Chlorophyll fluorimetry as a method for studying light absorption by photosynthetic pigments in marine algae*

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Abstract

Using laboratory cultures of algae and natural phytoplankton populations from Nhatrang Bay (South China Sea), the relationship between the chlorophyll fluorescence F_0, the chlorophyll a concentration C_a and light absorption capacities of algae cells was studied. It is shown that the ratio F_0/C_a depends mainly on the

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species composition of the algae population; hence, the concentration C_a can be measured with the fluorescence method with acceptable accuracy only when the species composition of algae populations varies over a rather narrow range. The fluorescence F_0 can, however, be a good index of the total absorption capacities of different phytoplankton species, because the intensity of F_0 depends on the sum total of light absorbed by all photosynthetic pigments in a plant cell. Thus, the fluorescence F_0 measures not only the concentration of chlorophyll *a*, but that of all photosynthetic pigment concentrations.

Introduction

Characterised by a high sensitivity and allowing rapid non-invasive assessment of several characteristics of phytoplankton, the methods of measuring chlorophyll fluorescence have become very popular in recent times (Matorin & Venediktov 1990, Falkowski & Raven 1997, Fadeev et al. 1999, Ostrowska et al. 2000a, b, Ficek et al. 2000, Ostrowska 2001, Woźniak et al. 2002). It was demonstrated some time ago that fluorescence intensity is correlated with phytoplankton concentration as measured by routine methods, for example, from the cell concentration or biomass (Karabashev 1987, Sirenko et al. 1988). However, the most frequently used characteristic of the phytoplankton content is the chlorophyll *a* concentration C_a (Vinberg 1969, Foy 1987), which has long been determined *in vitro* by measuring fluorescence in pigment extracts (Yentsch & Menzel 1963, Lorenzen 1966). Measurements of chlorophyll fluorescence, F_0, excited artificially by weak light, are now a common way of determining chlorophyll concentration *in situ*. Many authors have shown that F_0 correlates well with the chlorophyll concentration in natural water bodies. For example, Vedernikov et al. (1990) showed that, in the Black Sea, the correlation coefficients between these characteristics were equal to 0.7–0.85. Such a high correlation is not normally the rule, however. This may be due to the fact that the value of F_0 is proportional to the total content of photosynthetic pigments (carotenoids, chlorophylls *a*, *b*, *c*, etc.), whereas the fraction of chlorophyll *a* in pigments varies over a wide range, depending both on the taxonomic affiliation of the algae (Cullen 1982, Soo Hoo et al. 1986, Matorin & Venediktov 1990) and on such environmental conditions as irradiance, concentration of mineral nutrients, and pollutants (Kolber et al. 1988). We have been able to determine chlorophyll *a* concentrations much more accurately by using not only F_0 measurements but also our own physical models of light absorption and fluorescence in different natural plant communities (Woźniak et al. 1999, 2000, 2003, Majchrowski et al. 2000, Ostrowska et al. 2000a, b, Majchrowski 2001). Our method takes into consideration the fact that F_0 reflects the total concentration of photosynthetic pigments. Treated as a characteristic of light absorption by algae, it can then be used to determine

the photosynthetic production of phytoplankton (Antal et al. 1999, Ficek 2001, Ostrowska 2001, Woźniak et al. 2002).

The aim of the present work is to investigate and present the relationships between fluorescence F_0, chlorophyll a concentration C_a and the light absorption capacities of cell suspensions using laboratory cultures of algae and natural phytoplankton.

Theoretical background

The fluorescence F_0, excited by an artificial light source, has been measured in dark-adapted algae in which the PS II reaction centres are open, so that absorbed light energy can be converted into chemical bond energy with maximum efficiency (Klughammer 1992). The intensity of F_0 can be calculated from the equation

$$F_0 = G\ I_{fl}\ \overline{a}_{pl,\,PSP,\,fl}\Phi_{F_0},$$

where[1]

I_{fl} – total intensity of exciting flash (in our fluorimeter, $I_{fl}(\lambda)$ was nearly uniformly distributed over the 400–550 nm spectral range), (constant);

$\overline{a}_{pl,\,PSP,\,fl}$ [m^{-1}] – coefficient of absorption of the exciting flash by photosynthetic pigments, averaged over the 400–550 nm spectral range;

Φ_{F_0} – quantum yield of fluorescence in cells with open RC;

G – coefficient defined by the geometric characteristics and the sensitivity of the fluorescence light sensor, (constant).

