Rapid, Automated Analysis of Microplastics Using Laser Direct Infrared Imaging and Spectroscopy

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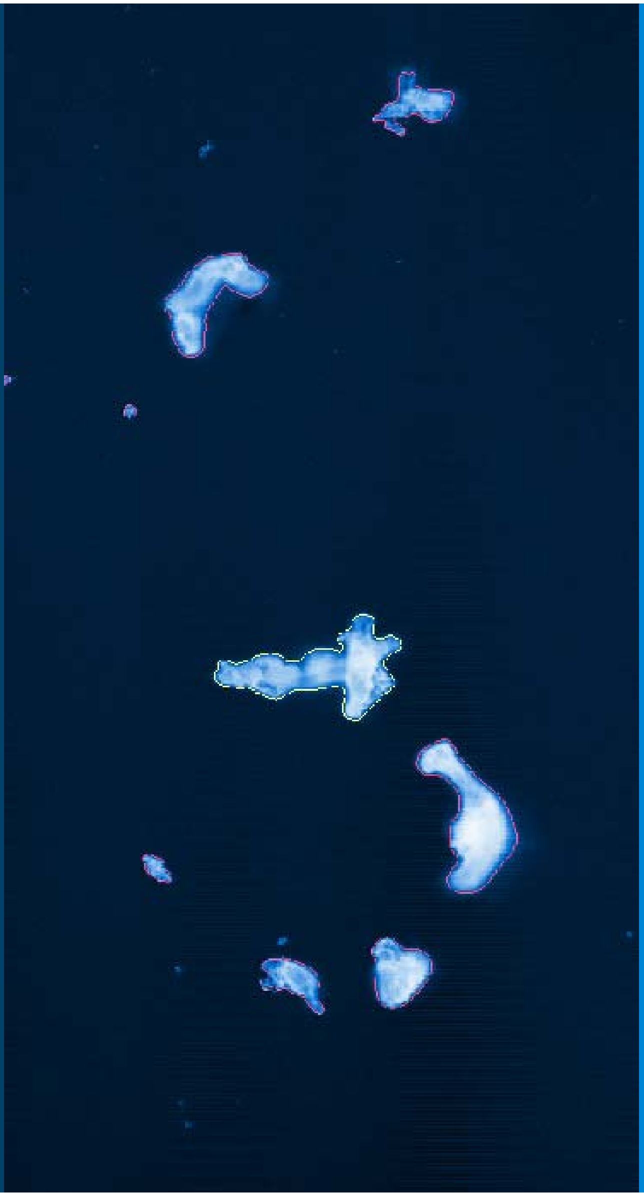

"Big Questions" in microplastics (MPs)

- From where did these plastics originate?
  - Have they penetrated the food chain?
- Are they harmful?
  - Have they absorbed harmful compounds?
- How can the impact be mitigated?

Fundamental questions in the routine analysis of microplastics

- Does my sample (drinking/waste water, food, environmental sample) contain microplastics?
- How many?
- What size are they?
- What are their chemical identities?

Best strategy for answering these questions
=
active area of research

- From where did these plastics originate?
- Have they penetrated the food chain?
- Are they harmful?
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- How can the impact be mitigated?

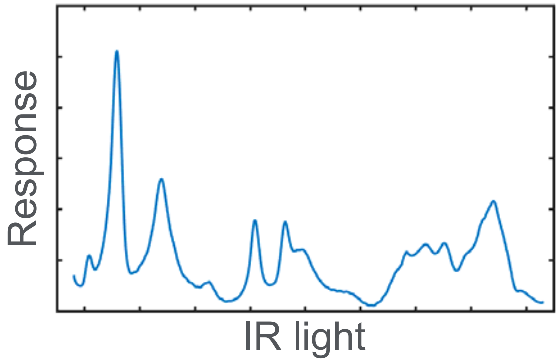
Strategies – what do you do once you've filtered the sample?

- Manual, by-eye approach
  - Inaccurate (unaided by analytical technique)
  - Time-consuming
  - Unlikely to determine chemical identity

- Wet chemistry & related methods
  - Physically intensive and time-consuming
  - Difficult for small or individual particles
  - Destructive – particle is gone, no opportunity for further analysis

- Optical micro-spectroscopy methods
  - Chemically-specific (get particle identities)
  - Non-destructive (leave the door open to further analysis)
  - Can be highly automated

What is optical micro-spectroscopy?



It's a microscope:

- Visible images of particles
- Particle count
- Particle size

It's an infrared (IR) spectrophotometer:

- Determine chemical identity of a chemical compound by measuring interaction with infrared light

Single combined platform

- MP counting, sizing, identification in one technology
- Non-destructive: can perform subsequent analysis
- Potential for automation or MP-specific workflows

