

# MICROPLASTICS



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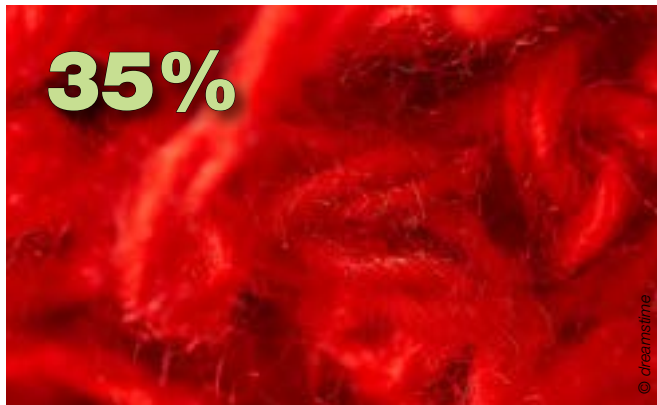


# What are microplastics?

## Where are they coming from?

**A study by the International Union for Conservation of Nature (IUCN)<sup>1</sup> identified the main sources of Microplastics and divided them into 7 main categories:**

### Synthetic textiles



Synthetic textiles are the single greatest contributors to engineered microplastics in the ocean, accounting for 35 percent of the total volume; indeed washing synthetic textiles frees engineered microplastics

through abrasion and shedding of fibers from the fabrics. This is due to the mechanical and chemical stresses that fabrics undergo during the washing process in a laundry machine. Browne et al.<sup>2</sup> showed that a single garment can release more than 1900 microplastics (<1mm) in each washing cycle and as there are more than 840 million washing machines globally<sup>3</sup> it is clear why synthetic textiles are the main source of microplastics.

### Tires



tire is metal and other compounds. Tires erode through heat and friction from contact with the road. The wind and rain spread the tire dust and wash it off the road. It enters tributaries, lakes and eventually the oceans.

### City Dust



of objects like synthetic soles of footwear, synthetic cooking utensils

Today, about 24% of a tire consists of synthetic rubber, a plastic polymer, and 19% natural rubber. Microplastics form a matrix of the synthetic polymers, giving the tire rigidity and providing traction. The rest of the

City dust, which accounts for 24 percent of microplastics in the oceans, comes from a variety of sources. While each is a small contributor, it adds up in a opulated area. City dust includes losses from the abrasion

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and of infrastructure like household dust, artificial turf, harbors and marina building coatings. It also includes particles from blasting, abrasives, weathering of plastic materials and use of detergents.

### Road Making



Crews apply road markings while building and maintaining roadways. Particularly in Europe these markings include polymer tapes and paints. These are thermoplastics that become soft and flexible

at warmer temperatures, allowing weathering or abrasion by vehicles to turn them into microplastics.

1. Boucher, J. and Friot D. (2017). Primary Microplastics in the Oceans: A Global Evaluation of Sources. Gland, Switzerland: IUCN. 43pp.  
2. Browne et al. ENVIRONMENT SCIENCE & TECHNOLOGY 21 p.9175 2011  
3. F. Salvador Cesa et al. SCIENCE OF THE TOTAL ENVIRONMENT 598 p.1116 2017

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### Marine Coatings



Operators apply marine coatings to all parts of seagoing vessels for protection. Coating's developers use several types of plastics for marine coatings, most commonly polyurethane and epoxy coatings, vinyl and lacquers. Weathering and spills during application, maintenance and disposal of these coatings cause the release of primary microplastics.

### Personal Care Products



Many personal care and cosmetic products contain a type of engineered microplastic known as microbeads. The products include scrubbing agents, shower gels and creams.

### Plastic Pellets



Manufacturers often produce primary plastic as small pellets or powders. These producers then transport the pellets to plastic transformers that make end products. Pellets can inadvertently spill into the environment during manufacturing, processing, transport and recycling. Plastic pellets make up 0.3 percent of the ocean's primary microplastics.

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# What are microplastics?

## Definition

The term microplastic was coined only in 2004 in a paper published by Thompson et al<sup>1</sup> in Science. In this pioneering work they observed the presence of microplastics for the first time in sediment coming from a UK beach close to Plymouth and their subsequent tests found microplastics in 17 other beaches. microplastics remained mainly an academic topic up to 2018 when the presence of microplastics was observed in bottled water<sup>2</sup> and human stools<sup>3</sup> raising a huge interest from the media.

Nowadays a universally agreed and official definition of "Microplastic" is still missing even if there is general agreement on what this term refers to within the relevant communities (Researchers, media etc.): **Microplastics are small pieces of plastic made from synthetic polymers.**

The National Oceanic and Atmospheric Administration, NOAA, defined in 2009 (Arthur et al<sup>4</sup>) an upper size limit in 2009: **"Piece of plastic particles smaller than 5 mm"**.

In 2015 the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP<sup>5</sup>), added a lower limit, including for the first time, nanoplastics (down to 1 nm): **Microplastics are particles in the size range 1 nm to < 5 mm.**

In our view, the definition which it summarizes all the others and provides an additional constraint around fibers (which are one of the main sources of microplastics in marine environments, see "where are they coming from?" section) is the one used by the European Chemical Agency in their Annex XV Restriction Report on Intentionally added Microplastics of August 2019<sup>6</sup>.

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*'Microplastic' means a material consisting of solid polymer-containing particles, to which additives or other substances may have been added, and where  $\geq 1\%$  w/w of particles have (i) all dimensions  $1\text{ nm} \leq x \leq 5\text{ mm}$ , or (ii), for fibr-es, a length of  $3\text{ nm} \leq x \leq 15\text{ mm}$  and length to diameter ratio of  $>3$ . Polymers that occur in nature that have not been chemically modified (other than by hydrolysis) are excluded, as are polymers that are (bio)degradable.*

1. Thompson et al, SCIENCE, 304 p.838 2004
2. Mason et al, FRONTIERS IN CHEMISTRY, 6 (article 407) p.1 2018
3. Schwabl et al, ANNAL INTERNAL MEDICINE, 171(7) p.453 2019
4. Arthur, C., J. Baker and H. Bamford (eds). 2009. Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Microplastic Marine Debris. Sept 9-11, 2008. NOAA Technical Memorandum NOS-OR&R-30.
5. GESAMP (2015). "Sources, fate and effects of microplastics in the marine environment: a global assessment" (Kershaw, P. J., ed.). (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). Rep. Stud. GESAMP No. 90, 96 p.
6. European Chemical Agency - Annex XV Restriction Report on "Intentionally added Microplastic"

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## What are microplastics?

We understand the definition of microplastics as small pieces of solid polymer particles etc., but it is important to make a step forward and identify which are the most common types of Plastics produced globally<sup>1</sup>.

Polyolefins (PP and PE based plastics) represent more than 50% of the global production (2015 data) as they have several advantages such as low production costs, good chemical/physical resistance, etc.; advantages that can turn into downsides when considering their lifecycle because they also degrade very slowly and can survive in the environment as microplastics for centuries, being one of the main components of city dust.

An additional differentiation of Microplastics widely used by the community, introduced first by Cole et al. in 2011<sup>2</sup>, is the separation between:

### Primary microplastics & Secondary microplastics.

**Primary microplastics** are directly released into the environment as small pieces of plastic. These are intentionally engineered particles, like those found in some consumer and industrial products. Cosmetics, for example, have used microplastics as abrasives and textiles use it for durability.

**Secondary microplastics** are the result of the degradation of large plastic waste, like plastic bags and bottles, into smaller plastic fragments when exposed to our environment.

1. Hannah Ritchie (2018) - "Plastic Pollution". Published online at OurWorldInData.org  
2. Cole et al., MARINE POLLUTION BULLETIN 62 p2588 2011

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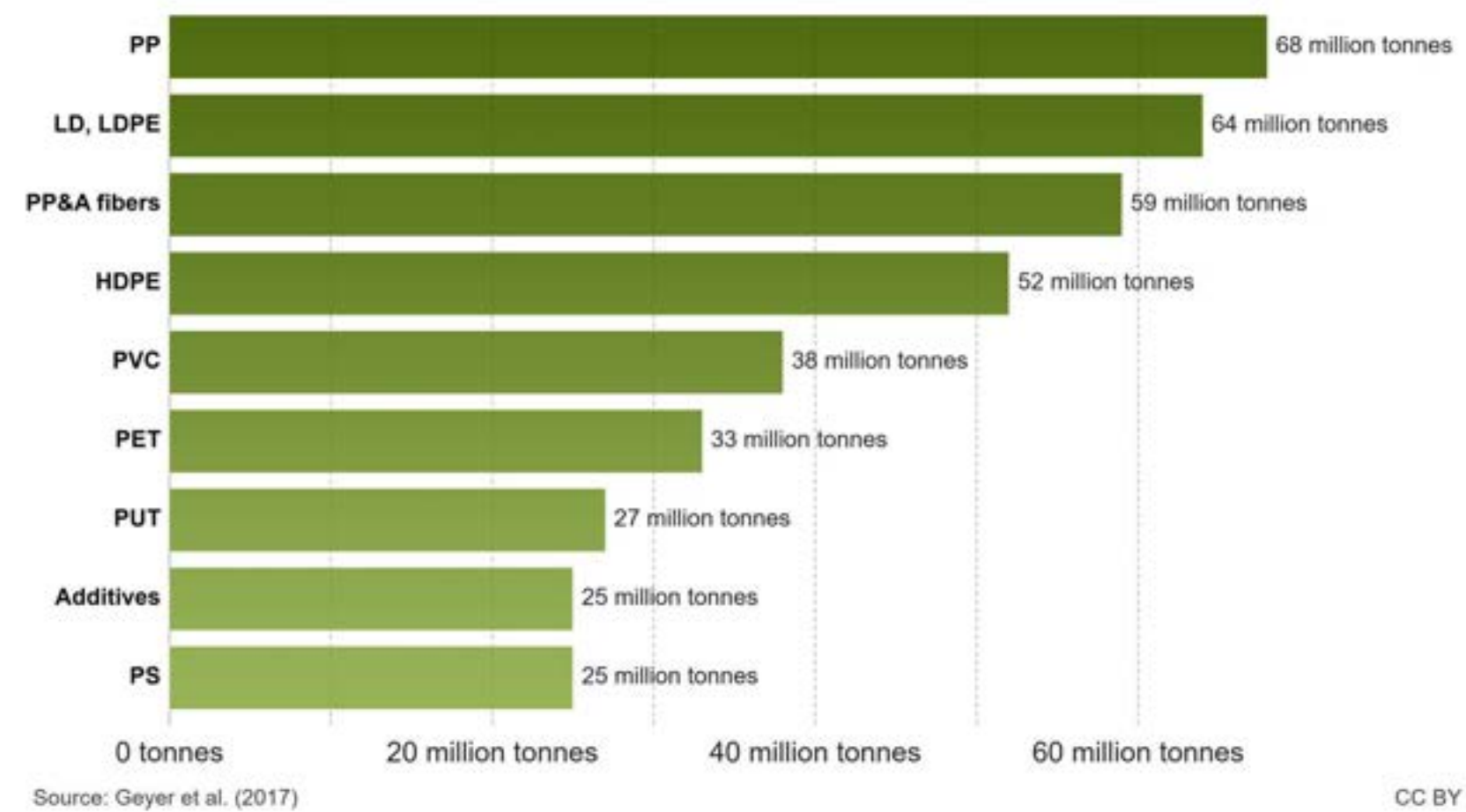


Fig. 1. Primary plastic production by polymer type, 2015  
Global primary plastic production by polymer type, measured in tonnes per year. Polymer types are as follows: LDPE (Low-density polyethylene); HDPE (High-density polyethylene); PP (Polypropylene); PS (Polystyrene); PVC (Polyvinyl chloride); PET (Polyethylene terephthalate); PUT (Polyurethanes); and PP&A fibers (Polyphthalamide fibers).

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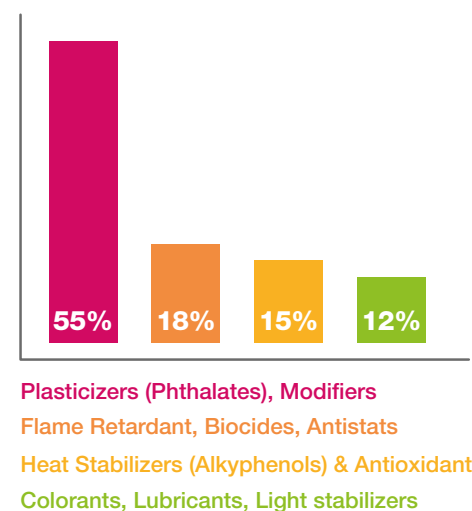
## Why are they a concern?

Microplastics are considered a great concern due to several reasons:

- **Plastic production** is increasing year over year and their degradation process is very slow. Plastics can remain in the environment, particularly the marine environment, for centuries\*.
- Microplastics on average contain 4% by weight<sup>1</sup> of other substances whose **human toxicity is well-known**, including:
  - Organics such as some Persistent Organic Pollutants (POPs), Polychlorinated Biphenyls (PCBs), Polycyclic Aromatic Hydrocarbons (PAHs), Phthalates etc.
  - Inorganics such as Titanium dioxide, Barium oxide etc.
  - Remaining monomers

*Microplastics can absorb and be an aggregation center for these types of substances dissolved in water due their higher chemical affinity with respect to water (higher hydrophobicity), increasing their load and potential toxicity.*

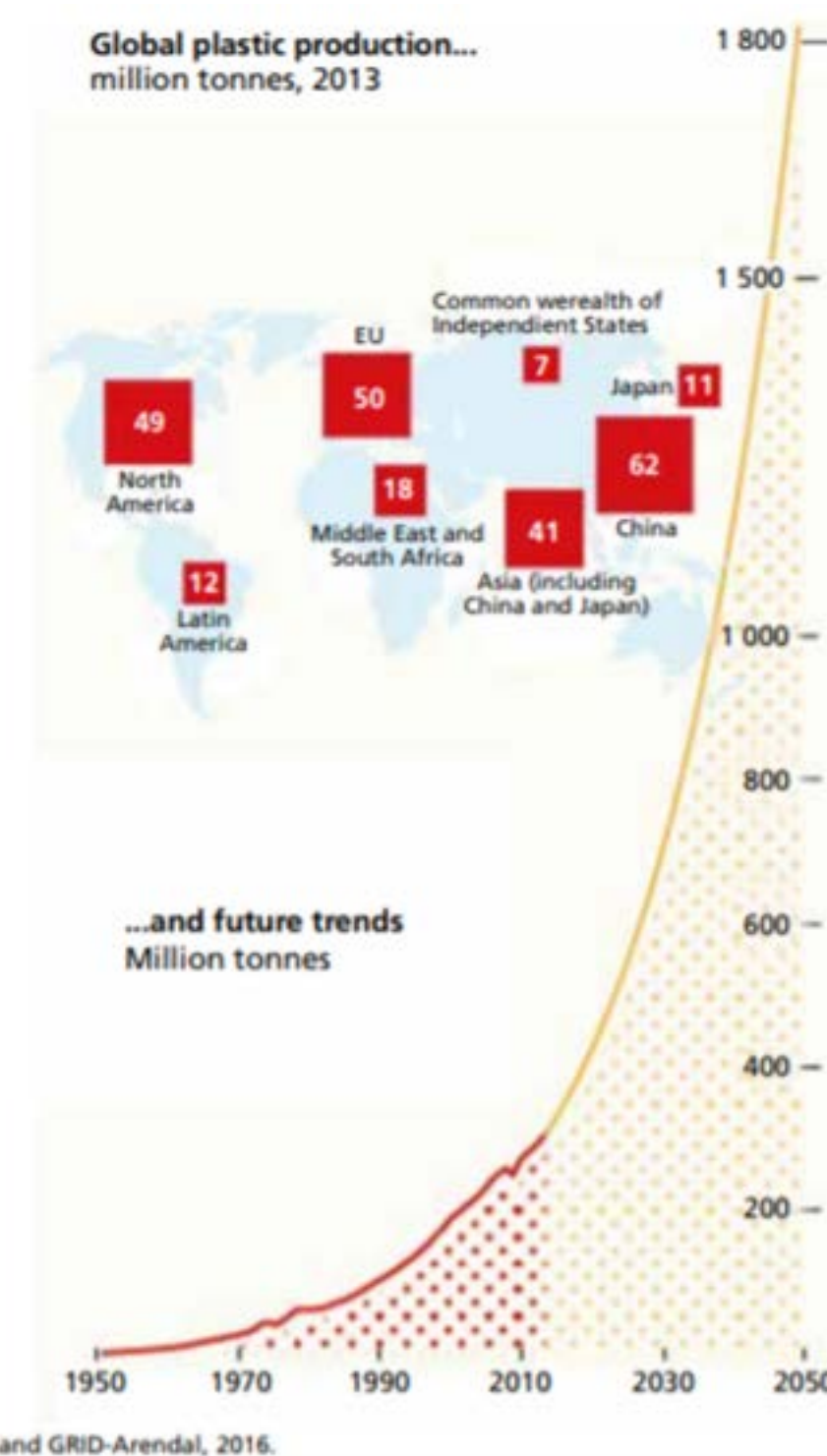
The plastic additives industry represents 10% (≈ 58 billion dollars) of the overall plastics value with plasticizers, modifiers and flame retardant being the main produced.



\* A high number of industries are strongly depended by plastics and several of their innovation were not achievable without them. The issue is not the Plastics but their recycling process, waste management and human behavior.

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Expected global plastics production up to 2050

Fig. 2. Trends in global plastic production. The projected exponential increase is the result of predictions based on increasing population and resulting demand and forecasting from the known curve

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## Why are they a concern?

Microplastics have been found in a huge number of species among all groups of wildlife (over 557 species<sup>2</sup>) and in several types of food (salts<sup>4</sup>, fish<sup>5</sup>, beer, honey<sup>6</sup>, tap and mineral water<sup>7</sup>, for example). The consumption of these foods can transfer microplastics and their additives into the human body.



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Exposure to microplastics in laboratory environment has demonstrated their potential toxicity, causing serious effects to marine animals<sup>3</sup> such as mortality, reduced feeding rate, body mass, and metabolic rate, decreased fertilization and larval abnormalities, neurotoxicity and others.

Recent studies have shown the presence of microplastics in human bodies:

- Schawbl et al.<sup>8</sup> in 2019 found microplastics in human stools, the number of samples was only 8 but each sample had a median of 20 plastic particles ranging from 50 to 500 µm in size. Nine polymer types were identified with polypropylene and polyethylene terephthalate the most abundant. The study of Schawbl demonstrates that microplastics can find a way through the human gut and potentially may move to the circulatory system,
- Ragusa et al.<sup>9</sup> detected plastic fragments in placenta samples collected from six patients with uneventful pregnancies, All the particles were less than 10 µm in size. The presence of microplastics in the placenta shows that they can reach the circulatory system and be transported to different organs.



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## Why are they a concern?

Notwithstanding the potential risk associated with microplastics it is difficult to predict their toxicity for human health due to the lack of studies providing in vivo data on the absorption of microplastics. Moreover, the few in vitro studies show that particle uptake by the human body (Lusher et al.<sup>10</sup> and references cited within) is expected to be limited and strongly linked to the size of the particles.

Only microplastics below 150 µm may translocate from the gut epithelium and a small portion of them, with sizes below 5 µm (nanoplastics being the more dangerous), may penetrate into other organs as demonstrated by the work of Ragusa.



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*Considering this, it is crucial to focus on the analytical techniques which allow the identification and characterization of the smallest particles. These include Raman Microscopy, SEM, AFM etc.*

1. Bouwmeester et al. ENVIRONMENT SCIENCE & TECHNOLOGY 49 p.8932 2015
2. Kühn, S., Rebolledo, E. L. B., & van Franeker, J. A. (2015). Deleterious effects of litter on marine life. In Marine Anthropogenic Litter (pp. 75-116). Springer, Cham.
3. Barboza et al., 2018. MARINE POLLUTION BULLETIN 133 p.336 2018
4. Yang et al., ENVIRONMENT SCIENCE & TECHNOLOGY 49 p.13622 2015
5. Rochman et al., SCIENTIFIC REPORTS 5 p.1 2015
6. Liebezeit et al., FOOD ADDITIVES & CONTAMINANTS: Part A 30 p.2136 2013
7. Mason et al., FRONTIERS IN CHEMISTRY, 6 (article 407) p.1 2018
8. Schwabl et al., ANNAL INTERNAL MEDICINE, 171(7) p.453 2019
9. Ragusa et al., ENVIRONMENT INTERNATIONAL 146 p.1 2021
10. Lusher et al., FAO Fisheries and Aquaculture Technical Paper. No. 615. Rome, Italy. 2017

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A good overview of the actual global regulations around plastics & microplastics is given by the United Nations Environment Programme (UNEP) which in 2018 published a review on the national laws and Regulations on the legal limits for single-use plastics and microplastics<sup>1</sup>.

This global review covers all the national legally-binding instruments, including bans and restrictions, and it separates them into three main sections:

- 1) plastic bags,
- 2) other single-use plastics,
- 3) microbeads.

### Plastic bags.

127 out of 191 countries have adopted plastic bag legislation but only 91 imposed some kind of ban and/or restriction on production, importation and distribution of plastic bags. Europe and Africa are the continents with most legislative activity.

