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Impact of Laser Induced Forward Transfering on Transfer of Escherichia Coli Bacteria

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ABSTRACT

Laser Induced Forward Transfer (LIFT) is a technique uses to print different materials with high spatial resolution for microarray preparation. In this work, the mechanism in which LIFT process effect *E.coli* bacteria used to produce microarray droplets from a liquid solution thin film coated a glass slide previously coated with gold of 65nm thick which was used as a laser absorbing layer. The transferred bacteria was achieved at laser fluence of 10 J/cm^2 and 100 µm distance between donor and receptor substrates.

The purpose of using such a technique was to assess the impact of laser on the viability of the transferred bacteria. Scanning electron microscope observation indicated that *E.coli* bacterial cells integrity was maintained during the process. The bacteria was viable as tested using MacConkey agar culture media.

Keywords: Laser Induced Forward Transfer (LIFT), Nd: YAG Q-Switching (SHG), Biosensor, Microarrays.

الانتقال الامامي المحتث بالليزر لنقل بكتريا *E.coli*

الخلاصة

النقل الامامي المحتث بالليزر تقنية تستعمل لنقل مختلف المواد وبدقة مكانية عالية لتحضير مجموعات بحجم مايكرومتر في هذا البحث , الية تاثير البكتريا E.coli المستعملة لانتاج قطيرات مايكروية من محلول سائل على شكل غشاء يغطي شريحة زجاجية قد طليت مسبقا بالذهب بسمك 65 نانومتر والتي تستعمل كطبقة ماصة لاشعة الليزر , طاقة اشعة الليزر المستعملة لنقل البكتريا كانت 10جول/سم2 و 100مايكرومتر المسافة بين المانح والطبقة القابلة . الغرض من استعمال هذه التقنية هو تقييم تاثير الليزر على قابلية النمو البكتريا المنقولة , اشار المجهر الالكتروني الماسح ان البكتريا المنتقلة سليمة والتي تم فحص النمو بواسطة الوسط المغذي.

INTRODUCTION

Laser Induced Forward Transfer (LIFT) was first proposed by Bohandy *et al.* in 1986 [1] and has been investigated by many researchers due to its unique process and the ability to fabricate microstructures. The technique was initially

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developed to transfer inorganic materials from precursor solid films [1-3]. Its mechanism of operation was thought to consist of the complete vaporization of a small portion of the film which further recondensate onto the receptor substrate in the form of a solid dot. The possibility of transferring liquids with LIFT process has allowed depositing biomolecules in solution, in this case the solvent acts as their transport vector from the holder to the substrate. Since the transferred bimolecular preserve their bioactivity during LIFT, the absence of vaporization was indicated, this prevents the biomolecules from decomposition. Among the biological materials that have been deposited with this technique without loss of bioactivity were proteins [4-6], DNA [7-9], and cells [10-12]. The work focused on the capacity of the technique for printing functional materials, basically consist in presenting the LIFT deposited features of different functional materials and then analyzing whether the properties suitable for a specific application were preserved or not. In this way, ink or pastes of electrochemical materials have been deposited for the fabrication of Ultra-capacitors [13], batteries [14-17], chemical sensors [18] and electrodes for organic thin-film transistors [19-20] . Different kinds of biomolecules have been deposited through LIFT, and after submitting them to hybridization or immunofluorescence assays, the preservation of their activity has been demonstrated. Actually quantitative analyses have revealed that the fraction of biomolecules damaged by the action of the laser pulse is not high [9-21].

The aim of our work to prove the feasibility of this technique to transfer different biological solutions without harm, such bacteria cells to prepare of microarrays of droplets of solution through LIFT at laser pulse energy, which makes it useful in many clinical applications, such as medical diagnostic[8], biosensor[10] and tissue engineering [11] .

