Chemical Sensors and Surface Plasmon Resonance Biosensors

Yuzuru Iwasaki†, *Tatsuya Tobita, Tsutomu Horiuchi, and Michiko Seyama*

Abstract

We have developed optical sensors for detecting biological samples using surface plasmon resonance (SPR), which is a basic platform for many kinds of chemical sensors. In this paper, we introduce the principle of chemical sensors, especially SPR sensors for biological substances. SPR can detect the specific binding of biological molecules but was previously unable to detect enzymatic reactions, which are a major class of biological reactions. To overcome this limitation, we developed electrochemical SPR sensor techniques and made an enzyme-based sensor array.

1. Introduction

Chemical substances affect our lives both directly and indirectly. Since all living things take in chemicals and air and then metabolize them, our vital functions are strongly dependent on the chemicals around us. For example, heavy metals can poison the food chain, acid rain damages trees, endocrine-disrupting chemicals are suspected of having long-term effects on human and animal health, and some people have allergic reactions to particular chemicals. Therefore, the detection and identification of chemicals is very important.

At the cellular level, all information is transmitted in the form of molecular signals, and genetic information is stored in the form of DNA sequences. These days, the information transmitted over telecommunication networks is acquired by physical devices such as keyboards, computer mice, microphones, and cameras. However, in the coming era of ubiquitous communication, this physical information will be supplemented by information about chemical substances (**Fig. 1**). For example, continuous monitoring of our personal health by measuring biomarkers will assist in the early diagnosis and treatment of lifestyle diseases. These personal health conditions vary from person to person and vary over time, so chemicals should be measured easily with realtime performance. To achieve such communication we need to develop devices to obtain information about these chemical substances, such as the class of chemical and its concentration. When we can identify and measure the concentration of chemical substances anytime and anywhere in the real world using a small (ubiquitous) sensor, we will be able to take innovative steps toward solving environmental and medical problems and improving our daily lives.

Our research group has studied chemical sensors designed to have a small size and high performance. To meet these two requirements, we have studied microfabrication technology to allow us to measure small sample volumes, surface science and biochemistry to improve detection sensitivity, and optical chemical sensing technologies to enable the integration of several different types of sensors, such as an electrochemical micro-sensor, enzyme-based sensor, and optical sensor. In this report, we introduce our recent development of an optical sensor for biochemical analysis that we constructed using surface plasmon resonance (SPR) as the first step towards ubiquitous chemical sensors. SPR sensors have already been built as analytical instruments and used as a tool for searching for candidates of pharmaceuticals. The main features of an SPR sensor are its simple struc-

† NTT Microsystem Integration Laboratories
Atsugi-shi, 243-0198 Japan
E-mail: iwasaki@aecl.ntt.co.jp

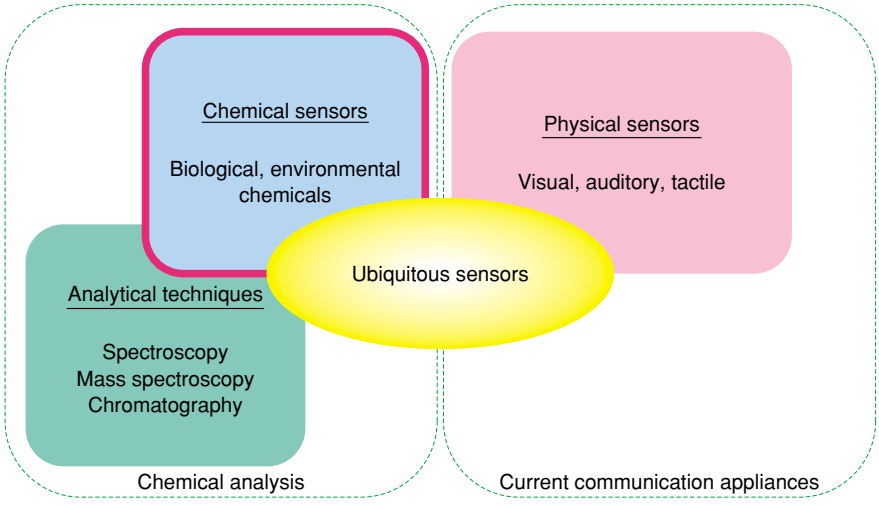

Fig. 1. Ubiquitous sensors.

