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Optical biosensors for probing at the cellular level: A review of recent progress and future prospects

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Abstract

New approaches are required to understand the complex processes taking place in the smallest unit of life. Recent years have seen an increasing activity in the use of optical devices and techniques for the investigation of the properties of single cells and also populations of cells including cell to cell communication. This article reviews relevant optical technologies to date as well as new advances in biosensor development, and goes on to explore reported applications in detection of biotargets and cellular signalling pathways inside individual living cells.

Keywords: Optical biosensor; Single cell; Review

List of abbreviations

ARROW: Anti-resonant reflecting optical waveguide
EDF: Epidermal growth factor
FRET: Fluorescence energy transfer
PEBBLE: Probes encapsulated by biologically localized embedding
SELEX: Systematic evolution of ligands by exponential enrichment
SPR: Surface plasmon resonance

1. Introduction

A biosensor is described as a compact analytical device, incorporating a biological or biomimetic sensing element, either closely connected to, or integrated within, a transducer system. The principle of detection is the specific binding of the analyte of interest to the complementary bio recognition element immobilised on a suitable support medium. The specific interaction results in a change in one or more physico-chemical properties which is detected and may be measured by the transducer. The usual aim is to produce an electronic signal which is proportional in magnitude or frequency to the concentration of a specific analyte or group of analytes, to which the biosensing element binds [1, 2]. Biosensors have been envisioned to play a significant analytical role in many sectors: biomedical, environmental and industrial monitoring, food safety and homeland security. Recent advances in nanosensor technology have begun to revolutionize other research fields like cellular biology, providing a more-reliable means of cellular and subcellular measurements.

Biosensors can be classified by their bio-recognition system or depending on the method of signal transduction. The main biological materials used in biosensor technology are the couples enzyme/substrate, antibody/antigen and nucleic acids/complementary sequences. In addition, microorganisms, animal and plant whole cells and tissue slices can also be incorporated in the biosensing system. Recent

advances in the molecular imprinting area offer an alternative approach involving the use of artificial biomimetic recognition systems. Optical transduction will be the focus of this article. In recent years, a key stimulus for the development of optical biosensors has been the availability of high-quality fibres and optoelectronic components at a reasonable cost. The advantages of optical biosensors are their speed, the immunity of the signal to electrical or magnetic interference and the potential for higher information content (spectrum of information available). Optical methods are readily multiplexed; samples can be interrogated with many wavelengths simultaneously without interfering with one another. The optical biosensor format may involve direct detection of the target of interest or indirect detection through optically labelled probes. The optical transducer may detect changes in the absorbance, fluorescence/phosphorescence, chemiluminiscence, reflectance, light scattering or refractive index. A large variety of optical methods have been used in biosensors however those devices based on fluorescence spectroscopy, surface plasmon resonance, interferometry and spectroscopy of guided modes in optical waveguide structures (grating coupler, resonant mirror) are the most common [3,4]. In practice, fiber optics can be coupled with all optical techniques, thus increasing their versatility.

2. Fluorescence-based detection

Fluorescence is a widely used optical method for biosensing due to its selectivity and sensitivity. A fluorescence-based device monitors the frequency change of electromagnetic radiation emission stimulated by previous absorption of radiation and subsequent generation of an excited state that only exits for a very short time. Single molecules could be repeatedly excited and detected to produce a bright signal easily measured even at single-cell level. There are three types of fluorescence biosensing. The first is direct sensing when a specific molecule is detected before and after a change or reaction takes place. The second form is indirect biosensing when a dye is added that will optically transduce the presence of a specific target molecule. The use of green fluorescent protein (GFP) is a powerful fluorescent tag that has enabled investigators to study the location, structure and dynamics of molecular events within living cells. However binding interactions between an activated signalling molecule and its target could be difficult to detect due to the difficulty of seeing this localized interaction over background fluorescence. A third type of fluorescence biosensing, called fluorescence energy transfer (FRET), can be used and it generates a unique fluorescence signal. In a typical fluorescence measurement, the fluorophore is excited by a specific wavelength of light and emits light at a different wavelength. However, when two fluorophores are paired in such a way that the emission wavelength of one overlaps with the excitation wavelength of the other, the excitation of one of them will stimulate fluorescence of the complementary pairing one (if they reside within about few Angstroms from each other). FRET has tremendous utility because the unique fluorescence signal generated under these circumstances can be used to visualize and quantify the position and concentration of interacting fluorophores. Two major strategies have been used to develop FRET biosensors: (i) two chain probes in which the fluorophores are on two different molecules resulting in intermolecular FRET when the two molecules come into proximity, or (ii) single chain probes in which different regions of a single molecule are tagged and undergo FRET due to intramolecular, conformational changes.

