MORPHOFUNCTIONAL STATE OF PERIPHERAL BLOOD ERYTHROCYTES AFTER FEMTOSECOND LASER TREATMENT

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The aim of the investigation was to assess femtosecond laser effect on morphofunctional state in peripheral blood erythrocytes exposed to radiation flux density variations.

Materials and Methods. We determined the level of malondialdehyde (MDA), the activity of superoxide dismutase (SOD), catalase and glutathione-transferase, and assessed topology and rigidity of erythrocyte membranes using scanning probe microscopy. In the experiment we used erbium fiber laser with pulse duration of $82 \cdot 10^{-15}$ s, peak and average power of 6 kW and 1.26 mW, respectively, and wavelength of 1.55 μm; radiation flux density being within the range from 0.10 to 2.70 J/cm^2.

Results. MDA level indicating lipid peroxidation intensity was found to change slightly varying from 430 to 509 μmol/l. The activity of antioxidant enzymes — SOD and catalase was revealed to depend on the energy density: its most pronounced activity increase was recorded for the radiation flux density of 0.10 J/cm^2. GT activity for all selected radiation energy densities was similar to that in control group. Scanning probe microscopy findings indicate erythrocyte topology modification as well as undulatory increase of erythrocyte membrane rigidity at increased energy density of femtosecond laser radiation.

Key words: femtosecond laser radiation; erythrocytes; scanning probe microscopy.

One of the promising directions in biomedical investigations is the use of new laser techniques in diagnosis and management based on such biological effects as photodestructive light action (in laser surgery) and photochemical light action (in therapy) [1–4]. Femtosecond laser characteristics are the following: short pulse duration, high average intensity during impulse action, high time and space coherence. High peak intensity of femtosecond laser radiation (FSLR) can result in cell death, though the causes of death may vary from thermocoagulation of membrane proteins to the damage of cell genetic apparatus. In medicine these lasers are applied as holographic forceps and an optical scalpel [5], as well as they are used for osseous tissue softening [6]. Their intended use in oncology will require significant increase of radiation dose [7]. All the above mentioned specifies the necessity to estimate the FSLR effect on macroorganism upon the whole, and primarily, on blood cells.

The aim of the investigation was to assess femtosecond laser effect on morphofunctional state in peripheral red blood cells exposed to radiation flux density variations.

Materials and Methods. The target of the research was donor red blood cells. In the investigation we used erbium fiber laser — a cooperative development of Scientific Center of Fiber Optics of Russian Academy of Sciences and Ulyanovsk State University (Russia). The laser has the following characteristics: pulse duration — $82 \cdot 10^{-15}$ s; average power — 1.26 mW; peak power — 6 kW; wavelength — 1.55 μm.

Calculation on energy flux density under femtosecond laser radiation. The cells were exposed in plastic trays 1 cm high. For the distance between the source of laser radiation and an erythrocyte surface of 3 cm (laser beam diameter 0.6 cm) and 5 cm (laser beam diameter 1 cm), and initial energy characteristics of the laser, energy density on biotissue (E) was calculated according to the formula [8]: $E=W/S$, where W — output radiation energy, J; S — laser spot area on biotissue, cm^2. We calculated the density values of average power of femtosecond laser depending on exposure time (Table 1).

Biochemical research techniques. The intensity of lipid peroxidation (LP) was assessed by the level of secondary

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Table 1

Energy density depending on exposure time and average power of femtosecond laser

Exposure time, min	1	1	3	5	3	10	5	10
Distance, cm	3	5	3	3	5	3	5	5
E_{av}, J/cm^2	0.10	0.27	0.29	0.48	0.81	0.96	1.35	2.70

product — malondialdehyde (MDA). We determined the activity of superoxide dismutase (SOD), catalase, glutathione- transferase (GT). Optical density of the study parameters was assessed by spectrophotometer Genesys UV-10 (Thermo Scientific, USA).

