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**.

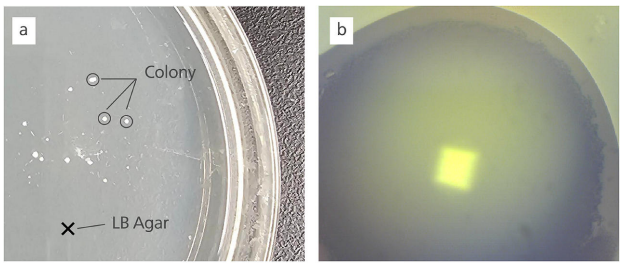
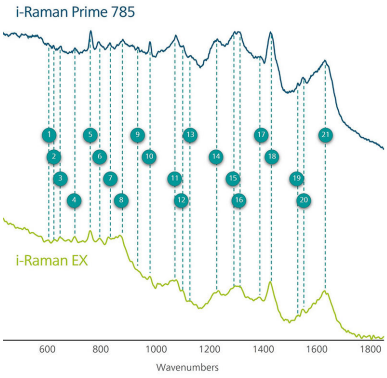


Figure 1. Bacterial colonies formed on the LB agar (a), with a magnified view of a colony under the BAC151C + 50x objective (b).

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].

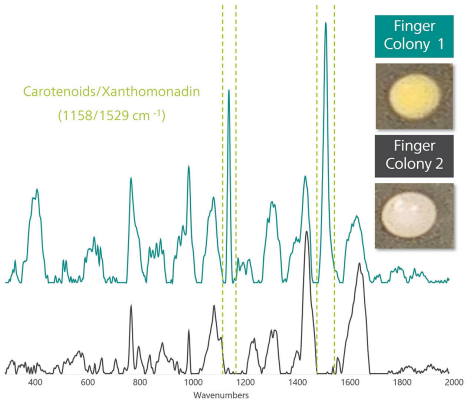


Figure 3. Raman spectra of yellow (teal line) and white (grey line) bacterial colonies formed on the LB agar. Spectra are baseline corrected. Raman peaks shown within the dotted lines may be associated with the yellow color of that particular colony.

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.

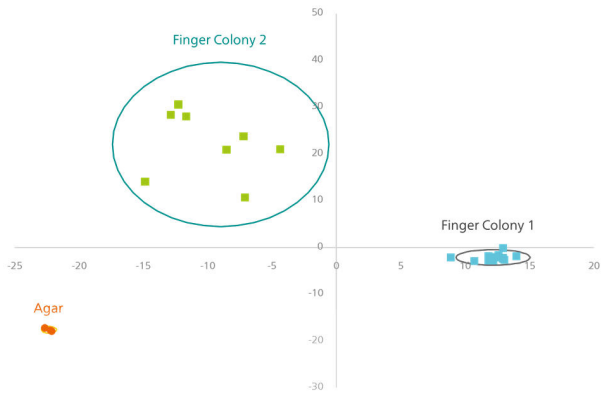


Figure 4. PCA plot of Raman spectra collected from white and yellow colonies formed on LB agar. Confidence ellipse 0.95.

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

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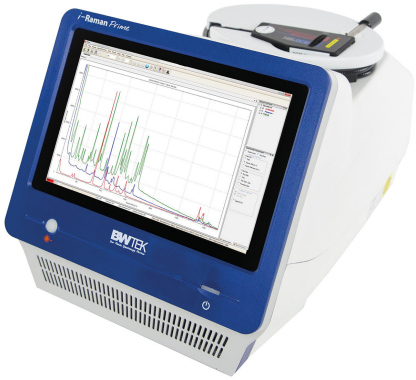
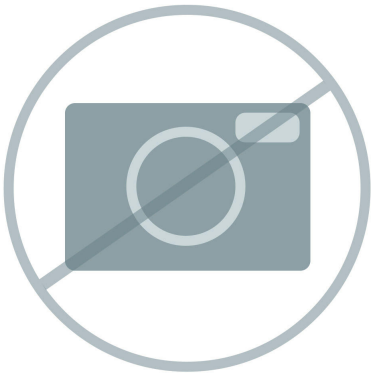
CONFIGURATION



i-Raman Prime 785S

该 i-Raman® Prime 785S 是一种高物料通过量、低噪声且完整集成的拉曼系统,内置平板电脑和一个光纤采样探头。该便携式拉曼光谱仪带有高量子效率、TE 深度冷却功能 (-25 ° C)、高动态范围的 CCD 阵列检测器,可实现研究级别的拉曼分析,包括实时定量和鉴定。由于具有较高的物料通过量,拉曼光谱提供了出色的信噪比,并借此可以测量更快速的过程,并且即使是微弱的拉曼信号也可以检测到细微的样品差异。

