

BIOLOGICAL AND ORGANIC MOLECULES PHOTOIONIZATION FOR BIOMOLECULAR DEVICE FABRICATION

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Abstract: This work aims to the development of a soft deposition technique for organic molecules and biological macromolecules, working in high vacuum rather than in solution so that environmental conditions compatible with the microelectronics fabrication processes can be achieved. Our technique is based on pulsed laser vaporization and ionization of the molecular species. Following ionization, electric fields can steer our material onto the substrate, and high resolution patterning can be achieved either by the insertion of removable masks obtained by e-beam lithography or by localized fields. The biological activity of the deposited species was proven, as well as the stability of our depositions. A characterization of surface smoothness and thickness of the deposit was effected. This technique was also applied to the deposition of photoionized metal atoms aiming to the fabrication of nanoscopic conductors.

Keywords: laser deposition, biosensor, micro device

INTRODUCTION

Direct writing using individual atoms and molecules is generally considered an almost ideal "bottom-up" assembly technique for nanoscale devices; unfortunately, in spite of the outstanding experiments performed in recent years, that prove the possibility of sliding single atoms and molecules in place with angstrom resolution^[1-3], the method employed so far, based on the direct interaction of the Scanning Probe tip with the molecule, is inherently slow. Furthermore, it can use only a limited number of single "bricks" in the construction of the designed architecture, drawn from a nearby limited reservoir adsorbed on the surface. We are therefore investigating a more generally exploitable technique, that can use any number and type of molecular "brick", while retaining the advantages of the bottom-up approach and nanometric resolution at the same time.

Laser vaporization/ionization techniques have been shown in recent years to supply atomic ions with great selectivity, and also molecular ions with little or no fragmentation at all^[4,5]. Most experiments on atoms and molecules, including fairly large organic molecules, use the resonance enhanced two photon ionization method, where the radiation is tuned on some intermediate atomic or molecular transition being slightly above one half the ionization threshold. If the radiation intensity is high enough to excite the atom or molecule with a second photon before it decays back to the ground state, selective ionization is achieved. Of course also lower energy intermediate transition can be used in principle, but more than two photons, or photons of different wavelengths, may be necessary. The reason behind the softness of the methodology lies mainly in the short duration of laser pulses^[6], that allows vaporization and/or ionization of the molecular species before energy redistribution can populate dissociative states. To achieve fragmentationless ionization of

complex molecules however, cooling of vaporized molecules in the supersonic expansion of an inert carrier gas is generally used^[4,5,7]. Similar methodologies have been used for mass analysis of peptides and even of large intact proteins in matrix assisted laser desorption ionization (MALDI)^[8]. In the latter case however, ionization is achieved by proton adduction to the protein; protons derive from the extensive photofragmentation of the matrix absorber molecules. The biological activity of laser deposited enzymes and antibodies was later demonstrated^[9].

Ionized atoms and molecules are subject to the action of an applied field and can thus be steered to impact a substrate in a spatially limited region. The spatial resolution that can be achieved depends on the method we use and also on the type of molecule that we want to deposit. The electric field can draw ions through removable masks or stencils onto the substrate; in this case resolution is limited by the masks (nanometers for e-beam lithography fabricated masks^[10]) and, more severely, by the adhesion of the removable mask to the substrate. Higher resolution should be achieved by use of a spatially well defined field such as that generated by a sharp Scanning Probe Microscope (SPM) tip; here the resolution is limited by the tip true size, by the ions velocities and by the tip to substrate distance.

