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Interface Design and Multiplexed Analysis with Surface Plasmon Resonance (SPR) Spectroscopy and SPR Imaging

Matthew J. Linman, Abdenmour Abbas, Quan Cheng*

Department of Chemistry, University of California, Riverside, California 92521

Abstract

Ever since the advent of surface plasmon resonance (SPR) and SPR imaging (SPRi) in the early 1990s, their use in biomolecular interaction analysis (BIA) has expanded phenomenally. An important research area in SPR sensor development is the design of novel and effective interfaces that allow for the probing of a variety of chemical and biological interactions in a highly selective and sensitive manner. A well-designed and robust interface is a necessity to obtain both accurate and pertinent biological information. This review covers the recent research efforts in this area with a specific focus towards biointerfaces, new materials for SPR biosensing, and novel array designs for SPR imaging. Perspectives on the challenges ahead and next steps for SPR technology are discussed.

1. Introduction

Originally popularized by Raether in the late 1980s,¹ surface plasmon resonance (SPR) is a label-free optical technique widely employed by chemists, biologists, and engineers alike. For analytical chemists in particular, SPR is greatly useful as a surface-sensitive technique that can be utilized to monitor chemical and biological interactions in real time. The real-time aspect of SPR measurements gives the technique a distinct advantage over endpoint binding assays, which are limited and have been gradually replaced by SPR since its inception in the 1990s. The phenomenon of surface plasmon resonance occurs at a metal/dielectric interface where one of the biological binding partners is immobilized on a metal surface (most often gold but can also be silver) while the other binding partner is allowed to flow across the sensing interface. SPR spectroscopy monitors the changes in refractive index occurring at the metal surface upon interaction between the two bio-specific ligands. A sample biosensor setup with a real-time sensorgram is depicted in Figure 1. All analyses require no labels, thus precluding the use of convoluted and sometimes disruptive labeling chemistry found in fluorescence methods. For a more comprehensive description of SPR theory there are a number of reviews available.^{2, 3} Given the clear analytical advantages SPR holds over many other optical methods, the use of surface plasmon resonance has grown substantially in recent years. Just in the last two years according to SciFinder Scholar 3,000 articles and reviews have been published on surface plasmon resonance analysis.^{4, 5}

*Corresponding author: Quan Cheng, Tel: (951) 827-2702, quan.cheng@ucr.edu, Fax: (951) 827-4713.

Despite the advantages as a label-free technique, SPR is a nonselective detection method. Nearly any binding moiety on the surface changes the local refractive index and thus the SPR signal. Therefore, reproducible and well-understood surface chemistry to create a selective sensing interface is an important research area for generating meaningful results and understanding biological interactions. There have been annual reviews on SPR application by the Myszka group at Utah and others as well.^{3, 4, 6} In this review, we will focus on the latest work involving rapid surface prototyping and unique designs for sensing of biologically significant molecules with SPR. In addition, we will highlight some of the exciting novel SPR substrates designed to improve existing gold planar platforms.

While highly useful for determining a variety of important kinetic and affinity parameters of biological interactions, one major drawback of SPR is its low-throughput nature. This problem has been largely circumvented with the advent of SPR imaging or SPRi technique. A comprehensive review of SPR imaging has appeared recently.⁷ Rather than a scanning-angle or scanning-wavelength measurement commonly employed in SPR spectroscopy, SPRi generally measures at a fixed angle where differences in reflectivity are monitored over time with both images and spectroscopic data, thus allowing for multiplexed detection for high-throughput bioanalysis, as shown in Figure 2. This review will also cover unique interface and array designs for SPR imaging that aim at advancing multiplexed analysis. Novel array fabrication methods will be highlighted as well, which may ultimately shape the future of high-throughput bioanalysis. In addition, new SPR and SPRi instrumentation with specific focus on design features for improving existing SPR/SPRi biosensor technology will be reviewed.

