

## Study & analysis of optical-Based Biosensors

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**ABSTRACT :** The scientifically proposed as well as successfully commercialized biosensor were those based on optical-based sensor for multiple analytes. Optical-based biosensors have been studied for a long time. Currently, transducers based on semiconductors and screen printed electrodes represent a typical platform for the construction of biosensors. Enzymes or enzyme labeled antibodies are the most common biorecognition components of biosensors. The principles of, and the most typical applications for optical-based biosensors are described in this review. The relevant systems are divided into three types according to the operating principle governing their method of measurement: Absorption and Reflectance Spectroscopy, Chemiluminescence and Fluorescence and Phosphorescence and the representative devices are described for each group. Some of the most typical assays are also mentioned in the text.

*Keywords: Absorption & Reflectance Spectroscopy, Chemiluminescence and Fluorescence & Phosphorescence*

### I. INTRODUCTION

Sensors are being developed to address the need for real-time analytical measurements in the field, as the physical characteristics of conventional analytical instruments (e.g. size, weight, and fragility) do not facilitate such measurements. The traditional process of sampling, preparations, and measurement creates a lapse between the time an analytical value is requested and when it is determined. The goal of sensor research is to provide continuous quantitative and/or, while preserving the precision and accuracy of laboratory analysis.

Optical fiber-based biosensors are one class of sensors that address this goal. As their name implies, optical transducers represent another major family of biosensors that have been exploited commercially. Optical biosensors, which are sometimes called 'optodes', have received considerable interest for disease / pathogen detection. The optical biosensor format may involve direct detection of the analyte of interest or indirect detection through optically labeled probes. In general, there are at least four types of biosensors using the

principles of optical technology. These are as follows: absorption / reflection; chemiluminescence; fluorescence; and phosphorescence [1, 2]. To a large extent, these all require some type of spectrophotometer to record the spectrochemical properties of the analyte [3]. It appears that sensors based on surface plasmon resonance and qualitative analysis, thereby eliminating the time lapse fluorescence principles are the two most common techniques of optical detection employed in biosensor studies [3]. In the imminent future. Recently, sufficient progress has been made in fiber-optic technology, laser miniaturisation and the reproducible fabrication of prisms / waveguides that optical biosensors may become a powerful tool in the imminent future.

### 1.1 Absorption & Reflectance Spectroscopy

When light (usually monochromatic) is passed through a sample several things can transpire. The light can either be reflected back or it can be transmitted through the sample. The process that occurs will depend on the wavelength of light, the composition (i.e., the type and concentration of molecules, etc) and thickness of the sample. The energy from the electromagnetic spectrum can be used to provide information about the changes in the local environment surrounding the analyte. Absorption spectroscopy is one technique that can be used to monitor the transmitted light intensity according to the Beer-Lambert Law. This is achieved by using a spectrophotometer to collect the absorption spectrum of the sample. The basic components of a spectrophotometer are as follows: light source (i.e., deuterium laser, laser, etc); wavelength selector; radiation transducer; and signal processor / readout device. The wavelength selector comprises various slits, lenses, mirrors, windows, gratings or prisms to isolate the radiation of interest. The radiation transducer, which is usually a semiconductor material, converts the photon energy into an electrical signal. However, in the case of an infrared spectrometer (IR) a dielectric material is sometimes employed to convert the heat energy into an electrical signal.

