Development of in Situ Continuous In-Flow Microplastic Monitoring Techniques Using Spectroscopic Techniques

分光分析によるマイクロプラスチック連続モニタリングシステムの開発

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Microplastics are serious pollutants in marine environments, and it is essential to know the distribution and composition of microplastics on local and global scales, as well as the temporal dynamic change, for a better understanding of pollution. However, very little data in deep waters is currently available due to the difficulty in accessibility. In addition, distributions/amounts of small plastics with a size of <100μm are not well known due to the limitation of measurement techniques. Aiming to monitor microplastics in water continuously, we have developed a novel in situ deep-sea analyser of marine particles by integrating holography and Raman spectroscopy. In addition, using coherent anti-Stokes Raman scattering (CARS), the classification of in-flow microplastics and other natural particles with a size of <100μm has been successfully demonstrated. The methods being developed in this research will enable continuous measurements of microplastics at much higher spatial and temporal scales than what is possible with the current methods and so allow for tracking dynamic variations of microplastics in terms of types, number densities and distributions.

世界規模の環境問題であるマイクロプラスチックについて，詳細な空間・時間スケールでの分布・変化の把握が急務である。しかし，現行のサンプリング調査では，アクセスや測定の困難さから深海や100μm以下の微小粒子の情報が乏しい。そこで，我々は，分光分析などを応用した水中粒子の連続モニタリング技術の開発を行っている。本研究によりサンプリング調査では難しかった詳細モニタリングが実現でき，汚染把握への貢献が期待できる。本論文では，ラマン分光分析とホログラフィ画像を統合した，海中現場型の計測装置の開発と，コヒーレント反ストークスラマン散乱法（CARS）を応用した，100μm以下の粒子の流路内連続測定手法について述べる。

Introduction

In recent years, pollution of marine environments by microplastics has become a global environmental issue and has come to the attention not only of researchers but also the wider public. Microplastic particles are transported all over the oceans like natural particles and are becoming a serious threat to various marine organisms[1]. As the density of plastic debris in the ocean has been selected as an indicator of the Sustainable Development Goals set by the United Nations for the year 2030[2], it is a global urgent task to understand dynamic temporal and spatial distributions of microplastics for a long-term scale.

Typically, the surveys of microplastics in the ocean are conducted by manually collecting and analysing samples in the laboratory. Microplastics on the sea surface are often collected using nets deployed from ships. The net mesh size of ~100 μm is typically used to collect samples as otherwise the nets are easily clogged. However, smaller microplastics can cause more serious impacts on marine animals[3], and measurement techniques for these smaller particles are required. Plastic surveys in deeper water layers are also a challenging task. Particles in the water column are typically collected by filtering sampled water in situ or on board or using sediment traps, as it is difficult to tow a net at a constant depth in the water column. Still, little is known about the distribution of microplastics in deep waters compared to the sea surface, as the sampling chances in deep waters are limited due to difficulty in accessibility.

In situ underwater measurement techniques have a large potential to increase survey efficiency. Conventional in situ techniques for marine particles use imaging for zooplankton and fluorescence analysis for phytoplankton detection. While the morphological or specific chemical (e.g. chlorophyll) information can be obtained in a non-contact manner using these devices, there are currently few methods that can directly measure the general chemical compositions to identify microplastics in water among other particles. Therefore, while it is crucial to survey the dynamic spatial and temporal changes of microplastics to understand the current situation of the pollution, methods for the surveys, particularly of the particles in the deep water column and with a size of <100 µm have not been established.

In our work, continuous non-contact, label-free and real-time monitoring methods of microplastics in water are developed by applying Raman spectroscopic-based and imaging techniques. The research consists of two topics:

1. Development of the in situ deep-sea marine particle analyser

2. Classification of microplastics by applying coherent anti-Stokes Raman scattering (CARS)

Development of the in situ deep-sea marine particle analyser

Digital in-line holography is a volumetric imaging technique that can take monochrome images of suspended particles by analysing interference patterns created by the collimated laser beam and scattered light at a suspended particle. While this has been widely applied to the imaging of underwater planktonic animals, it has been reported that 30 – 70 % of marine particles are not able to be identified only by morphological characteristic[4]. In our work, Raman spectroscopy, a molecular analytical technique that also observes scattered light directed at an object with shifted wavelengths due to molecular vibration/rotation/stretching modes, has been efficiently combined with holography to enable fast particle identification with both morphological and chemical information in a single, large-volume channel using a compact setup[5].

