INTEGRATED OPTIC CHEMICAL-BIOLOGICAL SENSORS

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ABSTRACT

Planar waveguides have evanescent fields sensitive to index of refraction changes in the volume immediately above the waveguide surface. Optically combining one guided sensing beam with a reference beam in an interferometric configuration generates measurable signals. Applying a chemically selective film over the sensing arm of the interferometer provides the basis for a chemical sensor. Tailored chemistries can be passive (e.g.; inducing swelling or dissolution in a film) or active (e.g.; containing reactive or binding sites). Fast and reversible chemistries are the goal, in most cases for both gaseous and liquid applications. Passive mechanisms are used when the target analyte is relatively inert, *i.e.* aromatic and chlorinated hydrocarbons. Active chemistries developed include tailoring the acid-base strength of the sensing film for pH or ammonia response, and antibody-antigen binding. Currently the integrated optic waveguide platform consists of thirteen interferometers on a 1x2-cm glass substrate. A different sensing film deposited on each channel allows for multiple analyte sensing, interferant cancellation, patterned outputs for analyte identification, or extended dynamic range. Sensitivities range from the low ppm to low ppb for both vapor and aqueous applications, 0.01 pH units and ng/mL for biologicals**.**

1.0 INTRODUCTION

The threat of chemical and biological (CB) attacks by hostile countries or terrorist organizations is a legitimate concern of the United States. The CB threat scenario associated with terrorist groups and extremist organizations is most difficult to counter and is likely to occur. Such hostile actions are inherently difficult to prevent and defend against in view of the relative ease with which small but dangerous quantities of CB materials may be covertly prepared and transported. Potential targets for terrorist attacks include cities with high population densities, public facilities such as transportation terminals, government buildings and business sites as well as military installations. In practically all instances, the first line of defense and response within the United States will involve local and state law enforcement agencies, fire departments, and medical emergency teams as well as members of federal agencies.

To mount an effective response to either the threat of an incident or an actual incident requires an integrated warning and response system. A threatened incident, even though a false alarm, can cause considerable disruption and economic loss. An actual incident could involve significant loss of life and even greater disruption of normal activities. Rapid detection and identification will be critical to any warning and response system. Ideally, the detection part of a system would have the ability to detect and identify agents or precursors at the manufacturing stage or during transportation and deployment. Similarly, the detection system must be capable of very quickly detecting and identifying an agent when agent release occurs. In practice high

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sensitivity, portability, multi-analyte detection capabilities, and rapid response are required for either of the scenarios. Practical early warning detection systems are required that are suitable for covert deployment at selected sites, installation as on-site monitoring systems within a facility and its air handling system or, alternatively, as an unattended sensor located around the perimeter of a facility or installation. Similarly, the detection system should be capable of functioning as a wearable unit for personnel exposure assessment

To date no one system has been identified as being capable of meeting all the requirements. The following sections of this paper discusses an integrated optic sensor system being developed for environmental, biomedical, and food safety applications that has the potential to fulfill many of the technical and performance demands. The sensor system is based on an integrated optic, multi-channel interferometer developed by the Georgia Tech Research Institute[1,2]. The sensor system and results are discussed in the following sections.

2. INTEGRATED OPTIC INTERFEROMETRIC SENSORS

A planar optical waveguide consist of a thin high refractive index surface film (the waveguide) deposited on the surface of an optically transparent substrate. An optical wave propagating through the waveguide is characterized by an exponentially decaying electric field (an evanescent field) that extends into the volume immediately above the waveguide surface. Due to the evanescent field, changes in the refractive index properties of the medium on or above the waveguide surface alters the phase or velocity of a guided wave. By interfering or beating the perturbed guided wave with a reference wave, surface index changes of less than 10^{-6} can be measured[1,2]. Thus adding a thin chemically selective film to the surface of an optical waveguide provides the basis for a highly sensitive chem/bio sensor in a chip format.

The basic IO sensor system configuration is illustrated conceptually in Figure 1. Key components include an IO chip, a diode laser, and a photodiode detector. The only sensor element not shown, but essential to a complete sensor system, is the signal processing electronics circuitry. Grating couplers are utilized for coupling light into and out of the waveguide. Grating couplers ensure a practical field instrument by overcoming the difficult problem of coupling light into the extremely thin waveguides required for sensitive integrated optic interferometers. Traditional end fire or butt coupling methods requires precision XYZ alignment and mode volume matching. Conversely, the grating method requires only a relatively coarse angular alignment and is sufficiently simple such that field replacement of an IO chip is possible.

