

# Plasmon-dispersion corrections and constraints for surface selection rules of single molecule SERS spectra

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The problem of extracting information from relative intensities of Raman peaks in surface-enhanced-Raman-scattering (SERS) is intimately related to several important topics in the technique. Among them: (i) the possibility (or sometimes impossibility) of observing surface selection rules in different situations, (ii) the role of analyte resonance conditions, (iii) the crucial inclusion of plasmon-resonance dispersion corrections in the analysis of relative Raman intensities among peaks, and (iv) the connection of these phenomena with (broader) issues like surface-enhanced fluorescence (SEF). This paper deals with the underlying connections among these (apparently disconnected at first sight) topics. The technique is now at a mature stage to review the aforementioned phenomena from a unified point of view; thus pinpointing the most important issues, clarifying concepts that have been historically confusing (or treated in isolation), and paving the road for future developments.

## I. Introduction and overview

With the advent of more reliable tools for single molecule surface-enhanced Raman scattering (SM-SERS) detection<sup>1–8</sup> several natural questions arise at this particular stage of the development of the field (some of them recently summarized in ref. 9). Among them, several of the (now) classic topics in SERS are brought back to life, but at the single molecule level.

One of them is the well established topic of *surface selection rules*, first studied by Moskovits for planar surfaces.<sup>10–12</sup> This topic is intimately related to the possibility of *determining molecular orientation at the single molecule level* which has been attempted with many different (yet unresolved)<sup>9,13</sup> issues in the past.<sup>14,15</sup> In addition, these problems have broader connections with other topics that are related to each other by an underlying theme, namely: how much information (about molecular orientation or otherwise) can we obtain from the relative intensities of Raman peaks under SERS conditions? The question is particularly relevant in the single molecule limit (to extend the concepts of surface selection rules to that domain), but its scope does not stop there.

Ref. 16–19, for example, have both suggested and attempted to extract information from the temporal fluctuations in relative intensities of Raman peaks in SERS spectra (under conditions which can be argued to be close to (or at) single molecule level). This has been attempted in many cases through 2D-intensity-correlation maps,<sup>19</sup> which show great promise for the visualization of correlated features in a sequence (temporal or spatial) of spectra. In completely different (and previous) studies,<sup>20–23</sup> there had been also attempts to study the orientation of *non-resonant* molecules,

that had been inferred from SERS spectra on substrates that go beyond the idealized cases<sup>10</sup> of a planar interface (molecules on metal sols, for example). It will turn out later that the distinction between the situation of *non-resonant* molecules in ensemble averages, or single molecules under *resonant* conditions, is crucial to understand how much information we can obtain from surface selection rules (in the different situations normally encountered in SERS). This is, in fact, one of the main underlying themes of the present paper.

It is possible, therefore, to claim that the determination of relative intensities among peaks in SERS spectra underpins several basic aspects of the technique.<sup>24,25</sup> Notwithstanding, the vast majority of SERS studies trying to obtain information from relative peak intensities avoid (or simply ignore) plasmon dispersion corrections to them.<sup>19</sup> Itoh *et al.*<sup>26</sup> have shown recently one of the best experimental determinations linking the plasmon dispersion (as observed in the extinction spectra) and the SERS intensities of different modes. This was done through a combination of dark-field illumination (to measure the extinction of different colloidal clusters) and Raman microscopy (to observe the SERS spectra of the same clusters).<sup>26</sup> With some limitations, the dispersion of the plasmon-resonance can be also monitored indirectly (but much more easily compared to dark-field microscopy) through the modification of the spectral profile of the surface-enhanced fluorescence signal (SEF) of the molecules.<sup>27–29</sup> Even though when this latter method is restricted to *resonant* molecules—which must still emit some residual fluorescence under SERS conditions—it provides a clear-cut demonstration of the importance of plasmon-resonance dispersion corrections, as we shall show later. In addition, this latter case is the most common situation found in the vast majority of single-molecule SERS experiments; which profit from resonant conditions to boost the intrinsic Raman cross section and make SM-SERS detection easier and feasible with lower SERS enhancements.

