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TRENDS

Surface plasmon resonance sensing: from purified biomolecules to intact cells

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Abstract

Surface plasmon resonance (SPR) has become a well-recognized label-free technique for measuring the binding kinetics between biomolecules since the invention of the first SPR-based immunosensor in 1980s. The most popular and traditional format for SPR analysis is to monitor the real-time optical signals when a solution containing ligand molecules is flowing over a sensor substrate functionalized with purified receptor molecules. In recent years, rapid development of several kinds of SPR imaging techniques have allowed for mapping the dynamic distribution of local mass density within single living cells with high spatial and temporal resolutions and reliable sensitivity. Such capability immediately enabled one to investigate the interaction between important biomolecules and intact cells in a label-free, quantitative, and single cell manner, leading to an exciting new trend of cell-based SPR bioanalysis. In this Trend Article, we first describe the principle and technical features of two types of SPR imaging techniques based on prism and objective, respectively. Then we survey the intact cell-based applications in both fundamental cell biology and drug discovery. We conclude the article with comments and perspectives on the future developments.

Keywords Surface plasmon resonance (SPR) · Biosensor · Label-free · Cell biology · Drug discovery

Introduction

The value of surface plasmon resonance (SPR) across multiple disciplines from basic researches to translational applications has been demonstrated since the invention of the first SPR-based biosensor in the 1980s [1, 2]. While label-based assays became mainstream technology in life science, SPR has made a splendid figure for manifold advantages, including real-time quantitative kinetic measurement with high temporal resolutions, single cell investigation with high spatial resolutions, noninvasive observation in native cells, compatibility with expanded devices, and most importantly, its intrinsic property of label-free [3–7].

Early attempts of SPR techniques for bioanalysis have been reported in numerous applications for molecules, such as interaction specificity and affinity, binding kinetics, and concentration determination of analytes. The binding kinetics of ligand-receptor or antibody-antigen could be analyzed with the corresponding optical readout [3, 4]. However, these biochemically based SPR assays rely on purified biomolecules and thus may not reflect the natural interactions in living beings. Besides, the purified proteins are heterogeneously expressed after labor-intensive purification procedures. Additionally, the throughput is limited by the signal acquisition component of the early SPR setups. Accompanied with the improvements on spatial and temporal resolutions of SPR imaging [SPRi) setups, both living cells and microarrays can now be studied [5–9]. Therefore, the SPRi technique has been rapidly applied in diverse biological areas, such as in situ binding kinetics [10], single bacterium analysis [11], cell-substrate interaction [12], cellular response to external stimuli [13] and drug discovery [14, 15]. Furthermore, a SPR microscopy (SPRM) system was configured to improve the spatial resolution and to eliminate image distortion recently [16–18].

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By using SPRM, cellular dynamic mass redistribution and even subcellular events were successfully investigated with unprecedented spatial resolution [19, 20].

In this Trend article, we first describe the principle and technical features of typical types of SPR imaging techniques. Then we survey its leading-edge applications from fundamental cell biology to pharmaceutical industry. We conclude the article with comments and perspectives on the future development of cell-based studies with SPR imaging techniques.

Typical types of SPR imaging techniques

SPR is the collective oscillations of free electrons that are excited at the dielectric–metal dielectric interface under Kretschmann configurations by a *P*-polarized light with certain incident angle (resonant angle). At the resonant angle, incident light is absorbed by surface plasmons, leading to a decrease in the reflected light intensity. The resonant angle is highly sensitive to the mass density of the surface layer in the medium-metal interface, which contains a wealth of information, such as molecular binding events taking place on or near the metal film (~200 nm), or the conformational change in the molecules bound to the film. These events can change the refractive index (RI) near the sensing surface as well. Without additional labels, researchers can study binding kinetics between a pair of biomolecules, such as protein–protein, lipid–protein, carbohydrate–protein, DNA–protein, DNA–DNA, and RNA–DNA [3, 4]. The ability of exploring living cells is crucial to cell biology and drug discovery. In the past decades, advances in both molecular biology and cell imaging techniques have made breakthroughs on multiplex areas. In parallel, SPR techniques have also been advanced to meet the increasing demands in characterizing molecules in living cells with high temporal and spatial resolutions, as well as with increasing throughput [5–7, 9, 21].

