

# Preservation of DNA and Protein Biofunctionality for Bio-MEMS & NEMS Fabrication

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## ABSTRACT

Microfabrication processes for the creation of MEMS and BioMEMS devices, especially in silicon are not compatible with biomolecules. Silicon processing is often performed at high temperatures and makes use of aggressive chemicals including acids, bases, oxidizing chemicals and organic solvents. Contacted to such chemicals biological materials are denatured, oxidized, hydrolyzed and their biofunctionality is destroyed. Therefore biological materials can only be combined with microdevices after the last microfabrication step was performed. The incompatibility of microfabrication with biological materials and molecules such as DNA or proteins is a limitation in regard of scalability, patterning and alignment of biological materials with microstructures and for the creation of integrated Biomicrodevices and BioMEMS. Recently we introduced a novel technology using a gold passivation mask to protect biomolecules during microfabrication [1]. Our process enables the integration of DNA and proteins into a mass scalable microfabrication process and preserving DNA and protein biofunctionality.

**Keywords:** BioMEMS, Biomicrodevices, Microfabrication, DNA, Protein

## INTRODUCTION

The introduction of silicon-based chemical analysers, total chemical analysis systems ( $\mu$ TAS) and microelectromechanical systems (MEMS) in the last decade demonstrated that the microfabrication methods developed in the silicon microelectronics industry can be used for the design and manufacture of so-called “lab-on-a-chip” devices for the chemical industry, research laboratories and environmental applications [2, 3]. The combination of such devices with biomolecules has the potential to revolutionize the future practice of bioanalytics and medicine by creating

Biomicrodevices and BioMEMS with powerful functionalities for diagnostics or medical treatment. Immobilization of DNA on solid supports including silicon wafers [4, 5] is now routinely performed in the fabrication of DNA-microarrays and immobilization of antibodies, enzymes and proteins for biosensors or protein-microarrays. Immobilization of DNA and proteins onto or into microdevices and BioMEMS is demonstrated as well. However, in all cases the biological molecules are immobilized or otherwise combined with the microfabricated MEMS device after the last microfabrication step, to finally create a BioMEMS device. Here we envisage a full integration of biological molecules into microfabrication processes, meaning the biological molecule can be combined with silicon, glass or polymer based substrates at any desired fabrication step to facilitate patterning, alignment, manufacturing of complex “Bio-Silicon hybrid” structures and a mass scalable process for future BioMEMS fabrication. In a first step towards this goal we demonstrated that DNA and proteins are fully protected during microfabrication (wet etching, metal deposition, and photolithography) by using a gold film as a biocompatible passivation mask.

## RESULTS AND DISCUSSION

Single stranded DNA oligonucleotides and NeutrAvidin (a biotin binding protein) were immobilized onto a silicon wafer with silicon dioxide surface. For oligonucleotide immobilization the silicon dioxide surface was silanized with 3-mercaptop trimethoxysilane (MPTS) and 5' thiolated oligonucleotides were bound via disulfide formation. For protein immobilization the silicon oxide surface was silanized with 3-aminopropyl trimethoxysilane (APTS), then treated with glutaraldehyde and protein were immobilized via Schiff's base formation. A gold film was evaporated onto the entire surface of the wafer to serve as a protection mask for the following microfabrication steps. Evaporation was chosen instead of sputtering to minimize the energy introduced to surface. The evaporation was

carried out below 50 °C. Gold has unique properties making it to a perfect material to serve as a biocompatible mask: it is very stable against oxidation, bases and acids; not permeable for gases; effective shielding UV and visible light; but at the same time gold can be removed chemically under mild conditions at room temperature by treatment with cyanide solution. In the presence of cyanide and oxygen (from the air) gold will be oxidized and forms a strong water soluble complex with cyanide ions. This gold removal process is compatible with most biomolecules.

