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Self-Assembled Monolayer Coating for Normalization of Surface Enhanced Raman Spectra

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ABSTRACT

We demonstrate that the use of a self-assembled monolayer, consisting of a thiol derivative of Dabcyl, can be used to normalize surface enhanced Raman signals (SERS) with respect to varying enhancement. Chaotic assemblies of gold nanoparticles exhibit large spatial variation in enhancement. Our work shows that in such a system the signals from the reporting molecules in the SAM co-vary with the signal from the analyte solution. With this knowledge, a normalization procedure was used to increase the precision of the analyte signal by 1 order of magnitude, to 8–13%, fully acceptable for quantitative work.

Surface enhanced Raman spectroscopy (SERS) is a very attractive technique for detection of various organic and inorganic molecules due to its sensitivity[1,2] and selectivity. To generate a surface enhancement, the molecule of interest must be attached or in the vicinity of a metal surface.[3] Different active surfaces with appropriate nanometer architecture have been developed over the years. They include electrochemical roughened electrodes,[4,5] island films,[6] colloids,[7] microfluidic formation of colloids,[8] and electron beam lithography,[9,10] each having their application and advantages. The optimum substrate is versatile and generates high and reproducible SER signals. Colloids present a great advantage since they can be synthesized in different sizes and shapes, thus, their optical properties can be controlled. Surface modifications can be performed to produce water-soluble particles,[11] and biofunctionalities can be attached to affect specificity.[12–14] However, colloids are sensitive to alterations in ionic strength and stabilizing agents.[15]

Reproducibility has been the weak point when SERS has been used for sensing purposes. The signal from analyte molecules will change not only because of the concentration but also due to changes in the metal substrate. Also, the contribution of the two enhancement mechanisms, chemical and electromagnetic enhancement, will effect the signal intensity and spectral shape.

Increase in the degree of order by controlled flow, aggregation, positioning, or averaging through large illumination area and long integration time, has been the most successful approach to reduce signal variation. The spatial variation for a substrate has, surprisingly, rarely been reported for far-field setups.

The use of self-assembled monolayers (SAM) is an efficient and known way to produce organic coatings onto gold and silver surfaces.[16,17] They have been used extensively for surface studies and chemical sensing such as surface plasmon resonance and quartz crystal microbalance. Several groups have also performed SERS measurements using different SAM coatings to characterize orientation and stability of adsorbates,[18] extraction of analytes,[5,19] and protection of the metal core.[20,21] There is a great flexibility using SAMs since the choice of coating molecules can be tailored to get specific chemical or physical properties such as functionality, hydrophobicity, charge, or bulkiness.

Our approach in this paper is to further contribute to the quantitative aim of SERS, not by increasing degree of order but rather the opposite. Independent of the surface roughness, positioning, orientation of particles, and colloidal aggregation, we aim to reveal the enhancement and normalize the intensities to this reference. If a reporting molecule is attached to the surface it will be enhanced by the local surface plasmon field (LSPF) in the same manner as other molecules in the vicinity. If the cooperative field from several colloids is beneficial for enhancement of an analyte molecule in the surrounding media, the coating consisting of reporter molecules will be influenced in the same way

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and, thus, the signals will co-vary, and the ratio between them will be constant.

In this communication we present signal normalization with the use of a reporting SAM layer consisting of a thiol derivative of Dabcyl (4-[4-(dimethylamino)phenyl]diazenyl}-N-(2-sulfanylethyl)cyclohexa-2,4-diene-1-carboxamide) with Rhodamine 6G (R6G) or Ru(2,2-bipyridyl)$_3$Cl$_2$ as the analytes. The recorded analyte spectra overlap with the Dabcyl signal. Consequently, no selective bands can be evaluated for Dabcyl. The normalization capabilities are therefore evaluated using multivariate (principal component analysis (PCA)[22] and partial least squares (PLS)) techniques.[23,24] These methods involve the whole spectral region and decompose the dataset based on its variations.

