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Research Progress of Raman Spectroscopy Technology in Biofluid Detection

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Abstract: ***Biofluids, serving as dynamic carriers reflecting physiological and pathological states of the organism, hold significant clinical value in disease screening, diagnosis, and monitoring. However, traditional biofluid detection technologies suffer from limitations such as cumbersome operation, long detection cycles, and insufficient sensitivity or specificity, making it difficult to meet the clinical demand for early, precise, and rapid diagnosis. Raman spectroscopy, leveraging its advantages of rapid detection, minimal sample requirements, and the ability to characterize at the molecular level, has been widely applied in the detection of various biofluids. Moreover, when combined with multivariate statistical methods and machine learning algorithms, Raman spectroscopy has demonstrated promising diagnostic potential across multiple disease areas, including tumors, central nervous system diseases, systemic diseases, and metabolic disorders. This article systematically reviews the research progress of Raman spectroscopy technology in detecting various biofluids, analyzes the challenges it faces during clinical translation, and proposes targeted solutions. It also looks forward to future development trends, such as its integration with multiple technologies, expansion of application scenarios, development of portable devices, and in-depth fundamental research, aiming to provide theoretical references and practical foundations for subsequent research and clinical translation in this field.***

Keywords: Raman spectroscopy, Biofluids, Clinical translation**.**

1. Introduction

Biofluids refer to mobile substances present within living organisms, primarily including blood, lymph, cerebrospinal fluid, urine, saliva, and other liquid media with rheological properties. Due to their ease of collection and low daily use costs, biofluids are ideal diagnostic media in clinical diagnosis and treatment [1]. These substances serve as crucial windows reflecting diseases and dynamic carriers of the body's physiological and pathological states. Human biofluid specimens are commonly used in clinical testing. Changes in their composition and substance content are closely associated with the maintenance of the body's health status and the occurrence and progression of diseases, making them central subjects in clinical disease screening, diagnosis, efficacy monitoring, and prognosis assessment [2].

However, traditional biofluid detection methods have numerous limitations. For instance, serum tumor marker testing exhibits relatively low specificity in cancer detection, with a high rate of false positives. Additionally, many markers are expressed across multiple types of cancer [3] and may also elevate in inflammatory conditions, benign diseases, or even normal physiological states. Moreover, many tumor markers only show significant elevation in advanced stages of cancer, limiting their value in early cancer diagnosis [4]. Furthermore, molecular biology testing, which has advanced rapidly in recent years, offers extremely high sensitivity. However, this very sensitivity often leads to false-positive results that are positive in detection but lack clinical significance. Coupled with high costs, lengthy testing times, and high technical operational barriers, these factors hinder its widespread adoption. Additionally, traditional detection methods generally suffer from insufficient sensitivity and specificity. They also have limited ability to identify low-concentration biomarkers, making it difficult to capture subtle molecular changes in the early stages of diseases. Moreover, they are highly susceptible to interference from sample matrices, resulting in elevated false-positive or false-negative rates, thereby impeding early and accurate disease diagnosis. Therefore, there is an urgent need for a rapid, highly accurate, and cost-effective diagnostic method.

Raman Spectroscopy (RS) technology, as a non-invasive spectral analysis technique based on molecular vibrational scattering effects, offers unique advantages such as rapid detection speed, minimal sample requirements, no need for complex pre-treatment, and precise molecular-level characterization [5]. Currently, the integration of Raman spectroscopy with biofluid detection has become a research hotspot in the fields of precision medicine and clinical diagnostics. Relevant studies have confirmed its feasibility and superiority in screening abnormal components in biofluids, identifying disease-specific biomarkers, and dynamically monitoring treatment effects. This technology holds the potential to overcome the limitations of traditional body fluid detection methods and advance clinical diagnostics toward "early, precise, and non-invasive" paradigms [6]. Building on recent domestic and international research progress, this article systematically reviews the current application status, technical advantages, existing challenges, and future prospects of Raman spectroscopy technology in the detection of various body fluids. The aim is to provide theoretical references and practical foundations for the further promotion, application, and subsequent research of this technology in the field of clinical diagnostics.

