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AlN piezoelectric micromachined ultrasound transducer (pMUT) for E. Coli detection

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Summary: This work presents the fabrication process and characterization of a pMUT intended for the detection of E. Coli in liquid samples. A piezoelectric transducer in aluminium nitride is integrated in a CMOS-based process and later functionalized with specific antibodies to achieve E.Coli detection through resonance frequency tracking in air using a Vector Network Analyser.

Keywords: pMUT, AlN, Biosensing, E. Coli

Introduction

E. Coli presence in surface water and drinking water is a global health and economic issue worldwide. Rapid and advanced biosensor are needed as, tests, currently available on the market based on PCR or bacterial culture, have response time far away from real-time detection. Real-time detection can be achieved using Micro-electromechanical systems (MEMS) which have proven their efficacity for mass sensing application [1]. However, mechanical biosensor have yet failed to provide market-ready solution [2]. To bring mechanical biosensor ready for on field application, we think that in the wide range of MEMS systems, pMUT using AlN is the most promising technology. Indeed the physical properties of AlN such as a low permittivity dielectric couple with pMUT advantages i.e no cavity structure, electrical sensing method, no voltage bias [3] make it a good choice for biosensing. This contribution presents a fabrication process of a membrane-based pMUT and dedicated experiments to detect E. coli based on mass sensing through microgravimetry.

Experimental details

The pMUT is a circular membrane of 75µm radii. This design is the result of a study to detect a few bacteria (at least one) on its surface. Figure 1 shows the process flow to make the device. The device is made using 4 inches of SOI wafers (with 3µm thick buried oxide and 1.5µm thick top silicon). On top of the SOI a stack of 200 nm of Mo, 1µm of AlN, and 200nm of Mo has been deposited. This stack is then processed to build the pMUT layout with the Mo as top and bottom electrodes of the AlN piezoelectrical layer. The first steps consists in patterning the top elctrode, the AlN and the bottom electrod as descibed in picture Fig1 a) to d). A lift-off of Ti/Ni/Au is then done to generate bond pads and a gold disk of 30 nm (made also by lift-off) is deposited on top of the membrane (Fig 1e) 1f)). This gold disk will be used for future specific functionalization of the pMUT. To get a fully functional device a backside deep reactive ion etching followed by an RIE is done to etch first silicon and then the SiO_2 buried oxide. Figure 2a) shows an optical micrograph of the pMUT sensor.The left pMUT is used for the detection meanwhile the right pMUT is used as an electrical reference (this pMUT is not open).

To detect E.coli, a two-step functionalization protocol is implemented. First, the device is incubated in a Phosphate Buffer Saline (PBS 1X) solution containing anti-Ecoli antibodies at 10µg/mL for 60 minutes. The device is then rinsed using PBS 1 X and incubated for 30 min with a solution of Bovine Serum Albumin (BSA) at 2% (w/w) in order to passivate non specific binding sites. The device is here again rinsed using PBS 1X and then in de-ionized water. Finally, the device is immersed in a suspension of E.coli (O157H7 strain with Syto-9, stained to be observed in green fluorescence) at 10^8 CFU/mL for 60 min at 300 RPM. The protocole is presented in Figure 2b). To avoid frequency fluctuation due to environement changes, a resonant frequency tracking was used in between each step for at least 5 minutes at room temperature : before the

functionalization step, between the functionalization and E. Coli incubation, and finally after this incubation.

Results and discussion

Figure 3 shows the COMSOL simulation for the membrane and the capacitance measurement using a vector network analyzer. The fundamental mode of resonance can be found at 986 kHz. At this frequency, the pMUT shows a capacitance of 1.2 pF. Although FEM simulations and experimental data are similar, there is a slight difference in the resonance frequency as well as the width of the resonance. These difference have been explained by a mismatch in the value of the silicon nitride layer stress between the actual deposited film and the parameters used in FEM computing. A second factor can be a variability in the membrane thickness due to deterioration during the buried oxide etching step.

