Integrated optical immunofluorescence multisensor for river pollution

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ABSTRACT

A 32-analyte integrated optical immunofluorescence multisensor system has been realised and tested for the first time. The sensor system is based upon bio/immuno-chemistry at the waveguide surface and fluoroimmunoassay in the evanescent fields of the optical waveguides, to enable rapid, simultaneous and high-sensitivity fluorescence detection of up to 32 pollutants in water, and automatic regeneration for immediate reuse. The system has been demonstrated for estrone and a detection limit of 13 ng/L has been achieved.

Keywords: Integrated optics; sensors; fluorescence; instrumentation

1. INTRODUCTION

Monitoring river water quality and identifying pollution sources are major tasks in the management of the river water environment, as rivers are a major source of water for human consumption. The aim of this work is to develop a cost-effective, on-line water-monitoring biosensor that will simultaneously measure a variety of organic pollutants with low molecular weight in a short time with remote control and surveillance. The biosensor reported here is part of the Automated Water Analyser Computer Supported System being realised by an EU-funded consortium (AWACSS)[1] which is intended to help meet the needs of both current and future water managers. The instrument will be networked so that pollution sources can be monitored remotely, and trend analysis and early-warning capabilities will be provided.

The AWACSS instrument employs fluorescence-based detection of the binding of fluorophore-tagged biomolecules to the surface of an optical waveguide chip. The fibre-pigtailed chip, driven by a semiconductor laser, consists of a waveguide circuit which distributes excitation light to 32 separate sensing patches on the chip surface. Bio/immuno-chemistry is used to sensitise each of the 32 patches to a specific analyte and a microfluidic system is used to automatically handle the sample injection over the sensor surface, enabling rapid, simultaneous and high-sensitivity fluorescence detection of up to 32 pollutants. A fibre-coupled detection array is used to monitor the 32 separate fluorescence signals, and software has been developed for control of the optics and fluidics and data acquisition and processing for the fluorescence signals, laser power, and ambient and chip temperature. The system is connected to a HTC-PAL autosampler and is capable of remote control via the internet interacting with the AWACSS' database.

In this paper we present a detailed description of the biosensor chip fabrication and operation with first results for detection of the pollutant estrone in water. The key optical components of the instrument, the modification of the sensor surface to render it specific to estrone, and the automated immunoassay procedure including surface regeneration, are described, and a characteristic immunoassay calibration curve has been generated, demonstrating a detection limit of 13ng/L estrone in water. Future prospects for this system in terms of ultimate detection limit, multianalyte operation, and regenerability will be discussed.

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2. SENSOR DEVICE FABRICATION AND SYSTEM OPERATION

2.1 Multisensor chip fabrication

The multisensor chip was fabricated by potassium ion-exchange in BK7 glass[2]. An aluminium masking film was deposited on the substrate, and the waveguide circuit was defined by opening tracks of 2.5 μm wide in this film using conventional photolithography, following the layout shown in Fig 1. Ion exchange was carried out by immersing the masked substrates in KNO_3 at 400°C for 2 hours to produce gradient index channel waveguides. This approach allows excellent control of the spatial distribution of optical power at the surface and offers the potential for more dense multisensor integration. Parabolic taper waveguides widening to 30 μm were introduced into each waveguide branch after the Y-junction splitters in order to reduce the optical power density at the waveguide surface, and hence the rate of photobleaching[3]. The ends of the chips were polished to allow fibre butt-coupling resulting in an overall length of approximately 67mm and width of 15mm. A silica isolation layer of approximate thickness 1 μm was sputtered on to the sensor chip, with 32 windows of 1.5 mm length and 0.3mm width being defined in this film over the waveguides photolithographically by positive lift-off, as shown in Fig 1. The isolation layer is to prevent excess losses due to contact with the environment, except in the sensing regions. In order to improve the sensitivity of the sensor, a 25 nm thickness high index film of Ta_2O_5 was deposited over the entire surfaces of the chip by reactive RF sputtering from a tantalum target, in an atmosphere containing 10% oxygen at a total pressure of 11mTorr[3]. A polarisation-maintaining (PM) fibre pigtail with PM connector was permanently bonded to the input end of the sensor chip with UV- cured epoxy.

