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Determination of photophysical parameters of chlorophyll *a* in photosynthetic organisms using the method of nonlinear laser fluorimetry

T.S. Gostev, V.V. Fadeev

***Abstract*. We study the possibility of solving the multiparameter inverse problem of nonlinear laser fluorimetry of molecular systems with high local concentration of fluorophores (by the example of chlorophyll *a* molecules in photosynthetic organisms). The algorithms are proposed that allow determination of up to four photophysical parameters of chlorophyll *a* from the experimental fluorescence saturation curves. The uniqueness and stability of the inverse problem solution obtained using the proposed algorithms were assessed numerically. The laser spectrometer, designed in the course of carrying out the work and aimed at nonlinear laser fluorimetry in the quasi-stationary and nonstationary excitation regimes is described. The algorithms, proposed in this paper, are tested on pure cultures of microalgae *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* under different functional conditions.**

Keywords: *compounds with high local concentration of fluorophores, fluorescence of chlorophyll a, nonlinear fluorimetry, algorithms for solving the inverse problem.*

1. Introduction

The fluorescence of chlorophyll *a* molecules, the basic pigment of photosynthetic organisms (PSOs), is widely used to obtain information about their functional condition [1]. To measure the photophysical parameters of PSO photosynthetic apparatus as a whole the methods of fluorescence induction [2] are used, among which the most advanced is the method of fluorescence induction and relaxation [3].

The performance capabilities of the fluorescent PSO diagnostics can be considerably improved if the determination of the photophysical parameters of PSO photosynthetic apparatus as a whole is complemented by determination of photophysical parameters of the chlorophyll *a* molecules (naturally, in the native PSO). At present such measurements can be carried out only using the method of nonlinear laser fluorimetry (saturation fluorimetry) [4].

T.S. Gostev, V.V. Fadeev Department of Physics, M.V. Lomonosov Moscow State University, Vorob'evy gory, 119991 Moscow, Russia; e-mail: onewhowaits@mail.ru

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The first studies of the possibility of measuring these characteristics using the method of nonlinear laser fluorimetry were performed in Ref. [5]. In Ref. [6] the two-parameter inverse problem of nonlinear laser fluorimetry was successfully solved using a few-parameter model to describe the fluorescence saturation curve of the algae. It appeared to be possible to measure two photophysical parameters, the so-called nonsaturated fluorescent parameter Φ_0 (the number of fluorescence photons in the absence of saturation, normalised by the linear reference value) and the saturation parameter A, equal to the product of three photophysical parameters of chlorophyll *a*, namely, the excitation cross section, the lifetime of the excited state and the rate of singlet–singlet annihilation.

In the present paper we report the theoretical and experimental study of the possibility of increasing the dimension of the inverse problem of PSO nonlinear laser fluorimetry (by the example of phytoplankton) and, as a consequence, the possibility of determining separately the photophysical parameters mentioned above.

2. Theory. Numerical modelling of the inverse problem of nonlinear laser fluorimetry of PSOs

Nonlinear laser fluorimetry [7] is based on registration of the nonlinear dependence of the fluorescence photon number $N_{\rm fl}$ on the photon flux density of the exciting laser radiation, i.e., the fluorescence saturation curve. The principal characteristic feature of PSOs as an object of fluorimetry is a high local concentration n_0 of fluorophore molecules (chlorophyll *a*) in the pigment–protein complex of a photosynthetic unit ($n_0 = 10^{19} - 10^{21}$ cm^{-3}). For this reason the saturation of fluorescence excited by nanosecond laser pulses manifests itself already at the photon flux density $F \approx 10^{21}$ cm^{-2} s^{-1}, which is low for the corresponding lasers. This saturation is mainly due to singlet–singlet annihilation [4] of the excited states of fluorophores.

At a further increase in the exciting radiation intensity, the dynamical depletion of the ground state begins too play a more important role. These two processes mainly determine the shape of the fluorescence saturation curve for the PSO, and the parameters, describing them, in principle can be derived from the experimental saturation curve by solving the proper inverse problem with the adopted model, describing the formation of a fluorescent response in fluorophores, excited by pulsed laser radiation with given parameters [4].