Taking into account the fact that $(G\ I_{fl})^{-1} = \mathrm{const}$, the coefficient of solar irradiance absorption by algae can be related to the fluorescence intensity as follows:

$$\overline{a}_{pl,\,PSP} = \mathrm{const}\ \Phi_{F_0}^{-1} A F_0,$$

where

$$A = \overline{a}_{pl,\,PSP}/\overline{a}_{pl,\,PSP,\,fl}$$

To simplify the analysis, we have assumed that $A \approx 1$, because the spectrum of the exciting light in our fluorimeter is similar to that of natural light in the sea. Thus, $\overline{a}_{pl,\,PSP} \approx \overline{a}_{pl,\,PSP,\,fl}$. In reality, parameter A can vary for different trophic types of sea and also with depth. This problem is discussed in Antal et al. (2001); see also Ostrowska et al. (2000a, 2001).

Material and Methods

The experiments were carried out with three cultures of algae: the green algae *Chlorella pyrenoidosa*, *Platymonas viridis*, and *Ankistrodesmus* sp.,

[1]See the appendix for the list of symbols used in the text.

the yellow-green alga *Nephrochloris salina*, the diatom *Thalassiosira weissflogii*, and natural phytoplankton from Nhatrang Bay (Vietnam).

Ch. pyrenoidosa (thermophilic strain CALU-175 from the collection of the Biology Institute, St. Petersburg State University, Russia) was grown in 1/5 Tamiya medium (pH 6.8) (Tamiya et al. 1961) at 32°C, under 60 μE m^{-2} s^{-1} background irradiation, and with moisturised air being bubbled through it. The culture was maintained in a log growth phase by daily dilution with fresh medium to maintain a cell density of about 1–10 $\times$ 10^5 cells ml^{-1}.

The marine algae *P. viridis*, *Ankistrodesmus* sp., *Th. weissflogii* and *N. salina* were grown at 20°C under indirect daylight with a maximum light flux density of 120 μE m^{-2} s^{-1} in an artificial medium described in Lanskaya (1971).

The mean effective absorption coefficient for blue light (spectrum similar to the spectrum of the flash-exciting F_0 in the fluorimeter) for cells in the algal suspension, $\tilde{a}_{pl,\,blue}$, were measured with a laboratory apparatus. Light from a KGM 150/24 halogen slide-projector lamp was passed through a blue-green filter SZS-22 with $\lambda < 600$ nm and a dark chamber $l = 0.2$ m in length filled with the sample, and the output quantum flux density was measured with a laboratory-made quantum sensor. The calculations were performed according to the formula

$$\tilde{a}_{pl,\,blue} = (I_{nc} - I_n)/(l\ I_{nc}) \text{ or } \tilde{a}_{pl,\,blue} = (\ln I_{nc} - \ln I_n)/l,$$

where

I_n [μE m^{-2} s^{-1}] – the intensity of light passed through the algal suspension of concentration n;

$I_{n,c}$ [μE m^{-2} s^{-1}] – the intensity of light passed through the suspension of algal cells bleached by illumination in the presence of 1 mM hydroxylamine.

The chlorophyll *a* concentration C_a in the algae samples was measured on a HITACHI-557-SPECTR (Japan) spectrophotometer using a routine method (Parsons & Strickland 1963).

Measurements of phytoplankton fluorescence were carried out over the whole area of Nhatrang Bay (Vietnam) from 23rd March to 5th April 1998. At each station, vertical profiles of fluorescence F_0 were measured *in situ* with a submersible fluorimeter. Functioning on the principle of the pump-and-probe method (Mauzerall 1972, Kolber et al. 1990), this fluorimeter was designed at the Faculty of Biology of the Moscow State University for the real-time measurement of initial and maximum fluorescences (F_0, and F_m), as well as underwater irradiation, temperature and pressure (depth) (Matorin et al. 1996, Antal et al. 1999). The fluorimeter was also used to measure the fluorescence of algae under laboratory conditions,

the fluorescence-exciting impulses being generated by an SSh-20 (MELZ, Russia) xenon lamp. The flashes were isolated from the sample by the blue-green filter SZS-22. The spectrum of the fluorescence excitation light was distributed practically uniformly within the range of wavelengths from 400 to 520 nm. The fluorescence signal was recorded by a photomultiplier-68 after having passed through a KS-17 cut-off glass filter ($\lambda > 680$ nm).

