Identifying the Removal Mechanism of Fouling Proteins In a Surface Acoustic Wave Biosensor: A Numerical Study and Comparison to Experiments

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Abstract — Biosensors typically operate in liquid media for measurement of biomarkers and suffer from fouling mechanisms such as nonspecific binding of protein molecules to the device surface. Using a novel numerical technique as well as experiments, we have identified that fluid motion induced by high intensity sound waves, such as those propagating in these sensors, can lead to the removal of the nonspecifically bound proteins, thereby eliminating sensor fouling. We present a computational and experimental study of the acoustic-streaming phenomenon induced biofouling elimination by surface acoustic waves (SAWs). The transient solutions generated from the developed coupled field fluid solid interaction (FSI) model were utilized to predict trends in acoustic-streaming velocity for various design parameters such as voltage intensity, device frequency, fluid viscosity and density. The model predictions were utilized to compute the various interaction forces involved and thereby identify the possible mechanisms for removal of nonspecifically-bound proteins. Our study indicates that the SAW body force overcomes the adhesive forces of the fouling proteins to the device surface and the fluid-induced drag and lift forces prevent its re-attachment. The streaming velocity fields computed using the finite-element models in conjunction with the proposed mechanism were used to identify the conditions leading to improved removal efficiency. Our research findings have significant implications in designing reusable and highly sensitive biosensors**.**

Keywords – Acoustic streaming; SAW; Biofouling; Finite element method

I. INTRODUCTION

SAW and other transducer devices used in biological-species detection suffer from fouling that result from binding of nonspecific protein molecules to the device surface. Nonspecific binding dramatically reduces the sensitivity and selectivity of biosensors [1]. Our experimental and numerical study indicates that the acoustic streaming phenomenon, *i.e.*, fluid motion induced from high intensity sound waves, can be used effectively to remove nonspecifically bound proteins to allow reuse of SAW biosensors (Fig. 1). The acoustic-streaming phenomenon can be used to remove these nonspecifically-bound proteins to allow more accurate determinations and reuse of these devices [2]. The generated sound fields cause tangential fluid motion along the inter-phase boundaries. These motions exert steady viscous stress on the boundary layer leading to liquid circulation near the boundaries. Although these stresses are not large, they are still significant enough to remove loosely-bound material on the surface of the device. Understanding the fluid dynamics in such a system is useful for efficient removal of nonspecifically-bound proteins. The application of this work broadly applies to all transducers used for biological species sensing that suffer from fouling and nonspecific binding of protein molecules to the device surface.
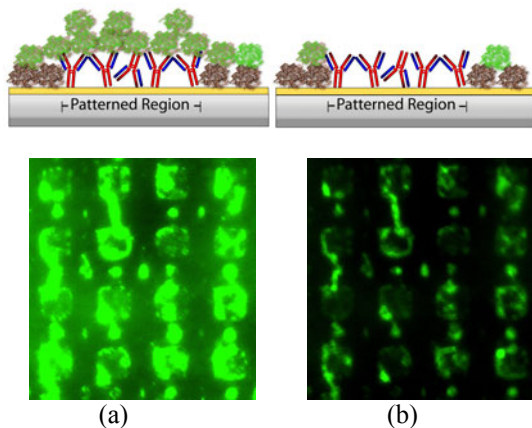

(a)   (b)

Figure 1. Nonspecifically-bound bovine serum albumin (BSA) fluorescent image (pseudo-colored) with illustration above of SAW device surface at end of experiment (RF power dose = 12,500 mW sec). The illustrations in (a) and (b) depict the removal of nonspecifically-bound proteins accomplished by acoustic-streaming phenomenon induced by the Rayleigh wave interaction with the fluid medium.

In this work, a finite element fluid solid interaction (FE-FSI) model of acoustic streaming phenomenon resulting from the interaction of SAWs (Rayleigh mode) with liquid loading is developed (Fig. 2). Parameters studied using the FE-FSI model include voltage intensity, device frequency, fluid viscosity and density. The transient solutions generated from the model were used to predict trends in acoustic streaming velocity. The generated streaming velocity fields are then utilized to predict the various adhesive and removal forces experienced by the specific and nonspecifically bound protein molecules located at various regions along the SAW biosensor.

