Micro-Raman study and identification of inactivated Bacillus endospores

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Under exogenous stress a few Gram-positive bacteria can metabolize into endospores. Among them is the soil-borne *Bacillus anthracis* as the etiological agent of the acute fatal disease anthrax in mammals. Thus, its potential as a biological warfare agent makes the detection and identification of bacterial endospores a vital concernment of human and veterinary medical interest.
Micro-Raman spectroscopy has an extensive history as a fast and sensitive tool for biological organism detection, classification, and identification[1]. Because Raman spectra provide a snapshot of the total molecular composition of single cells they inherently contain all the information needed to accurately identify microorganisms down to the subspecies level.Within the scope of point-of-care-testing a detection of intentionally released biosafety level (BSL) 3 agents, like *Bacillus anthracis* endospores, or their products, is attainable. But as a matter of fact, no Raman-spectroscopy compatible inactivation method for the notoriously resistant endospores has been elaborated so far.

In this contribution several physical and chemical killing methods for *Bacillus* endospores were assessed and evaluated in terms of sporicidal capacity and information conservation in the Raman spectra. The latter fact has been verified by successfully applying self-learning machines (like Support Vector Machines or Artificial neural networks) to identify *B. anthracis* related inactivated endospores with adequate accuracies within the range of the limited model database we employed in this work.
Furthermore, appropriate procedures of isolating the bio-agents from soil-like matrices have been determined to ensure a decent identification accuracy and taxonomic resolution[2].
With a custom-built, fully automated device the detection and actual micro-Raman measurements with visible laser excitation (532 nm) are performed. A comparable preceding model, which was developed in our work group before, proved to be a reliable device for the online monitoring and identification of bioaerosols[3]. As a new feature, actively fluorescence-stained endospores could be detected among remaining matrix to minimize the measurement efforts[4].

Acknowledgement
Funding of the research projects 13N9547 and 13N9549 from the Federal Ministry of Education and Research, Germany (BMBF) is gratefully acknowledged.

References:

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