

Integrated optical chemical and biochemical sensors

James S Wilkinson*

Optoelectronics Research Centre, University of Southampton

Highfield, Southampton, Hampshire, SO17 1BJ, UK

ABSTRACT

Planar optical waveguides may be used for the sensitive real-time interrogation of the optical properties of very thin films attached at surfaces, and microfabrication allows the definition of integrated optical circuits, including electrodes, for sensor arrays. Sensing techniques based upon thin-film absorption and refractive index changes are presented, including electrochemically-controlled surface reactions, and present work on fluoroimmunosensor arrays and instrumentation are discussed. In particular, integrated optical immunoprobes for environmental analysis are described.

Keywords: integrated optics, planar waveguides, biosensors, chemical sensors, surface plasmon resonance, fluoroimmunoassay, environmental monitoring, instrumentation.

1. INTRODUCTION

Integrated optics is a field of rapid growth in applications as diverse as telecommunications, tunable and short-wavelength laser light sources, and diagnostics in medicine, the environment, industrial control and food production. Application of integrated optics to chemical sensing at surfaces exhibits great potential for sensitive measurements incorporating a broad range of optical phenomena. The increasing complexity of such applications demands integration, so that approaches which allow the interrogation of arrays of sensors on microstructured surfaces where small volumes of analyte are controlled by integrated microfluidic systems are expected to find wide application. Planar optical waveguides embedded in flat dielectric substrates may be employed to guide light at a surface on which films sensitised to specific chemicals have been immobilised, allowing real-time interrogation of their optical properties during chemical reactions. This approach offers precise stable control of the optical interaction, reduction in interference from the bulk of the analyte, and potential for photolithographic production of dense sensor arrays.

The wide range of optical phenomena which may be used to observe reactions in thin films at surfaces has prompted the development of a range of sensor devices based upon the simple low-cost base technology of ion-exchange in glass. The intrinsic sensitivity of these waveguides to surface effects is lower than for waveguides formed from high-index thin films, due to their reduced optical confinement or large modal spot size. However they exhibit low loss, are well-suited to photolithographic formation of monomode channel waveguides, may readily be connected to instrumentation in a stable manner using pigtailed optical fibres, and the surface evanescent fields may be locally enhanced at sensing sites by incorporation of conducting or dielectric overlayers. The permanent connection of optical fibres is relatively costly when compared with "free-space" coupling of light into thin-film waveguides with gratings but brings the advantages of mechanical stability and ease of control of the spatial distribution of the evanescent intensity over the surface of the "chip". This approach is not aimed at cheap "single-shot" devices but at arrays of sensors for multiple analytes, where the surface chemistry may be regenerated *in situ* using electrochemical or chemical regeneration allowing repeated use. Transducers based on absorption [1,2], refractive index [3-5,7-9] and fluorescence [6] have been realised and their combination with electrochemical techniques using indium tin oxide (ITO)-coated [1,2] and silver-coated [4] devices demonstrated.

* Correspondence:

Email: jsw@orc.soton.ac.uk; Telephone: +44 (0) 23 8059 2792; Fax: +44 (0) 23 8059 3149;
WWW: <http://www.orc.soton.ac.uk/person/jsw.html>

In this paper, examples of each type of integrated optical sensor are discussed and recent results which emphasise research directed at realisation of sensor arrays, of electrochemical measurements at surfaces and of incorporation of these sensors in analytical instruments are given.

2. A WAVEGUIDE SURFACE PLASMON RESONANCE IMMUNOPROBE

The European Union (EU) has set limits on the concentrations of pesticides in ground and drinking waters of $0.1\mu\text{g/l}$ and $0.5\mu\text{g/l}$ for individual and total pesticides respectively. While laboratory-based techniques such as HPLC and GC-MS can readily reach these limits, they are expensive and time consuming. There is a need for low-cost portable instrumentation for rapid in-situ analysis of water samples for pesticide contamination. Immunoassay techniques offer an attractive approach to this application and when coupled to an appropriate transducer a selective immunoprobe may be realised. Several laboratory-based biosensor systems using this approach are commercially available, including the "Biacore" prism-based SPR system. The use of an integrated optical form of biosensor allows for ready miniaturisation and ultimately the realisation of densely-packed sensor arrays. The first integrated optical sensors based upon surface plasmon resonance (SPR) were described by Kreuwei et al. [10], and we have optimised these devices for the detection of immunobinding reactions in an aqueous environment. The configuration of the sensor, whose operation can be conveniently described in terms of coupled modes is shown in Fig. 1. The TM mode is excited in the monomode input waveguide and divided into transduction and reference paths with a 3dB splitter. The mode in the transduction arm partially couples to an optically lossy surface plasma wave (SPW) guided at the surface of the gold film at the interface with the superstrate medium. This coupling is governed by velocity-matching between the waveguide mode and the SPW, so that changes in the refractive index of the medium at the gold surface alter the power absorbed by the gold film. This change is monitored as a change in the power output of the transduction arm with respect to the reference arm to correct for input power drift. If a thin dielectric film, such as a protein monolayer, is adsorbed to the metal surface within the evanescent field of the SPW, the average index of the superstrate region is changed, which may be detected. In principle this may be used to monitor the real-time binding of unlabelled antigens to antibodies attached at the gold surface. A rigorous numerical model has been used to optimise the theoretical performance of this sensor [3,7] in water assuming homogeneous "slab" waveguides. Experimental optimisation of devices using diffused channel waveguides was undertaken using the well-characterised biotin-streptavidin system, which allows adsorption of the biomolecular layers to the gold surface without specialised surface chemistry.

