Moscow University
Physics Bulletin
Vol. 53, No. 4, pp. 60–72, 1998

Vestnik Moskovskogo
Universiteta. Fizika
UDC 535.372.2

NONLINEAR FLUORIMETRY AS A DIAGNOSTIC METHOD FOR NATURAL ORGANIC COMPLEXES

V. V. Fadeev

This paper summarizes the results of research into fluorescence saturation of natural organic complexes. Problems are analyzed that have to be solved in order to make nonlinear fluorimetry (saturation fluorimetry) a fundamentally new method of spectroscopy enabling one to determine molecular photophysical parameters of composite organic compounds and complexes *in vivo* and *in situ*. Two approaches are offered for solving inverse problems of saturation fluorimetry. The results of computer and actual experiments are presented that illustrate the possibilities of nonlinear laser fluorimetry in natural organic complexes diagnostics.

INTRODUCTION

The central problem of the remote laser diagnostics of natural organic complexes (NOC) in water and on land is to identify and determine the state of these complexes. Identification of an object is important not only by itself, but also for its quantitative study by optical methods, and in particular by means of the fluorimetry method widely used in NOC diagnostics.

There are at least three obstacles that have to be overcome in solving this problem:

(a) the large width of the NOC fluorescence bands and their overlap in natural media, in particular in natural waters; even in the case of excitation at wavelength $\lambda_{\text{exc}} = 266$ nm (fourth harmonics of Nd^{3+}:YAG-laser), when the situation is most favorable, this obstacle cannot be totally removed [1];

(b) the fact that the fluorescence bands of the representatives of the same NOC class are identical or close to one another, because, as a rule, fluorescence centers in them are identical: chlorophyll "*a*" in most of water-plants [2], one fluorophor in an aqueous humus substance (AHS) [3], two or three fluorescent amino acids in protein compounds [1], and obviously one fluorophor in a given class of oils or petroleum products [4];

(c) a change in the NOC state, as a rule, does not influence the form or position of the fluorescence band, but affects its intensity, especially in the case of phytoplankton [2].

After my twenty-year research into NOC fluorimetry I am convinced that it is impossible to overcome these obstacles and solve the NOC identification problem remaining only in the framework of a phenomenological approach, i.e., dealing only with spectra (radiation, fluorescence excitation, total luminescence spectra, etc.), though, of course, these spectra do provide a large amount of information. One has to go to the molecular level and, in addition to spectra, use molecular photophysical parameters, i.e., cross-sections of absorption, fluorescence, and excitation of fluorescent molecules and reaction rate constants for intramolecular transitions and intermolecular excitation energy transfer, under the indispensable condition that these parameters are measured *in vivo* and *in situ*.

Moscow State University, Faculty of Physics.

At present, the only method of measuring these photophysical parameters is nonlinear laser fluorimetry (saturation fluorimetry). It is important to emphasize that this approach is capable to solve not only applied problems of NOC diagnostics, but also fundamental problems: the mechanisms of photophysical processes (at the molecular level) in the interaction of laser radiation and NOC, the nature of fluorescence bands, the genesis and the transformation of NOC in water, e.g., the origin of aqueous humus (in particular, by measuring the parameters of organic compounds like melanoidins that participate in the Meyer reaction), or the transformation processes for oils and petroleum products in water, known as the "aging" process.

Thus, nonlinear fluorimetry is a fundamentally new approach in fluorimetry, the importance of which goes far beyond the NOC diagnostics.

In this paper I summarize the results obtained thus far by me and my team of researchers from Moscow State University in our study of NOC saturation fluorimetry.

SATURATION OF FLUORESCENCE: BASIC CONCEPTS, FIRST APPLICATIONS IN NOC DIAGNOSTICS

It was already in our first experiments in laser fluorimetry of aqueous media in 1975–1977 that we paid attention to fluorescence saturation of phytoplankton.

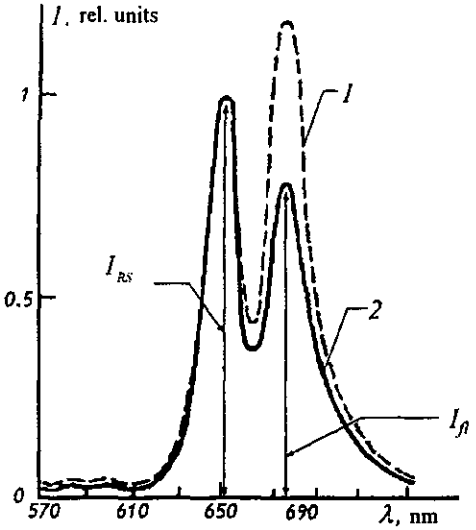

Fig. 1

Response spectra of an aqueous medium with phytoplankton for two values of the photon flux density of the exciting radiation F: F_1 (spectrum *1*) and F_2 (spectrum *2*); $F_2/F_1 = 3$. Bands with maxima at $\lambda = 651$ nm correspond to Raman scattering of water; those with maxima at $\lambda = 685$ nm are for phytoplankton fluorescence; excitation wavelength $\lambda_{\mathrm{exc}} = 532$ nm. Spectra are normalized to the intensity I_{RS} in the maximum of the Raman band.

Figure 1, which was first published in [5], illustrates this phenomenon graphically: with the growing photon flux density of exciting radiation F, parameter $\Phi = I_{\mathrm{fl}}/I_{\mathrm{RS}}$ decreases (I_{fl} and I_{RS} are the intensities of fluorescence bands for phytoplankton and of the Raman scattering for water, respectively). This fact indicates the saturation of phytoplankton fluorescence, since the intensity of spontaneous Raman scattering is a linear function growing with the increasing intensity of exciting radiation.

