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Mini Review

Surface Plasmon Resonance Biosensor Chips

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Abstract

In this study, we describe biosensors predicated on surface plasmon resonance (SPR), a phenomenon that has recently become a focus of interest in biomedical science research. SPR has been shown to occur on two-dimensional metal surfaces, which support the SPR-active substrate on a glass prism. The SPR biosensor, which exploits the SPR effect, is a label-free and surface-sensitive spectroscopic system that utilizes measured changes in the local refraction index upon adsorption. Based on the SPR phenomenon, other technologies have been developed, including SPR-MS, SPR microscopy, localized SPR, and SPR imaging. The development of an SPR imaging system has already begun to overcome one of the difficulties inherent to conventional SPR, namely its high-throughput limitation. In this review, the detection principles and properties of SPR biosensors are briefly elucidated, and the possible future biomedical applications of SPR biosensors, including high-throughput screening in drug discovery, analysis of biomolecular interactions, and proteomics research are also discussed. This discussion is particularly salient now that SPR imaging provides another useful tool for the performance of high-throughput assays.

Keywords: Surface plasmon resonance, SPR biosensor, SPR imaging, Gold chip, Label-free, Biomolecular interaction analysis

Introduction

Recently, several label-free analytical technologies have been developed and have been successfully coupled with protein microarray techniques[1,2]. Among these technologies, one of the most powerful label-free detection methods is surface plasmon resonance (SPR) technology, which is utilized for the analysis of biomolecular interactions[3-6]. SPR has been shown to occur on two-dimensional metal surfaces (typically gold or silver films) when the total internal reflection of incident light occurs at the interface of two different substances, one of which has a high refraction index and the other possessing a low refraction index[7]. The SPR system also features surface-sensitive spectroscopic characteristics and excellent real-time analysis ability. Thus far, remarkable advances have been achieved in SPR applications, covering points of interest in biomedical, biochemical, and environmental areas[8,9].

SPR biosensor technology allows for measurements of biomolecular interactions with a wide range of different molecular masses and binding affinities. Thus, the SPR system can provide us with important insights into molecular recognition phenomena, including receptor-ligand interaction. The monitoring of biomolecular interactions is not strictly limited to proteins. Interactions including DNA-DNA[10], protein-DNA[11,12], protein-polysaccharide[13], and protein-virus[14] can be also evaluated can be evaluated using this system. In addition, SPR biosensors may be applicable to functional proteomics studies, which seek to identify the functions of novel proteins, thereby providing possible new targets for drug discovery[15,16]. In this fashion, SPR technology not only provides a tool with which unknown proteins can be isolated, but also allows for the determination of their functional properties. Recently, a powerful analytical tool for the study of functional proteomics has been introduced, which involves the coupling of SPR technology to mass spectrometry (MS)[17-19], thus allowing for large-scale proteomics involving high-throughput screening and novel protein identification.

With regard to the first commercialized SPR-related devices, the Biacore SPR system, which includes both chips and instrumentation based on analytical technology, was introduced in 1990. This system was capable of protein-protein interaction analysis[20]. Although the SPR biosensor market has been dominated by the Biocore SPR system since its introduction, many competing SPR instruments have become available[21,22], including new systems that have been introduced by Texas Instruments (Dallas, TX)[23], Affinity Sensors (Franklin, MA)[24], Nippon Laser Electronics (Japan)[25], and many others (Table 1). The

Table 1. Commercially available SPR instruments.

SPR company	System	Internet-website
BIAcore AB (Sweden)	BIACORE	http://www.biacore.com
Texas Instruments (Dallas, TX)	TISPR	http://www.ti.com/spreeta
Affinity Sensors (Franklin, MA)	IASys	http://www.affinity-sensors.com
Quqntech Ltd (Eagan, MN)	FasTraQ	http://www.quantechltd.com
Nippon Laser Electronics (Japan)	SPR-670	http://www.rikei.com
Aviv (Lakewood, NJ)	PWR-400	http://www.avivinst.com
GWC Technologies (Madison, WI)	SPR 100	http://www.gwcinstruments.com
Artifical Sensing Instruments (Zurich, Switzerland)	OWLS	http://www.microvacuum.com
IBIS Technologies BV (Enschede, Netherlands)	IBIS	http://www.ibis-spr.nl

values of the SPR responses are expressed in resonance units (RU), such that 1000 RU corresponds to an angle change of ~0.1°. For the majority of proteins, binding of ~1 ng per square mm of protein at the dextran surface is required to induce a 1000 RU signal change. The exact relationship between RU and the ng amount of bound biomaterial depends on the properties of the sensor surface and the refractive index of the analyte. A common SPR biosensor chip is a glass slide which is covered with a gold thin film, approximately 50 nm in thickness. The sensor surfaces of SPR devices are coated with a carboxy-methylated dextran matrix layer that allows for the covalent coupling of proteins and other ligands, creating a hydrophilic environment in which biospecific interactions occur.

