Achieving Accurate Microplastics Characterization

The Agilent 8700 LDIR
Chemical Imaging System

Agilent
Trusted Answers

Contents

Introduction

Microplastics are everywhere.[1] They have been discovered in all areas of the natural environment, within the food chain, and in aquatic organisms, animals, and humans. Clearly, there is an urgent need to identify microplastics in the environment and to better understand how they move through air, soil, water, and into living organisms.

Extensive monitoring and studying of microplastics will help address the important questions of their long-term impact on the environment and ecosystems, and if they represent a long-term risk to health.

Many scientists have reported studies on the characterization of microplastics. However, due to the challenges of microplastic characterization, standard test methods are needed for the sampling, identification, and quantification of microplastics, and reporting of the data. Efforts are being made to standardize microplastics analysis around the globe through interlaboratory studies (ILS) involving multiple laboratories. Typically, each participating laboratory in the ILS will analyze the same samples using a technique and method of their choice.

The Agilent 8700 Laser Direct Infrared (LDIR) Chemical Imaging System has been used in two recent ILS designed to assess the performance of current testing methods.[2,3] LDIR and other techniques have also been used to perform microplastic characterization in various environmental matrices including groundwater, bottled water, air deposition samples, and fish intestines.[4-7]

In this ebook, we demonstrate why LDIR can be considered an accurate and reliable technique for the identification and characterization of microplastics in a full range of sample-types.

Agilent 8700 Laser Direct Infrared Chemical Imaging System and Agilent Clarity software.

Direct on-filter analysis

Standard operating procedures (SOPs) developed for the identification of material type of microplastic particles will include a section on sample preparation. Typically, a representative subsample of particles should be prepared either on a filter surface or on a glass slide. Users of LDIR can prepare samples on two types of substrates for the direct analysis of microplastics (Figure 1).

- Samples containing microplastics can be filtered through 25 mm diameter gold-coated filters using vacuum filtration apparatus (as shown in Figure 2). Two filters can be mounted on the sample holder for direct analysis by LDIR (Figure 1A).
- For the analysis of a high number of particles by LDIR, samples can be prepared for analysis on a large-area, low-emissivity (low-e) IR reflective slide. A 25 x 75 mm slide (MirrIR, Kevley Technologies) is shown in Figure 1B.

A **B**

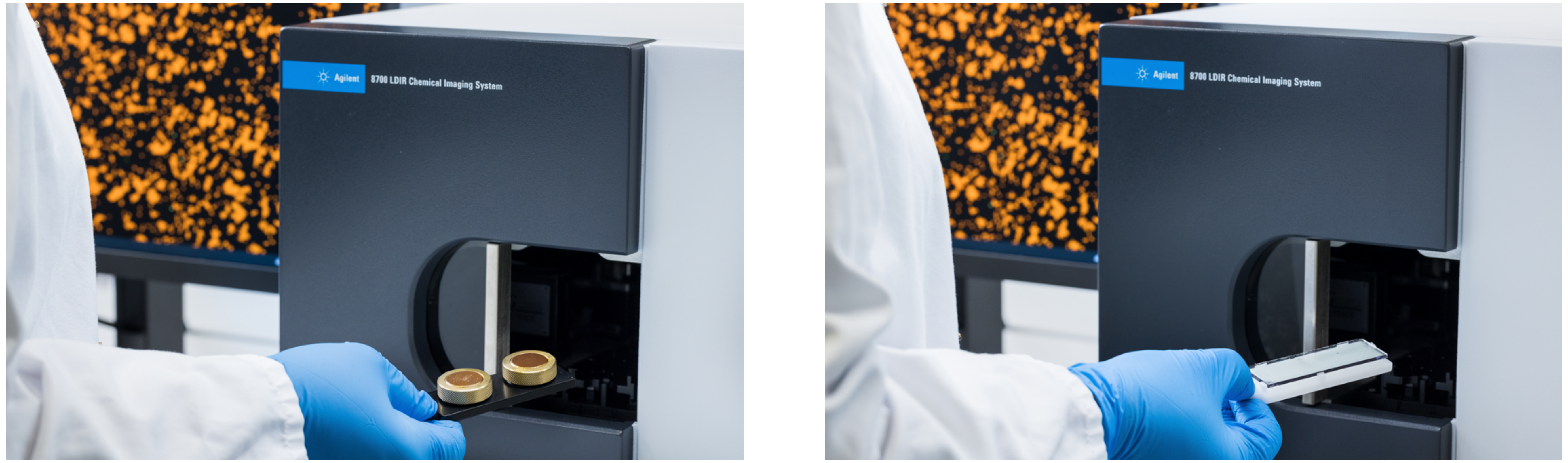

Figure 1. (A) Two gold-coated filters on holder; (B) low-e IR reflective slide. Samples can be prepared on either substrate, ready for direct analysis using the Agilent 8700 LDIR.

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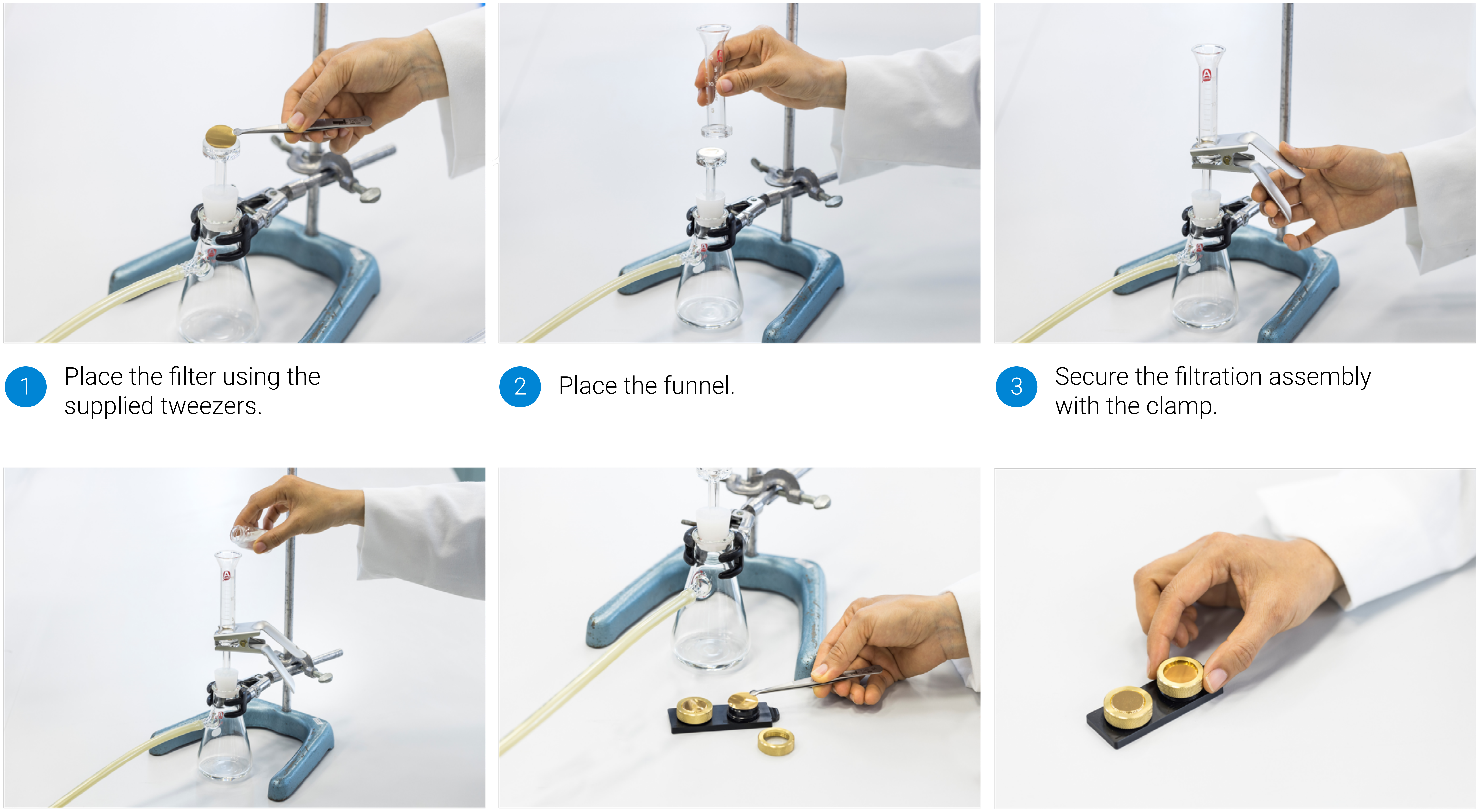

1. Place the filter using the supplied tweezers.
2. Place the funnel.
3. Secure the filtration assembly with the clamp.
4. Filter the sample.
5. Place the filter on top of the raised platform.
6. Thread the brass retaining ring.

Figure 2. Sample filtration equipment and steps for the preparation of samples for LDIR on-filter analysis.

Identification of the most common microplastics

To achieve accurate identification of polymer type in microplastic characterization studies, appropriate spectral reference libraries should be used.

The policy handbook recently published by the California Water Boards requires that the major types of polymers are identified correctly and that they can be distinguished from other natural materials.[8] The ISO technical committee ISO/TC 147 is also developing standards relating to the qualitative and quantitative analysis of commonly occurring microparticles.[9]

To aid identification of the composition of microplastics, the Agilent Clarity software includes a high-quality microplastics library for LDIR that contains spectral data of polymers and natural materials.[10, 11] The library was tested for accuracy by analyzing samples containing microplastics of known polymer type by the 8700 LDIR.

The polymers included polystyrene (PS), polypropylene (PP), polyethylene (PE), polycarbonate (PC), polyvinyl chloride (PVC), polyamide (PA), and polyethylene terephthalate (PET).

Plastics were ground into a powder using a metallic file. The particles were collected in a vial containing ethanol, shaken vigorously, and filtered using vacuum filtration glassware. The gold-coated membrane filter was then transferred to the filter holder. A selected area of each filter was analyzed by LDIR using the automated Particle Analysis workflow.

As summarized in Table 1, accurate identification of the major types of microplastics was achieved, as indicated by the hit quality index (HQI) results of >0.8 for 94–99% of particles. Figure 3 shows an example of the different types of microplastics measured using the LDIR method.

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Table 1. Identification of major types of polymers using the Agilent 8700 LDIR Chemical Imaging System (gold-coated filter analysis).

