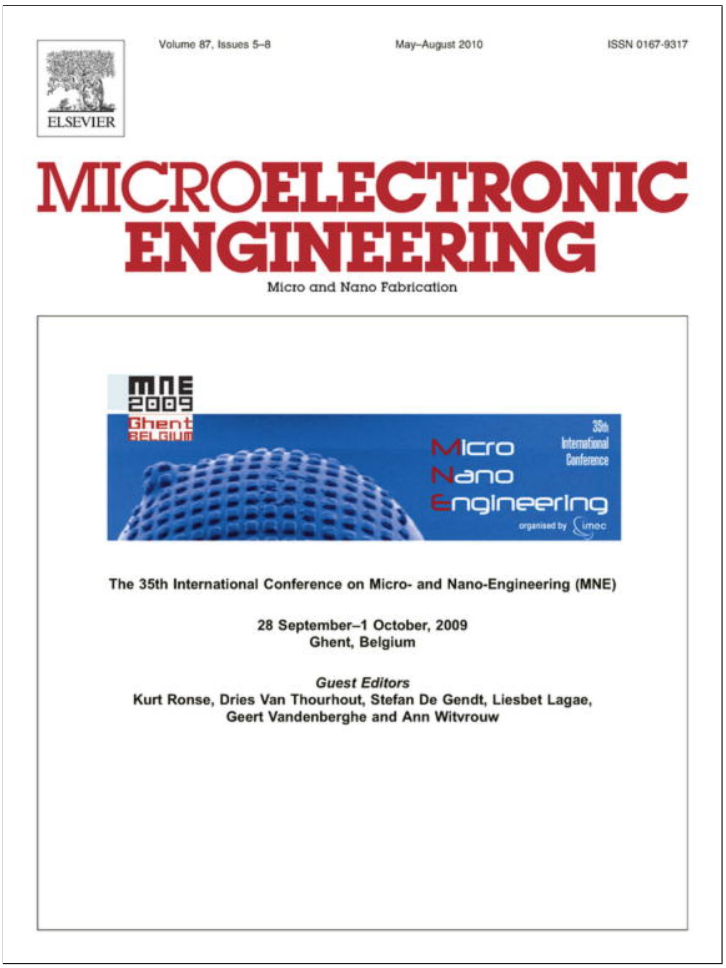

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Laser-assisted structuring of metal–polymer bilayers for protein patterning

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ABSTRACT

The fabrication of biomedical microdevices requires patterning techniques that can print structures ranging from hundreds of microns to sub-micron sizes, on large areas, with a low cost of ownership, and using essentially any material, but with relatively relaxed requirements for pattern precision. Addressing these specific needs and opportunities, we attempted to use the inherent micro/nano-level self-structuring of polymeric materials when exposed to large energies provided by laser micron-size focused beams. The proposed system comprises a layered system of a very thin, tens to hundreds nm thick, metal layer that is opaque to the laser light deposited on a transparent polymer. For this system, which allows the confinement of the polymer thermal processes in a micron-wide, sub-micron thick volume, we mapped the correlation between the microablation process parameters and material properties, on one hand, and the resolution and profile of the patterned micro/nano-structures, on the other. It has been found depending on the choice of polymer, metal and laser energies, one can obtain different sub-micrometre polymer profiles, ranging from domes to ripples and holes. We also probed the use of this technology for the fabrication of biomedical microdevices that require spatial addressable immobilisation of proteins, such as microarrays, biosensors and lab-on-a-chip devices, and found that the microablation can induce the selective adsorption of proteins on patterned surfaces.