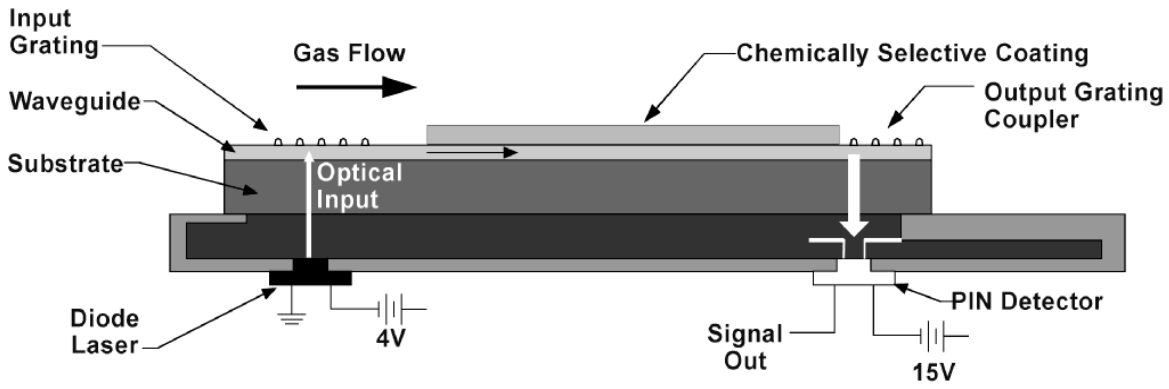

Figure 1. Cross-section of an IO interferometric sensor package.

The performance of an IO chip interferometer is critically dependent on the interferometer design and the optical waveguide properties. In a typical sensor configuration depicted in Figure 2, the waveguide is a thin layer (< 200 nanometers, nm) of an optically transparent high refractive index material deposited on the surface of an optically transparent substrate or buffer layer. The chemically selective layer may be a protein that binds with a specific antigen or antibody, a chemically active layer that undergoes a reversible reaction with the species of interest, or a selective film that undergoes a physical change such as swelling. Because the evanescent field of a guided optical wave penetrates the region immediately above the waveguide, the guided wave is sensitive to any changes in the thickness or refractive index of the chemically selective film. The overlap of the evanescent field and the chemically selective surface film defines sensor sensitivity.

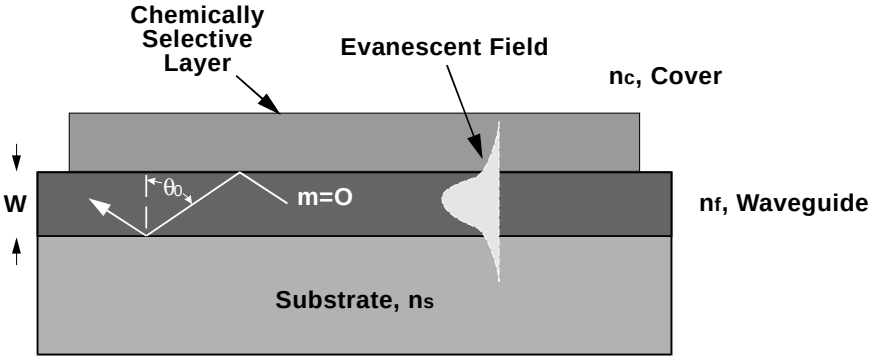

Figure 2. Diagram for single mode waveguide with buried evanescent field

The operation of the interferometer may be described quantitatively using a dispersion relationship that incorporates the step index waveguide parameters n_f (film index), n_c (cover film index), n_s (substrate index), and W (waveguide film thickness)[3]. A convenient model for describing the interaction is based on a "zig-zag" ray model for the reflection of an optical ray between the two waveguide surface boundaries. The dispersion relationship can also be derived from an electromagnetic boundary value condition where the guided wave is confined by two dielectric boundaries. The dispersion relationship is defined by the “transverse resonance condition”, which requires the sum of all phase shifts perpendicular to the direction of propagation in the waveguide to be a multiple of 2π radians ($2m\pi$, where m = 0, 1, 2, ...) for one "zigzag" period of the light ray propagating within the waveguide. Thus, the total phase shift associated with the transverse motion between the two boundaries must be an integer multiple of 2π for each full cycle. For one transverse passage through the waveguide, a phase shift of $kn_fW\cos\theta$ occurs ($k = 2\pi/\lambda$). One full period, however, requires two transverse passages. Additionally, phase shifts of $-2q_c$ and $-2q_s$ occur due to total internal reflection at the cover and substrate boundaries of the waveguide, as shown below:

$$(2kn_f W\cos\theta) - 2q_c - 2q_s = m(2\pi) \qquad (1)$$

where

$$\theta_c = \tan^{-1}\left[\frac{(n_f^2 \sin^2\theta - n_c^2)^{1/2}}{n_f \cos\theta}\right] \qquad (2)$$

and $$\theta_s = \tan^{-1}\left[\frac{(n_f^2 \sin^2\theta - n_s^2)^{1/2}}{n_f \cos\theta}\right] \qquad (3)$$

Because the waveguide is thin and the index differences are small, waveguiding occurs only at discrete values of θ. Each discrete angle corresponds to a propagating mode. The term $n_f \sin\theta$ is defined as the "effective mode index" (N_{eff}) and represents an effective refractive index the waveguide material system and the cover film exhibits for a propagating mode of a specific optical wavelength. If n_f, n_s, and W are held constant while n_c is varied (i.e., a refractive index change in the cover medium), then only θ can be varied to satisfy the conditions for propagation of the light ray through the waveguide. This change in the cover index manifests itself as a change in N_{eff}, which is easily detected using interferometric techniques. In practice, the minimal detectable change in N_{eff} is defined by the minimum phase shift detectable by the interferometer. The output of a waveguide interferometer operating at quadrature is described by the following equation:

$$I = \frac{I_o}{2}\left[1 + \cos\left[\frac{\pi}{2} + \frac{2\pi}{\lambda} L(\Delta N_{eff})\right]\right] \qquad (4)$$

In this equation, ΔN_{eff} represents the difference in the effective waveguide index induced by the surface index change due to the analyte interaction.

