Applications of surface plasmon resonance in biomedicine

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Abstract. As one of optical technique, surface plasmon resonance (SPR) shows a superb interdisciplinary usage for detection. A diverse of different SPR-based biosensors have been constructed and been used for various fields, such as biomedicine, environmental monitoring and food safety. This research outlines the basic concepts, the working principle of SPR and the applications of SPR in biomedicine. In addition, the discovery and development of SPR will be present, as well as the mechanism behind SPR instruments. It will also be examined in detail the application of SPR in drug analysis and early diagnosis of cancer. Some perspectives about the latest research advances and future development areas will also be discussed respectively. The advantages and disadvantages of SPR will be illustrated throughout this work.

Keywords: SPR, detection, biosensor, biomedicine, application.

1. Introduction

Throughout the last few decades, surface plasmon resonance (SPR) has evolved from a relatively arcane phenomenon into a widely-utilized optical instrument for scientific identification. As early as 1902, physicist Robert. W. Wood observed dark bands on the reflection spectra of subwavelength metallic grating, which is now referred to as Wood's anomaly. To account for such phenomenon, Lord Rayleigh initiated physical interpretation, which was further developed by Fano. However, it was not until 1968 that the explanation for the phenomenon became comprehensive and intelligible when Otto, Kretschmann and Raether concurrently recorded the excitation of surface plasmons, which is also interpreted as SPR.

In 1983, inspired by the ambition of developing physical methods for the label-free and real-time detection of bio-molecules, Liedberg, Nylander and Lundstrom demonstrated the application of SPR in biomolecular interaction monitoring. Deriving from a series of subsequent experiments, golf has been culled as the best inert metal film required for SPR. Further advance was made in 1990, during which studies shown the compatibility and efficiency of surface-immobilized caboxylated dextran as a subtrate for both a covalent immobilization of biomolecules and biomolecular interactions [1]. Such enhancement of sensor chips laid the foundation for the successful manufacture of the first SPR instrument, the Biacore instrument, by Pharmacia Biosensor in 1990. Since then, SPR has become the "gold standard" of transducer principle for analysis of biomolecular interactions, and the standard set by the Biacore instrument has long been pursued by other companies [3]. However, although many companies have endeavoured to seek for novel SPR system, such as the cuvette-based single-channel SPR system developed by IBIS Technologies. The Biacore instrument is still reckoned as the benchmark for SPR industry.

Since SPR detects differences in refractive index, no labelling procedure is needed in the detection. The SPR has already been utilized in many fields such as DNA detection and virus analysis [2]. Additionally, SPR only requires a minimal amount of sample to carry out the analysis. Such trait can be a significant superiority because scientists and health-care professionals are enabled to use less number of expensive materials, rendering such detection to be more affordable and accessible. What's

more, the sensor chip in the biosensing experiment can be reused, further reducing costs associated with SPR. With the determining effect of the quality and performance of the sensor chip on SPR analysis, the reusability of sensor chips is even more splendid. Additionally, SPR is eligible to handle complex samples and has the ability to repeat measurements. Even in situations when only crude samples are available for testing, SPR can still function in an appreciable manner. In general, SPR's capability of using reusable core component, running real-time detection, and detecting samples with limited amount and quality makes it irreplaceable in many fields. However, there are some limitations for the application of SPR biosensors, such as low sensitivity of direct label-free and instability of sensing surface in complex and coarse environments. To overcome these failings, researchers have endeavoured to combine SPR biosensors with materials science and nanotechnology to improve the sensitivity of SPR biosensors.

In recent years, device schematics, detection precision and practical feasibility of SPR biosensors have been refined, and SPR has solidified its status as one of the most important methods of researching the biomolecular interaction analysis. Energy conversion is one of the main applications to use nanophotonic. SPR plays an important role in hybrid nano-biostructures to improve absorption efficiency [3]. Due to the rapid development of technology and mankind's increasing demand for physical well-being, SPR technology has been widely practiced in the field of environmental monitoring and bio-medicine. Moreover, SPR technology has been proven to be indispensable in detection of a variety of interactions, including protein-protein, protein-DNA and antigen-antibodies and receptor-ligand, in the field of scientific research and drug screening.

To prepare for commercialization and further elevate sensitivity and specificity of SPR, most researchers are currently concentrating on developing miniaturized SPR devices. Herein, the research will outline the working principle of SPR and the applications of SPR in biomedicine.

