The accuracy, reliability, and interpretation of the results of *in vivo* laser fluorescence diagnosis in the spectral range of the fluorescence of endogenous porphyrins

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(Submitted April 28, 2009)
Opticheskiĭ Zhurnal **76**, 46–53 (November 2009)

Based on an analysis of various clinical data, the assumption was recently expressed that the often observed *in vivo* increased fluorescence of endogenous porphyrins in living biological tissues is a consequence of the status of chronic hypoxia in the tissues. Starting from this, this article discusses the accuracy, reproducibility, and information content of methods of *in vivo* laser fluorescence diagnosis (LFD) in actual clinical practice. It is shown that, despite the random error of single measurements in LFD of 30–40% established earlier, the accuracy and reliability can reach a fairly high level when the results of the diagnosis are interpreted. The formal random scatter in the results of single measurements is largely determined not by the instrumental error but by the methodological error and by the living and changeable character of the object of diagnosis, especially at the level of the blood-microcirculation system. © *2009 Optical Society of America*.

INTRODUCTION

Noninvasive (*in vivo*, *in situ* laser fluorescence diagnosis (LFD) is widely used today and is studied in various regions of medicine, such as oncology, dermatology, gastroenterology, etc.[1] The most interesting applications of LFD today are usually associated with the possibility of rapid and noninvasive optical detection of diseases and pathologies in tissues, especially malignant neoplasms, on the basis of an analysis of the endogenous fluorescence of various natural (endogenous) fluorophores in tissues, induced by external optical (laser) radiation.[2,3] This phenomenon is often called autofluorescence (AF) of biological tissues.[4] Some of the typical and most easily detected *in vivo* fluorophores in tissues are porphyrin and its derivatives—for example, protoporphyrin IX.[1] Porphyrins have a well-known, pronounced two-humped spectrum of red AF with maxima at wavelengths of 630 and 690 nm,[5] and this made it possible to observe strong AF of the porphyrins of necrotizing tumors already in 1924.[6,7] It was later established that increased and specific porphyrin (red) AF is possessed not only by the tissues of malignant neoplasms, but also by other living soft tissues, with various tumorous, inflammatory, purulent, and other destructive-degenerative processes.[5,8] However, unique theoretical, biological or biophysical prerequisites for the appearance of increased porphyrin AF in living tissues damaged by diseases are not yet altogether clear in many clinical situations.[1] A number of authors, for example, have indicated that the increased accumulation of porphyrins in living biological tissues, causing increased AF, may be the consequence of increased cellular proliferative activity, which is often observed in tumor tissues.[5,8,9] Other authors interpret the fact of the increased AF of porphyrins in tissues as a consequence of the utilization in them of a number of products of the metabolism of anaerobic microbes that are present there,[5,10] as a consequence of the reduction of the acidity index of the medium (the pH) in the tissues,[11] as a consequence of the increased uptake by pathologically altered biological tissues of porphyrins brought in by the blood flow,[1,12,13] etc. However, no unique approach to the explanation of the observed phenomenon has been developed.[1] Meanwhile, it is important in principle for the problem of medical interpretation of the LFD results, including problems of the development of software for diagnostic LFD systems, when, at the third, medical level of interpretation of the diagnostic results, it is necessary to give the physician definitive information on the monitor screen concerning the observed medicobiological processes.[14]

The assumption was recently expressed that the increased accumulation of endogenous porphyrins in the tissues is closely associated with the state of chronic hypoxia in the tissues.[15] In light of this hypothesis, it is important to evaluate the accuracy and reproducibility of LDF results in actual clinical practice. It is well known that the accuracy of any noninvasive spectrophotometric methods in medicine is still not very high, while the error of a method, for example LFD, can reach 35–40%.[16] The goal of this paper is therefore to investigate and discuss questions of the accuracy, reproducibility and reliability of the LFD results as applied to the problem of interpreting the LFD results in terms of chronic hypoxia of the tissues.

