Conference Paper

Laser Printing of Gel Microdrops with Living Cells and Microorganisms

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Abstract

We report the results of experiments on laser printing (wavelength λ=1064 nm) with gel microdrops acting as carriers of living microbial and cellular objects. The dynamics of transport processes with the help of high-speed optical video was studied, which allows to determine characteristics of the formed gel jets and to optimize the operating mode of the laser. It is shown that laser pulses of 4 to 20 ns duration and energy E ≤ 20 μJ should be used to minimize the negative effect on living systems. The results can be used to optimize the technologies of cellular printing and laser engineering of microbial systems (LEMS). LEMS technology is used to isolate hard-cultivated and non-cultivated by classical methods of microorganisms that can act as producers of new biologically active substances and antibiotics.

Keywords: laser printing, gel, microdrop, living cell, microbial

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1. Introduction

Laser induced forward transfer (LIFT) technology [1], is more and more widely used in biomedicine for the printing of biological materials [2], stem cells in the field of cell engineering, isolation of individual cells [3] has been developing actively. Most recently, LIFT technology was used to isolate hard-cultivated microorganisms (laser engineering of microbial systems - LEMS [4, 5]).

To implement laser transfer process, a thin layer of a gel substrate containing a filler (living cells or aggregates of cells, soil carriers of microorganisms) is applied to a donor plate - a glass plate with a thin absorbing (usually gold or titanium) thin film. Laser pulsed is focused on the absorbing layer, causing the removal of a the absorbing layer in irradiated zone of donor plate. As a result, a pressure jump occurs that leads to the transfer of the microdroplet of the gel substrate to the microbiological objects onto the

acceptor carrier. The characteristics of this transport process depend on the parameters of the laser pulse (wavelength, duration, energy, intensity, focusing parameters), the thickness of the absorbing layer and its material, as well as on the thickness of the gel substrate layer, its composition, uniformity, viscosity, surface tension, et al. [5].

A number of physical factors affect the cellular and microbial objects In the course of laser printing: laser pulse transmitted through the absorbing layer; shock waves; high temperatures; broadband optical radiation associated with the formation of plasma in the absorbing layer; nanoparticles of the absorbing layer, which together with the gel can be transferred to the acceptor plate [5]. The aim of the current work is to reveal the level the impact of these processes on living systems and to minimize negative impact.

2. Material and Methods

For laser printing process (Fig. 1), a pulsed fiber laser (1) YLPM-1-4x200-20-20 (IRE-Polus, Russia) was used. The operating wavelength is 1064 nm (M^2 <1.5), the pulse width was τ = 4 - 200 ns, the energy in the pulse E from 2 μJ to 1 mJ. For spatial laser beam control we used a two-mirror galvano-scanning head LscanH-10-1064 (AtekoTM, Russia) with F-theta lens SL-1064-110-160 (Ronar-Smith, Singapore) and focal length of 160 mm. The laser spot diameter in a focus was 30 μm. 200 ± 30 microns thick gel layer substrate was applied to a donor glass plate with a gold absorbing film 25, 50 or 100 nm in thickness. The gel substrate was based on hyaluronic acid (2% in water) with a viscosity of 15.5 ± 0.05 mPa·s. For the process of laser microsampling of carriers of microorganisms, soil particles with a size of <300 μm were added to the gel (200 mg of soil per 0.8 ml of gel). After carrying out the laser irradiation process, the gel microdroplets were collected on an acceptor glass plate mounted 1 mm from the underside of the donor plate.

Optical recording of laser-induced transport processes was carried out with a Fastcam SA-3 high-speed camera (Photron, Japan) at a speed of up to 60,000 frames per second. The backlight was provided by the light of a continuous laser diode with a wavelength of 660 nm (14) formed by a telescope (15). The spectrum of the accompanying optical radiation in the range of 200-1100 nm was recorded by the USB4000 spectrum analyzer (Ocean Optics, USA).

