Chapter 4

# Review on the Design Art of Biosensors

Shengbo Sang, Wendong Zhang and Yuan Zhao

Additional information is available at the end of the chapter

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## 1. Introduction

"Biological microelectromechanical systems" (BioMEMS) is a special class of Microelectromechanical systems (MEMS) where biological matter is manipulated for analyses and measures of its activity, characterisations under any class of scientific study. The BioMEMS-based devices are an attractive area of development based on microtechnology. The techonolgy has more exciting developments in the application of MEMS technology in recent decades. For scientific analysis and measurement, various novel sensor and detection platforms in the BioMEMS and microfluidic fields are required and have been reported, in addition to basic components, such as microchannels, micropumps, microvalves, micromixers and microreactors for flow management at microscopic volumes [1]. Any of the most important applications based on BioMEMS are: biomedical and biological analysis and measurements, micro total analysis systems (μTAS) and lab-on-a-chip systems [2-5], which will give new applications in biomedicine and biology, especially the ability to perform point-of-care measurements. The advantages of such systems are that they can deliver and process the biological or biomedical samples in microvolumes for testing and analysis in an integrated way therefore dramatically reducing the requirement to the manipulation steps and the samples, and improving data quality and quantitative capabilities. The BioMEMS technology also helps to reduce overall cost and time for the measurement. At the same time it improves the sensitivity and specificity of the analysis.

To the BioMEMS technology and application, biosensors play a critical role in the process of information gathering with the technologically advanced development of our civilization, demand for information. With new applications in the areas – genetics, diagnostics, drug discovery, environment and industrial monitoring, quality control as well as security and threat evaluation [6], the need for high throughput label-free multiplexed sensors for biological sensing has increased in the last decade.

A biosensor is a device for the detection of an analyte that combines a biological component with a physicochemical detector component [7]. In general, one concept of biological sensors encompasses two main features in addition to the associated signal processors used for the display of the results in a user-friendly way: the sensitive biological element which is a chemically receptive or selective layer, and the transducer or the detector element (it can work in a physico-chemical way, optical, piezoelectric, electro-chemical, etc.) that transforms the signal induced by the interaction between the analytes and the biological element into another more easily measured and quantified signal, Figure 1. The chemical layer provides specific binding sites for the target analyte of interest, such as molecules, proteins and cells. To most biological and chemical sensors, sensitivity has been increased tremendously in recent years, but it still has some deficiency and needs more improvement. The selectivity of the receptive layer can be designed employing principles of molecular and biomolecular recognition; for example antigen-antibody binding (i.e. any chemicals, bacteria, viruses, or pollen binding to a specific protein)[8]. Other surface functionalizations such as self-assembled monolayer [9, 10] and polymer coatings are also employed. The selectivity is then achieved by a specific chemical reaction on the functionalized sensor's surface. However, absolute selectivity remains a major challenge. In fact, most sensing technologies are faced with the issue of non-specific interactions which can complicate the sensor response, produce false positives, and affect the reproducibility and the suitability of the sensor system for a particular application. Therefore, the chemical layer must be designed to maximize the sensor's sensitivity to the specific response.

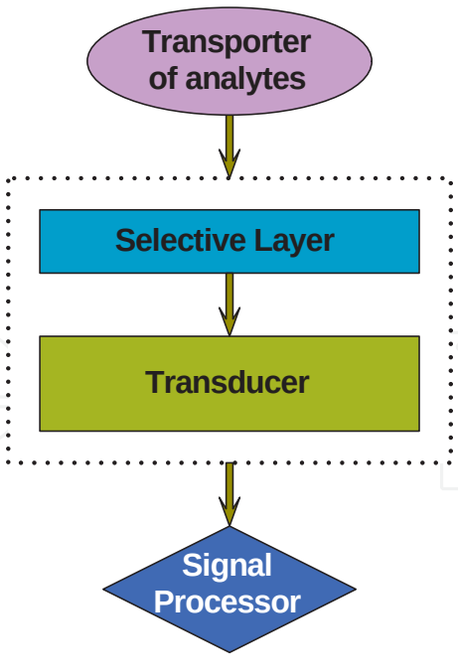


**Figure 1.** Generalized schematic representation of a biological sensor [11]

Once the analyte is recognized by the chemical layer, the transducer converts the chemical stimulus into a measurable output signal, as shown in Figure 1. Both the chemical layer and

the physical transducer impose limitations on the performance of a certain class of sensors. Nevertheless it is often the physical transducer which determines the limits of detection attainable. The search for new transduction principles is therefore constantly stimulated [8]. In fact, each step depicted in Figure 1 has an influence on the sensor's performance. From the mechanism that drives the analyte to the sensor (e.g. microfluidic, activated diffusion, etc.), to the instrument reading the output signal of the transducer; all stages are the subject of extensive research efforts.

Besides, microfluildic technology is a frequent technology which is used in biosensors. Microfluidic devices or components have emerged in the beginning of the 1980s and were quickly used in the development of inkjet print heads, Deoxyribonucleic Acid (DNA) chips, lab-on-a-chip technology and micro-thermal devices. Microfluidics can precisely control and manipulate fluids and analytes that are geometrically constrained to a small size, typically sub-millimeter, and scale. One of the most attractive applications of microfluidics has been in biomedical and life science diagnostics [12]. μTAS applications are attractive because of the potential of such systems to allow faster analysis of biological material. Further they can reduce the requirement to the amount of reagent and the number of processing steps. In addition, miniaturization of such systems can result in higher repeatability and precision of analysis, lower power consumption, and the potential to create portable diagnostic tools for on-site analysis. These advantages result not only in time and cost savings for diagnostic tests, but can also be life saving in time-critical environments such as critical medical diagnostics or biowarfare pathogen detection.

## 2. Design art of biosensors

The design art of biosensors can be broadly classified into label-free and label based on the detection technology. It can also be further classified as shown in Figure 2. Label-based techniques rely on the specific properties of labels like fluorescence, chemiluminescence etc. for detecting a particular target. However, the process of labeling and purification processes is associated with sample losses, which is critical when sample quantity is limited. Labeling processes can also have a detrimental effect on the functionality and stability of molecules like proteins. Mass spectrometry, surface plasmon resonance (SPR) and other optical method are label-free techniques, which can conquer these disadvantages. The following subsections are the detailed discussion to the respective advantages and disadvantages of every design art.