The thiol derivative of Dabcyl was prepared in the following way: 20 mg of 4-((4-(dimethylamino)phenyl)azo)-benzoic acid succinimidyl ester was mixed with 3 equivalents of cysteamine in 20 mL acetonitrile (ACN), under argon atmosphere. All glassware was rinsed with aqua regia and then with copious amounts of milli-Q water. The reaction was performed overnight at ambient temperature. The solvent was evaporated and the residue was dissolved in dichloromethane (20 mL) and extracted with 20 mL of acidic (0.1% formic acid) water three times. The solvent was evaporated and the product (Dabcyl-SH) was stored under argon in a freezer until further use. The identity of Dabcyl-SH was confirmed by electrospray ionization mass spectrometry (Micromass Quattro LC) operated in positive mode, coupled to an infusion pump with a 50:50 NH4Acetate/ACN and 0.1% formic acid as the running buffer. The molecular weight of (328 g/mol) could be confirmed. A water solution containing gold colloids with a diameter of 60 nm (Ted Pella, Redding) was mixed with an ACN solution of 0.25 mM Dabcyl-SH. The reaction was done overnight. Excess reactants were removed by sonication followed by centrifugation in ACN (3000 g, 10 min) and removal of the supernatant six times. The SAM-coated gold colloids were soluble in ACN for some time but precipitated overnight. The colloids became soluble again after they were treated with ultrasound.

Five μL of Dabcyl-coated gold colloids (optical density 0.32 at 550 nm) were dried onto a 0.11 mm cover glass. A 50 μL drop of 0.1 M phosphate buffer (pH 7.1) containing 1 mM Rhodamine 6G or 4.7 mM Ru(2,2-bipyridyl)$_3$Cl$_2$ was placed on the metal cluster. SER spectra were acquired by exciting the system with a 633 nm laser (400 μW at sample) via a 100× (NA 1.3 oil) objective, and the backscatter light was filtered, dispersed, and detected using a Labram INV microspectrometer (Jobin-Yvon) fitted with a cooled NIR enhanced CCD. For each sample, two XY maps consisting of 100−200 spectra were collected with a step size of 1 μm. In total 900 spectra between 400 and 1800 cm^{-1}, with a resolution of 4 cm^{-1}, were collected with an integration time of 1 s. Two maps of pure Dabcyl-coated clusters and pure R6G or Ru(2,2-bipyridyl)$_3$Cl$_2$ on gold clusters were collected in the same way.

All coated colloids yielded strong Dabcyl signals. These high signal intensities have to be attributed to the proximity
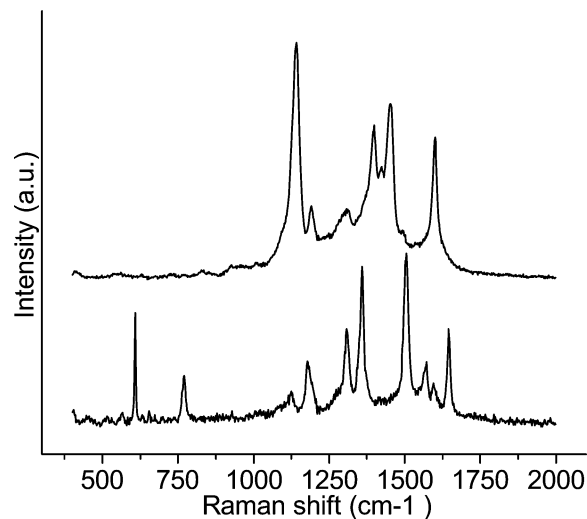

Figure 1. SERS spectra of a SAM layer consisting of a thiol derivative of Dabcyl (upper) and a SERS spectrum of the analyte Rhodamine 6G (lower). The spectra were recorded using excitation with 400 μW of 633 nm laser light for one second.
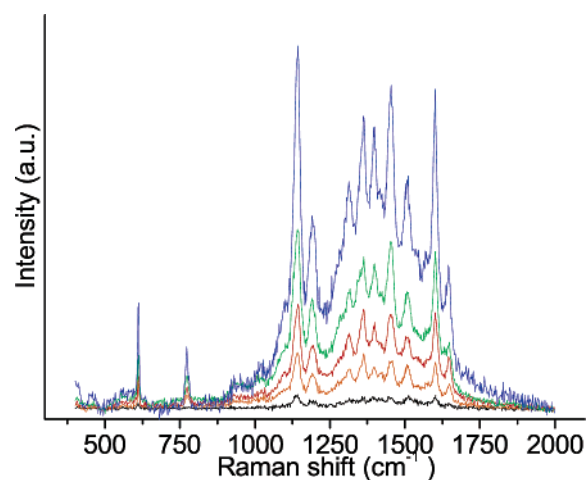

