Fast microplastics identification with stimulated Raman scattering microscopy

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Abstract
The abundance of plastic products in modern society has resulted in a proliferation of small plastic particles called “microplastics” in the global environment. Currently, spectroscopic techniques such as Fourier-transform infrared and spontaneous (i.e., conventional) Raman spectroscopy are widely employed for the identification of the plastic microparticles, but these are rather time consuming. Stimulated Raman scattering (SRS) microscopy, based on the coherent interaction of 2 different laser beams with vibrational levels in the molecules of the sample, would enable much faster detection and identification of microplastics. Here, we present for the first time an SRS-based method for identifying 5 different high production-volume polymer types in microplastics extracted from environmental or consumer product samples. The particles from the extracts were collected on a flat alumina filter, and 6 SRS images were acquired at specifically chosen wavenumbers. Next, we decomposed these spectral data into specific images for the 5 polymers selected for calibration. We tested the approach on an artificial mixture of plastic particles and determined the signal-to-noise and level of cross talk for the 5 polymer types. As a proof of principle, we identified polyethylene terephthalate particles extracted from a commercial personal care product, demonstrating also the thousand-fold higher speed of mapping with SRS compared with conventional Raman. Furthermore, after density separation of a Rhine estuary sediment sample, we scanned 1 cm² of the filter surface in less than 5 hr and detected and identified 88 microplastics, which corresponds to 12,000 particles per kilogram dry weight. We conclude that SRS can be an efficient method for monitoring microplastics in the environment and potentially many other matrices of interest.

KEYWORDS
environment, imaging, pollution, spectroscopy, SRS

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1 | INTRODUCTION

One major side effect of the abundance of plastic products in modern society is microplastic pollution, where small size polymer particles of diverse origins and types enter the environment. There is a growing public awareness and an increase in the body of literature on this subject, but much is still unknown about the full extent, ecotoxicological impact and environmental fate of this type of pollution. Microplastics are usually defined as plastic particles smaller than 5 mm in diameter with no lower limit. They constitute a wide range of chemically complex materials, often with additives and fillers influencing properties such as color, density, and durability. Moreover, they are hard to specifically label with, for instance, fluorogenic dyes. This diversity in properties and wide particle size range means that as a group of analytes, they are challenging to efficiently detect and identify in complex environmental matrices.

Analysis of microplastics in the micro-size range in environmental samples, for instance, in marine sediments, often relies on density separation, sample clean-up and filtration, followed by visual inspection. However, this identification method might result in large numbers of false positives, or false negatives, that is, classification of particles as microplastics when they are not, or missing plastic particles, respectively. For these reasons, identification methods that are specific for the polymers’ chemical structure are necessary for reliable monitoring. Therefore, spectroscopic techniques such as Fourier-transform infrared (FTIR), spontaneous (i.e., conventional) Raman spectroscopy, and pyrolysis-gas chromatography–mass spectrometry have been employed to identify microplastics. Although pyrolysis-gas chromatography–mass spectrometry is usually employed for confirmation of a few manually separated particles of interest, the optical based vibrational spectroscopic techniques FTIR and Raman scattering can scan the surface of a filter containing sample particles. Such spectroscopic techniques are noninvasive, so the particles remain available for further analysis if necessary.

Unfortunately, these methods can be very time consuming, especially when mapping large filter surfaces with a spatial resolution of the order of micrometers. Therefore, most methods based on FTIR or Raman micro-spectroscopy rely on visual inspection to preselect the particles suspected as microplastics for spectroscopic confirmation, rather than scanning the whole surface of the filter. The acquisition time of spontaneous Raman mapping is typically >1 s per pixel to be multiplied with the total number of pixels. Similarly, long mapping times are required for single-detector FTIR, but with a focal-plane array detector, the total image acquisition time with FTIR can be reduced by two or three orders of magnitude. Being able to analyze more samples within a reasonable analysis time would be crucial for providing reliable statistics on the occurrence of microplastics at a given sampling site.

