Saturation fluorimetry of complex organic compounds with a high local concentration of fluorophores (by the example of phytoplankton)

D.V. Maslov, E.E. Ostroumov, V.V. Fadeev

Abstract. **Saturation of fluorescence of complex organic compounds with a high local concentration of fluorescing molecules (fluorophores), when singlet – singlet annihilation makes a noticeable contribution to saturation, is considered. The fluorescence saturation curve is obtained analytically for the case of a rectangular temporal and spatial distribution of photons in a laser pulse. It is shown that the fluorescence saturation curve depends on the parameter Φ_0, which is proportional to the concentration of fluorescing molecules, and on the parameters A, B, and α describing the influence of singlet – singlet annihilation, bleaching of an optically thin layer, and nonstationarity of excitation, respectively. The fluorescence saturation curves are studied experimentally for compounds with a high local concentration of fluorescing molecules such as molecules of a monoculture of diatomic alga *Thalassiosira weissflogii*. The experimental fluorescence saturation curves are well described by the obtained analytic expression. The values of the parameter Φ_0, proportional to the concentration of chlorophyll *a*, and the parameter A (for the first time) are obtained from the alga fluorescence saturation curves.**

Keywords: *fluorescence, nonlinear fluorimetry, complex organic compounds with a high local concentration of fluorophores, phytoplankton.*

1. Introduction

Upon excitation of complex organic compounds (COCs) by laser pulses, fluorescence saturation can be observed as a nonlinear dependence of the fluorescence intensity on the excitation intensity [1]. This effect has been well studied for diluted solutions of organic dyes ($c \leqslant 10^{-9}$ M) when fluorescence saturation occurs due to the depletion of the ground state of molecules [2 – 6].

However, fluorescence saturation is manifested in a different way in the case of complexes with a high local concentration of fluorophores such as photosensitising organisms, in particular, microalgae – phytoplankton

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(PP). In this case, fluorescence saturation is caused by singlet – singlet annihilation whose elementary act consists in energy transfer between two identical excited molecules accompanied by the transition of one of the molecules to the ground state so that only one excited molecule remains [7, 8]. The first attempts to develop methods for determining photophysical parameters from fluorescence saturation curves for such objects were made in [9, 10]. This proved to be a complicated problem, which was not then solved completely. At the same time, the development of new methods for measuring molecular photophysical parameters, in particular, the method of saturation fluorimetry is of current interest both for studying the structure of fluorescing organic compounds [11, 12] and the elaboration of new approaches to the diagnostics of organic compounds in various media, including remote laser sensing of natural waters [13 – 15].

In this paper, we derive the expression describing the fluorescence saturation curve of a complex organic compound with a high local concentration of fluorophores in a broad range of the exciting radiation intensity. This expression allows one to find the specific unsaturated fluorescence intensity proportional to the fluorophores concentration and parameters determining the fluorescence saturation degree, which are equal to the product of molecular photophysical parameters of fluorophores.

2. Theory

The algorithm for determining photophysical parameters by the method of nonlinear fluorimetry (saturation fluorimetry) is based on measuring the nonlinear dependence $N_{fl}(F)$ of the number N_{fl} of fluorescence photons on the photon flux density F of exciting laser radiation. Note that N_{fl} is measured in experiments in relative units (photodetector counts). To pass to the quantities that can be measured in absolute units, the number N_{fl} of detected fluorescence photons is normalised to a reference signal N_{ref}, which linearly depends on the exciting photon flux density. As the reference signal, either the laser radiation itself directed with a beamsplitter to the second photodetector is used or the Raman scattering signal from solvent molecules (for example, water) is employed as the so-called internal reference [16]. The ratio $\Phi = N_{fl}/N_{ref}$ is called the fluorescence parameter, and the dependence $N_{fl}(F)$ [or $\Phi(F)$] is the fluorescence saturation curve. By solving the direct problem, i.e., by calculating the theoretical fluorescence saturation curve $\Phi(F)$ based on the accepted model,

we can solve the inverse problem of determining the model parameters.

