3

SENSING PRINCIPLES FOR BIOMEDICAL TELEMETRY

Athanasios Lioumpas, Georgia Ntouni, and Konstantina S. Nikita

School of Electrical and Computer Engineering, National Technical University of Athens, Athens, Greece

3.1 INTRODUCTION

During the last decades, the advances in technology have had significant impact on monitoring applications in fields such as the food industry, environmental monitoring, e-health, clinical diagnosis, and biomedical telemetry. Particularly for biomedical applications, besides other challenges, such as the demanding wired or wireless communication requirements, the successful development of these applications is highly related with the sensing devices, that is, the biosensors. These are charged with the challenging task of providing the physical, chemical, and biological data for the monitoring, which includes among others heart rate, blood oxygen saturation, physical activity, intracorporeal (e.g., intracranial) pressure, gastrointestinal parameters, and lumen visualization.

According to the International Union of Pure and Applied Chemistry (IUPAC), *a biosensor is an independently integrated receptor transducer device, which is capable of providing selective quantitative or semiquantitative analytical information using a biological recognition element (biochemical receptor)* (Thevenot et al., 1999). The biosensors aim to provide accurate and reliable information about the sensed parameter rapidly and in real time. The first biosensor was presented by Clark and Lyons in 1962, who utilized external electrode systems to record, quantitatively, blood pH,

Handbook of Biomedical Telemetry, First Edition. Edited by Konstantina S. Nikita.

oxygen, and carbon dioxide tensions and contents and intravascular electrodes for hydrogen detection and oxygen tension changes (Clark and Lyons, 1962).

Over the last years, novel sophisticated tools and new materials have made it possible to construct sensing devices with increased reliability and accuracy with respect to the sensed information. Based on the transduction process, biosensors can be divided into several categories, such as electrical, electrochemical, optical, piezoelectric, and thermal/calorimetric biosensors, while based on the receptor type, they can be characterized as enzymatic biosensors, genosensors, immunosensors or DNA-based sensors.

This chapter focuses on the operation principles of the biosensors in terms of both the receptor type and the transduction process. The different recognition and detection principles are reviewed and analyzed, providing insight into the biosensor design challenges.

3.2 BIOSENSOR STRUCTURE

Although there are numerous different types of biosensors and various operation principles, the fundamental elements of the biosensors are the following (Monošík et al., 2012):

1. The *bioreceptor*, which is an immobilized biological element able to recognize the target analyte, that is, the compound whose concentration is to be determined (e.g., enzyme substrate, complementary DNA, antigen). Among the biosensing elements that are utilized, enzymes are most commonly used.
2. The *transducer*, which is used to convert (translate) the (bio)chemical signal resulting from the interaction of the analyte with the bioreceptor, that is, the recognition event, into a measurable electric signal into one single sensor. The concentration of the analyte is directly related with the intensity of the generated signal. Based on the transducer's detection principle, they can be classified into electrochemical, gravimetric, calorimetric, or optical transducers with the electrochemical ones being widely used because of their low cost and small size.

The uniqueness of a biosensor is that the two components are integrated into one single device (Figure 3.1), a characteristic that distinguishes them from a bioanalytical system, which requires additional processes, such as a reagent (Sassolas et al., 2012). Some examples of usually employed bioreceptors and transducers are given in Table 3.1 (Grieshaber et al., 2008).

3.2.1 Design Constraints

Toward the construction of a biosensor able to measure the concentration of an analyte or interact with a specific conformation, several design principles should be taken into account, such as the choice for the biological element that will be used for the

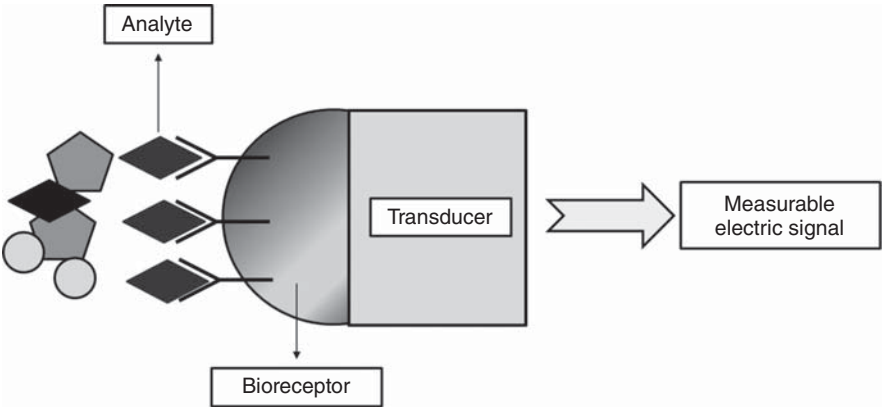

Figure 3.1 Components of a biosensor.

TABLE 3.1 Elements and Selected Components of Typical Biosensor

Samples	Bioreceptors	External System	Transducer	Interface
Analytes (e.g., glucose, lactate)	Enzyme Antibody Microorganism	Signal amplifier + signal processor + display	pH change Heat Light Mass change Electroactive substance	Semiconductor pH electrode Thermistor Photon counter Piezoelectric device Electrode

detection of the analyte, the chemical nature of the transducer, and the position into the protein to introduce the signal (Serra, 2011; Perumal and Hashim, 2013; Wang et al., 2009). The biological component is most usually a protein or nucleic acid, able to provide the stereospecificity that is necessary for the desired interaction with the analyte. The periplasmic binding proteins (PBPs) are able to bind a large number of analytes, such as carbohydrates, amino acids, ions, hormones, and heme groups (Medintz and Deschamps, 2006). The chemical nature of the transducer is a very critical parameter for the construction of a biosensor, and it must be carefully selected since the physiochemical properties of the transducer determine the quality of the generated signal (e.g., some signals are sensitive to the polarity of the solvent or the pH). Another important factor to be considered for the construction of a biosensor is its ability and efficiency to provide fast real-time reproducible measurements (Plaxco and Soh, 2011).

