Orthogonal Surface Acoustic Wave (SAW) Sensor for Cancer Biomarker Detection with Accelerated Binding Kinetics

Yuqi Huang[1], Maëlys Boucher[2], Theresa Evans-Nguyen[3], and Venkat R. Bhethanabotla[1]

[1]Department of Chemical & Biomedical Engineering, University of South Florida, Tampa, FL 33620 USA
[2]Department of Material Sciences, Engineering School of Nantes University, Polytech Nantes, Nantes, France
[3]Department of Chemistry, University of South Florida, Tampa, FL 33620 USA
evansnguyen@usf.edu
bhethana@usf.edu

***Abstract*—A device incorporating both Rayleigh wave and shear horizontal surface acoustic waves is made on a ST-Quartz substrate. The Rayleigh wave induced microfluidic mixing shows effects on accelerating the binding kinetics of real-time sensing between antibody and antigen, which is measured by phase change from the shear horizontal surface acoustic wave direction on the ST-Quartz. Preliminary results on this device show shortened response time and enhanced phase signal when the binding is accelerated by microfluidic streaming from the Rayleigh wave. The device can be fabricated using a low cost, single step photolithography method and can be combined with a small electronic sensor for data readout, which allows for a variety of surface-based biomarker detections on a portable platform. In this work, detection of Carcinoembryonic antigen (CEA) binding with functionalized capture antibody is studied to show the effects of mass loading amplification due to Rayleigh wave microfluidic streaming.**

Keywords—Immunoassay; biosensing; Rayleigh surface acoustic wave; ST-Quartz; Binding Kinetics

I. Introduction

Rapid screening of clinical cancer biomarkers with point-of-care methods and devices are significant for preliminary diagnosis and treatment tracking[1]. Traditional immunoassay such as western blotting, enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assay have drawbacks such as long sample preparation time, long incubation time, requiring trained personnel for laborious processes and need for costly instruments[2]. Surface acoustic wave (SAW) biosensors has been extensively studied for liquid-based biosensing applications and can track the binding between a ligand and a receptor[3]. The shear-horizontal SAW provides a surface-based solution for low-cost, efficient, and label-free biomarker analysis. The binding-induced mass loading from antigen-antibody pairs that are immobilized on sensor surface results in phase shift of SAW in the shear-horizontal direction[3, 4].

It is known that ST-Quartz can simultaneously propagate Rayleigh and surface skimming bulk waves (SSBW) in directions that are perpendicular to each other[5]. SSBW has the disadvantage of energy loss by propagating into the bulk substrate[6]. With selected waveguide layer deposited of the wave path, acoustic energy is confined into the waveguide layer and brought to the surface, and the energy loss is therefore minimized[7]. This orthogonal SAW device can provide microfluidic mixing from the Rayleigh wave to significantly reduce incubation time and required sample quantities, and the wave-guided SSBW can achieve biomarker quantifications via phase signal measurement[8].

In this work, we present a SAW device configuration by integrating Rayleigh wave microfluidic stream and phase signal measurement on the same substrate. Rayleigh wave streaming has shown to increase the phase signal, reduce the signal response time, and improve signal-to-noise ratio on the binding event between a surface-immobilized carcinoembryonic antigen (CEA) capture antibody and CEA antigen.

II. Sensor Configuration and System Design

The orthogonal SAW sensor consists of two pairs of symmetrical interdigital transducers (IDTs) that are parallel to the ST-Quartz wafer flat for SSBW, with one used for sensing and the other for noise-cancelling, and another pair of IDTs in orthogonal direction to the wafer flat for Rayleigh wave generation, as shown in Fig. 1(a). The IDTs were made on a single side polished 4-inch wafer. Each IDT contains 60 pairs of electrodes with a periodicity of 40 μm. The delay region between the SSBW IDTs is of 8000 μm long and 4000 μm wide. In between Rayleigh wave IDTs, 120 pairs of reflection electrodes are designed to have width of 10 μm. The orthogonal SAW devices were fabricated using photolithography with following steps: First, negative photoresist (NR0-1500PY, Futerrex) was spin coated on the wafer to achieve a uniform thickness of 2 μm. After prebake, the pattern was formed using a Karl-Suss mask aligner then followed by hard bake and soaked in developer. 80 nm of gold layer was deposited on top of the surface using E-beam evaporation (AJA) with a deposition rate of 0.05 nm/s. Photoresist lift-off was achieve by using acetone bath. 10nm gold layers were deposited in between SSBW IDTs as sensitive layers for antibody immobilization. The resonance frequency of SSBW is measured to be 121.3MHz from S_{21} parameter

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using a S5045 Copper Mountain Vector network analyzer (VNA), and 78.2 MHz for Rayleigh wave.

