Technology Networks

Innovative Solutions for Microplastics Analysis

Diving into Microplastics

Microplastic Identification and Characterization

Microplastics in Bottled Water

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Foreword

From bottled beverages and food packaging to household products and industrial components, plastics are ubiquitous in everyday life. As a result, their increasing prevalence in the environment is becoming a global concern.

Understanding the effects of microplastics pollution is a central challenge for environmental scientists, however the legislative environment surrounding microplastics research is constantly evolving. The development and implementation of techniques that help researchers identify, characterize and quantify microplastics contaminants in different matrices, will provide important insights to the legislative landscape in different geo-environment.

Thermo Fisher Scientific offers easy-to-use instruments, fit-for-purpose software and technical expertise to help scientists analyze microplastics and assess their impact on human health and the environment.

The development of techniques such as FTIR and Raman enables researchers to examine the chemical composition of polymers at molecular level. When combined with the morphological information provided by microscopy, these techniques can shed light on the origin, transportation and fate of microplastic particles found throughout the environment.

This eBook will explore microplastic pollution in detail and demonstrate the impact of innovative vibrational microspectroscopy techniques on this field of research.

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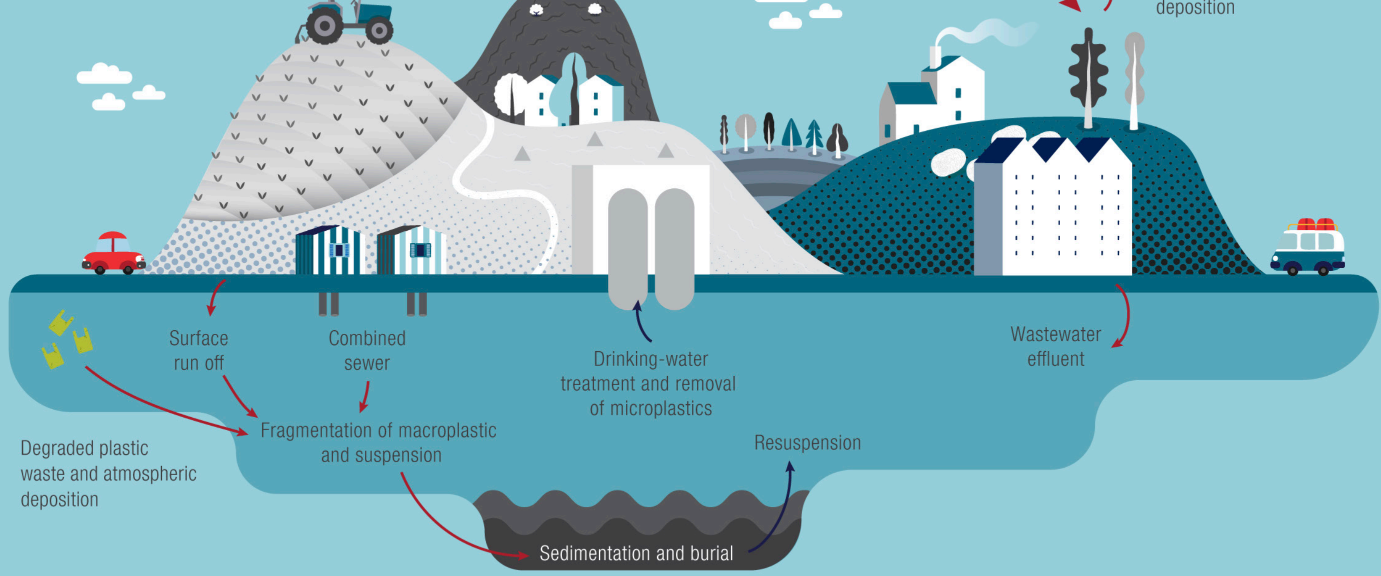
Diving into Microplastics

Microplastics are tiny plastic fibers and fragments (smaller than 5 mm in size) that are polluted into the environment from plastics used in everyday items and manufacturing processes.[1] They are predominantly made up of polystyrene, polypropylene and polyethene and can be divided into two types:[2]

Primary microplastics:
Directly released into the environment (e.g. microbeads in facial cleansers)[3]

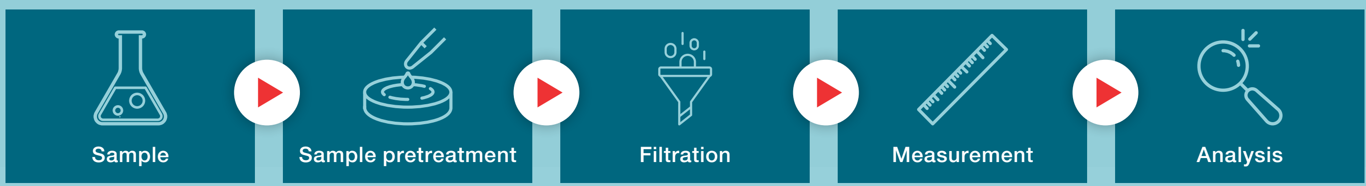
Secondary microplastics:
Originate from the degradation of larger plastic objects over time[3]

Microplastics enter water systems by:



How do we analyze microplastics?

There are many protocols for microplastic analysis. Since the complex matrix can contain both inorganic and organic material, it is essential that microplastics are pretreated to isolate the plastic components. Depending on the sample type, this can be achieved with chemicals or enzymes and must be done before analysis.[4]



5 Key identification techniques

- Visual microscopy
- Visual microscopy with Nile red
- FTIR
- Raman
- GCMS-Pyr

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The impact of microplastics: An interview with Dr Janice Brahney

Plastic pollution has become one of the most pressing environmental and social issues today. While microplastics research is still in its infancy, scientists are working hard to understand the sources of microplastics and their impact.

Dr Janice Brahney is an associate professor at the Environmental Biogeochemistry and Paleoliminology Lab, Utah State University. Her research focuses on the use of multi-displinary approaches to invesigate environmental processes and change. In this interview, she shares her insights into the sources of microplastics, their impact and the techniques that scientists are using to identify and characterize them.

Q: Can you provide a brief overview of microplastics and the biggest sources of micoplastic pollution?

Janice (J): Microplastics are pieces of plastic that range in size from 1 micron (µm) to 5 millimeters (mm). The smallest of the microplastics are not typically visible to the naked eye; Humans can generally see particles larger than 40 µm, but not smaller. For reference, the average width of a hair is 70 µm and a red blood cell is 8 µm in diameter. Microplastics are most commonly produced from the breakdown of larger pieces of plastic in the environment but some microplastics are made on purpose in this size range, these are called 'primary microplastics'. Examples of primary microplastics include microbeads, like those used in cosmetics, or nurdles. Nurdles are tiny pieces of plastic used in industry to manufacture plastic products. The most common form of microplastic in the environment are fibers from the breakdown of textiles and other industrial processes.

Q: What are the potential threats posed by microplastics?

J: Research on the effects of microplastics in the environment is still in its early stages as we don't have enough information yet to make clear predictions or statements of impact. However, studies have shown the effects of microplastics on both biological and physical processes. Organisms that accidently ingest microplastics can suffer consequences related to mechanical obstructions in the gut, a lack of nutrition, behavior changes, and possibly the transfer of contaminants from the plastics to the organism. Physical effects in the environment might include changes in soil properties such as the water retention, and soil structural stability. Plastics have also been shown to influence the microbial community composition in many locations, which can lead to altered biogeochemical cycling including reduced nutrient availability. At present, we do not have a grasp on the full extent of the impacts of microplastics in the environment, nor do we fully understand how different exposure rates or concentrations influence individual outcomes.

Q: Could you provide an overview of the traditional technologies used to analyze microplastics?

J: Microplastics are evaluated through several spectroscopic and spectrometric means. Fourier Transform Infrared Spectroscopy (FTIR) and Raman spectroscopy are common methods. These methods provide information on a particle-by-particle bases, identifying the polymer composition. However, they do not give us information on mass, which in some cases might be a more informative metric. In this case, techniques such as Pyrolysis- and Thermal desorption-Gas Chromatography Mass Spectrometry (pyr-GCMS, TD-GCMS) are more beneficial as they can be used to quantify the mass of polymer types within a given mixed or unmixed environmental sample. The limitation of here, however, is that you don't get particle count numbers, only total mass, as you are either completely pyrolizing the sample or heating the sample to evolve gases. I think one of the benefits of an approach such as TD-GCMS is that you can also get information about different contaminants that might be associated or other affiliated compounds with the sample. There are companies that build TD-GCMS instruments that can show if a piece of plastic came from a coke bottle from information on how sugary compounds affect the compounds. There is a lot of potential there.

Q: What are some of the challenges for microplastics analysis?

J: Some of the biggest challenges in microplastics analyses can be broken down into two categories 1) sample acquisition and identification, and 2) identification of polymer composition in the smallest size classes (< 10 microns).

Obtaining microplastic samples from different media, such as water, sediment, soil, or the atmosphere, is challenging. At the most basic level, how to sample each resource in a way that is representative can be difficult; you are not just sampling plastics, you're sampling organic matter and minerals as well, and then you need to isolate tiny particles from the rest of the sample using a physical or chemical process, which carry their own limitations. Often researchers will use a compound like hydrogen peroxide to digest the organic matter and then sieve to separate size classes and finally a density separation to isolate the plastics from the minerals. However, reagents can alter the form of some plastics, each processing step can come with plastic loss, and some plastic polymers are just as dense as minerals (like PTFE). There is a balance between having minimal processing and having a sample that is composed of a bunch of different components or not being able to quantify plastic pieces because they are mixed with natural items versus trying to isolate the plastic from your sample. Another challenge is how to take a representative sample from the media in question. To illustrate this challenge, most atmospheric samplers are calibrated to recover natural dusts, but plastics have lighter densities than minerals AND odd shapes (like fibers). Therefore, it is not clear that these active sampling techniques collect a representative sample from the atmosphere.

The second challenge relates to our ability to measure polymer composition of all the size classes found in the environment. The identification of the smallest microplastics is butting up against our technological limitations for most spectroscopic techniques. FTIR can generally only detect samples above 10 microns in size. A few methods have been developed to quantify smaller particles, even nanoplastics, but the technologies are not widely tested nor available to most plastic researchers. In addition to the limitation in size, there is also the issue in that spectroscopic techniques are surface measurements; our ability to identify plastic polymers is influenced by the presence of contamination on the surface of the plastic, photodegradation, or colonization by organic matter.

Q: Can you provide an overview of your research and how this has impacted the field?

J: My published research to date on microplastics has focused on the atmospheric transport and deposition of microplastics. Our research is focused on trying to understand not just how microplastics are bring deposited, but where they are coming from and how far they are traveling. Our first big contribution to the field was understanding microplastic deposition rates in remote locations. The sites we analyzed were 50-300 km from major urban centers in protected areas of the United States. We quantified the plastic fallout at a fairly high resolution over a 14-month period which involved looking at weekly wet deposition and monthly dry deposition in space and time. Using atmospheric models, we were able to show differences in transport distances for microplastics deposited wet versus dry. We found that microplastics deposited with rain (wet deposition) were sourced proximally from cities and soils. Whereas microplastics that are deposited under dry conditions are smaller, come from distal locations and are traveling higher in the atmosphere. We also used atmospheric models to identify the most probable sources of microplastics to the atmosphere to begin with. For microplastics in that size range, you need a physical mechanism to move plastic into the atmosphere and to transfer them over a long distance. Sources of plastics to the atmosphere are different than sources of plastics to the environment.

To understand how microplastics are getting into the atmosphere, we applied our understanding of how dust particles get into and are transported into the atmosphere, as they are within the same size range. We developed five different hypotheses and then used atmospheric models to compare them to our measured data to see which hypothesis was the most likely predictor of our data. We found that, globally, oceans are really important sources which makes sense as most of our plastics end up in the ocean. Gyre locations contain high concentrations of microplastics, which through wave action and bubble burst phenomena to generate plastic aerosols. Some are redeposited onto the ocean surface, and some make it into the rest of the environment. We discovered that the exchange from the oceans to the terrestrial environment is greater than the exchange from the terrestrial land to the oceans. This was initially surprising; however, we have been polluting the ocean for so many years that there is more legacy pollution available to be aerosolized than there is on the land. In the terrestrial environment, interestingly, cities didn't seem to be important sources; however, we found roads to be the most important mechanisms for moving plastics into the atmosphere. This is because the action of driving cars on roads provides the mechanical energy needed to emit particles high into the atmosphere; it's a very efficient mechanism. A few studies that have looked at road dust do see a lot of plastic particles – not just tire wear, but all kinds of commodity sources such as microbeads, fibers, and fragments. A lot of the major interstates in the US are littered with plastic, so it is easy to see how these would breakdown with car activity and be emitted into the atmosphere. Agriculture is also a potential source, which makes a lot of sense since agricultural soils end up in the atmosphere due to activities such as tilling – especially in drier areas in the West. There are also periods of time when the agriculture fields are fallow and so winds come along and erode the soil producing dust. All of the plastics that leave our home from our washing machine end up in wastewater treatment plants and more than 95% of that plastic gets retained in the solid fraction, which then gets turned into fertilizer and is applied on agriculture fields.

Having said all of that, our data is still pretty limited; we are using data from the Western US to make inferences about what is happened across the entire US, or even the world. We need a lot more information on microplastic concentrations in the atmosphere, as well as their levels and deposition rates. Our models are really an approximation that give us information on where to look and what to do next, however, they are by no means absolute.

Q: What can be done to reduce or even prevent microplastic pollution and where should research efforts be focused in the future?

J: It is really complicated; we need to stop using and producing single-use plastics and the only way that is going to happen is through policy and international agreements. Efforts should therefore be focused on legislation that reduces the use and production of single-use plastics. In addition, international treaties that ban the distribution of plastic waste need to be enacted, specifically from wealthy countries to those with limited infrastructure. It is difficult to put the onus on the consumer as it is virtually impossible to go to the supermarket and avoid purchasing anything plastic. We need the government to limit the creation of new plastic and stop the irresponsible shipping of plastic waste.

Dr Janice Brahney
Associate Professor,
Utah State University

Application note

Identification of Microplastics using the Nicolet RaptIR FTIR Microscope

Microplastics are a persistent and ubiquitous environmental pollutant, and their presence in aquatic sources, air, and the food chain is of growing concern.

