

**Foresight**

Infectious Diseases: preparing for the future

OFFICE OF SCIENCE AND INNOVATION

**S7: State-of-Science Review:  
Biosensors and Biomarkers**

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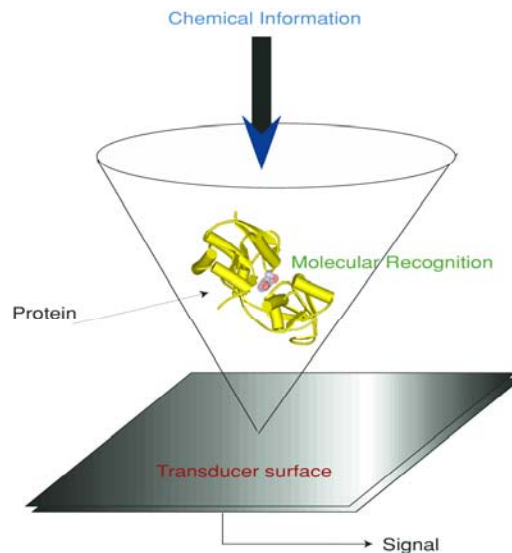
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## **References**

# 1 Introduction

information into electrical information via a process of biomolecular recognition (Figure 1). Operationally, we can characterise biosensors as being self-contained, portable and capable of unskilled/unattended operation (Cass 2002; Cooper and Cass 2004).

**Figure 1:** Biosensors act to convert chemical information to an electrical signal through a molecular recognition reaction on a physical transducer



The sensing or transduction element, although lying at the heart of the biosensor, is not the sole component of the device. 'Upstream' of the sensing element will be a sampling system, while 'downstream' we need signal conditioning, processing and transmission/storage elements. The sensing element in itself may also contain sample pre-treatment steps. These additional elements may move the biosensor towards a 'lab on chip' or micro total analytical system ( $\mu$ TAS), providing the operational characteristics referred to in the above paragraph are retained. But the distinction is of little importance to the end user.

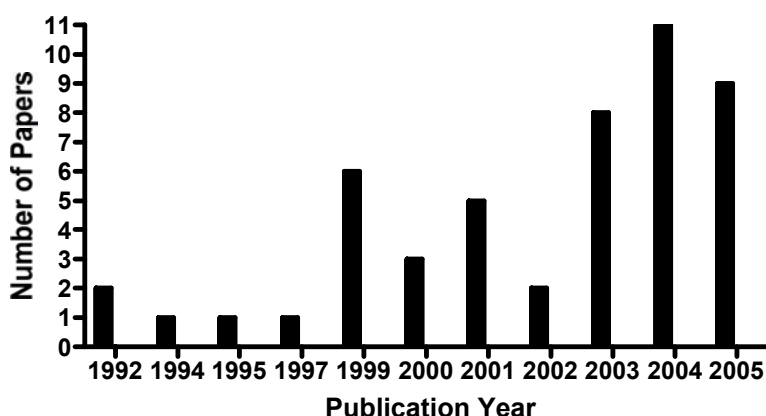
Later in this review, we will delve into more detail as regards to the molecules being sensed and the transduction methods available. At this stage, we make a particularly important distinction between single-use and continuous biosensors. Single-use biosensors are disposable devices to which a sample is added and a measurement made. They have the primary advantages that there are no concerns about sample carryover and contamination. Drift in the output of the sensor over time is not an issue, nor are the decrease in responsiveness due to fouling and loss of activity of the biological component. Single-use devices are typically employed where infrequent measurements are made (1–4 times per day or less).

Continuous biosensors, as their name implies, provide measurement results continuously (or at high frequency). Such devices need to be attached to the system being sampled and can suffer from problems of drift or loss of

responsiveness. Continuous biosensors are of particular use where long-term trends in analyte need to be followed or where rapid changes are anticipated.

The use of biosensors for the detection of infectious diseases is relatively recent. A search of the Web of Science with *biosensor* and *infectious* as keywords yielded 49 papers, with most of those (57%) having been published since 2003 (Figure 2). There have been previous reviews of biosensors for infectious or pathogenic micro-organisms, for example, Ivnitski et al. (1999) and Nakamura and Karube (2003).

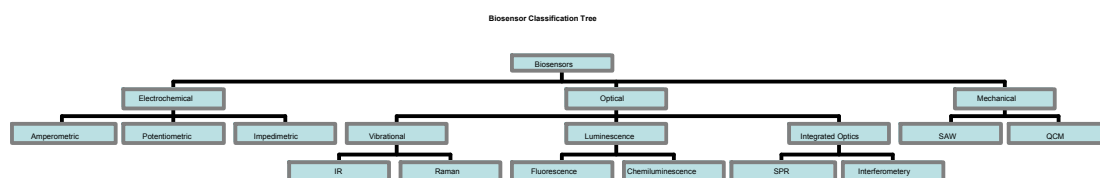
**Figure 2:** Distribution of papers found in the Web of Science with the keywords *biosensor* and *infectious*



### 1.1 Sensing modalities

An enormous range of physico-chemical transduction schemes has been employed in building biosensors. However, they can conveniently be classified into a biosensor ‘tree’, as shown in Figure 3. There is no necessary connection between the particular sensing modality shown in Figure 3 and the molecular recognition molecules discussed below.

**Figure 3:** The biosensor tree showing the different physical transduction most commonly employed in biosensors



A developing sensing modality is the use of arrays in which multiple sensors are combined into a single system. Sensing arrays may use a single modality with multiple different biorecognition molecules for multiple analytes or multiple modalities measuring the same analyte. In either case, advantages

accrue through increased richness of data, redundancy in the event of sensor failure and spatially distributed sensors.

