The Use of Polarization-dependent SERS from Scratched Gold Films to Selectively Eliminate Solution-phase Interference

Jason R. Anema · Alexandre G. Brolo

Abstract Polarization-dependent surface-enhanced Raman scattering (SERS) was studied for oxazine 720 molecules adsorbed on a scratched gold surface placed in situ and under electrochemical control. A quantitative method for evaluating the observed polarization dependence will be introduced. This method takes into account the polarization artifacts caused by optical elements in the light microscope used for Raman microscopy. Intensity of the SERS obtained from oxazine 720 adsorbed on scratches in gold showed a polarization dependence after correction was made for these artifacts. In contrast, intensity of the ordinary Raman signal obtained from perchlorate ions in the solution above a scratched gold surface was found to be polarization-independent. Therefore, polarization effects can be used to selectively remove solution-phase interference signals from the SERS spectrum of an adsorbed analyte. These polarization effects were found to be independent of the applied potential, meaning the methodology is applicable to electrochemical SERS studies.

Keywords SERS · Polarization-dependent SERS · Interference signal elimination · Scratched gold · Nanostructure · Nanowires · Spectroelectrochemistry · Oxazine 720

Introduction

Raman spectroscopy is based on the inelastic scattering of light by a molecule [1]. In surface-enhanced Raman

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spectroscopy (SERS), inelastic scattering occurs with 10^5–10^6 times more efficiency because of the molecule's proximity to the nanostructured surface of a free-electron metal [2]. This phenomenon results from interactions involving the molecule, the nanostructure, and the incoming and outgoing photons. Two mechanisms contribute to the SERS effect, one chemical (charge transfer or CT) [3] and one electromagnetic (EM) [2] in nature. It is generally agreed that the latter is dominant under conditions used to carry out most SERS experiments. In this case, surface plasmons (SPs) are initiated at the metal–dielectric interface by incident laser light. The SPs give rise to enhanced EM field in areas of high curvature and in the space between metallic structures [4–7]. The Raman signal of a molecule situated in such an area will be enhanced by the EM mechanism. The frequencies of the scattered light, in addition to that of the incident light, are usually within the SP resonance of the nanostructure, and this creates conditions for extra enhancement [2].

In 1997, two groups independently reported signal enhancement of about 10^{14} times and the detection of single molecules by SERS [8, 9]. Their work has opened the way for new possibilities in chemical analysis [10]. The identification of particular DNA and RNA oligonucleotides within mixtures, while at femtomolar concentration, is a good example [11].

A lack of reproducibility is generally viewed as the greatest obstacle to development of SERS-based analytical devices. Much work has been done to investigate new substrates for SERS, often with the goal of improving reproducibly [12]. Recently, with the advent of accessible nanofabrication methods, this problem is being solved by the deliberate engineering of nanostructures [13–15]. The disadvantage of this approach is that the techniques employed (e.g., focused ion beam milling, electron beam

lithography) are more cumbersome and more costly than traditional ones (e.g., colloid formation, electrochemical roughening).

The SERS substrate examined in this work is simply a set of scratches in a thin film of gold. A unidirectional scratch in a gold film, generated using a sharp tip, will consist of parallel nanostructures that resemble a set of randomly distributed nanowires with distinct diameters and shapes. The focus in this study is not on the reproducibility of the SERS signal obtained from this substrate but on the exploration of its interesting polarization properties.

It has already been demonstrated that, for molecules adsorbed onto a scratched gold surface, SERS intensity is dependent upon the direction of incident-beam linear polarization relative to the scratches [16, 17]. This phenomenon is not unique to scratched gold surfaces but may be found wherever a SERS substrate has linear features. In addition to scratched gold films, polarization effects have been reported for silver nanowires [18–20], silver nanorods [21, 22], and silver nanoparticle dimers [23].

This work examines the polarization-dependent enhancement effect associated with scratched gold films placed in situ and under electrochemical control. A quantitative method for evaluating the extent of a polarization effect will be proposed. In addition, proof of concept for application of this phenomenon to analytical devices will be given. It will be shown that the polarization effect can be used to eliminate the signal from solution-phase interferences without removing that of an adsorbed analyte.

