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Microplastics Analysis Using FTIR Spectroscopy and IR Microscopy

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Agenda

- Microplastics overview
- Overview of detection techniques
- Introduction to FTIR
- Macro FTIR and IR Microscopy
- Microplastic analysis examples
  - Clean samples – point mode detection
  - Dirty matrix – infrared imaging

Microplastics Overview

- Microplastics are polymeric particles < 5 mm in length. While these are ubiquitous across ecosystems, they're regarded as plastic pollutants.

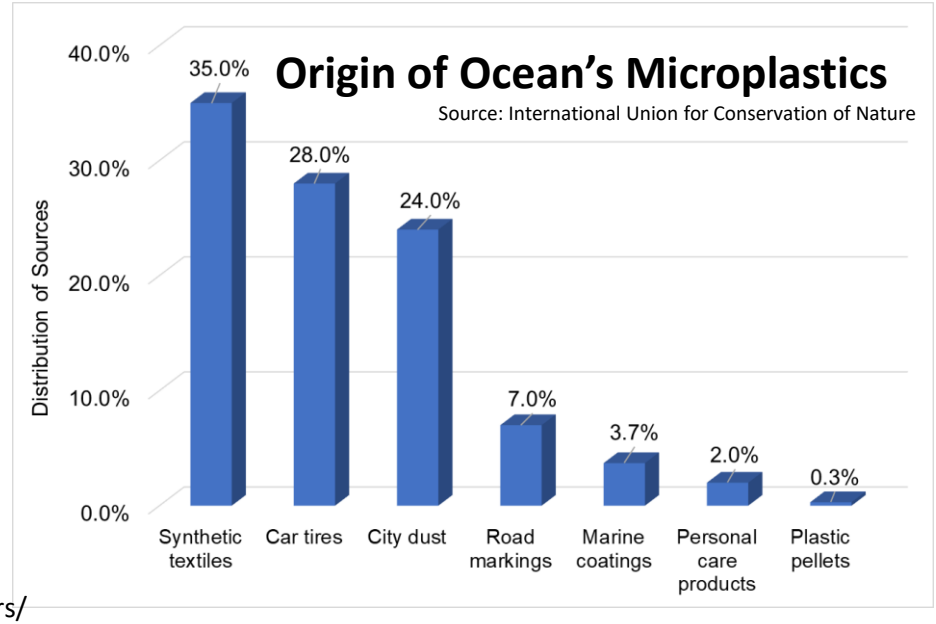
- Primary microplastics: plastic particles in products that are specifically designed to be of microscopic size
  - Cosmetic & toothpaste
  - Air blasting products
  - Commercial plastic powders
  - Glitter

- Secondary microplastics: small plastic particles that are generated by the breakdown of larger plastic items
  - Degradation of plastics in the environment
  - Fibers shed during washing of clothing

https://www.newscientist.com/article/2243731-we-may-have-missed-half-the-microplastics-in-the-ocean/

Top Polymer types

- Polyethylene terephthalate (PET) – plastic drinks bottles, textiles
- Polyethylene (PE) – grocery bags
- Polypropylene (PP) – packaging materials
- Polystyrene (PS) – food packaging (styrofoam)



Source: https://oceanconservancy.org/blog/2024/08/27/what-microplastics-microfibers/

Microplastics: Prevalence and Legislative Actions in US

- Microplastics are virtually everywhere: marine and fresh water, drinking-water, air, animals, food, human tissues, even on top of Mt. Everest and Antarctica.
- The sea floor contains more than 14 million tons of microplastics, about 25 x more than previously believed (Barrett et al., 2020).
- A Single load of laundry contains up to 18 million microplastics. In the last 60 years, 5.6 million metric tons of syntenic fibers have been released from cloths washing.
- 2024 research showed 16 common animal and plant protein sources all contain microplastics. Average American consumes 140 to 12,800 MPs per year (Baechler et al., 2024).

https://www.nationalgeographic.com/environment/article/microplastics-found-near-everests-peak-highest-ever-detected-world-perpetual-planet

- Microbead-Free Waters Act of 2015 – US federal ban on microbeads in "rinse-off" cosmetics
- 2018 - California first state in US to adapt sate-wide research strategy to identify early actions to reduce MP pollution. SOPs released in 2021/2022.
  - Senate bill No. 54 – "Solid waste: reporting, packaging, and plastic food service ware." 2022
- 2025 – Illinois introduce bill to require microfiber filtration in washing machines by 2030. CA, NJ have similar bills.
- 2024 – California Ocean Protection Council (OPC) created and signed into law
- ASTM D8332-20, D8401-24, D8489-23, and D8333 – 20: "Standard Practice for Preparation of Water Samples….for IR Spectroscopy…"
- Research is ongoing, and uncertainly remains regarding the impact on human health and well-being imposed by microplastics

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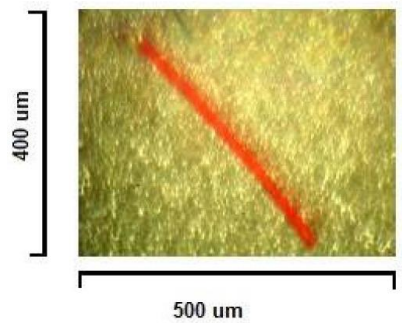
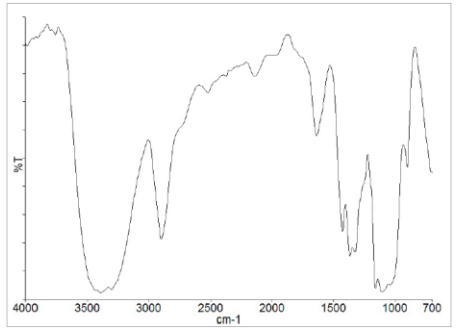
Methods of Microplastics Analysis

- Optical Identification
  - Naked eye, light microscopy, dye staining
- Scanning electron/energy dispersive X-ray microscopy
- Flow cytometry
- Spectroscopic techniques
  - FTIR spectroscopy
  - Hand-held, fiber-optic, ATR
  - FTIR microscopy
    - ATR imaging
  - Raman microscopy and imaging
- Thermal analysis techniques
- Thermal hyphenation, TGA-IR, TGA-IR/GCMS, PyGCMS

IR Microscopy

- Common analytical technique for the identification of polymers
- IR microscopy allows for the detection and identification of microplastics down to particles of only a few microns in size





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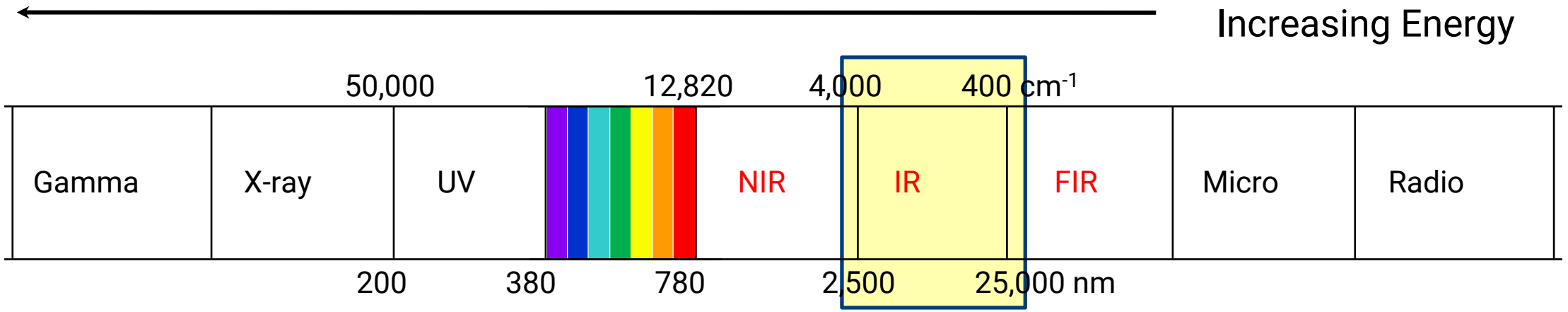
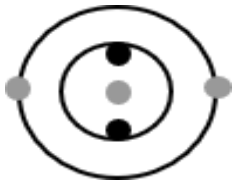
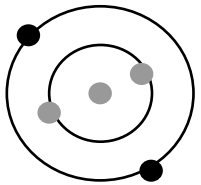
Introduction to FTIR: The Electromagnetic Spectrum





K- and L-shell electrons

X-ray diffr./fluor. NMR

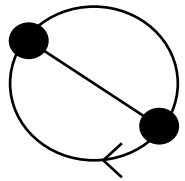


Valence electrons

UV-vis, fluor.

