# 15 Immunological Biosensors

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A principal impetus for developing biosensor systems has been the need to produce a simple, very rapid, sensitive, and easy-to-use analytical system that does not need trained specialists to produce results. An aim for many applications is to develop a small, portable unit into which the test sample can be applied directly, without pre-processing. The result should be obtained within seconds, and the answer should not be subject to interference or modification by the test-sample matrix, the user, or environmental factors. Sensors that use immunological detection methods are among the most advanced type of biosensor technologies due to the ubiquity of traditional immunoassay techniques and because high-detection specificity is readily obtained with receptor–ligand interaction chemistry.

Traditional 'rapid' tests for blood glucose, fertility hormones, and drugs of abuse are well proven and cost effective. Immunosensors have become more important in recent years as a result of progress in **point-of-care testing (POC)**, most of which are 'rapid' immunoassay technologies, and due to an increased focus on monitoring environmental biohazards.

POC technology made notable strides with the introduction of emergency room rapid assays for cardiac stress proteins (troponins, B-type natriuretic peptide – BNP) and in-clinic tests for infectious agents. Biosite Inc. developed a commercially successful emergency room POC assay for BNP, a biomarker for heart failure, as one recent example for acute cardiac care. Manufacturers are now marketing quick, low complexity assays of this type for a battery of cardiac markers. Several *in vitro* diagnostic companies offer a rapid optical immunoassay test designed to diagnose influenza A and B infection in the doctor's office to aid in prescription of neuraminidase inhibitors. (Mahutte, 1998; Key *et al.*, 1999; Tucker *et al.*, 2001; Azzazy and Christenson, 2002; Rodriguez *et al.*, 2002)

Detection of chemical and biological warfare agents is a primary driver of sensor development and many prototype detectors in this area utilize receptor–ligand or enzyme–reporter formats. One significant example is the work of Ligler and colleagues, who have produced an automated, portable, multi-analyte optical array biosensor for real-time biohazard detection. This sensor can detect a variety of chemical toxins and infectious agents in a complex background such as human serum. Applications for immunosensors related to biohazard detection also include pollution monitoring, food safety and industrial process control. (Iqbal *et al.*, 2000; Billman *et al.*, 2002; Sapsford *et al.*, 2004).

Continuous, *in vivo* sensing for diagnostic monitoring and drug delivery is probably the most technically demanding application for immunosensors and as such this field is in its infancy. A notable commercially-produced *in vivo* sensor is the Medtronic MiniMed Continuous Glucose Monitoring System. This prototype system consists of a subcutaneous sensor and an external monitor; in clinical studies it improved patient's glycemic control and lowered their hemoglobin $A_{1C}$ values. A similar but non-invasive sensor is the Glucowatch Biographer marketed by Cygnus. This device uses iontophoresis to extract sample through the skin and standard amperometric enzyme detection to measure glucose concentration. Both devices have received FDA approval but limitations in their stability, accuracy and longevity prevent widespread use. (Garg *et al.*, 2004; Kubiak *et al.*, 2004; Steil *et al.*, 2004; Abel and von Woedtke, 2002).

It is impossible to describe succinctly the rapidly developing field of immunosensors, so in this chapter we will focus on the core technical categories and provide a glimpse of especially promising nascent techniques and commercially significant efforts. Many excellent reviews exist for the reader who wishes a more in-depth discussion of a specific technology. Surface plasmon resonance (SPR) techniques, a very important sub-class of immunosensors, will not be addressed as they are treated at length in a separate chapter of this book (*see* CHAPTER 16, SURFACE PLASMON RESONANCE IN KINETIC, CONCENTRATION AND BINDING SITE ANALYSES) (Imoarai *et al.*, 2001; Fermann and Suyama, 2002; Hamilton *et al.*, 2002; Kratz *et al.*, 2002; Mastrovitch *et al.*, 2002; Phillips *et al.*, 2002;

The Immunoassay Handbook. *Third Edition*
D. Wild (Ed.)

Achyuthan *et al.*, 2003; Kabir, 2003; Murray *et al.*, 2003; Price, 2003; Armor and Britton, 2004; Donovan *et al.*, 2004; Gutierres and Welty, 2004).

## OVERVIEW

A biosensor uses a biological system to measure a substance and differentiate this from other substances in a test sample. It is a measurement device that is comprised of three components: a **biological receptor** of appropriate specificity for the analyte (or test material to be measured); a **transducer** to convert the recognition event into a suitable physical signal; and a **detection system**, including analysis and processing, that is usually electrical. The physical signal can, e.g. be acoustic, electromagnetic or mechanical. To bring together these three components for development of a biosensor, therefore, requires an integrated, multidisciplinary team of biologists, chemists, physicists, engineers, and computer experts. This blend of skilled personnel is not found in every establishment, so biosensor development has resulted primarily from inter-institutional collaborative projects, or within industry.

Most of today's analyses of biological samples take place in laboratories that use relatively expensive equipment and skilled personnel. Tests that involve many manipulations are increasingly being automated. Where the result is not obtained for many minutes or even several hours, attempts are made to decrease the time of analysis, often in automated systems. Microbiological determinations using conventional culture techniques can take several days or sometimes weeks, so modern alternative tests are being examined as rapid screens, particularly in the food industry in response to regulatory pressure, to satisfy consumer concerns about safety. The pharmaceutical industry is perhaps the biggest user of automated analytical screening techniques in its quest to develop better drugs, faster.