Among the basic components of SPR sensors, the optical coupling component plays a crucial role in the innovation of sensing styles from molecules to single living cell as classified in Fig. 1. In conventional SPR imaging systems, optical coupling components are mainly prisms with different refractive indices, which have limited spatial resolution. Yanase Y. et al. configured a prism-based SPRi setup as shown in Fig. 2 [22]. By replacing the prism with high numerical aperture objective, SPR microscopy (SPRM) achieves a spatial resolution up to the optical diffraction limit without image distortion, as shown in Fig. 3 [16–18]. It immediately leads our vision from the monitoring of molecular insentient interactions to the observation of whole cell dynamic mass redistribution [10, 18–20, 23]. Therefore, cellular and sub-cellular instantaneous activities could be explored whenever it comes to a stimulus, such as biological signal transduction, physiological microenvironment change, or drug exposure.

What is worth mentioning is that expanded devices made SPR imaging systems much more powerful. For example, by coupling with electrochemical impedance spectroscopy (EIS), plasmonic-electrochemical impedance microscopy (P-EIM) succeeded in an optical imaging of impedance. P-EIM maps cellular electrical polarizability and conductivity, which reflects the local information of cellular structure and ionic distribution [23–25]. The extensive optical and electrochemical data output by P-EIM could hardly be surpassed by any other microscopes till now. Besides, a surface plasmon-enhanced fluorescence microscopy (SPEF) was configured by a number of groups for several advantages, including the enhancement of fluorescence intensity by the resonance of plasmons on the metal surface, unchanged penetration depth of the evanescent field, and the lateral resolution [26, 27]. The SPR imaging data generated by SPEF configuration are much more comprehensive with fluorescent labels specific to certain cytoskeletons or functional proteins.

The detection depth of SPR sensors is generally around 200 nm from the metal surface, much smaller than the cell height [5–7]. Thus, changes of resonant angle from SPR sensors are much more sensitive to the area near the plasma membrane, whereas SPR sensors cannot detect the resonant angle changes among whole cells, especially in the upper area [5–7]. Fortunately, a type of electromagnetic waves, long-range surface plasmons, could be generated on a metal surface that is sandwiched between two dielectrics with equivalent refractive indices [28, 29]. The long-range surface plasmons resonance (LRSPR) sensors are able to provide both better evanescent field intensity and larger penetration depth compared with conventional SPR sensors. Thus, LRSPR sensors have the capacity for detecting biological events occurring inside living cells with penetration depth up to 1000 nm or more [28, 29]. Mejard and Thierry made a comparison between conventional SPR and LRSPR in 3T3 fibroblast cells cultured on SPR sensor chips. Important differences of intracellular responses resulting from biological stimulation have then been sensitively delved by LRSPR [29]. Furtheremore, a newly developed infrared SPR strategy is also able to penetrate deeply into the living cells by substitution of infrared light with the original visible light source [30, 31]. The penetration depth could reach up to 10 μm from the metal surface [29], and the cell radiation potentially caused in the visible light range of conventional SPR system may be reduced in the infrared SPR system [30, 31]. By cyclodextrins stimulation to HeLa cells cultivated on the sensing chip, infrared SPR effectively monitored dynamic cholesterol enrichment or depletion on cell membrane in a real-time, label-free, and noninvasive manner [30]. A mid-infrared range SPR efficiently detected the endocytic processes induced by transferrin in human melanoma cells [31]. These techniques give rise to an exciting new trend of cell-based SPR bioanalysis.