Molecular beacons are a recent technology that uses electronic energy transfer between a fluorescent molecule and a fluorescent quencher and has become an important tool in the study of biology [5]. Molecular beacons are single-stranded oligonucleotide

hybridization probes that form a stem-and-loop structure. The loop contains a probe sequence of DNA, RNA or PNA that is complementary to a target sequence. The stem structure holds the fluorophore and the quencher in close proximity, preventing the molecular beacons from emitting fluorescence. Binding to the target sequence induces conformational changes that open the loop and as a result the fluorescence is 'turned on'. A novel class of analytical probe has recently been reported that combines the sensitive signal transduction mechanism of molecular beacons with the specific protein-binding capability of aptamers [6]. Aptamers are single-stranded DNA or RNA molecules, generally 25-60 nucleotides in length, that have been selected in a process termed SELEX from a combinatorial library by their ability to bind to a specific target molecule with high affinity and specificity.

3. Refractive index detection

Sometimes labelling of molecules is not desired or possible, and anyway requires an additional preparation step. The following optical structures represent the majority of research activities in optical label-free sensor development: (1) surface plasmon resonance (SPR) based biosensors; (2) interferometer-based biosensors; (3) resonant mirrors. However, other emerging optical sensing technologies have been under investigation, such as optical ring resonators and photonic crystals [4]. Fibre Bragg grating sensors are refractive index sensors however these optical devices will be described in the fiber-optic biosensors section (section later).

3.1. Surface Plasmon Resonance

SPR is based on the interaction of light with a thin metallic film, typically gold or silver, coated on a transparent medium. When light of a certain wavelength impinges on a glass substrate, the amount of light that is reflected off the substrate increases with the angle of incidence until the critical angle, θc, is reached where the light is totally reflected back. This phenomenon is called total internal reflection and occurs for all angles greater than or equal to the critical angle. When the opposite surface of the substrate is coated with a thin metallic layer, there is another angle, greater than θc, where the light instead of being totally internally reflected back is 'coupled' into the metallic film, causing a minimum in the reflected light intensity. This angle at which this minimum occurs is called the SPR angle and the phenomenon arises because of oscillations of mobile surface electrons that propagate along the boundary of the metal film and the dielectric layer adjacent to it, causing the surface plasmon wave. At θ_{spr} the light is providing the correct quanta of energy to cause the metal layer to absorb this energy. The evanescent field of the resonance travels into the adjacent dielectric medium a few hundred nanometres above the metal surface and decays exponentially with the distance from the surface. So the frequency of the plasma wave and, hence θ_{spr} is found to be critically dependant not only on the physical nature of the metal boundary layer but, more importantly, it is also dependent on the dielectric properties of the adjacent medium directly in contact with the metal film. SPR is sensitive to local refractive index changes at or near the metal surface and can be used to study the specificity, affinity and kinetics of biomolecular interactions and measure the concentration levels of analytes in complex samples. The analysis is done in a label-free manner and in real time.

SPR sensors have been intensively developed and reviewed recently [7]. Today, the development of SPR sensors is geared toward the design of compact, low-cost and sensitive approaches. Prism coupling or Kretchmann's configuration has been found to be very suitable for sensing and it the most widely used geometry in SPR sensors.

