Single Molecule Detection Using Surface-Enhanced Raman Scattering (SERS)

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By exploiting the extremely large effective cross sections (10^{-17}–10^{-16} cm^2/molecule) available from surface-enhanced Raman scattering (SERS), we achieved the first observation of single molecule Raman scattering. Measured spectra of a single crystal violet molecule in aqueous colloidal silver solution using one second collection time and about 2×10^5 W/cm^2 nonresonant near-infrared excitation show a clear "fingerprint" of its Raman features between 700 and 1700 cm^{-1}. Spectra observed in a time sequence for an average of 0.6 dye molecule in the probed volume exhibited the expected Poisson distribution for actually measuring 0, 1, 2, or 3 molecules. [S0031-9007(97)02524-6]

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Detecting single molecules in solution with high sensitivity and molecular specificity is of great scientific and practical interest in many fields such as chemistry, biology, medicine, pharmacology, and environmental science [1,2]. For example, progress in human gene analysis will be greatly enhanced by methods for selective and rapid detection of single molecules [2]. One-photon [1–10]—and two-photon [11]—excited fluorescence are already useful techniques for single molecule detection.

Surface-enhanced Raman scattering (SERS) is a useful technique resulting in strongly increased Raman signals from molecules which have been attached to nanometer sized metallic structures. It is generally agreed that more than one effect contributes to the observed large effective SERS cross section. The enhancement mechanisms are roughly divided into electromagnetic and chemical effects [12,13]. The electromagnetic enhancement factor ${}^{\mathrm{em}}G_{\mathrm{SERS}}$ arises from enhanced optical fields due to excitation of electromagnetic resonances in the metallic structures. To a first approximation, ${}^{\mathrm{em}}G_{\mathrm{SERS}}$ can be expressed by the fourth power of the ratio of the total electric field $E(r_m, \nu)$ at the molecule location r_m to the incident excitation field $E_{\mathrm{inc}}(\nu)$ where ν is the laser frequency [14]

$$ {}^{\mathrm{em}}G_{\mathrm{SERS}}(r_m, \nu) = \left| \frac{E(r_m, \nu)}{E_{\mathrm{inc}}(\nu)} \right|^4 . \qquad (1) $$

Chemical SERS enhancement results from a metal electron-mediated resonance Raman effect via a charge transfer intermediate state [15] which takes place at so-called "active sites." However, a full understanding of SERS and quantitative estimate of total enhancement factors is not available.

Recently, we observed extremely large Raman cross sections on the order of 10^{-17} to 10^{-16} cm^2 per molecule in SERS experiments using near-infrared (NIR) excitation for dyes adsorbed on colloidal silver [16]. These enormous cross sections provided the hope of using SERS for single molecule detection as a complementary method to fluorescence which can offer exciting new aspects: (1) A SERS vibrational spectrum provides a high degree of structural information about the molecule. (2) Because of the shorter vibrational relaxation times compared to electronic relaxation times [17], the number of Raman photons per unit time which can be emitted by a molecule under saturation conditions will be higher than the number of fluorescence photons by about a factor of 10^3. This allows shorter integration times for detecting a molecule or higher rates for counting single molecules [18]. (3) SERS avoids photodecomposition of the probed molecules because the excitation energy is not in resonance with molecular transitions.

This report describes NIR-SERS experiments on crystal violet in colloidal silver solution at the single molecule level. The excitation source was an argon-ion laser pumped cw Ti:sapphire laser operating at 830 nm with a power of about 200 mW at the sample. Dispersion was achieved using a Chromex spectrograph with a deep depletion CCD detector. A water immersion microscope objective (×63, NA 0.9) was brought into direct contact with a 30 μl droplet of sample solution for both excitation and collection of the scattered light. Scattering volume was estimated to be approximately 30 pl [19]. The colloidal solution used in our experiments was prepared by a standard citrate reduction procedure [20]. NaCl was added in $10^{-2}M$ concentration to achieve optimum SERS-enhancement factors [21]. Electron micrographs of the sol taken before the addition of the targeted compound are shown in Ref. [22]. They show that the colloidal solution is slightly aggregated and consists of small 100–150 nm sized clusters. The sol extinction spectrum shows a maximum at about 425 nm [21]. Samples were prepared by adding 5×10^{-13} M crystal violet solution in methanol to this colloidal solution in a volume ratio of 1:15, resulting in a final sample concentration of 3.3×10^{-14} M, which means an average of 0.6 molecules in the probed 30 pl volume. From the total silver in the colloidal solution we estimate the number of individual silver clusters in the scattering volume to be about 100. The ratio of the number of dye molecules (0.6) to the

