Laser microprinting of liquid suspensions

Author: Albert Van Eeckhout Alsinet
Advisor: Pere Serra
Facultat de Física, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain.*

Abstract: Laser-induced forward transfer (LIFT) is a non-contact direct-write technique with a high spatial resolution and able to print a wide range of liquids in air and at room temperature. The versatility of LIFT has made it a promising alternative to lithography-based processes for the rapid fabrication of biomolecule microarrays. The aim of this work is the study of LIFT using femtosecond laser pulses and a solution suitable to act as a solvent for biomolecules. The influence of the main process parameter, laser pulse energy, on the morphological properties of the transferred droplets is analyzed. Circular and uniform droplets are obtained with good reproducibility. In addition, we observe a relationship between energy and droplet radius never identified before: a linear dependence of the radius to the fifth power with laser pulse energy.

I. INTRODUCTION

The development of highly integrated and miniaturized biosensors is increasingly growing due to recent advances in genetics and molecular biology. This is a competitive market in a constant demand for better strategies capable of increasing the speed, control, or resolution of the printing processes while in the same time decreasing the production costs. Pattern-transfer techniques such as photolithography are adequate for the large scale production of miniaturized biosensors, but in the field of rapid-prototyping applications they are not versatile enough. For these applications, an interesting alternative is direct-writing technologies, which allow a faster and less expensive production of the operative prototype. Although conventional direct writing techniques, like inkjet printing, are widespread [1,2], these techniques present limitations concerning the spatial resolution and the diversity of materials that can be used. Laser-induced forward transfer (LIFT) is a non-contact direct-write alternative to inkjet printing, being able to print a much wider range of liquid viscosities and rheologies, in air and at room temperature. In addition, LIFT has a potential higher spatial resolution. These characteristics make LIFT ideal for the development of highly integrated miniaturized biosensors.

In the LIFT technique, a laser beam is focused onto a precursor film (the donor film) to remove a part of the material from the donor and transfer it onto a receptor substrate (Fig.1). Due to the small separation between donor and receptor, and also to the highly focused laser beam, the desired high spatial resolution is obtained. Since its development by Bohandy *et al.* [3], LIFT using solid precursors has been widely demonstrated [4-6]. In this case the precursor is evaporated by the laser beam and then recondenses on the substrate. The problem of using solid precursors for depositing biomolecules is that these biomolecules suffer irreversible decomposition due to high temperature or simply direct interaction with the laser. The alternative of using a liquid precursor to transfer biomolecules in solution was first reported by Wu *et al.* [7] In the case of using a liquid precursor, the absorption of the focused laser beam generates a microbubble inside the liquid film which expands and creates a jet that transports the material to the receptor substrate, where it forms a drop (Fig.1.). In this case, the liquid solvent acts as transport vector for the biomolecules and prevents them from breakdown. Accordingly, various biological materials, including proteins [8-11], DNA [12-14], and cells [15-17] have been transferred without significant damage.

Although transferring microarrays of biomolecules in solution using LIFT has been considerable explored, the process has been studied mainly using nanosecond lasers [9,18,19,20]. The aim of this work is study LIFT using a femtosecond laser. In this study we analyze the influence of the main process parameter (laser pulse energy) on the morphological properties of droplets transferred using LIFT. The solution chosen to carry out the study is suitable to act as a solvent for biomolecules. In this work, we observe a relationship between energy and radius previously undetected.

II. EXPERIMENTAL

The liquid microprinting setup consists of a femtosecond laser, an optical system and a set of xyz translation stages. LIFT was carried out through the use of a pulsed ytterbium (YB:KYW) diode pumped laser (Amplitude Systemes, S-pulse), with 1027 nm wavelength and 450 fs pulse duration. The laser beam has a roughly circular Gaussian intensity distribution (Fig. 2). The optical system is composed of a crossed polarized attenuator and beam splitter for energy control and subsequent measurement, followed by a series of mirrors, whose purpose is to guide the laser radiation toward a microscope objective (50x, 0.55NA, 13 mm working distance). The objective tightly focuses the laser beam onto the donor substrate, a transparent glass slide coated with a titanium thin film with a thickness of around 50 nm, as shown in Fig.1. The transferred solution consisted of a mixture of water and glycerol, both at a concentration of 50% (v / v), and a surfactant [sodium dodecyl sulphate (SDS)] dissolved at a concentration of 1.0 mg/ml. The titanium coating acts as an absorbing layer. The use of absorbing layers of materials

* Electronic address:tfgac@ub.edu

such as Au [22], Ti [9,14,21], and Ag [22] has been already tested with positive results in the transfer of biomolecules and cells.

