

# Single Molecule Tip-Enhanced Raman Spectroscopy with Silver Tips

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Received: July 25, 2006; In Final Form: October 19, 2006

We present single molecule tip-enhanced resonance Raman spectra from brilliant cresyl blue (BCB) submonolayers adsorbed on a planar Au surface with Ag tips. A gap of 1 nm between a Ag tip and the Au substrate was employed to create a highly enhanced electric field and to generate Raman scattering from an area of  $\sim 100$  nm<sup>2</sup>. Three lines of evidence are presented to prove the single molecule sensitivity of our experiments: (1) Extremely diluted samples were used. Estimations show that at most a few molecules were excited by the Ag tip. (2) Spectroscopic fluctuations, including intensity fluctuations, frequency shifts, and line shape changes were observed. A histogram analysis of the intensity fluctuations of two different BCB coverages was carried out. The results clearly show the features of single molecule behavior. (3) Discrete signal losses also were observed. This is because of photochemical processes involving single molecules. Besides BCB, which shows a strong resonant absorption at 633 nm (the wavelength of the excitation laser), a self-assembled monolayer of benzenethiol, which does not strongly absorb at 633 nm, was studied. Good quality spectra were recorded with a short exposure time (10 s) and time-dependent spectral changes were also observed.

## Introduction

Tip-enhanced Raman spectroscopy (TERS) is a promising approach for nanoscale chemical analysis.<sup>1</sup> TERS employs a sharp metal tip, which is illuminated from the outside, to create a localized light source and excite the sample on a subwavelength scale. The original idea of this enhanced Raman spectroscopy came from surface-enhanced Raman spectroscopy (SERS), which was discovered 30 years ago<sup>2</sup> and has been well reviewed.<sup>3,4</sup> Classical electromagnetic theory predicts that the enhancement mainly stems from metallic nanostructures such as sharp protrusions or junctions between particles where the electric field can be highly enhanced.<sup>5</sup> On the basis of this idea, sharp metal tips have been suggested for the localized plasmon polaritons to be excited and thus to enhance the Raman scattering of molecules in close proximity.<sup>6</sup> This technique is called tip-enhanced Raman spectroscopy. Two types of TERS setups have been reported so far: TERS depending on the “lightning rod” effect of a sharp tip apex<sup>1,7–9</sup> and TERS based on the gap between a tip and a metal substrate,<sup>10–12</sup> which can give a higher enhancement factor than the former embodiment.<sup>11,13</sup> To date, TERS has been successfully used in the area of nanoscale analysis (i.e., the study of dye molecules),<sup>14</sup> carbon nanotubes,<sup>15</sup> DNA bases,<sup>16</sup> etc.

Studies of TERS not only open the door for nanoscale vibrational spectroscopy, but also could lead to a better understanding of the SERS phenomenon, especially the somewhat mysterious observation of single molecule SERS (SM-SERS). Single molecule SERS experiments were first reported one decade ago.<sup>17,18</sup> Intense studies by a number of groups<sup>19,20</sup> followed and the single molecule sensitivity has been confirmed, but SM-SERS still has problems, such as poor understanding of the origin of the giant enhancement and the strong spectral fluctuations.<sup>21</sup> It is difficult to solve these problems using normal SM-SERS experiments. In most SM-SERS experiments, Ag or

Au colloids were used as the field enhancement media. It is impossible to identify where the molecules stay, where the real “hot spots” are, and how large the enhancement factor is. On the contrary, these problems can be overcome with the TERS scheme in which the sample is much better defined, the location of the analyte can be determined by scanning probe microscopy (SPM), and in particular, the pure electromagnetic component of the enhancement can be unambiguously determined by comparing the result with and without the tip. However, TERS has its own problems: the enhancement factor is much lower than that of SM-SERS. One of the highest TERS enhancement reported so far is  $\sim 10^6$  times.<sup>10</sup> This was observed from the 1 nm gap between an Au tip and a planar Au substrate excited by a 633 nm laser. The sensitivity is already good enough to detect  $\sim 100$  molecules, which do not exhibit additional resonant enhancement, with a reasonable collection time (1 min).<sup>22</sup>

Our motivation is to improve the performance of this gap-mode TERS further, so that a shorter collection time and better sensitivity can be achieved; single molecule sensitivity should be possible. It is well known that an Ag electrode has a higher SERS enhancement than an Au electrode in the visible range, because the imaginary part of its permittivity is much smaller than that of other metals in the visible range<sup>23</sup> and, thus, the damping of the plasmon polaritons is smaller. In this paper, a “gap-mode” TERS setup with Ag tips is presented. Near-field Raman spectra from a benzenethiol (BT) self-assembled monolayer (SAM) on a planar Au surface collected with a short exposure time (10 s) and good spectral resolution (1 cm<sup>-1</sup>) are demonstrated. Furthermore, single molecule tip-enhanced resonance Raman spectra from brilliant cresyl blue (BCB) submonolayers were observed in the junction of an Ag tip and a planar Au substrate.