EXPERIMENTAL WORK

A pulsed Nd: YAG laser (Huafei Tongda Technology—DIAMOND-288 pattern EPLS) 532 nm wavelength of 10 ns pulse duration and energy of (20-100) mJ was used for absorption thin film deposition and LIFT process at fixed laser fluence of 10 J/cm^2. The laser was directed onto the target surface at an incident angle of 90◦, with the receiving substrate positioned below the target in different distances varied from 50 to 250µm (using Aluminum sheet spacers between the donor and the receptor substrates). Movement of the substrate was achieved via a translation stage with *X* and *Y* travel dimensions. The target consists of an optically transparent glass slides that has been coated with a gold thin film (thickness 65nm) which was deposited by pulsed laser deposition technique system as absorption layers as shown in Figure (1a) the choice of gold seemed appropriate since this element is biologically inert and also presents a good adhesion with glass. A solution of *Escherichia coli* bacteria was then spread on top of the absorption layer by means of spin coater, obtaining a film thickness of around 15µm (measured through the weight measurement). Bacterial culture coating solution volume was controlled by deposition via a micropipette. The *E. coli* cells were suspended in a solution of 80% standard Nutrient Broth (NB; 'Lab-Lemco' powder) and 20% glycerol (v/v), the presence of glycerol, reduced the evaporation rate of the solution. The receiving substrates were microscope slides which were rinsed with sterile water and ethanol than coated with poly-L-lysine Figure (1b). Under these conditions, microarrays was

prepared by displacing the stages 100µm between consecutive laser pulses, in such a way that every single laser pulse resulted in the deposition of a single droplet. The integrity of transferred bacteria was assessed with SEM imaging (TSCAN VAGA SEM, Czech repoplic). The viability of the transferred bacteria was tested using bacterial culture media (MacConkey Agar). This experiment was performed under the same conditions as mentioned above, but in this case a larger laser beam spot size were used, therefore the droplet diameters increase in this case and displacing the stages larger than 250 µm between consecutive laser pulses. The slide where the microarray deposited then immersed in a Petri dish containing 20 mg/ml of MacConkey agar culture media and incubated overnight at 37°C.

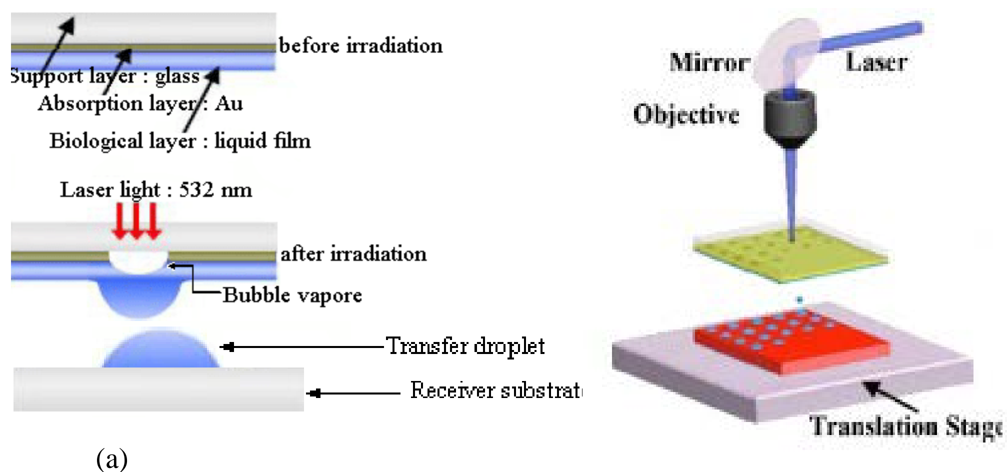


Figure (1) Scheme of the sample (a) Donor thin film before and after irradiation and Receiver substrate. (b) Laser induced forward transfer experimental setup.

RESULTS AND DISCUSSION

A highly ordered microarray of *E.coli* bacteria solution was generated on glass substrate coated with poly-L-lysine Figure (2) it was prepared at fixed film to substrate distance 100µm and fixed laser fluence 10 J/cm^2 and a liquid film thickness of about 15 µm. It is important to produce a uniform deposition having almost the shape and dimensions of the laser focal spot as shown in Figure (2) which has a good uniform deposited droplet of a shape close to the laser spot size and good amount of deposited material. Scanning Electron-Microscope (SEM) images of one droplet of the *E.coli* solution coated the gold thin film after transferred on the receiving substrate at 10 J/cm^2 laser fluencies is shown in figure 3. It appears from these images that *E.coli* cells separated and distributed through on the surface of the transferred gold thin film. It can be clearly seen that this process was effective in printing transfer of *E.coli* cells. One more observation of transferred *E.coli c*ells was that the structural integrity as array pattern, this indicate all bacteria that transferred on substrate weren't lose during transferred, otherwise cell integrity may random shape. The bacterial cells were imbedded in the gold thin film, but this impediment did not affect the cell integrity.

