Atomic force microscopy and surface-enhanced Raman spectroscopy.
I. Ag island films and Ag film over polymer nanosphere surfaces supported on glass

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The surface roughness and nanometer scale structure of Ag films used for surface-enhanced Raman scattering (SERS) are characterized using atomic force microscopy (AFM). Two important types of thin film based SERS-active surface have been examined in this study: (1) Ag island films (AgIF’s) on smooth, insulating substrates and (2) thick Ag films evaporated over both preroughened and smooth substrates. AFM is demonstrated to be capable of quantitatively defining the three-dimensional (3D) structure of these roughened surfaces. The effects of mass thickness, \( d_m \), and thermal annealing on the nanostructure of AgIF’s are studied in detail. Particle size histograms are calculated from the AFM images for both “as-deposited” and annealed IF’s with \( d_m = 1.8 \) and 3.5 nm. Quantitative measurements of the SERS enhancement factor (EF) are coupled with the AFM data and interpreted within the framework of the electromagnetic theory of SERS. AFM images for thick evaporated Ag films over a monolayer of polymer nanospheres (AgFON) shows the clear presence of “random substructure roughness” reducing their utility as controlled roughness surfaces. Similar roughness structures are observed for thick evaporated Ag films on smooth, insulating substrates. Nevertheless, AgFON surfaces are demonstrated to be among the most strongly enhancing thin film based surfaces ever studied with EF’s comparable to those found for electrochemically roughened surfaces. Applications of FON surfaces to ultrahigh sensitivity SERS, anti-Stokes detected SERS, and surface-enhanced hyper Raman spectroscopy (SEHRS) are reported.

I. INTRODUCTION

Surface-enhanced Raman spectroscopy (SERS) is a widely used technique for obtaining vibrational spectra at some roughened metal surfaces. Several recent review articles provide a comprehensive overview of the more than 2000 papers on SERS dealing with fundamental mechanistic studies of photon/molecule/surface interactions as well as applications in electrochemistry, surface science, materials science, and biochemistry. Recently, fundamental work has been far outpaced by applications development in spite of the circumstance that, in the opinion of the authors, a full, quantitative, and predictive understanding of all features of the SERS enhancement mechanism(s) has yet to emerge.

Currently, it is believed that two simultaneously operative mechanisms, a long range classical electromagnetic (EM) effect and a short range “chemical” (CHEM) effect are responsible for SERS. The partitioning of the total enhancement between these two mechanisms is still a matter of debate but at least for Ag surfaces excited with visible laser excitation wavelengths, it appears that the EM mechanism contributes about 10^4 with the CHEM mechanism contributing an additional factor of about 10^2. Schatz has reviewed the current state of the EM mechanism while Otto has reviewed the recent progress in conceptualizing the CHEM mechanism as a metal electron-mediated resonance Raman effect that involves increased electron-photon coupling at atomically rough surfaces and transient charge transfer into affinity levels of the adsorbate.

As a spectroscopic probe of surface chemistry and dynamics, SERS has its strengths. (i) The ability to determine the identity, bonding, orientation, and monitor the dynamics of adsorbed molecules. (ii) Extraordinary sensitivity and selectivity to the interfacial species. (iii) Adsorbate generality. (iv) Operation at solid/UHV, solid/gas, solid/liquid, and even solid/solid interfaces. (v) Operation at metal surfaces in addition to Ag, Cu, and Au: Li, Na, K, Cs, Rb, Al, Ga, In, Pt, and Rh. (vi) The scope of SERS has been extended to include other surface-enhanced spectroscopies (SES) such as the nonlinear processes of surface-enhanced second harmonic generation (SESHG) and surface-enhanced hyper Raman scattering (SEHRS).

SERS also has its weaknesses. (i) Surface roughness on a nanometer length scale is required thereby excluding the smooth, low index, single crystal surfaces commonly used in UHV surface science. (ii) The magnitude of the surface enhancement factor (EF) and, hence, the sensitivity of SERS is a strong function of the chemical and optical properties of the metal surface as well as its detailed surface morphology. (iii) It is not clear whether semiconductor surfaces are intrinsically SERS active or not. (iv) Insulator surfaces are not intrinsically SERS active. (v) The stability and reproducibility of most SERS-active surfaces
are far from ideal. (vi) A key weakness in the comparison between SES experiment and EM theory is the mismatch between the actual surface nanostructures used in experiments and the model surface nanostructures used for theory.6

One of our longstanding goals has been to ascertain whether these weaknesses are fundamental impediments to extending the scope of SERS or whether, through an improved understanding of the SERS enhancement mechanism(s) and/or appropriate experimental design, they can be overcome. Those problems which derive from the current lack of information concerning the detailed three-dimensional (3D) structure of SERS-active surfaces and its reproducible control will be specifically addressed in this paper.

Because SERS-active surfaces possess roughness on several characteristic length scales, x-ray diffraction and low-energy electron diffraction (LEED) methods are not generally applicable to surface structure determination. As a consequence, it has become customary in SERS studies to characterize surface structure (i.e., surface roughness) using scanning electron microscopy (SEM). Electrochemically roughened surfaces,27-31 island films,32,33 thick metal films over CaF, roughened surfaces,34 thick metal films over nanosphere (FON) surfaces,35-42 and lithographically fabricated 2D metal particle arrays16,43,44 have all been examined using SEM. While much useful information can be acquired from qualitative assessments of SEM micrographs and the measurement of particle size distributions as a function of surface preparation or SERS operating conditions, there are certain inherent limitations to SEM when it is applied to the SERS problem. For example, all of the studies cited above involve removal of the surface from the SERS operating environment and the placement of it in the vacuum chamber of the SEM instrument. Thus one must assume that there are no or minimal structural rearrangements that occur during this environment transfer. It would be far more desirable to carry out the microscopy under identical conditions to the spectroscopy. SEM requires electrically conducting samples. Thus SEM studies of metal island films, which are usually deposited on insulating substrates, requires overcoating the sample with thin continuous conducting films (e.g., Au). A more desirable situation would be to perform the microscopy on the "as-deposited" SERS-active surfaces. SEM has a typical resolution of 5-10 nm which is insufficient to resolve the complete distribution of roughness features in SERS which range from atomic scale roughness (ASR) to large scale roughness (LSR, particles with dimensions of 10 nm to 3 μm).

