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Study of terahertz-radiation-induced DNA damage in human blood leukocytes

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Abstract. **We have carried out the studies aimed at assessing the effect of terahertz radiation on DNA molecules in human blood leukocytes. Genotoxic testing of terahertz radiation was performed in three different oscillation regimes, the blood leukocytes from healthy donors being irradiated for 20 minutes with the mean intensity of 8–200 μW cm^{-2} within the frequency range of 0.1–6.5 THz. Using the comet assay it is shown that in the selected regimes such radiation does not induce a direct DNA damage in viable human blood leukocytes.**

Keywords: *terahertz radiation, human blood leukocytes, genotoxic effect, comet assay, safety levels.*

1. Introduction

The recent progress of techniques for generating and detecting terahertz radiation as well as the unique properties of electromagnetic radiation (EMR) within the frequency range 0.1–10.0 THz offer the challenge of its wide application in security, communication, medical diagnostics and therapy systems. In this connection a question arises about the dependence of biological effects on the physical parameters of the EMR in the terahertz range and on the estimates of the safety limits of its use. However, the presently existing hygienic standards, regulating the use of electromagnetic radiation sources in Russia [1] and abroad [2], are restricted to the frequency range up to 0.3 THz. At the same time, in the scientific community there is no universally recognised opinion on whether the EMR of the terahertz range can damage biological objects of different level of organisation [3–6]. According to the data of the review paper [7], summarising the work carried out by 37 research teams all over the world starting from the 1970s, in 43% of the studies no effects were observed at all, in 29% the effects were negative, in 14% they were positive, and 14% of the studies revealed differently directed effects on the same biological object. Such a variety of data is explained by different types of the radiation sources (pulsed or continuous-wave), different exposure duration, level of organisation and initial functional states of the biological objects under study.

At present there are two most widespread hypotheses on the interaction of terahertz radiation with biological objects. In the first of them it is assumed that the action of terahertz radiation on the biological tissues and cells is associated with their heating due to strong absorption of this radiation by water [3, 8–10]. If the heat is not specially removed during the experiment, then the heat release can give rise to uncertainties in other parameters measured in the experiment. The temperature increase in the given substance depending on the power and the frequency of the incident radiation can be calculated using the numerical solution of the heat conduction equation [11] for a disc, having the radius that corresponds to the radius of the terahertz radiation beam, normally incident on the exposed object, and the thickness that corresponds to the radiation penetration depth. The results of numerical solution of the heat conduction equation in the case of focusing terahertz radiation into a spot 0.5 mm in diameter have shown that under the continuous irradiation the temperature increment per a milliwatt of absorbed power is only 1.8 °C, which makes the thermal character of the radiation effect on the biological tissues doubtful for power values not exceeding 1 mW.

Within the framework of the second hypothesis, in spite of the low energy of terahertz quanta and, therefore, small probability of chemical bond rupture, the resonance interaction of terahertz radiation with molecules of deoxyribonucleic acid (DNA) is possible, which under certain conditions essentially affects the molecular dynamics and can induce DNA strand breaks [12–14]. The DNA strand breaks can lead to disturbance of gene expression, DNA replication and, as a consequence, to irreversible changes in cells of living organisms, occurrence of mutations and cancerogenesis. That is why it is of key interest to determine the threshold values of the energy parameters of terahertz radiation, within which the effect is reversible and safe for future vital activity of biological objects.

The aim of the present work was to determine the limits of biologically safe energy threshold for terahertz radiation by estimating its damaging effect on DNA of human blood leukocytes. An important role in the study of the terahertz radia-

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tion effect belongs to novel genome technologies that allow both the determination of cellular DNA damage and the analysis of definite genes expression and specific protein synthesis [15]. The simplest and operative method that allows detection of damage and modification of cellular DNA structure is the express method of molecular genotoxicology known as the comet assay (method of DNA comets or single cell gel electrophoresis). This method is recommended by the WHO for performing genotoxicity analyses, is certified and widely used in many countries [15, 16].

2. Experimental technique

The experiments were carried out using the general fraction of whole blood leukocytes from healthy donors aged 24±4. Samples of peripheral blood were taken from fingers of eight donors and placed into test tubes, containing phosphate buffered saline with 1 mM EDTA as an anticoagulant. To prepare the samples we used whole blood, diluted by 10 times. Seven microscope agarose slides were prepared from each blood sample, six of them later used for studying the effect of terahertz radiation and one being left for control. The control samples were subjected to simulation of the exposure: for this goal they were placed in the working area of the operating setups, but the radiation at the object was absent. The irradiation of the preparations was implemented in a humid chamber (Petri dishes with medical cotton wool wetted with water) to prevent drying.