The design constraints are highly related to the biomedical application and the particular use of the biosensor. The most stringent constraints are imposed by those applications involving an "invasive" sensing, where a penetration into the body is required. In such applications, the size, the power source, and the energy efficiency

of the implanted device play a decisive role. Apart from these issues, the most critical issue for the implanted biosensors is their biocompatibility with the individual patient, since the biosensor could be attacked by the immune system and lead to serious complications (Iniewski, 2012). Besides, the tissues conductivity may prevent the normal operation of the implanted device in case of their direct contact. In this sense, the used materials as well as the packaging of the biosensor and the implanted device play a key role in the design. Usually, the implanted or ingestible devices are encapsulated in a biocompatible material, such as porous silicon, thermal silicon oxide, polysilicon, silicon nitrate, titanium, and SU-8 (Guiseppi-Elie et al., 2005; Lopez-Martinez et al., 2009). Moreover, the electrical current sent to the device should be taken into account (e.g., through biocompatible wire sheathing) in order to avoid unintentional discharge or unnecessary current heating effects. Furthermore, proper biocompatible packaging and filtering could prevent incorrect signal detections because of the noise created by the compounds within the body (Guiseppi-Elie et al., 2005).

"Noninvasive" sensing applications, where the biosensor is attached to a wearable device, do not pose the same challenges as the invasive ones, since larger device sizes can be tolerated and conventional power sources can be utilized. The most challenging task is to effectively detect the biosignals on the exterior of the body and encounter the increased probability to sense undesired signals because of the noise and interference (Guiseppi-Elie et al., 2005). Wearable sensing devices usually employ advanced filtering and interference-mitigating techniques in order to achieve sufficient reliability.

3.3 ELECTROCHEMICAL BIOSENSORS

The principle of the first enzyme electrode with immobilized glucose oxidase was presented in 1962 by L. C. Clark, while the first commercial biosensor was produced by Yellow Spring Instruments in 1972 and was applied to the fast glucose assay in blood samples from diabetics (Grieshaber et al., 2008). The operation of a typical electrochemical biosensor is based on the presence of an appropriate enzyme in the biorecognition layer (baroreceptor), which is able to provide those electroactive substances to the physicochemical transducer in order to detect a measurable signal. Native enzyme can be used as the biorecognition element or enzymes can be additionally used as labels bound to antibodies, antigens, and oligonucleotides with a specific sequence, thus providing affinity-based sensors (Bakker, 2004). Depending on the analyte, different enzymes are employed for its detection, for example, glucose oxidase and glucose dehydrogenase for glucose assays, urease for urea, cholesterol oxidase coimmobilized with cholesterol esterase for the cholesterol assay, NADH-dependent lactate dehydrogenase, and lactate–cytochrome *c* oxidoreductase for lactate (Grieshaber et al., 2008). Electrochemical biosensors are classified to different categories, according to the transducer's operation principle, with the most common ones being the potentiometric, amperometric, and impedimetric transducers (Pohanka and Skladal, 2008).

3.3.1 Amperometric Electrochemical Biosensors

The operation of the amperometric electrochemical biosensors is based on the reaction between an enzyme immobilized at the surface of an amperometric electrode and a substrate. Clark oxygen electrodes represent the basis for the amperometric biosensors, which consist of a central Pt (platinum) cathode and an Ag/AgCl (silver) anode. When a reference potential is applied between the cathode and the anode, a current produced in proportion to the oxygen concentration is measured by the reduction of oxygen at the Pt electrode with respect to a Ag/AgCl reference electrode. If the current is measured at a constant potential, it is referred as *amperometry*, while if the current is measured during controlled variations of the potential, this is referred to as *voltammetry* (Chaubey and Malhotra, 2002). Figure 3.2 depicts the basic structure of an amperometric biosensor. The platinum cathode and the silver anode are placed in a solution (e.g., saturated potassium chloride) which is separated from the biocatalyst (e.g., glucose oxidase) by a membrane permeable to O_2, that is, a product after the chemical reaction between the enzyme and the analyte (e.g., glucose). The biocatalyst is separated from the analyte by another membrane permeable to specific products of the chemical reaction. For this specific example the chemical reactions that take place

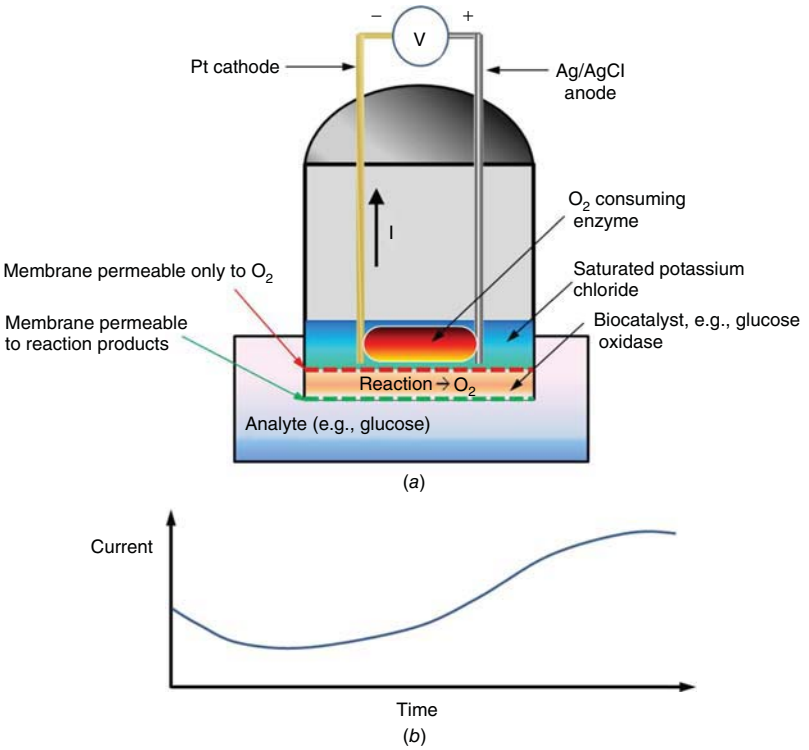

Figure 3.2 (*a*) Structure of simple amperometric biosensor and (*b*) current at electrodes after reaction between analyte and oxygen-consuming enzyme.

can be expressed as follows:

$$\text{Silver anode:} \qquad 4Ag^0 + 4Cl^- \rightarrow 4AgCl + 4e^-$$

$$\text{Platinum cathode:} \qquad O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

The amperometric biosensors that utilize two electrodes have some disadvantages with the most important being the limited control of the potential when higher currents occur, resulting in a shortened linear range. A solution to this problem involves the introduction of a third auxiliary electrode, so that the potential is applied between the reference and the working electrodes, while the current flows between the working and the auxiliary one.