Sufficient spatial resolution not only to resolve the ASR and LSR of SERS-active particles, but to determine highly detailed structural information about metal particles, can be obtained by using transmission electron microscopy (TEM) or high resolution electron microscopy (HREM). In the context of SERS, TEM has been applied to metal colloids,45-50 island films,51,52 and photographically derived Ag surfaces.2 HREM has been used to examine the internal structure of Ag and Au colloids although not specifically in the context of SERS.56,57 Here again there are inherent problems. Both TEM and HREM suffer from the environmental transfer problem cited above for SEM. In addition, these techniques require specimens that are semi-transparent to electrons. Therefore, specimen thicknesses must be <100 nm or the deposition of thin islands of SERS-active material must be on low atomic number substrates. It would be more desirable if such high resolution information could be obtained without the necessity of sample thinning or special substrates.

Since the invention of the scanning tunneling microscope (STM), it has been clear that its atomic resolution capabilities and environmental compatibility with solid/vacuum, solid/liquid, and solid/gas interfaces50-53 offered attractive possibilities for the study of surface enhancement phenomenon. Recently several research groups have used the STM to examine the morphology of silver surfaces: (1) 50 nm thick vapor deposited Ag films in air,64 (2) cold deposited and warm deposited Ag films in UHV,65-67 (3) Ag island films deposited on electrically conducting indium-tin-oxide (ITO) glass in UHV,68 and (4) electrochemically roughened surfaces.69-73 Although the relevance of the STM results to surface-enhanced optical processes was discussed in these papers, no SERS data were included. Several of these papers, however, have presented surface-enhanced second harmonic generation (SESHG) data.67,68,72,73 Byahut and Furtak74 have demonstrated strong SERS from electrochemically roughened and STM-analyzed Ag films; however, the SERS spectra were published without the concurrent STM images. To date, Ferrer and co-workers75,76 are the only researchers to publish both SERS spectra and STM data from the same surfaces. Although no STM images were reported, STM-derived surface roughness spectral power density functions are given. In their work, 45 nm thick Ag films were deposited over glass and over glass preroughened with CaF, films. However, they found no measurable enhancements from the Ag films on glass, and only weak enhancements could be obtained from the CaF, roughened surfaces when using benzic acid as the adsorbate. The main limitation to STM for the study of SERS-active surfaces is that like the SEM, it requires that the specimen be electrically conductive. Thus, the study of several popular types of SERS-active surface such as island films on insulator substrates and isolated particle arrays on insulating substrates are precluded from examination.

In this paper we report the first application of atomic force microscopy (AFM) to examine the 3D structure of a SERS-active surface. AgIF’s deposited on smooth glass substrates were chosen as the primary target surface for this combined AFM and SERS study because: their oblate structure is well suited to accurate topographic imaging by scanning probe microscopy, they are relatively stable and easy to fabricate, and they have played an important role in the development of SERS particularly in elucidating such properties of the EM enhancement mechanism as the long-range distance dependence, coverage dependence, and correlation with optical properties. AFM structural data on AgIF’s is combined with measurements
of their SERS enhancement factor (EF) and optical extinction spectra. These results are compared with published conclusions from the EM theory of SERS. In addition to the island film experiments, two other nanostructured surfaces have been imaged with the AFM: a thick (viz., about $d_m=250$ nm) Ag film deposited over a monolayer of polymer nanospheres supported on a glass substrate and, for comparison, an identical thick Ag film deposited on a smooth glass substrate. The purpose of this study was to test the hypothesis that the monolayer of polymer nanospheres would provide an easy to fabricate, reproducible, and variable sized, hemispherical template for the formation of a SERS-active metal overlay whose EM enhancement properties could be selectively "tuned" by changing the diameter of the nanospheres.38-45

II. EXPERIMENTAL TECHNIQUES

The fabrication of the roughened Ag films that are the subject of this research was carried out in a modified Consolidated Vacuum Corporation vapor deposition system. Pressures in the diffusion pumped chamber were $<10^{-6}$ Torr during the deposition. The deposition source consisted of a tungsten boat filled with the material to be deposited. Prior to deposition the substrates were cleaned by washing in 1 M NaOH followed by successive sonications in deionized water, methanol, and acetone. The temperature of the substrate, $T_s$, was not independently controlled in this apparatus but was continuously measured during the deposition by a thermocouple attached to the substrate.

The mass thickness of Ag films was measured with a homemade quartz crystal microbalance that was calibrated by two independent methods: cyclic voltammetry and STM. The cyclic voltammetric approach followed the work of Fornari.81 STM calibration of mass thickness was performed by masking half of a silver substrate and depositing silver over the other half. The STM tip was repetitively scanned over the boundary and the average height calculated.

Monodisperse colloidal suspensions of polystyrene spheres were purchased from three vendors: Interfacial Dynamics Corporation (Portland, OR), Bangs Laboratories (Carmel, IN), and Duke Scientific (Palo Alto, CA). The variation in sphere diameter was less than 4% for all polystyrene spheres used. Spheres were diluted by a factor of 2 to 5 in methanol and spin coated onto glass substrates. In all experiments, sphere surface coverage was roughly 1 monolayer.

