

Optimizing the Workflow for Microplastic Analysis by FT-IR Microscopy



Introduction

Microplastics are becoming a major global environmental concern with regular major newsworthy studies revealing the presence of plastics and microplastics in; remote geographic locations, or as contaminations in many different consumer products, especially food and beverages, and within the digestive systems of marine species. The sources of the microplastics can be primary microplastics, i.e. materials that are specifically designed or manufactured to be of small dimensions, or secondary microplastics that start off as larger materials but are broken down in the environment into smaller fragments. Microplastics were initially defined as being plastic materials below 5 mm in size, but the definition is now more commonly stated as plastic particles between the size 1 mm down to the micron level, although there is no globally accepted definition.

Bulk plastic pollution in the environment is a major visible concern and needs to be addressed. However, microplastics are of the size that is not always visible to the human eye, but their impact is significant to the health of aquatic and marine species and can ultimately end up in the human food chain.

Analysis of environmental samples containing microplastics is essential to determine their prevalence and their impact. A range of analytical techniques have been applied to the analysis of microplastics. Of the techniques adopted, infrared (IR)

spectroscopy, and more specifically IR microscopy, is the primary analytical technique for the detection and identification of microplastics.

The Microplastics Analysis Workflow for IR Microscopy

There are several steps involved in getting from the raw sample to answers, including the initial sampling through to data analysis. The steps involved may be different depending on the type of initial sample and the amount of sample cleanup required to prepare the sample for infrared (IR) analysis. The workflow is shown as Table 1.

Table 1. The steps involved in the microplastics analysis workflow.

| Sample Collection | Sample Cleanup | Sample Preparation for IR | Data Collection and Analysis |
|---|---|---|--|
| <ul style="list-style-type: none"> • Seawater • River Water • Sediments • Animal Digested • Consumer Related <ul style="list-style-type: none"> • Cosmetics • Domestic • Consumer Products | <ul style="list-style-type: none"> • Filtration • Flotation • Digestion <ul style="list-style-type: none"> • Acid Treatment • Peroxide • Enzymatic | <ul style="list-style-type: none"> • Filter Choice • Compatibility • Sample Size | <p>Sampling Modes</p> <ul style="list-style-type: none"> • Transmission • Reflectance • ATR <p>Measurement Modes</p> <ul style="list-style-type: none"> • Point Mode • Mapping • Imaging • Particle Detection |

There are a wide variety of different sample sources and sample types that need to be analyzed for microplastic content. Each different sample type has its own level of complexity for sample collection and sample clean-up. For example, the analysis of microplastics in bottled water requires no sample clean-up and just simple filtration to collect and prepare the sample for analysis. Whereas waste water or animal-ingested microplastics require extensive sample clean-up that can take several days to digest other organic materials allowing “clean” analysis of the microplastics present.

Sample Collection

The following is a brief summary of the different sampling strategies for the different sample sources.

Water Sampling

There are a wide variety of different water environments that contain microplastics, ranging from streams and rivers to lakes and the open seas. In addition, effluents from water treatment plants are known to contain microplastics. The requirements for sampling are similar, in that it is necessary to capture all of the microplastics present within a required range of particle size, and it is important know the volume of water sampled. With consistent sampling strategies it is possible to determine whether the trends for numbers and/or mass of microplastics are increasing or decreasing over time.

Seawater and freshwater have different requirements for sample collection primarily due to the different water densities. Most synthetic polymers have a density lower than seawater meaning the microplastics will mostly float on the surface, whereas many of the polymer types will be submerged in freshwater systems. The typical apparatus used for collecting seawater surface samples is a manta net with a known mesh size trailed behind a boat. For samples below the surface in the water column then suitable plankton nets are used. This approach is also applicable to lakes and inlets. The mesh size is an important parameter since too small a mesh will cause the nets to block reasonably fast during sample collection. Sample collection volume and area can be determined using flowmeters and based on the inlet dimensions of the net and the distance travelled during collection. For testing of river waters the nets are typically suspended at a fixed point in the river and the positioning of the nets can be set or adjusted for collection on the surface or at fixed depths below the surface.

