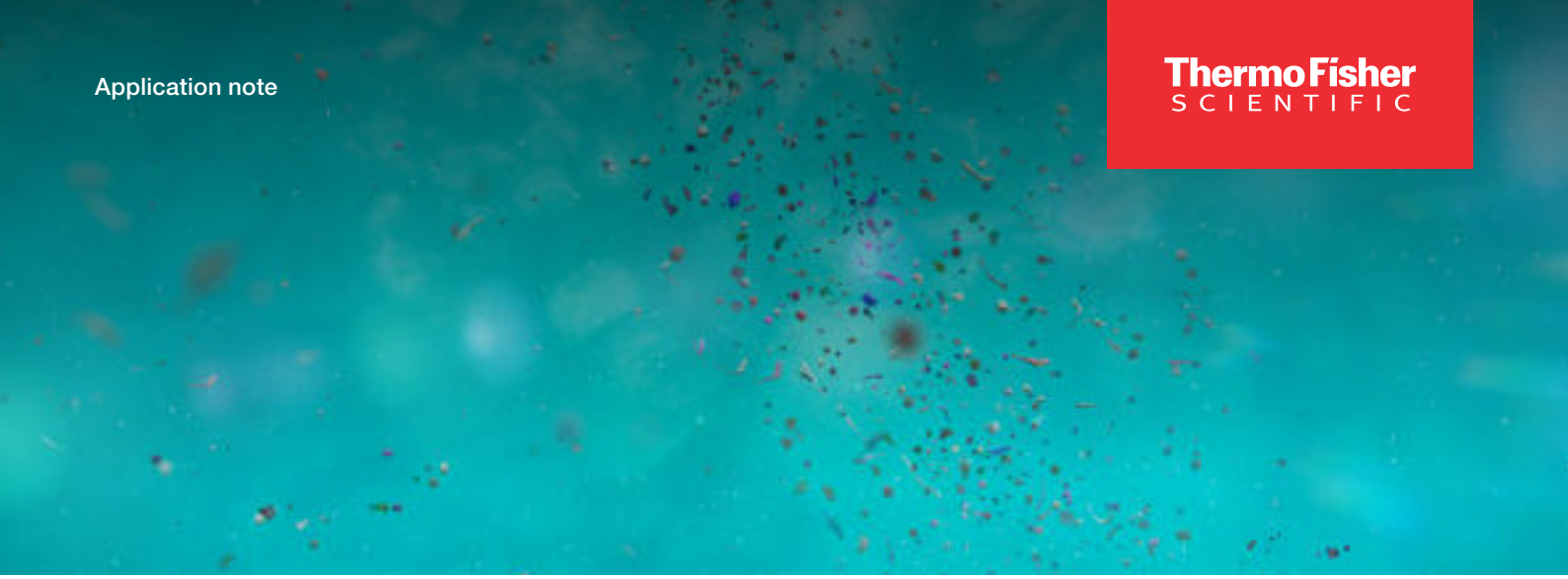
Application note



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.

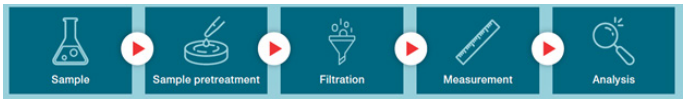


Figure 1. Summary of steps involved in isolation and analysis of microplastics.

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.

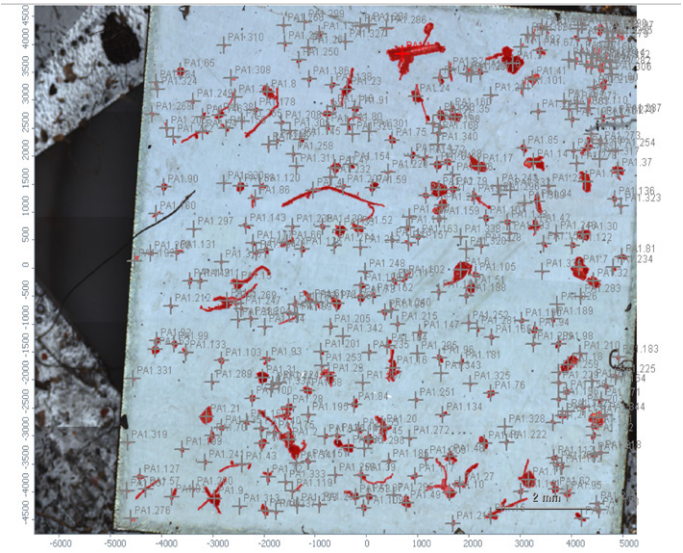


Figure 2. A silicon filter with atmospheric deposition of microplastics. Particles selected are between the size range of 25 µm – 1 mm.

