Research on Raman Imaging Technology Based on Surface Enhanced Raman Scattering

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Abstract. Raman scattering originates from the change of relative position between atoms in molecules or crystals, that is, vibration and rotation. It is an important analytical method to study the structure of molecules and solid materials. Compared with other analytical methods, Raman spectroscopy has been widely used in many fields such as chemistry, physics, materials science, life science, environmental science, medicine, geology, cultural relics archaeology, public security law and so on, because of its unique advantages such as abundant information, simple sample preparation and no damage to samples. In this paper, the principle and enhancement mechanism of SERS (Surface Enhanced Raman Scattering) are discussed, the Raman imaging technology based on SERS is introduced, and its applications in different imaging fields are discussed in detail. Finally, the existing problems of SERS biological imaging technology are discussed, and the research and application prospects of this technology are prospected.

1. Introduction

Scattering is a common phenomenon that light interacts with matter in nature. According to the frequency change of incident light and scattered light, it can be divided into elastic scattering and inelastic scattering. In the scattering process, only the direction of light changes, but its frequency does not change. The light without energy loss is called elastic scattering, such as Rayleigh scattering. Inelastic scattering is called when the frequency changes before and after scattering. Raman scattering discovered by Indian physicist C.V. Raman in 1928 when studying the light scattering phenomenon of liquid benzene belongs to one kind of inelastic scattering [1].

Raman effect is ubiquitous in all molecules, whether gas, liquid or solid, and its scattering frequency is usually the internal vibration or rotation frequency of molecules. Similar to the fact that everyone has fingerprints different from others, each substance (molecule) has its own characteristic Raman spectrum, so people can identify this substance according to its Raman spectrum [2]. In the conventional Raman detection of cells, tissues, etc., the interference of fluorescence signal to Raman signal is particularly serious, and it is difficult to obtain ideal Raman spectrum, so Raman spectrum measurement needs to utilize some enhancement effects. When sample molecules are adsorbed on some rough metal surfaces, Raman scattering signals of sample molecules will be enhanced by several orders of magnitude, which is called SERS (Surface Enhanced Raman Scattering). When gold, silver nanoparticles or metal core-
shell structures are combined with various biological proteins, ligands or antibodies, functionalized nanoparticles will have a targeting effect after entering cells, and can combine with specific substances in cells to play the role of SERS sensing and SERS spectral imaging, such as cell probes [3], SERS immunoprobes [4], etc. Nowadays, cell SERS spectral research has become one of the hot research directions in biomedical optics.

In this paper, the principle, application and research progress of SERS are discussed based on the progress of Raman imaging technology based on SERS in different imaging fields.

2. Principles of SERS
At present, the SERS enhancement mechanism is highly recognized, including electrical enhancement (EM) and chemical enhancement (CM). It is generally believed that both of them play a joint role on the substrate, and EM enhancement plays a dominant role, which has a dominant advantage in the contribution of SERS enhancement [5-6].

2.1. Electromagnetic field enhancement mechanism
Among the EM enhancement mechanisms, the most convincing one is the surface plasmon resonance effect [7]. When the external electromagnetic wave interacts with the free electrons on the metal surface, the electronic collective oscillation will occur at the interface between the metal surface and the electromagnetic wave, forming surface plasmon oscillation. Because the molecular Raman signal intensity is proportional to the fourth power of the electromagnetic field intensity, the molecular Raman signal in the electromagnetic field is greatly enhanced.

When plasma resonance occurs on the metal surface, the propagation mode of collective vibration wave of electrons is shown in Figure 1 [8]. When the size of metal particles is smaller than the wavelength of incident light, local-surface plasma on resonance (LSPR) will be generated under the action of external electromagnetic waves, and the vibration principle is shown in Figure 2[9].

