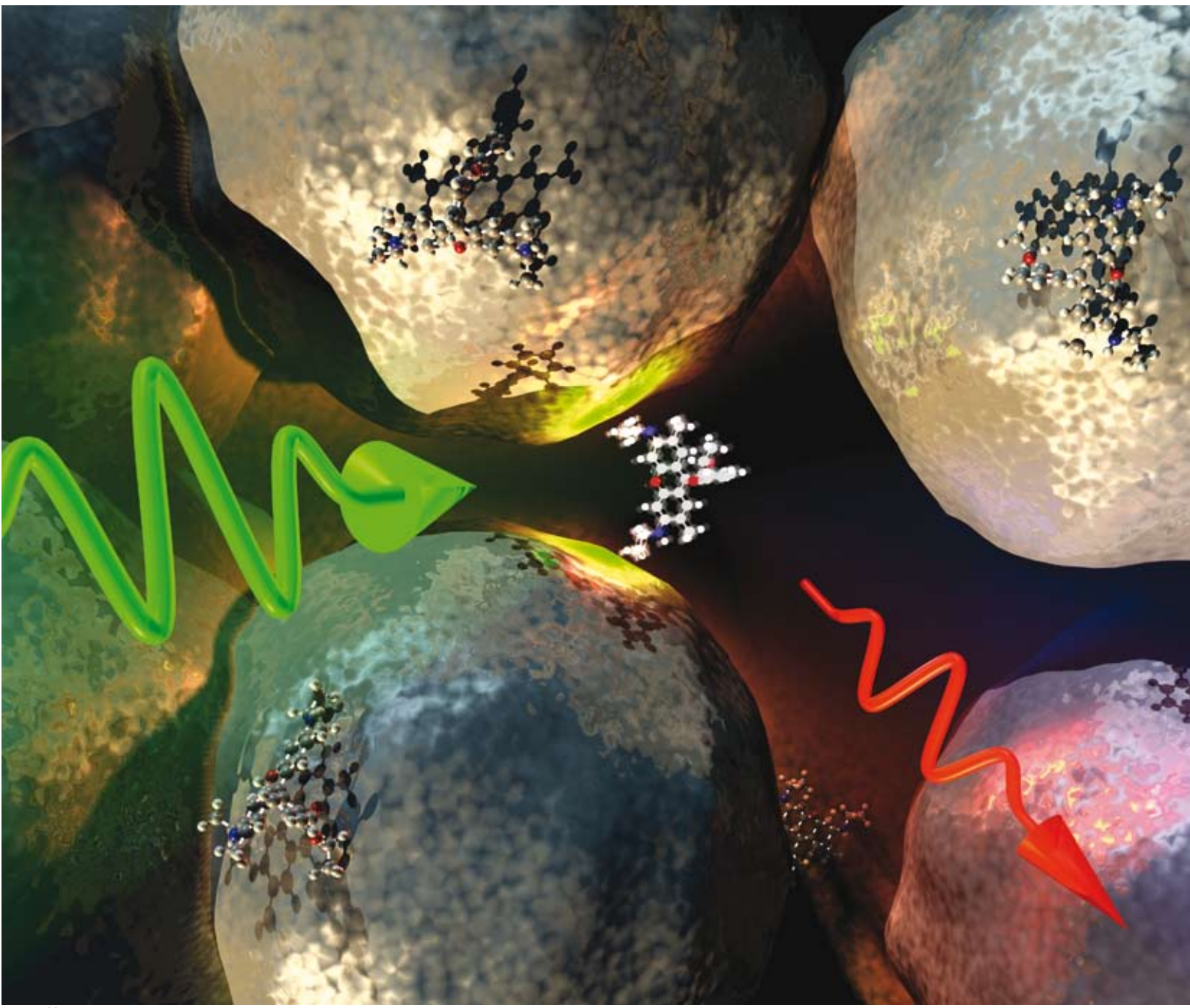
PCCP

Physical Chemistry Chemical Physics

www.rsc.org/pccp

Volume 10 | Number 40 | 28 October 2008 | Pages 6069–6200



ISSN 1463-9076

COVER ARTICLE
Etchegoin and Le Ru
A perspective on single molecule SERS: current status and future challenges

COMMUNICATION
Reid *et al.*
First observation of the elusive iodocarbene: ground state multiplicity and singlet–triplet gap of CHI

1463-9076(2008)10:40;1-Z

A perspective on single molecule SERS: current status and future challenges

P. G. Etchegoin and E. C. Le Ru*

Received 2nd June 2008, Accepted 21st July 2008
First published as an Advance Article on the web 9th September 2008
DOI: 10.1039/b809196j

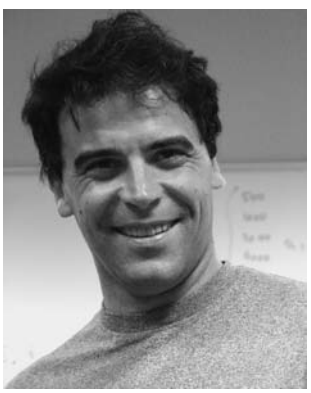
We present an overview of a some of the basic principles underlying current research in single-molecule surface-enhanced Raman scattering (SM-SERS). We summarize, by the same token, a series of conditions and characteristics that are common to most SM-SERS conditions, and discuss their implications for the understanding of data and for the comparison among different methods. We try to emphasize aspects of the problem that are not conventionally discussed in detail in the literature. In particular, we provide a full length discussion on the topics of: (i) the minimum SERS enhancement necessary to observe a single molecule, and (ii) the spatial distribution of the enhancement factor (EF) around hot-spots (which affects the statistics of SM-SERS events). A brief outlook into future perspectives of the different techniques used in SM-SERS and a few outstanding questions are also provided.

I. Introduction and overview

Surface enhanced Raman scattering (SERS) shares many aspects of other research topics in the fact that it has experienced "progress through contradictions and disagreements". SERS has been particularly notorious in that respect, for its conflicts of interpretations started, actually, with its discovery.[1–3] Over the years, many conflicts of interpretation have been resolved to a reasonable degree, for example, regarding the nature and origin of the SERS enhancement factors. But the story is arguably not yet complete on the fundamental aspects. Single molecule SERS (SM-SERS)—suggested originally in two pioneering papers in 1997[4,5]—has not been stranger to conflicts of interpretation either. In fact, many of the contradictions have survived to the present date. Some of the topics of discussion are still *very* basic; among them: (i) what constitutes a reliable proof of single molecule detection?; and (ii) can we extract other information from single molecule spectra (besides the mere fact that we are observing a single molecule)? The latter question is relevant to many interesting additional topics that we shall try to comment on briefly throughout the article. Among them: (i) can we infer the orientation of a single molecule on a surface with SERS?; (ii) can we use single molecule statistics to map the enhancement factor distribution?; (iii) is there evidence for subtler aspects of the SERS cross section (like non-radiative processes)?, *etc*.

In this feature article, we shall try to address some of these questions on SM-SERS. Nonetheless, we shall explicitly try to avoid reviewing the extensive literature in the field, and rather try to concentrate on *concepts* and *general aspects*, which are important as a starting point for a discussion on SM-SERS effects. Rather than repeating the arguments in the large number of SM-SERS papers that are appearing in the literature (including recent review articles specifically dedicated to the topic[6]), it seems more appropriate to concentrate on those aspects that are *not* conventionally explained in their full details (but make ideal material for a feature article). In this manner, we hope that our paper provides an additional insight but with a different

The MacDiarmid Institute for Advanced Materials and Nanotechnology, School of Chemical and Physical Sciences, Victoria University of Wellington, PO Box 600, Wellington, New Zealand. E-mail: Pablo.Etchegoin@vuw.ac.nz; Eric.LeRu@vuw.ac.nz



Pablo G. Etchegoin

Pablo G. Etchegoin is a Professor of nanotechnology at the MacDiarmid Institute and the School of Chemical and Physical Sciences, Victoria University of Wellington, New Zealand; with main interests in SERS, Raman spectroscopy of polymers, and isotopically substituted crystals (www.victoria.ac.nz/raman).

