Program Digest, 2019 IEEE International Ultrasonics Symposium (IUS)
Glasgow, Scotland, October 6-9, 2019

A Four Parameter Microfluidic Tandem SAW-IS Bio-Sensor

V. Yantchev
Advanced Engineering
Q-Arts Consulting EOOD
3000 Vratsa, Bulgaria
ventsi.yantchev@gmail.com

K. Kustanovich
Dept. Chemistry & Chem. Enginnering
Chalmers University of Technology
Goteborg, Sweden
http://orcid.org/0000-0002-7762-7157

A. Jesorka
Dept. Chemistry & Chem. Enginnering
Chalmers University of Technology
Goteborg, Sweden
aldo@chalmers.se

***Abstract*—Surface Acoustic Resonance (SAR) biosensing has recently been proposed as a highly compact and robust alternative to the conventional SAW delay-line based biosensing. The device can also be presented as a one-port high frequency alternative to the QCM, employing SAW resonance. It enables simple one-port measurements at low powers, while offering robust integration with microfluidics and implementation in integrated sensor arrays. Here we discuss the SAR approach as a key enabling and demonstrate its integration with the impedance spectroscopy (IS) concept in a single microfluidic device. The IS is integrated within a SAW reflector formed as interdigitated electrode (IDE) capacitor. A test fixture with SAW and IS ports is designed and fabricated. Four sensitive parameters are deduced from the tandem sensor readout and employed in a proof of principle study of liposome layers and their interaction with Ca2+ ions.**

Keywords—SAW, biosensing, impedance spectroscopy, low power, high sensitivity

I. Introduction

For decades the research on electro-acoustic biosensors have been focused towards the development of sensing device with capabilities exceeding that of the classical quartz crystal microbalance (QCM). Various MEMS based as well as surface acoustic wave (SAW) based topologies have been proposed with limited success. The problems with MEMS structures often are their fragility and high impedance [1]. The extensive development of the solidly mounted thin film bulk acoustic resonator (SMR) technology have demonstrated the utility of these devices in bio-sensing [1-3]. It was early recognized [4] that frequency scaling itself cannot bring advances in the resolution of gravimetric sensing. Measurements of SMR Bio-sensors near the GHz range have shown resolution at best comparable to QCM. The advantage however is in the miniaturization of the sensors which is associated with:

- Reduced amount of sample volume needed;
- Improved analysis and detection speeds;
- Compactness;
- Parallelization;

A problem limiting the commercialization of the SMR approach is the fact it uses tilted c-axis AlN films which are highly nonuniform and have poor stress control at present.

SAWs are also attractive towards sensor miniaturization owing to their planar technology and ability to easily scale the frequency by lithography techniques. The high frequency of operation is associated with smaller sensor dimensions, because the acoustic wavelength scales inversely proportional with the operation frequency. Typically, SAW sensor implementations rely on delay-line configurations [5-7] where the SAW propagates through a comparatively long distance between input and output transducers (two-port configuration) to accumulate sufficient time delay and phase shift. Thus, the frequency scaling effects are to a large extent compensated. As a result, SAW delay line sensors are relatively large and characterized by strong transmission loss when operated in-liquid, so that additional power and signal processing are often required.

We have recently introduced a microfluidics-integrated high frequency SAW sensor concept, combining the advantages of the 1-port measurement setup with wafer-scale, commercially viable fabrication processes and materials. The proposed resonant device is a modification of the 1-port SAW resonator topology in which the IDT es effectively protected form the electrical loading of the analyte, while operating with near 50Ohm impedance thus facilitating low power measurements [8]. Here we discuss the technological approaches to employ the surface acoustic resonance in Bio-sensing and the possibility to further integrate the SAR sensor with IS functionality in a 2-port single sensor microfluidic device.

II. The Surface Acoustic Resonance in Bio-sensing

The use of one-port SAW resonators in Bio-sensing is associated with number of potential benefits:

- Robust one-port measurements at low power;
- Operation at moderately high frequencies (<300MHz) for minimizing the impact of RF parasitics and simplifying the readout electronics;
- Matching to 50 Ohm impedance at resonance;
- Ability to use very cheap pocket VNAs operating in the defined low-power and frequency range;
- Ability to form compact solidly mounted sensor arrays and to use small analyte volumes for fast response;

- Commercially available technology and robust path towards thermal drift compensation and microfluidics integration;

Unlike QCM, SAW biosensors require specific design to avoid the electrical loading of the analyte. We have recently demonstrated a pioneering SAR design approach where driving and sensing units were separated [8-11]. More specifically it utilizes the reflective gratings of a one-port SAW resonator as sensing blocks, with the SAW IDT is protected from the measurement environment and act only as a read-out element. The optimum sensor performance has been derived from a trade-off between the ability of the IDT to probe the sensing blocks and their sensitivity determined by the amount of energy confinement [11]. This configuration achieves low susceptibility to damping, close to 50 Ohm device impedance and facilitates the integration in sensor arrays. The new surface acoustic resonance (SAR) sensor can detect mass and viscous loadings in liquid at a level comparable and better as compared to the state-of-art high frequency gravimetric sensors.