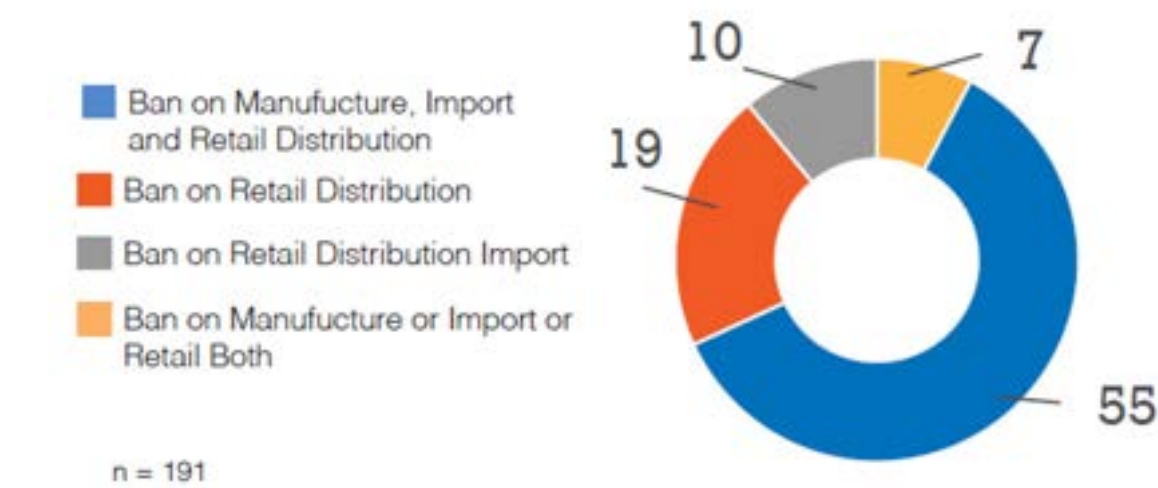
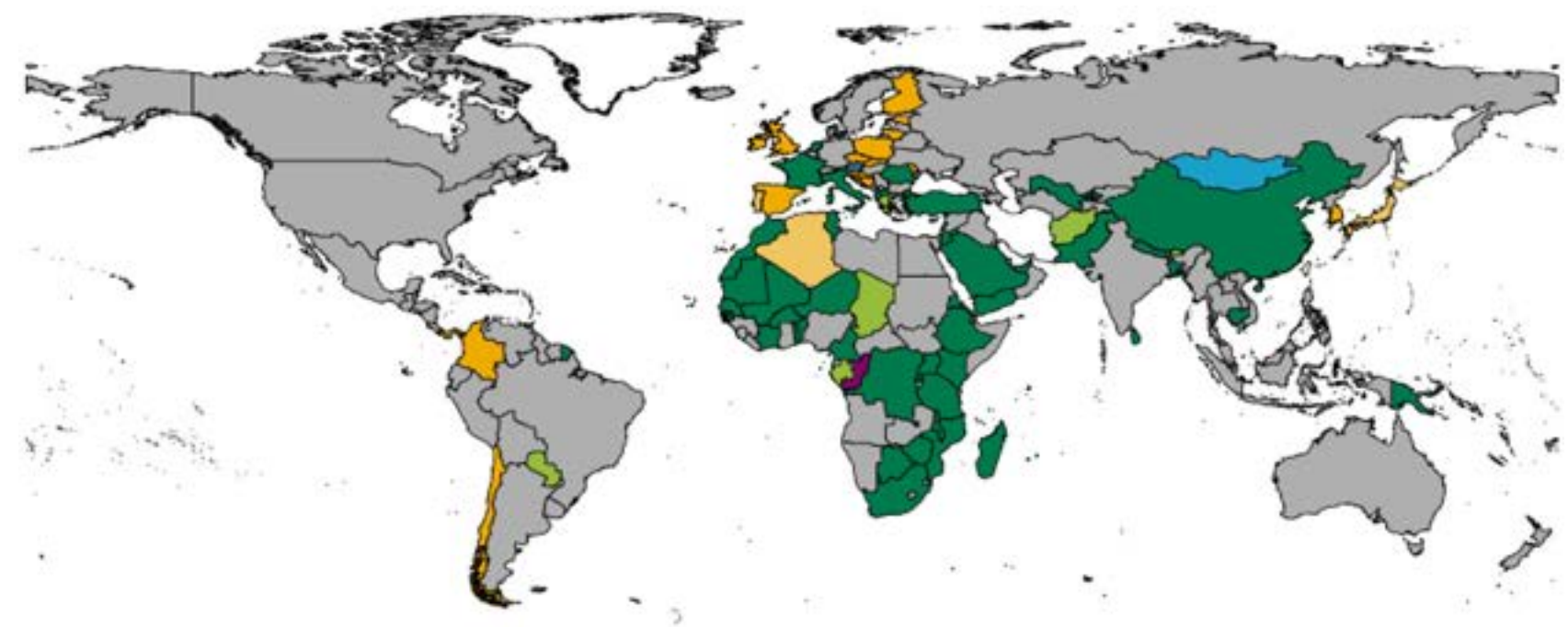


Fig. 3. Ban of plastic bags



Global overview of countries with bans on the manufacture, free distribution, and importation of plastic bags

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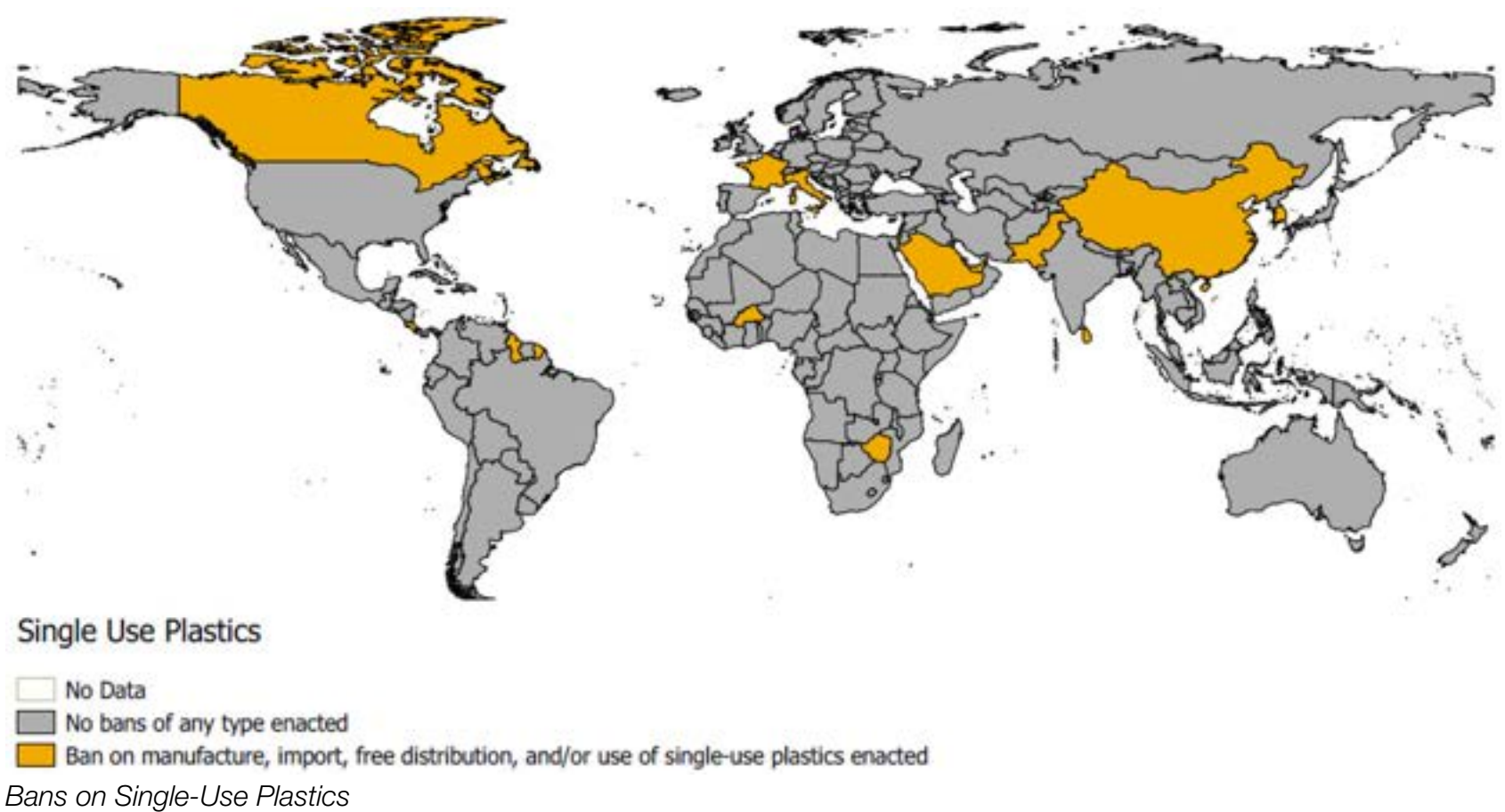


# What are microplastics?

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### Single-use Plastics.

For single use plastic the situation is different. Only 27 out of 191 countries have adopted single-use plastic legislation, imposing some kind of ban and/or restriction on production, importation and distribution of plastic bags.



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Fig. 4. Number of bans by type

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# What are microplastics?

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### Microbeads.

Only 9 countries out of 191 have adopted microbead legislation, imposing some kind of ban and/or restriction on production, importation and distribution of microbeads.

Microbeads are a primary microplastic, designed and intentionally engineered to be small, and are used for example in cosmetic products.

All countries define microbeads within their legislation, but it is possible to summarize all the different definitions with the following one: a “plastic microbead” is defined as any solid plastic particle that is — 5 millimeters or less in size.

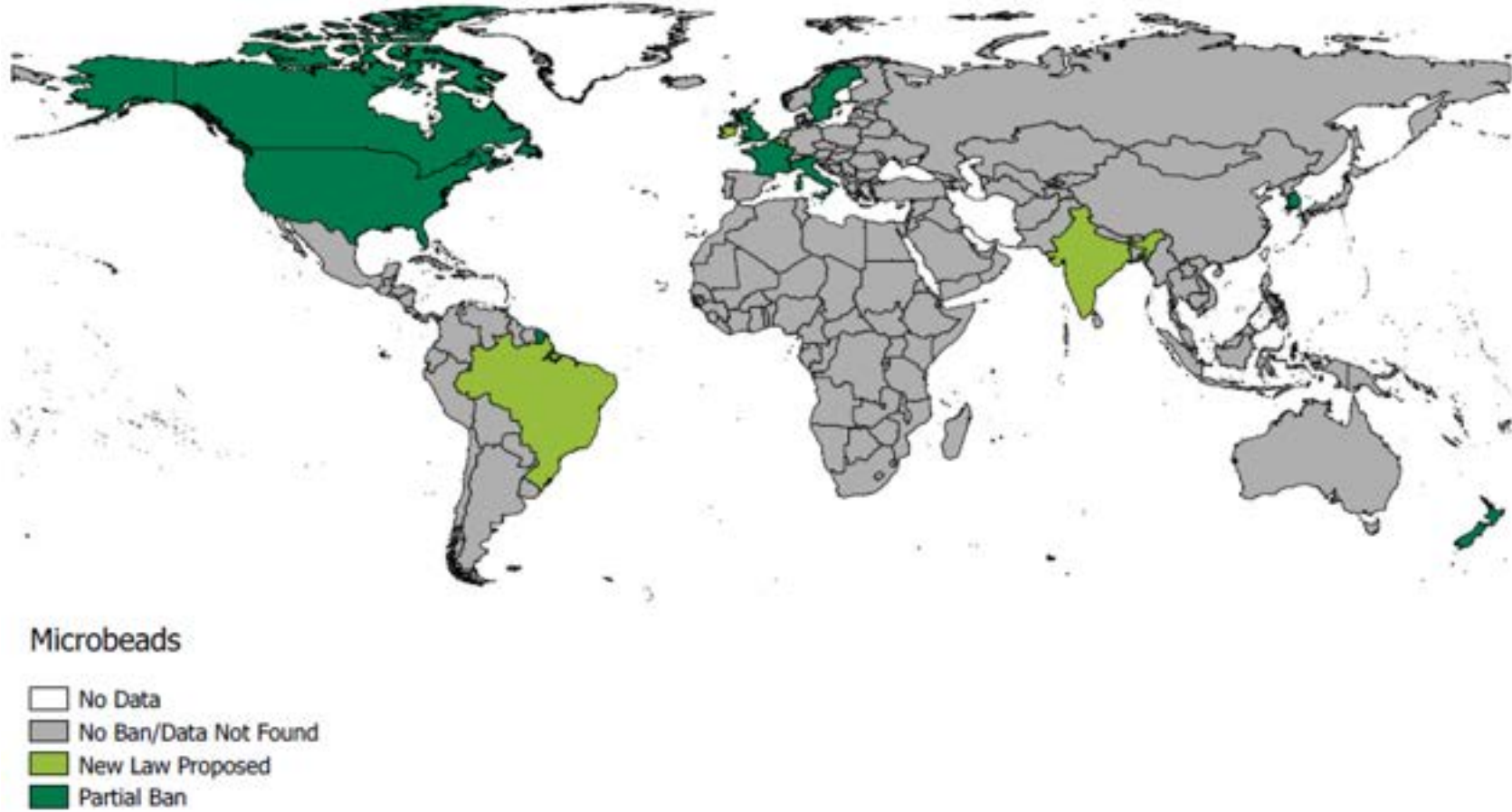
The Canadian legislation, microbeads in toiletries regulations (SOR/2017-111) of 2017, is the first to mention molecular spectroscopy techniques as a testing method.

Table 1. Regulatory landscape

Country	Law or Regulations Name
Canada	Microbeads in Toiletries Regulations (SOR/2017-111), June 2 2017
France	Reclaiming Biodiversity, Nature and Landscapes Act No 2016-1087 of 8, Article 124, August 2016
Italy	General Budget Law 2018: Law No 205 of 27, Art.1, Sections 543 to 548, December 20172
Republic of Korea	Regulations on safety standards for cosmetics [Annex 1] No. 2017-114, Notice, Article 3, Dec. 29, 2017
New Zealand	Waste Minimisation (Microbeads) Regulations 2017, under section 23(1)(b) of the Waste Minimisation Act 2008.
Sweden	Regulation amending Regulation (1998: 944) prohibiting etc. in certain cases in connection with handling, import and export of chemical products
UK	
England	The Environmental Protection (Microbeads) (England) Regulations 2017
Scotland	The Environmental Protection (Microbeads) (Scotland) Regulations 2018
Wales	The Environmental Protection (Microbeads) (Wales) Regulations 2018
Northern Ireland	The Environmental Protection (Microbeads) (Northern Ireland) Regulations 2018.
US	Microbeads-Free Waters Act of 2015

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Number of Countries with bans on Microbeads



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In the last few years, the European Community has made progress in this area and is developing a plan that intends by 2021 to regulate to restrict the use of intentionally added microplastics particles to all kinds of consumer and professional use products of all. Mineral and tap water regulation is in the scope of this plan.

The document (Annex XV1) released by the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) of the European Chemical Agency (ECHA) in 2019 was a first step towards final adoption.

In Annex XV it was identified that ‘intentionally added’ microplastics are used in various products such as consumer, agricultural and industrial and they include:

- Agriculture and horticulture (in fertilizers and plant protection products)
- Cosmetic products
- Detergents and maintenance products
- Paints, coatings and inks
- Chemicals used in the oil and gas sector
- Construction
- Medicinal products
- Medical devices
- Food supplements and medical food

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HORIBA France is actively involved in a group of experts within the French Standardization Association (AFNOR) currently working on establishing a regulation on the analysis of microplastics in drinking water, through spectroscopic techniques ( $\mu$ FTIR and Raman). This group, part of the T91M “Organic micropollutants” Commission, gathers various governmental, academic and industry organizations, including the Standardization Bureau for Plastics and Plastics Engineering (BNPP), with the objective of drawing up a new norm by the first half of 2021.

<https://www.echa.europa.eu/documents/10162/05bd96e3-b969-0a7c-c6d0-441182893720>

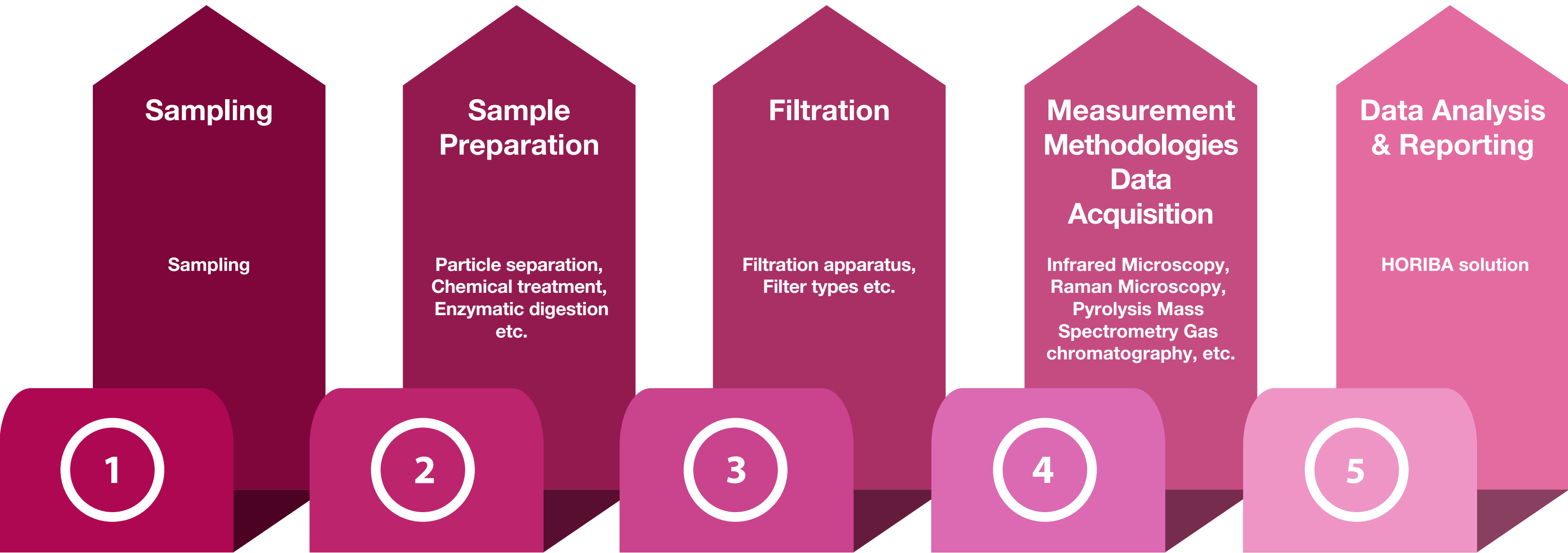


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A common analysis workflow for microplastics separation, counting and identification requires 5 main steps: Sampling; Sample preparation or sample pre-treatment ; Filtration, measurement methodologies and data acquisition; and finally, data analysis and reporting.





# Analysis Workflow

## Sampling

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Protocols  
Filtration  
Measurement & Acquisition

The appropriate sampling step is highly dependent on the matrix to be investigated/analyzed for the presence of microplastics. Considering the number of possible matrixes, it is tough to provide a complete picture, but we will touch the most important ones.

### Water Sampling.

Most important point for water (but also valid for sediment and other matrixes) is the representativeness of the sample collected. Ocean, sea and river water samples must come both from the water surface and the water column. Several studies (review of Hidalgo-Ruz et al.<sup>1</sup>) have demonstrated that the water surface has a higher number of microplastic items than the water column: Microplastics ranks from 0.022 to 8,654 items m<sup>3</sup> at the surface and from 0.014 to 12.51 items m<sup>3</sup> in the column.



The most common tools for water withdrawal are manta trawls (surface water) and plankton nets (water column) (Stock et al.<sup>2</sup> and papers cited within); the mesh of the net can vary between 50 to 3000 µm but the most common is 300 or 330 µm. Due to the mesh size most microplastics under 300 µm are lost. A mesh size net of 80 µm has also been used but the risk of clogging is high. A flow meter is usually used to measure the amount of water flowing through them for comparative and quantitative measurements.



Plankton Net



Manta Trawls

Alternative tools are: Continuous flow centrifuge which can collect particles down to 5 µm without clogging, but with a longer sampling time (1 hour for 1m<sup>3</sup> of water); Filter cascade with a fractionated pressure filtration, which guarantees fast measurement times and direct separation of the particles into size classes.

Some general guideline for water sampling, and also sediment and biota, in seas can be found in Guidance on Monitoring of Marine Litter in European Seas<sup>3</sup> by the Marine Strategy Framework Directive (MSFD).

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# Analysis Workflow

## Sampling

### Sediment Sampling.

For marine and freshwater sediment, the golden rule of ensuring the representativeness of the sample is still key. Some guidelines are provided in the MSFD document of the MSFD. Important are; the amount of the sample collected (often measured in volume L, weight kg or areal extension m<sup>2</sup>, analyzed); The location and the repetition for each location. The sampling depth is also an important parameter and can vary depending on the aim of the study but in many publications the upper 5 cm or less, is where most of microplastics are concentrated, and therefore has been used for microplastics monitoring. Sediments contain more microplastics than water, ranging from 0.21 to more than 77,000 items per m<sup>2</sup>.

Marine sediment, a part of the shoreline (beaches), can be differentiated by the location where they are collected in 3 different zones: Tideline or supralittoral, intertidal or eulittoral and sublittoral. In freshwater ecosystems the same differentiation does not apply, due to the minimal effects of tides. The tools for sediment collection are mainly mechanical, such as tweezers, table-spoons, hand picking and grabbers for deep sediment.

### Biota Sampling.

It's important to define the term Biota as a common starting point: Biota is the animal and plant life of an ecosystem.

Sampling methods are highly diverse and depend on the target and type of habitat: water column, sea surface, aquaculture etc. Lusher

et al.<sup>4</sup> wrote a wide and exhaustive review on this field, underling as the most important points: the avoidance of plastic contamination and handling of animals.

Handling stress can result in a loss, and therefore underestimation of microplastics due to gut evacuation. The safest methods of storage of the organisms, before their analysis are desiccation and freezing.

### Food Sampling.

Foods is more straightforward than sediment, water and biota sampling, They are readably available thorough the commercial chain; the key sampling factors in food sampling are the number of samples and repetitions, even if a recognized protocol is still missing. Below we summarize some examples of food sampling.

### Honey and Sugar.

Liebezeit et al.<sup>5</sup> collected mainly from Germany, 19 types of honey, both solid and liquid plus 5 types of sugar directly from the producers or supermarkets. Honey samples were filtered with a 40 µm sieve (the solid one after melting it), sugar was dissolved in deionized water and then filtered with a 0.8 µm cellulose filter.



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## Sampling

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### Salts.

Several studies on salts have been conducted since the first one of Yang et al.<sup>6</sup> but the one of Kim et al.<sup>7</sup> in 2018 is the most exhaustive. Kim collected 39 brands of table salt from supermarkets in 17 different countries over 6 continents, Salts were selected to provide a diverse range of sources (seawater, lake water) and manufacturing methods (solar-dried, refined or un-refined). A minimum of 500 grams for each salt were tested and duplicated.

### Tap water.

A recent study of Kosuth et al.<sup>8</sup> published in 2018 is a good example of tap water sampling. Kosuth collected 159 samples from 14 different countries. Samples were collected by running the tap water for 1 minute and then, while the water source was running, a bottle of 500 ml was filled and dumped twice before the final filling.

### Bottled water.

Manson et al.<sup>9</sup> in 2018 conducted a study on bottled water selecting 259 bottles from 11 brands in 27 lots, including leading global brands from various bottled water producers, purchased in 9 different countries. Bottled water came in bottles of different capacities (from 0.5 L up to 2 L) and several bottles were analyzed to reach a volume close to, or above, 5 L for each lot.

1. Hidalgo-Ruz, et al., ENVIRONMENT SCIENCE & TECHNOLOGY 46 p.3060 2012
2. Stock et al., TREND IN ANALYTICAL CHEMISTRY 113 p. 84 2019
3. <https://mcc.jrc.ec.europa.eu/documents/201702074014.pdf>
4. Lusher et al., ANALYTICAL METHODS 9 p.1346 2017
5. Liebezeit et al., FOOD ADDITIVES & CONTAMINANTS: Part A 30 p.2136 2013
6. Yang et al., ENVIRONMENT SCIENCE & TECHNOLOGY 49 p.13622 2015
7. Kim et al., ENVIRONMENT SCIENCE & TECHNOLOGY 52 p.12819 2018
8. Kosuth, M. et al. PLOS ONE 13 No. e0194970 2018
9. Mason at al., FRONTIERS CHEMISTRY 6 p.407 2018



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## Sample Preparation

Sample pre-treatment is the most important step for microplastics analysis because, if done correctly, it eliminates all types of possible organic contaminants that can affect microplastic chemical identification when using various techniques: Infrared Microscopy, Raman Microscopy, Pyrolysis Gas Chromatography/ Mass Spectrometry (GC/MS) etc. These contaminants are always present (even when analyzing bottled water) and their amount depends on the matrices analyzed.

There is extensive literature on pre-treatment protocols which vary depending by the type of matrix under investigation. There are some key aspects that must be considered independent of the analysis method :

### Integrity of the microplastic

Chemical treatment can modify both chemistry and sizes of microplastics if too aggressive. These two aspects are important to determine the potential toxicity of microplastics, so treatment must be carefully chosen to avoid changing the sample.

### Plastic contamination

Sample manipulation can cause additional plastic contamination from the laboratory environment. A blank, or reference, sample of just filtered deionized water is essential to understand plastic contamination and to avoid over-estimation of the microplastic content. Preparing samples under a laminar flow hood is highly recommended.

This section provides a general overview of the different methodologies and also gives detailed suggestions for some of the most common matrices. Due to the absence of standardized methods, the protocols suggested are the combination of our experience and the literature.

Apart from the organic contaminant removal protocols, additional treatments must be mentioned for sediment analysis. The first step is **physical separation** using various sieves to isolate Microplastics and inorganic materials depending on their size. The second step is **extraction**. Sediments contain other

inorganic materials, such as quartz sands and silicates. These must be separated from the microplastics to avoid interference during chemical identification. Extraction is done by means of density separation, exploiting the different densities of plastic and inorganic materials; the majority of polymers possess a lower density (usually from 0.8 to 1.6 see Table 1) than the inorganic constituents of the sediment. As an example, silicates density range from 2.196 for amorphous to 2.648 for  $\alpha$ -quartz.



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# Analysis Workflow

## Sample Preparation



Commonly density separation involves 4 steps as highlighted in the review of Hanvey et al.<sup>1</sup>:

- Introduction of an aqueous solvent with a specific density
- Mixing for defined periods of time
- Settling, or equilibration time
- Filtering to specific size fractions

By using an aqueous solvent with a higher density than plastics, they will float on the surface allowing them to be separated from inorganic materials. It is important to vigorously mix the solution to ensure that the microplastics can separate out during the settling step. It is highly recommended to repeat these steps at least two times.

The addition of salts increases the density of the aqueous solution and varying the types of salts allows the density to be tuned to meet specific requirements. Several salts (Hamm et al.<sup>2</sup> and references within) have been used in literature and the most common ones are listed in the following Table.

