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PACS 87.63.lt

INFLUENCE OF CONTINUOUS AND PULSED LASER RADIATION ON OPTICAL AND THERMAL PROPERTIES OF BIOLOGICAL TISSUES

An experimental installation is created to research the features in the optical and thermophysical properties of biological tissues under the action of continuous and pulsed laser radiation. The results obtained form a basis for the development of a complex technique aimed at estimating the influence of laser radiation and calculating the laser radiation dose obtained in the course of a laser therapy session. The data obtained may be useful to identify the pathologies of tissues and to accurately determine their boundaries already at the early stages of their evolution, which is important for the proper diagnostics and in surgery.

Keywords: laser diagnostics, femtosecond laser, fluorescent diagnostics, optical properties of biological tissues.

1. Introduction

Nowadays, a wide arsenal of lasers and laser systems has already been implemented in various domains of medical science and practice. Lasers are most used in surgery, urology, and ophthalmology, where the destructive effect of highly intense radiation is mainly applied at bloodless operations. Lasers are successfully applied to blood irradiation therapy and diagnostics of internal diseases. The basic physical effects that accompany the influence of laser radiation on biological tissues include fluorescence, backscattering, direct reflection, transmission, absorption, as well as acoustic waves, ablation, and thermal coagulation [1]. Different tissues are characterized by specific properties, both optical (spectral characteristics, the reflectance, the radiation penetration depth) and thermal (the thermal and temperature conductivities, the heat capacity). As a result, the results of a laser radiation action on them are also different. Hence, the irradiation mode and the relevant specific parameters – the wavelength, capacity, procedure duration, and radiation dose – should be selected separately to treat every type of biological tissues. Therefore, the necessity of researches concerning the optical properties of biological tissues is undoubted and, in turn, stimulates the development of new laser-based methods [2–4].

It is important to note that the existing methods of laser therapy are based on the integrated optical characteristics of biological tissues. They do not consider the local angular distributions of laser radiation transmitted through a tissue, which consider-

Fig. 1. Femtosecond complex. General view

ably restricts the calculation accuracy of radiation doses and, as a consequence, the efficiency of laser therapy. Therefore, this work aimed at studying the influence of laser irradiation in various modes – continuous and ultrashort (femtosecond) pulsed – on biological tissues in order to apply the obtained results in laser therapy and diagnostics.

2. Experimental Part

Experimental researches were carried out on the equipment of the Laser medical and biological center of the "Biophysics–Ukraine" Ltd. Company at the National Cancer Institute of the Ministry of Public Health of Ukraine (Fig. 1). The operating characteristics of the femtosecond complex were as follows.

The performance data of a laser Mira 900-F:

- pump source: radiation of a laser Verdi V-10;
- average output power: up to 1.5 W
- peak output power: up to 100 kW;
- radiation wavelength (tuning range): 700–900 nm;
- pulse duration: no more than 200 fs;
- pulse repetition rate: 76 MHz;
- instability of output power: less than 3%;
- wavelength instability: less than 2 nm;
- beam diameter: 0.8 mm;
- beam divergence: 1.7 mrad;
- beam polarization: horizontal;
- noise: no more than 2%;
- power consumption: less than 2 kW.

The performance data of a harmonic generator Model 0-050:

- wavelength of the first harmonic: 700–900 nm;
- polarization: horizontal;
- wavelength of the second harmonic: 350–450 nm;
- wavelength of the third harmonic:: 233–333 nm.

The output parameters of the laser complex – the power P and the wavelength λ – were controlled in the automatic regime. For power measurements, a Field Master$^{\text{TM}}$ GS energy/power analyzer was used, the semiconductor and thermal sensors of which allowed us to carry out measurements in wide ranges of wavelengths (from 0.19 to 10.6 μm), continuous radiation power (from 1 nW to 5 kW), and pulse energy (from 1 μJ to 20 J) [5]. Spectral characteristics were registered on a spectrometer HR2000+, which permitted measurements in a range from 200 to 1100 nm with a high resolution of 0.035 nm to be done [6]. In the wavelength interval 350–450 nm, the average output working power was equal to 200 mW, and the peak one to 17.5 kW; in the interval 700–900 nm, the corresponding values equaled 400 mW and 35 kW, respectively. To study the spectral characteristics of biological tissues (the transmission and reflection coefficients; the fluorescence, scattering, and transmission spectra) and their thermophysical parameters (the temperature distribution over the tissue depth and its behavior in time), an installation was created, the schematic diagram of which is exhibited in Fig. 2.