Fluorescence saturation in diluted COC solutions was studied in detail in papers [2–6]. The fluorescence signal was described by the kinetic equation for the population n of the first excited singlet state of complex molecules (by neglecting singlet–triplet conversion):

$$\frac{dn(t,r)}{dt} = F(t,r)\sigma[n_0 - n(t,r)] - \frac{n(t,r)}{\tau}. \tag{1}$$

Here, σ is the absorption cross section of molecules; τ is the lifetime of the first excited singlet state of molecules; n_0 is the concentration of molecules; and $F(t,r)$ is the photon flux density of exciting radiation.

Fluorescence saturation caused by the depletion of the ground state of molecules is described by the term $-F(t,r)\sigma n(t,r)$ in Eqn (1).

By assuming that the exciting pulse is rectangular in space and time and the solution under study is optically thin, we can obtain the analytic expression for the fluorescence saturation curve $\Phi(F)$

$$\Phi^{-1}(F) = \Phi_0^{-1}(1 + BF)\left\{1 + \frac{\alpha BF}{1 + BF}\right.$$

$$\left. \times \left[1 - \exp\left(-\frac{1 + BF}{\alpha}\right)\right]\right\}^{-1}, \tag{2}$$

where $B = \sigma\tau$ is the parameter determining the depletion degree of the ground state (the product BF is the ratio of the excitation rate σF of molecules to their deactivation rate $1/\tau$); $\alpha = \tau/\tau_p$ is the parameter characterising the degree of excitation nonstationarity; and $\Phi_0 = \lim_{F \to 0} \Phi(F)$ is the unsaturated fluorescence parameter, which is independent of F and is linearly coupled with the concentration n_0 of fluorescing molecules.

In the quasi-stationary approximation, when the laser pulse duration τ_p greatly exceeds τ (i.e., in the limiting case $\alpha \to 0$), expression (2) takes the form

$$\Phi^{-1}(F) = \Phi_0^{-1}(1 + BF). \tag{3}$$

The fluorescence saturation curves of diluted solutions of organic dyes obtained in the quasi-stationary case were well approximated by a linear function.

In the case of complexes with a high local concentration of molecules, the fluorescence signal is described by the kinetic equation

$$\frac{dn(t,r)}{dt} = F(t,r)\sigma[n_0 - n(t,r)] - \frac{n(t,r)}{\tau} - \gamma n^2(t,r), \tag{4}$$

in which the additional term $-\gamma n^2(t,r)$ appears compared to (1), which describes the deactivation of excited molecules caused by singlet–singlet annihilation (γ is the rate constant of singlet–singlet annihilation).

By assuming that the exciting pulse is rectangular in space and time and the solution under study is optically thin, we obtain the expression for the fluorescence saturation curve $\Phi(F)$

$$\Phi^{-1}(F) = \Phi_0^{-1}AF\left\{s\left[1 - \frac{1+v}{qv}\ln\left(\frac{1+v}{1+ve^{-q}}\right)\right]\right.$$

$$\left. - \alpha\ln\left(1 - \frac{s}{s+1}\right)\right\}^{-1}, \tag{5}$$

where $C = \tau\gamma n_0$; $A = BC = \sigma\tau^2 n_0$;

$$q = \frac{1}{\alpha}[(1 + BF)^2 + 4AF]^{1/2}; \quad s = \frac{1}{2}[-(1 + BF) + \alpha q];$$

$$v = \frac{\alpha q - (BF + 1)}{\alpha q + (BF + 1)}; \quad w = \frac{1 - e^{-q}}{1 + ve^{-q}}.$$

In (5) compared to (2), a new dimensionless parameter $C = \tau\gamma n_0$ appeared, although implicitly, which is the ratio of the maximum rate γn_0 of deactivation of excited states caused by interaction between excited molecules, resulting in singlet–singlet annihilation, to the intramolecular relaxation rate $1/\tau$. The parameter A appearing explicitly in (5) characterises the degree of influence of singlet–singlet annihilation on fluorescence saturation because the product AF is the ratio of the real (rather than maximal) deactivation rate due to singlet–singlet annihilation $\gamma n = \gamma\sigma\tau F n_0$ ($\sigma\tau F n_0 = n$ in the quasi-stationary approximation in the absence of bleaching) to the linear relaxation rate $1/\tau$.