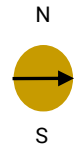
Molecular vibrations

FT-IR, Raman



Molecular rotations

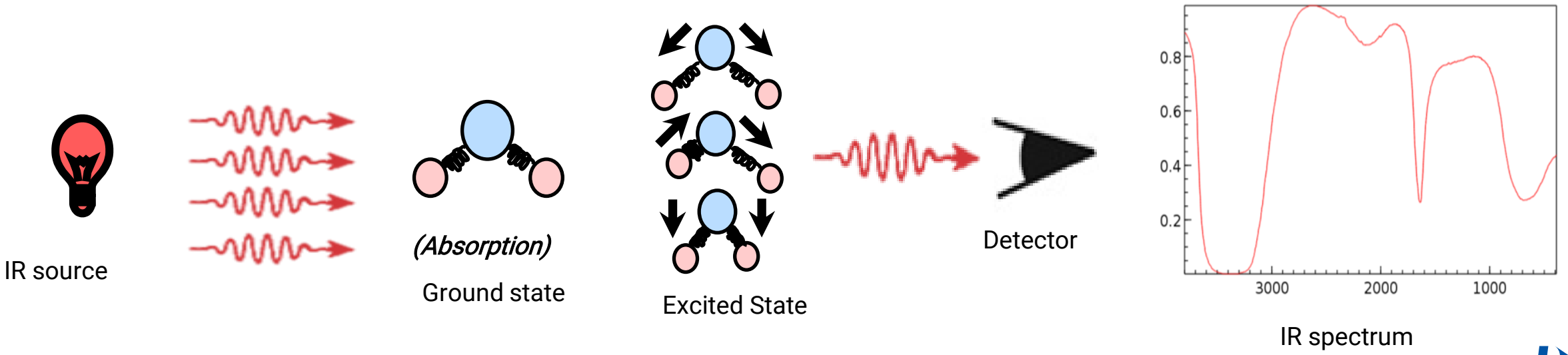
FT-IR



Nuclear spin transitions

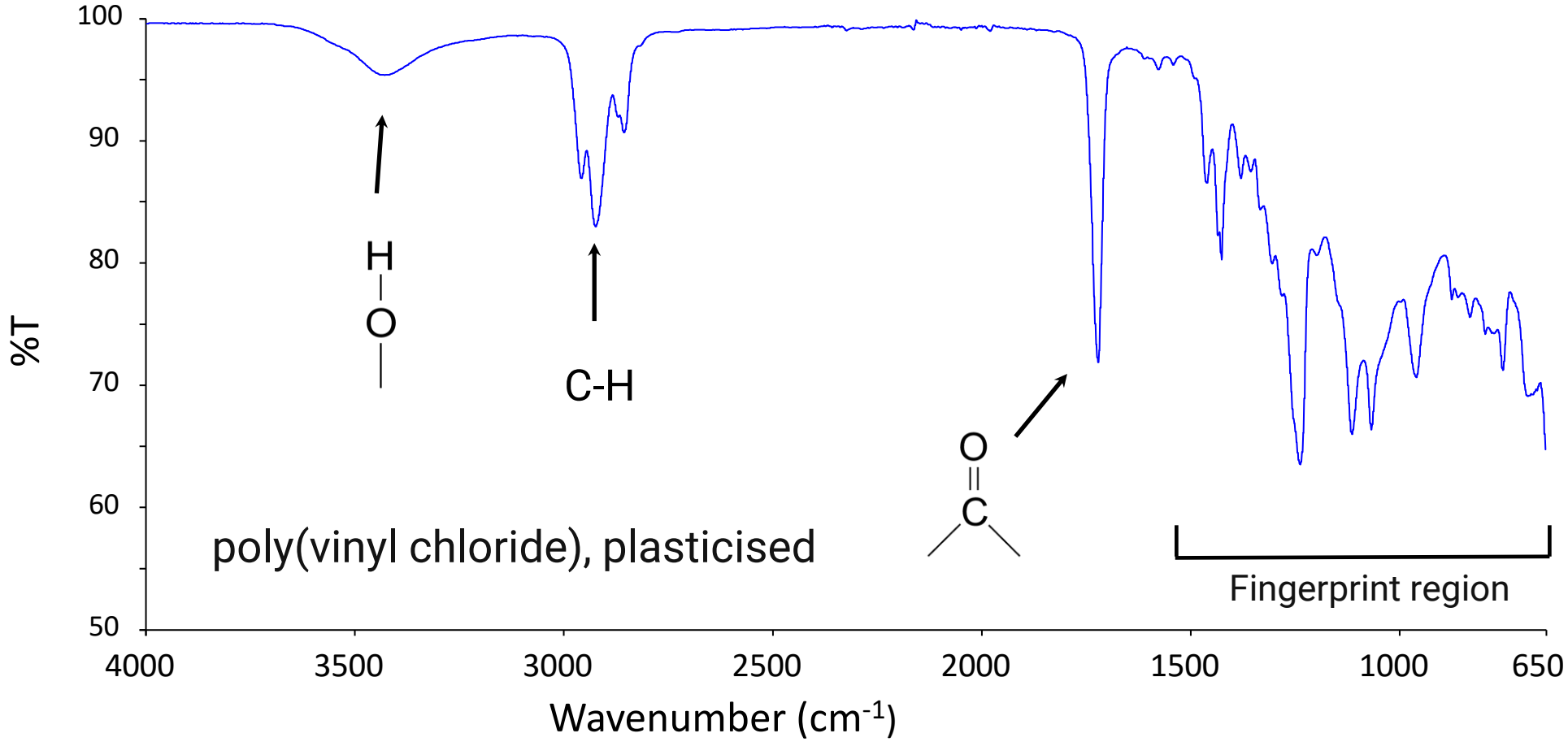
What Happens When Infrared Radiation Interacts With Matter?

- When a molecule is radiated with IR light, some of the wavelengths will be ***absorbed***
- Promotes molecule into excited vibrational state
- Molecules absorb IR light at discrete frequencies depending on the types of ***chemical bonds*** present



What Does the Infrared Spectrum of a Material Tell us?

- FTIR spectrum represents the chemical structure of sample
- Can be thought of as a '*fingerprint*' or '*barcode*' for a given compound



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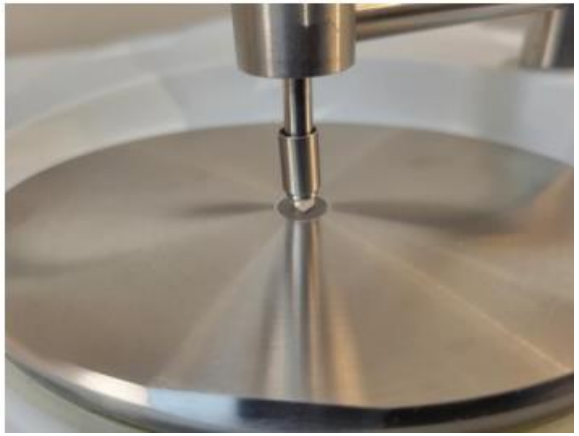
Macro-Sized Sample Analysis Using Benchtop FTIR and UATR

- Bench top FTIR and universal attenuated total reflectance (UATR) sampling
- Fast, easy, robust
- Ideal for measuring liquids, powders, solids, including “macro” plastics
- Good for measuring particles larger than ~1mm

UATR sampling accessory



Polymer bead on UATR crystal

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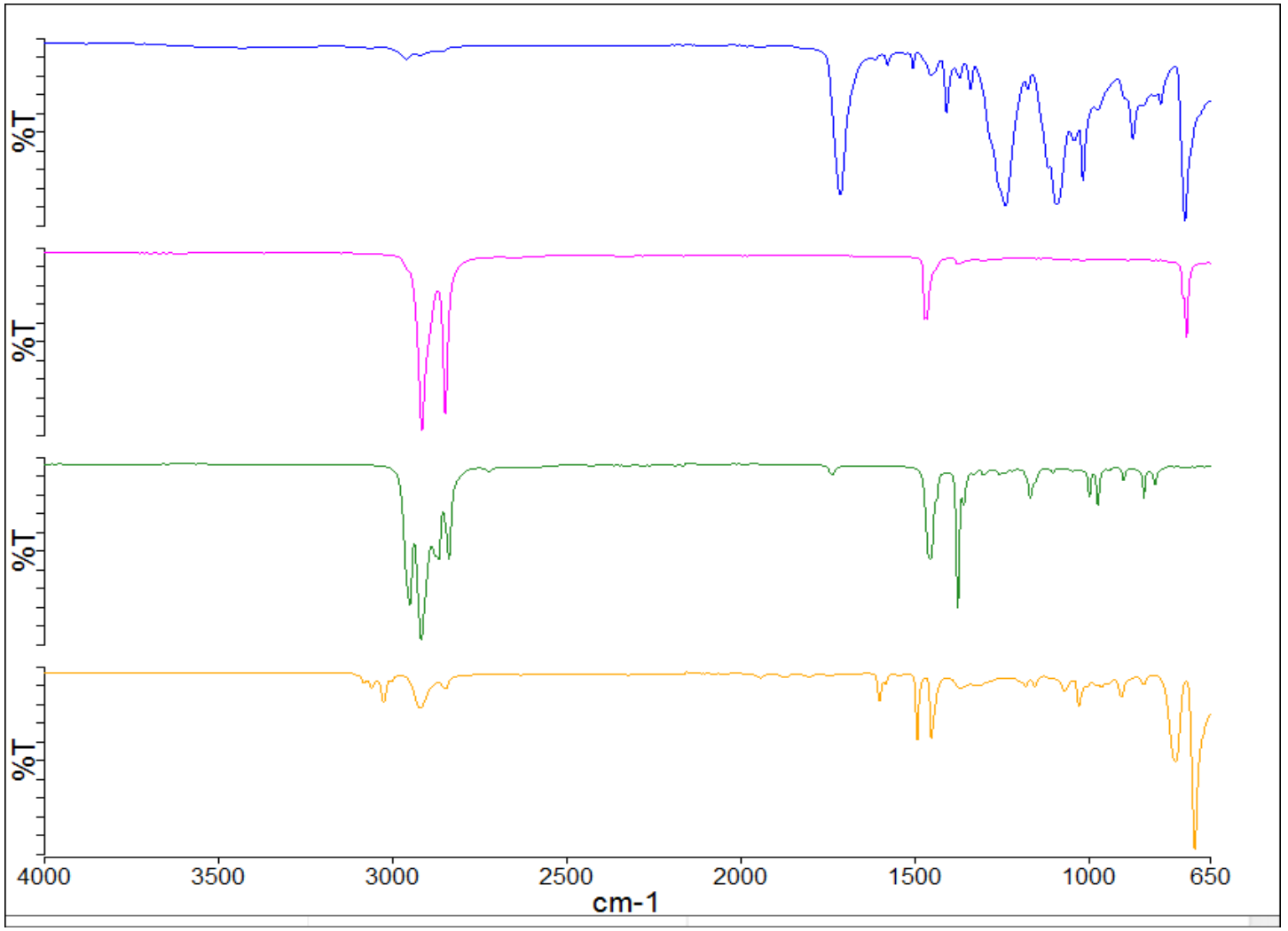
Library Search to Identify Most Common Plastic Fragments

Search Score 0.977896
Poly(ethylene terephthalate)

Search Score 0.991831
Polyethylene, low density

Search Score 0.991771
Polypropylene

Search Score 0.99229
Polystyrene



ATR spectra of larger (> 1 mm) plastic fragments Identified as PET, polyethylene, polypropylene and polystyrene

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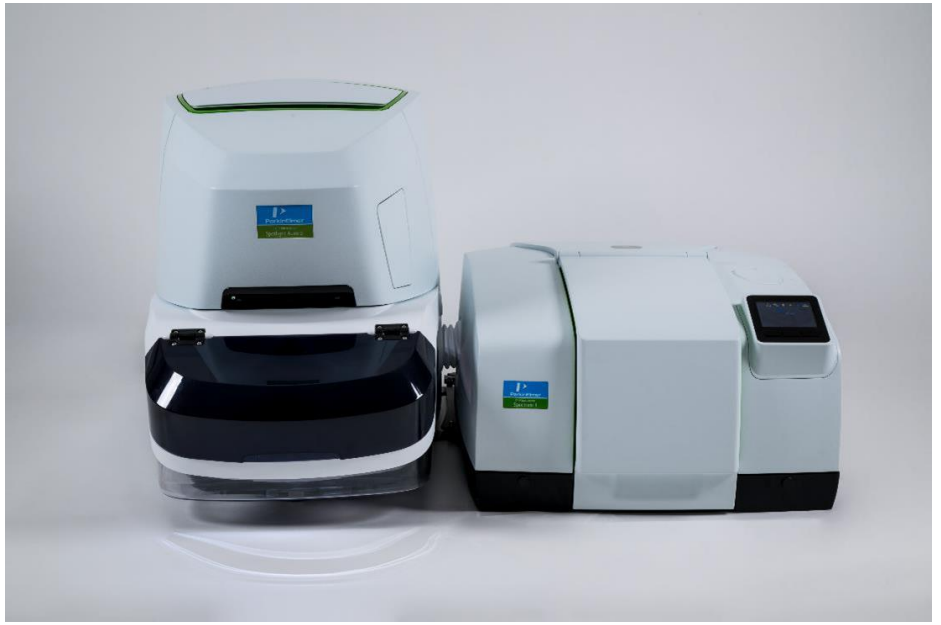
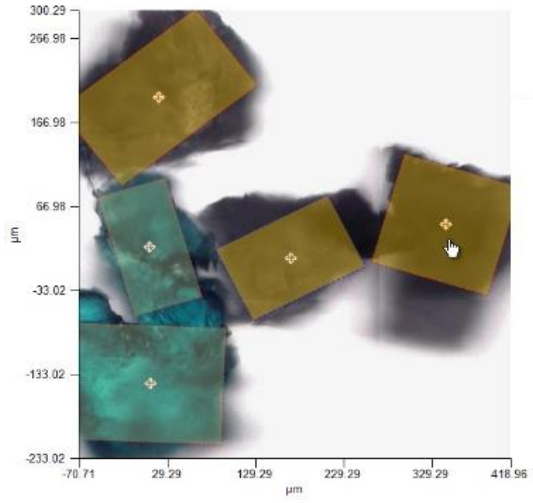
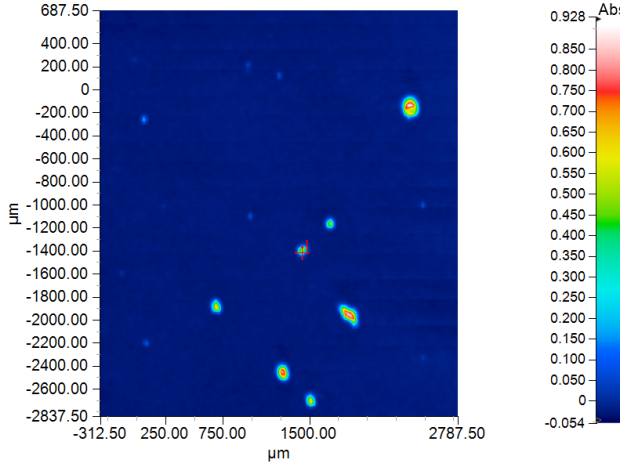
Introduction to IR Microscopy