Using the previous equations, IO interferometers based on specific material systems can be designed with enhanced sensitivity to surface index changes. In general, high sensitivity requires a thin high index waveguide film on a low index substrate. The objective is to maximize the overlap of the evanescent field with the chemically selective surface layer. Sensitivity as a function of waveguide film thickness is presented in Figure 3 for a silicon nitride (Si_3N_4) waveguide and fused silica substrate. The calculations were based on the binding of a 5 nm diameter protein (n = 1.48) to the waveguide surface functionalized with a 10 nm diameter antibody. The waveguide film index was assumed to have a refractive index of 1.90 and the substrate an index of 1.457. The results indicate a waveguide film thickness of approximately 140 nm (0.140 micrometers in Figure 3) offers maximum sensitivity. Detection sensitivity could be further enhanced by the use of a higher index waveguide film such as tantalum pentoxide. However, scattering effects due to the stronger interaction of the evanescent field with the surface chemistry will eventually limit utility.

3. IO SENSOR CHIP DESIGN, FABRICATION AND SENSOR SYSTEM DESCRIPTION

The layout of a single interferometer channel is illustrated in Figure 4. Key elements include grating couplers, the waveguide film, and beam combining/beam splitting elements. The optical input is coupled into the waveguide by the grating couplers and the propagating optical wave traverses the length of the device under the signal and reference channels defined on the surface of the waveguide. The beam splitting/beam combining elements divide and mix the reference and signal waves to generate the interference. Any phase delays due to interactions of an analyte with the surface chemistry on the signal arm is easily observed through the resulting changes in the interference pattern.

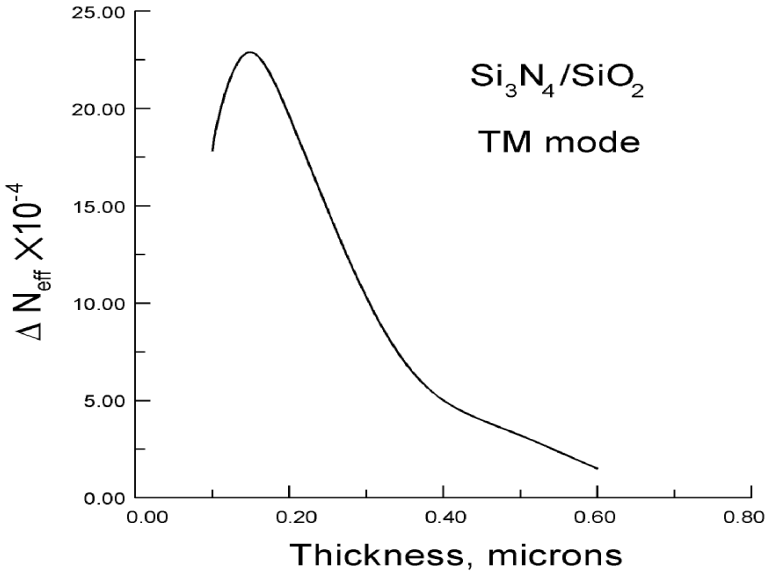

Figure 3. Si_3N_4 waveguide response to surface index changes as a function of thickness.

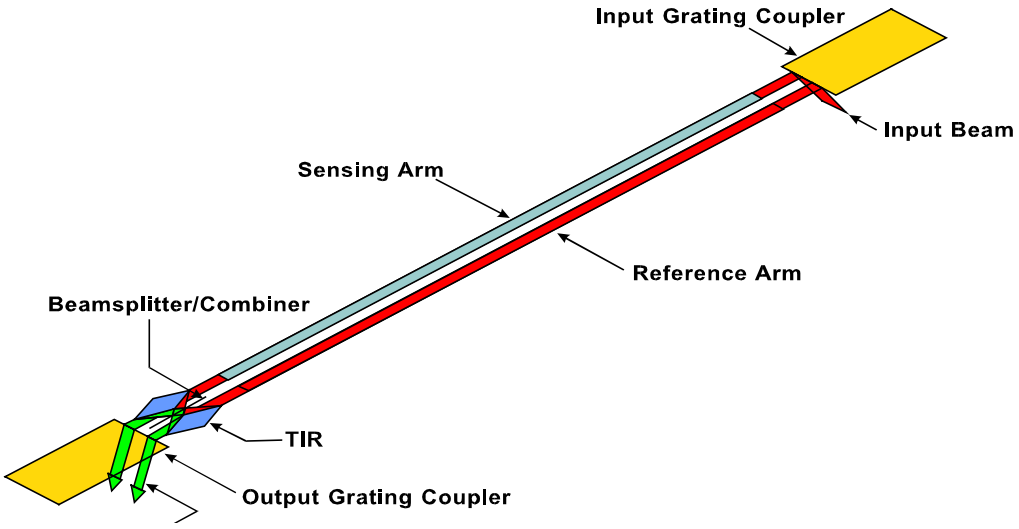

Figure 4. Single channel IO interferometer configuration.