Sediment Sampling

In many cases (micro)plastics can be observed on the surface of sediment samples such as on beaches or river banks. In such cases samples can easily be picked up and cleaned prior to analysis. However, microplastics are present deeper in sediments and a sampling strategy is required. Typically, a known mass or volume of sediment is collected and the mass or number of particles per unit volume is determined. Sediment samples can come from seabed, lake or river beds, or from beaches or river banks once tides or river levels are lower. Sediment samples require further sample cleanup in order to extract the microplastics for analysis as will be described later.

Animal Ingested Plastics Sampling

Plastics and microplastics are known to be present in the stomachs of a wide range of seabirds and marine species often leading to death.¹ Larger plastic materials can be physically picked from the opened stomachs of the species and cleaned prior to analysis. In order to determine the total amount of plastics including the microplastics it is necessary to completely remove the biological materials by digestion prior to analysis. The various methods of digestion will be discussed later. The digestion process will leave behind plastic materials and hopefully remove all other materials.

Domestic and Consumer Products Sampling

There are several sources of microplastics released within the home into the drainage system. The process of washing materials in a washing machine is known to generate thousands of fibers.² In addition, many consumer and cosmetic products such as toothpastes and body scrubs contain microbeads as constituents, although the use of microbeads is being phased out according to legislation in different countries and territories. Sampling of these microplastics from domestic products can be achieved using meshes of suitable mesh size on the water outlet of the washing machine or the outlet to the drainage system. In the case of exfoliants and body scrubs the majority of ingredients are water soluble, so mixing the sample with boiling water will typically remove all materials apart from the microplastics prior to filtration.³

Sample Cleanup

In order to obtain the best results from IR microscopic analysis it is essential to ensure the samples are clean and to remove any interfering materials such as biological matrix. The following is a brief summary of the different methods available and deployed for sample cleanup prior to IR analysis.

Density Separation (Flotation)

Plastics have a wide range of densities and thus some plastics will float in freshwater or seawater and others will sink. This principle of flotation can be utilised to separate plastic materials from other residual materials, such as sediments that will typically be more dense. By mixing a sample with a (higher density) saturated salt solution this will broaden the range of plastic materials that will float and the plastics can be removed from the upper portion of the liquid.

Plastics range in density from approximately 0.9 g cm³ for polypropylene (PP) and low density polyethylene (LDPE) up to 1.4 g cm³ for polyethylene terephthalate (PET) and polyvinyl chloride (PVC).⁴

Hence, for a freshwater or seawater sample with typical density of 1-1.05 g cm³ PP and LDPE will be separated by flotation from sand or sediment that will have significantly higher densities.

A range of saturated salt solutions have been deployed that maximise the density of the solution to allow a wider range of plastic materials to float.^{5,6,7} Sodium chloride, bromide and iodide with densities ranging from 1.2-1.8 g cm³ have been used as well as zinc chloride with a density of 1.7 g cm³.

The separation process involves agitating the sample, typically a sediment sample, and allowing the solution to settle. The upper portion of the sample can then be removed for filtration prior to analysis.

Sample Digestion

The aim of the sample digestion process is to remove biological, inorganic or organic materials that would interfere with the analysis of the microplastics but will not affect the microplastics themselves. Depending on the sample matrix a range of different sample digestion techniques can be applied. The materials used for digestion can be acidic, basic, oxidizing or enzymatic.^{8,9,10} For acidic digestion, hot nitric acid has been used but this will cause the degradation of some polymer types. Potassium hydroxide as a 10% solution has been used as a base material. Hydrogen peroxide solutions in the range 30-40% have been shown to be effective. However, this digestion can be slow, taking days for completion. An enzymatic digestion is effective using Proteinase K as the enzyme. This treatment is significantly faster and a two hour digestion at 50 °C removes significant biological materials from the sample and does not degrade the plastics themselves.

Filtration

In some sample types filtration is all that is required to separate the microplastics from the sample matrix, for example the collection and measurement of microplastics in bottled water. In many cases filtration is an extra step after the sample cleanup process. The filtration process needs to match the requirements of the analysis and can be used in itself as a sample cleanup stage. The use of meshes of different mesh size can tune the plastic collection to the sample size required for the analytical technique. For example, the initial use of a large mesh size can filter out the larger plastics present or can remove other larger debris to prevent blocking of the filters. Microplastics would be retained using smaller mesh sizes or filters. Larger plastics can be easily analyzed using a standard FT-IR spectrometer. However, the microplastics would more typically be analyzed using an FT-IR microscope. In some cases, FT-IR microscopic analysis of microplastics collected on a mesh can be performed directly on the mesh itself.³ Optimizing the filtration process will be described later.