2. Mechanism of SPR

2.1. SPR phenomenon

The occurrence of SPR generally ensues the hitting of a metal surface by a proton of incident light. It is common to select gold as the material of the metal surface because a SPR signal can be displayed by gold at combinations of angle of reflection and wavelength in a relatively convenient manner, because gold is inert to most reactants used in biological interactions. At specific incident angle, known as resonance angle, the coupling of a portion of the incident light energy with electrons in the metal surface results in the excitation of electrons, causing them to move around [5]. The movements of electrons are termed as plasmon, and their propagation is parallel to the surface. In accordance with the very basic principle of quantum mechanics, the transferring of energy to form surface plasmon can only happen at specific resonance wavelength of the incident light when the momentum of photons matches the one of plasmon. The absorption of optical light by the electrons on the metal surface is responsible for the dark band of reflection spectrum.

2.2. Principle of SPR instruments

To be excited by a beam of incident light, the wavevector of the incident light along the x-axis needs to be in conformity with the wavevector of the surface plasmons. However, as indicated in Figure 1, it is showcased explicitly that the wavevector of surface plasmons at a metal-dielectric interface is naturally greater than the wavevector due to incident light in the dielectric, illustrating the impossibility to excite surface plasmon by a sheer incident light beam on the surface. To accomplish such excitation, it is essential to increase the wavevector of an incident light.

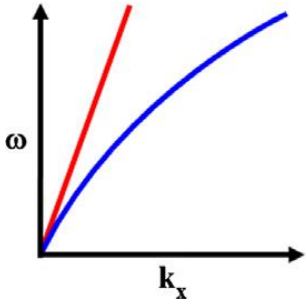


Figure 1. The red line shows the change in the wavevector of incident light in the dielectric with incident angle. The blue line showcases the variation of the wavevector of surface plasmons in the metal-dielectric interface with incident angle [14].

2.2.1. Prism coupling

One of the most common ways for wavevector accretion is passing the light through an optically denser medium, which is normally a prism with high reflective index. Such measure is termed as prism coupling. When a light beam travels from medium 1 to medium 2 (see Figure 2), with the refractive index of medium 1 larger than the one of medium 2 ($n_1 > n_2$), total internal reflection (TIR) can occur in medium 1 with the confinement that the incident angle, θ, is larger than critical angle, θ_c, which equals to

$$\theta_c \sin^{-1}(\frac{n_2}{n_1}) \tag{1}$$

In TFR, an electric field, which is known as evanescent wave, is induced on the opposite side (the side with lower refractive index) of the interface by the photons of the light beam, tunnelling through the gold film with appropriate thickness and thus exciting the surface plasmon [14]. The surface-parallel wavevector of the evanescent wave is represented as

$$k_x = \frac{2\pi}{\lambda} n_1 \, sin(\theta) \tag{2}$$

where λ is the wavelength of the incident light, n_1 is the refractive index of medium 1 and θ is the angle of incidence. Similarly, the wavevector of the surface plasmon, k_{SP}, is correlated with the dielectric constants of medium 2 and the gold layer [4]. Thus, the expression for k_{SP} can be written as

$$k_{SP} = \frac{2\pi}{\lambda} (\frac{{n_g}^2 {n_2}^2}{{n_g}^2 + {n_2}^2})^{\frac{1}{2}} \tag{3}$$

Since the conformity in momentum, which can be interpreted as the matching of k_{SP} with k_x, of the incident light beam and the plasmon is one of the most significant prerequisites for SPR to occur, the required angle for SPR, θ_{SPR}, can be deduced by combing Equation (2) and Equation (3):

$$sin(\theta_{SPR}) = \frac{1}{n_1} (\frac{{n_g}^2 {n_2}^2}{{n_g}^2 + {n_2}^2})^{\frac{1}{2}} \tag{4}$$

As indicated by the expression, the value of θ_{SPR} hinges on the refractive index of medium 2 and the gold film. Since the interactions, especially adsorption and desorption, by the gold layer have an influence on the reflective index of the interface, such interactions can be monitored and analyzed by recording changes in θ_{SPR}.

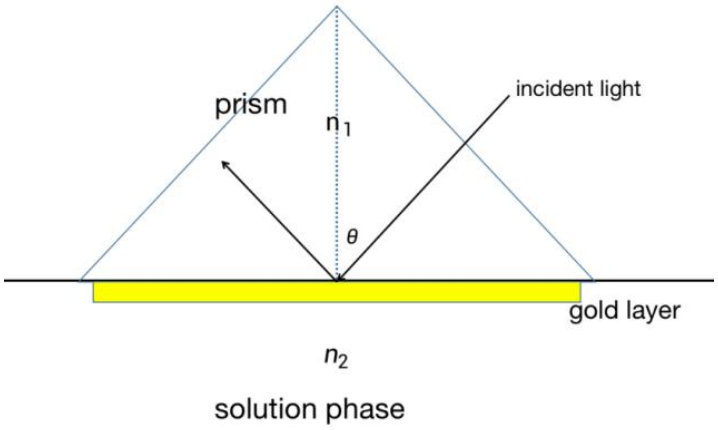


Figure 2. The general schematic of SPR.