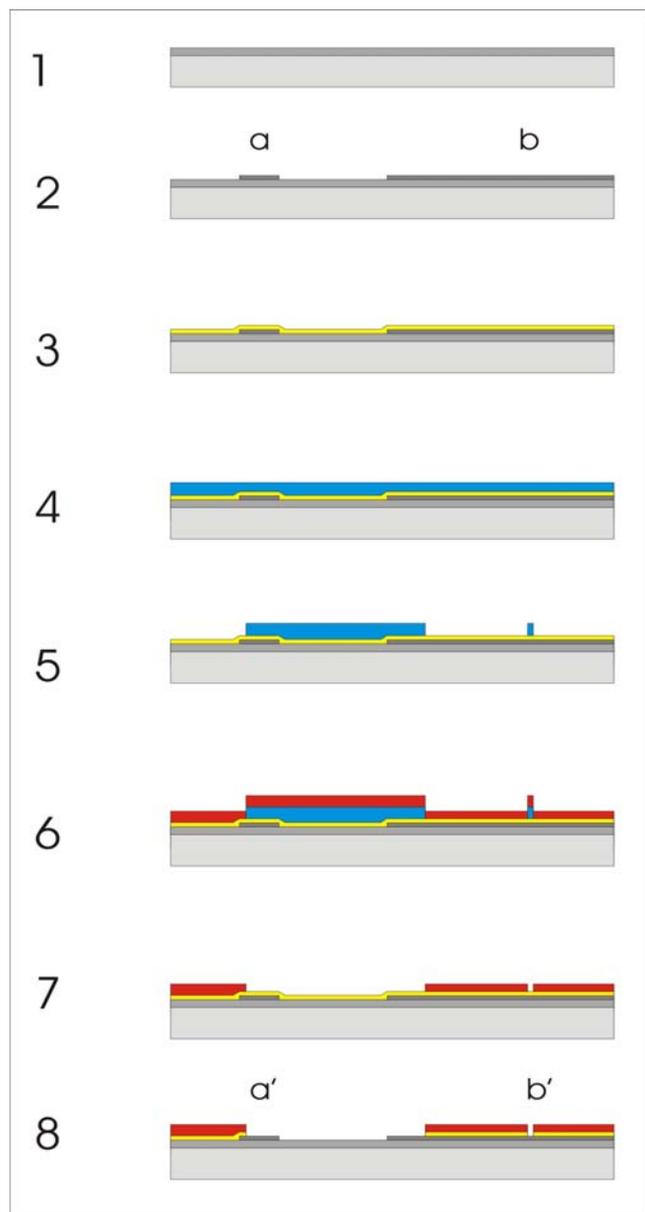


Figure 1 Fabrication of platinum electrodes onto a substrate with pre-immobilized oligonucleotides (DNA-microarray) using a gold film for oligonucleotides protection during photolithography, platinum sputtering and photoresist removal (lift-off process) with acetone.

However the cyanide may have a negative effect in some cases: Enzymes containing metal ions (e.g., Fe) or proteins containing hem groups may lose their biofunctionality due to complex formation with cyanide or cyanide may cleave certain covalent bonds used for immobilization.

Here we want to demonstrate the preservation of DNA during a process to make platinum electrodes (Fig 1). Instead of fabricating electrodes first and then spot a DNA-microarray onto electrodes or beside electrodes, we first created a DNA-microarray and protected the immobilized oligonucleotides (spots "a" in Fig 1-2) with a gold film (Fig 1-2 and 1-3). A photoresist was spin coated (Fig 1-4) and patterned by photolithography (Fig 1-5). Then 500 Å of TiW and 1000 Å of platinum were sputtered onto the wafer (Fig 1-6). Removing the photoresist with acetone resulted in platinum electrodes of different size and shape (Fig 1-7). In the last step the protective gold layer was removed to uncover the immobilized DNA. The process was performed on a 4 inch wafer resulting in a high number of platinum electrodes aligned or partly covering oligonucleotide spots of a DNA-microarray (Fig 2).



Figure 2 Wafer (4 inch) with platinum electrodes aligned or partly covering oligonucleotide spots of a DNA-microarray.