Figure 2. Several spectra of the analyte solution (Rhodamine 6G) in contact with a SAM-coated colloidal gold cluster. Both the analyte and the coating can be detected. The spectra are from different locations on the clusters and the signal strength varies due to spatial differences in the signal enhancement. The signal from the SAM layer co-varies with the signal from the analyte, and the ratio has been used to normalize the signal from the analyte.

of Dabcyl to the metal in combination with its high surface coverage. Azobenzene derivatives have been shown to form SAMs with large surface coverage.[25] Spectra from the Dabcyl coating are consistent with previous observations of similar molecules attached on gold surfaces.[26] The following major bands could be observed and assigned:[27] 1142 (phenyl-N=N stretch) and 1193 (C−H bend); 1399, 1423, and 1454 (−N=N− vibrations), and 1601 (benzene C=C stretch) (Figure 1).

To test the ability of the coating to compensate for the LSPF variations, a concentrated solution of an analyte with high cross section was added onto the dried metal cluster. The strong signal from Dabcyl requires a high concentration of analyte. This is in accordance with a previous report where the SAM layer signal was strong compared to the analytes.[20] Collection of Raman spectra over the colloidal gold clusters
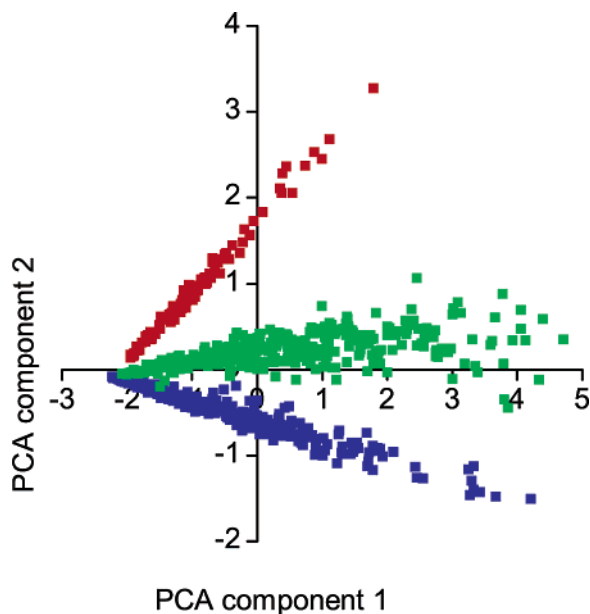
Figure 3. Scores from a principal component analysis of three data sets. Each color represents one composition: pure Dabcyl (red), pure Rhodamine 6G (blue), and a mixture of Dabcyl and Rhodamine 6G (green). Each point describes a SER spectrum, and a clear separation between compositions can be seen.

showed a clear relationship between the two signals, even if the signal strength varies substantially (Figure 2).

The variation in enhancement over the clusters is characterized with RSD values of 78−82% for the 703 cm^{-1} band area of R6G, respectively. Similar results were obtained for Ru(2,2-bibyridyl)$_3$Cl$_2$. The far-field setup generates a spot size (≈1 μm) that averages out some of the actual spatial variation. Larger variations on the nanometer scale could be expected theoretically.[3] Since the spectra of Dabcyl show no selective bands with respect to R6G, we applied a multivariate approach to normalize the SER signal. Furthermore, the large number of spectra, 800, promotes the use of chemometrics.

Three sets of SERS data were collected: pure Dabcyl coated colloids, pure R6G, and R6G in solution over Dabcyl colloids. Principal component analysis (PCA) was used to evaluate the collected spectra from each set of data and find points where the signal had saturated the detector or where the spectra were of unexpected shape or origin. Only a few spectra from each map had to be discarded. A linear background polynomial was subtracted from the spectra. In addition, the variation in each dataset was scaled to the same magnitude. A PCA on all three sets of spectra showed a clear separation between the pure spectra, with the mixture between (Figure 3).