- Common analytical technique for the identification of polymers
- IR beam focused down to small size using specialized optics and detectors
- Dedicated IR and visible imaging systems
- Able to detect and identify microplastics particles as small as a few microns
- Point or array detector options
  - Single spectrum of each particle (point-mode)
  - IR image of larger area (image mode)



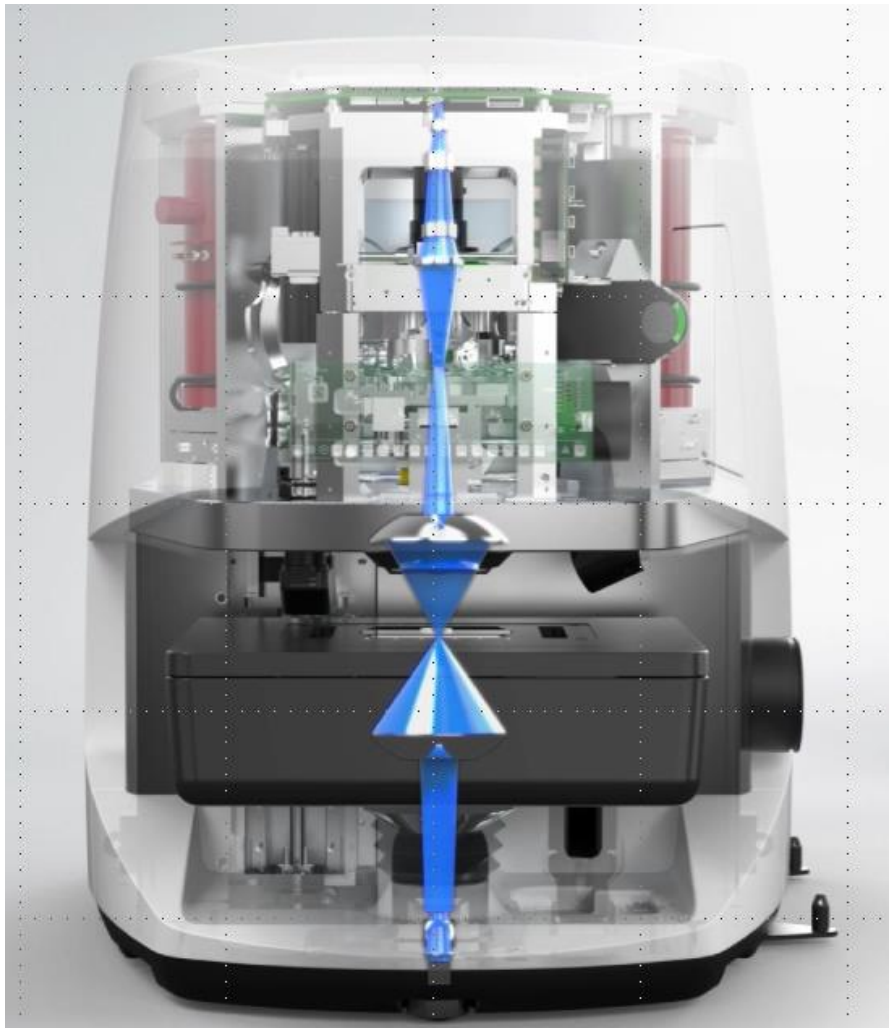


Point mode detector uses apertures to define area of interest



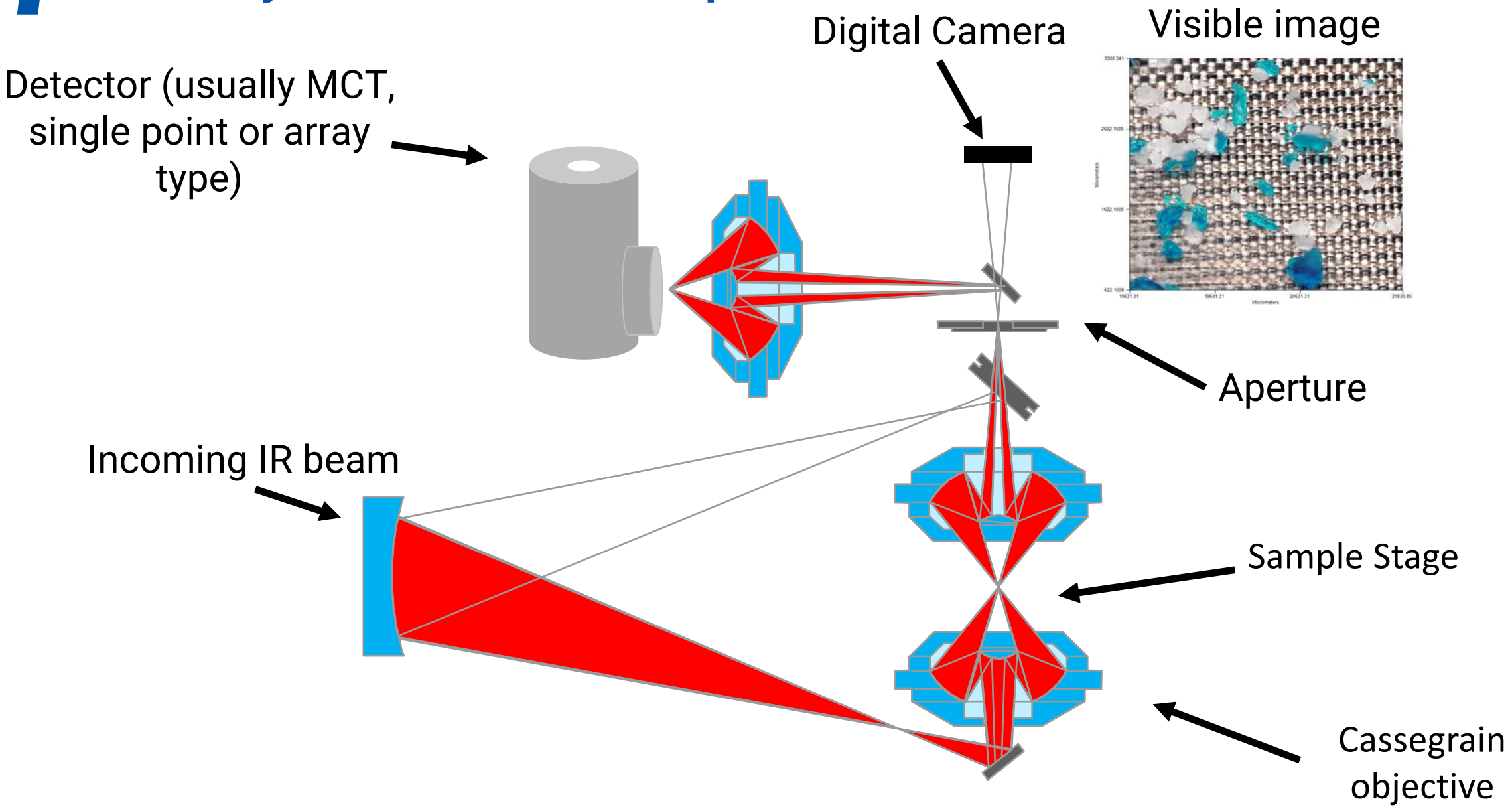
Array detector collects IR image of large areas

Introduction to IR Microscopy



- Measure modes include transmission, reflection, MicroATR, and ATRImaging
- Visible imaging system allows you to see samples/particles
- Motorized sample stage allow measurement of many particles or large areas automatically
- Manual and automatic data processing options
- Sample type and preparation plays a large role in determining best measurement approach

Anatomy of an IR Microscope



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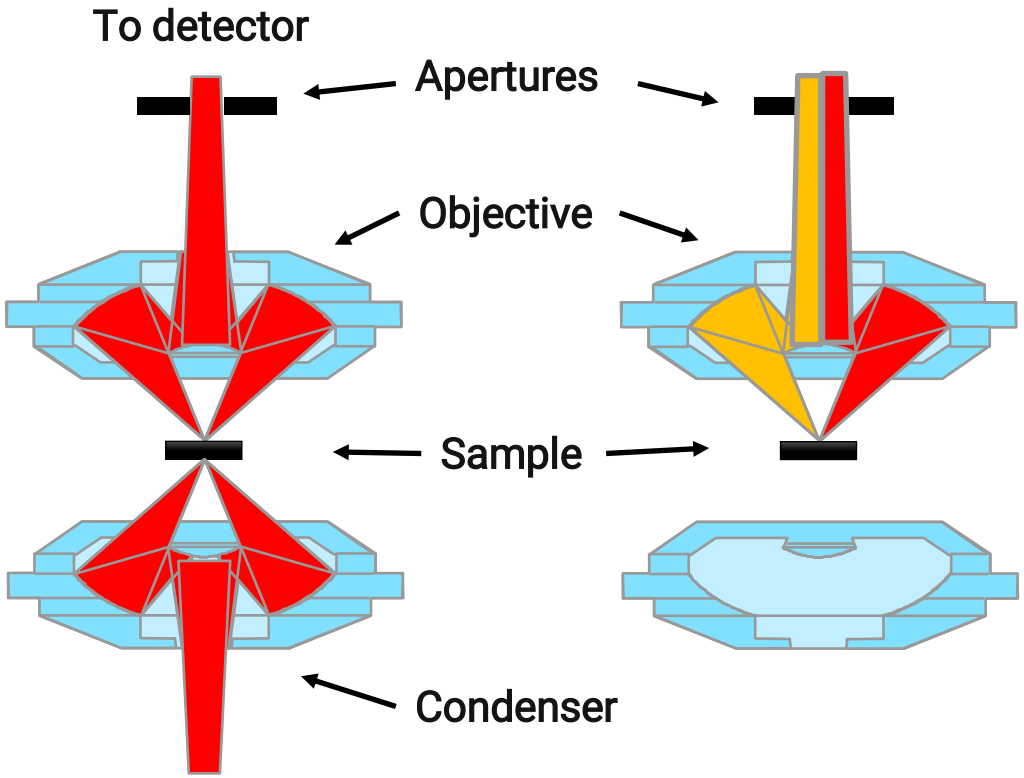
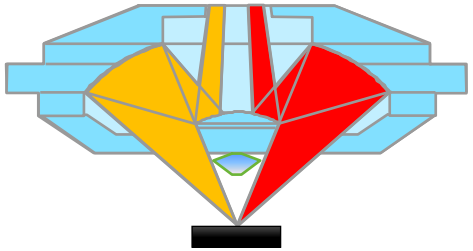
IR Microscope Operation Modes

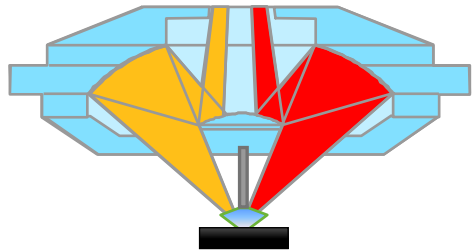
Transmission

Reflection

Micro ATR (Drop-down ATR)







* Imaging ATR – Highest Possible Spatial Resolution (~1.56 µm)