The 683 cm$^{-1}$ line of cobalt phthalocyanine (CoPc) was used as a SERS intensity standard for calculating the enhancement factors for the surfaces used in this study. The standard was fabricated by depositing 0.1 monolayer of CoPc on a thick Ag film over polymer nanosphere surface (see Sec. III B and the upper panel inset in Fig. 10). CoPc was chosen as the SERS intensity standard because of previous experience,82,83 ease of fabrication, specimen longevity, and its enormous intensity. Depositions of CoPc were carried out similarly to the method used by Carron.82,83 CoPc was sublimed simultaneously onto the SERS active substrate and six glass slides of known area. The CoPc was subsequently dissolved off each slide with pyridine and diluted to 5 ml and its concentration was measured by UV-visible absorption spectrophotometry using a Beckman DU-7 spectrophotometer. Coverages were calculated using $e=1.17\times10^4$ M$^{-1}$ cm$^{-1}$ and assuming 4×10$^{13}$ molecules per monolayer coverage.82,83 Preresonance Raman spectra of CoPc were acquired from a thick film sublimed onto a sapphire window. trans-1,2-bis (4-pyridyl)-ethylene (BPE) was chosen as an adsorbate because it adsorbs strongly to Ag in air and it is not resonantly enhanced in the visible. The BPE was spin coated onto the prepared substrates from a 2×10$^{-4}$ M solution in methanol.

A Spectra Physics model 171 Ar$^+$ laser was used both for Raman excitation, and to pump the tunable lasers used in determining excitation profiles. Two coherent dye lasers; a model 490 and model 590, were combined with three dyes; rhodamine 560, rhodamine 590, and DCM, to provide wavelengths between 535 and 700 nm. A Spectra Physics model 3900 Ti:Al$\text{2O}_3$ laser provided excitation wavelengths greater than 700 nm. A Spectra Physics series 3000 cw mode-locked Nd:YAG laser operating at 1064 nm and its second harmonic at 532 nm were used, respectively, for the experiment comparing SEHRS and SERS on thick film over polymer nanosphere surfaces. SER and SEHRS spectra were obtained on two detection systems: a SPEX-1400-II double grating monochromator equipped with a cooled RCA C31034A-02 photomultiplier tube, and an ACTON VM-505 single grating monochromator equipped with a Photometrics PM-512 charge couple device (CCD) camera.

AFM images were all taken in air using a Digital Instruments Nanoscope II. Commercial Si$_3$N$_4$ cantilever tips (Digital Instruments) with spring constants of approximately 0.12 nm$^{-1}$ were used. These tips are pyramidal in shape with a cone angle of 70° and an effective radius of curvature at the tip $R_c=20$-50 nm. The images reported here are raw, unfiltered data collected in the constant force mode (i.e., the tip-sample separation is adjusted to maintain a constant force equal to 15-25 nN) with a scan speed of 8 lines s$^{-1}$. All surfaces were imaged with at least two different tips to minimize the possibility of tip induced false images. Two scan heads, with ranges of 12 μm by 12 μm for large area scans and 640 nm by 640 nm for smaller areas, were routinely used.

III. RESULTS AND DISCUSSION

A. Island films on smooth glass substrates

"As-deposited" Ag island films were formed on glass substrates with mass thicknesses, $d_m$, of 1.8, 3.5, 8, and 16 nm using a fixed deposition rate, $r_d$, of 0.3 nm s$^{-1}$. Under these conditions, radiative heating of the substrate was minimal so that $T_s$ was approximately constant at about 300 K. "Annealed" Ag island films were prepared by a post-deposition heat treatment of the same specimens at $<10^{-6}$ Torr vacuum at $T_s=600$ K for 60 min.

Although all the island film surfaces reported in this paper were prepared with a fixed Ag $r_d=0.3$ nm s$^{-1}$, a systematic examination of the effect of deposition rate on
island film structure was carried out varying the rate from $0.05 < r_d < 2.0 \text{ nm s}^{-1}$. This study was carried out because, in previous work by Cotton,$^5$ a strong effect of deposition rate on film structure as determined by TEM, optical properties of the film determined by electronic extinction spectra, and SERS activity for both electronically resonant and nonresonant adsorbates was found. They prepared Ag island films of constant $d_m = 5.0 \text{ nm}$ on glass substrates at deposition rates from $0.003 < r_d < 0.5 \text{ nm s}^{-1}$. Films deposited with slow rates near $0.003 \text{ nm s}^{-1}$ were found to have larger, more well-separated particles and result in 5 or 6 times greater SERS activity than films deposited at high rates near $0.5 \text{ nm s}^{-1}$. The explanation offered was that at low deposition rates metal atoms migrate independently to their final surface sites. At higher deposition rates, additional metal atoms arrive within a capture area on the surface and influence the aggregate rate of migration to final surface sites. Thus the structure of the film would be a function of arrival rate at the substrate surface.

In our work, no such effect of $r_d$ on film structure as determined by atomic force microscopy, or optical properties, or SERS activity was found. Why there should be such disparity between our results and those of Cotton is not at all clear at this time. Two possible sources for this effect are (1) differences in $T_s$ caused by differences in radiative heating of the specimen in low vs high $r_d$ experiments and (2) differences in substrate surface energy caused by different pretreatments. Cotton et al. assumed that the distance between their sample and evaporation source (12 cm) was sufficient that $T_s$ was not influenced by the different radiative heat loads on the sample at different deposition rates and consequently, they did not measure $T_s$. If, in fact, $T_s$ was a function of $r_d$, the complex interplay between the rates of metal atom arrival, surface diffusion, nucleation of islands, growth of islands, and coalescence of islands would have to be modeled in detail to understand this phenomenon. Additional complexity enters this problem because the surface energies of the substrates used in our experiments are likely to be different from those in Cotton’s work as a result of the different surface pretreatments used. Consequently, the rates of surface diffusion, island growth, island coalescence, etc., would be modulated by differences in substrate surface energy. Clearly, further study is required to unravel the details of these two different observations concerning the effect of $r_d$ on island film structure and SERS EF values.