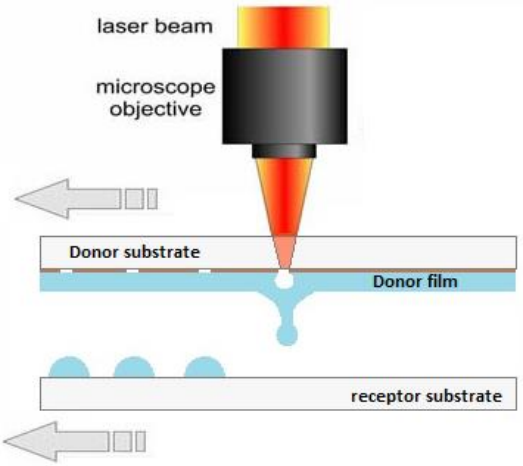

FIG. 1: Principle of operation of the LIFT technique.

A volume of 60 µl of the prepared solution was spread on the titanium thin film with a blade coater. The liquid film thickness, estimated through the measurement of the film weight, was approximately 40 µm. The system formed by the titanium-coated slide and the solution thin film has usually been referred to as the ribbon [23]. The receptor substrate is a commercially available glass slide treated with poly-*L*-lysine, a conventional receptor substrate for biomolecule microarrays production. The ribbon was placed parallel to the receptor substrate, with the liquid film facing the poly-*L*-lysine-treated surface at a distance of 50 µm.

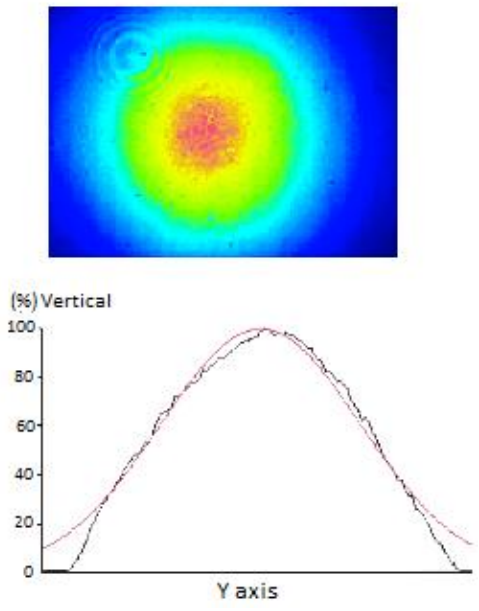

FIG. 2: Plot of the YB:KYW laser beam intensity distribution.

The ribbon-substrate system was laid on a *xyz* translation stage that permitted the precise translation of the ribbon-substrate system with respect to the laser beam. The transfer was carried out in such a way that each single laser pulse was responsible for the deposition of a single droplet of the solution onto the substrate. After the deposition of each droplet, the stage was displaced at a distance of 400 µm, and another droplet was transferred at another substrate position until each single row in the microarray was completed.

III. RESULTS AND DISCUSSION

Two microarrays of the described solution containing fifteen rows of 20 droplets each were deposited through LIFT at two different positions around the beam waist (10 µm above and below the waist). For each microarray the laser pulse energy was varied from one row to another. Once deposited, the microarrays were observed under an optical microscope to check the properties of the droplets and to measure their dimensions. Likewise, the spots produced by the laser beam on the titanium layer were also analyzed by optical microscopy. The obtained images for both droplets and spots are presented in Fig.3.

The titanium spots, which diameter increases with the laser pulse energy, can be used to determine the dimensions of the beam on the sample and the fluence ablation threshold of titanium. Magnified images of two selected spots, both in reflection, are presented in Fig. 4a. The spots are roughly circular and have a different shape depending on the focusing position. The spots obtained 10 µm above the beam waist are rather uniform, but those obtained 10 µm below the waist present several rings that are observed for all the analyzed energies. At 10 µm below the waist, the beam is focused inside the glass; therefore part of the energy can be absorbed by the glass. This absorption can modify the beam distribution on the metallic film and produce the rings and larger spots. For this reason, the sample used in the analysis is that corresponding to 10 µm above the waist, in which the beam is completely absorbed by the titanium coating.