The predicted mechanism of removal of nonspecifically bound proteins is based on an order of magnitude comparison of the various forces involved. Typically, there are mainly three different mechanisms for particle removal, namely, sliding, rolling and lift-off. For the sake of efficient removal across the entire SAW delay path, it is necessary to identify the dominant mechanism involved. This can be established by comparing the relative magnitudes of the various removal forces. The forces responsible for removal of particles are mainly characterized as the direct SAW forces, the lift and the drag forces that result from the mean velocity field in the fluid, whereas, the dominant adhesive forces for particles immersed in a liquid are the van der Waals and double layer forces. Based on the calculated streaming velocity fields, the interaction forces are analyzed for varying input parameters to identify conditions that lead to improved removal efficiency.

II. THEORY

A coupled-field FSI model of a SAW device based on a micron-sized piezoelectric substrate (YZ-LiNbO$_3$) in contact with a liquid loading was developed to study surface-acoustic-wave interaction with fluid loading [3]. A system of four coupled wave equations for the electric potential and the three component of displacement in piezoelectric materials are solved for the piezoelectric substrate or the solid domain [4]:

$$-\rho\frac{\partial^2 u_i}{\partial t^2} + c_{ijkl}^{E}\frac{\partial^2 u_k}{\partial x_j \partial x_l} + e_{kij}\frac{\partial^2 \phi}{\partial x_k \partial x_j} = 0 \tag{3.1}$$

$$e_{ikl}\frac{\partial^2 u_k}{\partial x_i \partial x_l} - \varepsilon_{ik}^{s}\frac{\partial^2 \phi}{\partial x_i \partial x_k} = 0 \tag{3.2}$$

These coupled wave equations can be discretized and solved for generating displacement profiles and voltages at each element/node. The piezoelectric material displacements obtained from the above equations are applied to the fluid domain at each time step.

Fluid domain was modeled using the Navier-Stokes equation; the arbitrary-Lagrangian-Eulerian approach was employed to handle the mesh distortions arising from the motion of the solid substrate.

$$\rho\left(\frac{\partial v_f}{\partial t}\right) + v_f \cdot \nabla v_f + \nabla P - 2\eta\nabla \cdot D = 0 \tag{3.3}$$

$$\nabla \cdot v_f = 0 \tag{3.4}$$

Here, v_f, P, ρ and η denote the fluid velocity, pressure, density, and viscosity, respectively. D is the rate of deformation tensor given by

$$D = \frac{1}{2}\left(\nabla v_f + (\nabla v_f)^t\right)$$

The fluid-solid coupling was established by maintaining stress and displacement continuity at the fluid-structure interface. The velocity v calculated from Eq. (3.3) and (3.4) contains harmonically varying terms and a "dc" term. The latter induces acoustic-streaming. When averaged over a relatively long time, the effect of the harmonically varying terms disappears and only the contributions from the dc part appear in the solution. The acoustic-streaming velocity ($\overline{v}_{a,i}$, i=x, y, and z) is therefore obtained by averaging v over a time period as follows:

$$\overline{v}_{a,i} = \frac{1}{T}\int_0^T v_i dt \text{ , i=}x,\ y \text{ and } z \tag{3.5}$$

where T is the time period of the wave propagation.

III. COMPUTATIONAL DETAILS

Insights into the dominant mechanism responsible for efficient removal of nonspecifically bound proteins are obtained from the estimates of various forces predicted using perturbation methods [5] and trends in the streaming velocity fields. For simplicity, the protein molecules are modeled as spherical particles. The different SAW-induced forces, which include linear forces (added mass (F_{AM}), drag (F_D), lift (F_L) and Basset forces (F_B), and nonlinear ones due to radiation pressure and drag (F_{ST}), are computed. Additionally, the magnitudes of forces are evaluated for various particle sizes. The combined effect of the various interaction (adhesive and removal) forces is utilized to gain insights into the removal mechanisms of nonspecifically bound proteins. The principal adhesion forces (van der Waals (F_{vdW}) and electrical double layer (F_{el}) forces) are used to model the interaction of the specific and nonspecifically bound proteins to the biosensor surface. The forces responsible for removal of particles are mainly characterized as the acoustic radiation forces as well as the lift and drag forces that result from the mean velocity field in the fluid. The streaming velocity field (Fig. 3) computed using the FE-FSI model is utilized to estimate the fluid induced drag and lift forces for a range of operating conditions and fluid properties. Comparison of the various removal forces to the adhesive forces that bind the specific and nonspecific proteins is used to estimate the removal efficiency on the SAW biosensor and to optimize conditions.