## 1.2 Biorecognition molecules

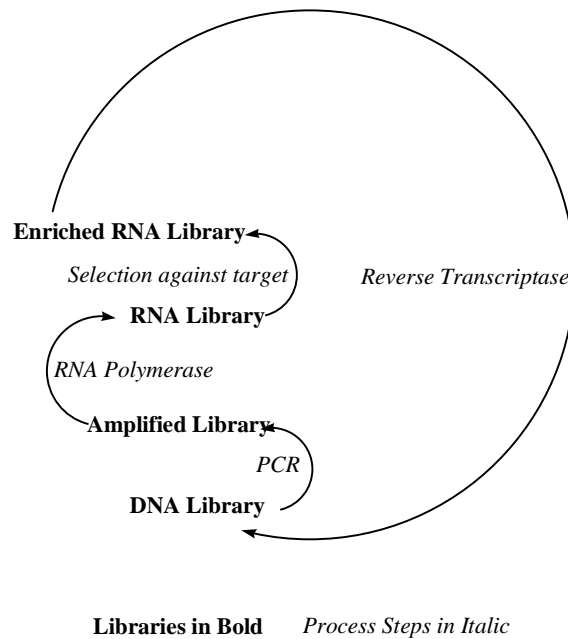
We can divide biosensors into two broad classes: *affinity* biosensors and *catalytic* biosensors.

In the former class, a non-covalent interaction between biomarker and recognition occurs that can give rise to a signal directly or can be 'developed' through a subsequent reaction. Catalytic biosensors use the chemical transformation of the biomarker as the source of signal. This distinction in turn determines the type of biorecognition molecule that can be employed. Affinity sensors require a biorecognition molecule that demonstrates a high affinity and specificity for the target biomarker. Classically, the commonest molecules of this class are antibodies as there is a long and well-established technology for generating antibodies against almost any desired target molecule.

The advent of recombinant DNA technology and antibody engineering opens up a plethora of modified antibodies, including single chain variable fragment antibodies (scFvs), bispecific antibodies and antibodies from species other than the conventional laboratory ones (both shark and camelid antibodies having been investigated in some detail). Recombinant DNA methods have also led to the engineering of other protein scaffolds with versatile binding characteristics. Among the more extensively investigated are Protein L and the so-called anticalins. These particular protein scaffolds offer the advantage of high stability when compared to conventional antibodies. Moreover, because they are selected for *in vitro*, they have advantages when the target ligand is of limited solubility or high toxicity. An additional advantage in using recombinant proteins is that they lend themselves to further modification through protein engineering approaches that can better tailor the molecules to sensing applications.

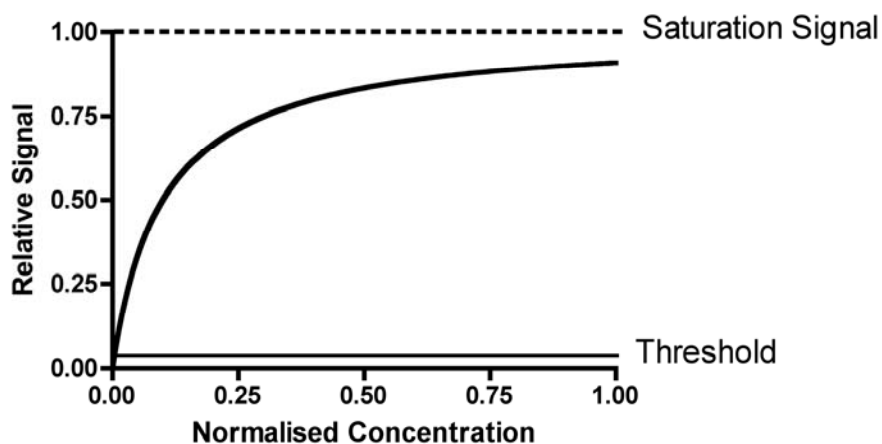
Alternative (bio)recognition molecules include RNA and DNA aptamers (Misono and Kumar 2005), molecularly imprinted polymers and templated surfaces (Hayden and Dickert 2001; Hayden et al. 2003). These materials have the advantage of being generally more stable than protein-based recognition molecules, and further work in this area is likely to result in improvements in specificity and affinity. DNA and RNA aptamers are particularly promising reagents as they can be prepared by automated synthesis; typical aptamer libraries can contain in excess of  $10^{15}$  different sequences. Given a particular target molecule, the library can be screened for particular sequences that exhibit an affinity for the target. The selection of binders is repeated a number of times (10–15) to enrich the library in a small number of sequences with the requisite affinity and specificity as shown in Figure 4. Interestingly, although there have been a number of publications on the use of aptamers for neutralising infectious agents, they have been little used, if at all, as diagnostic reagents for their detection.

**Figure 4:** Screening an aptamer library to select members with defined specificity and affinity



Catalytic biorecognition molecules initially undergo the same process of non-covalent complex formation. The complex then undergoes a reaction whereby the biomarker is transformed into a product, and it is this transformation that generates the signal. In both instances, the formation of the non-covalent (reversible) complex leads to a hyperbolic signal vs. analyte concentration curve (Figure 5).

**Figure 5:** Biosensor signal vs. concentration curve showing the threshold and saturation values



Unlike many biosensor applications where measurement of the concentration is important, biosensors for infectious diseases typically only need to indicate the presence or absence of the target marker. The signal saturation seen in

Figure 4 is less important than threshold detection. However, saturation may be important where the 'normal' levels of the marker are close to the saturation value making it more difficult to reliably assess an increase.

Biocatalysts commonly used in biosensors are either purified enzymes or whole cells/tissues. The latter are often easy to obtain but suffer from problems of cross-reactivity and the difficulty of controlling the loading of activity on the biosensor surface. Although widely used in biosensors for low molecular weight analytes, catalytic biorecognition molecules in the identification of infectious diseases are likely to be less common than affinity biorecognition molecules. The basis of this assertion lies in the observation that low-molecular-weight biomarkers for infectious agents/host responses have been less intensively investigated than high-molecular-weight biomarkers. Nevertheless, unique low-molecular-weight biomarkers in this context could offer an extra dimension to diagnosis. Like immune signatures, it may be the pattern of a variety of low-molecular-weight markers that provides a distinctive identification. There is some precedent for this idea where the markers are volatile molecules which are well known to be of use as a diagnostic for bacterial colonies. Examples here include tuberculosis, urinary tract infections and bacterial vaginosis.

