Prospects of using a microchip-laser with a wavelength of 1.5 μm in laser bioprinting

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Received May 4, 2025
Revised June 12, 2025
Accepted June 16, 2025

The study confirmed the feasibility of using a pulsed microchip-laser ($\lambda = 1.5\,\mu$m, $E \approx 300\,\mu$J, $\tau \approx 4$ ns, $f = 10$ Hz) for creating compact bioprinting systems using laser-induced forward transfer in sterile conditions. At optimal laser parameters ($E \approx 6\,\mu$J, $w_0 = 17 \pm 2\,\mu$m, $F \sim 1.3$ J/cm^2), the transfer of hydrogel microdroplets was successfully implemented. An important result was the detection of the positive feedback effect when using a Ti-absorbing layer on a donor substrate, which must be taken into account when developing new laser bioprinting systems.

Keywords: microchip-laser, laser-induced forward transfer, laser bioprinting, laser engineering of microbial systems.

DOI: 10.61011/TPL.2025.09.61826.8014

Laser bioprinting by laser-induced forward transfer is an advanced technology used in microbiological and medical research for various purposes, including for the fabrication of biomaterial structures [1], in regenerative medicine, tissue engineering, and microbiology. One promising trend in its development is laser engineering of microbial systems (LEMS) [2]. This technology was demonstrated to be highly efficient in isolating microorganisms that are difficult to cultivate using traditional methods [2], which is essential for the development of new antibiotics and biologically active substances.

In a traditional bioprinting setup, a nanosecond laser pulse (in most cases, near-infrared) is focused onto a transparent donor substrate with an absorbing metal film, onto which a hydrogel layer with living microobjects is deposited for transfer [3]. The absorption of pulse energy initiates the formation of a vapor-gas bubble, which produces a jet and induces the separation of a droplet containing living objects for transfer (living microorganisms and cells) [4,5].

The existing laser bioprinting systems are quite efficient, but often remain bulky and ill-suited for operation in sterile laminar flow units. At the same time, the scientific community is in need of compact and functional laser bioprinting and LEMS equipment adapted for operation in specialized microbiological laboratories. Such compact systems could become a new indispensable and effective tool in the hands of microbiologists [3]. One promising approach to solving this problem is to use microchip-lasers as the basis for a system of this kind.

The aim of the present study is to analyze the applicability of pulsed microchip-lasers with $\lambda = 1.5\,\mu$m in the design of an efficient laser bioprinting system. Such laser will not only make the system smaller, but also reduce its weight and cost and provide a higher level of safety.

A microchip-laser (Crylink, China) with $\lambda = 1.5\,\mu$m, pulse energy $E \sim 300\,\mu$J, pulse duration $\tau = 4$ ns, and pulse repetition frequency $f = 10$ Hz was used in experiments. Standard LEMS donor substrates in the form of glass plates with a deposited 50-nm-thick Ti layer were used.

At the first stage, the distribution of laser radiation intensity in the waist region was studied. Experiments were carried out using a setup with horizontal positioning of the laser beam and vertical positioning of the donor substrate (Fig. 1). Microchip-laser radiation was focused on the absorbing coating of the donor substrate by an aspherical lens with numerical aperture NA = 0.15 and a focal length of 18.4 mm (Thorlabs, United States). The laser pulse energy was adjusted using a polarizer (Edmund Optics, United States) and an energy meter (Gentec, Canada). A digital camera (ToupCam, China) and a high-speed camera (MindVision, China) were used for positioning and recording of dynamic processes. The single-pulse mode was set, which was established by shifting the metal-coated donor substrate in the direction perpendicular to the optical axis. The laser beam width in the far field was measured using the „knife“ method, and the beam width in the waist region was determined by means of a series of holes in the metal film. The temperature distribution in the exposed region of the Ti film was determined in accordance with the procedure outlined in [6].

At the second stage, the data obtained at the first stage were used to assemble a prototype laser bioprinter with vertical positioning of the laser beam and horizontal positioning of the donor substrate, which is standard for bioprinting systems and LEMS. A hydrogel layer (2% solution of medium molecular weight hyaluronic acid) with a thickness of $\sim 200\,\mu$m, which is often used in LEMS [3], was applied in this case to the donor plates. A Photron Fastcam SA3 high-speed camera with a framing rate up to 120,000 fps

