2. BIOSENSOR SYMPOSIUM TÜBINGEN 2001

Biosensors based on SPR Imaging

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Abstract

Surface plasmon resonance (SPR) belongs to the most sensitive detection methods and has been used for more than 20 years to study a variety of chemical and biochemical sensor developments. Now, with the recent introduction of biochips, SPR as a label-free technology for monitoring biomolecular interactions is a promising technique for rapid and parallel detection. SPR imaging using fast optical array detectors permits simultaneous measurements across an array of immobilized molecules. SPR imaging provides excellent spatial resolution at the same sensitivity as classical SPR. These features make SPR imaging a promising detection technology for biochips.

Introduction

Established transducer systems for biosensors such as electrodes or mass sensitive systems are increasingly complemented by optical methods. The interest in using optical transducers arise from their potential for parallel detection – often referred as array or imaging detection.

The development of biochips and high-throughput screening methods require both fast and highly sensitive imaging transducers. The majority of current optical transducers is based on fluorescence. Because most biomolecules exhibit only weak fluorescence, they have to be labeled by fluorescence markers in order to achieve the required sensitivity. Various problems related to labeling may be overcome by imaging transducers based on label-free detection [1].

SPR imaging is becoming increasingly interesting due to its potential for measuring interactions between unlabeled analyte molecules with species bound on the surface array [2]. Moreover, SPR allows the monitoring of thin film formation under variety of in-situ conditions [3,4]. In this review, we present a short introduction to SPR and SPR imaging, instrumentation required and new applications for biochips.

Surface Plasmon Resonance

SPR is an optical technique for the measurement of the intensity of light reflected at a dielectric covered by a metal layer of app. 50 nm thickness. The term “surface plasmon” is used to describe the charge-density propagating along the interface of the thin metal layer and the dielectric. Excitation of surface plasmons requires coupling devices, prism coupler or grating coupler. The typically wavelength range for excitation is between 630 and 1200 nm. Excitation schemes have been developed by Kretschmann [5,6] and Otto [7]. In the Kretschmann configuration, the optical substrate (prism) is coated with a thin metal layer. In the Otto configuration, a narrow dielectric layer is placed between the metal and the prism. Usually, gold or silver are the metals of choice. The wave vector of the surface plasmons is determined by properties of the prism, the metal, and the surrounding dielectric medium. Under appropriate conditions, the free electrons can come in resonance with light. When the angle of incident light or the wavelength reaches an appropriate value, the reflection decreases sharply to a minimum, corresponding to the resonance of surface plasmons. According to Maxwell’s law, changes in dielectric properties, e.g. thickness or refractive index, of the surrounding medium lead to changes in the wave vector and as a consequence there is a shift of plasmon resonance minimum of the reflected light. Because the plasmon resonance is extremely sensitive to dielectric properties and the fact that resonance occurs only in a small range (either wavelength or angle of incidence) the adsorption of molecules can be accurately detected. Only p-polarized light in plane of incidence with the electric field vector oscillating perpendicular to the plane of the metal film, is able couple to the plasmon mode. S-polarized light, with its electric field vector oriented parallel to the metal film, does not excite plasmons. Since s-polarized light is reflected by the metal surface, it can conveniently be used as a reference signal to improve the sensitivity.

Basically, two approaches have been used in SPR sensing. For chemical and biochemical sensing, the measurements of resonance angle has been most widely used. Measurement of the resonant wavelength induced by plasmon resonance has been often applied by using optical fibers as SPR sensor [8].

SPR Imaging

Coupling a microscope with an conventional SPR set-up has led to the collection of spatially resolved information based on a point-by-point rastering across the sample. The so called SPR-multi-channel measurement is used to study a small samples array (e.g. 4 x 4) of attached biomolecules [9]. In this way, the instrumentation being used is not suitable for collecting SPR images with more than 50 pixels (spatial points). Large acquisition time, which is typically 1 sec

per pixel, along with the instrumentation for sample position and systematic movement, restrict images with higher spatial resolution.

SPR imaging using CCD camera provides a method for overcoming this limitation. Knoll and Rothenhäusler from Max Planck Institute for Polymere Research in Mainz (Germany), were the first to work on SPR imaging. They call this approach SPR microscopy [10]. Fig. 1 shows the SPR imaging set-up in Kretschmann geometry.