Healthy and pathological tissues of human stomach and gullet were studied; 25 samples 10×10 mm^2 in dimensions in each group were used in researches. All the properties of biological tissues indicated above were analyzed at various wavelengths of laser radiation (375–450 and 750–900 nm) in the continuous and pulsed (femtosecond) regimes. The installation was capable of registering the scattering and reflection indicatrices. Before every measurement, the tissue samples were cooled down to room temperature (20 °C) in a physiological solution. The radiation scattered by the samples was registered by spectrometer 8. The temperature was measured using a digital multimeter DT 838 equipped with a thermocouple TP-01 (chromel-alumel, Ni–Cr vs Ni–Al). The measurement accuracy was 3%. The registered data were treated statistically with the use of the Excel 7.0 and Statistica 5.0 softwares [7–9], and their experimental statistical error was found to equal 5%. Each measurement was made three times.

3. Results and Their Discussion

3.1. Features of laser radiation transmission through biological tissues in the continuous and pulsed regimes

The researches were carried out on an experimental installation shown in Fig. 2. Laser radiation

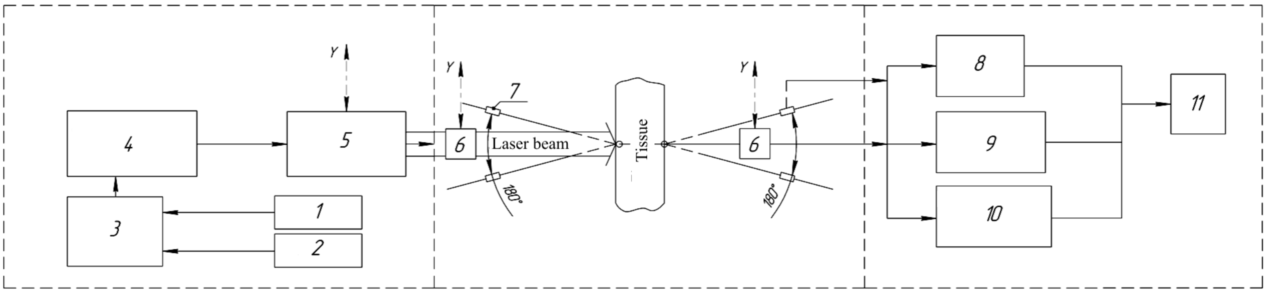


Fig. 2. Schematic diagram of experimental installation. Femtosecond complex: (*1*) power unit, (*2*) cooling block, (*3*) pumping laser Verdi V-10, (*4*) femtosecond laser Mira Optima 900-F, (*5*) harmonic generator Model 0-050. Measurement unit: (*6*) polarizer, (*7*) thermal and optical sensors. Registration unit: (*8*) spectrometer HR2000+, (*9*) power analyzer Field Master GS, (*10*) digital temperature meter (a multimeter DT 838 and a thermocouple TP-01), (*11*) personal computer

($\lambda = 800$ nm and $P = 400$ mW) passed through a sample and, after scattering, was registered in various directions using a movable power sensor located at a distance of 50 mm from the sample. This setup allowed us to determine the angular dependences of the transmission coefficient presented in Fig. 3.

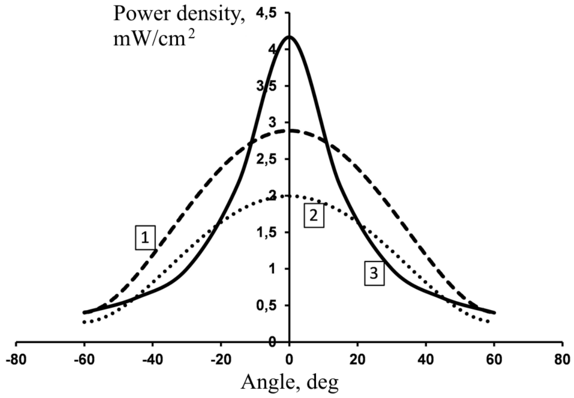
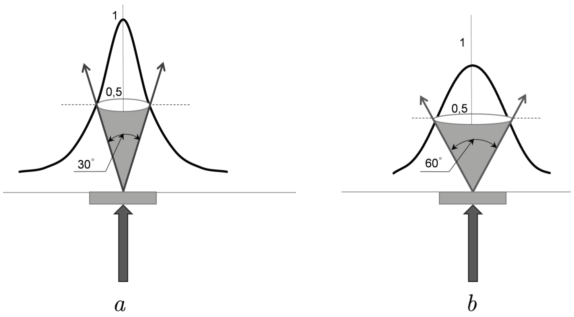