Results and discussion

Fig. 1 shows the dependence of F_0 on C_a, measured under laboratory conditions with a suspension of *Ch. pyrenoidosa* cells. It is evident that this dependence is nearly linear over the range of naturally occurring phytoplankton concentrations ($C_a < 16$ mg m^{-3}). Some deviation from linearity was observed only near the limits of the sensitivity ranges when the sensitivity of the sensors was changed. As follows from the curve, the F_0 signal can be automatically converted into chlorophyll *a* concentration units in the course of *in situ* measurements. However, this method yields only an approximate value of the chlorophyll concentration and can be used, for example, for a quick assessment of the trophic status of water bodies; for more accurate estimates of C_a content, one should take into account the fact that the F_0/C_a ratio is a function of the species composition of the microalgae. Fig. 2 shows F_0 as a function of C_a in four species of marine algae from three different taxa grown under laboratory conditions. Here we see that in the yellow-green (*N. salina*) and diatomaceous (*Th. weissflogii*)

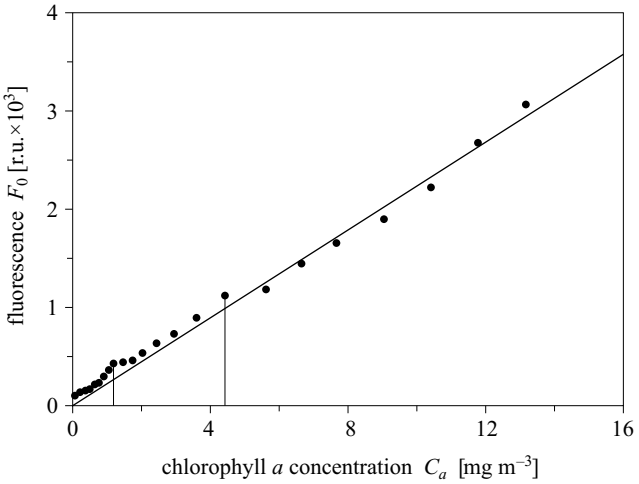

Fig. 1. Dependence of F_0 yield on chlorophyll *a* concentration in a suspension of *Chlorella vulgaris* cells. Vertical lines show the points at which the fluorescence sensor sensitivity was altered

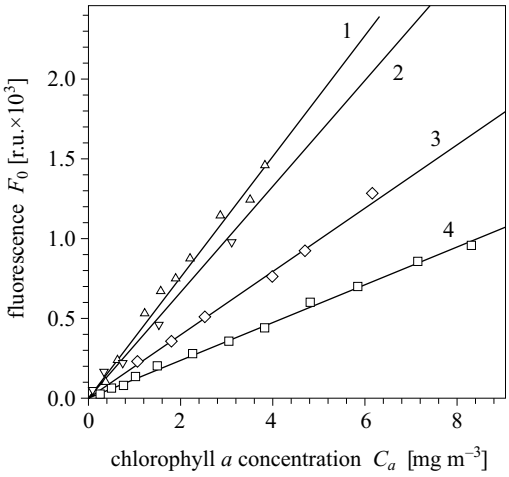

Fig. 2. F_0 vs C_a in suspensions of marine algae from three different taxa: diatom *Thalassiosira weissflogii* (1), yellow-green *Nephrochloris salina* (2) green *Ankistrodesmus* sp. and *Platymonas viridis* (3 and 4 respectively). The algae were grown under laboratory conditions

algae, in which chlorophyll comprises the least part of the light-harvesting pigments, the intensity of F_0 per unit of C_a concentration was about 2–3 times higher than in green alga, in which chlorophyll is the major light-harvesting pigment. The F_0/C_a ratio in these algae is correlated with the pigment index (the a_{430}/a_{663} ratio in a 90% acetone extract), which characterises the contribution of carotenoids to the total light absorption (Margalef 1963, Matorin et al. 1997).