Just before the submission of this manuscript, Raschke and co-workers published a study that claimed the observation of SM-TERS from malachite green.<sup>24</sup> Their claim is mainly based

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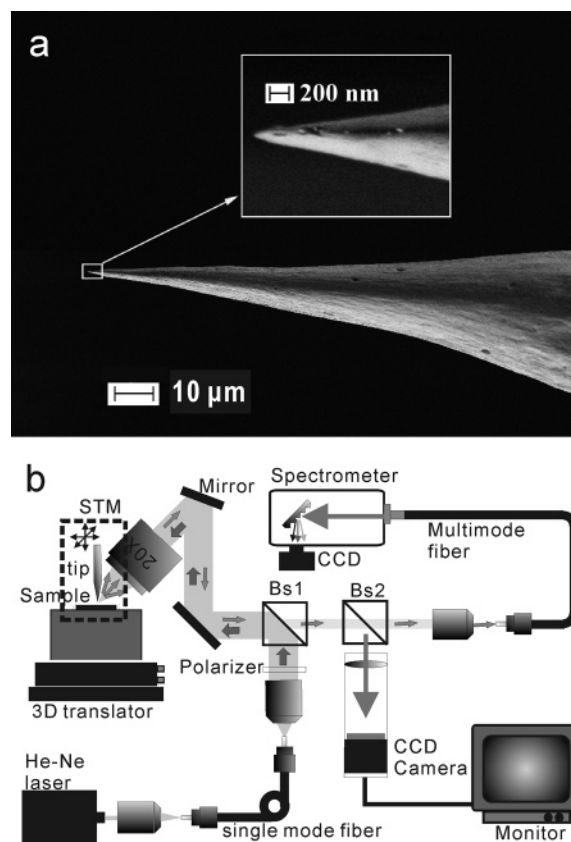
on the statistic analysis of the strongly fluctuating spectra, observed after the Au tip was approached to the sample surface. The electromagnetic enhancement factor reported was  $\sim 10^8$ , much lower than the estimated enhancement factor of  $10^{12}$  in SM-SERS experiments. Also, their spectra are very different from those reported by the Pettinger group.<sup>10</sup> New modes were observed without clear origin. Therefore, whether the signal truly came from single malachite green molecules and why single molecules could be observed at all with this relatively low enhancement is still unclear. In this work, SM-TERS from a different analyte, BCB was studied and an enhancement factor similar to that in ref 24 was found (vide infra). Our results are evaluated using three different criterias that all indicate that single molecule sensitivity can indeed be achieved with this relatively low electromagnetic enhancement in agreement with the conclusion in ref 24. Moreover, a higher spectral resolution ( $\sim 1 \text{ cm}^{-1}$ ) is used in our experiment. It allows us to investigate detailed spectral fluctuations (e.g., changing lineshapes and frequency shifts), which were not visible in ref 24.

### Experimental

Two types of samples were studied in this work: a benzenethiol SAM and BCB submonolayers on a planar Au surface. The benzenethiol SAM was used as a standard sample in our experiment because it is a well-defined system.<sup>25</sup> The gold substrates were made by annealing a 150 nm Au (99.99+%, Aldrich) layer, which was vapor coated onto freshly cleaved mica, with a butane microtorch. The Au substrates were checked with far-field Raman spectroscopy before use and no Raman signal was observed. The BT SAMs were made by soaking the Au substrates in a  $10^{-3} \text{ M}$  BT (99+%, ACROS) ethanolic solution for 3 min and rinsing thoroughly with ethanol afterward.<sup>22</sup> BCB was chosen here because it strongly absorbs the excitation laser (633 nm) that was used in our experiment.<sup>26</sup> The submonolayers were made by spin coating  $2.5 \mu\text{L}$  of diluted BCB (Fluka) ethanolic solutions ( $10^{-4}$  or  $10^{-5} \text{ M}$ ) onto Au layers.

