A DIGEST ON SURFACE-ENHANCED RAMAN SCATTERING: METHODOLOGY, MECHANISMS, AND SINGLE-MOLECULE DETECTION

by

MARIA ELENA PULIDO ANG

(Under the Direction of Richard A. Dluhy)

ABSTRACT

Surface-enhanced Raman scattering is recognized as a powerful tool both in chemical sensing and for studying surface dynamics. The SERS process is only partly understood, but currently accepted to be mainly an electromagnetic mechanism where Raman scattering is enhanced because of surface plasmon resonance at the excitation wavelength. Recent advances in nanofabrication and particle manipulation have been generating more insight into the fundamentals of this technique. Theoretical calculations and experiments suggest that the most intense electromagnetic fields are confined in the junctions of touching nanoparticles. Research on the single-molecule capability of SERS is intensive, providing more support for the chemical effect at work in the process. Currently, based on statistical analyses and experimentation, the typical "blinking" and spectral fluctuations common in "single-molecule" systems are attributed to signals from a few molecules. Research provides, however, more compelling evidence that SERS can soon strongly complement fluorescence spectroscopy as a single molecule technique.

INDEX WORDS: SERS, SPR, Electromagnetic mechanism, Nanofabrication, Nanowires, Charge-transfer, Chemical effect, Blinking, Single-molecule

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To my dearest Alan for his love and his beautiful heart and soul,

My family for their unconditional love and support, for the happy memories and many more we will all build together

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TABLE OF CONTENTS

Page

CHAPTER

CHAPTER 1

INTRODUCTION

Surface-enhanced Raman scattering (SERS) is certainly one of the most active research fields in the scientific community to date. The literature is rich in works that address the fundamental mechanism of the technique, as well as a vast selection of analytical application waiting to be optimized. Advances in laser and detection technologies plus better microscopy systems have been making these tasks possible.

Inelastic Raman scattering is known to have a low cross-section which is estimated to be about 10^{-29} cm^2/molecule [Kneipp, K., H. Kneipp, et al. (2002)]. One way of increasing the scattering cross-section is by utilizing excitation wavelengths that have corresponding resonances with the electronic absorptions of the molecule, a process called resonance Raman scattering (RSS). However, electronic absorptions typically occur in the visible range, which poses the drawback of a possible interfering broad fluorescence background that can mask sharp Raman lines. First observed in 1974 with pyridine on electrochemically roughened silver electrodes, it was found that Raman scattering can be also be enhanced tremendously on rough metal substrates. This technique of doing Raman scattering on rough metal substrates has since been called surface-enhanced Raman scattering (SERS). On such "SERS-active substrates", the fluorescence background problem at excitation wavelengths at or close to the molecule's electronic absorptions is minimized because of the available pathways for nonradiative decay on the metal. SERS plus electronic resonance is called surface-enhanced resonance Raman scattering (SERRS).

Since the discovery of SERS, significant progress has been achieved in the understanding of its fundamental mechanism, though a complete description is not yet at hand. Currently, the Raman enhancement, which is achievable on roughness features of tens to hundreds of nanometers, is believed to arise primarily from an enhancement in the electric field around the vicinity of the molecule. This is called the electromagnetic mechanism (EM) of SERS. The field enhancement is a consequence of exciting the surface plasmons (SPs) on the metallic substrate, their collective motion of which when in resonance with the incoming EM radiation, can enhance and concentrate the electromagnetic field locally. Another contributing mechanism called "chemical effect" is proposed, several advocates of which view as a distortion in the molecule's electron clouds as a result of strong interaction with the metallic surface that can lead to new bands and possibly excitable by the incoming EM field. These charge-transfer (CT) bands, when excited as in molecular resonance Raman scattering (RRS), can give additional enhancements.

Central to the SERS technique is thus the production of metallic surfaces that have reproducible roughness features with surface plasmon resonances (SPR) attuned to available laser wavelengths. There are research groups who mainly do fabrication of SERS-active substrates and understanding via probing their SPR and theoretical calculations using Maxwell's electromagnetic theory and various other methods like the discrete dipole approximation (DDA). Despite the lack of full understanding of the SERS mechanism however, the literature is abundant with applications in the field of biochemistry and environmental chemistry - detection of small and large molecules alike, like proteins, neurotransmitters, and DNA, to name a few [Kneipp, K., H. Kneipp, et al. (2002)]. Such tasks have been conducted mostly on colloidal substrates, which can be easily prepared or purchased commercially, and which give good average Raman enhancements enough to give attomole (10^{-18}) detection limits.

The excitement in the SERS community, however, lies in the observed single-molecule detection capability of SERS. This was fueled by separate reports by two groups, Kneipp *et al*. and Nie and Emory in 1997. Kneipp et al. [[Kneipp, K., H. Kneipp, et al. (1997)] reported large effective cross-sections of crystal violet estimated to be about 10^{-17} to 10^{-16} cm^2/molecule using NIR excitation on aqueous silver colloidal solution while Nie & Emory [Nie, S. M. and S. R. Emory (1997)] reported large enhancement factors on the order of 10^{14} to 10^{15} via SM-SERRS of Rhodamine 6G on large single silver particles about 110-120-nm in diameter. Today, single-molecule SERS (SM-SERS) addresses the observed issues such as the origins of the blinking and spectral fluctuations, and all seem to indicate a bright future for the technique. Researchers see the potential of SERS in DNA sequencing by the unique spectra of their nucleotide bases which were found to be unaffected by the sugar and phosphate bonds [Kneipp, K., H. Kneipp, et al. (1999)]. In this paper, a brief review of the significant findings made in SERS is presented: methodologies in general, SERS mechanism, and the current understanding of the big issues regarding the technique, like "hot spots" and single-molecule SERS.

CHAPTER 2

METHODOLOGY

First and foremost for the SERS experiment is the choice of the "SERS-active" substrate. SERS-active probes can be metallic colloids of various shapes (spheres, triangular, rods, or wires), used in solution or simply cast and dried on dielectric substrates. They can also be organized into various structural motifs like arrays (periodic or randomly distributed) and island films through vacuum evaporation on glass or other substrates. SERS requires that the probe molecule be adsorbed on the surface of metal nanostructures. Based on their optical properties, the metals silver, gold, and copper are the most efficient metals that work in the visible range. Silver, in particular, has been shown to give the largest enhancements, and thus is the most reported in the literature. Gold also gives comparable enhancement, but its attractiveness is on its relative inertness and biocompatibility.

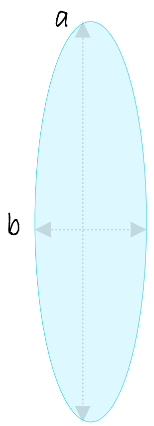
The first report of large Raman enhancement was achieved on electrochemically roughened silver substrates, the roughness (randomly distributed on the surface) of which was in the range of tens to hundreds of nanometers. To date, electrochemically roughened electrodes are still in use, primarily for studying interfacial or electrode processes, but several other methods of achieving nanometer roughness are already available. Wet chemical methods of fabrication abound but with the disadvantage of irreproducibility and matrix interferences. These rely on the chemical reduction of the metal salts in the presence of capping agents to provide stability by preventing aggregation.

Nanofabricated substrates prepared by lithography and vacuum deposition techniques offer a number of advantages. Reproducibility is expected to be higher in such substrates, avoiding polydispersity in shapes and sizes in order to provide better platforms for studying the fundamentals of SERS. Electron beam lithography can generate arrays of metal nanostructures of different shapes and arrangement in a very reproducible manner. Metal evaporation onto glass or silicon wafer substrates under vacuum can generate such arrays, too, but with lesser reproducibility. Shapes from spherical (aspect ratio=1) to rods (aspect ratio range of >1-20), and nanowires (aspect ratio >20) can be grown using either one of the techniques above. More complicated shapes like nanoshells and nanocrescent moons [Lee, L., Y. Lu, et al. (2005)], however, generally are prepared using a combination of "wet synthesis" and nanofabrication. Complicated shapes like these have been shown to generate large enhancements with excitation wavelengths tuned to the individual particles' SPR, and not to the resonance of coupled nanostructures as is often the case in colloidal nanoparticles (spherical in shape) [Jackson, J. B. and N. J. Halas (2004)].

Thin films of metals (7-10-nm) on glass substrates are also known to be SERS-active [Vo-Dihn, T., D.L. Stokes, et al. (1999)]. Thin films of metals which resemble fractals are usually prepared by vacuum deposition on either cold or heated substrates. These are usually described as "discontinuous films" or "metal island films" that possess the roughness features for effective SERS. Even the Tollens reaction has been used to fabricate silver films with fractal morphologies capable of generating large enhancements [Wang, Z. J. and L. J. Rothberg (2005)].

<u>Typical SERS-active Substrates</u>

Figure 2.1. Roughness features on the order of tens to hundreds of nanometers obtained by oxidation-reduction cycles (ORC).



Aspect Ratio, R

R= length of major axis/ length of minor axis
R= a/b

Sphere: R=1

Nanorods: 1< R < 20

Nanowires: R > 20

Figure 2.2. Diagram showing the aspect ratio of an elongated nanostructure and conventional definitions.

$$\text{Au or Ag salt} + \text{NaBH}_4 \xrightarrow{\textit{Citrate}} \text{Nanoparticles (spherical)}$$

$$\text{Nanoparticles} + \text{metal salt} + \text{ascorbic acid} \xrightarrow{\textit{CTAB}} \text{Nanorods/wires}$$

Figure 2.3. Diagram showing the typical synthesis of colloidal nanospheres, nanorods, or nanowires in solution [Murphy, C. J. and N. R. Jana (2002)]. The procedure by Lee and Meisel is commonly employed in most colloidal preparations [Xu, H. X., E. J. Bjerneld, et al. (1999)].

Colloidal solutions of gold and silver are the most commonly employed substrates for SERS. Salts of the metals are chemically reduced using reducing agents like sodium citrate and sodium borohydride. Their stability for storage is usually enhanced by the addition of poly (vinyl alcohol) or other aggregation-preventing additives [Aroca, R.F., Alvarez-Puebla, R.A., et al. (2005)], like inorganic salts. Nanospheres produced by chemical reduction can have diameters that vary from 10 to 80-nm. To prepare nanostructures of high aspect ratios like nanorods and nanowires, "seeding" is typically employed, whereby nanospheres about 3-5-nm in diameter serve as seeds onto which further growth is achieved by adding more of the metal salt, a weaker reducing agent like ascorbic acid, and rodlike micellar templates [Murphy, C. J. and N. R. Jana (2002)]. Since these are "one-pot" syntheses, there is usually a need to separate different shapes by centrifugation. Nonspherical shapes like triangular nanoplates of silver can also be prepared via wet synthesis by using N,N-dimethyl formamide (DMF) as a reducing agent as well as solvent for the reduction of $AgNO_3$ at 150 -180°C.

Nanowires, currently the most important one-dimensional nanostructure of interest, can be fabricated by various means. Nanowires of metals, semiconductor and multicomponent oxides can be fabricated using vapor-phase growth (e.g., vapor-liquid-solid growth (VLS) mechanism) or by chemical synthesis (e.g., polyol method) [Shankar, K. S. and A. K. Raychaudhuri (2005)], most of which still require optimization. For SERS substrates, metallic nanowires are commonly prepared by citrate reduction or by electrochemical deposition on porous templates like anodized alumina. Electrochemically deposited nanowires however, have to be freed by dissolution of the template, e.g., using phosphoric acid or sodium hydroxide. The polyol method is also often employed, in which the metal salt in ethylene glycol is mixed with hot poly (vinyl pyrrolidone) solution to grow nanowires of ca. 100-nm in diameter and several microns in length [Aroca, R. F., P. J. G. Goulet, et al. (2005)].

