Application Note

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Characterization of Microplastics in Environmental Samples by Laser Direct Infrared Imaging and User-Generated Libraries

Fast analysis of microplastics with an Agilent 8700 LDIR chemical imaging system and easily constructed spectral libraries

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Abstract

This application note identifies the chemical composition of microplastics derived from various large pieces of plastic collected from the beaches of Sorrento, Victoria, Australia. Chemical identification was achieved using a fully automated particle analysis method for the Agilent 8700 laser direct infrared (LDIR) chemical imaging system. The study also demonstrates the ease-of-use and simplicity of creating a library using the Agilent Clarity LDIR instrument control software.

Introduction

Microplastic contamination of waterways, soil, air, and drinking water is gaining significant public interest due to its emergence as an environmental threat.[1] The number of particles in water may span many orders of magnitude, so the potential harmful impact of exposure to microplastics is a hot topic in the scientific community.

Traditional methods of characterizing microplastics often involve microscopy, with Raman and Fourier transform infrared (FTIR) microscopy being the most commonly used techniques. However, both of these technologies require time-consuming data collection, often taking days to process a single sample. To overcome the challenge of sample analysis times, there is a need for more rapid, routine techniques for identifying polymers and providing data on particle count and size. The 8700 LDIR chemical imaging system provides an automated, easy, and quick way to characterize microplastics, enabling the routine testing of small contaminant particles in the environment (Figure 1).

The 8700 LDIR uses a semiconductor-based quantum cascade laser (QCL) as the infrared source. Using the QCL, electrons are tunneled through a series of quantum wells and emit light. These properties allow the QCL to be rapidly tuned through the wavenumber (λ^{-1}) range of 1,800 to 975 cm^{-1}. When combined with a single-point, thermoelectrically cooled mercury cadmium telluride (MCT) detector and rapid scanning optics, two useful modes of action arise. In the first mode, the LDIR selects a single wavelength and scans through the objective as it moves over the sample at high speed. In the second mode, the objective is parked at a single point, while the QCL sweeps through the whole wavelength range, obtaining a full spectrum in less than one second.

Figure 1. The Agilent 8700 LDIR chemical imaging system.

This study demonstrates the ease and simplicity of creating a library in the Agilent Clarity software to identify the microplastics generated from plastic samples collected from beaches in Australia. The microplastics were characterized using a fully automated particle analysis method for the 8700 LDIR that needed little input from the user.

Experimental

Library generation

To validate the identification confidence performance of the 8700 LDIR method, a small library was generated from 100% synthetic pure polymers. Various shapes and sizes of microplastic materials (sourced from SP2 Scientific Polymer Products Inc, New York State, USA; Polymer Sample Kit, part number 205) were created by grinding pellets of pure samples in a coffee grinder to produce an amorphous powder. The powders (~10 mg) were suspended in absolute ethanol (CAS No: 64-17-5) and transferred onto infrared reflective glass slides (7.5 × 2.5 cm; MirrIR, Kevley Technologies, Ohio, USA) using pipettes. The plastics included in the generated library are listed in Table 1.

Table 1. Polymers included in the LDIR user-generated library.

Type	Abbreviation	Cat No. Ref Material	Form as Supplied
Poly(tetrafluoroethylene)	PTFE	203	Powder
Polymethyl methacrylate	PMMA	377	Powder
Poly(ethylene terephthalate)	PET	138	Pellets
Polycarbonate	PC	035	Pellets
Polystyrene	PS	039A	Pellets
Polypropylene, isotactic	PP	130	Pellets
Nylon 6 [poly(caprolactam)]	PA	034	Pellets

For each type of polymer, two or three spectra obtained from different-sized particles were added to the library. The Clarity software allows users to easily manage libraries and spectra. After collecting LDIR data on samples or standards, the spectrum of interest can then be added to the library, as described in the following procedure and in Figure 2.

1. Click the "add library" icon (shown in Figure 2).
2. Enter a library name. In Figure 2, the library name is "Agilent Local Library".
3. To unlock the library and allow spectra to be added, click the lock icon.
4. After the analysis, select a spectrum of interest.
5. To add the spectrum to a library, tick the box next to the designated library name.
6. To check that the spectrum was added, open the spectral library.
7. From the drop-down menu, select the desired library.
8. Select the spectrum that was added in step 4 and rename it accordingly. In Figure 2, the spectrum was renamed to "polypropylene".

