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Microplastics analysis – automatic analysis by Dynamic Particle Imaging Analysis.

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In today's presentation

1. Microplastics analysis – ASTM
2. Analysis by Infrared (IR) spectroscopy
3. Analysis by Dynamic Imaging Analysis (DIA)
4. IR spectroscopy vs Dynamic Particle Imaging
5. Conclusions
6. Q&A

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ASTM

- 6 Work Items
- Focus on:
  - *Sample collection*
  - *Sample preparation*
  - *Sample analysis by*
    - IR
    - Raman
    - Pyr-GCMS
    - Dynamic Particle Imaging

Focus of my presentation

- Current data gaps WK67565 (IR)
- Initial developments WK72349 (Dynamic Particle Imaging)

- WK67563 - New Practice for **Collection** of All Matrices of High and Low Turbidity Water Samples for the Identification and Quantification of Micro-plastic Particles and Fibers.
- WK67564 - New Practice for **Preparation of Water Samples** with High, Medium or Low Suspended Solids for the Identification and Quantification of Microplastic Particles and Fibers using **Raman Spectroscopy, IR Spectroscopy or Pyrolysis-Gas Chromatography/Mass Spectroscopy (GC/MS).**
- WK67565 - Spectroscopic Identification and Quantification of Microplastic Particles and Fibers in all High and Low Turbidity Water Matrices including Municipal Wastewater Using **IR and Raman Spectroscopy**.
- WK67788 Identification of Microplastic Particles and Fibers in All Matrices of High and Low Turbidity Water Samples including Municipal Raw Wastewater using **Pyrolysis-GC/MS**.
- WK70831 The Production and Quantification of Microplastic Particles to be used in **Quality Control Reference Samples** in the Collection Practices, Preparation Practices and Identification Analysis Methods used on All Matrices of High and Low Turbidity Waters.
- WK72349 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**.

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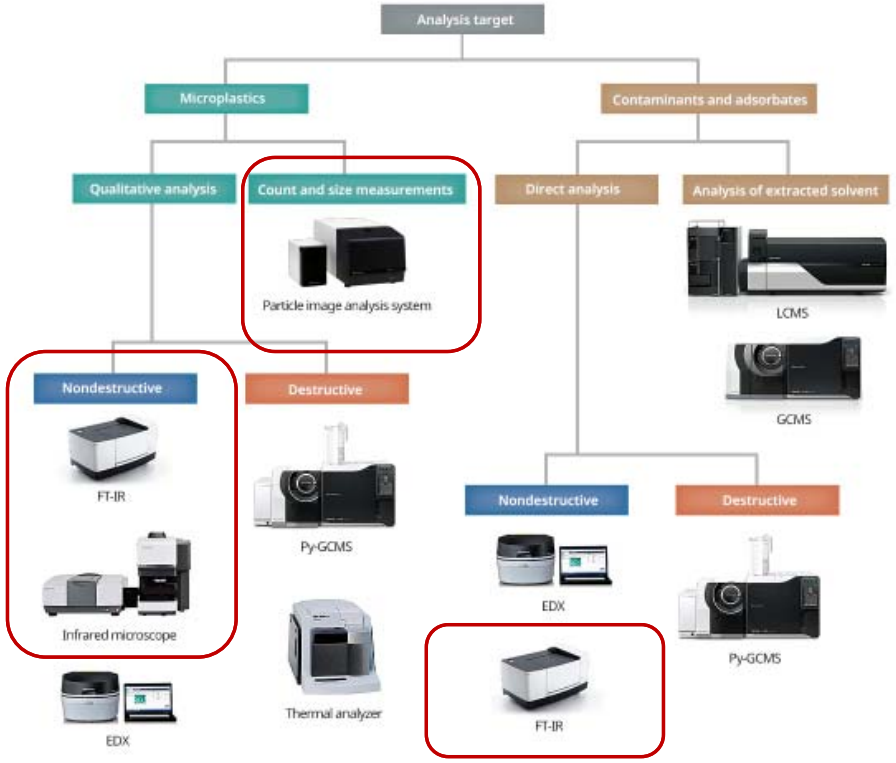
Analysis of Microplastics



Variety of complimentary techniques available.

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How does IR work?

- An IR spectrometer measures the absorption of IR radiation (wavelengths ~1 μm- ~40μm) by molecules in various states. Modern FTIR spectrometers often use Fourier transformed interferograms to generate IR spectra.
- In an IR spectrum the energy of IR radiation is usually represented in unit of reciprocal centimeter (cm^{-1}). Typically a Mid IR spectrum records from about 400 cm^{-1} to 4000 cm^{-1}
- Peaks in an IR spectrum reflect specific vibrational/rotational modes in molecules, therefore, can be used for qualitative/quantitative analysis of chemical species.
- FTIR microscopes can be applied to investigate samples on the microscopic scale. There are three operational modes: transmission, reflection or attenuated total reflection (ATR).

