1. Introduction

1.1 Biosensors

The combination of optical, electrochemical, piezoelectric, thermal and other physicochemical instrumental techniques with the specificity of a biological recognition system has resulted in a variety of new analytical devices known as biosensors. Biosensors are under intensive development worldwide because they have many potential applications, e.g. in the fields of clinical diagnostics[1], food analysis[2], environmental monitoring[3] and process control of industrial processes[4].

Biosensors are devices that transform biochemical information into an analytically useful signal. Three structural parts are essential, a recognition system, a detector element and a transducer that associates the two other components (Figure 1.1.1). The recognition site is usually a biological material, e.g. tissue, cell receptor, enzyme, antibody, protein or nucleic acid. The main function of the recognition system is to be highly *selective* for the analyte to be measured. The detector element measures physicochemical properties, such as small optical, piezoelectric electrochemical, thermometric, or magnetic changes. In contrast to the recognition system, the main purpose of the detector element is to offer a high *sensitivity*.

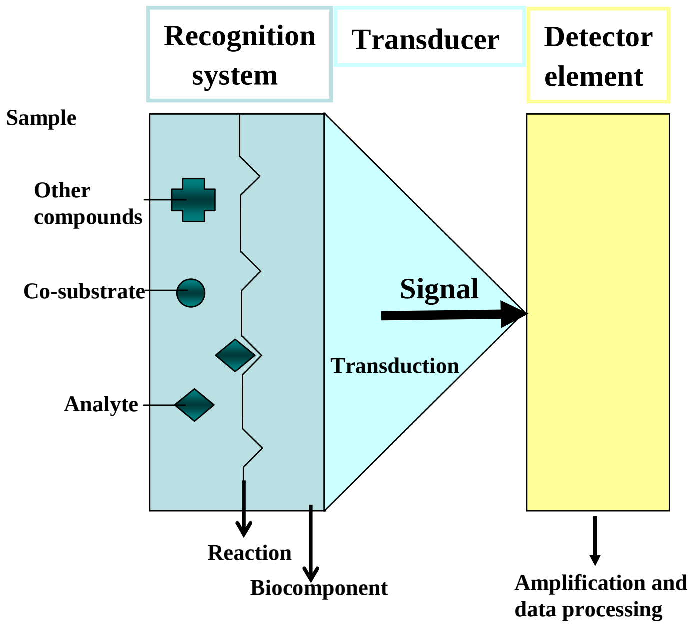

Figure 1.1.1: Principle of a biosensor.

Surface plasmon resonance (SPR) spectroscopy has become a routine technique in optical biosensor applications, where interactions between an analyte in solution and a biomolecular recognition element immobilized on the surface are probed[5, 6]. In 1990, the company Biacore introduced the first commercial SPR biosensing instrument where surface plasmon resonances are excited in a dense gold film and used to probe small changes in refractive index at the gold surface[7].

Much effort is spent on the development of more sensitive sensor platforms. One strategy for amplifying the sensitivity is to increase the amount of analyte binding sites. This can be realized by an enhancement of the sensor surface area. Subsequently, Biacore modified the flat gold film by attaching a three dimensional dextran hydrogel matrix, that allows high loading of analytes[8].

Other approaches try to influence the evanescent field of the plasmon wave to gain further sensitivity. The so-called long range surface plasmon, excited at the two sides of a metal layer in contact with two identical dielectric media[9], promises high resolution, as the field intensity at the interface is higher than in case of conventional SPR and the decay length of the evanescent field can be in the extended range of 400 - 800 nm[10]. Another approach to enhance the sensitivity is the use of localized surface plasmon phenomena[11, 12] for the detection of small molecules[13]. Novel fabrication methods for plasmonic materials are developed[14, 15].

1.2 Aim of the study

This study is divided into three parts. In the first part, nanoporous gold, as a new plasmonic material, is investigated in detail (chapter 4). In the second part, plasmonic features of gold/silica composite inverse opals are studied (chapter 5). While parts one and two mainly focus on fundamental research by looking into the properties of novel substrate materials in order to provide a basis for new optical biosensors, part three addresses an application of biosensing (chapter 6).

Generally, nanoporous gold, as a rough, but continuous gold membrane, shows features of both planar metal films that exhibit propagating-SPR (p-SPR) and nanostructured metal materials that exhibit localized-SPR (l-SPR), two kinds of optical excitations used in state-of-the-art optical sensing technologies. Therefore, nanoporous gold is an interesting substrate that can be incorporated into the recognition system of improved biosensors. Detailed analyses of the nanoporous gold are described in chapter 4.

In chapter 5, silica inverse opals are used as a substrate to host gold nanoparticles in order to investigate the optical features that may be created as a combinatory result of both the ordered macropores and the l-SPR from the nano metallic particles.

