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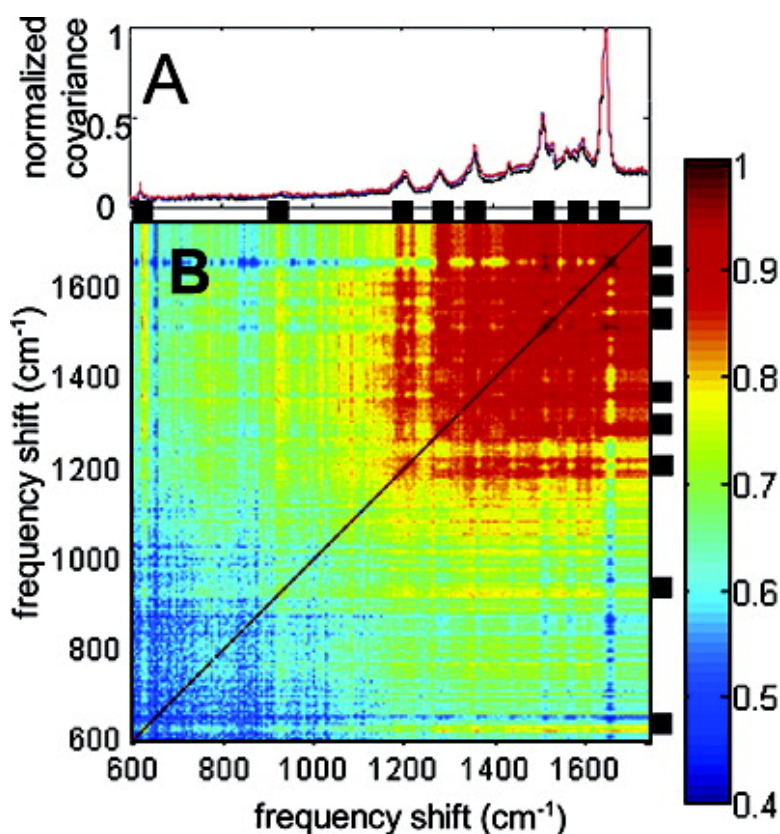
2D Correlation Analysis of the Continuum in Single Molecule Surface Enhanced Raman Spectroscopy

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2D Correlation Analysis of the Continuum in Single Molecule Surface Enhanced Raman Spectroscopy

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Observation of Raman spectra from single molecules on colloidal silver particles[1,2] has intensified interest in the mechanism of surface enhanced Raman scattering (SERS) on noble metal surfaces.[3,4] Single molecule SERS arises from colloidal clusters.[5,6] Ensemble measurements have revealed "active sites" with different binding energies and SERS enhancements.[7,8] Both ensemble[8] and single molecule SERS experiments[6,9,10] show that sharp Raman lines are invariably accompanied by an overlapping continuum, which may occur without sharp Raman lines.[6] The role and nature of the continuum associated with SERS in experiments to date are unclear.[10–14] Here, we demonstrate two-dimensional (2D) covariance and correlation analysis of single molecule SERS spectra and use it to correlate SERS peaks and the continuum for colloidal silver with and without rhodamine 6G. This analysis of spectra from a single SERS source provides unique insight into the relationship between SERS and the continuum.

The 2D covariance and correlation maps used here graph the statistical covariance[15] and correlation coefficient[15] between intensities for all pairs of frequencies. A fast Fourier transform-based algorithm calculates the covariance σ_{jk}^2 between intensities at Raman shifts ν_j and ν_k in sets of 256 or more SERS spectra. Two-dimensional covariance maps[16] are equal to "synchronous 2D correlation spectra".[17] Here, the 2D correlation map is $\rho_{jk} = \sigma_{jk}^2/(\sigma_{jj} \cdot \sigma_{kk})$.

For sufficiently large data sets, slices through the covariance at constant ν_j show the spectrum that fluctuates about the average in parallel with the intensity at ν_j. The correlation coefficient shows whether covarying fluctuations are diluted by other sources of fluctuating intensity. Spectral peaks that fluctuate together give large positive correlations. Conversely, spectral peaks that cannot occur simultaneously, such as those resulting from different conformations of a single molecule, will give negative anti-correlations. Unrelated intensity fluctuations should have zero correlation. For finite data sets, statistics can show that a correlation is not significant if $|\rho_{jk}|$ is less than that likely to arise from a set of independent measurements of completely uncorrelated spectra: with 256 independent spectra, all $|\rho_{jk}| < 0.16$ are *not* "highly significant" (99% confidence).[15] Chemical significance of a correlation often depends on a smaller number of physically independent events, for example, blinking of the spectrum.

