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Near IR Scanning Angle Total Internal Reflection Raman Spectroscopy at Smooth Gold Films

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Abstract
Total internal reflection (TIR) Raman and reflectivity spectra were collected for nonresonant analytes as a function of incident angle at sapphire or sapphire/smooth 50 nm gold interfaces using 785 nm excitation. For both interfaces, the Raman signal as a function of incident angle is well-modeled by the calculated interfacial mean square electric field (MSEF) relative to the incident field times the thickness of the layer being probed in the Raman measurement (DRS). The Raman scatter was reproducibly enhanced at the interface containing a gold film relative to the sapphire interface by a factor of 4.3−4.6 for aqueous pyridine or 2.2−3.7 for neat nitrobenzene, depending on the analyzed vibrational mode. The mechanism for the increased Raman signal is the enhanced MSEF at incident angles where propagating surface plasmons are excited in the metal film. The background from the TIR prism was reduced by 89−95% with the addition of the gold film, and the percent relative uncertainty in peak area was reduced from 15 to 1.7% for the 1347 cm−1 mode of nitrobenzene. Single monolayers of benzenethiol (S/N = 6.8) and 4-mercaptopyridine (S/N = 16.5) on gold films were measured by TIR Raman spectroscopy with 785 nm excitation (210 mW) without resonant enhancement in 1 min.

Disciplines
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Near IR Scanning Angle Total Internal Reflection Raman Spectroscopy at Smooth Gold Films

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Supporting Information

ABSTRACT: Total internal reflection (TIR) Raman and reflectivity spectra were collected for nonresonant analytes as a function of incident angle at sapphire or sapphire/smooth 50 nm gold interfaces using 785 nm excitation. For both interfaces, the Raman signal as a function of incident angle is well-modeled by the calculated interfacial mean square electric field (MSEF) relative to the incident field times the thickness of the layer being probed in the Raman measurement \( D_{RS} \). The Raman scatter was reproducibly enhanced at the interface containing a gold film relative to the sapphire interface by a factor of 4.3–4.6 for aqueous pyridine or 2.2–3.7 for neat nitrobenzene, depending on the analyzed vibrational mode. The mechanism for the increased Raman signal is the enhanced MSEF at incident angles where propagating surface plasmons are limited penetration into the sample. The depth over which the Raman scatter is generated \( D_{RS} \) depends on the incident angle of the illuminating light, the wavelength of light and the indices of refraction of the interfacial species \( (\eta_{sample}/\eta_{field}) \). \( D_{RS} \) is one-half the penetration depth of the evanescent wave and typically ranges from a hundred nanometers to a couple of micrometers. The number of Raman scatterers generating the signal is related to \( D_{RS} \) for a homogeneous sample.

Total internal reflection (TIR) Raman spectroscopy is a surface sensitive technique that provides information about the chemical content at an interface. In a typical TIR experiment, the illuminating light is directed onto an interface between a high refractive index prism or objective and a lower refractive index substrate. Under TIR, the Raman signal is confined to the interface as a result of the generated evanescent wave’s limited penetration into the sample. The depth over which the Raman scatter is generated \( D_{RS} \) depends on the incident angle of the illuminating light, the wavelength of light and the indices of refraction of the interfacial species \( (\eta_{sample}/\eta_{field}) \). \( D_{RS} \) is one-half the penetration depth of the evanescent wave and typically ranges from a hundred nanometers to a couple of micrometers. The number of Raman scatterers generating the signal is related to \( D_{RS} \) for a homogeneous sample.

