Application Note AN-RS-047

Rapid phenotypic identification of microorganisms with Raman

A simple and nondestructive method for bacterial analysis

Microorganisms are among the most diverse life forms on Earth. They exhibit unique characteristics and play crucial roles in ecological nutrient and material cycles. Microorganisms are essential to food production, including yogurt and alcoholic beverages, and in the remediation of environmental contaminants. Additionally, genetic modification of microorganisms facilitates production of valuable products like insulin. Given their importance, many countries maintain specialized repositories like the American Type Culture Collection (ATCC) and the Swiss Collection of Microorganisms (SCM) to preserve and accumulate microorganisms.

Traditionally, identifying microorganisms such as bacteria involved sequencing their genetic makeup. This expensive process requires specialized training and equipment. However, Raman spectroscopy is a potential tool for identifying bacteria and detecting metabolites produced by the culture, providing insights into the bioprocesses and function in an ecosystem. Metrohm's laboratory Raman portfolio contains options for 785 nm and 1064 nm Raman interrogation of bacterial cultures.

INTRODUCTION

Raman spectroscopy is used in microbiology for its potential to identify bacteria and monitor metabolites. All living organisms on Earth are composed of carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, and other trace elements. These elements bond together to form DNA, lipids, amino acids, and other biomolecules. The composition of these biomolecules varies between organisms. Some bacteria store metabolites (e.g., polyphosphate and glycogen) depending on environmental conditions. The Raman spectra of bacteria reflect these chemical differences, enabling their identification and elucidating their roles in bioprocesses.

EXPERIMENT

Lysogeny broth (LB) agar culture media was prepared by dissolving LB powder and agar powder in deionized water following manufacturer specifications (Sigma-Aldrich). After autoclaving, the mixture was poured into sterilized glass petri dishes and cooled. Once the LB agar solidified, fingers were pressed onto the surface to transfer bacteria to the media. The petri dish was then incubated at room temperature until bacterial colonies were observed.

The petri dish was placed on a BAC150B probe holder and BAC151C Video microscope, and Raman spectra were collected from colonies and the culture media (Figure 1). Instrument setup and acquisition parameters are summarized in Table 1.

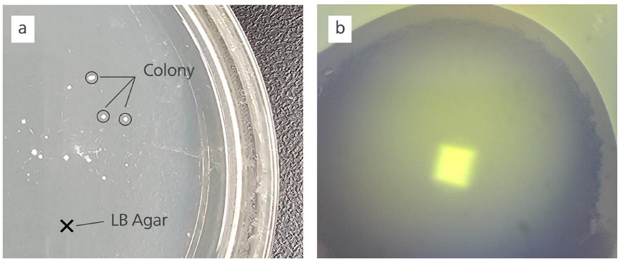
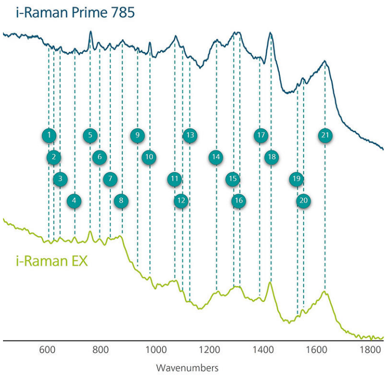


Figure 1. Bacterial colonies formed on the LB agar (a), with a magnified view of a colony under the BAC151C + 50x objective (b).

Table 1. Instrument setup used in this study Instrument setup and experimental parameters used in this study. * Acquisition parameters vary depending on the colony characteristics.