Figure 1. Schematic diagram of surface plasmon
2.2. Chemical strengthening mechanism

Most models for studying SERS physical enhancement mechanism are various simplified surface models built around different degrees of approximation to rough surfaces. For example, in reference [10], an ideal model consisting of a nanoscale central sphere and two conical tips was established to study the SERS enhancement mechanism of gold nanosatellites, and the model was simulated and calculated. The results show that SERS enhancement is the result of local enhancement caused by incident light with wavelength \( \lambda_m \) and Raman dipole emission with wavelength \( \lambda \). The enhancement factor, i.e. the ratio of SERS signal to Raman signal:

\[
SERS = \frac{\int_{\text{surface}} d^2s \Gamma(\lambda_{in})\Gamma(\lambda_{out})}{A \Gamma_0(\lambda_{in})\Gamma_0(\lambda_{out})}
\]  

In the formula, the integral is distributed over the surface of nanoparticles, \( A \) is the surface area of nanoparticles, \( \Gamma(\lambda) \) is the dipole emissivity of nanoparticle surface related to wavelength and position, and \( \Gamma_0(\lambda) \) is the dipole emissivity in air.

Early studies have shown that the SERS enhancement factor of \( 10^5 - 10^7 \) can be obtained by the combination of electromagnetic field enhancement and chemical enhancement. For some special SERS substrates, enhancement greater than \( 10^8 \) is not uncommon. Such high sensitivity makes SERS play a great role in qualitative analysis and detection in material chemistry, biochemistry, environmental science and other fields.

Literature [16] uses SPM to image nanostructures that can produce single molecule detection, which proves that this ultra-high sensitivity comes from the gap (gap<10 nm) of nanoparticle aggregates. In reference [10], the SERS enhancement effect of different regions of nanostructure aggregates was detected by destroying probe molecules with pulsed laser, and the following data were obtained (Table 1):
| Raman enhancement factor $\eta$ | Percentage of molecules | Percentage contribution to overall SERS signal |
|---------------------------------|--------------------------|---------------------------------------------|
| $< 2.8 \times 10^4$             | 0%                       | 0%                                         |
| $2.8 \times 10^4$ to $1 \times 10^5$ | 61%                      | 4%                                         |
| $10^5$ to $10^6$               | 33%                      | 11%                                        |
| $10^6$ to $10^7$               | 5.1%                     | 16%                                        |
| $10^7$ to $10^8$               | 0.7%                     | 22%                                        |
| $10^8$ to $10^9$               | 0.08%                    | 23%                                        |
| $> 10^{10}$                    | 0.005%                   | 17%                                        |

It can be seen from the table that most SERS signals come from less than 1% of the regions. It can be seen that there are some areas with huge electromagnetic field enhancement in SERS substrate, which are small in area (< 1%) and often exist in the gaps of some nanostructures. However, they contribute most to the generation of SERS signals. Theoretical studies have also shown that there is a region in the gap of aggregates that can provide huge electromagnetic field enhancement (Figure 3).

**Figure 3.** The electric field distribution on the surface of Au dimer is schematic, and the electric field intensity shows yellow, red and blue in turn from strong to weak

Although physical enhancement model plays a very important role in SERS effect, it cannot explain all SERS enhancement phenomena, and chemical enhancement is also a very important mechanism [10]. Among them, the charge transfer model is highly recognized [11]. There is an energy gap between full valence band and empty conduction band in semiconductor materials, so the charge transfer between semiconductor nanomaterials and molecules depends on the vibrational coupling between conduction band and valence band and excited state and ground state of molecules. Simply put, charge transfer in semiconductor-molecular system can occur through the following five ways:

1. Electrons occupying the molecular ground state are excited by incident light, and directly transfer from the highest occupied orbit to an energy level on the semiconductor conduction band. Then, the excited electrons immediately return to a certain ground state vibration energy level of the molecule and release a Raman photon.

2. Chemical bonds between molecules and semiconductors reduce the formation of charge transfer complexes, thus enhancing polarizability and Raman signals of original adsorbed molecules.
(3) Electrons in the valence band of semiconductors are excited to the highest occupied orbit of molecules, and then quickly return to the valence band, releasing a Raman photon.

(4) Electrons are first excited from the valence band of semiconductors to surface defects, thus forming surface states. Subsequently, electrons are further excited from the surface state of the molecule to the highest occupied orbit of the molecule, and a Raman photon is released by returning to the surface state.

