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Combined Application of SRS and Photoacoustic Imaging in Vivo Leaf Tissue in Situ Detection System

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Abstract. Absorption and scattering are two common methods to characterize the physical and chemical properties of substances. However, the combined application of the two techniques in situ detection is difficult. Taking biological tissues as an example, proteins and pigments absorb visible light strongly, and the scattering characteristics of soft tissues are obvious. Absorption and scattering can be used to characterize the physical and chemical properties of substances. Scattering characteristics of tissues can reveal information such as cell structure, and the absorption characteristics of tissues can often reflect information such as cell metabolism. The combination of light scattering and photoacoustic effect can extract all kinds of physical and chemical information of tissue and molecule at the same time and detect them in situ. Due to the influence of the detected objects, the combined application of the two in situ detection techniques is difficult. Therefore, in order to compensate for each other's shortcomings, the photoacoustic effect caused by optical absorption and Raman scattering are used to detect living tissues, and a combined experimental system of Raman and photoacoustic imaging in situ detection is proposed. Raman and photoacoustic signals are synchronously stimulated by high repetition frequency pulsed laser and multiplexed by optical system. On the basis of experiments of adjusting repetition frequency and power of pulsed laser, the effects of parameters of pulsed laser on Raman and photoacoustic signals are analyzed. The results are of great significance for analyzing optical and acoustic signals of living tissues.

Introduction

Active photoelectric detection technology is the main means to study the influence of space environment on the life of higher plants on the earth and to analyze the mechanism of physiological changes. According to the requirements and objectives of the roadmap planning for the space life sciences experiment proposal of the Chinese Space Station from 2020 to 2032, and in view of the planned requirements of "basic research in the fields of gravity biology and radiation biology of higher plants", the active detection technology scheme for cell and molecular level research of higher green plants is proposed and verified[1-2]. In this paper, photoacoustic and spectral imaging observations are used to characterize the effects and mechanisms of biological physiological homeostasis, providing basic research data for solving basic biological problems such as survival and adaptation of earth organisms in special environments. The strategic needs of China's future space life science include "basic research and exploration of space basic biology and cosmobiology supported by manned space activities". Relevant research in this field includes: phenomena of perception, response and adaptation of earth organisms to space environment; laws of influence of space environment on physiological stability of earth organisms; and exploration of origin, evolution and distribution of life in the universe[2-3]. In this paper, an active detection technology combining laser spectroscopy with photoacoustic effect is proposed. The technology includes Stokes Raman Scattering (SRS) and Photoacoustic Microscopy (PAM) caused by photoacoustic effect, in which SRS is the result of radiation transition caused by absorption of external energy by molecules, PAM is the result of non-radiative transition when molecules absorb external energy, at which time energy propagates in the form of heat energy.

In the 1980s, A.G. Bell first discovered the photoacoustic effect[4]. Because the photoacoustic effect is very sensitive to the composition and properties of substances, photoacoustic tomography (PAT) has been used in many ways: photoacoustic tomography (PAT)[5], photoacoustic Microscopy (PAM)[6], photoacoustic Doppler technology PAD[7]. Photoacoustic effect can be used to detect the absorption spectrum of substances, especially in biology to study the absorption and Photosynthesis of chlorophyll, carotene, etc.[8]. The application of photoacoustic technology in monitoring photoabsorption spectra of aquatic plants is reported for the first time in document[9].

If the material molecule is affected by the external radiation energy, the molecular energy transits from the ground state to the virtual state and returns to the higher or lower level from the virtual state, it will emit Stokes Raman Scattering (SRS) or Anti-Stokes Raman Scattering (ASRS). Stokes Raman scattering is generally referred to as Raman scattering[10]. The resonance Raman spectra of chlorophyll and other photosynthetic pigments were reported and their main characteristics were discussed. In reference[11], a Raman spectroscopy technique was proposed to study the structure and interaction of chromophores in photosynthesis, and its application in the field of photosynthesis was explained.

The combination of photoacoustic imaging and spectroscopy technology has achieved some research results at home and abroad, which also proves the feasibility of this technology in the observation and characterization of plant biological information. However, according to the development trend of space technology and mission requirements, in-situ detection of the same point has become a hot and difficult research topic in various fields. Because in-situ detection is the key to realize the correlation of various spectroscopic technologies, especially in the special environment (space station, etc.), the study of the different interaction mechanism between molecules and molecules, molecules and photons affected by external radiation. In this paper, we not only put forward a technical idea of photoacoustic and spectral imaging observation to characterize green plants' perception of special environment, but also realized the technical requirements of in-situ detection in the same place. The research results of this subject have clear scientific research significance and application prospects.

