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Short communication

Shear mode FBARs as highly sensitive liquid biosensors

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Abstract

Thin film bulk acoustic resonators (FBARs) operating in shear mode have been investigated for biosensing applications. Dynamic measurements in liquid were carried out and the adsorption of an antibody–antigen system was observed. Although this is the very first FBAR biosensor system operating in liquid environment it was found that the sensor performance ruled by the smallest detectable mass attachment, is already better (2.3 ng/cm^2) than that of QCMs. The ability of easy integration onto wafers together with readout-circuitry as well as the possibility of making up large arrays comprising pixels with different functionalities make these devices interesting for future acoustic biosensors.

Keywords: Biomedical transducers; FBAR; Shear mode; QCM

1. Introduction

Piezoacoustic devices are known to have excellent frequency stability and have therefore been applied in the form of quartz crystal resonators as tools for high precision determination of frequency and time in clocks or electrical transmitters. After Sauerbrey [1] demonstrated the direct correlation of frequency change and a thin film mass attachment their use extended into mass sensing applications. Since then their importance in this area has steadily been increasing making them today standard devices which are widely used in bioanalytics [2,3].

Nevertheless these devices have their limitations. Being manufactured top down, meaning that they are cut from the bulk quartz, and operating as a membrane, their maximum frequency is limited due to the minimum obtainable quartz thickness. So is their sensitivity which is directly related to frequency.

Piezoelectric thin film resonators that are manufactured bottom up are much smaller, can reach higher frequencies and can be manufactured with CMOS-compatible technologies [4]. These devices have been applied for filters for some years [5], yet their application as biosensors was lacking the ability of operation in shear mode, a prerequisite for low-loss operation in liquid. Measurements with longitudinal mode FBARs in liquid have been carried out [6] but their performance was poor.

By conducting bioexperiments in liquid and measuring the frequency shift in gaseous environment we showed earlier that FBARs are in principle suitable for biosensing [7]. Our work then focused on the development of an adequate piezoelectric layer allowing the excitation of transversal shear acoustic modes which exhibit a significantly lower energy dissipation in water [8]. In this publication, we will present first real-time measurements of the binding of antibody–antigen in liquid using a shear mode FBAR biosensor.

The sensor shows an excellent mass and time resolution exceeding that of a QCM-system which we obtained in a comparison measurement using the same reagents and fluidic system. For the sake of completeness, we also conducted dynamic measurements with an FBAR operating in longitudinal mode finding a performance clearly inferior as expected. Comparison of the outcome for all three systems is presented.

2. Experiment

2.1. Sensor principle

Fig. 1 shows a schematic cross-section of the sensor. A ZnO thin film with the *C*-axis 16° inclined relative to the surface normal forms the resonator (see SEM picture in Fig. 1). It was

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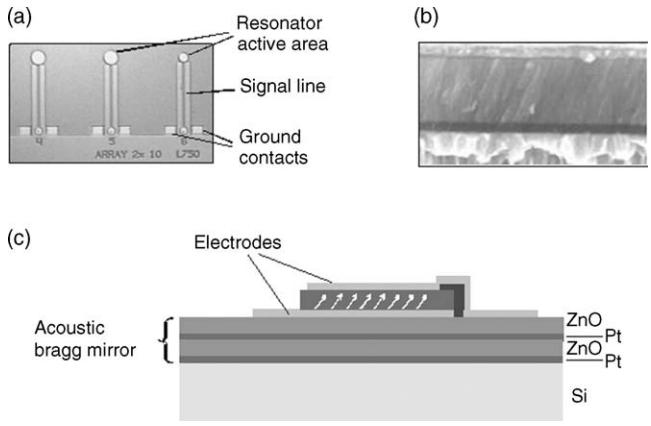

Fig. 1. Setup of the FBAR biosensor: (a) top view of the resonator and the electrical leads, (b) electron microscope picture of *C*-axis inclined ZnO and (c) schematic picture of the lateral structure comprising the resonator with two electrodes solidly mounted on an acoustic Bragg mirror.

successfully deposited by Link et al. in a modified magnetron dc pulsed reactive sputtering process by oblique particle incidence [8]. The inclination angle is needed to allow an excitation of transversal shear mode (TSM) acoustic waves.

The resonator layer has a thickness of about 500 nm so a ground resonance for a TSM mode can be excited at about 790 MHz. Longitudinal mode resonances can be likewise observed but because of the mode dependent acoustic velocity they are found at a much higher frequency. So relative mode purity is ensured.