Sample Preparation for Infrared Analysis

Infrared spectroscopy is the primary analytical technique for the identification and characterization of polymers. The infrared spectrum of a material gives a unique “fingerprint” for that material and can be compared against extensive spectral libraries for positive identification. Microplastic fibers and particles down to about 100 microns in size can easily be measured using standard FT-IR instruments and the sampling technique of Attenuated Total Reflectance (ATR). Small, portable FT-IR instruments (Figure 1) can be taken on board boats allowing immediate identification of collected samples.¹¹

For these larger samples measured by ATR generally there is no sample preparation required. The sample is placed directly onto the ATR accessory, pressure applied using the pressure applicator

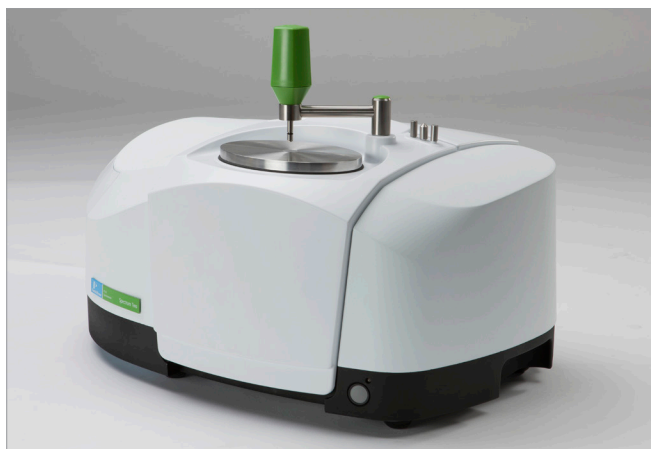


Figure 1. Spectrum Two FT-IR with ATR Sampling module.

and the sample scanned. However, it should be noted that plastics that have been present in the environment for considerable time will have weathered and the surface can be coated in biological film. ATR is a surface technique. So, in such cases it is recommended to slice the plastic sample and measure the internal bulk of the sample rather than the damaged/coated surface.

Smaller particles would be measured using an FT-IR microscope or FT-IR imaging system. In order to achieve the best results from these techniques it is essential to isolate the microplastics from the sample matrix. This has been discussed in the sample collection and sample clean-up descriptions previously. However, these procedures can be optimised for IR microscopy.

Optimizing the Filtration Process for IR Microscopic Analysis

Filtration of samples will isolate the microplastics onto a suitable substrate for analysis. Filters come in a wide variety of sizes, filter materials and pore sizes to optimise the filtration process. Some of the filter materials will have significant absorptions in the infrared region of the spectrum and these will mask the absorptions due to the particles of interest. It is therefore extremely important to use the most suitable filter materials. A range of different filter types and sizes have been evaluated to determine the optimum filter type(s) for analysis of microplastics by IR microscopy (Table 2).

Table 2. Range of filters evaluated for compatibility with IR microscopy.

| Filter | Diameter | Pore Size | Cost/Filter |
|---------------------------|--------------|--------------|-------------|
| Alumina Oxide | 13 mm | 0.2 Microns | \$8 |
| Glass Microfiber | 21 mm | Mixed | \$0.2 |
| Gold Coated Polycarbonate | 13 mm | 0.8 Microns | \$13 |
| PVDF | 25 mm | 0.45 Microns | \$2 |
| Silver | 13 and 25 mm | 5 Microns | \$8 |
| Silicon | 10 mm Square | 5 Microns | \$20 |

The filter diameter will influence the sample capacity and the filtration ability and should be kept down to a reasonable size to reduce the time required for IR imaging. The pore size will determine the smallest particle size to be retained, but should be kept to a size that will not block easily with some of the sample matrices. For compatibility with IR analysis the particles need to be larger than five microns (due to the approximate diffraction limit of IR analysis) and the available spectral range of the filter is extremely important. The relative cost per filter can be important, but for samples where the sample cleanup can take hours or days the filter cost is less important. For laboratories with higher sample throughput it should be an important consideration.