"Loose" nanostructures as-prepared using wet chemical syntheses are typically immobilized on flat substrates like glass and silicon wafer prior to SERS experiments. Needless to say, on their surfaces are adsorbed traces of matrix interferences that impart some anionic or cationic signatures which can influence the metal surface interaction with a probe molecule. Colloidal solutions as-prepared are usually diluted approximately at least three times prior to further treatment or incubation with probe molecules [Michaels, A. M., M. Nirmal, et al. (1999)]. Typical single-molecule SERS (SM-SERS) employ colloidal solutions by drop-casting them onto polylysine-coated glass substrates and allowing them to dry. However, some prefer to drop a few aliquots of the colloids (without drying), whereby the system is called a "solution" single-molecule (SM) SERS. The first report by Kneipp et al. of crystal violet Raman cross-section ca. 10^{-17} to 10^{-16} cm^2/molecule using NIR excitation made use of a 30-μL droplet of aqueous silver colloidal solutions. In such a system, the single-molecule behavior was inferred from statistical analysis of the observed signals such that a Poisson distribution instead of Gaussian was observed for the very few molecules (~1) that move to and from the illuminated probe volume [Kneipp, K., H. Kneipp, et al. (1999)].

Colloidal solutions of nanorods (NRs) and nanowires (NWs), however, are usually used as ordered assemblies on glass or silicon substrates. These substrates have shown large SERS enhancements and signals that are anisotropic. Several groups employ different methods of assembling them into flat arrays of aligned wires. The Langmuir-Blodgett (LB) technique was successfully used by Tao *et al*. [Tao, A., F. Kim, et al. (2003)] to assemble silver NWs on silicon wafer. The nanowires of pentagonal cross-sections and pyramidal tips were aligned parallel to each other. Even by simply depositing via drop-coating of electrochemically synthesized Ag NWs on oxidized Si wafer, Moskovits *et al.* [Moskovits, M., D. H. Jeong, et al. (2004)] came up with mainly well separated wires on the flat surface, but with wire bundles of compact roots which they called "rafts". The layer-by-layer (LBL) technique was also employed by Aroca’s group [Aroca, R. F., P. J. G. Goulet, et al. (2005)] to allow the self-assembly of Ag NWs on a dendrimer layer on glass.

Multilayers of nanostructures have also been used for SERS. The fabrication is usually accomplished by the LBL technique which exploits electrostatic interactions between the nanostructures and the sandwiching polymer. An example is a substrate with multilayers of positively-charged Au nanorods and negatively-charged poly (sodium styrenesulfonate), the plasmon band absorptions of which increased with the number of layers [Wang, E., S. Dong, et al. (2005)].

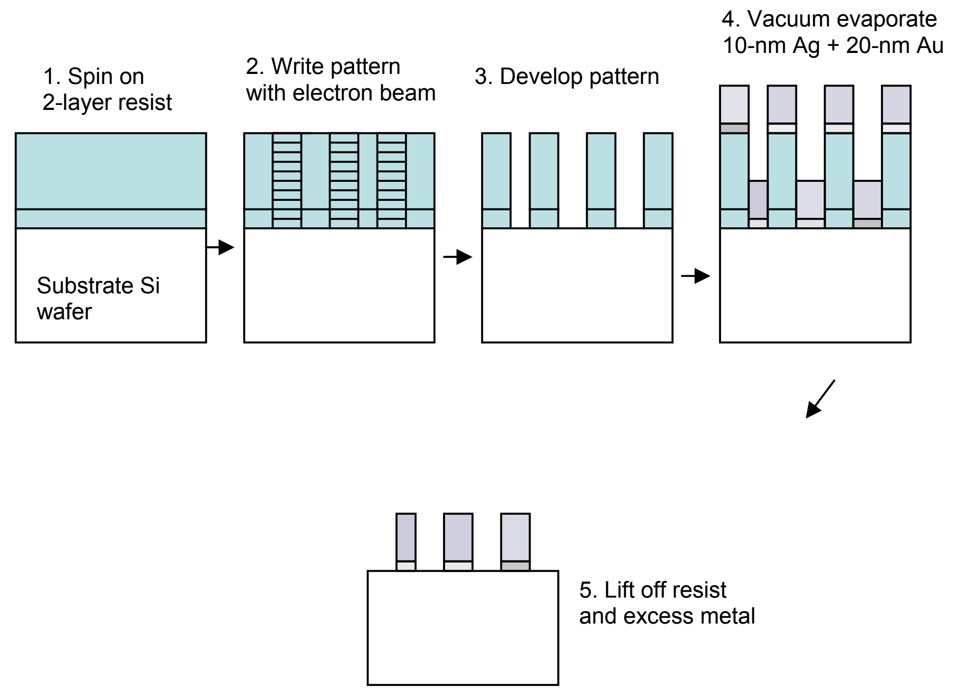


Figure 2.3. Diagram showing conventional electron-beam fabrication of nanostructure arrays; e.g., to obtain an array of rods of Au and Ag.

Electron-beam lithography (EBL) can fabricate arrays of NPs and NRs/NWs of the best reproducibility but are very expensive systems that cover typically only a few micrometers of area. EBL is used for the fabrication of more complicated shapes like a tunable plasmonic resonator made up of two silver discs separated by a silica layer [Zhang, X., K.Su , et al. (2006)]. Lithography techniques can be used to fabricate unusual shapes that can act as independent SERS substrates. The beauty of the technique, however, is best known as the capability to have precise control of particle dimensions and spacing in arrays. This is very beneficial in the current drive to have a full understanding of the SERS mechanism by probing the SPRs of the system.

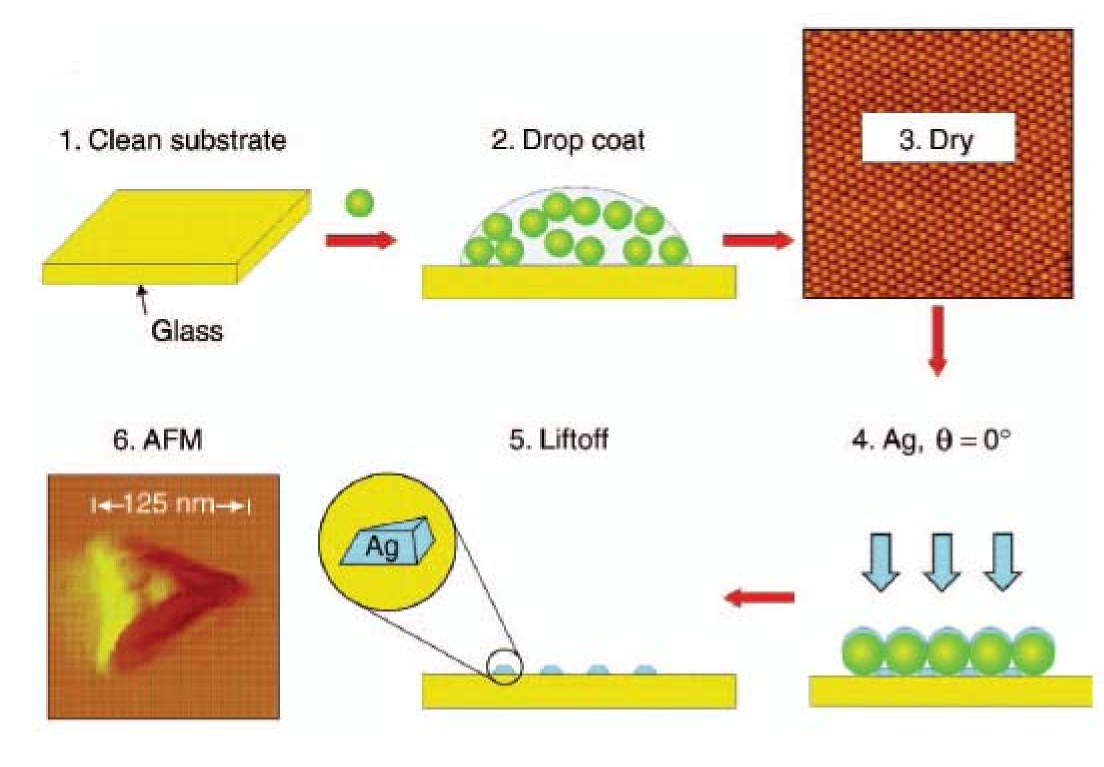


Figure 2.4. Diagram of the nanosphere-lithography (NSL) method [Haes, A.J., C. L. Haynes, et al. (2005)].

Nanosphere lithography (NSL), routinely performed in Van Duyne's group can fabricate metal nanoparticle (NP) arrays with SPR tunability, good reproducibility, and large enhancement factors. NSL is based on the self-assembly of polystyrene nanospheres into two-dimensional-hexagonal arrays onto a glass substrate, which then can serve as a template for depositing nanostructures of different metals. Prepared this way, the substrate is composed of nanostructures with triangular "footprints". Metal film over nanosphere substrates (MFON) prepared via this method have 30-50-nm roughness on top of each coated nanosphere, features that provide more enhancement in addition to the "humps" [Dieringer, J. A., A. D. McFarland, et al. (2006)].

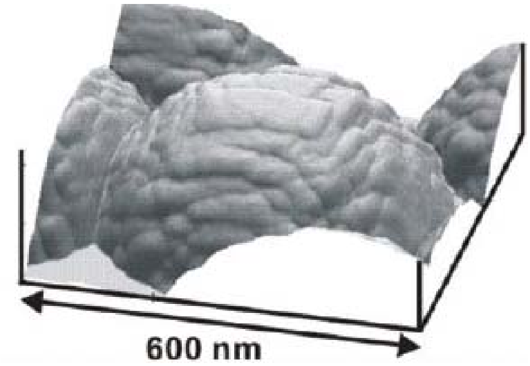


Figure 2.5. AFM image of one Ag film over polystyrene nanosphere (AgFON) showing substructure roughness [Dieringer, J. A., A. D. McFarland, et al. (2006)]

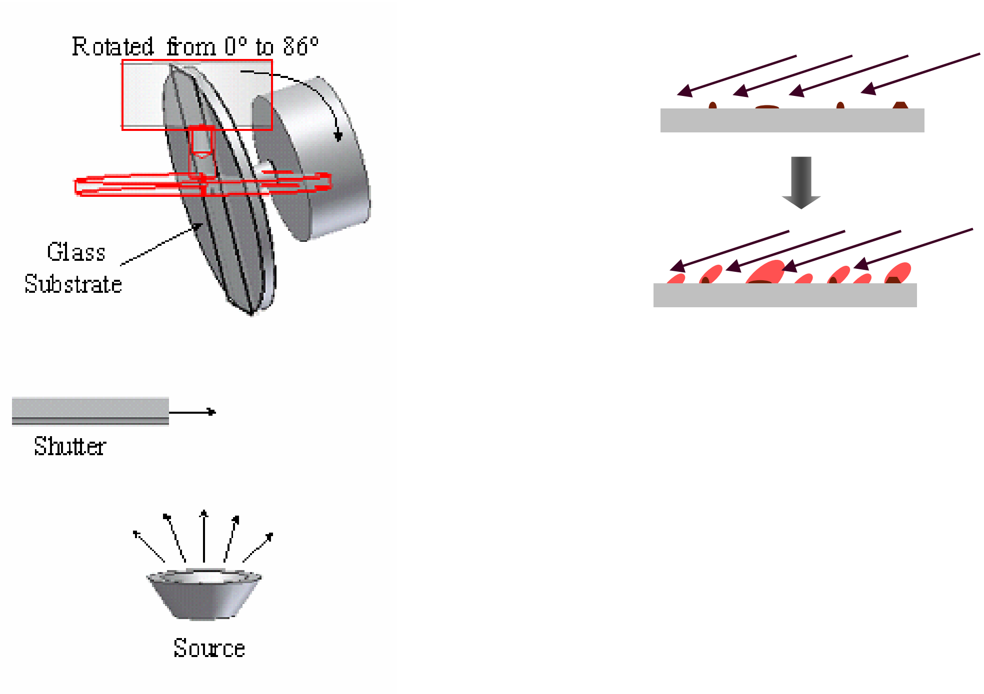


Figure 2.6. Diagram showing a typical glancing angle deposition (GLAD) setup [Chaney, S. B., S. Shanmukh, et al. (2005)].