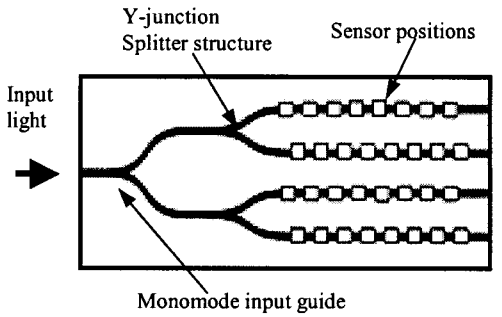

Fig 1. Schematic diagram of multisensor chip

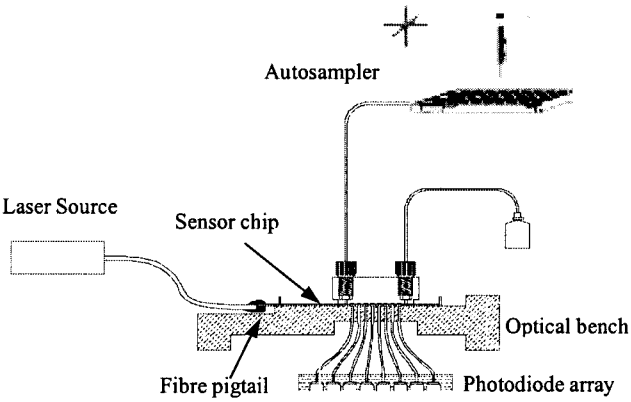

Fig 2. Experimental apparatus

2.2 Experimental system

The experimental system for immunoassay measurements is shown in Fig 2. Light from a semiconductor laser emitting approximately 5mW at 637±2nm is coupled into the input waveguide of the sensor chip using the polarization maintaining single mode fibre pigtail. This input power is divided equally into the four parallel waveguides using three Y-junction splitters and, in the 32 exposed sensing regions, the evanescent field is able to interact with the analyte, forming spatially separated sensing spots. If a fluorophore is brought within a few hundred nanometres of a sensing spot the evanescent field of the guided light will excite the fluorophore, resulting in fluorescence. The fluorescence is collected by an array of 1mm core diameter high numerical aperture polymer optical fibres located under the sensor chip, filtered to remove stray pump light, and detected by a silicon photodiode with integral amplifier having an NEP specified as $100fW.Hz^{-½}$. The signal is amplified to give a responsivity of ~54.6mV/pW and low-pass filtered with roll-off at 1.4Hz. A micro-flow-cell is affixed on top of the chip over the 32 patches to supply sequences of solutions to the sensor surface. The pumps and valves which supply the solutions, the laser, and the data acquisition system are controlled by an computer integral to the instrument. The fluorescence power from each sensing site, the laser power, the ambient temperature and the temperature at the sensor are recorded at a rate of 8 samples/s, and the data are analysed and presented by the computer system. The optical detection limit of the system, defined as 3×NEP, has typically been found experimentally to be equivalent to ~500fW of fluorescence power. This multisensor platform may be applied to a wide range of analytes according to the surface attachment protocols, described in detail elsewhere.

3. MEASUREMENT PROCEDURES

3.1 Sensor surface modification

Characterisation of chip and instrument performance for immunofluorescence sensing was carried out with the single analyte estrone, to allow direct patch-to-patch comparisons. The entire sensor surface was chemically modified in order to render the chip specific to estrone, to reduce non-specific binding and to enable repeated use of the sensor chip. The chip was cleaned with Piranha solution (H_2SO_4:H_2O_2) and a thin layer of trimethoxysilane was applied to the dried surface for 1 hour. The silanised surface was rinsed with dry acetone and was blown dry at room temperature. Aminodextran dissolved in water was coupled to the silanised surface for 2 hours. Analyte derivatives estrone carbonate acid (E1,3-CME) dissolved in N-Dimethylformamid (DMF) together with N,N¢-Dicyclohexylcarbodiimide (DCC) were attached to the sensor surface for 3 hours and then washed with DMF and water. This procedure leads to a high density of specific binding sites for antibodies to estrone at the sensor surface. Nonspecific binding is reduced to a minimum due to the shielding of the glass surface by the aminodextran[4].

3.1 Immunoassays

The performance of the sensor system was demonstrated by measuring the response to eight known concentrations of estrone in Milli-Q-water, ranging from 0ng/L (blanks) to 10µg/L. The blanks were repeated 9 times to yield a good statistical sample and all other concentrations were repeated thrice. Each standard solution was prepared in Milli-Q-water from a master solution of 10 µg/L estrone. To 900 µL standard solution of a concentration step 100 µL of the antibody stock solution containing 60 ng/mL polyclonal anti-estrone antibody, 150ng/mL ovalbumin in 10 fold phosphate buffered saline (PBS) were added and mixed thoroughly to allow the analyte to bind to the fluorescent-labelled antibody molecules (law-of-mass-action), according to its concentration, and so prevent them from subsequently binding to the sensor surface. The output of one sensing channel during a typical sensor test cycle using a Cy5.5 labelled anti-estrone concentration of 6 ng/mL, and a blank sample, is shown in Fig. 3. A constant flow of PBS was established through the micro-flow-cell, and the background signal, due to laser breakthrough and background fluorescence, was measured. While the incubated sample was being loaded and injected into the flow-cell, the laser was turned off to prevent the onset of photobleaching. After binding of the incubated sample at the sensor surface for 13 minutes, the cell was flushed with PBS again and the laser turned on.