After the library has been generated, previously collected data can be reanalyzed in an easy and quick process as described in the following procedure and in Figure 3.

1. Select the reprocessing arrow in the particle analysis file.
2. Select the desired library from the drop-down menu.
3. Click the tick icon.
4. A new particle analysis file appears, and the new statistical data can be checked.

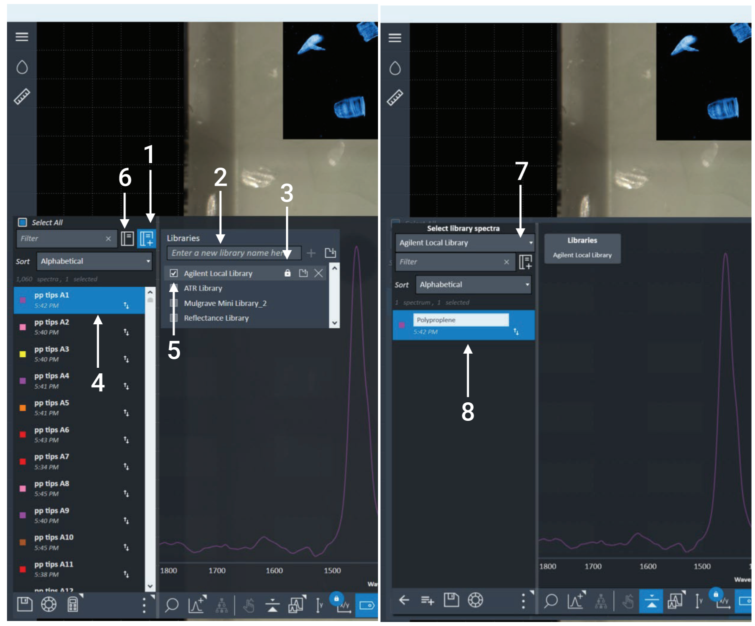

Figure 2. LDIR library generation steps using Agilent Clarity software.

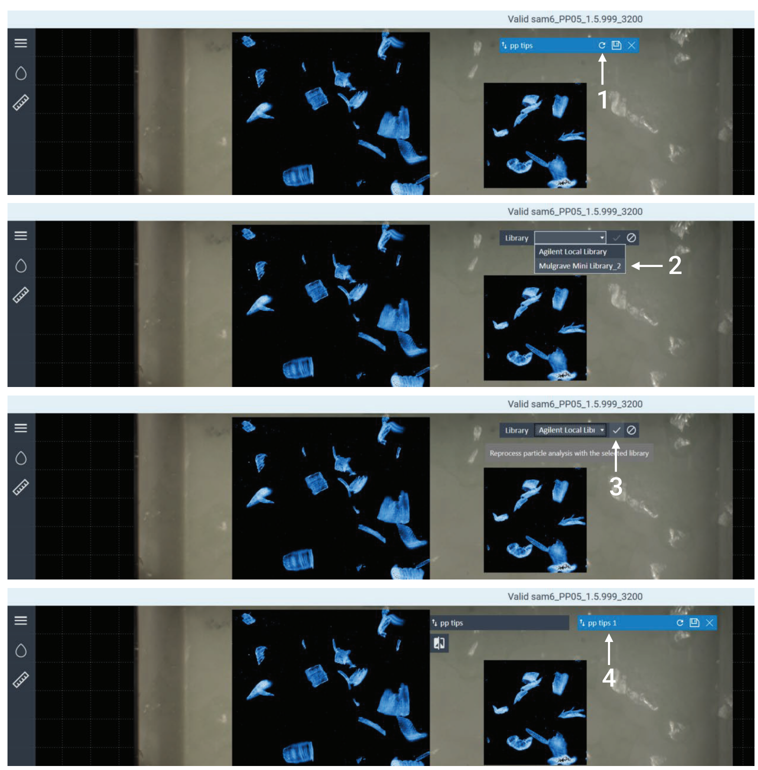

Figure 3. Data reprocessing steps of any generated/transferred library spectra using Agilent Clarity software.

Sample preparation

Plastic debris was collected at random from Sorrento beach, Victoria, Australia. Two samples that had visibly degraded in the environment were selected for this study (Figure 4).