Biacore AB is one of the companies that have successfully commercialized prism-based SPR sensor systems [8,9]. However the prism is bulky and difficult to integrate so optical fibers and waveguide structures have been proposed as good alternatives to transmit the surface plasmon excitation and reflection light (Fig. 1). Waveguide coupling is a robust and simple way to control the optical path in the sensing layer and is easier to integrate with other optical and electrical components, offering extra benefits like miniaturized devices and remote sensing. The integrated optical waveguide SPR structure consists of a channel waveguide locally coated with a planar layer structure supporting surface plasmons. The process of exciting a surface plasmon wave in an optical waveguide-based SPR sensor is, in principle, similar to that in the Kretschmann configuration. The light propagates in a waveguide through total internal reflection and generates an evanescent field at the waveguide-metal interface, which excites the surface plasmon wave if the phase velocities of the waveguide mode and that of the surface plasmon match. Optical fibers are two-dimensional waveguides, used to excite the surface plasmon wave in different configurations. Early fiber-SPR biosensors used single mode fiber with polished and metal-coated tip and the changes are measured by collecting the back reflected light from the fiber or the diffracted light from the polished end. Recently, to improve the coupling of light to the surface plasmon mode, micro-fabrication technology has been used to etch a micro-cone into the polished end of the fiber to form a micro-prism [10]. Another configuration is removing a small portion of the fiber and then coating this area with a layer of metal film.
Additionally the surface plasmon wave can also be excited by grating coupling that is a very low cost alternative for the mass production of biosensors. If a metal-dielectric interface is periodically distorted, the incident optical wave is diffracted forming a series of beams directed away from the surface at a range of angles. The component of the momentum of these diffracted beams along the interface differs from that of the incident wave by multiples of the grating wave vector. If the total component of momentum along the interface of a diffracted order is equal to that of the surface plasmon wave, the optical wave may couple to the surface plasmon wave. The SPR changes induced by the binding of target molecules to the sensing area can be detected by monitoring the resonant angle, resonant wavelength or resonant intensity change.

3.2. Interferometers

Another very highly sensitive way to read-out the change of the refractive index is an interferometric set-up. When light is propagated through an optical waveguide via total internal reflection, an evanescent field builds up at the core/cladding interface and extends into the surrounding area. If the waveguide surface is functionalized by immobilising specific target molecules, the sensing layer will cause a changing refractive index profile within the evanescence field and the phase velocity of the guided mode is decreased. In a typical configuration of the Mach-Zehnder interferometer, the guiding light is divided into two branches via a Y-junction. One branch is functionalized as the sensing arm, while the other serves as the reference arm. A second Y-junction recombines the two branches, which then interfere with each other showing a sinusoidal variation related to the change of refractive index of the surrounding medium (Fig. 2). Sensor chips can also consist of multiple Y-shaped stripe waveguides. In a typical design, large refractive index difference between the core/cladding layer and short-wavelength light source are required theoretically for achieving high detection sensitivity. HeNe laser commonly serves as the light source owing to its relatively short wavelength and strong interaction with most bio-molecules. Silicon-based materials are frequently used as the core/cladding materials of the waveguide because they can provide higher refractive index difference with high transparency. These materials can be patterned into the waveguide structures by

standard semiconductor techniques of chemical vapour deposition and plasma etching. An important design improvement for optic sensing is the use of ARROW (anti-resonant reflecting optical waveguide) structures. In this configuration, light confinement is based on anti-resonant reflections rather than total internal reflection. ARROW is a five-layer guiding structure where light is confined within the core layer by total internal reflection at the outer medium/core interface and by anti-resonant reflection (reflectivity around 99.96%) due to the presence of two cladding layers underneath the core [11].
In the literature other interferometer-based biosensors have been investigated based on the same principle of detection described but they differ in their design. In a Young's interferometer the single polarization light at the input is split into a reference arm and a sensing arm, however instead of recombining the arms, the optical output of the two arms combines to form interference fringes on a detector screen. To evaluate the position shift in the interference pattern, a Fourier transformation method is applied [12].