The analysis of the DNA damage in cells was carried out using the alkaline version of the comet assay with certain modifications [17]. The method is based on analysing the electrophoresis pattern of individual cells, whose DNA is stained with a fluorescent dye [18]. Keeping in mind the small depth of terahertz EMR penetration into the water-containing media, the prepared microscopic slides were made of two layers of 0.5% low-melting-point agarose (Serva, Germany) with cells, immobilised in the upper layer having the thickness of 50 μm. The cells included in the agarose slides were subjected to the terahertz irradiation from one of the three types of sources. Directly after the slides exposure the lysis procedure was performed during not less than 1 hour at room temperature in the darkness. Then the slides were transported in a humid chamber and were subjected to the comet assay procedures, following the *in vitro* protocol [16, 17]. The DNA damage was estimated by the percentage of the DNA in the 'comet tail'. As an indicator of condensation/decondensation of chromatin the nucleoid radius was used. The nucleoid is a structure that appears as a result of the lysis of a eukaryotic cell and consists of nuclear matrix proteins, to which the loops of DNA are attached. In each slide 40–60 images of nucleoids were recorded, which were then used to calculate the mean values of the mentioned characteristics. For each of the exposure conditions the mean values and the standard errors of the mean were calculated using the results of independent experiments (donors). The statistical analysis of the data was carried out using the Mann–Whitney U-test ($p < 0.05$).

The studies on the assessment of terahertz radiation effect on DNA of human blood leukocytes were performed using three experimental setups, differing in the terahertz radiation parameters, namely, the spectral range, the spectral intensity and the mean and peak powers. This allowed implementation of the experimental conditions routinely used in THz spectroscopic studies.

All the experimental setups were constructed following the same schematic diagram, presented in Fig. 1. The radiation from the femtosecond laser (*1*) served as a pump source for the terahertz radiation generator (*2*). Terahertz radiation was focused by the parabolic mirror or the lens (*3*) onto the surface of the cuvette (*4*). The cuvette was mounted in the holder (*5*), movable in the plane perpendicular to the optical axis. The power of the terahertz radiation was controlled by the Golay cell (*6*) with the cuvette (*4*) removed from the beam. The terahertz radiation spectra in all experimental setups are shown in Fig. 2.

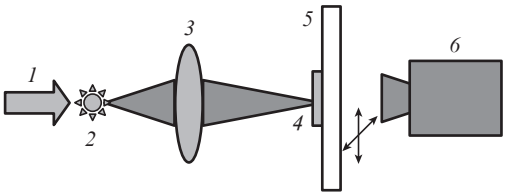

Figure 1. Scheme of the experimental setup: (*1*) radiation from the femtosecond laser; (*2*) terahertz radiation generator; (*3*) focusing unit; (*4*) cuvette with the sample; (*5*) cuvette holder on the two-coordinate translator; (*6*) detector (Golay cell).

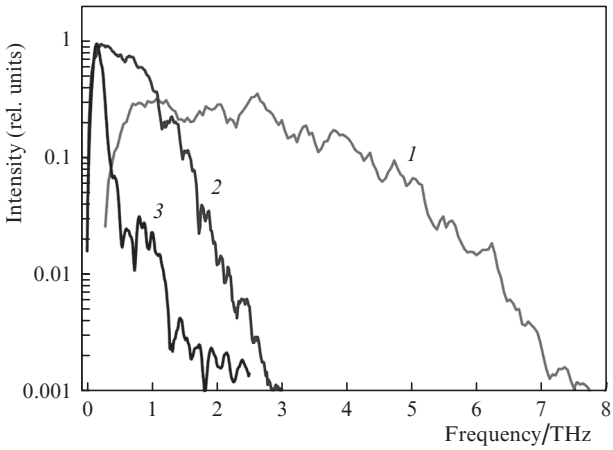

Figure 2. Spectra of terahertz radiation used in the experiments (the curve number corresponds to the number of the setups).

