Fourier Transform Surface Plasmon Resonance

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1 INTRODUCTION TO FOURIER TRANSFORM SURFACE PLASMON RESONANCE

Surface plasmon resonance (SPR) is a surface sensitive optical technique that is sensitive to the thickness and index of refraction of thin layers at the interface between a metal surface and bulk medium. As an instrumental method, it has found wide application for biochemists studying biological interactions.[1] The method focuses on the determination of biological activity rather than biological structure.[2] The picture it provides is not a classical vibrational spectrum showing what the molecule looks like, but rather what the molecule does in terms of interacting with other molecules in its environment. SPR can be used to characterize Langmuir–Blodgett films,[3] self-assembled organic monolayers,[4] adsorbed molecules,[5] and thin organic films at electrochemical interfaces.[6] Applications have been demonstrated in the areas of environmental monitoring,[7] medical diagnosis,[8] food testing,[9] and agriculture.[10] SPR spectroscopy has become widely used in the fields of chemistry and biochemistry to characterize biological surfaces and to monitor binding events.[1] The success of these SPR measurements is primarily due to three factors: (i) the kinetics of biomolecular interactions can be measured in real time; (ii) the adsorption of unlabeled analyte molecules to the surface can be monitored; and (iii) SPR has a high degree of surface sensitivity that allows weakly bound interactions to be monitored in the presence of excess solution species. SPR spectroscopy has been used to monitor such events as antibody–antigen binding, DNA hybridization, and protein–DNA interactions.[11] It should be emphasized that SPR measurements are particularly applicable to biomolecules that are not compatible with fluorescent labeling. In addition, SPR can be used for real-time in situ ellipsometry measurements of film thickness and index of refraction.[12]

The interface of a Fourier transform infrared (FT-IR) spectrometer to an SPR instrument provides the excitation light energy and readout for detecting adsorption changes at the chemical surface interface. By measuring changes in reflectivity in the near-infrared (NIR) spectral region, the Fourier transform surface plasmon resonance (FT-SPR) instrument provides information on the interactions occurring at the thin film interface. The FT-IR spectrometer has the advantages of high sensitivity, high ordinate and wavelength precision, and fast measurement time. FT-IR in the NIR is an established technique, and the extensive data acquistion and processing capabilities of these instruments provide an obvious interface to the type of data the SPR experiment produces.

While infrared reflection–absorption spectroscopy (IRRAS) has developed into one of the primary methods of monitoring the chemical structure and molecular orientation of thin films and monolayers adsorbed onto metal surfaces, FT-SPR provides a complementary method to IRRAS in that it provides information on the optical thickness and reaction kinetics of thin films. Both techniques can be used for measurements ranging from submonolayer films to films with a thickness of about 200 nm. A range of metal substrates can be used, and both methods are applicable to air/metal and water/metal interfaces.

2 SURFACE PLASMON RESONANCE IN THE NEAR-INFRARED

SPR is an optical phenomenon that allows an evanescent light wave to probe across a metallic surface layer. When
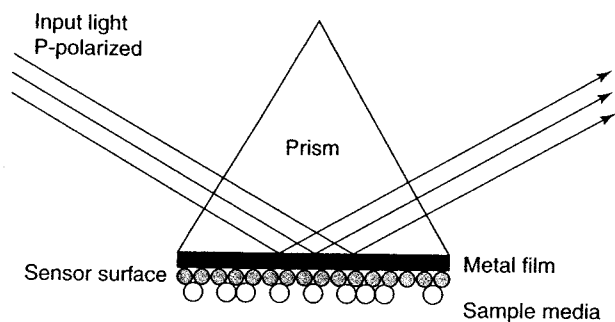
Figure 1. SPR prism configuration.