3.3. Resonant mirrors

The resonant mirror biosensor is also based on the evanescent field phenomenon and was developed to combine the simple construction of SPR sensors with the sensitivity of waveguide devices [13]. The resonant mirror biosensor consists of several layers: the sensing surface, the high refractive index dielectric resonant layer, the low index spacer layer and the high index substrate (normally a prism). Light enters the resonant layer by travelling through the prism (Fig. 3). The low-index coupling layer couples the light into the high refractive index layer that acts as a waveguiding structure and it is from this latter layer that the evanescent field extends into the bulk medium. Coupling the light into and out of the resonant structure is dependent on the angle of the incident light. Light coupled into the resonant structure undergoes a 90^{o} phase change. The evanescent wave associated with the resonant mirror is very sensitive to changes in surface refractive index. The commercially available Affinity Sensor IaSys system is based on the resonant mirror technology.

4. Fiber-optic biosensors

An optical fibre is a cylindrical waveguide made with silica that transmits light along its axis, by the process of total internal reflection. A fiber consists of a dielectric material (the core) surrounded by another dielectric material with a lower refractive index (the cladding). Even though most of the light is internally reflected, there is a small component of light, the evanescent field, which propagates into the cladding. In a standard fibre where the cladding is much thicker than the core, the evanescent field decays to near zero value at the fibre surface. However if the fibre is tapered down to a diameter less than the core diameter, the light is no longer guided by the core but is guided by the cladding, and the evanescent field will interact with the surrounding environment and also its magnitude is enhanced in the tapered region.
Fiber-optic biosensors can be classified into two categories: intrinsic and extrinsic sensors. In an intrinsic sensor, the interaction with the analyte occurs within an element of the optical fiber whereas in an extrinsic sensor the optical fiber is only used to couple light. Optical fiber biosensors can be used in combination with different types of optical techniques (e.g. absorbance, refractive index, fluorescence, chemiluminiscence measurements and other spectroscopic techniques) [14, 15].
Advances in nanotechnology have recently led to the development of optical fibers with submicron-sized dimensions opening a new possibility for microenvironments such as individual cell sensing. Fiber-optic devices offer many advantages for in situ monitoring applications due to the optical nature of the excitation and detection modalities, they are

not affected by electromagnetic interference from static electricity, strong magnetic fields or surface potentials.

4.1. Fibre Bragg gratings

Fibre Bragg gratings are periodic structures that are imprinted directly into the core region of optical fibers by UV radiation. Such structures consist of a periodically varying refractive index, typically over several millimetres of the fibre core. The specific characteristic of fibre Bragg gratings for sensing applications is that their periodicity causes them to act as wavelength sensitive reflectors. Light travelling down the fibre is partially reflected at each of the small refractive index variations, but at most wavelengths these reflections interfere destructively and light propagates down the fibre as if it were of uniform refractive index. However at a particular wavelength, called Bragg wavelength, the reflections from each successive period are in phase (constructive interference) and light is reflected back up the fiber. By monitoring shifts in the Bragg wavelength, the device functions as a refractive index sensor, which can be used for biochemical sensing [16]. There are different strategies to expose the evanescent field from a fiber Bragg grating: (i) create a surface grating on the side of the fiber, or (ii) chemical etching of the optical fiber down to its core. Major advantages of the fiber Bragg grating technology are the self-referencing capacity, minimal size, ease of multiplexing and distributed sensing possibilities.

5. Applications

Optical biosensors have been used widely over the past decade to analyse biomolecular interactions (such as enzyme-substrate, antigen-antibody reactions, DNA-DNA, peptide nucleic acid (PNA)-DNA hybridizations), providing detailed information on the binding affinity and kinetics of interaction. Only recent breakthroughs in optical biosensors and nanotechnology have allowed the use of this platform for living cell sensing, which is much more complex and dynamic. This section also discusses the applications of optical biosensor in cell signalling and cellular interaction.

5.1. Cell-surface interactions

One way to study cell signalling is to bring cells into contact with the biosensing surface, which could be achieved by cell culture. Due to the large dimension of cells compared to the short penetration depth of a biosensor, only the bottom portion of cells will be sensed. Mammalian cell attachment studies were conducted on a variety of common microchip surfaces for potential use in cell-based biosensors [17]. Data shows that there are differences in overall cell attachment between cell types but that most of the substrates will support attachment without the addition of any precoating protein and that transfection parameters are not significantly different between the surfaces that might be encountered in a biosensor. Optical waveguide lightmode spectroscopy that uses an optical grating to excite the guided modes of a planar waveguide is a powerful method for monitoring adsorption of protein, cell attachment and spreading processes [18]. Surface plasmon resonance-based biosensors have also been reported for the detection of cell-ligand interactions [19, 20].