In the first setup terahertz radiation was generated in the process of femtosecond laser-induced air breakdown. The setup and generation process are thoroughly described in Ref. [19]. In contrast to other setups, the studied sample was placed at some distance from the focal plane of the parabolic mirror (*3*), where the diameter of the beam was nearly 3 mm. In the second setup terahertz radiation was generated using a photoconductive antenna pumped by the radiation of the Femtolite FX-100i fibre laser having the average power 100 mW, the wavelength 805 nm and pulse duration 120 fs. This laser system can be used in most compact measuring scheme. Moreover, in this case the sample holder was fixed in an automated two-coordinate positioner. This provided an essential increase in the exposed area by moving the sample along the prescribed trajectory within the area 8×4 mm, which simplified the subsequent analysis of the exposure results. The time of passing the whole trajectory amounted to 2 min.

Table 1. Characteristics of the terahertz radiation sources used.

Setup	$\Delta\nu$/THz	f/MHz	τ_p/ps	W_p/pJ	d/mm	P_{av}/μW	I_{av}/μW cm^{-2}	P_{max}/W	I_{max}/W cm^{-2}
1	0.5–6.5	0.001	~1	550	3	0.55	8	550	7800
2	0.1–2.0	75	1	0.013	1 (scanning)	1	125	0.013	1.6
3	0.1–1.0	82	2	0.5	5	40	200	0.25	1.3

Notes: $\Delta\nu$ is the spectral range; f is the pulse repetition rate; τ_p is the pulse duration; W_p is the energy of the terahertz pulse; d is the spot diameter on the sample; P_{av} is the mean power; I_{av} is the mean radiation intensity on the sample surface; P_{max} is the peak power; and I_{max} is the peak radiation intensity on the sample surface.

In the third setup the source of terahertz radiation was a photoconductive antenna array pumped by radiation of a femtosecond Ti:sapphire laser. It differs from the other setups by the relatively high mean power and the narrowest spectrum of terahertz radiation.

All experimental setups were assembled on the basis of terahertz spectrometers, for which the principles of operation are described in Ref. [20]. The parameters of terahertz radiation for all setups used in the work are summarised in Table 1. The exposure duration in the experiments was 20 min.

3. Results and discussion

In the present study the genotoxic activity of terahertz EMR was tested in three different regimes of terahertz generation. The studies were performed using the *in vitro* exposure and the comet assay technique. This method, proposed in 1984 by Ostling and Johanson, is aimed at detecting the DNA damages in cells of living organisms and plants, caused by a variety of damaging agents of chemical, physical and biological nature. The method is based on microelectrophoresis of nucleoids of individual cells, in which the fragments of damaged DNA migrate towards the anode. After staining with a luminescent dye the DNA produces the electrophoresis pattern that reminds a comet with a compact fluorescing 'nucleus' (intact DNA) and bushy 'tail'; this likeness explains the origin of the term 'comet assay'. From the geometrical parameters and the fluorescence intensity of the stained DNA in the comet tail the degree of DNA damage is quantitatively evaluated.

The method allows revealing single-strand and double-strand breaks of DNA chain, alkali labile sites, DNA-protein cross-linkings, and, in combination with enzymatic processing of preparations, also oxidised purine and pyrimidine bases. The method also makes it possible to estimate the efficacy of DNA repair in cells of various organisms.

Figure 3 shows microphotographs of cell nucleoids after irradiation with terahertz EMR from three types of sources (setups 1–3). In the control preparations one can observe nucleoids having spherical shape and the size, normal for the human blood leukocytes. All images are characterised by the absence of the tail that could confirm the presence of DNA damages. The absence of comet tails in all preparations demonstrates the absence of direct DNA damages in cells under the action of terahertz radiation in the used regimes. An assessment of temperature changes in our study has shown that under the chosen conditions of irradiation the heating was smaller than 1 °C. Thus, the effect of radiation in all three regimes of sample exposure does not lead to inducing direct DNA damages, including single-strand and double-strand breaks of DNA chain and alkali labile sites, in viable human whole blood leukocytes and in nucleoids of previously lysed cells, obtained from healthy donors (data not presented).

