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Direct laser printing of biotin microarrays on low temperature oxide on Si substrates

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Laser Induced Forward Transfer (LIFT) technique is a direct-write method allowing the effective deposition of a wide range of materials with high spatial resolution. In this work, we present the direct printing of microarrays from a biotin solution by using a Nd:YAG laser (266nm wavelength, 4ns pulse duration). Standard materials of microelectronics such as Si and Low Temperature Oxide on Si (LTO/Si) were used as receiving substrates. For the LTO substrate, a detailed study for the optimum surface functionalization was carried out as it is a common passivation layer for biosensors. The laser beam parameters, such as the energy density and the beam size, were systematically varied in order to evaluate the effect on the transferred droplets. Furthermore, the energy density threshold for the ejection of the droplets was defined. Finally, the viability of the deposited droplets was demonstrated through the biotin–streptavidin reaction on silane coated LTO/Si substrates. This reaction was confirmed by fluorescence microscopy. These results have shown that the LIFT process can be used for biosensors and possibly for label-free detection by the combination of the method with capacitive sensors, sensors based on cantilevers or other sensors with electrical output.

1 Introduction The transfer of biomaterials, such as proteins, nucleic acids and oligonecleotides has a crucial role in the development of microarrays, biosensors, medical sensors and lab-on-chip devices. These applications present growing interest since they are advanced tools for the disease diagnosis, genome research and drug discovery. The conventional and more extended printing technologies rely on spotting [1], injection [2], and photolithography [3]. The LIFT is a technique which enables the controlled transfer of a thin film of a material, from a transparent carrier, to a receiving substrate. The receiving substrate is placed in parallel and in close proximity to the thin film source. LIFT was first demonstrated by Bohandy et. al. [4] for metal deposition. Since then, several groups have used LIFT in order to transfer a wide range of materials including oxides [5], superconductors [6], DNA [7], proteins [8, 9] and cells [10]. Furthermore, by using an intermediate sacrificial layer [11, 12] between a transparent carrier and a thin liquid film, liquid phase LIFT can be achieved [13, 14]. The printing of microarrays by liquid phase LIFT offers the advantages of the high spatial resolution, the contact less approach and of the biological material waste minimization.

In this work we present our results in liquid phase LIFT of biotin microarrays on Si and silane modified LTO/Si substrates. We have selected to study these substrates since they are standard material surfaces for microelectromechanical systems and therefore sensors. Additionally an extensive study of the LTO/Si surface chemical functionalization for the use of these substrates as biosensors has been accomplished in the framework of the presented work.

2 Experimental

2.1 Materials and methods The optimum biotin-streptavidin interaction efficiency on LTO/Si substrate was determined by comparing five types of aminosilanes. The evaluation of the different aminosilanes was achieved by reacting biotin with fluorescent streptavidin, and measuring the fluorescent signal. The LTO/Si flat substrates were coated with 3-[2-(2-Aminoethylamino)ethyl amino] pro-pyltrimethoxysilane (AEEPTMS) (Fluka), 3-(Aminopropyl) trimethoxysilane (APTMS) (Fluka), (3-Aminopropyl)tris(trimethylsiloxy)silane (APTTMS) (Fluka), polyamidoamine PAMAM dendrimer (4 generation) (Fluka) and aminopropyltriEthox-ysilane (APTES) (Fluka). All the above types of aminosilanes were formed on the surface by shaking the sample for 19 hours into the coating solution (1% aminopropyltriEthoxysilane in solution of 95% ethanol), washing the sample two times with 95% of ethanol solution and one time with distilled water. Afterwards the samples were dried at the specific temperature of 80°C for 2 hours.

We use biotin-NHS (Pierce) so as to be attached covalently to the amino- modified LTO substrate. The biotin-NHS was pipetted on the functionalized surface in three different concentrations (C_1 = 2.38 g/L, C_2 = 0.476 g/L, C_3 = 0.118 g/L) and then the samples were dried at 80 °C for 20 minutes. In order to remove the unbound biotin, the samples were washed two times with 50 mM Tris–HCl (pH 7.4) (Sigma) and four times with PBS (Phosphate Buffered Saline, 137mM NaCl, 2.7 mM KCl, 10mM Na_2HPO_4O, 2mM KH_2PO_4O, pH 7.4) (Sigma) .