MATERIALS AND METHODS

First of all, in light of the new hypothesis expressed here, the authors have once more analyzed their previous data from *in vivo* recording of the fluorescence spectra of endogenous porphyrins from normal biological tissue and tissue damaged by diseases of various origins, obtained earlier when solving a number of problems in three areas of medicine—oncology,[17,18] gastroenterology,[19] and occupational medicine.[20] An LÉSA-01 BIOSPEK optical-fiber laser

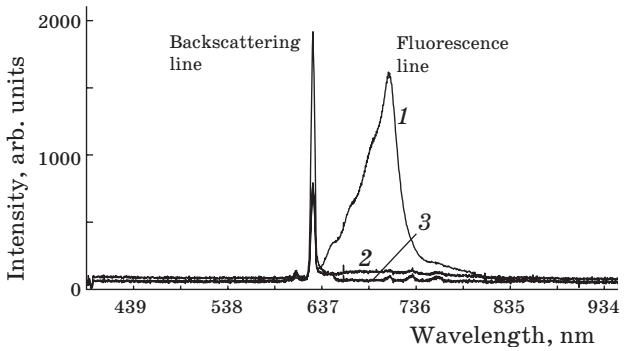

FIG. 1. Typical form of recorded backscattering and fluorescence spectra. *1*—Visible center of a tumor of the mucous membrane of the oral cavity, *2*—surrounding normal tissue, *3*—nonbiological light-scattering material (standard).

endoscopic spectrum analyzer was used in all these studies, with a base design by the group headed by Doctor of Physicomathematical Sciences V. B. Loshchenov.[8] A He–Ne laser (10 mW, 632 nm) was used as the fluorescence-excitation source. The distance between the illuminating and detecting optical fibers was 0.5 mm. The photodetector sensitivity was 10^{-11} W in the 600–750-nm region, while the SNR was at least 10:1.

The typical trace of the backscattering and fluorescence spectra recorded in these experiments is shown in Fig. 1. The intensity of the backscattered line (632 nm) in all the spectra recorded in this paper was reduced by about a factor of 1000 by a step-selective optical filter. However, the modified coefficient of fluorescence contrast K_f, introduced earlier, was used as the quantitative medical diagnostic criterion for analyzing these results; this is defined as

$$K_f = 1 + (\beta I_f - I_l)/(\beta I_f + I_l), \quad (1)$$

where I_f is the amplitude of the recorded signal at the maximum of the AF spectrum, I_l is the amplitude of the recorded signal in the backscattered pump-radiation line (of the He–Ne laser), and β is the attenuation factor of the filter ($\beta \approx 10^3$).

The previous experiments on the statistical evaluation of the LFD errors in the laboratory and under clinical conditions, in which the authors established the random errors of single measurements in LFD at the 3–5% level for nonbiological light-scattering media (instrumental error) and at the 30–40% level for actual patients with malignant neoplasms, were then repeated and analyzed once more.[16] At the same time, there was no possibility of giving a complete explanation of these results and of a number of other observed phenomena, for example, the recording of increased AF from healthy intact tissues in oncology patients. An attempt was made in this work to supplement these results with the data of other optical noninvasive methods of diagnosis, particularly the data of optical tissue oximetry (OTO) and laser Doppler fluorimetry (LDF). This was made possible by the creation of a multifunctional laser noninvasive diagnostic complex (MLNDC), which has three corresponding diagnostic channels (Fig. 2).[21]

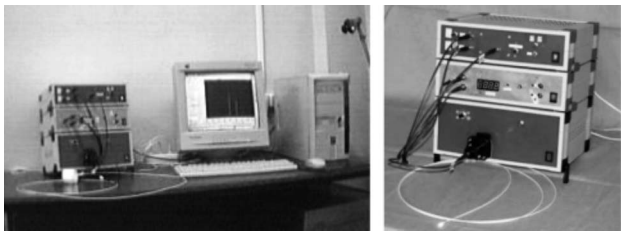

FIG. 2. Pilot sample of the MLNDC.