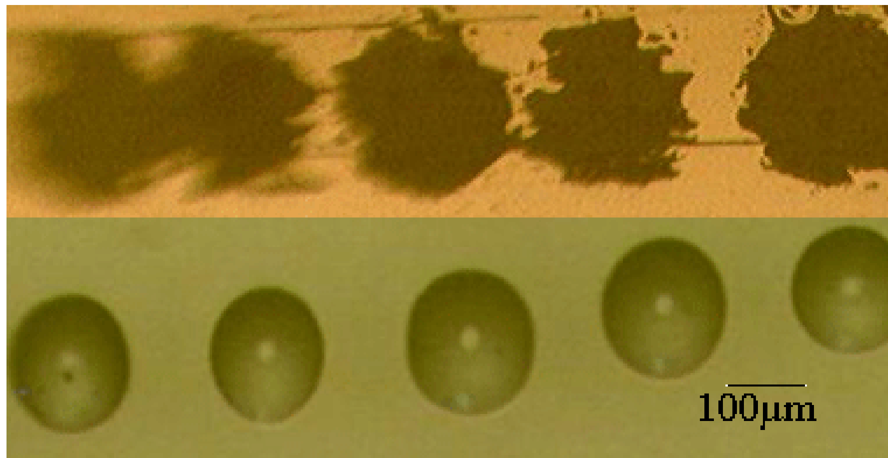


Figure (2) Optical microscopy images prepared at fixed laser Fluence (10 J/cm^2), the upper row refers to the donor thin film and the lower row refers to the receiver substrate (Transferred solution).

In order to assess the viability of the transferred bacteria, other microarrays were prepared. The bacterial deposition was carried out using a single laser shot per transfer spot. In this case, cells were found to be viable in the transfer area as indicated by colony formation within 24 h.