In Figure 1 the topology of the proposed sensor is outlined.

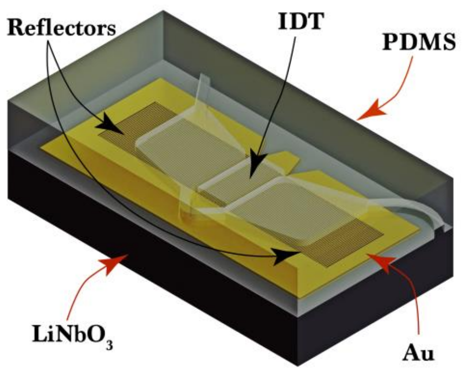

Fig. 1. Schematic design of the microfluidic integrated SAR sensor

The principles of operation and design optimization have been thoroughly addressed in our recent works [8-11]. It is of importance to note that this concept relies entirely on the ability to create microfluidic circuits which allows for efficient protection of the IDT from the analyte, while retaining the device response. The optimization is towards boosting the sensitivity since in classical SAW resonator design the SAW energy is rapidly decaying in the reflectors, thus limiting the portion of energy available for sensing. We have demonstrated that this limitation can easily be overcomed through a specific design promoting lateral energy confinement (LEC) in the sensing blocks [11]. The proposed structure employs IDT/Y-X LiNbO3 with grating pitch of about 10μm. Au electrode thickness of about 270nm and metallization ratio 0.5;

Alternatively, the SAR approach may be realized through a SiO_2/IDT/$LiTaO_3$ multilayer structure. More specifically SiO2 thickness of about 0.3λ will on one side promote significant compensation of the thermal sensitivity of frequency [12], while on the other will electrically decouple the sensor from the analyte owing to the substantial difference between the dielectric permittivity of LiTaO3 and fused silica. The idea is not entirely new but has not been practically implemented in Bio-sensing. Similar layered substrate was used in delay-line configuration with 0.1λ thickness of the oxide layer which seems still far from complete electrical decoupling and significant TCF improvements [13]. The proposed here 0.3λ-SiO_2/IDT/$LiTaO_3$ enables the use of continuous Au electrode on the surface, without loss in performance, facilitating thus the use of well-established bio-chemistry compositions. COMSOL simulations have been performed to briefly compare the two SAR approaches. In Figure 2 the particle displacement associated to the SH-SAWs in both the newly proposed 0.3λ SiO2/IDT/48Y-X $LiTaO_3$ and the IDT/Y-X $LiNbO_3$ structure proposed earlier [8-11]. These modes are very similar in nature but exhibit marked differences with respect to In-liquid performance regarding the electrical loading of the analyte. Table I summarizes the sensitivity features of both approaches as obtained by COMSOL eigen-frequency analysis. The structures were designed with about 300nm thick Au electrodes and the same grating pitch. Both operate at about 200MHz.

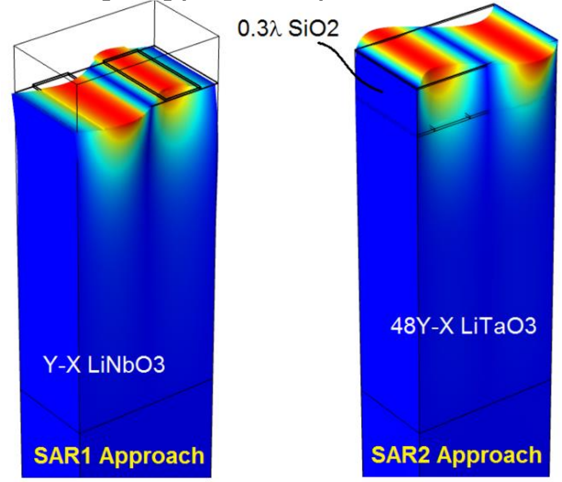

Fig. 2. Non leaky SH-SAWs displacements in structures enabling the surface acoustic resonance (SAR) biosensing. Significant surface confinement is achieved promoting thus a high sensitivity