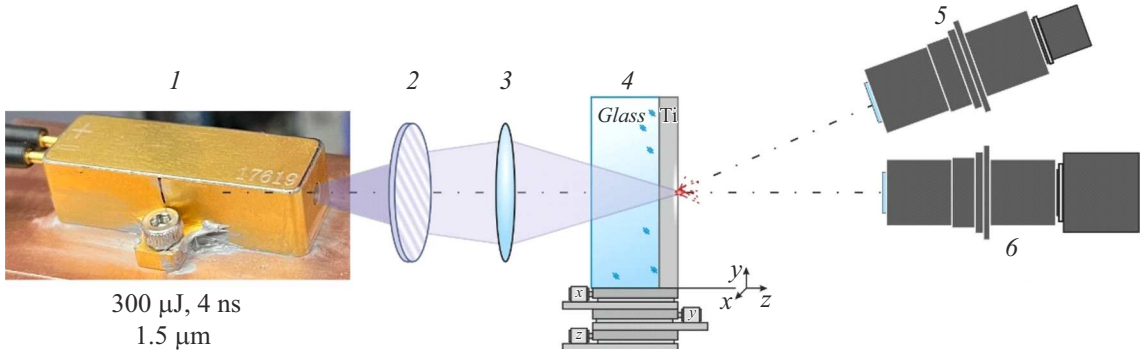

Figure 1. Functional diagram of the experimental setup with horizontal laser beam positioning. *1* — Microchip-laser, *2* — polarizer, *3* — focusing lens, *4* — donor substrate (glass plate with Ti-coating), *5* — high-speed camera, and *6* — digital camera.

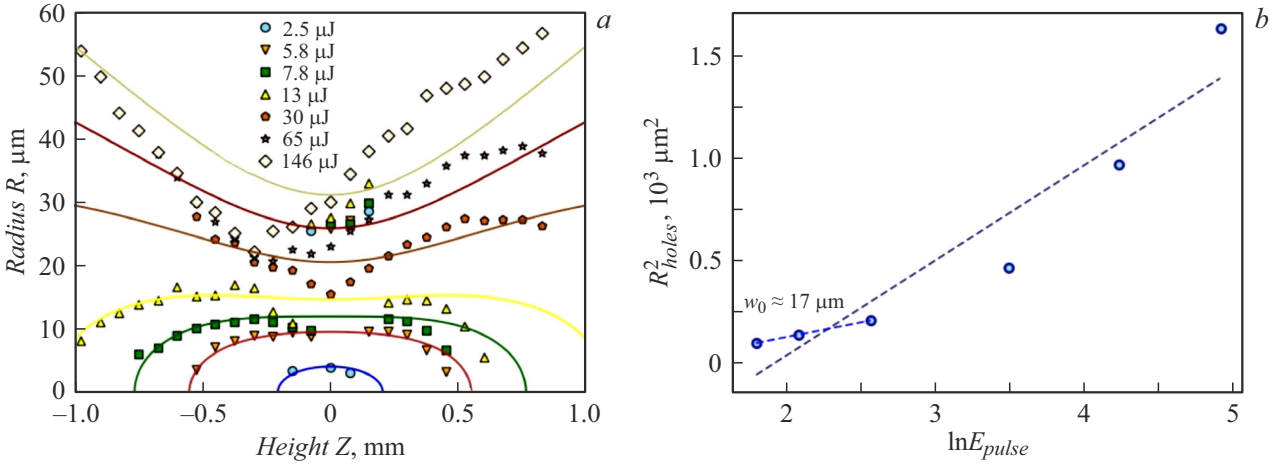

Figure 2. *a* — Radii of holes in the Ti film and intensity isolines in the laser waist region for different E and Z (from the origin of coordinates to the periphery): 117, 163, 153, 150, 86, 35, and 10 MW/cm^2. Pulse energies E for each series of holes are indicated. *b* — Dependence of the square of hole radius on the logarithm of pulse energy at $Z = 0.22 \pm 0.08$ mm.

was used additionally to record dynamic processes. The results are presented in the form of mean values with standard deviations.

Figure 2, *a* presents the isolines of radiation intensity in the laser waist region. The radii of holes in the Ti film of the donor substrate, which were obtained at different energies of laser pulses and with the substrate plane moving along the optical axis relative to the focal plane, are shown in the same figure. It can be seen that $E \sim 6\,\mu$J is the optimum mode. This energy, on the one hand, ensures resistance to defocusing within a wide range of displacements (~ 1 mm) and, on the other hand, provides high stability of the energy density (~ 1.3 J/cm^2) and, consequently, reliable transfer within the standard LEMS range [3].

Figure 2, *b* presents the dependence of the square of hole radius on the logarithm of pulse energy in the focusing region. It is evident that points do not form a single straight line, which is what should hold true for a Gaussian beam. Strong deviations from a linear dependence at high energies may be attributed to nonlinear processes [7]. The waist radius determined from the slope of the linear trend for low E values was $w_{0\,exp} = 17 \pm 2\,\mu$m. Note that this value corresponds to the standard LEMS parameters.

An intriguing effect associated with positive feedback (the „mirror“ effect) in the cavity formed by the laser's reflecting mirror and the parallel metal film of the donor substrate [8] was discovered when the matrix of holes was formed. This led to a sudden sharp increase in radiation intensity, the formation of „giant“ holes in the Ti film, and glass chipping at the center of the spot.

Figure 3 shows the scanning electron microscopy (SEM) images of „standard“ and „giant“ holes at $E = 3\,\mu$J. It can be seen that the edges of both holes are melted, which indicates that the melting point of Ti (1670 °C) has been reached at the edges. Approximation (Fig. 2, *b*) revealed that the energy increased spontaneously by a factor of 9 during the formation of a „giant“ hole.