IV. RESULTS AND DISCUSSION

A. Acoustic wave interaction with fluid medium

Simulation results predict strong coupling of ultrasonic surface waves on the piezoelectric substrate with the thin liquid layer causing wave mode conversion from Rayleigh to leaky SAW, which leads to acoustic-streaming as shown in Figs. 2 and 3. The leaky SAWs decay exponentially with distance from the source. Streaming velocity fields were studied for a range of input parameters such as input voltage, device frequency as well fluid properties such as density and viscosity. Our simulation results indicate that the induced streaming velocities typically vary from 1 µm/s to 1 cm/s with the exact values dictated by the device operating conditions as well fluid properties.
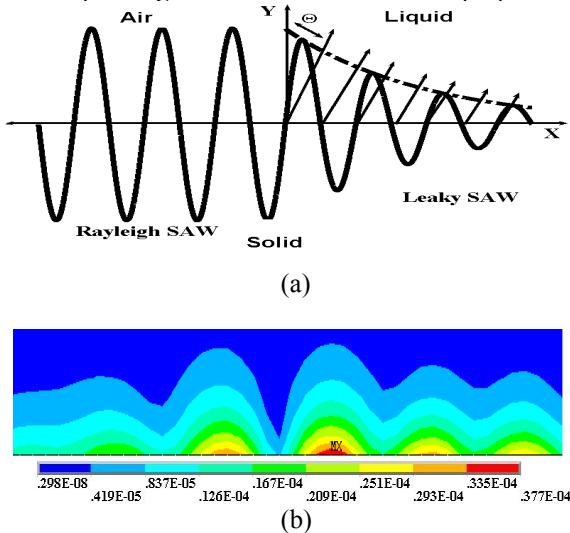

(a)

(b)

Figure 2. (a) Ultrasonic irradiation mechanism into the fluid medium (b) Simulated contours showing particle displacement profile (micrometers) in fluid region for an applied AC peak input voltage of 1 V in a SAW device operating at 100 MHz. The wave decays with distance from the interdigital transducer (IDT) fingers located at the center of the SAW device.

B. Acoustic streaming velocity

The SAW interaction with the fluid creates a net pressure gradient in the direction of sound propagation in the fluid which leads to an internal, acoustically induced streaming phenomenon [6-7]. The recirculation patterns resulting from the SAW motion gives rise to eddy formation as shown in Fig. 3. With increasing time, these eddies rise through the fluid and break into smaller ones, thereby dissipating their energy while new ones are created at the interface. Thus, the generated sound fields cause tangential motion along the inter-phase boundaries. The tangential motion results in

fluid induced shear stresses, which if larger than the adhesive forces, can be used to remove the nonspecifically bound proteins. Apart from this force, the motion of the acoustic waves also induces a removal force on the fouling proteins. This force acts at a Rayleigh angle (θ) as shown in Fig. 2(a).
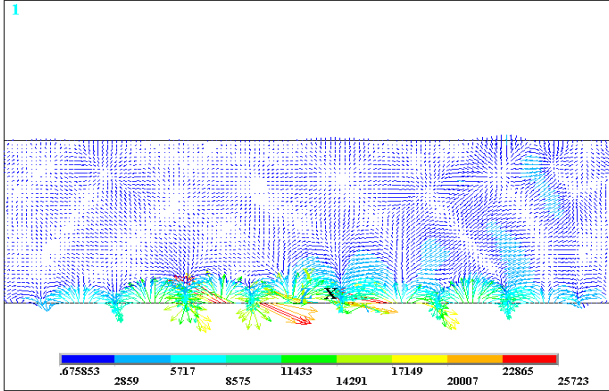

Figure 3. Induced streaming patterns in the fluid domain for a 100 MHz frequency SAW device. The calculated velocities are in μm/s. The displacements in the solid domain have been suppressed for clarity.

C. Mechanism of nonspecifically bound protein removal

An order of magnitude analysis of the various interaction forces was performed and the data for one such input condition are shown in Table I. Based on this analysis an approximate removal mechanism can be proposed. For micrometer- and sub-micrometer-sized protein agglomerates, the results in Table I illustrate that the fluid-induced removal forces are not significant enough to overcome the van der Waals forces. The SAW body force (F_{SAW}) is several orders of magnitude larger than the adhesion forces for the range of particle diameters considered in Table I.

TABLE I. FORCES (N) VERSUS PARTICLE RADIUS R (MICROMETERS) FOR A 100 MHz SAW DEVICE AND AN APPLIED INPUT VOLTAGE OF 1 V. THE FLUID VISCOSITY IS 1 CP. THE CALCULATED FORCES ARE BASED ON THE STREAMING VELOCITY FIELD GENERATED AT THE IDT REGION.