Two types of platinum electrodes were fabricated a) electrodes with a central hole containing a single oligonucleotide spot in the centre but not contacting the spot b) electrodes with a diagonal gap of 100 micrometer contacting the oligonucleotides spot (Fig 3). Figure 3 shows a platinum electrode with diagonal gap fabricated on top of an oligonucleotide spot. The left micrograph shows the electrode before removal of the gold film by cyanide solution. Removal of the gold film with cyanide solution uncovers the oligonucleotides. Biofunctionality was demonstrated by hybridization experiments with fluorescent labeled complementary oligonucleotides. The right fluorescent micrograph of Figure 3 demonstrates successful hybridization. It is clearly seen that two edges of the

oligonucleotide spot are covered by the platinum electrode, because the oligonucleotides were first spotted onto the wafer. Fluorescent light intensity is homogeneous suggesting no negative effect of the platinum or the sputtering process in close proximity to the platinum electrode edge.

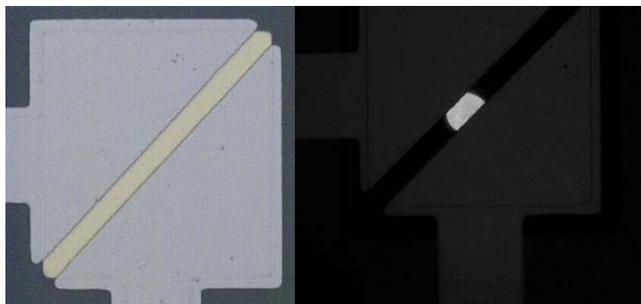


Figure 3 Platinum electrodes fabricated on top of an oligonucleotide spot. Left: electrode before the removal of the gold film (light microscopy micrograph). Right: electrode after removal of the gold film and hybridization of fluorescent labeled complementary oligonucleotides (fluorescent micrograph). The diagonal gap in the platinum electrode has a distance of about 100 micrometer.

Control experiments were carried out with non complementary oligonucleotides showing no hybridization and oligonucleotide spots not subjected to gold evaporation and platinum electrode fabrication to calculate the preservation of biofunctionality [1]. Biofunctionality for DNA/oligonucleotides is defined as the fluorescence intensity signal observed for hybridization of a fluorescent labeled complementary oligonucleotide after gold evaporation, microfabrication and gold removal compared to a signal observed from an experiment without gold evaporation, microfabrication and gold removal. About 85% of oligonucleotides biofunctionality was preserved by using our gold masking method.

## CONCLUSION AND OUTLOOK

Gold film passivation of oligonucleotides was demonstrated to fabricate platinum electrodes on top of a DNA-microarray by using photolithography and a lift-off process partly covering the oligonucleotides. 85% of DNA biofunctionality was preserved; demonstrating the full integration of DNA into silicon microfabrication with the potential to fabricate novel devices. DNA-microarray spotting and microfabrication was carried out on a 4 inch wafer and is potentially scalable for mass production. Electrodes with a gap aligned to an oligonucleotides spot are potentially useful for electrochemical detection strategies. Further studies were carried out to protect antibodies and other proteins. Our results demonstrate that DNA can be protected very efficiently and preliminary data indicate that the method is applicable to a variety of

proteins including antibodies. This would allow the integration of DNA and proteins by using one generic technology into fully integrated Biomicrodevices with the unique functionality, selectivity and sensitivity of such biomolecules.

## REFERENCES

- [1] Trau, D., Jiang J., Sucher J.N., *Langmuir* 2006, 22, 877-881
- [2] Vilknor, T.; Janasek, D.; Manz, A. *Anal Chem* 2004, 76, 3373-3386.
- [3] Liu WT, Zhu L, *Trends in Biotechnology* 2005, (4): 174-179
- [4] Lenigk, R.; Carles, M.; Ip, N. Y.; Sucher, N. J. *Langmuir* 2001, 17, 2497-2501.
- [5] Trau, D.; Lee, T. M.; Lao, A. I.; Lenigk, R.; Hsing, I. M.; Ip, N. Y.; Carles, M. C.; Sucher, N. J. *Anal Chem* 2002, 74, 3168-3173.