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# Application News

iSpect™DIA-10 Dynamic Particle Image Analysis System
AIMsight™ Infrared Microscope
IRTracer™-100/IRXross™ Fourier Transform Infrared Spectrophotometer

# Comprehensive Approach for Successful Microplastics Analysis

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## User Benefits

- Rapid measurement of total number of particles, size distribution, and shape in particles < 100 µm by Dynamic Image Analyzer.
- Qualitative and quantitative microplastics analysis for particles > about a few hundred µm by benchtop FTIR system.
- Qualitative and quantitative microplastics analysis for particles < about a few hundred µm by infrared microscope.

## ■ Introduction

Generally, microplastics are evaluated by observing their appearance, measuring their number and size, and qualifying the materials. The qualitative measurement of the material is one of the most important steps for identifying the origin of the microplastic; however, the size and size distribution of microplastics also needs to be evaluated, requiring the selection of appropriate analytical instruments.

Pyrolysis GCMS quantitates microplastic particles providing qualitative identification of individual plastics and a quantitative concentration (or mass) using gas chromatography mass spectrometry[1)]; however, this technique does not provide qualitative information such as total number of particles, size distribution, and shape. Raman or Infrared spectroscopy on the other hand, provides qualitative identification by using FTIR spectral analysis and enables actual counting and sorting of individual particles[2)].

A FTIR microscopy method in development at ASTM International[3)] differentiates the sorting and identification of plastics by particle size. The smaller, < a few hundred µm, particles are classified by size and shape, counted, and analyzed automatically using infrared microscope, and the larger, > a few hundred µm, particles are classified by size and shape, and counted using a stereomicroscope after which representative particles are transferred manually for identification with a benchtop FTIR system. The benchtop FTIR method is size limited by the ability of the analyst to accurately classify, count, pick up and transfer the smaller particles to a FTIR system.

This size limitation of the manual microscopy and benchtop FTIR method led us to develop ASTM D8489 – Test Method for the Determination of Microplastics Particle and Fiber Size, Distribution, Shape and Concentration in Waters with High to Low Suspended Solids Using a Dynamic Image Particle Size and Shape Analyzer, hereafter referred to as D8489. D8489 complements the manual microscopy and benchtop IR method by assisting in the estimation of particle size, size distribution, concentration, and shape. And D8489 focuses on smaller particles between the range of 5 - 100 µm.

## ■ Materials and Methods

A description of D8489, the apparatus used, the sampling and sample preparation procedure and the analysis procedure were thoroughly described previously[4)]. The data demonstrated acceptable precision and bias within the scope of the method, but only analyzed reference materials of plastic beads with a known diameter and concentration. In this Application News, we include analysis of real plastic particles after simulating sampling and preparation by ASTM Practices D8332 and D8333. Here, we examine particles in the 5 - 100 µm range by the Shimadzu iSpect DIA-10 Dynamic Particle Image Analysis System (Fig. 1), and use the Shimadzu IRTracer-100 Fourier Transform Infrared (FTIR) spectrophotometer system with QATR™ 10 single reflection type attenuated total reflection attachment integral with sample compartment (Fig. 2) to identify some of the larger particles. Additionally, we analyzed the entire < a few hundred µm fraction by FTIR microscopy using the Shimadzu IRXross FTIR spectrophotometer system with AIMsight infrared microscope (Fig. 3).

*Preparation of plastic particles*

Microplastic particles were prepared according to ASTM D8402[5)]; however, due to the lack of a cryogenic mill, only procedure A was used. Sheets of polypropylene (PP), pieces of polyethylene (PE), and a food container of polystyrene (PS) were obtained and manually shredded into fragments using a grater as shown in Fig. 4. Next, the plastic particles were mixed, and about 50 mg of the combined plastic was added to 1 mL methanol to create a synthetic D8333 mixture, then sieved through 212 and 100 µm.