According to recent reviews and numerous research articles, SPR is predicted to become one of the most potent technologies for applications in biomedical diagnostics and drug discovery via molecular interactions, without the need for any labeling. Thus, in this paper, we attempted to focus on the biomedical applications of SPR technology, including high-throughput screening in drug discovery, biomolecular interaction analysis, and proteomics research.

SPR Biosensor Theory

Surface plasmon resonance (SPR) biosensors are predicated on the behavior of surface plasmons (SPs), namely the phenomenon by which light of a specific wavelength turns into an electron density at the surface layer of a metal, typically a thin gold film, resulting in a collective electron oscillation[7,26]. The incidence of light can induce the excitation of the SPs in cases in which the momentum of the SPs matches that of the incident light. The frequency of the longitudinal oscillations in the metal surface is linked to the wave vector, k_{sp}, via a dispersion relation. In such cases, the surface plasmon resonance phenomenon occurs, as has been very well-characterized previously. As SPs waves evidence longitudinal characteristics, the waves can be coupled only with the transverse magnetic (TM) mode of the electromagnetic waves, the polarized direction of which is parallel to the incident plane. It has been shown that this wave-matching condition can be quite readily disrupted by even miniscule changes in the interface conditions. Thus, in cases in which a fixed light excitation condition is established, the SPR technique allows not only for the precise detection of changes in the refractive index or the thickness of the medium adjacent to the metal film, but also permits the detection of changes in the adsorption layer on the metal surface. As is shown in Figure 1a, the surface plasmons exhibit a larger wave vector than do light waves of the same energy $\leq \omega$. In order to photonically excite the SPs, the wave vector of the photons must be increased. Thus far, three basic SP apparatus have been exploited in order to achieve this phenomenon: 1. a prism coupler-based system[27], 2. a grating coupler-based system[28], and 3. a optical waveguide-based system[29]. However, the latter method is limited in regard to its application to multi-analyte assays, whereas the Kretschmann geometry of the Attenuated total reflection (ATR) method, which uses a prism, is currently the most frequently employed approach in SPR sensors[30]. This is a special case of ATR, in which a thin metal film exists at the ATR interface. As can be observed in Figure 1b, the light wave is completely reflected at the interface, and excites the SPs through the evanescent field. Detection is thus accomplished via the monitoring of changes in the resonance angle (under the wavelength fixed system), the wavelength (under the angle fixed system), or the reflectivity (under the wavelength/angle fixed system).

