Competition Between Extinction and Enhancement in Surface-Enhanced Raman Spectroscopy

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ABSTRACT: Conjugated metallic nanoparticles are a promising means to achieve ultrasensitive and multiplexed sensing in intact three-dimensional samples, especially for biological applications, via surface-enhanced Raman scattering (SERS). We show that enhancement and extinction are linked and compete in a collection of metallic nanoparticles. Counterintuitively, the Raman signal vanishes when nanoparticles are excited at their plasmon resonance, while increasing nanoparticle concentrations at off-resonance excitation sometimes leads to decreased signal. We develop an effective medium theory that explains both phenomena. Optimal choices of excitation wavelength, individual particle enhancement factor, and concentrations are indicated. The same processes that give rise to enhancement also lead to increased extinction of both the illumination and the Raman-scattered light. Nanoparticles attenuate the incident field (blue) and at the same time provide local enhancement for SERS. Likewise, the radiation of the Raman-scattered field (green) is enhanced by the nearby sphere but extinguished by the rest of the spheres in the suspension upon propagation.

SECTION: Physical Processes in Nanomaterials and Nanostructures

Several methods for using surface-enhanced Raman scattering (SERS)† have emerged for biomedical applications, ultrasensitive sensing, and multiplexed analyses. In particular, nanoparticles have been the focus of recent efforts toward in vitro and in vivo molecular sensing.‡−§ Nanoparticles can dramatically increase the electric field intensity near and at their surface, providing useful SERS-based probes,|| especially for deep tissue imaging at varying concentrations.7 Typically, a nanostructured particle is bioconjugated and employed in the same manner as conventional fluorescent probes are used for molecular imaging. SERS probes are postulated to offer bright and stable signals and extensive multiplexing,|| while it has been assumed that experimental best practice parallels that of fluorescent probes, that is, that one should excite at the strongest resonance and use a high concentration. In fact, it has recently been recognized even in single-particle enhancement of fluorescence that peak signals are observed to be red-shifted from the plasmon reference.9 Thus far, the design of nanoparticle-based SERS experiments has focused on maximizing the local electromagnetic field enhancement in or around an individual particle.||,10 This strategy fails to take into account the physics of propagation in the bulk medium where the same processes that give rise to enhancement also lead to increased extinction of both the illumination and the Raman-scattered light. Particles provide enhanced fields for Raman scattering, and the same particles form an effective medium with corresponding absorption. The importance of absorption of the Raman-scattered light is recognized in ref 12. However, they do not describe the necessary link and competition between the enhancement and the extinction. For example, it is commonly known to experimentalists that gold nanospheres exhibit a plasmon resonance at 520 nm and should produce a large local field enhancement when illuminated at 532 nm; yet, no appreciable Raman signal is observed upon 532 nm excitation commonly ascribed to interband transitions in gold.13 Away from the plasmon resonance frequency maximum, the Raman signal is again observed and actually increases as the excitation wavelength becomes longer.

In this Letter, we address the issue of extinction by a suspension of nanoparticles in SERS experiments through an effective medium approach. It is shown that extinction and enhancement are tied to each other and compete in such a way that peak signals are acquired off-resonance and that, at any wavelength, an optimal particle concentration exists to maximize the Raman signal. We provide verification of the model with experiments in which the particle concentration is varied.

Propagation of light in a dilute suspension of identical particles is well-approximated by propagation through a...
homogeneous medium with an effective refractive index \( \tilde{m} \), given by

\[
\tilde{m} = m \left[ 1 + \frac{2\pi \rho}{k^3} S(0) \right]
\]  

(1)

where \( m \) is the refractive index of the medium in which the particles are embedded, \( k = \omega/c \) is the wavenumber in the medium, \( \rho \) is the number of particles per unit volume, and \( S(0) \) is the scattering amplitude in the forward direction. The absorption coefficient in a medium with a complex refractive index is \( \alpha = 2k \text{Im} \tilde{m} \). For a suspension with small identical particles, the absorption coefficient is given by \( \alpha = 4\pi \rho k^3 \text{Re} \left[ S(0) \right] / mC_{\text{ext}} \), where \( C_{\text{ext}} \) is the extinction cross section of a single particle in the suspension, proportional to the real part of the forward-scattering amplitude. The attenuation of a well-collimated beam propagating through the effective medium is described by Beer’s law,

\[
(\text{absorption cross section}) = \rho C_{\text{ext}} \]

