DESIGN AND FABRICATION OF AN ACOUSTIC MICROMIXER FOR BIOLOGICAL MEDIA ACTIVATION

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Introduction

The bioassay of infinitesimal quantities of protein markers in biological samples is the way to early cancer detection. However, this detection can be limited by the diffusion of these macromolecules (analytes) from the bulk to the sensor chip (surface of ligands). Here, we propose a new method to overcome this drawback by the activation of the biological media during the detection step. The principle consists in using ultrasonic vibrations in order to disrupt the equilibrium states of such biomolecular reactions and performing simultaneous detection inside an acoustic micromixer. Technological realization and initial characterizations of the device have been performed and are presented in this work. Moreover, the capacity of our system to enhance the biosensors performances was studied and validated through a biological model related to breast cancer.

1. Design and operating principle

The biological fluid is activated thanks to acoustic vibrations, at the low frequency range, generated by a bulk Lead-Zirconate-Titanate (PZT) fixed on a silicon membrane.

Acoustic micromixer design

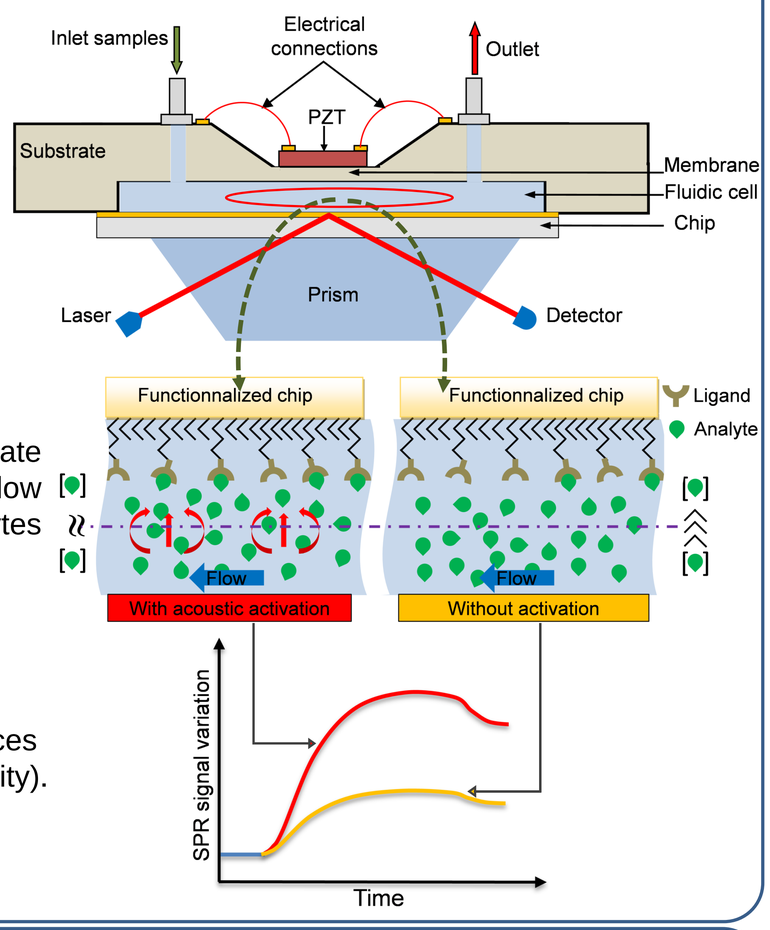
The developed device allow both activation and detection using label free techniques as surface plasmon resonance imaging (SPRi) and surface-enhanced Raman spectroscopy (SERS).

Activation effect

Acoustic radiation pressure create turbulences in the fluidic cell which allow more interaction between free analytes and immobilized ligands.

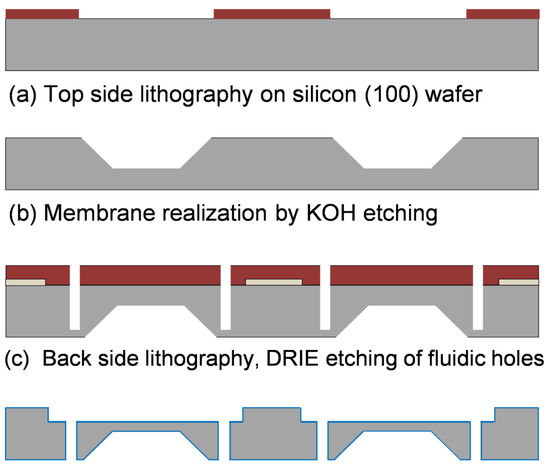
Goal

Increasing the biosensors performances (sensitivity, accuracy and reproducibility).