Table 2. Density values for the most common polymers. The ones highlighted in grey represent more than 80% of global plastic production.

Polymer type	Density (gr/cm <sup>3</sup> )
Poly(propylene), PP	0.861
Poly(ethylene), PE (Low to High density)	0.854-0.96
Poly(vinyl chloride), PVC	1.388
Poly(ethylene terephthalate), PET	1.333
Thermoplastic Polyurethane PUR	1.23-1.35
Polystyrene, PS	1.052
Polytetrafluoroethylene, PTFE or Teflon	2.2
Poly(amide) 6, PA6	1,06-1,16
Poly(vinylidene fluoride), PVDF	1.675
Polychloroprene, Neoprene	1.243
Poly(methyl acrylate), PMA	1.224
Poly(isobutene)	0.864
Poly(caprolactam)	1.084
Poly(Bisphenol A carbonate), PC	1.206
Poly(lactic acid, PLA	1.248
Poly(ethylene glycol), PEO, PEG	1.128
Poly(methyl methacrylate), PMMA	1.159
Poly(vinyl alcohol), PVOH	1.300
Poly(vinyl acetate), PVA PVAC	1.190
Poly(ethylene-vinyl acetate) PEVA	0,92 - 0,94

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This table also provides the cost, an important consideration for sediment analysis, and the potential safety issues correlated with the handling of some of them.

Table 3. List of salts for density separation process.

Salt		Maximum density $\rho$ (g/cm <sup>3</sup> )	Amount (Kg)	Cost (euro)	Amount (gr/L) for Maximum $\rho$	Safety
Sodium Chloride	NaCl	1.2	1	35,9	311	no effect
Sodium Iodide	NaI	1.8	0,5	287	797	
Zinc Chloride	ZnCl <sub>2</sub>	1.9	1	116	1373	
Zinc Bromide	ZnBr <sub>2</sub>	1.7	0,5	139	1125	
Calcium Chloride	CaCl <sub>2</sub>	1.4	0,5	25	558	
Sodium Polytungstate	3 Na <sub>2</sub> WO <sub>4</sub> 9WO <sub>3</sub>	3 (1.55)	0,1	216	5671 (798)	no effect

- Eye, skin and respiratory tract irritation;
- Corrosive;
- Possible burns.

Sodium Chloride is the readily available and safe solution but the maximum density achievable is only 1.2 g/cm<sup>3</sup> which does not allow separation of high-density plastics such as PVC and PET. A minimum density of 1.5 to 1.55 is needed to recover more than 90% of the plastics.

Sodium Polytungstate is probably the best technical solution because the density can be easily tuned up to 3 g/cm<sup>3</sup>, it has a low viscosity, it can be reused and additionally it has no safety constraints, but its main drawback is the cost which is more than 2000 euros per kilograms. According to Coppock et al.<sup>3</sup> and considering all the aspects, we suggest as the best balanced solution Zinc Chloride; care must be taken with handling, but the price is reasonable and densities up to 1.9 g/cm<sup>3</sup> can be reached.

After this overview of physical separation and extraction methods we can move on to organic contaminant removal protocols or the digestion step. Most of the digestion protocols envisage the use of concentrated acids and alkali solution which destroy proteins, carbohydrates and fats (the main constituents of organic residues in sediment and marine water samples and also in foods). These are the main interreference agents for microplastic chemical identification using the common analytical methods e.g. Fourier Transform Infrared/ Raman Microscopy and Pyrolizer GC-MS.

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The review of Hamm et al.<sup>2</sup> of 2018 (and papers cited within) provides an exhaustive picture of digestion protocols and a summary of their efficiency vs. their unwanted ability to degrade Microplastics. A visual representation of this summary is depicted in figure here after.

Acid treatments are highly effective for removing organic residues reaching an efficiency above 80% in several cases, but they can easily damage microplastics preventing their chemical identification.

**Alkali treatments can have different effects:**

- 1. 30% and 35% aqueous solutions of H<sub>2</sub>O<sub>2</sub> are an effective treatment but they can chemically damage some types of plastics (such as PVC and Polyamide 6-6/6 – Nuelle et al.<sup>4</sup>) and moreover they can also modify the shape and size of the particles. Temperature and incubating time are also important parameters to be considered, increasing them we have a positive impact on the digestion efficiency but a negative one on the particle chemistry/shape/size.

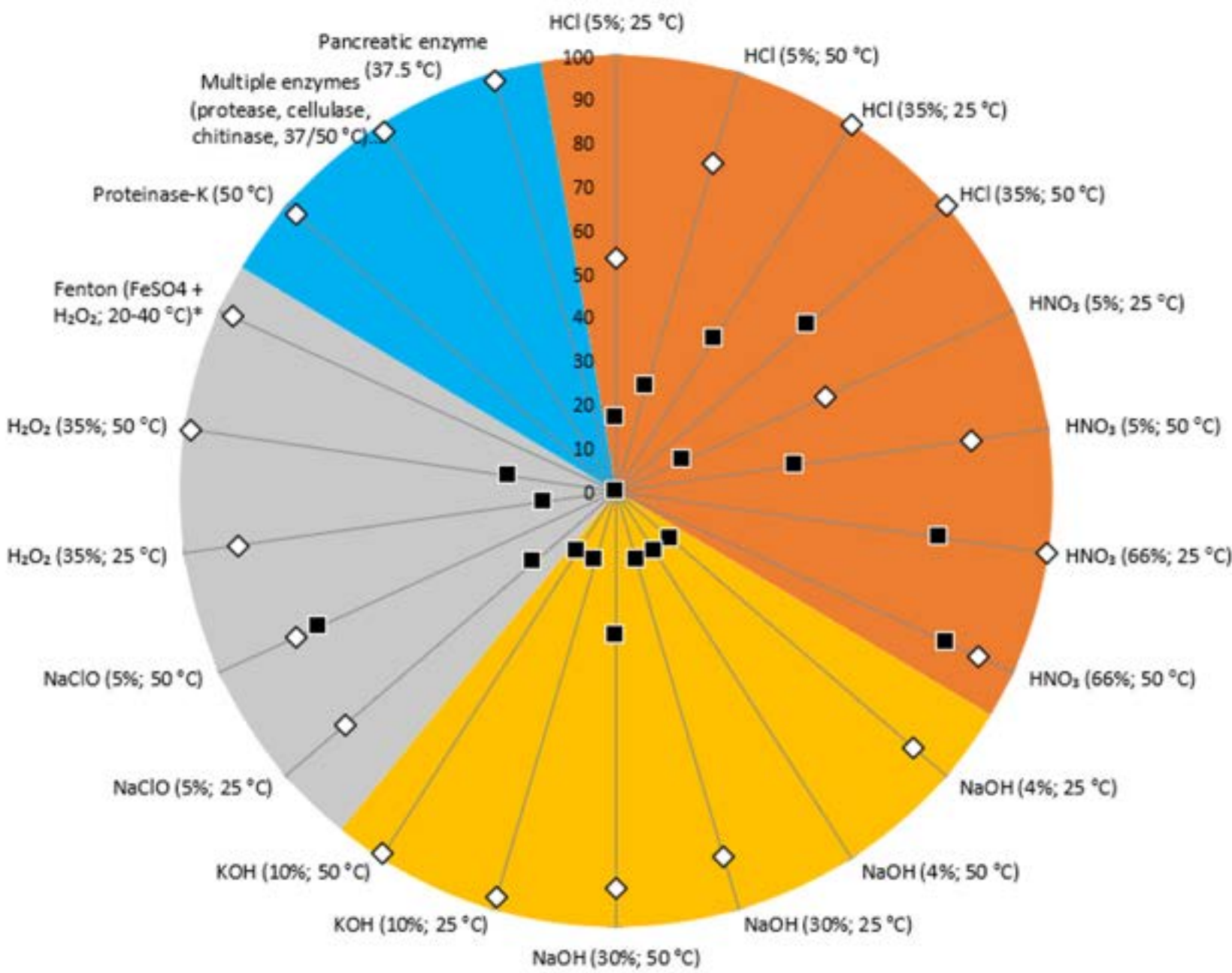


Fig. 5. (■) Max. % of Microplastic negatively affected by treatment; (◇) Effectiveness of the treatment in %. (Image provided by Claudia Lorenz, University of Aalborg)

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2. 10% aqueous solution of KOH provides better results than H<sub>2</sub>O<sub>2</sub> as demonstrated by Karami et al.<sup>5</sup>. Karami tested this solution at different temperatures and incubation times, the table below summarizes the results.

Table 4. Treatment efficiency in function of conditions

Digesting Solution 10% KOH	Temperature (°C)	Incubation Time (hours)	Efficiency / Recovery Rate
	25	96	97.1
	40	48	98.6
	50	36	98.9
	60	24	97.61

The treatment efficiency (obtained by averaging the values for all the polymers tested) is above 97% with all combinations but at 50°C and 60°C, Karami observed some degradation of PVC, PET and Polyamide 6-6/6. The best condition balancing temperature / speed (i.e. incubation time) was at 40°C for 48 hours, where only PVC shows a recovery rate below at 93%.

Three additional digestion processes are important to mention:  
Fenton’s Reagent (Tagg et al.<sup>6</sup>); Mono-Enzymatic treatment (Cole et al.<sup>7</sup> used Proteinase-K, while Courtene-Jones et al.<sup>8</sup> used Trypsin); Basic and Universal Enzymatic Purification Protocol (BEEP-UEEP) which combines a Multi-Enzymatic treatment with an oxidizing agent (H<sub>2</sub>O<sub>2</sub>) and a detergent Sodium Dodecyl Sulfate (SDS), this protocol is usually employed for protein denaturation (Loder et al.<sup>9</sup>)

**Fenton’s reagent** is prepared by mixing solutions of 30% H<sub>2</sub>O<sub>2</sub> and FeSO<sub>4</sub>·7H<sub>2</sub>O to reach final FeSO<sub>4</sub>·7H<sub>2</sub>O concentrations of: 3.33, 6.67 and 10 mg/ml. Its efficiency was demonstrated with infrared microscopy and even PVC and Polyamide didn’t undergo to any modifications. The major advantages of Fenton’s reagent stressed by Tagg et al. is the rapid digestion time of only around 10 minutes, much faster than Alkali treatments and the fact that it works at room temperature.

We observe only one issue connected to Fenton’s reagent digestion that can affect microplastic identification by Raman microscopy (the key technique for analyzing microplastics below 5/10 microns): the presence of Iron leads to the formation of fluorescence compounds that interfere with the chemical identification of polymers by Raman.

**Enzymatic treatment.** Both the mono-enzymatic digestions were tested on marine biota, bivalve species for Cole and mussel for Courtene-Jones, and they show very high efficiency and no degradation of any plastics. Efficiency was 97% for Proteinase-K and 88% for Trypsin.



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As some biogenic material remains undigested, using Trypsin, Courtene-Jones et al.<sup>8</sup> proposed the use of an additional enzyme such as chitinase to remove the residual parts. The protocol developed by Cole<sup>7</sup> for bivalves differs on one aspect: the sample was homogenized with a solution of 400 mM Tris-HCl buffer, 60 mM EDTA, 105 mM NaCl and 1% SDS before adding the enzyme while Courtene-Jones<sup>8</sup> used a solution of Trypsin, made with deionized water, directly on the sample.

These enzymatic protocols are particularly useful for marine biota and marine sediments, their main drawback is the relatively high cost of the purified enzymes.

**Basic and Universal Enzymatic Purification Protocol (BEEP-UEEP).** Loder et al.<sup>9</sup> starts from the approach of Cole<sup>7</sup> and Courtene-Jones<sup>8</sup> but develops a complete protocol (BEEP), including a density separation step, combining multi-enzymatic digestion (Protease, Cellulase and Chinase) and oxidative treatments. Moreover, he successfully evaluated the protocol for its efficiency and applicability for infrared microscopy and for Microplastics with dimensions down to 20 µm.

UEEP is a further optimization of the BEEP protocol that widens its versatility for different environmental sample matrices (BEEP was developed first for seawater samples) by adding two additional enzymes (Lipase and Amylase). Loder<sup>9</sup> developed one of the most complete sample pre-treatment protocols (particularly suited for marine environments – biota, sediment and water) where all the possible interferents (such as chitin-containing materials, plant residues, planktonic organisms and cell residues) for microplastics identification are selectively attacked and, notwithstanding the use of enzymes, he was able to reduce the cost compared to Cole<sup>7</sup> by using technical grade enzymes.

One potential drawback of the BEEP/UEEP protocols is the incubation time needed for all the enzyme steps which bring the overall pre-treatment time to between 10-12 days. Furthermore, the presence of several steps is an additional potential source of unintended plastic contamination and/or particle loss.



1. Harvey et al., ANALYTICAL METHODS 9 p.1369 2017
2. Hamm et al. (2018) Microplastics in Aquatic Systems – Monitoring Methods and Biological Consequences. In: Jungblut S., Liebich V., Bode M. (eds) YOUMARES 8 – Oceans Across Boundaries: Learning from each other. Springer, Cham. [https://doi.org/10.1007/978-3-319-93284-2\\_13](https://doi.org/10.1007/978-3-319-93284-2_13)
3. Coppock et al., ENVIRONMENTAL POLLUTION 230 p.829 2017
4. Nuelle et al., ENVIRONMENTAL POLLUTION 184 p.161 2014.
5. Karami et al. SCIENCE OF THE TOTAL ENVIRONMENT 578 p.485 2017
6. Tagg et al., CHEMICAL COMMUNICATION 53 p.372 2017
7. Cole et al. SCIENTIFIC REPORTS 4:4528 2014
8. Courtene-Jones et al., ANALYTICAL METHODS 9 p.1437 2017
9. Loder et al., ENVIRONMENTAL SCIENCE & TECHNOLOGY 51 p.14283 2017

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# Analysis Workflow

## Protocols

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Following the overview of the sample pre-treatment workflows, in this section we will propose protocols for various matrices, starting with bottled and tap water. This section will be updated twice a year and new detailed protocols will be added for different matrices as a result of advances in the literature and HORIBA experience.

### Bottled Tap Water.

Bottled water sample can be analyzed without any pre-treatment, but we recommend the protocol developed by Oßmann et al.<sup>1</sup> as the treatment is rapid and the removal of many non-plastic particles can reduce the total measurement time. The same treatment can also be used for tap water.

The method uses:

- Ethylenediaminetetraacetic salt (EDTA): EDTA is well-known to reduce the water hardness by complexing metal ions such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ;
- Sodium Dodecyl Sulfate (SDS): SDS is an anionic surfactant that improves plastic suspension and provides better homogeneity.

EDTA (250 g/L solution) is added in an equimolar amount depending on the content of Calcium and Magnesium ions indicated on water bottle label.

EDTA must be left for 15 minutes. This treatment reduces the number of Calcium and Magnesium carbonate particles speeding up the full analysis time; since Raman and infrared microscopy identify plastics by analyzing each particle individually (see “Measurements Methodologies” section) removing the inorganic ones in advance reduces the overall acquisition time.

Following EDTA, 3 ml of SDS (100g/L) is added per litre of water. After the filtration step, SDS, must be removed with a solution of 50% ethanol (ultrapure ethanol) in deionized water. This SDS step is optional.

Prior to using any solution, filtering using a 0.1  $\mu\text{m}$  mesh will limit plastic contamination from the lab environment.

1. Oßmann et al., WATER RESEARCH 141 p.307 2018



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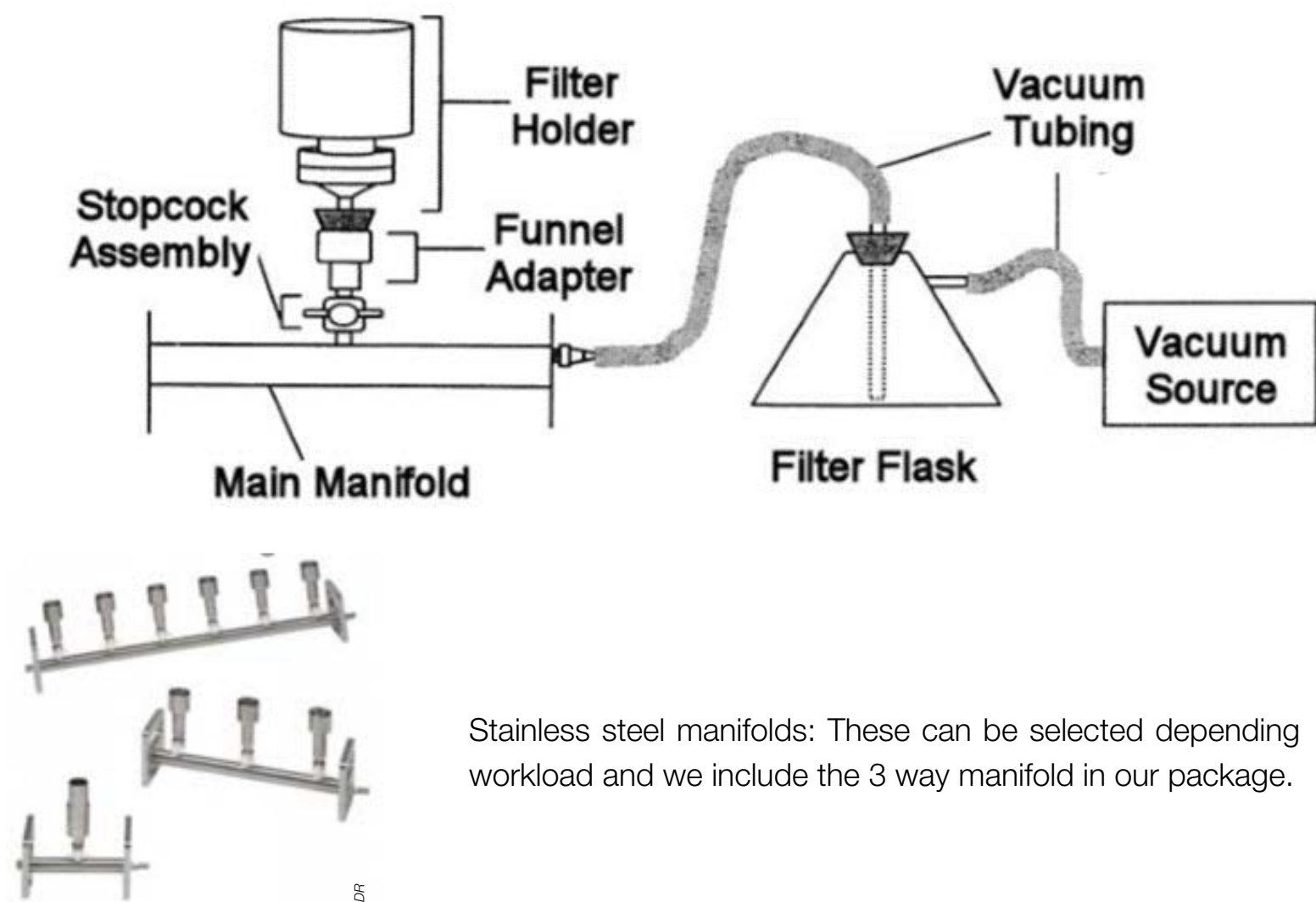
## Filtration

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### Filtration apparatus.

There are several choices of set-up but the main point to keep in mind is to avoid, as far as possible, plastic parts since they can be an unintended source of microplastics. The HORIBA choice (the parts depicted below are offered in our “Microplastic package” see HORIBA Solution section) for the filtration apparatus is:



Stainless steel manifolds: These can be selected depending on the workload and we include the 3 way manifold in our package.

*Filtration is the last step prior to the identification of the microplastics by the technique of choice (FTIR microscopy, Raman microscopy and optical microscopy) and two points must be addressed in this section: Filtration apparatus and filter types.*

We have selected Sterlitech (<https://www.sterlitech.com/>) as our preferred filtration apparatus supplier, and the parts are:



Glass funnel (available up to 1L, 100 ml in our package), a sintered glass support base with 13 mm available filtration area, a silicone stopper and a 1L borosilicate glass flask with side arm.

A diaphragm vacuum pump chemically resistant and completely oil-free.

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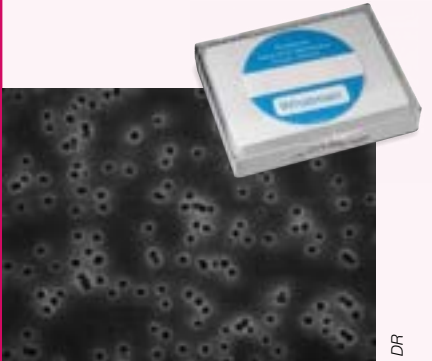
Filters.

There is a wide choice of filter/membrane and several of them have been tried and tested microplastics analysis. The three important characteristics are: filter size (13, 25, 47, 55 mm in diameter), filter material (polycarbonate, polytetrafluoroethylene PTFE, alumina, silicon etc. and pore size (0.2, 0.7, 1.6, 4.2 µm etc.). Of course, these features must be tuned depending on the microplastic sizes of interest and also on the techniques that will be used to identify them.

Our focus for filter choice is on the microscopy techniques (FTIR, Raman and optical microscopy) which are the most commonly used and seem to provide the most complete microplastics picture allowing: Chemical identification (true for Raman and FTIR), counting (number and size distribution) and quantitative estimation (number and mass).

The most commonly used filters are: Borosilicate glass fibers, Alumina, Polycarbonate (un-coated and coated with various metal layers) and Silicon. The table below summarizes the pros and cons of each of them including: **optical quality** (for microscope visualization); **mechanical resistance and handleability**; **interference** for microplastic chemical identification with Raman and Infrared Microscopy; and price.

Table 5. Filters pros and cons (part 1)

Filter Type	Optical Quality	Handleability	Interference	Unit Price per filter (euro)
 <b>Borosilicate Glass Fiber</b> (no binder) available with different pore sizes (lowest 0.6 µm)	Rough surface can reduce ability to identify microplastics (most significant for small particles, below 10 µm). White membrane → low contrast for transparent plastics	No issue	Possible interference signals for Raman and Infrared Microscopy.	0,25 to 14 Depending on filter size (up to 257 mm in diameter available) and grade
 <b>Polycarbonate Uncoated</b> available with different pore sizes (lowest 0.2 µm)	Flat surface. White membrane → low contrast for transparent plastics	Issue in case of Alkali treatment (KOH)	Strong interference with Raman and Infrared Microscopy. Polycarbonate shows strong bands both in Raman and Infrared. Not usable for Transmission Infrared Microscopy	0,6 to 13 Depending on filter size (up to 142 mm in diameter available)





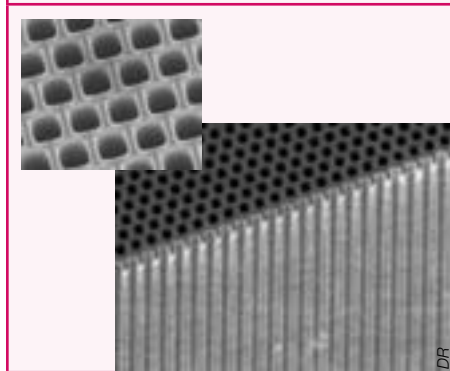
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Table 6. Filters pros and cons (part 2)

Filter Type	Optical Quality	Handleability	Interference	Unit Price per filter (euro)
	<b>Polycarbonate Coated</b> available with different pore sizes (from 0.2 to 5 µm) and different metal coating: gold,silver Flat surface and high relectivity and good contrast (Highly textured surface for Silver)	Issue with Alkali treatment (KOH)	Less interference than uncoated, but still present if metal is thin and for particles below 5 µm. Not useable for Transmission Infrared Microscopy	8 to 23 for silver and 18 to 30 for gold Depending on filter size (up to 47 mm in diameter available for both)
	<b>Alumina (Anodisc)</b> supported (surrounded by a polypropylene ring) and unsupported available with different pore sizes (from 0.02 to 0.2 µm) Flat surface. White membrane low contrast for transparent plastics	Highly fragile, careful handling required	Low interference for FTIR (peak intensity change over the filter) and for Raman (broad spectral feature) - Useable for Transmission Infrared Microscopy but no signal below 1250 cm <sup>-1</sup>	5 to 15 Depending on filter size (up to 47 mm in diameter available)
	<b>Silicon</b> with different pore sizes (from 1 to 18 µm) Flat surface. High relectivity and good contrast	Easy handling, possible fragility along crystalline direction. Squareshaped (dedicated holder needed)	Raman (silicon peaks do not interfere with plastic peaks) - FTIR (possible interference from silicon oxide) - Useable in Transmission Infrared Microscopy	14 to 24 Depending on volume

Regarding polycarbonate filters one research group<sup>1</sup> tried different metal coatings, not commercially available, and obtained very good results with Raman microscopy and Aluminum coated polycarbonate. Aluminum can enhance Raman scattering by a factor of 4<sup>2</sup>, thus improving detectability.