It is important to note that remarkable saturation of phytoplankton fluorescence is observed already at very low (for pulsed lasers) photon flux densities: $F \cong 10^{22}$ cm$^{-2}\cdot$s^{-1} with corresponding power density ~ 1 kW/cm^2 [6]. For the values of the absorption cross-section typical of composite organic compounds (like dye-staffs or pigments of photosynthesizing organisms), i.e., $\sigma_{\mathrm{abs}} \cong 10^{-16}$ cm^2, and for the lifetime of the excited singlet state equal to $\tau_3 \cong 10^{-9}$–10^{-8} s, the quasistationary population of the excited state at $F \cong 10^{22}$ cm^{-2}·s^{-1} amounts to $n_3 \cong (0.01\text{–}0.001)n_0$, where n_0 is the concentration of fluorescent molecules. Therefore, the trivial saturation mechanism, i.e., dynamic depletion of the ground state at $F \cong 10^{22}$ cm^{-2}·s^{-1} is not yet apparent [7]. Now we know what causes such a high saturation of fluorescence of organic complexes like phytoplankton at low levels of F: this is a singlet-singlet (S–S)-annihilation, which becomes apparent at a high local concentration of fluorescent molecules (for $C \geq 10^{-3}$ M); concentration of fluorescent molecules (chlorophyll "a") in the pigment-protein complexes of photosynthesizing organisms is highly above this bound: $C^{\mathrm{loc}}_{\mathrm{chl}\,\text{“}a\text{”}} \cong 0.1\text{–}1$ M. It is reasonable to assume that it can reach high enough values in other NOC forms (see [3, 4]). Thus, it became necessary to correct the derived values of parameter $\Phi = I_{\mathrm{fl}}/I_{\mathrm{RS}}$ (intensity of the Raman scattering band for water is used as an internal reference point for the continuous calibration the NOC fluorescence band intensities [8]) with respect to the saturation effect in order to restore parameter $\Phi_0 = I^0_{\mathrm{fl}}/I_{\mathrm{RS}} = \lim I_{\mathrm{fl}}/I_{\mathrm{RS}}$ at $F \to 0$, the parameter that is used to determine fluorescent molecules concentration. The method of this correction was first reported in [7] and is being improved constantly [2, 6].

At the same time the fluorescence saturation effect can be employed for determining photophysical characteristics of composite organic compounds and complexes: parameters of the saturation curve are functions of these characteristics. For the first time this problem was formulated in 1979 [9], and later elaborated in [7, 10, 11]. During that stage of research, the results of which were summarized in [12], we used a simple approach based upon analytical expressions for the fluorescence saturation factor, which was derived as a result of solving a system of rate equations for molecule populations in the monomolecular approximation, i.e., ignoring the influence of intermolecular interactions on the fluorescence saturation curves. We showed that for low-concentration dye-staff solutions, this approach yielded an absorption cross-section in the absence of information on concentration and enabled us to measure the lifetime of the excited singlet level with the use of exciting pulses, whose duration by far exceeded this time. In the case of the classical absorption and kinetic spectroscopy this determination is impossible.

During the course of my dissertation [12], I applied this approach to one of the most widely spread and interesting natural organic complexes, namely, to an aqueous humus substance (AHS), despite a possible incorrectness of this application, because one might expect intermolecular interactions to appear in it. Estimation of the absorption cross-section (cross-sections of fluorescent centers in the general case) and comparison of its values for various dimensional fractions of AHS resulted in the formulation the first version of the hypothesis on the nature of the AHS fluorescence band (this hypothesis was further evolved in [3, 13]).

It was obvious, that the analytical expressions derived in the framework of the monomolecular approximation with very stringent assumptions did not completely describe complicated photophysical processes that take place in NOC. Therefore, in 1992 work on the development of a rigorous method for nonlinear fluorimetry was initiated [14, 15]. It was based upon the canonical procedure of solving inverse problems including the construction of an adequate physico-mathematical model, solving a direct problem on its basis (using numerical methods in the general case), and a search for an optimal algorithm of solving an inverse problem that might provide for stability of a solution and minimal possible errors in reconstructing desired parameters with given experimental errors.

PHYSICO-MATHEMATICAL MODELS FOR PHOTOPHYSICAL PROCESSES IN NOC AND A GENERAL PROCEDURE FOR CALCULATING AND PLOTTING FLUORESCENCE SATURATION CURVES (DIRECT PROBLEM)

A saturation curve (functional dependence of intensity I_{fl} or the number of fluorescence photons N_{fl} on the photon flux density of the exciting radiation F) can be obtained by solving (analytically or numerically) a system of kinetic equations for populations of the energy states of fluorescent molecules.

The principle point of this procedure is the rational choice of a physical model that describes interaction between laser radiation (with its characteristic parameters) and a set of fluorescent organic molecules or complexes. In mathematical formulation, this is to fix the number of parameters that have to be determined in solving the inverse problem. The singularities of the NOC saturation curves (see [16]), suggest that the number of these parameters should not be greater than five.

In the case of a monomolecular solution of noninteracting organic compounds (of the dye-staff type), the main parameters that influence the saturation curve are [17] (see Fig. 2):

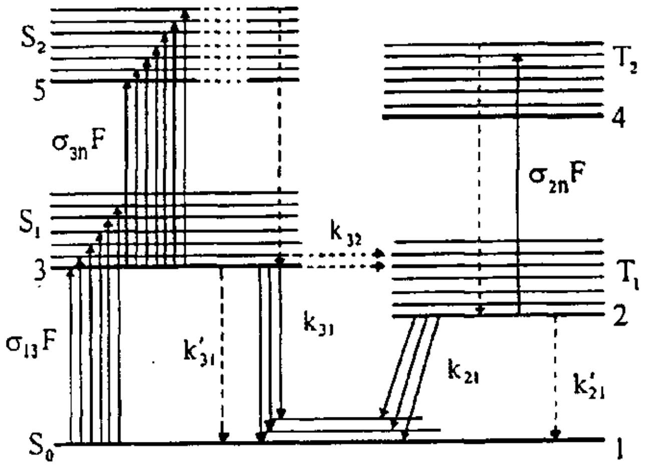

Fig. 2

Model of photophysical processes in complex organic molecules without incorporation of intermolecular interactions.