To produce microplastic particles with irregular shapes and sizes, the samples were grated using a blade. The particles were then transferred into vials and suspended in 1 mL absolute ethanol (CAS No: 64-17-5) without further processing. The particle/ethanol suspension was transferred onto two infrared reflective glass slides (7.5 × 2.5 cm; MirrIR, Kevley Technologies, Ohio, USA) using pipettes. The pipette tip was manually cut to widen the aperture to accommodate particles up to a maximum size of 1,000 µm. All microplastic particle preparation steps were performed in a laminar airflow fume hood to minimize contamination by air.

8700 LDIR chemical imaging system

An 8700 LDIR chemical imaging system controlled using Clarity software was used in this study. The infrared reflective glass slides loaded with microplastic particles from each sample were analyzed by the 8700 LDIR using the fully automated "Particle-analysis" method in the Clarity software. The method setup parameters used for data acquisition are shown in Table 2. Instrument parameters were all set to the instrument default settings.

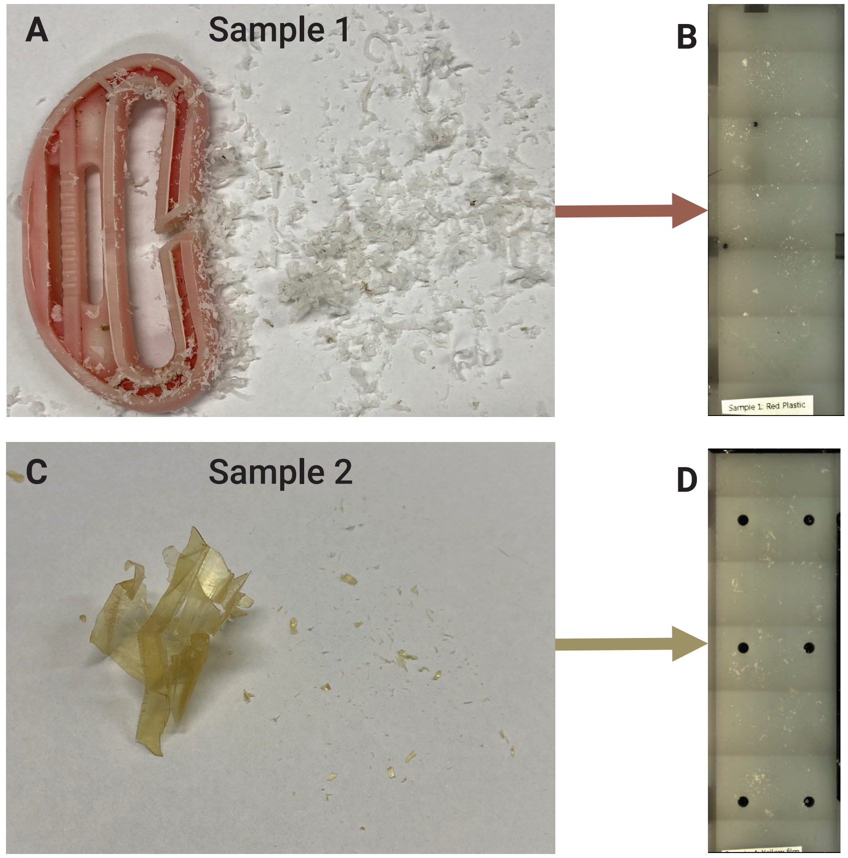

Figure 4. (A) Microplastic generated from sample 1: a red and white plastic. (B) Sample 1 microplastics transferred onto infrared reflective glass slides. (C) Microplastics generated from sample 2: a yellow film. (D) Sample 2 microplastics transferred onto infrared reflective glass slides.

Table 2. Parameters used for the Agilent 8700 LDIR automated method analysis of microplastics.

Parameter	Setting
Method	Particle-analysis
Library Used	Performance proof – user-generated library
Minimum Particle Size to be Detected (µm)	20
Maximum Particle Size to be Detected (µm)	600
Collect Visible Images	Yes
Size Classification Range (µm)	0 to 30 30 to 50 50 to 100 100 to 200 200 to 300 300 to 600
Scan Speed	Default (8)
Sweep Speed	Default (3, high speed)
Focus Offset	0
Polarization (Degree)	Default (0)
Attenuation (%)	Default (0)/Auto

Cary 630 FTIR coupled with an ATR module

To further confirm the identity of samples 1 and 2, an Agilent Cary 630 FTIR spectrometer coupled with a diamond attenuated total reflectance (ATR) module was used (Figure 5). The FTIR spectral library "ATR Polymers and Polymer Additives" available from Agilent (part number G8045AA option 106) was used in a library search method applying the "Similarity" search algorithm using the parameters shown in Table 3. This spectral library contains 7,974 spectra of selected polymers, plastics, polymer additives, plasticizers, and packing materials.