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Experimental Conditions

IR	
Detector	Liquid nitrogen cooled MCT detector
Objective mirror	15x reflection objective mirror
Optical system mode	Reflection and ATR
Resolution (cm^{-1})	8
No. of Scans averaged per spectrum	Variable, 64 (typical)
Intensity mode	Absorbance
Range (cm^{-1})	700-4000
Mirror speed (mm/s)	9
Apodization	HappGenzel

Sample ID	True diameter (µm)	Material
10 µm	10.23	Polystyrene
20 µm	20.10	Polystyrene
50 µm	50.56	Polymethacrylate
100 µm	100	Polystyrene

Filter Type	Description
Stainless steel mesh	5 µm pore size
Cellulose ester	25 mm diameter, 0-6 µm pore size
Glass fiber	25 mm diameter, grade 934-AH, 1.5 µm pore size

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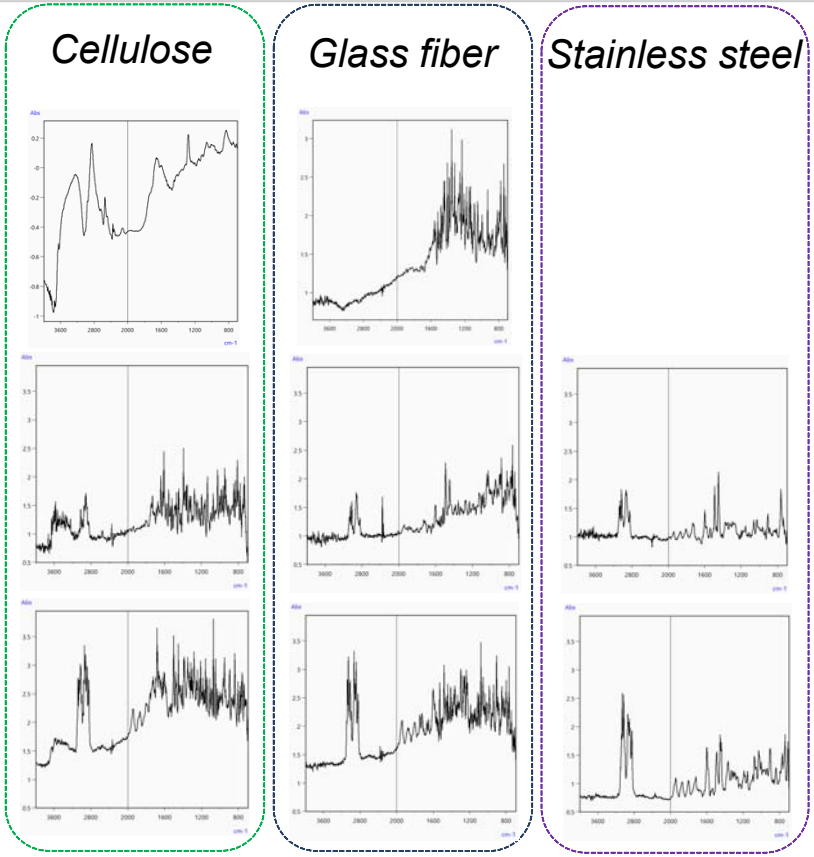
Results: Background and importance of filter material



- Analysis performed in reflection mode
- Spectra from cellulose and glass fiber filter presents absorbance across the measuring region
- Stainless steel is highly reflective and does not show absorbance
- Qualitative results demonstrates the negative impact of filter background on sensitivity

Replication sample analysis: n=5

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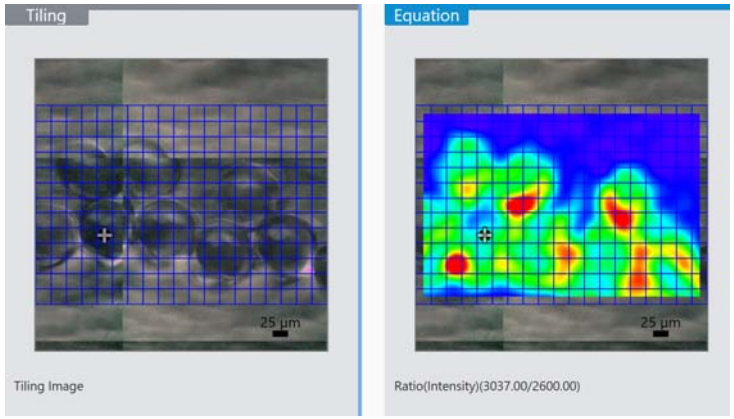
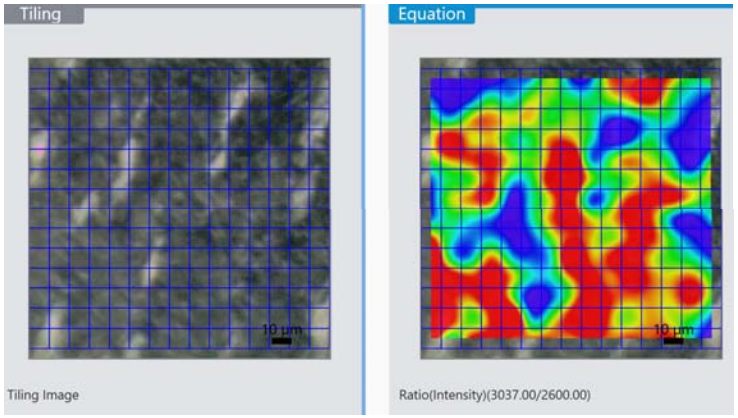
Results: Mapping and its value

100 µm



10 µm



- Analysis performed in reflection mode
- Qualitative initial screening:
  - *Identify areas of interest*
  - *General assessment of size distribution*
- Sizes of particles from 100 to 20 µm were unequivocally determined
- Reflection from neighboring particles interfered in the analysis of 10 µm