### 2.1. Label-free biosensor

#### *2.1.1. Surface Plasmon resonance (SPR) biosensors*

Surface Plasmon Resonance (SPR): SPR-based biosensors measure the refractive index near a sensor surface through an optical method for getting some information. When a light beam impinges onto a metal film at a specific (resonance) angle, the surface plasmons can be res-

onated with the light. As a result, it can induce the absorption of light. For the widely used Kretschmann configuration, a beam is focused onto the metal film. There is a range of incident angles provided focused light and the reflected beam will have the same range of the angles while the projection of the beam forms a band. A dark line will appear in the reflected band if the SPR occurs within the spread angles. An intensity profile of this band can be monitored and plotted against the range of angles as shown in Figure 3.

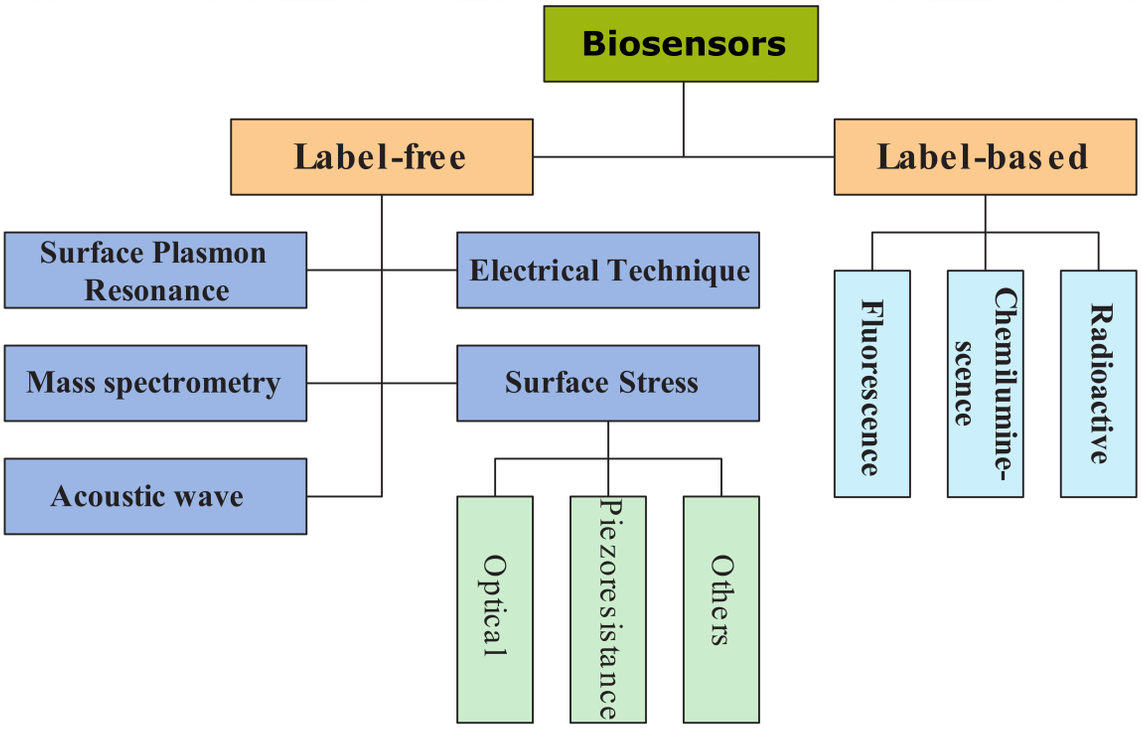


**Figure 2.** Classification of the design art of biosensors based on the detection method [11]

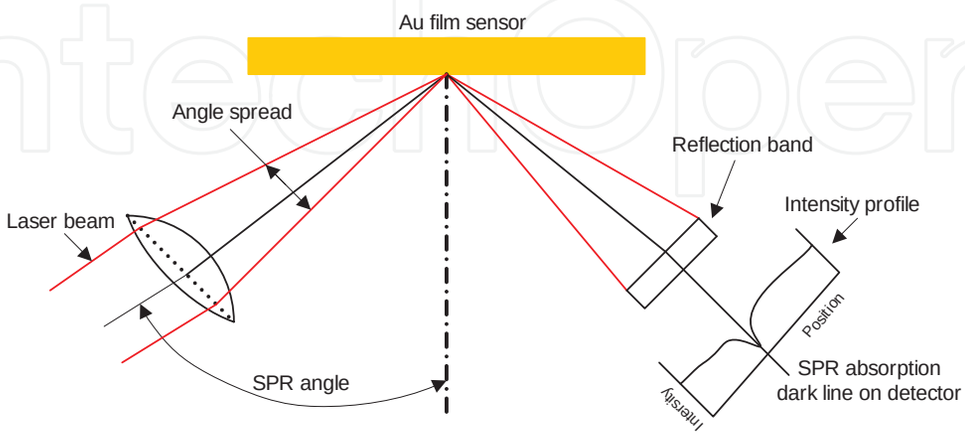


**Figure 3.** Principle of SPR detection in the mode of measuring the SPR angular shift[11]

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Many researchers have worked on developing SPR biosensors for studying various kinds of biological reactions, and many reports have been published. The first application of SPR in biosensor was demonstrated in 1982 and the first commercial SPR sensor was introduced in 1990's [13, 14]. Biosensing Instrument Incorporated uses a different approach to detect the SPR angle change, for example, using the position-sensitive detector. Only the position shift of the dip is measured, so it offers a highly sensitive detection scheme to measure extremely small angle changes of the SPR. In the range of the SPR angle spread, the system delivers exceptionally high angular resolution in its measurement [15]. Like Figure 4 illustrates, SPR is observed as a sharp shadow in the reflected light from the surface at an angle that is dependent on the mass of material at the surface [15]. When biomolecules bind to the surface and change the mass of the surface layer, the SPR angle will shift (from I to II in the lower left-hand diagram). This change in resonant angle can be monitored non-invasively in real time as a plot of resonance signal (proportional to mass change) versus time [15].