An alternative label-free technique that could provide very fast mapping speeds is stimulated Raman scattering (SRS), where two laser beams coincide on a sample. The signal is then generated when the beams’ photon energy difference matches a vibrational state of the molecules in the focal volume. Detection is usually achieved by amplitude modulating one of the beams before the sample and detecting the modulation transfer imposed on the other beam. The signal intensities at different wavenumbers follow the spontaneous Raman spectrum of the target analyte. SRS is often used for label-free imaging, because the signal acquisition time per pixel, and as a result also the total mapping time, can be reduced by several orders of magnitude compared with spontaneous Raman. With the help of very fast electronics, even video rate acquisition has been demonstrated. In SRS imaging, the signal response of the chemical components in the focal volume at a specific wavenumber serves as the contrast mechanism. For instance, in several biological applications, the wavenumber difference of the two laser beams was tuned to the C–H stretching vibration at approximately 2,850 cm⁻¹ to image the lipid distribution.

In the context of microplastics, Cole et al. used a technique similar to SRS, coherent anti-Stokes Raman scattering, to track the ingestion of well-defined plastic particles by zooplankton by imaging a spiked sample. To the best of our knowledge, SRS (or coherent anti-Stokes Raman scattering) has not yet been used for the detection of a range of microplastics in an environmental sample. However, because of the wide variety of different types of polymers and copolymers potentially present in plastics in environmental samples, SRS analysis at a single wavenumber setting would not be sufficient to detect all polymer materials. There is no single vibrational frequency that is specific for all polymers and at the same time selective over other compounds.

In this paper, we propose an SRS microscopy approach for faster detection of plastic microparticles with only a limited number of measurements at different wavenumbers. We selected five polymers with high global production volumes: polyamide 6,6, polyethylene terephthalate, polystyrene, polypropylene, and high-density polyethylene, noted as Nylon, PET, PS, PP, and PE; respectively. To determine the optimal wavenumbers, we first measured conventional Raman spectra of these selected plastics. A method using the full Raman spectra was used to determine the wavenumbers that provide the best discrimination. After calibrating with these five reference compounds at six SRS
wavenumber settings, we could then automatically identify these polymer species in subsequent samples.

We first test this approach on an artificial test mixture of the five polymers, by evaluating the cross talk terms between the identification channels. Next, we illustrate this concept with the detection of microplastics from a commercial glitter nail polish product (containing PET particles as stated on the label) and show how the automated data processing offers both identification and size determination of the particles collected on a flat filter. We also show the relative gain in overall mapping speed in comparison with conventional Raman. We then demonstrate the identification of five types of microplastic particles from a sediment sample from the Rhine estuary using SRS and multiplexed data processing.

2 | EXPERIMENTAL

2.1 | Sample preparation

An artificial test mixture of the five polymers was made by grinding each polymer type with sand paper and by placing one or a few grains, of each polymer, in close proximity on a glass slide. The order from left to right was Nylon, PET, PS, PP, and PE. After applying water for refractive index matching on the glass slide, a cover slip was placed on top. Glitter nail polish (multidimensional topcoat “a cut above,” Essie) was subjected to microwave destruction with nitric acid as described by Bettinelli et al.\[30\] followed by direct filtration on an Anodisc alumina filter membrane (pore size: 0.2 μm, Whatman). The filter was placed between a microscope slide and a cover slip and wetted to improve refractive index matching necessary for SRS imaging.