Experimental

The scheme of our experiment is reported in fig.1. For mass analysis of molecular species, the ionization laser beam is directed between the entrance electrodes of a time of flight mass spectrometer, in a high vacuum vessel; for deposition experiments these electrodes are replaced by a millimeter sized electrode, facing the deposition substrate covered by a removable metal stencil;

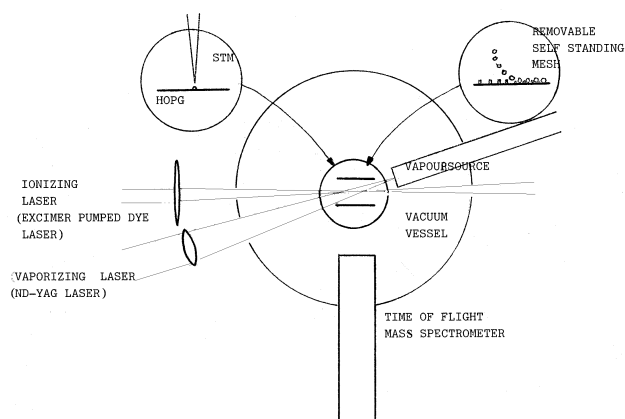


Fig. 1 Sketch of the experimental layout

for higher resolution depositions, that we perform in a different UHV vessel, the electrodes are replaced by a Scanning Tunnelling Microscope (STM) tip facing a conductive sample (highly oriented pyrolytic graphite (HOPG) or gold coated silicon wafers). The mass spectrometer is a home built 85 cm linear time of flight analyzer, with two microchannel plate ion detectors in series.

The distance between the vapour source and the ionization region is of the order of 15 mm; this requires a careful selection of the delay between the two laser pulses, that, in order to minimize the delay jitter, is achieved by two standard delay generators, both triggered by the Nd-YAG laser (the vaporizing laser) flashlamp starting pulse and operating separately on both the Nd YAG Q-switch, that lets the vaporizing pulse through, and on the excimer laser trigger.

Laser induced vaporization and ionization of atoms and molecules

Depending on the molecule we want to ionize we prepare the sample following different protocols. As we have already noted, cooling of the laser vaporized molecules is in general necessary,

by means of a supersonic adiabatic expansion of an inert carrier gas, to obtain both selectivity in the ionization and unfragmented molecular ions. We found however that a suitable sample preparation, aimed at maximizing the heating rate^[6] and minimize direct molecular absorption of the vaporizing radiation pulse, yields

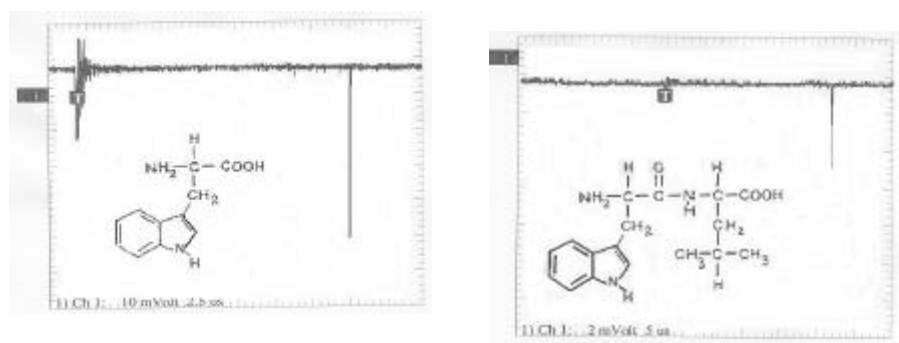


Fig. 2 The time of flight mass spectrum of the aminoacid tryptophan vaporized by a 355 nm laser pulse from a frozen water/alcohol solution doped with hydroxybenzaldehyde. Ionization is effected by an excimer laser pumped dye laser tuned at 286 nm. The oscillating noise at short times is due to the excimer laser firing and marks the origin of the flight time. Only the tryptophan parent ion at 18 microseconds is observable.

Fig. 3 The time of flight mass spectrum of the peptide tryptophyllucine. Details as for fig 2. The parent ion is at 22.4 microseconds.

intact aromatic aminoacids and peptides (figs 2 and 3), with even less fragmentation than reported in the literature for jet cooled samples^[5]. In this case the best results are obtained using a solution of our molecule doped with a suitable absorber (absorbing at wavelengths different from the absorption band of our molecule), frozen on the tip of a cold

finger. Molecules are vaporized by 8 ns laser pulses from different harmonics of the Nd-YAG laser, depending on the sample to vaporize and/or the absorber. Fairly strict focussing is required in the vaporization step in order to maximize the heating rate of the sample. The two photon ionization step is achieved by an excimer pumped dye laser, tuned on an intermediate electronic transition of molecules or atoms. The 8 ns pulse is mildly focussed between the electrodes or at the STM tip to sample gap. If the laser radiation is tuned on the peak of the molecular electronic transition, if its intensity is sufficiently low to not allow further absorption from the ionizing state, and if the delay between the vaporizing and the ionizing pulse is correctly tuned to ionize the early part of the molecular gas pulse, molecular fragmentation is minimized.

