

Looking towards label-free biomolecular interaction analysis in a high-throughput format: a review of new surface plasmon resonance technologies

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Surface plasmon resonance (SPR) biosensors have enabled a wide range of applications in which researchers can monitor biomolecular interactions in real time. Owing to the fact that SPR can provide affinity and kinetic data, unique features in applications ranging from protein–peptide interaction analysis to cellular ligation experiments have been demonstrated.

Although SPR has historically been limited by its throughput, new methods are emerging that allow for the simultaneous analysis of many thousands of interactions. When coupled with new protein array technologies, high-throughput SPR methods give users new and improved methods to analyze pathways, screen drug candidates and monitor protein–protein interactions.

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Introduction

Surface plasmon resonance (SPR) is an optical biosensor technique that measures molecular binding events at a metal surface by detecting changes in the local refractive index. The depth probed at the metal–aqueous interface is typically 200 nm [1], making SPR a surface-sensitive technique ideal for studying interactions between immobilized biomolecules and a solution-phase analyte. SPR offers several advantages over conventional techniques such as fluorescence or ELISA (enzyme-linked immunosorbent assay). First, because the measurements are based on refractive index changes, detection of an analyte is label free and direct. The analyte does not require any special characteristics (scattering bands) or labels (radioactive or fluorescent) and can be detected directly,

without the need for multistep detection protocols (sandwich assay). Second, the measurements can be performed in real time, allowing the user to collect kinetic data, as well as thermodynamic data. Last, SPR is a versatile technique, capable of detecting analytes over a wide range of molecular weights and binding affinities [2]. Because of its unique features, SPR has become a powerful tool for studying biomolecular interactions. For example, SPR has been used to investigate protein–peptide interactions [3], cellular ligation [4], protein–DNA interactions [5,6], and DNA hybridization [7,8]. At the current time, most commercially available SPR sensors offer a small number of independent sensing channels (<20). Such systems are very useful for performing detailed studies on a small set of analytes, but are not practical for high-throughput applications.

An emerging technology within the proteomics arena, which is beginning to build a presence in the marketplace, is planar protein arrays. The goal of these arrays is to take existing proteomic methods (ELISA, for instance), which have limited throughput, and make them parallel. Based on the pioneering work from Snyder's laboratory [9,10], a handful of commercial protein arrays are available. Most notably, Invitrogen currently offers its ProtoArrayTM line of high-density protein arrays, and Sigma has begun to produce antibody arrays. A promising new method for generating a protein array utilizes *in situ* protein expression and capture from cDNA on a chip surface [11^{**}]. Although not yet to market, this method provides a powerful new tool by which a whole proteome, or some significant subset, can be generated on one array. Regardless of the content or production method, all of these arrays rely on standard labeling techniques and give only end-point determination (i.e. no kinetic data).

The recent development of SPR imaging (or microscopy) combines the advantages of traditional SPR (kinetic and affinity data) with high-throughput capabilities, thereby allowing researchers to simultaneously monitor thousands of biomolecular interactions. When combined with protein arrays, SPR imaging technology has the potential to become an invaluable tool for a broad range of applications that require high-throughput analysis of biomolecular interactions, such as proteomic analysis, drug discovery and pathway elucidation.

Theory and instrumentation

SPR exploits surface plasmons, special electromagnetic waves that can be excited at certain metal interfaces, most notably silver and gold [1]. When incident light is coupled with the metal interface at angles greater than the critical angle, the reflected light exhibits a sharp attenuation (SPR minimum) in reflectivity owing to the resonant transfer of energy from the incident light to a surface plasmon. The incident angle (or wavelength) at which the resonance occurs is highly dependent upon the refractive index in the immediate vicinity of the metal surface. Binding of biomolecules at the surface changes the local refractive index, which results in a shift of the SPR minimum. By monitoring changes in the SPR signal, it is possible to measure binding at the surface in real time.

Traditional SPR spectroscopy sensors, which measure the entire SPR curve as a function of angle or wavelength, have been widely used, but offer limited throughput. The development of SPR imaging [12,13] allows for the simultaneous measurement of thousands of biomolecular interactions. Typically, an SPR imaging apparatus consists of a coherent p-polarized light source expanded with a beam expander and consequently reflected from an SPR active medium to a detector. A CCD (charge-coupled device) camera collects the reflected light intensity in an image. SPR imaging measurements are performed at a fixed angle of incidence that falls within a linear region of the SPR dip, such that changes in light intensity are proportional to the changes in refractive index caused by binding of biomolecules to the surface. As a result, gray-level intensity correlates with the amount of material bound to the sensing region (Figure 1) [14,15].

