

A Conclusive Demonstration of Vibrational Pumping under Surface Enhanced Raman Scattering Conditions

R. C. Maher,^{*,†} P. G. Etchegoin,^{§,‡} E. C. Le Ru,^{§,⊥} and L. F. Cohen[†]

The Blackett Laboratory, Imperial College London, Prince Consort Road, London SW7 2BZ, United Kingdom, and The MacDiarmid Institute for Advanced Materials and Nanotechnology, School of Chemical and Physical Sciences, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand

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We provide a conclusive demonstration of vibrational pumping under Surface Enhanced Raman Scattering (SERS) conditions by performing anti-Stokes/Stokes ratio measurements over a large spatial area and low power density, down to 10 K with dried silver colloids, the dye rhodamine 6G, and 676 nm laser excitation. The method we propose allows for the measurement of the cross sections for different modes and also provides the determination of the asymmetry between the anti-Stokes and Stokes SERS cross sections for each mode.

After its discovery in the 1970s, Surface Enhanced Raman Scattering (SERS) became a subject of intense research up to the mid-1980s with varied applications.¹ A resurgence in interest occurred after two independent reports^{2,3} in 1997 on the observation of single molecule SERS, with potential advantages over fluorescence-based techniques.

Kneipp and co-workers first proposed in 1996⁴ the possibility of vibrational pumping via SERS. The presence of vibrational pumping was inferred mainly from two observations. First, they showed that for a number of dye molecules the SERS spectrum contained anti-Stokes emissions so intense that they could not be accounted for in terms of thermal population of excited vibrational modes. Haslett et al.⁵ challenged that conclusion and provided evidence to suggest that this observation was also consistent with resonance phenomena in the dye molecules. This conclusion was also reported by other authors.⁶ The second evidence was the observation of a quadratic power dependence of the aS/S ratio. Although some attempts to confirm this dependence were unsuccessful,⁵ the results have been reproduced.⁷ Note that the possibility that this dependence results from heating effects and not pumping has also been previously discussed.⁶ For these reasons, the subject is one of ongoing debate and review in the literature.⁸ The importance of pumping lies not only in fundamental aspects of molecular spectroscopy but also in the potential of the technique for metrology applications and the determination of SERS cross sections.⁴ Recently we have built on this prior work and demonstrated that in fact the anti-Stokes/Stokes ratio can be used to map out the underlying resonances in the system for the case of silver colloids and R6G dye molecules.⁹

In this work we use the silver colloid-R6G molecule system, where the underlying resonances have been previously characterized, and we introduce a new approach, based on measurements of the aS/S ratio as a function of temperature down to 10 K, that allows for a conclusive and reproducible signature

for vibrational pumping in SERS. Furthermore, we show that we can extract the cross section by two independent methods which yield values that are completely in agreement with each other. Our experiments are also performed at low laser power and over a large area reducing the possibility of sample heating.

First we review the theoretical background to nonlinear processes in aS/S ratio. Consider a single vibrational level in a molecule at temperature T . A laser with intensity I_L [W/cm²] creates occupation of the level through pumping with a rate proportional to both the Raman–Stokes cross section σ_S [cm²] and the intensity. The population of the level is in part also determined by the thermal equilibrium condition defined by the molecular temperature T . Vibrations remain in a specific level for a finite lifetime τ [s], which takes into account all the possible relaxation mechanisms starting from that level, either through intramolecular vibrational relaxation, IVR (anharmonic processes), or external relaxation mechanisms. The rate equation for the average phonon population n of the vibrational mode is

$$\frac{dn}{dt} = \frac{\sigma_S I_L}{\hbar\omega_L} + \frac{n_B(\hbar\omega_\nu/k_B T)}{\tau} - \frac{n}{\tau} \quad (1)$$

where $\hbar\omega_L$ and $\hbar\omega_\nu$ [J] are the energies of the laser and vibration, respectively, and $n_B(\hbar\omega_\nu/k_B T)$ is the Boltzmann factor for that vibration. The second term corresponds to thermal excitation and has been adjusted so that in the steady state ($dn/dt = 0$) $n = n_B(\hbar\omega_\nu/k_B T)$ when $I_L = 0$. When $I_L \neq 0$