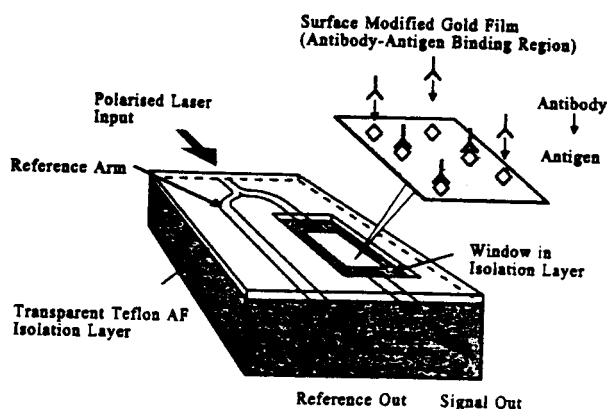


Figure 1 Waveguide Surface Plasmon Resonance Immunosensor

Waveguides were fabricated by potassium ion-exchange in Pyrex substrates at 385°C for 8.5 hours, with a nominal waveguide diffusion mask opening of $2\mu\text{m}$. The gold film was deposited over a 2mm length of the transduction arm by thermal evaporation, and patterned photolithographically. A 700nm thick isolation layer of Teflon AF1600 was then thermally evaporated and patterned by lift-off leaving a window over the gold film. Gold film thicknesses of 36nm, 48nm, and 65nm ($\pm 3\text{nm}$) were used, covering the range for maximum sensitivity predicted by theory, with the gold and Teflon films being removed and redeposited between experiments.

TM polarised light from a 10mW 633nm He-Ne laser was end-fire coupled into the device and the ratio of the two outputs recorded as a function of bulk superstrate index before and after adsorption of a dual layer of biotinylated bovine serum

albumin (BSA-Bi) and polystyrene-avidin (pSA). A wall-jet flow-cell was attached to the sensor and a flow-injection analyser was used to pump aqueous sucrose solutions over its surface and to control injection of the biochemicals. The normalised output of the sensor with 65nm gold, as a function of superstrate index, is shown in Fig. 2 before and after attachment of the biomolecular film. This figure clearly shows the resonance between the waveguide mode and the SPW and also shows that the device is operating close to the steepest part of the resonance curve when in water ($n = 1.33$). The lowest detection limit achieved was estimated to be equivalent to a bulk index change of 3×10^{-5} . However, this was limited by the mechanical stability of the apparatus and improvements in mechanical, fluidic, and electronic aspects of an instrument are expected to result in substantial improvements.

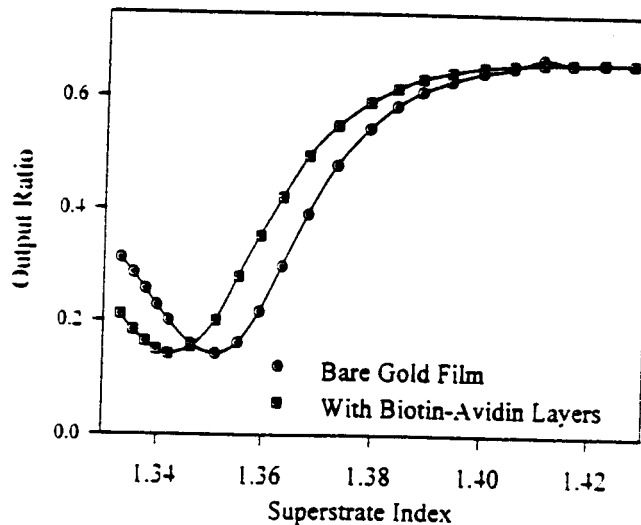


Figure 2 Waveguide SPR response to bulk index before and after biolayer absorption

A similar device employing a 3mm long, 53nm thick, gold film was then modified as a specific immunoprobe for simazine. The gold surface was activated by treatment with 0.1% mercaptopropionic acid in ethanol to form a carboxy-functionalised surface. Amino dextran was attached to this surface [22] to reduce non-specific binding and to provide a 3-dimensional matrix for immobilisation of the ligand. A triazine derivative with high affinity to antisimazine antibodies (4-chloro-6-(isopropylamino)-1,3,5-triazine-2(6'-amino) caproic acid) was coupled to the remaining amino groups [8] rendering the surface activated for the binding of antisimazine antibodies or Fab fragments. The activated immunoprobe was stable at room temperature for at least one month at this point, an important benefit for practical devices. A wall-jet flow-cell connected to a flow-injection analyser was again clamped to the sensor chip and a background stream of phosphate buffered saline (PBS) pumped around the fluidic circuit.

The immunoprobe was tested using a binding inhibition assay for simazine as follows. Antibodies were mixed with aqueous simazine solutions of known concentration and, after incubation, the remaining free antibody concentration was determined by injecting the resultant solution into the stream flowing over the sensor surface. For low antibody concentration and high density of binding sites on the sensor surface this results in a constant rate of binding to the sensor surface, which may be related to the concentration of free antibody and then to the concentration of simazine in the original sample. Following such a measurement, the sensor surface was automatically chemically regenerated by pumping pepsin and an aqueous solution of acetonitrile and propionic acid through the flow cell, allowing repeated measurements.

900 μ l PBS samples with simazine concentrations in the range 0 - 100 μ g/l were incubated for 5 minutes with 100 μ l aliquots of 0.35 μ g/ml antisimazine IgG antibodies. Subsequently this procedure was repeated replacing the antibodies with Fab fragments at 0.35 μ g/ml. This procedure was repeated three times for the antibody assay and twice for the Fab fragment assay. The temporal response of the immunoprobe corresponding to one set of whole antibody measurements is shown in Fig 3. The curve with the steepest slope corresponds to zero simazine concentration and the shallowest slope corresponds to the solution containing 100 μ g/l simazine. The immediate fall in signal on injection of the sample and immediate increase on pumping the sample out of the cell is due to the difference in bulk refractive index of the circulating PBS and the sample. There are clearly significant fluctuations in the binding rate during incubation of the sample at the sensor surface, leading to

errors in the measurement which degrade the lower limit of detection (LOD). It is believed that these fluctuations may result from non-optimised flow-cell design. Figure 4 shows the calibration curves generated by plotting the slopes of these binding curves against known simazine concentration. The circled data points show the averaged triplicate calibration curve using whole antibodies and the square data points show the average of two calibration curves generated using the anti-simazine Fab fragments. The lower limit of detection is estimated from the error recorded on the blank samples to be $0.16 \mu\text{g/l}$ simazine when using whole antibodies and $0.11 \mu\text{g/l}$ simazine when using Fab fragments. The cycle time for simazine measurement and regeneration of the sensor was 20 minutes, allowing repeated rapid determinations to be made.