Experimental section

Glass slides plated with 5nm of chromium and 100nm of gold were obtained from Evaporated Metal Films (EMF). These slides were scratched by running a 10μm metal tip along the surface of the gold. AFM images of scratches produced in this way are published elsewhere [16, 17]. They show that a set of parallel gold nanowires, with a random distribution of sizes and shapes, are formed within the scratches.

Oxazine 720 (from Lambdachrome) was dissolved in ethanol (from Commercial Alcohols Inc.) to create a 10-μM solution. A slide was held at an angle while a single drop of the oxazine 720 solution was added to its gold surface and allowed to run overtop of the scratched area. After the solvent evaporated, the slide was rinsed with ultrapure water (18.2MΩ cm, from a Barnstead NANOpure Diamond water purification system).

The Au slide, modified with oxazine 720, was placed in a custom-made Teflon electrochemical cell prepared in house. The cell was filled with a 0.1M $NaClO_4$/0.1M NaCl

aqueous electrolyte solution and placed under an Olympus BHT optical microscope set up to collect Raman spectra in a backscattering geometry (Fig. 1).

A 35mW HeNe laser from Melles Griot provided 632.8nm light. The beam passed through a 1/2-wave plate, entered the microscope through an optical inlet, was reflected by the scratched gold surface, and exited the microscope through an optical outlet. An Olympus MS Plan 50× ultra-long working distance microscope objective with numerical aperture 0.55 was used to focus the laser onto the sample. This same lens was used to collect scattered and reflected light. A Kaiser super-notch filter was used to exclude most of the Rayleigh light. The remaining light, which contained Raman information, was focused by a Kaiser Holospec f/1.4 spectrograph onto an Andor DV-401-BV CCD. The diameter of the laser spot was approximately equal to the scratch width, about 10μm.

A potential difference was established between the scratched gold film and the electrolyte solution so that the polarization effect could be examined over a range of applied potentials. The gold surface of the slide acted as the working electrode, and a circular platinum wire acted as the counter electrode. A silver wire coated with silver chloride acted as the reference electrode. The electrical potential of the working electrode was controlled by a Hokuto Denko Limited potentiostat/galvanostat, model HAB-151.

A broad fluorescence background signal was obtained for slides modified with oxazine 720. It is well known that fluorescence from molecules in contact with a metal surface is efficiently quenched [24, 25], so the fluorescence background implies that a multilayer of oxazine 720, rather than a monolayer, was deposited by using the procedure described above. To minimize this signal, the area to be investigated was irradiated with HeNe laser light for 1h before accumulation of SERS data began. Due to photo-bleaching of the dye, a steady decline in fluorescence
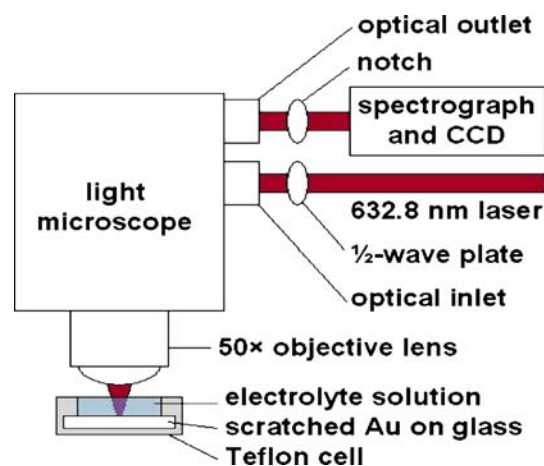
Fig. 1 A microscope was used to focus the laser onto the scratched gold surface. The gold-plated glass slide, immersed in an aqueous solution, resided in a spectroelectrochemical cell

intensity was observed during this time. The fluorescence background was substantially decreased and time-stable after this treatment.

For the blank experiments, scratched gold surfaces without oxazine 720 adsorbed were immersed in a 0.2M NaClO$_4$ aqueous electrolyte solution.