2. Interface Design for Rapid Sensing of Biologically Significant Molecules

2.1 Food and Bacterial Sensor Developments with SPR

The detection of bacterial pathogens and related protein toxins in food matrices is of extreme importance to human health. While novel interface design is important, food matrices often present a significant obstacle in the realization of reliable quantitative detection. Recent applications in this area have been extended to in-depth microbial analysis including fingerprinting and serotyping of the pathogens. Mazumdar *et al.* reported the use of an SPR-based sandwich immunoassay for serotyping *Salmonella*.⁸ The *Salmonella* are captured on the chip using a polyclonal antibody, and SPR sensorgrams are obtained for the immunoreactions of the somatic (O) and flagellar (H) surface antigens of the captured bacteria to their respective antibodies. This group was able to completely serotype *Salmonella Enteritidis* using this SPR-based method. In addition, *Salmonella* belonging to serogroups B, C and D were successfully assigned to their respective serogroups. This work is important because it is quantitative and not prone to false-positives as often seen in the standard slide agglutination test (SAT) for bacterial pathogens. Another area of food safety importance is the detection of toxins that cause food-borne illnesses. One example is parasitic shellfish poisoning (PSP) from shellfish, which is transferred through the food chain.⁹ Human and mammalian consumption of shellfish contaminated with PSP toxins can result in illness or death if exposed to sufficient levels. PSP toxins are neurotoxins that bind

to voltage-dependent sodium channels, resulting in the blockage of ion transport, which may lead to paralysis followed by death. An SPR bioassay was developed using the carboxymethylated dextran (CM5) chip to bind saxitoxin binding protein in a quantitative manner.⁹ Detection capabilities fall well within governmental standards and also coincide with other analytical results based on ELISA.

In other food related work a group recently detected colostrum in bovine and caprine milks by identifying IgG.¹⁰ Colostrum, which is detected by the presence of IgG, is a biomarker for bovine pregnancy and is essential for dairy industries to avoid negative economic consequences. In their assay a CM5 chip from Biacore was used to immobilize the IgG antibody and quantitative detection of IgG was performed at the low ng/mL level. Raz *et al.* has focused on food safety to specifically monitor antimicrobial drug residues in foods. This group developed a microarray-based method with SPR and SPRi detection for quantitative and simultaneous immunodetection of different antibiotic residues in milk.¹¹ Model compounds from four major antibiotic families were detected using a single sensor chip by immobilizing corresponding antibodies for the antibiotics. By multiplexing seven immunoassays in a competitive format, the authors were able to measure all the target compounds at parts per billion (ppb) levels in buffer and in 10×diluted milk. Sample preparation for this method is minimal and it is compatible with commercial instrumentation, making this potentially useful for a high-throughput screening method for antibiotics and other significant biomolecules in milk in a label-free fashion.

2.2 Lipids, Carbohydrates, and Aptamers

Lipids and carbohydrates make up some of the most important biomolecules often implicated in biological recognition events. For instance, carbohydrate-protein interactions play key roles in modulating intracellular traffic, endocytosis, cell-cell recognition, and signal transduction processes. Improper or mal-function of these interactions has been linked to a number of disease states including cancer. Thus their analysis and detection is vital to human health. The label-free nature of SPR makes it an ideally suited analytical technique for this application. However, the weak interaction nature of these species demands careful and effective design of the interface before meaningful results can be extracted. Our group has reported the fabrication of a novel sensing interface using biotinylated sialosides for probing lectin-carbohydrate interactions with SPR spectroscopy.¹² A diagram of our biointerface is shown in Figure 3. Briefly, the attachment of carbohydrates to the surface using biotin-NeutrAvidin interactions and the implementation of an inert hydrophilic hexaethylene glycol spacer (HEG) between the biotin and the carbohydrate has been utilized in our work. This approach resulted in a well-defined interface, enabling desired orientational flexibility and enhanced access of binding partners. The specificity and sensitivity of this interface was demonstrated through lectin binding and altering carbohydrate structure. We observed that upon changing just one functional group or sialyl linkage on the carbohydrates, significant differences in binding to specific lectins occur. We later transformed this surface to an array format with a 400-spot carbohydrate microarray chip with SPRi detection.¹³ Differential binding was also observed with high sensitivity for the biotinylated sialosides. The importance of carbohydrate analysis with emphasis on surface chemistry has been recognized by several groups including Ratner's who studied

carbohydrate array surfaces based on mixed self-assembled monolayers (SAMs) to examine protein binding.¹⁴ Surface glycan density has been related to biological function, which is carried out with lectin binding to SAMs of varying densities of carbohydrates using SPR imaging.

