Guest Forum

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New Developments in Infrared and Raman Spectroscopy
– Principles and Applications of Surface-Enhanced Raman Scattering –

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This lecture focuses on the Raman spectral measurement of a single molecule using Surface-Enhanced Raman Scattering (SERS), as an example of a new development of infrared and Raman spectroscopy. In particular, the relation between the Surface Plasmon Resonance (SPR) and SERS will be reported.

Introduction

Raman spectral measurements can be performed from a single molecule using Surface-Enhanced Raman Scattering. Given the reports regarding hemoglobin applications among others, it would not be an exaggeration to say that this method will become the ideal high-sensitivity analytical technique of the future, allowing more high-sensitive analyses based on SERS. Since the success of single-molecule SERS measurements, rapid progress has been made in elucidating the mechanism of SERS. In connection, we are beginning to understand how to measure the SERS spectrum with higher sensitivity and with better reproducibility. One can say that we're a step closer to the practical application of SERS.

Features and Advantages of SERS

SERS has the following features:
- SERS takes place on silver, gold, platinum, and nickel surfaces; enhancement is most profound on silver and gold surfaces.
- Some molecules show a significant increase in Raman scattering intensity by SERS, and others do not.
- SERS intensity depends on the molecule orientation adsorbed on the metal surface. It is also dependent on the distance from the metal surface.
- Roughness of the metal surface is somehow involved with manifestation of SERS.
- SERS intensity shows dependence on the excitation

wavelength.

Before we discuss the reasons behind such features, a summary of its benefits and advantages:

Advantages of SERS:

- Very high sensitivity (a typical Raman scattering intensity increase of 10^3-10^6 can be expected).
- High selectivity. There are two meanings to "selectivity." One is that only the adsorbed molecules on a metal surface indicate the SERS effect. The other is that the Raman band of adsorbed molecules in specific regions indicates particularly strong SERS effect.
- Because the fluorescence of molecules adsorbed on metal surfaces become quenched, the fluorescent background that appear in SERS spectra loses its intensity, and so the Raman band that was hidden by the fluorescence become easier to observe.

An advantage of SERS as a surface and interface approach, unlike other methods that require high vacuum, is that it can be applied in situ to the interface between liquid phase and solid phase.

Because of these benefits and advantages, SERS, from its early research stages, has been used to study the catalytic action on metal surfaces and the chemical reaction mechanism on electrode surfaces.

Single Molecule SERS (Raman Spectrum Measurement from a Single Molecule)

Kneipp group measured the Raman spectrum of a single crystal violet molecule adsorbed on silver colloid with excitation wavelength of 830 nm, while Nie group measured the Raman spectrum of a single rhodamine 6G (R6G) molecule also on a silver colloid system, using excitation wavelength of 514.5 nm. It was confirmed that Raman scattering from R6G is definitely from the single molecule, by measuring the Raman spectrum from a single silver nanoparticle (assuming that a ratio of R6G to a silver nanoparticle is 1:1).

Features of Single Molecule SERS Spectrum

A single molecule SERS spectrum has the following unique features. Figure 1 shows an example of SERS spectrum measurement of a single molecule (R6G) indicating those features.

- Raman spectrum varies with time, Raman band shifts, and relative intensity of the band changes.
- Raman bands "blink". In other words, the overall Raman scattering intensity increases and decreases.
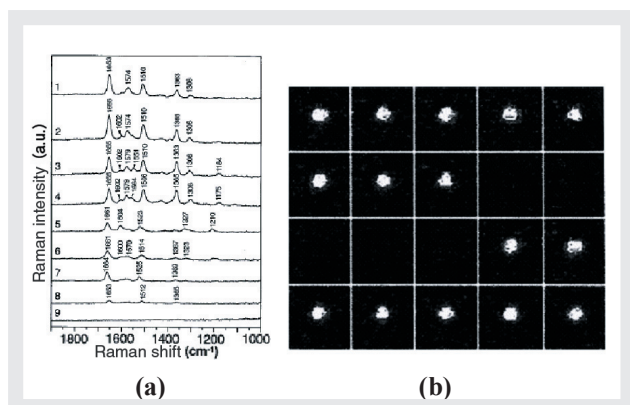

