Micro-Raman spectroscopy of single bacterial cells

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A fast and reliable identification of bacteria is necessary in order to provide an appropriate therapy for patients. Here the cultivation time should be kept to a minimum. For a fast and unambiguous identification of microorganisms Raman spectroscopy has proven to be a valuable tool. Micro-Raman spectroscopy with an excitation wavelength of 532 nm yield a spatial resolution of approx. 1 µm which is quite in the size range of single bacterial cells. [1] Since this laser wavelength is non-resonant to any electronic excitation such as DNA/RNA the Raman spectra obtained from the bacteria reveal spectral information of the various molecules present inside the cell. Therefore, this average molecular information results into phenotypic identification whereas excitation wavelengths in the UV-light range results into genotypic identification. [2]
The localization of microorganisms biotic but also abiotic complex sample matrixes like e.g. air or food samples is often complicated, since many particles are similar in size and shape compared to microorganisms. Therefore, a localization routine was established which allows for the differentiation of biotic and abiotic particle.
One possible approach is the use of fluorescence dyes. Since fluorescence is several orders of magnitude stronger than Raman spectroscopy the fluorescence signal of the dyes may mask the Raman spectrum completely. Nevertheless, using fluorescence labels with absorption maxima far away from the Raman excitation wavelength allows to selectively localize bacteria and identify them in a second step by Raman spectroscopy. In this contribution we show a combination of fluorescence imaging and Raman spectroscopic identification of microorganisms on a single cell level. [3]

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References

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