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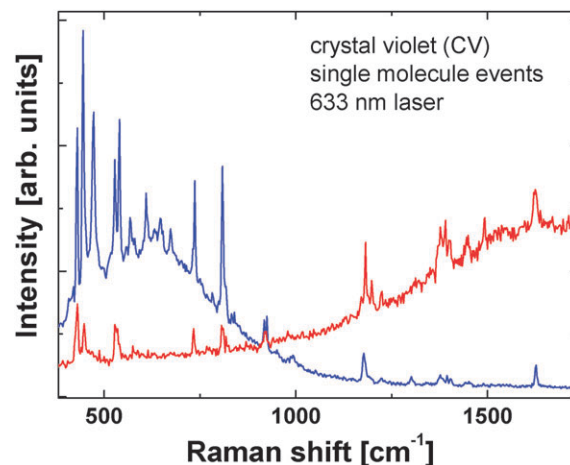
Accordingly, the bottom line is that there is a family of topics that are linked to each other and revolve around the issue of extracting information from relative intensities of peaks under SERS conditions. This paper is around the mutual connections of these topics, and the overriding influence that plasmon dispersion corrections can have on the answer to a specific question based on relative intensities of SERS peaks. We shall show explicitly how ignoring plasmon dispersion corrections can lead to a wrong (or misleading) result, and shall demonstrate how the symmetry information for single molecules in SERS is (more often than not) affected by resonance effects. The connection with the phenomena of surface enhanced fluorescence (SEF) will arise as natural in this context. Finally, we shall put all these phenomena within the scope of surface selection rules (and local fields) and derive a set of restrictions that apply to them under standard conditions often found in SERS experiments.

This paper relies on several concepts that are accepted as known here. Affirmations like: *the intrinsic symmetry of the Raman tensors is washed out by resonance conditions* are part of the “folklore” of Raman resonance phenomena; and are either taken and accepted for granted by the reader or require a long detour into the existing literature.<sup>30,31</sup> The same applies to assertions regarding the influence of the local field in SERS which, although basic, have seen a fair amount of disagreement in the literature. The latter topic has been treated in full length recently in ref. 32 (and for further details, see ref. 25).

## II. Resonance dispersion and surface-enhanced fluorescence

One way to reveal the dispersion of the resonance affecting the SERS spectrum, *i.e.* the fact different peaks at different Raman shifts are enhanced by different amounts, is through the background<sup>27–29</sup> of the spectra which is mostly present for *resonant* molecules. This means that these molecules would have emitted plenty of fluorescence if it had not been for the fact that the surrounding metallic SERS substrate provides non-radiative channels to quench the emission (see ch. 4 of ref. 25 for more details). The quenching is not complete and, in fact, most situations seen in SERS of coexistence of Raman signals with fluorescence are different special cases of competition between the radiative and non-radiative aspects of light emission in close proximity to metals.<sup>25</sup> Ref. 27 shows explicitly that the SEF background should follow the *radiative enhancement factor*  $M_{\text{rad}}(\omega_S)$ , evaluated at the scattered photon energy  $\omega_S$ . The SERS enhancement factor in the  $|E|^4$ -approximation,<sup>33</sup> on the contrary, is composed of two parts: the local-field enhancement factors at the laser ( $M_{\text{loc}}(\omega_L)$ ), and scattered energy ( $M_{\text{loc}}(\omega_S)$ ), respectively.  $M_{\text{loc}}(\omega_L)$  is fixed by the choice of laser excitation, but  $M_{\text{loc}}(\omega_S)$  will vary for different modes at  $\omega_S$ . Taking into account that  $M_{\text{loc}}(\omega_S)$  and  $M_{\text{rad}}(\omega_S)$  are expected to follow the same resonance dispersion,<sup>25</sup> we can take the SEF background as a measure of the dispersion of  $M_{\text{loc}}(\omega_S)$  and correct the intensity of Raman modes with it.

The connection between the dispersion of the SEF background and the varying intensities of the Raman peaks in typical SERS spectra is sometimes very evident when



**Fig. 1** Two clear examples of SM-SERS spectra where the correlation between the underlying background and the Raman intensities of the peaks above can be observed. In this particular case, these are two crystal violet (CV) SM-spectra taken from a bi-analyte SERS experiment<sup>1</sup> between CV and Nile Blue (NB) on a *dry* substrate with Ag-colloids (at room temperature), as described in ref. 1 and 34. Therefore, the two spectra (red and blue) represent two different single-molecule events of CV at two different points on the sample, while NB (not present in these spectra) is only used as a “contrast signal” (to identify single-molecule cases of CV) in the spirit of the bi-analyte SERS technique.<sup>1</sup> The cases displayed here show two (relatively narrow) plasmon resonances centered on the low and high Raman-shift regions of the spectra, respectively. Resonances on these dry samples tend to be “narrower” on average compared to those found in colloidal liquids, and reveal a more visual connection among the dispersion of the resonance, its effect on the underlying background,<sup>27–29</sup> and its connection to the varying intensities of Raman peaks at different energies.

