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New Generation Biosensors Based on Ellipsometry

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1. Introduction

1.1 What is a biosensor?

There is a big demand for the fast, reliable and low-cost systems for the detection, monitoring and diagnosis of biological molecules and diseases in medicine (Sharma, 1994; D`Orazio, 2003; Mohanty and Kougianos, 2006). Of course, this demand is not restricted only in the field of medicine; for environmental pollutant monitoring, detection of food borne pathogens and potential danger of bioterrorism. Therefore, researchers from various fields such as; physics, chemistry, biology, engineering and medicine interested in the developing, constructing and manufacturing of new sensing devices to get more efficient and reliable information (Figure 1.); (Mohanty and Kougianos, 2006).

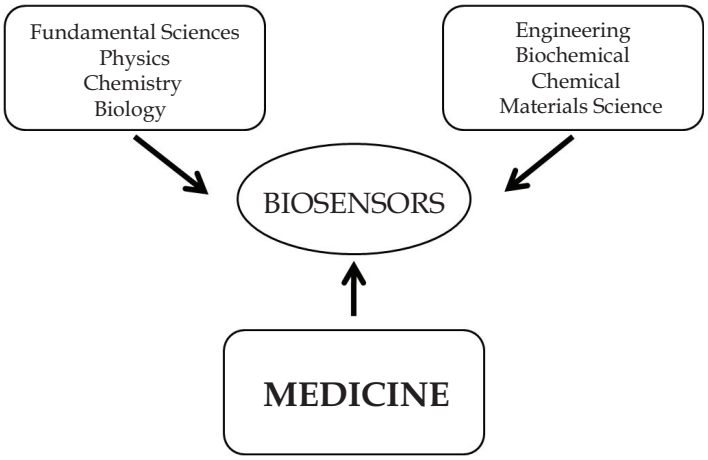

Fig. 1. Biosensors; an excellent example of multi and interdisciplinary research area.

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Biosensors are the most impressive and useful devices which correspond to these purposes. In the literature about biosensors, there are two common articles which describe the term biosensor as "a biosensor is a chemical sensing device in which a biologically derived recognition entity is coupled to a transducer, to allow the quantitative development of some complex biochemical parameter," and "a biosensor is an analytical device incorporating a deliberate and intimate combination of a specific biological element (that creates a recognition event) and a physical element (that transduces the recognition event)" by S.P.J. Higson and D.M. Fraser, respectively (Fraser, 1994; Higson, 1994; Mohanty and Kougianos 2006). In another words, a biosensor is commonly defined as an analytical device which is used mostly for the recognition of target biological molecules, macromolecules, and atoms which can also be named as analytes. Typically, a biosensor consists of three main components: first one; a biological recognition part that is responsible for the specific interaction with analyte second one; a transducer which transduces the biological interaction to an electrical signal and third one; an output system (Vo-Dinh and Cullum, 2000). In figure 2, main parts of a typical biosensor are schematized.

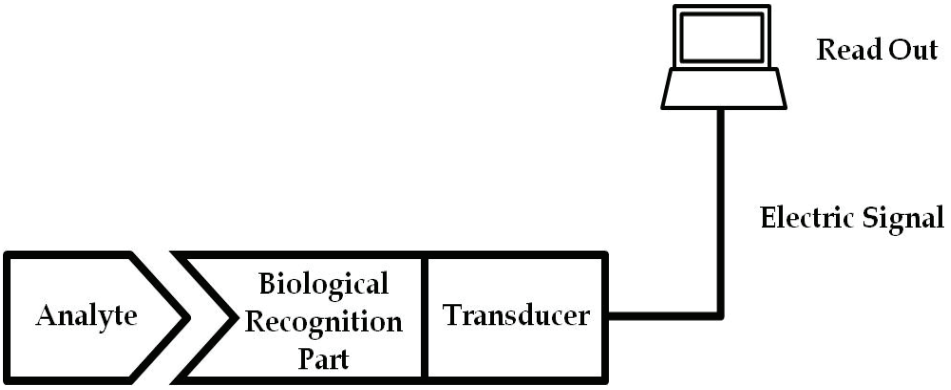
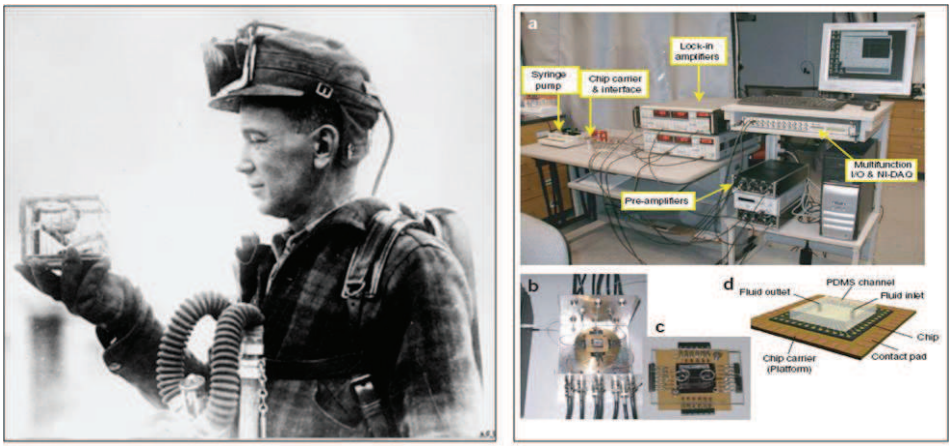


Fig. 2. Shematic representation of main parts of a typical biosensor.