The sampling modes for IR microscopy will be discussed in greater detail later, but for analysis of particles on filters the choice is generally limited to Transmission or Reflectance. ATR can be used but is prone to sample carryover when automated measurement of multiple particles is performed. The filter type either needs to transmit or reflect IR radiation without any significant absorptions. Infrared transmission and reflectance spectra were recorded for the filters shown in Table 1 and the useable range for each type determined. The summary is shown in Figures 2a for transmission and 2b for reflectance.

The gold-coated polycarbonate filter has very good energy in reflectance but no transmitted energy, the PVDF filter shows significant absorption bands in both transmission and reflectance so is unsuitable.

The recommendation is to use silicon filters to perform transmission analysis and to use silicon, silver membrane or gold-coated polycarbonate for reflectance analysis.

The only negative points about the silicon are the relative cost and the “non-standard” sizes (rectangular dimensions) that are not directly compatible with standard filtration systems.



Figures 2a and 2b. Transmission and Reflectance ranges of filter types.

Example spectra for two different filter types are shown as Figure 3.

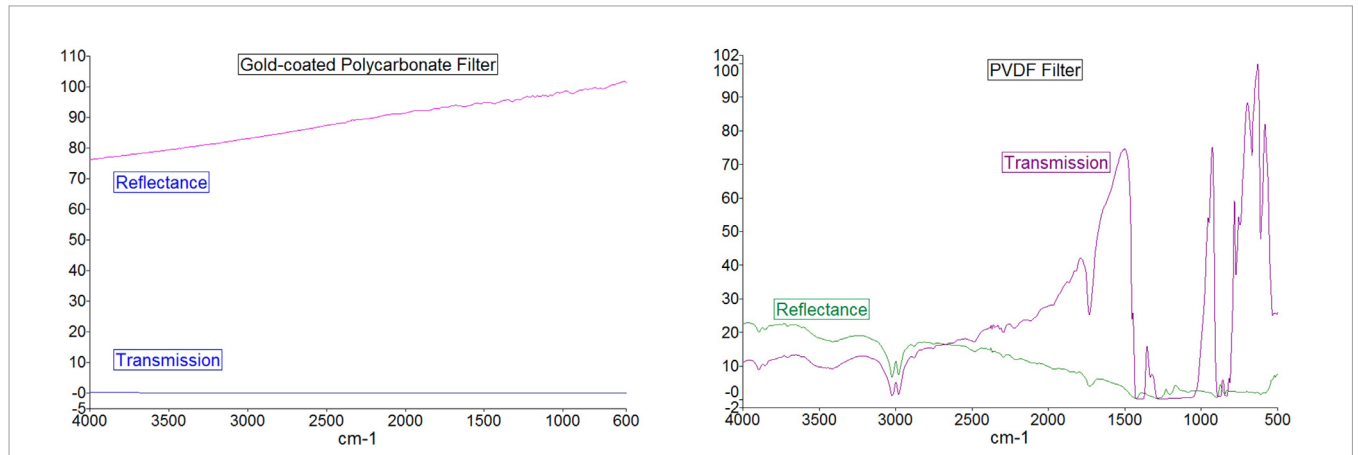


Figure 3. Transmission and Reflectance ranges of filter types.

Analysis by IR Microscopy

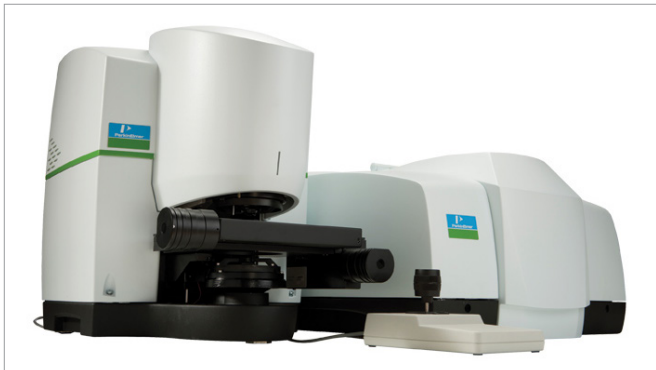


Figure 4. PerkinElmer Spotlight 400 FT-IR Imaging system

Sampling Modes

The same principles of measurement that apply to measuring routine samples by infrared analysis apply to the measurement of microplastic samples using an FT-IR microscope. The sampling modes are transmission, reflectance or ATR. The same benefits and limitations equally apply.