A sediment sample from the Rhine estuary was prepared using a procedure similar to the one described by Karlsson et al.\[31\] In short, we mixed 26 g of dry sediment with saturated salt solution (density 1.2 g cm\(^{-3}\)) and let it settle for >5 hr. Next, saturated NaCl solution was slowly pumped into the bottom of the flask (with a thin layer of 6 ml of ethanol on top to prevent microplastics from adhering to the glassware), until the surface layer was raised past a valve. We then separated the supernatant and filtered it with a vacuum pump over a 25-mm diameter Anodisc filter membrane. This filter was selected because of its low Raman background\[32\] and its flatness, which is crucial for the narrow focal depth of SRS. The filter’s effective membrane surface diameter was measured to be 21 mm. Afterwards, we applied 20 ml of pentane to remove oil residues, followed by 20 ml of 30% hydrogen peroxide for >20 min in order to remove biotic residues. We rinsed the filter with >100 ml of Milli-Q\(^*\) analytical grade water, and while wet, we placed it between a microscope slide and a coverslip and sealed it at the edges to avoid evaporation. We took standard precautions to avoid contamination of the filter while handling, such as wearing protective lab gear, covering glassware with aluminum foil after cleaning, and working in a fume hood. Reagent-only measurements (i.e., without sample) were included to determine procedural blanks. Quantitative aspects of the sample preparation procedure, including high recoveries, were reported earlier by Karlsson et al.\[31\]

2.2 | SRS setup

The SRS microscopy setup was mainly as described previously\[33\] and is shown in Figure 1. Briefly, a frequency-doubled Nd:YAG laser (Plecter Duo, Lumera) with 80 MHz repetition rate, 8 ps pulses, and 532 nm output pumps an optical parametric oscillator (OPO, Levante Emerald, APE) with 790–950 nm tunability range. A second output beam of the laser at 1,064 nm was amplitude modulated with an acousto-optical modulator (3080194, Crystal Technology) at 3.636 MHz. The laser and OPO beams were overlapped temporally with a delay stage, combined with a dichroic mirror and sent into a laser scanning microscope 7MP(Zeiss) with a 32× water immersion objective (C-achroplan W, numerical aperture [NA] = 0.85) to scan the sample. A single frame has a size of 380 × 380 μm. For scanning larger areas, an x-y raster stage was used (tile scan). The distance between

FIGURE 1  Scheme of stimulated Raman scattering setup. OPO = optical parametric oscillator; DS = delay stage; AOM = acousto-optical modulator; DM = dichroic mirror; LSM = laser scanning microscope; O = objective; S = x-y raster stage; C = condenser; F = short-pass filter; D = photo-detector; LIA = lock-in amplifier; PC = computer. Samples are scanned frame by frame (380 × 380 μm), and together with the x-y raster stage a larger area of the filter can be mapped.
neighboring measurement points (i.e., pixel size) was 3 μm, as a compromise between spatial resolution and overall measurement time. The average applied powers on the sample were 20 mW of the 1,064-nm beam, and 10 mW of the OPO output beam. At these powers, no photo-induced damage was observed with SRS.

The light was collected with a water immersion condenser (NA = 1.2) below the sample, after which the 1,064-nm beam was blocked. The stimulated Raman loss signal was detected at the OPO wavelengths with a photodetector (DET36A, Thorlabs) integrated with a home built trans-impedance amplifier, reaching shot noise limited detection. The signal was demodulated with a lock-in amplifier (HF2LI, Zurich instrument) with 100 μs time constant and used to reconstruct images with the microscope software (ZEN2011). This time constant is a balance between the imaging speed and the signal-to-noise ratio (SNR) from some of the calibration polymers.

2.3 | Calibration

In order to be able to identify five polymers with a limited number of SRS measurements, we first needed to select five Raman wavenumbers for maximum discrimination. A conventional Raman spectrometer (inVia Reflex, Renishaw) was used to acquire reference spectra of five polymers: Nylon, PET, PS, PP, and PE. The spectra were verified by comparing them with the literature. Differences between spectral intensities were corrected using standard normal variate as shown in Figure 2. Then, we used sparse partial least squares discriminant analysis as described by Cao et al. to find the five most discriminating wavenumbers to separate this set of polymers. Note that this method does not select the purest peaks, that is, peaks that only have a signal for a single polymer. Instead, the method favors some overlap, as seen in Figure 2, because more nonzero data points per polymer will result in a better discrimination. For example, the method selected the wavenumber 1,631 cm⁻¹ on the slope of the amide peak, in order to classify Nylon (high signal) and also to confirm PS and PET (low signal) and to exclude PE and PP (no signal).

