A Love-wave sensor for direct detection of biofunctionalized nanoparticles

L. El Fissi, J-M. Friedt
SENSEOR
Parc de Haute Technologie – Lot n°3
694, Avenue du Docteur Maurice Donat
06250 MOUGINS France
lamia.elfissi@femto-st.fr
jmfriedt@femto-st.fr

V. Luzet, F. Chérioux, G. Martin S. Ballandras
Institut FEMTO-ST, CNRS UFC ENSMM UTBM,
Besançon, France
ballandr@femto-st.fr

Abstract: **In this work, we have designed and manufactured Love wave sensors equipped with a liquid cell for the detection of bio-functionalized nano-particules in water. Measurements using these devices are based on the determination of velocity and propagation loss changes of a guided acoustic shear wave generated by Inter-Digital Transducers (IDTs) patterned on a (YXlt)/36°/90° cut of quartz and passivated by a silica overlay. The so-called Love mode acoustic wave sensors are known to be compatible with operation in liquid medium but requires and adapted fluidic packaging. The SU-8-based liquid cell is patterned atop the SiO_2 guiding layer. We then describe the use of these sensors for the time resolved identification of the presence of nanoparticles in suspension. Silane surface chemistry for non-selective detection of most bio-functionalized nanoparticles health hazards is demonstrated by analyzing the gravimetric signal associated with the binding of avidin-functionalized gold nanoparticles.**

I- Introduction

Love mode acoustic sensors have been widely demonstrated as sensitive transducers for detecting surface binding of molecules in liquid media [1-3]. They particularly can be applied for direct detection and sensing for which no preliminary preparation of the analyzed sample is required. More specifically, sensors used for detecting biological species must be able to operate in liquid media [4-6]. Shear surface-guided acoustic waves then represent an advantageous solution in that purpose, as they provide actual capabilities for the detection of low levels of analytes in liquid samples.

As manufacturing technologies of such devices have considerably progressed these last years, a wide range of modern applications are then addressable along this approach [7-9]. Recently, nanomaterials, such as nanoparticles, have emerged as a new class of building blocks for many promising devices (electronic, sensors, mechanics…). However, despite of their potential properties, they are currently perceived as a potential environmental and health hazard, mainly in the case of nanoparticules coated with biocompatible layers. Their direct detection is required for continuously monitoring for instance waste water before and after treatment in order to assess the cleaning efficiency. More generally, an accurate control of nanoparticule environmental dispersion is an issue to address for promoting their use and explotation for public applications. In this work, we investigate the interest of a Love-wave-based microbalance to detect such particules and to potentially identify their nature by an appropriate wave-guide chemical preparation. We briefly recall the sensor pinciple and structure, and we describe the associated fluidic structure built on the wave-guide to allow for the localisation of the analyzed liquid in the reaction area. We finally describe the chemical preparation of the sensor surface for the detection of non selective streptavidin molecule adsorption.

II- Description of the Love-Wave-Based Microbalance

Love-wave devices consist in delay lines built on (AT,Z) cut of quartz. The wave guidance is achieved by depositing a 2.5 µm thick silica layer atop an AT-cut of quartz (a trade-off between an optimal gravimetric sensitivity and a reasonably simple and reproducible sensor fabrication). The Love wave is excited and detected using IDTs composed of 50 pairs of 4-finger-per-wavelength electrodes made of 200 nm thick Aluminium strips depositied by evaporation and patterned using a lift-off process. The grating period is 10µm, i.e. a wavelength close to 40 µm, yielding a frequency operation in the vicinity of 125 MHz. A 3.2 mm long cavity is achieved in between the two delay line IDTs, corresponding to the location where biochemical reactions are assumed to take place (the so-called sensing area). The acoustic aperture is 3.5 mm (about 90 wavelength). The silica overlays used here as guiding layers are patterned to access the bonding pads. Figure 1 shows a scheme of the delay line and figure 2 shows a photo of the dual (differential) delay line. One delay line is dedicated to the sensing operation, whereas the other is used as phase and magnitude reference.