TABLE I. SENSITIVITY FEATURES OF SAR ENABLING STRUCUTRES NEAR 200 MHZ

Load	SAR Sensitivity and Electromechanical Coupling		
	IDT/Y-$LiNbO_3$ (SAR1)	*SiO_2/IDT/48Y $LiTaO_3$ (SAR2a)*	*150nm Au on SiO_2/IDT/48Y $LiTaO_3$ (SAR2b)*
Surface short circuiting	1030ppm	0.1ppm	0ppm
Mass Loading (10nm PMMA)	300ppm	164ppm	430ppm
Coupling	20%	6%	3%

The inherently lower electromechanical coupling of the $LiTaO_3$ determine lower coupling of the SAR2 structure as compared to SAR1. Yet, the achieved couplings are more than enough for high performance SAR biosensors with 50 Ohm matching. The use of 0.3λ thick SiO_2 practically makes the device purely mass sensitive with no interference with the electrical properties of the analyte. Higher mass sensitivity in the SAR2 structure is readily achievable through a continuous top Au electrode which improves the surface confinement of the wave at the expense of electromechanical coupling. Unlike SAR1 structure, the SAR2 can adopt the classical 1-port SAW resonator topology in a straight-forward manner. The microfluidic container will be covering the total resonator surface, thus simplifying significantly the integration with microfluidics.

SAR based bio-sensing provide also opportunities to extend the sensor performance beyond the gravimetry. Impedance spectroscopy (IS) integration with acoustic wave bio-sensing opens new possibilities for studies on cells and pathogenic microorganisms, where the bio-membrane at the cell-surface interface, and the integrity of the bulk structure can be simultaneously monitored. Examples for either technique applied individually have been reported earlier [14, 15]. A combination of pathogen detection and drug response determination could, for example, be an application of the tandem IS-Acoustic Wave sensor. Initially, IS integration with acoustic wave sensing has been demonstrated and found useful with the QCM platform. The proposed schemes feature an additional reference electrode, not functional part of the QCM. The large size of the QCM sensor limits the ability to work with small analyte volumes, as well as the sensor parallelization. The SAR platform enable robust integration of both sensing principles within one sensing device [16]. We employed the SAR1 structure in combination with the sensing topology in Fig. 1 to demonstrate such an integration for the first time [16]. IS integration with SAR2 structure also seems feasible.

III. SAR-IS Integration and Test Fixture

The tandem SAR-IS sensor employs the SAW reflecting gratings as sensing element not only for SAW but also for IS. This functionality is achieved through transforming the SAW reflective gratings in an IDE capacitor (see Fig. 3). It is important to note that IDE must use splitted 4 strips per λ or 3 strips per λ gratings. Such type of electrode connection will not change the SAW propagation characteristics under the reflective gratings. By contrary, the use of regular 2 strips per λ may affect SAW propagation upon changes of the electric properties of the media (analyte).

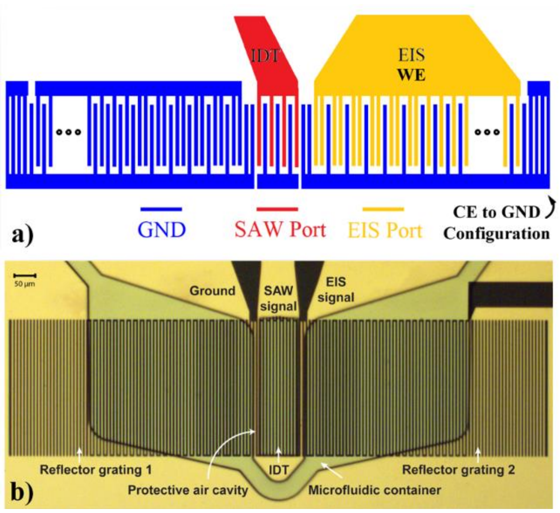

Fig. 3. Integration between impedance spectroscopy and surface acoustic resonance. a) schematic view b) practical implementation with bonded PDMS microfluidics.

In the structure proposed here (Fig. 3), 2 per λ strip grating is used far from the IDT, where SAW is significantly damped. VNA based measurements have shown more than 60dB rejection between the SAW port (IDT) and the IS port. This is a prerequisite for interference free tandem operation.The SAR-IS tandem sensor is complemented with a robust test fixture enabling chip size reduction and automated measurements through a spring-loaded contacts applied to the SAW and IS ports. It is noted that test-fixtures are widely used in commercial QCM based Bio-sensors. With this development, the SAR sensor measurement becomes more robust as compared to the widely used GSG probe supported measurements.