Additionally, a sixth wavenumber was included at 1,800 cm⁻¹, where there are no vibrations for the targeted polymers, in order to be able to correct for SRS artifacts.

We constructed a matrix X by measuring the five reference polymers at these six wavenumbers with SRS and used ordinary least squares to construct B, (see Equation 1).

\[
B = \left( X^T X \right)^{-1} X^T Y,
\]

where matrix B (with dimensions 6 × 5) contains the regression coefficients, \(X^T\) is the transpose of matrix X, and Y (5 × 5) is a dummy binary class matrix where the rows correspond to the spectrum and the columns to the polymer type.

2.4 | Acquisition and data processing

For microplastics detection with SRS, we acquired six images of a sample on the filter at the six chosen wavenumbers. At each location (pixel), the six intensities were joined into a vector and multiplied with the matrix B, resulting in a five-element vector. The vector set was then decomposed into five images, each representing a different polymer. The pixel intensities are scores for the identification of the polymers.

We rejected false positives generated by noise and artifacts in the SRS signal by thresholding the polymer images, thus creating binary images showing the detected polymers on a dark background. To set the threshold, we took the standard deviation above the average of each image, multiplied with a factor obtained at the wavenumber with no expected SRS signal (1,800 cm⁻¹). This factor is defined as the ratio between the SRS signal intensity at 1,800 cm⁻¹ and the value of the standard deviation above the average at the 1,800 cm⁻¹ raw image, with a minimum of 1. The threshold rejects the false identification of polymers due to random noise intensity combinations in the raw SRS images, and the factor addition helps to reject spurious signals that can originate from the Kerr effect or transient absorption from, for example, soot particles. In addition, we removed small pixel clusters with an opening...
image processing function \cite{36} with a kernel of $4 \times 4$ pixels. In other words, a particle is counted only if several adjacent pixels have a score above the threshold. Finally, we multiplied the binary images with the scored images.

3 | RESULTS

3.1 | Artificial test mixture

An area of 1.1-mm width and 3.6-mm length of the five polymers sample was measured at the six aforementioned wavenumbers with SRS and processed as described in the image processing section. To appreciate the SRS signal strength, we show in Figure 3a,b the raw SRS images of a PET particle and a PS particle at 1,616 and 1,465 cm$^{-1}$, respectively. The corresponding cross section traces are shown in Figure 3c,d, where the signal is expressed as the ratio between the generated stimulated Raman loss power and the pump power on the detector. The SNR of the PET at its peak (1,616 cm$^{-1}$) is 223, whereas the SNR of PS at the slope of one of its weaker bands (1,465 cm$^{-1}$) is only 2.16. The latter case suggests that the limiting factor in acquisition speed is the low SRS signal for some of the polymers at suboptimal wavenumbers. The full SNR information is shown in Table S1 for all polymers at all wavenumbers. The signal of a polymer was calculated from an averaged area at the same location of an identification of this polymer, as shown in Figure 3j. Signal cross section traces of all polymers at all wavenumbers are shown in Figure S1.

In order to evaluate the identification of the particles and the cross talk between them, we normalized the five element vectors, which represent the five detected polymers in each corresponding pixel, and plotted it in Figure 3e−i with a scale between −1 and 1. Each image shows the part of the sample that contains the polymer type that it meant to detect. A value of 1 (white) indicates a perfect identification, whereas a value of 0 indicates detection in other channels (i.e., high cross talk). Negative values are associated mainly with noise. A complete evaluation of the identification and cross talk for all five particles, from Figure 3e−i, is shown in Table 1, together with an evaluation of the identification scores from the background.