To control laser-induced dynamic processes, an optoacoustic method was used [6, 7]. Acoustic signals were recorded with a (Precision Acoustics, UK) microphone (1-mm

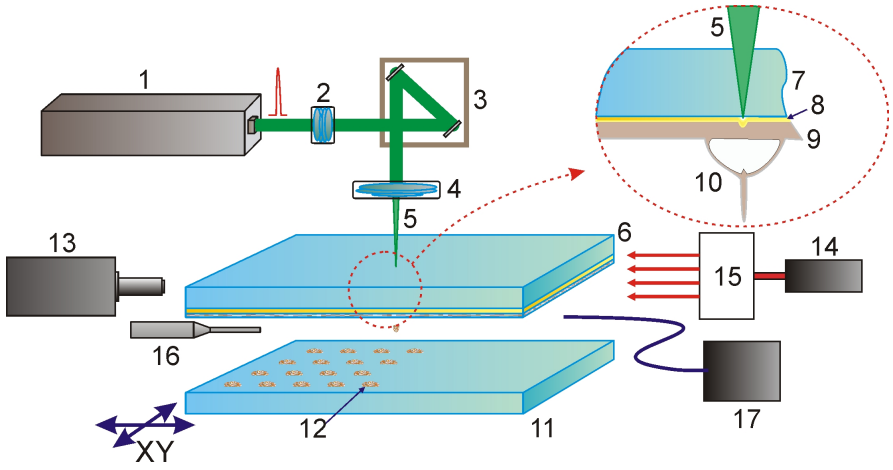

Figure 1: Scheme of installation for laser-induced transfer of gel microdroplets. *1 - pulse laser, 2 - beam shaper, 3 - galvano-scanner, 4 - lens, 5 - focused beam, 6 - donor plate, 7 - glass plate, 8 - Au film, 9 - gel substrate layer, 10 - substrate jet, 11 - acceptor plate, 12 - microdrops, 13 - high-speed camera, 14 - continuous wave laser, 15 - telescope, 16 - acicular hydrophone, 17 - fiber spectrometer.*

in diameter) with a preamplifier; acoustic signals were detected in the range from 10 kHz to 50 MHz The energy of laser pulse was monitored by S310C and S144C sensors and a PM100D (Thorlabs) power meter. The samples were analyzed by an optical 3D microscope HRM-300 Series, (Huvitz, Korea) and a scanning electron microscope PHENOM ProX (Phenom World, the Netherlands) with an energy-dispersive detector module. Gold nanoparticles in gel droplets on an acceptor plate were observed using SEM and the original threshold algorithm of SEM images processing.

3. Results and Discussions

Focused the laser pulse causes the transfer of a small amount of gel substrate from the donor to the acceptor plate. In the gold-absorbing coating on the donor plate, holes are formed, which are clearly observed by an optical microscope. The structure of the holes in gold coating is clearly visible in the SEM images (the tab in Figure 2a). Holes are almost circular in shape; inside they contain darker areas in the form of concentric circles or rings. Analysis of SEM images showed that the rings in the inner part of the hole consist of gold nanoparticles (Au NP) with a size of 10-200 nm. As the energy of the laser pulse - **E** increases, the size of the holes in the absorbing gold layer and the diameters of the rings (outer and inner) increases (Figure 2a). With increasing **E**, the diameter of the microdrops on the acceptor plate also increases (Fig. 2b). As can be seen from Fig. 2b, the size of the microdroplets is inversely proportional to the thickness of the absorbing Au film.

