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Teze doktorské disertační práce
k získání vědeckého titulu "doktor věd"
ve skupině věd technických

Optical Biosensors Based on Spectroscopy of Surface Plasmons

název disertace

Komise pro obhajoby doktorských disertací v oboru
Elektrotechnika, elektronika, optoelektronika a fotonika

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Resumé

Poslední desetiletí bylo svědkem mohutného rozmachu bádání na rozhraní vědních oborů. Jedním z příkladů tohoto trendu jsou optické biosenzory, které jsou rozvíjeny interdisciplinární spoluprací fotoniky, elektroniky, chemie a biologie.

Předkládaná disertační práce je zaměřená na oblast optických biosenzorů založených na rezonanci povrchových plasmonů (surface plasmon resonance – SPR) a představuje vybrané výsledky autorova výzkumu v této oblasti publikované v posledních dvanácti letech. Práce je tematicky zaměřena na výzkum a vývoj SPR biosenzorů pro detekci chemických a biologických látek a soustřeďuje se především na metodu SPR, teorii SPR senzorů, nové optické platformy pro SPR biosenzory a využití SPR biosenzorů pro detekční aplikace v oblastech analýzy potravin, lékařské diagnostiky a monitorování životního prostředí.

Hlavní autorovy příspěvky k *rozvoji SPR metody a teorie SPR senzorů* diskutované v rámci disertační práce jsou následující: příspěvek do teorie SPR senzorů propojující funkční charakteristiky SPR senzorů s jejich konstrukčními parametry, multikanálové SPR senzory založené na multiplexování v oboru vlnových délek pro vývoj kompaktních multikanálových SPR senzorů se spektrální modulací, multi-plasmonová spektroskopie pro informačně bohaté SPR senzory, metoda spektroskopie povrchových plasmonů založená na optické excitaci povrchových plasmonů a spektrálním rozkladu světla na stejném optickém elementu a spektroskopie povrchových plasmonů s dalekým dosahem pro SPR senzory s vysokou citlivostí. Disertační práce rovněž přináší výsledky *výzkumu a vývoje nových optických platforem pro SPR senzory*, především SPR senzor založený na jedno-módovém optickém vlákně, který dnes představuje nejvyšší stupeň miniaturizace SPR senzorů, SPR senzor založený na integrovaně-optickém vlnovodu a SPR senzory s vysokým počtem měřících kanálů (>100) pro vysoce paralelizované studium molekulárních interakcí. Pozornost je zároveň věnována propojení těchto originálních optických platforem s vhodnými molekulárními receptory a *metodami pr*o imobilizaci molekulárních receptorů na površích SPR senzorů. V této oblasti se kromě tradičních imobilizačních metod uplatňují i originální metody vyvinuté pro specifické typy SPR senzorů, jako jsou například senzory s multiplexováním v oboru vlnových délek a se spojitě navazujícími detekčními oblastmi. Uplatnění *SPR biosenzorů pro detekci chemických a biologických látek* je ilustrováno na příkladech SPR biosenzorů pro detekci protilátek a hormonů pro lékařskou diagnostiku, biosenzorů pro detekci patogenů a toxinů pro analýzu potravin a biosenzorů pro detekci chemických látek narušujících činnost žláz s vnitřní sekrecí pro monitorování znečištění životního prostředí.

Výsledky výzkumu optických biosenzorů s povrchovými plasmony prezentované v této disertační práci ukazují nejen obrovský potenciál optických biosenzorů, ale i vysokou společenskou aktuálnost tohoto výzkumu. Lze proto očekávat, že výzkum SPR biosenzorů se bude nadále dynamicky rozvíjet a přispěje k rozvoji dalších oborů jako jsou genomika, proteomika, lékařská diagnostika, monitorování životního prostředí a kontrola kvality potravin.

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1. Introduction

Diffusion of inorganic and biological worlds represents an important paradigm of modern science and technology [1]. Biophotonics stands out as emerging field of research at the crossroad of physical, chemical and life sciences. Integration of photonics, biology and nanotechnology leads to a new generation of devices that makes it possible to characterize chemical and other molecular properties as well as discover novel phenomena and biological processes occurring at the molecular level. Biophotonics is widely regarded as the key science upon which the next generation of clinical tools and biomedical research instruments will be based.

The last two decades have witnessed an increasing effort devoted to research and development of optical biosensors and biochips worldwide. Recent scientific and technological advances have demonstrated tremendous potential such devices hold for applications in areas such as genomics, proteomics, medical diagnostics, environmental monitoring, food analysis, agriculture, and security. Label-free optical biosensors present unique technology that enables direct observation of molecular interaction in real-time and allows study of molecular systems without the use of labels. Optical label-free biosensors measure binding-induced refractive index changes and are typically based on interferometric transducers, such as integrated optical Mach-Zehnder interferometer, integrated Young interferometer, and white light interferometer, and transducers based on spectroscopy of guided modes of dielectric waveguides, such as resonant mirror sensor and grating coupler sensor, or metal-dielectric waveguides, such as surface plasmon resonance (SPR) sensor. Since the first demonstration of surface plasmon resonance (SPR) method for study of processes at the surfaces of metals [2] and sensing [3] in early 1980s, SPR sensors have received a great deal of attention and made vast advances both in terms of technology and applications [4]. SPR biosensors have been also increasingly developed for detection of chemical and biological species and numerous SPR biosensors for detection of analytes related to medical diagnostics, environmental monitoring, food safety and security have been reported.

The Thesis concerns with research and development of SPR biosensors and consists of three main parts. The first part (Chapter 2) describes fundamentals of the SPR biosensor technology. The second part provides a brief review of the current state of the art of this field (Chapter 3). The last part presents the main contributions of the author to the field of SPR biosensors, specifically, to the SPR method (Chapter 5), optical platforms for SPR sensors (Chapter 6), and SPR biosensors for specific applications (Chapter 7).

2. Fundamentals of surface plasmon resonance (SPR) sensors

The first observation of surface plasmons was made in 1902 by Wood who reported anomalies in the spectrum of light diffracted on a metallic diffraction grating [5]. Fano has proven that these anomalies are associated with the excitation of electromagnetic surface waves on the surface of the diffraction grating [6]. In 1968 Otto demonstrated that the drop in the reflectivity in the attenuated total reflection (ATR) method is due to the excitation of surface plasmons [7]. In the same year, Kretschmann and Raether observed excitation of surface plasmons in another configuration of the attenuated total reflection method [8]. In the following years, systematic research of surface plasmons has been carried out and electromagnetic theory of surface plasmons has been established [9-11].

Surface plasmon is a special mode of electromagnetic field which may exist at the interface between a dielectric and a metal which behaves like free-electron plasma. The electromagnetic field of a surface plasmon reaches its maximum at the metal-dielectric interface and decays exponentially into both media. The penetration depth of a surface plasmon at the interface between gold and an aqueous medium increases with an increasing wavelength and ranges from 100 to 600 nm for the wavelengths between 600 nm and 1000 nm. If the metal film has a limited thickness, surface plasmons may exist at both the interfaces. For thin metal films, there is coupling between surface plasmons at each interface, giving rise to mixed modes, symmetric and antisymmetric surface plasmons, which are sometimes referred to as long-range and short-range surface plasmons, a reference to their relative propagation lengths [12-14].

SPR affinity biosensors are sensing devices which consist of a biorecognition element that recognizes and is able to interact with a selected analyte and an SPR transducer that translates the binding event into an output signal, Figure *1*.

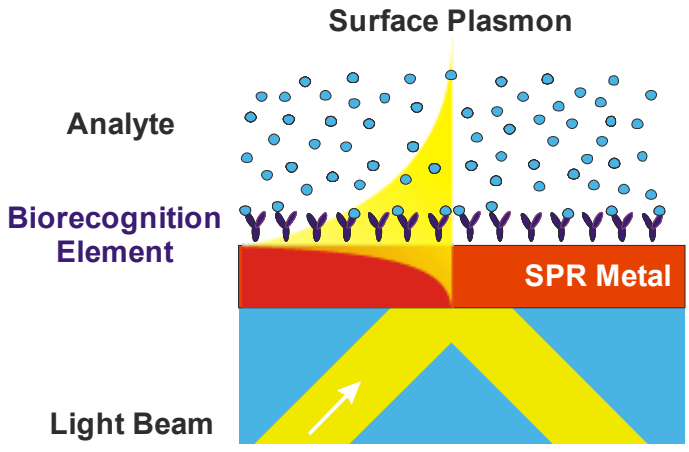

Figure 1. Concept of SPR biosensing.

