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Review 综 述

拉曼光谱检测微生物的研究方法和进展

刘坤香[1]，刘博[1]，薛莹[2]，黄巍[3*]，李备[1,2*]

1 中国科学院长春光学精密机械与物理研究所，吉林 长春 130033
2 长春长光辰英生物科学仪器有限公司，吉林 长春 130033
3 牛津大学工程科学系，英国 牛津 OX1 3PJ

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摘　要：快速准确地识别和鉴定微生物对于环境科、食品质量以及医学诊断等领域研究至关重要。拉曼光谱(Raman spectroscopy)已经被证明是一种能够实现微生物快速诊断的新技术，在提供微生物指纹图谱信息的同时，能够快速、非标记、无创、敏感地在固体和液体环境中实现微生物单细胞水平的检测。本文简单介绍了拉曼光谱的基本概念和原理，重点综述了拉曼光谱微生物检测应用中的样品处理方法及光谱数据处理方法。除此之外，本文概括了拉曼光谱在细菌、病毒和真菌中的应用，其中单独概括了拉曼在细菌快速鉴定和抗生素药敏检测中的应用。最后，本文阐述了拉曼光谱在微生物检测中的挑战和展望。

关键词：拉曼光谱；微生物鉴定；抗生素药敏检测

*Corresponding authors. E-mail: HUANG Wei, wei.huang@eng.ox.ac.uk; LI Bei, beili@ciomp.ac.cn
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Methods in the detection of microorganisms by Raman spectroscopy

LIU Kunxiang[1], LIU Bo[1], XUE Ying[2], HUANG Wei[3*], LI Bei[1,2*]

1 Changchun Institute of Optics, Fine Mechanics and Physics, Chinese Academy of Sciences, Changchun 130033, Jilin, China
2 Hooke Instruments Co., Ltd., Changchun 130033, Jilin, China
3 Department of Engineering Science, University of Oxford, Oxford OX1 3PJ, United Kingdom

Abstract: Rapid and accurate identification and characterization of microorganisms is essential for the research in environmental science, food quality, and medical diagnostics. Raman spectroscopy has been shown to be a new technique that enables rapid microbial diagnosis, providing microbial fingerprinting information while enabling rapid, non-labeled, non-invasive, and sensitive detection of microorganisms at the single-cell level in both solid and liquid environments. This paper briefly introduced the basic concepts and principles of Raman spectroscopy and focused on a review of the sample handling methods and spectral data processing methods in the application of Raman spectroscopy for microbial detection. Besides, this paper outlined the application of Raman spectroscopy in bacteria, viruses, and fungi, among which the application of Raman in rapid bacterial identification and antibiotic drug sensitivity detection was outlined separately. Finally, this paper described the challenges and prospects of Raman spectroscopy in microbial detection.

Keywords: Raman spectroscopy; microbial identification; antibiotic drug sensitivity testing

微生物包括细菌、真菌和病毒等，是自然界中重要的生物资源，在医疗卫生、生物能源、食品制造及食品安全等领域与人类生活息息相关。因此，对微生物的鉴定是微生物研究的基础。以病原菌检测为例，传统的检测方法主要有细菌学诊断和免疫血清学诊断两类。其中细菌学诊断的依据是病原菌的形态(大小、形状、排列及核质分布情况等)、细菌成分、代谢产物和核酸等。形态学的诊断准确率太低，而细菌成分、代谢等的研究往往成本太高、操作复杂、耗时太久。免疫血清学诊断则需要对细菌进行标记，且需要昂贵的科学仪器。这些方法大多需要经过细菌培养才能完成微生物鉴定，拖延了检测流程[1]。而分子生物学方法(聚合酶链式反应、全基因组测序、基因芯片等)主要针对微生物群体进行分析且需要破坏微生物结构后进行检测，难以用于原位状态下低丰度微生物检测和微生物群体内的细胞表型异质性研究[2-4]。所以，一种无标记、免培养、非接触、单细胞水平的快速微生物检测方法是目前微生物研究所需要的。

拉曼光谱(Raman spectroscopy)是由于入射光子与物质相碰撞时发生了能量交换，引起分子的振动或转动能级的改变[5-6]，每种分子都有其特征拉曼光谱，包括一切气体、固体和液体分子。因此拉曼光谱可以鉴别各种物质的特性和结构，且不需进行任何预处理，可以避免预处理过程对信号的破坏。拉曼光谱作为一种光学检测方法，具有无标记、所需样品量少的优点，能够提供样