1. Transmission

To measure a sample in transmission mode the sample should be placed on a suitable infrared transmitting substrate. The sample should typically be less than 50 microns thick to avoid total absorption of the strongest bands in the spectrum. If the analysis consists of a small number of particles and it is possible to “pick” the particles then the best approach is to position the particle onto a 13 mm KBr window in a sample slide holder in the microscope. This ensures that the particle is isolated and will generate a pure spectrum of the particle. If the particle is thicker than 50 microns then the sample can be placed in a micro diamond anvil cell, compressed to a thinner dimension and measured in transmission on the microscope stage. However, in most cases the samples are either too thick or not easy to isolate even using microscope tools. Suitable filters that transmit IR radiation, as described previously, can be used without requiring sample preparation or removal of particles onto other substrates. Also, in most cases there are a variety of particles with a variety of dimensions, some sub 50 microns, others larger. Removal of a large number of particles would be time consuming and difficult.

2. Reflectance

Reflectance measurements (direct specular reflectance) are not commonly performed on bulk polymers when the goal of the analysis is identification. The spectra obtained will consist of a mixture of spectral components, namely surface reflections and transmission/reflectance components. These lead to spectral distortions especially in the stronger bands in the spectrum and would interfere in the spectral library searching process. However, the transmission/reflectance component can often be the dominant spectral contribution and produce identifiable spectra. When the IR beam hits the sample, some of the beam

will reflect directly off the sample surface, the remainder will pass (transmit) into or through the sample. If the sample is placed on a highly reflective substrate, such as a gold mirror or reflective filter then the beam will reflect off that substrate and back through the sample, effectively giving a double transmission. So, good quality spectra can be obtained from reflectance measurements but the strongest bands may be very intense. For a range of sample thicknesses reflectance will work well, better than transmission.

3. ATR

ATR has become the standard technique for the simple measurement and identification of samples on an FT-IR instrument. It requires no sample preparation and can work with a range of sample dimensions including extremely thick samples that would not work in transmission or reflectance. It is a surface technique and thus the spectrum obtained is the spectrum of the surface of the material, not the bulk. In addition, the effective sample thickness measured is in the region of 1 or 2 microns, leading to weak infrared spectra. However, the spectral intensities achieved are sufficient to identify the material or major components of the material. IR microscopes can be fitted with micro ATR crystals to perform automated ATR measurements on microparticles. If the sample is on a rigid solid substrate, such as a gold mirror, window material or microscope slide and contains a very small number of particles then ATR could be the technique to use *as long as the ATR crystal is cleaned after every measurement/particle*. The ATR principle involves the compression of the sample between the substrate and the ATR crystal. After the measurement, when the pressure is released, the sample will often remain on the ATR crystal, not back on the substrate. So, contamination would be a major issue if multiple particles were to be measured without cleaning the crystal. For this reason, ATR is not commonly adopted as the sampling mode.

ATR Imaging is an alternative if the total sampling area is less than 1.5 mm. In ATR imaging an ATR crystal with a significantly larger crystal surface is in contact with the sample and allows ATR measurements across the entire crystal surface. In practice, most real sample measurements require significantly larger areas to be measured than the crystal surface area.

Measurement Modes in IR Microscopy

IR microscopes are capable of measuring single microscopic particles but they have the added advantage of being capable of running in fully automated modes to measure multiple particles in a sample, and also to map or image the entire sample, such as a complete filter. The automation applies to each of the different sampling modes described previously. The microscopes are also equipped with visible cameras to allow the operator to see the samples they are working with and to setup the positions to perform the analysis.

Point Mode

In point mode the software allows the user to select the measurement position or positions corresponding to the particles. The IR microscope will then drive the stage to the positions for measurement, perform the scan, then move to the next sample position. If the sample has a small number of particles this can be a very fast method for collecting spectra. For each position the software controlled aperture blades should visually enclose the particle to avoid stray light. As in standard FT-IR measurements a suitable background scan is required and on the IR microscope this should be performed using the same aperture dimensions as used for the sample scan. For transmission the background should be measured in a clear area with no sample. For reflectance the background should be recorded on a clean area of the reflective substrate. For ATR the background should be measured using a clean crystal.