number of silver clusters (~100) makes it unlikely that more than one dye molecule will be adsorbed on the same cluster. Repeated checking of the extinction spectra of the sample solution during and after SERS measurement time showed no change implying no further aggregation after the addition of crystal violet. Therefore we can assume that we probed individual silver clusters, and that Brownian motion of these silver clusters transports single dye molecules into and out of the scattering volume. The average residence time of a particle in the probed volume can be roughly estimated to be between 10 and 20 seconds [23], which is at least ten times longer than the measurement time. Therefore it is most probable that a "1-molecule-Raman spectrum" is generated by the same molecule and that all properties of the spectrum are a function of the individual properties of this molecule.

Figure 1 shows 100 surface-enhanced Raman scattering (SERS) spectra measured in time sequence from a scattering volume which contains an average of 0.6 crystal violet molecules.

Figure 2(a) displays the peak heights of the 1174 cm^{-1} line for the 100 SERS spectra. Figure 2(b) illustrates the background level of the colloidal solution with no dye present. The horizontal line at 14 counts/s is the mean background signal. Using a common rule of thumb [1], we set the threshold for signal detection to 25 counts/s which is three times the standard variation in the mean background signal. Figure 2(a) shows that about 40 signals measured in the presence of dye molecules meet this criterion.

For comparison, Fig. 2(c) shows an analogous measurement for the 1030 cm^{-1} Raman line of 3M methanol in colloidal silver solution (about 10^{14} molecules of methanol in the scattering volume). We adjusted the methanol concentration to achieve approximately the same count rate for "many" molecules as for a single crystal violet molecule in order to compare statistics at approximately the same signal-to-noise levels (see next paragraph). Previous experimental data showed no indication of any SERS enhancement of the methanol Raman signal [24]. Since there are about 10^{14} times more molecules of methanol than of crystal violet in the scattering volume, the same signal strengths for the methanol Raman line and for the crystal violet SERS line confirm an enhancement factor of about 10^{14} and cross sections on the order of 10^{-17} to 10^{-16} cm^2/molecule, agreeing with the estimate obtained in Ref. [16] from the observed vibrational pumping.

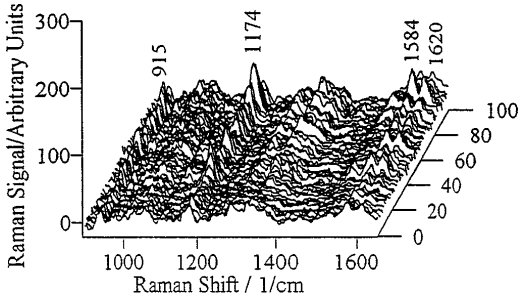

FIG. 1. 100 SERS spectra collected from a 30 pl scattering volume containing an average of 0.6 crystal violet molecules, displayed in the time sequence of measurement. Each spectrum is acquired in 1 second.

Figure 3 presents a statistical analysis of the Raman signals measured in time sequence using 20 bins whose widths are 5% of the maximum of the observed signals (x axis). The y axis displays the frequency of the appearance of the appropriate signal levels of the bin.

Figure 3(a) gives the statistical analysis of 100 "normal" Raman measurements of 10^{14} methanol molecules in the probed volume. As expected, the Raman signal of many methanol molecules shows a Gaussian statistical distribution.

Figure 3(c) displays statistical analysis of 100 SERS measurements (signal of the 1174 cm^{-1} Raman line) of 0.6 crystal violet molecules in the probed volume. In contrast to the Raman signal of many molecules, the statistical distribution of the "0.6 molecules SERS signal" exhibits four relative maxima which are reasonably fit by the superposition of four Gaussian curves [25] whose areas are roughly consistent with a Poisson distribution for an average number of 0.5 molecule. This reflects the