Eric C. Le Ru

Eric C. Le Ru is a Lecturer at the MacDiarmid Institute for Advanced Materials and Nanotechnology and the School of Chemical and Physical Sciences, Victoria University of Wellington, New Zealand; with main interests spanning SERS and related spectroscopies, plasmonics, and electromagnetism at the nanoscale (www.victoria.ac.nz/raman).

perspective. We refer the reader to the relevant papers when required, to gain further information into the specific details of individual topics. Moreover, with the aim of stimulating the discussion and debate around SM-SERS, we shall also express our views on some of the controversial issues.

II. Brief history of SM-SERS

The initial reports on SM-SERS were basically based on the concept of *ultra-low concentrations*[4,5] typically combined with very small sampling volumes (or areas), as achieved in Raman microscopes. Simple arithmetic indicates that there is less than one molecule on average in the scattering volume (by concentration) and, therefore, spectral fluctuations in the Raman signals are expected to be related to the "single-molecule character" of the signal. This approach, however, is not without some basic shortcomings, which are discussed in detail (for example) in the introduction of ref. 7. The two main shortcomings are: (i) the poor control and large uncertainties in the actual number of adsorbed molecule in the probed area/volume; and (ii) the poor statistics of SM-SERS events: only those molecules that adsorbed at a site of sufficient enhancement produce a detectable signal. Both issues contribute to ambiguities in the interpretation and hamper a systematic study of SM-SERS events.

Several improved strategies have therefore been devised to overcome some of the limitations of ultra-low concentration studies. These include: (i) the technique of Langmuir–Blodgett films,[8–11] providing a much better estimate of the concentration of molecules on the SERS substrate and, therefore, a new layer of confidence on the link between the observed signals and their single molecule character, (ii) the technique of tip enhanced Raman scattering (TERS)[12–14] which achieves the best possible control on the characteristics and spatial localization of a single hot-spot producing the signal, and (iii) the bi-analyte SERS technique (BiASERS),[7,15–19] which works basically as a *contrast* method, to pin down single molecule signals of one dye in the background of the signal produced by the other(s). This latter technique solves the problem of the poor statistics: it is possible to work at a relatively larger concentration, whilst retaining the ability to identify single molecule events.

In the next few sections, we shall overview the main characteristics and ideas behind these techniques. The main aim is to provide a basic map of current concepts in SM-SERS. Nonetheless, we shall start with some rudimentary (but very important) characteristics common to *all* SM-SERS situations, which will help us to clearly define the problem and set the ground for a brief comparison of the different methods toward the end.

III. SERS enhancement factors for single-molecule detection

A How much enhancement do we need to see a single molecule?

A question that lingered for almost a decade since the initial reports of SM-SERS is related to the *minimum enhancement* needed to see SM-SERS. Curiously enough, there are still conflicting (and erroneous) claims in the literature that associate the ability of SERS to detect single molecules to the existence of "remarkably high enhancement factors" (of the order of $\sim 10^{14}$). Such figures have been dramatically revised recently by several authors to more reasonable enhancement factors of the order of $\sim 10^7$–10^8.[6,10,17,20,21] It seems, therefore, appropriate to discuss this issue in some detail.

In order to address the question of the minimum enhancement to observe a single molecule, we need to answer first the question of: what is the minimum signal we can distinguish experimentally?, and then relate this value to a reference sample of known Raman differential cross section ($d\sigma/d\Omega$). Hence, the question *does not involve SERS at all* in the initial step; it is a *sensitivity* question (with respect to a known standard); we then know the minimum differential cross section we can measure $(d\sigma/d\Omega)_{min}$. This figure is, inevitably, somewhat "system-dependent", but it will not vary by more than a factor of ~ 10 among typical optimized Raman systems. If we then require $(d\sigma/d\Omega)_{min}$ to come from *one* molecule, we need to compare this value with the bare (*i.e.* non-SERS) differential Raman cross-section $d\sigma/d\Omega$ of the particular molecule we would like to detect. The ratio of $(d\sigma/d\Omega)_{min}$ and the bare $d\sigma/d\Omega$ gives then the *minimum enhancement factor* to observe SM-SERS in the given experimental conditions. Up to this point, no SERS experiment is involved yet. The answer will not be unique either; it depends crucially on the molecule under study (mostly through its bare Raman cross-section), but also on how far we can push the sensitivity in the first place, and how we define the "minimum detectable signal". A standard practice is to define a minimum sensitivity by using a signal-to-noise ratio (S/N-ratio) criterion. For example, a peak with an intensity above the root-mean-square (rms) amplitude of the noise by a predetermined factor (~ 2) is considered to be "a signal". Everything below is considered to be buried in the noise (or is statistically unreliable). However, there is plenty of room still to define the minimum sensitivity, as we shall explain in what follows.

The "minimum distinguishable signal" for a given typical Raman system with a CCD detector depends primarily on: (i) the incoming laser power density; and (ii) the integration time (τ). To these, one can add a long list of details like the exact optical layout of the collecting optics (numerical aperture (NA), confocality, *etc*) or the particular grating/CCD efficiency (blazing) of a specific system. These latter factors are—to a large degree—secondary, and they will not vary much among different systems (up to a factor of less than ~ 10, with the *numerical aperture* playing the most important role). SM-SERS experiments are typically performed mostly on Raman microscopes with high-NA objectives (to reduce the scattering volume as much as possible). As a result, there are "characteristic values" for the overall efficiencies in different reports that are not expected to be dramatically different. Accordingly, the main factors are: (i) how much power we deliver to the sample, and (ii) how long it is integrated for.

A weak signal (low S/N-ratio) can be integrated for longer to improve its visibility above the noise level. We can also use obviously more laser power. However, there are limitations to these parameters for SM-SERS applications. SERS signals in general, and SM-SERS signals in particular, tend to be unstable on time scales above ~ 1 s for a variety of reasons, which include: (i) changes in the SERS substrate configuration (Brownian motion of colloids, for example); (ii) photo-bleaching; (iii)

molecular surface diffusion; (iv) photo-chemical effects, *etc.* Many of these effects (or combinations thereof) are sometimes hidden under the broad label of "blinking". $\tau \sim 1$ s is, therefore, a characteristic "maximum" integration time (longer τ's may be acceptable, but at the expense of a lower laser power). Along these lines (and strongly linked with the previous list) the power density cannot be increased indefinitely without generating a variety of undesirable photo-induced processes; with photo-bleaching being the main obvious limitation for resonant or pre-resonant molecules. *The maximum usable power density is strongly linked to* τ. It also depends on whether *resonant* or *non-resonant* excitation is being used. However (as with the case of the minimum sensitivity of a system), it is possible to define some "maximum characteristic values" for the power density too; at least for standard SERS probes that are used in many studies. We do so in the following sub-section.

B A specific example

We choose to show some of the previous underlined concepts with a specific example of sensitivity determination and minimum SM-SERS enhancement estimation. To this end, we choose the case of SM-SERS in solution (Lee & Meisel Ag-colloids,[22] 10 mM KCl) for a specific molecule: a methyl ester version of rhodamine 6G (ref. 19) which we shall call RH6M. This particular molecule has optical properties identical to the most commonly used ethyl ester version (rhodamine 6G; RH6G) and can be isotopically substituted in the phenyl moiety to achieve the "partner" molecule d_4-RH6M which has been used recently for bi-analyte SERS studies.[19] In addition, there are a number of easily accessible Raman cross section standards in liquids, with which the $d\sigma/d\Omega$s can be quantified.

