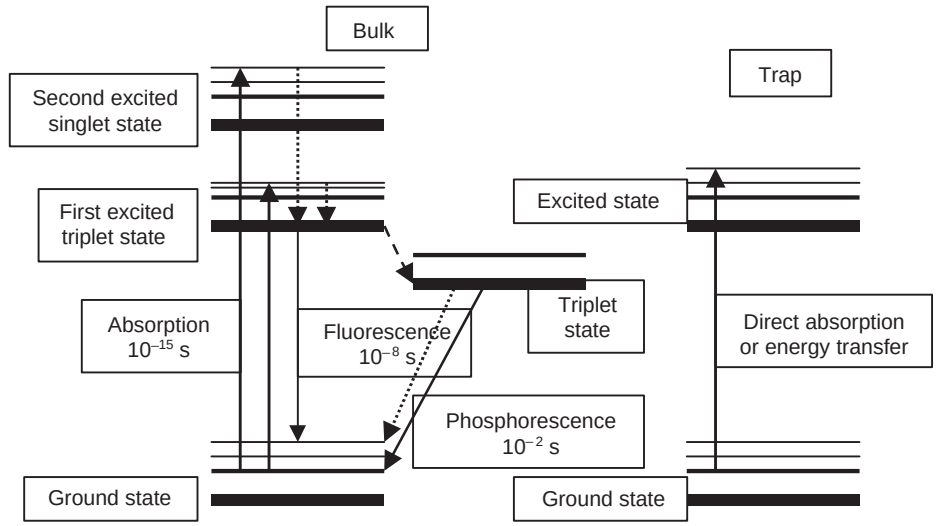

Figure 1 Energy level diagram for 'bulk' chlorophyll (left) and PSII trap molecule (right) after Govindjee (1975). Bold, horizontal lines represent the energy levels of the main electronic states, and the light lines the vibrational/rotational levels. Solid vertical lines represent electronic transitions (absorption and emission) and dashed lines represent radiationless internal conversions.

applies only to fluorescence from antenna chlorophyll or Chl*a* *in vitro*. Different mechanisms operate for the 'fluorescence' emanating from the photosynthetic apparatus. Much of the historical research refers to the 'fluorescence' from photosystems I and II in higher plants but the basic processes relate equally to marine and freshwater algae. Research on higher plants, leaves, and isolated chloroplasts has shown the relationship between fluorescence and the bioenergetics of photosynthesis. Marine applications are recent developments of this work.

In vivo, Chl*a* fluoresces at 683 nm and longer wavelengths (690 and 720 nm) depending on its photochemical form and its involvement in the photosynthetic process. In solution the fluorescence wavelength is shorter (670 nm), as the Chl*a* is detached from the light-harvesting antenna complex of the photosynthetic apparatus. *In vitro* chlorophyll-*b* and chlorophyll-*c* fluoresce at shorter wavelengths (660 and 642 nm, respectively) and the degradation products pheophytin-*a*, -*b*, and -*c* fluoresce at 676, 662, 665 nm, respectively. This can be a hindrance in the quantitative assay of Chl*a* concentration *in vitro* when using simple filter fluorometers. Research dating from the 1960s showed consistent emission of fluorescence (at 670 nm) when excited at 450 nm for the spectrofluorometric analyses of several phytoplankton species (extracted in 85% acetone). This provided the specifications to convert a 'G.K. Turner' filter fluorometer to give quantitative measurements of Chl*a* with improved limits of detection compared to standard spectraphotometry. All pigments have much greater fluorescence yields in solution ($\sim 30\%$ *in vitro* compared to $\sim 3\%$ maximum *in vivo*) and are shifted to shorter wavelengths. All the chlorophylls and their derivatives (chlorophyllides, pheophorbides) fluoresce at similar red wavelengths as a consequence of their common unique structure, the conjugation of the double bonds of the four pyrrole rings. These characteristic fluorescences disappear only when these bonds are destroyed (by hydrogenation, for example). Thus measurement of the fluorescence of chlorophyll *in vitro*, with a simple filter fluorometer, measures all the chlorophyllous compounds, albeit dominated by Chl*a*. The other chlorophylls and their degradation products are usually in smaller concentrations ($< 5\%$ of Chl*a*) and have smaller fluorescence yields. Despite its utility, errors associated with the 'Turner' fluorometer were evident from early usage, especially the acidification procedure for estimating pheopigments and particularly when chlorophyll-*b* was present. Many of the measurements of pheopigments in ocean waters by this method are considered erroneous. Pigment measurements by high-pressure liquid chromatography (HPLC) hardly ever detect pheopigments in sizable quantities, except in known circumstances, such as in estuaries or from accumulations due to zooplankton grazing on phytoplankton populations in the thermocline. Modification of the filter fluorometer can reduce the errors by change of the lamp and excitation filter and by use of a narrow-band red filter to accept only Chl*a* fluorescence. Chl*a* determined by the modified method compares closely with determinations by HPLC. The acidification

protocol for pheopigments is not used in the modified procedure.

