
Preface

Biosensor Technologies

A biosensor is defined by the International Union of Pure and Applied Chemistry (IUPAC) as “a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals” [1]; all biosensors are based on a two-component system:

1. Biological recognition element (ligand) that facilitates specific binding or biochemical reaction with the target analyte.
2. Signal conversion unit (transducer).

Since the publication of the first edition of this book in 2009, “classical” biosensor modalities such as electrochemical or surface plasmon resonance (SPR) continue to be developed. New biosensing technologies and modalities have also been developed, including the use of nanomaterials for biosensors, fiber-optic-based biosensors, genetic code-based sensors, and field-effect transistors and the use of mobile communication device-based biosensors. Although it is impossible to describe the fast-moving field of biosensing in a single publication, this book presents descriptions of methods and uses for some of the basic types of biosensors while also providing the reader a sense of the enormous importance and potential for these devices. In order to present a more comprehensive overview, the book also describes other biodetection technologies.

Dr. Leland C Clark, who worked on biosensors in the early 1960s, provided an early reference to the concept of a biosensor by developing an “enzyme electrode” for glucose concentration measurement using the enzyme glucose oxidase (GOD) [2]. Glucose monitoring is essential for diabetes patients, and even today, the most common clinical biosensor technology for glucose analysis is the electrochemical detection method envisioned by Clark more than 50 years ago. Today glucose monitoring is performed using rapid point of care biosensors made possible through advances in electronics that have enabled sensor miniaturization. The newest generation of biosensors includes phone-based optical detectors with high-throughput capabilities.

The Use of Biosensors

Biosensors have several potential advantages over other methods of biodetection, including increased assay speed and flexibility. Rapid, real-time analysis can provide immediate interactive information to health-care providers that can be incorporated into the planning of patient care. In addition, biosensors allow multi-target analyses, automation, and reduced testing costs. Biosensor-based diagnostics may also facilitate screening for cancer and other diseases by improving early detection and therefore improving prognosis. Such technology may be extremely useful for enhancing health-care delivery to underserved populations and in community settings.

The main advantages of biosensors include:

Rapid or real-time analysis: Direct biosensors such as those employing surface plasmon resonance (SPR) enable rapid or real-time label-free detection and provide almost immediate interactive sample information. This enables facilities to take corrective measures before a product is further processed or released for consumption.

Point of care detection capabilities: Biosensors can be used for point of care testing. This enables state-of-the-art molecular analysis without requiring a laboratory.

Continuous flow analysis: Many biosensors are designed to allow analysis of bulk liquids. In such biosensors, the target analyte is injected onto the sensor using a continuous flow system immobilized in a flow cell or column, thereby enhancing the efficiency of analyte binding to the sensor and enabling continuous monitoring.

Miniaturization: Increasingly, biosensors are being miniaturized for incorporation into equipment for a wide variety of applications including clinical care, food and dairy analyses, agricultural and environmental monitoring, and in vivo detection of a variety of diseases and conditions.

Control and automation: Biosensors can be integrated into online process monitoring schemes to provide real-time information about multiple parameters at each production step or at multiple time points during a process, enabling better control and automation of biochemical facilities.

Biosensor Classification

In general, biosensors can be divided into two groups: direct recognition sensors in which the biological interaction is directly measured and indirect detection sensors which rely on secondary elements (often catalytic) such as enzymes or fluorescent tags for measurements. Figure 1 illustrates the two types of biosensors. In each group, there are several types of

