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PHYSICAL INSTRUMENTS FOR ECOLOGY, MEDICINE, AND BIOLOGY

An Apparatus for Studying the Laser Radiation Effects on Biotissue

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Abstract—An apparatus for studying the processes of laser action on various materials, including biological tissue, is described. The system makes it possible to obtain thermograms of the sample surface, conduct high-speed video recording, and record acoustic signals in a wide frequency range during experiments. The system was tested using a pulsed nanosecond high-frequency laser source with a wavelength of $3.03 \,\mu$ m. The samples were exposed to pulses with a duration of 1.5 ns and a frequency of 8 MHz. It is shown that it is possible to use a laser system to obtain incisions on biological tissues of various types without carbonization. The obtained experimental data made it possible to clarify the mechanism of the action of laser radiation on the surface of water-saturated biological tissues.

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1. INTRODUCTION

The study of the features of the interaction of laser radiation with biological tissue is one of the urgent tasks of laser medicine. When carrying out medical manipulations performed with the help of various laser sources and with various parameters of laser radiation, various mechanisms of interaction of radiation with biological tissue can be involved and various results can be obtained. [1]. For example, when performing endovenous laser coagulation of veins using radiation with working wavelengths $\lambda = 970$, 1560, and 1940 nm, due to significantly different values of the absorption coefficient of laser radiation in water, the ongoing processes differ significantly [2].

To develop a particular medical technology, preliminary experiments are carried out on samples of biological tissues and/or their phantoms. Such studies help to find the necessary modes of laser exposure and determine the optimal parameters (wavelength, power, pulse duration, intensity in the laser spot, as well as the algorithm for moving the laser spot) for performing medical manipulations, which makes it possible to form technical specifications for the design and improvement of laser devices for developers of laser equipment. When studying laser action on biological tissues, a set of data on dynamic processes, temperature, and acoustic fields is required. Therefore, the experimental setup should contain a combination of devices for video filming, high-speed video filming (information on the dynamics of surface deformation and substance removal), thermogram recording (information on the dynamics of temperature fields), and broadband acoustic signals (shock and acoustic waves). At the same time, in order to increase the reliability of the studies, it is desirable that the measurements be carried out by several devices simultaneously.

The aim of the work is to create a compact installation that allows for promptly carrying out complex diagnostics of processes during laser exposure to biological tissue. The setup was tested on a pre-series sample of a pulsed nanosecond high-frequency laser radiation source with a wavelength of $3.03 \,\mu$ m, which is intensely absorbed in water-saturated biological tis-



Fig. 1. The schematic diagram of the setup (1) Source of laser radiation, (2) galvanic scanning system, (3) thermal imaging camera, (4) backlight for high-speed camera, (5) needle hydrophone, (6) high speed camera, (7) camera for visual control, (8) personal computer, (9) storage oscilloscope, (10) water drop, (11) surface of the biological tissue. On inserts are photographs from the displays of the respective devices obtained as a result of various experiments.

sues. It is important to note that the setup is universal and can be used with various sources of laser radiation.

2. INSTALLATION DESIGN

The schematic diagram of the setup for studying laser effects on biological tissues is presented in Fig. 1.

Experimental equipment, including the laser source and diagnostic instruments, is placed on a honeycomb optical plate. To obtain data on the distribution of temperature fields on the surface of the samples, a FLIR A655sc thermal imaging camera (3) (FLIR Systems, United States) with an additional Close-up IR Lens 5.8x lens was used, which made it possible to provide a spatial resolution of approximately 100 μ m/pixel. Using the camera, thermograms were recorded on the surface of the samples up to a temperature of 160°C with a frame rate of up to 50 fps.

To determine the time-resolved dynamics of substance removal and deformation of the sample surface in the region of laser action, a Fastcam SA-3 highspeed camera (6) (Photron, Japan) with shooting speed up to 100000 fps was used. The registration of processes was carried out in the shadow video mode with front illumination using a fiber optic illuminator (4). In the experiments, video data was recorded from a region of size 2×1.5 mm at 10 000 fps.

Registration of acoustic pulses and shock waves was carried out using a needle hydrophone 5 (Precision Acoustics, United Kingdom) with preamp with a bandwidth of 10-50 MHz. Signals from the preamplifier were recorded with a GOS 72304 storage oscilloscope (9) (GW Instek, Taiwan) with a maximum sampling frequency of 300 MHz.

Optical control of the sample surface and its location relative to laser radiation was carried out using a digital camera (7) (Eakins, China) with an additional LED illumination mounted on a micro lens. The camera provided images in Full HD quality (1920×1080 pixels) size 16×10 mm with a frequency of 60 Hz.

The proposed setup makes it possible to test various laser sources irradiating the sample, both in the contact mode (using an optical fiber) and by focusing the laser beam. In this paper, the capabilities of the facility are demonstrated using a S-3050-P new laser source (1) (IRE-Polus, Russia) as an example. Its feature is the generation of radiation at a wavelength of $3.03 \,\mu m$. This wavelength, as is known, corresponds to the peak of the main absorption of water in the IR range with an absorption coefficient $\mu_A = 9824 \text{ cm}^{-1}$ [3]. The laser operates in a pulsed mode with a frequency of 8 MHz, a pulse duration of 1.5 ns, and a pulse energy of up to 0.8 µJ. The laser action on the samples was carried out by bursts of pulses either with a given number of pulses or with a given burst duration. The movement of radiation in space over the surface of the sample was carried out using a single-mirror galvanic scanning system 2 installed in the laser head. The laser radiation was focused on the sample surface by a sapphire lens with a focal length of 71 mm, which was installed after the galvanoscanner.