the optical conditions for creating the resonance are met, an evanescent light wave will penetrate beyond a metal film to a thickness of approximately 200 nm. Figure 1 depicts the geometry required for SPR. This geometry using a prism is referred to as the Kretschmann coupling arrangement.[13] A light beam of a particular wavelength is incident on the glass prism. The incident light is polarized parallel to the plane of incidence (P-polarized) at an angle just above the critical angle. The prism is arranged so the light strikes the face at near-normal incidence and reflects through the adjacent side when at the critical angle. The hypotenuse of the prism is coated with a thin metallic film such as gold. When the light incident angle is increased to several degrees above the critical angle, a minimum in the reflected light intensity is observed with the reflectivity changing from greater than 90% to 1% or less. As the incident angle is again increased the reflected light returns to a high level. The angular range over which the reflectivity minima occurs is about 1°. This minimum in the reflectivity is termed *surface plasmon resonance* and the angle is the *resonant angle*.[14] Figure 2 shows a plot of measured reflectivity versus angle of incidence using several different laser wavelengths as the excitation source.[15] At an angle of approximately 51°, a cusp in the reflectivity curves is observed, which is caused by the critical angle of the prism. Beyond the critical angle, a minimum in the reflectivity occurs, which is the SPR resonant angle.

SPR is the result of an evanescent wave propagating through the metal and exciting surface plasmons on the surface. Surface plasmons are electron charge waves that propagate along the surface of a metal. When the wave vector of the light beam exactly matches the wave vector of the metal plasmons, a resonance occurs and the radiation is scattered (rather than reflected) at that particular angle. For surface plasmons to exist at such an interface the dielectric permeabilities of the two media must be of opposite sign. This condition is met in the infrared–visible region for air/metal and water/metal interfaces (where the dielectric permeabilities of metals are negative and that of air or
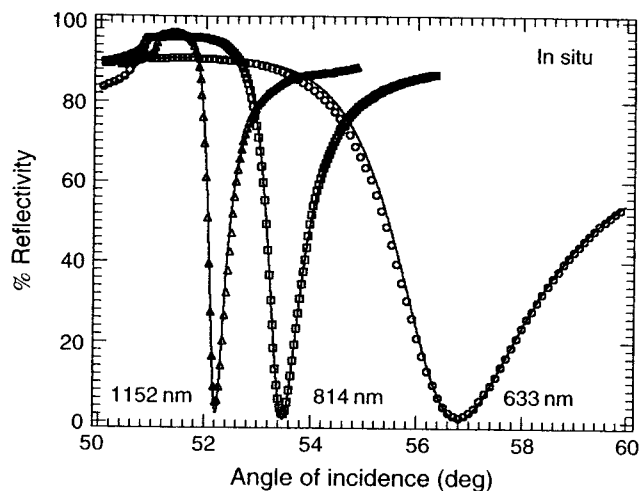

Figure 2. SPR reflectivity curves as a function of incident angle for an SF10 prism/Au/water assembly with 1152-, 814-, and 633-nm excitation. Symbols are measured data and lines are results of Fresnel calculation. [Reprinted with permission from B.P. Nelson, A.G. Frutos, J.M. Brockman and R.M. Corn, *Anal. Chem.*, **71**, 3928 (1999). Copyright 1999 American Chemical Society.]

water is positive). The resonance will occur only for light reflected above the critical angle and polarized in the plane of incidence.[13]

The resonance is sensitive to both the light wavelength and angle of incidence. By fixing the angle and varying the input light wavelength, the minimum in reflectivity occurs at the *resonant wavelength* (Figure 3). The resonant condition is also very sensitive to the index of refraction of the prism, the metal structure and thickness, and the optical characteristics of the medium in contact with the metal surface. Because the resonance is sensitive to the index of refraction and thickness of the medium, SPR is very useful as a sensing technique for measuring changes at the metal surface. A change in optical thickness as a result of adsorbed material at the surface will cause a shift in the resonant wavelength. By monitoring the shifts in the SPR wavelength, the rate and amount of adsorbed material
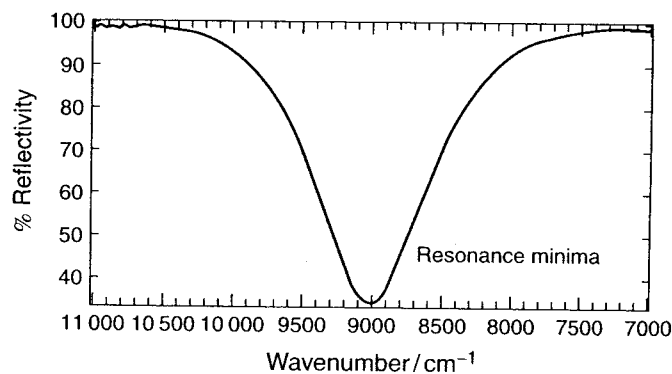

Figure 3. Reflectivity spectrum of the resonant wavelength with an SF10 prism/Au/water assembly.

can be monitored. When the surface is exposed to a sample medium of analyte molecules that bind to the surface via a specific interaction, the amount of binding is proportional to the concentration of the analyte in the sample.