It might have been expected that biosensors would be ideally suited to address some areas requiring rapid analysis. They offer the potential to use relatively inexpensive equipment to provide results almost instantaneously outside of the laboratory, where unskilled personnel may handle a sample. Over the past 10 years, however, the biosensors that have been commercialized have succeeded only in addressing niche markets; some of the reasons will be considered below.

There are many technologies that could potentially be used for biosensing (Schultz, 1991). The range of these devices, with their many overlapping principles, adaptations and modifications, makes classification and discussion of all types quite difficult. Therefore, only those devices using **immunological or enzymatic components**, and which are likely to have some commercial potential, will be mentioned here.

Generally, in clinical chemistry analyses, a lower threshold of detection at around micromolar concentrations is satisfactory for most analytes. The demands for hormone measurements in the endocrinology clinic pushed the threshold for detection to around nanomolar concentrations and then below this to the picomolar range. These measurements were performed almost exclusively with biological binding assays, often immunoassays. For some more recent applications, lower detection limits are needed, for example with DNA fragments or pesticide residues.

The ability to detect a small amount of material in a test sample is determined by the signal-to-noise ratio. Jackson and Ekins (1983) have shown that the detection limit of an immunoassay system is directly proportional to the relative error in the signal, and inversely proportional to the equilibrium constant of the binding reagent. So, a decrease in the measurement error and a high-affinity constant (a low-valued equilibrium constant) would contribute to a low detection-limit for the analysis (*see* CHAPTER 1 – PRINCIPLES). Most immunological methods of analysis are subject to more-or-less severe interference from other components in the complex sample matrix. Washing or other additional manipulations are often used to minimize interference effects.

The design features of a biosensor are little different from those of any modern laboratory instrumentation system. The concept of an ideal portable biosensor would probably feature most of the following characteristics:

- Instruments should be small, self-contained, cheap, and robust, capable of interfacing with existing central laboratory systems.
- The user interface should be simple, for use by unskilled operators.
- No volumetric measurement of the specimen, e.g. by pipette, should be necessary.
- The test specimen alone should be added, with no further reagent addition being required.
- Results should be unaffected by the test-specimen matrix, e.g. water, whole blood, serum, urine, or plasma.
- The time between presentation of the specimen and final result should be rapid, and ideally less than 5 min.
- Built-in standardization and controls are required.
- An easily understood record of the results should be available.
- The detection-limit should be appropriate to the analyte, and in the sub-picomolar range for the most sensitive systems.
- A wide analytical range is required if the same biosensor is to be adapted to many different analytes: for a generic analytical system, a capability for immunochemistry, clinical chemistry, enzymology, DNA probe measurements and a variety of other applications is desirable.
- The potential for simultaneous measurement of multiple analytes should be considered.

- There should be a good correlation of results with already-established test methods.
- Biosensor consumables must be cheap to manufacture in bulk and readily available (Vo-Dinh and Cullum, 2000; Luppa *et al.*, 2001; Raiteri *et al.*, 2001; Abel and von Woedtke, 2002; Cruz *et al.*, 2002; Kusnezow and Hoheisel, 2002; O'Sullivan, 2002; Peppas and Huang, 2002; Porwal *et al.*, 2002; Raman *et al.*, 2002; Schoning and Poghossian, 2002; Albers *et al.*, 2003; Dickert *et al.*, 2003; D'Orazio, 2003; Frederix *et al.*, 2003; Ganter and Zollinger, 2003; Hierlemann and Baltes, 2003; Hierlemann *et al.*, 2003; Ikai *et al.*, 2003; Robinson *et al.*, 2003; Selvaganapathy *et al.*, 2003; Yuqing *et al.*, 2003; Monk and Walt, 2004; Sadik *et al.*, 2004; Sapsford *et al.*, 2004; Turner and Magan, 2004).

# ELECTROCHEMICAL SENSORS

## AMPEROMETRIC SENSORS

Enzymes provide an attractive method of signal amplification. The continual turnover of a substrate generates a cascade signal that is large and can therefore be measured quite easily. Amperometric biosensors generally use reduction–oxidation (redox) enzyme systems. In the simplest case, a redox enzyme is immobilized by some convenient procedure at an electrode surface. The electrode is held at a fixed potential, adjusted so that electrons arising from an oxidized substrate are transferred to the electrode (or *vice versa* for a reduction reaction), and this regenerates the active form of a cofactor for another redox cycle by the enzyme. The specificity of the reaction is determined by the enzyme. Because the rate of enzymic reaction at a fixed temperature and pH is directly proportional to the substrate concentration, the current produced at the electrode is proportional to the rate of modification of the substrate by the enzyme (Figure 15.1).

