PHOTOTHERMAL
SPECTROSCOPY CORP

Sub-micron IR and Raman spectroscopy for chemical identification of microplastics

Microplastics have recently gained increased scientific and political exposure and are widely recognized as a pressing environmental and health issue. Research into microplastics has seen exponential publication growth with a corresponding increase in funding to investigate both human health and global environmental impacts.

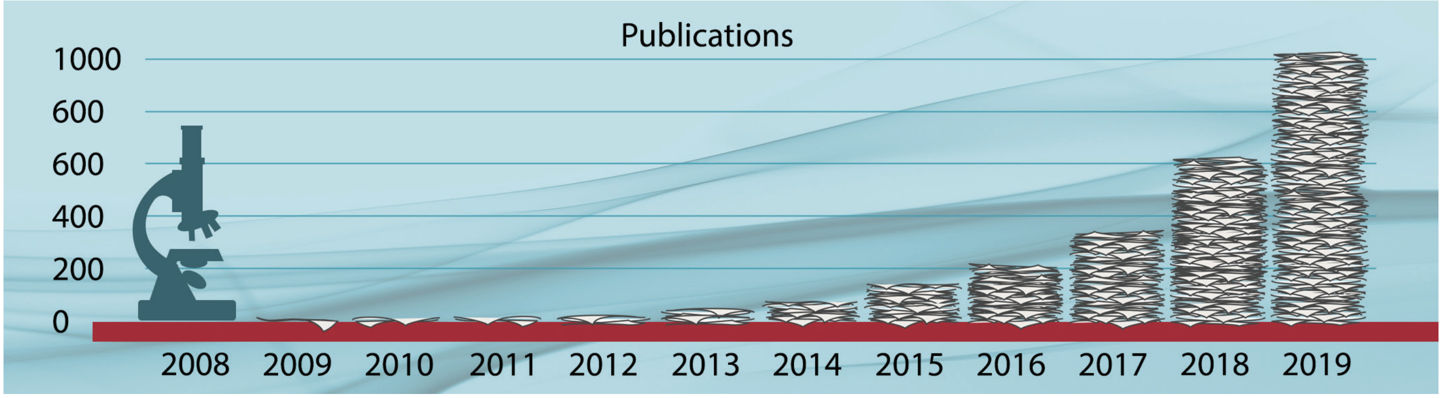
- O-PTIR uniquely provides accurate automated IR measurement of microplastics from sub-micron to 100µm's
- O-PTIR provides artifact free spectra that are directly searchable against existing FTIR databases
- Automated measurement and ChemID wth featurefindIR and micro-chemID
- O-PTIR overcomes many of the limitations of Raman while providing complementary and confirmatory results
- O-PTIR and Raman spectra of microplastic particles collected simultaneously
- Co-located fluoresence microscopy and O-PTIR provides a unique combination to accurately and quickly locate and identify only polymeric particles thus increasing sample throughput


Microplastics in the ocean

The microplastics size range with the most potential to cause biological harm is less than 20µm

→ **This concern has** driven US federal and state governmental bodies to enact new legislation and regulation, such as California Senate Bill 1422 (2018) which requires analysis of microplastics in water sources for drinking water. Additionally, the recent European Drinking Water Directive introduces new regulations for monitoring microplastics. Similar requirements are expected to be adopted internationally in coming years, driving increased demand for microplastics testing.

There are three key drivers for chemical and morphological analysis of MP particles: firstly, to understand the origins of microplastics to help take steps to reduce their release; Secondly, to assess potential hazards of microplastics to the environment and human health; and finally, to identify potential ways to remove microplastic particles from the environment.



Microplastics scientific publications/year[1]

Recent studies[1-4] have suggested that when we consider the size of microplastics, the size range with the most potential to cause biological harm is the smaller size fraction (<20µm), which is therefore an important threshold to achieve.

A range of techniques are used for characterizing the many different aspects of microplastics. The most used techniques for chemical analysis of microplastics are conventional Fourier Transform Infrared (FT-IR) and Raman spectroscopy, but these techniques have several limitations in terms of spatial resolution, measurement accuracy, throughput, photodamage, fluorescence interference or other issues that prevent widespread use of these techniques for <20µm microplastic particles.

Optical Photothermal Infrared (O-PTIR) spectroscopy is an emerging but rapidly growing super-resolution technique that provides chemical analysis via infrared spectroscopy, but with a spatial resolution of ~500nm, around 10-30X better than conventional FT-IR microspectroscopy without any spectral artifacts caused by particle morphology (shape and size). O-PTIR also avoids the issues of low sensitivity, fluorescence interference, or photodamage issues that negatively impact Raman.

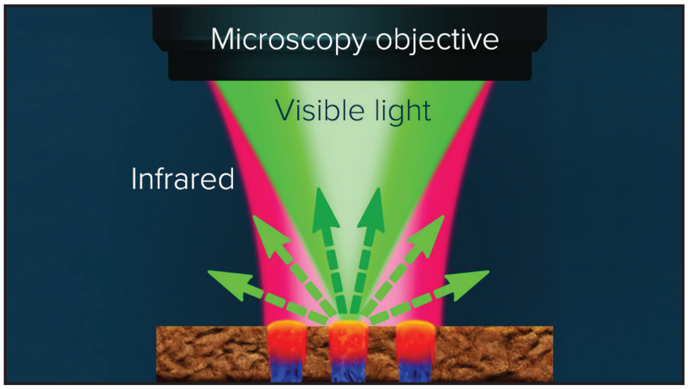


Figure 1: A pulsed tunable, IR source is focused on sample. Absorbed IR light causes the sample to heat up, creating a photothermal response. The visible probe laser centered in the diameter of the IR illuminated area detects the photothermal response from the sample upon IR absorption