1. Power density and integration time limitations. We first define the limitations in integration time (τ) and laser power density (I_0) for this specific situation. The main limitation for increasing τ in these cases is *colloid diffusion* through the scattering volume. A typical τ to observe SM-SERS in this case is $\sim$0.1 s.[19] Moreover, if the waist w_0 of the (Gaussian) laser beam and the incident power P are measured, the maximum power density I_0 (at the center of the beam) follows from $I_0 = 2P/\pi w_0^2$.[17] The limitations in the maximum I_0 will depend on the specific probe, the nature of the substrate, and the laser wavelength (resonant/non-resonant conditions). It is also intimately linked to the chosen τ. The photo-stability of the probe is typically affected by both: an increase in I_0 and τ, but will be further affected by the SERS enhancement factor. This aspect must be assessed on a case-by-case basis. We report data here at $I_0 = 5 \times 10^9$ W m^{-2}; which corresponds to $P = 3$ mW (at 633 nm) focused through a $\times$100 immersion objective with a beam waist of $w_0 = 625$ nm.[17]

2. Minimum detectable differential cross section. Once the appropriate maximum I_0 and τ have been chosen (limited by the photo-stability of the probe and/or the changing nature of the SERS substrate), we can now define $(d\sigma/d\Omega)_{min}$. Fig. 1(a) shows two Raman spectra of RH6M and d_4-RH6M taken under the described experimental conditions (in a bi-analyte SERS experiment[7]). These signals are indeed single molecule,

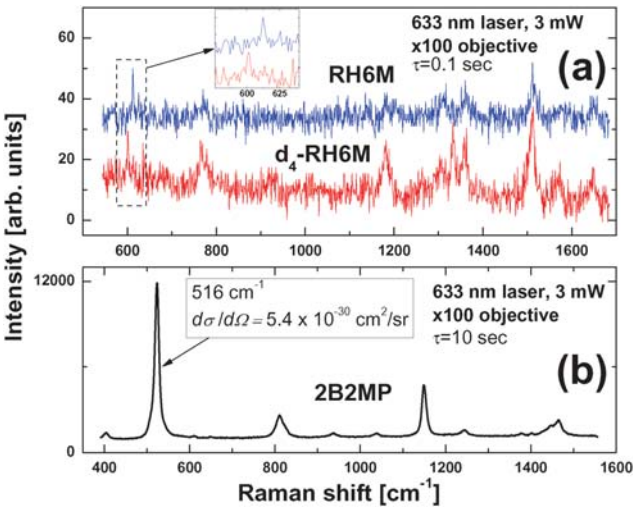


Fig. 1 (a) Two examples of "minimum detectable signals". The examples shown here are indeed single molecule events using the bi-analyte technique[7] with isotopically substituted dyes (RH6M and d_4-RH6M from ref. 19), but this distinction is irrelevant as far defining the minimum detectable signal. The inset in (a) shows the region of the low frequency "fingerprint" modes of RH6M and d_4-RH6M, which define the *minimum detectable signal*. Once this value has been defined (by a S/N-ratio criterion), it has to be compared to a standard of known $d\sigma/d\Omega$ and known number of molecules in the scattering volume. We use here the 516 cm^{-1} mode of 2-bromo-2-methyl-propane,[17] shown in (b), which has a well characterized differential cross section of $d\sigma/d\Omega = 5.4 \times 10^{-30}$ cm^2 sr^{-1}. Hence, the comparison between the data in (a) and (b) defines the minimum differential cross section $(d\sigma/d\Omega)_{min}$ we can measure. This value is afterward required to come from *one* molecule in SM-SERS. The ratio of $(d\sigma/d\Omega)_{min}$ to the bare differential cross section of the molecule we want to detect defines the *minimum enhancement factor* needed to see SM-SERS. See the text for further details.

but this distinction is irrelevant at this stage; we are only interested in the sensitivity of the system. The two signals in Fig. 1(a) are chosen for their "weakness", but they could be coming from any other arbitrary Raman signal. They satisfy the criterion that the signal is $\sim$2 times the rms of the noise level for the fingerprint modes at 612 and 600 cm^{-1} of RH6M and d_4-RH6M, respectively.[19] These modes are explicitly shown in the inset of Fig. 1(a). From the integrated intensity of these peaks we define our minimum detection level to be $\sim$350 counts s^{-1} in the given experimental conditions ($\tau = 0.1$ s, $P = 3$ mW, $w_0 = 625$ nm, laser wavelength = 633 nm). This can now be compared with the integrated intensity of a standard; we take the 516 cm^{-1} mode of 2-bromo-2-methyl-propane (2B2MP)[17] shown in Fig. 1(b). This mode has $d\sigma/d\Omega = 5.4 \times 10^{-30}$ cm^2 sr^{-1}—fully characterized in ref. 17—and it produces an integrated signal of $\sim$1370 counts s^{-1} under identical experimental conditions. If the effective scattering volume is accurately measured (13 μm^3 in our system[17]), and taking into account the density (1.22 g cm^{-3}) and molecular weight (137 g mol^{-1}) of 2B2MP, this signal can be viewed as that produced by $\sim 7 \times 10^{10}$ molecules subject to the same power density I_0 as that in the center of the beam[17] (this is, in fact, the meaning of an "effective scattering volume"). This means that an observable signal above the noise in Fig. 1(a)

corresponds to a differential cross-section of $(d\sigma/d\Omega)_{min} = 9.5 \times 10^{-20}$ cm^2 sr^{-1}. This figure is mostly defined by the sensitivity of the system and the combination of I_0 and τ. The width and shape of the Raman peaks may affect slightly this value (by a factor of ~2–3), but it is otherwise independent of the exact molecule we are measuring.

3. Minimum SERS enhancement factor. If this $(d\sigma/d\Omega)_{min}$ is to be produced by a *single* molecule, we need an enhancement factor F from the bare $d\sigma/d\Omega$ of the molecule we want to detect. The enhancement factor, therefore, bridges the gap between the (typically small) bare cross section of a single molecule and the minimum cross section we can observe. Here we arrive at the very important step of normalizing with respect to the real (experimentally determined) bare $d\sigma/d\Omega$ of the probe; a step that has not always been properly considered.[17] Among other things, bare $d\sigma/d\Omega$s of different SERS probes can differ by many orders of magnitude,[17] depending on their structure, size, and—most importantly—resonance condition. If the normalization is not properly done, errors of several orders of magnitude can arise in the estimation of F. For the 612 and 600 cm^{-1} of RH6M and d_4-RH6M we know the bare $d\sigma/d\Omega$s at 633 nm, and both are $d\sigma/d\Omega \sim 0.5 \times 10^{-27}$ cm^2 sr^{-1}.[19] Therefore, for a single molecule of either RH6M or d_4-RH6M to produce $(d\sigma/d\Omega)_{min} \sim 9.5 \times 10^{-20}$ cm^2 sr^{-1} and be observable above the noise level, we need a minimum enhancement factor of $F_{min} \sim 2 \times 10^8$.

C Minimum and maximum SERS enhancement factors for SM-SERS

It is worth reminding again that F_{min} is *not* unique and in particular strongly dependent on the analyte under consideration and the excitation wavelength. It can moreover (in principle) be lowered by improved experimental conditions. Under special circumstances, in particular in resonant Raman conditions where the bare $d\sigma/d\Omega$ can be as large as ~10^{-24} cm^2 sr^{-1},[21] it could go down to $F \sim 10^5$–10^6 (depending on the photo-stability). This is an important conclusion: enhancement factors of the order of ~10^8 are more than enough to see single molecules in SERS, at least for resonant or pre-resonant probes with bare $d\sigma/d\Omega$s of the order of ~10^{-27} cm^2 sr^{-1}. Similar conclusions have been reached by other authors,[6,21] including fully theoretical arguments.[20] Following the same reasoning, the detection of a single non-resonant molecule, with typical differential Raman cross-sections of ~10^{-30} cm^2 sr^{-1}, would require a minimum enhancement of the order of 10^{10}–10^{11}.

As a matter of fact, there is substantial experimental and theoretical evidence for SM-SERS enhancement factors up to maximum values of the order of ~10^{10}–10^{11};[17,23–26] with values perhaps close to ~10^{12} under some truly optimized conditions. These Fs can be fully justified with conventional electromagnetic theory in the $|E|^4$-approximation.[27] Enhancements of the order of ~10^{10}–10^{11} are typical of (so-called) *hot-spots*, arising for example at gaps between two closely-spaced metallic objects. Hot-spots play an important part in SM-SERS, and they are the aspect of the technique that can be best controlled in TERS. Note that enhancement factors of the order of ~10^{11} can, in principle, compensate for molecules with intrinsic $d\sigma/d\Omega$s that are ~10^3 times smaller than the example of RH6M. Therefore, it is indeed possible to achieve SM-SERS even for *non-resonant molecules*, for which the bare $d\sigma/d\Omega$s are of the order of ~10^{-30} cm^2 sr^{-1}. The detection of non-resonant molecules, however, is normally not pursued for a variety of experimental complications that are not related to the enhancement factor itself. Contamination problems and the presence of unwanted photo-products with comparable cross sections to the ones we want to detect[28] are some of the experimental issues that make single molecule detection of non-resonant species a lot more challenging than a simple "rescaling" of the problem.