Morphological research techniques. The topology and rigidity of erythrocyte membranes was assessed by scanning probe microscopy (SPM) (Smena A, NT-MDT Company, Zelenograd, Russia). We used silicon probes with rigidity of 0.2 N/m, tip bending radius was approximately 50 nm. Red blood cells were scanned in tapping mode. Membrane rigidity was assessed by Young's modulus calculated according to Hertz theory [9].

To determine the differences between the data obtained in the experiment and those in the control group we used nonparametric Mann–Whitney U test (Statistica 6.0). Findings were considered statistically significant when $p \leq 0.05$.

Results and Discussion. The performed investigations showed MDA level in red blood cells after FSLR under various energy flux density (from 0.10 to 2.70 J/cm^2) to have no statistical significance varying from 430 to 509 μmol/l.

The enzymatic components of anti-oxidant system (AOS) — SOD and catalase — are assumed [10] to be laser radiation acceptors, therefore, it is of interest to explain the FSLR effect on the activity of these enzymes in red blood cells. When exposed to radiation, erythrocytes were found to have decreased catalase activity, with energy density being 0.27 J/cm^2. If the density increased, the enzyme activity was lower than the control values (Fig. 1). The change of catalase activity may be due to conformational alterations in its active centre under laser radiation.

The increase of SOD activity after FSLR was dose-dependent (Fig. 2). The highest values were recorded at 0.10; 0.27; 0.48 and 0.81 J/cm^2 of energy density; at0.29 J/cm^2 the enzyme activity went down sharply. Further, activity variations persisted, though were less obvious.

After FSLR using all energy flux density values, GT activity did not statistically significantly differ from that before the exposure.

Thus, the obtained findings suggest possible anti-oxidant effect of FSLR on red blood cells *in vitro*.

The works of L.V. Korsi et al [11] showed that the deformability of erythrocytes exposed to different spectral ranges of semiconductor lasers, dye lasers, and soled titanium:sapphire lasers is resonant, similar to absorption spectrum bands of molecular oxygen under high pressure. The scanning of intact erythrocytes using SPM revealed mainly normocytes (Fig. 3).

Erythrocyte cytoarchitectonics changed after FSLR exposure at energy flux density of 0.29 J/cm^2. A scanned image shows reversibly deformed forms — echinocytes (Fig. 4). 3D-image (Fig. 5) demonstrates echinocytes to form under physiological conditions due to the change of membrane ion permeability, and channel malfunction; spherocytes — at the doses of 0.81 and 0.96 J/cm^2.

Exposed to the doses of 1.35 and 2.70 J/cm^2 almost all erythrocytes on scanned images are spherocytes. They can be of irregular form with modified linear dimensions (Fig. 6).

Scanning probe microscopy technique has been frequently used in biomedical researches since the 1990-s enabling to study cell parameters without resort to long-term and complicated fixation and avoid misrepresentation of information obtained. The technique enables to measure elastic properties of cell surfaces [12]. The studies carried out using a scanning probe microscope Smena A indicate that FSLR causes viscoelasticity reduction of erythrocyte membranes accompanied by the increase of rigidity values (Table 2).

Thus, FSLR changes erythrocyte rigidity and topology dose-dependently.

Scanning probe microscopy findings indicate erythrocyte topology modification as well as undulatory increase of erythrocyte membrane rigidity in increased energy density of femtosecond laser radiation.

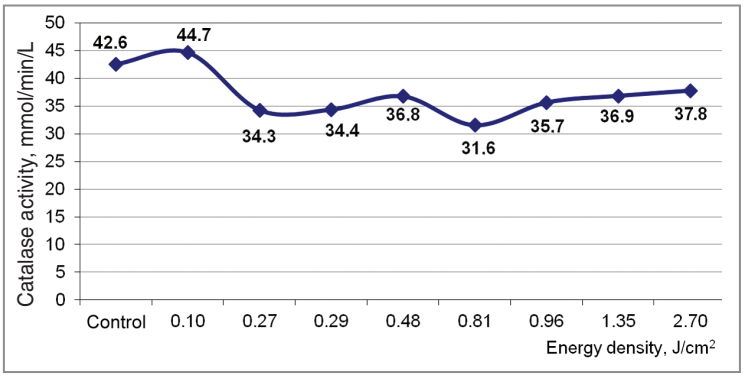


Fig. 1. Catalase activity in erythrocytes depending on energy density of femtosecond laser radiation