The simple structure and operation principles of the amperometric biosensors have allowed their mass production and have made them the most commonly used sensors for biomedical applications. Several commercial amperometric biosensors exist, with the glucose biosensors being the most known.

Furthermore, several attempts have been made toward the further simplification of amperometric biosensors (Belluzo et al., 2008). For example, an outstanding approach has been proposed to determine glucose using glucose oxidase directly "plugged" to the electrode taking advantage of the controlled electron transference through a carbon nanotube (Patolsky et al., 2004). Moreover, biosensor research is highly motivated by the need for miniaturized clinically useful devices where microscale biosensor devices react with the analyte and generate a measurable response within the same compact body. A good example is the enzymatic biosensor to detect lactate in saliva in a one-step analysis (Schabmueller et al., 2006).

3.3.2 Potentiometric Electrochemical Biosensors

The operation of the potentiometric electrochemical biosensors is based on potentiometry, which detects the ion activity during an electrochemical reaction. The biosensor measures the cumulative potential between two electrodes in an electrochemical cell when the current flowing through the electrodes is equal to or near zero. Usually, ion-selective electrodes (ISEs) and ion-sensitive field effect transistors (ISFETs) are employed, with the output signal resulting from the ions that are accumulated at the ion-selective membrane. The relationship between the ion concentration and the potential is governed by the *Nernst equation*, also referred to as the electromotive force (EMF), that is,

$$\text{EMF or } E_{\text{cell}} = E^0_{\text{cell}} - \frac{RT}{nF} \ln Q \tag{3.1}$$

where E_{cell} represents the observed cell potential at zero current, E^0_{cell} a constant potential contribution to the cell, R the universal gas constant, T the absolute temperature in degrees Kelvin, n the charge number of the electrode reaction, F the Faraday constant, and Q the ratio of ion concentration at the anode to ion concentration at the cathode (Buerk, 1993).

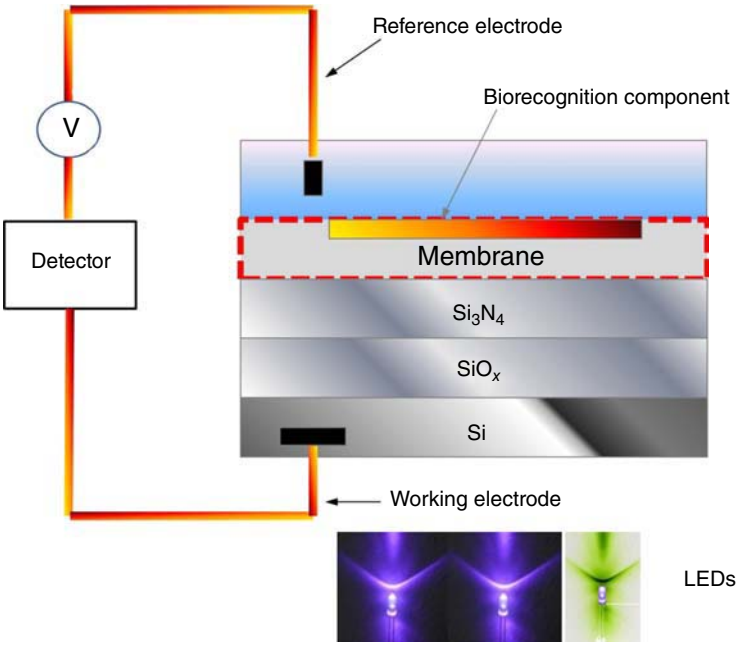

Figure 3.3 Structure of simple potentiometric biosensor.

Semiconductor-based physicochemical transducers are among the most commonly used potentiometric biosensors and more particularly the ISFETs and light addressable potentiometric sensor (LAPS).

In potentiometric biosensors employing the LAPS, a light-emitting diode (LED) activates the semiconductor (Yoshinobu et al., 2005). Figure 3.3 illustrates a simple biosensor based on the LAPS principle. In such structures an *n*-type Si is coated with 30 nm of SiO_x, 100 nm of Si_3N_4, and indium–tin oxide (ITO). The LAPS measures a voltage change as a function of medium pH in the LED-activated zone. A commercial biodetector device (Smiths Detection, Warrington, UK) based on the LAPS-type biosensor is found in mobile laboratories for automated eight-channel analysis of biological agents (Pohanka and Skladal, 2008).

3.3.3 Impedimetric Electrochemical Biosensors

The impedimetric biosensors measure the ability of an analyte to conduct an electrical current between electrodes or reference nodes and follow either impedance (Z) or its component resistance (R) and capacitance (C). The impedance biosensor is commonly a functional part of the Wheatstone bridge. They are also called conductometric devices, because of the inverse value of resistance, that is, the conductance. These devices are usually related to enzymes, where the ionic strength of a solution between two electrodes changes as a result of an enzymatic reaction.

A common application of the impedimetric biosensors is the assay of urea using urease as a biorecognition component. In general, impedimetric biosensors are not as popular as the potentiometric or amperometric biosensors. Nevertheless, they have contributed to important research studies, such as the hybridization of DNA fragments previously amplified by a polymerase chain reaction, or to the monitoring of microorganism growth due to the production of conductive metabolites.

3.4 OPTICAL BIOSENSORS

The demanding requirements for fast and accurate detection of any type of substances have accelerated the development of a large variety of biosensors. For most applications it is desirable to have a compact biosensor with high sensitivity, fast response, and the ability to perform measurements in real time. Optical methods for the detection of biomolecular interactions and their biomedical applications have attracted great research interest, showing that they meet these requirements. Moreover, the cost reduction of high-quality fibers and optoelectronic components has played a key role for the development of optical biosensors (Velasco-Garcia, 2009). These biosensors have many advantages that stem from the optical technology, such as the immunity to external electromagnetic interference, while they are characterized by the increased speed of biodetection. Furthermore, thanks to the increased bandwidth at the optical frequencies, they are suitable for biomedical applications with a large volume of information. Besides, the optical may directly detect a target of interest or indirectly through optically labeled probes. Most usually the operation of the optical biosensors is based on the detection of changes in (Gauglitz, 2005; Velasco-Garcia, 2009):

- Absorbance
- Fluorescence/phosphorescence
- Chemiluminiscence
- Reflectance
- Light-scattering or refractive index

The optical methods for detection are based on fluorescence spectroscopy, surface plasmon resonance, interferometry, and spectroscopy of guided modes in optical waveguide structures (Passaro et al., 2007; Fan et al., 2008). The advantages of optical sensors become more visible with the utilization of photonic integration, where multiple photonic functions are integrated on a single optical circuit, thus improving the functionality, sensitivity, and resistance of the biosensor and enabling the ability for mass production and reduced costs.