An acoustic Bragg mirror being made up of alternating layers of high and low acoustic impedance (in this case platinum and zinc oxide) ascertains the acoustic energy to be confined in the top layers [7]. These layers have a thickness that corresponds to a quarter of the acoustic wavelength which is therefore in the order of some hundred nanometers.

2.2. Liquid measurements

To be suitable as a biosensor, efficient operation of the sensor in liquid must be assured. As shown in Fig. 2, a flow cell is positioned on the wafer. A viton strip in between ensures it to be sealed and the fluid flow inside the cell to be well controlled. It is composed of a chamber made from acrylic glass with an inflow and outflow that can be connected to a fluidic system. The wafer is designed in a way that the resonator active area is situated inside the flow cell whereas the electrical contacts are located outside.

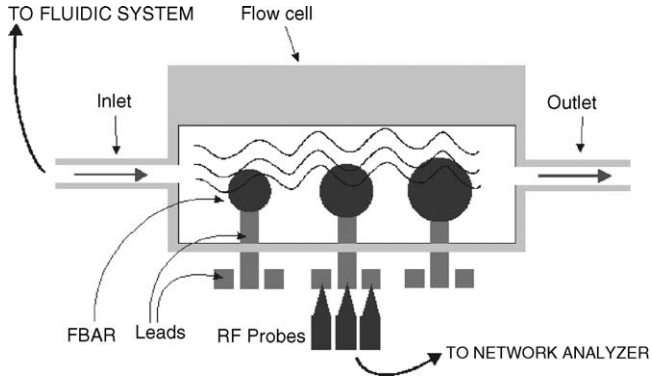

Fig. 2. On-wafer measuring setup: flow cell with connections to the fluid system is placed on the wafer. The active resonator area is completely immersed in water, electrical readout is done outside the flow cell with an RF-tip.

The sensor was characterized with the help of an HP 8513A Network Analyzer. Frequency sweeps covered a range of 30 MHz around the resonance with a 200 point resolution. Measurements were taken every 14 s. The data obtained were read out by the computer and converted to impedance values. The resonance frequency was calculated from the impedance phase.

In a next step a biolayer system was flushed over the sensor and the sensor response to the consecutive binding processes observed. A sensor with circular shape and a diameter of 200 μm was selected. The reagent samples of 200 μl for each injection were pumped through the flow cell via an injection valve with a flow rate of 36 μl/min.

The model system used was an antibody/antigen pair comprising a sequence of avidin (the antigen), with a concentration of 100 mg/l followed by bovine serum albumin (BSA, 1 g/l) and anti-avidin (105 mg/l, diluted in 1 g/l BSA). All these reagents have been dissolved in a buffer solution ("HBS buffer", containing 150 mM NaCl and 10 mM HEPES at a pH of 7.46) that also served as a reference solution stabilizing the biosystem between each injection. Each injection was performed in duplo and the signal was stabilized with the buffer solution in between the injections.

3. Results and discussion

3.1. Sensor performance in liquid

The performance of an acoustic mass sensor is principally ruled by the device sensitivity and the quality-factor. The resonance frequency is usually determined from the phase of electrical impedance of the piezoelectric resonator. Assuming a minimum phase $\Delta\Phi$ that can be resolved (due to phase noise in the device and/or a limited resolution of the evaluation circuit) it follows from the definition of the quality-factor that the minimum frequency shift that can be resolved is:

$$\Delta f = \frac{f_0}{2}\frac{\Delta\Phi}{Q} \tag{1}$$

Dividing by the sensitivity ($s = \Delta f/(\Delta m/A)$) gives us the minimum detectable mass. Hence, the figure of merit (FOM) determining the noise equivalent mass resolution of the sensor is defined by:

$$\mathrm{FOM} = \frac{f_0}{Q \cdot s} \tag{2}$$

To get some theoretical value for the sensitivity the following approach was applied. As shown in the first section the sensor is composed of a multi-layer stack. The thicknesses of these layers are of the same order of magnitude, so none of the layers can be neglected for the acoustic performance. Therefore, theoretical models (Sauerbrey, etc.) to predict the sensor sensitivity

Table 1
Resonator performance in liquid: with a theoretical value for sensitivity the resonator figure of merit can be calculated for an approximate comparison of the different resonators mass resolution

	L-FBAR	S-FBAR	QCM
Frequency (MHz)	2000	790	10
Simulated sensitivity, s (Hz cm^2/ng)	2500	800	0.22[a]
Q in liquid	10	100–150	2000
FOM (cm^2/ng)	80000	6580–9880	22730

[a] Quartz sensitivity according to Sauerbrey.

cannot be applied as they assume systems of maximum two layers. Instead, simulations of the acoustic stack of the FBAR have been carried out. Using a one-dimensional model and varying the top layer thickness an estimation for the mass sensitivity could be obtained [9]. It is approximately 800 Hz cm^2/ng which is more than three orders of magnitude higher than the sensitivity of a 10 MHz quartz crystal microbalances (0.22 Hz cm^2/ng calculated with Sauerbrey). These results are shown in Table 1.