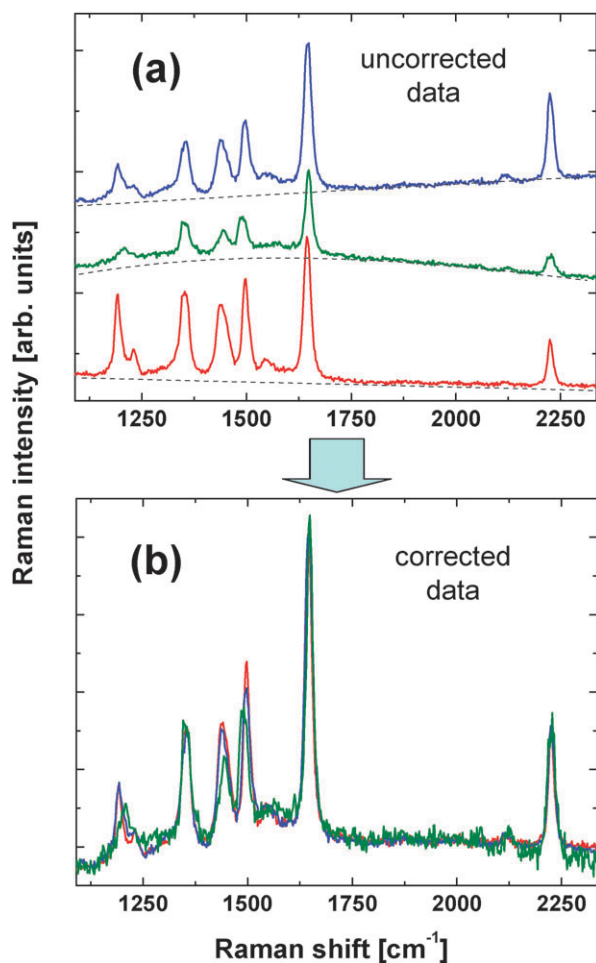
relatively narrow resonances can be achieved. An example of this is given in Fig. 1 for SM-SERS spectra of crystal violet (CV) taken under bi-analyte SERS conditions<sup>1</sup> (with Nile Blue (NB) as a partner) at 633 nm laser excitation on *dry* clusters (as in ref. 1). In this situation, relatively narrow resonances spanning a range that is smaller than the typical fingerprint Raman region ( $\sim 1700 \text{ cm}^{-1}$ ) can be achieved.<sup>35</sup> Fig. 1 shows two examples of resonances centered towards the low and high energy sides of the fingerprint region, respectively. It is obvious from examples like that in Fig. 1 that the relative intensity of peaks needs to be corrected by the underlying dispersion of the resonance if there is any chance to extract further information from there (for example, on surface selection rules). We show in the next Section examples of resonance dispersion corrections in colloidal liquids under SM-SERS conditions.

## III. Experimental

Experiments are performed in two different molecules that have a residual background in SERS, and under conditions that have been previously explored in terms of analyte concentration (5 nM) and integration times (0.2 sec) to be in the SM-SERS regime. Experimental conditions are identical to those reported in ref. 6; we therefore keep details to a bare minimum. Samples were prepared with citrate-reduced Ag

(Lee & Meisel) colloids.<sup>36</sup> We used 633 nm as excitation wavelength and take time sequences using an immersion objective ( $\times 100$ ) index-matched to water. The characterization of the scattering volume of our microscope has been thoroughly reported in the supporting information of ref. 37.

