



Short communication

Self-aligned process for the development of surface stress capacitive biosensor arrays

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ABSTRACT

A new fabrication process and first experimental results of a surface stress based capacitive biosensor array are presented. Flexible membranes and a fixed electrode on the substrate constitute the capacitive sensors. Probe molecules are immobilized on the membrane surface and the surface stress variations during biological interactions force the membrane to deflect and effectively change the capacitance between the flexible membrane and the fixed substrate. The array consists of 60 sensors and thus is suitable for parallel sensing. The process is characterized by the self-alignment of the sensitive flexible membranes and the use of silicon fusion bonding to fabricate the complete device. First experimental results on biosensing indicate that the sensors are able to detect the hybridization of beta-thalassemia oligonucleotides.

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1. Introduction

Surface stress based biosensors have attracted considerable interest as a new approach for detecting biomolecular interactions through direct and simple methods. Their main advantages are label-free sensing and consequently reduced cost and simplified sample preparation, small size and ability for parallelization into arrays for high throughput analysis. The sensing element is a flexible structure, usually a cantilever or a membrane. The biosensor response depends on the surface stress changes induced during the interaction between the probe molecules immobilized on the flexible structure's surface and the appropriate target molecules.

In most cases, surface stress based biosensors are microcantilevers with optical or piezoresistive readout [1]. However, optical setups are difficult to implement and are usually expensive and bulky. In addition, optical detection is difficult in opaque liquids such as blood. On the other hand, piezoresistive detection is temperature dependent and less sensitive. The capacitive readout is ideal for easy and real time monitoring; it is highly sensitive, requires low power consumption and is very suitable for miniaturized and portable systems. Nevertheless, capacitive detection is not feasible in cantilever biosensors due to faradic currents between the capacitor plates in an electrolyte solution. An alter-

native approach is to replace cantilevers with an ultrathin Si membrane effectively creating a sealed capacitor which prevents liquid insertion between its plates [2–4]. In that work, two special wafers were required to successfully build the device: one SOI wafer and one wafer with strain compensated SiGeB epitaxial layer grown on one surface.

Herein, a novel fabrication process is presented for the fabrication of the capacitive biosensor array. This process is simpler and subsequently cheaper than in [3] and relies on the use of boron implantation through a SiO₂ window to create a highly boron doped region which will result in the sensing element thin flexible membrane. In addition, the use of implantation results in a rather rough sensor surface, which is expected to facilitate probe immobilization [5] and enhance the sensing performance of the membranes [6,7]. First experimental results for biological diagnostics are presented utilizing the beta-thalassemia CD19 mutation.

2. Materials and methods

2.1. Biosensor array description

The biosensor array consists of 60 membranes which are used as sensing elements for multiple target detection. Each sensing element is a capacitor comprised of two electrodes (Fig. 1a). The flexible electrode is a boron doped ultra thin silicon membrane suspended over a cavity. The counter electrode on the substrate is formed by phosphor implantation and is common for all sensing elements. The distance between the capacitor plates is determined

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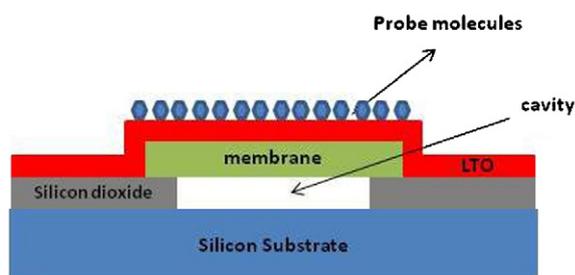


Fig. 1. Schematic of the capacitive sensing element. The cavity etched in the silicon dioxide layer allows for the deflection of the silicon membrane electrode. All the sensing area is passivated by a low temperature oxide (LTO) on which the immobilization of the probe molecules takes place.

by the thickness of the silicon dioxide layer that supports the membranes peripherally. A passivation layer of silicon dioxide deposited at low temperature (LTO) offers electrical isolation and also constitutes a suitable surface for the immobilization of the biomolecules.