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.

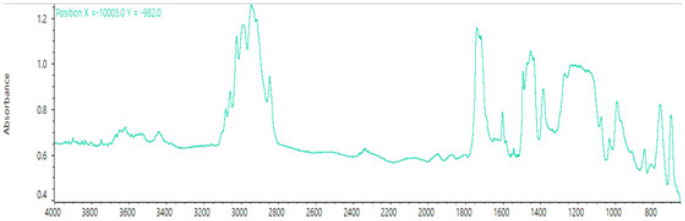


Figure 3: A single scan spectrum of a particle on a filter. Particle measured in reflectance; 16 scans coadded at 8 cm^{-1}.

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

Columns

(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	

Particle Distribution Component PE with Additives X-axis Area (µm²)

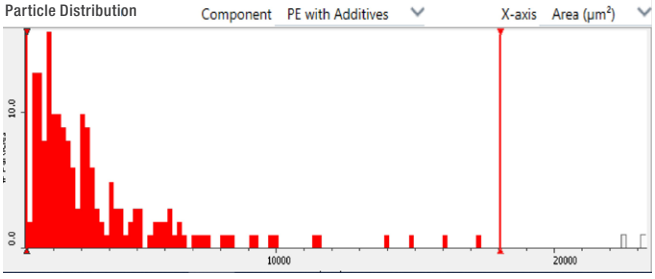


Figure 4(a). Report with full image, identification, particle size, and magnified particle image providing a complete picture. (b) Size distribution of each identification type.

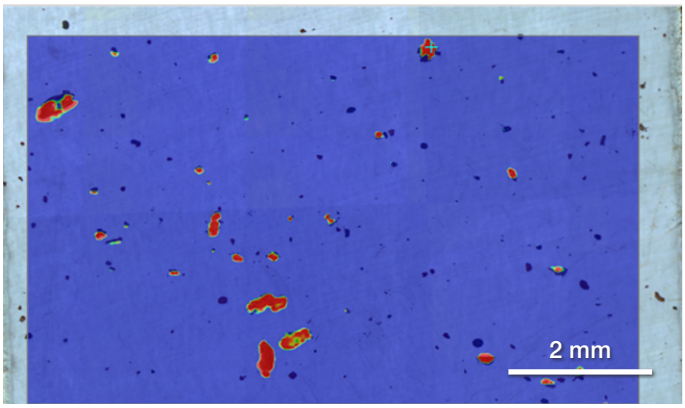


Figure 5. Silicon filter collected with microplastics analyzed with area mapping. Red regions are particles of PE correlated to the entire map.

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).

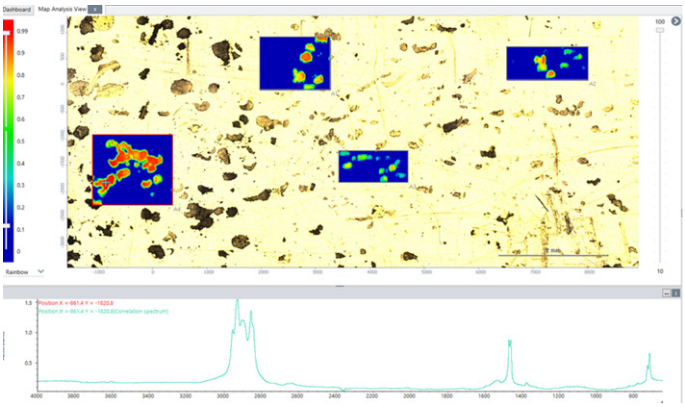


Figure 6. Reflective gold surface covered with microplastic particles. Four regions were selected for analysis. Regions highlighted.

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.

References

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