Glancing-angle vapor deposition (GLAD) is a vapor deposition technique that can generate arrays of tilted metal nanorods (or nanocolumns, as they are not perfect rods), among others. Zhao's laboratory has fabricated such dense arrays of Ag rods on thin Ag films that have shown large SERS enhancements and anisotropic response. The deposition is highly dependent on a systematic control of the substrate rotation with respect to the substrate normal (thus the incident angle) and the deposition rate [Chaney, S. B., S. Shanmukh, et al. (2005)].

Typical Characterizations Prior to SERS

Once the nanostructures are generated, the next step is typically morphological analysis of the SERS-active substrates via scanning probe microscopies like AFM and SEM and UV-Visible absorption spectroscopy in transmission mode. The peaks in the absorption spectra correspond to absorption of the surface plasmons (SPs), when the collective oscillations of the metal's conduction electrons resonate with the excitation wavelength. Surface plasmon resonance (SPR) is the star player in the currently accepted electromagnetic mechanism (EM) of SERS, which is briefly described in the following chapter. Some research groups also look into the (elastic) Rayleigh scattering spectra of the substrates by using white light illumination plus the Raman spectrometer without holographic notch filters.

Typical extinction (absorption plus scattering) spectra of colloidal solutions of silver and gold nanospheres contain a broad diffuse peak centered at the visible wavelength, ca. 420-nm for Ag and ca. 500-nm for Au [Aroca, R.F., Alvarez-Puebla, R.A., et al. (2005)]. The more uniform the sizes of nanoparticles are, the sharper the absorption peaks become. Deviation from spherical shapes leads to the observance of another absorption peak at longer wavelengths. This peak can be tuned from the visible to NIR regions by controlling the aspect ratio of the nanorods: experiments show that the longer the nanorod is, the more red-shifted the longer wavelength band becomes. Nonspherical structures have at least two peaks in their absorption spectra. Such bands separated and of small full width at half heights (FWHH) indicating size uniformity are shown in Figure 2.7.

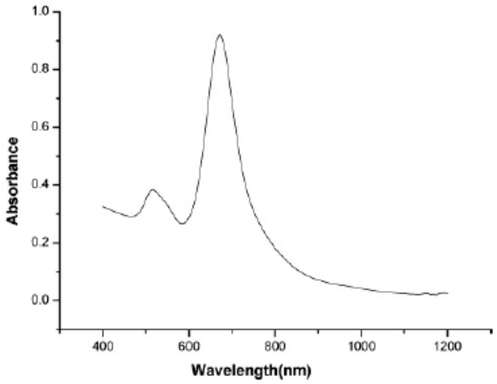


Figure 2.7. UV-Vis absorbance of Au nanorods (aspect ratio 3) in water [Hu, X. G., W. L. Cheng, et al. (2005)]. Transverse (short-wavelength) and longitudinal (longer wavelength, also called extended plasmon resonance EPR) bands appear together.

The extinction spectra of regular arrays of metal structures usually show two bands that essentially have the same features as those of colloidal solutions of Ag and Au. In these arrays, in cases where there is a wide separation between the immobilized particles, these two bands can be excited independently depending on the polarization of the incident light [Mulvaney, Juste, Santos, Marzan (2005)]. Representative spectra are shown in Figure 2.9. With "unpolarized" light (all polarizations present), a superposition of the two is usually seen. Strong interparticle couplings make the extinction spectra of arrays more complex, in addition to causing the substrate to show anisotropic response, i.e., either an increased or decreased absorption when the electric field is oriented perpendicular or parallel to the long axis of the nanorods or to the axis of interacting particles (e.g., dimer). Arrays of strongly interacting nanorods (See Figure 2.10) and nanowires [Tao, A., F. Kim, et al. (2003)] display such anisotropy.

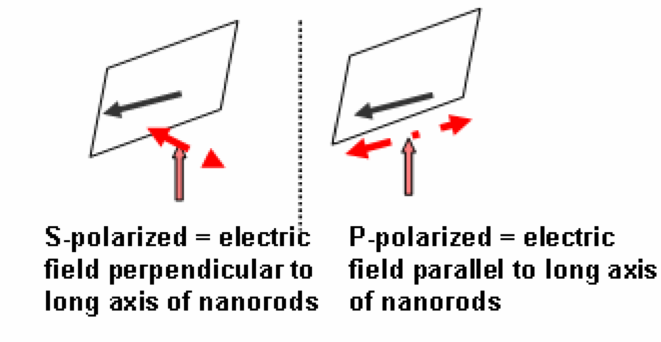


Figure 2.8. Diagram showing parallel and perpendicularly-polarized configurations in SERS setups.

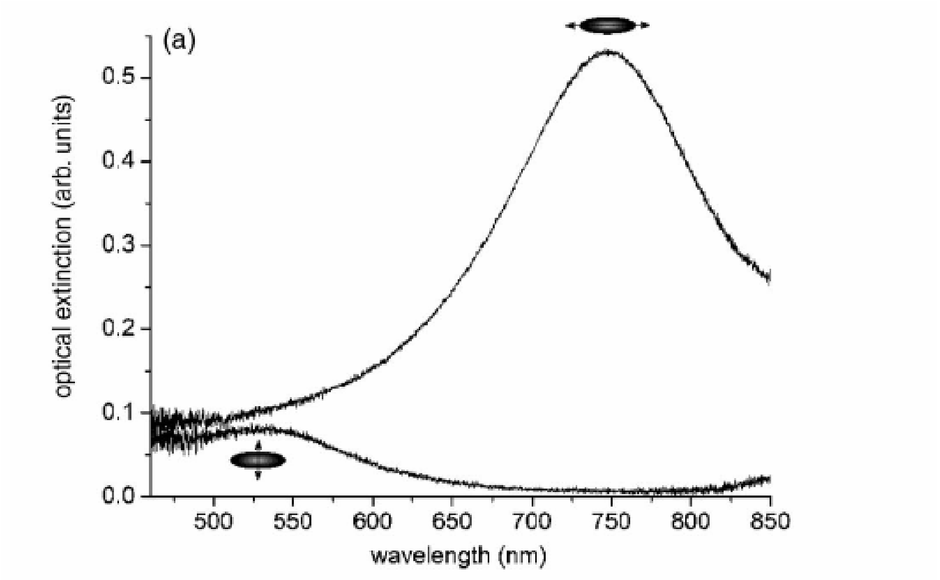


Figure 2.9. Extinction spectra of an EBL-fabricated ellipsoidal particle array for 2 polarization directions of incident light, parallel and perpendicular to the major axis [Grand, J., M. L. de la Chapelle, et al. (2005)].

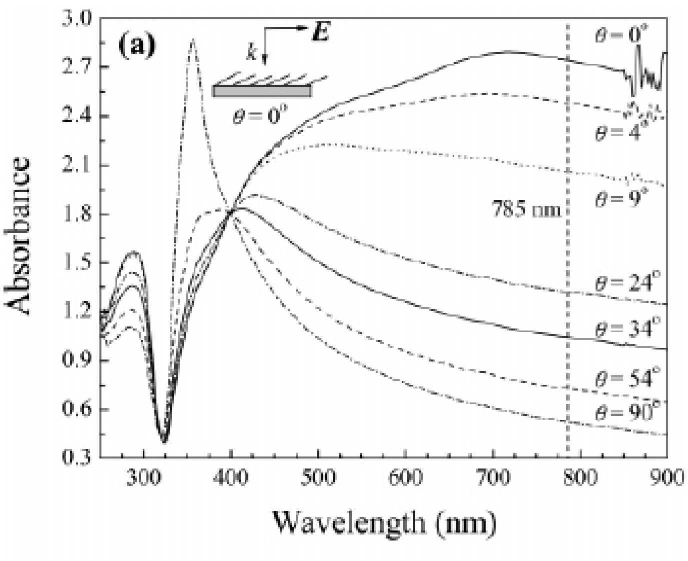


Figure 2.10. Polarized absorption spectra of Ag nanorods (aspect ratio ~9, spacing 177-nm, 71.3 degree tilt) in dense arrays [Source: Zhao, Y. P., S. B. Chaney, et al. (2006)].

Typical Probe Molecules

The probe molecules commonly employed in the fundamental studies of SERS are molecules with extensive pi electron systems. Fluorescent dyes like Rhodamine 6G (R6G) and crystal violet are commonly employed in single molecule studies. In average "ensemble" SERS approach of analytical applications, as opposed to single-molecule SERS, a wealth of various small molecule probes as well as large molecules are utilized: pyridine, salicylic acid, anions like perchlorate, hemoglobin, and DNA, among others [Baker, G.A., Moore, D.S. (2005)]. In fundamental studies, the choice of probe molecule is normally dictated by the characteristic absorption properties of the probe, how well-defined its Raman spectra is (e.g., how well-understood its orientation is on the metal surface), its relative insensitivity to orientation effects, and the strength of its interaction with the SERS-active substrate. Nitrogen-containing probe molecules like R6G are known to show preferential adsorption on silver surfaces, while probes with sulfur atoms (e.g., 2-napthalenethiol) show preference for gold surfaces. Trans-1, 2-bis (4-ptridyl) ethane, also known as BPE, is also a favorite probe, its Raman spectral features having been studied theoretically [Van Duyne, R., W.Yang, et al. (1996)]. Perylene dyes are a favorite in Aroca's research group for single-molecule studies, because of its terminal amino group that facilitates chemisorption on Ag surfaces [Lemma, T., Aroca, R.F. (2002)].

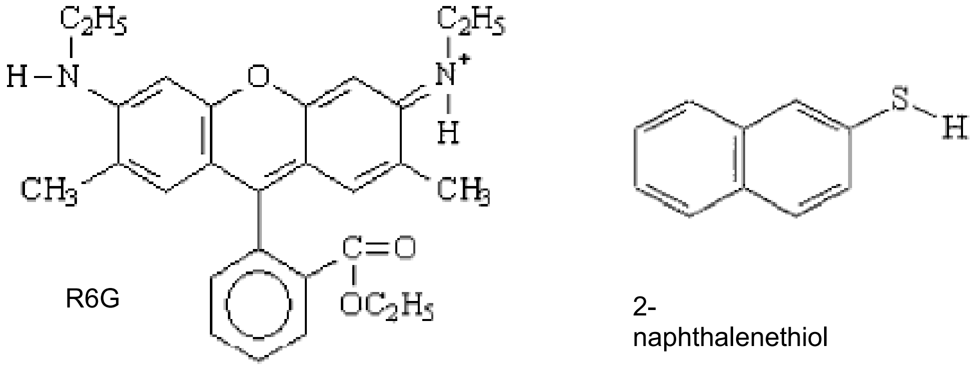


Figure 2.11. Structures of Rhodamine 6G and 2-napthalenethiol, two common probes in SERS.