Replication sample analysis: n=1

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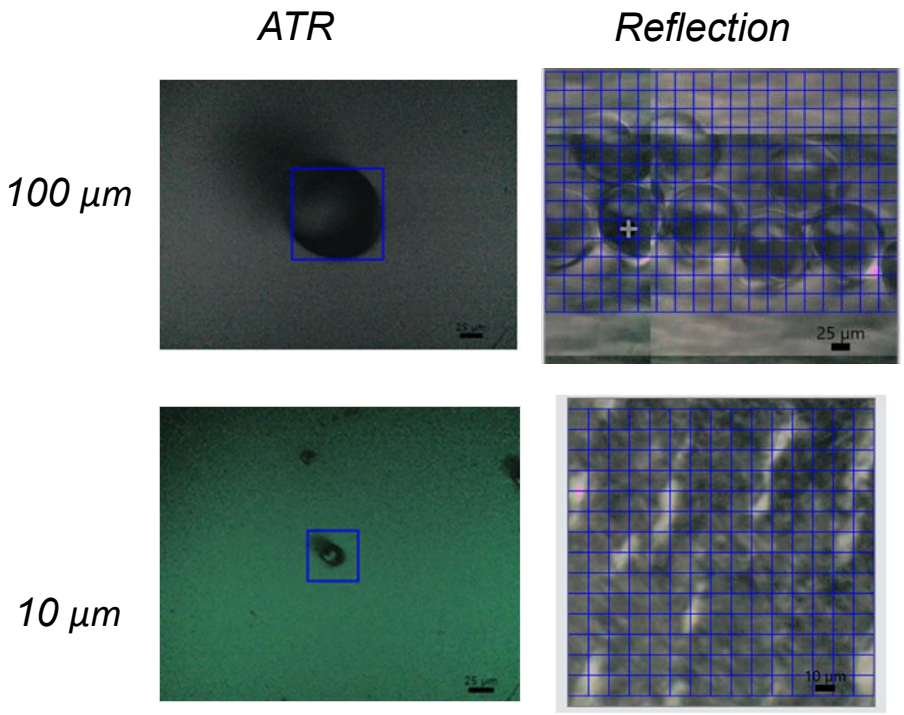
Results: ATR vs reflection mode



- ATR mode presents higher specificity than reflection mode
- Sizes of particles from 100 to 20 µm were unequivocally determined
- 10 µm particles were also identified
- Increase in sensitivity when using ATR mode was qualitatively demonstrated

Replication sample analysis ATR: n=3

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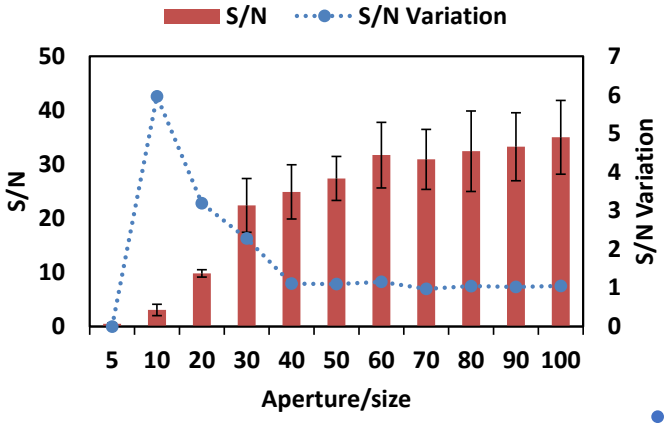
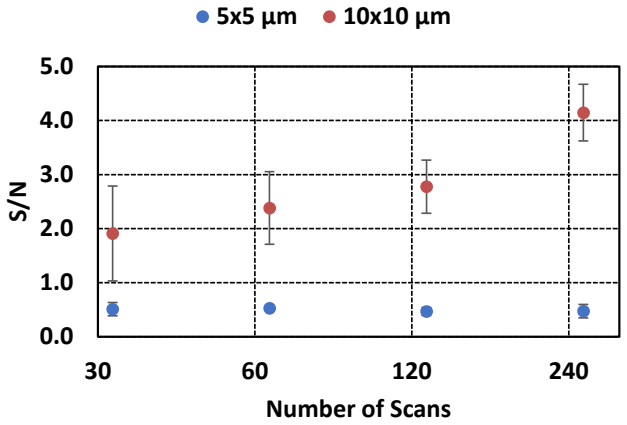
Results: Quantitation of detection threshold





- Analysis performed in reflection mode ("worse" mode)
- Decreasing particle size mimicked by decreasing aperture size from 100x100 µm to 5x5 µm
- Results at 10x10 µm:
  - *S/N≥3*
  - *Relevant increase in S/N when increasing # of scans to 256*