$$n = \frac{\tau\sigma_S I_L}{\hbar\omega_L} + e^{-\hbar\omega_\nu/k_B T} \quad (2)$$

where the first and second terms account for vibrational pumping and thermal effects, respectively. This expression can be justified more rigorously.⁸ This is the weak pumping regime where vibrational pumping might compete with the thermal population of the level but n remains small. The Stokes–Raman signal is independent of n and it is simply $I_S = N\sigma_S I_L$, where N is the number of molecules. In contrast, the anti-Stokes signal does

* Address correspondence to this author. E-mail: robert.maher@imperial.ac.uk.

† Imperial College London.

§ Victoria University of Wellington.

‡ E-mail: pablo.etchegoin@vuw.ac.nz.

⊥ E-mail: eric.leru@vuw.ac.nz.

depend on n and it is given by $I_{aS} = nN\sigma_{aS}I_L$. The anti-Stokes/Stokes ratio (ρ) of a specific vibration is then

$$\rho = I_{aS}/I_S = \frac{\sigma_{aS}}{\sigma_S} n = A \left[\frac{\tau\sigma_S I_L}{\hbar\omega_L} + e^{-\hbar\omega_v/k_B T} \right] \quad (3)$$

where $A \equiv \sigma_{aS}/\sigma_S$ is the *asymmetry parameter* taking into account that in SERS we have $\sigma_{aS} \neq \sigma_S$ in general, due to the presence of underlying frequency-dependent plasmon resonances.⁹ If the first term (pumping) in eq 2 dominates over the second (Boltzmann factor), this scenario produces a Stokes signal $\propto I_L$ and an anti-Stokes one $\propto I_L^2$, while ρ is linear in I_L . Moreover, the slope of ρ vs I_L provides $A\tau\sigma_S/\hbar\omega_L$. If A is known or estimated, and if a judicious estimation of τ can be made, the SERS Stokes cross section can then be gained. This description led to a series of claims on the order of magnitude of the SERS cross sections.⁴ The quadratic dependence of I_{aS} on I_L at room temperature was the origin of the original claim on SERS pumping.⁴

Note that there remains an issue concerning the number of active molecules N represented in the Stokes and anti-Stokes intensities (as clearly set out in ref 5). It is clear that only the molecules with the highest cross-sections will experience pumping and contribute to the aS signal whereas the situation with the Stokes signal is rather case specific.¹⁰ In the simple model presented here, we assume that all the molecules experience the same SERS enhancement (and have the same cross section). In reality, the SERS enhancements are highly nonuniform, with a few places of extremely high enhancements (hot spots).

From eq 3 we can see clearly how a power dependence might originate from purely heating effects.⁶ If the first term is negligible compared to the second one in eq 3 (normal situation) then $\rho \propto e^{-\hbar\omega_v/k_B T}$. If the laser causes local heating, the molecule is at a higher temperature $T^* = T + \Delta T \equiv T + \beta I_L$. When $\Delta T \ll T$, expanding this expression, we obtain $\rho \propto \exp(\hbar\omega_v\beta I_L/k_B T^2)$, i.e., an exponential or linear dependence on I_L (depending on the value of $\hbar\omega_v\beta/k_B T^2$) can be expected. These dependencies have indeed been observed,¹¹ and they can easily be confused with pumping.