The ratio F_0/C_a is also different in natural phytoplankton in the same water body. For example, deep-sea and shore stations in Nhatrang Bay (Fig. 3) and in the Baltic Sea (data not shown) differed significantly in their F_0 dependence on chlorophyll a concentration. These results show that the light-harvesting complexes of algal cells from shore regions were richer in chlorophyll a than algae from deep-water regions. This may be related to a shift in the ratio of dominant algal species and to the high levels of mineral nutrients in shore waters, and also to photo-acclimation to the changing light environment. Previously, we showed that a deficiency in the main mineral nutrients (nitrogen, phosphorus) in the culture medium led to a smaller contribution of chlorophyll a to the light-harvesting complex (Matorin et al. 1997).

Earlier, F_0 was shown to correlate more closely with another phytoplankton characteristic – the light absorption capacity of the photosynthetic pigments in PS II centres (Ostrowska et al. 2000a, Antal et al. 2001). In principle, the intensity of F_0 is proportional to $\overline{a}_{pl,\,PSP}$,

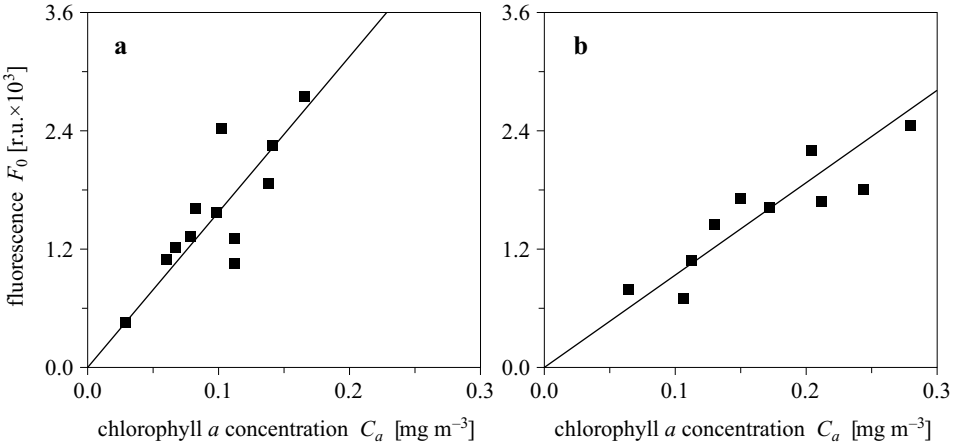

Fig. 3. Dependences of F_0 on C_a in deep water (a) and coastal (b) stations of Nhatrang Bay and the results of the linear regression of these dependences. The values of F_0 and C_a are averaged over the water column

the constant of proportionality being dependent on Φ_{F_0}, if the spectral distribution of the light exciting F_0 is similar to that of the natural light in the sea, i.e. $A = 1$ (see Ostrowska et al. 2000a, Antal et al. 2001). These assumptions were confirmed by studies of the relationship

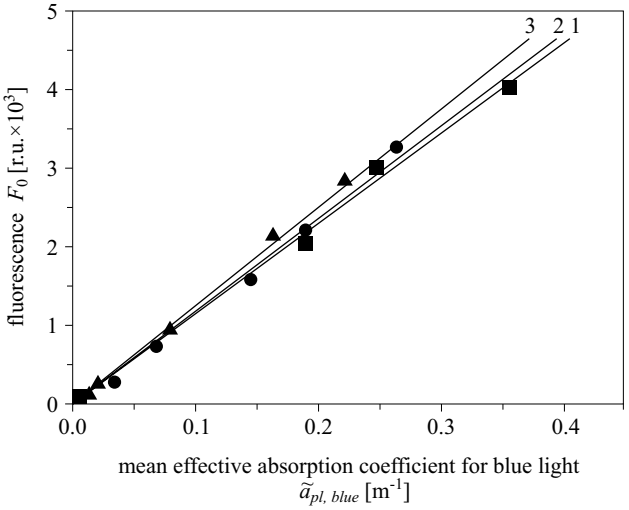

Fig. 4. Dependences of F_0 on $\tilde{a}_{pl,\,blue}$ in suspensions of marine algae from three different taxa: diatom *Thalassiosira weissflogii* (square), green *Chlorella pyrenoidosa* (circle) and yellow-green *Nephrochloris salina* (triangle). The results of the linear regression of these dependences are marked 1, 2 and 3 respectively. The algae were grown under laboratory conditions