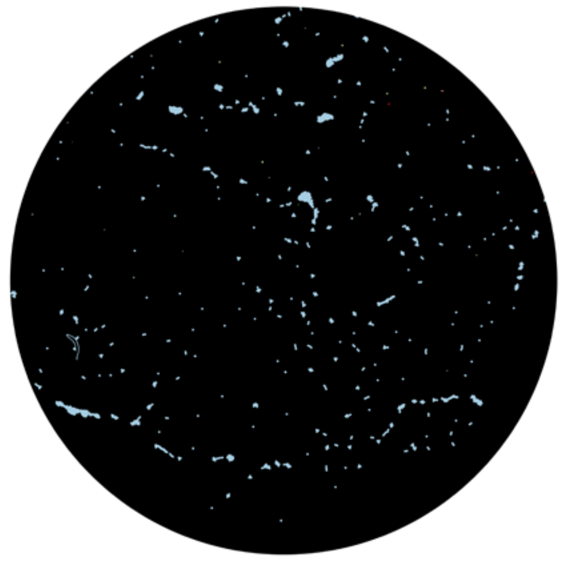
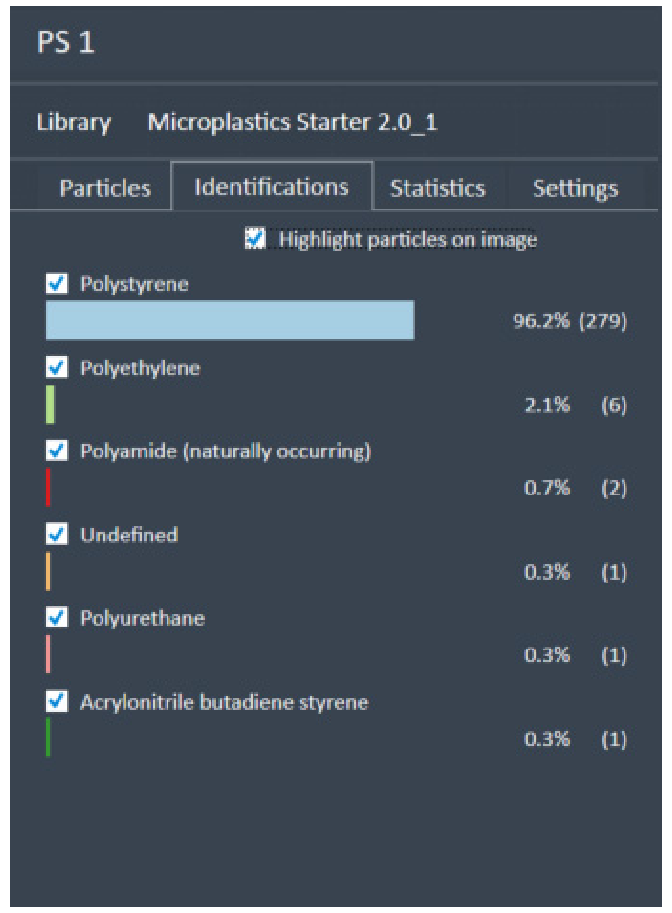
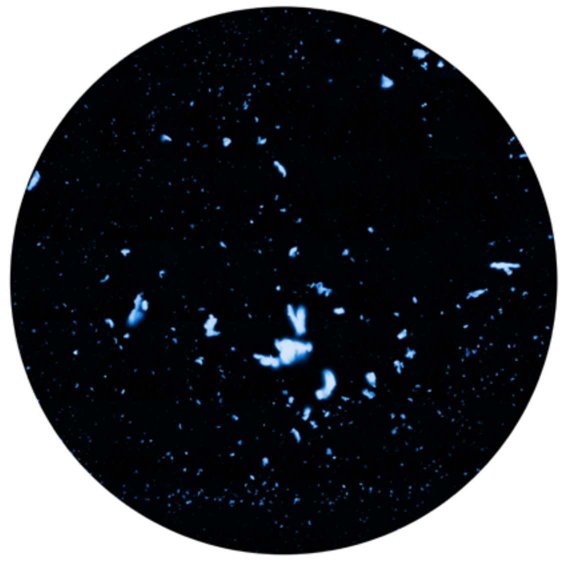
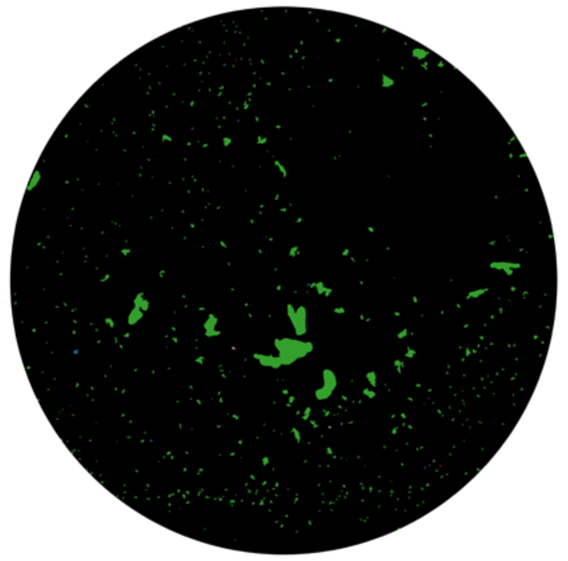
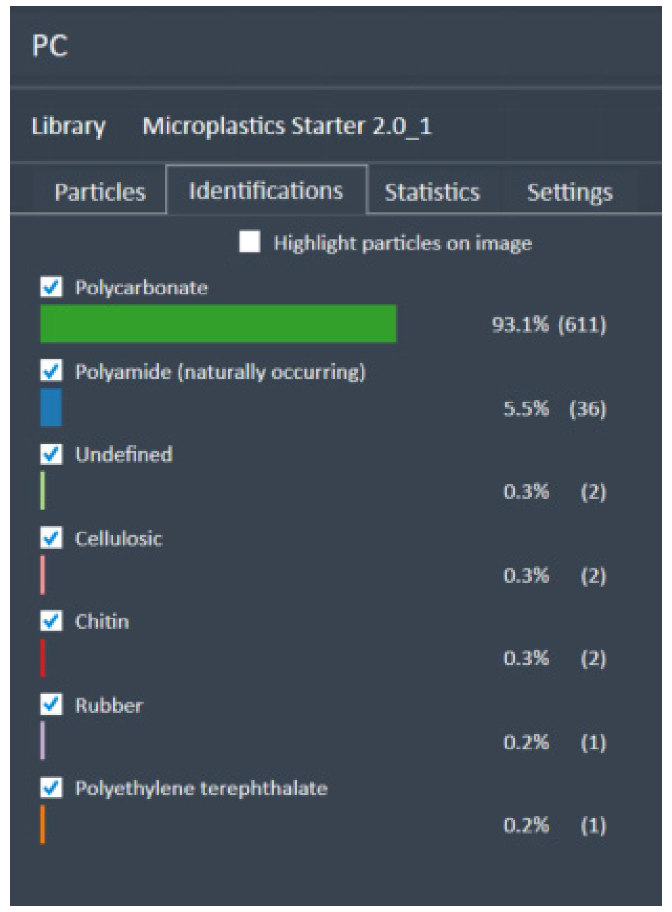
<table>
<tr><th>Type of Polymer</th><th>Infrared Image</th><th>Identification Image</th><th>Statistical Overview</th><th>Quality of the Data</th></tr>
<tr><td>Polystyrene</td><td></td><td></td><td>
</td><td>Total number
of particles: 290
> 0.8 HQI: 284 (98%)
< 0.8 HQI: 6 (2%)</td></tr>
<tr><td>Polycarbonate</td><td></td><td></td><td>
</td><td>Total number
of particles: 656
> 0.8 HQI: 635 (96.8%)
< 0.8 HQI: 21 (3.2%)</td></tr>
</table>

Table 1. Identification of major types of polymers using the Agilent 8700 LDIR Chemical Imaging System (gold-coated filter analysis).