ture and its potential to be made small enough to be integrated into a headset or cellular phone by means of optical microfabrication technologies in the future. Several classes of small sensors, such as immunosensors, DNA sensors, and enzyme-based sensors, have already been developed and arrays of sensors of one class have also been developed. The SPR sensor can detect the specific binding of biological molecules such as antigen-antibody and DNAs, but it has not been able to detect enzymatic reactions. However, a ubiquitous chemical sensor must be able to integrate different kinds of detection schemes in a single platform. We show that electrochemical SPR sensor technology can be used to overcome this limitation, and we apply it to an array of enzyme sensors.

2. Chemical sensors

2.1 Overview

When detecting molecules we want to identify a specific molecule and quantify its amount. A sample obtained from the environment or a sample of body fluid is a mixture of many molecules, so a critical step in conventional chemical analysis is to separate the different molecules. Molecular separation techniques such as chromatography and mass spectroscopy have been developed. They allow the precise measurement of natural samples, and they are already contributing to the medical, environmental, and fundamental chemical analysis needs of a number of industries. However, these separation techniques require large instruments. A chemical sensor, on the other hand, can detect a specific molecule without separation.

Chemical sensors consist of a molecular recognition unit and a transducer unit. Instead of separating all the molecules, the molecular recognition unit identifies only a specific group of molecules by means of a chemical reaction. The physical change resulting from the reaction is converted into a physical signal by a transducer (**Fig. 2**). The physical signals used by chemical sensors include current, voltage, light intensity, and refractive index. Chemical sensors can be made small and used at the location where the specimen is sampled. NTT Microsystem Integration Laboratories has recently developed small chemical sensors for an environmental gas monitoring system [1], for an odor recognition sensor [2], and for a realtime monitoring system for cardiac patients [3]. The molecule identification performance of chemical sensors depends on the molecular recognition unit, whereas the sensitivity depends mainly on the transducer unit. The former can be improved by using biological molecules and the latter can be improved by using a technique for measuring the optical refractive index.

2.2 Measurement of biomolecular interactions

Biological reactions are primarily controlled by the specific binding reaction of two molecules. For example (**Fig. 3**), an antibody finds a specific molecule with a characteristic structure (antigen) and binds firmly to it. This binding triggers a chain of immunoreactions. A sequence of single-strand DNA binds with a complementary sequence of DNA to form double-strand DNA. These natural biological-molecule-specific reactions, which are called biomol-

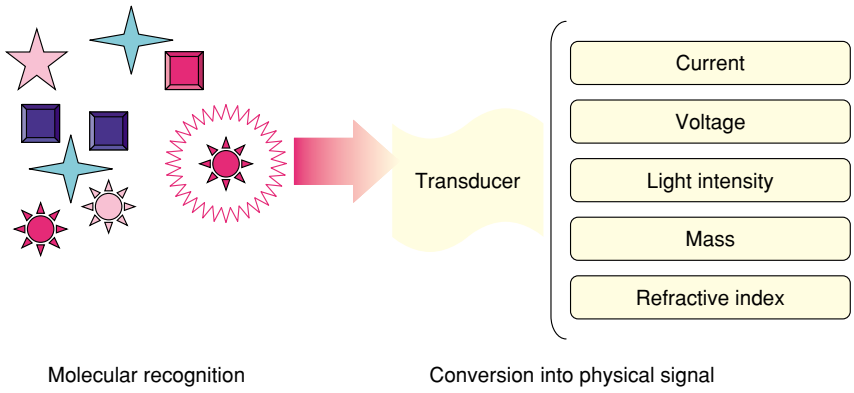

Fig. 2. Elements of chemical sensor.

