Application Note

Environmental

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

Direct analysis of particles on infrared reflective glass slide and gold-coated filters by the Agilent 8700 LDIR chemical imaging system

Authors

Wesam Alwan, David Troiani, and Darren Robey
Agilent Technologies, Inc.

Abstract

Microplastics derived from polyethylene terephthalate (PET) bottles were analyzed on infrared reflective glass slide and gold-coated membrane filters using the Agilent 8700 Laser Direct Infrared (LDIR) chemical imaging system. Direct particle analysis by the 8700 LDIR is suitable for the routine testing of microplastics in environmental samples. Using a straight-forward experimental design, the LDIR method provided high identification accuracy, significant time savings compared to other techniques, and easy implementation by nonexpert operators.

Introduction

Microplastics are small plastic pieces less than 5 mm long that can be found in different natural habitats, foods, and drinking water. To understand the negative implication of microplastics on ecosystems, proper characterization of these particles is highly desirable.[1] Spectroscopic characterization of microplastics in different matrices in terms of number of particles, size, shape, and polymer type is critical in building objective scientific knowledge in this field.[2]

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. In this application note, we demonstrate the ability of the Agilent 8700 LDIR chemical imaging system (Figure 1) in providing fully automated microplastic particle analysis over slides or filters containing large numbers of particles. Large areas of the slides or filters containing particles can be analyzed within minutes to hours by the 8700 LDIR, rather than days to weeks required by other techniques. Microplastics derived from PET bottles were analyzed on both infrared reflective glass slide and gold-coated membrane filters using the 8700 LDIR chemical imaging system. Being able to analyze all particles present on a sample relatively quickly by LDIR results in comprehensive data on fully representative sample populations, which greatly aids research into microplastics.

Figure 1. Agilent 8700 LDIR chemical imaging system and screenshot of the Agilent Clarity software Particle Analysis workflow. Monitor showing a representative image of microplastics in an environmental sample.

Highly automated instrumentation

The 8700 LDIR uses a tunable quantum cascading laser (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 used for identification. All known organic materials, to which all known microplastics belong, are MIR active in this region, and each type of molecule contributes several unique 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, as it is not strictly required 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. Users can also analyze many more samples, over a much larger area, and in much less time using the 8700 compared to other techniques.

Experimental

Sample preparation

Part of a PET bottle was ground into a fine powder using a metallic file 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 5 mL of absolute ethanol (Scharlau ET00052500; CAS No: 64-17-5) without further processing to create a microplastic solution.

The microplastic solution was then prepared for analysis in two ways:

1. On-IR reflective glass slide analysis

Multiple 10 µL aliquots of the microplastic solution were transferred onto an IR reflective glass slide (7.5 × 2.5 cm; MirrIR, Kevley Technologies, Ohio, USA, Figure 2) using pipettes. The slide was then left to dry at room temperature to allow the ethanol to evaporate before analysis. A similar sample preparation procedure was used in a previous study for the analysis of surface water and treated effluent by LDIR.[3]

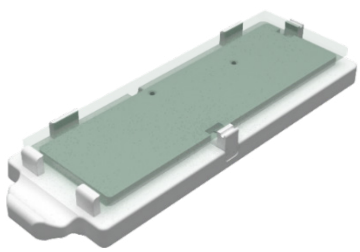

Figure 2. Infrared reflective glass slide holder used in the 8700 LDIR chemical imaging system.

2. On-filter analysis

5 mL of the microplastic solution was filtered using vacuum filtration glassware that was attached to a pump. The gold-coated membrane filter was then transferred to the filter holder, 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 JAVAC (Model: CC-81 vacuum pump (Victoria, Australia). The pump was also equipped with a manual vacuum regulator (SMC Metric M6 port 140 L/min, Figure 3). Polyester (PETG) Gold-Coated Membrane Filters, 0.8 µm pore size, 100/0 nm Coating, 25 mm diameter, from Sterlitech Corporation (Auburn, Washington USA), were used to extract particles from solution. Due to the delicate nature of the membrane filters, a gentle vacuum pressure of 700 mbar (–30 kPa) was applied during the filtration of each particle solution (see Figure 3). The vacuum pressure setting translated to approximately 15 mL filtration in 30 seconds (filtration rate ~ 34 minutes per liter).