Particle detection algorithms within the software are capable of analyzing the visible image to find the presence of particles within the sample. The software will then automatically scan the spectra of all of the particles and appropriate backgrounds. This offers significant speed advantages over manually selecting positions to analyze, or significant time savings if the whole sample was mapped or imaged. The Particle Finder tool is shown in Figure 5.

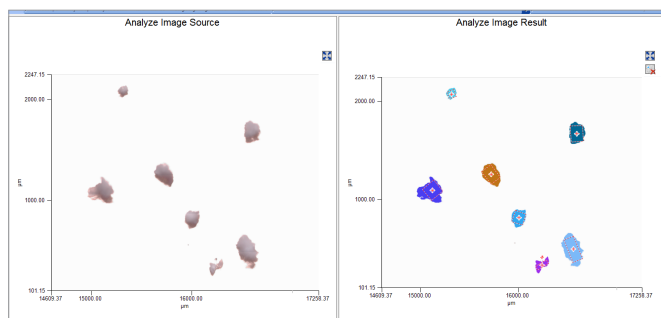


Figure 5. Analyze Image finds the particles present.

Mapping

A mapping experiment involves defining an area of the sample over which to perform the measurement (this can be several mm) and defining the X,Y spacing of the measurements over that whole sample. For example, if the sample was 0.7 mm x 1 mm and the measurement should be performed every 100 microns the mapping experiment would perform 70 measurements (7 x 10). At each point an IR spectrum would be collected and over the entire area an IR map of the sample generated.

Mapping experiments utilize the single element detector present in the IR microscope (typically an MCT detector) and will measure a single spectrum, move the stage, measure a spectrum, move the stage. For small sample areas or large XY spacing this is sufficient. However, for large sample areas such as filters, or to measure very small particles at small XY spacing, the mapping experiment can be extremely slow and the amount of time required prohibitive.

Imaging

The imaging experiment is similar to the mapping experiment with the significant difference that the imaging experiment uses detectors with arrays of elements instead of a single detector element, measuring multiple points simultaneously, resulting in a significantly faster overall measurement. The array detectors can be either linear arrays or focal plane arrays. The linear arrays are of the geometry $n \times 1$, where n is typically 16 or 32, whereas focal plane arrays are of the geometry $n \times n$, where n is typically 16, 64 or 128. The focal plane array detectors are significantly more expensive and have a spectral cutoff of about 950 cm^{-1} that misses some vital spectral information, whereas linear array detectors offer the full MCT spectral range down to 600 cm^{-1} .

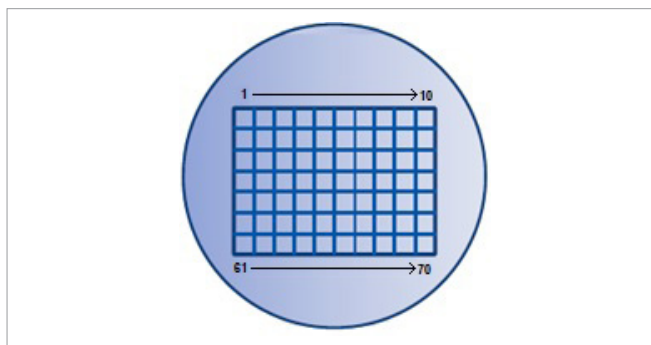
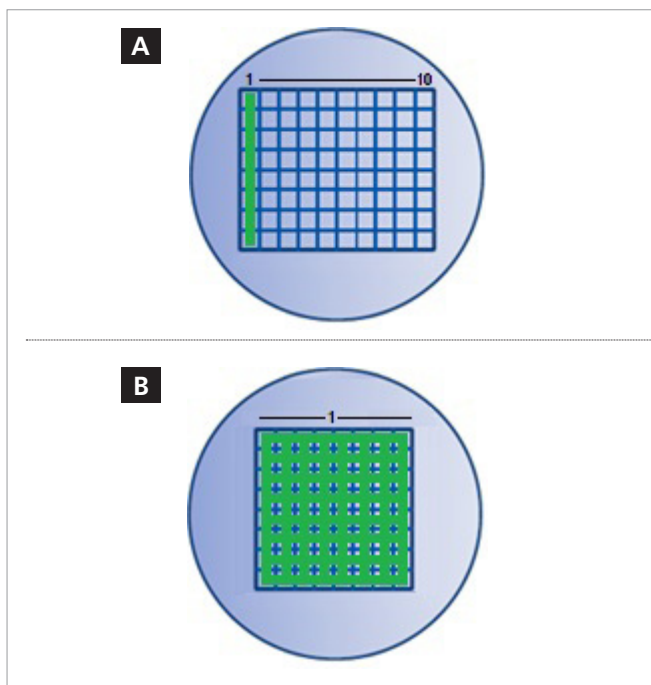