Results and discussion

Preliminary experiments to confirm the polarized nature of the scratches were conducted before the in situ spectroelectrochemical ones. These preliminary experiments are summarized in Fig. 2. Two sets of scratches, with direction perpendicular to each other, were produced in a gold film and coated with the Raman-active dye molecule oxazine 720. A pair of microscope objectives focused HeNe laser light onto the scratched gold surfaces, through air, in a forward scattering geometry (Fig. 2a). A 1/2-wave plate was used to rotate the plane of incident-beam polarization through angles, which are defined in Fig. 2b. In Fig. 2c, a polar plot shows the dependence of SERS intensity on the relative orientation between incident-beam polarization and scratch direction for the 596cm^{-1} band of oxazine 720. Maximum SERS intensity was obtained at approximately 0 and 180° for scratch orientation a (diamonds) and at approximately 90 and 270° for scratch orientation b (squares).

A photon in free space and a photon bound to the surface of a noble metal (a SP) do not normally interconvert because the former does not possess as much momentum as

the latter [26]. If a grating is present in the surface of the metal, this momentum mismatch can be overcome [26].

The preliminary results in Fig. 2c indicate that the parallel nanostructures within a scratch can act as a crude grating. In other words, the random parallel nanowires can be thought of as a Fourier superposition of a number of oriented gratings of different periodicity that maintains the polarization properties of the original set. When the polarization of incident light is perpendicular to the long axis of the scratches, SPs may travel across the corrugations, and enhanced EM field will occur in areas of high curvature and in the space between metal nanostructures. Raman-active molecules adsorbed in these areas will give a strong SERS signal, as shown in Fig. 2c.

When the polarization of incident light is parallel to the long axis of the scratches, SPs are not expected and SERS should not be observable because the crude grating is absent. Parallel-alignment SERS peaks were found to be less intense than the corresponding perpendicular-alignment ones;, however, signal intensity obtained for the parallel-alignment was non-zero (diamond and square data points in Fig. 2c do not pass through the center of the polar plots). A small amount of field enhancement does occur in the parallel orientation because some of the nanometer-sized features within the scratched area are randomly oriented. These can be regarded as defects, which arise from the simple procedure employed for scratch generation. In addition, alignment between the scratches and the incident beam is subject to some error, and this explains the tilt-angle of about 10° in the polar plots.

Fig. 2 a The apparatus used to carry out preliminary experiments is illustrated. b The angle of rotation for incident-beam polarization is defined. c Polar plots show the dependence of SERS intensity on incident-beam polarization for scratch orientation a (diamonds) and orientation b (squares)
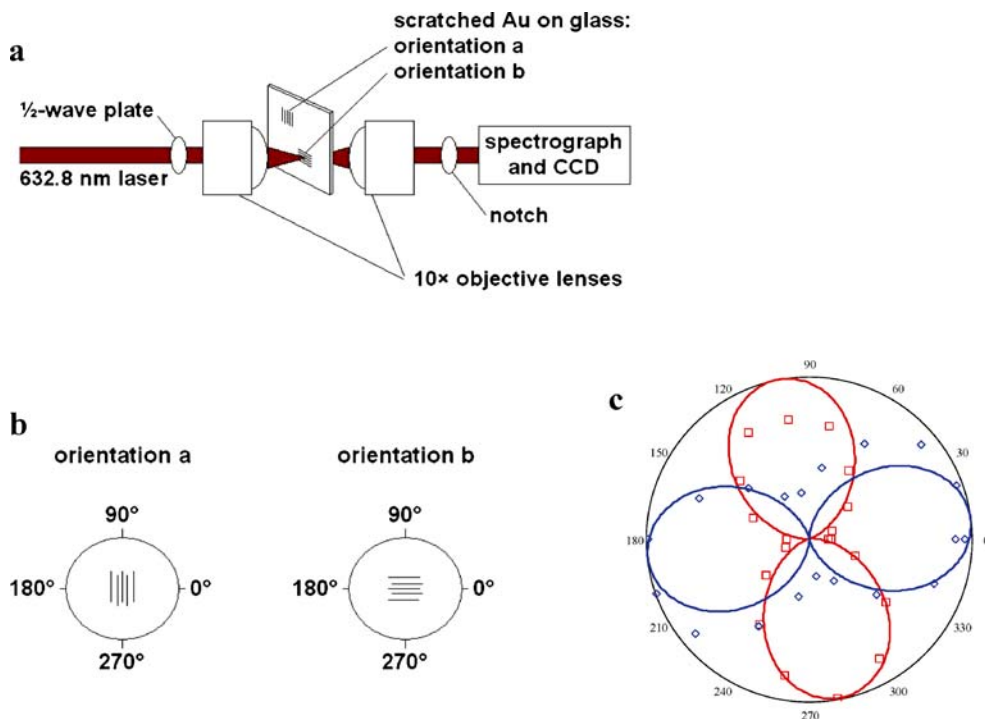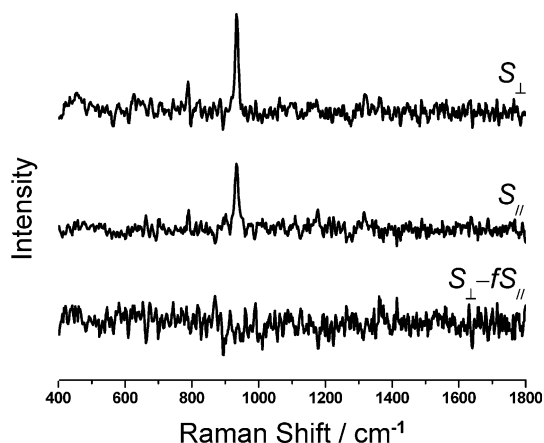
Fig. 3 $S_\perp$, $S_{//}$ and $S_{analytical}$ for perchlorate, which represents an interference in the solution above the analyte. Acquisition time was 45 s. Before application of Eq. 4, $S_\perp$ and $S_{//}$ were smoothed and baseline corrected. The interference signal is eliminated by use of Eq. 4 (see text)