As a result, various environmental pollution regulation agencies across the globe are gearing up to establish methods and limits for microplastic characterization. Challenges in establishing a method arise from the size, diversity and multi-dimensionality of these contaminants.[1,2]

The current definition adopted by the California State Water Resources Control Board (SWRCB) for microplastics in drinking water categorizes these particles as large (100-5,000 µm), small (1-100 µm), submicron (100-1,000 nm), and nano plastics (1-100 nm) based on size. A standardized protocol is now available from the California SWRCB and the Southern California Coastal Water Research Project (SCCWRP), where FTIR and Raman are the specified methods for analyzing microplastics[3]. Traditionally FTIR and Raman spectroscopies are the go-to instrumentation for analysis and QC of polymers, so it is natural that they are the de facto techniques employed to identify microplastics.

The identification of traditional polymers is carried out using appropriate spectral databases which is ideal for non-degraded polymers from production. However, microplastics are polymers that may have gone through environmental degradation, making their identification potentially challenging. Oxidation and other exposure events, like UV exposure, may cause spectral changes.

For individual particles, micro-attenuated total reflectance (ATR) analysis of particles is a preferred method. However, automated micro-ATR is not as useful for multi-particle microplastic analysis because when the micro-ATR tip moves from particle to particle there can be cross-contamination and adhesion of material to the tip. This is why reflection mode is preferred, as nothing touches the sample. However, most commercial libraries are collected in transmission or ATR mode. When the sample spectra are collected in reflection mode, it is ideal to utilize reflection libraries to optimize spectral matches. In this note, we will discuss the availability and use of a polymer library collection in reflection mode.

Currently, various labs are working to automate FTIR (and Raman) data collection and analysis for microplastics. The challenge of analyzing samples automatically involves finding the particulates and providing coordinates for the analysis.

Thermo Fisher Scientific has pioneered techniques for locating materials of interest for many years, driven by a strong electron microscopy and software business. This technology is now available for use in FTIR microscopy through the Thermo Scientific™ OMNIC™ Paradigm™ Software launched with the Thermo Scientific™ Nicolet™ RaptIR™ FTIR Microscope. The FTIR microscopy system includes tools for image capture of each particle and the determination of the form factors (shape and size) for each particle. The aperture size for each particle is determined by the form factors, optimizing the data quality of the overall analysis. This then couples with the FTIR search capability to yield the identity of the particles. The full software report provides all details from the analysis, completing the process.

Method

Sample preparation

Microplastics in water or wastewater were processed as per the SCCWRP/California SWRCB protocol.[3] The final filtrate (1-50 µm) was filtered using an appropriate filter such as silicon, gold-coated polycarbonate, Al_2O_3, or stainless steel. Figure 1 summarizes the steps involved in the isolation and analysis of microplastics.

Particles from the air were collected by leaving the filter/ slide exposed for a prolonged time to the outdoor environment.

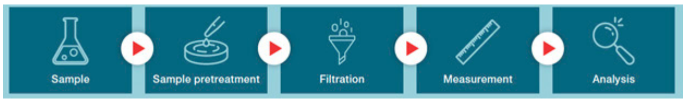


Figure 1. Summary of steps involved in isolation and analysis of microplastics.

FTIR analysis

Two types of data collection features are described in this note. The first approach utilizes particle analysis and involves collecting data from only the particles distributed across a filter (find the particles first, then collect and analyze). The second approach involves collecting a chemical map of the entire filter (find the particles using the FTIR spectra). For both approaches done here, a single point LN_2 cooled MCT detector was used for data acquisition at a resolution of 8 cm^{-1}, and each spectrum resulted from the coaddition of 8 or 16 scans.

In the particle analysis method, an image analysis is performed on the visual image of the filter, and particles are located based on the contrast against the filter itself. The software automatically moves the microscope stage to all these positions, and spectra are collected and analyzed against a set of appropriate libraries. Once the measurement is completed for the hundreds or thousands of particles on the surface, a complete particle analysis report with identity, size, shape, and distribution is generated.

In the chemical mapping method, the entire area of the filter can be analyzed as an image. This type of analysis is beneficial when there is minimum contrast between the particles and filter backdrop. The downside is a large number of 'empty' data pixels (points with no particles) which waste collection time and memory storage. Multiple regions in a large sample can also be analyzed (instead of all the area in one map). Once the data is obtained, then correlation, multivariate curve resolution (MCR), or principal component analysis (PCA) algorithms can be applied to the maps to find and identify the particles.

Microplastics reflectance library

A microplastics reference library was created using the CMDR Polymer Kit 1.0 from Hawaii Pacific University (HPU)[6], Polysciences microbead standards, and polymer standards from Sigma-Aldrich.

Small size particles were created using a lab grinder or metal scraper. Reflection spectra from at least 3 particles were collected at a resolution of 8 cm^{-1} and 32 scans. The list of materials in the microplastics reference library is shown in Table 1. The library includes 30 of the most common polymers and 5 common contaminants found in laboratories.

	Name	Source
1	ULDPE (Ultra low density polyethylene)	HPU
2	LDPE (Low density polyethylene)	HPU
3	LLDPE (Linear low density polyethylene)	HPU
4	MDPE (Medium density polyethylene)	HPU
5	HDPE (High density polyethylene)	HPU
6	PP (Polypropylene)	HPU
7	PEST (Polyester poplin fabric)	HPU
8	PET1 (Polyethylene terephthalate)	HPU
9	PET2 (Recycled polyethylene terephthalate)	HPU
10	EVA (20% Ethylene-vinyl acetate)	HPU
11	ABS (Acrylonitrile-butadiene-styrene)	HPU
12	EPS (Expanded polystyrene foam)	HP U
13	PS (Polystyrene)	HPU
14	PA6 (Nylon 6)	HPU
15	PA66 (Nylon 6,6)	HPU
16	PVC 1 (Polyvinyl chloride)	HPU
17	PVC 2 (Polyvinyl chloride with phthalates)	HPU
18	CR (Crumb rubber from used tires)	HPU
19	CA* (Cellulose acetate)	HPU
20	PBS (Polystyrene-block-polybutadiene-block-polystyrene)	Sigma-Aldrich
21	Skin cells	lab
22	Cellulose fiber	lab
23	Soil/silica	lab
24	Nitrile gloves (blue)	lab
25	Hair	lab
26	PE (Polyethylene)	Polysciences
27	PE (Polyethylene with additive)	Polysciences
28	PMMA (Poly [styrene-co-methyl] methyl acrylate)	Sigma-Aldrich
29	PET (Polyethylene terephthalate)	Sigma-Aldrich
30	PC (Polycarbonate)	Sigma-Aldrich
31	PTFE (Polytetrafluoroethylene ethylene)	Sigma-Aldrich
32	PVDF (Polyvinylidene fluoride)	Sigma-Aldrich
33	Silicone	Sigma-Aldrich
34	Epoxy resin	Sigma-Aldrich
35	PU (Polyurethane)	Sigma-Aldrich

Table 1. Materials represented in the microplastics reference library used for particle identification. Materials found locally are marked with "lab" as the source.

Results and Discussion

Particle Analysis Method: Analysis of visually located particles

The Nicolet RaptIR FTIR Microscope powered with OMNIC Paradigm Software allows the microplastics analysis to be completed in 3 simple steps. First, a region is selected for analysis. Figure 2 shows a 10x10 mm silicon filter with a wide range of particle sizes. The red highlighted points were automatically selected for analysis; these can be edited by the user.

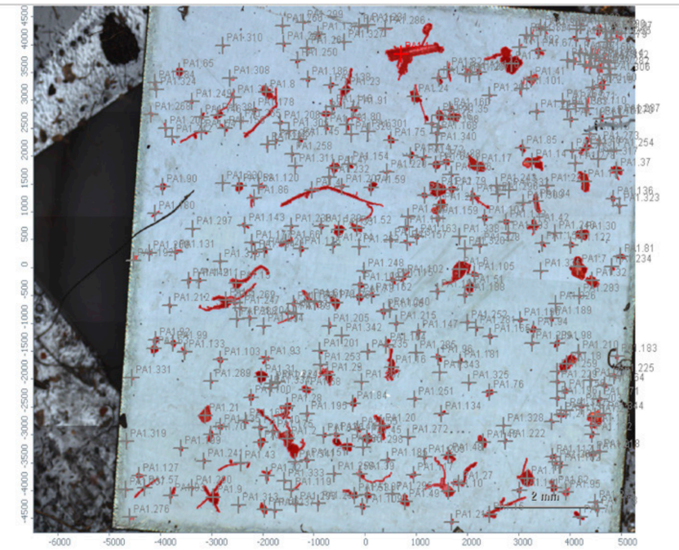


Figure 2. A silicon filter with atmospheric deposition of microplastics. Particles selected are between the size range of 25 µm – 1 mm.

Step two is the automatic measurement of the FTIR spectra of all particles. The software chooses the aperture sizes needed, collects backgrounds, then follows an optimal path through the field of particles for the data collection. Depending on the number of particles selected and the number of scans being accumulated, this can take a few seconds to several minutes. Figure 3 shows a typical spectrum measured in this mode.

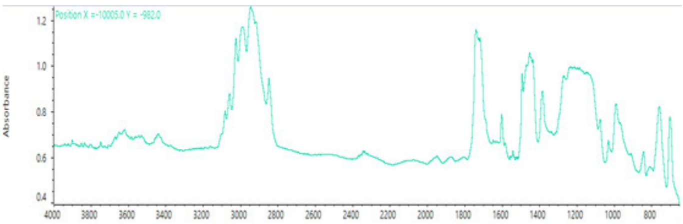


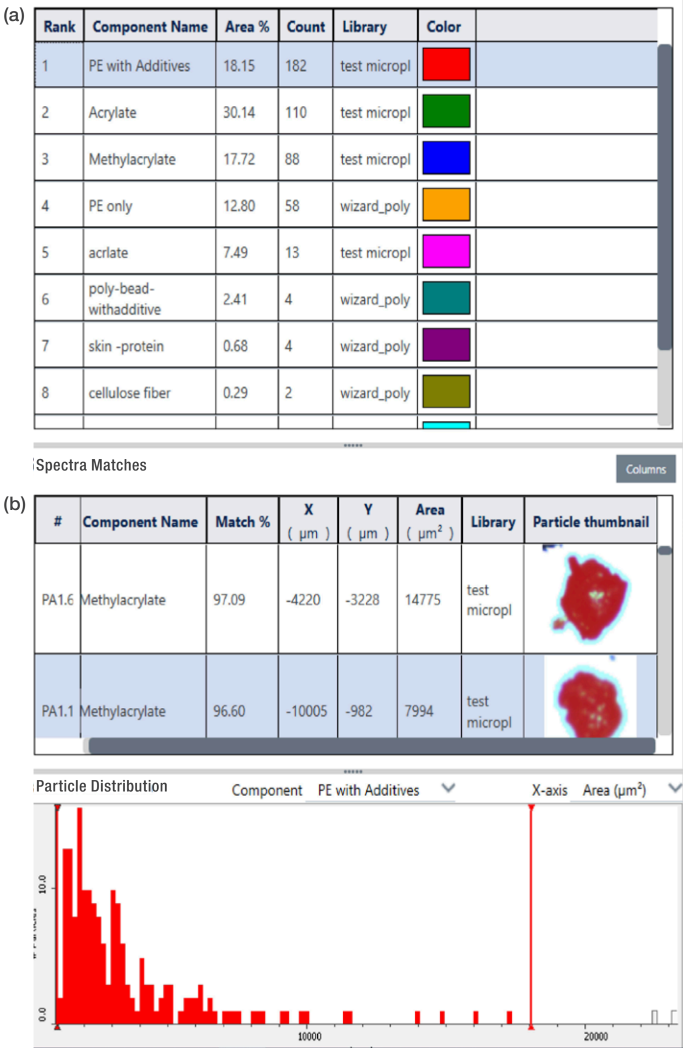
Figure 3: A single scan spectrum of a particle on a filter. Particle measured in reflectance; 16 scans coadded at 8 cm^{-1}.

Step three takes place after the measurement is complete; the particle analysis is performed with the selected library. For this example, the microplastics reference library compiled from the sources listed in Table 1 was used.

Figure 4(a) shows the report with particle identity size and a zoomed-in view of the particle itself. Figure 4(b) shows the particle size distribution for each of the identified particle types. Libraries used for the identification of polymers can be customized and applied to stored spectral data.

This enables researchers to analyze stored filter data sets with newer polymer reference libraries.

The complete report from the software provides insights into the total particles, the relative numbers or different materials found, and - from the shape - some indication of the effect of environmental forces (abrasion, etc.) on the particles. The speed, simplicity and automation of this microscope ensures minimal wasted time and enables novices to achieve the same results as described in this application note.



(a)

Rank	Component Name	Area %	Count	Library	Color
1	PE with Additives	18.15	182	test micropl	
2	Acrylate	30.14	110	test micropl	
3	Methylacrylate	17.72	88	test micropl	
4	PE only	12.80	58	wizard_poly	
5	acrlate	7.49	13	test micropl	
6	poly-bead-withadditive	2.41	4	wizard_poly	
7	skin -protein	0.68	4	wizard_poly	
8	cellulose fiber	0.29	2	wizard_poly	

Spectra Matches

(b)

#	Component Name	Match %	X (µm)	Y (µm)	Area (µm²)	Library	Particle thumbnail
PA1.6	Methylacrylate	97.09	-4220	-3228	14775	test micropl	
PA1.1	Methylacrylate	96.60	-10005	-982	7994	test micropl	

Figure 4(a). Report with full image, identification, particle size, and magnified particle image providing a complete picture. (b) Size distribution of each identification type.

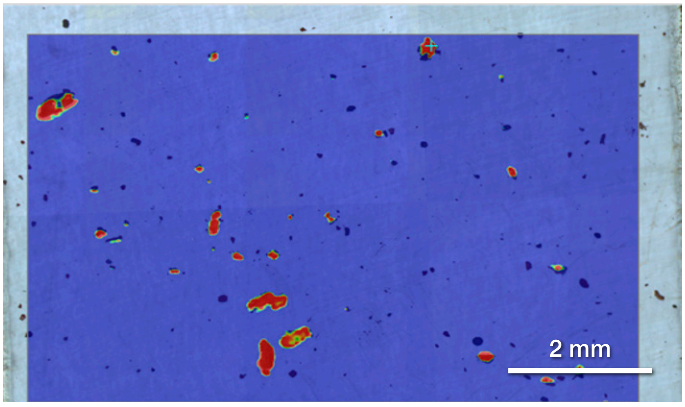


Figure 5. Silicon filter collected with microplastics analyzed with area mapping. Red regions are particles of PE correlated to the entire map.

Chemical Mapping Method: Analysis of the full filter

In the chemical mapping approach, an infrared image is collected from the entire region of interest, in which every pixel contains an infrared spectrum. The entire filter can be selected and fully mapped or the user can set up several selected regions of interest and then collect and analyze those regions individually. Figure 5 shows an example of a silicon filter where the entire 10X10 mm area is mapped as one complete map. The regions highlighted in red show the correlation to polyethylene spectra. Figure 6 shows an example of multi-region mapping on a gold slide. The correlation shown is to polyethylene (PE).