Numerous studies have been devoted to developing SPR imaging technology, and a handful of instruments are available commercially. An instrument employing a broad-band light source combined with Fourier-transform algorithms was developed by Corn's laboratory and later commercialized by GWC Technologies [16]. HTS

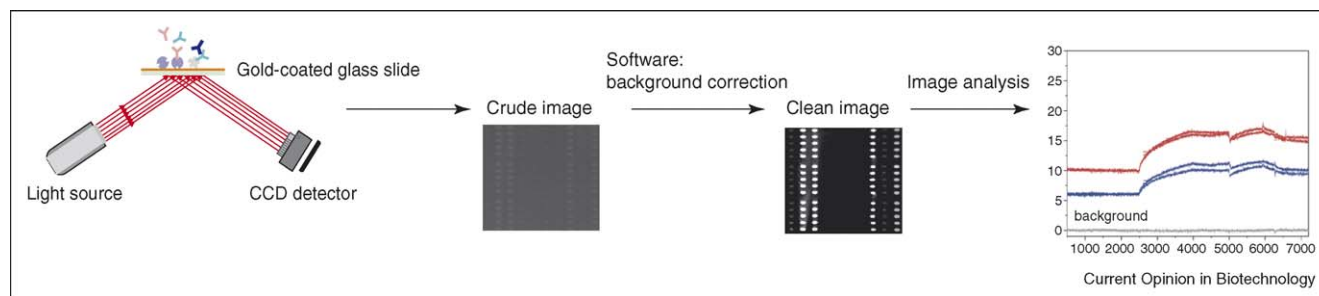
Biosystems designed an SPR imaging platform utilizing grating-couplers, which has since been purchased by BIAcore [17,18]. Systems have been reported using various light sources, including a broad-band light source [19,20], an LED [21], and a He-Ne laser. [15] Other researchers have also reported the use of SPR imaging systems as a microarray reader [22], and have described their use in combination with fluorescence labeling [23].

One of the factors determining the sensitivity of an SPR imaging system is the intensity of the light source. The signal strength from the metal surface is linearly proportional to the incoming light strength, so a laser light source is preferred over LEDs and halogen lamps. However, expanding a spot Gaussian profile from a laser source using optical elements does not necessarily provide homogeneous illumination, which results in signal variation across the spot (i.e. sensing area). This requires a background correction that can limit the sensitivity of detection. Additionally, the interference patterns, which are inherent to coherent laser sources, can also limit the resolution and sensitivity of spot detection. Taking advantage of Lumera's proprietary technology, we have developed the ProteomicProcessorTM, an SPR imaging instrument that uses a scalable light source engine to project laser power to the designated area. This approach provides a more flexible, homogeneous and significantly larger illumination area without generating any interference patterns. The spatial resolution has been experimentally measured to be approximately 10 μm , giving the capability to resolve more than 10 000 spots in a 1.4 cm^2 microarray. Figures 2 and 3 show a demonstration protein/antibody array where it was possible to acquire real-time binding curves and calculate binding and dissociation constants.

Applications

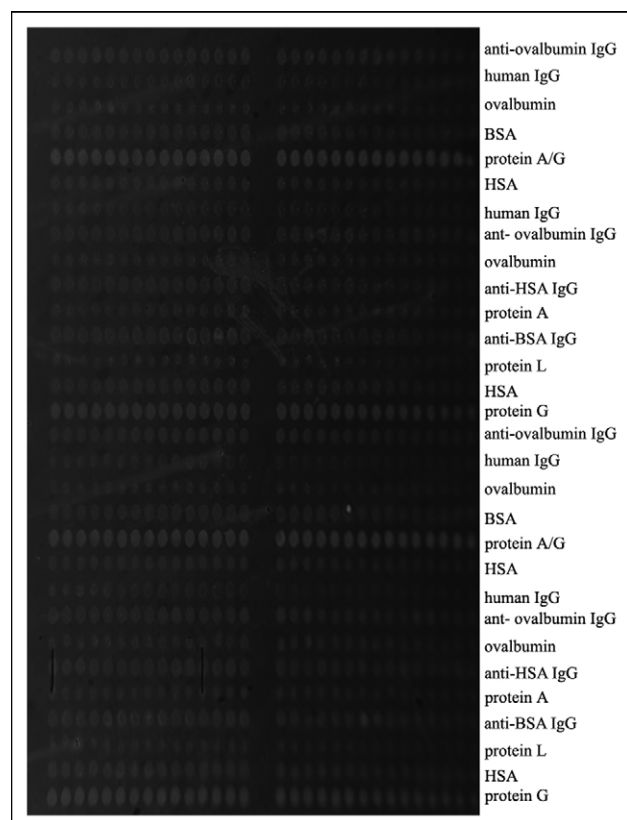
The past few years have seen dramatic growth in the number of studies reporting the use of SPR technology, with over 1000 new publications each year. For a detailed