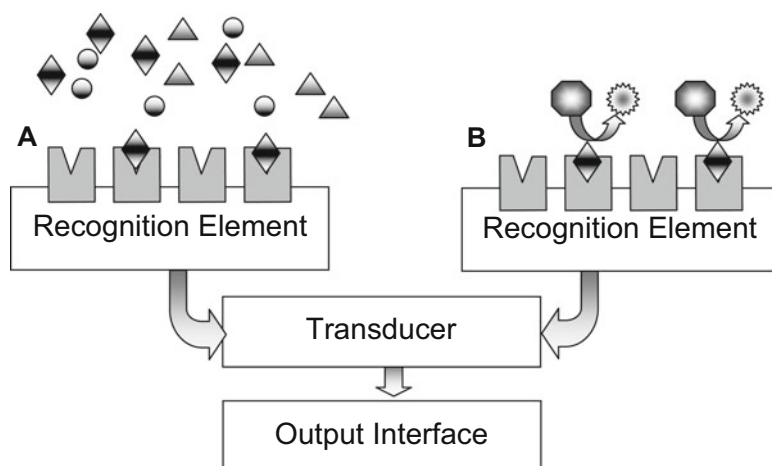


Fig. 1 General schematic of biosensors. (A) Direct detection biosensors where the recognition element is label-free and (B) indirect detection biosensors using “sandwich” assay where the analyte is detected by labeled molecule. Direct detection biosensors are simpler and faster but typically yield a higher limit of detection compared to indirect detection systems

optical, electrochemical, or mechanical transducers. Although the most commonly used ligands are antibodies, other ligands are being developed including aptamers (protein-binding nucleic acids) and peptides.

There are numerous types of direct and indirect recognition biosensors, and the choice of a suitable detector is complex and based on many factors. These include the nature of the application, type of labeled molecule (if used), sensitivity required, number of channels (or area) measured, cost, technical expertise, and speed of detection. In this book, we describe many of these detectors, their application to biosensing, and their fabrication.

The transducer element of biosensors converts the biochemical interactions of the ligand into a measurable electronic signal. The most important types of transducer used today are optical, electrochemical, and mechanical.

Direct Label-Free Detection Biosensors

Direct recognition sensors, in which the biological interaction is directly measured in real time, typically use non-catalytic ligands such as cell receptors or antibodies. Such detectors typically measure directly physical changes (e.g., changes in optical, mechanical, or electrical properties) induced by the biological interaction and do not require additional labeled molecules (i.e., are label-free) for detection. The most common direct detection biosensors are optical biosensors including biosensors which employ evanescent waves generated when a beam of light is incident on a surface at an angle yielding total reflection. Common evanescent wave biosensors are surface plasmon resonance (SPR) or resonant mirror sensors. Other direct optical detectors include interferometric sensors or grating coupler. Nonoptical direct detection sensors are quartz resonator transducers that measure change in resonant frequency of an oscillating piezoelectric crystal as a function of the mass (e.g., analyte binding) on the crystal surface, microcantilevers used in microelectromechanical systems (MEMS) measuring bending induced by the biomolecular interactions, or field-effect transistor (FET) biosensors, a transistor gated by biological molecules. When biological molecules bind to the FET gate, they can change the gate charge distribution resulting in a change in the conductance of the FET.

Indirect Label-Based Detection Biosensors

Indirect detection sensors rely on secondary elements for detection and utilize labeling or catalytic elements such as enzymes. Examples of such secondary elements are the enzyme alkaline phosphatase and fluorescently tagged antibodies that enhance detection of a sandwich complex. Unlike direct sensors, which directly measure changes induced by biological interaction and are “label-free,” indirect sensors require a labeled molecule bound to the target. Most optical indirect sensors are designed to measure fluorescence; however, such sensors can also measure densitometric and colorimetric changes as well as chemiluminescence, depending on the type of label used.

Electrochemical transducers measure the oxidation or reduction of an electroactive compound on the secondary ligand and are one common type of indirect detection sensor. Several types of electrochemical biosensors have been developed including amperometric devices, which detect ions in a solution based on electric current or changes in electric current when an analyte is oxidized or reduced. Another common indirect detection biosensor employs optical fluorescence, detecting fluorescence of the secondary ligand via CCD, PMT, photodiode, and spectrofluorometric analysis. In addition, visual measurement such as change of color or appearance of bands (e.g., lateral flow detection) can be used for indirect detection.

