Application note

Improved Analysis of Microplastics and Other Microlitter Components in Environmental Samples

Authors

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Industry/Application

Microplastics analysis in environmental samples

Products Used

Thermo Scientific™ Nicolet™ iN10 MX Infrared Imaging Microscope

Goals

Demonstrate advantages of the automated function of WIZARD particle analysis for a faster and accurate analysis of microplastics

Key Analytes

Microplastics, Particle analysis

Key Benefits

- Reduce time and errors in microplastics analysis
- Increase cost benefits by eliminating a lot of time for spectra acquisition and identification
- Provide complete integration solution for simplified analysis

Introduction

In the analysis of microplastics (MPs) in real environmental matrices, it is required that the particles be unambiguously identified[1-7] to distinguish them from particles of biogenic origin (e.g., particulate organic matter) and particles belonging to the microlitter in general (i.e., non-plastic artificial and natural fibers, or plastic additives). Optical microscopy using dye staining and electron and fluorescence microscopy are the standard non-invasive techniques employed to quantify suspected MPs since the unambiguous identification of polymers is not provided[8-10]. On the other hand, vibrational spectroscopy, which employs Fourier Transform infrared (FTIR) and Raman spectrometers, is a well-known non-destructive technique that allows the characterization of polymers and other particles. Therefore, after the optical investigation, some selected particles are chosen to be further analyzed, usually by means of an FTIR spectrometer. Larger particles or fibers (>500 µm) can be analyzed by attenuated total reflection - FTIR (ATR - FTIR) since overestimation/underestimation of MPs can occur. Particle quantification (microscopic counts) and subsequent analysis of selected particles to be unequivocally identified is significantly time-consuming. Besides this drawback, selecting only a few particles may not be representative of what is really present in the sample. On the other hand, while punctual analysis of particles using techniques such as micro-FTIR and micro-Raman can be equally time-consuming, particle analysis via micro-FTIR allows MPs and other microlitter components, especially those below 100 µm (e.g., small microplastics, SMPs), to be unequivocally identified and simultaneously and reliably quantified by microscopic count, thus avoiding over- or underestimation[3,4,6,11,12]. Particle analysis, using the Particles WIZARD section of Thermo Scientfic™ OMNIC™ Picta™ Software, the instrument software of the Thermo Scientific™ Nicolet™ iN10 MX Infrared Imaging Microscope, can be performed on any filter suitable for the analysis of microplastics (e.g., aluminum oxide filters, silicon oxide filters, etc.).

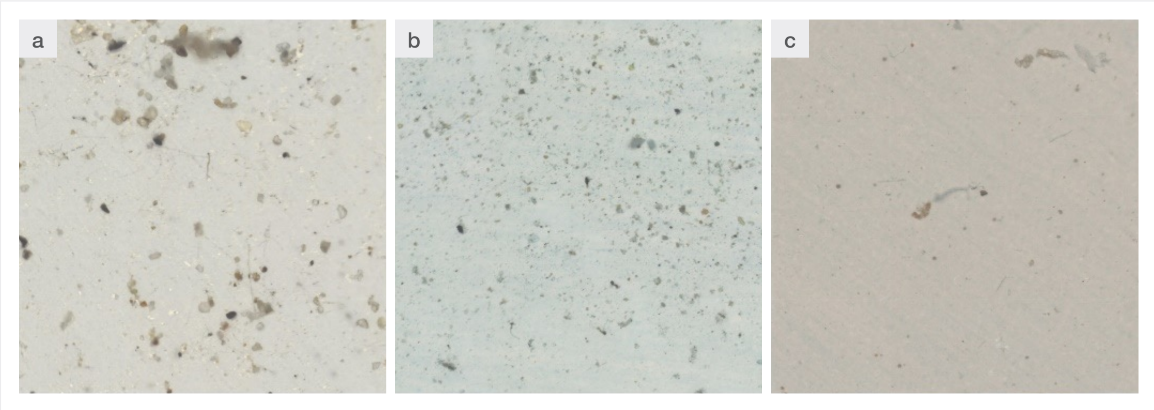

Figure 1. Examples of mosaic or count field: a) in permafrost sample, b) in soil sample, c) in seawater.