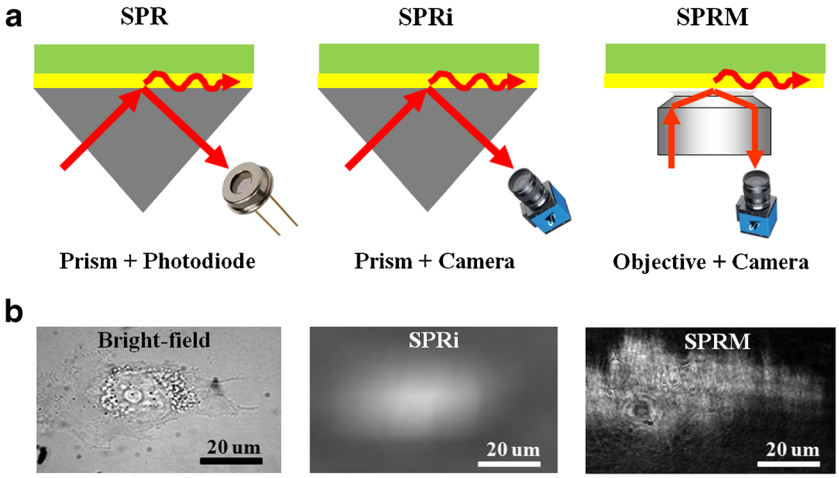

Fig. 1 Typical types of SPR techniques. **(a)** Three types of SPR setups invented in the past three decades. Imaging capability is incorporated by the introduction of a camera. Improved spatial resolution is achieved by replacing the prism with a high numerical aperture objective. **(b)** A bright-field image of a single cell acquired by normal optical microscope; SPRi static images with 1- to 5-fold magnification acquired by prism-based setups for the same cell; SPRM static images with 60- to 100-fold magnification acquired by objective-based setups for the same cell

Applications in cell biology

The cell is the functional minimum unit of living organisms, whether for *Escherichia coli* in Prokaryota or Homo sapiens in Eucaryotae. Understanding the cellular basic components and how cells work is fundamental to life sciences, including molecular biology, cell biology, physiology, and so on. There are increasing demands in label-free, noninvasive, and real-time observation of status and functions of living cells for basic research in medical fields such as physiological process,

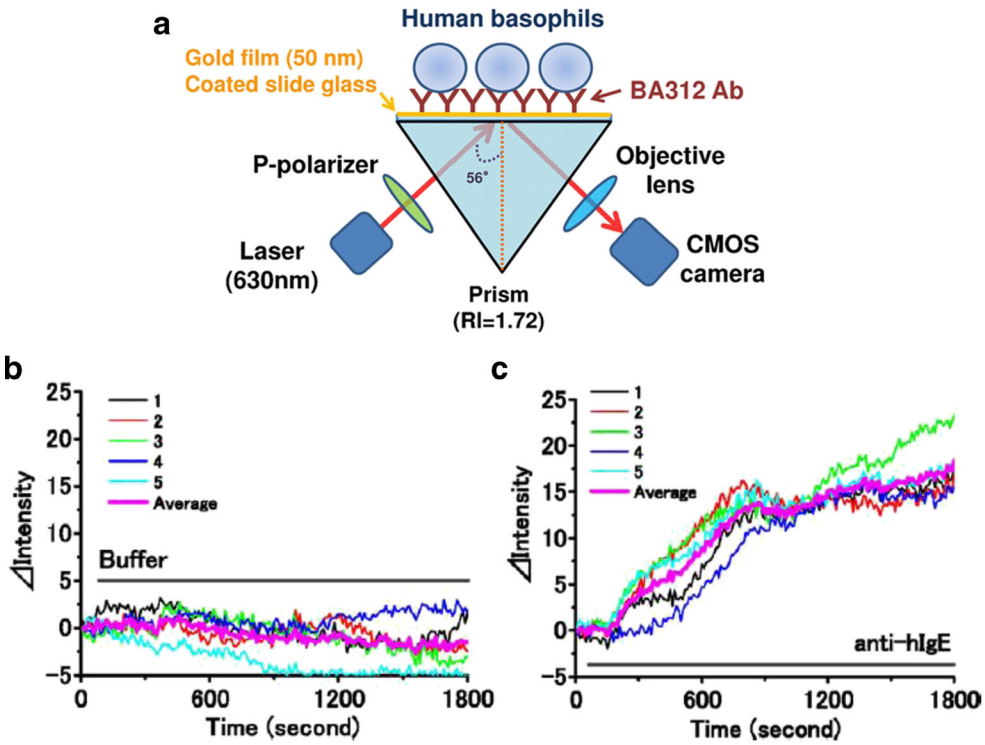

Fig. 2 A prism-based SPRi setup for cellular analysis. **(a)** The sensor is composed of a diode laser emitting at the wavelength of 630 nm, *P*-polarizer, S-LAL-10 glass prism, sensor chip (S-LAL-10) coated with gold film (50 nm), objective lens (2× or 4×), and CMOS camera. Basophils isolated from peripheral blood were fixed to the surface of sensor chip via BA312 antibody. The light was directed to the prism surface at the incident angle 56 degree. Two-dimensional images were created from the light reflected at the interface between gold film and glass using objective lens and CMOS camera. Living cells were visualized by the difference of RI between buffer solution and living cells. **(b)** and **(c)** Time course of RI changes in five randomly selected cells (samples 1–5) and their average (pink bold line) is plotted every 10 s. Similar results were obtained from three independent experiments [22]

