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Laser Tweezers Raman Spectroscopy and its application in bio-chemical sensing

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Abstract. Laser Tweezers Raman Spectroscopy (LTRS) technology combines Raman spectroscopy and optical tweezers technology, providing a new way to research in single cell analysis and microbial classification. Driven by the development of precision spectroscopy and deep learning techniques, LTRS plays an increasingly important role in biomedical research, clinical diagnosis and environmental research. For example, it can be used to detect cancer cells, analyse bacterial infections, and monitor harmful environmental substances. In this paper, we review the basic principles of LTRS technology through literature reading and case studies, explain how the technology combines the advantages of optical tweezers and Raman spectroscopy in order to provide a non-destructive, label-free and susceptible single-cell analysis method, summarize the latest development of LTRS technology in recent years, as well as its applications in the field of biochemical sensing, and finally discuss the problems it is currently facing and look forward to its future development direction. Finally, the current problems faced by LTRS are discussed, and the direction of its future development is anticipated.

Keywords: Raman Spectroscopy, optical tweezers, LTRS, bio-chemical sensing

1. Introduction

Biochemical sensing refers to techniques in biology and chemistry that enable the conversion of relevant biological or chemical information into specific signals that can be measured. Raman spectroscopy in biochemical sensing can provide information specific to biological or different chemical molecules. However, the drawback is that Raman spectra of individual cells or molecules do not have sufficient integration time to obtain a clear Raman spectrum due to the movement of molecules and cells. To address this problem, the combination of optical tweezers makes fixing a cell or molecule at a specific location possible to obtain an effective Raman spectrum. The new technique that combines Raman spectroscopy and optical tweezers is Laser Tweezers Raman Spectroscopy(LTRS).This article mainly uses the methods of literature review and case study. The article first starts with the basic principles of LTRS technology, introduces the two primary technologies within it, optical tweezers technology and Raman spectroscopy, and explains the basic structure of LTRS, and then discusses the latest research progress of LTRS, analyses the features, key technologies and specific applications of LTRS in the field of biochemical sensing. In fact, there are many aspects of LTRS applications in each field, and the most representative ones will be selected in

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this paper. Finally, the overall development status of LTRS at present is summarised and the development prospects for LTRS are envisaged. It is hoped that it will provide some help to future scholars who are interested in researching LTRS technology.

2. Technical Principles of LTRS

2.1. Optical tweezers technology

Optical tweezers are a technique for manipulating tiny particles using a highly focused laser beam. The essence of how optical tweezers work is the interaction of light with an object. According to De Broglie's theory, light has wave-particle duality[1]. A laser beam can be regarded as a photon moving in a directional direction, and when the photon collides with the surface of the object, scattering and refraction will occur. The photon's state of motion will change when it transfers its momentum to the object in the form of a force to change the object's state of motion. The working principle of optical tweezers is based on two main forces: scattering force and gradient force. Scattering force is the force generated by the scattering of photons by an object to push the object to move in the direction of the beam propagation. In contrast, gradient force is the force generated by the refraction of photons by an object and by the gradient of the intensity of the light field to push the particles to the region of higher intensity of light, i.e., to the focal point of the laser. Scattering forces tend to destroy the gradient potential well, so only when the gradient force is greater than the scattering force can a stable optical potential well can be formed at the focus of the laser, thus confining the object at the centre of the focus.

2.2. Raman spectroscopy

Raman spectroscopy is a spectroscopic technique for non-destructive analysis based on the principle of Raman scattering. When a laser strikes an object, most photons are scattered with the same frequency as the original photons This is elastic scattering, also called Rayleigh scattering, which dominates the scattering. A small portion of the photons interacting with the object will change its energy, thus making the frequency of the scattered light different from that of the incident light. This can be divided into Stokes scattering, which is the scattering of excited molecules from a virtual energy state back to vibrational energy level with a lower energy than the initial state, and the energy of the scattered photons is lower than that of the incident photons, so the scattered photons have a longer wavelength. Anti-Stokes scattering is a molecule in the absorption of photons before the vibrational energy state has been excited. It can be from the virtual energy state jump to a lower energy state, this time the energy of the scattered photons is higher than that of the incident photons, so the scattered photons have a shorter wavelength. For the same substance, the frequency change in the scattered light is specific and depends on the chemical structure of the molecule and its vibrational modes. Information about the vibrational modes of a molecule can be obtained by measuring the difference between the frequency of the scattered light and that of the incident light. Each chemical bond and functional group has unique Raman scattering characteristics, so Raman spectroscopy can be used as a "fingerprint" of a molecule or biological cell.

