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

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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!

Measure the response for each wavelength

**Match:** Polystyrene!

Compare our spectrum against a library of known spectra
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
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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
Training set samples – polycarbonate microplastics

- 41 identified particles over 25mm²
- 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 126mm²
- 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

• Filtration and enzymatic digestion (protease, cellulase, chitinase, H₂O₂) 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
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!