# NON-LINEAR FLUORIMETRY FOR DIAGNOSTICS OF THE PHYTOPLANKTON STATE

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## ABSTRACT

In this paper a new approach for phytoplankton diagnostics based on the non-linear fluorimetry method (saturation fluorimetry method) is proposed. This approach can be realised both in a sample probe mode and in a remote sensing mode.

The procedure for the determination of the non-saturated fluorescence parameter $\Phi_o$ which is proportional to concentration of chlorophyll *a* molecules and the photophysical parameter *A* has been elaborated. Parameter *A* is given by $A=\sigma\tau^2\gamma n_o$, where $\sigma$ is the excitation cross-section of chlorophyll *a* molecules, $\tau$ is the lifetime of the excited chlorophyll *a* molecules taking into account all processes of a deactivation of excitation except the singlet-singlet annihilation, and $\gamma n_o$ is the maximum rate of the singlet-singlet annihilation.

Laboratory experiments with the axenic culture of eurihaline *Thalassiosira weissflogii* have shown that *A* is strongly dependent on the functional state of phytoplankton. The state was changed by treatment with herbicide DCMU and by actinic light.

**Keywords:** phytoplankton, non-linear fluorimetry, laser remote sensing

## INTRODUCTION

At present, for diagnostics of the phytoplankton state the following methods are used: Pump-and-probe (1), fast-repetition-rate (FRR) (2), pump-during-probe (3), and pulse-amplitude-modulation (PAM) (4).

However these methods in their classical variants can be realised only on water samples (or using submergible probes) and the problem of searching for remote sensing methods for phytoplankton diagnostics is still relevant. Remote sensing of water areas using pulse lasers reveals phytoplankton fluorescence saturation since the intensity of pulse laser excitation on the water surface is mostly higher than 1 kW/cm$^2$. Then the dependence of fluorescence intensities on chlorophyll *a* concentrations becomes non-linear. The determination of the non-saturated value of fluorescence intensity has been shown to be a difficult problem (5). On the other hand fluorescence saturation can be used for the determination of molecular photophysical parameters of phytoplankton (5,6).

In previous papers (reviewed in (5)) a strong possibility for using the non-linear fluorimetry method for phytoplankton diagnostics has been demonstrated. However, this research did not lead to a practical algorithm for the determination of phytoplankton photophysical parameters.

In this paper a practical realisation of the method of non-linear fluorimetry is presented. The algorithm for the determination of the non-saturated fluorescence parameter $\Phi_o$ and of the parameter *A* are theoretically substantiated and experimentally verified. It is shown that parameter *A* is extremely sensitive to the functional state of phytoplankton.

## THE ANALYTICAL EXPRESSION FOR PHYTOPLANKTON FLUORESCENCE SATURATION

The algorithm of determination of photophysical parameters by the method of non-linear fluorimetry is the following. At the beginning a non-linear dependence of the fluorescence photon number $N_{fl}(F)$ on the photon flux density of the exciting laser radiation *F* is measured. Under typical values

cal values of the photon flux density for pulse lasers, a deviation of this function from the linear dependence appears, and this holds to a different degree for various organic compounds. For phytoplankton this deviation reveals itself at $F \geq 10^{21}$ $cm^{-2}s^{-1}$. The dependence $N_{fl}(F)$ is called the fluorescence saturation curve.

Then the inverse problem is solved: the values of the model parameters are determined using the experimental saturation curve $N_{fl}(F)$. Solving of the inverse problem is carried out in two stages. In the first stage the direct problem is solved: for certain sets of the values of the model parameters the dependencies $N_{fl}(F)$ are calculated. The number of emitted fluorescence photons $N_{fl}$ is connected to the concentration $n$ of the excited chlorophyll *a* molecules:

$$N_{fl}(F) = k_{fl} \times \iiint_V \mathrm{d}^3 r \int_{-\infty}^{\infty} n(r,t;F)\, dt \quad , \tag{1}$$

where $V = \iiint \mathrm{d}^3 r$ is the volume from which the optical signal is detected, and $k_{fl}$ is the rate of the emitting deactivation of the excited chlorophyll *a* molecules.

In the second stage the inverse problem is solved: the experimental saturation curve is compared with theoretical curves calculated for the different sets of the parameters values. With the results of this comparison the search parameters are determined.