Optical photothermal IR (O-PTIR) spectroscopy

The O-PTIR technique overcomes the IR diffraction limit associated with traditional IR techniques microscopy by illuminating the sample with a mid-IR pulsed tunable laser and detecting the infrared absorption with a second visible laser probe beam (532nm). When the mid-IR laser is tuned to a wavelength that excites molecular vibrations in the sample, absorption occurs, thereby creating photothermal effects, e.g., sample surface expansion and a change in refractive index. The visible probe laser, focused to ~ 0.5µm spot size, detects this photothermal response via a modulation induced in the scattered visible light, as shown in Figure 1. The IR laser can be swept through the wavenumber range in two seconds or less to obtain an IR spectrum.

Comparison of O-PTIR and FT-IR spectra for plastics

To understand the correlation of O-PTIR with standard FT-IR, Boke et al,5 undertook a significant evaluation of a wide range of plastics. The researchers determined that O-PTIR spectra were in very good agreement with the FT-IR spectra types, as highlighted in Figure 2. Full details of the comparison can be viewed in the publication.

O-PTIR spectroscopy of submicron plastics

As a demonstration of O-PTIR capability to measure sub-micron IR plastics, a range of plastic beads were analyzed by the mIRage system. Figure 3 shows an optical image and corresponding IR spectra of Polystyrene (PS) plastic beads from ranging from 500nm to >4µm in diameter.

Sub-micron IR shape and size independent spectra

As a demonstration of O-PTIR capability to measure various particle shapes and sizes with artifact-free data collection in the presence of highly scattering salt crystals, we created a mixed model sample consisting of Polystyrene (PS) beads (900nm, 2µm, 4.5µm and 10µm) and Poly(methyl methacrylate) (PMMA). When using an FT-IR/QCL system, not only would these particles sizes be too small to measure, but the range of different particle sizes and the nearby presence of salt crystals would generate dispersive scatter artifacts which can significantly alter the spectra making accurate identification more difficult. To further demonstrate the relative immunity to scattering artifacts, these mixed polymer bead samples were suspended in salt water and deposited on to a CaF2 substrate, so that the polymer beads would be interspersed with salt crystals as shown in figure 4.

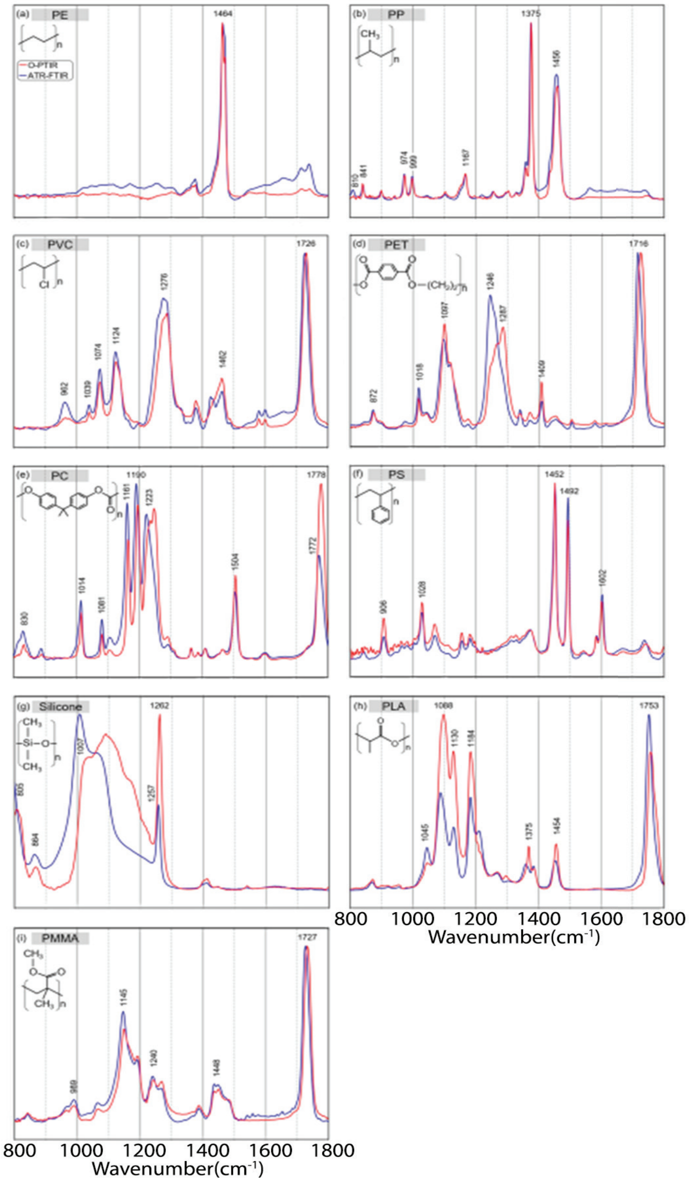


Figure 2: Normalized IR spectra from 1800 to 800 cm^{-1} of different plastics: polyethylene (PE) (a), polypropylene (PP) (b), polyvinyl chloride (PVC) (c), polyethylene terephthalate (PET) (d), polycarbonate (PC) (e), polystyrene (PS) (f), silicone (g), polylactide acid (PLA) (h), and polymethylmethacrylate (PMMA) (i), collected with standalone FT-IR system (blue) and the O-PTIR instrument collected in refection mode (red)