The input/output couplers are produced by reactive ion etching (RIE) a 70 nm period grating into the substrate surface. The waveguiding film is then deposited on the substrate using a plasma enhanced chemical vapor deposition process (PECVD). A 140 nm Si_3N_4 film is typically used for the waveguides. The beam splitting/beam combing elements consist of total internal reflecting (TIR) mirrors and a beam splitter (BS). The TIR is fabricated by etching a 0.40 millimeter wide trough completely through the Si_3N_4 film to the substrate surface. The beam splitter is generated by etching a 4000 nm wide trough approximately 50 nm into the waveguide film. The entire surface area of the planar waveguide structure, with the exception of the sensing region of the waveguide surface, is then overcoated with a 500 nm thick silicon dioxide (SiO_2) film. The SiO_2 isolates the grating couplers, the TIR mirrors, and the beam splitters to ensure that their performance is insensitive to environmentally induced changes at or on the waveguide surface. The performance of a TIR mirror is critically dependent on the existence of optically smooth walls that vertical with respect to the substrate surface to within $\pm$ 1 arc degree.

Integrated interferometric based sensors have been developed to the prototype level for environmental, biomedical and food safety applications. Current prototype systems are based on an optical chip incorporating 13 interferometric sensing channels. In practice one sensing channel is always used as a reference to ensure proper system performance and calibration while the 12 remaining channels are typically used for different analytes or alternatively to provide redundant information to minimize the potential for false positives or false negatives.

A fully packaged IOS system designed for food safety applications (exclusive of a flow cell) is shown in Figure 5. Dimensions of this package are approximately 2.5 x 3.0 x 6.5 inches. All electronics and optics, exclusive of display, are contained within the package. Key system components include the following:

- beam shaping optics and source, a 670 nm or 780 nm wavelength diode laser.
- a 128 element self scanned photodiode array (TSL1401, cost approximately $7) for optical to electronic signal conversion.
- IOS sensing chip.
- Electronic board providing signal processing capabilities, communication with external devices, clock and control signals for the 128 element detector, and active control of diode laser output power.

Current prototype systems provide serial readout and are designed to interface with a laptop computer. The data-sampling rate is limited to 1 Hz with that limitation defined by the processing power of the microprocessor (an Enhanced Am486DX Processor by Advanced Micro Devices). At the 1 Hz rate, the output from all 13 channels (26 optical outputs as each interferometer provides two optical outputs) is processed, the analyte defined, and its concentration determined (ppbv, ppb or ng/ml).

4. SURFACE CHEMISTRY AND SENSOR PERFORMANCE

The chemical selectivity and sensitivity of an IO sensor is dependent on the surface chemistry. Unlike other microsensor technologies such as the surface acoustic wave devices, the IO sensors is more than a mass sensor or device that measures a change in a simple physical parameter. The IO sensor functions by detecting refractive index changes occurring on the waveguide surface. The source of the index changes can originate from simple physical mechanisms (absorption and