Experimental conditions follow ref 18 with changes below. "Blinking" single molecule spectra were obtained from silver colloids incubated with rhodamine 6G (R6G) at a concentration equivalent to 0.3 molecules per particle. Other spectra were obtained from native silver colloids with no added probe molecule. Integration times were 1 s for single colloids or clusters in a $1/e^2$ beam radius of 1 μm; 0.5 mW excitation power yielded a spatially averaged photon flux between 10^{22}–10^{23} photons/(cm^2 s), comparable to that used in ref 2.

Figure 1 shows covariance slices (A) and the 2D correlation map (B) of R6G on a silver colloid. The 2D contour maps have a color
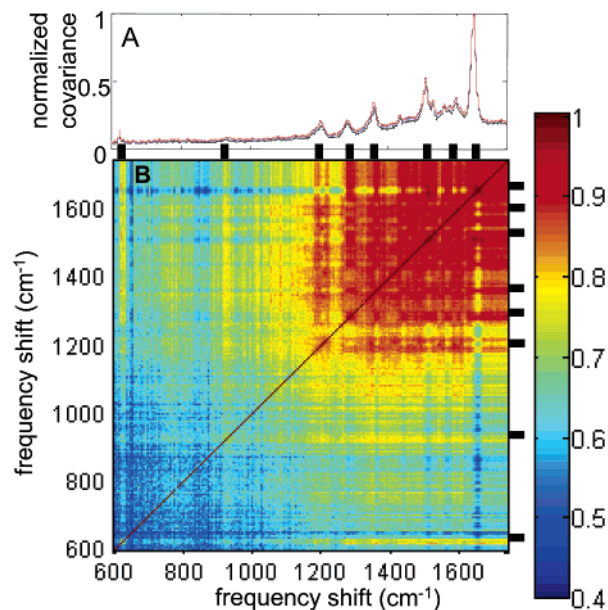
Figure 1. (A) Normalized covariance slices of rhodamine 6G on Ag colloids at frequencies corresponding to R6G Raman peaks: black, 1650 cm^{-1}; blue, 1510 cm^{-1}; red, 623 cm^{-1}. (B) Two-dimensional correlation map of the same molecule with Raman peaks at 1650, 1600, 1575, 1361, 1291, 1214, 940, and 623 cm^{-1}, marked by ticks.

bar starting at red (highest correlation) and passing through yellow to blue (lowest correlation). In Figure 1B, the diagonal ($\nu_j = \nu_k$) correlation is 1, and the width of spectral peaks gives high correlation off the diagonal. This occurs both for sharp peaks and the broad continuum above $\sim$1200 cm^{-1}. Raman shifts of the sharp R6G Raman peaks are similar to those previously reported,[7] and some are marked in the figure. Cross-peaks ($\nu_j \neq \nu_k$) occur when spectral peaks with different frequencies are correlated. The cross-peaks connecting the diagonal R6G Raman peaks and continuum with the Raman peak at 623 cm^{-1} are seen as yellow stripes in Figure 1B.

Figure 1A shows covariance slices at three frequencies corresponding to R6G SERS peaks: 1650, 1510, and 623 cm^{-1}. Slices are normalized to the 1650 cm^{-1} peak to compare the relative peak heights and strongly resemble R6G spectra,[7,10] including the continuum between $\sim$1200 and 2500 cm^{-1} observed by others.[2,10] The correlation map in Figure 1B and the normalized covariance slices in Figure 1A show that *all* of the R6G SERS lines are highly correlated to the *same* continuum in a region beginning at $\sim$1200 cm^{-1} and extending toward larger Stokes shifts even when the amplitude of the continuum immediately below the peak is low (e.g., 623 cm^{-1}). For this SERS source, 2D correlation maps covering a $\sim$6000 cm^{-1} spectral range are consistent[19] with reports that the continuum associated with the R6G peaks, which has a maximum at $\sim$1600 cm^{-1}, is occasionally[10] accompanied by a
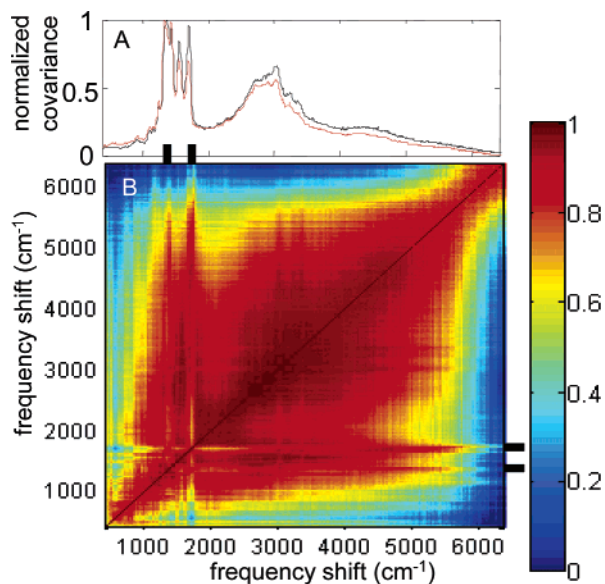
Figure 2. (A) Normalized covariance slices of citrate prep Ag colloids with no added adsorbate at two frequencies corresponding to Raman peaks: black, 1717 cm^{-1}; and red, 1340 cm^{-1}. (B) Two-dimensional correlation map of the same data with the Raman peaks sampled in part A marked by ticks.

