Application Note

Environmental

Rapid, Large-Area, On-Filter Analysis of Microplastics from Plastic Bottles Using Laser Direct Infrared Imaging

Direct analysis of particles on gold-coated filters by the Agilent 8700 LDIR chemical imaging system

Authors

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Abstract

In this study, microplastics derived from polyethylene terephthalate (PET) bottles were analyzed on gold-coated membrane filters using the Agilent 8700 laser direct infrared (LDIR) chemical imaging system. The direct on-filter analysis of particles by the 8700 LDIR is suitable for the routine testing of microplastics in environmental samples. Using a simple experimental design, the LDIR method provided high identification accuracy, significant time savings compared to other techniques, and easy implementation by nonexpert operators.

Introduction

Awareness of the impact that industrialized economies are having on the environment is driving investment in more sustainable practices, such as renewable energy, reduction of waste, and re-use of materials. Plastic waste is a problem, with recent calculations estimating that more than 5.25 trillion plastic particles float in the world's oceans.[1] As plastic breaks down over time, waste is also a major source of microplastic pollution in the environment.[2] While many researchers agree that micron-sized particles (between 1 µm and 5 mm) can have negative implications on ecosystems, more needs to be known about the primary sources of the particles.

The characterization of microplastics is challenging due to the large number of particles to be analyzed in each sample, and the large area over which the particles are distributed in typical samples. Environmental samples, such as water, contain many thousands of microplastic particles that are mixed with different types of non-microplastics, such as sand and organic waste matter. It is possible to analyze a subset of the microplastic particles and extrapolate the results to simulate a large sample population. However, the extrapolated results might not be an accurate representation of the whole sample, because of the random nature of the selection of the subset.

Rapid analysis of a full sample population

Various technologies have been used to analyze all the content of large-area microplastic samples containing thousands of particles, with varying degrees of success. Techniques such as Fourier transform infrared (FTIR) microscopy can be used to image or map large-area samples. However, with a limited 100 × 100 µm field of view, it can take several days to over a week to map a sample as large as a microscope slide. Typically, Raman microscopy is faster than FTIR, but it is limited by molecular fluorescence interference and difficulties identifying certain microplastics.

Agilent has developed the 8700 LDIR chemical imaging system with quantum cascade laser (QCL) technology, which overcomes the limitations of FTIR and Raman microscopy. The 8700 LDIR provides fully automated microplastic particle analysis over large samples in minutes to hours, rather than days to weeks, for samples containing thousands of particles. Being able to analyze all particles present on a sample relatively quickly by LDIR means that comprehensive results on fully representative sample populations can be obtained, aiding research into microplastics.

Highly automated instrumentation

The 8700 LDIR uses a tunable QCL IR source. The ultrabright laser can sweep through the mid infrared's (MIR) fingerprint region (1,800 to 900 cm^{-1}) at any location in the sample to provide molecular-specific spectral signatures that are used for identification. All known organic materials are MIR-active in this region, and each type of molecule contributes several unique, sharp spectroscopic signatures through vibrational, rotational, and translational modes. The MIR activity of organic materials in this fingerprint region makes LDIR an ideal technique for the characterization of microplastics. The broader, generic absorbance bands found elsewhere in the greater MIR region (3,300 to 1,800 cm^{-1}) can sometimes assist with the analysis of microplastics. However, the lack of band specificity in this region relegates the data to a supporting role and means that it is not needed for the identification of microplastics.

The QCL source of the 8700 can raster rapidly across entire samples at specific wavenumbers, enabling the LDIR to quickly locate microplastic particles anywhere on the sample. Particle size information is also reported, which can be automatically categorized, or divided into user-defined groups, depending on the aims of the application. The 8700 is equipped with two high-quality visual cameras – one with low magnification and one with high magnification – that are fully controlled by the Agilent Clarity software. The 8700 LDIR provides a fully automated IR microscopy solution without the significant training requirements associated with traditional IR and Raman microscopic techniques. Also, scientists can analyze many more samples, over a much larger area, and in much less time using the 8700 compared to other technologies.

In this study, microplastics derived from PET bottles were analyzed on gold-coated membrane filters using the 8700 LDIR chemical imaging system.