2. Fundamentals of Raman Spectroscopy Technology and Its Adaptability in Biofluid Specimen Detection

2.1 Principles of Raman Spectroscopy

The core theoretical foundation of Raman spectroscopy is the

Raman scattering effect, discovered by Indian scientist C. V. Raman. This effect is a form of inelastic scattering resulting from the interaction between light and molecular substances. As illustrated in Figure 1, the fundamental principle involves the interaction between a monochromatic light beam (typically laser light) and molecular structures within a sample. Upon irradiation, laser photons collide with molecules of varying structures in the sample. Most of the scattered photons maintain the same frequency as the incident light, a phenomenon known as Rayleigh scattering (also called elastic scattering), which does not contain molecular structural information and is typically filtered out. A small portion of the scattered light, however, undergoes a frequency shift, differing from the incident light frequency; this is termed Raman scattering (or inelastic scattering). This frequency shift is associated with the vibrational energy levels of molecules and specifically reflects the vibrational characteristics of the molecules, providing crucial molecular structural information for the qualitative detection of target substances. When the scattered light frequency is lower than the incident light frequency, it is referred to as Stokes scattering; when the scattered light frequency is higher, it is termed anti-Stokes scattering. The difference between the scattered light frequency and the incident light frequency is known as the Raman shift.

The magnitude of the Raman shift is strictly correlated with the vibrational and rotational modes of molecules. It is independent of the incident light frequency and determined solely by the intrinsic structural characteristics of the molecules themselves. Different types of molecules and chemical bonds with varying structures exhibit distinct vibrational modes, resulting in correspondingly unique Raman shifts. These differences manifest as characteristic absorption peaks in the Raman spectrum, often referred to as the "molecular fingerprint" [7]. Leveraging this property, Raman spectroscopy can accurately capture information about molecular chemical bond types, spatial conformations, and composition, providing a reliable theoretical basis for qualitative analysis and structural characterization of samples. This capability constitutes the core prerequisite for its application in biological sample detection, disease diagnosis, and other related fields. In summary, the working principle of Raman spectroscopy is based on the interaction between photons and molecular substances. By measuring the frequency changes in scattered light, it reveals vibrational and rotational information of molecules, thereby enabling in-depth investigation of material structure and properties.

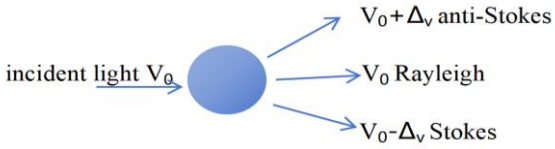

Figure 1: Principle of Raman Spectroscopy

2.2 Classification of Raman Spectroscopy

Raman spectroscopy can be categorized based on its fundamentals into Conventional Raman Spectroscopy (CARS), Fourier Transform Raman Spectroscopy (FT-Raman), and Resonance Raman Spectroscopy (RRS), as illustrated in Figure 2. Based on enhancement types, it can be classified into Surface-Enhanced Raman Spectroscopy (SERS), Tip-Enhanced Raman Spectroscopy (TERS), and Spatially Offset Raman Spectroscopy (SORS). In terms of imaging and dynamics, it includes Coherent Anti-Stokes Raman Spectroscopy (CARS) and Stimulated Raman Scattering (SRS). Based on optical configurations, it can be divided into Transmission Raman Spectroscopy (TRS) and Micro-Raman Spectroscopy. Currently, the most commonly used types in disease detection are Conventional Raman Spectroscopy and Surface-Enhanced Raman Spectroscopy. Conventional Raman Spectroscopy is the most fundamental technique, which does not require enhancement substrates and directly acquires molecular structural information by detecting the Raman scattering signals of biofluid samples. Its key features are simplicity of operation and the absence of special sample pretreatment, enabling qualitative and quantitative analysis of common biomolecules in biofluids, such as proteins, nucleic acids, and lipids. Surface-Enhanced Raman Spectroscopy (SERS) relies on metallic nanoparticles, such as silver or gold nanoparticles, to construct enhancement substrates. Utilizing the surface plasmon resonance effect of metals, it amplifies the Raman scattering signals of molecules in biofluid samples by a factor of 10^6 to 10^{14}. SERS offers extremely high detection sensitivity, allowing for the detection of biomarkers at low concentrations or even at the single-molecule level, making it ideally suited for detecting trace disease-related molecules in biofluids. It is currently the most widely applied Raman spectroscopy technique in the field of biofluid detection.