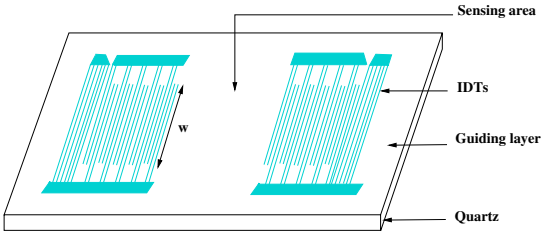

Figure 1 : Scheme of the Love-wave based delay line

Figure 3 shows the typical response of Silica based Love-wave delay lines. The insertion loss is observed near 22 dB.

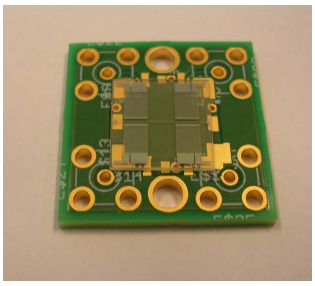

Figure 2 : Photo of the dual delay line

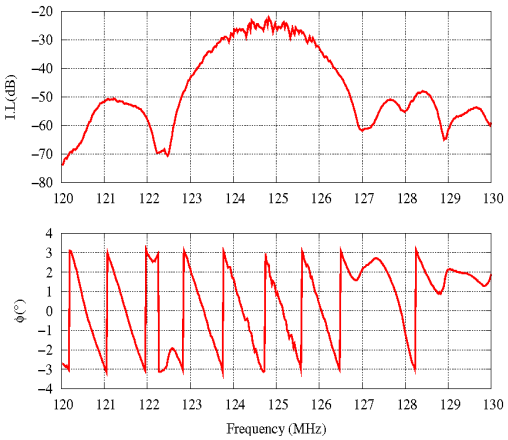

Figure 3 : Transfer function of delay line exploiting Silica guiding overlays

III- Microfluidic Packaging

A liquid cell fabricated using the SU-8 photo-resist has been developed to prevent the presence of liquid onto the transducers and to isolate the sensing area. The photo-resist SU-8 is chosen here fo many reasons: (i) once polymerized, it exhibits excellent mechanical and chemical properties, (ii) it is biologically inert and resistant to solvents used during the surface functionalization and cleaning steps (organic solvents and acids), (iii) it enables the formation of thick ridges and extremely rigid structures simply by standard UV photolithography, (iv) it is a relatively inexpensive technology for implementing high aspect ratio structures compared to other techniques, e.g. LIGA or dry etching techniques.

Two aspects have been considered for the design of the liquid cell. First, the open well configuration is chosen for an easy access to the sensing area. Second, the SU-8 walls must cover the smallest length of the acoustic path in order to obtain the minimum acoustic attenuation and the best liquid tightness. In order to resolve the second point and to find the correct dimensions for the wall, several wall widths have been tested to evaluate the influence of this parameter on the attenuation of the acoustic wave.

Figure 4 shows the effect of the presence of thick SU-8 photoresist walls on the acoustic path. According to these curves, we find out that the acoustic wave is significantly affected by the presence of the SU-8 walls and the insertion loss increases when increasing its width. According to these results , the 80 µm width configuration was selected to build our fluid system to keep insertion losses as close as possible to 30 dB.

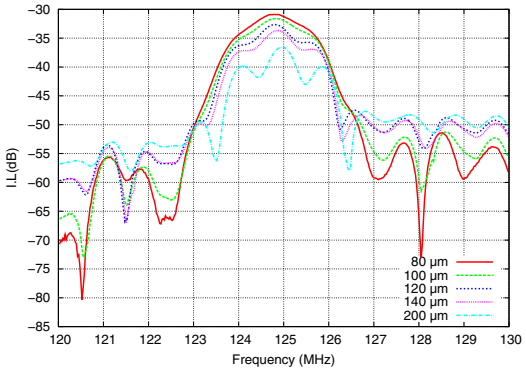

Figure 4 : Evolution of the insertion loss versus the width of the walls deposited on the acoustic path

We present now the basic steps of the SU-8 technology developed here for the fabrication of this liquid cell. the overall thickness of the structure is about 120 µm. The adhesion of a thick SU-8 layer onto silica overlays is known to be poor if cleaning and drying procedures are not carefully respected. The SU-8 2075 resist from Microchem Corp. (MA, USA) has been used here.