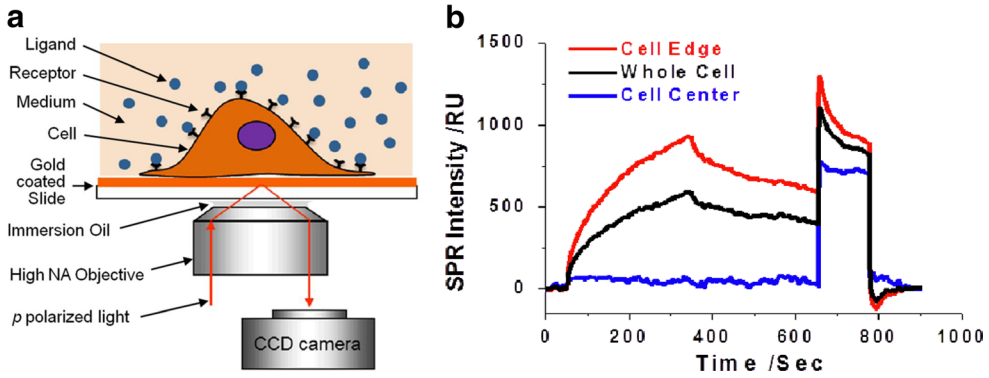

Fig. 3 An objective-based SPRM setup for cellular analysis. **(a)** A *P*-polarized laser beam is directed onto a gold-coated glass coverslip via an oil immersion objective to create surface plasmon resonance (SPR) on the gold surface, which is imaged with a CCD camera. Conventional bright field images of the same sample can also be taken via illumination from the top of the sample. Human epithelial SH-EP1 cells were attached on the gold coated slide before analysis. **(b)** Wheat germ agglutinin (WGA) as ligands could specifically bind to *N*-acetylglucosamine as receptors expressed on the cell membrane. The exposure of culture medium containing WGA to the SH-EP1 cells could results in local SPR intensities. SPR sensorograms of the entire cell region (black curve), cell edge region (red curve), and cell central region (blue curve) during the binding and dissociation of WGA [10]

disease progress, and clinical diagnosis. As listed in Table 1, SPR technology stepped into all of the medical fields mentioned above in the past three decades, and made extraordinary findings as the technology itself improved rapidly [5–7].

To date, two different assay types are commonly applied for cellular SPR analysis. The difference between these two types is whether cells are fluidic in the assays. Fluidic cellular SPR is the earlier analysis pattern for living cells. Antibodies specific to the antigens expressed on the surface of living cells are routinely immobilized on the surface of the sensor chip, and the antibodies would catch the cellular antigens when the mobile phase containing living cells or cellular components are flowing over. The cells or cellular components pulled down by antibodies lead to the changes in the resonant angle of SPR, and the signals are recorded and calculated as reflective index or SPR intensity. Both prism-based and objective-based SPR apparatus have been reported in this type of assays [36–38, 40, 41]. Macromolecules like proteins are massive enough to cause detectable changes of resonant angle. Immobilized cellular SPR is the other type of assay that antibodies or ligands solved in the mobile phase bind to the antigens or receptors expressed in the outer membrane of living cells immobilized on the sensor chip, or researchers take

Table 1 The applications of SPR techniques in the cell biology and drug discovery

Types of SPR	Optical coupling components	Signal acquisition components	Magnifications	Applications in cell biology	Applications in drug discovery
Conventional SPR	Prism	Photodiode	No spatial resolution	-	-
SPRi	Prism	Camera	1–5 folds	Subcellular activities [25, 32, 33], Interactions with ECM [12], Cell morphology [13, 34], Cell proliferation [35], Stem cells research [36–38], Cancer research [34, 39], Allergic disease diagnosis [22], Blood type tests [40], Cell sorting [41].	Efficacy evaluation [42], Drug resistant analysis [43], Drug delivery [44], Distribution and interaction kinetics of drug targets [45], Proliferation inhibitors [35], Drug induced secretion [46], Neuronal response to drug stimulation [47].
SPRM	Objective	Camera	60–100 folds	Subcellular activities [19, 23], Interactions with ECM [20], Cell proliferation and cancer research [10].	-

observations focused on internal/comprehensive events of the immobilized cells directly [10, 12, 13, 19, 20, 23, 25, 32–35]. In comparison with the primal SPR sensors based on prism and photodiode, SPR devices assembled with high speed camera and high numerical aperture objective are much more adept for immobilized cellular SPR assays owing to their superior temporal and spatial resolutions for resolving intact cells [10, 18–20, 23].

