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## **Development of a combined surface plasmon resonance / surface acoustic wave (spr/saw) device for characterization of biomolecules**

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### **Abstract**

It is known that acoustic sensor devices, if operated in liquid phase, are sensitive not just to the mass of the analyte but to various other parameters, such as size, shape, charge and elastic constants of the analyte as well as bound and viscously entrained water. This can be used to extract valuable information about a biomolecule, particularly if the acoustic device is combined with another sensor element which is sensitive to the mass or amount of analyte only. The latter is true in good approximation for various optical sensor techniques. This work reports on the development of a combined surface plasmon resonance / surface acoustic wave sensor system which is designed for the investigation of biomolecules such as proteins or DNA. Results for deposition of neutravidin and DNA are reported.

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## Introduction

In the field of biosensing both optical and acoustic sensors have been widely used in the past. Optical sensors are sensitive to, e.g., the refractive index of the analyte which has been shown to be linearly related to the deposited mass [1]–[4]. Despite the existence of some reports [5]–[7], currently available optical technology does not generally allow for the detection of the conformation of an analyte and the way it is bound to a surface. Therefore, optical techniques cannot supply much information about the analyte beyond its mere presence. On the other hand, mass is detected reliably and the amount of analyte can easily be quantified.

In contrast, acoustic sensors are sensitive not just to the presence of the analyte but to other physical parameters, such as size, shape, charge and elastic constants of the analyte as well as bound and viscously entrained water [8]–[12]. Consequently, valuable information can potentially be extracted from a measurement using this type of sensor; however, because of the multiple parameters influencing the measurement, a single output signal often cannot be unambiguously interpreted.

Different strategies exist to circumvent this problem. One approach is to use two acoustic parameters, such as acoustic frequency and dissipation, which will respond in different ways to different analytes. Provided a theory exists to describe the dependence of these output parameters on the relevant physical characteristics of the experiment, conclusions about the latter can be drawn [11], [13]. However, since both acoustic frequency and energy dissipation are non-linearly related to some physical characteristics such as viscosity, density and conformation of the bound analyte, acoustic measurements still do not provide a direct means to exactly quantify the bound mass the way optical sensors do.

Therefore, many attempts have been made to combine optical and acoustic sensors into a single set-up. Rishpon *et al.* used a combination of ellipsometric, acoustic, and electrochemical detection on a single surface to study the growth of a polyaniline film [14]. Kößlinger *et al.* combined a QCM (quartz crystal microbalance) with SPR (surface plasmon resonance); the latter is a well established optical technique for detection of biomolecules in solution. With this set-up they were able to detect the binding of bovine serum albumin and antibodies to the sensor surface [15]. However, their system used two different sensor chips for acoustic and optical detection; in this case, differences in the fabrication and preparation of the two sensor surfaces could lead to discrepancies in the results.

Laschitsch *et al.* used a QCM with surface corrugation which served for optical coupling of a light beam in a SPR grating configuration set-up. This approach was used to follow streptavidin binding [16]. While their system permits optical and acoustic detection on a single surface, the surface corrugation will influence the signal measured by the acoustic sensor [8], [17]. Remarkably, the value

for the streptavidin layer thickness measured by the acoustic device was lower than expected, a result which could not be unambiguously explained [16].

Thus, another approach was taken by combining a SH-SAW (shear horizontal surface acoustic wave) sensor with SPR in the conventional Kretschmann configuration [18]. This configuration permits the use of a single smooth surface for simultaneous optical and acoustic detection [19]. The combination with another experimental technique such as electrochemical detection is easily possible. This set-up was used to detect electrodeposition of copper and deposition of S-layer proteins [19].

