

SMART ACOUSTIC SENSOR FOR THE DETECTION OF BACTERIA IN MILK

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ABSTRACT

A smart sensor has been designed that comprises a pair of shear horizontal (SH) surface acoustic wave (SAW) devices with one open delay line and one electrically shorted through a ground plane. The delay lines have been fabricated on a 36° Y-cut X-propagating lithium tantalate piezoelectric wafer using UV microlithography and have an operating frequency of *ca.* 60 MHz. The dual SH-SAW sensor employs no biological layer and so the detection principle is based simply upon its response to certain physical properties of the analyte, such as conductivity, permittivity, and visco-elastic coupling. An investigation has been carried out to see whether the sensor can detect the presence of bacteria in dairy milk. It was observed that the smart sensor was sensitive to concentrations of *S. aureus* and *S. uberis* in milk down to the level of *ca.* 100 cfu/ml. This type of acoustic biosensor could potentially offer a simple, low-cost and robust (repeatable) way to assay bacterial loads in complex liquids.

KEY WORDS

Biosensors and transducers, microtechnology and BioMEMS

1. Introduction

A novel dual SH-SAW sensor has been designed by Warwick University (UK) based upon previous work jointly with Pennsylvania State University (USA) [1]. The sensing device comprises of two SAW delay lines – one delay is open with the acoustic waves both electrically and mechanically coupled to the liquid whilst the other is shorted by a ground plane and so is only mechanically coupled to the liquid. Figure 1 shows a basic arrangement of the dual delay-line liquid sensing system.

The perturbation theory [2,3] shows that the changes in the velocity and attenuation of the waves in the presence of a liquid may be approximated by:

$$\frac{\Delta v}{v} \approx -\frac{K_s^2}{2} \frac{(\sigma' / \omega)^2 + (\varepsilon_0 \varepsilon_r' - \varepsilon_0 \varepsilon_r)(\varepsilon_r' \varepsilon_0 + \varepsilon_P^T)}{(\sigma' / \omega)^2 + (\varepsilon_r' \varepsilon_0 + \varepsilon_P^T)^2} \quad (1)$$

$$\frac{\Delta \alpha}{k} \approx \frac{K_s^2}{2} \frac{(\sigma' / \omega)(\varepsilon_r \varepsilon_0 + \varepsilon_P^T)}{(\sigma' / \omega)^2 + (\varepsilon_r' \varepsilon_0 + \varepsilon_P^T)^2} \quad (2)$$

where, K_s^2 is the electromechanical coupling coefficient when the unperturbed liquid is loaded on the free surface, ε_P^T is the effective permittivity of the SAW crystal, ε_r is the relative permittivity of the reference liquid (distilled water), ε_r' and σ' are the relative permittivity and conductivity (related to loss) of the measurand, respectively. From the above equations permittivity and conductivity of the liquid under test can be determined, while theory related to mechanical perturbation of SH-SAW on a metallized surface [3] shows that the changes in the velocity and attenuation on the shorted delay line can be related to viscosity and density of the liquid.

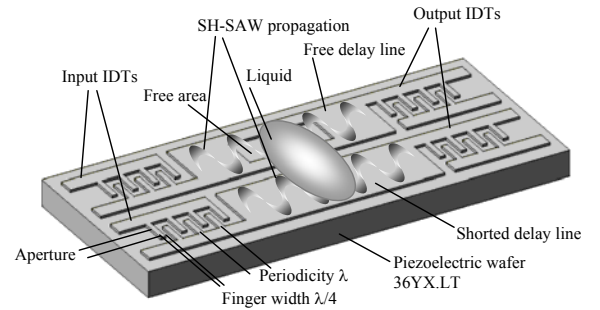


Fig. 1: Schematic of dual-delay line SH-SAW liquid sensor

Devices with gold input-output IDTs with a width of 17 μm and spacing of 34 μm were patterned on the surface of a 3" 36° Y-cut X-propagating lithium tantalate piezoelectric wafer (Figure 2a). A simple etching process was employed with the steps illustrated in Figure 3.

A PTFE micro-cell was machined and mounted above the SAW delay lines which were on a printed circuit board header. The general arrangement is shown in Figure 2b with the volume of liquid cell being a maximum of 100 μL .

