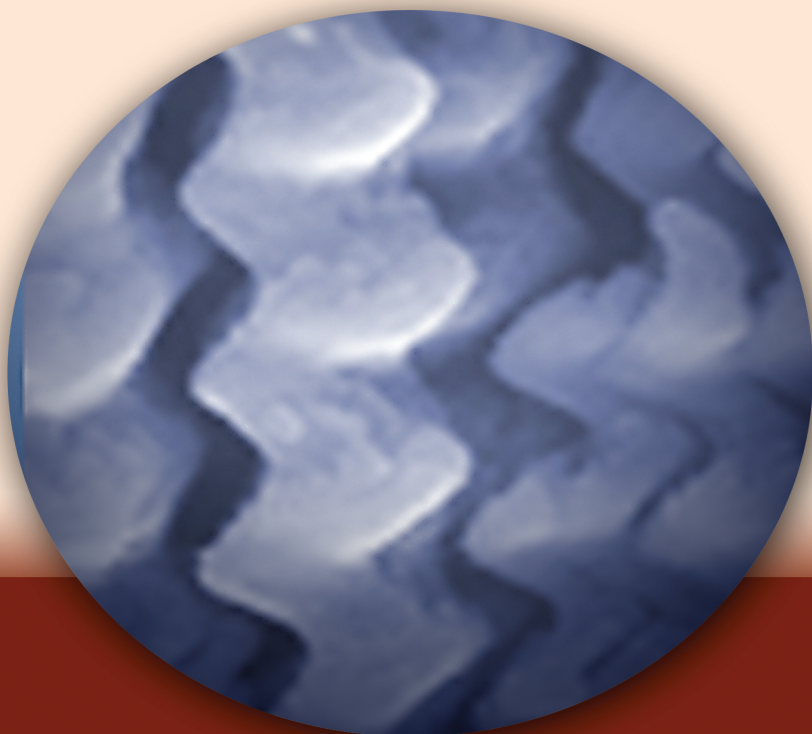


Pan Stanford Series on the High-Tech of Biotechnology  
Volume 4

# NANOMATERIALS FOR WATER MANAGEMENT

Signal Amplification for Biosensing from Nanostructures

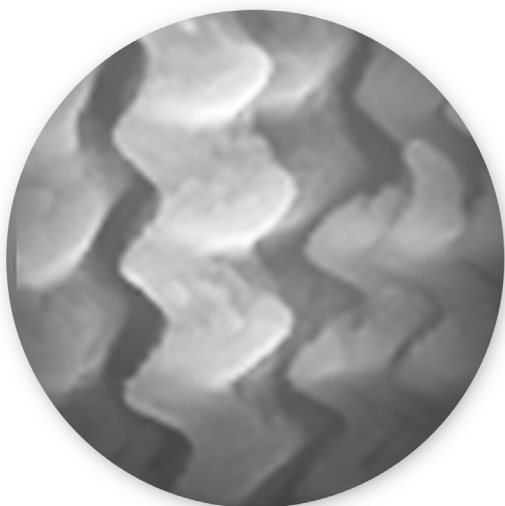


edited by

Ibrahim Abdulhalim  
Robert S. Marks



# NANOMATERIALS FOR WATER MANAGEMENT



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# NANOMATERIALS FOR WATER MANAGEMENT

Signal Amplification for Biosensing from Nanostructures

edited by

Ibrahim Abdulhalim  
Robert S. Marks



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# Preface

Water is one of the key world resources, considered a limited valuable commodity, for which its sustainability has been an essential issue until the present time and for which a myriad of technologies are being used. At their forefront is the use of nanometer-scale technologies, which exhibit tremendous opportunities, including water management to improve water quality, detection, and remediation. An important class of nanomaterials is metallic nanostructures, which exhibit remarkable plasmonic properties at the nanoscale. The newly emerging field of nanoplasmonics, including surface plasmon resonance (SPR), takes advantage of related phenomena in metallic structures at the nanoscale, such as those found in nanoparticles, nanoholes, nanowires, and other combinations of different geometrical nanoscale shapes. One of the most important prerequisites to improve water quality is to be able to monitor the concentrations of critical pollutants in water. In this regard biosensors in thin-film form based on SPR-related technologies have been developed in various configurations and formats for sensing a variety of target samples in water, including pesticides, deleterious organic chemicals, biological pathogens, and biotoxins. The sensitivity and detection limit of SPR-based sensors continue to improve, so samples of ever-smaller volumes can be detected with enhanced reliability and specificity, in particular when combined with spectroscopic probes such as in surface-enhanced Raman scattering (SERS), surface-enhanced fluorescence (SEF), and surface-enhanced infrared absorption (SEIRA) spectroscopies.