Generally the number of photophysical parameters, describing the two mentioned processes, approaches ten,

because the photosynthetic apparatus includes a number of excitation and relaxation channels for the excited state of chlorophyll *a* molecules. For typical PSO fluorescence saturation curves (Fig. 1) the inverse problem with such a large number of variable parameters cannot be solved (i.e., the problem is incorrect). Therefore, the authors of paper [8] proposed a few-parameter model, describing the fluorescence saturation curve of PSOs as objects with high local concentration of fluorophores. The model involves three generalised (in a certain sense) photophysical parameters: σ, the excitation cross section of chlorophyll *a* molecules, taking into account both the direct absorption of light by these molecules and the energy transfer to them from the molecules of auxiliary pigments; τ, the effective lifetime of the chlorophyll *a* molecules, which takes into account all processes of deactivation of the excited state, except singlet–singlet annihilation; γn_0, the maximum rate of the singlet–singlet annihilation (γ being the rate constant).

The population n of the first excited singlet state of the chlorophyll *a* molecule in the framework of this model satisfies the following kinetic equation:

$$\frac{\mathrm{d}n(t,\boldsymbol{r})}{\mathrm{d}t} = F(t,\boldsymbol{r})\sigma[n_0 - n(t,\boldsymbol{r})] - \frac{n(t,\boldsymbol{r})}{\tau} - \gamma n^2(t,\boldsymbol{r}), \qquad (1)$$

where $\boldsymbol{r}$ is the radius-vector in the laser beam cross section, z is the coordinate in the direction of laser pulse propagation. The number of fluorescence photons from the excited volume of the medium is given by the expression

$$N_{\mathrm{fl}} = k_{\mathrm{fl}} \iiint_V \mathrm{d}^2\boldsymbol{r}\mathrm{d}z \int_{-\infty}^{\infty} n(\boldsymbol{r},z,t;F)\mathrm{d}t, \qquad (2)$$

where k_{fl} is the rate of radiative decay of the excited state of the fluorophore; $n(\boldsymbol{r},z,t;F) = n(\boldsymbol{r},z,t;F(\boldsymbol{r},t),\sigma,\tau,\gamma,n_0)$ is the solution of Eqn (1); V is the volume, from which the fluorescence signal is received. Note that the dependence of the photon flux density of the exciting radiation F on the coordinate z can be neglected, because in the real experiment the concentration of microalgae is small, and the attenuation of the laser radiation is negligible (the approximation of an optically thin layer).

As a rule, the dependence of the relative number N_{fl} of fluorescence photons on the photon flux density F of the exciting laser radiation is measured in the experiment. To proceed to the quantities expressed in absolute units, it is necessary to normalise the registered number of fluorescence quanta N_{fl} with respect to a certain reference signal N_{r}, linearly depending on the photon flux density of the exciting radiation. Following Ref. [7], we will call the ratio $\Phi = N_{\mathrm{fl}}/N_{\mathrm{r}}$ the fluorescent parameter and the dependence $\Phi^{-1}(F)$ the fluorescence saturation curve. The fluorescent parameter Φ_0 in the absence of saturation is related with $\Phi(F)$ by the obvious formula

$$\Phi_0 = \lim_{F\to 0} \Phi(F).$$

The dimension of the nonlinear fluorimetry inverse problem (the maximum number of unambiguously determined parameters) depends on the degree of the polynomial approximating the fluorescence saturation curve with given accuracy. The saturation curve of PSOs, measured in the range of photon flux density from $10^{21} - 3\times10^{25}$ cm^{-2} s^{-1}, is perfectly approximated by a polynomial of the third degree, which means the possibility of determining up to four parameters from it. In paper [6] only the initial part of the saturation curve was used ($F = 10^{20} - 10^{22}$ cm^{-2} s^{-1}), where the dominant mechanism of nonlinearity is the singlet–singlet annihilation, while the dynamical depletion of the ground state does not manifest itself yet. As a result, the degree of the approximating polynomial reduces to one, and the dimension of the inverse problem to two. The parameters determined in this case are the nonsaturated fluorescent parameter Φ_0 and the saturation parameter $A = \sigma\tau^2\gamma n_0$. Then the saturation curve can be described by the following approximate analytical expression:

$$\Phi^{-1}(F) = \Phi_0^{-1}(\alpha_1 + \sqrt{\alpha_2 + \alpha_3 AF}), \qquad (3)$$

if the quasi-stationary excitation regime is implemented, when the laser pulse duration is an order of magnitude larger than the characteristic time of deactivation of the excited states of chlorophyll *a* molecules in the PSO cells, which does not exceed 1 ns [9]. In Eqn (3) α_i are the calculated numerical coefficients, depending on the spatio-temporal distribution of the laser radiation intensity (the coefficients α_1 and α_2 satisfy the condition $\alpha_1 + \sqrt{\alpha_2} = 1$, which follows from the definition of the parameter Φ_0). Figure 1 demonstrates this specific feature of the initial part of the saturation curve in a quasi-stationary excitation regime: the saturation curves for different values of σ and τ, but similar values of A, coincide with high accuracy. Note, that in the nonstationary excitation regime the situation is quite different, as it can be seen from Fig. 1. These properties are explained by the specific features of physical processes, responsible for the formation of fluorescent response in different excitation regimes, and are used in the present work to construct a two-stage algorithm for solving the inverse problem (see below).

Maslov et al. demonstrated [10] that the saturation parameter A of the alga is highly sensitive to the species

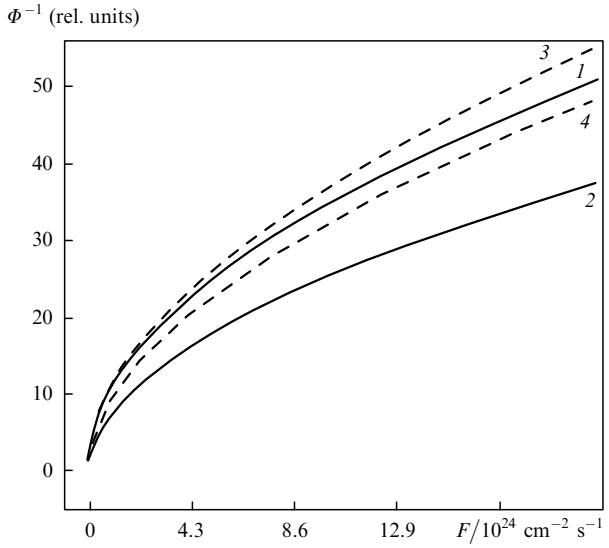

Figure 1. Model fluorescence saturation curves at a fixed saturation parameter ($A = 10^{22}$ cm^2 s), $\sigma = 1\times10^{-16}$ cm^2, $\tau = 1\times10^{-9}$ s, $\gamma n_0 = 1\times10^{12}$ s^{-1} (*1*, *2*), $\sigma = 8\times10^{-16}$ cm^2, $\tau = 0.5\times10^{-9}$ s, $\gamma n_0 = 0.5\times10^{12}$ s^{-1} (*3*, *4*), the laser pulse duration 25 ns (quasi-stationary regime; *1*, *3*) and 0.3 ns (nonstationary regime; *2*, *4*).

Table 1. Results of numerical modelling of the nonlinear fluorimetry inverse problem, obtained using version 1 of the two-stage algorithm ($F = 10^{21} - 3 \times 10^{25}$ cm^{-2} s^{-1}).

Model curve	The value of $A/10^{-23}$ cm^2 s		The value of $\sigma/10^{-16}$ cm^2		The value of $\tau/10^{-10}$ s		The value of $\gamma n_0/10^{11}$ s^{-1}	
	Given	Recovered	Given	Recovered	Given	Recovered	Given	Recovered
1	1.13	1.15	2.50	2.67	3.00	2.84	5.00	4.78
2	1.17	1.18	7.30	6.78	9.00	9.47	4.00	3.83
3	1.21	1.22	4.80	5.13	5.00	4.76	1.00	0.96

Note. The errors in determining A, σ, τ and γn_0 are 1 %, 7 %, 5 % and 4 %, respectively.

it belongs to (i.e., to the composition of auxiliary pigments) and the condition of photosynthetic apparatus. However, it appeared impossible to extract the individual contribution of each photophysical parameter of model (1) into the variation in the saturation parameter. This was a limiting factor for applying the method to the investigation of photophysical processes in PSOs and diagnostics of the photosynthetic apparatus condition.