The adaption of the Turner fluorometer to the measurement of Chl*a* fluorescence *in vivo* has had even greater impact on the determination of phytoplankton distributions globally, because of the simplicity of the method. Since the early 1970s, fluorometric measurements of pumped surface sea water while the vessel is under way have been acquired on nearly every research cruise. The amount of data on local, seasonal, interannual, and basin-wide distributions of phytoplankton acquired has been immense, exceeded only by that from satellite remote sensing (Coastal Zone Color Scanner, 1979–86). Even so, the method has required careful calibration for different phytoplankton assemblages in different sites and seasons. The *in vivo* fluorescence (IVF) yield of chlorophyll has been reported to vary with species composition, size category, and time of day (IVF yields may be greater by a factor of 5 at night). Near-surface phytoplankton, in high light and with high photosynthetic activity, have reduced (quenched) IVF yield. At night, quenching is inactive and the IVF yield is maximized. IVF has been reported to vary with physiological condition, for example, nutrient stress and previous light history. Variations of IVF *in situ* have been detected in response to rapid fluctuations of light. The wide range of environmental responses is mostly due to differences in phytoplankton physiological condition or photosynthetic activity.

One approach to deal with the variable fluorescence yields, copied from terrestrial ecology, has used the herbicide 3-(3,4-dichlorophenyl-1, 1-dimethyl urea (DCMU), which blocks the electron transfer process and chemical conversion. Addition of DCMU maximizes the IVF yield, giving a quasi-constant response for range of phytoplankton species. In laboratory and field studies, DCMU-enhanced IVF yield has been found to be an index of photosynthetic activity, though with no robust relationship.

The causes of the variability of chlorophyll IVF yield are known and arise from a misunderstanding of the source of Chl*a* fluorescence. At room temperature, 683 nm fluorescence originates mostly from electron–hole recombination processes in photosystem II (PSII) and is more accurately described as luminescence. Bulk antenna chlorophyll contributes only a small fraction. The luminescence process has a high but variable efficiency, inversely related to photosynthetic rates, yet it still dominates stimulated fluorescence from antenna chlorophyll by a factor of 10 or more (efficiency is 1–4% compared to 0.1–0.4%). Understanding these processes is key to the theory of active fluorescence, discussed in a subsequent section.

Subsurface 'quenching' by sunlight is reduced and often negligible by depths of 20–30 m (5% or less of ambient light levels) and fluorescence measurements provide reliable values for chlorophyll concentration (when calibrated). For near-surface measurements, the effects of fluorescence quenching can be estimated from vertical profiles or vertical sections obtained with towed undulating sensor systems, such as the Undulating Oceanographic Recorder (UOR), Sea-Soar, or Batfish. Other ancillary sensors, such as transmissometers or light sensors, can show the true vertical structure of phytoplankton biomass and provide a correction of the fluorescence measurements when subject to quenching.

The calibration of fluorometers for the measurement of Chl*a* *in vivo* or *in situ* is an issue, in the context of the variability of IVF yield. As IVF yield may vary with site, season, phytoplankton assemblage, and physiological state, vicarious calibration is usual, whereby the fluorometer output is calibrated against contemporaneous discrete water samples, filtered, extracted in solvent, and assayed chemically. Given the day-to-night variations of IVF yield, this procedure shows consistency at night and scattering by day that is unavoidable. As discussed above, the data must be interpreted in this context. A bivariate calibration of Chl*a* concentration against fluorescence and incident PAR has been shown to work well both for surface layer measurements and for vertically profiled data.

Measurements of Chl*a* fluorescence *in situ* use fluorometers that are generally low-powered, self-contained, and often self-logging, suitable for profiled, towed, or moored applications. Since the late 1970s these have been manufactured commercially (e.g., Chelsea Instruments 'Aquatracka'). Smaller and lower cost instruments have been developed, expanding the applications. Versions have been adapted for DOM fluorescence and the phycobiliproteins in cyanobacteria and an *in situ* spectrofluorometer has been manufactured (SAFIRE, Wet Labs Inc.).