Biological molecules are in principle more difficult to manipulate, as they can both fragment and undergo conformational changes that may inhibit their functional properties. The simple fact that they can maintain their mass after the MALDI process does not necessarily mean they are still conformationally unchanged. Furthermore, the time of flight mass spectrum refers to the molecule at the time of its acceleration in the spectrometer, while the macromolecule may decompose or undergo structural changes on impact on the surface of the detector. It was proven in recent years that a large amount of biomolecules do retain their functional properties even in the immobilized state after being ionized, accelerated and deposited on the surface^[9]. Here, we tackle the problem of assessing the stability of our laser deposited biomolecules in solution. A test of the enzymatic activity of horseradish peroxidase was performed by dipping peroxidase coated specimens in a solution of a standard substrate (ABTS) and by measuring the oxidized state absorption of the latter. This gives us a measure of the amount of active enzyme molecules that are present on the surface when the specimen is dipped in the solution. On repeating this measure several times, each one with a new solution, for constant dipping times, we see in fig.4

that the ABTS oxidation rate decreases while the enzyme is lost from the glass specimen in each solution .

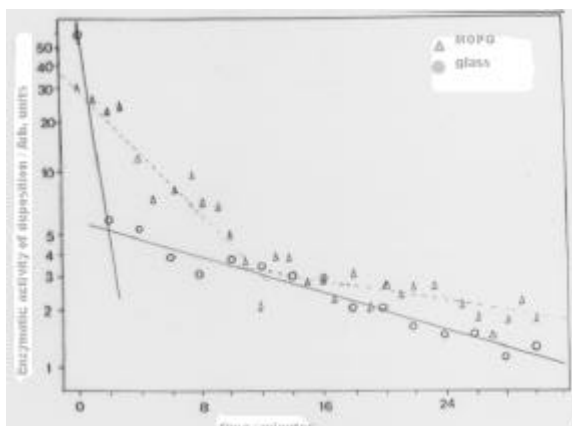


Fig. 4. The enzymatic activity of peroxidase coated specimens vs. time of permanence in water

Two distinct decrease times are found. This behaviour may be associated with the different solubility of the protein when in contact a) with other protein molecules and b) with the surface. Thus, the adhering monolayer would have a much lower solubility compared to the other layers. To prove this interpretation we repeated the solubilization experiment on a patterned deposition, whose thickness could be measured by an atomic force microscope (AFM). The pattern is obtained by covering the surface with a lithographic self standing mesh that is then removed after the deposition. The mesh has 1 μ m square holes and 1.5 μ m wires. Each AFM line scan thus measures both the uncovered surface and the protein deposit, allowing a sure measurement of the film thickness.