Figure 1



SPR imaging. A light source projects light onto a gold surface, on which a biomolecular interaction is occurring, and the reflected light is imaged onto a CCD detector. This process is performed over a period of time and a collection of images obtained. A background correction is performed using software to generate a clean image, from which binding curves are generated.

Figure 2



An antibody/protein microarray containing a series of immobilized proteins, imaged via Lumera's ProteomicProcessor™ SPR. The image is a snapshot at a point in time as the array is being probed with anti-HSA. The spots are $\sim 150 \mu\text{m}$ in diameter.

review of the SPR literature, see the annual reviews published by Myzyska's group [24,25,26]. Highlighted below are a few select applications where the use of SPR has provided unique data that might not be accessible by traditional methods.

Drug screening

SPR is capable of detecting interactions between small molecules and immobilized protein targets [27,28], making it a useful tool for screening drug candidates. For example, Rich *et al.* [29] thoroughly characterized the binding of the small molecule warfarin to immobilized human serum albumin (HSA) and showed that the measurements correlated well with solution-phase data. On the basis of the results, they developed a generalized method for screening and evaluating interactions between small molecules and HSA. Researchers from the same group also measured the binding interactions of small molecules to the ligand-binding domain of a human estrogen receptor [30]. Through detailed measurements of the association and dissociation rate constants, the authors were able to clearly distinguish between specific ligands of the receptor and nonligands.

Cell signaling pathways

Transforming growth factor- β (TGF- β) plays a role in many cell signaling processes and its overexpression has been linked to many human pathologies, including fibrotic disease and metastasis [31]. TGF- β signaling is mediated through its interaction with cell-surface receptors RI, RII and RIII. De Crescenzo and colleagues used a *de novo*-designed heterodimerizing coiled/coil system [32] to induce dimerization of the cell-surface receptors, and utilized SPR to study how the dimerization affected binding to TGF- β [33,34]. The use of SPR allowed the authors to calculate kinetic and thermodynamic constants. On the basis of these calculations, they concluded that dimerization of the cell-surface receptors stabilized the interaction with TGF- β .

Kinase analysis

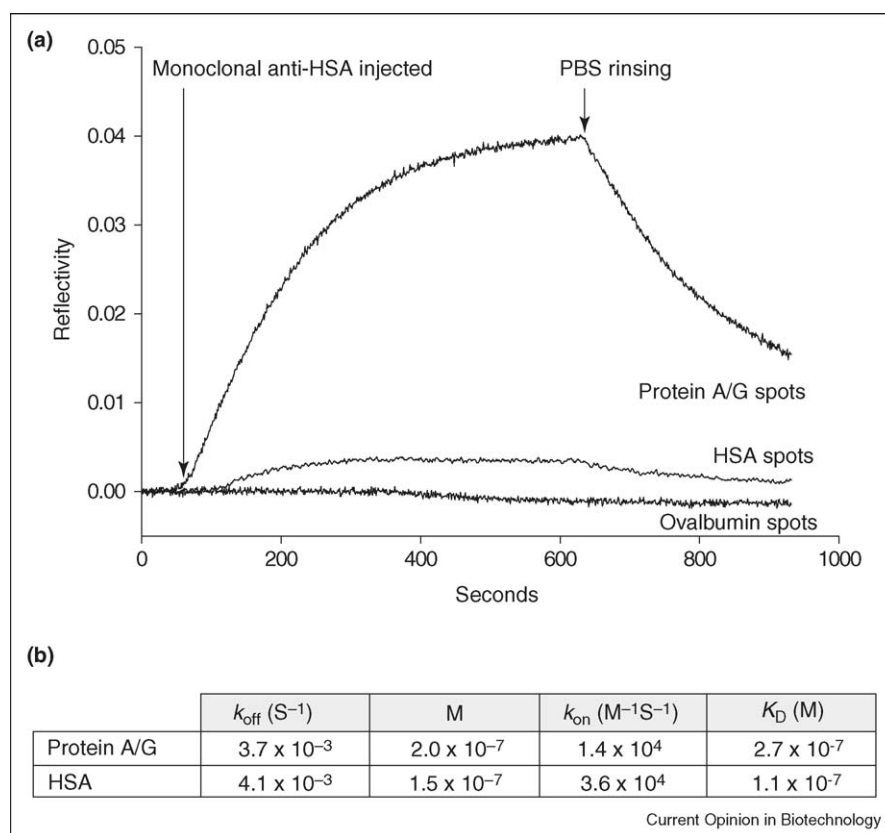
Human protein kinases have a central role in regulating a wide variety of signaling pathways, which may contribute to several diseases such as diabetes and cancer. As such, kinases are intriguing drug targets, resulting in a strong interest in identifying new kinase proteins, inhibitors and substrates. Although several high-throughput kinase microarray studies have been reported [9,10], they rely on traditional labeling techniques that only provide end-point analysis. By contrast, SPR has been used to fully characterize the affinities, kinetics, and thermodynamics of a handful of smaller kinase systems [3,35]. For example, Casper *et al.* [36] investigated the binding of small-molecule inhibitors to p38 α and, in a similar fashion, Nordin and coworkers [37] studied small-molecule binding to a set of eight different serine/threonine and tyrosine kinases.