The Q-factor of the device is determined by a complicated set of factors. It is defined as the ratio of stored energy to the energy lost in the device. Such energy losses occur as acoustic and electric losses. Acoustic losses comprise losses by friction which are mainly caused by crystal imperfections (grain boundaries, dislocations, etc.) and acoustic scattering at the interfaces of different layers. The latter can be scattered away from the resonator, the acoustic energy thus being lost. All these losses certainly play a considerably more important role in the FBAR device than they do in quartz crystals. This is due to the acoustic wavelength which is smaller than a micrometer reaching the range of possible grain sizes and surface roughness.

When the system is immersed in liquid an additional channel of energy leakage emerges. To quantify these losses, measurements in liquid have been carried out. For the FBAR Q-factors of maximum 156 where found in water compared to 285 in air. So the quality-factor is reduced to 55% due to immersion into water, which means that the acoustic energy loss due to dissipation into water is doubling relative to the acoustic loss within the resonator alone. For a quartz crystal microbalance the reduction of Q-factor is from 20,000 to 2000 or to 10%, which means that the acoustic dissipation is increasing by a factor 10. So the negative impact on sensor performance from immersion into liquid is much stronger for the QCM than for the FBAR.

To get an idea of which mass resolution can be theoretically obtained in water, the figure of merit (according to Eq. (2)) was calculated and is shown in the last row of Table 1. These values show the FBAR performance to be superior to that of the QCM. The (mass resolution equivalent) figure of merit is about 6500–9900 and more than two times better than that of the QCM (22,730). So the much lower Q-factor is more than compensated by the higher sensitivity coming from the higher operation frequency. The expected performance of the longitudinal mode FBAR on the other hand is about 10 times worse which is mainly because of a much lower Q.

It has to be mentioned that this figure of merit is only proportional to the minimum detectable mass under the conditions of a constant phase resolution $\Delta\Phi$. Therefore, further experiments

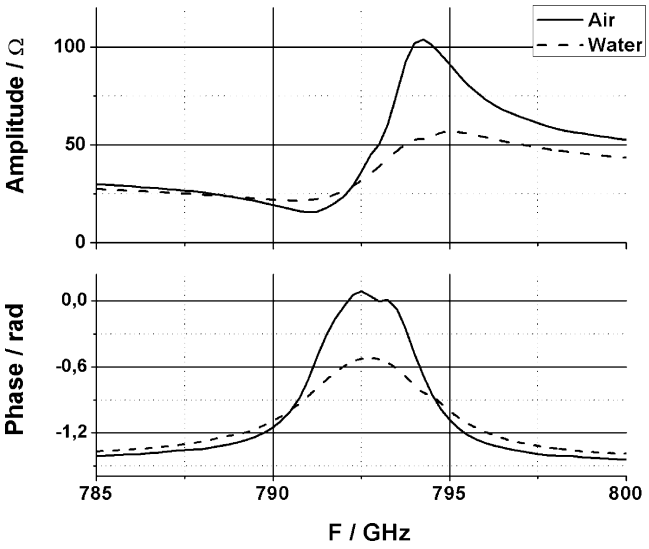

Fig. 3. Impedance characteristic (amplitude and phase) of the resonator used in the biosensor experiments.

have been conducted to evaluate the sensor performance under practical conditions.

3.2. Immunosensor experiments

The FBAR sensor has been connected to a fluidic system, and a measurement of the model system avidin/anti-avidin as described in the previous section was carried out. A comparison of the impedance characteristic in water and air of the resonator used in the experiments is shown in Fig. 3. Fig. 4 shows a complete time evolution for the consecutive bindings of avidin, BSA and anti-avidin, where the values obtained for the parallel resonance are being plotted in the upper graph. Every injection causes a significant sensor response and the beginning of the injection is marked with a vertical dashed line, respec-