The interest in finding reliable and highly sensitive sensors for water quality control has grown recently, empowered by the explosion of cutting-edge technologies such as nanotechnologies, optoelectronics, and computing on the one hand and by the increasing need for more secure control of water quality on the other hand. This book highlights a number of modern topics in the field of biosensing, particularly for water sensing in which the signal is enhanced. It starts with surface-enhanced spectroscopies using plasmonic nanostructures such as SERS, SEIRA, and SEF.



Then SPR-enhanced detection is highlighted in two chapters and addressed using signal processing and the use of color of solutions as a result of modification of the LSPR properties of nanoparticles. Porous materials are another field of research where enhancement is achieved due to the increase of the area-to-volume ratio. Good examples are the two emerging fields of porous Si and sculptured thin films prepared by the oblique deposition technique. One of the long-standing problems is bacteria detection in water, which is addressed thoroughly with emphasis on the problems usually encountered in detecting large bioentities.

The book is an important multidisciplinary addendum, authored by a selection of researchers belonging to different disciplines, targeting advanced undergraduate- and graduate-level students in nanotechnology, engineers and scientists in the industry, and researchers involved in biosensing, nanotechnology, chemistry, biology, and medicine, especially those with an interest in biosensing.

The editors would like to acknowledge all the contributors to this book for spending the time and effort in writing their chapters and for their patience until this book got published. We are grateful to the students as well as the research collaborators who were involved in obtaining some of the research results presented in this book.

The initiation of this book was encouraged by Prof. Ma Jan of the School for Materials Science and Engineering at the Nanyang Technological University in Singapore, who, sadly, passed away on June 26, 2012, before seeing it published. We mourn the loss of an extraordinary scientist and friend. This book is dedicated to his memory.

**Ibrahim Abdulhalim**

**Robert S. Marks**

## Chapter 1

# Nanoplasmonic Structures and Enhanced Spectroscopies for Water Sensing

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In this chapter, we will address our recent work on the optimization of surface-enhanced Raman scattering (SERS) substrates for biosensor applications. This kind of sensor is of great interest concerning the metrology of the environment. It is a very promising technique that can allow in situ or on-site measurements to ensure

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water surveillance and monitoring. The optimization of SERS performances is considered in two aspects, increasing of SERS sensitivity by using the molecule adhesion layer between gold nanostructures and glass and nonpolarization-dependent SERS detection with apolar plasmonic nanostructures. All the works are based on the optimization of localized surface Plasmon resonance (LSPR) of metallic nanostructures; thus the methods could also be generalized to other surface-enhanced spectroscopies (SESS).

## 1.1 Introduction

The latest European regulations (directives 2000/60/EC, 2006/118/EC, and 2006/11/EC) on water quality aim to achieve good ecological and chemical status for water bodies by 2015. To ensure water surveillance (fresh, surface, ground, coastal, and transitional waters), a measurement network has been set up to conduct sustainable monitoring of aquatic environments, in particular to assess the impacts of human activities generating industrial and agricultural pollutant emissions.

The most common approach to contaminant measurements consists of sporadic sampling by laboratory analysis. This approach remains unsatisfactory for several reasons. First, it is costly as it requires time and human resources. Second, sample contamination, a frequent occurrence due to adsorption onto sampling tubes, distorts the quantities present in samples. Furthermore, changes in temperature, pressure, and CO<sub>2</sub> and O<sub>2</sub> content cause alterations in the pH, redox potential, and species status. In addition to these factors, microbial activity during sampling and the transport of samples can also affect the speciation of original samples. In hardly accessible areas, the large sample volume required can sometimes be prohibitive.