To calculate theoretical fluorescence saturation curves a three-parametrical model of phytoplankton fluorescence response under pulse laser excitation (7) is used (pulse duration $\tau_{pulse} \approx 10$ ns, pulse repetition rate $f \approx 10$ Hz). This model is described by the following kinetic equation for concentration $n$ of the excited chlorophyll *a* molecules:

$$\frac{\mathrm{d}\, n(t,r)}{\mathrm{d}\, t} = F(t,r) \cdot \sigma \cdot \left(n_o - n(t,r)\right) - \frac{n(t,r)}{\tau} - \gamma \cdot n^2(t,r)\,. \tag{2}$$

In Eq. (2) $n_o$ is the local chlorophyll *a* concentration in the chloroplasts, and $F$ is the photon flux density. The parameters of the model are the following:

- $\sigma$ is the excitation cross-section of chlorophyll *a* molecules; it takes into account both light absorption by chlorophyll *a* molecules and energy transfer to chlorophyll *a* from accessory pigments;
- $\tau$ is the time of a linear deactivation of the excited chlorophyll *a* molecules, taking into account the intramolecular deactivation and the energy transfer to the reaction centres.
- $\gamma$ is the rate constant of singlet-singlet annihilation of the excited chlorophyll *a* molecules (sometimes it is preferable to use the product $\gamma n_o$, which is the maximum rate of singlet-singlet annihilation).

It was shown in (7,8) that $\tau$ is the effective lifetime of the excited state of the molecules. It does not depend on photon flux density $F$ with some restrictions on the rates of energy transfer from the light-harvesting complex to reaction centres in different states (open PIQ, closed $PIQ^-$ and two intermediate $P^+IQ^-$ and $P^+I^-Q^-$).

The fluorescence photon number $N_{fl}$ is measured experimentally in relative units because the procedure of measuring absolute values of $N_{fl}$ is rather complicated. To obtain parameters, which can be measured in absolute units, a normalisation of $N_{fl}$ to some reference signal $N_{ref}$ is carried out which should be proportional to photon flux density $F$. As reference signal one can use, for example, the laser emission directed by a beam splitter to a second channel of the registration system, or the water Raman scattering signal (the so called “internal standard” (9)). The ratio $\Phi = N_{fl} / N_{ref}$ is called the fluorescence parameter. The dependencies $N_{fl}(F)$ and $\Phi(F)$ are called fluorescence saturation curves in different modes (Figure 1).

If it is supposed that excitation of an optically thin layer of an alga cell suspension is performed by laser pulses with a "rectangular" intensity distribution in time and in cross-section, it is possible to obtain an analytical expression for the dependence of the fluorescence parameter $\Phi$ on the photon flux density $F$. This expression is very complex so that it is not dealt with in this paper.

However, exciting phytoplankton fluorescence with 10 ns laser pulses, as in our experiments, one can use a quasi-stationary approximation because the pulse duration $\tau_{pulse}$ is much longer than the lifetime $\tau$ of the excited chlorophyll *a* molecules of ≈300 ps in case of open reaction centres and ≈1 ns in case of closed reaction centres (10). In the quasi-stationary approximation the expression for dependence $\Phi(F)$ is simplified to:

$$\Phi^{-1}(F) = \frac{\Phi_o^{-1}}{2}\left(1 + BF + \sqrt{(1+BF)^2 + 4AF}\right) , \qquad (3)$$

where

- the parameter $A=\sigma\tau^2\gamma n_o$ characterises fluorescence saturation due to the process of singlet-singlet annihilation;
- the parameter $B=\sigma\tau$ characterises fluorescence saturation due to the dynamic decrease in the number of the chlorophyll *a* molecules in the ground state;
- $\Phi_o = \lim_{F\to 0} \Phi(F)$ is the non-saturated fluorescence parameter; it does not depend on the photon flux density $F$.