Raman spectrometer | Fourier transform Raman spectroscopy | Resonance Raman spectrum

Figure 2: Classification of Raman Spectroscopy

2.3 Application of Raman Spectroscopy

In practical applications, Raman spectroscopy technology is often integrated with machine learning algorithms to enhance diagnostic capabilities. Machine learning demonstrates unique advantages in analyzing large-scale, complex spectral datasets, enabling the rapid and efficient transformation of vast amounts of biological data into characteristic information with diagnostic value, thereby facilitating subsequent analysis.

Simultaneously, the spectral information we collect is highly susceptible to influences from external environments, testing conditions, and instrument performance. Therefore, raw spectral information not only includes component data of the measured sample but also contains irrelevant details such as sample background, instrument noise, and environmental stray light. To eliminate these interfering factors and extract effective spectral information, preprocessing of the spectral data is necessary. Effective preprocessing is crucial for isolating and extracting intrinsic Raman bands to achieve reliable analysis [8]. The results of spectral preprocessing are illustrated in Figure 3.

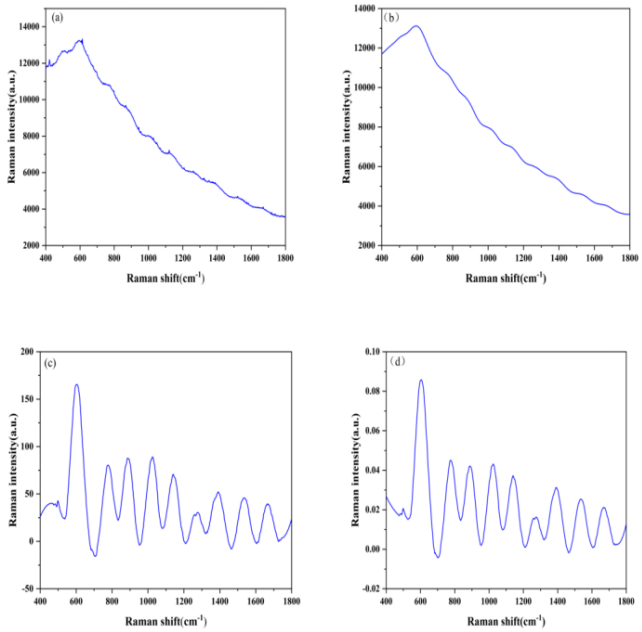

Figure 3: RS spectral preprocessing results; (a) Raw spectrum; (b) The spectrum of the raw spectrum after denoising; (c) Baseline corrected spectrum;(d) Normalized spectrum

Baseline drift is a common issue in Raman spectroscopy measurements. It reduces spectral quality and adversely affects the qualitative and quantitative analysis of spectra [9]. The causes of baseline drift are multifaceted, with fluorescence background interference being the most significant factor [10, 11]. Therefore, to mitigate the impact of baseline drift on spectral analysis, baseline correction is a critical step before Raman spectral analysis. Spectral normalization is a method for standardizing spectra to a uniform scale for data analysis. Since spectral quality is influenced by factors such as sample concentration, detection environment, and instrument parameters, direct comparisons cannot accurately reflect the differences between datasets. Thus, spectral data need to be mapped to a range of 0–1 for comparative analysis [12]. Spectral normalization removes the influence of dimensionality, enabling more efficient and faster analysis. During Raman spectroscopy measurements, spectral signals are affected by external environmental and instrument-related noise, resulting in a significant amount of random noise in the spectral data. Smoothing the spectral data can improve the signal-to-noise ratio and reduce random noise, making spectral smoothing an essential preprocessing method in Raman spectral analysis [13]. Currently, common smoothing methods include interpolation and polynomial fitting, with the Savitzky–Golay polynomial smoothing method being the most widely used and effective approach [14].