Various technologies are available for the construction of photonic biosensors, with the silicon technology being one of the most practical and promising tools. In silicon photonics the construction of the devices is based on silicon materials using microelectronics technologies in order to integrate all elements on a single chip. The propagation of light for biosensor applications is based on the total internal reflection

(TIR) in flat or lateral waveguides, concave waveguides, antiresonant reflecting optical waveguides (ARROWs), or waveguides apertures.

3.4.1 Integrated Optical Biosensors

A fiber is a waveguide that transmits light along its axis by the process of total internal reflection, and it consists of a dielectric material (the core) surrounded by another dielectric material with a lower refractive index (the cladding). Although most of the light power is confined within the waveguide, a small amount of this power is radiated and can interact with the environment. This type of optical sensor has important advantages, such as small size, high sensitivity, and low cost. In a large number of integrated optic sensors that have been proposed, the presence of the analyte either causes a change in the refractive index of the medium that covers the waveguide (homogeneous detection) or determines the thickness of the molecular layer that is placed over the interface between the waveguide and the cover medium (surface detection). Both phenomena affect the propagating optical mode effective index, measured in various ways, depending on the architecture of each biosensor.

3.4.2 Interferometric Architectures

The interaction between the sample (analyte) and the optical signal that propagates through the sensor causes a change in the propagating optical mode effective index and therefore in the phase. The transduction of this phase change to amplitude change width is based on the interferometric architectures. Among these architectures, the Mach–Zehnder approach is the most commonly used to determine the relative phase shift variations between two collimated beams and ensures high sensitivity. In this kind of integrated optical sensor (Figure 3.3), the optical input signal is separated into two signals by a Y splitter. These two signals propagate in the reference and sensing arms, and the interaction between the sample and the optical signal is carried in the sensing arm. Thereafter, the two optical signals acquire a phase shift $\Delta\phi$. If the input power P_{in} is unequally divided between the two arms [the sensing and reference arms receive an optical power equal to γ_1 and $(1-\gamma_1)P_{\text{in}}$], the output power will be the sum of the optical power at the sensing arm multiplied by γ_2 and the optical power at the reference arm multiplied by $1-\gamma_2$, resulting in an output power–input power ratio that is expressed as (Passaro et al., 2007)

$$\frac{P_{\text{out}}}{P_{\text{in}}} = \gamma_1\gamma_2 + (1-\gamma_1)(1-\gamma_2) + 2\sqrt{\gamma_1\gamma_2(1-\gamma_1)(1-\gamma_2)}\ \cos(\Delta\phi) \tag{3.2}$$

where $\Delta\phi = 2\pi(L/\lambda)(n_{\text{eff}}^S - n_{\text{eff}}^R)$ with L being the length of the reference and sensing arm and λ the optical signal wavelength and n_{eff}^S and n_{eff}^R are the propagating optical mode effective index at the sensing and reference arms respectively.

During the last two decades, a large number of integrated optical biosensors have been proposed and implemented based on the Mach–Zehnder interferometer (MZI)

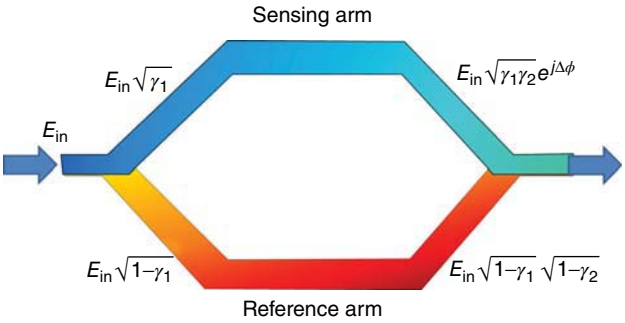

Figure 3.4 Mach–Zehnder interferometer.

architecture (Figure 3.4). The technologies that are employed for the construction of such biosensors is complementary metal–oxide–semiconductor (CMOS) compatible (guiding field on silicon, silicon nitrides, or silicon oxynidrite), but also glass and III–V semiconductor material have been proposed.

The sensitivity S of the MZI-based biosensors can be expressed as

$$S = -\frac{2\pi}{\lambda} L S_W \tag{3.3}$$

where S_W is the waveguide sensitivity, which for the case of homogeneous sensing is defined as

$$S_W = -\frac{\partial n_{\text{eff}}}{\partial n_C} \tag{3.4}$$

while for the case of surface sensing as

$$S_W = -\frac{\partial n_{\text{eff}}}{\partial \rho} \tag{3.5}$$

where ρ is the thickness of the molecular layer, which is placed at the interface between the guiding film and the cover medium of the wavelength.

Furthermore, the sensitivity of the MZI-based integrated biosensors depends on the length, L, of the sensing arm and therefore can be reduced by the unavoidable changes of the propagating optical mode effective index (e.g., because of temperature variations). Prieto et al. (2003) implemented an optical MZI biosensor capable of detecting the immune reactions between antibodies–antigens with detection limit equal to 7×10^{-6} with respect to the minimum detectable change of the refractive index of the wavelength cover medium. A detection limit of 1.5×10^{-6} was achieved by Drapp et al. (1997) using a wavelength with glass substrate BGG36.

Other types of interferometric optical sensors include the Young interferometers with four channels, which achieve the simultaneous and independent detection of up

to three antigen–antibody reactions (Ymeti et al., 2003). In this device, the interference pattern is monitored by a charge-coupled device (CCD) camera and is analyzed with a fast Fourier transform algorithm, resulting in a detection limit equal to 8.5×10^{-8}.

