# WAVEGUIDE SURFACE PLASMON RESONANCE BIOSENSOR FOR THE AQUEOUS ENVIRONMENT

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We report the fabrication and performance of gold coated waveguide surface plasmon resonance biosensors. Biotin-avidin binding reactions at the sensor surface were observed. The output power of the sensor showed a decrease of 32 % on binding a dual layer of biotin-avidin.

## 1. Introduction

The use of guided-wave optical biosensors, and their market possibilities, is well documented[1]. Many optical transducer mechanisms are possible and they are currently generating a great deal of excitement as the potential of such devices is realised in the laboratory and, increasingly, commercially. Optical biosensors are generally small, light, and rugged, offering the portability required for a field monitoring system. Integrated optical sensors maybe connected by optical fibres and allow for the fabrication of multiple sensors in a single substrate using photolithographic techniques.

The exploitation of a surface plasmon resonance (SPR) to form the basic transduction mechanism of a chemical sensor has been known for some years[2]. A surface plasmon is usually a transverse magnetic (TM) electromagnetic mode guided by the interface between two media whose dielectric constants have real parts of opposite sign. For visible light this requirement can be fulfilled by a dielectric and a metal. The formation of a surface plasmon can be achieved by using a 'bulk' optical component such as a prism, and equipment employing this approach is now commercially available[3]. Another option is to employ the distributed coupling between a planar waveguide and a surface plasmon in a metal-coated waveguide. The use of SPR transduction in an optical sensor allows for the use of the metal film as an electrode for electrochemical control of sensing reactions.

In this paper we demonstrate the use of a simple waveguide SPR sensor to measure the binding of several layers of a biotin-avidin system onto the surface of the sensor. This device represents a portable sensor realisation of the otherwise laboratory-based 'bulk' SPR technique.

## 2. Waveguide SPR Sensor Design and Operation

The general configuration of the sensor whose operation can be described in terms of coupled modes is detailed in figure 1. A single TM mode is excited in the waveguide and this mode then couples to the surface plasmon mode, guided by the interface between the metal layer and the dielectric superstrate, if the two modes are closely phase matched. If a thin dielectric film is adsorbed to the metal surface then the effective index of the surface plasmon mode will be altered, changing the coupling condition between the waveguide and surface plasmon modes. This change can then be monitored, for example, as a function of wavelength or a change in the output intensity of the sensor.

A rigorous model has been used to evaluate the performance of the type of sensor shown in figure 1 when monitoring the adsorption of a thin dielectric film to the surface of the metal layer. A typical SPR curve is given in figure 2, for a waveguide SPR sensor based on a low refractive index glass substrate, a potassium ion-exchanged waveguide, and a thin gold film. The figure shows that the SPR is located at an analyte index close to that of water, indicating that it is not strictly necessary to employ a buffer layer in the sensor design to enable operation in an aqueous

environment. However extensive design work[4], has revealed that the use of a buffer layer in the sensor design should lead to the greater sensitivity for the device in an aqueous environment. Modelling also shows that the transverse electric (TE) polarisation is not significantly affected by the adsorption of thin films and may be used as a reference.

## 3. Experimental Measurement of Biotin-Avidin Binding Reactions

The waveguides were fabricated by potassium ion-exchange in low index glass (n=1.471) substrates at a temperature of 385 °C for 18 hours. A thin gold film was deposited on top of the waveguides by thermal evaporation. The length of the gold film varied from 1.5 mm to 5.4 mm, in 100 μm steps, over a total of 40 waveguides each with a nominal width of 2 μm. Two samples were fabricated with identical ion-exchange parameters but with gold thicknesses of 64 and 36 nm. The variation in the thickness of the gold films was determined to be ± 10 % of the quoted thicknesses over the area of both of the films. Light from a 10 mW linearly polarised, intensity stabilised, He-Ne laser, operating at a wavelength of 632.8 nm was end-fire coupled into the waveguide under test so that both TM and TE modes were excited in the device. The ratio of the TM output power to the reference TE output power was taken, to correct for instrumental drift, and recorded on a chart recorder.