The Ag tips used in our experiment were fabricated by electrochemical etching.<sup>27</sup> A mixture of perchloric acid (70%, Riedel-de Haën) and ethanol with a volume ratio of 1:4 was used as etchant. A gold ring with a diameter of 1 cm was employed as the negative electrode and the Ag wire (99.99+%, Aldrich) with a diameter of 0.25 mm was used as the positive electrode. During etching, a constant voltage of 1.6 V was applied; the part of the Ag wire close to the etchant surface was etched the fastest. When the lower part of the Ag wire dropped off, a sharp tip was formed (Figure 1a). The tips were rinsed with methanol after etching. We found that the tip shape was sensitive to the etching geometry. In our case, a 10 mm length of Ag wire was immersed in the solution, and the Au electrode was positioned just below the surface of the etchant. It also has been reported that the shut-off time of the etching voltage after the lower part of the metal wire drops off determines the sharpness of the tip.<sup>28</sup> Our method was to use short pulses instead of a constant voltage just before the drop off, so that only a small amount of charge passed the tip end after the drop off.

Our TERS setup, as illustrated in Figure 1b, was composed of a homemade micro-Raman system and a commercial STM system (EasyScan, NanoSurf) which was mounted on a translation stage. The excitation laser (633 nm, He-Ne laser, Thorlabs) was guided in by a single mode optical fiber. P polarized light (parallel to the tip axis) was chosen using a polarizer; half of the laser power was reflected by a beam splitter (BS) and

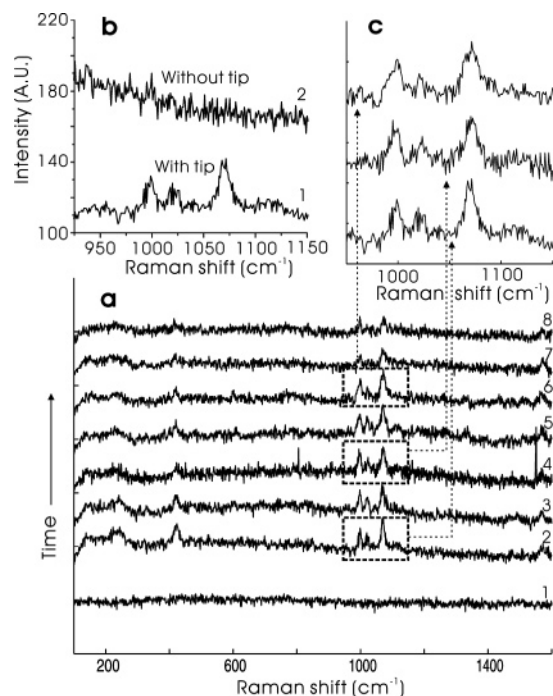


**Figure 1.** SEM images of a typical Ag tip (a). The inset of (a) shows a zoom of the tip apex, which is sharper than 50 nm. The lower part (b) is the schematic diagram of our setup, which is composed of a home-built micro-Raman system and a commercial STM.

focused onto the tip-sample gap by a long working distance microscopic objective (N.A. = 0.35, Olympus) with an angle of  $\sim 45^\circ$  with respect to the tip axis. The scattered light was collected by the same objective and coupled into a Raman spectrograph (Holospec VPT, Kaiser) by a multimode fiber. Spectra were recorded by a liquid nitrogen cooled charge-coupled device (CCD) camera (LN/CCD-2500, Princeton Instruments). We would like to point out that this system is not fully optimized. The two 50–50% BSs decreased the signal by a factor of 4 times and the laser power reaching the sample was only about 0.5 mW. Nevertheless, it will be shown below that the enhancement reached by our gap TERS system was so large that clean spectra could be observed with short exposure times even with this unoptimized system.