Figure 6. A mapping experiment collects one row of data points then moves to the next row until complete.



Figures 7a and 7b. (a) A Linear Array detector collects 1 "column" of data points then moves to the next "column". (b) A Focal Plane Array detector collects columns and rows of data points in one measurement.

An example IR image is shown as Figure 8.

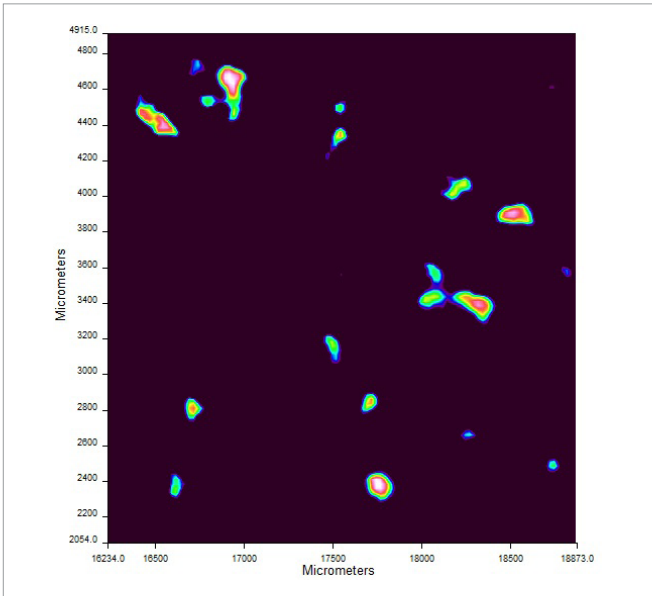


Figure 8. Total IR absorbance image of microplastic particles filtered from a cosmetic formulation.

Each pixel in the IR image has a full IR spectrum associated with it. When working in Point Mode the system will generate one spectrum per particle. When working in Image Mode the system will generate 1 spectrum per pixel resulting in significant amounts of data per experiment. For example, a 10 mm x 10 mm image measured at 6.25 μm pixel size will consist of over 2.5 million spectra. Various software tools allow for the data interpretation step to be simplified, the most powerful being Principal Components Analysis (PCA). In the PerkinElmer Spectrum Image software this is invoked by selecting the Show Structure function. This function will use PCA to look for different sources of variation within the samples, such as different chemical species. Different PCA scores will represent different materials present and generate images for the different scores indicating where different materials are distributed within the sample. An example is shown in Figures 9a-d.