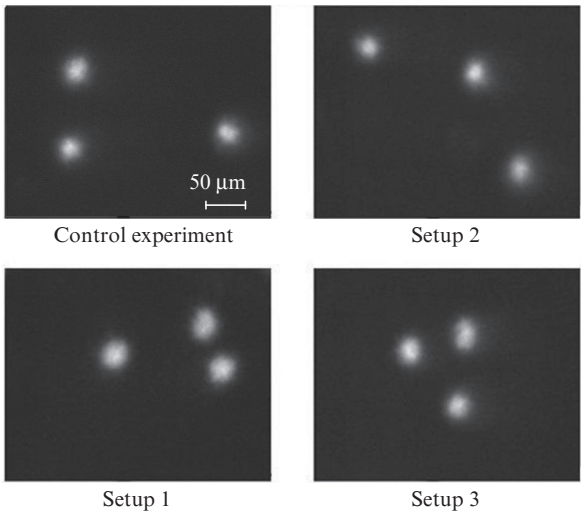

Figure 3. Characteristic microphotographs of the control cell nucleoids and nucleoids after exposure of cells to terahertz radiation.

Similar results were obtained within the framework of the THz-Bridge project, where the effect of terahertz radiation on human blood leukocytes was also studied [21–23]. For this aim the samples of blood from healthy donors were subjected to the action of terahertz radiation having the frequencies of 120 and 130 GHz for 20 min. Similar to our study, the alkali version of comet assay was used to evaluate DNA damage. As a result, the authors concluded that the terahertz radiation with the used parameters does not lead to toxic or genetic modifications in blood leukocytes and does not affect the cell cycle kinetics. It is worth noting, that both the calculated and the measured temperature changes did not exceed 1 °C.

Opposite effects were observed under the action of continuous radiation having the frequency of 0.1 THz on the human blood lymphocytes during 2 and 24 hours [24]. By means of the fluorescent method the increase in aneuploidy of chromosomes 11 and 17 in the process of cell division was detected, which causes the genome instability and can lead to cancer development. Significant reduction of cell viability was found under the action of continuous radiation having the frequency of 3.688 THz on the human blood lymphocytes for 90 min [25]. In the lymphocytes of the same sample that remained viable after the exposure the enhancement of cell division was observed. According to Refs [3, 8], this effect is associated with the increase in temperature during irradiation. The calculated increase in the temperature was 1.5 °C [3].

In our study the effect of terahertz EMR from different sources did not lead to significant changes in the nucleoid size (Fig. 4). In the histogram of the nucleoid size distribution no essential difference from the control distribution was found (Fig. 5). The shift of the histogram to the left or to the right, i.e., towards smaller or larger nucleoid size, could evidence in

favour of the condensation or decondensation of chromatin, respectively. In the present case the distributions are close to normal, and no significant shift of them along the abscissa axis with respect to the control distribution is observed. Thus, no condensation or decondensation of chromatin is observed under the chosen conditions of irradiation.

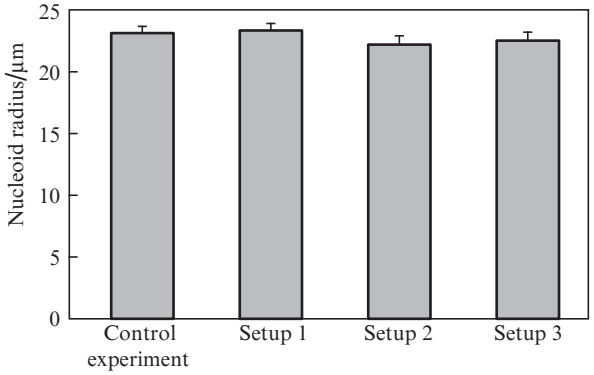

Figure 4. Sizes of control cell nucleoids and nucleoids after the action of terahertz radiation.

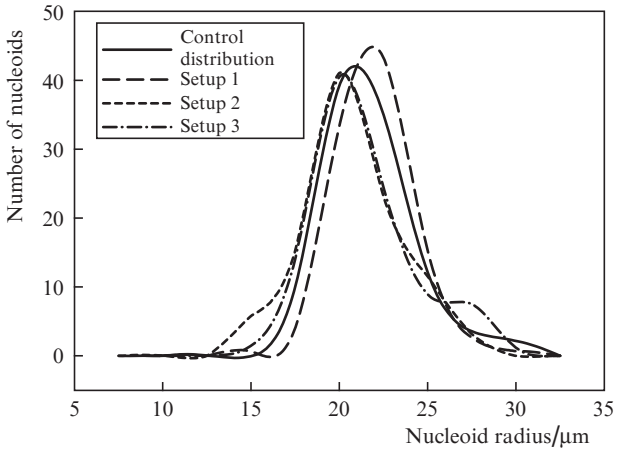

Figure 5. Histograms of the size distribution of the control nucleoids and the nucleoids affected by the terahertz radiation (the number of nucleoids $n = 180–200$).