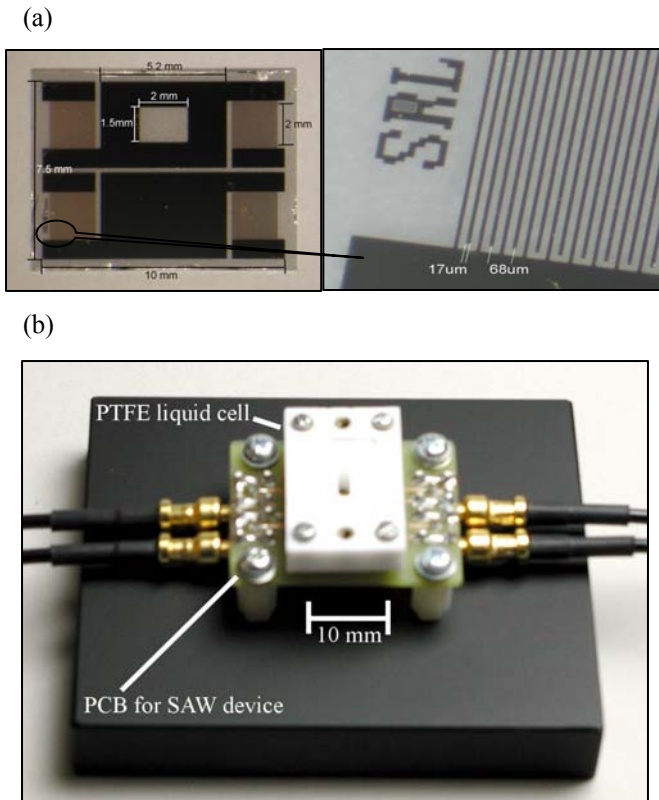


Fig. 2: Design of dual SH-SAW sensor for liquid analysis: (a) dual SH-SAW device and (b) sensing system.

The two input IDTs were driven at the same frequency of *ca.* 60 MHz whilst the attenuation and phase delays were measured at the output IDTs using a vector voltmeter (HP8505A). Previous work has reported on the use of this sensor to discriminate between first different liquids (e.g. orange juice, milk, water) and secondly the fat content of dairy milk [4].

Here we investigate the use of the dual SH-SAW sensor to determine the bacterial loading in milk. High levels of bacteria in milk are associated with mastitis and clearly form a health hazard to cows and an economic loss to farmers.

2. Preliminary experiments on milk

Replicate samples were taken from each of four different milk samples. The first two samples of milk were taken from a single cow at different stages of its oestrus cycle. One sample of fresh milk corresponding to a time when the progesterone level was high and another when it was with a low progesterone level, i.e. close to bulling. The second two samples were fresh milk loaded with either 10^5 or 10^7 colony forming units (cfu) per ml of *S. uberis*. Five replicate samples of each milk were taken and the total set randomly tested with the attenuations (~ 10 db) and phase delays logged by the dual SH-SAW sensor system.

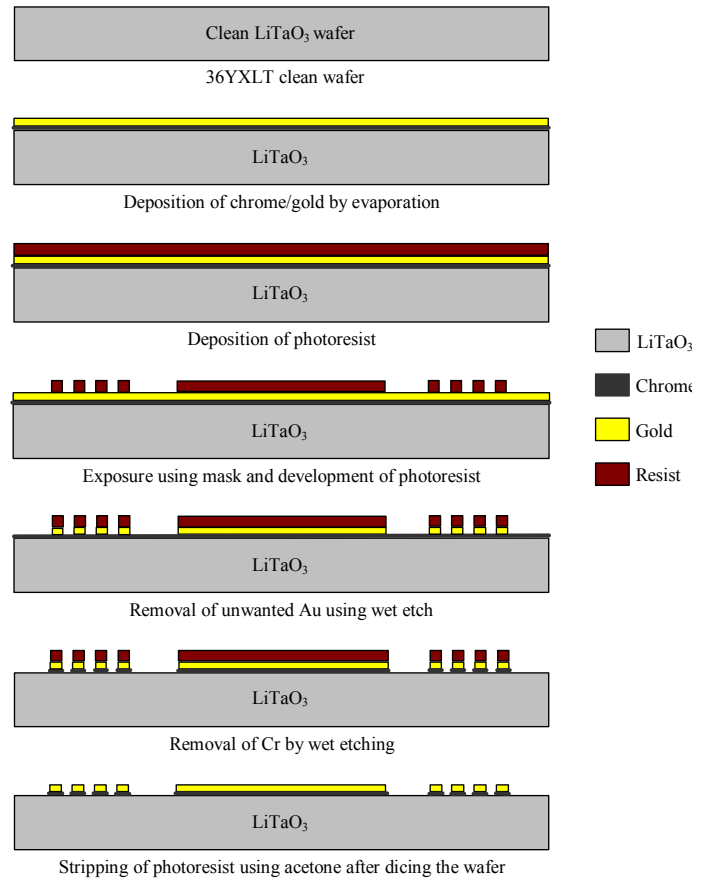


Fig. 3: Processing of lithium tantalate wafer to make SH-SAW sensing devices. For full details see [5].