The biorecognition elements are immobilized in the proximity of the surface of a metal film supporting a surface plasmon. Analyte molecules in a liquid sample in contact with the SPR sensor bind to the biorecognition elements, producing an increase in the refractive index at the sensor surface The change in the refractive index gives rise to a change in the propagation

constant of the surface plasmon which through the coupling condition alters the characteristics of the light wave coupled to the surface plasmon (e.g. coupling angle, coupling wavelength, intensity, phase). Based on which characteristic of the light wave modulated by a surface plasmon is measured, SPR sensors are classified as sensors with angular [15], wavelength [16], intensity [3], or phase [17] modulation.

3. Present state of the art

SPR biosensors represent a highly interdisciplinary field of research requiring concerted effort in a broad range of scientific disciplines including photonics, electronics, chemistry, and molecular biology. This Section is intended to present main research directions and give illustrating examples in the research areas within the scope of this Thesis rather than to provide a comprehensive coverage of this complex and fast moving field. For extensive review on SPR biosensors, the reader is kindly referred to a recent book on the subject matter [18].

3.1. Optical platforms for SPR sensors

Research into optical platforms for SPR sensors has been driven by several needs: high performance (sensitivity, resolution), large number of sensing channels (high-throughput screening), and miniaturization/portability (SPR systems for field use). Advances in these directions are discussed in the following sections.

Most of the SPR sensors developed to date use prism coupling and the attenuated total reflection method to couple light to a surface plasmon [18]. Prism coupling is convenient and can be readily combined with any type of modulation. In the early 1990s an angular modulation-based SPR sensor consisting of a light-emitting diode (LED), a glass prism and a detector array with imaging optics was introduced [19]. A divergent beam produced by the LED was collimated and focused by means of a cylindrical lens to produce a wedge-shaped beam of light which illuminated a thin gold film on the back of a glass prism containing several sensing areas (channels) [19]. This design has been adopted by Swedish company Biacore (now Biacore is a part of GE Healthcare) and resulted in a family of commercial SPR sensors with a resolution down to 1×10^{-7}RIU (RIU – refractive index unit) and up to 4 sensing channels [20]. In the effort to improve performance of SPR sensors, phase-modulation-based SPR sensors have been widely researched. Nikitin's group (Institute of General Physics, Moscow) demonstrated two SPR sensor platforms based on phase modulation and (i) the interference of the TM-polarized signal beam with the TE-polarized reference beam [21] and (ii) Mach–Zehnder interferometer combining TM-polarized signal and reference beams [22]. The achieved refractive index resolution was in the order of 10^{-7} RIU [22]. Later, Wu et al. demonstrated a phase-modulation SPR sensor based on common-path, heterodyne interferometry and the refractive index resolution of 2×10^{-7} RIU [23]. Naraoka and Kajikawa reported a phase-modulation SPR sensor based on a rotating analyzer method [24]. In their approach, a phase difference

between the TE and TM components of the reflected light were determined from the dependence of the reflectivity on the angle of analyzer. Refractive index resolution of their system was estimated to be 2×10^{-7} RIU [24]. In recent years, SPR sensors with phase modulation have been extensively studied by the researchers at the Chinese University of Hong Kong and City University of Hong Kong. In 2004 Wu et al. reported an SPR sensor with phase modulation based on Mach–Zehnder interferometer with modulated optical path and with a refractive index resolution better than 6×10^{-8} RIU [25]. This work represents the best refractive index resolution achieved to date using conventional surface plasmons. A concept of miniature SPR sensor based on integration of all electro-optical components in a monolithic platform was developed by Texas Instruments (USA) in mid-1990s [26]. Their Spreeta 2000 SPR sensor consists of a plastic prism molded onto a microelectronic platform containing an infrared LED and a linear diode array detector. Initial version of this platform exhibited a refractive index resolution of 5×10^{-6} RIU [27]. A portable multichannel SPR instrument based on Spreeta 2000 design was reported by Furlong's group (University of Washington, Seattle) who achieved a refractive index resolution of 3×10^{-6} RIU [28]. Another compact, portable SPR sensor platform was developed by Kawazumi et al. [29]. Their system provided four independent sensing channels, however, a rather poor refractive index resolution of 10^{-4} RIU [29]. In late 1980s the potential of the SPR method for spatially resolved measurements was recognized and the first surface plasmon microscopes were reported [30]. In the following years, the idea of SPR imaging was further advanced, yielding SPR sensing devices with a large number (>100) of sensing channels. Corn's group (University of California, Irvine) has researched SPR imaging for over a decade. In 1997 they introduced an SPR imaging instrument with an incoherent light source and a NIR narrow band-pass filter [31] and demonstrated a refractive index resolution in the 10^{-5} RIU range [32]. Subsequently, they reported SPR imaging with a special multilayer structure supporting long-range surface plasmons, however, the use of long-range surface plasmons led only to a minor sensitivity improvement compared to the conventional SPR imaging [33]. Campbell's group (University of Washington, Seattle) reported an SPR imaging system with a controllable angle of incidence [34]. They demonstrated that their sensor is able to measure simultaneously in 120 sensing channels with a refractive index resolution down to 5×10^{-6} RIU [35].

Light can be also coupled to a surface plasmon via diffraction on a metal-coated diffractive grating. Although this approach offers several attractive features such as design flexibility and compatibility with mass production, it has not been used in SPR sensors as widely as the prism coupling. The potential of diffractive gratings for construction of SPR sensors was realized at the University of Cambridge, Cambridge, by Cullen and coworkers in late 1980s [36]. Later, Sambles' group (University of Exeter, Cornwall) demonstrated wavelength-modulated SPR sensors [37] and intensity-modulated SPR sensor on a disposable plastic diffraction grating [38]. In 1995 the same group described an interesting approach to SPR sensing based on the wavelength modulation and the use of an

acousto-optic modulator and demonstrated a refractive index resolution below 10^{-6} RIU [39]. In 2001 Brockman and Fernandez presented a high-throughput SPR imaging device in which a collimated monochromatic light beam was made incident onto a plastic chip with an array of 400 sensing channels and then projected onto a 2-dimensional CCD array [40]. This concept was further developed by HTS Biosystems (USA). In 2005 this FLEXChip technology was acquired by Biacore.

Direct excitation of surface plasmons by modes of optical fibers presents an interesting approach to development of miniature SPR sensing devices. The first SPR sensor based on a multimode optical fiber was reported by Yee's group (University of Washington, Seattle) in 1993 [41]. This wavelength modulation-based SPR sensor used a polymer clad silica (PCS) fiber with partly removed cladding and a metal film deposited symmetrically around the exposed section of fiber core. This sensor was shown to be able to detect refractive index variations with a resolution of 5×10^{-5} RIU [42]. A similar geometry, in which the sensing area is formed not at the tip but in the middle of an optical fiber, has been used for the development of an intensity modulation-based SPR sensor by RonotTrioli et al. [43, 44]. In this configuration, a collimated monochromatic light beam is launched into a fiber in such a way that only modes with propagation constants within a narrow range are efficiently excited. Refractive index resolution of this sensor was demonstrated to be 8×10^{-5} RIU. Homola's group developed the first fiber optic SPR sensor based on a single-mode optical fiber (see a more detailed discussion in Section 6.1). Later, Chiu et al. reported a fiber optic SPR sensor based on a D-shape single-mode optical fiber and heterodyne interferometry [45]. Their sensor measured refractive index changes down to 2×10^{-6} RIU. In 2007 Lin et al. extended this approach to multimode fibers and reported an SPR sensor based on a side-polished multimode optical fiber and wavelength modulation with a refractive index resolution of 3×10^{-6} RIU [46].

