Love Wave Fully Integrated Lab-on-chip Platform for Food Pathogen Detection - LOVE-FOOD

(Contract No 317742 – Starting Date: 1 September 2012)

Deliverable 2.5

Report on biochips evaluation to multiple DNA and nanoparticles sensing

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DELIVERABLE SUMMARY SHEET

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Executive summary

Work performed within tasks 2.1 and 2.2 (work package 2) revealed the optimum combination of operating frequency/waveguide that could be selected for the final array chip. Evaluation of the sensitivity of the dual devices was based on mass and viscous loading measurements as illustrated in D2.1 and D2.2 which were used for the design and fabrication of the final biochip as described in D2.3.

Current report deals with the evaluation of the fabricated biochips to liquid sensing. Evaluation requires investigation of: a) signal quality differences of the 4 channels among different biochips, b) variation in liquid measurements among the different biochips, c) variation in liquid measurements among the sub-divisions of one sensor area when the flow cell is placed on top of the biochip.

The performed initial evaluation was based on measurements in air and in liquid with glycerol and protein solutions. It is also planned to complete the evaluation by performing measurements with amplified DNA and functionalized nanoparticles. The first batch of biochips had a short life-cycle (could be easily scratched and damaged) which caused a delay in the completion of the planned performance evaluation.

Main text

A. Signal quality of biochips in air

Acoustic signal in air for four different biochips each one consisted of four separate sensing areas operating at 155MHz (fig. 1) was initially recorded (fig. 2).

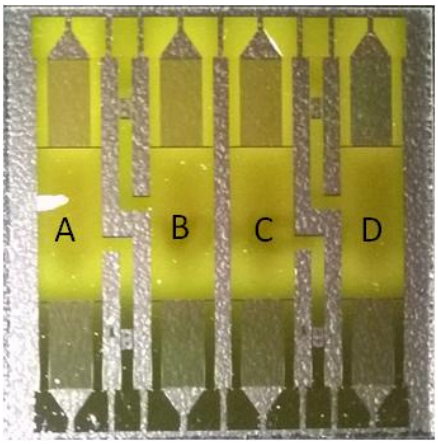

Figure 1: Acoustic biochip operating at 155MHz with four sensing areas (A-D)

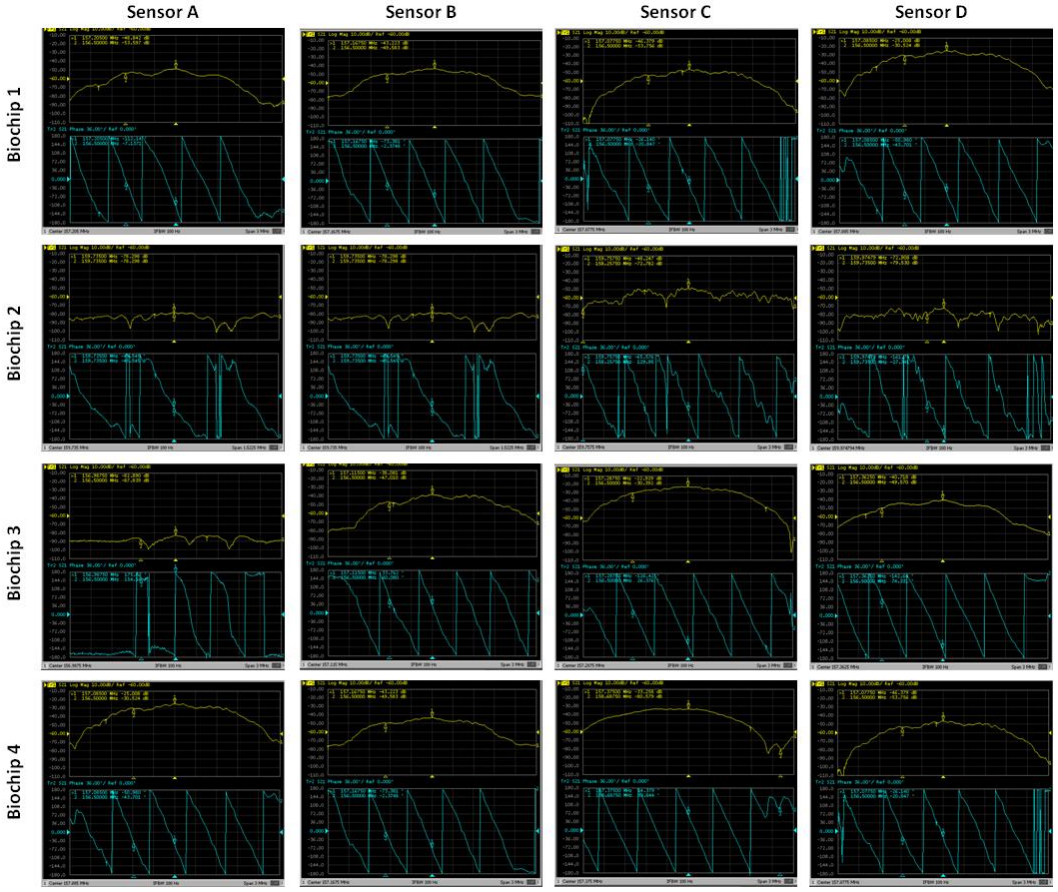

Figure 2: Recorded acoustic signal (amplitude and phase) in air. Working frequency 155 MHz, span 3 MHz.