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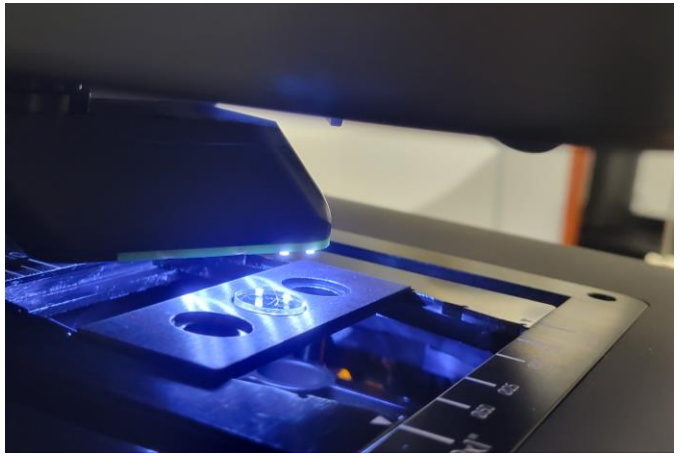
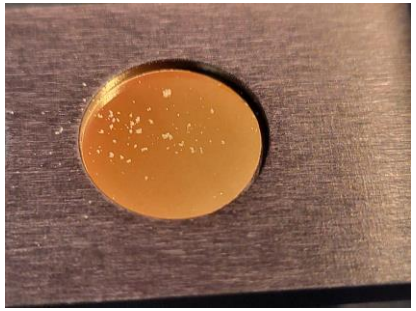
Case Study 1: Microplastics Prepared Standard

- Polypropylene (PP) and polyethylene (PE) particles of approximately 50 µm in diameter
  - Particles mixed then transferred to gold coated filter for reflectance and a KBr window for transmission
- PerkinElmer Spotlight Aurora IR microscope
  - Visible images collected with 1x1mm high-def camera
  - “Analyze Image” software process was used to identify particles and set aperture dimensions
  - IR data collected at 8 cm^{-1} resolution, 32 accumulations, 1 cm/sec OPD scan speed from 4,000 to 600 cm^{-1}

PerkinElmer Spotlight Aurora IR microscope



Sample on KBr window in microscope stage



Gold coated filter

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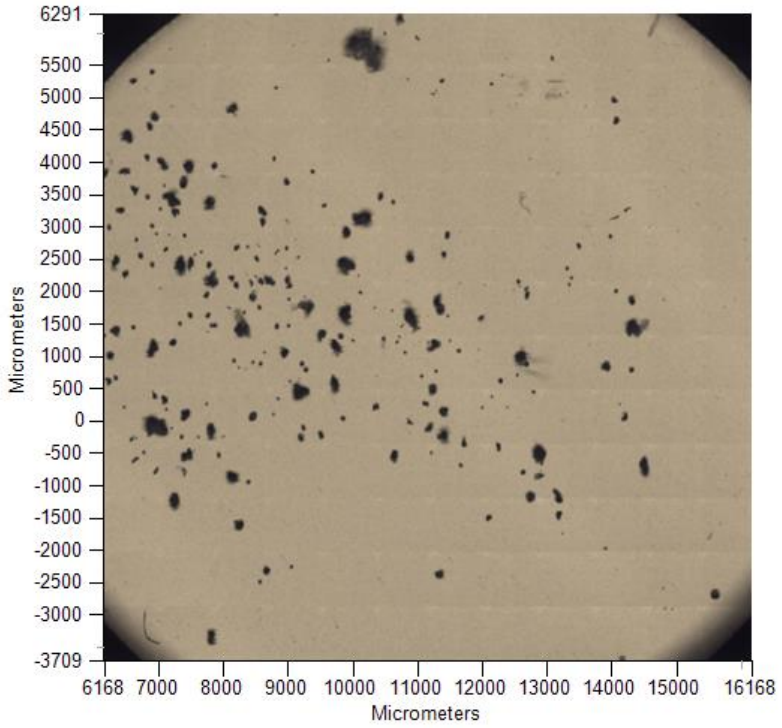
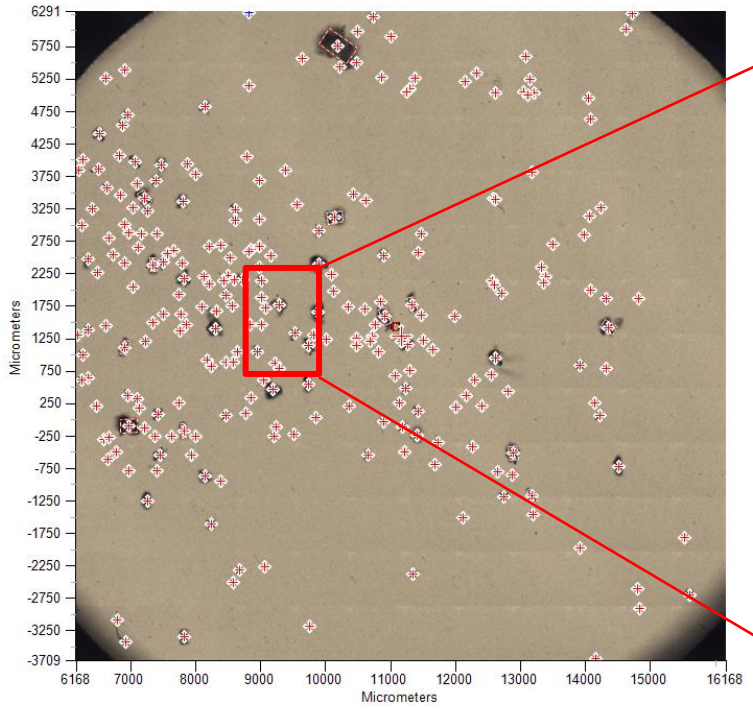
Visible Imaging to Identify Particles

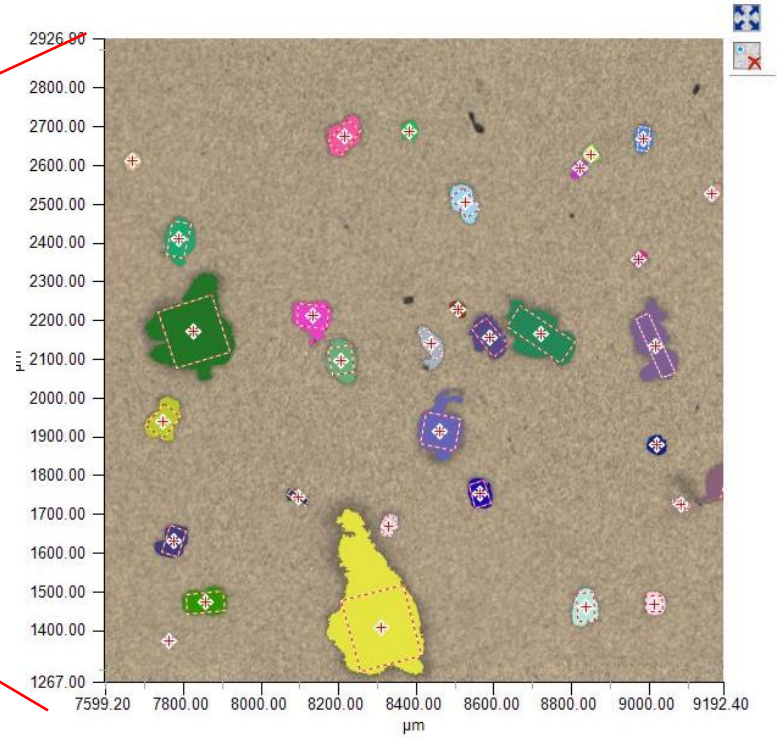
- Automated stage allow for large visible image collection followed by automatic IR data collection on all particles



Collect visible image



“Analyze Image” process will identify each particle as area of interest and specify aperture dimensions



Particle size and shape information collected

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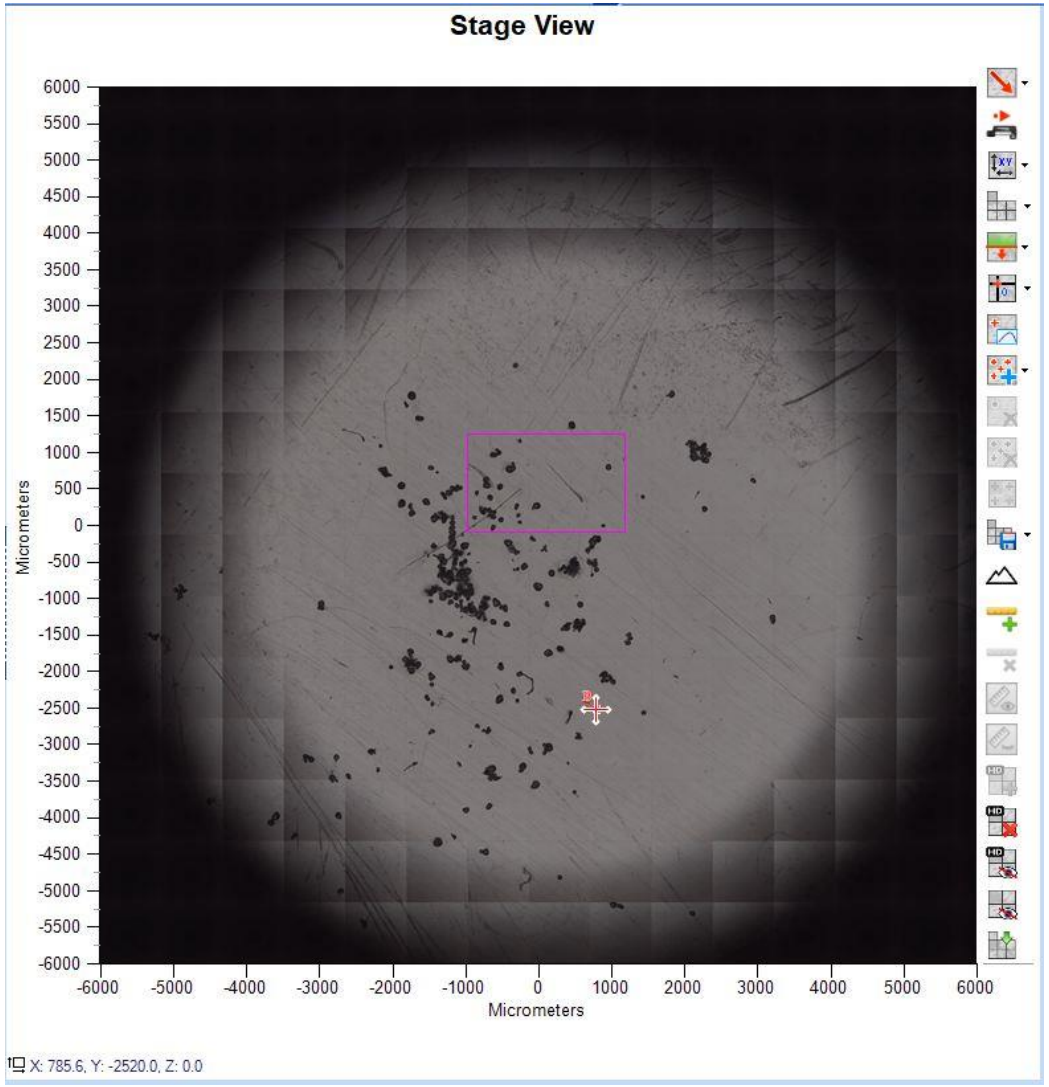
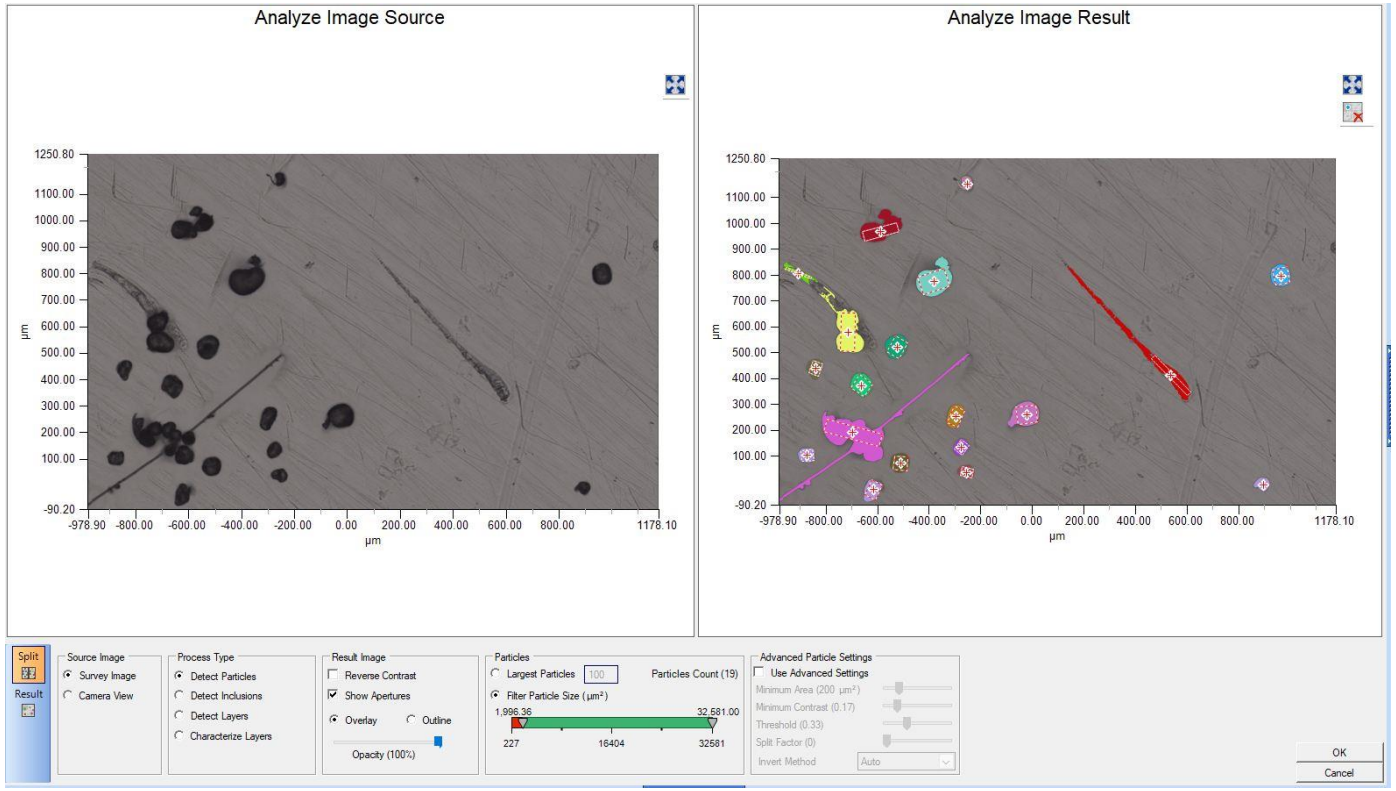
Microplastics on KBr Window - Transmission