Research into integrated optical waveguide SPR sensors was pioneered by the research group of Lambeck (University of Twente, Enschede) in the early 1990s [47]. Subsequently, integrated optical SPR sensors were investigated by Wilkinson's group (University of Southampton, Southampton) who demonstrated SPR sensing devices using slab [48] and channel [49] single-mode integrated optical waveguides. An integrated optical SPR sensor with intensity modulation and one sensing and one reference channel was reported by Mouvet et al. [50]. The signal from the sensing channel was normalized to the signal from the reference channel, resulting in an increased stability and a refractive index resolution of 5×10^{-5} RIU [51]. In 2006 an SPR sensor based on a strip waveguide consisting of a germanium-doped silicon dioxide waveguiding layer on a silicon substrate and wavelength modulation was reported by Huag et al. The sensor was demonstrated to provide a refractive index resolution as low as 1×10^{-6} RIU [52]. In conventional waveguide-based SPR sensors, the resonant coupling between a surface plasmon and a waveguide mode occurs for refractive indices of sample considerably higher than the refractive index of a typical aqueous sample. Various approaches to

controlling the operating range of waveguide-based SPR sensor, so that it includes aqueous environments, have been proposed. They include an integrated optical waveguides fabricated in low refractive index glass [49] or a waveguide with a low-refractive index buffer layer [48]. In 2006 Skorobogatiy and Kabashin proposed to overcome this limitation of conventional waveguides by employing a photonic-crystal waveguide in which the effective index of a mode confined in the waveguiding layer can be made considerably smaller than the refractive index of the waveguiding layer material, enabling phase matching with a surface plasmon at any wavelength [53].

3.2. Biorecognition elements and their immobilization

Numerous types of molecules have been employed as biorecognition elements [28, 54]. Antibodies offer relatively high affinity and specificity and therefore have been widely used in SPR biosensors. Other types of biorecognition elements include peptides, aptamers and polymers with molecular imprinting. Given the complexity and variability of biorecognition elements, there is no universal immobilization method and the choice of immobilization chemistry is therefore made based on the properties of a specific biorecognition element. A detailed review of immobilization methods for SPR biosensors can be found in [18].

Biorecognition elements are either immobilized directly on the surface or in a three-dimensional matrix [55] (*e.g.* carboxymethylated dextran [56]). Direct immobilization on the sensing surface is often performed via self-assembled monolayers (SAMs) of alkanethiolates [57]. To provide a desired surface concentration of biomolecular recognition elements and non-fouling background, mixed SAMs of long-chained alkanethiolates terminated with functional group for attachment of biomolecular recognition elements and oligo(ethylene glycol)-terminated shorter-chained alkanethiolates resisting non-specific adsorption have been developed [58, 59]. Proteins are most frequently immobilized via covalent bond between the nucleophilic functional groups supplied by amino acids of the protein and electrophilic groups on the sensor surface [60]. Alternative approach is based on streptavidin immobilized on the sensing surface (covalently or via pre-immobilized biotin) and subsequent attachment of a biotin-conjugated protein. Antibodies can be also immobilized via interaction between the Fc region of the antibody and Protein A or Protein G. This method provides good access to the binding site of the antibody, however, in order to control orientation of the antibody, orientation of protein A itself needs to be controlled [61]. Immobilization of peptides can be performed using similar strategies as those used for proteins. Immobilization of oligonucleotides is most frequently performed using the streptavidin-biotin chemistry and biotinylated oligonucleotides [62]. For inhibition assay-based detection of small molecules, the small molecules have to bee immobilized on the SPR sensor surface. Those with functional groups (amines, thiols, aldehydes or carboxylic groups) can be covalently linked to corresponding groups on the

sensor surface while small molecules without suitable functional groups need to be derivatized prior to their immobilization [63].

3.3. Applications of SPR affinity biosensors

Urgent need for sensitive, specific, and rapid bioanalytical technologies exists in numerous important sectors including medical diagnostics, environmental monitoring, food safety and security. This is why development of SPR biosensors for detection of specific biological and chemical analytes related to these application areas has been one of the priority directions in the SPR biosensor research [18].

Analytes implicated in food safety and security have been receiving most attention. Numerous groups have engaged in research into SPR biosensors for foodborne pathogens and toxins and SPR-based detection of Escherichia Coli O157:H7 [64, 65], Salmonella enterica [66], Listeria monocytogenes [67], Staphylococcus aureus [68] and toxins, such as staphylococcal enterotoxins [69] and domoic acid [70], has been reported. Other analytes related to food safety and targeted by SPR biosensors include drug residues, vitamins, hormones, antibodies, chemical contaminants, and allergens.

Medical diagnostics has been perceived as one of the most significant field for applications of SPR biosensor technology. Therefore SPR biosensors for detection of molecules related to medical diagnostics have been widely researched and analytes, such as antibodies (antibodies against herpes simplex virus type 1 and type 2 [71]), hormones (chorionic gonadotropin hormone [72], 17ß-estradiol [73]), cancer markers (carcinoembryonic antigen [74], prostate-specific antigen [75]), allergy markers, and drugs have been demonstrated.

Applications of SPR biosensors in environmental monitoring have been also reported. Analytes of environmental concern targeted by SPR biosensors include pesticides (simazine [76], chlorpyrifos and carbaryl [77]), aromatic hydrocarbons (benzo[a]pyrene [78]), phenols (bisphenol A [79]), 2,4,6-trinitrotoluene, polychlorinated biphenyls and metal ions.

4. Scope of the Thesis

This Thesis presents selected results of research into surface plasmon resonance (SPR) (bio)sensors and their applications for detection of chemical and biological species [80, 81]. The main areas of research coved by this Thesis are:

- theory of SPR sensors and new approaches to SPR sensing,
- novel optical platforms for SPR (bio)sensors,
- SPR biosensors for applications in food analysis, medical diagnostics and environmental monitoring.

5. Surface plasmon resonance method

5.1. Theory of SPR sensors

Performance of an SPR sensor is described by several performance characteristics, of which sensitivity and resolution are the most important ones.

Sensitivity of an SPR sensor is to a large extent predetermined by the design of the sensor. Therefore, sensitivity of SPR sensors and its relationship to the main design parameters have been systematically investigated [82-84]. Theoretical analysis of sensitivity of SPR sensors based on prism coupling of light to surface plasmons and wavelength modulation was established in 1997 [82]. Attention was focused on two cases which are of interest in SPR biosensors – bulk refractive index sensitivity (change in the refractive index occurs within the whole sample) and surface refractive index sensitivity (change in the refractive index occurs only within a very short distance from the sensor surface). Analytical expressions for the bulk and surface refractive index sensitivities have been derived enabling better understanding of the factors influencing sensitivity of SPR sensors. The analysis demonstrated that in order to achieve a higher sensitivity, the SPR sensor should employ a metal with a higher magnitude of the real part of the dielectric constant and excite surface plasmons at longer wavelengths [82]. Subsequently, theoretical analysis of sensitivity of SPR sensors has been extended to SPR sensors with prism couplers and angular modulation and SPR sensors with diffraction couplers and angular or wavelength modulation [83]. Analytical expressions for sensitivity of these SPR sensor configurations have been derived and the influence of the major design parameters of the sensing structures on the sensitivity of the sensor has been determined. It was established that grating-based SPR sensors using wavelength modulation are less sensitive than their prism coupler-based counterparts. In case of sensors with angular modulation, sensitivity of SPR sensors was predicted to be comparable for grating and prism-coupler based systems [83]. Later, relationship between surface and bulk refractive index sensitivities was established [84]. It was concluded that the surface refractive index sensitivity is proportional to the bulk refractive index sensitivity and the ratio of the thickness of the layer within which the refractive index change occurs and the penetration depth of the surface plasmon [18]. This explains the observed spectral dependence of the ratio of surface and bulk refractive index sensitivity which is a prerequisite of multi-surface-plasmon spectroscopy for discrimination of surface and bulk refractive index changes (Section 5.3.).