Alternatively, it is possible to use CaF<sub>2</sub> and/or ZnSe windows (usually with a diameter of 13 mm); these are not filters but windows widely used in Infrared and Raman microscopy. A solution of microplastics can be concentrated to few millilitres by evaporating the solvent and then it can be poured onto the window and left to dry before spectroscopic analysis.

A last important point, it is simple but is key to getting good results, is the amount of microplastics in the solution to be analyzed. The filter must not be tightly packed with material otherwise optical identification and further analysis of the particles will be complicated if they overlap. In this case just prepare a more dilute solution before filtration as was done in the literature<sup>3</sup>.

1. Oßmann et al., WATER RESEARCH 141 p.307 2018  
2. Kamemoto et al., APPLIED DPECTROSCOPY 64 p.255 2010  
3. Bergmann et al., ENVIRONMENTAL SCIENCE & TECHNOLOGY 51 p.11000 2017

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The last step following sampling, sample preparation and filtration is identification of the microplastic using one or more different techniques.

Five main techniques are used for this purpose:

- 1. Fluorescent staining with Nile Red, coupled with Fluorescence microscopy
- 2. Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX)
- 3. Infrared Microscopy
- 4. Raman Microscopy
- 5. Pyrolysis Gas Chromatography Mass Spectrometry (Pyr-GCMS)

They are complementary with each other but Infrared and Raman provide a more detailed picture. Raman being more flexible (all sizes of plastics can be analyzed) since it is able to detect microplastics below 10  $\mu\text{m}$ . These represent the main threat/concern for Human health (Ragusa et al.<sup>1</sup> observed plastic pieces below 10  $\mu\text{m}$  in the placenta).



### Nile Red staining/Fluorescence microscopy.

Nile Red is a fluorescent dye (see absorption curve, dashed line, and emission curve, below) widely used to localize and quantify lipids but it can also selectively bind to most plastics, allowing them to be identified by looking at the fluorescence in both the green as well as in the red.

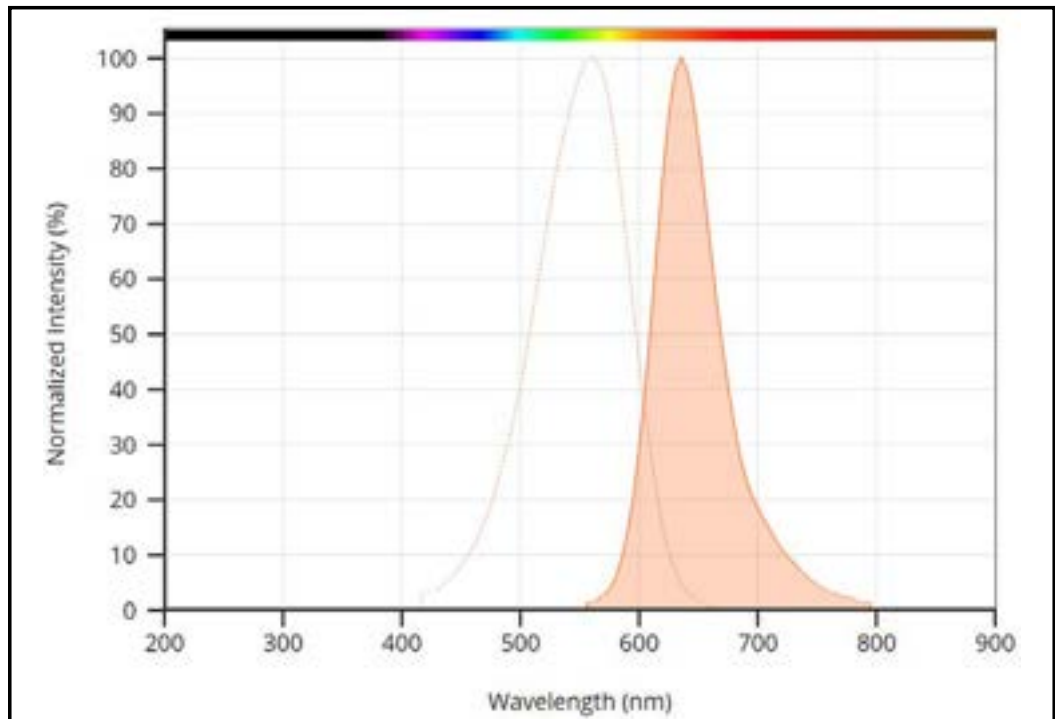


Fig. 6. Absorption curve (dashed line) and emission curve of Nile Red fluorescent dye

Since Nile Red also binds to lipids, environmental samples, careful sample preparation (complete digestion of the biogenic material<sup>2</sup>) is key to successful analysis, as the presence of biological residues can lead to an overestimation of the amount of microplastics.

For non-environmental samples such as bottled/tap water<sup>3</sup> where digestion is not needed Nile Red can be used directly. After staining microplastics can be easily and automatically counted by looking at the green or red colored particles with a fluorescence microscope. The best approach, as demonstrated in the literature<sup>2</sup>, is to use green / yellow fluorescence (excitation/emission 450–490/515–565 nm) as this avoids natural lipids which emit deeper into red (higher wavelength). A Nile red concentration of ranging from 0.1 and 2 µg/mL is typically used.

*The advantage of this technique is speed and simplicity, the main drawbacks are the lack of chemical identification of the polymer types and the possible presence of false positives. Erni-Cassola et al.<sup>3</sup> validated this method by also using Raman microscopy, which notwithstanding the presence of the dye, can be still used, to chemically identify the polymer.*

### SEM-EDX.

Scanning Electron Microscopy stand alone allows the complete morphological characterization of the particles down to the nanometer range (which is a strong advantage in comparison to the other techniques) but it is not able to provide chemical information and samples, in almost all cases (Fries et al.<sup>4</sup>), must undergo to additional treatments due to the high vacuum in the test chamber and to avoid charge accumulation. Moreover, the filters suitable for Infrared and Raman microscopy cannot be used, instead the microplastics must be dried and then transferred onto double-sided adhesive carbon tabs on aluminum SEM stubs.

SEM combined with the energy dispersive X-ray spectroscopy can give additional information by providing the elemental composition of the sample. Elemental information allows:

- Inorganic and carbon-based material to be distinguished (the full digestion of organic contaminants is essential to assign carbon-based material to plastic);
- Some polymer types to be identified, such as PVC due to the presence of Chlorine<sup>5</sup>;
- Identification of the presence of other elements<sup>6</sup> such as Al, Ca, Mg and Si on the plastics which can be the signature of polymer additives.

Summarizing, SEM-EDX is a technique which unlike can provide detailed morphological information down to nanometer range (morphology may influence the diffusion of microplastics within the human body) but it cannot be used alone as it does not provide comprehensive chemical information.

### Infrared Microscopy.

Infrared microscopy is currently the most widely used technique for microplastic analysis and all the commercial instruments are also combined with optical microscopy. In some instruments the optical microscopy is limited by the use of infrared objectives only, which cannot provide the flexibility and high magnification of standard visible objectives and this limits the identification of small particles.

Infrared microscopy is a non-destructive technique and can provide morphological information (by the analysis of the optical and/or chemical image), quantitative analysis (in terms of number of particles) and chemical identification of the microplastic (by comparing the collected infrared spectra with the ones in commercial libraries). Like Raman microscopy one of the most complete techniques. The main drawback/limitation of Infrared microscopy is its inability to identify particles smaller than 10 µm. Zhu et al.<sup>7</sup> in a recent review of June 2020 mentions that the smallest particle size determined with infrared microscopy is 20 µm.



There are two main approaches to analyze microplastic with infrared and both start with an optical image acquisition of the filter used in the filtration step:

1. In the first approach, the particles' size, shape and their location on the filter are determined by analyzing the optical image, the location is then used to individually measure each particle by moving the infrared beam to the required location;
2. In the second approach, the particles' size and shape are determined by the analysis of the optical image and then the whole filter is chemically imaged with the infrared beam (This approach collects many spectra so needs a specialized detector such as a Focal Plane Array (FPA) or a linear array, to reduce the acquisition time as much as possible). The size and shape of the particles can also be determined by analyzing the chemical image, but accuracy can be limited by the resolution of the image.

### Raman Microscopy.

Raman microscopy is the second most common technique and like Infrared it also includes standard optical microscopy. Raman instruments use visible objectives that are available with a full range of magnifications, so image quality and morphological information is uncompromised, Raman microscopy is a non-destructive, non-contact technique that provides morphological information (by analyzing the optical image), quantitative analysis (number of particles) and chemical identification of the microplastic (by comparing the collected Raman spectra with the ones in commercial libraries).

The biggest advantage of Raman microscopy compared to infrared is the ability to measure and identify particles of 1  $\mu\text{m}^8$  and below in size. This point is crucial since the biggest concern for human health seems to come from particles below 10  $\mu\text{m}$  because they can migrate within our body<sup>1</sup>.

One drawback of Raman microscopy is the interference caused by fluorescent materials such as environmental and/or plastic pigments, additives and pollutants; fluorescence which can overlap with the Raman spectrum, limiting the ability to identify the microplastic. The presence of fluorescent material does not always limit plastic identification (Enri-Cassola et al.<sup>2</sup> successful measured Nile red stained particles) and additionally several excitation wavelengths are available to assist avoiding fluorescence interference (the use of a near infrared excitation source, i.e. 785 nm, often limits the fluorescence signal).

The main approach to analyze microplastics with Raman microscopy is:

*First step is the acquisition of an optical image of the filter from which the particles' size, shape and location are determined; second step, is to move the laser beam to each identified location, and acquire a Raman spectrum of each particle.*

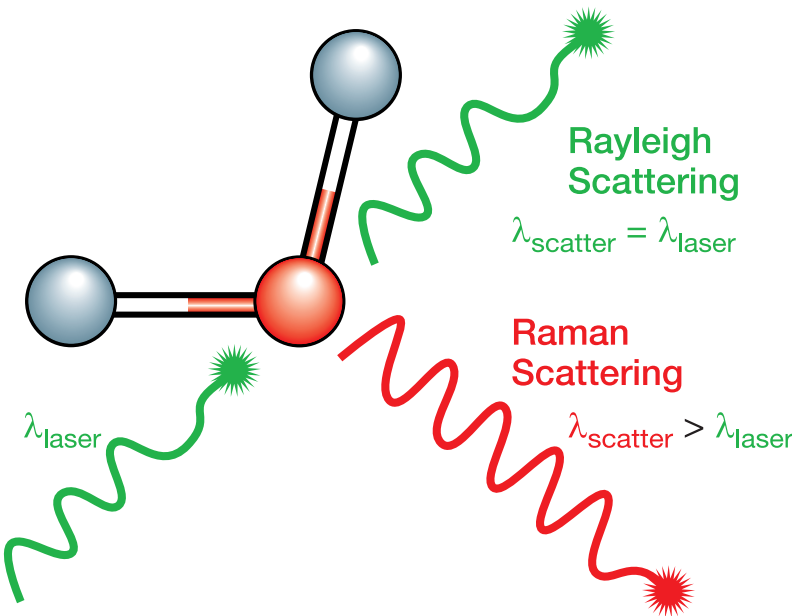


Fig. 7. Raman principle

# Analysis Workflow

## Measurement methodologies

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**Pyr-GCMS.**

Pyrolysis combined with Gas Chromatography Mass Spectrometry can determine the chemical composition of the microplastic by analyzing their pyrolysis products (Pyrograms). Similar to Infrared and Raman, which use spectral libraries for chemical identification, the pyrograms obtained are compared with reference ones of known polymers. The chemical identification is not as detailed as for vibrational spectroscopy techniques, in particular, the polymer<sup>9</sup> sub-type (such as Low density vs. High density polyethylene) cannot be discriminated and, in case of complex matrices, the identification can be misleading.

The main advantages of Pyr-GCMS are the quantitative analysis of Microplastic in terms of weight per polymer type for polymers which exceed the quantification detection threshold, and the low amount of material needed (5 µg can be enough) although this small quantity may not be representative for complex environmental matrices.

The drawbacks of Pyr-GCMS are:

- Destructive technique: Samples cannot be re-analyzed;
- Lack of information on particle morphology: size and shape, which are well known to influence the risk assessment of microplastics;

Table 7. In the table, we have summarized the main advantages and disadvantages of the different techniques.

Technique	Nile Red & Fluorescence Microscopy	Scanning Electron Microscopy & Energy Dispersive X-ray Spectroscopy (SEM-EDX)	Infrared Microscopy	Raman Microscopy	Pyrolysis Gas Chromatography Mass Spectrometry (Pyr-GCMS)
Pros	Fast and Simple  Low-cost  Morphological information  Quantitative analysis (Number of Particles)	Particle down to nanometer size  Discrimination between Inorganic and carbon-based material  Elemental analysis	Non destructive  Morphological information  Chemical identification  Quantitative analysis (Number of Particles) and Quantitative per polymer type	Non contact and non destructive  Morphological information  Chemical identification  Quantitative analysis (Number of Particles) and Quantitative per polymer type  Particles down to 1 micron and below	Quantitative analysis (weight of particles)  Partial Chemical identification
Cons	False positives  No chemical identification  No Quantitative analysis per polymer type	Additional preparation needed  No chemical identification  High cost	Sensitive to particle dimension (bigger particles cannot be analyzed in transmission)  Smaller particles (<10 micron) cannot be analyzed	Interference by fluorescent material	No Morphological information  Destructive

1. Ragusa et al., ENVIRONMENT INTERNATIONAL 146 p.1 2021

2. Erni-Cassola et al., ENVIRONMENTAL SCIENCE & TECHNOLOGY 51 p.13641 2017

3. Mason et al., FRONTIERS IN CHEMISTRY, 6 (article 407) p.1 2018

4. Fries et al., ENVIRONMENTAL SCIENCE PROCESSES & IMPACTS 15 p.1949 2013

5. Wang et al., SCIENCE OF THE TOTAL ENVIRONMENT 603-604 p.616 2017b

6. Dehghani et al., ENVIRONMENTAL SCIENCE POLLUTION RESEARCH 24 p.20360 2017

7. Zhu et al. ANALYTICAL METHODS 12 p.2944 2020

8. Oßmann et al., WATER RESEARCH 141 p.307 2018

9. Dehaut et al., ENVIRONMENTAL POLLUTION 215 p.223 2016a

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After considering the optimal microplastic analysis workflow and the needs and challenges of the people approaching this topic. HORIBA has developed a full solution to for this application that will help our existing and future customers by providing all the tools needed in a single bundle:

1. This **Booklet**, which will be updated with new releases twice a year to keep you up to date on microplastic (new protocols, new regulations etc.)
2. **Filtration apparatus**
3. **Filters & Filter Holder**
4. **Raman microscope** stand alone (with detector options available) or coupled with a Fluorescence microscope in a single instrument
5. **Video Raman Matching** with GPS like technology to reliably locate particles on the filter
6. **Reference standard sample** to validate your analysis
7. **ParticleFinder™**: Fully automated particles analysis software which allows
  - Viewing and locating the particles from the optical images
  - Characterization by size/shape
  - Analysis using Raman microscopy
  - Chemically identification using dedicated libraries
  - Result reporting with statistics on shape, size etc.
8. **KnowItAll**. Software for spectral identification with a dedicated polymer library built-in that includes over 150 spectra.

The provided KnowItAll HORIBA edition includes advanced capabilities (Multicomponent search etc.) with the additional ability to build your own library.

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## Filtration apparatus

Included in the starter kit are:

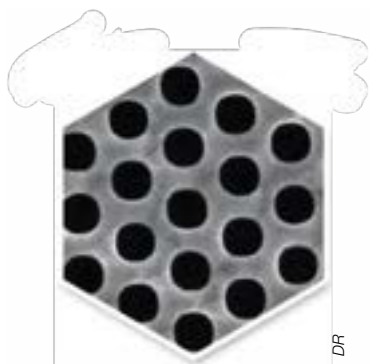
Three way Stainless Steel Manifold

- 100 ml Glass funnel
- Sintered glass support with
- 13 mm filtration area
- Silicone stopper
- 1L Borosilicate glass flask
- Diaphragm vacuum pump



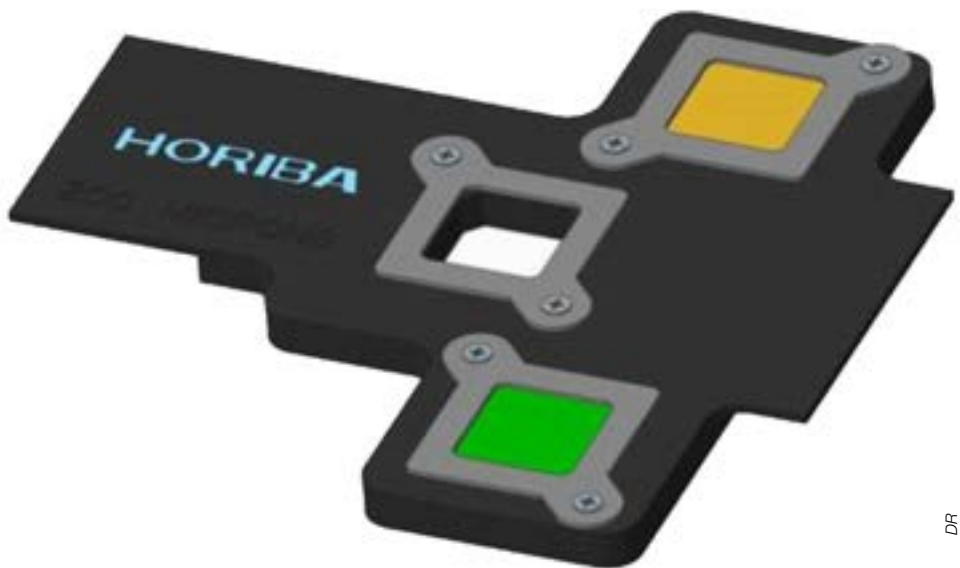
## Filters & Filter holder

The filters we have selected for this microplastic starter kit are square shaped Silicon filters from SmartMembrane (<http://www.smartmembranes.de/en/>)



Filter size	10x10 mm <sup>2</sup>
Pore diameter	2.5 micron
Interpore distance	4.2 micron
Filter thickness	500 micron

Together with a box of 25 filters we provide a filter holder specifically developed to hold them. The holder has places for 3 filters (picture below), allowing efficient use of the system as up to 3 filters can be analyzed without needing the user to be present to change the sample.





## Raman microscope

Two platforms are available:



**XploRA™ PLUS**

Both Raman microscopes can be equipped with a standard detector (CCD - Charge Couple Device) or an Imaging detector (EMCCD - Electron Multiplied Charge Couple Device). For microplastic analysis using our dedicated ParticleFinder™ software our standard detector is the perfect choice, providing excellent performance for this application in a cost-effective package. For multipurpose systems that will acquire large maps, the imaging detector may reduce measurements times for these maps, while not degrading microplastic analysis performance.

All platforms/configurations include 5X, 20X, 50X-LWD (Long Working Distance) and 100X-LWD objectives and two excitation laser lines: 532 nm and 785 nm.

**LabRAM Soleil™**



The XploRA™ PLUS is also available with Fluorescence illumination to combine two measurement methodologies widely used for microplastic analysis: Nile Red staining/Fluorescence microscopy and Raman microscopy. Nile Red can be the fast-screening technique for particle counting and shaping and Raman can validate the results by chemically identifying a subset of the particles: We added the 785 nm excitation laser to optimize the collection of Raman spectra on Nile Red stained particles. It is also useful to reduce fluorescence interference from organic contamination.

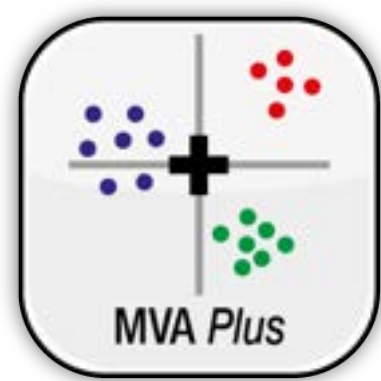
To ensure full flexibility, Fluorescence illumination is provided with band-pass excitation filter from 460-490 nm and emission in the green (515-555 nm), where plastics fluoresces more selectively (as mentioned in the “Measurement Methodologies” session), and with an excitation filter at 535 nm with emission in the red (610 nm).

To complete the visual configuration both Raman microscopes are equipped with at least a 5 megapixel 5MP CMOS video camera, upgraded to a 6 megapixel version with the Fluorescence option. The LabSpec 6 software platform is common to both systems and provides complete instrument control, data processing and this microplastic package also includes, in addition to ParticleFinder™ and KnowItAll which will be described in detail later, a number additional tools:

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## MVAPlus



Multivariate Analysis module which includes a number of multivariate (chemometric) methods, providing additional tools for data analysis such as PLS (Partial Least Square), CLS (Classical Least Square), PCA (Principal Component Analysis), MCR (Multivariate Curve Resolution) and Cluster Analysis.

## ViewSharp™ and NavSharp™



ViewSharp and NavSharp which provide a clear view of the sample's surface and guarantees the highest focal quality in Raman Images (autofocusing during Raman collection) and particle images. It provides a topography image and allows 3D chemical visualization.

## Video Raman Matching (VRM) with nano-GPS Technology

Video Raman Matching is the perfect tool to reliably move to your particles even when transitioning from low to high magnification (do the mosaic with a 10x and measure the particle confidently with higher magnification). It also perfectly matches the chemical information and the visible image. VRM technology is based on GPS technology, a patented tag allows locations to be identified and to accurately position the sample and /or particles. This technology is the HORIBA gateway to Correlative Microscopy.

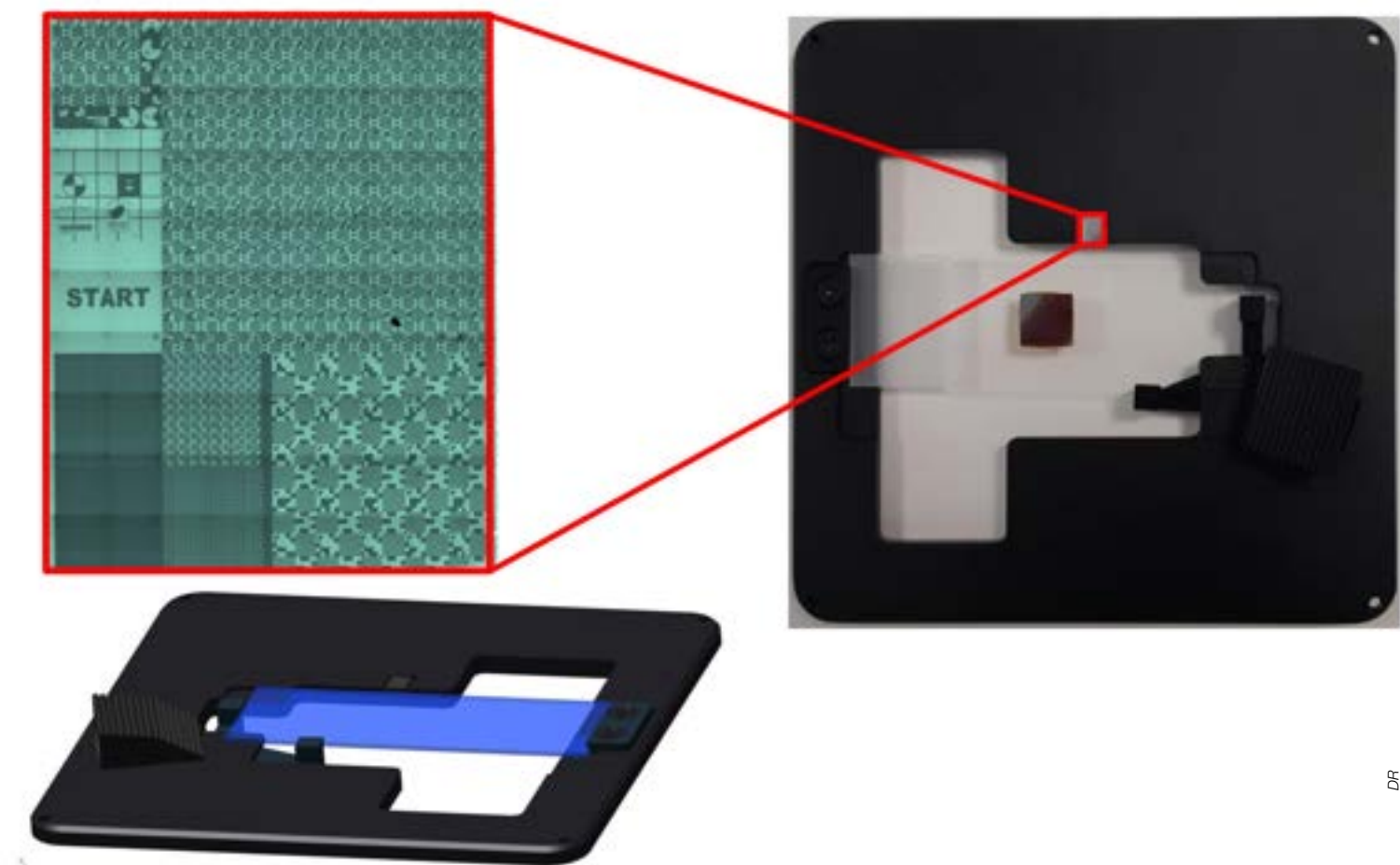


Fig. 8. Video Raman Matching (VRM)

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## Reference standard sample

As a part of samples of microplastics in order to confidently approach the microplastic analysis and to validate your workflow and your lab environment. The standards are delivered in tablet form with a detail receipt and instruction on handling and usage.

