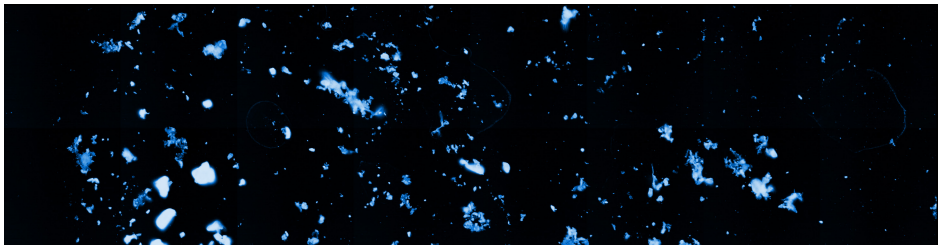
Technical Overview

Optimizing Microplastic Characterization by LDIR: Automated versus Manual Workflows

Evaluation of the Agilent 8700 LDIR's measurement mode on minimum detectable particle size



Introduction

The Agilent 8700 LDIR chemical imaging system with Agilent Clarity control software provides automated and manual workflows for the characterization of microplastics isolated from a wide range of matrices.

The Particle Analysis workflow is specially designed for routine testing, enabling fast, fully automated characterization of particles within a default size range of 20 to 500 µm. This range can be adjusted based on user methodology and analytical requirements. Once a region of interest has been defined on the substrate and a spectral library has been selected in the software, the 8700 LDIR automatically detects particles, acquires infrared (IR) spectra, matches the spectra against the library, and updates results in real time.

The manual analysis workflow allows users to adjust parameters, acquire spectra, and collect high-magnification images of particles as small as a few micrometers, providing greater flexibility for method development and research applications.

This technical overview evaluates the performance of both types of workflow using NIST-traceable polystyrene (PS) latex beads of 2, 5, and 10 µm diameter. Measurements were performed on both low-E slides and aluminum-coated (Al-coated) filters (0.8 µm pore size) to assess the influence of the substrate background on the minimum measurable particle size.

Experimental

Substrates

Low-E infrared (IR) reflective slide (25 × 75 mm, Agilent part number M7300-68010) and Al-coated polyester filters (25 mm, 100/0 nm coating, 0.8 µm pore size, Agilent part number M7300-68011).

Sample preparation

NIST-traceable polystyrene (PS) latex bead suspensions containing 2, 5, and 10 µm particles in 15 mL bottles were bought from Applied Physics USA (Alamosa, Colorado, USA). Before use, the suspensions were shaken thoroughly, and the first drop was discarded to ensure consistency. A total of six samples were prepared as follows:

- **2 µm beads:** One drop of the NIST PS stock suspension was diluted in 10 mL ethanol (EtOH). From this dilution, 3 × 10 µL were applied to a low-E slide and 1 × 10 µL to an Al-coated filter.
- **5 µm beads:** One drop of the stock suspension was diluted in 5 mL EtOH. From this dilution, 3 × 10 µL were applied to a low-E slide and 1 × 10 µL to an Al-coated filter.
- **10 µm beads:** Two drops of the stock suspension were diluted in 5 mL EtOH. From this dilution, 3 × 10 µL were applied to a low-E slide and 1 × 10 µL to an Al-coated filter.

Workflow

The manual analysis was performed following the steps described in Figure 1.