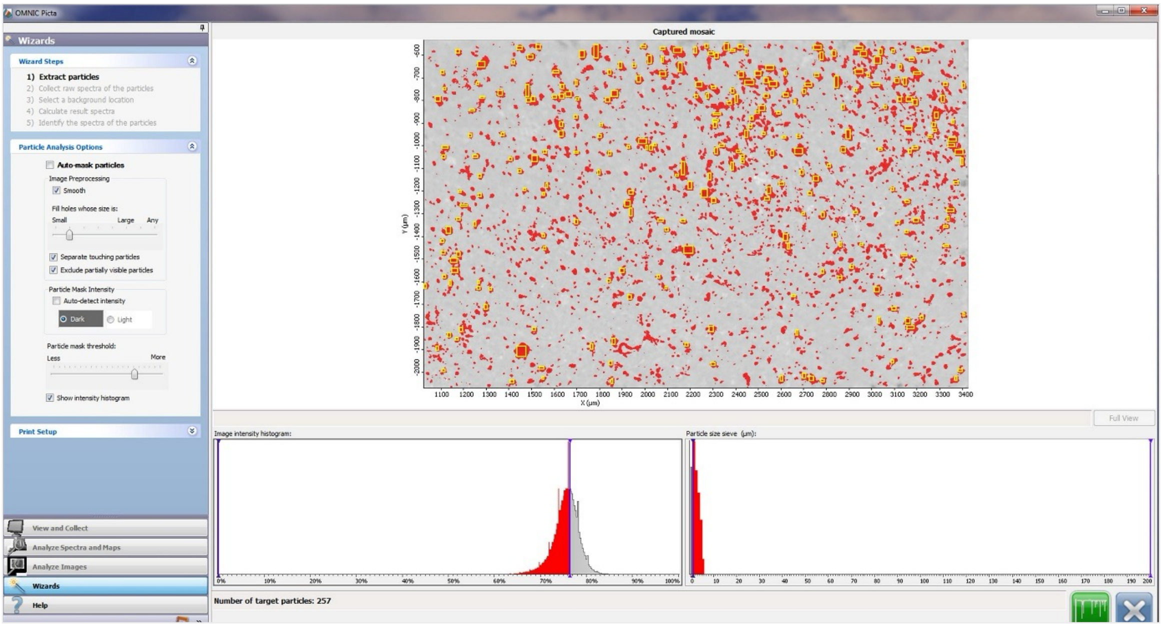

Figure 2. Particle Analysis via WIZARD: Example of how to select the particles on the count field.

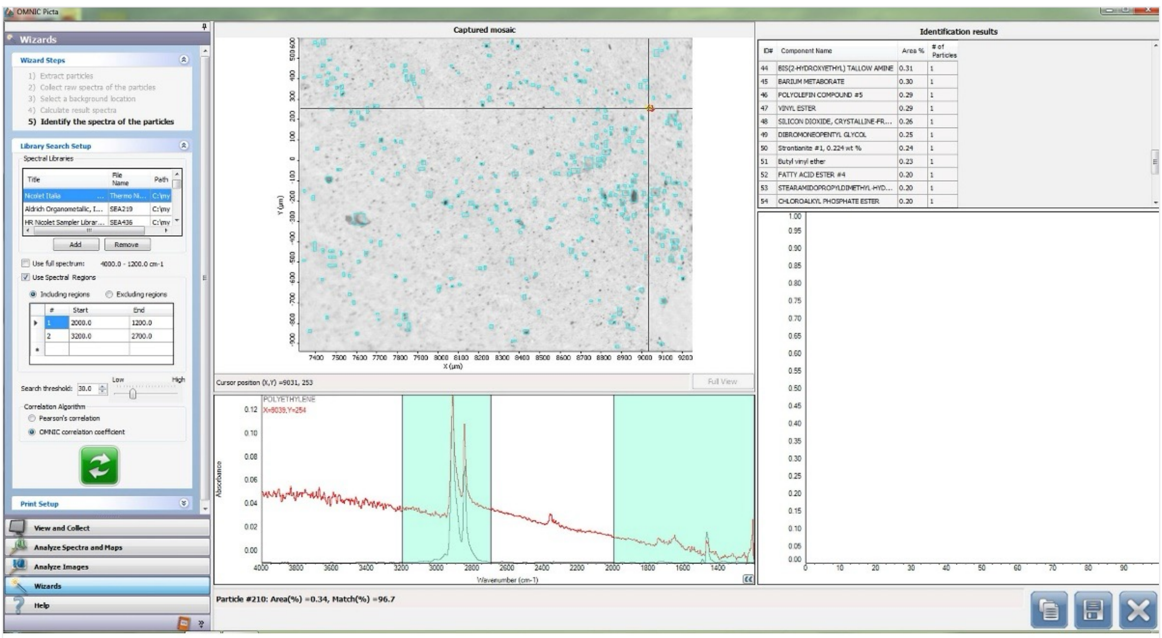

Figure 3. Particle Analysis via WIZARD: The spectra of the particles are identified.