品的生化信息，实现微生物的快速鉴定，在食品认证、微塑料鉴定、药物分析和肿瘤诊断等领域都有广泛应用[7-12]。

拉曼光谱能够反映微生物的单细胞表型分子组成，包含 DNA/RNA、蛋白质、脂质和碳水化合物，以及物种非特异性组分(例如类胡萝卜素)的许多不同振动模式的整体振动曲线。不同的微生物的分子组成会有所不同，例如蛋白质组、脂质、碳水化合物或细胞壁的组成，因此表现出略有不同的拉曼光谱[13]。这提供了样品之间微小生化差异的特异性，并能够在物种甚至亚种水平上表征、区分和鉴定细菌、真菌和酵母[14]。本文从方法和应用上全面介绍了拉曼在微生物检测中的研究进展。主要包括微生物样品处理方法，光谱数据处理方法，以及拉曼光谱检测细菌、病原菌(耐药菌)、病毒和真菌的研究进展。

1 拉曼光谱在微生物领域检测方法

拉曼光谱可以提供细菌的核酸、蛋白质、脂质等生物大分子成分信息，在单细胞水平上形成表型特征，是研究微生物成分、种类、生理机能及代谢的一种新工具。目前拉曼光谱检测微生物的一般流程为样品处理-拉曼采集-光谱分析。流程图如图 1 所示。在样品处理阶段一般会采用过滤和离心、免疫测定、液液(固相)萃取(图 1A)，以及光镊和微流控等方法实现微生物样品的富集与捕获(图 1B)，最终通过拉曼光谱采集系统实现固体或液体中微生物的光谱采集(图 1C)。在对得到光谱数据进行滤波、基线校正、归一化

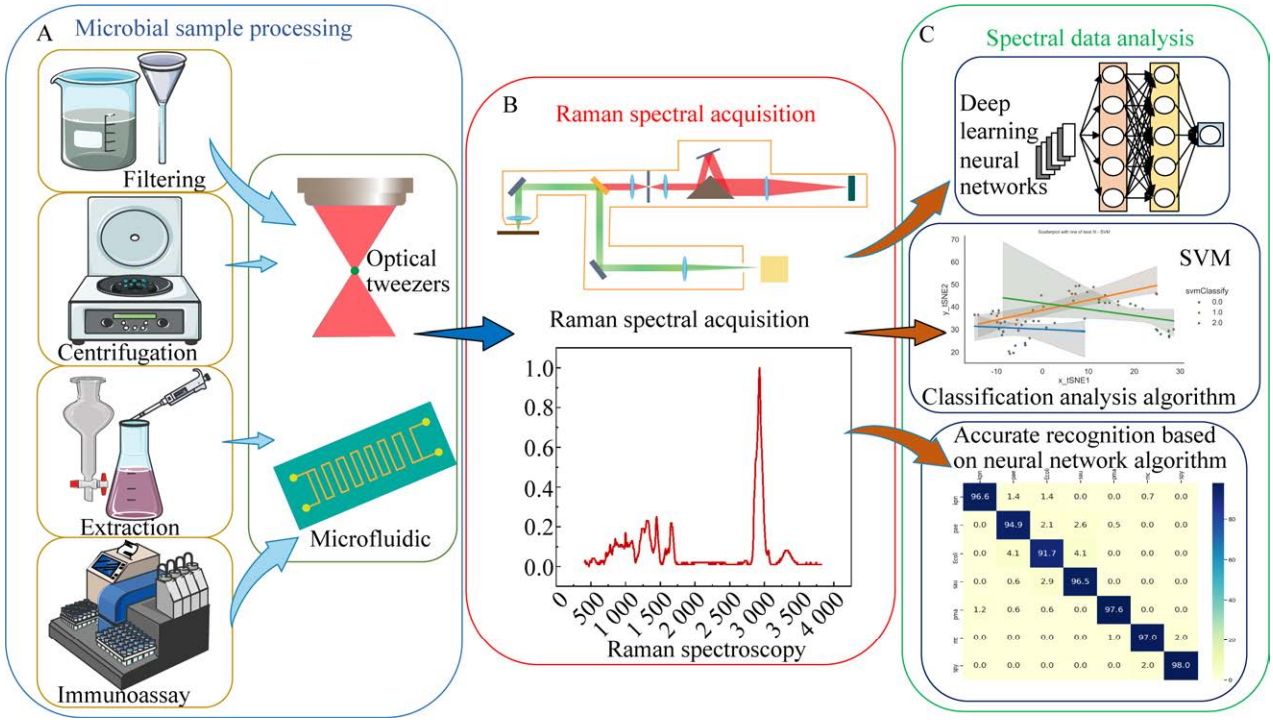

图 1 拉曼微生物检测流程

Figure 1 Flow of Raman microbial detection. A: Microbial sample processing. Including filtration, centrifugation, extraction, immunoassay, optical tweezers and microfluidic methods. B: Raman spectral acquisition. C: Spectral data analysis. The spectral analysis and rapid identification of microorganisms are achieved by statistical, machine learning and deep learning methods.