SPR Biosensor Characteristics

SPR biosensors have been recognized as one of the

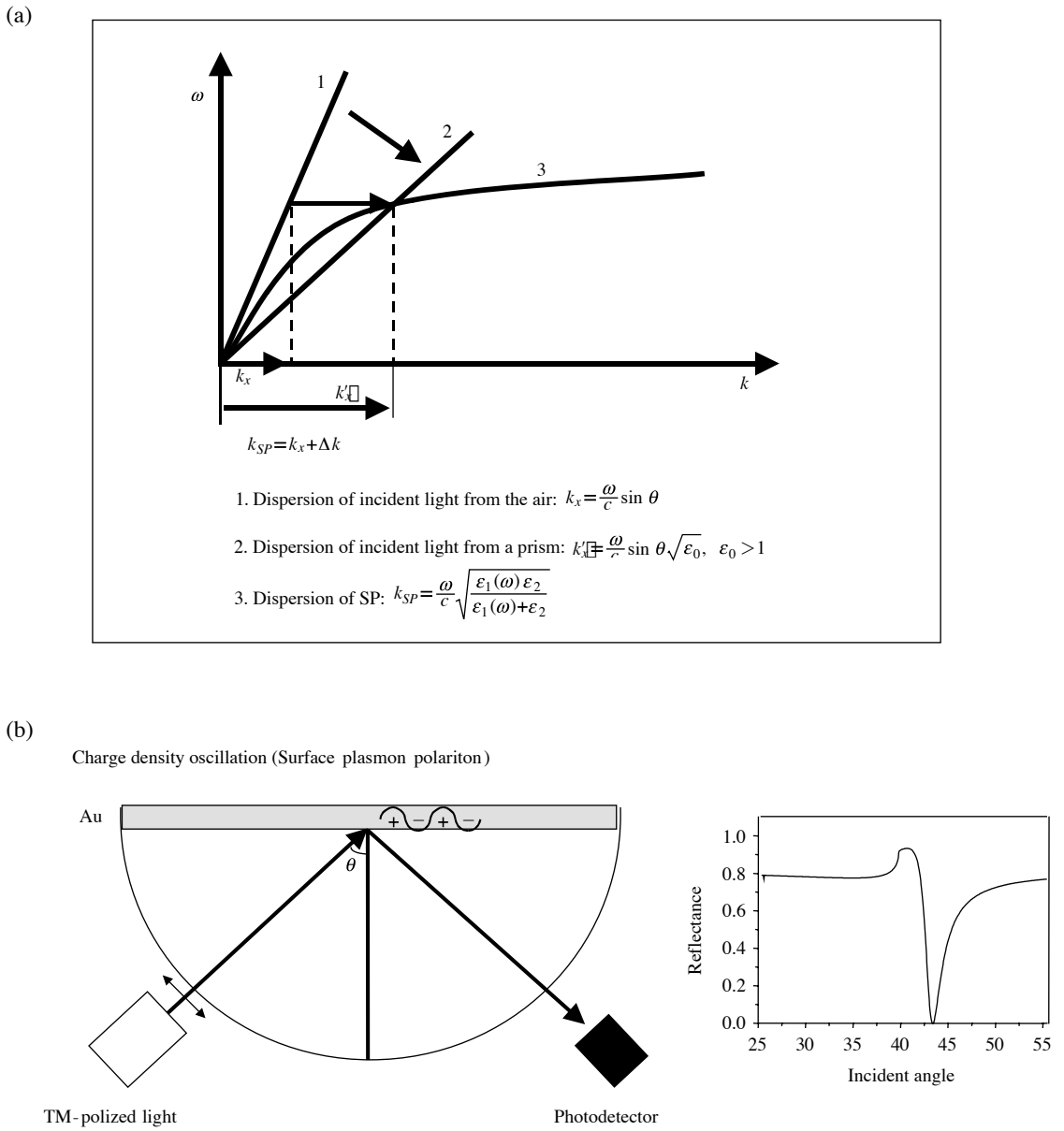

Figure 1. Principle of SPR biosensor. (a) The dispersion relation of non-radiative SPs, and (b) the configuration of the ATR method. See text for details.

most promising optical systems in the fields of biomedical science, analytical biochemistry, and experimental biology, due largely to their unique capabilities of real-time and label-free affinity-based measurement. After its appearance in 1991, as reported first by Jonsson *et al.*[20], the applications of SPR biosensors have increased immensely, and these devices are now used in an enormous range of applications. Above all, the applications of SPR technology in biomedical science are of particular note.

Conventional commercial SPR biosensors are single parameter instruments based on measurements in the shift of resonance angles. In recent decades, newly-designed SPR devices have been proposed and have been seen in the relevant literature, usually as the result of theoretical developments in the field. One such intriguing approach is the coupling of SPR and mass spectrometry (SPR-MS), which may prove extremely useful in the field of functional proteomics[17-19,31,32]. This SPR-MS proteomics research system constitutes a tool for the fast and effective identification of specific proteins in complex biological

mixtures. The other noteworthy SPR-related technology involves the application of a detection technology based on an imaging format, referred to as SPR imaging. SPR imaging using rapid optical array detectors allows for simultaneous detections via an array of surface-immobilized biomolecules, and may thus allow for high-throughput screening in drug discovery protocols[1,33]. Moreover, SPR imaging technology may potentially enable next-generation clinical diagnostic protocols in a variety of diseases, including human cancers. Presently, commercially available SPR instruments are large in size and not particularly economical, which renders SPR biosensors somewhat irrelevant to applications that require portability and affordability, including the U-health care system. Parallel to this trend in the development of SPR biosensors, attempts have been made to miniaturize SPR instruments in order to develop a hand-portable system[34,35], such that remarkable advances in the miniaturization of SPR biosensor systems have been made in parallel with other components, in an effort to develop more improved SPR instruments.