2.4 Adaptability Advantages of Raman Spectroscopy in Biofluid Detection

Raman spectroscopy technology requires no complex sample pretreatment, can detect low-concentration metabolites, and is capable of identifying various biomolecules in body fluids, including proteins, lipids, nucleic acids, adenine, and thymine, among others. The most abundant component in human biofluids is water, yet water exhibits weak Raman signals, minimizing interference with Raman spectroscopy detection. This allows Raman spectroscopy to more effectively detect changes in other substances. Compared to traditional biofluid detection methods, Raman spectroscopy offers improvements in detection speed, specificity, non-invasiveness, sample volume requirements, and sensitivity to varying degrees.

3. Research Progress of Raman Spectroscopy in the Detection of Various Biofluids

3.1 Serum

Blood is one of the most direct biological samples reflecting the physiological and pathological states of the body. In Raman spectroscopy detection, serum is typically chosen for analysis over whole blood. The primary objective is to reduce interference from components such as blood cells, thereby improving detection accuracy and better aligning with the technical characteristics of Raman spectroscopy. Currently, Raman spectroscopy has been widely applied in serological diagnostic research for various diseases.

Bahreini [15] et al.employed Partial Least Squares Discriminant Analysis (PLS-DA) to detect and analyze Raman spectra from serum samples of 40 healthy individuals and 20 gastric cancer patients. The results showed that the diagnostic model achieved a correct identification rate of 87.5% ± 2.5% in distinguishing healthy individuals from gastric cancer patients, indicating that Raman spectroscopy holds potential as a screening tool for gastric cancer.

Gao [16] et al. combined Surface-Enhanced Raman Spectroscopy (SERS) technology with feature selection techniques and a deep learning framework to develop a diagnostic model for ovarian cancer detection based on serum component analysis. Serum samples were collected from clinically diagnosed ovarian cancer patients, healthy individuals, and patients with ovarian endometriosis. Using the Light Gradient Boosting Machine (LightGBM) algorithm and a Deep Neural Network (DNN), the DNN algorithm

achieved an accuracy of 92.03% in the five-fold cross-validation for the classification of the three sample types after feature selection. Moreover, in the evaluation on an independent test set, the accuracy remained as high as 86.96%. These findings demonstrate that the integration of serum SERS with the powerful LightGBM-DNN algorithm provides a promising strategy for clinical ovarian cancer screening.

Cheng [17] et al. collected a total of 47 samples from patients with chronic renal failure and 54 control samples. They employed Raman spectroscopy combined with six different algorithms to classify serum from chronic renal failure patients and controls. The diagnostic accuracies achieved were 70.4%, 71%, 83.5%, 86.9%, 89.7%, and 82.8%, respectively. The results demonstrate the potential of Raman spectroscopy in distinguishing between controls and patients with chronic renal failure.

In addition to the diseases mentioned above, Raman spectroscopy has also been applied in the detection of serum for conditions such as hematologic disorders [18], breast cancer [19], lung cancer [20], and liver cancer [21], with relatively promising research outcomes achieved.

3.2 Cerebrospinal Fluid

Cerebrospinal fluid (CSF) is a colorless liquid secreted by the central nervous system (CNS) and isolated from peripheral blood circulation by the blood-brain barrier. This unique anatomical and physiological feature makes CSF a more direct indicator of physiological and pathological changes in the central nervous system compared to other biological samples.

In the context of infectious diseases, R. Sathyavathi [22] et al. obtained silicate-related Raman signatures from cases positive for Mycobacterium tuberculosis. Building on this, they utilized Raman spectroscopy to analyze silicate-associated spectral data in cerebrospinal fluid for the rapid diagnosis of tuberculous meningitis, a medical emergency. Their algorithm achieved a sensitivity of 91% and a specificity of 82%, demonstrating the potential of this approach as a supplementary screening tool.