等基础的预处理流程后，可以直接分析微生物平均拉曼光谱的某些特征拉曼峰来进行初步的生化成分判定。若要通过拉曼光谱鉴定不同种类、不同生长状态的微生物，则需要借助统计分析、机器学习或深度学习的数据处理方法构建识别模型，实现微生物拉曼光谱的快速、准确识别(图1D)。目前拉曼光谱在细菌、真菌、病毒鉴定，细菌的抗生素药敏检测中都有广泛应用。下面对拉曼微生物应用中微生物样品的处理方法、光谱数据分析方法及应用进行重点介绍。

1.1 微生物样品处理方法

1.1.1 过滤和离心

从液体中分离固体颗粒的最简单方法之一是过滤。过滤可以作为唯一的样品制备技术，也可以作为进一步样品处理的重要中间步骤。例如，Stöckel 等[15]利用两个连续的过滤步骤从动物饲料中分离出细菌。分离颗粒的另一种简单有效的技术是离心，有 2 种不同类型的离心：差速离心和密度梯度离心。离心后的细菌可以通过单细胞拉曼测量来鉴定细菌[8,16]。Kloß 等[17]使用离心和随后的拉曼测量来检测患者尿液样本中 10^3 cells/mL 范围内的病原体。离心和过滤步骤也可以结合起来进行有效的样品制备[18-19]。

1.1.2 免疫测定

当直接检测液体样品中微生物的拉曼光谱时，可能会因为低灵敏度和低检测量使检测变得困难。提高拉曼检测灵敏度和速度的一种方法就是用抗体功能化的表面捕获目标样品[20]。已有研究表明，抗原-抗体相互作用与拉曼和表面增强拉曼光谱(surface-enhanced Raman scattering, SERS)的结合优于单独的光谱方法[21-22]。值得一提的是，通过使用涂有能够捕获目标分子/细胞的合成或生物聚合物(包括抗体)的磁珠来捕获目标检测微生物也是一种比较新型有效的微生物富集方式[23-26]。

1.1.3 液液萃取(liquid-liquid extraction, LLE)

LLE 是利用物质在两种不混溶溶剂中具有不同溶解度的一种常规的样品分离方法。通过重复添加萃取剂并重新分离，可以几乎完全分离所需的产物，随后通过拉曼容易地测量[20]。例如，Thien 等[27]开发了一种测量装置，结合了微流体和拉曼显微光谱的优点，可以有效地确定 LLE 数据。

1.1.4 固相萃取(solid phase extraction, SPE)

SPE 可通过在固相(吸附剂)上的特定相互作用来富集，浓缩和隔离分析物。拉曼光谱通常需要在检测和定量之前对小体积进行预浓缩[20,28-29]。

1.1.5 新方法——微流控

在实际样品检测中，为满足样品小体积需求和自动化分析的要求，微流控芯片或微流体平台被广泛应用于病原体的拉曼检测中。微流体平台通常与介电电泳结合使用，以便从液体样品中捕获细菌。这种方法也适用于拉曼检测，并且可以自动控制对生物安全需求增加的病原体[30-34]。

1.1.6 新方法——光镊

通过光镊技术操作微颗粒的方法已经被广泛应用于生物学和医学中。光镊作为样品制备步骤，富集、操纵或捕获激光焦点内的目标物，并记录拉曼光谱[35]。光镊是实现液体中微生物单细胞分析的一种重要工具。众多研究表明，拉曼光镊能够在 10 min 直接鉴定来自液体样品的微生物[36]，包括废水[37]和人尿[38]，能够检测抗生素的抗性[39]和形成生物膜[40]的能力；能够描述微生物的代谢变化[41]和细菌裂解[42]。

除此之外，研究表明，在微生物样品制备过程中，某些介质对微生物指纹有重大影响(Roosvelt-park institute medium, CHROMagar)，不应用于获取拉曼光谱[43]。然而，至少有两种方法可以克服介质干扰：(1) 将细胞重新悬浮在水中或简单缓冲液中；(2) 在不捕获细胞的情况

下获取培养基的拉曼光谱，并通过数据处理去除培养基背景。常见的固定剂如戊二醛和乙醇会对细菌的拉曼光谱产生显著变化，而甲醛和叠氮化钠在保留光谱特征方面更好[44]。

1.2 光谱数据分析方法

不同样品之间的拉曼光谱存在差距，但是同种微生物不同状态下的拉曼光谱差距十分微小，很难通过人眼直观地将它们区分开来。所以我们通常会借助统计学方法、机器学习或深度学习等计算机科学技术来帮助我们更好的快速识别和区分拉曼光谱。

拉曼光谱在采集时会受到光谱仪的变化以及环境条件的改变的影响，使得采集到的拉曼光谱中含有大量与待测目标无关的噪声信息，而噪声信息往往随着样本、仪器或者环境的改变而产生波动。在实验研究中会导致光谱分析普适性及光谱识别准确率的下降。除了光谱采集设备的优化，改进光谱数据处理的流程和方法是解决含噪声光谱数据一种重要手段(图 2)。