3.4.3 Biosensors Based on Antiresonant Reflecting Optical Waveguides

The ARROW is a five-layer waveguide where the optical confinement is based on the total internal reflection at the air–core interface and a very high reflectivity, of 99.96%, at the two interference cladding layers underneath the core (Figure 3.5) (Prieto et al., 2000).

The ARROW waveguides are usually designed with CMOS-compatible technology and the material commonly used is silicon for the substrate, oxide silicon for the core, and the second cladding layer and nitride silicon for the first cladding layer. ARROW structures exhibit low loss, allowing greater dimensions than conventional waveguides based on TIR and are characterized by their high sensitivity. These features are quite attractive for sensoring applications.

3.4.4 Biosensors Based on Surface Plasmon Resonance

The principle behind surface plasmon resonance (SPR) is the interaction between light and a thin metallic film (e.g., Au or Ag) coated on a transparent medium. When the opposite surface of the substrate is coated with a thin metallic layer, there is an angle θ_{SPR} greater than the critical angle θ_C (i.e., the angle of incidence at which the light is totally reflected back) where the light, instead of being totally internally reflected back, is "coupled" into the metallic film, resulting in a minimum in the reflected light intensity (Figure 3.6). This angle is called the SPR angle and is the result of the oscillations of the surface electrons that propagate along the boundary of the metal film and the dielectric layer, causing the surface plasmon wave.

It has been shown that θ_{SPR} depends not only on the physical properties of the metal boundary layer but also on the dielectric properties of the medium, which is

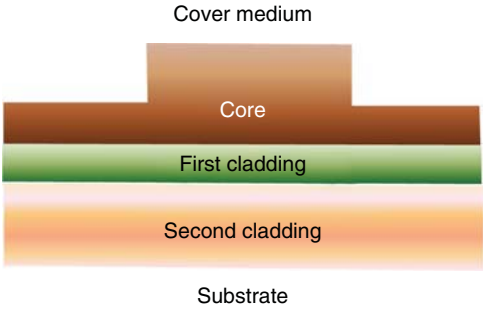

Figure 3.5 ARROW waveguide.

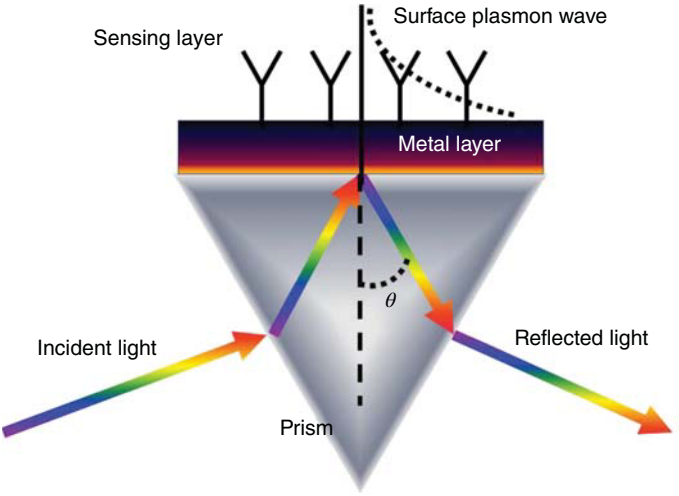

Figure 3.6 Structure of biosensor based on SPR.

directly in contact with the metal film. SPR can be used to study the biomolecular interactions and measure the concentration levels of analytes in complex samples (Hoa et al., 2007). The development of SPR biosensors is motivated by the need for compact, low-cost, sensitive devices. Prism coupling or Kretchmann's configuration has been found to be very suitable for sensing and is the most widely used geometry in SPR sensors. Biacore AB is one of the companies that have successfully commercialized prism-based SPR sensor systems (Homola, 2003). Nevertheless, because of the size constraints of the prism, it is not always easy to integrate in compact devices, and therefore optical fibers and waveguides have been proposed as good alternatives to transmit the surface plasmon excitation and reflection light.

Waveguide coupling is a robust and simple way to control the optical path in the sensing layer and is easier to integrate with other optical and electrical components, offering extra benefits like miniaturized devices and remote sensing (Velasco-Garcia, 2009). The excitation of a surface plasmon wave in an optical waveguide-based SPR sensor is similar to that in the Kretschmann approach, since the light propagates in a waveguide through total internal reflection and generates an evanescent field at the waveguide–metal interface. The surface plasmon wave is excited if the phase velocities of the waveguide mode and that of the surface plasmon match. Recently, microfabrication has been employed in order to improve the coupling of light to the surface plasmon mode.

3.5 THERMAL/CALORIMETRIC BIOSENSORS

A property that all biological reactions taking place inside living organisms share is the absorption or evolution of heat, reflected to the surrounding environment as a

change in the temperature. Thermal biosensors exploit this property and constitute a promising tool with various applications (e.g., detecting pathogenic bacteria), especially since the enzyme thermistor (ET) was invented (Danielsson and Mosbach, 1988). Among their advantages are their small size, long-term and high stability, and non–chemical contact measuring (Syam et al., 2012).

Calorimetry involves measuring the heat following a biochemical reaction. Defining Q as the total heat absorbed or produced during a chemical reaction, n_p as the amount of moles produced, ΔH as the molar enthalpy change, C_p as the heat capacity of the system, and ΔT as the temperature shift caused by the heat evolution or absorption, the first law of thermodynamics, which deals with energy conservation, is expressed as

$$Q = -n_p \sum \Delta H \tag{3.6}$$

$$Q = C_p \, \Delta T \tag{3.7}$$

The basic part of a thermal biosensor is a temperature sensor that measures the temperature and immobilized enzyme molecules. When these enzymes come into contact with the analyte, a biochemical reaction takes place and the temperature shift is measured. The enthalpy change that a biochemical reaction will cause depends on the specific enzyme-catalyzed reaction, namely on the enzyme and the substrate used, and the heat capacity of the system on the organic solvent of which the surrounding environment consists. These can be specified by tables available in the literature. Thus, the amount of product molecules created can be calibrated against the temperature change (Lammers and Scheper, 1999).