The local fluence (energy per unit area) distribution for a Gaussian laser beam is (Fig.2b) is given by

$$F(r) = \frac{2E}{\pi\omega^2} e^{-2r^2/\omega^2}, \qquad (1)$$

where r is the spot radius, E the laser pulse energy and ω the beam radius (distance around the beam axis from which the local fluence decreases a factor $1 / e^2$).

Accordingly, and assuming that titanium has a laser ablation threshold F_0 the radius of the spots should scale with laser pulse energy as:

$$r^2 = \frac{\omega^2}{2}\left(lnE - ln\frac{\pi\omega^2 F_0}{2}\right) \qquad (2)$$

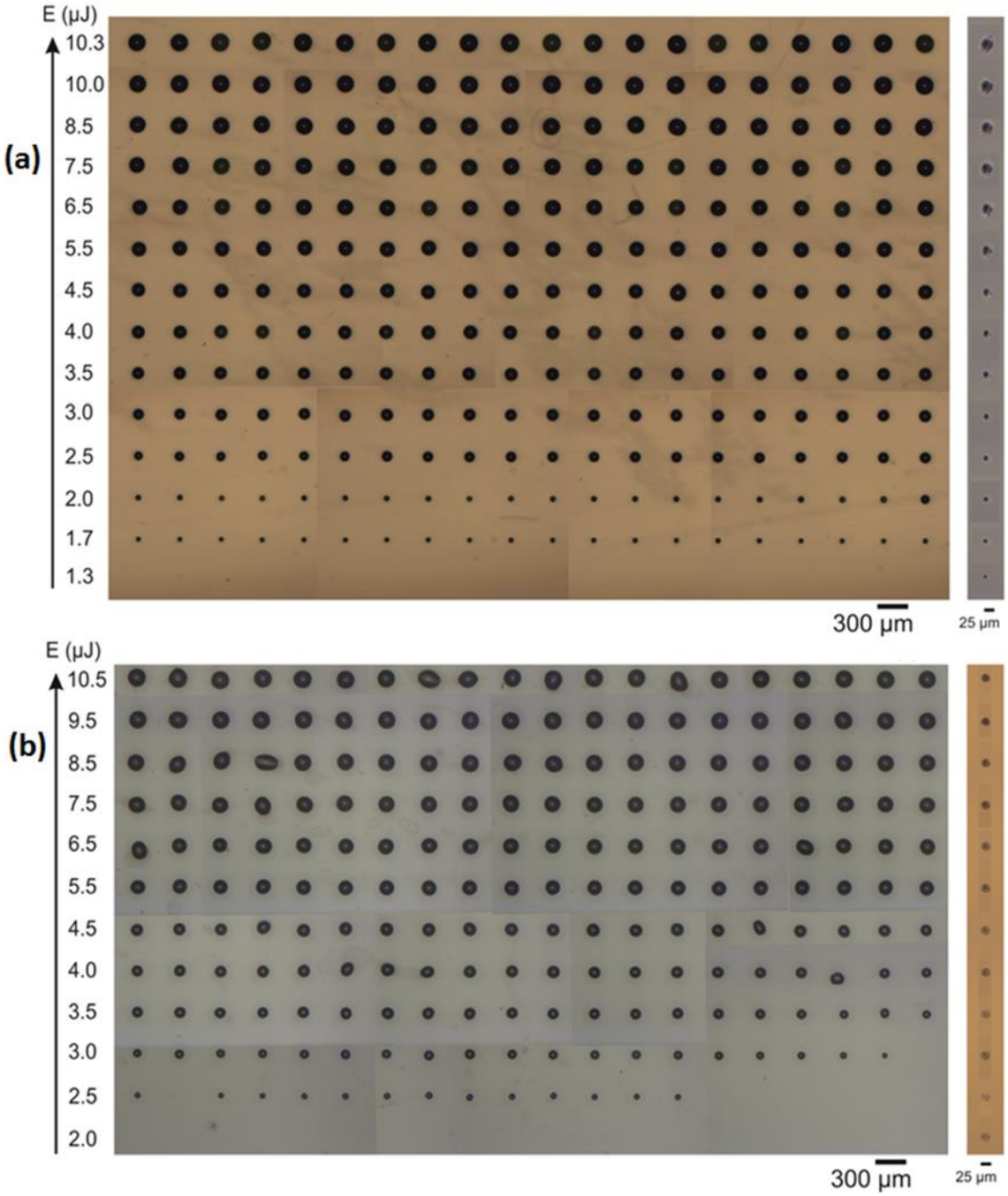

FIG. 3: Optical microscopy images of the microarrays prepared at a) 10 μm above and (b) 10 μm below the laser beam waist. All the droplets in each row were transferred at the laser pulse energy indicated in the left part of the image. A selection of the spots ablated on the titanium film is shown in the right.