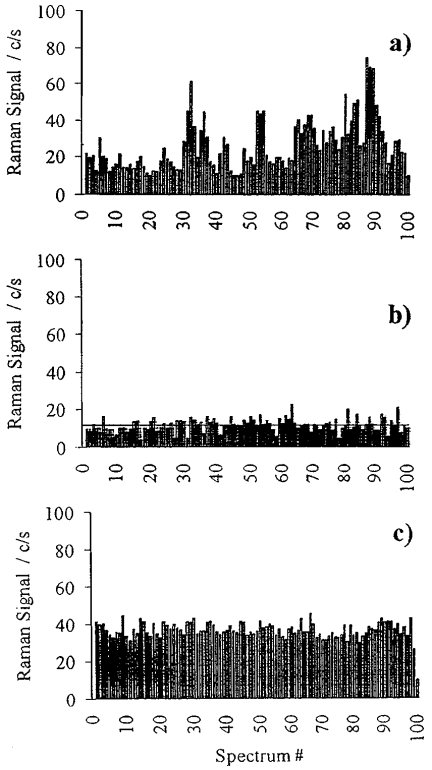

FIG. 2. (a) Peak heights of the 1174 cm^{-1} line for the 100 SERS spectra shown in Fig. 1. (b) Signals measured at 1174 cm^{-1} for 100 spectra from a sample without crystal violet to establish the background. (c) Peak heights of the 1030 cm^{-1} Raman line for 100 spectra measured from 3M methanol, experimental conditions were as before.

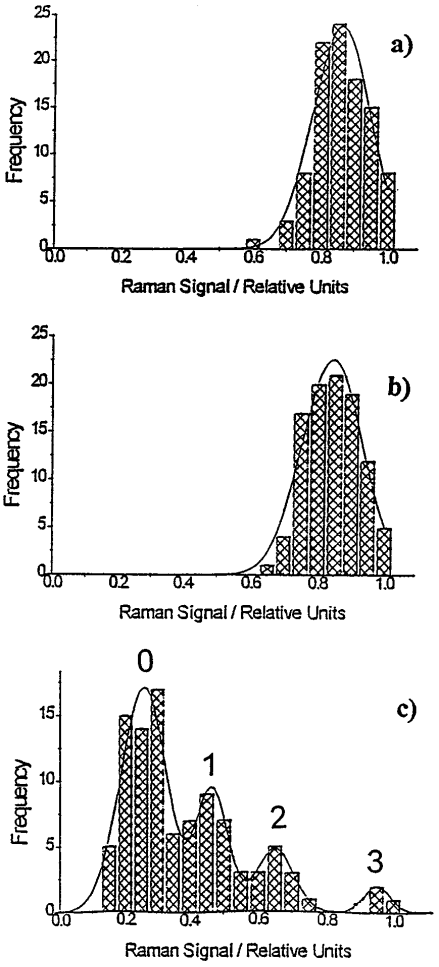

FIG. 3. (a) Statistical analysis of 100 "normal" Raman measurements at 1030 cm^{-1} of 10^{14} methanol molecules. (b) Statistical analysis of 100 SERS measurements (1174 cm^{-1} Raman line) of six crystal violet molecules in the probed volume. The solid lines are Gaussian fits to the data. (c) Statistical analysis of 100 SERS measurements (1174 cm^{-1} Raman line) for an average of 0.6 crystal violet molecules in the probed volume. The peaks reflect the probability to find just 0, 1, 2, or 3 molecules in the scattering volume.

probability to find 0, 1, 2, or 3 molecules in the scattering volume during the actual measurement. Comparing the Poisson fit with the 0.6 molecule concentration/volume estimate we conclude that about 80% of molecules are adsorbed.

Figure 3(b) shows that the characteristic Poisson distribution vanishes and the statistics of the SERS signal becomes more Gaussian if we increase dye concentration by a factor of 10.

The change in the statistical distribution of the Raman signal from Gaussian to Poisson when the average number of dye molecules in the scattering volume is 1 or less is evidence for single molecule detection by SERS.

Our measurements require extremely large SERS cross sections, which we found at NIR excitation, and whose appearance seems to be connected with the existence of 100–150 nm sized silver clusters and with low concentration "NaCl activation" of the colloidal solution. The relatively well "quantized" signals for 1, 2, or 3 molecules suggest relatively uniform enhancement mechanism(s) despite the nonuniform shape and size (~10–40 nm) of the silver particles forming the clusters. The large SERS enhancement can be understood by favorable superposition of a very strong electromagnetic enhancement due to the silver clusters, which is particularly effective at NIR excitation [26] coupled with a strong chemical enhancement [15].

Currently we are studying SERS enhancement factors of various molecules. It is likely that there are relatively many molecules with SERS enhancement factors sufficient for single molecule detection.

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