Several high-conductivity metals can be used for SPR, with gold being the most common as it has the best overall characteristics for resonant coupling and oxidation resistance, and is suitable and highly characterized for various chemical surface attachment methods. Other possible metals include silver, copper, and aluminum. When the medium in contact with the metal is air (ex situ), BK7 ($n = 1.52$) is often used as the prism glass. When the sample medium is aqueous (in situ), BK7 or SF10 ($n = 1.72$) are used. Both glass materials are supplied by Schott Glass and have high light transmission and good chemical resistance. With a gold film thickness of approximately 40 nm, resonance will occur in the visible or NIR regions of the spectrum.

To determine the angle and wavelength at which resonance will occur, calculation involving the Fresnel equations can be utilized to model SPR reflectivity curves for different combinations of wavelength and angle.[16] Figure 4 shows theoretical plots of wavelength versus angle of incidence for a 410-Å gold film_in both ex situ and in situ configurations. Each curve or contour represents a region of constant reflectivity from 1 to 50%. The dashed line in each plot represents the wavelength dependence of the critical angle for the BK7/air interface or the SF10/water interface. These contours were calculated using three-phase Fresnel calculations and take into account the dispersion of the prism, gold, and water. Theoretical scanning angle SPR curves (reflectivity versus angle) can be generated from these contour plots by taking slices parallel to the *x*-axis at a fixed wavelength. As shown in Figure 4(a), for a 410-Å gold film in contact with air, an SPR curve with a minimum reflectivity of 1% could be obtained by using a fixed wavelength between ~900 and 1100 nm. Figure 2 shows both the measured data and Fresnel calculation SPR reflectivity as a function of incident angle at three wavelengths. The curves sharpen with increasing wavelength, and the measured and calculated reflectivities fit very closely.

Resonant wavelength curves are generated from the contour plots by taking slices parallel to the *y*-axis at a fixed angle. The change in the SPR reflectivity curves (reflectivity versus wavelength) with angle of incidence is different for samples in contact with air (ex situ) and water (in situ). For example, notice that as the vertical cuts through the contours move toward smaller angles of incidence, the SPR curves become narrower for the in situ configuration, but broaden ex situ. Specifically, the full width at half height (FWHH) of the 50% contour line in the ex situ configuration shown in Figure 4(a) broadens from 150 nm at an angle of incidence of 42.6° to 366 nm at 42.1°; conversely, the
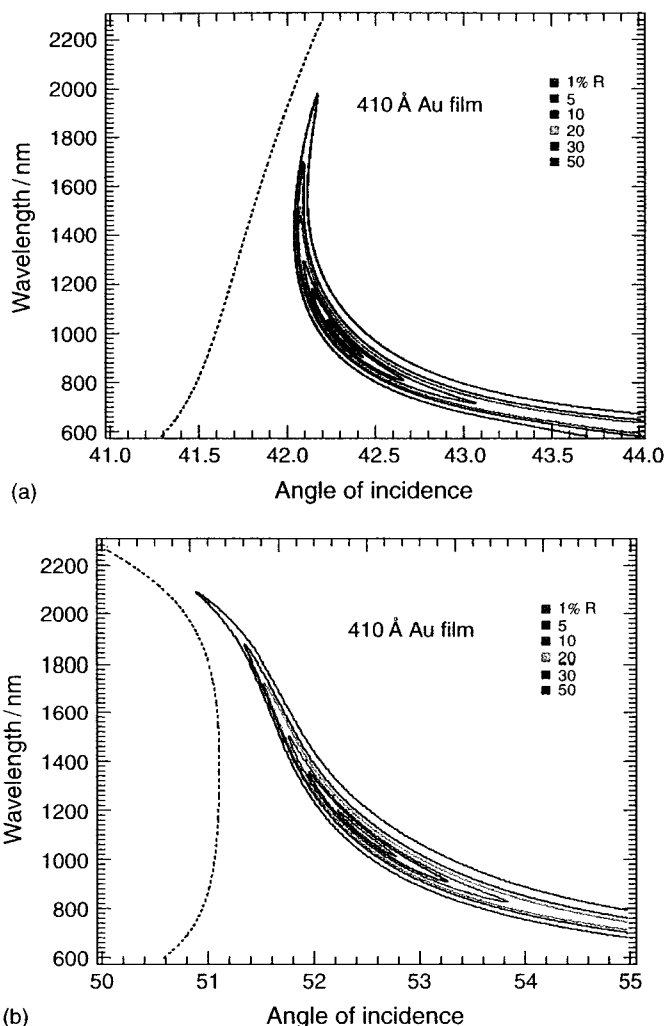