Activities of subcellular organelles in the living cells are exceptionally attractive to cytologist. Early studies based on SPR technology have made great efforts on exploring subcellular processes since 2002 [32]. Hide M. et al. applied the SPR based biosensor to study interactions between living mast cells and dinitrophenol-human serum albumin. They demonstrated that biologically strong and steady SPR signals were monitored in the IgE sensitized mast cells in a dose-dependent manner to the antigens [32]. Yanase Y. et al. studied the SPR signals for movements and membrane ruffling and cytoskeleton rearrangement of rat basophilic leukemia cells and mouse keratinocyte cells, as well as when activated by antigen and epidermal growth factors. They concluded that resonant angle changes reflect intracellular events to extracellular stimuli rather than changes in the size of the area to which cells adhere observed by ordinary light microscope [33]. Recently, by using a SPRM apparatus, Yang Y. et al. successfully tracked nano- and micrometer-sized subcellular structures in the native state of the cells with nanometer precision. Nanometer steps of organelle (e.g., mitochondria) transportation were observed along neurite microtubules in primary neurons, from which the 3D structure of neurite microtubule bundles was then reconstructed [19]. Furthermore, in order to identify cellular electrophysiological properties explicitly, Liu X-W et al. have developed a SPR imaging technique to study transient electrical activities in single neuron cell coupled with electrochemical impedance. This coupled technique is on the basis of converting electrical signals into plasmonic signals, which is sensitively recorded in a label-free manner. This technique would play a role in the study of bioelectric activities in basic cellular processes such as action potentials [25].

The mechanisms of interactions between cells and extracellular matrices (ECM) at a single cell level are essential to exploring various cellular activities, from cell adhesion, detachment, migration, and growth to cell differentiation. Since 1999, Giebel et al. have started employing SPR techniques to observe the contacts between cells and ECM on an aluminum-coated glass prism. In order to obtain resonance curves, cells were imaged at different angles of incidence. The distances between different cells and substrates were quantitatively analyzed [12]. By introducing hypertonic and hypotonic conditions artificially, the entire dynamic process of the cellular responses to extracellular osmotic pressure was studied on a SPRM device in 2012. We successfully measured the cell-substrate connections under label-free and noninvasive conditions. It could be considered as a promising method for studying the mechanisms of interactions between cells and ECM [20].

Cell morphology is critical in identifying the shape and size of cells. To investigate the mechanism of SPR signal changes in different cell induced by activation of epidermal growth factor (EGF) receptor, Hiragun T. et al. monitored a diversity of responses against EGF in various carcinoma cell lines by SPR. Different carcinoma cell lines displayed distinguishing changes of SPR signals and the pattern of SPR signals was independent of the concentration of EGF [34]. Yanase Y. et al. developed an SPRM to analyze refractive index of individual living cells and their changes upon stimuli. This SPRM setup could distinguish reactions of different types of cells co-cultured on a sensor chip and their responses to either specific or non-specific stimuli, such as antigen, phorbol ester, or epidermal growth factor, with or without their inhibitors [13].

Cell proliferation is the process that results in an increasing number of cells, and is commonly defined as the balance between cell divisions and cell death. Deng S. et al. designed a SPR time-lapse imaging system with a microfluidic device for long-term living cell monitoring. In this study, proliferation process of Hela cells was detected by SPRi and the SPR signals displayed in four phases with different growth rates [35]. Cell apoptosis is a process of programmed cell death accompanied by manifold morphological changes, including cell shrinkage, cell blebbing, nuclear fragmentation, and chromatin condensation. We observed the apoptotic process of cervical cancer cells with high spatial and temporal resolutions at sub-micron and millisecond levels, respectively. The dynamic images with high quality resolutions make it possible to resolve the sub-cellular structures and processes in real time but without labels [10].