Resolution is another key performance characteristic of an SPR sensor. Resolution of an SPR sensor is defined as the smallest change in the bulk refractive index that produces a detectable change in the sensor output. SPR sensors of all the modulation approaches measure changes in the intensity of light wave coupled to a surface plasmon to determine the sensor output. Therefore, their resolution is limited by the noise in the intensity of the detected light. Sources of noise in an SPR sensor and

propagation of the noise through one of the most frequently used data processing method, the centroid algorithm, have been investigated [85]. A method for predicting SPR sensor output noise from the noise of SPR spectra has been developed and used to optimize parameters of the centroid method to yield the best signal to noise ratio. Assuming Lorentzian profile of the SPR dip and the optimum threshold at the half of the SPR dip depth [85], analytical formula interrelating design parameters of the sensor and the noise of the sensor output has been derived [86]. This theory indicates that resolution of an SPR sensor is proportional to the ratio of the noise at the threshold and the depth of the SPR dip (difference of intensities between the dip minimum and threshold) and depends only weakly on the choice of the coupler and modulation [86].

5.2. Multichannel SPR sensors based on wavelength division multiplexing

Multichannel SPR sensors are desired for two main reasons. First, they enable simultaneous detection of multiple analytes at a time using independent sensing channels of the sensor. Second, SPR biosensors are based on measurement of binding-induced refractive index changes and therefore effects causing refractive index changes (*e.g.* sample composition changes) can interfere with the SPR biosensing. Simultaneous measurements in sensing and reference channels (with and without appropriate biorecognition elements) make it possible to compensate for the interfering effects.

In late 1990s the concept of multichannel SPR sensors with wavelength division multiplexing (WDM) of sensing channels was conceived. In this approach, signals from multiple surface plasmons excited in different areas of the sensing surface are encoded into different regions of the spectrum of the light wave. These signals are decoded using a single-channel spectrum analyzer. This allows increasing the number of sensing channels without increasing complexity of the optical detection system.

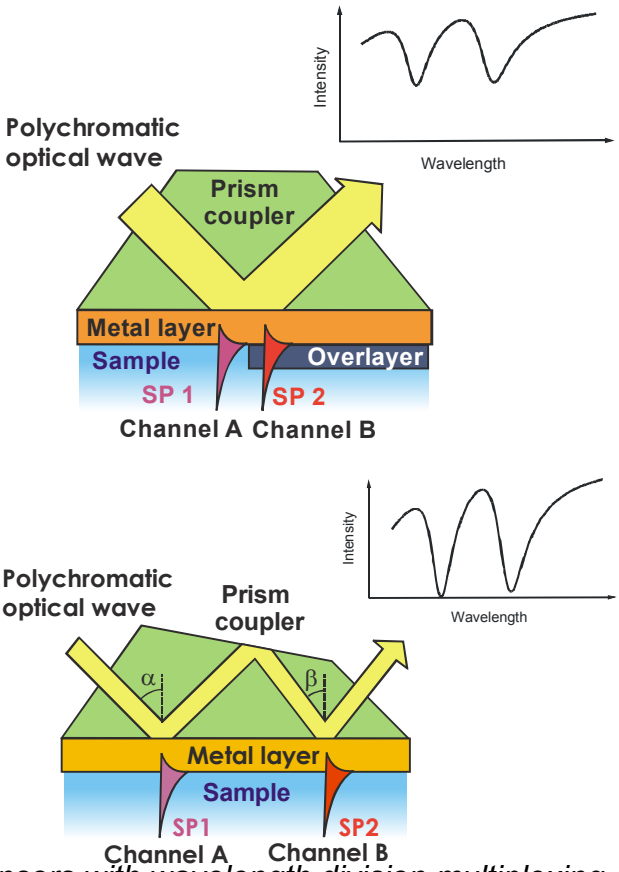

Figure 2. SPR sensors with wavelength division multiplexing (WDM) of sensing channels. WDM by means of a high refractive index overlayer (upper figure) [87]. WDM by means of a sequential excitation of surface plasmons at different angles of incidence (lower figure) [88].

Initially, the wavelength division multiplexing was achieved by using a high refractive index overlayer [87]. In this configuration, a wide parallel beam of polychromatic light is made incident onto a sensing surface consisting of a thin gold film a part of which is coated with a thin dielectric film (Figure 2). As the presence of the thin dielectric film shifts the coupling wavelength to a longer wavelength (compared to the bare gold), the reflected light exhibits two dips associated with the excitation of surface plasmons in the area with and without the overlayer [87]. Later, an alternative method has been developed in which a polychromatic light was launched into a special prism coupler and made incident on different areas of the sensing surface at different angles of incidence (Figure 2b) [88]. Due to the different angles of incidence, the surface plasmons in different regions are excited with different wavelengths of the incident light and the spectrum of transmitted light contains distinct dips associated with surface plasmons in different areas of the sensing surface. This approach was successfully combined with the parallel architecture, yielding an eight-channel SPR sensor with a resolution of 1×10^{-6} RIU [88]. Potential of this approach for simultaneous detection of multiple analytes was demonstrated [89]. In 2003 this approach to WDMSPR sensing was granted a Czech patent.

5.3. Multi-surface-plasmon spectroscopy for information-rich biosensing

The idea of a WDMSPR sensor, which introduced surface plasmons of different field profiles into a single platform and the demonstration that these surface plasmons exhibit different sensitivities to surface and bulk refractive index changes have opened possibilities for development of a new type of SPR sensors capable of providing information about the spatial distribution of the measured refractive index changes [90]. This capacity has *not* been available in the existing SPR sensors and presents an important step towards SPR biosensors for analysis of complex molecular assemblies and detection of analytes in complex samples in interfering environments [90]. In the following years, the concept of multiple-surface-plasmon spectroscopy has been implemented in several platforms. In 2006 an SPR sensor based on spectroscopy of multiple surface plasmons generated on a special multidiffractive grating was reported [91]. In this sensor, a polychromatic light beam is made incident onto a special metallic grating with a grating profile composed of multiple harmonics. The presence of these individual gratings allows simultaneous excitation of surface plasmons of different field profiles by different spectral component of the incident light. Therefore, the reflected light contains multiple SPR dips, one for each grating period. Ability of the sensor to distinguish surface and bulk refractive index changes has been demonstrated [91]. Another approach to multi-surface-plasmon spectroscopy of surface plasmons is based on exploitation of long-range and short-range surface plasmons excited by different spectral components of incident light wave on a thin metal film [92, 93]. In this configuration, the attenuated total reflection was used to excite long-range and short-range surface plasmons on a thin gold film supported by a low-refractive index buffer layer. Using this approach, sensor response to the binding of target analyte was distinguished from a refractive index change occurring on the background [93].

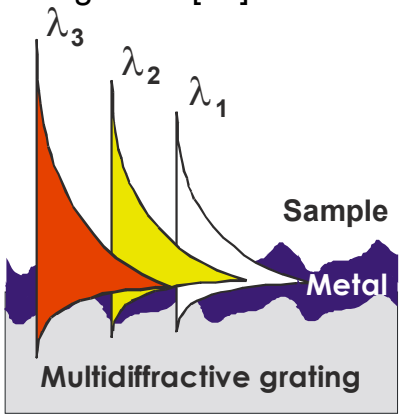

Figure 3. Simultaneous excitation of multiple surface plasmons on multidiffractive grating [91].

Most recently, the approach of multi-surface-plasmon spectroscopy has been extended to Bragg-scattered surface plasmons excited on a special metal-coated diffractive structure. In this approach surface plasmons excited on a harmonic grating are Bragg-scattered by an additional modulation of the diffractive structure with the spatial frequency equal to

two values of the propagation constant of the surface plasmon. The resulting Bragg-scattered surface plasmons (BSSPs) exhibit fields with different penetration depths into the sample and therefore exhibit unequal sensitivities to the presence of (bio)molecular films on the surface of the metal. As BSSPs exhibit an order of magnitude larger difference in the penetration depths than conventional surface plasmons excited at the same wavelengths, they allow a more accurate discrimination of surface and bulk refractive index changes. Moreover, as the BSSPs are excited at rather close wavelengths, the effect of dispersion of a biolayer is rather small, which improves accuracy of the method even further [94].

