White Paper

Environmental

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Best Practice for On-Filter Analysis of Microplastics Using the Agilent 8700 Laser Direct Infrared (LDIR) Chemical Imaging System

Introduction

Everyday plastic items can degrade into small fragments and particles through use, wear, weathering, or improper disposal of waste. Plastic particles that range in size from 1 to 5 mm in diameter are often referred to as microplastics. These small-sized plastic particles are ubiquitous substances in the environment and are considered as emerging contaminants by the World Health Organization.[1] More research is needed into the impacts of microparticles on human health, especially as microplastics have been found in drinking water, wastewater, and foods.[2-5]

To understand the behavior of microplastics in the environment and in food chains, accurate methods are needed that can identify, characterize, and quantify different polymers.[6] Efforts are being made to standardize microplastic analysis methods around the globe by performing interlaboratory studies (ILS). So far, however, the results of ILS have shown significant variation, due in part to differences in methodology, including inconsistent use of quality assurance procedures.[7,8]

To achieve accurate and reproducible analysis of microplastics, certain practical aspects should be considered. This white paper describes the best practices for performing accurate on-filter microplastic analysis using the Agilent 8700 LDIR Chemical Imaging System (Figure 1).

Before filtration: minimizing sample contamination

To ensure accurate microplastic analysis, potential contamination from the laboratory environment should be controlled.[8] This section describes multiple sources of contamination (e.g., air quality, personal protective equipment, and glassware) and how to minimize contamination using practical steps.

- The airflow in the lab must be controlled to maximize air purity and minimize the levels of airborne contaminants.
- Sample preparation/filtration should be performed in a laminar workflow (fume hood).
- Lab coats made from natural materials (e.g., cotton) or particle-free materials are recommended, so contaminants (such as threads) originating from lab coats are easy to identify. A lint roller can be used to remove small fibers from clothing, minimizing contamination.
- Disposable laboratory gloves are a potential source of contamination (e.g. stearates in latex gloves) and should not be worn unless needed for safety purposes.
- Analysts can minimize contamination by thoroughly washing their hands, avoiding the use of skin creams and make up, and by containing hair.
- Before setting up the vacuum filtration apparatus, such as the Sigma-Aldrich vacuum filtration assembly, all glassware and the fume hood must be thoroughly cleaned. Use high-purity water or high-purity ethanol (EtOH), according to the lab's cleaning protocols.
- The quality of water used to clean, rinse equipment, or for sample preparation should be checked by analyzing blanks to estimate or characterize the level of water contamination. Contaminants include microplastics and non-microplastics such as cellulose and naturally occurring polyamides (animal and plant fibers).

Figure 1. Agilent 8700 LDIR Chemical Imaging System with monitor showing a screenshot from the Agilent Clarity software Particle Analysis workflow.

During filtration: assuring sample flatness and best handling of filters

Best practices are described for sample filtration, filter handling, and the use of filter holders to achieve accurate microplastic analysis, especially particle detection.

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

1. Samples should be kept in clean glass containers. Plastic containers and pipettes should be avoided.
2. Glassware should be cleaned thoroughly with high-purity water and covered with aluminum foil to avoid contamination from the lab environment.
3. Filters (Figure 2) should be kept in a clean container to reduce the risk of contamination from the lab environment. The filter box should be opened only when the filter is needed. Examples of the specifications of filters used for LDIR analysis are available from Sterlitech:
4. Gold-coated filters are delicate, so careful handling of the filters is required. Use the tweezers, which are supplied, to transfer the filters onto the filtration stem (Figure 2). Do not use damaged filters and do not reuse filters.