### Results and Discussion

Figure 2 shows the tip-enhanced Raman spectra from a BT SAM on the planar Au substrate. They were collected during a tip approach with a 10 s exposure time per line (Figure 2a). At the beginning, when the tip was  $\sim 0.5 \mu\text{m}$  away from the sample, a flat baseline was observed. After the tip was brought into tunneling feedback ( $\sim 1 \text{ nm}$ ), clean peaks from BT appeared. In the spectral range of our instrument, five fingerprint modes of BT molecule are observed. Table 1 gives the assignments<sup>29</sup> showing a comparison between TERS and SERS data. An intensity decrease and a broadening of these modes also are observed (Figure 2c) after long exposure times. To obtain a distinguishable far-field spectrum and to estimate the enhancement factor, a much longer collection time (10 min) was used (Figure 2b), but still no distinguishable peak could be observed. Thus, the only way to estimate the enhancement factor is to



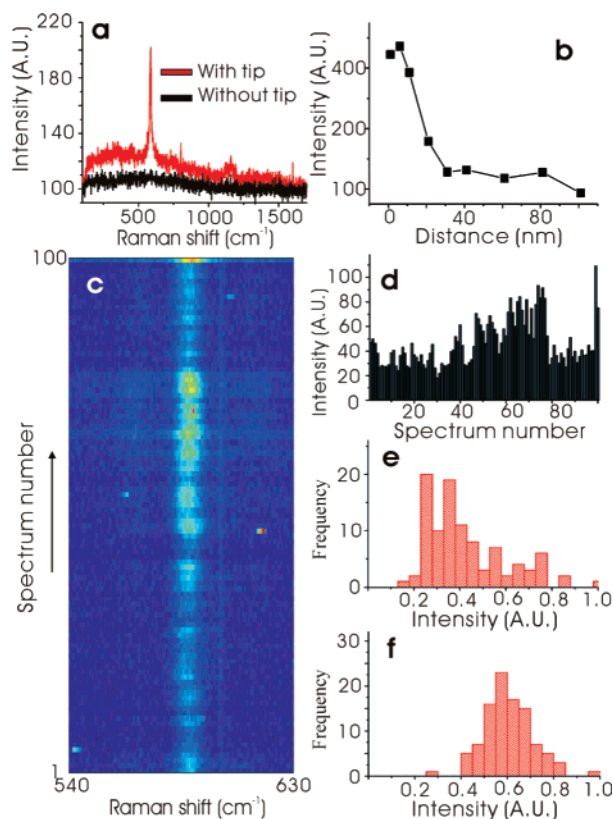
**Figure 2.** Tip-enhanced Raman spectra from a BT SAM on a planar Au surface. Spectra were collected continuously before and after the tip approaching with an exposure time of 10 s per frame (a). Line 1 was collected before the tip was brought onto the sample and lines 2–8 were collected with the tip in tunneling feedback. After the tip approached to the sample, peaks from BT were observed with a 10 s exposure time (line 1 in (b)). Without the tip, no distinguishable peak was observed even with a 10 min exposure time (line 2 of (b)). The intensity of the signal decreased and the line shape also changed with time. Figure (c) shows the details of the modes at  $\sim 1000$   $\text{cm}^{-1}$  corresponding to the parts boxed in (a).

**TABLE 1: Assignment of Benzenethiol Vibrational Modes**

vibrational assignment	frequency ( $\text{cm}^{-1}$ )	
	TERS	SERS <sup>29</sup>
7a(a <sub>1</sub> ), $\nu_{\text{CS}} + \beta_{\text{CCC}}$	424	417
12(a <sub>1</sub> ), $\beta_{\text{CCC}}$	998	999
18a(a <sub>1</sub> ), $\beta_{\text{CH}}$	1020	1022
1(a <sub>1</sub> ), $\beta_{\text{CCC}} + \nu_{\text{CS}}$	1069	1073
8a(a <sub>1</sub> ), $\nu_{\text{CC}}$	1568	1573

assume that the signal was below the level of the background noise. The illuminated area was around  $5 \mu\text{m}^2$ , estimated using a laser scanning image of isolated Ag nanoparticles with the same micro-Raman system. The diameter of the tip-enhanced area can be estimated by the formula  $(2Rd)^{1/2}$ , where  $d$  represents the size of the gap and the  $R$  represents the radius of curvature of the tip end.<sup>26,30</sup> In our case,  $d$  was  $\sim 1$  nm and  $R$  was smaller than 50 nm as estimated from the scanning electron microscopy (SEM) image (Figure 1a). The enhanced area thus was smaller than  $100 \text{ nm}^2$ . The peak intensity in the far-field spectrum obtained with 10 min exposure time was lower than the noise level ( $\sim 10$  counts), and the peak intensity in the near-field spectrum from a 10 s exposure was 30 counts (i.e., the enhancement factor is  $\sim 9 \times 10^6$ ). Here the peak intensity is used to estimate the enhancement factor. A similar estimation based on band integrals would yield a more meaningful enhancement factor. However, this was difficult to do with our far-field spectrum and in any case, an even higher enhancement factor would result.

