Some results of multiwave in situ autofluorescence diagnostics

Tchernyi V.V.[a], Rogatkin D.A.[b], Bychenkov O.A.[b], Polyakov P.Yu.[b]

[1)] Institute of General Physics of Russian Academy of Science,
Department of medical & ecological devices
Vavilova str. 36, Moscow, 117924, RF
Tel/Fax: 7-095-135-01-58, email: chernyv@bk.ru

[2)] Department of Radiology of Moscow Regional Research & Clinical
Institute "MONIKI", Shepkina str., 61/2, Moscow, 129110, RF
tel. 7-095-684-56-23, fax. 7-095-315-12-84, email: laserrog@mtu-net.ru

ABSTRACT

The laser “in vivo” autofluorescence diagnostics is now widely studied and applied in different areas of medicine, such as an oncology, dermatology, etc. Recently we have reported of created new professional multiwave laser diagnostic system (MLDS) for this purpose under the international scientific research and development project #1001 supported by the International Scientific and Technology Center. This presentation lights some results of application of the MLDS in a real clinical practice at Moscow Regional Research and Clinical Institute “MONIKI”, Department of Radiology. With the use of MLDS we investigated a skin and oral cavity cancer endogenous fluorescence before, during and after standard radiotherapy treatment. A statistical analysis showed that the best radiotherapy result was achieved for the patients with a small initial porfirines’ autofluorescence and a great initial flavines’ one from irradiated tumor tissues. It was shown that each radiotherapy procedure has an influence on a tissues’ autofluorescence intensity. The tendencies in porfirines’ fluorescence during a treatment course can be an additional prognostic factor for the prediction of the efficacy of a radiotherapy treatment. Moreover, it was estimated that a number of non-cancerous skin disease has a typical “cancer” initial autofluorescence, that makes it difficult to distinguish them one from another with the use of only the fluorescence diagnostics, but opens the way to investigate the non-cancerous tissues diseases with the help of tissues endogenous fluorescence phenomenon.

Keywords: laser, fluorescence, cancer, tumor, noninvasive diagnostics, radiotherapy.

1. INTRODUCTION

The laser “in vivo” autofluorescence diagnostics is now widely studied and applied in different areas of medicine, such as an oncology, dermatology, etc.[1-3]. Recently we have reported of a created new professional multiwave medical laser diagnostic system (MLDS) for this purpose under the international scientific research and development project #1001 supported by the International Scientific and Technology Center (ISTC)[4,5]. With the use of MLDS a different medical research was carried out at Moscow Regional Research and Clinical Institute “MONIKI”, Department of Radiology. Concerning the laser “in situ” fluorescent diagnostics in oncology we were interesting to find out a new or additional prognostic diagnostic criteria for a radiotherapy treatment on the basis of studying the cancer autofluorescence phenomenon before, during and after the radiotherapy treatment course.

It is widely known today that any cancerous soft tissue has some specific and strong autofluorescence spectra that can be an additional diagnostic parameter for a doctor[6]. But not only the cancer existence or absence in a tissue is interesting in means of the use of laser fluorescence diagnostics at clinics. When the cancer already presents a noninvasive fluorescence technique can be applied as an additional tool for monitoring a dynamics of processes in a tissue, especially in the case of radiotherapy treatment. First of all, it is very interesting on a recently lighted way of probability of existence of correlation between concentration of porphirines molecule in a tumor and a total chronic hypoxia state into that[7], because one of the main and well-known reasons of a low efficacy of a radiotherapy treatment is the hypoxia

of the cancerous cells. Additionally, it must be interesting a dynamics of fluorescent spectrum from cancerous tissues during the irradiation course to find out any reasons for the spectrum changing[6]. And, in third, the initial different fluorescence intensity corresponding to different endogenous fluorochromes concentration into a cancerous tissue probably could correlate with the proliferative cells activity, the examination of which is today a main but very expensive prognostic factor for the radiotherapy treatment. So, the studying of endogenous fluorescence of normal and cancerous tissues in means of application it to radiotherapy is very perspective way for the real radiology practice.