Raman cross sections are smaller than most fluorescence cross sections, and fewer molecules are probed in the TIR geometry compared to non-TIR illumination. High backgrounds generated in the TIR prism also remain problematic for many TIR Raman measurements. As a result, TIR Raman spectra often have low S/N ratios, despite the TIR geometry theoretically providing modest signal enhancement relative to non-TIR illumination when the same number of molecules are probed. With 785 nm excitation at a sapphire/sample interface, the increase in the electric field at the critical angle is calculated to be between \( \sim 5 \times (\eta_{sample} = 1.57) \) or \( \sim 7 \times (\eta_{sample} = 1.33) \) the incident electric field. The majority of TIR Raman studies use visible excitation sources, and monolayer detection is possible using a high laser power (e.g., 1 W) and large incident angles to decrease \( D_{RS} \). Near-IR excitation is preferred for applications where high background from fluorescent compounds may be present or where photodegradation is a concern, such as the case for many biological samples. With near-IR excitation, however, TIR Raman signal at large incident angles is low even for molecules with large Raman cross sections due to the frequency dependence of Raman scatter, and several minute acquisition times have been required. To decrease overall analysis time, increasing the signal from small probe depths with near-IR excitation is required.

Common enhancement strategies for linear Raman spectroscopy are surface enhanced Raman spectroscopy (SERS), resonance Raman spectroscopy (RRS), or in combination surface enhanced resonance Raman spectroscopy (SERRS). These enhancement techniques have limitations that reduce their broad applicability. SERS requires an analyte interaction with a nanoparticle or roughened noble metal surface and reproducibility is poor in most SERS experiments. Nano-fabricated SERS substrates have increased the reproducibility of SERS measurements significantly however intra- and intersubstrate signal differences remain a focus of current research.
TIR Raman, in contrast to SERS, provides enhanced interfacial Raman signals that extend further than a couple of nanometers from the substrate, which may be beneficial for some applications. For example enhanced SERS signals are limited to molecules, biomolecules, and thin polymers at the surface whereas TIR Raman can be used to measure polymer layers several hundred nanometers thick. One key issue is that the SERS signal is not easily modeled, limiting the ability to obtain quantitative measurements. For resonance Raman spectroscopy, the analyte must have an electronic transition that coincides with the energy of the excitation source. To date, many TIR Raman studies have utilized the resonance Raman effect.

An alternative approach to enhance Raman signals under TIR is to use smooth metal substrates at the interface, akin to those used in surface plasmon resonance (SPR) spectroscopy. Hereafter this is referred to as SPR Raman spectroscopy. Gold is the most commonly used metal for SPR spectroscopies because of its chemical stability and optical properties in the visible and near-IR region of the spectrum. The largest MSEF is generated at the gold/sample interface at the SPR angle. This is the angle with the greatest attenuation of the reflected light intensity and is associated with the excitation of surface plasmon polaritons. Recently, 633 and 647 nm excitation wavelengths were used to measure the resonant Raman scatter from Nile blue at sapphire/50 nm gold/water and glass/50 nm gold/air interfaces, respectively. The predicted MSEF enhancement at a gold interface is on the order of \(10^4\) at the SPR angle compared to non-TIR illumination Raman measurements, and the MSEF enhancement is \(\sim 2\times\) larger for near-IR versus visible excitation wavelengths due to gold’s smaller real and larger imaginary refractive index components in the near-IR. While the expected signal enhancement with SPR Raman spectroscopy is smaller than many values reported for SERS, the signal is predicted to be both reproducible and well-modeled by Fresnel calculations. Additionally, SPR Raman measurements probe deeper into the interface than SERS allowing SPR Raman measurements of thicker films (\(\sim 100\) nm).

Until recently, SPR Raman measurements have primarily used Ag films in the Kretschmann configuration. The predicted enhancement using a Ag film is \(\sim 2\times\) larger than predicted for a gold film using near-IR excitation. Ag films, however, are not chemically stable, which increases surface roughness and makes the SPR Raman data irreproducible and difficult to model. Smooth gold films provide a stable substrate for reproducible signal enhancement.

The goals of this work are: to measure the TIR Raman scatter for neat and aqueous nonresonant analytes at a smooth gold film with near-IR excitation, to compare the Raman scatter generated at gold and sapphire interfaces, to model the Raman signal obtained at different incident angles, and to demonstrate a monolayer of analyte can be measured by nonresonant SPR Raman spectroscopy. The work presented herein will provide a method for reproducible nonresonant TIR Raman signal enhancement with near-IR excitation and information to generate a fundamental understanding of other plasmon enhanced Raman scattering techniques.

