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Development of an Orthogonal Dual Delay Line Biosensor with Acoustic Mixing for Enhanced Sensitivity on a ST-Quartz Substrate

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Summary:
This study discussed the development of an orthogonal dual-delay line biosensor fabricated on an ST-Quartz substrate, which combines of Rayleigh wave SAW propagation in orthogonal to the SH-SAWs was used to enhance biosensing sensitivity and binding kinetics through Rayleigh wave streaming. The SH-SAW waveguides were optimized and demonstrated reduced insertion loss and maximized acoustic energy confinement to improve sensor's performance. The implementation of a dual-line configuration also provides a reference sensing line that minimized thermal variation and environmental noises presented in sensing data. Binding experiments between Protein G and rabbit anti-mouse IgG showed that SAW mixing can lead to a stronger binding affinity compared to the non-mixing case. These results highlight the efficacy of Rayleigh wave mixing in reducing assay time, improving binding efficiency, and enhancing sensor sensitivity, and demonstrate a low-cost sensor with the potential to facilitate experiments where multiple affinity bindings need to be performed at low concentrations.
Keywords: surface acoustic wave (SAW), binding affinity, Rayleigh wave, biosensors, acoustic streaming

Background, Motivation an Objective

The Surface acoustic waves (SAWs) have been widely applied in RF filters, sensors, nebulizers, and actuators, leveraging the piezoelectric effect to convert energy between electrical signals and mechanical waves [1]. Among SAW modes, shear horizontal (SH) SAWs are particularly suited for label-free biosensing, as their sensitivity to surface perturbations allows detection of biological conjugation [2]. However, challenges regarding of biosensors include non-specific adsorption leading to noise, and the need for enhanced sensitivity and faster binding kinetics to reduce assay time. Acoustic streaming, driven by Rayleigh waves, can improve mass transport and accelerate reaction times in small-scale bioreactors but may introduce heating that could degrade biological samples.

This study introduces a dual-mode SAW biosensor combining Rayleigh waves and SH-SAWs on an ST-Quartz substrate. The Rayleigh wave interdigital transducers (IDTs) facilitate mixing, while orthogonal SH-SAWs with optimized waveguide thickness enhance sensitivity. Thermal effects were analyzed to select optimal power for mixing experiments, and dual SH-SAW delay lines minimized thermal variations. Binding kinetics were assessed through equilibrium binding constants, revealing that Rayleigh wave mixing significantly enhances binding affinity. This platform demonstrates potential for improving sensitivity, reducing assay time, and advancing label-free biosensing applications.

Description of the method and materials

The orthogonal dual delay line SAW device operates with Rayleigh wave and SH-SAW center frequencies of 78 MHz and 123 MHz, respectively. It features symmetrical SH-SAW IDTs for sensing and noise cancellation, oriented orthogonally to Rayleigh wave IDTs. Fabrication involved spin-coating photoresist, UV exposure, metal deposition, and lift-off. Achieving waveguide thicknesses between 1.1 μm and 3.5 μm were used for sensor optimization. A 10 nm gold biorecognition layer was coated, followed by oxygen plasma etching for antibody immobilization – Fig.1.

A PDMS chamber was designed to stabilize liquid samples. Fabricated using SU-8 molds, the chamber was integrated with the SAW sensor through covalent bonding, enhanced by

a silica layer and plasma treatment. Device performance was characterized using a VNA, thermal camera, and syringe pump-controlled microfluidic system.

For antibody-Protein G binding studies, Protein G was immobilized on gold-coated regions and incubated overnight. Antibody solutions (0.67 to 67 nM) were introduced sequentially at 5 µL/min, with PBS buffer cleaning between concentrations. The mass-loading effect was monitored via phase changes in SAW signals, comparing sensing and reference lines. Minimal antibody desorption was observed, confirming mostly irreversible binding. This device integrates acoustic streaming for enhanced binding kinetics, offering a stable platform for biosensing applications. Details of binding kinetics are presented in the results section.

