

Laser fluorimetry of mixtures of polyatomic organic compounds using artificial neural networks

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Abstract. New possibilities of laser fluorimetry offered by the use of algorithms for solving inverse problems based on artificial neural networks are demonstrated. A two-component mixture of polyatomic organic compounds is analysed by three methods of laser fluorimetry: a direct analysis of the fluorescence band, the kinetic fluorimetry (when durations of the laser pulse and the detector gate pulse are comparable with the fluorescence lifetimes or exceed them), and the saturation fluorimetry. The numerical experiments showed that the use of artificial neural networks in these methods provides a high practical stability of the solution of inverse problems and ensures a high sensitivity and a high accuracy of determining the contribution of components to fluorescence and of measuring molecular photophysical parameters, which can be used for the identification of components.

Keywords: nonlinear fluorimetry, kinetic spectroscopy, polyatomic organic compounds, inverse problems, artificial neural networks.

1. Introduction

Fluorimetry is an efficient method for studying polyatomic organic compounds (POCs) [1]. Interest in the development of the methods for studying and assay of POCs is caused by their key role in many problems of science and technology, for example, in laser physics [2], as well as by their wide abundance in the nature, for example, in natural waters [3]. The main advantage of fluorimetry of POCs is its high sensitivity, while its only important disadvantage is low selectivity related to a large width (several tens of nanometres) of the structureless (or weakly structured) fluorescence bands of POCs observed under usual conditions. This disadvantage is distinctly manifested in the analysis of mixtures of POCs whose fluorescence bands are overlapped, especially, when the component to be determined makes a considerably lower contribution to the total fluorescence band than other (background) components. This problem is

now very important, for example, in the fluorescence diagnostics of the oil pollution (OP) of natural waters, when a small contribution of oil pollution to the total fluorescence band, which is mainly determined by aquatic humic substance (AHS), should be measured [3]. It seems likely that the problems of separating fluorescence contributions from several POCs and identifying the latter are encountered quite often.

In this paper, we consider three methods for determining contributions from the integrated fluorescence bands of components in POC mixtures and for measuring some photophysical parameters of POCs. All these methods use the technique of artificial neural networks (ANNs). Using ‘numerical experiments’, we analyse the possibilities of the following methods: analysis of the fluorescence spectrum of a mixture of fluorescing components, first of all for separating a small contribution of one of them; kinetic fluorimetry when the durations of the exciting pulse and the detector gate pulse are comparable with the fluorescence lifetimes or longer; and nonlinear fluorimetry. All the three approaches assume laser excitation, which is either essential (third approach) or improves the quality of the initial data (first and second approaches). The numerical simulation was performed for the values of parameters corresponding to the real characteristics of the laser fluorimeter equipped with a gated optical multichannel analyser used in our laboratory and to the characteristics of real objects: organic dyes and natural organic complexes in water. We also used the experimental fluorescence spectra of AHS and oil.

2. Solution of inverse problems using ANNs

Mathematical algorithms and methods based on the use of ANNs permit the efficient solution of a variety of problems in the field of prediction, estimates, classification, and recognition of patterns of different types [4]. Recently, ANNs were also applied in laser spectroscopy [5]. The efficiency of ANNs in the solution of problems of saturation fluorimetry was demonstrated in papers [6–8].

It is known [4] that the accuracy of the reconstruction of parameters by the trained network is estimated using the examination data set. To verify the network operation in the presence of noise in the input data, we produced additional ‘noisy’ examination data sets by adding the noise with the amplitude from 1 to 10% of the fluorescence intensity to each channel of the main examination data set (at each point of the kinetic curve or the fluorescence saturation curve). This procedure was performed ten times for each main examination data set, which provided the required averaging

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over the noise realisations in the calculations of statistical quantities.

The correctness of the choice of the ANN architecture and of its training was controlled, for example, by the value of δ calculated for the examination data set, which represents the root-mean-square error of the parameter reconstruction normalised to the range of variation of the corresponding variable. Note that the training of the network using noisy data can substantially reduce the effect of the input data noise on the algorithm performance. The training is performed by presenting to the network the patterns to which a random noise from the specified noise amplitude range is added each time.

Two essentially different ANN-based formulations of the inverse spectroscopy problem are possible, one of them being based on the experiment and the other one on simulations. In the first case, the network is trained using the experimental data set for which the solutions of the inverse problem are known a priori. The main advantage of this approach is an automatic consideration of all the factors affecting the shape of the observed curve, while its main drawback is the difficulty of providing the required representativity of the training set.