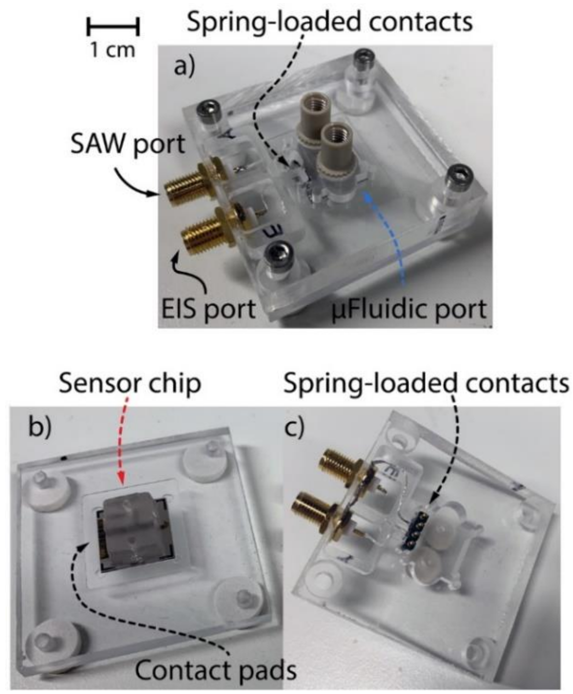

Fig. 4. Measurement test fixture for the SAR-IS integrated Bio-sensor. a) overall view b) and c) detailed images of the test fixture building blocks.

IV. SAR-IS Response

The simultaneous response of the SAR-IS tandem sensor is shown in Fig. 5 and Fig. 6. Four sensitive parameters were identified. Two are based on the acoustic wave characteristics (resonance frequency and conductance peak magnitude), while the other two are impedimetric parameters from the Randle's cell (Leak resistance and constant phase element (CPE_{Dl}) of the double layer Dl). All four parameters are describing states at the interface between the electrode grating and the analyte.

In Table II we summarize the SAR-IS sensor response upon loading with HEPES buffer solution, liposome layer and its interaction with Ca^{2+}. Upon Ca^{2+} interaction, both the acoustic and the IS response have shown certain level of signal recovery towards the base HEPES levels. The SAW resonance frequency and R_{Leak} has shown a moderate recovery, while the magnitude of peak conductance and CPE_{Dl} recovered almost to the levels of HEPES. Although the behavior can be provisionally separated in two pairs with different sensitivities, the underlying phenomena however are of completely different nature.

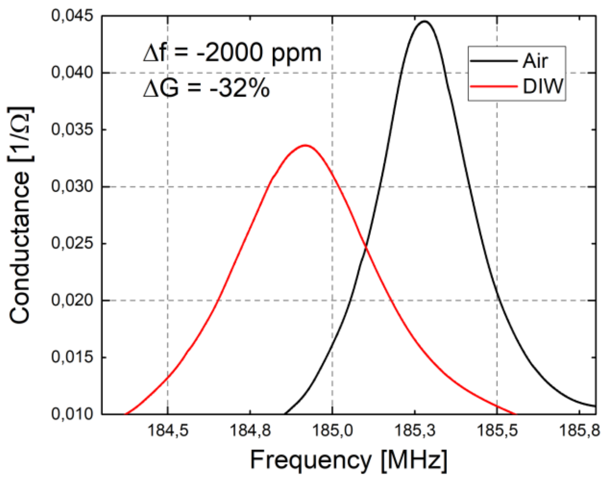

Fig. 5. Close-in resonance SAR response of the IS-SAR sensor in DIW load.

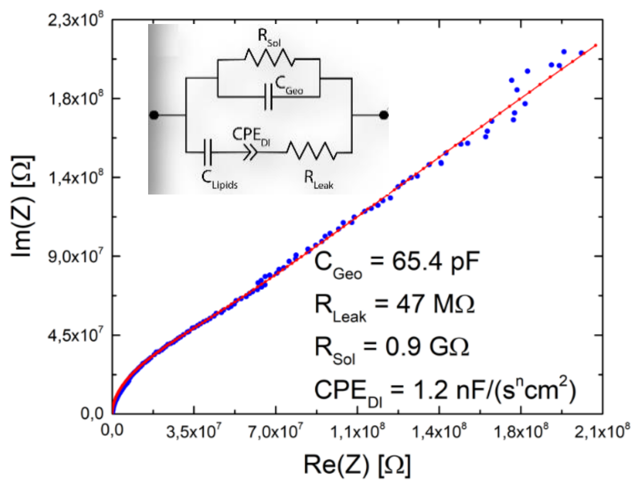

Fig. 6. Nyquist plot of IS response of the tenadem sensor immersed in DIW. Randle's cell parameters are obtained from fitting to the measured response.