1. Atomic force microscopy

A set of AFM images with (400 nm)$^2$ field of view is shown in Fig. 1 for “as-deposited” Ag island films of various $d_m$ on glass substrates. These images provide an overview of “as-deposited” island film morphology as a function of mass thickness. For purposes of discussing the geometry of the particles in these AFM images, as well as making contact with the EM theory of SERS based on spheroids,$^6$ the films shown in Figs. 1(a) and 1(b) with $d_m = 1.8 \text{ nm}$ and 3.5 nm since they are composed of regularly shaped, individual particles. The 2-contrast scale provides a qualitative
indication of the particle heights for these films which range up to 8 and 10 nm, respectively. When the mass thickness is increased to 8 nm [Fig. 1(c)], the film is still composed of individual particles but there is now great irregularity to the particle shape and the distribution of particle sizes and shapes has increased. The z-contrast scale shows that particle height now ranges upward of 15 nm. This is presumably due to the onset of particle–particle coalescence. At $d_m=16$ nm [Fig. 1(d)], the thickest films imaged in this series, the Ag particles appear more spheri- cal but now strongly overlap each other. Particle heights, which really correspond to film thickness now that the particles overlap, are somewhat lower than those in the 8 nm film with a range up to 12 nm. Particle–particle overlap and/or coalescence [Figs. 1(c) and 1(d)] reduces the ease of particle geometry interpretation in terms of a random array of spheroids.

A corresponding set of $(400 \text{ nm})^2$ AFM images that reveals the overall behavior of Ag island films subjected to post-deposition, vacuum annealing to $T_v=600$ K is shown in Fig. 2. These images show that annealed 1.8 nm [Fig. 2(a)] and 3.5 nm [Fig. 2(b)] island films are still composed of individual spheroidal particles but that they have grown in diameter and height compared to those in the corresponding “as-deposited” film. The 8 nm [Fig. 2(c)] annealed film still shows the irregular particle shapes produced by coalescence but they are larger and higher than those of the “as-deposited” film. The particles composing the 16 nm annealed [Fig. 2(d)] film, in contrast to those of the 8 nm annealed film, are reduced in diameter with particle height or film thickness now much smaller at approximately 3 nm after annealing.

In order to quantify the effect of mass thickness and thermal annealing on particle geometry, a series of higher resolution AFM images for these same films in both their “as-deposited” and annealed states were acquired. Figures 3 and 4 show AFM images with $(100 \text{ nm})^2$ and $(200 \text{ nm})^2$ fields of view, respectively, for 1.8 nm and 3.5 nm films along with selected AFM line scans which provide an independent measure of the particle height distribution. Since these surfaces are composed of isolated, regularly shaped particles, it was possible to obtain histograms of the particle diameter distribution which are shown in Fig. 5. The insets to Fig. 5 schematically illustrate the particle geometry. Particle diameters, $a$, are given as the mean for 40–60 particles in the field of view. Particle heights, $b$, are derived from the peaks in AFM line scan data and are reported as average values from at least five randomly se-
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FIG. 4. AFM images and linescans of Ag island films on glass slides. Depositions performed at room temperature, \( r_\text{p} = 0.3 \text{ nm s}^{-1} \) and \( d_\text{m} = 3.5 \text{ nm} \) (a) unannealed and (b) annealed at 600 K for 60 min.

Selected lines in the field of view. These values and their standard deviations are also given in Table I. Figures 6 and 7 present higher resolution AFM images and line scans for the 8 and 16 nm mass thickness films. Particle diameter histograms were not computed for these surfaces due to the small number, irregular shape, and/or overlapping of the particles. Estimates of \( a \) were obtained by measuring the diameter of a circle drawn to circumscribe the particle on at least ten particles. Particle height was again taken directly from the AFM line scan maxima. Table I summarizes all the particle diameter, height, and shape, data obtained for these films. Several trends in particle structure are clearly revealed in these studies as a function of increasing \( d_\text{m} \). In “as-deposited” films the average particle diameter increases; distribution of particle diameters is quite narrow and nearly Gaussian (\( d_\text{m} = 1.8 \text{ and } 3.5 \text{ nm films} \)); particle height increases more rapidly than the diameter (the 16 nm film is an exception probably due to the effects of particle–particle overlap); and particle shape becomes increasingly oblate with the minor-to-major axis ratio systematically varying by a factor of 5. Thermal annealing to 600 K for 60 min produces the following changes in particle structure: particle diameter increases by about 35%, 45%, and 90% for the 1.8, 3.5, and 8 nm films, respectively, but decreases by a factor of 2.5 for the 16 nm film; particle diameter distributions are significantly broadened; particle height doubles for the 1.8 and 3.5 nm films, increases by only 50% for the 8 nm film, and actually decreases for the 16 nm film; oblate particle shape is retained with particles in the 1.8 and 3.5 nm films becoming significantly more prolate.

This reexamination of the structure of Ag island films from the new vantage point afforded by atomic force microscopy shows that the AFM is a powerful tool for nonconducting specimens with lateral resolution superior to that for SEM and comparable to that for TEM. In addition the clear advantage of the AFM is its capability of independently measuring both particle diameter and particle height. Thus, we are able to easily extract the particle shape parameter, \( R \), that is so important in determining the optical properties of these surfaces and, in particular, their SERS activity.

FIG. 5. Histograms of size distributions from AFM micrographs for \( d_\text{m} = 1.8 \text{ and } 3.5 \text{ nm Ag island films} \). Schematic illustrations of these surfaces are shown in the insets.

FIG. 6. AFM images and linescans of Ag island films on glass slides. Depositions performed at room temperature, \( r_\text{p} = 0.3 \text{ nm s}^{-1} \) and \( d_\text{m} = 8.0 \text{ nm} \) (a) unannealed and (b) annealed at 600 K for 60 min.

### Table I. Hemispheroidal particle size and shape properties for Ag island films.

<table>
<thead>
<tr>
<th>Film thickness (nm)</th>
<th>As-deposited</th>
<th>Annealed</th>
</tr>
</thead>
<tbody>
<tr>
<td>( d_\text{m} )</td>
<td>( a ) (nm)</td>
<td>( b ) (nm)</td>
</tr>
<tr>
<td>1.8</td>
<td>10.0±1.3</td>
<td>5</td>
</tr>
<tr>
<td>3.5</td>
<td>14.6±2.1</td>
<td>8</td>
</tr>
<tr>
<td>8.0</td>
<td>33±4</td>
<td>12</td>
</tr>
<tr>
<td>16.0</td>
<td>100±10</td>
<td>12</td>
</tr>
</tbody>
</table>
FIG. 7. AFM images and linescans of Ag island films on glass slides. Depositions were performed at room temperature, $r_p=0.3 \text{ nm s}^{-1}$ and $d_m=16 \text{ nm}$ (a) unannealed and (b) annealed at $600 \text{ K}$ for $60 \text{ min}$.  