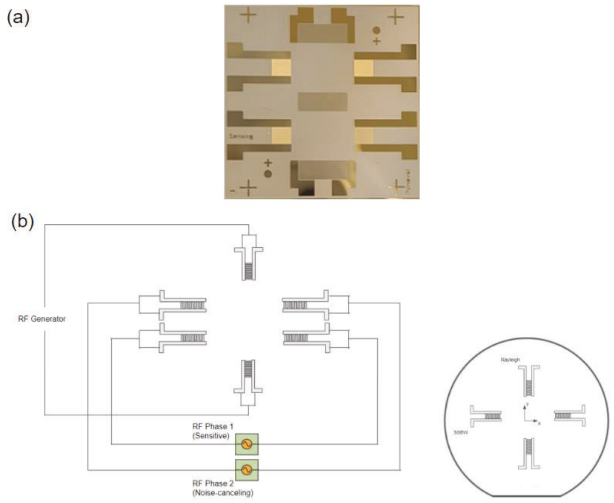

Fig. 1. (a) Photo of an orthogonal SAW chip; (b) Schematic of phase detection and SAW streaming setup with different SAW modes on a ST-Quartz wafer.

The signal measurement of phase shift was obtained with the copper mountain VNA model S5045, where the phase change and insertion loss were monitored and recorded in real-time during the conjugation between antibody and antigen. The recorded data were then subtracted by controlled baseline that contains no antigen in the solution, which minimizes phase drift caused by temperature variations from environmental effects and Rayleigh wave streaming. To import RF signal to the Rayleigh wave IDTs, a signal generator (Rohde & Schwarz SMA100A) was connected to a high-power RF amplifier (LZY-22+, Mini-circuits) for Rayleigh wave streaming of the sample droplet. A schematic of system design is shown in Fig. 1 (b).

III. Experimental

A. Materials

All materials and reagent utilized in this experiment are of analytical grade: CEA antigen (Abcam, ab742), CEA capture antibody (Fitzgerald, 10-C10D), PBS (Life Technologies, pH 7.4), Recombinant Protein A (abcam).

B. Sensor Preparation and CEA Sensing

After coating the 10 nm gold sensitive layer, 15 µL of Protein A at a concentration of 100 µg/mL was added to the gold film and incubated for 1 hour. After that, PBS solution was used to wash away unbound Protein A. Then 15 µL of CEA capture antibody at a concentration of 200 µg/mL was added to the sensitive layer to conjugate with Protein A for 1 hour, followed by PBS wash. From there, two methods were used for CEA antigen sensing to study the mixing effect from Rayleigh wave. A range of CEA concentrations from 1-100 µg/mL were sequentially added to the sensor surface, with a droplet volume of 15 µL. The schematic of immunoassay sensing is illustrated in Fig. 2. It is noted that prompt sample exchange was maintained every time when a sample is removed and new sample is added to the surface, in order to keep the sensor surface wet. The other group was conducted with same experimental procedures, with the addition of Rayleigh wave excitation from the RF signal generator and amplifier set to 25dBm.

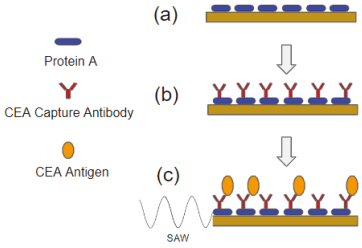

Fig. 2. Schematic of CEA antigen detection: (a) Protein A is functionalized on gold surface; (b) CEA capture antibodies is attached to Protein A; (c) CEA Antigen is bond to antibody with and without Rayleigh wave mixing.

IV. Results and Discussion

The real-time sensor response of CEA antigen binding to the capture antibody without Rayleigh wave streaming is shown in Fig 3 (a). The phase shift from 1 µg/mL CEA antigen sample is within the noise signal, and therefore is not obvious. Slight phase shift starts to appear after adding 10 µg/mL sample and the 1 degree change at 100 µg/mL. In comparison, Fig 3 (b) shows the phase change from CEA antigen binding under SAW streaming. It is worth noting that compared to dry surface, the temperature increase from Rayleigh wave could be higher when liquid is presented in the path of wave propagation[9, 10], due to the kinetic and potential energy from acoustic energy radiated into the sample droplet. The temperature rises results in an increase in phase drift that magnifies with higher RF power, and is cancelled by subtracting the phase change recorded from the noise-canceling unit measuring change from 15 µL PBS buffer solution.