Replication sample analysis: n=4

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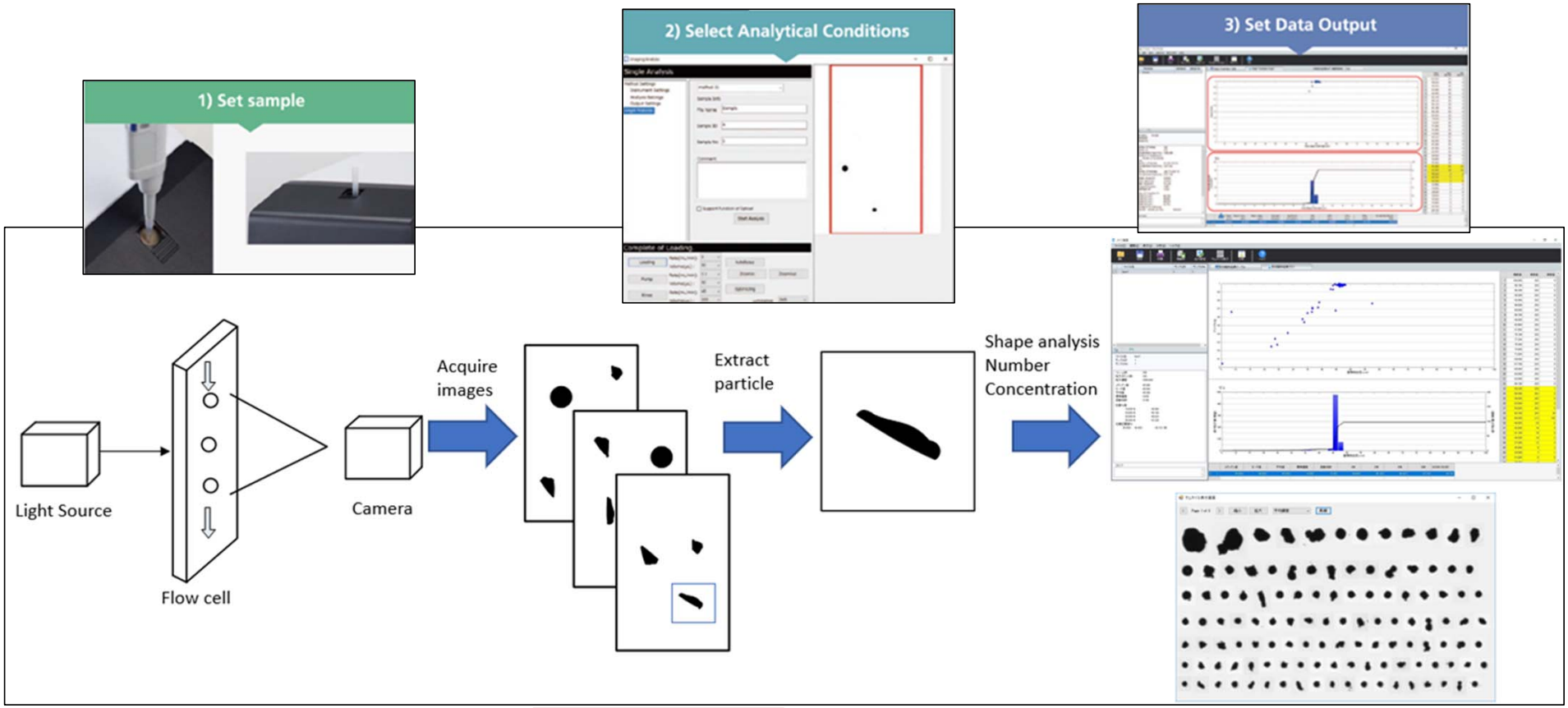
How does DIA work?



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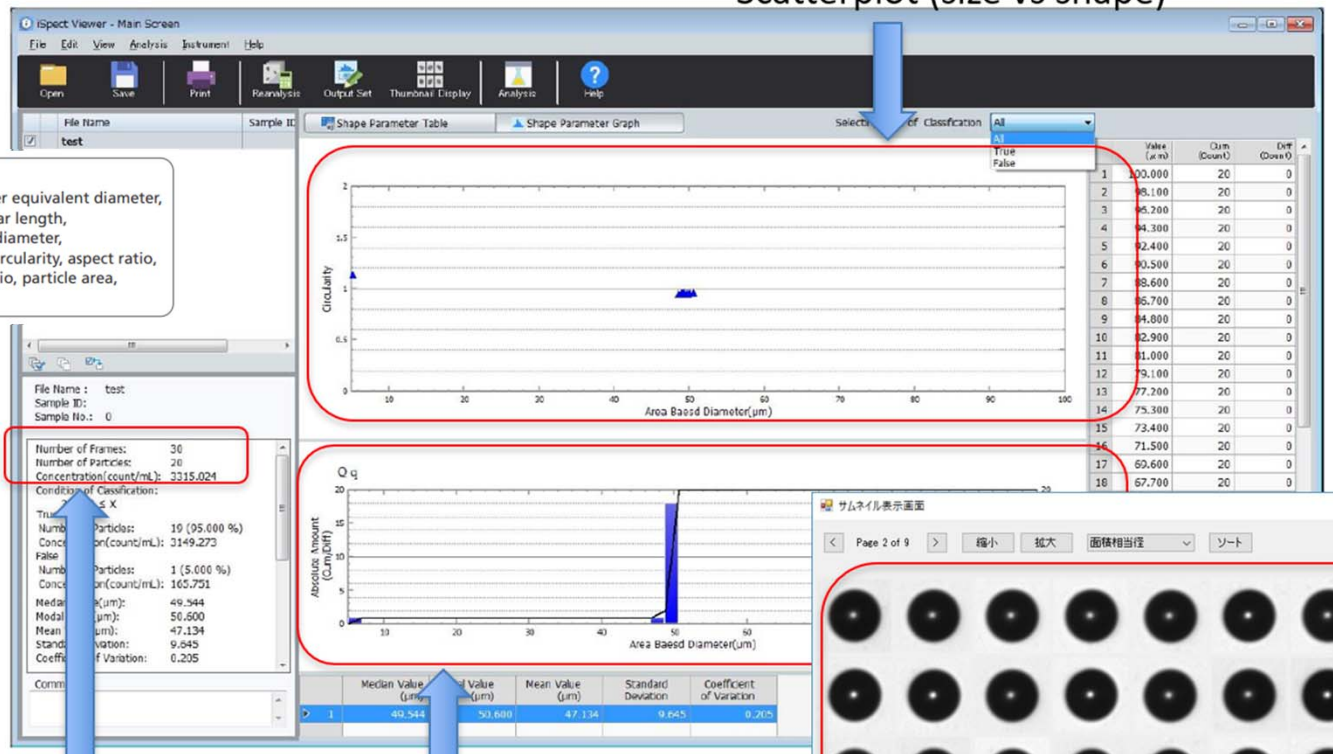
How does DIA work?