除了其移动式结构形式外,该 i-Raman Prime 785S 具有宽光谱范围和高分辨率的特点,并进而可以进行 150 cm^{-1} 至 3350 cm^{-1} 的测量。该 i-Raman Prime 可使用蓄电池运行,并进而方便运输。因此,无论什么地方都可以进行研究级别的高精度以及高质量和高定量的高质量拉曼分析。该系统经过优化,可与我们的 STRaman® 技术搭配使用,可用于通过非透明包装进行分析。



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显微镜物镜,无限校正,50 倍放大,工作距离 (mm) = 9.15,焦距 (mm) = 4,数字光圈 (NA) = 0.55。
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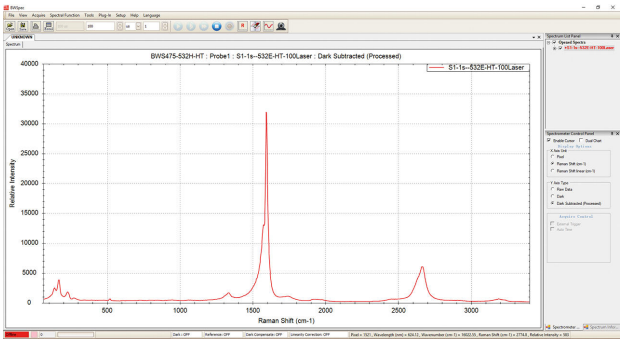

BWSpec

BWSpec® 是一款 B&W 的常规光谱软件,适用于仪器控制和数据采集,包括实时波峰分析和趋势。BWSpec 是购买所有 B&W Tek 便携式拉曼系统和光谱仪产品随附的一款操作软件。其适用功能广泛,只需点击一个按钮即可执行复杂的测量和计算。其支持多重数据格式并能提供选项优化测量参数,比如积分时间和激光输出功率控制。除了数据采集和数据处理外,它还提供自动暗去除、光谱平滑、基线校正以及峰值监测和趋势分析。

i-Raman EX

i-Raman® EX 是我们屡获殊荣的 i-Raman 便携式拉曼光谱仪系列之一,具有专利的 CleanLaze® 激光 1,064 nm 激光激发。采用了高敏感度的 InGaAs 系列检测器,具备深层 TE 冷却、高动态范围,以及高通量光谱设计功能,这款便携式拉曼光谱仪能够提供高信噪比,而无需使用自体荧光剂,这使其能够测量多种天然产品、生物样本(如细胞培养)以及染色样本。

i-Raman EX 能够提供光谱覆盖范围,从 100 cm^{-1} 至 2,500 cm^{-1},帮助您测量整个指纹范围。该系统的设计体积小、重量轻且能耗低,能够确保在任何位置进行科研等级的拉曼分析。借助其扩展性分析能力,它可与我们的专利性 Vision 软件以及 BWIQ® 多元分析软件和 BWID® 识别软件一起使用。使用 i-Raman EX,您始终可以获得高精度的拉曼解决方案,无需荧光即可进行定性和定量分析。

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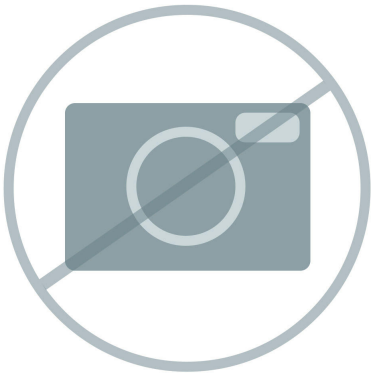

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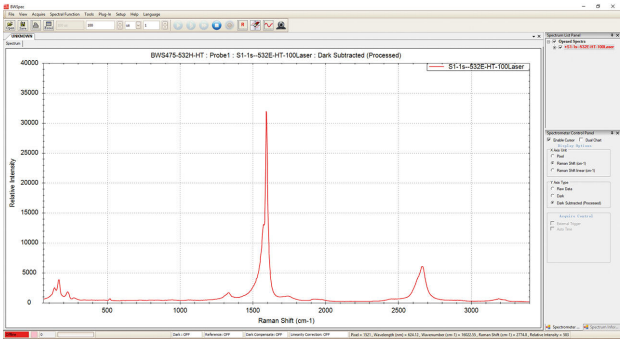

BWSpec

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