D Comparison with fluorescence

Fluorescence spectroscopy is often considered as the "rival" of SERS when it comes down to SM detection, and much has been written on the "competition" between both. As far as the minimum differential cross section for detection is concerned, there is an important difference between the two that is worth highlighting, namely, the importance of the *spectral width* of the emission. The differential cross section $d\sigma/d\Omega$ is a *spectrally integrated* quantity. If we need the signal to emerge above the rms of the noise level, what we need to compare is the *spectrally resolved* differential cross sections in a given range. In other words, the fact that Raman peaks are much narrower in spectral width compared to fluorescence plays in favor of the Raman effect at the time of defining $(d\sigma/d\Omega)_{min}$. A typical width (FWHM) of a Raman peak of a dye will be ~15–20 cm^{-1}, while a typical fluorescence emission will span for ~1000–2000 cm^{-1}. Thus, SM-SERS detection is in principle possible for $d\sigma/d\Omega$s of the SERS peaks which are a factor of ~10^2 times lower than the differential fluorescence cross-sections required for SM-fluorescence. In the previous section, for example, we determined that $(d\sigma/d\Omega)_{min}^{Raman} = 9.5 \times 10^{-20}$ cm^2 sr^{-1} ~ 10^{-19} cm^2 sr^{-1}. This implies that in the same experimental conditions we will have $(d\sigma/d\Omega)_{min}^{fluo} \sim 10^{-17}$ cm^2 sr^{-1}. This is not a problem for most (good) fluorescent dyes, which have differential fluorescence cross sections of the order of ~10^{-17} cm^2 sr^{-1} or more (*i.e.* total fluorescence cross sections ~10^{-16} cm^2 or above). Note also in this context that for a freely diffusing fluorophore, the diffusion time in the scattering volume can be quite small (smaller than colloids in particular, due to the mass difference). Therefore, τ must be reduced, even though this can be partly compensated by a larger I_0.

We hope this first section of the paper clarifies the long standing (inaccurate) "myth" in SM-SERS that enhancement factors or the order of ~10^{14} are needed to see single molecules. These latter values are not only inaccurate, but also very difficult to justify theoretically; Fs that are 10^6 times smaller than that are already enough for SM-SERS. This reinforces a conclusion put forward in ref. 7, which states that *SM-SERS is actually a lot more common than was originally assumed.*

IV. General properties of large SERS enhancements

A The spatial distribution of SERS enhancements

Another aspect that is not sufficiently emphasized in most SM-SERS reports (and is arguably universal) is the issue of the spatial distribution of the enhancement and the presence of a long-tail distribution. One way or another, SM-SERS is always associated with large enhancements above $\sim 10^7$–10^8, possibly up to $\sim 10^{10}$–10^{11}. We emphasize here a few very general properties of the spatial distribution of F at hot-spots, which have very important consequences for the statistics of SM-SERS signals.

With some limitations (that are well understood[27]), a good yardstick figure for F can be obtained from the so-called $|E|^4$-approximation. Fig. 2 shows an example of the spatial distribution of the enhancement factor F within this approximation at a gap formed by two spheres (colloids) with the dielectric properties of gold.[29] The calculation is performed in the electrostatic approximation, which tends to over-estimate F except in the smallest sized objects, but none of the main conclusions here depend on this. Similar results can be obtained with exact generalized Mie theory as in ref. 26. The calculation in Fig. 2 is performed for polarization parallel to the main axis of the dimer. The main point here is the drastic spatial variation of F over the surface of one of the colloids (where a single molecule could be adsorbed). Furthermore, as can be appreciated in Fig. 2, the enhancement can vary by an order of magnitude over distances comparable to a few molecular dimensions ($\sim$2–4 nm). The calculation is for λ = 559 nm, which provides the maximum value for F on the axis of the dimer (on the surface of the colloid) for this particular geometry in the electrostatics approximation, but the conclusions are fairly general. Note that a maximum F of $\sim 10^8$ is normal for gold at this wavelength. Gold is more "lossy" than silver in the blue/green part of the visible range. It is only when localized surface plasmon resonances are pushed above (in wavelength) $\sim$600 nm that Au becomes comparable to Ag and delivers maximum Fs of the order of $\sim 10^{10}$–10^{11}. In general, one would perform experiments in gold for lasers with wavelengths larger than 600 nm, to profit from these larger enhancements.

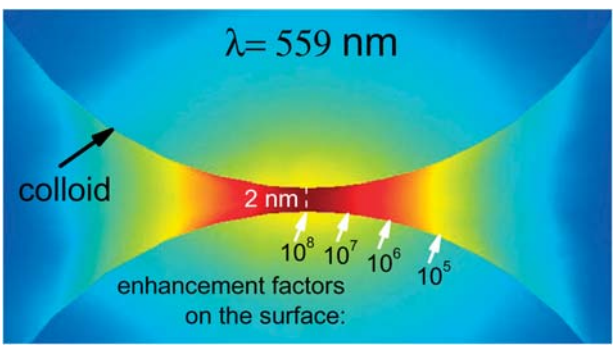


Fig. 2 Enhancement factor (F) distribution in the region of the gap (2 nm) between two gold colloids (radii = 30 nm) calculated in the electrostatic approximation with finite-element modeling (for polarization along the vertical axis of the dimer). The dielectric properties of gold are taken from ref. 29 and F is calculated in the $|E|^4$-approximation.[27] The enhancement is presented in a logarithmic color scale, with red (blue) being the most (least) intense. The calculation is performed at λ = 559 nm, where F exhibits its maximum value ($\sim 10^8$) at the surface of one of the colloids. Several values of F along the surface at different distances from the vertical axis are shown; they can vary substantially over distances comparable to a characteristic molecular size. This is a universal characteristics of gap hot-spots resulting in a long-tail probability distribution that determines most of the basic characteristics of SM-SERS statistics.

The example in Fig. 2 is far from unique. Fig. 3 displays an equivalent example of hot-spot for a gold tip over a planar gold surface (a typical situation in TERS). Calculations are also performed for vertical polarization (along the axis) in the electrostatic approximation. Several values of F are explicitly shown on the underlying surface; these would be the Fs experienced by molecules lying on the surface in a TERS experiment.[12,13] The SERS enhancement in Fig. 3 decreases by a factor of $\sim$2 at a distance of 1.7 nm from the maximum (at the axis of the tip) on the surface. This position can still be

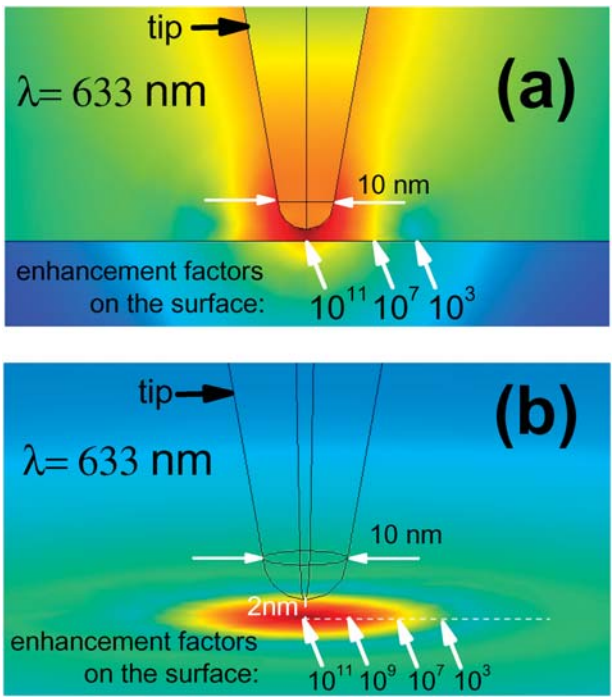


Fig. 3 TERS enhancement factor in the $|E|^4$-approximation for a gold tip on top of a gold surface. The tip is separated by a 2 nm gap from the surface (in air). The enhancement factors are calculated in the *electrostatic approximation* at 633 nm with the parametrized dielectric function of gold from ref. 29 and vertical polarization. The enhancement factor is plotted on a logarithmic color scale as in Fig. 2. In (a) we show a side view of the tip (10 nm in diameter) with the enhancement distribution in the plane cutting the tip through its center, while (b) displays an "elevated view" over the surface underneath the tip, and displays the enhancement factor on the surface with the same color scale. This latter plot would be the distribution of Fs experienced by molecules on the surface. Several specific values of F at different positions are explicitly given. F decreases already by a factor of $\sim$2 at a distance of $\sim$1.7 nm from the center, thus showing the extreme sensitivity to position; a widespread property of gap plasmon resonances producing hot-spots which results in a long-tail distribution probability for F. See the text for further details.

considered to be "under the tip", taking into account that its radius is 5 nm. Further away at $\sim$7 nm from the center of the tip on the surface, F has decayed already by an order of magnitude. In the case of the dimer in Fig. 2, the signal at the hot-spot (in the center of the gap on the surface) drops by a factor of $\sim$2 at a distance of $\sim$2.5 nm from the center (along the surface). Large variations in enhancements therefore occur over distances that are comparable to a characteristic size $\sim$1 nm of a dye molecule (like RH6M, for example).