Study of lipid interactions with SPR is an emerging area that has significant biological impact as lipid-protein interactions mediate a number of processes and specific recognition of a lipid or lipid receptor in a membrane is typically the key regulatory step of protein action.¹⁵ We have reported a microfabrication approach to generate well defined, addressable, and regenerable lipid membrane arrays in poly(dimethylsiloxane) (PDMS) microchips for label-free analysis of lipid-protein interactions with SPRi.¹⁶ The array-based detection uses a tethered bilayer membrane array built in parallel microchannels. Protein detection at the nanomolar level for cholera toxin (CT) is realized and the membrane can be effectively removed by mild detergent with minimal loss of surface functionality. Others have examined lipid-protein interactions with SPR including a study between Ca^{2+} and C-reactive protein (CRP).¹⁷ CRP plays an important role in host defense but its interactions with other biomolecules is largely unexplored. In this work, CRP was immobilized on a sensor chip, and increasing concentrations of Ca^{2+} or phosphocholine were injected. Binding of Ca^{2+} induced a 10-fold higher signal than expected from the molecular weight of Ca^{2+} due to a conformational change upon binding. It was interpreted to result from the conformational change with two different binding sites which were traced back to the three-dimensional structure of CRP. This work marks an important advancement in SPR biosensor technology because typically low molecular weight molecules are difficult to detect with SPR. The use of conformational change is a clever approach as it clearly allows detection and quantification of the binding of very small molecules or ions to immobilized proteins.

Protein interaction with nucleic acid aptamers has also been studied regularly with SPR in the last few years. Recently an aptamer-based sensor for detection of human immunoglobulin E (IgE) was developed using SPR.¹⁸ In this work various oligo(ethylene glycol) mixtures were used for the construction of self-assembled monolayers and then using biotin/avidin chemistry a streptavidin tagged aptamer for IgE was bound to the surface for detection of IgE. The limit of detection was in the low ng/mL range with a dynamic range spanning three orders of magnitude. Other groups have also found the utility of aptamers for SPR biosensors. Specifically, an SPR sensor was reported for retinol binding protein 4 (RBP4) in serum samples, which is a useful biomarker in the diagnosis of type 2 diabetes.¹⁹ A single-stranded DNA (ssDNA) aptamer that showed high affinity and specificity to RBP4 was selected. This RBP4-specific aptamer was immobilized on a gold chip using tosyl-activated magnetic beads. Compared to traditional immunogenic assays, the SPR method gave better dose-dependent responses and was more sensitive.

It is worth mentioning that biotin/avidin chemistry has a particularly useful application in SPR sensing interface construction. A recent study reports the binding of apolipoprotein A-I to high density lipid (HDL) states with SPR by biotinylating HDL on the streptavidin-coated SPR chip.²⁰ The partitioning of apolipoprotein A-I (apoA-I) molecules in plasma between HDL-bound and -unbound states is an integral part of HDL metabolism. Thus the study helped determine binding kinetics previously undetermined for apoA-I/HDL interactions.

Our group has also shown the use of biotinylated initiator and ATRP-based polymerization method to enhance SPR signal and examine protein interactions down to the low femtomolar level.²¹ The high sensitivity through signal enhancement enables protein detection and analysis with SPR at a new level. It is expected that SPR analysis becomes a burgeoning field with technical advancement.

2.3 Small Molecule Detection

Finally, biologically relevant small molecules have been studied by SPR using a variety of surface immobilization approaches. We have recently demonstrated the incorporation of a water-soluble deep cavitand into a membrane bilayer assembled onto a nanoglassified surface¹⁵ for study of molecular recognition in a membrane-mimicking setting. This cavitand retains its host properties, and real-time analysis of the small molecule guests (< 400 Da) via surface plasmon resonance has been realized.²² In this case the SPR signal was generated by a perturbation in the local membrane environment, but more commonly small molecules are detected by an enhancement method such as nanoparticles, enzymes, or polymers. Nanoparticles, in particular AuNPs, have been used for signal enhancement in SPR for the detection of *Hg(II)* ions,²³ single stranded DNA (ssDNA),²⁴ and protein binding small molecules.²⁵