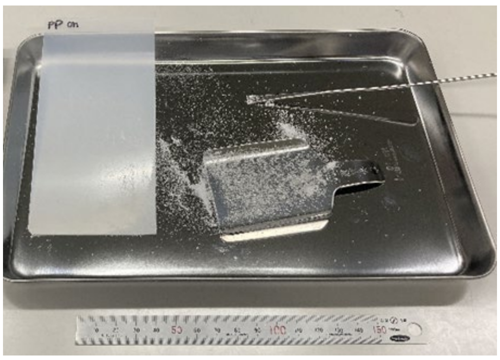


Fig. 4 Preparation of PP microplastic particles

Fig. 1 iSpect™DIA-10

Fig. 2 IRTracer™-100 and QATR™ 10

Fig. 3 IRXross™ with AIMsight™

## ■ Experimental

The <100 µm fraction of particles <100 µm was analyzed by the Shimadzu iSpect DIA-10 Dynamic Particle Image Analysis System for determination of size distribution, shape, and counting of particles between 5 - 100 µm. A 250 µL aliquot of the < 100 µm fraction was added to 2250 µL of 50% methanol and 50% glycerin according to D8489. Seven 150 µL replicates were introduced, captured, and analyzed.

Fig. 5 and 6 show a Particle size distribution and scattergram, respectively, with particle size distribution of one of seven replicates of the < 100 µm fraction. Table 1 lists the concentration and repeatability data of all seven replicates differentiated by size. Fig. 7 shows a thumbnail display of particle images.

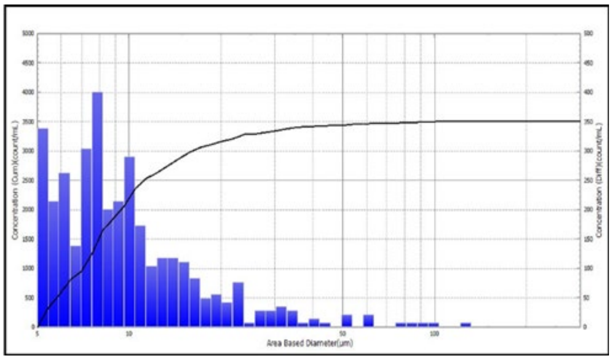



Fig. 5 Particle Size Distribution

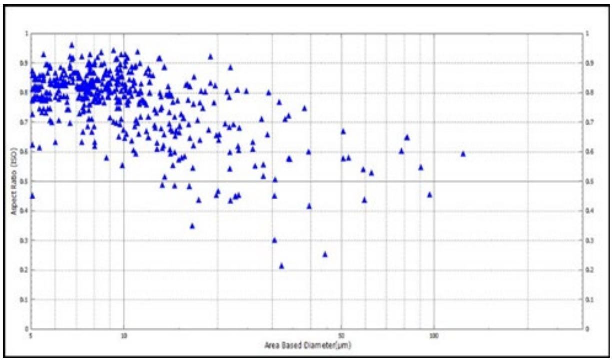



Fig. 6 Scattergram of area based diameter (x-axis) and aspect ratio (y-axis)

Table 1 Concentration and repeatability data of all seven replicates differentiated by area based diameter

| Replicate number | Concentration (count/mL)* | | | | | |
|---|---|---|---|---|---|---|
| | All | 5-10 µm | 10-25 µm | 25-50 µm | 50-100 µm | > 100 µm |
| 1 | 3253 | 1623 | 1250 | 214 | 83 | 83 |
| 2 | 3667 | 2051 | 1140 | 283 | 69 | 124 |
| 3 | 3764 | 2037 | 1271 | 290 | 90 | 76 |
| 4 | 3640 | 2141 | 1140 | 228 | 76 | 55 |
| 5 | 3419 | 1837 | 1167 | 235 | 111 | 69 |
| 6 | 3508 | 2051 | 1105 | 242 | 76 | 35 |
| 7 | 3101 | 1761 | 1050 | 186 | 62 | 41 |
| Average | 3479 | 1929 | 1160 | 240 | 81 | 69 |
| S.D. | 238.4 | 190.1 | 77.9 | 36.7 | 15.8 | 30.1 |
| RSD | 6.85% | 9.86% | 6.72% | 15.31% | 19.54% | 43.59% |

* Concentration is calculated from Number of Particles, Number of Frames and flowcell volume.