The added mass resulting of the fonctionnalization step is shown in Figure 4a), this step results in a mean resonance frequency shift of -978 Hz. After E. coli incubation the mean resonance frequency shifts of -100 Hz. This result has been coupled with fluorescence microscopy images (Fig 4b) which clearly show two E. Coli on top of the membrane. The theorytical mass sensitiviy calculated of our pMUT is -20Hz/pg. As a bacteria weight arround 1pg we should get -40Hz instead of -100Hz after the incubation which means that others compounds have been deposited. The work paves the way for the detection of E.Coli directly in aqueous media. Additional experiments are being conducted in aqueous media and supplementary data will be presented during the conference.

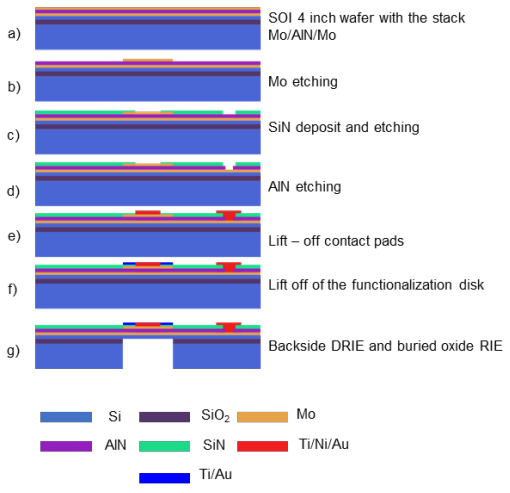

Figure 1: Process flow Description of the main technological steps of the micro- fabrication process used to fabricate the proposed pMUTs

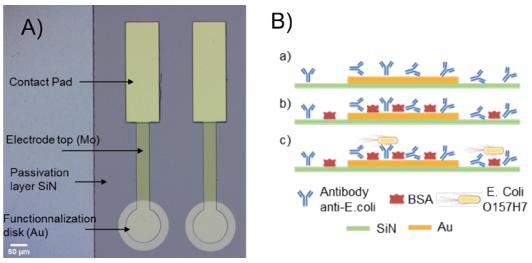

Figure 2: A) Optical micrograph of a two pMUT. The left one is used for the detection meanwhile the right one is used for electrical reference. B) Functionnalization steps : a) antibodies anti-E. coli b) BSA. c) Incubation of E. Coli prior detection

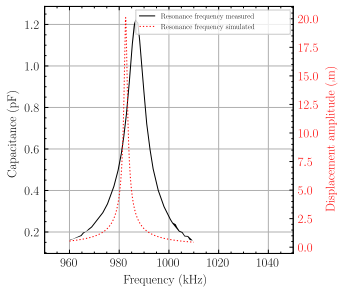

Figure 3: Resonance frequency measured (black curve) vs resonance frequency simulated using COMSOL (red dotted curve)

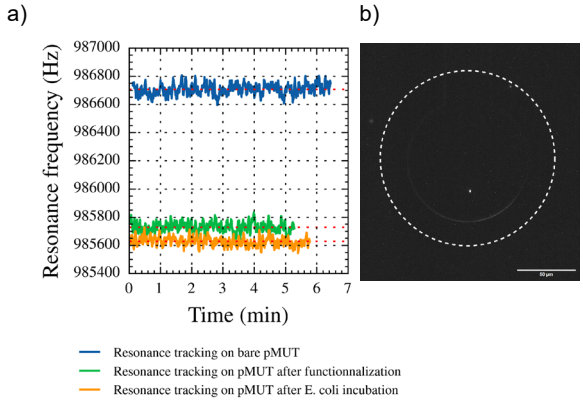

Figure 4 a) Real-time measurement of the frequency resonance: bare pMUT (blue curve), after functionalization (green curve) and after E. coli incubation(yellow curve). b) Fluorescence micrograph of the pMUT after E. coli incubation, the white dotted circle delimits the perimeter of the membrane.

References

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