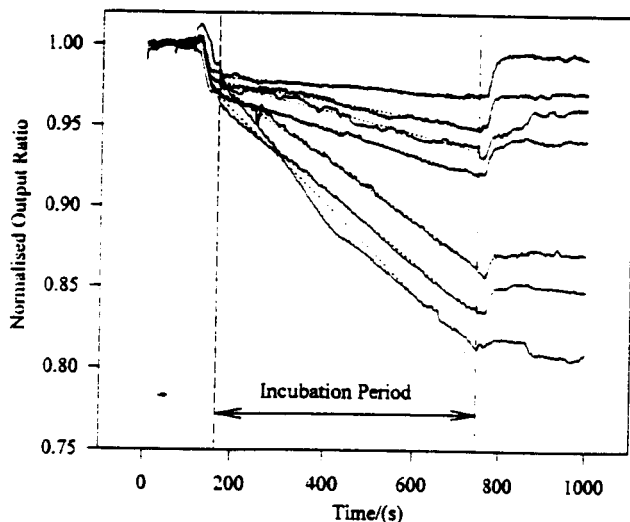


Figure 3 Real-time SPR immunoprobe responses

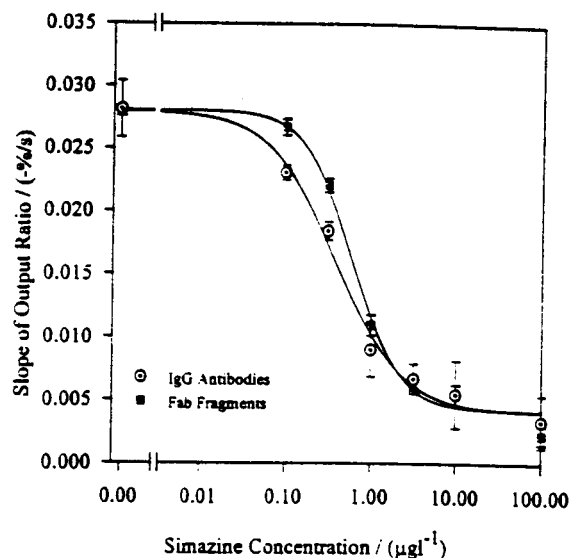


Figure 4 SPR triplicate simazine calibration curve

This waveguide immunoprobe has exhibited LOD close to that required by the EU although many aspects of the sensor, flow-cell, sample-handling, and instrumentation design require optimisation. The use of Fab fragments rather than whole IgG antibodies may be advantageous as they do not contain the Fc portion, reducing non-specific adsorption to the sensor surface. The sensitivity of these devices could be improved by inserting a low-index buffer layer between the waveguide and the gold film, shifting operation in water to the steeper rising edge of the SPR curve [3]. However, this would lead to increased complexity of manufacture and reduced yield and is not warranted until improvements have been made to procedures and to the surrounding instrumentation. The active area of these devices is of order $0.01\text{mm} \times 2\text{mm}$, potentially allowing the use of small sample volumes and reduced quantities of biochemicals and the integration of multisensor arrays in small areas. The use of a gold surface is convenient for stable biochemical attachment and a device prepared in a similar way to that reported here was used repeatedly without disassembly for more than 250 measurements over a three-week period without significant reduction in surface activity [5]. The gold film may also be employed as an electrode for electrochemical control and measurement in sensing reactions and, while results have previously been obtained for cationic electroadsorption at a silver-coated waveguide surface [4], these devices show rather poor stability. Preliminary results have been obtained for the more stable gold-coated devices optimised for an aqueous environment discussed above and will be briefly discussed.

3. INTEGRATED OPTICAL MACH-ZEHNDER SENSORS

In common with the SPR sensor, the integrated optical Mach-Zehnder interferometer (MZI) is an evanescent refractometer which must be chemically modified to render it sensitive to specific chemical species. In applications where there is risk of deterioration of gold films through abrasion in repeated use and re-modification of the sensor surface, the MZI is an advantageous approach, although surface modification for immunoassay applications is not as well established. In principle the MZI is more sensitive, primarily because it does not employ coupling to a lossy waveguide, although exploitation of this enhanced sensitivity may require longer sensitive regions. Furthermore the MZI may be rendered sensitive over a wider range of superstrate indices, as it does not employ "resonant" coupling between two dissimilar waveguides but effectively directly measures modal velocity changes due to refractive index variations in the evanescent field of an unisolated waveguide compared with a waveguide isolated from the analyte. Highly sensitive MZI immunosensors have been

demonstrated, for example by Heideman et al [11], and while we have demonstrated immunosensing with ion-exchanged MZI's using a similar approach to that described above [12], our work has concentrated upon achieving sensitive operation over a wide range of indices, enhancing the sensitivity of ion-exchanged devices using high-index overlayers, and investigating the simple and reliable incorporation of these sensors into instrumentation.

A schematic diagram of a typical device and measurement system is shown in Figure 5. Light is coupled into the sensor chip using a monomode fibre pigtail, is divided for referencing purposes, and is then split again into the two arms of the interferometer. The relative phase of the light in the two paths combining at the 3x3 coupler depends upon the refractive index of the analyte in the window in the isolation layer otherwise covering the device. In a conventional MZI the light is recombined in a simple Y-junction, resulting in an output which varies periodically upon relative phase, with excess power being scattered into the substrate. Sensitive transducer response to small changes in analyte index requires that the device operates in a sensitive region of the response curve, which cannot be satisfied over the entire range of indices for a single-output MZI, which shows maxima and minima in the interference function. The use of a three-waveguide coupler results in three outputs with interference functions which are shifted by nominally 120° with respect to each other. In this way at least one output yields a sensitive response to small index changes whatever the starting value [13] and, assuming negligible waveguide loss, the individual signals may be referenced to the sum of the outputs, removing the effects of source intensity fluctuations. In the presence of optically absorbing analyte media the independent reference output may be used to estimate the optical absorption of the analyte as well as its refractive index.