(1) absorption cross-section $\sigma_{\text{abs}} \equiv \sigma_{13}$ that determines the rate of molecule transition from the ground singlet state S_0 (from level 1) to the first excited singlet state S_1 (level 3) under the influence of the photon flux with density F ($\text{cm}^{-2}\cdot\text{s}^{-1}$);

(2) molecule lifetime τ_3 in the state S_1, i.e., on the level 3;

(3) quantum emergence of molecules to the lower triplet state T_1 (to level 2) as a result of intercombinative conversion $S_1 \to T_1$ ($\eta_T = k_{32}/k_3$, where $k_3 = k_{31} + k'_{31} + k_{32}$; k_{31} and k'_{31} being the rates of radiative and nonradiative transitions from S_1 to S_0; k_{32} is the rate of transition $S_1 \to T_1$; $k_3^{-1} = \tau_3$).

In some cases, these parameters can be supplemented by cross-sections for the absorption of exciting radiation by molecules in excited states S_1 and T_1. This effect manifests itself differently in optically thin and optically thick layers. Its influence on the saturation curve is substantially different for molecules whose transition $S_1 \to S_n$ is rapidly followed by their return to S_1, and for those with high probability of transition directly from S_n to S_0.

To calculate saturation curves for dye-staff fluorescence saturation we have so far used a model with the first three parameters, which corresponds to the following system of kinetic equations:

$$\begin{cases} \dfrac{\partial n_1(t,\mathbf{r},z)}{\partial t} = (k_3 - k_{32})n_3(t,\mathbf{r},z) - F(t,\mathbf{r},z)\sigma_{13}n_1(t,\mathbf{r},z), \\ \dfrac{\partial n_2(t,\mathbf{r},z)}{\partial t} = k_{32}n_3(t,\mathbf{r},z), \\ \dfrac{\partial n_3(t,\mathbf{r},z)}{\partial t} = F(t,\mathbf{r},z)\sigma_{13}n_1(t,\mathbf{r},z) - k_3 n_3(t,\mathbf{r},z), \\ n_1 + n_2 + n_3 = n_0, \end{cases} \tag{1}$$

where z is a coordinate in the direction of laser radiation propagation, $\mathbf{r} = \{x, y\}$ are coordinates in the direction across the beam. Here, transitions $T_1 \to S_0$ are not incorporated (and, correspondingly, parameter k_{21} is absent), which is justified where the laser pulse duration $\tau_p \ll k_{21}^{-1}$, $k_{21}^{-1} = \tau_2$ being a triplet level lifetime. For pulsed lasers, which are frequently used in nonlinear laser fluorimetry with $\tau_p = 10$ ns, this condition is valid *a priori*.

For the NOCs discussed, i.e., phytoplankton, AHS, protein compounds, and oil films, all with high ($\gtrsim 10^{-3}$ M) local concentrations of fluorescent molecules, participating in forming a fluorescent response, one has to introduce parameters that describe the processes of energy transfer between molecules. The molecules can be divided into three groups: (1) fluorophors as proper fluorescent molecules, (2) molecules that absorb exciting radiation and transfer energy to fluorophors (we call them "auxiliary" by analogy with auxiliary pigments in photosynthesizing organisms [2]), and (3) molecules, to which excitation energy can be transferred by fluorophors (extending the terminology used to describe photosynthesizing organisms to all types of NOC, we may call them "reaction centers"). Energy transfer may also take place between singlet excited molecules of one group (e.g., between fluorophor molecules). In each of these interaction acts, one of the two excited molecular states, i.e., excitons, is "destroyed", which is the reason why this process has been called singlet-singlet (S–S) annihilation [17]. Hence, in the case of NOC, the following parameters should be added:

— reaction rate for singlet-singlet annihilation $k_{\mathrm{SS}} \equiv \gamma$;

— rate of energy transfer from "auxiliary molecules" ("AM") to fluorescent ones $k_{\mathrm{AM,fl}}$;

— rate of energy transfer from fluorescent molecules to "reaction centers" ("RC") $k_{\mathrm{fl,RC}}$.

In this case the system of kinetic (rate) equations for populations of the energy levels of fluorophor molecules becomes considerably more complicated:

$$\begin{cases} \dfrac{\partial n_1}{\partial t} = -\sigma_{13} F n_1 + (k_3 - k_{32}) n_3 + \dfrac{\gamma}{2} n_3^2 + k_{\mathrm{fl,RC}} n_3 n_1^{\mathrm{RC}}, \\ \dfrac{\partial n_2}{\partial t} = k_{32} n_3, \\ \dfrac{\partial n_3}{\partial t} = \sigma_{13} F n_1 - k_3 n_3 - \dfrac{\gamma}{2} n_3^2 + k_{\mathrm{AM,fl}} n_3^{\mathrm{AM}} n_1 - k_{\mathrm{fl,RC}} n_3 n_1^{\mathrm{RC}}. \end{cases} \tag{2}$$

This system needs be supplemented by equations for populations of the energy levels of "auxiliary molecules" n_i^{AM} and of "reaction centers" n_i^{RC}.

For pigment-protein complexes of water-plants (photosynthetic units), all of the three mechanisms were fairly well determined, and parameters γ, $k_{\mathrm{AM,fl}}$, and $k_{\mathrm{fl,RC}}$ were defined concretely (see [2, 17]). For the remaining NOCs this has yet to be done.

