

Novel Love mode surface acoustic wave based immunosensors[☆]

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Abstract

Love mode surface acoustic wave (SAW) immunosensors were developed. The Love mode guiding layers in these sensors are ZnO and SiO₂ thin films. It is shown that mass sensitivity of the devices with ZnO layer are larger than that of with SiO₂ guiding layers. A system comprising dual delay line, based on ZnO/90° rotated ST-cut quartz crystal devices, was set up for conducting the immunosensing experiments. In these experiments adsorption of rat immunoglobulin G onto the active area of the sensor was monitored. Upon exposure to solutions containing IgG, the operational frequency of the system changed due to the interaction of IgG and a gold surface on the active area of the sensor. Mass sensitivities were measured and frequency responses to various IgG concentrations are presented.

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1. Introduction

Crystals that allow the propagation of leaky-SAW or surface skimming bulk wave (SSBW) can be used for the fabrication of Love wave liquid phase sensors [1–3]. By depositing waveguiding layers onto these substrates, the propagating acoustic waves become more confined into the layers. Such a phenomenon generally increases the electromechanical coupling coefficient and the particle displacement on the active area of the device. As a result, a larger mass sensitivity can be obtained from the layered device structures compared to their blank SAW counterparts. Depositing optimal thicknesses of piezoelectric and/or non-piezoelectric materials can increase the sensitivity to its maximum value. This optimum value is 0.15 of the operational wavelength for a single layer device [4]. For layered SAW devices, the sensitivity depends on the physical properties of the films and the substrates.

Layered SAW devices were fabricated. These SAW devices are based on SiO₂/90° rotated ST-cut quartz and ZnO/90° rotated ST-cut quartz crystal structures. A system was set up for immunosensing applications. This system comprises dual delay line transducers, with the first trans-

ducer operating as the sensor and the other as a reference. The system has high performance liquid chromatography (HPLC) pump that directs buffer solutions of different IgG concentrations into the liquid cell. The selective layer is a gold film.

In this paper, a brief theoretical discussion regarding layered SAW devices will be presented. The system will be introduced, results of mass sensitivity measurements will be shown, then the frequency measurement results will be presented.

2. Theory and mass sensitivity measurements

Mass sensitivity (S_m) for acoustic wave sensors is defined as the incremental frequency change occurring in response to an incremental change in mass per unit area on the surface of the device [5]:

$$S_m = \lim_{\Delta m \rightarrow 0} \frac{\Delta f/f_0}{\Delta m}, \quad (1)$$

where Δm is the uniformly distributed mass per unit area added to the surface of the device, f_0 is the unperturbed frequency of the device and Δf is the change in the operational frequency that occurs due to mass loading. If the thickness of the film is less than one-tenth of the acoustic wavelength, then first-order perturbation theory can be used

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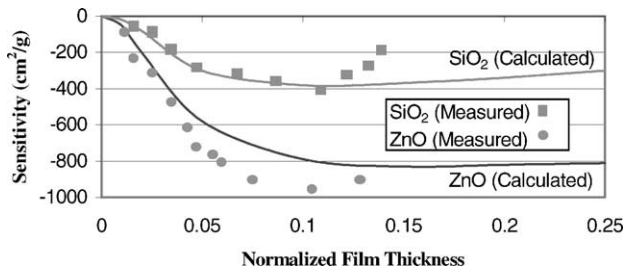


Fig. 1. Mass sensitivity versus normalized layer thickness: comparison of theoretical calculations and measurements.

to calculate the change of the operational frequency. Thus the change in frequency is given by [4]

$$\Delta f = -\frac{V}{4} f_0 \rho d \left[1 - \left(\frac{V}{V_{S2}} \right)^2 \right] |\hat{v}|_{z=0}^2. \quad (2)$$

From Eq. (2), the frequency shift depends on the acoustic phase velocity in the media (V), the operational frequency f_0 , ρd which represent the mass loading per unit area, the term $1 - (V/V_{S2})^2$, which represents a reduction in mass sensitivity with the increase of the thickness of the layer and on $|\hat{v}|_{z=0}^2$ the particle velocity at the surface. The frequency change approaches a peak at a thickness of approximately 0.15 of the magnitude of the wavelength. After the peak, due to the effect of the term $1 - (V/V_{S2})^2$, the frequency shift becomes smaller as the layer thickness increases.