Figure 1 An Example of Single Molecule (R6G) SERS Spectrum Measurement
(a) Time-varying single molecule (R6G) SERS Spectra (measured with each second)
(b) Single molecule (R6G) SERS spectrum "blinking"

Electromagnetic Mechanism of SERS

Figure 2 shows the TEM images of SERS-active silver nanoparticle aggregate. The silver nanoparticle, depending on the size and shape, shows a strong resonance from the visible range to the near infrared range. The resonance is caused by collective oscillation of conduction electrons within the silver particle, which is referred to as Localized Surface Plasmon Resonance (LSPR) or simply as Surface Plasmon Resonance (SPR). The periphery of the silver

nanoparticle is covered in a strong electric field. If two silver nanoparticles come closer to each other, a very strong, augmented electric field is generated at the contact point. If one or more molecules become adsorbed on the contact point, a profound SERS effect occurs. Figure 3 illustrates this concept schematically.
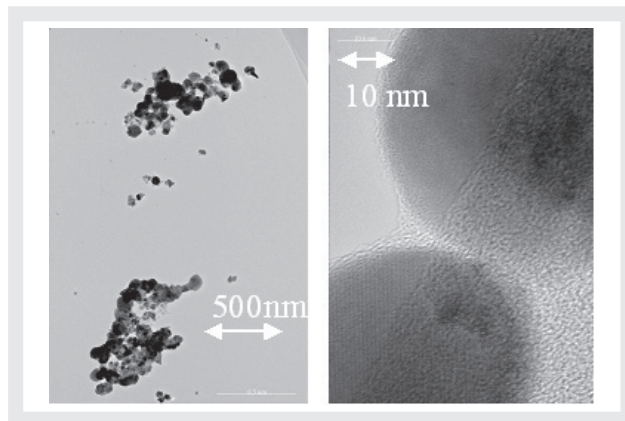

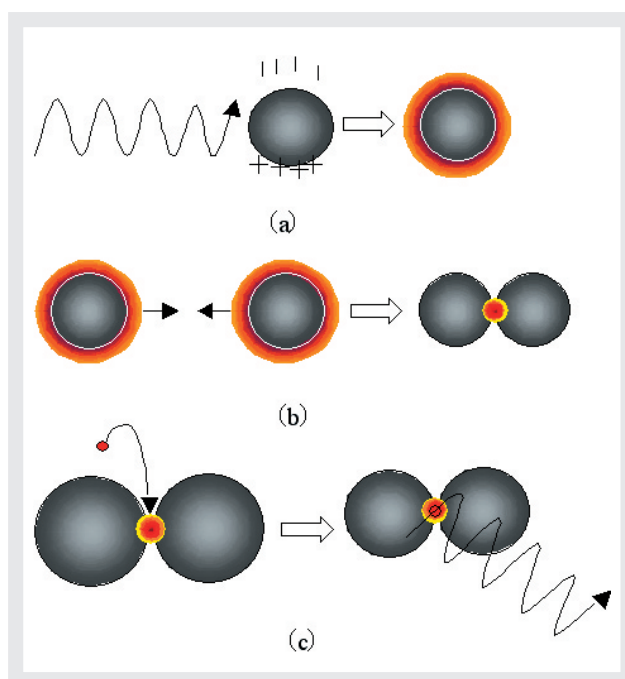Figure 2 TEM Images of SERS-Active Silver Nanoparticle Aggregate

Figure 3 The Concept of SERS Mechanism (Schematic)
(a) Light hitting a nanoparticle provokes oscillation of conduction electrons within the nanoparticle, so that it becomes covered in a strong electric field.
(b) A very strong augmented electric field is generated at the contact point of two particles shrouded by strong electric fields.
(c) When a molecule is adsorbed at the contact point, SERS is generated.