The rate of an enzymic reaction is dependent on the temperature, pH, ions, cofactors, and competitive or non-competitive inhibitors (or activators) present in the test sample. Any redox compound present, such as oxygen, ascorbate, thiols or certain drugs can obviously interfere with the reaction. To circumvent some of these interference effects, chemical electron-acceptors are used as mediators. Thus, for example, the ferrocene–ferricinium redox system has been used in the mediation of electron transfer from glucose oxidase to graphite electrodes. The pen-sized glucose biosensor produced originally by MediSense Inc. is based on this system, and physiological concentrations of glucose in the millimolar concentration range may be measured in whole blood (Hill and Sanghera, 1990). Q3

Immunoassay methods are normally required for measurements of analyte concentrations in the sub-millimolar range. Various methods of detection of antibody–antigen interactions using enzyme-labeled reagents have been tried, coupling the enzymic redox reaction to an amperometric detection system (Foulds *et al.*, 1990; Heineman and Halsall, 1987). Q4 However, the efficiency of coupling of the biological electron-generating steps to the electrode is not fully characterized. Additionally, the effects of interfering substances are greater when the substrate concentration falls below millimolar. These and other technical difficulties, such as attaching the reagents to the membrane or electrode, have hindered the development of systems based on this principle for immunosensing (Porter, 2000; Warsinke *et al.*, 2000; Dijksma *et al.*, 2001; Liu *et al.*, 2001a; Liu, *et al.*, 2001b; Pemberton *et al.*, 2001; Porter *et al.*, 2001; Lefeber *et al.*, 2002; Li, *et al.*, 2002b; Mittelmann *et al.*, 2002; Sarkar *et al.*, 2002; Albers *et al.*, 2003; Darain *et al.*, 2003; Fahnrich *et al.*, 2003; Lei *et al.*, 2003; Zeravik *et al.*, 2003; Zhou *et al.*, 2003; Dai *et al.*, 2004; Lei, *et al.*, 2004; Zacco *et al.*, 2004).

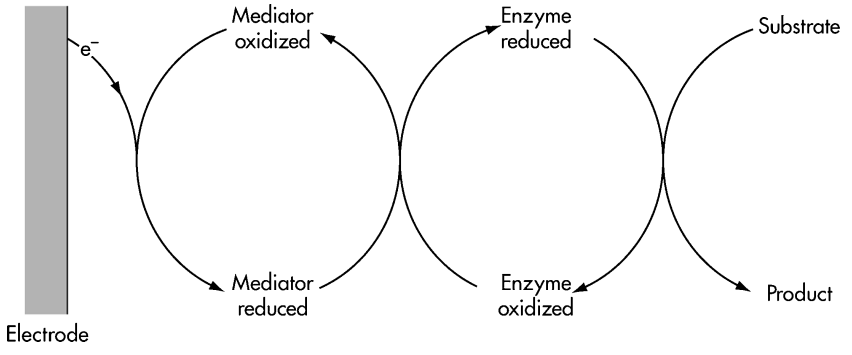


**Fig. 15.1** An amperometric biosensor arrangement. A mediator is used to transfer electrons from an electrode to an enzyme-catalyzed redox reaction.

## POTENTIOMETRIC SENSORS

Potentiometric devices rely on the measurement of changes in potential that arise from reaction of an analyte with a specific receptor. An extensive range of configurations has been described in which receptor molecules have been immobilized on ion-selective electrodes.

Advances in technology now allow silicon semiconductors to be coupled to a biological receptor, offering the potential of cheap, miniature, mass-produced biosensors. Several reports on the use of ion-sensitive field-effect transistors (ISFETs) as biosensors have appeared and Japanese workers are particularly active in this area. However, commercialization of these devices has been restricted because of technical difficulties associated with the reproducibility of depositing enzymatic material, its stability, and the relatively high cost of the devices compared with other systems (Figure 15.2).

When applied to immunoassay-based detection systems, this technology has also encountered problems similar to those of amperometric biosensors. Model systems can be shown to work in buffer solutions, but the interference effects that occur from materials present in the actual test specimens have restricted their more widespread application. (Holt *et al.*, 2002; Perez-Luna *et al.*, 2002; Schoning and Poghossian, 2002; Selvanayagam *et al.*, 2002; Zayats *et al.*, 2002; Besselink *et al.*, 2003; Hierlemann and Baltes, 2003; Hirano *et al.*, 2003; Yuqing *et al.*, 2003; Sadik *et al.*, 2004).

## NANOMECHANICAL SENSORS

## PIEZOELECTRIC MASS SENSORS

Mass detection sensors are among the most widely used microanalytic technologies. These methods rely, in general, on measuring the changes in vibrational resonant frequency of piezoelectric quartz oscillators that result from changes in mass on the oscillator's surface (see Figure 15.3).