Agilent 8700 LDIR Chemical Imaging System

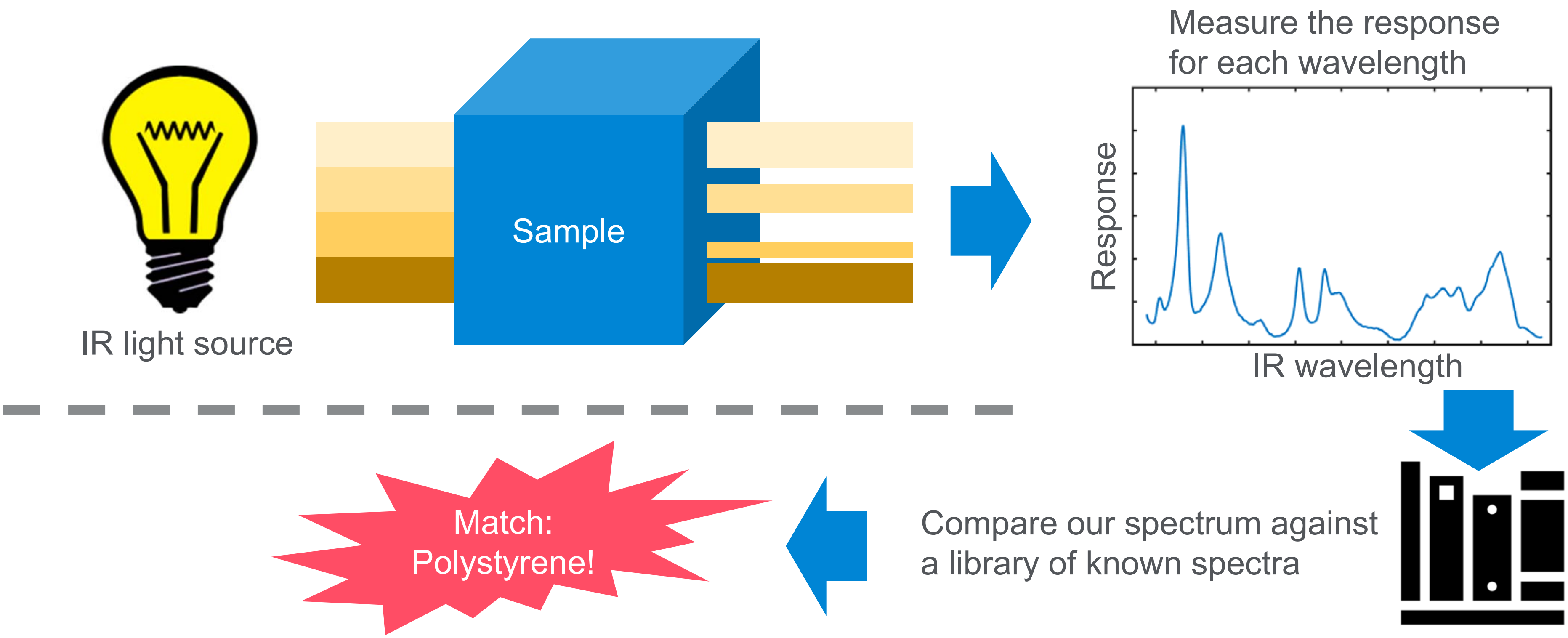
Routine, robust, automated microplastics analysis by non-experts

- Fully-automated infrared microscopy platform
- Utilizes a new *laser light source*
  - Fast, crisp infrared images
  - Laser-focused spectroscopy – ideal for small particles
- Removes many of the ‘chores’ associated with traditional infrared microscopy
  - No liquid nitrogen, autofocus, auto collection of background data, etc.

IR Micro-Spectroscopy – what does it *actually do?*

A material's *response* (absorbance) to a wide range of infrared wavelengths produces a spectrum (signature) unique to each compound.

Thus, IR spectroscopy can be used for chemical identification!



IR Micro-Spectroscopy – what does it *actually do?*

A traditional IR micro-spectroscopy instrument spreads light over an area.

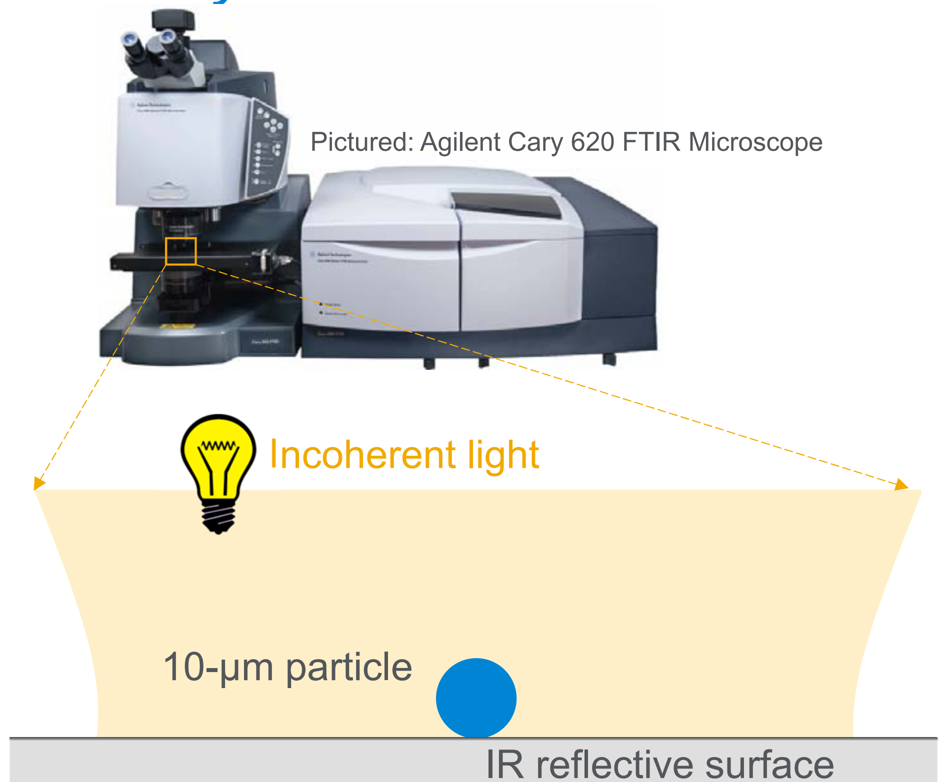
The good:

- Look at multiple particles simultaneously

The bad:

- Weak signals, slow analysis
- Measures ‘empty space’ between MPs
- Significant data processing
- >30 seconds per spectrum typical

Solution: use a laser!



Only a tiny fraction of the light can be absorbed.

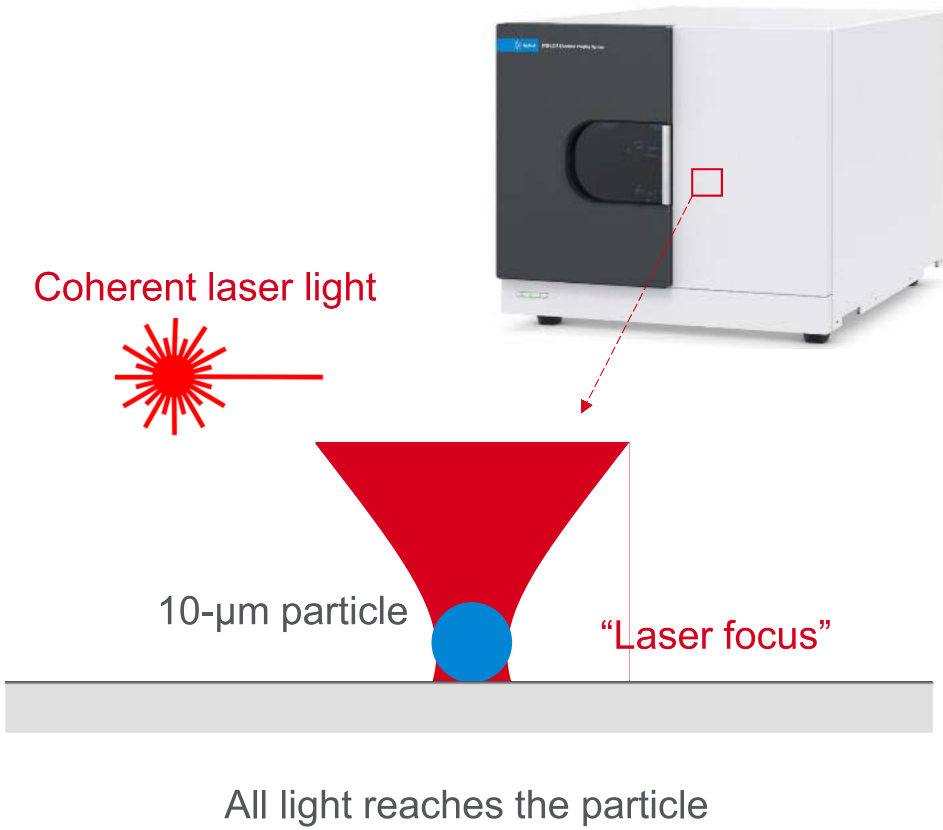
Laser Direct Infrared (LDIR) Spectroscopy

New instrument architecture

- Bright, focused infrared laser source
- Full automation provides a tailored particle-specific workflow
- Intuitive software and operation reduces user influence and enhances consistency

Other advantages

- One second per spectrum
- Targeted analysis – near-zero time measuring empty space
- Real-time data processing and feedback