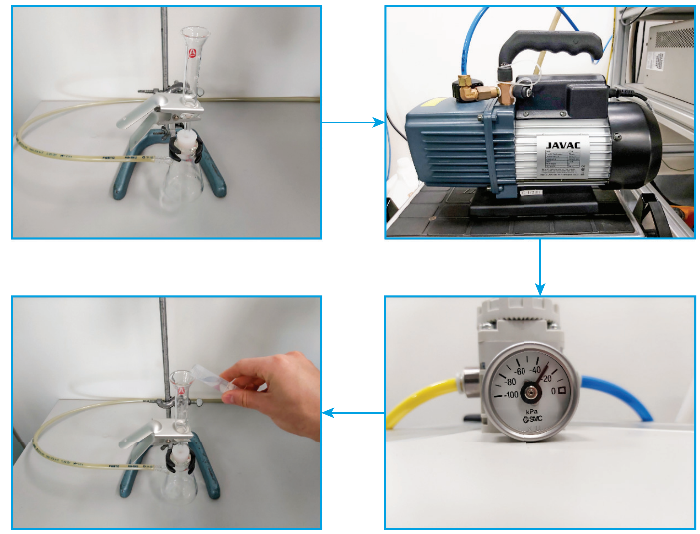

Figure 3. Vacuum apparatus used for vacuum filtration of particles and vacuum pressure applied to filtration.

Filter processing

Once the filtration had completed and the filter had sufficiently dried, the filter was transferred to the filter holder (Figure 4), using the following steps:

1. Remove the brass component from the filter holder.
2. Carefully remove the filter from the glassware using tweezers and gently place the filter on top of the raised platform of the filter holder.
3. Slowly thread the brass component back onto the holder, being careful not to disturb the filter's position.
4. Tighten the brass so that it secures the filter in place and keeps the filter flat.
5. Insert the sample holder into the 8700 LDIR.

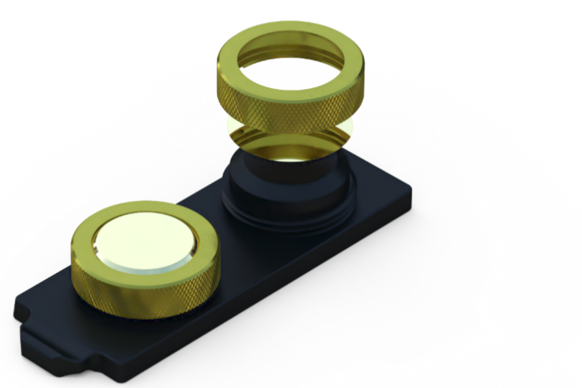

Figure 4. Filter holder used in the 8700 LDIR chemical imaging system, accommodating two gold-coated polyester membrane filters.

Ideally, when preparing samples for analysis by the 8700 LDIR, there should be no more than a 10 µm height difference (localized area of 3 × 3 mm) in the surface topography. However, it is possible for samples to have up to 50 µm surface height difference and still produce acceptable results.

Particle analysis workflow

The automated Particle Analysis workflow in the Clarity software was used to analyze the infrared reflective glass slide and two filter samples using the 8700 LDIR. 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 then performs a user-defined library search to confirm each particle's identity based on its IR spectrum. Operating parameters of the 8700 LDIR are listed in Table 1.

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

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 library, it is only intended to demonstrate the instrument's capabilities rather than be used as an extensive reference library. It is recommended that users generate their own libraries built on plastic and polymer standards that are readily available from multiple vendors to ensure that the highest-quality data are obtained in microplastics analyses. The analyses performed in this study used a library that had been developed specifically for microplastics identification.