### MATERIALS AND METHODS

#### Chemicals.

Nitrobenzene, benzenethiol, 4-mercaptopypyridine (Sigma-Aldrich, St. Louis, MO), and pyridine (Fisher Scientific, Waltham, MA) were used as received. 1.25 M Pyridine was prepared in deionized water from an 18.2 MΩ EasyPure II filtration system.

#### Instrumentation.

All experiments were performed using a previously reported scanning angle TIR Raman microscope that was further modified as outlined below and in the Supporting Information Figure S1.\(^{16}\) The s-polarized output from a 785 nm 0.5 W single frequency laser (Toptica Photonics, Victor, NY) was directed through an OD 0.4 neutral density filter before it was directed vertically to a focusing lens and a variable angle mirror mounted on separate motorized translation stages with the direction of travel perpendicular to the plane of the optical table. The variable angle mirror directs the now p-polarized excitation beam to the prism/sample interface that is centered on the stage of an inverted wide-field optical microscope. A 1280 × 1024 CMOS camera (Thor Laboratories, Newton, NJ) mounted in the front port of the microscope is used to align the laser onto the center of the prism. The intensity of the reflected beam from the prism/sample interface is measured using a 1 cm\(^2\) photodiode (Thor Laboratories, Newton, NJ) fitted with a OD 1.4 neutral density filter and mounted on a third vertically oriented motorized translation stage opposite the variable angle mirror. The Raman scatter generated at the prism/sample interface is collected with a 10× 0.30 NA objective lens and focused from the side port of the microscope to an f/1.8i imaging spectrometer (Kaiser Optical Systems, Ann Arbor, MI) fitted with a 1340 × 400 near-infrared enhanced CCD detector (Princeton Instruments, Trenton, NJ). The movement of the translation stages and variable angle mirror are controlled through an in-house developed program with LABVIEW 8.6.

The excitation power at the sample was 210 mW and all Raman spectra were collected by binning 160 vertical pixels (i.e., the nonspectral dimension) on the CCD with an integration time of 60 s, unless otherwise noted. The intensity of the reflected light from a sapphire/water interface was collected to calibrate the instrument’s incident angle and to measure the incident angle spread as previously reported.\(^{16}\) These parameters are extracted from the reflectivity data through modeling with Fresnel calculations,\(^{35}\) and are required to model the Raman signal at varying incident angles. For the instrument used in these studies, the incident angle spread varies from \(\pm 0.25\) to \(\pm 0.50\) degrees. Replicate measurements were acquired by making consecutive scans through the entire angle range required for a particular interface and the gold films used were from a single batch deposition either on sapphire or SF-11 glass.

Peak areas and intensities for the measured Raman modes were calculated by fitting them to Gaussian curves with the “Multipeak fitting 2” algorithm in IGOR Pro 6. The reflected light intensity from the interface was modeled with Fresnel calculations using macros developed in IGOR Pro 6 that allow the incident angle spread to be varied.\(^{16}\) Fresnel calculations were also used to calculate mean square electric fields at the interface. Signal-to-noise ratios were calculated as the maximum peak intensity after background subtraction divided by the standard deviation of the noise measured in a 50 cm\(^{-1}\) region of the spectrum where no analyte peaks were present. The percent relative uncertainty in peak area is reported as the standard deviation divided by the mean peak area.