Common configurations are the **quartz crystal microbalance (QCM)** and the **surface acoustic wave (SAW) device**. The QCM device consists of a quartz crystal disk driven by electrodes on either face. The mass of analytes that bind to the sensor is measured as a change in the crystal's resonant frequency. This type of sensor is also known as a **thickness-shear mode (TSM)** device. The mass determination in a TSM sensor is given in terms of the Sauerbrey relation:

$$\Delta M = (A \text{ sqrt } (\rho\mu)/2F^2)\Delta F$$

In the SAW sensor an acoustic wave is created by applying an alternating voltage to a metallized, interdigitated electrode plated onto one end of the thin piezoelectric planar substrate of the device. The acoustic wave acts on a symmetric transducer at the opposite end of the substrate, where the energy is converted back into an electrical signal. The SAW device can be altered for particular applications by varying the interdigital spacing

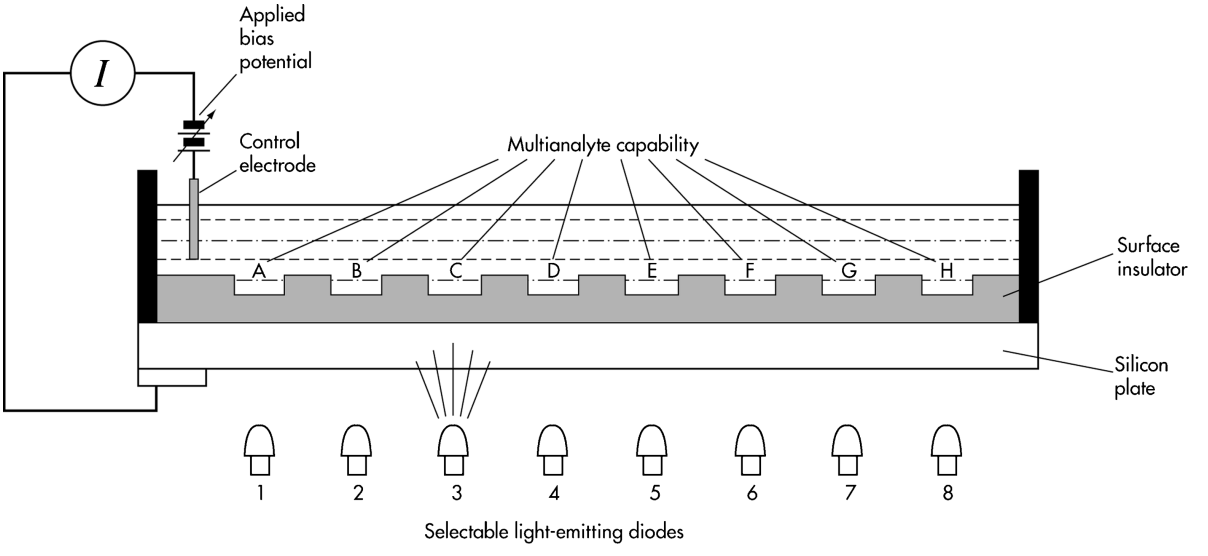


**Fig. 15.2** Diagrammatic form of a light-addressable potentiometric sensor (LAPS). The underlying silicon plate has a surface insulator layer (shaded) of oxynitride. Different detection systems are located in the channels that become photo-responsive only when selectively illuminated by the light-emitting diodes. The alternating photocurrent (I) in the external circuit depends on the applied bias potential. (Redrawn from Hafeman *et al.* (1988)).

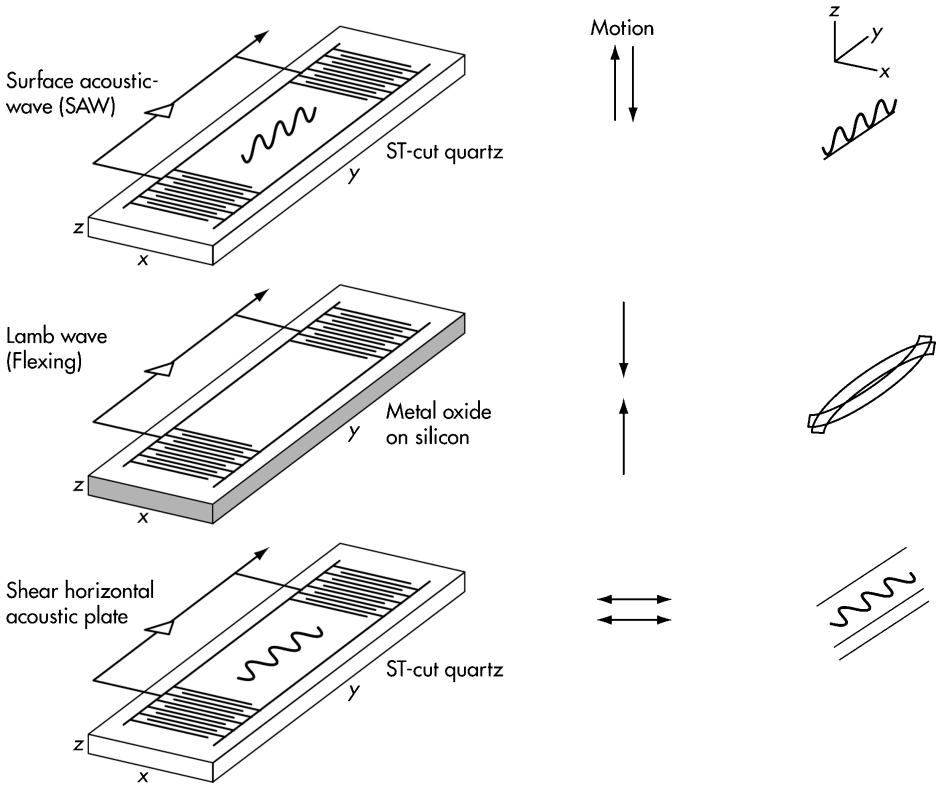


**Fig. 15.3** Three different forms of piezoelectric biosensors. (Redrawn from Ward and Buttry (1990)).

of the transducers, the distance between the transducers, and the thickness of the substrate.