In the field of neurodegenerative diseases, Alzheimer's disease (AD) causes characteristic changes in various molecular components within cerebrospinal fluid (CSF). Related studies have detected Raman spectra of certain biomarkers in CSF and constructed a unified diagnostic model based on the overall composition of CSF utilizing multiple biomarkers [23]. Techniques such as Principal Component Analysis (PCA), genetic algorithms, and other multivariate statistical methods were employed for feature extraction and classification of the Raman spectral data. The results indicate that this method can accurately distinguish AD patients from healthy individuals, providing a novel spectroscopic approach for the early and objective diagnosis of AD.

3.3 Urine

Urine serves as a vital carrier for biomarker research, containing a wealth of proteomic information and post-translational modification data. To date, over 2, 600 glycoproteins have been identified in urine, and abnormal changes in their glycosylation levels are closely linked to the onset and progression of various diseases [24]. Urine samples offer notable advantages such as ease of collection and non-invasiveness, though their composition is relatively complex, and their origins are multifaceted [25]. However, urine specimens can be collected continuously and non-invasively, and they directly reflect renal filtration function, making urine an ideal sample for disease diagnosis, dynamic monitoring, and prognosis assessment [26]. Moreover, compared to blood samples, urine is not under the strict regulation of homeostatic mechanisms and has a smaller dynamic range of proteins, granting it a unique advantage in capturing early, subtle molecular changes. This provides a solid foundation for biomarker discovery.

In the context of kidney disease detection, Chen [27] et al. explored the diagnostic value of Raman spectroscopy technology for chronic renal failure. The study involved urine samples from 48 patients with chronic renal failure and 44 individuals with normal renal function. A convolutional neural network (CNN) combined with a support vector machine (SVM) algorithm was employed to analyze the spectral data. After external validation, the diagnostic accuracy reached 84.6%, indicating the potential of this method for rapid screening of kidney diseases using urine samples.

In the field of metabolic diseases, Jian [28] et al. collected Raman spectral data from urine samples of 37 diabetic patients and 37 healthy volunteers. After employing Principal Component Analysis (PCA) for feature dimensionality reduction, three classification models—ResNet, Support Vector Machine (SVM), and Linear Discriminant Analysis (LDA)—were used to distinguish diabetic patients from healthy controls. The results showed that the ResNet model exhibited the best classification performance. After five-fold cross-validation, the average accuracy, recall, and F1-score reached 84.28%, 86.20%, and 84.02%, respectively, with the area under the Receiver Operating Characteristic (ROC) curve at 0.93. This confirms the application value of Raman spectroscopy combined with deep learning models in the non-invasive diagnosis of diabetes.

Li Ying [29] et al. applied Raman spectroscopy to the early screening of obesity-related metabolic abnormalities. The study included 31 normal-weight individuals, 27 individuals in the pre-obesity stage, and 8 obese subjects. Raman spectroscopy analysis revealed significant molecular differences in the urine composition of overweight individuals, which may reflect underlying metabolic changes in the body. The research demonstrated that Raman spectroscopy combined with Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) can effectively capture subtle metabolic changes in pre-obesity populations, providing an important reference for the development of personalized health management strategies.

3.4 Saliva

Saliva, as a biofluid obtainable non-invasively, is primarily secreted by the salivary glands and can be collected through

stimulated or unstimulated methods. Its composition is stable and rich in various biomolecules, making it a valuable material for non-invasive disease diagnosis and biomarker research.

In the field of oral cancer diagnosis, Wen [30] et al. developed an explainable artificial intelligence (xAI)-assisted label-free surface-enhanced Raman spectroscopy (SERS) platform for the analysis of salivary exosomes, enabling the non-invasive diagnosis of oral squamous cell carcinoma (OSCC). The model achieved an accuracy of 90.63% in distinguishing OSCC patients from healthy subjects and 86.63% in distinguishing non-metastatic from metastatic OSCC cases. This provides a new technological approach for the early screening, disease staging, and prognosis assessment of oral squamous cell carcinoma.