1. Introduction

Presently, there are two, quite distinct, development paths for patterning technologies that emerged from semiconductor manufacturing. The progress of classical lithography (optical, e-beam and ion-beam), and of new patterning technologies, such as nanoimprint, addresses the needs of the semiconductor device-orientated market, which are centred on the ever-increasing resolution and the use of electrical- and electronic-functional materials. On the other hand, various emerging markets, in particular biomedical microdevices (and to some extent MEMS devices) require large patterned areas, independence in choosing the processed materials and low cost of ownership [1]. There are however many situations when both sets of applications require both micro-level patterning on large areas and nano-level patterning on small areas on the same chip surface, which resulted in many attempts for more flexible patterning technologies [2]. The biomedical microdevices market, with its requirement for low cost of ownership, is however resistant to the use of the mix-and-match patterning and printing technologies, which are widely used in semiconductor devices market. In this context, laser microablation can produce patterns ranging from several tens of microns to few hundreds of nanometres, in virtually any non-transparent material [3], and on large areas, which is an extremely attractive mix of benefits, provided that the requirements for the definition of the patterns and the fabrication throughput are relaxed [4,5].

Following previous work [6], the objective of the present study is twofold. First, we attempt to map the correlation between the microablation process parameters and the material properties, on one hand, and the resolution and profile of the patterned micro/nano-structures, on the other. Second, we probe the use of this technology for the fabrication of biomedical microdevices that require spatial addressable immobilisation of proteins, such as microarrays, biosensors and lab-on-a-chip devices.

2. Materials and methods

Glass coverslips (22 × 22 mm, 1 mm thick) were purchased from Corning Glass. Poly(methyl methacrylate) – PMMA; Poly(-tert-butyl methacrylate) – PTBMA, Poly(styrene) – PS and hexamethyldisilazane (HMDS) were purchased from Sigma Aldrich and used as received. Cyclo-olefine copolymer – COC was a gift from TOPAS Advanced Polymers GmbH. Propylene glycol methyl ether

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acetate (PGMEA), ethanol and propanol were purchased from Sigma Aldrich and toluene was purchased from Fisher Scientific.

The coverslips were thoroughly rinsed and sonicated in an ethanol–propanol mixture and de-ionized water, respectively, then primed by exposure to HMDS vapours at 200 °C for 2 h. The spin-coating of COC, PS and PMMA (4% w/w solutions in toluene) and PTBMA (1% w/w in PGMEA) was carried out using a spin-coater (Laurell), first at 500 rpm then at 3000 rpm for 1 min. The soft baking was carried out for 8 h at temperatures slightly above the glass transition temperature of each respective polymer. Metals, i.e., Au, Pt, and Al, have been deposited onto the polymer surfaces by electron beam evaporation using a Balzers BAK550 system, operated below 1.10^{-7} mbar and powers of 7, 15 and 40 W. Using these parameters and a deposition rate of 0.3 nm s^{-1} we obtained metal films of 50, 100 and 200 nm.