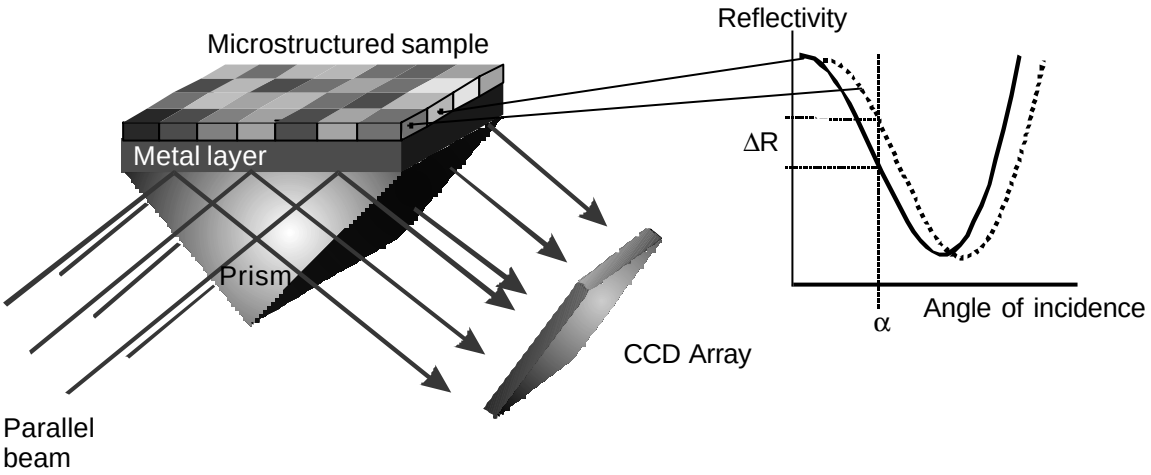

Fig. 1 Schematic of SPR imaging. A parallel beam is incident on a prism near the SPR resonance angle. The reflected light is detected by a CCD Array. Contrast in SPR image arises from variation in the reflected light intensity ? R.

The parallel beam of monochromatic light with a certain angle of incidence is coupled into surface plasmons. The reflected light is focused by a simple glass lens and examined by means of a CCD camera. Finally, a polarizer permits measurements both with p-polarized and s-polarized light, the latter being used as reference. In conventional SPR, usually the angle of incidence is observed, at which most energy is transferred to the surface plasmons. For a given sample arrangement and a given wavelength of the light, the resonance coupling appears as sharp minimum in the angular distribution of the reflected light. The minimum shifts upon slightest changes of refractive index or layer thickness of the sample. If the dielectric medium in contact to the metal layer is patterned, the resonance angles will be different for different areas of the metal film, or in other words: the reflected intensity keeping this angle constant will be changed. This feature forms the contrast mechanism in SPR imaging, showing in fig. 1.

Real optical surfaces are not perfectly smooth. Due to this surface roughness, the plasmons can couple with photons on the sample side of a metal film, and scattered light can emerge on the sample side of the imperfect metal film. If the metal film is covered by a microstructured analyte layer, the intensity of the scattered light depends both on the roughness of the metal surface and on the analyte properties. For this reason, both the light scattered to the air side and the light reflected into the glass prism can be used for imaging purposes [10]. However, reflected light is preferred in general because it enables in-situ measurements.

To date, several commercial SPR systems are available. The BIAcore systems [a] is one of the most used commercial SPR device. Although the market is dominated by the BIAcore systems, some new SPR instruments, often aimed at specific segments of the market, are being introduced as well [b,c]. No commercial SPR imaging instrument is currently offered.

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Data processing and quantification

Usually, a CCD camera is connected with a frame grabber and a computer for reading and displaying images. The basic operations like grey scaling, smoothing, gamma correction, contrast enhancement and peak height ratios are relatively simple. In many cases these procedures are adequate for quantitative analysis. Performing a reference measurement with s-polarized light is often used to improve the contrast and to eliminate artifacts. In addition, noise determines the accuracy of measurements. In practice, the most used approach is to increase the intensity of the light source or the frame rate (time integration) of the camera and the number of co-added frames. Both frame rates and co-added frames determine the total collecting time of the array. Very low frame rates may lead to pixel saturation even at low illumination intensity because the intensity is integrated.

For better quantitative analysis, Fresnel calculations has to be used [11]. In these calculations, small changes in refraction index and thickness of the molecular layer can be used for determination of interaction between molecules on the metal surface. To obtain a significant improvement of the quantitative results, a five-phase Fresnel calculation may be applied. Fig. 2 schematically shows the arrangement of five optical phases.