Various alternative approaches have been proposed. Manaka *et al.* have combined a QCM with a simple and rugged optical fiber set-up to provide a compact, low-cost system for biosensing [3]. Wang *et al.* have combined QCM-D (QCM with dissipation monitoring) with a modified reflectometry set-up [4]; this system was used to study lipid, protein and peptide binding by evaluating acoustic frequency, dissipation, and the reflectometer response [20]. These investigations have demonstrated that quantitative extraction of physical parameters other than the amount of analyte is indeed possible with suitable combinations of acoustic and optical sensor elements.

In this work the design of such a set-up for biosensing applications is discussed. There is a particular need for the combined acoustic / optical approach in biosensing for various reasons:

- Many biochemical reactions have to be conducted in liquid phase.
- Water will be coupled to most biomolecules in the form of a rigidly attached hydration shell and / or in the form of viscous drag [2], [10], [21].
- As a result of the latter, acoustic sensors are sensitive to size and shape of the target molecule [10], [11], [22]. This is important since the conformation and shape of a biomolecule can change as a result of a biochemical reaction or a change in environmental conditions [6], [10], [11].

The system developed for this work is based on a commercially available SPR system. Neutravidin and various double-strand DNA molecules have been used to measure the performance of the set-up. Questions addressed include whether or not an acoustic waveguide should be used, and whether acoustic dissipation should be exploited as a third signal parameter.

## Materials and Methods

### *Chemicals and biochemicals*

Three biotinylated ds (double strand) DNA molecules of different chain lengths were used in this work. Two of them, 132 and 198 bp (base pairs), respectively, were prepared from pBR322 plasmid using PCR (polymerase chain reaction) as described previously [11]. A straight 90 bp dsDNA molecule was also used and its nucleotide sequence is given in [11]. Neutravidin was obtained from Pierce. Tris buffer was prepared by dissolving 50 mM tris-(hydroxymethyl) amino methane pH 7.5 (Biomol, “pure” grade), 10 mM MgCl<sub>2</sub> (Sigma, anhydrous), and 10 mM KCl (Riedel-de-Haën, p.a.) in deionized and filtered (Barnstead Nanopure) water; the buffer was autoclaved before use. Poly(methyl methacrylate) (PMMA) powder (Aldrich, medium molecular weight) was dissolved in 2-ethoxyethyl acetate (Aldrich, >99%).

### *Apparatus*

The SPR instrument used for this work was a Reichert SR 7000 refractometer. A new flow cell was designed in-house and fabricated by Reichert Analytical Instruments. It exposes a slightly larger surface area (5.6 mm × 4.4 mm) to the liquid than the companies standard flow cell and accommodates the electrical contacts needed for operation of the acoustic device. A network analyzer (Hewlett Packard 8753ES) was used to generate the acoustic wave and to detect frequency and dissipation; the time gating function of the instrument was activated. Measurements were conducted under flow conditions using a peristaltic pump (Gilson Minipuls 3) and a flow rate of about 50 µl/min.

### *Sensor devices*

The SPR/SH-SAW sensor devices were designed in-house and fabricated at Nottingham Trent University. The acoustic transducers (IDTs) and the metal surface needed for reflection of the SPR light beam were produced in a single step by depositing 10 nm Ti and 50 nm Au on a substrate of 0.5 mm thick 42.5°-rotated Y-cut, 90° X-propagation quartz. The sensor employs a single delay line design with a wavelength of 48 µm, corresponding to a frequency of operation of 106 MHz. Each IDT contains 41 pairs of split fingers. PMMA (about 1 µm thick) was spin-coated onto the IDTs only, followed by heating to 195°C for 2 hours. A schematic of the sensor design is shown in figure 1.

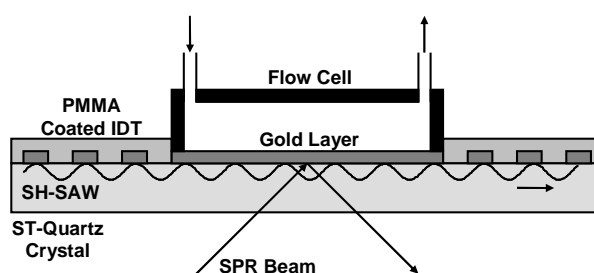


Figure 1: Cross-sectional view of the SPR/SH-SAW sensor device with flow cell (not drawn to scale).