Fig. 3. Indicatrices of the coefficient of laser radiation transmission through biological tissue samples: (*1*) femtosecond regime, healthy tissue; (*2*) femtosecond regime, pathological tissue; (*3*) continuous regime, healthy tissue



a *b*

Fig. 4. Schematic illustration of laser radiation transmission through a healthy biological tissue in the (*a*) continuous and (*b*) femtosecond regimes

Figure 4 schematically illustrates the features in the angular distribution of the laser radiation transmittance for a healthy tissue sample in various radiation regimes. Namely, let us distinguish those directions, in which the intensity of scattered radiation exceeds half the transmitted intensity maximum. Then those directions formed a cone with an opening angle of 30° in the continuous regime and 60° in the femtosecond one. The total intensity scattered in those cones amounted to 48 mW in the continuous regime and 67 mW in the femtosecond one, i.e. 12 and 17%, respectively, of the incident radiation intensity.

3.2. Laser radiation backscattering by biological tissues in various regimes

The corresponding researches were carried out on an experimental installation shown in Fig. 2. Laser radiation was scattered by the samples of biological tissues in various regimes (continuous and pulsed) and registered with the use of a power meter sensor located in front of the sample at a distance of 50 mm from it. The backscattering indicatrices for the samples of healthy and pathological biological tissues are exhibited in Fig. 5.

The analysis of Figs. 3 to 5 allows the following conclusions concerning the features of the laser radiation transmission through and backscattering by healthy and pathological tissues of various types to be drawn:

– samples of healthy tissues absorb weaker than samples of pathological ones;

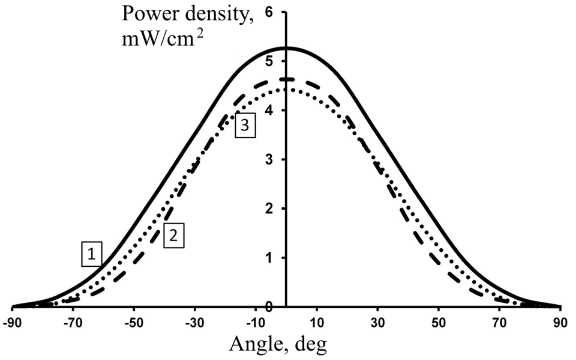
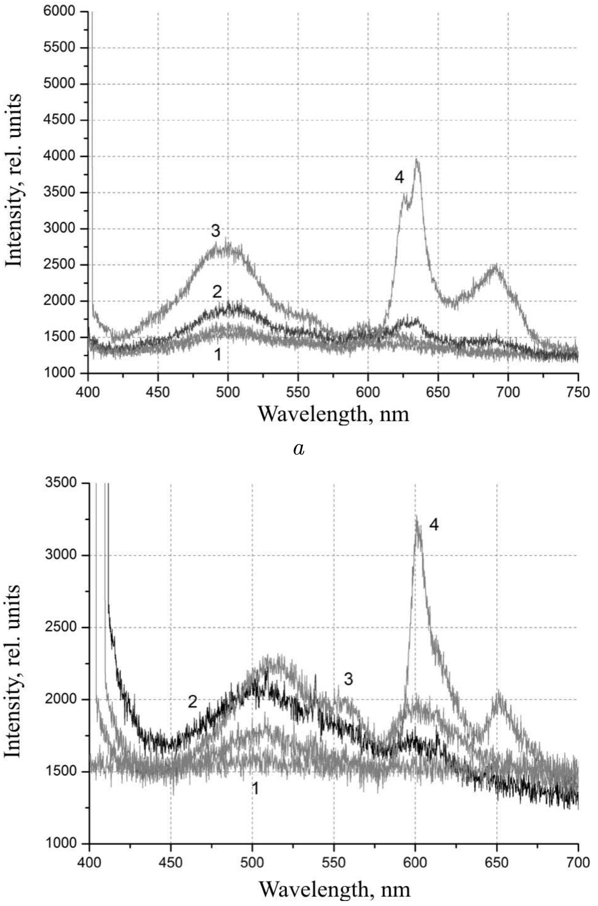