In the second case, the network is trained using the data obtained by numerical solving the direct problem. The main advantage of this approach is the possibility of providing the required representativity of the training set both over the range of variations of parameters and in the number of examples. The main disadvantage of this method is the difficulty of developing the adequate analytic model for the solution of the direct problem, which could take into account all the factors affecting the observed curve, including the type and amplitude of noise.

In this paper, all the results were obtained using the architecture of a five-layer perceptron trained with the learning rate 0.01 and the moment 0.9 using the error back-propagation algorithm [4]. We found that the results are mainly determined not by the ANN architecture or the methods of its training but by the method of the formation of the data set and by the fundamental properties of the ANN as an algorithm of data processing.

3. Separation of contributions from the POC mixture components by analysing its fluorescence band using the ANN

A standard procedure for separating the contributions of the components of a mixture to the total spectrum is based on the construction of difference spectra [1]. It is obvious that in this case, the reliable results can be obtained if the intensities of the difference spectra exceed the noise and the error of measurements. Variations in the parameters of the fluorescence bands of components in the mixture (for example, the bandwidth and the distance between the bands) cause large errors in the measurements of contributions from the components. Let us elucidate whether the ANN technique has any advantages over the method of difference spectra.

We will study this problem for the example of the OP–AHS mixture in seawater. To find out the limiting possibilities of ANNs in the determination of fluorescent contributions by direct analysis, we performed the following numerical experiment. We used the fluorescence spectra of model solutions of fulvic acids (FAs) and oil emulsion in

water. These base fluorescence spectra are shown in Fig. 1 together with the Raman band of water, which can be used as an internal reference to calibrate the fluorescence band [9]. In this case, it is convenient to use the fluorescent parameter $\Phi_0 = N_{\text{flu}}^0/N_{\text{RS}} = \sigma_{\text{flu}}n_0/(\sigma_{\text{RS}}n_{\text{RS}})$, where N_{flu}^0 is the number of fluorescence photons (in the absence of fluorescence saturation [6]); N_{RS} is the number of Raman photons; σ_{flu} and n_0 are the fluorescence cross section and the concentration of fluorophore molecules, respectively; and σ_{RS} and n_{RS} are the Raman cross section and the concentration of Raman-active water molecules, respectively.

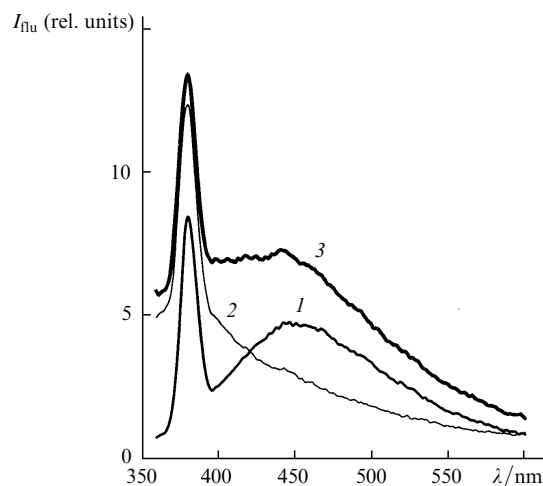


Figure 1. Fluorescence spectra of model solutions of FA (1), light oil (2), and their mixture (3) in distilled water ($\lambda_{\text{exc}} = 337$ nm).

The problem was solved assuming the absence of interaction between the components. The spectra of the mixture were simulated as linear superpositions of the initial (base) spectra of the components with variable weight coefficients. As these coefficients, the partial values of the fluorescent parameter Φ_0 were used. Using this model we calculated all the required data sets by varying the parameter Φ_0^{fa} from 0.01 to 20 (for FAs) and the parameter Φ_0^{op} from 0.01 to 20 (for OP). Using the ANN, we reconstructed the parameters Φ_0^{fa} and Φ_0^{op} and determined their average relative deviations ε_{fa} and ε_{op} from real values (i.e., the errors of the reconstruction) (Fig. 2).