The laboratory setup is shown in Figure 1 (a). The measurement process is the following: a 20 cm measurement cell where the water flows using a pump is constantly illuminated using a collimated laser beam. Using the beam, holographic images are captured at a high frame rate (several tens of Hz) to detect a particle. When a particle is detected, the pump stops to trap the particle and a Raman measurement is initiated using a laser beam at the same beam path as holography. After the Raman measurement, which typically takes several to several tens of seconds, the pump and holographic imaging start again to wait for the next particle. The advantages of this measurement method are that both image and chemical information of particles can be taken in a large volume of water; the whole process can be fully automated; and the system is compact and simple without a filter or mesh to collect particles, which does not require frequent maintenance and is ideal for a long-term in situ deployment. Using a laboratory setup, different plastic pellets and representative marine particles (i.e. polypropylene (PP) pellet, polyethylene (PE) pellet, PE fragment collected from the sea, zooplankton, foraminifera), were measured, as shown in Figure 1 (b). Both holographic images and Raman spectra were successfully obtained for each particle.

The 3000 m depth-rated in situ device, called “RamaCam”, as shown in Figure 2 was developed and deployed at a water depth of 1000-2000 m during the research cruise of KM24-03 conducted by the research vessel (R/V) Kaimei, and KS-24-11 conducted by the R/V Shinseimaru, in 2024. While the detailed data analysis obtained during the cruises is ongoing, automatic holography and Raman measurements of marine particles in the deep water were successfully performed for the first time using the device.

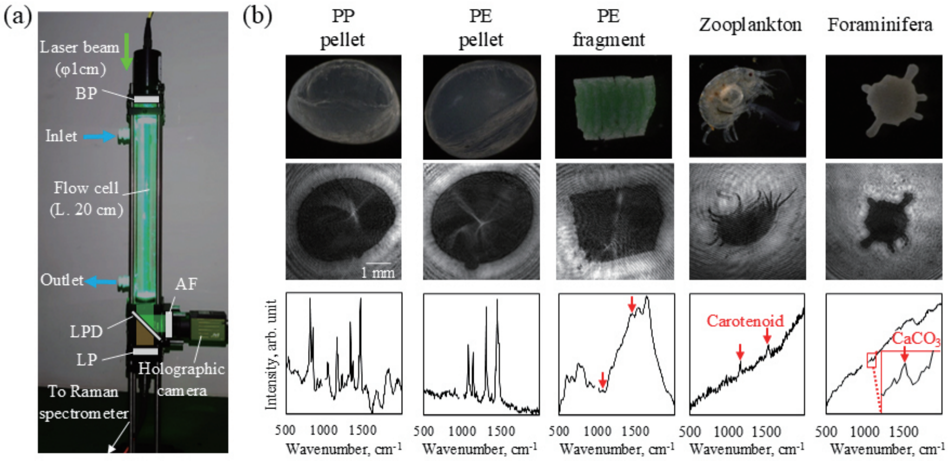

Figure 1 (a) Experimental setup of the integrated system of holography and Raman spectroscopy and (b) the data obtained for typical marine particles using the setup. BP: bandpass filter; LPD: longpass dichroic filter; LP: longpass filter; AF: attenuation filter.

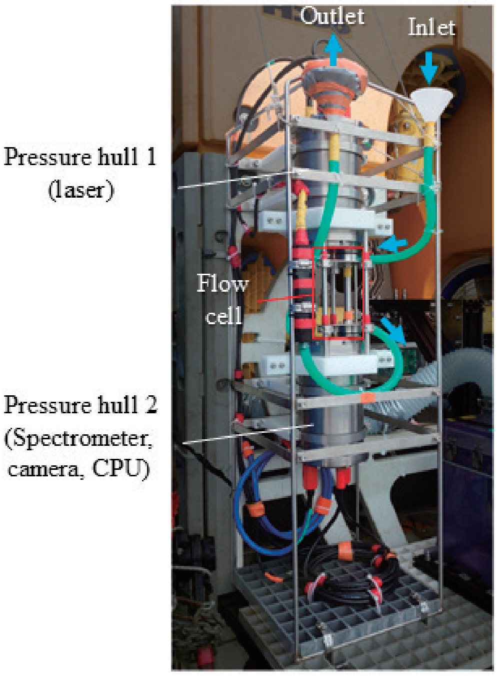

Figure 2 Deep-sea in situ marine particle analyser.