### **1.3 Sampling**

Reliable sampling is often the key to accurate measurement and takes account of the ease and convenience of drawing the sample, the concentration of target analyte and the presence of background or interfering substances. The role of sampling is twofold: to draw material for analysis and to present this to the sensor for subsequent measurement. Detection of disease will be based on two broad sampling strategies, either: fluids drawn from the individual organism being assessed; or ambient sampling of the atmosphere.

Individual sampling is the more direct, makes a link between infection and measurement and has an element of pre-concentration by the individual. A number of more or less invasive samples can be taken, the choice depending on both the pathology of the infection and the acceptable level of invasiveness.

Ambient sampling has attracted considerable interest in the context of providing an early warning (e.g. in the event of a bioterrorism attack). The levels of infectious agent will be orders of magnitude lower in an ambient sample and the background will be much more variable. Both of these factors place considerable demands on both sensor performance (especially in terms of limit of detection and specificity) as well as on sample acquisition (Settles 2005).

Between the individual and ambient sampling regimes is the so-called 'portal sampling' whereby the local atmosphere around a single individual (or small group) is analysed (Figure 6).

**Figure 6:** A portal for explosives detection that pre-concentrates and analyses air samples (Courtesy Barringer Industries)



Individual sampling can be non-invasive (e.g. exhaled breath (Robinson et al. 1999), urine (Guernion et al. 2001) or stool samples), minimally invasive (e.g. throat swab, sputum or microneedle sampling (Zahn et al. 2005)) or fully invasive (e.g. blood samples).

#### **1.4 Biomarkers**

The molecular recognition that occurs within the sensing element requires the disease-indicating biomarker and the recognition molecule to undergo some kind of reaction that generates a specific signal. Recognition molecules are generated based on a knowledge of the relevant biomarker, which can be either pathogen- or host-derived (He et al. 2003; Yi et al. 2004; Zhang et al. 2005). The former are likely to be highly specific, while the latter may be either specific or non-specific. Often host-derived biomarkers also offer an amplification advantage in that many molecules of the host response marker may be generated for each infectious particle. Biomarkers can be broadly classified into nucleic acids, proteins and low-molecular-weight metabolites.

Pathogen-derived biomarkers can be highly specific. But there is still considerable debate about the utility of host-derived biomarkers (with the exception of circulating antibodies against the pathogen) in that many of those proposed have been shown to be quite non-specific, in many cases indicating a stress response rather than a pathogen-specific response. In the latter case, panels of biomarkers may offer increased discriminatory power.

In addition to antigenic molecules as biomarkers, the sensing of particular enzyme activities derived from infectious agents is also a valuable diagnostic aid. Here, examples include the high-urease activity in *Helicobacter pylori* or the  $\beta$ -D-glucuronidase activity of *Escherichia coli*. Host-derived changes in enzyme activity that have been used as general indicators of bacterial infection include serum lysozyme and myeloperoxidase, as well as 'stress proteins' such as interleukin-6 and C-reactive protein. Host-derived biomarkers, although useful as indicators of infection, are less suited to identifying a particular infectious agent, even though they may be capable of distinguishing bacterial and viral causes.

Nucleic-acid-based biomarkers (pathogen-derived) are, in principle, extremely powerful both in terms of the accuracy of their identification and the limit of detection that can be achieved using polymerase chain reaction (PCR) amplification. Rapid PCR equipment has made great strides in the last few years, and there has been considerable effort put into integrating PCR methods into  $\mu$ TAS devices. The thermal cycling intrinsic to PCR means that the rapid heat transfer characteristics of  $\mu$ TAS devices fits particularly well and offers very short heating and cooling cycles resulting in rapid amplification. Chip-based PCR formats include both flowing and non-flowing. In the former, the reaction mixture is moved in channels between different temperature zones. Chip-based, non-flowing methods are closer to conventional PCR, where a stationary sample is cycled in very small volumes. Both approaches have been recently reviewed (Zhang et al. 2006). This chip-based approach means that nucleic-acid-based biosensors can be effective generic platforms, although they cannot distinguish between live and dead cells and the nucleic acid may be rapidly degraded in the latter case.

## **2 Biosensor platforms**

The sensor tree shown in Figure 3 illustrates the wide range of sensing mechanisms that have been coupled to molecular recognition. In this section, we look at these in more detail, in the specific context of detecting infectious pathogens. All of the biomarker types described in Section 1.4 have been detected using biosensors, although there are inevitable trade-offs between ease of use, speed to result and detection limit. Each sensing modality will also be differently sensitive to background interferences.

### **2.1 Electrochemical sensors**

The broad class of electrochemical sensors encompasses both amperometric and potentiometric devices. The former measure the flow of current due to the oxidation or reduction of a redox active species at the electrode under the influence of an applied potential. The latter measure the potential difference between two phases as a consequence of the difference in ionic concentrations in the two phases.

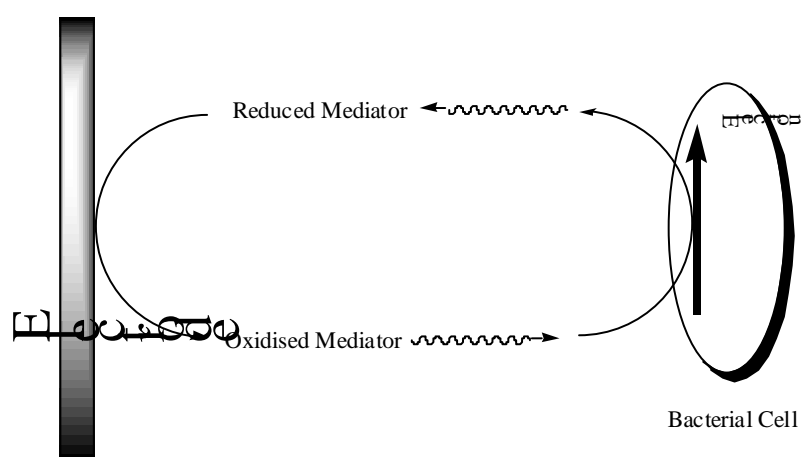
A familiar example of an amperometric sensor is an oxygen electrode where the oxygen concentration is determined by its reduction at a platinum

electrode. The archetypal potentiometric sensor is the pH electrode. These base chemical sensors are then modified by coating them with biomolecules.