In this paper we propose a two-stage algorithm that allows high-precision separate determination of all photophysical parameters of chlorophyll *a*, involved in the model described by Eqn (1). The algorithm is implemented in two modifications: using only one fluorescence saturation curve, recorded in the quasi-stationary excitation regime (version I) and using two saturation curves, one of which is recorded in the quasi-stationary regime and the other – in the nonstationary excitation regime (version 2).

In version 1 the fluorescence saturation curve is measured using laser pulses with the duration greater than 10 ns (quasi-stationary excitation regime), but in a much wider range of photon flux density values ($F = 10^{21} - 3 \times 10^{25}$ cm^{-2} s^{-1} than in [6]. In principle, this allows determination of all four parameters $\Phi_0, \sigma, \tau, \gamma n_0$ by solving the inverse problem. However, as shown by numerical modelling, the stability of the solution against the input data noise (saturation curve) is not high (the input data noise level of ~ 5 % gives rise to the mean-square error of the determined parameters approaching 45 %).

To reduce the errors, we propose a two-stage algorithm, based on the *a priori* information that follows from the analysis of photophysical processes in systems with high local concentration of fluorophores and consists in the fact that at the initial part of the saturation curve (at $F = 10^{21} - 8 \times 10^{23}$ cm^{-2}s^{-1}) the dynamic depletion of the ground state is small, so that one can neglect it and process the saturation curve using a quasi-stationary approximation and Eqn (3). As a result, the parameters Φ_0 and A are determined. Having fixed these parameters, we solve again a two-parametric inverse problem numerically, now using the whole fluorescence saturation curve. At this second stage the parameters to be determined are the generalised time τ of the deactivation of the excited state and the excitation cross section σ. Then, using the value of the parameter A found at the first stage, we calculate the parameter γn_0.

Table 1 summarises the estimation results of uniqueness of the inverse problem solution using the proposed method. For this goal at the stage of solving the direct problem three saturation curves are generated differing by no more than 1 % from each other at any point. Then, in the course of solving the inverse problem, the parameters of the model and their estimation error are derived from the noiseless saturation curves. The solution of the inverse problem was implemented using the Levenberg – Marquardt method [11].

One can see from Table 1 that the estimation error of parameters is significantly smaller than their difference for any two curves. This fact characterises the uniqueness of the inverse problem solution. For simplicity all curves were generated with the nonsaturated fluorescent parameter Φ_0 taken to equal 1; the error of its estimation did not exceed 1 %.

To estimate the stability of the inverse problem solution against the input data noise the model curves were made noisy following the law

$$\Phi_{\text{noise}}^{-1}(F) = \Phi^{-1}(F)\left[RA(F_{\min})\sqrt{\frac{F_{\min}}{F}}\right], \qquad (4)$$

where R is a random number in the interval $[-1, 1]$; $A(F_{\min})$ is the noise amplitude in the minimum of the saturation curve at $F = F_{\min}$.

This mathematical model provides the accurate approximation of the noise of the optical detectors used in our experiments. Each dependence was subjected to noise independently ten times with the given noise amplitude, after which the inverse problem was solved and the standard deviations from the value, used in the generation of the model curves at the stage of the direct problem solution, were determined for each estimated parameter. The results of the modelling are presented in Table 2.

As seen from the presented results, at the input data noise ~ 5 %, which can be achieved under the condition of a

Table 2. Stability of the inverse problem solution, obtained using the two-stage algorithm, against the input data noise.

Input noise amplitude (%)	Version of the inverse problem solution	Error in determining σ (%)	Error in determining τ (%)	Error in determining γn_0(%)
1	1	9	6	5
	2	7	5	5
3	1	16	11	14
	2	14	9	13
5	1	21	16	23
	2	17	15	19
10	1	34	29	36
	2	31	29	34

real experiment, the error in determining the photophysical parameters does not exceed 23 %. This is acceptable for a number of applications, particularly in the cases, when the used values are not the absolute values of the parameters, but their variations under the action of certain factors.

