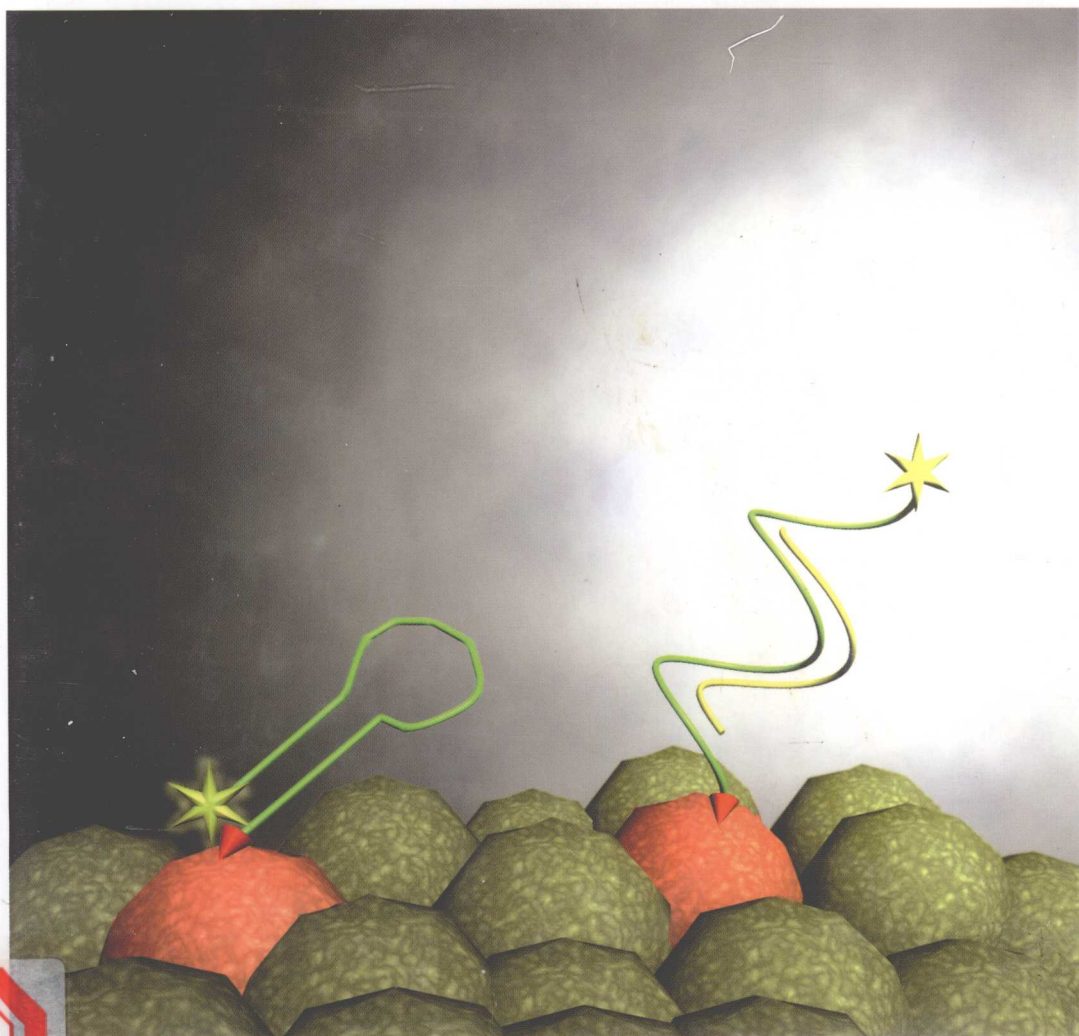


# Surface Enhanced Raman Spectroscopy

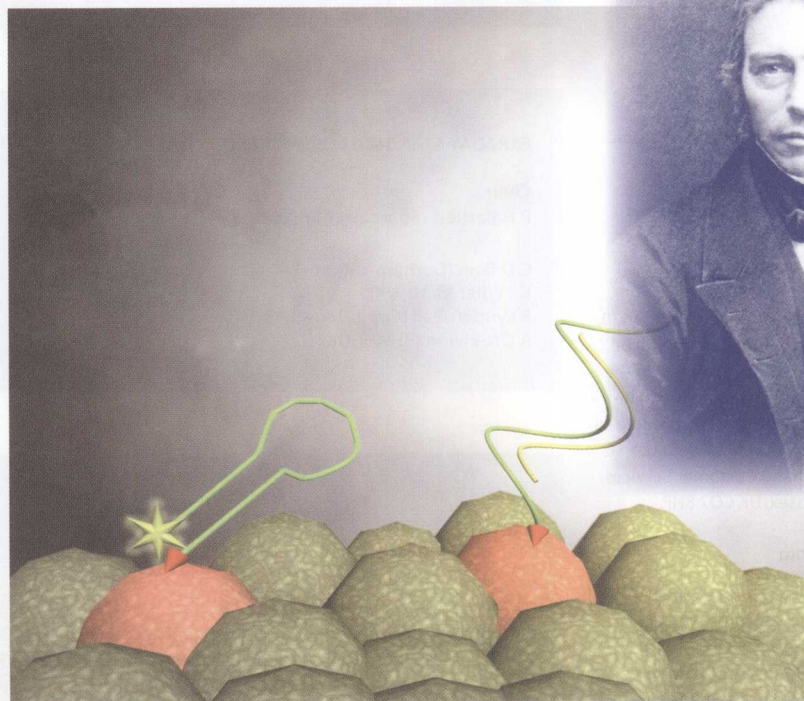


# Surface Enhanced Raman Spectroscopy



30805388

Imperial College London  
19–21 September 2005



## FARADAY DISCUSSIONS

Volume 132, 2006

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# Surface Enhanced Raman Spectroscopy





## Faraday Discussions 132

This volume focuses on Surface Enhanced Raman Spectroscopy (SERS). SERS offers unique functions such as the ability to detect, identify and characterise simple and complex analytes in a variety of matrices, at very low concentration levels. It is for these reasons that SERS is an increasingly active area for research and study. Work is presented in the following areas:

- Fundamental research into the physical/chemical origins of the SERS effect