This diagnostic system also uses a bundle of optical fibers as the working sensor with approximately identical geometry as in the LÉSA-01 BIOSPEK complex; therefore, the technique by which all the laboratory and clinical experiments were performed was identical to that used earlier. However, new simulation standards that reproduce all the main optical properties of biological tissues, including their endogenous porphyrin AF, are used as a nonbiological object for diagnosis in the new series of experiments.[22] These standards are made from a light-scattering material—the base, and the spectrally selective light-absorbing, light-scattering, and fluorescent layers (Fig. 3), which allow them to be simultaneously used not only for the LFD method, but also for the other diagnostic channels of the MLNDC, for example, for the OTO channel. The authors used these standards to again carry out a statistical estimate of the mathematical expectation of the result of diagnosis on a nonbiological object (M), the rms deviation (σ), and the relative random error of measurements $\delta = 100\sigma/M$ in percent in a series of thirty repeated identical single (instantaneous) measurements. In addition, an attempt was made to answer the following questions:

Is it possible to reproduce the preceding statistical results (from 10 years ago!), using the new diagnostic equipment and new standards?

Is the previously established 3–5% random error of measurements for the LFD method on nonliving standards and 30–40% for living biological tissues with pathology specific only to the LFD method, or does it show up for the other diagnostic channels of the MLNDC—in particular, for the OTO channel—and is it the objectively existing error for such methods and objects of diagnosis?

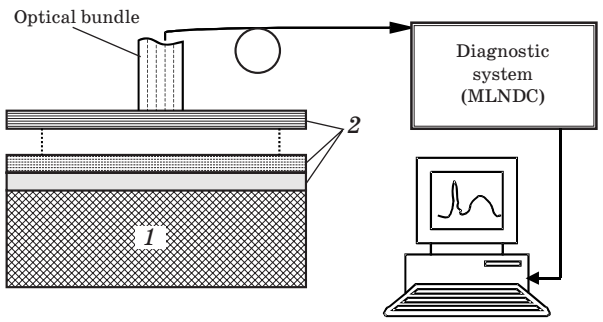

FIG. 3. Layout of a laboratory experiment and structure of simulation standards. *1*—Light-scattering base, *2*—various light-absorbing, light-scattering, and fluorescing film layers.

TABLE I. Statistical LFD results for single (instantaneous) measurements.

Object of investigation	Statistical parameters	Recorded physical parameters (signals), arb. units		Computed K_f
		I_l	I_f	
standard	M	1918	227.9	0.212
	σ	37.6	9.3	0.007
	δ, %	1.96	4.08	3.21
healthy tissue	M	1207	57.95	0.091
	σ	30.39	4.73	0.006
	δ, %	2.52	8.16	6.85
malignant process (cancer)	M	768	341.5	0.621
	σ	35.88	40.13	0.047
	δ, %	4.75	11.75	7.61

TABLE II. Statistical OTO results for single (instantaneous) measurements.

Object of investigation	Statistical parameters	Recorded physical signals, mV			Computed medical parameters, rel. units	
		V_G	V_R	V_{IR}	S_tO_2	V_b
standard	M	897.8	2264	2114	0.89	0.22
	σ	16.23	38.79	24.12	0.04	0.01
	δ, %	1.81	1.71	1.14	4.49	4.55
healthy tissue	M	821.9	2052	1358	0.81	0.13
	σ	65.71	73.95	47.13	0.09	0.02
	δ, %	7.99	3.59	3.47	11.1	15.3
malignant process (cancer)	M	774.5	2678	1594	0.93	0.16
	σ	39.76	83.18	40.51	0.06	0.01
	δ, %	5.13	3.11	2.52	6.45	6.25

How can one explain the authors' previous results in the light of the new hypothesis that the AF spectra of endogenous porphyrins in tissues are correlated with the state of chronic tissue hypoxia?

In the light of this hypothesis, is the 30–40% error of the LFD method for living biological tissues with pathology critical for making a diagnosis in practical health care, or does it not strongly influence the final medical conclusion from the results of the diagnosis, and can it be explained by some other objective causes that also have a definite diagnostic significance for the doctor?