Fang *et al.* have been using resonant-waveguide grating sensors to examine the dynamic mass redistribution of human epidermoid A431 cells in response to diverse β2-adrenoceptor ligands, cellular functions of cholesterol in cells, cytoskeleton modulation and cell signalling mediated through epidermal growth factor (EDF) receptor or G-protein-coupled receptor [21-24].

5.2. Detection of cell proliferation/apoptotic signalling in single living cells

Optical nanobiosensors have been used for monitoring the onset of the mitochondrial pathway of apoptosis in a single living cell by detecting enzymatic activities of Caspases. These cysteine proteases are activated during apoptosis in a self-amplifying cascade. Minimally invasive analysis of single live human breast adenocarcinoma MCF-7 cells for caspase-9 activity was demonstrated by immobilizing a non-fluorescent enzyme substrate, Leucine-GlutamicAcid-Histidine-AsparticAcid-7-amino-4-methyl coumarin (LEHD-AMC), onto a fiber-optic. LEHD-AMC was cleaved during apoptosis by caspase-9 generating free AMC. An evanescent filed was used to excite cleaved AMC and the resulting fluorescence signal was detected. By monitoring the changes in fluorescence signals, caspase-9 activity within a single living cell was detected [25]. Kintzios *et al.* created a miniaturized biosensor system combining the electrophysiological response of immobilized Vero cells with superoxide-sensing technology, optical and fluorescence microscopy allowing for the simultaneous detection of seven different cell parameters and the prediction of division- or death associated events [26].

5.3. Monitoring biochemicals in single living cells

Using optical nanosensors it would be possible to detect individual chemical species in specific locations throughout a cell. Vo-Dinh *et al.* have recently developed nanometer-scale fiber optic biosensors capable of being inserted into individual cells and measuring toxic chemicals (such as benzo[α]pyrene tetrol and benzo[α]pyrene), via fluorescence spectroscopy. The carcinogen benzo[α]pyrene is a polycyclic aromatic hydrocarbon of great environmental and toxicological interest. Benzo[α]pyrene tetrol is an important biological compound, which was used as a biomarker of human exposure to benzo[α]pyrene. In order to perform these measurements, antibodies to the particular antigen of interest are attached to tips of nanofibers coated with silver [27-29]. Sensors based on optical waveguide lightmode spectroscopy were also demonstrated to response of mouse osteoblastic MC 3T3-E1 cell line cells to the presence of sodium hypochlorite [30].

5.4. Genetic sensors

Optical cellular biosensors have also been used to determine which genes are key modulators for health or disease and demonstrate their specific function [31]. Single mammalian cells are versatile biosensors that give robust and reproducible signals. To construct a reporter-tagged library composed of millions of cells, specific tagging markers are introduced in the cells. The library of cells can be stimulated and subjected to fluorescent-activated cell sorting to separate clones where a tagged gene is expressed. Sequential rounds of sorting can be used to isolate cell clones where tagged genes are induced or repressed by different agents including receptors, ligands, drug candidates, infectious agents or toxins. The sensors can be applied to high throughput compound screening during early stages of drug delivery, to toxicological and diagnostic methodologies and to basic cell biology research.