#### Sample Configuration.

A custom-made flow cell was designed for the Kretschmann configuration. For TIR Raman measurements, a 25.4 mm diameter hemispherical sapphire prism (ISP Optics, Irvington, NY) was pressed to a 25.4 mm diameter × 400 μm thick sapphire disk ensuring optical contact with index matching fluid (\(n_i = 1.7800\) Cargille Laboratories, Cedar Grove, NJ). The sapphire disk composed the top of the
flow cell under which the analyte solution was introduced through channels in a KEL-F disk using a syringe. For SPR Raman measurements, the sapphire or SF-11 disk was coated on one side with 2 nm Cr followed by 47 nm gold (Evaporated Metal Films Corporation, Ithaca, NY for films on sapphire or GWC Technologies, Madison, WI for films on SF-11). The rms roughness of the gold films was 0.639 nm measured by atomic force microscopy (Supporting Information Figure S2). The sapphire discs and prisms were cleaned by immersion in piranha etch (5:1 H₂SO₄/H₂O₂) to remove organic residues, rinsed with deionized water and dried with N₂. [Caution: Piranha etch is aggressive and explosive. Never mix piranha waste with organic solvents. Check precautions before using it.] The flow cell was cleaned by sonication in aqueous Alconox detergent and ethanol, then dried with N₂ before each experiment.

**RESULTS AND DISCUSSION**

**TIR Raman Spectra at a Sapphire Interface.** Figure 1A and B illustrate the dependence of $D_{RS}$ and MSEM on the incident angle at the sapphire/1.25 M pyridine or sapphire/nitrobenzene interface, respectively. The critical angle for the sapphire/nitrobenzene interface is 61.2 degrees. At this angle both $D_{RS}$ (578 nm) and MSEM (5.3) are at a maximum, so Raman scatter is predicted to be the largest at the critical angle. Compared to the nitrobenzene interface, the sapphire/1.25 M pyridine interface is associated with a slightly higher MSEM of 6.9 and $D_{RS}$ of 693 nm at the 49.9 degree critical angle. As expected, the largest signal for all Raman peaks is measured at the critical angle for both interfaces (Figure 1C and 1D).

Spectra obtained near the critical angle or using non-TIR illumination, referred to hereafter as the bulk spectra, for pyridine and nitrobenzene are shown in Figure 2A and B. In all cases, the Raman spectra were collected using p-polarized incident light, and the spectra were normalized to the 1003 (pyridine) or 1347 (nitrobenzene) cm⁻¹ peak in the spectrum in order to make relative intensity comparisons. Several differences are observed between the bulk and TIR Raman

**Figure 1.** Calculated mean square electric field relative to the incident electric field (gray) and $D_{RS}$ (black) for (A) a sapphire/1.25 M pyridine or (B) sapphire/nitrobenzene interface with a (A) ± 0.25 or (B) ± 0.50 degree incident angle spread. The measured Raman spectra of (C) 1.25 M pyridine or (D) nitrobenzene at a sapphire interface are shown at varying incident angles.

**Figure 2.** Bulk (black), TIR (black dotted), and SPR (gray) Raman spectra for (A) pyridine and (B) nitrobenzene normalized to the largest analyte peak in the spectrum. TIR Raman spectra were acquired near the critical angle. $D_{RS}$ is 580 nm for pyridine and 414 nm for nitrobenzene. SPR Raman spectra were acquired at the SPR angle. $D_{RS}$ is 156 nm for pyridine and 112 nm for nitrobenzene. Peaks at 643 and 748 cm⁻¹ are assigned to sapphire. The spectra in A and B have been offset for clarity. TIR (black dotted) and SPR Raman (gray) spectra from 0.5 M pyridine (C) using a SF-11 prism at a 56 degree incident angle show a large decrease in the SF-11 background spectrum in the SPR Raman configuration. The TIR spectrum is multiplied by 1/5 for comparison and the scale bar represents 20 counts per second (cps).
spectra. Sapphire prisms can be used in TIR Raman spectroscopy with near-IR excitation to limit spectral background. Despite the overall low background compared to other prism materials, intense peaks at 643 and 748 cm\(^{-1}\) are observed in the TIR Raman spectra obtained at the sapphire interface. There are also changes in peak intensities under TIR compared to the bulk spectra. The nitrobenzene \(v_{8a}, v_{8b}, v_{9a}, v_{9b}\) and \(v_{6b}\) (Wilson Notation, spectral assignments shown in Supporting Information Table S1) in plane modes are relatively more intense in the TIR compared to the bulk spectrum. The predominate peaks in the pyridine TIR spectrum with higher relative intensities compared to the bulk are the \(v_{8a}, v_{8b}\) and \(v_{9a}\) modes. In all cases, these peaks are nontotally symmetric modes characterized by large depolarization ratios. The electric field generated under TIR is enhanced in the Z direction, perpendicular to the interface, and to a minor extent in the X direction, parallel to the interface with p-polarized incident light. Depolarized Raman scatter is more efficiently collected than polarized Raman scatter in this case, which explains the measured differences in peak intensity.