Fig. 2 shows examples of SERS spectra taken for rhodamine 800 (RH800)<sup>38</sup> ( $\lambda_{\text{max}}^{\text{abs.}} = 686 \text{ nm}$ ). Monitoring events in a colloidal liquid implies that we are sampling different hot-spots over time as the clusters move in and out of the scattering volume of the microscope by Brownian motion. Therefore, the spectra reveal over time the different types of resonances (in different hot-spots) that the colloidal solution is capable of producing. These resonances will favour different SERS peaks for different cases, depending on the typical dispersions present. The dispersion of the resonances found



**Fig. 2** Examples of plasmon-resonance dispersion corrections for rhodamine 800 (RH800)<sup>38</sup> (see the text for experimental details). In (a) we show three individual spectra with three different types of backgrounds, revealing resonances with a positive (top), bow-like (middle), and negative (bottom) slopes. These underlying resonances (revealed by the dispersion of the SEF background,<sup>27</sup> schematically shown with dashed black lines) alter the Raman (SERS) intensities of the different modes; with the most obvious changes seen for the  $\sim 2230 \text{ cm}^{-1}$  cyano bond of RH800 with respect to the other modes in the *fingerprint region* ( $< 1700 \text{ cm}^{-1}$ ). However, after the intensities are corrected by the plasmon dispersion, the spectra converge to a single result (b) in which all the intensities of the peaks are mostly correlated.

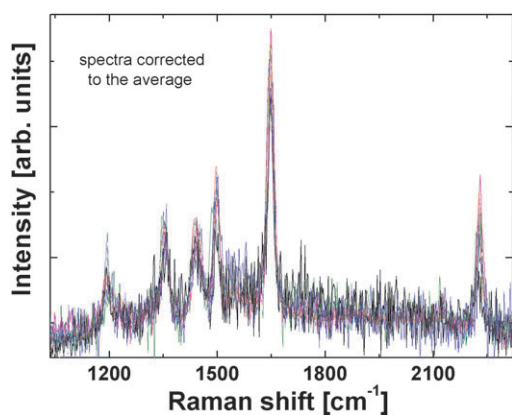
in most events in these colloidal liquids are not as narrow as the ones sometimes found on dry clusters (see Fig. 1), but rather adopt the typical shapes of a: (i) negative, or (ii) positive slope, or (iii) a bow-like (quadratic) underlying dispersion. Three examples of these cases are explicitly shown in Fig. 2(a). The fact that the relative intensities of peaks are affected from one example to another is particularly visible in RH800 by the fact that the molecule has a Raman active mode at  $\sim 2230 \text{ cm}^{-1}$  (the  $\text{C}\equiv\text{N}$  cyano bond). This increases slightly the normal window spanned by the fingerprint region ( $< 1700 \text{ cm}^{-1}$ ) of most molecules, and allows a better appreciation of the underlying resonance dispersion. This is, in fact, the reason why we are using this molecule for the present purposes.

The natural questions that arise at this stage are: how much of the relative intensities among peaks can be attributed to the dispersion itself? and: is there any other effect left over after the correction has been made? In order to correct the spectra by the presence of the dispersion a careful removal of the background is needed. This is done in a first step by experimentally subtracting a water background obtained under identical experimental conditions. In addition, events of low intensities (where the difference between background and signal is not clear) are discarded for the analysis using a signal-to-noise criterion. The next problem to be tackled is that we do not have access to the bare Raman spectrum of the molecule (precisely because of resonance effects). We do have access however to the average SERS spectrum in the colloid and we study departures of the resonance dispersion (and how it affects the intensity of the Raman peaks) with respect to this average. As a matter of fact, due to the relatively smooth types of backgrounds present in this particular system (unlike those in Fig. 1), a *quadratic correction factor* that accounts for the difference between the dispersion of a specific event (with respect to the average) is enough to bring all spectra to the average. This proves then that the “apparently different relative intensities” seen among peaks (in the spectra of Fig. 2, for example, between the cyano-bond vibration at  $\sim 2230 \text{ cm}^{-1}$  with respect to the mode at  $\sim 1650 \text{ cm}^{-1}$ ) is to a very good approximation nothing but a consequence of the plasmon dispersion. The choice of a quadratic function is based on the observation of the typical plasmon-resonance dispersions in the experimental data (for this particular system). More complicated background dispersions exist in other systems, but a quadratic approximations proved to be sufficient to account for the typical cases observed in colloidal liquids. Needless to say, a rule of thumb in general is to use the simplest analytical representation of the characteristic backgrounds with the minimum number of parameters and assumptions.