The device operation relies on the surface stress changes induced on the membrane surface due to biomolecular interactions. In particular, probe molecules are immobilized on the membrane surface and waiting for binding with their respective target counterparts. The surface stress changes that are mainly caused by the target–probe interactions, result in the membrane bending and therefore in a change of the capacitance between the flexible and the counter electrode. When different elements of the same array accommodate different probe molecules the signal of each sensing site contributes to a high throughput analysis.

2.2. Fabrication process

For the fabrication of the biosensors we need two wafers, the membrane wafer and the substrate wafer, which are silicon fusion bonded. The process begins by first forming a 5000 Å thick thermal silicon dioxide layer (Fig. 2a) on the membrane wafer. Circular sensor cavities are then formed in the thermal oxide using optical lithography and CHF₃ anisotropic dry etching (Fig. 2b). Boron ion implantation follows for the creation of a highly boron doped region which will subsequently form the flexible electrode of the capacitive sensing element (Fig. 2c). The ion implantation and annealing conditions determine the thickness of the final membranes and these conditions are selected based on simulation results and/or wet etching test experiments. The chosen conditions were 2×10^{16} ions/cm² with energy 150 keV, followed by thermal annealing at 1050 °C for 1 h and resulted in the formation of 0.8 μm thick membranes after wet etching in EDP (ethylenediamine/pyrocatechol/H₂O) solution.

On the surface of the substrate wafer, phosphor implantation with 10^{15} ions/cm² dose and 20 keV energy, followed by thermal annealing at 1000 °C for 20 min, results in the formation of the fixed substrate electrode (Fig. 2d). Afterwards, RCA cleaning takes place and a 200 Å thick silicon dioxide film covers the fixed phosphor doped electrode in order to provide isolation in case a membrane touches the substrate.

After the preparation of these two wafers, silicon fusion bonding (SFB) between the two takes place for the formation of one final Si wafer which will accommodate the capacitive micro-membrane arrays (Fig. 2e). The membrane wafer is then lapped and the wafer thickness is reduced from 500 μm to about 50 μm (Fig. 2f). For this step a 12 μm calcined aluminium oxide powder in a plane iron tray is used. The rest of the wafer is etched with EDP and the membranes are patterned as wet etching stops at the highly boron doped regions (Fig. 2g).