The standards are prepared by the Norwegian Institute for Water Research (NIVA); NIVA is the Norway’s leading institute for fundamental and applied research on marine and freshwaters and was founded back in 1958. NIVA has an extensive experience in the microplastic monitoring and evaluation.

The tablets contain a well defined individual microplastic polymers or a polymer mixture (Polyethylene, Polystyrene, Polyvinyl Chloride and Polyethylene Terephthalate) ranging from 50 to 355 µm. HORIBA’s full solution will provided also reference standards in micron size.

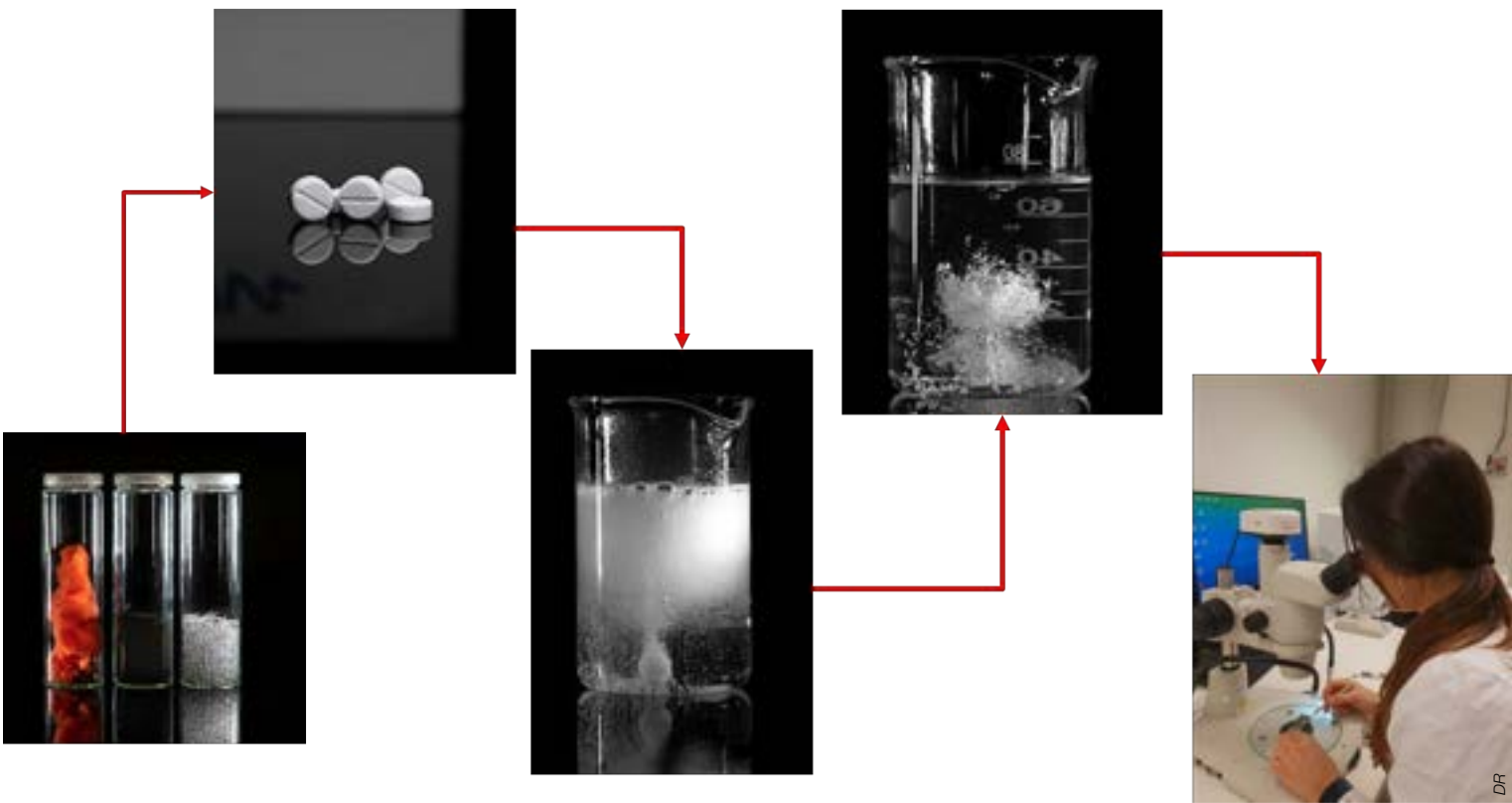


Fig. 9. Reference standard sample preparation

### ParticleFinder™

This software app is described in a dedicated section



### KnowItAll – Building Library

This software app is described in a dedicated section





**ParticleFinder™** is the perfect software solution for particle analysis. It provides a step-wise, automated routine to locate, characterize and chemically identify, through Raman analysis, particles. ParticleFinder™ is ideal to analyze microplastics but can also be used for other environmental applications (e.g. Particulate analysis) and also for pharmaceutical applications (powder dispersion) and in any field where chemical and morphological characterization of particles is needed.

ParticleFinder™ routine has 6 consecutive steps:

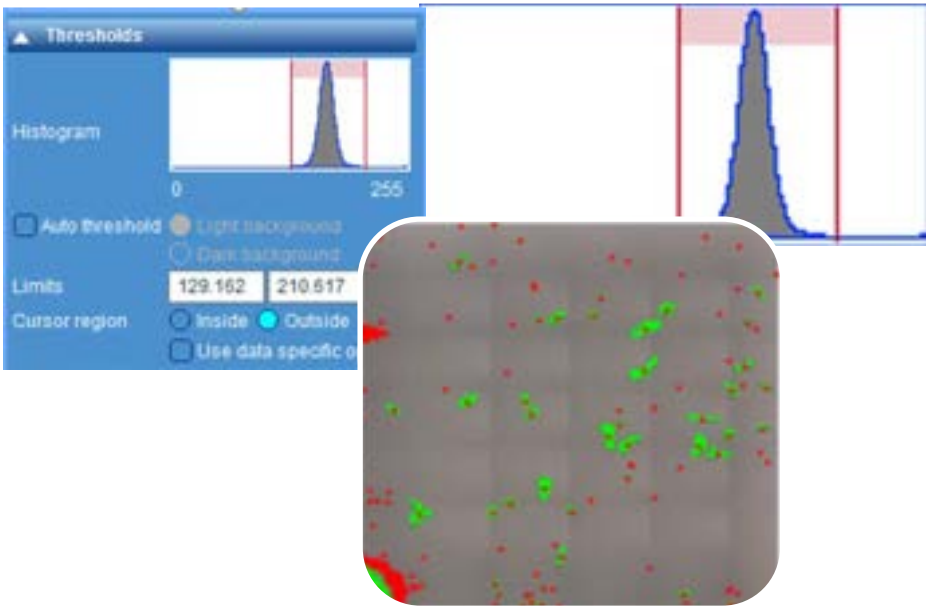
**Image acquisition** (Single field of view) or Mosaic

**Image acquisition**  
Single image, or  
montaged or  
mosaic wide field  
of view image



**Threshold & Process** Auto-thresholding with dark or light backgrounds or manual-thresholding and processing such as removing touching particles, filling holes etc.

**Process**  
Erode/dilate/open/  
close/majority, fill  
holes, remove  
edge particles



**Characterize & Locate** Full morphological characterization of the particles (x-y position, diameter, perimeter, major and minor axis, aspect ratio, circularity, brightness)

**Characterize**  
Position, size,  
shape, circularity,  
brightness and  
much more

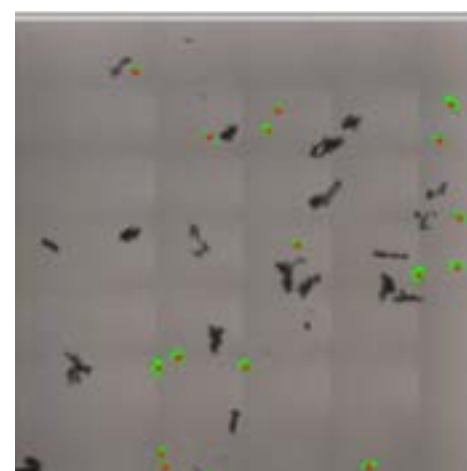




**Select** Define the particles to be chemically analyzed using any of the available morphological information. Selection can also be done first, before image acquisition.

## Select

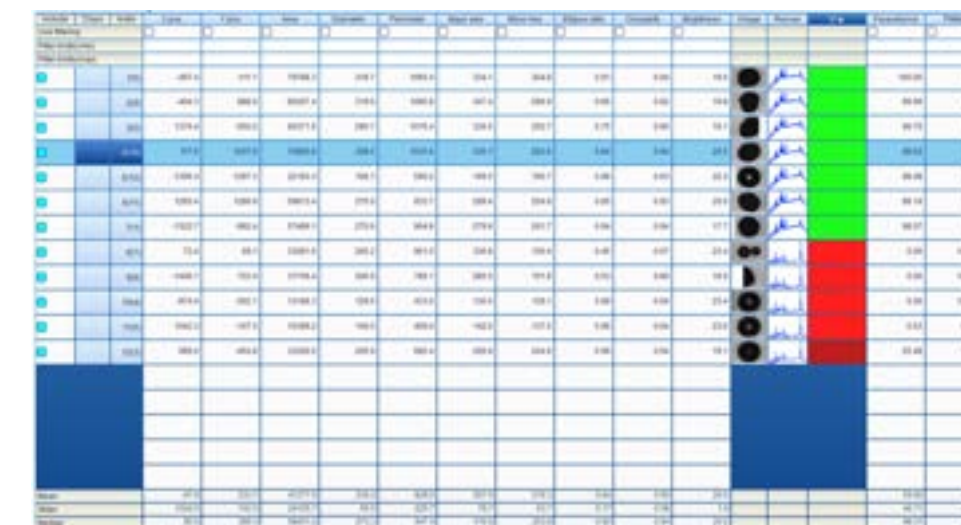
Screen particles  
based on position,  
size or shape



**Report and Chemically identify.** Several reporting options are available along with chemical identification by means of KnowItAll and the HORIBA Microplastic library.

## Identify and Report

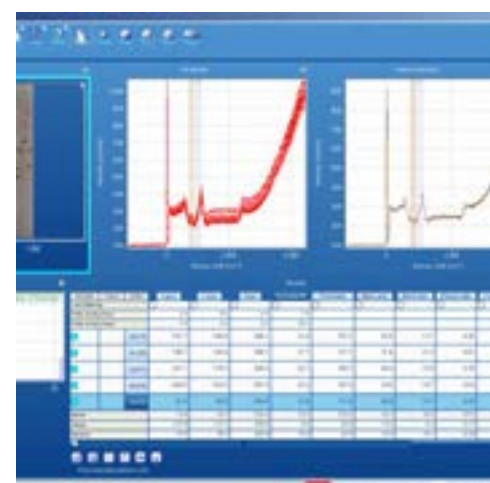
Chemically identify  
each particle by  
dedicated libraries



**Acquire Raman** Collect a Raman spectrum for each particle. It is possible to: a) Do a single spectrum; b) Do multiple spectra and averaging them to get one; c) Do small maps to get full picture of big particles.

## Acquire Raman

Automatically  
analyze/identify  
each particle



Moreover, ParticleFinder™ can be used with 2 different approaches to maximize performance in any laboratory condition - Static mode or Dynamic mode.

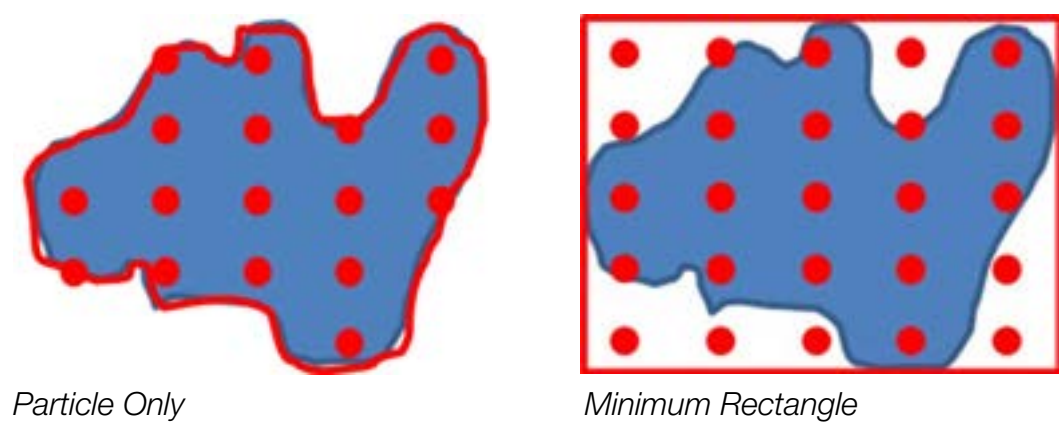
In static mode an image is acquired, which can be a single Field Of View FOV (the size of the FOV depends on the magnification of the objective) or a mosaic (where several tiles, i.e. single images, are stitched together to create a wider image of your sample), after imaging these are processed to characterize the particles' shape/size/location, as described above, and analyzed to chemically identify the particles. Summarizing the Static Mode works by following the 6 steps in order: 1 → 2 → 3 → 4 → 5 → 6 or, if selection is done first, 4 → 1 → 2 → 3 → 5 → 6.

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*In Step 5, Raman Acquisition, we would like to add some additional information on the acquisition options available for each particle to allow you to appreciate the extreme flexibility of ParticleFinder™.*

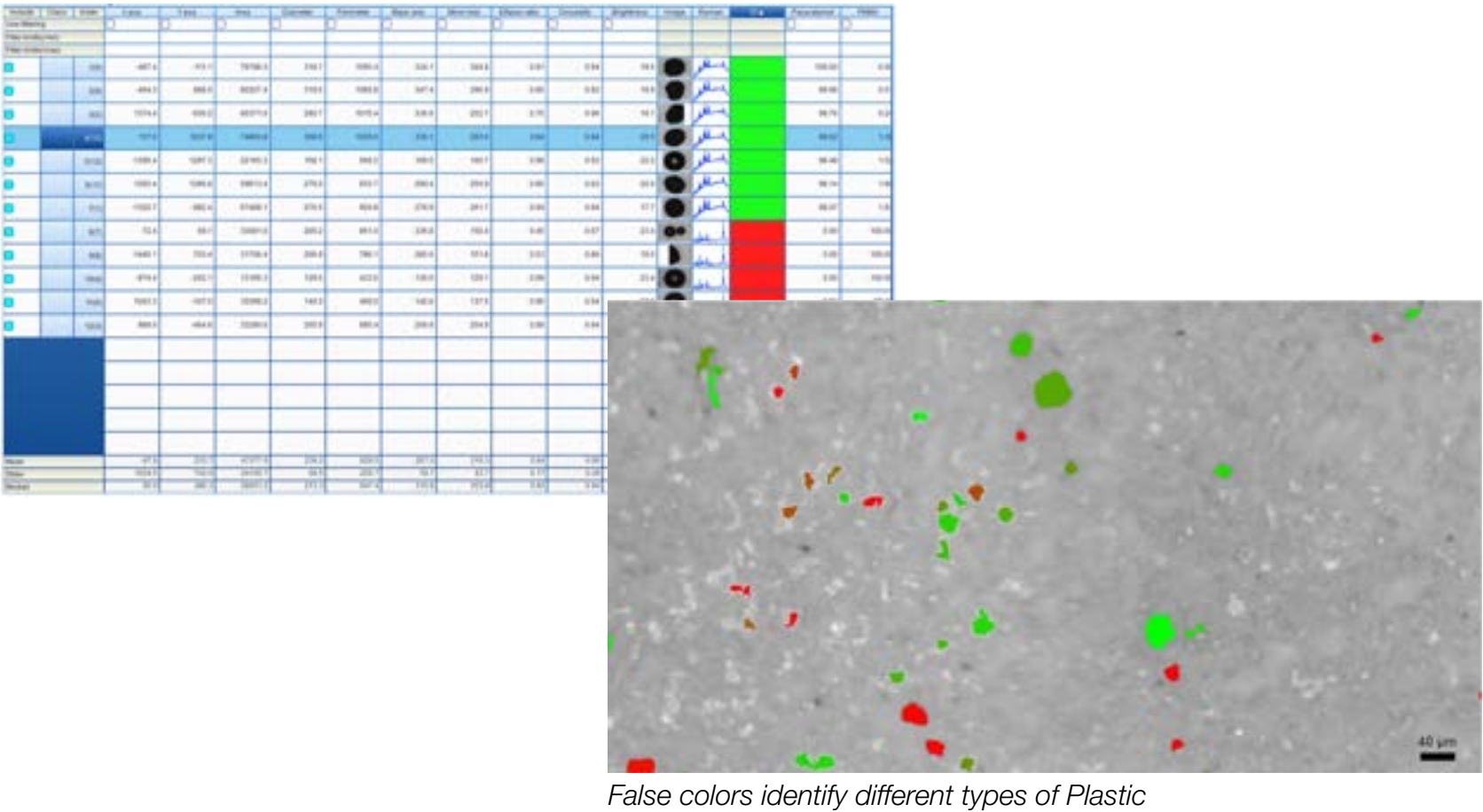
Each particle can be identified by collecting a single spectrum, several spectra (a matrix of points 2X2, 3X3 etc. can be defined) and averaging them, a small map which can be “Particle only” or “Minimum rectangle” as depicted in the picture below.



In Dynamic mode, to reliably analyze your particles even in tough conditions (instability due to the bench, laboratory environment etc.), for mosaics (multiple tiles) the software does not acquire the full image but completes the full routine on each tile. So firstly we select the particles of interest (step 4) for example all particles in the range 1 to 20 µm) and the required thresholding/processing (for steps 2/3) are setup just once (it does not need to be done for each tile individually). Then the first tile (single image) is acquired

(step 1), it is then processed (step 2), the particles characterized (step 3) and have Raman spectra/maps taken of them (step 5). The software then moves to the next tile and the process (steps 1 → 2 → 3 → 5) is automatically repeated. The particles are then characterized and the data reported (step 6).

Chemical identification of the particles can be also visually represented by false coloring the results table and map by plastic type: Each color is assigned to a different material (PE, PP, PET, etc.)





## ParticleFinder™

The best solution for **Microplastic identification** with HORIBA instruments (XploRA™ PLUS and LabRAM Soleil™)

- Automatic Workflow
- Intuitive Interface
- Maximum Flexibility



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# Applications

## Marine Water

As application example to show the full workflow of ParticleFinder™ software we used a marine water sample. All the data are collected by using the LabRAM Soleil™.



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## Image acquisition

The mosaic image is acquired with a 10x objective by using the flatfield correction in order to avoid the stitching effect that could affect the particles selection. The Raman spectra are acquired with a 50x long working distance objective.

Our VRM (Video Raman Matching) coupled with nanoGPS technology allow us a fast and precise calibration enabling multi-magnification Raman Imaging to optimize your analysis time.

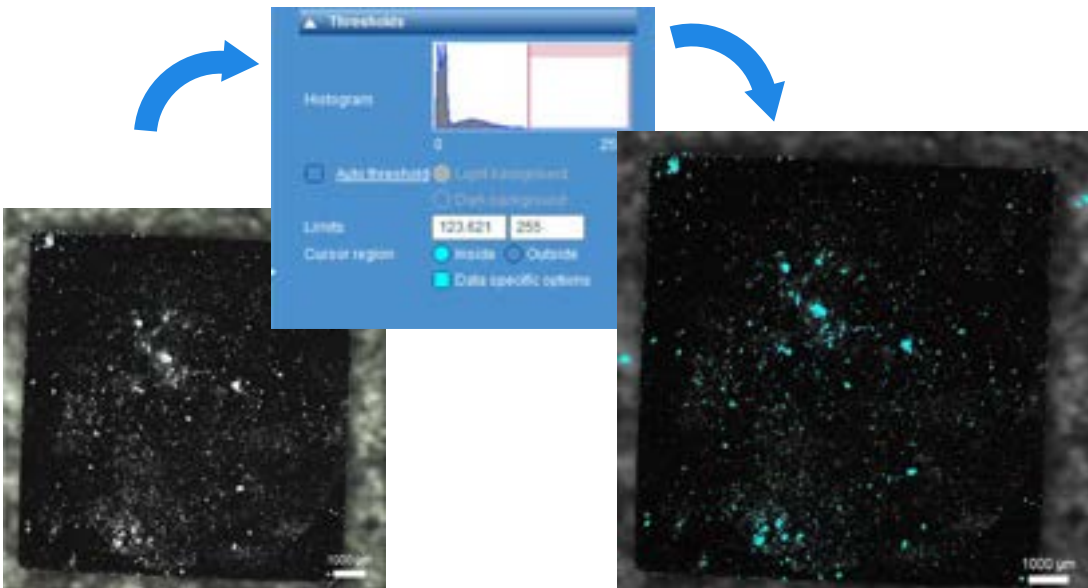
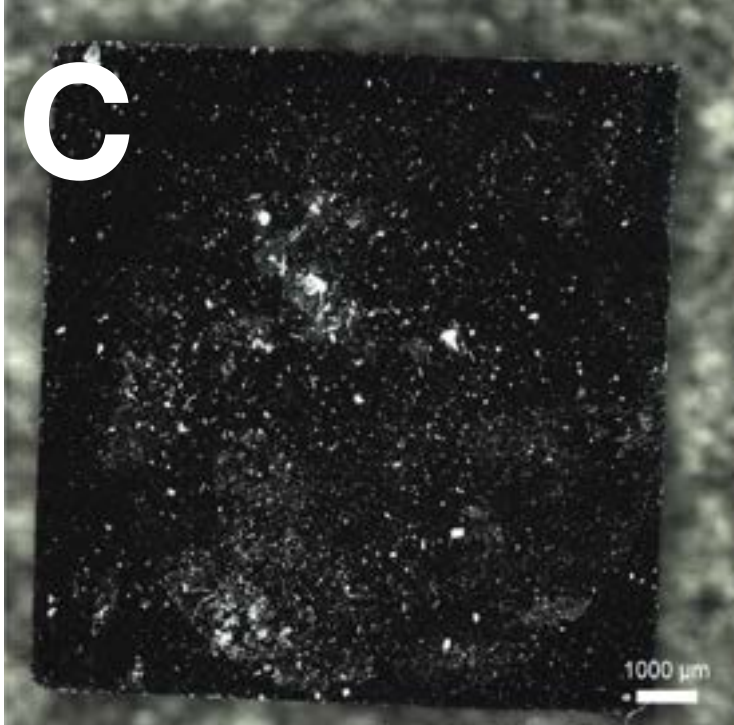
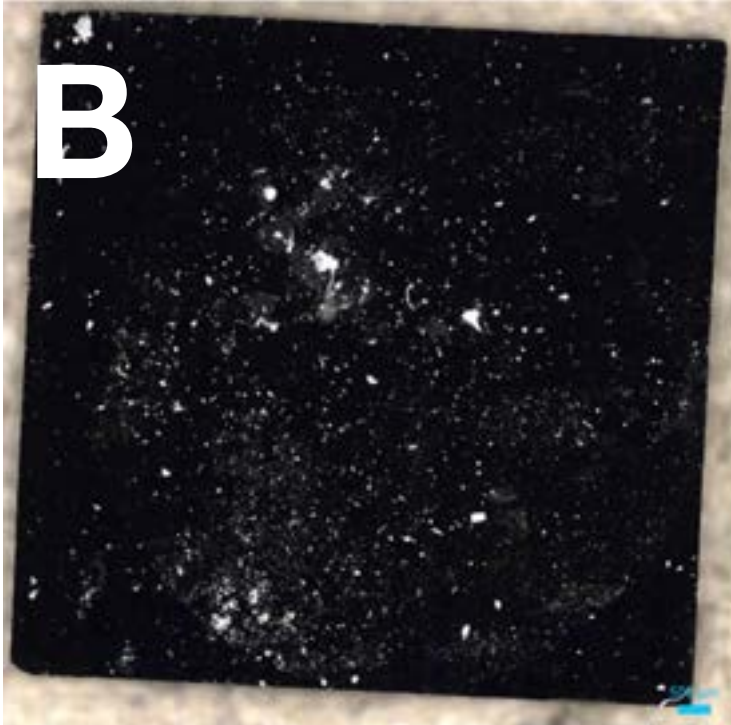
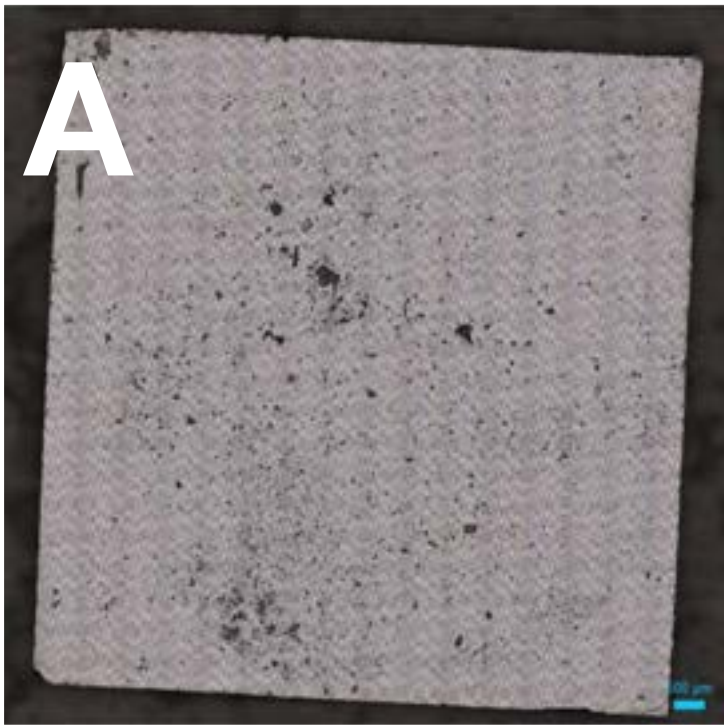
*The sample was pre-treated with KOH to remove the organic residues and a silicon filter with pore size of 1 micron was used for the filtration step.*