For the spectra shown in figure 4, it is noteworthy that despite the spectra being collected from differently sized

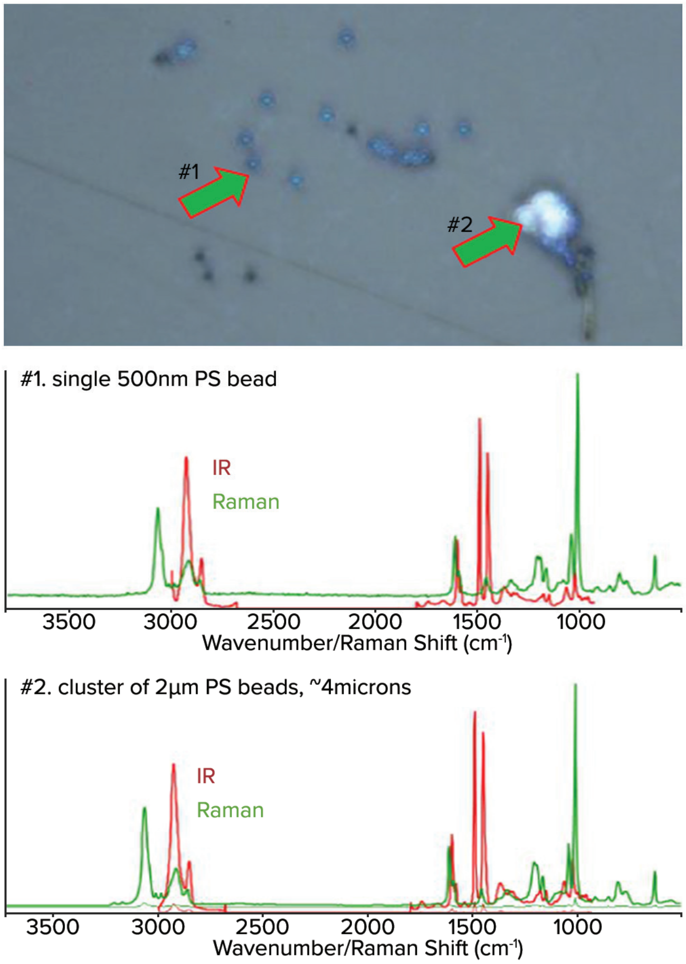
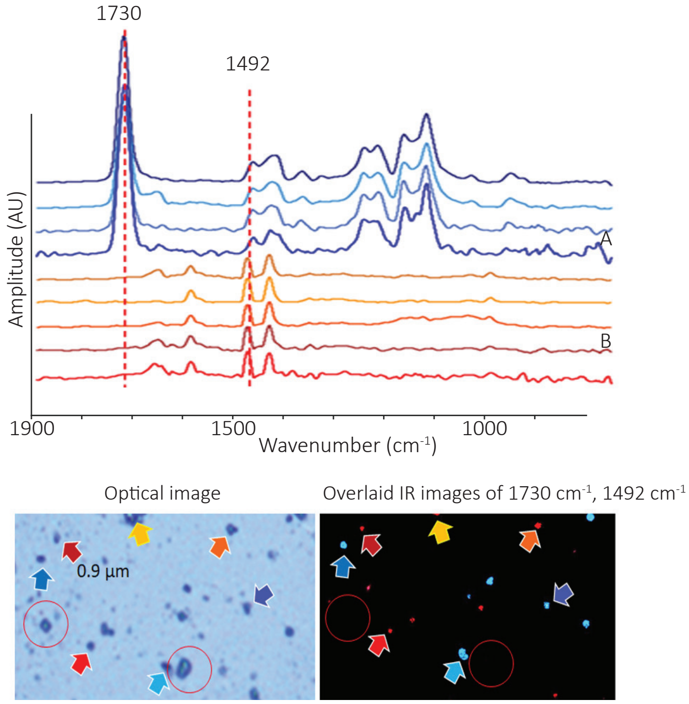


Figure 3: Top, *Optical image of various sizes of small PS beads.* Middle, *The corresponding O-PTIR spectra from a 500nm PS bead* Bottom, *Spectra from a 2µm cluster of beads of approximately 4µm in size. The IR spectra from single 500nm bead or a cluster of 2µm beads all look the same. The spectra are raw and unfiltered.*

beads the spectra all look very consistent with their polymer type (i.e., no dispersive scatter artifacts are present). This ensures that correct identification can be determined, regardless of particle shape and size.

O-PTIR single frequency imaging

Single frequency imaging can be very advantageous to speed up the process of particle identification, especially if some key absorbing spectral features are known and distinct from other components. In this example in Figure 4, we have used the main bands from PS (1492cm-1) and PMMA (1730cm-1). A key point unique to O-PTIR to highlight is that a spectral response is only generated from a sample absorbance, other scattering particles do not confound the image. This is very evident in figure 4 in which the location of salt crystals is circled. They are clearly visible in the optical image and in fact could be confused for polymer beads. However, in the single frequency image, only the polymer beads are highlighted, with salt crystals returning no response. This is in stark contrast with traditional FT-IR and direct QCL microscope methods where the presence of salt crystals would severely distort the chemical images and show up as scattering artifacts in the spectra of particles in the proximity of the salt crystals.