The fundamental question addressed in chapter 6 is the development of a binding assay to probe the protein/protein interaction of the calcium binding protein centrin with the heterotrimeric G-protein transducin. Therefore, a commonly evaporated, flat/dense gold film was used to support a propagating surface plasmon mode.

1.3 References

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2. Methods for surface characterization

2.1 Theoretical Background – Surface Plasmon Resonance (SPR) Spectroscopy

During the last 15 years surface plasmon resonance (SPR) spectroscopy has become a popular technique for optical immunosensor applications[1]. In general, it is a method for measuring the refractive index of very thin (order of nm)[2] layers of material adsorbed on metals. A change in the refractive index can be detected close to the metal, typically within a distance of 200 - 300 nm to the sensor surface[3]. Material adsorption can be converted into mass and thickness with knowledge of the respective refractive indices. The following chapter outlines the principles of SPR.

2.1.1 Excitation of Propagating Surface Plasmons (p-SPR)

2.1.1.1 Optical properties of materials

Plasmons are collective oscillations (non-radiative) of free electron gas at optical frequencies. Surface plasmons are confined to surfaces and occur at the interface of a material with a positive dielectric constant ($\varepsilon_{dielectric}$) and a material of a negative dielectric constant (ε_{metal}). Surface plasmons can be excited on metallic surfaces under certain conditions (depending on material, incident angle, polarization and wavelength of the incident light). The conditions depend on the dielectric constants (≅ refractive indices) of the metal, dielectric and dielectric adsorbate layers, and are consequently reliant on the excitation wavelength. Equations 2.1 and 2.2 describe the relationship between the dielectric constant and refractive index.

$$\varepsilon' = n^2 - k^2 \qquad \text{--- 2.1}$$

$$\varepsilon'' = 2nk \qquad \text{--- 2.2}$$

Optical properties of materials can be described by the refractive index, n which describes the real part and the absorption coefficient, k which describes the imaginary part. Alternatively the dielectric constants ε' (real part) and ε'' (imaginary part) are used.

The electromagnetic field of the surface plasmon decays exponentially into both the dielectric and the metal, with the highest intensity at the interface. The plasmonic dispersion relation (between angular frequency ω and wave vector k) reveals that the longer the excitation wavelength λ nm (smaller ω_L, since $\omega = 2\pi(c/\lambda)$), the smaller the k vector needed to match the plasmon excitation condition (cf. Figure 2.1.1).

The k_{ph} of the incident light (also called momentum) can be tuned/magnified using either prism or grating coupling, so that photons are not coupled directly to the metal/dielectric interface, but via high-index prism (with $\varepsilon_{prism} > \varepsilon_{dielectric}$)[3]. The wavevector k_{SP} is described by $k_{SP} = (2\pi/\lambda)\cdot n_{prism}\cdot \sin\theta_{PSP}$.

$$K_{SP} = \frac{\omega}{c}\sqrt{\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}} \geq K_{Ph} = \frac{\omega}{c}\sqrt{\varepsilon_d} \qquad \textbf{--- 2.3}$$

Plasmonic dispersion relation: At any angle θ $|k_{sp}|$ (k surface plasmon) is larger than $|k_{ph}|$ (k photon, air).