Results and discussion

The Clarity Particle-analysis method used both LDIR scan and full-spectrum modes. Scanning mode was used first to rapidly scan the sample area at a single wavenumber. The resulting IR image was used to both locate particles in the sample and determine each particle's boundary. Once located, the LDIR then rapidly and automatically moved to each particle and acquired a full spectrum over the wavelength range. These spectra were then compared to the microplastics spectral library in real time. The best fit match for the spectrum was determined and reported for each particle. The 8700 used a large field-of-view camera to obtain a whole view of the sample, and a microscope-grade objective to capture high-magnification visual images.

Figure 5. Agilent Cary 630 FTIR spectrometer coupled with a diamond ATR module.

Table 3. Agilent Cary 630 FTIR–ATR operating parameters.

Parameter	Setting
Method	Library search
Library Used	ATR Polymers and Polymer Additives
Search Algorithm	Similarity
Spectral Range	4,000 to 650 cm^{-1}
Background Scans	64
Sample Scans	64
Spectral Resolution	16 cm^{-1}
Background Collection	Air

The types of microplastics that were identified in sample 1 included polypropylene (74.8%), undefined (17.0%), polyamide (6.8%), and polyacrylamide (1.4%). For sample 2, the most abundant polymer was polyvinyl chloride (85.4%), polyamide (10.9%), polyacrylamide (2.2%), and undefined (1.5%).

The Clarity software automatically generates statistics on all identified microplastics, and the data are automatically updated during the analysis. The particles can be highlighted in the scanned area and the particles can be color-coded based on the identification of the type of microplastic, as shown in Figures 6 and 7. The statistics include the particle size distribution, according to user-defined ranges. Figures 6D and 7D show summary of particle size range distribution plotted against the number of particles observed per sample. Within the studied size range of 20 to 600 µm, most of the particles ranged between 20 to 200 µm, as shown in Figures 6 and 7.

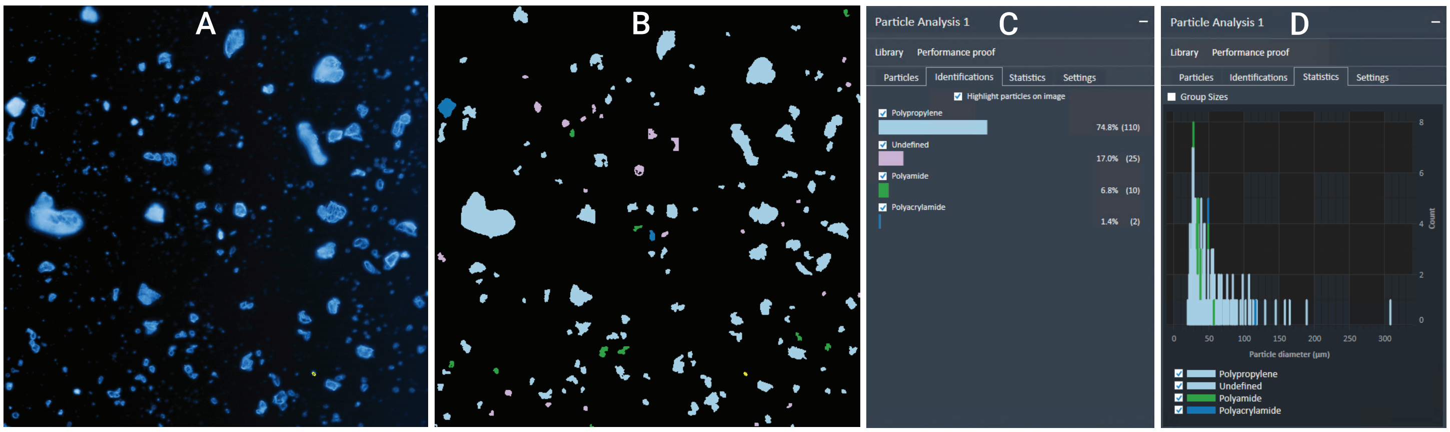

Figure 6. Microplastics characterization of sample 1 using the Agilent 8700 LDIR. (A) IR image of sample 1 scanned at 1,800 cm^{-1}. (B) Highlights of particles found in sample 1 — the particles are colored based on the identification of the type of microplastic. (C) Automatic statistical data of sample 1 generated based on the identification of microplastics. (D) Statistical data of microplastic particles based on various size ranges for sample 1.