5.4. Long-range surface plasmons for high-performance SPR sensors

The use of long-range surface plasmons represents one of the most promising approaches to further improving resolution of SPR sensors. The potential of long-range surface plasmons for high-sensitivity SPR sensors was demonstrated in 2001 [95]. In this work, long-range surface plasmons were excited by means of the attenuated total reflection on an optical multilayer consisting of buffer layer (Teflon AF-1600 or MgF_2), thin metal layer (gold) and sample (aqueous medium). Sensitivity of the long-range surface plasmon resonance sensor was demonstrated to be seven times higher than that of the SPR sensor using a conventional surface plasmon. However, the demonstrated refractive index resolution (2×10^{-7} RIU) was not better than that of the SPR sensor using a conventional surface plasmon [95]. Recently, the sensing structure was further optimized and combined with a more sophisticated sensor readout optics employing a superluminescent diode as a light source and a special collimating optics. This improved system was demonstrated to be able to resolve refractive index changes as small as 3×10^{-8} RIU which is the best resolution achieved with an SPR sensor to date [96].

5.5. SPRCD approach to spectroscopy of surface plasmons

In 2006 a new approach to spectroscopy of surface plasmons was developed [97]. In this approach a collimated beam of polychromatic light is made incident on a special diffraction grating. A portion of the incident light is coupled to a surface plasmon at the metal-dielectric interface via the second order of diffraction of the grating. Simultaneously, the light diffracted into the first diffraction order is dispersed and the light components of different wavelengths are directed to different areas of a position-sensitive detector. As both the coupling of light into a surface plasmon and dispersion of light are performed on a single diffractive structure, this structure is referred to as an SPR coupler and disperser (SPRCD), Figure 4. The coupling of light into a surface plasmon results in a drop in the intensity of diffracted light, which is observed as a narrow dip in the spectrum of diffracted light. An SPR sensor based on this approach has been developed and demonstrated to be able to resolve refractive index changes as small as 3×10^{-7} RIU [97].

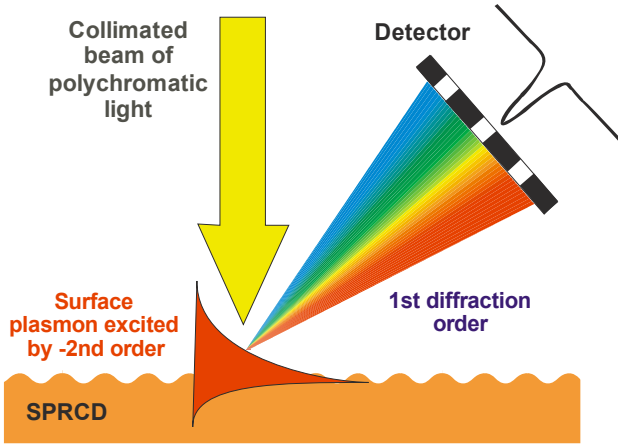

Figure 4. SPR sensor based on simultaneous excitation of surface plasmons by polychromatic light and the dispersion of light on a special grating coupler (SPRCD) [97].

6. Optical platforms for SPR sensors

6.1. Fiber optic SPR sensors

The idea of fiber-optic SPR sensor based on a single-mode fiber was conceived in early 1990s. Propagation of light in a side-polished single-mode optical fibers with a thin metal layer on a side-polished region of the fiber was studied and, based on this analysis, a fiber optic SPR sensor was designed [98]. Experimental proof of the concept was given in 1996, when a laboratory prototype of an SPR sensor based on a side-polished single-mode optical fiber was developed and it was demonstrated that changes in the refractive index of a sample in contact with the metal layer result in changes in the intensity of transmitted light [99]. Later, this geometry was reduced to a miniature SPR fiber optic probe [100] which represents the highest degree of miniaturization of SPR sensors achieved so far. In 1997 a fiber optic SPR sensor with wavelength modulation was demonstrated [101]. In order to shift the operating range of the sensor to the refractive index of media typically encountered in SPR biosensing, the design of the fiber optic sensing structure was altered and a thin high refractive index dielectric overlayer was introduced. Using a thin layer of tantalum pentoxide as a tuning layer, intensity and wavelength modulated SPR fiber optic sensors for aqueous media have been demonstrated [102]. A weakness of this SPR sensor design was that fluctuations in the polarization of light interacting with surface plasmons (*e.g.* due to deformations of optical fiber) produce fluctuations in the sensor output and therefore, polarization of light in the optical fiber needs to be precisely controlled to ensure stable output. First attempt to address this issue consisted in the use of depolarized light and wavelength modulation [103]. This fiber optic SPR sensor was demonstrated to exhibit a considerably improved resistance to the effects of fiber deformations and offered a refractive resolution of 5×10^{-7} RIU (under no deformations) and 3×10^{-5} RIU (under moderate deformations) [103]. In 2003 sensitivity of the sensor to deformations of the fiber was further reduced by the introduction of polarization-maintaining fibers, Figure 5. The SPR sensor based on a side-polished polarization-maintaining fiber was

demonstrated to exhibit a refractive index resolution of 4×10^{-6} RIU even under moderate deformations [104].

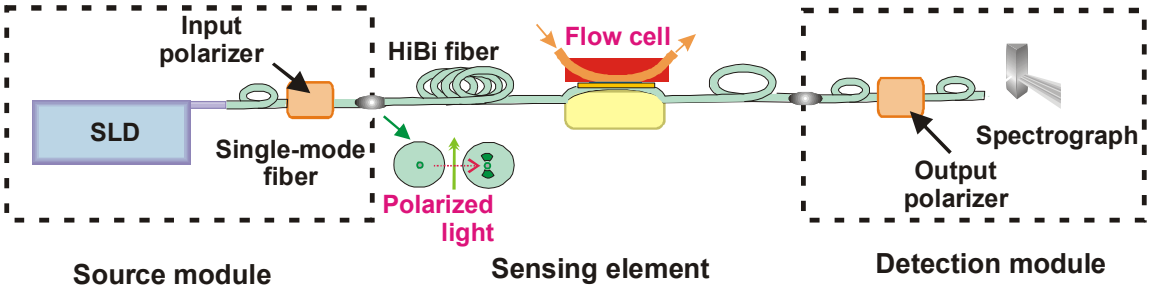

Figure 5. Wavelength-modulation fiber optic SPR sensor based on a polarization-maintaining fiber [104].

6.2. Integrated-optical SPR sensors

SPR sensors based on integrated-optical waveguides have been also investigated. Initially, theoretical analysis of propagation of light through an SPR sensor consisting of a $K^+\leftrightarrow Na^+$ ion-exchanged waveguide and gold overlayer was carried out using the modal approach and the mode expansion and propagation method. Based on the results of the theoretical analysis, an intensity-modulated integrated optical SPR sensor was designed, *Figure 6*. A laboratory prototype of the sensor was manufactured and demonstrated to be able to detect refractive index changes of 5×10^{-5} RIU [105].

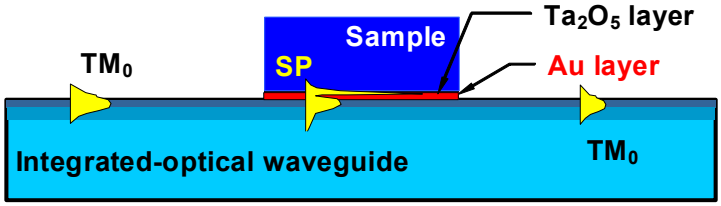

Figure 6. SPR sensor based on an integrated optical waveguide [109].

More rigorous analysis of the integrated optical SPR sensor was performed using the eigenmode propagation and matching technique [106]. In 1999 interlaboratory comparison of simulation approaches to modeling properties of integrated optical SPR sensors was performed. Rigorous approach based on a bi-directional mode expansion and propagation method demonstrated that the back-reflections in the structure are rather weak and most of the transmitted power is associated with only a limited number of modes [107]. Later, it was demonstrated that the operating range of the integrated optical SPR sensor with wavelength modulation can be controlled by a thin high refractive index overlayer [108]. Finally, in 2001 a wavelength-modulation integrated-optical SPR sensor for aqueous media was developed and its refractive index resolution was demonstrated to be 1×10^{-6} RIU [109].