Fig. 5. Indicatrices of laser radiation backscattering: (*1*) femtosecond regime, healthy tissue; (*2*) femtosecond regime, pathological tissue; (*3*) continuous regime, healthy tissue



a

b

Fig. 6. Fluorescence spectra of biological tissue samples of (*a*) human gullet and (*b*) human stomach excited by femtosecond radiation: (*1*) healthy tissues, (*2*) in the tumor bulk, (*3*) near the tumor boundary, (*4*) at the tumor boundary

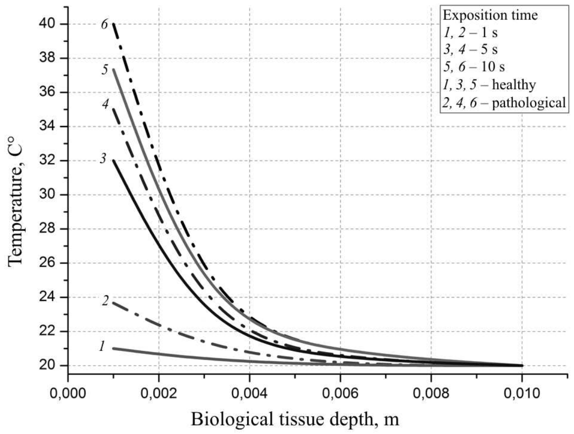


Fig. 7. Temperature distributions along the transverse cross-section of healthy (*1, 3, 5*) and pathological (*2, 4, 6*) human gullet tissue samples at various times of their exposition to femtosecond laser radiation

– femtosecond radiation is absorbed weaker than continuous one.

Those conclusions were also confirmed by the results of temperature researches of biological tissues presented in Section 3.4. It should be noted that the indicated features were observed for the majority of kinds of soft biological tissues.

3.3. Researches of fluorescence spectra of biological tissues

Fluorescence spectra of healthy and pathological biological tissues (human gullet and stomach) at their excitation with laser radiation in the continuous and pulsed (femtosecond) regimes were registered on an experimental installation shown in Fig. 2. Fluorescence signals were registered at a distance of 10 mm from the tissue samples at various angles with respect to the horizontal axis. The most informative turned out the fluorescence spectra measured in the angular interval from -15 to $+15°$. The analysis showed that, when the samples were subjected to the action of laser radiation in the wavelength interval 760–840 nm, the spectra of scattered and transmitted radiation coincided with the spectra of incident radiation. When the laser radiation wavelength was in the interval 380–420 nm, no transmitted signal was registered behind the samples, and the spectral structure of scattered radiation had a number of peculiarities.

In Fig. 6, the fluorescence spectra of pathological and healthy tissues of human gullet and stomach excited by laser radiation with $\lambda = 400$ nm are depicted. One can easily see that the fluorescence signal registered from a sample of the tumor boundary under the influence of pulsed radiation has the largest intensity in comparison with other spectra. From the medical viewpoint, this fact is quite reasonable, because it is at the tumor boundary that essential biochemical variations take place. The processes in the tumor bulk can be considered as already finished, and, as a result, the fluorescence signal from the tumor bulk is much weaker than from its boundary. Hence, the fluorescence spectra obtained while exciting the biological tissue samples with pulsed femtosecond radiation can be considered as a sound factor for the identification and the determination of pathology boundaries in biological tissues.

3.4. Influence of pulsed laser radiation on the thermal characteristics of biological tissues

The temperature distribution in the samples of healthy and pathological gullet tissues was measured on an experimental installation shown in Fig. 2. For irradiation, laser pulses of femtosecond duration with the wavelength $\lambda = 800$ nm and the power $P = 400$ mW were used. The exposition time intervals were 1, 5, and 10 s. The temperature in the biological tissue samples subjected to irradiation was measured at depths of 1, 3, 6, and 10 mm with the help of a thermocouple, which was moved along the transverse cross-section of the sample.