2. MAIN EXPERIMENTAL SETUP AND TECHNIQUE

In our research we used a new multiwave medical laser diagnostic system (MLDS) as the main instrument for the fluorescence registration “in situ”. A general view of MLDS is presented in Fig.1.

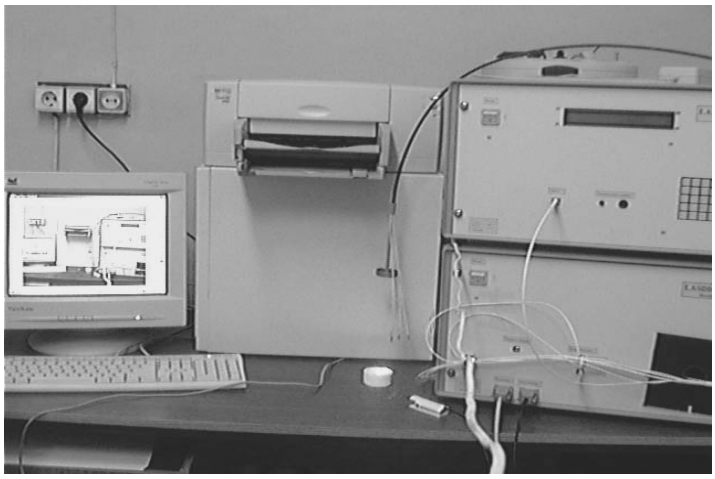


Fig.1. New medical laser noninvasive multiwave diagnostic system (MLDS).

It consists[5] of a laser source unit (upper unit on Fig.1), unit of the polychromator and a photodetector’s array, electronic unit, optical fiber as a handle probe for a doctor and a personal computer as a main control and data processing unit. With the use of MLDS a diagnostic procedure for a doctor consists of a location of a fiber on a tested tissue surface in a slight contact with one. Then the tissue is automatically illuminated with the different laser light and the fluorescence signal in a different spectral range is automatically registered. It takes not more than 5-10 sec to register full spectrum fluorescence data. Fig.2 shows such procedure.

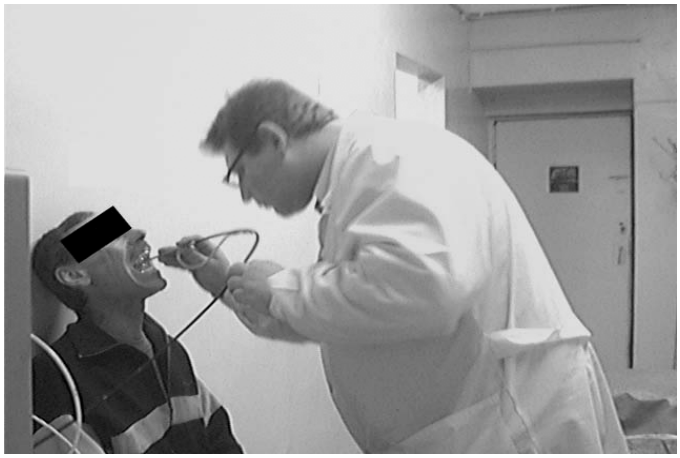


Fig.2. Typical diagnostic procedure with the use of MLDS optical fiber probe.

To excite and research an autofluorescence into biotissue the different lasers and spectral ranges is used in MLDS – 377 nm, 405 nm, 532 nm, 632 nm and 670 nm. All lasers are work alternately in time during 1-2 sec, so the full spectral autofluorescence data can be alternately and fast collected from the tested tissue's surface point. The depth of effective light penetration the typical soft tissue is around 1-10 mm for different waveband, so the tested tumor volume under the tissue's surface can be around 1-8 mm^3. The typical spectra of an oral mucosa tumor autofluorescence registered by MLDS in a case of 632 nm excitation wavelength is presented in Fig.3.