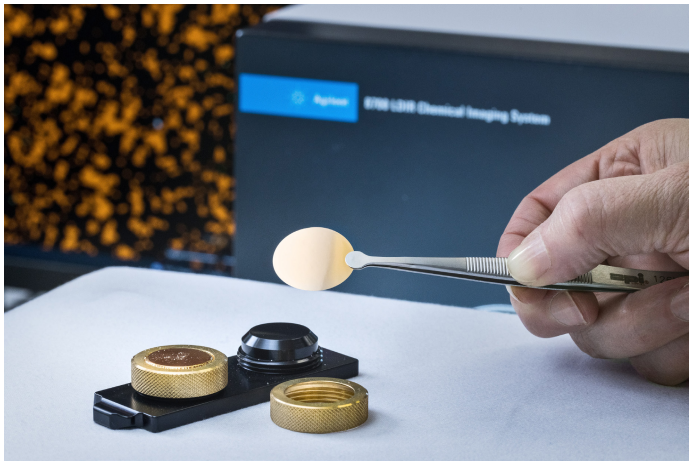

Figure 2. LDIR filter holder and polyester gold-coated membrane filter (PETG), 0.8 µm pore size, 100/0 nm (top/down) coating, 25 mm diameter.

5. To avoid damaging or deforming the filter, the following steps are recommended:
   a. Place the filter onto the filtration stem supporting material (Figure 3, image 1).
   b. Switch on the vacuum and adjust to a gentle vacuum pressure of 700 mbar (–30 kPa).
   c. Finally, place the funnel and secure the vacuum filtration assembly with the clamp (Figure 3, images 2 and 3). Alternatively, the gold filters can be soaked in high-purity EtOH, then transferred to the vacuum filtration apparatus before applying vacuum pressure.
6. The filtration stem supporting material is coarse (fritted glass). To avoid trapping silica particles underneath the filter, wipe the surface with EtOH before use. Alternatively, cellulose pads can be used as a support surface for the filter. Stems made from smoother materials can also be used (e.g., stainless steel support screens or PTFE gaskets). Examples of filter support materials are available from Sterlitech.
7. Once the filtration of the sample has finished (Figure 3, image 4), remove the funnel, switch off the vacuum, and let the filter dry at room temperature for ~2 minutes.
8. After the filter has dried sufficiently, it can be transferred to the filter holder, using the following steps:
   a. Keep the filter holder next to the vacuum filtration apparatus (Figure 3, image 5).
   b. Remove the brass retaining ring from the filter holder.
   c. Clean the raised platform of the filter holder with EtOH to remove any particles present on the stage that may create a hump in the filter, affecting the flatness of the sample.
   d. Carefully remove the filter from the glassware using tweezers and gently place the filter on top of the raised platform. The filter can be centered using the tweezers.
   e. Slowly thread the brass retaining ring back onto the holder (Figure 3, image 6).
   f. Tighten the brass so that it secures the filter in place and keeps the filter flat.
9. The sample (filter) should be covered to minimize the risk of contamination from the lab environment.
10. The filter holder is clearly labeled I and II. If only one filter is being measured, it should be placed in position I, and not position II. If two filters are being measured, the two filters should be made from identical materials. Gold-coated and silver filters should not be placed in the same filter holder.