Fig. 1 shows both theoretical values and measurements of the mass sensitivities of devices with different thicknesses of ZnO and SiO₂ layers as a function of normalized layer thickness for a device with the periodicity of 50 μm . Substrates are 90° rotated ST-cut quartz crystals. For SiO₂ layers, experimental data agrees with theoretical predictions for thicknesses up to 0.1 of the wavelength.

Love mode devices with ZnO layers should show mass sensitivities two times higher than that of SiO₂. However, measurements of Love mode devices with ZnO layers show mass sensitivities 20% higher than the theoretical values. In the opinion of the authors, this difference may be due to the porosity of the ZnO surface. Love mode devices with SiO₂ layers show a maximum sensitivity value of 400 cm²/g and devices with ZnO layers show a significantly higher sensitivity of 950 cm²/g. Both devices have their peak values at a layer thickness of 0.12 wavelength. After the peak values, the mass sensitivities decrease faster than Eq. (2) predicts. This is not surprising, as the perturbation theory was applied for the calculation of mass sensitivities, which is accurate only for small layer thicknesses.

The difference between the theoretically calculated and measured mass sensitivities is even larger when the piezoelectricity of the media increases and substrates allow leaky-SAW propagation rather than SSBW. Using numerical methods, such as finite element, more reliable results can be obtained. The authors have applied ANSYS software package in 3D environment to simulate operation of layered

SAW devices and calculate the mass sensitivities. The outcome of this investigation will be published soon.

3. Sensor fabrication

Love wave devices were fabricated on single side polished, 0.5 mm thick ST-cut quartz substrates, with propagation direction normal to the crystallographic x -axis. Interdigital transducers (IDTs) consist of 64 finger pairs at the input port and 36 finger pairs at the output port, with a periodicity of 50 μm . IDTs were patterned using conventional photolithographic techniques. Metal layers were deposited by the electron beam evaporation technique. They comprised of a 30 nm chromium layer followed by a 30 nm Gold layer. The center-to-center separation of the IDTs was 80 wavelengths with an aperture of 2.5 mm. A 4 μm ZnO layer was deposited using an r.f. magnetron sputterer. An additional 20 nm gold/10 nm chromium film was deposited on the active area of the sensor as the selective layer.

4. System set-up

A system was set up for conducting immunosensing experiments in order to compare the sensitivity of different guiding films (Fig. 2).

This system consists of a dual delay line transducer in a feedback loop along with broadband amplifiers. The first transducer operates as the sensor and the other as a reference. The combinations of the amplifiers and the delay lines oscillate at frequencies which are functions of the acoustic wave velocity within the delay lines. The operating frequencies of the oscillator circuits are approximately 89 MHz. The system uses an HPLC pump which provides liquid flow of different rates.

5. Measurements

Experiments were conducted in order to analyze the direct adsorption of rat IgG onto the gold surface, and hence measure the mass sensitivity of the sensor in liquid. IgG itself has a high affinity with gold and can be adsorbed directly onto the gold surface [6]. This capability was used to measure the frequency response of the system to different concentrations of IgG in phosphate buffered saline (PBS) dissolved in DI-water.

