Study of Combined One- and Two-photon Excited SERS-Nanosensors for Bio-applications

Marina Gühlke, Zsuzsanna Heiner, Janina Kneipp

Department of Chemistry, Humboldt-Universität, Brook-Taylor-Str. 2, 12489 Berlin, Germany

Biological tissues and cells are transparent and fluorescence-free at wavelengths between 800 and 1200 nm making this range an excellent choice for the combined use of one- and two-photon near-infrared excited laser spectroscopy techniques for *in vivo* study. Surface-enhanced Raman scattering (SERS) can provide chemical fingerprints at the single molecule level thanks to the extremely high local field enhancement factors in the close vicinity of plasmonic nanostructures. Therefore, combining Raman signature capability with scanning microscopy has generated an array of imaging modalities for material and biological studies at the nanometer scale. Surface-enhanced hyper-Raman scattering (SEHRS), which is a two-photon excited analogue of SERS, can offer additional chemical and structural information because of the altered selection rules and a better spatial resolution. In addition, the inherently low multi-photon cross sections can easily be overcome near metal surfaces by exploiting the nonlinear scaling of the signal with the intensity of the excitation field [1]. Moreover, using SEHRS, the detection wavelength range can conveniently fall into the visible, where cameras exhibit better sensitivities and signal-to-noise ratios than in the near-infrared. In order to exploit all these advantages, we employed a near-infrared excited SERS and SEHRS-based microspectroscopic approach, simultaneously, for the investigation of the applicability of various metal nanostructures prepared by different procedures. Here, we demonstrate its excellent feasibility of our combined approach for all-optical pH nanosensing over a wide pH-range.

The vibrational modes of para–mercaptobenzoic acid (pMBA) change upon protonation and deprotonation which makes pMBA a useful pH-probe in cells [2]. In our study, we compare one- and two-photon excited non-resonant Raman spectra of pMBA in the local fields of various plasmonic nanostructures in the pH range of 2-12 [3]. We found that the combination of SERS and SEHRS spectra excited at 1064 nm allow more robust and precise pH sensing than SERS measurements alone in the 532-850-nm excitation range, where SERS microscopy is typically used. Investigating the plasmonic properties under different experimental conditions, we find that multimodal SERS-SEHRS based fluorescence-free sensing is possible in the low femtoliter range with extremely high enhancement factors and without multiple tagging. Our results demonstrate that a combined one- and two-photon excitation together with the tunable optical properties of plasmonic nanoparticles opens up new possibilities for microscopic bio-sensing.

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References

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