Raman spectroscopy and microscopy based on mechanical force detection

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The Raman effect is typically observed by irradiating a sample with an intense light source and detecting the minute amount of frequency shifted scattered light. We demonstrate that Raman molecular vibrational resonances can be detected directly through an entirely different mechanism—namely, a force measurement. We create a force interaction through optical parametric down conversion between stimulated, Raman excited, molecules on a surface and a cantilevered nanometer scale probe tip brought very close to it. Spectroscopy and microscopy on clusters of molecules have been performed. Single molecules within such clusters are clearly resolved in the Raman micrographs. The technique can be readily extended to perform pump probe experiments for measuring inter- and intramolecular couplings and conformational changes at the single molecule level. © 2011 American Institute of Physics. [doi:10.1063/1.3652760]

The Raman effect¹ is one of the most widely used phenomenon in chemical spectroscopy. Over the past 80 years, this effect has been measured by irradiating the sample with intense monochromatic light and detecting the minute amount (one part in 10^9) of frequency shifted scattered light. A typical Raman setup utilizes a high rejection, low insertion loss, long pass filter to reject the incident pump light. This is followed by a sensitive detector coupled to a high resolution spectrometer to record the molecular vibrational spectrum. We present initial results on an entirely different mechanism for detecting the Raman effect. It is based on the force interaction between a Raman excited molecule and a scanning probe microscope probe tip. The ability to measure the Raman effect using a non-optical channel is important because the weak Raman signal can be detected in zero optical background, providing similar sensitivity advantages to that offered by photoacoustic spectroscopy.² Furthermore, since we do not need a spectrometer for the measurement, the resolution of the technique can be improved by several orders of magnitude as compared with conventional techniques, since it is only limited by the spectral bandwidth of the lasers used and not by the spectrometer resolution; for diode lasers, this bandwidth can be as narrow as 100 kHz. We present images from our *Raman Probe Force Microscope* of molecular clusters where single molecules can be discerned within them due to their orientation differences.

The concept of our scheme can be understood by referring to Figure 1. Two collinear optical beams—a pump beam at frequency ν_1 and a stimulating beam at frequency ν_2 —are focused to a diffraction limited spot on the sample surface to efficiently stimulate molecular vibrations at frequency $(\nu_1 - \nu_2)$. The stimulating beam at frequency ν_2 is tunable, allowing $(\nu_1 - \nu_2)$ to be tuned through the various Raman vibrational resonances. We probe the force interaction between the excited oscillating molecules and a cantilevered, gold coated, scanning probe microscope tip approached

within nm range of the sample surface as the force interaction is modulated at frequency f_m . The force and force gradient at f_m modulates the cantilever fundamental mechanical resonance (frequency f_0) creating sidebands at $f_0 + f_m$ and $f_0 - f_m$. The $f_0 + f_m$ sideband is detected at the second mechanical resonance of the cantilever using standard AFM methods^{3,4} (see supplemental information¹² b).

We can understand the theory of parametric force detection by resorting to a classical model. Assume that the sample (or molecule) being studied occupies the space between tip and substrate. If the electric field at the tip is $\mathbf{E}$, then the force $\mathbf{F}$ on the tip due to its interaction with the sample (or molecule) is

$$\mathbf{F} = (1/2)\alpha_0 \nabla E^2 + (1/2)\partial\alpha/\partial q|_{q=0} Q \cos 2\pi\nu_R t \nabla E^2, \quad (1)$$

where α_0 is the linear polarizability of the sample, $\partial\alpha/\partial q|_{q=0}$ $Q \cos 2\pi\nu_R t$ is the non-linear polarizability term, q is the nuclear vibration coordinate, Q is the nuclear vibration amplitude, and ν_R is the Raman resonance frequency. Alternatively, in terms of linear susceptibility χ_0 and non-linear susceptibility $\partial\chi/\partial q|_{q=0} Q \cos 2\pi\nu_R t$ of the sample, if area of tip end is A

$$F = 0.5A\epsilon_0 E^2 [1 + \chi_0 + \partial\chi/\partial q|_{q=0} Q \cos 2\pi\nu_R t].$$

For incident fields oriented along the tip axis, we can write $\mathbf{E} = E_z = (E_1 \cos 2\pi\nu_1 t + E_2 \cos 2\pi\nu_2 t)$, E_1 being the pump field amplitude at ν_1 and E_2 being the stimulating field amplitude at ν_2 .