### 2.1.1 Amperometric biosensors

These devices have been used for detecting micro-organisms by coupling the electrode to the cellular electron transport chain via low-molecular-weight *mediators*. These are redox species that transfer electrons between the electrode and a redox active biomolecule via an alternate oxidation/reduction reaction (Figure 6). While such an approach can certainly detect bacteria, its detection limit is rather high and the method has limited capacity to differentiate different bacteria.

**Figure 6:** Mediators as electron shuttles between an electrode held at a given potential and redox active biomolecules



The discrimination could be enhanced by using a capture molecule such as an antibody (Muhammad-Tahir and Alocilja 2003), lectin (Ertl et al. 2003) or aptamer directed to a cell surface marker to enrich the target organism on the electrode surface and then to detect the viable cells through the mediated scheme shown in Figure 6. Such an approach would have improved selectivity due to the close proximity of the target cells to the electrode plus the amplification offered by the mediator recycling.

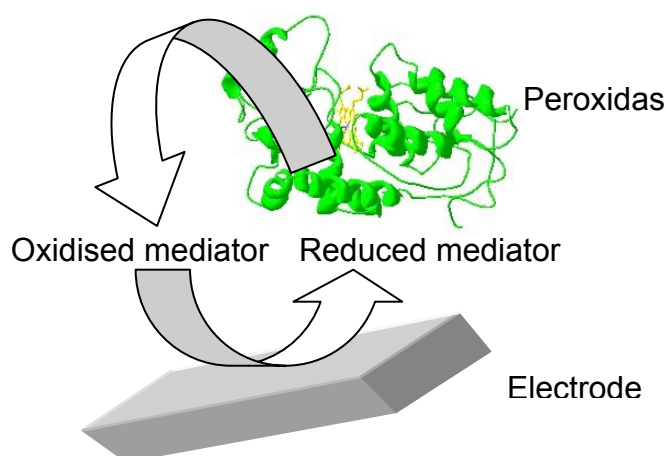
Electrochemical sandwich immunoassays have been used in this fashion, whereby the cells are captured on the electrode through a specific antibody and then stained with a second, peroxidase-conjugated antibody, that generates an electroactive product (Muhammad-Tahir and Alocilja 2004) (Muhammad-Tahir and Alocilja 2003; Safina et al. 2005). Peroxidase is a good enzyme label in this application as it is readily detected electrochemically through the production of an oxidised product that can be electrochemically reduced, thereby generating a catalytically enhanced current (Figure 7).

These approaches are essentially conventional binding assays that use an electrochemical endpoint rather than an optical one. As such, they suffer from

many of the disadvantages of binding assays that use a label to detect the reaction (label-free methods will be covered later in this review), namely, primarily the complex multistep procedures of incubating and washing combined with the unfavourable geometry often found in traditional immunoassay formats such as those based on microtitre plates.

Many of these problems should be highly amenable to automation through microfluidics. Microfluidic devices (also called 'lab on a chip') exploit the rapid heat and mass transfer that occurs in microchannels to automate and miniaturise procedures as well as to achieve unparalleled integration. The price that is paid for such miniaturisation is low signal levels arising from detection volumes that may only be a few pence. The use of microfluidic devices will be discussed later in this review as they are generally applicable to many types of signal generation and readout.

**Figure 7:** Peroxidase-mediated electron transfer



Peroxidase is also a useful host-derived biomarker for bacterial infections, which can be measured amperometrically in an exactly similar way to when it is used as a label. Salivary peroxidase levels have been measured using an amperometric electrode as an indicator of periodontal infections (Ivnitski et al. 2004).

Amperometric methods have also been used extensively in DNA biosensors (Drummond et al. 2003) to detect hybridisation of a probe strand on the electrode to a target strand in solution (Caruana and Heller 1999). These methods, although giving good limits of detection, especially when combined with nucleic acid amplification methods such as PCR, necessitate considerable pre-processing of the sample (cell lysis, DNA extraction, DNA denaturation). Here, again, microfluidics should offer a higher level of integration (Cady et al. 2005; Zaytseva et al. 2005).

Where better detectability is required than can be provided by current measurements, a hybrid measurement platform – electrochemiluminescence (Yamaguchi et al. 2001) – offers the advantages of sensitive light detection with electrochemical generation of the excited state. Typically, ruthenium complexes are used as a label, and the technique is suitable for both antibody- and nucleic-acid-based methods.

### **2.1.2 Potentiometric biosensors**

Again, these have been most widely used to detect the product of an enzyme label in an immunoassay or DNA hybridisation assay, and the logarithmic relationship between potential and ion concentration offers a very large dynamic range. The particular type of potentiometric sensor that has been most widely applied is the ion-sensitive field-effect transistor (ISFET), which comprises a metal-oxide semiconductor field-effect transistor (MOSFET) with a chemically sensitive gate rather than a gate electrode (Wroblewski et al. 2004; Martinoia et al. 2005). ISFETs are potentially the most integratable of the sensing platforms as the silicon processing allows circuit elements to be incorporated on chip (Chung et al. 2004; Milgrew et al. 2004; Stroschio and Dutta 2005). Moreover, by operating in the 'weakly inverted' region of the i-V curve, considerable power savings can be realised, an important consideration where remote monitoring is foreseen (Shepherd and Toumazou 2005). A further consideration with ISFETs operating in this region is that they are highly amenable to analogue processing methods, and here, too, power consumption can be much reduced. Moreover, analogue processing allows one to take advantage of the common underlying physics that relate the (Nernstian) interfacial chemistry to the underlying device physics (Shepherd and Toumazou 2005).