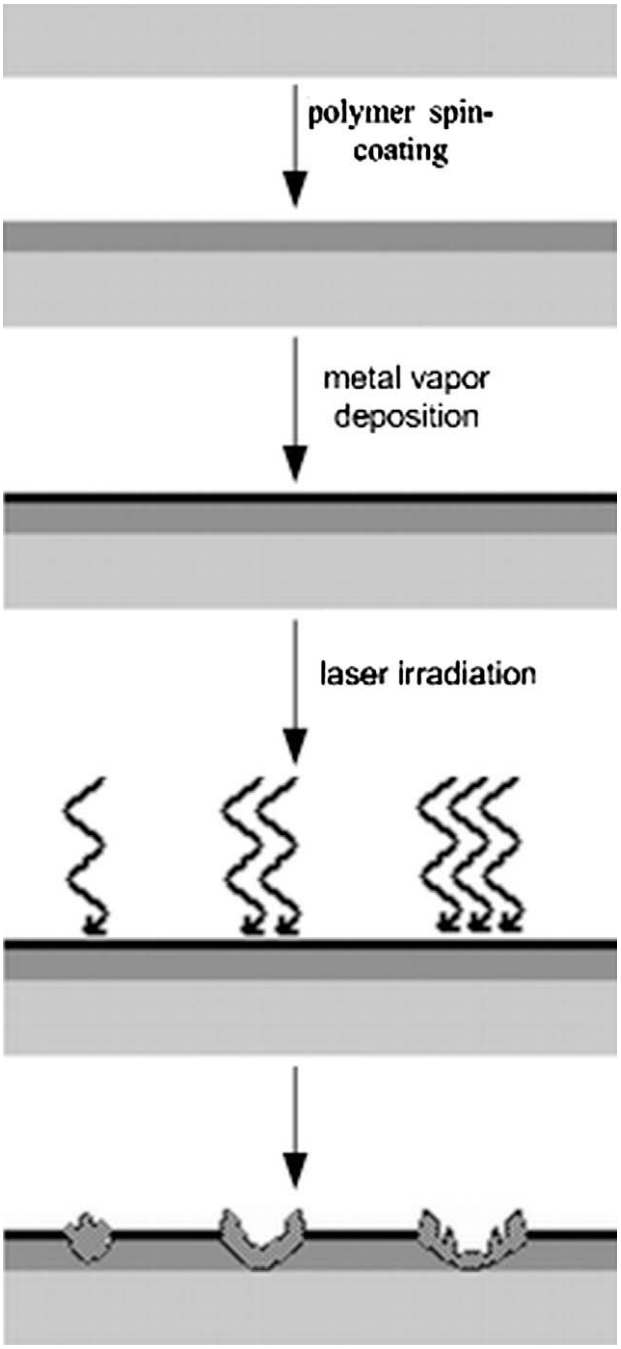

Fig. 1. Process diagram of the microablation of thin metallic films deposited on polymer surfaces.

The microablation patterning used a laser milling system (New Wave Research) comprising a ns-pulsed solid state Nd:YAG laser, working in doubled and tripled frequency regime. The pulse frequency ranges from 1 to 50 Hz, with a typical pulse width of 3–4 ns. Pulse energies of 0.6 J/pulse can be reached and the energy delivered to the surface can be as high as 25 J cm^{-2} in doubled frequency mode (at 532 nm wavelength). The beam was focused through a 20× dry objective resulting in a theoretical minimum feature of 2 μm. The system is equipped with a motorized *xy*-stage with a 1 μm stepwise resolution and programmable moving speed. For each metal–polymer bilayered sample, different energies (up to 25 J cm^{-2}) have been used to print 24 test areas, each comprising dots and lines with dimensions ranging from 0.5 to 4 μm. The micro/nano-fabrication process diagram is presented in Fig. 1.

The site-specific protein adsorption was carried out, for Au-covered samples only, using fluorescently labelled bovine serum albumin (f-BSA) and two patterning procedures. First, the patterned surface was incubated with a solution in PBS buffer of the FITC fluorescently labelled protein (1 mg mL^{-1} concentration) for 30 min at 30 °C. An alternative route was to incubate the sample, prior to patterning, with a green (FITC) fluorescence labelled blocking protein (BSA) followed by the incubation of the patterned samples with the solution of the Rhodamine fluorescently labelled protein, similarly with the procedure described above.

Optical and atomic force microscopy were employed to characterise the patterned features. Optical microscopy was carried out using a Zeiss LSM 5 laser scanning confocal inverted microscope in reflected light, DIC and fluorescence mode. An atomic force microscope (CP-II SPM, Veeco) was used to obtain the profiles of the micro-ablated surfaces.

3. Results and discussion

3.1. Pattern profile and dimensions

The interaction between the microablation parameters (variable laser beam energy; fixed wavelength, pulse rate and duration, and magnification of the optical system) with the substrate characteristics (materials used, both metals and polymers, and the thickness of the top ablatable metal layer) leads to a large variety of micro/nano-structure dimensions and profiles (Fig. 2). Apart of the large range of technological parameters, this nearly-combinatorial variability of the microstructure geometries is the result of the complex process of flash-pyrolysis of the polymer beneath the metal layer during microablation. This process is confined in a "micro/nano-furnace" which is few microns-wide and few hundreds of nanometres-deep.