One important consequence is that—in order to have a homogeneous SM-SERS signals that will allow a "quantization" of the intensity (*i.e.* the observation of a Poisson distribution of SERS intensities)[5]—molecules would have to be positioned with a precision comparable to their size, and in hot-spots that are *always* the same (*i.e.* they have exactly the same maximum F within a factor of less than $\sim$2).[30] This situation simply does not happen in reality, and it is one of the main (but not the only one) counter-arguments against the interpretation of SM-SERS evidence through "quantized intensities" as extensively discussed in ref. 30. Further discussions on this specific topic for the particular case of SM-TERS signals are provided in ref. 31 and 32.

The data in Fig. 2 and 3 are complemented with the additional information in Fig. 4, where we show: (i) the wavelength dependence of F for the dimer and tip (in Fig. 4(a)), and (ii) the distance dependence of the enhancement from the maximum (the main symmetry axis) for both cases (Fig. 4(b)). Distances are measured along the surface (where molecules would be adsorbed). The data in Fig. 4(a) justifies the choice of wavelengths in Fig. 2 and 3; *i.e.* the enhancement distribution is studied at the maximum values of F for the dimer or tip, respectively. Note that the resonance for the tip case (Fig. 4(a)) is shifted further into the red compared to the colloids and, hence, the maximum F increases to $\sim 10^{11}$. As mentioned before, this is due to Au becoming less "lossy" in this range, and delivering Fs which are comparable to those obtained in Ag. An interesting comment on Fig. 4(b) is that the spatial distribution of the enhancement is actually not much dependent on wavelength. Despite the fact that we are showing the spatial distributions for the wavelengths where the maximum F occurs (in both cases), similar decays can be obtained at other wavelengths. The drastic spatial variations is *not* a characteristic of the wavelength at which F is maximum, but rather a general property. Note also that in Fig. 4(b) the enhancement decreases away from the hot-spot up to a certain value where it starts increasing again. This type of behavior also appears in the exact solution of the problem for dimmers studied in ref. 26. Such a detail is only visible on a log-scale, as shown in the plot, but is otherwise irrelevant to the SERS signals, since it relates to EFs that are $\sim$8 orders of magnitude smaller than those obtained around the hot-spot.

In summary, the two important aspects highlighted in these simple examples of enhancement distributions are the fact that: (i) the surface area where the largest enhancements occur (hot-spot) is small compared to the total surface area of the substrate, typically $\sim$1% or less, and (ii) F can vary drastically as a function of position. These conclusions are robust and independent of the approximations used to solve the electromagnetic problem. They are also not restricted to a single

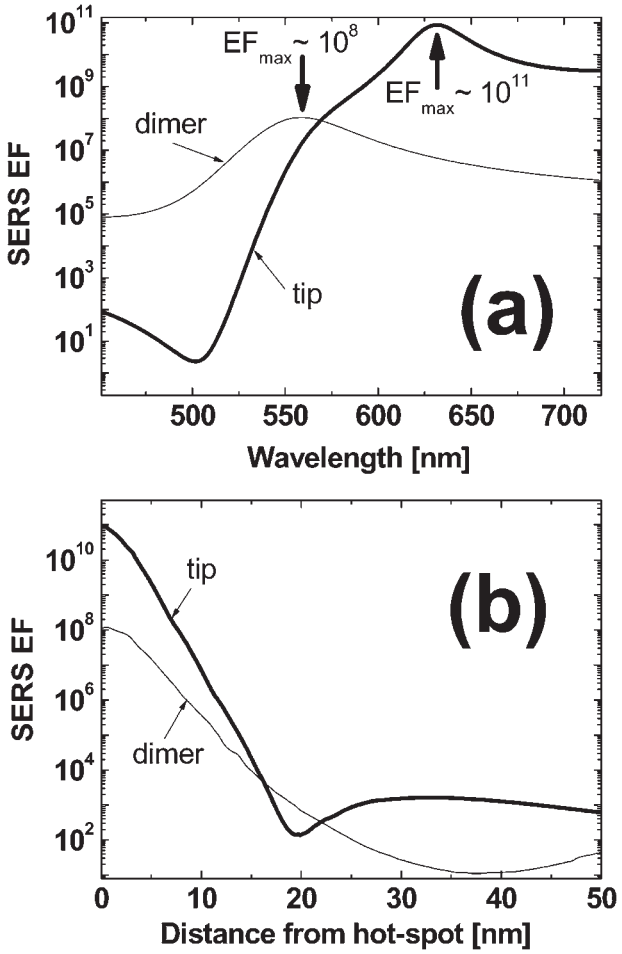


Fig. 4 (a) Wavelength dependence of the enhancement factor F at the gap (axis) on the surface (in both cases) for the dimer in Fig. 2 and the tip over a plane in Fig. 3. Note that the gap coupled plasmon resonance of the tip is pushed further into the red (to $\sim$633 nm) and, therefore, it becomes considerably stronger than the one on the dimer. This can be accounted for by the lower absorption of Au in this range.[29] In (b) we show the spatial distribution of the enhancement F as a function of distance (on the surface) from the axis. Note that the vertical axis is logarithmic. In both cases, F decreases by a factor of $\sim$2 at distances comparable to typical molecular dimensions. At a few nanometers further away from the axis ($\sim$10 nm), F can be more than an order of magnitude smaller than the maximum.

peculiar geometry. The two cases shown here (for pedagogic purposes) are only two examples of characteristics that can be considered truly general of electromagnetic hot-spots. Any attempt to understand the physics of these hot-spots has to address, in one way or another, their huge spatial inhomogeneities and the way this characteristic affects the statistics of SM-SERS signals.

B Extreme statistics at hot-spots

An important problem in the understanding of the statistic of SM-SERS events is the inversion problem of the enhancement distribution into a probability density. Assuming, then, that molecules will be randomly distributed at different positions on the surface, this will be the probability density that dictates the observed statistics of events. As an illustration, let us

consider the case of the tip on top of the surface in Fig. 3 (the case of the dimer of colloids has been extensively studied in ref. 26). Consider individual molecules spread at random over the surface of the plane in Fig. 3. We restrict ourselves to distances within a radius of 15 nm from the central axis (on the surface), for this is the region where the interesting (most intense) enhancements occur. Fig. 5 shows the probability density distribution $p(F)$ *vs.* enhancement F obtained from the numerical solution of the problem. The probability distribution is plotted on a log–log scale to reveal its long-tail nature. The analogies with the cases of dimers studied by exact Mie theory in ref. 26 are obvious. The probability distribution is determined by two main parameters: (i) the exponent "k" (see Fig. 5), and (ii) the maximum enhancement (cut-off). The exponent k, for example, is remarkably similar for many cases (geometries and materials); a point raised already in ref. 26. The cut-off, however, is more dependent on the specific resonance/non-resonance condition.