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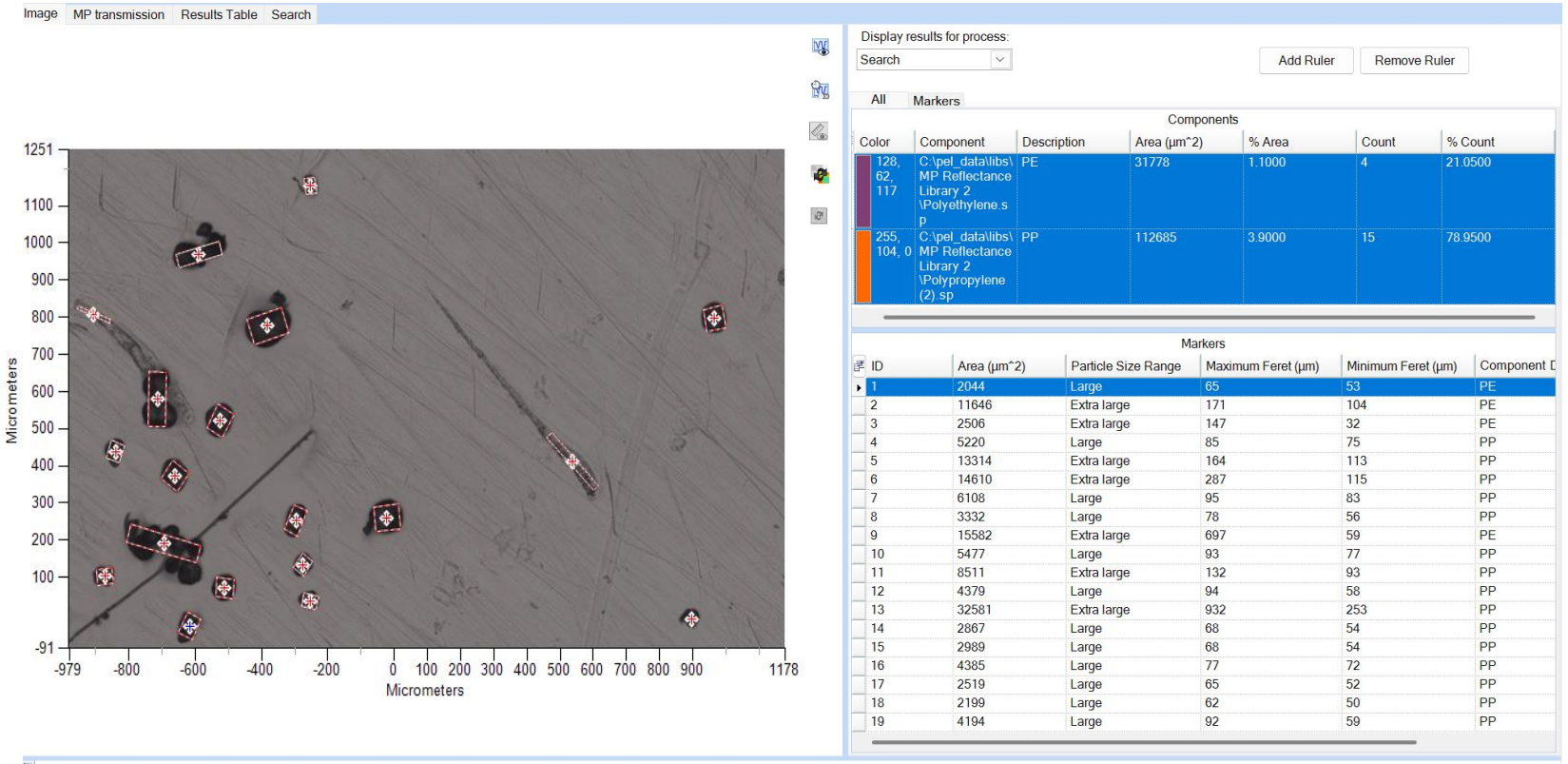
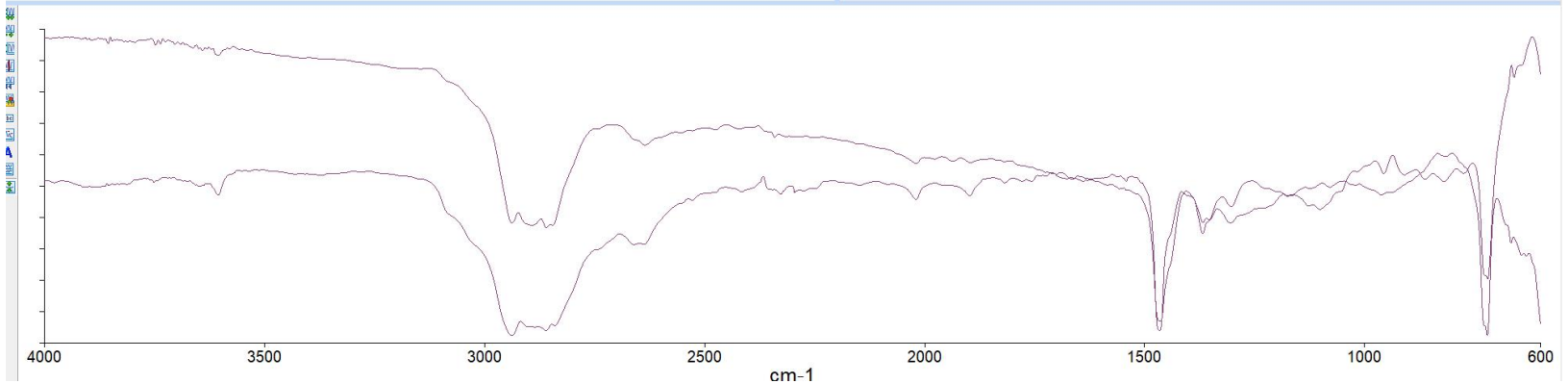
Microplastics on KBr Window - Transmission





- Area in um^2
- Count of identified particle type
- % Count of identified particle type
- Particle Size Range

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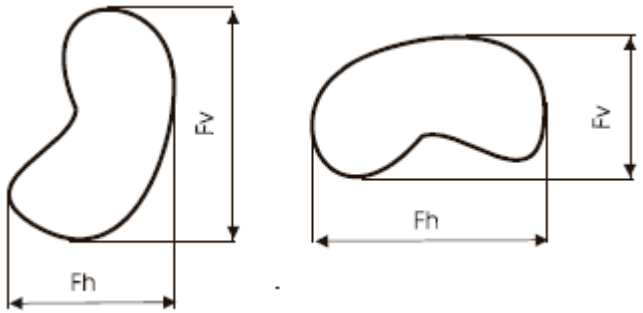
Microplastics on KBr Window - Transmission

- Feret diameter
  - The Feret diameter or Feret's diameter is a measure of an object's size along a specified direction. In general, it can be defined as the distance between the two parallel planes restricting the object perpendicular to that direction.
- Maximum Feret
- Minimum Feret



Components

Color	Component	Description	Area (µm^2)	% Area	Count	% Count
128, 62, 117	C:\pel_data\libs\MP Reflectance Library 2\Polyethylene.sp	PE	31778	1.1000	4	21.0500
255, 104, 0	C:\pel_data\libs\MP Reflectance Library 2\Polypropylene (2).sp	PP	112685	3.9000	15	78.9500

Markers

ID	Area (µm^2)	Particle Size Range	Maximum Feret (µm)	Minimum Feret (µm)	Component Des	Search Score
1	2044	Large	65	53	PE	0.888752
2	11646	Extra large	171	104	PE	0.884403
3	2506	Extra large	147	32	PE	0.127813
4	5220	Large	85	75	PP	0.956821
5	13314	Extra large	164	113	PP	0.901681
6	14610	Extra large	287	115	PP	0.955945
7	6108	Large	95	83	PP	0.961361
8	3332	Large	78	56	PP	0.93637
9	15582	Extra large	697	59	PE	0.138848
10	5477	Large	93	77	PP	0.960834
11	8511	Extra large	132	93	PP	0.937313
12	4379	Large	94	58	PP	0.955424
13	32581	Extra large	932	253	PP	0.959181
14	2867	Large	68	54	PP	0.953615
15	2989	Large	68	54	PP	0.910249
16	4385	Large	77	72	PP	0.95375
17	2519	Large	65	52	PP	0.957547
18	2199	Large	62	50	PP	0.914263
19	4194	Large	92	59	PP	0.943714