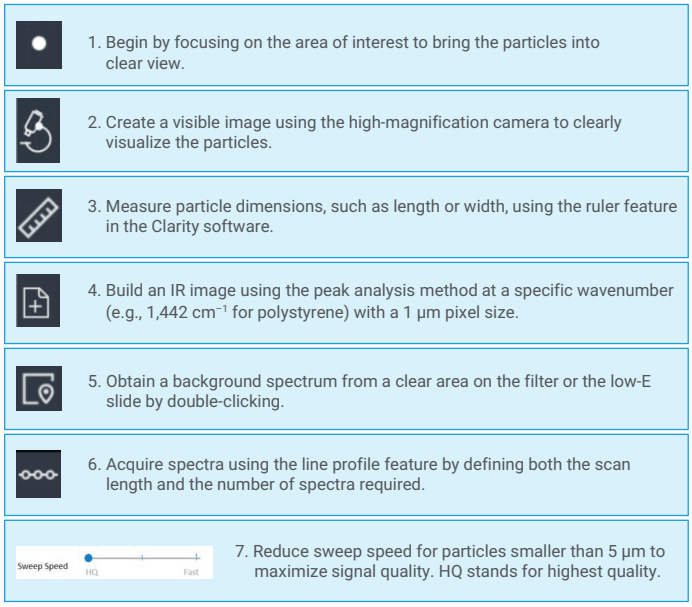


Figure 1. Manual Analysis Workflow. The icons are located in the main menu of the Agilent Clarity software. IR image resolution can be adjusted to pixel sizes of 1, 3, 5, 10, or 20 µm.

The automated Particle Analysis workflow was performed using the parameters listed in Table 1.

Table 1. Parameters used for the Agilent 8700 LDIR chemical imaging system automated method analysis of microplastics.

Parameter	Setting
Method	Particle Analysis
Library Used	Microplastics Starter 2.1
Collect Visible Images	Yes
Particle Sensitivity	Automatic
Hit Quality Index (HQI) Ranges	Hit quality describes how closely the spectrum of the sample matches that in the reference library. For this experiment, classification ranges (i.e., the characterization of spectral match quality by "high," "medium," and "low") were set to: – Low confidence (0.65 to 0.75) – Medium confidence (0.75 to 0.85) – High confidence (0.85 to 0.99) Any particles falling outside this range (i.e., < 0.65) were classified as "undefined"
Minimum Size (µm)	Adjusted to the respective PS beads size (i.e., 5 and 10 µm).

Results and discussion

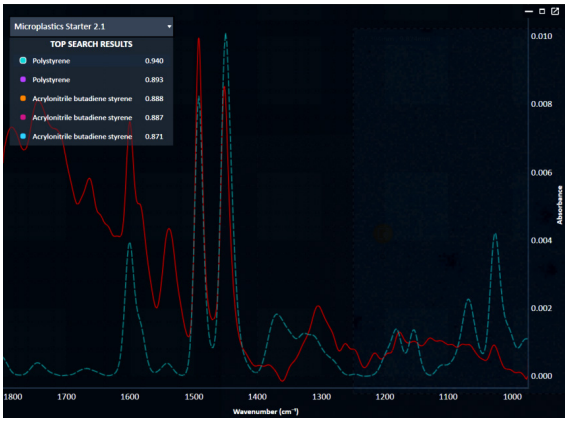
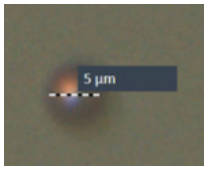
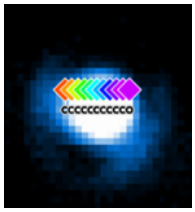
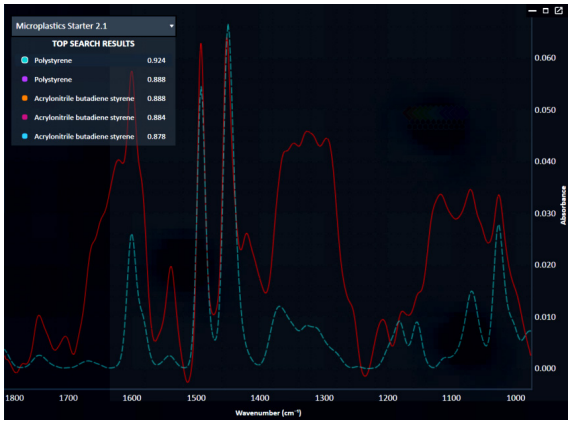
Manual workflow