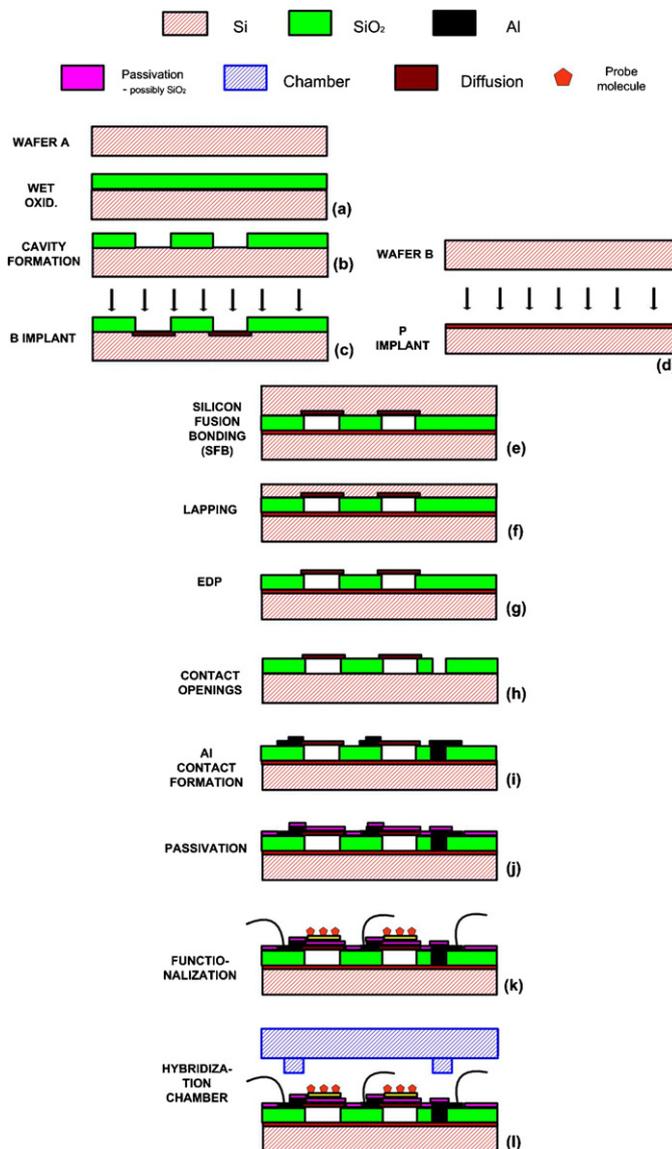


Fig. 2. Capacitive biosensor array fabrication process.

Next, optical lithography and anisotropic dry etching of silicon oxide follows for the formation and opening of the substrate contacts (Fig. 2h). Afterwards, a 5000 Å thick Al layer is deposited and the desired Al areas are defined with optical lithography (Fig. 2i). In order to create the passivation layer, which also serves as the probe molecules immobilization layer, a SiO₂ film (low temperature oxide, LTO) is deposited with chemical vapor deposition (Fig. 2j). For the removal of the LTO from the Al pads, optical lithography is used, followed by wet etching with BHF for 1 min and dry etching with SF₆ for 5 min. After this step, a batch of biosensor arrays of ultrathin Si membranes has been fabricated. The developed sensors have membrane diameters 150, 200 or 250 μm. These arrays were finally cut in 12 mm × 12 mm dies. In each array the membranes occupy 5 mm × 5 mm and this area will be referenced as the sensing area. Afterwards, each array can be functionalized (Fig. 2k) and measured in a specially designed chamber that isolates the sensing area (above which the biological solutions must flow) from the electrical connections (Fig. 2l).

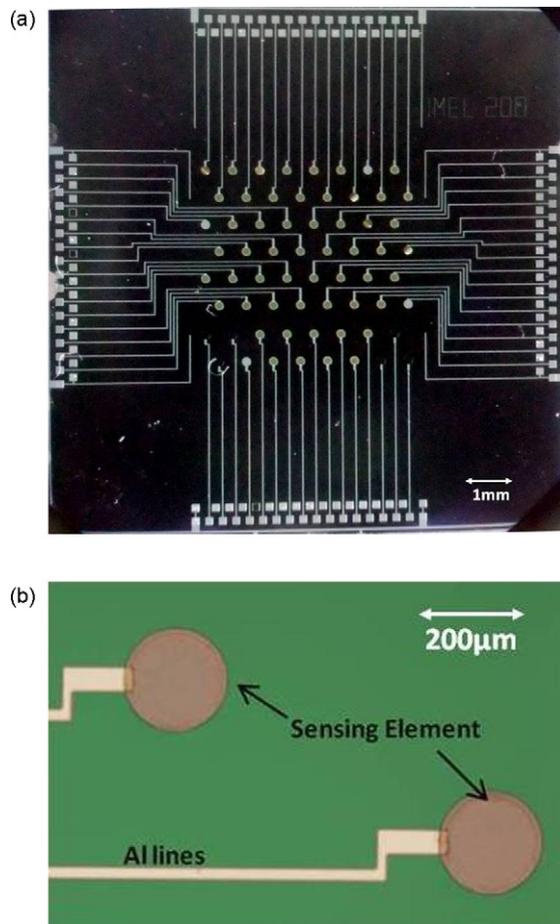


Fig. 3. (a) Microphotograph of a fully processed biosensor array and (b) closer view of the circular membranes.

3. Results and discussion

In Fig. 3 a fully processed biosensor array micro-photograph is shown. The long Al lines are necessary for the isolation of the electrical connections from the sensing area, which is enabled through a specially designed gasket in the hybridization chamber. Fig. 3b shows part of the biosensor array with two sensing elements.