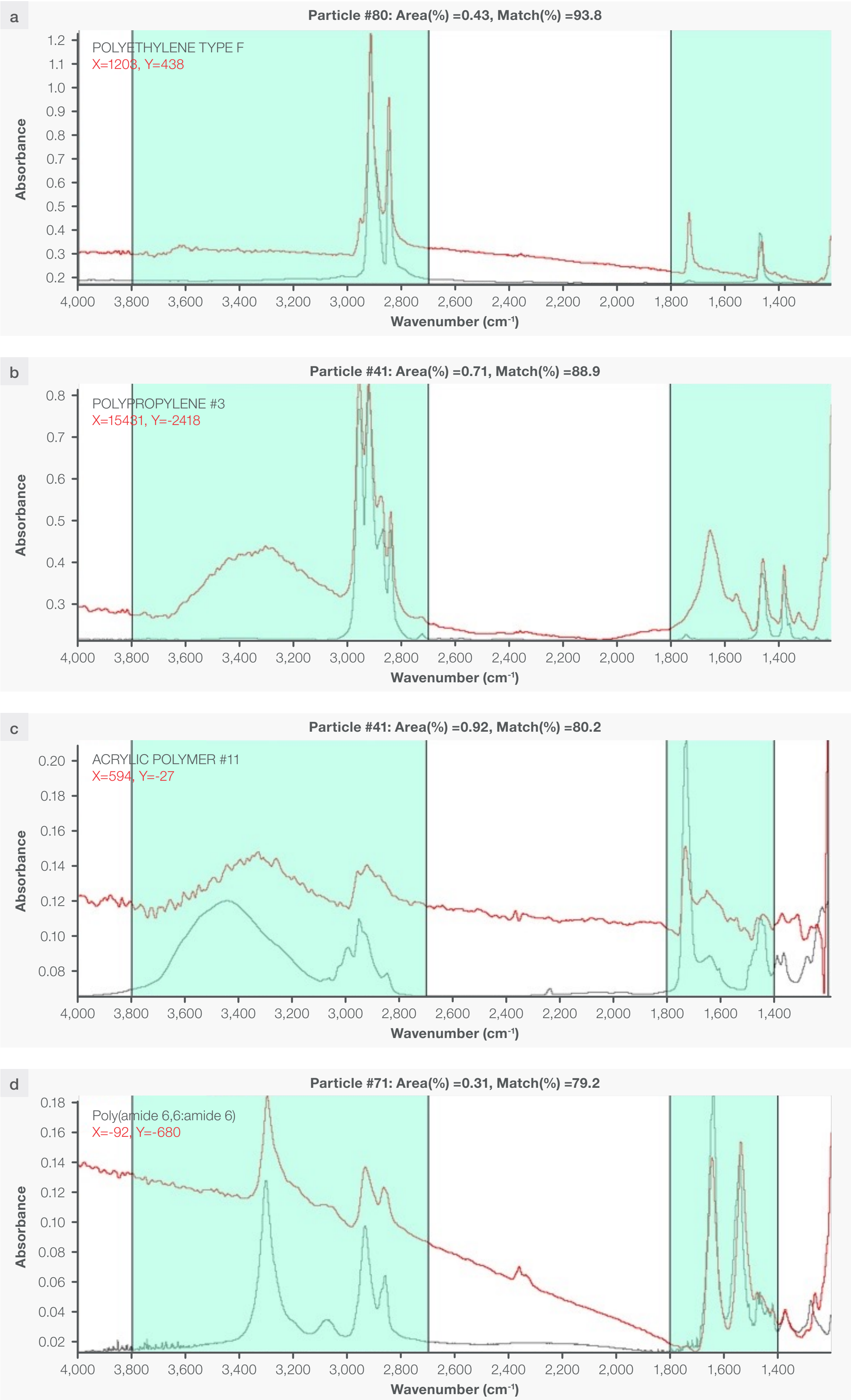

Figure 4. Some exemplary spectra of polymers optimally identified with a match percentage > 80%:
a) polyethylene, b) polypropylene, c) acrylic, d) polyamide 6.