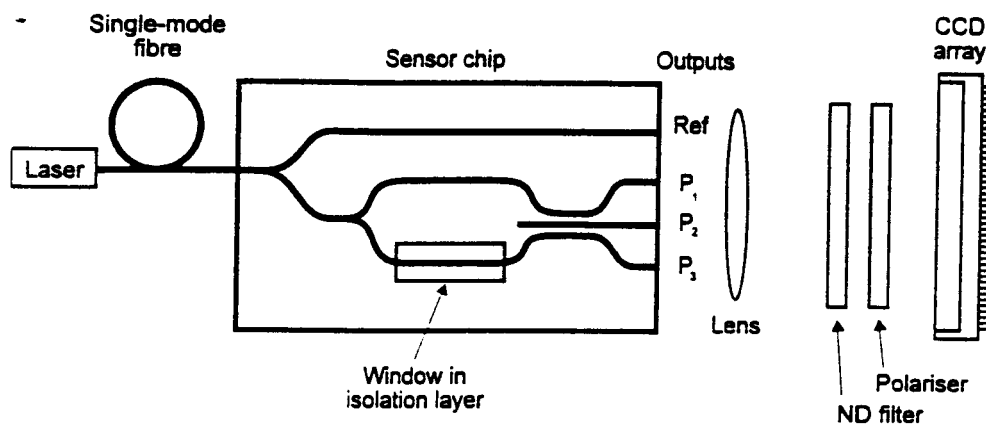


Figure 5 Experimental Mach-Zehnder sensor system

The analyte refractive index range of interest spans 1.32 to 1.6, at wavelengths close to 600nm, for applications in product quality control, requiring the use of a substrate glass of commensurately high index. We have fabricated waveguides by silver ion-exchange in BGG36 glass (Schott) which has an index of approximately 1.6 at 600nm. Typically, ion-exchange is carried out in a 10% $\text{AgNO}_3/\text{NaNO}_3$ melt at 316°C for 10 minutes, resulting in a surface index change of 0.075; the devices are then annealed at 250°C for 1 hour to reduce losses and improve coupling to optical fibre. Teflon FEP was used as the isolation layer and the window, defined by lift-off, is typically $0.15\text{mm} \times 10\text{mm}$. Initial device characterisation was carried out using sucrose solutions pumped through a flow-cell as described above, and the variation of the three MZI outputs with analyte index is shown in Fig 6. The three outputs are clearly phase-shifted, however not by 120° ; this is because the device was designed for use with a silica isolation layer ($n \approx 1.46$) rather than with a Teflon isolation layer ($n \approx 1.30$). The sensitivity to bulk refractive index changes is estimated to be 5×10^{-6} by comparison with fluctuations in the background signal when PBS is flowing over the sensor surface.

Characterisation of the sensor for thin-film biochemical measurements was carried out using the biotin-avidin system used for the SPR sensor with a laser input wavelength of 786nm. The untreated glass surface was initially incubated for 8 minutes with BSA-Bi, followed by alternate 8-minute incubations with pSA and BSA-Bi. Each incubation step was followed by a 2 minute washing stage to flush the unbound material out of the flow-cell with PBS. Figure 7 shows an example of the response of output 3 of the sensor to the addition of two complete BSA-Bi-pSA protein adlayers in terms of

interferometer phase shift. It can be seen that fast binding takes place upon injection of a solution due to the high protein concentration and the high affinity constant. As further layers are added the phase change becomes smaller and levels out as the layers fill the evanescent region. This allows estimation of the protein layer index at $n \approx 1.40$ by comparison with the bulk index measurements and estimation of the dual protein layer thickness of 24nm. Commonly employed estimates of protein monolayer density [14] may be combined with our estimated sensitivity to phase changes resulting in a limit of detection for protein loading of order 5pg/mm^2 . Immunosensing measurements on simazine have been conducted using this device, in a similar manner to those described above for the waveguide SPR sensor, resulting in a detection limit marginally below $0.1\mu\text{g/L}$.