To overcome this obstacle of environmental metrology, original field measurement chains are very useful for the detection of contaminants in aquatic environments. In this aim, optical spectroscopies are well-adapted techniques for on-site or in situ measurements [1]. The innovative aspect of this kind of method lies in the coupling of a (bio)chemical sensor, able to collect the contaminants from water, to SERS selected for its qualitative and quantitative analytical performance.

In situ analytical tools will enable a better estimation of the spatial and temporal variability of water contaminants. Over the long term, it could support decision making by public authorities in the event of a spill, whereby the response time is of crucial importance.

To reach this goal, surface-enhanced spectroscopies (SESS) are being developed. In the recent past, metallic nanoparticles and nanostructures with pronounced localized surface Plasmon resonance (LSPR) had been exploited in kinds of SESS for sensing applications. Spectral signals amplified by LSPR give rise to surface-enhanced Raman scattering (SERS) [2–5], surface-enhanced resonance Raman scattering (SERRS) [6], and surface-enhanced infrared absorption (SEIRA) [7, 8], as well as surface-enhanced fluorescence spectroscopy [9]. For decades, SERS was the most widely used SES as an extremely sensitive analytical technique for chemical or bioanalytical applications. For SERS detection, two main strategies are proposed, metal colloids and lithographed nanostructures. The solution-state SERS substrate is problematic—for instance, SERS activity strongly depends on the nature of the chemical agents, and aggregation of colloids leads to poor reproducibility from experiment to experiment. In contrast, it would be advantageous to exploit regular metallic nanostructures as SERS-active substrates to reach high repeatability and reproducibility. However, for sensor application, high sensitivity to achieve low concentration detection is still a challenge, although single-molecule detection has already been predicted [2, 5]. In most of the cases, giant enhancements of the Raman signal were reached for coupled nanostructures such as at interstitial sites in nanoclusters or nanostructured surfaces with closely spaced features [5, 10–12]. However, SERS performance at so-called “hot spots” showed poor repeatability and reproducibility that are not suitable for biosensor exploitation. Therefore, it is more meaningful to improve the SERS sensitivity in a more controllable way for realization of biosensors [13].

In this chapter, we will address our recent works and results on the optimization of SERS measurements for deliverable biosensor applications. The improved SERS performances are based on the optimization of the LSPR properties of the metallic nanostructures [14]. First, we will show that SERS sensitivity could be greatly improved due to stronger near-field enhancement by using the molecule adhesion layer between gold nanostructures and the glass substrate, with good mechanical robustness. Then, we present one

kind of complex nanostructure with apolar behavior that makes SERS detection easier and more flexible. The discussion will be focused on electron beam lithography (EBL)-fabricated SERS substrates, but the methods could be generalized to other SESs that are based on electromagnetic field enhancements due to excitation of LSPR around the metallic nanostructures.

## 1.2 Improved SERS Sensitivity Using the Molecule Adhesion Layer

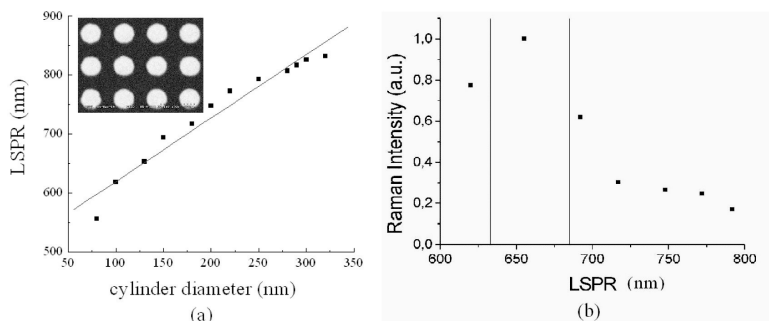
### 1.2.1 Optimization of the SERS Process by LSPR Control

The enhancement of the electromagnetic field around the metal surface due to excitation of LSPR in metallic nanostructures is the basis for SERS. According to Wokaun's theory [15], the total Raman enhancement factor could be expressed as  $G_{\text{SERS}} = |f(\lambda_0) \cdot f(\lambda_R)|^2$ , where  $f(\lambda_0)$  and  $f(\lambda_R)$  are the near-field enhancement at the excitation and Raman wavelengths, respectively. Thus, SERS efficiency shows a strong dependence on the LSPR of metallic nanostructures. In the case of nanocylinders or nanotriangles, investigations have demonstrated that the best Raman enhancement is achieved for an LSPR position located between the excitation and Raman wavelengths [16–18]. Thus, for SERS measurements precisely control of LSPR is the key point to get the best Raman enhancement.