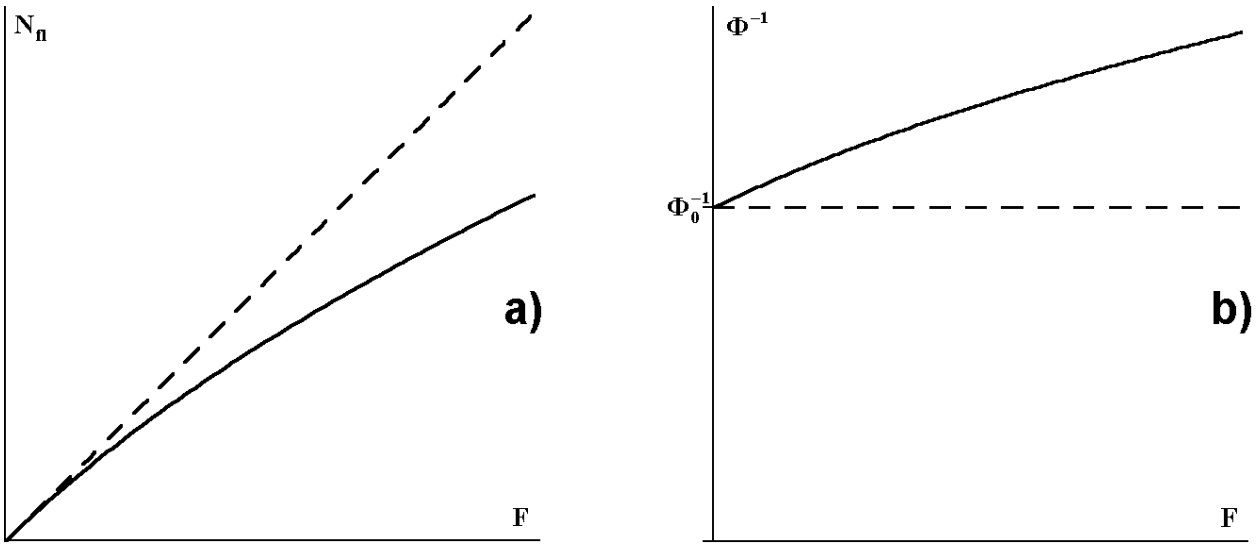


*Figure 1. A fluorescence saturation curve in different modes: a):* $N_{fl}(F)$*, b):* $\Phi^{-1}(F)$*. The dependencies in the absence of fluorescence saturation effects are displayed by dash lines.* $N_{fl}$ *is the fluorescence photon number;* F *is the photon flux density of the exciting laser radiation;* $\Phi=N_{fl}/N_{ref}$ *is the fluorescence parameter;* $N_{ref}$ *is a reference signal.*

The quantity $\Phi_o$ plays an important applied role: it is proportional to the concentration of chlorophyll *a* molecules $C_{Chl\text{-}a}$. If we perform the procedure of calibration i.e. find the constant of proportionality between $\Phi_o$ and $C_{Chl\text{-}a}$ we can use $\Phi_o$ for the determination of $C_{Chl\text{-}a}$.

The important peculiarity of phytoplankton is the high local concentration of chlorophyll *a* molecules in chloroplasts (≈0.1÷1 M). Therefore the maximum rate of singlet-singlet annihilation $\gamma n_o$ is much higher than the rate of linear deactivation $1/\tau$: $\gamma n_o >> 1/\tau$, $A >> B$. Hence in the range of photon flux densities where the following inequality holds,

$$B^2F^2 << 4AF \quad \text{or} \quad F << \gamma n_o/\sigma, \qquad (4)$$

the dependence $\Phi(F)$ is further simplified:

$$\Phi^{-1}(F) = \Phi_o^{-1}\left(0.5 + \sqrt{0.25 + AF}\right) \quad (5)$$

Experimental fluorescence saturation curves are measured usually in the following range of photon flux densities: $F \approx 10^{20} \div 10^{25}$ cm$^{-2}$s$^{-1}$. The lower limit is due to the limited sensitivity of the registration system, the upper limit is caused by the negative influence of intense laser emission on phytoplankton cells. The parameters $\sigma$ and $\gamma n_o$ have the following typical values: $\sigma \approx 10^{-16}$ cm$^2$, $\gamma n_o \approx 10^{12}$ s$^{-1}$. Therefore in the practical range of photon flux densities the inequality given in Eq. (4) holds, and the dependence of $\Phi(F)$ is described by Eq. (5).

In Figure 2a fluorescence saturation curve, which was calculated using Eq. (5), is shown. From Eq.(5) and Figure 2 one can see that in the range of photon flux density $AF >> 0.25$ the fluorescence saturation curve $\Phi^{-1}(F)$ can be approximated by the square root function $\Phi^{-1}(F) \approx \Phi_o^{-1}\left(0.5 + \sqrt{AF}\right)$. However, if values of F are reduced the linear approximation of $\Phi^{-1}(\sqrt{F})$ is not correct and the curve attains the stationary level $\Phi^{-1}(\sqrt{F}) \approx \Phi_o^{-1}$.