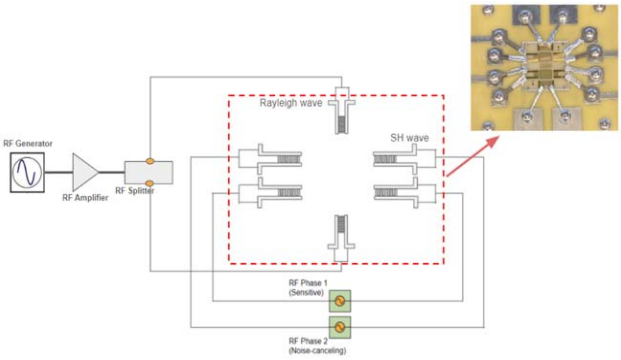

Fig. 1. The scheme of the experimental set-up

Results

The real-time phase change data from the noise-canceled signal for Protein G binding to rabbit anti-mouse IgG are presented in Fig.2. The control group without Rayleigh wave mixing is in Fig.2(a). For both with and without SAW group (Fig.2(b)), the phase change corresponding to the binding event stabilizes once the IgG concentration is sufficiently high, indicating that the reaction has reached equilibrium between the association and dissociation of Protein G and IgG. The buffer wash performed between each concentration group did not reduce the cumulative phase change, suggesting that the binding event is irreversible within the observed time frame.

By extracting the cumulative phase change for each concentration, a comparison between IgG concentration and cumulative phase responses is plotted in Fig.3. The sensor response under Rayleigh wave streaming demonstrates a steeper curve and amplified signal at each concentration group. The sensitivity calculated from the calibration curve shows about threefold enhancement. These indicate higher sensitivity achieved through the mixing effect, which enhances the binding efficiency between Protein G and IgG. As a result, greater mass loading is observed at equivalent concentration levels.

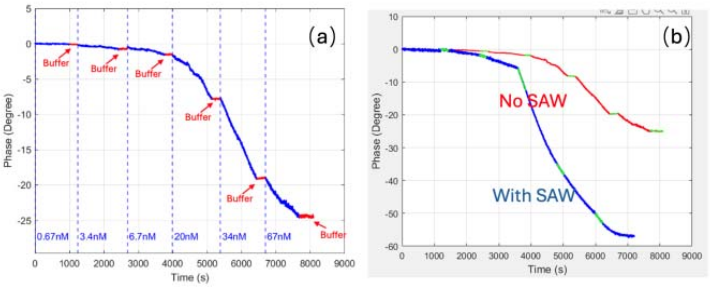

Fig. 2. (a) Real-time data of IgG to protein G binding without Rayleigh wave mixing, rabbit anti-mouse IgG at concentrations from 0.67 nM to 67 nM were bond to the conjugated protein G at a concentration of 50 µg/mL; (b) Comparison of the real-time binding curves with and without SAW mixing

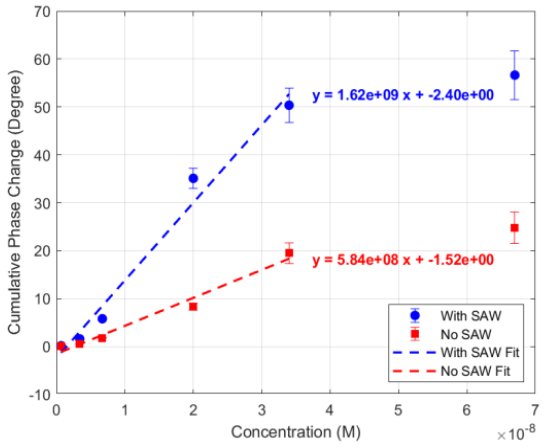

Fig. 3. Improved phase response and sensitivity with Rayleigh wave streaming

Conclusions

This study developed an orthogonal dual-delay line SAW biosensor combining Rayleigh wave mixing and SH-SAW sensing, enhancing binding kinetics and sensitivity. The device demonstrated faster and stronger Protein G-antibody interactions with minimized thermal noise, showcasing its potential for sensitive, low-cost biosensing applications and advancing biomolecular diagnostics at low concentrations.

References

[1] Y. Huang, P. K. Das, and V. R. Bhethanabotla, "Surface acoustic waves in biosensing applications," *Sensors and Actuators Reports,* vol. 3, pp. 100041, 2021/11/01/, 2021.

[2] S. Li, Y. Wan, Y. Su *et al.*, "Gold nanoparticle-based low limit of detection Love wave biosensor for carcinoembryonic antigens," *Biosensors and Bioelectronics,* vol. 95, pp. 48-54, 2017.