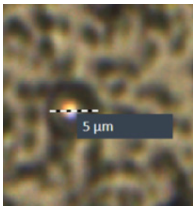
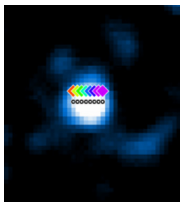
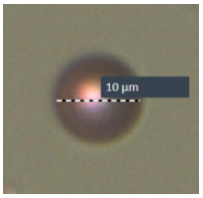
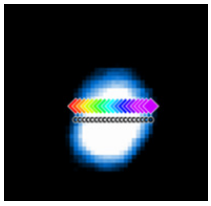
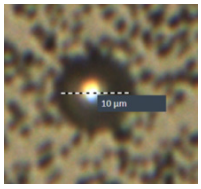
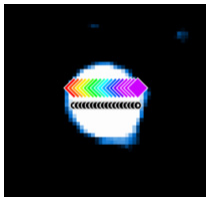
2 µm PS beads: High-magnification visible images were obtained for the 2 µm PS beads on the low-E slide by focusing on individual particles and using the ruler feature in the Clarity software to measure the length (Table 2). IR images were generated at 1,442 cm^{-1} with a pixel size resolution of 1 µm, resolving the 2 µm beads. Using the line profile feature and a reduced acquisition (sweep) speed, 20 spectra were acquired. The averaged spectrum (automatically obtained after data collection) was compared to reference spectra in the Microplastics Starter 2.1 library. The particle was correctly identified as polystyrene with a hit quality index (HQI) of 0.940. The 2 µm particles were successfully detected and identified on low-E slides only. Background interference from the 0.8 µm pores of the Al-coated filters prevented data collection from the 2 µm particles.

5 µm PS beads: High-magnification visible and IR images were successfully obtained for the 5 µm PS beads on both types of substrate. The spectra were collected in line profile mode using a faster data acquisition rate than for the smaller particles. The beads were accurately identified as PS with HQIs of 0.924 and 0.947 for the low-E slide and Al-filter, respectively (Table 2).

10 µm PS beads: The 10 µm PS beads were easily imaged and identified on both substrates with improved HQIs of 0.972 for the low-E slide and 0.968 for the Al-coated filter (Table 2).

Table 2. Agilent 8700 LDIR chemical imaging system's manual workflow results for detection and identification of 2, 5, and 10 µm PS beads on low-E slides and Al-coated filters.

PS Bead Size (µm)	Substrate	High-Magnification Visible Image	IR Image at 1,442 cm^{-1}	Identification
2	Low-E slide			
5	Low-E slide			

PS Bead Size (µm)	Substrate	High-Magnification Visible Image	IR Image at 1,442 cm^{-1}	Identification
5	Al-coated filter			
10	Low-E slide			
10	Al-coated filter			

Automated workflow

2 µm PS beads: Because the automated Particle Analysis workflow uses a fixed IR image pixel resolution of 5 µm, it is not possible to detect 2 µm particles in this mode. The 5 µm pixel resolution setting is optimized to enable rapid microplastic analysis and minimize overall processing time. Increasing the pixel resolution would enhance the detection of smaller particles, but it would also significantly increase the time required for particle detection.

5 µm PS beads: Although the automated method is at the lower limit of the Particle Analysis workflow's resolution for the analysis of 5 µm particles, most particles are detectable by the 8700 LDIR. However, for identification purposes, any results for particles with a low HQI should be further validated.

In this study, automated detection of 5 µm PS beads was successfully achieved on the low-E slide and Al-coated filter.

Out of a total of 1,083 particles that were detected on the low-E slide, 506 particles were identified as PS with 124 particles (high confidence), 142 particles (medium confidence), 136 (low confidence), and 104 particles (undefined) below the confidence threshold.

A total of 693 particles were detected on the Al-coated filter (Figure 2). The majority of particles (377) were identified as PS with 199 particles (high confidence), 102 particles (medium confidence), and 76 (low confidence). A further 11 particles (undefined) were also identified as PS but with an HQI below the confidence threshold.

Examples of 5 µm PS particles identified on both substrates using the automated workflow are shown in Figure 3.