This relationship gives rise to the representation of the square radius of the spots vs the logarithm of the energy presented in Fig. 4b. The spots dimensions were determined using appropriate software to identify the circle best suited to the spot perimeter. The figure represents a clear linear dependence. From this dependence the values of F_0 and ω can be obtained. These are, respectively, 0.8 J/cm^2 and 13 μm.

In all the microarrays in Fig. 3 the droplet dimensions increase with the laser pulse energy. Therefore, for a fixed laser beam the amount of deposited material from the liquid film is larger the higher the energy of the beam is. It can be observed at low energies that there is a minimum energy E_{min} below which there is no deposited material. This minimum energy is different for the two positions, being higher for the sample located 10 μm below the beam waist. This is consistent with the aforementioned possible interaction between laser and glass: since a fraction of the pulse energy is absorbed by the glass of the donor substrate, a higher energy is required to propel a droplet.

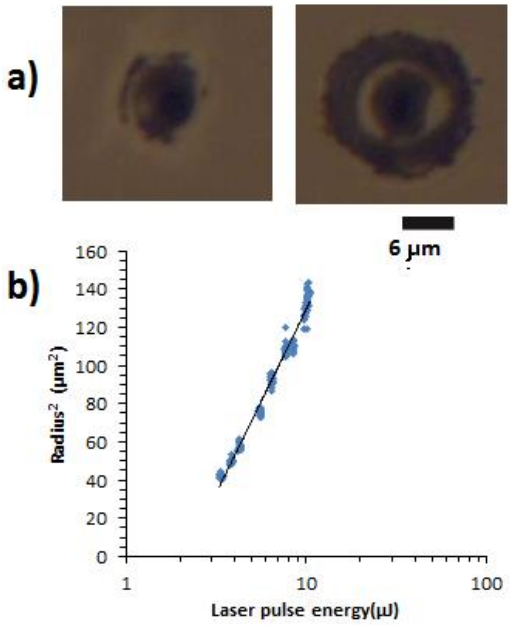

FIG. 4: a) Images of the spots for the two positions: 10 µm above (left) and below (right) the laser beam waist. Both spots are generated at a laser pulse energy of 3.5 µJ. b) Plot of the radius squared of the laser spots vs laser pulse energy.

Circular and uniform droplets are obtained with good reproducibility, and the smallest droplet diameter obtained is approximately 50 µm. Thus, a precise control of the droplet diameter can be exerted. However, for the lowest energies in Fig. 3b some voids are obtained in conditions where droplets should have been printed. This is probably due to the fact that the layer of liquid is not perfectly uniform. Actually, creating a completely uniform liquid layer is very difficult with the method used here; the process of depositing the liquid layer was sometimes repeated over five times until obtaining a suitable liquid layer. Also, for energies lower than E_{min}, there are spots in the Ti layer in the absence of drops. A possible explanation is that here is enough energy to make a spot, generate a microbubble and consequently generating a jet, but this jet does not have enough energy to reach the substrate, as it has been found previously through time-resolved imaging of the process [19].

To better understand the relationship between the amount of material deposited and the laser pulse energy, the radius of all droplets (R) deposited on both arrays is measured and plotted versus the laser pulse energy (E) for the two analyzed positions around the beam waist (Fig. 5a). The relationship is clearly not lineal. As in previous studies using a nanosecond laser a linear dependence between the volume of the droplets and laser pulse energy [11,18,20]was found, R^3 is plotted versus E (Fig. 5b). Two different trends could be claimed for, one for high energies and one for low energies. A similar behavior was found out before [24], and it was attributed to a sudden change in the contact angle of the droplet. However, the evolution in the present case for each section (low and high energies) is not as linear as it was in that previous work.