In Fig. 4 a scanning electron microscope (SEM) image of the cross-section of a fully fabricated sensing element is shown. The membrane rim over the SiO₂ layer is less than 0.5 µm and is cre-

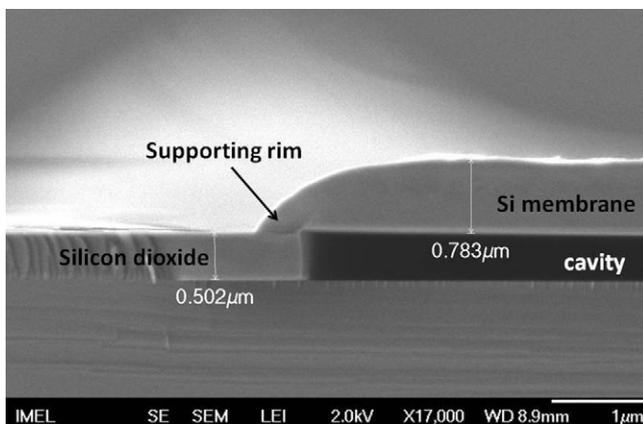


Fig. 4. SEM image of the Si membrane and its silicon dioxide support layer.

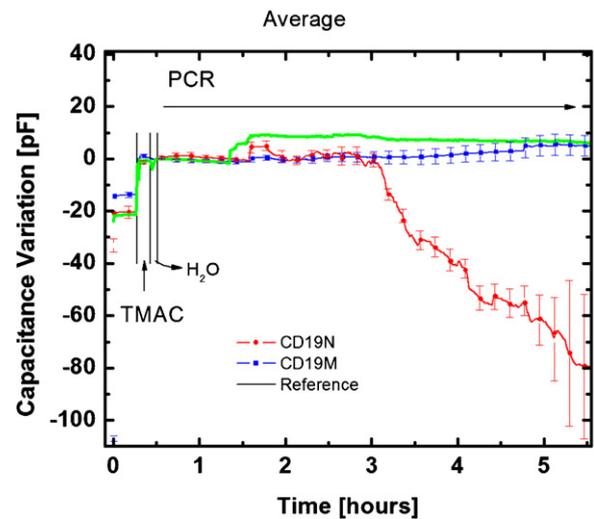


Fig. 5. The average response of the biosensor array during DNA hybridization.

ated by the lateral diffusion of boron into the Si. The narrow rim, on which the membrane stands, hinders that the membrane is not fully clamped on its edges and thus it can deflect easier.

After fabrication, the biosensor array was tested using the beta-thalassemia related mutation CD19 and arrays with sensor membranes of 250 µm diameter. During the experiment, two different oligonucleotide types, CD19 normal (CD19N) and CD19 mutated (CD19M), were immobilized on the functionalized surface of selected sensing elements on the same array. Some membranes of the array were left without probe molecules on their surface in order to be used as reference. The polymerase chain reaction (PCR) product was intended to hybridize only the CD19N oligonucleotides and has a single nucleotide mismatch with the CD19M probes. The two different amino-modified oligonucleotides, CD19N and CD19M, were immobilized using a micropipette. The functionalization procedure and the measuring setup are described in detail in Ref. [4].

In Fig. 5 the average response along with the corresponding error of several sensing elements that have been spotted with the CD19N and CD19M oligonucleotides during hybridization is shown together with the response of a reference membrane. The PCR product concentration is 18 nM and the immobilized CD19 probe molecules concentration was 100 µM. The average value of the capacitance variation due to the biomolecular interaction is extracted out of the response of the individual sensing elements in the same array with CD19N and CD19M oligonucleotides. The response of the sensing elements spotted with the CD19M probe oligonucleotides and the reference sensor show negligible capacitance variations compared to the average capacitance change of the CD19N spotted sensors thus indicating the hybridization of the CD19N oligonucleotides. These results compare well with previous studies [4] and indicate the ability of the biosensor arrays to detect hybridization events. Moreover, they offer considerable advantages in terms of simplifying the fabrication process and lowering fabrication costs.