Hence, the basic idea of plasmon dispersion correction here is to show that a simple smooth quadratic dispersion relation is solely responsible for most (if not all) of the relative Raman intensity fluctuations among peaks. As can be appreciated from Fig. 2b, which shows the corrected spectra, the results converge (to a very good approximation) to the same spectrum (which is the average spectrum). The small differences in relative intensities among cases are in most cases small imperfections of the dispersion correction process and we shall

come back to them later. Fig. 2b shows only the corrected versions of the three cases in Fig. 2a, to avoid excessive over-cluttering of the figure, but in fact, we can show a much larger group of spectra in Fig. 3, where a collection of fifteen different single molecule events have been corrected by a quadratic dispersion to the average. These spectra showed, originally, dispersions that were either, negative, positive, or bow-like as a function of energy (Raman shift). It can be seen from Fig. 3 that the correction of the resonance dispersion accounts for most of the differences among spectra. It also shows that most (if not all) of the relative fluctuations among Raman peaks are *not* an independent problem from the dispersion of the background itself, for they can be corrected simultaneously with the latter under the simple assumption that the spectrum is being modified “smoothly” throughout a given energy range by an underlying dispersion which affects both the Raman peaks and the background itself. In fact, such an effect has already been demonstrated clearly (but not in SM-SERS conditions) in the case of uniform arrays of metallic particles, for which the underlying plasmon resonance is well-defined across the array and can be characterized independently by extinction measurements.<sup>28,29</sup>

The result in Fig. 3 can be considered, in fact, to be the main message (in its most visual version) of the paper: background dispersions account (in most cases) for the most important, if not dominant, contribution to relative peak intensities. The latter cannot be neglected and, despite the apparent simplicity of the claim, this has been a source of problems in many previous studies. We believe, for example, that 2D-correlation maps like those reported in ref. 19 do not represent faithfully intrinsic properties that can be derived from relative intensities of Raman peaks, but rather properties related to the different



**Fig. 3** Fifteen different spectra (originally with different positive, negative, or bow-like underlying dispersions) corrected to the average spectrum by a single quadratic dispersion factor (different for each spectrum). The correction brings *both* the background itself and the Raman peaks to a spectrum that resembles the average SERS signal. This is a demonstration that both the background and Raman peaks are affected by the same phenomenon (the dispersion of the resonance) and that relative intensity fluctuations among Raman peaks that are far away from each other are an “artifact” of the dispersion. The result also highlights the fact that Raman peaks of resonant molecules under SM-SERS conditions display (in general) the same symmetry, which is dominated by the resonance itself. See the text for further details.

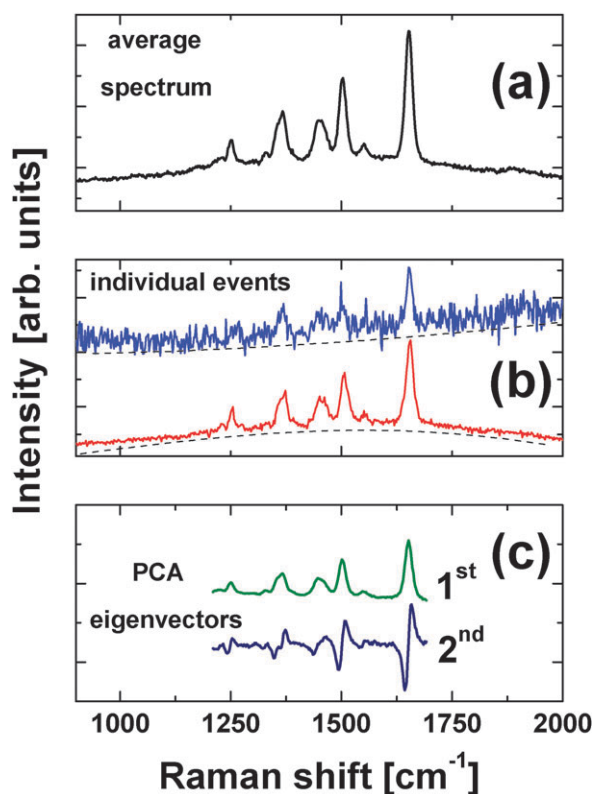
types of plasmon resonances (and their dispersions) found in a specific system.