The temperature sensor most commonly used for measuring the temperature in the reaction medium is an ET. (See Figure 3.7.) It consists of an external thermostated box surrounded by a foam which insulates it from the environment and the internal cylinder as well. The cylinder inside the box has two cavities for two columns which are inserted inside it. Each column has a probe attached to it, where the immobilized enzymes can be placed. If different enzymes are placed on the probes, two different analytes can be measured by the device, but the thermistor can also be used with an immobilized enzyme on the first column and a reference column with an inactivated enzyme or just support material. Two separate small thermistors connected to a Wheatstone brigde are fixed on each of the two columns respectively measuring the temperature of the sample inserted in the probe connected to its column.

The buffer certifies the current flow of a stream inside the cylinder to which samples are injected and pumped to the thermistor. When the thermistor probe where the immobilized enzymes are placed reacts with the analyte, the temperature change caused is detected by the measuring thermistor. The differential temperature signal of the measuring and reference thermistor is the temperature shift, which according to equations (3.6)–(3.7), corresponds to a substrate concentration.

The characteristic that distinguishes thermistors from other temperature sensors is that they work over a relatively small temperature range. As resistance decreases exponentially with the increase of temperature, thermistors are considered highly

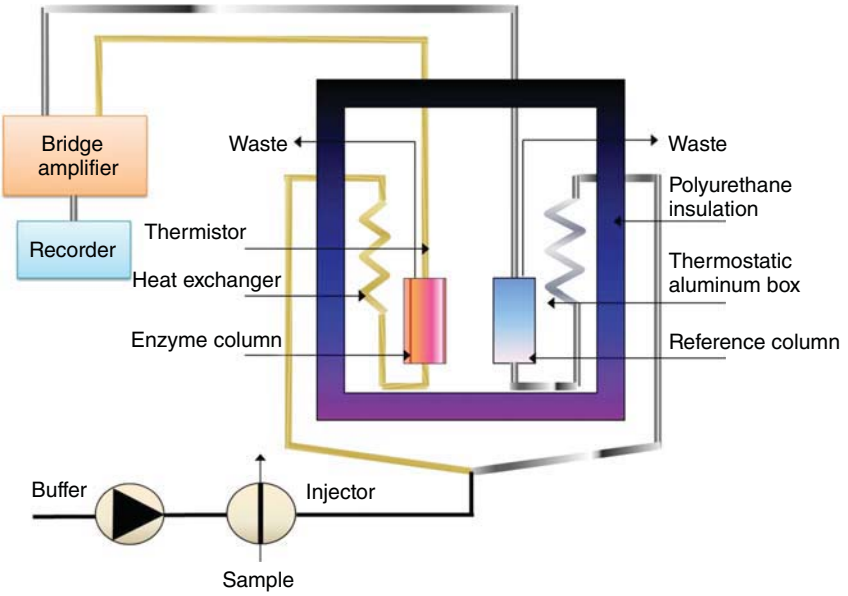

Figure 3.7 Schematic setup of enzyme thermistor.

sensitive devices within their working range, which constitutes another advantage accompanying their low cost and small size.

Thermal biosensors were recently applied as devices for detecting pesticides and pathogenic bacteria (Syam et al., 2012), and of course they are still used as detectors for the presence of particular substances or for measuring biological parameters.

3.6 PIEZOELECTRIC BIOSENSORS

The fundamental principle on which piezoelectric biosensors base their operation is the piezoelectric effect connected with their basic component, namely the piezoelectric crystal. Piezoelectricity is the electric charge in crystals and biological matter (e.g, DNA, proteins) resulting from changes in pressure. In our case, when the substance on the surface of the piezoelectric crystal reacts with a substrate, its mass changes, resulting in an oscillation of the crystal's characteristic reasonant frequency. Thus, a piezoelectric biosensor can be defined as a device that measures pressure by converting it to an electrical charge utilizing the piezoelectric effect.

Assuming a crystal with a thin, uniform, and purely elactic surface, Sauerbrey (1959) derived the basic equation describing the relationship between the reasonant frequency of an oscillating piezoelectric crystal (f in Hertz) and the amount of mass

deposited or removed from the crystal's surface (Δm in grams) as follows:

$$\Delta f = \frac{Kf^2 \ \Delta m}{A} \tag{3.8}$$

where K is a constant of the crystal which depends on its structure, A is the crystal's surface area in square centimeters, and Δf is the change of the reasonant frequency in Hertz.

The most common piezoelectric sensor used in biomedical applications consists of a thin crystal with two electrodes fixed to the two opposite sides. An electrical field applied to the crystal through the electrodes makes the crystal oscillate near its resonant frequency. When the substance immobilized on the crystal's surface reacts with the analyte, the mass on the crystal changes, causing a frequency shift that is easily detected by the oscillator signal.

Choosing a crystal with proper reasonant frequency is critical for the detection of the desired biomolecule. Especially for small molecules, such as antigens, amplification and other types of signal processing may be necessary in order to obtain a direct and observable signal if the reasonant frequency is not the appropriate one.

Apart from being small and inexpensive, piezoelectric biosensors have many advantages. Crystal materials are not very sensitive and offer long-term stability as well as almost excellent temperature behavior. Above all they are capable of giving a rapid response signal, which makes them especially attractive when compared to other classical devices. The major drawback of these sensors is that they cannot be used for static measurements as they only detect changes of a variable.

Piezoelectric biosensors can be used as part of a flow injection analysis (FIA) system (Figure 3.8), but they have other applications as well. They are devices that can detect substances in aqueous solutions or directly and continuously monitor their

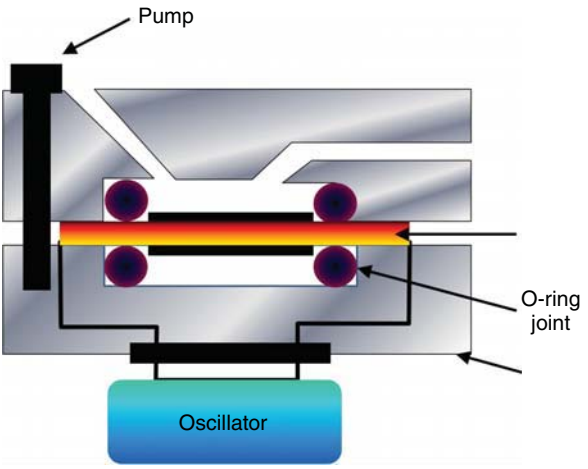

Figure 3.8 Schematic setup of piezoelectric biosensor.

concentration. In one of their recent and most attractive applications they were used for point mutation detection in human DNA (Del'Atti et al., 2006).