4. Conclusions

A new fabrication process for a biosensor array with Si membranes as sensing elements is demonstrated. Flexible membranes and a fixed electrode on the substrate constitute capacitive biosensors based on surface stress variations. The process is characterized by simplicity and lower cost that are achieved through the self-alignment of the membrane. First experimental

results on biosensing indicate that the sensors are able to detect the hybridization of beta-thalassemia oligonucleotides in 18 nM concentration.

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Biographies

Dr. Vasiliki Tsouti was born in 1980. She received the BS degree in 2002 and MSc degree in solid state physics in 2005, both from the Department of Physics at the University of Athens, Greece. In 2009 she received the PhD degree from the Department of Applied Sciences of the National Technical University of Athens and the Institute of Microelectronics at NCSR “Demokritos”. Her undergraduate and MSc thesis were focused on the electrical properties of SOI structures. Her PhD work included the development of chemical and biological micromechanical sensors. She is currently post-doctoral researcher of the Institute of Microelectronics at NCSR

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Myrto-Kyriaki Filippidou was born in 1985 in Athens, Greece. She received her BSc degree in 2007 from the National Kapodistrian University of Athens, Physics Department and her MSc in microelectronics in 2010 from the National Kapodistrian University of Athens, Telecommunications and Informatics Department. Her undergraduate thesis was a study on silicon targets using charged particles and X-rays, which was elaborated on the Institute of Nuclear Physics at NCSR “Demokritos”. Her MSc thesis focused on biosensors and included the detection of DNA hybridization. The last two years she is working in the Institute of Microelectronics at NCSR “Demokritos” on the development and optimization of capacitive silicon sensors for biological applications.

Christos Boutopoulos was born in 1982. He received his BS in applied physics from the National Technical University of Athens in 2005 and his MSc in microsystems and nanodevices from the same University in 2008. His MSc thesis was focused on the development of protein microarrays via direct laser printing. He is now working at NTUA on his PhD thesis and his current research is focused on the combination of direct laser printing methods and sensing microsystems for the development of biosensors and chemical sensors.

Panagiotis Broutas was born in 1983. In 2005 he graduated from the applied physics faculty of the School of Applied Mathematical and Physical Sciences of the National Technical University of Athens. In 2007 he got a masters from the “Control Systems and Robotics” direction of “Automation Systems” master studies program of the same university. He is currently working on his PhD thesis on mechanical strain sensors at the Institute of Microelectronics of the National Center for Scientific Research “Demokritos” (NCSR) in Athens, Greece.

Dr. Ioanna Zergioti (assistant professor physics, National Technical University of Athens) has more than 20 years experience in phenomena related to the interaction of electromagnetic radiation with matter and the development of laser materials processing for applications in nanotechnology and microdevices. During her PhD she worked at the University of California, Berkeley, and after her PhD she did research work at Max Planck Institute fuer Biophysikalische, and then at Philips CFT working on “Stereostiction of passive ceramic electronic 3-dimensional structures using inkjet and laser sintering technologies”. One of her main research achievement was the use of laser pulses to direct print biomolecules on biosensors and polymers on sensors. She was involved in many research projects funded by the EC as a project coordinator and as research team member, during the last 18 years. She has more than 120 publications in international peer reviewed journals and publications in conferences and she has contributed three chapter books on the laser printing technology.

Dr. Stavros Chatzandroulis received the bachelor degree and the MSc in electronic automation from the Department of Physics at the University of Athens in 1990 and 1993, respectively. In 1999 he received the PhD degree from the same department for his work on integrated silicon sensors. Since then, he has been with the Institute of Microelectronics at NCSR “Demokritos” where he now holds the position of researcher. His interests include silicon micromachining technology, sensor and sensor electronic interface design and wireless networking. He has worked on the development of a number of sensors, including pressure sensors, chemical sensors and biosensors and their electronic readout.