The first experiment was conducted by exposing the Love mode sensor to different concentrations of IgG (Fig. 3). IgG in PBS with the concentration of 400 ng/ml was pumped into the liquid cell with a flow rate of 25 $\mu\text{l}/\text{min}$. The device showed -7 kHz frequency shift after 400 s. A higher concentration of IgG in PBS (1 $\mu\text{g}/\text{ml}$) was then pumped into the cell, to which the sensor showed a frequency shift equal to

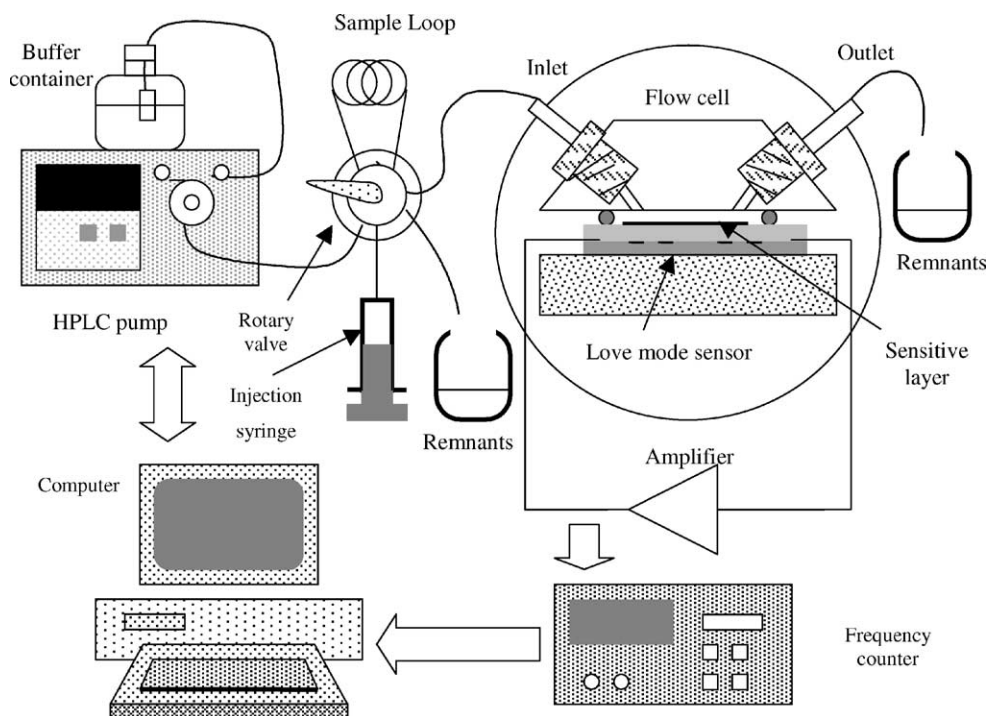


Fig. 2. System set-up.

–19 kHz after 400 s. This shows that the concentration of 400 ng/ml did not saturate the sensing system. Consequently, when the device was exposed to higher concentrations, IgG covered up the gaps between the bounding sites resulting in a larger frequency shift.

The system response to different IgG concentrations in PBS was repeatable. A higher flow rate resulted in a slower response due to molecules having insufficient time to bind onto the gold surface. The frequency changes of the Love mode sensor for various IgG concentrations in PBS are

shown in Fig. 4. The frequency shift was measured after 500 s with a flow rate of 20 $\mu\text{l}/\text{min}$ of IgG in PBS into the liquid cell.

The primary advantage of the Love mode devices with ZnO layer is the low detection limit which is achievable with these sensors. They exhibited a frequency shift of –250 Hz for 10 ng of IgG in 1 ml PBS. This means that with a frequency stability better than 12.5 Hz a mass detection limit of 500 pg/ml IgG is achievable. This agrees with the mass sensitivities calculated from Eq. (2).