- Synthesis and characterisation of novel SERS substrates
- Quantitative detection at ultra-low concentrations
- The *in-situ* combination of SERS with other probe microscopy techniques
- The future challenges for SERS
- Comparison of the strengths and weaknesses of competing technologies

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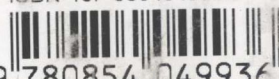
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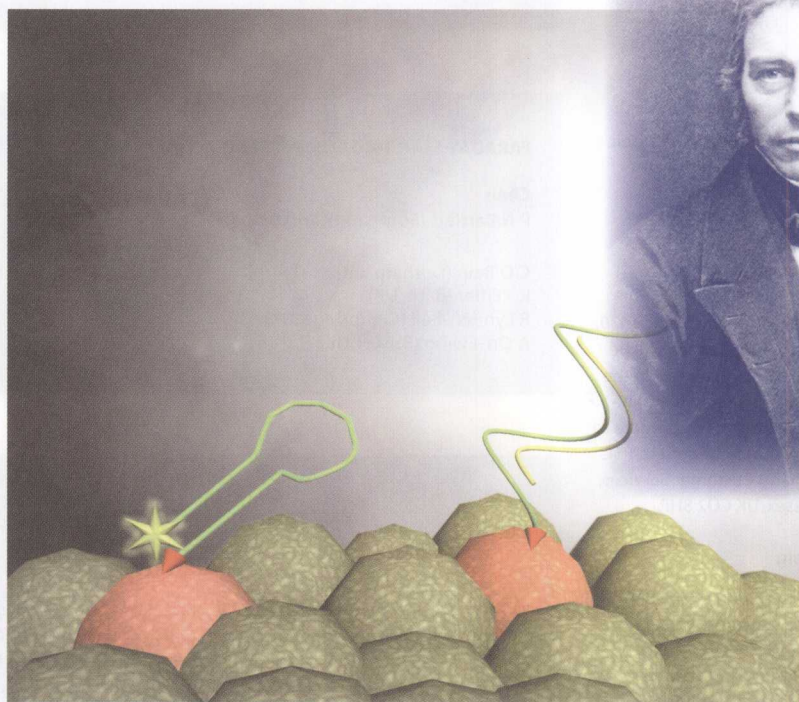
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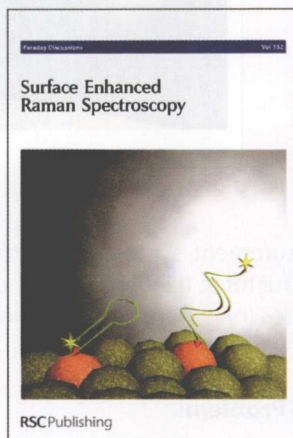
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A SERRS Beacon immobilised on a silver nanoparticle with fluorescence quenched and SERRS active (red nanoparticle) and an opened SERRS Beacon with fluorescence emission and SERRS activity.