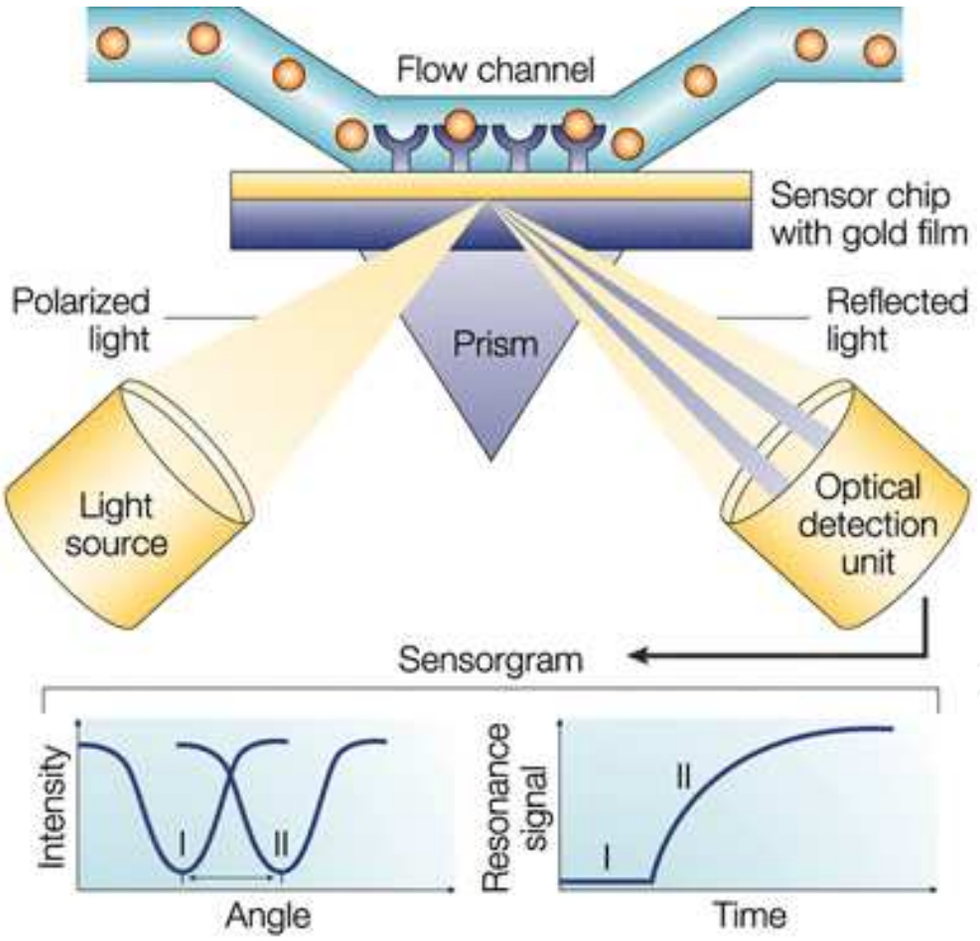


**Figure 4.** Schematic of SPR sensing method [15]

Earlier works were focusing mainly on antigen-antibody interactions [16], the streptatividin-biotin reaction [17], and some Immunoglobulin G (IgG) examinations, especially to test new algorithms in biospecific molecular interaction analysis, to characterize newly developed

SPR set-ups [18]. Current researches include far more advanced ways to improve the sensitivity of biosensor through functionalization layer [19] and silver mirror reaction which the biosensor based on Ag/Au film could make the resonant wavelength move to longer wavelength following with the sensitivity enhancement of the SPR biosensor [20]. One of the new areas is the examination of protein–protein or protein–DNA interactions [21], even detecting conformational changes in an immobilized protein [22]. A domain within the tumor suppressor protein adenomatous polyposis coli(APC) has been examined regarding its biochemical properties [23], as well as the binding kinetics of human glycoprotein with monoclonal antibodies [24]. Work has been done on the activator target in the Ribonucleic Acid(RNA) polymerase II holoenzyme [25].

SPR biosensor technology exhibits various advantageous features. Versatility generic SPR sensor platforms can be tailored for detection of any analyte, providing a biomolecular recognition element; Analyte does not have to exhibit any special properties such as fluorescence or characteristic absorption and scattering bands because label-free binding between the biomolecular recognition element and analyte can be observed directly without the use of radioactive or fluorescent labels; The speed of analysis binding event can be observed in real-time providing potentially rapid response and flexibility SPR sensors can perform continuous monitoring as well as one time analyses [26].

However, SPR biosensors exhibit two inherent limitations: On the one hand, specificity of detection specificity is solely based on the ability of biomolecular recognition elements to recognize and capture analyte. Biomolecular recognition elements may exhibit crosssensitivity to structurally similar but nontarget molecules. If the nontarget molecules are present in a sample in a high concentration, sensor response due to the nontarget analyte molecules may conceal specific response produced by low levels of target analyte. On the other hand, sensitivity to interfering effects similar to other affinity biosensors relying on measurement of refractive index changes, SPR biosensor measurements can be compromised by interfering effects which produce refractive index variations. These include nonspecific interaction between the sensor surface and sample (adsorption of nontarget molecules by the sensor surface), and background refractive index variations (due to sample temperature and composition fluctuations) [26].

*2.1.2. Mass spectrometry biosensors*

Mass spectrometry (MS) is an analytical technique that can be used to measure the mass-to-charge ratio of charged particles, the mass of particles, the elemental composition of a sample and the chemical structures of molecules by ionizing chemical compounds.

In order to measure the characteristics of individual molecules, a mass spectrometer converts molecules to ions so that they can be moved about and manipulated by external electric and magnetic fields. Mass spectrometers are generally composed of three fundamental parts, namely the ionization source (a small sample is ionized, usually to cations by loss of an electron), the mass analyzer (the ions are sorted and separated according to their mass and charge) and the detector (that registers the number of ions at each m/z value) [27].A typical procedure usually contains five steps: A sample is loaded onto the MS instrument and

undergoes vaporization. The components of the sample are ionized by one of a variety of methods (e.g., by impacting them with an electron beam), which results in the formation of charged particles (ions).The ions are separated according to their mass-to-charge ratio in an analyzer by electromagnetic fields. The ions are detected, usually by a quantitative method. Finally, the ionsignal is processed into mass spectra.