3.1.1. Modulation of microstructures by the laser beam parameters

Two types of microstructure profiles have been obtained: (i) spikes, i.e., profiles presenting multiple, steep elevations in the ablated area; and (ii) wells, sometimes presenting multiple, curtain-like walls. For the process parameters used, the dimensions of the structures are usually in the micron range, but the minimum dimensions reach 350 nm laterally and 50–250 nm vertically. The structure dimensions are also profile type-specific: the base diameter of the spikes does not exceed 500 nm, while the smallest walled-wells have a well diameter starting from 500 nm. The same geometry classes are present on all substrates, with the laser beam energy being the primary modulating factor: (i) the spikes form at low laser energies; (ii) more complex geometries are present at moderate energies; and (iii) above an energy threshold only round cut wells are present (with occasional roughness at the bottom of

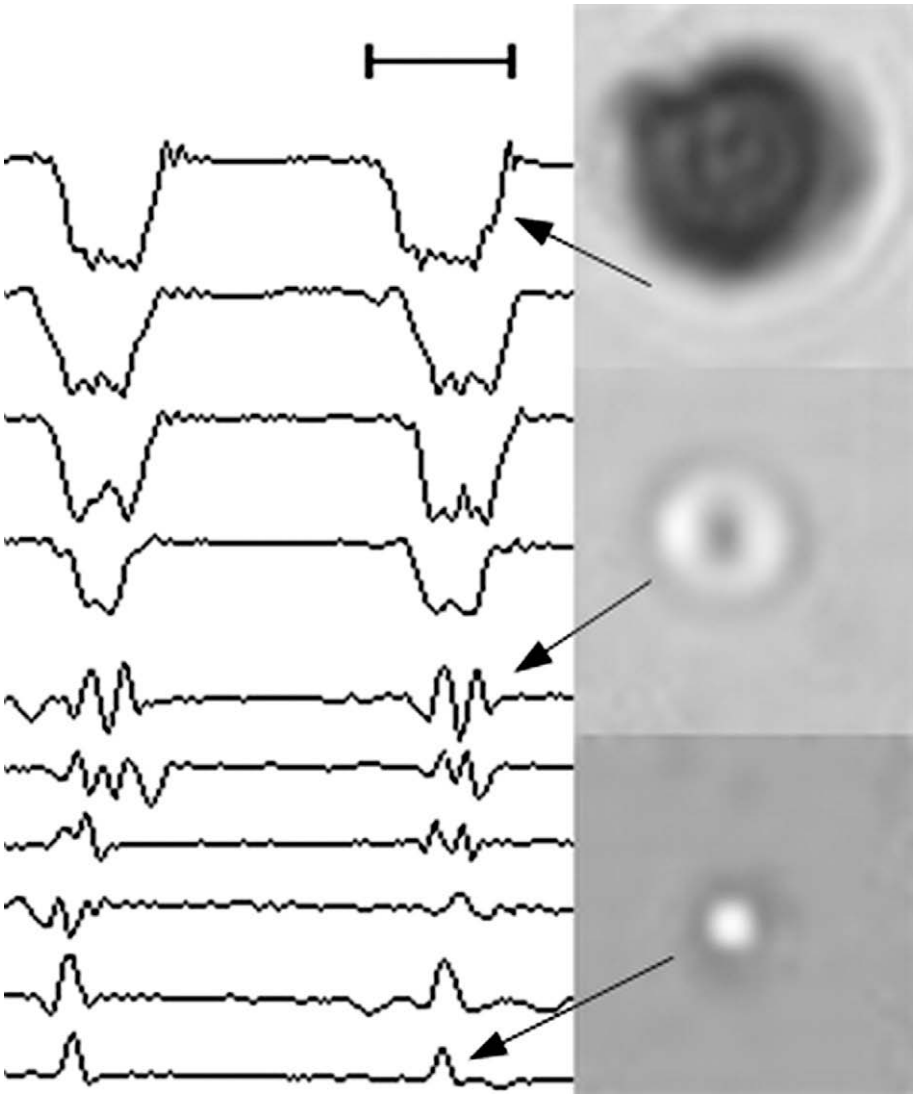

Fig. 2. Cross-section line profiles of 50 nm Pt layer deposited on COC (the energy increases from bottom to top); the scale bar is 5 μm.

the well). The geometries obtained at mid-range energies, i.e., multiple-walled-wells or ridges, are of particular interest because this sub-structuring process is a facile and versatile route for the fabrication of sub- and deep-sub-micron, quasi-organised structures, but using a multi-micron patterning technology.