1.2 History of biosensors

Most probably the first biosensors were canaries which have been used in coal mines since 1911 to monitor gas leakage. Up to early 1960`s the developments in this field were mostly in chemical sensing devices as glass electrodes for sensing hydrogen ion concentration and oxygen electrodes. In 1962, Clark made the first study on biosensors which was an amperometric enzyme biosensor for the detection of glucose (Table 1 summarizes the historical development of biosensors) (Clark, 1962). In this historical study, Clark used platinum (Pt) electrodes to detect oxygen. The enzyme glucose oxidase (GOD) was entrapped by using a piece of dialysis membrane. The change at the enzyme activity depending on the surrounding oxygen concentration was monitored by this amperometric enzyme electrode biosensor (Clark, 1962). After this milestone study, there are numerous studies have been done to monitor the interaction of target biomolecules (enzymes, Deoxyribonucleic acid (DNA), antibodies, cells, viruses etc.) and molecules (amino acids, metal ions, etc) with their complementary molecules (Justino, 2010). Today, in the nanotechnology era, quantum dots, nanowires, nanotubes and nanoparticles are being used

as new biosensing devices to get more efficient and reliable information from diagnosis and monitoring of biomolecules (Figure 3) (Yogeswaran and Chen, 2008; Ghoshal, 2010, Palma, 2010).



(a) (b)

Fig. 3. A survey of progress in biosensing devices at 100 years (a) The first biosensors were canaries which have been used in coal mines (Copyrighted Google), (b) Silicon nanowire biosensors are the latest biosensing devices (Copyrighted Nature Publishing Group).

1.3 Recognition parts and immobilization

The recognition part probably is the most crucial part of a biosensor, because the selectivity relies on the interaction between the target biomolecules (analytes) and the molecules immobilized on the surface of a biosensor. Analytes mostly in biosensors are such as; enzymes, antibodies, DNA, oligonucleotides, oligopeptides, carbohydrates, cells, bacteria, microorganisms, viruses and tissues (Vo-Dinh and Cullum, 2000). However, sometimes analytes can be macromolecules, chemicals and even if atoms, especially in biosensors that monitor the environmental pollutants. The complementary of biomolecules are immobilized on the surface of biosensors and sometimes biomimetic structures, polymers (molecular imprinted), synthetic molecules (especially in drug research using computational chemistry), oligonucleotides (ODNs) and atoms can be used to interact with the target biomolecules (analytes). Table 2, shows the specific interactions of biomolecules which are commonly used in biosensing devices to increase specificity and discriminate between different substances exist in the same environment (Vo-Dinh and Cullum, 2000).

There are several factors which affect the performance of a biosensor such as sensitivity, calibration, background signal, hysteresis, long-term stability, dynamic response and biocompatibility (Justino, 2010). Immobilization of biomolecules (molecules those are responsible for the specific interactions with the analytes) is one of the important steps of constructing a biosensing device that directly or indirectly influence the performance of a biosensor. Immobilization is a technique used for the fixation of biomolecules such as enzymes, antibodies or other proteins, oligopeptides, oligonucleotides, DNA, cells, microorganisms, organelles, physically or chemically onto (or into) a solid support for

increasing the stability, repeated and long term usability of biosensors (Tang, 2002; Chou, 2002; Manelli, 2003).

Year	Type of Sensor	References
1911	Canaries have been used as biosensors	Hernandez, 2008
1922	First glass pH electrode	Hughes,1922
1956	First oxygen electrode	Clark, 1957
1962	First biosensor: an amperometric enzyme electrode for glucose	Clark, 1962
1969	First potentiometric biosensor: urease immobilized on an ammonia electrode to detect urea	Guilbault and Montalvo, 1967
1970	First ion selective Field Effect Transistor (ISFET)	Bergveld, 1970
1975	First fiber-optic sensor : an indicator immobilized to measure carbon dioxide or oxygen	Opitz and Lübbers, 1975
1975	First commercial biosensor (Yellow springs instruments glucose biosensor)	Magner, 1998
1980	First fiber optic pH sensor for in vivo blood gases	Peterson, 1980
1982	First fiber optic-based biosensor for glucose	Schultz, 1982
1983	First surface plasmon resonance (SPR) immunosensor	Liedberg, 1983
1990	SPR based biosensor by Pharmacia BIACore	Jonsson, 1991
2000	Ellipsometric Biosensors	Arwin, 2001
Current	Quantum dots, nanoparticles, nanowires, nanotubes, etc	Ghoshal, 2010

Table 1. Historical development of biosensors.

Analyte	Complemantary
Antibody	Antigen
Enzyme	Substrate
Carbohydrates	Receptors
Cells	Receptors
Drug	Receptors
ssDNA	Complementary DNA
Metal ions	Aminoacids

Table 2. The specific interactions of biomolecules which are commonly used in biosensing devices.