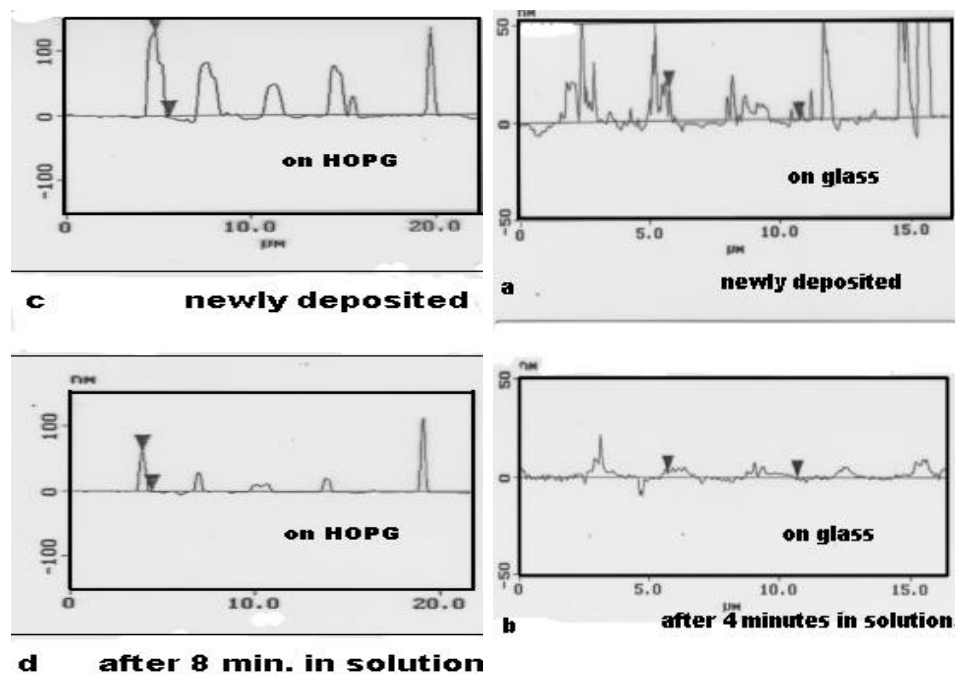
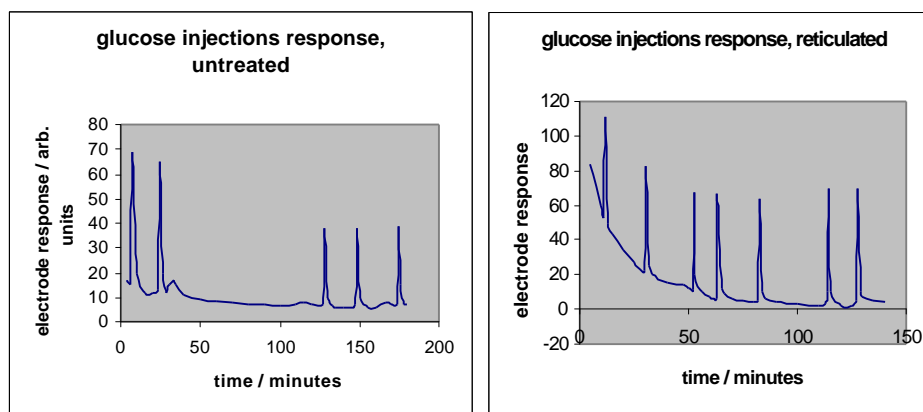


Fig. 5 Single line AFM scans accross a patterned deposit of horseradish peroxidase, newly deposited and after some permanence in water

As we see from fig 5a, the deposit is initially about 50-100 nm thick. The sample is then retracted from the cantilever region after recording the stage coordinates, and a 100 μ l water drop is deposited on it and gently stirred taking care not to touch the surface. After 4 minutes the water drop is drained away and the sample is dried with a stream of warm air for ten minutes, after which it is repositioned at the original coordinates and a second AFM scan is performed over the same protein spots. As we can see (fig. 5b) the film thickness has now decreased to an average of about 5 nm, not very dissimilar from the protein globule diameter which is calculated at about 3.5 nm. The film is thus almost reduced to a monolayer in the time the enzymatic activity

changes its decay rate. This confirms our hypothesis on the low solubility of the adhering monolayer. The experiment was repeated on a glass substrate and on a HOPG substrate (fig 5c,d); in agreement with the data of fig. 4, the conductive substrate data yield a longer solubilization time, that has been interpreted as a consequence of a stronger effective electric field drawing the molecules to the surface. Indeed, we expect the insulating substrate to charge up while positive ions are deposited, thus establishing an opposing field that decreases the kinetic energy with which ions hit the surface. This may lead to a lower adhesion of the layers, although the exact mechanism of adhesion must still be investigated. Further tests on the deposit stability were also effected by a flow injection method on glucose oxidase deposited on macroscopic platinum electrodes, measuring the response to glucose injections. These tests were effected on the raw laser deposit and after reticulation with glutaraldehyde. The results are reported in fig. 6. We can see that the peak intensity decreases to about 60% in three hours for



the untreated deposit while it remains constant for the reticulated sample.