Indirect detection can be combined with direct detection to increase sensitivity or to validate results; for example, the use of secondary antibody in combination with an SPR immunosensor. Using a sandwich assay, the analyte captured by the primary antibody is immobilized on the SPR sensor and generates a signal which can be amplified by the binding of a secondary antibody to the captured analyte.

Ligands for Biosensors

Ligands are molecules that bind specifically with the target molecule to be detected. The most important properties of ligands are affinity and specificity. Of the various types of ligands used in biosensors, immunosensors—particularly antibodies—are the most common biosensor recognition element. Antibodies (Abs) are highly specific and versatile and bind strongly and stably to specific antigens. However, Ab ligands have limited long-term stability and are difficult to produce in large quantities for multi-target biosensor applications where many ligands are needed.

Other types of ligands such as aptamers and peptides are more suited to high-throughput screening and chemical synthesis. Aptamers are protein-binding nucleic acids (DNA or RNA molecules) selected from random pools based on their ability to bind other molecules with high affinity. Peptides are another potentially important class of ligand suitable for high-throughput screening due to their ease of selection. However, the affinity of peptides is often lower than that of antibodies or aptamers, and peptides vary widely in structural stability and thermal sensitivity.

New Trends in Biosensing

While the fundamental principles and the basic configuration of biosensors have not changed in the last decade, this book expands the application of these principles using new technologies such as nanotechnology, integrated optics (IO) bioelectronics, portable imaging, new fluidics and fabrication methodologies, and new cellular and molecular approaches.

Integration of nanotechnology: There has been great progress in nanotechnology and nanomaterial in recent years. New nanoparticles have been developed having unique electric conductivity and optical and surface properties. For example, in several chapters, new optical biosensors are described that integrate nanomaterials in SPR biosensor configurations such as localized surface plasmon resonance (LSPR), 3D SPR plasmonic nanogap arrays, or gold nanoparticle SPR plasmonic peak shift. In addition to SPR biosensors, nanomaterials are also applied to fluorescence detection utilizing fluorescence quantum dot or silica nanoparticles to increase uniform distribution of enzyme and color intensity in colorimetric biosensors or to improve lateral flow detection. In addition to optical sensors, gold nanoparticles (AuNPs) have been integrated into electrochemical biosensors to improve electrochemical performance, and magnetic nanoparticles (mNPs) have been used to improve sample preparation. Nanoparticle-modified gate electrodes have been used in the fabrication of organic electrochemical transistors.

Bioelectronics: Several chapters described the integration of biological elements in electronic technology including the use of semiconductors in several configurations of field-effect transistors and light-addressable potentiometric sensors.

Application of imaging technologies: The proliferation of high-resolution imaging technologies has enabled better 2D image analysis and increases in the number of analytical channels available for various modalities of optical detection. These include two-dimensional surface plasmon resonance imaging (2D-SPRi) utilizing CCD cameras or 2D photodiode arrays. The use of smartphones for both fluorescence and colorimetric detectors is described in several manuscripts.

Integrated optics (IO): Devices with photonic integrated circuits are presented which integrate several optical and often electronic components. Examples include an integrated optical (IO) nano-immunosensor based on a bimodal waveguide (BiMW) interferometric transducer integrated into a complete lab-on-a-chip (LOC) platform.

New fluidics and fabrication methodologies: Fluidics and fluid delivery are important components of many biosensors. In addition to traditional polymer-fabricated microfluidics systems, inkjet-printed paper fluidics are described that may play an important role in LOCs and medical diagnostics. Such technologies enable low-cost mass production of LOCs. In addition, several chapters describe the use of screen printing for device fabrication.

Cellular and molecular approaches: Molecular approaches are described for aptamer-based biosensors (aptasensors), synthetic cell-based sensors, loop-mediated DNA amplification, and circular strand displacement for point mutation analysis.