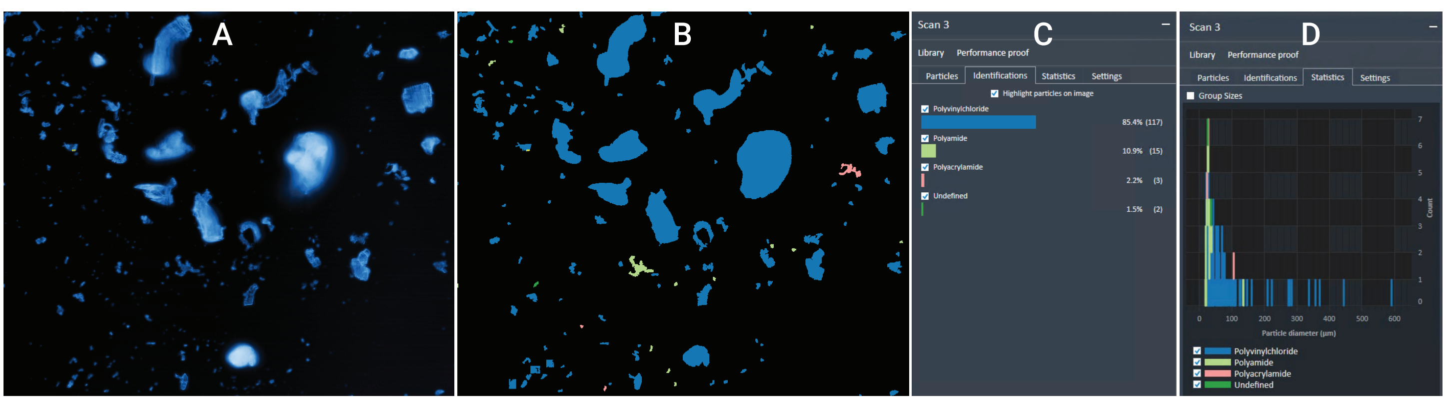

Figure 7. Microplastics characterization of sample 2 using the Agilent 8700 LDIR. (A) IR image of sample 2 scanned at 1,800 cm^{-1}. (B) Highlights of particles found in sample 2 — the particles are colored based on the identification of the type of microplastic. (C) Automatic statistical data of sample 2 generated based on identification of microplastics. (D) Statistical data of microplastic particles based on various size ranges for sample 2.

Identification confidence of samples 1 and 2

Users can verify the quality of identification 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. 71.4% of microplastics in sample 1 and 78.8% of microplastics in sample 2 had an HQI value >0.8, as summarized in Table 4.

The LDIR automated microplastic analysis method provided high HQI values for both small particles (~20 µm) and large particles (200 µm). As an example, for sample 1, particles A128 (23 µm) and A2 (189 µm) were identified as polypropylene with a hit quality of 0.946 and 0.963, respectively (Figure 8). For sample 2, particles A130 (20 µm) and A10 (210 µm) were identified as polyvinyl chloride with a hit quality of 0.934, as shown in Figure 9.

Table 4. Agilent 8700 LDIR automated method analysis of samples 1 and 2.