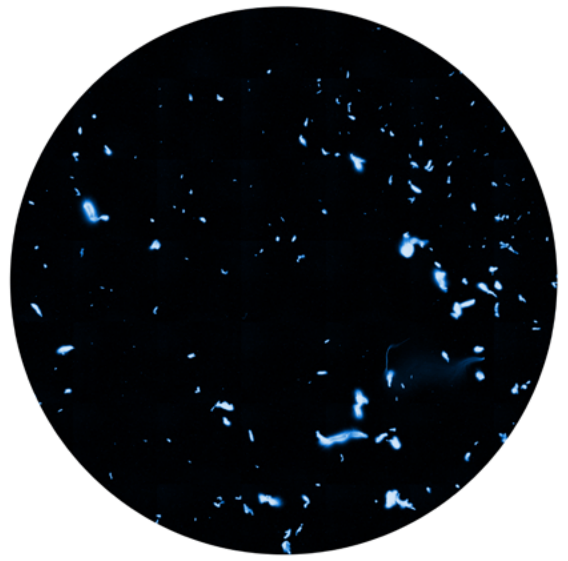
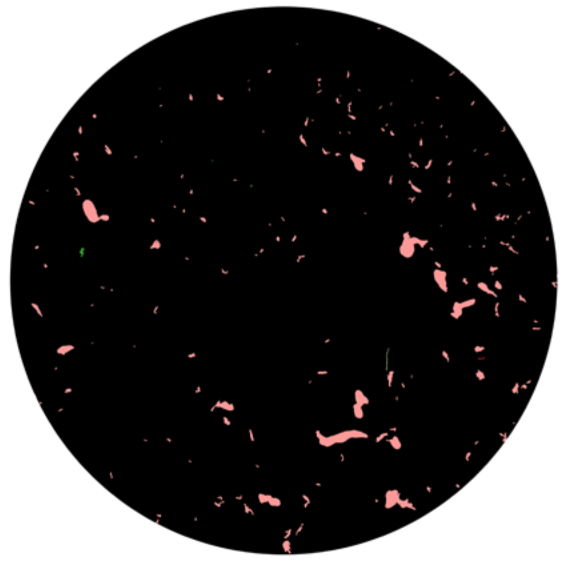
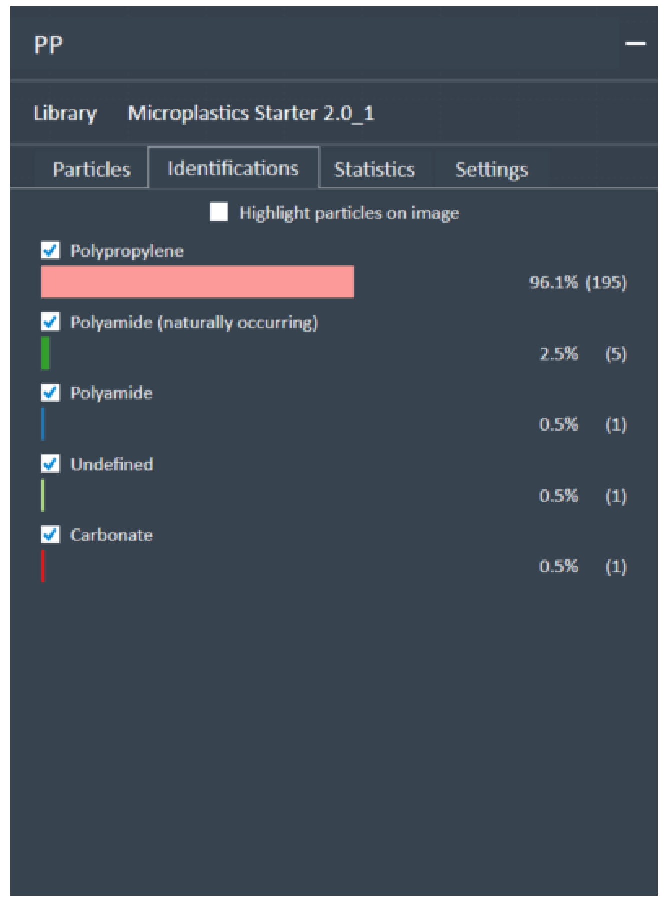
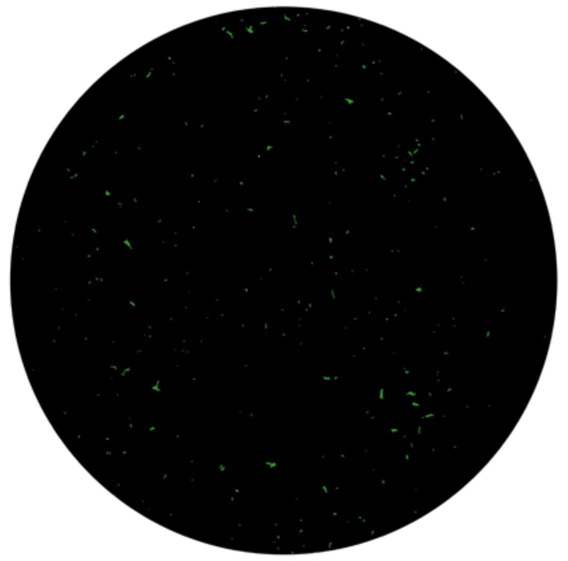
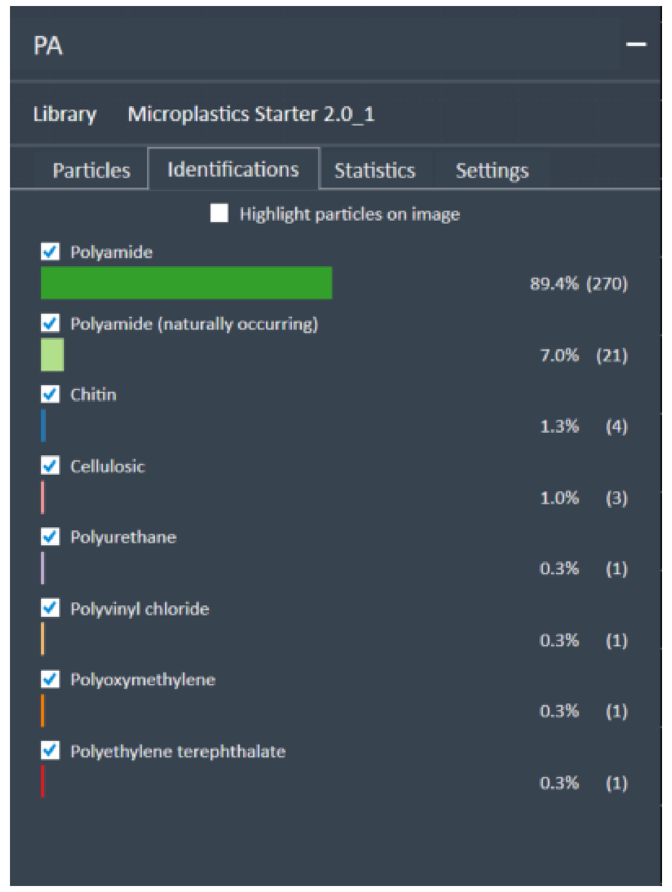
<table>
<tr><th>Type of Polymer</th><th>Infrared Image</th><th>Identification Image</th><th>Statistical Overview</th><th>Quality of the Data</th></tr>
<tr><td>Polypropylene</td><td></td><td></td><td>
</td><td>Total number of particles: 203
> 0.8 HQI: 201 (99%)
< 0.8 HQI: 2 (1%)</td></tr>
<tr><td>Polyamide</td><td></td><td></td><td>
</td><td>Total number of particles: 302
> 0.8 HQI: 298 (98.6%)
< 0.8 HQI: 4 (1.4%)</td></tr>
</table>

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Table 1. Identification of major types of polymers using the Agilent 8700 LDIR Chemical Imaging System (gold-coated filter analysis).