2. Extinction spectra

The optical properties of submicron structures composed of metal particles have been extensively studied.\textsuperscript{35,36,87-95} It is well known, for example, that the optical absorbance of Ag island films composed of oblate spheroidal particles change dramatically as a function of $1 \text{ nm} < d_m < 16 \text{ nm}$.\textsuperscript{35,36} Normal incidence, unpolarized extinction spectra of the "as-deposited" Ag island films studied above by AFM are shown in Fig. 8(a). The 1.8 nm thick film shows a maximum extinction at 510 nm. Since the particles in this film are oblate with $R=0.50$, this extinction maximum can be assigned to surface-plasmon excitation along the major axis. As $d_m$ increases to 3.5, 8.0, and 16 nm, the surface-plasmon resonance energy red shifts and its width broadens significantly. These results, when combined with our AFM structural data (Table I) which showed a systematic decrease in $R$ with increasing $d_m$, are consistent with EM theory (see, for example, Fig. 3 in Ref. 67).

The corresponding set of normal incidence, unpolarized extinction spectra for Ag island films after post-deposition annealing at $T_a=600 \text{ K}$ for 60 min is shown in Fig. 9(a). Three features are observed: (1) the extinction maxima for the annealed samples are blue-shifted and reduced in intensity with respect to those for the "as-deposited" samples, (2) the surface-plasmon resonance widths are decreased, and (3) there is a general loss of light absorption in the red for all samples. Blue-shifted extinction or absorption maxima have been previously reported for thermally annealed Ag island films on rock salt\textsuperscript{97} and on glass\textsuperscript{98} substrates. McCarthy measured Ag particle shape and height using the Pt shadow casting technique with TEM observation. He interpreted the blue shift to be a consequence of thermally induced Ag particle breakup and regrowth at new nucleation sites resulting in Ag particles with increased height. Aussenegg did not characterize annealed AgIF's using microscopic methods and did not offer a structural interpretation of the blue shift. Red-shifted extinction or absorption maxima have also been observed for thermally annealed Ag island films.\textsuperscript{36,87} The red shifts were interpreted as being consistent with EM theory based on SEM and TEM micrographs showing the particles in the annealed film to be larger in...
diameter than those in "as-deposited" films. In order for this to be the case for oblate spheroidal particles, the particle shape factor, \( R \), would have had to be approximately constant before and after annealing. No independent measurements of the particle height were made to vary the constancy of \( R \).

Our results for low \( d_m \), thermally annealed Ag island films on glass are similar to those of McCarthy on rock salt. The AFM shows that annealed AgIF's are composed of larger but significantly less oblate particles than "as-deposited" films. This change in the minor-to-major axis ratio of the Ag particles accounts for the blue shift in the extinction spectra according to EM theory.

Why the annealing of Ag island films in some experiments should be dominated by increases in particle height, whereas, the annealing of similar films should be dominated by increased particle diameter is not clear. One possible explanation centers on substrate energies. Different substrates and/or pretreatments for the same substrate are likely to result in different substrate surface energies and consequently contact angles between the metal overlayer and the substrate. If, for example, the Ag islands in the Royer experiments were to "wet" the substrate more effectively at higher temperatures, then they will grow in diameter and decrease in height producing the red shift as they are annealed. If, on the other hand, the Ag islands in our experiments or those of McCarthy and Aussenegg were to "wet" the substrate less effectively at the annealing temperature, the opposite would be expected to occur.

3. SERS enhancement factor

In this section we examine the dependence of the surface enhancement factor for AgIF's on film thickness and laser excitation wavelength in light of the nanostructural information provided by the AFM. Correlations with optical properties characterized by extinction spectra and with the results of the EM theory of SERS are also made. trans-1,2-bis(4-pyridyl)ethylene (BPE), rather than the more familiar pyridine system, was chosen as the adsorbate molecule for these island film EF measurements because it adsorbs to Ag more strongly than pyridine; adsorbs irreversibly on Ag as compared with the reversible adsorption of pyridine; has a larger spontaneous Raman cross section than pyridine; and is a nonresonant adsorbate-like pyridine when excited at excitation wavelengths in the visible and near infrared. A representative SER spectrum of BPE on the "as-deposited" AgIF's as a function of \( \lambda_{ex} \) and \( d_m \) are listed in Table II as the mean and standard deviation for 6 replicate surfaces at each film thickness. The wavelength dependencies of the EF, which are also plotted in Fig. 8(b) as excitation profiles to facilitate the visualization of the trends. The following characteristics are observed in the 488 < \( \lambda_{ex} < 722 \) nm range: (1) a systematic increase in EF as \( \lambda_{ex} \) is swept to the red, (2) no well-defined EF maxima are observed, and (3) the largest EF = 5.3 x 10^5 for \( d_m = 8.0 \) nm and \( \lambda_{ex} = 722 \) nm.

The EF of BPE adsorbed on AgIF's was obtained by first measuring the ratio of its SERS intensity to that of a thin film SERS intensity standard based on the 683 cm\(^{-1}\) line of cobalt phthalocyanine (CoPc).\(^{82,83}\) Combining this ratio with the enhancement factor for the CoPc intensity standard excited at 753 nm and the ratio of the bulk, normal Raman intensities for BPE and CoPc we get the desired EF from the following identity:

\[
\text{EF}_{\text{BPE}, 683 \text{ cm}^{-1}}(\lambda) = \frac{I_{\text{surf, BPE}}(\lambda)}{I_{\text{surf, CoPc}}(\lambda = 753 \text{ nm})} \times \frac{I_{\text{bulk, CoPc}}(\lambda = 753 \text{ nm})}{I_{\text{surf, CoPc}}(\lambda = 753 \text{ nm})}.
\]

The EF for the 683 cm\(^{-1}\) CoPc standard is calculated to be 1 x 10\(^5\) from the spectra shown in Fig. 11. The inset in Fig. 11(b) shows the electronic absorption spectrum for an 80 monolayer thick film of CoPc on glass and reveals that the unenhanced Raman reference spectrum is actually an electronically resonant Raman spectrum.