A classical electromagnetic calculation reveals that a strong augmented electric field is generated at the contact point. The augmented electric field generated between the two adjacent silver nanoparticles (diameters 60 nm and with a gap of 1 nm) was calculated using the Finite Difference Time Domain (FDTD) method. The enhanced electric field corresponds to an increase of the Raman scattering cross section by 10^8-10^{10} times, which

well explains the enhancement of the Raman scattering intensity by SERS. In the Raman scattering measurement of a single molecule, the cross-sectional enhancement is 10^{14}-10^{15} times. The difference between 10^{14}-10^{15} and 10^{8}-10^{10} can be explained by the difference between enhancement of the resonance Raman Effect (10^3-10^4 times) and the charge transfer (CT) effect (up to 10^3 times). Another point is that the enhanced electric field shows strong dependence on polarized light (Figure 4), since with the two silver nanoparticles coming closer to each other, SPR splits into the long axis and short axis directions of the two particles.
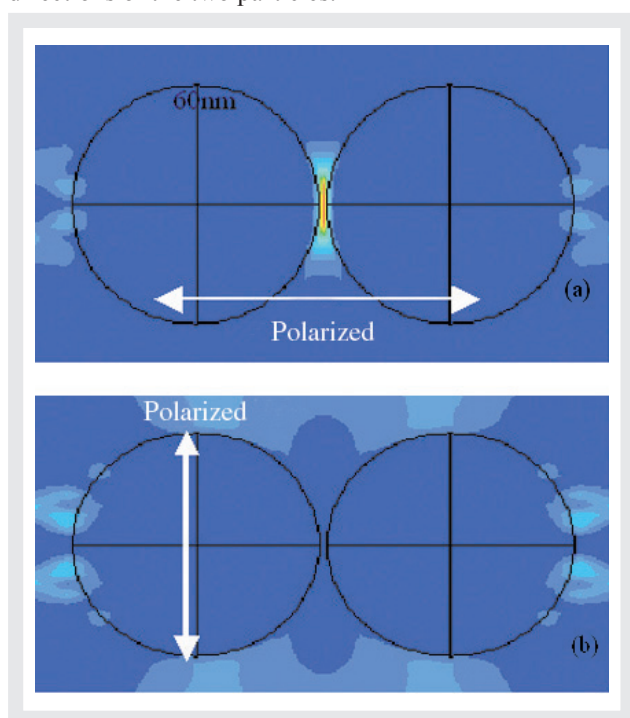

Figure 4 Augmented Electric Field Generated between Two Adjacent Silver Nanoparticles Calculated by the FDTD Method
   (a) A strong augmented electric field is generated when the direction of polarized light is in the same direction as the long axis of the two particles.
   (b) Almost no electric field is generated when the direction of polarized light is in the same direction as the short axis of the two particles.

SPR/SERS Microspectroscopic System

On the premise that SERS should have the same strong polarization properties as SPR, Itoh et al. from our laboratory, set up a system (Figure 5) where both SPR and SERS spectra can be measured on the same single nanoparticle system, and found that both spectra have the same polarization properties[1]-[5]. The system is so designed that SPR exciting light and SERS exciting light irradiate the same location, and in addition, a pinhole is set at the point where the light from the specimen converges to focus into an image, so that SPR and SERS

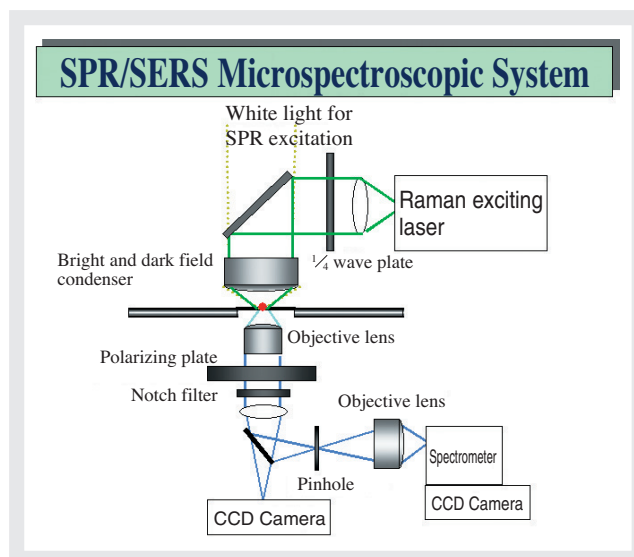spectra can be measured only for a silver particle system of about several hundred nanometers.

