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Integrative technology-based approach of microelectromechanical systems (MEMS) for biosensing applications

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Abstract— **In this work we simultaneously aim at addressing the design and fabrication of microelectromechanical systems (MEMS) for biological applications bearing actuation and read-out capabilities together with adapted tools dedicated to surface functionalization at the microscale. The biosensing platform is based on arrays of silicon micromembranes with piezoelectric actuation and piezoresistive read-out capabilities. The detection of the cytochrome C protein using molecularly imprinted polymers (MIPs) as functional layer is demonstrated. The adapted functionalization tool specifically developed to match the micromembranes' platform is an array of silicon cantilevers incorporating precise force sensors for the trim and force measurements during deposition of biological materials onto the sensors' active area. In either case, associated analog electronics is specifically realized to deal with specific signals treatment fed through the MEMS-based devices.**

I. Introduction

The microelectromechanical systems for biological applications (a.k.a. bioMEMS) are stirring the MEMS scientific interest for more than 10 years now [1]. Despite considerable efforts, the bioMEMS domain is still looking for success-stories similar to MEMS-based accelerometers or pressure sensors. The reason of this uncomfortable conclusion is due to all complex issues related to biological sensing that can be successfully addressed when considered separately but may become a real dead-end when put together at the microscale.

State-of-the-art bioMEMS with detection limits of clinical relevance have been proposed [2] making use of basic functionalisation methods which eventually reduce the multiplexing capabilities. Conversely, massively parallel arrays of cantilevers has been developed for localized biological functionalization purposes over large areas with sub-100 nm feature size control [3] whereas all but fluorescence or scanning probe microscopy read-out methods are inadequate for such high-density patterns.

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To both answer the biosensing and localized functionalization issues, we aimed at addressing the design and fabrication of MEMS bearing actuation and read-out capabilities together with adapted tools dedicated to surface biopatterning at the microscale. The biosensing platform presented here is based on arrays of silicon micromembranes integrating piezoelectric actuation and piezoresistive read-out capabilities. One specific application is addressed in this paper: the detection of the cytochrome C protein using molecularly imprinted polymers (MIPs) as functional layer. The adapted functionalization tool specifically developed to match the micromembranes' platform is an array of silicon cantilevers incorporating precise force sensors for the trim and force measurements during deposition of biological materials onto the sensors' active area. In either case, associated analog electronics is specifically realized to address sensing purposes related to the aimed applications.

II. Fabrication of biosensors array and associated electronics scheme

A. Biosensors array

Arrays of 5 silicon-based circular micromembranes were realized by standard microfabrication techniques from 4 inch (100) N-type silicon-on insulator (SOI) wafers (5 µm device layer, 1 µm buried oxide, 525 µm handle). Each membrane is actuated by a 1 µm-thick 54/46 $PbZr_xTi_{1-x}O_3$ (PZT) patch patterned on top of the membrane [4]. The detection of the membrane's vibration is realized by a piezoresistor (20° angular aperture, 200 *nm* thick and 10 *µm* width) located at its very periphery. To prevent common mode phenomena from being measured, the differential method of measurement is chosen. For this to be done, one of the five micromembranes on the chip is used as reference. Fig. 1 shows one chip (7.2 x 6.4 mm²) of 5 micromembranes fabricated as described above.

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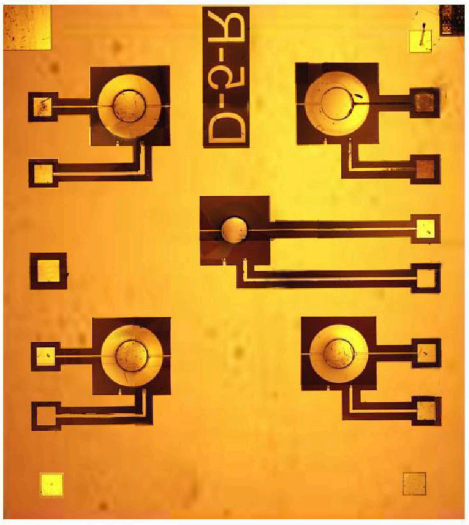

Figure 1. Optical micrograph of the complete micromembranes' chip.