2.3. LTRS Technology

In biochemical research, people often need to examine individual cells or molecules. Raman spectroscopy provides unique structural information about a cell or molecule, it is highly sensitive and specific, and the sample is not destroyed or altered during the test. These features make Raman spectroscopy an important research tool in biomedical, chemical analysis and other fields. However, Raman scattering is a weak process, combined with the inherent weak scattering of many biological materials, we need long integration time or high excitation power to obtain a clear Raman spectrum. Additionally, Brownian motion and cellular motion make it difficult to integrate the scattering from a single cell for a long enough time under normal conditions, and increasing the excitation power will linearly increase the rate of photodamage. Therefore, optical tweezers were combined with Raman

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spectroscopy to overcome the above mentioned limitations, and LTRS technology was born. Optical tweezers are used to immobilise a single cell, giving the researcher enough time to collect a clear Raman spectrum. In contrast, the researcher captures the cell at the focal point of the laser beam, optimising the scattering light path and improving the signal-to-noise ratio. LTRS technology inherits the advantages of both techniques and is a technology with the ability to detect biochemical processes in real time.

2.3.1. LTRS Structure

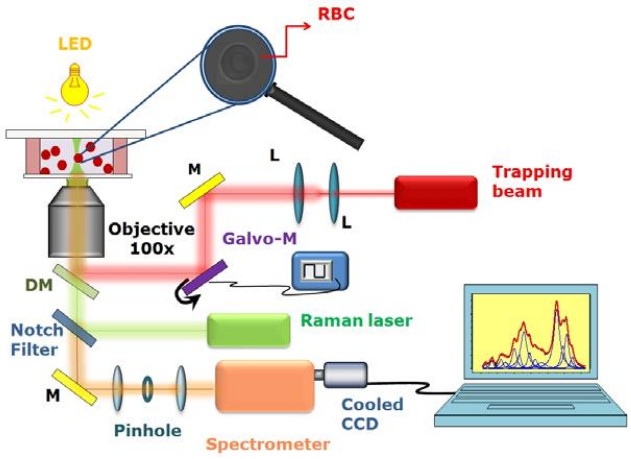

Figure 1. Structure of LTRS with dual light sources for the study of erythrocytes[2]

As shown in Fig. 1, which demonstrates a LTRS structure with dual light sources for the study of erythrocytes [2], and using this figure as an example, this paper will introduce one of the most prevalent LTRS structures, the dual-source LTRS structure. The system consists of laser, lens, objective, reflector, dichroic mirror, filter, spectrometer, and detector CCD. In the example a 1064 nm Nd:YAG laser is used to capture cells, another 532 nm Nd:YVO frequency-multiplying laser is used for Raman excitation, and a yellow LED light source is used for microimaging. The light from the two lasers is mixed through a dichroic mirror and converged into the microscope objective to act on the cells separately, while the yellow light in the figure represents the inelastically scattered light that is reflected through the filter and ultimately passes through the spectrometer to be received by the detector and analysed. This system using dual light sources simultaneously, significantly enhances the efficiency of signal collection without increasing sample damage, and provides greater flexibility at the operational level, allowing the manipulation of a cell. At the same time, excitation is applied to a specific location of the cell, thus obtaining signals characteristic of the specific region.

2.3.2. LTRS Advances. In recent years, combining the LTRS technique with other techniques has been one of the paths of its continuous improvement and upgrading. The traditional method of Raman spectral classification relies on large reference databases. However, method may not be applied when there are fewer spectra available or the signal-to-noise ratio of the spectra is low. Bo Liu et al. combined LTRS with a Progressive Growing of Generative Adversarial Nets (PGGAN) and a Residual network (ResNet) as a new method for microbial classification[3]. They used LTRS to obtain Raman spectra of individual bacteria, which is used in PGGAN to generate many high-resolution Raman spectra. At the same time, ResNet is trained to generate discriminative models from these large numbers of spectra, which can accurately classify Raman spectra with a low signal-to-noise ratio. The method overcomes the traditional difficulties by improving classification accuracy and reducing the need for large amounts of experimental data, making it particularly suitable for analysing samples with low signal-to-noise ratios. In addition, the technique speeds up the intermediate process, reducing the spectral data collection time by two-thirds without compromising accuracy. Another approach combining LTRS with artificial intelligence for analysing microorganisms was proposed by Weilai Lu

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et al. [4]. They used LTRS to acquire Raman spectra of individual microbial cells and used these measurements to train a convolutional neural network (ConvNet) model. The model achieved more than 90% accuracy, proving its effectiveness. In addition, they proposed an occlusion-based Raman spectra feature extraction(ORSFE) method to determine which Raman spectral features are more important for differentiation of different species. ORSFE extracted Raman spectral features for ConvNet to differentiate between different species, a precious method for diagnosing fast, culture-independent microorganisms.