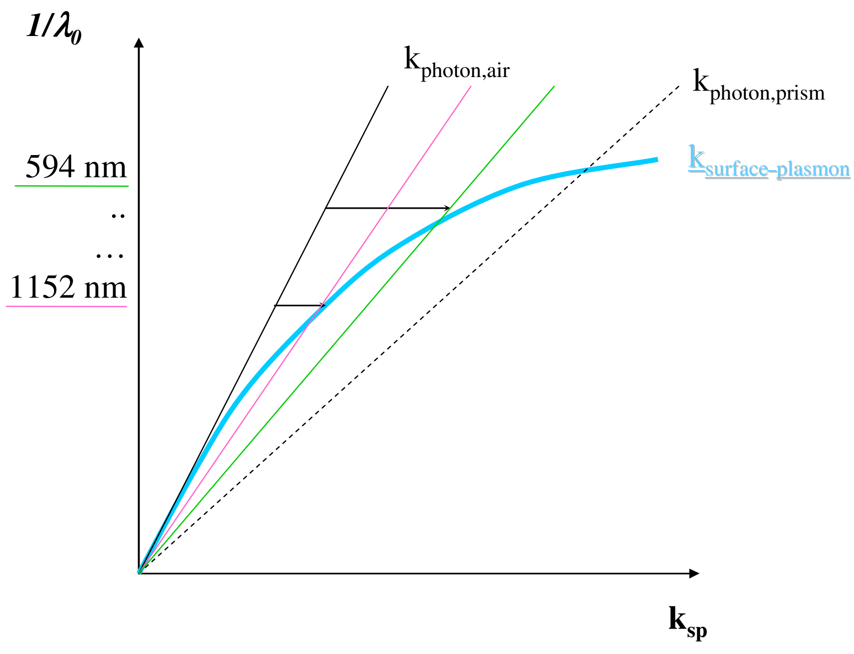

Figure 2.1.1: Dispersion relation demonstrates the enhancement of k using a high index prism, e.g. LaSFN9. There is no intersection for $k_{photon,air}$ and $k_{surface\ plasmon}$, while $k_{photon,prism}$ intersects $k_{surface\ plasmon}$. It also becomes apparent that the light coupling needs smaller k for longer excitation wavelength.

When the energy <u>and</u> the momentum of the photon are just right, it interacts with the free electrons of the metal. The incident p-light (transversal magnetic, TM- or p-polarized) photons are absorbed and converted into surface plasmons.

2.1.1.2 Prism coupling

Different configurations of SPR devices are capable of generating and measuring propagating surface plasmons: devices that use prism coupling[4, 5] or grating coupling[6]. Prism based SPR was firstly described in 1959 by Turbadar[7, 8]. One decade later, prism coupling was further developed and split in two coupling variations, known as the Otto[5] and the Kretschmann/ Raether configurations[4]. In this thesis, only prism coupling in the Kretschmann/Raether configuration was used, in this arrangement the photons travel through a high index prism and couple through a gold film, that is in contact with the dielectric medium (Figure 2.1.2).