6. Future technology developments

The emergence of nanoparticle technology is opening new horizons for the probing in the smallest environments such as individual living cells, allowing for measurements of chemical species in specific locations within the cell. The use of these nanoparticles could provide the basis for ultrasensitive detection at a nanoscale level in any area of interest especially within the cell, avoiding the diffusion problems of indicator dyes. Kopelman *et al.* described the smallest stand-alone chemical sensor, consisting of

multicomponent nano-spheres with radii as small as 10nm [32, 33]. The sensor probe encapsulated by biologically localised embedding (PEBBLE) is delivered into a cell by minimally-invasive techniques. The PEBBLE composition, including the matrix, the fluorophore and other components, are optimised for the task at hand and PEBBLEs have been prepared for pH, calcium, magnesium, potassium and oxygen sensing. Sensor PEBBLEs can be prepared for those analytes with no fluorescent indicator dyes sufficiently selective for intracellular measurements by pairing non-fluorescent selective ionophores with fluorescent pH indicators and other additives within the boundaries of the hydrophobic polymer. The confinement of the sensor reporter dyes enables the differentiation of nano-optode locations from those of auto fluorescence centres in the cell and also makes possible the simultaneous observation of several analytes. PEBBLEs also show very good reversibility and stability to leaching and photobleaching and exhibit short response times. This technology holds much promise for intracellular sensing.

There is also considerable interest in the use of quantum dots as luminescent inorganic fluorophores. A quantum dot is a nanoscale crystal of semiconductor material that glows or fluoresces when excited by a light source. Quantum dots have the potential to overcome some of the functional limitations encountered by organic dyes in fluorescence labelling applications (such as narrow excitation bands and broad emission spectra, low resistance to chemical degradation and photodegradation). Luminescence emission from quantum dots is detected at concentrations comparable to organic dyes by using conventional fluorescence methods and individual quantum dots and quantum dots –bioconjugates are easily observable by confocal microscopy. Quantum dots could become very powerful tagging agents. Researchers are attempting to modify the surface chemistry of quantum dots so they can be attached to cells, proteins and nucleic acids [34- 36]. Quantum dots can be also designed to emit light at any wavelength from infrared to visible to ultraviolet, allowing for multiplexed assays.

Recently several research groups have begun to explore alternative strategies for the development of optical biosensors based on noble metal nanoparticles. Single nanoparticle biosensors have been developed to detect individual receptor molecules, map the distribution of receptor and measure the binding kinetics and affinity of single protein molecules on single living cells for an extended period of time (hours), using single nanoparticle optical microscopy and spectroscopy and measuring localized surface plasmon resonance spectra of single nanoparticles [37]. Localized surface plasmon resonance spectroscopy operates in a manner totally analogous to propagating surface plasmon resonance sensors by transducing small changes in refractive index near the noble metal surface, but the localised variant yields ultrasensitive biodetection due to the short electromagnetic field decay length [38]. Another type of sensor fabricated by nanosphere lithography relies on surface-enhanced Raman scattering [39]. Advantages of this vibrational spectroscopic method are the low detection limit, both qualitative and quantitative analysis capabilities, high degree of specificity and capability for multi-analyte detection.

The ability to generate patterns of proteins and cells on surfaces is crucial for the development of successful biosensors and also for fundamental studies of cell biology. Many research groups are investigating techniques that are compatible for patterning biomolecules at a molecular level, placing biological ligands at well-defined locations on substrates. Novel soft lithographic technologies can be used to pattern a variety of planar and non-planar substrates with ligands, providing a simple and cost-effective procedure. Soft lithography does not require stringent control over the laboratory

environment and offers control over both the surface chemistry and the cellular environment. These methods use an elastomeric stamp or mold, prepared by casting the liquid prepolymer of an elastomer against a master that has a patterned relief structure. Most of the research has used poly(dimethylsiloxane) as the elastomer because it is biocompatible, permeable to gases, optically transparent down to about 300 nm and it can contact non-planar surfaces conformally. Soft lithographic techniques can be used to make three-dimensional microstructures. The main techniques are: (i) microcontact printing, (ii) microfluidic channels and (iii) laminar flow patterning [40].

In order to perform many optical measurements, it is necessary to bring the sample solution to the sensing surface of the device. Often for that purpose bulky cuvette systems are used but microfluidics are being successfully implemented in various sensing platforms and offer several advantages: (i) faster response due to shorter mixing times, (ii) smaller sample volumes and (iii) easy to miniaturize and integrate the entire analytical sensing system (pumps, valves, mixers), resulting in a compact sensing device [41, 42]. Two of the crucial components of a microfluidic system are micropumps and microvalves and among many with different activation principles, the ones formed by soft lithographic methods have attracted considerable interest [43].