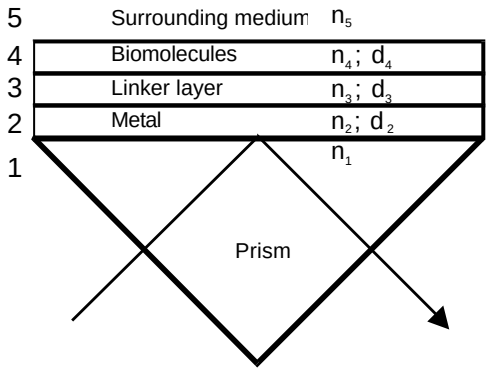

Fig. 2 Five-phase model for Fresnel calculations. Each phase is determined by its refractive index (n). In addition, three layers are also denoted by the thickness d.

It is quite difficult to exactly determine the refractive index and the thickness of layer of biomolecules (4). However, the proportional relation between refractive index and number of attached molecules is a well suited assumption for modeling [12]. Further developments also involve patterned recognition methods to distinguish between specific and non-specific adsorptions.

Methods for enhancement of sensitivity at high spatial resolution

Several methods for contrast enhancement are known in conventional microscopy. The simplest seems to be the dark-field technique which can also be used for SPR imaging [13]. A dark-field image is obtained when a small diaphragm is placed at the axial focal point of the first lens that collects the reflected light as shown in fig. 3. The diaphragm eliminates the zero order diffraction maximum, e.g. the specularly reflected light. As a result, the contrast of the SPR imaging is enhanced. Fig. 4 shows both a conventional and a dark-field SPR image of a silicon test pattern of 5 nm thickness. In case of dark-field method, a pronounced improvement of contrast can be seen. [14]

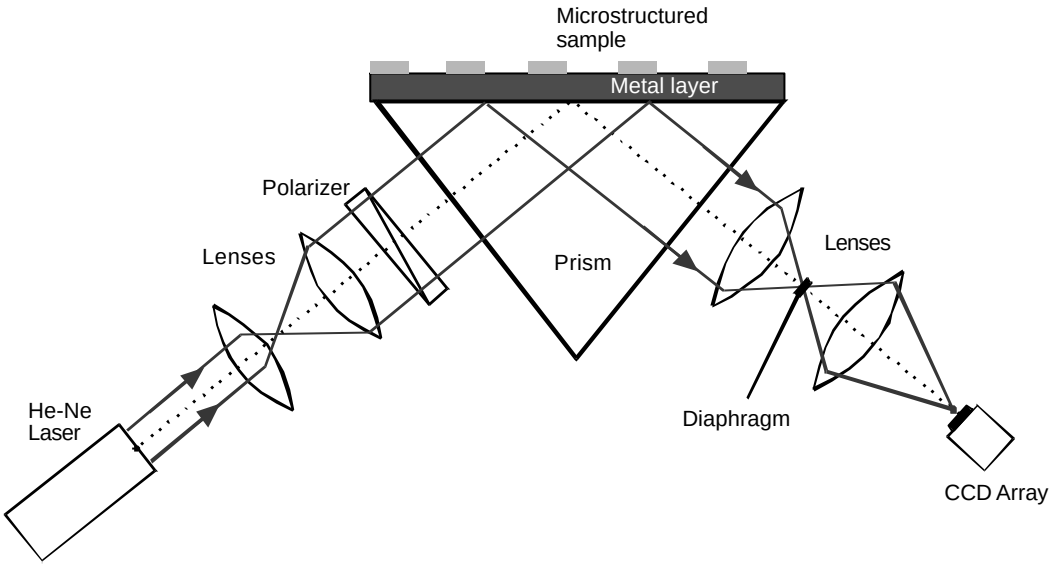

Fig. 3 Schematic representation of the set-up for SPR dark-field imaging. The diaphragm at the axial focus point will block the zero order diffraction maximum.

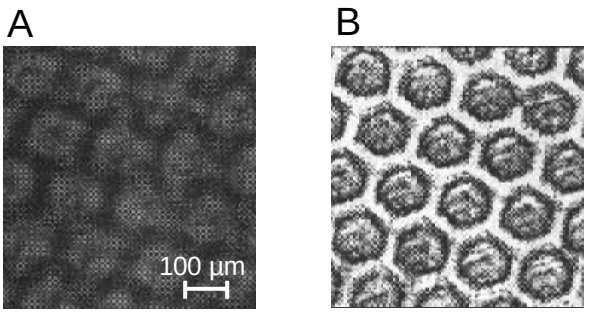

Fig. 4 SPR images (A) acquired without diaphragm and (B) acquired with diaphragm. Both images show exactly the same sample position.