A setup with a vertical optical path was assembled (Fig. 4, *a*) based on the parameters of spatial transfer of gel microdroplets obtained at the first stage. Digital camera *5*

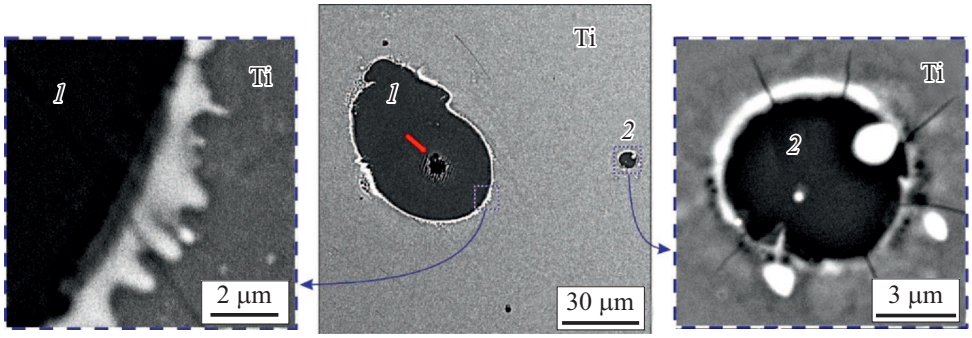

Figure 3. SEM images of „giant“ (*1*) and „standard“ (*2*) holes in the Ti film positioned in the focal plane that were obtained at the same set pulse energy ($E = 3\,\mu$J). The arrow points at a crater on the glass plate surface.

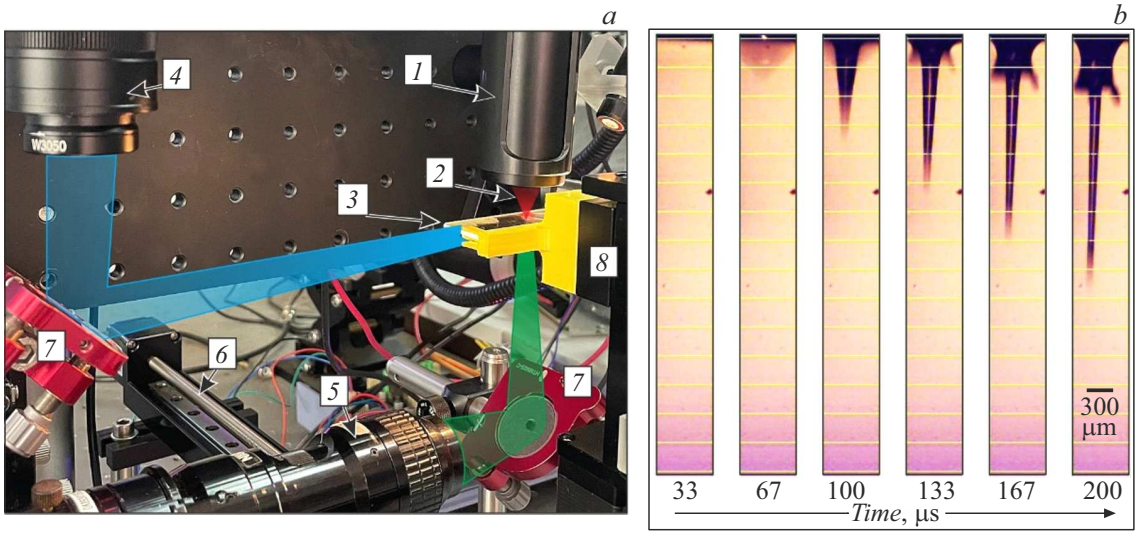

Figure 4. *a* — Image of a part of the experimental setup with a vertical optical path. *1* — Telescope with a focusing lens, *2* — laser radiation, *3* — donor substrate with a Ti film, *4* — high-speed camera, *5* — digital camera, *6* — motorized adjusters, *7* — mirrors, and *8* — donor substrate holder. *b* — Successive frames from high-speed video recording of the formation of a hydrogel microjet. $E = 6\,\mu$J.

was used to monitor the shift of the donor plate. Microjets were recorded using high-speed camera *4* with a framing rate of 30,000 fps. Figure 4, *b* presents an example of a hydrogel microjet with an initial transfer velocity of 84 ± 5 m/s.

The obtained results verified the applicability of a pulsed nanosecond microchip-laser with $\lambda = 1.5\,\mu$m in the design of a compact laser bioprinting system. Stable transfer of hydrogel at a rate of 84 ± 5 m/s was demonstrated at the optimum parameters ($E \sim 6\,\mu$J, $w_0 = 17 \pm 2\,\mu$m, and an energy density of ~ 1.3 J/cm^2). The production of a mobile bioprinting and LEMS system of this kind will significantly expand the capabilities of specialized biomedical laboratories that are in need of precise, efficient, and convenient tools for working with living cells in sterile laminar flow units.

Funding

This study was carried out under the state assignment of the National Research Center „Kurchatov Institute.“

Conflict of interest

The authors declare that they have no conflict of interest.

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Translated by D.Safin