The smart sensor signals were pre-processed and then vector decomposed using principal components analysis (PCA). Figure 4 shows a 3-D principal components plot of the set of acoustic signals for each milk with the fresh and bulling milk samples repeated twice. The results indicate that the milk samples loaded with *S. uberis* can be linearly discriminated from fresh milk samples as seen by the distinct clusters for both low and high loads.

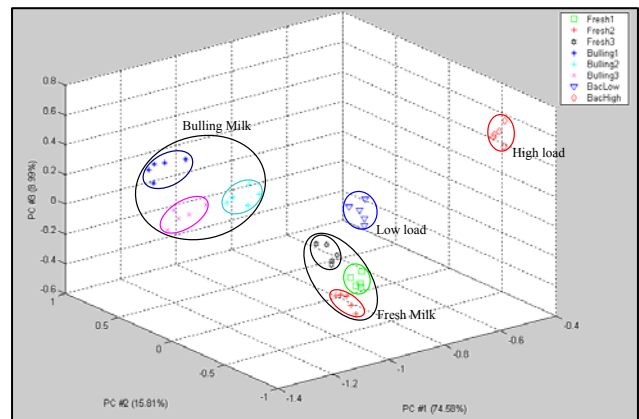


Fig. 4: PCA plot of fresh, bulling and milks loaded with *S. uberis* bacteria (kept in frozen storage).

The milk samples studied had been stored at a constant temperature of $-80\text{ }^{\circ}\text{C}$ and so some concern existed that the freezing process could affect the physical properties of the milk. Accordingly the experiment was repeated with both unfrozen and frozen milk samples. The results are shown in Figure 5 and indicate that the freezing process did not significantly affect the PCA results with loaded milks fresh and frozen appearing close to each other again in distinct clusters.

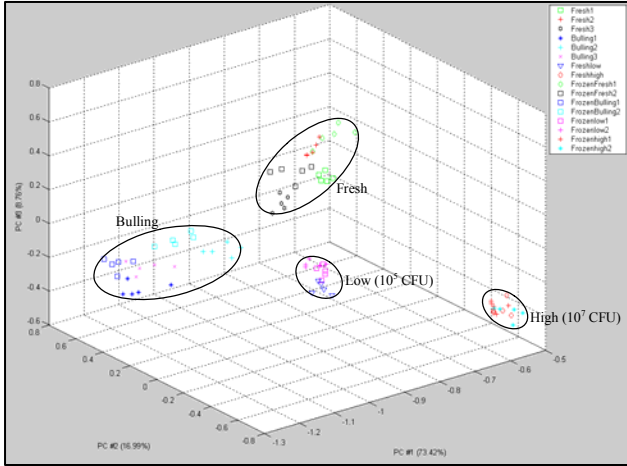


Fig. 5: PCA plot of fresh, bulling and milks loaded with *S. uberis* bacteria. Frozen and non-frozen samples lie within the class as denoted by the ellipses).

3. Sensitivity tests on bacterial loading

The limit of detection was then determined by creating a dilution series of stocks of *S. uberis* 10 001 strain and *S. aureus* 072 strain. These were used to inoculate fresh milk and samples taken every hour from 2 h to 9 h. A negative control of phosphate buffered saline (PBS) was added in which the dilution series were made. The total set of samples and approximate cell concentrations are given in Table 1 below:

Table 1. Approx. cell concentrations for the dilution tests on milk loading.

Time (h)	<i>S. aureus</i> (cfu/ml)	<i>S. uberis</i> (cfu/ml)
2	5	2
3	40	8
4	80	53
5	800	350
6	4.85×10^3	3.7×10^4
7	4.7×10^4	7×10^4
8	2.27×10^5	3.73×10^5
9	2×10^6	3.03×10^6

Figures 6 and 7 show a PCA plot of the effect of the bacterial loading of 10^2 to 10^7 cfu/ml of *S. uberis* and *S. aureus* in milk, respectively. In both cases the lowest two loadings overlap while all the other concentrations are distinct.

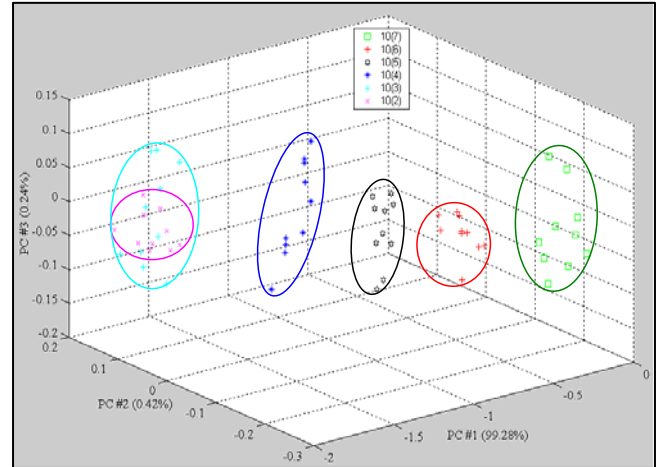


Fig. 6: PCA plot of different levels of *S. uberis* bacteria in milk samples. Only samples taken at times 2 h and 3 h (see Table 1) overlap.