Another approach for detection of small molecules is the indirect detection through antibodies or proteins. One such example involves the direct detection of antibodies against the human growth hormone (anti-HGH) using SPR has been reported.²⁶ A deficiency of HGH produces a variety of health problems and the early detection of HGH in complex matrices is important. In this work the sensing surface was modified by covalent coupling of the human growth hormone (HGH) to a self-assembled monolayer (SAM). The specific binding of monoclonal anti-HGH antibody on the HGH-modified surface indicated a limit of detection in the low nanomolar range. Another group recently used the same type of interface design to detect the presence of cortisol in saliva.²⁷ Cortisol, a small molecule used as a marker for stress and various disease states, was covalently coupled to the sensor surface through a SAM and then a primary antibody to cortisol was bound with detection from a secondary antibody. The limit of detection in buffer was in the low pg/mL range and it was also detected in saliva across the whole spectrum of physiological concentrations. For a more in-depth discussion of small molecule detection with SPR and other optical methods readers are referred to a recent review.²⁸

3 SPR and SPRI: New Substrates for Sensing

3.1 Carbon-Coated Metal Substrates for SPR Sensing

An interesting new substrate fabricated for biomolecule immobilization with SPR detection involves a carbon-on-gold substrate using amorphous carbon.²⁹ The carbon-on-gold substrates employ a lamellar structure in which an SPR conducting gold thin film was evaporated onto a high-index glass substrate and then an amorphous carbon overlayer was sputtered onto the gold. The amorphous carbon was then hydrogen terminated with inductively coupled hydrogen plasma and UV photofunctionalization. To demonstrate the utility of this substrate Smith and coworkers created an oligonucleotide array synthesized on

the substrate in a base-by-base fashion using photolithographic chemical methods. Complementary base detection using a commercial SPR imager was realized for a variety of oligonucleotides. The synthetic process is reproduced in Figure 4 along with other SPR substrates mentioned later in this section. While this design offers promise for surface functionalization, the authors note that sensitivity can be decreased by as much as 42% by the addition of the carbon film. These authors later tried to address this problem by optimizing the fabrication process to limit the thickness of the amorphous carbon.³⁰ Beyond the use in oligonucleotide arrays, they have demonstrated the identification of RNA accessible sites on these arrays as well.³¹ Another group has used this technology but altered the fabrication process slightly by coating the gold (Au) or silver (Ag) substrate with 5-nm thin amorphous silicon-carbon alloy films.³² $\text{Ag}/a\text{-Si}_{1-x}\text{C}_x\text{:H}$ and $\text{Au}/a\text{-Si}_{1-x}\text{C}_x\text{:H}$ multilayers realized a sensitivity enhancement 2.8 times over gold and 2 times over silver for SPR refractive index sensing. While still in development, these carbon-on-gold surfaces offer a possible alternative to conventional SPR gold substrates.

3.2 Glass-Coated Gold Substrates for SPR Sensing

One disadvantage of conventional gold coated substrates for biosensing is the rather limited surface chemistry available. In an effort to rectify this issue we have developed stable, nanometer-scale glass (silicate) layers on gold substrates via a layer-by-layer (LbL) approach for SPR analysis.³³ This surface is especially beneficial for lipid-based analyses given that lipid vesicles directly fuse to glass forming a stable lipid bilayer while on gold they stay in vesicle form. Additionally, because the glassy layer is very thin the detection sensitivity is not compromised as compared to a planar gold substrate. We have expanded on this idea in the SPR imaging mode to examine cholera toxin/GM1 interactions on a supported membrane interface.³⁴ While the initial LbL deposition method was effective, we felt the method could be improved as the silicate coating at very low thickness often shows nanoscale fractures. Recently we have demonstrated the fabrication of the silicate coated-gold SPR substrates via a paint gun technique.¹⁵ This high-volume, low-pressure (HVLP) paint gun technique offers high precision and better control of surface morphology through pressurized nitrogen gas. The resulting silicate coated substrates are mainly fracture-free and stable in solution for a long period of time. To demonstrate the utility of these ultrathin, fracture-free substrates lipid bilayer membranes were directly fused on the silicate coated gold and lipid-protein interactions were studied successfully at the nanomolar level. The Corn group has recently demonstrated the coating of gold nanorods with a thin silica film and then functionalized them with single-stranded DNA (ssDNA).³⁵ Coating the nanorods with 3–5 nm of silica improve their solubility and stability while also allowing SPR and SPR imaging to be performed. In their work, amine-modified ssDNA is attached to the silica-coated gold nanorods which are capable of hybridization with the complementary ssDNA sequence either immobilized onto a planar gold surface or attached to another nanorod. It should be noted that the technique of silicate-coated SPR gold substrate we developed has found applications beyond SPR as has been recently demonstrated by our group as a unique substrate allowing direct mass spectrometric analysis without the need for organic matrices.