Biomolecules can be immobilized physically through; hydrophobic, ionic or Van der Wall's interactions to a solid matrix or by covalently immobilized to chemically activated surfaces. Physical attachment of molecules can be very effective in many cases; however, in some applications it fails due to weakness of the interaction of biomolecules with the biosensor

surface. Covalent immobilization is often used for the molecules those do not adsorb, weakly adsorb or improperly adsorbed by using physical immobilization. In covalent immobilization, bioactivity of molecules (enzymes, protein 3D structure) can be sustained, shows less non specific adsorption and greater stability of the immobilized biomolecules (Tang, 2002; Chou, 2002; Su and Li, 2004). Proteins are much more sensitive to their physiological environments and can be easily denaturized by physical and chemical effects. Protein`s 3D conformation must not change during immobilization procedure. However, on the other side, DNA molecules are much more stable and durable to harsh physical and chemical conditions (Sharma and Rogers, 1994; Pişkin and Garipcan 2004; Pişkin 2009,).

As stated above the text, immobilization procedure is very important step for manufacturing biosensing devices so that, immobilization reaction should have several characteristics for effective, sensitive, selective and long-term usable biosensors. These are;

- The coupling reaction of biomolecules and activated groups on the biosensor surface should occur rapidly, to use low amounts of reagents for feasible and economical immobilization,
- The chemistry which will be used for immobilization should require little, if any, no post-synthetic modification of biosensor surface before immobilization,
- Immobilized molecules must be in an oriented and homogeneous manner, because orientation of biomolecules during the immobilization procedure is very important for proper interaction of target biomolecules and probe molecules (molecules responsible to interact with the target biomolecules) on the surface of the biosensor. As an example, antibody-antigen interaction occurs from the antigen binding regions of antibodies. If, the antigen binding regions of an antibody interact with the biosensor surface, proper interaction will not occur between antibody and antigen molecules.
- Surface density of the probe molecules should be optimized.
  Low density surface coverage of probe molecules will decrease the interaction of probe molecules with target biomolecules and resulted in low detection signal value,
  High surface densities may result steric hindrance between the covalently immobilized probe molecules and the target biomolecules,
- If necessary, to avoid steric hindrance and for correct orientation of the probe molecules on the surface and using a spacer arm can be critical and make the probe molecules available to interact with the target biomolecules,
- During immobilization procedure, it should be avoided for any deformation on the 3D-structures of the probe molecules (especially in the case of protein-based probes),
- None or minimum nonspecific interactions of the probe molecules with the biosensor surface will be desired, immobilization reaction should be only occur via specific functional groups (amino, carboxylic acid, aldehyde, epoxy, etc.) on the biosensor surface and probe molecules (Pişkin and Garipcan, 2004; Pişkin 2009).

1.4 Transducers

When a target biomolecule interacts with the immobilized probe molecules on a biosensor surface, depending on the application; a physical, chemical or biological change is observed. This change is converted to a measurable signal by a detecting device which is called, a transducer. These physical, chemical and biological changes can be pH, electro-active substance formation and/or consumption, heat, light, mass and viscosity. The magnitude of

the measurable electrical signal is proportional with the concentration of the target biomolecules. Transducers are the key components of the biosensors. Transducers can be categorized according to the fundamentals of the physical or chemical changes as optical, electrochemical, acoustic (mass based) and thermal transducers (Vo-Dinh and Cullum, 2000).

Optical transducers are one of the most common types of transducers used in biosensors which are based on the measuring of the changes in light. After the interaction of the target molecules and probe molecules, a change in light intensity, polarization, phase, peak position, and angular wavelength will be observed and this change can be measured and converted to an electrical signal by optical transducers (Borisov and Wolfbeis, 2008). As mentioned above, optical transducers are widely used in biosensors; however electrochemical transducers are also very common due to simplicity of construction and low cost. A change at electrical potential, current, conductance and impedance can be measured and converted to an electrical signal by electrochemical transducers (Ronkainen, 2008). Also, Field Effect Transistors (FETs) based biosensors which use one type of electrochemical transducer become very promising when integrated with semi-conductor nanowires (Patolsky, 2007; He, 2010) and Carbon Nanotubes (Yang, 2007; Hu, 2010) due to their high selectivity and low detection levels. Acoustic transducers are a relatively new concept in biosensing applications that their principle is based on responding to mass accumulation on the biosensors surface. Piezoelectric crystals (Quartz Crystal Microbalance Biosensors) are the most common acoustic transducers which involve the generation of electric currents from a vibrating crystal. The frequency of vibration is affected by the mass of material adsorbed on its surface, which could be related to changes in a reaction (Cooper and Singleton, 2007; Karamollaoğlu, 2009). There are also thermal and micro cantilever based transducers are being used as detection devices which are based on a processes measuring the production or absorption of heat and the change in the resonant frequency of the cantilevers (Micrometer-sized cantilevers, started to be used for sensing purposes shortly after the invention of the atomic force microscope (AFM) in 1986), respectively (Ricciardi, 2010; Muhlen 2010).

