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.

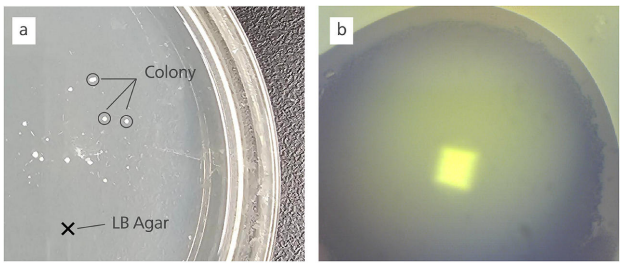
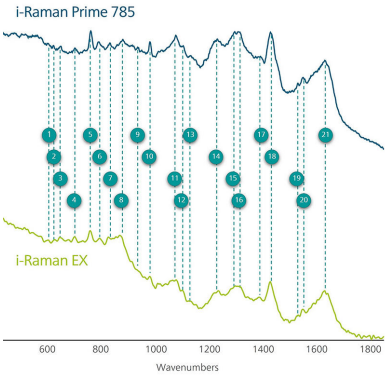


Figure 1. Bacterial colonies formed on the LB agar (a), with a magnified view of a colony under the BAC151C + 50x objective (b).

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].

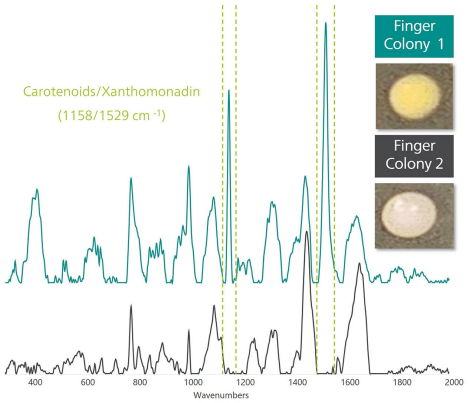


Figure 3. Raman spectra of yellow (teal line) and white (grey line) bacterial colonies formed on the LB agar. Spectra are baseline corrected. Raman peaks shown within the dotted lines may be associated with the yellow color of that particular colony.

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.

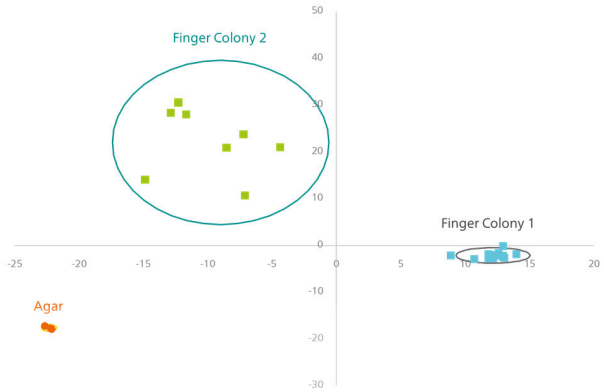


Figure 4. PCA plot of Raman spectra collected from white and yellow colonies formed on LB agar. Confidence ellipse 0.95.

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

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

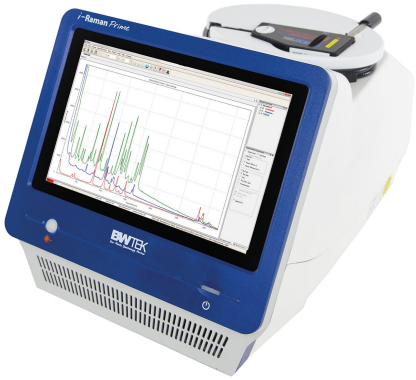
1. Paret, M. L.; Sharma, S. K.; Green, L. M.; et al. Biochemical Characterization of Gram-Positive and Gram-Negative Plant-Associated Bacteria with Micro-Raman Spectroscopy. *Appl Spectrosc* **2010**, *64* (4), 433–441. DOI:10.1366/000370210791114293

CONTACT

瑞士万通中国
北京市海淀区上地路1号院
1号楼7702
100085 北京

marketing@metrohm.com.cn

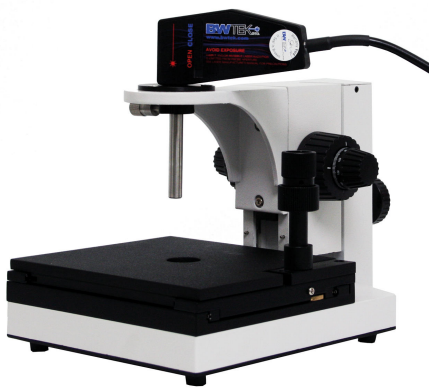
CONFIGURATION



i-Raman Prime 785S

i-Raman® Prime 785S 是一高物料通量、低噪声且完整集成的拉曼系,内置平板和一个光采探。便携式拉曼光有高量子效率、TE 深度冷却功能 (-25 ° C)、高范的 CCD 列器,可研究的拉曼分析,包括定量和定。由于具有高的物料通量,拉曼光提供了出色的信噪比,并借此可以量更快速的程,并且即使是微弱的拉曼信号也可以到微的品差。