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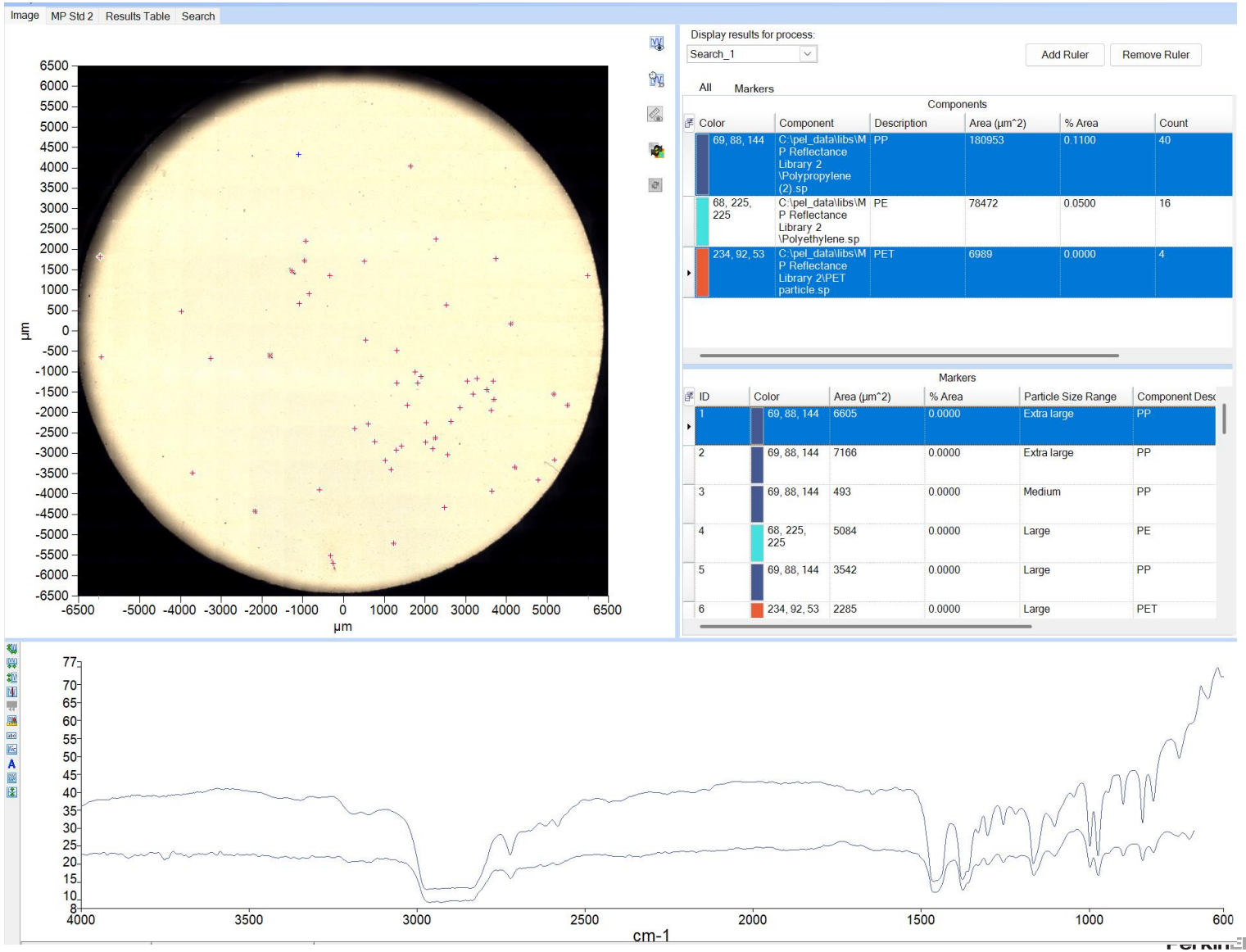
Microplastics on KBr Window - Reflection

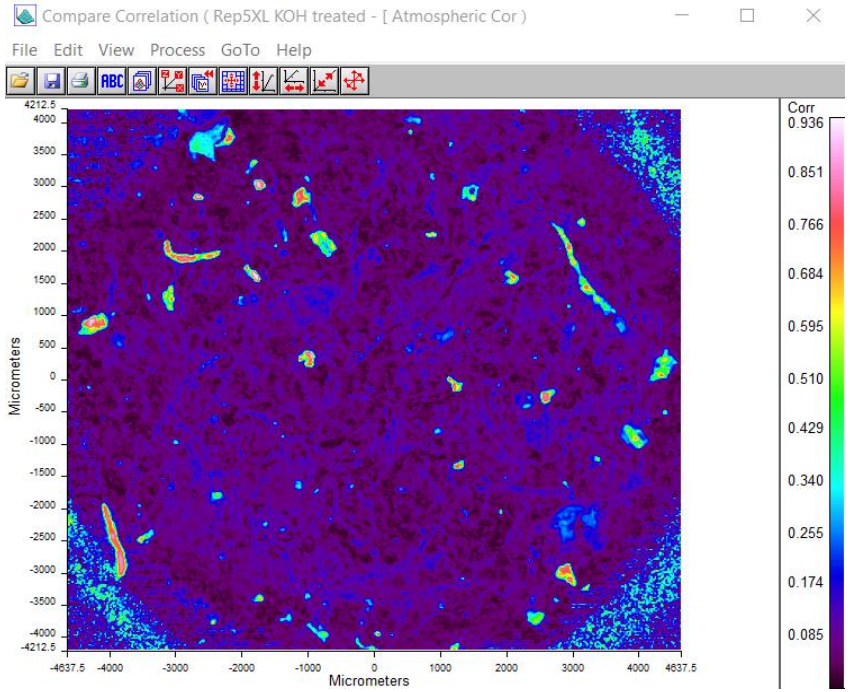
- Particles transferred to gold coated filter
- Gold is an excellent substrate for reflectance
- Filtering and measuring directly on gold filter eliminates transfer step to KBr window or other substrate



Case Study 2: Microplastics in Clean Water and Wastewater

- Two microplastic samples were prepared and measured using IR microscopy
- One clean water spiked with microplastics
- One wastewater sample
- This study illustrates the use of IR imaging to identify microplastics from different water sources

PerkinElmer Spotlight 400 IR Microscope with Spectrum 3



IR Image of Filtered Microplastics

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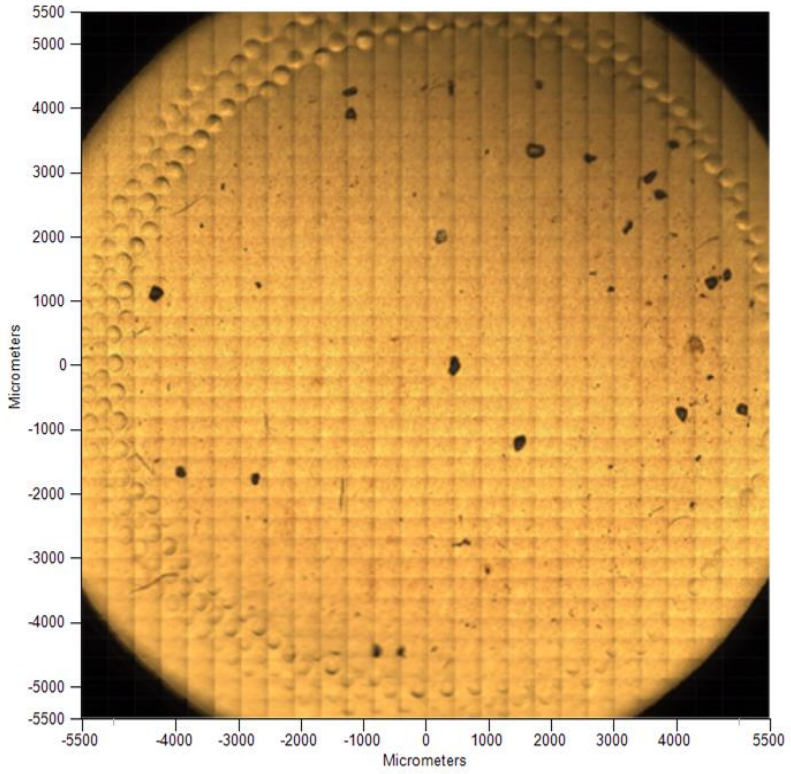
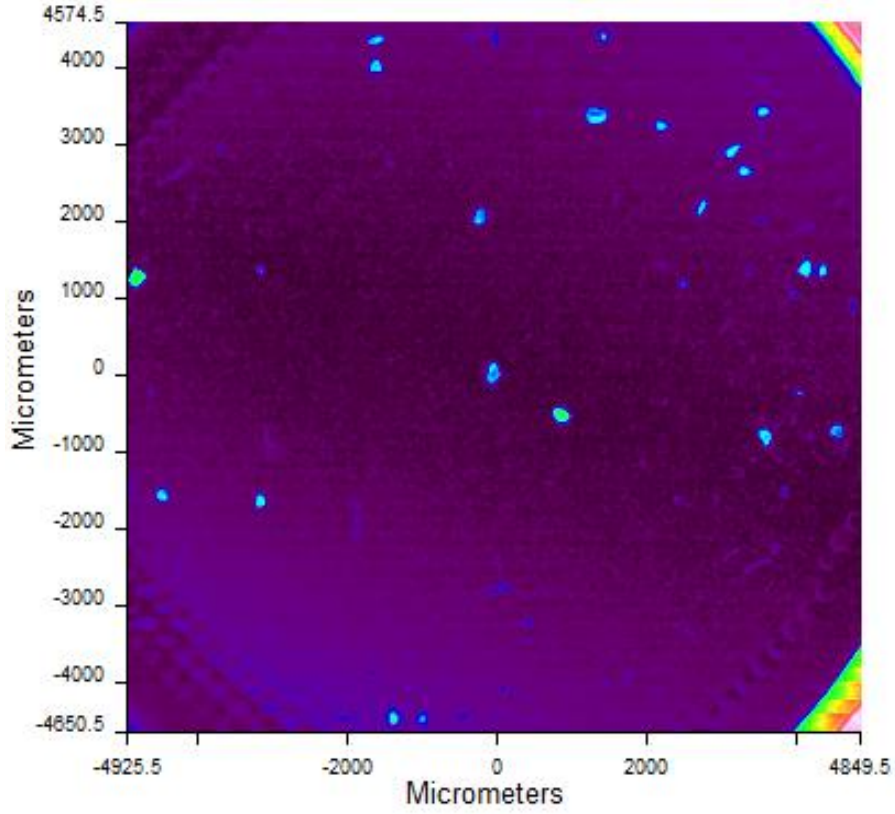
Spiked Clean Water Sample

Visible Image



False-Color IR Image



- Spotlight IR Microscope combines visual imaging with IR imaging
- High-sensitivity array detector collects high-resolution IR images in reflectance mode
- Each pixel in IR image represents an IR spectrum