Unlike (2), expression (5) describes the saturation curve in the general case when fluorescence saturation is caused simultaneously by bleaching and singlet–singlet annihilation. In the absence of singlet–singlet annihilation (i.e., in the limiting case $C = \tau\gamma n_0 \to 0$), expression (5) transforms to (2).

In the quasi-stationary approximation, the expression for the fluorescence saturation curve is simplified:

$$\Phi^{-1}(F) = \frac{1}{2}\Phi_0^{-1}\{1 + BF + [(1 + BF)^2 + 4AF]^{1/2}\}. \tag{6}$$

Figure 1 presents the saturation curves calculated from expression (6) for different values of the parameter C. One can see from this figure and (6) that the parameter C (or A) most strongly affects the dependence $\Phi^{-1}(F)$ in the range of F where the inequality $(1 + BF)^2 \ll 4AF$ is satisfied.
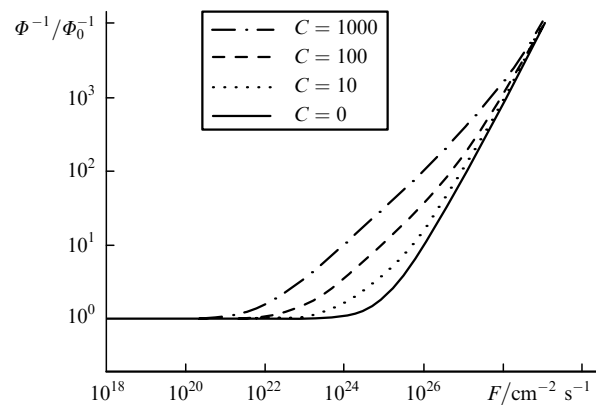

Figure 1. Fluorescence saturation curves $\Phi^{-1}(F)$ calculated for different values of the parameter $C = \tau\gamma n_0$. For $C = 0$, the dependence $\Phi^{-1}(F)$ was calculated by expression (3); $B = 10^{-25}$ cm^2 s.

If $A \gg B$ ($\tau\gamma n_0 \gg 1$ or $C \gg 1$), the fluorescence saturation in this range of F is caused only by singlet–singlet annihilation, and the saturation curve is described by the approximate expression

$$\Phi^{-1}(F) = \frac{1}{2}\Phi_0^{-1}[1 + (1 + 4AF)^{1/2}]. \qquad (7)$$

As F is increased, bleaching begins to make a contribution to fluorescence saturation; as F is further increased, when $(1 + BF)^2 \gg 4AF$, the contribution of singlet–singlet annihilation becomes small, fluorescence saturation is mainly caused by bleaching, and the dependence $\Phi(F)$ can be calculated in the approximation (3).

If $A \ll B$ ($\tau\gamma n_0 \ll 1$ or $C \ll 1$), the inequality $(1 + BF)^2 \gg 4AF$ is valid for any F. In this case, fluorescence saturation in the entire range of F is caused by bleaching, and the dependence $\Phi(F)$ is described by expression (3).

A typical object with a high local concentration of fluorescing molecules is phytoplankton. PP is the association of photosynthesising microalgae. The room-temperature fluorescence spectrum of PP excited at a wavelength of 532 nm is a band of width 20 nm with a maximum at 685 nm belonging to chlorophyll a (Chl a) molecules [17].

The fluorescence response of PP excited by 10-ns laser pulses can be described by kinetic equation (4) in which the physical meaning of parameters σ and τ is changed [18] assuming that σ is not the absorption cross section but the excitation cross section for Chl a molecules, which takes into account both the direct absorption of light by Chl a molecules and energy transfer to them from auxiliary pigment molecules, and τ is the relaxation time of excited states of Chl a molecules determined by the intramolecular deactivation and energy transfer to reaction centres.