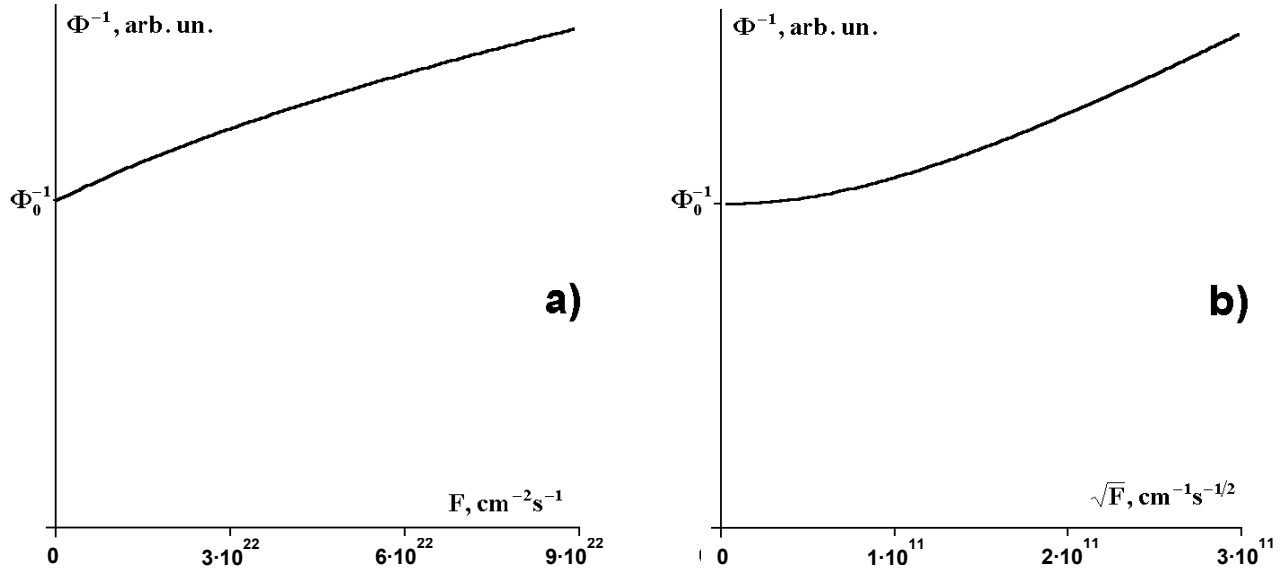


*Figure 2. The theoretical fluorescence saturation curve calculated using Eq.(5) in the modes* $N_{fl}(F)$ *(a),* $\Phi^{-1}(F)$ *(b). For the calculation a value of* $A = 9 \cdot 10^{-24}$ *cm$^2$s was used.*

Eq. (5) is obtained for the case of a "rectangular" intensity distribution of the exciting laser pulse over time and cross-section. In general, when there is an arbitrary spatio-temporal photon distribution of the exciting laser pulse the expression $\Phi^{-1}(F)$ becomes complicated. However, the shape of the fluorescence saturation curve is not changed in principle; in the practical range of photon flux densities the dependence $\Phi^{-1}(F)$ can be approximated by an analogous expression of Eq. (5):

$$\Phi^{-1}(F) = \Phi_o^{-1}\left(\alpha_1 + \sqrt{\alpha_2 + \alpha_3 AF}\right) \ , \quad (6)$$

where the coefficients $\alpha_1$, $\alpha_2$ and $\alpha_3$ depend only on the type of distribution (here: $\alpha_1 + \sqrt{\alpha_2} = 1$). If the intensity distribution of the exciting laser pulse over time is a "hyperbolic tangent", and "rectangular" over its cross-section, the coefficients $\alpha_1$, $\alpha_2$ and $\alpha_3$ have the following values: $\alpha_1$=0.64, $\alpha_2$=0.13 and $\alpha_3$=0.43.

Hence, the non-saturated fluorescence parameter $\Phi_o$ and the photophysical parameter $A = \sigma\tau^2\gamma n_o$ can be determined from experimental fluorescence saturation curves of phytoplankton.

## MATERIALS AND METHODS

To obtain fluorescence saturation curves an experimental set-up with a block diagramme as shown in Figure 3 was developed. To excite fluorescence of chlorophyll *a* molecules a pulsed Nd:YAG laser with frequency doubling ($\lambda$=532 nm) is used. The fundamental laser emission at $\lambda$=1064 nm is absorbed by an optical filter. The laser radiation at $\lambda$=532 nm wavelength has the following characteristics: pulse energy $E$=10 mJ, pulse duration $\tau_{pulse}$=12 ns, pulse repetition rate $f$=10 Hz.