As far as the main message of the paper is concerned, the story can be finished here. There is, however, the possibility of adding additional analysis tools to the problem. A few pending questions at this stage are the following: (i) even if the background correction accounts for the biggest fraction of relative intensity fluctuations, do we still have small fluctuations afterwards that still retain a physical meaning?, and (ii) can we explain the experimental fact that all peaks seem in our examples to be “correlated” with each other (*i.e.* not affected by surface-selection rules)? To address the first question, one could study 2D-correlation maps of the plasmon-dispersion-corrected spectra. There is, in fact, an additional and perhaps more powerful way of investigating whether there are additional correlations/anti-correlations in the peaks (after the background correction has been performed) by using Principal Component Analysis (PCA) over a much larger set of spectra (>1000) than what we can show in a single figure (like Fig. 3). We show this explicitly in Fig. 4 for another resonant molecule: rhodamine 700 (RH700) ( $\lambda_{\text{max}}^{\text{abs.}} = 641 \text{ nm}$ ). Once the low intensity events have been discarded and the peaks have been corrected by the dispersion of the resonance, we can apply PCA to the resulting series of spectra and analyze the intensity correlations among different peaks. If all the residual intensities of the peaks are fully correlated, they appear as a single (most important) eigenvalue in the PCA method, with the second most important one representing additional details of the peaks (like frequency shifts). The latter has a characteristic “derivative shape” around peaks that cannot be confused with a contribution to the intensity. If there are remaining anti-correlations (or independent components fluctuating with respect to each other) there is the need of *more than one* eigenvector to represent the intensity fluctuations. What is observed in Fig. 4c is the former case: the most important eigenvector resembles the average spectrum in Fig. 4a, while the second eigenvector in 4(c) can only account for frequency shifts of the peaks. The fact that this is so indicates that the apparent relative intensity fluctuations in Fig. 4b are only caused by the dispersion of the resonance. Once removed, the peaks are basically correlated in intensity.

This latter situation of peaks entirely correlated in intensity once the dispersion of the resonance has been accounted for is, actually, the rule rather than the exception in most *resonant* SM-SERS situations we have studied. There are a few possible exceptions but, more often than not, resonant molecules will show fully-correlated SERS peaks, which in the framework of surface selection rules correspond to Raman peaks with an underlying single symmetry.

We can summarize the results of this section in the following statement: under SM-SERS conditions in resonance the biggest fraction of the fluctuations among relative intensities of Raman peaks comes from the dispersion of the resonance. The latter can be made evident through the SEF-background that characterizes resonant excitation conditions in SERS. If the intensities are adequately corrected by the dispersion of the resonance, we are left with peaks that are fully correlated in intensity, pointing out to a single underlying symmetry. In the





**Fig. 4** Plasmon dispersion corrections for rhodamine 700 (see the text for experimental details). (a) Average SERS signal over 6000 spectra. (b) Two examples of SERS spectra with a positive (top) and bow-like (bottom) plasmon resonance dispersion (revealed by the underlying SEF background). (c) Principal Component Analysis (PCA) of 1072 background-corrected spectra (discarding the low intensity events from the original 6000). Once the intensities of the Raman peaks are corrected by the dispersion, PCA can help reveal any additional correlation/anticorrelation in the system. The first eigenvector corresponds to the “average spectrum” as expected for fully correlated SERS peaks. This eigenvector results from the fact that there are small imperfections in the corrections to the peak intensities (to the average) by the background dispersion and, therefore, PCA still needs an eigenvector accounting for the average intensity. Independent components (or anti-correlations among peaks) will appear from the 2<sup>nd</sup> eigenvector onwards. However, the 2<sup>nd</sup> eigenvector accounts for a *frequency shift* of the spectrum as a whole. Any correlation/anticorrelation among peak intensities are relegated to higher order eigenvectors, with negligible importance (as judged by the eigenvalues of the covariance matrix in PCA.<sup>39</sup>)

next section, we discuss the implications and consequences of this observed phenomenology.

#### IV. Discussion and conclusions

At the single molecule level in SERS hot-spots, any residual effect of orientation (surface selection rules) can only come from the projection of the Raman tensor along the highly uniaxial field characteristic of these situations. This point is discussed in full length in ref. 32. If modes retain different symmetries at the single molecule level, they should show relative fluctuations in intensity depending on the orientation of the molecule with respect to the local field. The question

remains then of why the peak intensities in all the examples shown in the previous section are fully correlated. The simplest answer to this is that the Raman peaks indeed have a common symmetry under resonance Raman conditions, *i.e.* their intrinsic (non-resonance) symmetries are washed out in this case. While this is part of a standard jargon, and many practitioners of resonant Raman spectroscopy will take it from granted, it is an important point in this context and it is intimately related to the possibility (or impossibility) of observing surface selection rules at the single molecule level in SERS.