## Methods

Immediately prior to the start of each experiment the sensor chip was subjected to plasma cleaning for 1 min (Harrick Plasma Cleaner PDC-002, “HI” setting). The entire experiment was conducted in tris buffer. At the beginning of the measurement the sensor was kept under buffer flow until a stable baseline was recorded. Then the flow system was switched to neutravidin solution (1.7  $\mu\text{M}$  in tris buffer). The neutravidin was allowed to deposit onto the gold surface, followed by buffer flow. Next, biotinylated dsDNA strands were added in various concentrations as stated below, followed again by buffer flow. After each experiment the device was rinsed with detergent. All experiments were carried out at room temperature (25°C).

## Results and Discussion

Quartz was chosen as the substrate material for the sensor chip since its refractive index is within the range required by the SPR instrument. Figure 2a shows the acoustic transmission spectrum for the ST-quartz sensor chip. In the experiments described below no waveguide was deposited in the area between the IDTs in order to avoid interference with SPR operation (see figure 1). However, this leads to a high insertion loss of the acoustic signal. This detrimental effect has been minimized by depositing a waveguide material (PMMA) only on the IDTs where it does not interfere with SPR operation [23]. Figure 2a shows that a total insertion loss of 32 dB can be achieved in air. In liquid environment this value increases by 1.5 to 2 dB. Thus, the resulting signal level is sufficient for network analyzer operation.

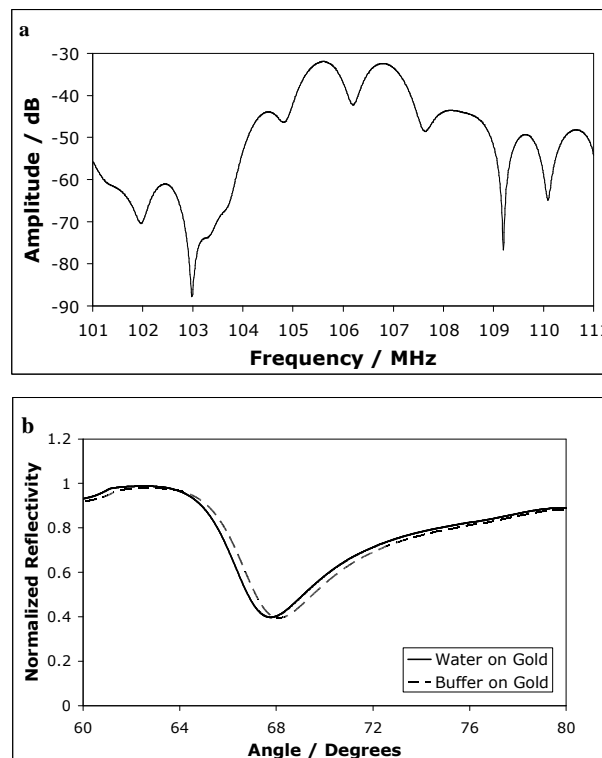


Figure 2: Acoustic transmission spectrum for the ST-quartz sensor chip in air, given for a frequency band around the SH-SAW frequency of 106 MHz (a). Normalized SPR angle scans for the same chip in water and tris buffer (b).

Because of the birefringence of quartz a wide angle scan of the SPR instrument shows an interference pattern which depends on the orientation of the quartz substrate with respect to the plane defined by the incident and reflected SPR beam [19]. This distortion is minimized if the orientation of the sensor chip is chosen such that the SH-SAW is propagating parallel to the above plane. Figure 2b shows the SPR angle scans for water and tris buffer, normalized to air, in the relevant range of angles. The minimum in the scans is due to coupling of the SPR beam to the surface plasmon; it is the position of this minimum which is tracked in order to monitor deposition of analyte molecules onto the surface. In this range of angles no distortion due to birefringence is observed. The remaining interference pattern is restricted to higher angles and, therefore, does not interfere with SPR operation. Even misalignment of the sensor chip by one or two degrees can easily be tolerated.