Thanks to modern nanofabrication techniques, the shape and size of individual nanoparticles as well as the interparticle distance can be well controlled and then the LSPR of metallic nanoparticle arrays could be precisely tuned. In our case, metallic nanostructures were fabricated by the EBL technique, which was achieved by a 30 kV Hitachi S-3500N scanning electron microscope (SEM) equipped with a nanometer pattern generation system (NPGS). Here we show an example of gold nanocylinders with tunable LSPR properties and SERS performance. As shown in Fig. 1.1a, the LSPR position of Au nanocylinder arrays could be tuned in the whole visible wavelength range by varying the cylinder diameters.

Figure 1.1b gives the relative SERS intensity (trans-1,2-bis(4-pyridyl)ethylene [BPE] band at  $1200 \text{ cm}^{-1}$ ) versus the LSPR positions for the excitation wavelengths at  $\lambda_0 = 632.8 \text{ nm}$ . A maximum of Raman enhancement was reached for the LSPR position located around 650 nm, exactly between the excitation wavelength  $\lambda_0 = 632.8 \text{ nm}$  and

the Raman wavelength  $\lambda_R = 685$  nm. The result is in good agreement with Wokaun's model and confirms that the EBL-fabricated SERS substrates could be optimized by tuning the LSPR position via choosing the appropriate size and shape of the nanoparticles.



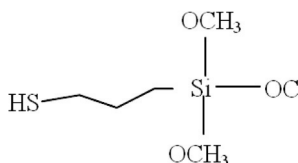
**Figure 1.1** LSPR wavelength and cylinder diameter (a) (Inset: SEM image of 280 nm gold nanocylinder); Raman intensity of the  $1200\text{ cm}^{-1}$  BPE line, and LSPR of the nanocylinders. (b) The two lines indicate the excitation wavelength and Raman wavelength, respectively.

## 1.2.2 Improved SERS Sensitivity by MPTMS

### 1.2.2.1 Limitation of SERS sensitivity by chromium

Gold is widely exploited as a molecule label, a marker in chemical or biological sensors for its chemical stability and potential biocompatibility. In terms of on-chip biosensors, an adhesion layer is needed to guarantee good bonding between Au nanostructures and a glass (or Si) surface. Usually, a thin adhesion layer (Cr, Ti, or Ni typically) of a few nanometers is commonly used to improve the adhesion between a glass (or Si) surface and an Au, Ag film or nanoparticles [19, 20]. However, the Cr layer degrades the optical properties by shifting and broadening the LSPR of gold nanostructures [21]. As a consequence, the spectral signal, for example, SERS, will be decreased because of the damping of LSPR, which brings limitations to the improvement of sensor sensitivity. This problem could be resolved by using a molecule adhesion layer (3-mercaptopropyl trimethoxysilane [MPTMS]), instead of Cr, as demonstrated in our recent work on SERS optimization. MPTMS is

expected to work as an adhesion layer because its silane moieties could covalently bind to the glass surface through siloxane bonds, while the thiol groups attach strongly to the evaporated gold coating through the well-known Au-S binding (Fig. 1.2). More importantly, MPTMS could improve the optical properties of gold nanostructures on glass and thus optimize the SERS performance.