Fig. 5 also shows the distances (from the center of the tip) at which different Fs happen on the surface. Once again we see how $p(F)$ decreases drastically for decreasing enhancements. This is a general characteristic of long-tail distributions. The truncated Pareto distribution, discussed in ref. 26 in the framework of hot-spots produced by dimers, is one such example. The Pareto distribution was introduced to describe economic systems and led to the so-called "Pareto principle" which states that "20% of the population holds 80% of the wealth". In the SERS enhancement factor problem this statements translates into: "a small fraction of the surface holds the largest part of the enhancement". As a matter of fact, the hot-spot distribution is a lot more extreme than those used in economics: this was stated on a specific example of a hot-spot in ref. 26: "2% of the (randomly adsorbed) molecules contribute to 98% of the SERS signal". Very recently, there have been experimental attempts to measure the long-tail distribution of enhancements in SERS.[33]

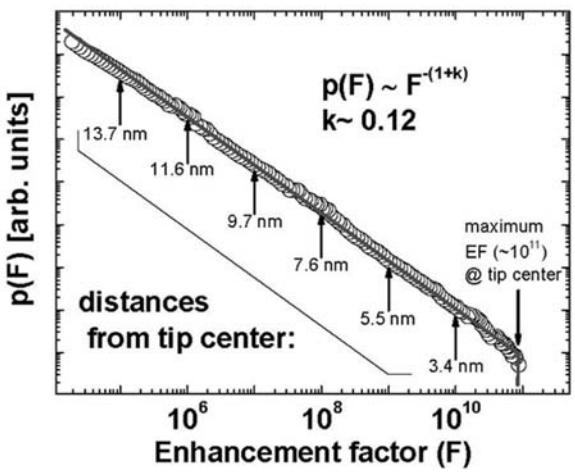


Fig. 5 Probability density $p(F)$ as a function of the enhancement for the "tip over the plane" problem in Fig. 3. Molecules are randomly distributed over the surface (within a circle of radius 15 nm) and the histogram of enhancements is evaluated from the numerical solution of the electrostatic problem at $\lambda = 633$ nm. Note that both axes are logarithmic. The long-tail nature of the distribution ($p(F) \sim F^{-(1+k)}$, with $k \sim 0.12$) is obvious from the plot. The distribution stops however at a maximum value (cut-off) which in this case is the enhancement directly underneath the tip (on the surface). We also provide the distances (from the central axis) at which a specific F occurs. A rapid decrease of F away from the maximum is a general characteristic of gap hot-spots. The solid line represents a fit with a truncated Pareto distribution.[26]

Due to the generality of these phenomena, an heuristic approach would be to assume the existence of a long-tail distribution (with a certain exponent and a cut-off) in most hot-spot situations and try to deduce the consequences imposed by its presence. Long-tail distributions have very "unusual" properties compared to more standard ones (like, say a Gaussian distribution). For example, they can have a standard deviation that is much larger than the mean! This implies that the magnitude of signal fluctuations can also be much larger than the mean signal. The extreme nature of the fluctuations of SM-SERS is something that will not be strange to most practitioners in the field. Their origin, is intimately linked to the peculiar nature of the enhancement factor probability distribution. A related key issue is that of *sampling*; when fluctuations are comparable to the mean, insufficient sampling with a few hundred spectra might not be good enough to gain any statistical confidence on the results, as shown extensively in ref. 26. The issue of sampling has also been a matter of debate around the claims of quantized SM-SERS intensities, as further discussed in ref. 30.

V. Experimental approaches

Having introduced some of the main aspects of the problem, we are now in a good position to revisit briefly the main techniques mentioned in section II for SM-SERS and pinpoint quickly their advantages and limitations in the light of these general properties. Let us first briefly consider the possible approaches to the SM-SERS problem.

A Single molecule detection *vs.* single molecule analysis

There are basically 3 different scenarios that can arise in SM-SERS, depending on whether the emphasis is put on the mere demonstration of single-molecule detection sensitivity or on the use (analysis) of the information that this type of spectroscopy can provide (*i.e.*, one step further from the demonstration of single molecule sensitivity). We can tentatively divide the problem into:

1. Problem 1: demonstrating SM-SERS detection. This refers simply to the demonstration that single molecule detection is possible with SERS; even if the occurrence of SM-SERS events is random, uncontrollable, or very sparse for a combination of reasons. This is in fact a pre-requisite before investing efforts into the next two problems.

2. Problem 2: identifying a large number of SM-SERS events. This problem goes one step further and it basically aims at obtaining as much statistics as possible on SM-SERS events to gain additional insight into the problem, for example the nature of the hot-spots producing these events, the enhancement distribution, or to study molecular properties such as adsorption geometry or homogeneous broadening.

3. Problem 3: SM-analysis or detection of every single molecule. A more ambitious (arguably more difficult) problem is to aim at the detection of *every* possible molecule of a specific type for analytical purposes. This require exact positioning of the target molecule at the hot-spot position (and therefore also a precise control of its geometry). These capabilities would transform SM-SERS into the ultimate analytical tool; but unfortunately only partial answers to the question exist at present.

With this in mind, let us now revisit the various experimental approaches to the SM-SERS problem.

B Ultra-low concentrations

The pioneering studies of ultra-low concentrations[4,5] have importance for historical reasons and are aimed at addressing *problem 1* in the previous list. However, after more than ten years of research, it is well understood that its main conceptual simplicity and intuitive appeal are, at the same time, its greatest weakness. A big part of the problem is the extreme statistics at hot-spots (section IVB) which renders any study based on the ''scaling with concentration'' almost impossible. A *very* small proportion of the surface of the SERS substrate contributes to hot-spots capable of showing SM-SERS. The convolution of ''rare sites'' with ultra-low concentrations produces a very poor statistical reliability. This problem was evident for example in ref. 4. Only a small proportion of particles (so-called ''hot'' particles) exhibited a detectable SERS signal, most likely the ones where a molecule adsorbed by chance at a hot-spot. This opens up the possibility of alternative interpretations of the results. There is for example a chance that we are observing exceptional places, and their statistical ''rarity'' is precisely a consequence of this. This is particularly hard to rule out in SERS substrates like Lee & Meisel colloids,[22] which are notorious for their size and shape inhomogeneities. Hence, it is perfectly reasonable to doubt the statistical significance of rare events, for it is possible that they come from equally ''rare'' nano-particles that accumulate more dye than others for a combination of reasons (surface area, shape and overall size, surface ''traps'', *etc*). These issues are exacerbated by the fact that exact concentration estimates (on which the SM-SERS identification ultimately relies) can be difficult and the source of further uncertainties. Nevertheless, the ultra-low concentration approach did go some way to convince researchers that *problem 1* of the previous list had been addressed, and this triggered an enormous interest in the field and a revival of its study.

C Langmuir–Blodgett films

The technique of Langmuir–Blodgett films (LB-films)[8–11] represents a definite step forward to address *problem 1* in the previous list. Due to the fact that the concentration of adsorbed analyte is precisely controlled by the LB-film (which ensures an even spatial distribution). This provides a much better confidence in the interpretation of the results, but still within the ultra-low concentration approach and therefore susceptible to problems of poor statistics.

D Bi-analyte SERS

1. Principle. The bi-analyte method (extensively treated in ref. 7, 15, and 19) aims at solving *problem 2* of the previous list, and by the same token provides a more direct evidence for *problem 1*. It can, moreover, be combined with the LB-film approach.[10] The principle is to increase the molecule concentration in order to improve the statistics and the sampling. The aim is to target a concentration that ensures that one molecule (on average) is adsorbed at most hot-spots. But we then potentially loose the ability to know if the signal truly originates from a single molecule at a hot-spot, rather than from many molecules at positions of lower enhancements. This is where the concept of using *two* analytes as a contrast method comes into play. By studying the statistics of the *relative intensities of the two molecules*, it is possible to infer the single-molecule nature of the signal, irrespective of the enhancement factor distribution over which we have little control.

This remains a statistical approach, and the SM-nature cannot be inferred from a single event, but only from the statistics of many spectra, as follows: At sufficiently large analyte concentrations, the signals originate from many molecules, and should therefore be (statistically) a mixture of both analyte spectra (''mixed spectra''). Contrariwise, at sufficiently low concentrations only single-molecule events should be observed (albeit with a poor statistics). All spectra are then those of either one analyte or the other (''pure spectra''). In practice, one should start from a low (or ultra-low) concentration and gradually increase the two-analyte concentrations until ''mixed'' spectra start to appear. This maximum concentration is then the best one for a SM-SERS study for the system under consideration. Because of the convolution between hot-spot localization and random analyte adsorption, the concentration is much larger than the ultra-low values that would be required in a standard single-analyte approach. Hence, it allows for a much better sampling and statistical analysis of events. The identification of the various regimes from single-molecules to many-molecules using bi-analyte methods are discussed further in ref. 15.