	Sample 1	Sample 2
Sample Description	Red and white plastic	Yellow film
Area scanned (mm^2)	3.04 × 3.06	3.76 × 4.42
Number of Particles Detected	147	137
Sizes of Microplastics Detected Based on Diameter		
0 to 30 µm 30 to 50 µm 50 to 100 µm 100 to 200 µm 200 to 300 µm 300 to 600 µm	36 (24.5%) 57 (38.8%) 41 (27.9%) 12 (8.2%) 1 (0.7%) 0 (0.0%)	42 (33.7%) 39 (28.5%) 32 (23.4%) 14 (10.2%) 5 (3.7%) 5 (3.7%)
Types of Microplastics Detected	Polypropylene 110 (74.8%) Undefined 25 (17.0%) Polyamide 10 (6.8%) Polyacrylamide 2 (1.4%)	Polyvinyl chloride 117 (85.4) Polyamide 15 (10.9%) Polyacrylamide 3 (2.2%) Undefined 2 (1.5%)
Hit Quality		
0.9 to 1.0 0.8 to 0.89 0.7 to 0.79 <0.7	92 (62.6%) 13 (8.8%) 8 (5.4%) 34 (23.1%)	63 (46.0%) 45 (32.9%) 24 (17.5%) 5 (3.7%)

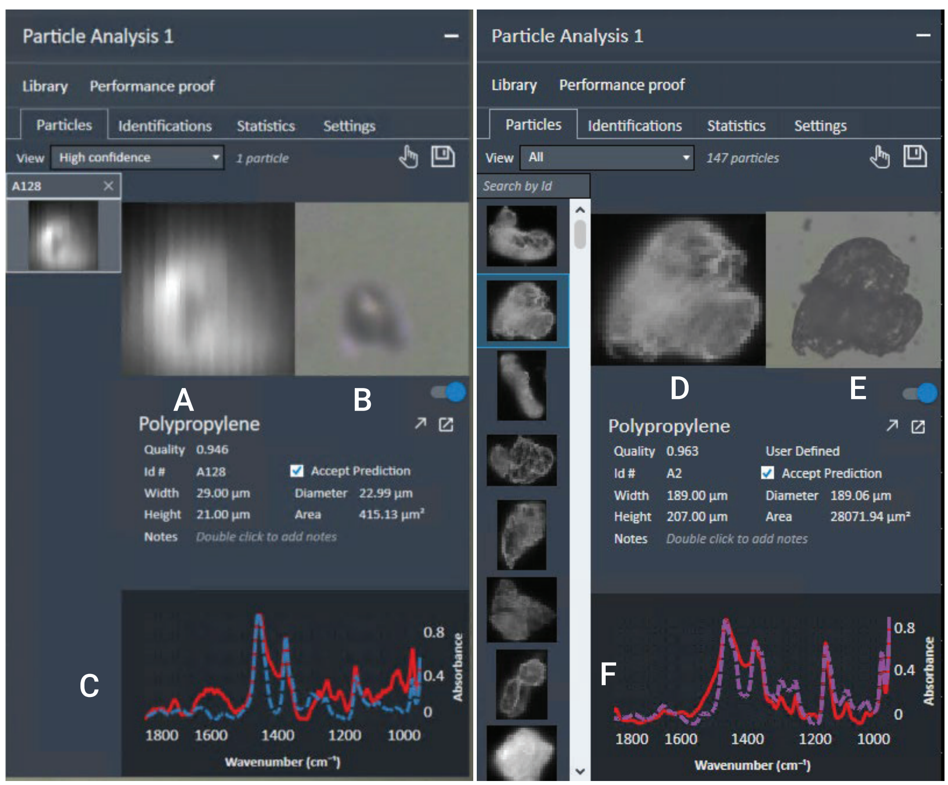

Figure 8. Sample 1 particles hit quality and size information obtained from Agilent Clarity software. (A) IR image of particle A128. (B) Visible image of particle A128. (C) Overlap of A128 spectrum (red line) and matched library spectrum (blue dashed line). (D) IR image of particle A2. (E) Visible image of particle A2. (F) Overlap of A2 spectrum (red line) and matched library spectrum (purple dashed line).

Material ID confirmation by Cary 630 FTIR

Further confirmation of material type for samples 1 and 2 was performed using the Cary 630 FTIR spectrometer coupled with a diamond ATR. Powdered samples were placed on the ATR crystal and data was collected using the parameters listed in Table 3. The collected spectrum was compared with the ATR Polymers and Polymer Additives to confirm the samples' identity using Microlab software. Sample 1 was identified as a blend of polypropylene and poly(propylene co-ethylene) with a hit quality of 0.922, as shown in Figure 10. Sample 2 was identified as polyvinyl chloride with a hit quality of 0.969, as shown in Figure 11. The ID obtained for samples 1 and 2 agreed with the data obtained using the 8700 LDIR chemical imaging system.