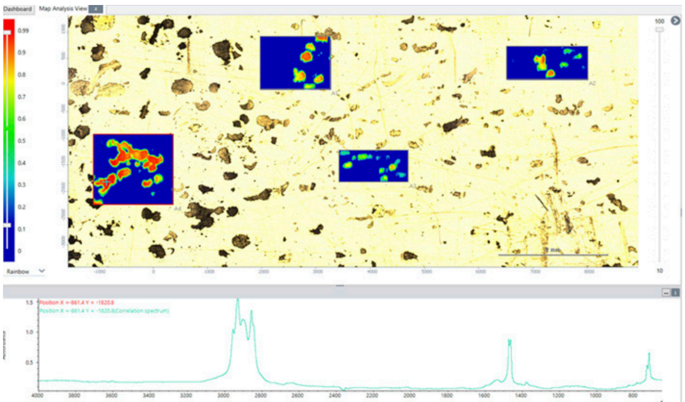


Figure 6. Reflective gold surface covered with microplastic particles. Four regions were selected for analysis. Regions highlighted.

This type of analysis can be applied to filters which have very dense particle groupings or when there are any concerns with very low visual contrast polymers on the surface of the filter. It is also helpful to do mapping analysis for fibers and films collected on filters. For an in-depth understanding of laminated film or degradation analysis of an environmentally exposed particle, this type of chemical approach can be used.

Conclusions

The analysis of microplastics has been greatly simplified by the Nicolet RaptIR FTIR Microscope and OMNIC Paradigm Software. Automation and sensitive particle finding tools have been combined with outstanding visual and IR optics to allow users from novices to experts to obtain excellent results. The extraction of the shape and size factors allows investigations of the origin and environmental evolution of the particles. This is a complete and reliable solution.

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Smart Notes

Q A

Is fast, automated particle analysis of microplastics achievable with FTIR microscopy?

Microplastics are a persistent and ubiquitous environmental pollutant; their presence in aquatic sources, air, and food chains is of growing concern across the globe. Working to address the concern, regulatory agencies monitoring environmental pollution aim to establish the scope and methodology for microplastic characterization. Yet the size and diversity of these micro-contaminants present challenges to such method development. How can this work be established and done efficiently?

Fourier-transform infrared spectroscopy (FTIR) is a widely accepted analytical tool for identifying polymers, and it's well-suited for detecting large numbers of microplastic particles in a simple, straightforward manner. The Thermo Scientific™ Nicolet™ RaptIR™ FTIR Microscope provides automation to quickly extract the information about microparticles. Let's walk through the short workflow for analyzing microplastics.

Methodology

Microplastics on appropriate filters such as silicon, gold-coated polycarbonate, or aluminum oxide (Al_2O_3) on stainless steel can be easily evaluated using the Particle Analysis feature in Thermo Scientific™ OMNIC™ Paradigm Desktop Software.

Image analysis of the filter surface first locates the particles based on their visual contrast. Next, the software uses the stored locations to move the sampling stage and collect a spectrum of each particle which is then analyzed against appropriate spectral libraries. This workflow is particularly efficient because the Nicolet RaptIR FTIR Microscope can collect hundreds or thousands of particle spectra without user supervision. Also, the collection time is minimized because only the spectra of particles, not the filter, are collected.

Finally, a report is created presenting results for each particle, including identification and size.

Nicolet RaptIR FTIR Microscope with the Thermo Scientific™ Nicolet™ iS50 FTIR Spectrometer

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Particle analysis in a few clicks

The Nicolet RaptIR FTIR Microscope is driven by its OMNIC Paradigm Software. The Particle Analysis tool allows microplastics analysis to be done with three simple steps.

1. Choose the target analysis region and define the size range for particles. See Figure 1.
2. Collect spectra from the selected set of particles with one click (typically, 16 scans at 8 cm^{-1}).
3. Request particle analysis report. Each particle's match to library spectra of known polymer materials and size characteristics are calculated and presented in the report.

Complete results in report format

An example particle analysis report is shown in Figure 3. The sample analysis picture is complete with a listing of each particle's image, size, and identification. The report can also be customized to show specific particle measurements and histograms of size distributions for each particle material.

Answer to the question

Fast, automated particle analysis with FTIR microscopy is not only achievable, but simple to perform. Hundreds of microplastics can be analyzed in minutes with the Nicolet RaptIR FTIR Microscope, depending on the resolution, scan number, and the number of particles being analyzed. The IR microscope has tremendous tools and flexibility designed for fast, accurate analysis of microplastics.

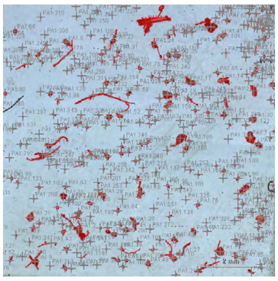


Figure 1: Silicon filter with atmospheric deposition of microplastics. Particles were selected in the size range of 25 µm – 1.5 mm.

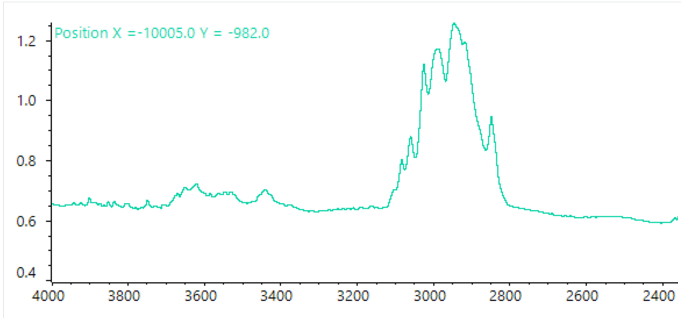


Figure 2: A single scan of a representative particle.

Rank	Component Name	Area %	Count	Library	Color
1	soil-silicate	6.79	11	test micropl	
2	MINIUM	0.29	5	HR Minerals	
3	Poly (tetrafluoroethylene)	2.77	4	Hummel Polymer and Additives	
4	PE with Additives	1.88	4	test micropl	
5	Poly(N-methyl acrylamide)	1.02	4	Hummel Polymer and Additives	
6	Polyethylene, LD	0.64	4	Hummel Polymer and Additives	
7	Polymerized, oxidized organic	3.72	3	Hummel Polymer and Additives	

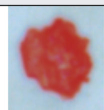
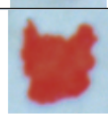
#	Component Name	Match %	Area (µm²)	Library	Particle thumbnail
PA1.99	Poly(N-methyl acrylamide)	76.97	10238	Hummel Polymer and Additives	
PA1.100	Ethylene glycol stearate	73.70	10188	Hummel Polymer and Additives	

Figure 3: Typical particle analysis report.

Learn more about the FTIR microscope that allows users to analyze large samples efficiently at **thermofisher.com/raptir**

Guide to the identification of microplastics by FTIR and Raman spectroscopy

Introduction

The presence of microplastics in the environment and our food-chain is of growing concern. This has led to increased testing for the presence of microplastics in a variety of samples including bottled, ocean and fresh water, which has brought about tougher legislation to limit the amount of plastics entering the ecosystem. Fourier Transform Infrared (FTIR) and Raman spectroscopies have long been used for the analysis of polymers, and it is, therefore, natural that they be the de facto techniques to identify microplastics. This note provides an overview of FTIR and Raman techniques as applied to the identification of microplastics.

Microplastics – common materials

A microplastic is a small piece of plastic that is <5 mm in size.[1] A list of common polymers found in microplastics is provided in Table 1.

Of these materials, polypropylene and polyethylene are particularly prevalent in the environment due to their production in vast quantities for consumer packaging applications. The humble plastic bag is made from polyethylene, while polypropylene is used for candy wrappers and bottle caps. These polymers float on both fresh and salt water, enabling them to travel long distances from the initial source of pollution.

Name	Abbreviation	Typical Density (g/cm^3)
Expanded Polystyrene	EPS	0.02
Polypropylene	PP	0.89
Polyethylene	PE	0.96
Acrylonitrile-butadiene-styrene	ABS	1.05
Polystyrene	PS	1.06
Polyamide (Nylon)	PA	1.14
Polymethyl methacrylate	PMMA	1.18
Polycarbonate	PC	1.21
Cellulose Acetate	CA	1.3
Polyvinyl chloride	PVC	1.39
Polyethylene terephthalate	PET	1.39
Polytetrafluoroethylene	PTFE	2.2

Table 1: Common polymers (densities derived from Teegarden[2])

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The infrared (IR) and Raman spectra of polyethylene and polypropylene are shown in Figures 1 and 2, respectively. Although both polyethylene and polypropylene are simple polyolefins, they can be readily identified and distinguished by both FTIR and Raman instruments, which are commonly used techniques throughout the polymer and plastics industries. The other polymers listed in Table 1 are also identifiable by their IR and Raman spectra.

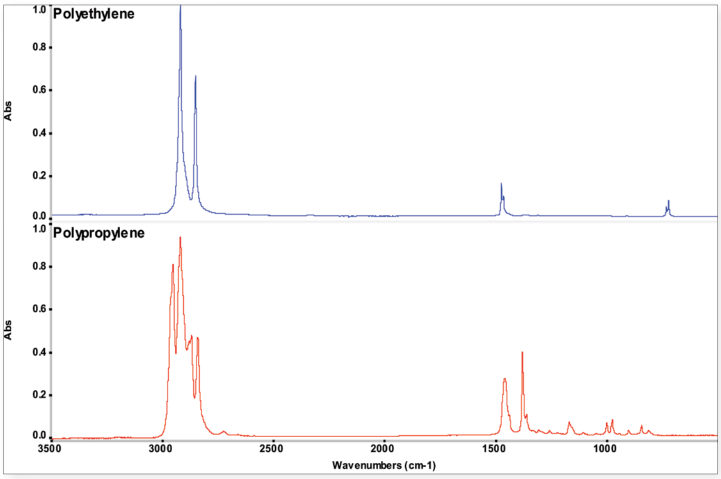


Figure 1: IR spectra of Polyethylene and Polypropylene.

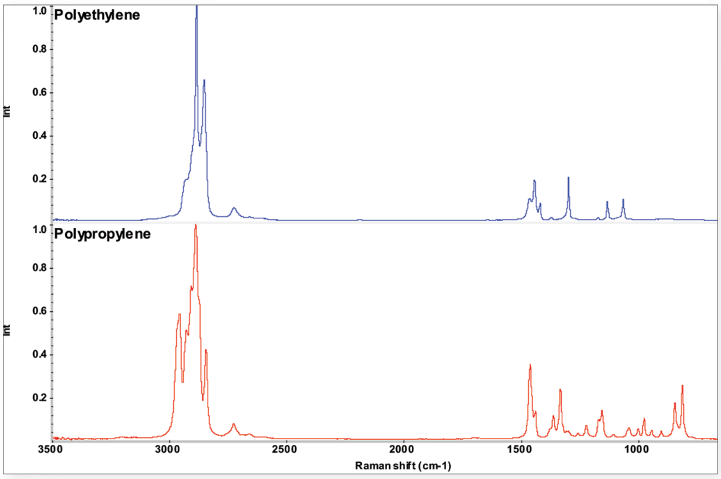


Figure 2: Raman spectra of polyethylene and polypropylene.

Microplastics – spanning a range of sizes

To be classified as a microplastic, the piece of plastic in question has to be small. How small? The National Oceanic and Atmospheric Administration (NOAA) defines a microplastic as being less than 5 mm long. Many particles of concern are smaller than this, typically between 100 µm and 1 µm. This is quite a range of sizes; from objects that are easily visible to the naked eye to small particles or fibers that can only be observed with a high-quality microscope.

Some microplastics are deliberately engineered to be small. These are termed “primary” microplastics. Primary microplastics are a target for legislative control. An example is the US ban on microbeads in personal care products enacted under the Microbead-Free Waters Act of 2015 (H.R.1321). Other microplastics start off as larger items that get broken down to smaller particles in the environment. These are designated as secondary microplastics. Both primary and secondary microplastics, spanning the range of particle sizes, are of concern in the environment due to their potential impact on marine life.

Concerns around the threat posed by microplastics to the health of organisms throughout the food chain include ingestion by marine organisms, e.g., zooplankton,[3] the presence of toxic materials used in the manufacture of the plastics, for example bisphenol-A, (BPA)[4] and the transport of persistent organic pollutants (POPs) by the microplastic particles.[5] While both FTIR and Raman can identify a long list of plastic materials, a number of instrument choices come into play when addressing the range of particle sizes. As the size of the particle decreases, the sophistication and cost of the equipment needed for its analysis increases. Therefore, the first consideration should be given to the size of microplastics to be studied when selecting the appropriate analytical platform. These considerations and more will be discussed throughout this paper, providing an overview and general guidance on spectroscopic instruments for microplastics analysis.

Analysis of particles from 5 mm to 100 microns

Particles in the size range of 5 mm to 100 microns are visible to the eye and can, with a steady hand, be manipulated with tweezers. As these are easy to see and handle, the spectroscopic system required for their analysis is relatively simple. By far the most common spectroscopic technique for the analysis of polymers is an FTIR spectrometer coupled with an Attenuated Total Reflection (ATR) accessory. The ATR allows the IR spectrum of a material to be obtained simply by pressing the sample against a transparent crystal, commonly diamond. The infrared light passes through the crystal into the sample where energy is absorbed by the sample, and the light is reflected back into the crystal to generate a spectrum. A Thermo Scientific™ Nicolet™ Summit FTIR Spectrometer equipped with a Thermo Scientific™ iD7 Diamond ATR is shown in Figure 3. The diamond is a couple of mm in diameter and does not need to be

Figure 3: Nicolet Summit FTIR Spectrometer with the iD7 ATR accessory in the sample compartment.

completely covered by the sample, making it ideal for the analysis of samples in this size range. One caveat with an ATR measurement is that it will detect materials that are on the surface of the sample. This is advantageous if a surface coating, such as an absorbed toxin, is of interest. However, if a sample has been weathered (irregular surface), this may interfere with its identification. If this occurs, the surface should be removed prior to analysis by slicing or polishing.

The ATR accessory pictured in Figure 3 does not allow the sample to be viewed after it has been sandwiched between the ATR arm and the diamond crystal. This presents no issue when dealing with samples in the 5 mm to 1 mm range. However, for samples smaller than this, it is preferable to be able to see the sample throughout its placement on the accessory and subsequent measurement. There are ATR accessories available that can provide viewing and magnification, facilitating analysis of samples in the 1 mm to 70 micron range. An example of such an accessory is the Czitek SurveyIR® Microspectroscopy Accessory as pictured in Figure 4.