(5) Some dye molecules are easily excited by visible light to the highest occupied orbital energy level, and then electrons are injected into the matching energy level in the semiconductor conduction band by resonance tunneling effect. The electrons finally return to the ground state vibration energy level of the molecule and release a Raman photon.

3. Application of Raman imaging technology based on SER S in different imaging fields

3.1. The application of SERS in fingerprint recognition

Fingerprint has become the most powerful evidence in forensic science because of its "unique" characteristics, and the identification of exogenous substances such as drugs, drugs, explosives, etc. in fingerprints can often become the key to crime tracking. Surface-enhanced Raman imaging is a non-destructive spectral chemical imaging technology, which can simultaneously obtain fingerprint optical images and the information of exogenous substances carried by them, and has great application potential in forensic evidence collection [12].

Literature [13] prepared adhesive nanoparticle adhesive tape film (ANF as SERS substrate, using surface enhanced Raman imaging technology, realized the extraction and imaging of large-area living fingerprint.

Gold nanoparticle monolayer was prepared by gas-liquid two-phase membrane method, and then transferred to the surface of adhesive tape to prepare flexible and adhesive SERS substrate. The substrate not only has excellent SERS activity and uniformity, but also has adhesive and flexibility of adhesive tape, which can realize on-site sampling and transfer of trace substances on different object surfaces. It is an ideal and practical SERS substrate suitable for sampling.

Sampling of living fingerprint by sticking flexible substrate. Two-dimensional distribution of fingerprints can be observed under an optical microscope. SERS detection found that the signal at 1640 cm$^{-1}$ in the spectrum belongs to the vibration of phthalamine I band of protein [14]. A clear fingerprint image can be obtained by SERS scanning the substrate with 1640 cm$^{-1}$ characteristic peaks.

Therefore, the viscous flexible SERS substrate combined with surface enhanced Raman imaging technology can be applied to fingerprint recognition, and the fingerprint image containing the first-level, second-level and third-level features of fingerprints can be obtained and the distribution of exogenous substances in fingerprints can be monitored. To sum up, fingerprint recognition can be realized by using large-area adhesive nanoparticle monolayer (ANF), which can be extended to the sampling and imaging of latent fingerprints on different object surfaces, and has great application potential in the field of crime tracking. The determination of exogenous substances and in-depth analysis of fingerprints will be reported in detail at the conference.

3.2. The application of SERS in biological imaging

3.2.1. Raman imaging technology based on SERS. Raman imaging technology is an advanced detection method to obtain detailed chemical images of samples by collecting Raman signals in certain sample areas. Taking confocal Raman imaging as an example, this method obtains Raman spectrum of each point in the detected area, and then draws a pseudo-color image according to the intensity of specific Raman peak, beam or half-width of peak. Amplifies Raman signals of active molecules to detect targets more sensitively; At the same time, SERS is also used to enhance many other properties of the substrate, such as its affinity for biological samples, which can reduce the repulsion between the substrate and organisms.
Compared with other detection methods, SERS technology, as a means of biological imaging analysis and detection, has the following advantages. First, the detection sensitivity is high, and the enhancement factor of SERS can reach $10^{14}$ at the highest, which can realize single molecule detection; Secondly, the selectivity is high, and SERS technology can select and enhance target molecules in complex systems by using the surface selection rule and the selectivity of resonance enhancement. Third, the multiplexing ability is strong, and the target can be detected in multiple ways, and the efficiency is improved; Fourthly, the detection conditions are loose, SERS technology can be easily applied to aqueous solution system, and samples can be solid, liquid or gaseous. At present, researchers have observed SERS effect at various interfaces, which shows that SERS technology has good universality. Typical SERS labeling imaging uses SERS nanotags composed of SERS active metal nanoparticles, Raman active molecules and polymer coating materials to realize labeling imaging of samples. SERS imaging method has the characteristics of high intensity and high sensitivity, which can overcome the shortcomings of traditional Raman imaging, so it is favored in the diagnosis of tumor diseases. The introduction of this method opens up a new way for the application of Raman spectroscopy in biomedical field.

