Multivariate classification of bacteria utilizing LIBS and Raman Spectroscopy

A conventional discrimination and identification of bacteria and bacterial strains is complicated and time consuming procedure. The process can be simplified and accelerate by using spectroscopic methods and multivariate data analysis.

Here the performance of LIBS and Raman spectroscopy for bacteria discrimination is studied. Moreover, the performance of merged data is also examined. For data discrimination the PCA was employed and for data identification the artificial neural networks (namely Kohonens self-organazing maps) was utilized.

Experiment setup

The measurements were performed directly on agar plate. In case of LIBS, the laser energy was 50 mJ per pulse and the spot size diameter varied from 0.2 to 0.6 mm. For each sample the region with high bacteria concentration was chosen. Within this region 250 × 10 points with step size 0.65 mm was measured. This resulted in 250 unique LIBS spectra. Detector gate delay was 1.2 μs and gate width was 50 μs.

The Raman spectroscopy measurements were performed on commercially available device inVia Reflex (Renishaw, UK). We used a laser on wavelength 785 nm. The laser radiation was focused on the sample using a Leica objective (50×, NA 0.5). The spot size was 30 × 10 μm. Scattered radiation was led in optical spectrometer via collection optics. 10 repetitions of a measurement were performed for each sample, i.e. 10 Raman spectra were obtained.

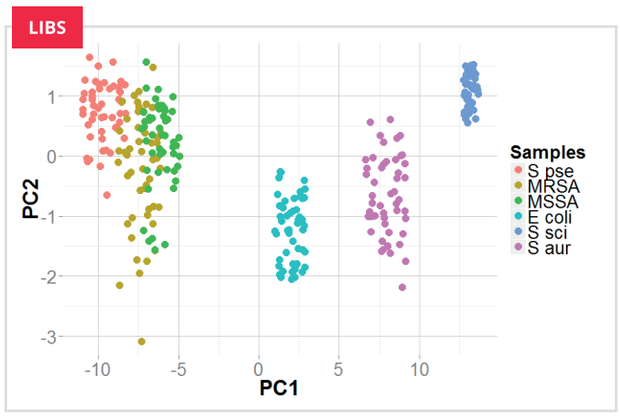
Results

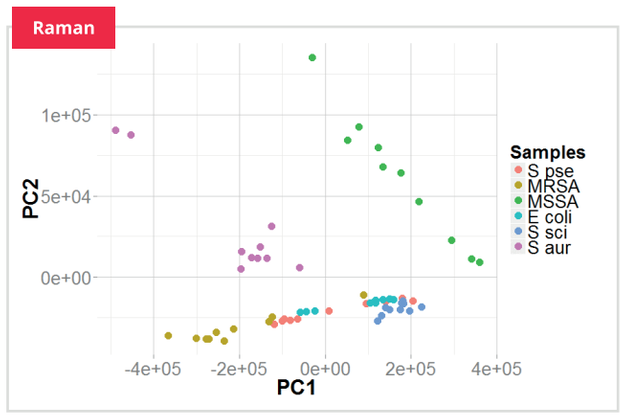
A score plot of PC1 vs PC2 for LIBS data (a), Raman data (b) and merged data (c) is presented in figures bellow. It can be clearly seen that PCA of LIBS data is able to discriminate 3 bacteria, PCA of Raman spectroscopy data discriminate only 2 bacteria strains and merged data are able to discriminate all bacteria.

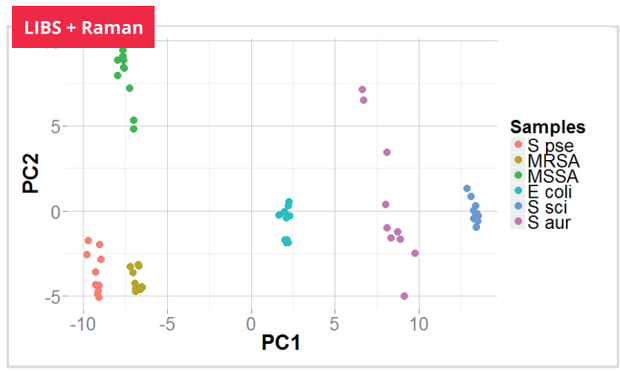
The classification success rate is presented in Tab 1.

Tab. 1: The classification succes rate.

Bacteria strain/classification success	LIBS	Raman	Merged data
Staphylococcus pseudointermedius (S pse)	70 %	50 %	100 %
Staphylococcus aureus CCM 4750 - methicillin resistant (MRSA)	45 %	75 %	100 %
Staphylococcus aureus CCM 3953 - methicillin sensitive (MSSA)	75 %	100 %	100 %
Escherichia coli CCM 3954 (E coli)	100 %	100 %	100 %
Staphylococcus sciuri (S sci)	100 %	100 %	100 %
Staphylococcus aureus CCM 4223 (S aur)	100 %	100 %	100 %





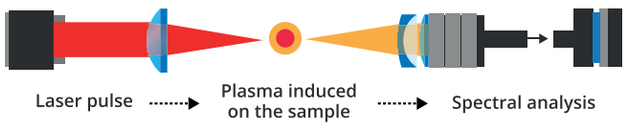


Supplementary materials

The procedure and results are described in detail in D. Prochazka, *et al.*, Spectrochim. Acta B **139**, 6-12 (2018).

LIBS measurement with the Sci-Trace

Laser-Induced Breakdown Spectroscopy (LIBS) is a modern and flexible analytical technique. It is a combination of laser ablation and atomic emission spectroscopy. Pulsed laser rejects a small part (down to few nanograms) of analyzed material and creates a microplasma.



Spectral analysis of the laser-induced plasma radiation provides a qualitative and quantitative data about the chemical composition of the analysed sample.

It is possible to analyze solid, liquid or gaseous samples without any special sample preparation in a matter of seconds.

LIBS is sensitive to the majority of chemical elements, including light elements, with limits of detections as low as about 1-10^2 ppm.

Measurements from this application list were performed utilizing the **Sci-Trace** - configurable analytical instrument specialized on the LIBS technique.

Sci-Trace is designed by scientists for scientists and can be configured to meet the requirements not only of novice or experienced LIBS researchers but also of any analytical laboratory user. Sci-Trace is easily extendable with a number of specialized modules, therefore it is always ready for experimentator`s diverse ideas.

Sci-Trace includes advanced spectra processing software: the **AtomAnalyzer**.