Figure 5 SPR/SERS Measuring Apparatus for a Single Silver Nanoparticle

Figure 6 shows the procedure for preparing samples of the SERS-active nanoparticle agglomerate.

One of the excellent features of this spectroscopic system is that SPR and SERS measurements can be performed on the same single metal nano-aggregate under the same conditions. A second feature is that measuring scattered light from a single metal nanoparticle selectively under the dark field microscope enables simple and easy spectroscopic measurement of a nanoparticle with the size below the diffraction limit, without having to use the near field. This allows for a straightforward understanding of the SPR-SERS relationship. A third feature is that SERS spectroscope can be easily performed in the electron resonance region or in the non-resonance region of molecules with multi-wavelength.

Polarization Angle Dependence of Single Silver Nanoparticle Aggregate for SERS and SPR Bands

Figure 7 shows the polarization angle dependence of silver nanoparticle aggregate for SERS and SPR. The polarization angle dependence was measured by rotating the polarizing plate in Figure 5.
As shown in Figure 7(c), the polarization angle dependence curves for the SERS and SPR bands match each other well. This indicates that the optical anisotropy of the SPR band has induced that of SERS's. In addition, the SPR band that synchronizes with the SERS band always was caused by the long axis of the silver nanoparticle aggregate (the contact point direction of the
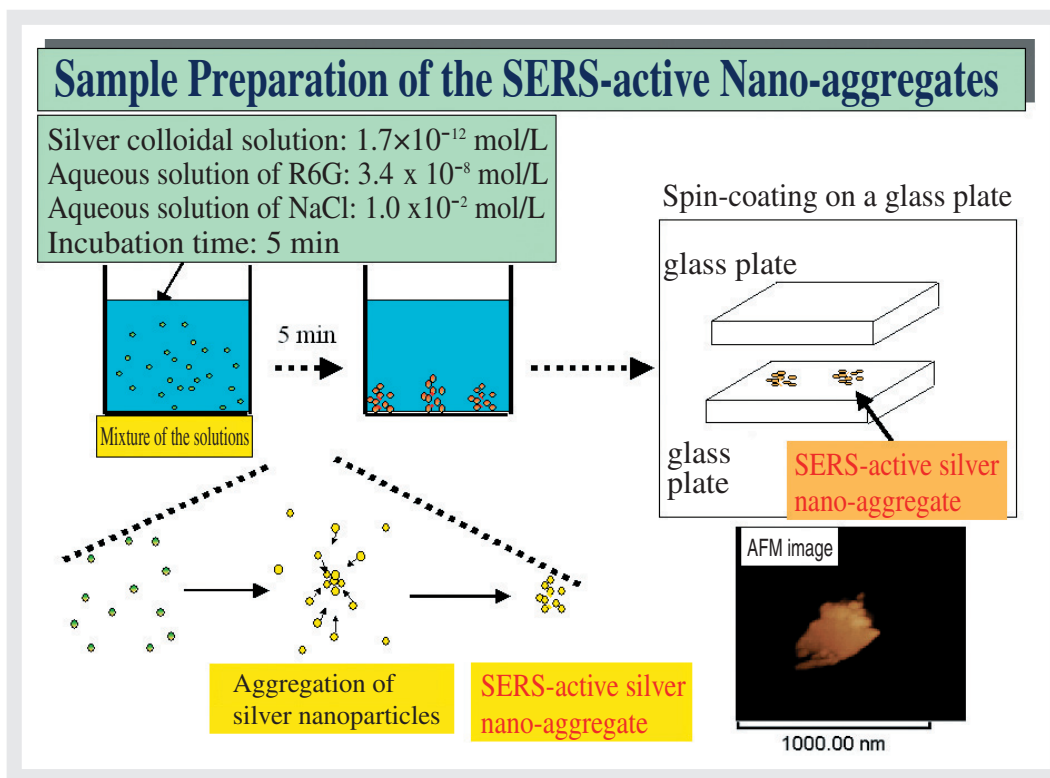
Sample Preparation of the SERS-active Nano-aggregates