3.2.2. Construction of SERS probe molecule. The Raman signal of most biomolecules is very weak, which can be captured only by SERS enhanced substrate. The SERS substrate is mainly made of nano materials, and nano materials have good modifiability, so the strategy of forming probe molecules with SERS substrate and Raman dye molecules came into being. Literature [15] constructed a SERS probe for specific detection of folic acid by antigen-antibody reaction. Firstly, gold nanoparticles and silver nanospheres were prepared as SERS enhanced substrates, and then folic acid antigen and antibody were added into the suspensions of gold nanoparticles and silver nanospheres, respectively, so that the surface of gold nanospheres had antibodies. Literature [16] constructed a DNA-driven self-assembled pyramidal probe molecule for quantitative detection of telomerase activity. Firstly, spherical gold nanospheres with a diameter of 15 nm were synthesized, and then mixed with single-stranded DNA with specific sequence to make them self-assemble into pyramid shape. The detection limit of telomerase activity by this method can reach $6.2 \times 10^{-15}$ IU, and its linear range is $1 \times 10^{-14} - 5 \times 10^{-11}$ IU. It can also quantitatively detect telomerase activity in situ in cells.

Literature [17] constructs SERS probes for quantitative detection of bisphenol A by using aptamers. Firstly, rod-shaped gold nanoparticles and spherical gold nanoparticles were synthesized respectively, and the spherical gold nanoparticles were connected with Raman dye molecules to make them have certain SERS signals. Then, gold nanospheres are connected with bisphenol A aptamer, and gold nanorods are connected with complementary single-stranded DNA of bisphenol A aptamer. The aptamer is paired with its complementary single-stranded DNA, so that gold nanorods and gold nanospheres are close together to enhance Raman dye molecules on gold nanospheres, thus obtaining stronger signals.

The SERS substrate is combined with Raman dye molecules, and then skillfully designed to make the probe, which makes use of the specificity of antigen-antibody, single-stranded DNA binding specificity and adaptation specificity to give the probe high specificity, which also shows that the SERS substrate has very good plasticity and has a very broad application prospect.

3.3. The application of SERS in polymer

With the application of CCD probe and optical fiber in FT- Raman spectroscopy, the signal-to-noise ratio, spectral range and precision are greatly increased. With the rapid development of generalized two-dimensional FT- Raman correlation spectroscopy and near-infrared FT- Raman spectroscopy with dispersive instrument and multichannel detector, and the application of multivariate analysis, Raman spectroscopy can be applied to process monitoring and quantitative analysis, which makes Raman technology play an increasingly important role in polymer science. With the development of optical fiber FT Raman spectroscopy, it can be applied to real-time monitoring of long-distance industrial
processes, which can not only get accurate results, but also ensure the safety of operators, and is expected to be widely used in industry.

### 3.3.1. Monitoring of polymer curing process

DSC is often used in laboratory analysis of polymer curing process. DSC has a small amount of samples and a thin sample, which can quickly and effectively dissipate the heat generated by polymerization and control the reaction in a pseudo-isothermal state. However, most polymers and composites are thick in industry, which makes the heat generated by polymerization unable to dissipate quickly. Reference [3] designed a new probe, which increased the signal-to-noise ratio, so that the fiber Raman spectroscopy can directly monitor the curing reaction in real time, quickly obtain spectral data, and then determine the reaction temperature and chemical structure, and quickly and quantitatively determine the curing percentage by multivariate technology. The same results can be obtained by studying the curing process of FRP reinforced composites in industry. In the process of laboratory and industrial polymerization, the standard deviation of curing percentage can be obtained by multivariate analysis, which is less than 0.52% for laboratory samples and less than 0.82% for industrial samples. After 99% curing percentage, due to the increase of internal stress, the obtained Raman signal has a large deviation. If the design of probe and the treatment process of polymer are changed, the measurement of curing percentage can be more than 99%. Higher mold temperature can reduce polymer viscosity, accelerate injection speed and improve measurement accuracy.