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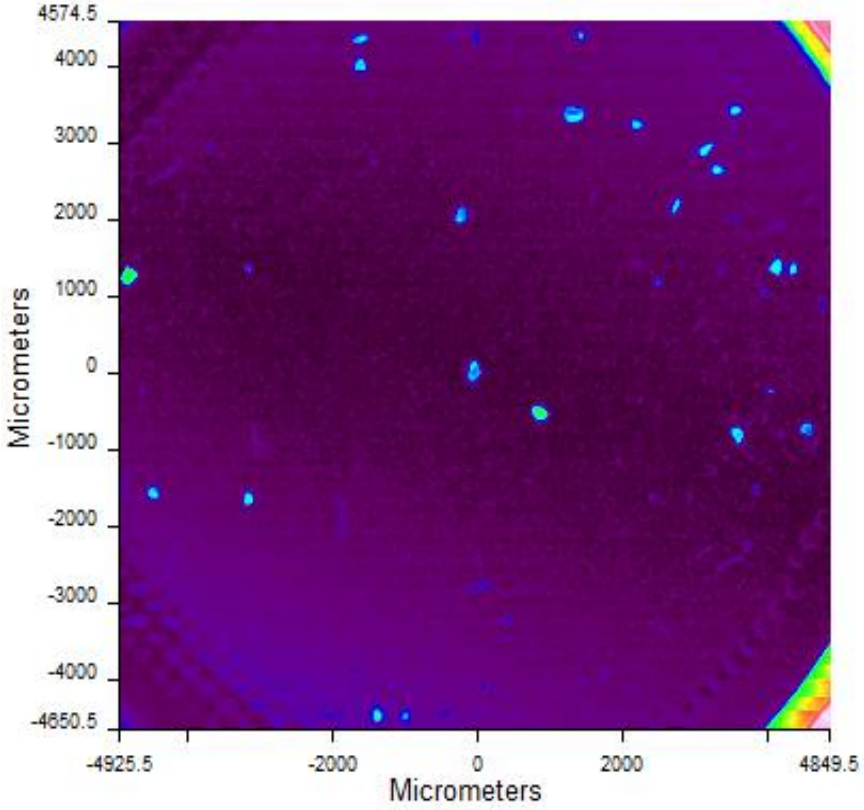
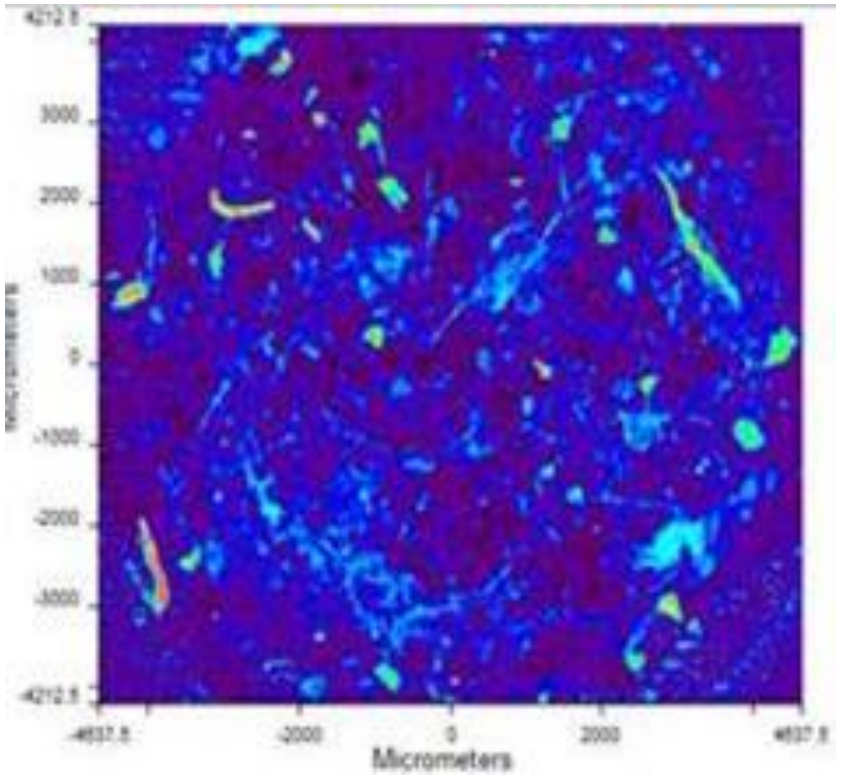
Spiked Clean Water *vs* Wastewater

IR Image Clean Water



IR Image Wastewater



- Spiked clean water shows well defined particles
- Wastewater sample has complex matrix
- Sample treatment helps; however, samples are challenging

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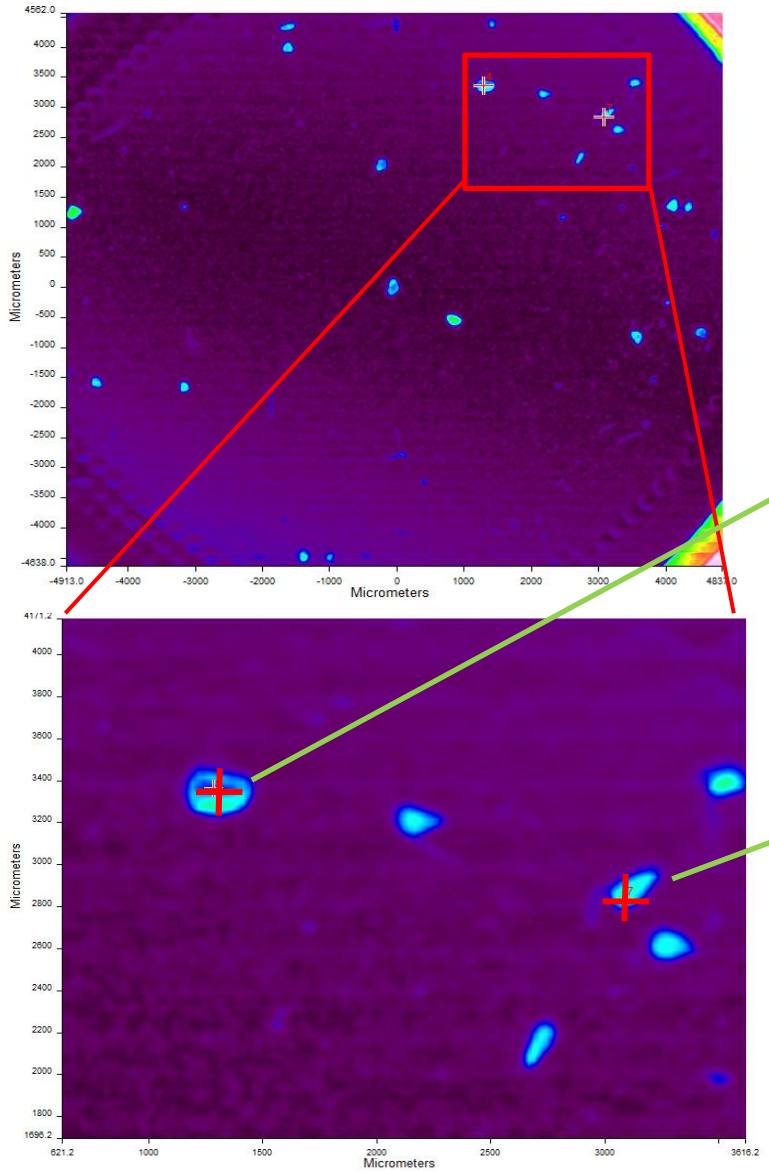
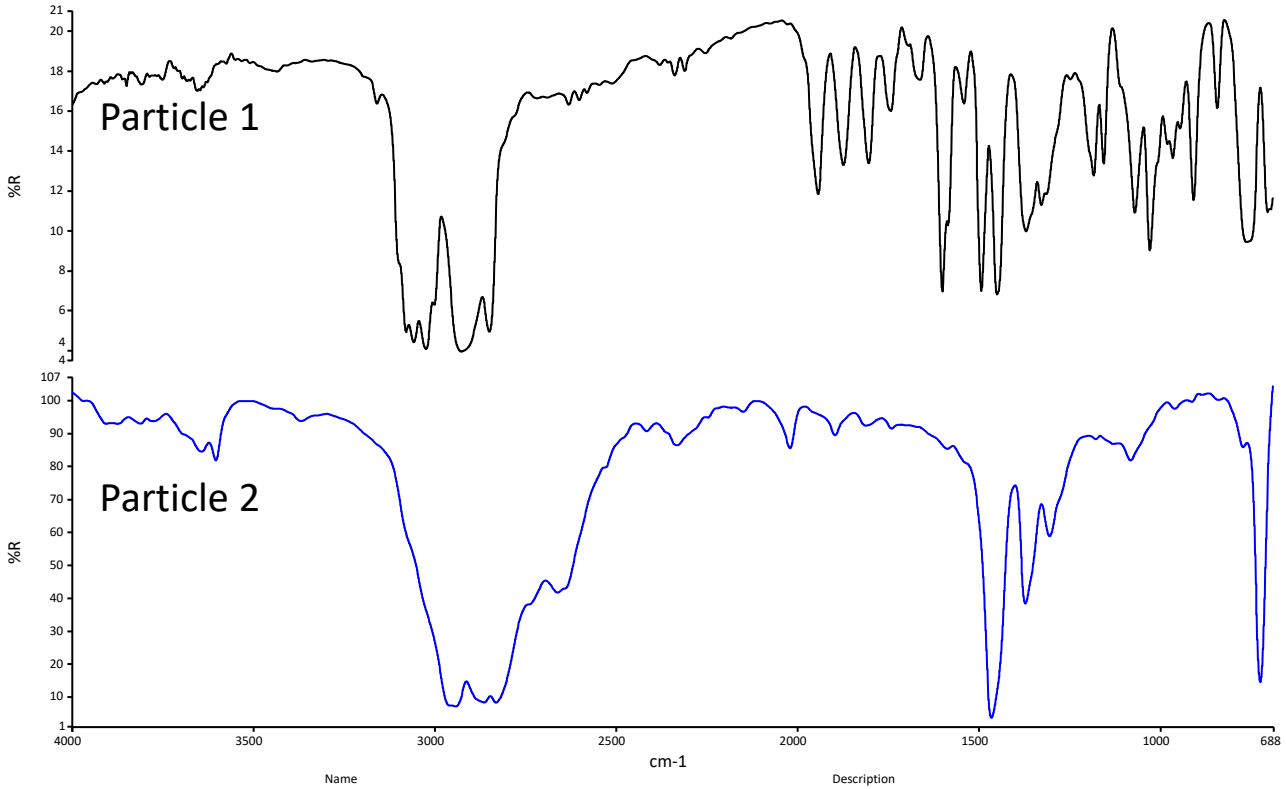
Data Analysis: IR Images Contains Thousands of Spectra