Experimental considerations

Selection of particles

Using the objective of the micro-FTIR with a spatial resolution of 100 µm, an area is framed. Then, a mosaic of definite dimensions is drawn: For example, in environmental samples, the dimensions will be 2000 µm x 1400 µm. This mosaic will then be the "count area" or "count field" (Figure 1). The mosaic is saved, and the Particles Analysis function, via the WIZARD section of OMNIC Picta software, can start. The particles present on the surface of the filter, on the specific count area, are detected in relation to the brightness value (defined here as the brightness ratio of each particle in contrast to the background (Figure 2). The first thing a user should do is to uncheck the option Auto-mask particles. In Image preprocessing, "Smooth," "Separate touching particles," and "Exclude partially visible particles" should be selected. The latter two are particularly essential for microscopic counting. Then, in Particle Mask intensity, while "Auto-detect intensity" should be unchecked, "Show intensity histogram" should be selected.

The particles, which will be later analyzed, can be selected through the intensity histogram. The particles are enclosed in rectangles called bounding boxes. The image intensity histogram is needed to be able to select an adequate and significant number of particles since the amount of microplastics in that field is not known *a priori*. By using the Particle size sieve function, any potential interference signal can be diminished (Figure 2); when spectral interferences and/or background interferences are present, the brightness ratio is affected, and the software detects a lower number of particles.

Following detection, raw spectra of particles are collected. After collecting spectra, a background location is chosen on the count field. By the combination of the raw spectra and the background spectra, the resulting spectra of the particles are calculated. Finally, the resulting spectra are identified, through comparison with reference libraries. A match percentage is used identifies each spectrum of each particle (Figure 3). In addition to this, each particle's coordinates in the count field are retrieved, allowing each particle to be univocally identified.

According to the instrumental characteristics, the optimal range of match percentage is ≥ 65%; however, the match percentage can be > 80% or even higher (Figure 4), depending on the pretreatment employed. Spectral and background interferences should be eliminated during pretreatment, especially when a purification procedure is applied during the filtration.[3,4] This allows particle selection to be efficient, and the identification match percentage of each particle to be enhanced over the optimal range. When the identification match percentage is <65%, particles cannot be optimally identified and are not counted. Therefore, MPs' abundance is underestimated.

Microscopic counting for microplastics and microlitter

Microscopic counting has been employed for bacteria, phytoplankton, pollen, spores, and MPs.[3,4,6,13-22] A significant advantage of microscopic counting is that it eliminates doubt about how many organisms, cells, or particles are present within valid computable limits and degrees of chance.

Filters can be easily employed as a support for counting. These filters can be round or square. Analyzed filter areas (i.e., counting areas or count fields) need to adequately represent the entire filter to avoid issues regarding representativeness and reproducibility. Representative measurement areas of the same size can be chosen on the surface of the filter in one of the approaches shown in Figure 5.

The approach employed in the Bürker chamber can be applied to square filters. For the analysis of MPs, the number of filtering areas (i.e., count areas or count fields) analyzed must be equal to or greater than 20 in order to obtain meaningful and robust quantification. Since the loading of the filters cannot be known in advance, count areas with different abundances should be considered to avoid issues regarding the accuracy of extrapolation of findings of microplastics, organisms, cells, or bacteria. Because of this guideline, the randomized approach without overlapping (Figure 5d[3-5,7]) proves to be the most suitable. The microscopic count is considered representative when a reliable and significant number of particles is analyzed, which should never be less than 4000 particles. When these two conditions are met, the microscopic count is robust, and consequently, the quantification is unencumbered by under- or overestimation.