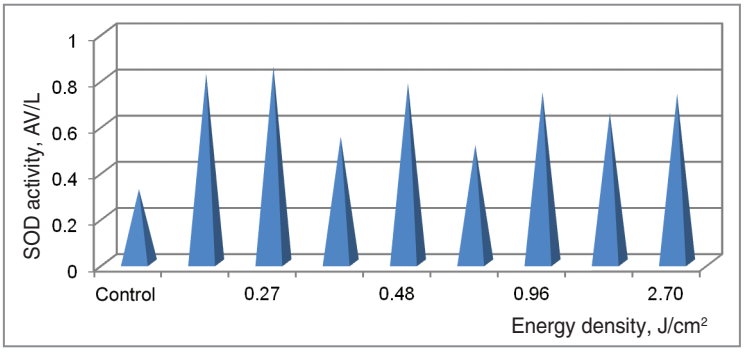
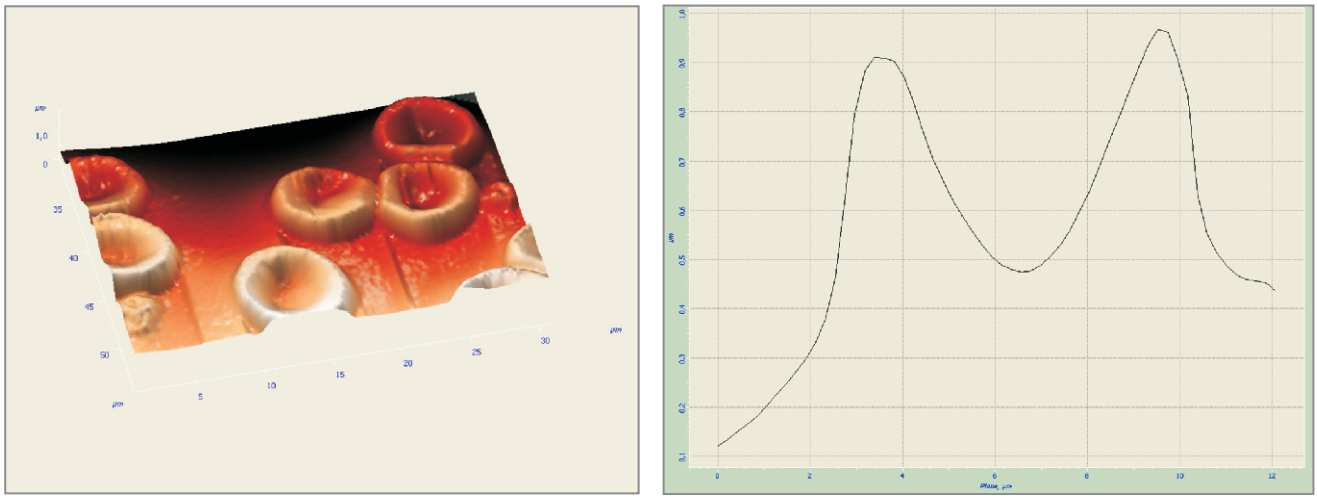
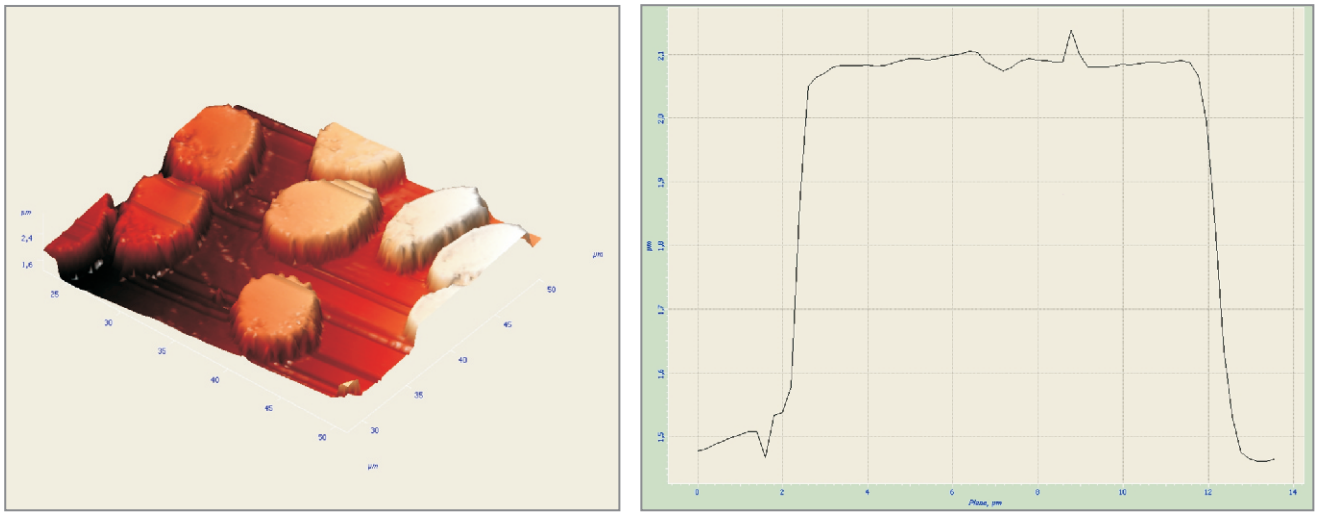