BCB submonolayers were studied next. As a dye molecule showing strong resonance Raman effects, BCB is often used to demonstrate the performance of TERS.<sup>1,26</sup> Figure 3a shows the



**Figure 3.** Tip-enhanced Raman spectra from BCB submonolayers. The peak at  $585 \text{ cm}^{-1}$  was greatly enhanced after the tip approached the sample surface (a). The collection time was 10 s. To make sure that it is a pure near-field effect, Raman intensity as a function of tip–sample distance was measured (b). The signal is enhanced only if the tip–sample distance is smaller than 30 nm. Time-resolved TERS measurements also have been done. A tip-enhanced spectral series of one hundred exposures from a BCB submonolayer made by spin coating a  $10^{-5}$  M solution on an Au substrate (c) were collected continuously with an exposure time of 5 s per spectrum. The peak intensity  $\sim 585 \text{ cm}^{-1}$  shows a random fluctuation (d). It shows a broad distribution of the intensity. The same experiment was done with a sample made by spin coating a  $10^{-4}$  M BCB solution on an Au substrate, and its histogram of the peak intensity (f) shows a completely different pattern from (e).

comparison of a tip-enhanced Raman spectrum and the corresponding far-field Raman spectrum from a sample made by spin coating  $2.5 \mu\text{L}$  of a  $10^{-5}$  M BCB solution onto an Au substrate with a size of  $\sim 1 \text{ cm}^2$ . A large peak around  $585 \text{ cm}^{-1}$  appeared when the tip was in feedback, whereas no signal was observed when the tip was retracted from the sample. Peaks at  $\sim 720 \text{ cm}^{-1}$  and  $1150 \text{ cm}^{-1}$  also are distinguishable, but their intensities are much smaller than the peak at  $585 \text{ cm}^{-1}$ . This is similar to the results reported by Pettinger et al.<sup>26</sup> An approach curve (Figure 3b) also is recorded to ensure that the enhancement is due to a near-field effect. The data shows that the signal is enhanced only when the tip–sample distance is within 30 nm.

The number of BCB molecules in the “hot” area can be estimated from the average coverage. If we assume a uniform coverage, there were on average 15 BCB molecules in a  $100 \text{ nm}^2$  area, which was the size of the enhanced area according to the discussion above. However, during sample preparation a considerable part of the BCB molecules are lost during spin coating (i.e., the real coverage is significantly lower than this estimation) on the order of only a few molecules in the hot area. Despite the relatively short exposure time (10 s), single molecule sensitivity can be reached by our setup because of the good signal-to-noise ratio. This is also expected from the

good quality spectra obtained for the BT monolayer, where  $\sim 100$  molecules are in the enhanced area ( $\sim 100 \text{ nm}^2$ , assuming that one molecule occupies an area of  $1 \text{ nm}^2$ ). Considering that BT does not absorb at  $633 \text{ nm}$ , whereas BCB absorbs strongly and thus benefits from additional resonance enhancement, single molecule sensitivity of tip-enhanced resonance Raman spectroscopy is possible. Further evidence for this claim is given below.

**Low Surface Coverage.** As described above, only a few BCB molecules were in the “hot” area created by the gap. However, one may argue that the BCB molecules can form clusters or nanocrystals during sample preparation. Thus, the signal observed in our experiment could stem from these (i.e., from a large number of molecules). To rule out this possibility, many tip-enhanced spectra were collected at different positions chosen randomly. The results showed that the samples were homogeneous on the millimeter scale, and only very few locations did not show a large signal intensity. Considering the low overall BCB coverage, there should be large areas without sample molecules if BCB clusters had indeed formed, and a homogeneous average coverage would not be observed.

To further ensure that the signal originates from single molecules rather than clusters, the BCB sample described above was rinsed with ethanol, and tip-enhanced spectra were recorded again at different locations. Decent spectra could still be observed with intensities similar to the ones obtained before rinsing. However, spectra due to BCB were now only found at very few locations; no signal was observed at most other positions. For the rinsed sample, clusters of BCB would be washed away. Also, the coverage of BCB was greatly decreased, because (i) the solubility of BCB in ethanol is good, and (ii) there is no strong chemisorption interaction between BCB and the Au surface. Taken together, these observations prove that the sensitivity of our system has reached the single molecule level.