Experimental

Reagents and Equipment

According to the principle of photoacoustic imaging, 532 nm pulsed laser irradiates biological tissues for excitation, which absorbs laser and generates heat. Because of the inhomogeneous absorption of light in tissues, the absorber generates thermal expansion and radiates sound waves outward, which is the mechanism of photoacoustic signals. Photoacoustic signals penetrate biological tissues and transmit to the surface, which is detected by ultrasound probes and converted into electrical signals. The amplified electric signal is collected by the acquisition card and stored in the computer. The system is equipped with a scanning module to obtain two-dimensional photoacoustic information.

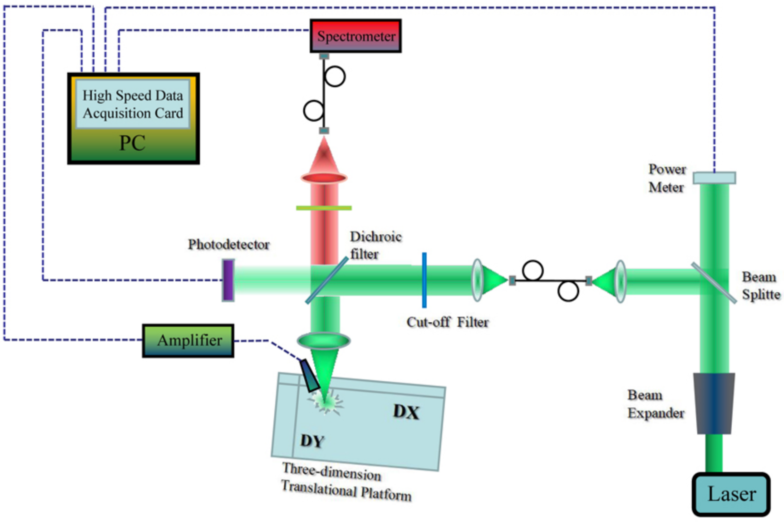

Figure 1. Schematic diagram of experimental facility

Experimental Process

When the data acquisition card is in the acquisition state and the scanning function is normally turned on, the waveform of the photoacoustic signal appearing in the first sampling time will be refreshed and displayed in real time. Using this visualization technology, the state and change of photoacoustic signals can be easily observed. Figure 2 shows the data collected when no photoacoustic signal is generated, and Figure 3 shows the photoacoustic signal generated and collected. By selecting specific part of the data, the photoacoustic signal can be magnified and viewed in a specific short period of time. By this way, the change of photoacoustic signal (40MHz) in units of 25ns can be seen. This value is much larger than the maximum spectrum of effective photoacoustic signal (10MHz). In this way, the complete change of photoacoustic signal in a laser pulse cycle can be completely restored. Fig. 4 is the original data of the photoacoustic signal, and Fig. 5 is the denoising pretreatment of the photoacoustic signal.

Figure 2. Primary data of non-photoacoustic signals

Figure 3. Generate and collect photoacoustic signals

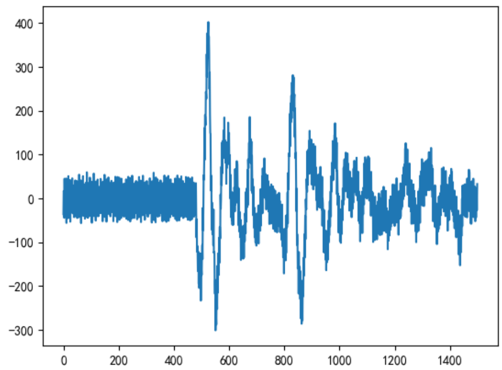

Figure 4. Primary data of photoacoustic signals

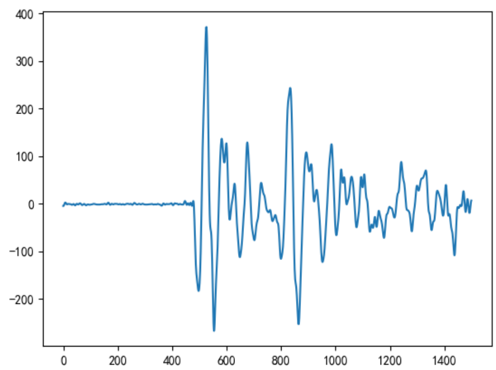

Figure 5. Noise Reduction of Photoacoustic Signals

Results

The sample is scanned by the above experimental device, and the point signal is reconstructed by two-dimensional image. The intensity of photoacoustic signal is the most important parameter of image reconstruction. As shown in Figure 6, the two-dimensional scanning image of photoacoustic imaging is presented.