On the infrastructure of LTRS Tobias Dahlberg et al. designed a multi-track measurement method for the case of weak Raman scattering signals [5]. The collection efficiency of the LTRS is doubled by simultaneously collecting both forward and backwards scattered light. This multi-track measurement combines the precise spatial selectivity of confocal Raman spectroscopy with the wide range of sensitivity of non-confocal Raman spectroscopy, resulting in a more detailed and efficient spectral analysis. This approach reduces the integration time required by the detector and the power of the light source, which is extremely helpful in reducing the thermal damage effects on biological cells. In this study, they also added a new sensitivity, polarisation sensitivity. This provides additional biochemical information by analysing how Raman scattering affects the polarisation of light.

3. Specific applications of LTRS in biochemical sensing

3.1. Biological Research Applications

LTRS are widely used in the biological field, where researchers have used them to combine the capabilities of optical capture and Raman spectroscopy, enabling sophisticated analysis at the single-cell level. Below are some specific applications related to biological research.

3.1.1. Applications in lipidomics. Lipidomics, as a subfield of biology, focuses on the systematic study of intracellular lipids, which play key roles in various biological functions, including energy storage, maintenance of cellular structures, signalling, and intercellular communication. One of the hot topics in recent years is new energy fuels, which are related to bioenergy fuels. Huawen Wu et al. explored a new method for analysing microalgal lipids in applying LTRS in lipidomics [6]. This approach allows direct and quantitative analysis of lipid properties in individual living cells, providing insight into their chemical composition and kinetics under various conditions. They used LTRS to capture high-quality real-time Raman spectra from individual microalgae cells and analysed real-time intracellular mean lipid chain length and lipid unsaturation. This real-time data, researchers allows researchers to screen and manipulate algal strains under different environmental conditions to promote lipid production. In contrast to traditional fluorescent probe methods that use probes that are potentially altered in cellular properties, LTRS allows for non-destructive detection and provides immediate chemical information. This approach provides a more precise and more accurate way to understand cellular lipid content and composition, which not only advances the field of lipidomics but also provides a valuable tool for the bioenergy industry and ensures efficient algal biofuel development.

3.1.2. Applications in the dynamic real-time evolution of single cells. LTRS has important applications in studying the dynamic real-time evolution of single cells. In addition to using of LTRS to pr obe the lipid properties of microalgae cells in real time, Ashwini V. Bhat et al. used LTRS to probe the occurrence of photodamage in bacterial membranes in real time [7]. They used LTRS to capture real-time Raman spectra of Bacillus subtilis cells to observe the changes that occur due to laser-induced photodamage. They found the phenomenon that the rotation of the bacterial flagellum due to its cell rotation stops as the membrane photodamage continues to rise and related this behaviour to the changes in the Raman spectral peaks representing phospholipid and cytosine biomolecules in the cell, establishing a correlation between the changes in Raman spectra and photodamage to explore the extent of photodamage in the cell at different times and the changes in the

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structure of the biomolecules. Sun et al. explored using LTRS to monitor the yeast spore germination process in real time [8]. They found that the Raman spectral peaks associated with DNA and proteins did not change significantly at the early stage of germination, while these peaks increased significantly at the growth stage, indicating active DNA replication and protein synthesis. In addition, the peaks associated with lipids and intracellular alginate decreased during the growth phase, suggesting lipid depletion while alginate may be converted to glucose and thus taken up by the cells. This study confirms that the LTRS technique can effectively reflect the metabolic activities of biomolecules within spores and provide detailed information on material changes during spore germination. It can advance our understanding of micro-scale cellular processes and reveal dynamic cellular component changes during key biological events.