Experimental

Sample preparation

Part of a PET bottle was ground into a fine powder using a diamond-coated metallic tool that is available at most hardware stores. The particles were collected into a vial containing ethanol, shaken vigorously, and left overnight. Small-volume aliquots of the solution were pipetted into de-ionized (DI) water to create working microplastic solutions. The microplastic solutions were then filtered as described in the next section.

Vacuum filtration apparatus

Due to the delicate, flexible nature of the gold-coated membrane filters, a small-pore glass frit vacuum filtration filter base was used as the supporting structure for the filters. The high pore density and solid structure of the glass frit helped to distribute the

vacuum pressure more evenly and prevent the gold-coated membrane filters from deforming irreversibly during vacuum filtration. This vacuum filtration glassware was attached to a Vacuubrand ME 2 NT oil-free diaphragm vacuum pump (Wertheim, Germany) capable of 70 mbar maximum vacuum. The pump was also equipped with a manual regulation valve and an analog glycerine-filled manometer (Figure 1). Gold-coated polycarbonate membrane filters from SPI Supplies Inc (West Chester, PA, USA), 25 mm diameter, 0.8 µm pore size were used to extract particles from solution.

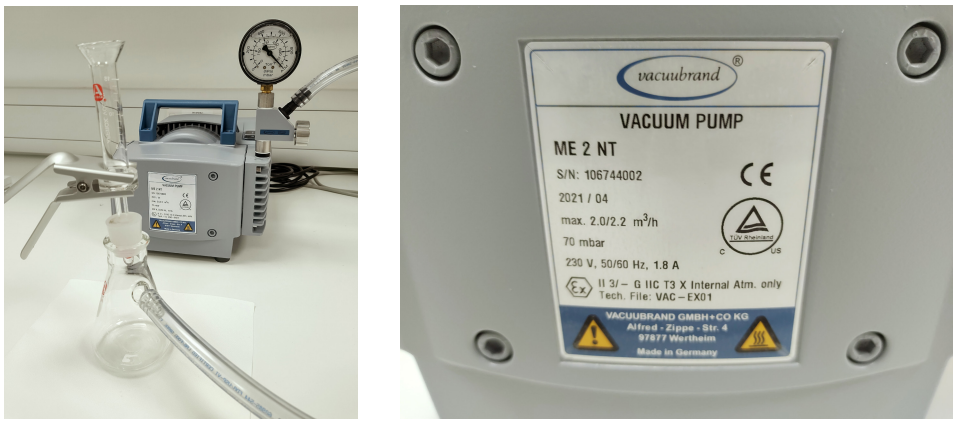

Figure 1. Vacuum apparatus used to perform vacuum filtration of particles.

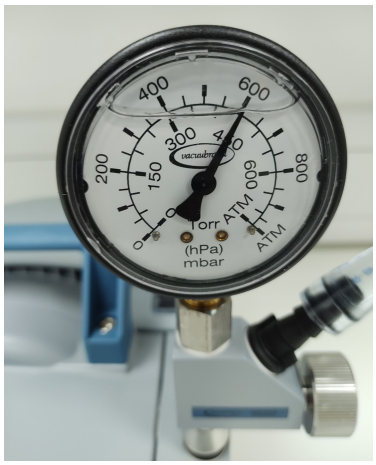

Figure 2. Vacuum pressure applied to filtration.

Filter processing

The filters were premoistened with solvent (DI water) before filtration. Due to the delicate nature of the membrane filters, a gentle vacuum pressure of 600 mbar was applied to filter each particle solution (see Figure 2). This setting translated to approximately 15 mL filtration in 90 seconds. While the solution was being filtered, a 3M 468MP double-sided adhesive patch was cut to fit on a microscope slide (75 × 25 mm), as shown in Figure 3.

A

B

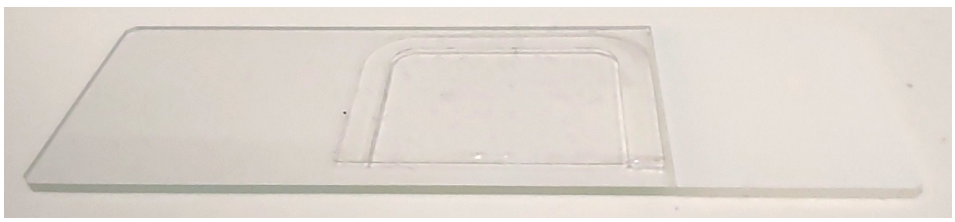

Figure 3. A microscope slide (75 × 25 mm) fitted with double-sided adhesive. (A) Adhesive showing the upper protective layer. (B) Adhesive with the upper protective layer removed, ready for attachment of the filter.