The mass spectrometry can be applied to identify and, increasingly, to precisely quantify thousands of proteins from complex samples, which is believed to have a broad impact on biology and medicine [28]. But, the size of these equipments is large in general, which makes them unfeasible for field applications which require portable devices, especially for biosensors.

*2.1.3. Acoustic wave biosensors*

The detection mechanism of acoustic wave sensors is an acoustic (mechanical) wave. The velocity and/or amplitude of the acoustic wave can be affected by the changes of the characteristics of the propagation path when the acoustic wave propagates through or on the surface of the material [39]. Based on the sensor, the changes of velocity can be monitored by measuring the frequency or phase characteristics, and then it can be analyze based on the corresponding physical quantity being measured [29]. Acoustic wave based biosensors offer a promising technology platform for the development of label-free, sensitive and cost-effective detection of biomolecules in real time.

Emerging applications for acoustic wave devices as sensors include as torque and tire pressure sensors [30~33], gas sensors [34~37], biosensors for medical application [38~41], and industrial and commercial applications such as: vapor, humidity, temperature, and mass sensors [42~44]. Additional capabilities of acoustic wave sensors include remote operation and passive interrogation [44].

Surface acoustic wave sensors, as a class of MEMS, are widely used recently. The sensor can transform an input electrical signal into a mechanical wave which can be easily influenced by physical phenomena. Then, the changed mechanical wave is transduced back into an electrical signal. The presence of the desired phenomenon can be detected through the difference between the input and output electrical signal (amplitude, phase, frequency, or time delay). The basic surface acoustic wave device consists of a piezoelectric substrate, an input interdigitated transducer (IDT) on one side of the surface of the substrate, and a second output interdigitated transducer on the other side of the substrate.

As shown in Figure 5, Self-assembled monolayer (SAW) devices have the interdigitated transducers (IDTs) excitation electrodes fabricated on the one side of the piezoelectric film. As a result, the SAW devices have the acoustic waves propagating along the surface of the piezoelectric substrate. The SAW device could be resonator or delay line depending of the design of the IDTs. For SAW resonators the IDTs are fabricated in a central position and reflectors are added on both sides of the input and output IDTs to trap the acoustic energy within a cavity. The surface between the IDTs is coated with antibodies sensitive to the analyte to be detected. The analyte molecules binding to the immobilized antibodies on the sen-

sor surface influence the velocity of the SAW and hence the output signal generated by the driving electronics.

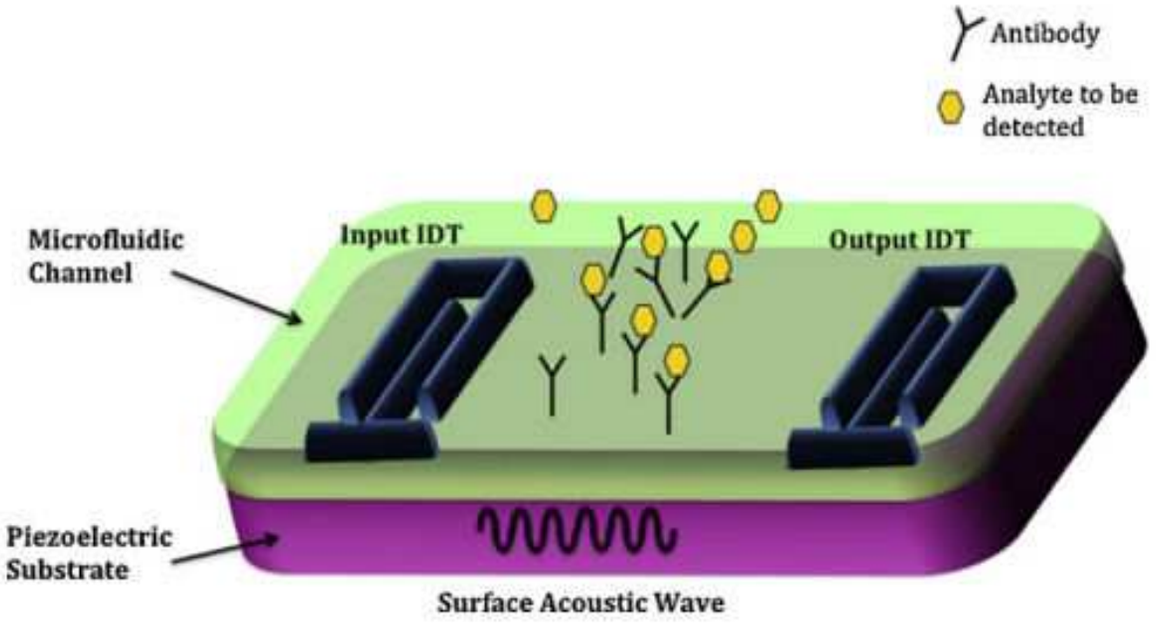


**Figure 5.** SAW delay line biosensor integrated in a microfluidic channel[45]

Virtually all acoustic wave devices and sensors use a piezoelectric material to generate the acoustic wave. The technology has been utilized in the commercial range for more than 60 years. And the high mass sensitivities of acoustic wave devices make them an attractive platform for monitoring immunochemical and other biomolecular recognition events. However, not all acoustic wave devices are suitable for liquid operation. If the sensor has surface normal deformations and the velocity of acoustic wave is greater than the compressional wave velocity of sound in liquid, then they can couple to compressive waves in the liquid and cause severe attenuation of the sensor signal. In contrast, devices in which surface particle motion is parallel to the sensor surface dissipate energy into the liquid primarily by viscous coupling, which does not produce severe losses and therefore are suited for liquid phase sensing.

*2.1.4. Electrochemical biosensors*

Biosensors based on electrochemistry provide an attractive means to analyze the content of a biological sample due to the direct conversion of a biological event to an electronic signal. Electrochemical biosensors build a bridge between the powerful analytical methods and the recognition process of the biological specificity.

