Combined Electrochemical Surface Plasmon Resonance for Angle Spread Imaging of Multielement Electrode Arrays

Andrew C. Hillier
Iowa State University, hillier@iastate.edu

Chang Hoon Choi
Iowa State University

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Abstract
A surface plasmon resonance imaging system combined with a multielement electrode array is described. An optical system with shaping optics is used to direct a wedge of light onto a gold-coated sample. The reflected light is detected in the form of an angle-spread image of the surface, with one direction denoting a variable incident angle and the other showing a span of locations along one lateral direction of the sample surface. At the proper incident angle, the angle-spread image shows the complete surface plasmon resonance curve over a span of locations on the surface. This imaging system is combined with a sample configuration consisting of a series of gold microelectrode bands, each with independent electrochemical control. In solution, this system can be used to perform high-throughput and dynamic electrochemical experiments. Simultaneous measurement of electrochemical and surface plasmon resonance can be quantitatively performed on each of the electrode surfaces either by holding each electrode at a different potential value or by scanning the applied potential. The sensitivity of this configuration is demonstrated by monitoring oxide formation and removal at a gold electrode in an aqueous electrolyte. A second example, with the use of a thin poly(aniline) coating, illustrates the ability to monitor film changes, including thickness, dielectric properties, and associated electrochemically induced polymer oxidation/reduction on multiple electrodes. This represents a simple and compact method for combining the sensitivity of surface plasmon resonance into an array-based, high-throughput electrochemical system.

Disciplines
Biological Engineering | Chemical Engineering

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Combined Electrochemical Surface Plasmon Resonance for Angle Spread Imaging of Multielement Electrode Arrays

Chang Hoon Choi and Andrew C. Hillier*

Department of Chemical and Biological Engineering, Iowa State University, Ames, Iowa 50011

A surface plasmon resonance imaging system combined with a multielement electrode array is described. An optical system with shaping optics is used to direct a wedge of light onto a gold-coated sample. The reflected light is detected in the form of an angle-spread image of the surface, with one direction denoting a variable incident angle and the other showing a span of locations along one lateral direction of the sample surface. At the proper incident angle, the angle-spread image shows the complete surface plasmon resonance curve over a span of locations on the surface. This imaging system is combined with a sample configuration consisting of a series of gold microelectrode bands, each with independent electrochemical control. In solution, this system can be used to perform high-throughput and dynamic electrochemical experiments. Simultaneous measurement of electrochemical and surface plasmon resonance can be quantitatively performed on each of the electrode surfaces either by holding each electrode at a different potential value or by scanning the applied potential. The sensitivity of this configuration is demonstrated by monitoring oxide formation and removal at a gold electrode in an aqueous configuration is demonstrated by monitoring oxide formation and removal at a gold electrode in an aqueous configuration.

Optical measurements based upon surface plasmon resonance (SPR) have become increasingly popular for the analysis of solid–liquid and solid–gas interfaces and in the development of various sensor platforms. Excitation of surface plasmons can be achieved at continuous metal films, bound nanoparticles, colloidal suspensions, and at various nanostructured or nanopatterned surfaces. SPR sensing has been extensively used in biosensor development, including label-free detection platforms and for proteomics, immnosensing, drug discovery, detecting DNA hybridization, and protein–DNA interactions.

Some of the earliest applications of SPR for the analysis of surfaces and films involved electrochemical systems, including measurement of oxide formation and ion adsorption on noble metal electrodes. Subsequently, SPR combined with electrochemical measurements have been exploited in a variety of contexts, including the study of electrochemically modulated processes associated with self-assembled monolayers, biopolymer adsorption, and processes involving electrochemically induced adsorption and redox processes in various biomolecules. Various label-free detection strategies have also been developed for biosensing applications based upon combined electrochemistry with SPR detection. Simultaneous electrochemical and SPR analysis has been extensively used in the characterization of various conducting and electroactive polymer films to provide information about polymer assembly and redox transformations and electrochemically catalyzed processes.

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*To whom correspondence should be addressed.