Prior to resist coating, the quartz wafer with SAW sensor must be dehydrated at 200 °C for 30 minutes. The SU-8 2075 is spread at 300 rpm (acceleration fixed to 100 rpm s^{-1}) during 60 s, followed by a spin-coating at 1600 rpm (acceleration 300 rpm s^{-1}) during another 60 s in order to achieve a layer thickness of 120 µm. After spun operations, an initial soft-bake is carried out on a hotplate at 95 °C for 35 minutes to remove the photoresist solvents. This layer now can be patterned by standard photolithographic techniques to form the walls. A second bake is then performed at 95 °C for 15 minutes to finish the cross-linkage of the SU-8. The unexposed epoxy is then dissolved in propylene glycol monomethyl ether acetate (PGMEA). Figure 5 summarizes this fabrication process. Once these operations achieved, the wafer must be diced to release each sensor with its patterned SU-8 fluidic structure. Figure 6 presents a scanning electron microscope (SAM) image of the final SAW sensor equipped with its SU-8 based liquid cell.

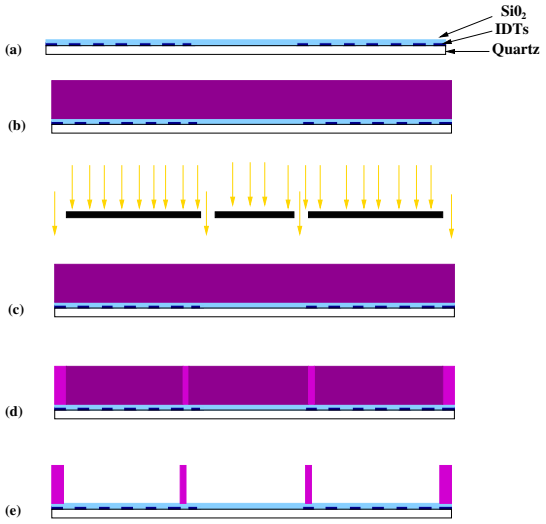

Figure 5 : SU8 walls fabrication steps. (a) Love wave delay line with SU8 guiding layer ; (b) spin coating of SU8 2075 and soft-bake ; (c) soft contact lithography and post-bake ; (d) development of SU8. Transfer function of delay line exploiting Silica guiding overlays

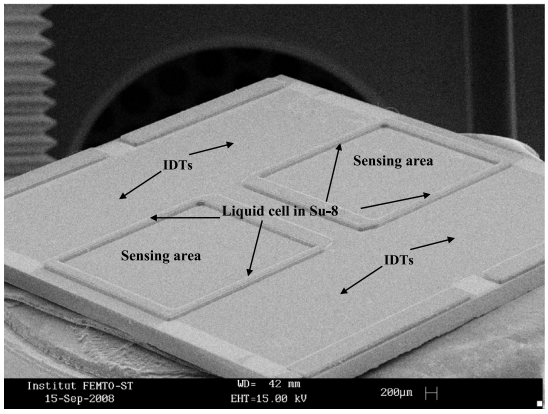

Figure 6 : SEM image of the SAW device coated with a patterned thick SU8

The resulting sensor is glued on a FR4 epoxy substrate with electrical connections being performed by conducting silver loaded epoxy between the bonding pads and the copper tracks on the circuit (see figure 7).

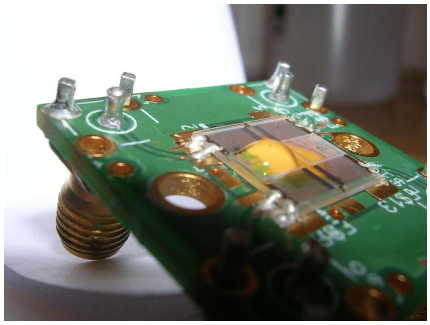

Figure 7 : SAW sensor equipped with its liquid cell

Figure 8 shows the typical response of our fluidic system with SU-8 walls. According to both figures 3 and 8, we note that equipping the sensor with the proposed fluidic system generates more insertion loss (8 dB more) compared with the initial acoustic response of our delay line. The final insertion loss actually is observed at 32 dB.