Fig. 6 The enzymatic activity of a glucose oxidase coated specimen, as deposited by our technique (left) and after reticulation with glutaraldehyde (right)

This deposition technique was also applied to the miniaturization of a glucose biosensor, obtained by the fabrication of a platinum electrode by a hybrid electron beam lithography and laser deposition technique. The first qualitative data are published elsewhere^[11]

Metal atoms are easier to handle, raising no fragmentation nor conformational problems. However, the vaporisation step, obtained by focussing the laser on the surface of the bulk metal, easily generates a plasma^[12]; the amount of ions is normally very high, leading to possible distortions of the applied field, so that a slower heating is necessary. We found that pulses of a few tens of microseconds at 1064 nm, obtained by running our Nd YAG laser without Q-switching, give good results with lead. Fig 7 shows the ionization signal obtained tuning the ionizing radiation on the 2833.5 Angstrom transition of lead, and its very narrow dependence on wavelength, typical of atomic transitions.

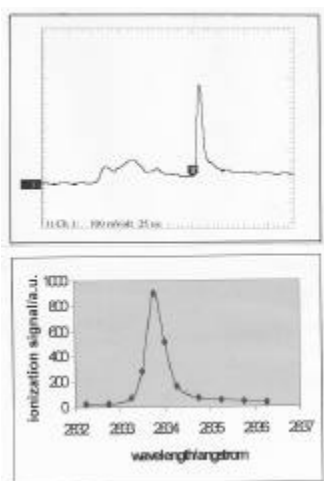


Fig. 7 The oscillogram of the ionization signal from lead atoms vaporized by a free running 1064 nm Nd-YAG laser pulse from bulk lead and ionized by a finely tuned 283.38 nm dye laser pulse is reported in the upper part of the figure. Note ions associated with the vaporizing pulse (the low oscillating signal). The sharp peak depends on the presence of both lasers. The spectral distribution of the sharp peak is reported in the lower part, assuring that the signal is related to lead.

Ions arising from the vaporizing laser pulse can be observed as a broad oscillating structure in the upper part of the figure, while the photoionization generated by the dye laser is clearly visible as a sharper peak, that can be easily recognized in the experiment as it is present only when both laser operate at the correct delay. The wavelength dependence of the sharp peak assures that it is relative to the lead vapour, as is shown in the lower part of the figure.

Other patterned depositions of organic molecules have been obtained using self standing meshes, like the one reported in fig. 8, or localizing the electric field by means of a thin tip; the tip may also be moved following any arbitrary pattern.

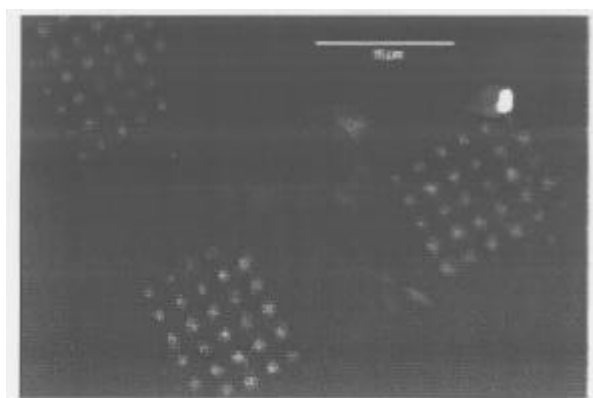


Fig. 8 *Reticular patterns of Fluorescein isothiocyanate on glass, as observed by the green fluorescein emission in a confocal microscope.*

In fig 9, tryptophan molecules are deposited on highly oriented pyrolytic graphite by the field generated by a tip moved along a "V" pattern by a commercial, low resolution xy stage.