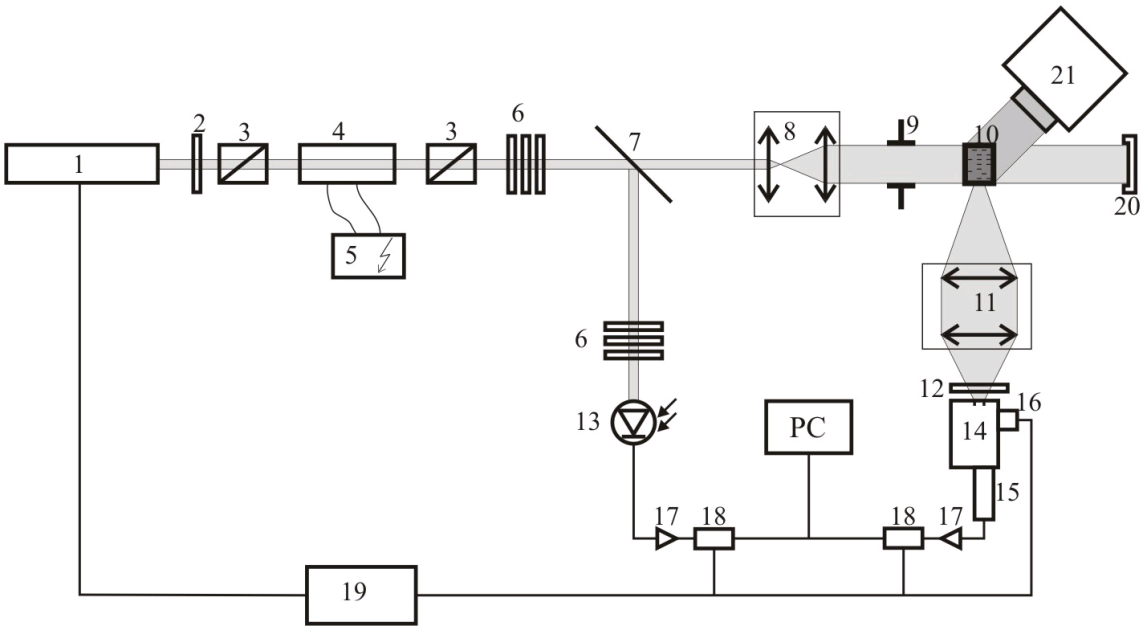


*Figure 3. Experimental setup. 1: Nd:YAG laser with frequency doubling (emission wavelength 532 nm), 2: optical filter, 3: polarizer, 4: electro-optical cell, 5: high voltage source, 6: set of attenuating optical filters, 7: beamsplitter, 8: lens telescope, 9: diaphragm, 10: sample cuvette, 11: lens objective, 12: optical filter; 13: photodiode, 14: monochromator, 15: photomultiplier, 16: step motor, 17: amplifier, 18: A/D conversion, 19: block of synchronous, 20: pyroelectric sensor for short light pulses, 21: slide projector.*

The laser beam passes through an electro-optical cell, a set of attenuating optical filters, a lens telescope and falls into a diaphragm, which selects a central part of the beam. Attenuating filters are used to set a selected value of maximal photon flux density $F_{max}$, relatively to which attenuation of radiation is performed using an electro-optical cell.

The emission excited in the cuvette is collimated and focused by a lens objective onto the input slit of the first channel of a registration system. The registration system consists of monochromator and photomultiplier. The signal from the photomultiplier is fed to a high frequency amplifier and then to a 12-bit A/D converter. The A/D-converter output is connected to the computer. During the measurement of the saturation curve the monochromator is tuned at the maximum wavelength of the phytoplankton fluorescence band (685 nm). The monochromator slit width is 1.5 mm corresponding to a 3 nm spectral bandwidth.

The second channel of the registration system is used to measure the reference signal. The laser radiation directed by the beam splitter to a photodiode is used as a reference signal. The signal from the second channel is stored by the computer simultaneously with the basic signal.

The minimum value of photon flux density (when the signal in the basic channel can be surely registered) is determined by the average concentration of chlorophyll *a* molecules, i.e. by content of phytoplankton cells in the sample. The phytoplankton content in the sample is selected on the one hand so that a reliable registration of fluorescence signal is ensured and on the other the condition of an optically thin layer is met. To increase the signal/noise ratio an averaging over 128 laser pulses is used.

Before measurements the maximum value of the photon flux density $F_{max}$ was detected. To do this the laser pulse energy $E_{max}$, pulse duration $\tau_{pulse}$ and beam cross-sectional area $S$ were measured. The maximum photon flux density $F_{max}$ was calculated with

$$F_{max} = E_{max} /(\hbar\omega \cdot S \cdot \tau_{pulse})$$

where $\hbar\omega$ is photon energy.

The shape and duration of the laser pulse were measured using a high-frequency coaxial photoelement and a high-frequency oscilloscope. It turned out that the shape of the laser pulse can be approximated by a "hyperbolic tangent" distribution

$$g(t) = \begin{cases} \frac{1}{\ln(2)} \cdot \frac{th\left(2 \cdot t/\tau_{pulse}\right)}{ch^2(t/\tau_{pulse})}, & t \geq 0 \\ 0, & t < 0 \end{cases} \qquad (7)$$

with a pulse duration half width $\tau_{pulse}$=12 ns. The intensity distribution of the beam after the diaphragm was similar to a "rectangular" distribution; the beam diameter S was 7 mm.

To measure the laser pulse energy we used a pyroelectric sensor OPHIR PE25, which was calibrated according to a NIST standard. The error of the pulse energy measurement was 5% at wavelength 532 nm. Simultaneously with the maximum pulse energy $E_{max}$ the number $N_{ref}^{max}$ of counts in the reference channel was detected.

After measuring $F_{max}$ and $N_{ref}^{max}$ the fluorescence saturation curve was measured. The first experimental point was registered when the photon flux density was at its maximum $F_{max}$. Then the laser radiation was attenuated with the electro-optical cell. In every point of the saturation curve the signals $N_{fl}$ and $N_{ref}$ in both channels of the registration system were measured. The photon flux density $F$ was derived from the reference signal: $F = F_{max} N_{ref} / N_{ref}^{max}$ . The experimental cycle was finished by measurements with the minimum value of the photon flux density.

To check the degree of negative influence of intense laser radiation on the alga cells a second experimental cycle in inverse order was performed. In almost all cases the saturation curves obtained under attenuation (first cycle) and amplification (second cycle) of photon flux densities coincided within the limits of the measuring accuracy, which proves the invariability of the sample state during the experiments.