After the identification procedure, the five binary images were combined to one overlay image, shown in Figure 3j, with the following color map: Nylon is red; PET, orange; PS, green; PP, magenta; and PE, yellow. The placement of the five plastic microparticles in the artificial sample was in the same order.

3.2 | Nail polish

A nail polish sample was extracted and filtered as described above. A filter area of 3.4-mm width and 6.8-mm length was measured with SRS at the six aforementioned wavenumbers and processed as described in the image processing section. The five binary images were combined to one overlay image with the same color code as for Figure 3. The sample after preparation should only contain particles that are inert under the conditions of the

![FIGURE 3](image-url) Stimulated Raman scattering imaging of the artificial test mixture. Raw stimulated Raman loss (SRL) images of polyethylene terephthalate (PET) and polystyrene (PS) particles at 1,616 and 1,465 cm$^{-1}$, respectively, are shown in (a) and (b). Figures (c) and (d) are signal cross section graphs along the white lines in (a) and (b), respectively. Signal values are indicated as the ratio between the stimulated Raman loss light power and the pump power on the detector. (e) to (i) are identification images of Nylon, PET, PS, polypropylene, and polyethylene, respectively, where perfect identification is expressed in white. In the stimulated Raman scattering overlay image (j), five binary versions of the five identification images were color coded and overlaid as follows: Nylon: red; PET: orange; PS: green; PP: magenta; and PE: yellow. Scale bar: 100 μm (same for graphs [e] to [i]).
preparation process. As indicated in the nail polish product description, an abundance of PET particles was expected.

Figure 4c shows the overlay SRS image, where most particles, 103, were identified as PET, one identified as Nylon, and one as PS. The latter two are very small and hardly visible in Figure 4c; see Figure S2 for an enlarged view. The objects’ spatial distribution mostly corresponded to the white light image shown in Figure 4a. However, some objects that did appear in Figure 4a were missing or appear eroded in Figure 4c. This can be explained by the nonlinear SRS signal acquisition: SRS is a multiphoton process where the signal is only generated in the overlapping focal volumes, and as a result, the out-of-focus regions are not shown in the SRS image.

The identification of one particle was verified by comparing its spontaneous Raman spectrum with a PET reference spectrum (Figure 4b). A subset from Figure 4c is shown in Figure 4e in grayscale, where the intensity represents the score of the identification. This can be compared with spontaneous Raman mapping, shown in Figure 4d, which was fitted to a reference PET spectrum. The separation between Raman measurements points

![Figure 4](image)

**FIGURE 4** Stimulated Raman scattering (SRS) and Raman mapping of a nail polish extract. The tile scanned white light image of the measured area is shown in (a); scale bar: 500 μm. A particle (indicated by the orange arrow) in this image was measured with conventional Raman for confirmation, and its spectrum is shown in (b; orange spectrum, particle). The blue curve is a Raman reference spectrum of polyethylene terephthalate (PET) for comparison. In the SRS overlay image (c), five binary versions of the five identification images were color coded and overlaid as follows: PET: orange; Nylon: red; polystyrene: green; and polypropylene and polyethylene were not found; scale bar: 500 μm. (d) is a spontaneous Raman mapping from the area marked with a white square in (a) and (c), fitted with direct classical least squares to a reference spectrum of PET. (e) is the same area of the PET identified image with SRS, with greyscale values indicating the identification scores. Scale bars in (d) and (e): 200 μm

| Nylon channel | PET channel | PS channel | PP channel | PE channel |
|---------------|-------------|------------|------------|------------|
| Nylon         | 0.89        | -0.02      | -0.01      | -0.07      | 0.09       |
| PET           | 0.00        | 0.96       | -0.03      | -0.01      | 0.11       |
| PS            | -0.08       | -0.11      | 0.92       | 0.04       | 0.09       |
| PP            | -0.09       | -0.05      | -0.07      | 0.90       | 0.04       |
| PE            | 0.02        | 0.02       | 0.03       | -0.03      | 0.90       |
| Background    | -0.02       | 0.01       | -0.01      | -0.01      | 0.00       |