(2)

where \( C_{\text{ext}} = \sum_{N=1}^{\infty} N P_{N} \int d\omega' \{ E_{\omega'}(r, \omega) f(\omega') \}^{2} p(r, N) \)

(3)

where \( C_{\text{ext}}(\omega) \) is the Raman signal from one molecule absent the particle and \( G \) is the Raman enhancement factor and generally depends on \( p(r, N) \) and \( P_{N} \). For systems in which the particle placement is independent of the number of particles, the sum and the integral may be carried out independently, the sum yielding the average number of molecules \( \langle N \rangle \), and the integral resulting in a \( G \) independent of the number of molecules.

The enhancement factor for a small sphere of radius \( a \) \( (a \ll \lambda) \) with a uniform probability of molecule placement over the surface of the sphere can be calculated in closed form

\[
G(\omega, \omega_{0}) = \left[ \left( 1 + 2g(\omega_{0}) \right) \right] \left[ 1 + 2g(\omega) \right]^{2}
\]  

(4)

where \( g = (p^2 - 1)/(p^2 + 2) \), \( \omega_{0} \) is the frequency of the incident field, and \( \omega \) is the frequency of the Raman-scattered field.

The enhancement calculated by eq 4 is shown in Figure 1 alongside the extinction using the optical constants obtained by Johnson and Christy. It is clear that enhancement and extinction are closely linked and that when the enhancement is strong, the correspondingly strong extinction must be taken into account. The light falling on a single particle is attenuated by propagation through the suspension and arrives with amplitude attenuated by the factor \( \exp[-\int_{0}^{\infty} \text{d}z' \rho(z')] mC_{\text{ext}}(\omega_0)/2 \). The local Raman signal is then \( \langle N \rangle R(\omega) \rho(z) \exp[-\int_{0}^{\infty} \text{d}z' \rho(z')] mC_{\text{ext}}(\omega) \). In transmission mode, this signal must then propagate out through the medium to \( z = h \), and the intensity is attenuated by a factor of \( \exp[-\int_{0}^{\infty} \text{d}z' \rho(z')] mC_{\text{ext}}(\omega) \). The total signal is a sum over the signal from all particles, so that

Figure 1. (blue) The normalized extinction cross section \( C_{\text{ext}} \) from eq.

2. Extinction by gold spheres of 5 nm radius in aqueous suspension as a function of the wavelength of the incident light. (red) The normalized Raman enhancement, \( G(\lambda) \) from eq 4, versus the excitation wavelength for gold spheres of radius much smaller than the wavelength evaluated for a Raman shift of 0 nm.
The expression for the Raman signal is slightly altered versus the concentration for two different incident wavelengths; the radius of the spheres is 15 nm.

From this expression, it is seen that there are two competing processes that determine the size of the Raman signal, the enhancement, \( G \), and the extinction that results in an exponential decay of the signal. The same processes that increase the enhancement also increase the extinction. The attenuation due to extinction depends not only on the frequency but also on the concentration of the nanospheres. This is illustrated in Figure 2a, where it is shown that for increasing concentration, the peak of the signal is shifted farther away from the resonant wavelength. This result explains the absence of Raman signal at the plasmon resonance where extinction is so strong that no signal is observed.