Under certain assumptions, one can perform "convolution" of the parameters, in particular, introduce effective cross-section σ^* for the excitation of fluorescent molecules, which is controlled by the absorption of exciting radiation by the fluorescent molecules themselves (by cross-section σ_{13}) and by the energy transfer from "auxiliary molecules" to them (in the case of phytoplankton, from auxiliary pigments). Introducing cross-section σ^* and including energy transfer from fluorescent molecules to "reaction centers" into constant k_3 (denoting it by k_3^*), one can simplify system (2):

$$\begin{cases} \dfrac{\partial n_1}{\partial t} = -\sigma^* F n_1 + (k_3^* - k_{32}) n_3 + \dfrac{\gamma}{2} n_3^2, \\ \dfrac{\partial n_2}{\partial t} = k_{32} n_3, \\ \dfrac{\partial n_3}{\partial t} = \sigma^* F n_1 - k_3^* n_3 - \dfrac{\gamma}{2} n_3^2. \end{cases} \tag{3}$$

Solving this system of kinetic equations, we find the value of population for the first excited level n_3, and calculate the number of fluorescence photons, emitted from the volume V:

$$N_{\mathrm{fl}} = k_{13} \int_0^\infty dt \int_V dV\, n_3(t, \mathbf{r}, z), \tag{4}$$

where $dV = d\mathbf{r}dz$. In most of our calculations, we used a thin optical layer approximation, when $F(z) \cong$ const, which corresponded with laboratory experimental situations, even though this may not hold for the remote sensing of the whole water stratum (we will return to this problem at the end of the paper).

Different photophysical parameters have different effects on the fluorescence saturation curve, which is illustrated in Fig. 3, where saturation curves are plotted in two coordinate systems: (N_{fl}, F) and (Γ, F), where $\Gamma = \Phi_0/\Phi$ is a fluorescence saturation factor. As seen from Fig. 3, for large local concentrations of fluorescent molecules, S–S-annihilation becomes apparent at considerably lower levels of F, than does the bleaching of the optically thin layer. Comparison of the curves displayed in Fig. 3 with the experimental saturation curves, obtained for phytoplankton and a low-concentration solution of

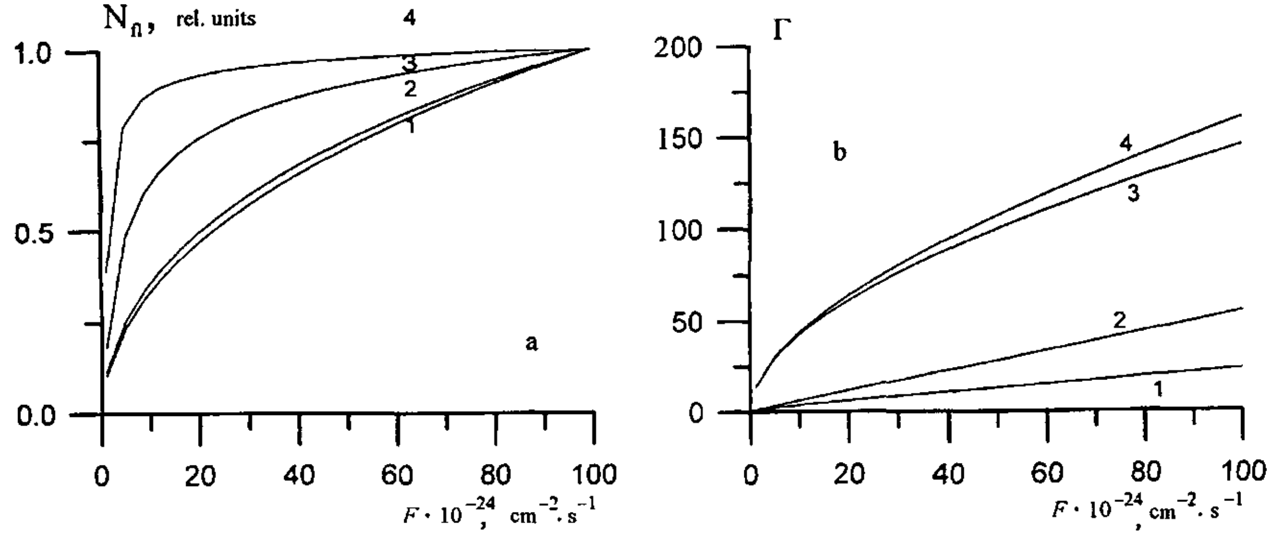

Fig. 3

Effect of various photophysical parameters on the form of the fluorescence saturation curve. The curves were calculated with the help of system (3): $\sigma^* = 10^{-16}$ cm^2, $\tau_3 = (k_3^*)^{-1} = 6$ ns (for all curves); $\eta_T = 0, \gamma n_0 = 0$ (curve *1*); $\eta_T = 0.5, \gamma n_0 = 0$ (*2*); $\eta_T = 0, \gamma n_0 = 10^{11}$ s^{-1} (*3*); $\eta_T = 0.5, \gamma n_0 = 10^{11}$ s^{-1} (*4*).

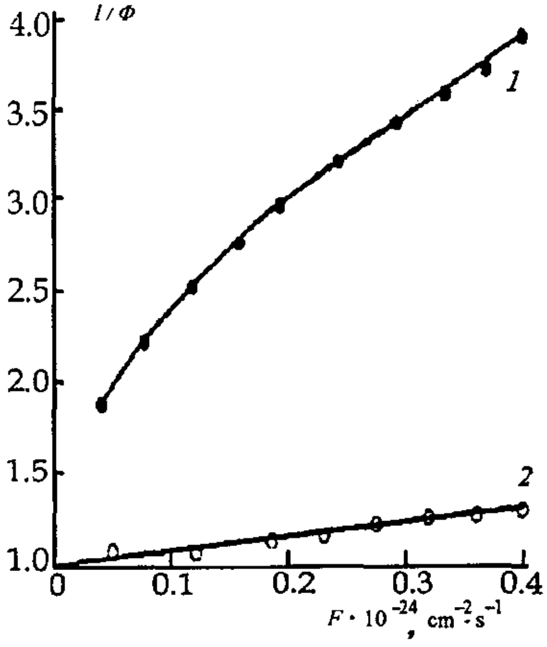

Fig. 4

Experimental fluorescence saturation curves of phytoplankton (*1*) and a low-concentration solution of chlorophyll "*a*" in acetone (*2*).

chlorophyll "*a*", the principle pigment of photosynthesizing organisms (Fig. 4), demonstrates that in the case of the phytoplankton the contribution of S–S-annihilation to photophysical processes is substantial, and absent in the case of the chlorophyll "a" solution (as well as in the case of low-concentration dye-staff solutions and related organic compounds).