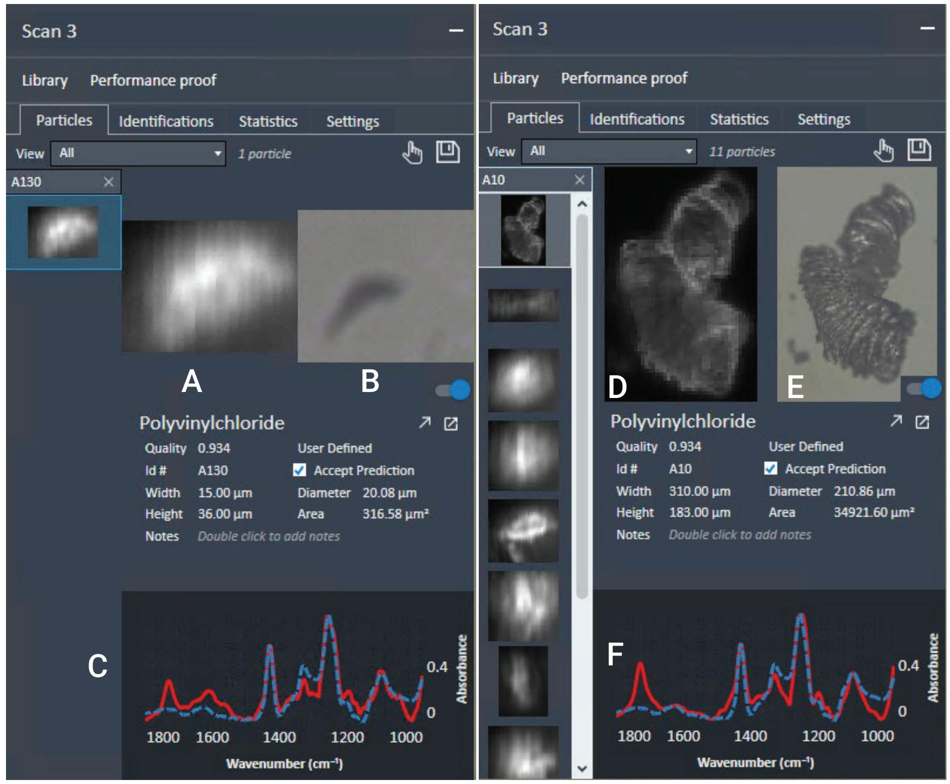

Figure 9. Sample 2 particles hit quality and size information obtained from Agilent Clarity software. (A) IR image of particle A130. (B) Visible image of particle A130. (C) Overlap of A130 spectrum (red line) and matched library spectrum (blue dashed line). (D) IR image of particle A10. (E) Visible image of particle A10. (F) Overlap of A10 spectrum (red line) and matched library spectrum (blue dashed line).