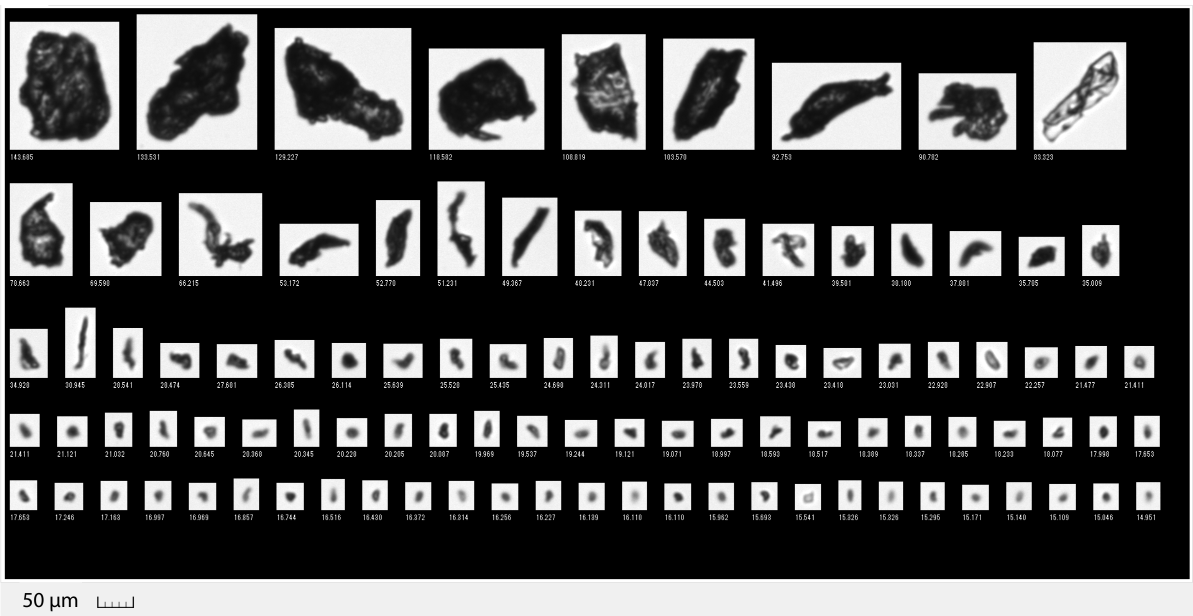



Fig. 7 Thumbnail Display of particle images and ordered by area based diameter (µm)

Next, we selected a few of the larger particles that did not pass through the 100 µm sieve for qualitative analysis using the FTIR system with attenuated total reflectance attachment. IRTracer-100 operating conditions are shown in Table 2, and three examples of photographs, maximum length, and infrared spectra are shown in Fig. 8.

Table 2 FTIR Measurement Conditions

| | |
|---|---|
| Instruments | : IRTracer-100, QATR 10 |
| Resolution | : 4 $cm^{-1}$ |
| Number of Scan | : 30 |
| Apodization function | : SqrTriangle |
| Detector | : DLATGS |