In the ideal case (in the absence of the input data noise), the ANN was capable of determining the OP contribution with the fluorescent parameter Φ_0^{op} down to 0.02 against the FA fluorescence background with the parameter Φ_0^{fa} up to 20.0. In this case, the error of measurement of the parameter Φ_0^{op} did not exceed 10%. The error decreased with increasing Φ_0^{op} , so that the error averaged over the range of variation of Φ_0^{op} and Φ_0^{fa} was about 2%, both for Φ_0^{op} and Φ_0^{fa} . The addition of the 3% noise to the input data considerably deteriorated the result. In this case, we could measure with the 10% error Φ_0^{op} down to ~ 1 , which nevertheless corresponds to a rather high accuracy and sensitivity of the algorithm. The effect of the input data noise can be strongly decreased by training the network using the noisy spectra (cf. curves 2 and 3 in Fig. 2).

Note that for light oil and for petroleum product emulsions in water, the parameter $\Phi_0^{\text{op}} = 0.02$ corresponds, according to our measurements, to the concentration of about $0.1\text{--}0.4 \mu\text{g litre}^{-1}$ and $\Phi_0^{\text{op}} = 1$, to the concentration 5--

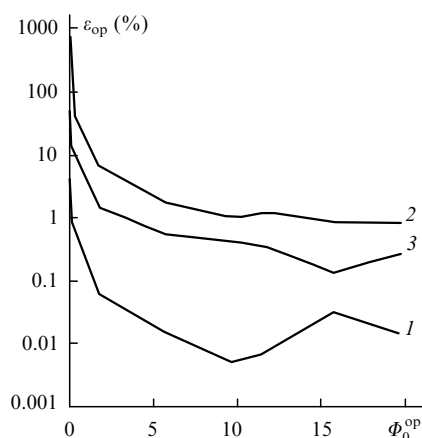


Figure 2. Dependences of the error ε_{op} of measurement of the parameter Φ_0^{op} on Φ_0^{op} for $\Phi_0^{fa} = 19.7$ and for the presentation of the data without noise (1) and the data with the 3% noise (2, 3) to the network trained without noise (1, 2) and with noise (3).

20 $\mu\text{g litre}^{-1}$, whereas the permissible concentration of OP in natural waters is 50 $\mu\text{g litre}^{-1}$ [10]. For AHS (and FA), the parameter $\Phi_0^{op} = 20$ corresponds to the concentration of the order of several milligrams per litre, which is typical for coastal seawaters.

The high sensitivity and accuracy of the method were achieved because we used the quasi-model approach by constructing the model spectra using a combination of the experimental base spectral components. In this way, we obtained a large training data set, and the data obtained with the help of the same model were presented to the network. In this case, the sensitivity and the accuracy of the method were restricted by the properties of the network only, which played the role of the instrumental limit.

4. Stability of solutions to input data errors and variations in the model parameters

The problem of determining the contributions of spectral components to the total fluorescence band that we are solving here belongs to a class of inverse problems. Consider the practical stability [11] of the solution of our problem to the input data noise and to variations in the model parameters. We will follow the approach developed in Ref. [8] for the inverse problem of nonlinear fluorimetry. As mentioned above, the noise in the spectrum presented to the ANN results in a drastic increase in the error of measuring of the parameter Φ_0^{op} . By training the ANN using noisy spectra, the error can be somewhat reduced. These effects are the manifestations of the practical instability and they illustrate one of the methods for eliminating this instability (Fig. 3).

As the elements of the model whose variation can result in the solution instability with respect to the model, we considered the widths of the fluorescence bands of the components of the OP–AHS mixture and the distance between the maxima of these bands. The shape of the band was distorted so that its FWHM $\Delta\lambda$ changed, while the wavelength dependence found by the group method of data handling [12] was preserved. Fig. 4 shows the errors $\delta(\Phi_0^{fa})$ and $\delta(\Phi_0^{op})$ of the parameter reconstruction for the examination set as functions of the relative change in the width of the fluorescence band of FA. Qualitatively and quantitatively

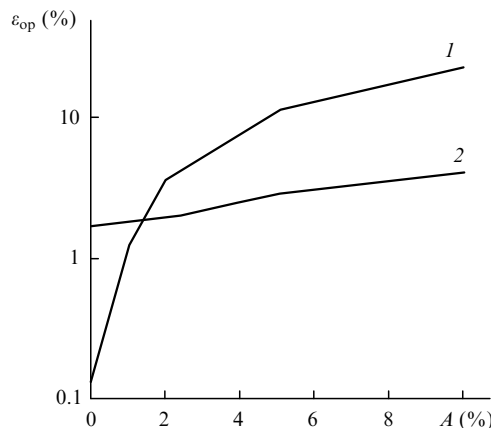


Figure 3. Dependences of the error ε_{op} of measurement of the parameter $\Phi_0^{op} = 1.7$ on the input data noise amplitude A for $\Phi_0^{fa} = 9.7$ for the ANN trained without noise (1) and with noise (2).

veley similar results have been obtained for changes in the width of the fluorescence band of FA. Qualitatively and quantitatively similar results have been obtained for changes in the width of the fluorescence band of OP.