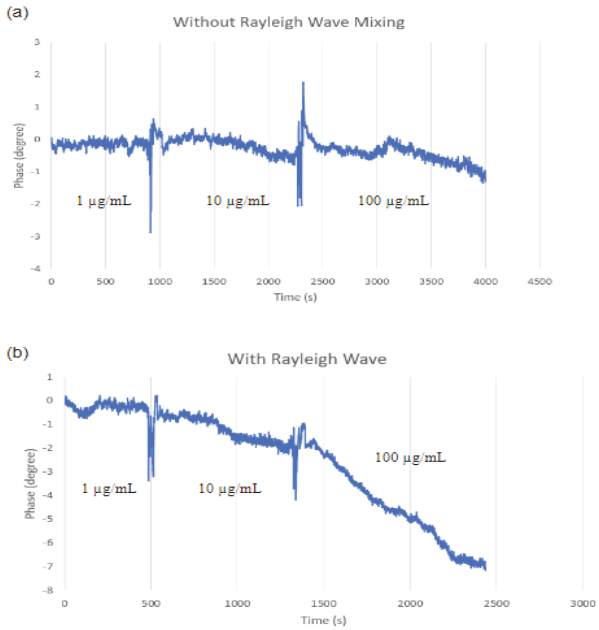

Fig. 3. (a) Real-time phase change from antigen-antibody binding; (b) Real-time data from antigen-antibody binding under Rayleigh SAW mixing

It is seen that all CEA concentration groups have higher sensitivity under the Rayleigh wave mixing. Comparing the two trends in Fig. 3, there is slight phase change starting at 1 µg/mL, and faster phase response for higher antigen concentrations. It is noted that the phase disturbances at sample injection for all concentrations are artifacts that are inevitable. The slopes of phase change at 100 µg/mL indicates higher binding efficiency with mixing presents (Fig. 4), and results in 5 times higher sensor response with shorter amounts of incubation time needed (Fig. 5).

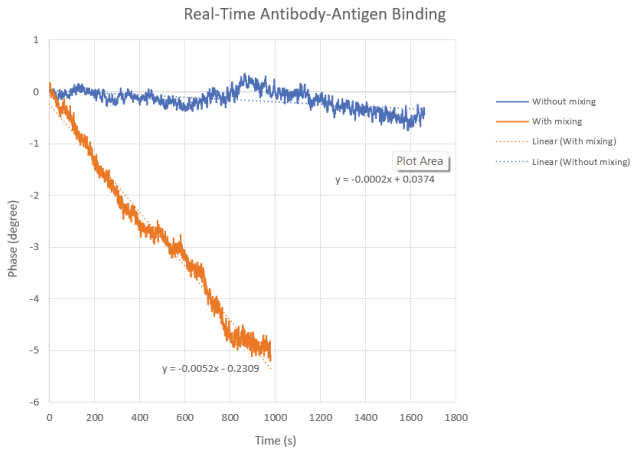

Fig. 4. Real-time phase change from 100 µg/mL antigen sample binding, blue curve represents no mixing involved and orange curve represents mixing.

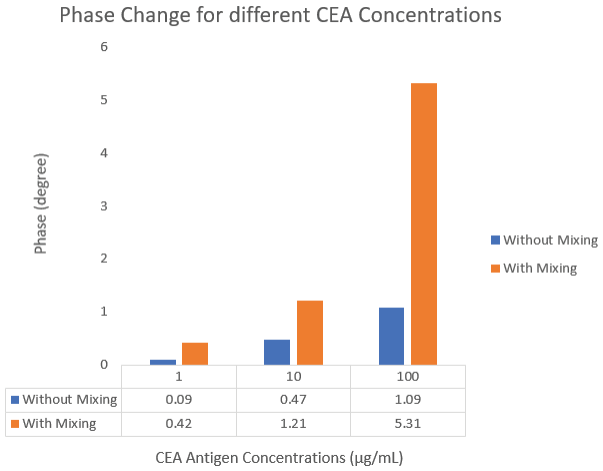

Fig. 5. Phase shifts of different CEA antigen concentrations.

V. Conclusion

In this work, we present a low-cost orthogonal SAW device that can simultaneously achieve Rayleigh wave induced microfluidic streaming and immunosensing using SSBW delay region. By SAW mixing on the analyte sample, faster incubation time is achieved with enhanced signal response. More importantly, only very small amount of sample volume is needed since SAW mixing facilitates the ligand-receptor binding event. This sensor can be easily integrated to a portable system when combined with handheld vector network analyzer and RF signal generator, which can be meaningful for researching novel analytical methods on immunosorbent assays and clinical tests of cancer biomarkers.

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