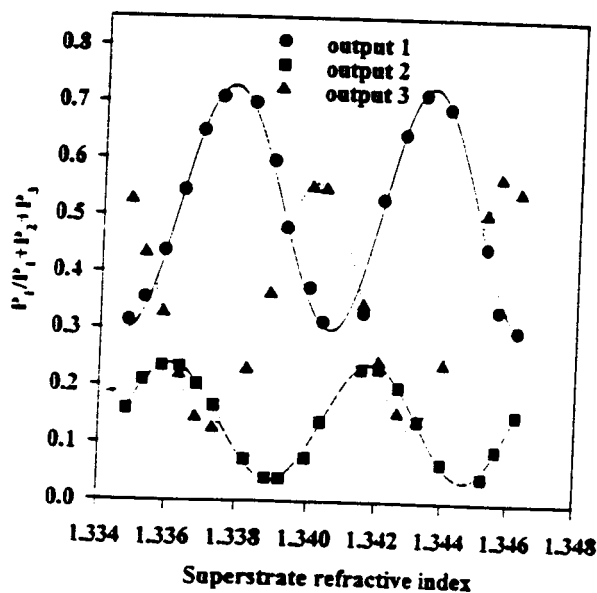


Figure 6 Variation of MZI outputs with superstrate index

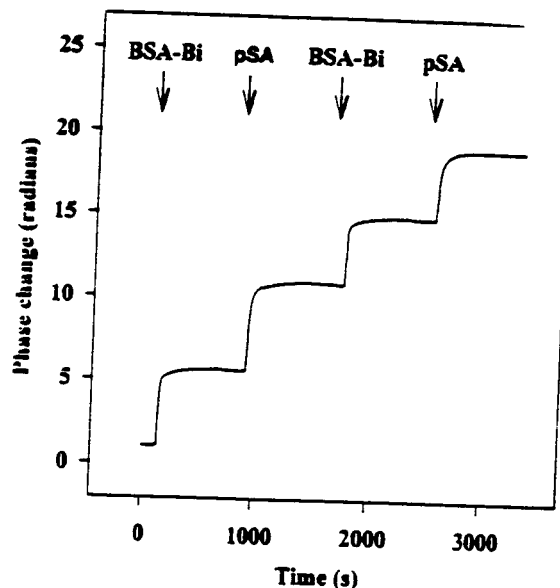


Figure 7 Phase response of MZI to BSA-Bi-pSA films

The greater the proportion of guided power that passes through the analyte the greater the sensitivity of the guided modes to changes in the analyte, causing many workers to use thin high-index waveguides to achieve high sensitivity [15,19]. These devices are often subject to higher losses than ion-exchanged waveguides and techniques such as prism-coupling and grating coupling are used to excite these waveguides. The proportion of power carried in the superstrate region of low confinement waveguides may be increased by the addition of a thin high-index film, as proposed by Stewart et al. for the case of fibre sensors [16]. An alternative approach to achieving high sensitivity is therefore to use low-loss fibre-compatible ion-exchanged waveguides incorporating non-guiding high-index films. The addition of a high-index film will also increase losses, due to scattering between the coated and uncoated regions and due to the relatively lossy nature of the materials used. In order to assess the enhancements achievable using this approach we realised four MZI devices with tantalum pentoxide films ($n \approx 2.1$) of thickness 0, 20, 30 & 40nm deposited on the waveguide in the window in the isolation layer and compared their response to changes in bulk analyte index [17]. In this case waveguides supporting a single mode at 633nm were formed by ion-exchange in soda-lime glass by immersing in molten KNO_3 at 400°C for 25 minutes, through $3\mu\text{m}$ wide openings in an aluminium mask, and a 500nm thick Teflon FEP film was used as an isolation layer defining a 10mm long interaction region. Radiation from a He-Ne laser at 633nm was launched into the polished end of the waveguide and the signal from the single MZI output was divided by a reference output. A wall-jet flow-cell was clamped to the sensor surface and sample solutions of sucrose in water ($1.334 \leq n \leq 1.360$) were sequentially injected into a continuous flow of water using a flow-injection analyser as described above. Over the small range of refractive indices used the phase change experienced through the MZI interaction region is expected to vary approximately linearly with superstrate index. The interferometer phase shift determined from the experimental response of the device to each pulse, is plotted against sucrose pulse index in Fig 8 for the TE polarisation. The gradients of these lines correspond to the relative sensitivities of the interferometers, showing that use of a 40nm film increases sensitivity by a factor of more than 50. However, the losses induced by films of this thickness reduce the interferometer contrast so that, by comparison with the "baseline" noise measured with constantly flowing water, the limit of detection is optimum for the device having a 30nm Ta_2O_5 overlayer. In this case the limit of detection for refractive index changes is determined to be 4×10^{-7} , an enhancement by a factor of 14 when compared with the MZI having no Ta_2O_5 overlayer.

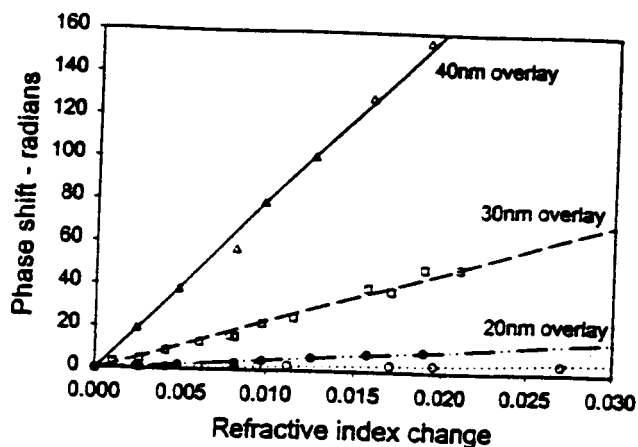


Figure 8 Phase response to superstrate index of overlaid MZI

While deposited Teflon is a useful isolation material for experimental devices as it has a low refractive index, low porosity and is easy to pattern, it has not so far proved acceptable for use in sensors which are to be handled repeatedly as it is easily damaged. Development of these devices for prolonged use in an instrument has required optimisation of sputtered silica films as robust isolation layers. However, these must be deposited at low temperature and annealed in air or oxygen at no more than 250°C - 300°C to improve transparency while avoiding significant diffusion of the underlying ion-exchanged waveguide. We are working with the Kyoto Electronic Manufacturing Company to explore the feasibility of a low-cost instrument based upon fibre-pigtailed MZI arrays with robust surface materials for beverage analysis [18]. One application under investigation is to excite the interferometer with light from an LED emitting at 590nm and to focus eight output signals onto a low-cost 1D CCD array. While signal levels are low, the system is very stable and initial measurements show that sufficient light is coupled from standard packaged LED's to render their use with the current instrumentation a practical possibility. A readily measurable signal is detected from each of the signal outputs and reduction in waveguide losses, presently dominated by those of the silica isolation layer, improvements in fibre coupling efficiency and the expected availability of high-brightness LED's are expected to lead to further improvements in signal-to-noise ratio (SNR). The use of an LED at the industrially important wavelength of 590nm has shown that measurable signals may be obtained using CCD array imaging and that interferograms may be readily generated. However, further work is required on silica deposition, as the SNR is degraded by isolation layer losses (to which a sensitive device is particularly prone) and the sensitivity is degraded by diffusion of the waveguide during annealing, reducing the interaction of the evanescent field with the analyte. Preliminary measurements on absorbing liquids have shown that absorption may be estimated by comparison of the MZI outputs with the reference output, and algorithms will be developed to extract the real and imaginary parts of index from these measurements.

MZI sensors show promise for sensitive detection of bulk and thin-film index and absorption changes over a wide range of analyte indices and are ideal as a platform for measurements on a wide range of sensing films. In common with the SPR sensors, limits of detection may be dramatically improved by the stable incorporation of the sensors in an instrument, allowing individual sensors to be more rigorously compared. In order to more fully exploit the inherent sensitivity of waveguide sensors and to explore the practical deployment of waveguide sensors beyond the research laboratory we have embarked on the establishment of a demonstrator which is yielding promising results.

4. WAVEGUIDE IMMUNOFLUORESCENCE SENSORS

While label-free devices detecting changes in refractive index of films at sensor surfaces offer simplicity in operation, sensors relying on labels such as fluorophores generally offer lower limits of detection as only the target molecule yields the required signal. Label-free approaches are more prone to non-specific binding of molecules to the transducer surface and fluctuations in analyte index due to compositional and temperature changes in the bulk medium. Excellent sensitivities have recently been reported for fluorescence-based immunosensors, for example by Duvencek et al. [19].