The deposited by LIFT solution consisted of a solution of biotin-NHS in PBS (2.38 g/L) mixed with glycerol, both at a concentration of 50% (v/v). Adding glycerol in the solution prevents the evaporation of the thin liquid film on the carrier, improves the quality of the transferred droplets and offers better wettability of the solution on the carrier [15, 16].

The reactive groups of the substrate were blocked using 1% BSA (bovine serum albumin) (Sigma) in PBS solution at room temperature for 1h. After BSA blocking, the samples were washed five times with PBS solution. The interaction of biotin-streptavidin was achieved by treating the samples with streptavidin-R-phycoerythrin (Pierce) solution (C=5mgr/L) for 1h at room temperature and after they have been washed with PBS for three times.

Quartz plates coated with Cr were used as carriers. The Cr thin film (40 nm thick), deposited by sputtering, acts as radiation absorber and prevents the damage of the transferred material. The biotin solution was spread on Cr coated quartz plates in order to form a 5μm thick film as it was estimated through mass measurements. After the biotin droplets were deposited on the LTO/Si substrates, an immobilization procedure of the transferred biotin, as well as a treatment with a streptavidin-R-phycoerythrin, were carried out as described above.

The microarrays were observed by optical microscopy (Leica) and the statistical analysis on the transferred droplets was performed by means of a commercialy available image processing software. The fluorescence signal was detected by a Leica fluorescence microscope.

2.2 Experimental setup The LIFT experiments were carried out by means of the experimental apparatus depicted in Fig. 1. It is comprised of a pulsed Nd:YAG laser (266 nm wavelength, 4 ns pulse duration) and a high power imaging micromachining system. A multi-target holder was fixed onto a computer-controlled x−y translation stage, allowing a maximum 25 mm x 25mm movement and having a 1μm positioning resolution. An in-house program was used in order to synchronize the x-y motion with the laser. The laser beam was attenuated and expanded to irradiate a rectangular or circular variable mask. The microarrays were prepared using a 15 X (OFR) objective to focus the laser beam onto the Cr coated quartz plates. The transfer was carried out in such way that each droplet was deposited by a single pulse and the distance between the liquid thin film and the substrate was about 100 μm. The resulting spot diameter on the Cr coated quartz plates was varied from 10 to 40 μm.

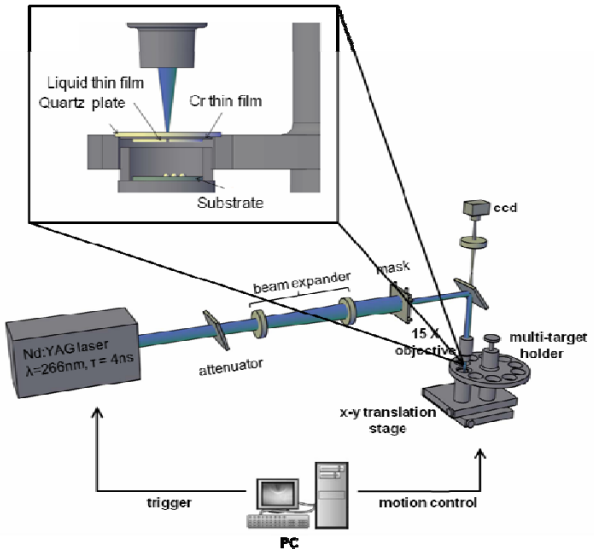

Figure 1 Laser induced forward transfer experimental setup.