A variant on the ISFET, also based on silicon fabrication, is the light-activated potentiometric sensor (LAPS) (Tu et al. 2002; Schoning 2005). In this device, the silicon responsiveness to a change in pH is modulated by light. Using the LAPS device to determine the urease activity conjugated to an antibody after immunofiltration, the instrument could be used to detect Newcastle disease virus (Lee et al. 2000).

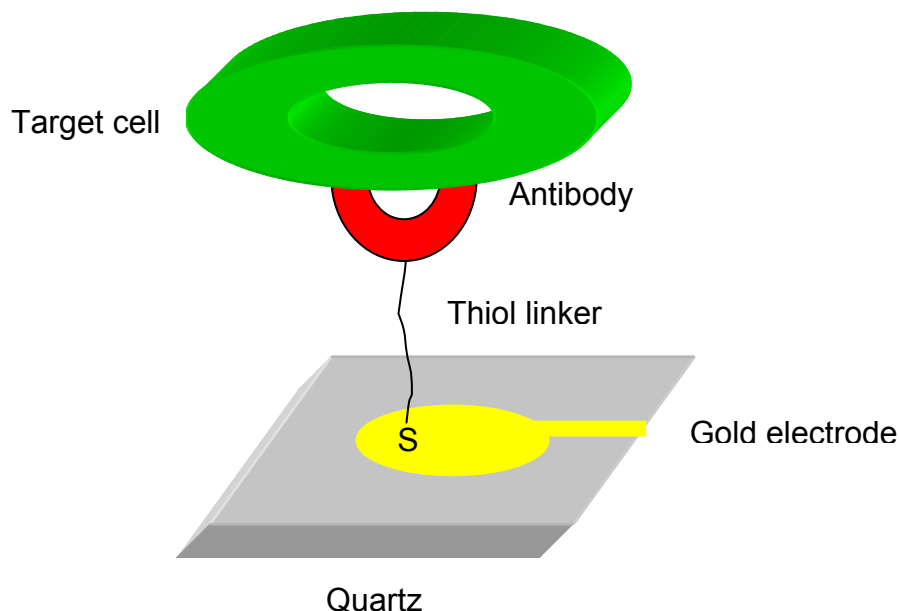
A very different approach to potentiometric sensing has recently been described, whereby a pair of nanogap gold electrodes are fabricated on a silicon dioxide/silicon substrate and the surface covered with a monolayer of gold nanoparticles coated in a specific antibody against hepatitis C virus (HCV). The current flowing between the pair of electrodes is small (<1pA). However, when a 'sandwich' of HCV and a second gold nanoparticle antibody conjugate is deposited on top, there is a significant increase in current and a change in the IV curve of the nanogap electrodes (Tsai et al. 2005). As the size at which nanotechnology can now create defined features is comparable to that of viruses, it is very likely that further devices based on this size similarity will be developed. Some indications are that the earliest of these will be based on synthetic or semi-synthetic nanopores, where specific molecular interactions within the pores act to 'gate' the flow of ions through them, causing a transient 'blockade' of the pore and an increase in its resistance (Jia et al. 2002; Nakane et al. 2002). This principle has been used for many years

on the micron scale in the form of the Coulter counter, wherein the transit of a cell through a narrow orifice is detected through a sudden and transient increase in resistance. On this scale, there is no indication which cells are being counted as there is no molecular discrimination. In contrast, the nanopores have a *molecular gating* and are therefore capable of giving molecular information (Winters-Hilt and Akeson 2004).

## 2.2 Mass-sensitive (mechanical) sensors

Any binding reaction occurring on a surface will give rise to a mass change, and several instruments have been developed to measure these very small fluctuations in mass loading. Target analytes for mass sensing can be either molecular components, such as proteins or nucleic acids, or whole cells. The quartz crystal microbalance (QCM) illustrates mass sensing devices. The sensing element consists of an AT cut quartz crystal with a gold electrode deposited on its surface. When an alternating voltage of the right frequency (typically in the MHz region) is applied to the electrode, a mechanical oscillation is set up in the quartz crystal, the resonant frequency of which depends on the mass loading. When the latter shifts due to the target binding at the surface, so does the resonant frequency. Typically, the gold electrode provides the surface onto which antibodies are immobilised, often via thiol-containing self-assembled monolayers, as shown in Figure 8.

Figure 8: **The quartz crystal microbalance as a sensor for bacteria and viruses**



The detection of cells by the QCM is rapid and requires no labelling of the target. It does, however, suffer from a lack of sensitivity (typically  $10^4$  cfu/ml) (Hayden et al. 2003; Su and Li 2004; Lee et al. 2005; Pohanka and Skladal 2005). As in many of the biosensor platforms, the measurement principle is quite generic. The specificity for particular bacteria, spores or viruses depends

to a very great extent on the characteristics of the capture molecules (specificity, affinity and stability). One important characteristic of the QCM (and some other mechanical sensors) is the damping of the oscillations in aqueous solution, which means that, for the highest detection capability, the measurement of resonant frequency was often made in air. Recently, systems that also perform well in aqueous solution, using microfluidic channels, have been described (Godber et al. 2005) and the energy dissipation (damping) can itself contain useful information (Edvardsson et al. 2005)

Other mass-sensitive mechanical sensors include surface acoustic wave (SAW) (Berkenpas et al. 2004) and Love-wave devices (Branch and Brozik 2004). Considerable improvements in detection limits can be achieved with mass sensors if they are reduced in size, and cantilevers have proven to be particularly effective in this regard. Microfabricated silicon nitride cantilevers have been used to detect both single cells of *E. coli* O157 (Ilic et al. 2001) and single virus particles (Ilic et al. 2004; Louie 2004) where the signal generation is based on detection of the bending stress in the cantilever under the influence of an applied mass.