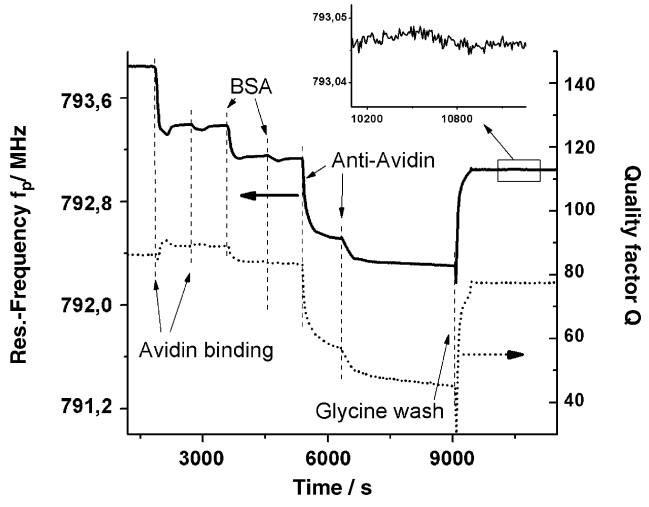

Fig. 4. Dynamic measurement for the avidin/anti-avidin system obtained with a shear mode FBAR. The upper graph shows the parallel resonance frequency f_p as a function of time, giving information about the mass attachment. The lower graph shows the Q-factor obtained from the derivative of the phase revealing details about the top layer visco-elastic properties.

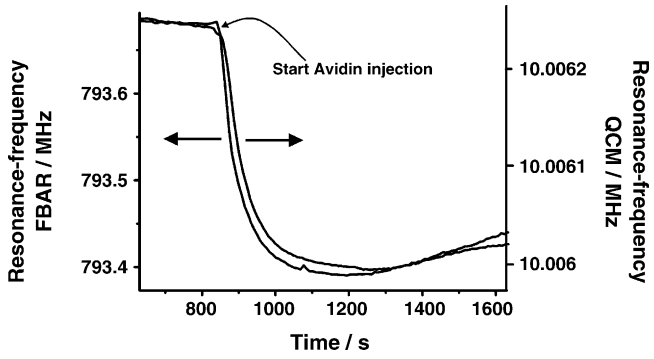

Fig. 5. Comparison between avidin-binding measured with QCM-system and FBAR: the same qualitative characteristics are observed, events with a high curvature seem to be better resolved by the FBAR (see arrow).

tively. In general an injection consists of period of declining resonance frequency when the reagent is binding and the sensor mass increasing. This is followed by a period of stabilization when the reference fluid is flushed over the sensor and possibly removing loosely bound molecules. The first two injections comprise avidin which is binding directly to the sensor gold surface. The second of those injections has the task to fill binding sites that may have been blocked before by loosely bound avidin molecules. Therefore, the second injection shows a much lower frequency shift of just 10 kHz.

Bovine serum albumin is a rather small molecule and has the task to fill the gaps between the avidin molecules. This impedes anti-avidin in the following injection to bind directly to the sensor surface. The overall frequency shift caused by BSA is about 253 kHz after two injections. Detachment processes are much less pronounced here.

Finally, at a time of about 5400 s, anti-avidin is injected and causes a frequency shift of about 618 for the first and about 190 kHz for the second injection.

The frequency shifts detected are pretty much in the range of what is being expected for these bindings and an excellent signal stability is obtained (below 1 kHz; see inset of Fig. 4). Reaction dynamics can be extracted and details such as two stage processes (see the kink in the first avidin-binding indicating the binding of a second layer with a lower rate) can be discerned.

From the gradient of the impedance phase the quality-factor of the resonator/top layer complex could be determined and is shown in the lower curve in Fig. 4. As the properties of the resonator itself are not altered by the attachment of additional layers, changes in quality-factor are attributed to changes in top layer elastic properties and therefore provide valuable information about the different biolayers attached. We see that the Q-factor is strongly affected by the biolayers (variation is between 30 and 90) and also here an excellent signal stability (Q-noise is determined to 0.078) is obtained.

Fig. 5 shows a plot of the resonance frequency taken dynamically for the avidin-binding. The surface of the FBAR sensor was subjected to a pretreatment with a piranha solution (29% H_2O_2 and 70% H_2SO_4) obtaining thereby a hydrophilic character of the surface. One curve shows the binding process as it was seen with the shear mode FBAR whereas the other curve shows the result obtained with a conventional QCM. It clearly shows that the result obtained from both devices is qualitatively the same. A sharp decline in resonance frequency when avidin is flushed over the resonator is observed, the speed of the adsorption yet declining throughout the process. The adsorption is lasting for 350 and 420 s for the FBAR and QCM, respectively, before a slight increase of the resonance frequency begins. This time coincides with the duration of the avidin-injection, so the increase can be attributed to a detachment of loosely bound avidin molecules. The maximum reaction speed could be determined to 2.9 Hz/s or 5.4 ng/(cm^2 s) for QCM and 4.4 kHz/s or 7.6 ng/(cm^2 s) for the FBAR. The agreement of all qualitative features observed shows that although the devices are operating at very different frequencies (the FBAR operation frequency is 80 times higher than that of QCM), the same binding processes are observed in the same way. Both curves continue to rise slowly and with nearly constant speed until the next injection. So for this hydrophobic-surface case the binding of avidin to the surface seems to be less stable than for the hydrophilic case as it was shown in Fig. 4. There the curve becomes flat after only 200 s.