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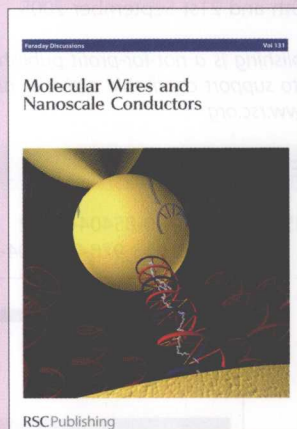
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# Surface enhanced Raman spectroscopy: new materials, concepts, characterization tools, and applications

Jon A. Dieringer, Adam D. McFarland, Nilam C. Shah, Douglas A. Stuart, Alyson V. Whitney, Chanda R. Yonzon, Matthew A. Young, Xiaoyu Zhang and Richard P. Van Duyne\*

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Surface-enhanced Raman spectroscopy (SERS) is currently experiencing a renaissance in its development driven by the remarkable discovery of single molecule SERS (SMSERS) and the explosion of interest in nanophotonics and plasmonics. Because excitation of the localized surface plasmon resonance (LSPR) of a nanostructured surface or nanoparticle lies at the heart of SERS, it is important to control all of the factors influencing the LSPR in order to maximize signal strength and ensure reproducibility. These factors include material, size, shape, interparticle spacing, and dielectric environment. All of these factors must be carefully controlled to ensure that the incident laser light maximally excites the LSPR in a reproducible manner. This article describes the use of nanosphere lithography for the fabrication of highly reproducible and robust SERS substrates for both fundamental studies and applications. Atomic layer deposition (ALD) is introduced as a novel fabrication method for dielectric spacers to study the SERS distance dependence and control the nanoscale dielectric environment. Wavelength scanned SER excitation spectroscopy (WS SERES) measurements show that enhancement factors  $\sim 10^8$  are obtainable from NSL-fabricated surfaces and provide new insight into the electromagnetic-field enhancement mechanism. Tip-enhanced Raman spectroscopy (TERS) is an extremely promising new development to improve the generality and information content of SERS. A 2D correlation analysis is applied to SMSERS data. Finally, the first *in vivo* SERS glucose sensing study is presented.

## 1. Introduction

It is most appropriate that Faraday Discussion 132 focuses on the topic of surface-enhanced Raman spectroscopy (SERS). Faraday's monumental contributions to electromagnetism include his posing of the central problem of small particle (*viz.*, nanoparticle) optics.<sup>1</sup> Faraday reasoned that there might be "some value in observing the action of light on material particles which in turn were also small compared with the wavelength of light." He then proceeded to carry out an amazing variety of experiments with nanoparticles of gold, silver, platinum, and many other metals. In retrospect, the intersection of these prescient studies by Faraday, the seminal work of Gustav Mie on the theory of nanoparticle optics,<sup>2</sup> and the discovery of vibrationally inelastic light scattering by Sir. C. V. Raman<sup>3</sup> clearly foreshadowed the emergence of SERS.

The existence of surface-enhanced Raman spectroscopy was recognized nearly thirty years ago.<sup>4,5</sup> SERS has since been demonstrated to be a powerful analytical tool for the sensitive and selective detection of molecules adsorbed on nanostructured (*i.e.* roughened), coinage metal surfaces. More than 5000 primary research articles,  $\sim 100$  review articles, and several books on SERS have appeared in the literature. This voluminous research database provides ample testimony of the

Department of Chemistry, Northwestern University, Evanston IL 60208-3113, USA.