Hou [31] et al. developed a silver nanoparticle-based Surface-Enhanced Raman Spectroscopy (SERS) platform aimed at identifying DNA differences in saliva between nasopharyngeal carcinoma (NPC) patients and healthy individuals. By adding an aggregating agent ($MgSO_4$), high-quality nucleic acid SERS signals with single-base resolution were obtained. The feasibility of the platform was validated using standard DNA sequences and actual saliva samples, and a machine learning-based NPC classification model was constructed, achieving a diagnostic sensitivity of 73.8% and a specificity of 76.7%. The findings indicate that saliva SERS combined with machine learning holds promise as a potential tool for the non-invasive screening of nasopharyngeal carcinoma.

3.5 Tear Fluid

Tear fluid, as a biological sample that can be conveniently and non-invasively obtained, provides a valuable source for biomarker screening of ocular and systemic diseases. Its proteomic research has been widely applied in the discovery of diagnostic and prognostic biomarkers for various diseases. Tears are a semi-transparent three-layered liquid covering the ocular surface, and their function on the avascular corneal surface is analogous to that of blood in other parts of the body. This includes transporting oxygen and nutrients, clearing metabolic waste, defending against viral invasion, and repairing ocular surface damage [32]. This fully demonstrates that tears contain rich clinical information, not only closely related to the state of the ocular surface but also capable of reflecting physiological and pathological changes in other parts of the body.

In the detection of ocular diseases, Wei [33] et al. collected tear fluid samples from 16 keratitis patients, 13 conjunctivitis patients, and 46 healthy subjects, and performed comparative analysis of their Raman spectra. The study employed Principal Component Analysis (PCA), Partial Least Squares (PLS), and Minimum Redundancy Maximum Relevance (mRMR) for feature extraction, and the processed spectral data were input into deep learning models, including Convolutional Neural Network (CNN) and Recurrent Neural Network (RNN), for classification. The results showed that after PLS-enhanced processing, the classification accuracies between healthy subjects and keratitis patients, healthy subjects and conjunctivitis patients, and between keratitis and conjunctivitis patients reached 94.8%, 95.4%, and 92.7%, respectively. This indicates that combining large-sample tear fluid Raman spectroscopy data, PLS feature extraction, and deep learning algorithms holds significant potential for clinical screening of keratitis and conjunctivitis.

In the field of non-invasive diagnosis for systemic diseases, Fan et al. [34] developed a diagnostic model based on tear fluid Raman spectroscopy combined with machine learning classification algorithms to achieve rapid, non-invasive differentiation between cerebral infarction and cerebral ischemia. The study collected tear samples from 30 cerebral infarction patients, 10 cerebral ischemia patients, and 30 healthy volunteers. They employed three feature extraction methods combined with four machine learning classification models, constructing a total of 12 classification models. The results showed that all 12 models achieved high classification diagnostic accuracy, exceeding 85%, with the Partial Least Squares–Probabilistic Neural Network (PLS-PNN) model achieving 100% classification accuracy and superior operational efficiency. The experimental findings confirm that tear fluid Raman spectroscopy combined with machine learning classification models offers excellent screening performance for cerebral ischemia and cerebral infarction, providing a promising new technological pathway for the non-invasive and rapid clinical diagnosis of cerebrovascular diseases in the future.

4. Challenges and Solutions in the Clinical Translation of Raman Spectroscopy in Biofluid Detection

Although Raman spectroscopy has demonstrated significant potential in the detection of various biofluids, offering novel pathways for non-invasive and rapid disease diagnosis, its translation into clinical practice still faces several challenges that require targeted solutions.