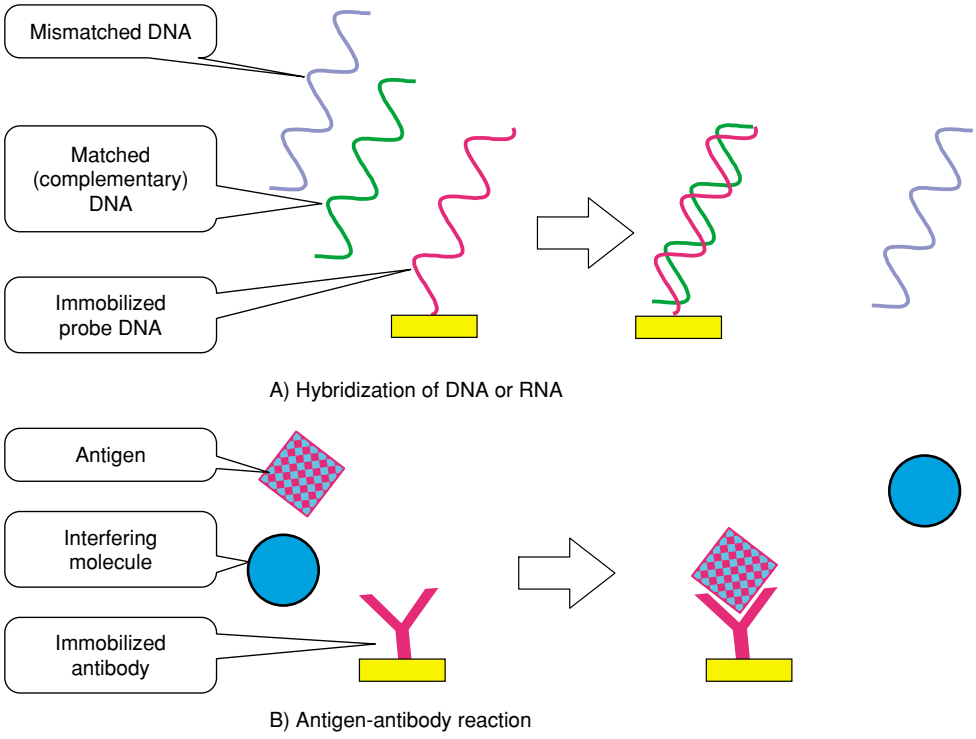

Fig. 3. Increase in refractive index caused by binding reaction.

ecular interactions, can be used to detect a specific molecule without a separation step. A sensor based on this idea is called a biosensor [4]. When one molecule of the pair is immobilized on a substrate and a sample solution containing the other molecule of the pair is brought into contact with it, the immobilized molecule captures the complementary molecule by a molecule-specific recognition reaction such as an antigen-antibody reaction, DNA hybridization, or an enzymatic reaction. Molecules that are not recognized by these reactions are not held on the substrate. As a result, the target molecule is concentrated on the substrate and then detected by a transducer unit. For example, a detection scheme using a fluorescent molecule and a photodetector as a transducer unit is widely used in biological analysis. A fluorescent molecule is chemically bonded to all the molecules in a sample before they come into contact with the substrate. When the sample reacts with the substrate, fluorescence is observed on the substrate if the sample con-

tains the target molecule. The molecular concentration can be calculated from the fluorescence intensity. This method does not require a separation step but does need the pre-reaction or pretreatment of the molecules in the sample with fluorescent molecules.

Because the binding of a pair of molecules causes an increase in molecular density, the recognition reaction can be detected by measuring the change in refractive index of the substrate surface. In a liquid sample, a bound molecular complex has a higher refractive index than an unbound molecular set. This is a simple procedure, but it requires high-resolution measurement of the refractive index.

2.3 Refractive index measurement using SPR

The refractive index of a solution correlates with its concentration. A commonly used method of determining the refractive index is to measure the critical angle. The measurement sensitivity can be improved by using surface plasmon resonance (SPR) measurement. SPR is a strong coupling phenomenon between light and the free electrons in a metal surface [5], and it also refers to the measurement method. The most commonly used metal for sensor applications is gold. The principle of SPR is shown in **Fig. 4**. A gold sensing film is formed on a prism having a high refractive index. The gold film is 40 to 50 nm thick and is semi-transparent. The refractive index of the gold surface (sensing layer) is measured. The gold surface is illuminated from the prism side with monochromatic light and the reflection intensity is measured as a function of the angle of incidence. Because the refractive index of the prism is higher than that of the sensing layer, the incident light undergoes total reflection. However, the reflected p-polarized light (the electric field of the light is parallel to the plane including the incident and the reflected light path) is diminished at a certain incidence angle when thin gold film is present at the prism surface. As shown in **Fig. 5**, depending on the refractive index of the sensing layer, the curve of reflectivity versus incidence angle moves to a larger angle as the refractive index of the sensing layer increases. This curve is called an SPR curve and the incidence angle of the minimum reflectivity is called the SPR angle. When the prism material is BK7, a refractive index of up to 1.5 can be measured. Because many biological molecules in solution phase have refractive indexes between 1.0 and 1.4, SPR can detect a wide range of biological molecules. The dependence of the SPR angle shift on the refractive index change is determined by a Fresnel multilayer reflectivity calculation. As shown in