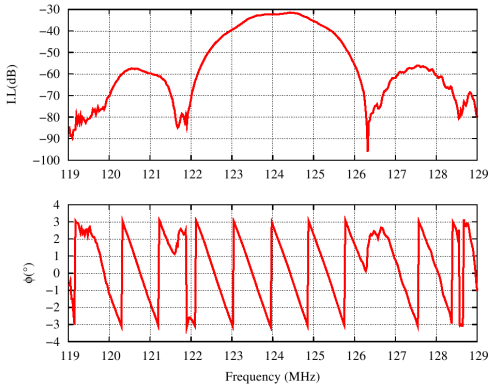

Figure 8 : Transfer function of SAW senser with liquid cell

IV-Chemical Functionalization and Detection Reactions

In order to check if the acoustic device can be used as chemical sensor, the functionalization of the sensing area has been achieved by an adequately design organic monolayer. The target analyte is commercially available core-shell nano-objects based on a gold nanoparticule (20nm) surrounded by strepavidins. Strepavidin coated gold nanoparticles have superior stability and biocompatibility. They are suitable for numerous biomedical applications. These particles have well controlled size with narrow size distribution. However, if nanomaterials/nanoparticles are finding popularity in many therapeutic and research applications due to their versatility, ease of synthesis, and unique properties, few studies are focused on their effect on human health and the environment [10]. Therefore, the detection of small amounts of these bionanoparticules are of interest.

The SAW sensor with its fluidic cell was treated under plasma-ozone for 30 minutes. This oxidation set-up induces the formation of hydroxyl functions atop the SiO_2 guiding layer. This step was followed by an overnight stay in 3-Aminopropyl-diethoxy-methylsilane (Aldrich) diluted in ethanol as shown in Figure 9.

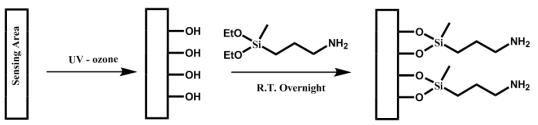

Figure 9 : Functionalization of SiO_2 sensing area by amino-ended

single layer

The main purpose of these operations is to ensure the formation of free reactive radicals atop the sensing area which can then be used for the grafting of new functionalities onto the sensor surface.Next, the sensors were rinsed with large amounts of ethanol and heated during 30 minutes at 80°C. After that, we have run the second step of the functionalization with Gluteraldehyde solution (Sigma-Aldrich) (see figure 10).

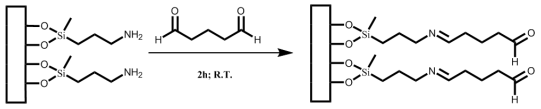

Figure 10 : Functionalization of the sensing area by a Gluteraldehyde solution

During this step, the sensing area is functionalized and the sensor response (IL and phase) is monitored for 2 hours (figure 11).

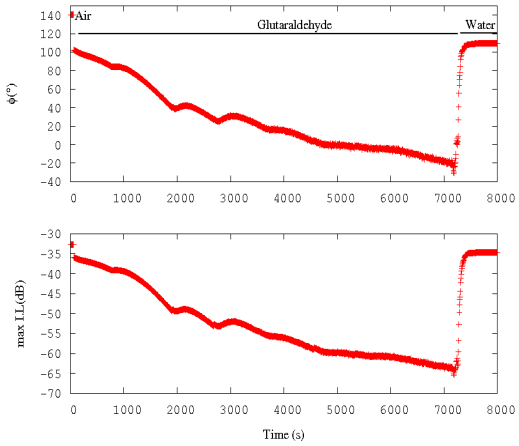

Figure 11 : Magnitude & phase measurements monitored during Gluteraldehyde functionlization

After these 2 hours of surface functionalization, the fluidic cell is rinsed with DI water several time to clean the sensing area and to define the initial baseline before running the adsorption of Streptavidin-gold (Sigma-Aldrich) (figure 13).

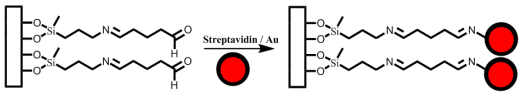

Figure 12 : Adsorption the streptavidin-gold on the sensing area

This step is the continuation of the stage of the functionalization of the sensing area with the Gluteraldhyde solution. The magnitude and the phase are again monitored to check the evolution of the surface grafting.