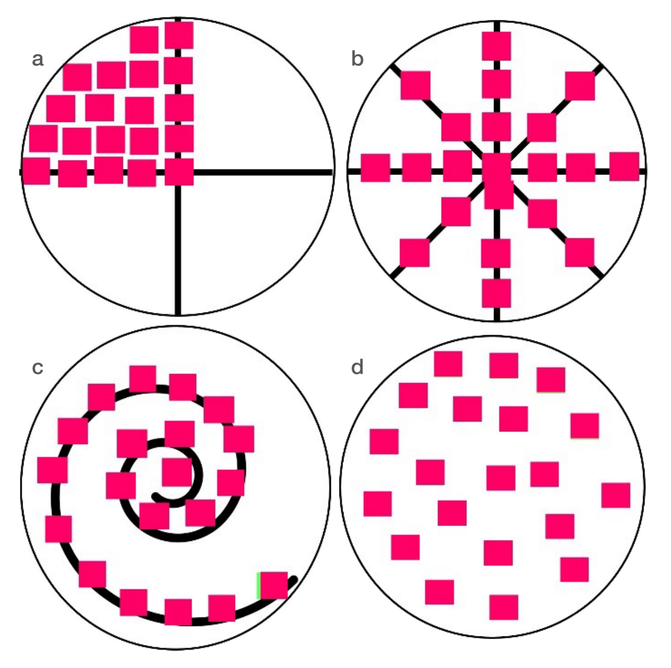

Figure 5. Different approaches may be used for representative measurement areas on filters; at least 20 count areas or count fields should be considered. These approaches can be employed on filters of different diameters and materials (e.g., aluminum oxide, silicon oxide, PTFE). Example a) represents a quarter of the filters; b) represents the cross-section of the four axes c) represents a helical assembly; and d) represents a randomized assembly.

The total counts from each filter must be multiplied by appropriate microscope conversion (optical factor F) and volume or dilution factors[3-5,7] to provide the absolute abundance (number of MPs per L, number of MPs per kg, number of MPs per m, etc.). The equations for calculating the abundances[3-5,7] are as follows:

$$N_{MPs}\ L^{-1} = \frac{n * 1000 * F}{V}$$

Equation 1.

$$N_{MPs}\ kg^{-1} = \frac{n * 1000 * F}{W}$$

Equation 2.

Where $N_{MPs}\ L^{-1}$ or $N_{MPs}\ kg^{-1}$ are the total abundance in the samples analyzed; V is the volume of water analyzed, W is the weight of sediments, soil, etc. analyzed; n is the sum of all the plastic particles in the count fields analyzed, F is the factor which is calculated as follows:

$$F = \frac{\text{Area of the filter}}{\text{Area of a count field} * \text{number of all the count fields}}$$

Equation 3.

Density, size, and shape are essential characteristics of particles and fibers that affect their transport and permanence in the environment as well as their ingestion and/or inhalation by humans and other animals. As observed for aerosol particles, microplastic particles and fibers can have different irregular shapes, which shape descriptors can then describe[23-26].

Selection and counting of particles using WIZARD

Using the Particles Analysis function on the iN10's OMNIC Picta software, particles are not only identified and counted, but the length and width of each particle are also retrieved during the analysis. When particles are selected in the software (Figure 1), they are enclosed in rectangles. These rectangles are called bounding boxes because they correspond to the smallest rectangles enclosing the shape of each particle. The particles' shapes are then categorized based on the aspect ratio of the bounding boxes.

Calculation of aspect ratio and volume of MPs

Aspect ratio (AR) is defined as the ratio between the maximum length (L) and the maximum width (W) of the bounding box enclosing the shape:

Particles are considered spherical when the AR values are ≤1. When AR ≥ 2, particles are ellipsoidal, When AR ≥ 3, particles are considered cylindrical. Therefore, thanks to the AR, volumes of particles and fibers are calculated according to their geometrical shape (i.e., sphere, ellipse, or cylinder). Since the particles have been optimally identified, their density can be retrieved; thus, each particle's weight can be calculated.

$$AR = \frac{L_{max}}{W_{max}}$$

Equation 4.

Conclusions

When analyzing MPs, it is paramount to discern the polymers from the rest of the microlitter components and any other particles in the environmental matrix under exam. Individual analysis of hundreds of particles to obtain robust quantification would take a very long time; it can take up to several days, to analyze all the FTIR spectra retrieved. Additionally, performing a count of MPs separately from analysis by vibrational spectroscopy would significantly contribute to the length of time for the analysis, possibly adding as much time as performing a complete analysis of the whole filter.

Instead, in each field count, a single particle is unambiguously located by its own spatial coordinates, its spectrum is unambiguously and optimally identified, and its sizes (width and length) are also collected. When operating in Particles Analysis mode, quantification by microscopic count is carried out simultaneously with spectral identification. Each count field can be saved with the filename extension .map, which allows a subsequent analysis of each particle in a precise count field to confirm/verify spectral identification. Therefore, Particles Analysis software makes microplastics analysis significantly less time-consuming and markedly more robust.

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