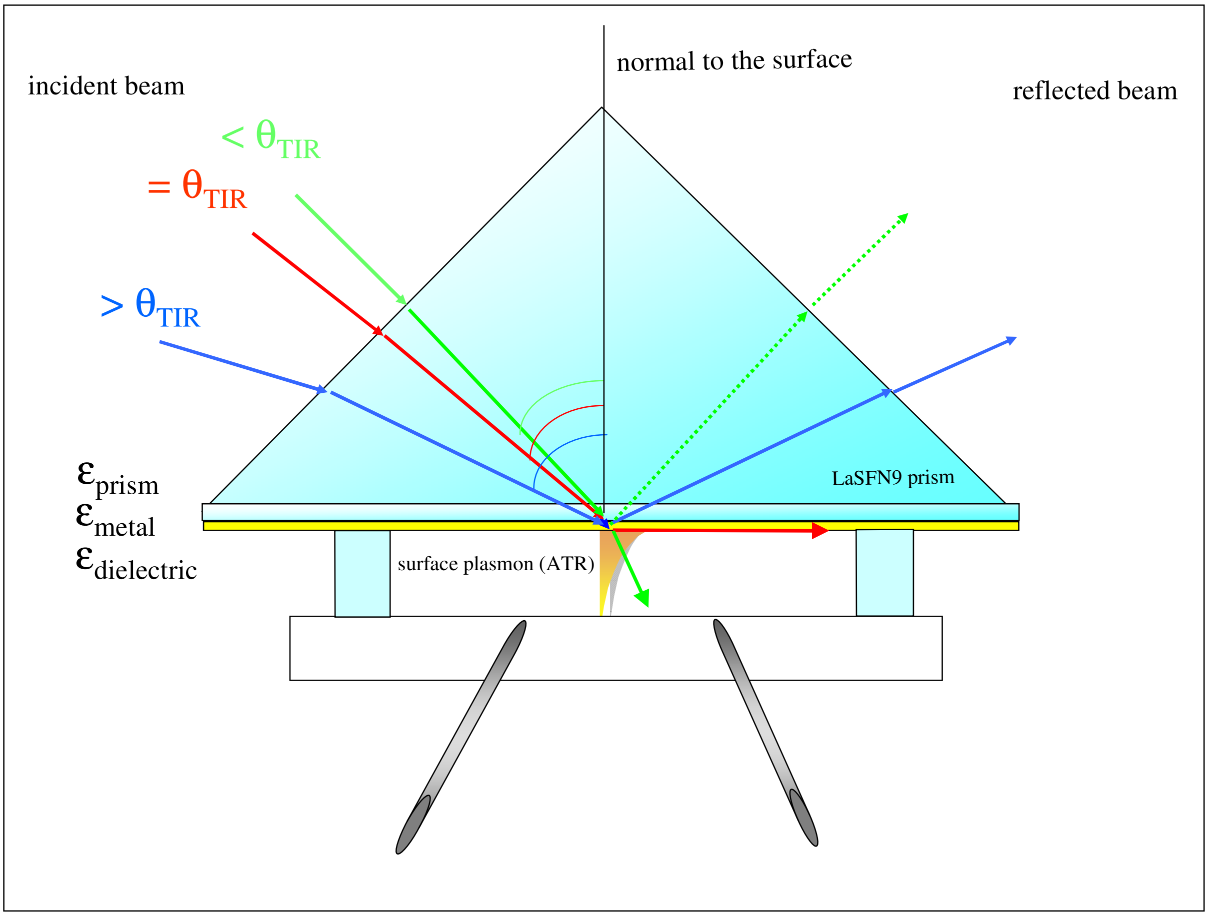

Figure 2.1.2: Prism coupling in the Kretschmann/Raether configuration was used in this thesis to excite propagating surface plasmon resonances. Both the angle of incidence and the angle of reflection are defined as the angle between the corresponding light beam and the normal to the surface, so the reflected beam is detected at 2θ.

The incident laser beam passes into the prism and is reflected, partially transmitted or absorbed at the base of the prism (Figure 2.1.2). Below θ_{TIR} (angle of the total internal reflection) most of the light is transmitted (green line). At θ_{TIR} the transmitted light propagates parallel to the surface (red line). If the angle of incidence is greater than θ_{TIR}, no light is transmitted. The surface plasmon occurs at higher angle θ dependent on the properties of the materials (gold film: thickness, ε`, ε``,…).

2.1.1.3 SPR signal

The SPR signal can be derived by monitoring the intensity of the reflected light as a function of the incident angle[7, 8]:

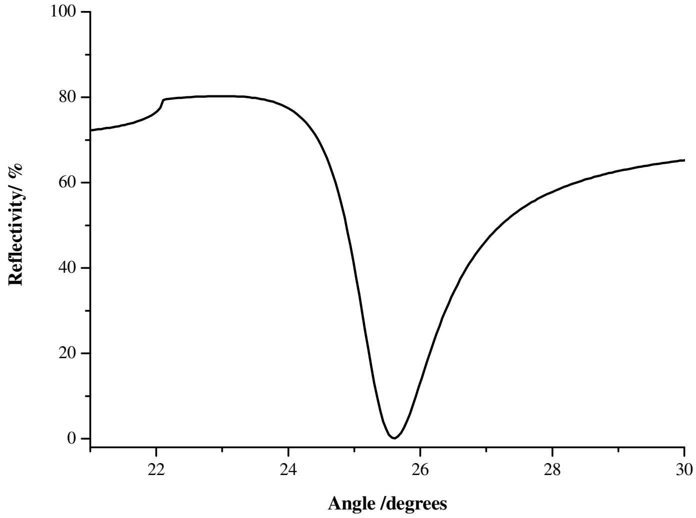

Figure 2.1.3: Simulated surface plasmon resonance signal (Winspall, version 2.20): triangular prism, 50 nm Au film, air; 632.8 nm excitation in p-polarization.

Due to absorption, the plasmon phenomenon is also called the *attenuated* total internal reflection (minimum of reflectivity). The thickness of the gold film, as well as the excitation wavelength influences the coupling angle and the coupling efficiency.

2.1.1.4 Influence of the excitation wavelength to the SPR signal

The SPR signal is strongly influenced by the excitation wavelength. With larger wavelengths the resonances become narrower and the angle of total internal reflection increases:

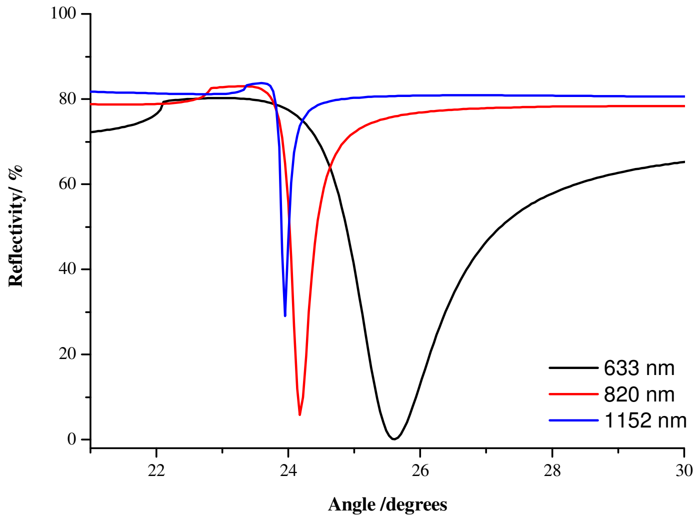

Figure 2.1.4: Simulated surface plasmon resonance signals (Winspall, version 2.20) demonstrate the influence of the excitation wavelength for three different wavelengths. All other parameter stayed the same as in Figure 2.1.3.