3.1.2. Modulation of the microstructures by the material properties

There is a strong coupling between the metal and polymer properties (Table 1, Supplementary information) that impact on structure geometries. At one end of the spectrum, PTBMA samples have an unpredictable behaviour, possibly due to the 'explosive' nature of the polymer pyrolysis. At the other end of the spectrum, predictably, the energies required to produce similar patterns (e.g., multi-walled-wells) is lower for thinner than thicker metal films.

Within this technological range, there are a number of simple guiding rules for the fabrication of quasi-regulated sub-micron structures. In general, Al-coated samples are sub-structuring at higher energies than Au and Pt-coated ones, presumably the result of considerably lower optical absorption (Table 1, Supplementary information).

On the polymer side, the thermo-reactivity of the polymers, expected to range in increasing order PTBMA > PMMA > PS > COC, couples with the energy accumulated in the metal layer and transferred to the polymer, the latter modulated by the thermal conductivity of the metal and glass transition temperature of the polymer. Thus the higher thermal conductivity of Au and Al, compared with Pt helps the sub-structuring to occur within a larger range of ablation energies. The moderate reactivity of the PMMA and PS, as opposed to the high reactivity of PTBMA and very low thermal conductivity of COC (an aliphatic polymer) makes PMMA (and to a lesser extent PS) and Au the best pair of materials for achieving laser-induced sub-structuring (Table 2 Supplementary information). Further experiments, using smaller apertures and lower wavelengths, coupled with detailed simulation, are in progress and are expected to deliver a more advanced understanding of the sub-structuring process (Fig. 3).

3.2. Positive and negative-tone protein patterning

The discussion above focused on the physical aspects of the patterning process, but, due to the very small lateral (and vertical) dimensions of the microstructures, the experimental evidence regarding the actual surface chemistry of the polymer surface can be only inferred. An interesting experimental possibility is the hydrophobicity-specific adsorption of the proteins. For instance, when the samples are micro-ablated at low to moderate energies the resultant metal-free polymer surface is hydrophilic due to the oxidative processes incurred during ablation. Conse-

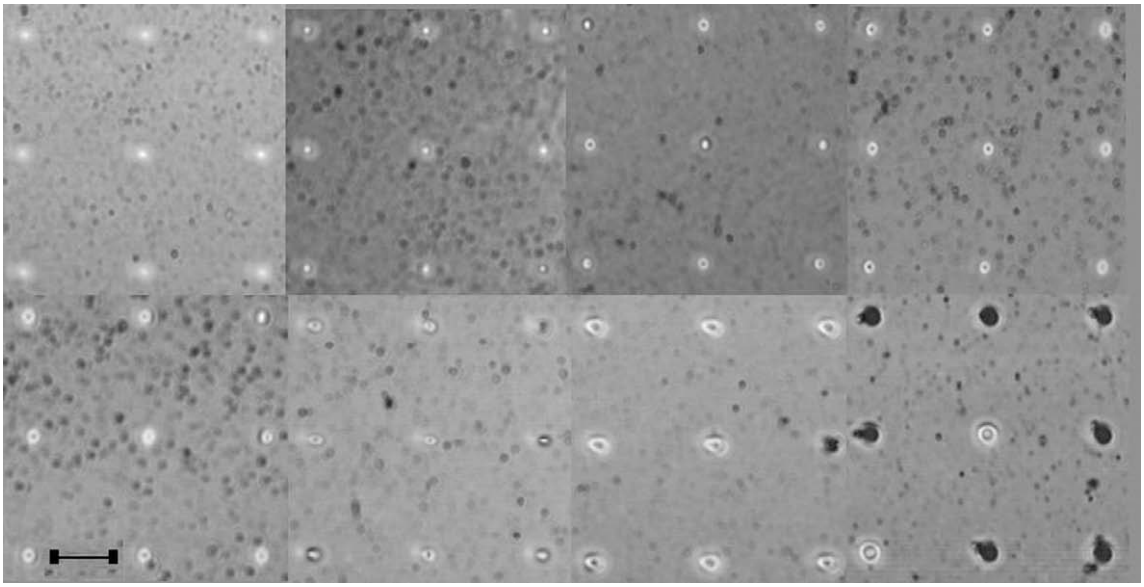

Fig. 3. Optical micrograph series of 50 nm Pt on PS after laser irradiation; beam energy increases from left to right; the scale bar is 5 μm.