Workflow

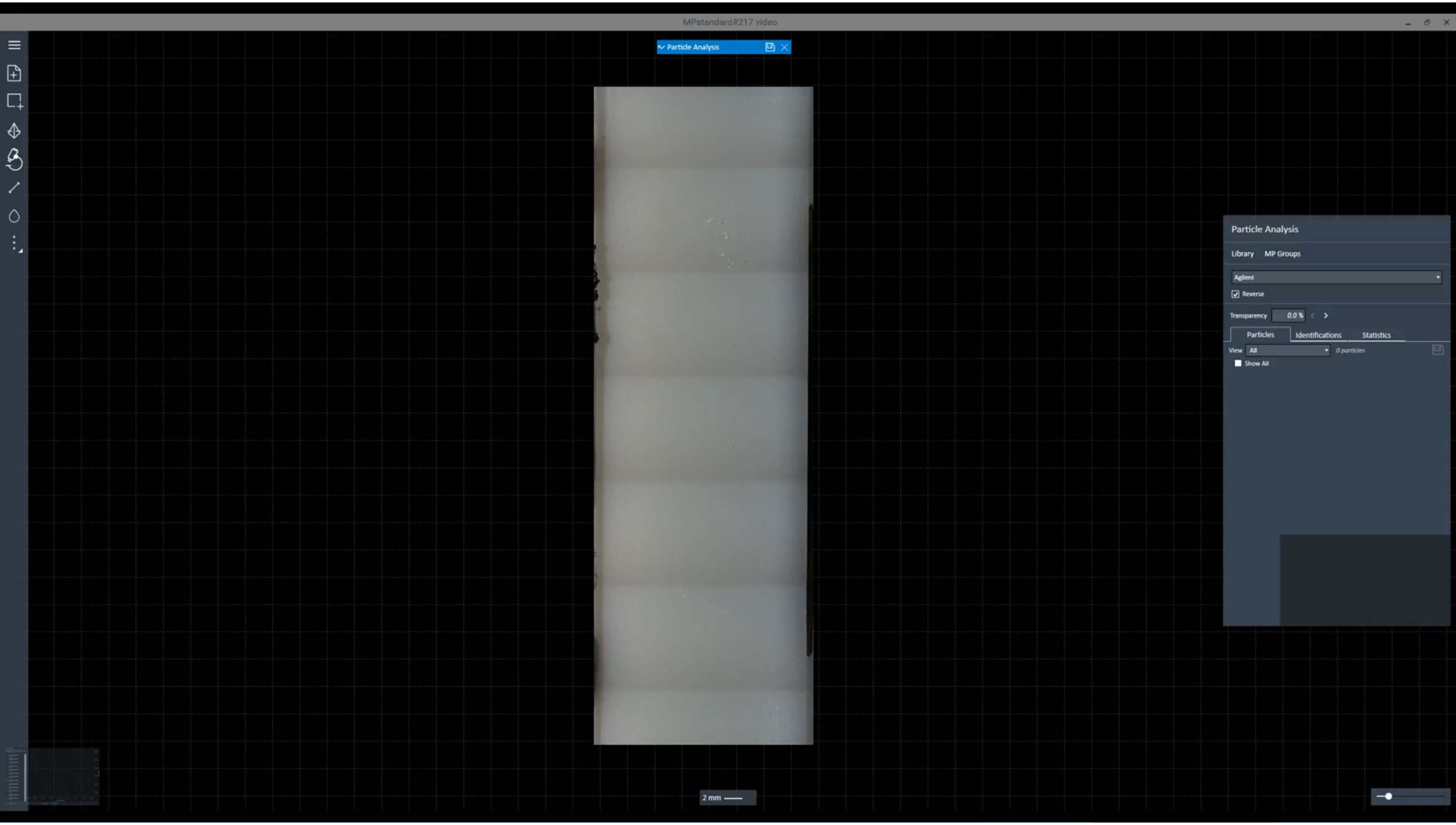
Sample Preparation

Filter, digest, etc. → Suspend in Ethanol and Sonicate → Aliquot on reflective microscope slide

Analysis Workflow – 8700 LDIR + Clarity software

Insert Sample → Locate particles → Acquire Spectrum → Compare to library → Update Statistics

These steps are repeated for each microparticle located



MPstandard#217 video
Particle Analysis
Particle Analysis
Library
MP Groups
Agilent
Reverse
Transparency
0.0 %
Particles
Identifications
Statistics
View
All
0 particles
Show All
2 mm

Workflow

- Insert the sample
- Select an on-board microplastic library
- Draw a box over an area of interest
- Software acquires an image, automatically identifies particle locations
- User may specify a size range (ex. exclude very large or small particles)
- Software automatically takes a spectrum of each particle, performs a library match, continually updates statistics of all particles!

LDIR imaging provides excellent contrast for identifying particle count, locations, sizes