Results and discussion

The 8700 LDIR chemical imaging system was successful in identifying PET microplastic particles from a PET bottle on both the IR reflective glass slide and directly on two gold-coated filters. The highly reflective coatings of both the glass slide and gold-coated polyester filters provided excellent spectral response and contrast, as well as sharp IR and visible images of particles.

Method A: Particle analysis workflow on infrared reflective glass slide sample

The number of particles detected by the 8700 LDIR on the infrared reflective glass slide totaled 7,949, spanning a size range of 10 to 486 µm in diameter. Out of the detected particles, 95.2% (7,566) were correctly identified as PET, 4.6% (362) were polyamide, and insignificant numbers of other trace contaminants (polyurethane, polypropylene, and few others). The results are shown in Figure 5.

The benefits of performing the particle analysis on infrared reflective glass slides include the large scan area (around 60 × 22.5 mm in this example) and characterization of large numbers of particles. Also, there is minimal input needed from the analyst once the sample has been inserted into the LDIR and the parameters set.

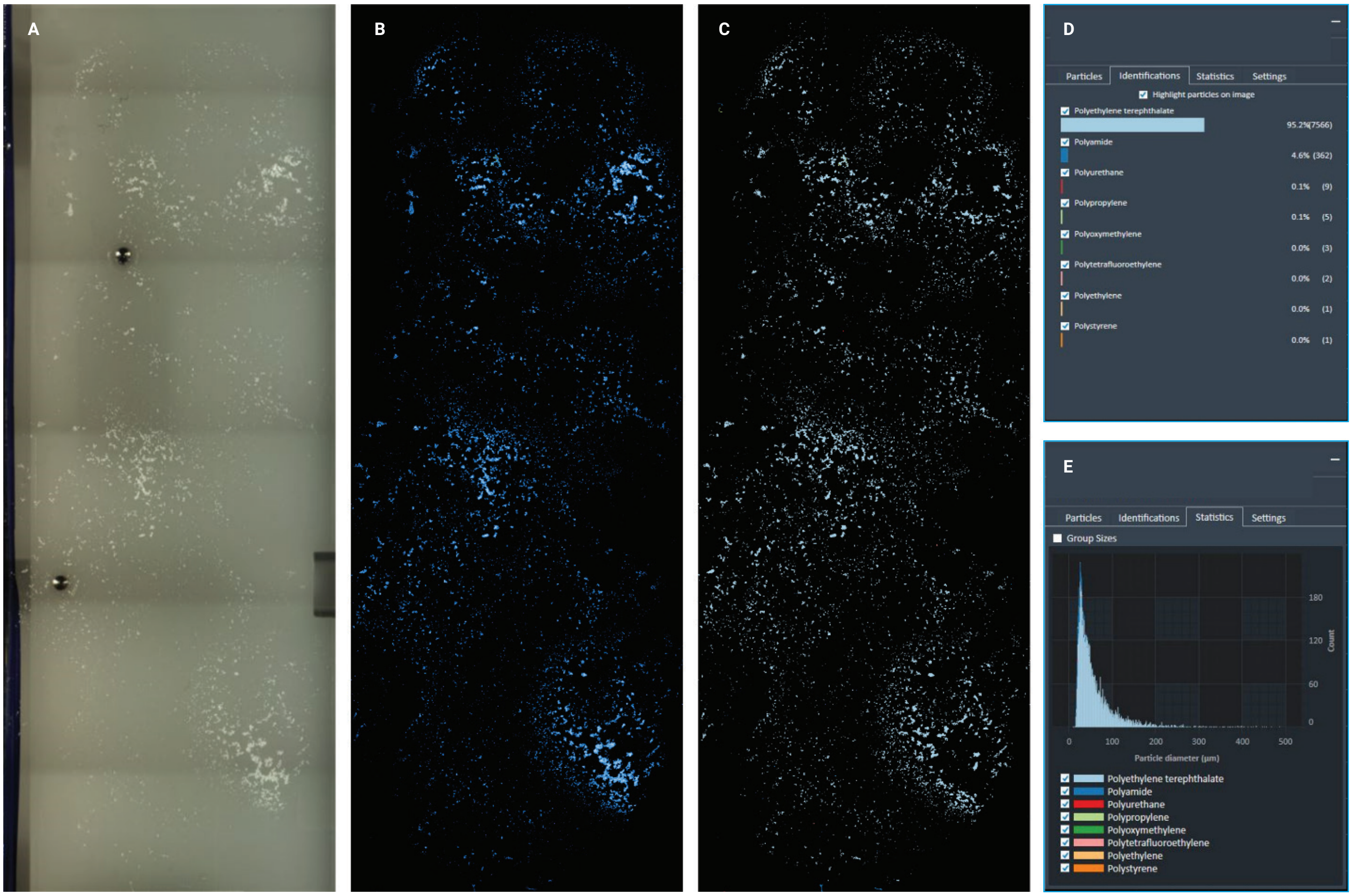

Figure 5. Identification and classification data of microplastics analyzed directly on infrared reflective glass slide using an Agilent 8700 LDIR. (A) Visible image. (B) IR image scanned at 1,442 cm^{-1}. (C) Highlights of particles found—the particles are colored based on the identification of the type of microplastic. (D) Automatic statistical data generated based on the identification of microplastics. (E) Statistical data of microplastic particles based on various size ranges.

Method B: Particle analysis workflow on two gold-coated filter samples

The number of particles detected by the 8700 LDIR on the first filter totaled 4,384 with a particle size range of 11 to 413 µm in diameter. Out of the detected particles, 99.2% (4,347) were correctly identified as PET, and 0.8% were identified as polyamide, polypropylene, and others (Figure 6). On the second filter, 5411 particles were detected with 98.4% (5,324) identified as PET and < 1.6% identified as polyamides and others (Figure 6).

The benefits of direct on-filter analysis outweigh the laborious, multistep process of transferring particles to slide. The direct method also significantly reduces the potential for contamination as it requires less sample handling and fewer preparation steps. Analysts have the option of analyzing two filters under one Particle Analysis workflow or using individual workflows for each filter. The Particle Analysis workflows can also be automatically queued for both filters, allowing the Clarity software to analyze them in series. Automating the method increases the efficiency and productivity of the analysis. Figure 5 shows two 25 mm diameter gold-coated polyester membrane filters mounted on the same filter holder.