We also would like to emphasize the fact that the estimation of the surface coverage in TERS experiments is much more precise than in the case of SERS. As pointed out by Le Ru et al.,<sup>31</sup> the surface coverage in SM-SERS experiments with colloids is easily underestimated because of the inaccurate method used for estimating the colloid concentration. In the case of TERS, the estimation is more direct; therefore, it can be used as a satisfactory proof.

**Spectral Fluctuations.** Spectral fluctuations were also observed; they usually serve as an indicator for single molecule behavior. In the past decade, such “blinking” phenomena in single molecule SERS have been studied by different groups and several different mechanisms have been proposed: molecules moving in and out of the laser focal volume (in solution),<sup>17</sup> molecules hopping into and out of the “hot” spot,<sup>32</sup> changes in orientation,<sup>33</sup> fluctuations of charge transfer between the molecule and metal substrate,<sup>34</sup> etc. Although there is still no comprehensive theory for the fluctuation today, it is clear that the intensity of SERS or TERS signals can be influenced by many factors simultaneously. If there is one molecule in the tip substrate junction in our experiment, the signal will depend on its position, orientation, and adsorption geometry. At room temperature, these parameters all change on the time scale of the experiment or faster, because of thermal motion (i.e., the spectrum is expected to show fluctuations) and the intensity distribution will be broad.

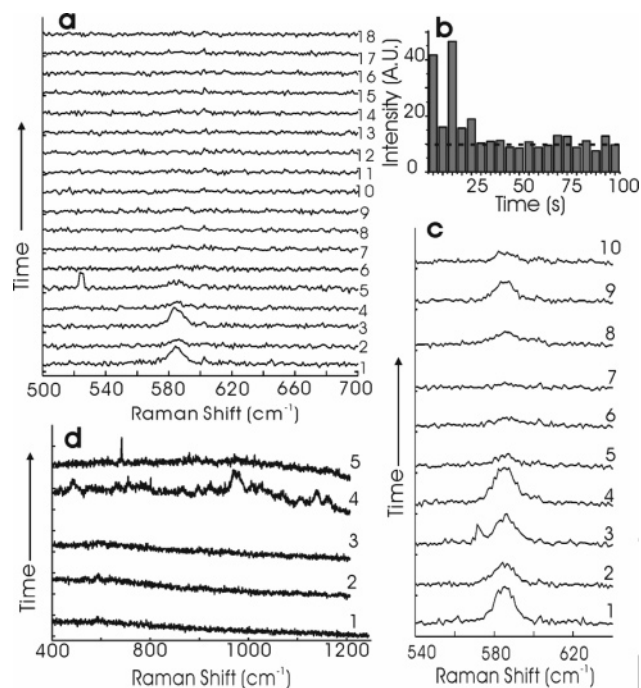
Figure 3c shows a typical example of the spectral fluctuations in our TERS studies. One hundred spectra were collected continuously from a sample made from a  $10^{-5} \text{ M}$  BCB solution

with a collection time of  $5 \text{ s}$  per exposure. The intensity of the BCB band at  $\sim 585 \text{ cm}^{-1}$  shows obvious fluctuations as demonstrated in Figure 3d. We can assign these fluctuations to the spectroscopic behavior of single molecules. The fluctuation behavior in SERS or TERS is different from that in single molecule fluorescence experiments. For fluorescence experiments, single molecules often show two-state behavior (“on” and “off”).<sup>35</sup> However, for single molecule SERS or TERS, the signal is not determined by a bright and dark state of the molecules but by several different mechanisms, which were listed above. Thus, the signal intensity in single molecule SERS experiments always shows broad and unstructured intensity fluctuations.

To analyze intensity fluctuations for single molecule SERS experiments in solution, a statistical method was proposed by Kneipp et al.<sup>17</sup> In their work, the signal intensity was normalized to the maximum value and a histogram of this normalized signal intensity distribution was constructed. If only one molecule enters the laser focal volume during an acquisition, the histogram of the normalized signal will show a broad peak; if several molecules can enter the focal volume, the histogram will show several isolated peaks that correspond to the probability to find 0, 1, 2, or 3, ... molecules in the detection volume. When the concentration of the sample is high, the number of molecules in the detection volume will be fairly constant and the intensity fluctuations will be averaged out. Thus, the histogram will show a narrow peak.