Electrochemical biosensors are normally based on enzymatic catalysis of a reaction that produces or consumes electrons (such enzymes are rightly called redox enzymes).Electrochemical sensor consists of biological materials as sensitive components, electrode (solid

electrodes, ion selective electrode, gas sensor electrode etc) as a conversion components, electric potential or current as the detection signal. The sensor substrate usually contains three electrodes: a reference electrode, a working electrode and a counter electrode. The target analyte is involved in the reaction that takes place on the active electrode surface, and the reaction may cause either electron transfer across the double layer (producing a current) or can contribute to the double layer potential (producing a voltage). We can either measure the current (rate of flow of electrons is now proportional to the analyte concentration) at a fixed potential or the potential can be measured at zero current (this gives a logarithmic response). Note that potential of the working or active electrode is space charge sensitive and this is often used. Further, the label-free and direct electrical detection of small peptides and proteins is possible by their intrinsic charges using biofunctionalized ion-sensitive field-effect transistors [46].

Electrochemical sensors can be classified into amperometric, potentiometric or conductometric sensors based on whether current, potential or resistance is being measured during an electrochemical reaction (oxidation or reduction) between the analyte of interest and the electrode surface.

Macro scale electrochemical sensors have been used in chemical and biological sensing for a very long time. Recently, due to many electrochemical sensor researches, sensors utilizing nanoscience and nanomaterials exploit unique properties (i.e. nanoporous electrodes, nanoparticles, nanotubes, etc.). A microbiochip that is based on an electrical detection system has been used for the detection of alpha-fetopro-tei (AFP) antigen [47].

The inherent advantages of electrochemical biosensors are their robustness, easy miniaturization, excellent detection limits, also with small analyte volumes, and ability to be used in turbid biofluids with optically absorbing and fluorescing compounds [48, 49]. The main adverse problems is long term stability and reliability associated with incorporation of liquid electrolytes, life time and cycle time issues due to small amount of reactants (consumable electrodes like Ag/AgCl). Secondly, non specific reactions taking place between the electro active impurities on the surface and the sample also limit the sensitivity of these sensors [50].

### *2.1.5. Surface stress sensors*

Surface stress [51, 52] is a macroscopic quantity that is governed by microscopic processes. Although being a macroscopic quantity, the measurement of the surface stress involved in a system can lead to insight into the microscopic mechanisms basic for the generation of surface stress without detailed knowledge of the atomistic processes involved. Recent investigations of surface reconstruction, interfacial mixing, and self-organization at solid surfaces have renewed interest in the study of surface stress [53-57]. Now the surface stress existing between biological molecules, cells and some special functional materials has been used in the biosensors for biological and medical research based on the surface stress analysis.

Biological sensing of numerous analytes based on surface stress can be achieved using cantilever or membrane as the sensitive element of sensors. It is possible to sensitize one surface

of the sensititive element differently than the opposing surface. When the analytes of interest interact with the sensitized surface, a surface stress is induced, and the cantilever or membrane bends due to the different surface stresses acting on both sides of the cantilever or membrane. The sensor's specificity, i.e. the sensitivity of the sensor to a specific analyte, is determined by the chemical functionalization of the sensitized surface of biosensors. Very specific surface functionalizations can be achieved using molecular self-assembled monolayers (SAMs) as sensing layers assembled on the surface of biosensor sensitive element. Thiol-chemistry has been favored as a versatile method of sensitizing a surface.

Microcantilever and micromembrane surface stress sensors have lots of applications in numerous flieds. Integrated microfluidics enabled individual cantilever/membrane addressing in the array for selective functionalization. Hansen *et al.* were able to show that microcantilevers are sensitive enough to detect single base-pair mismatch in DNA hybridization [58]. Wu *et al.* developed a Prostate-specific antigen (PSA) detection assay using a single microcantilever at clinically relevant levels in a large background concentration of human serum proteins – albumin and plasminogen [59]. Yue*et al.* [60] showed that passivation of the backside with inert coatings like polyethylene glycol (PEG) is absolutely necessary in order to make reliable protein interaction measurement in cantilever detection system. Si-Hyung "Shawn" Lim developed a 2-D multiplexed cantilever sensor array plat-form for high-throughput target specific coating material search, which performed chemical sensing experiments using toluene and water vapour [61]. In addition, misun Cha demonstrated the capability of the thin membrane transducer (TMT) for detecting biomolecular reactions such as hybridization, single nucleotide polymorphism (SNP), and aptamer–protein binding [62]. Vasiliki Tsouti reveals the structure characteristics that should be considered in the design of the biosensors in the case of flat Si membranes based on the simulation results [63]. Srinath Satyanarayana presents the design and fabrication of a novel parylene micro membrane surface stress sensor that exploits the low mechanical stiffness of polymers, measuring the sensor response to organic vapors like isopropyl alcohol and toluene [64]. But all these kinds of biosensor must associate with some detection methods to measure and analyse the usable information gotten from the analyte, for example, optical method, piezoresistance, and capacitive method.

*2.1.5.1. Optical detection methods*

The invention of the atomic force microscope (AFM) in 1986 [65] and its impact on the fields of biotechnology and nanotechnology has created a new modality of sensing: the cantilever. The most simple way of measuring cantilever deflection resulting from surface stress between the analytes with cantilever to get some measured information is by optical beam deflection as in most AFM instruments [66].

In the optical beam deflection technique, a laser diode is focused on the end of the free cantilever and the reflected laser beam is monitored using a position sensitive photodetector, as shown in Figure 6 [9]. The typical displacement sensitivity achieved using this technique is on the order of $10^{-9}$ m [8]. Fritz *et al.* demonstrated DNA immobilization and hybridization using microcantilever measured by optical deflection detection [67]. And Yue *et al.* demon-

strated a 2D cantilever array with integrated microfluidics using a single laser source and a Charge-coupled Device (CCD) camera for simultaneous interrogation of several hundred cantilevers for DNA and protein sensing [68]. The advantages are its simplicity, linear response, and lack of electrical connections. However it suffers some limitations. A calibration is needed in order to obtain the recorded signal in terms of the actual cantilever deflection. Index of refraction changes of the surrounding medium of the cantilever can produce artificial deflection and the technique cannot be used in opaque media such as blood.