为了提高模型对未知样本的预测能力，已有研究主要关注在光谱预处理、特征选择和建模方法等方面的提升(图 2)[45]。数据预处理去除了光谱信号中不必要的变化和伪影的干扰。典型的预处理方法包括去宇宙射线、基线校正和平滑去噪(滤波)等。常用的滤波方法是 Savitzky-Golay (SG)滤波和去噪自动编码器[46]。光谱数据包含大量的无效信息，直接对全谱进行分析可能会影响诊断效果，增加计算成本。除了根据先验知识手动截谱或选择特征峰外，常通过算法对原始光谱进行特征选择和光谱提取。

而建模的过程则是为了实现对不同样品的识别诊断。在微生物拉曼光谱的研究中，最常见的是主成分分析-线性判别分析(principal component analysis-linear discriminant analysis, PCA-LDA)，偏最小二乘-判别分析(partial least squares-discriminant analysis, PLS-DA)和支持向量机(support vector machines, SVM)等[47-48]。除此之外，深度学习的应用也提高了细菌识别的速度和准确率。例如，Ho 等[49]利用 Resnet 识别了病原菌，Liu 等[36]通过生成对抗网络和 Resnet 识别了深海微生物。表 1 中展示了 14 篇开源研究中使用的拉曼光谱数据处理方法。

大多数研究利用 PCA 对光谱数据进行降维，这样能够减少后续数据分析过程中的数据量，提高模型的训练和识别速度。但是 PCA 在对数据进行降维的同时，损失了拉曼光谱数据的特征。LDA、SVM、DA、DFA、DT、KNN、RF、GB 等机器学习分类方法在一般的光谱分类任务中能够得到很好的识别效果，但是这些方法对于光谱预处理过程的依赖性很强，不同的设备及环境干扰、不同的预处理方法都会造成识别结果的差异，模型鲁棒性较差。除此之外，机器学

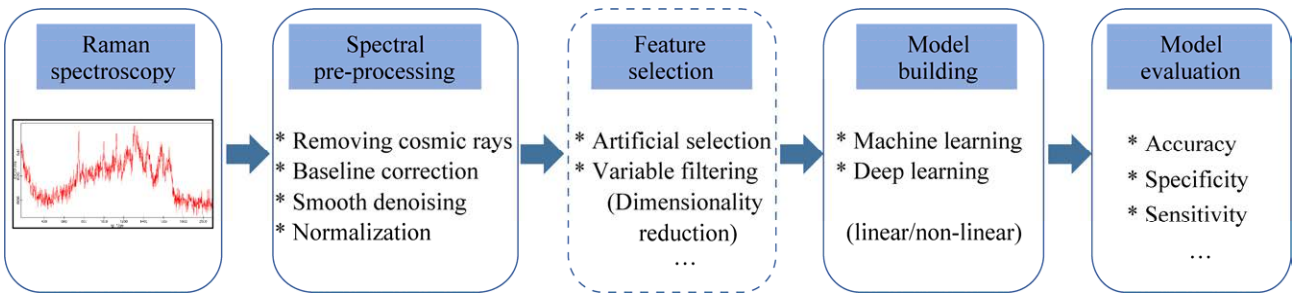

图 2 拉曼光谱数据分析流程

Figure 2 Flow of Raman spectroscopy data analysis.

表 1　拉曼微生物检测应用中的光谱数据处理方法

Table 1　Spectral data processing methods in the application of Raman microbe detection

Research objectives	Spectral pre-processing	Feature selection	Model building	Model evaluation	Reference
Identification of Gram-positive bacteria	Nor	PCA	/	/	[50]
Identification of bacteria and fungi in blood culture media	/	PCA	LDA ANN	Accuracy	[51]
Improves spectral signal-to-noise ratio and identifies bacteria	Nor, SG, Wavelet transform, FIR- Filtering, Factor Analysis, Weighted spectral reconstruction	PCA	SVM	ROC, Accuracy, Sensitivity, Specificity	[52]
Identification of bacterial surface markers	CRR, BC, Filtering	PLS	DA	/	[53]
Identification of *Burkholderia mallei*	CRR, BC, Nor	PCA	SVM	Accuracy	[54]
Detection of pathogens at the single-cell level	Filtering, BC, Nor	PCA	/	Accuracy	[55]
Individual bacterial identification	Filtering, Nor	/	SVM	Accuracy	[56]
Identification of *Acinetobacter*	SNV, SG	PLS	DA	Accuracy	[57]
Identification of pathogenic *Escherichia coli*	BC, Nor, Filtering	PCA PLS	DFA DA	Accuracy, Sensitivity, Specificity	[58]
Identification of foodborne pathogens	SG, SNV, MSC, SG 1st Der	/	DT, ANN, FDA	ROC	[59]
Identification of urinary tract pathogens	BC, SG, CRR	PCA	LDA	K-value	[60]
Detection of drug-resistant *Staphylococcus* species	Nor	PCA	KNN, DT, RF, GB, SVM, AdaBoost, GNB, QDA, CNN, LSTM	Accuracy, Recall rate, F1-score, MCC, KAPPA	[61]
Rapid identification of pathogenic bacteria	Nor	/	CNN-Resnet	Accuracy	[49]
Classification of marine bacteria	CRR, BC, Nor	PGGAN	CNN-Resnet	Accuracy	[36]
Rapid diagnosis of bacterial pathogens	CRR, BC, Nor, DAE	PCA	LDA	Accuracy	[46]