The main variation in the datasets is described by the first PCA component, and it corresponds mainly to the signal strength. The variation along the second PCA component described the spectral variation.
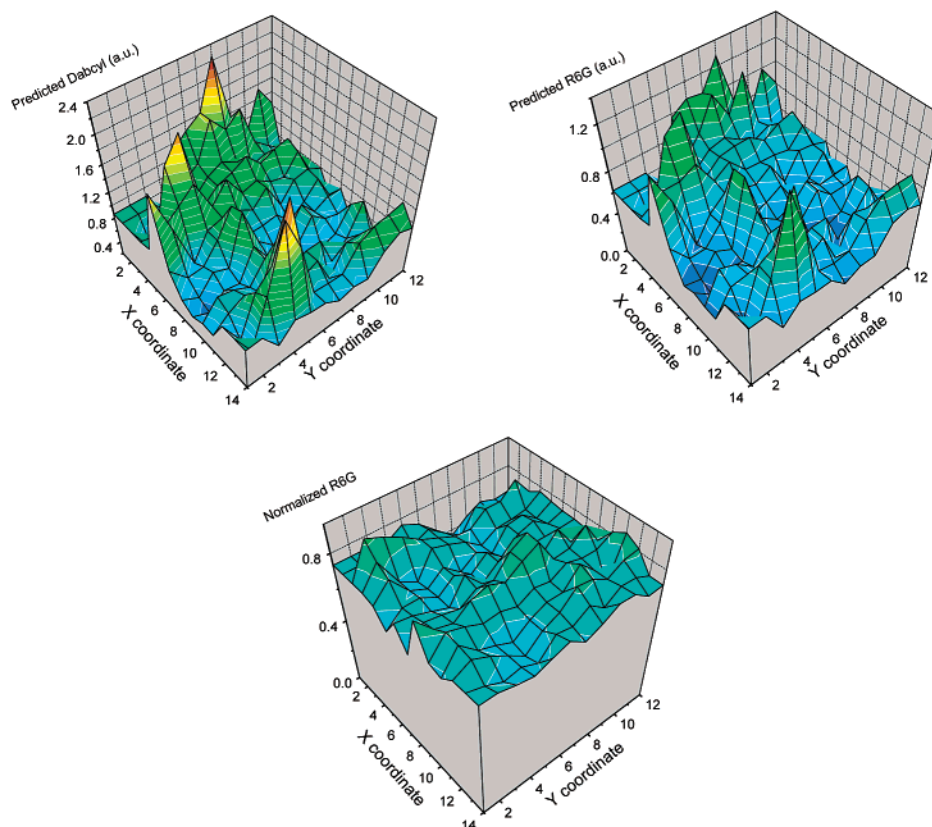

Figure 4. SERS maps over a Dabcyl coated colloidal Au cluster with an analyte (Rhodamine 6G) in solution. The large spatial signal variations for the coating and the analyte are covariant (upper left, upper right) and can be used to normalize the signal intensity (lower) and thereby increase the precision with 1 order of magnitude. The signal contribution from each substance is estimated by a partial least squares (PLS) model, and the ratios between the coating and the analyte are described with RSD values of 8−13%.

To estimate the signal contribution of the two substances in each spectrum, PLS regression was used. A model design with the following combinations of Dabcyl and R6G was constructed: high/low, low/high, high/high, and low/low. Three spectra with similar intensity from each of the pure compounds and the mix were selected and assigned an arbitrary intensity value. Three spectra with low intensities were also selected and assigned zero intensity. The model consisted of two components and explained 82% of the intensity variation. The remaining spectra (752) were then predicted, and the signal contributions of Dabcyl and R6G were used to calculate the ratio between them, and, thus, for the measure of the signal normalization capability. Spectra with signal intensities below the limit of quantification (LOQ) were discarded. LOQ was determined as the average estimate of Dabcyl in pure R6G samples, plus 10 times the standard deviation.

The two maps of R6G in solution over Dabcyl coated gold clusters showed a large spatial signal variation. A univariate evaluation of one selective R6G band (703 cm^{-1}) gave RSD values of 78% and 83% (Figure 4). Multivariate PLS regression on R6G improved these to 38% and 50%, respectively. The normalization procedure based on the ratio between PLS estimates of the SAM coating and the analyte decreased the variation with 1 order of magnitude down to 8% and 13% respectively (Figure 4). The co-variation between the signals facilitated a stable analyte signal, even if the SERS active substrate is chaotically assembled with strong heterogeneity and altering local enhancements.

To improve the function of the SAM, it could be useful to change the properties of the reporting molecule to give spectrally selective bands, and possibly, fewer bands in total. Furthermore, the signal strength of Dabcyl is high, and therefore it would be preferable to choose a reporting molecule with lower cross section. Since the electromagnetic enhancement is strongly dependent on distance, it could be a better choice to use a shorter molecule, which would allow the analyte closer to the surface.