Silver colloidal solution: 1.7×10^{-12} mol/L
Aqueous solution of R6G: 3.4×10^{-8} mol/L
Aqueous solution of NaCl: 1.0×10^{-2} mol/L
Incubation time: 5 min

Spin-coating on a glass plate

glass plate

5 min

Mixture of the solutions

glass plate

SERS-active silver nano-aggregate

AFM image

1000.00 nm

Aggregation of silver nanoparticles

SERS-active silver nano-aggregate

Figure 6 Sample Preparation of the SERS-active Nano-aggregates
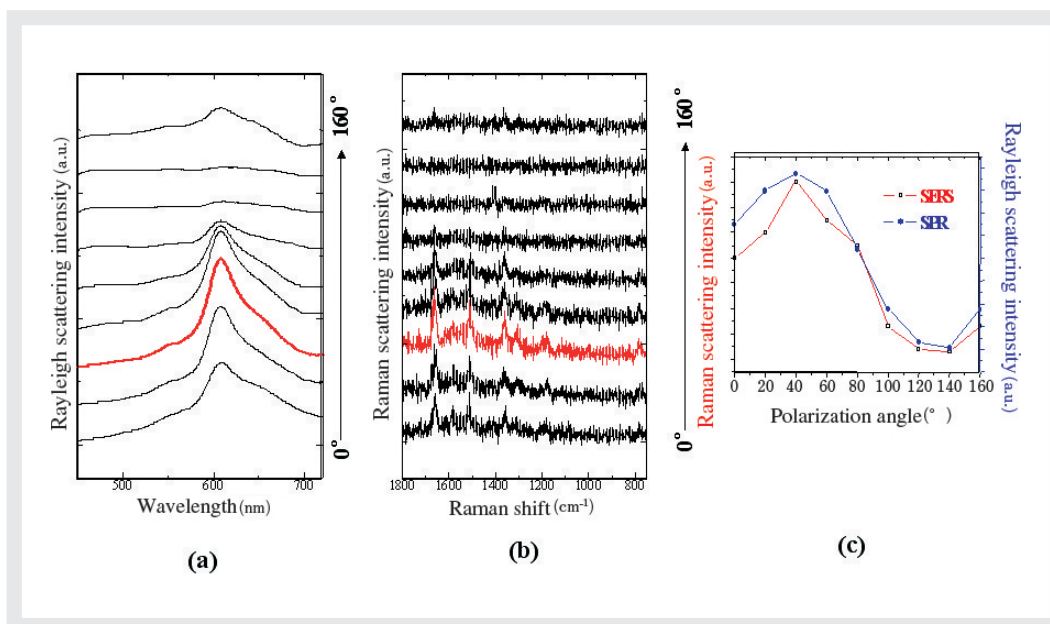
(a)

(b)

(c)

Figure 7 Polarization Angle Dependence of the Single Silver Nano-aggregate
　　　　(a) SPR band
　　　　(b) SERS band
　　　　(c) Polarization angle dependence of the peak intensity for SPR band (blue curve) of 680 nm and that for SERS band (red curve) of 1660 cm^{-1}

aggregate). This result strongly supports the model that R6G molecules adsorbed at the aggregate contact point of the silver nanoparticles interact with the enhanced electric field to generate SERS.

SERS/CT Emission Intensities and SPR

Unlike regular Raman scattering, broadband emissions always overlap in SERS. This emission is considered an emission transition from CT level formed by the adsorbed molecules, or a normal emission that is combined with SPR and then enhanced. It is not yet known which causes this emission, but some groups have presented results indicating CT emission, so we assume CT emission in the following discussion. Recently, we selected the single nanoparticle aggregate with SPR in the dipole mode and tried to compare SERS and CT emission intensities and the resonance Q-values, to examine generation mechanism of SERS.