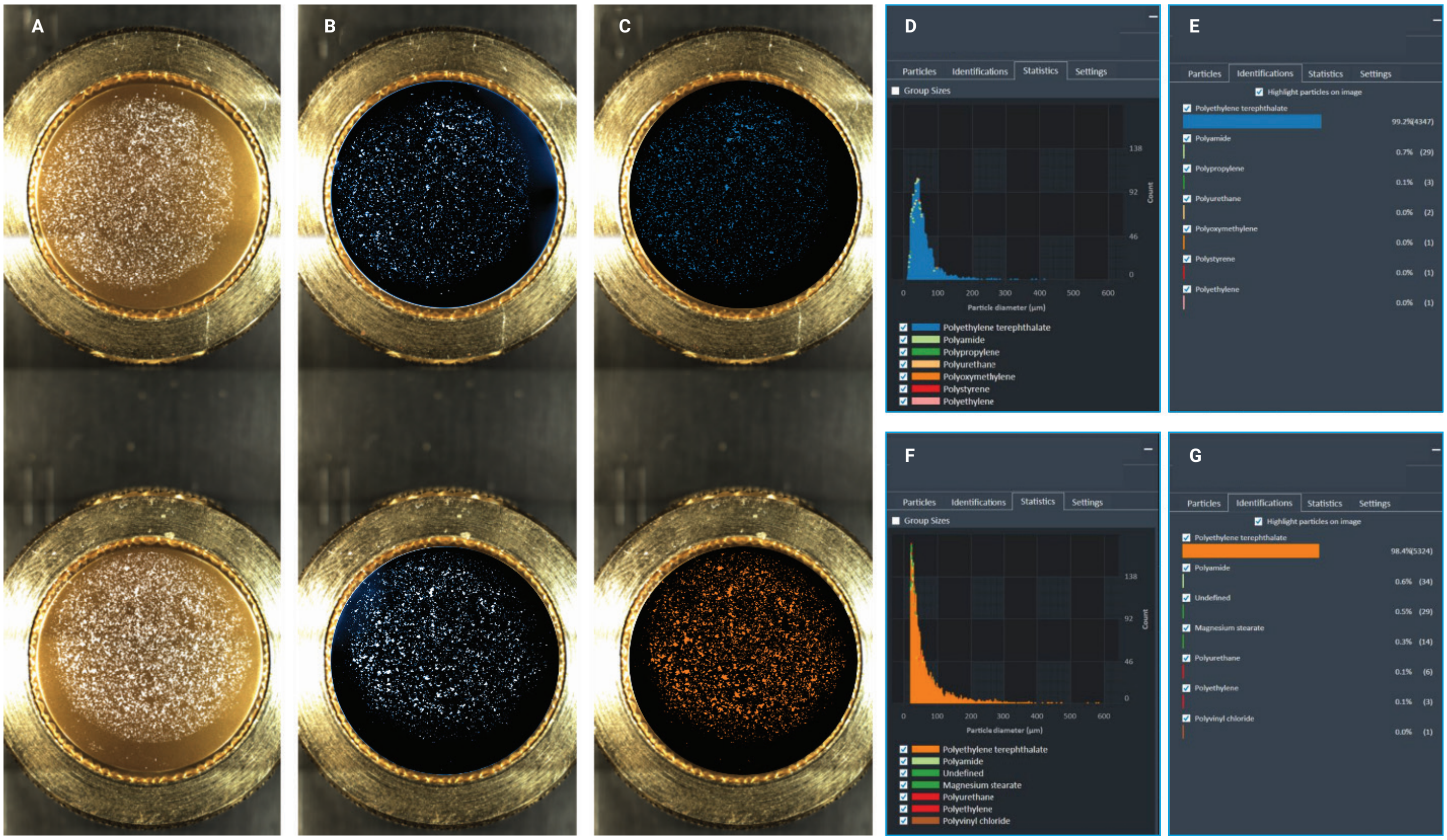

Figure 6. Identification and classification data of microplastics analyzed directly on gold-coated polyester membrane filters (~18 mm diameter) using an Agilent 8700 LDIR. (A) Visible image of both filters. (B) IR image scanned at 1,442 cm^{-1} of both filters. (C) Highlights of particles found—the particles are colored based on the identification of the type of microplastic of both filters. (D) Statistical data of microplastic particles based on various size ranges of filter 1. (E) Automatic statistical data generated based on the identification of microplastics of filter 1. (F) Statistical data of microplastic particles based on various size ranges of filter 2. (G) Automatic statistical data generated based on the identification of microplastics of filter 2.

High hit quality index across various particle size ranges

Analysts 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.

Figures 7 and 8 show the particle identification and classification data for both infrared reflective glass slide and gold coated membrane filters, respectively. Particles with diameters as small as ~10 µm were identified correctly as PET, with high confidence. The Clarity software allows users to create as many Particle Analysis workflows as needed, with each one having its own independent sampling areas and identification libraries.