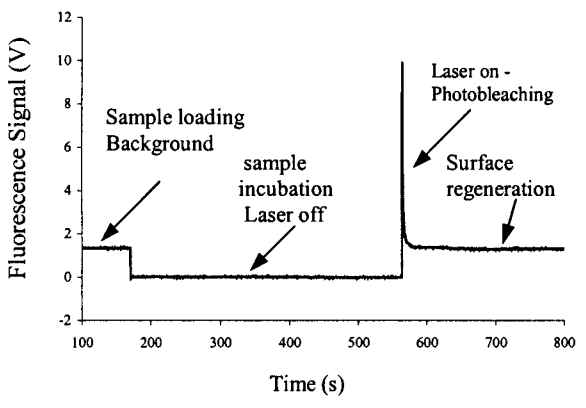

Fig 3. .Sensor test cycle for blank with 6ng/mL anti-estrone

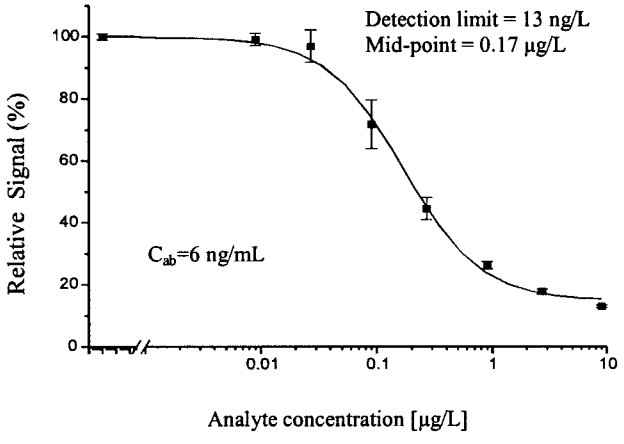

Fig 4. Averaged calibration curve for estrone

Fig 3 shows that, once the background has been subtracted, a peak signal of ~155pW was obtained and that this was bleached in a few seconds. Nonetheless, there is no difficulty in acquiring this signal, and the optical signal to noise ratio is well over 1000. After bleaching, the surface was regenerated by 0.5% SDS (sodium dodecyl sulphate) adjusted with HCL to pH 1.8 so that another measurement could be carried out, with the entire cycle time taking less than 20 minutes.

It has been found that surface regeneration may be carried out up to 400 times without significant degradation of the surface chemistry. Calibration curve was obtained by measuring this procedure using the same concentration of dye-labelled antibodies (6 ng/mL) and estrone-spiked water samples in the concentration range of 0-10ppb (μg/L), prepared as described above (9x blanks and 3x the concentration steps). The averaged data have been fitted to a logistic function, representing a close approximation to the actual shape of a typical immunoassay calibration curve, and an example is given in Fig 4.

Fig 4 presents the estrone calibration curve for one sensing patch normalised to the mean of the signals for the blanks; data for other patches are similar and a full statistical analysis of the data is underway. The detection limit for estrone for this sensing patch was found to be 13 ng/L, and the mid-point of the response curve occurred at a concentration of 0.17 μg/L. The detection limit was taken to be the concentration at which the signal has fallen below the mean blank value (100%) by three times their standard deviation on the calibration curve in Fig 4 .

As the concentration of estrone in the sample increases, more fluorescent-tagged anti-estrone is bound to free analyte in the incubation phase and therefore prevented from binding to the sensor surface, with the result that the fluorescent signal is lowest for the highest concentrations of estrone. The detection limit of 13ng/L is nearly an order of magnitude better than that required by EU legislation for organic pollutants, and it is expected that improvements in sample handling and signal processing will reduce this further. The device and instrument are particularly suitable for an alarm system to be triggered when a concentration rises to an unacceptable level. The ability to automatically regenerate the sensor after an assay as part of an automated protocol means that the sensor and system may be left unattended for weeks or months, in normal operation, before a chip may need to be replaced. There is significant potential for further miniaturisation, increased integration, and reduced usage of reagents as the fluorescent signal is obtained from an area less than 50×10^{-3} mm^2 and a volume less than 20pL. This instrument is being produced in portable form where it can be taken to a site and connected to the internet for remote control and data handling, and initial field trials are underway.

4. CONCLUSIONS

A novel 32-analyte integrated optical fluorescence-based multisensor chip has been realised, and integrated with fluidics, detection system, surface chemistry, immunochemistry, and computer for control and signal processing. The capability of the resultant automated water analyser computer supported (AWACSS) system has been demonstrated on the pollutant estrone. A detection limit of 13 ng/L was achieved, with the mid-point of the calibration curve occurring at 0.17 μg/L. Ongoing tasks include the use of spotting technology to modify the individual patches on the sensor surface to allow measurement of multi organic pollutants, and application of the system to real samples in the field.

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