The main properties of PP as an object for fluorimetry are as follows. First, because the fluorescence lifetime τ of PP changes from $\sim 3 \times 10^{-10}$ s (in the case of open reaction centres) up to $\sim 10^{-9}$ s (in the case of closed reaction centres) [17, 19], the quasi-stationary approximation is valid upon excitation by 10-ns pulses.

Second, the local concentration of Chl a molecules in PP chloroplasts achieves $0.1 - 1$ M [20], corresponding to $n_0 \approx 6 \times 10^{19} - 6 \times 10^{20}$ cm^{-3} (while the average concentration of Chl a molecules in the alga suspension is considerably lower); in this case, the inequality $A \gg B$ or $C = \tau\gamma n_0 \gg 1$ is valid. Therefore, in the range of F where the inequality $(1 + BF)^2 \ll 4AF$ is valid, the dependence $\Phi(F)$ is described by expression (7).

The experimental fluorescence saturation curves for PP are measured, as a rule, in the range of photon flux densities $F \approx 10^{20} - 10^{25}$ cm^{-2} s^{-1}. This range is bounded below by the detection sensitivity and is bounded above by the negative influence of a high-power laser radiation on PP cells. Because the typical values of parameters are $\sigma \sim 10^{-16}$ cm^2 [17] and $\gamma \sim 10^{-8}$ cm^3 s^{-1} [7], the inequality $(1 + BF)^2 \ll 4AF$ is obviously satisfied in the working range $F \approx 10^{20} - 10^{25}$ cm^{-2} s^{-1}, and the dependence $\Phi(F)$ is described by expression (7).

Figure 2 presents the fluorescence saturation curve for PP calculated by expression (7). One can see from (7) and Fig. 2 that the fluorescence saturation curve for PP is linear in coordinates $\Phi^{-1}(F)$ in the region $AF \gg 0.25$. However, as F is decreased, the linearity is violated and the curve $\Phi^{-1}(\sqrt{F})$ gradually tends to a stationary level $\Phi^{-1}(\sqrt{F}) \approx \Phi_0^{-1}$.
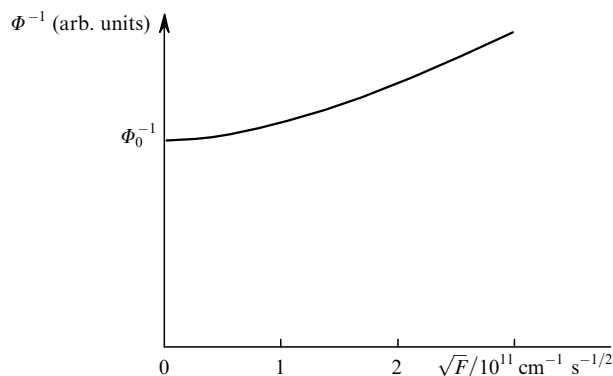

Figure 2. Fluorescence saturation curve for phytoplankton in coordinates $\Phi^{-1}(\sqrt{F})$; $A = 9 \times 10^{-24}$ cm^2 s.

This allows us to determine correctly the unsaturated fluorescence parameter Φ_0 from the fluorescence saturation curve. In earlier papers devoted to the fluorescence diagnostics of PP, where fluorescence saturation was taken into account [21], singlet–singlet annihilation was neglected. The fluorescence saturation curve was measured within a narrow region of F ($\sim 10^{24}$ cm^{-2} s^{-1}) and the dependence $\Phi^{-1}(F)$ was erroneously approximated by a straight line. By extrapolating this straight line to the intersection with the ordinate axis, the value of Φ_0^* was obtained, which was not equal to Φ_0, as became clear later [16]. It was pointed out in [9] that the parameter Φ^{-1} linearly depends on $\sqrt{F}$ in a rather broad range of the photon flux density and it was proposed to determine Φ_0 by extrapolating a straight line $\Phi^{-1}(\sqrt{F})$ to $F = 0$. It follows from (7) that the value of Φ_0 determined in this way is half the real value. To determine Φ_0 correctly, the fluorescence saturation curve should be measured in the range of F where the saturation curve passes from the linear region $\Phi^{-1}(\sqrt{F})$ to the stationary level $\Phi^{-1}(\sqrt{F}) \approx \Phi_0^{-1}$, and then it should be approximated by expression (7).