When the nature and significance of chlorophyll fluorescence has been established, fluorometric methods can be used to examine the distribution of phytoplankton biomass at oceanographic structures, either vertically or at fronts between different oceanographic water masses. **Figure 2** shows the UOR contoured vertical sections of temperature, salinity and chlorophyll fluorescence (calibrated in units of $mg\,m^{-3}$) across the equatorial front in the North Atlantic at 20°W, from 2°N to 1°S, September 1996. North of the front, the surface mixed

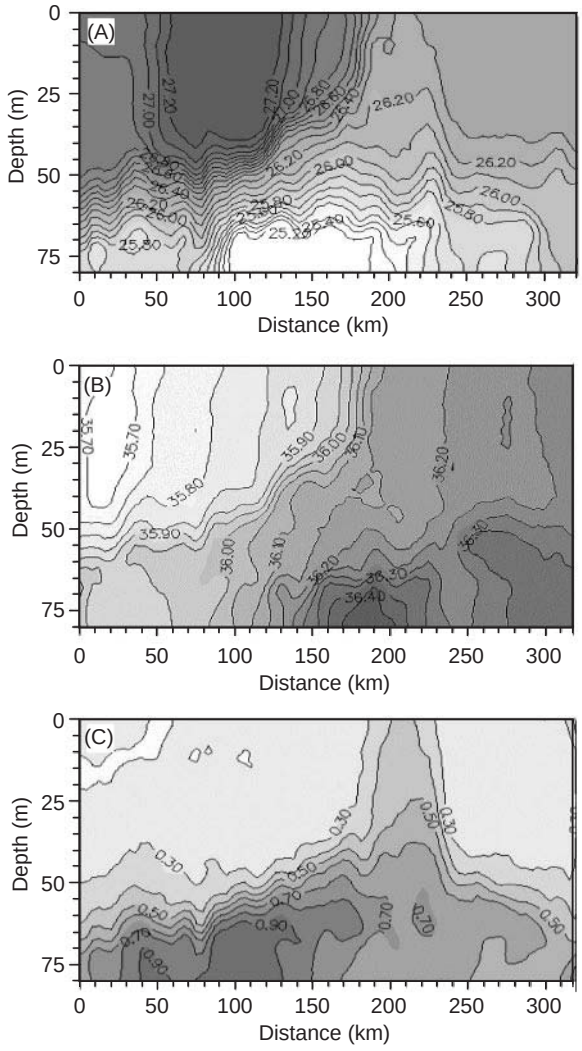

Figure 2 UOR contoured vertical sections of temperature, salinity and chlorophyll fluorescence (calibrated in units of $mg\,m^{-3}$) across the equatorial front in the North Atlantic at 20°W, from 2°N to 1°S, September 1996.

layer depth was 40 m, temperature ~ 27.2°C, and salinity ~ 35.8 above a sharp thermocline. Chlorophyll was ~ 0.2 at the surface, increasing to 0.9 in the thermocline. South of the front, the surface mixed layer was deeper, cooler (26.3°C), and of higher salinity (> 36.2) with a lower concentration of chlorophyll in the thermocline. The convergence between the two counterflowing water masses, north and south of the Equator, induced an upwelling, drawing up the phytoplankton thermocline population to the surface at the Equator.

Despite the limitations of the measurement of chlorophyll fluorescence for phytoplankton biomass *in vivo* or *in situ*, it is the simplest method and provides the most widely mapped variable in biological oceanography.

Passive Solar Stimulated Fluorescence (PSSF), Airborne and Satellite Observations

The emission of fluorescence at 683 nm, stimulated by sunlight, can be observed as a peak in the upwelling radiance signal, measured by a radiometer *in situ* through a subsurface phytoplankton population in the thermocline. The signal is equivalent to the stimulated fluorescence measured by standard *in vivo* or active fluorometers and is subject to the variability and problems of interpretation discussed above. It has been proposed that the PSSF relates to photosynthetic rates, but there is no mechanistic justification for this. The PSSF signal at 683 nm can be measured remotely from ships or aircraft (or helicopters), most notably with the Airborne Oceanographic Lidar (AOL), an active–passive laser system, or the Compact Airborne Spectrographic Imager (CASI), a high-resolution CCD array imager. The AOL also measures the laser-stimulated fluorescence of chlorophyll (LSFC) at 683 nm using a nadir-pointing laser at 532 nm. The PSSF and LSFC measurements are almost identical, suggesting (though not proving) that their mechanisms of origin are the same. The AOL passive upwelling radiances at 443 and 560 nm can be used with a remote sensing (RS) algorithm to provide a measurement of Chl*a*, equivalent to the observations from satellite sensors CZCS and SeaWiFS. The PSSF and LSFC measurements agree well with the RS determinations, which are free from the variability of IVF yield. The AOL also measures the laser-stimulated fluorescence from phycoerythrin and DOM, providing a useful technique for the determination of the distributions of these biogeochemically active variables in the marine environment. A development of the AOL provides measurements of the photosynthetic quantum efficiency from aircraft; see section below on active fluorescence.