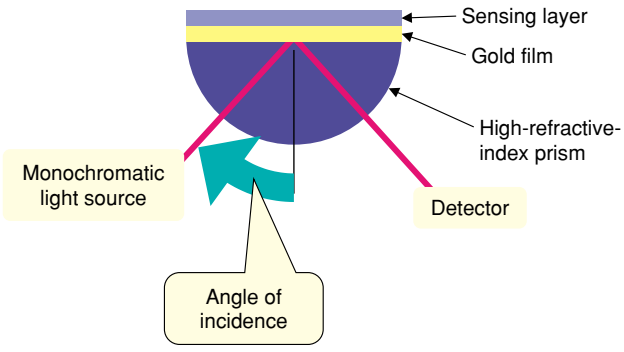

Fig. 4. Measurement of refractive index by SPR.

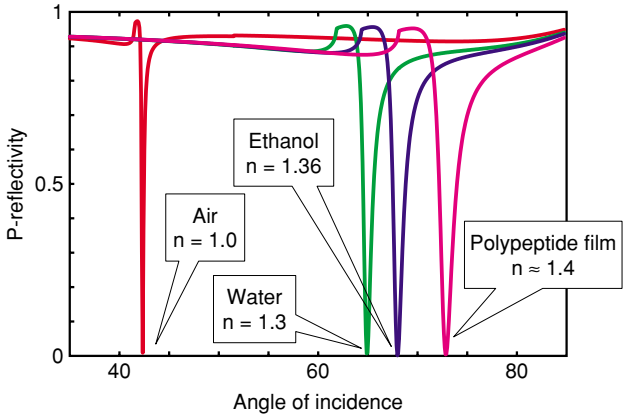

Fig. 5. SPR curves for different molecules (n = refractive index).

Fig. 6(a), the SPR curve shows a large reflectivity change in a narrow incidence angle range, and the SPR angle can be determined with high resolution. Moreover, the refractive index of the sensing layer is directly related to the SPR angle, as shown in **Fig. 6(b)**.

A practical optical setup for SPR experiments is shown in **Fig. 7**. An inexpensive LED (light emitting diode) light source is commonly used. After being collimated with a lens system, the light emitted from the LED enters the prism and is focused on its center with a certain range of incidence angles. The reflected light is imaged by an array photodetector such as a CCD (charge-coupled device) camera. In this optical setup, the incidence angle corresponds to the position of the array detector, and the reflectivity is determined from the measured brightness of the image. The SPR angle can be calculated from the image. As shown in Fig. 7, SPR can be carried out using a sim-

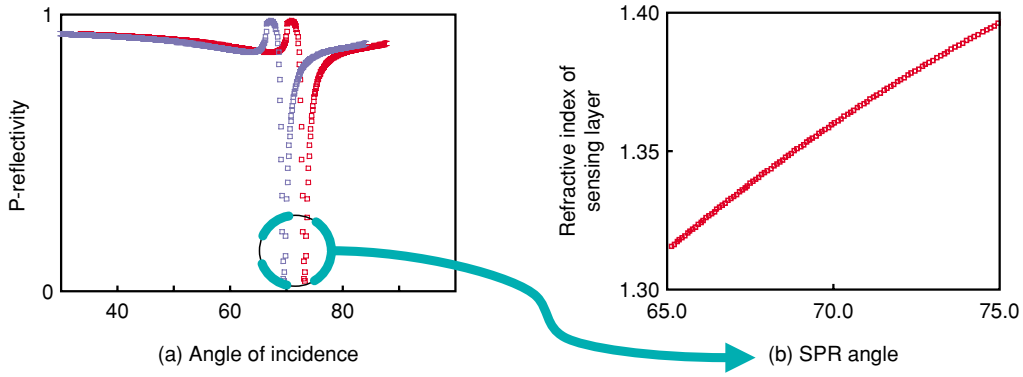

Fig. 6. Refractive index and SPR angle.