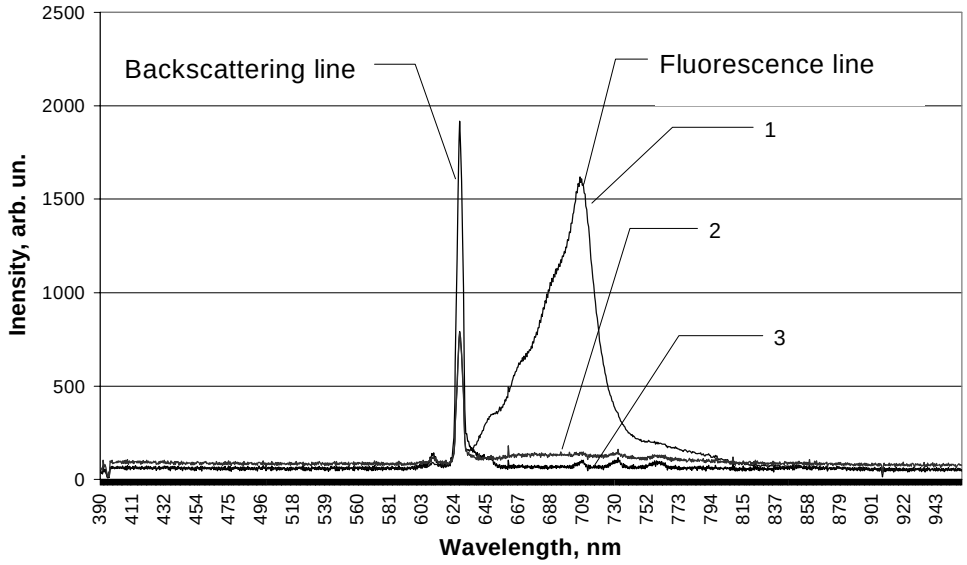


Fig.3. Typical registered by MLDS spectra of an oral mucosa tumor autofluorescence (excitation line – 632 nm). 1- visible center of mucosa tumor; 2 – surrounding intact mucosa; 3 - standard (non-alive) scatter.

At the step of diagnostic data processing in our investigation we have used our standard methodology of the fluorescence contrast coefficients[3,7,8], when the coefficient K_f

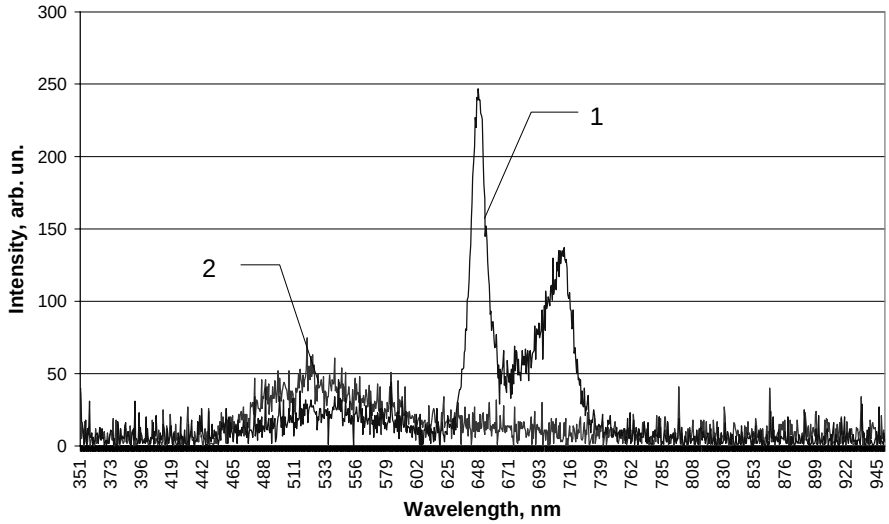
$$K_f = 1 + (I_f \cdot \beta - I_l)/(I_f \cdot \beta + I_l), \quad (1)$$