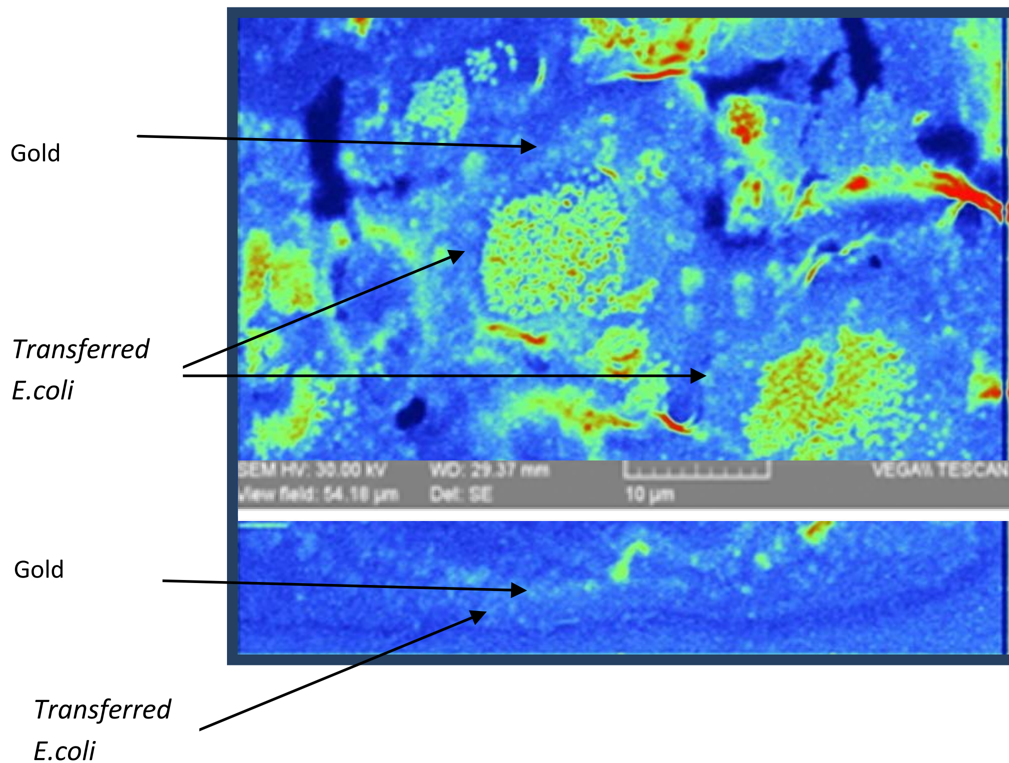
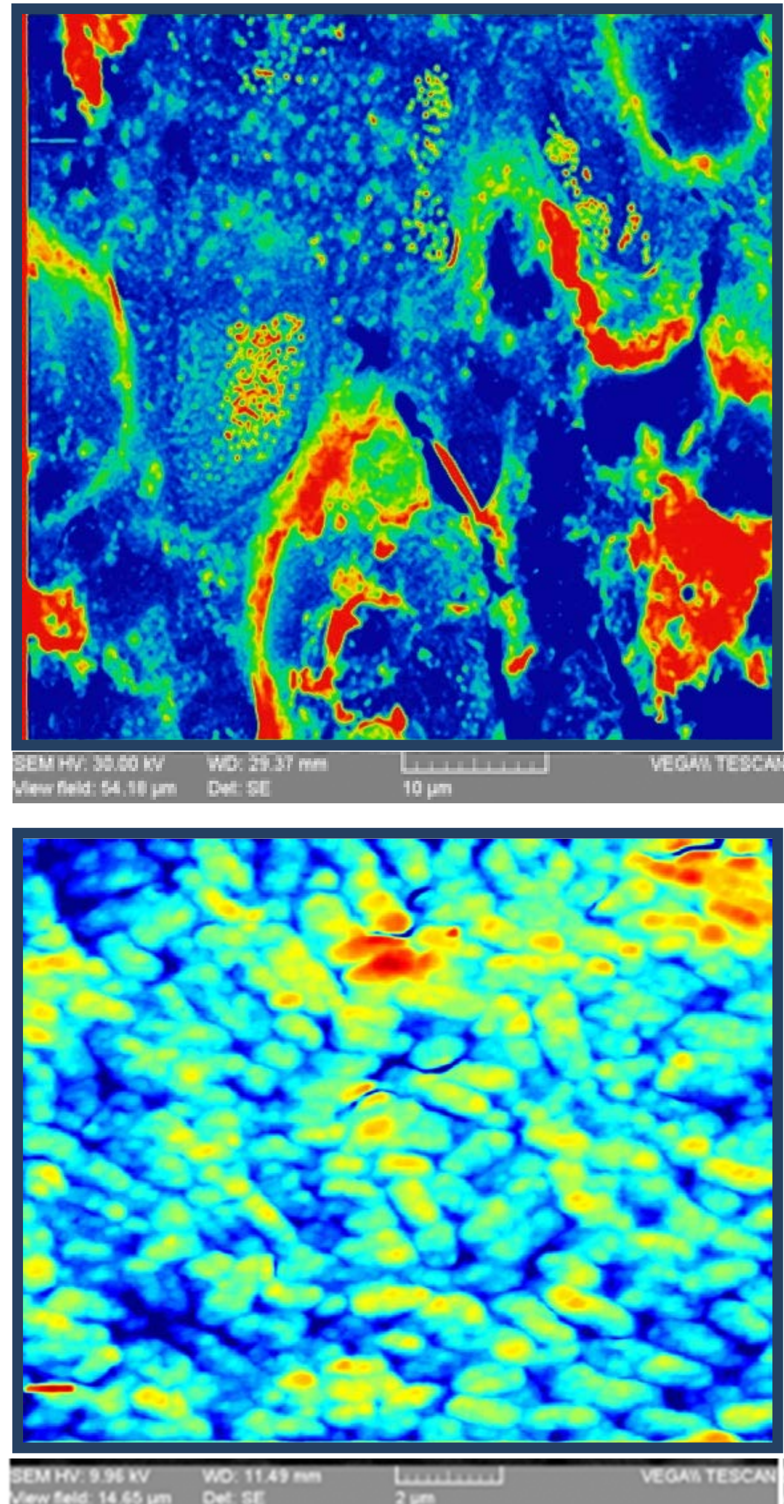