One can see from Fig. 4 that the error of measurements of Φ_0^{fa} and Φ_0^{op} increases from 0.3 to 10% as the fluorescence bandwidth increases by 65% for Φ_0^{fa} and by 35% for Φ_0^{op} and when the fluorescence bandwidth decreases by 25% for Φ_0^{fa} . The stability of the solution to the variation in another parameter of the model, the distance $\Delta\lambda_{max}$ between the maxima of the fluorescence bands of OP and AHS, also proved to be rather high: the error of measurements of fluorescent parameters Φ_0^{fa} and Φ_0^{op} increased to 10% when $\Delta\lambda_{max}$ changed by 20–30 nm.

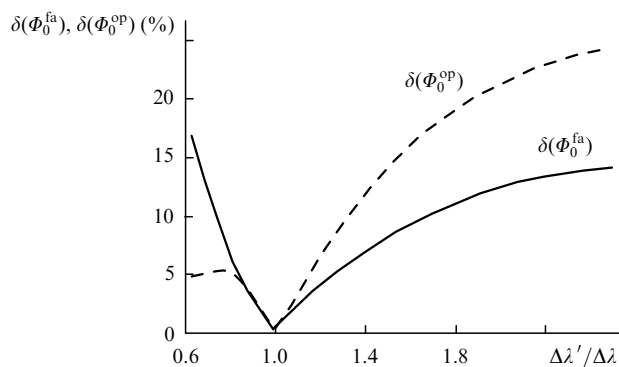


Figure 4. Dependences of the errors $\delta(\Phi_0^{fa})$ and $\delta(\Phi_0^{op})$ of measurement of the fluorescence parameters of FA and light oil on the ratio of the widths of the distorted ($\Delta\lambda'$) and initial ($\Delta\lambda$) fluorescence bands of FA.

Therefore, the obtained results considerably decrease the requirements to the accuracy of a priori information on the shape and the mutual position of the fluorescence bands of the mixture components.

5. Analysis of POC mixtures by the method of kinetic fluorimetry

In the variant of fluorimetry considered below, the information on the mixture components is obtained from the kinetic curve, which represents the dependence of the number N_{flu} of fluorescence photons integrated over the spectrum in

the gate pulse of a detector (the gate-pulse duration is τ_g) on its delay t_d with respect to the laser pulse exciting fluorescence. For a mixture of two fluorophores, the parameters to be measured are the fluorescence lifetimes τ_1 and τ_2 and the ratio of their fluorescent parameters Φ_{01}/Φ_{02} .

The kinetic curve is represented in the form (for a rectangular gate pulse)

$$N_{\text{flu}}(t_d) = \int_0^{\infty} d\lambda \int_{t_d}^{t_d+\tau_g} I_{\text{flu}}(t, \lambda) dt, \quad (1)$$

where $I_{\text{flu}}(t, \lambda)$ is the fluorescence intensity at the wavelength λ at the instant of time t . A standard procedure for measuring parameters τ_1, τ_2 and Φ_{01}/Φ_{02} from the detected dependence (1) is based on the minimisation of the residual between experimental and calculated kinetic curves by varying the required parameters [1]. The stronger the inequalities

$$\tau_p < \tau_{1,2}, \quad \tau_g < \tau_{1,2}, \quad (2)$$

where τ_p is the laser pulse duration, the better the result of this procedure. As τ_p and τ_g approach $\tau_{1,2}$, the errors of measurement of τ_1, τ_2 , and Φ_{01}/Φ_{02} drastically increase. Our aim was to find out how the application of the ANN for the solution of the inverse problem will affect the accuracy of measurements τ_1, τ_2 and Φ_{01}/Φ_{02} when the conditions (2) are violated.

