Development of Super-Resolution Infrared Microscopy and Ultrafast Infrared Spectroscopy

超解像赤外顕微鏡および超高速赤外分光法の開発

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Infrared spectroscopy is a standard analytical method for identifying microplastics. However, conventional techniques, such as Fourier-transform infrared spectroscopy (FTIR) and FTIR microscopy, have inherent limitations. Traditional FTIR measurements are constrained to a throughput of up to approximately 100 spectra per second, rendering them unsuitable for high-throughput analysis. Furthermore, FTIR microscopy has a spatial resolution limited to several micrometers, making it challenging to analyze fine plastic particles. To address these limitations, we developed ultrafast infrared spectroscopy techniques and super-resolution infrared microscopes, paving the way for large-scale microplastic analysis and the detection of nanoplastics.

赤外分光法はマイクロプラスチックの同定に利用される標準手法であるが，フーリエ変換赤外分光法(FTIR)や顕微FTIRといった従来の技術には，それぞれ性能上の限界が存在する。従来のFTIRでは，計測スループットが毎秒100スペクトル程度に留まるため，高スループット計測には適していない。また，従来の顕微FTIRでは空間分解能が数マイクロメートルに限られ，微細なプラスチックの計測が困難である。我々は，超高速赤外分光法および超解像赤外顕微鏡を開発し，これらの技術的制約を克服することに成功した。これらの新技術は，マイクロプラスチックの大規模計測やナノプラスチックの検出を可能にする新たな道を切り拓くものである。

Introduction

Microplastics have infiltrated ecosystems through the food chain, raising serious concerns about their impact on human health. In response, governments and research institutions worldwide are advancing studies to develop reliable methods for assessing microplastics. Broadband vibrational spectroscopy in the mid-infrared region (2.5–25 μm) is considered one of the most promising identification techniques, offering unique molecular fingerprints for various polymers and enabling non-contact, non-destructive measurement and analysis of microplastics. Currently, commercial Fourier-transform infrared spectrometers (FTIR) and FTIR microscopes are commonly used to analyze particles. Although these conventional methods are widely recognized as effective for measuring microplastics, they are insufficient to address the challenges posed on a global scale. This research seeks to propose advancements from two critical perspectives.

Firstly, we examine the impact on human health. Nanoplastics smaller than 1 μm pose a significant concern as they are difficult for the body to eliminate, leading to accumulation in organs such as the heart and brain via the bloodstream. This buildup can trigger inflammation, increasing the risk of conditions such as heart attacks and strokes. Traditional FTIR microscopes, with a spatial resolution limited to several micrometers, are incapable of detecting nanoplastics. As this limitation is dictated by the diffraction limit of mid-infrared light, there is an urgent need for new technologies that operate on alternative principles.

Secondly, we address the challenge of acquiring large-scale data for statistical analysis. Microplastics are not only found in rivers and seas but also in drinking water and food, infiltrating agriculture, industry, and residential areas. Addressing this global issue requires extensive sampling from diverse locations and the accumulation of robust datasets. Flow particle measurement, capable of analyzing thousands of particles per second, is a promising method for generating such data. In cell biology and medical research, flow cytometry leverages high-speed fluorescence measurements to analyze cells. However, this technique is unsuitable for microplastics, which require molecular vibrational spectroscopy without the need for fluorescent staining. Conventional FTIR measurements, with a maximum throughput of approximately 100 spectra per second, remain inadequate for flow-based analyses. To date, high-throughput flow measurement using infrared spectroscopy has not been demonstrated, underscoring the urgent need for substantial advancements in measurement rates.

Super-resolution infrared microscopy

FTIR microscopes have been utilized as standard infrared microscopes for many years. Recently, infrared microscopes employing quantum cascade lasers—a type of semiconductor laser operating in the infrared range—have been developed, significantly improving the signal-to-noise ratio (SNR). However, the spatial resolution of these methods is constrained by the diffraction limit determined by the wavelength of infrared light, achieving only several micrometers of spatial resolution.