Thus, for compounds with a high local concentration of fluorophores at which the rate of singlet–singlet annihilation exceeds by an order of magnitude and more the rate of intramolecular relaxation of the excited state (for example, in alga cells) the fluorescence saturation curve has a complicated character in the region of very low F, which resulted in errors in measurements of Φ_0 in previous papers.

In a more general case of an arbitrary spatiotemporal distribution of photons in a laser pulse, expression (7) becomes more complicated. However, the principal character of the fluorescence saturation curve does not change, and the dependence $\Phi^{-1}(F)$ can be approximated with an accuracy of a few percent by the expression

$$\Phi^{-1}(F) = \Phi_0^{-1}[\alpha_1 + (\alpha_2 + \alpha_3 AF)^{1/2}], \qquad (8)$$

which is similar to (7). Here, the coefficients α_1, α_2 and α_3 depend mainly on the distribution type ($\alpha_1 + \sqrt{\alpha_2} = 1$) and weakly depend on the ranges of F and parameters σ, τ and γn_0. If the temporal shape of the laser pulse is described by the hyperbolic tangent distribution

$$g(\tau/\tau_{\mathrm{p}}) = \begin{cases} \dfrac{1}{\ln 2}\dfrac{\tanh(2\tau/\tau_{\mathrm{p}})}{\cosh^2(\tau/\tau_{\mathrm{p}})}, & \tau \geq 0, \\ 0, & \tau < 0, \end{cases}$$

and the spatial distribution is rectangular, the coefficients α_1, α_2 and α_3 are approximately 0.64, 0.13, and 0.43, respectively. This was the case in our measurements of fluorescence saturation curves.

3. Experiment

3.1 Experimental setup

Figure 3 shows the scheme of our experimental setup for studying fluorescence of objects with a high local concentration of fluorophores. Fluorescence was excited by 10-mJ, 12-ns, 532-nm pulses from a frequency-doubled Nd:YAG laser with a pulse repetition rate of 10 Hz. The fundamental laser radiation at 1064 nm was suppressed with a cutoff optical filter.

The laser beam propagates through a Pockels cell, a set of attenuation filters, a lens telescope, and is incident on an aperture separating the central part of the beam. The attenuation filters are used to select the maximum flux density F_{max} with respect to which the radiation is continuously attenuated with a Pockels cell.

Fluorescence excited in the cell with a sample is collected with a lens objective and is focused to the input of the first detection channel consisting of a 1200-lines mm^{-1} MUM monochromator with a reciprocal linear dispersion of 3 $nm\ mm^{-1}$ and a FEU-100 photomultiplier operating in the current regime. The output signal of the photomultiplier was amplified, transformed in an analogue-to-digital converter and fed to a computer.

The fluorescence spectra were scanned with a step motor of the monochromator. The saturation curve was measured by setting the monochromator to the fluorescence band maximum (in our experiments, to the maximum of Chl a fluorescence in PP at 685 nm). The spectral width of the monochromator slit was 3 nm.

The second detection channel is used to obtain the reference signal, which is produced by directing a part of laser radiation with a beamsplitter to an FD-7K photodiode (operating in the photodiode regime). The output signal from the second channel is fed to the computer simultaneously with the main signal. This detection system developed in our laboratory is described in [22].

The minimum photon flux density F at which a signal in the main channel can be reliably detected is determined by the average concentration of Chl a molecules, i.e., by the amount of PP cells in the detected sample volume. We selected the PP amount in a sample to provide, on the one hand, the reliable detection of fluorescence and, on the other, to ensure the condition of an optically thin layer. The signal-to-noise ratio was increased by averaging over 128 laser pulses.