3.7 OTHER TYPES OF BIOSENSORS

3.7.1 Magnetic Biosensors

Magnetic biosensors were used in the past but have been disregarded mainly because of their size and power consumption. The magnetic biosensors generated recently overcome these problems by the use of giant magnetoresistance material (GMR) and promise detection of biomolecules in small samples with low concentration with great sensitivity, selectivity, speed, and economy (Wang and Guanxiong, 2008).

The sensing technique is based on magneto-nanodetection of magnetic nanoparticles that biomolecules have. The sensors consist of the GMR detector, conductors carrying the current by which the permanent magnetic field is created, and immobilized microbeads. (See Figure 3.9.) These microbeads develop a dipole field when magnetized by the external field and can be used in order to detect small fields. It should be noted that the sensitivity is maximized when the size of the sensor is matched to the microbead's size.

The fact that this technology is scalable makes magneto-nano-GMR biosensors very promising. They can be applied for molecular diagnostics of various diseases, such as cancer, cardiac problems, or infectious diseases.

3.7.2 Pyroelectric Biosensors

Pyroelectic biosensors were named after the basic substance on which their operation is based. Pyroelectric materials have the ability to generate voltage or create a current

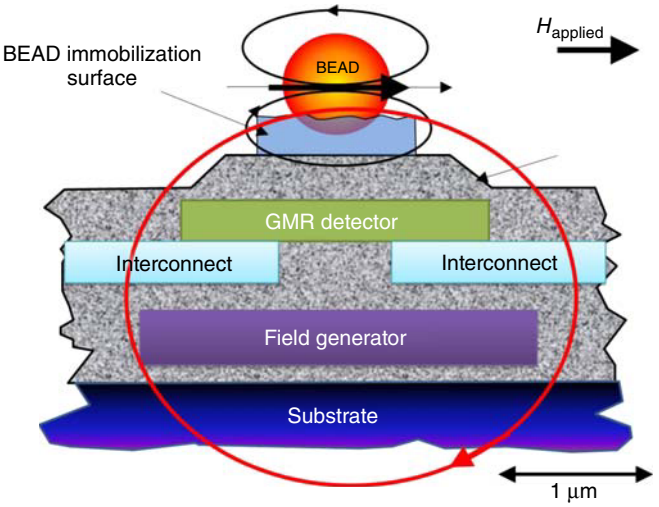

Figure 3.9 Schematic setup of magnetic biosensor.

whenever their temperature changes as the temperature shift modifies the position of their atoms, resulting in a polarization change. The sensing technique involves measuring the change in the pyroelectic material polarization, expressed as a generation of a voltage, when the temperature changes.

Assuming a pyroelectric material on which constant pressure (σ) and electric field (E) are applied, its pyroelectric coefficient (p) is expressed as

$$p = \left(\frac{\partial P}{\partial T}\right)_{E,\sigma} \tag{3.9}$$

where T stands for the temperature and P for the polarization.

3.7.3 Ion Channel Biosensors

Biological ion channels are membrane proteins whose main role is to regulate the flow of ions across the cell membrane and regulate the cell volume and its electrical and biochemical activities. The fact that they are present in the membranes of all cells makes these biosensors suitable for detecting molecules of interest, such as drugs with low molecular weight, large proteins, or microorganisms (Krishnamurthy et al., 2010).

A sensor of this type has a proper molecule fixed to a gold surface. When two nonconductive monomers align, forming a conductive dimer, the ions start flowing. The electrical charge generated when the analyte meets the immobilized molecule is the detection signal, which can also be used to feed an external device. Then it may be processed and provide analytical data, for example, about the concentration of the molecule of interest. The most important part of this type of biosensor is the switching mechanism used to disrupt the ion flow when the analyte will be detected.

This type of biosensor is very useful as it can be used with multiple immobilized molecules on it. Thus, it makes it possible for the sensor to detect a number of different analytes that contribute to ion channel signal delivery. One of its most common uses is for the detection of influenza and viruses (Syam et al., 2012).

3.8 CONCLUSIONS

The development of novel sophisticated tools and new materials as well as the advances in microelectronics over the last years have resulted in a major transformation of sensing devices, which are nowadays characterized by increased reliability and accuracy with respect to the sensed information. Several types of biosensors exist—electrical, electrochemical, optical, piezoelectric, and thermal/calorimetric—each with different advantages and disadvantages. The construction of a biosensor is a complex task where several design principles must be taken into account, including the selection of the biological element that will be used for the detection of the analyte, the chemical nature of the transducer, and the position in the protein to introduce the signal. Another important factor

to be considered is the requirement for fast real-time and reliable reproducible measurements.

Modern biomedical applications, which require miniaturized medical devices to be implanted into human bodies, dictate even more stringent requirements, including the size, energy efficiency, and biocompatibility of the device with the individual patient. Biosensor research is particularly motivated by the need for miniaturized clinically useful devices where microscale biosensor devices react with the analyte and generate a measurable response within the same compact body. In the future the further miniaturization of the implantable devices is expected to enable on-organ monitoring and highly specific treatment delivery without prohibiting the normal functioning of surrounding organs and tissues. Furthermore, in addition to more sophisticated neuroprosthetics and artificial organs, the expected developments in brain–computer interfacing will enhance our ability to investigate and alter cognitive or sensori-motor functions in humans.

REFERENCES

Bakker E. 2004. Electrochemical sensors. *Anal. Chem.* 76:3285–3298.

Belluzo MS, Ribone MÉ, Lagier CM. 2008. Assembling amperometric biosensors for clinical diagnostics. *Sensors* 8(3):1366–1399.

Buerk D. 1993. *Biosensors. Theory and Applications*. Lancaster: Technomic Publishing.

Chaubey A, Malhotra BD. 2002. Mediated biosensors. *Biosensors Bioelectron.*, 17(6):441–456.

Clark LC, Lyons C. 1962. Electrode systems for continuous monitoring in cardiovascular surgery. *Ann. NY Acad. Sci.*, 102(1):29–45.

Danielsson B, Mosbach B. 1988. Enzyme Thermistors. *Methods Enzymol.*, 137:181–197.