Note. PET = polyethylene terephthalate; PS = polystyrene; PP = polypropylene; PE = polyethylene.
was 3 \( \mu m \) to match the acquisition resolution of SRS. Acquisition time of the Raman mapping was 1 s per spectrum and a total of 24 hr for the image in Figure 4d, which is significantly slower than the total time needed for an overlay image with SRS acquisition. To illustrate this difference when mapping larger areas, it took 4.5 hr to acquire a 1-cm\(^2\) overlay image with SRS (for instance, the full zoomed-out area mapped originally for Figure 5a-e), whereas with conventional Raman, one could map the same area in 116 days. However, it should be noted that the spectral information contained in the conventional Raman mapping can potentially be used to identify many more polymer species.

### 3.3 | Sediment sample from Rhine estuary

Analysis of a 1-cm\(^2\) area of the filter holding the solid residues from a Rhine estuary sediment sample resulted in the identification of 88 plastic microparticles in total. The number of particles detected per polymer type is presented in Table 2. Figure 5a–e shows some of the detected particles, with greyscale indicating the scores of the identification strength. Figure 5f is the white light image of a particle identified as polystyrene (in Figure 5e). We verified the identification of the particle as PS by acquiring its spontaneous Raman spectrum, as shown in Figure 5g. Interestingly, the conventional Raman spectrum of the particle in Figure 5g also shows a strong fluorescence background, which does not play a role in SRS.

By extrapolating these results of detected particles from 1 cm\(^2\) to the total effective filter surface (3.46 cm\(^2\)), and the sampled weight to 1 kg, the number of particles can be expressed as the number of particles per kilogram dry sediment, resulting in a total of \( \sim12,000 \) particles per kilogram dry weight in the Rhine estuary sediment sample for all target polymer types combined as shown in Table 2. This is slightly higher than the values reported by Leslie et al.\(^{[1]}\) on microplastics particles in sediment from the

![Fig 5](image)

**FIGURE 5** Identification of particles from sediment sample from the Rhine estuary. Nylon, polyethylene terephthalate, polypropylene, polyethylene, and polystyrene (PS) particles are shown in (a), (b), (c), (d), and (e), respectively. The greyscale indicates the scores of the fits, where white is a perfect fit, and the background is suppressed to zero, as described in Section 2.4. Bars are 50 \( \mu m \). (f) is a white light image of the same area as in (e). For confirmation, the particle’s conventional Raman spectrum (g, in green) is compared with a reference Raman spectrum of PS in blue

**TABLE 2** Numbers of microplastic particles identified with stimulated Raman scattering from 26 g of Rhine estuary dry sediment sample on a 1-cm\(^2\) filter surface and their two-dimensional area. Considering the effective surface of the filter (3.46 cm\(^2\)), we can extrapolate to the number of particles per kilogram of sediment (dry weight)

| Polymer | Number of identified particles | Area (\( \mu m^2 \)) of particles found | Identified particles per kilogram |
|---------|-------------------------------|----------------------------------------|---------------------------------|
| Nylon   | 20                            | 4,000                                  | 2,700                           |
| PET     | 7                             | 1,200                                  | 1,000                           |
| PS      | 14                            | 8,400                                  | 1,900                           |
| PP      | 9                             | 1,500                                  | 1,200                           |
| PE      | 38                            | 9,200                                  | 5,200                           |
| Sum     | 88                            | 24,300                                 | 12,000                          |

*Note.* PET = polyethylene terephthalate; PS = polystyrene; PP = polypropylene; PE = polyethylene.
same location. In comparison, suspended matter (SPM) samples collected from the river Rhine further upstream at the Dutch–German border contained 1,700–4,900 particles per kilogram SPM dry weight, which with a typical SPM level of 20 g m\(^{-3}\) would correspond with 30–100 particles per cubic meter of river water.