Stem cells, which play a fundamental role in developmental biology, are able to differentiate into terminal cells or divide to more stem cells. Characterization of the lineages and exploring the differentiation process of stem cells are major projects to scientists in the fields of stem cell and developmental biology. Kuo YC et al. developed a SPR-based biosensing apparatus for real-time detection of osteogenic differentiation in live mesenchymal stem cells (MSCs). By coating OB-cadherin antibody on the sensing surface, the SPR signals are able to evaluate osteogenic maturation of MSCs in a noninvasive and label-free manner [36]. Specific membrane glycan patterns are classic characteristics for identification of stem cells. Nand et al. applied a SPRi device plus a lectin microarray for rapid and label-free profiling of cells including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and mouse embryonic fibroblasts (MEFs). The SPR affinity analysis of stem cells to eight different lectins was sensitive and reliable for the assessments of stem cell pluripotency [37]. The epithelial–mesenchymal transition is an essential process in tissue regeneration. The identification of cell differentiation at the early stage could be meaningful for predicting the regeneration of certain tissues. By fixation of

vascular endothelial cadherin (VE-cadherin) antibody on the surface of SPR sensor chip, Fathi et al. evaluated the differentiation course of human MSCs to endothelial lineage. The SPR signals boosted along with the growing expression level of VE-cadherin on the cell membrane in the differentiation process. It makes endothelial differentiation at the early stage sensible in a label-free form, whereas fluorescence microscopy and flow cytometry could hardly do so at the same time. These SPR techniques could accurately study intact stem cell differentiation but relying on purified antibodies [38].

Attempts that researchers explore the feasibilities of SPR biosensors for cancer diagnosis are carried out at living cell levels step by step. Specifically, overexpressed membrane proteins exhibit the hallmark of cancer cells and become more and more important in both cancer diagnosis and therapy. Recently, a SPRM based real time detection of membrane proteins was conducted by us in human epithelial SH-EP1 cells cultivated on sensor chips [10]. This plasmonic microscopy method precisely mapped real time distributions and in situ binding kinetics of different membrane proteins in single living cells without labels. The polarization of the membrane proteins on the cell surface during chemotaxis was studied at the same time [10]. Furthermore, Hiragun T. et al. detected the responses of various carcinoma cell lines stimulated by epidermal growth factor (EGF). They demonstrated that SPR biosensors would be developed for real time diagnosis of various cancers [34]. Moreover, Yanase Y. et al. made a conceptual improvement of SPR diagnosis from cancer cells in vitro to cancer tissues in vivo. By fixation of basophilic leukemia cells onto the tip of an optical fiber coated with gold thin film, a steady increase of SPR signals was monitored when the cells were stimulated by an antigen [39].

Besides investigation of cell morphology, adhesion, proliferation, differentiation, apoptosis, sub-cellular activities, and cancer diagnosis reviewed above, SPR biosensors are valuable for many other studies in life science. Representative cases are the applications for blood type tests [40], allergic disease diagnosis [22], cell sorting [41], and so on.

Applications in drug discovery

The path a pharmaceutical drug travels from laboratories to medicine cabinets is distant, usually 10 years and more. It is noteworthy that the high attrition rates in drug research and development are largely due to poor pharmacokinetic or pharmacodynamic profiles [15, 48]. In order to discover ideal drug candidates with high efficacy, low toxicity, and proper bioavailability, a diversity of screening technologies have been enrolled in the early phase of a drug discovery journey, such as fluorescence resonance energy transfer (FRET), homogeneous time-resolved fluorescence (HTRF), multi-color fluorescent chemical dyes, and stable reporter protein expression cell lines [15, 48]. Although label-based strategies, both chemically/radioactively linked labels and genetically engineered fluorescent/ luminescent fusion proteins, are commonly adopted by both big pharmaceuticals and biotech companies, advances in SPR technology enabled many of these studies to be carried out with high spatial and temporal resolutions, and more importantly in a native state without labeling [14, 35, 42–47, 49–59].