New satellite ocean color sensors, MODIS on TERRA (launched late 1999) and MERIS on Envisat (to be launched mid-2001) have bands at 678 nm, 681 nm, respectively, specifically to detect PSSF. Preliminary data from MODIS have shown distributions of PSSF that are different from the Chl*a* maps derived from the RS passive color observations, adding a new option to the interpretations from satellite sensors.

Automatic Flow Cytometry (AFC)

AFC has been developed from medical applications to count, identify, and sort cohorts of single cell

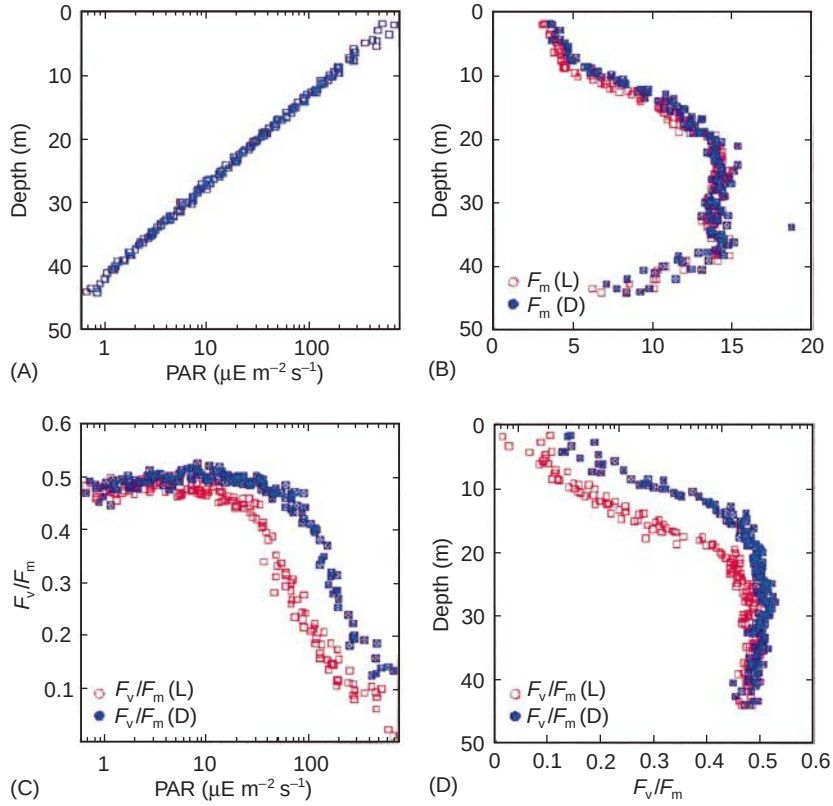

Figure 3 Vertical profiles of FRRF data for May 2000: (A) PAR versus depth; (B) F_m(L) and F_m(D) versus depth; (C) F_v/F_m versus PAR; (D) F_v/F_m versus depth. PAR = photosynthetically available radiation (1E = 1 mol photons); D and L refer to dark chamber and ambient light data, respectively.

types from mixed phytoplankton assemblages using laser (488 nm) generated optical signals. Phytoplankton can be counted and sorted on the basis of forward and 90° scattering (size- and shape-dependent) along with the fluorescence at 683 nm from chlorophyll and 580 nm from phycoerythrin. The optical classification can be used to apply an electrical charge to each class and deflect the cells out of the flow for subsequent analysis. 'Cytobuoy' is a recent *in situ* AFC development.