In picture A,B and C we collected the same optical image in three different modes: A. Brightfield mode; B, Brightfield mode with visible polarizer in cross-polarization; C, Darkfield mode.

The flexibility of our platforms (XploRA™ PLUS and LabRAM Soleil™) in term of optical imaging to visually identify Microplastic and thus correctly measure them is a key aspect. For this sample we selected the Darkfield image mode (highlighted in yellow).

## Threshold and Process













Threshold & Process step allow the visual selection of Microplastic. The selected Microplastic particles are highlighted in the picture on the right.





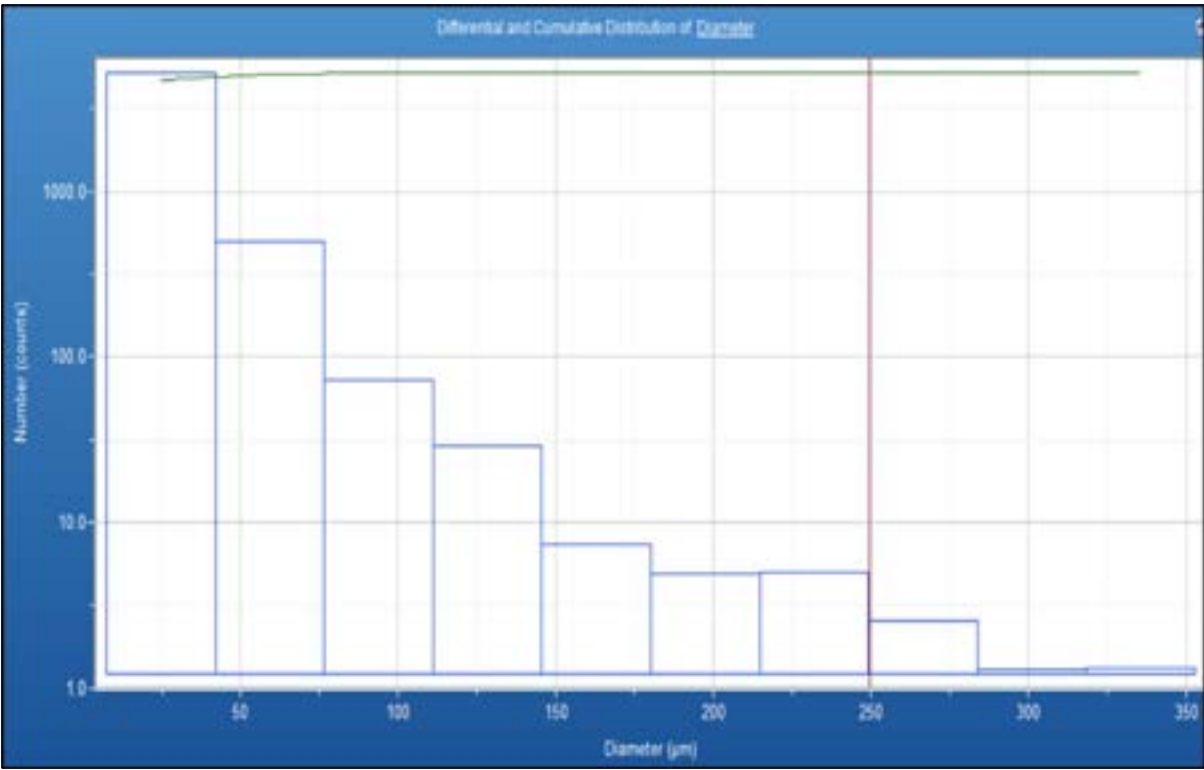
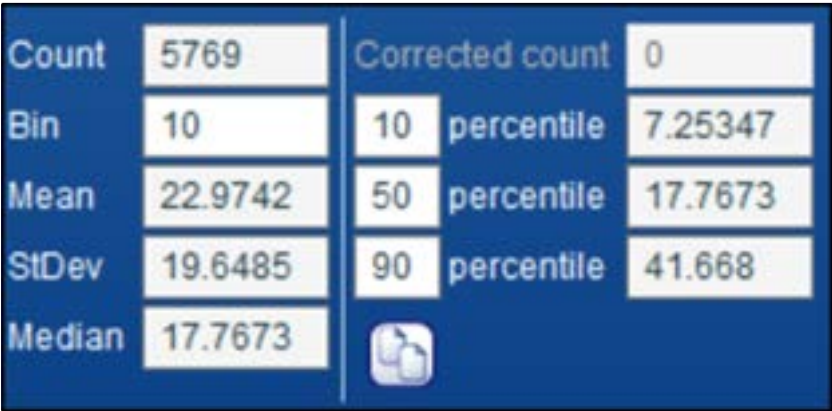
Characterize and Locate

A table is generated after the particles selection where particles and their morphological characteristics (such as area, circularity, diameter etc...) are listed.

Include	Class	Index	X pos	Y pos	Area	Diameter	Perimeter	Major axis	Minor Axis	Ellipse ratio	Circularity	Image
Use filtering												
Filter limits(min)			0.0	-4401.6	7.0	8.0						
Filter limits(max)			0.0	5939.3	8.0	10.0	0.0					
<input checked="" type="checkbox"/>		1527(5403)	-1462.0	5149.1	237.6	17.4	64.7	19.7	15.4	0.78	0.84	
<input checked="" type="checkbox"/>		1528(3590)	-3126.6	2951.8	237.6	17.4	60.2	21.6	14.0	0.65	0.91	
<input checked="" type="checkbox"/>		1529(1511)	3222.1	-75.5	309.9	19.9	64.7	21.0	18.7	0.89	0.96	
<input checked="" type="checkbox"/>		1530(2679)	-210.6	879.6	309.9	19.9	67.4	22.0	18.3	0.83	0.93	
<input checked="" type="checkbox"/>		1531(4037)	138.5	3549.0	144.6	13.6	55.7	16.7	11.0	0.66	0.77	
<input checked="" type="checkbox"/>		1532(114)	2906.4	-4052.0	712.8	30.1	113.2	44.5	20.6	0.46	0.84	
<input checked="" type="checkbox"/>		1533(5088)	-1653.6	4805.4	1952.5	49.9	200.6	55.8	48.7	0.87	0.78	
<input checked="" type="checkbox"/>		1534(543)	-3989.8	-2465.9	124.0	12.6	51.1	14.2	10.8	0.76	0.77	
<input checked="" type="checkbox"/>		1535(961)	-2788.5	-1367.6	454.5	24.1	85.9	33.0	17.7	0.54	0.88	
<input checked="" type="checkbox"/>		1536(1400)	-2766.0	-276.0	836.8	32.6	137.0	55.1	22.3	0.40	0.75	
<input checked="" type="checkbox"/>		1537(1062)	-2438.0	-1011.4	433.9	23.5	82.1	29.3	18.9	0.65	0.90	
<input checked="" type="checkbox"/>		1538(5744)	2870.7	5708.9	454.9	24.3	82.1	29.2	20.2	0.69	0.93	
Mean			-1000.4	1642.7	717.6	23.0	92.4	29.7	19.3	0.76	0.84	
StDev			2528.1	2638.1	2682.6	19.6	116.7	32.2	16.6	0.19	0.11	
Median			-1902.8	1928.2	247.9	17.8	64.7	20.5	15.5	0.81	0.87	

The majority of Microplastic particles are in the range between 0 to 40 microns underlying even more the importance of a tool like Raman Microscopy which allows to analyse particles down to the sub-micron range.

This picture shows the overall number of particles counted by Particle Finder™ for this marine sample: 5769.



The histogram shows the size distribution of the 5769



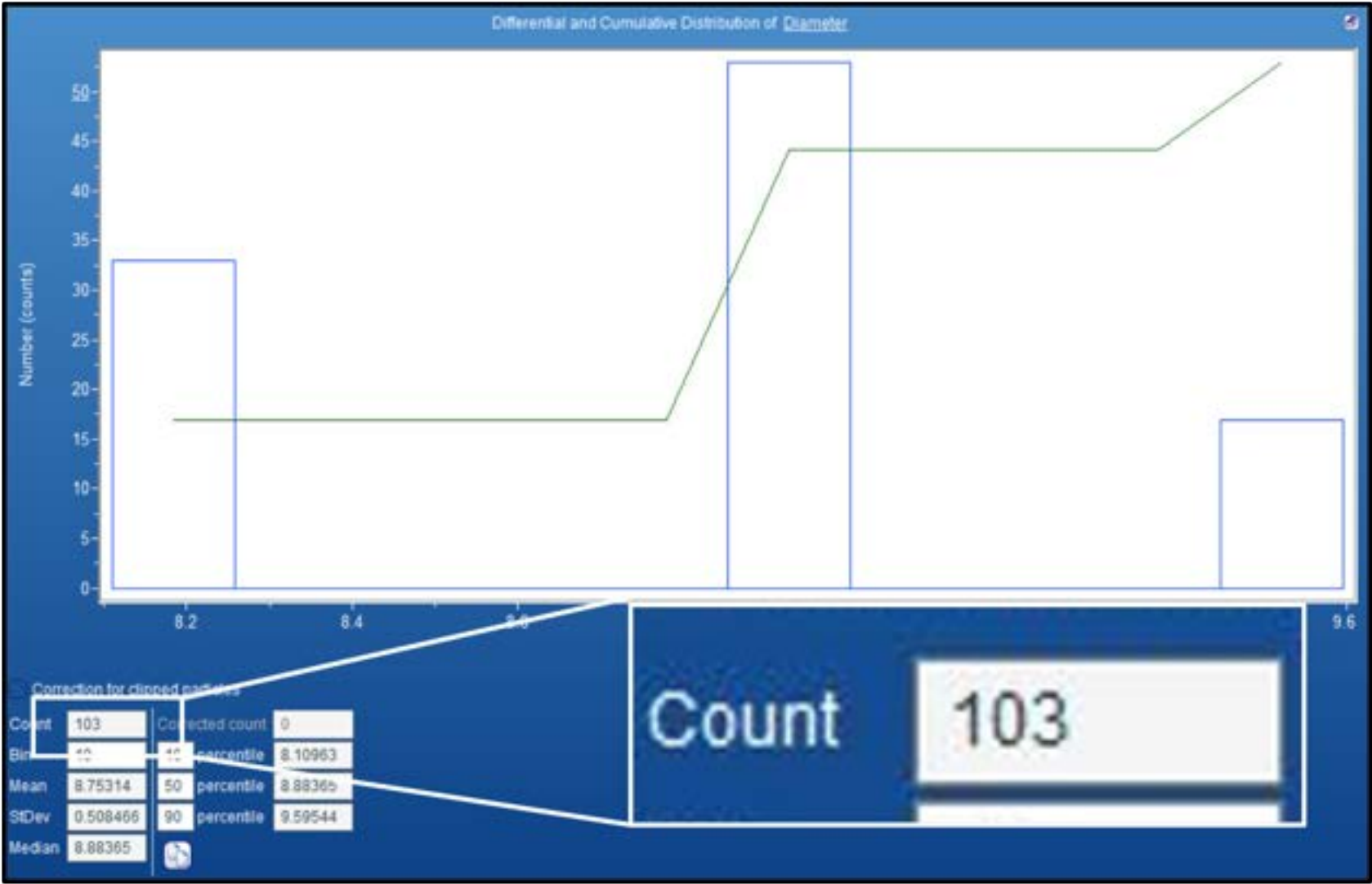
Select

To show the high flexibility of ParticleFinder™ we select two particles size range to be chemically identified with Raman for this sample :

- Particles in the size range 8 to 10 microns;
- Particles in the size range 7 to 15 microns.

The overall number of particles are 103 and 1807 respectively for the different size ranges.

Results							
Include	Class	Index	X pos	Y pos	Area	Diameter▼	Perimeter
Use filtering			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Filter limits(min)						8.0	
Filter limits(max)						10.0	
<input checked="" type="checkbox"/>	4	703			51.7	8.1	38.8
<input checked="" type="checkbox"/>	4	704			51.7	8.1	38.8
<input checked="" type="checkbox"/>	4	705			51.7	8.1	27.3
<input checked="" type="checkbox"/>	4	706			51.7	8.1	43.3
<input checked="" type="checkbox"/>	4	707(5230)	-1423.9	4952.5	51.7	8.1	38.8
<input checked="" type="checkbox"/>	4	708(4493)	-2667.0	4047.2	51.7	8.1	38.8
Mean			-1230.8	1849.9	60.4	8.8	44.9
Stdev			2821.0	2569.4	7.0	0.5	8.3
Median			-1382.7	2034.9	62.0	8.9	49.8



The particle size range can be easily selected by typing the desired number in the table (picture on the left for 8 to 10 micron).

The histogram below is providing the number of particles according to their size distribution.

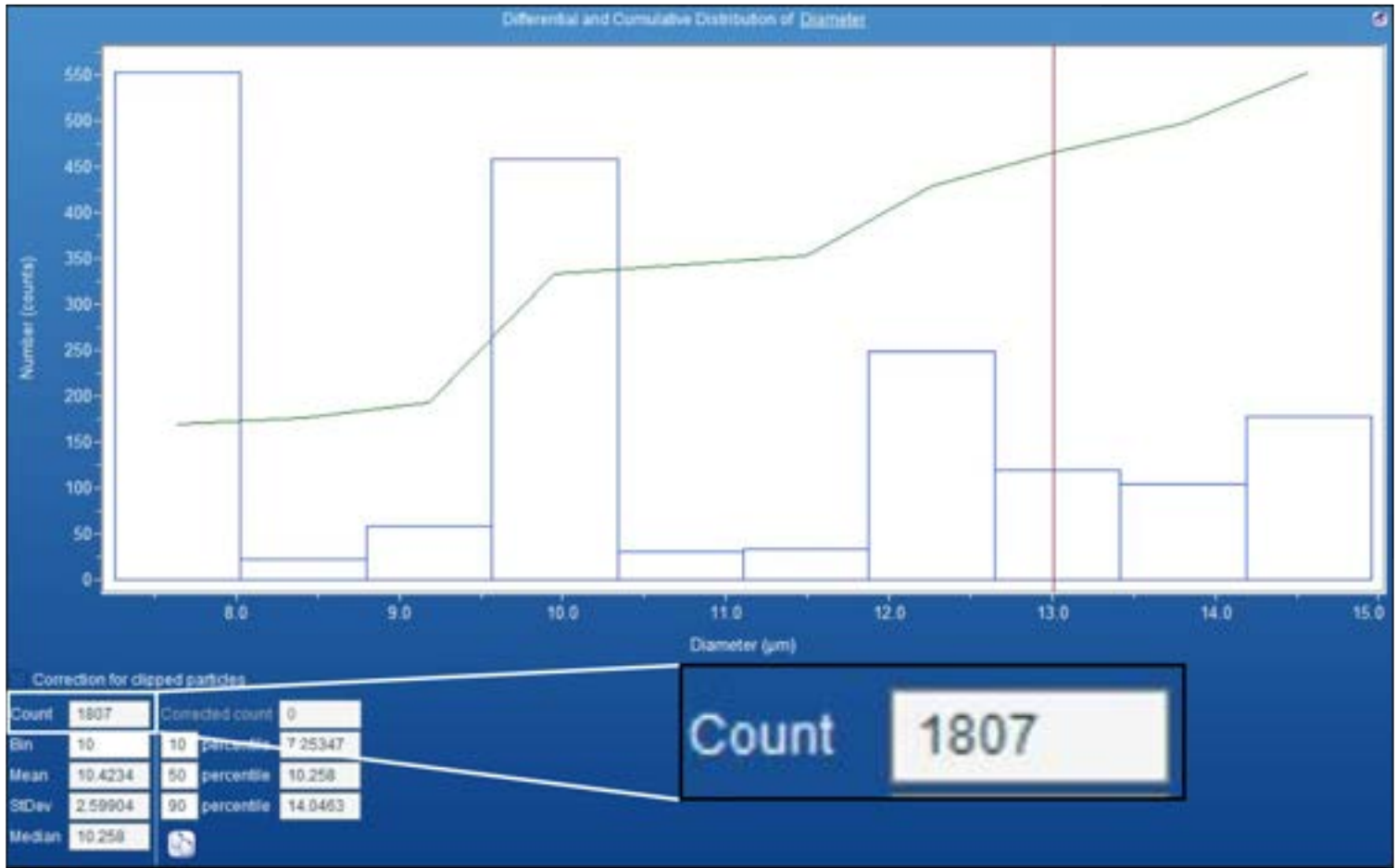
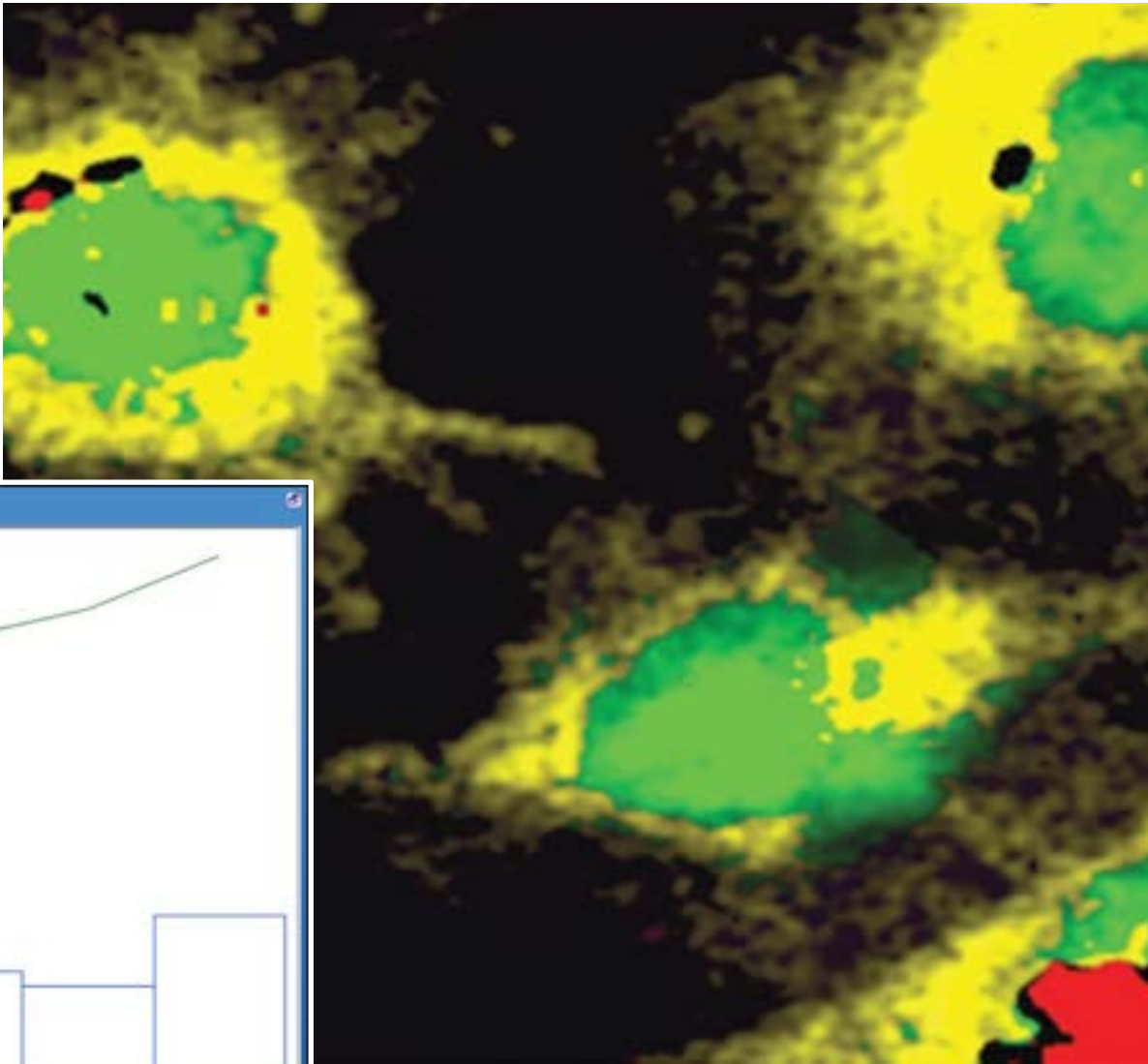


On the next two pictures we are showing the particles selection for the range between 7 to 15 micron and the histogram with the particles size distribution for the 1807 particles.