Pharmacokinetics is dedicated to determine how the body affects a specific chemical after administration, including absorption, distribution, metabolism, and excretion. Since 2000, scientists started to use SPR technology for studying drug-like properties of chemicals [58, 59]. More than 10 years have passed, and SPR technology has now set foot in calculation or prediction of many pharmacokinetic properties, including the affinity and the association/dissociation kinetics with a specific target, the half-life (t1/2) of drug-serum protein complexes, cell membrane permeability, drug–drug interactions, and even structure-activity relationship (SAR) studies [14]. These studies are mainly limited to biomolecular binding assays out of living cells, which monitor the local refraction index changes on the sensing surface when flowing small molecular compounds bind to the immobilized purified membrane liposomes or serum proteins [14, 58, 59]. Instead of immobilization of proteins in binding assays, the whole living cell adhesion on the sensing surface would provide on-site signals from drug–protein interactions and drug–drug interaction. Therefore, the pharmacokinetic parameters delivered by the cell-based assays would be more accurate and reliable than the biochemically binding assays. As we focus on the cell-based SPR studies, these assays without living cells for pharmacokinetic researches will not be comprehensively reviewed here.

Traditional assays for pharmacodynamic evaluation rely mainly on the use of chemical dyes and genetically engineered reporter cell lines to investigate pharmacodynamic profiles [15, 48]. However, any type of labels could harbor side effects on the interactions being measured and would lead to false positive or false negative results, which are fatal to drug candidates. Furthermore, labeling needs additional assay development time/steps as well as additional samples consumptions. Increasing emphasis is being placed on SPR platforms for drug screening techniques in label-free patterns. Numerous drug targets have been reported by using the binding assays in the absence of living cells, like G protein-coupled receptors (GPCRs) [55, 56], vascular endothelial growth factor (VEGF) [50], Kelch-like ECH-associated protein 1 (KEAP1) [51], tumor necrosis factor alpha (TNFα) [52], α-glucosidase (GAA) [54], glucagon-like peptide 1 (GLP-1) [49], signal transducer and activator of transcription 3 (STAT3) [57], and programmed death-ligand 1 (PD-L1) [53]. The indications vary from cancer, neurodegenerative disease, and diabetes to viral infections [18, 35, 42, 45–47, 49–57]. Just as in pharmacokinetic studies, implementation of SPR techniques in the biomolecular binding assays exceed that in cell-based assays for several years. However, introduction of cell-based SPR assays for pharmacodynamic

evaluation has been reported recently by different research groups for a great variety of drug targets, as listed in Table 1, [35, 42–47].

In an early case for drug screening in 2010, Nishijima H. et al. developed a quantitative assay based on a SPRi setup for efficacy evaluation of anti-liver cancer drugs [42]. HepG2 cells were cultured on a sensor chip in this assay, and the differential SPR angle changes depended on the concentration of anti-cancer drugs. The comparison between the changes of SPR angle and cell viability calculated by trypan blue staining showed a good correlation (R = 0.928) [42]. In order to map the distribution and interaction kinetics of EGFR in living cells, both EGFR-positive cell lines (A431, HeLa, and A549) and EGFR-negative (HEK 293) cell lines, cultured on sensing surface, were investigated by Zhang F. in 2015 [45]. The SPRi signals were triggered by anti-EGFR monoclonal antibodies. The results demonstrated that SPRi is a useful tool for label-free quantification of membrane protein distribution and ligand binding kinetics in a single cell [45]. Furtheremore, real-time detection of cell behaviors under different doses of two cytotoxic chemotherapy agents, paclitaxel and cisplatin, was successfully performed in a SPRi system integrated with a two-compartment microfluidic component. The SPR signals revealed inhibitions of Hela cell proliferation by two drugs with distinct kinetics [35]. By immobilizing both prostate cancer cells and antibodies against prostate specific antigen (PSA) and beta-2-microglobulin (B2M) on the senor chip, Berthuy et al. made an achievement of detection for drug induced cellular secretion on a gold surface. Following dihydrotestosterone induction, PSA and B2M secreted by prostate cancer cells were successfully detected on the same chip, and different kinetics of these two secreted proteins were then precisely determined in real time [46]. To explore the pharmacodynamic responses of neuronal cells to nerve growth factor (NGF) and muscarine, a nonselective agonist of the muscarinic acetylcholine receptor, Mir et al. cultured neuronal precursor cell lines (PC12) on a sensing chip. The signals generated by a two-dimensional SPRi system revealed that PC12 cells treated with NGF showed a remarkable enhancement of SPR response to stimulation by muscarine [47].

