Raman Microspectroscopy for Non-invasive, Three-dimensional Analysis of Biofilms

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Confocal Raman Microspectroscopy (RM) enables the three-dimensional, chemical characterization of transparent samples with particles in the µm range. Especially sensitive and complex structures, e. g. biofilms, are suited for the analysis by RM. Few sample preparations are needed: mechanical strain does not significantly affect the sample during the measurement and water does not interfere with the spectroscopic analysis. In their natural habitat, microbial communities tend to develop biofilms. Aquatic microorganisms predominantly occur in this form of appearance [1]. Non-invasive, three-dimensional, potential time-resolved analyses of biofilms offer the potential for access to fundamental information about interactions between microorganisms, the occurrence of pathogens or the flow of water quality-related pollutants.

Our work with artificial biofilm models indicates the high spatial resolution of RM in all three dimensions. Microparticles and bacterial cells (e. g. E. coli) could be chemically differentiated 0.5 mm below the sample surface. Further, the combination of Stable Isotope Probing (SIP) and Surface-Enhanced Raman Spectroscopy (SERS) was explored as shown in Figure 1. The ability to detect the incorporation of stable isotopes features another striking advantage of the RM for the analysis of biological samples [2-4]. By evaluating red-shifted signals three-dimensionally it is possible to gain a more detailed insight into the fate of stable isotope labeled compounds or relations between microbial communities. Special focus is supposed to be on *Legionella pneumophila*-containing biofilms, which is the causative germ of the Legionnaires' disease. Unicellular host organisms not only facilitate growth, survival and recovery of Legionella but also enhance virulence of this pathogen [5]. The three-dimensional analysis potential of RM might promote new leads into this parasitic relationship and its impact.

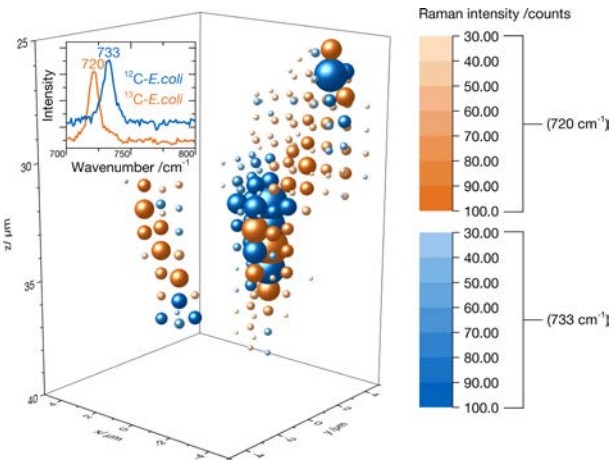

Figure 1. SERS map of an artificial biofilm (^{12}C and ^{13}C labeled *E. coli* with Ag nanoparticles in an agarose matrix) and embedded SERS spectra of single ^{12}C and ^{13}C *E. coli* cells.

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