Extra care was taken to ensure that the cut edges of the adhesive were flat and did not protrude higher than any other part of the adhesive patch. Once filtration had completed and the filters were sufficiently dried, the filter was carefully removed from the glassware using tweezers, and gently placed on the microscope slides. The outer perimeter of the filter was delicately patted with the tweezers to ensure it was flat on the adhesive. If a vacuum wand is used to transport the filter from glassware to microscope slide, there is less need to pat the edges of the filter to ensure flatness. The filters were left to rest for at least 30 minutes to allow any temporary deformation during vacuum filtration to relax and regain a flat profile (Figure 4). The goal for 8700 LDIR is to prepare samples with a surface of no more than 10 µm difference in surface topography. However, it is common to see up to 50 µm difference across the surface when working with filters and still produce acceptable results. The microscope slides were placed on the 8700 LDIR sample holder and inserted into the 8700 LDIR one at a time for analysis.

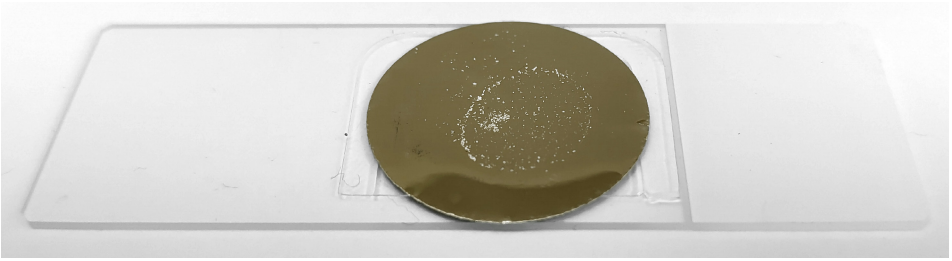

Figure 4. Gold-coated membrane filter set on a microscope slide.

Table 1. Agilent 8700 LDIR operating settings and data acquisition parameters.

Parameter	Value
Analysis Workflow	Particle/peak analysis
Backgrounding Method	Auto/filter background
Focusing Method	Manual focus on filter
Scan Speed	Default (8)
Sweep Speed	Default (3, high speed)
Focus Offset	0
Polarization (Degree)	Default (0)
Attenuation (%)	Default (0)/Auto
Library	User-generated microplastics library
Total Number of Particles Analyzed	949
Total Analysis Time	1.5 hours (IR images only)
Time Per Particle	6 seconds (IR images only)

On-filter analysis of particles

The automated Particle Analysis workflow in the Clarity software was used to analyze the filter samples using the 8700 LDIR. Once the preloaded microplastics analysis method has been selected, the Particle Analysis workflow automatically identifies all particles within a user-defined area of the sample, draws boundaries around each particle, photographs, and identifies each one. The software performs a library search to confirm each particle's identity based on its IR spectrum. The user can select which spectral library to use. Operating parameters of the 8700 LDIR are listed in Table 1.

As the 8700 LDIR is a relatively new technique for microplastics analysis, library-selection can significantly impact the accuracy of the microplastic-identification results. Although the Clarity software includes a basic Microplastics Starter Library, this library is only intended to demonstrate the instrument's capabilities rather than be used as an extensive reference library.

To ensure the best quality data, it is recommended that users generate their own libraries built on plastic and polymer standards that are readily available from multiple vendors. Kevley (low-e IR reflective) slides can be used as substrates for the plastic and polymer standards, and initial comparisons demonstrate excellent compatibility with spectra obtained from the gold-coated membrane filters. Unfortunately, spectra contained within standard off-the-shelf FTIR ATR libraries differ too greatly from the 8700 LDIR's specular reflection spectra to be reliably used for identification. On-going work in this area is attempting to address this inconvenience. The analyses performed in this study referenced an internal Agilent library that was developed specifically for microplastics through analysis of known pure plastic and polymer standards on Kevley slides.