Polarization Angle Dependence

Figure 8(a) shows SPR Rayleigh scattered light spectrum and SERS and CT emission spectra. Two SPR bands having peak wavelengths of 650 nm and 450 nm, respectively, are seen in the SPR Rayleigh scattered light spectrum. Figure 8(b) shows the polarization angle dependence of SPR band intensities that appeared on the short and long wavelength sides. The both curves fit

each other with $\cos(2\theta)$ and each phase angle differs by 90°. This result indicates that each band is the SPR bands corresponding to short axis and long axis of the aggregate, respectively. Figure 8(c) shows the SPR band of the aggregate's long axis and polarization angle dependence of SERS and CT emission intensities. It is evident that this SPR band has the same polarization properties as SERS or CT emissions. This result indicates that both SERS and CT emissions combine with SPR (that is corresponding to the long axis) and are enhanced.

Resonance Q-Value of the SPR Band and SERS/CT Emission Intensities

We studied the correlation of Resonance Q-value (Q-value = Eres /G, where Eres: peak energy, 1/G: relaxation time, and G: line width of the SPR band) of the SPR band with SERS intensity and CT emission intensity. With increasing Q-value, radiation deactivation efficiency also increases against no radiation deactivation efficiency, and SERS-generation capability increases as well. Figure 9(a) shows the resonance Q-values of SPR bands on the long and short axis side. For the same aggregate, the Q-values on the long axis side are always larger than those on the short axis side. This is considered to be the reason why SPR and SERS on the long axis side combine efficiently, and their polarization angle dependence levels are equal. Further, Figure 9(b) and Figure 9(c) show the relation of the Q-value to the 4th power with the CT emission
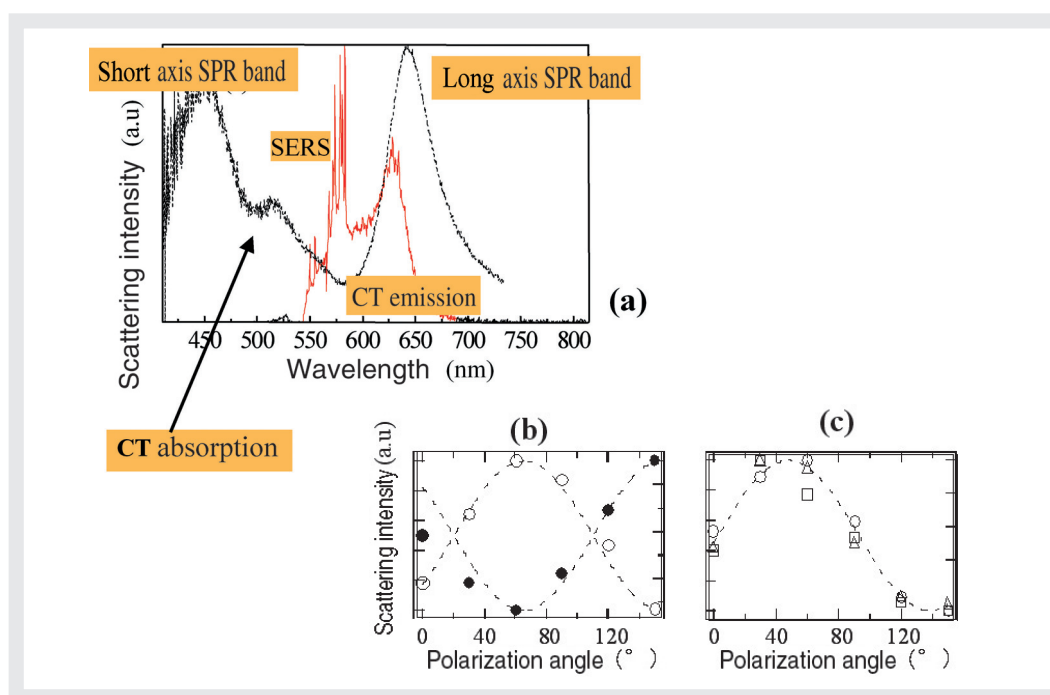