With this in mind, the general conclusions derived from this study can be summarized as follows:

- Most of the intensity fluctuations that happen in SM-SERS under resonant or near resonant conditions (as in the examples of the previous section) can be accounted for by the dispersion of the resonance. With some limitations, this is achievable through a correction using the SEF-background<sup>28</sup> observed simultaneously with the SERS spectrum.<sup>25</sup> In the absence of such a correction, any conclusion on relative peak intensities fluctuation will be flawed. This is, in fact, one of the main messages of the present study.

- There is a basic conundrum as far as surface selection rules in SERS are concerned. If non-resonant molecules are measured, Raman modes conserve their intrinsic (in principle different) symmetries, and orientation information of the molecules with respect to the local field is in principle available. This is the classic problem studied originally by Moskovits,<sup>10</sup> and generalized to SERS hot-spots in ref. 32. This, however, implies that single molecules cannot be easily measured (because non-resonant molecules will have much smaller intrinsic cross sections). Moreover, a certain degree of averaging—for example with respect to typical orientations found on the surface—might be necessary to understand the results.

- If resonant molecules are used, SM-SERS becomes possible. The local field here is normally the highly uniaxial field of hot-spots, and information of single molecule orientation would be (in principle) possible to retrieve from relative SERS peak intensity fluctuations. However, molecules under resonant conditions tend to have their symmetry washed out and dominated by the characteristics of the electronic state producing the resonance itself. Accordingly, the intensities become correlated and the only fluctuations left are intensity fluctuations of the spectrum as a whole. We believe any conclusion regarding the surface selection rules of single molecules must account for resonance dispersion in the first place, and the effect of resonance on the intrinsic symmetry of the modes in addition.

- The symmetry washing-out at resonance does not preclude completely the possibility that some molecules might show surface selection rules at hot-spots in SM-SERS. For example, competitions between two different resonances can preserve symmetry information even in resonance Raman conditions, while still providing a high intrinsic cross-section. However, one of the main messages of this paper is that these possible situations are *special cases*, and need to be treated on a case-by-case basis. A rule of thumb for the vast majority of resonant molecules used in SM-SERS is that the intrinsic

symmetries of the modes will be washed out by resonance conditions and that most of the relative intensity fluctuations come from the dispersion of the resonance. In that sense, the examples shown in this paper tend to be the rule rather than the exception in SM-SERS.

• For non-resonant molecules, it is still necessary to correct for the plasmon dispersion, even if the single molecule level is not aimed at. The problem in this case is that the dispersion cannot be obtained (even if approximately) from the background, for the molecules emit no residual fluorescence. It might be possible with some care to use extrinsic fluorescent signals (from impurities) to obtain experimentally the dispersion of the resonance. But this is a procedure that needs to be done with utmost care, and the quality of the result needs to be judged critically. In an ideal (background-less) situation for non-resonant molecules, it is impossible to obtain the dispersion from the SERS spectrum in the same experiment, and one might have to resort to extinction experiments with dark-field illumination (as in ref. 26).

Overall, we hope our study here will help to clarify several issues that have been the source of confusion in more than one occasion in the past. The contribution of the dispersion, and the effect of resonance Raman conditions on the intrinsic symmetry of modes, cannot be ignored if any meaningful information on surface selection rules is sought from relative Raman intensities in SERS. The problem is particularly acute in the single-molecule limit, where resonance effects are most of the time exploited to boost the intrinsic Raman cross sections and make SM-SERS accessible at lower enhancement factors ( $\sim 10^8$ – $10^9$ ). It is conceivable also that many examples of surface selection rules from the early days might have to be revisited in the future with an improved understanding, and that the lack of dispersion correction for relative intensities might have produced many problems in the analysis (and understanding) of many experiments in the past.<sup>18,19</sup> In methods like the bi-analyte SERS technique,<sup>1</sup> which was specifically developed for SM-SERS,<sup>39</sup> the complications of resonance dispersion are avoided by choosing narrow spectral windows with fingerprint modes of the two partner molecules. But in other applications the use of narrow spectral windows might not be possible, and corrections by the dispersion of the resonance become mandatory for any meaningful conclusion.

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