Results							
Include	Class	Index	X pos	Y pos	Area	Diameter	Perimeter
Use filtering			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Filter limits(min)						7.0	
Filter limits(max)						15.0	
<input checked="" type="checkbox"/>	4	1739(3538)	-5568.9	4115.9	175.6	15.0	65.3
<input checked="" type="checkbox"/>	4			42.0	175.6	15.0	49.2
<input checked="" type="checkbox"/>	4			36.4	175.6	15.0	49.2
<input checked="" type="checkbox"/>	4			34.3	175.6	15.0	78.4
<input checked="" type="checkbox"/>	4			30.4	175.6	15.0	49.2
<input checked="" type="checkbox"/>	4			27.9	175.6	15.0	49.2
<input checked="" type="checkbox"/>	4			29.0	175.6	15.0	60.2
<input checked="" type="checkbox"/>	4	1746(2024)	-771.7	1624.4	175.6	15.0	49.2
<input checked="" type="checkbox"/>	4	1747(3642)	-3386.1	4245.0	175.6	15.0	49.2
<input checked="" type="checkbox"/>	4	1748(4107)	-2918.7	4989.2	175.6	15.0	49.2
<input checked="" type="checkbox"/>	4	1749(95)	-3479.7	-3908.4	175.6	15.0	53.0
<input checked="" type="checkbox"/>	4	1750(1115)	-5612.4	-237.7	175.6	15.0	49.2
Mean			-1590.2	1828.6	90.6	10.4	39.3
Stdev			2467.5	2575.0	43.5	2.6	9.9
Median			-1859.0	2117.1	82.6	10.3	33.7

Raman acquisition

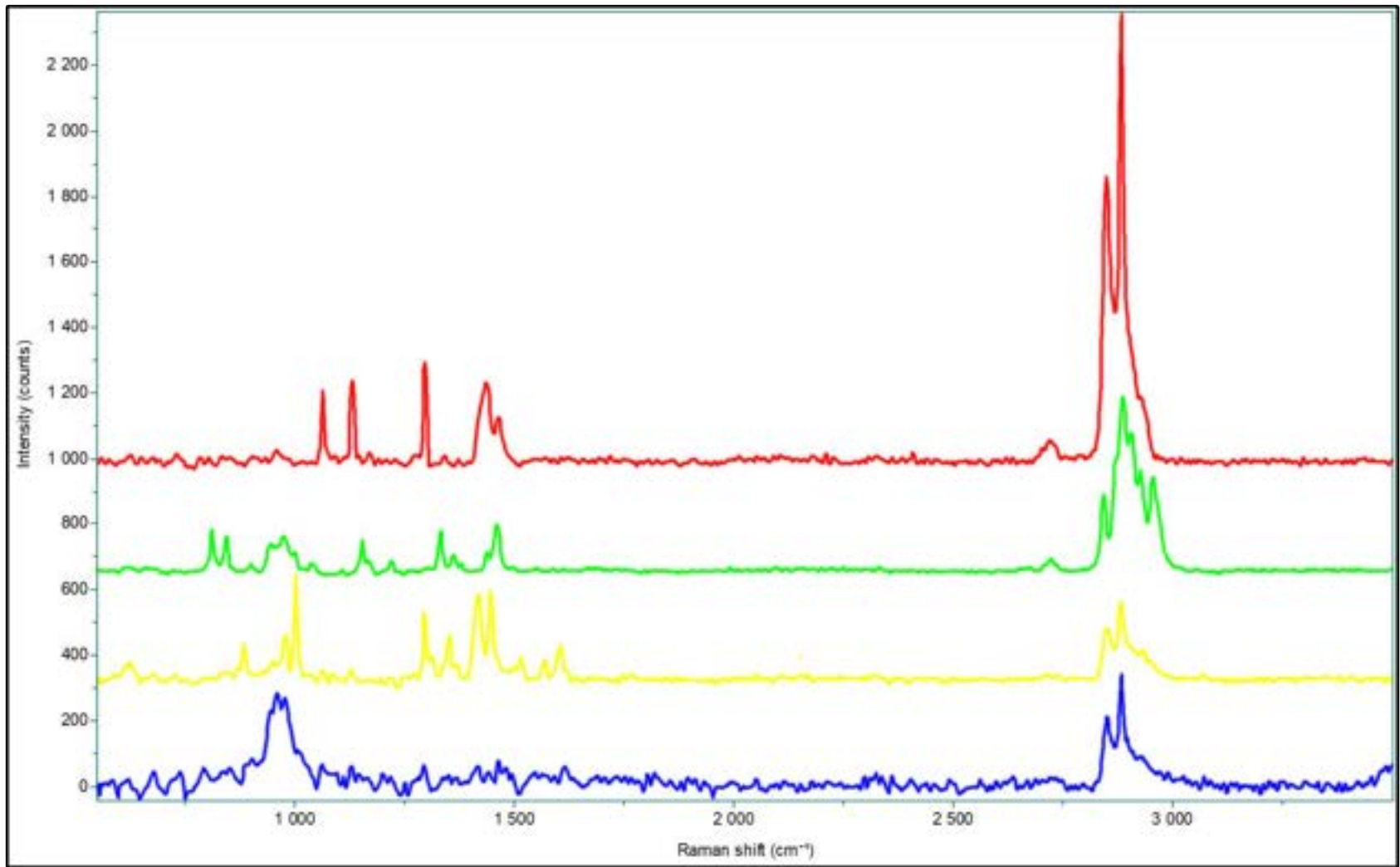
After the particles selection Raman spectra are collected for each particle. In this case we used a 532 nm laser, a 50x long working distance objective and an acquisition time of 7 seconds per spectrum. Spectra are collected in the range from 50 to 3500 cm<sup>-1</sup>.





Chemical identification and reporting

The chemical identification by exploiting the HORIBA polymer library identify four main polymer types which spectra are displayed below: Polypropylene [PP], High Density Polyethylene [HDPE], Polyethylene (low density) [PE] and Polytetrafluoroethylene [PET].

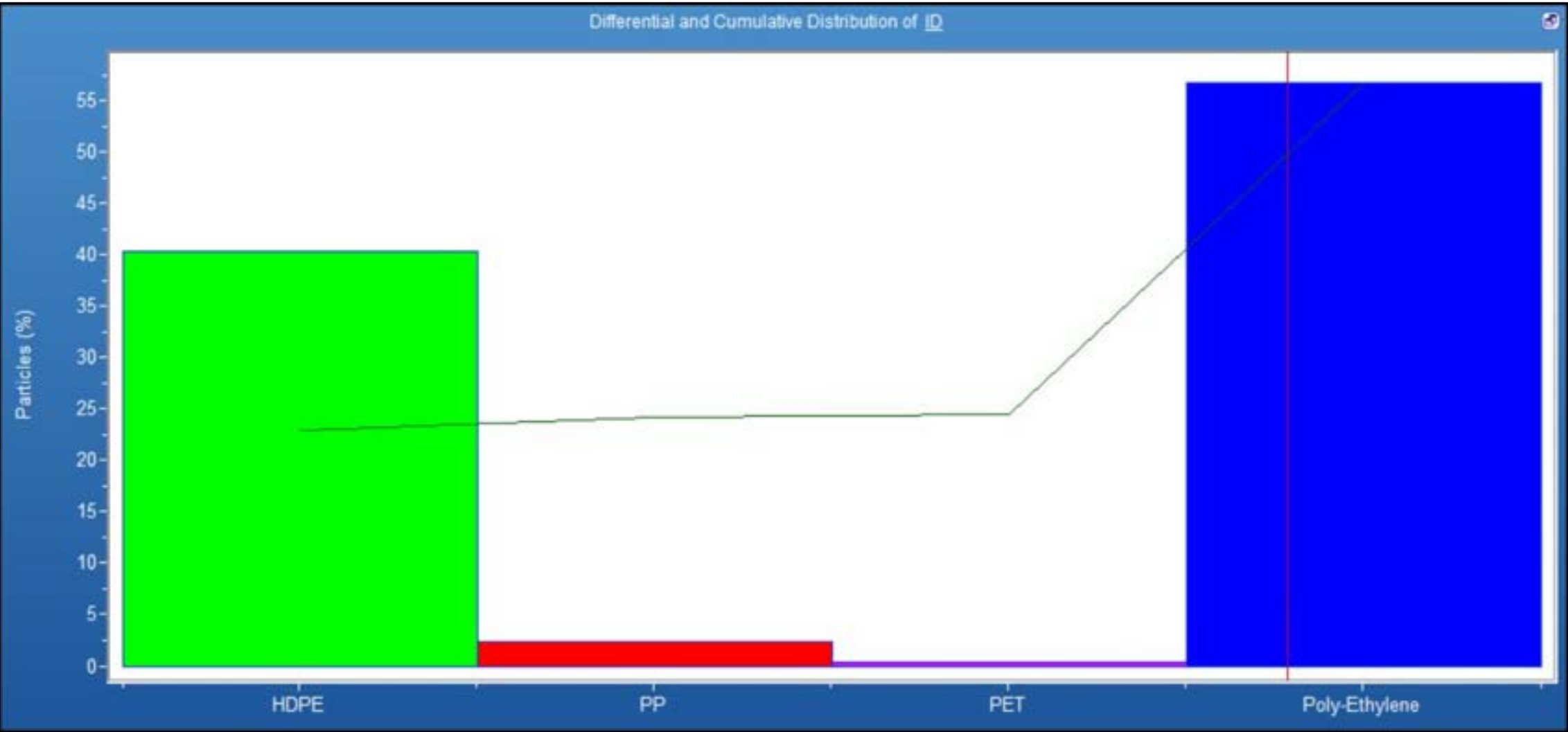


The table generated at the end of the acquisition reports two additional columns respect to the one obtain in the Characterise & Locate step:  
Raman spectrum (Raman column) and chemical identification (ID column). Chemical identification is colour coded in the table to quickly visually/identify Particle vs. Polymer Type.

Results											
X pos	Y pos	Area	Diameter	Perimeter	Major axis	Minor Axis	Circularity	Image	Raman	ID	
0.0	-4401.6	7.0	7.0	0.0	0.0	0.0	0.00				
0.0	5939.3	8.0	15.0	0.0	0.0	0.0	0.00				
-1069.2	4998.8	82.6	10.3	33.7	11.1	9.1	0.96				
-3325.0	5010.0	82.6	10.3	33.7	11.1	9.1	0.96				
-1419.7	697.1	82.6	10.3	33.7	11.1	9.1	0.96				
857.0	697.1	82.6	10.3	33.7	11.1	9.1	0.96				
-3146.5	5021.3	82.6	10.3	33.7	11.1	9.1	0.96				
-2733.3	-3686.5	82.6	10.3	33.7	11.1	9.1	0.96				
1730.0	5039.0	82.6	10.3	33.7	11.1	9.1	0.96				
-577.2	5037.4	82.6	10.3	33.7	11.1	9.1	0.96				
1352.2	668.2	82.6	10.3	33.7	11.1	9.1	0.96				
-985.6	652.2	82.6	10.3	33.7	11.1	9.1	0.96				
797.5	5048.6	82.6	10.3	33.7	11.1	9.1	0.96				
-2942.3	-3753.9	82.6	10.3	33.7	11.1	9.1	0.96				
-1590.2	1828.6	90.6	10.4	39.3	11.1	9.3	0.84				
2467.5	2575.0	43.5	2.6	9.9	3.6	2.7	0.12				
-1859.0	2117.1	82.6	10.3	33.7	11.1	9.1	0.85				

The images of the particle look pixelate because the mosaic is collected with a low magnification objective to speed up the mosaic collection time (better image can be easily obtained by using a 50x objective)

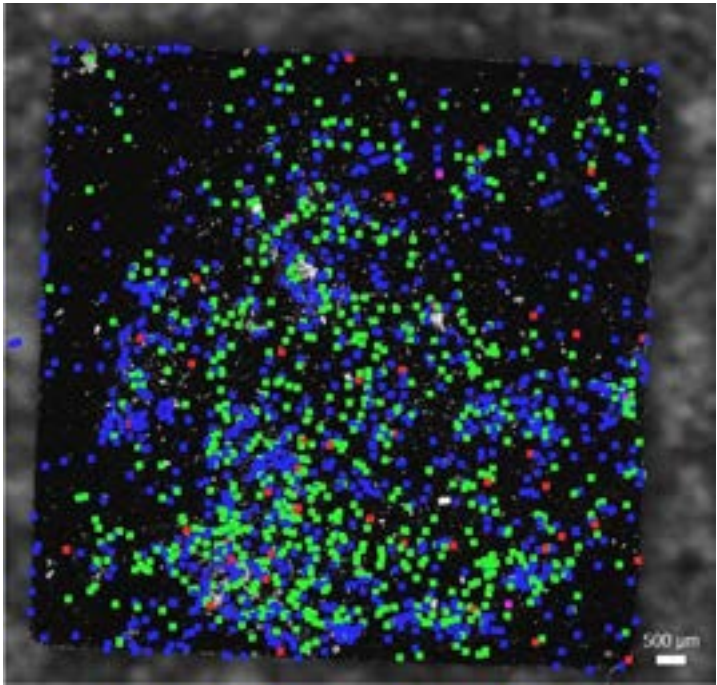
Generated the table and finalized the chemical identification several data treatments and data visualization are available to display the information of the particles. We report below just two of the option available for the size range ranging from 7 to 15 microns.



HDPE 38,74% (700 particles) in green;  
PP 2,43% (44 particles) in red;  
PE 58,55% (1058 particles) in blue;  
PET 0,58% (5 particles) purple.

The histogram above shows the percentage of particles by polymers type (a colour is assigned to the different polymers):

At the same time it's possible to displays the particles on the optical image by using the colour code of the histogram.



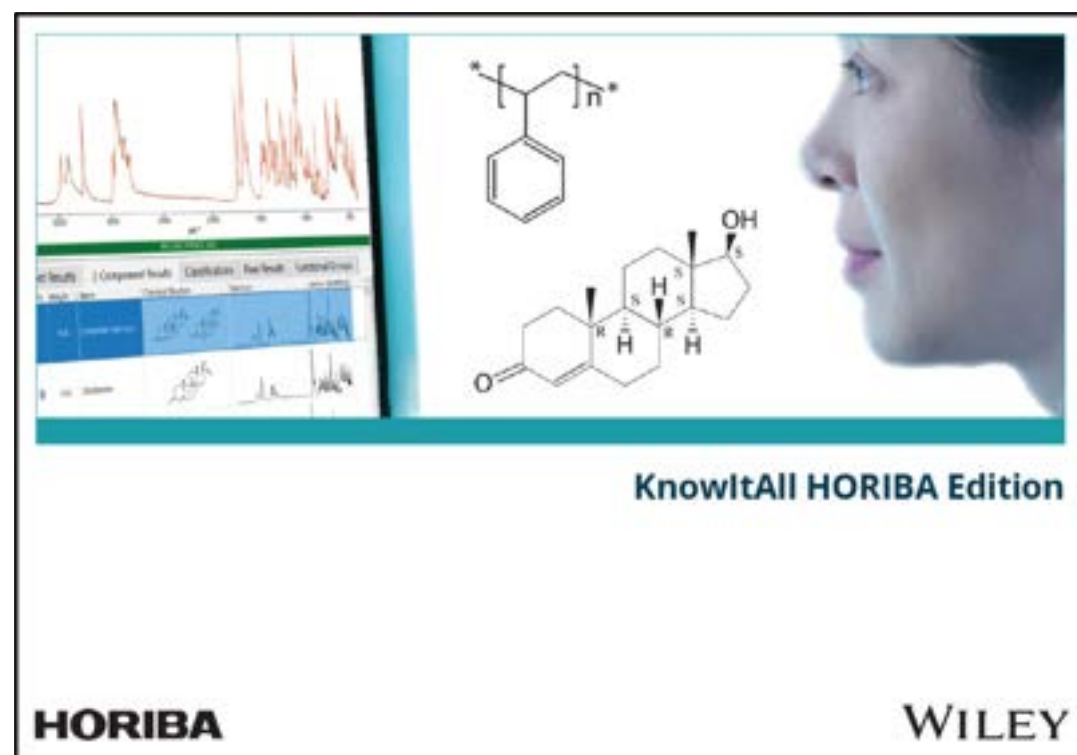
We are working on other matrices (sediment, bottled water, salt etc..) and more application will be added on the next release of the Booklet.



KnowItAll HORIBA Edition integrates spectral data and tools into a single interface, so chemists can perform multiple tasks in relation to that data and ultimately extract greater knowledge from it. Easily transfer information from one tool to another and move from one task to the next, without having to leave the main interface or open another program. **KnowItAll HORIBA Edition offers solutions to identify, analyze, and manage Raman spectral data and supports multiple file formats.**

Table 8. KnowItAll Solutions to identify, analyze, and manage Raman spectral data and supports multiple file formats.

Data Toolbox	
<b>ID Expert™</b>	One-click spectral identification tool: to perform applicable basic analyses (single and multi-component search, peak search, and functional group analysis) on an unknown spectrum
<b>SearchIt™</b>	Advanced database searching including mixture analysis, pure compound search, simultaneous multi-technique search
<b>Minelt™</b>	Multi-technique spectral database building and data mining. Includes patented Overlap Density Heatmap technology to visualize similarities and dissimilarities in datasets.
<b>QC Expert™</b>	Perform a QC comparison of a sample Raman or IR spectrum against a reference spectrum



**KIA/Minelt** is the tool which allows you to create your own databases.

Users can:

- Build databases with one or more analytical techniques
- Build databases with multiple spectral scans in the same record
- Import analytical data from different instruments in the laboratory
- One-click import of common native instrument file formats and \*.csv format (spreadsheet)
- Enhance each record with peak information, structures, and properties, such as sample source, boiling point, etc.
- Import multiple structure formats (with stereochemical bonds and identifiers)
- Use “Batch Import and Export” for efficient handling of spectra, structures, and property files

*Researchers can build searchable databases that include one or more analytical techniques (Raman, IR, MS, Near IR, NMR, UV-Vis), chemical structures, and other metadata by using the Minelt tool.*

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- Supports unrestricted spectral range and resolution - Store spectra at the extract range and resolution at which each spectrum was measured rather than having to use a fixed range and resolution
- “Auto-Property” computes values such as formula, molecular weight, etc. for entire datasets
- Make databases more powerful by attaching spreadsheets, MSDS, and other documents or adding hyperlinks to web pages
- Create cross-references to data from other techniques; i.e., a Raman spectrum can be linked to an IR spectrum
- Quickly add properties and structures from PubChem to your database

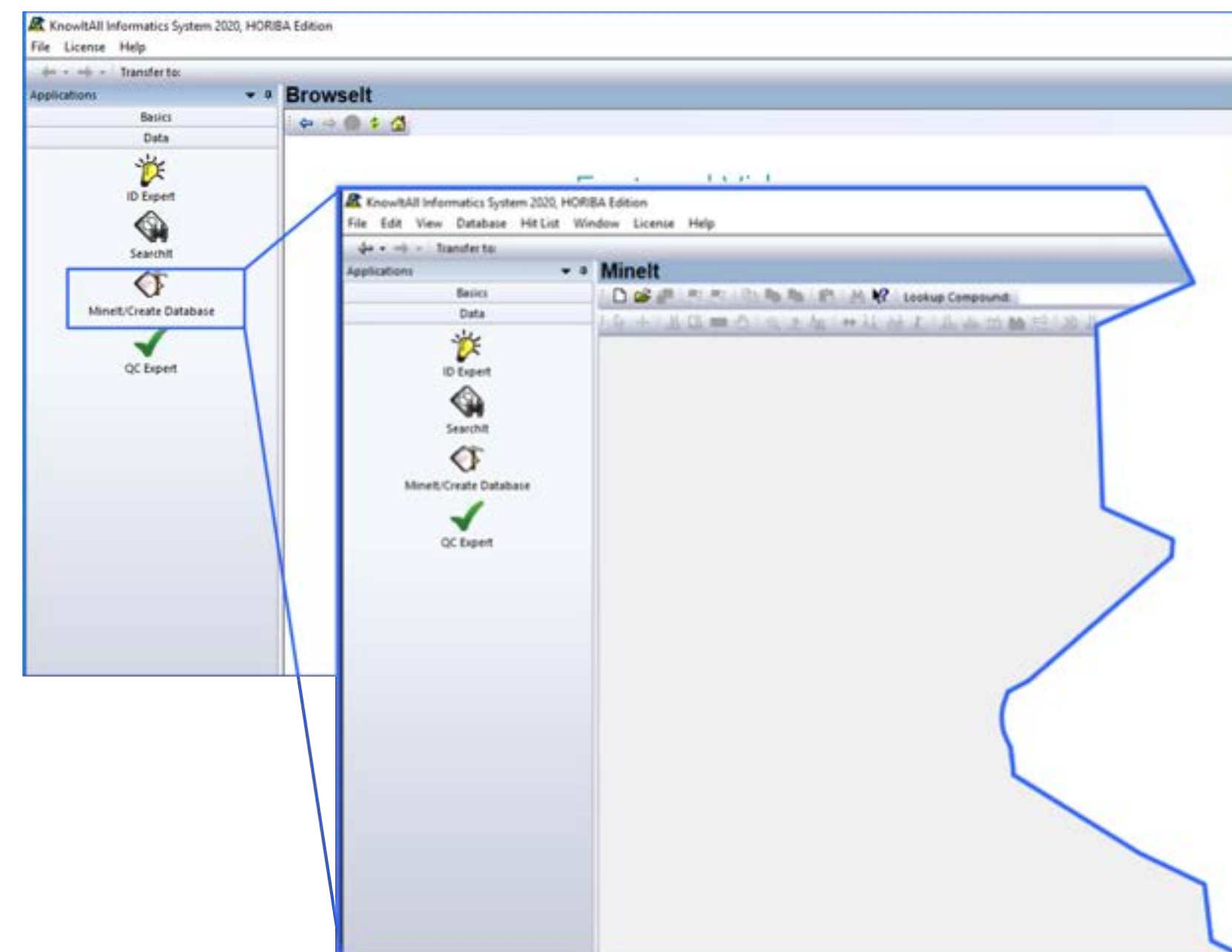
Moreover, databases can be customized:

- Databases can be customized to meet laboratory specifications
- Users can create custom fields to store associated metadata relevant to their work
- Choose from three types of property fields: text, numeric, hyperlink
- Generate “preferred property” forms so users enter properties consistently
- Set spectral parameters such as x- and y-resolution

The next section provides a visual guide of how to build your own database with KIA/Minelt.