Dilute solutions of the probe molecules are typically incubated with appropriately diluted colloidal metal solutions prior to casting on polylysine-coated glass (or microscope slides) or polymer-coated silicon wafer for SERS experiments. In average "ensemble" SERS, with ca. 10^{-6} M of the analyte incubated and appropriately diluted with the colloid, only about 10^{-15} mole of the analyte is probed [Garrell, R. L. (1989)] in a few μL aliquot of the solution cast on glass. Typically on metal arrays, low coverage of the probe is achieved by drop-coating of its dilute solution onto the surface, then allowing for spontaneous coverage of a few micrometers of the substrate with complete evaporation of the solvent. Depending on the stability of the SERS-active substrate and the properties of the probe molecule, often the sample preparation consists of incubation of the probe molecule with the substrate for hours, then deposition of the resulting solution onto polymer-coated slides, followed by rinsing with copious amounts of water to remove loosely bound analytes. An example is a system consisting of adenine as a probe molecule deposited as 5 x 10^{-10} mole on silver island films, where washing extensively with water was done prior to spectral acquisition [Giese, B. and D. McNaughton (2002)]. Several probes and substrates are amenable to washing, but generally, washing is not done with dyes as probes. The LB technique also seems to be an effective method for depositing monomolecular layers of probe molecules on a variety of substrates. Constantino *et al*. observed SERRS of the dye AzoPTCD on a fatty acid matrix deposited on silver island films prepared by vacuum evaporation [Constantino, C.J.L., Lemma, T.et al. (2001)]. In single-molecule studies on colloidal solutions, taking into account typical concentration of metal particles of 10^{11} particles/mL after synthesis and appropriate dilution, the volume of aliquots cast on glass substrates, and the optical focus, an average of one R6G molecule is adsorbed per silver particle at 10^{-10} M R6G concentration [Nie, S. M. and S. R. Emory (1997)]. At concentrations lower than 10^{-11} M, there is a higher probability that the number of probe molecule is very small, close to one. In the "solution" SERS performed by Kneipp *et al*., [Kneipp, K., H. Kneipp, et al., (1997)], a 10^{-13} M of crystal violet was diluted with the colloidal in a 1:15 ratio prior to spectral acquisition. Chloride salts (1-10-mM) are usually added to the colloids, especially in single-molecule studies, to generate more SERS-active particles.

- SM-SERS: e.g., 1:15 Volume Ratio of 10^{-13} M crystal violet: colloidal solution [Kneipp, K., H. Kneipp, et al., (1997)]
- Incubation prior to immobilization on glass surface
- Cast aliquot of colloidal solution with probe molecule and air-dry

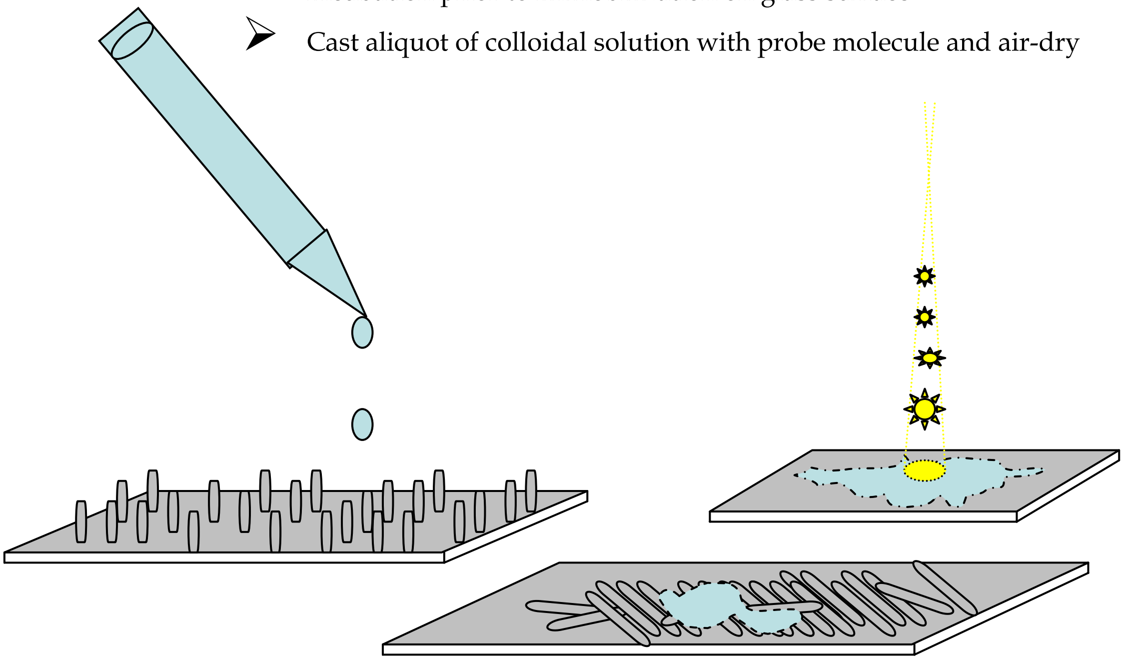


Figure 2.12. Deposition of Probes on SERS-active Substrates prior to spectral acquisition.

Typical SERS Setup

SERS spectral acquisition is usually done on Raman systems equipped with confocal microscopy and grating spectrometers coupled to CCD detectors, in back-scattering geometry. Confocal measurements ensure that the scatter is coming from a small region of the illuminated spot and that spectral interferences from out-of-focus regions are minimized. In typical SERS experiments using colloidal substrates, SERS-active particles are identified by choosing the clusters that show bright scatter when viewed through a Raman microscope. Typical Raman setups make use of low laser power (microwatts to tens of milliwatts) to prevent sample degradation on essentially ca. picoliter focal volumes. At such configurations, the power density at the samples is at least 10^3 W/cm^2 for 633-nm excitation [Pieczonka, N.P.W., Aroca, R.F. (2005]. At larger probe areas (e.g., 120- µm x 1-mm) illuminated by 35-mW of 514.5-nm, the power density is ca. 30-W/cm^2 [Michaels, A. M., M. Nirmal, et al. (1999)]. Current single molecule systems probe areas of ca. 1-µm^2 [Constantino, C.J.L., Lemma, T.et al. (2001)]. It is usually a choice between capturing the single molecule spectral behavior by using low integration times and moderate laser intensities and attaining good signal to noise ratios by more accumulations.

The collection of scattered photons is also maximized typically by situating the observation angles around 55°-60° relative to the surface normal [Wei, A., B. Kim, et al. (2001)]. In addition, the incidence angle is also optimized to ensure efficient excitation of the SPs, so several systems work around 60°-80° [Michaels, A. M., M. Nirmal, et al. (1999)] incidence with respect to the substrate normal. Laser excitation wavelengths are mainly from the visible and NIR regions, with 514.5-nm, 532-nm, 633-nm and 785-nm more commonly employed. Single molecule studies that make use of dyes like Rhodamine 6G typically employ either 514.5-nm or 532-nm excitation where the probe molecule has electronic resonances, whereby the technique is called SERRS. Small molecules are typically probed far from their absorption bands.

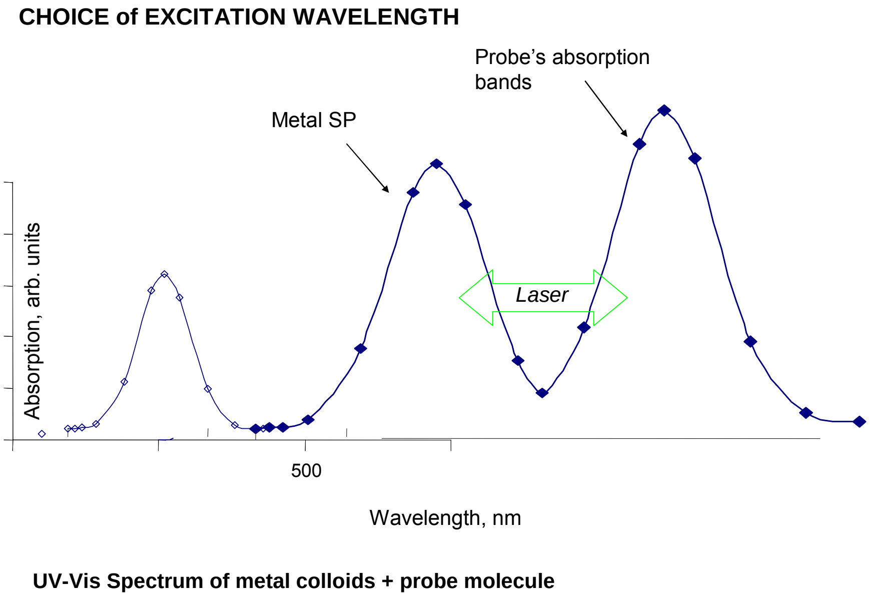


Figure 2.13. An illustration of a typical UV-visible spectrum of metal colloids plus the probe molecule. SP and molecule's absorption bands are typically both excited in the visible range.

The extinction spectra of the typical metal colloids containing the probe molecule usually contain a superposition of the metal SPs and the molecule's absorption bands. Instances where the excitation wavelength is capable of exciting both such resonances, a "double resonance" is possible, effecting larger enhancement in Raman scattering.

Enhancement Factor

Treatment and analyses of the data acquired from morphological analyses of the substrates, extinction spectroscopy, and Raman scattering usually start with calculation of the enhancement factor (EF) of the system. Empirical SERS enhancement factors (EFs) are estimated by comparing the normalized intensities of the Raman lines acquired via SERS versus those acquired using normal Raman. According to Kneipp et al., a better estimate can also be done by performing vibrational pumping experiments [Kneipp, K., H. Kneipp, et al. (1999)]. There is no absolute standard (e.g., against which the "surface" enhancement is compared) for EF calculations, and typically, the number of molecules that contribute to the Raman scatter is mostly based on mere estimates with the assumption of their fairly uniform deposition or surface coverage on the substrate surface. In colloidal substrates, for example, the density of the metal colloids is approximated based on the original starting amounts and the size of the colloids [Le Ru, E. C., M. Meyer, et al. (2006)]. The enhancement factor (EF) is usually calculated using this formula:

EF (of a Raman line) = $(I_{surface}/N_{surface})/(I_{volume}/N_{volume})$, where $I_{surface}$ refers to the normalized intensity of the signal acquired on the SERS-active substrate; I_{volume} refers to the normalized intensity of the signal acquired via normal Raman (assumed as liquid or neat sample); N refers to the number of molecules in the probed volume; and $N_{surface}$ refers to the number of molecules in the probed area.

EF estimates reported in the literature are sometimes calculated using the EM theory. The EM mechanism can predict field enhancements for various structures and arrays. There is usually a good correlation between EF estimates from experiments and those from theory. Earlier calculations had been based on simple shapes like spheres and ellipsoids in isolation, but current advanced calculations can now treat more complicated shapes including interparticle coupling effects. Theoretical calculations usually start with the input of the dimensions acquired from structural characterizations (like AFM or SEM) into Maxwell's equations. Afterwards, the absorption and Raman intensities are calculated based on known dielectric properties of the material [Yang, W., G. C. Schatz, and R.P. Van Duyne. (1995)]. The Raman enhancement factor is estimated as the 4th power of the field enhancement around the nanostructure:

$(E/E_0)^4$ =Raman enhancement factor, where $\mathbf{E_0}/\mathbf{E}$ refers to the local field enhancement in the electromagnetic field, $\mathbf{E_0}$ being the incident and **E** the enhanced electric fields around the nanostructure.

Traditional structures like electrochemically roughened electrodes and island films give average SERS enhancements of ca. 10^6 and 10^4-10^5, respectively [Haynes, C. L., A. D. McFarland, et al. (2005)]. These average enhancement factors correspond to responses from an ensemble of molecules which are predicted to generate in general, 10^3-10^7 EFs for most types of SERS-active substrates, be they metallic colloids or nanostructure arrays [Pieczonka, N.P.W., Aroca, R.F. (2005)]. Current nanofabricated arrays can give at least 10^7 empirical Raman enhancement factor. NSL-fabricated Ag nanoparticle arrays generated a Raman EF of ~10^7 for benzenethiol [Haes, A.J., C. L. Haynes, et al. (2005)]. Silver nanorod arrays fabricated by oblique angle deposition gave an empirical EF of ~10^8 for BPE using 785-nm [Chaney, S. B., S. Shanmukh, et al. (2005)]. The enhancements on nanowire assemblies are currently not large, but an assembly of Ag NWs of pentagonal cross-section was estimated to give ~10^9 EF for SERRS of R6G [Tao, A., F. Kim, et al. (2003)].