Antibody development

Therapeutic antibodies for the diagnosis and treatment of cancer represent one of the fastest growing segments of the pharmaceutical market, but their application has been limited because of problems with immunogenicity. In recent years, SPR has proven a valuable tool for evaluating therapeutic antibodies. For example, Ritter *et al.* [38] used SPR to measure the antibody response in the serum from 44 patients that had been treated with humanized anti-A33, an antibody that targets colon cancer. In another report, Gonzales *et al.* [39] used an SPR-based competitive assay to compare the immunogenicity of a set of genetically engineered variants of a potential therapeutic antibody against TAG-72, a protein expressed by a variety of carcinomas. In both cases, the researchers were able to directly test patient serum for an immune response to the therapy, without the need for labels or secondary reagents. These results suggest that beyond being used as a tool for developing therapeutic antibodies, SPR also has the potential to measure treatment efficacy.

Phage display has proven to be a powerful tool for the development of large antibody libraries. Evaluation of

Figure 3



The injection of monoclonal anti-human serum albumin (anti-HSA) and phosphate-buffered saline (PBS) across the 1032 spot array shown in Figure 2. (a) Three select curves demonstrate the selectivity of anti-HSA to various well-studied proteins (protein A/G, HSA and ovalbumin). As expected, HSA has the fastest K_{on} , whereas protein A/G is second and ovalbumin does not bind. (b) Kinetic constants calculated from the kinetic curves shown in (a).

such libraries requires extensive screening, which can be labor-intensive and time-consuming. Recently, Wassaf *et al.* [40[•]] developed an SPR-based array for screening a library of Fabs generated against human tissue kallikrein 1 (hK1), a serine protease linked to inflammation. The authors printed 96 unique Fabs (in triplicate) per array, and employed SPR to simultaneously measure kinetic constants. In total, they ranked almost 200 Fabs by their affinity towards hK1. In addition, SPR measurements were used to classify the Fabs according to their ability to recognize different active sites.

Conclusions and future directions: merging protein arrays with surface plasmon resonance

Realizing the full potential of SPR imaging will require seamless integration of SPR with protein array technology. Towards this goal, Lumera is marrying protein arrays with high-throughput SPR based on the combination of its NanoCaptureTM-HPT peptide tag and ProteomicProcessorTM SPR tool. NanoCaptureTM-HPT, a highly selective capture mechanism that is

compatible with both printed and *in situ* expression arrays, involves the highly efficient and specific ($K_{\text{d}} \sim 60$ pM) interaction between two synthetic peptide coils. Expressed proteins are terminated with one peptide coil (E Coil), while the surface is modified with the other peptide coil (K Coil). Interaction between the two coils results in selective protein immobilization on the array surface.

As protein arrays emerge as a viable technology, it is clear that high-throughput SPR techniques are crucial to enabling their full value. We believe that integration of these two technologies will not only accelerate discovery in existing applications, but will also play a significant role in driving the development of new applications.

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