TABLE II. THE FOUR PARAMETERS SENSITIVITY OF THE SAR-IS TANDEM SENSOR

Load	**Extracted SAW-IS sensing quantities**			
	Δf, ppm	***ΔG, %***	***R_{Leak}, MΩ***	***CPE_{DI}, nF/($s^n cm^2$)***
DIW to HEPES	-118	-2.1%	47 to 4.2 (-91%)	1.2 to 1.6 (+30%)
HEPES to Lipids	-86	-2.3%	4.2 to 2.9 (-31%)	1.6 to 2.9 (+45%)
Addition of Ca^{2+}	+19	+2.1%	2.9 to 3.3 (+14%)	2.9 to 1.8 (-38%)

CONCLUSIONS

Surface acoustic resonance (SAR) was proposed as a robust approach in Bio-sensing. Two different structures enabling the one-port SAR sensing are presented in comparative manner. The one-port SAR sensor has been further integrated with impedimetric sensing feature and complemented with a dual port test fixture for automated measurements. Our initial results demonstrate the rich set of sensing parameters that can be used to describe a given bio-sensing interaction in a more specific manner.

REFERENCES

[1] I. Katardjiev and V. Yantchev, "Recent developments in thin film electro-acoustic technology for biosensor applications," Vacuum, vol. 86, no. 5, pp. 520-531, 2012

[2] M. DeMiguel-Ramos et al., "Gravimetric biosensor based on a 1.3 GHz AlN shear-mode solidly mounted resonator, Sensors and Actuators B - Chemical, vol. 239, pp. 1282-1288, Feb 2017.

[3] M. Nirschl et al., "CMOS-Integrated Film Bulk Acoustic Resonators for Label-Free Biosensing," Sensors, vol. 10, no. 5, pp. 4180-4193, 2010

[4] J. R. Vig et. al., "On acoustic sensor sensitivity", IEEE Trans. Ultrason. Ferroelectr. Freq. Control, vol. 38, p.311, 1991

[5] K. Lange, B. E. Rapp, and M. Rapp, "Surface acoustic wave biosensors: a review," Analytical and Bioanalytical Chemistry, vol. 391, no. 5, pp. 1509-1519, Jul 2008.

[6] J. Kondoh, Y. Matsui, and S. Shiokawa, "New Biosensor Using Shear Horizontal Surface-Acoustic-Wave Device," Japanese Journal of Applied Physics, vol. 32, no. 5b, pp. 2376-2379, May 1993.

[7] K. Mitsakakis, A. Tserepi, and E. Gizeli, "SAW device integrated with microfluidics for array-type biosensing," Microelectronic Engineering, vol. 86, no. 4-6, pp. 1416-1418, Apr-Jun 2009.

[8] K. Kustanovitch, "Integration of Surface Acoustic Wave and Microfluidic Technologies for Liquid Phase Sensing Applications", Ph.D Thesis, Dept. Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg, Sweden 2019.

[9] K. Kustanovich, V. Yantchev, A. Jesorka, "Design and Characterization of Surface Acoustic Wave Resonance (SAR) System for In-liquid Sensing", 2017 Joint Conference of the European Frequency and Time Forum and IEEE International Frequency Control Symposium, pp. 652-655, 2017.

[10] K. Kustanovich et. al., "A high-performance lab-on-a-chip liquid sensor employing surface acoustic wave resonance", J Micromech Microeng, vol. 27, art.no. 114002, 2017.

[11] K. Kustanovich et. al., "A high-performance lab-on-a-chip liquid sensor employing surface acoustic wave resonance: part II", J Micromech Microeng, vol. 29, art.no. 024001, 2019.

[12] S. Matsuda et. al., "Investigation of SiO2 film properties for zero temperature coefficient of frequency SAW devices" , 2010 IEEE Int. Ultrasonics Symp., pp. 633 – 636, 2010

[13] J Freudenberg, M. von Schickfus, S Hunklinger, "A SAW immunosensor for operation in liquid using a SiO2 protective layer", Sensors and Actuators B Chemical, vol.76, no. 1, pp. 147-151, 2001

[14] Z. Rácz et. al., "Cell-based surface acoustic wave resonant microsensor for biomolecular agent detection", 2011 Int. Solid-State Sensors, Actuators and Microsystems Conference, pp. 2168–2171, 2011.

[15] Y.C. Xu et. al., "A review of impedance measurements of whole cells", Biosens. Bioelectron. Vol. 77, pp. 824–836, 2016

[16] K.Kustanovich et. al., "A microfluidics Integrated impedance/surface acoustic resonance tandem sensor", Sensing and Bio-Sensing Research, vol. 25, art.no. 100291, 2019