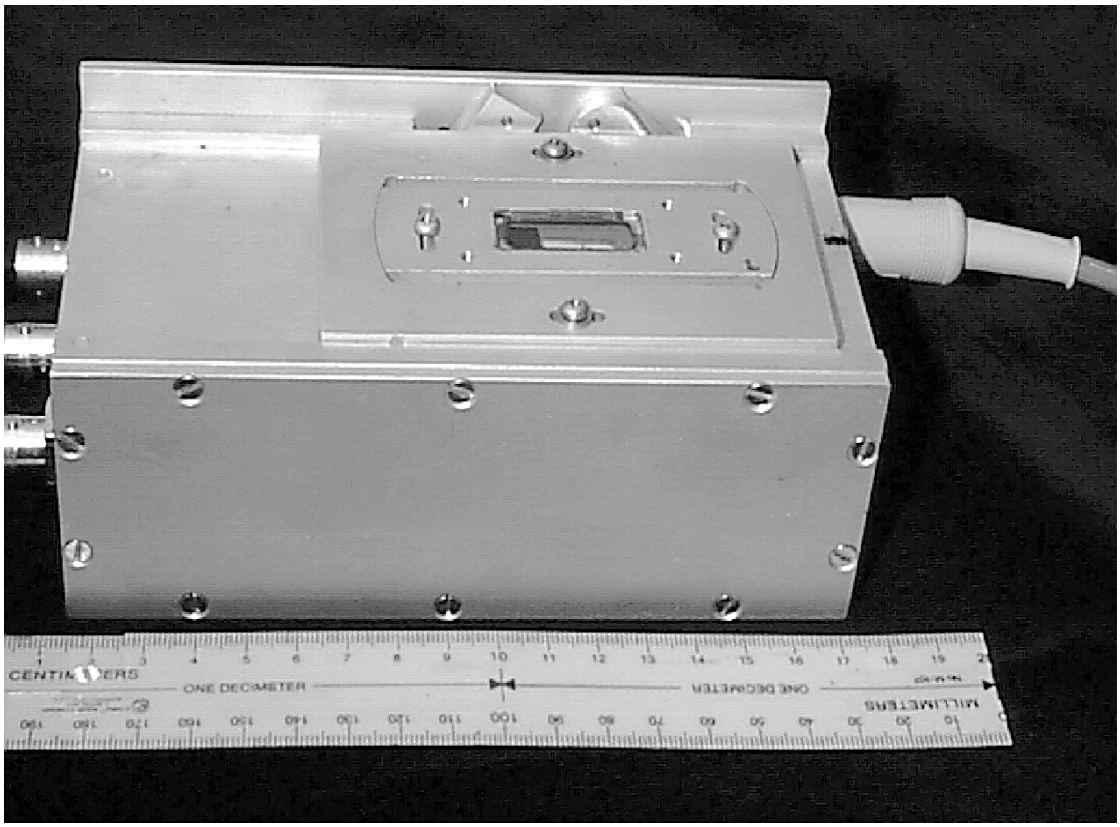

Figure 5. Packaged biosensor system minus sample delivery cell.

polymer swelling for example) or more importantly from electronic changes at the molecular level. The latter offers important advantages as this approach opens the door to an entirely new class of surface sensing chemistries and, as prior results have shown, an avenue for "chemically amplifying" the effective refractive index change[4]. Thus the reactive surface chemistries offers improved selectivity and enhanced sensitivity.

To provide a specific chemical sensing function, the waveguide surface is functionalized with surface films varying in thickness from a monolayer to tens of nanometers. By utilizing sensing films with thicknesses of 500 nm to 700 nm, the evanescent field associated with the guided waveguide remains buried in the sensing layer. This not only optimizes detection sensitivity, but also allows the device to operate in very dirty and harsh environments (media that is highly absorbing at the source optical wavelength). It is this feature that permits the sensors to be utilized in sewer systems for example. Typically for chemical agent detection, reversible equilibrium driven surface chemistries are utilized. Biological agent detection generally employs immunoassay based chemistries. In this approach, antigen binding is directly detected without the need for any labeling steps. More recently, reactive surface chemistries have been combined with immunoassays to provide and indirect detection scheme offering enhance detection limits for biological agents. Direct detection offers fastest response while the indirect methods offer highest sensitivity.

4.1 Biological Agent Detection

Direct detection methods are based on selective binding of an antigen (a biological agent) to antibodies covalently attached to the waveguide surface (note the reverse situation may also be employed where the antigen is used as the specific capture site on the waveguide surface). The binding step alters the phase velocity of a guided optical wave which is easily detected interferometrically. This approach is suitable for detecting toxins, viral agents and bacteria. Typically the signal arm of the interferometer is functionalized with a specific antibody while the reference arm is functionalized with a non-related antibody (see Figure 4). This compensates for nonspecific binding and nulls bulk refractive index changes. Direct detection requires the agents to exist in a hydrated state; thus, if airborne samples are to be monitored, they must be collected and introduced into an aqueous medium. This mode of operation is still consistent with concept of an unattended perimeter monitoring system as the quantities of reagents involved are in the tens of microliters range.

Direct detection techniques are capable of detecting bacteria at approximately 5 $x10^3$ colony forming units/milliliter (cfu/ml), proteins (such as a toxin) at approximately 10 picograms/millilter (pg/ml), and viruses at $<10^4$ plaque forming units/milliliter (pfu/ml)[5]. Figure 6 represents a typical response curve for *Salmonella.* The response exhibits an initial sharp increase in signal followed by a leveling off, indicating an equilibrium condition. The equilibrium condition is reached in approximately 10 minutes. Analyte concentration is proportional to both the initial slopes of the response curve as well as the equilibrium value. Figure 7 presents a dose response curves for the protein HCA (human chorionic gonadotropin)[5] using both initial slope and equilibrium conditions. Use of the slope response provides concentration data in only a few minutes versus more than ten minutes required using the equilibrium condition. Note the minimum detection limit observed for hCG is approximately 10 pg/ml.