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Although most of the combined electrochemical and SPR studies utilized uniform electrode surfaces with traditional SPR detection, there have been several examples of combined electrochemical systems with SPR imaging, where the optical response of various locations on the electrode surface are investigated simultaneously. Examples include imaging of two-dimensional patterns induced by surface electric fields and localized phenomena associated with surface patterns or arrays. Indeed, multielement electrode configurations have become increasingly popular in the design of lab-on-a-chip devices.

One of the key benefits of SPR-based detection methods is that it can readily be modified to incorporate imaging optics in order to perform SPR microscopy or SPR-based imaging. SPR imaging methods have been most frequently used in conjunction with array-based sample formats to achieve high-throughput sample analysis. Multichannel and array-based sensing platforms allow for simultaneous interrogation of a large number of samples. These array-type sensors have been most widely applied to perform high-throughput sensing of protein–surface interactions. SPR imaging of protein microarrays is used to deduce complex interactions of proteins with DNA and other biomolecules.

The majority of SPR-based imaging systems have used collimated optics where differences in reflectivity at various surface locations on a surface are used to detect adsorption. In this work, we describe a combined electrochemical-surface plasmon resonance imaging system that exploits an optical format that creates an angle-spread image. A multielectrode sample configuration using several well-known electrochemical examples, including oxide formation on gold and poly(aniline) redox switching.

**MATERIALS AND METHODS**

**Materials and Reagents.** Cyclohexane, toluene, acetone, trichloroethylene, isopropyl alcohol, hydrogen peroxide (Fisher Scientific, Pittsburgh, PA), gold (99.999%, Ernest Fullam, Latham, NY), 3-mercaptopropyl trimethoxysilane (MPTS), sulfuric acid, sodium sulfate, aniline (Sigma-Aldrich, St. Louis, MO), Microposit photoresist, developer, and remover (Shipley Company, Marlborough, MA) were used as received. Piranha solution (75% H₂SO₄ + 25% H₂O₂) and aqua regia (75% HCl + 25% HNO₃) were made by mixing appropriate ratios of these chemicals. All solutions were prepared with 18 MΩ deionized water (NANOPure, Barnstead, Dubuque, IA).

**Electrode Preparation.** Gold electrodes were prepared on 1 in. × 3 in. commercial glass slides (Fisher Scientific) that were cut to size, cleaned in 2% detergent solution (Neutrad, Decon Laboratories), and then immersed in piranha solution (Caution: Piranha solution reacts violently with organic compounds and must be handled with extreme care) at 50 °C for 30 min. This was followed by copious rinsing with deionized water and drying under a stream of nitrogen. An adhesion layer was then formed by incubating the cleaned glass slides in a 5 mM solution of MPTS in toluene for 6 h. The slides were rinsed in toluene to remove unbound MPTS and dried in nitrogen. The slides were subsequently placed in a vacuum chamber for deposition of ∼45 nm of gold by resistive heating (Model Bench Top Turbo III, Denton Vacuum, Moorestown, NJ) at a rate of 0.1 Å s⁻¹. The gold-coated slides were subsequently cleaned by exposing their surfaces to an oxygen plasma (model PDC-001, Harrick Plasma, Ithaca, NY) for 2 min prior to use. This plasma treatment served to remove surface contaminants and create a reproducibly hydrophilic surface.

Multi (4, 8, 16, or 32) element electrode band arrays were prepared by further processing of the gold-coated glass slides. The gold surfaces were patterned with a custom electrode design via photolithography. A thin layer of the photoresist (Microposit S1813 Photoresist, Shipley, Marlborough, MA) was coated on the gold/glass substrate by spin coating at 1000 rpm. The substrate was soft-baked in a convection oven at 100 °C for 30 min. The substrate was then exposed to ultraviolet light for 30 min through a custom patterned mask. The substrate was developed immediately (Microposit MF-319 Developer, Shipley, Marlborough,
the lamp was collimated with a biconvex lens (focal length written in Matlab (Mathworks, Inc., Novi, MI).