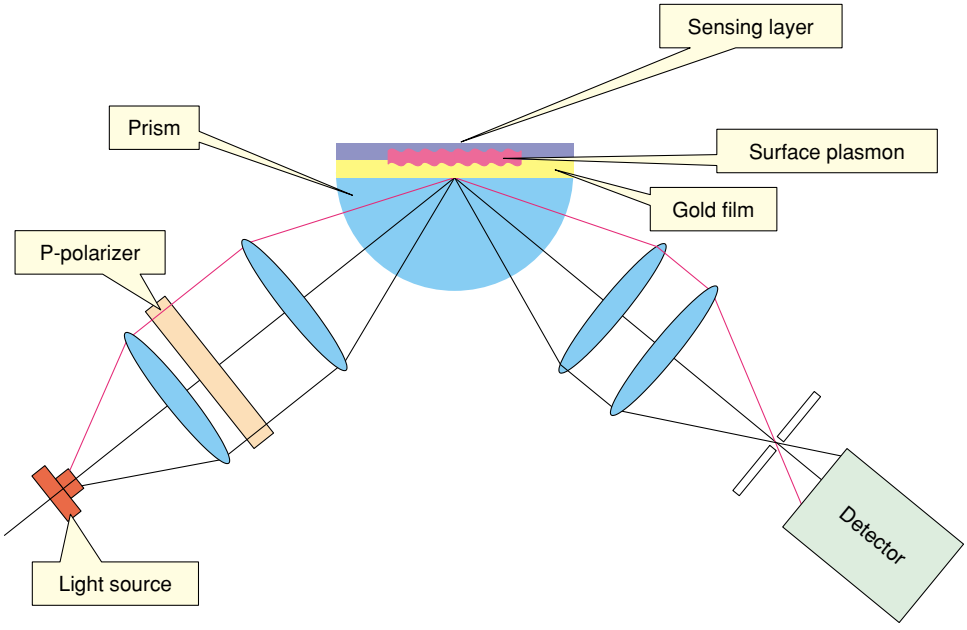

Fig. 7. Optical setup for SPR.

ple optical setup. Moreover, the measuring system is small enough to fit in the palm of your hand. In the future, it will be possible to integrate all of the optical elements using optical fabrication technologies.

2.4 Refractive index mapping using SPR

For biological analysis, it is desirable to measure many samples in a single operation. A chemical sensor can detect not only a specific molecule, but also multiple different molecules if it is configured as an array of chemical sensors for different targets. Furthermore, an array of a single type of chemical sensor can be used to measure the spatial distribution of a biological reaction such as cell metabolism. The optical setup shown in **Fig. 8** enables SPR to be used for this purpose. In this setup, an area of gold film is illuminated with collimated light with a fixed incidence angle and the reflected light forms the image of the reflecting surface on an imaging device. The incidence angle is set slightly smaller than the SPR angle, and a small refractive index change or variation is observed as a change in reflection intensity. For example, in **Fig. 9**, the SPR curve shows the reflection minimum incidence angle at 63.15° when the refractive index of the sample layer was 1.32 (red curve). The biological binding reaction increased the