1.5 Classification of biosensors

Biosensors can be classified according to their recognition part [enzyme, antibody (immunosensors), nucleic acid, tissue, microbial, polysaccharide, etc] or transducers (optical, electrochemical, acoustic, thermal, etc.) (Justino, 2010). Classification according to transducers seems much more logical then recognition part, because using only the biological component does not give much information about the biosensing device. Hence using both recognition part and transducer (even if using the sub type of the transducer) together is the best way to describe the type of biosensors, as an example Ellipsometry based DNA biosensors (Figure 4) (Demirel, 2008).

Table 3 gives an overview of biosensors which are classified according to transducer and recognition parts. A brief summary of the transducer fundamentals and literature will be discussed in this section. As mentioned, electrochemical transducers are also very common due to simplicity of construction and low cost (Ronkainen, 2008). Ion et al, have chosen organophosphate pesticides as target molecules and acetylcholinesterase as probe molecules and constructed voltammetric enzyme biosensors (Ion, 2010) where voltammetry refers to

the measurement of current resulting from the application of a potential (Kissenger and Heineman, 1996; Ronkainen, 2008). In amperometry, changes in current generated by the electrochemical oxidation or reduction are monitored directly with time while a constant potential is maintained at the working electrode with respect to a reference electrode. It is the absence of a scanning potential that distinguishes amperometry from voltammetry (Barlett, 2008; Ronkainen, 2008).

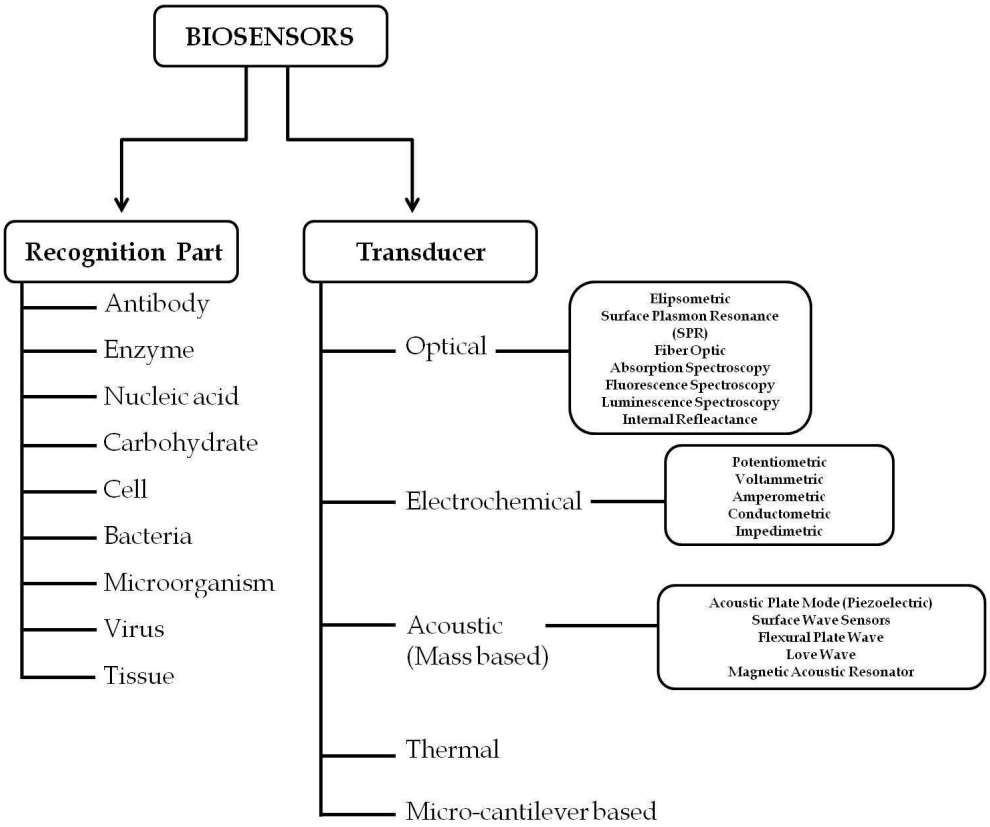


Fig. 4. The classification of biosensor according to recognition parts and transducers.

Salazar et al., have designed an amperometric enzyme biosensor for the detection of H_2O_2 in brain fluid by immobilizing Prussian blue on the biosensor surface (Salazar, 2010). Potentiometry is the branch of electroanalytical chemistry in which potential is measured under the conditions of no current flow (Eggins, 2002; Ronkainen, 2008). A DNA biosensor was developed by Wu et al (Wu, 2009) and a cell electrochemical biosensor for monitoring hydroquinone cytotoxicity on conductive polymer modified electrode surface by Wang et al (Wang, 2010) were two examples of potentiometric electrochemical biosensors. Impedimetry is an ac method that describes the response of an electrochemical cell to small amplitude sinusoidal voltage signal as a function of frequency (Prodmidis, 2010). An impedimeric