To enhance the spatial resolution of infrared microscopy, various techniques have been proposed. These include methods leveraging near-field effects using nanoscale probes and far-field imaging techniques that combine fluorescence imaging or utilize vibrational sum-frequency generation. While these methods successfully achieve super-resolution capabilities surpassing the diffraction limit of infrared light, they face certain limitations. The former requires physical contact between the probe and the sample, while the latter depends on fluorescence or second-order nonlinear optical effects, inherently restricting the range of measurable samples. Recently, advancements in mid-infrared photothermal microscopy have addressed these challenges.

The principle of mid-infrared photothermal microscopy is as follows: when monochromatic infrared light irradiates a sample, molecules with resonant vibrations absorb the infrared photons and start vibrating. The vibrational energy is rapidly dissipated, transferring to surrounding molecules and generating heat—a phenomenon known as the photothermal effect. This localized temperature increase causes a change in the refractive index of the material. By detecting and quantifying this refractive index change using visible light microscopy, the system achieves image contrast corresponding to infrared absorption, with spatial resolution defined by the wavelength of visible light. Figure 1 illustrates the principle of mid-infrared photothermal (MIP) microscopy.

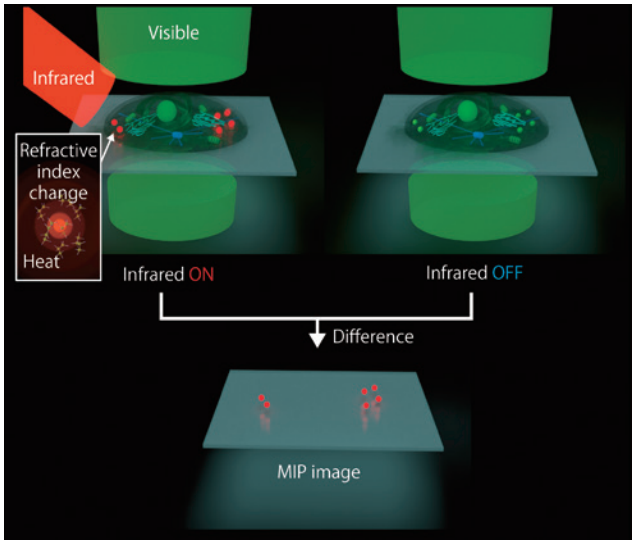

Figure 1 Principle of mid-infrared photothermal microscopy.

We have demonstrated wide-field mid-infrared photothermal (MIP) microscopy techniques. Initially, we utilized a commercially available phase-contrast microscope and successfully validated the proof-of-concept[1]. However, phase-contrast microscopy introduces image artifacts, such as halo and shade-off effects, which also affect MIP images. To address this, we replaced the phase-contrast microscope with a quantitative phase microscope, enabling the accurate measurement of phase shifts caused by refractive index changes without image artifacts[2]. Furthermore, we demonstrated three-dimensional imaging by applying the principle of optical diffraction tomography[3]. By incorporating a single-objective imaging configuration and aperture synthesis technology, we further enhanced spatial resolution, achieving 120 nm (Nyquist resolution) or 175 nm (full width at half maximum of the point spread function)[4].

We have also prioritized improving the SNR in MIP microscopy. Initially, quantum cascade lasers were employed, and their low pulse energies limited the SNR. To overcome this, we developed a nanosecond optical parametric oscillator capable of delivering pulse energies two orders of magnitude higher. By pairing this with a high-full-well-capacity image sensor, we achieved more than two-orders-of-magnitude improvement in SNR, enabling the world's first video-rate measurements[5]. Furthermore, we expanded the dynamic range of MIP quantitative phase imaging through the application of wavefront control technologies[6].