Scatterplot (size vs shape)



* Measurement Parameters (13 Types)

Area circle equivalent diameter, perimeter equivalent diameter, maximum length, maximum perpendicular length, vertical Feret diameter, horizontal Feret diameter, particle perimeter, envelope perimeter, circularity, aspect ratio, horizontal bounding rectangle aspect ratio, particle area, average brightness

Number concentration (particles/mL)

Size distribution

Particle image

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Experimental Conditions

Measurement Size Range	5 - 100 µm
Sample Volume	50 - 1000 µL
Flow Cell Size	120 µm deep x 1 mm wide
Analysis Flow Rate	0.1 µL/min (30 s – 10 min analysis)
Measurements	Particle size, distribution, count, concentration, shape, morphology, brightness (grayscale)

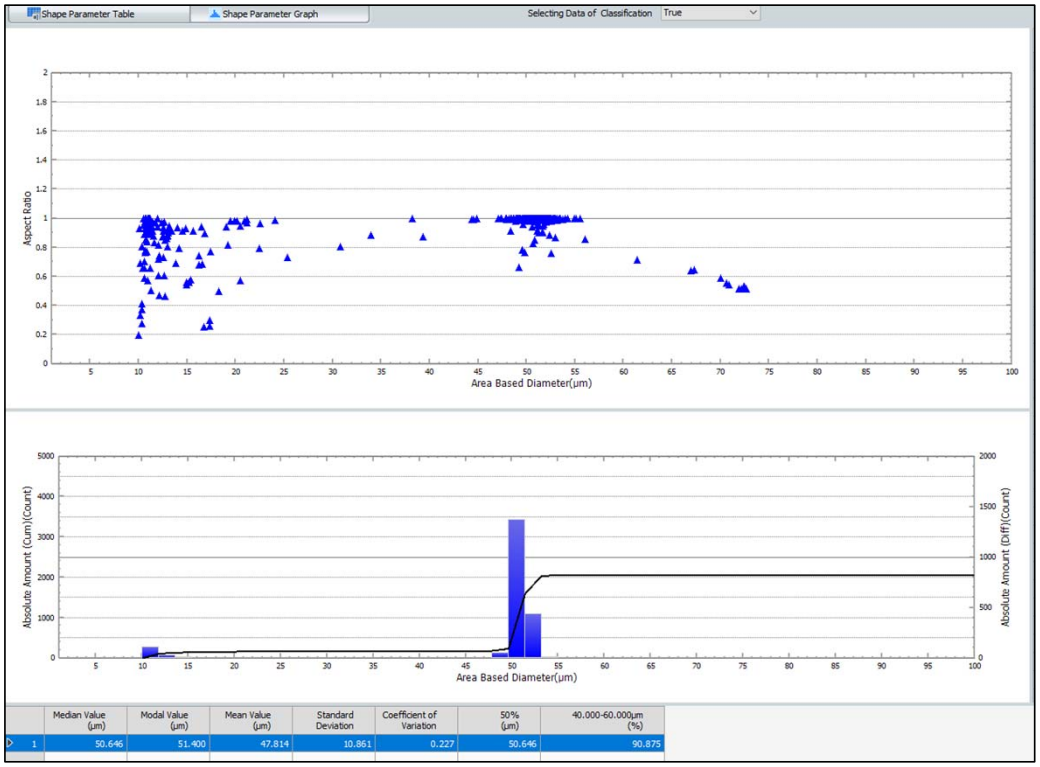
Sample ID	True diameter (µm)	Material	Analysis time (frames)	Output filters (exclude <)
10 µm	10.23	Polystyrene	5 min (2,100)	<8 µm
20 µm	20.10	Polystyrene	5 min (2,100)	<10 µm
50 µm	50.56	Polymethacrylate	5 min (2,100)	<10 µm
100 µm	100	Polystyrene	2 min (1,200)	<10 µm
NIST-20 µm	20.06	Polystyrene	5 min (1,800)	<10 µm

Example Data Output

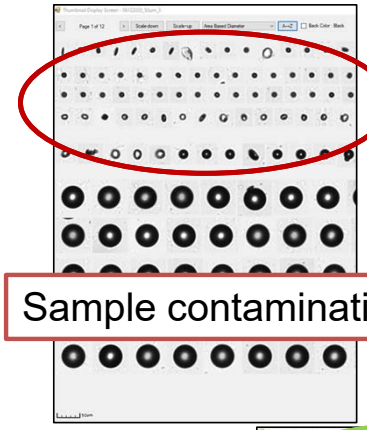
File Name : 06122020_50um_3
Sample ID: 06122020_100_3
Sample No.:

Number of Frames:	2100
Number of Particles:	2069
Concentration(count/mL):	4899
Condition of Classification:	
10.000 ≤ X	
True	
Number of Particles:	2069 (100.000 %)
Concentration(count/mL):	4899.132
False	
Number of Particles:	0 (0.000 %)
Concentration(count/mL):	0.000
Median Value(µm):	50.646
Modal Value(µm):	51.400
Mean Value(µm):	47.814
Standard Deviation:	10.861
Coefficient of Variation:	0.227
Value of Cumulative %	
50.000 % (µm) :	50.646
Cumulative % of Interval	
40.000- 60.000 µm (%):	90.875