Overall, good substrates typically provide average "ensemble" 10^6 total EF without electronic resonance, and up to 10^{10} in the presence of electronic resonance (SERRS) [Dieringer, J. A., A. D. McFarland, et al. (2006)]. However, the enormous enhancements of about 10^{-14} to 10^{-15} achieved using colloidal solutions of silver, both far from and at electronic resonances of the dye molecules R6G and crystal violet indicate that ensemble averaging can be removed and used to probe molecules at single molecule limits in SERS. Since its realization in 1997 by Kneipp *et al*. and Nie & Emory, this has become a subject of current intense investigation in SERS. At the center of this is the question of where these large enhancements are situated in a vast field of nanostructure roughness features that are available as adsorption sites.

The enhancement is highly dependent on the excitation wavelength as a consequence of the SPR. Nanorods with higher aspect ratios are predicted to generate larger enhancements. Typically, the excitation wavelength is tuned to match the SPR of the metallic substrate in order to observe large Raman enhancements. Calculations show that "the SERS excitation profile (wavelength-dependent enhancement of the Raman scattered intensity) correlates well with the field enhancement at the surface" [Garrell, R. L. (1989)]. However, some experimental data show otherwise. Wei *et al.* [Wei, A., B. Kim, et al. (2001)] observed that there was no strong correlation between the excitation wavelength and the extinction maxima of the substrate of planar close-packed arrays of Au nanoparticles using resorcinarene tetrathiol as the probe. Using R6G on colloidal silver, Michaels *et al.* [Michaels, A. M., J. Jiang, et al. (2000)] found that the Raman excitation spectrum follows the absorption profile for R6G. Using reflectivity data showing plasmon-related absorptions of nanostructured voids on Au substrate, Baumberg *et al.* [Baumberg, J. J., T. A. Kelf, et al. (2005)] found strong correlations between the SPRs and the Raman enhancements. Despite the minor differences, it was shown in different systems that the best enhancements are obtained when the localized SPR of the SERS-active substrate is between the excitation wavelength and the vibrational line [Haynes, C. L., R. Van Duyne (2002)].

Enhancements are significantly affected by factors such as angle of incidence and angle of collection [Pieczonka, N.P.W., Aroca, R.F. (2005)]. Vibrational modes show different SERS enhancements at different detection angles as a result of the angular plasmon energy dispersion [Baumberg, J. J., T. A. Kelf, et al. (2005)]. The alignment of the electric field in relation to the nanostructures' axes also affects the Raman enhancement. Theoretical calculations predict that for dimers of spheres, maximum field enhancement is attained when the electric field is parallel to the dimer axis [Andersen, P. C. and K. L. Rowlen (2002)]. Experiments show that close assemblies of interacting nanorods and nanowires show anisotropic responses with polarization.

The amount of coverage of the probe molecule on the substrate surface also affects the enhancement significantly. Higher EFs are achievable with very low coverage (~monolayer) [Zeman, E.J., Carron, K.T., et al. (1987)]. This seems to relate to the homogeneous broadening of peaks at high coverage, causing the Raman signals to become buried in a broad background; however this can also be due to the absence of a "first layer effect" for the majority of the molecules unadsorbed at high coverage. Low monolayer coverage via the Langmuir-Blodgett technique allowed the observation of overtones and combinations in SERRS of perylenetetracarboxylic diimides (PTCDs) adsorbed on Ag metal island films. Aroca et al. reported SERRS (514.5-nm) as a single molecule detection method for a perylene derivative using Ag and Au colloids cast onto glass slides [Aroca, Constantino, and Tolaieb. (2004)].

CHAPTER 3

ELECTROMAGNETIC MECHANISM

Since its discovery in the early 1970s, the SERS process has only been partly understood; however electromagnetic enhancement is recognized as the major contribution to the enhancement process. To date, the electromagnetic (EM) mechanism is the main driving force in the intensive research involving SERS. The majority of what is found in literature consists of efforts to understand the fundamental mechanism of SERS by way of more systematic synthesis and assembly of nanostructures assisted by theoretical calculations.

The EM mechanism is based on the enhancement of the fields around nanostructures or roughness on surfaces upon illumination. In the presence of such nanostructures, the coupling of the metal with the field is enhanced, resulting in the enhancement of both the incident field and the Raman emitted fields. The coupling is facilitated by so-called surface plasmons (SPs) which are collective oscillations of conduction electrons in the metal as a response to the electromagnetic field. In turn, the surface plasmons also improve the coupling of the EM field with the adsorbed molecule. Both localized and propagating plasmons exist on metal surfaces, but in SERS, it is the "localized" SPs that provide the major enhancement. Surface plasmon resonances are dependent on particle sizes, shapes, interparticle couplings (array structures and spacings), and the dielectric media.

At the surface plasmon resonance (SPR), the metal exhibits increased absorption and Rayleigh scattering, which shows up as extinction peaks in conventional UV-Vis absorption measurements. The noble metals gold, silver, and Cu have these resonances at visible wavelengths and have been shown to give large SERS enhancements. The plasmon resonance of a spherical metal is described as a dipolar peak, the lowest order resonance [Gersten, J. I., D. A. Weitz, et al. (1980)], but nonspherical shapes give at least 2 peaks. Ellipsoid-like structures, like a nanorod, have two plasmon resonances that correspond to plasmons excited along the minor and the major axes. The transverse mode corresponds to the excitation of plasmons along the minor (short) axis while the longitudinal mode corresponds to those along the major (long) axis. The transverse mode is at the higher energy side and is essentially fixed (around 520-nm for Au and 410-nm for Ag). The longer wavelength resonance is found to be very sensitive to polarization and corresponds to excitations of higher multipolar charge distributions [Sosa, I. O., C. Noguez, et al. (2003)]. These charge distributions, also called multipolar resonances, are believed to arise from the inhomogeneous charge distributions around the nanostructure as a consequence of the dimensions becoming closer to the wavelength of light. The larger the deviations are from a spherical shape, the more "multipoles" can arise. The peak of the longitudinal band can be tuned from the visible to NIR regions by controlling the aspect ratio (the length/width) of the nanorods. Calculations predict the shifting to shorter wavelengths of the transverse mode with increasing aspect ratio, while the longitudinal absorption band red-shifts with increasing aspect ratio [Van Duyne, R., W.Yang, et al. (1995)]. In general, there is a linear relationship between the absorption maximum of the longitudinal plasmon resonance and the mean aspect ratio of Au nanorods [Link, S., M. B. Mohamed, et al. (1999)].

The EM mechanism predicts sharp edges or points of high curvatures to be the sites of largest field enhancements, essentially because of accumulation of charges at the tips. A single elongated rod would have more of the photons scattered at the tips thus generating enormous fields. This is called the "lightning rod effect", a term that is almost already synonymous to the EM mechanism. Theoretical calculations suggest that EM fields degrade exponentially with a characteristic decay length of ~2-nm [Haynes, C. L., A. D. McFarland, et al. (2005)]. The EM field is considered to be highest perpendicular to the surface. The component of the EM field perpendicular to the surface is much larger than the components parallel to the surface, causing vibrations along the direction perpendicular to the surface to become more enhanced than those that are parallel to the surface. This leads to the preferential enhancement of the in-plane vibration modes of those molecules adsorbed standing up compared to the out-of-plane modes [Mahmoud, M. A., Y. Badr (2006)].

Some of the SERS studies indicate the main role of EM in the SERS effect by taking note of the predominance of in-plane vibrations in the enhanced Raman spectra in cases where the orientation of the adsorbed molecule is known. At this point in SERS fundamental studies, however, orientation effects are not yet very well-understood for most systems. The major influence of EM mechanism in SERS is supported by the observed synchronous variation ($\cos^2\theta$ dependence) with polarization of both the lower-energy SPR bands and SERS intensities in arrays of high-aspect ratio nanostructures. Generally, clusters of nanoparticles display similar anisotropies of both the SPR and the SERS intensities [Itoh, T., K. Hashimoto, et al. (2003)], which is also observed in arrays of ellipsoidal particles of spacing distances large enough to prevent strong interparticle couplings [[Grand, J., M. L. de la Chapelle, et al. (2005)]. Arrays of strongly interacting nanostructures with high aspect ratios, however, sometimes show differences in the anisotropies [Zhao, Y. P., S. B. Chaney, et al. (2006)]. The EM mechanism is estimated to contribute at the most 10^{12} Raman enhancement factor, corresponding to a field enhancement, $\mathbf{E}/\mathbf{E}_0$, of 10^3 [Kneipp, K., H. Kneipp, et al. (2002)].

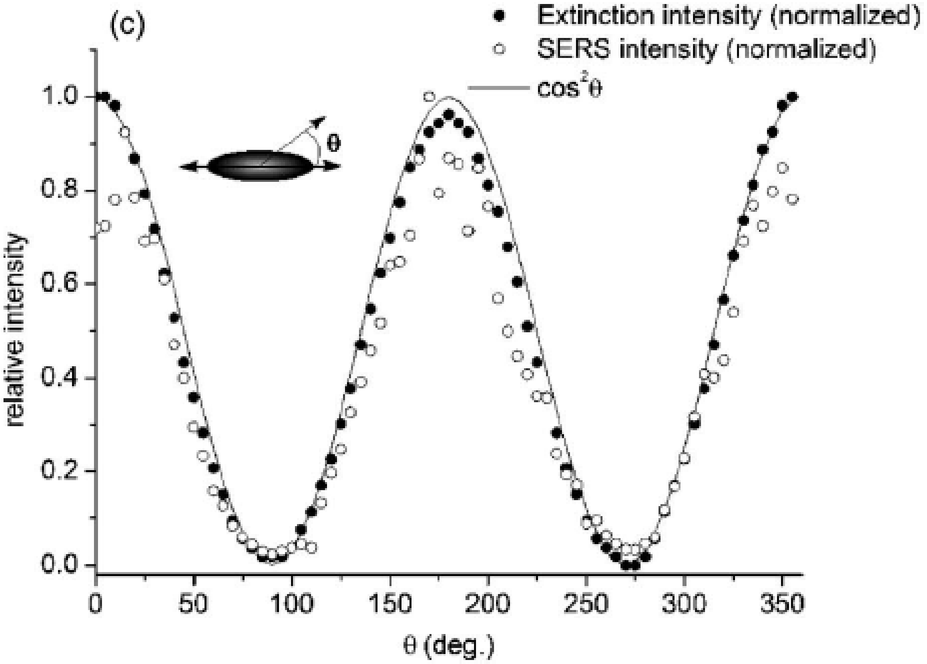


Figure 3.1. Spectra showing similar anisotropies of SPR and SERS. Raman (open circle) and LSPR (mode along the major axis located at 750-nm of EBL-fabricated ellipsoidal particle array, full circle) intensity plotted against the polarization angle (0 ° is the polarization parallel to the major axis); the solid line is the cos² fit [Grand, J., M. L. de la Chapelle, et al. (2005)].