Figure 4. SPR reflectivity contour plots. These contours were generated from three-phase Fresnel calculations and show regions of constant reflectivity from 1 to 50% as a function of wavelength and incident angle. The contours in (a) are for an ex situ configuration and (b) for an in situ configuration. The dashed line shows the wavelength dependence of the critical angle. In each case, the outermost contour is for 50% R and the innermost contour is for 1% R. [Reprinted with permission from A.G. Frutos, S.C. Weibel and R.M. Corn, *Anal. Chem.*, **71**, 3935 (1999). Copyright 1999 American Chemical Society.]

FWHH between the 50% contour line in the in situ configuration shown in Figure 4(b) narrows from 190 nm at 52.6° to 171 nm at 51.6°. Sensitivity to measuring changes at the resonant spectral region is greatly increased with increasing extinction of the reflected light and with decreasing width of the resonant spectral curve.

For each metal film thickness, there exists an optimum angle to perform the SPR wavelength shift measurement. The optimum angle is selected to provide a resonant curve with minimum width, maximum extinction, in a spectral region providing high signal-to-noise ratio, and to avoid regions near an optical cutoff.

Another feature that can be observed in the in situ contour plot is that the reflectivity minimum associated with the SPR angle narrows considerably at longer wavelengths. This is illustrated clearly in Figure 2, which shows the reflectivity curves sharpen and shift closer to the critical angle. A theoretical analysis of this narrowing has been explained in detail.[17] This band narrowing improves the sensitivity of SPR measurements as shifts in the resonant wavelength position are detected more readily at the longer wavelengths.

3   EXPERIMENTAL FOURIER TRANSFORM SURFACE PLASMON RESONANCE

Because the SPR occurs in the NIR region of the spectrum, it is practical to take advantage of the available light sources, optics, and detectors compatible with this wavelength region. In addition, if SPR is to be useful as a biosensing technique, it requires materials that are suitable for an aqueous environment. FT-IR spectrometers optimized for NIR operation have become standard instruments, with optical quality for achieving high baseline stability. The infrared (IR) source is tungsten–halogen, and the beamsplitter substrate is either quartz or calcium fluoride. The optimum detector is indium gallium arsenide (InGaAs) covering the spectral range from 12 000 to 5500 cm^{-1}.

The instrument layout is depicted in Figure 5. The light beam from the external port of a Fourier transform near-infrared (FT-NIR) spectrometer is focused with a lens onto an aperture and recollimated with a second lens. The additional collimation of the beam exiting the spectrometer is required because of the high sensitivity of the resonance minima to the angle of incidence on the metal film. The bandshape of the reflection minimum is broadened with

reduced intensity as the incident light beam diverges from ideal collimation. The aperture size and the focal lengths of the two lenses should be selected to optimize the light throughput and angular beam divergence.

The light beam is then polarized with a NIR film polarizer. The polarizer must be efficient over the range 6000–12 000 cm^{-1}. The polarizer is attached to a rotating mount to provide control of the output polarization state. A background spectrum is acquired with the light polarized perpendicular to the incident plane, and the subsequent sample spectra are acquired with light paralled to the plane of incidence, with each sample spectrum ratioed to the background. Because the desired data are polarization difference spectra, polarization artifacts in the optical components should be minimized.