/: The relevant method is not mentioned in the study. ANN: Artificial neural network; AdaBoost: Adaptive boosting; BC: Baseline correction; CRR: Cosmic ray removal; CNN: Convolutional neural network; DFA: Discriminant Function analysis; DT: Decision Tree; FIR: Finite Impulse Response; FDA: Fisher Discriminant analysis; GB: Gradient boosting; GNB: Gaussian Naive Bayes; DAE: Denoising autoencoder; KNN: K-nearest neighbor; LSTM: Long short-term memory; MCC: Matthews correlation coefficient; MSC: Multivariate scattering correction; Nor: Normalization; QDA: Quadratic discriminant analysis algorithm; RF: Random forest; PGGAN: Progressive growing of generative adversarial networks; SNV: Standard normal variate transform; SG 1st Der: First derivative of SG algorithm.

习在应对样本种类较多的识别任务时，效果往往较差。ANN、CNN、LSTM 等深度学习的数据分类方法可以不依赖数据处理方法，数据训练过程中的光谱特征是由神经网络自动捕获的。但是，深度学习的方法往往需要大数据集来训练识别模型；且深度学习是一个黑盒子，其特征识别的过程无法可视化，即无法确定对哪些拉曼特征峰最能体现样本之间的差异。值得一提的是，Liu 等[36]通过 PGGAN 对光谱数据进行了数据增强，降低了深度学习分类模型训练需要的数据量。

2 拉曼光谱在微生物检测领域的应用

2.1 拉曼光谱在细菌检测中的应用

我们以“拉曼”和“细菌”为关键词进行文献检索，筛选了发表于 2022 年的 10 篇开源文章(IF>6)。总结了其研究目标、光谱采集技术和分类方法，见表 2。其中包括利用拉曼分析细菌成分，利用拉曼光谱结合机器学习区分不同种类细菌以及研究致病菌的耐药性等研究。

2.1.1 拉曼光谱用于细菌代谢产物的检测

拉曼光谱能够提供细菌样品的生化信息。例如 DNA、RNA、蛋白质、脂质、碳水化合物，这通常被称为全生物指纹[70-72]。拉曼光谱同样能够提供微生物色素的相关信息。例如普遍存在于微生物中的类胡萝卜素的拉曼特征峰为 1 004、1 157、1 520 cm^{-1} (C=C 伸缩振动) [73-75]。参考文献的补充表中总结并提供了生物分子的拉曼谱带分配[76]。

拉曼光谱应用于单一细菌分析和活细菌研究，助力微生物生理学。通过显微拉曼光谱检测单个细菌化学信息技术的不断发展，为生物机体各组成部分的功能及实现其功能的内在机制的研究提供了一种全新的研究方法。此外，研究细菌的生理学过程对食品、医学、微生物学、制药以及环境领域意义重大且有巨大的潜在应用价值。Huang 等[72]通过研究证明了不同生长期的细菌谱图之间有差异，并且能够对此进行鉴定。更重要的是，他们初步指出不同生长期之间存在的差异不会影响菌种水平上的鉴定。显微拉曼光谱也能够用于研究纳米颗粒对细菌产生的损伤以及环境污染物对细菌生理状态产生的影响。例如，Sahoo 等研究发现，氮化镓纳米颗粒能够有效抑制生物膜的形成[73,77]。除此之外，拉曼光谱还能够原位无损地鉴定生物膜中不同细菌以及相互的作用，也能够确定微生物群体的复杂结构与空间分布，为研究微生物生物膜的生成机理及复杂结构提供技术支持[78-79]。