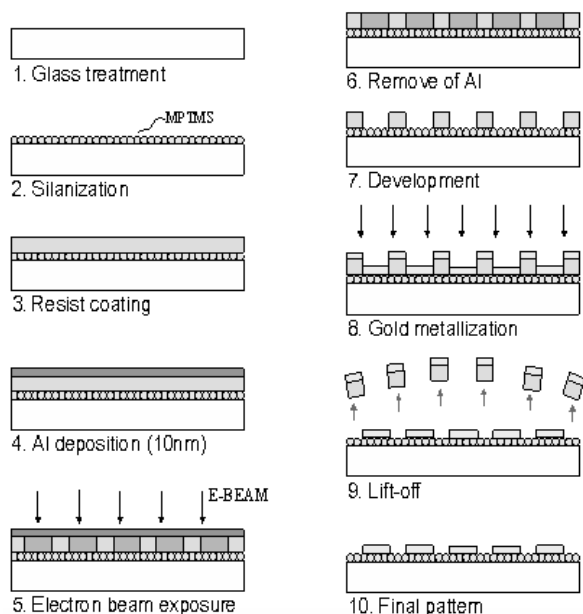
**Figure 1.2** Molecular structure of MPTMS.

Nanoscratching tests performed on nanostripes (data not shown here) proved that the MPTMS layer has similar mechanical properties as the Cr one and that MPTMS could actually act as a molecular adhesion layer sufficient for gold nanostructures on glass for sensing applications. Gold nanoparticles do not stick to glass and are removed by a soft water flush. With the MPTMS adhesion layer, nanoscratch proved that mechanical robustness of the adhesion layer is stronger than the adhesion between the glass and the gold. It is necessary to make trenches in glass to remove the adhesion layer [22].

### 1.2.2.2 Introducing MPTMS to the EBL process as an adhesion layer between glass and Au nanostructures

Enlightened by the method of immobilizing colloidal particles on a glass surface, [23] we introduce MPTMS to the EBL process as a molecular adhesion layer between the glass surface and gold nanostructures. The scheme of such an EBL process is shown in Fig. 1.3. The glass functionalization with MPTMS was processed according to the method proposed by Charles A. Goss [24] with modifications. Briefly, glass slides were immersed in a freshly prepared “piranha” solution (1:3 H<sub>2</sub>O<sub>2</sub> 30%:H<sub>2</sub>SO<sub>4</sub> 98%) for 30 minutes and then rinsed with distilled water, dried under a nitrogen stream, and then placed on a hotplate at 100°C for about 10 minutes. After this procedure the glass surfaces were terminated by hydroxyl groups, -OH. Consequently, the silanization solution was prepared by adding 2 mL MPTMS and 2 mL H<sub>2</sub>O to 80 mL of 2-propanol. Then the pretreated glass slides were immersed in the mixture, which was heated to boiling. After the solution boiled, the glass slides were

kept in the solution for an additional 10 minutes, and then they were carefully rinsed with enough 2-propanol, blow-dried under a nitrogen stream, and then cured at 110°C for 8 minutes. This procedure was repeated three times. With the MPTMS molecules covalently bound to the surface, the functionalized glass was coated with a high-resolution resist (polymethyl methacrylate [PMMA]) and then prebaked at a temperature of 170°C for about 15 minutes. To get good quality of EBL on the nonconductive glass surface, a layer of 10 nm aluminum was evaporated on the top of the resist. Then the sample was loaded in the SEM chamber to make the electron beam “expose” the designed patterns with expected nanostructures. After exposure, the patterns were developed using methyl-isobutyl-ketone (MIBK):isopropyl alcohol (IPA) (1:3), and then an accurate thickness of gold was evaporated on the top. After the lift-off process, we finally got the expected Au nanostructures well adhered to the glass surface with the MPTMS as the adhesion layer.



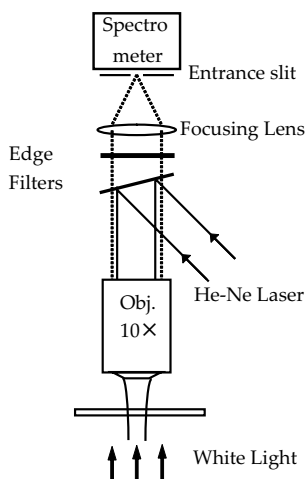
**Figure 1.3** Schematic presentation of the EBL fabrication process with MPTMS. (1) Pretreatment of glass, (2) silanization with MPTMS, (3) resist coating and prebaking, (4) Al deposition, (5) exposure with the electron beam, (6) removal of the Al layer, (7) development, (8) metallization, (9) lift-off, and (10) the final pattern with MPTMS as the adhesive.