2.2.2. Grating coupling

The utilization of diffraction grating can contribute to the increase of the wavevector of incident light. The incident light is firstly scattered by the diffraction grating, and its wavevector along the x-axis, k_x, is thus altered by an integer multiple of the grating number, N, which depends on the order of diffraction [14]. Assuming the order of diffraction is d^{th}, then the coupling condition for the resonance to happen is:

$$k_d = k_x + Nd = \frac{2\pi}{\lambda_0} n_d sin(\theta) + \frac{2\pi d}{\Lambda} \quad (5)$$

where k_d is the surface-parallel wavevector of the diffracted light, n_d is the dielectric refractive index and Λ represents the period of grating. Under grating coupling principle, detection can be made by plotting the incident light's reflected intensity versus incident angle.

2.2.3. Waveguide coupling

As a guided mode which propagates along the dielectric waveguide travels into the area coated by thin metal layer, its evanescent wave is able to penetrate the metal film [14]. It is then feasible for resonance to happen if the guided mode's wavelength-dependent wavevector along the surface matches the wavevector of the surface plasmons which are located at the outer metal-dielectric boundary. Since such conformity in phase can only be achieved for a narrow range of wavelength, a dip should occur on a graph where wavelength is plotted against transmitted power.

3. Applications of SPR for Pharmaceutical Analysis

Nowadays, identifying new biologic therapies focused on cancer treatment is a challenge for most pharmaceutical companies. And thus, SPR is used to analyze the biotherapeutic agents in the early phase clinical development rapidly.

3.1. Pharmacokinetic Drug Profiling

One of the most important aspects of pharmacokinetic studies relates to the management of drug transport into the systemic circulation because drugs are considered to be absorbed only when they enter the capillaries. And at the same time, drug absorption also depends on many physicochemical factors, which lipophilicity and solubility are important. Therefore, the characteristics of drug penetration into bio membranes are usually characterized by SPR biosensors that immobilized liposomes or cell membrane fragments directly bonded to the sensor surface.

When monitoring the interaction between several drugs and immobilized liposomes and compared with the proportion absorbed by the human body, liposomes were directly attached to the surface of the SPR biosensor. Although the distribution coefficients measured in liposome systems may not

always reflect biosmosis and may have an impact on drug-induced membrane effects, they reflect drug-surface interactions and can identify most drugs with high cellular uptake [6].

3.2. High-Throughput Screening (HTS)

High-throughput screening (HTS) is defined by a large number of compounds tested for ligand discovery of receptors, enzymes, ion channels and other pharmacological targets, as well as pharmacological analysis of cellular and biochemical pathways of interest. Today, the trend among pharmaceutical companies to focus on the screening of drugs and drug fragments has identified a new and advantageous position for biosensors as high-information-content screening tools capable of detecting multiple drug candidates [7].

Recently, Brooker announced at the SLAS 2022 International Conference and Exhibition that the multi-channel HTS detection method takes SPR high-throughput screening technology to a whole new level. The innovative microfluidic system of the Bruker Sierra SPR-32 Pro uses a unique 8×4 array that can read measurement data from 32 measurement sensor points in parallel per injection with excellent stability. This configuration considers the flexibility of biomacromolecule and small molecule drug candidate development while ensuring high throughput. For example, we can screen 31 antibodies simultaneously with 1 antigen, or screen 8 small molecule drugs with 3 target proteins, and each channel can set a separate reference.

The Sierra SPR-32 Pro's multichannel capabilities can triple the measurement time, and its highly sensitive SPR technology enables experiments where the analyte and target differ in size by three orders of magnitude. This will make high-throughput drug screening become an essential technology for future drug development.