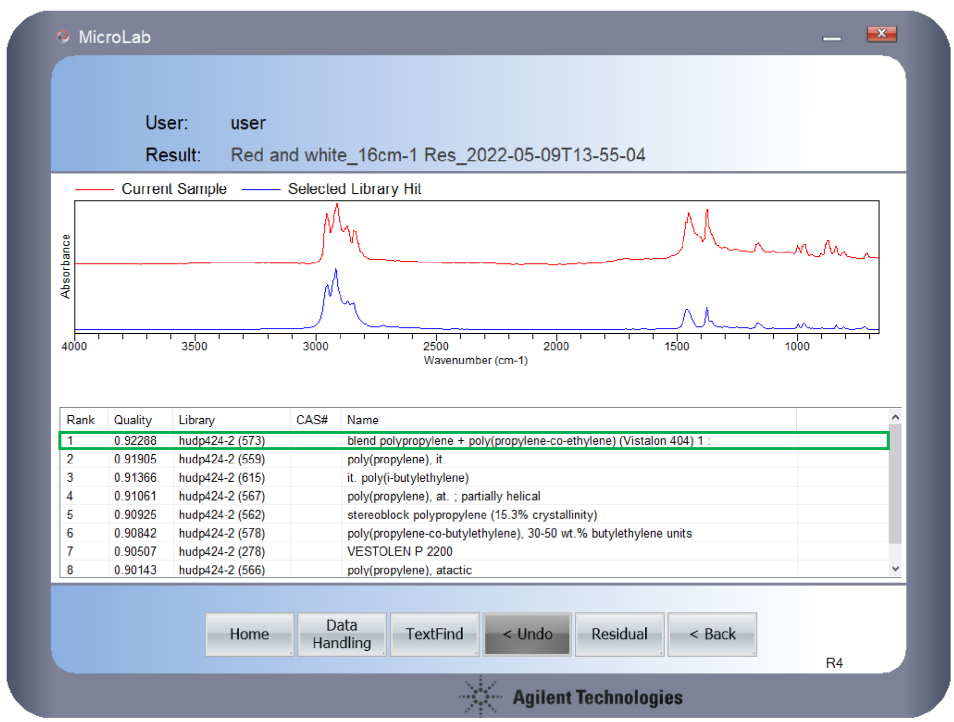

Figure 10. Agilent Cary 630 FTIR qualitative analysis of sample 1 (red trace); library hit (blue trace). The table shows the hit quality, library used, and hit name.

Conclusion

This study shows that the Agilent 8700 LDIR chemical imaging system can successfully classify and differentiate microplastics obtained from environmental samples with minimal interaction required from the user. The fully automated particle analysis method within the Agilent Clarity software is an efficient way for users to obtain information on particle sizes, distribution, and identification of microplastics. Users can quickly create their own libraries by following a few easy-to-follow steps in the Clarity software.

The 8700 LDIR with a user-generated library obtained high-quality identifications of most microplastics analyzed in the samples. The identities of the microplastics were verified using the Agilent Cary 630 FTIR ATR, confirming the accuracy of the 8700 LDIR method.

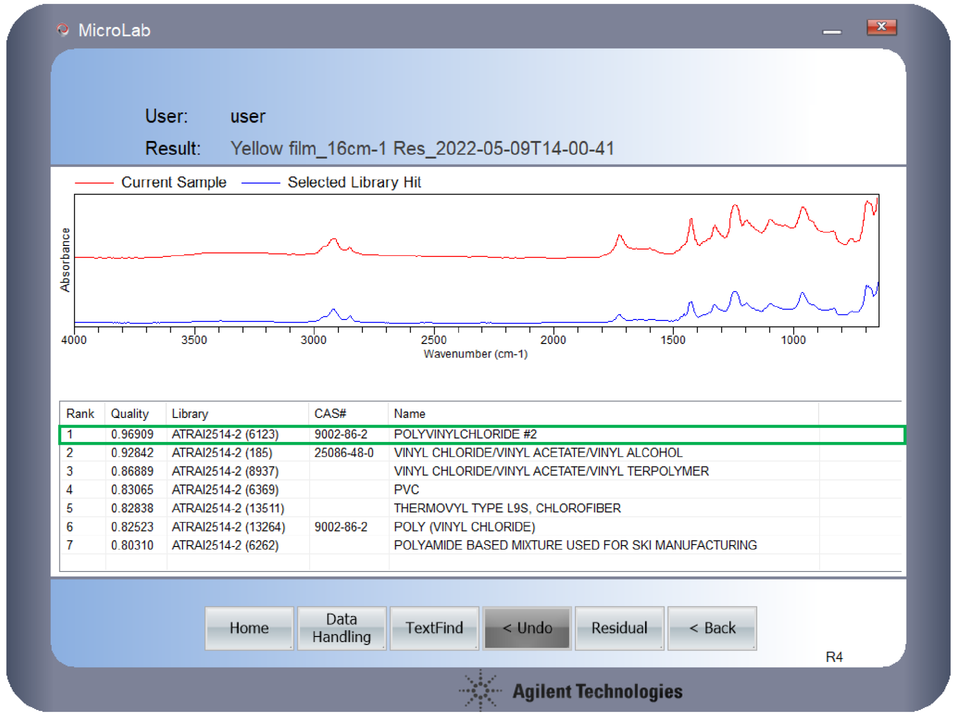

Figure 11. Agilent Cary 630 FTIR qualitative analysis of sample 2 (red trace); library hit (blue trace). The table shows the hit quality, library used, and hit name.

Reference

1. Laskar *et al.* Plastics and Microplastics: A Threat to Environment. *Environmental Technology & Innovation* **2019**, *14*, 100352. 10.1016/j.eti.2019.100352, https://doi.org/10.1016/j.eti.2019.100352

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