between F_0 and the absorption coefficient of blue light, $\tilde{a}_{pl,\,blue}$ in cell suspensions of various groups of marine algae. Experimental relatinships of F_0 as a function of $\tilde{a}_{pl,\,blue}$ were measured for green (*Ch. pyrenoidosa*), diatomaceous (*Th. weissflogii*) and yellow-green (*N. salina*) algae over the range of natural concentrations (Fig. 4). The algae were grown under optimum conditions (for example, at a low irradiance level): we may assume, therefore, that the values of Φ_{F_0} in these species of algae are similar. Determined for each group from the linear regression method, the $F_0/\tilde{a}_{pl,\,blue}$ ratios were almost identical, whereas the F_0/C_a ratios varied greatly among the algal taxa (see Fig. 2). Small differences in the $F_0/\tilde{a}_{pl,\,blue}$ ratios may be due to the difference between the light absorption capacity of whole cells and that of the PS II photosynthetic pigments, i.e. $\tilde{a}_{pl,\,blue} \neq \overline{a}_{pl,\,PSP,\,fl}$. The so-called packaging effect could also be the reason for such differences. This problem has been analysed in detail in our previous papers (Woźniak et al. 1999, 2000).

As noted above, the relationship between $\overline{a}_{pl,\,PSP}$ and F_0 depends on Φ_{F_0} and factor A, which can vary. Previous studies of natural phytoplankton populations have shown that in some polluted areas, near the surface, the quantum yield of F_0 was suppressed (Antal et al. 2001); nevertheless, this was observed rather rarely, and the value Φ_{F_0} can be assumed constant (Ostrowska et al. 2000a). We also showed that the spectrum of underwater irradiation in natural water bodies from a depth of 5 m to the lower limit of the euphotic zone is very close to that of the exciting F_0 light of the

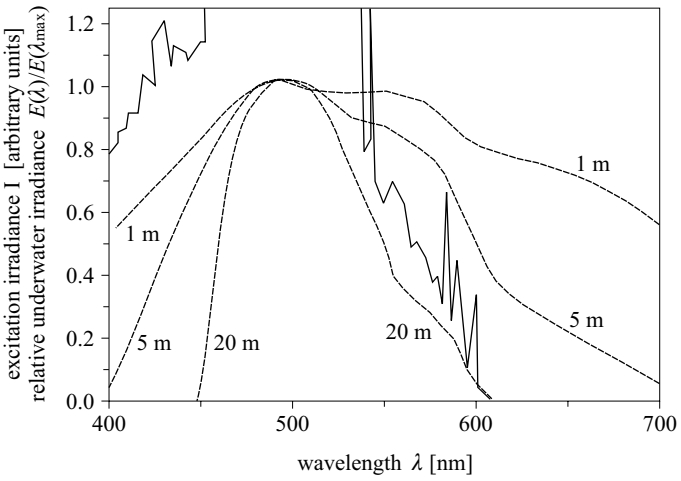

Fig. 5. Spectrum of the light used to excite chlorophyll fluorescence in the PrimProd fluorimeter (solid line), and the spectral distribution of underwater irradiance in the sea at different depths (after Antal et al. 2001)

fluorimeter (400–600 nm), i.e. $A \sim 1$ (see Fig. 5). Hence, estimates of the light absorption capacity by measuring F_0 are probably correct over most of the photosynthetic zone. However, this assumption may be not true for highly eutrophic waters ($C_a > 10$ mg m^{-3}), in which the fraction of blue light ($\lambda < 500$) decreases at a depth of 10 m and more because it is absorbed by yellow substances, and green light with its spectral maximum at 550 nm is dominant (Dera 1995).

Conclusions

We analysed the relationships between fluorescence F_0 and chlorophyll a concentration as well as between fluorescence F_0 and total absorption earlier for a comprehensive empirical *in situ* database of different natural plant communities in the World Ocean (Fig. 6) (Ostrowska et al. 2000a, b). The correlation coefficients for these empirical data are $r = 0.916$ for $F_0 = f(C_a)$ and $r = 0.942$ for $F_0 = f(\overline{a}_{pl,\,PSP})$. Evidently, the general tendency in algae from different seas is that the fluorescence F_0 is more closely related to light absorption capacities than to the chlorophyll concentration. The results of the analyses presented in this work for laboratory cultures and natural phytoplankton populations confirm our earlier results.