where: K_f - fluorescence contrast coefficient ($0<K_f<2$); I_f – registered radiation intensity in the maximum of fluorescence line (Fig.3); I_l - intensity of registered backscattered laser radiation in the excitation wavelength line (Fig.3); β - device's reduction coefficient ($\beta \approx 1000$ for 632 nm excitation line), is calculated for each excitation wavelength and each fluorescence waveband. As it was recently shown[8] K_f is directly proportional to the average fluorophores content into a tested tissues volume and its effectiveness of fluorescence emission. All calculation in MLDS runs automatically just after the each diagnostic procedure ends.

There were around 150 oncologic and another patients and around 50 healthy volunteers under our medical noninvasive multiwave laser diagnostic examination. For each healthy volunteer the normal autofluorescence data were collected from the different skin areas and oral mucosa areas. For each oncologic patient the data were collected from the center of the visible area of the presented cancer and from the normal (intact) soft tissue next to the tumor area. For the oncologic patients, for which a radiotherapy treatment was applied, all diagnostic data were collected before (initial data) and several times during the radiotherapy course. It must be special mentioned that in our real clinical practice not all patients, which had started with us our investigation, could be under our observation up to the end of the necessary medical care. For example, some of them have aborted theirs radiotherapy treatment on different stages to have a surgery operative intervention. So, not all of our experimental data per each of our patients contain a full data set obtained from the beginning to the end of the planned radiotherapy treatment. Nevertheless, the collected data allows us to state some interesting results and to do some intermediate conclusions.

3. SOME CLINICAL RESULTS

First of all, among the total obtained clinical data, we'd like to mark a number of more interesting results in means of questions formulated in Section 1 about diagnostic effectiveness of autofluorescent monitoring in radiology. We already had recently reported[6] that each radiotherapy irradiation procedure has a visible influence on a registered tissue's autofluorescence spectra. But in our previous investigation we had observed that in a red waveband only. In present research we used the different spectral ranges. Nevertheless, such phenomena in a great manner were observed for a fluorescence of endogenous porphirines (red region of spectrum) in a cancer as well as it was previously. For fluorescence of flavine-involving molecules (region of 480-600 nm) or NADH (region of 380-450 nm) especially for normal surrounding tissues it was observed more seldom. For example, Fig.4 shows a fluorescence spectra before (Fig.4a) and after (Fig.4b) ionizing distance-photon irradiation (IDPI) when in both regions of spectra we have saw a reduction of tissue's autofluorescence intensity from a cancer area, but not from a normal (surrounding) tissues.



a)

b)