Particle classification method using images and spectra

For the interpretation of multimodal data obtained from the setup of the integrated holography-Raman spectroscopic analyser, a data fusion analysis of images and spectra was developed[6]. While data fusion applications have been expanded to a wide range of multi-sensory data analysis[7], the previous methods have not been applied to the identification of marine particle types/materials due to the limitation of multiple sensory applications to analyse particles. We investigated autoencoder-based unsupervised feature learning approaches to group the different particle types. Autoencoders are a generic type of unsupervised feature learner that has been well-established for analysing imagery, including holographic images[8]. They consist of an encoder network, which reduces the input data down to smaller latent representations, and a decoder network that attempts to reconstruct the original data from the compressed latent representation. The latent representations, through optimising both networks to minimise the difference between the original inputs and their reconstructions, can be used as features for clustering and classification tasks[9]. A key advantage is that they are unsupervised and can flexibly manage different sizes and dimensionality of data inputs as well as the size of the latent feature space representations they output, without significant modification of their underlying form, which is suitable for multimodal data[7]. Figure 3 illustrates the proposed multimodal holographic image and Raman spectrum feature learning. For holographic images with a large data size (227 × 227 pixels, downsized from the original image size prior to the feature learning to reduce the computational time), a convolutional autoencoder, which can handle a complex dataset was used to extract features.

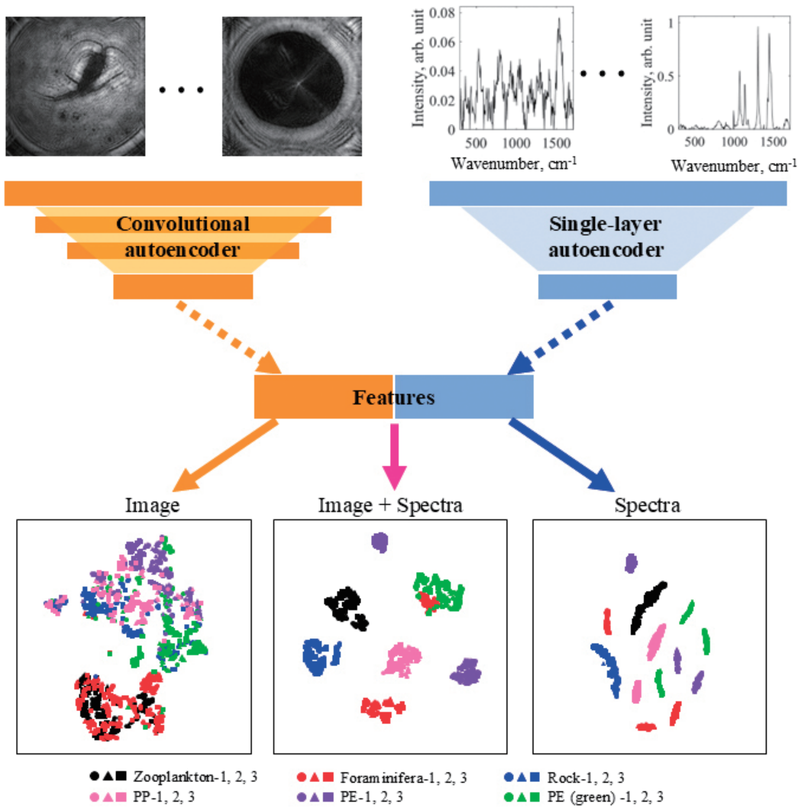

Figure 3 Classification algorithms using features extracted from holographic images and Raman spectra and the t-SNE visualisation of features extracted from holography (left), blended features (middle), and extracted from Raman spectra (right).

For Raman spectra with a small data size (309 × 1 pixels) compared to images, a simple single-layer autoencoder was used. The extracted latent representations from each image and spectra were blended using the t-distributed stochastic neighbour embedding (t-SNE), a method to non-linearly reduce the dimensions of the data to two.

The classification was performed for the data of six typical marine particles (three different individuals per type) taken using the laboratory setup introduced in the previous section. As seen in Figure 3, only two main clusters were formed when only holographic images were used, while too many clusters were formed when only Raman spectra were used for classification. This might be because some types of particles are morphologically too similar to distinguish each other, while the features of Raman spectra pick the difference between individuals. When the combined features were used, the classification accuracy was enhanced by 44 % compared to the accuracy obtained through the analysis only of holographic images or Raman spectra.

Classification of microplastics by applying CARS

Although the device introduced in the previous section is a novel and powerful tool to identify marine particles in situ in the ocean, the measurable size is limited to >1 mm due to the weak Raman scattering light. The diameter of the collimated beam is ~1 cm to scan a large volume of water without filtering, while this can be a disadvantage for Raman spectroscopy as the laser power density at a target is weak, which linearly affects the intensity of the Raman scattering light.