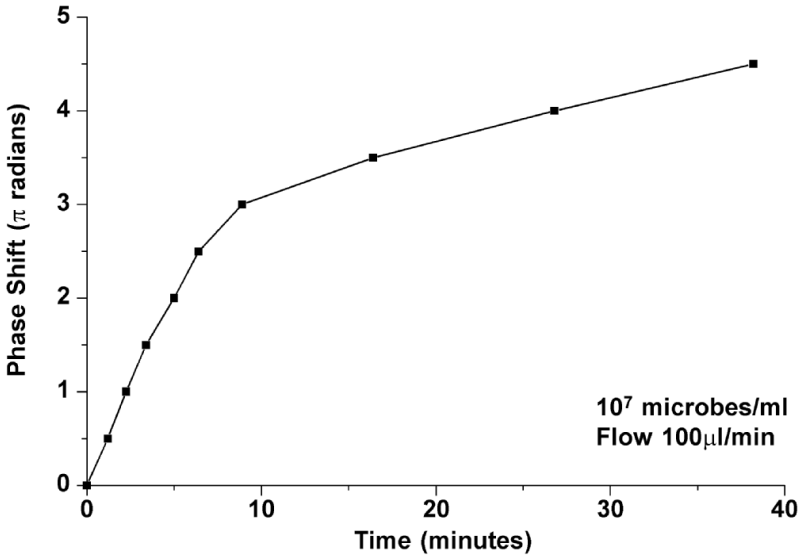

Figure 6. Direct detection response curve for *Salmonella.*

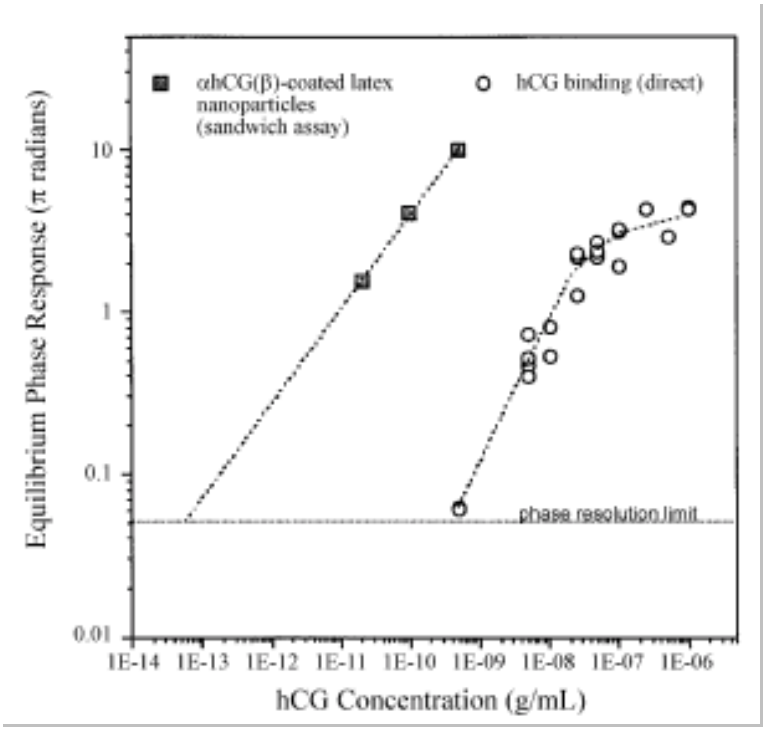

Figure 7. Direct detection dose response curve for hCG.

An indirect detection method based on enzymatic reactions has shown a dramatic improvement in sensitivity relative to the direct detection method. The indirect method offers an electronic ELISA with rapid response. In this process, an enzyme (urease) acts as a catalyst, converting a specific substrate (urea) to a specific product (ammonia). Implementing a sandwich assay, a urease labeled antibody is introduced to a surface bound antigen. This complex is exposed to urea, generating ammonia. Using a highly sensitive NH_3 IO sensor[5], trace levels of NH_3 are easily detected at the low part per billion levels. The process is illustrated in Figure 8. With this method, a small sample cartridge (a few hundred microliters volume) containing a functionalized bead matrix is injected with a test sample containing suspected agents. After agent binding to the antibody-coated beads, an appropriate enzyme labeled antibody (an antibody with a urease label) is introduced and allowed to react. Following a wash to remove any unbound enzyme labeled antibody, urea would be introduced to react with the bound urease, causing the release of ammonia. This method has the advantage that the reaction continually generates a product (such as ammonia) as long as unreacted urea is available. Also the design is such that the product is concentrated at the waveguide surface. These features combined with the highly sensitive NH_3 IO sensor provides outstanding detection sensitivity. This approach has been utilized with proteins and demonstrated to have sub pg/ml or Atamolar (10^{-18} moles) detection capabilities. Furthermore, this methodology results in a reusable sensor with only the sample cartridge requiring replacement. This approach does require multiple chemistry processing steps and reagents, increasing overall processing time to 5-10 minutes. Three reagents are also required, these being a wash, the urease labeled antibody and the urea. Because the beads provide a three dimensional matrix, time delays due to diffusion kinetics are substantially reduced, thus the relatively short processing time even though multiple processing steps are required.