So, a new dependence is assayed:

$$R^5 = K(E - E_0) \qquad (3)$$

where K and E_0 are constants. The plot (Fig. 5c) surprisingly reveals a clear linear relation between R^5 and E.

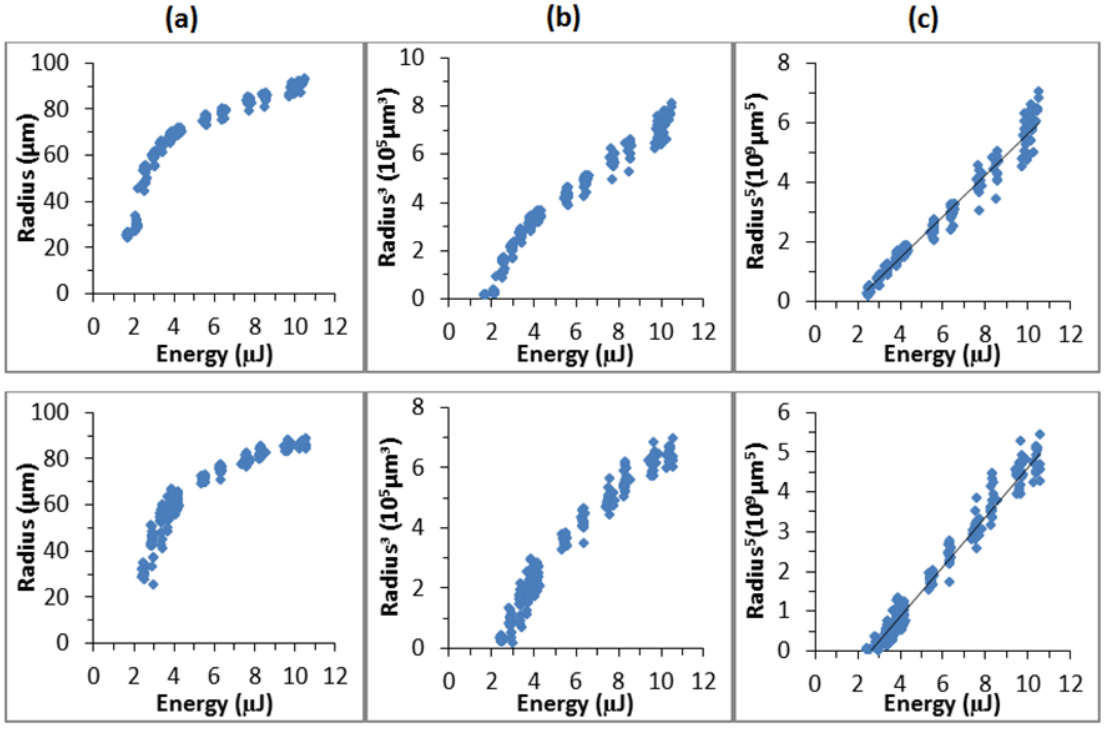

FIG. 5: Plots of a) the transferred droplet radius, b) radius to the third power and c) radius to the fifth power versus laser pulse energy for the two positions: 10 µm above (top plots) and below (bottom plots) the laser beam waist.

The proportionality constant K can be interpreted as the efficiency of the process. K values are similar for both focusing positions: $7.0 \cdot 10^8$ $\mu m^5/\mu J$ for 10 µm above and $6.3 \cdot 10^8$ $\mu m^5/\mu J$ for 10 µm below the beam waist. E_0 values are similar to E_{min} and also to each other, being 1.9 µJ and 2.6 µJ. Such astonishing dependence had never been found before; since there are practically no published works with femtosecond pulses, further investigation will be required.

IV. CONCLUSION

The observation by means of optical microscopy of the microarrays prepared using LIFT with femtosecond laser pulses reveals that circular and uniform droplets are obtained with good reproducibility. Therefore, it is possible to control the droplet diameter with high precision by focusing the laser in the right position and using the right laser pulse energy. The smallest droplet diameter obtained is approximately 50 µm, a value well suited for the production of miniaturized biosensors.

The analysis of the dimensions of the deposited droplets reveals an astonishing dependence of the droplet diameter with laser pulse energy not found before: a linear dependence of the radius to the fifth power with laser pulse energy. The determination of the origin of such dependence would require further investigation since the amount of work on LIFT with femtosecond pulses is still scarce.

Acknowledgments

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