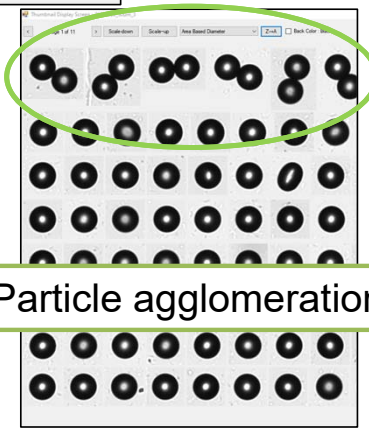
Comment
500uL in 100mL stirred



	Median Value (µm)	Modal Value (µm)	Mean Value (µm)	Standard Deviation	Coefficient of Variation	50% (µm)	40.000-60.000µm (%)
1	50.646	51.400	47.814	10.861	0.227	50.646	90.875



Sample contamination



Particle agglomeration

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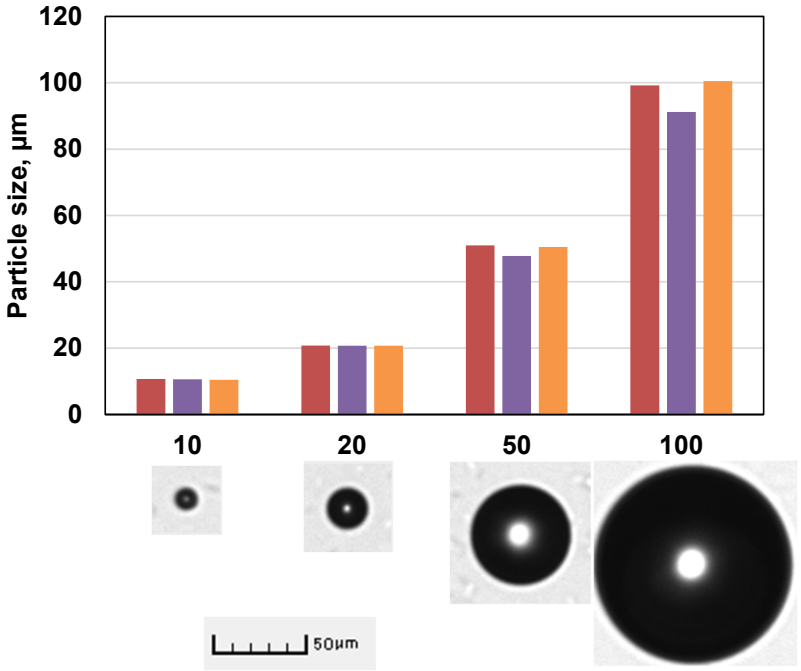
Results: Sizing particles



- Individual samples prepared with each microplastics standard
- Mean size calculated from all particles present in the sample
- Accurate and precise determination of particle size from 10 to 100 µm:
  - *10.6 µm ± 1.2%*
  - *20.7 µm ± 0.2%*
  - *49.8 µm ± 3.4%*
  - *97.0 µm ± 5.2%*

Replication sample analysis: n=3

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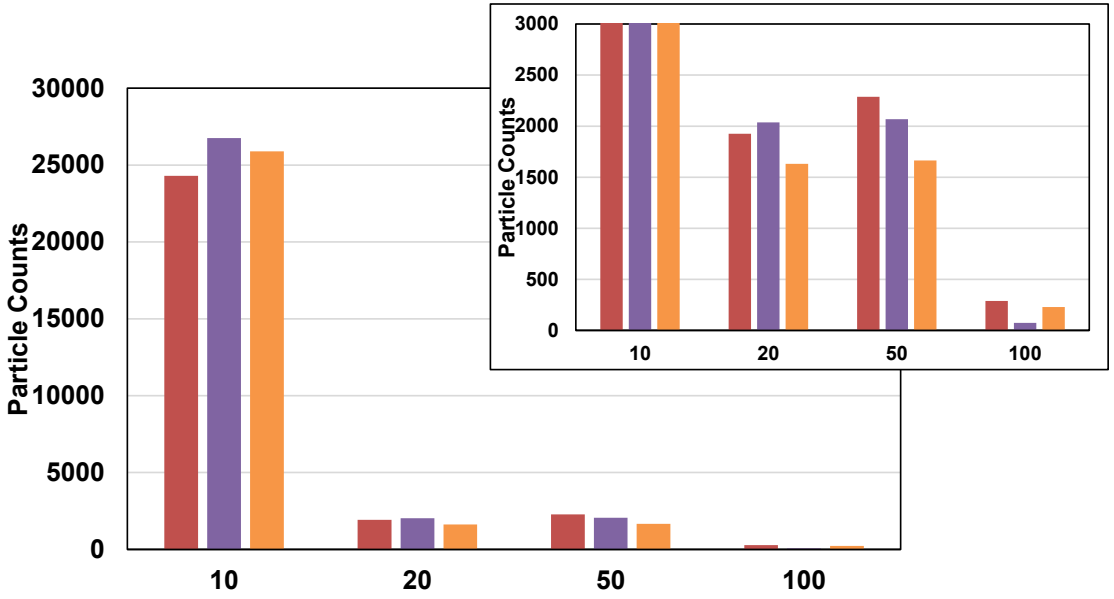
Results: Counting particles