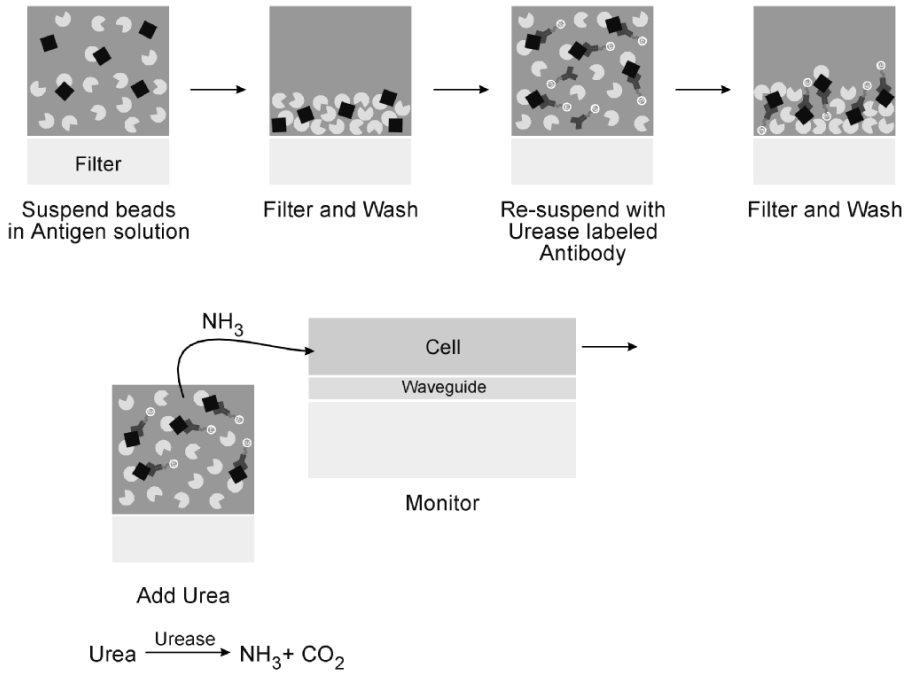

Figure 8. Indirect detection method for biological agents.

4.2 Chemical Agent Sensing: Active Chemistries

Active chemistry relies on a chemical reaction in the sensing film to identify the analyte. pH and vapor phase ammonia sensors have been developed using active chemistries that do not involve a change in color or fluoresence but rather a change in index of refraction of the sensing layer. Acid-base chemistry either on the waveguide surface or incorporated in the polymer is tailored for either a broad pKa response for pH or a narrow pKa response for ammonia. The reference arms are comprised of the same polymer as the sensing arm, with an inert acid-base chemistry for the pH sensor or an unreactive pKa for the ammonia sensor.

The pH sensor employs amino acids as the acid-base sensing moiety. Amino acids have nominally two acid/base groups with the possibility of additional groups present in the side chain. Figure 9 shows the pH response of a covalently attached monolayer of glutamic acid. As a proton is removed from the glutamic acid, small dipole changes occur. The evanescent field couples with these dipoles producing a response. The response is small, .8π radians, over a 6-10 pH range.
But by placing a proton conducting polymer over the glutamic acid the signal is enhanced ten-fold. It is believed that increased charge separation producing a much larger dipole is the basis of the enhanced response. The polymer, poly(2-hydroxyethyl methacrylate), contains water. This allows for a proton cascade to take place within the polymer when the pH changes in the surrounding solution. This leads sequentially to proton abstraction from the glutamic acid. The counterion does not diffuse through the polymer but is retained outside creating the large charge separation. The enhancement brought about by the proton conducting polymer is shown in the comparison between polymer coated and uncoated glutamic acid in a response to a pH change in Figure 10.