As it can be seen, biochip 2 could not be used for measurements in liquid because the signal quality was too low. The same applies to sensor A of biochip 3 although in this case the remaining three sensing areas were functional.

B. Signal quality of biochips in liquid

We proceeded with measurements on three of these biochips (1, 3, 4) since the signal of biochip 2 did not allow further measurements. Signals were recorded a) in air, b) after the placement of the fluidic cell and c) after the addition of buffer. The evolution of signals upon the addition of the fluidic cell and buffer for biochips 1, 3 and 4 respectively are summarized in table 1.

	Amplitude (dB)				
	Sensor A	**Sensor B**	**Sensor C**	**Sensor D**	
Biochip1	-48,8	-43,2	-46,4	-25	air
			56,8		flowcell
			66,4		buffer
Biochip2	X	X	X	X	air
					flowcell
					buffer
Biochip3	X	-38,1	-22,9	-40,7	air
			-30,7		flowcell
			-32,7		buffer
Biochip4	-25	-43,2	-33,3	-46,4	air
	-29		-47,2		flowcell
			-49,9		buffer

Table1: Acoustic signal evolution measurements. Air – flowcell – buffer

Regarding the fluidic cell devoted to the biochip, the inlet and outlet pairs of tubing corresponding to four separate areas defined by the fluidic cell can be seen in fig. 3.

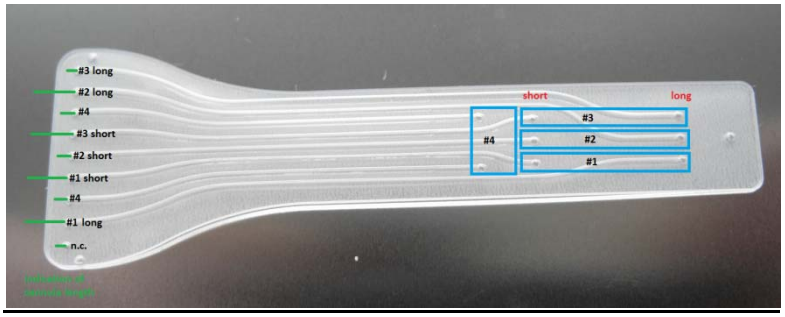

Figure 3: Plumbing layout; inlet –outlet pairs of tubing and corresponding microfluidic channels.

As previously mentioned, the biochip comprises 4 different sensor elements designated as A, B, C and D. Microfluidic channels 1, 2, 3 divide the surface of each one of the sensor elements A, B, C in 3 sub-areas, e.g. 1A, 2A, 3A.

Microfluidic channel 4 is dedicated entirely to sensor D. Thus, an array of 10 sub-areas is formed on which 10 distinct and independent experiments can be carried out.

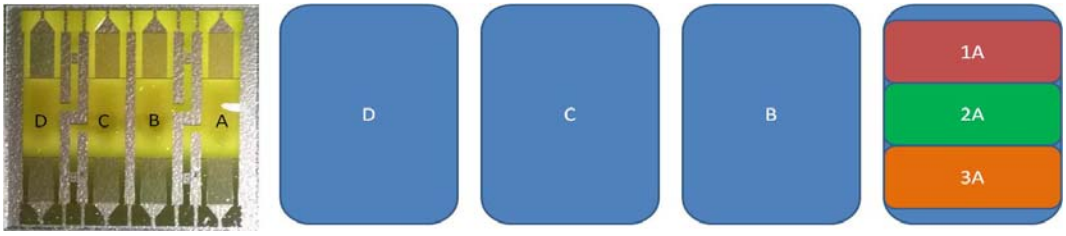

Figure 4: Biochip with 4 sensing elements marked as A, B, C, D. Microfluidic channels (1, 2, 3, 4) divide each one into three sub-areas apart from D which remains undivided.

Variation in measurements among different biochips

Physical adsorption of a protein on these three biochips was monitored by injecting 200 μg/mL neutravidin solution in the microfluidic channel 2 (fig. 3). PBS buffer was used in the protein solution and for the rinsing.

Area measured	Biochip 1, sensor C, channel 2	Biochip 3, sensor C, channel 2	Biochip 4, sensor C, channel 2
ΔPhase (deg)	5	6.3	8.2
ΔA (dB)	0.3	0.4	0.4

Table 2: Changes in phase and amplitude upon physical adsorption of neutravidin solution measured with three different biochips.