Results and discussion

The 8700 LDIR chemical imaging system was used for the direct on-filter analysis of microplastic particles from a PET bottle. The highly IR-reflective coating of the gold-coated polycarbonate filters provided excellent spectral response and contrast, as well as sharp IR and visible images of particles (Figure 5). Although attention is required at some points during sample preparation, the benefits of direct on-filter analysis outweigh the laborious, multistep process of transferring particles from filter to slide. The direct method also significantly reduces the potential for contamination as it requires less sample handling and fewer preparation steps.

The number of particles detected by the 8700 LDIR on the filter totaled 978, spanning a size range of 20 to 478 µm in diameter. Out of the detected particles, 88% (863) were correctly identified as PET, 9% (89) were undefined, and 1% (14) were identified as cellulose; there was an insignificant number of other trace contaminants (poly-methyl-methacrylate, polyacrylamide, and a few others).

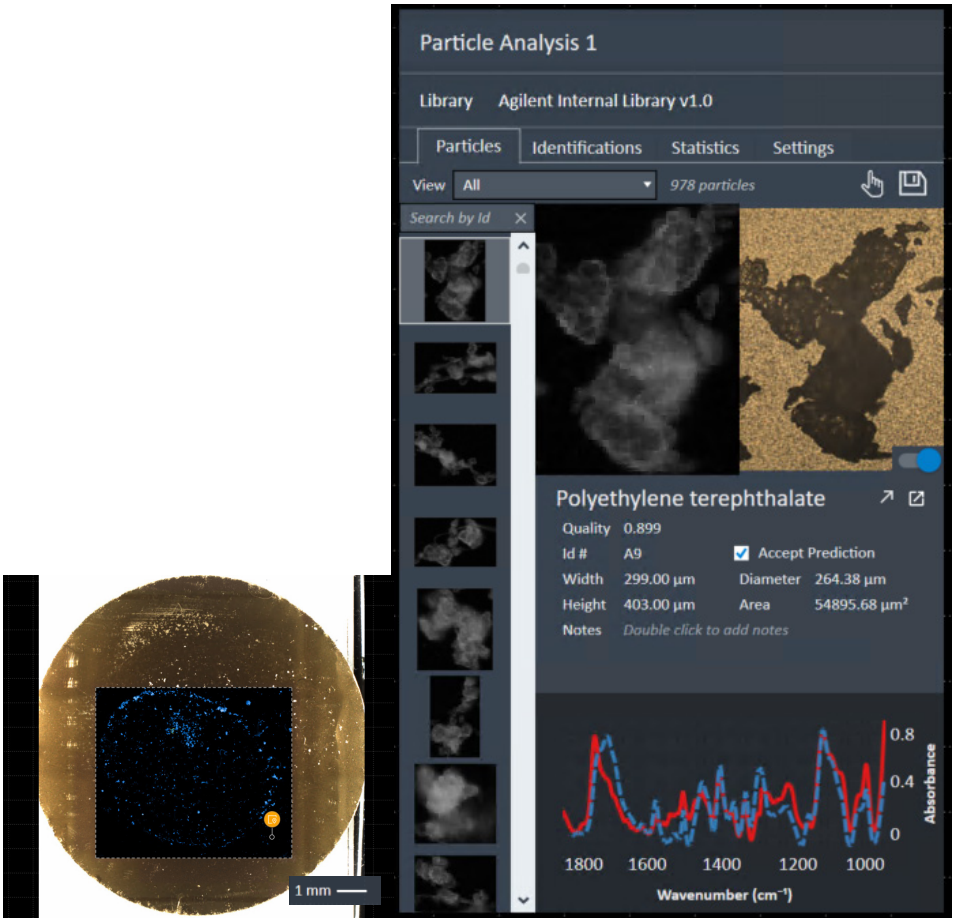

Figure 5. Visible and IR images of microplastics directly on gold-coated polycarbonate membrane filters.

Users can verify the quality of identification of particles in the Clarity software based on high-, medium-, or low-confidence, and a Hit Quality Index (HQI) score, where 1.0 is an identical library match. The HQI score was higher than 0.8 for most of the correctly identified PET particles, which places the identified PET particles in the high-confidence group. Figure 6 shows the Clarity software summary of the particle identification and classification data.