A somewhat different use of mechanical/acoustic coupling to measure binding reactions is to increase the amplitude of the vibrations and effectively shake off the bound molecule and measure the acoustic energy released (Cooper et al. 2001).

## **2.3 Optical sensors**

Optical sensing platforms are among the most diverse. They comprise integrated optical devices, spectroscopy-based platforms and scattering methods. The development of optoelectronic components, driven by the telecoms industry, has had a major impact through the provision of compact light sources and detectors as well as optical fibres (Kostov and Rao 2000). In this area in particular, the distinction between 'classical' biosensors and microfluidic devices is particularly blurred.

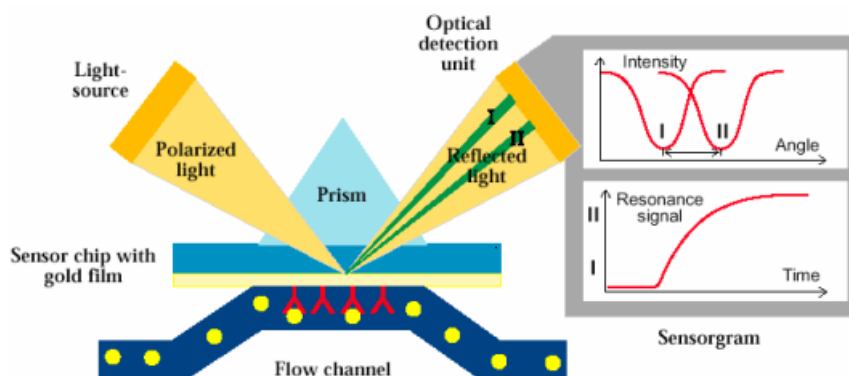
### **2.3.1 Integrated optical sensors**

In many ways, these are the optical equivalents of the mass sensors described above, being responsive to changes in the mass on the sensor surface. However, rather than using the mechanical properties of the underlying transducer, they are based on the refractive index of the sensing layer.

One of the first examples, and still the most popular, is surface plasmon resonance (SPR) (Oh et al. 2005; Perkins and Squirrell 2000). In SPR, the key component is a dispersion element (a prism or grating) onto which is coated a thin metal film (usually 100nm of gold). When light is shone onto the grating at a certain angle of incidence, the momentum of the photons is converted into a collective motion of the electrons in the metal called surface plasmons. This results in a decrease in the intensity of the reflected light. The angle of incidence at which resonance occurs depends on the optical thickness (essentially refractive index) of the layer that is within 300nm of the

diffraction component surface (the evanescent field). SPR measurements in the laboratory are often made with the chip in a flow cell to allow for controlled delivery of reagents and sample (Figure 9).

**Figure 9:** A surface plasmon resonance ‘chip’



A variety of formats have been described for SPR instruments, including planar and optical-fibre-based instruments. The relatively short penetration depth at which the SPR measurement occurs means that, when being used to directly detect bacteria, most of the cell will be outside this region and hence sensitivity is relatively low. SPR has proved more effective in detecting either host responses (from antibody profiles or more general immune signatures) or specific biomarkers derived from an infectious agent.

In a rather different format that allows the evanescent wave to penetrate the entire volume of an immunocaptured bacterium, (Malins et al. 2004) (the ‘metal-clad leaky waveguide sensor’) an improved limit of detection of about three-fold over SPR was measured (Zourob et al. 2005).

Interferometry has also been employed in a similar fashion (Schneider et al. 1997).

As with all ‘label-free’ sensing formats, non-specific binding is a constant consideration. Lack of labelling can significantly simplify the assay. However, any species that binds non-specifically to the surface will bring about mass or refractive index changes and so must be minimised. This is of particular importance where the target analyte is being determined in the presence of a large excess of background molecules. In a comparative study investigating the relative sensitivity of SPR and QCM in detecting *E. coli* O157: H7, the latter had the better performance with a detection limit of  $10^5$  cfu/ml compared to  $10^6$  cfu/ml (Su and Li 2005).

### 2.3.2 Optical absorbance/reflectance sensors

The absorbance or reflectance of visible light makes for a very simple sensing format, as the readout system (at least qualitatively) is the human visual system. Until relatively recently, the usual labels for such a readout would be

dyes, often in the form of dye-loaded beads. This still forms the basis of a powerful and simple immunodiagnostic platform: the lateral flow immunoassay. Developments in nanotechnology have led to the introduction of new materials based on metal nanoparticles, where light is absorbed or reflected through interactions with surface plasmons in the metal (Jain 2003; Emerich 2005; Fortina et al. 2005; Jain 2005; Kricka et al. 2005; Vaseashta and Dimova-Malinovska 2005).

The use of lateral-flow immunodiagnostics is well established in many areas, most notably pregnancy and fertility testing. The instrumentation necessary to make a numerical, rather than visual assessment of the result is now sufficiently cheap (by the standards of developed nations) to be 'bundled' with the test strips. Once the results are captured in a digital form, they can be stored (as an example for trend analysis), transmitted and archived.

Functionalised nanoparticles have been used as sensing elements and undergo a variety of interactions with visible light.

Fluorescence will be discussed in the next section, so here we confine the discussion to resonance light scattering (RLS). Under comparable conditions, particle-based RLS appears to offer a significant detection advantage over fluorescence when probing isolated molecular interactions, and it seems likely that this would also translate to viral and possibly bacterial detection schemes (Aslan et al. 2005).

### **2.3.3 Fluorescence sensors**

Fluorescence is an immensely versatile sensing modality which has been extensively employed in many detection schemes. Most often, fluorescent materials, either organic dyes, semiconducting quantum dots or nanoparticle entrapped rare earth complexes, have been used as labels to quantify the amount of material present in a binding assay. Here, again, antibodies directed to surface antigens and labelled with a fluorescent reporter moiety have proven to be the most popular approach. In developing this into a complete biosensor device, the work of Ligler and her colleagues at the Naval Research Laboratory (NRL) in Washington DC has resulted in field deployable devices. Although aimed primarily at biowarfare agents, the platform is sufficiently generic to be applicable to many other infectious organisms (Rowe-Taitt et al. 2000). The NRL system uses coated optical fibres in a sandwich format, relying on the evanescent field to couple light into and out of the fibre and to reject any fluorescent molecules not within 250nm of the surface, thereby avoiding the need to wash away excess detection antibody (Figure 10).