According to the data of the study carried out and to the literature sources, in Table 2 the biologically safe energy parameters of terahertz radiation are summarised. The effects of radiation on cells depend on multiple factors, including both the parameters of the incident radiation, such as the mean and peak power, radiation intensity in the exposed area, duration and periodicity of exposure, and the method of assaying the results of the action and the state of the irradiated object itself.

According to the actual hygienic standards [1], for the EMR with the frequency no greater than 300 GHz the safety level of intensity is 200 μW cm^{-2} for one hour of irradiation. Our studies have shown that the action of pulsed picosecond terahertz radiation in the frequency range of 0.1–6.5 THz on human blood leukocytes does not induce DNA damage during the 20 min exposure with the mean intensity up to 200 μW cm^{-2} and peak intensity of 7.8 kW cm^{-2}. In this case the heating of the irradiated sample does not exceed 1 °C. Thus, acting on leukocytes of peripheral blood of healthy donors, the above energy parameters of the pulsed terahertz radiation can be accepted as safe for the exposure durations no greater than 20 min. However, one should keep in mind that an increase in the pulsed radiation intensity can lead to inducing DNA damage [23]. When using continuous-wave regime, the radiation with the power 20 mW and intensity about 40 mW cm^{-2} becomes definitely unsafe for living organisms [25], since the absorbed energy in this case can essentially exceed the safety limits.

In the present paper the discussion is mainly focused on the energy parameters of the terahertz EMR, and the conclusion is made about the existence of a certain threshold value of the radiation intensity, beyond which the effect of the radi-

Table 2. Effect of terahertz radiation on human blood leukocytes at different parameters of the sources used [(p) stands for a pulsed source and (cw) – for a continuous-wave source].

Frequency/THz	Intensity/μW cm^{-2}	Exposure time/min	ΔT/°C	Effects	References
0.5–6.5 (p)	8	20	<1	no	
0.1–2.0 (p)	125	20	<1	no	
0.1–1.0 (p)	200	20	<1	no	
0.12 (p) 0.13 (p)	50 30	20	<0.3	no	[22]
0.12 (p) 0.13 (p)	50 30; 160; 230	20	0.35	no	[21]
0.13 (p)	150 250	20	0.3	no	[23]
0.13 (p)	2000 5000	20	0.3	yes[1]	[23]
0.1 (cw)	31	60 120 1440	<0.3	no yes[2] yes[3]	[24]
3.68 (cw)	39800	30 90	<1.5[2]	yes[4] yes[5]	[25]
0.0003–0.3	200	60			[1]
0.003–0.3	10000				[2]

Notes: ΔT is the temperature increase during irradiation; [1] is DNA damage; [2] is the 40% increase in asynchronous replication; [3] is the 50% increase in asynchronous replication; [4] is the 10% decrease in cell viability; and [5] is the 50% decrease in cell viability.

ation may be unfavourable. In this connection it is worth noting, that an important role in inducing biological effect can belong to other radiation parameters, including the duration of exposure, the spectrum of carrier and modulation frequencies. The possibility of hydrogen bond breaking in the DNA double helix under the action of low-intensity EMR with the frequencies, close to those of the natural phonon modes in the DNA (i.e., in the terahertz range), was theoretically analysed in Ref. [13]. It is assumed that the terahertz EMR can exert resonance influence on the inherent dynamical behaviour of the DNA molecule, introducing changes at the level of gene expression and DNA replication [12]. In this way the functional modifications of DNA activity can be induced and, as a consequence, a number of functional features of the entire cell may be changed. Based on the results of the studies on biological effect of EMR with extremely high frequencies, it is logical to suppose that the biological effects of the terahertz EMR can be strongly dependent upon the functional state of the studied object, i.e., the action of the radiation with the same physical parameters can be safe for healthy organism and dangerous when acting on the organism in the modified functional state, e.g., as a result of disease or joint action of different physical and chemical factors.

Particular attention should be paid to the problems, related to strong absorption of terahertz EMR by water-containing media. In this connection it is necessary to create special conditions, providing the availability of cells and tissues to direct action of terahertz EMR and the possibility of precise dosimetric control of the EMR absorption. In our experiment the irradiation was applied to cells, immobilised in the agarose layer with the thickness of 50 μm, i.e., the conditions for minimal EMR attenuation in the process of cell irradiation were created. Discussing the results of other experiments, one should account for the specific features of the terahertz EMR absorption and estimate the fraction of radiation energy that can directly reach the biological object under study.