NEW STATISTICAL RESULTS

Some of the new statistical results for the LFD and OTO methods, obtained in single (instantaneous) measurements on a nonbiological simulation standard under laboratory conditions and in patients, as well as voluntary healthy subjects in the clinic, are shown in Tables I and II, respectively. In the OTO method, the physical parameters to be analyzed were the recorded signals from the photodetector (a photodiode) in millivolts in various wavelength ranges [as presented in Table II, in the green (V_G), red (V_R), and near infrared (V_{IR}) regions). However, the medical parameters to be computed were the average tissue arteriovenous oxyhemoglobin saturation of the blood of a microcirculatory vessel (S_tO_2) and the mean volume fraction of the overall hemoglobin (of the blood) in a test volume of biological tissue (V_b), i.e., the usual parameters in the practice of OTO.[26]

ANALYSIS OF THE EARLIER CLINICAL DATA

As reported earlier,[17,18] for oncology patients, the authors often observed strong and objective differences in the AF spectra for different patients and different types and stages of development of the tumors. However, at the same time, the authors found no correspondence in the various observed AF spectra with differences in localization, morphological shape of the cancer, its stage of development, etc. The most varied spectra were recorded not only for different forms of cancer and stages of its development, but also for completely identical ones. In a number of clinical cases (about 30%), no visible AF signal was noted for advanced cancer of the mucous membranes of the oral cavity before beginning a course of treatment. A number of patients (about 40%) possessed elevated AF not from the entire surface of the tumor, but only from 1–2 points around the center. A very variable character of the AF spectra was observed in the course of radiotherapy. On the whole, it was established that the mean initial value of K_f over all patients with increased initial porphyrin AF from a tumor is at the level $K_f = 0.6-0.7$, at the same time that $K_f < 0.1$ for healthy subjects.

For patients with long-term, slow-healing erosive-ulcerous lesions (EULs) of the gastrointestinal tract (GIT), the mean initial value of K_f was established at 0.25–0.35.[19] The smaller the individual initial value of K_f recorded from the region of the EUL of the GIT, the more effective laser therapy was for these patients, by the end of the course evening out the K_f values for the region of pathology and intact mucous tissues at a level of 0.05–0.08.

The study of endogenous porphyrin AF for the skin of the fingers, in patients with occupational vibration syndrome (VS), which is caused by the prolonged action of manufacturing vibration on the worker's hand, also showed a stable increase of the value of K_f.[20] At the second stage of VS, with pronounced trophic lesions of the skin of the fingers, the mean value of K_f was determined to be at a level of 0.23 ± 0.08. The K_f value was also increased for the initial (1st) stage of VS, but not so significantly—to the level of 0.17 ± 0.08, when the K_f values in a control group of voluntary subjects were 0.08 ± 0.05.

What can explain all these clinical results in light of the problem under consideration—the medical reliability and interpretation of LFD data? If it is assumed that the elevated accumulation of endogenous porphyrins in biological tissues is caused by increased cellular proliferation, it is impossible to explain the results in the area of gastroenterology[19] and occupational medicine,[20] since, for example, it is well known that there is no increased cellular proliferation in the skin of the fingers with VS. There also is none in the case of a long-term slow-healing EULs of the GIT. These results cannot be explained by a microbial etiology, since in general it is not applicable to patients with VS.

In the authors' opinion, all these results can be explained from a common standpoint with no clear inconsistencies in only one way—by the presence of chronic tissue hypoxia and its variability in the course of treatment, especially courses of radiotherapy, making the hypothesis that chronic hypoxia affects the process of accumulation of porphyrins in biological tissues. In all the cases described above, tissue hypoxia is a fundamentally important, common, and objectively present factor. For example, in accordance with general radiobiological prerequisites, malignant neoplasms often have a stable fraction of hypoxic cells, which are a factor in the radioresistance of tumors.[23] It is often observed not over the entire volume of the tumor but in separate segments of it and oscillates in the course of radiation treatment. Chronic hypoxia can affect the metabolism of porphyrins in tissues.[13,15]

Therefore, after once more analyzing all the results obtained earlier in order to create a well-substantiated system of medical interpretation of the LDF data that takes into account the hypothesis that the LFD results are affected by tissue hypoxia, the following preliminary classification of the K_f values obtained by the authors was established in terms of tissue hypoxia:

- $K_f < 0.1$: the absence of noticeable chronic hypoxia;
- $K_f = 0.1-0.2$: initial (light-stage) hypoxia;
- $K_f = 0.2-0.4$: chronic hypoxia of medium seriousness;
- $K_f > 0.4$: serious stage of chronic hypoxia of the tissues.