Infrared spectroscopy has recently become a popular tool for the detection of microorganisms such as *Escherichia coli*, *Staphylococcus*, *Candida albicans*, *Mycobacterium fortuitum*, etc [4, 5, 6]. An infrared spectrometer (IR) is able to provide a fingerprint of the microorganism by revealing changes in the molecular bond vibrations, noting that IR is based on the principles of absorption spectroscopy. Raman spectroscopy, another form of vibrational spectroscopy, has also been employed to identify a number of microorganisms [7, 8, 9]. In Raman spectroscopy the sample is irradiated with a powerful laser source (i.e., argon ion, helium / neon, etc) and the scattered radiation is measured by the spectrometer. Both infrared and Raman spectroscopy are able to provide rapid information on the molecular composition of a sample [5]; however, these techniques are not sensitive enough to directly detect pathogens in a complex matrix such as blood. In fact, the sample generally needs to be subjected to a lengthy concentration and/or culturing step before it can be measured by IR or Raman spectroscopy. Nonetheless, a recent article has appeared in the literature on the use of Raman spectroscopy as a glucose biosensor [10]. Alternatively, reflectance spectroscopy can be used, which measures the light reflected back from a surface. A commonly used method of reflectance spectroscopy for disease detection is surface plasmon resonance (SPR). This detector is able to detect subtle changes in the refractive index, which occur when cells binds to receptors immobilized on the transducer surface. SPR is a phenomenon that has been known for over 25 years and occurs during optical illumination of a metal surface (usually gold). This method is particularly attractive for direct label-free detection of pathogens and arises when light is reflected under certain conditions from a conducting film at the interface between two media of different refractive index. A thin layer of gold (~600 Å thick) is used to ensure that a quasi-electron cloud is established [4]. The generation of plasmons, which represent the 'excited' free electron portion of the surface metal layer, causes a reduction in the intensity of reflected light at a surface metal layer, causes a reduction in the intensity of reflected light at a specific angle of reflection. An evanescent wave is created at the interface of two transparent materials, which have different refractive indices (or dielectric constants), and the coupling of the electromagnetic energy of this wave with a plasma wave travelling along the surface of a thin metal film. The penetration depth of the evanescent wave is roughly 300-400 nm. Figure 1 shows a

schematic diagram of a typical SPR detector. When molecules in the sample bind to the sensor surface, the refractive index at the surface changes and the SPR signal is monitored.

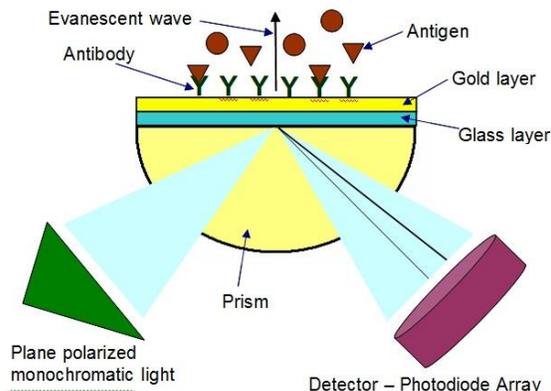


Fig. 1 A surface plasmon resonance biosensor.

SPR is a simple technique that provides non-invasive real-time kinetic data on association and dissociation rates along with equilibrium binding constants for receptor/ ligand systems [4]. It has the advantage that it can measure complex formation without labeling the reactants, and it can analyse samples from crude preparations [11]. However, some reports suggest that the SPR signal is very sensitive to non-specific physical binding on the surface, and this can severely limit this approach [4]. In addition, the technique is not sensitive enough when the molecular weight of the compound / biochemical species is less than 5000 Daltons. Notwithstanding, SPR technology has been widely applied and embraced for immunochemical sensing in the environmental, pharmaceutical, food and medical fields [12, 13, 14]. Similarly, a number of papers have shown that SPR is a powerful tool for pathogen / disease identification [15, 16, 17, 18, 19, 20]. Gomara et al. (2000) developed an immunosensor, which uses synthetic peptides for the detection of the hepatitis A virus in human serum [16], whereas Koubova et al. (2001) used an SPR-based immunosensor to detect various bacteria (i.e., *Salmonella enteritidis* and *Listeria monocytogenes*) [21]. More importantly, these reports suggest that the sensitivity of SPR is comparable to ELISA [16, 17]. Gomes et al. used SPR to study the effects of combining multiple amino acid replacements within the sequence of the antigenic GH loop of foot-and- mouth disease virus [22]. Recently, Canziani and coworkers (2004) demonstrated that it is possible to screen many antibodies from hybridoma culture samples using the

same SPR sensor surface [11]. Various other groups have developed SPR biosensors for the detection of DNA [15, 18].

### 1.2 Chemiluminescence

Luminescence is a term normally applied when discussing emission of radiation. It occurs when light is emitted from atoms or molecules due to an electronic transition from an excited state to a lower energy state or the ground state [1]. Chemiluminescence is a technique that can be used to detect specific biochemical reactions. It involves the generation of light as a result of a chemical reaction between the analyte and the chemiluminescent species (e.g. Rhodamine B). In fact, some chemical reactions can create an excited intermediate and in the process emit light, noting that no external light source is needed to instigate the reaction. A photomultiplier tube is used to detect the emitted light. Some reports suggest that the chemiluminescence transducer is the most suitable optical method for detecting antigen-antibody reactions [23], whereas recent studies by Chen and coworkers have used this transduction method to detect DNA from calf thymus and herring sperm [24, 25]. More importantly, it was revealed that chemiluminescence is a very sensitive method, noting that a detection limit of  $\sim 6.5 \times 10^{-6} \mu\text{g/ml}^{-1}$  was obtained for calf thymus DNA and  $\sim 4.3 \times 10^{-8} \mu\text{g/ml}^{-1}$  for herring sperm DNA [24, 25]. However, very few studies have been undertaken using chemiluminescence detection compared to other optical methods. In addition, this technique is time consuming and is not amenable for real time monitoring in blood.