Figure 3 shows typical experimental data. The acoustic frequency response is shown in figure 3a, and the simultaneously measured SPR response in  $\mu\text{RIU}$  (micro refractive index units) is given in figure 3b. In this case the DNA was added in two successive steps. Concentrations of 1.46 and 3.1  $\mu\text{g/ml}$  of 132 bp dsDNA, respectively, were used. Binding of the biomolecules is reflected in each of the two signals shown, but the two signals respond in different ways to the analytes. This can be seen when comparing the ratio of the responses of DNA to neutravidin.

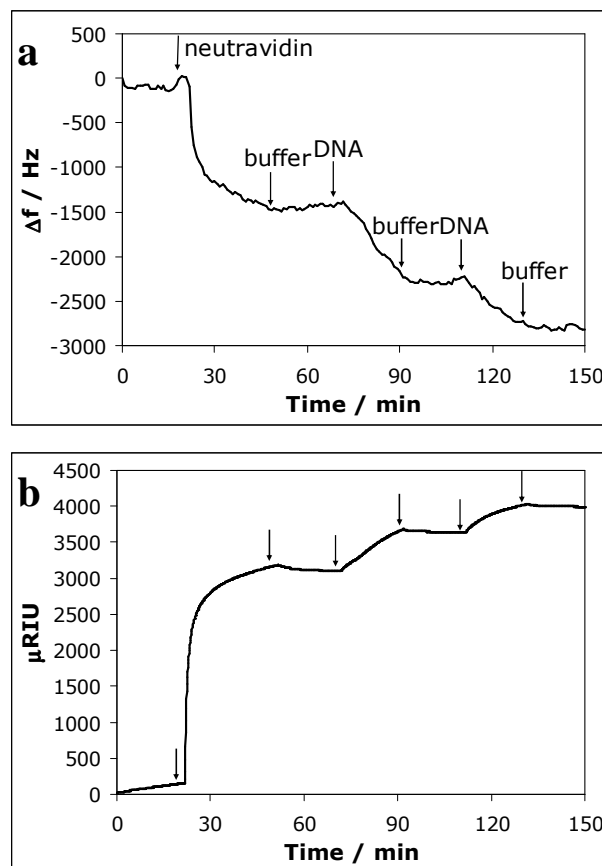


Figure 3: Deposition of neutravidin and binding of 132 bp dsDNA on a SPR/SH-SAW sensor chip at the times indicated in the graph. The frequency response of the SH-SAW (a) and the SPR response (b) are shown for the same experiment.

The latter becomes more evident when plotting the frequency response versus the SPR response, as demonstrated in figure 4. In this experiment, neutravidin deposition was followed by binding of 90 bp dsDNA (1.07  $\mu\text{g/ml}$ ). Data points are fitted with linear curves, the slopes of which are given in the graph. Based on the assumption that the SPR signal is proportional to the mass of the analyte, the slopes in figure 4 are proportional to the ratio of total vibrating mass, including hydration shell and viscously entrained water, to the mass of the analyte only. Figure 4 indicates that the ratio of vibrating mass over analyte mass for dsDNA is a factor of 2.87 higher than for neutravidin. This is in good agreement with the factor of 2.7 obtained in [10] for a QCM by comparing the vibrating mass in liquid environment with the dry mass of the analyte. In order to obtain an error estimate the experiment was repeated many times with DNA samples of two different sizes (132 bp and 198 bp, see below).