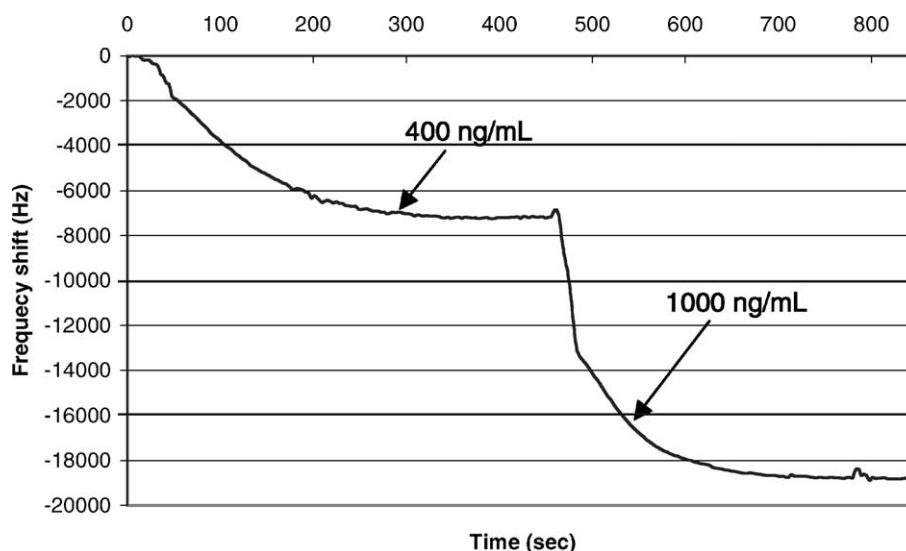


Fig. 3. Response of the bare gold Love mode SAW device to different IgG concentrations.

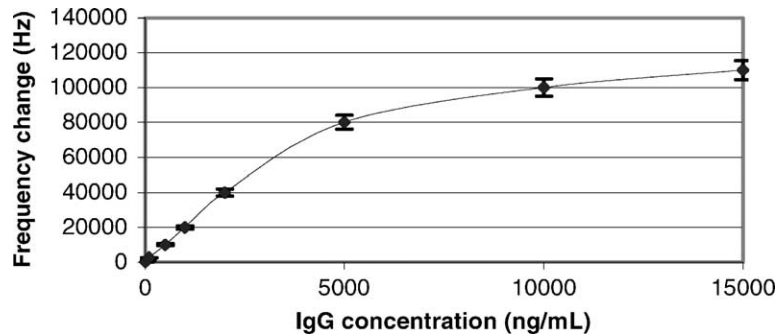


Fig. 4. IgG concentration versus frequency change of the Love mode sensor with ZnO layer (gold as the selective layer). Bars show the error (less than 10%) deviations.

It is of interest to note that for an affinity sensor operating in liquid, the mass adsorption due to the molecular binding is always accompanied by significant interfacial viscosity interference [7,8]. In addition, the effective mass of the bounding layer will usually be increased by water entrapment [8,9]. However, for the low IgG concentrations used in these experiments, such an increase in the response was not observed.

6. Conclusions

In this study, novel Love mode immunosensors based on ZnO/90° rotated ST-cut quartz crystal structures, were developed. With these immunosensors, it was possible to detect IgG concentrations as low as 500 pg/ml. For a ZnO guiding layers with the thickness equal to 0.12 of the wavelength the mass sensitivity was as high as $0.95 \text{ (cm}^2/\text{ng)} \times \text{(Hz/MHz)}$. The authors believe that the mass sensitivity of such a device is higher than any previously reported acoustic wave device, operating at the same frequency, in the literature. The sensor is a promising candidate for immunosensing applications.

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Biographies

Kourosh Kalantar-Zadeh received his Bachelor of Science and Master of Science from Tehran University, Tehran, Iran and Sharif University of Technology, Tehran, Iran, respectively. He majored in Telecommunications. He received his PhD from RMIT University, Melbourne, Australia in 2002. Kourosh is a lecturer at RMIT University, Melbourne, Australia. He is currently undertaking research on Surface Acoustic Wave (SAW) Biosensors as part of a project with the CRC for Micro-technology, Australia. His previous research and development involvements include: SONAR systems, MRI signal processing and Ionospheric Surveillance systems. He has published more than 30 scientific papers in refereed journals and in the proceedings of international conferences and holds 2 patents.

Wojtek Wlodarski has worked in the areas of sensor technology and instrumentation for over 30 years. He has published four books and monographs, over 300 papers and holds 28 patents. He is a professor at RMIT University, Melbourne, Australia, and heads the Sensor Technology Laboratory located within the School of Electrical & Computer Engineering.

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