Lead atoms were also deposited in the tip induced electric field. In fig 10 we tried to establish the spot size dependence on the tip to

substrate distance; the spot size varies from 2 to 0.5 μm approximately, as shown by the Scanning Tunnelling Microscope images, on varying the distance from 3.5 to 0.14 μm . However no quantitative relation has been obtained so far due to deposits of



neutral material on the tip, that broaden and distort the field.

Fig. 9 "V" shaped tryptophan deposition. The AFM scan is 8x8 μm

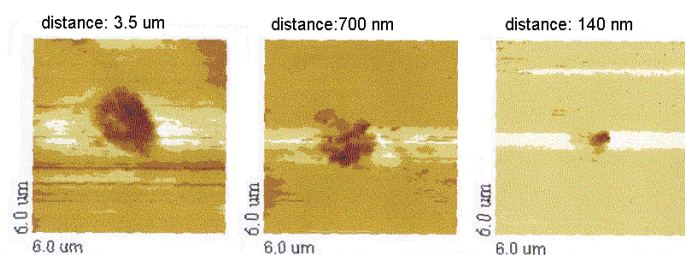


Fig 10 Spots of lead on HOPG for different tip to surface distance

Conclusions

We have shown that laser induced ionization can be used for high resolution patterning of atoms and molecules. In the case of

biological macromolecules, which maintain their biological activity as demonstrated in previous work, we observe that this method yields effective immobilization of the first monolayer adhering to the surface. Patterning has been obtained mainly by masking the surface with removable self standing lithographic stencils; first attempts with electric field localization have been performed, but resolution is so far limited to hundreds of nanometers, mainly due to cluster deposits on the tip altering the electric field. Applications of this method range from microbiosensor fabrication, to ultra high density DNA or protein sequencing devices, to molecular electronics. In this respect a particularly interesting feature offered by the laser/SPM technique is that the same apparatus can deposit semiconducting molecules, organic conductors and metal nanowires and effect a test of the functional properties of each molecular device by recording current-voltage characteristics. Due to the compatibility of this technique with the microelectronics fabrication processes, hybrid bioelectronic and silicon based microelectronic devices are easily envisaged.

References

- 1) Eigler, D.M. and Schweizer, E. K. (1990) *Nature* 344, 524
- 2) Gimzewski, J.K.; Jung, T.A.; Cuberes, M.T.; Schlittler, R.R. (1997) *Surf. Sci.* 386,101
- 3) Tang, H.; Cuberes, M.T.; Joachim, C. and Gimzewski, J.K. (1997) *Surf. Sci.* 386, 115
- 4) Cable, J.R.; Tubergen, M.J. and Levy, D.H. (1987) *J. Am. Chem. Soc.* 109, 6198
- 5) Grotemeyer, J. and Schlag, E.W. (1988) *Org. Mass Spectrom.* 23, 388

- 6) Beuhler, R.J.; Flanigan, E.; Greene, L.J.; Friedman, L (1974) J. Am. Chem. Soc. 96, 3990
- 7) Meijer, G.; de Vries, M.S.; Hunziker, H.E. and Wendt, H.R. (1990) Appl. Phys. B 51, 395
- 8) Hillenkamp, F.; Karas, M.; Beavis, R.C. and Chait, B.T. (1991) Anal. Chem. 63, 1193A
- 9) Morales, P.; Pavone, A.; Sperandei, M. Leter, G.; Mosiello, L.; Nencini, L.; Grifoni, L. and Santucci, S. (1995) Mat. Sci. Eng. C2, 173
- 10) Di Fabrizio, E.; Grella, L.; Baciocchi, M.; Gentili, M.; Ascoli, C.; Cappella, B.; Frediani, C. and Morales, P. (1997) J.Vac. Sci. Technol. B15, 1
- 11) Di Fabrizio, E.; Gentili, M.; Morales, P.; Pilloton, R.; Mela, J.; Santucci, S. and Sese, A. (1996) Appl. Phys. Lett. 69, 3260
- 12) Ready, J.F. (1971) *Effects of High Power Laser Radiation;* Academic Press