The MSEF at the interface depends on the sample index of refraction \((n_{\text{sample}})\) and this parameter must be known in order to model the Raman data at varying incident angles. The reflected light from the sapphire/sample interface is simultaneously collected with the Raman scatter to measure \(n_{\text{sample,785nm}}\) (Figure 3, top panel). Fresnel calculations model the Raman peak area of the 1.25 M pyridine solution are larger than those measured for nitrobenzene since fewer pyridine molecules are within the probed area. This results in a lower S/N ratio for the pyridine spectra: 63 (pyridine) versus 132 (nitrobenzene) for the most intense peak in the spectrum at an incident angle near the respective critical angle. In a previous report, the Raman peak area from a ZnSe/benzonitrile interface was modeled by the \(D_{\text{RS}}\) curve without consideration of the MSEF.\(^{16}\) Since the MSEF does not significantly change over the studied incident angle range for the bare prism interface, the \(D_{\text{RS}}\) curve provides an acceptable fit to the Raman peak area.

Figure 3. Reflected light intensity (top, symbol) and TIR Raman peak areas (bottom, symbol) for a (A) sapphire/1.25 M pyridine or (B) sapphire/nitrobenzene interface. The reflected light intensity is modeled using Fresnel calculations with a (A) \(\pm 0.25\) or (B) \(\pm 0.50\) degree incident angle spread (solid line). The Raman peak areas are modeled by the calculated MSEF times \(D_{\text{RS}}\) (solid line). Error bars represent standard deviation from 3 replicate measurements.

The MSEF at the interface depends on the sample index of refraction \((n_{\text{sample}})\) and this parameter must be known in order to model the Raman data at varying incident angles. The reflected light from the sapphire/sample interface is simultaneously collected with the Raman scatter to measure \(n_{\text{sample,785nm}}\) (Figure 3, top panel). Fresnel calculations model the Raman peak area of the 1.25 M pyridine solution are larger than those measured for nitrobenzene since fewer pyridine molecules are within the probed area. This results in a lower S/N ratio for the pyridine spectra: 63 (pyridine) versus 132 (nitrobenzene) for the most intense peak in the spectrum at an incident angle near the respective critical angle. In a previous report, the Raman peak area from a ZnSe/benzonitrile interface was modeled by the \(D_{\text{RS}}\) curve without consideration of the MSEF.\(^{16}\) Since the MSEF does not significantly change over the studied incident angle range for the bare prism interface, the \(D_{\text{RS}}\) curve provides an acceptable fit to the Raman peak area.

In most cases, the sapphire peaks can be observed in TIR measurements at a gold interface, but their intensity decreases by 89–95% compared to the bare sapphire interface (Figure 2A and 2B). A more dramatic example of the reduction in the prism’s contribution to the spectral background at a gold interface is evident using a SF-11 prism, which is a high index of refraction glass commonly used for SPR and TIR fluorescence spectroscopies (Figure 2C). Large broad peaks across the fingerprint region in the SF-11 Raman spectrum, which is divided by 5 to appear on the same scale as the spectrum obtained at the gold film, obscure analyte bands and limit its use in TIR Raman spectroscopy. At the gold interface the prism background is significantly reduced and analyte peaks are evident. In this manner, thin gold films can be used to mask or reduce background signals from the prism material, which represents a significant challenge in most TIR Raman measurements.\(^{15,16}\) Other than the reduced prism background, the TIR Raman and SPR Raman relative peak intensities are similar when the spectra are normalized to the most intense analyte peak in the spectrum (Figure 2). The low S/N ratio for the 1.25 M pyridine SPR Raman spectrum, as a result of the smaller 156 nm \(D_{\text{RS}}\) value at this interface, explains why some peaks appear in the TIR Raman spectrum but not the SPR Raman spectrum.