DISCUSSION OF THE RESULTS

As far as the reproducibility of the LFD data (Table I) is concerned, it is obvious that the result obtained for the relative measurement error was of the same order as earlier. The small decrease of the overall relative error for each of the objects of diagnosis can be explained by the improved diagnostic equipment over that used 10 years ago. In general, however, the situation is completely qualitatively reproduced in which the random error of single (instantaneous) measurements increases as one goes from a nonliving to a living object of diagnosis and from the norm to pathology (for example, a malignant process on the mucous membrane of the oral cavity).

It is more important that the same result was recorded for the OTO method (Table II); i.e., the given regularity is not specific to the LFD method. Moreover, the result obtained for OTO shows that the computed medicobiological parameters in OTO have substantially more perceptible random error (the scatter of the data from measurement to measurement) than do the recorded physical parameters (signals). This suggests that the computational algorithms of the OTO methods and devices substantially contribute to the total measurement error in the form of methodological error.[1)] Moreover, a significant part of this methodological error is apparently formed at the very concluding stages of the computations when solving the inverse problem and directly determining the S_tO_2 and V_b parameters from the computed optophysical characteristics of the medium, since the direct problem on the absorption and scattering of light in biological tissue can be solved fairly accurately today for most cases in OTO that are important in practice.[24] However, neglecting the computational algorithms for both the LDF and the OTO methods, the overall instrumental error evaluated on the simulation standards from the levels of the recorded primary physical data is not very substantial (1–2%) and is of the same order of magnitude as was obtained earlier.[16]

Although the overall random error did not exceed 20% in this series of experiments on a living object of interest, nevertheless, it is very important from the viewpoint of metrology to estimate the significance and possible influence of this biologically induced error on the final medical findings from the results of a diagnostic examination of a patient by the LFD method. In the classification of K_f values in terms of hypoxia proposed in the preceding section, the numerical values of K_f change from stage to stage by a factor of 2 or more; therefore, it is obvious that even an error of 30–40% in determining K_f will have no substantial significance. The fact of increased random error could itself apparently be the object of additional detailed study.

What can cause the increase of the random error of measurement in the case of living biological tissues? The new combined diagnostic data obtained using the OTO and LDF methods today make it possible to give a definite answer to this question. It is well known that pronounced rhythms in the microcirculation of blood for living biological tissues are objectively observed in classical LDF.[25] The authors have more than once observed the same rhythms by OTO methods.[15,17] Therefore, with single (instantaneous) measurements, for example, of the S_tO_2 parameter, carried out from time to time, the physician can observe the pattern imaged in Fig. 4a. He may interpret this result as random scatter in the diagnostic data.

However, if the measurements are made continuously in time, as shown in Fig. 4b, the S_tO_2 parameter, represented as a function of time, will be interpreted by the doctor as the appearance of rhythms of microhemodynamics in the blood-microcirculation system—i.e., as an absolutely correct and explicable result. Thus, if the living and changeable character of the object of diagnosis is not kept in mind, especially at the level of the blood-microcirculation system, it can be easily and falsely interpreted as random error of the method.

The same thing can probably be said of the LFD method. The blood in a microcirculatory vessel of biological tissue is a strong spectral absorber of light, especially in the blue and green regions of the visible spectrum, as well as a strong light-scattering component in the red region. Therefore, volume oscillations of the blood in the microvessels of biological tissue (V_b) and the different degree of its oxygenation (S_tO_2) can strongly affect the recorded signals in LFD. The more variable the perfusion of the tissues with blood, the larger the random scatter in the recorded LFD data that can be observed *in vivo* in a clinical experiment. The results in Table I clearly reflect this tendency.