No signal was obtained from oxazine720 adsorbed onto a smooth gold surface. An electronic absorption band of oxazine 720 is centered at 620nm, and this transition is in resonance with the laser excitation [27]. As resonance Raman scattering from molecules on the smooth surface is expected, the absence of signal implies that a very small amount of dye was left on the metal by the application procedure reported in this paper.

To explore the polarization effect described above for background suppression, the scratched gold film was placed in a custom made Teflon cell, and the cell was filled with an aqueous solution of sodium perchlorate electrolyte (Fig. 1). Oxazine 720 was used as the adsorbed Raman-active molecule, and it represents some potential analyte in an electrochemical SERS experiment. The perchlorate anion is also Raman-active, but it is not adsorbed onto the gold surface, and it represents a solution-phase interference.

The premise of the proposed methodology for background subtraction is that the polarization-dependent enhancement will be observed for Raman-active molecules adsorbed onto the surface but not for those in the solution phase. This is because SPs, which cause the enhancement, are basically surface-confined. As the perchlorate anion is not adsorbed, it does not give rise to a polarization-dependent SERS signal, and only an ordinary Raman signal is observed. A caveat for this is the fact that the SP field decays exponentially with distance from the surface [26]. When the polarization of the light is perpendicular to the scratched metal surface (and SERS is operative), some of the solution species will be within the SP field. The main contribution to the overall signal will come from the adsorbed species, however, because the SP field is at maximum at the surface and decays fast within a few nanometers. The SERS is not as significant when polarization of the laser is parallel to the scratches. In this case, the

contribution from the adsorbed species is drastically reduced, but the ordinary Raman signal from the solution species is not significantly changed.

Figure 3 shows the Raman spectra of perchlorate in solution above a scratched gold surface that was not treated with oxazine 720. The electrochemical potential was held at 0mV. The $934cm^{-1}$ band is due to the completely symmetric stretch of the ion. The spectra $S_\perp$ and $S_{//}$ were obtained with the incident beam polarized across and along the scratches, respectively.

It can be seen, by comparing spectra $S_\perp$ and $S_{//}$ in Fig. 3, that perchlorate band intensity changes with the direction of incident-beam polarization relative to the direction of the scratch. There are two possible reasons for this change. It may be that some perchlorate anions, although in solution, still lie within the decaying field of the SPs and are therefore subject to a small polarization effect. The other possibility is that some optical anisotropy is introduced by the microscope.

To determine if the microscope introduces an artificial polarization effect, the laser spot was focused on a smooth gold surface. A "real" polarization effect is impossible here because the scratches that give rise to polarization-dependent SERS are absent. When the 1/2-wave plate was rotated to positions used for perpendicular- and parallel-geometry data acquisition, a difference in intensity between the spectra $S_\perp$ and $S_{//}$ was indeed observed. This means that optical components within the microscope result in a polarization bias, likely because s- and p-polarizations are reflected from them with different efficiencies [28].