The prism is configured so the light beam will transmit through the first surface at near-normal incidence and undergo critical reflection at the prism/metal film interface. The metal film is actually deposited onto glass slides and then brought into optical contact with the prism using an index matching fluid. The prism/metal film assembly is mounted on a rotation stage, which allows control of the incident angle. The light reflected from this assembly is focused onto the InGaAs photodiode detector. The detector lens and photodiode assembly angular position is controlled with a gear stage coupled to the rotation stage. For in situ adsorption measurements, a low-volume liquid flow cell is mounted to the back of the prism/metal sample assembly with an O-ring sealing against the metal surface.

SPR angle shift measurements require a rotating stage with an angular precision of 0.001°. This high precision is required for measuring small refractive index changes. However, by taking advantage of the wavelength dependence of SPR the mechanical requirements of the rotation stage are greatly reduced. By maintaining a constant angle of incidence and measuring the resonance
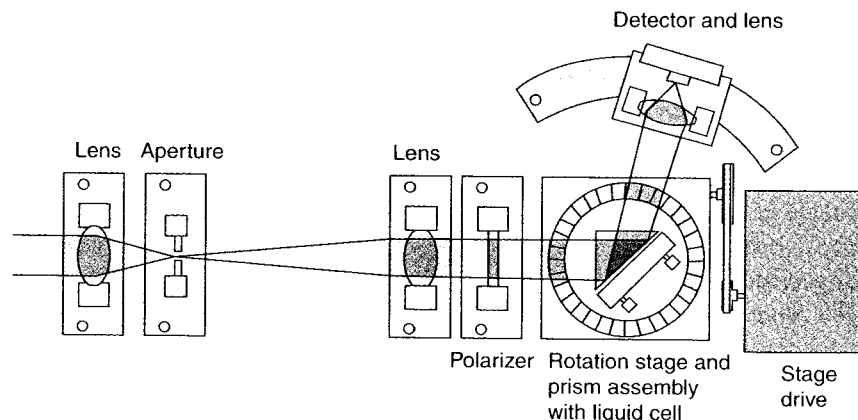

Figure 5. Optical diagram of the GWC Instruments FT-SPR 100 instrument model. The instrument uses the collimated external output beam of the FT-IR spectrometer.

wavelength shift, the wavelength precision of $0.01\,\mathrm{cm^{-1}}$ typically achieved with an FT-IR spectrometer will produce high-precision SPR measurements with a rotating stage of reduced mechanical precision. The requirement of high angular precision with angle shift measurements becomes a requirement for high wavelength precision with wavelength shift measurements.

A BK7 prism/air sample geometry will have a resonance at an angle of approximately 43° with an Au film 425 Å thick. For in situ measurements, SF10/water will produce a resonance at approximately 53°. The BK7/water geometry will have a resonance at approximately 68°, with the more oblique angle reducing the detector signal. With the lower refractive index of BK7 (1.52 versus 1.72 for SF10) a larger variety of index matching materials are available, however the lower refractive index of the prism moves the resonance to a much higher angle when the sample medium is aqueous.

To demonstrate the spectral range of the FT-SPR, and to show how the SPR wavelength minimum can be tuned by changing the angle of incidence, reflectivity curves at a series of different angles were obtained from a 410-Å Au film in contact with water.[16] The reflectivity curves were collected in increments of −0.13°, starting at 53.9°. The SPR curves (Figure 6) in this in situ configuration sharpen with decreasing angle of incidence. As predicted by the contour plots, the minimum moves to longer wavelengths with decreasing angle of incidence.

The capability to tune the angle for a desired resonant spectral region is useful in the situation where interfering adsorption bands of the thin films must be avoided.
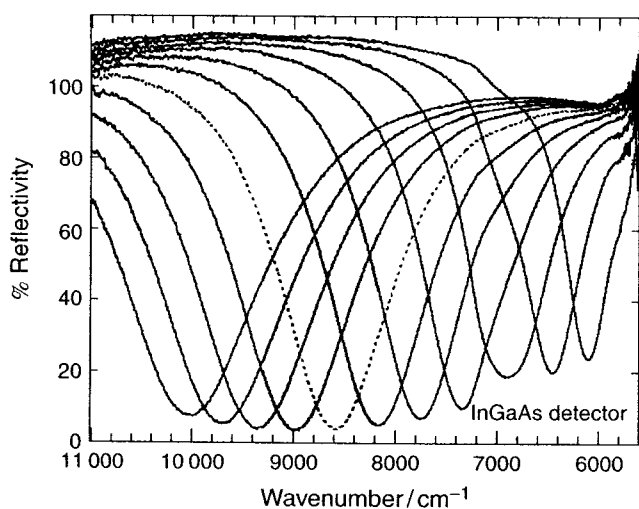

Figure 6. In situ FT-SPR reflectivity curves obtained at a range of incident angles for a 410-Å gold film. [Reprinted with permission from A.G. Frutos, S.C. Weibel and R.M. Corn, *Anal. Chem.*, **71**, 3935 (1999). Copyright 1999 American Chemical Society.]