It should be re-emphasized perhaps that one of the important contributions of the bi-analyte method has been not only in providing a proof of single molecule sensitivity[7] but also in the quantification of the effect.[17] The SERS signal that is measured in given experimental conditions is always a result of the convolution of the distribution of SERS EFs (or cross sections) with the number of molecules and their positions in this distribution. Based on the concepts of the previous sections (in particular the extreme nature of the spatial distribution of the enhancement), it is impossible in general to deduce precisely how many molecules are contributing to the signal. The bi-analyte method provides a statistical solution to this problem, thus allowing the identification of cases where we can have a higher confidence on the number of molecules contributing to the signal (one!). All quantifications of the SERS differential cross sections follow from this fact; it also demonstrates why it has been very difficult for many years to have definite answers about the magnitude of the enhancement, when the number of molecules contributing to the signal

was unknown or was only roughly estimated from concentration arguments.

As before, the bi-analyte technique does not address *problem 3* of the previous list. We have typically no control over the position of the molecules to be analyzed. The method still works through ''blind statistics'' (though with a reliable sampling) to infer the single molecule nature of the signals.

2. Isotopic editing. Isotopic labeling[18,19] has recently added a new level of sophistication to bi-analyte SM-SERS by providing probes that have *identical* surface chemistries for all practical purposes while having at the same time distinguishable Raman signals. Isotopic editing of dyes had been tried before in the context of SERS,[34] but without emphasizing the SM-SERS aspects of the problem. Isotopic editing could provide new standards in years to come in the practical implementation of SM-SERS through bi-analyte techniques. An extensive discussion on these topics is provided in ref. 19.

3. Analysis tools. The heavy reliance on statistics over thousands (and in most cases tens of thousands) of spectra to sample properly some of the main characteristics of the SM-SERS problem (like the enhancement distribution) calls for the development of special analysis tools that can extract the information in a simple and unbiased way. Modeling of the spectra through fits in situations of widely varying intensities (characteristic of the enhancement distribution, section IVA) and complicated line-shapes of the peaks can be particularly problematic. Techniques like the modified principal component analysis (MPCA)[15] have been specifically design to gain information from bi-analyte SM-SERS spectra without any assumption or modeling of the peaks. This method is an *add-on* to the bi-analyte SERS technique, but by no means it is necessarily linked to it. Analysis methods like MPCA are not a prerequisite to understand and analyze SM-SERS data, but rather provide an alternative tool with which statistical information can be gained in a fast and unbiased way.

E Tip-enhanced Raman scattering (TERS)

One of the milestones of SM-SERS has been undoubtedly the demonstration of TERS,[12–14] which added a new layer of confidence and understanding, and could play a role in tackling *problem 3* of our list. TERS is, in fact, the most advanced type of control to date over the characteristics of a single hot-spots producing a SERS signal. This property gives it a life of its own and opens the very interesting possibilities of combining SERS with some from of tip-based microscopy (STM, or AFM). TERS is in some ways complementary to the LB-films approach: rather than controlling the molecule concentration, we can now control the position (and characteristics) of the hot-spot. There remains the problem of having the molecule precisely at the hot-spot (or rather the hot-spot at the molecule). In practice, some sort of compromise can be achieved (by scanning the tip, for example), but this makes *problem 2* particularly acute. It is very difficult to gain statistics (even over a few hundred events) with TERS, but this could in principle be solved by combining it with a two-analyte approach.

It is worth highlighting the fact that TERS is a technique that truly measures the properties of a *single spot* on the SERS substrate. If different molecules are seen always with the tip at the same position, what we are measuring is the true statistical properties of the enhancement distribution of *one hot-spot*. This is in contrast with the bi-analyte method, for example, where the ability to control hot-spots is sacrificed for the sake of statistics. In this latter case, single molecule cases measure the statistical properties of the enhancement factors of a certain class of hot-spots (with some characteristic properties). The meaning of the different ensemble averages of signals that can be defined in both cases can represent, accordingly, different things.

A recent related development has been the attempt to study simultaneously SM-SERS and current conduction through a single molecule (using the tip as a contact).[35] It is early days to decide what new insight this combination will bring, but it is the sort of example that can only be achieved with tip-based techniques. There are several possibilities related to the study of dissipation processes at the single molecule level, and even vibrational pumping.[36] But more work is clearly needed before some of these ideas are materialized and fully understood.

Another definite step toward further progress in TERS has been demonstrated recently in ref. 37 where the reduced photobleaching of molecules—achieved in the typical UHV conditions needed for TERS—have provided enough photostability to produce *tip-enhanced Raman imaging*; undoubtedly a very fundamental development in the field.

VI. Outstanding issues

In the spirit of the previous section, we provide some brief comments on areas we feel are outstanding issues in SM-SERS.

A Do laser forces play a role?

Laser forces, either on the substrate (*e.g.* colloids) or on the analyte molecules themselves, have been speculated for some time to play a role in SERS in general, and in SM-SERS in particular.[38] These may have an influence in the statistical interpretation of SM-SERS events (in particular in liquids).[39] Effects of ''laser radiation pressure'' on the movement of colloids in solution are relatively easy to see experimentally. Laser trapping of metallic particles is a bit more involved, but still possible; it has been recently used, in fact, in an experiment to create hot-spots on demand for SM-SERS.[40] But direct optical forces affecting the molecules themselves (pushing them toward hot-spots, for example) are a lot more difficult to demonstrate. A ''killer experiment'' is missing in this specific area. One would naively speculate that a simple incident laser power scaling should be able to demonstrate the existence of laser forces on the molecules. But the situation in reality is a lot more complicated and convoluted with additional photo-induced effects that need to be discriminated before a convincing proof is presented. This should be an interesting possibility to keep in mind in the future.

B Can we measure the orientation of a single molecule?

The determination of the orientation of single molecules from SM-SERS data is also an open field at the moment. This is the equivalent of the classic problem on surface selection rules (introduced by Moskovits on planar surfaces[41]) but for a single molecule in a hot-spot. In the past, attempts to determine the orientation of single molecules at hot-spots[42,43] have been hampered by the overriding effect of the *local field polarization*.[44,45] This does *not* mean that information on the orientation of the molecule cannot be retrieved (in principle). A discussion of surface selection rules at hot-spots has been given recently,[46] and information can in principle be obtained if the different Raman tensors of specific vibrations are known. But clear experimental demonstrations are complicated by a variety of causes. Among them: (i) the underlying dispersion of the localized surface plasmon resonance (which affects the relative intensities of the peaks),[47–49] and (ii) the exact knowledge of the symmetry of the Raman tensors for a specific probe, which can be affected by resonance conditions and/or by adsorption on the metal. As in the previous case, a clear-cut experiment with unambiguous interpretation is awaiting to be done.

C Non-radiative cross sections and vibrational pumping

An often forgotten aspect of the SERS cross section (which is arguably the most important characteristic) is that it is composed of a *radiative* and a *non-radiative* component.[17] One could argue that the non-radiative component of $d\sigma/d\Omega$ is irrelevant, because it is never observed in the far-field. There is, however, one exception to the rule: SERS vibrational pumping,[36] where the *total* (*i.e.* radiative + non-radiative) cross section is the relevant one. Non-radiative processes (*i.e.* Raman processes that do happen in the molecule but are not detected in the far-field because they are absorbed in the metal) do contribute to the population measured in the anti-Stokes in SERS pumping conditions. In principle, the cross section deduced from the Stokes signal and that deduced by vibrational pumping from the anti-Stokes/Stokes ratio should agree with each other if non-radiative processes are not important. Combined with the technique of bi-analyte SERS, it is possible to observe vibrational pumping in single molecules,[36] thus providing an ideal testing ground for these concepts. The resulting $d\sigma/d\Omega$s tend to be *larger* than the radiative estimations obtained from the Stokes signal. This is a strong indication that $d\sigma/d\Omega$s measured by vibrational pumping, *i.e.* the *total* SERS cross-section, is an over-estimate of the standard radiative SERS cross-section, because of a large non-radiative component. Measuring the non-radiative cross section is an extremely difficult task, and SM-SERS vibrational pumping could—in principle—provide the means. This would allow an estimation of the non-radiative component, which plays an important role in the related phenomena of surface enhanced fluorescence and fluorescence quenching. We have, indeed, data suggesting this possibility, that will be reported elsewhere.[50]

VII. Conclusion

Undoubtedly, the SM-SERS story is not yet finished. A balanced view of current trends would suggest that the Langmuir–Blodgett, TERS, and bi-analyte SERS approaches stand as the most promising avenues to explore the SM-SERS problem at present; each of them complementing each other and providing insights into different aspects. They should, for example, contribute to a better understanding of some of the most pressing outstanding issues, like those discussed in section VI.