Instrument	Probe holder (BAC150B)	Video microscope (BAC151C)
i-Raman Prime 785	BAC102-785HT	50x objective
i-Raman EX	BAC102-1064HT	50x objective
BWSpec Software		
Acquisition Parameters*		
Laser power (%)	30–100	
Integration time	3–60 s	
Averages	3–5	

RAMAN SPRECTRA OF BACTERIAL COLONY



Position	cm^{-1}	Assignments
1	622	Phenylalanine
2	642	Tyrosine
3	669	Guanine
4	723	Adenine
5	781	Guanine, cytosine, citric acid
6	811	Tyrosine
7	854	Tryptophan δ-ring
8	903	COC stretch
9	957	CN Stretch
10	1002	Phenylalanine
11	1101	Carbohydrate
12	1128	=C-C= unsaturated fatty acid
13	1156	Tyrosine
14	1253	Amide III
15	1321	Amide III
16	1337	Adenine
17	1419	COO- symmetric stretch
18	1454	CH2 bending (lipids)
19	1554	Tryptophan
20	1572	EPS
21	1654	Amide I

Figure 2. Raman spectra of a bacterial colony formed on the LB agar measured using the i-Raman Prime 785 (teal line) and i-Raman EX (green line). Raman peaks that correspond to reported features are marked with dotted lines and assigned in the table on the right [1].

Raman spectra of the bacterial colony (**Figure 2**) contained peaks representing various amino acids (1001, 1156, and 1654 cm^{-1}) and DNA (723, 669, and 1337 cm^{-1}). These features, commonly observed in bacteria, confirm the success of i-Raman Prime 785 in microbial analysis [1].

Raman excitation at 785 nm provided stronger and sharper peaks than excitation at 1064 nm. This is attributed to the higher scattering power of the 785 nm laser and better resolution of the silicon CCD detector compared to the InGaAs array detector with a lower pixel density. However, 1064 nm excitation may mitigate fluorescence associated with darkly colored substrates, such as chocolate agar or blood agar.

DIFFERENTIATING BACTERIA

Bacteria with two distinct morphologies (white and yellow) formed on the LB agar, suggesting that they are different organisms (Figure 3). The Raman spectra of these two bacteria were markedly different, with the yellow bacteria displaying peaks associated with colored pigments commonly found in plants and microorganisms [1].

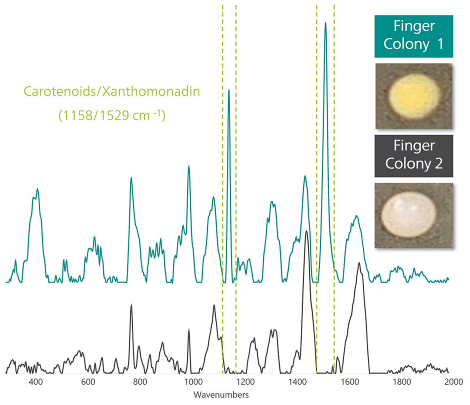


Figure 3. Raman spectra of yellow (teal line) and white (grey line) bacterial colonies formed on the LB agar. Spectra are baseline corrected. Raman peaks shown within the dotted lines may be associated with the yellow color of that particular colony.

Principal Component Analysis (PCA) may be suitable for differentiating bacteria with distinct phenotypic features in small bacterial communities, as in this experiment (Figure 4). However, researchers typically develop machine-learning algorithms to detect subtle differences in minor peaks for more detailed characterization.

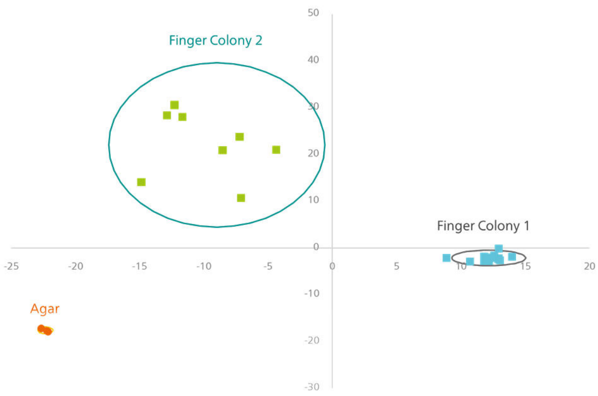


Figure 4. PCA plot of Raman spectra collected from white and yellow colonies formed on LB agar. Confidence ellipse 0.95.