The solution to this problem comes from SERS measurements at temperatures much lower than any reported previously.^{12,13} The quantity $\tau\sigma_S I_L/\hbar\omega_L$ is normally $\ll 1$ and it is completely negligible under normal Raman scattering conditions. Under SERS conditions, this quantity is still small but can become comparable to $\exp(-\hbar\omega_v/k_B T)$ if I_L is sufficiently high,⁴ in which case heating effects can complicate the interpretation, or if T is sufficiently small. There is then a crossover from an anti-Stokes/Stokes ratio dominated by the thermal population ($\rho \propto \exp(-\hbar\omega_v/k_B T)$) to one dominated by pumping ($\rho \propto \tau\sigma_S I_L/\hbar\omega_L$). This crossover is easily observed in the following experiments at temperatures in the range ~ 10 – 150 K depending on the analytes and the specific mode under consideration. The temperature at which the crossover happens depends on mode energy due to the rapid variation of the exponential term. This will occur at higher temperatures the larger the Raman shift of the mode, with small variations from one case to another depending on the magnitude of the first term in eq 3, which depends on the specific σ_S and τ .

The reason vibrational pumping is very hard to identify at higher temperatures is because it involves the study of small departures in mode populations from an already existing thermal population in the levels. By going to very low temperatures,

departures from zero population in the levels become more evident. Once the crossover for different modes has been reached, it is possible to observe anti-Stokes signals which are not related at all to a thermal population and only depend on the relative σ 's and τ 's. At sufficiently low temperatures, all main Raman peaks on the anti-Stokes side become independent of T , and have relative intensities which are not related to a Boltzmann factor. For example, at 10 K, we can observe modes with $\hbar\omega_v \sim 1500$ – 1600 cm^{-1} with a measurable intensity in a few seconds on the anti-Stokes side (the Boltzmann factor for these modes at 10 K is $\sim 10^{-100}$) while modes with smaller energies are much weaker in intensity or, in some cases, barely visible. The breakdown of relative intensities and the non-Boltzmann distribution of signals on the anti-Stokes side are both confirmation of SERS pumping. It can also be said that each mode has an effective temperature T_{eff} that is different for each mode, thus showing explicitly the breakdown of internal thermal equilibrium of the molecule.

We concentrate now on the experimental evidence for the much studied dye rhodamine 6G (RH6G). Silver colloids were produced as described in ref 14. SERS samples were prepared to a final 1 μM concentration of RH6G in a 50%–50% solution of colloids and 20 mM KCl. The sample was then dried onto a Si substrate and mounted on a closed-cycle He-cryostat (CTI-cryogenics) with temperature control in the range 10–300 K. Raman measurements have been performed for several laser lines of Kr^+ and Ar^+ ion lasers; we shall concentrate here only on a subset of these measurements to prove the point we raised. The laser was focused to a 20 μm spot and the signal was collected by a high-numerical aperture photographic zoom lens (Canon, $\times 10$ magnification) through the quartz window of the cryostat and onto the entrance slit of a high-dispersion double-additive U1000 Jobin-Yvon spectrometer coupled to a N_2 -cooled CCD detector. The wider spot area compared to those used in Raman microscopy has the triple advantage of (i) reducing photobleaching to a negligible level, (ii) reducing any indirect laser heating effect, and (iii) improving the averaging over cluster geometries.

Figure 1 shows anti-Stokes/Stokes spectra for two strong Raman modes widely separated in energy (610 and 1650 cm^{-1} , respectively) for 676 nm laser excitation (Kr^+ ion laser, $I_L = 1.6 \times 10^8$ W/m^2) at three different temperatures. At 300 K the peaks show the normal anti-Stokes/Stokes ratio (except for the asymmetry factor A , which is a small correction compared to the effect of the exponential in (3)). The Stokes side remains relatively constant throughout the measurement as can be seen in the figure. At $T = 150$ K an anomalous relative intensity between the two peaks on the anti-Stokes side can already be seen while the anti-Stokes signal of the 610 cm^{-1} mode has decreased (as expected by the lower temperature) and the one for the 1650 cm^{-1} mode still remains visible. This is because, at this T , the pumping term already dominates (1st term in eq 3) while the former is still responding to temperature changes (2nd term in eq 3), as we shall show in the next figure. At $T = 10$ K, the anti-Stokes signals of both peaks are almost comparable in size. The Boltzmann factors for these two peaks at 10 K are $\sim 10^{-38}$ (610 cm^{-1}) and $\sim 10^{-100}$ (1650 cm^{-1}), i.e., the only reason they are still seen is SERS pumping.