To determine parameter $\Phi_o$ and the $A$ values from the obtained experimental saturation curve $\Phi^{-1}(F)$ the following routine was used. The experimental curve was approximated by the model function $\Phi^{-1}(F) = \Phi_0^{-1}(\alpha_1 + \sqrt{\alpha_2 + \alpha_3 AF})$, where the coefficients $\alpha_1$, $\alpha_2$ and $\alpha_3$ are fixed and depend only on the spatio-temporal distribution of the exciting laser pulse, $\Phi_o^{-1}$ and $A$ are varied parameters. To solve the inverse problem the least square method was used. It was supposed that the photon distribution of the exciting laser pulse in time is a "hyperbolic tangent", its cross-section is "rectangular" and therefore we used the following values: $\alpha_1$=0.64, $\alpha_2$=0.13, $\alpha_3$=0.43.

The axenic culture of eurihaline *Thalassiosira weissflogii* (Grunow) Fryxell et Hasle (Bacillariophyta) was used. The algae were grown in seawater (salinity 18 ppt) and with nutrients according to the f/2 medium (11). The chlorophyll *a* concentration of *T. weissflogii* is 0.8 - 4 pg/cell typically, depending on culture conditions.

A slide projector was used for illumination of the alga cells by continuous white light.

## RESULTS AND DISCUSSION

In Figure 4 the experimental fluorescence saturation curves obtained with the alga *T. weisflogii* in different functional states are presented, with a range of photon flux density of

$4 \cdot 10^{20} \le F \le 4 \cdot 10^{22}$ cm$^{-2}$s$^{-1}$ (Figure 4a) and $6 \cdot 10^{19} \le F \le 6 \cdot 10^{21}$ cm$^{-2}$s$^{-1}$ (Figure 4b). The saturation curves had a stable form and were well reproduced.

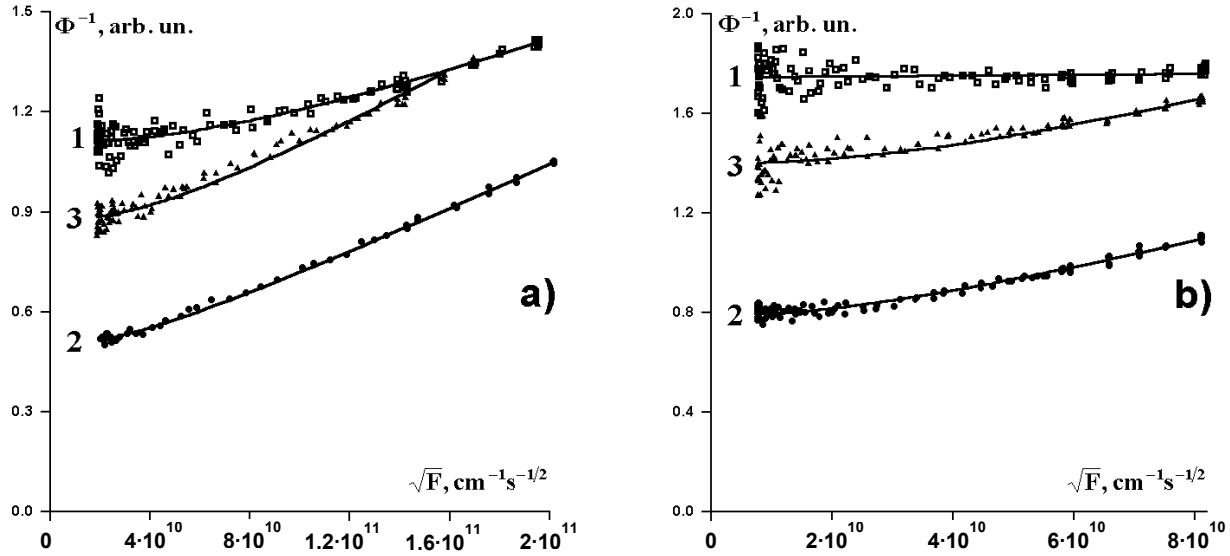


*Figure 4. The experimental fluorescence saturation curves of the alga* T. weisflogii *in different functional states. 1: the alga after dark adaptation (in state with open reaction centres), 2: the alga after addition of DCMU (in state with close reaction centres), 3: the alga was illuminated by continuous white light with an intensity of 50 W/cm$^2$. Each point is a result of averaging over 128 laser pulses. The solid curves are results of an approximation. The ranges of photon flux densities are $4 \cdot 10^{20}$ - $4 \cdot 10^{22}$ cm$^{-2}$s$^{-1}$ (a), $6 \cdot 10^{19}$ - $6 \cdot 10^{21}$ cm$^{-2}$s$^{-1}$ (b).*

It can be seen from Figure 4 that the experimental fluorescence saturation curves have the same features as the model curve displayed in Figure 2: when $F$ decreases the dependence of $\Phi^{-1}(\sqrt{F})$ declines from linear and reaches a stationary level. The qualitative coincidence of the experimental fluorescence saturation curves with the model demonstrates adequacy of the approximate analytical expression Eq. (6) to fluorescence saturation curves.