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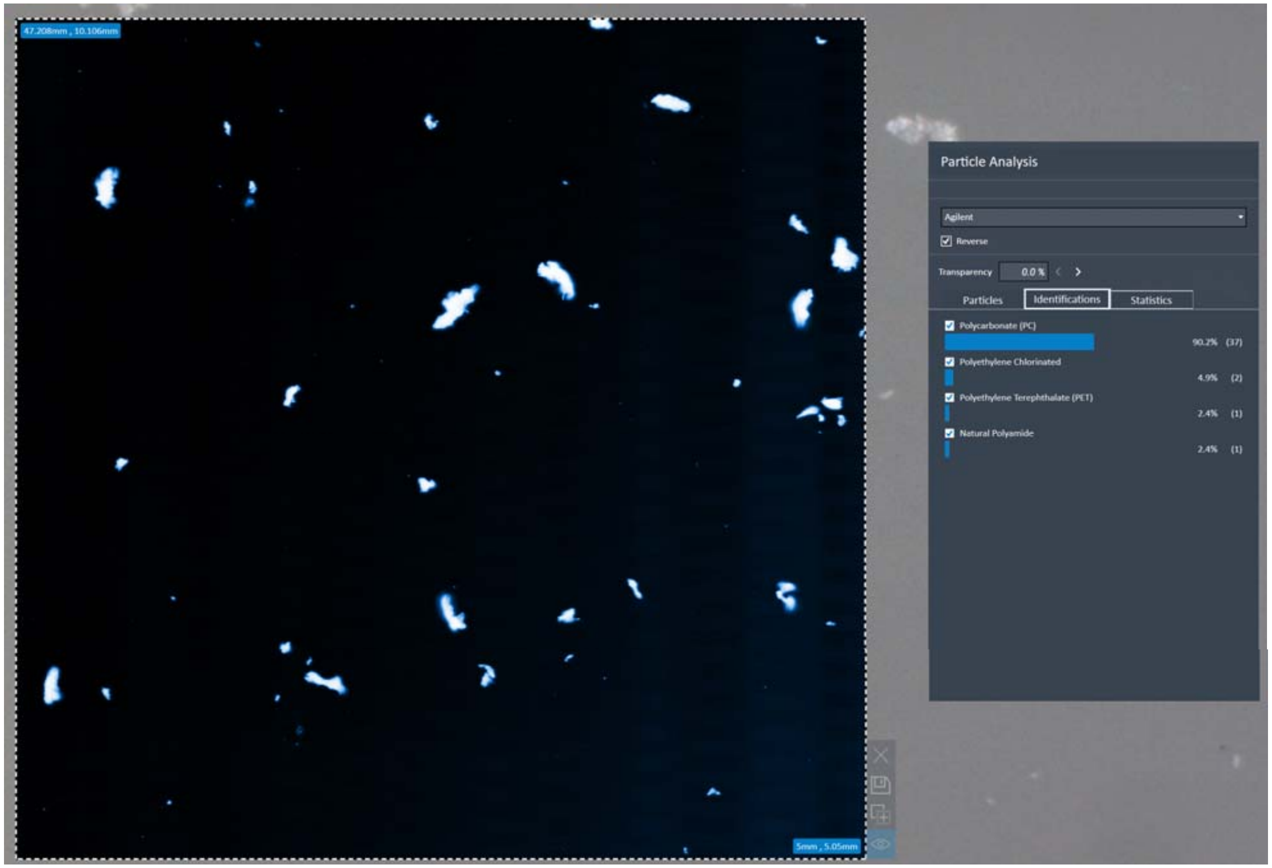
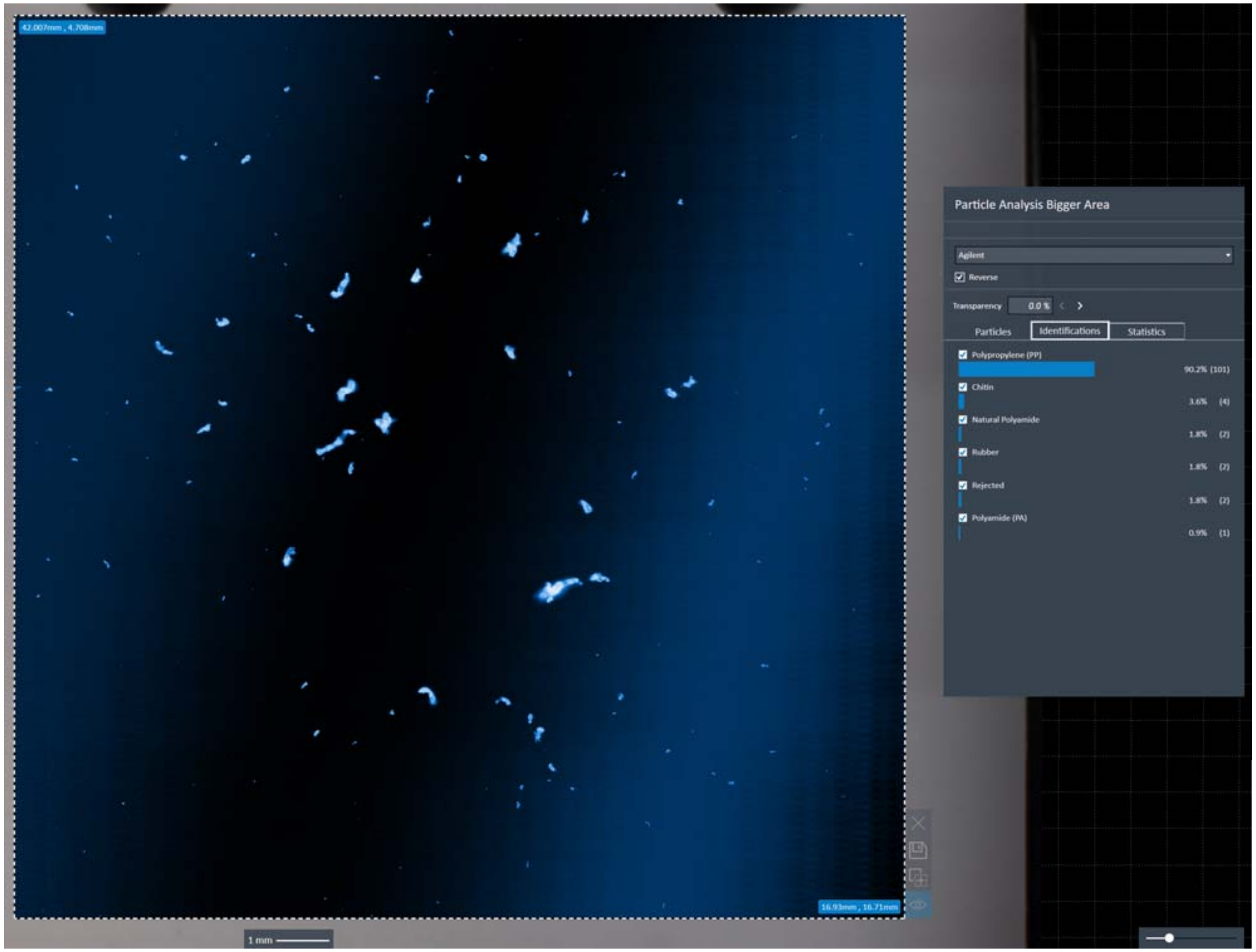
Training set samples – polycarbonate microplastics