We remark, that in a certain sense the above classification of saturation mechanisms is conventional. For example, S–S-annihilation leads not only to the "destruction" of one of two excited states (excitons), but also to the contraction of the molecule return time from state S_1 to S_0, i.e., it counteracts the mechanism of depleting the ground state.

In all of the algorithms for solving inverse problems in saturation fluorimetry, it is very important that the form of presenting the saturation curve is chosen in a rational way. Most informative is a saturation curve in coordinates (N_{fl}, F), where N_{fl} is the number of fluorescence photons, and F is the photon density of exciting laser radiation. However, it is very difficult to measure N_{fl} in absolute units: when a real experiment is going on, this is a practically hopeless task. This obstacle is usually overcome (in diagnostics of NOC in aqueous medium) by the use of an internal reference, a signal of Raman scattering in water, i.e., by representing the saturation curve in coordinates $(\Phi = N_{\text{fl}}/N_{\text{RS}}, F)$ or in coordinates (Φ^{-1}, F). Another way to represent a saturation curve is to use coordinates (Γ, F), where $\Gamma = N_{\text{fl}}^0/N_{\text{fl}} = \Phi_0/\Phi$ is the saturation factor with $\Phi_0 = \lim \Phi(F)$ at $F \to 0$. This representation displays the form of a saturation curve but does not provide any information of the absolute number of fluorescence photons. To determine Γ in an experiment one has to find the value of Φ_0, which can be done by means of extrapolating the measured segment of the curve $\Phi^{-1}(F)$ to the point $F = 0$. In the case of weak dye solutions, this procedure is fairly simple, yet in the case of NOC, due to S–S-annihilation appearance, this is a rather complicated problem (see Fig. 3). For phytoplankton, where S–S-annihilation is high (and, possibly, for other NOC), parameter Φ_0 can be found from an experimental saturation curve plotted in somewhat unexpected coordinates $(\Phi^{-1}, N_{\text{fl}})$, where experimental saturation curves can be fitted well by polynomials [2]:

$$\begin{aligned} \Phi^{-1} &= \Phi_0^{-1} + AN_{\text{fl}} && \text{for } F \le 5{\cdot}10^{23}\ \text{cm}^{-2}{\cdot}\text{s}^{-1}, \\ \Phi^{-1} &= \Phi_0^{-1} + C_1 N_{\text{fl}} + C_2 N_{\text{fl}}^2 && \text{for } F \le 5{\cdot}10^{25}\ \text{cm}^{-2}{\cdot}\text{s}^{-1}. \end{aligned} \tag{5}$$

However, the use of saturation curves in coordinates (Γ, F) is not always productive. In particular, function $\Gamma(F)$ is almost linear for weak dye-staff solutions: $\Gamma \cong 1 + \beta F$ up to the value $F = 10^{25}\ \text{cm}^{-2}{\cdot}\text{s}^{-1}$. Consequently, only a one-parameter inverse problem can be solved, i.e., determination of coefficient β. In every particular case for every particular algorithm of solving an inverse problem, one has to employ its own optimal form for representing a saturation curve. In some cases, one can use several representations at a time.

STABILITY AND UNIQUENESS OF INVERSE PROBLEMS IN SATURATION FLUORIMETRY

In the general case, an inverse problem in saturation fluorimetry is a Hadamard ill-posed problem [18]. However, using *a priori* information on photophysical processes that form the fluorescence of complex organic compounds and NOC, one can limit the number of parameters in a model and their measurement range, so that the problem goes over to the class of conditional well-posed or properly well-posed problems, according to Tikhonov [19]. In our previous experiment [20] done in collaboration with A. V. Tikhonravov and K. V. Popov from the Research Computer Center of Moscow State University, the solution of a three-parameter inverse problem for a monomeric solution of noninteracting molecules (dye solution) was proved to be unique and stable, i.e., the problem was well-posed, according to Tikhonov. It is reasonable to believe that the addition of singlet-singlet annihilation will not result in the loss of this property, even though this has to be verified.

We tested two methods of solving saturation fluorimetry inverse problems. We carried out a detailed study of saturation curves calculated for low-concentration solutions of three dye-staffs with known photophysical parameters $\sigma_{\text{abs}} \equiv \sigma_{13}$, τ_3, and η_T. The values of these parameters for dye-staffs that were used in solving saturation fluorimetry inverse problems are shown in parentheses in Tables 1 and 2. One of these methods was used to solve a problem for experimental fluorescence saturation curves for three NOC representatives: phytoplankton, AHS, and petroleum films.

Table 1

Dye-Staff Parameters Determined from Fluorescence Saturation Curves Using the Method of Least Squares (MLS) and Artificial Neural Networks (ANN) (Results of Numerical Experiment)

Noise level, %	MLS		ANN	
	$<\sigma_{13}> \cdot 10^{16}$, cm^2	$<\tau_3>$, ns	$<\sigma_{13}> \cdot 10^{16}$, cm^2	$<\tau_3>$, ns
Rhodamine 6Zh				
	(2.50)	**(4.0)**	**(2.50)**	**(4.0)**
0	2.50	4.0	2.50	4.0
1	2.50	4.0	2.49	4.0
3	2.50	4.0	2.51	4.0
5	2.50	4.0	2.51	4.0
10	2.54	4.0	2.51	4.0
Eosine				
	(1.10)	**(1.4)**	**(1.10)**	**(1.4)**
0	1.10	1.4	1.10	1.4
1	1.10	1.4	1.10	1.4
3	1.10	1.4	1.10	1.3
5	1.10	1.4	1.10	1.3
10	1.10	1.4	1.10	1.4
Rose Bengale				
	(1.20)	**(0.10)**	**(1.20)**	**(0.10)**
0	1.20	0.10	1.20	0.10
1	1.20	0.10	1.20	0.11
3	1.21	0.11	1.21	0.06
5	1.21	0.12	1.21	0.09
10	1.22	0.14	1.22	0.16

True values of parameters are given in parentheses.