From the consideration of the mechanism of dynamic depletion of the ground state it is clear that this effect is expected to manifest itself stronger when the fluorophores are excited by laser pulses with the duration shorter than the relaxation time of their excited states (nonstationary case). This is qualitatively illustrated by Fig. 1. The variations in the excitation cross section σ and the relaxation time τ in this case lead to a greater difference between the saturation curves than in the case of quasi-stationary excitation, which indicates a greater stability of the inverse problem solution against the random errors.

This physical *a priori* information forms the base for version 2 of the two-stage algorithm, which is one more step towards increasing the precision of estimation of photophysical parameters. In this version the first stage coincides with that of version 1, i.e., the initial part of the saturation curve, obtained in the quasi-stationary excitation regime, is used and the parameters Φ_0 and A are determined. At the second stage the fluorescence saturation curve in the nonstationary excitation regime is used, when the pulse duration is 0.3 ns and the photon flux density varies within the same limits, as in the quasi-stationary regime ($F = 10^{21} - 3\times 10^{25}$ cm^{-2} s^{-1}). The exciting pulse durations 25 and 0.3 ns, used in the modelling, correspond to the parameters of the laser spectrometer used in the experiments (see below).

Numerical estimation of the stability of the inverse problem solution against the input data noise within the framework of this approach has shown that the error of the parameter estimation is smaller than in version 1, particularly for the parameter σ. From physical considerations one could expect that the advantage of version 2 will be even greater in solving the problem, where the processes of the energy transfer between the auxiliary pigments and chlorophyll *a* in a light-collecting antenna and from the antenna to the reaction centre are considered separately (in our model they are taken into account in the generalised parameters σ and τ). An advantage of version 2, expected and confirmed by numerical modelling, is the stability of the inverse problem solution against the variations in laser pulse characteristics, which are fixed parameters of the model. In particular, the variation in the laser pulse duration within 20 % leads to the variation in the error in the estimation of photophysical parameters from the solution of the inverse problem no greater than by 5 %.

3. Experiment

3.1 Experimental setup

To implement the algorithms described above, a laser spectrometer was developed based on a two-stage solid-state Nd^{3+}:YAG laser (M.F.Stelmakh Polyus Research and Development Institute) emitting 25- or 0.3-ns pulses. The output radiation at the fundamental wavelength 1064 nm was converted into the second harmonic (the wavelength 523 nm) with the pulse energy that could be smoothly varied from zero to the maximum value of 12 mJ by means of a Pockels cell.

The fluorescence signal was detected using the H5784-20 photomultiplier (Hamamatsu) with enhanced sensitivity in the red spectral region; the spectral selection was implemented using a narrow-band filter with the transmission maximum at 685 nm, which corresponds to the position of the maximum of the chlorophyll *a* fluorescence band. The concentration of algae used in our experiments was such that the contribution of the Raman scattering band of water (with maximum at the wavelength 651 nm and the FWHM of 20 nm) into the registered signal could be neglected.

The fluorescence signal was normalised to the signal in the reference channel, proportional to the intensity of laser radiation. The detection in the reference channel was implemented using the pin-photodiode with a built-in S8746-01 preamplifier (Hamamatsu). A similar detector was used in the ADC triggering system.

Two multifunctional USB-6211 devices (National Instruments) with 16-bit ADCs were used to digitise the signals coming from detectors. With the noise and the detector nonlinearity taken into account, the registration system provided the effective dynamical range of ~ 5000, which allowed registration of the fluorescence saturation curve of the microalgae in the range of the exciting radiation photon flux densities $F = 6 \times 10^{21} - 3 \times 10^{25}$ cm^{-2} s^{-1}.

3.2 The samples

To test the proposed algorithms of the inverse problem solution experimentally, we measured the photophysical parameters of chlorophyll *a* molecules of two test objects, the pure cultures of microalgae *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* in different functional conditions: of the dark-adapted (DA) microalgae in the active state; of the light-adapted (LA) microalgae under the light stress conditions; of the microalgae, treated with the diuron herbicide (DCMU) in the molar concentration of 10^{-6} M and having the chain of electron transport in reaction centres blocked. The chosen functional conditions are characteristic for PSO and are a subject of detailed study in biophysics of photosynthesis [12].