7. Conclusions

The combination of nanotechnology, molecular biology and photonics opens the possibility of developing nanodevices which have the potential for a wide variety of diagnostic and therapeutic uses at the molecular and cellular level. Optical nanosensors for living cell analysis are promising analytical tools that needs to mature. The technology remains some way from being widely accepted in fundamental biological research and drug discovery, but over the next decade its impact on whole cell probing is likely to continue to grow and strengthen.
In biosensor development, there are a number of important goals that have not been discussed in this review but need careful attention, such as the surface immobilization chemistry, the integration of the whole sensing system and data analysis. Continuous advances are being made in these areas.

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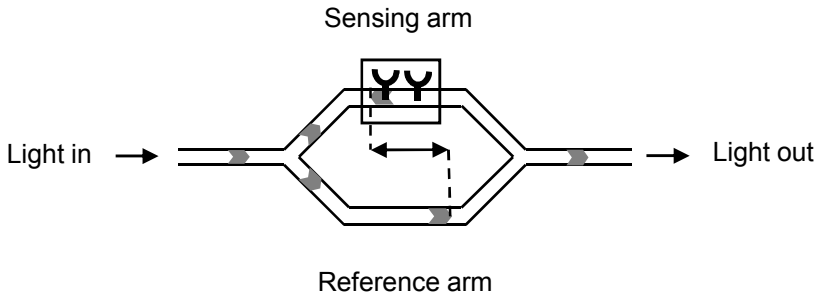
Figure legends

Fig.1. Surface plasmon resonance sensor configurations: (a) prism coupling, (b) waveguide coupling, (c) optical fiber coupling and (d) grating coupling.

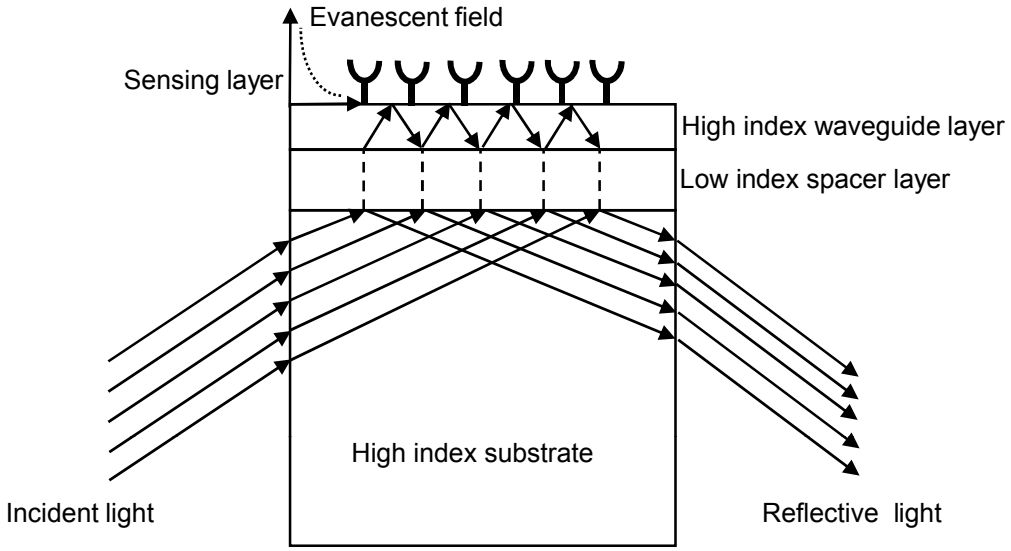
Fig.2. Mach-Zehnder interferometer sensor.

Fig.3. Resonant mirror sensor.