It is also interesting to point out that these later data make it possible to take a new look at the authors' previous results, obtained long before this article was written, and to

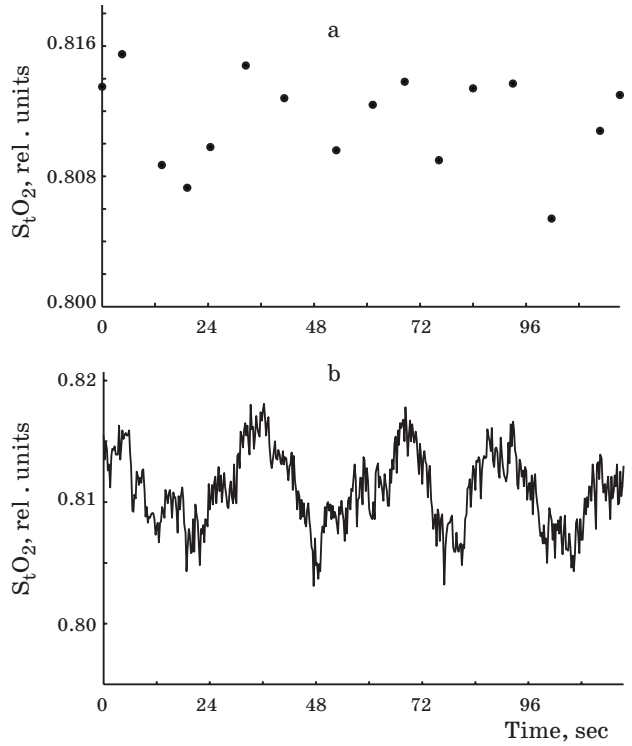

FIG. 4. Various forms of "random" scatter in the results of (a) single and (b) continuous-in-time results of measurements.

give a number of new explanations of the phenomena observed earlier, which formerly did not receive the necessary study. Thus, for example, it was reported 10 years ago that increased porphyrin AF was observed in a number of oncological patients from the intact tissues surrounding the tumors and even from other healthy tissues over the entire organism.[16] Stage III–IV tumors were being studied at that time, and the increased K_f values from intact (healthy) tissues could have been a consequence of the general intoxication of the patient's organism at the terminal stage of development of the cancer, caused by general ischemia and by hypoxia of the tissues over the patient's entire organism. The changeable character of the AF of the tumors observed in the course of radiotherapy[17] can reflect the development of destructive processes in the blood-microcirculation system under the action of strong ionizing radiation; this is well known in theoretical radiology (breakdown of the microvessels of the tumor, neoangiogenesis, etc.). The overall reduction of K_f by the end of a course of radiotherapy[18] could be evidence of the complete breakdown of the microvessels of the tumor, if one assumes the hypothesis that porphyrins penetrate into the tumor cells with the blood flow or, conversely, that the processes of microcirculation of the blood in the tumor undergo renewal and strengthening with an overall reduction of the level of tissue hypoxia. In any case, it is possible at present to note a clear correlation of the LFD data with the processes of microhemodynamics and the oxygen exchange in the tissues.

CONCLUSION

The data on the studies presented here show that today there is a complete basis for asserting that LFD results in any laboratory and clinical experiments are fairly reliable from a medical viewpoint and are easy to reproduce. Moreover, even though there is a formal "random" error of the single (instantaneous) measurements in LFD at the 20–40% level, the accuracy of the diagnostic result in light of the detected correlation of the LFD data with the state of chronic hypoxia in the tissues can be fairly high. The formal "random" scatter in the results of single measurements that is often observed in clinical experiments is largely determined not by instrumental, but by methodological error and by the living and changeable character of the object of diagnosis, especially at the level of the blood-microcirculation system. Accordingly, the more information the doctor has concerning various features of the blood-microcirculation process in the test volume of biological tissue, the more accurate and correct the interpretation of the observable LFD data he can provide.