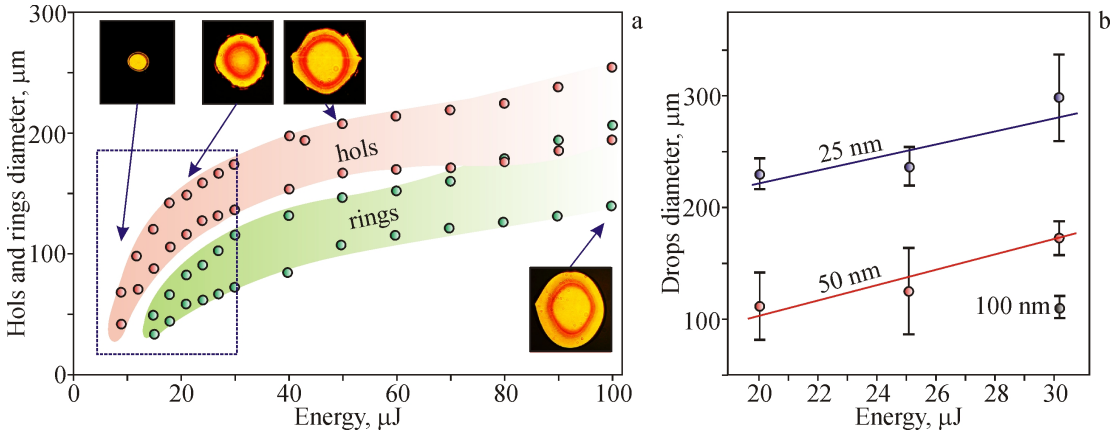

Figure 2: Dependences of the diameters of the holes and rings of nanoparticles in the gold absorbing layer on the donor plate (a) and the size of the droplets of the gel on the acceptor plate (b) on the energy of the laser pulse. *The thickness of the Au film: a - 50 nm, b - 25, 50 and 100 nm. Laser pulse duration is τ = 8 ns. A rectangle marks the area with the most accurate and stable holes in the absorbing film. The insets of Fig. 2a show the corresponding SEM images of the holes and rings in the gold absorbing layer.*

Our measurements have shown that a significant part of the laser pulse energy passes through the gold absorbing layer. Figure 3 demonstrates the dependence of the transmittance of the gold layer of the donor plate on the laser pulses duration τ (pulse energy **E** = 20 μJ). Transmission monotonically increases from ~ 10% to ~ 45%. with increasing τ value from 4 to 200 ns.

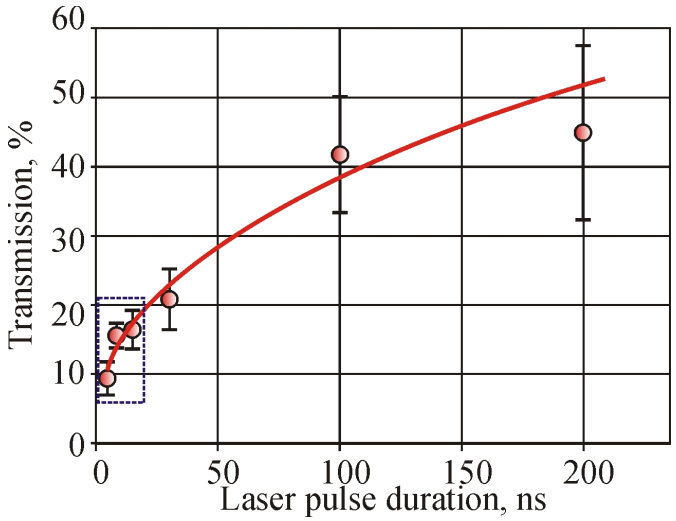

Figure 3: Dependence of the transmission of pulsed laser radiation on the duration of pulses. *Energy of laser radiation in a pulse E = 20 μJ. A rectangular region is selected with the lowest transmission values.*

When the gel substrate is transferred from the donor plate to the acceptor one, a number of nanoparticles from Au film are carried along with the jet. Fig. 4 shows an example of images illustrating the process of laser-induced segregation of gold nanoparticles from a droplet of gel with soil microparticles. The results of mathematical processing of SEM images showed that the amount of gold in droplets of gel in the form

of nanoparticles and microparticles increases on average with increasing the energy pulse E. In the example shown in Fig. 4, the total area of Au NP was 1.6% of the area of the drop.