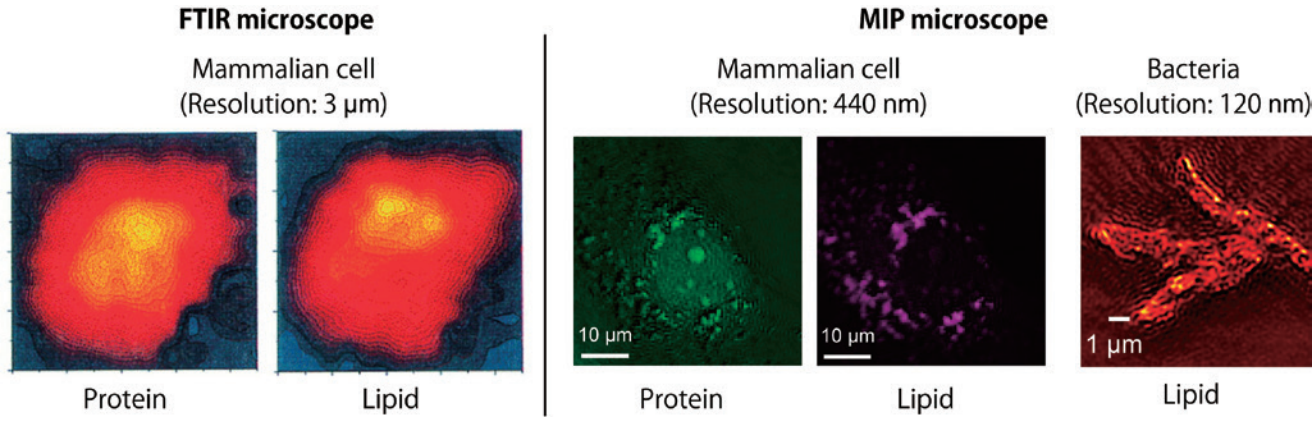

Figure 2 Comparison of cellular MIP images captured with a conventional FTIR microscope and MIP microscopes. (Left) Cellular images acquired using a conventional FTIR microscope with a spatial resolution of 3 μm[7]. (Right) MIP images of a mammalian cell with a resolution of 440 nm and bacteria with a resolution of 120 nm.

Figure 2 compares infrared microscopic images of cells obtained using a conventional FTIR microscope and our MIP microscope. The intracellular structures of cells are not visualized with FTIR microscopy but are clearly visible with MIP microscopy. The high spatial resolution allows the observation of finer structures inside bacteria. This novel technology enables the non-destructive imaging of detailed chemical information with unprecedentedly high spatial resolution, paving the way for new studies, such as investigating the interactions between nanoplastics and cells.

Ultrafast infrared spectroscopy

Fourier-transform infrared spectroscopy (FTIR) has been the standard method for infrared spectroscopy for over half a century. This technique enables the acquisition of broadband vibrational spectra but requires a relatively long measurement time—typically one second per spectrum—making it best suited for static measurements. For capturing dynamic changes in vibrational spectra, rapid-scan FTIR is often used. This approach enables high-speed measurements at approximately 100 spectra per second with commercially available instruments.

In recent years, we developed a faster FTIR technique, known as phase-controlled FTIR, capable of achieving measurement speeds of 10^4 to 10^5 spectra per second[8]. This breakthrough was enabled by the integration of a high-speed delay scanner utilizing an angle-scanning mirror and a spectral phase manipulation technique. Additionally, dual-comb spectroscopy—employing two optical frequency combs with slightly detuned repetition rates—provides a mechanical-scanner-free, high-speed FTIR technology[9]. This approach achieves the maximum measurement rate of approximately 10^6 spectra per second, allowing for time resolutions of 1 microsecond. Applications of these advanced techniques include studies of protein structural changes and combustion dynamics.

The measurement speed of dual-comb spectroscopy is fundamentally limited by the signal-to-noise ratio (SNR), posing challenges for further acceleration. Frequency-swept spectroscopy (FSS), however, offers higher SNR than Fourier-transform spectroscopy (FTS), including dual-comb spectroscopy, under equivalent conditions of measurement time, sampling rate, and detected light power. The SNR of FSS increases proportionally to the square root of the number of spectral components. Figures 3(a) and 3(b) show the SNR versus the number of spectral elements and measurement time under typical conditions. For example, with 1,000 spectral elements, the SNR for FTS is approximately 1 for a measurement time of 1 μs. In contrast, for FSS, the same SNR is achieved with a measurement time of about 1 ns, corresponding to a measurement rate of approximately 1 GHz. This highlights the potential of FSS for even faster measurements.