The presented normalization procedure makes it possible to obtain quantitative data from chaotic assemblies. An interesting application of the combination of SAM coated nanoparticles and Raman spectroscopy, is to use it as a detector. The size of such a detector could be in the 5−100 nm range, well below the typical scale of microfluidic assay formats.

References

(1) Kneipp, K.; Wang, Y.; Kneipp, H.; Perelman, L. T.; Itzkan, I.; Dasari, R.; Feld, M. S. *Phys. Rev. Lett.* **1997**, *78*, 1667.
(2) Xu, H.; Bjerneld, E. J.; Käll, M.; Börjesson, L. *Phys. Rev. Lett.* **1999**, *83*, 4357.
(3) Moskovits, M. *Rev. Mod. Phys.* **1985**, *57*, 783.
(4) Fleischmann, M.; Hendra, P. J.; McQuilla, A. J. *Chem. Phys. Lett.* **1974**, *26*, 163.
(5) Mosier-Boss, P. A.; Lieberman, S. H. *Appl. Spectrosc.* **2003**, *57*, 1129.
(6) Seki, H. *J. Vac. Sci. Techn.* **1981**, *18*, 633.
(7) Vonraben, K. U.; Chang, R. K.; Laube, B. L. *Bull. Am. Phys. Soc.* **1981**, *26*, 378.
(8) Keir, R.; Igata, E.; Arundell, M.; Smith, W. E.; Graham, D.; McHugh, C.; Cooper, J. M. *Anal. Chem.* **2002**, *74*, 1503.
(9) Gunnarsson, L.; Petronis, S.; Kasemo, B.; Xu, H.; Bjerneld, J.; Kall, M. *Nanostruct. Mater.* **1999**, *12*, 783.
(10) Kahl, M.; Voges, E.; Kostrewa, S.; Viets, C.; Hill, W. *Sens. Actuators, B* **1998**, *51*, 285.
(11) Templeton, A. C.; Chen, S. W.; Gross, S. M.; Murray, R. W. *Langmuir* **1999**, *15*, 66.
(12) Berggren, C.; Johansson, G. *Anal. Chem.* **1997**, *69*, 3651.
(13) Freire, R. S.; Pessoa, C. A.; Kubota, L. T. *Quimica Nova* **2003**, *26*, 381.
(14) Gooding, J. J.; Hibbert, D. B. *Trac-Trends Anal. Chem.* **1999**, *18*, 525.
(15) Rodger, C.; Rutherford, V.; White, P. C.; Smith, W. E. *J. Raman Spectrosc.* **1998**, *29*, 601.
(16) Abraham, U. *Self-assembled monolayers of thiols*; Academic: San Diego, 1998.
(17) Whitesides, G. M.; Grzybowski, B. *Science* **2002**, *295*, 2418.
(18) Zhang, Z. J.; Hou, S. F.; Zhu, Z. H.; Liu, Z. F. *Langmuir* **2000**, *16*, 537.
(19) Shafer-Peltier, K. E.; Haynes, C. L.; Glucksberg, M. R.; Van Duyne, R. P. *J. Am. Chem. Soc.* **2003**, *125*, 588.
(20) Olson, L. G.; Lo, Y. S.; Beebe, T. P.; Harris, J. M. *Anal. Chem.* **2001**, *73*, 4268.
(21) Tao, A.; Kim, F.; Hess, C.; Goldberger, J.; He, R. R.; Sun, Y. G.; Xia, Y. N.; Yang, P. D. *Nano Lett.* **2003**, *3*, 1229.
(22) Wold, S.; Esbensen, K.; Geladi, P. *Chemom. Intell. Lab. Syst.* **1987**, *2*, 37.
(23) Sjöström, M.; Wold, S.; Lindberg, W.; Persson, J. Å.; Martens, H. *Anal. Chim. Acta* **1983**, *150*, 61.
(24) Geladi, P.; Kowalski, B. R. *Anal. Chim. Acta* **1986**, *185*, 1.
(25) Yu, H. Z.; Wang, Y. Q.; Cheng, J. Z.; Zhao, J. W.; Cai, S. M.; Inokuchi, H.; Fujishima, A.; Liu, Z. F. *Langmuir* **1996**, *12*, 2843.
(26) Ye, Q.; Fang, J. X.; Sun, L. *J. Phys. Chem. B* **1997**, *101*, 8221.
(27) Itoh, T.; McCreery, R. L. *J. Am. Chem. Soc.* **2002**, *124*, 10894.