Following the statistical method described above, a histogram of the signal intensity, Figure 3e, was constructed with a binning value of 0.05 (the signal intensity was normalized to the maximum). It shows a broad, unstructured distribution, which covers the range from 0.2 to 1.0. As a comparison, spectra also were collected from samples with higher BCB coverage (Figure 3f), made by spin coating  $2.5 \mu\text{L}$  of a  $10^{-4} \text{ M}$  BCB solution on an Au substrate ( $150 \text{ molecules}/100 \text{ nm}^2$ ). The histogram is completely different from that in Figure 3e; the normalized intensity is concentrated in the range of  $0.5\sim 0.7$ . As discussed in the previous paragraph, this comparison clearly demonstrates the effect of averaging: when the sample coverage is low and only one or very few molecules are in the tip-substrate junction, the intensity will greatly fluctuate and the intensity distribution will be broad; when the number of molecules increases, the fluctuations will be averaged out and the intensity distribution will be confined to a smaller range. This comparison clearly indicates that the fluctuations observed in our experiments are due to single or very few molecules.

It is important to note that the histogram presented here differs significantly from the result reported by Kneipp et al.,<sup>17</sup> which shows a profile with isolated peaks resembling a Poisson distribution. There are several reasons for this difference. First of all, the Poisson distribution requires a good uniformity of the SERS intensity from different molecules.<sup>31</sup> In the case of Kneipp’s work, the experiment was done in solution<sup>17</sup> and the presence or absence of a molecule in the detection volume dominates the signal intensity so that this requirement is satisfied. On the other hand, our experiments were done with dry samples. As mentioned above, several mechanisms can simultaneously influence the Raman intensity in this case. Therefore, the signal is not expected to be uniform from molecule to molecule. Furthermore, the Poisson distribution needs a large number of intensities. One hundred intensities are not sufficient to define a Poisson distribution. As pointed out by Le Ru et al., “a sample of 100 intensities following a log-normal distribution often exhibits oscillations similar to that



**Figure 4.** (a) and (b) are examples for single-step bleaching of the tip-enhanced Raman scattering. The spectral series was collected with 5 s per exposure (a) and the peak intensity sequence (b) shows an obvious discrete decrease after the first 5 frames. The dashed line denotes the background level. Panel (c) shows the fluctuation of the line shape of BCB. Sometimes the tip-enhanced signal even shows random fluctuation (d) irrelevant to the sample molecule BCB. The collection time used in (c) and (d) was 10 s per exposure.

shown in Kneipp's work.<sup>31</sup> Therefore, a broad and unstructured distribution in our experiment is reasonable.

**Discrete Signal Losses.** A discrete signal decrease was often observed in our experiments and serves as further evidence for single molecule detection.<sup>35</sup> Figure 4a,b show one continuous spectral sequence. The intensity of the main band at 585 cm<sup>-1</sup> exhibits large intensity and spectral fluctuations during the first 5 scans and then the band vanishes. This irreversible decrease of Raman intensity can be explained by either photoinduced dissociation or photoinduced desorption of a single BCB molecule. This signal drop should be as sudden as in the case of single-step photobleaching observed in single molecule fluorescence experiments. However in SM-TERS experiment, it may not be recognizable as such, because the intensity of Raman scattering is influenced by many separated mechanisms simultaneously and will fluctuate, as discussed above.

On the basis of these three lines of argumentation, we conclude that SM-TERS phenomenon was indeed observed in our experiments. In the remainder of this paper, other experimental phenomena related to single molecule detection and high field enhancement by the tips are discussed.

Besides the intensity fluctuation, SM-TERS bands of BCB also show changing lineshapes, linewidths, and frequency shifts. Like the study of the intensity fluctuation above, we concentrate on the area around the peak at 585 cm<sup>-1</sup> because of its high intensity. To obtain clean spectra, a 10 s exposure time was used instead of 5 s and 10 spectra were collected continuously (Figure 4c). The shape of the main peak at ~585 cm<sup>-1</sup> changes with time: in line 1, it is symmetric; in line 2, 9, and 10, it shows an asymmetric shape; and in line 4 and 8, there is a shoulder to the red. The most unusual spectrum is in line 3, which has a peak at 570 cm<sup>-1</sup> that does not show up in any of the other spectra. A frequency shift of ~2 cm<sup>-1</sup> also can be

observed in Figure 3b in which the trace of the peak is not a straight line. One possible reason for these fluctuations is thermal drift of the molecules under the tip. When the local environment changes, the vibrational modes of the molecules will shift, and as a result any motion, such as rotation or translation, which may happen at room temperature, can induce these spectral fluctuations.