- 41 identified particles over 25mm^2
- Size range: 220µm – 25µm
- 90% matched as polycarbonate
- Of the others:
  - One was truly polyamide (dust)
  - One was polyester fiber (possibly from clothing)



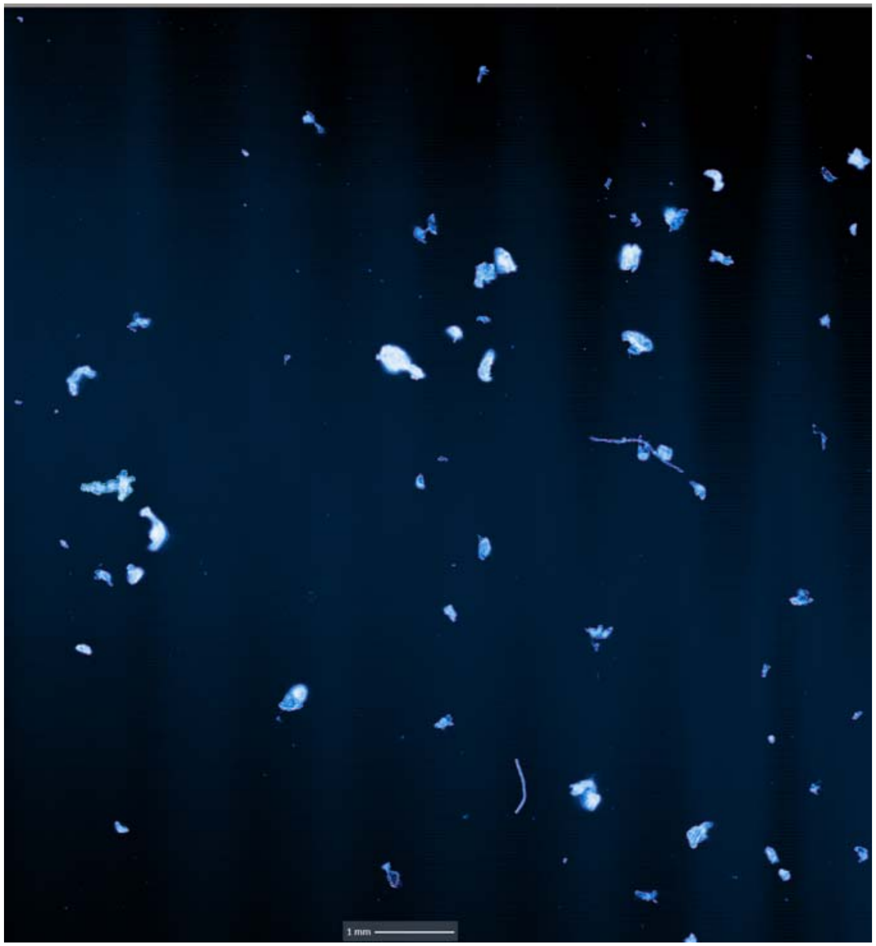
Training set samples – polypropylene microplastics

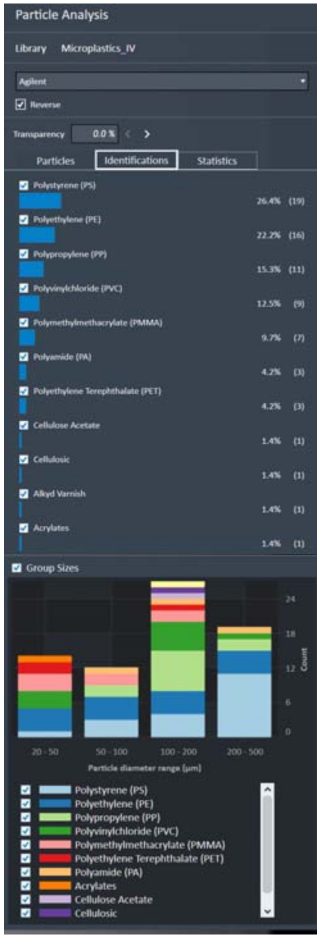
- 112 identified particles over 283mm^2
- Size range: 495µm – 25µm
- 90% matched as polypropylene
- Of the others:
  - Several were truly polyamide (dust)
  - Other contaminants



Testing set samples – mixed microplastics

- Blend of 7 known components
- 72 identified particles over 126mm^2
- Size range: 435µm – 25µm
- 94% match with known components in the blend
- Some particles were organic contaminants (cellulose-based)