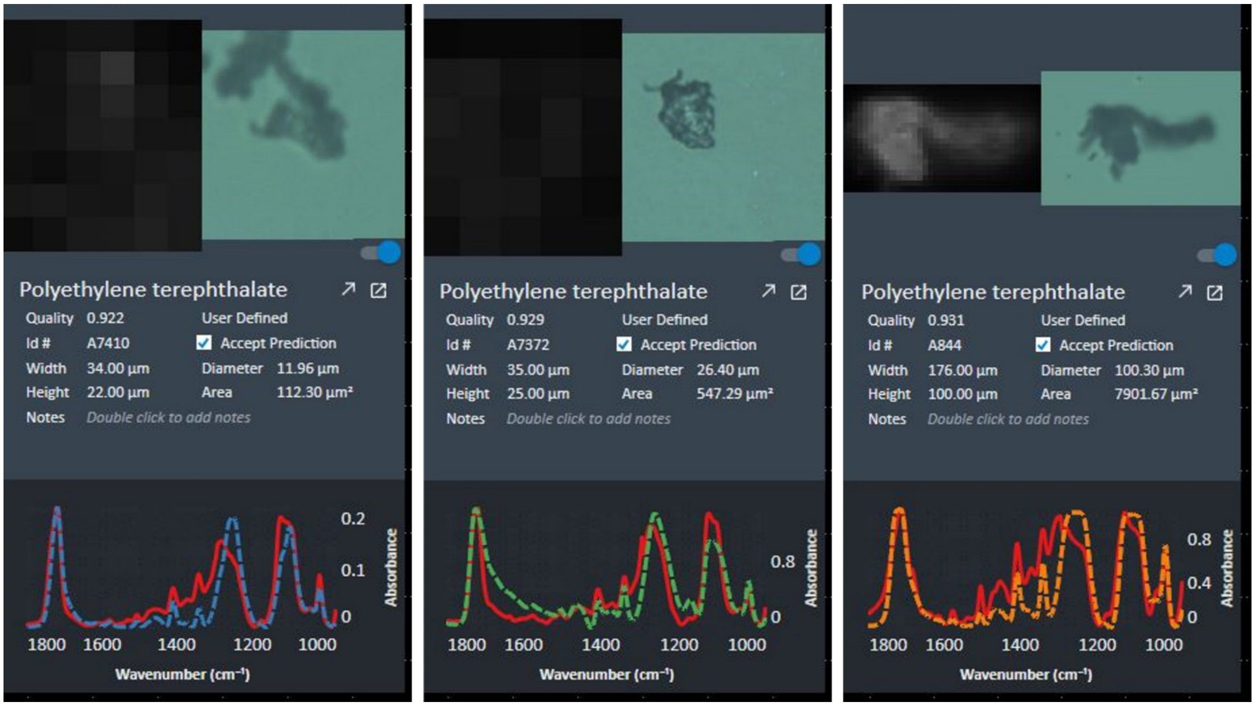

Figure 7. Hit quality and size information of particles analyzed on infrared reflective glass slide obtained from Agilent Clarity software. Particle information such as: IR image, visible image, hit quality index, and overlap of spectrum (red line) and matched library spectrum (dashed lines) can be displayed.