The fluorescence saturation curves of the alga *T. weisflogii* after dark adaptation (i.e. in a state with open reaction centres) are marked with 1, and data are shown as squares in Figure 4. The value of the parameter $A$ is $A_{open}=1.6 \cdot 10^{-23}$ cm$^2$s in this case.

The fluorescence saturation curves of the alga *T. weisflogii* after addition of 3-(3,4-di-chlorophenil)-1,1-dymethylurea (DCMU) and switching on weak background illumination with intensity $I \approx 1$ W/m$^2$ (i.e. in a state with closed reaction centres) are marked with 2, data are given as circle in Figure 4. The calculated value of the parameter $A$ is $A_{close}=1.5 \cdot 10^{-22}$ cm$^2$s, i.e. the value of the parameter $A$ is increased by 9 times.

As is known, DCMU blocks the electron transfer after the primary quinone $Q_a$ that leads to an increase of the deactivation time $\tau$ of the excited chlorophyll *a* molecules. If it is assumed that the parameters $\sigma$ and $\gamma n_o$ are not changed by the addition of DCMU then the increase of $A=\sigma\tau^2\gamma n_o$ by a factor of 9 means that the deactivation time $\tau$ is increased by 3 times: $\tau_{close}/\tau_{open}=3$.

In Figure 5 the change of alga fluorescence intensity after the addition of DCMU is shown. One can see that fluorescence is increased by 2.5-3 times. The photon flux density of the probing laser pulses is $F \approx 7 \cdot 10^{20}$ cm$^{-2}$s$^{-1}$. At that value of photon flux density no fluorescence saturation is seen (Figure 4) and one can suppose that $N_{fl} \sim \sigma\tau F$. Therefore the observed change of the alga fluorescence means that the deactivation time $\tau$ is increased by 2.5-3 times: $\tau_{close}/\tau_{open}=2.5 \div 3$, assuming that $\sigma$ is not changed. This estimation within the experimental accuracy coincides well with the estimation based on measurements of $A$ values by non-linear fluorimetry.

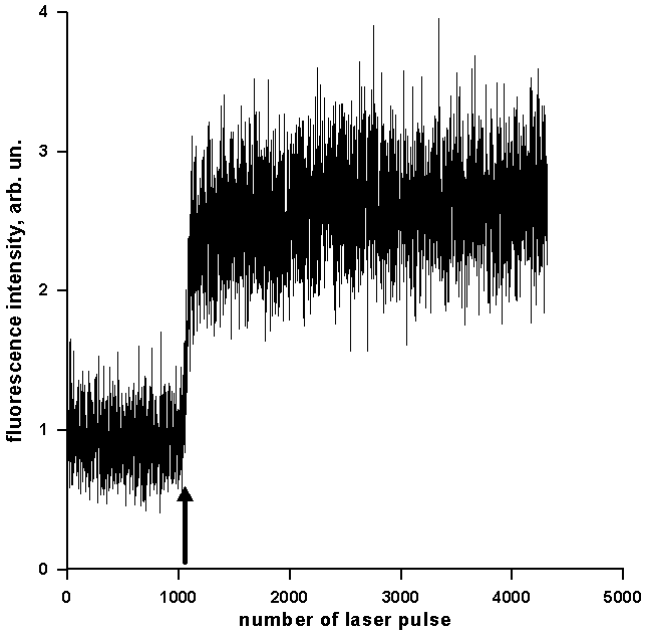


*Figure 5. Change of the alga* T. weisflogii *fluorescence intensity after addition of DCMU. The arrow is the moment of addition of DCMU in concentration $10^{-5}$ M and switching on weak background illumination (1 W/cm$^2$). The photon flux density of probing laser pulses is $\approx 7 \cdot 10^{20}$ cm$^{-2}$s$^{-1}$.*

The fluorescence saturation curves obtained under illumination of the alga by continuous white light with intensity 50 W/m$^2$ correspond to curves 3 and to the triangle data points in Figure 4. The value of the parameter $A$ is $A_{light}=5.7 \cdot 10^{-23}$ cm$^2$s in this case. This value of A is 3.5 times higher than $A_{open}$ calculated for the alga with the open reaction centres.

In Figure 6 the change of fluorescence intensity of the dark-adapted alga after switching light on is shown. The photon flux density of the probing laser pulses is $F \approx 8 \cdot 10^{20}$ cm$^{-2}$s$^{-1}$; therefore it is possible to neglect by fluorescence saturation and one can suppose that $N_{fl} \sim \sigma \tau F$. From Figure 6 one can see that at as soon as the light is switched on the fluorescence intensity increases, then it decreases and reaches a stationary level which is about 15-20% higher than the initial fluorescence level.

Therefore an interesting and important result is obtained for diagnostics of phytoplankton: under illumination of the algae with continuous white light (50 W/m$^2$) the steady-state level of the fluorescence is almost the same (within 15-20%) as initial one in the open reaction centre state while the value of the photophysical parameter A is increased by 3.5 times.