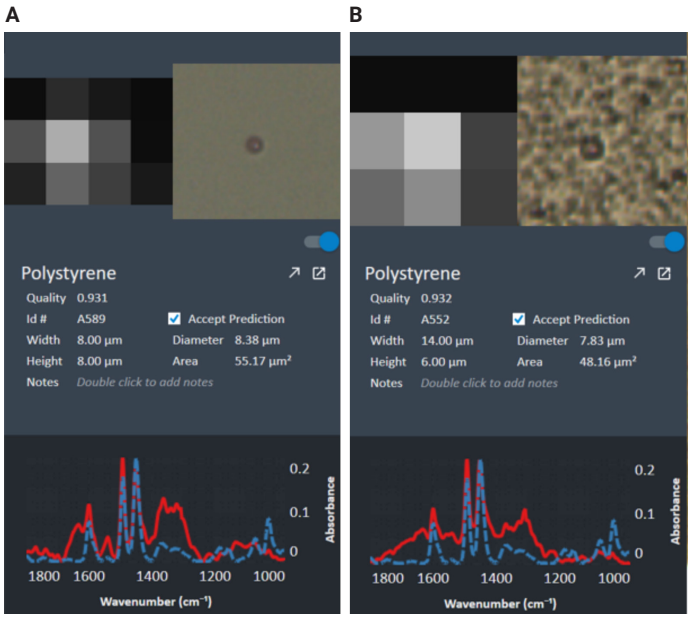


Figure 3. Examples of data acquired for 5 µm PS beads on a low-E slide (A) and Al-coated filter (B) using the Agilent 8700 LDIR chemical imaging system's automated Particle Analysis workflow.

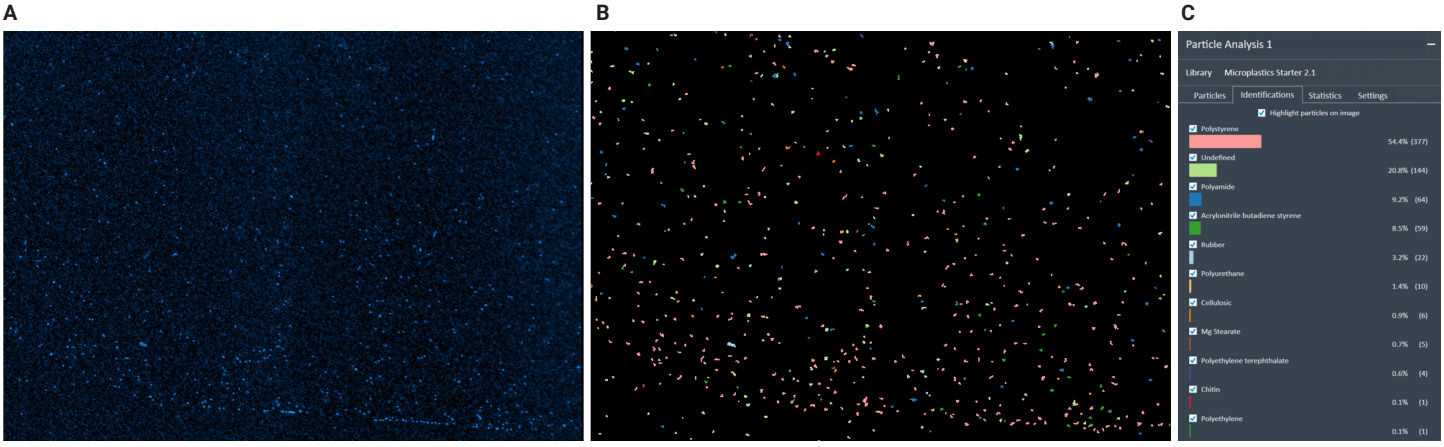


Figure 2. Identification and classification data for 5 µm PS beads analyzed on an Al-coated filter using the Agilent 8700 LDIR chemical imaging system's automated Particle Analysis workflow. (A) IR image scanned at 1,442 cm^{-1}. (B) Highlights of particles found—the particles are colored based on the identification of the type of microplastics. (C) Automatic statistical data generated based on the identification of microplastics in the 5 µm PS bead sample.

10 µm PS beads: The automated workflow delivered excellent performance on both substrates for the 10 µm PS bead samples, as the particle size exceeds the IR image pixel resolution of 5 µm. On the low-E slide (Figure 4), 654 particles were detected, with 640 identified with high confidence as PS (HQI > 0.91). On the Al-coated filter (Figure 5), 389 particles were detected, of which 366 were identified as PS with high confidence (HQI > 0.85).

Across both substrates, single-particle analysis demonstrated good size accuracy, with an average particle size of 12 µm (SD = 2 µm) from approximately 200 distinct, non-overlapping particles analyzed per substrate.