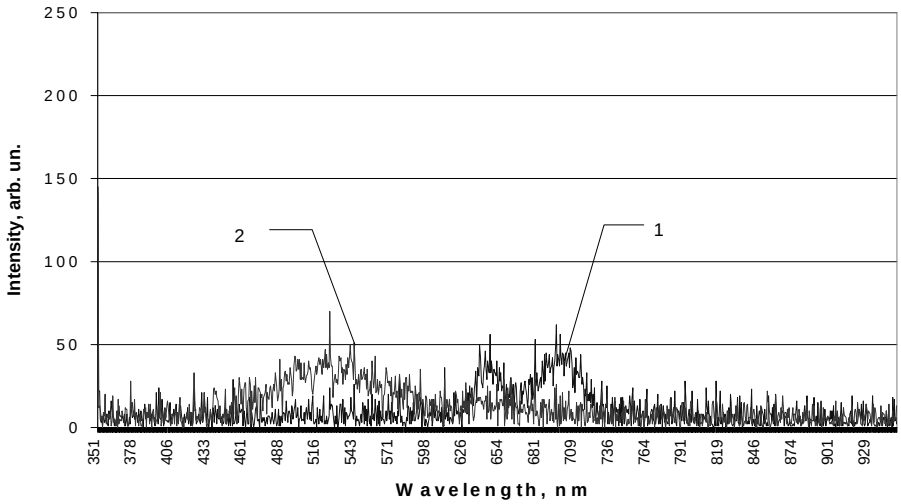
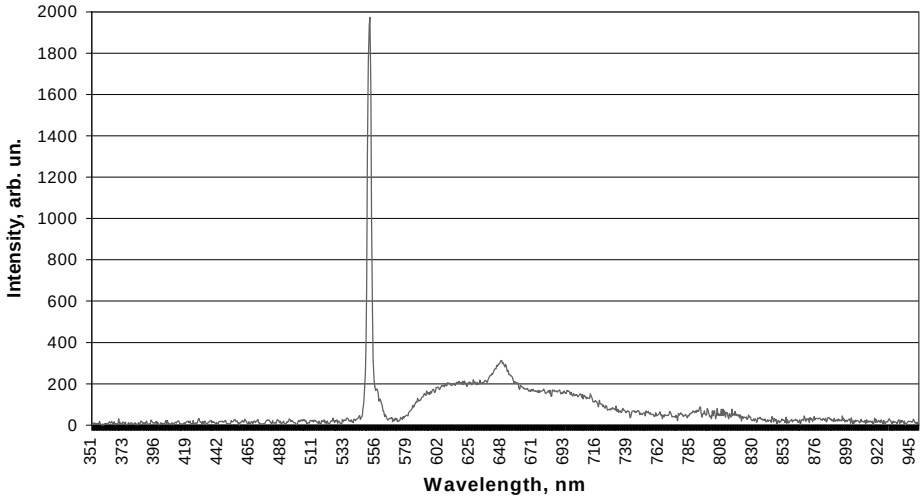


Fig.4. Spectra before (a) and after (b) treatment irradiation. Excitation wavelength - 405 nm. The backscattered line are not presented. 1 – Center of visible oral mucosa tumor and porphirines fluorescence lines. 2 – Surrounding normal tissue and flavines fluorescence line.

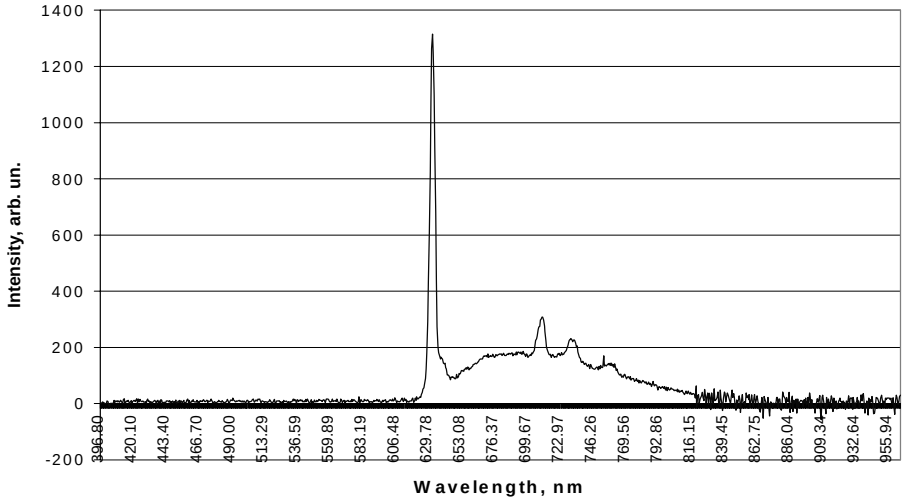
It is well visible that after IDPI all fluorescence lines (both porphirines' and flavines' ones) for the cancer area became more less, than they were before IDPI. And vice versa: for a normal tissue the flavines' fluorescence doesn't become

more less after IDPI (IPDP, of course, was applied on a cancer area only). Today we still can't explain such phenomenon (increasing or decreasing of amplitudes of autofluorescent signal right after the IDPI) but can observe it.