In reflection mode, there is always a contribution from the front layer of the sample that is not attenuated, and therefore, the expression for the Raman signal is slightly altered

\[
R = \langle N \rangle A R^{(0)} G \int_0^h dz \rho(z) \exp[-h \int_0^z dz' \rho(z')] \times m C_{\text{ext}}(\omega_0) \exp[-h \int_0^h dz' \rho(z') m C_{\text{ext}}(\omega)]
\]

where \( A \) is the integral over the transverse beam profile normalized to the peak value, the effective transverse area of the beam. When the concentration \( \rho(z) \) does not depend on \( z \), the integrals can be computed in closed form with the result

\[
R = \langle N \rangle A R^{(0)} G \frac{e^{-m C_{\text{ext}}(\omega_0) h} - e^{-m C_{\text{ext}}(\omega) h}}{m C_{\text{ext}}(\omega) - m C_{\text{ext}}(\omega_0)}
\]

From this expression, it is observed that the Raman signal is equal to or very close to, \( C_{\text{ext}}(\omega_0) \), the optimal concentration becomes \( \rho_{\text{opt}} = 1 / (\omega \pi h) \). The strong nonlinearity with concentration that these competing phenomena impose on the recorded signal is also a caution in the development of practical assays and must be taken into account to correctly quantify results across samples. Hence, this physics-based analysis enables quantitative molecular imaging for SERS-based microscopy.

The model presented in this paper is validated by measuring the SERS signal of 4,4’dipyridyl Raman reporter molecules attached to gold nanoparticles. Spectra were acquired from the nanoparticles in suspension using a high-resolution Raman spectrometer (LabRAM, Horiba) with a 90 s acquisition time. The Raman shift from 1000 to 1800 cm\(^{-1}\) was collected at 10 cm\(^{-1}\) resolution with 10 mW laser power at the sample. Transmission Raman measurements were collected by focusing laser light through a 1 cm cuvette with a 50 mm focal length lens and collected with a 100 mm focal length lens to collimate the transmitted light and direct it to the spectrophotometer.

The integrated SERS signal under three different bands (476, 1076, and 1600 cm\(^{-1}\)) is compared for different concentrations of the gold spheres when excited at 632 nm. The SERS spectra from 4,4’dipyridyl for increasing concentrations is illustrated in Figure 3a. The three boxes indicate the Raman bands for which the signal is investigated as a function of concentration. The signal is obtained by integrating the Raman band of interest over the width of the box, as shown in Figure 3b. It is seen that there is a concentration that maximizes the signal. This optimal concentration, \( \rho_{\text{opt}} \), can be found by differentiating eq 6 and equating it to zero, giving the following expression

\[
\rho_{\text{opt}} = \frac{\ln[C_{\text{ext}}(\omega)/C_{\text{ext}}(\omega_0)]]}{\omega h [C_{\text{ext}}(\omega) - C_{\text{ext}}(\omega_0)]}
\]

When the extinction cross section, \( C_{\text{ext}}(\omega) \), equals, or is very close to, \( C_{\text{ext}}(\omega_0) \), the optimal concentration becomes \( \rho_{\text{opt}} = 1 / (\omega h) \).
not focus on achieving greater local enhancement but instead might strive for designs that maximize total signal by separating the single-particle enhancement and absorption peaks or otherwise tailoring the shape of the enhancement and absorption curves to maximize the gap between absorption and enhancement at frequencies away from resonance. A move toward using thin samples with large areas of collection is also suggested. We see that the signal is increased by moving away from resonance and, in some cases, by lowering the concentration of particles. While we focused on nanospheres, our results apply broadly to particle-based Raman enhancement with nonspherical particles as well.

**EXPERIMENTAL METHODS**

Gold nanospheres of 15 nm radius were synthesized by the boiling citrate method. For stability against aggregation, 100 mg of bis(p-sulphonatophenyl)phenylphosphine dihydro dipotassium salt (BSPP) was added to 100 mL of as-synthesized nanoparticles. The mixture was left to stir overnight (12–16 h), and excess reagents were removed by two centrifugation cycles (3000 RCF, 20 min). For 4,4′-dipyridyl complexation, 1 mL of 10 mM 4,4′-dipyridyl in water was added to 9 mL of BSPP-stabilized gold nanoparticles and left to complex overnight. Excess reagents were removed by two centrifugation cycles (3000 RCF for 20 min). For final purification, we diazoyzed the solutions in Thermo Scientific G2 Slide-A-Lyzer G2 cassettes against 4 L of Barnstead E-Pure water for 48 h.

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Notes
The authors declare no competing financial interest.

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