Active Fluorescence

At room temperatures, 683 nm fluorescence emanates from photosystem II (part of the photosynthetic apparatus). It is the fraction of energy originally absorbed by the accessory pigments that is unused in the photosynthetic process because of a limiting factor such as nutrient deficiency. This variable inefficiency leads to the variation of chlorophyll IVF yield measured by a 'standard' fluorometer *in vivo*, often termed 'fluorescence quenching.' The pump and probe fluorometer (PPF) was the first oceanographic instrument, designed to exploit the measurements of the 'variable fluorescence' of phytoplankton, to determine the activity and efficiency of photosystem II. Laboratory experiments show that when photosystem II is 'pumped' with saturating intensities of incident radiation, all the PS2-traps are closed. Following a delay of 80–100 μs after saturation, the fluorescence yield, measured by a low-intensity 'probe' pulse (about 0.1% of saturating) is maximal (F_m). A 'probe' in ambient light by a low-intensity pulse before the 'pump' gives the fluorescence yield (F_0), which is proportional to the fraction of PSII-traps 'open,' i.e., inactive. The activity of PSII in ambient conditions is proportional to the fraction of PSII-traps closed, equal to $(F_m - F_0)/F_m$ or F_v/F_m, where $F_v = (F_m - F_0)$. F_v is termed the variable fluorescence and F_v/F_m the photochemical quantum efficiency. F_0 is akin to the fluorescence stimulated in a standard fluorometer or passive solar-stimulated fluorescence and is highly variable. F_v/F_m is a dimensionless number proportional to phytoplankton productivity and with suitable models can be used to derive phytoplankton photosynthetic parameters (such as maximum rate of photosynthesis (p_{max}), the light saturation parameter (E_k) and the light-limited rate of photosynthesis (α)).

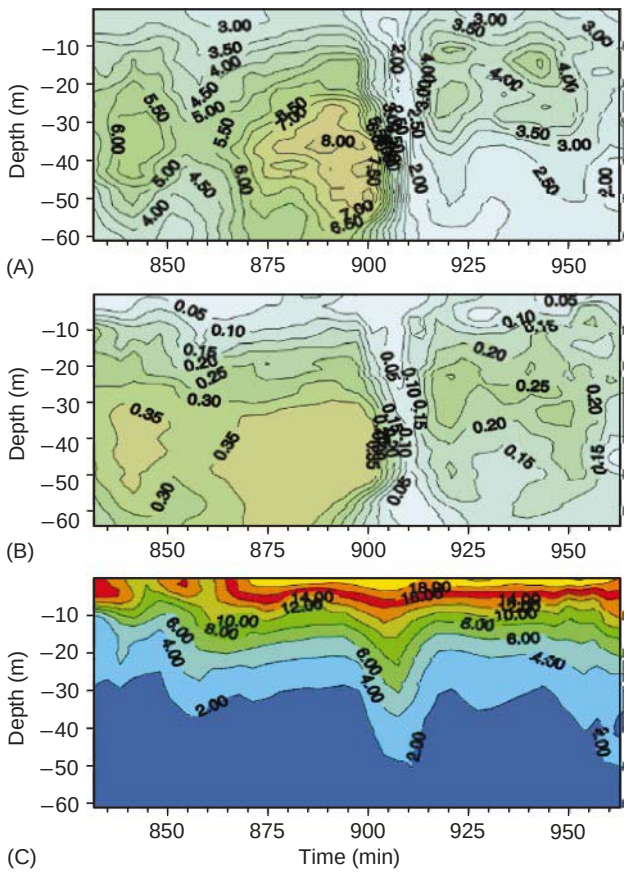

Figure 4 Contoured vertical sections from 0 to 60 m of (A) F_m, (B) F_v/F_m, and (C) E_{d490} for UOR tow A514 Oct. 1997.

The fast repetition rate fluorometer (FRRF) was developed at the Brookhaven National Laboratory from the simple PPF technique by devising a complex flash sequence to derive other phytoplankton photosynthetic parameters *in situ*, rapidly, and non-destructively. By 'pumping' PSII to saturation with a rapid sequence of 'flashlets' of subsaturating intensity, the size of the cross-section of PSII (σ_{PSII}) is determined from the slope of the F_v/F_m curve to saturation. After saturation, the frequency of the flashlets is decreased and the saturation of PSII decays, with a slope proportional to the turnover τ time of PSII. Both σ and τ are determined by fitting an exponential function to the data. A mechanistic model and the operational protocols for the PPF and FRRF are given in the key reviews.