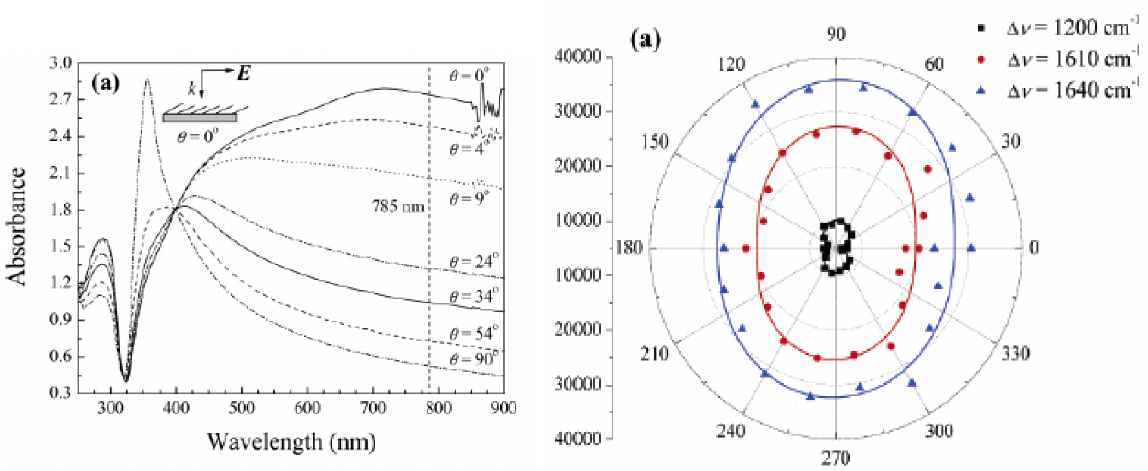


Figure 3.2. Spectra showing different anisotropies of the SPR and SERS responses (Left)Polarized absorption spectra of Ag nanorods, aspect ratio ~9, spacing 177-nm, 71.3° tilt; (Right) Polar plots of integrated intensities of BPE bands on Ag nanorods [Source: Zhao, Y. P., S. B. Chaney, et al. (2006)].

CHAPTER 4

CHARACTERISTICS OF SERS SPECTRA

SERS spectra typically have a diffuse background that is observed only in the presence of the probe molecule. Even molecules that do not have electronic resonances with the excitation wavelength display such a background. In the absence of the probe molecule, the spectra contain some Raman lines of residual matrix elements in colloidal solutions but without the background. A closer look into this diffuse background also called "electronic continuum" by those who do single-molecule SERS has led some to associate this with the creation of electron-hole pairs in the metal, a process which is not normally possible in the absence of a scattering center. Brus's group (Columbia University, NY) that leads single molecule SERS studies of R6G on colloidal silver with chloride ion activation ascribes such scattering center to the probe molecule itself, R6G. For this to work, strong chemisorption of the probe molecule is required.

Several explanations have been proposed to identify the source of the continuum. Many believe this to be amorphous carbon, either ubiquitous in silver substrates or a result of photodegradation of probe molecules [Pieczonka, N.P.W., Aroca, R.F. (2005)]. Studies with R6G on silver colloids have shown this background to "blink" with the Raman lines of R6G. Currently, this "electronic continuum" is proposed as arising from some modes of plasmon dephasing brought about by transient localization of ballistic electrons on the adsorbed molecule that creates an anionic R6G. Anionic adsorbed molecules caused by transient electron localization are known to photoluminesce [Michaels, A. M., J. Jiang, et al. (2000)].

Though enhanced fluorescence is also observed in noble metals [Andersen, P. C. and K. L. Rowlen (2002)], SERS allows fluorescence quenching enough for the observance of Raman lines even when strongly fluorescent probes are used. This advantage is especially effective when using low coverage of the probes such that there a stronger interaction between the molecule and the metal for quicker and more efficient energy transfer and nonradiative energy dissipation to occur. Another common feature in SERS spectra are the "new" bands which are typically not observed in normal Raman- a consequence of the so called "relaxation of surface selection rules in SERS". Some studies also report depolarization of Raman lines in the enhanced spectra [Kneipp, K., H. Kneipp, et al. (2002)].

Frequency shifts are frequent in SERS spectra. Raman bands are shifted relative to the unenhanced (normal) Raman spectra, as a result of the molecule’s interaction with the metal surface. Such shifts are more apparent in small molecules [Pieczonka, N.P.W., Aroca, R.F. (2005)]. In single-molecule SERS studies, spectral fluctuations are frequently observed, typically within 10 wavenumbers. Also, Raman spectra from different particles have some slight variations in frequencies, suggesting that the probe molecules are situated on different adsorption sites [Nie, S. M. and S. R. Emory (1997)]. Frequency fluctuations, however, are not limited to "single-molecule" systems, i.e., fluctuations were also observed even on systems that consist of high concentration of dyes [Le Ru, E. C., M. Meyer, et al. (2006)].

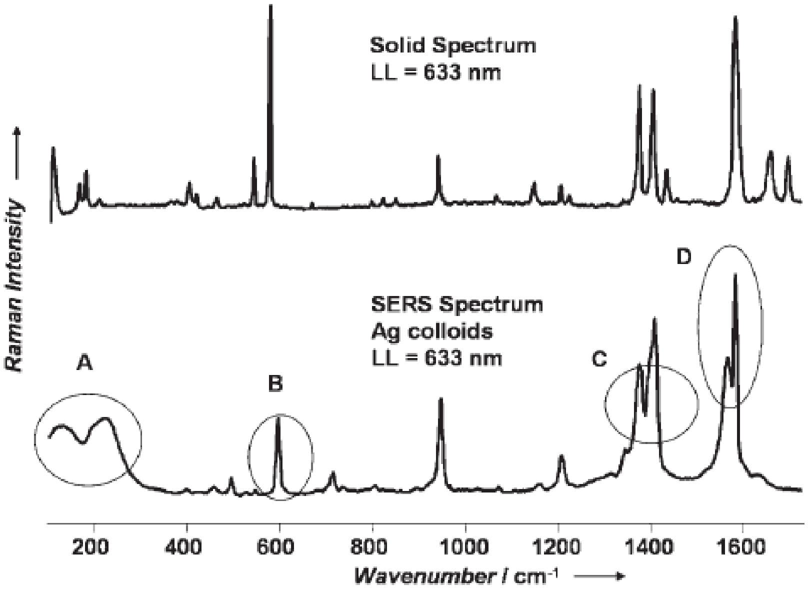


Figure 4.1. Spectra showing some common features of SERS compared to normal Raman, such as frequency shifts, change in relative band intensities and new bands for the molecule naphthalimide on colloidal Ag [Pieczonka, N.P.W., Aroca, R.F. (2005)].

Some SERS-active substrates also show strong SERS anisotropies. As mentioned in the previous chapter, such SERS anisotropies usually resemble the anisotropies of the surface plasmon resonances, but strongly interacting particles display otherwise, though only one case of the latter was noted [Chaney, S. B., S. Shanmukh, et al. (2005)]. Assemblies of nanowires, in particular, have also shown striking polarization dependences of SERS signals. An important finding about nanowires, according to Schider *et al.*, is the ease by which the plasmon modes corresponding to excitation along the short axis (modes that were found to respond only to higher energies) can be excited by visible light [Schider, G., J. R. Krenn, et al. (2003)].

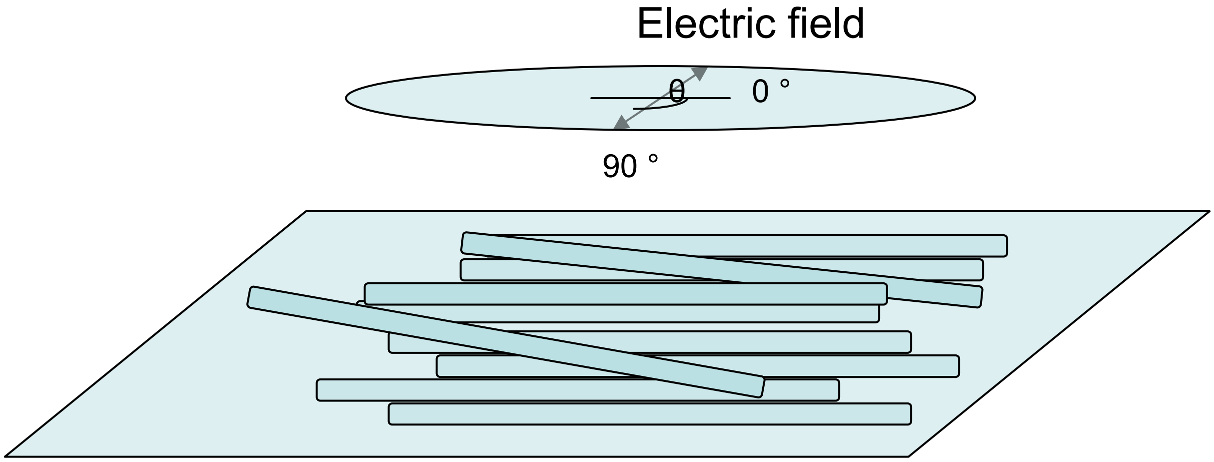


Figure 4.2. An assembly of nanowires showing the polarization of electric field.

On aligned films of Ag NWs [Yang,P. and Tao, A. (2005)], SERS using 1-hexadecanethiol at 532-nm excitation showed polarization dependence of the intensities of all the Raman bands: the SERS intensities were maximum when the electric field was perpendicular to the long axis of the NWs. They found this dependence to be stronger with better alignment of the wires. Similarly, on flat arrays of Ag NWs using R6G as a probe at 514.5-nm, Moskovits *et al.* [Moskovits, M., D. H. Jeong, et al. (2004)] observed 10x more intense signals when the electric field vector was across the nanowires.

Such observations enumerated above among others are fueling increased interest in the SERS community. Current efforts are directed into the identification of the nature of "hot spots", the chemical contribution to the total SERS enhancement which is commonly known as the "chemical effect", and most importantly, single-molecule SERS. Such issues are all addressed in experiments where sometimes, the separation of the EM enhancement from the chemical effect is attempted.

CHAPTER 5

"HOT SPOTS"

Hot spots are sites of enormous field enhancements on SERS-active structures. The EM mechanism identifies such sites as sharp tips or protrusions and even large curvature regions on isolated nanostructures. However, concerted efforts to explain enormous Raman enhancements achieved on colloidal nanoparticles have generated stronger evidence that "hot spots" are situated in between interacting nanoparticles. This is a consequence of inter-particle couplings that can concentrate enormously amplified fields in between. Once in the spot, the molecule experiences the tremendous force that greatly increases its polarizability. The size of the hot spot is estimated to be in the nanometer regime [Kneipp, K., H. Kneipp, et al. (2002)].

With typical power densities (30 W/cm^2) in micro-Raman experiments, at the gap the size of a few nanometers between a Ag spheres of ca.90-nm in diameter, the concentrated EM field can reach as much as 3 x 10 ^{6} W/cm^2 [Michaels, A. M., J. Jiang, et al. (2000)]. An estimate of the Raman enhancement is ca. 10^{11} at the middle of two spherical Ag or Au of interparticle distance of 1-nm [Kneipp, K., H. Kneipp, et al. (2002)].

The stimulus of course in all these undertakings is the first reports, using SERS and SERRS almost a decade ago, of the very high Raman cross–sections of dye molecules (at least ~10^{-16} cm^2/molecule, comparable to that of fluorescence cross-sections and estimated to be 14 orders of magnitude higher than typical Raman cross-sections) [Kneipp, K., H. Kneipp, et al. (2002)]. Since then, the literature has accumulated vast SERS data on varied types of substrates. One basic indication of the existence of "hot spots" is generally the small enhancement factors achieved for isolated silver, and higher EFs for Ag clusters [Andersen, P. C. and K. L. Rowlen

(2002)]. Vosgrone *et al.* [Vosgrone, T. and A. J. Meixner (2005)], on their SERRS studies of several Rhodamine dyes on Ag colloids cast on glass, found intense signals only when aggregates of hundreds of particles were probed, and no activity was detected with isolated single particles. The SM-SERRS paper by Nie and Emory in 1997, however, reported the active substrates to be "well-separated large single particles about 110-nm to 120-nm in diameter". Another indication of hot spots is the larger EFs with decreased interparticle spacings in arrays of nanostructures, which has been well supported by theoretical calculations and experiments. In addition, in clusters of colloidal particles, only a few percentage of the population are identified as SERS-active.