SPR Imaging-Based Biosensors

SPR imaging biosensor systems detect changes in the reflectivity of incident light, via the binding of biomolecules to the chip surfaces at a fixed angle of incidence, unlike other SPR systems that involve the measurement of shifts in the SPR angle or the wavelength[1]. The SPR images are captured at a slightly smaller incident angle than the SPR angle of the bare chip surface, at which the difference in the intensity between both SPR images is at a maximum. Thus, the brighter areas on the SPR images indicate the affinity binding of the target molecules to the gold surface. Figure 2 shows the instrumental design of an SPR imaging system.

Although SPR biosensors represent powerful and promising tools for the monitoring of biomolecular interactions, they also evidence a significant drawback, namely an inability to conduct multiplex analyses[36]. The numbers of different analytes that can be analyzed at one time using conventional SPR devices is limited to less than four samples on average. This indicates that these systems are neither practical nor efficient for use in high-throughput assays. In order to overcome this drawback, a newly revised SPR version has been required for the measurement of hundreds or thousands of samples in parallel. Along this line, the current trend in SPR-related technology for high-throughput screening (HTS) involves the formation of array chips and microfluidics. In particular, the application of the concept of an array chip in an SPR imaging system makes it possible to analyze parallel screening of many samples on a single chip. The unique characteristics of SPR imaging biosensors include the ability of the system to monitor simultaneously a number of biomolecular interactions, in a manner equivalent to quantitative kinetics studies, as well as qualitative

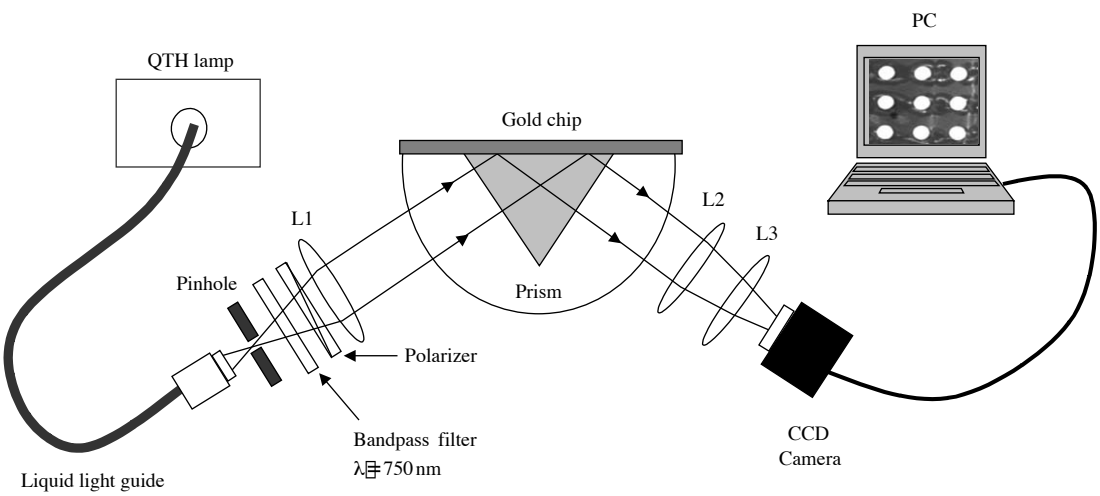

Figure 2. SPR imaging system. A quartz tungsten-halogen lamp (150 W) was employed as a light source, and the light was delivered to the goniometer arm using a liquid light guide. The light was collimated by the lenses, and passed through a narrow interference filter (750 nm, $\Delta\lambda$=2 nm) and a polarizer, in order to convert the monochromatic and linear polarized beams, respectively. A p-polarized and monochromatic light beam was focused directly on a prism coupler (FD110, n~1.76). The image reflected from the surface of the gold chip was photographed with a 1/2 inch monochromatic CCD camera. A combination of lenses (L2, L3) was positioned in front of the bare CCD in order to acquire a clear image. This image was then digitally stored in a personal computer using a B/W frame grabber, and further analyzed using SPR imaging software.

measurements of biomolecule binding. With regard to detection parts, imaging platforms for detection applied to SPR biomolecular interaction analysis render it simpler to determine the differences between the binding analytes and the non-binding analytes. Additionally, economical prices and comparable detection limits may also be other positive aspects of SPR imaging biosensors.