The IronEx II experiment in the Equatorial Pacific in 1994 was the first notable exploitation of the FRRF *in situ*, towed in the UOR. The site (high-nutrient, low-chlorophyll) was surveyed before, 3 days after iron-fertilization, and 12 days later. Following fertilization, F_v/F_m increased from an initial value of ~ 0.25 to ~ 0.5 within 20 h. A phytoplankton bloom developed, reaching biomass concentrations of $0.5\,mg\,m^{-3}$ after 3 to 5 days, before dispersing owing to subduction after 12 days. The rapidity of enhanced photosynthetic activity suggests that an increase of F_v/F_m should be a precursor indicator of imminent phytoplankton blooming.

Figure 3 shows the vertical profiles (Celtic Sea, May 2000) of (A) photosynthetically available radiation (PAR) versus depth and FRRF ambient light (L) and dark chamber (D) data for (B) F_m versus depth, (C) F_v/F_m versus PAR and (D) F_v/F_m versus depth. The F_m(L) and F_m(D) versus depth equate to Chl*a* biomass profiles, with quenching by sunlight in the surface 20 m, as a standard fluorometer. The F_v/F_m(L) is always less than F_v/F_m(D), indicating that the residence time (~ 1 s) in the dark chamber is sufficient to produce a different response (light reactions stop in < 1 s). The F_v/F_m versus PAR profile is analogous to a productivity (P) versus irradiance (E) curve. The 'constant' values of F_v/F_m at low light (below about $50\,\mu E\,m^{-2}\,s^{-1}$) or depth (below about 20 m) are equivalent to the linear, low-light regime of photosynthesis P versus E curve (of slope α). The region of decreasing F_v/F_m at high light (shallow depths) equates to the photosaturated (P_{max} value) or photoinhibited regime. The point of inflection between the two regimes equates to the PAR value of the light saturation parameter, E_k.

FRRF systems have been used extensively on the Atlantic Meridional Transect (AMT) cruises between the United Kingdom and Antarctica, southward in the Boreal fall and Austral spring and northbound the following April and May. Data from ATM-5 (Oct. 1997) showed that photosynthetic activity and parameter values changed significantly across 'frontal' structures in natural systems in this region of the Sub-Antarctic convergence, from $\sim$ 33–45°S. This area has extreme heterogeneity, formed by the confluence of the warm, southerly-flowing, Brazil Current and the cold, northerly-flowing Falklands Current, an extension of the Antarctic circumpolar current. The interaction produces large-area detached warm-core 'rings' of Brazil Current water, surrounded by small, cold-core dipoles or multipoles. Satellite imagery of sea surface temperature (AVHRR) and height (TOPEX) show these features. **Figure 4** shows the contoured vertical sections (0 to 60 m) of F_m, F_v/F_m, and downwelling irradiance at 490 nm (E_{d490}), for UOR tow A514, starting in a cold-core and crossing a sharp front along the edge of warm-core water. Before the front, biomass (F_m) was twice as high as