We have demonstrated a regenerable integrated optical fluorescence immunosensor for environmental monitoring suitable for rapid multianalyte determinations in small sample volumes using ion-exchanged waveguides with thin high-index overlays for signal enhancement [20,21]. The system has been designed to use Cy5.5 dye labelled antibodies which absorb

at a wavelength of 635nm and emit at approximately 700nm. This approach allows the use of readily-available semiconductor lasers and photodiodes, and the long wavelength avoids significant background fluorescence. Typically, monomode waveguides were formed in soda-lime glass by ion-exchange in potassium nitrate for 25 minutes at 395°C. One aim of this work was to demonstrate the feasibility of realising a linear array of fluorescence sensors for six different analytes. The chip was designed so that the input radiation was split into a reference and signal arm and six 2mm long windows separated by 2mm were opened in the isolation layer along the reference arm. These defined six separate sensing patches, each of active area 0.02mm². The optical absorption due to the attachment of dye-labelled antibodies at each patch is expected to be negligible so that pump reduction due to binding at an "upstream" patch is not expected to cause significant cross-talk. In this case the isolation layer was formed from silica sputtered in a reactive atmosphere and annealed in air at 250 °C, resulting in an acceptable excess loss of 1dB/cm. A series of 2mm long 20nm thick patches of Ta₂O₅ was defined at the sensing patches before deposition of the silica film.

Fluorescence was excited by radiation from a 633nm He-Ne laser coupled into the waveguide and the collected by NA polymer fibres located under the sensor chip, filtered to remove stray pump light and detected by a silicon photodiode. A mechanical shutter controlled by a PC (which also controlled the flow-injection analyser (FIA) and acquired the data) was used to control exposure of the dye-labelled antibodies attached to the sensor surface. In present experiments the laser and shutter have been replaced by a pigtailed laser diode. The sensor surface was modified in a similar manner to the SPR sensor described above so that all patches were rendered specific for anti-simazine [22]. Work on committing each sensor patch to a different analyte [23] is beyond the scope of this paper. A flow-cell was attached to the sensor surface and a sequence of binding inhibition immunoassay cycles, including regeneration as described above, were carried out under control of the FIA. In this case the antibodies were labelled with Cy5.5 dye. The high pump intensity at the sensor surface caused substantial photobleaching of the dye so that, rather than monitoring fluorescence while the incubated sample was injected into the cell, the sample was allowed to dwell in the cell for 400s to allow binding before washing out excess material in flowing PBS and then opening the excitation shutter. The fluorescence signal was integrated for approximately 60s after opening the shutter, by which time fluorescence was largely bleached and a background signal, obtained by integrating for 60s after regeneration with PBS flowing, was subtracted. Calibration curves for two sensing patches, showing the normalised integrated signal are shown in Figure 9. Separate experiments have shown that the high-index film causes an enhancement of peak fluorescence by a factor of up to 10; however rapid photobleaching reduces the enhancement of the integrated signal.

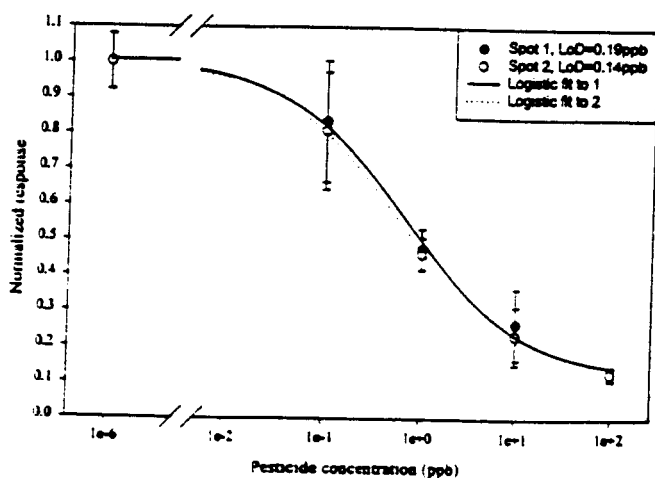


Figure 9 Simazine triplicate calibration curves

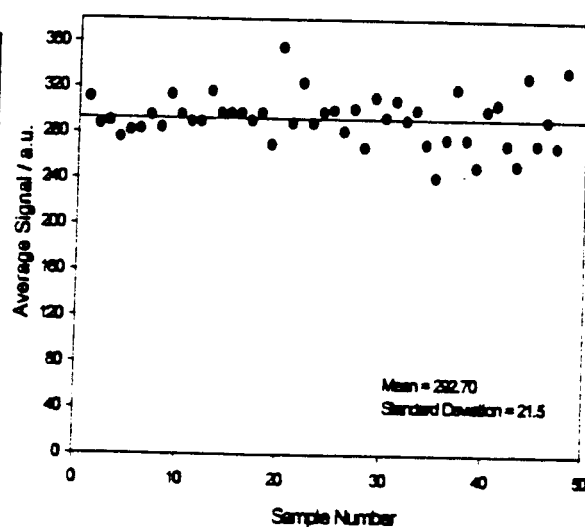


Figure 10 Response to 50 repeated test-cycles

Figure 9 shows that the detection limit for simazine is 0.14µg/l – 0.19µg/l, a similar result to those obtained for the refractometer-based sensors. This reinforces the view that limits of detection in these systems, calculated conventionally by reference to the standard deviation of (rather few) measurements on water samples containing no simazine, are dominated

by issues of sample preparation, mechanical stability, surface regeneration, and fluid circuit design. To test the stability of the surface modification a device with 30nm Ta_2O_5 was subjected to an extended series of binding and regeneration cycles. Over a period of 1 day, approximately 50 test cycles were carried out and Figure 10 shows the integrated fluorescent signals for each sample. No significant degradation of the response occurred over the course of these measurements, indicating that Ta_2O_5 is an acceptable material for the surface chemistry applied. In all, approximately 150 measurements were carried out over a three-day period with no apparent degradation of the surface chemistry.

