Label-free in situ Raman and SERS imaging of biofilms

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Biofilms are communities of microorganisms embedded in a matrix of extracellular polymeric substances (EPS), which represent the predominant mode of microbial life. EPS (biopolymers such as polysaccharides, proteins, nucleic acids, lipids, and humic-like substances) play a major role in the formation and maintenance of the biofilm structure and in the protection of the embedded microorganisms against environmental stress. Information about physical and chemical properties of the biofilm matrix is relevant in various fields, such as medicine, industry, and technological processes. However, the precise structure, chemistry, and physiology of the biofilm vary with the nature/behavior of its resident microbes, the local environment, and the biofilm age [1]. Therefore, the characterization of the biofilm matrix requires the establishment of a rapid nondestructive analytical tool that reveals detailed chemical information at high spatial resolution, sensitivity, and reproducibility.

Raman microscopy (RM) combines spectroscopic and optical methods and provides whole-organism vibrational fingerprint spectra of the biological samples with spatial resolution in the μm-range. It allows for label-free, noncontact, and nondestructive analysis with little or no interference from water (major component of biofilms). Additionally, Raman imaging provides information on the spatial distribution of different molecular species within heterogeneous samples. We apply RM for the *in situ* chemical characterization of multispecies heterotrophic biofilms and find a heterogeneous composition (polysaccharides, proteins, nucleic acids, and carotenoids) of microbial constituents and EPS. However, RM suffers from low sensitivity, making Raman imaging time consuming. Moreover, the Raman spectra of biofilms typically exhibit only a few bands and therefore available chemical information is rather limited. The application of surface-enhanced Raman scattering (SERS) for biofilm analysis allows us to overcome these drawbacks [2].

Surface-enhanced Raman scattering is a promising technique for the chemical characterization of microbiological systems. It has high sensitivity in comparison with normal Raman spectroscopy, yields highly informative spectra, and can be applied directly in aqueous environment [3]. We use hydroxylamine hydrochloride reduced [4] colloidal silver nanoparticles for label-free *in situ* SERS analysis of multispecies biofilms. Good SERS measurement reproducibility, along with a significant enhancement of Raman signals by SERS ($>10^4$), and the highly informative SERS signature, enables rapid SERS imaging (1 s for a single spectrum) of the biofilm matrix [5]. Thus, SERS has great potential for *in situ* biofilm analysis, including the detection of different constituents and the determination of their distribution in a biofilm even at low biomass concentration (e.g. initial biofilm formation).

References

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