Figure 4. Nicolet Summit FTIR Spectrometer with the SurveyIR® Accessory in the sample compartment.

An FTIR spectrometer with an ATR accessory is simple to use and relatively inexpensive. Further, the small form factor of the Nicolet Summit Spectrometer allows it to be moved close to where microplastics are to be collected and studied. This can be an advantage in environmental studies done outside the laboratory.

Analysis of particles from 100 microns to 1 micron

Once the particle size falls much below 100 microns, some magnification is required. There are two options here; IR microscopy and Raman microscopy (both techniques are also referred to as microspectroscopy). For particles less than 10 microns in size, Raman microscopy is the preferred choice.

Infrared Microscopy

Infrared (IR) microscopy enables the identification of particles down to 10 microns or less. There are several options available for IR microscopy in terms of both the **sampling technique** used and the **degree of automation** desired for the analysis.

Sampling techniques used in infrared microscopy include transmission, reflection and ATR. Transmission generally results in the best quality spectra, but often requires the sample to be pressed or otherwise processed to be less than 100 microns thick in order to allow the infrared light to pass through the sample. Reflection is, in principle, the easiest technique, as it requires no sample preparation or interaction between the microscope and the sample. However, it can result in distorted spectra, which may complicate the identification of polymer components. An ATR sample measurement works as described in the previous section. A downside of using ATR with microscopy is the potential of cross contamination between consecutive measurements because the ATR element comes into contact with the sample. This is not an issue with manual ATR systems where the crystal is easily cleaned between measurements. However, in automated microscope systems, in which the ATR comes into repeated contact with the sample without being cleaned between measurements, sample carry-over can present a problem. The choice of sampling technique is, therefore, largely dependent upon the nature of the sample.

The **degree of automation** available on an infrared microscope runs from simple point-and-shoot analysis of a single spot to fully-automated imaging covering a larger area of the sample and measuring multiple particles.

Figure 5 shows the Thermo Scientific™ Nicolet™ iN5 Infrared Microscope attached to the Nicolet iS20 FTIR Spectrometer. This is a point-and-shoot IR microscope designed for simplicity of operation. Example spectra

Figure 5. Nicolet iN5 IR Microscope attached to the Nicolet iS20 FTIR Spectrometer.

of microbeads, a primary microplastic in consumer products, were obtained using this IR microscope system shown in Figure 6.

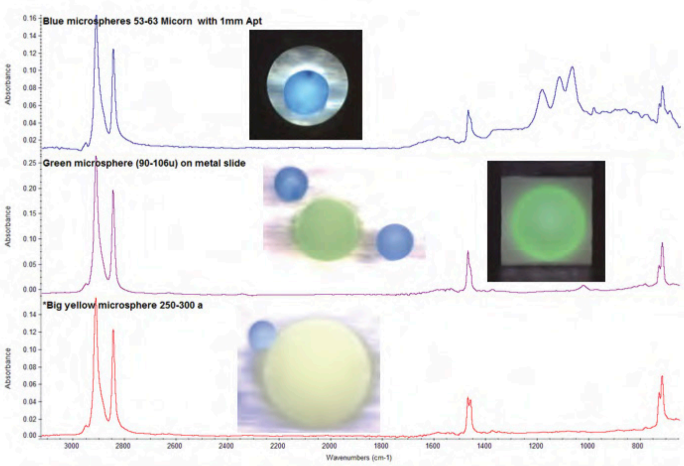


Figure 6. Spectra of microbeads obtained on a Nicolet iN5 IR Microscope. All spectra match polyethylene. The spectrum of the blue sphere also indicates the presence of barium sulfate.

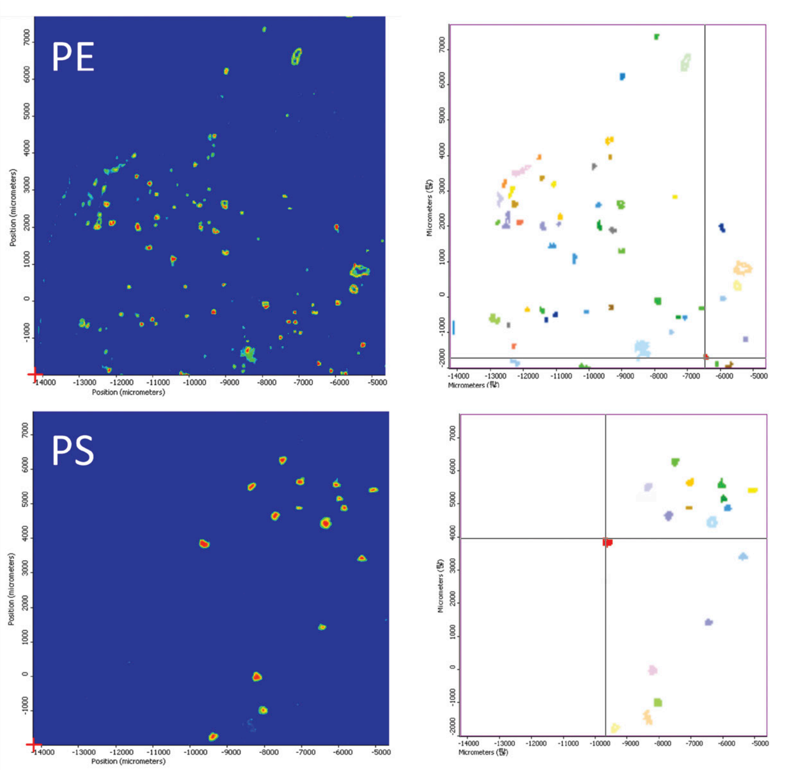
Point-and-shoot or single-point analysis is ideal for situations in which only a small number of particles are to be located and analyzed. The cost of this system is relatively low and, due to the few operator controls, it is easy to learn and use. However, if large numbers of particles are to be analyzed, some level of automation is desirable.

Filtration is frequently used as a final step in the isolation of microplastics from their matrix. Many particles may be captured on a filter surface where analyzing these filtered particles one by one is a laborious process. Thus, some level of automation is desirable when analyzing particles across the filter surface. Data collection and analysis can be automated through the use of a microscope equipped with a motorized stage and associated software. The Thermo Scientific™ Nicolet™ iN10 MX FTIR Imaging Microscope, pictured in Figure 7, provides this useful level of automation.

Figure 7: Nicolet iN10 MX FTIR Imaging Microscope with ATR accessories.

There are two main approaches to collecting data from particles distributed across a filter. The first is discrete particle analysis. In this approach, image analysis is performed on the video image of the filter to locate the positions of the particles. The system then automatically collects IR spectra from each location and then identifies each particle from its spectrum.

The second approach is imaging. In this case, an infrared image is collected from the entire region of interest, in which every pixel contains an infrared spectrum. This provides a chemical 'picture' of the filter. Automated analysis of this image by software can produce information about the identity, number and sizes of the individual particles. Such an analysis is shown in Figure 8. Here, two types of particles are identified from their IR spectra. Image analysis provides information about the number and dimensions of these particles. While, in principle, this is an elegant solution, there are some drawbacks compared to discrete particle analysis. The first is that the image may contain



Feature Sizing Results

Feature #	Area (µm²)	Length (µm)	Width (µm)	Aspect
1	29998	302.40	150.00	2.0
2	14999	75.00	100.00	0.8
3	27499	149.88	150.00	1.0
4	39998	187.18	150.00	1.2
5	19999	92.68	150.00	0.6
6	17499	92.68	100.00	0.9
7	182491	1055.18	399.99	2.6
8	22499	100.00	150.00	0.7
9	17499	85.35	150.00	0.6
10	14999	75.00	100.00	0.8
11	19999	278.07	150.00	1.9
12	14999	75.00	100.00	0.8
13	14999	75.00	100.00	0.8
14	17499	85.35	150.00	0.6
15	67497	331.50	299.99	1.1
16	19999	92.68	100.00	0.9
17	14999	75.00	100.00	0.8
18	17499	85.35	150.00	0.6
19	22499	108.13	100.00	1.1
20	19999	100.00	100.00	1.0
21	19999	110.35	150.00	0.7
22	37498	142.67	199.99	0.7
23	24999	110.35	150.00	0.7
24	59997	195.71	249.99	0.8
25	137493	495.07	449.99	1.1
26	34998	160.35	199.99	0.8
27	27499	150.00	150.00	1.0
28	27499	160.35	150.00	1.1
29	29998	133.93	199.99	0.7
30	29998	145.71	150.00	1.0
31	37498	153.03	199.99	0.8

Feature Sizing Results

Feature #	Area (µm²)	Length (µm)	Width (µm)	Aspect
1	57497	203.83	249.99	0.8
2	79996	520.11	299.99	1.7
3	59997	192.67	249.99	0.8
4	64997	211.16	249.99	0.8
5	39998	150.80	150.00	1.0
6	47498	175.69	249.99	0.7
7	64997	213.10	249.99	0.9
8	94995	263.38	349.99	0.8
9	59997	198.74	249.99	0.8
10	19999	100.00	100.00	1.0
11	39998	153.03	150.00	1.0
12	32498	142.56	150.00	1.0
13	42498	167.67	150.00	1.1
14	52497	178.03	199.99	0.9
15	47498	168.48	199.99	0.8
16	44998	195.71	249.99	0.8
17	49997	178.03	249.99	0.7
Total image area	94082712			
Total feature area	899955			
Feature area percentage	0.96%			

Figure 8. Chemical images of a filter (left) obtained on a Nicolet iN10 MX FTIR Imaging Microscope showing polyethylene (PE) and polystyrene (PS) particles, together with particle size statistics derived from the chemical images.

a substantial amount of redundant data. There may be only a small percentage of the image data set that contains information about the particles, the rest being the filter. As the image contains at least four dimensions of data (x-positon, y-position, spectral wavelength and absorption) the size of the 'data cube' containing many thousands of spectra can be excessive. The second factor is that the array detectors used for imaging are more expensive than the simple, single-point detectors used for discrete particle analysis. The approach chosen is situation dependent on 1.) how many particles are to be analyzed 2.) in what time period and 3.) over how large an area. Fortunately, the Nicolet iN10 MX IR Microscope provides options for all modes of sampling and automation.

Raman microscopy

Both Raman and FTIR spectroscopy are capable of identifying microplastics. However, Raman spectroscopy does have three distinct advantages when applied to microscopy. The first is that Raman spectroscopy uses sub-micron wavelength lasers as its light source and, as such, is capable of resolving particles down to 1 micron and less. FTIR microscopy uses mid-infrared light as its source, resulting in a wavelength range that loses the ability to identify particles much below 10 microns. The second is that, unlike IR systems, Raman microscopes are built around research-grade, white-light microscopes, which facilitate easy viewing of the particles. The third is the ease of sampling. There is no need to choose between transmission, reflection and ATR sampling techniques required for FTIR measurements. The Raman system laser is focused on the sample, and the spectrum is simply acquired by collecting the scattered light.

So, why is Raman microscopy not always the best choice for microplastics analysis? To balance the advantages, there are three key disadvantages to Raman microscopy. The first is the body of knowledge. FTIR spectroscopy has been around longer than Raman spectroscopy as a common analytical technique, and it evolved as the polymer industry grew. As such, there is a greater amount of historical data around IR spectroscopy than Raman spectroscopy for the analysis of polymers. However, this gap is decreasing as time goes on, and there are certainly enough reference spectra available to enable the identification of common microplastics. The second is cost. Typically, due to component costs, research-grade Raman systems are more expensive than their IR equivalents. The third drawback is fluorescence. Some samples exhibit fluorescence when irradiated by a laser. Fluorescence can obliterate the useful analytical Raman signal. This may be mitigated by the selection of appropriate laser wavelengths, but sample fluorescence is an issue in Raman microscopy that is not encountered in FTIR microscopy. With all this said, Raman microscopy is still the technique of choice for particles less than 10 microns in size due to the wavelength of the probing radiation.

As discussed above, sampling options for Raman microscopy are generally trivial. Unlike FTIR microscopy, where the quality of the spectrum is critically dependent upon the sampling technique, Raman microscopy simply measures the laser light scattered from the sample with no special sampling required. The key considerations for Raman microscopy are around the choice of laser, which affects signal strength and sample fluorescence.

As with IR microscopy, Raman microscopy offers a choice of automation options, from simple point-and-shoot for discrete particle analysis to high-speed imaging. The Thermo Scientific™ DXR3 Raman Microscope shown in Figure 9 is a fully automated Raman microscopy system. Just as with IR microscopy, the cost, complexity and sophistication of data analysis increases with the degree of automation required. The analysis of microplastics on a filter by Raman is shown in Figure 10.

Figure 9: DXR3 Raman Microscope for analysis of Microplastics.

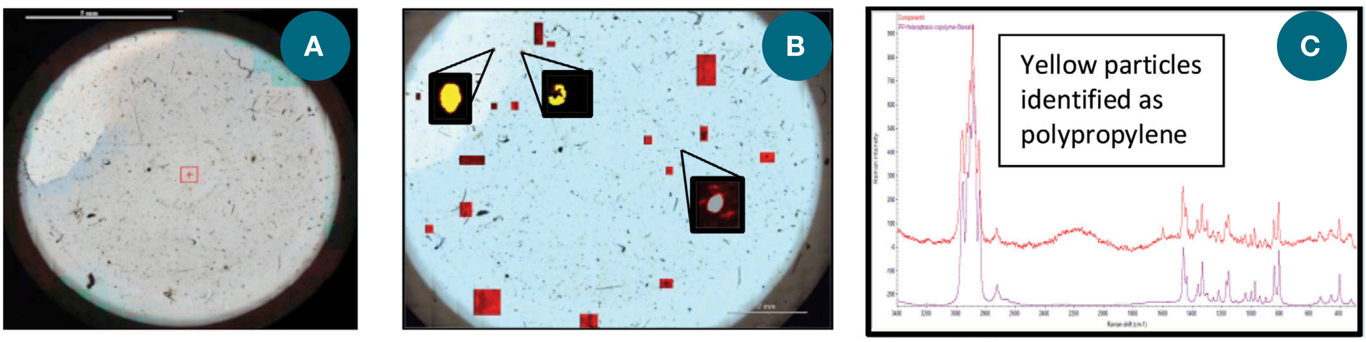


Figure 10: An example of microplastics analysis using the DXR3xi Raman Imaging Microscope. (A) Video image of the alumina filter with microplastic particles; (B) Chemical image of the filter with microplastic particles; and (C) Spectrum of one of the yellow particles compared to the library spectrum of polypropylene.