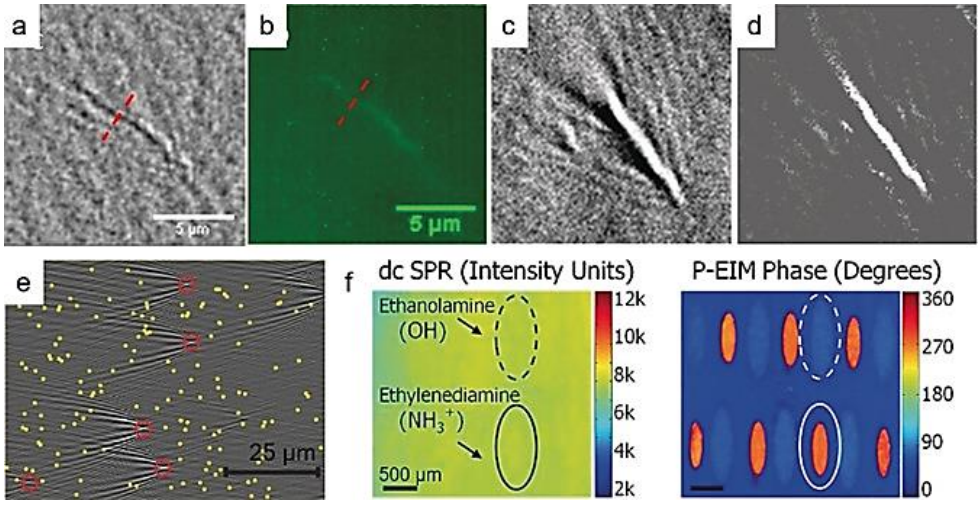


Figure 3. The differential SPR method for optical imaging and detecting the DNA length [2].

4. Applications of SPR for cancer diagnoses

Significant expectations are endowed upon SPR-based biosensors on cancer diagnoses. This is normally termed “liquid biopsy”. What is noticeable is that the sensor needs to sensitive enough, since there are numerous similar proteins and glycoproteins in our body [9]. In this way, the SPR can only react to the marker that we want and not to have a false positive result [2]. And differ from conventional tissue biopsy, liquid biopsy can detect biomarkers of the sample at one time [8]. There are already some validated biosensors and related analytical procedures in the detective fields, as shown in Figure 3. Protein, which is the major marker in detection and also large molecules, such as PSA, CEA or CA-125/MUC16 can be detected by SPR. By detecting different varieties of protein, we can identify the type of cancer or diseases. In spite of that the rate of the markers can determine the process of the cancer.

Quantifying the rate of both CTCs and ctDNA will provide a more accurate insights of dynamic fluctuations and characteristics of the tumor disease, in order classify the cancer. However, due to the figures of CTCs and ctDNA, the detection of them can be inconvenient. As a result, the researchers find a way using the highly specific surface-immobilized probe with a complementary sequence to the target one. But unfortunately, until now, there is still not a research report on simple SPR in detecting CTCs and ctDNA. And due to the plasmonic coupling and the second structure of mutated ctDNA, the LSPR are not able to detect the ctDNA yet [8].

SPR are expected to take over the position of the previous ones. Conventional clinical test methods have varieties of other drawbacks [11] [12]. Standard process of diagnosing cancer is now tissue biopsy. This method will take the specific tissues from human bodies by special needles or surgery to observe the structure and the features of invasive tumor lesions of the sample. However, there are few researches show that not all the tumor cells can be collected due to their position and in some cases the test itself might even increase the possibility of metastatic lesions [15]. What's more, the surgery is time-consuming and costly. Thus, they will delay the treatment and prognosis of the patients. But if we put SPR into clinical uses, this situation can be changed. Firstly, we can get the result by SPR in a real short time and the expenses will be lower thanks to the reusable chips of the detector. Even more momentous, patient don't have to take the surgeries for the tissue biopsy [13].

Since the medical industry is still in lack of a tool that can diagnose the cancer in a definite way, we still have to refine several different detection methods altogether to get a more accurate result.

5. Conclusions

As mentioned in prior sections, SPR is now been increasingly considered as an effective tool in both pharmaceutical analysis and cancer detection. SPR biosensors can monitor almost considerable kinds of molecular interaction between different types while it can observe the concentration of component in real time. In drug profiling, we can analyze the characteristics of drug penetration into bio-membrane by SPR biosensors. To sum up, SPR system is easy to predict quantitative measurements of passive transport, and this provides huge convenience for the study pharmacokinetic.

Cancer markers are now increasingly considered as diagnostic targets. SPR bio-sensors have more advantages than other methods in the determination of cancer markers. The adorable features of SPR include non-destructive analysis, simpler miniaturization, label-free, rapid and real-time monitoring of the target biomolecule, superb selectivity, and cost-efficacy. In addition, the utilization of SPR may contributes to the discovery of novel cancer markers. In general, SPR has become a practical tool for early and selective detection of cancers. In this review, we focused on the advantages of SPR being a detective technique in cancer therapy and compare them with the previous clinical ones, especially to the surgery.

6. Authors' Contributions

Yangtao Du, Xiaoping Qu and Guanzhong Wang complete this work together.

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