### 1.2.2.3 Improved LSPR properties and SERS sensitivity using MPTMS as a molecule adhesive

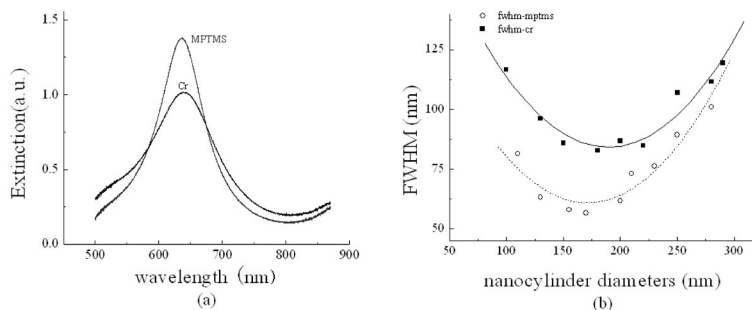
As confirmed by scratching tests MPTMS has similar mechanical robustness as the Cr adhesion layer, and it is even worthy to mention that only MPTMS can improve the optical properties of gold nanostructures on glass. Here let us focus on the improved LSPR properties and SERS sensitivity of Au nanocylinders by using MPTMS as an adhesion layer.

In all the patterns, the gap between the particles has been kept constant around 200 nm since this distance is large enough to avoid any near-field coupling. Extinction and SERS measurements were performed with a Jobin-Yvon micro-Raman spectrophotometer (Labram) (Fig. 1.4). The extinction spectra were recorded in transmission configuration with a  $\times 10$  objective (NA = 0.25) by removing the edge filters. To achieve SERS measurements, the substrates were immersed in a  $10^{-3}$  M solution of BPE for one hour and dried with nitrogen. Raman measurements were carried out with the 632.8 nm line of a He-Ne laser, and Raman spectroscopy was recorded with a  $\times 100$  objective (NA = 0.90) in backscattering geometry.



**Figure 1.4** Experimental setup for extinction and SERS measurements. The extinction spectrum is recorded in a transmission configuration with a  $\times 10$  objective (NA = 0.25) by removing the edge filters. Raman spectroscopy is recorded with a  $\times 100$  objective (NA = 0.90) in backscattering geometry.

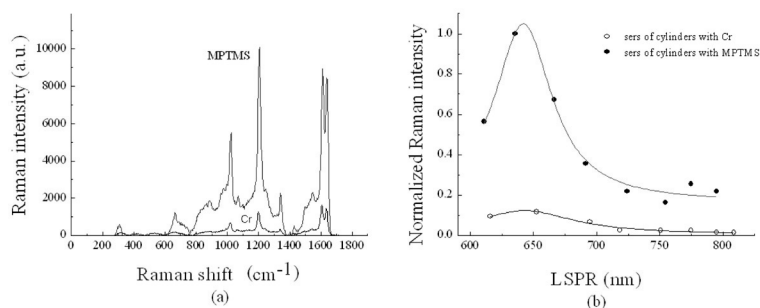
The extinction measurements revealed obvious optimized LSPR properties of Au nanocylinders with MPTMS as the adhesion layer—compared with the broad resonance of Au nanocylinders with a Cr layer, a much thinner LSPR line width and a significant increase of peak intensity were observed in the same-sized Au nanocylinders (Fig. 1.5a). Moreover, as shown in Fig. 1.5b, a decrease of the LSPR line width (full width at half maximum [FWHM]) of about 25% was observed in Au nanocylinders with the MPTMS layer for all diameters. The decrease of line width is a competitive advantage since the near-field enhancement factor  $f$  is inversely proportional to the LSPR width [25, 26]. A thinner LSPR width presents a larger quality factor and a better resonance efficiency of the metallic nanostructures.



**Figure 1.5** Extinction spectra of Au nanocylinders (130 nm) with Cr and MPTMS as the adhesive layer. (a) FWHM of the LSPR spectra versus cylinder diameters for Cr (black squares) and MPTMS (white squares) layers. (b) The fits are represented to guide the eyes.