Figure (3) to be continued



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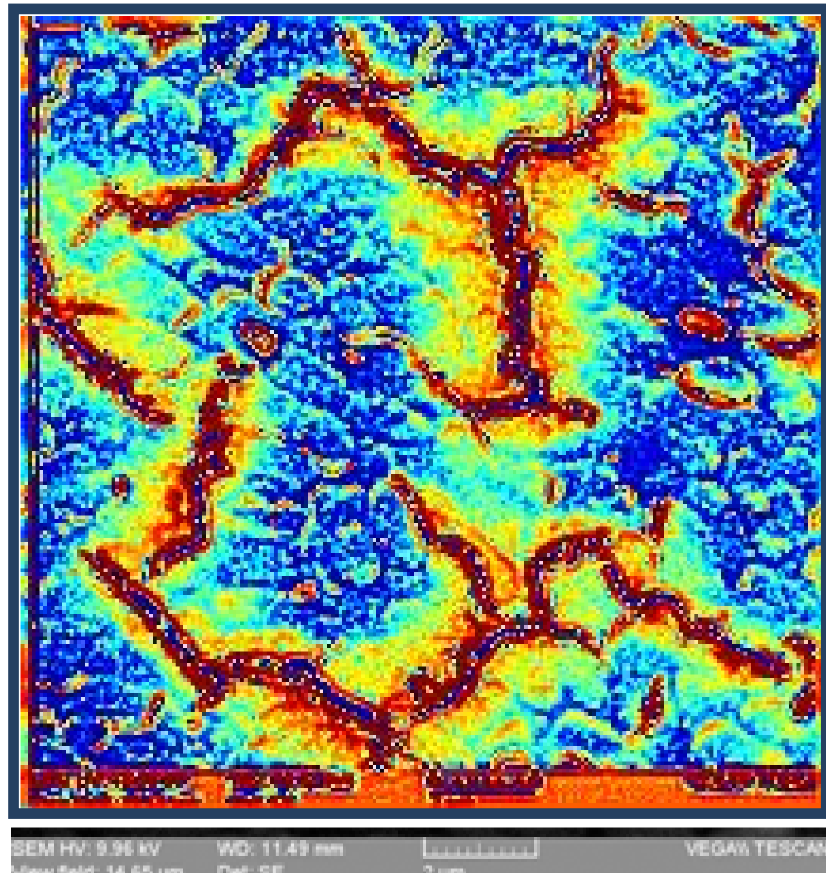

Figure (3) SEM images of E.coli cells transferred by LIFT process at fixed laser fluence 10 J/cm^2.

CONCLUSIONS

For future production of biosensor systems, a large number of biomaterials will need to be accurately placed onto small areas. LIFT is an excellent technique for this type of work, as it is able to transfer very small volumes of material in a precise and accurate manner. At 100μm, well-defined and circular droplets with clean counter were obtained. The pink spots of the deposited droplets indicate that the bioactivity of the transfer droplets was kept during the LIFT process at laser fluences 10 J/cm^2.

REFERENCES

[1]. Bohandy, J. B. F. Kim, and J. Adrian, Metal deposition from a supported metal film using an excimer laser, J. Appl. Phys. **60**, 1538 (1986).

[2]. Fogarassy, E. C. Fuchs, F. Kerherve, G. Hauchecorne, and S. Perriere, Laser-induced forward transfer: A new approach for the deposition of high Tc superconducting thin films, J. Appl. Phys. **66**, 457 (1989).

[3]. Kántor and T. Szörényi, Z. Dynamics of long-pulse laser transfer of micrometer- sized metal patterns as followed by time-resolved measurements of reflectivity and transmittance, J. Appl. Phys. **78**, 2775 (1995).