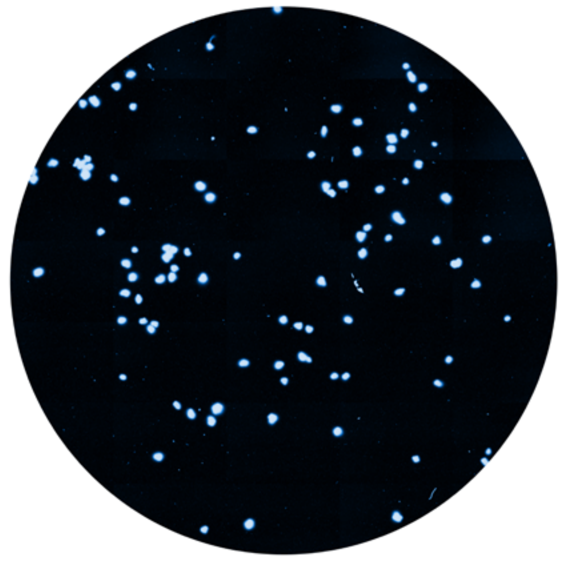
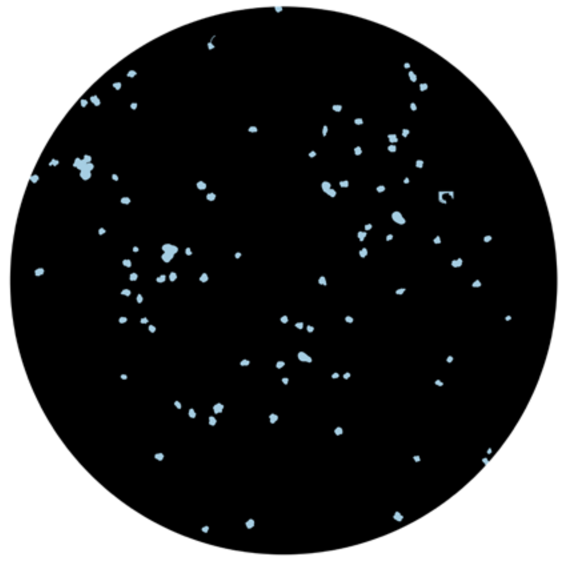
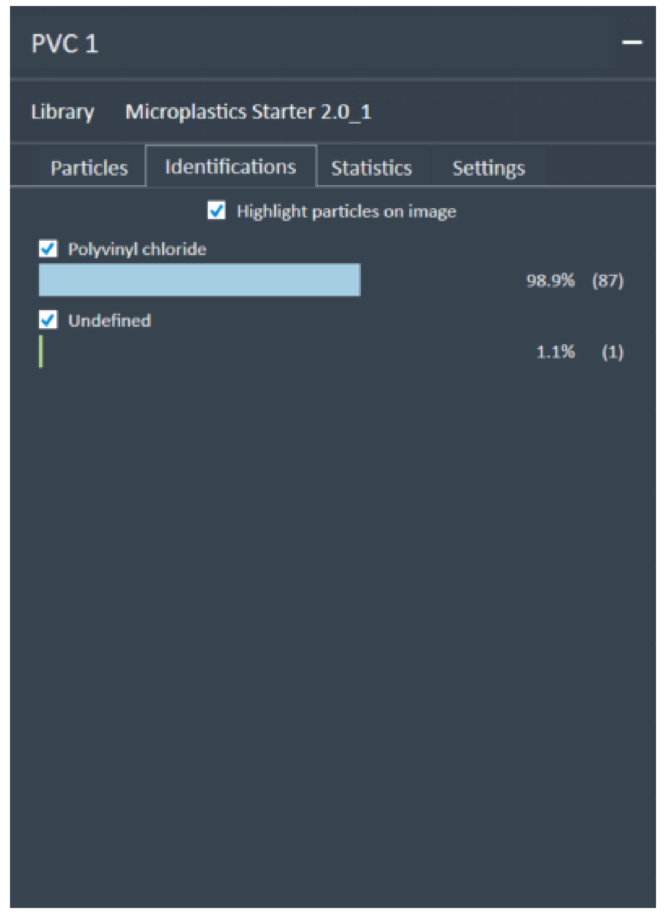
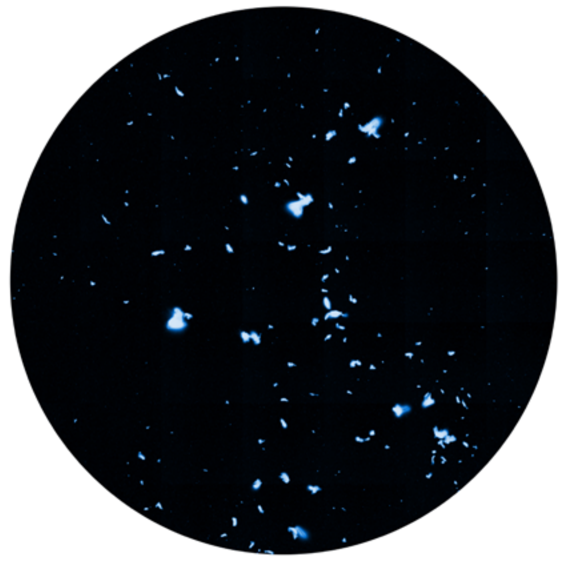
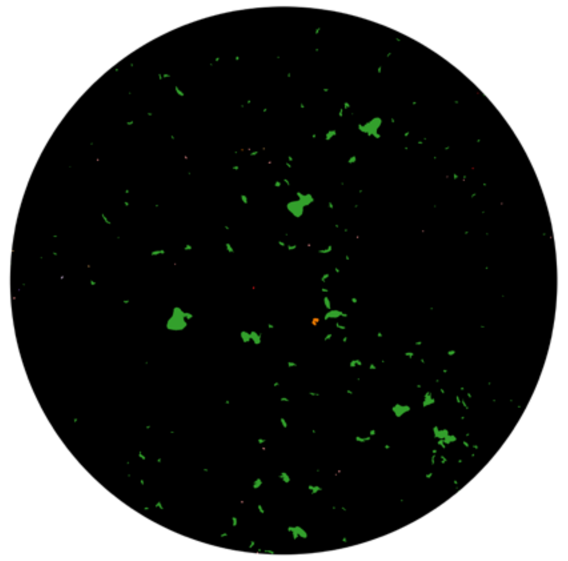
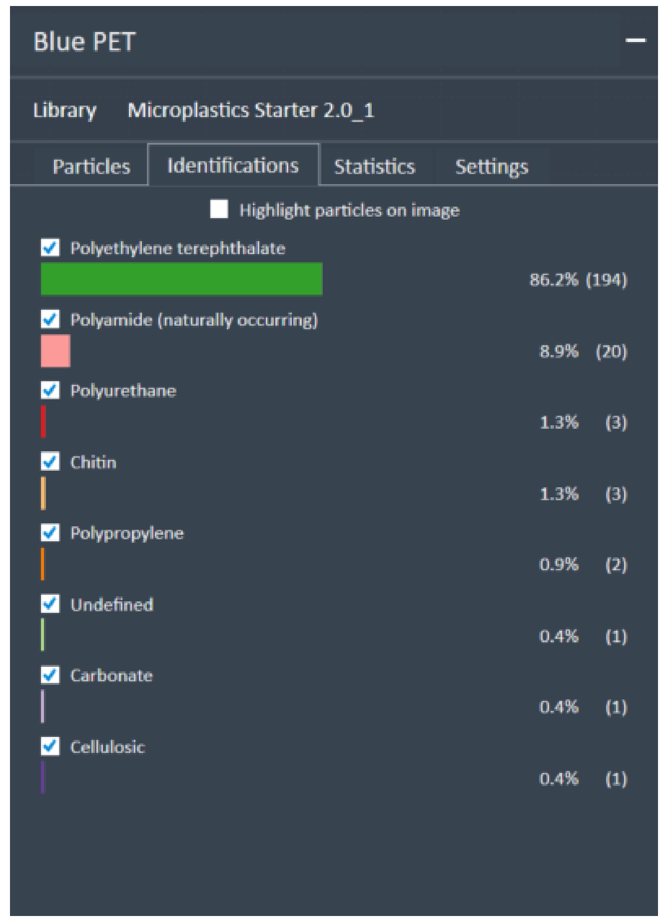
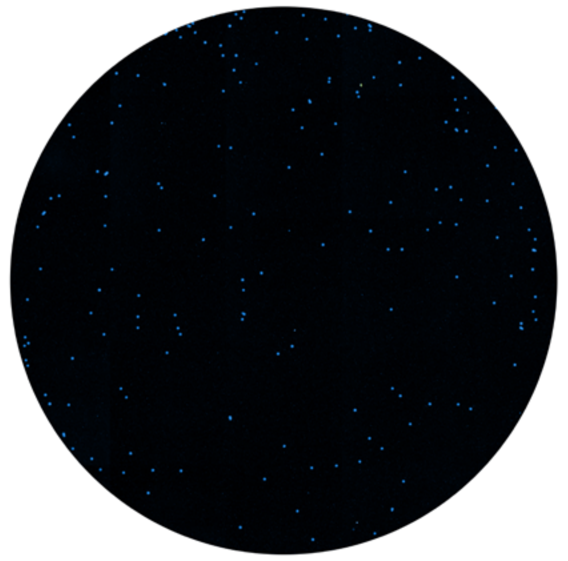
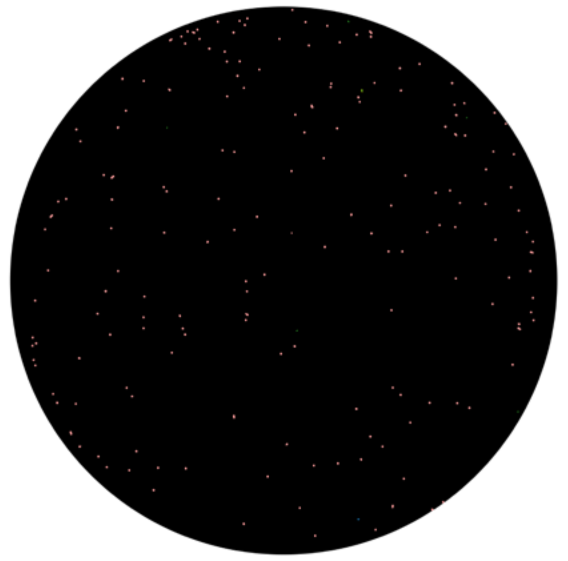
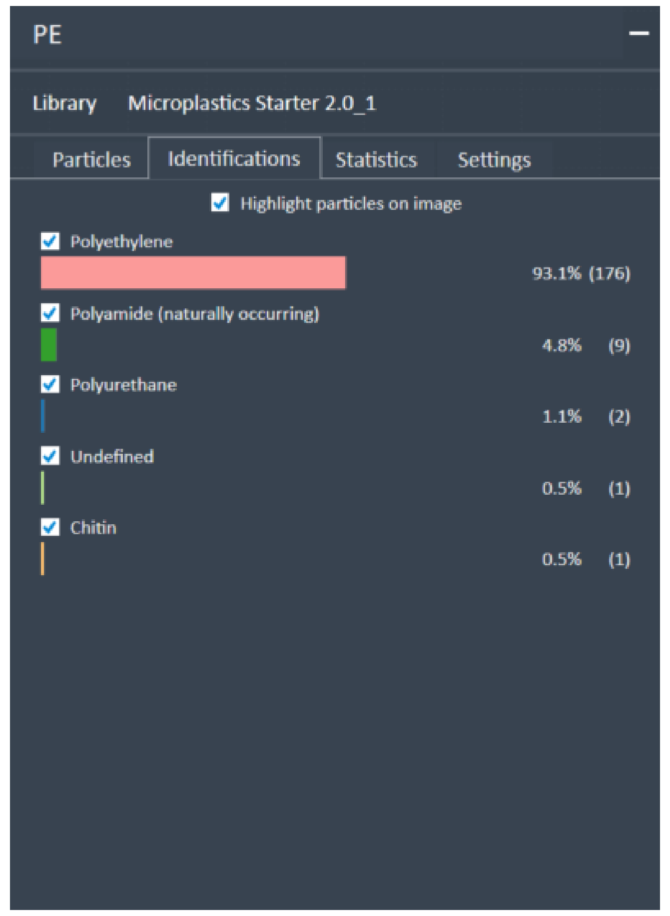
Type of Polymer	Infrared Image	Identification Image	Statistical Overview	Quality of the Data
Polyvinyl chloride				Total number of particles: 88 > 0.8 HQI: 87 (99%) < 0.8 HQI: 1 (1%)
Polyethylene terephthalate				Total number of particles: 225 > 0.8 HQI: 211 (94%) < 0.8 HQI: 14 (6%)

Table 1. Identification of major types of polymers using the Agilent 8700 LDIR Chemical Imaging System (gold-coated filter analysis).

Type of Polymer	Infrared Image	Identification Image	Statistical Overview	Quality of the Data
Polyethylene				Total number of particles: 189 > 0.8 HQI: 185 (98%) < 0.8 HQI: 4 (2%)