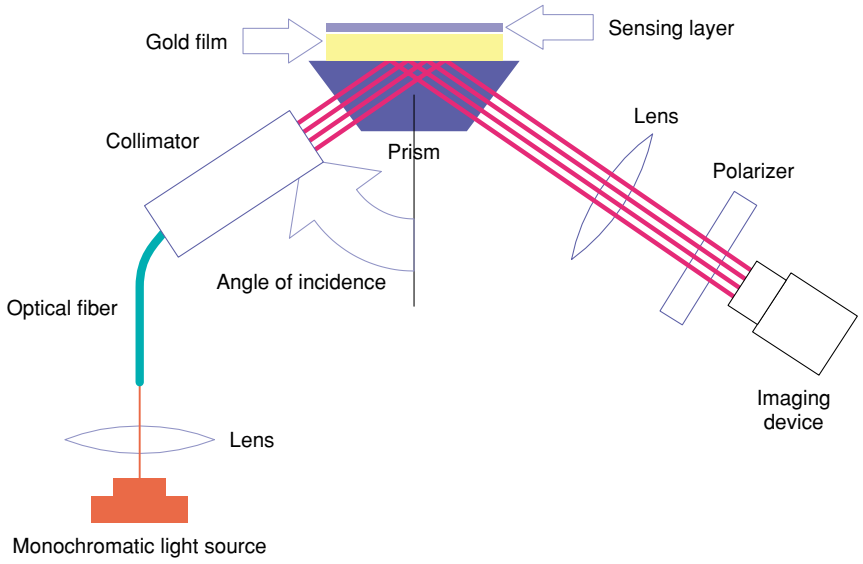

Fig. 8. Optical configuration for imaging SPR.

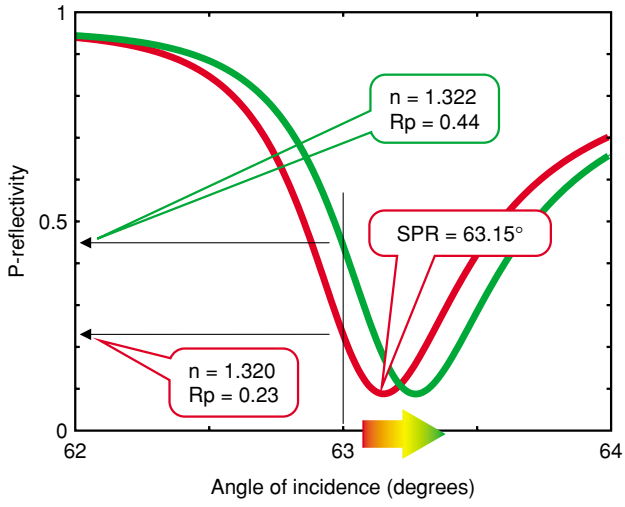

Fig. 9. Refractive index mapping.

refractive index of the sample to 1.322, and the SPR curve shifted to the right (green curve). The observed reflectivity increased from 0.23 to 0.44 when the reflected light was observed at an incidence angle of 63.0°. Therefore, small variations in the refractive index can be mapped or imaged. Although many biological molecules are transparent at visible to near-infrared wavelengths, a slight change in a biological reaction can be detected and visualized using this method based on refractive index difference.

3. SPR enzyme-based sensor

Molecule-specific binding reactions can be detected by SPR. This is because the binding constants of antigens and antibodies and of the complementary sequences of DNA are very high, so the lifetimes of the complexes are sufficiently long for them to be detected by SPR as a refractive index increase. Enzymatic reactions are another major class of molecular recognition reactions in biological systems. An enzyme recognizes its specific substrate molecule and forms an enzyme-substrate intermediate, which then catalyzes the chemical reaction of the substrate and releases the molecule. The lifetime of the intermediate is too short to provide a sufficient difference in the refractive index, so previous efforts to detect enzymatic reactions by SPR were ineffective. Nevertheless, since SPR is already used as an immunosensor and DNA sensor, if it were possible to detect enzymatic reactions by SPR, then all the major biological reactions could be detected by SPR.

We have already developed a novel electrochemical reaction analysis method using SPR. By using gold film as a working electrode for an electrochemical reaction, we were able to perform a realtime *in-situ* analysis of the surface status of the gold electrode [6].

Because electrochemical reactions involve a change in the electron states of a molecule and a rearrangement of the double-layer structure on the surface of the gold film, the rate of the electrochemical reaction can be determined by SPR-based refrac-