Optical image

Overlaid IR images of 1730 cm^{-1}, 1492 cm^{-1}

Figure 4: Top, spectra of PS and PMMA beads ranging in size from 0.9µm to 10µm. Bottom left, corresponding optical image. Bottom right, overlaid chemical image collected at 1492 cm^{-1} and 1730 cm^{-1} highlights both materials. Colors of the arrows correspond to the colors of the spectra. Salt crystals are highlighted in red circles. Note how no O-PTIR signal is observed in the IR image from the salt crystals

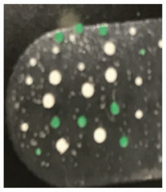
Artifact-free IR spectroscopy of large size and colored microplastics

Figure 5 shows O-PTIR measurements of a range of larger microplastics sizes and colors, highlighting O-PTIRs capability to measure from sub-micron to <1mm size particles. The spectra are artifact free with no band saturation effects that would be demonstrated in traditional FTIR techniques, despite being almost 1mm in size.

Microplastics characterization with simultaneous IR and Raman and dual IR/Raman database searching

In addition to the O-PTIR measurements, the visible probe laser also generates Raman scattered light from the measurement area, which when collected, allows for the simultaneous acquisition of both IR and Raman spectra, with the same submicron spatial resolution, at the same location and at the same time. A schematic diagram for this optical beam path is shown in figure 6.

When combined on one system, co-located and simultaneous O-PTIR and Raman spectroscopy can provide a powerful combination for a broader range of microplastics



Particle Type	Type 1	Type 2	Type 3
Morphology	sphere	sphere	sphere
Color	white	white	green
Size range	370-450μm dia	770-920μm dia	320-680μmdia
Count	10	7	9
Material	polyethylene	polyethylene	polyethylene

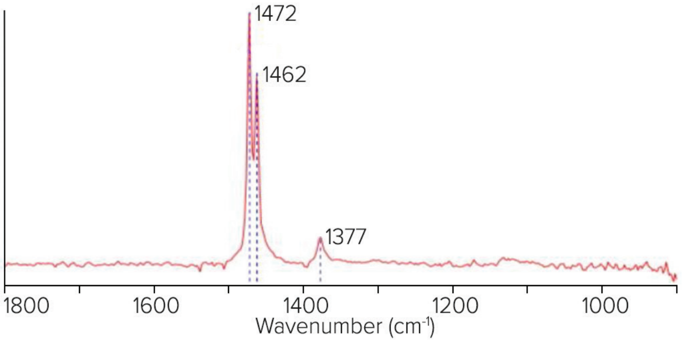


Figure 5: Top left, optical image shows location and color of the microplastics. Top right, table shows the types of microplastics. Bottom, spectra demonstrate artefact free measurements of the larger microplastic samples

measurements, provide complementary and confirmatory spectral information for more accurate identification as well as higher productivity of measurements.

A demonstration8 of this powerful combined and correlative approach can be found when one considers a new two dimensional spectroscopy approach where both the IR and Raman Spectral Hit Quality (HQI) are displayed in 2D scatter plot as shown in figure 7(rop right) and (bottom right). We have two particles (particle 1 and 2) of sub 20µm particles observed on the surface of the 0.8µm – 20µm size fraction filters. The corresponding simultaneously captured O-PTIR and Raman spectra are then searched using the dual IR/Raman searching capabilities of the Wiley KnowItAll® software. As we can see, the output of the search is a scatter plot where the most likely matches are in the top righthand corner of the plot, with the highest combined hit quality index (HQI) for both IR and Raman.