Spectral changes were also observed in TERS experiment of BT including changes in intensity, width, and frequency of the peaks. As opposed to the result from BCB, the intensities of the BT modes, especially the C–S stretching mode at 417 cm<sup>-1</sup><sup>29</sup> show only a continuous decrease without obvious fluctuations. This can be interpreted with photoinduced dissociation or desorption of the SAM due to the highly enhanced field in the tip–sample junction. Figure 2c shows a zoom of the boxed parts in Figure 2b. Broadening of the peaks at 999 cm<sup>-1</sup> and 1073 cm<sup>-1</sup> can be clearly seen. One possible reason for this phenomenon is the tip-induced thermal effect.<sup>36</sup> In our experiment, the thermal effect may be important for two reasons. First, the enhancement of the electric field for the particular tip used in this experiment was very large. Joule heating by the electric field is proportional to  $\sigma|E|^2$  where  $E$  is the electric field and  $\sigma$  is the conductivity of the material. In our experiments,  $|E|^2$  is enhanced by more than 1000 times, as estimated from the enhancement factor of Raman signal ( $\sim 10^7$ ), which is proportional to  $[g(E)]^4$  in which  $g(E)$  is the enhancement of the field intensity. Second, the heat generated around the gap cannot be dispersed away efficiently because the Ag tips are thin and the Au layers are also only 150 nm thick. As a result, an elevated local temperature under the tip is expected.

Another type of spectral fluctuation, showing random structures unrelated to the sample molecules, was also observed in a few cases. Figure 4d is a typical example of such fluctuations. This spectral series was collected from the ethanol rinsed BCB sample described above. The spectrum shows a sudden increase of signal strength, and many peaks unrelated to BCB appeared (line 4). One interpretation is that photochemical processes occur, such as photodissociation induced by the high intensity of the field in the gap, and that the Raman spectra of the photodissociation products is observed. Another possibility is that some contamination from the environment reached the gap by chance and gave a completely different spectrum. In our opinion, the second explanation is more likely. First, our experiments were done in an ambient environment. There is some chance that ambient molecules adsorb in the tip–sample junction. Second, no direct relation between the spectrum and the sample molecules was found. Last, if the spectral jump was caused by photodissociation, the spectrum should be reproducible because the products of such a reaction should always be the same; however, it was completely random.

Some influences from degradation of the Ag tips were also found. We observed that the enhancement of the Ag tips decreased over half a day, probably because of oxidation of the Ag. More than 50% of freshly made tips gave reasonable enhancement but after half a day, tips rarely were still hot. Thus, freshly made tips were always used in our experiment. Second, the tips can be contaminated by sample molecules during experiments. When molecules have more than one functional group with affinity to Ag, the chance of picking up matter by the tip increased. This also was reported by Ren et al.<sup>22</sup> For BCB multilayer samples, the tips were sometimes contaminated. In our experiments, the tips were always checked with a clean Au surface to make sure that the signal did not come from tip contamination.

In summary, single molecule tip-enhanced resonance Raman spectra from BCB were observed in the gap between an Ag tip and a planar Au surface. The electromagnetic field was greatly enhanced due to excitation of the localized plasmon polaritons. Fluctuations of the intensities and the line shapes of Raman bands were observed, indicating that there were only one or a few molecules under the tip. This experiment confirms the SM-TERS observations by Raschke and co-workers and may lead to a better understanding of the SM-SERS phenomenon. Moreover, with such a large enhancement, tip-enhanced Raman spectra of a BT SAM adsorbed on Au surface were collected with low excitation power and only 10 s collection time. This opens up the possibility to perform the real-time TERS investigation of chemical reactions with nanoscale spatial resolution.

**Acknowledgment.** The authors acknowledge the SEM analysis by Dr. Frank Krumeich (ETH Zurich). This work is supported by the Gebert–Rüf Foundation (Grant No. P-085/03) and the Deutsche Forschungsgemeinschaft (grant awarded to Thomas Schmid).

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