We performed the following numerical experiment. The kinetic curve $N_{\text{flu}}(t_d)$ for the mixture was represented by a sum of kinetic curves for individual components. The fluorescent parameters Φ_{0i} ($i = 1, 2$) were defined by the expressions

$$\Phi_{0i} = \int_0^{\infty} N_{\text{flu}i}(t) dt / N_{\text{RS}}, \quad (3)$$

where N_{RS} is the number of Raman photons for water or other solvent.

By varying the times τ_1, τ_2 and the ratio Φ_{01}/Φ_{02} , we obtained a set of kinetic curves $N_{\text{flu}}(t_d)$ for training the ANN. Thus, we used the model approach (see section 2). We considered the case when the time τ_1 was varied from 1 to 13 ns, while the time τ_2 was varied from 8 to 20 ns (but the condition $\tau_2 > \tau_1$ was always fulfilled), and the ratio Φ_{01}/Φ_{02} was varied from 1 to 10.

In the calculation of model kinetic curves, the laser pump pulse was described by a Gaussian with the width $\tau_p = 10$ ns, while the gate pulse was assumed a rectangular pulse of duration $\tau_g = 10$ ns. We presented the calculated kinetic curves to the trained network and determined the parameters τ_1, τ_2 and Φ_{01}/Φ_{02} . Fig. 5 shows the relative error ε of measurements of the parameters averaged over the entire examination set as a function of the input data noise amplitude. The analysis of these dependences and other results obtained for this problem lead to an important and quite unexpected conclusion.

The error of measurement of fluorescence lifetimes τ_1 and τ_2 is very small (3%–5% for the spectra without noise and no more than 8% for the spectra with noise) nearly over the entire range of variation of τ_1 and Φ_{01}/Φ_{02} . Note once more that these results were obtained for the case when the durations of the laser and gate pulses (10 ns) were longer than τ_1 and of the order of τ_2 , while the gate-pulse position step was 2 ns, i.e., of the order of τ_1 . The error of measurement of the ratio Φ_{01}/Φ_{02} of fluorescent contributions is

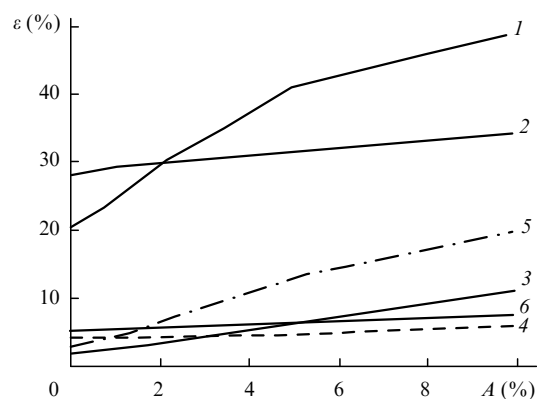


Figure 5. Dependences of the relative error ε on the noise amplitude A in the input data for the measurements of the parameters Φ_{01}/Φ_{02} (1, 2), τ_1 (3, 4), and τ_2 (5, 6) using the ANN trained without noise (1, 3, 5) and with noise (2, 4, 6).

much greater than that for τ_1 and τ_2 and strongly depends on the range where the values of τ_1 and Φ_{01}/Φ_{02} fall.

6. Possibilities of saturation fluorimetry in the analysis of mixtures of organic compounds

In Refs [6–8], nonlinear fluorimetry (saturation fluorimetry) was studied as a method for measuring photophysical parameters of POCs, such as the absorption cross section, the excited-state lifetime of POCs, the triplet-state quantum yield, etc., in one-component solutions. The best results in the solution of the corresponding inverse problems were provided by ANNs trained on model fluorescence-saturation curves. The photophysical parameters determined in this way can be used for identification of POCs. It would be attractive to apply this method to POC mixtures, at least to two-component mixtures. Obviously, this problem in its full-scale statement is much more complicated than for one-component objects [6]. A minimal set of parameters required for diagnostics of fluorophore complexes (mixtures) includes the excited-state lifetimes τ_1 and τ_2 of the fluorophores, their absorption cross sections σ_1 and σ_2 , and the ratio Φ_{01}/Φ_{02} of their fluorescent contributions.

Such a five-parametric inverse problem is a very complicated one to be solved sufficiently accurately ‘in a single step’, even with the help of an ANN. However, a combination of two spectroscopic methods, kinetic spectroscopy and fluorescence saturation spectroscopy, allows the solution of this problem. As an example illustrating this approach, we present the results of the solution of a two-parametrical inverse problem of saturation fluorimetry, in which the absorption cross sections σ_1 and σ_2 for a two-fluorophore system (or a mixture of two organic compounds) are determined. It is assumed that the values of τ_1, τ_2 and Φ_{01}/Φ_{02} are known and used as fixed parameters of the model. They can be preliminary measured by the method of kinetic fluorimetry (see section 5).