Another method is based on SPR interferometry (SPRI) [15]. When plasmon excitation is maximal, the phase of the reflected light changes abruptly. This jump-like behavior of the phase is used for sensing with ultra-high sensitivity. Fig. 5 illustrates the detection principle of imaging technique by using a Mach-Zehnder interferometer. SPRI can also be performed with a standard

ellipsometry instrumentation. Fig. 6. demonstrates the enhanced sensitivity of SPRI imaging. Image A was obtained with the Mach-Zehnder interferometer while in the image (B) the reference beam was inhibited (conventional SPR imaging). Only the SPRI image displays the edge of a silicon film [14].

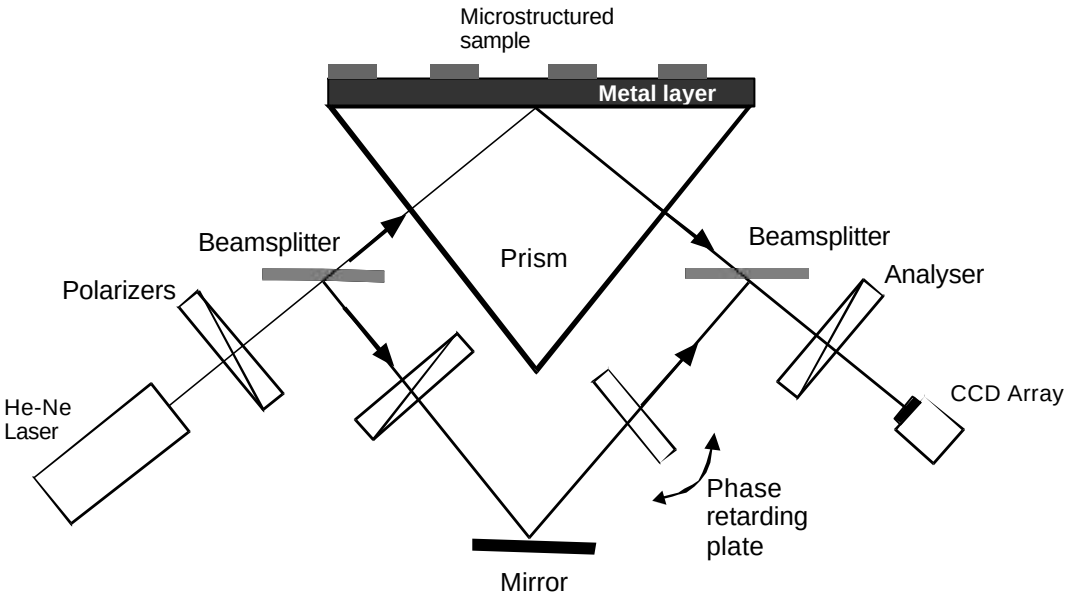

Fig. 5 A simplified schematic of SPR interferometry imaging.

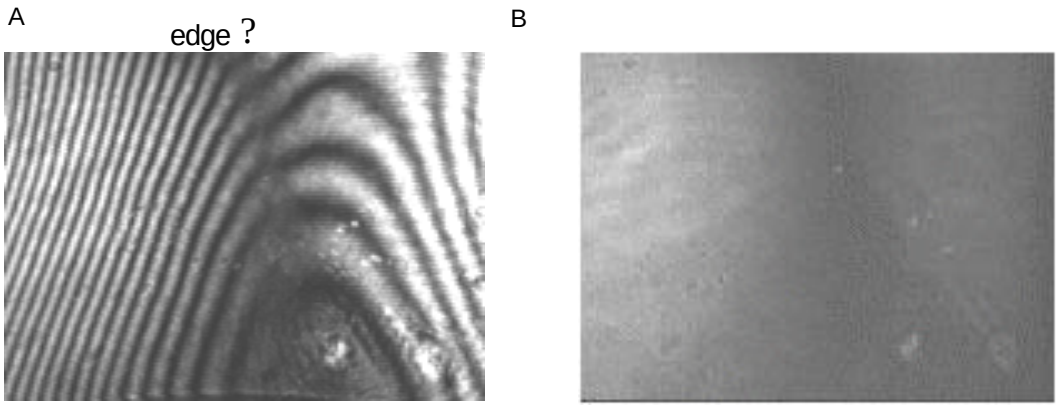

Fig. 6 SPR images of silicon layer of 0.5 nm thickness. (A) SPR interferometry image and (B) conventional image at the same sample area.