The cantilever cannot respond at optical frequencies. To calculate the Raman force, we need to calculate the time averaged force $\langle F_z \rangle$ from Eq. (1):

$$\begin{aligned} \langle F_z \rangle = & (1/2)\alpha_0 \nabla [E_1^2/2 + E_2^2/2] + (1/2)\partial\alpha/\partial q|_{q=0} \\ & \times Q \nabla [E_1 E_2 \langle \cos 2\pi(\nu_1 - \nu_2)t \cos 2\pi\nu_R t \rangle]. \end{aligned} \quad (2)$$

The first term is simply the force resulting from the average power incident on the molecule interacting with the linear

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FIG. 1. (Color online) Schematic of the Raman probe force microscope.

polarizability of the sample while the second term is the force resulting from the stimulated Raman oscillations *parametrically down converted* to a detectable mechanical frequency. At the condition for stimulated excitation $(\nu_1 - \nu_2) = \nu_R$, we generate a Raman force from the second term $\langle F \rangle_{\text{SRaman}} = (1/4) \partial \alpha / \partial q|_{q=0} Q \nabla [E_1 E_2]$.

This term is modulated at f_m by modulating the power in beam E_1 at f_1 and E_2 at f_2 , such that $f_1 - f_2 = f_m$. It should be noted that Eq. (2) only provides a lower bound for the Raman force as it does not include image force effects of the sample interacting with the tip.³

For Raman force detection, the ratio of the first term to the second term in Eq. (2) is $\alpha_0 / [Q \partial \alpha / \partial q|_{q=0}]$. From far field optical stimulated Raman data, we estimate the value of $\alpha_0 / [Q \partial \alpha / \partial q|_{q=0}]$ to be in the range of 0.053–0.017 (see supplemental information¹² a). By relating this value to our previous work on force detection of molecules driven at their electronic resonance³ (i.e., the response due to the first term in Eq. (2)), we estimate the stimulated Raman force to be around 10^{-11} N—an order of magnitude higher than our experimental observations.

For our very first successful experiment, we created the force modulation at f_m by modulating E_1 at f_m . However, the power modulation of $E_1(\nu_1)$ created an unwanted modulation of the force signal at f_m through the first term of Eq. (2); we nulled the effect of this modulation by introducing a collinear “balancing” optical beam $E_0(\nu_0)$ at $\lambda_0 = 633$ nm (dotted line in Figure 1), where $(\nu_0 - \nu_2)$ was outside any Raman resonances and whose power was adjusted and modulated at f_m in anti-phase with E_1 . While this scheme worked, it was cumbersome and we soon realized and implemented a much simpler detection scheme shown in Figure 1, where we eliminated the third beam $E_0(\nu_0)$ altogether by modulating E_1 at f_1 and E_2 at f_2 such that $f_m = f_1 - f_2$, thereby generating a true background free Raman signal. All results shown here were done with this second scheme.

The force detection is based on a modified AFM platform working in the vibrating tapping mode.⁵ Two collinear CW single mode diode laser beams, $\lambda_1(\nu_1) = 594.1$ nm and $\lambda_2(\nu_2) = 656$ nm (bandwidth of 70 GHz), were focused onto an object on a glass cover slide using an oil immersion objective (NA = 1.45). The focused lasers excited a Raman

resonance of the object. A 10 nm radius gold coated silicon tip was approached to the object to detect the force and force gradient on the tip at the modulation frequency f_m . To further enhance the force interaction, we used a radial polarizer (ARCOptix) at the entrance pupil of the objective to ensure that the optical electric field oscillation was along the axis of the probe tip. The optical power of each beam at the entrance pupil of the objective was nominally adjusted to be approximately 200 μ W. We chose a cantilever with $f_0 = 65$ kHz (quality factor = 115, stiffness constant $k = 3$ N/m). We chose $f_1 = 860$ kHz and $f_2 = 500$ kHz so that $f_1 - f_2 = 360$ kHz and the upper sideband $f_0 + f_m = 425$ kHz coincided with the second mechanical resonance of the cantilever. This sideband was detected using a lock-in amplifier while the sample was raster scanned to record an image.

Experiments were performed using Coomassie brilliant blue G250 dye. We pipetted a 30 μ L drop of 0.01 mM dye molecules dissolved in ethanol onto a clean glass microscope cover slide and allowed it to dry. The dye aggregated into molecular clusters with an average dimension of approximately 25 nm while the smallest topography features observed were on the order of 10 nm as shown in Figure 2(a). The 10 nm feature on the top right corner of Figure 2(a) and several others (indicated by arrows) are inferred to be single molecules from the topography scans, which show a height change of less than 500 pm corresponding to the estimated height of a Coomassie blue molecule.