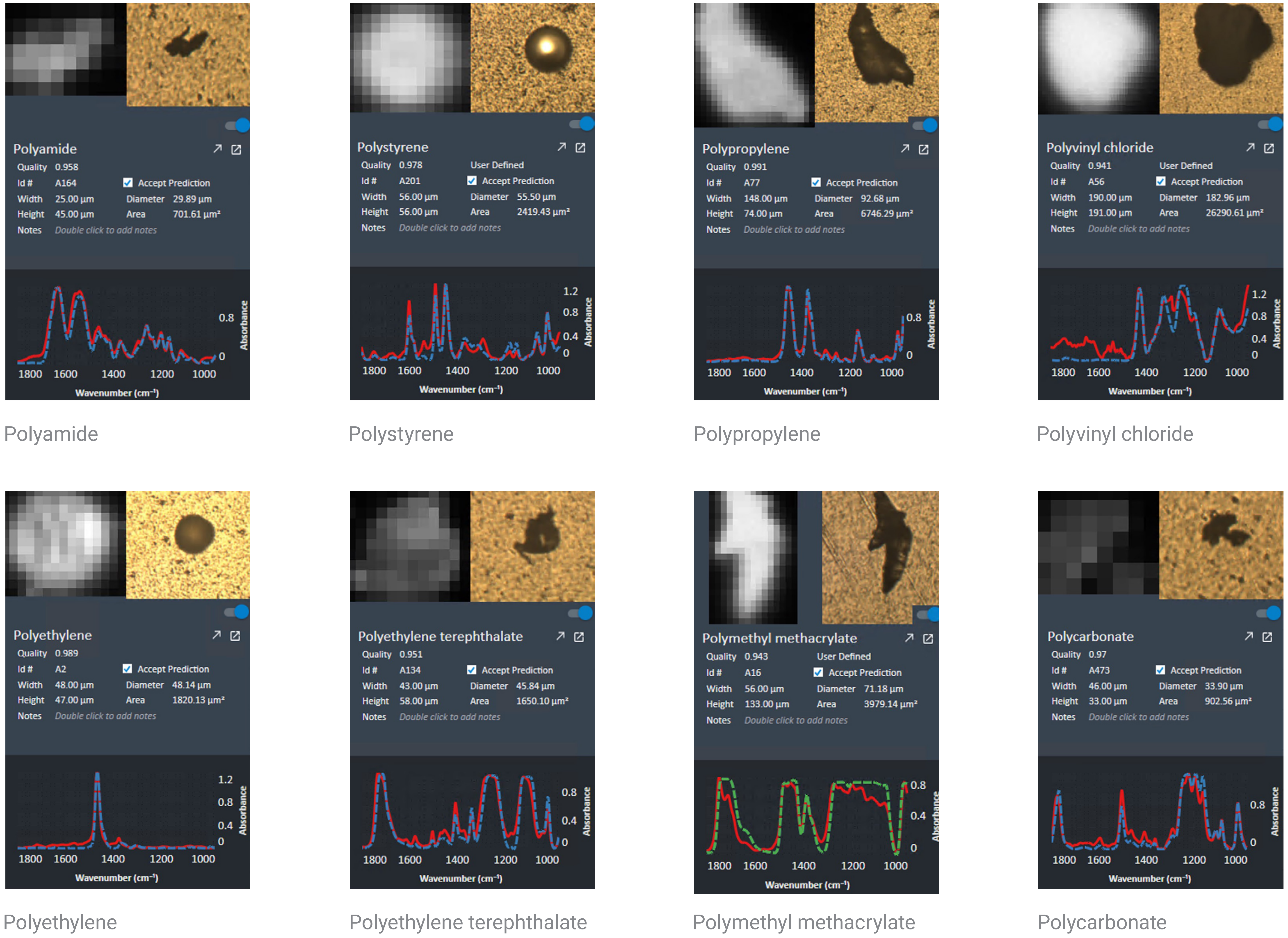


Figure 3. Examples of major types of microplastics polymers analyzed using the automated Particle Analysis workflow for the Agilent 8700 LDIR

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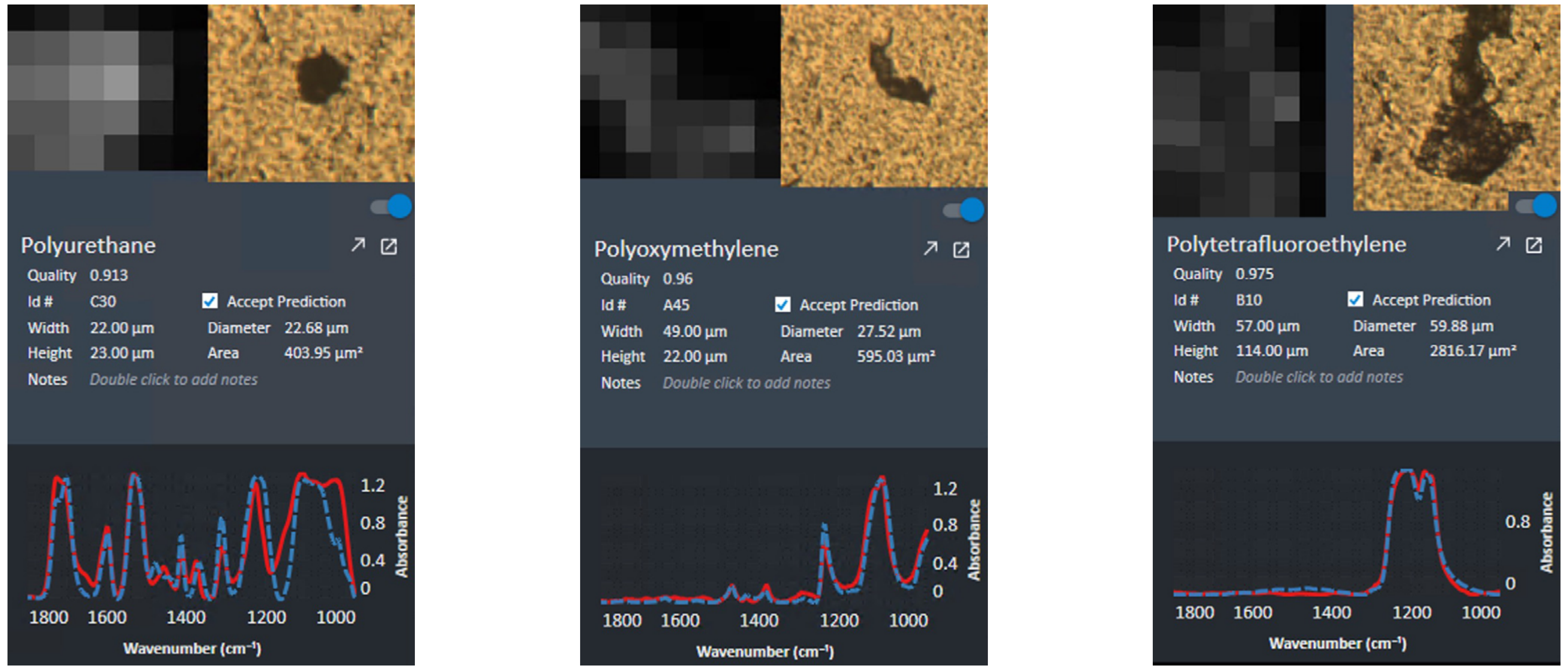

Polyurethane | Polyoxymethylene | Polytetrafluorethylene

Figure 3. Examples of major types of microplastics polymers analyzed using the automated Particle Analysis workflow for the Agilent 8700 LDIR.

Identification of non-microplastics: natural materials and minerals

Materials other than microplastics, e.g., rubber, naturally occurring polyamides, chitin, cellulosic materials, carbonates, and stearates are commonly present in environmental samples and may lead to false identification of microplastics.

To avoid false identification (false positives and negatives) of microplastics and to ensure accurate overall analysis of microplastics, the Clarity software microplastics library contains various spectral data of non-microplastics.

Figure 4 shows a few examples of natural materials that have been detected and identified with HQIs > 0.8 using LDIR on both gold-coated filters and low-e slides

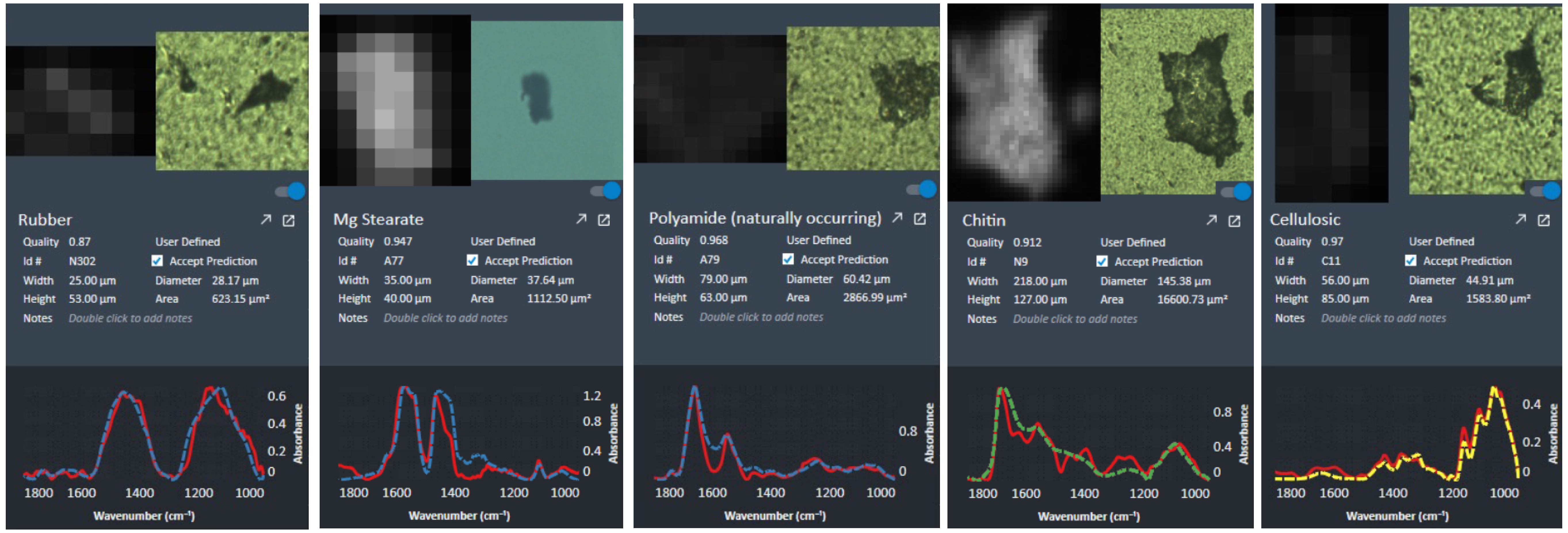

Figure 4. Examples of non-microplastics particles detected and identified with high hit quality index results using the Agilent LDIR Chemical Imaging System.

Particle sizing

It is necessary to check the accuracy of particle sizing data generated by the analytical method used for the analysis of microplastics. Best practice guides require information on the smallest particle size that can be analyzed using the method.

The 8700 LDIR was used to measure NIST traceable 20 µm polystyrene latex beads on a gold filter. As shown in Figure 5, 120 particles were detected with an average size of 23.4 µm (standard deviation = 1.8 µm) was achieved by the 8700 using the automated Particle Analysis workflow. The method also correctly identified the type of particles, with an HQI of > 0.98, demonstrating the characterization capabilities of LDIR for particles down to 20 µm.