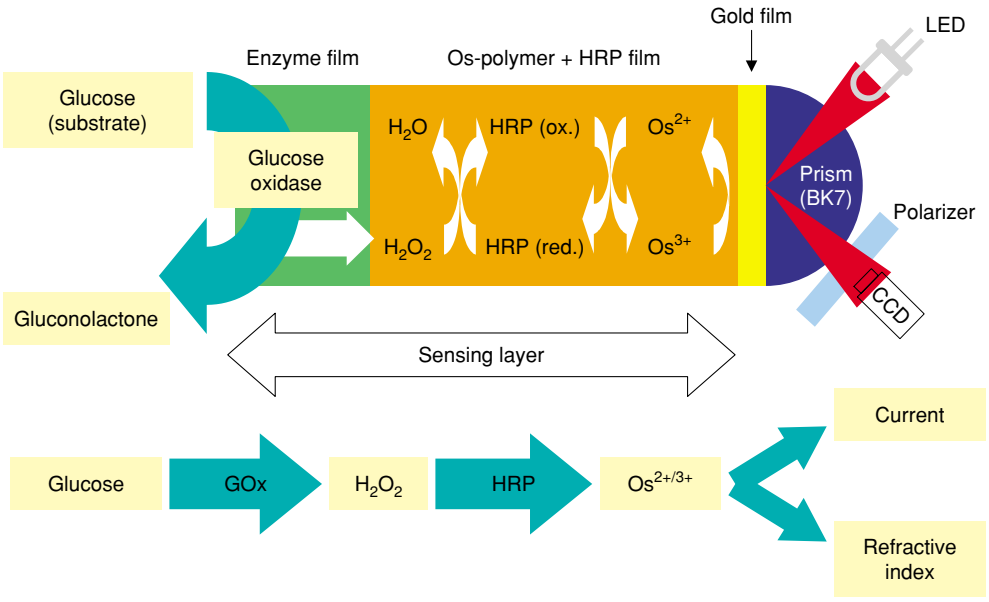

Fig. 10. Structure of SPR enzyme-based sensor.

tive index measurement. We used this method to construct an enzyme-based sensor. As an example, we constructed a glucose sensor. The determination of glucose concentration is important in the treatment of diabetes and the analysis of food. We constructed multiple enzyme layers and an electron mediator layer in the sensing layer of the SPR sensor, as shown in **Fig. 10**. The optical setup was the same as that shown in Fig. 7. As the electron mediator, we used an osmium ion complex that shuttles electrons between the gold and horseradish peroxidase (HRP), which is an enzyme that catalyzes the reduction of hydrogen peroxide. On the HRP layer, we formed a layer of glucose oxidase (GOx), which recognizes the glucose molecule, catalyzes the oxidation of glucose, and produces hydrogen peroxide. On the gold electrode, the osmium ion complex underwent a redox reaction, and the refractive index of the film measured by SPR exhibited a large change. SPR enables the redox status of the electron mediator film to be monitored in real time [7]. When a sample containing glucose was introduced on the sensor surface, GOx produced hydrogen peroxide at a rate proportional to the glucose concentration. The hydrogen peroxide reacted with the HRP in the sensing layer, and the HRP reacted with the osmium ion complex. As a result, the redox state of the osmium complex was quantitatively changed at a rate proportional to the glucose concentration. Using this scheme, we were able to determine the glucose concentration from the rate of change of the redox state of the osmium complex layer measured by SPR [8]. The sensitivity of this sensor to glucose was 10^4 times higher than when the concentration was determined from the refractive index increase of the glucose concentration itself. This result shows that an enzymatic reaction can be measured by SPR, which is the first step towards the total integration of biological sensors.

4. SPR imaging of biochemical reactions

Using the optical setup shown in Fig. 7 and the SPR enzyme sensor described in the previous section, we can visualize a biological reaction or construct an enzyme sensor array. The glucose sensor structure shown in Fig. 10 was constructed on a gold thin film. The HRP and osmium ion complex layer were formed on the surface. Then a GOx layer was spin-coated onto the surface. The resulting multi-layer film was observed. The refractive index changes of this sensor film are shown in **Fig. 11** [9]. The osmium ion complex layer was visualized as a ring-shaped region. The reflected light intensity corresponds to the refractive index. By controlling the redox state of the film with the electrode potential and differentiating two images taken at different redox states, we were able to visualize the redox state of the osmium ion complex layer (Fig. 11(a)). The taller regions correspond to high redox reaction activity while the lower regions represent little or no redox reaction.