As expected, the largest Raman peak intensity is not obtained at the critical angle, rather at the predicted SPR angle for a gold/1.25 M pyridine (Figure 4C) or gold/nitrobenzene (Figure 4D) interface. The reflected light intensity from these interfaces show the expected attenuation at the SPR angle (Figure 5, top panel). The reflectivity data has been modeled by Fresnel calculations with the appropriate angle spread described above. In all cases the Fresnel calculations predict the location of the critical angle and the SPR angle to within 0.3 degrees.

The same Raman modes used to generate the data shown in Figure 3 were used to generate the data shown in Figure 5.
for 1.25 M pyridine and nitrobenzene at the gold interface. Near the SPR angle, the Raman peak area is approximately the mirror image of the SPR curve, and the maximum Raman peak area is measured at the SPR angle. Similar to the modeled reflectivity data, there is a decent fit between the experimental Raman peak area and the MSEF × DRS curve. The similarity between the shape of the reflectivity curve and the Raman peak area curve suggests that measurements at interfaces where the reflectivity data can be modeled will result in Raman peak areas that can be modeled, and that the reflectivity data can be used to predict the collected Raman signal. Differences between the predicted Raman peak area and the measured Raman peak area are attributed to gold films with a thicker than desired (2 nm) Cr adhesion layer and possible contamination of the gold film during the deposition process.

Comparison of Raman Signals at Sapphire and Gold Interfaces. As a result of the larger MSEF, more Raman scatter is predicted at the gold/aqueous than at the gold/organic interface assuming the same number of scatterers are probed. The theoretical enhancement factors were $\sim 11 \times$ (aqueous pyridine) and $\sim 9 \times$ (nitrobenzene) at a gold film compared to the sapphire interface. Measured enhancements are indicated in Figure 6, which shows plots of the Raman peak intensity divided by the calculated $D_{RS}$ for each incident angle. As expected, the TIR Raman signal is a relatively flat line since this data representation should mimic the MSEF curves shown in Figures 1 (sapphire interface) or 4 (gold interface). The increase in the SPR Raman signal with respect to the TIR Raman signal represents signal enhancement when the same number of Raman scatters are probed. The appreciably enhanced modes of pyridine are $\nu_1$ and $\nu_{12}$ with enhancement factors of 4.6 and 4.3. The $\nu_{8a}$, $\nu_{8b}$, $\nu_1$, $\nu_{12}$ and NO$_2$ symmetric modes of nitrobenzene gave enhancements of 3.7, 2.2, 2.4, 2.8, and 3.7, respectively. Average uncertainties in the Raman peak areas at the gold interface are smaller than at the sapphire interface for nitrobenzene’s $\nu_{8a}$, $\nu_{8b}$, $\nu_1$, $\nu_{12}$ modes and NO$_2$ symmetric stretch; and pyridine’s $\nu_1$ mode. Analysis of the spectra reveals that all of these peaks have S/N ratios greater than 21. For example, the percent relative uncertainty of nitrobenzene’s 1347 cm$^{-1}$ peak area decreases from 15% at sapphire to 1.7% at the gold interface. Uncertainties in Raman peak areas did not improve at the gold interface for modes with lower S/N ratios.

The measured Raman enhancement is 2.4–4.1× lower than expected at the gold film. Previous studies have indicated a 10× lower than expected enhancement for pyridine at an Ag electrode in a similar experimental configuration. This may be because of a combination of suboptimal gold films, small changes in the collection efficiency at different excitation angles, and the small increase in the background as surface plasmons are excited in the

Figure 4. Calculated MSEF (gray) and $D_{RS}$ (black) for a (A) sapphire/gold/1.25 M pyridine interface or (B) sapphire/gold/nitrobenzene interface with a (A) ± 0.25 or (B) ± 0.50 degree incident angle spread. The measured Raman spectra of (C) 1.25 M pyridine or (D) nitrobenzene at a sapphire/gold/analyte interface are shown from 50 to 55 and 65–70 degrees, respectively.