Dell'Atti D, Tombelli S, Minunni M, Mascini M. 2006. Detection of clinically relevant point mutations by a novel piezoelectric biosensor. *Biosens Bioelectron.*, 21(10):1876–1879.

Drapp B, Piehler J, Brecht A, Gauglitz G, Luff BJ, Wilkinson JS, Ingenhoff J. 1997. Integrated optical Mach-Zehnder interferometers as simazine immunoprobes. *Sensors Actuators B: Chem.*, 39(1):277–282.

Fan X, White IM, Shopova SI, Zhu H, Suter JD, Sun Y. 2008. Sensitive optical biosensors for unlabeled targets: A review. *Anal. Chim. Acta*, 620(1):8–26.

Gauglitz G. 2005. Direct optical sensors: Principles and selected applications. *Anal. Bioanal. Chem.*, 381(1):141–155.

Grieshaber D, MacKenzie R, Vörös J, Reimhult E. 2008. Electrochemical biosensors—Sensor principles and architectures. *Sensors*, 8(3):1400–1458.

Guiseppi-Elie A, Brahim S, Slaughter G, Ward KR. 2005. Design of a subcutaneous implantable biochip for monitoring of glucose and lactate. *Sensors J., IEEE*, 5(3):345–355.

Hoa XD, Kirk AG, Tabrizian M. 2007. Towards integrated and sensitive surface plasmon resonance biosensors: A review of recent progress. *Biosensors Bioelectron.*, 23(2):151–160.

Homola J. 2003. Present and future of surface plasmon resonance biosensors. *Anal. Bioanal. chem.*, 377(3):528–539.

Iniewski K (ed.). 2012. *Biological and Medical Sensor Technologies*. Boca Raton, FL: CRC Press.

Krishnamurthy V, Monfared SM, Cornell B. 2010. Ion-channel biosensors—Part I: Construction, operation, and clinical studies. *IEEE Trans. Nanotechn.*, 9(3):303–312.

Lammers F, Scheper T. 1999. Thermal biosensors in biotechnology. In Scheper T. (ed.). *Thermal Biosensors, Bioactivity, Bioaffinitty*. Springer: Berlin/Heidelberg, pp. 35–67.

Lopez-Martinez MJ, Campo EM, Caballero D, Fernandez E, Errachid A, Esteve J, Plaza JA. 2009. Versatile micropipette technology based on deep reactive ion etching and anodic bonding for biological applications. *J. Micromech. Microeng.*, 19(10):105013.

Medintz IL, Deschamps JR. 2006. Maltose-binding protein: A versatile platform for prototyping biosensing. *Curr. Opin. Biotechnol.*, 17(1):17–27.

Monošík R, Stre'anský M, Šturdík E. 2012. Biosensors—classification, characterization and new trends. *Acta Chim. Slov.*, 5(1):109–120.

Passaro V, Dell'Olio F, Casamassima B, De Leonardis F. 2007. Guided-wave optical biosensors. *Sensors*, 7(4):508–536.

Patolsky F, Weizmann Y, Willner I. 2004. Long-range electrical contacting of redox enzymes by SWCNT connectors. *Angew. Chem. Int. Ed.*, 43(16):2113–2117.

Perumal V. Hashim U. 2013. Advances in biosensors: Principle, architecture and applications. *J. Appl. Biomed.*, 11.

Plaxco KW, Soh HT. 2011. Switch-based biosensors: A new approach towards real-time, in vivo molecular detection. *Trends Biotechnol.*, 29(1):1–5.

Pohanka M, Skladal P. 2008. Electrochemical biosensors—principles and applications. *J. Appl. Biomed.*, 6(2):57–64.

Prieto F, Llobera A, Calle A, Lechuga LM. 2000. Design and analysis of silicon antiresonant reflecting optical waveguides for evanscent field sensor. *J. Lightwave techn*., 18(7):966.

Prieto F, Sepulveda B, Calle A, Llobera A, Domínguez C, Abad A, Lechuga LM. 2003. An integrated optical interferometric nanodevice based on silicon technology for biosensor applications. *Nanotechnology*, 14(8):907.

Sassolas A, Blum LJ, Leca-Bouvier BD. 2012. Immobilization strategies to develop enzymatic biosensors. *Biotechn. Adv.*, 30(3):489–511.

Sauerbrey G. 1959. Verwendung von Schwingquarzen zur Wägung dünner Schichten und zur Mikrowägung. *Zeitschrift für Physik* 155(2):206–222.

Schabmueller CGJ, Loppow D, Piechotta G, Schütze B, Albers J, Hintsche R. 2006. Micromachined sensor for lactate monitoring in saliva. *Biosensors Bioelectron.*, 21(9):1770–1776.

Serra PA (ed.). 2011, Biosensors for health, environment and biosecurity. *InTech Open Access*.

Syam R, Davis KJ, Pratheesh MD, Anoopraj R, Surej Joseph B. 2012. Biosensors: A Novel Approach for Pathogen Detection. *Vetscan*, 7:14–18.

Thevenot DR, Toth K, Durst RA, Wilson GS. 1999. Electrochemical biosensors: Recommended definitions and classification. *Pure Appl. Chem.*, 71(12):2333–2348.

Velasco-Garcia MN. 2009. Optical biosensors for probing at the cellular level: A review of recent progress and future prospects. In *Seminars in Cell & Developmental Biology*, Vol. 20, No. 1, San Diego, CA: Academic Press, pp. 27–33.

Wang, SX, Guanxiong L. 2008. Advances in giant magnetoresistance biosensors with magnetic nanoparticle tags: Review and outlook, *IEEE Trans. Magnetics*, 44(7):1687–1702.

Wang H, et al. 2009. Recent progress in strategies for the creation of protein-based fluorescent biosensors. *Chembiochem*, 10(16):2560–2577.

Ymeti A, Kanger JS, Greve J, Lambeck PV, Wijn R, Heideman RG. 2003. Realization of a multichannel integrated Young interferometer chemical sensor. *App. optics*, 42(28):5649–5660.

Yoshinobu T, Iwasaki H, Ui Y, Furuichi K, Ermolenko Y, Mourzina Y, Schöning MJ. 2005. The light-addressable potentiometric sensor for multi-ion sensing and imaging. *Methods* (San Diego, Calif.), 37(1):94.