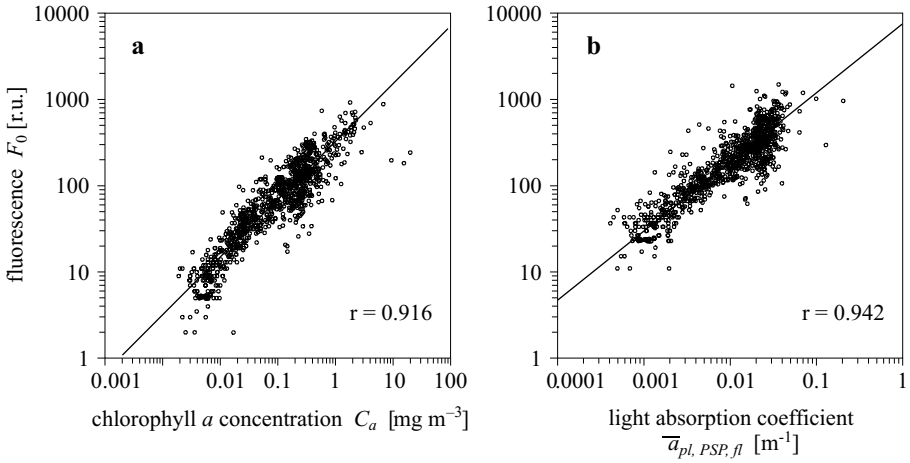

Fig. 6. The relationship between fluorescence F_0 and chlorophyll a concentration (a) and fluorescence F_0 and the light absorption coefficient $\overline{a}_{pl,\,PSP,\,fl}$ (b), for data from different regions of the World Ocean (after Ostrowska et al. 2000b)

Our data show that measurement of F_0 for *in situ* estimation of chlorophyll a content should be performed after fluorescence calibration in a particular area, i.e. when the spatial and temporal variations in the species composition of phytoplankton are not significant. The fraction

of chlorophyll a in the total photosynthetic pigments in algal cells can be estimated by calibrating F_0 on C_a, thus enabling the dominant algal groups in the region to be characterised. It is more correct to use F_0 for estimating the light absorption capacity by phytoplankton cells because the fluorescence better reflects this characteristic. The light absorption capacity is a more appropriate measure of phytoplankton abundance than C_a, because it is proportional to the concentration of the total photosynthetic pigments. In addition, the light absorption capacity can be used to estimate photosynthetic production in phytoplankton (Antal et al. 2001, Ostrowska 2001).

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Appendix

List of the main symbols and abbreviations used in this paper

Symbol	Denotes	Units
$\tilde{a}_{pl,\,blue}$	mean effective absorption coefficient for blue light	m^{-1}
$\overline{a}_{pl,\,PSP,\,fl}$	mean coefficient of exciting flash absorption by PSP, averaged over the 400–550 nm spectral range	m^{-1}
$\overline{a}_{pl,\,PSP}$	mean coefficient of light absorption by PSP, averaged over the 400–700 nm spectral range (PAR)	m^{-1}
A	ratio of the mean absorption coefficient: mean in PAR range and averaged over the 400–550 nm spectral range	dimensionless
C_a	sum of chlorophylls a + pheo, or total chlorophyll (chl a + divinyl chl a) concentrations	mg tot.chl a m^{-3}
$E(\lambda)$	spectral scalar irradiance	Ein m^{-2} s^{-1} nm^{-1}
F_0, F_m	*in vivo* phytoplankton fluorescence yield induced by a weak probe flash in the dark (initial), and following a saturating flash (maximum), measured in a light-adapted state	relative units
G	coefficient defined by geometric characteristics and sensitivity of the fluorescence light sensor (constant)	relative units
I_n	intensity of light passed through a suspension of algae of concentration n	μEin m^{-2} s^{-1}
$I_{n,c}$	intensity of light passed through a suspension of algae of concentration n bleached by illumination in the presence of 1 mM hydroxylamine	μEin m^{-2} s^{-1}
$I(\lambda)$	spectrum of light excitation – depends on the light source used by the instrument	quanta m^{-2} nm^{-1} s^{-1}
I_{fl}	total intensity of exciting flash (constant)	quanta m^{-2} s^{-1}
PAR	photosynthetically available radiation	
PSP	photosynthetic pigments	
PS II	photosystem II	
RC	reaction centre	
Φ_{Fo}	quantum yield of fluorescence in cells with open RC	dimensionless
λ	light wavelength	nm