New designs of fluorescence immunosensor chips are being prepared for use in a compact demonstrator instrument which incorporates permanent stable fibre pigtailed, six collection fibres with receiver circuitry and an integrated flow-injection system. These designs will incorporate the low-loss robust silica isolation layers and high-index films reported here together with new approaches to reducing the effects of photobleaching to allow exploitation of the advantages of the high surface intensities achievable with these devices.

5. WAVEGUIDE EVANESCENT ABSORPTION SENSORS

Evanescent absorption spectroscopy of thin chemically-specific films on optical waveguides offers another example of the use of integrated optical transducers for chemical sensing. In particular these demonstrate how a strong well-controlled optical signal may be obtained from thin films which would yield weak absorption in conventional transmission spectroscopy but which respond rapidly in diffusion-dominated reactions. We have concentrated in exploring thin films which may be controlled electrochemically to yield reversible pseudo-continuous sensors through electrochemically-controlled oxidation and reduction or through electroassisted extraction into a polymer film containing a complexing agent for a metal ion allowing colorimetric determination.

In an early example of this kind of sensor an ITO electrode sandwiched between a thin film of lutetium biphthalocyanine and a planar waveguide was used to detect dissolved chlorine in water [1,2]. The spectral changes which occurred when chlorine oxidised lutetium biphthalocyanine were measured through evanescent field absorption in the film; electrochemical control was used to restore the film to its original state ready for a new measurement. The sensor consisted of a potassium ion-exchanged waveguide in soda-lime glass, fabricated as described above, coated with a 200nm sputtered silica buffer layer, a 10nm thick film of indium tin oxide (ITO) deposited by reactive evaporation, and a 10nm thick film of lutetium biphthalocyanine ($Lu(PC)_2$) defined over a 4.5mm length of the waveguide. Light from a tungsten halogen lamp was passed through a monochromator and polariser. A silica cell was affixed to the sensor surface, filled with aqueous 0.05M NaCl buffered to control the pH at 7, and a platinum counter electrode and an Ag/AgCl reference electrode were placed in the cell to allow use of a conventional potentiostat.

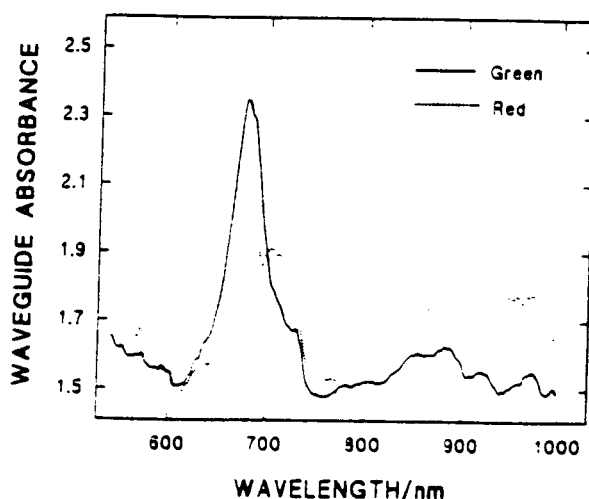


Figure 11 Experimental absorption spectra for $Lu(PC)_2$ -loaded waveguides

The Lu(PC)₂ film was repeatedly oxidised and reduced by cycling the potential between -0.1V and +0.8 V. Waveguide absorption spectra were obtained by ratioing the waveguide output spectrum to the excitation fibre output spectrum for both the TE and TM polarisation. These spectra are shown for the TE polarisation for both the reduced (green) and oxidised (red) state in Figure 11. Strong absorption changes may be observed at around 700nm and 980nm. Chlorine concentration measurements were made by introducing 10µl aliquots of stock solution of NaClO, and measuring the waveguide absorption change at 950nm upon electrochemically reducing the film after allowing the film to oxidise for 10minutes. A linear dependence of this absorption change with chlorine concentration was observed.

More recently thin PVC films containing a complexing agent for lead ions deposited onto an ITO electrode have been used to demonstrate sensing using a new electrochemically-assisted solvent extraction method. An anodic potential was applied to the film, extracting lead ions from aqueous solution and resulting in an increase in absorbance at 550nm which was linearly dependent upon the concentration of lead in the range 0-5ppm. The sensing film was reset for a fresh measurement by applying a cathodic potential. In parallel, optimisation of very thin ITO films for waveguide sensors, where there are stringent requirements for low loss and low sheet resistance, has been carried out [24].

6. CONCLUSIONS

Integrated optical sensors for refractive index changes, fluorescence and absorption in thin specific films attached to waveguide surfaces have been discussed and examples of their application to environmental and chemical sensing given. The sensors described employ a common base technology with different waveguide circuit layouts and thin-film overlayers used for specific applications. Further advances will require consideration of complete sensor systems and development of chemistries for reagent-free continuous monitoring. Approaches to incorporating electrochemical control and sensing in these devices and steps towards application of these devices in compact and stable demonstrator instruments have been outlined. Waveguide sensors exhibit high sensitivity, are well-adapted for use in flow systems, and may be applied flexibly to a wide range of chemistries. The realisation of practical instruments incorporating these devices will depend upon the further development of re-useable sensor arrays cheaply and reliably interfaced with optoelectronic components.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the UK Engineering and Physical Sciences Research Council, the European Union, and the Kyoto Electronic Manufacturing Co Ltd for supporting this work, and the many colleagues and collaborators who have contributed to this programme.