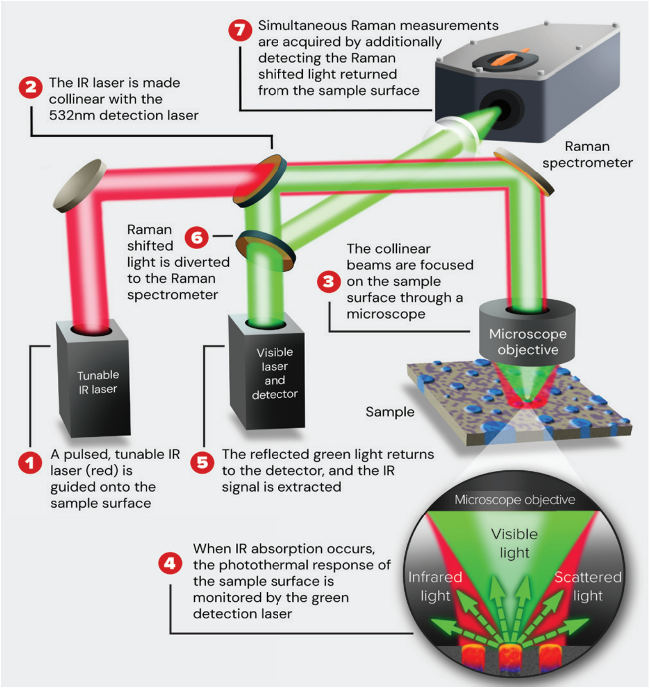
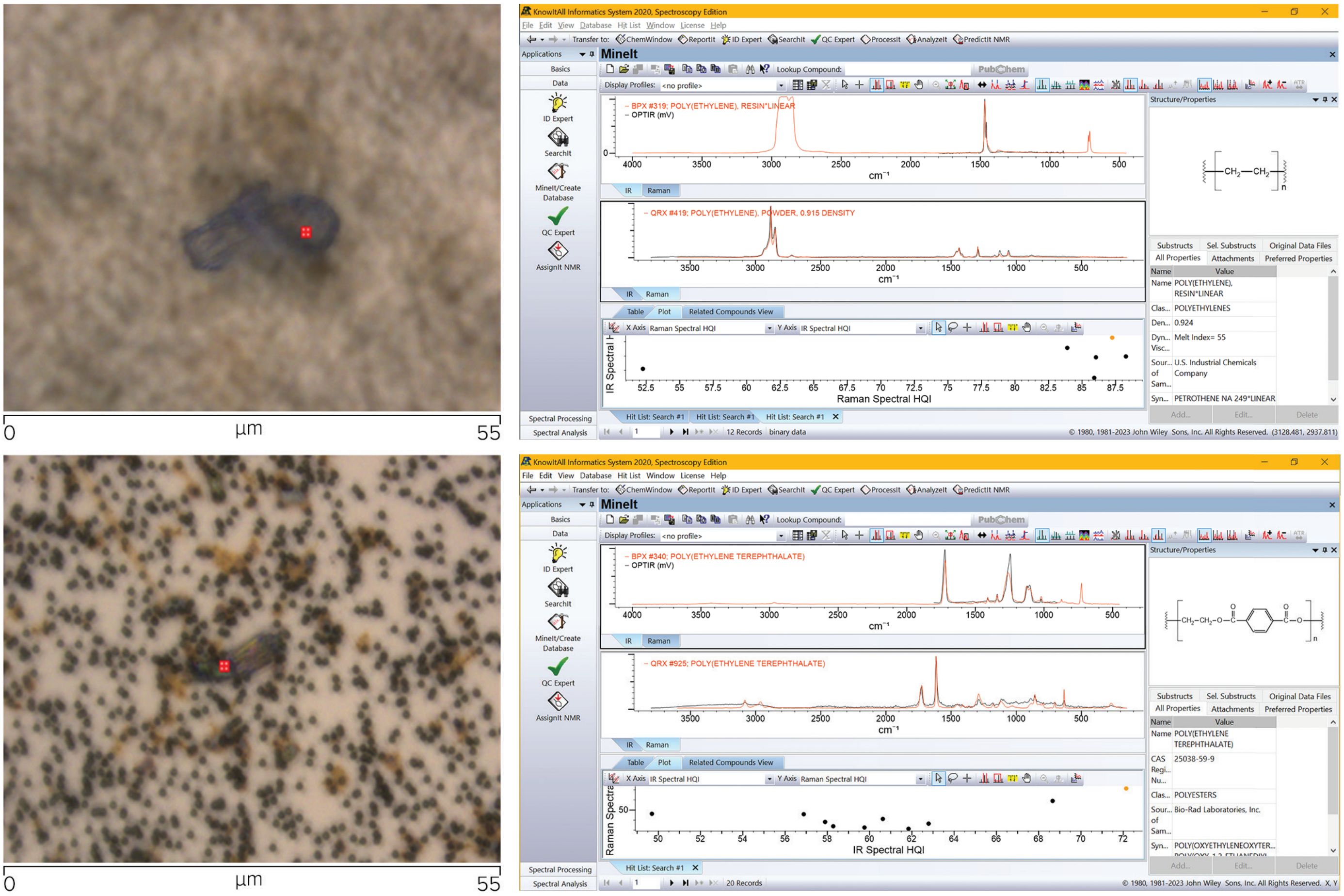


Figure 6: Schematic representation of the beam paths for the generation of simultaneous IR and Raman



– BPX #319; POLY(ETHYLENE), RESIN*LINEAR
– OPTIR (mV)

– QRX #419; POLY(ETHYLENE), POWDER, 0.915 DENSITY

X Axis Raman Spectral HQI
Y Axis IR Spectral HQI

Name	Value
Name	POLY(ETHYLENE), RESIN*LINEAR
Clas...	POLYETHYLENES
Den...	0.924
Dyn... Visc...	Melt Index= 55
Sour... of Sam...	U.S. Industrial Chemicals Company
Syn...	PETROTHENE NA 249*LINEAR

12 Records binary data

© 1980, 1981-2023 John Wiley Sons, Inc. All Rights Reserved. (3128.481, 2937.811)

– BPX #340; POLY(ETHYLENE TEREPHTHALATE)
– OPTIR (mV)

– QRX #925; POLY(ETHYLENE TEREPHTHALATE)

X Axis IR Spectral HQI
Y Axis Raman Spectral HQI

Name	Value
Name	POLY(ETHYLENE TEREPHTHALATE)
CAS Regi... Nu...	25038-59-9
Clas...	POLYESTERS
Sour... of Sam...	Bio-Rad Laboratories, Inc.
Syn...	POLY(OXYETHYLENEOXYTER...

20 Records

© 1980, 1981-2023 John Wiley Sons, Inc. All Rights Reserved. X, Y

Figure 7: Top left, Optical image of particle 1 (40 x Cassegrain objective). Bottom left, Optical image of particle 2 (40 x Cassegrain objective). Top right, Wiley KnowItAll dual IR/Raman database search result for O-PTIR/Raman spectra collected on particle 1. Bottom right,Wiley KnowItAll dual IR/Raman database search result for O-PTIR/Raman spectra collected on particle 2

The database search is able to sort through a wide range of possible identifications and specifically in this case the Raman HQI can be used to eliminate many of these similar materials giving much more confidence in the results of the search.

