

New Software Tool for Automated FT-IR Microspectroscopic Analysis of Micro-colonies

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Infrared spectra of microorganisms provide complex information about the cell structure and composition as well as the metabolic state of the cells. These spectral features are often used to differentiate, classify or even identify bacteria (for review see [1]). Recently, IR-microspectroscopy was successfully employed to reduce the time exposure for microbial analysis dramatically [2]. For this, microcolonies, incubated for 6-8 hours on agar plates, were transferred to IR-transparent ZnSe-plates by a modified replica technique providing a spatially accurate imprint. The dried microbial spots, containing the topmost 2-3 layers of the primary microcolonies, were afterwards spectroscopically analyzed by an FTIR-microscope equipped with a computer controlled xy-stage. For automation of this technique we developed a software (OBJECT), which ideally can be used in combination with the established OPUS/VIDEO and OPUS/MAP packages. For the FTIR-investigation of the imprinted microcolonies the user has merely to define an interesting area on the sample plate. Subsequently, an visible overview image of the defined image is automatically generated. After performing several image processing functions the microcolonies are automatically detected and localized. The found positions are used to accomplish the FTIR measurements of the microcolonies. The size and the position of the detected microcolonies are stored with the associated spectra.

In summary, this software could help to simplify the usage of FTIR-microscopy for microbial investigations. Moreover, it could help to accelerate this promising technique and to increase the throughput.

[1] D. Naumann, D. Helm und H. Labischinski, 'Einsatzmöglichkeiten der FT-IR-Spektroskopie in Diagnostik und Epidemiologie', Bundesgesundheitsblatt, 33, 1990, 387-393

[2] N.A. Ngo Thi, C. Kirschner and D. Naumann, 'FT-IR Microspectrometry: A new tool for characterizing micro-organisms' in: A. Mahadevan-Jansen, G.J. Puppels (eds.) Biomedical spectroscopy: Vibrational spectroscopy and other novel techniques, Proceedings of SPIE vol. 3918, pp 36-44, Bellingham, Washington, (2000).