**Figure 10: An evanescent waveguide-based sandwich fluoroimmunoassay**

QuickTime™ and a  
TIFF (LZW) decompressor  
are needed to see this picture.

This work has recently been extensively reviewed and its advantages, limitations and future challenges clearly described (Taitt et al. 2005).

Antibodies are not the only molecules that can be used as fluorescent sensors, Gram-negative bacteria can be identified through the presence of lipopolysaccharide (LPS) and lipid A (LA) (the bioactive component). Mutants of green fluorescent protein (GFP) were created that bound to LPS/LA and showed a quenching of the GFP fluorescence in both live cells and endotoxin-contaminated samples (Goh et al. 2002a, 2002b). Such engineered fluorescent proteins are likely to become more prevalent in the future as they offer a single reagent approach which obviates the need for the additional washing and incubation steps typical of sandwich immunoassays.

Quantum dots (QDs) have attracted a lot of interest since their discovery as replacements for organic fluorescent dyes in view of their narrow emission peaks, resistance to photobleaching and excitation by white light. Early work on their use as biological labelling reagents was hampered by a lack of stability in aqueous solutions, but this has now been largely overcome. In a recent paper, Hahn et al. (2005) showed that, when conjugated to a specific antibody, they were capable of detecting a single *E. coli* O157: H7 cell and distinguishing it from a common laboratory strain. The detection limit was around two orders of magnitude better than for a fluorescent dye in the same assay format. Others have used lectin-conjugated QDs to label the surface glycoproteins of bacteria (Rothert et al. 2005). Silica nanoparticles containing luminescent materials showed a similar capability to detect single bacterial cells (Tan et al. 2004).

In a report in late 2004, it was shown that QDs conjugated to either aptamers or antibodies display a dramatic wavelength shift (>100nm) when bound to bacteria (Dwarakanath et al. 2004).

Another area of spectroscopy capable of great limits of detection as well as being very rich in data is Raman scattering, particularly in its surface enhanced mode (that is, surface-enhanced Raman scattering, SERS). It has been used to detect and identify micro-organisms in a non-invasive fashion (Rosch et al. 2003; Harz et al. 2005; Premasiri et al. 2005; Rosch et al. 2005; Zhang et al. 2005), and compact Raman instrumentation is currently being developed by a number of companies.

### 3 Array sensing

Conventionally, the identification of micro-organisms is based on the determination of one or a small number of biomarkers. Where multiple organisms are to be detected, some multiplexing of a biomarker panel is carried out. The question that arises is whether a much larger multiplex confers any advantages in the detection and identification of infectious diseases.

Multiplex assays on a single sample are usually achieved through a set of spatially distinct measurements in an *array*. We can broadly distinguish two types of array: those where each element detects a specific component of the sample and each component reacts with a single element of the array; and those with elements that react with multiple components of the sample and where each component reacts with multiple elements. These cross-reactive arrays have a sensing modality akin to that of the olfactory system, and interpretation of their outputs requires complex data processing. The one-to-one arrays have been typically used for DNA and, more recently, protein analysis, while the cross-reactive arrays have been used for low-molecular-weight biomarkers. There has been a long tradition of diagnosis from detection of exhaled volatiles and this method is being further developed using an array of instrumental methods.

#### 3.1 DNA and protein microarrays

DNA microarrays have been used to genotype pathogens and evaluate antibiotic resistance (Chen et al. 2005; Stabler et al. 2005), and also to evaluate changes in host-gene expression in peripheral blood cells. It has been proposed that such an approach could be an early warning of a developing disease, either as a result of an epidemic or a hostile act (Lin et al. 2003). Protein microarrays have been used to follow changes in host (human) proteins in response to a SARS infection (Yip et al. 2005), while lectin microarrays coupled to electrochemical detection have been used to distinguish different strains of *E. coli* (Ertl et al. 2003).

#### 3.2 Chemical and device microarrays

Chemical, and more generally 'device', arrays sacrifice some of the specificity of biological arrays for the robustness and fabrication capabilities of wholly synthetic materials (Milgrew et al. 2004). Chemical arrays, often referred to as 'electronic noses', have gone in and out of popularity as tools for analysing infectious organisms based on profiles of volatile metabolites. There are numerous examples in the literature of this approach, which seems to be undergoing something of a renaissance at the time of writing this review (Guernion et al. 2001; Hockstein et al. 2005).

## 4 Microfluidics

As discussed in the introduction, although the biosensor is a key component in the detection and identification of infectious diseases, if it is to move from the laboratory/hospital to community or personal care, a systems approach to device design will be needed. Microfluidics will be a key element in terms of providing the link between the sample and the sensor, as well as providing a means for sample concentration, pre-treatment and calibration. Indications from the current literature show the direction in which work in this area is going. An example is the combination of microfluidics with PCR, lateral-flow separation and upconverting phosphor labels described by (Wang et al. 2006) using genomic DNA from *Bacillus cereus* as the test sample.

Microfluidics devices can also be used for the miniaturisation and automation of 'conventional' micro-organism detection methods. A recent example of this latter approach is a microfluidic device for DNA isolation amplification and detection from spores in a fully autonomous fashion (Song et al. 2005). A liposome amplification scheme has been incorporated into a microfluidic device for pathogen detection (Zaytseva et al. 2005). The incorporation of a fluorescence sensing scheme into a standard semiconductor fabrication process (complementary metal-oxide semiconductor or CMOS) also used for ISFET fabrication, nicely illustrates the move towards higher levels of integration (Song et al. 2005). The same group has also described an integrated capillary electrophoresis device (Song et al. 2003).