E-mail: vanduyne@chem.northwestern.edu



impact of SERS on both fundamental and applied studies in fields as diverse as chemistry, physics, materials science, surface science, nanoscience, and the life sciences.<sup>6–18</sup>

As has been the case with other scientific fields, the storyline of SERS does not follow a monotonically rising arc to detailed understanding, extensive application, and widespread acceptance. Rather, SERS has experienced its “ups” and “downs.” The first “up” period of SERS lasted from its inception to the mid 1980s. During this time research on SERS was largely populated by representatives of the condensed matter physics and chemical physics communities. Intense research activity was squarely focused on achieving a mechanistic understanding of the primary observation—the  $10^6$  fold intensity enhancement observed for normal Raman scattering. The 1985 review article by Martin Moskovits,<sup>18</sup> eloquently summarizes the majority view of that time that the Raman enhancement factor,  $EF_{\text{SERS}} = 10^6$  could be understood as the product of two major contributions: (1) an electromagnetic (EM) enhancement mechanism and (2) a chemical (CHEM) enhancement mechanism. Of course, there really are no other choices since the intensity of Raman scattering is directly proportional to the square of the induced dipole moment,  $\mu_{\text{ind}}$ , which, in turn, is the product of the Raman polarizability,  $\alpha$ , and the magnitude of the incident electromagnetic field,  $E$ . As a consequence of exciting the localized surface plasmon resonance (LSPR) of a nanostructured or nanoparticle metal surface, the *local* electromagnetic field is enhanced by—say a factor of 10. Since Raman scattering approximately scales as  $E^4$ , the EM enhancement factor is of order  $10^4$ . The CHEM enhancement factor of  $10^2$  was viewed as arising from the excitation of adsorbate localized electronic resonances or metal-to-adsorbate charge transfer resonances (*viz.*, resonance Raman scattering (RRS)). It is also worthwhile noting that surface-enhanced resonance Raman scattering (SERRS) with combined SERS and RRS enhancement factors in the  $10^9$ – $10^{10}$  range was already known.<sup>4</sup>

During the next ten-twelve years, SERS developed into a relatively mature field. Many of the condensed matter physics researchers turned their attention elsewhere and were replaced by a broad spectrum of researchers interested primarily in applications of SERS to problems in electrochemistry, heterogeneous catalysis, polymer science, biochemistry of surface immobilized proteins, and many others. Perhaps it is not quite fair to characterize this as a “down” period; but, it was not one where the level of excitement matched that of the early days.

In marked contrast, the last eight years have seen an enormous resurgence of interest in SERS driven in large part by the remarkable discovery of single molecule SERS (SMSERS).<sup>19,20</sup> In addition, the explosion of activity in nanoscience and nanotechnology has fueled strong interest in SERS. It is actually difficult to find a paper on nanoscale optical properties that does not offer SERS as the primary example for applications. Today, there is an extraordinary amount of research going on that is exploring new nanoscale optical materials, new computational methodologies, and new measurement techniques. Broadly speaking, the goal of this work is to learn how to control, manipulate, and amplify light on the nanometer length scale using the properties of the collective electronic excitations in noble metal films or nanoparticles, known colloquially as surface plasmons. Consequently, a new term has entered the SERS vocabulary—Plasmonics. The interactions between adsorbed molecules and plasmonic nanostructures (*i.e.*, molecular plasmonics<sup>21</sup>) is likely to have a significant impact on many applications, including localized surface plasmon resonance (LSPR) spectroscopy for chemical and biological sensing,<sup>22–27</sup> sub-wavelength optical microscopy,<sup>28,29</sup> and nanolithography<sup>30</sup> as well as SERS.

This article will focus on some of the new materials and fabrication methods, concepts, characterization tools, and applications that have been developed in the Van Duyne Group over the past few years. In the materials section, we review the versatility of nanosphere lithography (NSL) as a simple means to fabricate structurally well-defined SERS-active surfaces that provide large enhancement factors, size- and shape- tunable localized surface plasmon resonance spectra, long shelf-life, temporal stability in solution, gas phase, or vacuum environments, thermal stability, and electrochemical stability. Next, we highlight the use of atomic layer deposition (ALD) as a powerful means for tuning the wavelength maximum of the localized surface plasmon resonance and carrying out high precision “spacer” or distance dependence measurements. The new concept we wish to discuss herein is the detailed relationship between the LSPR spectroscopy and wavelength scanned SER excitation spectroscopy (WS SERES) of NSL-fabricated nanoparticle surfaces. With regard to new characterization tools, we report some of our first results on tip-enhanced Raman spectroscopy (TERS) and single molecule SERS (SMSERS). Both TERS and

SMSERS are likely to have profound impacts on our ability to obtain detailed chemical information on the nanometer length scale. Finally, we will discuss one of our newest applications areas for SERS—in vivo glucose sensing.