Figure 2 shows the experimental anti-Stokes/Stokes ratios ($\ln(\rho)$) for three different modes in RH6G as a function of temperature for 676 nm laser excitation. A clear crossover between the two regimes dominated by the first or second term in eq 3 can be seen in the data. The crossover happens at higher

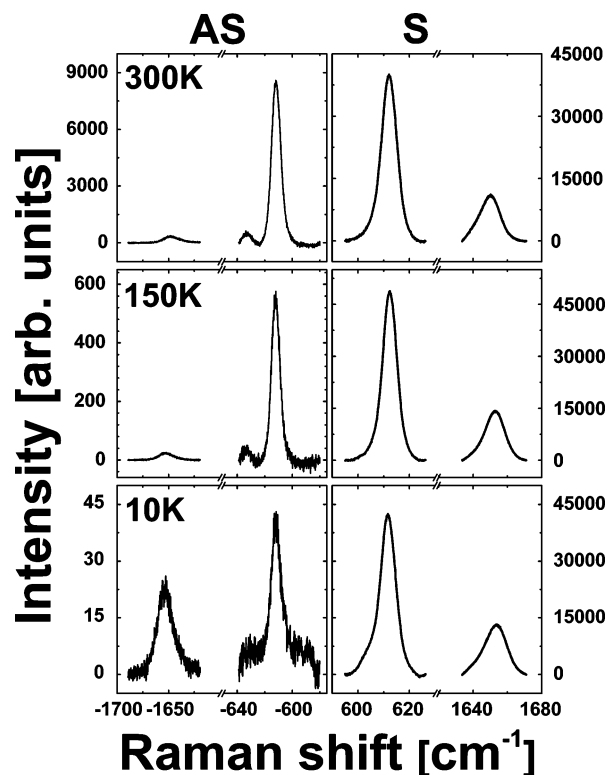


Figure 1. Anti-Stokes (left) and Stokes (right) spectra for two widely separated modes of RH6G (610 and 1650 cm^{-1}) under 676 nm laser excitation. At room temperature the normal ratio is observed (slightly modified by the asymmetry parameter A). At 150 K the anti-Stokes intensity of the 1650 cm^{-1} mode is already anomalous with respect to 610 cm^{-1} . At 10 K the anti-Stokes signals of both peaks are comparable in size and only exist because of vibrational pumping. This is equivalent to having both modes with different effective temperatures (Table 1).

temperatures for higher energy modes, as expected. It is convenient to fit the experimental data to

$$\ln(\rho) = a + \ln[b + e^{-\hbar\omega_{\nu}/k_{\text{B}}T}] \quad (4)$$

where $a = \ln(A)$, and $b = \tau\sigma_{\text{S}}I_{\text{L}}/\hbar\omega_{\text{L}}$. This is then a fit with two parameters, from where $\tau\sigma_{\text{S}}$ can be gained for a known I_{L} . Fits to eq 4 are explicitly shown in Figure 2. The agreement between theory and experiment is excellent. From here we can obtain $\tau\sigma_{\text{S}}$ and eventually σ_{S} if the lifetime is estimated.

The need to obtain the relaxation time τ for each level might be seen as a drawback. For a start, relaxation times could be ultimately determined by invoking a different type of spectroscopy (time resolved) if needed, but even within the present framework they can also be estimated from the fwhm (Γ) of the peaks. The plateaus in Figure 2 at low temperatures suggest that τ remains constant (within a few percent) in this temperature range, in accordance with the almost constant Γ observed. If the broadening is homogeneous,¹⁵ τ follows directly from $\tau \sim \hbar/\Gamma$. Molecular vibrations have such strong couplings with other vibrations in the molecule that contributions from inhomogeneous broadenings are negligible. Even in SERS experiments where the single molecule limit is approached¹⁰ (which would be more sensitive to inhomogeneous broadening) the fwhm does not change by more than 1 – 1.5 cm^{-1} in peaks with a typical Γ of ~ 15 – 20 cm^{-1} . An estimation of τ from Γ is expected to be accurate within ~ 10 – 15% , accordingly. An almost constant τ at low temperatures is also expected from general considerations on anharmonic interactions where Γ is expected to follow $\Gamma = \Gamma_0(2n_{\text{Bose}}(\hbar\omega_{\text{av}}/k_{\text{B}}T) + 1)$, with $\hbar\omega_{\text{av}}$ being a characteristic