### 3.3.2. Study on water structure and intermolecular force in polymer aqueous solution and gel system

The relaxation time of water molecules in liquid phase is $10^{-11}-10^{-12}$ s, while the relaxation time of Raman vibration is $10^{-13}-10^{-14}$ s, so the vibration structure of molecular orientation of water molecules can be studied by Raman spectroscopy [8]. Because Raman spectrum is very sensitive to the environment in which molecules live, the instantaneous relative position information of water molecules can be obtained from the spectrum.

The interaction between water and polymer chains has aroused great interest of polymer chemists. In polymer aqueous solution, some properties of water show unusual characteristics, and there are some special phase transition phenomena in polymer aqueous solution. In the low molecular weight region, hydrogen bond defects mainly exist in the hydration layer of the polymer chain and its counterions, that is, only the water molecules close to or colliding with the polymer chain and counterions break away from the hydrogen bond network. In this molecular mass region, the molecular mass has little influence on the structure of water. Because PAA and PAAm can form intramolecular hydrogen bonds [10], the crosslinking effect of hydrogen bonds also increases the entanglement of chains. When that space enclose by the fake network is small enough and the entanglement time supporting the fake network structure is long enough for the water molecules to recombine, the structure of the water enclosed by the fake network change and the number of hydrogen bonds between molecules decreases.

Study on the interaction between molecules and ions in PAN and PMMA-based gel electrolytes by Raman spectroscopy shows that [13]: the mixed system of polar solvents ethyl carbonate (EC) and propyl carbonate (PC) shows short-range dipole-dipole interaction. PAN is an active matrix, which interacts with solvents and solvated ions to change the state of electrolyte. However, PMMA is an inert matrix, which neither produces special interaction which can be measured by spectrum nor affects the state of electrolyte, but only increases the macroscopic rigidity of the system.

The study of water absorption of polyvinyl alcohol by Raman spectroscopy shows that [14]: at 75% relative humidity, water molecules break the hydrogen bonds between polymer chains and form stronger hydrogen bonds with polymer chains; At 100% relative humidity, liquid water clusters exist in polyvinyl alcohol crystals.

### 3.3.3. Raman chemical imaging

Raman chemical imaging microscopy provides unique aberrations in molecular composition without using dyes. Raman aberration comes from the distinct characteristics of vibration spectrum of materials, which is very sensitive to the configuration, dynamic mechanics and
chemical environment of materials. Raman imaging can be carried out with little or no sample preparation, so it is widely used in material research and defect analysis.

Using liquid crystal tunable filter (LCTFS) and CCD detector, high resolution Raman images can be obtained. Literature [15] has done a lot of work using acousto-optic tunable filter (AOTE) and liquid crystal tunable filter. The spectral accuracy of NOTES is one order of magnitude worse than that of ordinary Raman photometer, and its spatial accuracy is 2.5 times of diffraction limit. Later, it was changed to LCTF, which is superior to fluorescence microscope and has a wide spectral bandwidth, which limits its application in Raman imaging to a certain extent.

For multi-component Raman imaging of multi-layer polystyrene film containing KN₃, TiO₂, WO₃ [7], LCTF can be adjusted to the characteristic vibration frequency of each component to collect bright-field Raman images, which can easily distinguish each component. LCTF Raman imaging can also be used to analyze polymer blends. According to the characteristic vibration signals of each component, the structure and molecular composition of polymer blends can be determined quickly and non-destructively. Similarly, Raman spectroscopy can also be applied to quantitative analysis of pathology, and the Raman characteristics of molecules have a potential role in the diagnosis and evaluation of tissue structure.

4. Summary
To sum up, this paper discusses the basic principle and enhancement mechanism of SERS, and focuses on the application of SERS-based Raman imaging technology in different imaging fields. SERS imaging, as a fast, efficient, non-destructive and highly sensitive detection method, has shown great application potential in biomedicine, medicine, food detection and other fields, especially in the research of cell imaging and tumor recognition in biomedicine.

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