There are at least two ways of interpreting this result. One of them gives rise to the conclusion that under illumination of the algae by intense continuous white light the parameter $\sigma$ is decreased by 3.5 times. The second interpretation is to conclude that the maximum rate $\gamma n_o$ of singlet-singlet annihilation is increased 3.5-times. This increase can be connected with conformational changes of the chloroplasts.

We shall put off the detailed discussion of this question until the results of further experiments are obtained. This shall be done with a simultaneous application of non-linear fluorimetry and the PAM method, which allows to determine separately the degree of photochemical and non-photochemical quenching of fluorescence of phytoplankton.

Now we note that the parameter $A$ turned out to be very sensitive to changes in the state of the photosynthetic apparatus of the algae. Values of $A$ derived from the experiments reported here are presented in Table 1.

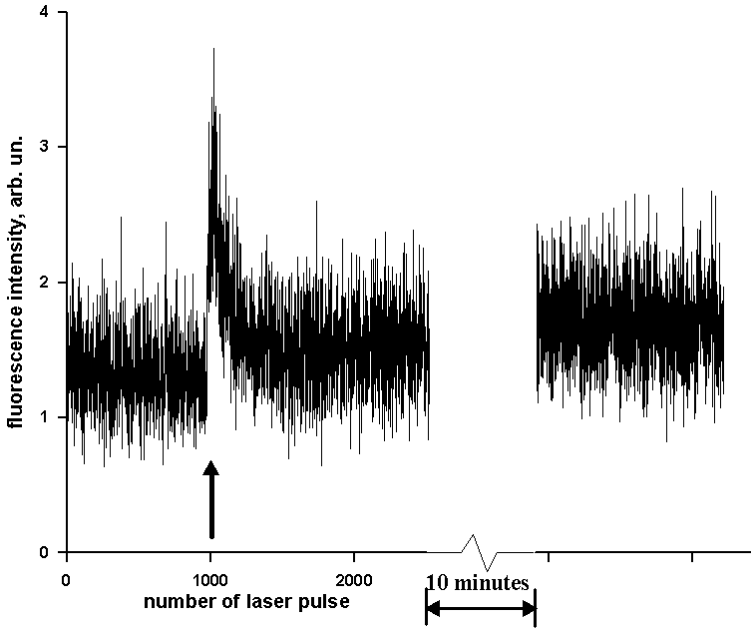


*Figure 6. Change of the alga* T. weisflogii *fluorescence intensity after switching on continuous white light with intensity 50 W/cm$^2$. The arrow denotes the switching on of the illumination. The photon flux density of probing laser pulses is ≈8·10$^{20}$ cm$^{-2}$s$^{-1}$.*

*Table 1. The values of the parameter A of the alga* T. weisflogii *in different functional states.*

| **Functional state** | **A /cm$^2$s** |
|---|---|
| Algae after dark adaptation (in state with open reaction centres) | 1.6·10$^{-23}$ |
| Algae which is illuminated by continuous white light with intensity 50 W/cm$^2$ | 5.7·10$^{-23}$ |
| Algae after addition of DCMU (in state with close reaction centres) | 15·10$^{-23}$ |

## CONCLUSIONS

In this paper the procedure for determining the non-saturated fluorescence parameter $\Phi_o$ which is proportional to the concentration of chlorophyll *a* molecules and of the parameter *A* which is the product of three photophysical parameters of chlorophyll *a* molecules in native chloroplasts has been developed. The laboratory experiments which were carried out with the axenic culture of eurihaline *Thalassiosira weissflogii* have shown that *A* depends on the state of the photosynthetic apparatus of the algae which was changed by treatment with DCMU or by illumination with intensive white light. The revealed sensitivity of A to the state of the photosynthetic apparatus gives possibilities to apply the method of non-linear laser fluorimetry for diagnostics (including remote sensing) of phytoplankton.

The advantages of the developed approach will be revealed under diagnostics of phytoplankton with remote sensing, which can be realised only using powerful pulse lasers. At present no other method of determining these photophysical parameters can be realised with remote sensing.

At the same time the results presented in the paper indicate only principal possibilities of the use of non-linear fluorimetry for *in situ* diagnostics of phytoplankton. The practical realisation of these possibilities is a separate complicated problem. Its solution depends on concrete experimental conditions and practical tasks. Once more it is noted that laser remote sensing of phytoplankton makes it necessary to take into account a possible appearance of fluorescence saturation and to search ways of correction of the fluorescence intensity (or the measured parameter $\Phi_o$) on this effect. One of the possible algorithms of correction (i.e. the determination of the non-saturated fluorescence parameter $\Phi_o$) is presented in the paper. On the other hand fluorescence saturation can be used for the estimation of molecular photophysical parameters, which contain information

about the state of the photosynthetic apparatus and taxonomic composition of algae. One of the possible algorithms for the determination of these parameters is proposed in the paper.

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