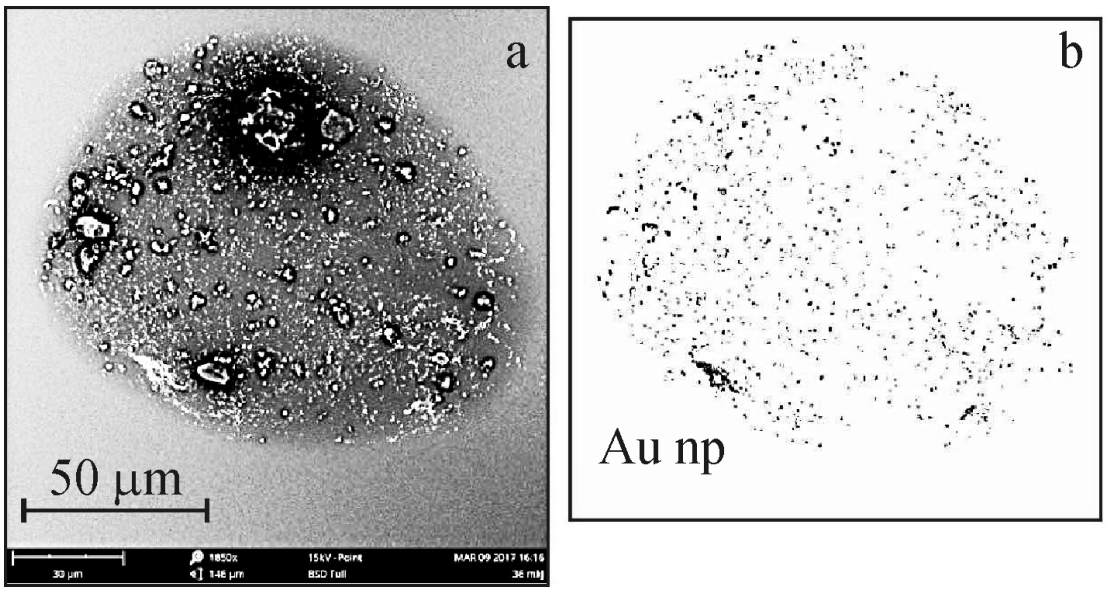

Figure 4: An example of a SEM image of a droplet of a gel with soil microparticles (a) and the distribution of Au NP in this drop (b). **τ** = *8 ns,* **E**=*20 μJ*.

Study of transport process of gel substrate with a high speed video showed that the transfer of gel is accomplished with microjets (2 in Fig. 5). Microjets are formed by the laser pulse energy exceeding the threshold value $\mathbf{E}_{th}$. A small bubble is emitted at the bottom of microjet (1 in Figure 5). The velocity of the microjets increases with increasing energy **E**. For a pure gel, the threshold value $\mathbf{E}_{th}$ = 12±1 μJ, the velocity is **V** = 30 ± 10 m/s at **E** = 15 μJ, and is **V** = 50 ± 16 m/s at **E** = 30 μJ. Addition of soil particles to the gel changes the parameters of the laser transfer significantly. From the comparison of Fig. 5a and Fig. 5b it can be seen that the addition of soil microparticles to the gel leads to a thickening of the jet (2) and of the bubble at its bottom (1). Also. our estimation showed about 5-6 fold decrease in the microjet velocity.

The mechanism of laser-induced transfer is associated with the initial absorption of laser pulse in the gold layer (Fig. 1). At a low laser pulse intensity with **λ** = 1064 nm, ~ 0.15% of the energy passes through the absorbing gold layer 50 nm in thickness [5]. In our case, the transmission is much larger. It depends both on the energy and on the duration of the laser pulse. When **E** = 20 μJ the transmittance monotonically increases from ~ 10% to ~ 45% (Fig. 3) with increasing **τ** from 4 to 200 ns). From the point of view of minimizing the impact on living systems, a range of τ from 4 to 20 ns can be recommended (Fig. 3).

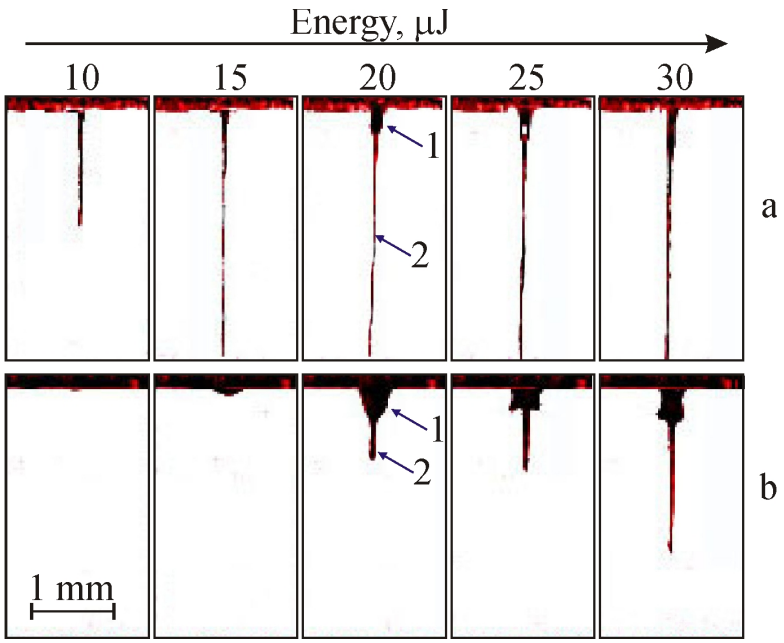

Figure 5: Generation of microjets when laser with different energies of laser pulses exposes a donor plate with gel (a) and gel with soil microparticles (b). 1 - bubble; 2 - jet of pure gel or gel with filler. *Pulse duration* **τ** *= 8 ns, pulse energy* **E** = 10 μJ.