The video images were performed using a custom software code (focal length

730 nm and a full width at half-maximum (fwhm) of 10 nm was

rotating linear polarizer (Edmund Optics, Barrington, NJ) was

used as the white light source (360

A tungsten halogen lamp (LS-1, Ocean Optics, Dunedin, FL) was

setup was used to perform angle-spread SPR imaging (Figure 1).

Surface Plasmon Resonance Imaging. A custom-built optical

E. Electrochemistry. Electrochemical experiments were performed using a multielectrode potentiostat (model CHI1030, CH Instruments Inc., Austin, TX), which could control eight working electrodes simultaneously with a single counter and reference electrode. The working electrode leads were connected to 8 of the pins of a 20-pin dip-clip (Pomona Electronics, Pomona, CA), which was attached directly to the substrate electrode. A wound Pt–Ir wire counter electrode and a Hg/HgSO₄ reference electrode were used in the solution cell during electrochemical experiments. The electrochemical cell consisted of a custom-built Teflon compartment with a Viton O-ring to provide a liquid-tight seal with the gold/glass substrate electrode.

RESULTS AND DISCUSSION

The experimental setup used for angle spread SPR imaging consisted of a custom-built optical train (Figure 1A) that introduced a converging sheet of light onto the back side of a gold-

coated glass slide. Reflected light emerged as a diverging sheet that was focused on a two-dimensional CCD detector. The sample was oriented at a specific incident angle (θ), and the converging light beam covered a spread of angles (Δθ) about that base angle (Figure 1B). The wedged shape of the incident light beam exposed a one-dimensional slice of the substrate of approximately 1 cm in length along the sample surface. Images were acquired in the form of relatively reflectivity (Rₑ/ₚₑ) as a function of angle (θ) and position (x) along the sample. The angle spread image interrogates a length of the sample's surface and provides the ability to investigate a larger region of the sample surface.

Figure 1B depicts a schematic of a sample configuration that combines the angle spread SPR imaging system with an electrochemical cell and a substrate consisting of a multielement electrode array for high-throughput electrochemical experiments. In this configuration, the substrate consists of a microfabricated electrode pattern consisting of multiple gold electrode bands. The substrate is mounted to a Teflon cell, which can be filled with liquid and contains a counter and reference electrode. Combined with an external multielectrode potentiostat, this configuration allows for simultaneous SPR imaging of each electrode element.

**Figure 1.** (A) Schematic of optical setup used for angle spread surface plasmon resonance imaging showing the light source, optical components, sample, and detector. (B) Schematic of the electrochemical cell for the SPR angle spread imaging system using an eight-element microelectrode sample with counter and reference electrodes for independent electrochemical control of each electrode band.
in combination with independent electrochemical control of the various electrodes.

An optical image of an eight-element band electrode shows the geometry of the electrode pattern (Figure 2A). Each electrode is approximately 1 mm wide by 20 mm long and has two terminal pads for electrical contact. The spacing between adjacent electrodes is \( \sim 1 \) mm but could be much smaller. Several versions of this substrate configuration have also been developed that include 4, 8, 16, and 32 electrode bands (see the Supporting Information).

An angle spread SPR reflectivity image of the band electrode taken with the electrochemical cell filled with water (Figure 2B) depicts reflectivity as a function of the \( x \)-position along the sample over an angle spread of \( \sim 13^\circ \). The image shows alternating stripes that reflect the response of both glass and gold regions on the sample. The glass regions show an increase in reflectivity at low angles and then a plateau in reflectivity near \( R_p/R_s \sim 1 \), indicating the onset of total internal reflection as the angle of incidence is increased. The gold regions show an increase in reflectivity with increasing angle, but then a significant dip in reflectivity occurs, corresponding to the excitation of surface plasmons. A quantitative reflectivity curve (Figure 2C) extracted from each of the glass and gold regions shows the onset of total internal reflection at \( \sim 61^\circ \) over the glass and a SPR minimum angle of \( 67.1^\circ \) over the gold. These values are consistent with the optical configuration of the sample at an excitation wavelength of 730 nm, which includes a glass prism (BK7 glass, \( n = 1.51 \)), glass (BK7), or gold (45 nm, \( n + ik = 0.135 + 14.33 \)), and water (\( n = 1.33 \)). Each of the glass and gold regions show responses that are identical to the others of their kind under these conditions.