Fig. 2. SOD activity in erythrocytes depending on energy density of femtosecond laser radiation



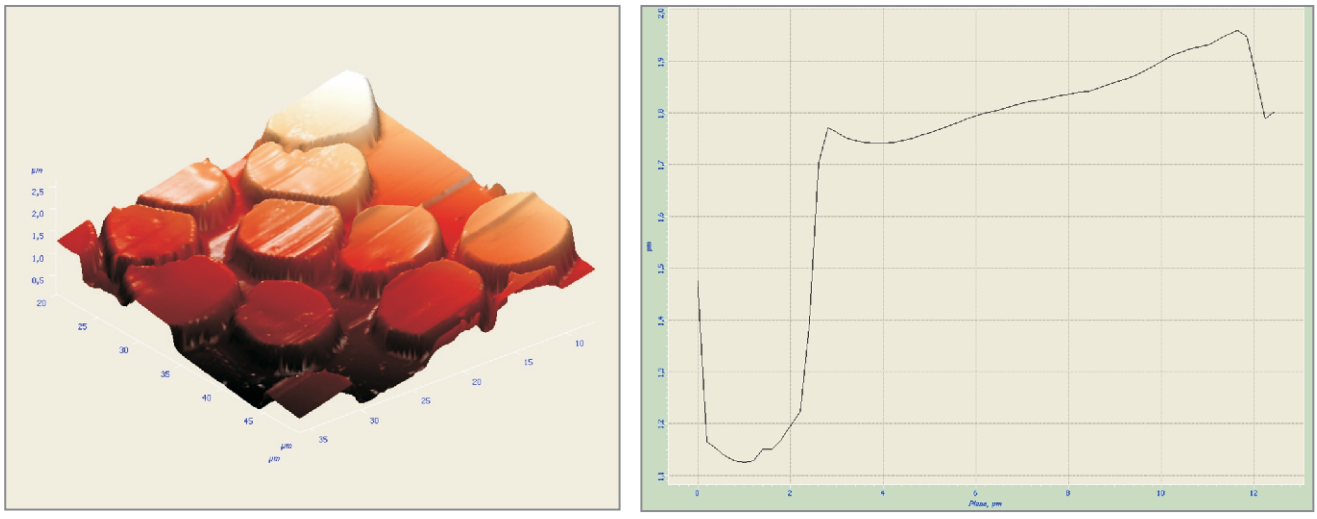
a b

Fig. 3. 3D-image (*a*) and lateral cross-section (*b*) of intact erythrocytes



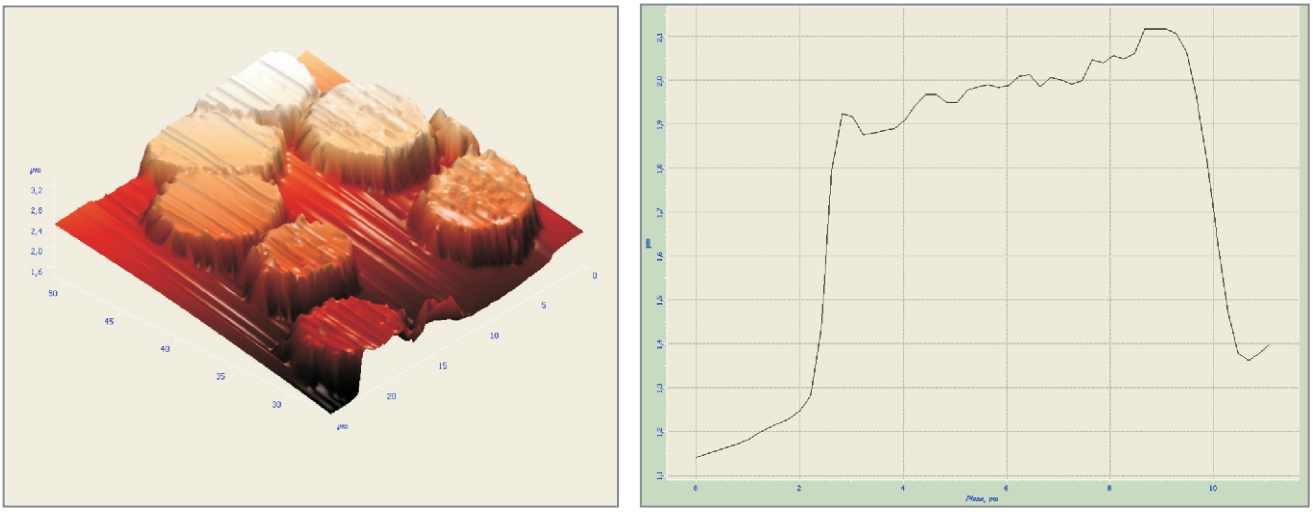
a b

Fig. 4. 3D-image (a) and lateral cross-section (b) of erythrocytes after femtosecond laser radiation at energy density of 0.29 J/cm2



a b

Fig. 5. 3D-image (*a*) and lateral cross-section (*b*) of erythrocytes after femtosecond laser radiation at energy density of 0.96 J/cm^2



a b

Fig. 6. 3D-image (*a*) lateral cross-section (*b*) of donor erythrocytes after femtosecond laser radiation at energy density of 2.7 J/cm^2

Table 2

Erythrocyte rigidity when exposed to femtosecond laser radiation

	Energy density, J/cm^2								
	Control	**0.1**	**0.27**	**0.29**	**0.48**	**0.81**	**0.96**	**1.35**	**2.70**
Rigidity, Pa	360.80± 14.13	561.70± 22.88*	515.90± 14.96*	569.60± 22.62*	550.10± 29.95*	481.60± 19.32*	496.30± 26.43*	382.00± 17.72	937.40± 33.09*

* — findings statistically significantly differ from those in control group.

Conclusion. Femtosecond laser radiation with flux density from 0.10 to 2.70 J/cm^2 does not change significantly MDA level in erythrocytes, though increases the activity of such anti-oxidant enzymes as catalase and SOD. The effect is the most pronounced at radiation flux density of 0.10 J/cm^2 that suggests an anti-oxidant effect of femtosecond laser radiation. Scanning probe microscopy findings indicate erythrocyte topology modification as well as undulatory increase of erythrocyte membrane rigidity at increased energy density of the studied radiation.

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