3.2 Measuring method

Before measurements, we calibrated readings (the number of counts N_{reper}^{max}) in the reference channel at the maximum photon flux density F_{max}. The laser pulse shape and duration were measured with a coaxial FK-15 photocell (1-GHz pass band) and a fast Tektronix TDS5104B oscilloscope (1-GHz pass band). The laser pulse shape was well approximated by the hyperbolic tangent distribution with the pulse FWHM $\tau_p = 12$ ns.

The radiation intensity distribution in the beam cross section behind the aperture was close to rectangular and the beam diameter in a cell was 7 mm. The laser pulse energy was measured with a pyroelectric PE25 detector (OPHIR, USA) calibrated according the NIST standard with an accuracy of 5 %.

After determining F_{max} and N_{reper}^{max}, we measured the fluorescence saturation curve. The first experimental point was detected at the maximum photon flux density F_{max} and then laser radiation was attenuated with the help of a Pockels cell. The signals N_{fl} and N_{reper} were measured at each point of the saturation curve in both detection channels. The photon flux density F was determined from the reference signal $F = F_{max}(N_{reper}/N_{reper}^{max})$. Special tests showed that alga cells were not damaged in experiments.

The parameters Φ_0 and A were found from the experimental fluorescence saturation curve $\Phi^{-1}(F)$ as follows.
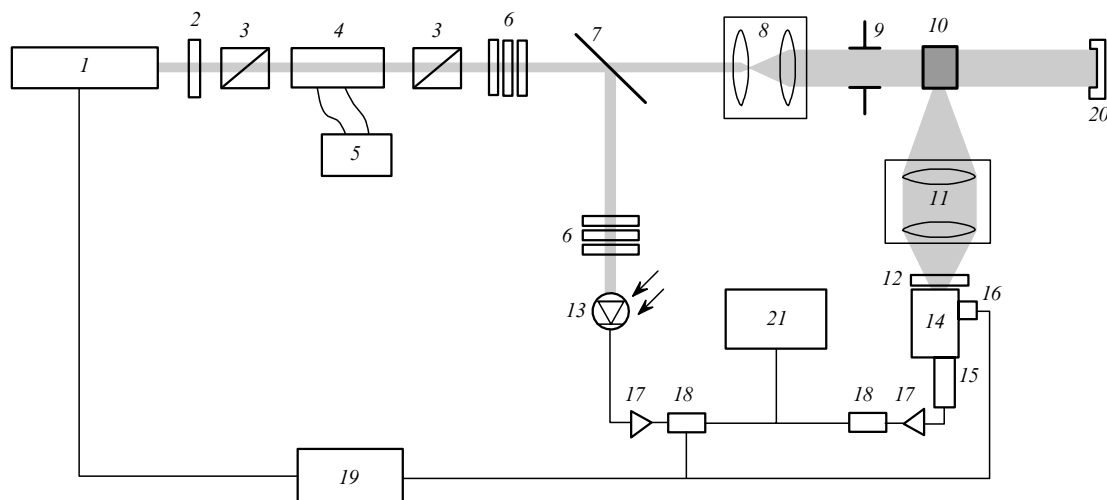

Figure 3. Scheme of the experimental setup: (*1*) frequency-doubled 532-nm Nd:YAG laser; (*2*) cutoff optical filter; (*3*) Glan prism polariser; (*4*) DKDP electro-optic element; (*5*) controllable high-voltage power supply; (*6*) set of attenuating filters; (*7*) beamsplitter; (*8*) lens telescope; (*9*) aperture; (*10*) sample cell; (*11*) lens objective; (*12*) cutoff optical filter; (*13*) photodiode; (*14*) monochromator; (*15*) FEU-100 photomultiplier; (*16*) step motor; (*17*) amplifier; (*18*) ADC; (*19*) synchronisation unit; (*20*) pyroelectric power meter; (*21*) computer.