The sensors is first loaded with water. Once its response stabilized (3dB loss more due to water absorption [11]), corresponding to a stable baseline at about 34.7dB/109°, a droplet of streptavidin-gold with a concentration of 10% of the commercial solution (0.14g/l) is deposited on the sensing area of the SAW device (figure 13). The large shift of the phase and the insertion loss caused by the filing of a second droplet of the solution indicates the beginning of the formation of a streptavidin-gold film on the sensitive surface. Once the fluidic cell rinsed many times with DI water, a new baseline (34.8dB/107°) is observed for a DI water load. The phase and insertion loss between the initial and current baselines is associated with streptavidin-gold grafted onto the surface.

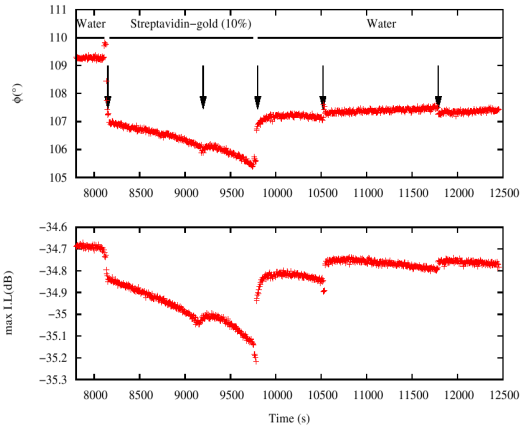

Figure 13 : Magnitude & phase measurements monitored during Streptavidin-gold adsorption

V- Results and Discussion

Four aqueous solutions with different concentrations (10, 5, 1 and 0.5%) of the commercial solution of Streptavidin-gold were analyzed by the fluidic system.

The sensors used to detect the different concentrations are prepared along the same steps of functionalization described above. Figure 14 shows the phase monitoring for the tested concentrations.

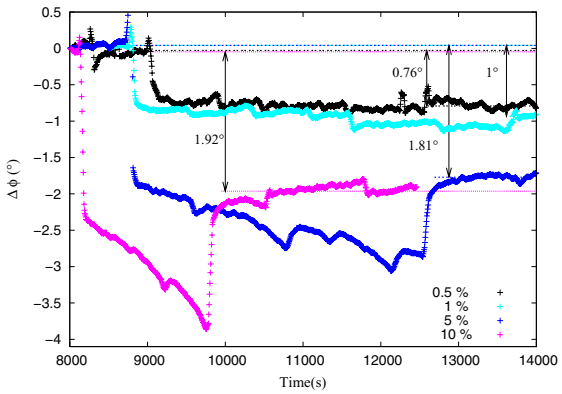

Figure 14 : Time resolved measurements of the phase shift of a 125 MHz Love mode acoustic wave sensor upon adsorption of different streptavidin-gold concentrations

Figure 15 shows the variation of the phase shift for the tested concentrations of the commercial solution of

streptavidin-gold. These curves show that surface saturation is nearly reached for the 5% concentration solution because the phase shift is identical for a 10 or 5 % solution.

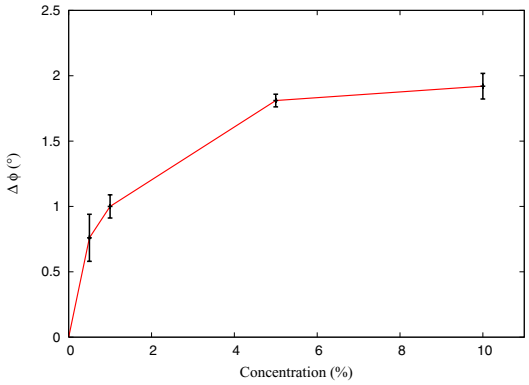

Figure 15: Variation of the phase shift for different concentrations of Streptavidin-gold solution

The detection limit of Love mode SAW sensors is in the 60 ng/cm^2 range, deduced from the intrinsic gravimetric sensitivity of the transducer [12] (250 cm^2/g), the phase-frequency slope dφ/df =3.7 10^{-4} °/Hz,which is consistent with the expected value of dφ/df = 360 .L/V=3.7 10^{-4} °/Hz where V= 5000 m.s^{-1} and L= 5.23 mm (the center-to-center distance between the interdigitated transducers).