electrochemical DNA biosensor was designed by Bonani et al., for detection of Single Nucleotide Polymorphism (Bonanni, 2010). Conductometric detection relys on the changes in the electrical conductivity of the the solution (Anh, 2004; Ronkainen, 2008). Korpan et al, used an conductometric enzyme biosensor for the detection of formaldeyhde by using formaldeyde dehydrogenase as probe molecule (Korpan, 2010). The quartz crystal microbalance, QCM, and is undoubtedly the oldest and the most recognized acoustic sensor. QCM technique involves the generation of electric currents from a vibrating crystal. The frequency of vibration is affected by the mass of material adsorbed on its surface, which could be related to changes in a reaction (Cooper and Singleton, 2007; Karamollaoğlu, 2009). In a study by Wang et al, a QCM immunosensor was developed for the detection of γ-Aminobutyric acid (Wang and Muthuswamy, 2008), in an another study QCM immunosensor for monitoring Aflatoxin B1 was developed by Wang et al (Wang and Gan, 2009a). Karamollaoğlu et al was constructed an interesting DNA QCM biosensor for the detection of Genetically Modified Organisms (GMOs) (Karamollaoğlu, 2009). Love wave sensors are acoustic devices that employ Love waves, propagating shear-horizontal acoustic waves that are confined to the surface region of a substrate by applying a thin overlayer that acts as a waveguide. In common with many other acoustic sensors, the principle of measurement is that the propagation of the acoustic wave through the solid medium of the sensor is affected by changes in the adjacent medium that contains the analyte of interest (Dinh, 2010). An acoustic Love wave immunosensor was developed by Saitakis at al, for the detection of major histocompatibility complex class I HLA-A2 proteins (Saitakis, 2008). Micrometer-sized cantilevers, started to be used for sensing purposes shortly after the invention of the atomic force microscope (AFM) in 1986. A change in the resonant frequency of the cantilevers is caused by a change in mass and/or stiffness of the cantilever, and this change can be measured (Ricciardi, 2010; Muhlen 2010). An microcantilever based immunosensor was designed by Muhlen at al, for the detection of Activated Leukocyte Adhesion Molecule (ALCAM) (Muhlen, 2010). In an another study by Ricciardi et al, immunosensor and receptor based microcantilever biosensors were developed for angiopoietin using angiopoitein antiboy and protein A probe molecules, respectively. (Ricciardi, 2010). Wang et al, have used imaging ellipsometry as an immunosensor in a model study to monitor the interaction of bovine serum albumin (BSA), fibrinogen and immunoglobulin- G with their antibodies (Wang and Jin, 2003). In another, study by Demirel et al, have shown that ellipsometry could also be used to monitor DNA hybridization (Demirel, 2008). Surface plasmon resonance (SPR) biosensors are also very well known optical biosensors which have been found many applications in this field. Milkani et al have constructed a SPR based DNA biosensor for oligonucleotide mismatch detection (Milkani, 2010) and Frasconi have shown that SPR based biosensors can also be used as a drug sensor (Frasconi, 2010). Fiber-optic biosensors (FOBS) use optical fibers as the transduction element, and rely exclusively on optical transduction mechanisms for detecting target biomolecules where as Kapoor et al, have detected trophic factor by immobilizing the Anti- signal transducer and activators of transcription 3 (STAT-3) antibody on an optical fiber (Kapoor, 2004). Not only biomolecules can be detected, but chemicals like 1-2 dichloroethane was sensed with enzyme immobilized fiber optic biosensors (Derek and Müller, 2006). A more detailed description on ellipsometry and SPR biosensors will be given in next section.

Transducer	Recognition Part	Target Molecules	Probe Molecules	Ref.
Optical/ Ellipsometry	Immunosensor	Bovine Serum Albumin (BSA) Fibrinogen Immunoglobulin-G	Anti-BSA Antibody Anti-Fibrinogen AntibodyAnti-Immunoglobulin-G Antibody	Wang, 2003
Optical/ Ellipsometry	DNA	Oligonucleotide	Complementary Oligonucleotide	Demirel, 2008
Optical/SPR	DNA	Oligonucleotide mis match detection	Complementary and non-complementary Oligonucleotide	Milkani, 2010
Optical/SPR	Drug	Neomycin, Kanamycin, Streptomycin Antibiotics	Imprinted Boronic acid functionalized Au nanoparticles	Frasconi, 2010
Optical/ Fiber Optic	Immunosensor	Trophic factor	Anti- signal transducer and activators of transcription 3 (STAT-3) antibody	Kapoor, 2004
Optical/ Fiber Optic	Enzyme	1,2 Dichloroethane	Haloalkane dehalogenase	Derek, 2006
Electrochemical/ Voltammetric	Enzyme	Organophosphate pesticides	Acetylcholinesterase	Ion, 2010
Electrochemical/ Amperometric	Enzyme	H_20_2 in brain fluids	Prussian Blue	Salazar, 2010
Electrochemical/ Potentiometric	DNA	DNA hybridization	Complementary DNA	Wu, 2009
Electrochemical/ Potentiometric	Cell	Hydroquinone cytotoxicity	Conductive polymers	Wang, 2010
Electrochemical/ Impedimetric	DNA	Single Nucleotide Polymorphism	Complementary Oligonucleotide	Boranni, 2010
Electrochemical/ Conductometric	Enzyme	Formaldehyde	Formaldehyde dehydrogenase	Korpan, 2010
Acoustic/ QCM Quartz Crystal Microbalance	Immunosensor	γ-Aminobutyric acid (GABA)	Anti-GABA Antibody	Wang, 2008