second continuum peaking at 3000 cm^{-1} that reduces the correlation in the 2D map.

Figure 2 shows covariance slices (A) and the 2D correlation map (B) of SERS spectra taken from a native silver colloid over a wider spectral range. (Similar spectra were often observed while hunting for R6G SERS.) In Figure 2B, sharp Raman lines can be seen between 1750 and 1300 cm^{-1}. These sharp lines are less correlated to the near continuum than to the continuum beyond 3000 cm^{-1}. Covariance slices through the Raman lines at 1717 and 1340 cm^{-1} in Figure 2A show the continuum associated with vibrational Raman enhancement peaks at 3000 cm^{-1}, not immediately under the vibrational Raman peaks. This scenario parallels the SERS enhancement of the R6G Raman lines at 623 and 940 cm^{-1} in that the Raman lines are located on the tail of the correlated continuum. Reduced correlation near the sharp Raman lines is due to an uncorrelated continuum at ~1600 cm^{-1}.

Continua with Raman-like and luminescence-like excitation frequency dependence have both been reported accompanying SERS from silver colloids.[14] The polarization of the 1600 cm^{-1} continuum is the same as that of the sharp molecular R6G Raman lines,[10] and it resembles electronic Raman scattering from rough metal surfaces;[13,20] it has been suggested that this continuum is electronic Raman scattering from the metal.[10,13,20] On the basis of Raman gain studies, Heritage[11] argued the continuum is luminescence because near the cyanide SERS peak "no more than 25% of the continuum strength can be Raman effect". We have observed that the continuum correlated to molecular SERS enhancement can peak at a Raman shift far from the enhanced molecular Raman peaks. It is thus possible that Heritage did not observe Raman gain near the cyanide peak because the Raman scattering continuum associated (covarying) with the molecular SERS enhancement had low amplitude near the sharp peaks, with luminescence dominating the continuum there. The limited data set in Figure 2a of ref 14 suggests a correlation between sharp Raman peaks and a continuum with a Raman-like excitation frequency dependence that supports the above hypothesis. Thus, while our 2D maps alone do not indicate the associated continuum is Raman or luminescence, they do indicate

that the luminescent continua in refs 11 and 12 are consistent with a Raman continuum associated with SERS suggested by other data.[10,13,14,20]

The covariance and correlation maps of the R6G and native colloid SERS data unambiguously show that the molecular SERS peaks from these different molecules are correlated to different continua, one peaking near 1600 cm^{-1} (R6G) and one peaking near 3000 cm^{-1} (native). As both continua occur without sharp Raman lines, the continua are properties of the colloids. This suggests a hypothesis that two distinct active sites (or surface species) on the silver colloids produce the two continua, and that R6G is enhanced at a different site than the molecule(s) responsible for the SERS peaks from the native colloid. As both continua are observed for each sample, both sites are present on a single SERS source. Assuming a two-photon process, SERS and the associated continuum are most likely competing re-radiation channels from a coupled "molecule in active site" system. Uncoupled metal hot spots can give rise to noncovarying continua, so the total continuum has a variable appearance.

Brus[10] has suggested the 3000 cm^{-1} continuum is electronic Raman scattering from a metal hot spot not involved in R6G SERS enhancement. The absence of single molecule R6G SERS spectra associated with the 3000 cm^{-1} continuum (in both the literature and our data) suggests this site cannot enhance R6G SERS or can do so only weakly. The new observation of SERS associated with this continuum implies a molecular specificity to the colloidal SERS active sites, such as a chemical surface modification or a size condition for a molecular junction between particles. Once understood, such specificity might be exploited to design and synthesize SERS substrates that specifically enhance a targeted group of analytes.

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JA043651U