The same we can say about the differences of observed spectra in different spectral wavebands with the use of different excitation lasers. For example, as it follows from the theory of biotissue endogenous fluorescence, in a green excitation region there is not any noticeable absorption of effective tissues' fluorophores. But in our investigations with the use of 532 nm laser light (power 5 mW) the strong tissue autofluorescence was observed in parallel to the red fluorescence of porfirines under the 632 nm or 405 nm excitation illumination. Fig. 5 shows both the "green" and the "red" fluorescence from the center of the same oral mucosa tumor presented in Fig.4a.



a)



b)

Fig.5. The "green" and the "red" fluorescence of the same oral tumor and in the same time like presented in Fig.4a. a) Excitation line - 532 nm, b) Excitation line - 632 nm.

Total dynamics of fluorescence intensity during radiotherapy treatment we have studied in a red region of spectra under the 632 nm excitation light. Namely this region is very interesting to understand a hypoxia influence on a porphyrin content into a tissue[7,8] as well as to study a prognostic efficacy of the laser fluorescent diagnostics (LFD)[6,8]. In "MONIKI" a standard radiotherapy course consists of two stages of ionizing irradiation (the so-called "Dynamic Multifractioning Schedule (DMS) of the radiation doze"). From the diagnostic point of view a very important step in DMS is the beginning of the second stage of irradiation. On this step a doctor must formulate a final decision of the efficacy of applied therapy – to have to continue it or not? (On this step the total doze of ionizing irradiation is still not

more than a conventional the so-called “pre-operative” doze often used in oncology before the surgery intervention). So, the seeking a powerful and convenient diagnostic tool to make such final decision is a very perspective and interesting scientific task. In our previous research[9] it was shown that the reduction of the fraction of proliferating cells in tumor by the beginning of the second stage of irradiation up to 80% and more as compared with the initial value of that is the good prognostic factor to continue a radiotherapy. We had estimated that around 70±5% of patients usually have good chances to continue a treatment course. But in our mentioned research all diagnostic data were obtained with the use of well-known autoradiography (ARG) method, which is very expensive and long in a real clinical practice. The laser fluorescence diagnostics (LFD) being a noninvasive and real time method would be more simple procedure if could give a similar result. In spite of there are not today any evident facts that LFD can detect the cells proliferation we had tested the possibilities of LFD to reflect the cells processes during the radiotherapy irradiation. For this purpose the fluorescent data for a group of our patients (100 men) were collected before start and during an ionizing radiation treatment. We observed and studied a reduction of porphirines fluorescence in comparison with the initial (before treatment) fluorescence data. Some our intermediate and such collected results are presented in Fig.6 and Table 1.