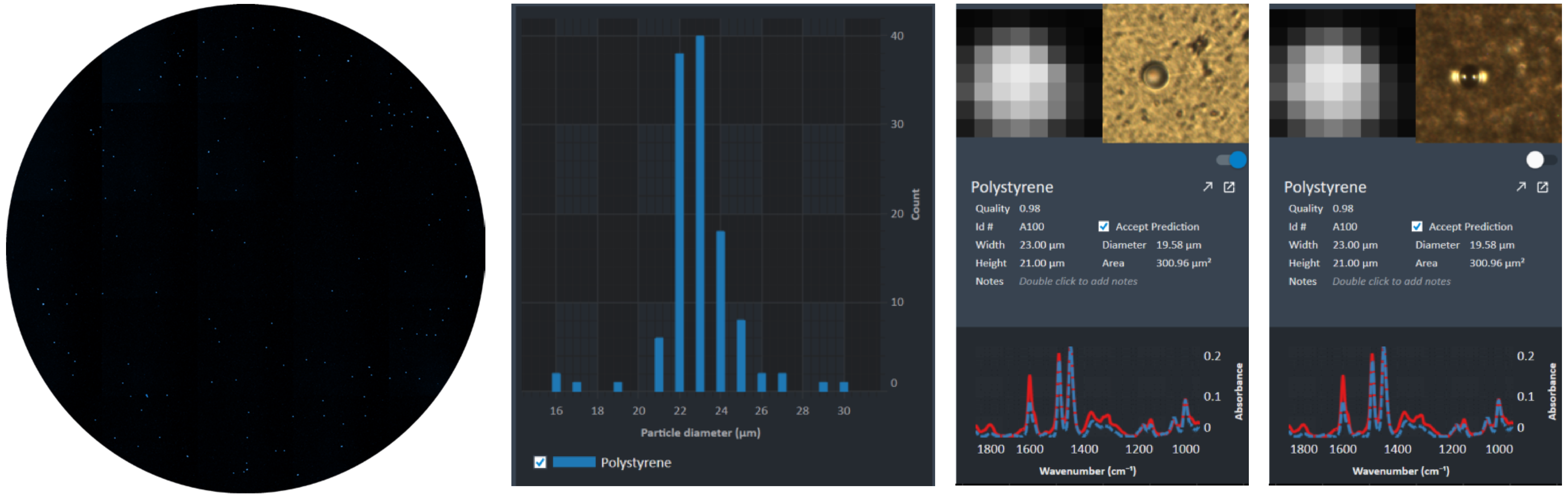

Figure 5. Examples of 20 µm polystyrene beads (NIST traceable) measured using the automated Particle Analysis workflow for the Agilent 8700 LDIR Chemical Imaging System.

Differentiation between microplastics and interferences

The infrared spectral similarity between synthetic and natural polyamide is a potential source of false positive identification. [11] To overcome this challenge, the Clarity software library contains multiple spectral data that can easily differentiate between synthetic and natural polyamide. Figure 6 shows that accurate differentiation was achieved for both polymers (HQIs >0.94) using LDIR.

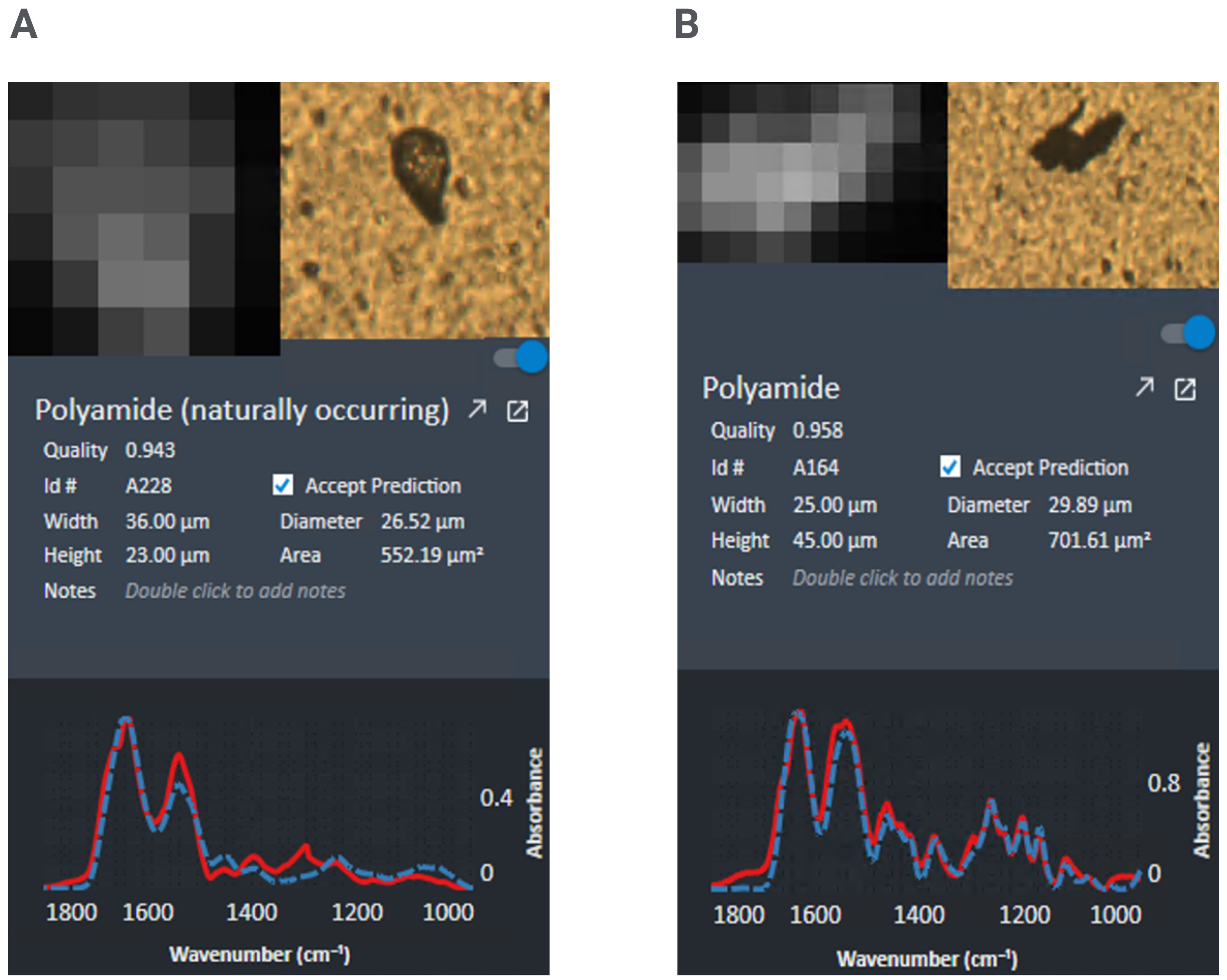

Figure 6. Example of accurate differentiation of microplastics and interferences using the Agilent 8700 LDIR Chemical Imaging System. (A) polyamide (naturally occurring). (B) synthetic polyamide.

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Another interference that could lead to inaccurate microplastic analysis is the similarity in IR spectra of long carbon chains and polyethylene. Clarity software uses first derivative spectral treatment as a matching algorithm, enabling the accurate identification of magnesium stearate and clear differentiation from polyethylene with LDIR (Figure 7). [12]

It is difficult for conventional spectroscopic techniques such as Raman microscopy to classify colored pollutants, which is a key requirement in ecological impact studies. In Raman microscopy, dyes and pigments typically have sharper, higher intensity peaks compared to polymers, which can lead to inaccurate identification of microplastics.[13, 14]

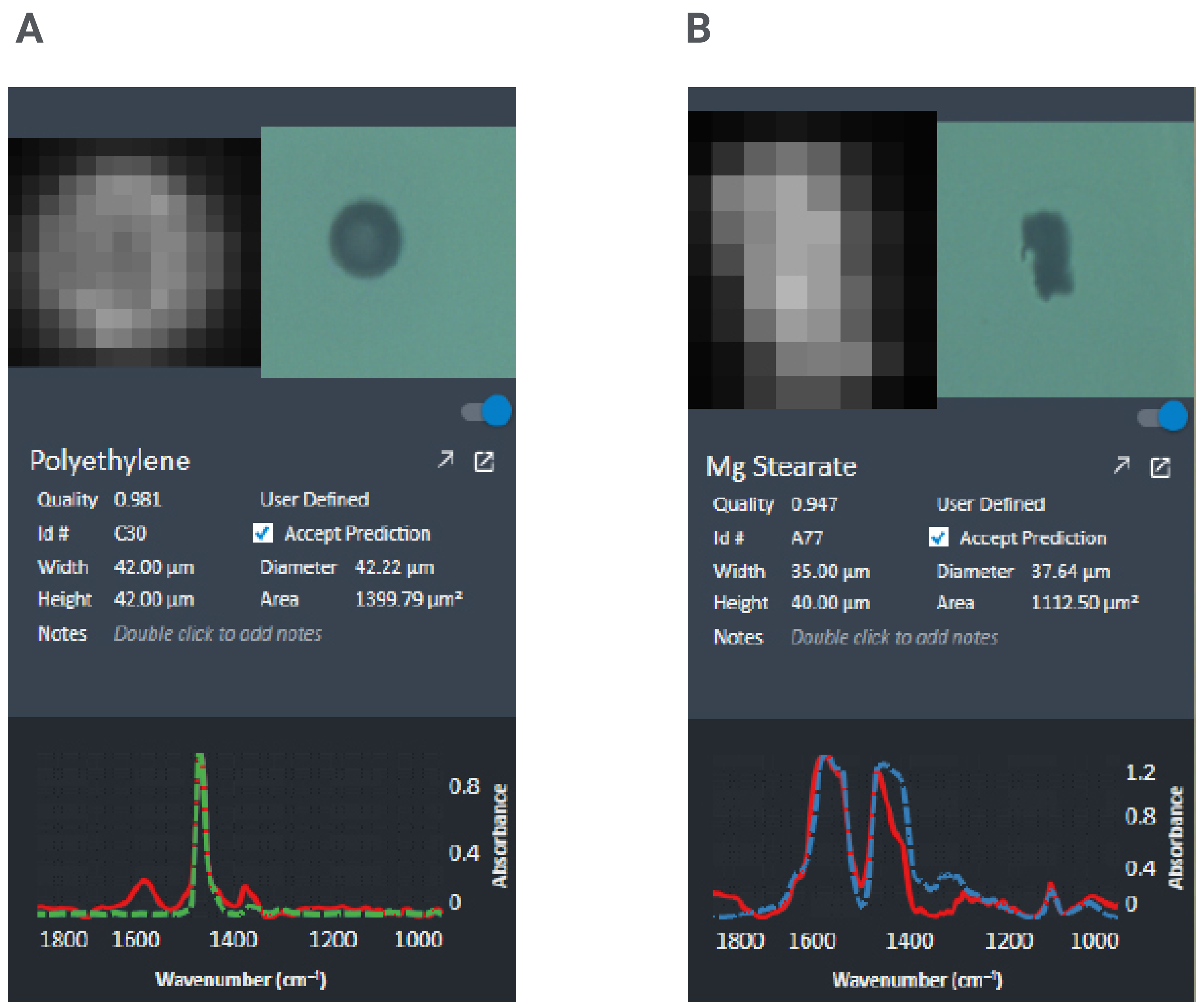

Figure 7. Example of accurate differentiation between microplastics and interferences using the Agilent 8700 LDIR Chemical Imaging System. (A) polyethylene. (B) magnesium stearate.

Since the 8700 LDIR measurements are based on infrared, colorants (dyes and pigments) added to polymers have no effect on the characterization of microplastics. Colored polyethylene terephthalate microplastics were accurately identified (HQIs > 0.9) using the automated microplastics analysis workflow for the 8700 LDIR, as shown in Figure 8.