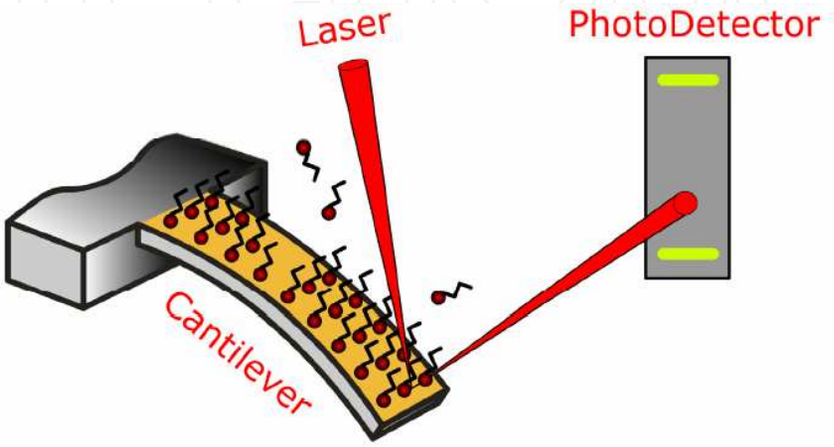


**Figure 6.** The optical beam deflection technique is used to monitor the deflection of the cantilever [9]

Another optical method which can attain better performance is interferometry [69]. When using a fiber optic interferometer [69, 70], the interference signal from the reflected light of the cleaved end of the fiber optic and of the cantilever surface is a direct measure of the average cantilever displacement in the field of view. Deflection in the range of $10^{-11}$ m to $10^{-13}$ m can be measured [71]. Fiber optic interferometer is a mature technology and has many advantages, good performance, low loss, high bandwidth, safety and relatively low cost, for example, which is suitable for biosensors. The principle is as schematically shown in diagram Figure 7, the interference is formed inside an optical fiber. When the laser diode light passes at the fiber end-face, a portion is reflected off at the fiber/air interface ($R_1$) and the remaining light still passed through the air gap (L) with a second reflection occurring at the air/membrane interface ($R_2$). $R_1$ is the reference reflection named the reference signal ($I_1$) and the sensing reflection is $R_2$ called sensing signal ($I_2$). These reflective signals interfere constructively or destructively in the fiber due to the difference of the optical path length between the reference and sensing signals, which is called the interference signal [70]. Therefore, small deflection of the membrane causes a change in the air gap (L), which changes the phase difference between the sensing and reference signals producing fringes.

However, optical detection systems for cantilever arrays are still typically large and are more suited for bench-top applications than for portable handheld use. Nonspecific adsorption on the back side (non functionalized side) of the cantilever because of sensor immersion in liquid sample during measurement is a significant source of noise in these sensors.

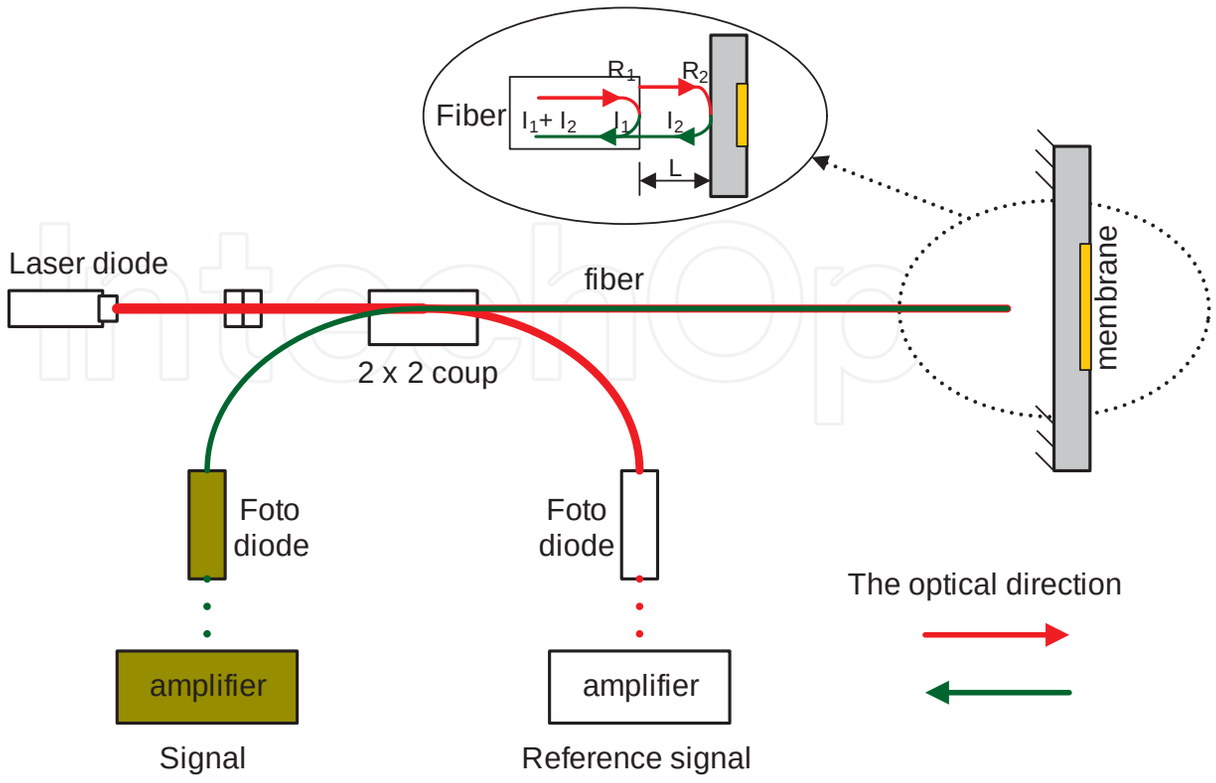


**Figure 7.** Schematic diagram of a fiber interferometer [72]

*2.1.5.2. Piezoresistance detection methods*

Piezoresistivity is the variation of the bulk resistivity under applied stress. When a silicon cantilever is stressed because of its bending caused by surface stress, a highly doped region will change resistance in a sensitive way. The variation of cantilever resistance is typically measured using a Direct Current (DC)-biased Wheatstone bridge. The advantage of piezoresistivity technique is that the sensor and the detection scheme can be easily integrated into lab-on-a-chip type devices. In addition it is more compatible with large array formats. Marie at al developed a cantilever system using piezoresistive detection instead of optical deflection method for sensing DNA hybridization [73].