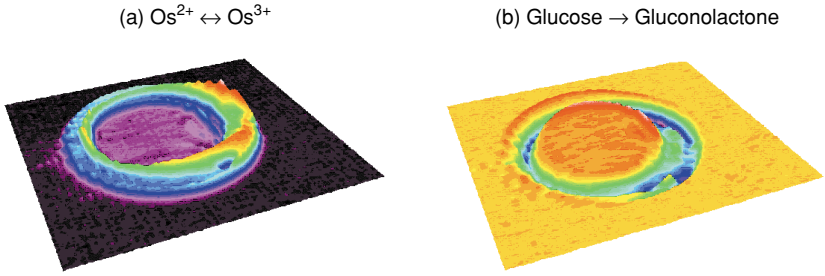

Fig. 11. Visualization of enzyme reaction.

Initially, we fully reduced the film electrochemically and then applied a glucose solution to the sensor surface. The glucose gradually reacted with the enzymes on the sensor and the osmium ion complex was oxidized. The difference between the refractive indices of the initial state (before enzymatic reaction) and final state (after the reaction) is shown in Fig. 11(b). The zero level is shown as dark yellow (fringe area) in this picture. When other sugars that are not recognized by GOx were used, no spatial variation was observed in this experiment, so the structure shown in this picture visualizes the enzymatic reaction. This result also shows that we could measure an enzymatic reaction in a region as small as a single pixel of the CCD camera we used and that a sensor array can be formed using this technique.

5. Summary

This paper explained the principle of chemical sensors and described an SPR sensor for detecting biological molecules. By combining it with electrochemical methods, we were able to use SPR to detect both the binding reactions of biological molecules and enzymatic catalytic reactions. Furthermore, we demonstrated the visualization of an enzymatic reaction, which is a fundamental technique for an integrated biological sensor array. SPR is now commonly used in the field of bioinformatics research. A portable system and an SPR imaging system have already been commercialized by the NTT group [10]. SPR sensors can be made small and inexpensive and further improvements in sensitivity and usability will make this sensor a platform for ubiquitous chemical sensors.

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Yuzuru Iwasaki

Research Scientist, NTT Microsystem Integration Laboratories.

He received the B.E. and M.E. degrees in polymer science and bio-electrochemistry from Tokyo Institute of Technology, Tokyo and the Dr.Eng. degree in synthetic chemistry from the University of Tokyo, Tokyo in 1986, 1989, and 1992, respectively. He joined NTT Basic Research Laboratories in 1992 and moved to NTT Environmental and Energy Technology Laboratories, NTT Lifestyle and Environmental Technology Laboratories, and finally to NTT Microsystem Integration Laboratories in 2002. He has been involved in R&D of electrochemistry including the analysis of electron transfer reactions of proteins, development of ion transfer battery materials, and optical biosensors.

Tatsuya Tobita

Engineer, Materials Analysis Center, NTT Advanced Technology Corporation.

He received the B.E. and M.E. degrees from Shinshu University, Nagano in 1993 and 1995, respectively. Since joining NTT Advanced Technology Corporation in 1995, he has been involved in the development of an optical fiber coupler machine and SPR measurement equipment.

Michiko Seyama

Network Hardware Integration Laboratory, NTT Microsystem Integration Laboratories.

She received the B.S., M.S., and Ph.D. degrees in applied chemistry from Waseda University, Tokyo in 1995, 1997, and 2004, respectively. She joined the NTT Integrated Information and Energy Systems Laboratories, Tokyo in 1997. She moved to the Lifestyle and Environmental Laboratories in 1999 and to the Microsystem Integration Laboratories in 2002. She has researched optical chemical sensing and odor sensing systems. She is a member of the Electrochemical Society of Japan, the Japan Society for Environmental Chemistry (JEC), and the Society of Polymer Science (Japan). She received a research promotion award and paper award from JEC in 1999 and 2001, respectively.

Tsutomu Horiuchi

Senior Research Scientist, Network Hardware Integration Laboratory, NTT Microsystem Integration Laboratories.

He received the B.S. and M.S. degrees in physics from Waseda University, Tokyo and the Ph.D. degree in chemistry from Keio University, Kanagawa in 1983, 1985, and 1994, respectively. He joined NTT Ibaraki Electrical Communication Laboratories in 1985. He has been engaged in research of electrochemistry and biosensors and chemical sensors. He was a visiting scientist at New Mexico State University, Las Cruces, NM, USA, in 1997. He is a member of the Japan Society of Applied Physics.