Figure 4 shows the Raman spectrum for oxazine 720 drop-coated onto a scratched gold slide, then placed in situ and under electrochemical control (0mV). The difference between $S_\perp$ and $S_{//}$ was much greater for oxazine 720 (Fig. 4) than it was for perchlorate anion (Fig. 3), and this indicates that oxazine 720 molecules are located in areas where the
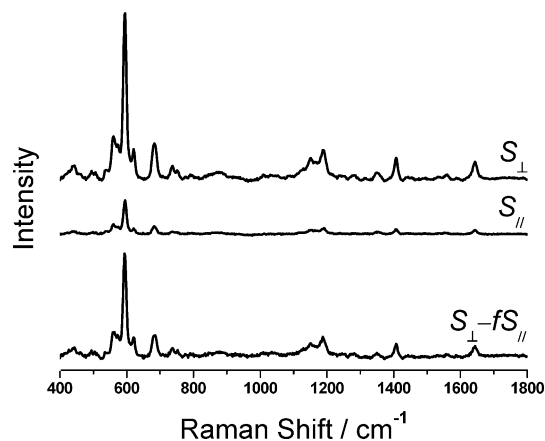
Fig. 4 $S_\perp$, $S_{//}$ and $S_{analytical}$ for oxazine720, which represents an analyte on the surface of the metal. Acquisition time was 20°s. Before application of Eq. 4, $S_\perp$, $S_{//}$ were baseline corrected. The signal is retained (see text)

polarization-dependent surface-enhancement effect is operative (i.e., SERS was observed for oxazine 720, whereas an ordinary Raman spectrum was observed for perchlorate).

Perchlorate was present in the aqueous solution above the slide, but its signal is masked by the far greater one from oxazine 720. The experimental setup was the same for oxazine 720, as it was for perchlorate, so the anisotropy introduced by the microscope is expected to affect $S_{\perp}$ and $S_{//}$ in Fig. 4 to the same extent as it did $S_{\perp}$ and $S_{//}$ in Fig. 3.

Equation 1 is now proposed in an attempt to quantify the extent of an observed polarization effect.

$$p_{observed} = \frac{I_{\perp} - I_{//}}{I_{\perp} + I_{//}} \qquad (1)$$

Here $I_{\perp}$ is the intensity of a Raman band obtained using a perpendicular alignment of incident-beam polarization with scratch direction, and $I_{//}$ is the intensity of the same band obtained using a parallel alignment. This approach allows an empirical polarization effect to be quantified on a scale ranging from 0 to 1. The best possible polarization effect would yield a large value for $I_{\perp}$ and a null value for $I_{//}$; therefore, the ideal value for $p_{observed}$ is 1. No polarization effect would yield $I_{\perp} = I_{//}$, and a $p_{observed}$ value of 0.

However, a correction factor f is required to compensate for artificial polarization effects from the microscope.

$$f = \frac{I'_{\perp}}{I'_{//}} \qquad (2)$$

In Eq. 2, a prime indicates that the intensity must be measured under conditions where a "real" polarization effect is impossible (a smooth area on the surface of the metal must be used).

Equation 1 thus becomes Eq. 3, and $p_{observed}$ ranges from 0 to 1 in the microscope experiment.

$$p_{observed} = \frac{I_{\perp} - f \cdot I_{//}}{I_{\perp} + f \cdot I_{//}} \qquad (3)$$

As stated earlier, the polarization-dependent enhancement effect can be used to eliminate the Raman band of a solution-phase interference (represented here by perchlorate) without removing the SERS band of some adsorbed analyte (represented by oxazine 720). It is a proof of concept that we present in this work—clearly, there is no overlap between the 934cm^{-1} perchlorate band and any of the higher intensity oxazine 720 bands. The background subtraction is accomplished by obtaining a mathematically manipulated spectrum for analytical purposes, $S_{analytical}$, using Eq. 4.

$$S_{analytical} = S_{\perp} - f \cdot S_{//} \qquad (4)$$

Here, $S_{\perp}$ is the entire spectrum obtained using a perpendicular alignment, $S_{//}$ is the entire spectrum obtained using a parallel alignment, and f from Eq. 2 is a constant

applied to each data point in $S_{//}$. In this study, f values obtained for perchlorate in solution over a smooth gold surface were used to correct $S_{//}$ for both oxazine 720 and perchlorate.