4 APPLICATIONS OF FOURIER TRANSFORM SURFACE PLASMON RESONANCE MEASUREMENTS

One of the original applications of SPR utilized the technique for gas sensing.[18] In this application, a thin layer of a gas chromatography stationary phase was attached to the metal film. When a gas specific to this column material was exposed to the metal surface, resonant angle shifts were measured. This experiment demonstrated quantitative results from several parts per million to 1000 ppm.

A large number of SPR applications have been developed for biomolecular interactions.[9] The following section summarizes an experiment demonstrating the application of FT-SPR for monitoring and quantifying adsorption of biopolymers from solution.

Sequential adsorption measurements of biopolymers onto modified Au surfaces were made.[16] It has been shown that multilayer films can be formed in a layer-by-layer electrostatic assembly by exposing charged surfaces to solutions containing oppositely charged polymers. For example, by starting with a negatively charged carboxylate-terminated surface, multilayer films can be formed by alternating layers of positively charged poly(L-lysine) (PL) and negatively charged poly(L-glutamate) (PG). In this experiment, an InGaAs detector was used, and the angle of incidence was fixed at 52.3°. A sample with a 410-Å Au film was modified with a monolayer of the alkanethiol 11-mercaptoundecanoic acid (MUA) and then assembled in an in situ SPR flow cell in contact with water. The SPR reflectivity of this surface was obtained and is shown as the dotted curve in Figure 7. A layer of PL was then adsorbed from solution onto this MUA-modified Au surface, resulting in a shift of the SPR reflectivity minimum of $-127\,\mathrm{cm^{-1}}$ as shown by the first solid curve in Figure 7. This Au/MUA/PL surface was subsequently modified by the adsorption of a layer of PG, which resulted in an additional shift of $-63\,\mathrm{cm^{-1}}$ (first dashed curve). This series of PL/PG adsorption steps was repeated seven times (for clarity, only the first set of three adsorption steps are shown in the figure). The shift in wavenumber minimum for each adsorption step was used in conjunction with Fresnel calculations to determine the thickness of each adsorbed layer.

The shift in minimum upon adsorption of each successive polypeptide (PL/PG) layer shown in Figure 7 was correlated with a change in thickness by using a five-phase (prism/Au/MUA/polypeptide/water) Fresnel calculation. The theoretical wavenumber minimum versus monolayer thickness is plotted in Figure 8(a) as the solid line. Also shown in the figure as the circles and squares are the experimentally observed minima after each layer of PL and
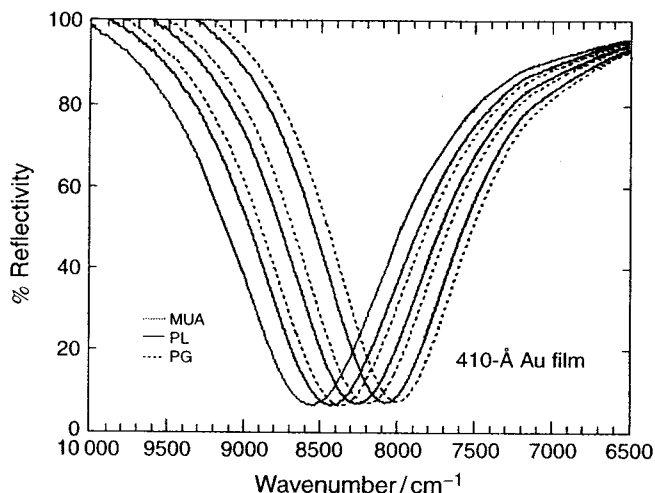
Figure 7. FT-SPR spectra for in situ adsorption measurements. These curves were taken at a fixed angle during the sequential adsorption of alternating layers of positively charged PL and negatively charged PG onto an MUA-modified Au film. [Reprinted with permission from A.G. Frutos, S.C. Weibel and R.M. Corn, *Anal. Chem.*, **71**, 3935 (1999). Copyright 1999 American Chemical Society.]