Applications of the SPR Biosensor

Analysis of biomolecular interaction. Real-time monitoring predicated on SPR technology has proven useful for the study of biomolecular interactions. Particularly, with regard to the application of SPR biosensors to biomedical purposes, SPR biosensors are very well-suited to a variety of biological research areas, including biomolecular affinity binding analysis, high-throughput assays, and functional proteomics studies. The most widely utilized SPR biosensor application is the affinity binding analysis of biomolecules, which provides us with important clues regarding the manner in which a given protein performs its functions. Using this approach, a variety of biological assays have been achieved in binding processes on protein microarray biochips, including those involving dynamic analysis of antigen-antibody interactions[37,38], ligand-receptor interaction kinetics[39-43,45-47], differential protein expression profile analyses[48-50], enzyme-substrate interaction analyses[51,52], epitope mapping[53], and real-time monitoring of DNA manipulations such as DNA hybridization or endonuclease-mediated DNA cleavage[54]. Moreover, the SPR signal intensity has been shown to be profoundly affected by changes in the optical thickness of the sensor surface, as well as by changes in the refraction index occurring on the gold chip surface at a distance of ~200 nm[55-58]. Now that these optical indicators can be affected by conformational changes in surface-immobilized proteins, SPR biosensors have also been applied to the monitoring of structural transitions in proteins when they interact with small molecules[59] or exist under diverse environmental conditions[60,61].

SPR-MS and proteomics research. The identification of the interaction partners of target proteins provides critical information for determining the biological functions of the proteins, and also facilitates the discovery of protein biomarkers for clinical diagnostics and drug screening[62-64]. Recently, a combined apparatus consisting of an SPR biosensor system coupled with mass spectrometry (SPR-MS) has been utilized successfully for functional proteomic studies, including a 'ligand fishing' assay using a complex biological mixture[18]. This SPR-MS-mediated ligand fishing technique allows for the identification of molecules that bind to the ligand of interest, which includes drug candidates, novel proteins, and clinical biomarkers. In this experiment, the binding of unknown molecules, normally proteins, to the immobilized ligand of interest on the surface of a sensor chip can be detected first via SPR technology, followed by a detailed characterization of the captured proteins via mass analysis. Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) measurements have been used extensively for this purpose recently[65-67]. Both techniques, SPR and MS, require no target molecule labeling. This non-invasive technique, therefore, is considered to be one of the more effective approaches to the identification of novel ligand binding proteins. Recently, electrospray ionization (ESI) mass spectrometry has also been coupled with the aforementioned SPR technology for the same purpose, further enhancing the potential of the method for the characterization of bound biomolecules[68]. This combined SPR-MS system is expected to provide a powerful tool in the field of quantitative analysis in functional proteomics, including large-scale 'ligand fishing' assays[18,69,70].

High-throughput screening. SPR biosensors can be applied not only to dynamic analyses of ligand-receptor interaction kinetics, but also for drug discovery and development protocols. Using an SPR-based binding approach, compounds can be rapidly and readily identified in accordance with their specificity at the binding site of a target protein. Thus, SPR technology shoud be considered a future trend in screening methods for drug discovery. There are several different formats for SPR biosensors, including the array format, multi-channel unit format, and SPR imaging format, which allow for simultaneous and continuous detection for the performance of hundreds to thousands of affinity binding events on a chip surface. Despite the excellent benefits inherent to SPR technology, SPR biosensors have a serious limitation in their inability to support multiplex analysis, as less than four analyses with conventional SPR instruments make such parallel operations feasible. On the contrary, SPR imaging technology as a multi-analyte biosensor permits not only a high-throughput approach, but also evidences a similar degree of sensitivity as can be achieved with conventional SPR biosensors. For those reasons, SPR imaging systems without any labeling requirements are more relevant to high-throughput screening, particularly in drug discovery, than any other optics-based detection techniques (Figure 3).