SPR techniques were also applicable for drug resistant analysis, especially for large biomolecules studies [43]. In an assay involved with a perfusion device, SPRi signals were recorded from the binding of the drug Herceptin to different lines of cancer cells (drug Herceptin-sensitive and -resistant cell lines; drug target Her2-positive and -negative cell lines) cultured on the sensing surface. We measured the in situ Herceptin-Her2 binding kinetics in individual intact cells for the first time, and observed significantly weakened Herceptin-Her2 interactions in Herceptin-resistant cells, compared with those in Herceptin-sensitive cells. The results indicated that the steric hindrance of Mucin-4, a membrane protein, was responsible for the altered drug-receptor interactions. The effect from a third molecule to the drug-receptor bindings could barely be investigated by traditional purified protein methods, illustrating the importance of the intact cell-based label-free analysis by SPR techniques [43].

Nanomaterials as drug carriers could assist in navigating through physiological barriers in human bodies. Determining how the nano-conjugates interact with membrane receptors has aroused great interest in pharmaceuticals. By a prism-based plasmonic imaging configuration, Yin L. designed a SPRi study of the binding kinetics of Herceptin-conjugated gold nanoparticles with Her2-expressing cancer cells attached on the sensing surface. The findings would assist pharmacologists in optimizing the design and selection of nanomaterials for drug delivery [44].

Outlook

SPR technology has served life science for more than three decades, and investigated the fields of cell biology as well as drug discovery and development from a brand new point of view. Real-time quantitative analysis in a label-free manner with high spatial resolutions makes SPR superior to many other techniques, especially for bioanalysis. Taking fluorescence spectroscopy for example, additional fluorescent labels could affect the natural functions of certain targets and even generate false positive or false negative signals; fluorescent dyeing is usually performed with invasive processes in living cells and even requires dehydration and fixation, leading up to dead cells in advance; and fluorescent labeling needs extra assay development time/steps as well as sample consumptions. However, the applications of SPR technology has also been limited in some aspects, including sensitivity, resolution, and biological data interpretation. Fortunately, sensitivity may be increased by system optimization because sensitivity is mostly determined by the stability of setups, including light source, temperature control, as well as environmental and mechanical noises; the resolution could be improved by advanced instrumentation [60] and image processing [61]; and the data could be well interpreted case by case with proper experimental design, such as settings of control groups, introduction of specific antibodies, or antagonists/agonists.

Although great contributions have been made, as the application of SPR technology advances from purified biomolecules to individual intact cells, more scientific questions need to be answered. While previous works usually recorded signals in whole-cell and even whole-surface fashion, new attempts could focus on the optical changes from subcellular organelle activities such as mitochondria [19]. Former SPR-based pharmacokinetic studies were commonly operated by chemical or immunological binding assays in molecular level; in the nest stage, more attention should focus on cellular or subcellular pharmacokinetic assays Pharmacodynamic and toxicodynamic evaluation by SPR in a higher throughput would be more helpful to pharmacologists, when combining with components like microfluidics and

automatic liquid handling. Unknown substances could be identified by SPR likewise with the help of instruments like mass spectrometry and Raman imaging. Whether electrophysiological properties, such as action potential and electrophysiological communications, of cardiomyocytes or neurons could be probed is still a challenge for the present capacity of SPR techniques, even coupled with electrochemical impedance and other devices. In 2016, by right of accuracy, stability, and reproducibility, SPR technique has been collected by the part of immunological test methods, general information, United States Pharmacopoeia, and also part of biotechnological/biological products, general information, Japanese Pharmacopoeia. Even so, SPR instrumentation, including software, should be regulatory compliant (like 21 CFR Part 11) and should be amenable to validation. These requirements are important because they help ensure the integrity of both data acquisition and data evaluation for the pharmaceutical industry.

Technically, the sensing range of SPR technique is usually less than 500 nm from the surface where cells attached. Although the range has been lengthened by LRSPR with a better evanescent field penetration depth into the sensing area about 1000 nanometers, it could rarely reach the top of every individually cultured cell from different tissues or organs. On the other side, the sensing sight of SPR technique is insufficient for spotting most small molecules. Taking an example in drug discovery, SPR signals are limited from interactions between large biomolecules, such as antibody-antigen, ligand-receptor, or the movements of large molecules themselves. Small molecules, like low molecular weight chemical compounds, are mainly investigated in an indirect manner. When antagonists block ligands binding to receptors, SPR signals are generated from the larger ligands rather than the smaller antagonists. With technical breakthrough of both sensing range and sensitivity in the future, we expect that a vast scientific territory with broad applications can be explored during the adventure of SPR technology moving from purified molecules to intact cells.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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