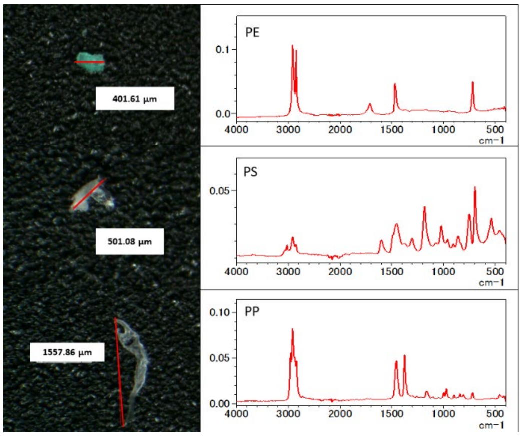


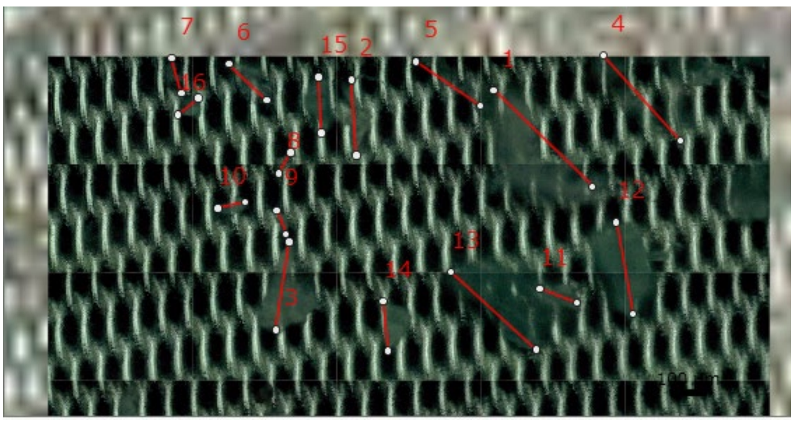

Fig. 8 Photographs, maximum length, and infrared spectra

Finally, we analyzed two < a few hundred µm fraction aliquots using the Shimadzu IRXross FTIR system with the AIMsight infrared microscope. For this test, 200 µL of the D8333 mixture of microplastics in methanol was transferred onto a 25 mm diameter 15 µm stainless steel mesh screen and filtered. The amount of sample filtered was estimated to contain about 3000 - 4000 particles. Three individual measurement areas of 1.232 $mm^2$ were selected per aliquot from a total filter area of 490.625 $mm^2$. The IRXross FTIR system was configured according to the conditions in Table 3.

Table 3 Infrared Microscope Measurement Conditions

| | |
|---|---|
| Instruments | : IRXross, AIMsight |
| Resolution | : 8 $cm^{-1}$ |
| Number of Scan | : 5 |
| Apodization function | : SqrTriangle |
| Aperture size | : 50 µm × 50 µm |
| Measurement interval | : 50 µm |
| Mapping range | : 850 µm × 1,450 µm |
| Detector | : T2SL |

Fig. 9 shows the AIMsight infrared microscope view of a measurement area of one aliquot with particle length as determined by the Shimadzu AMsolution software and the chemical identification of each particle as determined by the FTIR spectrum. Fig. 10 shows mapping images identifying the chemical identity of the individual plastic particles found. Areas where large numerical values were obtained for the plastic component are shown in red, while areas with small values are shown in blue. Fig. 11 shows the spectra of the three plastics.




| No. | Length(µm) | Material |
|---|---|---|
| 1 | 382 | PP |
| 2 | 209 | PP |
| 3 | 245 | PP |
| 4 | 319 | PS |
| 5 | 217 | PS |
| 6 | 145 | PS |
| 7 | 100 | PS |
| 8 | 67 | PS |
| 9 | 70 | PS |
| 10 | 78 | PS |
| 11 | 110 | PS |
| 12 | 257 | PE |
| 13 | 319 | PE |
| 14 | 138 | PE |
| 15 | 156 | PE |
| 16 | 74 | PE |