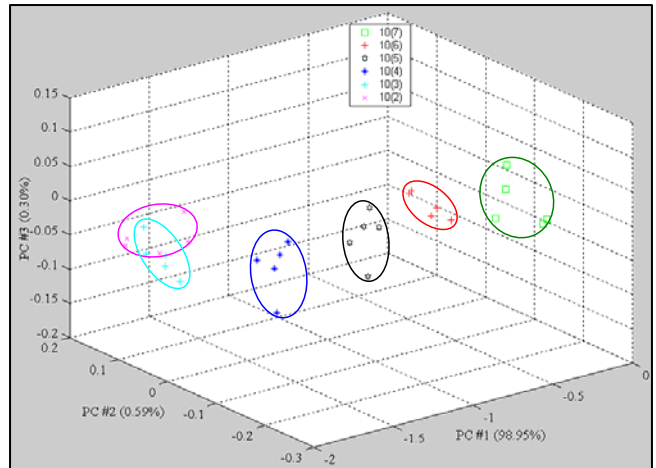


Fig. 7: PCA plot of different levels of *S. aureus* bacteria in milk samples. Only samples 2 and 3 (see Table 1) overlap.

4. Experiments on mastitic milk samples

Finally, nine mastitic milk samples were tested using the smart dual SH-SAW sensor system. The samples were now taken from nine different cows in which three samples contained coagulase-negative *staphylococci*, five contained *S. aureus* and one contained both *S. aureus* and *streptococci*. Three blank samples were also used; these were taken from two other cows that come from a

different herd to the mastitic samples and finally one from a bulk milk tank, i.e. mixture of milk from many cows.

Figure 8 shows the results for these different cows. The PCA plot shows considerable spread of the results that cannot be attributed simply to the bacterial loading of the milk. The difference between the individual cow's milk and that taken from the bulk tank suggests that there is significant variation from cow to cow in the acoustic properties of milk. This is not an unexpected result because the levels of, for example, calcium, fat, lactose, are known to vary.

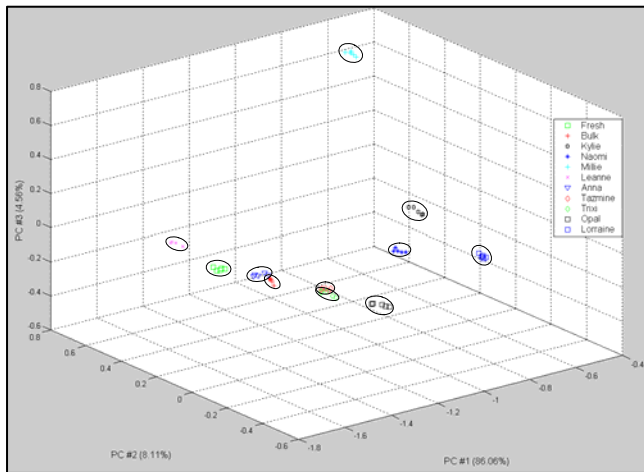


Fig. 8: PCA plot of milk from nine mastitic cows, fresh milk and bulk milk showing considerable cow-to-cow variation in acoustic properties.

5. Conclusion

A novel smart dual SH-SAW sensor system has been developed and used to study the effect of bacterial loading on milk taken from dairy cows.

Our preliminary studies suggest that an acoustic method is able to detect the presence of bacteria in milk down to a concentration of *ca.* 100 cfu/ml.

We postulate that the bacteria are altering the cellular structure of the milk and that in turn results in both an increased level of calcium ions and a lower viscosity. Both of these effects would change the acoustic coupling of the SAW with the milk samples and thus affect the attenuation and phase delay. Interestingly, the conductivity and pH of the milks was measured using commercial sensors and these parameters alone could not discriminate between the different milk samples [5].

Considerable variation in the acoustic properties of milk taken from different cows has been observed. Thus any practical implementation of the biosensor will require the

continual sampling of the milk taken from each cow. A possible methodology would be to take the milk from a healthy cow and use this as a baseline or reference signal. Then the measured deviation from the norm can be attributed to some other factor, such as a fall in progesterone level or an increase in bacterial concentration. Clearly, bioassays would be needed to calibrate and thus validate the sensing system.

We believe that the acoustic sampling of liquids is an attractive methodology and the dual sensor approach allows the rejection of some common interference, such as temperature sensitivity [5]. It has been shown elsewhere [6] that the smart sensor system can be used to discriminate between the four basic tastes of sweet, sour, bitter and salty. Thus its applicability could be much wider than demonstrated here.

6. Acknowledgements

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