Conclusions

FTIR and Raman spectroscopy are powerful analytical tools to identify microplastics in the environment and bottled water. Many solutions are available, from simple point-and-shoot devices to sophisticated imaging systems. The choice of system depends upon the size of particles under investigation, where the analysis is to be performed, and the degree to which automation is required. This information is summarized in the instrument selection guide shown in Table 2.

	Microplastic size	FTIR + ATR	FTIR + Small Spot ATR	Point-and-Shoot FTIR Microscope	FTIR Imaging Microscope	Raman Microscope
Configuration		 Nicolet Summit FTIR Spectrometer and iD7 ATR Accessory	 Nicolet Summit FTIR Spectrometer and SurveyIR Accessory	 Nicolet iS20 FTIR Spectrometer and Nicolet iN5 IR Microscope	 Nicolet iN10 MX IR Imaging Microscope	 DXR3 Raman Microscope
Measureable Particle Size	5 mm	■				
	1 mm	■	■			
	500 µm	■	■			
	100 µm		■	■	■	
	10 µm			■	■	■
	1 µm					■
Manual Sample Placement Only		Yes	Yes	Yes	No	No
Automated Analysis of Filters		No	No	No	Yes	Yes
Immunity to Sample Fluorescence		Yes	Yes	Yes	Yes	No
Relative Cost		$	$$	$$$	$$$$	$$$$$

Table 2: Analytical instruments for microplastics analysis.

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Microplastic identification and characterization by Raman imaging spectroscopy

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Key words: DXR3xi Raman Imaging Microscope, Microplastic identification, Raman, Marine environment, Marine Ecosystem, Plastic debris, gold filter, Raman microspectroscopy

Introduction

Plastic pollution in aquatic environments is extremely harmful and is at present widespread all over the world. The huge, 299 million tons/year, worldwide production of plastics,[1] which is linked to an increased use of disposable goods, combined with low degradability of polymers have contributed to the accumulation of plastic debris in natural habitats.[2] Despite the durability of synthetic polymers, large plastic items can undergo fragmentation processes, mainly as a consequence of mechanical breakdown caused by abrasion by sand and other materials or wave action and is promoted by photochemical processes triggered by UV-B light.[3,4,5] The smaller plastic fragments, having diameters of 5 mm or less, have been categorized as microplastics.[6,7] This category has been further divided in large microplastic (L-MPP, ranging between 1 and 5 mm) and small microplastic particles (S-MPP ≤ 1 mm), with diameters of 1 mm or less.[8]

Microplastics can absorb both persistent organic pollutants (POPs) and heavy metals from water and sediments, and the smallest particles can enter the food web,[9,10] posing a serious health risk to wildlife and ultimately to humans.

Identification and quantitation of microplastics are important analytical challenges, and the lack of official analytical methods makes the comparison of different studies difficult. Most of the research studies carried out to date begin by visually sorting particles under a stereo-microscope to separate potential microplastics from other debris.[11]

The principal limitation of visual sorting is particle size, as the smaller the size, the greater the difficulty of discriminating microplastics from interfering particles.[12, 13] Therefore, it is highly recommended to analyze sorted particles with techniques that enable proper identification of plastics, such as spectroscopic techniques or pyrolysis-GC/MS (py-GC/MS), even if these approaches are less efficient and miss the microplastic particles discharged by visual sorting.

A recent study[14] indicated that spectroscopic techniques such as FTIR, NIR, and Raman in microplastics identification and quantification increase the sensitivity and the accuracy of the analysis, but few methods exist that enable fast and reliable analysis. Generally, spectroscopic approaches are single-point analyses that are not automated; only a few semi-automated filter analysis studies have been conducted, employing single MCT detector-μFTIR-chemical mapping[15,16] to analyze only a few sub-areas of the filter surface and, more recently, using Focal Plane Array (FPA)-based imaging μFTIR to scan the whole surface of a small filter with diameters greater than 10 mm.[17] Even though this last approach provided very good results, showing high lateral resolution and allowing the detection of particles down to a size of 20 μm, smaller microparticles and sub-microparticles cannot be analyzed by μFTIR techniques, due to diffraction phenomena which occur below 10 μm in FTIR. Another drawback is a long analysis time, which can be on the order of tens of hours.[18] To overcome these limitations, the most promising technique is Raman imaging microscopy, which combines high spatial resolution, typical of Raman microscopy technique, with the speed of an imaging technique.

This paper discusses a simulation of the analysis of microplastic particles by using reference materials to provide an ideal analytical model of potential environmental samples.

Experimental

Materials and methods

Three different reference materials were used to simulate microplastic debris with different particle sizes: irregularly shaped polyethylene particles sieved at 74 microns (Sigma-Aldrich S.r.l.), polystyrene-divinylbenzene PS-DVB (2% of DVB - Sigma-Aldrich) microspheres sieved between 37 -74 microns, and polyethylene-titanium dioxide PE-TiO_2 microspheres with a size range of 27-45 micron (white polyethylene, opaque polymer microspheres beads; density 1.25 g/cm^3 - Cospheric LLC). A few milligrams of each type of particles were mixed with 0.5 L of water and filtered through a gold-coated polycarbonate membrane (Whatman® Nuclepore™ Track-Etched Membrane 800195) with a diameter of 13 mm and a pore size of 0.8 µm. After filtration, the membrane was dried in a furnace at 60°C for 2h before Raman microscopy measurements to avoid shrinking the filter under laser illumination. The filter was then blocked between two thin glass cover slips (180 microns thick) in order to improve its flatness and was measured in confocal mode with the Thermo Scientific™ DXR™2xi Raman Imaging Microscope. The gold filter was chosen due to its versatility since it can be used for Raman as well as for infrared microscopy measurements.

Raman analysis

Raman data were collected using the DXR3xi Raman Imaging Microscope and the accompanying Thermo Scientific™ OMNIC™xi Raman Imaging Software. The DXR3xi Raman Microscope is capable of collecting up to 600 spectra/second, enabling the analysis of the whole surface of a filter. This approach greatly expands the analytical possibilities for microplastics and sub-microplastics down to the nanoplastics range, and also significantly reduces the analysis time. This increase in acquisition speed means that collection of large-area Raman images is now, not only practical, but can be routine. The OMNICxi Software also represents an evolution in software specifically designed for imaging, providing a convenient and easy-to-use graphical interface for harnessing all additional data. The software also contains powerful analytical algorithms to process the data into informative Raman images in real time. During data acquisition, Multivariate Curve Resolution (MCR) is the technique used to calculate the number, relative concentration and distribution of the components of an unknown mixture without prior information.

Raman imaging result

The 13 mm gold filter has an active 10 mm diameter area that can easily be imaged with the DXR3xi Raman Microscope. An area of 10 mm x 10 mm was selected, as shown by the red frame in Figure 1A, and a 10x objective was used to scan the entire active surface. The acquisition speed was 400Hz (400 spectra/seconds) and 13 exposures were collected at each point using a 532 nm laser, a laser power of 10 mW measured at the sample, and a pinhole aperture of 25 µm. The image pixel size was 10 µm and more than 600,000 spectra were acquired. Figure 1B, C, and D each show the distribution of the microplastic particles; PE, PS-DVB, and PE-TiO_2 respectively, on the filter surface after the acquisition of the Raman spectral image. Identification of the particles was accomplished by using the library search tool within the OMNICxi Software.

The collection mode of the OMNICxi Software permits the acquisition of one exposure over the full region of interest, allowing a quick visualization of the chemical information all over the image. The quality of the image can be improved by co-adding exposures to the first one with the same collection mode. This feature allows the user to look at different chemical information such as peak height, peak area, peak position, peak area ratio, peak height ratio and MCR during the collection and to stop the experiment as soon as the desired information is obtained.

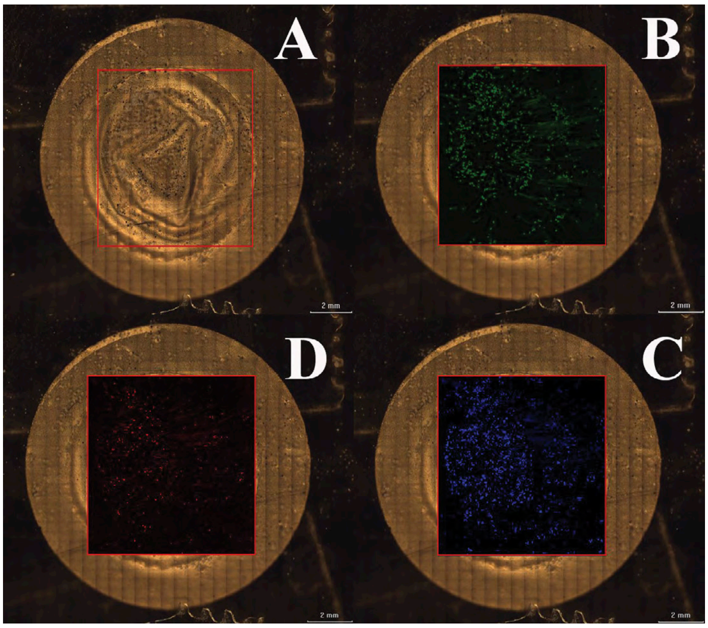

Figure 1: A, optical image of the gold filter; **B**, distribution of irregular shaped PE particles, which are highlighted with green; **C**, distribution of PS-DVB, which are highlighted with blue; **D**, distribution of PE-TiO_2 microspheres, which are highlighted with red.

The spectra of the three reference microplastic particulate materials are shown in Figure 2 and were used to create a library. The search capability of the OMNICxi Raman Imaging Software, which allows the identification of the particles by using built-in libraries, is illustrated in Figure 3.

The analysis of the Raman spectra underlines the value of Raman microscopy as compared to infrared microscopy. Not only does Raman microscopy have better spatial resolution than infrared microscopy, but it also enables the identification of the plastic additive, titanium dioxide (TiO_2) in this specific case, since the low wavenumber range down to 50 cm^{-1} is accessible with Raman spectroscopy. The identification and quantitation of TiO_2 are important because of recent studies on its potential toxicity[18] and due to the fact that it is widely used in packaging material as well as in cosmetics such as scrubbing cream, toothpaste, and other common and widely used products. The FPA infrared detector, which is most commonly used for this application, has a cut-off at 900 cm^{-1} and, thus is not suitable for the determination of TiO_2.

The map analysis using the MCR algorithm calculates the distribution and size of the three standard particles and facilitates visualization of each reference material with a different color. Figure 1 shows the complete picture of the filter with the particulate materials identified, while Figure 4B shows an enlargement of a specific region where it is possible to appreciate how Raman analysis is able to identify small particles.

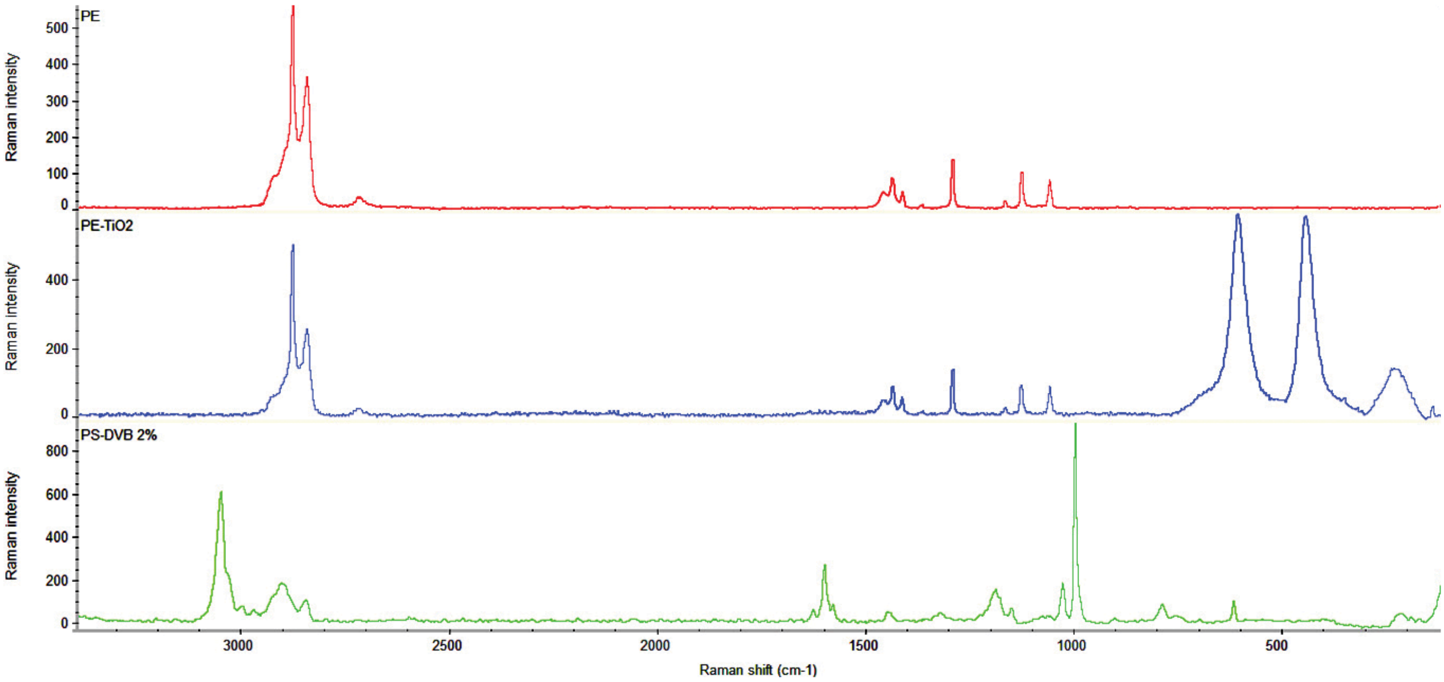

Figure 2: Raman spectra of the microplastic standards: PE - polyethylene; PE-TiO_2 polyethylene-titanium dioxide; PS-DVB – polystyrene-divinylbenzene.