FIELD TEST NOTE

- Using glass petri dishes avoids spectral contributions from plastic.
- Raman spectra of colonies may change after low-temperature storage and extended culturing.
- A video microscope is used with 1064 nm laser excitation to visualize the laser spot

CONCLUSION

Raman spectroscopy can be used to acquire spectra of bacterial colonies directly from solid culture media. Raman spectra collected with 785 nm excitation provides higher resolution, while excitation at 1064 nm reduces fluorescence from culture media.

Simple bacterial colonies can be differentiated using PCA models, but advanced machine-learning algorithms can be used to characterize more complex microbial communities.

Users can easily export the spectral files from i-Raman instruments for further analysis using BWSpec software or other more advanced machine learning tools.

REFERENCE

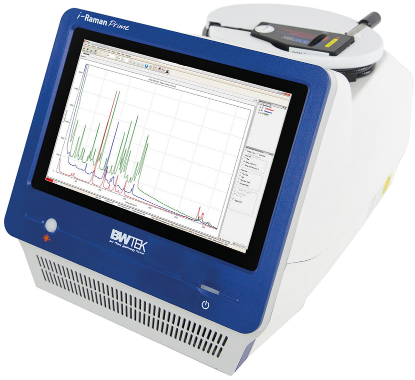
1. Paret, M. L.; Sharma, S. K.; Green, L. M.; et al. Biochemical Characterization of Gram-Positive and Gram-Negative Plant-Associated Bacteria with Micro-Raman Spectroscopy. *Appl Spectrosc* 2010, *64* (4), 433–441. DOI:10.1366/000370210791114293

CONTACT

Metrohm Siam
Phyathai
10400 Bangkok

info@metrohm.com

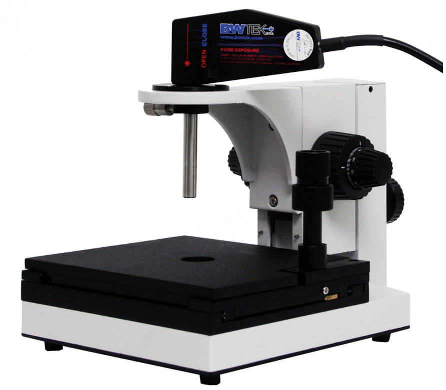
CONFIGURATION



i-Raman Prime 785S Portable Raman Spectrometer

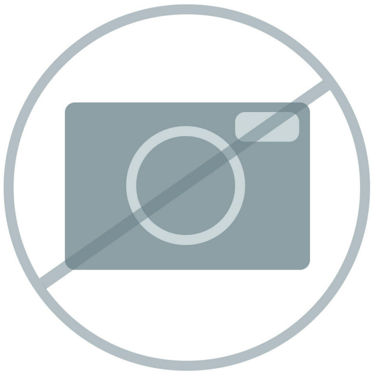
The i-Raman® Prime 785S is a low-noise, high-throughput, and fully integrated Raman system with an embedded tablet computer and a fiber-optic sampling probe. Using a high-quantum-efficiency CCD array detector with TE deep cooling (-25 °C) and high dynamic range, this portable Raman spectrometer delivers research-grade Raman analysis capabilities, including real-time quantitation and identification. The high throughput gives Raman spectra with excellent signal-to-noise ratio, making it possible to measure rapid processes and to measure even the slightest Raman signals, detecting subtle sample differences.

In addition to its portable construction, the i-Raman Prime 785S features the unique combination of wide spectral coverage and high resolution, thus enabling measurements from 150 cm^{-1} to 3,350 cm^{-1}. The i-Raman Prime can be battery-operated for easy portability, providing research-grade Raman analysis capabilities for high-precision qualitative and quantitative work wherever needed. The system is optimized for use with our STRaman® technology for analyses through non-transparent packaging.