Figure 5. Reflected light intensity (top, symbol) and SPR Raman peak areas (bottom, symbol) for a (A) sapphire/gold/1.25 M pyridine or (B) sapphire/gold/nitrobenzene interface. The reflected light intensity is modeled using Fresnel calculations with a (A) ± 0.25 or (B) ± 0.50 degree incident angle spread (solid line). The Raman peak areas are modeled by the calculated MSEF times $D_{RS}$ (solid line). Error bars represent standard deviation from 3 replicate measurements.

(bottom panel) for 1.25 M pyridine and nitrobenzene at the gold interface. Near the SPR angle, the Raman peak area is approximately the mirror image of the SPR curve, and the maximum Raman peak area is measured at the SPR angle. Similar to the modeled reflectivity data, there is a decent fit between the experimental Raman peak area and the MSEF × $D_{RS}$ curve. The similarity between the shape of the reflectivity curve and the Raman peak area curve suggests that measurements at interfaces where the reflectivity data can be modeled will result in Raman peak areas that can be modeled, and that the reflectivity data can be used to predict the collected Raman signal. Differences between the predicted Raman peak area and the measured Raman peak area are attributed to gold films with a thicker than desired (2 nm) Cr adhesion layer and possible contamination of the gold film during the deposition process.
gold. Unlike typical SERS data, it has been shown that the SPR Raman signal is well-modeled. None of the spectra shown herein have been background subtracted nor baseline corrected.

**SPR Raman Measurement of a Monolayer without Resonant Enhancement.** For SERS, the analyte must be adsorbed or very close to the metal surface and surface selection rules must be considered when interpreting the spectra; whereas, the SPR Raman signal is predominately from analyte that is not adsorbed to the metal. To test whether a monolayer of adsorbate could be measured using SPR Raman spectroscopy with 785 nm excitation, a monolayer of benzenethiol or 4-mercaptopyridine was prepared on a gold film and probed under TIR at the SPR angle. Figure 7 shows the SPR Raman signal for the sapphire/gold/benzenethiol monolayer/water or sapphire/gold/4-mercaptopyridine monolayer/air interfaces.

The acquisition times range from 1 to 3 min using an incident power of 210 mW. The S/N ratios for the spectra obtained in 1 min are 6.8 and 16.3 for benzenethiol and 4-mercaptopyridine, respectively. A comparison of the integrated peak areas of the 1002 cm$^{-1}$ mode of benzenethiol from a monolayer or neat benzenethiol solution reveals that roughly 2% of the signal comes from adsorbate at the SPR angle, when the difference between the mean square electric field at the aqueous or benzenethiol interface (a factor of 2.4×) is considered (Supporting Information Figure S3). In SERS, chemical enhancement results from interaction between the metal and adsorbate species. Since the adsorbed species contribute little to the overall SPR Raman signal, chemical enhancement is negligible when the signal is dominated by solution species. These spectra reveal that the SPR Raman technique is suitable for monolayer measurements with near-IR excitation wavelengths where no resonant enhancement is expected.

**CONCLUSIONS**

The detection of a monolayer of nonresonant molecules at a gold film with near-IR excitation is a significant step toward the broader application of the SPR Raman method. Near-IR excitation and the use of gold films in the TIR configuration provides a lower background from system optics, an overall decrease in the uncertainty of most Raman peak intensities and monolayer level sensitivity. The use of other noble metals (e.g., Ag) with more favorable plasmonic properties in the near-IR could provide a larger MSEP at the metal/sample interface and lead to measurements of weak Raman scatterers including biological samples; however, the chemical stability of the Ag film needs to be considered. Currently, additional methods to
increase the MSEF at the interface without introducing surface roughness to the metal are being pursued.

■ ASSOCIATED CONTENT

1 Supporting Information

Instrument schematic, AFM image of the smooth gold substrates, monolayer, and neat SPR spectra from benzenethiol and table of Raman peak assignments available. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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