The EF's for BPE on the "as-deposited" AgIF's as a function of \( \lambda_{ex} \) and \( d_m \) are listed in Table II as the mean and standard deviation for 6 replicate surfaces at each film thickness. The wavelength dependencies of the EF, which are also plotted in Fig. 8(b) as excitation profiles to facilitate the visualization of the trends. The following characteristics are observed in the 488 < \( \lambda_{ex} < 722 \) nm range: (1) a systematic increase in EF as \( \lambda_{ex} \) is swept to the red, (2) no well-defined EF maxima are observed, and (3) the largest EF = 5.3 x 10^5 for \( d_m = 8.0 \) nm and \( \lambda_{ex} = 722 \) nm. The film thickness dependence of EF at constant \( \lambda_{ex} \) shows a systematic increase with \( d_m \) from 1.8 to 8.0 nm followed by a fall-off as \( d_m \) is further increased to 16.0 nm. In order to interpret this behavior within the framework of the EM theory of SERS for isolated oblate spheroidal particles, it is
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necessary to keep in mind that the EF for each AgIF is actually characterized by the two independent particle structure parameters, $a$ and $R$, rather than a single $d_m$ value. For convenience the $a$ and $R$ structural parameters from Table I are reproduced in Fig. 8(b). The EM theory of SERS predicts that, for a fixed value of the particle's major axis, $a$, the plasmon frequency will shift to the red as the spheroid becomes more oblate (decreasing $R$) increasing the Raman EF with red laser excitation compared to blue, and, similarly, for a fixed particle shape, $R$, the plasmon frequency will also shift to the red as the spheroid's major axis, $a$, becomes larger again increasing the Raman EF with red laser excitation compared to blue. Thus the systematic increase in EF as $\lambda_{ex}$ is swept to the red observed in Fig. 8(b) for AgIF's with $d_m=1.8$, 3.5, and 8.0 nm can be understood as the consequence of increasing the major axis length and decreasing the $R$ parameter for the film's constituent particles. The EF for the $d_m=16.0$ nm film does not follow this trend. This is probably a consequence of the high packing density of the particles in these films. Densely packed particle arrays, either periodic or random, exhibit surface plasmon resonance features that are broadened considerably by dipole–dipole interactions and may have either red- or blue-shifted excitation spectra compared with those of isolated particles. Thus, for $\lambda_{ex}$ that produces a large EF in a system of well isolated particles, a diminished EF is likely to result from increased particle–particle interactions in an otherwise similar system.

Two previous studies of the SERS enhancement properties of AgIF's provide relevant data for comparison. Otto$^1$ (see Fig. 8) reported excitation spectra for $d_m=2.2$, 6.0, and 15.6 nm. AgIF's prepared in UHV that exhibit EF maxima $= 1.4 \times 10^4$ at 475 nm, $1.4 \times 10^4$ at 540 nm, and $0.6 \times 10^4$ at 575 nm, respectively. Optical transmission spectra were reported for each of these UHV-prepared AgIF's but no nanostructural characterization was carried out. As far as we are aware, our observation of EF=$5.3 \times 10^5$, is the largest yet reported for a AgIF. Although EF's of this magnitude are commonly found for electrochemically roughened surfaces most AgIF EF's are about $1 \times 10^4$ as found by Otto. Otto's results in comparison with ours clearly attest to the high degree of sensitivity that the enhancement properties of a SERS-active surface have with respect to its method of preparation and therefore its detailed nanostructure. In addition, they suggest that it would be profitable to examine the $700 < \lambda_{ex} < 1000$ nm range more extensively to search for an optimum excitation wavelength that would maximize the SERS EF. Cotton$^5$ found a linear relationship between SERS intensity and optical density for AgIF's with constant $d_m=5.0$ nm but deposited with varying $r_d$. However, no quantitative characterization of particle structure vs $r_d$ was reported. Our results show that although SERS intensities are generally stronger for more strongly absorbing films there is no quantitative linear relationship manifested. Our conclusion is that one must supplement the measurement of an island film's optical absorption with the direct measurement of nanostructural parameters to provide a firm basis for the interpretation of its surface enhancement properties.

The EF's for BPE on the annealed AgIF's as a function of $\lambda_{ex}$ and $d_m$ are listed in Table III as the mean and standard deviation for six replicate surfaces at each film

![FIG. 11. Surface enhanced and preresonance Raman spectra of CoPc. (a) SER spectrum of 0.10 monolayer of CoPc. Inset in (a) shows a schematic illustration of the surface composed of 200 nm of Ag evaporated over a monolayer of 542 nm polymer nanospheres. Substrate composed of 200 nm of Ag evaporated over 542 nm polystyrene microspheres. 1.0 mW of $\lambda_{ex}=753.2$ nm. (b) Preresonance Raman spectrum of an 80 monolayer thick CoPc film. 300 mW of $\lambda_{ex}=753.2$ nm. Inset in (b) is UV-vis absorption spectrum of 80 monolayer thick CoPc film on glass.](image-url)

![TABLE II. SERS enhancement factors (EF) for BPE from "as-deposited" Ag island films at various laser excitation wavelengths (nm).](table-url)
TABLE III. SERS enhancement factors (EF) for BPE from annealed Ag island films at various laser
excitation wavelengths (nm).