表 2 拉曼微生物检测相关应用

Table 2 Applications related to Raman microbe detection

Research objectives	Spectral acquisition technology	Analysis method	References
Single-cell level study of 12 bacterial clinical isolates	Raman+IR	PCA, Clustering	[62]
Distinguish between bacteria and biofilm that cause otitis media	Raman+Optical Coherence Tomography (OCT)	/	[63]
Effect of microbial factors on SERS spectra	SERS	PCA	[64]
Rapid diagnosis and drug sensitivity testing of pathogenic bacteria in urine	Raman Spectroscopy+Excited Raman Scattering Microscopy	CNN	[65]
Increase the speed of single cell Raman spectroscopy analysis	/	Conditional generation of adversarial networks	[66]
Distinguish surface imprints of *E. coli* and *B. cereus*	Raman+Scanning Probe Microscopy	PLS-DA	[53]
Rapid determination of *Mycobacterium tuberculosis* infection and drug resistance	SERS	CNN, GRU, LSTM, MLP, RF, SVM	[67]
Detection of stx2 from Shiga toxin-producing *Escherichia coli* (STEC)	SERS	/	[68]
Identification of foodborne pathogenic bacteria	/	PCA, DT, ANN, FDA	[59]
Phenotypic convergence of bacterial adaptation to sublethal antibiotic therapy	Flow Cytometry+Raman	PCA, DA	[69]

/: The relevant method is not mentioned in the study. GRU: Gate recursive unit; MLP: Multilayer perceptron.

除此之外，拉曼光谱能够定性和半定量的反映微生物代谢产物的种类和含量。徐健等通过分析微藻的拉曼光谱，揭示了单细胞中拉曼敏感代谢物的生物合成谱，并通过拉曼组内相关分析(intra-Ramanome correlation analysis, IRCA)检测了它们的相互转化[80]。王霞等[81]利用拉曼光谱对运动发酵单胞菌不同发酵过程中发酵液的拉曼信号进行建模分析，成功预测了发酵过程中葡萄糖、木糖、乙醇和乳酸浓度。

2.1.2 拉曼光谱在细菌快速鉴定中的应用

拉曼光谱能够反映出不同种微生物之间的差异，能够提供细菌的全景式指纹图谱信息，从而实现细菌的鉴定。同时，由于拉曼可以在单细胞水平进行检测，无需冗长的培养富集，在临床复杂样品细菌数量少的情况下仍然适用[82]。

拉曼光谱结合机器学习、深度学习等分类方法能够实现细菌种类的快速鉴定。例如，拉曼光谱能够区分革兰氏阳性菌与革兰氏阴性菌，与革兰氏阴性菌相比，革兰氏阳性菌在 540 cm^{-1} 和 1 380 cm^{-1} 处的某些峰具有显著差异[83]。Ho 等[49]利用深度学习成功识别了 30 种常见的病原体，在低信噪比频谱上实现了超过 82%的平均分离水平精度和 97.0%±0.3%的抗生素治疗识别精度。同时，这种方法以 89%±0.1%的准确度区分了甲氧西林抗药性和敏感性金黄色葡萄球菌分离株(methicillin-sesistant *Staphylococcus aureus*, MRSA; methicillin-susceptible *Staphylococcus aureus*, MSSA)。就病原菌的拉曼检测而言，由于原位检测条件下细菌浓度较低，目前大多数的研究都是将提取到的细菌在琼脂平板中培养[83-84]。但是也有部分研究在原位开展，例如 Kloß 等[85]使用拉曼光谱和化学方法成功识别了腹水中的革兰氏阳性细菌，Maquelin 等[51]使用拉曼光谱在自动血液培养系统中快速鉴定了细菌和真菌病原体。

常规拉曼的信号通常较弱、灵敏度低，一般需要较长的采谱时间，使用 SERS 可以很好地解决这一难题。SERS 利用纳米金或银的强大电磁场增强效应，可将待测物质的拉曼信号增强 6–14 个数量级[86]。为了改善细菌数量较低时血液和尿液等临床样品中的弱拉曼信号，SERS 技术的应用促进了细菌病原体的无培养鉴定。例如，NI 等[87]成功检测了来自尿路感染患者尿液样本中的细菌病原体。尽管 SERS 是一种非常有前途的分析技术，但它尚未在临床实验室中用作常规诊断方法，其最大的限制是 SERS 独特特征的合适衬底的制备相对复杂[88]。因此，开发新的成本低、可重复使用的 SERS 基底也将大大提高 SERS 的灵敏度和准确度，从而使该技术得到更广泛的应用。

2.1.3 拉曼光谱在抗生素药敏检测中的应用

拉曼光谱所提供的生化信息能够灵敏反映细菌在不同抗生素作用下的表型响应、生理状态和代谢活性的变化[89-91]。Zhou 等[92-93]通过在细菌表面原位合成银纳米粒子对水中的细菌进行了检测，并进一步研究了抗生素作用下不同活性的细菌 SERS 谱图差异，证明该方法可以鉴定细菌的活性。Münchberg 等[94]成功鉴定了大肠杆菌(*Escherichia coli*)的抗微生物菌株和铜绿假单胞菌(*Pseudomonas aeruginosa*)样品，并验证了增加抗生素浓度对鉴定性能没有影响。