Fig. 9 Unit area and length (µm) of each particle with ID as determined by FTIR

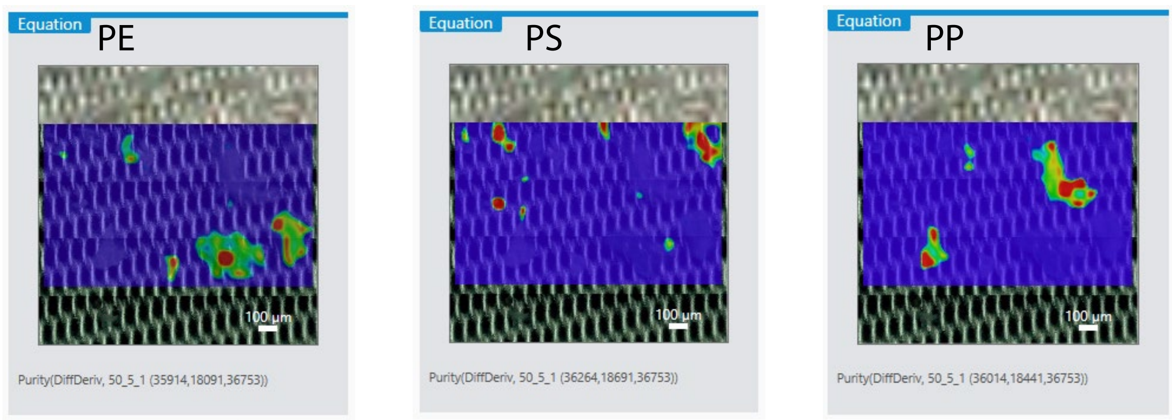



Fig. 10 Mapping images identifying each plastic in the unit area

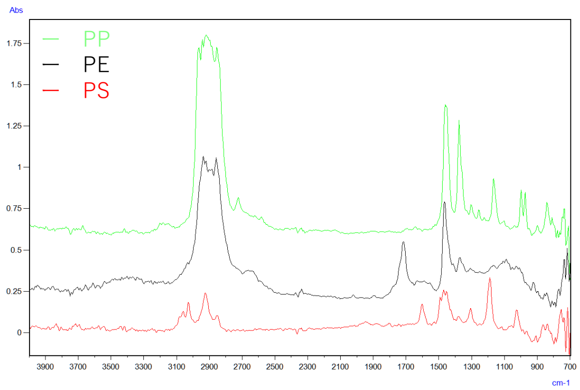



Fig. 11 Color coded FTIR Spectra of the individual plastics

The plastics in each measurement area were tabulated by chemical identity and count, averaged, and then calculated using the following equation to estimate the total plastic particles in the aliquot filtered.

Total particles = Number of particles in measurement area x filter area / measurement area

For sample 1 and sample 2, the average per measurement area and calculated number of microplastic particles per filter are shown in Table 4.

Table 4 Average particles per measurement area and total particles per filter

| | Sample 1 | | | | Sample 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | PE | PS | PP | Total | PE | PS | PP | Total |
| Average Particles per measurement area | 4.3 | 3.3 | 2.3 | 10.7 | 5.3 | 1.3 | 1.7 | 8.3 |
| Total Particles per filter | 1725 | 1327 | 929 | 4246 | 2110 | 517 | 663 | 3317 |

## ■ Conclusion

In this Application News we demonstrated use of the Shimadzu iSpect DIA-10 Dynamic Particle Image Analysis System and followed ASTM D8489 in the analysis of particle shape, size, and size distribution of real plastics between 5 and 100 µm. In addition, we followed ASTM work item WK87463 to measure and identify microplastic particles using microscopy and IR Spectroscopy. Here, we used the Shimadzu IRTracer-100 FTIR system with a QATR 10 to identify some of the larger, > a few hundred µm, particles.

Next, with assistance of the Shimadzu AMsolution length measurement and mapping software, we used the Shimadzu IRXross FTIR system with the AIMsight infrared microscope to classify, enumerate and identify the < a few hundred µm particles. Using ASTM standards developed for sampling, sample preparation, and analysis for microplastics in water, the portfolio of Shimadzu Instruments enable a very comprehensive characterization of the distribution and composition of microplastics in water.

<References>

1) Shimadzu App News GCMS-2202, An Automated Workflow for Quantitative Analysis of Microplastics in Environmental Samples via Pyrolysis-GC/MS
2) Shimadzu App News 01-00396-en, Analysis of Microplastics Using AIRsight Infrared/Raman Microscope
3) WK 87463 Standard Test Method for Spectroscopic Identification and Quantification of Microplastic Particles in Water Using Infrared (IR) Spectroscopy
4) Shimadzu Whitepaper, New Standard Determination of Microplastics Particle and Fiber Size, Distribution, Shape and Concentration in Waters with High to Low Suspended Solids Using a Dynamic Image Particle Size and Shape Analyzer, September 2022 Whitepaper-STP-DIA.pdf (shimadzu.com)
5) ASTM D8402, Standard Practice for Development of Microplastic Reference Samples for Calibration and Proficiency Evaluation in All Types of Water Matrices with High to Low Levels of Suspended Solids



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