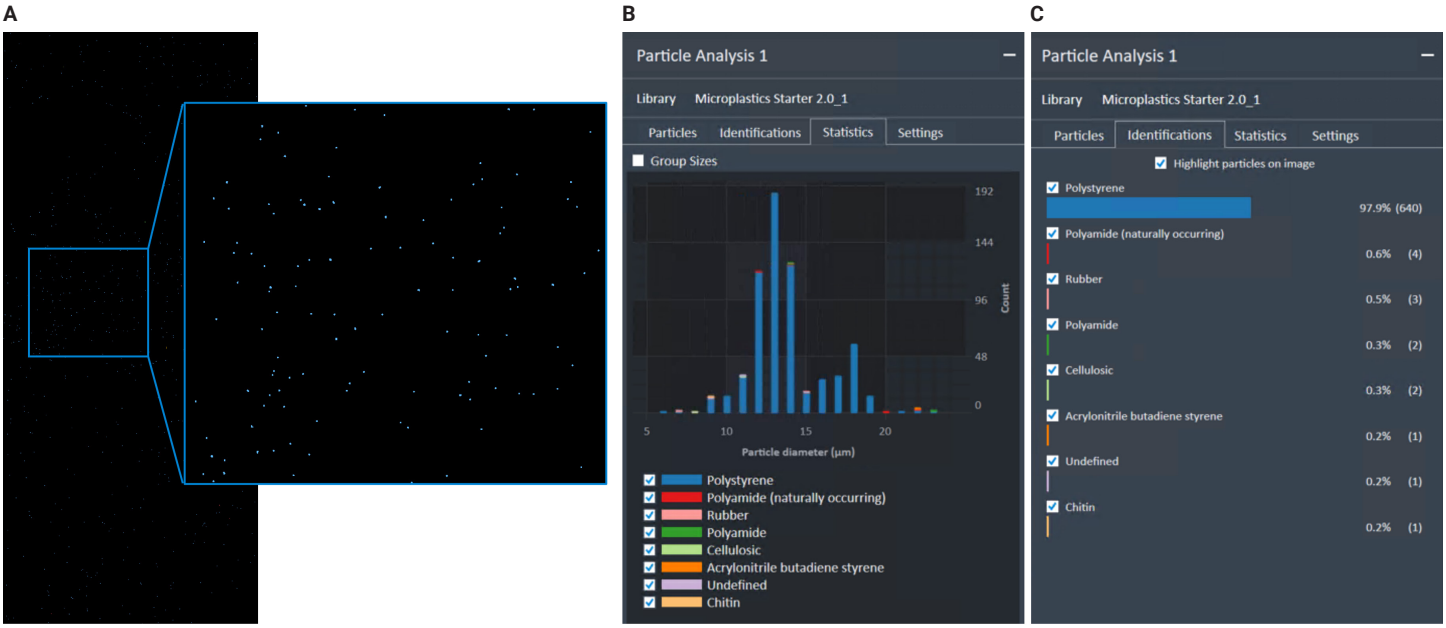


Figure 4. Identification and classification data for 10 µm PS beads analyzed on a low-E IR slide using the Agilent 8700 LDIR chemical imaging system's automated Particle Analysis workflow. (A) Highlights of particles found—the particles are colored based on the identification of the type of microplastics. (B) Automatic statistical data generated based on the particle size (µm), count, and identification of microplastics in the 10 µm PS bead sample. (C) Automatic statistical data generated based on the identification of microplastics.