[4]. Ringeisen, B.R. P.K.Wu, H. Kim, A. Pique´, R.Y.C. Auyeung, H.D. Young, D.B. Chrisey, Picoliter-scale protein microarrays by laser direct write, Biotechnol. Prog. **18**, 1126 (2002).

[5]. Serra,P. J.M. Ferna´ndez-Pradas, F.X. Berthet, M. Colina, J. Elvira, J.L. Morenza, Laser direct writing of biomolecule microarrays, Appl. Phys. A **79**, 949–952 (2004).

[6]. Barron, J.A. H.D. Young, D.D. Dlott, M.M. Darfler, D.B. Krizman, B.R. Ringeisen, Printing of protein microarrays via a capillary-free fluid jetting mechanisms, Proteomics **5**, 4138 (2005).

[7]. Fernández-Pradas, J.M. Colina, P. Serra, J. Dominguez and J.L. Morenza, Laser induced forward transfer of biomolecules, Thin Solid Films, **4**, 453, 27–30 (2004).

[8]. Serra, P. M. Colina, J.M. Ferna´ndez-Pradas, L. Sevilla, J.L. Morenza, Preparation of functional DNA microarrays through laser-induced forward transfer, Appl. Phys. Lett. **85**, 1639 (2004).

[9]. Colina, M. P. Serra, J.M. Fernández-Pradas, L. Sevilla, J.L. Morenza , DNA deposition through laser induced forward transfer, Biosens. Bioelectron. **20,** 1638 (2005).

[10]. Barron, J.A. R. Rosen, J. Jones-Meehan, B.J. Spargo, S. Belkin, B.R. Ringeisen, Biological laser printing of genetically modified Escherichia coli for biosensor applications, Biosens. Bioelectron. **20**, 246 (2004).

[11]. Barron, J.A. B.R. Ringeisen, H. Kim, J. Spargo, D.B. Chrisey, Application of laser printing to mammalian cells, Thin Solid Films **453- 454**, 383 (2004).

[12]. Hopp, B. T. Smausz, Zs. Antal, N. Kresz, Zs. Bor, D. Chrisey, Absorbing film assisted laser induced forward transfer of fungi (Trichoderma conidia), J. Appl. Phys. **96,** 3478 (2004).

[13]. Arnold, C.B. R.C. Wartena, K.E. Swider-Lyons, A. Piqué, Direct-write planar microultracapacitors by laser engineering, J. Electrochem. Soc. **150**, 571 (2003).

[14]. Arnold, C.B. T. Sutto, H. Kim, A. Piqué, Direct-write laser processing creates tiny electrochemical systems, Laser Focus World **40**, S9 (2004).

[15]. Arnold, C.B. H. Kim, A. Piqué, Laser direct write of planar alkaline microbatteries, Appl. Phys. A. **79**, 417 (2004).

[16]. Piqué,A. C.B. Arnold, H. Kim, M. Ollinger, T. Sutto, Rapid prototyping of micropower sources by laser direct-write, Appl. Phys. A **79,** 783 (2004).

[17]. Kim, H. R.C.Y. Auyeung , A. Piqué, Laser-printed thick-film electrodes for solid-state rechargeable Li-ion microbatteries, J. Power Sources **165,** 413 (2007).

[18]. Boutopoulos , C. V. Tsouti , D. Goustouridis, S. Chatzandroulis, I. Zergioti, Liquid phase direct laser printing of polymers for chemical sensing applications, Appl. Phys. Lett. **93,** 191109 (2008).

[19]. Kim, H. R.C.Y. Auyeung , S.H. Lee , A.L. Huston, A. Piqué, Laser forward transfer of silver electrodes for organic thin-film transistors, Appl. Phys. A **96**, 441 (2009).

[20]. Kim, H. R.C.Y. Auyeung, S.H. Lee, A.L. Huston, A. Piqué, Laser-printed interdigitated Ag electrodes for organic thin film transistors, J. Phys. D: Appl. Phys. **43,** 085101 (2010).

[21]. Dinca,V. A. Ranilla, M. Farsari, D. Kafetzopoulos, M. Dinescu , A. Popescu, C. Fotakis, Quantification of the activity of biomolecules in microarrays obtained by direct laser transfer, Biomed. Microdevices, **10,** 719 (2008).