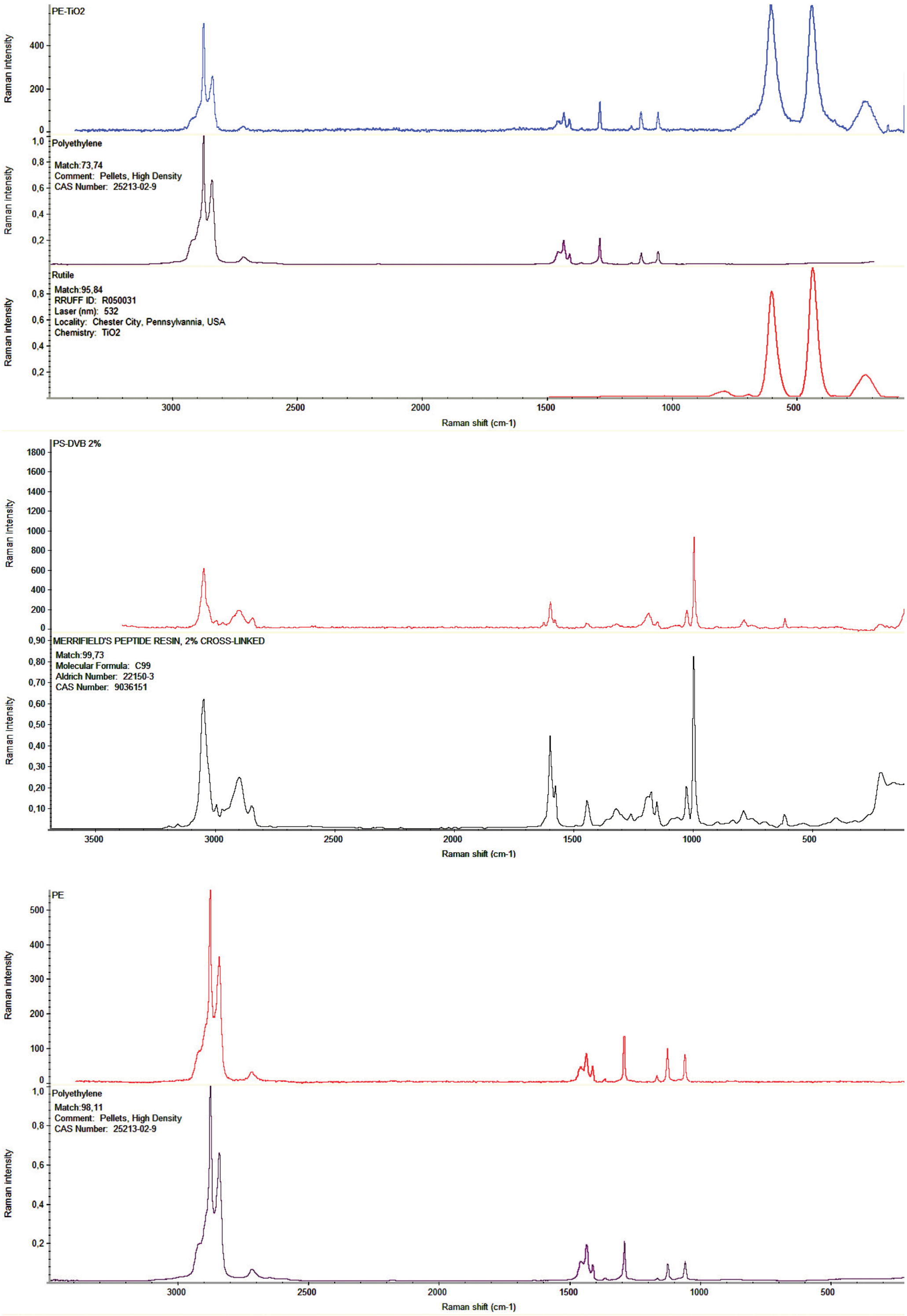

Figure 3: Identification of unknown particles through library search of the microplastic standards

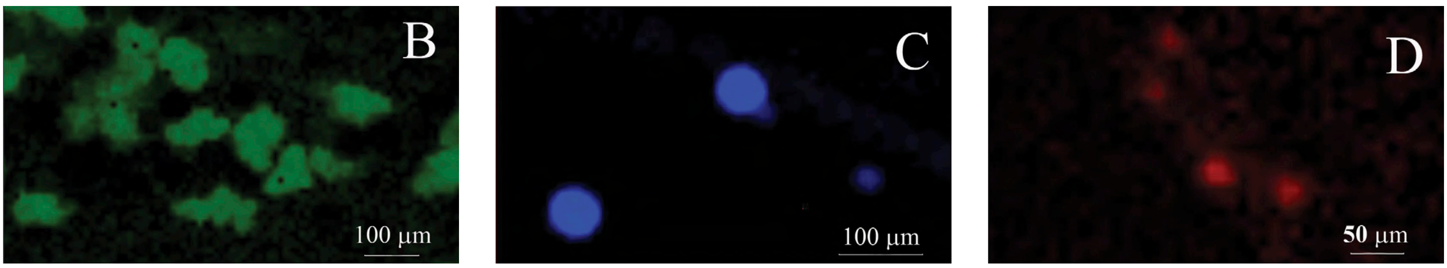

Figure 4: B, distribution of irregular shaped PE particles; **C**, distribution of PS-DVB; **D**, distribution of PE-TiO_2 microspheres. Enlargement of specific region of Figure 1 to emphasize the chemical image resolution reachable with 10 micron pixel size.

Figure 5 shows a map of approximately 1.5 mm x 2 mm acquired with a smaller image pixel size of 3 microns and a 50x long working distance objective. The same acquisition parameters were used; three exposures were collected for each point and more than 400,000 spectra were acquired. The smaller image pixel size improves the quality of the image and enables correct identification of the circular shape of the smallest standard microspheres of PE-TiO_2, as shown in Figure 5D. These particles were not well defined in the previous map. OMNICxi Software allows the user to see the chemical image superimposed on the optical image and to control its transparency allowing a direct correlation between image and chemical information.

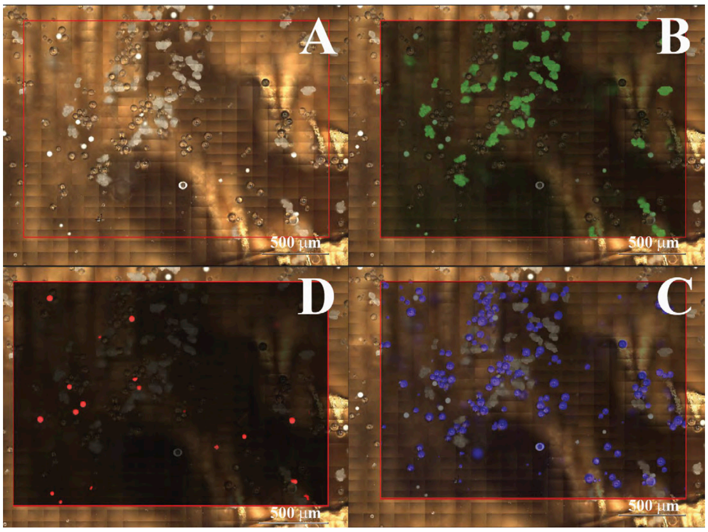

Figure 5: A, optical image of the gold filter; **B**, distribution of irregularly-shaped PE particles, which are highlighted with green; **C**, distribution of PS-DVB, highlighted with blue; **D**, distribution of PE-TiO_2 microspheres, which are highlighted in red. Superposition of the optical and chemical images for a direct correlation between the sample image and the chemical information

Conclusion

This paper illustrates how Raman imaging microscopy can be used for the analysis and identification of small microplastic particles. It showcases a valid alternative to, and in some cases, a more attractive technique than infrared imaging microscopy. In fact, Raman microscopy allows the analysis of particles below 1-micron size and the correct identification of plastic materials as well as inorganic materials such as titanium dioxide or other additives.

Acknowledgement

We would like to thank the National Research Council, Padova Unit (CNR-IDPA, Venice) for providing the microparticle standard and for fruitful scientific discussions.

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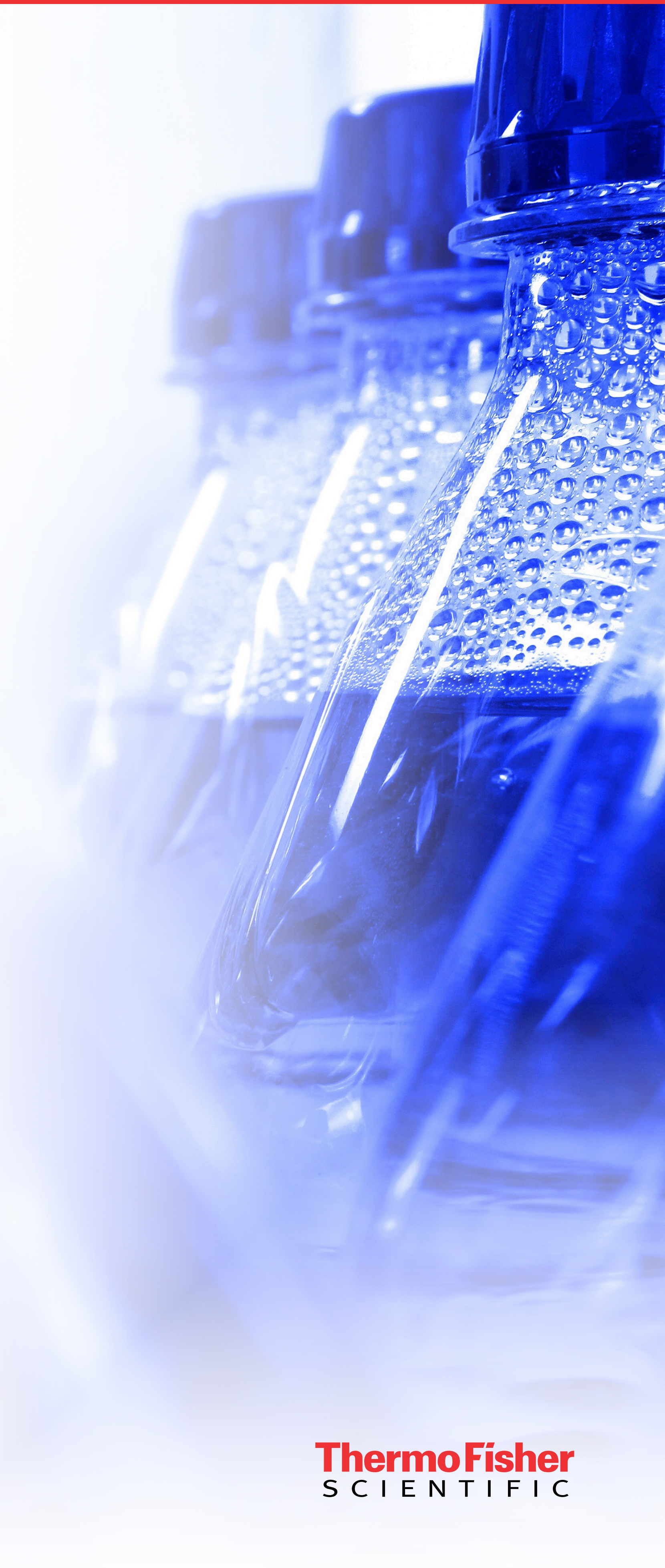
Microplastics in bottled water

Abstract

Microplastic pollution in water, land or even air has become an increasing concern for society. In addition to its detrimental impact on ecosystems and human health, recent revelation that many bottled water and other beverages products contain microplastic contamination also put many corporations at risk of negative publicity and brand erosion. Combating the microplastic pollution requires a thorough understanding of its sources and pathways, which starts from the detection and identification of microplastics in different types of samples. The combination of the Thermo Scientific™ Nicolet™ iN10 MX Infrared Imaging Microscope, Thermo Scientific™ DXR3xi Raman Imaging Microscope, Thermo Scientific™ OMNIC™ Picta™ Software, and polymer libraries from Thermo Fisher Scientific offers a complete solution to address the challenge in analyzing microplastics of a wide size range (1-5000 µm).

Microplastics…a growing global concern

The widespread presence of microplastic material (small synthetic plastic particles <5 mm) in the environment has gained considerable attention in recent years, as regulators, researchers, and manufacturers race to understand its sources and pathways, to assess its impact on ecosystems and human health, and to develop effective means to tackle the issue. Products ranging from simple synthetic fibers in clothing to plastic microbeads in consumer products have, over time, left residual materials in the environment, especially in the aquatic environment. Starting from the accumulation of microplastics in ocean water, microplastics are infiltrating fresh water, land and even air. Recent studies have revealed a much more pervasive problem than many feared. Overall, 83% of the tested tap water samples were contaminated with plastic fibers, of which the US has the highest contamination rate at 94% and Europe has the lowest at 72%. The research at the State University of New York at Fredonia indicates that 93% of tested bottled water showed signs of microplastic contamination, and the contamination is at least partially coming from the packaging and bottling process. Microplastic fibers and fragments were also found in beer, honey, sugar and air.[1]



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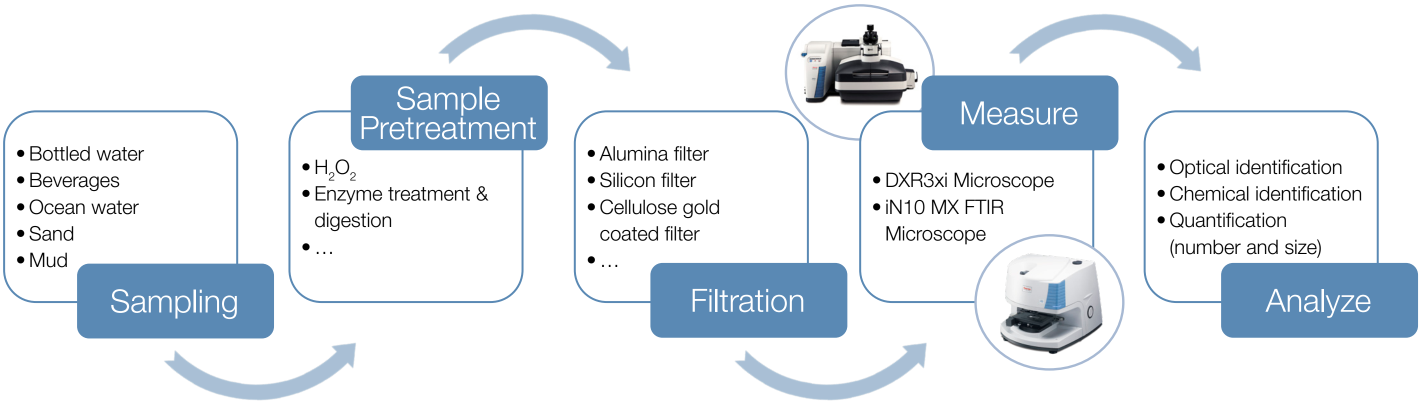

Figure 1. Schematic showing the typical workflow for microplastics analysis.

The Thermo Fisher Scientific solution

Figure 1 illustrates a typical workflow for microplastics analysis. After pre-treatment, where necessary, to remove biogenic material, aqueous samples are passed through filters. Dried filters are then directly placed onto the sample stage of microscopes for microspectroscopic analysis.

