Nano-engineering of BIO-ARRAYS with Vacuum Ultraviolet Light

E. Sarantopoulou, Z. Kollia, A. C. Cefalas,
National Hellenic Research Foundation. TPCI, 48 V. Constantinou Avenue, Athens 11635 Greece

A. M. Douvas, M. Chatzichristidi, P. Argitis, K. Misiakos, Z . Petrou. S. Kakabakos,
NCSR "Demokritos",Athens, 15310, Greece

Abstract

157nm laser surface processing is a new methodology for bio-array patterning based on laser ablation of polymeric films with the overall goal to strengthen protein binding on bio-surfaces and to increase writing density. The 157 nm F_2 laser is enhancing photo-chemical over thermal processing during patterning and is improving the resolution capabilities. Nano-fabrication and control at 157nm allows atomic resolution depth control with limited chemical changes under certain conditions on the remaining substrates with the most prominent application of DNA micro-array fabrication.
The technology is used for nano/micro-engraving of bio-substrates enhancing –probe binding strength and detection sensitivity. Microfabrication efficiency depends on both light and matter parameters.

Experimental

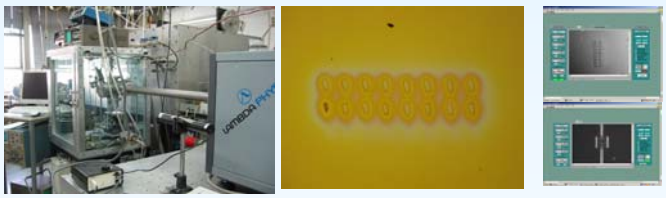
Reagent

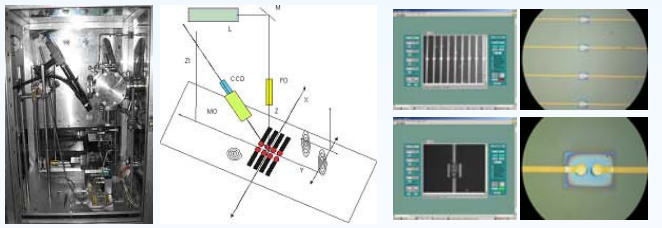
- Rabbit IgG, bovine serum albumin (BSA, Cohn fraction II, III, RIA grade) and 2,2-azinobis(3-ethylbenz-thiazoline sulfonic acid) diammonium salt (ABTS)
- Goat anti-rabbit IgG AF 488 & streptavidin AF 546
- Biotinylated BSA (B-BSA)
- Microtitration plates
- AZ5214, a novolac-diazonaphthoquinone-type photoresist
- Poly(methyl methacrylate) (PMMA, M_w=350,000).
- Epoxy novolac (EPN), (M_n=1,277, M_w=2,438, I=M_w/M_n=1.9)
- Methyl(3,3,3-trifluoropropyl)methylvinyl-siloxane (MTFPMVS)
- Poly(2,2,2-trifluoroethyl-methacrylate) (PTFEMA, M_w=114,700, M_n=45,900, I=2.5, & T_g=68.5°C). Free radical polymerization of 2,2,2-trifluoroethyl-methacrylate using 2,2-azobis (2-methylbutyronitrileas initiator.

Sample no.	PAB	Exposure (at 254 nm)	% Adsorption
1	120°C, 30 min	–	0
2	150°C, 30 min	–	0.7
3	200°C, 30 min	–	14.3
4	120°C, 30 min	15 min	7.9
5	150°C, 30 min	15 min	12.2
6	200°C, 30 min	15 min	14.3

Film Preparation

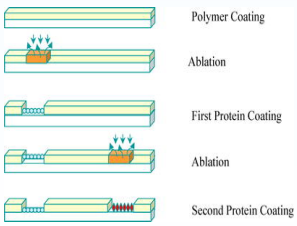
- The polymeric films were applied by spin coating on a silicon wafer that was covered by a thick film (200-250 nm) of a protein-adsorbing layer (photoresist AZ5214). These films were thermally treated after spin coating at temperatures in the range 90-200°C for a period of 5-30 min.
- Further treatment of the polymeric films was applied in certain cases, as indicated in Table. Film exposures were performed by using a Hg-Xe exposure tool and a 254nm filter .



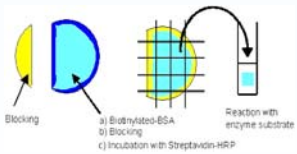


Laser ablation of polymeric films

- Polymers were ablated by hitting the target with laser energies from 0.5 to 5 mJ cm^{-2} per pulse at 20Hz rep.rate at a background pressure of 1×10^{-5}mbar and Nitrogen.
- The laser light was focused on the target with a CaF_2 lens having 5cm focal length. The CaF_2 lens was protected from the ablation products with a 1-mm-thin CaF_2 window,
- Fine patterning of the laser beam on the target was carried out by moving the substrate in the X-Y plane and by fine focusing in the Z-axis with a micrometric translation stage controlled by a PC.