PG, respectively. Notice that the shift in wavenumber minimum for each PL layer is larger than for each PG layer. The shift in minimum of $-127\,\mathrm{cm^{-1}}$ upon adsorption of the first PL layer corresponds to a change in thickness of 17.5 Å, and the additional shift of $-63\,\mathrm{cm^{-1}}$ upon the subsequent adsorption of the first PG layer corresponds to a thickness change of 9 Å. The change in thickness for each PL and PG layer versus layer number is shown in Figure 8(b). Notice that each PL layer is thicker than the corresponding PG layer, and the thickness of both layers increases with layer number. A shift in wavenumber/thickness can be estimated from the slope of the best-fit line through the points in Figure 8(a) and is approximately $50\,\mathrm{cm^{-1}\,nm^{-1}}$. Since the spectra were acquired with a resolution of $8\,\mathrm{cm^{-1}}$, shifts in the wavenumber minimum are easily measured to less than $4\,\mathrm{cm^{-1}}$, which corresponds to a thickness change of ~ 1 Å. The wavenumber minimum shift can be calculated to a higher precision by using center-of-gravity or least-squares curve fitting methods. This would improve the sensitivity of FT-SPR for measuring thickness changes of less than 1 Å.

FT-SPR can distinguish between an in situ film thickness change or bulk refractive index change at the surface.[19] By making wavelength shift measurements at various angles of incidence for the two types of changes, the following experiment demonstrates that the slope of the shift in the resonance minimum is positive for a change in ionic strength and negative for the adsorption of a monolayer.

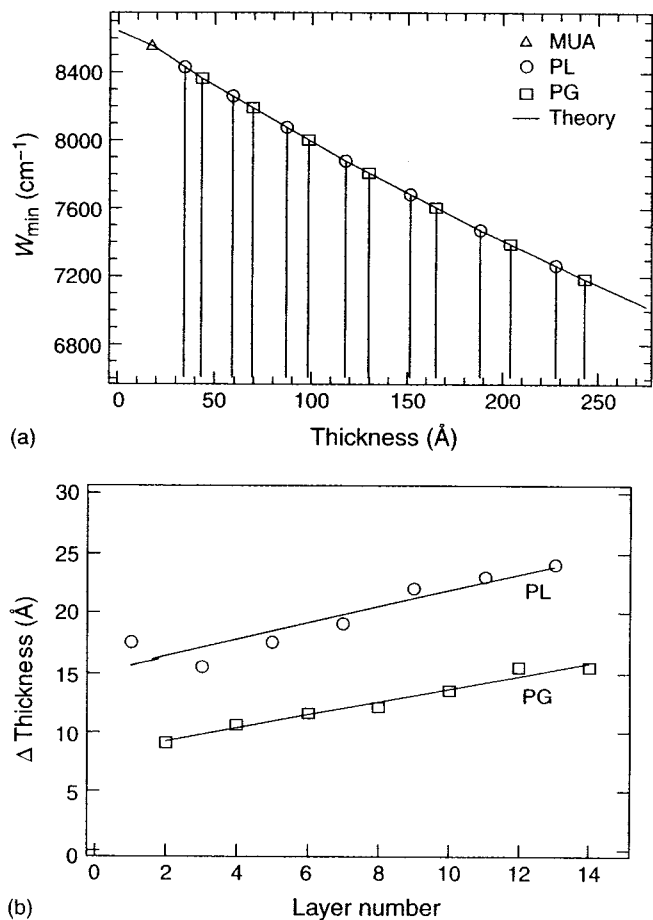Figure 6 shows that there is a change in the SPR wavelength minimum for various incident angles. A series of

Figure 8. Analysis of in situ adsorption experiments. (a) Shift in frequency of the SPR minimum upon the sequential adsorption of PL and PG layers. The observed shift is converted to a change in thickness by using a five-phase Fresnel calculation indicated by the line. (b) The change in thickness of each layer is plotted versus layer number. Note each PL layer is thicker than each PG, and the thickness of each of these layers increases with layer number. [Reprinted with permission from A.G. Frutos, S.C. Weibel and R.M. Corn, *Anal. Chem.*, **71**, 3935 (1999). Copyright 1999 American Chemical Society.]