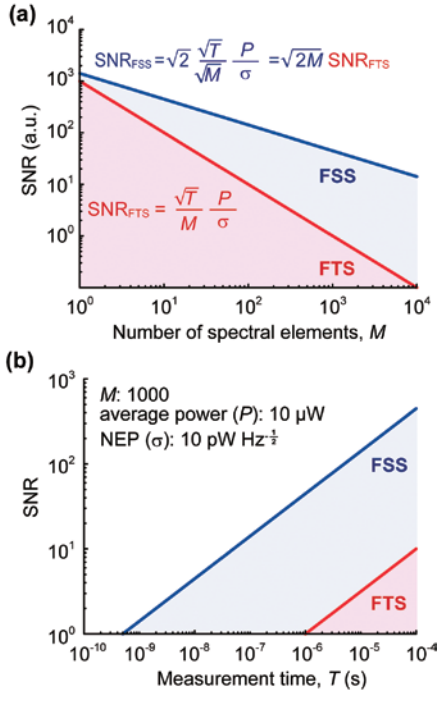

Figure 3 SNR comparison between FSS and FTS as a function of (a) the number of spectral elements and (b) the measurement time under typical conditions.

Time-stretch spectroscopy is a high-speed FSS technique that continuously measures the spectrum of each pulse from a high-repetition-rate ultrashort pulsed laser. The pulses are temporally stretched to generate chirped pulses with significant wavelength dispersion. When second-order dispersion dominates, the temporal intensity waveform directly corresponds to the spectral shape of the pulse. By recording this waveform with a high-speed photodetector and an oscilloscope, the spectrum of each pulse can be measured. However, this method had been limited to the near-infrared region, particularly within telecommunications wavelengths. Implementing time-stretch spectroscopy requires substantial dispersion, typically achieved using optical fibers typically 10 km in length. While ultra-low-loss optical fibers are readily available for telecommunications wavelengths, those suitable for the mid-infrared region suffer from high losses and are impractical. Furthermore, high-speed photodetectors with ~10 GHz bandwidth are commercially available for telecommunications wavelengths, but mid-infrared photodetectors are restricted to ~1 GHz bandwidth. These limitations had thus precluded the application of time-stretch spectroscopy in the mid-infrared region.

We pioneered time-stretch spectroscopy in the mid-infrared region by utilizing a free-space time stretcher and a high-speed quantum cascade detector[10]. Figure 4(a) illustrates the experimental setup. Femtosecond pulses from a mid-infrared pulsed laser (a femtosecond optical parametric oscillator) with an 80 MHz repetition rate were temporally stretched using a free-space angular-chirp-enhanced delay (FACED) system. This free-space time-stretcher enabled time-stretch spectroscopy in the mid-infrared region. The stretched pulses passed through the sample were captured by a quantum cascade detector with ~5 GHz bandwidth and then digitized using a 16 GHz oscilloscope. Figure 4(b) presents the time-stretched spectra of phenylacetylene, showing approximately 30 spectral components with a resolution of 15 nm (7.7 cm^{-1}). Continuous spectral measurements at 80 MHz, governed by the laser repetition rate, were successfully demonstrated.