## Step 1 - Database selection

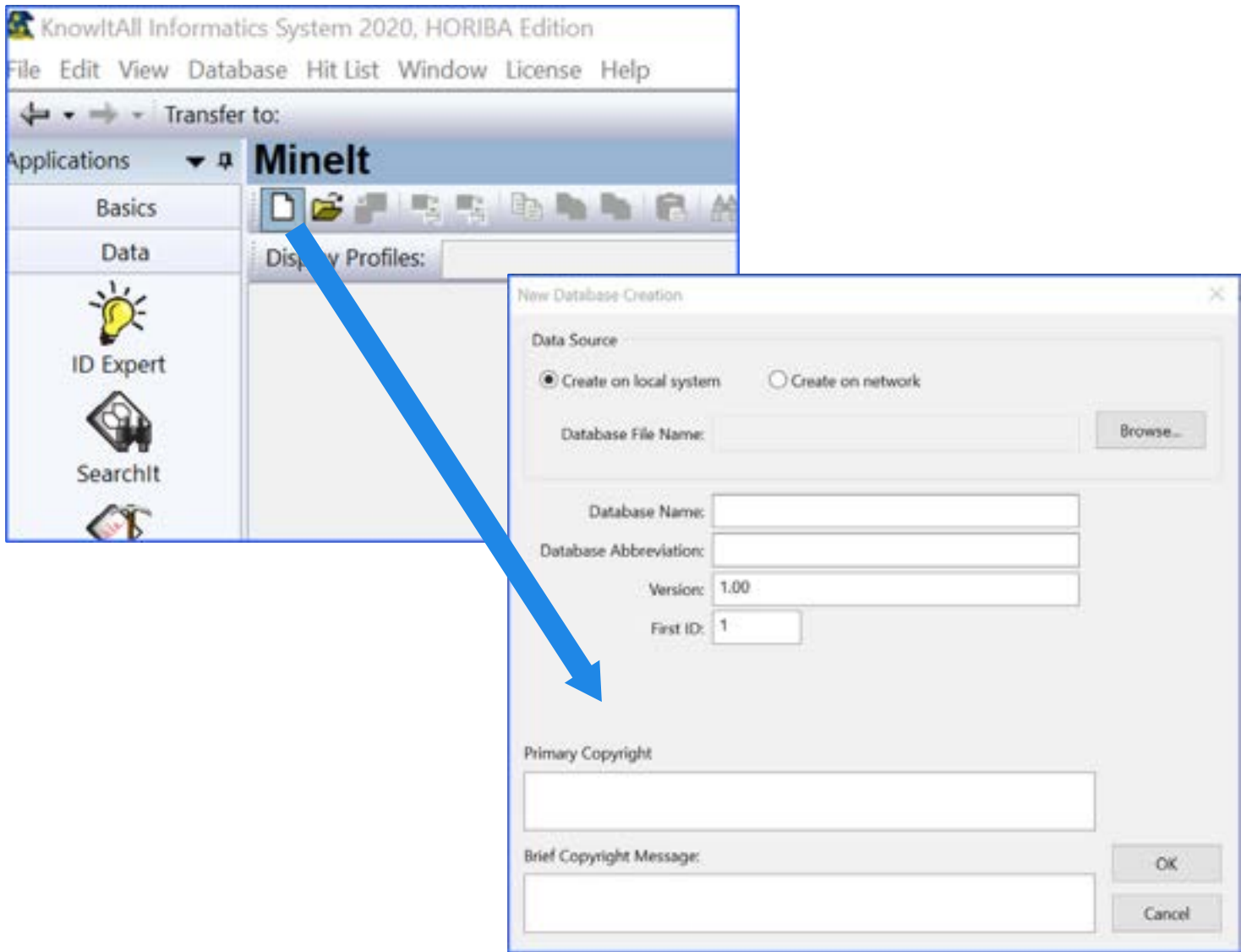
Select the Minelt/Create database option from the menu on the left side on the main page of KnowItAll





## Step 2 - Database creation

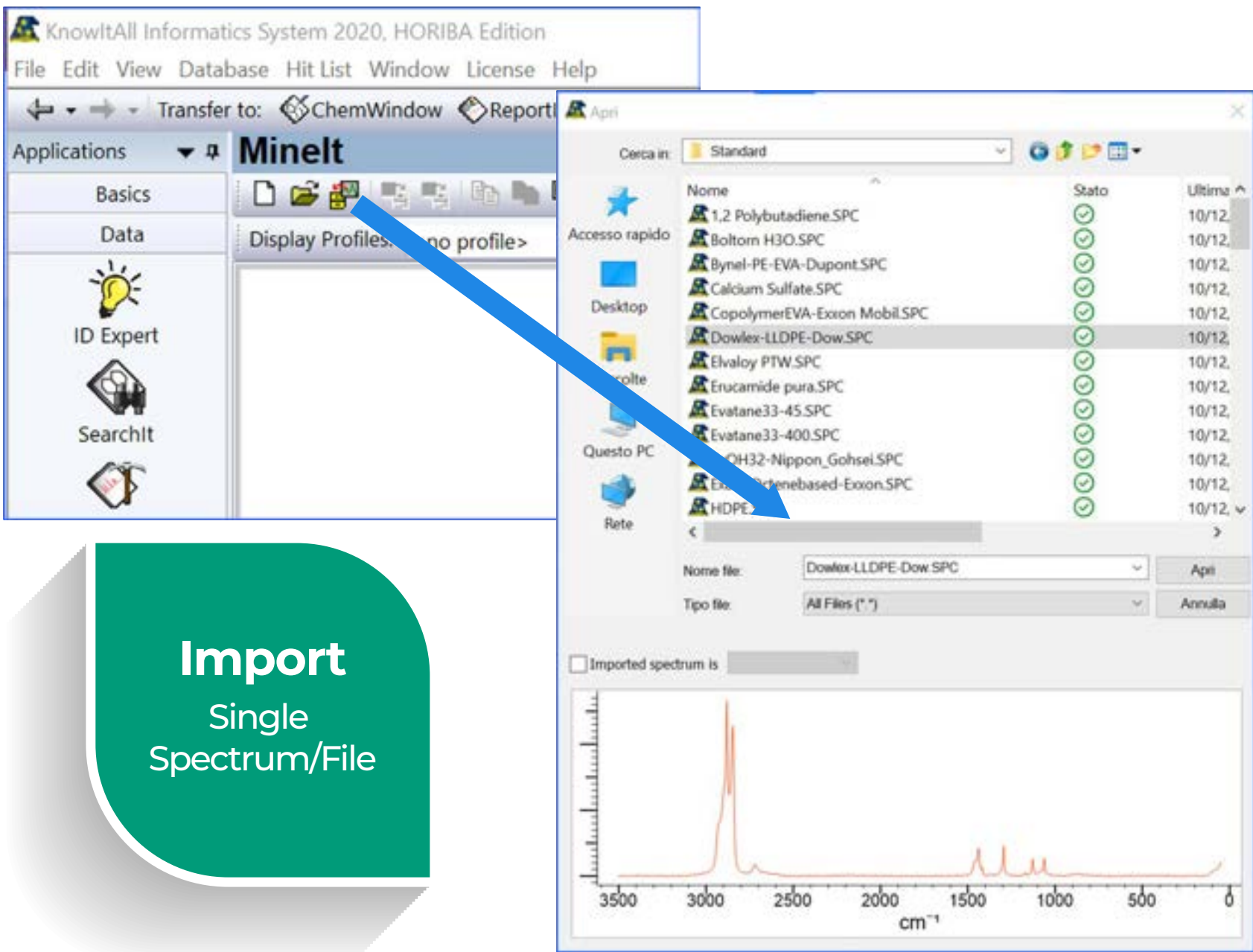
Select create new user database



Browse to the location where you want to store the database, on your local computer or on a network location. Define a name and an abbreviation for the database to allow easy identification.

## Step 3 - Import spectra

Spectra can be imported one at a time (Import) and/or all together by using the batch import option



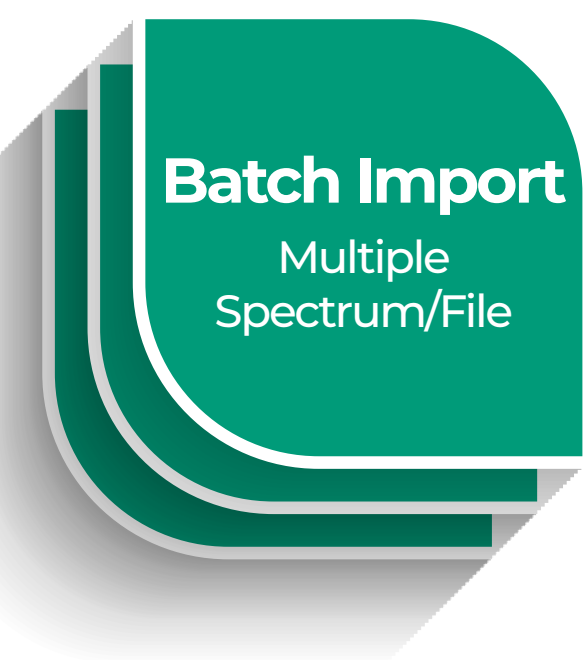
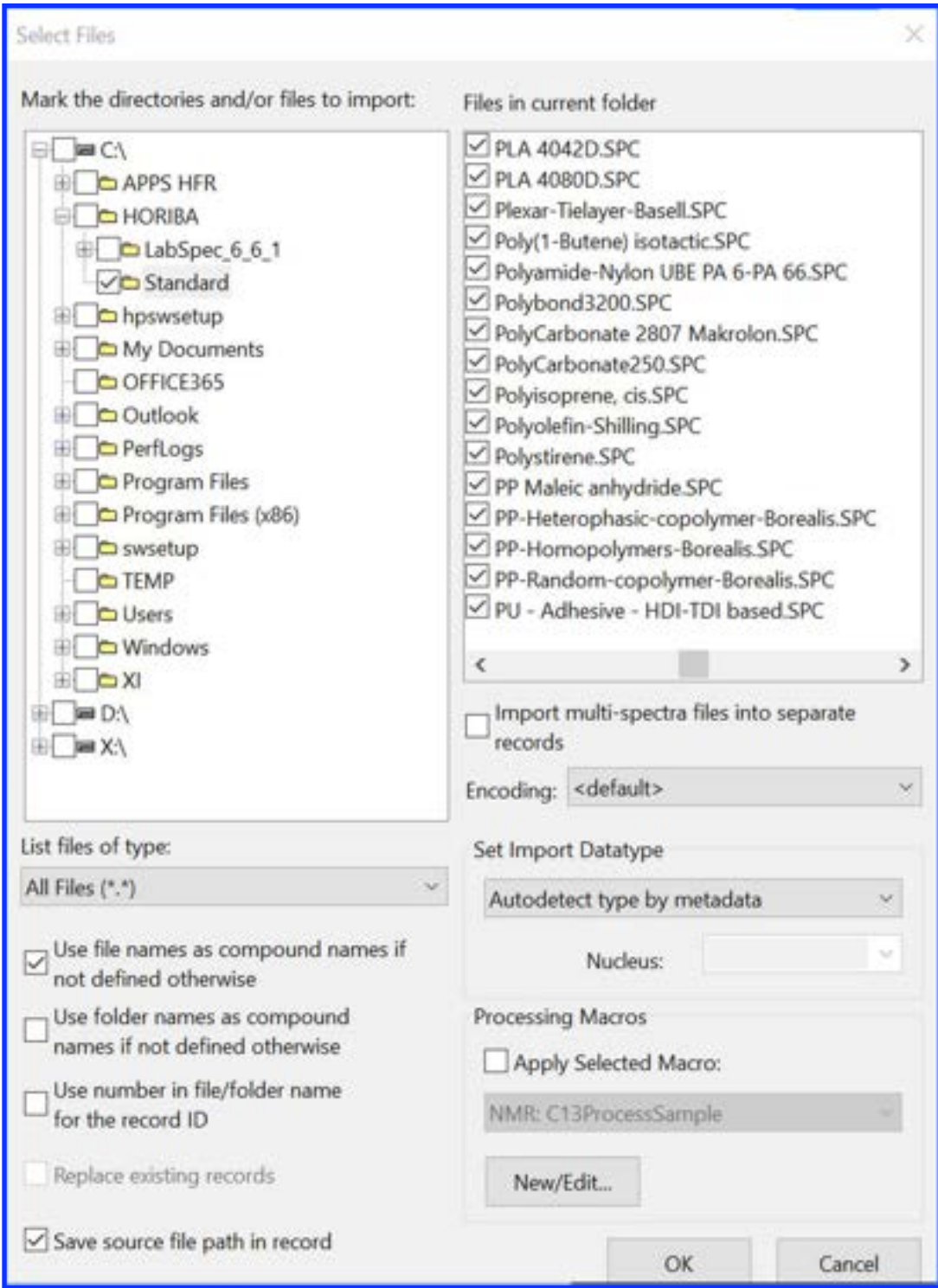
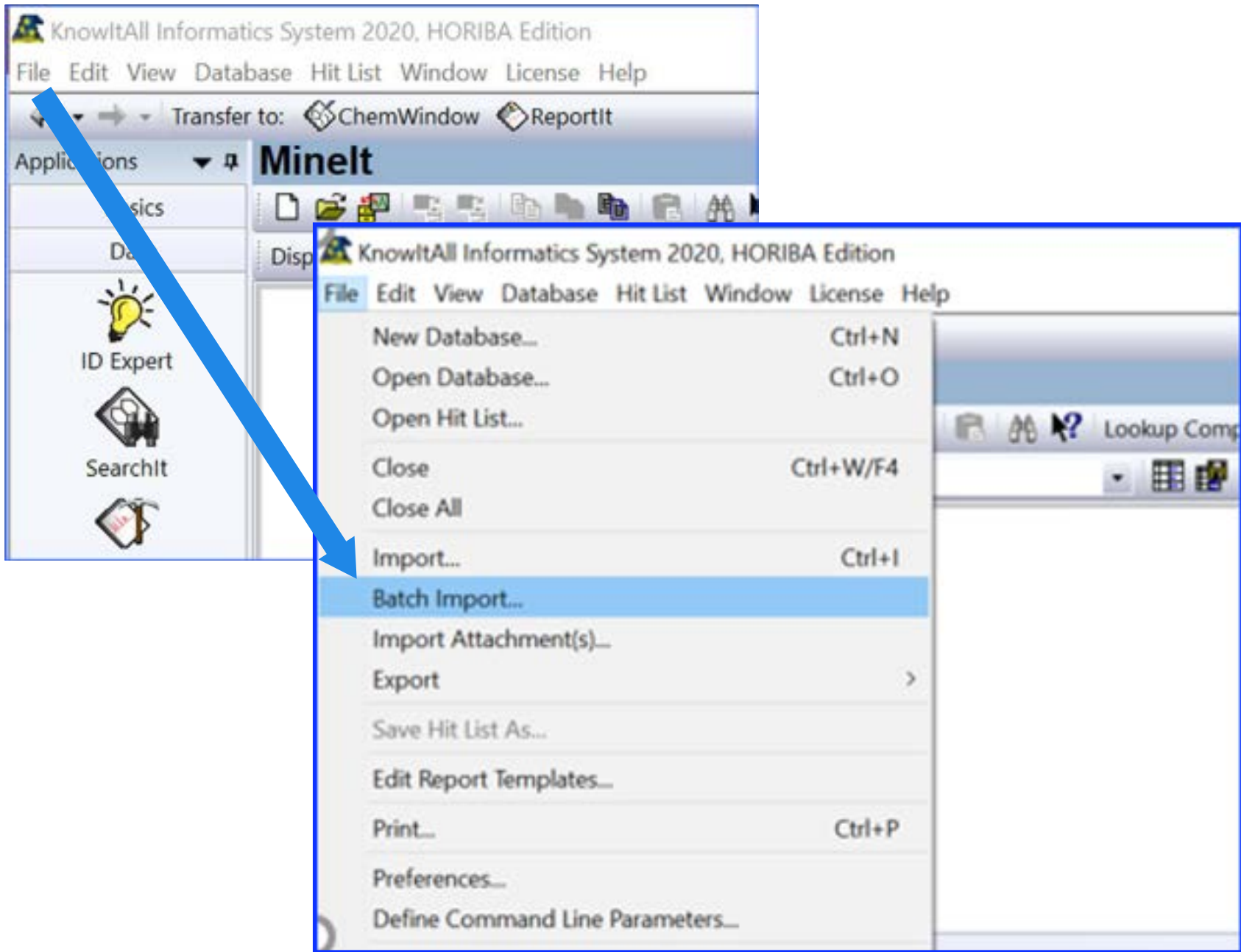
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# Know It All - Building a library

Home  
Database selection  
Database creation / import spectra

**Batch import**  
Addition to library / Library  
customization



The batch import option allows multiple spectra to be selected and metadata types are automatically recognized.

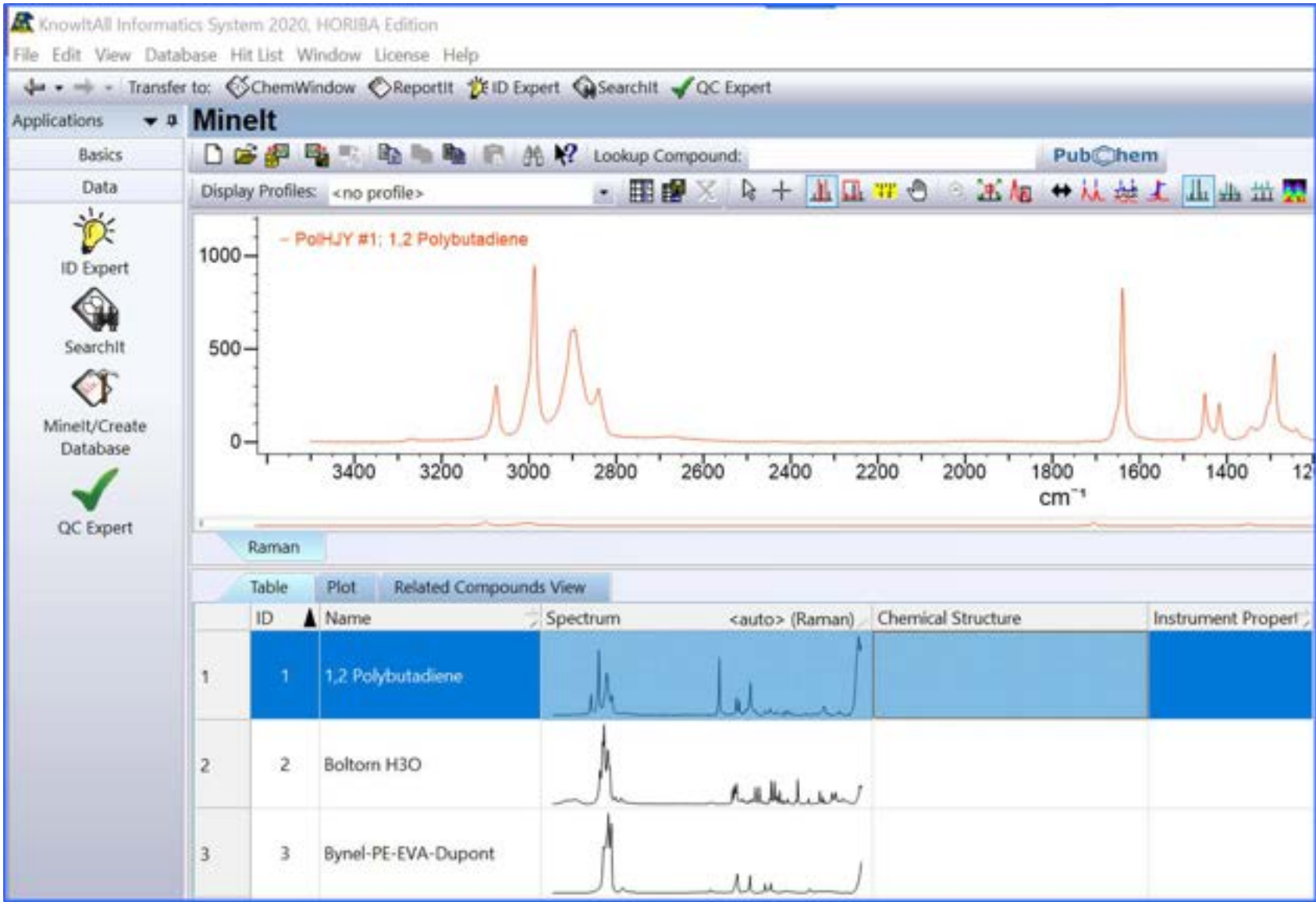
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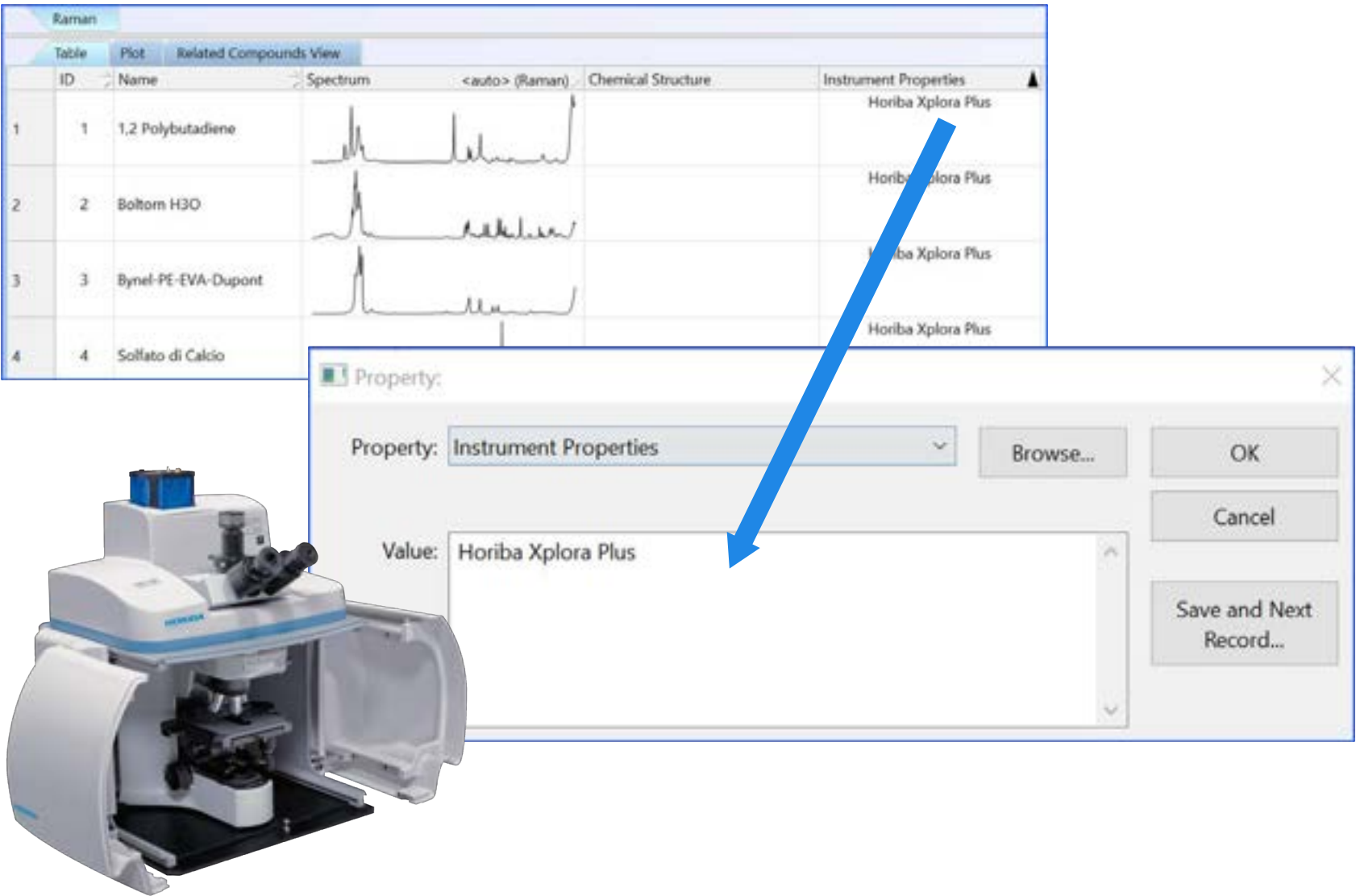
## Step 4 - Addition to the library

Once the spectrum/spectra are imported the library is ready to be used. However, users can also customize the library by adding other information (such as physical, chemical and instrumentation parameters, flags, names, etc.) by adding as many columns as needed.



## Step 5 – Library customization

The existing information on each column (for example “Instrument Property” in the picture shown below) can be modified for each spectrum by double clicking inside the column; the modification can also be assigned to all spectra and not just to a single one. Here we specified that the instrument used for data collection was the HORIBA Xplora PLUS.



Adding a column to see additional information is also straightforward: right click on the table and select “add column”.

Raman

TablePlotRelated Compounds View

	ID	Name	Spectrum	<auto> (Raman)	Chemical Structure	Instrument Proper
1	1	1,2 Polybutadiene				
2	2	Boltorn H3O				
3	3	Bynel-PE-EVA-Dupont				

Add Column...

Insert Column...

Delete Column...

Edit Columns...

A submenu will open-up, allowing a new column to be select from an extensive list (see the example on the right)

Choose Single Column

Choose a Column:

AllUserPrimarySecondaryIR/UV-VisNMR

Name

Unit

Literature Reference 3

Literature Reference Author

Literature Reference Citation

Literature Reference DOI

Literature Reference Issue

Literature Reference Journal

Literature Reference Page

Literature Reference Volume

Literature Reference Year

Melting Point

Molecular Weight

Reagent Gas

Recommendation(s)

Related CAS Registry Number(s)

RTECS Number

Sadtler Reference Number

Solubility

Solvent

Source File Reference

Source of Sample

Source of Spectrum

Spectrum

SPI ASH

Choose Single Column

Choose a Column:

AllUserPrimarySecondaryIR/UV-VisNMR

Name

Unit

Absolute Peak Position

Absorption Maximum

Accessory

Acquisition Delay

Acquisition Mode

Acquisition Time

Acronyms

Active Ingredients

Activity Category ER\_RBA

Activity mg

Activity mmol

Actual Reference Signal Gain

Actual Signal Gain

Actual Signal Gain 2nd Channel

Additional Data Treatment

AddToParent

Adhesive

Algorithm

Analog Signal 1

Analog Signal 2

Aniline Point

Aperture Setting

API Gravity

OK

Cancel



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