B. *Associated electronics*

Fig. 2 shows the block diagram of the associated electronics allowing the micromembranes' resonant frequencies measurement in air or liquid media.

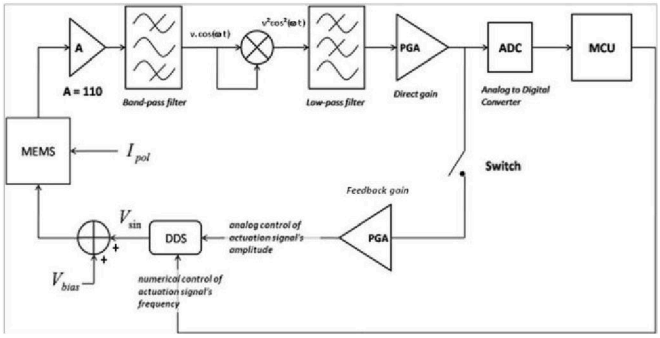

Figure 2. Scheme of the electronic set-up for multiplexing, amplification and treatment of the piezoresistors' output signals. MEMS stands for the micromembranes' chip, Ipol represents the polarization current injected through the measurement and the reference piezoresistors, A is the amplification factor, PGA stands for Programmable Gate Array, ADC is the Analog-to-Digital Converter used to drive the microcontroller unit (MCU), DDS is the Direct Digital Synthesizer, Vsin stands for the output voltage provided by the DDS and Vbias is the static voltage eventually used for optimum actuation conditions of the piezoelectric module

First, a compensation protocol allows canceling the eventual piezoresistors' mismatches before resonant frequencies measurements being done [5]. The resulting signal is then amplified and treated through a wide band-pass filter to eliminate low frequency and digital noise. The signal is multiplied by itself and low-band filtered so that an image of the square of its amplitude is given. This last signal is then amplified by a settable gain (called direct gain) and a microcontroller is used to detect the locals' maximum or minimum, signing either a resonance or an anti-resonance phenomenon. In order to increase the resonance peaks quality factors, a closed-loop chain can be activated with a switch. The amplitude of the oscillating output potential read-out on the direct gain amplifier is derived and amplified by a second settable amplifier. This balances the analog amplitude signal provided by the Direct Digital Synthesizer (AD9832, Analog Device, USA) which is re-injected to the micromembrane for actuation purpose.

III. FUNCTIONALIZATION TOOL

Fig. 3 shows a schematic of the cantilevers array chip dedicated to the localized functionalization of the micromembranes previously described.

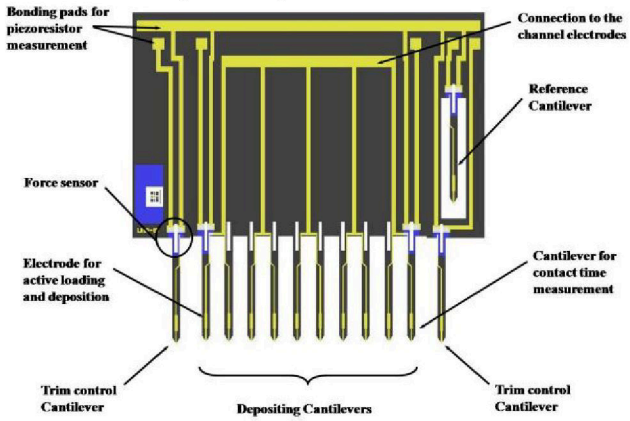

Figure 3. Drawing of the cantilevers array chip.