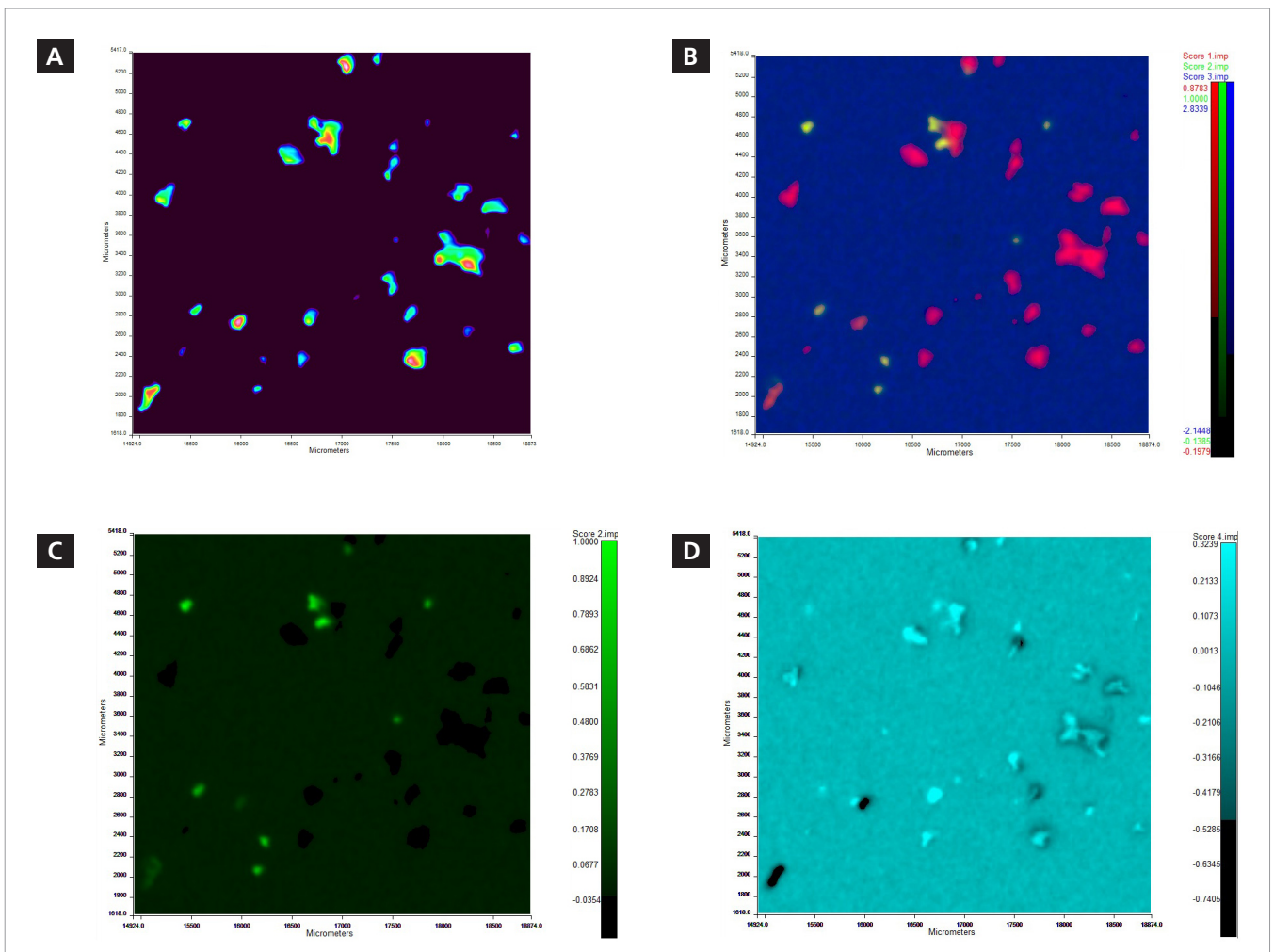


Figure 9a-d. (a) Total Absorbance IR Image. (b) PCA analysis showing mixed scores. Different scores are in different colors, separating different chemical types. (c) Scores 2 image. (d) Scores 4 image.

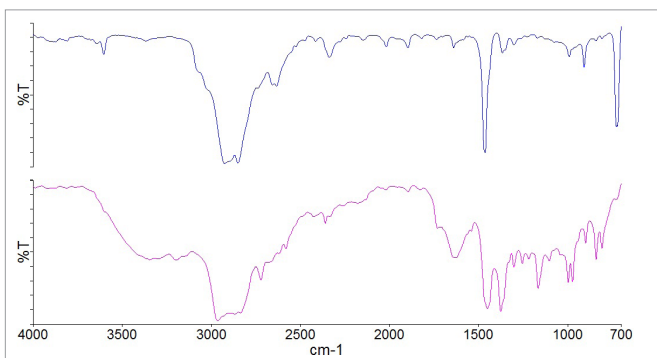


Figure 10. Spectra of particles observed in; Scores 4 image, identified as polyethylene (top), and Scores 2 image, identified as polypropylene (bottom).

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