Transducer	Recognition Part	Target Molecules	Probe Molecules	Ref.
Acoustic/ QCM Quartz Crystal Microbalance	Immunosensor	Aflatoxin-B1	Anti- Aflatoxin-B1 Antibody	Wang, 2009a
Acoustic/QCM Quartz Crystal Microbalance	DNA	Genetically ModifiedMicroorganisms (GDOs)	Complementary Oligonucleotide	Kara-mollaoğlu 2009
Acoustic/ Love Wave	Immunosensor	Major histocompatibility complex classI HLA-A2 proteins	Anti- HLA-A2 protein Antibody	Saitakis, 2008
Acoustic/LSAW Leaky Surface Acoustic Wave	Peptide-DNA	Human papilla virus	Complementary Oligonucleotide	Wang, 2009b
Microcantilever based	Immunosensor	Activated Leukocyte Cell Adhesion Molecule (ALCAM)	Anti-ALCAM Antibody	Muhlen, 2010
Microcantilever based	Immunosensor and Receptor	Angiopoietin-1	Anti-Angiopoeitin-1 Antibody Protein A	Ricciardi, 2010

Table 3. Overview of biosensors and transducers.

2. Ellipsometry based biosensors

In this chapter, we will specifically focus on the new generation biosensor systems based on ellipsometry for the detection of biological molecules (i.e. DNA and protein). Before discussing the sensor applications, it is useful to give some basic principles of ellipsometry for further understanding. Traditionally, ellipsometry is an optical and reflection-based technique which is mostly used for determining optical properties of materials and micro-structural parameters such as layer thicknesses, porosity and crystal orientation through ellipsometric data (Azzam and Bashara, 1972; Azzam and Bashara, 1977). In an ellipsometric measurement, fundamentally, the change in polarization, or more precisely, the polarization states after and before reflection which depend on surface properties are measured (Figure 5).

The incident light is not only reflected on the thin film surface but also penetrates into the outermost substrate material under the film surface. As a result, it reflects and refracts further at each interface and obtained ellipsometric data include information for investigated material within the penetration depth of the light (Poksinski and Arwin, 2006). In an ellipsometry, two experimental parameters (also called ellipsometric angles), ψ and Δ, defined as the relative amplitude and phase difference for p- and s-polarized light, before and after reflecting on sample surface are usually measured. They are defined by the ratio ρ

of the complex reflection coefficients R_p for light polarized parallel and R_s for perpendicular to the plane of incidence as,

$$\rho = \frac{Rp}{Rs} = \tan\psi \exp(i\Delta) \tag{1}$$

Ellipsometry does not provide the relevant informations about the structure and the investigated materials directly. In most cases, an appropriate optical model has to be established and nonlinear regression has to be applied to obtain reliable data for investigated materials. In the presence of biological molecules, further ellipsometric modeling is also needed because of their low refractive indexes and nanometer range thicknesses. More detailed informations for ellipsometry and data analysis can be found elsewhere (Poksinski and Arwin, 2006; Arwin, 2001; Arwin, 2000; Aspnes and Palik, 1985).
There are various types of ellipsometer for measuring two ellipsometric parameters, such as fixed polarizer, rotating polarizer, nulling and phase modulating. Ellipsometers can also utilize fixed wavelength or multiple wavelength light source. In monochromatic ellipsometers, typically a diode laser is used. Some versions utilize two or more diodes in order to expand measurement capability. More sophisticated ellipsometers utilize polychromatic light source and a monochromator for spectrophotometric measurements, which is more versatile than single wavelength ellipsometers. Additionally, angle modulation is necessary for an ellipsometric measurement. Angle modulation is performed either by automatic motorized controller or by manual adjustment. For angle modulation this two arm, light source and detector parts, are assembled on a goniometer, of which complexity also determine the type/price of the ellipsometer. Finally, if a monochromatic light source is used in the ellipsometer system, one may use an optical setup and preferably a CCD camera for monitoring and mapping of the surface, which system called as "imaging ellipsometer".