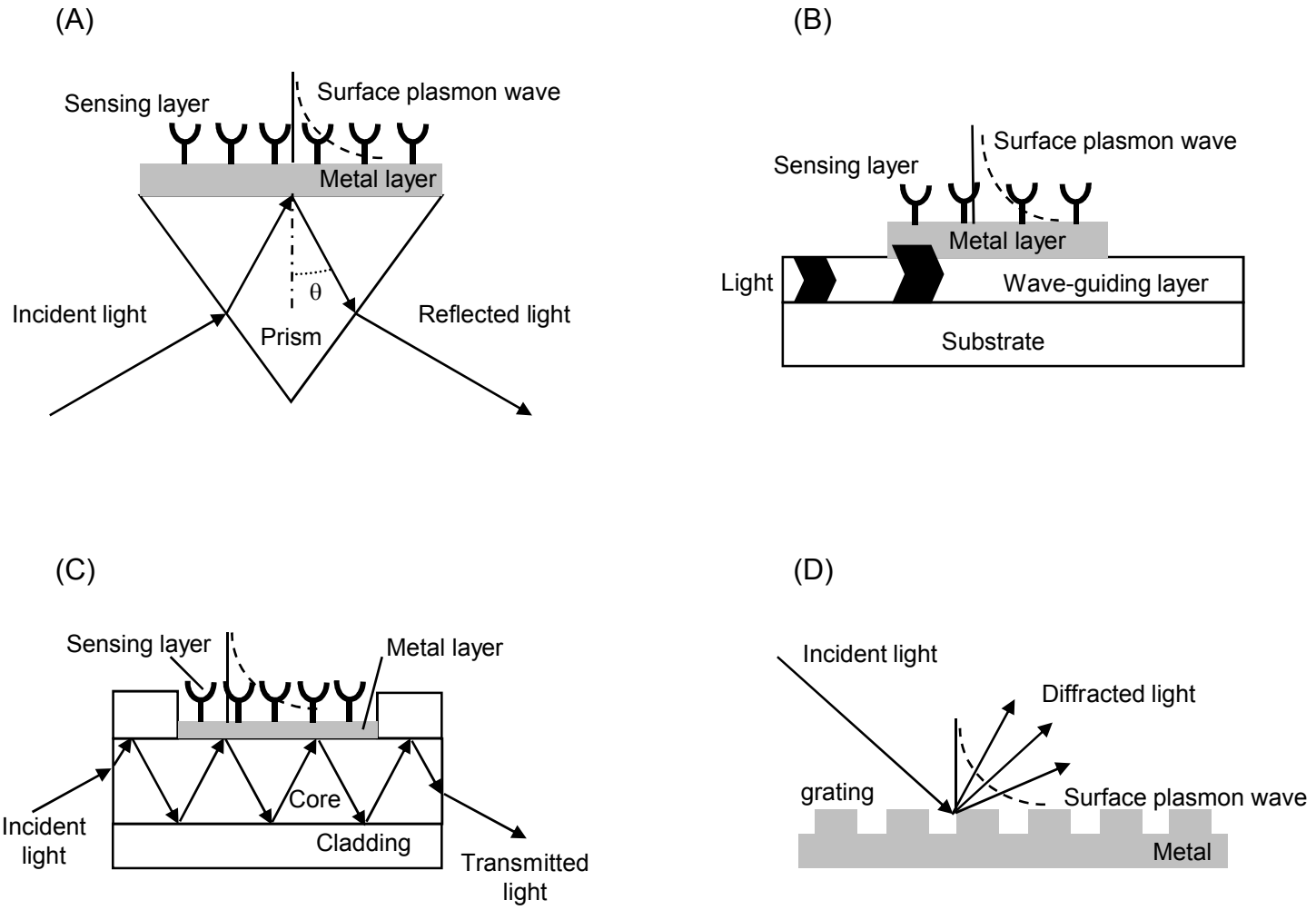
Figure



Figure


Evanescent field
Sensing layer
High index waveguide layer
Low index spacer layer
High index substrate
Incident light
Reflective light

Figure


(A)
Sensing layer
Surface plasmon wave
Metal layer
θ
Incident light
Prism
Reflected light
(B)
Sensing layer
Surface plasmon wave
Metal layer
Light
Wave-guiding layer
Substrate
(C)
Sensing layer
Metal layer
Core
Incident light
Cladding
Transmitted light
(D)
Incident light
Diffracted light
grating
Surface plasmon wave
Metal