## 5 Connectivity

Unlike most other diagnostic measurements, the diagnosis of an infectious disease has societal as well as individual implications. Once a biosensor has generated an electronic signal that carries information about the biomarker(s) present in the sample, this has implications for what happens to the data. Measurement of the biomarker does not place the quantitative demands on the analysis that many other clinical diagnostics have, offering the opportunity to do most of the data processing locally, either on the device itself or on a PDA or computer to which the sensor device would communicate wirelessly (Hammond et al. 2005). The concentration determined by the biosensor would be reduced to a low, borderline or high output. Similarly, if trend analysis were desirable, this could be done 'on device', and the output would be decreasing, constant or increasing.

An important aspect of connectivity where multiple measurements are made is in the area of decision support i.e. the automated interpretation of data to aid in reaching a conclusion about the cause of the observed data. Decision support systems have been developed for the identification of infectious diseases and in 2004 the Centers for Disease Control (CDC) published an assessment of both detection and decision support systems in the context of bioterrorism (Bravata et al. 2004). One example of a web-based decision support system that integrates clinical data, microbiological information and geographical data is GIDEON (Berger 2005). Currently, this relies on

physician-entered data, but it can be readily envisioned that automated entry of sensor data would allow for a surveillance and prediction capability to be added.

## **6 Conclusions**

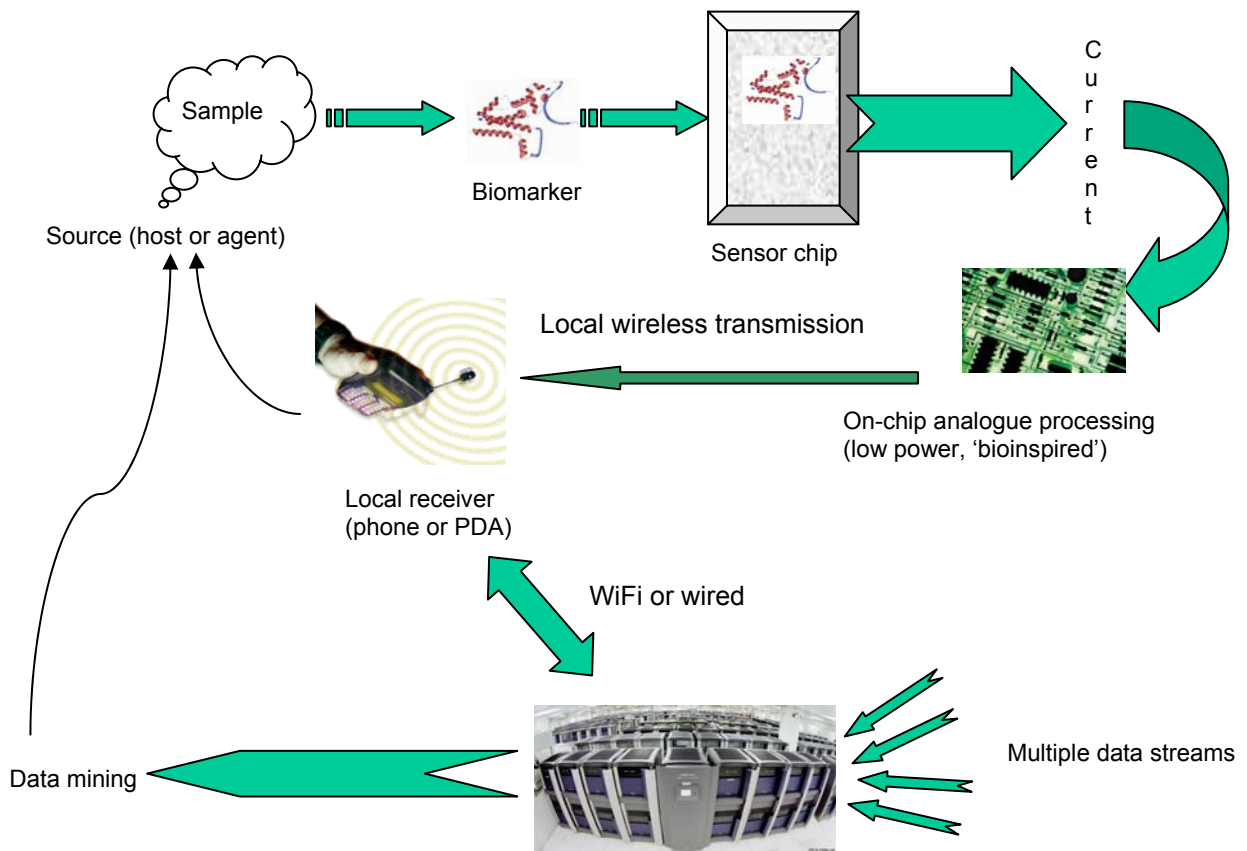
Although they have been established for some 40 years and commercialised on a large scale for 15 years, the widespread use of biosensors has been limited to a small number of applications. Recent technology developments in protein engineering, nanotechnology, microfabrication and wireless telecommunications are revivifying this field, while advances in genomics and proteomics are identifying new biomarkers for disease, including infections.

Two major technology trends are likely to have the biggest impact on biosensors of infectious disease diagnosis and monitoring. The first of these is nanotechnology. The recent publication of several reviews on the role of nanotechnology in diagnostics points in this direction (Emerich 2005; Fortina et al. 2005; Jain 2005; Kricka et al. 2005; Rosi and Mirkin 2005; Vaseashta and Dimova-Malinovska 2005) and a discussion of the factors that influence what sort of detection limits that can be achieved has recently been published (Sheehan and Whitman 2005).

The second key technology is the increasing interconnectedness of electronic devices that will offer the potential for the automated measurement, tracking and treatment assessment of infectious diseases. The convergence of these developments is summarised in Figure 11.

This technology, however, comes at a price, both in cost terms and societal impacts, and its adoption will depend on factors beyond the simply technological.

**Figure 11:** The integration of technologies for advanced biosensing, decision support and data fusion



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