Particles contained within the 0 to 20 µm diameter size range are challenging for any MIR instrument to identify correctly. These tiny particles approach the physical size of the MIR wavelengths used in measurements, and the relative sizes of particles and wavelengths mean that the MIR beam patch is often significantly larger than the particle itself. The negligible number of cellulose particles present can be attributed to contamination during sample handling, likely arising from human skin cells or hair. The poly-methyl-methacrylate and polyacrylamide particles are likely contaminants from articles of clothing.

It is possible to analyze multiple filters on the same microscope slide. The Clarity software allows users to create as many Particle Analysis methods as needed, with each one having its own independent sampling areas and identification libraries. If multiple Particle Analysis methods are used, analysts have the option of analyzing multiple filters under one Particle Analysis workflow or having individual workflows for each filter. Figure 7 illustrates two 25 mm diameter filters mounted on the same microscope slide, which can be analyzed independently. In theory, users could mount several smaller filters on the same slide for higher analysis efficiency.

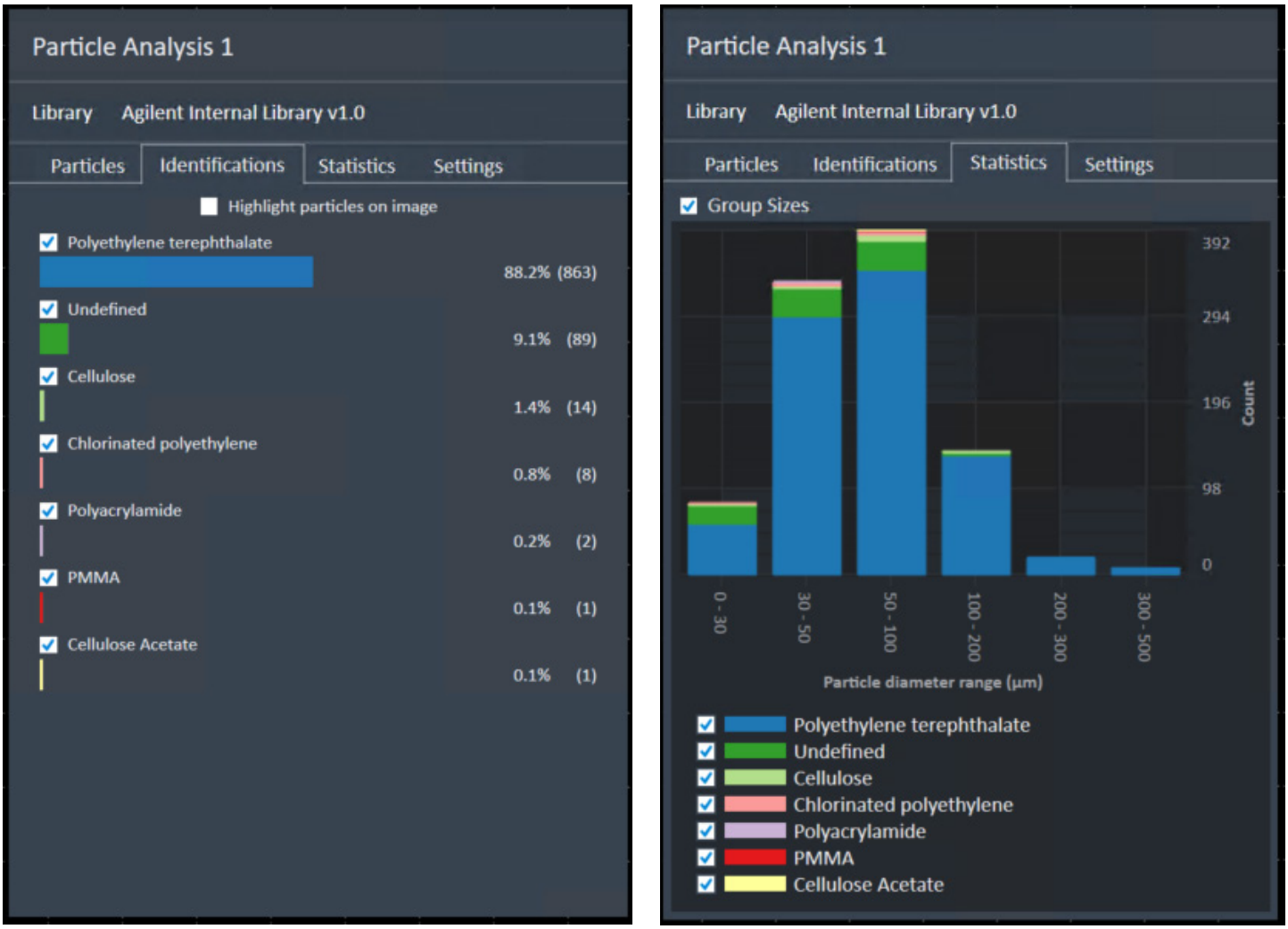

Figure 6. Identification and classification data of microplastics analyzed directly on gold-coated polycarbonate membrane filters using an Agilent 8700 LDIR.

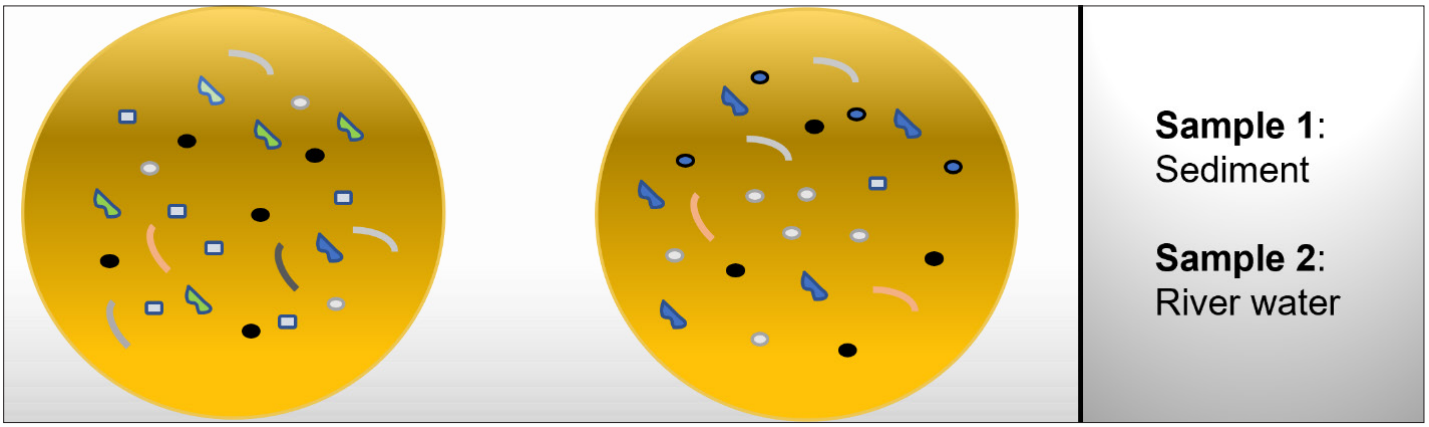

Figure 7. Microplastics from different samples on separate filters mounted on the same microscope slide for analysis by Agilent 8700 LDIR.

Conclusion

The Agilent 8700 LDIR chemical imaging system was used for the direct on-filter analysis of microplastic particles derived from a polyethylene terephthalate (PET) bottle. The particles were trapped on gold-coated polycarbonate filters that were mounted directly on microscope slides. Using the automated Particle Analysis method in the Agilent Clarity software and an Agilent-generated spectral library, high levels of identification accuracy and confidence were achieved for the PET particles.

The vacuum filter sample preparation procedure and LDIR method provided significant time savings and reduced potential for sample contamination. The method is also easy to implement in routine analysis settings compared to traditional microscopic techniques.

The speed and simplicity of the 8700 LDIR will help microplastic research activities, which involve high numbers of samples and fast sample throughput. Due to a high degree of automation and intuitive software, the 8700 requires no training in microscopy or IR spectroscopy to use successfully. Scientists will also benefit from the large area analysis, automated particle detection, identification and classification, ability to reprocess results with new libraries, and visible and IR images of all detected particles.

References

1. Eriksen, M. *et al.* Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. *PLoS ONE* **2014**, 9.
2. XiaoZhi, L. Microplastics Are Everywhere—But Are They Harmful? *Nature*, 4 May **2021**, https://www.nature.com/articles/d41586-021-01143-3.

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