Real-world environmental sample

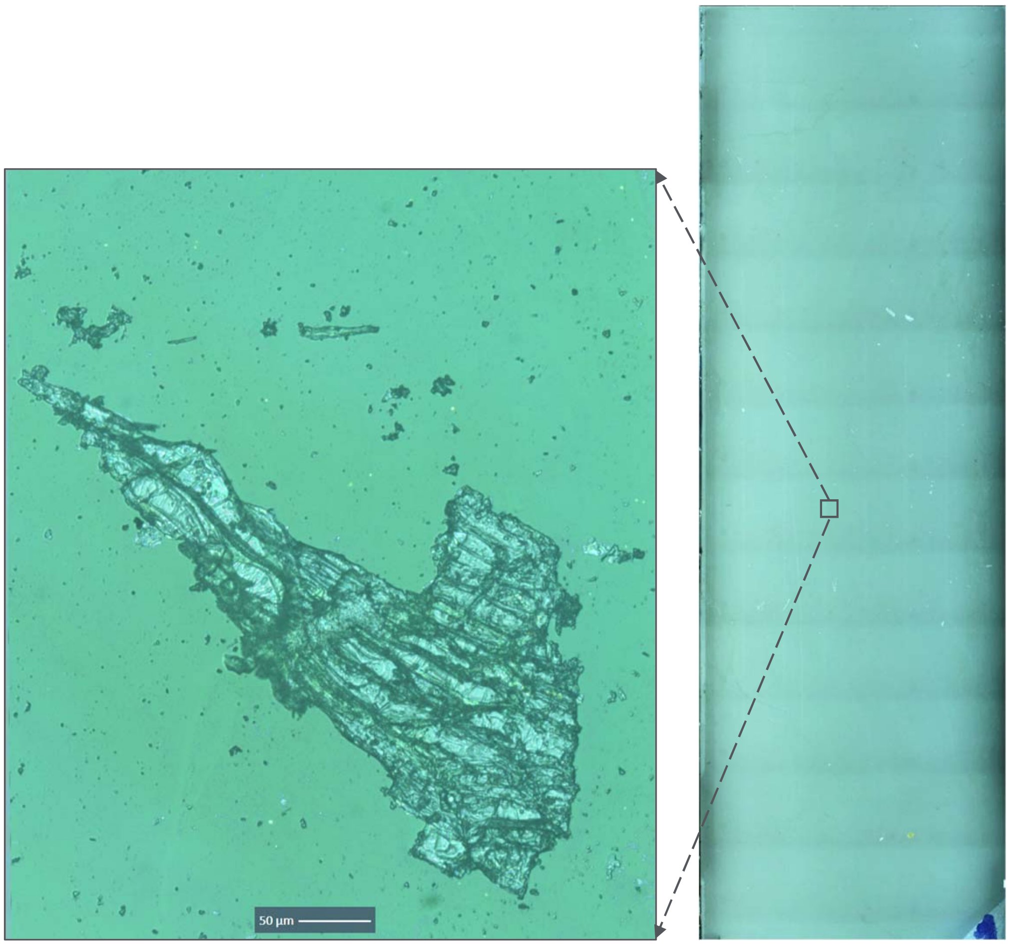
Effluent from wastewater treatment plant

Sewage is used as fertilizer -
route for microplastic contamination

Sample preparation protocol[1]

- Filtration and enzymatic digestion (protease, cellulase, chitinase, H_2O_2) to remove organic debris
- Density separation to remove inorganics like sand

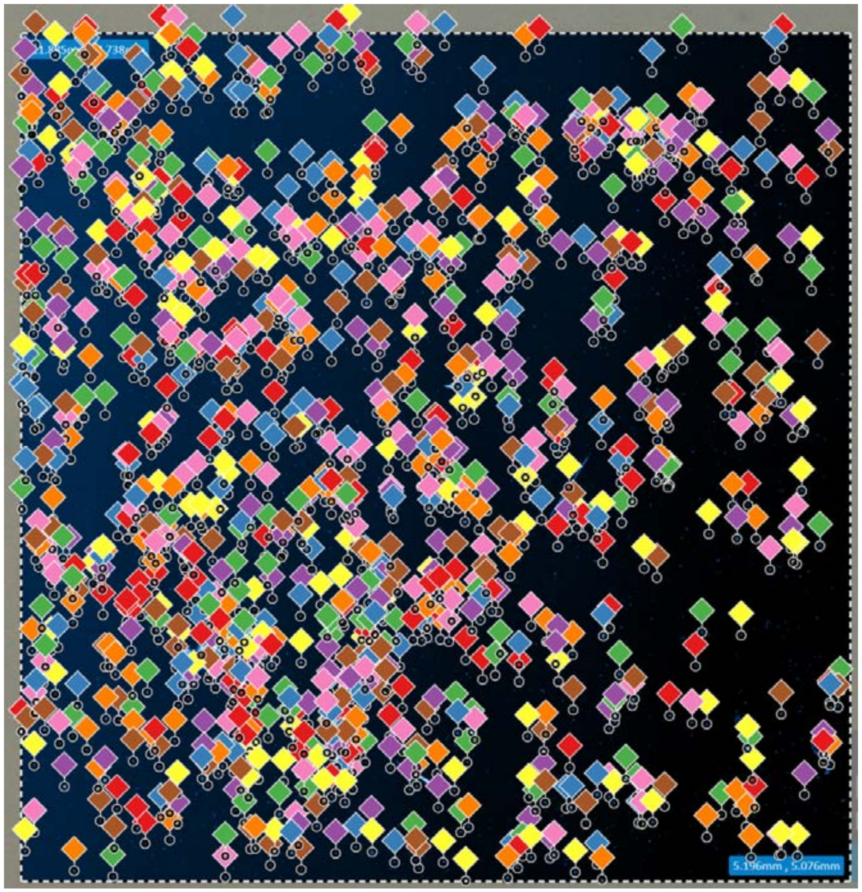
Retained particles are suspended and aliquoted onto a slide for analysis.