The first experiment, employing the device with a 64 nm thick gold film, was performed by measuring the ratio of the TM/TE output signals from the sensor as a function of the analyte refractive index before and after adsorbing a combined layer of thermally denatured biotinylated bovine serum albumin (BSA) and polystreptavidin. The analyte index was varied by attaching a flow cell on the sensor and using a peristaltic pump to flow sucrose solutions over the gold film. By dissolving increasing weights of sucrose in de-ionised water, solutions of varying refractive index could be readily produced. The refractive index of each sucrose solution used was measured using an Abbé refractometer at $\lambda$ = 589.3 nm. The sensor surface was then washed with phosphate buffered saline (PBS), (0.1 mol/l $KH_2PO_4$, 0.15 mol/l NaCl, pH 7.5), and incubated for 10 minutes with a solution of 40 μg/ml BSA in PBS. The surface was then washed again with PBS before being incubated with a solution of 40 μg/ml polystreptavidin in PBS. Finally the sensor surface was washed with PBS solution. The measurements of the ratio of the TM/TE output signals of the sensor were then repeated using sucrose solutions with the same index as used previously.

The second experiment used the sensor with a 36 nm thick gold film. The ratio of the TM and TE output signals from the sensor was measured as a series of biotin-avidin films were adsorbed on the gold layer of the sensor. The peristaltic pump was replaced by a flow injection analyser pump and six way valve. The sensor was initially washed with PBS (n=1.337 at $\lambda$=589.3 nm) at a flow rate of 0.186 ml/min (used for all solutions) to provide a system baseline. Subsequently 40 μg/ml BSA in PBS solution was passed over the sensor surface for approximately 10 minutes. Following this, a solution of 40 μg/ml polystreptavidin in PBS was passed over the sensor surface for the same period of time. The biotin-avidin solutions were alternately passed over the sensor surface to build up several complete biotin-avidin layers. Finally PBS was passed through the flow cell to ensure that the biotin-avidin layers were firmly attached to the sensor.

## 4. Results and Discussion

Plots of the TM/TE ratio as a function of superstrate index, before and after adsorbing a combined biotin-avidin layer are shown in figure 3 for the sensor with a gold thickness of 64 nm and an interaction length of 3.3 mm. The SPR in the absence of biotin-avidin is centred at a superstrate index of 1.355. At superstrate indices below this, the curve has its greatest slope at an index of 1.348, representing the most sensitive operating point of the sensor. However the slope of the SPR curve at the refractive index of water is substantial and indicates that the device will operate as a sensor, while not fully optimised.

The second plot in figure 3 shows the SPR curve for the sensor after a biotin and an avidin layer have been adsorbed to the gold surface. It is believed that the biotin and polystreptavidin layers have thicknesses of approximately 3 nm and 6 nm respectively[5]. The centre of the resonance has

now shifted to an index of 1.351, leading to a decrease in the TM/TE ratio at a superstrate index of 1.333 of approximately 16.5 %, showing that the sensor adequately detects the adsorbing of biotin and then avidin to the sensor surface in an aqueous environment.

Figure 4 shows the variation in theTM/TE ratio as a series of biotin and then avidin layers are adsorbed to the surface of the sensor having a 36 nm thick gold film and an interaction length of 4 mm. The change caused by the adsorption of the first biotin layer to the sensor surface is 14 %. However the slope is greatest when the second biotin layer is bound to the sensor, with a decrease in the TM/TE ratio of 35 %. The location of this maximum at a point other than on the attachment of the first molecular layer also indicates that the sensor is not fully optimised for water. A similar biotin-avidin protocol has also been applied to an integrated optical Mach-Zehnder interferometer which has also been interrogated by measuring the change in the output power of the sensor as the biotin-avidin layers adsorb to the surface of the device[5]. It is clear that both types of sensor are capable of monitoring a binding sequence of biotin-avidin layers and exhibit similar performance, although neither device is fully optimised.

The curve plotted in figure 4 was extracted from the chart recorder trace displayed in figure 5, which details the real-time variation in the output of the SPR waveguide sensor as the biotin and avidin solutions were pumped over the sensor surface. The plot shows the first 3 biotin, and 2 avidin, layers attached to the device. The gap in the trace was caused by an air bubble which was visually observed to enter the flow cell at that time.

## 5. Conclusions

Gold coated waveguide SPR sensors have been fabricated and used to monitor the attachment of biotin-avidin layers to the surface of the sensors in the aqueous environment. Fabrication was kept as simple as possible, leading to reliable devices which are not, however, fully optimised for operation in water. It is expected that the incorporation of a low refractive index buffer layer into the sensor design will lead to devices with a greater sensitivity, if required.

Further studies are also being carried out into the experimental optimisation of the gold film parameters and the form of the detection system. Utilising the gold film as an electrode to allow the possibility of performing electrochemical experiments also remains to be investigated and is a potential advantage over other, dielectric based, integrated optical biosensors. It is intended that this type of waveguide SPR biosensor will be employed to monitor low concentrations of organic pollutants in ground water.

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