SAW devices respond to changes in the surface wave amplitude or, more commonly, in the velocity of the acoustic wave when it interacts with molecules bound to the surface of the substrate. The thin layer of bound material alters the elasticity, density, viscosity, and conductivity of the SAW substrate. In some early work, it is apparent that the exquisite temperature sensitivity of these devices was not taken into account. There are many modes of acoustic wave that can propagate on the SAW devices, which can be used variously for sensing applications of gases, liquids or deposited solids.

Alternative acoustic modes induce particle motion on *both* surfaces of the substrate; these devices are sometimes referred to as **acoustic waveguide (AWG)** devices. The modes can be grouped into families and, by suitable selection of the type of mode, high sensitivity, and minimization of non-specific interferences can be achieved. Over the past decade, it has become clear that, for applications such as immunosensors, SAW devices should operate in shear horizontal (SH) or surface transverse wave (STW) modes (Collings and Caruso, 1997).

The so-called **Love wave device**, sometimes referred to as a surface-skimming bulk wave, is obtained when a layer of the appropriate thickness and acoustic properties is deposited over a conventional SH device. The energy of the bulk wave is concentrated in the guiding layer because the shear acoustic velocity in this layer is lower than in the substrate, leading to the alternative name of the surface-guided SH wave. The sensitivity to mass loading is increased by focusing the energy in this layer, which is dependent on the layer thickness and its acoustic properties.

The latter devices have the advantage of avoiding radiation losses in liquids, yet have much better sensitivity to mass loading by concentrating the energy near the surface. The SH wave may be guided along device surfaces by gratings as well as over-layers, as either SH waves or as STW. The improved sensitivity compared with simple SH devices, without much greater complexity in fabrication, holds much promise for their use in liquid-phase biosensing. This is because the viscous loading contribution of the solvent, in its attenuation of the shear wave in other modes, shifts the resonance of the transducer with consequent alteration in sensitivity.

Using conventional antibody–antigen interactions on thin substrates, detection limits of nanomolar down to

picomolar concentrations have been claimed with 'ideal' test samples, though this is perhaps unlikely to be achieved with typical clinical specimens. Manufacture of the devices to produce cheap, disposable units with uniform characteristics may be relatively easy, although provision of multi-analyte analysis on a single unit would provide a challenge (Cavic *et al.*, 1999; O'Sullivan and Guilbault, 1999; O'Sullivan *et al.*, 1999; Kaiser *et al.*, 2000; Shen *et al.*, 2000; Uttenthaler *et al.*, 2001; Zhou and Cao, 2001; Chou *et al.*, 2002; Eun *et al.*, 2002; He and Zhang, 2002; Li *et al.*, 2002a; Liss *et al.*, 2002; Sota *et al.*, 2002; Wong *et al.*, 2002; Aizawa *et al.*, 2003; Killard and Smyth, 2003; Kim *et al.*, 2003a; Kim *et al.*, 2003b; Kim and Park, 2003; Marx, 2003; Ruan *et al.*, 2003; Stubbs *et al.*, 2003; Tamarin *et al.*, 2003; Killard and Smyth, 2004; Laricchia-Robbio and Revoltella, 2004; Schaible *et al.*, 2004).

## MICROCANTILEVER SENSORS

The microcantilever is an emerging and particularly versatile class of sensor which is unique in its combination of simplicity, sensitivity, and potential for miniaturization. Microcantilevers can sense and quantitate biological proteins, nucleic acids, and a variety of organic and inorganic chemical species. The principal mechanism of action for sensing is a nanoscopic deflection caused by receptor–ligand-induced stress on one face of the microcantilever. The deflection signal can be recorded with an optical lever, an interferometer or a piezoresistive element. When operated in 'active mode', cantilever sensors are induced to oscillate at their resonant frequency and function very much like the mass-detection sensors described above.

Gimzewski and colleagues produced the first cantilever array immunosensor, which could distinguish species-specific binding of protein A to rabbit IgG. The same sensor design could also detect single base-pair mismatches in DNA oligonucleotide hybridization experiments. Another iteration of this device, a 'nanomechanical nose', used an array of non-specific polymer probes to distinguish hydrogen, primary alcohols, natural flavors, and water vapor in air (Baller *et al.*, 2000; Fritz *et al.*, 2000; Lang *et al.*, 2002; Arntz *et al.*, 2003; Yue *et al.*, 2004).

Majumdar and co-workers produced a prototype POC cantilever immunosensor for prostate specific antigen (PSA). This device detected physiologic levels of free PSA in a high background of albumin and plasminogin human serum proteins serum (Wu *et al.*, 2001; Majumdar, 2002; Yue *et al.*, 2004).