SOLVING SATURATION FLUORIMETRY INVERSE PROBLEMS BY A VARIATION METHOD WITH THE USE OF A QUASI-NEWTONIAN METHOD OF MINIMIZING RESIDUAL FUNCTIONAL

The first method we used to solve saturation fluorimetry inverse problems was chosen from the class of variation methods [20] based upon minimizing the discrepancy between experimental and theoretical data. In using variation methods one is faced with technical problems related to a multi-extreme discrepancy functional, as well as to its intricate "ravine" structure. One of the effective methods to solve these problems is a quasi-Newtonian method [21]. We used this method to calculate parameters σ_{13} and τ_3 in the framework of a two-parameter problem without incorporating intermolecular interactions. With the use of the system

Table 2

Simultaneous Determination of Three Photophysical Parameters of Dye-Staffs with the Use of ANN (Numerical Experiment)

Noise level, %	$<\sigma_{13}> \cdot 10^{16}$, cm^2	$<\tau_3>$, ns	$<\eta_T>$
Rhodamine 6Zh			
	(2.50)	**(4.0)**	**(0.002)**
0	2.49	3.9	0.004
1	2.56	3.8	0.0039
3	2.85	3.5	0.0036
5	3.02	3.5	0.0038
10	3.10	3.8	0.0053
Eosine			
	(1.10)	**(1.4)**	**(0.68)**
0	1.14	1.4	0.94
1	1.20	1.3	0.89
3	1.42	1.2	0.78
5	1.52	1.1	0.75
10	1.56	1.1	0.67
Rose Bengale			
	(1.20)	**(0.095)**	**(0.95)**
0	1.85	0.083	0.53
1	1.83	0.086	0.54
3	1.67	0.098	0.66
5	1.53	0.109	0.77
10	1.17	0.137	1.17

True values of parameters are given in parentheses.

of equations (1) a numerical experiment was carried out, where parameters σ_{13} and τ_3 were determined for three dye-staffs that had essentially different values of parameter η_T (see Table 2). We simulated experimental saturation curves with artificial noise from a random number generator (noise amplitude was varied between zero and ten percent). Mean values and variances of the parameters were determined [20]. The mean values of parameters, obtained as a result of solving the inverse problem, were found to deviate from the true ones by not more than 2–3 percent (with the exception of quantity $<\tau_3>$ for a rose bengale dye, whose deviation from the true value of τ_3 amounted to 10–40 percent at the noise level 3–10 percent, see Table 1); the variance of the reconstructed parameters was not greater than the noise level in the experimental saturation curves.

Table 3

Photophysical Parameters of Natural Organic Complexes
Determined from Experimental Saturation Curves
by Means of the ANN Method

Samples	λ_{exc}, nm	$\sigma \cdot 10^{17}$, cm^2	$\gamma n_0 \cdot 10^{-8}$, s^{-1}
Nitzschia	532	230	1200
Phaeodactylum tricornutum	532	420	2400
Platimonas viridis	532	220	11 800
Diesel fuel	355	10	8.8
Shaimskaya petroleum crude	532	8.0	5.3
AHS from Baltic sea	308	2.9	3.0
AHS from Moskva river	308	6.0	2.6
AHS from Gulf of Mexico	308	5.7	6.5

SOLUTION OF INVERSE SATURATION FLUORIMETRY PROBLEMS USING ARTIFICIAL NEURAL NETWORKS

Artificial neural networks (ANN) provide powerful means for effectively solving various problems of pattern recognition, classification, and prediction. A unique property of neural networks is their ability to learn in examples, and not only to remember but also to generalize the information provided, discover hidden regularities and classify data presented [22]. There are some publications about the application of neural networks in spectral pattern recognition for organic complexes in water [23] and in remote sensing [24].

The ability of neural networks to extract important characteristics from the entered data and reconstruct parameters even when the influence of these parameters on a signal is unknown or altered by noise enables one to use the neural network technique for solving ill-posed inverse problems.

Below follow the first results we obtained from using ANN for solving fluorescence saturation inverse problems. More details can be found in [25]–[28].

We solved inverse problems for saturation curves with two parameters (σ_{13} and τ_3) and three parameters (σ_{13}, τ_3 and η_T). These curves were calculated with the help of equations (1) for the three dyes, described in Tables 1 and 2. With the exception of parameters τ_3 for rose bengale and η_T for rhodamine 6Zh, the variance of the reconstructed parameters and their deviations from the true values, both in two- and three-parameter problems did not exceed the errors of experimental saturation curves (see Tables 1 and 2).

The ANN method was also used to determine parameters σ^* and γ using experimental saturation curves for three types of water-plants, optical thin petroleum and diesel fuel films, and AHS in various types of natural water. The resulting saturation curves are depicted in Figs. 4–6, and the results of solving the inverse problem are listed in Table 3 (here, n_0 is the local concentration of fluorescent molecules in NOC, therefore γn_0 is the maximum value of the S–S-annihilation rate for the given value of γ). In our calculations, the form of the photon flux density distribution for the exciting radiation $F(t, \mathbf{r})$ was chosen to be rectangular in space and Gaussian in time, which was a rather rough approximation for the realistic distribution of $F(t, \mathbf{r})$ in the laser beams used in the experiments.