6.3. SPR sensors for high throughput screening

Polarization phenomena occurring at surface plasmon resonance have been investigated and polarization control scheme for spectral surface plasmon resonance (SPR) sensors has been developed which allows observing SPR as a peak in the spectrum of the reflected light rather than a dip as common in conventional SPR sensors. This approach also allows reducing the effect of light reflected from the areas of the sensing surface where the coupling condition is not fulfilled [110].

Recently, polarization properties of SPR have been used to develop a novel high-throughput SPR sensor [111]. In this configuration, a prism coupler with a special patterned multilayer structure was placed between two crossed polarizers. Two types of SPR multilayers with opposite sensitivities to refractive index were employed. The reflected light was imaged on a CCD matrix detector and the ratio of the intensities corresponding to the two neighboring multilayers was used as a sensor output. This provides a sensor output immune to fluctuations in the intensity of the light source and dramatically improves performance of this type of SPR sensor compared to conventional SPR imaging. In addition, the output polarizer blocks all the light reflected from the (inactive) areas outside the sensing multi-layers, generating high-contrast images which are ideally suited for automated image analysis. The sensor was demonstrated to provide a refractive index resolution of 5×10^{-6} RIU in more than 100 sensing channels [111]. Recent optimization of the design resulted in the improvement of resolution down to 2×10^{-6} RIU [112].

An alternative approach to development of high-throughput screening SPR sensors is based on angular spectroscopy of surface plasmons on an array of diffraction gratings, Figure 7 [113]. In this configuration, a collimated beam of mono-chromatic light is focused with a cylindrical optics on a row of gold-coated diffraction gratings and reflected under nearly normal incidence. The angular spectra are transformed back to a collimated beam and projected onto a CCD matrix detector. Rows of gratings are read sequentially by moving the beam splitter and cylindrical optics with respect to the sensor chip. A refractive index resolution of 5×10^{-6} RIU was achieved for simultaneous measurements in 200 sensing channels [113]. Through further optimization of the sensor, refractive index resolution was improved down to 5×10^{-7} RIU for 120 sensing channels [114].

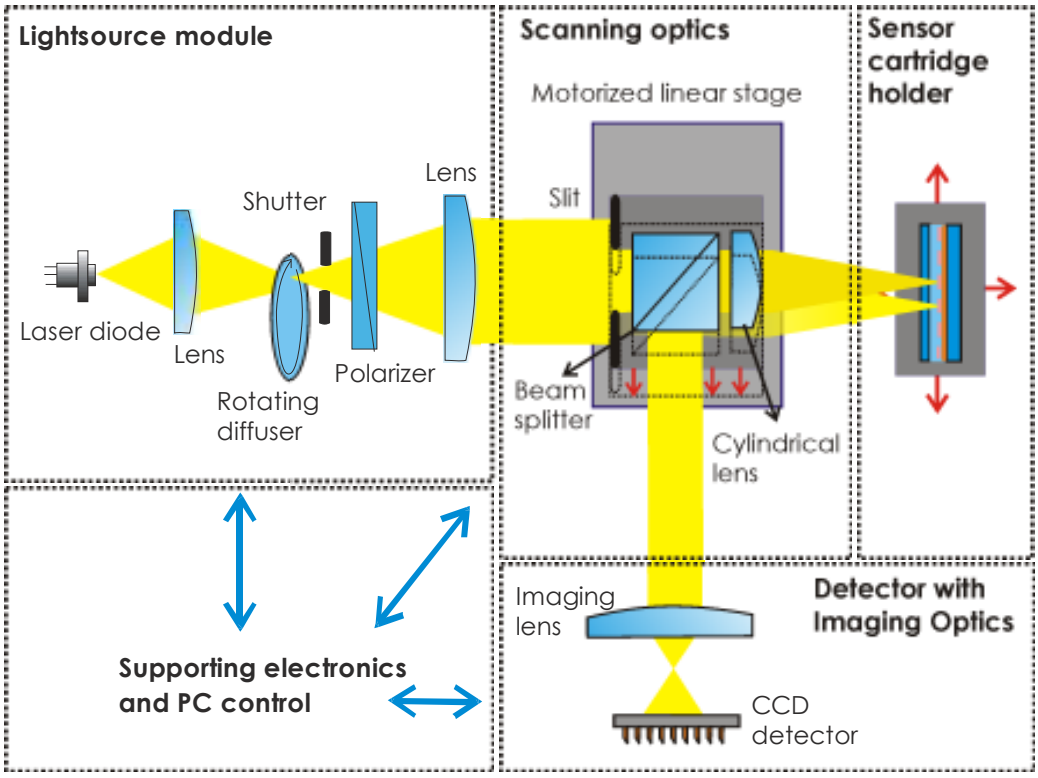

Figure 7. High-throughput SPR sensor based on angular spectroscopy of surface plasmons on an array of diffraction gratings [113].

7. SPR biosensors for specific applications

7.1. Immobilization methods for SPR biosensors

In order to provide the developed SPR sensor platforms with desired biosensing functionality, immobilization methods based on hydrophobic and electrostatic interactions [115], covalent coupling [116], and attachment of biotinylated biorecognition elements via biotin-streptavidin interaction [117] have been applied. Functionalization of multichannel SPR sensor was typically performed by the fluidic addressing in which different sensing channels were functionalized through independent fluidic channels. This approach is, however, not suitable for functionalization of SPR sensors with wavelength division multiplexing by means of a dielectric overlayer [87] and high-throughput screening SPR sensor systems [111, 113] for which alternative methods had to be developed.

For functionalization of WDMSPR sensors with adjacent sensing channels [87], an approach based on orthogonal self-assembled monolayers has been developed [118]. In this approach, the gold side of the chip is functionalized with a mixed self-assembled monolayer of polyethylene oxide (PEO) and biotin terminated alkanethiolates, whereas the tantalum pentoxide side of the chip is coated with PEO terminated silanes. The PEO terminated alkanethiolates and silanes serve as a protein resistant background, while the biotin terminated alkanethiolates are used for subsequent attachment of streptavidin and biotinylated biorecognition elements. Hence, the gold side of the chip is used for the detection of target analytes and the tantalum pentoxide side acts as a reference channel [118].

Protein contact printing has been demonstrated to be also applicable to functionalization of WDMSPR sensors with adjacent sensing channels and to provide a simple, efficient, and versatile alternative to the method based on the orthogonal self-assembled monolayers [119].

Two approaches have been researched for spatially resolved functionalization of high-throughput screening SPR sensors: DNA-directed immobilization and microspotting of biomolecules on a prefunctionalized sensing surface. The first approach takes advantage of established DNA chip technology which provides exceptionally stable pattern of single-stranded DNA (ssDNA) sequences and uses it for the immobilization of proteins. This method is based on site-directed immobilization of protein-DNA conjugates onto a mixed self assembled monolayer (SAM) composed of ssDNA alkanethiolates and oligo(ethylene glycol) (OEG) terminated alkanethiolates [120, 121]. The protein-conjugates consist of an antibody chemically linked to a ssDNA target with a sequence complementary to the surface-bound ssDNA probes and are immobilized on the surface via sequence-specific hybridization. The second approach uses a robotic microspotter to deliver biotinylated probes to streptavidin-coated surface of the sensor. The microspotting technique was used to functionalize SPR imaging sensor with polarization contrast with biotinylated oligonucleotides [112]. It was demonstrated that the optimized functionalization method based on microspotting provided even higher surface concentration of short oligonucleotide probes than the conventional flow-through functionalization method [112].

7.2. Detection of foodborne pathogens and toxins

Development of SPR biosensors for detection of foodborne pathogens and toxins have been pursued since the late 1990s and have become a subject of intensive collaboration of the researchers at the Institute of Photonics and Electronics ASCR (Prague), University of Washington (Seattle) and U.S. Food and Drug Administration (College Park).