除了菌种的鉴定，拉曼光谱还能够直接检测菌株的耐药性。NI 等收集了尿路感染患者的脓尿标本，同时进行培养和拉曼光谱测定，培养后进行药敏检测，发现产超广谱 β-内酰胺酶的大肠杆菌会在 729 cm^{-1} 处比头孢菌素敏感的大肠杆菌呈现更低的峰值[1,87]。Kirchhoff 等[95]利用拉曼和介电泳研究细菌对环丙沙星的药敏检测发现，细菌在 1 458 cm^{-1} 和 1 485 cm^{-1} 处拉曼峰的强度比值可以反映细菌生长处于正常或抑制状

态。Premasiri 等[96]利用 SERS 实现了尿路感染中分离菌株的药敏结果快速判定。

单细胞拉曼显微光谱与稳定同位素探测(stable isotope probing, SIP)结合使用可以直接揭示单个微生物的功能。迄今为止，大多数使用 SIP-Raman 的研究都集中在 ^{13}C，^{15}N 和 ^{2}D 上，以分别替代它们的原始同位素(^{12}C、^{14}N 和 ^{1}H)[76]。当细菌摄入同位素标记物后，细胞内蛋白、脂类及细胞色素等拉曼谱峰会出现与同位素摄入量成正比的红移现象，因此可以反映出细菌活性或功能。拉曼结合同位素标记已经实现了固氮菌、光合菌、解磷菌及活着但不可培养菌的单细胞检测[97-102]。特别是当病原体在重水(D_2O)存在下与抗生素孵育时，由于烟酰胺腺嘌呤二核苷酸磷酸(nicotinamide adenine dinucleotide phosphate, NADPH)和代谢活性，抗抗生素细菌将显示 C-D 带[103]。虽然病原体对抗生素敏感，但 C-D 带将缺失。C-D 拉曼带的位置约为 2 173 cm^{-1}，位于单细胞拉曼光谱的“静默区”(1 847–2 668 cm^{-1})。无声区通常是平坦的，没有任何拉曼背景信号。因此，C-D 拉曼带的外观清晰且易于识别。Tao 等[91]和 Song 等[104]利用拉曼结合重水标记，考察了不同抗菌剂对细菌活性的响应，并发展了基于细菌代谢活性的最小活性抑制浓度指标。

2.2 拉曼光谱在病毒检测中的应用

病毒可以导致传染性疾病，严重影响人类的健康和生活。常规检测病毒的方法步骤繁琐、灵敏性较低、耗时耗材且检测昂贵。随着 SERS 技术的不断进度，拉曼光谱能够高分辨率的检测和分析极小的分子物质，因此同样能够检测病毒病原体。Cao 等[105]获得了 A/B 型肝炎病毒、艾滋病毒、埃博拉病毒、天花病毒的特异性拉曼图谱。Fan 等[106]利用 SERS 快速准确地识别区分了 7 种食物和水中的病毒。

自全球暴发冠状病毒(COVID-19)以来，各种利用拉曼光谱进行新冠病毒 SARS-CoV-2 快速检测的研究被广泛开展。例如，Carlomagno 等[107]通过唾液拉曼光谱分析，成功区分了健康个体与感染患者(准确率>95%)；Yin 等[108]通过分析血清样品的拉曼光谱，也成功区分了两者(准确率 90%)。

除此之外，拉曼光谱同样在寨卡和登革热病毒、流感病毒、M13 噬菌体、人类免疫缺陷性病毒、禽流感病毒、呼吸道合胞病毒及乙肝病毒等病毒的检测中有着十分广泛的应用[109]。但是由于病毒非常容易发生变异，遗传物质结构不稳定，也给这一领域的工作带来了挑战[110]。

2.3 拉曼光谱在真菌检测中的应用

与细菌、病毒不同，真菌是真核生物，有完整的细胞核、核膜、细胞质、细胞膜和细胞壁[111]。真菌在自然界中广泛存在，当人体免疫系统受损时，真菌会入侵人类身体并造成真菌感染。艾滋病、糖尿病、肿瘤以及新冠肺炎感染都伴随着真菌感染[112]。真菌鉴定的主要手段为表型鉴定、血清学鉴定及分子生物学鉴定。但是拉曼光谱检测方法的优势之一是不需要对样品进行复杂的前处理，因而能够避免或减少前处理过程中真菌失活。且能够在接近自然的状态下研究生物大分子的结构及其变化。目前拉曼光谱技术已应用于检测念珠菌(*Candida*)、曲霉菌(*Aspergillus*)、皮肤癣菌等(*Dermatophytes*)，但还须不断扩充光谱数据库[113-117]。