It is now well accepted that SERS is capable of single-molecule detection and that SM-SERS is even more common than originally thought in many typical situations. The next logical step—an important one for applications—is to devise now methods to detect, not only *a* single molecule that happened to be at the right place, but *the* single molecule that we want. This requires the positioning of this molecule with extreme precision and, to some degree, a precise control on the geometry of the SERS substrate (hot-spot) itself. TERS may play a role for the latter, but new tools and approaches will undoubtedly be needed to tackle the former drawing perhaps resources from many research areas such as laser forces, micro/nanofluidics, surface functionalization, self-organization, and other recent advances in nano-sciences. As far as we can foresee it at the moment, the future of SM-SERS is still wide open.

Acknowledgements

Both authors are indebted to the Royal Society of New Zealand (RSNZ) for financial support through a Marsden grant. We are indebted to M. Meyer, C. Galloway, and E. Blackie for useful discussions.

References

1 M. Fleischmann, P. J. Hendra and A. J. McQuillan, *Chem. Phys. Lett.*, 1974, **26**, 163.
2 D. L. Jeanmaire and R. P. Van Duyne, *J. Electroanal. Chem.*, 1977, **84**, 1.
3 M. G. Albrecht and J. A. Creighton, *J. Am. Chem. Soc.*, 1977, **99**, 5215.
4 S. Nie and S. R. Emory, *Science*, 1997, **275**, 1102.
5 K. Kneipp, Y. Wang, H. Kneipp, L. T. Perelman, I. Itzkan, R. R. Dasari and M. S. Feld, *Phys. Rev. Lett.*, 1997, **78**, 1667.
6 N. P. W. Pieczonka and R. F. Aroca, *Chem. Soc. Rev.*, 2008, **37**, 946.
7 E. C. Le Ru, M. Meyer and P. G. Etchegoin, *J. Phys. Chem. B*, 2006, **110**, 1944.
8 C. J. L. Constantino, T. Lemma, P. A. Antunes and R. Aroca, *Anal. Chem.*, 2001, **73**, 3674.
9 P. J. G. Goulet, N. P. W. Pieczonka and R. F. Aroca, *J. Raman Spectrosc.*, 2005, **36**, 574.
10 P. J. G. Goulet and R. F. Aroca, *Anal. Chem.*, 2007, **29**, 2728.
11 N. P. W. Pieczonka and R. F. Aroca, *Chem. Soc. Rev.*, 2008, **37**, 946.
12 B. Pettinger, G. Picardi, R. Schuster and G. Ertl, *Single Mol.*, 2002, **5–6**, 285.
13 B. Pettinger, B. Ren, G. Picardi, R. Schuster and G. Ertl, *J. Raman Spectrosc.*, 2005, **36**, 541.
14 W. Zhang, B. S. Yeo, T. Schmid and R. Zenobi, *J. Phys. Chem. C*, 2007, **111**, 1733.
15 P. G. Etchegoin, M. Meyer, E. Blackie and E. C. Le Ru, *Anal. Chem.*, 2007, **79**, 8411.
16 Y. Sawai, B. Takimoto, H. Nabika, K. Ajito and K. Murakoshi, *J. Am. Chem. Soc.*, 2007, **129**, 1658.
17 E. C. Le Ru, E. Blackie, M. Meyer and P. G. Etchegoin, *J. Phys. Chem. C*, 2007, **111**, 13794.
18 J. A. Dieringer, R. B. Lettan II, K. A. Scheidt and R. P. Van Duyne, *J. Am. Chem. Soc.*, 2007, **129**, 16249.

19 E. Blackie, E. C. Le Ru, M. Meyer, M. Timmer, B. Burkett, P. Northcote and P. G. Etchegoin, *Phys. Chem. Chem. Phys*., 2008, **10**, 4147.
20 A. Otto, *J. Raman Spectrosc*., 2006, **37**, 937.
21 S. Shim, C. M. Stuart and R. A. Mathies, *ChemPhysChem*, 2008, **9**, 697.
22 P. C. Lee and D. Meisel, *J. Phys. Chem*., 1982, **86**, 3391.
23 H. Xu, E. J. Bjerneld, M. Käll and L. Börjesson, *Phys. Rev. Lett*., 1999, **83**, 4357.
24 H. Xu, J. Aizpurua, M. Käll and P. Apell, *Phys. Rev. E*, 2000, **62**, 4318.
25 P. Johansson, H. Xu and M. Käll, *Phys. Rev. B*, 2005, **72**, 035427.
26 E. C. Le Ru, P. G. Etchegoin and M. Meyer, *J. Chem. Phys*., 2006, **125**, 204701.
27 E. C. Le Ru and P. G. Etchegoin, *Chem.Phys. Lett*., 2006, **423**, 63.
28 E. J. Bjerneld, F. Svedberg, P. Johansson and Mikael Käll, *J. Phys. Chem. A*, 2004, **108**, 4187.
29 P. G. Etchegoin, E. C. Le Ru and M. Meyer, *J. Chem. Phys*., 2006, **125**, 164705.
30 P. G. Etchegoin, M. Meyer and E. C. Le Ru, *Phys. Chem. Chem. Phys*., 2007, **9**, 3606.
31 K. F. Domke and B. Pettinger, *Phys. Rev. B*, 2007, **75**, 236401.
32 C. C. Neacsu, J. Dreyer, N. Behr and M. B. Raschke, *Phys. Rev. B*, 2007, **75**, 236402.
33 Y. Fang, Nak-Hyun Seong and D. D. Dlott, *Science*, 2008, **321**, 388.
34 D. Zhang, Y. Xie, S. K. Deb, V. J. Davison and D. Ben-Amotz, *Anal. Chem*., 2005, **77**, 3563.
35 D. R. Ward, N. J. Halas, J. W. Ciszek, J. M. Tour, Y. Wu, P. Nordlander and D. Natelson, *Nano Lett*., 2008, **8**, 919.
36 R. C. Maher, C. M. Galloway, E. C. Le Ru, L. F. Cohen and P. G. Etchegoin, *Chem. Soc. Rev*., 2008, **37**, 965.
37 J. Steidtner and B. Pettinger, *Phys. Rev. Lett*., 2008, **100**, 236101.
38 H. Xu and M. Käll, *Phys. Rev. Lett*., 2002, **89**, 246802.
39 F. Svedberg and M. Käll, *Faraday Discuss*., 2006, **132**, 35.
40 F. Svedberg, Z. Li, H. Xu and M. Käll, *Nano Lett*., 2006, **6** 2639.
41 M. Moskovits, *J. Chem. Phys*., 1982, **77**, 4408.
42 T. O. Shegai and G. Haran, *J. Phys. Chem. B*, 2006, **110**, 2459.
43 A. M. Michaels, J. Jiang and L. Brus, *J. Phys. Chem. B*, 2000, **104**, 11965.
44 P. G. Etchegoin, C. Galloway and E. C. Le Ru, *Phys. Chem. Chem. Phys*., 2006, **8**, 2624.
45 E. C. Le Ru, J. Grand, N. Félidj, J. Aubard, G. Lévi, A. Hohenau, J. R. Krenn, E. Blackie and P. G. Etchegoin, *J. Phys. Chem. C*, 2008, **112**, 8117.
46 E. C. Le Ru, M. Meyer, E. Blackie and P. G. Etchegoin, *J. Raman Spectrosc*., 2008, **39**, 1127.
47 E. C. Le Ru, M. Dalley and P. G. Etchegoin, *Curr. Appl. Phys*., 2006, **6**, 411.
48 T. Itoh, K. Yoshida, V. Biju, Y. Kikkawa, M. Ishikawa and Y. Ozaki, *Phys. Rev. B*, 2007, **76**, 085405.
49 E. C. Le Ru, P. G. Etchegoin, J. Grand, N. Félidj, J. Aubard, G. Lévi, A. Hohenau and J. R. Krenn, *Curr. Appl. Phys*., 2008, **8**, 467.
50 C. Galloway, E. C. Le Ru and P. G. Etchegoin, unpublished.