3.2. Medical Applications

3.2.1. Applications in clinical microbiology. Clinical microbiology, a science that studies the relationship between microorganisms and human health, is an important part of infectious diseases. It focuses on how pathogens cause disease and how to detect and combat these microbes. Related work is critical to diagnosing, treating and preventing infectious diseases, and it requires a rapid, reliable and cost-effective diagnostic tool. LTRS enable direct, non-contact analysis of microbial cells in human body fluids without the need for time-consuming cultures. This property has a high potential for application in clinical microbiology. OND ˇREJ VACULÍK et al. presented a proof-of-concept for the use of LTRS in conjunction with advanced processing methods for the rapid identification of pathogens in serum [9]. They highlighted examples of the extremely high efficiency of LTRS in identifying common pathogens such as Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli and Candida albicans directly from human sera. They proposed a system for identifying microorganisms using an automated spectroscopy-based classification method. Such a system reduces the need for extensive manual processing and potentially reduces the possibility of human error. The whole approach is expected to improve the speed and accuracy of pathogen detection in medical diagnostics, thus enhancing patient care and infection management. Katarina Rebrosova et al. specifically investigated the application of LTRS for rapid microbial identification in clinical samples [10]. For bloodstream infections that cause fatalities, they selected 28 species and 305 strains of microorganisms as pathogens of bloodstream infections for correlation analysis. In their experiments they used algorithms such as Support Vector Machine (SVM) and Principal Component Analysis (PCA) to classify the spectra, the Support Vector Machine model was trained on a subset of the data and tested on a separate validation set to assess its accuracy. The accuracy of over 90 per cent reinforces the importance of LTRS in the field and its potential.

3.2.2. Applications in cellular drug resistance. The study of drug resistance in cells is of great importance in medicine and drug development, which can help scientists to overcome or bypass the resistance mechanisms of diseased cells in order to develop new therapeutic approaches and drugs. LTRS can play an important role in related research by providing a unique approach to explore and understand cellular response and resistance mechanisms to drugs at the single-cell level. Qian Zhang et al. discussed the application of LTRS in distinguishing drug-resistant and non-drug-resistant chronic myeloid leukaemia cells [11]. Their experiments found that the results of LTRS reflected the spectral differences between drug-resistant and non-drug-resistant cells, especially in terms of biochemical components such as proteins, lipids and nucleic acids. They quantified these differences using principal component analysis and regression tree algorithms, and specific results showed a sensitivity and specificity of more than 90% for distinguishing between the two cell types, thus confirming the validity of LTRS. Based on the Raman spectral changes of drug-resistant cells, they proposed three metabolic pathways associated with drug resistance, pointing out that the spectra can be used as a reliable indicator determining of drug resistance and pointing to new avenues for clinical diagnosis and treatment planning.

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3.3. Applications in environmental studies

The application of LTRS in environmental research is of great significance for the precise manipulation and detailed chemical composition analysis of airborne particles. Among them are aerosols, as solid or liquid particles suspended in the atmosphere, the study of the physicochemical behaviour of atmospheric aerosols is of far-reaching significance in the fields of environmental science, climate change, human health, and atmospheric chemistry. Luc Boussekey et al. applied LTRS to the study of the photochemical properties of airborne microdroplets [12]. The experiments used microdroplets containing sodium nitrate solution under conditions simulating the composition and humidity of the atmospheric gas phase. They suspended the droplets and exposed them to narrow UV radiation to induce photochemical reactions, and collected Raman spectra before and after the photochemical reactions, which were used to analyse the photochemical behaviours and distributions of the photoproducts within the droplets. This study marks the first example of photochemical experiments and Raman phasing on optically suspended droplets. It provides new tools and methods for the detailed study of aerosols in air and their dynamics under ambient conditions.

4. Conclusion

In this paper, LTRS technology and its applications in biochemical sensing are discussed in depth, the basic principles and system structure of its combination of optical tweezers and Raman spectroscopy are described, and the latest research progress of related technologies is introduced. Detailed case studies demonstrate the diverse applications of LTRS technology in biological research, clinical diagnosis and environmental science. LTRS technology provides an efficient and precise means of analysis due to its non-contact and non-invasive characteristics, especially in studying single-cell structure and its dynamic change process, which shows great practical value. At the same time, it has a very high sensitivity and specificity allowing it to achieve accurate recognition at the single molecule and single cell level. However, despite the significant advantages of LTRS technology, its practical application is still facing some challenges, such as the weakness of the Raman signal and the low signal-to-noise ratio, and it is difficult to process a large amount of sample data in a short time. Future development may improve the signal-to-noise ratio problem from the laser quality enhancement and the improvement of the basic system structure, as well as the processing speed and analysis efficiency through automation and multi-beam technology. This article only focuses on a few key research directions in the biological, medical and environmental fields. It does not include all of them, so expect more discussion in other articles in the future. In conclusion, LTRS technology shows its great potential in scientific research and practical applications, and predicts that it will play a more important role in biomedical research, disease diagnosis and environmental research. With the continuous progress and optimisation of related technologies, the application scope and influence of LTRS are expected to be further expanded.

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