3 拉曼光谱应用于微生物研究的挑战

毫无疑问，拉曼光谱是一种新的快速、无标记、无损和非侵入性微生物检测的方法。与常规方法和分子方法相比，样品制备简单且不需要昂

贵的耗材。此外，拉曼结合光镊、微流控及免疫标记等多种分离或富集微生物的方法，从而实现了微生物的单细胞分析以及非培养微生物的检测和鉴定。

近几年来，通过检索关键词“拉曼”和“细菌鉴定”，发现相关的研究成果不断增加。但是，基础研究与实际应用之间仍然存在差距，这也是拉曼技术成为实验室常规检测技术的限制。表 3 总结了当前拉曼光谱应用于微生物研究的优缺点。例如，拉曼光谱收集的过程中受到外界干扰严重，加之不同光谱数据预处理方法不同，导致同一样品的光谱有所不同。也就是说，在不同研究中呈现的拉曼光谱是特定于当前研究的，这使得难以形成统一的标准数据光谱和数据库[88,118]。为了促进微生物拉曼光谱标准化数据库的建立，应该尽可能降低样品制备流程复杂度，并对采集到的拉曼光谱数据进行标注。这样不仅可以降低噪声信号，还可以提高拉曼实验的可重复性[119]。此外，在今后的工作中，应引入样品制备建议和数据处理指南。

此外，微生物样品荧光信号会影响拉曼光谱的数据分析，但这个问题可以通过选择 785 nm 或 830 nm 波长的近红外激光激发来避免[120]。较强的激光辐射会导致样品发热和样品破坏，将样品放置在水溶液中会有所改善[121]。

在利用人工智能相关方法处理拉曼光谱数据时，往往会存在模型过拟合的问题，导致所建立的模型对于外部数据的识别效果不理想。此外，利用机器学习方法辅助识别拉曼光谱时需要建立完善的微生物光谱数据库，这个数据库里面不只需要包含实验室培养条件下的光谱数据，同时需要包含环境中直接提取或原位检测的光谱数据，因为数据库涵盖的光谱范围会直接影响模型识别结果。为了识别模型得到更好的、普适性更强的识别结果，需要有更接近真实情况的数据集，且数据集的大小能够满足模型的训练和测试需求。此外，识别模型的算法选择和参数优化对拉曼光谱数据分析也是十分重要的。

除此之外，目前拉曼光谱设备的价格相对较高，仪器的操作难度较大，自动化和小型化光谱仪可能更有利于未来潜在的应用。随着拉曼光谱技术的不断进步，小型化、便携式的拉曼光谱仪也在其他行业有着众多应用，相信不久就会在微生物检测领域有众多进展。如果解决了这些问题，拉曼光谱设备将在未来引起临床诊断微生物学研究的革命[26]。

表 3　拉曼光谱应用于微生物检测研究的优缺点[26]

Table 3　Advantages and disadvantages of Raman spectroscopy applied to microbial detection research[26]

Advantages	Disadvantages
Fast and sensitive	No standard database
Non-destructive, non-invasive	Relatively expensive equipment
Simple sample preparation	Poor universality of recognition models
Suitable for non-culturable microorganisms	Need to incubate when sample concentration is low
Allows single cell analysis	High interference level
Uncovering bacterial metabolism	Semi-quantitative research

4 总结与展望

拉曼光谱可以提供微生物表型信息，区分微生物种类，实现微生物识别与鉴定，反映微生物代谢与活性，在病原菌鉴定、抗生素耐药性研究等方面都有广泛应用。虽然大多数研究都是基于纯细菌分离株开展的，但是越来越多的研究已经尝试和探索微生物的原位拉曼光谱检测，而这也是拉曼光谱微生物研究的研究趋势和最终应用。虽然目前的相关研究还在实验阶段，但是随着新型拉曼技术、纳米结构材料、计算方法和标准化流程的不断深入研究，拉曼光谱终将应用于实际环境中的微生物检测研究。

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黄巍，博士，英国牛津大学教授。合成生物学以及单细胞拉曼领域的世界前沿研究者，以微生物研究为核心，利用合成生物学的工具以及光学仪器工具开展了单细胞领域非常前沿的研究，尤其在单细胞拉曼检测领域。负责主持由 EPSRC、BBSRC、NERC、Royal Society、欧盟、瑞士、美国 NSF 以及中国科技部等支持的 30 余项科研项目，是世界单细胞拉曼技术的先驱者之一，BBSRC、EPSRC、NERC、Wellcome trust、Leverhulme Trust、英国皇家工程院、美国国家科学基金会等申请评审专家；*Nature* 等顶级杂志审稿人。目前开展四个方向的课题研究：发展单细胞(simcell)作为合成生物学的基础平台—EPSRC；工程化单细胞用于绿色工程化学—EPSRC and BBSRC；工程化编辑单细胞用于疾病诊断和治疗—EPSRC；发展拉曼单细胞分选技术—NERC。黄教授在单细胞拉曼领域经过十几年的基础研究，沉淀了一套属于自己的单细胞研究体系，在获得学术成果的同时，也培养了很多青年骨干，传承了他的科研思维和独立承担精神，在相应的领域也已取得了突出成果。