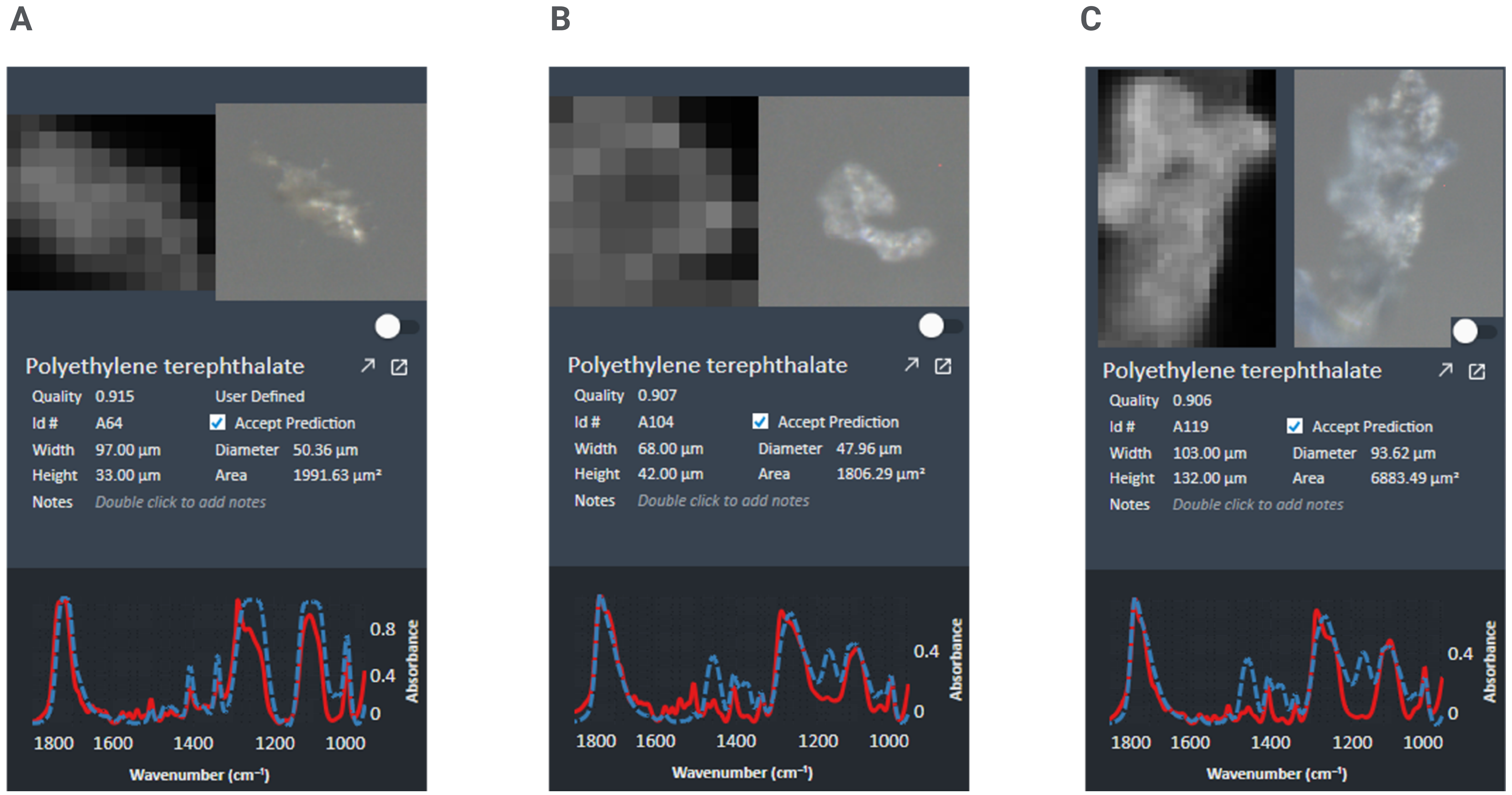

Figure 8. Examples of colored polyethylene terephthalate microplastics analyzed using the Agilent 8700 LDIR. Dyes/pigments had no effect on the accurate identification of particles: (A) Brown PET. (B) White PET. (C) Blue PET.

Measuring high numbers of particles

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.

The 8700 LDIR chemical imaging system with quantum cascade laser (QCL) technology overcomes the limitations of FTIR and Raman microscopy. The 8700 LDIR provides fully automated microplastic particle analysis over large samples in minutes to hours for samples containing thousands of particles, rather than days to weeks. 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.

As shown in Figures 9 and 10, large area and high numbers of particles on both substrates (low-e reflective slide and gold-coated filters) were measured easily and accurately using LDIR.[15]

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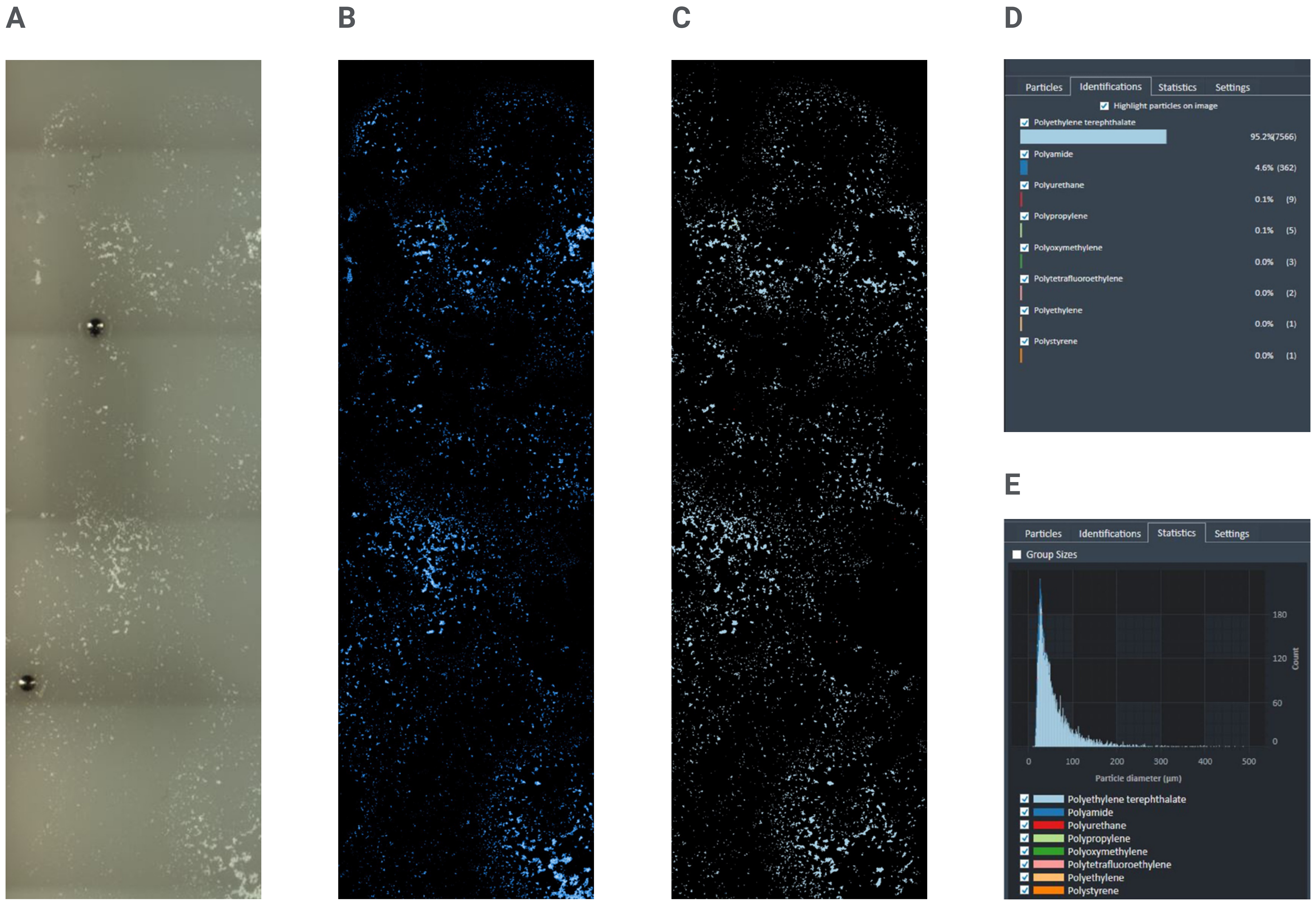

Figure 9. Identification and classification data of microplastics from plastic bottles analyzed directly on an infrared reflective glass slide using the Agilent 8700 LDIR. (A) Visible image. (B) IR image scanned at 1,442 cm^{-1}. (C) Highlights of particles found—the color of the particles is based on the identification of the type of microplastic. (D) Automatically generated statistical data based on the identification of microplastics.(E) Statistical data of microplastic particles based on various size ranges.