Although this method enabled time-stretch infrared spectroscopy, challenges such as losses from multiple mirror reflections in the free-space time-stretcher limited the number of spectral elements and resolution. Since time-stretch spectroscopy achieves superior SNR compared to FTIR, particularly with a larger number of spectral elements, an improved approach was demanded. To overcome these limitations, we employed a nonlinear wavelength conversion (upconversion) technique to map mid-infrared spectra into the near-infrared region[11]. This allowed for low-loss time stretching using telecommunications optical fibers. Furthermore, the use of high-speed,

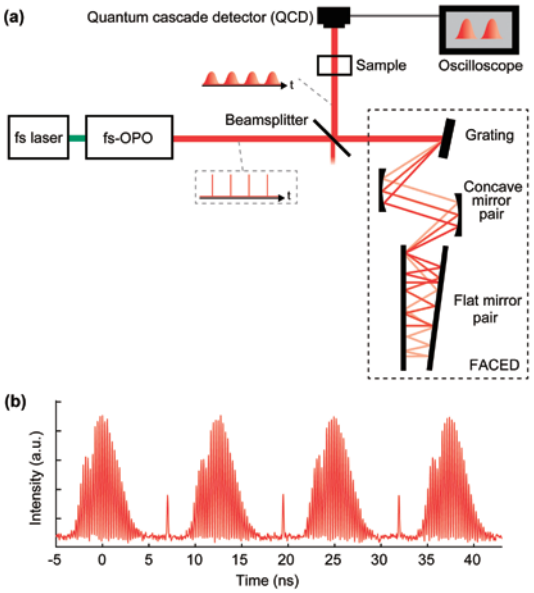

Figure 4 (a) Schematic diagram of time-stretch infrared spectroscopy utilizing a free-space time stretcher. (b) Time-stretched spectra of phenylacetylene.

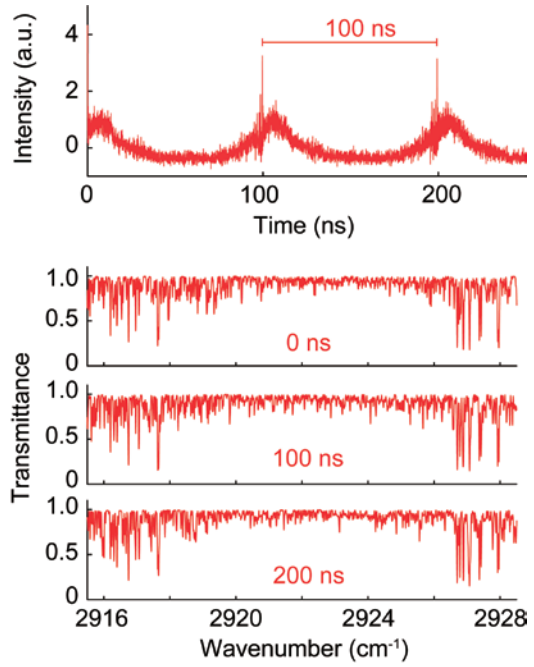

Figure 5 Methane gas spectra measured using upconversion time-stretch infrared spectroscopy.

high-sensitivity photodetectors in the telecommunications range significantly enhanced both spectral resolution and SNR. Figure 5 illustrates methane gas spectra measured using upconversion time-stretch infrared spectroscopy, employing a 60-km dispersion-compensating fiber. This setup achieved a measurement rate of 10 MHz, a spectral resolution of 0.017 cm^{-1}, and captured 1,000 spectral elements. Fiber-based time stretching improved resolution by more than two orders of magnitude, enabling high-speed infrared spectroscopy for gas molecules.

Conclusion

The super-resolution infrared microscope and ultrafast infrared spectroscopy developed in this research have thus far been primarily demonstrated through proof-of-concept measurements of cells and liquid or gaseous molecular samples. Looking ahead, these technologies are envisioned to be applied to the measurement of nano- and microplastics. In super-resolution microscopy, it will become possible to analyze nanoplastics absorbed by cells, advancing research into the effects of nanoplastics on biological functions—a field that has been challenging to study until now. For ultrafast infrared spectroscopy, its application can be extended to large-scale flow measurements in combination with microfluidic channels. High-speed infrared spectroscopy for flow particle analysis will enable high-throughput measurements of water samples from various sources, facilitating the identification of regions potentially at risk from microplastic contamination.

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