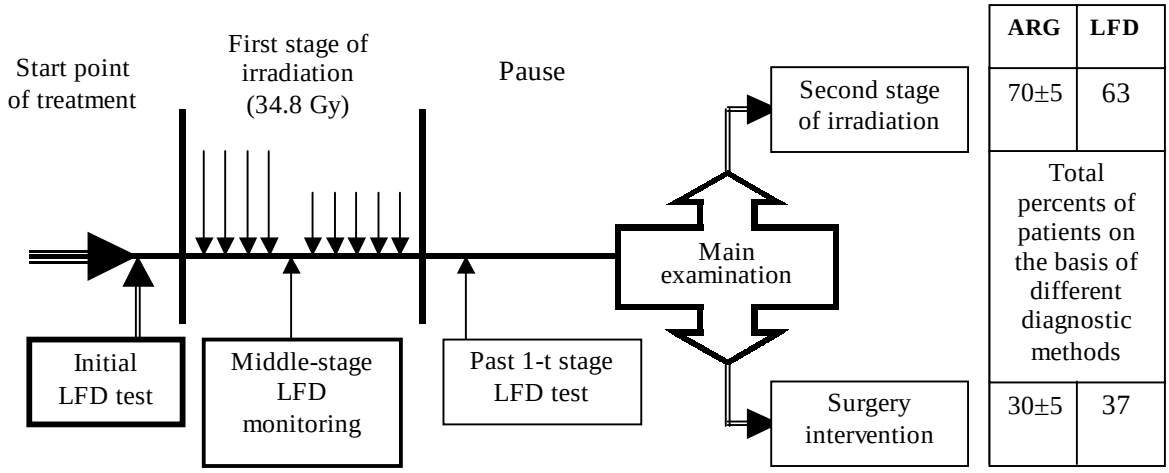


Fig.6. The schedule of investigation and radiotherapy treatment course and some results.

Table 1. K_f in red region of spectra (fluorescence of porphirines) for different pathologies and different stages of treatment. Excitation line - 632 nm.

Groups of investigation	Number of cases	Intact tissue	Process before treatment	Middle of the 1-t stage of irradiation	The end of the 1-t stage	Beginning of the 2-d stage
Normal skin	150	0,11±0,06	-	-	-	-
Normal oral mucosa	50	0,09±0,05	-	-	-	-
Metatypical, squamous cell, non-differentiated cancers of oral cavity	47	0,15±0,08	0,63±0,32	0,62±0,31	0,36±0,25	0,30±0,14
Metatypical, squamous cell, non-differentiated cancers of tongue	38	0,18±0,11	0,68±0,39	0,60±0,40	0,32±0,27	0,27±0,18
Skin basaliomas	34	0,11±0,08	0,54±0,26	0,49±0,22	0,28±0,16	0,15±0,09
Skin psoriasis scales	8	0,13±0,09	0,37±0,14	-	-	-
Skin pappilomas	6	0,09±0,06	0,10±0,07	-	-	-
Ceratoma of skin	5	0,12±0,09	1,03±0,47	-	-	-
Cylindroma	1	0,14	0,13	-	-	-

Some preliminary results of comparing a total efficiency of radiotherapy with the initial fluorescence in red and blue regions of spectra (initial tumor autofluorescence associated with porphirines and flavines) for a group of 20 patients are presented in Table 2.

Table 2. Initial autofluorescence in tumor tissues and a total efficiency of radiotherapy.

	Number of cases	Initial coefficients of fluorescent contrast (K_f)			
		Flavines, intact tissues	Porphirines, intact tissues	Flavines, tumor	Porphirines, tumor
Normal tissues	20	0,52±0,23	0,10±0,06	-	-
Positive dynamics of radiotherapy treatment	14	0,50±0,21	0,16±0,09	0,43±0,20	0,25±0,13
Absence of positive dynamics	6	0,51±0,25	0,18±0,1	0,12±0,09	0,78±0,35

4. DISCUSSION AND CONCLUSION

As we can see, all obtained results show the high potential of LFD in application to oncology and radiology. A multiwave LFD allows a doctor to study and display different cells processes and the dynamics of that into a tumor tissue in spite of not all of them are well understudied yet. First of all, it concerns a dynamics of fluorescent spectra after each irradiation procedure (see Fig.4). Moreover, it was estimated that a number of non-cancerous skin disease has a typical “cancer” initial autofluorescence (see Table 1) that makes it difficult to distinguish them one from another with the use of only the fluorescence diagnostics. But it opens the way to investigate the non-cancerous tissues’ diseases with the help of tissues’ endogenous fluorescence phenomenon. For example, Fig.7 shows the autofluorescence of foot skin and nail for one of our patients who had II-stage diabetes as an accompanying decease.