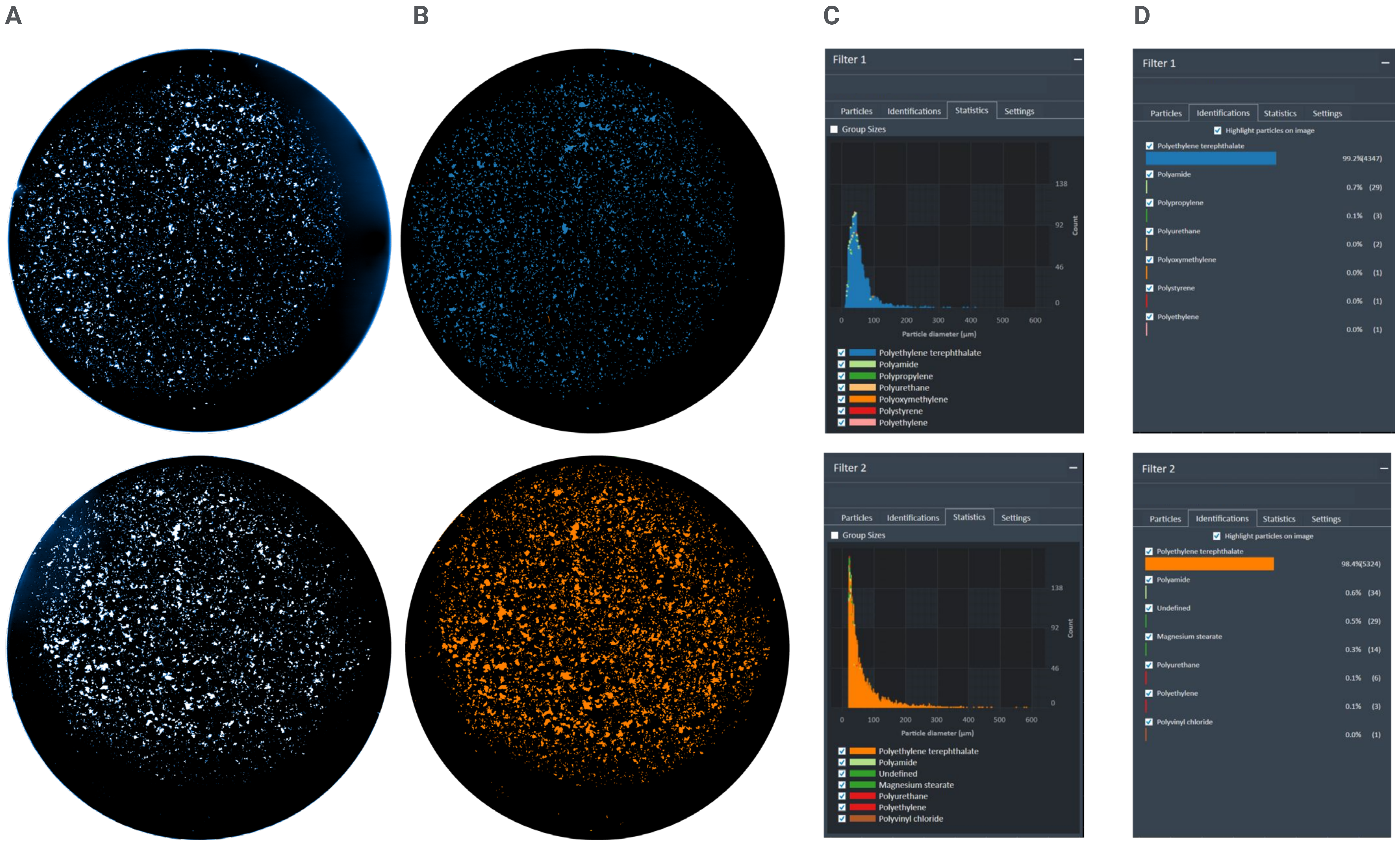

Figure 10. Identification and classification data of microplastics from plastic bottles analyzed directly on gold-coated polyester membrane filters (~16 mm diameter) using an Agilent 8700 LDIR. (A) IR image of both filters scanned at 1442 cm^{-1} of both filters. (B) Highlights of particles found—the particles are colored based on the identification of the type of microplastic of both filters. (C) Statistical data of microplastic particles based on various size ranges of both filters. (D) Automatic statistical data generated based on the identification of microplastics of both filters.

Automated particle analysis workflow

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 975 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 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 (Figure 11). 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 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. Figure 10 shows the sequential analysis of two gold-coated filters with PET microplastics.

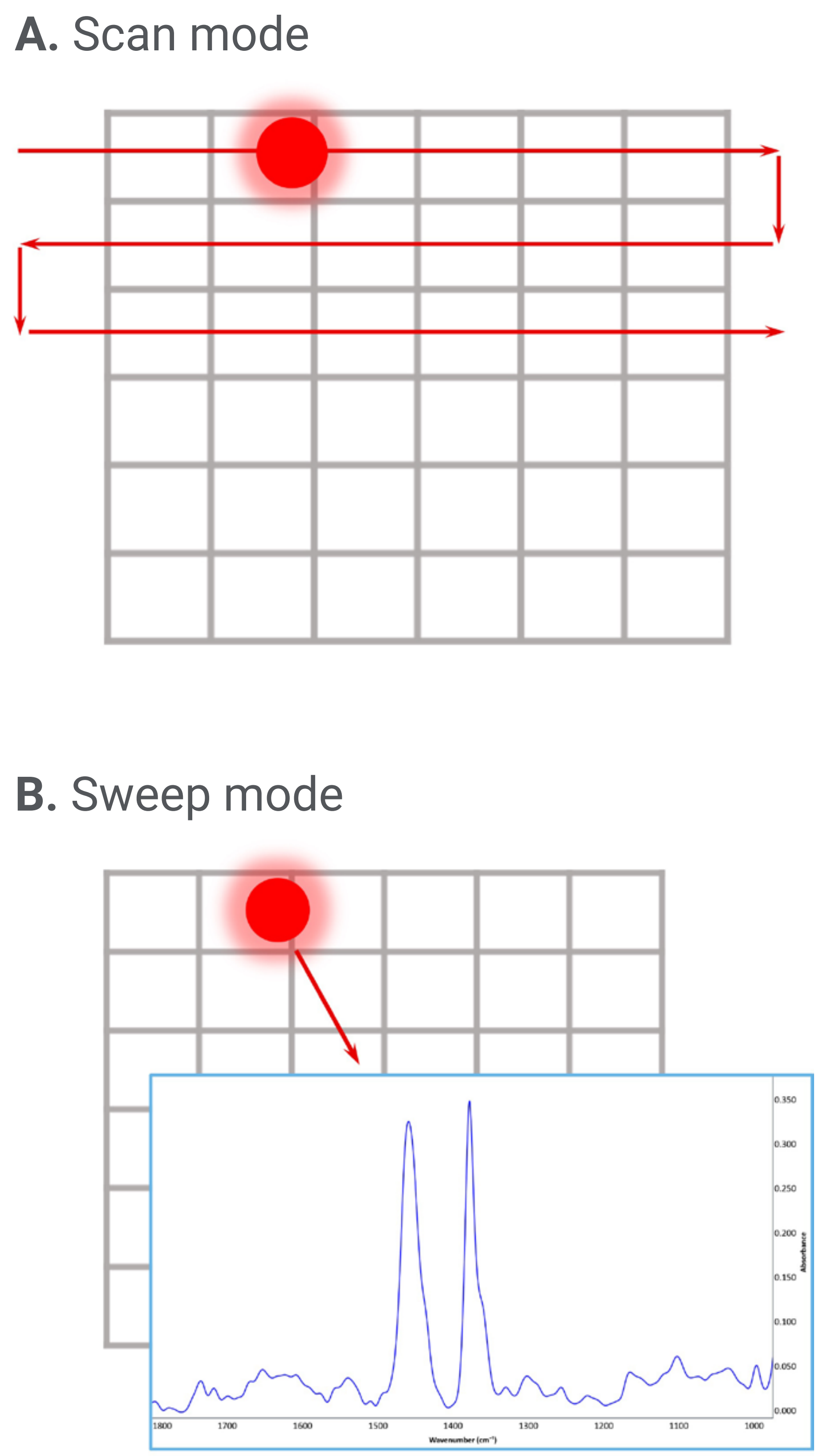

Figure 11. The QCL source of the Agilent 8700 LDIR Chemical Imaging System. can raster rapidly across an entire sample at specific wavenumbers, enabling the instrument to quickly locate microplastic particles anywhere on the sample.

Data processing and reporting

Data processing and reporting are critical steps in the comprehensive analysis of microplastics. The 8700 LDIR and Clarity software provide automatic real-time data processing for microplastics. Once the spectrum of a sample has been collected, the best fit match for each particle in the sample is automatically determined and reported. These automatic features eliminate the need for post processing of spectral data using another program once the last spectrum has been collected.

Following the analysis of a sample containing microplastics using the 8700 LDIR, the Clarity software provides comprehensive information (Figure 12), including:

- Total number of particles detected.
- Total number of particles identified/unidentified.
- Total particle count within each size fraction.
- Type of polymer for each particle identified.
- Statistical overview of the identified particles color-coded based on their identification.
- Size ranges.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	#	Id	Width (µn	Height (µm)	Diameter (µm)	Aspect Ratio	Area (µm²)	Perimeter (µm)	Eccentricity	Circularity	Solidity	Identification	Notes	Match Type	Quality	Is Valid
2	136	A136	32	66	45.22632333	0.483799877	1606.469364	183.4010314	0.875289104	0.600176193	0.907668	Polypropylene		Auto	0.989182309	true
3	1187	N160	30	25	29.04343641	1.2	662.5	95.35533845	0.593723077	0.915599871	1	Polypropylene		Auto	0.988142941	true
4	1457	P9	84	46	44.48404777	1.811320484	1554.169845	464.5337838	0.67340319	0.090505242	0.511458	Polypropylene		Auto	0.986195172	true
5	1058	N31	45	109	66.69425946	0.409728698	3493.548612	295.2764086	0.892176977	0.503522817	0.8869	Polyvinyl chloride		Auto	0.984782141	true
6	1366	N339	15	35	22.56758334	0.428571429	400	88.28427076	0.800696062	0.644916053	0.941176	Polyethylene		Auto	0.984609453	true
7	722	B10	43	84	44.55184594	0.514751476	1558.910878	336.0091002	0.765732289	0.173511823	0.531656	Polypropylene		Auto	0.984072136	true
8	635	A635	20	25	22.21216612	0.8	387.5	75.35533845	0.57691853	0.857538291	0.96875	Polypropylene		Auto	0.983210414	true
9	1039	N12	124	117	114.7050223	1.064931471	10333.67382	474.5188353	0.710734849	0.576710291	0.859654	Polypropylene		Auto	0.982491071	true
10	1033	N6	199	127	140.5186246	1.565273437	15508.06675	630.4570881	0.608798103	0.490293868	0.829895	Polycarbonate		Auto	0.981200019	true
11	1788	Z9	54	43	33.09729244	1.251956148	860.3493127	402.5195673	0.681442654	0.066728396	0.495013	Polypropylene		Auto	0.979425796	true
12	16	A16	162	282	196.3089538	0.57290026	30267.0503	1393.702997	0.831195256	0.195812086	0.803308	Polypropylene		Auto	0.979344044	true
13	385	A385	23	48	27.92595963	0.491803282	612.5	143.6396092	0.847632573	0.373050415	0.662162	Polyethylene terephthalate		Auto	0.976482834	true
14	852	G4	157	217	182.5185397	0.726839294	26163.98264	701.2129183	0.729164188	0.668673173	0.929609	Polypropylene		Auto	0.974727215	true

Figure 12. Screenshot of the report that can be generated at the end of the Clarity Particle Analysis workflow for the Agilent 8700 LDIR Chemical Imaging System.

References

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