### 3.4 Blank measurements

Three procedural blank samples were prepared, measured, and processed the same way as the sediment sample. The objects detected in the blank could either be the result of misidentification because of noise, SRS artifacts such as Kerr effect or transient absorption, or could be due to contamination with real microplastics during the preparation process. The average blank detection rate was 12 objects per square centimeter; blank correction was not applied.

### 4 DISCUSSION AND CONCLUSION

In this proof-of-principle study, we applied SRS microscopy to detect and identify microplastics by sequentially scanning at six wavenumber settings a selected region of solid sample residues on a filter and by decomposing this multiplexed information into polymer identification data at each pixel. This procedure does not rely on visual inspection; thus, it eliminates the bias that might arise from subjective interpretation of a given inspected area. After illustrating the approach for the detection of five plastic particles from an artificial test sample, and detecting PET particles in nail polish sample, we used this method to detect 88 microplastic particles from a 1 cm\(^2\) filter area of an extract of 26 g of the Rhine estuary sediment. This corresponds to 12,000 microplastic particles per kilogram sediment dry weight. Our approach for mapping this filter area is not only bias free but also relatively fast (approximately 4.5 hr per 1 cm\(^2\)). Furthermore, analysis of the five polymers mixture showed high detection scores and very low cross talk between the detection of the different polymers, confirming its suitability for microplastics detection.

Other optically based vibrational spectroscopic techniques are slower in comparison. As shown in Figure 4d, mapping with conventional Raman is at least three orders of magnitude slower, which is why conventional Raman (and also single detector FTIR) is often used after presorting by visual inspection. The optimized imaging speed reported by Löder et al.\(^{[18]}\) for FTIR with a focal-plane array detector was approximately 11 hr for a 1.1 cm × 1.1 cm filter surface area at a similar step size, only somewhat slower than our SRS approach.

The identification of polymers in microplastic particles with SRS has some drawbacks when compared with linear optical spectroscopic techniques. In our approach with signal acquisition at six wavenumbers, the method we applied was optimized only for the five calibrated polymers, which significantly limits the spectral information. Moreover, the sharp depth of focus can hinder detection or cause deformation of images of particles that are not in the mapping plane. Additionally, the opening image processing function effectively limits the resolution to 12 μm, because it removes small pixel clusters below that size. Nevertheless, mapping with six wavenumbers was sufficient for the detection of five of the most prevalent plastics with typical particle sizes of a few tens of microns, and the depth of focus effect was minimized by choosing a flat surface filter.

To overcome the drawbacks outlined above, the future development of this method can take different approaches. The focal point in this method was smaller than the separation between neighboring points. Using a lower NA objective with a wider focal spot that matches the step size between measurement points, and increasing the power to maintain the same power density on the sample, should lead to an increased SNR for particles larger than 3 μm. The better SNR should add to the reliability of the identification or, alternatively, would allow the use of a smaller kernel of the opening function and, thus, the detection of smaller particles. Currently, the signal collection efficiency is limited by the commercial laser scanning microscope optics, and we will attempt to achieve a higher collection efficiency in the future (in forward scattering mode). An alternative way to reduce the overall measurement time would be to quickly scan the total surface with two wavenumbers, followed by mapping only the areas of interest with the other wavenumber settings. This approach will require automated data processing and operation of the microscope stage, which requires further development.

In addition, by using the scores of detection rather than thresholding, we expect that other polymers with some Raman response at these six chosen wavenumbers can also be detected once added to the calibration set. Another approach could be the use of broadband SRS for data acquisition, thus combining greater spectral information with the sensitivity of SRS.\(^{[37,38]}\) In conclusion, we expect that SRS-based methods will play an increasingly important role in monitoring microplastic particles in the environment and potentially many other matrices of interest.

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