Combination of surface enhanced Raman and hyper-Raman imaging using hyperspectral analysis

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Combination of surface enhanced Raman scattering (SERS) with its two-photon analogue, surface enhanced hyper-Raman scattering (SEHRS), provides complementary structural and chemical information due to the different corresponding selection rules. Depending on the molecular symmetry, SEHRS may probe IR active modes or additional “silent” modes, which are seen neither in Raman nor in IR spectra [1]. In a SEHRS experiment, thanks to the near infrared excitation, deeper penetration and femtoliter-range probed volume can be achieved, resulting in an improved resolution for imaging compared with SERS. Because technically SERS and SEHRS can be detected quasi-continuously within one micro-spectroscopic setup, it is straightforward to combine these complementary methods to study nanoparticle-molecule interactions [2,3]. When applying multivariate signal processing techniques (e.g., hierarchical cluster analysis, HCA, or principal component analysis, PCA) to one- and two-photon excited SERS spectra, the sensitivity in the analysis can be substantially increased [4].

Here, we investigated the SEHRS and SERS spectra and the spatial distributions of crystal violet (CV) and malachite green (MG) on immobilized plasmonic surfaces. Each sample type consisted of two connected regions, either a CV and CV-MG mixture or an MG and CV-MG mixture, respectively. The SERS and SEHRS spectra were recorded by scanning many small, microscopic areas across the border regions and also at the distinct non-border regions on both sides on a macroscopic (millimeter) scale. Because CV and MG structurally differ only in one dimethylamino group, their one- and two-photon excited spectra exhibit a high degree of similarity. To utilize all of the spectral differences between the spectra of the studied dyes and their mixtures, we successfully applied PCA to map the distribution of the molecules. The presented hyperspectral mapping technique constitutes a novel method for multiplex imaging of complex biological systems. As the first real demonstration of this hyperspectral imaging, we show that combined SERS and SEHRS fingerprints can also be collected and studied from single living cells which will help SEHRS hyperspectral imaging to become an important new direction in future all-optical nanosensing as well as in nanobiophotonics.

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