To prevent sedimentation of the microalgae cells on the bottom of the cuvette during the experiment the studied volume of the medium was permanently stirred with a magnetic stirrer. To check the absence of PSO photoinhibition under the action of laser pulses with the photon flux densities, used in the experiment, two test saturation curves were successively registered for one sample with the stirring in the cuvette switched off. The curves obtained in this case coincided up to the experimental error.

3.3 Experimental results and their discussion

Figure 2 demonstrates the fluorescence excitation and emission spectra of one of the chosen objects, the microscopic alga *Chlorella pyrenoidosa* in different functional conditions. The spectra were obtained using the Fluoromax-4 lamp spectrofluorimeter (HORIBA Jobin Yvon). It is seen that the light adaptation leads to significant reduction of fluorescence yield, which is associated with the development of non-photochemical quenching processes [13] of excited states of chlorophyll *a* in the photosynthetic apparatus, which is one of mechanisms of PSO photoadaptation. The shape of the fluorescence excitation band in this case is slightly changed, indicating an insignificant change in the pigment composition. The treatment with diuron, the inhibitor of electron transport, practically does

not affect the shape of the excitation band, but leads to a significant increase in the fluorescence yield. This fact has the following generally accepted interpretation: diuron blocks the channel of the energy transfer from the chlorophyll *a* molecules to the reaction centre.

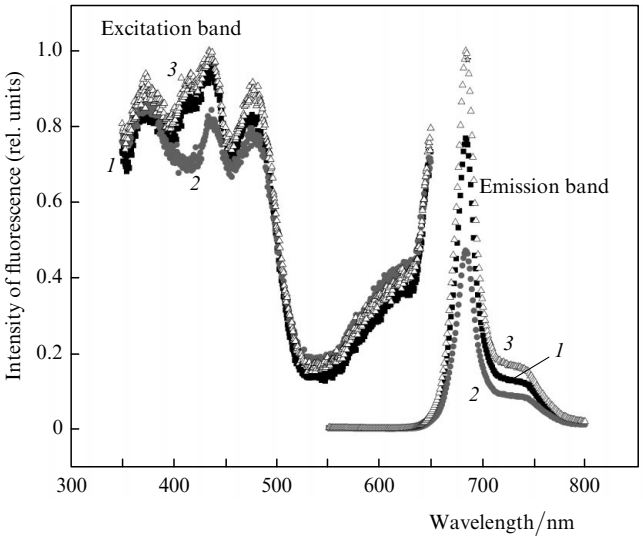

Figure 2. Fluorescence excitation and emission spectra of the alga *Chlorella pyrenoidosa* in the functional conditions DA (*1*), LA (*2*), and DCMU (*3*).

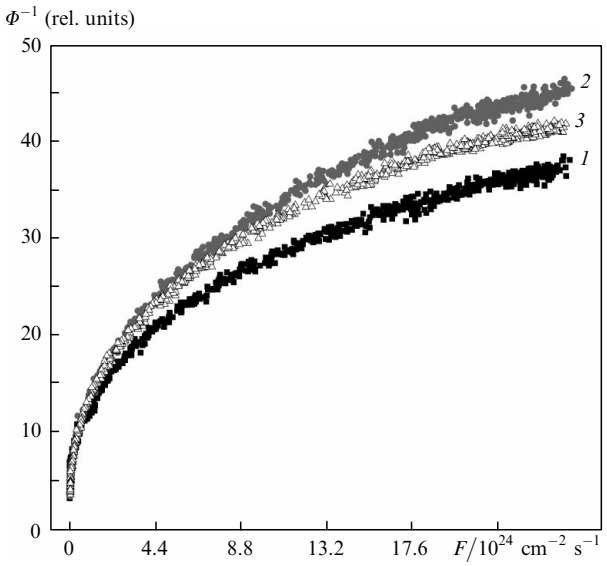

Figure 3. Fluorescence saturation curves of the alga *Chlorella pyrenoidosa* in the functional conditions DA (*1*), LA (*2*), and DCMU (*3*), obtained in the quasi-stationary excitation regime (duration of laser pulses 25 ns at $F = 6 \times 10^{21} - 3 \times 10^{25}$ cm^{-2} s^{-1}).