Thundat and colleagues have used single cantilever nanosensors to detect heavy metal ions, neurotoxins, and glucose. The latter application utilized cantilever-bound glucose oxidase enzyme as a detector–reporter element (Cherian *et al.*, 2002; Stevenson *et al.*, 2002; Cherian *et al.*, 2003; Yang *et al.*, 2003; Pei *et al.*, 2004).

Baltes and co-workers developed techniques for manufacturing cantilever sensors using a CMOS process, which provides the potential advantage of mass production for commercial applications. Their fully integrated device contains a Wheatstone bridge to sense cantilever bending, eliminating the need for external optics (Franks *et al.*, 2002; Hierlemann and Baltes, 2003; Hierlemann *et al.*, 2003).

Similarly, Boisen *et al.* have produced an active cantilever array system with completely integrated on-chip actuation and deflection sensors, highlighting the miniaturization potential that gives cantilever sensors an advantage over many competing technologies (Boisen *et al.*, 2000; Grogan *et al.*, 2002; Davis *et al.*, 2003).

Startup companies Protiveris, Cantion, and Concentris are all attempting to perfect and commercialize cantilever technology (Luckham and Smith, 1998; Moulin *et al.*, 2000; Hansma, 2001; Ilic *et al.*, 2001; Allison *et al.*, 2002; Marie *et al.*, 2002; Sepaniak *et al.*, 2002; Arntz *et al.*, 2003; Dutta *et al.*, 2003; Kooser *et al.*, 2003; Liu *et al.*, 2003a; Rasmussen *et al.*, 2003; Ilic *et al.*, 2004; Zhang and Ji, 2004).

## MICROMAGNETIC SENSORS

This sensor technology is less mature than others but has shown promise in proof-of-concept studies. Magnetically based nanomechanical sensors make use of the weak interaction of magnetic fields with the sample and are related to magnetic bead-based assays that are common in molecular biology. Experiments that measure receptor–ligand binding forces make use of magnetic particles to precisely control applied unbinding forces. The relatively inert magnetic particles can be translocated and rotated in a sample to measure force in a variety of directions and when coated with the appropriate sensor element can even probe receptor–ligand interactions inside single cells (Kausch and Bruce, 1994; Schalkhammer, 1998; Ptak *et al.*, 2001; Richardson *et al.*, 2001; Tanaka and Matsunaga, 2001; Graham *et al.*, 2003; Kim *et al.*, 2003a,b; Liu *et al.*, 2003b; Puckett *et al.*, 2003; Ruan *et al.*, 2003; Hong *et al.*, 2004; Weizmann *et al.*, 2004).

## OPTICAL SENSORS

As with the other types of biosensor outlined above, there are now a large number of developments that make use of the physical properties of light in various ways to detect small changes in analyte concentration. In an immunosensor configuration, optical methods often rely on fiber-optic input and collection elements, with the receptor–ligand pair bound to the fiber surface or in the nearby fluid. Detection and quantitation typically make use of fluorescent tags or bio-/chemiluminecent reporters.

## FLUORESCENT EVANESCENT WAVE SENSORS

This technique relies on the properties of light when it is reflected at a surface between two transparent media of different refractive indices. A light beam is totally internally reflected within an **optical waveguide** (e.g. an optical fiber) and, at the points of reflection, part of the light enters the external, lower refractive index medium before it returns to the waveguide. This results in an **evanescent field** which penetrates only a fraction of a wavelength of the light into the surrounding medium, and which decays exponentially with distance from the surface (Figure 15.4). Because there are multiple reflections within an optical fiber, it is essentially covered by this evanescent field, whose external influence is limited to within a few 100 nanometers from the fiber surface. Thus, if the optical fiber is dipped into a solution containing a fluorophore with an appropriate excitation wavelength, only those molecules in the evanescent field near the surface will fluoresce (Sutherland *et al.*, 1984).

When appropriate receptors or antibodies are attached to the fiber surface, then either competitive or immunometric assays can be performed using fluorophore-labeled reagents. An immunoglobulin G (IgG) antibody has dimensions of $5 \times 15$ nm, i.e. an average of about 10 nm. Although the influence of the evanescent field will extend a few 100 nanometers into the medium, the concentration of unbound fluorophore will be low, so a majority of the fluorescence will arise from specifically bound fluorophore at the fiber surface. Detection limits at nanomolar concentrations have been achieved with measurement times of only a few seconds.

A major deficiency with most fluorescent measurement techniques is that the fluorophore and exciting light are both influenced by the test specimen. When the specimen is serum, plasma or whole blood, light absorption and fluorescence quenching can significantly decrease the measured signal in a specimen-dependent manner. Alternatively, interference can occur from endogenous fluorophores present in the specimen; this is normally minimized by introducing a washing step. Although fluorescent techniques have the potential for great sensitivity, and hence even lower detection-limits, the interference effects have generally limited the attainment of very low detection-limits in real samples. Despite this limitation, commercial developments have been investigated by several companies (Squillante, 1998; Spiker and Kang, 1999; Cui *et al.*, 2000; DeLisa *et al.*, 2000; Marks *et al.*, 2000; Moreno-Bondi *et al.*, 2000; Blair and Chen, 2001; Anderson and Nerurkar, 2002; Balcer *et al.*, 2002; Neumann *et al.*, 2002; Vo-Dinh, 2002; Krioukov *et al.*, 2003; Liebermann and Knoll, 2003; Liu *et al.*, 2003b; Tedeschi *et al.*, 2003; Ekgasit *et al.*, 2004; Garden *et al.*, 2004; Monk and Walt, 2004).