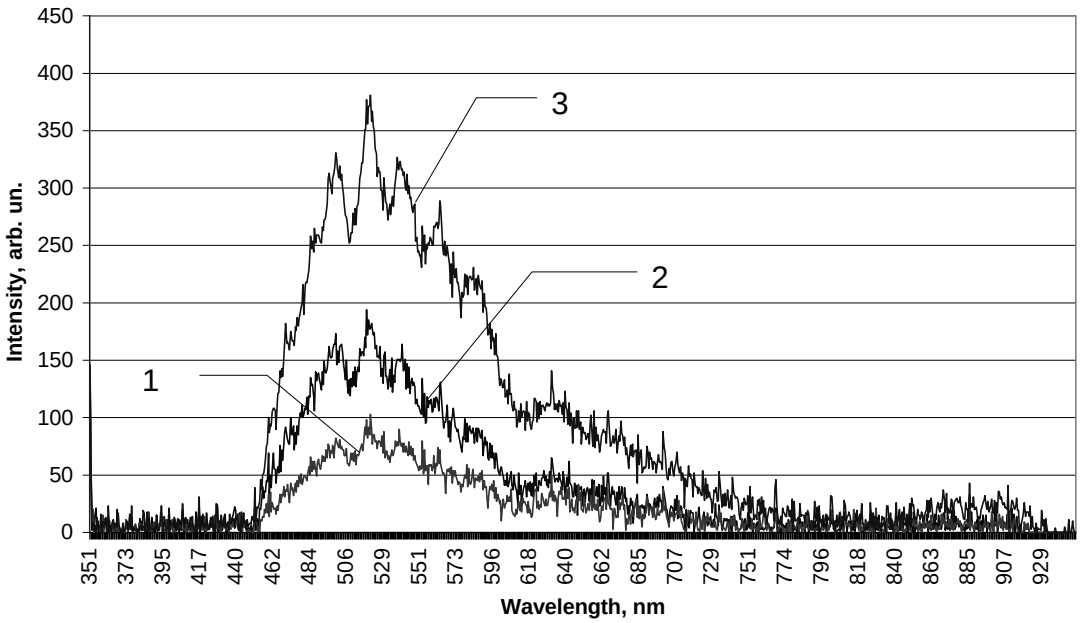


Fig.7. The fluorescence of skin and nail of a “diabetes foot”. Excitation wavelength - 405 nm. Backscattering line is not shown. 1 – normal skin; 2 – skin of a “diabetes foot”; 3 – nail of a “diabetes foot”.

We can't today answer in detail why, in instance, the diabetes nail has so strong fluorescence in a region of a flavines activity. But it is already clear from Fig.7 that for diabetes patients, who has a blood microcirculation disorders on theirs hands and/or legs, the LFD would detect any initial stages of such disorders. And the looking for any biophysical reasons for abnormal tissues accumulation of different fluorophores for different disorders could throw light on the specialties of them pathogenesis.

The same difficulties appear when analysis of curves of the tumor fluorescence spectra in cases of different excitation wavelength is done (see Fig.4a, 5a, 5b). Everyone can estimate the similarity of viewed spectra of porphirines' fluorescence, but not a full equality of them. What hides in differences?

As to prognostic efficiency of the multiwave LFD in radiology, we obtained, assuredly, only preliminary but very perspective two results. First of all, our results to prediction an efficacy of a second stage of irradiation courses lie close to the ARG results, so we concluded a good correlation between them. In our opinion a dynamics of autofluorescence intensity during a radiotherapy treatment can be an additional diagnostic parameter for a doctor to monitoring an individual action (result) of ionizing irradiation, even every step of irradiation. But, unfortunately, today we can talk about it in means of statistical data only, not like about a personal (individual) result for every patient and for every treatment procedure. For every concreted patient a reduction (or increasing) of fluorescent tissues' activity by the beginning of the second stage of therapy could be not so evident and predictable. All tendencies have appeared after statistical analysis of all collected data only. Probably, it says of not enough good hardware and methodological approaches to our patient examination with the use of our MLDS. Otherwise, a statistical analysis assuredly showed (see Table 2) that the best radiotherapy result could be predicted for patients with a small initial autofluorescence of endogenous porphirines and a great initial autofluorescence of endogenous flavines from the malignant tissues.

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