3 Results and discussion

3.1 Functionalization of the LTO/Si substrate Amine functionalization of the LTO/Si surfaces was performed by chemical treatment with five type of aminosilanes: AEEPTMS, APTMS, APTTMS, PA-MAM and APTES. The ester-terminated biotin was covalently attached onto amino-silanated substrate surface and, afterwards the reaction with fluorescent streptavidin was performed. The intensity of the fluorescent signal for the biotin-streptavidin reaction and for the different types of aminosilane coatings of the LTO/Si substrate is depicted in Fig. 2. Moreover in Fig. 2, we present the dependence of the fluorescent intensity on the concentration of the immobi-

lized biotin solution. It can be seen that the AEEPTMS coated LTO/Si substrates offer the highest interaction signal for all three different concentrations of immobilized biotin. In addition the AEEPTMS coating presents the lowest background signal. Consequently, according to the above results we choose to use AEEPTMS coating to prepare LTO/Si substrates for the LIFT experiments.

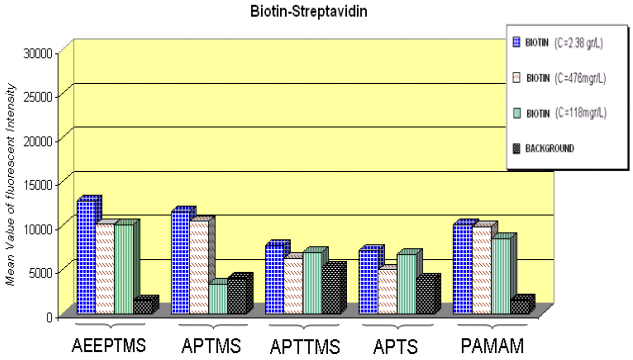

Figure 2 Fluorescent intensity for the biotin-NHS - streptavidin-R-phycoerythrin (5 mg/L) reaction for five different aminosilane coatings of the LTO/Si surface.

3.2 LIFT process An optical microscopy image of a 5 x 8 microarray of the biotin solution is depicted in Fig. 3. Each droplet was transferred on the silane coated LTO/Si substrate by a single laser pulse at a laser pulse energy density of 260 mJ/cm^2, while the spot size on the Cr thin film was 20 μm. The size and the shape of the transferred droplets were quite similar and no satellite droplets were observed. However, a small misalignment of the droplet's position is observed. This is due to the low resolution of the motors. Additionally, this may be enhanced by some thickness ununiformity of the target liquid phase thin film. The effect of both laser energy density and laser beam spot size on the droplets size and shape was investigated. Fig. 4 shows the variation of the droplet size versus the energy density for 20 μm, 30 μm laser spot size on LTO/Si substrate and 30 μm, 40 μm on Si substrate. The mean value of the energy density threshold for the droplets ejection was 140 mJ/cm^2 for three different spot sizes. For both LTO/Si and Si substrates and for lower energy densities, the droplets size presents a linear dependence on the energy density, while at higher energy densities reaches a plateau. The smallest droplets, having a diameter of about 8 μm, were transferred on Si substrate at 150 mJ/cm^2 and for 10 μm laser beam spot size on the Cr thin film. It is mentioned that above of the ablation threshold of the Cr thin film, the transferred droplets were irregular and surrounded by small satellites.

In the case of the LTO/Si substrates, the deposited droplets were found to have larger diameters compared with those deposited on Si substrates for the same laser energy density and spot size. This is attributed to the higher hydrophilicity of the LTO/Si substrates compared to the hydrophobic Si surface.

3.3 Biological activity test The bioactivity of the transferred biotin droplets was confirmed by their reaction with fluorescent streptavidin-R-phycoerythrin (5 mg/L). A fluorescent image of a transferred microarray on silane coated LTO/Si is depicted in Fig. 5. The fluorescent round spots are uniform and present negligible distribution of the fluorescence signal. The droplets were transferred from a biotin-NHS in PBS (2.38 g/L) solution mixed with glycerol both at a concentration of 50% (v/v). The fluorescence signal revealed the viability of the transferred droplets.