In 2001 an SPR biosensor for detection of *Salmonella enteritidis* and *Listeria monocytogenes* was demonstrated. The sensor used prism coupling and wavelength modulation and a double layer of antibodies physisorbed on the sensing surface and crosslinked with gluteraldehyde, and was demonstrated to detect *S. enteritidis* and *L. monocytogenes* down to 10^6 cells/ml and 10^7 cells/ml, respectively [122]. Effect of various sample treatment methods on ability of SPR biosensors to detect bacterial pathogens was examined [123]. In this work the same SPR sensor platform was used and monoclonal antibody was immobilized on a mixed -COOH and -OH terminated self-assembled monolayer (SAM) of alkanethiolates via amine coupling chemistry. Detection of *E. coli* O157:H7 was performed in the sandwich detection format using a secondary polyclonal antibody. Detection limits for detergent-lysed bacteria, heat-killed bacteria and untreated bacteria were determined to be 10^4 cell/ml, 10^5 cell/ml and 10^6 cell/ml, respectively [123]. Most recently, simultaneous detection of E. coli O157:H7, C. jejuni, S. typhimurium and L. monocytogenes has been carried

out using a 4-channel SPR sensor with prism coupling and wavelength modulation [124]. Simultaneous detection of individual bacteria in the mixtures showed good agreement with detections of individual bacteria in buffer. Detections of individual bacteria and mixtures were also performed in apple juice samples. Limits of detection for these four different detection scenarios were established at 10^4 cell/ml, 5×10^4 cell/ml, 5×10^4 cell/ml and 10^4 cell/ml for E. coli O157:H7, C. jejuni, S. typhimurium and L. monocytogenes, respectively [124].

In 2002 SPR-based detection of staphylococcal enterotoxin B (SEB) in buffer and milk was demonstrated [125]. The SPR biosensor was based on prism coupling and wavelength modulation and covalent attachment of antibodies on a mixed self-assembled monolayer via activated carboxyl groups. The biosensor was demonstrated to be capable of directly detecting concentrations of SEB in buffer as low as 5 ng/ml. In sandwich detection mode, the lowest detection limit was determined to be 0.5 ng/ml for both buffer and milk samples, *Figure 8* [125]. Later, detection of SEB was also demonstrated using a fiber optic SPR sensor based on a side-polished single-mode optical fiber and wavelength modulation. For specific detection of Staphylococcal enterotoxin B, the SPR sensor was functionalized with a covalently crosslinked double-layer of antibodies against SEB. The sensor was demonstrated to be able to detect SEB in ng/ml-levels [116].

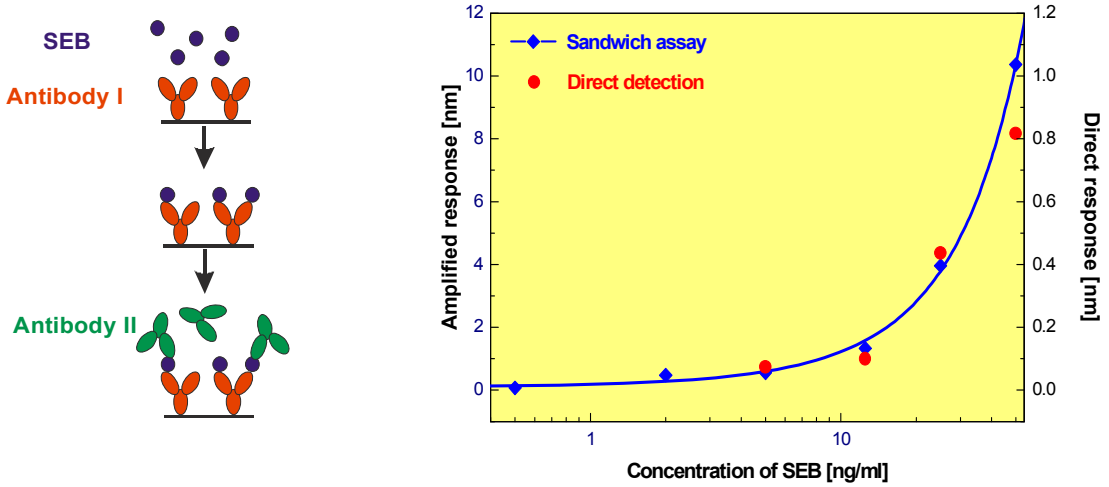

Figure 8. Detection of staphylococcal enterotoxin B using sandwich assay (left). Response of SPR biosensor to SEB as a function of SEB concentration (right) [125].

Detection of a low-molecular-weight analyte, domoic acid (DA), has been demonstrated using an SPR sensor based on prism coupling and wavelength modulation and inhibition assay [126]. DA was immobilized on a mixed SAM of oligo (ethylene glycol) (OEG)-containing alkanethiolates using amine coupling chemistry. The binding of monoclonal anti-DA antibodies onto the sensor surface was measured and the concentration of the antibody used in the inhibition assay was optimized to provide the desired detection limit and operating range. The lowest limit of detection of DA was established at 0.1 ng/ml [126].

7.3. Detection of diagnostic markers

Human chorionic gonadotropin hormone (hCG) is known to be related to abortion and preterm delivery during early pregnancy. This hormone has been a target of several SPR sensor platforms and immobilization chemistries. For instance, detection of hCG has been demonstrated using an SPR biosensor with the wavelength division multiplexing and immobilization of antibodies by means of the orthogonal self-assembling. This sensor was demonstrated to be able to detect hCG at concentrations down to 5 ng/ml [118]. Detection of hCG has been also carried out using a wavelength-modulated SPR sensor and DNA-directed antibody immobilization method which consisted of non-covalent attachment of streptavidin to a biotinylated SAM of alkanethiolates followed with the binding of biotinylated oligonucleotides to available streptavidin binding sites. Antibodies chemically modified with oligonucleotides with a complementary sequence were finally attached to this surface via DNA-hybridization. The limit of detection for hCG was determined to be 0.5 ng/ml [121].

Detection of anti-viral antibodies plays important role in medical diagnostics. An SPR biosensor for detection of antibodies against the Epstein-Barr virus (anti-EBNA) has been developed. The sensor is based on prism coupling, wavelength modulation and wavelength division multiplexing. Detection of the antibody was performed using the immunoreaction between anti-EBNA and a respective synthetic peptide, which was conjugated with bovine serum albumin (BSA-EBNA) and immobilized on the sensor surface, Figure 9. Three immobilization chemistries based on covalent coupling, electrostatic and hydrophobic interactions were evaluated for the attachment of BSA-EBNA. The developed SPR biosensor functionalized with the optimal immobilization method was demonstrated to be able to detect anti-EBNA at concentrations down to 0.2 ng/ml both in buffer and 1% human serum [127].

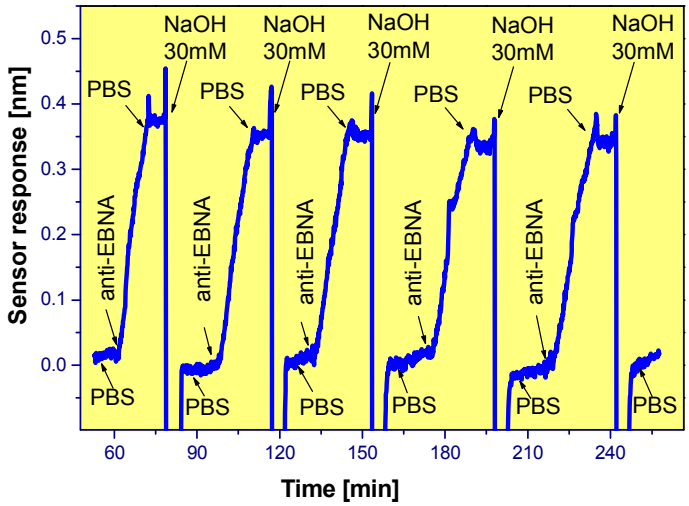

Figure 9. Detection of antibodies against the Epstein-Barr virus (anti-EBNA). Response of SPR biosensor to repeated detection of anti-EBNA [127].

