Time resolved schlieren study of sub-pecosecond and nanosecond laser transfer of biomaterials

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Abstract

A comparative study of the effect of ultrashort (0.5 ps) and short (15 ns) pulses on the laser forward transfer of DNA molecules is presented in this paper. We use femtosecond laser pulses to directly print a wide range of biomaterials, in complicated patterns and structures. The ultrashort laser pulses reduce the thermal effects, thus allowing the effective deposition of sensitive biomaterials at high spatial resolution for micro-fabricating patterns. This direct laser printing process enables gentle and spatially selective transfer of biomaterials and facilitates application possibilities for the fabrication of biosensors and arrays for multi-analyte assays. Here, we present the direct micro-printing of biomaterials such as enzyme patterns by laser-induced forward transfer method using 500 fs laser pulses emitted at 248 nm. Furthermore, the dynamics of the process was investigated by stroboscopic schlieren imaging for time delays up to 3 μs following the laser irradiation pulse.

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1. Introduction

The spherical view of biological systems is revolutionizing the life sciences. A common mission unites pharmaceutical companies, academia, biotech, and diagnostic firms to employ massive analysis in order to develop products that improve quality of life. The fabrication of advanced micro-devices (biosensors, lab-on-chip, medical sensors, and actuators) will provide better health studies and fast detection of spread and rare illnesses. Previous techniques that have been developed for deposition of biomaterials are
A variety of methods are currently available for depositing biopolymers in biosensor systems. According to most of the published works [4], biopolymer materials are applied onto their binding or adsorbing substrates in solution by simple dispensing or soaking techniques. For achieving localized deposition a variety of micro-fluidic, gel micro-casting, and localized activation methods have been described for recent examples. The vast majority of methods for depositing a plurality of biopolymer samples onto solid substrates are liquid deposition processes. High density arrays of nucleic acids and proteins samples on a porous membrane have been produced by the “dot–blot” approach by Kafatos et al. [5]. In these methods, the biopolymers in solution are delivered on a retaining porous membrane by a pipette while a vacuum manifold facilitates the transfer. Dot–blot methods are unsuitable for deposition on non-porous substrates and inadequate for producing dots smaller than 1 mm. Spots of biological samples at high densities have been produced by the use of pins or capillary dispensers that are dipped into the solution of the biological material and then used for generating spots on either porous materials or solid supports [6]. The contact spotting methods using pins produce spots of variable size due to differences between the pins and variations in the rheological properties of the liquid samples. Additionally, the impact of pins on the substrate surface limits the durability of the spotting tools and may cause imperfection of the substrate.

Liquid non-contact printing methods, such as the “inkjet” and “drop-on-demand” approaches, have also been used for spotting of biological liquid samples on various substrate surfaces [7]. These methods are uneconomical for valuable biopolymer samples and inherently limited by the viscoelastic properties of the samples.

Alternative methods to deposition are the direct synthesis of distinct oligonucleotides on solid supports [3], which involve combinatorial schemes of chemical synthesis at discrete regions of the solid support. These methods require relatively expensive photolithographic and/or micro-fluidic processing equipment and are limited to the production of relatively short oligomers.

On the other hand, laser rapid prototyping has been used for the fabrication of three-dimensional periodic micro-structures of metals and oxides, which are important for creating photonic [8] and electronic structures [9]. In the laser based direct methods, such as the direct laser printing method (DLP), the structures are built directly without the need of masks, enabling rapid prototyping. The DLP method [10] utilizes pulsed lasers to remove and transfer material from a thin film of metal, oxide, or polymer on an optically transparent support, onto a substrate placed in close proximity. In our previous work, we have published the micro-printing of DNA [14] using 0.5 ps laser pulses and we have proved that the proteins [11] maintain their properties and biological functions and thus can be practically used as biosensors.