Microplastics localization with fluorescence microscopy

Nile Red staining, combined with fluorescence has been used in the past to locate and count the number of polymeric particles in a sample, however it lacks the ability to identify polymer types6. The Nile Red stain is lipophilic and so it preferentially interacts with the hydrocarbon (CH) chains present in most polymeric materials and allows for fluorescence imaging to identify the location of microplastics7. By locating the position of only the polymer particles and not the other particles, not of interest, like sand, biological etc, it provides a throughput enhancement by allowing the system to not waste time with non-polymeric samples and in doing so, it can also relieve the pressure for ensuring near perfect sample preparation and clean-up. However, Nile Red staining does not provide any chemical identification of the particles observed in imaging, therefore, further chemical analysis is required to definitively identify the material.

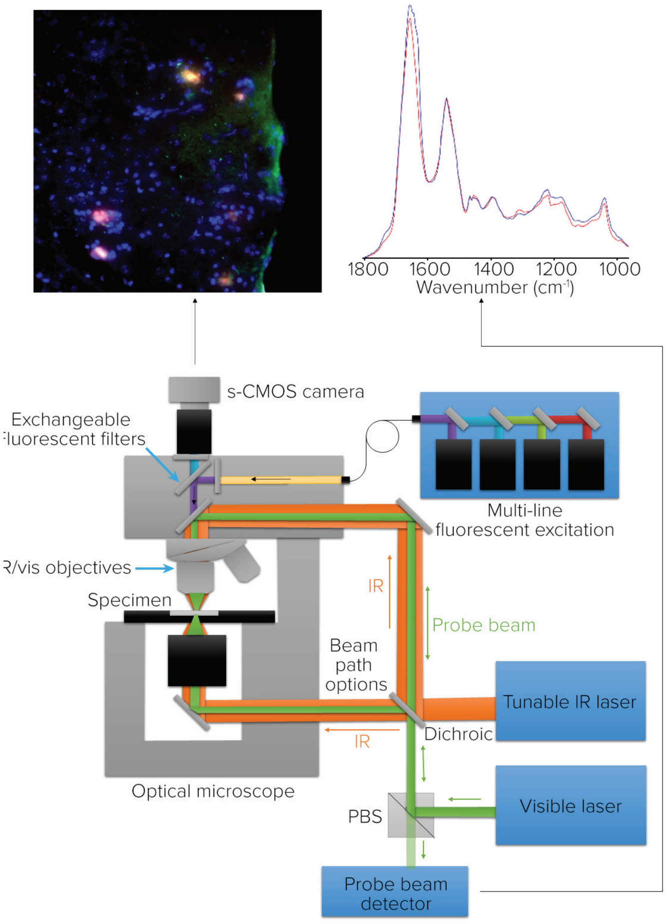


Figure 8: Microscope schematic for O-PTIR and co-located fluorescence microscopy

The mIRage-LS system supports co-located O-PTIR and fluorescence microscopy capability to enable more productive chemical identification of microplastics

mIRage-LS principal of operation: Co-located O-PTIR and fluorescence microscopy

The system layout for a combined O-PTIR sub-micrometer IR microscope, co-located with fluorescence microscopy is illustrated in Figure 8. mIRage-LS works in both reflection mode and new high resolution Counter propagating mode. In counter-propagating mode, the pulsed IR beam is directed through the underside of the sample. The fluorescence and visible probe beam paths are co-located through the microscope objective.

Using the mIRage-LS co-located O-PTIR and fluorescence option the system can collect a fluorescence image and then collect O-PTIR spectra of the particles identified from the Nile Red-stained imaging. As the exact same objective is used for fluorescence imaging as well as O-PTIR data collection, there are no sample or image registration issues. Figure 9 shows a fluorescent image acquired using a filter cube, with excitation at 497nm and emission at 535nm, highlighting multiple particles (circled in red). We also observe several particles on the surface of the filter that do not exhibit fluorescence (circled in green). Nile Red staining isolates microplastics from non-microplastic materials thus eliminating the need for measurement and possible mis-identification of non-microplastics. Eliminating unneeded measurements reduces analysis time and improves accuracy.

Although Raman spectra can be simultaneously collected, they are not useful due to the large fluorescence background signal caused by the Nile Red staining. Fortunately, the O-PTIR measurements are not affected by fluorescence. Furthermore, as the concentration of Nile Red dye is typically too low, its presence does not change the O-PTIR spectra. Figure 9 (right; top, middle, bottom) shows the Wiley KnowItAll database searching results for the O-PTIR spectra collected from the highlighted particles that were identified via fluorescence. The results of the searching indicate there are three different microplastics identified; PS, PE and PMMA.