7.4. Detection of nucleic acids

Sensitive detection of nucleic acids is of great interest in fields such as medical diagnostics, forensic analysis, food safety and security. Direct SPR-based detection of low concentrations of short oligonucleotides has been carried out using an 8-channel WDMSPR sensor functionalized by the covalent attachment of streptavidin to alkanethiolate self-assembled monolayer via amine coupling chemistry and spatially-controlled delivery of probes to sensor surface via microfluidics. The sensor detected complementary 23-mer oligonucleotides at concentrations down to 100 pM [117]. Recently, detection of short oligonucleotides has been performed using a high-throughput SPR sensor based on polarization contrast and excitation of surface plasmons on spatially patterned multilayers. The sensor was demonstrated to be able to monitor hybridization simultaneously in 64 independent channels with the same limit of detection [112].

7.5. Detection of endocrine disrupting compounds

Endocrine disrupting compounds (EDCs) pose a substantial environmental threat. SPR biosensor for simultaneous detection of pollutants exhibiting endocrine-disrupting activity, namely atrazine, benzo[a]pyrene, 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-nonylphenol, has been developed. The biosensor utilizes a multichannel SPR sensor based on wavelength modulation and wavelength division multiplexing of sensing channels, and inhibition detection format. In this format, a fixed concentration of antibody is mixed with a sample containing an unknown concentration of EDC. Then, the mixture is injected in the flow-cell of the SPR sensor and flowed over the sensor surface to which EDC-conjugate is immobilized and non-complexed antibodies are detected as they bind to the analyte molecules immobilized on the sensor surface. Immobilization of EDC-conjugates with bovine serum albumin and ovalbumin was performed via activated carboxylic groups on a mixed SAM of alkanethiolates assembled on the sensor surface. Limits of detection were established at 50, 70, 160 and 260 ng/ml for benzo[a] pyrene, atrazine, 2,4-D and 4-nonylphenol, respectively, Figure 10 [128].

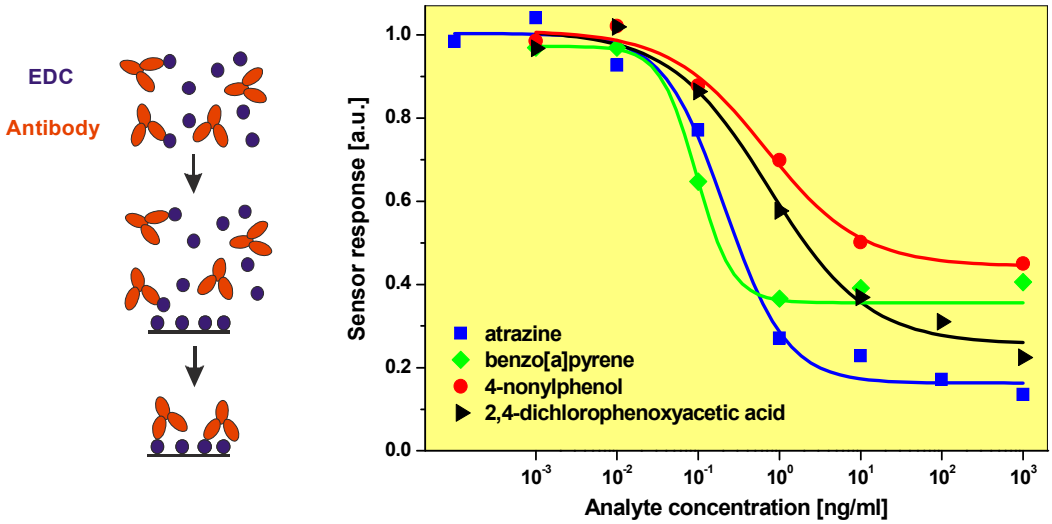

Figure 10. Detection of atrazine, 2,4-dichlorophenoxyacetic acid, benzo[a]pyrene, and 4-nonylphenol using inhibition assay (left). Response of SPR biosensor to the analytes as a function of their concentration (right) [128].

8. Summary

This Thesis covers various areas of research in the field of optical biosensors based on surface plasmon resonance (SPR), including SPR method, optical SPR sensor platforms, functionalization of SPR sensors, and application of SPR biosensors for detection of chemical and biological species. In the research of SPR method and optical platforms of SPR sensors, which are key areas of author's research, several important achievements have been made.

- A *comprehensive theory of performance characteristics of SPR (bio)sensors* has been developed. This theory connects main performance characteristics (sensitivity and resolution) of an SPR sensor with its design parameters, and thus facilitates design and optimization of SPR biosensors.
- *SPR sensors* have been, for the first time, combined *with the wavelength division multiplexing* method to provide a new generation of multichannel SPR sensors with potential for development of compact analytical systems for field use.
- *Spectroscopy of multiple surface plasmons* has been developed and established as a tool which makes it possible to gain more complex information about the analyzed processes and discriminate different sources of contribution to the response of an SPR sensor.
- *Novel method of spectroscopy of surface plasmons* based on proprietary SPR coupler and disperser (SPRCD) technology has been invented. This approach holds great potential for development of simple, compact, yet high-performance spectroscopic SPR sensing devices.
- *Sensors based on spectroscopy of long-range surface plasmons* have been realized and demonstrated to provide performance which is superior to that of SPR sensors using conventional surface plasmons.
- *Novel fiber optic SPR sensor based on a single-mode optical fiber* has been developed. This sensor belongs to miniaturized SPR sensors with the best performance and offers mechanical stability superior to other fiber optic SPR sensors.
- Original *high-throughput SPR sensor platforms* have been developed which bring benefits of high-performance SPR sensing to highly parallelized detection formats.

Original optical SPR sensor platforms have been combined with the state-of-art immobilization chemistries and biorecognition elements for detection of selected chemical and biological species.

- SPR biosensor-based *detection of bacterial pathogens Salmonella enteritidis and Listeria monocytogenes* has been demonstrated. This has been one of very first works reporting successful direct detection of bacterial pathogens using SPR.
- *Detection of antibodies against the Epstein-Barr virus* by means of SPR biosensor technology has been reported for the first time.
- The developed SPR biosensor for *detection of staphylococcal enterotoxin B* (SEB) represents one of the most sensitive SPR

biosensors for SEB demonstrated so far and one of successful examples of application of SPR biosensors in complex food matrices.

9. Conclusions

Surface plasmon resonance (SPR) biosensors represent one of the most advanced label-free biophotonic sensing technologies. SPR biosensors have become a mainstay of both life science and pharmaceutical research and hold potential for a wide range of bioanalytical tasks in sectors such as medical diagnostics, environmental monitoring, food safety, and security.

The Thesis covers more than a decade of systematic research into SPR sensors and reports research and development efforts on several fronts of SPR (bio)sensor science and technology. These include advances in theory of SPR sensors, novel approaches to SPR method and SPR platforms, functionalization methods, and applications of SPR biosensors. The novel approaches to SPR method and SPR platforms have given rise to SPR sensors with rich information content (sensors based on spectroscopy of multiple surface plasmons), improved performance (sensors based on spectroscopy of long-range surface plasmons), high throughput (sensors based on spectroscopy of surface plasmons on an array of diffraction gratings and SPR imaging on multilayer structure in polarization contrast), remote sensing capability (fiber optic SPR sensors), and portability (SPR sensors with wavelength division multiplexing of sensing channels, sensors based on simultaneous coupling and dispersing of light coupled to a surface plasmon - SPRCD). SPR sensor platforms have been also combined with appropriate immobilization chemistries and biorecognition elements and applied to detection of chemical and biological analytes, such as foodborne pathogens and toxins, hormones, antibodies, nucleic acids, and endocrine disrupting compounds. Small and medium-size analytes have been detected at sub-ng/ml levels, illustrating the potential of SPR biosensor technology for development of rapid and sensitive detection devices.

It is envisioned that the developments reported in this Thesis will increase utility of SPR biosensors in traditional applications, open new application areas and contribute to shaping vision for SPR instrumentation of future.

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List of publications constituting the Thesis (in the order they appear in the Thesis)

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