When Eq. 4 was applied to the ordinary Raman signal obtained from perchlorate in the solution above the scratched gold surface, the perchlorate peak was eliminated (Fig. 3). When it was applied to the SERS signal obtained from oxazine 720 adsorbed onto the scratched gold surface, however, oxazine 720 peaks persisted (Fig. 4).

Although areas of the metal treated with oxazine 720 were irradiated with the HeNe laser for 1h before any spectra were accumulated from them, some oxazine 720 fluorescence remained (see "Experimental section"). It should be noted that this fluorescence persists in $S_{analytical}$ if $S_{\perp}$ and $S_{//}$ are not baseline-corrected.

The 596cm^{-1} peak was used to calculate $p_{observed}$ values for oxazine 720, and the 934cm^{-1} peak was used for perchlorate. In each case, $p_{observed}$ values were fairly constant and reproducible over the potential range investigated: +300 to −300mV for oxazine 720 and +300 to −900mV for perchlorate (Fig. 5). Potentials below −300 mV were not examined for oxazine 720 because electrochemical reduction and desorption, evidenced by an increase in fluorescence, was found to occur there [27]. The $p_{observed}$ values of about 0.4 for oxazine 720 and 0.0 for perchlorate indicate that a polarization effect exists for the former and not the latter.

Figure 5 indicates that our procedure (Eq. 4) is applicable to electrochemical studies provided the interfering species (perchlorate in our case) is not adsorbed or electrochemically active within the potential range investigated. The study of water structure in the double layer region by SERS is a good candidate for our methodology, as the SERS signal from water is inherently weak and can
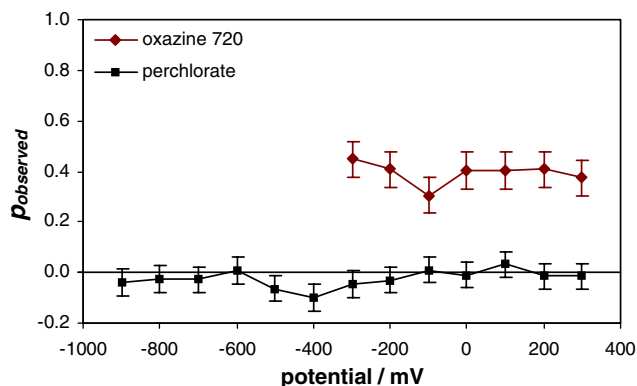

Fig. 5 Values of $p_{observed}$ for oxazine 720 and perchlorate remain fairly constant and reproducible with changes in potential. Each point is an average of two, and error bars mark the standard deviation obtained from all $p_{observed}$ values for oxazine 720 or all $p_{observed}$ values for perchlorate

sometimes be overwhelmed by the ordinary Raman signal from the solvent. These types of applications are now being explored by our group.

Conclusions

We successfully demonstrated that scratched gold slides and a polarized excitation source may be used to eliminate solution-phase interference signals in electrochemical SERS studies. The SERS spectra obtained from oxazine 720 adsorbed on scratches in gold were found to be polarization-dependent over the potential range investigated. The ordinary Raman spectra obtained from perchlorate ions in the aqueous solution above a scratched gold surface were found to be insensitive to the direction of incident-beam polarization for all applied potentials.

Polarization artifacts can arise from the light microscope employed in Raman microscopy; however, it is easy to remove these artifacts mathematically by using a reference signal from a smooth surface.

The use of polarization-dependent SERS for removal of solution-phase interference signals, as described in this work, is preferable to the more customary subtraction of a solution spectrum (obtained using a smooth surface) from one containing both SERS and solution-phase information (obtained using a rough surface). This is because rotation of a 1/2-wave plate is less awkward and subject to less error than locating and relocating a particular micrometer-scale area on an inhomogeneous SERS substrate. Automation, and therefore real-time measurement, is more easily accomplished using our method because signal and background are accumulated from the same location.

Finally, the gold-plated glass slides used in this study are cheap, and the scratches are easy to fabricate. Therefore, scratched metallic films of this type might be appropriate for analytical devices that require a disposable SERS substrate.

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