Another direct write (DW) technique which combines the laser-induced forward transfer (LIFT) method with the matrix assisted pulsed laser evaporation (MAPLE) has been demonstrated by Ringeisen et al. [12] and Pique and Chrisey [13] who deposited patterns of active proteins, viable Escherichia coli, and mammalian Chinese hamster ovary cells and also have generated patterns of viable E. coli by using nanosecond laser pulses. The laser printing of biomaterials by means of MAPLE/DW method requires the assistance of transferring matrix materials. In our work, we present a novel technique for depositing biomaterials by laser direct transfer avoiding the use of any matrix material. The method achieves high spatial resolution by the means of ultrafast laser pulses that avoid melting and vaporization and allow material transfer with narrow angular divergence. A further advantage of the method is that labile biomaterials are deposited without significant damage, since the use of ultrafast laser pulses minimizes the adverse thermal effects of the process and lowers the required energy threshold for transfer.

Here, we present the direct micro-printing of biomaterials such as enzyme patterns by laser-induced forward transfer method using 0.5 ps laser pulses emitted at 248 nm. We also compare the sub-picosecond to the nanosecond of laser transfer of DNA thin films by using stroboscopic schlieren imaging. We experimentally demonstrate that the ejected material using ultrashort (sub-picosecond) pulses compared with the use of short (nanosecond) pulses is highly directional with narrow angular divergence enabling high spatial resolution of the micro-deposited features.
2. Experiment

The experimental printing setup is depicted in Fig. 1a and described in more details in a previously published work [14,15]. The apparatus illustrated in Fig. 1a is comprised of a laser source generating ultrashort pulses and an image projection micro-machining optical system, which performs mask projection onto the target on a large reduction basis (×30). The edge resolution of the laser micro-machining system is 0.5 μm. A hybrid distributed feedback dye laser/KrF excimer laser, delivering ~0.5 ps duration pulses of 10 mJ/pulse, at λ = 248 nm, is used for the printing procedure. A holder able to support a plurality of biomaterial targets is also used for the fabrication of different isolated structures or repetitions of adjacent features forming arrays or localized coatings, respectively. The biomolecular solution was first deposited on transparent quartz wafers by spin on disk method resulting in 250 nm thick film. The biomaterial films were selectively ablated and subsequently deposited on the substrates (receiver wafers), which were positioned in close proximity with the target materials at spot sizes from 10 to 100 μm.

The dynamics of the process is studied under atmospheric pressure by stroboscopically probing the ejected micro-plume. The experimental setup is illustrated in Fig. 1b. A second laser (XeCl excimer laser, 308 nm, 30 ns) is used as the probe laser to

![Fig. 1. Experimental setup. (a) The micro-printing workstation. The 248 nm (0.5 ps) laser pulsed beam is attenuated and expanded to irradiate a rectangular aperture mask. The mask is imaged on the target substrate by the use of a microscope objective. A special holder supports a variety of target substrates, while the target and the receiver substrate are situated placed on computer controlled X–Y motor stages. (b) The stroboscopic schlieren imaging setup. The target substrate is irradiated by the micro-printing workstation objective. A pump XeCl excimer laser illuminates the ejected plume, which is imaged on a CCD detector by a knife edge spatially filtered, two lens optical system.](image-url)
obtain stroboscopic images of the ejected microplume at delay times starting from 40 ns up to 3 μs. The pulse energy of the probe laser is kept below 4 μJ. The probe beam experiences absorption and scattering while propagating through the ejected material and then is focused by a short focal length lens. To enhance the ejected micro-plume visibility, the probe beam is partially filtered out by a knife-edge positioned on the back focal plane of the lens. This schlieren image filtering method is sensitive to local changes in refractive index, and thus to the density and pressure gradients of the surrounding media. A second lens is used to image the plume onto the CCD detector. A frame grabber is used to capture the image from the CCD camera after triggering a pulsed delay generator, which in turn triggers the probe and the pump laser. At least three images are obtained at each delay time in order to certify the reproducibility of the results.

3. Results and discussion

In order to improve the flexibility of the method to a diverse range of biological systems, we present the micro-printing of enzymes on glass slides by laser transfer. Fig. 2 shows the enzymatic staining reaction (sodium acetate 0.095 M, H₂O₂ 0.04%, citric acid 0.065 M, manitol 0.027 M, 3,3,5,5-tetramethyl-benzidine 1.2 mM) of the deposited horseradish peroxidase, demonstrating that the enzyme remains active after the printing process. This example shows the versatility of the methods for a variety of biomaterials (nucleic acids, proteins, and enzymes) that could be easily extended to different other substances, such as carbohydrates, lipids, etc. This enzyme is relatively sensitive and the stain developed over the deposited enzyme demonstrates that the enzyme remains active after the direct laser printing process. This is very important for the development of applications where both printing resolution and biological activity are required, such as antibody or antigen micro-arrays.