The saturation curve was approximated by the model function of type (8), where Φ_0^{-1} and A are the variable parameters and the coefficients α_1, α_2 and α_3 are equal to 0.64, 0.13, and 0.43, respectively. By minimising the root-mean-square deviation between the experimental and model curves (the residual function), we found the values of parameters Φ_0 and A.

3.3 Object

We studied monocultures of euryhaline diatomic alga *Thalasssiosira weissflogii*. The alga was cultivated at natural light in the medium prepared based on sea water with a saltiness of 18‰.

Photosynthesising organisms, in particular PP, are interesting objects for laser-induced fluorescence studies both due to their known role in nature and from the methodological point of view. The properties of PP to change its photophysical parameters under the action of various factors opens up broad possibilities for testing new methodological approaches, in particular, nonlinear fluorimetry. It is known [17, 19], for example, that the treatment of PP by 3-(3,4-dichlorophenil)-1,1-dymethylurea (DCMU) results in a change in the excited-state lifetime of Chl *a* molecules. Such a possibility to test the method being developed was used in our paper. Attention was also given to the outlook for the development of laser diagnostics of the photosensitising apparatus of algae by measuring photophysical parameters by the method of nonlinear fluorimetry. This aspect of our work is beyond the scope of this paper and our considerations concerning this question are of preliminary character.

3.4 Experimental results and discussion

Figure 4 shows the fluorescence saturation curves $\Phi^{-1}(\sqrt{F})$ obtained in experiments with alga *T. weissflogii* in two functional states: after dark adaptation and after the addition of DCMU.

One can see from Fig. 4 that the fluorescence saturation curves have, in accordance with analytic expression (8), the following features: for 'large' values of F ($AF \gg \alpha_2/\alpha_3$), the fluorescence parameter Φ^{-1} linearly depends on $\sqrt{F}$; as F decreases, the linearity is violated and the dependence $\Phi^{-1}(\sqrt{F})$ tends to the stationary level $\Phi^{-1}(\sqrt{F}) \approx \Phi_0^{-1}$.

Figure 4 shows that the values of the parameter Φ_0 are different for the fluorescence saturation curves obtained for the same sample (i.e., with the same concentration of Chl *a* molecules) in different states. This is explained by the fact that the quantum yields of fluorescence (fluorescence cross sections) are different in the two states of alga.

The fluorescence saturation curve of *T. weissflogii* in the state with open reaction centres (after preliminary dark adaptation) is shown by squares in Fig. 4. The parameter A_{open} calculated in this case is $(1.6 \pm 0.2) \times 10^{-23}$ cm^{2}s.

The fluorescence saturation curve for the alga with closed reaction centres (after the addition of DCMU and weak background illumination) is shown by circles in Fig. 4; $A_{close} = (1.5 \pm 0.2) \times 10^{-22}$ cm^2 s.

After the addition of DCMU, the electron transport chain in reaction centres is blocked [17, 19], resulting in an increase in the excited-state lifetime τ of Chl *a* molecules. If we assume that the parameters σ and γn_0 do not change after the addition of DCMU, then, because $A = \sigma \tau^2 \gamma n_0$, the increase in the parameter A by a factor of nine means that the lifetime increases by a factor of three: $\tau_{close}/\tau_{open} = 3$.
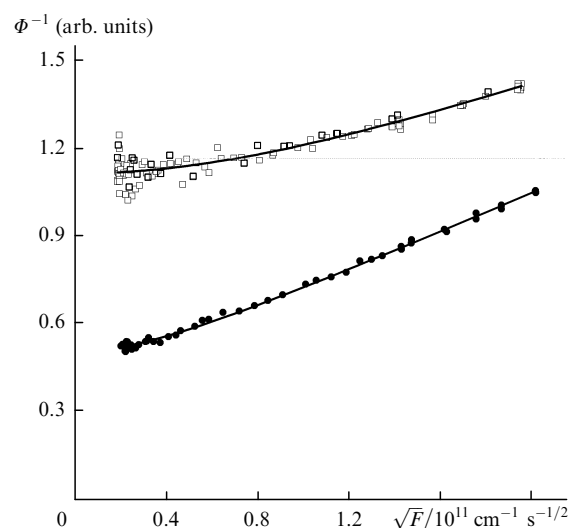
Figure 4. Fluorescence saturation curves $\Phi^{-1}(\sqrt{F})$ for alga *T. weissflogii* in two functional states. Squares correspond to the alga in the state with open reaction centres (after preliminary dark adaptation), circles correspond to the alga in the state with closed reaction centres (after the addition of DCMU and weak background illumination). Each point is the result of averaging over 128 laser pulses. The solid curves are approximations. The photon flux density F was changed in the range from $4 \times 10^{20} - 4 \times 10^{22}$ cm^{-2} s^{-1}.