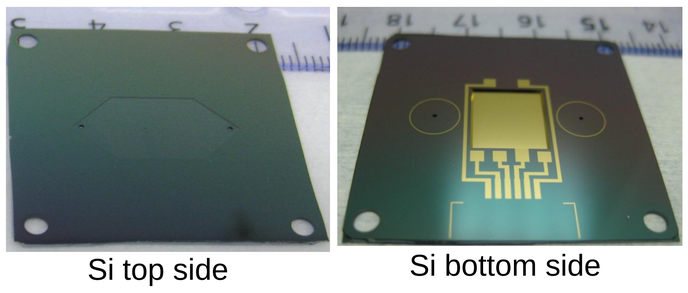


2. Microfabrication and integration

The silicon part is realized by using standard micromachining process as follow:



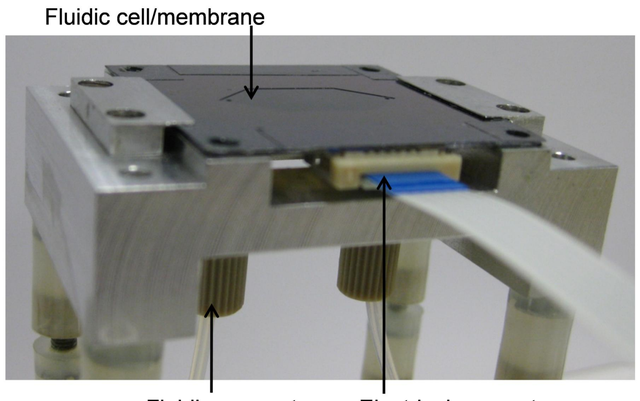
Process flowchart.



Si top side Si bottom side

Picture of the micromachined part.

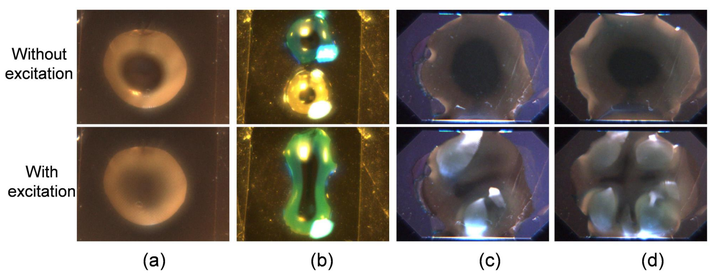
Both electrical and fluidic connectors are integrated on the same silicon substrate.



Realized prototype.

3. Test and validation

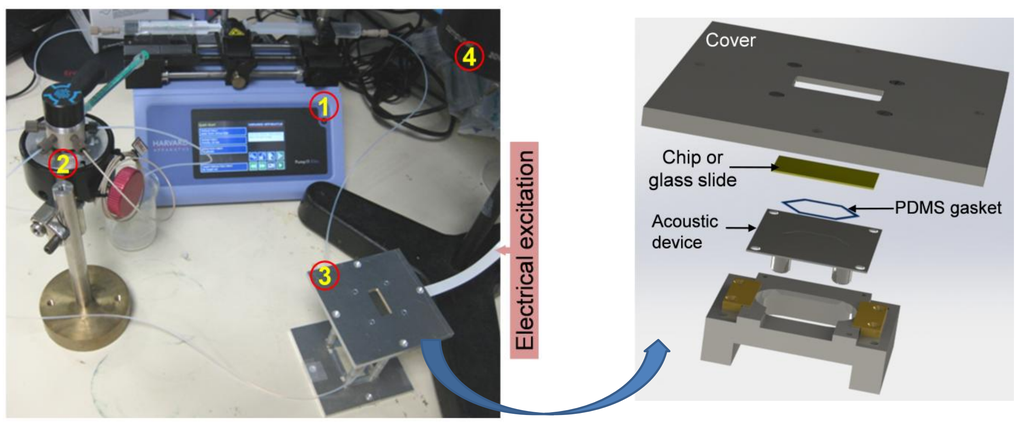
To evaluate mixing efficiency of the device, a solution of colored water of about 50 µl is manually deposited on the top side of the membrane. Then, the PZT element is excited at a frequency range from 1 to 30 kHz.