It should be particularly emphasized that, in the clinical studies that have been carried out, no special goal has been set to study any biochemical or biophysical mechanisms for the accumulation of porphyrins in biological tissues at the molecular or cellular level. Only the diagnostic possibilities of LFD have been experimentally studied in the clinic to explain from a unified theoretical standpoint the observable experimental data from the viewpoint of the information content of the diagnosis and their medical interpretation. Nevertheless, the results obtained in this paper make it possible with a definite measure of reliability to speak today of the possibility of the biophysical mechanisms of the increased accumulation of porphyrins in tissues. They can be associated with the state of chronic hypoxia in the tissues and with the mobilization of porphyrins under these conditions from the flow of blood circulating in the tissues. Of course, this does not eliminate other mechanisms that affect the metabolism of porphyrins in the cells—for example, the mechanism associated with reducing the pH level in the cells and tissues, often discussed in the literature. However, it is fairly easy to show that the initial factor and the mechanism that triggers the change of the pH in the tissues and cells is also chronic tissue hypoxia.[27,28]

This work was carried out with the financial support of the Russian Fund for Fundamental Research, Grant No. 08-02-00769.

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[1]It should be especially emphasized that the authors specially developed and proposed the K_f diagnostic criterion for the LFD method in order to reduce the overall error of that method.[16]

[1]M. A. Mycek and B. W. Pogue, eds., *Handbook of Biomedical Fluorescence* (Marcel Dekker Inc., New York, 2003).
[2]D. M. Harris and J. Werkhaven, "Endogenous porphyrin fluorescence in tumors," Lasers Surg. Med. **7**, 472 (1987).
[3]H. Kato, K. Aizawa, J. Ono, C. Konaka, N. Kawate, K. Yoneyama, K. Kinoshita, K. Nishimiya, H. Sakai, and M. Noguchi, "Clinical measurement of tumor fluorescence using a new diagnostic system with hemato-

