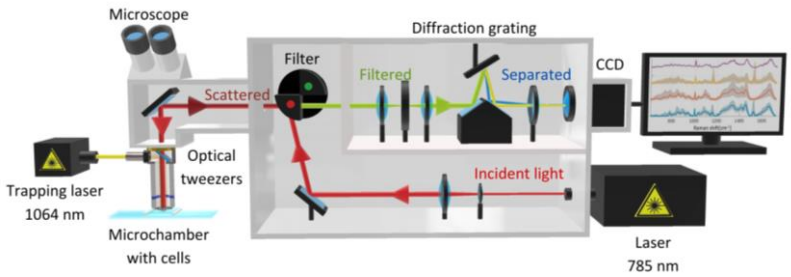
RAMAN TWEEZERS

Applied and Integrated Photonics Group

Institute of Scientific Instruments
The Czech Academy of Sciences

Application Note

Raman tweezers, a combination of Raman spectroscopy and compact optical tweezers, are an optical tool that allows single-cell analyses and manipulations. This is very advantageous in microbiology since it facilitates studies of individual microbial cells such as bacteria or yeasts. This way, the microbial cells can be obtained from samples where they are present in low numbers; they can be moved to specific locations and cultured and/or treated with antimicrobial agents. Also, this way, it is possible to study the metabolic changes of individual microbial cells. Combined with microfluidic platforms, Raman tweezers represent a powerful tool for microbial studies at the single-cell level.



Visualization of Raman tweezers, courtesy of Martin Kizovsky, ISI CAS, Brno, CZ.

Compact optical tweezers module attached to Renishaw inVia Raman microscopy system.

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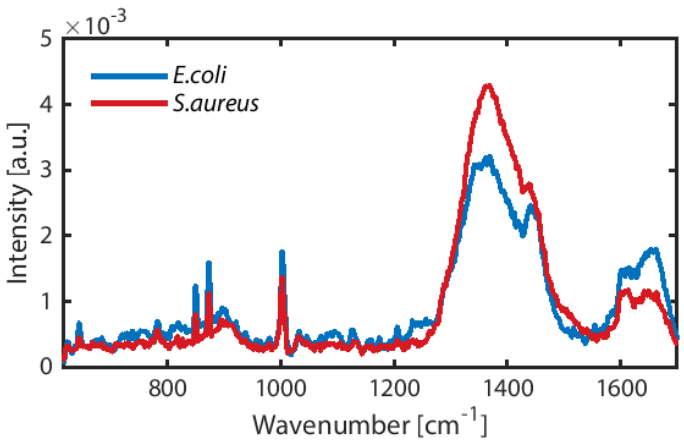
Experiment

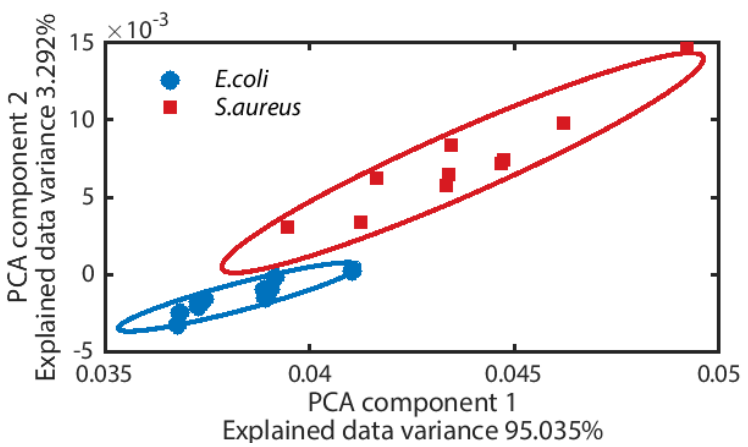
Microbial cells were cultured for 24 h at 37 °C on Mueller-Hinton (MH) agar plates. The strains were obtained from the Czech Collection of Microorganisms, Brno, CZ.

Before the experiment, one loop full of bacteria (1 µl) was transferred into 1.5 ml of deionized water. 20 µl of the sample was then transferred onto CaF_2 glass (Crystan, UK) and covered with a cover glass. To avoid drying, a special tape was used. A 1064 nm laser was used to capture the cells. In the course of the experiment, a commercial Renishaw Raman spectrometer (Renishaw inVia Raman Spectrometer, Renishaw Plc., Wotton-under-Edge, UK), using a single-mode diode laser at wavelength 785 nm as the excitation source was used, which was provided and operational within the experimental setup. The diameter of the circular laser beam was approximately 2 µm. The system included a microscope objective (Olympus, 100x, PLAN chromat, immersion objective). The spectral acquisition was performed using 20 accumulations of 1 s. Ten spectra per strain were collected.

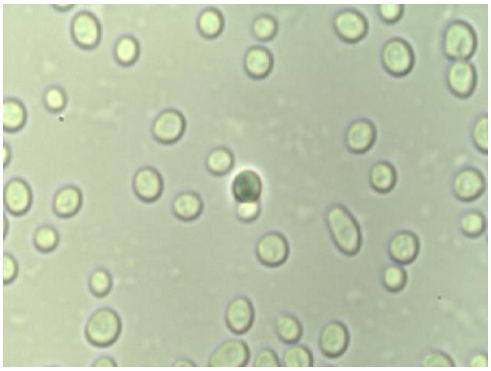
Acquired Raman spectra were treated with the Savitzky–Golay filter (2nd order, 7 width) coupled to the advanced rolling filter background removal routine (10 passes, 700 px circle radius). Subsequently, they were analyzed using in-house written analysis software using MATLAB (MathWorks, MA, USA).



Raman spectrum of E. coli and S. aureus obtained by Raman tweezers, courtesy of Katarina Mlynarikova, Faculty of Medicine, Masaryk University, Brno.



Principal component analysis (PCA) plot of the first two principal components. Ellipses around the principal components were plotted using a Mahalanobis distance of 2.15, courtesy of Katarina Mlynarikova, Faculty of Medicine, Masaryk University, Brno.



Yeast C. albicans trapped with compact optical tweezers.

Commercial Renishaw Raman spectrometer with compact optical tweezers attached.

Compact optical tweezers module attachable to a Raman microscope.