The variation in phase changes for protein adsorption is approximately 25% and for amplitude changes is calculated around 15%. Acoustic ratios though are less than 14%. Since protein adsorption on unmodified surfaces is not well controlled, it is important to repeat the inter-chip variation measurements with a more controlled procedure such as DNA immobilization through biotin-avidin interactions.

Variation among the sub-divisions of one sensor area

For the evaluation of the variation in the acoustic data across the three sub-areas of the same sensor element we have measured three aqueous glycerol solutions: 5, 12.5 and 25%. Such solutions do not absorb on the surface and are removed completely upon water rinsing. The solutions were injected in microchannels 1-3 (with water rinsing in between) and the response of sensor A was recorded (fig. 5). It was calculated that the variation in the acoustic measurements in both amplitude and phase changes was less than 10% which is a promising result.

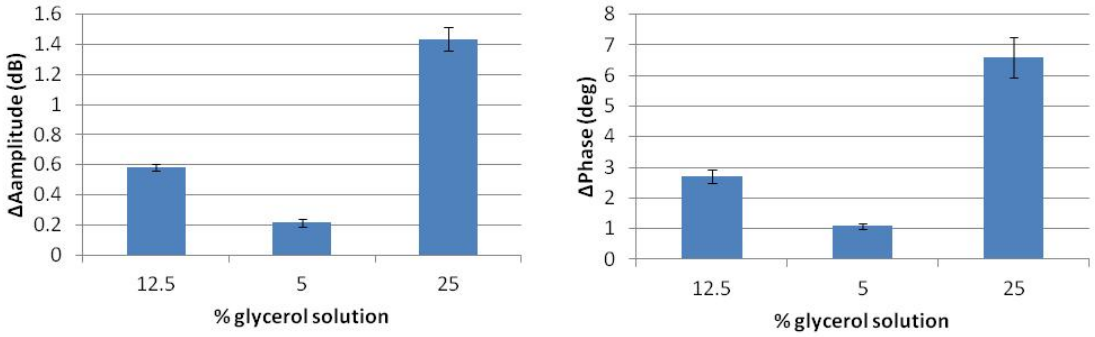

Figure 5: Variation of amplitude and phase among the different sub-areas of sensor A as formed by the microfluidic channels 1-3.

Sensor D was also tested by injecting the same solutions of glycerol in microchannel 4 (fig.6)

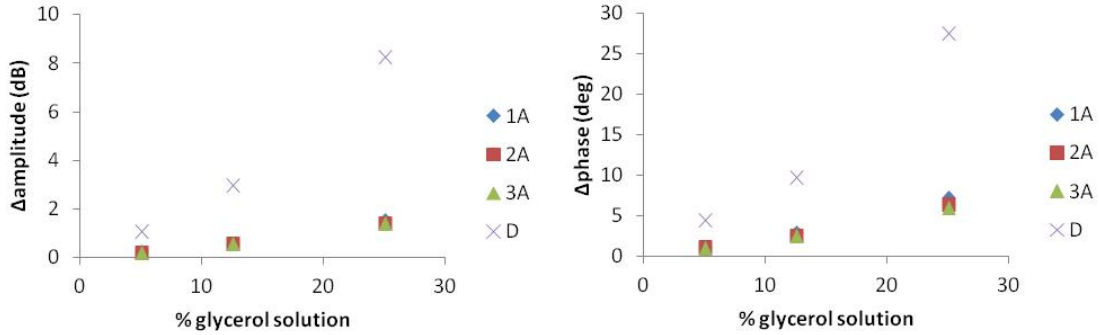

Figure 6: Change in amplitude and phase for sub-areas 1A, 2A, 3A of sensor A and sensor D upon the addition of glycerol solutions (5, 12.5 and 25%).

Sensor D resulted in approximately 5x more amplitude change and 4x more phase change compared to the measurements derived from the 3 areas of sensor A. This was not a surprise since the gaskets of the flow cell apply pressure at multiple parts on the sensing area which results in more than 3x drop in absolute phase and amplitude changes.

Conclusions

The performed initial evaluation was based on measurements in air and in liquid with glycerol and protein solutions. Inter-chip variation for protein adsorption ranges from 15 up to 25% while variation within sub-divisions of the same sensor area is less than 10% when glycerol solutions were used.

It is planned to complete a more thorough evaluation by performing measurements with amplified DNA and functionalized nanoparticles which are both closer to the operation conditions of the LOC platform. The delivery of this full report is scheduled for M30. Such a thorough investigation was impossible to have been completed at this point because the first batch of biochips had a short life-cycle. In fact it was very easy for the pads to get damaged even after only one or two measurements. Scratching of the pads was caused upon contact with the pins of the device holder resulting in acoustic signal loss. Senseor partner is aware of the issue and is going to prepare a new batch of biochips with the addition of gold on the pads to increase resistance to scratching.