The literature shows that there are those groups that identify "hot particles" as being fractal-like (non-compact aggregates of particles) while some identify them as compact. Fractal-like substrates are typically fabricated by vacuum deposition on either cold or hot glass substrates, and their morphologies can be simply described as a system of interacting particles. It is argued that fractal-like substrates can have many hot spots because of the localization of high EM fields in small regions. Even dendritic structures (commonly derived from electrochemical fabrication of high aspect ratio rods or wires) are being studied because of "the fractal phenomenon" possibility with dendrites [Yang, S., X. Wen, et al. (2006)]. Brus's group labels such "hot particles", from morphological analysis, to be compact aggregates with a minimum of two individual particles [Michaels, A. M., J. Jiang, et al. (2000)]. Fractal or compact, the findings strongly suggest the hot spots to be junctions of interacting particles.

Active research by Brus's group, concentrated on understanding the chemical contribution to the total SERS enhancement, has been generating substantial insights into the nature of the hot spot. The probe molecule usually employed is R6G and the SERS-active substrates being Ag colloidal solutions activated with chloride ion. Some of the more important contributions come from their (a) observation of the increase in the number of SERS-active particles when R6G concentration is increased, not an increase in the intensity of the SERS signals [Michaels, A. M., M. Nirmal, et al. (1999)] which suggests the existence of rare chemisorption sites; (b) depolarization studies where they found the depolarization ratios to be highly dependent on wavelength [Jiang, J., K. Bosnick, et al. (2003)], and strong polarization dependence of SERS intensities [Bosnick, K. A., J. Jiang, et al. (2002)], both of which suggest the molecule is situated in a junction; and (c) the insensitivity of the resonant Rayleigh scatter with the polarization angle, suggestive of the existence of hot spots in extremely small confinement [Bosnick, K. A., J. Jiang, et al. (2002)]. Polarization dependence is a very strong indication of local confinement of EM fields.

Current observations of the strong polarization dependence of SERS intensities for assemblies of Ag NWs also suggest the hot spots to be the junction of interacting particles [Yang,P. and Tao, A. (2005)]; Moskovits, M., D. H. Jeong, et al. Journal of Physical Chemistry B (2004)]. In Yang *et al.*'s experiments, the polarization-dependence of the low-frequency Raman bands was fit to $\cos^2\theta$ function (θ being the angle between the polarized electric field and the long axis of the NWs). This observation provided an indirect evidence of the localization of the large EM fields in the interstices between adjacent nanowires. Their model for the generation of large enhanced fields in between the wires is based on the opposing polarization charges coming from each particle, from both sides of the gap. The excitation of plasmons associated with interwire coupling (coupled plasmon mode) was supported by the preferential efficiency of the process using 532-nm, far from the 380-nm plasmon band of a single Ag wire. A system consisting of bifurcated nanowires (35-nm in diameter, 10-μm in length) prepared by electrochemical deposition onto porous alumina also showed polarization dependence of the SERS intensities of R6G on silicon wafer using 514.5-nm excitation [Moskovits, M., D. H. Jeong, et al. Chemical Physics Letters (2004)]. The signals were found to be most intense when the electric field vector was "across" the cleft.

The strong SPR and SERS anisotropy in a system of dense nanorod arrays, irregularly deposited using oblique angle deposition (OAD), was also attributed to strong EM coupling [Chaney, S. B., S. Shanmukh, et al. (2005); Zhao, Y. P., S. B. Chaney, et al. (2006)]. The enhanced coupling was facilitated by the dense array of high aspect ratio rods (~6) with sharp features, tilted, and some possibly intertwined, creating an inherent anisotropy in the system. The SERS intensities were found to be most intense when the electric field was perpendicular to the long axis of the nanorods.

Two-dimensional mapping experiments using SERRS of LB-deposited perylene dye on thin Ag films provided additional indirect evidence of the existence of local electromagnetic hot spots [Goulet, P.J.G., Pieczonka, N.P.W., Aroca, R.F. (2005)]. The rarity of such hot spots was inferred from brightness fluctuations in several mapping images of samples containing various (decreasing down to single-molecule limits) concentrations of perylene mixed with arachidic acid.

The experimental findings mentioned above all support that hot spots are possibly junctions of interacting nanostructures. The large EM fields probably arise from the interaction of the EM dipoles from each nanostructure that can lead to a concentration of electric fields at the junction. Once in the hot spot, the probe molecule’s Raman scattering cross-section is enhanced.

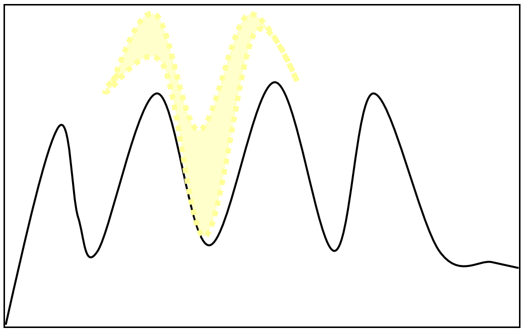


Figure 5.1. Representation of a "hotspot" in between nanostructure roughness features.

CHAPTER 6

CHEMICAL EFFECT

Studies on the single-molecule capability of SERS lend support to the chemical effect that is believed to be operative in SERS. Several indications of the chemical effect have already been observed early in SERS history: the influence of electrode potential to SERS enhancements, the observed large enhancements for highly conjugated molecules, the difference in the SERS signal intensities between CO and N_2 despite identical free-Raman scattering cross-sections, [Michaels, A. M., M. Nirmal, et al. (1999)], just to name a few. The chemical effect is viewed as a charge-transfer (CT) interaction between the molecular orbitals of the adsorbed molecule and the metal's electrons. The interaction leads to the shifting of the original electronic levels in the molecules or to the appearance of new bands. As in resonance Raman scattering, the excitation of such CT bands changes the polarizability of the bands that enhances the scattering. The chemical effect resembles resonance enhancement because molecules like citrate and water do not experience enhancement [Doering, W. E. and S. M. Nie (2002)]. An essential assertion in the effect is the presence of strong chemisorption sites on the metal surface that can act as "surface-active sites". One such site can provide a lower work function for the metal, such that some electrons are left weakly coupled to bulk states. Because of the commonly observed enhancing role of halide ions in single-molecule SERS systems, Nie *et al.* proposed such SERS-active sites to be high-affinity binding sites (65 kJ/mol) associated with adsorbed anions such as chloride and bromide [Nie, S. and J.T. Krug (1999)].

To date, several other indications of a chemical effect have been reported. The role of chloride ions in activating SERS systems is a mainstay. At typical concentrations used (1-10-mM), no significant aggregation is visible in such systems. But a comparison of the effect of the aggregating agent polylysine on a Ag colloidal system to that of chloride ions suggests that the role of the chloride ions is to cause aggregation and effect "activation" [Michaels, A. M., J. Jiang, et al. (2000)]. In a study of the effect of different anions on colloidal Ag, Doering *et al.* [Doering, W. E. and S. M. Nie (2002)] found a trend in the anions' effect in activating the nanoparticles immobilized on glass - by monitoring the time of activation (about 10-30 mins.), they found that the more stable silver-halide complexes activated the system more easily. The surface-active sites were associated to residual Ag cations which can be stabilized by the halides. The role of an oxygen atmosphere in the observance of "dynamic" hotspots has also been reported [Etchegoin, P. G., H. Liem, et al. (2002)]. Oxygen is viewed as a mediator for efficient coupling of the EM field with the molecule. The observance of dependence of SERS enhancement with shapes and crystal planes of Ag colloids can also be considered as an indication of a chemical effect [Zhang, J. T., X. L. Li, et al. (2005)]. The efficiency of the charge-transfer was related to the activity of the exposed crystal plane, i.e., to get thermodynamic stability, the crystal planes with higher free energies interact preferentially with the adsorbed molecules.

Research in Brus's group (Columbia University, NY) has been actively generating more insight into the chemical effect. Their system is usually composed of colloidal Ag activated with chloride ion and R6G as a probe. One observation from their experiments that strongly indicates the presence of a process other than the EM mechanism was the lack of direct correlation between Rayleigh scattering spectra and SERS intensities. The group postulated that if it were only an EM mechanism behind SERS, then all the particles that show strong Raman scatter should also have strong Rayleigh scatter. However, there were particles that showed strong complex Raman scattering at 514.5-nm but the corresponding Rayleigh spectra of which were not strong. Some particles with strong Rayleigh scatter did not show any SERS activity at all. There was no correlation with SERS both in the intensity and the frequency of the Rayleigh scatter, but all such SERS-active particles showed resonances at 514.5-nm excitation. The existence of "strong chemisorption sites" was also inferred from the observance of an increase in the number of SERS-active particles when the concentration of R6G was increased, instead of an expected increase in Raman signal intensities: an increase in the [R6G] 100 times increased the number of SERS-active particles 5 times [Michaels, A. M., M. Nirmal, et al. (1999)].

The chemical effect in SERS takes into account the observed diffuse background or "electronic continuum". One proposed model of the chemical effect is that of a dynamic CT between the molecule and the electrons of the metal. This essentially incorporates the EM mechanism, as called chemical (electronic) contribution to SERS by Otto [Otto, A. (2005)]. It is also sometimes known as a "first layer effect" where only the first layer effect of molecules that are adsorbed and trapped in small gaps (<1-nm) experience efficient dynamic CT to undergo single-molecule SERS [Otto, A. (2001)]. This dynamical charge-transfer can be described by the following steps [Kneipp, K., H. Kneipp, et al. (2002)]:

(a) excitation of an electron into a "hot-electron" state, with consequent photon annihilation;
(b) transfer of the hot electron into the LUMO of the molecule;
(c) transfer of the hot electron from the LUMO back to the metal, with some changes in the molecule’s molecular vibrations; and
(d) return of the electron to its initial state and Stokes-photon creation.

The correlation between spectral fluctuations and changes in the shape of the diffuse background in SERRS of R6G on Ag nanocrystals can be attributed to concerted CT resonance and resonance Raman/EM mechanism according to Weiss *et al.* [Weiss, A. and G. Haran (2001)]. They argue that both mechanisms can selectively enhance Raman bands, i.e., resonance Raman/EM mechanism enhancing those in-plane modes while CT resonance enhancing those that are out-of-plane, with the assumption that the R6G is standing on the metal surface. The chemical effect is estimated to contribute about 10^2-10^3 contribution to the total SERS effect. Its influence does not extend beyond a few nanometers from the surface, as modeled in Otto's "first layer effect".

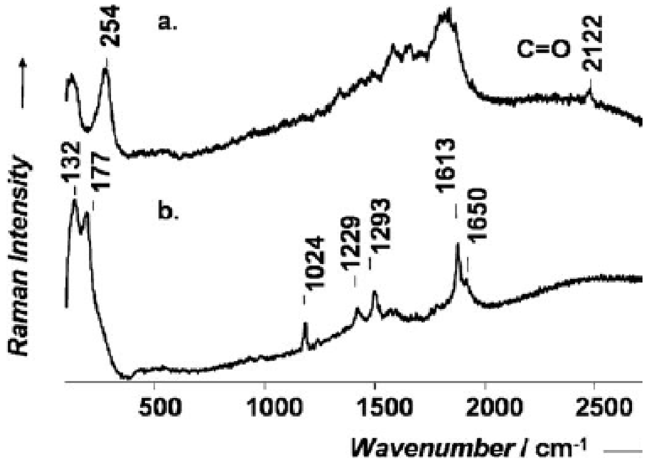


Figure 6.1. Spectra showing typical diffuse backgrounds in SERS. Raman background of Au colloids cast on glass (a); and in solution (b) [Pieczonka, N.P.W., Aroca, R.F. (2005)].