Nevertheless, this method possesses electrical connections which need to be protected for experiments performed in liquids and requires current to flow through the cantilever. This results in heat dissipation and thermal drifts which causes parasitic cantilever deflections.

*2.1.5.3. Others*

There are some less widely used and readout schemes existed methods, such as the capacitive method, piezoelectric method and electron tunneling. More recently, displacement detection methods for nanoscale cantilevers were implemented Cleland et al. developed a scheme based on capacitively coupling a nanobeam to a single electron transistor achieving sensitivity down to $10^{-14}$ m[73].

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## 2.2. Label based techniques

Label based techniques use 'tags' or labels to detect a particular analyte in a background of other materials. Fluorescence, chemiluminescence and radioactive are three popular label based techniques in biosensors.

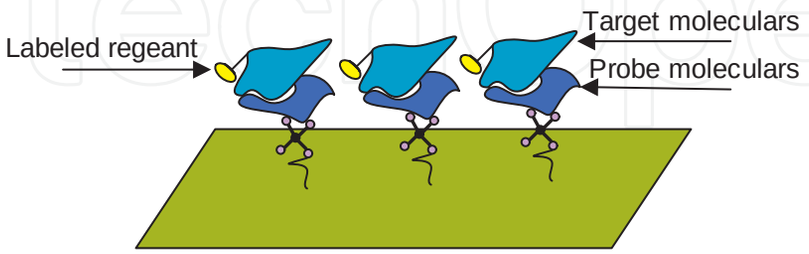


**Figure 8.** Schematic diagram of fluorescence: Surface with different probe molecules is exposed to solution with pre-labeled target molecules, presence of fluorophores on the surface indicates a specific binding reaction and the presence of a target molecule [11]

### *2.2.1. Fluorescence*

Fluorescence is the short-time (< 1 μs) category of luminescence, which is mostly exploited as an optical phenomenon in cold bodies. For doing this, the molecular component used absorbs a photon and can conesecutively emit a photon with a longer (less energetic) wavelength. Molecular rotations, vibrations or heat can be produced because the absorbed photons have different energy; with the emitted photons, for example, the emitted light can be in the visible range even if the absorbed photon is in the ultraviolet range. The phenomenon depends on the absorbance and Stokes shift of the particular fluorophore.The fluorescence sensor's principle can be shown via an example in Figure.8: the probe molecules, such as antibodies, are immobilized onto the surface of probing microchamber using cross-linkers or covalent methods; the target moleculars with labelled reagent, such as antigens with fluorophore are loaded on the microchamber and they will bind to probes; and then the bound targets can be detected. Fluorescence is generally preferred and the most widely used detection method for reasons of sensitivity, stability, and availability of fluorescent scanners tailored for microarray use [74].

The disadvantage of this technique is that most fluorophores are bleached quickly upon exposure to light and are very sensitive to environment conditions such as solution's hydrogen ion concentration(pH) value. And both direct and indirect labeling methods have also their disadvantages. Indirect labeling is more complicated and time consuming, while fluorescence tags in direct labeling may be less stable and more disruptive to the labelled proteins as compared to small molecule tags in indirect labeling.

### *2.2.2. Chemiluminescence*

Chemiluminescence or chemoluminescence is the phenomenon of light emission as the result of a chemical reaction, which leads to limited emission of heat (luminescence).

Chemiluminescence (CL) analysis promises high sensitivity with simple instruments and without any light source, so chemiluminescence has become an attractive detection method in μTAS in recent years [75-78]. On the other hand, many flow sensors based on CL reaction and molecular recognition using enzymes, which have great sensitivity in environmental, biomedical and chemical analysis, have been developed [79-84]. However, limited feature resolution because of signal bleeding and limited dynamic range is the reported drawbacks of this method [85]. Furthermore, sensing with chemiluminescence can be performed only once, unlike fluorescence-based methods which can be archived for future imaging.

### *2.2.3. Radioactivity*

Radioactivity-based detection method is comparable to fluorescence method except that the labels are radioisotopes instead of fluorophores. Techniques using radioactive labels offer robust and reproducible protocols in applications that require ultimate sensitivity and/or resolution. Quantification of results is possible by the following exposure of signal to autoradiography film or reusable storage phosphor screens in automated imaging systems. However, automated liquid handling of radioactivity is difficult due to the need for safe handling and disposal of radioactivity, and is generally limited to manual, low-throughput applications.

## 3. A novel surface stress-based biosensor

Surface stress-based membrane biosensors, whose bottom surface can be sealed by the biological solutions, are ideal for the development of novel surface stress based biosensors with capacitive readout. Capacitive readout has the advantage of easy and accurate detection as well as it is suitable for device miniaturization. However, it is not applicable in the cantilever biosensors due to faradaic currents in the electrolytic solutions.

Due to the advantage of membrane biosensors, a new biosensor is proposed by Micro Nano System Research Center (MNSRC), which can be used to detect cells. It consists of micro-fluidics, sensitive membrane and capacitive readout unit. The sensitive element of this biosensor—membrane (Figure 9) is formed by the Polydimethyl siloxane (PDMS) thin film with part gold coated on its surface and the trapezoidal structure in the silicon substrate. The surface stress changes when analyte species adhere to the probes which are immobilized on the membrane surface. The change of surface stress causes an out of plane deflection that alters capacitance.

The parameters of membrane (Table 1) have been decided based on the FE(finite element) analysis software ANSYS just like reference [86] and the fabrication techniques. The dimension of one whole bio-sensor chip is 13×7mm$^2$(length×width).