Absorption of the laser pulse leads to heating of the absorbing film and the adjacent layers of the substrate and gel to high temperatures (>>10^3K). The depths of thermal heating of these layers increases with time t: for glass $d_T^G = \sqrt{4 \cdot D_T^G \cdot t}$, where D_T^G=3.4·10^{-7} m^2/s - thermal diffusivity of glass; for gel (water) $d_T^W = \sqrt{4 \cdot D_T^W \cdot t}$, where D_T^W =1.4·10^{-7} m^2/s - thermal diffusivity of water [5]. In the gel, bubble formation and cavitation collapse occurs due to the explosive boiling of water [7, 8], (insert in Fig. 1, Fig. 5). At a temperature T_2≈7000 °K (critical temperature of gold T_c^{Gold}=7250 °K) an explosive boiling of the gold film takes place, with the formation of an expanding bubble of atoms and Au NP. The kinetic energy passes into the acceleration of its jet (Fig. 5) its velocity is directed from the plate perpendicular to it because of the presence of a solid boundary. The dynamics of the jet depends essentially on both its exciting energy and on the parameters of the substrate (thickness, viscosity). The addition of soil particles to the gel causes the increase in viscosity in a significant decrease in the jet velocity (Fig. 5).

Evaporation of a part of gold film and removal of Au NP from the region of exposure leads to the formation of holes in the absorbing layer (Fig. 2a). With E value rises, both the dimensions of these holes (Figure 2a) and the diameter of the microdrops on the acceptor plate (Fig. 2b) increases. The region with the most accurate and stable holes in the absorbing layer (7 μJ≤E≤30 μJ) is shown in Fig. 2a with a rectangle. The

resulting inverse dependence of microdroplet size on the absorbing film thickness (Fig. 2b) can be rationalized as follows. For the constant absorbed energy, an increase in the thickness of the absorbing layer leads to a gradual decrease in the temperature jump. The decrease in temperature will accordingly reduce the energy of hydrodynamic processes.

The processes described above can have a significant effect on living cells in the gel. Another factor of influence in a laser microprinting can be gold microparticles and nanoparticles that have come from the donor plate to the acceptor plate and are trapped by a drop [9]. As it was shown in [5] at **τ** = 8 ns, the percentage (in area) of the Au NP content in the drops of a pure gel increases almost linearly with **E**. When using a gel with soil particles in the operating regime of energies and durations, a certain amount of Au NP is transferred. At **E** = 20 μJ, **τ** = 8 ns (Figure 4), the total area of Au in the microdroplet of the gel was ~ 1.6% of the droplet area (Fig. 4). When optimizing the laser exposure, E ≤ 20 μJ can be recommended, since Au NP can have undesired toxic effects on microorganisms and cell systems [9].

Thus, laser transfer technology allows transferring carry a precise amount of desired substances (individual cells and their agglomerates, microorganisms, particles). Such approach in our LEMS technology makes it possible to realize the process of laser isolation of microbial objects from soil carriers in order to isolate hard-cultivated and noncultivated microorganisms. They can be used in biomedicine and pharmaceuticals as sources of new biologically active substances and antibiotics.

4. Summary

Transport processes were studied in the course of the transfer of gel microdrops under the action of laser pulses with a wavelength of 1.064 μm, duration in the range **τ** 4-200 ns, and energy **E** from 2 μJ to 1 mJ. the processes of explosive boiling of water and gold are shown to play an important role in the mechanism of laser-induced transfer. It is revealed that for operating energy values the transmission of laser radiation λ = 1.064 μm through the donor plate increases with increasing pulse duration. From the point of view of minimizing the impact on living systems, a range of τ from 4 to 20 ns is recommended. It is shown that the volume of transferred Au NP increases with energy of the laser pulse. Therefore, to reduce the undesirable negative effect of Au NP on living systems, it is recommended to use the values of **E** ≤ 20 μJ.

Acknowledgments

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