The Nicolet iN10 MX FTIR Microscope is ideally suited for the analysis of microplastic particles >10 µm. An example is demonstrated in Figure 2. The visual image (Figure 2A) consists of more than 200 video captures combined into a mosaic covering approximately 1 cm2. A total of ~17,500 spectra were collected in ~30 min (50 µm steps and 0.1 s/spectrum). Representative spectra from the filter itself and the spheres are shown in Figure 2B. Using the polyethylene spectrum from a reference standard, a correlation map relating to each spectrum in the map was constructed (Figure 2D), where the red spots are strongly correlated to polyethylene while the blue field is uncorrelated. The particle wizard of the Picta Software can complete the analysis automatically. After a region from the video image is selected, the software identifies the target particles and proceeds to produce spectra for each particle. These spectra are then searched against a spectral library, and a report catalogs the number of particles in the inspection area. The data can then be back extrapolated through the volume of filtered liquid to provide a semi-quantitative measure of the particulate concentrations.

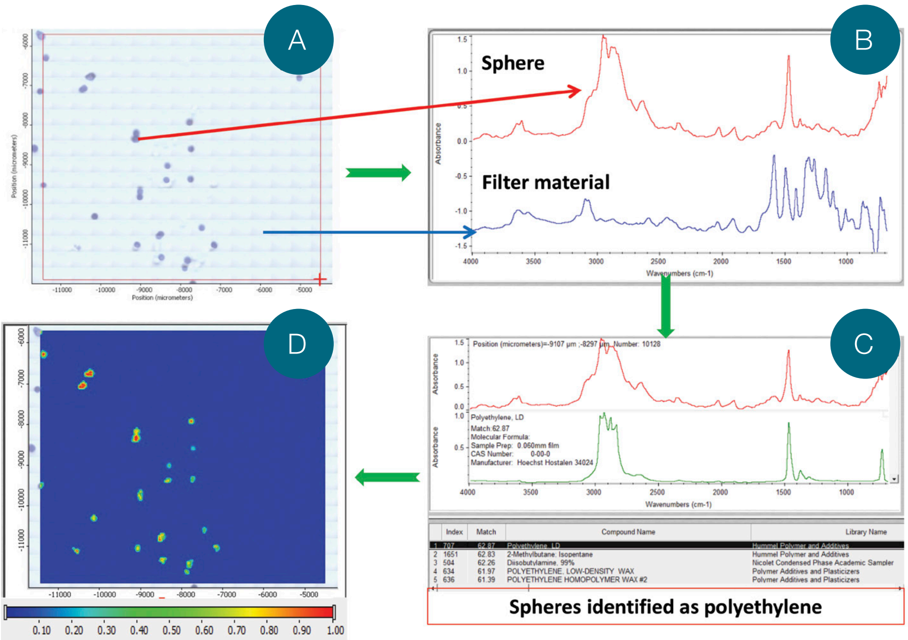

Figure 2: An example of using the Nicolet iN10 MX FTIR Microscope for microplastics analysis. (A) A visual image of the filter showing particles; (B) Spectra of the particles and the filter paper; (C) Library searching results of the particles; and (D) Correlation map to the spectrum of the particles.

For particles <10 µm, the DXR3xi Raman imaging Microscope offers a powerful solution with a spatial resolution down to 0.5 µm. Figure 3 shows the analysis of a sample of ocean water collected from the Pellestrina beach in the Lagoon of Venice. The software for the DXR3xi Raman Microscope recognized and located several particles on the alumina filter from the optical image (Figure 3A). Only the regions of interest, based on predefined criteria, were then selected for spectral acquisition, which effectively minimized the total analysis time. During spectral acquisition, a real-time MCR (Multivariate Curve Resolution) allowed chemical identification of the particles, but also provided direct visualization of the particles of different chemical origins (Figure 3B). For example, all three particles outlined in Figure 3A have a size between 5 to 10 µm. The yellow particles were identified as polypropylene, and the grey one was identified as PV23 Hoechst Laser pigment.

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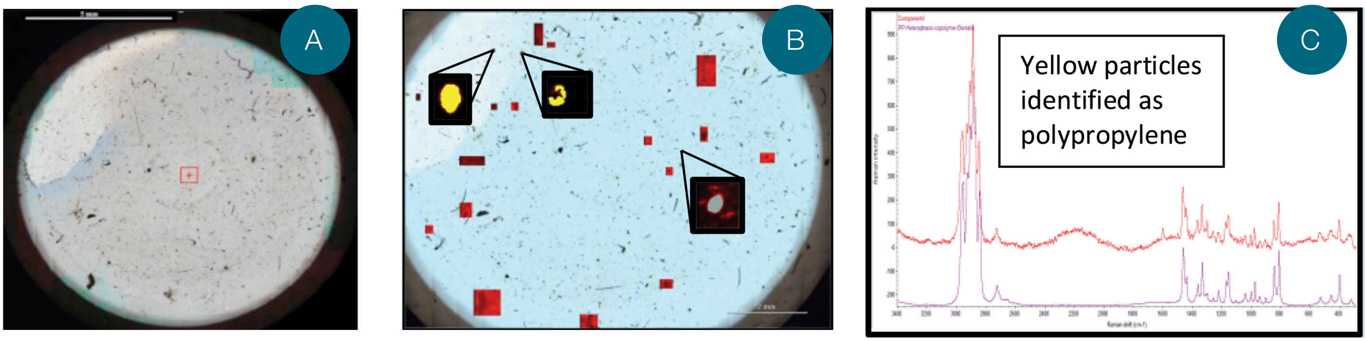

Figure 3. An example of microplastics analysis using the DXR3xi Raman Microscope. (A) Video image of the alumina filter with microplastic particles; (B) Chemical image of the filter with microplastic particles; and (C) Spectrum of one of the yellow particles compared to the library spectrum of polypropylene.

Conclusions

The wide size range of microplastics (1-5000 µm) poses a particular challenge for their analysis. Thermo Fisher Scientific is uniquely positioned to tackle that challenge with an industry-leading product portfolio that includes 1.) the Nicolet iN10 MX FTIR imaging Microscope with Picta Software for particle sizes >10 µm and 2.) the DXR3xi Raman imaging Microscope for particles >1 µm in diameter. The intelligence built into both Picta and OMNICxi Software can be used to facilitate the analysis of samples where microplastics are present. Ultimately, the Nicolet iN10 MX FTIR Microscope and the DXR3xi Raman Imaging Microscope provide tools to maximize analytical efficiency and minimize analysis time of microplastics found in our water supply.

Reference

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APPLICATION NOTE

No. AN53167

Straightforward identification of microplastic particles in bottled water

Using FTIR & Raman Spectroscopy

Plastics can be formed into an endless variety of shapes with different physical characteristics and that makes them attractive materials for a wide assortment of products. Large and diverse usage results in a significant amount of plastic materials eventually ending up as refuse. The size of the plastic waste can range from large, easily visible, easily handled, and easily detectable macroplastics down to microplastic particles that may be too small to detect with normal vision. The European Chemical Agency (ECHA) defines microplastic particles as particles with dimensions from 5 mm to 1 nm. The source of these microplastics is from primary sources where the plastics have been produced as small particles (such as in some cosmetic products and cleaners) or as secondary products that arise from the mechanical, photochemical, or thermal break down of larger plastic products. Many studies on microplastics have centered on contamination in marine environments and related areas (sea salt, seafood, and aquatic animals) they also appear in tap water and even in commercial products such as bottled water and other beverages. The detection and identification of microplastic particles are important for determining the possible sources of these contaminants and for evaluating the environmental impact and health risks associated with the different types of materials. There are ongoing efforts to understand the full impact of these microplastics.

One of the biggest challenges with the analysis of microplastics involves distinguishing microplastic particles from other types of materials and making sure that misidentification does not lead to over-estimating the amount of microplastics in a sample. While there can be all types of matrices that contain microplastic particles this report will focus on microplastics in a dilute water suspension. The particles are isolated by filtration. After filtration the sample consists of a collection of particles on a filter and at that point the analysis requires a facile way of distinguishing and identifying microplastic particles within a field of other particles.

FTIR and Raman spectroscopy (two different forms of vibrational spectroscopy) are widely used for the unambiguous identification of different types of polymeric materials. The molecular information reflected in the vibrational spectra of polymer materials provides a molecular fingerprint that can be used with spectral databases to positively identify different types of polymer materials. The spectral information supplied by infrared and Raman spectroscopy is similar but not exactly the same. Infrared spectroscopy depends on changes in dipole moments and is particularly useful for detecting polar functional groups found in many different types of polymer materials (hydroxyl, amines, amides, carbonyls, etc.). Raman spectra depend on a change in polarizability and thus are good for looking at polymer backbone structures as well as where there are delocalized electrons such as alkenes and aromatics. Raman spectroscopy can be more sensitive to differences in molecular structures. Infrared spectroscopy is more useful for identifying some types of polymers and Raman spectroscopy is better for other types of polymers but when used in conjunction they deliver complementary data that is more useful than either technique alone. Both FTIR and Raman spectroscopy can be used to analyze microscopic-sized samples. This makes vibrational spectroscopy an indispensable tool for the identification and analysis of microplastic particles.

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The analysis of microplastic particles described here can be called a targeted discrete point analysis approach. In this approach a visible image is obtained from multiple fields of view through the microscope stitched together to form a visual mosaic. This visual mosaic is used to automatically select possible particles for analysis as well as to provide the information on the particle size and shape. Once all the potential particles have been targeted, vibrational spectra are collected from these particles and used to determine which particles are truly microplastics. The keys to this approach are a good visual image of the particles and a filter material that has minimal spectral interferences. This approach has an advantage over just collecting a Raman image of the whole filter in that imaging such a large area at a reasonable resolution requires an incredible amount of spectral data. If the distribution of particles on the filter is relatively dilute then a majority of the spectral data shows only the filter and no particles.

Experimental

The sample used in this investigation was a commercially available 500 ml bottle of drinking water. The drinking water was filtered using the Thermo Scientific™ Microparticle Sample Preparation Kit. The kit utilizes a 10 x 10 mm silicon filter that can be used for transmission FTIR analysis, reflection FTIR analysis, or Raman analysis of the isolated particles.

The silicon filters were then analyzed using both a Thermo Scientific™ Nicolet™ iN10 MX FTIR Imaging Microscope and a Thermo Scientific™ DXR3 Raman Microscope. Both of these instruments utilized software options specifically designed for automated particle analysis. The Nicolet iN10 MX FTIR Imaging Microscope uses the Particle Wizard option in the Thermo Scientific™ OMNIC™ Picta™ Software and the DXR3 Raman Microscope uses the Particle Analysis option that is part of the Thermo Scientific™ Omnic™ Atlus Software and the Thermo Scientific™ Omnic™ for Dispersive Raman Software.

The analysis using the iN10 MX FTIR Imaging Microscope was done in transmission mode using an MCT A detector and 64 scans per spectrum. The background position was selected as an area clear of particles near the center of the silicon filter. The apertures were automatically selected as part of the Particle Wizard routine and are based on the particle size. The number of potential particles was determined from an image analysis of the visible mosaic image and refined through adjustments made to the sensitivity and particle size parameters.

The analysis using the DXR3 Raman Microscope was done using a 10X objective and a 532 nm laser. Each spectrum was collected using a 2s exposure and 10 exposures. The number of potential particles were selected using the Particle Analysis routine and like the iN10 MX FTIR Imaging Microscope analysis this is based on an image analysis of the visual mosaic along with sensitivity and size parameters.

Results and discussion

The workflows using both the iN10 MX FTIR Imaging Microscope and the DXR3 Raman Microscope are very similar. In both cases the analysis starts with defining and collecting a visual mosaic of the area on the silicon filter containing the particles filtered from the drinking water. Then an image analysis of the visible mosaic is used to select possible particles. This is based on the contrast in the image and the sensitivity can be adjusted to determine what visual features are selected as particles. There is also a sieve based on size that can be used to adjust what sizes of particles are chosen for analysis. These parameters help determine the number of potential particles selected for spectroscopic analysis. Figure 1 shows the process of particle selection using the Particle Wizard software in Picta Software with an iN10 MX FTIR Imaging Microscope and a silicon filter with particles filtered from bottled drinking water.

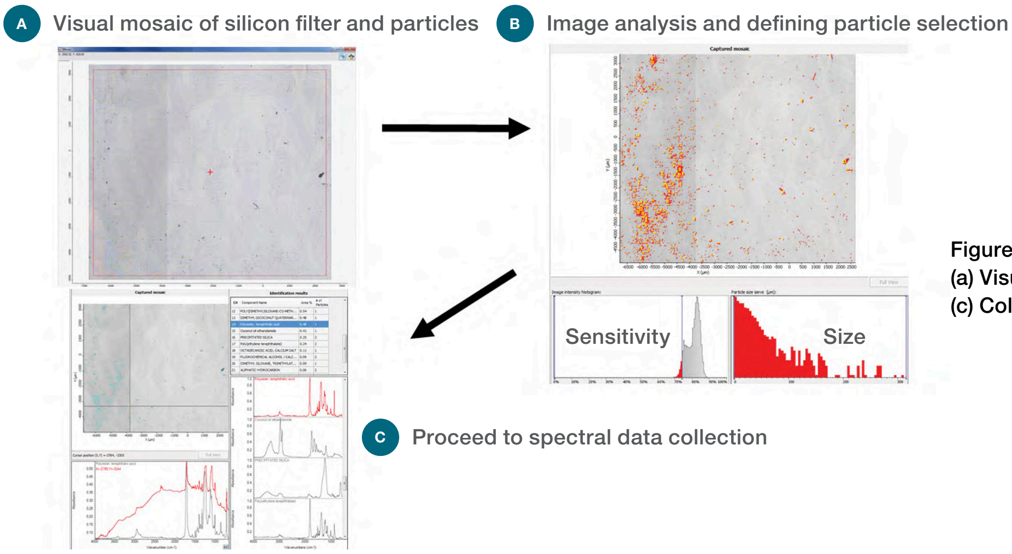

Figure 1. Workflow for particle analysis: (a) Visual Image, (b) Selecting potential particles, (c) Collect spectral data and report results

Once the potential particles have been selected, the next step is to collect spectral data to identify the microplastic particles. Not all the features visually identified as particles are actually particles. They could arise from things like scratches or discoloration of the filter as well as other visual differences that are not actually different materials. It is also possible to have particles that are biological or mineral based. These types of particles while interesting are not the goal of this analysis. Some particle materials such as metals would not be either Raman or infrared active and thus would not provide a vibrational spectrum. Actually a small portion of the features are microplastics and the goal of the analysis is to ferret out these particles.