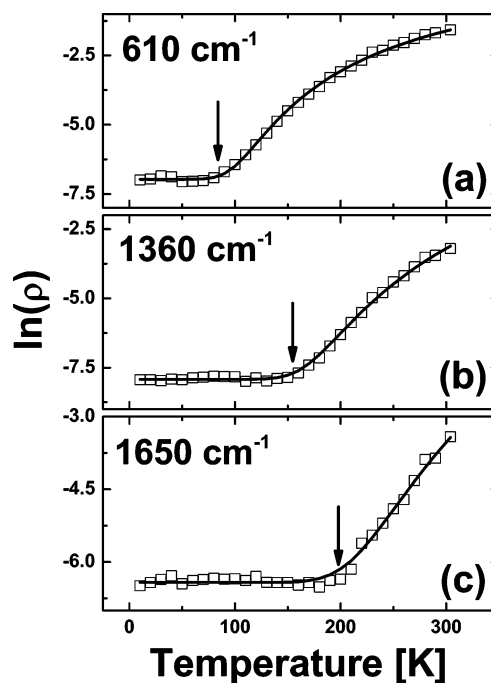


Figure 2. Anti-Stokes/Stokes ratio ($\ln(\rho)$) as a function of T for three modes in RH6G (676 nm laser excitation). Note that the crossover happens at different temperatures for different modes (vertical arrows), being at higher temperatures for larger vibrational energies. The crossover temperature is roughly equivalent to the effective mode temperature T_{eff} . The solid lines are fits with eq 4. From the two parameters of the fit, the fwhm of the peaks, and the laser intensity I_{L} , the SERS cross section can be readily estimated.

TABLE 1: SERS Cross Sections for the Modes in Figure 2 (676 nm laser) with Lifetimes Estimated from the fwhm of the Peaks for Rhodamine 6G^a

mode (cm^{-1})	σ_{S} (cm^2)	σ_{aS} (cm^2)	$\tau \sim \hbar/\Gamma$ (ps)	b	T_{eff} (K)
610	6.0×10^{-15}	2.2×10^{-14}	0.8	2.6×10^{-4}	102
1360	5.2×10^{-16}	1.4×10^{-14}	0.5	1.3×10^{-5}	174
1650	1.2×10^{-15}	9.5×10^{-14}	0.32	2.1×10^{-5}	220

^a σ_{aS} is obtained from σ_{S} by means of the asymmetry factor A obtained from the fit. The effective mode temperature T_{eff} is obtained from data at 10 K and is mode dependent, thus showing explicitly the breakdown of thermal equilibrium.

energy in the density of states of the molecule. For typical molecular vibrations in organic dyes this can be neglected at low temperatures. By taking these considerations into account, we produced the individual SERS cross sections of the three modes shown in Figure 2 in Table 1. The accuracy of these values in terms of the assumptions made for the estimation of the lifetimes is the subject of a full forthcoming paper in preparation and will be reported elsewhere.