The initial data are the fluorescence saturation curves for a mixture of fluorophores, which were calculated by the method developed for nonlinear fluorimetry of single-fluorophore system [6]. The saturation curves were calculated for σ_1 and σ_2 varied from 10^{-17} to 10^{-16} cm². The results of the solution of this problem are presented in Table 1, where are also given the results of the solution of another inverse prob-

Table 1. Root-mean-square errors of measuring parameters σ_1 and σ_2 and fluorescence lifetimes τ_1 and τ_2 of fluorophores for different noise amplitudes of the fluorescence saturation curve for the mixture of fluorophores.

Noise amplitude (%)	ε_{σ_1} (%)	ε_{σ_2} (%)	ε_{τ_1} (%)	ε_{τ_2} (%)
0	6.6	5.5	2.6	2.4
1	7.4	6.0	3.2	2.8
3	9.8	7.8	6.0	4.8
5	12.0	10.5	8.2	6.5
10	14.0	15.2	14.7	12.5

lem of nonlinear fluorimetry of a two-fluorophore mixture in which the lifetimes τ_1 and τ_2 were determined for fixed values of σ_1 , σ_2 and Φ_{01}/Φ_{02} . (The values of τ_1 and τ_2 varied between 1 and 4 ns and between 4 and 15 ns, respectively, while the fixed parameters were $\sigma_1 = \sigma_2 = 10^{-17}$ cm² and $\Phi_{01}/\Phi_{02} = 1$.)

The results presented in Table 1 were obtained using ANNs that were trained by adding the noise. One can see from Table 1 that the use of ANNs allows one to solve two-parametric inverse problems of saturation fluorimetry with good accuracy, in particular, in the presence of the input data noise with the amplitude up to 10%.

7. Conclusions

The results of analysis performed in this paper demonstrate a great potential of the ANN technique for applications in three typical methods of laser fluorimetry of POC mixtures. All these problems are inverse problems of laser fluorimetry. The ANN technique provides a high practical stability of the solutions of these problems. Numerical simulations showed that the use of ANNs in a direct analysis of the shape of the fluorescence band of a mixture allows one to determine the fluorescent contribution that is lower than 1% of the dominant-component contribution for the noise and the error of measurement of the spectrum exceeding 3%. In kinetic fluorimetry, this technique provides the measurement of the lifetimes of fluorophores no longer than 1 ns in a two-component mixture with an error of less than 10% upon excitation by a 10-ns laser pulse and using a 10-ns detector gate pulse with a positioning step of 2 ns.

In this paper, we have formulated for the first time the problem of measuring photophysical parameters of a two-fluorophore system (mixture) by the method of saturation fluorimetry and have obtained the first promising results of the solution of this inverse problem using the ANN. We have shown that, within the framework of two-parametric inverse problems, either the absorption cross sections of fluorophores can be determined (even when their concentrations are unknown) or the excited-state lifetimes of molecules with the errors that exceed the error of measurement of the saturation curve no more than by a factor of 1.5. Obviously, the possibilities of the fluorescent analysis of POC mixtures increase when several approaches are used.

A high stability and a low error of the ANN solutions are probably explained by the properties of the ANN as an algorithm for data processing. First, the ANN solves the problem by using not a few obvious and easily defined quantities, such as the positions of spectral components, their width, etc., but a great number of features distinguished by the ANN itself during its training. Second, during training, the ANN acquires the information contained not in a single curve under study but in the entire

training data array, by separating significant and reproducible information and discarding insignificant and noisy variations in the input data. This is an important advantage of the ANN technique over the methods using other algorithms. For example, variation algorithms use the minimisation of the residual by fitting a small number of preliminarily determined parameters, this procedure being performed separately for each data pattern (curve). The use of the model approach, if possible, and introducing the noise to the data during training increases the effective number of the patterns acquired by the network, which additionally facilitates the problem of separation of significant information and improves the quality of the network operation.

The results obtained in this paper demonstrate the features of ANN-based algorithms for solving the inverse problems of laser fluorimetry and can be considered as a basis for further studies in this field.

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