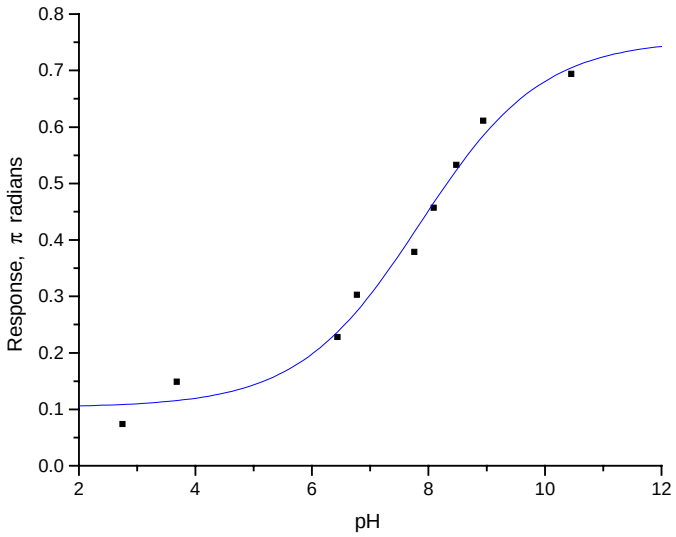

Figure 9. pH response of a monolayer of glutamic acid

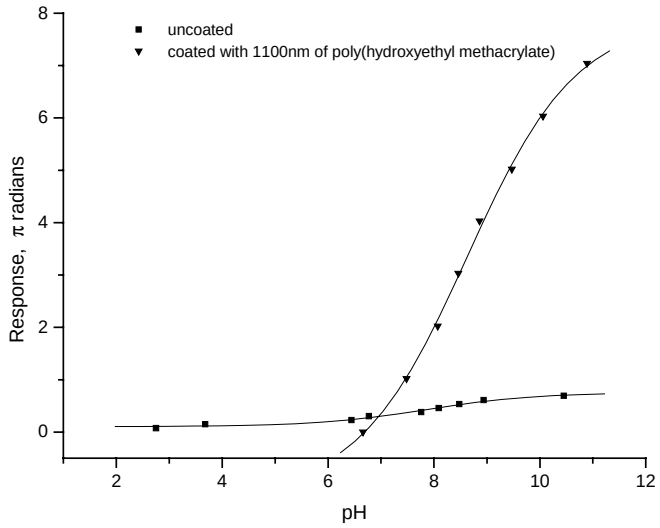

Figure 10. Comparison of coated and uncoated pH response of glutamic acid

The ammonia sensor uses two different pKa's. One arm of the interferometer has a pKa which lies above that of ammonia and on the other arm, a pKa that lies below that of ammonia. Ammonia will go into both arms equally but will react only with one to produce dipoles. A basic polymer, polyethyleneimine-80% ethoxylated, is titrated with citric acid, a triprotic acid that serves as both the sensing and the reference chemistries. The reference arm contains polyethyleneimine-80% ethoxylated titrated to pH 8.0 so that no acid protons are left on the citric acid. The sensing arm is titrated with citric acid to pH 6.0 leaving one proton on each citric acid capable of reacting with the ammonia. Ammonia reacts reversibly with the remaining citric acid proton. The response to various ammonia concentrations is shown in Figure 11. Sensitivities are in the sub 100 ppbv range and show that active chemistries provide substantial improvement over passive chemistries.

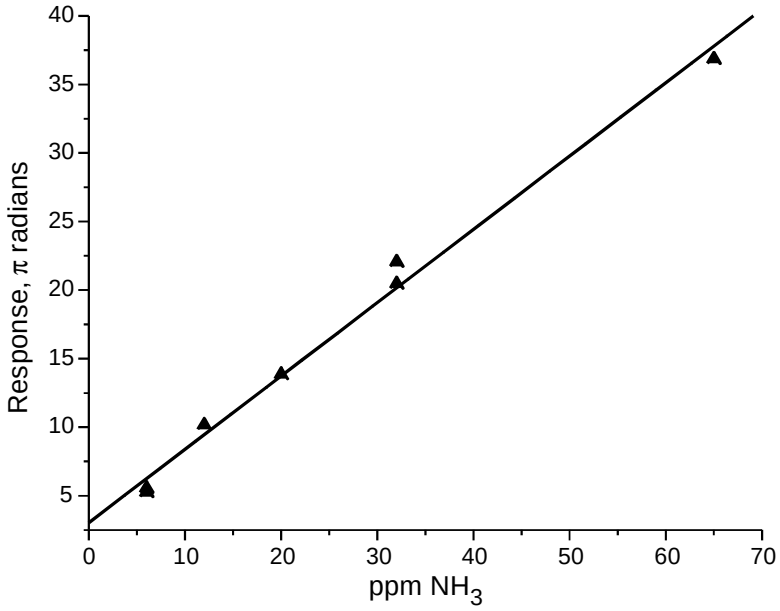

Figure 11. Sensor response to gaseous ammonia.

In Table I detection levels for various agents tested to date date using an IO interferometric sensor are summarized. Note these values do not represent lower limits, but reflect only the target values identified for the specific applications. In practice the sensitivity can be increased significantly by using phase locked detection schemes.

TABLE I
MEASURED DETECTION LIMITS FOR SPECIFIC ANALYTES

Chemical Agents		
Analyte	Aqueous Phase	Vapor Phase
Benzene	100 ppb	<1 ppmv
Xylene	<50 ppb	300 ppb
Toluene	100 ppb	<1 ppmv
TCE	<7 ppb	1 ppmv
Chloroform	<100 ppb	1 ppmv
pH	Range 0-14, $\pm$ 0.001 units	
Biological Agents		
Bacteria (*Salmonella*)		500 cfu/ml
Virus		$<10^4$ pfu/ml
Protein (Toxin)		10 pg/ml

5. SUMMARY

Integrated optic interferometric sensor systems have been developed to a prototype level suitable for field testing and evaluation. The IO sensor chip offers sensitivity, response and an affordable system package. The design of the IO chip provides a highly sensitive transducer providing chemical and biochemical sensing capabilities that are useful for both vapor and aqueous phase measurements. Detection of viral agents, bacterial agents, and proteins has been demonstrated at low concentration levels. Similarly chemical contaminants have been detected at ppbv for vapor phase and ppb levels for aqueous media. The IO sensor is capable of quantitative measurements and in many instances is suitable for real time in-situ measurements. Current sensor packages exhibit a volume of 2 inches x 3 inches by 6 inches. By going to silicon substrates and integrating elements such as detectors and signal processing circuits onto the wafer, future package dimensions can easily be reduced to pocket size instruments.

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