To monitor smaller particles, particularly with a size of <100 µm, we applied CARS to the measurement of flowing particles as a proof of concept[10]. CARS is an advanced Raman spectroscopic-based method where a specific Raman scattering is enhanced by two laser beams with a wavelength difference equal to the wavelength of the scattering light. In the field of biomedical science, CARS is known as a technique for non-destructive and high-speed analysis of living cells, including in-flow measurements in microfluidic devices[11]. While CARS is promising for measuring suspended particles in water, few studies using CARS to aim at continuous monitoring of microplastics in natural environments have been reported, possibly because of strict flow control to align a wide size range of particles in a microfluidic device. We demonstrated the detection of in-flow microplastics with wide size ranges in a relatively large channel (500 µm depth) to avoid clogging by proposing reconstruction analysis of two-dimensional CARS line scanning with a wide view (0.5 mm width, 1.6 times wider than typically used views for CARS images). Selective detection of polystyrene (PS), Poly(methyl methacrylate) (PMMA), and low-density polyethene (LDPE) beads with the size of several tens to hundreds of µm flowing and with the speed of 4 mm/s was successfully performed when the CARS signals of the corresponding frequencies (3050 cm^{-1} for PS, 2940 cm^{-1} for PMMA, and 2840 cm^{-1} for LDPE) was detected as shown in Figure 4 (a) and (b). With this method, the number density and diameters of flowing particles can be calculated. We also demonstrated the classification of flowing microplastics (PMMA) and bio-organic particles (algae) by taking CARS and two-photon excited autofluorescence (TPEAF) signals simultaneously.

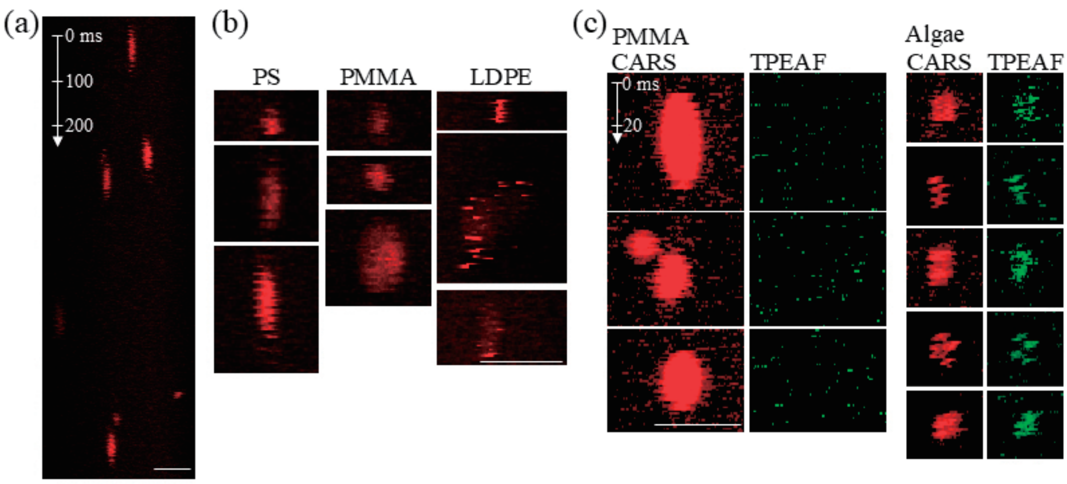

Figure 4 (a) Example of CARS images of PS beads in flow. (b) Zoomed CARS images of different microplastics detected with the corresponding frequencies. The scale bars indicate 100 µm. (c) CARS (red) and TPEAF (green) signals for PMMA and alga particles in flow. The scale bar indicates 50 µm. The scan speed and direction are indicated in (a) and (c). Adapted with permission from Ref [10]. © *2021 American Chemical Society.*

The average intensity of both PMMA and alga particles in the CARS signals at the frequency for C-H bonds (2940 cm^{-1}) was higher than the background level, while only algae emit TPEAF signals because of the existence of chlorophyll. Classification of PMMA and alga particles in flow has been successfully performed by simultaneous detection of CARS and TPEAF signals, as shown in Figure 4 (c).

Conclusions

In this paper, we first reported the development of the integrated device of Raman spectroscopy and holography for in situ deep-sea particle measurements. Different marine particles, including plastic pellets with a size of ~1 mm, were successfully identified using the laboratory setup. The classification algorithm of marine particle types using both holographic images and Raman spectra was also proposed. In 2024, the in situ device was developed and deployed in the sea, and fully automated in situ measurements of marine particles were successfully performed at the water depth of 1000-2000 m. Secondly, for measurements of smaller microplastics with a size of <100 µm, a method based on CARS was reported. In-flow microplastics and bio-organic particles (algae) were successfully detected and classified by simultaneous detection of CARS and TPEAF signals.

This research opens new possibilities for monitoring of microplastics. Measurements that are currently made manually by sampling will be performed in situ and continuously. With the method being developed in this research, the efficiency of surveys of microplastics in the ocean can significantly be increased, which will be game-changing for future surveys. Acquisition of global-scale chemical data on microplastics with high spatial and temporal resolution will be possible, which will lead to the accurate estimation of microplastic pollution. It is hoped that this will also feed back into the public awareness for the protection of our oceans and the establishment of government policies.

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