One interesting consistency check for the value of the product of the SERS cross section and the lifetime ($\sigma_{\text{S}}\tau$) is to reproduce the original method proposed for SERS pumping,^{4,7,16} i.e., a power dependence of I_{S} and I_{aS} , and compare the values with those obtained from a temperature scan fitted with eq 4. This is done in Figure 3 for the same laser line (676 nm, Kr^+ ion laser) and one mode (610 cm^{-1} of RH6G). We can stop at a single temperature where the first term in eq 3 dominates (we chose $T = 20$ K) and study the anti-Stokes and Stokes intensities as a function of incident power. This is shown in Figure 3a,b. The anti-Stokes signal is quadratic as a function of incident power and is fitted with $I_{\text{aS}} = \gamma I_{\text{L}}^2$. A plot of I_{aS} on a log–log graph (inset of Figure 3a) shows indeed that it has a linear slope

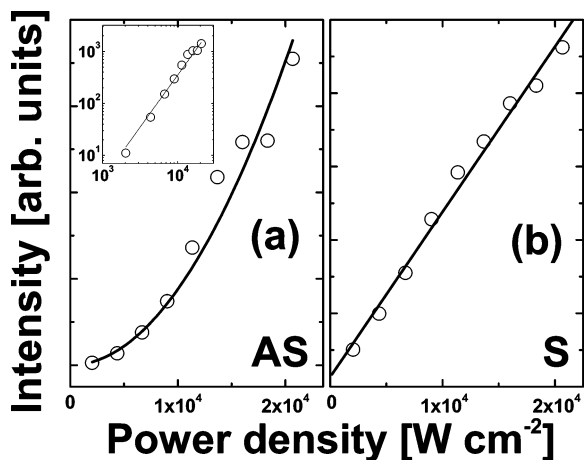


Figure 3. (a) Anti-Stokes signal power dependence at $T = 20$ K with the corresponding Stokes intensity in part b. From a quadratic fit of the data in (a) and a linear fit of the data in (b) the SERS cross section can be obtained. The inset in (a) shows the power dependence of I_{AS} on a log-log plot, from where a linear fit with a slope of $\sim 2.1 \pm 0.07$ is obtained, thus showing the expected quadratic behavior. The cross section determined by this method shows an excellent internal consistency with the temperature scan in Figure 2.

of 2.1 ± 0.07 , thus showing the quadratic dependence on I_L . The Stokes signal is linear in I_L and fitted to $I_S = \kappa I_L$. From the ratio $\gamma/\kappa = A\sigma_S\tau/(\hbar\omega_L)$ and a knowledge of the asymmetry factor A and the lifetime τ , the Stokes cross section σ_S can be obtained. The asymmetry factor A can be obtained from either a measurement at room temperature (where the second term in eq 3 dominates) or by using the A obtained from the temperature scan. From here we obtain $\sigma_S = (7.6 \pm 2.2) \times 10^{-15} \text{ cm}^2$, in excellent agreement with the temperature scan $\sigma_S = (6.0 \pm 1.3) \times 10^{-15} \text{ cm}^2$. This shows an internal consistency of the method and its interpretation, with SERS cross section being consistent by different approaches. Values obtained at 647 nm (not shown here) are also slightly larger than those at 676 nm, consistent with the idea of an underlying resonance.⁹

We have observed similar pumping effects in most of the important modes of other classical SERS probes such as crystal violet (CV) and 3,3'-diethyloxadicarbocyanine (DODC). Differences in SERS cross sections for a single analyte (the crossover temperature) can also be seen by changing the excitation to other lines of the Kr⁺ laser (647 nm), or lines of the Ar⁺ laser (514 nm); these results will be reported elsewhere in an extended paper. Another important issue is the measurement of the number of active molecules involved in the pumping process, which could, for example, be addressed by performing

a concentration dependence set of measurements. The reproducibility and clarity of our current temperature-dependent aS/S ratio methodology would allow such experiments to be performed.

We believe our results establish a new methodology (different from the original proposal of power dependence of the anti-Stokes signals at room temperature) to accurately obtain the SERS cross sections of different modes. For dyes where the normal cross section (σ^n) can be measured for a given laser, i.e., in situations where the normal Raman signal of a concentrated solution can be obtained without interference from fluorescence, this method also provides an accurate determination of the SERS enhancement factor for a specific substrate: $G_{\text{SERS}} \equiv \sigma^{\text{SERS}}/\sigma^n$. We do not doubt that there will be many practical applications of this type of experiment for the determination of SERS cross sections of important analytes/substrates.

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