<table>
<thead>
<tr>
<th>Film thickness (nm)</th>
<th>488.0 (nm)</th>
<th>514.5 (nm)</th>
<th>641.3 (nm)</th>
<th>722.4 (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8</td>
<td>0.70±0.10×10^4</td>
<td>1.2±0.3×10^4</td>
<td>0^a</td>
<td>0^a</td>
</tr>
<tr>
<td>3.0</td>
<td>0.10±0.20×10^4</td>
<td>1.4±0.4×10^4</td>
<td>0.66±0.16×10^4</td>
<td>0.20±0.07×10^4</td>
</tr>
<tr>
<td>8.0</td>
<td>7.8±2.5×10^4</td>
<td>4.0±2.0×10^4</td>
<td>7.8±2.3×10^4</td>
<td></td>
</tr>
<tr>
<td>16.0</td>
<td>8.0^b</td>
<td>0^b</td>
<td>0^b</td>
<td>0^b</td>
</tr>
</tbody>
</table>

^aEF < 10^2.
^bNo data available.

thickness. Excitation profiles are plotted in Fig. 9(b). The EF's for annealed films are essentially unchanged at the
low d_m and blue λ_ex end of the data set but attenuated
by factors as large as 40 at the high d_m and red λ_ex end of the
data set compared to “as-deposited” films, show very little
wavelength dependence, have no maxima, and have the
largest value equal to 7.8×10^4 for d_m=8.0 nm. The film
thickness dependence of EF at constant λ_ex shows a sys-
tematic increase with d_m from 1.8 to 8.0 nm followed by a
falloff to EF < 10^2 as d_m is further increased to 16.0 nm.
For the d_m=1.8 and 3.5 nm films, the attenuation of EF in
Fig. 9(b) can be understood in terms of an annealing in-
duced change to significantly less oblate particle shapes
with only slightly increased major axis size. This is consis-
tent with the annealing induced blue shift observed in the
extinction spectra. For d_m=8.0 nm film, the annealing
doubles the major axis size while leaving R little changed
thus offsetting the blue-shift trend and maintaining a rela-
tively large EF. The lack of SERS activity for the d_m=16.0
nm film is likely to be due to a shift, probably to the red, of
the plasmon resonance caused by a combination of
R=0.08 and the effects of dipole–dipole interactions in
densely packed random particle arrays. So far as we are
aware there have been no previous studies of SERS EF's
for annealed Ag island films for comparison; however,
Aussenegg25 has reported the effects of island film anneal-
ing on the fluorescence intensity and lifetime for adsorbed
dye molecules. One practical consequence of this annealing
study, which should be noted in addition to the important
results connecting AFM structural data, SERS EF's, and
EM theory, is that AgIP's are viable candidates for SERS-
active surfaces in high temperature applications such as in
situ heterogeneous catalytic studies and combined thermal
desorption/SERS investigations in UHV experiments.

B. Thick films over polymer nanospheres

It is clear from the experiments presented earlier that
even with the improved structural information afforded by
AFM, island films are still not quite ideal surfaces either
for studying more detailed aspects of the EM theory of
SERS or for preparing optimized, reproducible surfaces for
purposes of chemical analysis. Comparison of theory and
experiment for island films is complicated by the distribu-
tion of particle sizes and shapes in thin films with isolated
particles as well as the overlapping particle problem in
thicker films. Analytical studies are complicated by the
need to control the many deposition parameters that con-
trol the details of the particle size, shape, and spacing dis-

FIG. 12. AFM images of $d_m=250$ nm thick Ag film vacuum evaporated over close-packed 542 nm diameter polystyrene nanospheres. $r_{gm}=0.3$ nm s$^{-1}$. (a) 2 $\mu$m by 2 $\mu$m image showing patterning caused by spheres and (b) 200 nm by 200 nm image of "random substructure roughness" on a single sphere.

field of view showing that the individual 40 nm particles are free of substructure roughness down to about the 1 nm level.

SERS intensities for CoPc adsorbed on sphere coated substrates were measured using $\lambda_{ex}=753.2$ nm from a cw Ti:$\text{Al}_2\text{O}_3$ laser as a function of $D$, $d_m$, and nanosphere coverage. The values used were $D=121, 250, 542,$ and 870 nm and $0<d_m<400$ nm. SERS EF's were then calculated from the ratio of the intensities of the 683 cm$^{-1}$ line in the surface and bulk samples. Figure 14(a) shows the dependence of SERS intensity on Ag film thickness for various nanosphere sizes. The maximum intensity was obtained for $d_m=200$ nm and $D=542$ nm. This intensity maximum corresponds to an EF$=1 \times 10^7$. Given that the AFM images for Ag film over polymer nanosphere surfaces clearly show two distinct roughness scales, the question naturally arises as to their relative contributions. This question was addressed in two ways. First, EF's were compared for the $d_m=200$ nm and $D=542$ nm film over polymer nanosphere system with a $d_m=200$ nm film directly deposited on glass. These numbers were EF$_{total}=1 \times 10^7$ and EF$_{film only}=1 \times 10^5$. Therefore the incremental enhancement is EF$_{nanospheres}=1 \times 10^2$. Next the dependence of EF$_{total}$ on nanosphere coverage and size were determined. Figure 14(b) shows the coverage data for two sphere sizes. This incremental enhancement due to the nanospheres is approximately linear in sphere coverage and clearly size dependent. From these studies we conclude that EF$_{total}=1 \times 10^7$ may be partitioned into a $10^5$ contribution from the RSR of the thick film and a size dependent contribution from the roughness defined by the nanospheres. It is likely that this incremental enhancement due to the large scale but well-defined roughness of the nanospheres is the consequence of extended or delocalized, propagating surface plasmon polaritons (SPP). Studies of the wavelength dependence of EF$_{nanospheres}$ along with theoretical and experimental examination of the gratinglike properties of these surfaces will be required to further elucidate the role of SPP's in the incremental enhancement mechanism.

Before moving on to the section dealing with applications of film over polymer nanosphere surfaces, a comment is directed to the observation that $d_m=200$ nm Ag films directly deposited on glass at room temperature support SERS EF's on the order of $10^5$. While this may not be the first observation of this effect, we were unable to readily locate literature references to previous studies. The closest was the case of cold-deposited thick Ag films which are well-known to be SERS active.$^{15}$ In fact, the usual impression is that thick Ag and Au films are too smooth to support SERS. Clearly this is also highly dependent on depo-
sition and substrate conditions. That thick films of Ag and Au support SERS directly may be extremely relevant to studies of the molecular structure of alkanethiol-based self-assembled monolayers (SAM).