After the spectral data is collected, the spectra are searched against spectral databases to find matches and identify the particles. A summary of the number of particles of each type is included as part of the analysis report along with the spectra from the individual particles and the dimensions of the particles. Analysis reports can be exported as text document that can be incorporated into other software programs or can be printed in hard copy or electronic format. Table 1 shows the summary of the identified microplastic particles from the silicon filter both for the FTIR and Raman analysis. The size of the particles was about 10–200 microns and length and width information is provided for each particle in the full report. An example of a particle from the Raman analysis is shown in Figure 2. This is a polypropylene fiber that is approximately 16 microns wide and 78 microns long.

Table 1. Summary of the microplastic particles identified on the silicon filter by both the FTIR analysis and Raman analysis

	FTIR (total particles – 801)	Raman (total particles – 1065)
PTFE	5	9
Polyester (PET)	3	3
Polystyrene	0	3
Polypropylene	3	3
Unspecific long chain aliphatic hydrocarbon containing materials	2	1
Polyethylene	2	1

While the FTIR and Raman analysis did not identify exactly the same number of microplastic particles the majority of the materials are the same. The differences can be explained by the differences in the selection rules, the way the spectral data is collected, and differences in the spatial resolution. Some materials are better suited to identification by each technique. A combination of the two is advantageous for optimizing the analysis of a wider range of particles.

As indicated previously, only a small number of the potential particles are actually microplastics. Particles of other materials such as minerals (talc, quartz, and titanium dioxide) and biological-based materials (cellulose, proteins, and beta-carotene) were also identified. A majority of the features selected by the image analysis routine were not identified. Many of these are simply visual features that did not represent real particles. Some are from materials that are not Raman or FTIR active. The spectra from these areas show no features beyond those associated with the filter itself. However, since the goal was to identify microplastics particles the fact that these other materials are not clearly defined does not matter for this application. While the collection of spectral data from particles that are not microplastics might be seen as slowing down the analysis, the fact that the data collection and most of the analysis is automated means that the arduous and involved task of separating microplastics from non-microplastic particles does not require manual subjective visual sorting and thus saves working hours.

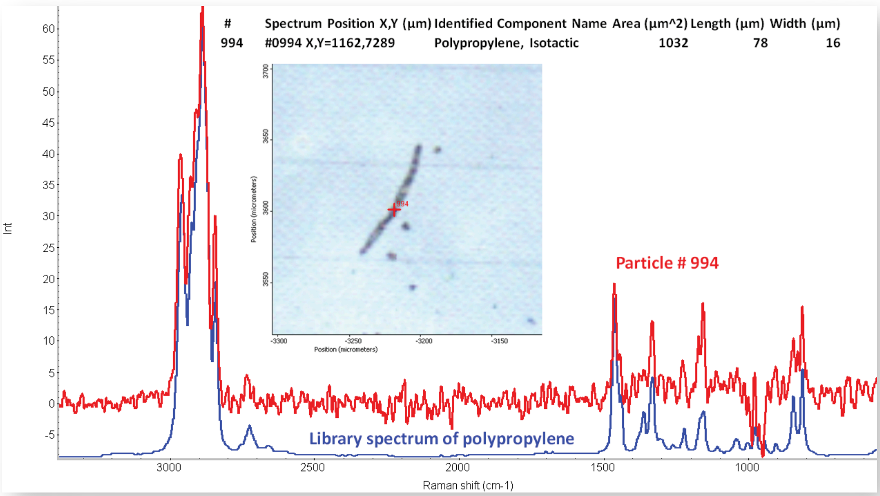

Figure 2. Example of a microplastic particle from the Raman particle analysis of the silicon filter. The particle is a small polypropylene fiber approximately 78 microns long and 16 microns wide. The Raman spectrum of the fiber matches a library spectrum of polypropylene.

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Summary

Microplastics have been discovered in a wide variety of locations ranging from sea life to food and beverages. While the majority of microplastics are likely to be biologically benign with the scope of the distributions and the amounts of the materials being as large as they are it is important to understand the total impact. To that goal, it is important to be able to isolate, detect, and identify microplastic particles. For spectroscopic analysis it is important to select an isolation technique that allows for subsequent spectroscopic analysis. We have found that silicon-based filters provide certain advantages for both FTIR and Raman analysis. Vibrational spectroscopy provides access to molecular information and can identify a wide variety of materials including polymeric materials. Micro-spectroscopy allows for analysis of microscopic particles and is ideal for the analysis of microplastic particles. While infrared and Raman spectroscopy both provide molecular structure information they do excel at identifying different aspects of polymer materials. Where one is weak the other is strong so that together they provide exceptional coverage for identifying a wide variety of different materials.

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MICROPLASTICS No. TN53168

Use and development of the Microparticle Sample Preparation Kit

Isolating microplastics and other microparticle contaminants for spectroscopic identification

The isolation and identification of particles, specifically microplastic particles, is an area of increasing interest. The discovery of microplastic particles in sea life, natural waterways, and in consumable products like bottled water and other beverages has sparked concern for the potential environmental and toxicological impact of these contaminants. The isolation of particles from liquid suspensions involves filtration and identification of the resulting particles is accomplished using either FTIR or Raman microscopy, or both. While there are a wide variety of choices for filter materials, it is important that the filter material be compatible with vibrational spectroscopic methods. This report provides details on the development and use of the Thermo Scientific™ Microparticle Sample Preparation Kit for dilute suspensions of particles in liquids such as water.

The first step of the development process was to select a filter material that would allow for both FTIR and Raman analysis. The characteristics of the filter material that were considered most important for this evaluation were the following:

1. The filter material should allow for FTIR microscopic analysis preferably in transmission as well as reflectance modes and should be suitable for Raman analysis as well.
2. The filter material should provide for a clear visual image of the particles. The surface texture cannot interfere with analysis routines that use visual image analysis for targeting potential particles for spectroscopic identification as well as using the visual image for determining the size and shape.
3. The filter needs to lay flat for analysis. While there are ways to mount more flexible filter materials to get them flat and there are ways to accommodate uneven filter surfaces these are just complications that, if they can be avoided, simplify the analysis.
4. Any spectroscopic contributions from the filters should be very small or limited and well defined so they do not interfere with the analysis of the particles of interest.
5. The filter material should be reasonably non-reactive with the liquid matrixes of interest.
6. The filters should be readily available and not prohibitively expensive.

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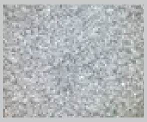
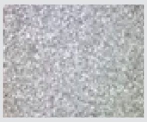
Results from a recent study using common types of filters can be seen in Table 1.

Table 1. Summary of advantages and disadvantages of different filter materials

Filter type	Advantages	Disadvantages	FTIR	Raman
Gold-coated polycarbonate 	• Readily available • Can be used with filter apparatus without gaskets	• Does not lie flat • Highly reflective surface may hinder contrast (particle recognition) • Expensive	Good choice for reflection	• Possible to see polycarbonate peaks through gold • Some broad baseline offset with some lasers
Silver 	• All metal • Less expensive than gold coated	• More rigid than gold-coated PC • More of a textured surface at high magnification • More reactive – reported problems with pH of carbonated water	• Reasonable for reflection – less reflective than gold	• Some spectral artifacts from filters themselves
Al_2O_3 	• Readily available • More rigid • Transmitted light possible if intense enough • Less expensive option	• Delicate – easily broken • Visual images – contrast an issue – surface not clearly defined. Some features on surface that might be detected as particles	• Can be used in transmission but limited to > 1,250 cm^{-1} • Some spectral peaks and some variation in peaks over the filer. • Reflection weak	• Some Raman spectral contributions from the filters – broad features • Baseline offsets • Laser light transmits through
Silicon 	• Rigid • Good visible images	• Square • Needs gasket development • Fragile • Expensive	• Transmission • Some variation across filter (filter background: (Si-O)) – broad baseline offset • Reflection not as good as gold but possible	• Silicon peaks

We chose the silicon filter because it has the majority of the desired traits. Silicon allows for transmission analysis with the FTIR microscope and while silicon has strong Raman spectral features, they are reasonably sharp, well defined, and can be accounted for during Raman analysis. A Raman spectrum of the filter itself can be subtracted from all of the particle spectra in the map. That removes the spectral features of the silicon filter from the particle spectra but there may be some residual spectral features that remain due to incomplete subtraction. The spectral regions containing these residual features can be avoided when doing spectral searching; to be sure they do not interfere with particle identification.

Since the silicon filters are square and rigid it was necessary to adapt a standard filtration apparatus designed for 13 mm diameter circular filters. Gaskets were used to adapt the square filters to the apparatus designed for larger circular filters, to prevent leaks, and to provide some cushion for the brittle silicon filters to prevent breakage. The filter apparatus used for this investigation is shown in Figure 1.

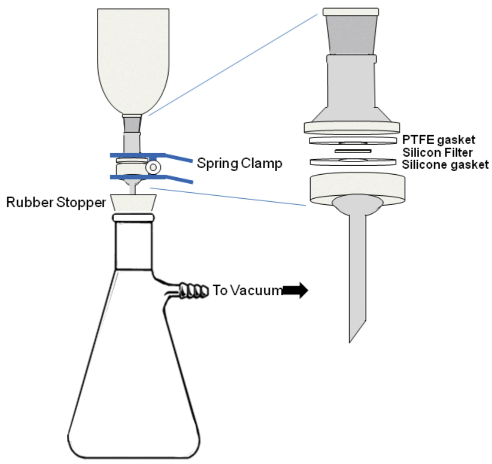


Figure 1. Apparatus for filtering microplastic particles from drinking water for vibrational spectroscopic analysis

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Two types of gaskets were used. A polytetrafluoroethylene (PTFE) gasket (1-inch diameter with an 8 mm diameter hole) was used on top of the silicon filter. These gaskets were sonicated for 50 minutes in ultra-filtered deionized water in an attempt to remove any particles from the filter itself. The gasket underneath the silicon filter was silicone. The use of the silicone gasket (1-inch diameter and 9 mm diameter hole) provided a better seal and the filter was less likely to break compared to using two PTFE gaskets. However, care must be taken to center the silicon filter on the silicone gasket to avoid contact between the backside of the silicon filter and the silicone gasket in the area exposed by the hole in the PTFE gasket. The reason for this is that the silicone gasket can leave some residue on the backside of the filter and if the filter is analyzed in transmission mode on an FTIR microscope these residues can be detected. Figure 2 shows a transmission map of the silicone filter and defines the usable area of the silicon filter that was not in contact with the silicone gasket. If the gasket and filters are properly centered then the residue should be outside the area occupied by the filtered particles (8 mm circular area). This is not an issue when analyzing the filter using Raman microscopy or FTIR microscopy in reflectance mode.

To be sure all the particles on the filter are coming from the bottled water it is important to avoid introducing additional particles from either the glassware or the environment. This was only partially successful, and particles could be detected on new filters after re-filtering previously filtered water. Controlling the conditions and careful treatment of the apparatus is crucial for reproducible and meaningful sampling.

The particles on the silicon filters can then analyzed using a Thermo Scientific™ Nicolet™ iN10 MX FTIR Imaging Microscope or a Thermo Scientific™ DXR3 Raman Microscope, or both. These instruments both have software options specifically designed for automated particle analysis. The Nicolet iN10 MX FTIR Imaging Microscope uses the Particle Wizard option in the Thermo Scientific™ OMNIC™ Picta™ Software and the DXR3 Raman Microscope uses the Particle Analysis option that is part of the Thermo Scientific™ Omnic™ Atlus Software and the Thermo Scientific™ Omnic™ for Dispersive Raman Software.

Summary

The Microparticle Sample Preparation Kit was developed to allow for the isolation of particles from dilute suspensions in liquids and subsequent vibrational identification of the particles using FTIR and Raman spectroscopy. The silicon filters were chosen to provide good visual images and allow for both transmission and reflectance FTIR analysis, as well as Raman analysis. A commercially available filter apparatus designed for 13 mm circular filters was adapted to use these rigid, square filters using gaskets to provide leak-free operation and protection for the thin, brittle silicon filters. Together with the iN10MX FTIR Imaging Microscope and the DXR3 Raman Microscope this kit provides a streamlined path for identifying a wide variety of particle contaminants.

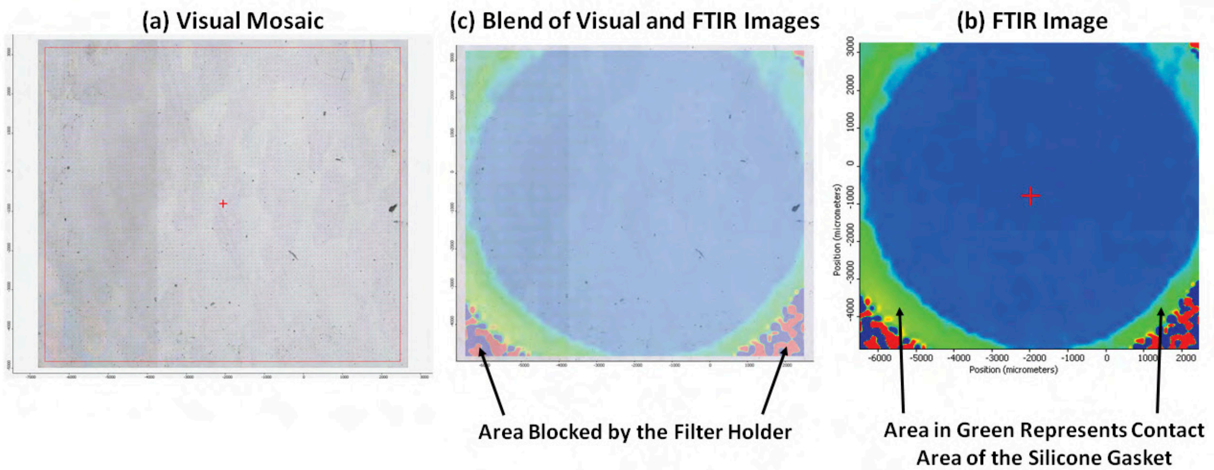

Figure 2. Illustrating the usable filter area with an FTIR transmission image of the filter. (a) The visual mosaic image, (b) The FTIR transmission image, and (c) A blend of the visual and FTIR images. The lighter blue area in the center of the image represents the usable filter area for transmission FTIR analysis.

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- Researching microparticles in liquefiable or dispersible samples

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Thermo Scientific DXR3xi Raman Imaging Microscope

Thermo Scientific™ DXR3xi Raman Imaging Microscope

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Microparticle Analysis Starter Kit

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- Gaskets and filters for 50 analyses

Raman Polymer Library, Raman Forensics Library

Best applications:

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- Researching microparticles in liquefiable or dispersible samples

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