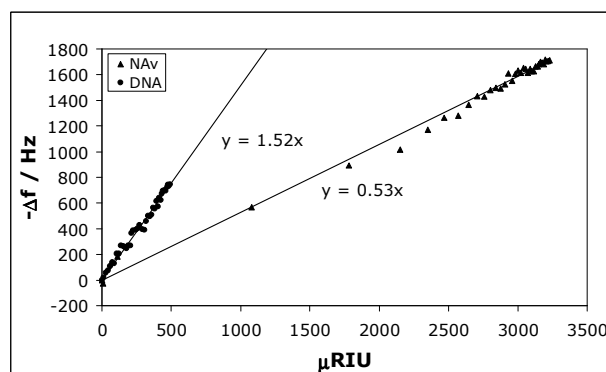


Figure 4: Ratio of the (negative) acoustic frequency to SPR signal for deposition of neutravidin (NAv) and successive binding of 90 bp dsDNA. The data points are shown as symbols; the lines are linear fits.

Note that for dsDNA the data points in figure 4 fall on a straight line. This indicates that the amount of vibrated mass per molecule does not depend on surface coverage, in agreement with [11] and [22]. The data points for neutravidin also fall on a straight line, although a small curvature appears in the beginning of the deposition, conceivably indicating structural rearrangements.

Fig. 5 shows the acoustic and SPR responses for various concentrations of 132 bp dsDNA. Note that the neutravidin-biotin binding is irreversible. According to Fig. 5, different DNA concentrations will therefore result in different slopes (kinetics) but approach the same plateau value if left long enough. This behavior is equally reflected in the acoustic and SPR signals.



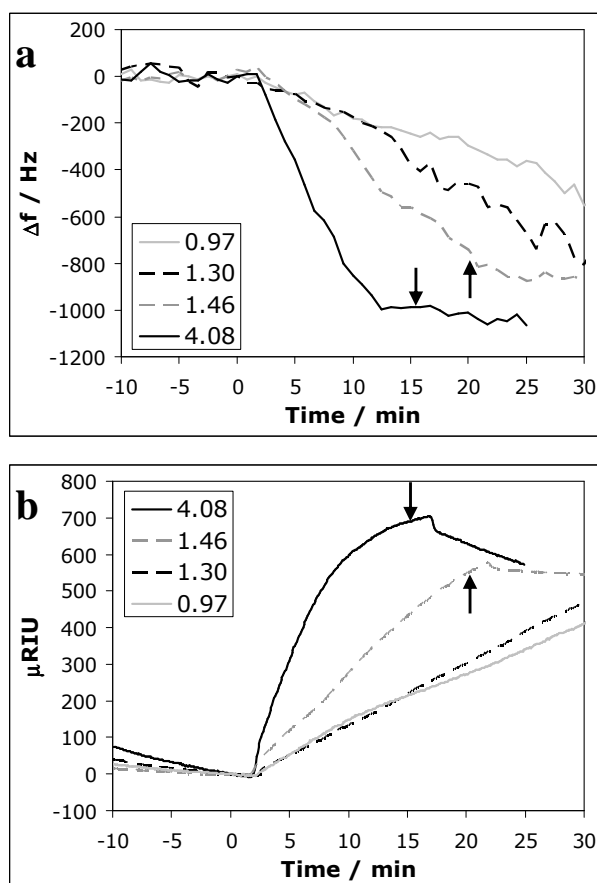


Figure 5: Acoustic frequency response (a) and SPR response (b) for binding of 132 bp dsDNA at the indicated concentrations (in  $\mu\text{g/ml}$ ) to neutravidin (neutravidin deposition is not shown). The flow system was switched to DNA at time 0 min. Rinsing with buffer is indicated by arrows for the higher concentrations.