Figure 8 SERS/CT Emission Intensities and SPR
(a) SPR Rayleigh scattered light spectrum (black), and SERS and CT emission spectrum (red)
(b) Polarization angle dependence of the long axis SPR band (○) and the short axis SPR band (●)
(c) Polarization angle dependence of the long axis SPR band (○), and SERS (△) and CT emission (□)
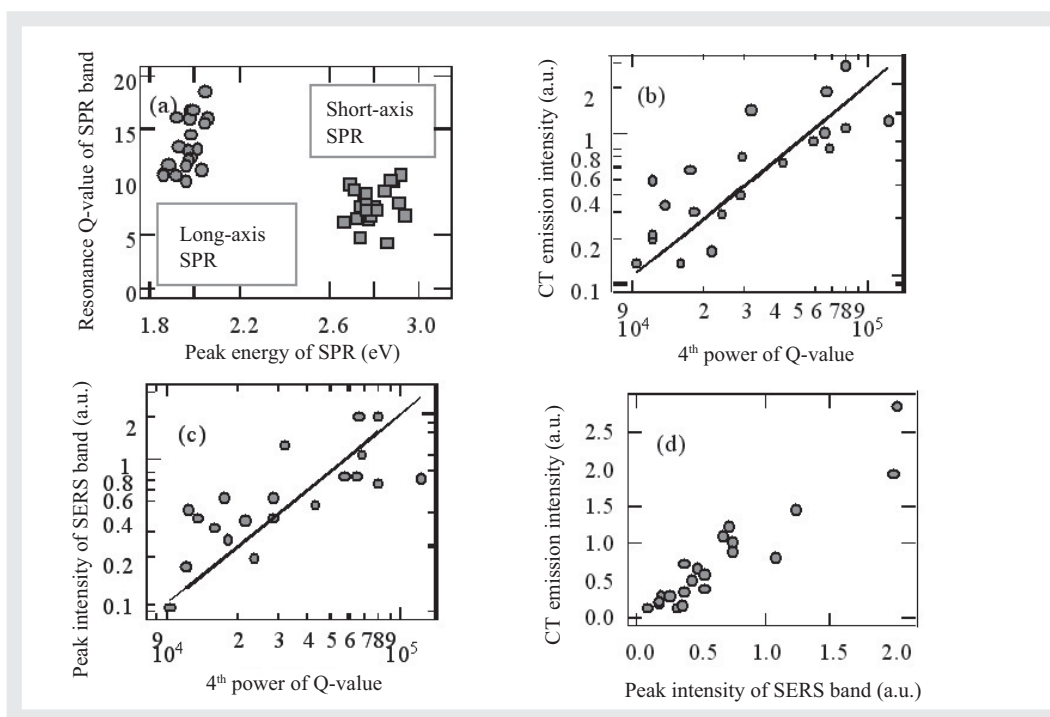
Figure 9 Resonance Q-value of the SPR Band, and SERS and CT Emission Intensity
(a) Resonance Q-value of the SPR band
(b) Relation between CT emission intensity and SPR band resonance Q-value
(c) Relation between SERS intensity and SPR band resonance Q-value
(d) Relation between SERS intensity and CT emission intensity

intensity and SERS intensity; SERS intensity is expected to be proportional to the resonance Q-value to the 4th power from the conventional SPR models. It clearly shows that both have positive correlation with the Q-value to the 4th power. In addition, Figure 9(d) shows that the CT emission intensity is proportional to the SERS intensity. This indicates that both have the same source. From these results, it can be said that SERS is: The CT resonance Raman scattering that is enhanced by the high intensity electric field generated from the aggregate, combined with SPR and radiated. The result further indicates that not only the local region where the augmented electric field causing SERS exists, but also that the entire structure of the silver nanoparticle aggregate that gives the high Q-value are essential to achieve high efficiency in molecule analysis using SERS.

Conclusion

Infrared and Raman spectroscopy are very old methods of spectroscopy; the infrared method has been in use since the 19th century. Nevertheless, the spectroscopy has always been very important and have been state-of-the-art throughout the ages. In particular, infrared and Raman spectroscopy apparatuses have evolved greatly, ranging from the light sources to detectors. Effective use of these will help in the studies of the most advanced sciences. What has been discussed is just one of the examples.

<Excerpt from the Screening Committee Lecture for Masao Horiba Awards, May 10, 2005 (Publication members have responsibility for the translation)>

Reference

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