## 2. Experimental

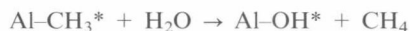
### 2.1 Nanosphere lithography

Nanosphere lithography was used to fabricate monodispersed, surface-confined Ag nanoparticles.<sup>31,32</sup> Glass substrates were cleaned in a piranha etch solution (3 : 1 H<sub>2</sub>SO<sub>4</sub>/30% H<sub>2</sub>O<sub>2</sub>) for 30 min at 80 °C. After rinsing with water, the substrates were sonicated for 60 min in 5:1:1 H<sub>2</sub>O/H<sub>2</sub>O<sub>2</sub>/NH<sub>4</sub>OH in order to create a hydrophilic surface on the substrate to facilitate self-assembly of the nanosphere masks. Finally, the substrates were rinsed and stored in water for future use. 2D self-assembled monolayer masks of nanospheres were fabricated by drop-coating approximately 2.5 µL of undiluted nanosphere solution (10% solid) on the pretreated substrates. The nanospheres were allowed to dry in ambient conditions. Ag was deposited by electron beam (e-beam) deposition in a Kurt J. Lesker Axxis e-beam deposition system (Pittsburg, PA) with a base pressure of 10<sup>-8</sup> Torr. The mass thickness and deposition rate (1 Å s<sup>-1</sup>) was monitored using a Sigma Instruments 6 MHz gold plated QCM (Fort Collins, Colorado). After the Ag deposition, the nanosphere masks were removed by sonication in absolute ethanol for 3 min.

### 2.2. Atomic layer deposition

Al<sub>2</sub>O<sub>3</sub> films were fabricated on NSL Ag nanoparticles by atomic layer deposition. The reactor utilized in these experiments is similar to that described in previous publications.<sup>33</sup> TMA and deionized H<sub>2</sub>O vapors were alternately pulsed through the reaction chamber utilizing N<sub>2</sub> as the carrier gas at a mass flow rate of 360 sccm and a pressure of 1 Torr using a growth temperature of 50 °C. Al<sub>2</sub>O<sub>3</sub> ALD proceeds on a hydroxylated surface according to the pair of self-limiting reactions shown in Scheme 1.<sup>34</sup>

Scheme 1



The asterisks (\*) signify the surface species. One complete AB cycle is 42 s: (1) TMA reactant exposure time = 1 s, (2) N<sub>2</sub> purge following TMA exposure time = 10 s, (3) H<sub>2</sub>O reactant exposure time = 1 s, (4) N<sub>2</sub> purge following H<sub>2</sub>O exposure time = 30 s. Long purge times are necessary at low temperatures to prevent chemical vapor deposition (CVD) of Al<sub>2</sub>O<sub>3</sub>.<sup>34</sup>

### 2.3 SERS distance dependence

Experiments were performed to map the distance decay function of SERS. Silver film over nanosphere (AgFON) samples were prepared by depositing 200 nm of silver over 390 nm polystyrene spheres from an electron beam evaporation source (Kurt J. Lesker AXXIS System). Alumina was then deposited by a custom fabricated atomic layer deposition (ALD) system at Argonne National Laboratory in thicknesses of 0.0 nm, 1.6 nm, 3.2 nm and 4.8 nm. The SERS spectrum of 50 mM pyridine in 100 mM NaCl/water was measured over the AgFON and Alumina AgFON samples ( $\lambda_{\text{ex}}$  = 532 nm,  $P$  = 1.0 mW, acquisition time = 300 s). The excitation was performed in an epi configuration through a 20× objective (Nikon, NA = 0.5) on an inverted microscope (Nikon TE300). The SERS signal was collected through the same objective in 180 degree backscattering geometry.