The mass sensitivity S for acoustic wave sensors is defined as the incremental frequency change occurring in response to an incremental change in mass per unit area A on the surface of the device as follows (ne faut-il pas citer Sau:

$$S = \frac{\Delta f}{f_O} \cdot \frac{A}{\Delta m} (cm^2/g)$$

where Δm is the uniformly distributed mass per unit area added to the surface of the device, f_0 (125MHz) is the unperturbed resonance frequency of the device and Δf is the change in the operational frequency due to mass loading effects.

The variation of the phase shift corresponding to 0.5 and 10% of the concentration of the streptavidine-gold solution are respectively 0.76 and 1.92° (see figure 15) and the absorbed mass corresponding to these concentrations are respectively 64.8 and 166 ng.cm^{-1} .

VI- Conclusion

In this work, we have shown the possibility to graft streptavidin-gold molecules onto a Love-wave sensor surface in a controlled way, to demonstrate the capability of the sensor to detect nanoparticules in aqeous media.

We have reported the fabrication of a Love-wave-based sensor consisting in a delay line equipped with a fluidic cell fabricated using the SU-8 photoresist. The proposed fabrication approach allows for a wafer level batch packaging with optimized acoustic losses and exhibiting a liquid tightness compatible with long chemical reaction processes (4 hours or even more). The validation of the system operation has been proved by the detection of streptavidin-gold nanoparticles in aqueous solutions for different concentrations (between 0.5 and 10%).

References

[1] E. Gizeli, Biomolecular Sensors, E. Gizeli and C. Lowe, Eds. Taylor & Francis: London, 2002.

[2] T.M.A. Gronewold, “Surface acoustic wave sensors in the bioanalytical field: Recent trends and challenges”, Anal. Chim. Acta, vol. 603, no. 2, pp. 119-128, 2007.

[3] B. Jakoby and M. Vellekoop, “Viscosity sensing using a love-wave device” Sensors and Actuators A, vol. 68, pp. 275–281, 1998.

[4] K.Länge, G. Blaess, A. Voigt , R. Götzen , M. Rapp, “Integration of a surface acoustic wave biosensor in a microfluidic polymer chip” Biosensors and Bioelectronics, vol. 22, pp. 227–232, 2006.

[5] V. Raimbault, D. Rebière, C. Dejous, M. Guirardel, “ Acoustic love wave platform with PDMS microfluidic CHIP”, Sensors and A ctuators : A Physical, vol. 142, pp. 160-165, 2007.

[6] K. Mitsakakis, A. Tserepi, E. Gizeli, “Integration of microfluidics with a Love Wave Sensor for the fabrication of a multisample analytical microdevice “ journal of Microelectromechanical Systems, Vol. 17, pp. 1010-1019, 2008.

[7] E. Gizeli, M. Liley, C.R. Lowe, H. Vogel,”Antibody Binding to a Functionalized Supported Lipid Layer: A Direct Acoustic Immunosensor” Anal. Chem. vol. 69, pp. 4808-4813, 1997.

[8] T. Wessa, M. Rapp, H.J. Ache,”New immobilization method for SAW-biosensors: covalent attachment of antibodies via CNBr” Biosensors & Bioelectronics, vol. 14, pp. 93–98, 1999.

[9] T.M.A. Gronewold, U. Schlecht, E. Quandt “Analysis of proteolytic degradation of a crude protei mixture using a surface acoustic wave sensor” Biosensors and Bioelectronics vol. 22, pp. 2360–2365, 2007.

[10] B. rothen-rutishauser, R. N. Grass, F. blank, L. K. limbach, C. Mühlfeld, C. Abrandenberger, D . O. Raemy, P. gehr, W. j. Stark “ Direct Combination of Nanoparticle Fabrication and Exposure to Lung Cell Cultures in a Closed Setup as a Method To Simulate Accidental Nanoparticle Exposure of Humans” Environ. Sci. Technol., 2009, 43 (7), 2634-2640,

[11] L. El fissi, J.-M. Friedt, S. Ballandras ”Modeling the Rf Acoustic Behavior of Love-Wave Sensors Loaded with Organic Layers” Proceeding Ultrasonics Symposium, IEEE; 28-31 Oct. 2007 Page(s):484 - 487

[12] J.-M. Friedt,K.H.Choi, ; F. Frederix, A. Campitelli. J. Elect. Soc. 2003, *150*, H229-H234.