SPR imaging systems can be utilized as efficient

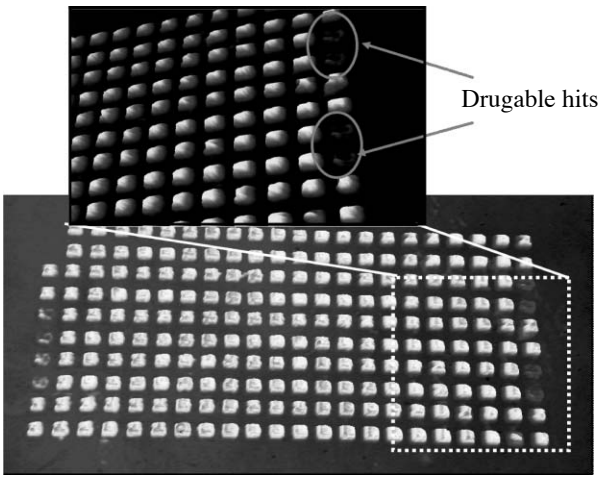

Figure 3. High-throughput drug screening using an SPR imaging protein chip system. The bright image indicates protein-protein interaction on a gold surface. Upon the binding of inhibitor to target protein, protein-protein interactions are disrupted, resulting in changes in SPR imaging signal intensity, darker image.

methods for the quantitative determination of biomolecular interactions, including DNA-DNA hybridizations and DNA-drug interactions[71]. The properties of DNA have been exploited for a variety of studies involving DNA-DNA hybridization. For example, with DNA-DNA duplexes, it has been determined that SPR imaging biosensors enable the monitoring of structural alterations imposed by single-base mismatches in kinetic analyses of DNA-DNA hybridization[72]. Another interesting application of SPR imaging systems involves the monitoring of the real-time affinity binding of target proteins to their cognate double-stranded DNA-coated chip surfaces in large-scale analyses. Maillart *et al*. have described an SPR imaging system-based HTS approach for multiple analyses of interactions between the p53 transcription factor and its corresponding cis-acting DNA elements[73].

The traditional well-based format using 96-well or 384-well microplates remains limited with regard to its ability to screen low-molecular-weight compounds, as a significant quantity of small compounds (chemicals or drugs) as well as their cognate target proteins are required for these assays. Thus, protein chip technology, which requires only a nanoliter-scale low volume sample with a few picograms of the target proteins, has been considered a promising method for HTS in drug discovery. In addition, more recently, a protein chip-based HTS assay combined with an SPR imaging system has been developed for the screening of anti-cancer drug candidates, as described by Ro *et al*.[74]. In that study, the interaction of the retinoblastoma tumor suppressor RB protein with the human papillomavirus (HPV) E7 protein was employed as a protein target, in order to determine whether or not the SPR imaging system made feasible screening for low-molecular-weight inhibitors that could block RB-E7 interactions. The RB-E7 interaction was evaluated by the array-spotting of the RB protein in the presence of PepC, the RB-binding peptide, in an array format on a gold chip surface. The SPR imaging results evidenced a concentration-dependent effect of PepC on the RB-E7 interaction, indicating that the SPR imaging-based HTS system may constitute a promising method, which may allow for high-throughput drug screening, potentially enhancing the speed of the drug discovery process.

Conclusions

The SPR biosensor has become a representative instrument in the fields of biomedical, biochemical, and biophysical research. In particular, biosensors have become user-friendly and common monitoring health care instruments, even for non-patient users. SPR biosensors provide a very useful method for the performance of proteomics research in the field of biomedical science. With the minimal detectable change in mass of 1 ng/mm^2, detection on a pico-molarity (10^{-12} M) scale is possible. This notion makes it possible to discover attractive small molecules as new drug candidates, and to develop a promising method for point-of-care diagnostics on the basis of the immuno-biosensor concept. Also, SPR provides a label-free system which helps the analyte to remain in its native conformation, and is also a non-invasive method which causes no damage to target analytes, thereby allowing for rapid and real-time detection. Moreover, another recent trend in drug discovery is the application of SPR imaging-based protein chip technology to high-throughput screening (HTS) efforts, as compared to traditional drug screening methods, owing to its simplicity, low cost, and high speed. Based on this recent tendency toward drug discovery, there is no doubt that SPR imaging-based HTS technology could potentially provide a versatile tool for the selection of useful small molecule inhibitors in a protein chip format.

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