The decrease of the LSPR line width indicates a higher local field enhancement that predicts improved sensitivity of all SESs that are based on electromagnetic field enhancements around the metallic nanostructures. As given in the BPE SERS spectra obtained with nanocylinders of 130 nm diameter (Fig. 1.6a), the Raman intensity was 1 order of magnitude stronger for the nanocylinders with MPTMS as the adhesion layer than that with a Cr layer. It is worthy to mention that SERS signal enhancement with MPTMS compared to the Cr layer is observed for all LSPR positions (Fig. 1.6b). This means that the improvement of SERS is then intrinsic to the change of the adhesion layer but not an effect of the LSPR position. Also shown

in Fig. 1.6b, the Raman intensity (BPE band at  $1200\text{ cm}^{-1}$ ) versus the LSPR position shows the same trend for the Au nanocylinders with Cr and MPTMS as the adhesion layer, respectively—each gave a maximum of Raman signal at about 645 nm, exactly between the excitation wavelength (632.8 nm) and the Raman wavelength (685 nm). Such results are in good agreement with Wokaun's model and our previous work [18, 27], as mentioned in Section 1.2.1. As it is well accepted that the SERS process could be optimized by tuning LSPR at a proper position, results shown in Fig. 1.6 demonstrated that the SERS signal could be further amplified by using MPTMS as the adhesion layer instead of Cr. We attribute the improvements of SERS sensitivity mainly to the optimization of LSPR by using MPTMS as the adhesion layer and more especially to a thinner line width of LSPR, inducing a higher near-field enhancement.



**Figure 1.6** Raman spectra with Au nanocylinder of 130 nm using Cr (black) and MPTMS (gray) as the adhesive layer. (a) SERS intensity (BPE  $1200\text{ cm}^{-1}$ ) versus the LSPR position for Au nanocylinders with Cr (circle) and MPTMS (dot) as the adhesion layer. (b) The solidlines are the Lorentz fitting of the measured data.

Besides the electromagnetic mechanism, other factors may also have effects on SERS efficiency, such as the chemical interactions between Raman molecules and a metal surface. When Cr was used as the adhesion layer, it could diffuse along the grain boundaries to the metal surface. Such interdiffusion or alloy formation between Cr and Au may change the surface properties of metallic particles, which could also restrain the SERS signal [28]. Such negative effects induced by Cr could be avoided by using MPTMS as the adhesion layer; thus further optimization of SERS could be achieved.

## 1.3 Easier for Measurements: Apolar Plasmonic Nanostructures

### 1.3.1 Limitation from SERS Sensors with Polarization Dependence

As discussed in Section 1.2, the excitation of the LSPR of metallic nanostructures plays a key role in the SERS process. As a consequence, the SERS signal will strongly depend on the excitation polarization that greatly controls the excitation of LSPR around the metallic nanostructures. For example, for SERS measurements with nanoellipses, intense Raman signals could only be obtained when the excitation polarization is parallel to the major axis of nanoellipses [18]. The strong dependence on the excitation polarization brings many limitations to the practical applications of SERS sensors. It is necessary to precisely put the SERS substrate in the right orientation with regard to the polarization of the excitation laser. Good management of polarization in the measurements setup and professional training for the operator will be required. Cylinder- (or spherical)-shaped nanoparticles showed an independent optical response on the excitation polarization. However, in such structures the tuning of LSPR is not so “efficient.” For example, getting a plasmon resonance beyond 700 nm requires the diameters of Au nanocylinders to be larger than 150 nm, excluding the efficient lightning rod effect, which is also an important enhancement factor for the local electromagnetic field. In fact, it is not easy to get the best LSPR position, the most effective local field enhancement, and an apolar response in a single nanostructure scheme. Here we will review our recent work on the design and fabrication of one kind of complex nanostructure that shows apolarity as well as effective near-field enhancement. The apolar behaviors of nanostructures are experimentally confirmed by extinction and SERS measurements, as given in the following sections.

### 1.3.2 Apolar Behavior of Nanoparticle with $C_n$ Symmetry

With group theory, one can demonstrate that any complex nanoparticle with  $C_n$  symmetry (with  $n \geq 3$ ) has an optical response