Raman Microspectroscopic Characterization of Pathogens in Planktonic and Biofilm State

Dragana Kusić[a]; Bernd Kampe[a]; Petra Rösch[a]; Jürgen Popp[a,b]

[a] Institut für Physikalische Chemie und Abbe-Zentrum für Photonics, Friedrich-Schiller-Universität Jena, D-07743 Jena, Germany
[b] Institut für Photonische Technologien e.V. (IPHT), D-07745 Jena, Germany

Biofilms are complex communities of microorganisms characterized by structural heterogeneity and an extracellular matrix of polymeric substances which provides a habitat and nutrients, increases bacterial resistance to antibiotics and detergents allowing the survival and multiplication of bacteria outside a host cell [1, 2]. Waterborne pathogens such as *Legionella* and *Pseudomonas* are naturally present in water environments and persist mostly in a viable but nonculturable state or in association with protozoa and complex biofilm formations [3]. Isolation and afterwards identification of these pathogens from environmental samples by using common identification procedures based on cultivation are extremely difficult and prolonged. The development of a fast and sensitive method based on the cultivation-free identification of bacteria is necessary. In this study, Raman microspectroscopy is used to discriminate between different species of bacteria grown both in planktonic state and biofilms and to investigate spectral differences between those two forms of the same organisms. Our findings suggest that Raman microspectroscopy combined with support vector machines is a fast and reliable method to distinguish between seven species of bacteria grown in tap water in both planktonic form and biofilms. For further investigations, it is important to adapt the isolation procedure to the use of Raman microspectroscopic analysis on the single cell level in order to create a Raman database of these waterborne pathogens isolated out of protozoa and biofilms.

Acknowledgements
Funding of the research project RiMaTH (02WRS1276E) from the Federal Ministry of Education and Research, Germany (BMBF) is gratefully acknowledged. We are very grateful to Prof. Dr. Eberhard Straube and Svea Sachse for providing us with the bacterial strains and for useful discussions.

References:

[1] R.M. Donlan, *Emerging Infectious Diseases*. 8, 881-890 (2002).
[2] P.S. Stewart , J.W. Costerton, *Lancet*. 358, 135-138 (2001).
[3] B. Fields, R. Benson, R. Besser, *Clinical Microbiology Reviews*. 15, 506-526 (2002).