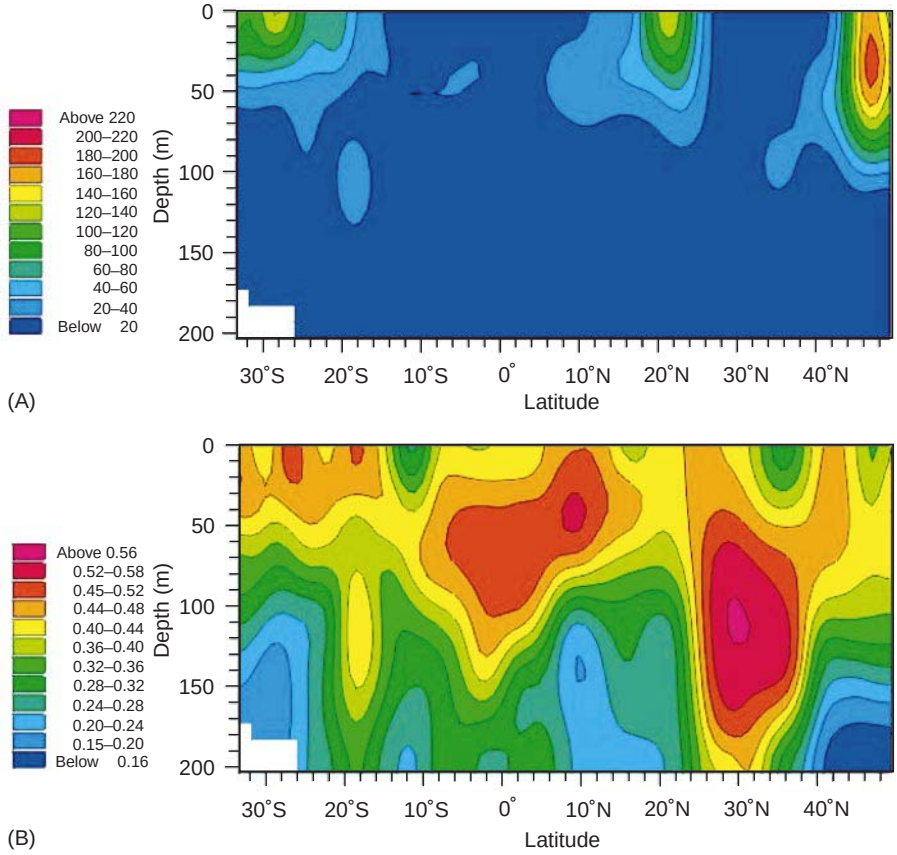

Figure 5 The contoured vertical sections of (A) F_m and (B) F_v/F_m, from 35°S to 50°N for AMT-6.

after. Within the front, biomass dropped to near zero, and light (E_{d490}) penetrated almost twice as deep, indicating a zone of plant-free water, probably upwelled from depth by convergence between the counterrotating water masses. The photosynthetic quantum efficiency (F_v/F_m) was ~ 0.4 in the cold water, reducing to about 0.25 in the warm water, just less than the changes induced by the iron-fertilization in IronEx II. This variability of photosynthetic activity in natural systems can be determined only by the FRRF.

On AMT-6 (May 1998), FRRFs measured F_v, F_m and F_v/F_m of pumped surface sea water (7 m) in flow-through mode (dark-adapted), on the CTD (conductivity, temperature, and depth) casts to 200 m and in the UOR. **Figure 5** shows the contoured vertical sections of F_m and F_v/F_m for 39 CTD stations from Cape Town (35°S) to the western English Channel (50°N). F_m represents biomass, except for the 'quenching' in the surface 10–20 m. There were three regions of high biomass and productivity: the Benguela (35–20°S), the West African upwelling (18–25°N), and the Celtic Sea shelf break. Subsurface maxima of F_v/F_m were observed in the North Atlantic Equatorial region (0–16°N) and the subtropical gyre (26–38°N). The along-track F_m and F_v/F_m for the surface water were very high in the Benguela, often close to 0.65, the assumed theoretical maximum. By contrast, the values in oligotrophic areas were much lower, 0.2 to 0.3. These basin-scale data of phytoplankton photosynthetic parameters derived *in situ* and nondestructively by the FRRF provide a unique method to characterize productivity in biogeochemical provinces globally. For biological oceanography, their impact should be comparable to that of the Turner fluorometer three decades earlier.

DOM Fluorescence

Both autochthonous (produced *in situ*) and allochtonous DOM of terrestrial, riverine, origin fluoresce across the UV and visible spectrum when excited by UV and blue light. DOM have many polar and carbon-chain compounds, both labile and highly refractory, of molecular weights up to several thousand. Only aromatic ring compounds have optical properties (absorption and fluorescence). Single-ring compounds (benzenes) absorb at 250–280 nm and fluoresce at 280–300 nm; double-ring compounds

(naphthalenes) have fluorescence maxima at 300–320 nm; three- and four-ring compounds have fluorescence maxima at 340–380 nm; and compounds with five or more rings have fluorescences at > 400 nm, typically 440 nm. These characteristics can be determined by spectrofluorometers with diode-array spectrometers, providing 3D excitation–emission matrices, to differentiate the components of the sea water. DOM is highly reactive to UV in the surface ocean, breaking down high-molecular-weight compounds and releasing biogenic gases, CO, CO_2, COS, CH_4, many of which are greenhouse gases.

Crude and refined petrochemicals have chemically similar compounds to DOM, and can be detected by ship-borne or airborne laser fluorometers at very low concentrations (1 in 10^{13}) and used to track 'oil' spills. The fluorescence signals from DOM and crude or refined oils are indistinguishable, although the fractional composition of one-, two-, three-, and four-ring aromatics in the oil can provide a unique identifier of its source or geographical origin.