- Individual samples prepared with each microplastics standard
- Particles present in the sample counted
- Reproducible counting results from different size particles.
  - *25654 ± 4.9%*
  - *1865 ± 11.3%*
  - *2007 ± 15.6%*
  - *196 ± 56.5%*



Replication sample analysis: n=3

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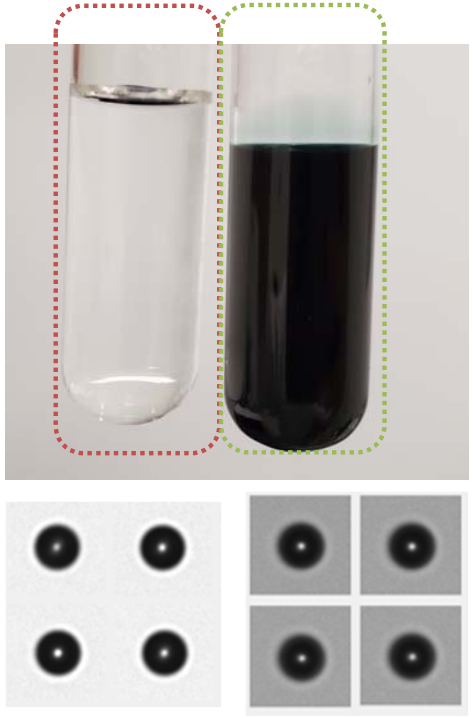
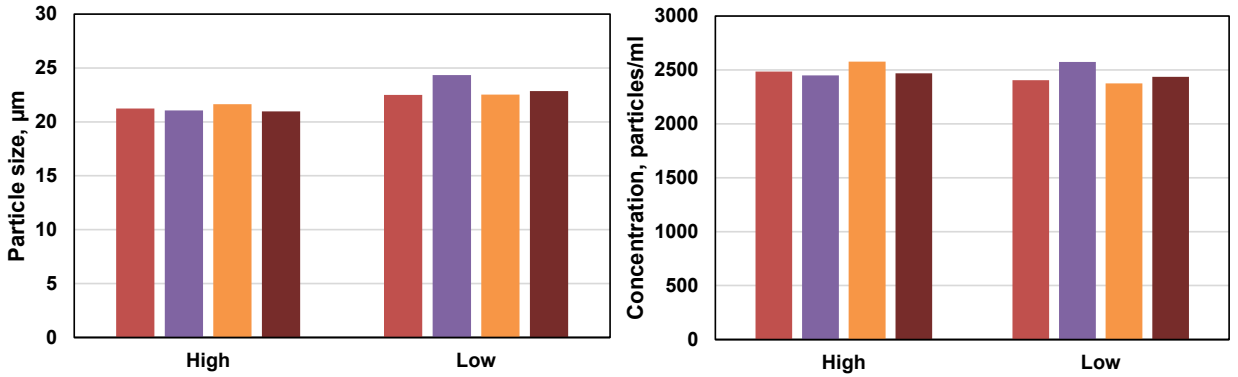
Results: Impact of variable brightness conditions



NIST Traceable standard 20 µm, acceptable concentration range: 2,430 – 3,630 particles/mL

- Light source intensity can be adjusted to improve brightness and contrast in dark or turbid solutions

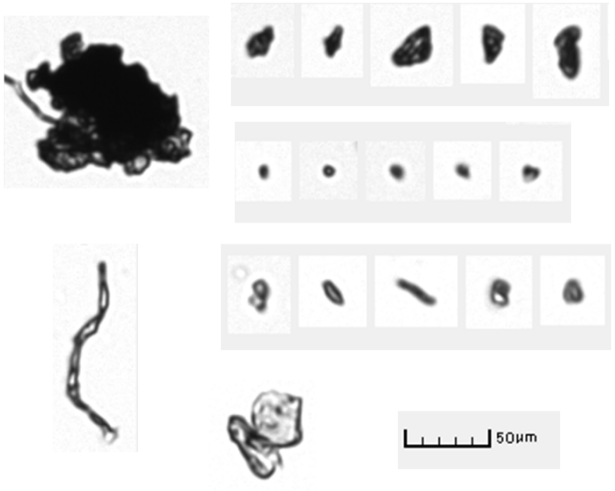
High Brightness Low Brightness

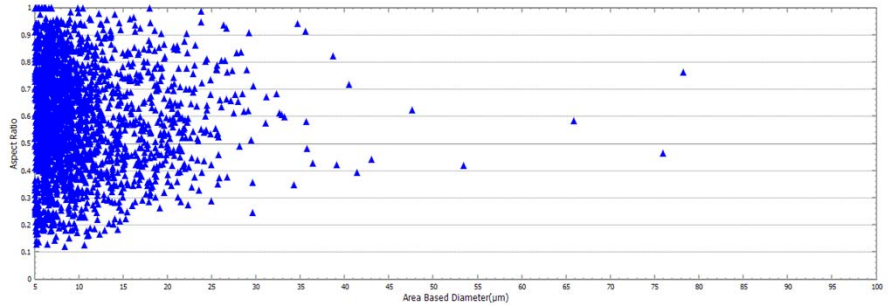


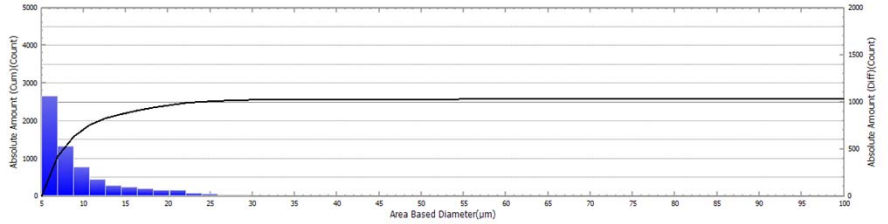
- Results (size and concentration) accurate, precise and reproducible under both brightness conditions.