While “classic” transducer modalities such as SPR, electrochemical, or piezoelectric remain the predominant biosensor platforms, new technologies such as nanotechnology, integrated optics, or advanced fluidics are providing new capabilities and improved sensitivity.

Aims and Approaches

This book attempts to describe the basic types, designs, and applications of biosensors and other biodetectors from an experimental point of view. We have assembled manuscripts representing the major technologies in the field and have included enough technical information so that the reader can both understand the technology and carry out the experiments described.

The target audience for this book includes engineering, chemistry, biomedical, and physics researchers who are developing biosensing technologies. Other target groups are biologists and clinicians who ultimately benefit from development and application of the technologies.

In addition to research scientists, the book may also be useful as a teaching tool for bioengineering, biomedical engineering, and biology faculty and students. To better represent the field, most topics are described in more than one chapter. The purpose of this redundancy is to bring several experimental approaches to each topic, to enable the reader to choose an appropriate design, to combine elements from different designs in order to better standardize methodologies, and to provide readers more detailed protocols.

Organization

The publication is divided into two volumes. Volume I (Springer Vol. XXX) focuses on optical-based detectors, while Volume II (Springer Vol. XXX) focuses on electrochemical, bioelectronic, piezoelectric, cellular, and molecular biosensors.

Volume I (Springer Vol. XXX)

Optical-based detection encompasses a broad array of technologies including direct and indirect methods as discussed above. Part I of Volume I describes various optical-based direct detectors, while Part II focuses on indirect optical detection. Three types of direct optical detection biosensors are described: evanescent wave (SPR and resonant waveguide grating), interferometers, and Raman spectroscopy sensors.

The second part of Volume I describes various indirect optical detectors as discussed above. Indirect directors require a labeled molecule to be bound to the signal-generating target. For optical sensors, such molecules emit or modify light signals. Most indirect optical detectors are designed to measure fluorescence; however, such detectors can also measure densitometric and colorimetric changes as well as chemiluminescence, and detection depends on the type of label used. Such optical signals can be measured in various ways as described in Part II. These include various CCD-based detectors which are very versatile, inexpensive, and relatively simple to construct and use. Other optical detectors discussed in Part II are photodiode-based detection systems and mobile phone detectors. Lateral flow systems that rely on visual detection are included in this section. Although lateral flow devices are not “classical” biosensors with ligands and transducers, they are included in this book because of their importance for biosensing. Lateral flow assays use simple immunodetection (or DNA hybridization) devices, such as competitive or sandwich assays, and are used mainly for medical diagnostics such as laboratory and home testing or any other point of care (POC) detection. A common format is a “dipstick” in which the test sample flows on an absorbant matrix via capillary action; detection is accomplished by mixing a colorimetric reagent with the sample and binding to a secondary antibody to produce lines or zones at specific locations on the absorbing matrix.

Volume II (Springer Vol. XXX)

Volume II describes various electrochemical-, bioelectronic-, piezoelectric-, cellular-, and molecular-based biosensors.

In Part I of Volume II, we describe several types of electrochemical and bioelectronic detectors. Electrochemical biosensors were the first biosensors developed and are the most commonly used biosensors in clinical settings (e.g., glucose monitoring). Also included are several electronic/semiconductor sensors based on the field effect. Unlike electrochemical sensors, which are indirect detectors and require labeling, electronic/semiconductor biosensors are label-free.

In Part II, we describe “mechanical detectors” which modify their mechanical properties as a result of biological interactions. Such mechanical direct biosensors include piezoelectric biosensors which change their acoustical resonance and cantilevers which modify their movement.

Part III describes a variety of biological sensors including aptamer-based sensors and cellular and phage display technologies.

Part IV describes several microfluidics technologies for cell isolation. In addition, a number of related technologies including Raman spectroscopy and high-resolution micro-ultrasound are described.

The two volumes provide comprehensive and detailed technical protocols on current biosensor and biodetection technologies and examples of their applications and capabilities.

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