Terms Used

α	Light limited rate of photosynthesis ($mg\,C\,m^2\,mol\,quanta^{-1}$)
σ_{PS2}	Functional cross-section of PS2 ($m^2\,quanta^{-1}$)
τ	Turnover time of electron transport (μs)
AFC	Automatic flow cytometry
AMT	Atlantic meridional transect, research ship programme
AOL	Airborne oceanographic lidar, laser fluorosensor
AVHRR	Advances very high resolution radiometer, satellite sensor for SST
Chl*a*	Chlorophyll *a*, ubiquitous phytoplankton pigment ($mg\,m^{-3}$)
CTD	Conductivity temperature and depth
CZCS	Coastal zone color scanner, satellite sensor for Chla
DCMU	(3-(3,4-dichlorophenyl)-1,1-dimethylurea)
DOC/DOM	Dissolved organic compounds/material
E_k	Light saturation parameter ($\mu E\,m^{-2}s^{-1}$)
Envisat	European Space Agency Earth Observing Satellite carrying MERIS
F_m	Maximum fluorescence, after a saturating pulse of photons (arbitrary)
F_0	Initial fluorescence (arbitrary units)
FRRF	Fast repetition rate fluorometer
F_v	Variable fluorescence $= F_m - F_o$ (arbitrary units)
F_v/F_m	Photochemical quantum efficiency (dimensionless)
HPLC	High performance liquid chromatography, assay for pigments
IronEx II	Iron fertilization experiment II, Equatorial Pacific, May–June 1995
IVF	*In vivo* fluorescence (arbitrary units)
MERIS	Medium Resolution imaging spectrometer on Envisat
MODIS	Moderate imaging spectrometer, on TERRA
PAM	Pulse amplitude modulation
PAR	Photosynthetically available radiation ($\mu E.m^{-2}s^{-1}$)
PPF	Pump and probe fluorometer
P_{max}	Maximum rate of photosynthesis ($mg\,C\,h^{-1}$)
PS2	Photosystem 2
PSSF	Passive solar stimulated fluorescence
SeaWiFS	Sea-viewing wide field-of-view sensor, satellite sensor
SSH	Sea surface height
SST	Sea surface temperature
TERRA	NASA polar platform carrying MODIS
TOPEX	Altimeter satellite sensor for SSH
UOR	Undulating oceanographic recorder, a towed sensor system
UV	Ultra violet radiation

Phytoplankton Pigments

Chlorophylls	Chlorophyll-*a*, -*b*, -*c*, chlorophyllides
Carotenoids	Photosynthetic (e.g. fucoxanthin), photoprotective (e.g. zeaxanthin)
Pheopigments	Pheophytins, pheoborides
Phycobilliprotines	Phycoerythrin, phycocyanin

See Also

Bio-optical Models. Fluorometry for Chemical Sensing. Inherent Optical Properties and Irradiance. Ocean Color from Satellites. Optical Particle Characterization. Phytoplankton Blooms.

Further Reading

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FLUOROMETRY FOR CHEMICAL SENSING

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Introduction

In the context of ocean sciences, 'chemical sensing' denotes the acquisition of data on the concentration of certain chemical species (the analytes) in sea water. This acquisition process may be achieved by a 'chemical sensor' in the strict sense, i.e., a device that continuously and reversibly exhibits a change in some of its properties as a function of the concentration of a respective analyte. As an alternative, sensing may be done directly, without the detour over the properties of a sensor, by measuring some effect displayed by the analyte itself. Intermediate between these two methods is the use of an indicator that is added to a sample of sea water and changes its properties depending on the analytes' concentration. An example of a sensor is the Clark electrode, which delivers a current proportional to the concentration of oxygen. An example of direct sensing is the determination of salinity by conductivity measurements. The well-known use of litmus to monitor pH is an example for the indicator method.

'Fluorometry' refers to the measuring of fluorescence, which is the reemission of light from a compound upon exposition to light of a shorter wavelength (in this context, phosphorescence is included under the term fluorescence, although strictly speaking these are two different processes). Fluorescence occurs from a singlet excited state, while phosphorescence is originating from a triplet. This emission is characteristic for the emitting compound, but may be subject to modification by the environment. Thus, fluorometry may be applied to both sensing schemes outlined above: In a sensor device, its characteristic fluorescence properties may be modified by the concentration of the analyte. Direct sensing preferentially will be applied if the analyte itself is fluorescent.

Fluorescence

The fluorescence emission displays a number of general characteristics. Except for atoms in the vapor phase, the emission is shifted to lower wavelengths relative to the excitation. The energy loss between excitation and emission, the so-called 'Stokes shift', occurs owing to rapid decay of excitation energy to the lowest vibrational level of the excited state, as well as from subsequent decay to higher vibrational levels of the ground state. As the relaxation to the lowest vibrational level usually occurs within 10^{-12} s, the fluorescence emission spectrum does not depend on the excitation wavelength. The fluorescence can be described using the parameters spectral