CHAPTER 7

SINGLE-MOLECULE SERS (SM-SERS)

Single-molecule detection is currently an active area in SERS. Systems believed to show single-molecule capability have the trademark "blinking" and spectral fluctuations. SM-SERS research started when independent reports of enormous enhancements in Raman cross-sections came out in literature : by Kneipp *et al.* [Kneipp, K., H. Kneipp, et al. (1997)], of crystal violet, estimated to be about 10^{-17} to 10^{-16} cm^2/molecule on a 30-μL droplet of chloride-activated aqueous colloidal Ag using an excitation wavelength that matched the SPR of the system (830-nm) ; and by Nie & Emory [Nie, S. M. and S. R. Emery (1997)] , 10^{14} to 10^{15} via SM-SERRS (514.5-nm) of R6G on single Ag particles (110-120-nm in diameter) cast on polylysine-coated glass. SERRS (514.5-nm) enhancement was also reported for hemoglobin using Ag particles immobilized on polymer-coated silicon wafer [Xu, H. X., E. J. Bjerneld, et al. (1999)].

In SM-SERS, the statistical averaging is removed, in contrast to ensemble averaged data with many molecules. This results to the observance of several attributes that can be simply described as a display of "frequent jumps from average values". Typical single-molecule spectra are characterized by blinking, intensity fluctuations of the Raman lines also called "spectral wandering" around a central frequency [Nie, S. and W.E. Doering (2003)], splitting [Vosgrone, T. and A. J. Meixner (2005)], and a background (often called electronic continuum) that blinks with the Raman signals.

Systems that show "single-molecule" capability are known to display a "blinking" phenomenon and considerable fluctuations in frequency. Blinking refers to fluctuations in the signal intensity from a very low to a measurable magnitude, usually occurring in time scales of milliseconds to seconds. The spectral fluctuations observed are typically attributed to a process other than the EM mechanism. This is because the EM properties (thus, the optical constants) of single Ag nanoparticles for example cannot change in a random, abrupt manner, especially with laser-induced heating at intensities employed in typical Raman setups [Doering, W. E. and S. M. Nie (2002)]. At the laser power intensities used in typical SERS wide-field Raman imaging, about 2 orders of magnitude less than 1 kW/cm^2, the geometry of two touching particles is hard to change [Hallock, A. J., P. L. Redmond, et al. (2005)]. Both the observance of the blinking and spectral fluctuations is automatically attributed to single molecules because there is a very low probability for many molecules to have the same orientations at the same time.

SM-SERS active substrates are typically colloidal aggregates of Ag activated with chloride ions, as in Brus's group, or vacuum-evaporated Ag films on heated glass [Goulet, P.J.G., Pieczonka, N.P.W., Aroca, R.F. (2005)]. Low concentrations ($\leq 10^{-10}$ M) of the probe molecule diluted with the colloidal solution are drop-cast on silanized surfaces then allowed to dry. The Langmuir-Blodgett technique is often used to transfer monolayers of probes on substrates like nanostructured films [Goulet, P.J.G., Pieczonka, N.P.W., Aroca, R.F. (2005)]. Unlike fluorescence spectroscopy with established criteria for single molecule detection, however, the single molecule detection capability in SERS is only inferred from statistical estimates of the number of molecules in the probed volume by appropriate dilutions and the observance of "blinking" and frequency fluctuations. At probe molecule (e.g., R6G) concentrations below 10^{-11}M, typical Ag colloidal solutions contain an average of close to 1 molecule per metal particle [Nie, S. M. and S. R. Emory (1997)]. Current issues addressed in single-molecule SERS are the origins of the blinking, the electronic continuum, and whether single-molecule SERS is real or not. The high variability in each " single-molecule " system brings doubts about whether or not SM-SERS is really being observed. Even systems that have many molecules show blinking behavior and spectral fluctuations [Le Ru, E. C., M. Meyer, et al. (2006)].

Several explanations for the blinking have been proposed. Though movements of the molecule and/or the particles are both possible, most attribute the fluctuations to the movement of molecules in and out of hot spots. The literature seems to have two groups of proponents: those that support lateral motion (by diffusion) of the molecule from the hot spots and those that attribute such fluctuations to modifications in the CT interactions (probably tangential not lateral motion) between the adsorbed molecule and the metal. In addition, there is always the consideration of possible photo-induced or thermal molecular transformations or rearrangements [Futamata, M., Y. Maruyama, et al. (2004)]. The "lateral motion of molecule" explanation is usually inferred from viscosity-dependence, temperature-dependence [Futamata, M., Y. Maruyama, et al. (2004)], and laser power-dependence of the fluctuations. However, SERS of R6G on compact Ag colloidal aggregates showed no correlation between the frequency fluctuations and the laser intensity [Michaels, A. M., J. Jiang, et al. (2000)]. SERS of R6G on Ag colloidal nanoparticles immersed in glycerol showed less fluctuation rates than when the system was immersed in water [Haran, G. (2004)]. Weiss & Haran [Weiss, A. and G. Haran (2001)] attributed the large spectral fluctuations on the time scale of seconds observed on the SERRS spectra of R6G adsorbed on Ag nanocrystals to lateral motion of the adsorbed molecule on the surface as a consequence of photodesorption events made feasible by electron tunneling between the metal and the molecule. The change in local configuration explanation is usually inferred from the timescale of the fluctuations, i.e., such millisecond time scale indicates local heating that can cause changes in the local geometry [Etchegoin, P. G., H. Liem, et al. (2002)]. Such changes in colloid configurations from Brownian motion can also cause fluctuations in intensity and spectral shapes observed in "single-molecule SERS" of mixed solutions of dyes [Le Ru, E. C., M. Meyer, et al. (2006).]

The explanation supporting the modifications of CT interactions is usually inferred from observations of actively fluctuating low-frequency bands and a relatively quiescent group of bands. Jiang *et al.* [Jiang, J., K. Bosnick, et al. (2003)], on their depolarization studies of R6G deposited on Ag colloids, observed synchronous fluctuations in the average depolarization ratio suggestive of a single dipole attached to the substrate. From this analysis, the fluctuations were assigned to modifications in the R6G adsorption-desorption kinetics on top of the metal particle. Doering & Nie [Doering, W. E. and S. M. Nie (2002)] explained the blinking in R6G bands on colloidal Ag as possible interruptions in electronic couplings between the molecule and the metal. The mechanism of the CT modification, however, is currently not understood. Also, the common single-molecule dyes R6G and crystal violet seem to show different behaviors [Sharaabi, Y., T. Shegai, et al. (2005)]. Haran's system [Haran, G. (2004)] of R6G on colloidal Ag which showed viscosity-dependent fluctuations, also had spectral peaks divided into two groups: one that was fluctuating and polarization-dependent, and another that fluctuated less. The observations seemed to support both modes causing the fluctuations, i.e., both lateral motion and CT modifications.

Perhaps the most controversial issue in SERS is the question of whether the claims of single-molecule observance are indeed real. Some of the best examples in the literature are those provided by Goulet *et al.* [Goulet, P.J.G., Pieczonka, N.P.W., Aroca, R.F. (2005)] and Le Ru et al. [Le Ru, E. C., M. Meyer, et al. (2006)]. Goulet *et al*. had used thin films of Ag ("nanostructured island films") to do SERRS of a perylene derivative LB-deposited as monolayers with arachidic acid. Several concentrations of the probe molecule approaching the single-molecule limit showed spectral features characteristic of SM detection limits: at higher probe concentrations the peaks (FWHH, frequencies, and intensities) were essentially stable, but started to show variations in typical peak parameters towards approaching the single molecule level. Le Ru et al. [Le Ru, E. C., M. Meyer, et al. (2006)] used two analytes, R6G and benzotriazole simultaneously, both at relatively high concentrations (100-nM) to test for single-molecule sensitivity. They observed several spectra (out of a series of 1000 SERS spectra collected at 0.2-second integration) that had signals composed only of the bands of one type of dye despite the presence of both in relatively large amounts: one time it is of R6G's; another it is benzotriazole's; or a mixture of both. Such observation indicated that the signals were coming from a very small number of molecules. Two of the most common SM-SERS systems were used, cast and dried colloids, containing the probe molecules and colloidal solutions, both of which suggested that single or few –molecule (<10) signals are very common in SERS, both in liquids and on dry substrates. Both of the groups mentioned above suggest the existence of electromagnetic hotspots that are easily achievable in typical substrates but the rarity of single-molecule SERS arises from the relatively low probability of putting the molecule at the right spot.

Sharaabi et al. [Sharaabi, Y., T. Shegai, et al. (2005)] showed that at least more than one molecule is needed to observe the single-molecule detection limit in small clusters of silver colloids (50-nm average particle size) with 632.8-nm excitation. From multiple SERS spectra of solution concentrations ranging from 10^{-6} M to 10^{-10} M (molecule: colloid ratios of ca. 10^5 to 10, respectively), they observed significant decreases in intensity in going from 10^{-6} M and lower that reached a plateau below 10^{-8} M. Sasic et al. [Sasic, S., T. Itoh, et al. (2006)] used principal component analysis (PCA) to check trends in SERRS spectra of R6G molecules adsorbed on isolated Ag colloidal particles, by varying the molecule: particle ratios (1:1 to 1:1000). Cluster analysis showed the separation of the SERRS spectra from substrates on four different particles that had adsorbed R6G molecules of >1000.

Current studies suggest that it is a "few-molecule" system contributing to SM-SERS. However, molecular dynamics in SM-SERS, i.e., the origins of the blinking, is still not understood. Research continuously provides more compelling evidence that SERS can soon strongly complement laser-induced fluorescence in single-molecule detection. The optical properties of nanoparticles need further understanding, however, as blinking is apparent even in close-packed systems where diffusion is minimized [Nie, S. and W.E. Doering (2003)].

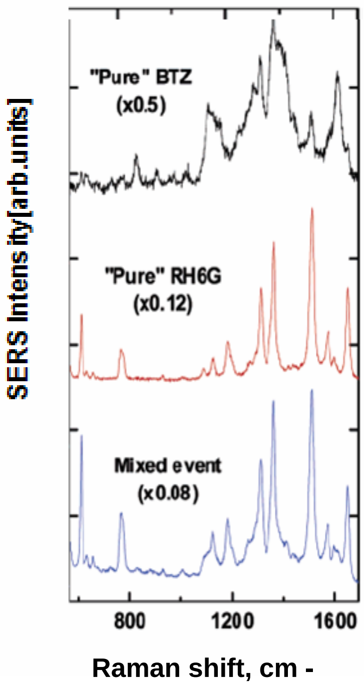


Figure 7.1. Representative spectra from a solution of dyes R6G and BTZ showing individual and mixed events [Le Ru, E. C., M. Meyer, et al. (2006)].

CHAPTER 8

CONCLUSIONS

Surface-enhanced Raman scattering (SERS) has been shown to generate large enhancements for highly sensitive detection. The literature is rich in analytical applications of the technique using simple colloidal silver substrates that give average enhancements acceptable for trace detection. Nanofabrication has been providing excellent substrates for both the fundamental studies of the SERS mechanism and SERS-based sensing. Currently, single-molecule SERS appears to be commonly associated with colloidal aggregates of Ag or Au cast on glass and with dye molecules. Experimental results based both on compact colloidal systems and typical fractal systems from thin films suggest that the most intense electromagnetic fields are confined in the junctions of touching particles. Current data from assemblies of nanowires and arrays of nanorods also support the claim. Hot spots are junctions of interacting nanostructures whose electromagnetic dipoles couple to generate giant EM amplifications in between. Several reports, including statistical analyses, suggest that SERS is frequently capable of detecting a small number of molecules in a probe area and that single-molecule SERS is not a rare phenomenon. Single-molecule-SERS deserves further attention as several nanofabricated SERS platforms generate large enhancement factors close to those reported for colloidal systems. Advances in particle manipulation with microscopy hopefully will provide stronger evidence of the capability of SERS in single-molecule detection. Currently, there are still a lot of questions regarding SM-SERS dynamics.

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