### 2.4 Wavelength scanned SERS excitation spectroscopy

**2.4.1 Instrumentation.** All optical measurements were performed using a Nikon Eclipse TE300 inverted microscope (Fryer Co., Huntley, IL) equipped with a 20× objective (NA = 0.5). Substrates were mounted on a piezoelectric stage (model P-517.3CD, Polytech PI, Auburn, MA) to allow for



sample positioning and raster-scanning during spectral acquisition. The light scattered by the samples was analyzed with a TriplePro three-stage spectrograph equipped with a liquid nitrogen-cooled, deep-depletion Spec-10:400BR CCD detector (Roper Scientific, Trenton, NJ). A color video camera was also attached to the front port of the microscope to facilitate laser alignment and positioning of the samples. Laser excitation was provided by the following systems: (1) a Spectra-Physics (Mountain View, CA) Millennia Xs ( $\lambda_{\text{ex}} = 532$  nm), (2) a Spectra-Physics Tsunami with GWU harmonic generator ( $\lambda_{\text{ex}} = 350\text{--}500$ ,  $700\text{--}1000$  nm), and (3) a Coherent (Santa Clara, CA) model 590 dye laser ( $\lambda_{\text{ex}} = 610\text{--}700$  nm). The laser light from the tunable laser systems was filtered using Pellin-Broca prisms or a diffraction grating to ensure monochromatic illumination of the sample. For the NSL-fabricated triangular nanoparticles, *in situ* measurement of the LSPR spectrum was achieved by illuminating the sample with the microscope lamp and analyzing the transmitted light with a fiber-optically coupled miniature spectrometer (model SD2000, Ocean Optics, Dunedin, FL). Atomic force micrographs of the SERS substrates were collected in non-contact mode using a Molecular Imaging (Tempe, AZ) PicoPlus microscope.

**2.4.2 Methods.** During spectral acquisition, the substrates were linearly scanned over a range of  $100\text{ }\mu\text{m}$  at a rate of  $2\text{ Hz}$  to prevent sample degradation. It is worthwhile to note that all illumination powers reported in this work were the laser powers incident on the microscope beamsplitter, not the power incident on the sample. Based on experimental measurements, approximately  $5\text{--}10\%$  of the reported power is incident on the sample; however, because of the intensity standard the absolute power at the sample is not a critical measurement.

In order to correct for any variation of the SERS intensity not due to the enhancement by the substrate, the  $1444\text{ cm}^{-1}$  normal Raman scattering band of neat cyclohexane was used as an intensity standard. This standard was used to correct for the inherent  $\nu^4$  behavior of Raman scattering, spectral dependence of the detection system, and differences in the illumination power. This was accomplished by mounting each sample face down as the bottom window of a transparent flow cell. When the flow cell was filled with cyclohexane, the nanoparticle array with an adsorbed benzenethiol monolayer was not in contact with the cyclohexane liquid. In this way, following each SERS acquisition, an intensity standard spectrum of cyclohexane could be taken by translating the inverted microscope objective  $\sim 400\text{ }\mu\text{m}$  vertically.

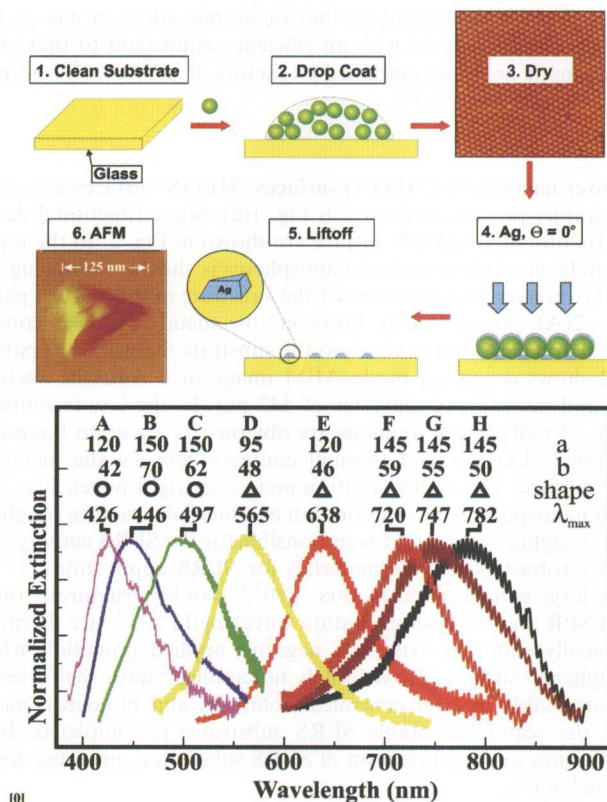
### 3. Results and discussion

#### 3.1 SERS substrates fabricated by nanosphere lithography

The most critical aspect of performing a SERS experiment is choice and/or fabrication of the noble metal substrates. Because the SERS intensity is dependent upon the excitation of the LSPR, it is important to control all of the factors influencing the LSPR in order to maximize signal strength and ensure reproducibility.<sup>35</sup> These factors, which include material, size, shape, interparticle spacing, and dielectric environment, must be chosen carefully to ensure that the incident laser light excites the LSPR. We have used the technique of nanosphere lithography to fabricate two different types of SERS substrates: (1) metal nanoparticle arrays and (2) metal film over nanosphere (MFON) surfaces.