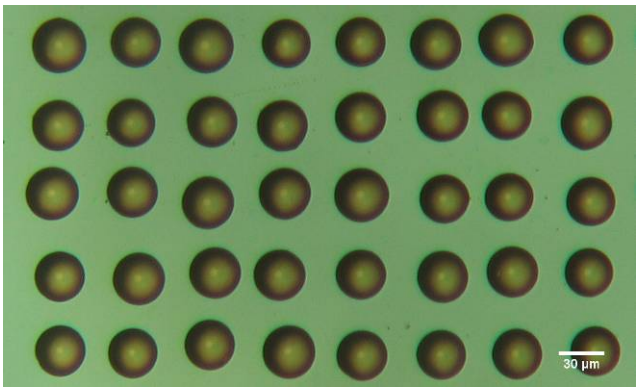

Figure 3 Optical microscopy image of a biotin microarray on a silane coated LTO/Si substrate (Φ = 260 mJ/cm^2, 20 μm spot size).

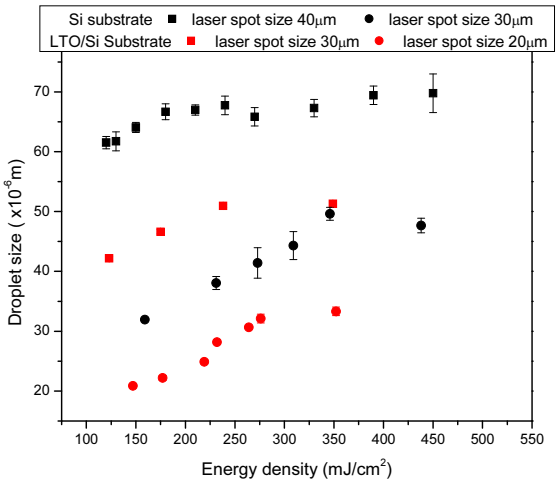

Figure 4 Plot of the diameter of the transferred droplets versus the laser energy density for different laser spot sizes and substrates.

The intensity of the fluorescent signal from the LIFT droplets was compared to the signal from deposited by pipette control spots and was found to be similar. The control spots were prepared by pipetting 3 μL of the biotin solution. We have used the same solution for the LIFT experiments and the control spots.

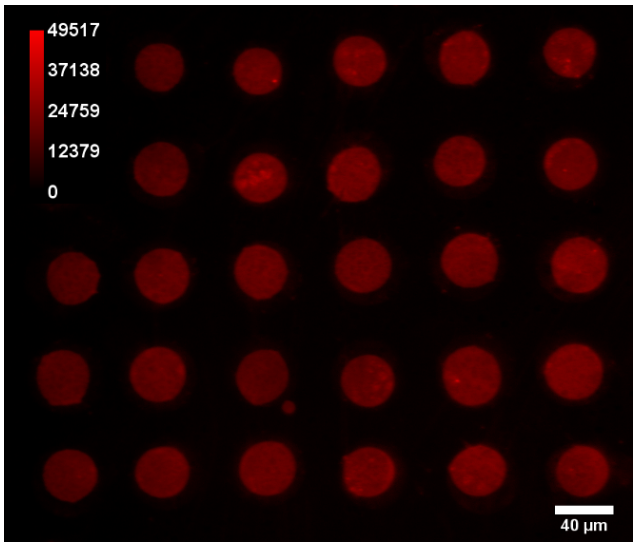

Figure 5 Fluorescence microscopy image of the transferred droplets (Φ = 120 mJ/cm^2, 30 µm spot size) obtained after the biotin-streptavidin reaction on a silane coated LTO/Si substrate.

4 Conclusion The LIFT process was used in order to transfer biotin microarrays on Si and silane coated LTO/Si substrates. A detailed study for the surface functionalization of the LTO/Si substrates proved that the 3-[2-(2-Aminoethylamino)ethyl amino] pro-pyltrimethoxysilane (AEEPTMS) silane coating is the optimum for the immobilization of the transferred biotin droplets. Round and uniform droplets were transferred for energy densities lower than the Cr thin films ablation threshold. The viability of the transferred droplets was confirmed through their interaction with fluorescent streptavidin. Our work revealed that a standard biological reaction (biotin-streptavidin) can be performed efficiently on LTO/Si substrates by using the LIFT process for the biotin printing. The combination of the LIFT process with sensors having LTO/Si surfaces and electrical read out would lead to label free detection and this will be the subject of our future work.

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