Figures 2(a) and 2(b) are simultaneously recorded topography and Raman images, respectively, when the laser diode frequency ν_2 was temperature tuned so that $(\nu_2 - \nu_1)$ coincided with the 1625 cm^{-1} vibrational mode of the Coomassie blue molecule. Rich detail is visible in the Raman image as compared with the topography image. Figures 2(c) and 2(d) show corresponding images taken with $(\nu_2 - \nu_1)$ tuned away from this vibrational mode showing no

FIG. 2. (Color online) Topography and Raman images of Coomassie brilliant blue G250 dye molecules on glass. (a) Topography images with $(\nu_1 - \nu_2)$ tuned to the 1625 cm^{-1} molecular resonance. (b) Simultaneously recorded stimulated Raman force gradient image corresponding to (a). Inset shows single molecules resolved within molecular clusters where contrast changes are attributed to molecular orientation changes; 3.5 nm spectroscopic features are visible in these images. (c) Topography image with $(\nu_1 - \nu_2)$ tuned off the 1625 cm^{-1} molecular resonance. (d) Simultaneously recorded Raman image corresponding to (c) showing no image contrast.

FIG. 3. (Color online) Comparison of recorded Raman spectrum with the probe over a molecular feature in image (b) obtained by tuning $(v_2 - v_1)$ through the 1625 cm^{-1} Raman resonance (solid squares), compared with the Raman spectrum from a thick layer of Coomassie blue molecules recorded using a commercial Renishaw spectrometer (solid line).

information in the Raman image but essentially the same topography information aside from a small thermal drift.

On closer examination of Figure 2(b), we notice that individual molecules within the molecular clusters are clearly visible in the Raman image while they are not at all resolved in the topography image of Figure 2(a). The inset (right column) in Figure 2(b) shows clusters of one, two, three, and four and five molecules imaged using our Raman probe. We notice that some molecules in these clusters appear very bright while others are less bright. We believe that these contrast variations arise from the different orientations of the various molecules within a cluster giving rise to different excitation efficiencies. The inset (bottom of Figure 2(b)) shows several clusters exhibiting single molecule contrast. The resolution in the Raman image (3.5 nm) is superior to that in the topography image (10 nm) due to the fact that in our Raman probe, both the excitation dipole and the interaction force field are localized to the spatial dimensions of the probe tip since both are near-field interactions. This results in a narrower point spread function compared with other Raman imaging schemes—such as Tip Enhanced Raman Spectroscopy⁶—where the excitation is in the near-field but the detection is done in the far-field. The S/N of the Raman signal was estimated to be 35 dB in 1 Hz bandwidth—currently limited by laser pointing instabilities. Following the steps described previously,³ we calculate that the detected force was $0.85 \times 10^{-12} \text{ N}$ and the force gradient was $3.7 \times 10^{-5} \text{ N/m}$.

To further validate our imaging concept, we located the probe over one of the Raman features and recorded the force

response as a function of $(v_2 - v_1)$ by temperature tuning the laser at v_2 while using an optical feedback loop to maintain a constant laser power at v_2 . Figure 3 shows the recorded Raman spectrum (squares) as compared with a spectrum acquired using a Renishaw inVia spectrometer (solid line); the agreement is excellent. The resolution of the spectrum was 2 cm^{-1} , comparable to the resolution of the Renishaw spectrometer.

In conclusion, we have shown that the Raman effect can be detected through an entirely different channel—namely, by measuring the force gradient between a Raman excited molecular feature and a gold coated probe tip in a scanning probe microscope. While apertureless near-field microscopes^{7–10} based on light scattering from tips have had great success in imaging nanoscopic objects from the visible to the infrared, including the Raman effect through fluorescence, the signals are very weak since they are based on far-field optical detection of a near-field interaction. Force detection techniques are capable of measuring forces 6 orders of magnitude weaker than those reported here.¹¹ We believe that our approach can be readily extended to perform pump-probe experiments on single molecules where it should be possible to use it to measure inter- and intramolecular couplings at the single molecular level and to track conformational changes within a molecule. Furthermore, we see no impediment to achieving atomic resolution Raman spectroscopy and imaging with this method.

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¹²See supplementary material at <http://dx.doi.org/10.1063/1.3652760> for estimation of the detected near-field Raman force.