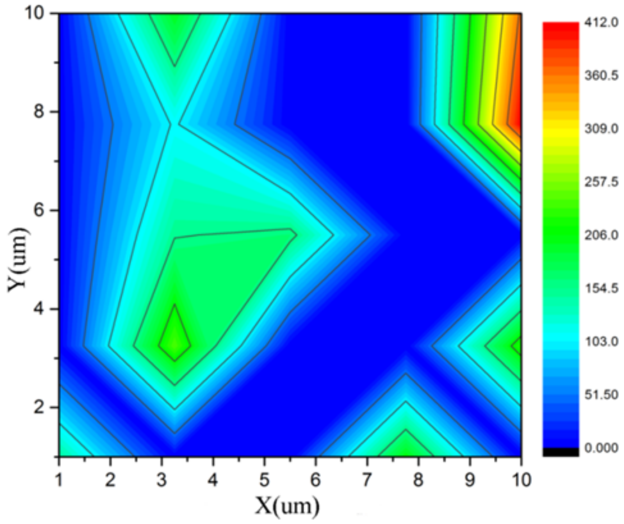

Figure 6. 2-D Scanning Map of Photoacoustic Imaging

In order to verify the feasibility of multiplexing the photoacoustic effect with Raman spectroscopy, the two-dimensional planar imaging of sample photoacoustic microscopy was realized. The photoacoustic signal was collected at the same time and the pulse Raman spectroscopy of the point was extracted. As shown in Figure 7, the Raman spectra (black line) stimulated by the pulsed laser at the location where the photoacoustic signal is detected are also collected.

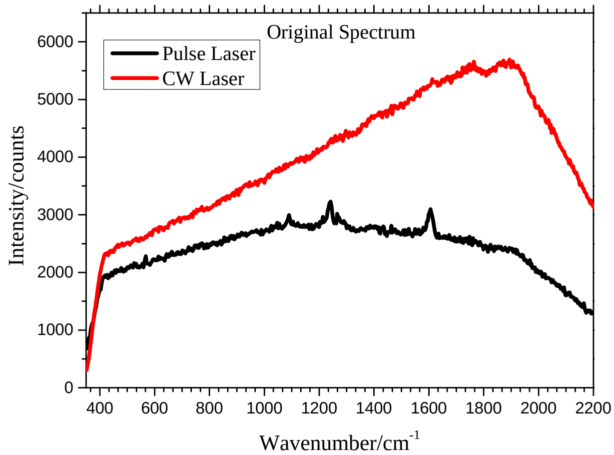

Figure 7. Raman and Continuous Raman Spectrum Data of Blade Pulse

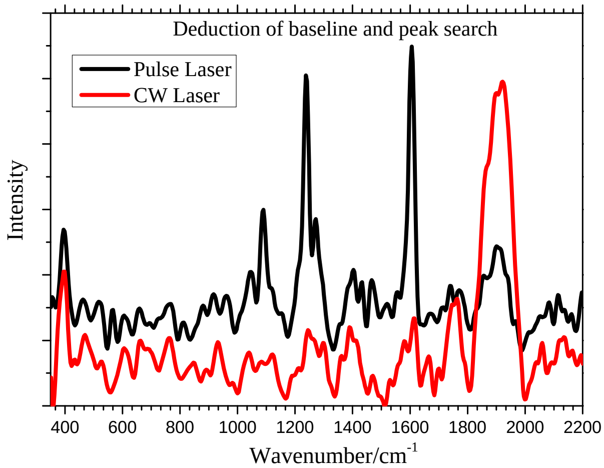

Figure 8. Debaseline Data of Blade Pulse Raman and Continuous Raman Spectra

Discussion

At present, the combined detection method of continuous laser Raman spectroscopy and pulsed laser-induced photoacoustic signals is mainly used for blade detection. This method is not only difficult to detect in-situ and in-situ living bodies, but also difficult to analyze each other, thus reducing the technical advantages of the combined application of the two technologies. To solve this problem, this paper proposes to use pulsed laser to excite light at the same time. The detection methods of photoacoustic signal (absorption) and Raman signal (scattering) can realize in situ in vivo detection of the same point of the two technologies. In addition, continuous Raman is more likely to produce strong fluorescence spectra, as shown in Figure 7 (red lines). After baseline processing of the original data, the spectra shown in Figure 8 are obtained. Compared with pulsed Raman spectra, continuous Raman has lost part of the Raman spectrum information in the strong fluorescence background.

Conclusion

Scattering characteristics of tissues can reveal information such as cell structure, and absorption characteristics of tissues can often reflect information such as cell metabolism. Photoacoustic effect is the result of the thermal interaction between tissues after absorbing light energy. Light scattering is the result of the electron cloud oscillation of histomolecular chemical bonds caused by incident light. The combination of light scattering and photoacoustic effect can realize the simultaneous extraction and in-situ detection of various physical and chemical information of tissues and molecules. Absorption and scattering can be used to characterize the physical and chemical properties of substances. The experiment uses high repetition rate pulse laser to synchronously stimulate Raman and photoacoustic signals, and realizes multiplexing of optical system. The results are of great significance to the analysis of tissue optical signal and acoustic signal detection in vivo.

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