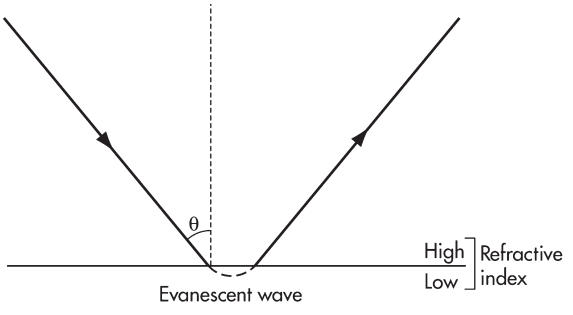


**Fig. 15.4** Diagrammatic representation of the evanescent wave. The evanescent field decays exponentially with distance away from the high refractive-index surface.

## INTEGRATED OPTICAL SENSORS

As mentioned above, Ligler and co-workers have developed and field tested an integrated optical immunosensor which is capable of detecting a variety of toxic substances and pathogens in real-world situations. This prototype is fully automated and portable, which is essential for continuous monitoring applications. Efforts in this area now focus on miniaturizing the optics and detector elements so that the entire device can be handheld. Further, most of the detection chemistry is antibody-based and new efforts are needed to expand the range of probe chemistries to accommodate a wider array of analytes (Rowe-Taitt *et al.*, 2000a; Rowe-Taitt *et al.*, 2000b; Rowe-Taitt *et al.*, 2000c; Sapsford *et al.*, 2001; Delehanty and Ligler, 2002; Holt *et al.*, 2002; Sapsford *et al.*, 2002; Taitt *et al.*, 2002; Sapsford *et al.*, 2003; Sapsford *et al.*, 2004).

One integrated optical sensor that has made it to the clinic is the **optode continuous intravascular blood gas monitor**. An example of this type of device is the FDA-approved Paratrend 7 from Diametrics Medical, which is a disposable, sterile, single-use fiber-optic sensor for continuous measurement of pH, $pCO_2$, $pO_2$ and temperature. This device is used in critical care situations to provide real-time oxygenation, ventilation, and metabolic data. Sensors of this type use a variety of biochemical reporter methods including chemically sensitive dyes and compound selective membranes; they are also compatible with affinity probe chemistries and enzyme-based reporter systems (Mahutte, 1998; Ganter and Zollinger, 2003).

## QUANTUM DOTS

Quantum dots are luminescent semiconductor nanocrystals that behave similarly to fluorescent reporter molecules. They are unique in that the emitted light is confined to a very narrow frequency band making them ideal for use in multiplex assays. Also, quantum dots are much more physically robust than fluorescent molecules, which suffer from photobleaching and other undesirable

photochemistry. Goldman and colleagues have conjugated antibodies to quantum dots for use in multiplex immunosensing. They detected and quantitated biotoxins including cholera toxin, ricin, shiga-like toxin 1, and staphylococcal enterotoxin B in a microtiter format (Cunin *et al.*, 2002; Medintz *et al.*, 2003; West and Halas, 2003; Goldman *et al.*, 2004).

## CONCLUSIONS AND FUTURE DIRECTIONS

The benefits and deficiencies of the various types of biosensor systems discussed above are summarized in Table 15.1. The amperometric and potentiometric sensors have been intensively investigated over the past 35 years since Clark and Lyons, 1962 described the first glucose sensor. It is notable that the most advanced commercial glucose sensors still use electrochemical detection methods. In studies to date electrochemical methods seem to be less versatile than other techniques, but they are competitive in applications where the proper chemistry exists, while ISFET efforts have great miniaturization potential.

Nanomechanical sensors such as microcantilevers, or one of the several optical devices described are the most likely candidates as general-purpose bioanalytical sensors of the future. They are easy to produce and have potential for use over a wide range of analyte concentrations. They also approach the low detection limits required for immunosensing, while the reproducibility of results is similar to that achieved by conventional immunoassays. These devices rely on relatively well-understood physical principles, and the associated instrumentation is quite simple to assemble. The analyte-detection elements in the optical or the piezoelectric sensors are relatively cheap and easy to construct.

The fluorescent methods used with the optical sensors, although intrinsically more sensitive than the other optical methods, suffer from potentially greater interference effects arising from the test-specimen matrix. It is for this reason that the SPR or integrated optics sensors have been commercialized as the next generation of general-purpose analytical tools. These devices have not yet met all the criteria listed in this chapter for an 'ideal' biosensor, particularly with regard to miniaturization; but they have met most of these. Future developments are likely to extend their flexibility and deployment within the next 20 years.