Raman Probe Holder

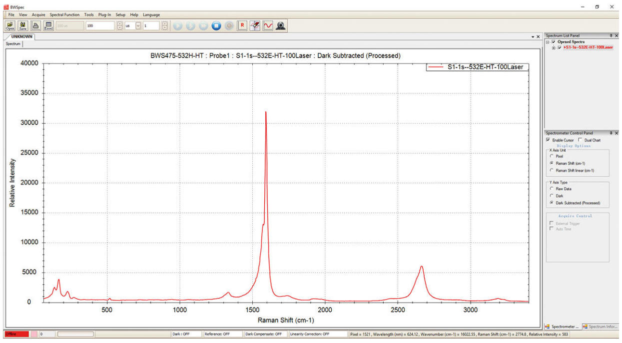
Probe holder for use with B&W Tek's lab-grade Raman probes. Provides manual coarse and fine XYZ adjustments.



Video Microscope Objective, 50x (RML150A)

Microscope objective, infinity-corrected, 50x, working distance (mm) = 9.15, focal length (mm) = 4, numerical aperture (NA) = 0.55.

RML150A



BWSpec Software

BWSpec® is B&W Tek's general spectroscopy software for instrument control and data acquisition, including real-time peak analysis and trending. BWSpec is the operating software included with the purchase of all B&W Tek portable Raman systems and spectrometer products. It is designed with features for broad range applications, performing complex measurements and calculations at the click of a button. It supports multiple data formats and provides the option to optimize measurement parameters, such as integration time and laser output power control. In addition to data acquisition and data processing, it also offers automatic dark removal, spectral smoothing, baseline correction, as well as peak monitoring and trending.

i-Raman EX Portable Raman Spectrometer

The i-Raman® EX is part of our award-winning series of i-Raman portable Raman spectrometers with our patented CleanLaze® laser with 1,064 nm laser excitation. Using a high-sensitivity InGaAs array detector with deep TE cooling, high dynamic range, and a high throughput spectrograph design, this portable Raman spectrometer delivers a high signal-to-noise ratio without inducing autofluorescence, making it possible to measure a wide range of natural products, biological samples (such as cell cultures), and colored samples.

The i-Raman EX provides a spectral coverage range from 100 cm^{-1} to 2,500 cm^{-1}, enabling you to measure across the entire fingerprint region. The system's small footprint, lightweight design, and low power consumption ensure research-grade Raman analysis capabilities at any location. For expanded analysis capabilities, it can be used with our proprietary Vision software as well as BWIQ® multivariate analysis software and BWID® identification software. With the i-Raman EX, you always have a high precision Raman solution for qualitative and quantitative analysis without fluorescence.

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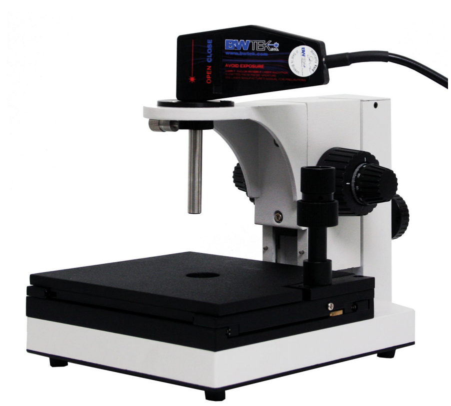

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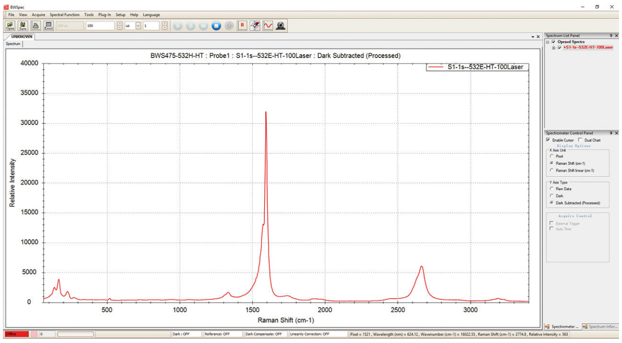
RML150A



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