C. Applications for film over polymer nanosphere surfaces

Studying the EM enhancement mechanism is only one use for nanosphere roughened substrates. Because they support large enhancements, these surfaces can be used for a variety of other studies. The fact that the enhancements are greatest in the red are a further advantage. In many cases it is desirable to study adsorbates at longer wavelengths to avoid electronic resonances and reduce laser degradation of adsorbates—particularly with biological molecules.

In addition, the introduction of new detectors, most importantly, the charge coupled device (CCD), have enabled efficient detection of photons into the near IR. Coupled with CCD detections systems, nanosphere surfaces can be valuable for improving detection limits of SERS, and studying scattering processes weaker than Stokes Raman; i.e., anti-Stokes Raman, and hyper-Raman processes.

Figure 15 shows a SERS spectra of BPE taken with only 5 μW of input 753 nm radiation. A data acquisition time of 1000 s resulted in a S/N>100. Previously, the lowest reported laser power for a SERS experiment was 0.7 mW at 632.8 nm for a monolayer of benzoic acid adsorbed on a stochastic Ag particle array surface yielding a spectrum with S/N=4–5 (data acquisition time not given). A 100-fold reduction in the minimum laser power requirements for Raman spectroscopy has been achieved. This suggests that μW power level SERS may permit experiments on adsorbates that readily decompose by photothermal or photochemical pathways. In addition, low power diode lasers or even nonlaser light sources should now be usable for SERS.

The 10^7 enhancements for SERS also increases the intensities of an adsorbate’s anti-Stokes spectrum. Classical Raman theory predicts that both the Stokes and anti-Stokes branches contain the same vibrational information, but the relative intensities are related as

\[ \frac{I_s(v_K)}{I_{ac}(v_K)} = \frac{e^{(h v_L/v_K)T}}{e^{(h v_L/v_K)T}} \left( \frac{(v_L-v_K)^2 + \Gamma^2}{(v_F-v_K)^2 + \Gamma^2} \right) \]

where \( I = \) intensity, \( v_L = \) laser frequency, \( v_K = \) vibration frequency, \( h = \) Planck’s constant, \( k = \) Boltzmann’s constant, and \( T = \) temperature. The term in large parentheses is a correction factor for heating induced by the resonance Raman effect, where \( v_r \) is the frequency of the absorption for the resonance and \( \Gamma \) is the full width at half maximum (FWHM) for the resonance. With the exception of colloidal systems, the importance of this correction is difficult to quantify. Furthermore, although this correction factor has been used by Pemberton and co-workers to measure adsorbate temperatures, the correction was small. SERS spectra of the anti-Stokes bands of BPE at 1609 and 1610 cm⁻¹ demonstrate the large intensities obtainable.
FIG. 16. SERS of 1.0 monolayer of BPE adsorbed on a 200 nm Ag film over 542 nm diameter polymer nanosphere surface in the 1600 cm\(^{-1}\) range using a CCD detector. (a) Stokes region, 30 mW of \(\lambda_{\text{exc}} = 753\) nm, 1 s integration. (b) Anti-Stokes region. 30 mW of \(\lambda_{\text{exc}} = 753\) nm, 30 s integration.

At room temperature the anti-Stokes bands are predicted to be approximately \(10^3\) weaker than the Stokes intensities for 753 nm excitation.

Large enhancements from nanosphere roughened substrates are not limited to experiments in air or vacuum. Figure 17 reveals that not only SERS spectra, but also surface-enhanced hyperRaman spectra (SEHRS), can be readily acquired in electrochemical environments. It is significant to note that although comparable SERS intensities are seen for SERS spectra taken in air and electrochemical environments, SEHRS was only obtainable when the surface was under electrochemical control. It is possible that electromagnetic enhancements are greatly increased at 1064 nm excitation for electrochemical systems and not ones in air. However, SERS spectra taken by FT-Raman indicate that enhancements actually decrease between 730 and 1064 nm.\(^{105}\) Another possibility is that chemical enhancement mechanisms are major contributors to hyperRaman enhancements. Further studies are underway to determine the relative role of enhancements in SEHRS.

IV. CONCLUSIONS

This is the first study in which atomic force microscopy has been used to directly probe the three-dimensional nanostructure of SERS-active surfaces. AFM is clearly demonstrated to be a powerful new tool for determining the shape factor, \(R\), for isolated particles. The direct measurement of \(R\) afforded by AFM greatly simplifies the interpretation of the SERS EF's for Ag island films in terms of the electromagnetic theory of SERS for isolated oblate spheroids.

This is one of the first AFM studies of island film formation and nanostructural modification upon thermal annealing. Since many island films are grown on insulating substrates, these systems would normally require shadow coating with conductive metal for conventional scanning electron microscopy or scanning tunneling microscopy work.

The SERS activity of Ag island films is not lost when they are subjected to quite vigorous thermal annealing conditions (viz., 600 K for 60 min.). EF's with blue excitation are essentially the same as "as-deposited" surfaces while EF's with red excitation are attenuated from the \(5 \times 10^5\) level down to the \(10^4\) level.

AFM studies show that Ag film over polymer nanosphere systems do not possess the well-controlled roughness characteristics desired in nanofabricated surfaces. However, SERS EF measurements show that they are extremely active. Under "optimized" conditions EF's in the \(10^3\) range can be obtained. The \(\text{EF}_{\text{total}} = 10^7\) is partitioned into a \(10^5\) contribution from random substructure roughness supporting localized surface plasmons and a \(10^2\) contribution which is nanosphere size dependent and may by the consequence of delocalized surface plasmon polaritons. This is the first thin film based SERS-active surface with an EF comparable to that found for electrochemically roughened surfaces.

Ag film over polymer nanosphere surfaces are shown to have applications in ultrahigh sensitivity SERS experiments involving only 5 \(\mu\)W of laser power, detecting anti-Stokes SERS signals for \(\omega_{\text{ob}} \leq 1650\) cm\(^{-1}\), and surface-enhanced hyper-Raman scattering (SEHRS).

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