The optical setup used in this SPR imaging system allows significant flexibility in the imaging resolution. Both the range and resolution of the spatial and angular imaging components can be readily modified. The measured angle spread in Figure 2 is \( \sim 13^\circ \). This range can be increased or decreased by changing the focal length of the hemicylindrical lenses, which will change the shape of the incoming/exiting light wedge. The spatial extent of the SPR image in Figure 2 of \( \sim 1 \) cm can also be changed by increasing or decreasing the magnification of the lens used at the detector. The flexible sample format allows a range of electrode configurations to be employed. The electronics we employ in our current system allow the potential of up to eight electrodes to be independently controlled. However, one could readily construct multiplexed current amplifiers to allow the recording of a much larger group of electrodes. Indeed, commercial potentiostat systems exist that can measure up to 100 independent electrode currents from a single sample.

In the presence of an electrolyte solution, the electrochemical potential at each gold electrode can be independently controlled and the behavior monitored via measurement of the current and optical response. A direct comparison of the electrochemical current (Figure 3A) and SPR minimum angle (Figure 3B), as extracted from the SPR images during potential scanning, can be used to elucidate the processes occurring at the gold surface (see the Supporting Information). The cyclic voltammogram of an unmodified gold electrode (Figure 3A) in an aqueous solution of 0.01 M H\(_2\)SO\(_4\)/0.1 M Na\(_2\)SO\(_4\) shows the characteristic response of this surface in an acidic solution. At low potentials, the current response is flat and reflects double layer charging at the electrode surface. As the electrode potential increases above \( \sim 0.8 \) V, the current increases substantially as a gold oxide layer forms. The current decreases when the potential is reversed and a large reduction current is observed at \( \sim 0.6 \)
V, corresponding to reduction of the gold oxide layer. Further lowering of the potential leads to a flat current response in the double layer region. A small reduction peak is seen at −0.5 V, which reflects the onset of proton reduction.

The SPR minimum angle changes in accordance with the electrochemical response (Figure 3B). In the double layer region between −0.5 and 0.5 V, the minimum angle increases slightly with increasing potential. This increase has been interpreted as being caused by the adsorption of bisulfate ions from solution onto the gold surface as the potential exceeds the potential of zero charge (pzc).46 A significant increase in SPR angle occurs as the gold oxide layer forms at potentials >0.5 V. The total anodic charge passed during gold oxide formation in this scan is 701 µC cm⁻², which corresponds to an oxide thickness of ∼0.46 nm.47 The SPR minimum angle shifts during this experiment by ∼0.4°. The use of a refractive index for the gold oxide of 3.24 + 11.09i predicts an oxide layer thickness of ∼0.5 nm based upon the SPR angle change, which is consistent with the electrochemical results. The SPR angle remains nearly constant as the potential is reversed at 1.1 V, reflecting a constant gold oxide thickness. As the electrode potential is decreased below 0.8 V, the SPR minimum angle decreases rapidly in accordance with the oxide reduction peak, indicating removal of the oxide layer. The SPR angle then decreases slowly to arrive at a value consistent with the original surface.

As a further illustration of the utility of this combined electrochemical-SPR imaging platform, we examined the electrochemical response of a poly(aniline)-coated surface. Poly(aniline) films have been previously studied using combined electrochemical and SPR measurements in order to develop a greater understanding of the film thickness and optical changes that occur during film growth and redox switching.21,27,30,31,49 Fabrication of a poly(aniline)-coated electrode was achieved here by cycling the electrode potential in an aniline containing solution (see the Supporting Information). During deposition, an increase in the SPR angle of ∼0.7° was observed after a single cycle, which can be attributed to deposition of the polymer layer. This increase in minimum angle can be used to estimate the polymer thickness using the refractive index of the reduced film (n = 1.6), giving a film thickness of ∼2.2 nm.31,49