porphyrin derivative, laser photoradiation and spectroscopy," Lasers Surg. Med. **4**, 49 (1984).
[4]S. G. Demos, R. Gandour-Edwards, R. Ramsamooj, and R. de-Vere White, "Near-infrared autofluorescence imaging for detection of cancer," J. Biomed. Opt. **9**, 587 (2004).
[5]S. Yudenfrend, *Fluorescence Analysis in Biology and Medicine* (Mir, Moscow, 1965).
[6]A. Policard, "Etudes sur les aspects offerts par des tumeurs experimentales examinés a la lumière de Wood," C. R. Soc. Biol. **91**, 1423 (1924).
[7]F. N. Ghadially and W. J. P. Neish, "Porphyrin fluorescence of experimentally produced squamous cell carcinoma," Nature (London) **188**, 1124 (1960).
[8]D. A. Rogatkin, "Noninvasive laser fluorescence diagnosis in medicine," Lazern. Med. **4**, No. 1, 30 (2000).
[9]K. A. Askarov, B. D. Berezin, R. P. Evstigneeva, E. V. Bystritskaya, and N. S. Enikolopyan, *Porphyrins: Spectroscopy, Electrochemistry, Application* (Nauka, Moscow, 1987).
[10]A. Orenstein, G. Kostenich, C. Rothmann, I. Barshack, and Z. Malik, "Imaging of human skin lesions using multipixel Fourier transform spectroscopy," Lasers Surg. Med. **13**, No. 4, 112 (1998).
[11]A. E. Sorokatyĭ, D. M. Yagudaev, and M. V. Markova, "Photodynamic therapy in urology," Lazern. Med. **10**, No. 6, 58 (2006).
[12]M. M. H. El-Sharabasy, A. M. El-Wassel, M. M. Hafez, and S. A. Salim, "Porphyrin metabolism in some malignant diseases," Br. J. Cancer **65**, 409 (1992).
[13]A. A. Kubatiev, *Porphyrins, Vitamin B-12, and Cancer* (Priokskoe Izd., Tula, 1973).
[14]D. A. Rogatkin, "Basic principles of the organization of system software of multifunctional noninvasive spectrophotometric diagnostic devices and complexes," Med. Tekh **2**, 8 (2004).
[15]R. V. Gorenkov, V. N. Karpov, D. A. Rogatkin, and V. I. Shumskiĭ, "Chronic hypoxia as one of the factors of increased fluorescence of endogenous porphyrins in living biological tissues," Biofizika **52**, 711 (2007).
[16]D. A. Rogatkin, O. A. Prisnyakova, L. G. Moiseeva, and A. S. Cherkasov, "Analysis of the accuracy of laser clinical fluorescence diagnosis," Izmer. Tekh. **7**, 58 (1998).
[17]D. A. Rogatkin, P. Yu. Polyakov, O. A. Bychenkov, and E. A. Stepanenko, "Noninvasive fluorescent diagnostics in radiotherapy of mucosal oral tumors," Proc. SPIE **4707**, 236 (2002).
[18]V. V. Tchernyi, D. A. Rogatkin, O. A. Bychenkov, and P. Yu. Polyakov, "Some results of multiwave in situ autofluorescence diagnostics," Proc. SPIE **5693**, 336 (2005).
[19]G. I. Klebanov, D. A. Rogatkin, and S. G. Tereshchenko, "Measuring the surface fluorescence of endogenous porphyrins in the process of laser therapy of ulcers of the stomach and of the 12-ring intestine," Biofizika **49**, 941 (2004).
[20]P. N. Lyubchenko, R. V. Gorenkov, D. A. Rogatkin, M. L. Ginzburg, and V. N. Karpov, "Using laser methods of diagnosis for evaluating trophic damage in the distal sections of the tissues of the upper extremities in patients with vibration syndrome," Lazern. Med. **9**, No. 3, 38 (2005).
[21]V. V. Sidorov and D. A. Rogatkin, "Multifunctional laser noninvasive diagnostic system for medicine," in Proceedings of the Russian-Bavarian Conference on Biomedical Engineering, Moscow: MIET, 2008. P. 362–364.
[22]L. G. Lapaeva and D. A. Rogatkin, "Simple working standards for calibrating and checking devices of noninvasive spectrophotometry," in Materials of the Ninth International Conference on Measurement, Control, Information (IKI-2008), ed. O. I. Khomutov and L. I. Suchkova (AGTU im. I. I. Polzunova, Barnaul, 2008), pp. 145–149.
[23]C. Harting, P. Peschke, K. Borkenstein, and C. P. Karger, "Single-cell-based computer simulation of the oxygen-dependent tumour response to irradiation," Phys. Med. Biol. **52**, 4775 (2007).
[24]D. A. Rogatkin, "Concerning the features in determining the optical properties of turbid biological tissues and media in calculational problems of medical noninvasive spectrophotometry," Med. Tekh **2**, 10 (2007).
[25]A. I. Krupatkin and V. V. Sidorov, eds. *Laser Doppler Flowmetry of the Microcirculation of the Blood* (Meditsina, Moscow, 2005).
[26]M. V. Amzina, A. A. Micheev, D. A. Rogatkin, and V. V. Sidorov, "Combined medical diagnostic system with separated laser-Doppler and reflectance oximeter channels," Proc. SPIE **6163**, 616317 (2006).
[27]O. Soyemi, M. Shear, M. Landry, D. Anunciacion, and B. Soller, "In vivo, noninvasive measurement of muscle pH during exercise using near infrared spectroscopy," Proc. SPIE **6007**, 60070N (2005).
[28]F. A. Gallagher, M. I. Kettunen, S. E. Day, Hu De-En, J. H. Ardenkjær-Larsen, R. Zandt, P. R. Jensen, M. Karlsson, K. Golman, M. H. Lerche, and K. M. Brindle, "Magnetic resonance imaging of pH in vivo using hyperpolarized ^{13}C-labelled bicarbonate MR images reveal tumour pH," Nature (London) **453**, No. 7197, 940 (2008).