If the reaction is aborted in order to obtain various DNA surface coverages, the ratio of acoustic frequency response to SPR response can be plotted as a function of surface coverage. This was done in figure 6. Within the scatter of the data points there is no significant deviation from a linear fit, again indicating that the amount of vibrated mass per molecule does not depend on surface coverage. The slope of the resulting linear fit is  $1.38 \text{ Hz}/\mu\text{RIU}$ , with an error of  $\pm 0.10 \text{ Hz}/\mu\text{RIU}$  (standard deviation /  $\sqrt{n}$  for  $n=6$  data points). For neutravidin a value of  $0.50 (\pm 0.01, n=7) \text{ Hz}/\mu\text{RIU}$  was obtained. This results in a value of  $2.75 (\pm 0.21)$  for the ratio between 132 bp dsDNA and neutravidin. For 198 bp DNA a similar ratio of  $2.64 (\pm 0.14, n=7)$  was obtained (results not shown). Both ratios are in excellent agreement with the factor of 2.7 obtained in [10].

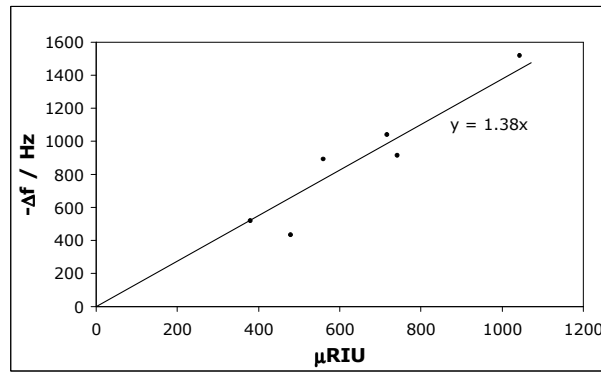


Figure 6: Ratio of the (negative) acoustic frequency to SPR signal for various surface coverages of 132 bp dsDNA. Filled circles indicate the final plateau value for each individual experiment. The curve is a linear fit with slope indicated in the graph.

## Conclusions

In this work a combined SPR/SH-SAW sensor design has been presented which permits simultaneous optical and acoustic detection of biomolecules on a single surface. A smooth gold surface is used and no waveguide is deposited between the IDTs in order to ensure a reliable, repeatable response which is easy to interpret.

The results obtained support the conclusion that the optical response reflects the mass of the analyte only while the acoustic response is more complex reflecting the involvement of hydrodynamics at the interface. While the distinction between analyte mass and total vibrating mass can be made using a single acoustic device by following a wetting / drying procedure [10], the device presented here is designed to perform this task in real-time as the layer of biomolecules is deposited. This is useful if the conformation of a biomolecule is changing in the course of an experiment. It was demonstrated that this goal can be achieved without the need for a waveguide in the sensing area which is in contact with the liquid, and without evaluating the acoustic dissipation. It was found that deposition of a waveguide only on the IDTs (which are located outside the flow cell) is a useful means to reduce the insertion loss of the device to an acceptable level.

However, while the system presented here can distinguish between the optical and acoustic masses of the analyte, it has been stated before that the ratio of acoustic dissipation to acoustic frequency contains additional information about the size and shape of a biomolecule [11], [22]. For the current set-up the signal-to-noise ratio for the acoustic dissipation is low, resulting in a significant change in dissipation only for DNA but not for neutravidin (results not shown). Therefore, it is desirable to modify the current design in order to increase the acoustic sensitivity. This can be done by increasing the acoustic frequency and / or by applying a waveguide not only on the IDTs but on the entire delay line of the device [24], [25]. These design modifications will also permit the use of an electronic set-up which is more compact and more affordable than the network analyzer used for this

study. Since the penetration depth of the SPR (~200 nm) is smaller than effective waveguide thicknesses ( $> 1 \mu\text{m}$ ), the SPR beam will have to be reflected not on the substrate / waveguide interface but on the waveguide / liquid interface. Consequently, the waveguide must have a refractive index near that of quartz, and the gold layer has to be deposited on top of the waveguide. This is technically feasible, and experiments with different waveguide materials are currently being carried out. Such a design modification will extend the range of applications accessible to the SPR/SH-SAW device and further increase the amount of information supplied by the system.

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