**3.1.1 Nanosphere lithography (NSL).** NSL is a surprisingly powerful, yet simple, approach to the fabrication of nanoparticle arrays with precisely controlled shape, size, and interparticle spacing<sup>32</sup> as well as metal film over nanosphere surfaces that provide extremely robust surfaces for SERS applications.

The fabrication process, see Fig. 1(a), begins with the self-assembly of size-monodisperse nanospheres of diameter,  $D$ , to form a two-dimensional colloidal crystal deposition mask. A substrate is prepared so that the nanospheres freely diffuse until they reach their lowest energy configuration. This is achieved by chemically modifying the nanosphere surface with a negative charge that is electrostatically repelled by a negatively charged substrate such as mica or chemically treated glass. As the solvent (water) evaporates, capillary forces draw the nanospheres together, and they crystallize in a hexagonally close-packed pattern on the substrate. As in all naturally occurring crystals, nanosphere masks include a variety of defects that arise as a result of nanosphere



**Fig. 1** (a) Schematic representation of the nanosphere lithography (NSL) fabrication process. (b) Size- and shape-tunable localized surface plasmon resonance spectra of Ag nanoparticles fabricated by NSL. The wavelength of maximum extinction,  $\lambda_{\max}$ , is changed by variation in-plane width,  $a$ , and out-of-plane height,  $b$ , of the nanoparticles.

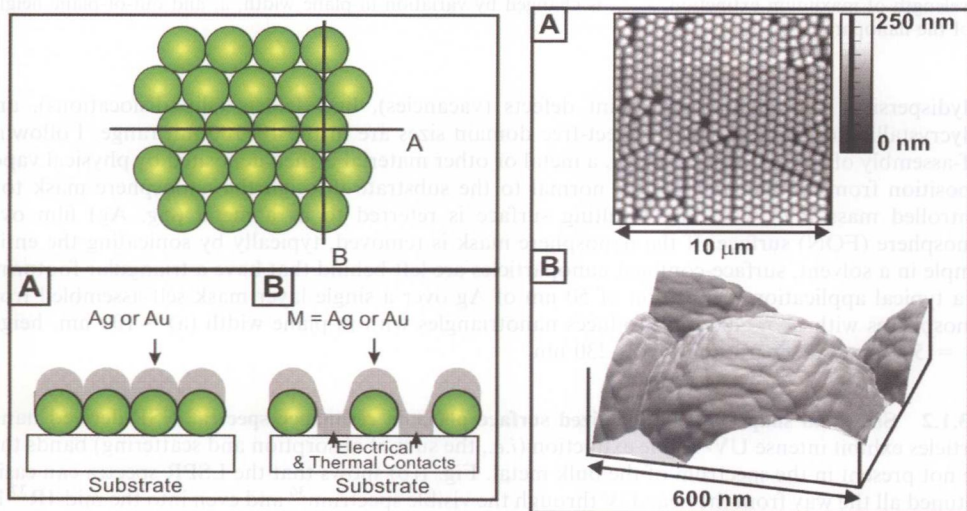
polydispersity, site randomness, point defects (vacancies), line defects (slip dislocations), and polycrystalline domains. Typical defect-free domain sizes are in the 10–100  $\mu\text{m}$  range. Following self-assembly of the nanosphere mask, a metal or other material is then deposited by physical vapor deposition from a collimated source normal to the substrate through the nanosphere mask to a controlled mass thickness. The resulting surface is referred to as a metal (e.g. Ag) film over nanosphere (FON) surface. If the nanosphere mask is removed, typically by sonicating the entire sample in a solvent, surface-confined nanoparticles are left behind that have a triangular footprint. In a typical application, deposition of 50 nm of Ag over a single layer mask self-assembled from nanospheres with  $D = 400$  nm produces nanotriangles with in-plane width (a)  $\sim 100$  nm, height (b) = 50 nm, and separation ( $d_{\text{ip}}$ )  $\sim 230$  nm.