Figure 5 demonstrates the increase in the fluorescence intensity of alga by a factor of $2.5 - 3$ after the addition of DCMU. The photon flux density of probe laser pulses was $F \approx 7 \times 10^{20}$ cm^{-2}s^{-1}. For such a value of F, no fluorescence saturation was observed, and we can assume that the number of fluorescence photons is $N_{fl} \sim \sigma \tau F$ [1]. Therefore, this result means that the lifetime τ (assuming that σ is constant) increases: $\tau_{close}/\tau_{open} \approx 2.5 - 3$. This value coincides within the experimental error with the estimate made by measuring the parameter A by the method of nonlinear fluorimetry.

The fluorescence saturation curves were measured earlier [9, 10] in the range of 'large' values of F where the dependence $\Phi^{-1}(\sqrt{F})$ was linear. It was pointed out that
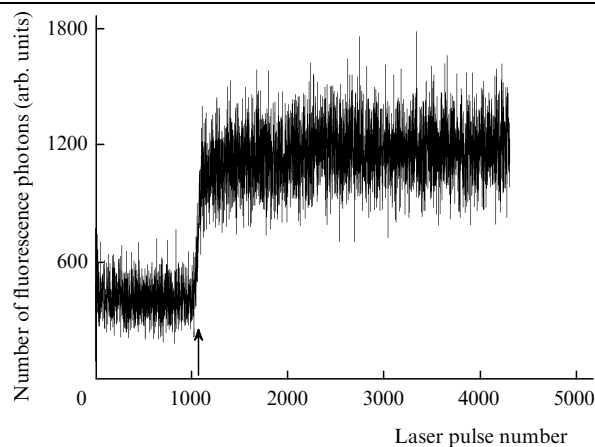
Figure 5. Change in the fluorescence intensity of the alga after the addition of DCMU. The arrow indicates the instant of DCMU addition at a concentration of 10^{-5} M and switching on weak background illumination. The laser pulse repetition rate is 10 Hz and the photon flux density is $F \approx 7 \times 10^{20}$ cm^{-2} s^{-1}.

the dependence $\Phi^{-1}(\sqrt{F})$ began to deviate from linear with decreasing F. However, this part of the saturation curve was not used. At the same time, as our study has shown, it is this part of the fluorescence saturation curve that seems to be most informative for determining the parameters A and Φ_0.

4. Conclusions

We have studied theoretically the saturation of fluorescence of complex organic compounds with a high local concentration of fluorophores. The analytic expression for the fluorescence saturation curve has been obtained in the case of excitation by rectangular laser pulses. It has been shown that the fluorescence saturation curve in the quasi-stationary approximation depends on three parameters, Φ_0, A and B of which the first one is proportional to the concentration of fluorophores and the remaining two are the products of photophysical parameters σ, τ and γn_0: $A = \sigma\tau^2\gamma n_0$, $B = \sigma\tau$.

The fluorescence saturation curves have been obtained for complexes with a high local concentration of fluorophores such as the monoculture of diatomic alga $T.$ $weissflogii$. It has been shown that the experimental fluorescence saturation curves are close to the corresponding curves calculated within the framework of the proposed model. The parameter A has been determined from the saturation curves for alga $T.$ $weissflogii$ in two different states.

The procedure for measuring the unsaturated fluorescence parameter Φ_0 from the fluorescence saturation curve has been developed.

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