During the main experiments, pig biological tissue was used as samples (11): muscle tissue and prostate tissue. The samples were placed on a three-coordinate stage under the laser head so that their surface lay in the region of the focal plane. To form incisions on the surface of the biological tissue, successive exposure to a given point with laser pulses was carried out, after which the beam was moved to the next point along the selected trajectory. This manipulation was repeated the required number of times. The following parameters were used: pulse energy $0.8 \,\mu$ J, duration of a pulse



Fig. 2. Photos of holes in titanium film test samples obtained at different energies of laser pulses (0.8 and $0.4 \,\mu$ J) and a pulse burst duration of 100 ms. The images were obtained in reflected and transmitted light.

burst from 1 to 100 ms, distance between individual points when moving in space 150 μ m, and time delay between pulse bursts from 10 to 100 ms.

Quartz glasses 300 μ m thick with a titanium film 80 nm thick were used as samples to determine the geometric parameters of the laser waist. Separate experiments on laser action on water (10) included the measurement of thermograms and acoustic signals with the registration of ongoing processes using highspeed video recording. An additional study of the areas of laser action on the samples was carried out using an optical 3D microscope HRM-300 (Huvitz, Korea).

3. RESULTS AND DISCUSSION

The diameter of the laser spot on the sample surface is the most important characteristic that determines the geometric and energy parameters of laser exposure. To determine the waist radius w_0 in a thin titanium film of test samples, several holes were formed at two different energy values E_1 and E_2 (Fig. 2). As can be seen from Fig. 2, the sizes of the holes formed depend essentially on the energy in the pulse. To determine the diameter of the laser spot, a five-fold determination of the average values of the diameters d_1 and d_2 at energies E_1 and E_2 , respectively, was carried out. The laser waist radius was calculated using the well-known expression

$$w_0 = \sqrt{\frac{d_2^2 - d_1^2}{2\ln\left(E_2/E_1\right)}}.$$
 (1)

The waist radius calculated in this way was $w_0 = 92 \pm 14 \,\mu\text{m}$.

The absorption of laser radiation in biological tissue in the studied wavelength range (3 μ m) is mainly determined by the absorption of laser radiation in water. Therefore, test experiments were initially performed on laser action on the surface of a water drop (10 in Fig. 1). The dynamics of temperature fields, the dynamics of the ejection of water microdroplets from the surface, and shock waves were studied. An example of a thermogram of the water surface, a frame of high-speed shooting with an emerging microdrop, and an acoustic signal spectrum obtained in the experiment are presented in Fig. 3.

According to Fig. 3, laser pulse action on the water surface led to its heating and to active dynamic processes, accompanied by the generation of a broadband acoustic signal. Figure 3b illustrates the departure of a microdroplet from the liquid surface and its movement in the vertical direction at a speed of approximately 2.5 m/s. Note that, in the case of the presence of a biomaterial in a liquid medium, such a drop will carry living objects. Therefore, this source can be used as a kind of bioprinter [4].

Acoustic methods are a convenient tool for studying the processes occurring in biological tissues under laser irradiation. Thus, the spectrum of the acoustic signal (Fig. 3c) clearly shows two local maxima F_1 and



Fig. 3. Results of laser action on the surface of a water drop: (a) thermogram of the water surface (1) heating of the water surface area, (2) needle hydrophone; (b) high-speed shooting frame (the dashed line marks the water surface); (c) acoustic signal spectrum (F_1 and F_2 are local maxima in the ranges of 10–100 kHz and 10–30 MHz, respectively).



Fig. 4. Results obtained when performing laser incisions on the surface of biological tissue: (a) photograph of a biological tissue sample with laser incisions; (b) temperature dynamics at the first point of laser exposure as a function of time (the inset above shows examples of thermograms for each of the points forming the incision; below the thermograms, the numbers of laser exposure points in space are indicated); (c) example of a histological section of a biological tissue in the area of a laser incision.

 F_2 . This indicates that, upon absorption of pulsed laser radiation, high-frequency acoustic pulses are periodically generated. The first peak F_1 corresponds to the repetition frequency of these pulses, and the peak F_2 corresponds to the high-frequency impulses themselves. We believe that the periodic generation of high-power highfrequency pulses is associated with explosive boiling events in a thin, near-surface layer of the drop [5].

At the next stage, laser-induced processes were studied using biological tissue samples as targets. Figure 4 shows a photograph of a biological tissue sample after laser incisions, a graph of the temperature at the first point of laser exposure versus time, and the results of histological studies.

According to the presented graph of the temperature dependence (see Fig. 4b), the temperature on the surface of the biological tissue exceeds 100°C in some cases. In this case, it should be taken into account that the presence of both time and temperature restrictions during the registration of thermograms leads to an underestimation of the obtained values. Therefore, real temperatures on the surface of a biological tissue can be much higher.

As shown by the results of histological studies (see Fig. 4c), a thin layer of biological tissue near the border of the formed laser incision is denatured, but there are no visual signs of carbonization. We believe that this effect was achieved due to the fact that, under the action of short laser pulses under conditions of strong absorption, the most heated tissue microparticles were periodically ejected. Such a release, as shown by acoustic studies, could be caused by the explosive boiling of water in the bulk of the biological tissue.

4. CONCLUSIONS

The paper presents a new experimental setup that allows the study of processes during laser action on biological tissue. The dynamics of the processes caused by laser heating was recorded using a thermal imaging camera, high-speed photography, and acoustic methods. The system was tested on the example of a new pulsed laser source with a radiation wavelength of $3.03 \,\mu\text{m}$. The conducted studies made it possible to understand the dependence of the efficiency of forming the sizes of laser incisions on the surface of a biological tissue on the parameters of laser exposure and to clarify the mechanism of action of laser radiation. It has been shown that the laser source under study makes it possible to perform laser incisions without significant carbonization of the biological tissue.

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