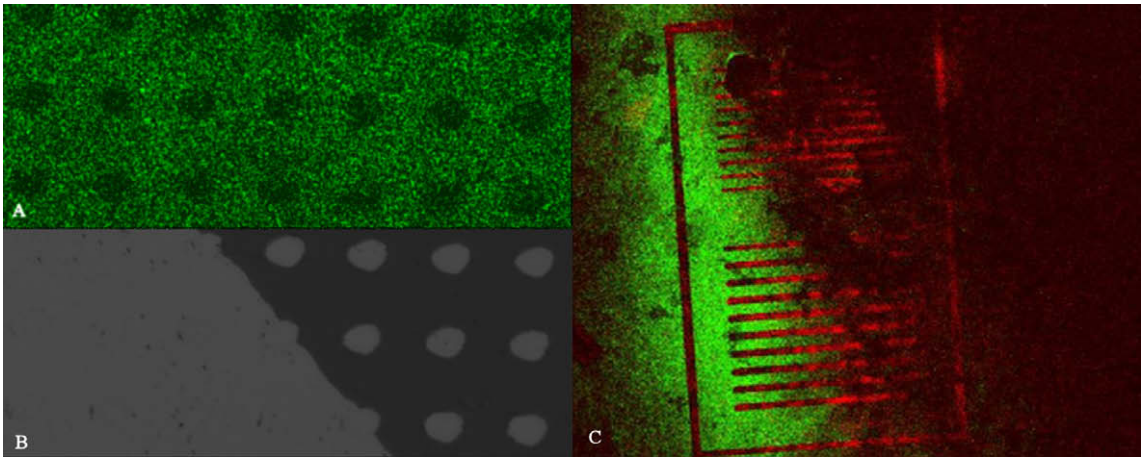

Fig. 4. (A) Fluorescent image of negative tone patterned BSA adsorbed on micro-ablated 50 nm Au on PMMA. The protein is relatively uniform adsorbed on bare polymer and Au-covered surfaces (see image (B) further), but not on hydrophilic ablated areas (approximately 5 mm diameter). (B) The bright light image of the same area as in image (A). (C) Fluorescent images of the positive tone patterned BSA (red fluorescence) preferentially adsorbed on hydrophobic lines (10 mm width) patterned by high microablation energies. The green areas are due to the green fluorescently labelled blocking protein adsorbed prior to microablation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

quently, the proteins will adsorb preferentially on the non-ablated areas (Fig. 4A and B) resulting in a negative tone image. Conversely, when large energies are used, the polymer degradation is more advanced leading to more hydrophobic surfaces. Consequently, and if the non-exposed areas are blocked before ablation by a protein-repelling layer (e.g., BSA), a positive image is obtained, i.e., proteins adsorbed on micro-ablated areas, for high ablation energies (Fig. 4C).

Apart of offering an insight into the chemistry of the micro-ablated structures, this methodology could be easily imported for the fabrication of biomedical microdevices that require a specific, spatially addressable protein immobilisation.

4. Conclusions

Laser-assisted structuring of metal–polymer bilayers appears as an advantageous method for topographical and chemical surface engineering, primarily intended for microarray fabrication. The method is cost-effective, relying on the use of readily available materials and established fabrication techniques; it generates features in the micron and sub-micron range, with additional availability for surface chemistry generation/modification. The proposed system allows the deep-sub-micron level structuring under micron-sized high energy beams, depending on the choice of polymer, metal and laser energies. Protein adsorption tests showed that interaction of the laser with the bilayers also modified the surface chemistry demonstrating that both negative and positive tone protein patterning are possible with this methodology.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mee.2009.12.016.

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