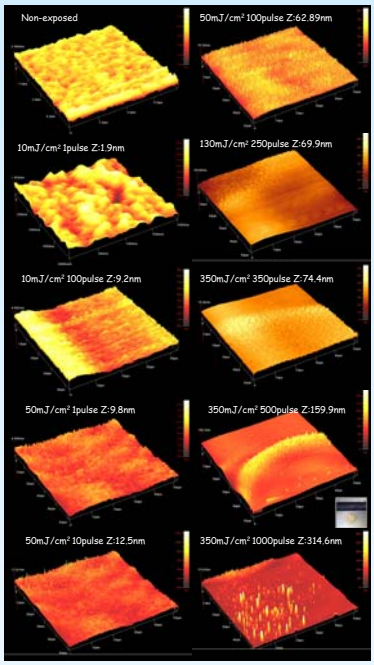


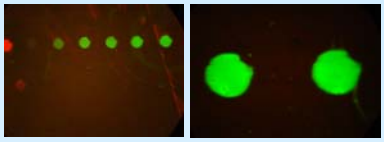
Schematic representation of laser ablation process for creating spots of different biomolecules on the same substrate



The protein adsorption resistance of the polymeric films was evaluated through a model binding-assay.

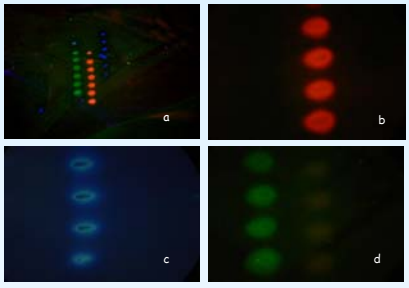
Result and Discussion



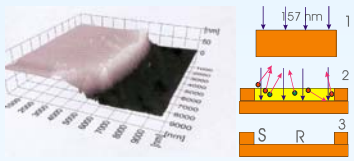


Microarrays consisted of two proteins, rabbit RIgG (green) and B-BSA (red), deposited successively after ablation of PTFEMA film and creation of approximately 400μm spots. Spots were visualized after reaction with anti-rabbit IgG antibody labeled withAlexa Fluor 488 (green) and streptavidin labeled with Alexa Fluor546 (red)

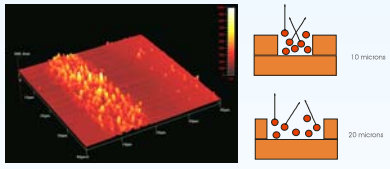
Laser ablation results of PTFEMA films: **(a)** remaining thickness of PTFEMA film versus ablation dose, **(b)** microscope image of two typical spots created by ablation, **(c)** spot depth profiling obtained by profilometry



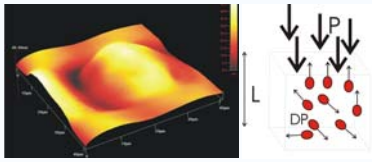
Fluorescence detection of three proteins deposited on laser ablated spots Spotted-microarray consisted of three (a) one (b), (c) & two protein (d) respectively.



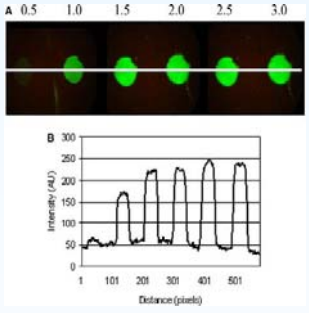
AFM image & Schematic lay out of the 157nm etching (laser beam dimension >20μm).



AFM image of irradiated area where stacking of dissociated products on the surface of the polymer is indicated. When the laser beam was ~10μm, the dissociated products were stuck on the polymer surface forming pillars due to enhanced collision rate. The nano/micro-size of the pillars is increasing under prolonged irradiation.



AFM image & Schematic lay out of ablation process following 157nm laser irradiation with 10 μm laser beam The laser radiation (P), penetrates the PTFEMA within the distance (L) specified by the absorption coefficient followed by photo-dissociation of polymer chains and fast increasing of pressure within the polymer volume from fast moving dissociated photo-fragments (DP).



Fluorescence image **(a)** and fluorescence intensity **(b)** of the spots created on PTFEMA film for ablation times of 0.5, 1.0, 1.5, 2.0, 2.5, & 3.0 min, respectively. Spot diameter is approximately 400μm

Conclusions

- A new methodology is proposed for the fabrication of multi-protein/DNA micro-arrays based on microfabrication with lasers at 157nm.
- Spots dimension < 5 μm depending on material.
- This approach is based on controlled laser ablation (at 157 nm) of PTFEMA polymeric film. This film has been selected because it prevents protein binding. An under layer of commercial AZ5214 photoresist has been used owing to its high protein adsorption capability.