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3.3 Stimuli Responsive Polymers-Coated Gold Substrates for SPR Sensing

Stimuli-responsive polymers (SRPs) represent one of the most exciting topics in polymer science. These materials are capable of physical, conformational and chemical changes after external stimulation by a modification in the physical or chemical environment including temperature, pressure, electric/magnetic field, pH and solvent polarity. An increasing number of applications have been reported.³⁷ An interesting applications of SRPs to surface plasmon resonance sensors was reported by I. Tokareva et al.³⁸ They constructed a sensor for gold nanoparticle enhanced transmission surface plasmon resonance spectroscopy. The sensor is based on the real-time determination of swelling/shrinking of poly(2-vinylpyridine) responsive ultrathin polymer caused by pH modification. In another work, Mitsuishi et al. assembled gold nanoparticles with poly(*N*-isopropylacryl-amide) thermosensitive polymer on a modified glass substrate.³⁹ The heating/cooling of these hybrid nanoassemblies induce stretching/shrinking motion, which is transduced by localized surface plasmon resonance (LSPR). They demonstrated the reversible and reproducible optical modulation of SRPs-Au nanoparticles assemblies based on spatially sensitive LSPR at the tens-of-nanometers scale.

Silver nanoparticles have been used by Endo et al. as a LSPR transducing element for a stimuli-responsive hydrogels.⁴⁰ In this experiment, glucose oxidase was immobilized into the hydrogel. When a glucose solution was applied to the surface of this hybrid composite, the interparticle distances of the silver nanoparticles were increased, and thus the absorbance strength of the LSPR was decreased. This work could lead to a new kind of biosensor for glucose detection based on surface plasmon resonance. Similar studies were conducted by Qian et al. using gold nanocrystals and a pH-sensitive polymer.⁴¹

3.4 Plasma Polymerized Films-Coated Gold Substrates for SPR Sensing

During the last decade, plasma polymerization has become a very effective method for the deposition of thin polymer films in a dry process onto a wide variety of substrates. Plasma polymerized films (PPF) have been successfully used for biomolecule immobilization and functionalization for many kinds of sensors.^{42, 43} The use of PPF as an interface design for SPR biosensors has been a focus of the groups of Knoll and Forch. Zhang et al. demonstrated the possibility of tuning the film deposition to obtain films either resisting protein adsorption or having a strong protein affinity.⁴⁴ They used SPR biosensors to analyze the attachment of fibrinogen, bovine serum albumin, and immunoglobulin on plasma polymerized di(ethylene glycol) monovinyl ether. Recently, they realized DNA probe immobilization using the streptavidin–biotin assembly coupled with a non-fouling PPF.⁴⁵ The resulting DNA sensors showed good resistance against adsorption of both BSA and fibrinogen, and were employed to discriminate different DNA sequences from protein-containing sample solutions, using surface plasmon enhanced fluorescence spectroscopy.

Muguruma et al., demonstrated the performance of a sensor chip based on PPF deposited on gold substrates for kinetic analysis of biomolecular interactions.⁴⁶ They showed that these films are less dominated by the transport limited condition than carboxymethylated dextran-based commercial chips, and claimed that the advantages of PPF increases in the analysis of large molecular weight analytes (10^6 M.W.) and in systems involving a fast rate constant ($k_a > 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$ and $k_d < 10^{-2} \text{ s}^{-1}$). Later, they proposed a novel ethylenediamine plasma-

polymerized film matrix that is deposited on gold surfaces for use in SPR immunosensing.⁴⁷ The obtained SPR chip is reported to have a better sensor response than a conventional design partly because biomolecules are densely and two-dimensionally immobilized onto the surface of the plasma-polymerized film.