The behavior of this thin polymer film can be analyzed using both electrochemical and SPR measurements. Under acidic conditions, poly(aniline) can be readily transformed between its reduced and oxidized state via potential cycling. The cyclic voltammogram (Figure 4A) shows a clear oxidation/reduction peak at ∼0.2 V, indicating the transition between the fully reduced leucoemeraldine and half-oxidized states of the poly(aniline) film. This transition between oxidation states is accompanied by swelling of the film due to an ingress of solution anions as well as a substantial increase in film conductivity.50 A simultaneous swelling of the film due to an ingress of solution anions as well as a substantial increase in film conductivity.50 A simultaneous increase in film conductivity is due mainly to an increase in the polymer’s conductivity, which increases the imaginary part of its refractive index to produce a broader resonance response.49 Notably, the SPR response of thicker poly(aniline) films is typically dominated by the increase in conductivity and its impact on the imaginary part of the polymer’s refractive index.27,30,31 Although these results give a quantitative picture of the film behavior during redox switching, SPR angle spread images can provide a more rapid signature of the differences.

Figure 4C,D depicts two different angle spread SPR images of poly(aniline)-coated electrodes. In these images, all eight of the electrodes are coated with a ∼2.2 nm thick poly(aniline) film and images with all electrodes held at −0.3 V have been subtracted. In the left image (Figure 4C), the electrodes are held at potential values starting with −0.3 V for band 1 and increasing at 0.15 V intervals up to 0.75 V for band 8. A clear contrast is seen in the images. This reflects the ready oxidation of the polymer film and the clear difference in thickness and dielectric properties of the reduced and oxidized polymer that occurs with increasing applied potentials. In Figure 4C, the contrast in each band becomes more significant at each increasing potential value. The right image (Figure 4D) reflects bands 1–4 held at a potential of −0.3 V and bands 5–8 held at a potential of 0.75 V. In Figure 4D, there is a discrete change in contrast between the electrodes at −0.3 V in the reduced state and the electrodes at 0.75 V, where the polymer is in the oxidized state. This is just one example of how the multielectrode sample can be used to perform combined electrochemical and surface plasmon resonance measurements. A large variety of these measurements can be performed here and with

![Figure 4](image-url)

**Figure 4.** (A) Cyclic voltammogram for single gold band coated with 2.2 nm poly(aniline) film in a solution of 0.05 M H₂SO₄/0.1 M Na₂SO₄. (B) SPR reflectivity curves at −0.3 and 0.75 V. Angle spread SPR difference images with electrodes held at potentials noted for (C) increasing values in 0.15 V increments and (D) at two different values for poly(aniline)-coated electrodes. The images were generated by subtracting images of the same surface with all electrodes held at −0.3 V.
other systems to develop novel sensor designs and high-throughput opto-electrochemical measurements.

**CONCLUSIONS**

This technical note describes a combined electrochemical-SPR imaging system designed to perform angle-spread SPR imaging on multielectrode sample configurations. One of the unique features of this angle-spread format is that it provides a complete reflectivity curve at each surface location instead of simply a difference in reflectivity as would be measured in traditional SPR imaging. Also, the ability to simultaneously measure quantitative reflectivity curves at numerous regions on a sample surface with independent electrochemical control of the potential of multielement electrode bands allows rapid mapping of potential-dependent phenomena occurring on electrode surfaces. Thus, one can readily evaluate the impact of applied potential on an electrode surface or electrode coating by imaging the changes in SPR response, simultaneously with the corresponding electrochemical current, on a multielectrode surface. In the examples reported here, the oxidation of gold was easily measured with subnanometer sensitivity. The ability to evaluate a multiple, poly(aniline)-coated electrodes in a single experiment was also demonstrated. This simple, yet robust method could be readily extended to other electrochemical processes and systems.

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**SUPPORTING INFORMATION AVAILABLE**

Optical configuration and surface plasmon resonance data for uniform gold film, surface plasmon resonance images of 4, 8 and 16-element electrode band samples, electrochemical surface plasmon resonance data for uniform gold film versus potential for gold band, and cyclic voltammetry and resonance angle change during poly(aniline) deposition. This material is available free of charge via the Internet at http://pubs.acs.org.

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