The technique is performed over a wide range of biomaterials. The deposited material is of the order of 0.1 μg/mm². The retained enzyme mainly depends on the binding capacity of this surface.

Our printing results demonstrate that the direct laser printing process is wide applicable and can be used to directly write micro-patterns of biological molecules, such as DNA, proteins, and enzymes without the assistance of any transferring matrix material.

The mechanisms governing this printing method were studied by means of time-resolved stroboscopic schlieren imaging [16], using a second laser (308 nm, 30 ns), as a probe, for delay times between 0.05 and 4 μs following the laser printing pulse. Fig. 3A depicts a series of stroboscopic images of the DNA material ejection following the 0.5 ps laser irradiation at various delay times. The images clearly illustrate the blast wave in air and the ejected material propagation. We detect the emerging material at ~100 ns propagating through air at a speed of 430 ± 11 m/s. The lateral expansion of the material versus time is minimal, corresponding to an angular divergence of 2°. For comparison purposes, we

Fig. 2. Active enzyme staining reaction. The enzyme deposited on the glass surface is incubated in a solution containing sodium acetate 0.095 M, H₂O₂ 0.04%, citric acid 0.065 M, D-mannitol 0.027 M, 3,3,5,5-tetramethyl-benzidine 1.2 mM, and horseradish peroxidase activity results the precipitation of the chromogenic compound. The developed staining is visualized by confocal laser fluorescent scanning.
investigated the dynamics of the process using 15 ns laser pulses, and in Fig. 3B, a series of stroboscopic images of the ejected DNA material is depicted. The lateral expansion of the material is high and the angular divergence is about 30°. The 0.5 ps laser pulse duration is less than all relaxation times of the material. It is likely that the short laser pulse induces high pressure in the thin film, which is released by a shock wave, propagating towards the air–material boundary and after reflection as a rarefaction wave propagating in the opposite direction [17]. The whole material thin film is removed when the rarefaction wave reaches the material–substrate boundary. In this case, the faster than all relaxation times energy deposition in the thin biomaterial layer, leads to a rapid, within a few tens of picosecond, shock assisted detachment of the thin film material. The high directionality of the ejected material for ultrafast laser printing pulses, as revealed in our experiment, leads to higher spatial printing resolution (down to 10 μm) and superior printing quality of the biomaterials. The use of ultrafast laser pulses for the biomolecules propels material with narrow angular divergence, avoiding melting and vaporization, and thus, produces features with minimum spread and high spatial resolution (down to 10 μm). A further advantage of using ultrafast laser pulses is that they minimize the adverse thermal effects of the process and lower the required energy threshold for transfer; thus, thermally labile biomaterials are not damaged during transfer, particularly when using a pulse width of below nanosecond regime. Another advantage of the present work is the simplicity of the method wherein biomaterials absorb the energy of the ultrafast laser pulses and can be deposited on the substrates without the assistance of any transferring matrix material.

4. Conclusions

The ultrafast UV laser-induced forward transfer is a direct process wherein biomaterials absorb the energy of a single sub-picosecond UV laser pulse and are
subsequently deposited on a substrate without the assistance of any transferring matrix material. This simple, one-step process of fabrication of DNA and protein micro-arrays, in a repetitive and reproducible manner, facilitates the in vitro study of protein function on a genome-wide level. The process can be also expanded to a widely applicable method for depositing patterns of various biopolymer materials on a variety of solid substrates. In conclusion, we have experimentally demonstrated that the use of sub-picosecond laser pulses for the laser transfer of biomolecules is efficient with a minimal spread of the ejected material. These results show that the laser transfer of confined foils can be successfully applied for the fabrication of complex patterns with high spatial resolution in one-step process.

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