As one can see from Fig. 7, the temperature in the healthy gullet tissues subjected to femtosecond irradiation for 1 s appreciably changed only in a layer 3 mm in thickness. If the irradiation lasted 10 s, the temperature reached 37 °C at a depth of 1 mm and smoothly fell down to a depth of 8 mm following the exponential law. At the same time, the temperature in the pathological gullet tissues exposed to femtosecond irradiation for 1 s appreciably changed only in a layer 6 mm in thickness, whereas for an exposition of 10 s, the temperature reached 40 °C at a depth of 1 mm and smoothly fell down exponentially to a depth of 9 mm. From Fig. 7, it follows that the temperature in the pathological gullet samples at a depth of 1 mm was higher than in the healthy ones by about 3 °C at various exposition times, but did not differ substantially at a depth of 6–10 mm. Hence, in the pathological tissue, its temperature raises considerably even at the first second of interaction with laser radiation. This fact originates from biochemical variations in the pathological tissue giving rise to a distortion of its morphological and histological parameters, so that it becomes capable of heat absorption and retention. Hence, in order to identify healthy or pathological biological tissues and to calculate the radiation doses at laser therapy, the determination of the temperature at depths not smaller than 1–3 mm is optimal.

4. Conclusions

While studying the optical characteristics of biological tissue samples (human stomach and gullet), the following results were obtained.

– Fluorescence spectra demonstrate reproducible peaks. A distinct difference between the fluorescence spectra of pathological and healthy biological tissues was observed. In particular, the spectral intensity for pathological tissue is higher, and it grows by a factor of 3 with respect to the intensity for healthy tissue when moving from the tumor bulk toward its boundary. Laser irradiation of the tissue region between the healthy and pathological sections in the short-wave interval 380–400 nm gives rise to the appearance of additional peaks in the long-wave region (600–700 nm) of the fluorescence spectrum.

– The intensity of radiation scattered by healthy biological tissues is 20–30% higher in comparison with pathological ones, since the pathological tissue has a higher absorption factor. As a result, pathological tissue becomes more heated during its exposition to laser radiation.

– There exist the angular regularities in the propagation of continuous and femtosecond laser radiation through biological tissue, which are different for different tissue types. Namely, the transmitted radiation with the intensity higher than or equal to half the maximum value is propagated within a cone with an opening angle of 30° for the continuous regime and 60° for the femtosecond one.

Our researches of the thermal characteristics of biological tissues (human gullet and stomach) showed that pathological tissues are able to accumulate a larger amount of heat owing to the variations in their morphological and histological parameters. Hence, the thermal properties can be used to diagnose tissue pathology. Thus, the complex approach, which is based on studying the fluorescence spectra and the intensities of scattered radiation and radiation transmitted through biological tissues, makes it possible to distinguish pathological tissues from healthy ones and determine the boundaries of pathological neoplasms, which is essentially important, when a surgical intervention is required. In turn, the analysis of the angular distribution of the laser radiation propagation in biological tissues allows the power density to be determined for various tissues, which is important for the calculation of a radiation dose at laser therapy.

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Received 26.02.14.
Translated from Ukrainian by O.I. Voitenko

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ОСОБЛИВОСТІ ВПЛИВУ НЕПЕРЕРВНОГО ТА ІМПУЛЬСНОГО ЛАЗЕРНОГО ВИПРОМІНЮВАННЯ НА ОПТИЧНІ ТА ТЕПЛОФІЗИЧНІ ХАРАКТЕРИСТИКИ БІОЛОГІЧНИХ ТКАНИН

Резюме

Створено експериментальну установку для дослідження особливостей оптичних та теплофізичних характеристик біологічних тканин під дією неперервного та імпульсного (фемтосекундного) лазерного випромінювання. Отримані результати є передумовою для створення комплексного підходу, що дасть змогу оцінити вплив та розрахувати дози лазерного опромінення при проведенні сеансів лазерної терапії, а також дозволить виявляти патології тканин та визначати їх чіткі межі вже на ранніх стадіях розвитку, що є вкрай необхідним як в діагностичних цілях, так і при необхідності хірургічного втручання.