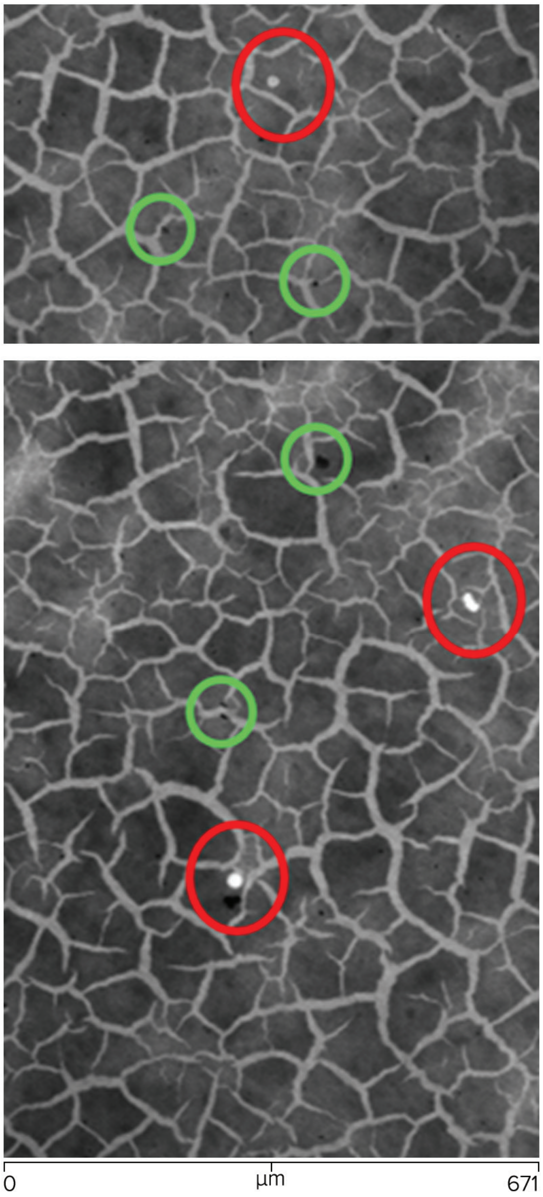
Conclusion

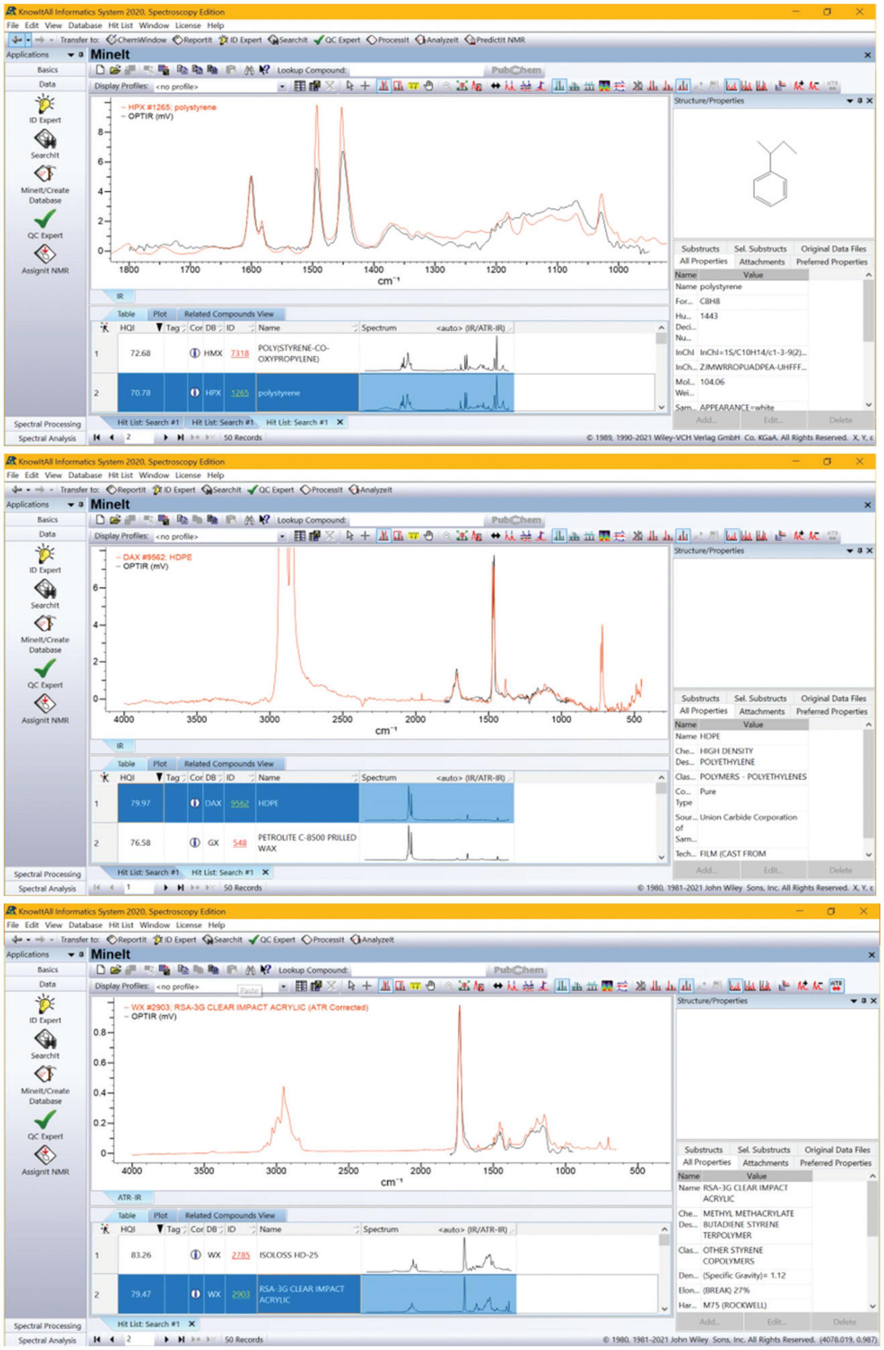
O-PTIR spectra have shown excellent correlation to FT-IR reference spectra across a wide range of type of microplastics. The mIRage and mIRage-LS microscopes have demonstrated the ability to analyze and identify a range of microplastics sizes from >500µm to sub-micron microplastics including 500nm PS beads, sub 10µm microplastics in a water sample and a mixed model sample of different polymer types and sizes amongst salt crystals without any spectral artifacts from sample size/size dependent dispersive scattering (Mie scattering).