4. Conclusions

Thus, as a result of the investigation carried out it was shown that the used sources of terahertz radiation in the frequency range of 0.1–6.5 THz with the intensity of 8–200 μW cm^{-2} do not cause genotoxic effects at the level of direct DNA damage of human blood leukocytes. Special thorough studies should be devoted to investigating the dependences of terahertz EMR biological effect upon other parameters, such as the exposure duration, the carrier and modulation frequency spectrum, as well as to determining the precise energy threshold of the impact.

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References

1. *Fizicheskiye factory proizvodstvennoy sredy 1996-08-05. SanPiN 2.2.4/2.1.8.055-96* (Physical Factors of Production Environment 1996-08-05. Sanitary Rules and Regulations 2.2.4/2.1.8.055-96) (Moscow: State Sanitary and Epidemiologic Inspectorate, 1996).
2. *IEEE Standard for Safety Levels with Respect to Human Exposure to Radio Frequency Electromagnetic Fields, 3 kHz to 300 GHz. IEEE Std C95.1* (2005).
3. Wilmink G., Grundt J. *J. Infrared, Millimeter, Terahertz Waves*, **32**, 1074 (2011).
4. Hintzsche H., Jastrow C., et al. *Radiation Research*, **179**, 38 (2012).
5. Fedorov V.I. *Biomed. Radioelektron.*, (2), 17 (2011).
6. Fedorov V.I., Popova S.S., Pisarchik A.N. *Intern. J. Infrared and Millimeter Waves*, **24** (8), 1235 (2003).
7. Hintzsche H., Stopper H. *Crit. Rev. Environ. Sci. Technol.*, **42**, 2408 (2012).
8. Wilmink G.J., Rivest B.D., Roth C.C., et al. *Lasers in Surgery and Medicine*, **43**, 152 (2010).
9. Berry E., Walker G.C., Fitzgerald A.J., et al. *J. Laser Appl.*, **15**, 192 (2003).
10. Ramires M.L.V., Nieto de Castro C.A., Nagasaka Y., et al. *J. Phys. Chem.*, **24**, 1377 (1995).
11. Kristensen T.T.L., Withayachumnankul W., Jepsen P.U., Abbott D. *Opt. Express*, **18**, 4727 (2010).
12. Alexandrov B.S., Gelev V., Bishop A.R., et al. *Phys. Lett. A*, **374**, 1214 (2010).
13. Chitanvis S.M. *J. Polymer Sci B: Polymer Phys.*, **44** (18), 2740 (2006).
14. Zhuang W., Feng Y., Prohofsky E.W. *Phys. Rev. A*, **41**, 7033 (1990).
15. Gun-Sik Park et al. *Convergence of Terahertz Sciences in Biomedical Systems* (Dordrecht: Springer, 2012).
16. Tice R.R., Agurell E., Anderson D., et al. *Environ. Mol. Mutagen.*, **35**, 206 (2000).
17. Gapeyev A.B., Romanova N.A., Cheremis N.K. *Biofiz.*, **56** (4), 688 (2011) [*Biophys.*, **56** (4), 672 (2011)].
18. Ostling O., Johanson K.J. *Biochem. Biophys. Res. Commun.*, **123**, 291 (1984).
19. Borodin A.V., Esaulkov M.N., Kuritsyn I.I., et al. *J. Opt. Soc. Am. B*, **29**, 1911 (2012).
20. Nazarov M.M., Shkurinov A.P., Kuleshov E.A., Tuchin V.V. *Kvantovaya Elektron.*, **38**, 647 (2008) [*Quantum Electron.*, **38**, 647 (2008)].
21. Zeni O., Gallerano G.P., Perrotta A., et al. *Health Phys.*, **92**, 349 (2007).
22. Scarfi M.R., Romano M., Di Pietro R., et al. *J. Biol. Phys.*, **29**, 171 (2003).
23. Doria A., Gallerano G.P., Giovenale E., et al. *Infrared Physics & Technology*, **45**, 339 (2004).
24. Korenstein-Ilan A., Barbul A., Hasin P., et al. *Radiat Res.*, **170** (2), 224 (2008).
25. Fedorov V.I., Klement'ev V.M., Khamoyan A.G. et al. *Millimetrovye volny v biologii i meditsine*, No. 102 (53–54), 88 (2009).