Replication sample analysis: n=4

Results: Analysis of simulated microplastics solution







Average diameter: 7.737 µm
Concentration: 7141 count/mL
(2585 particles observed)

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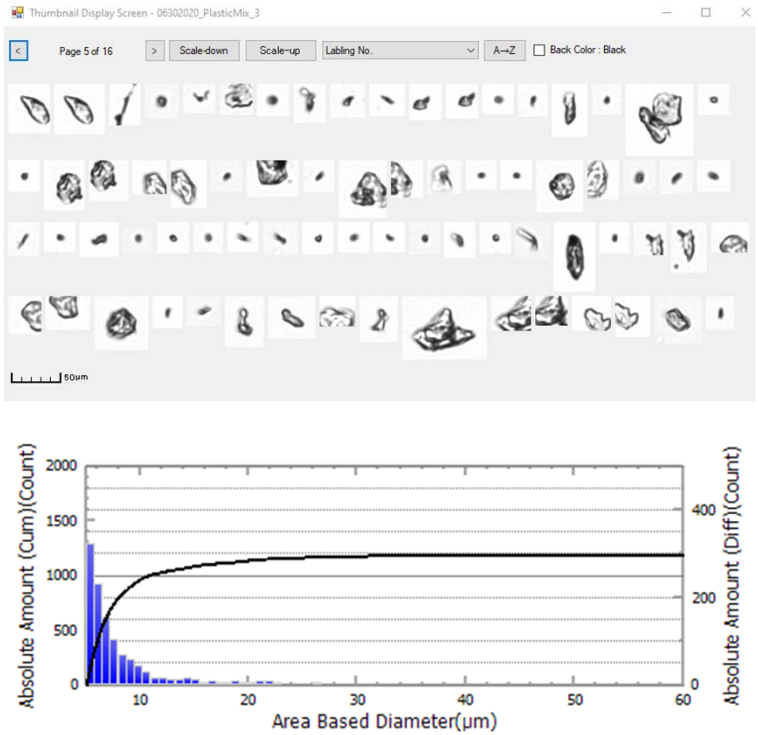
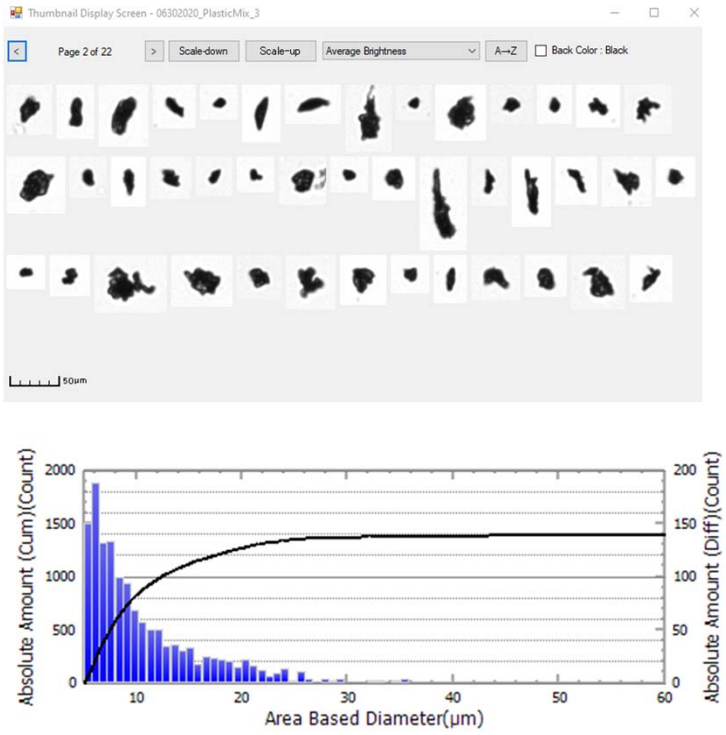
Results: Analysis of simulated microplastics solution

- Data can be processed based on particle brightness, in addition to particle size.





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IR & DIA - applicability

	IR	DIA
Sample Matrix	Filtered liquid sample on filter or dried sample on microscope slide	Liquid sample (prep steps feasible)
Minimum particle size (µm) with current measuring conditions	10	10
Information obtained	• Size, shape and agglomeration • Plastic type after entering unique spectral characteristics parameters in software	• Size, shape and agglomeration • Results can be filtered for flexible data analysis and stats analysis (ex. particle distribution)

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Conclusions

- Detection threshold for particle size by IR (in reflection and ATR modes) was qualitatively determined: 10 µm.
- Stainless steel filters demonstrated to be the most suitable ones for IR analysis.
- Detection threshold for particle size by IR in reflection mode (less sensitive than ATR) was quantitatively determined: 10 µm, with S/N ≥ 3. Number of scans >124 increased S/N.
- Initial demonstration of performance by DIA was completed:
  - *Accurate and precise determination of particle size from 10 to 100 µm.*
  - *Reproducible counting results from different size particles.*
  - *Results (size and concentration) accurate, precise and reproducible under variable brightness conditions.*

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