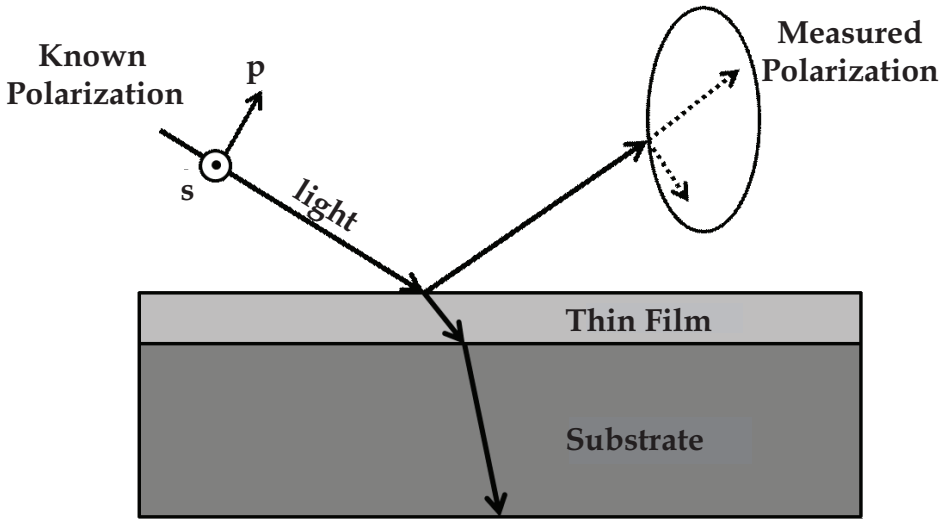


Fig. 5. The fundamental of ellipsometry.

Some of the advantages and disadvantages of ellipsometry are tabulated in Table 4. Ellipsometry has remarkable features such as high precision of the measurement, very high thickness sensitivity, fast measurement, wide application area, real-time observations, feedback control of processing and no contact with the investigated materials. Beyond these superiorities, it has also some drawbacks. The most important drawback of ellipsometry is the necessity of an optical model in data analysis. Another problem is the spot size of a light beam used for ellipsometry. Typically, they are several millimeters and caused to the low spatial resolution of the measurement. Characterization of small absorption coefficients is also rather difficult (Arwin, 2001).

Advantages	**Disadvantages**
- Non-destructive measurement	- Indirect analysis
- Large measurement range (nm to μm)	- optical model for data analysis
- Real Time monitoring	- low spatial resolution
- Fast Measurements	- Difficulty in the characterization of low absorption coefficients
- High Thickness sensitivity	
- No reference necessity	

Table 4. Some important advantages and disadvantages of Ellipsometry

Since the first application of ellipsometry to monitor antigen and antibody interactions (Rothen, 1945), ellipsometry based sensor systems have been attracted more interest for variety of applications due to the superior features, recently. The main reason of the using ellipsometry in sensor application is about reflection based technique and therefore, highly sensitive to changes taking place on the surface because of it only measures polarization change of light beam and blind to light scattering or absorption in the beam path (Arwin, 2001). As a result, any reference material is not needed like in many other techniques. Ellipsometry can also be used in explosive, corrosive or high temperature environments due to the non-electric technique. With well-collimated lasers it is possible to develop systems for remote sensing. Ellipsometry is a label-free technique and no markers are needed. In sensor applications, multi-sensing is also possible due to the each ellipsometric measurements provide two data which gives additional information (Arwin, 2001).

Basically, different sensing principles can be used in ellipsometry based biosensor systems. The simplest one is the based on affinity mechanism. In this case, a sensing layer, mostly antigen, aptamer or single stranded DNA, is formed on a substrate via chemical or physical modification methods. The changes in the Ψ and Δ depending on the interaction with target molecules are then monitored. Another possibility is to use a thin polymer layer. This princibles is based on the swelling or shrinking of the polymer layer and thereby to changes in the film optical properties and thickness. In porous materials, pore filling by adsorption on the inner walls of pores or capillary condensation are also useful sensing mechanisms (Arwin, 2001).

Beyond the conventional applications of ellipsometry, recently, total internal reflection ellipsometry (TIRE) is used for monitoring the ultrathin films in aqueous environments which is essential for biosensor and other *in situ* applications. A known technique, Surface Plasmon Resonance (SPR) is an evanescent wave technique which consists of a coupler to interact evanescent wave with surface-dielectric interface (Sutherland and Dahne, 1987). The

detection system of a SPR sensor essentially consists of a monochromatic and p-polarized (i.e. electrical vector of light is parallel with the plane of incidence) light source, a glass prism (used as coupler), a thin metal film in contact with the base of the prism (plasmon source) and a photodetector. In order to couple an evanescent wave, a total internal reflection mechanism is used. A useful and widely used coupler configuration is Kretschmann configuration (Kretchmann and Raether, 1968). Obliquely incident light on the base of the prism exhibits total internal reflection for angles larger than the critical angle. This causes an evanescent field to extend from the prism into the metal film (Figure 6.). Intensity of this evanescent field logarithmically decays from the coupler surface into the next media. Generally, effective intensity of evanescent waves in Kretschmann configuration is maintained up to half of the wavelength of incident light (i.e. 250 nm for 500 nm – green - incident light).