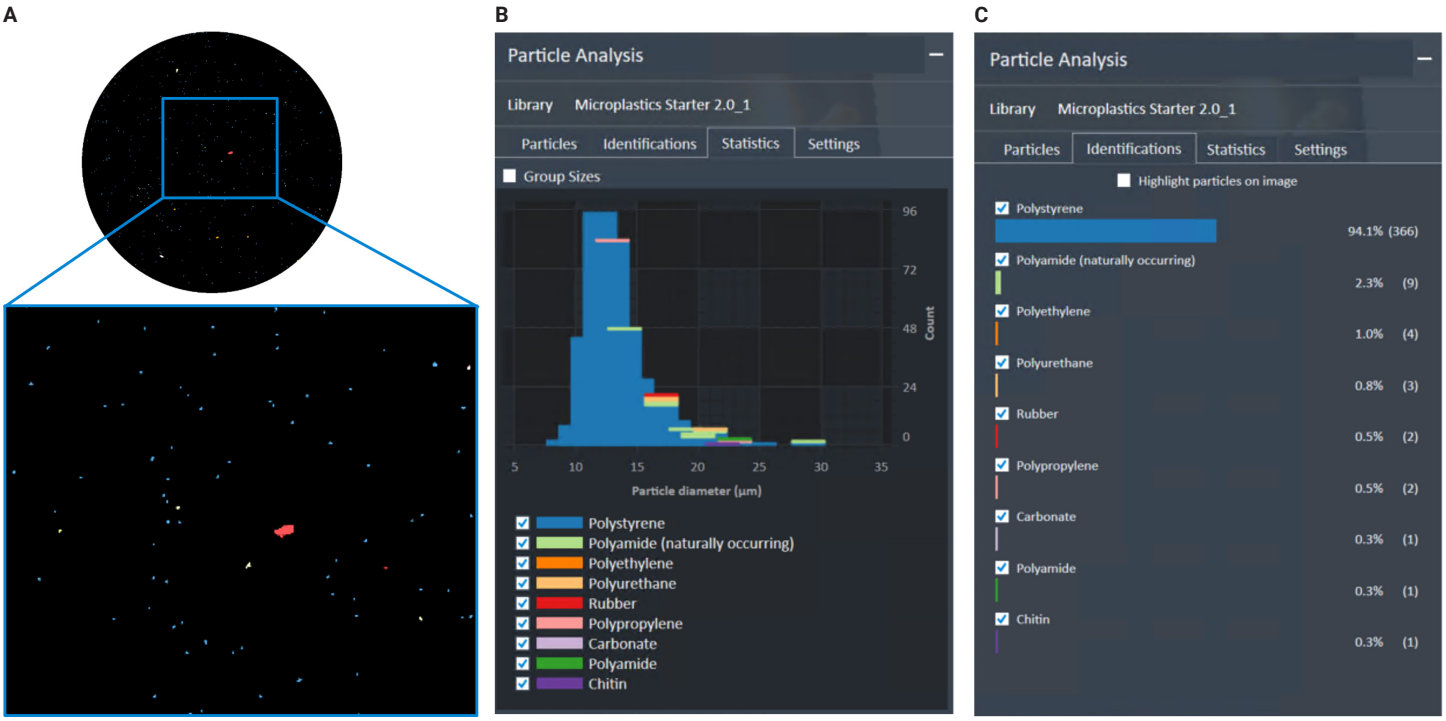


Figure 5. Identification and classification data for 10 µm PS beads analyzed on an Al-coated filter using the Agilent 8700 LDIR chemical imaging system's automated Particle Analysis workflow. (A) Highlights of particles found—the particles are colored based on the identification of the type of microplastics. (B) Automatic statistical data generated based on the particle size, count, and identification of microplastics in the 10 µm PS bead sample. (C) Automatic statistical data generated based on the identification of microplastics.

Table 3 shows a comparison of the manual and automated workflows for the detection of 2, 5, and 10 µm PS beads. Manual analysis enables the characterization of particles as small as 2 µm on low-E slides, while the automated workflow provides robust performance for particles ≥ 10 µm. The performance of the gold-coated filter is equivalent to that of the Al-coated filter.

Table 3. Comparative summary of manual and automated modes of measurement for the characterization of microplastics using the Agilent 8700 LDIR chemical imaging system.

Mode	2 µm PS Beads	5 µm PS Beads	10 µm PS Beads
Manual Analysis	Detection and identification on low-E slides only.	Detection and identification on both low-E slides and filters.	Detection and identification on both low-E slides and filters.
Automated Particle Analysis	Not possible (below 5 µm IR image pixel resolution).	Most particles can be detected. Identification results with low HQI should be checked manually.	Reliable detection and identification on both substrates.

Conclusion

The Agilent 8700 LDIR chemical imaging system is capable of analyzing microplastics as small as 2 µm on a low-E slide using a manual workflow and 5 µm and above using the automated Particle Analysis method. However, the automated workflow provided the most reliable identification and highest-confidence library matching results for particles ≥ 10 µm.

Together, the two workflows offer complementary capabilities—automation for the rapid, routine analysis of larger particles and manual control for smaller particle characterization and research applications.

Further information

- Agilent 8700 LDIR chemical imaging system
- Agilent Clarity Software
- Microplastics Technologies FAQs
- Microplastics Analysis in Water

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