REFERENCES

- 1 "Optoelectrochemical Thin-Film Chlorine Sensor Employing Evanescent Fields on Planar Optical Waveguides", C.Piraud, E.K.Mwarania, G.Wylangowski, K.O'Dwyer, D.J.Schiffirin, & J.S.Wilkinson, *Analytical Chemistry*, v.64, pp.651-655, 1992.
- 2 "Optoelectrochemical Transduction on Planar Optical Waveguides", C.Piraud, J.Yao, E.K.Mwarania, K.O'Dwyer, D.J.Schiffirin, & J.S.Wilkinson, *Journal of Lightwave Technology*, v.10, n.5, pp. 693-9, 1992.
- 3 "Waveguide Surface Plasmon Sensors", R.D.Harris & J.S.Wilkinson, *Sensors & Actuators B*, v.29, pp.261-267, 1995.
- 4 "Electrochemically-Controlled Waveguide-Coupled Surface Plasmon Sensing", C.R.Lavers, R.D.Harris, S.Hao, J.S.Wilkinson, K.O'Dwyer, M.Brust & D.J.Schiffirin, *Journal of Electroanalytical Chemistry*, v.387, pp.11-22, 1995.
- 5 "Determination of Simazine in Water Samples by Waveguide Surface Plasmon Resonance", C.Mouvet, R.D.Harris, C.Maciag, B.J.Luff, J.S.Wilkinson, J.Piebler, A.Brecht, G.Gauglitz, R.Abuknesha & G.Ismail, *Analytica Chimica Acta*, v.338, n.1-2, pp.109-117, 1997.
- 6 "Immunofluorescence sensor for water analysis", A.Klotz, A.Brecht, C.Barzen, G.Gauglitz, R.D.Harris, G.R.Quigley, J.S.Wilkinson and R.Abuknesha, *Sensors and Actuators B*, v. 51, pp.181-187, 1998.

- 7 "Theory and modelling of optical waveguide sensors utilising surface plasmon resonance", J.Ctyroký, J.Homola, P.V.Lambeck, S.Musa, H.J.W.M.Hoekstra, R.D.Harris, J.S.Wilkinson, B.Usievich and N.M.Lyndin, *Sensors and Actuators B*, v. 54, pp.66-73, 1999.
- 8 "Integrated Optical Surface Plasmon Immunoprobe for Simazine Detection", R.D.Harris, B.J.Luff, J.S.Wilkinson, J.Piehler, A.Brecht, G.Gauglitz & R.A.Abuknesha, *Biosensors & Bioelectronics*, v. 14, pp.377-386, 1999.
- 9 "Integrated Optical Sensor System for Beverage Analysis", B.J.Luff, K.Kawaguchi & J.S.Wilkinson, *Proc. SPIE International Symposium on Industrial and Environmental Monitors and Biosensors*, Boston, USA, November 1st - 6th, 1998, pp.10-16.
- 10 "Surface Plasmon Dispersion and Luminescence Quenching Applied to Planar Waveguide Sensors for Measurement of Chemical Concentrations", H.J.M.Kreuwel, P.V.Lambeck, J. van Gent & T.J.A.Popma, *Proc SPIE 789 (1987)* 218-224.
- 11 "Performance of a Highly Sensitive Optical Waveguide Mach-Zehnder Interferometer Immunosensor", R.G.Heideman, R.P.H.Kooyman & J.Greve, *Sensors & Actuators B*, v.10, pp.209-217, 1993.
- 12 "Integrated Optical Immunosensors for Pesticide Analysis", B.J.Luff, R.D.Harris, J.S.Wilkinson, J.Piehler, A.Brecht, G.Gauglitz & U.Hollenbach, in *Applications of Photonic Technology 2*, G.A.Lampropoulos & R.A.Lessard, Eds., Plenum, NY, 1997, pp. 909-914.
- 13 "Integrated Optical Mach-Zehnder Biosensor", B.J.Luff, J.S.Wilkinson, J.Piehler, U.Hollenbach, J.Ingenhoff & N.Fabricius, *J. Lightwave Technol*, v.16, pp.583-592, 1998.
- 14 "Ellipsometry as a Tool to Study the Adsorption Behaviour of Synthetic and Biopolymers at the Air-Water Interface", J.A.DeFeijter, J.Benjamins & F.A.Veer, *Biopolymers*, v.17, pp.1759-1772, 1978.
- 15 "Integrated Optical Difference Interferometer as Refractometer and Chemical Sensor", C.Stamm & W.Lukosz, *Sensors & Actuators B*, v.11, pp.177-181, 1993.
- 16 "Optical Waveguide Modelling and Design for Evanescent Field Chemical Sensors", G.Stewart & B.Culshaw, *Optical and Quantum Electronics*, v.26, pps249-S259, 1994.
- 17 "Sensitivity Enhancement of Integrated Optical Sensors by Thin High-Index Films", G.R.Quigley, R.D.Harris & J.S.Wilkinson, *Applied Optics*, in press.
- 18 "Integrated Optical Sensor System for Beverage Analysis", B.J.Luff, K.Kawaguchi & J.S.Wilkinson, *Proc SPIE*, v.3539, pp.10-16, 1998.
- 19 "Novel Bioaffinity Sensors for Trace Analysis Based on Luminescence Excitation by Planar Waveguides", G.L.Duveneck, M.Pawlak, D.Neuschäfer, E.Bär, W.Budach, U.Pieles & M.Ehrat, *Sensors & Actuators B*, v.38-39, pp.88-95, 1997.
- 20 "Integrated Optical Immunofluorescence Sensor for Environmental Monitoring", R.D.Harris, G.R.Quigley, J.S.Wilkinson, A.Klotz, C.Barzen, A.Brecht, G.Gauglitz & R.A.Abuknesha, *Proc. Fifth World Congress on Biosensors*, Berlin, 3-5 June 1998, p. 72.
- 21 "Sensitivity Enhancement of Transducers for Total Internal Reflection Fluorescence", A.Klotz, C.Barzen, A.Brecht, R.D.Harris, G.R.Quigley, J.S.Wilkinson & G.Gauglitz, *SPIE Proc. Photonics West*, 23-29 Jan 1999.
- 22 "Surface Modification for Direct Immunoprobes", *Biosensors & Bioelectronics*, J.Piehler, A.Brecht, K.E.Geckeler & G.Gauglitz, *Biosensors & Bioelectronics*, v.11, pp.579-590, 1996.

- 23 "Optical Immunoprobe Development for Multiresidue Monitoring in Water", A.Brecht, A.Klotz, C.Barzen, G.Gauglitz, R.D.Harris, G.R.Quigley, J.S.Wilkinson, P.Sztajn bok, R.Abuknesha, J.Gascón, A.Oubina & D.Barceló, *Anal.Chim.Acta.*, v362, pp.69-79, 1998.
- 24 "Indium Tin Oxide Overlayered Waveguides for Sensor Applications", B.J.Luff, J.S.Wilkinson & G.Perrone, *Applied Optics*, v.36, pp.7066-7072, 1997.