The fabrication of the cantilever arrays based on standard micromachining techniques. The process includes 8 photolithographic steps. The starting substrate is a 100 mm, (100), n-type SOI wafer, with a 1 µm thick buried oxide layer and a 5 µm thick top silicon layer. The use of SOI wafer enables the precise control of the cantilever thickness, ensuring the consistency of their mechanical properties. The first step consists in creating the piezoresistor in the bulk silicon. In order to optimize the piezoresistor sensitivity, the cantilevers are patterned along crystal axes for which the longitudinal coefficient is maximum, i.e. along the <110> direction in the case of a p silicon piezoresistor. After removing the oxide masking layer in buffered oxide etch (BOE), the cantilever shape is created by a deep reactive ion etch (RIE) of the top Si layer. The final shape of the piezoresistor is realized with this anisotropic dry etch process. The channel and the reservoir are also fabricated during this step. Contact holes are opened and a 200 nm thick AlSi layer is sputtered and patterned in order to obtain electrical contacts for the resistors. Metal annealing is then performed at 450 °C for 20 min. The cantilevers are finally released by a deep reactive ion etch (DRIE) of the back side of the silicon wafer, followed by a RIE of the buried silicon dioxide layer.

Each cantilever is 1500 µm long, 120 µm wide, and 5 µm thick, the channel is 200 µm long, 4 µm wide, and 5 µm thick, and the optional reservoir is 200 µm long, 24 µm wide and 5 µm thick. The volume of the channel and the reservoir is approximately 4 pL and 24 pL, respectively. The 10 depositing cantilevers are separated from each other by 320 µm. To ensure that the trim control cantilevers touch the surface first when approaching the array to depositing

surfaces, they are shifted 30 µm forward the depositing cantilevers. Each chip is then packaged to allow fixing it to the positioning apparatus and connecting it to the piezoresistor readout electronics. For that purpose, the chips are glued and bonded to a printed circuit board (PCB). The chip is designed to incorporate ten cantilevers for parallel liquid deposition [6]. Two of these cantilevers incorporate a force gauge to measure and monitor the contact time and force during deposition. Two similar force sensing cantilevers are added on each side of the array for the trim control. An additional piezoresistive cantilever is integrated in the body of the chip for reference purpose as it undergoes the environmental changes but is free from contact with the surface. The fluidic part of each cantilever comprises a slit that opens out into the extremity of the cantilever tip and a reservoir in the beam body.

The electronics designed for the cantilevers chip incorporates one modified Wheatstone bridge (consisting in replacing the two static resistors of the bridge with controlled current generators bridge per piezoresistive cantilever), using the reference cantilever as the reference resistance in the four bridges. The electronic components used in the realization of the parallel bridges are incorporated onto a detection board which the packaged chip is directly connected to. Fig. 4 shows the electronic diagram of the detection board. For packaging issues, this electronic board does not incorporate all the electronic components and is then connected to a main driving board. This board includes a final amplification stage, means to compensate the resistance dispersions (using offset voltages on the second amplification stage), bias driving, and A/D converters for the measurement of each piezoresistive cantilever. It also includes a microcontroller that allows the operator to fix the voltages and currents required for an optimized operation.

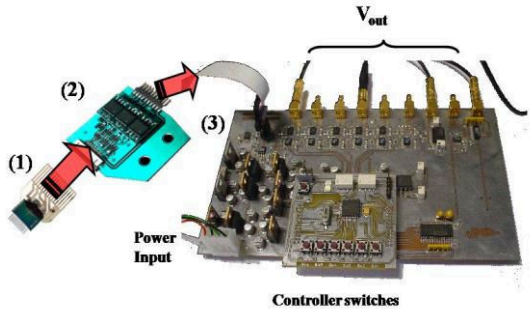

Figure 4. Complete electronic setup including: (1) the cantilevers' chip on a printed circuit board, (2) the detection board allowing the chip to be mounted onto the robot, and (3) the main driving board.