A major development in the biosensor field is directed at designing a reaction cell in which both the kinetics of analytical reaction and flow characteristics at the sensor surface are optimized for ease of manufacture and use. The key to reproducible manufacture and consequent ease of use of these biosensor systems is the uniformity of physical and chemical properties of the biophysical interface. Instrumentation developments center around miniaturization, improved signal processing, and addressing the possibility of analyzing multiple analytes simultaneously on the same reaction-cell sensor surface. The chemistry of reagent deposition, assay format and other aspects related to the use of the reagents are, in general, not very different from those encountered with immunoassay systems. Attention has to be given to using

**Table 15.1.** Biosensors: a comparison of technologies.

| Sensor technology | Main advantages | Main disadvantages |
|---|---|---|
| Amperometric | Proven technology<br>Clinical chemistry analytes (micromolar) | Susceptible to interference<br>Limited low detection limit<br>Immunosensing difficult |
| Potentiometric | Potentially cheap microchip manufacturing technology | High set-up manufacture costs<br>Limited low-detection limit<br>Limited reproducibility |
| Light-addressable potentiometric | Proven technology<br>Potentiometric stability<br>Multi-analyte capability | Slow near lower detection limit<br>Multiple steps, including washing |
| Fluorescence evanescent-wave | Rapid (<5 min) | Lower detection limit limited by sample interferences<br>Non-homogeneous format |
| Surface acoustic wave | Theoretical lower detection limit is picomolar | High cost of manufacture of identical units<br>Temperature sensitivity<br>Non-specific effects |

assay formats in which the signal change is large enough to measure. The assay system should be a homogeneous one, with no separation or measurement step required, so the design of the assay format becomes in some respects easier than for a conventional immunoassay.

Much of this discussion has focused on methods to detect a receptor–ligand binding event that is compatible with real-time, continuous monitoring. These technologies have become relatively sophisticated and each has strengths and weaknesses that vary by application. In the future we expect more research emphasis on design of the biochemical receptor elements themselves. Most current sensors use canonical antibody–antigen chemistry or something very similar. There is great need for probes which are more stable, more versatile in terms of recognition while retaining high specificity and which can be regenerated *in situ*. Some examples of new probe chemistries include molecularly imprinted polymers (MIPs), 'smart-polymers' and RNA aptamers (de Wildt *et al.*, 2000; Iqbal *et al.*, 2000; Kaiser *et al.*, 2000; Mishra and Schwartz, 2002; O'Sullivan, 2002).

We also expect more advanced probe design to be accompanied by signal processing informatics that compensate for the unavoidable non-specific background found in real-world biosensing situations (Baller *et al.*, 2000; Reder *et al.*, 2003; Turner and Magan, 2004).

Array-based approaches are already very popular in nucleic acid detection and much effort is being expended to produce reliable protein–protein recognition arrays. Arrays would allow 'super multiplex' analysis of samples and are needed to capture the complex patterns of disease states; this aspect of molecular diagnostics is severely limited by today's single- or few-analyte rapid immunoassay methods (Blank *et al.*, 2003; Niemeyer *et al.*, 1994; Rowe-Taitt *et al.*, 2000b; Delehanty and Ligler, 2002; Kusnezow and Hoheisel, 2002; Abedinov *et al.*, 2003; Albrecht, *et al.*, 2003; Arntz *et al.*, 2003; Pavlickova *et al.*, 2003; Peluso *et al.*, 2003).

Integrated immunosensors require some form of sample handling and for the miniaturization necessary, this will involve microfluidics. Microfluidics is a broad and promising field with many iterations of tiny fluid circuits, pumps, separation devices, etc. One successful commercial example is the Caliper Technologies LabChip, which allows very high density formats for traditional ligand–receptor and enzyme-relayed assays. The primary market for this device is drug discovery but in time this technology and its derivatives will move to the clinical laboratory and replace large immunoassay machines and other traditional instruments (Wells, 1998; Dutton, 1999; Nachamkin *et al.*, 2001; Christodoulides *et al.*, 2002; Schmut *et al.*, 2002; Wu *et al.*, 2003).

Cost per test/datapoint is very important when comparing immunosensors to traditional immunoassay technology. Standard ELISA assays are very sensitive and inexpensive, which creates a high hurdle for sensor technologies in standard applications. Thus, we expect immunosensors to emerge first in applications where real-time continuous sensing is a chief concern and cost/sensitivity vs. traditional techniques is less important. We have described a number of efforts to use semiconductor-like manufacturing technologies to produce immunosensors cheaply and in quantity. This, along with further miniaturization, will be necessary to make immunosensors competitive with current ELISA-type assays in many diagnostic applications (Hierlemann *et al.*, 2003; Lee, 2003).

Continuous, *in vivo* biosensing would revolutionize the diagnosis of disease and the controlled delivery of therapeutics. The blood glucose sensing technology already mentioned in this chapter and the arterial blood gas optode mark early but notable efforts. In reality, researchers have just begun to address the many substantive hurdles which exist in this area: these include complete sensor/readout integration, biocompatibility and long-term, *in vivo* sensor stability. Finally, the widespread use of biosensors is limited in many cases not by technology development but by questions about the utility of various biologic and environmental markers (Gerritsen *et al.*, 1999; Trettin *et al.*, 1999; Frost and Meyerhoff, 2002; Musham and Swanson, 2002; Nicolette and Miller, 2003; Yancy, 2003; Coradini and Daidone, 2004; Mark and Felker, 2004; Roongsritong *et al.*, 2004).

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