R	0.1	1	10
F_{vdW}	-2×10^{-9}	-2×10^{-8}	-2×10^{-7}
F_{SAW}	4×10^{-6}	4×10^{-4}	4×10^{-2}
F_L	3×10^{-20}	3×10^{-18}	3×10^{-16}
F_{ST}	2×10^{-14}	2×10^{-13}	2×10^{-12}
F_{AM}	1.5×10^{-13}	1.5×10^{-10}	1.5×10^{-7}
F_B	6×10^{-14}	6×10^{-12}	6×10^{-10}

It therefore appears that the SAW direct force causes the removal or detachment of the nonspecific proteins from the SAW surface and moves the particle away from the region of influence of the adhesive forces. The van der Waals adhesion forces decrease rapidly with distance from the SAW surface. However, the SAW direct forces also decay rapidly with distance into the fluid. Hence, a steady force is required to cause the removal of the detached nonspecific proteins. The horizontal streaming-induced F_{ST} helps to push the nonspecific proteins away from the fouled area. The vertical streaming force or F_L helps prevent re-adhesion of the proteins to the surface. Thus, the

fluid-induced drag and lift forces result in a net displacement of the detached proteins. The mechanism of removal is depicted in Fig. 4.
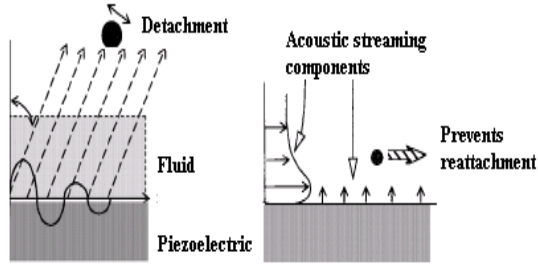

Figure 4. Predicted mechanism based on adhesive and removal forces calculated for known velocity fields. Body force arising from motion of SAW is responsible for detaching nonspecifically bound proteins. Streaming induced drag and lift forces prevent re-attachment of the detached nonspecific proteins to the SAW device surface.

D. *Efficiency of nonspecifically bound protein removal*

The main aim of this study is to achieve complete removal of nonspecifically bound proteins along the entire SAW delay path. Based on the removal mechanism predicted using our simulation model, the dominant force responsible for removal appears to be the SAW body force. The magnitude of this force decays rapidly as we move away from the IDT region, with the extent of decay depending on the applied voltages, device frequency and fluid properties such as density and viscosity. A decay length can be defined which represents the region beyond which removal forces are not strong enough to eliminate biofouling. Based on the forces calculated for various locations along a SAW delay path, it was found that the decay length for sub-micron sized particles is approximately 100 microns for the above device. Similar analysis was carried out for the SAW device operating at higher fluid viscosity of 10 cP. We find that at the MHz device frequencies simulated in the present work, the variation in streaming velocity for a fluid viscosity change from 1 to 10 cP is significant, especially at regions along the delay path located far away from the IDT region. We find that when the fluid viscosity is increased from 1 to 10 cP, the calculated decay length rapidly decreases from 100 microns to 60 microns. Therefore, our study indicates that for increasing fluid viscosity in the simulated range, the removal of nonspecifically-bound proteins that can be efficiently carried out by SAW-induced streaming phenomenon decreases rapidly with increasing distance along the SAW delay path.

V. CONCLUSIONS

Fluid-solid interaction finite element models were used to predict the mechanism of removal of nonspecifically bound proteins in SAW biosensing application. The generated streaming velocities were used to compute the various adhesive and removal forces involved. Our study indicates that the SAW body force overcomes the adhesive forces of the fouling proteins to the device surface and the fluid-induced drag and lift forces prevent its re-attachment. The streaming velocity fields computed using the finite-element models in conjunction with the proposed mechanism were used to identify the conditions leading to improved removal efficiency. In particular, the results of our numerical analysis show that higher amplitude and high frequency SAWs can clean biosensor surfaces most effectively in media with fluid properties similar to those of water, *i.e.,* lower viscosity and density, when (1) the SAW wavelength can be made comparable to the particle radius to promote effective acoustic-particle interaction; (2) the viscous boundary layer is thin and (3) the non-linear acoustic streaming forces exceed typical adhesion forces which is true for MHz frequencies. Based on the simulation analyses, possible mechanisms of nonspecifically bound protein removal are discussed and interpreted in terms of the experimental observations of SAW biosensor surface cleaning. Predictions of the model are in good agreement with those of simple analytical theories as well as the experimentally observed trends of nonspecifically bound protein removal in typical SAW biosensing operation.

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