At the technical level, the primary challenge lies in the high operational threshold, as there is currently a lack of unified detection standards and standardized operational protocols. This leads to poor comparability of results across different laboratories and instruments, hindering large-scale clinical application. Additionally, for Surface-Enhanced Raman Spectroscopy (SERS) technology, the stability of substrate batch preparation is insufficient, and it is susceptible to variations in preparation processes and environmental factors, resulting in poor detection reproducibility and limiting its clinical adoption. To address these issues, potential solutions could focus on the development of miniaturized and cost-effective instruments, simplifying operational procedures to reduce reliance on specialized personnel; establishing unified detection standards and quality control systems to standardize all stages, including sample collection, preprocessing, spectral detection, and data analysis; optimizing SERS substrate preparation processes, exploring novel and stable substrate materials, and combining automated preparation technologies to enhance batch consistency and detection reproducibility.

At the level of clinical validation and application, the majority of current research on Raman spectroscopy-based biofluid detection is characterized by single-center, small-sample

designs. There is a notable lack of multicenter, large-sample clinical trial validation, making it difficult to fully confirm its clinical applicability and reliability. Moreover, comparative studies between this technology and conventional clinical detection methods are scarce, and the clinical relevance of detection results still requires further clarification. This leads to limited acceptance of the technology among clinicians, hindering its integration into existing clinical diagnostic workflows. Additionally, the accuracy of the technology in distinguishing different disease subtypes, such as the staging of lung adenocarcinoma or the subtypes of acute myeloid leukemia (AML), still requires further investigation. At present, it remains challenging to meet the demands of clinical precise subtyping and personalized treatment.

The aforementioned issues can be addressed through the following approaches: (1) Conducting multicenter collaborations to carry out large-sample, long-term follow-up clinical trials, systematically comparing the diagnostic efficacy of Raman spectroscopy with conventional clinical detection methods. (2) Strengthening collaboration with clinicians to optimize detection protocols based on actual clinical needs, thereby enhancing the clinical utility of the technology. (3) Leveraging artificial intelligence technologies such as machine learning and deep learning to identify disease subtype-specific features in biofluid Raman spectra, refining classification models to improve the accuracy of disease subtype differentiation. This will facilitate the clinical translation of Raman spectroscopy technology and empower the advancement of precision medicine.

5. Conclusions and Outlook

Raman spectroscopy technology, leveraging its notable advantages such as simple operation, low cost, label-free detection, rapid analysis, minimal destructiveness, and high precision, has been extensively studied in the detection of various biofluids, including blood, cerebrospinal fluid, urine, saliva, and tears. The integration of Raman spectroscopy with machine learning algorithms has demonstrated promising potential for classification, diagnosis, and screening across multiple disease domains, such as oncology, central nervous system disorders, metabolic diseases, systemic conditions, and ocular diseases. This provides a novel technological pathway for the early, precise diagnosis and dynamic monitoring of clinical diseases.

However, during the clinical translation of Raman spectroscopy technology in biofluid detection, dual challenges at both the technical and clinical levels remain: Technically, the high operational threshold of instruments, lack of unified detection standards, and insufficient stability in the batch preparation of SERS substrates persist. Clinically, there is a shortage of multicenter, large-sample clinical trial validations, insufficient comparative studies with conventional detection methods, limited recognition among clinicians, and ongoing optimization needed for the accuracy of disease subtype differentiation. To address these challenges, strategies such as developing miniaturized instruments, establishing unified detection standards, optimizing SERS substrate preparation processes, and enhancing multicenter clinical collaboration alongside refining detection models are required to advance technological breakthroughs and clinical translation.

In the future, as Raman spectroscopy integrates more deeply with microfluidics, nanotechnology, and artificial intelligence, its detection performance will be further enhanced, and its application scenarios will continue to expand. This holds the potential for one-stop, non-invasive screening of a wider range of diseases and biofluid types. Simultaneously, the deepening of fundamental research and the development of portable, intelligent detection devices will accelerate the transition of Raman spectroscopy technology from research to clinical application, providing crucial support for the advancement of precision medicine. In summary, Raman spectroscopy technology holds broad application prospects in the field of biofluid detection. However, its clinical translation requires multidisciplinary collaborative efforts to overcome existing bottlenecks and achieve precise alignment between technological value and clinical needs.

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