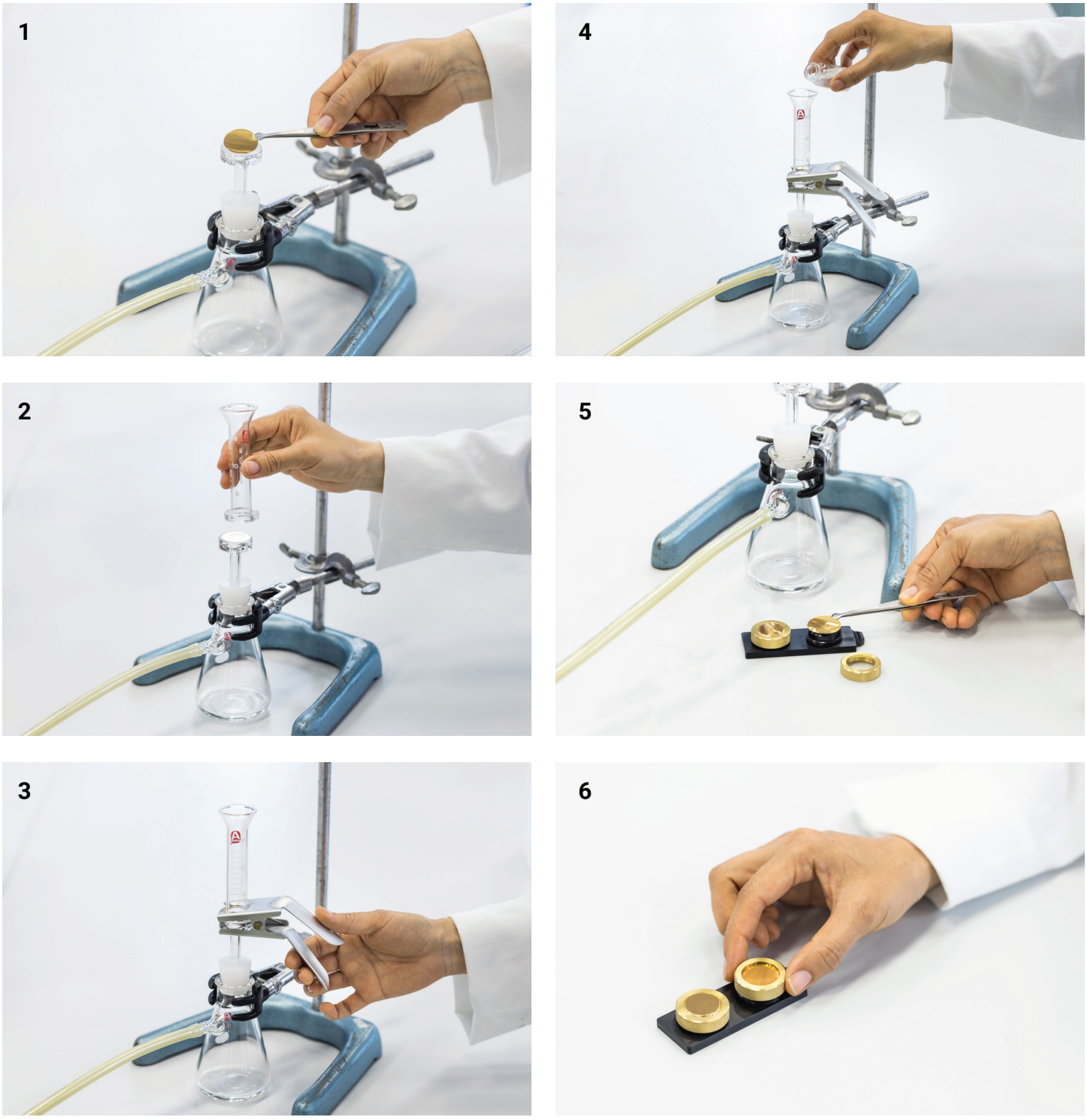

Figure 3. Sample filtration equipment and steps for the preparation of samples for LDIR on-filter analysis.

After filtration: achieving high quality results

Once the filters have been loaded into the filter holder, the holder can be inserted into the 8700 LDIR (Figure 4). Do not tilt the sample holder.

Figure 4. Sample insertion into the Agilent 8700 LDIR Chemical Imaging System.

To ensure accurate and efficient microplastic analysis using the Clarity instrument control software, consider the following points:

- To start any type of analysis, focus on the highest point within the filter holder, ideally on an empty area in the center of the filter.
- Before running the automated Particle Analysis workflow, a peak analysis at 1,442 cm^{-1} (5 µm pixel size) covering the whole filter area is recommended. The scan data can be used to check if the sample is overloaded and help select the best area of the filter to be analyzed.
- The Particle Analysis workflow automatically detects all particles within a user-defined area of the sample and draws boundaries around each particle. Clarity software displays a warning if the area selected does not meet the flatness threshold (flatness is needed to ensure that all particles within a selected area are detected). If a warning is given, the Particle Analysis workflow will still continue. However, the data should not be reported, and the sample should be reinserted or reprepared.
- If Auto Scan within the Particle Analysis workflow is enabled, the software will perform the full analysis automatically. If the user wishes to preview the found particles, adjust the particle sizes or sensitivity, include, or exclude particles, Auto Scan should be disabled.
- Auto Scan will automatically collect visible images for each particle. This high-magnification image is used to improve the accuracy of particle size measurements. Users can speed up the analysis by disabling this option and using particle sizing data from the infrared images generated for each particle.
- The Particle Analysis workflow contains a particle sensitivity slider. Increasing the sensitivity will detect smaller and fainter (lower absorbance) particles in the scanned area. The sensitivity setting will be adjusted automatically in the Particle Analysis workflow.
- Hit quality describes how closely the spectrum of the sample matches that in the reference library. The classification range used for the identification of microplastics in a sample uses a hit quality index (HQI) score system, where a score of 1 represents the highest quality result. Users can adjust the classification range criteria according to their data reporting requirements. The following example of classification ranges (i.e., the characterization of spectral match quality defined as "high," "medium," and "low") are based on HQI scores between 0.65 and 0.99:
  - 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 the range, i.e., <0.65, would be classified as undefined.
- The default setting of the Particle Analysis workflow considers a particle with a minimum size of 20 µm to be characterized (guaranteed specification). Users can change the minimum size to be detected and check the results manually.
- Once the analysis has been performed, filters should be transferred into a Petri dish, covered, and stored.

Data processing and reporting

Once the sample has been analyzed using the 8700 LDIR system, the Clarity software automatically provides the analyst with the following statistical data:

- Total number of particles detected.
- Total number of particles identified and 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 the identification of each particle.
- Size ranges.
- Infrared and high-magnification visible images for each particle detected.

The Clarity software generates a full report containing all relevant information for all particles analyzed, as shown in Figure 5. The data can be used for further statistical analysis.

Analytical results of microplastics can be reported according to analyst requirements and the purpose of the analysis. Two examples of microplastic data reporting techniques include:

- Exclude all non-microplastics particles (e.g., natural polyamide, stearates, cellulosic materials, carbonates, etc.), and report all other particles (microplastics particles) based on selected HQI criteria. The laboratory should consider manual verification of any polymers that are subject to interference from other materials.
- Include all particles detected in the report (microplastics and non-microplastics) based on selected HQI criteria. This method of reporting can be used to study the ratio of microplastics/non-microplastics or for quality assurance, e.g., presence of non-microplastics in air, water blanks, and contamination control.

Complimentary analysis using FTIR

To further investigate the potential source of microplastics found in a sample, larger pieces of plastic (minimum size of ~2 mm) can be analyzed and identified using an Agilent Cary 630 FTIR spectrometer with a diamond ATR module (Figure 6).

Figure 6. The Agilent Cary 630 FTIR spectrometer can help to identify the source of larger sized pieces of plastic.

The Agilent MicroLab software uses instructive pictures to navigate the user through each step of the analysis including sampling and cleaning procedures. The MicroLab software automatically performs the library search and provides the user with a list of the best library matches in an easy-to-understand result display format—making getting answers with the Cary 630 FTIR simple and straightforward.

Agilent provides a wide selection of ready-to-use, application-specific libraries that can be used with the MicroLab software. Alternatively, specialized spectral libraries can easily be created, maintained, and managed in the MicroLab software.[9]

	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 5. Screenshot of the report generated by the Agilent Clarity software at the end of the Particle Analysis workflow.

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Further information

- Agilent 8700 LDIR Chemical Imaging System
- Agilent Clarity Software
- Microplastics Technologies FAQs
- Microplastics Analysis in Water
- Agilent Cary 630 FTIR Spectrometer
- Agilent MicroLab Software
- ATR-FTIR Spectroscopy Overview

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