IV. RESULTS AND DISCUSSION

Synthetic receptors capable of binding a target molecule with similar affinity and specificity to antibodies or enzymes have been a long-term goal in biosensor development. One technique that is being increasingly adopted for the generation of such biomimetic receptors is molecular imprinting of synthetic polymers. This is a process where functional and cross-linking monomers are co-polymerized in the presence of an imprint molecule (the target molecule or a derivative thereof) that acts as a molecular template. The functional monomers initially form a complex with the imprint molecule, and following polymerization, their functional groups are held in position by the highly cross-linked polymeric structure. Subsequent removal of the imprint molecule reveals binding sites that are complementary in size and shape to the template. In that way, a molecular memory is introduced into the polymer (molecularly imprinted polymer, MIP), which is now capable of selectively rebinding the target with a specificity and selectivity similar to an antibody or an enzyme.

For the application aimed here, MIPs against proteins (cytochrome C) were synthesized. It has to be noted that MIPs for the specific detection of proteins remain a challenge because of the size and the 3-dimensional configuration of proteins. We first studied the individual functionalization of micromembranes with a MIP Cytochrome C and his corresponding Non-Imprinted Polymer (NIP), acting as a control. The micromembranes were individually coated with MIPs and NIPs by using the cantilever array based deposition tool previously described [7-8] (Fig. 5).

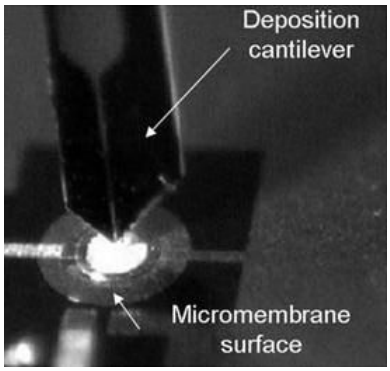

Figure 5. A cantilever loaded with MIP precursor solution during deposition step onto a micromembrane.

After the polymers' deposition, the micromembranes' chip was placed in a N_2-saturated atmosphere to allow the UV-polymerization (365nm) of the mixtures. Subsequently, the resonant frequency of the functionalized membranes was monitored in real-time to study the stability of the deposited MIPs. As shown on Fig. 6, drifts of the resonant frequency (due to thermal sensitivity of the polymer) are clearly visible. In addition, these frequency shifts depend on the volume of deposited polymer. Preliminary cytochrome C detection experiments exhibited coherent results in terms of frequency shifts of the micromembranes. Indeed, a resonant frequency increase of 4 kHz was observed on the MIP after the template's elution, while a decrease of 8 kHz was measured

after the incubation of the surface in a 50µg/ml solution of cytochrome C.

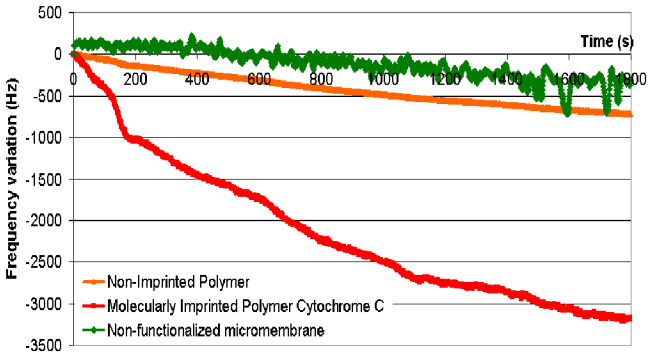

Figure 6. Real-time monitoring of the resonant frequency of micromembranes functionalized by a MIP against cytochrome C, corresponding NIP and a non-functionalized membrane.

In summary, the use as biosensors of piezoelectric circular micromembranes functionalized with MIPs by means of a MEMS-based patterning tool was investigated. It is reasonably expected that 5 to 10 years from now the integration level and low power consumption of such biomimetic sensing platforms could offer new opportunities for portable instrumentation dedicated to biological analyses.

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