A slight difference between the FBAR and the QCM-curve is observed when the decline of the resonance frequency starts (see arrow): it the case of the FBAR it is sharper. A possible explanation for this is the smaller size of the FBAR active area (0.04 mm^2 compared to 64 mm^2 for the quartz crystal microbalance) which brings about a quicker reaction time for the processes taking place as the fluid containing the reagent needs less time to flow over the sensor.

3.3. Quantitative sensor performance

In the following the quantitative FBAR sensor performance has been assessed in practical biosensing applications and compared to the QCM and longitudinal FBAR device under similar conditions. The main parameter of interest is the mass resolution, which needs an evaluation of the frequency noise. Fifty measurements of the resonance characteristic were taken over a time range of about 700 s while the rest of the system was held stable (see inset of Fig. 4). A buffer solution flow of 36 μl/min and a chip temperature of 25 °C were employed. The threefold standard deviation of this frequency was regarded as the minimum frequency shift that can be measured. It was determined to be 2580 Hz for the shear mode FBAR compared to 46,200 Hz for the longitudinal mode FBAR. There was thus a drastic improvement due to the much lower energy dissipation in water. In order to get an experimental value for the sensitivity of our device the avidin-binding was taken as a reference. Through reference measurements[1] the attached mass for avidin was about 420 ng/cm^2. In consequence a sensitivity of 585 Hz cm^2/ng and a mass detection limit of about 2.3 ng/cm^2 was derived. In the same way data were obtained for the longitudinal mode FBAR and QCM, as summarized in Table 2. This table thus provides a direct comparison of the performance of the different sensor systems under practical conditions.

[1] Reference Measurements were Performed with Surface Plasmon Resonance Technique (Biacore 3000).

Table 2
(Bio-)sensor performance of different bulk acoustic wave devices

	L-FBAR	S-FBAR	QCM
Frequency (MHz)	2000	**790**	10
Experimental sensitivity (Hz cm^2/ng)[a]	937.5	**585**	0.54
Measured noise level (Hz)	15400	**864**	1
Minimum detectable mass (ng/cm^2)[b]	21	**2.3**	5.2

Note that the performance ruled by the detection limit is already better for the shear mode FBAR device (bold values) than for QCM!

[a] The frequency shift evoked by the avidin-binding was taken as a reference.

[b] The mass was determined with the three-fold frequency noise as frequency resolution.

According to the present assessment the shear mode FBAR biosensor is already twice as good as the conventional QCM-system. Though the noise level of the device is much higher than for QCM (864 Hz compared to 1 Hz), a better mass resolution is achieved because of the superior mass sensitivity.

The performance obtained with a longitudinal mode FBAR is quite poor, as expected. Such devices do not need to be considered for practical biosensing applications directly in liquid. The results for the relative performance of FBAR and QCM agree reasonably with the semi-theoretical considerations made earlier (see Table 1). That is, the mass resolution as obtained from biosensing experiments corresponds to the figure of merit as obtained from the sensor performance in liquid.

Nevertheless, there is a difference between theoretical and experimental sensitivity (as given in Tables 1 and 2). The experimental sensitivity for QCM is higher whereas for the FBAR it is lower than the respective theoretical values. Here side effects deriving from top layer elastic properties and with frequency dependent impact on sensor sensitivity may play a role. These influences will be subject to future investigations.

4. Conclusion

We have demonstrated for the first time the suitability of shear mode FBARs for biosensing in liquid. Based on an avidin/anti-avidin system it was shown that the performance in terms of mass resolution is already better than that of a QCM system, as determined under the same conditions. Since this is the very first description of a functioning shear mode FBAR biosensor device so far, the performance is expected to improve through further sensor optimization. Factors such as the manufacturing of the *C*-axis inclined layer, the frequency readout and the overall sensor design can all be further optimized. Because of its manifold advantages, ranging from production costs over the suitability for multi-array-testing to CMOS-compatibility, this sensor provides a platform for various future applications in biosensing. Research will be continued to investigate the sensor characteristics deriving from the higher frequency as well as its multi-layer design.

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