For each of the mentioned objects the saturation curves were measured three times (examples are presented in Fig. 3). The mean values of photophysical parameters, determined from the experimental curves, are summarised in Table 3. From this Table one can see the expected dependence of the excitation cross section σ of the algae on the species they belong to, the difference in σ being in agreement with the difference in the fluorescence excitation spectra. It is more interesting to trace the influence of the environmental factors on the photophysical parameters.

Continuous illumination of algae with intense light (light adaptation) leads to a significant change in the parameter τ, the generalised lifetime of the excited state of chlorophyll *a* molecules. One of the reasons is the well-known reduction of the energy transfer rate to the reaction centres as a consequence of their transition to the closed state [14]. Besides, as seen from Table 3, the light adaptation increases the rate of singlet–singlet annihilation, which can be associated with conformational changes in the photosynthetic apparatus not noticed before. The excitation cross section changes insignificantly, which agrees well with the absence of noticeable deformation of the fluorescence excitation spectrum (Fig. 2) and indicates the conservation of the auxiliary pigment composition of the photosynthetic apparatus.

The addition of diuron, as already mentioned, blocks the energy transfer to the reaction centre, which leads to the increase in the lifetime of the excited state of chlorophyll *a* molecules. Simultaneously, one more parameter, namely, the rate of singlet–singlet annihilation, is changed. This can be associated with changes in the pigment–protein matrix of the light-collecting complex of the photosynthetic apparatus, to which no attention was drawn before.

Table 3. Molecular photophysical parameters of chlorophyll *a* in the microalgae *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* in different functional conditions (DA – adapted to darkness, LA – adapted to light, DCMU – treated with diuron)

Microscopic alga	Version of inverse problem solution	Φ_0 (rel. units)	$A/10^{-24}$ cm^2 s	$\sigma/10^{-16}$ cm^2	$\tau/10^{-10}$ s	$\gamma n_0/10^{11}$ s^{-1}
Chlorella pyrenoidosa (DA)	1	0.2	5.7	5.6	5.8	3.2
	2			5.8	6.7	3.4
Chlamydomonas reinhardtii (DA)	1	0.3	2.4	3.3	4.5	3.9
	2			2.6	4.6	3.6
Chlorella pyrenoidosa (LA)	1	0.4	23.2	6.1	11.2	3.7
	2			5.5	8.8	3.9
Chlamydomonas reinhardtii (LA)	1	0.4	5.5	3.5	7.2	2.7
	2			3.6	8.3	3.6
Chlorella pyrenoidosa (DCMU)	1	0.4	16.7	6.9	12.4	1.9
Chlamydomonas reinhardtii (DCMU)	1	0.5	7.6	3.9	9.9	2.1

4. Conclusions

In the present paper we propose and implement a two-stage algorithm for solving a four-parameter inverse problem of nonlinear laser fluorimetry pf photosynthetic organisms (by an example of microalgae) as representatives of molecular systems with a high local concentration of chromophores and fluorophores, causing high rate of intrermolecular transfer of the excitation energy. By means of numerical modelling and laboratory experiment the possibility is demonstrated to determine the fluorescent parameter Φ_0, the excitation cross section σ, the generalised lifetime of the excited chlorophyll *a* molecules, and the maximum rate of singlet – singlet annihilation of their excited states γn_0.

It is found that these parameters depend on the algae species and are influenced by the environmental factors, which alter the physilological condition of the algae cells. The analysis of the results (even rather superficial from the viewpoint of photosynthesis biophysics) shows that the determination of photophysical parameters of chlorophyll *a* molecules in native PSOs opens new possibilities to study the mechanisms of photophysical processes in the photosynthetic apparatus and for diagnostics of the environmental influence on them.

The results of the experimental evaluation of the nonlinear laser fluorimetry method are in good agreement with the literature data, obtained using other methods, in particular, the method of fluorescence induction and relaxation, and, at the same time, complement them.

The obtained results demonstrate (by the example of a natural object) the unique potential of nonlinear laser fluorimetry that allows simultaneous determination of several photophysical characteristics of fluorophores in complex organic compounds within the framework of a single alromithm and using one device.

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