The values of σ^* and γn_0 are considered to be preliminary, because they will be corrected in the course of improving the method and testing new models. Comparing the values of σ^* and γn_0 for various classes of NOC and for different representatives of the same class suggested to the following conclusions. One of them is that the values of σ^* and γn_0 for phytoplankton were in satisfactory qualitative agreement with the expected values of σ^* (the values of this parameter *in situ* were obtained for the first time) and with

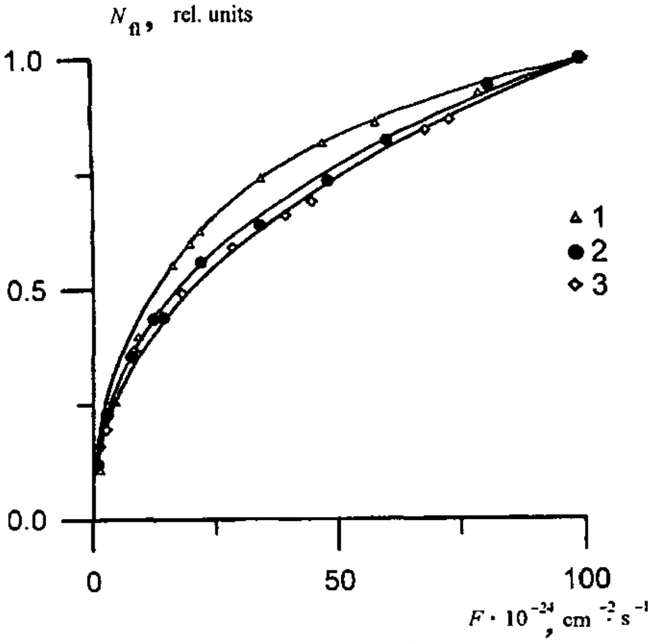

Fig. 5

Experimental saturation curves for various samples of AHS from the Baltic Sea (*1*), Gulf of Mexico (*2*), the Moskva river (*3*); excitation wavelength 308 nm. The curves are normalized to the values of N_{fl} at the maximum value of F.

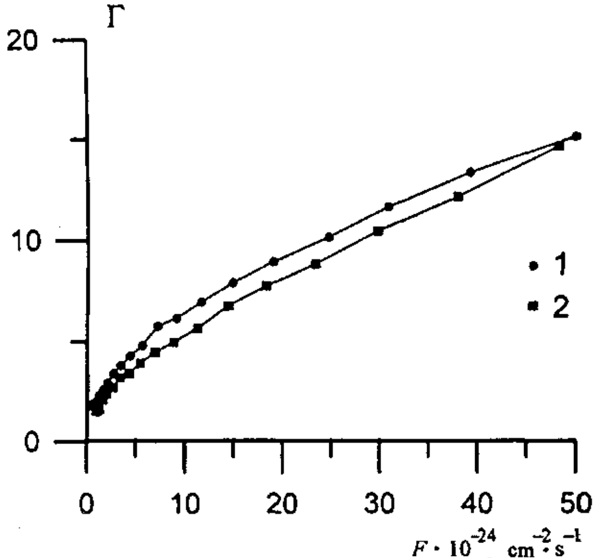

Fig. 6

Experimental fluorescence saturation curves: (*1*) diesel fuel films (λ_{exc} = 355 nm); (*2*) Shaimskaya oil films (λ_{exc} = 532 nm).

the values of γ obtained by picosecond laser spectroscopy [29]. These values confirm the important role of energy migration in the formation of a fluorescent response of phytoplankton to optical excitation.

As to the other two classes of NOC, we had no experimental data to compare their values of σ^* and γn_0. These values provided evidence for the existence of a much lower concentration n_0 of fluorescent molecules in these complexes, compared to phytoplankton, and indicated that the contribution of the process of energy transfer was lower. This conclusion, though, has to be taken as preliminary, because it was derived for a model that did not incorporate singlet-singlet conversion ($\eta_T = 0$) or any other processes that might contribute to fluorescence saturation.

POSSIBILITIES OF USING SATURATION FLUORIMETRY FOR NOC IN A REMOTE MODE

There are several obstacles that need be overcome in applying saturation fluorimetry in the remote mode for investigating and detecting NOC in aqueous media and on land.

One of them is due to the fact that one has to know exact absolute values of the photon flux density for the laser radiation F_i, at which the number of fluorescent photons $N_{\text{fl}i}$ or parameters $\Phi_i(F_i) = N_{\text{fl}i}/N_{\text{RS}i}$ are measured. This is not a simple problem even for laboratory measurements on samples. In the remote mode this is much more difficult, in particular for the case, where a fluorescence signal is received not from the surface (as in the case of petroleum film sensing), but from the whole water thickness or from a deeply lying water layer. The best method to determine F_i is to use a "standard" organic compound, as was proposed in [12]. In analyzing samples, this compound may be any dye-staff with known values of σ_{abs}, τ_3, and η_T (it is better if $\eta_T = 0$). In remote sensing of aqueous media, the only candidate for the role of a standard compound is AHS. The possibility of employing AHS depends on how well the photophysical parameters of AHS are known and to what extent they are steady, at least in a given water area.

The second problem is the violation of the optical thin layer condition in the case when the laser pulse duration τ_p and gate duration τ_g do not satisfy the following conditions: $c\tau_p \leq \varepsilon^{-1}$, $c\tau_g \leq \varepsilon^{-1}$, where ε is the total attenuation coefficient for water at the laser radiation wavelength. When a layer with an arbitrary optical thickness is probed, in addition to the fact that the response over the whole thickness need be integrated (this procedure was performed in [30]), all other of the above mentioned saturation mechanisms (in particular, pumping absorption in excited states) should be incorporated. Theoretical and experimental studies need be performed in this connection, so that the optimal conditions for sensing could be found.

CONCLUSION

In this paper I analyzed the results of the research into NOC fluorescence saturation that has been conducted by our team since 1975 and examined various approaches to solving NOC saturation fluorimetry inverse problems. The main conclusions are that (1) this effect should be taken into account in interpreting the results of the laser sensing of aqueous media and (2) it is possible to measure NOC photophysical parameters with satisfactory accuracy by the saturation fluorimetry method using at least two algorithms for solving inverse problems.

Photophysical parameters of phytoplankton, AHS, and petroleum films were measured *in vivo* and *in situ* for the first time.

