Laser-based inactivation of pathogenic microorganisms

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Abstract. In the context of studying the possibility of inactivation of pathogenic microorganisms, the dynamic transmission spectra of femtosecond laser radiation in the mid-IR range by bacteria of the *Pseudomonas aeruginosa* culture planted on a silicon substrate were investigated. Irradiation revealed a blue shift in the characteristic absorption bands of bacteria, indicating the breaking of hydrogen bonds responsible for the formation of the secondary and tertiary structure of the proteins.

1. Introduction

Currently, the task of combating pathogenic microorganisms is more urgent than ever. A large number of disinfection methods are known: from traditional UV radiation, which is the most widespread method, to modern nanotechnological methods using photosensitive materials and inactivation by femtosecond laser radiation of the visible and near-IR ranges \[1, 2, 3, 4, 5\]. However, each of the above methods has its drawbacks: UV causes mutations in mammalian cells, leading to cancer, nanoparticles and other photosensitizers can be toxic, the method of direct laser inactivation by femtosecond radiation requires a long exposure time. IR radiation has also been considered in the context of disinfection. The effect of inactivation of spores and microorganisms has been shown \[6, 7\]. In this paper, we consider the possibility of inactivation of pathogenic microorganisms by resonant vibrational excitation of characteristic vibrations of proteins of a bacterial cell by femtosecond laser radiation in the mid-IR range. The mechanism of inactivation is the selective denaturation of proteins as a result of the destruction of their secondary and tertiary structure, and, consequently, the inactivation of their function, which is of fundamental importance for the proliferation of a bacterial cell \[8\].

2. Experimental details

In this study, the transmission of bacterial samples excited by femtosecond laser pulses in the C-N and C = O region of vibrations of amide I and II (1550 - 1750 cm\(^{-1}\)) (Fig.1). Bacteria of the *Pseudomonas aeruginosa* culture taken from the collection of the Gamaleya National Research Center for Epidemiology and Microbiology, were planted on n-doped silicon plates 0.5 mm thick as a submonolayer.
Figure 1. Stationary spectra of optical density of non-irradiated P. aeruginosa and spectrum of femtosecond laser pulse. The inset depicts dynamical spectra of laser pulses for bare Si and Si with bacterial coating.

Figure 2. Experimental setup.

The sample was placed in front of the slit of an IR spectrometer (Solar TII MS2004) along the normal to the optical axis of radiation focused on the slit by a spherical mirror with a focal length of 150 mm. Mid-IR laser radiation (central wavelength 5.8 μm, half-width 0.6 μm) with a duration of 130 fs, an energy per pulse of 2 μJ, and a repetition rate of 1 kHz was obtained by parametric transformation of Ti: Sapphire laser (Spitfire HP, Spectra-Physics, center wavelength - 800 nm, repetition rate - 1 kHz, pulse duration at half maximum - 50 fs) in a parametric amplifier with a difference frequency generator “OPA TOPAS-C + nDFG” (Light Conversion) (Fig. 2). The peak intensity of ultrashort pulses with a wavelength of 6 μm on the sample surface was 5 GW/cm² without filters and 0.3 GW/cm² after attenuation with a set of neutral metallized filters on BaF2 substrates. The obtained spectra of transmitted ultrashort pulses for samples with bacteria were normalized to the spectrum of transmitted radiation for a control pure silicon wafer, giving a dynamic transmission spectrum of the bacterial coating. Stationary optical density spectra in the range 400 - 4000 cm⁻¹ were obtained using a Vertex V-70 IR Fourier spectrometer (Bruker).

3. Results
Vibrational spectra of bacteria in the range 1800 - 1500 cm⁻¹ (6 μm) contain characteristic C = O bands of stretching lipid vibrations (~ 1740 cm⁻¹), amide I bands (1695, 1685, 1675, 1655 and 1637 cm⁻¹) and amide II (1550 - 1520 cm⁻¹) of proteins and peptides. The amide I and amide II bands provide a structural information about α-helix and β-sheet and random coil conformations in proteins. Nucleic acids and phospholipids have absorption bands in the spectral region 1500 – 1200 cm⁻¹: amide III (1310 – 1240 cm⁻¹) and P=O symmetric stretching modes of >PO₂ phosphodiesters (Fig. 3).
Figure 3. Optical density spectrum of P. aeruginosa and characteristically bands of vibrations of cell’s components at the range 1200 – 1800 cm\(^{-1}\).

The dynamic transmission spectra of bacteria under 20-second exposure to laser radiation showed an increase in characteristic absorption in the 1550-1900 cm\(^{-1}\) region and a blue shift of the spectral bands, which may be associated with the destruction of hydrogen bonds (Fig. 4).

Figure 4. Dynamical and FT-IR spectra of optical density of P. aeruginosa.

Figure 5. Stationary spectra of optical density of non-irradiated and irradiated P. aeruginosa.

Examination of the samples by FT-IR spectroscopy showed a difference in the spectrum of the irradiated bacterial layer from the control sample (Fig. 5).
4. Conclusion
In conclusion, as a result of fs irradiation with mid-IR laser pulses (5 - 6.6 \( \mu \)m) with intensities from 0.1 to 10 GW / cm\(^2\) of a submonolayer of P. aeruginosa bacterial cell culture, a blue shift was demonstrated in the region of characteristic absorption bands of proteins and nucleotides of the bacterial cell. This indicates the breaking of hydrogen bonds responsible for the formation of the secondary and tertiary structure of the protein. Differences were found in the FT-IR spectra of irradiated and non-irradiated bacteria, which may also indicate the inactivation of pathogenic microorganisms. Further research is in progress.

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