wavelength shift measurements was taken at a range of incident angles for two different events: (i) the adsorption of single-stranded binding (SSB) protein onto a surface functionalized with single-stranded DNA oligonucleotides; and (ii) a change in the solution index of refraction due to a change in the ionic strength by introducing a 300 mM NaCl aqueous solution. For each event, the shift can be shown either as a function of incident angle or SPR wavelength minimum. In the experiment, after starting at a particular angle the SPR minima were recorded over a range of incident angles. The incident angle was then returned to the initial value, and the shift due to protein binding was measured at the same angular positions. Again the incident angle was returned to the initial value and the shift due to the ionic strength change was measured at the same angular positions.
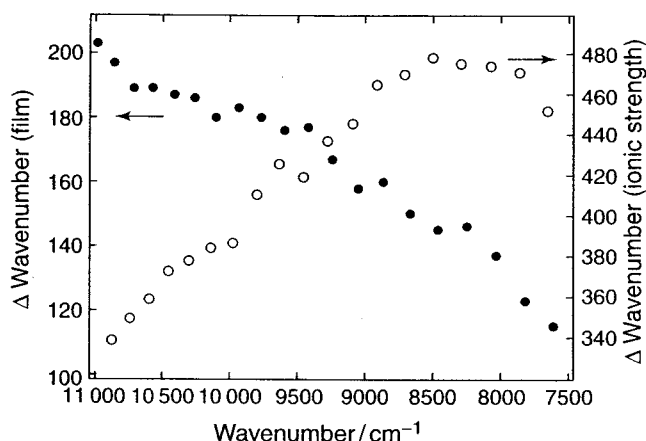
Figure 9. In situ FT-SPR multiangle resonance shift measurements of protein adsorption (●) and ionic strength change (○). (Reproduced from Corn *et al.*[19] by permission of the authors.)

Figure 9 shows the resulting shift in wavenumber for each event as a function of the SPR wavenumber minimum. The shift in the resonance minimum decreases as the incident angle decreases (wavenumber minimum shifts to a lower frequency) with adsorption of the protein. However, for an increase in ionic strength, the resonance minima shift increases as the wavenumber of the resonance minimum shifts to a lower frequency.

By comparing experimental results with the shifts predicted by Fresnel calculations, one can distinguish between a shift due to surface adsorption and a change in ionic strength or a shift due just to a change in ionic strength. The results of the two different shift experiments can be explained based on the properties of the evanescent field that penetrates through the Au film during SPR excitation. The precise decay length of the evanescent field depends upon the wavelength of the incident light that produces the SPR effect: as the wavelength of the light increases, the decay length of the evanescent field also increases. For the case of the ionic strength change, the wavenumber shift increases as the resonant wavelength increases because the ionic strength is changing within the entire evanescent wave and extending consistently further out into the solution. For the SSB protein adsorption measurement the film thickness has changed only within a finite area near the Au surface. The majority of the evanescent wave does not see the thickness change. As the resonant wavelength increases, the adsorption thickness change becomes smaller relative to the evanescent field length, resulting in a reduced shift in the SPR minima.

5 SUMMARY

SPR measurements are becoming widely used as a technique for characterizing surface adsorption of a variety of

monolayers and thin films. Making these measurements in the NIR with an FT-IR spectrometer has the potential to improve the sensitivity and versatility of the technique. Other areas where FT-SPR has application include: (i) measuring in real time the kinetics of surface reactions to determine binding constants;[20] (ii) monitoring surface changes produced by electrochemical reactions in an aqueous environment;[21] and (iii) probing the silicon/metal interface at wavelengths longer than 1100 nm.[22]

ABBREVIATIONS AND ACRONYMS

FT-SPR	Fourier Transform Surface Plasmon Resonance
InGaAs	Indium Gallium Arsenide
MUA	11-Mercaptoundecanoic Acid
PG	Poly(L-glutamate)
PL	Poly(L-lysine)
SPR	Surface Plasmon Resonance
SSB	Single-stranded Binding

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