So far, we consider our studies only as a demonstration of the possibilities of laser saturation fluorimetry, stimulating further larger scale and more profound investigations, as well as applications in NOC diagnostics. The serious problems, indicated in the paper, are to be solved on this way. However, these efforts are justified by the prospects of using this fundamentally new approach, whose significance extends beyond the framework of NOC laser diagnostics.

I am grateful to the members of my research team, T.A.Dolenko, E. M. Filippova, S. M. Glushkov, and V. V. Chubarov for their help in preparing the manuscript and useful discussions.

This work was supported by the Russian Foundation for Basic Research (grant 96-02-18175) and by the Program "Fundamental Spectroscopy" of the State Committee of Russian Federation for Science and Technology.

REFERENCES

1. E.M. Filippova, V.V. Chubarov, and V.V. Fadeev, *Canadian J. Appl. Spectr.*, vol. 38, no. 5, p. 139, 1993.
2. V.V. Fadeev, D.K. Bunin, and P.S. Venediktov, *Kvant. Elektron.*, vol. 23, no. 11, p. 963, 1996.

3. E.M. Filippova, V.V. Fadeev, and V.V. Chubarov, *Proc. SPIE.*, vol. 2370, p. 651, 1994.
4. Taer Abd Deidan, S.V. Patsaeva, V.V. Fadeev, and V.I. Yuzhakov, *Vestn. Mosk. Univ. Fiz. Astron.*, no. 2, p. 51, 1994.
5. V.V. Fadeev, *Kvant. Elektron.*, vol. 5, no. 10, p. 2221, 1978.
6. I.G. Ivanov and V.V. Fadeev, *Kvant. Electron.*, vol. 15, no. 1, p. 191, 1988.
7. V.V. Fadeev, A.M. Chekalyuk, and V.V. Chubarov, *Dokl. Akad. Nauk SSSR,* vol. 262, no. 2, p. 338, 1982.
8. D.N. Klyshko and V.V. Fadeev, *Dokl. Akad. Nauk SSSR,* vol. 238, no. 2, p. 320, 1978.
9. V.V. Fadeev, A.M. Chekalyuk, A.A. Demidov, et al., *Proc. Third Conf. on Luminescence.*, Hungary, 1979, p. 149.
10. L.L. Maslyukova, A.A. Demidov, V.V. Fadeev, et al., *Proc. 10th Conf. on Coherent and Nonlinear Optics* (in Russian), Kiev, 1980, Part 2, p. 97.
11. A.M. Chekalyuk, V.V. Fadeev, G.M. Georgiev, and Zh.S. Nickolov, *Opt. Commun.*, vol. 38, no. 3, p. 177, 1981.
12. V.V. Fadeev, *D. Sci. (Phys.-Math.) Thesis* (in Russian), Moscow State University, Faculty of Physics, Moscow, 1983.
13. E.M. Filippova, *Cand. Sci. (Phys.-Math.) Thesis* (in Russian), Moscow State University, Faculty of Physics, Moscow, 1994.
14. S.Ya. Dzhasim, N.Ya. Serov, V.V. Fadeev, and A.M. Chekalyuk, *Zh. Prikl. Spektr.*, vol. 56, no. 2, p. 252, 1992.
15. N.Ya. Serov, V.V. Fadeev, and A.M. Chekalyuk, *Vestn. Mosk. Univ., Fiz. Astron.*, no. 5, p. 48, 1992.
16. V.V. Fadeev, S.A. Dolenko, T.A. Dolenko, et al., *Kvant. Elektron.*, vol. 24, no. 6, p. 571, 1997.
17. J. Lakovich, *Foundations of Fluorescent Spectroscopy* (Russian translation), Moscow, 1986.
18. A.N. Tikhonov, V.I. Dmitriev, and V.B. Glasko, *Mathematical Methods in Mineral Exploration* (in Russian), Moscow, 1983.
19. A.N. Tikhonov and V.Ya. Arsenin, *Methods of Solving Ill-Posed Problems* (in Russian), Moscow, 1980.
20. T.A. Dolenko, O.V. Kozyreva, K.D. Popov, et al., *Proc. All-Russian Conf. "Physical Problems of Ecology (Physical Ecology)"* (in Russian), Moscow, vol. 2, p. 29, 1997.
21. A.V. Tikhonravov, ed., *Computers in Laboratory* (in Russian), Moscow, 1992.
22. R.P. Lippman, *IEEE ASSP Mag.*, vol. 3, no. 2, p. 4, 1987.
23. Yu.V. Orlov, S.P. Rebrik, I.G. Persiantsev, and S.M. Babichenko, *Proc. SPIE,* vol. 2503, p. 150, 1995.
24. Y.C. Tzeng, K.S. Chen, W.-L. Kao, and A.K. Fung, *IEEE Trans., Geosci. Remote Sensing,* vol. 32, p. 1096, 1994.
25. S.A. Dolenko, T.A. Dolenko, V.V. Fadeev, et al., *Proc. Conf. on Adaptive Computing in Design and Manufacture,* Plymouth, UK, p. 54, Apr. 1998.
26. E.M. Filippova, I.V. Boichuk, T.A. Dolenko, and V.V. Fadeev, *Proc. Third EARSeL Workshop on Lidar Remote Sensing of Land and Sea,* p. 51, Tallinn, Estonia, 1997.
27. V.V. Fadeev, G.L. Rubin, and Ya.V. Usenkov, *Proc. Third EARSeL Workshop on Lidar Remote Sensing of Land and Sea,* p. 133, Tallinn, Estonia, 1997.
28. V.V. Chubarov, V.V. Fadeev, and S.M. Glushkov, *Proc. Third EARSeL Workshop on Lidar Remote Sensing of Land and Sea,* p. 163, Tallinn, Estonia, 1997.
29. O.V. Braginskaya, *Cand. Sci. (Phys.-Math.) Thesis* (in Russian), Moscow State University, Faculty of Physics, Moscow, 1984.
30. E.V. Baulin and V.V. Fadeev, *Izv. Akad. Nauk SSSR, Fiz. Atmos. Okeana,* no. 1, p. 105, 1985.