New developments are expected in the field of PPFs-coated SPR. Many practical advantages make these chips attractive, including the one-step fabrication process. Furthermore, fabrication of the sensor chip starting from the metal film to the PPF layer in a plasma deposition reactor offers the possibility of mass production.

4. SPR Imaging Based Interfaces: Designs for Multiplexing

4.1 Microfluidic Based SPR Imaging Arrays

There are multiple new avenues in the SPR imaging research arena, especially when it comes to array fabrication. Specifically, microfluidics offers the distinct advantage of possible individual addressability, which in turn enables multiple chemical environments to be explored in a multiplexed fashion. Additionally, by creating arrays within a microfluidic environment the whole process takes place in-situ and thus is especially advantageous for lipid based arrays that would lose functionality when exposed to air. An interesting work in this area is an electrokinetic label-free biomolecular screening chip (Glass/PDMS) to screen up to 10 samples simultaneously using SPRi.⁴⁸ It builds off a previous method where they demonstrated a first generation electrokinetically driven SPRi chip.⁴⁹ The glass/PDMS hybrid chip allows for as many as 90 ligands to be immobilized on the surface. As proof of principle a variety of proteins were bound to the surface using conventional covalent coupling and this array design overall substantially reduces analysis time. Ouellet et al. have used soft lithography techniques to create an array consisting of 264 element-addressable chambers isolated by microvalves.⁵⁰ The resulting 700 pL chamber volumes, combined with a serial dilution network for simultaneous interrogation of up to six different analyte concentrations all at once, allow for further minimizing detection times. To test their system the human α -thrombin/anti-human α -thrombin IgG interaction pair was analyzed with equilibrium constants closely mirroring those found in the literature. While this design is certainly unique the complexity of the microfluidics somewhat limit its widespread application. Other groups have slightly simplified the approach just using a large mold of PDMS with flowchannels built-in to create an array on a standard SPR substrate on a platform known as the microfluidic flow cell array (MFCA).⁵¹ This array system was used to deliver sample solutions with continuous flow in 24 channels in parallel for rapid microarray creation and binding analysis while using SPRi for analyzing antibody-antibody interactions.⁵¹

4.2 Pin-Spotting Based Designs for SPR Imaging

A more simplistic approach to array fabrication for SPR imaging is contact printing. One major drawback of patterning with microfluidics is that elaborate patterns with features more complex than simple cross patterned strips, such as arrays in high density, are hard to achieve without additional fabrication steps.⁵² Contact printing has been widely used and many pin-type arrayers are readily available, which use different designs and delivery

mechanisms. A popular method of contact printing employed is the use of solid pins. Advantages include easy deposition of viscous solutions, simple design that enables reproducible and efficient printing, and a simple cleaning procedure for the pins.⁵³ We have employed this method of array deposition to create a 400-spot high-density carbohydrate microarray mentioned earlier in this review.¹³ Yan's group also created a fine carbohydrate microarray with pin-printing to analyze low volume glycoprotein interactions using as little as 50 nL.⁵⁴ Several labs have utilized pin-spotters to make high quality arrays for SPR imaging analysis including the work that spotted various aptamers for virus coat protein detection.⁵⁵ Thiolated aptamers were deposited on a gold substrate with subsequent binding of virus coat proteins indicative of apple stem pitting virus (ASPV) in plant extracts detected with SPRi. Hook *et al.* recently used pin-printing to deposit functionalized polymers to study the adsorption and desorption of a variety of proteins.⁵⁶ The spots were also incubated with an adherent cell line, enabling insight into the underlying mechanisms of cell attachment to the polymers.