### 1.3. Fluorescence & Phosphorescence

Fluorescence is slightly different from chemiluminescence, in that it requires an external light source to initiate electronic transitions in an atom or molecule. In this process light is used to excite electrons to a higher energy state and as the electrons return to a lower energy level this causes light to be emitted at a longer wavelength. On a similar note, when a phosphorescent material is illuminated with light, some light is absorbed, exciting it into a higher energy state [26]. The energy is then transferred (normally called quenching) either by light emission or some other process. It is important to note that phosphorescence is generally a slower process and is not as popular compared to fluorescence detection. Since, neither antigens nor

antibodies exhibit any fluorescence properties, a conjugate is used to generate a fluorescent signal. In fluorescent immunoassays, fluorochrome molecules are used to label immunoglobulins. The fluorochrome absorbs short-wavelength light and then emits light at a higher wavelength, which can be detected using fluorescent microscopy or a spectrophotometer (i.e., Fluorometer). Fluorescein isothiocyanate and rhodamine isothiocyanate-bovine serum albumin are the most common fluorochromes used to tag antibodies [27]. Fluorescence measurements are of particular interest in biochemical systems due to their high sensitivity. Today, most of the DNA chips are used with fluorescent markers [28].

A recent review has shown that this method is very appealing for nucleic acid detection [29]. Similarly, immunosensors that involve fluorescence transduction are probably one of the most sensitive methods of detection [23]. Rowe-Taitt and coworkers have developed a fluorescence-based multianalyte immunosensor array to simultaneously detect various microorganisms / toxins (i.e., Bacillus anthracis, Francisella tularensis, Cholera toxin, Ricin, Staphylococcal enterotoxin, Botulinum toxins, etc) [30, 31]. It is reported that this technology exhibits comparable sensitivity to the standard ELISA method [30]. In addition, it has led to the fabrication of a portable biosensor, which essentially consists of a patterned array of biological recognition elements (i.e., antibodies, receptors) immobilised on the surface of a planar waveguide, and a fluorescence assay is performed on the patterned surface, which yields an array of fluorescent spots [32]. Signal transduction is achieved by using a diode laser for fluorescence excitation and a CCD camera to capture the image [32]. Recently, other workers have used the fluorescence detection platform for Bacillus anthracis and Escherichia coli [33]. Lichlyter et al. (2003) developed a novel immunosensor technique based on fluorescence resonance energy transfer [34]. Moschou et al. (2004) report that fluorescence-based glucose detection is a growing class of sensors [35]. However, a major drawback of fluorescence technology is that it is relatively expensive, often gives rise to a reaction that is time consuming, exhibits lower selectivity and is not amenable for real time monitoring [23, 29]

Table 1. Properties of transducer technologies used in biosensors.

Fluorescence	Expensive	Difficult	Very Good	Excellent	Indirect	Time consuming
Chemiluminescence	Expensive	Difficult	Very Good	Excellent	Indirect	Limited to
Surface Plasmon Resonance	Expensive	No	Good	Good	Direct	Versatile

## 2. Conclusion

Optical based biosensors have been the focus of a large volume of research in the past 2 year. Fluorescence optical based biosensors continue to be the most commonly reported type; however, the number of publication on immunoassay, nucleic acid, and chemiluminescence optical based biosensors has greatly increased. Immobilization of the biological recognition element has been a common focus for optical based biosensor research, and has ultimately led to increases in sensitivity, selectivity, and in some cases, reversibility. Optical based biosensors are beginning to address issues associated with shelf life and long-term stability; some of the sensors reported in this review were stored for several months, or were used for periods up to a month while retaining over 75% of their initial response. Advances have been made in lowering detection limits as well; several papers reported sensors with detection limits comparable to more sophisticated large bench-top analytical instrumentation. Despite these advances, much more work needs to be done before optical based biosensor become commercially viable options for sensing.

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