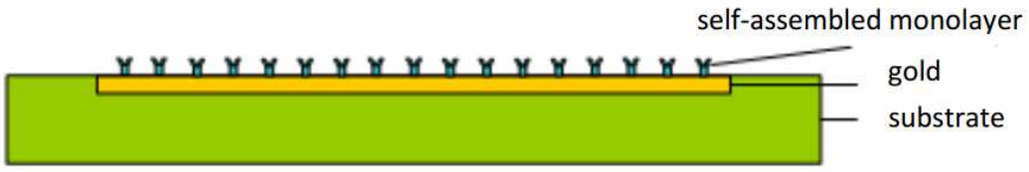


**Figure 9.** Schematic diagram of membrane[86].

| Material | Length(μm) | Width(μm) | Thickness(μm) |
|---|---|---|---|
| **PDMS** | 400 | 400 | 1 |
| **Gold** | 390 | 390 | 0.02 |

**Table 1.** The membrane parameter [87]

Based on the parameters, the biosensors were fabricated [87].One biosensorchip contains two micro-membranes, one acting as the active membrane and the other as reference, as shown in Figure 10. The active membrane is sensitized to react with specific analytes. The selective biochemical reactions between the analytes and the membrane will induce a surface stress change that causes the membrane to deflect. The analytes will not present on the reference membrane, it is only used to remain sensitive to other environmental factors that can also result in a deflection of the active membrane, such as temperature variations (bimetallic effect), laminar or turbulent flow around the membrane, vibrational (including acoustic) noise, non-specific binding (including the swell induced by solution) and so on. The differential signal is solely due to the interaction of the analytes with the membrane. This design has very sensitive surface stress measurements for analytes detection, which is one main novelty of the biosensor.

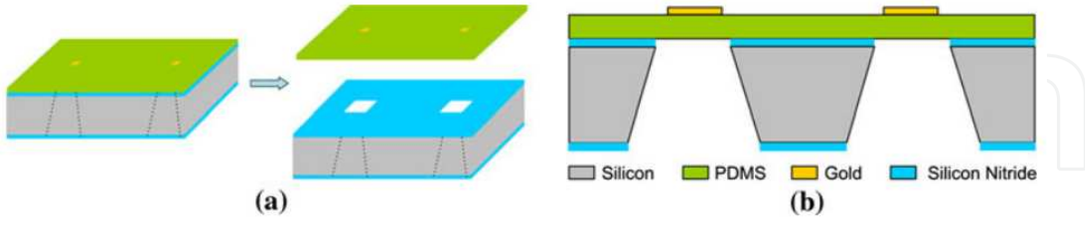


**Figure 10.** The schematic structure of biosensor(a: 3D schematic diagram of biosensor, b: Cross-section view of biosensor) [87]

One biosensor test systems was set up based on the fiber optic interferometer (FOI) debugged for the test [88]. Figure 11 shows the comparison of active signals and reference signals obtained from FOI-based biosensor test system. Lines 1 and 2represent the basic reference signals when 20μl pure medium (no E.coli) was loaded on the membranes. The voltage had a big change at the loading point because the membrane deflections were first mainly

caused by the medium weight. With the evaporation of medium, the influence factors of weight and reflex became smaller and the signals increased again. The active signals shown by lines 3 and 4 were achieved when20μl medium with living and with dead E. coli (1.7 × $10^3$cells/μl)was loaded into the reservoirs, respectively. The primary deflections also came from the weight and the reflex of E. coli medium, so at the loading point the active signals became smaller as well.

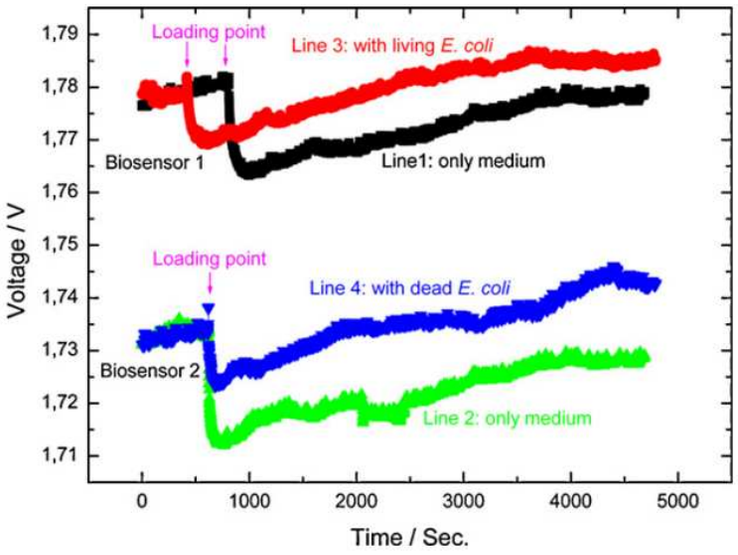


**Figure 11.** The comparative signals between only medium and the medium containing living or dead E. coli[88]

For E.coli detection, the experimental results show that the conditions of E. coli can be detected based on the deflection tests. For the biological and medical applications, other kinds of cells or molecules can also be detected based on the biosensor test systems. The analysis of some pathological changes of cells or molecules can be carried out based on the alteration of membrane deflection. Furthermore, the deflection signal can be translated into electric signal though adding an electrode on the substrate, which is still promoting for portable sensitive biosensor by us.

## 4. Conclusion

Labeling of biomolecules with fluorescent or other similar tags for detection can result in sample losses during the labeling and purification process and occasional loss of functionality, especially in proteins and other small molecules. Label-free detection technique can conquer the disadvantage, and the optical interference method is easy and simply to operate and high precision to detect the deflection of membrane for getting the information/properties of the biological analytes. Label-free detection measurements of biosensors are becoming a promising alternative approach to traditional label-based methods. Surface stress-based micro membrane biosensor is a relatively new class of sensor, this kind of biosensor

allows the detection in opaque media and the miniaturization of the sensor to incorporate it in portable devices for point-of-use sensing. The technique is advantageous since the process does not require any labeling of target molecules or the addition of redox probes.

PDMS attracts extensive attention worldwide as a membrane of sensors. One kind of PDMS micro membrane biosensors was successfully fabricated through conquering many challenges, e.g., integration of PDMS processing with conventional micro-fabrication processes and the fabrication of perfect PDMS thin film.

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## Author details

Shengbo Sang[1,2*], Wendong Zhang[1,2] and Yuan Zhao[1,2]

1 MicroNano System Research Center, Taiyuan University of Technology, Shanxi, China

2 Key Lab of Advanced Transducers and Intelligent Control System of the Ministry of Education,Taiyuan University of Technology, Shanxi, China

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