[1] Löder *et al.*, Enzymatic Purification of Microplastics in Environmental Samples. Environmental Science & Technology 2017 51 (24), 14283-14292

Sample courtesy of Dr. Jeff Prevatt, Deputy Director, Treatment Division, Pima County Regional Wastewater Reclamation Dept., Tucson, AZ

LDIR Analysis of Wastewater Sample



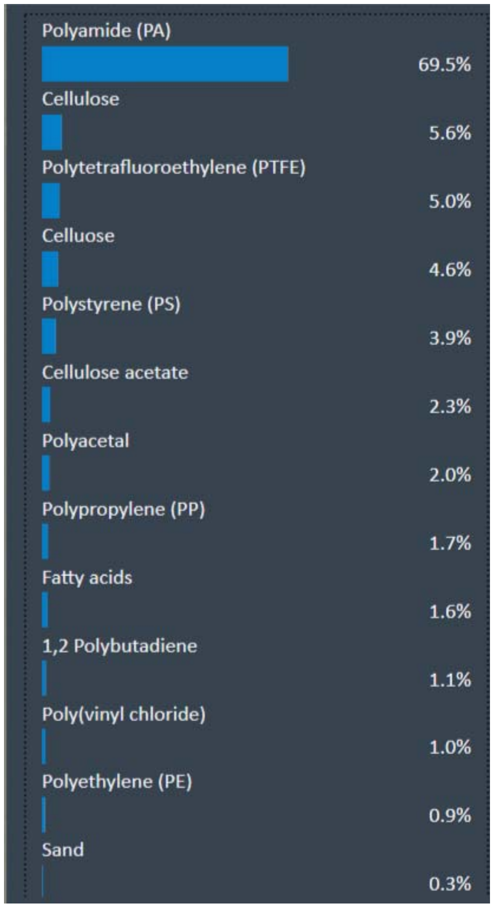
5 mm × 5 mm analyzed

1028 particles found

2.5 hours total time

- 8 seconds per particle
- Actual spectrum time 1 second
- All automation, data processing overhead included in this figure
- No operator required for bulk of analysis

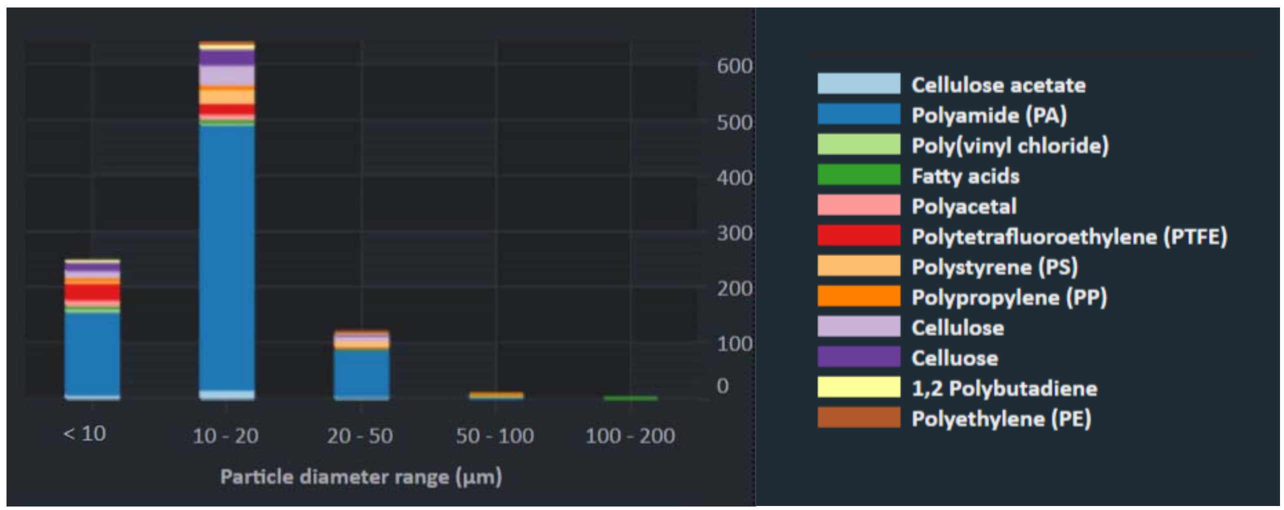
Wastewater particle identification (1028 particles)



Polyamides were most prevalent

- Wool, silk, nylon from textiles
- Undigested protein-rich debris

Followed by cellulosic particles, PTFE (Teflon), polystyrene



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Agilent 8700 LDIR Chemical Imaging System

Routine, robust, automated microplastics analysis by non-experts

Recently announced; shipping later this year.

Meanwhile…

- Improving speed
- Pushing to even smaller particles
- Enhancing statistical analysis and reporting
- Validating on real-world samples from diverse sites in external labs

Workflow still in R&D—we need your feedback!

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Thank you!