Applications

Applications of SPR imaging can be divided into two large areas: biosensing and analysis of thin films [13]. Here we discuss applications in biosensing only.

SPR has been used to study a variety of processes, including changes in conformation of proteins [16], interaction between antibody/antigen [8,17], enzyme/substrate, DNA/RNA [14,18], DNA/protein [19] and protein/protein [20]. SPR turned out to have a high sensitivity in detecting small amounts of unlabeled biomolecules. Thus, SPR imaging has an attractive format as optical transducer for DNA or protein microarrays [21]. Thin microstructured polymer films are often used as patterned and linker layers for biomolecules. Hence, one of the first applications of SPR imaging was the investigation of thin polymer films on metal surfaces [22]. The formation of self assembly monolayers (SAM) on gold and silicon surfaces has been one of the most successful chemistry for modification of surface in order to immobilize complex biomolecules for biosensing. The specific adsorption of biological target molecules onto a metal surface can occur either on a homogeneous SAM or on a patterned layer. In both cases the quality of the SAM has to be probed. Often, a two- or more-step surface modification is required for the attachment of

biomolecules which employs the surface modification with SAM, followed by the covalent attachment of various synthetic polypeptides or amine. Fig. 7 shows the SPR image of condensed ?-benzyl-L-glutamate-N-carboxyanhydride onto a hexagonal pattern SAM of alkanethiols onto gold [22]. The size of the hexagons is 70 µm across.

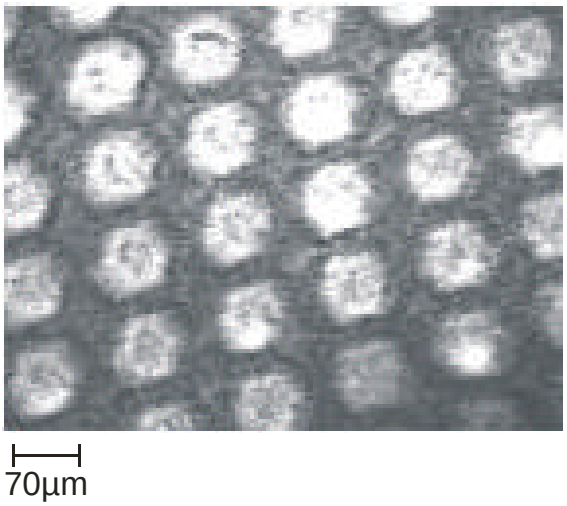

Fig. 7 SPR image of a ?-benzyl-L-glutamate-N-carboxyanhydride on a patterned thiol SAM.

Researchers at the University of Wisconsin have done some of the early work to apply SPR imaging for detection of biomolecular interactions [d]. The adsorption of biotinylated oligonucleotides, single or double stranded DNA, proteins and polypeptides were probed with SPR imaging [23-25]. The detection limit of unlabeled RNA and DNA oligonucleotides is as low as 10 nM [14]. Because of the ability of SPR to monitor biomolecular interactions of wavelengths > 1100 nm [26,27] the characterization of biochips on silicon becomes interesting. All these results demonstrate that SPR imaging is well suited to follow in-situ adsorption of biomolecules onto a patterned array and to quantify adsorption information as well as to study any sample-to-sample variations.

Conclusion

The ability of SPR imaging to characterize the formation of patterned arrays as a basis for the attachment of unlabeled biomolecules and to detect interactions between biomolecules makes this technique to a sensitive and versatile optical transducer for biochips. SPR imaging has a high potential in parallel or multi-channel biosensing and is capable of real time monitoring of adsorption and hybridization kinetics. For biochemical and medical applications, these advantages will support the development of biochips with label free detection. In future, the SPR imaging techniques may find applications for medical diagnostics.

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[b] http://www.ti.com

[c] http://gwcinstruments.com

[d] http://corninfo.chem.wisc.edu

Acknowledgment

The authors gratefully thank the Deutsche Forschungsgemeinschaft (DFG) for financial support within the “Sonderforschungsbereich Reaktive Polymere” (SFB 287) and acknowledge financial support by the “Fonds der Chemischen Industrie”. We thank Dr. Valdas Sablinskas (Vilnius University, Lithuania) for his support in performing SPR imaging.