- Moving cursor displays IR Spectrum



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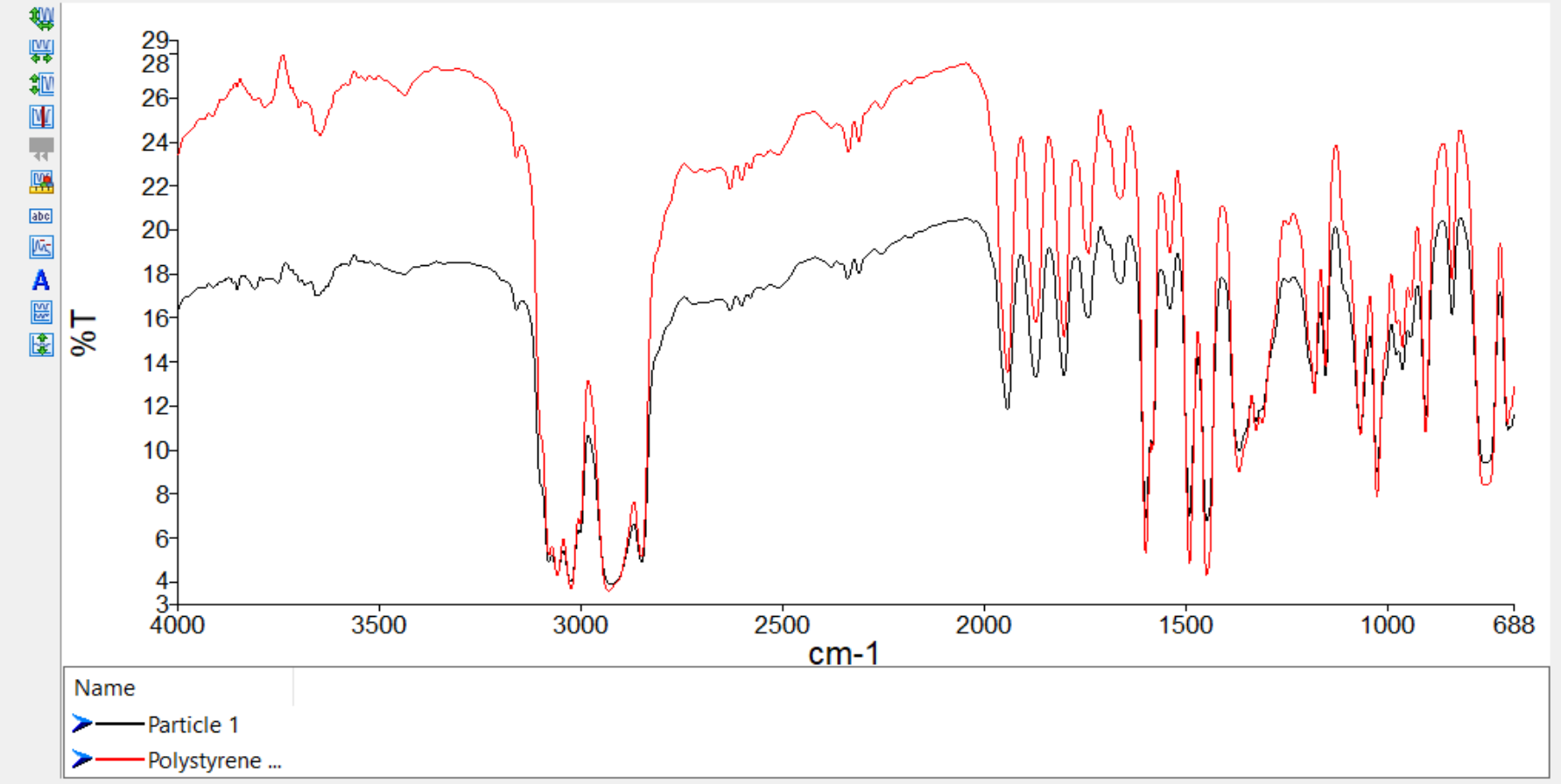
IR Library Search

Source Spectra Search Results:

	Search Score	Sample Name	Search Best Hit Description
1	0.994618	Particle 1	Polystyrene
2	0.815523	Particle 2	Polyethylene



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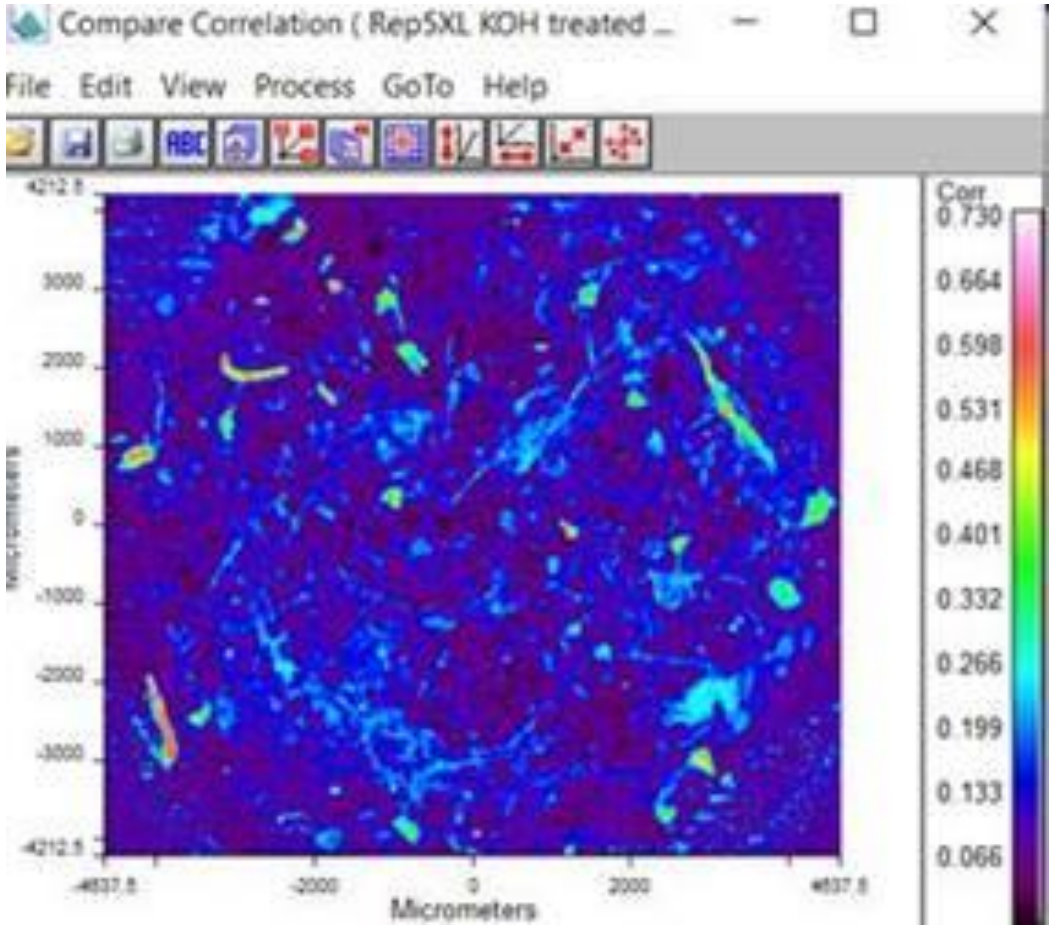
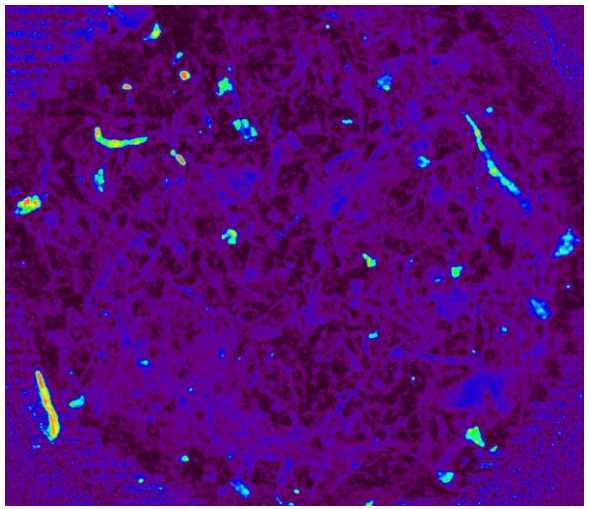
Compare Algorithm to Isolate Particles of Same Identity

IR Image



polyethylene



PET

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Results - Microplastics in Clean Water and Wastewater

Spiked Clean Water Results

Polymer Type:	Polyethylene	PET	Polystyrene	Total
Min size (µm)	54	50	66	50
Max. size (µm)	251	250	202	251
Count	9	7	9	25

Waste Water Results

Polymer Type:	Polyethylene	PET	Total
Min size (µm)	48	47	47
Max. size (µm)	398	415	398
Count	20	10	30

- IR Microscopy can determine identity, count, and size of microplastics from various sources
- Sample treatment and preparation for IR microscopy are critical steps
- Wastewater sample illustrates the difficulty of interference due to high organics loading

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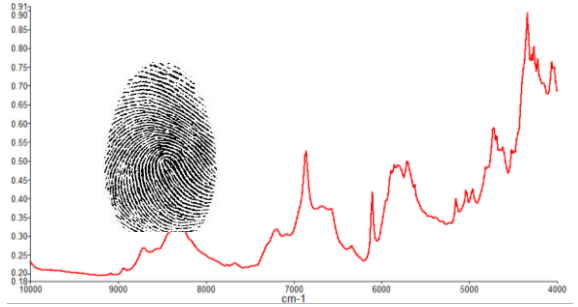
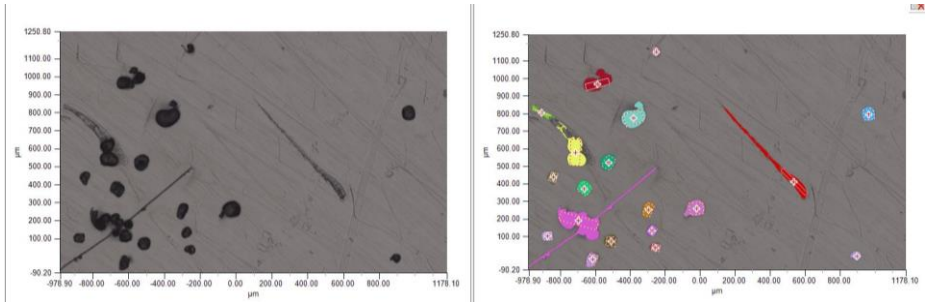
Summary

- Microplastics overview
  - Microplastics are everywhere
  - Research ongoing
  - SOPs, methods, and legislate is in progress
- Infrared spectroscopy
  - FTIR can identify polymers
  - Large “macro” samples can be measured with traditional bench-top IR and UATR
- Infrared microscopy
  - <1mm requires IR microscope
  - Visible images and IR data to determine identity, count, and size
  - Point mode (single-element detector) vs. infrared imaging (array-detector) approaches
  - Difficult matrix increase reliance on data processing tools

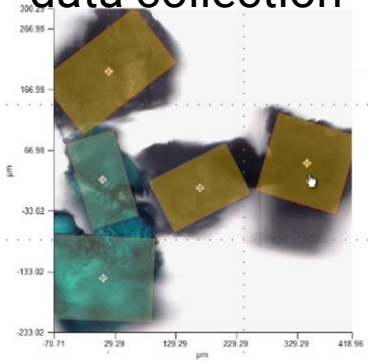
Infrared spectrum



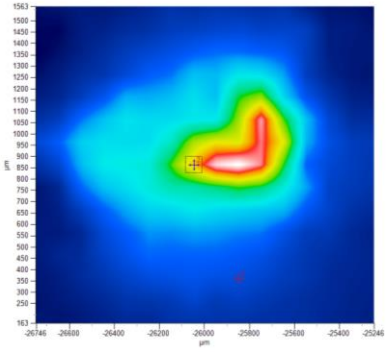
Visible image



Point mode data collection



Infrared image



Thank You!

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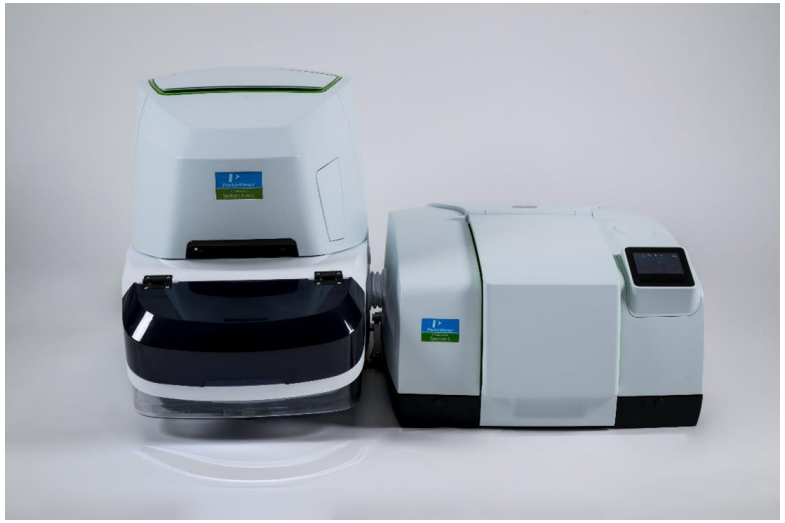

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