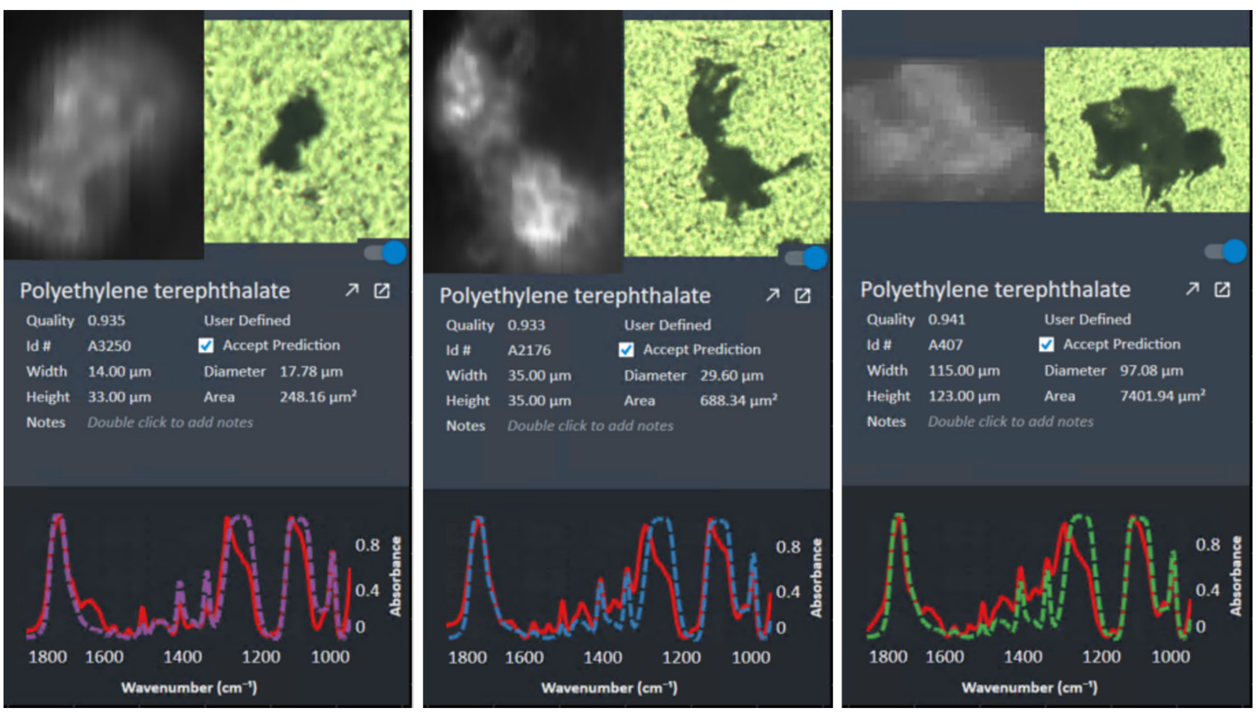

Figure 8. Hit quality and size information for particles analyzed directly on gold-coated polyester membrane filters obtained from Agilent Clarity software. Particle information such as: IR image, visible image, hit quality index, and overlap of spectrum (red line) and matched library spectrum (dashed lines) can be displayed.

Conclusion

The Agilent 8700 LDIR chemical imaging system was used for the analysis of microplastic particles derived from a PET bottle and suspended in ethanol. The samples were prepared for analysis using an infrared reflective glass slide and two gold-coated polyester filters that were then mounted directly on a filter holder. Data was acquired from the glass slide and filters using an automated Particle Analysis method in the Agilent Clarity software and a spectral library developed specifically for microplastics analyses.

High levels of identification accuracy and confidence were achieved for the PET particles on the infrared reflective glass slide (95.2% accuracy) and two gold-coated filters (99.2 and 98.4% accuracy). The results demonstrate the flexibility of the technique for the analysis of microplastic particles prepared using different methods.

Compared to Raman and Fourier transform infrared (FTIR) microscopy techniques, both the glass slide and vacuum filter sample preparation procedures and LDIR methods provide significant time savings. The infrared reflective glass slide provides a larger scanning area, so more particles can be investigated. However, the direct-filter LDIR method requires less sample handling than the glass slide method, reducing the potential for sample contamination. Both methods are easy to implement in routine analysis settings compared to those more traditional microscopic techniques, with the on-filter method offering excellent accuracy and higher sample throughput.

The speed and simplicity of the 8700 LDIR will help microplastic research activities, which involve high numbers of samples and fast sample throughput. With a high degree of automation and intuitive software, the 8700 requires little-to-no training in microscopy or IR spectroscopy to operate successfully. Users will also benefit from the large area analysis, automated particle detection, identification, classification, and visible and IR images of all detected particles.

References

1. XiaoZhi, L. Microplastics Are Everywhere—But Are They Harmful? *Nature*, 4 May **2021**, https://www.nature.com/articles/d41586-021-01143-3
2. Schymanski, D. *et al.* Analysis of Microplastics in Drinking Water and Other Clean Water Samples With Micro-Raman and Micro-Infrared Spectroscopy: Minimum Requirements and Best Practice Guidelines. *Anal. Bioanal. Chem. 413*, 5969–5994, **2021**, https://doi.org/10.1007/s00216-021-03498-y
3. Danence, L. *et al.* Quick and Easy Characterization of Microplastics in Surface Water and Treated Effluent, Agilent publication number 5994-3932EN

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