O-PTIR has also successfully demonstrated measurement of microplastics >500µm with no band saturation.

Simultaneous O-PTIR and Raman measurements have demonstrated higher productivity, accuracy and a confirmatory approach to identifying a range of microplastic types.

O-PTIR spectra have been exported to the KnowItAll® database for digital searching enabling researchers to understand the entire composition of the smallest microplastics.





– HPX #1265: polystyrene
– OPTIR (mV)

HQI	Tag	Cor	DB	ID	Name
72.68			HMX	7318	POLY(STYRENE-CO-OXYPROPYLENE)
70.78			HPX	1265	polystyrene

Name polystyrene
For... C8H8
Mol... 104.06
Sam... APPEARANCE=white

© 1989, 1990-2021 Wiley-VCH Verlag GmbH Co. KGaA. All Rights Reserved.

– DAX #9562: HDPE
– OPTIR (mV)

HQI	Tag	Cor	DB	ID	Name
79.97			DAX	9562	HDPE
76.58			GX	548	PETROLITE C-8500 PRILLED WAX

Name HDPE
Che... HIGH DENSITY
Des... POLYETHYLENE
Clas... POLYMERS - POLYETHYLENES
Sour... Union Carbide Corporation
Tech... FILM (CAST FROM

© 1980, 1981-2021 John Wiley Sons, Inc. All Rights Reserved.

– WX #2903: RSA-3G CLEAR IMPACT ACRYLIC (ATR Corrected)
– OPTIR (mV)

HQI	Tag	Cor	DB	ID	Name
83.26			WX	2785	ISOLOSS HD-25
79.47			WX	2903	RSA-3G CLEAR IMPACT ACRYLIC

Name RSA-3G CLEAR IMPACT ACRYLIC
Che... METHYL METHACRYLATE
Des... BUTADIENE STYRENE TERPOLYMER
Clas... OTHER STYRENE COPOLYMERS
Den... (Specific Gravity)= 1.12
Elon... (BREAK) 27%
Har... M75 (ROCKWELL)

© 1980, 1981-2021 John Wiley Sons, Inc. All Rights Reserved.

Figure 9: Left, *Two areas of fluorescence image (Nile red stained) acquired using a GFP filter cube with excitation at 497 nm and emission at 535 nm.* Right, *Wiley KnowItAll database search results identifying each of the particles identified by imaging*

O-PTIR and co-located fluorescence imaging with Nile Red stain was used to distinguish microplastics from non-plastic materials and provide more productive approach to measuring microplastics.

Overall, O-PTIR provides reliable and accurate sub-micron IR spectroscopy for a wide range of microplastics, from 100µm's down to sub-micron size and includes seamless integration other multi-modal techniques.

This capability makes O-PTIR a powerful and flexible technique for researchers to achieve accurate measurements in a highly productive manner.

References

1. Qin, F., et al., Bibliometric profile of global microplastics research from 2004 to 2019. International Journal of Environmental Research and Public Health, 2020. 17(16): p. 5639.
2. Hale, R.C., et al., A Global Perspective on Microplastics. Journal of Geophysical Research: Oceans, 2020. 125(1): p. e2018JC014719.
3. Wong, C.S.C., Scott, Standard Operating Procedures for Extraction and Measurement by Infrared Spectroscopy of Microplastic Particles in Drinking Water. 2021, Southern California Coastal Water Research Project Authority and California State Water Resources Control Board.
4. Wong, C.S. and S. Coffin, Standard Operating Procedures for Extraction and Measurement by Raman Spectroscopy of Microplastic Particles in Drinking Water. 2021, Southern California Coastal Water Research Project Authority and California State Water Resources Control Board.
5. Böke, J.S., Popp, J. and Krafft, C. Optical photothermal infrared spectroscopy with simultaneously acquired Raman spectroscopy for two-dimensional microplastic identification. Sci Rep 12, 18785 (2022). https://doi.org/10.1038/s41598-022-23318-2
6. Myers, N., Catarino, A. I., Declercq, A. M., Brenan, A., Devriese, L., Vandegehuchte, M., De Witte, B., Janssen, C., Everaert, G., 2022. Microplastic detection and identification by Nile red staining: Towards a semi-automated, cost- and time-effective technique. Sci. Total Environ. Volume 823, 153441. https://doi.org/10.1016/j.scitotenv.2022.153441
7. Maes, T., Jessop, R., Wellner, N., Haupt, K., Mayes, A. G., 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Sci Rep 7, 44501. https://doi.org/10.1038/srep44501

Acknowledgments

PSC acknowledges Dr Rae McNish for supplying the Nile Red-stained samples.

Further reading O-PTIR for Microplastics
View our publications page and search for microplastics:
Publications using the mIRage microscope with O-PTIR and IR+Raman (photothermal.com)

Watch our microplastics webinar:
Webinar - October 2021 - Live demo – Accurate microplastics identification - View on-demand - Optical photothermal infrared and Raman spectroscopy with the mIRage

PHOTOTHERMAL
SPECTROSCOPY CORP

325 Chapala Street, Santa Barbara, CA 93101
(805) 845-6568 info@photothermal.com www.photothermal.com