Variants of pin spotting have also been introduced recently with a pin electrospeoting method allowing distinct sensing layers on the SPRi chip.⁵⁷ Specifically, proteins modified with an aryl-diazonium adduct are addressed to the SPRi chip surface through the electroreduction of the aryl-diazonium to create the arrayed spots. A specially designed setup enabled this instrument to directly observe the mass increasing at the sensor surface while the proteins were electrografted. As proof of principle of the method various antibody-antigen interactions were detected at the low nanomolar level. Finally, Lausted *et al.* recently reported a high density antibody based 792 spot microarray created with pin-spotting to measure the concentrations of four serum proteins.⁵⁸ Quantification of these proteins compared favorably to ELISA methods of analysis and this work was taken a step further to compare the serum protein profiles from three liver cancer patients and three non-liver cancer patients. Using the SPRi data and hierarchical clustering these samples were easily distinguished representing an exciting assay for both protein profile and expression levels.

4.3 Bulk Flowcell Array Based SPRi Interfaces

A compromise between a microfluidic method and contact printing, bulk flowcell array method keeps the assay simpler than microfluidics and the whole assay runs in solution unlike contact printing methods. The Corn group has recently demonstrated its utility in the fabrication of a single-stranded DNA(ssDNA) microarray by attaching amine-modified oligonucleotides to a monolayer of poly(L-glutamic acid) (pGlu) that is electrostatically adsorbed onto the chemically modified gold film on a spotted chip.⁵⁹ This array can examine hybridization adsorption of complementary ssDNA onto mixed ssDNA microarray elements and also the adsorption of single-stranded binding protein (SSB) onto fully and partially hybridized DNA microarray elements in a quantitative manner. Grant *et al.* also used the similar chips to create an array of *Salmonella* disaccharide epitopes to probe antibody binding with SPRi.⁶⁰ Minute differences in the stereochemistry of the immobilized, modified disaccharides are shown to greatly influence the binding of a monoclonal antibody. Finally, Jiang and coworkers recently reported the use of a home-built SPR imager with a bulk flow cell to create an antibody array for the detection of cancer biomarker candidates.⁶¹ Antibodies are spotted onto a self-assembled monolayer (SAM) surface for the detection of

two cancer biomarker candidates, activated leukocyte cell adhesion molecule/CD 166 (ALCAM) and transgelin-2 (TAGLN2). Both of these biomarkers are detected in buffer and in 10% human serum with limited cross-reactivity.

5. Conclusions

Through the use of unique surface chemistry and advanced substrate design, the scope of SPR study has changed considerably in the past few years. Especially in regards to substrate construction, the interdisciplinary strategies that bring in more engineering approaches to SPR interface design have opened up a whole new avenue of micofabrication techniques and metal deposition methods. Concurrently, array systems continue to advance at a rapid pace. Whether they are made *in-situ* with microfluidics or offline with contact printers, there is a vast array of literature out there on array patterning techniques. Additionally, the area of instrument design continues to grow for SPRi platforms. The utility of SPR and SPRi for small molecule detection may expedite due to its importance in the pharmaceutical industry.

While much progress has been made in this area the fundamental problem of individual addressability in an array format using a simplistic assay design has still yet to be fully addressed. The transport limitation issue in kinetic analysis has continuously attracted attention. New developments in interface design are expected to overcome this barrier as well as problems in minute solution delivery in a reliable manner. Suppression of non-specific background signal is of most importance and remains a challenging task. The ultimate goal for SPR biosensing technology is to effectively compete with more popular labelled techniques such as fluorescence methods. Despite the challenges, with the advent of new technologies, interdisciplinary approach to biosensor design, and increasing support from industry, the future of SPR biosensors is very promising.

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Figure 1. Sample biosensing interface for SPR with corresponding reflectivity curve and sensorgram reproduced from Linman et al.⁶²

Figure 2. Schematic of the SPR imaging process. The measurement is carried out by using a camera at a fixed angle (upper left), and images show differences in reflectivity observed at an array interface (lower right).

Figure 3. Carbohydrate-functionalized interface based on the interaction between the biotinylated sialosides and corresponding lectin (green).¹²

Figure 4. Fabrication methodology of new SPR substrates. Synthetic protocol for a) nanoglassified gold;¹⁵ b) silicate-coated gold nanorods;³⁵ c) carbon-on-metal;³⁰ d) silicon-carbon alloy films;³² and e) gold-nanoparticle coated polymer brushes.³⁸