(a) Fluid mixing at 9 kHz (fundamental mode)
(b) Mixing of 2 fluids, fundamental mode
(c) Fluid mixing, mode (2,1) @ 21 kHz
(d) Fluid mixing, mode (2, 2) @ 28 kHz

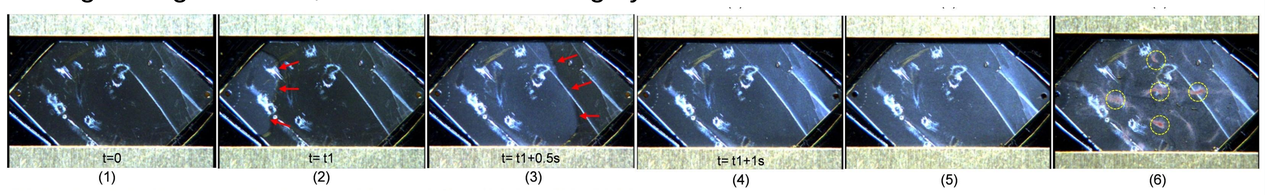
Views of different mixing modes of the membrane.

To test the realized prototype in continuous-flow mode, an experimental setup is established.



Fluidic experimental setup.(1) Syringe pump, (2) Loop, (3) Acoustic device, (4) Camera CCD.

The fluidic test is performed using a diluted blood cell solution. The following images, taken through the glass slide, show the functioning system.



Views of the fluidic cell showing both injection and activation processes in continuous flow mode.

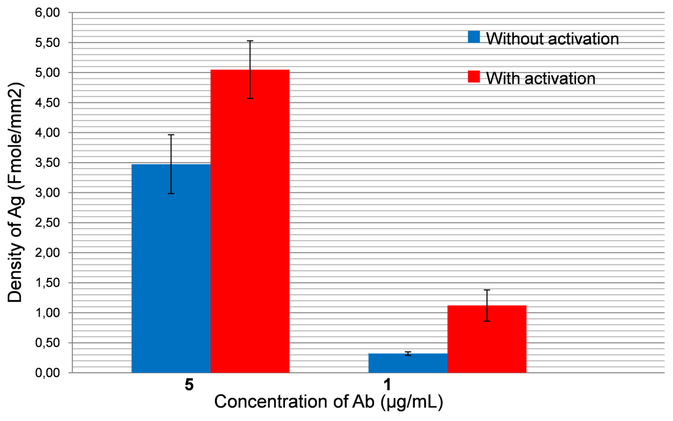
(1) The cell before fluid injection (volume ≈ 25 µl), (2) Arrival of the fluid at a rate flow of 3 ml/min to avoid air bubbles, (3) Fluidic cell partially filled (red arrows indicate the fluid front), (4) Cell completely filled, (5) Injection of diluted red blood cell solution (dilution 200x) at 50 µl/min, (6) Fluid under acoustic excitation (yellow rings show agglomerations of blood cells due to acoustic actuation).

4. Biological experiment

For this experiments, we grafted the monoclonal antibody A9H12 directed against lymphocyte-activation protein 3 (α-LAG3), a candidate-marker of breast cancer, at low concentrations (5 and 1 µg/ml) with and without acoustic activation.

The efficiency of the Abs grafting has been indirectly measured by SPRi (SpriPlex II apparatus from Horiba) by injecting its protein target at a concentration of 27.5 nM.

Results show an amplification of the biorecognition rate by a mean ratio of 1.5 to 3. This experiment highlights the effect of the active mode onto the biochemical reaction between the Abs and the chemically functionalized chips.



Average density of captured LAG3.

Conclusion

We reported the design, the fabrication and the functioning of an acoustic micromixer. The realized device is mainly composed of vibrating element (PZT) and a silicon membrane stacked together in order to create acoustic radiation in integrated fluidic cell which contain the biological media. The efficiency of fluid mixing is demonstrated by mixing small fluid volumes at different vibrations modes of the membrane in the range of low frequency. Then, the activation performance is studied through identified biological model, the analysis of results shows that in the active mode the rate of biorecognition is increased by a mean ratio of 1.5 to 3 depending on the initial concentration of Ab. The work is in progress for integrating a simultaneous label free detection technique based on SPRi & SERS.

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