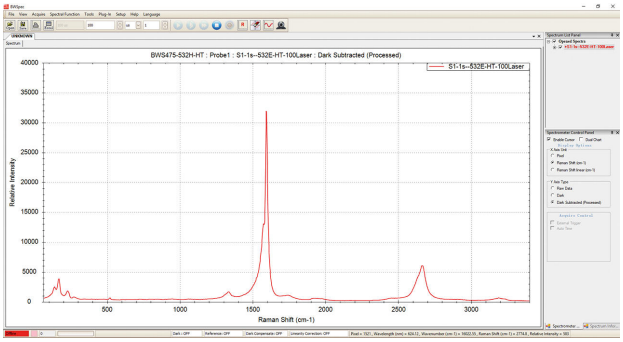
除了其移式形式外, i-Raman Prime 785S 具有光范和高分辨率的特点,并而可以行 150 cm^{-1} 至 3350 cm^{-1} 的量。 i-Raman Prime 可使用蓄池行,并而方便。因此,无什地方都可以行研究的高精度以及高量和高定量的高量拉曼分析。系化,可与我的 STRaman® 技搭配使用,可用于通非透明包装行分析。



探支架,搭配 B&W Tek 的室量拉曼探一起使用。提供手 XYZ-粗和精。

50

微物,无限校正,50 倍放大,工作距 (mm) = 9.15,焦距 (mm) = 4,数字光圈 (NA) = 0.55。
RML150A



BWSpec

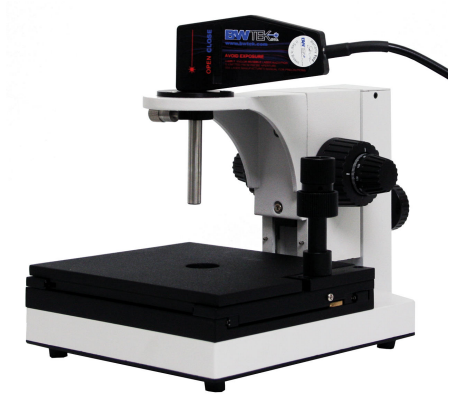
BWSpec® 是一款 B&W 的常光件,用于器控制和数据采集,包括波峰分析和。BWSpec 是所有 B&W Tek 便携式拉曼系和光品随附的一款操作件。其用功能广泛,只需点一个按即可行的量和算。其支持多重数据格式并能提供化量参数,比如分和激光出功率控制。除了数据采集和数据理外,它提供自暗去除、光平滑、基校正以及峰和分析。

i-Raman EX

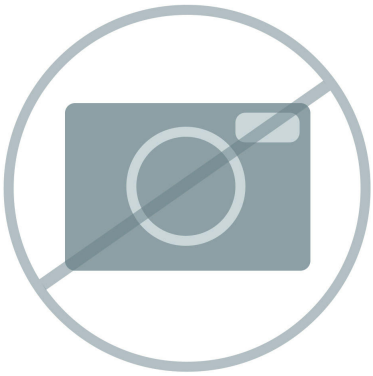
i-Raman® EX 是我屡殊的 i-Raman 便携式拉曼光系列之一,具有利的 CleanLaze® 激光 1,064 nm 激光激。采用了高敏感度的 InGaAs 系列器,具深 TE 冷却、高范,以及高通量光功能,款便携式拉曼光能提供高信噪比,而无需使用自体光,使其能量多天然品、生物本(如胞培)以及染色本。

i-Raman EX 能提供光覆盖范,从 100 cm^{-1} 至 2,500 cm^{-1},助量整个指范。系的体小、重量且能耗低,能保在任何位置行科研等的拉曼分析。借助其展性分析能力,它可与我的利性 Vision 件以及 BWIQ® 多元分析件和 BWID® 件一起使用。使用 i-Raman EX,始可以得高精度的拉曼解决方案,无需光即可行定性和定量分析。

BWS485III



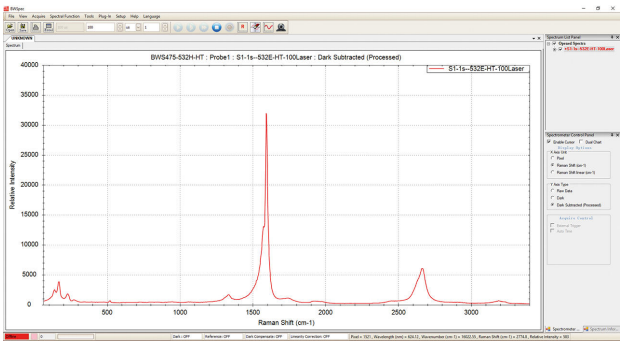
探支架,搭配 B&W Tek 的室量拉曼探一起使用。提供手 XYZ-粗和精。



50

微物,无限校正,50 倍放大,工作距 (mm) = 9.15,焦距 (mm) = 4,数字光圈 (NA) = 0.55。

RML150A



BWSpec

BWSpec® 是一款 B&W 的常光件,用于器控制和数据采集,包括波峰分析和。BWSpec 是所有 B&W Tek 便携式拉曼系和光品随附的一款操作件。其用功能广泛,只需点一个按即可行的量和算。其支持多重数据格式并能提供化量参数,比如分和激光出功率控制。除了数据采集和数据理外,它提供自暗去除、光平滑、基校正以及峰和分析。