**3.1.2 Size- and shape-tunable localized surface plasmon resonance spectra.** NSL-derived nanoparticles exhibit intense UV-visible extinction (*i.e.*, the sum of absorption and scattering) bands that are not present in the spectrum of the bulk metal. Fig. 1(b) shows that the LSPR spectra can easily be tuned all the way from the near-UV through the visible spectrum<sup>36</sup> and even into the mid-IR<sup>37</sup> by changing the size or shape (triangle or hemisphere) of the nanoparticles. Note that the LSPR bandwidth does not change significantly as the wavelength at peak maximum,  $\lambda_{\max}$ , is tuned. Additionally, several other surprising LSPR optical properties have been discovered for NSL-derived Ag nanoparticles: (1)  $\lambda_{\max}$  shifts by 2–6 nm per 1 nm variation in nanoparticle width or height;<sup>36</sup> (2) the molar decadic extinction coefficient is  $\varepsilon = 3 \times 10^{11} \text{ M}^{-1} \text{ cm}^{-1}$ ;<sup>36</sup> (3) the LSPR

oscillator strength per atom is equivalent to that of atomic silver in gas or liquid phases;<sup>36</sup> (4) resonant Rayleigh scattering<sup>38,39</sup> occurs with an efficiency equivalent to that of  $10^6$  fluorophors,<sup>40</sup> and (5) local electromagnetic fields are amplified by factors of  $|E|^2 \approx 10^4$ , leading to intense signals in all surface-enhanced spectroscopies.<sup>12</sup>

**3.1.3 Metal film over nanosphere (MFON) surfaces.** MFON surfaces are produced in step 4 of the nanosphere lithography process as shown in Fig. 1(a). Some functional details and a close-up view of the surface structure of a AgFON surface are shown in Fig. 2. In the top portion of the left panel of Fig. 2, a monolayer of close-packed nanospheres is shown. Following along line A, it can be seen that the metal overlayer does not contact the substrate in this perfect packing arrangement, see left panel of Fig. 2(A). Along line B, however, the metal overlayer contacts the substrate, providing both thermal and electrical contacts to the substrate material, left panel of Fig. 2(B). The right panel of Fig. 2 shows a contact mode AFM image of a AgFON electrode with Ag mass thickness of 200 nm and nanosphere diameter of 542 nm. In the low-resolution image, see right panel, Fig. 2(A), areas of well packed domains are obvious, as are grain boundaries and vacancies which provide additional electrical and thermal contacts between the metal overlayer and the underlying “smooth” surface. The high resolution image, see right panel, Fig. 2(B), shows that the top of each Ag-coated nanosphere is not smooth but exhibits substructure roughness features of 30–50 nm in size. It is this roughness scale that is responsible for the SERS activity of AgFON surfaces.

AgFON surfaces are robust plasmonic materials for SERS applications.<sup>41–47</sup> We have demonstrated that they have large enhancement factors  $\sim 10^7$ ,<sup>10</sup> can be structurally tuned to optimize the overlap between the LSPR and the laser excitation wavelength,<sup>10,46,47</sup> are thermally stable to  $\sim 500$  K,<sup>41</sup> are electrochemically stable at extremely negative applied potentials where the traditional electrochemically roughened surfaces are definitely not stable,<sup>42</sup> have shelf-lives of  $> 40$  days after manufacture,<sup>47</sup> and are stable in electrochemical solutions and biological media for at least 10 days.<sup>46</sup> In our view, the search for stable SERS substrates is completed. In order to make a significant new contribution to the fabrication of SERS substrates, one must demonstrate a surface that exceeds these benchmarks.



**Fig. 2** Metal film over nanosphere (MFON) surfaces. (Left panel) Illustration of electrical and thermal contacts for MFONs. (top) Close-packed array of nanospheres, (A) view along a row of nanospheres, and (B) view along a column of nanospheres. (Right panel) Ambient, contact mode, atomic force microscope image of 200 nm Ag over 542 nm diameter polystyrene spheres. (A) Array of spheres ( $10\ \mu\text{m} \times 10\ \mu\text{m}$ ) and (B) image ( $600\ \text{nm} \times 600\ \text{nm}$ ) of one sphere showing substructure roughness.