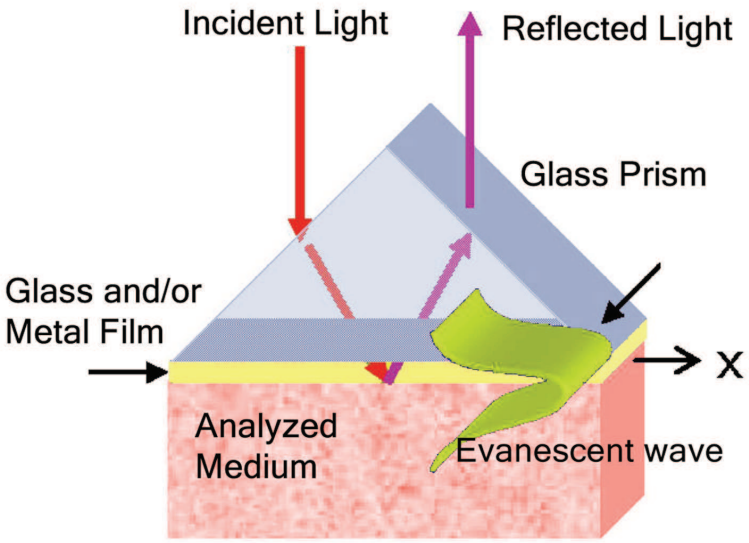


Fig. 6. The Principles of SPREE.

In conventional SPR systems, this evanescent field can couple to an electromagnetic surface wave, a surface plasmon at the metal/liquid interface. Coupling is achieved at a specific angle of incidence, or specific wavelength. In particular, reflected light intensity goes through a minimum at resonance angle for angle modulation. It should be noted that evanescent field is used for various applications such as intensity enhancement by nanoparticles. Plasmon resonance is highly sensitive to change in refractive index, or dielectric constant of the analyzed medium adjacent to the metal surface. Any change in the local refractive index and therefore the permittivity (ε) either by way of bulk index change or, as for instance in the case of biosensor, by the binding of an analyte to the surface plasmon polaritions active interface thus changes the SPR excitation conditions. If the ellipsometric parameters are measured with attenuated total reflection coupling of surface plasmon waves, this technique called as surface plasmon resonance ellipsometry (or surface plasmon resonance enhanced ellipsometry, SPREE) (Arwin, 2004). SPREE shows several

similarities to SPR techniques. A major and advantageous difference is that in SPR only the intensity information for reflection of p-polarized light is measured. However, in ellipsometry, properties of both p-polarized and s-polarized light are measured. The polarization state change at the probed interface (analyzed medium) is primarily due to the reflectance associated with total internal reflection (TIR) at a dielectric interface with composition change at interface. Particularly for biosensing, the binding of analytes to the surface cause thickness changes (t) and changes in complex refractive index (N=n-iκ) which are likely be determined by Δ and ψ parameters measured by ellipsometry (Venketosubbaro, 2006). Ellipsometry is more complex technique than SPR but has some advantages over SPR techniques. The s-polarization provides a reference for the overall intensity transmittance and with Δ parameters, phase information is also utilized, in addition to amplitude (intensity) information.

Another exciting application of evanescent waves with ellipsometry is Localized Surface Plasmon Resonance (LSPR) enhanced ellipsometry (Caglayan, 2009). In the first group of plasmonic ellipsometry sensors, the system based on propagating surface plasmons in thin metallic layers, so called Surface Plasmon Polaritions (SPPs). The second group utilizes metal nanostructures. Similarly to flat metal films, metal nanoparticles exhibit charge density oscillations giving rise to very intense and confined electromagnetic fields so called LSPRs. In this method, TIRE measurements are likely enhanced by immobilizing metal nanoparticles on sensor surface within useful depth of evanescent field. However, the basis of SPR-TIRE and LSPR-TIRE are generally confused with total internal reflection ellipsometry (TIRE). The TIRE, in principle, is based on spectroscopic (or more primitively single wavelength) ellipsometry performed under condition of total internal reflection. It should be noted that, in TIRE method which is proposed by Poksinski, there is no ultrathin metal film coated below the coupler, the latter is needed for SPR conditions (Poksinski and Arwin, 2006). Thus, for TIRE measurements there is no need a plasmon coupling at the coupler-analyzed medium interface. TIRE configuration is similar to Kretschmann configuration and utilizes TIR. This configuration is suitable for monitoring and analysis of thin semitransparent films, even they are in aqueous media, which is common for biosensor applications.

3. Conclusion

Ellipsometry techniques have several unique advantages for biosensor applications not only it does not require labeling of molecules as do fluorescence measurements, but also it can provide high precision of the measurement, very high thickness sensitivity, fast measurement, wide application area, real-time observations, feedback control of processing and no contact with the investigated materials etc. Beyond the current applications of ellipsometry in immunoassays and DNA sequencing, we believe that if multiplexing reading, in-field using, affordable price and scale up protocols could be solved for ellipsometric detections, these systems would be useful for next generation sensor systems. Moreover, integrated ellipsometry techniques, such as optical fibers, AFM and waveguide systems, will be appeared the future researching priorities. The integration with MEMS (or NENS) system to enable the multiplexing and miniaturizing will be another trend for ellipsometry based biosensors. Multifunctional biosensor which not only sense refractive index variation or phase shift but also other critical parameters, such as molecule structure and orientation change, will also attracting more and more interests.

4. Acknowledgment

We would like to gratefully acknowledge the Gazi University for the financial support (Project No. 05/2010-83 and 05/2010-17).

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New Perspectives in Biosensors Technology and Applications

Edited by Prof. Pier Andrea Serra

ISBN 978-953-307-448-1
Hard cover, 448 pages
Publisher InTech
Published online 24, June, 2011
Published in print edition June, 2011

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How to reference

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