Application note

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Morphologically targeted Raman analysis of microplastic particles

The microplastics problem

The use of plastics and polymers has increased in recent decades and we are discovering an abundance of microplastics in the environment from food chains to our oceans and watercourses. These particles are typically not biodegradable, are potentially harmful to the ecosystem and can ultimately end up in humans. It is vital to be able to identify such particles to understand their toxicity and monitor any larger impact.

Raman analysis provides a non-destructive, chemically specific technique to enable particles to be investigated individually in an automated way. Raman has the advantage over other techniques, such as infra-red, of having the capacity to analyse a range of particle sizes from as low as 1 µm to several 100 µm in diameter. It also provides excellent specificity between even highly similar materials.

The range of sampling locations is vast, from natural waterways and oceans, to processed animal intestines, drinking water and the atmosphere.

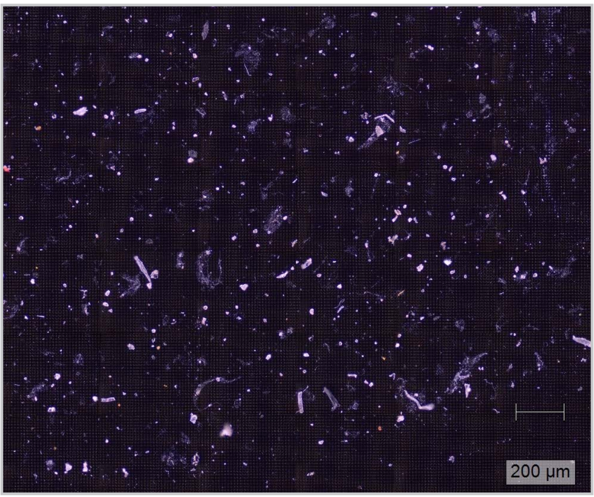

Figure 1 - A darkfield image showing the particles on the filter.

Here we used an inVia™ Raman microscope and Particle Analysis software to determine the composition and morphology of particles in a commercially available bottle of mineral water.

Targeted, automated analysis

We used an etched silicon filter[1] and a vacuum pump to filter the bottled water. We analysed an area 2.5 mm × 2.1 mm on the filter surface with an inVia confocal Raman microscope as the filtration process, in this case, produced a very high concentration of particles.

1. A large darkfield image (Figure 1) was collected using the inVia microscope's 20× objective and high resolution 5 Mpixel camera. This provided excellent optical contrast between the particles and the substrate
2. The image was analysed using the Renishaw's Particle Analysis module to produce a list of 5,663 particles for potential analysis.
3. In this case, the particles to target for analysis were chosen based on their size relative to the filter holes. The minimum particle area was limited to 4 µm^2 (2.3 µm in diameter) producing 1,026 particles. The largest particle (Figure 2), fibre-like in aspect ratio, had a length of 147 µm.

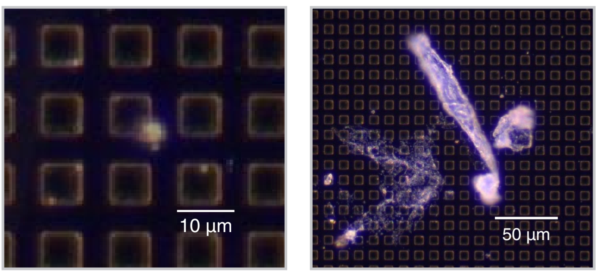

Figure 2 - Two of the 1,026 particles that were selected for analysis. One of the smaller particles (left) and the largest particle (right).

Each particle was analysed sequentially and automatically using 532 nm laser excitation and a 50× objective. The data collection parameters were set using a pre-defined template, with each spectrum having an acquisition time of just 1 s. The MS30 stage, with its high repeatability and accuracy, moved to each particle in an optimised direction ensuring highly positional accuracy. Focusing was automated using LiveTrack™ focus technology. This ensured that the particle was accurately positioned at the very small collection volume of the high optical efficiency 50× objective.

Data processing and analysis were performed using a dedicated user predefined 'Chain'. This automated process performed intelligent fitting baseline subtraction and library searching. It was not practical to review each individual result because of the large number of particles.

Instead, the library search hit quality index was used to quickly select a subset of particles for review. Several particles were then re-targeted for additional data collection, thereby ensuring the data were thorough and complete.

Equivalent circle diameter / µm	**Total**	**0 - 4**	**4 - 8**	**8 - 12**	**12 - 16**	**16 - 20**	**20 - 24**	**24 - 28**	**28 - 32**	**32 - 36**	**36 - 40**
Polypropylene (PP)	183	30	45	40	29	21	10	4	2	1	1
Calcium carbonate	271	74	111	45	26	8	3	2	1	0	1
Hostafine yellow (yellow dye)	13	5	6	0	1	0	0	0	1	0	0
Rutile (TiO_2)	1	1	0	0	0	0	0	0	0	0	0
Beta-carotene	154	87	47	13	4	2	0	0	0	1	0
Chromophtal blue (blue dye)	2	1	1	0	0	0	0	0	0	0	0
Calcium sulfate	5	5	0	0	0	0	0	0	0	0	0
Polystyrene (PS)	4	2	2	0	0	0	0	0	0	0	0
Polyethylene (PE)	12	6	3	1	0	1	0	0	1	0	0
Talc (hydrated magnesium silicate)	3	0	0	2	1	0	0	0	0	0	0
Polyurethane (PU)	1	1	0	0	0	0	0	0	0	0	0
NYLON 6-10	1	0	1	0	0	0	0	0	0	0	0
NYLON 12	8	4	3	0	1	0	0	0	0	0	0
Anatase (TiO_2)	3	2	1	0	0	0	0	0	0	0	0
Irgalith blue (blue dye)	5	4	1	0	0	0	0	0	0	0	0
NYLON 6-6	1	0	1	0	0	0	0	0	0	0	0
Hostaperm blue (blue dye)	1	1	0	0	0	0	0	0	0	0	0
Poly(tetrafluoroethylene) (PTFE)	3	2	1	0	0	0	0	0	0	0	0
Other materials	1	0	0	0	1	0	0	0	0	0	0
Total	**672**	**225**	**223**	**101**	**63**	**32**	**13**	**6**	**5**	**2**	**2**

Figure 3 - The Particle Analysis module report detailing chemical composition and equivalent circle diameter for the particles. This highlights the broad range of chemicals present and allows correlation between type and size.

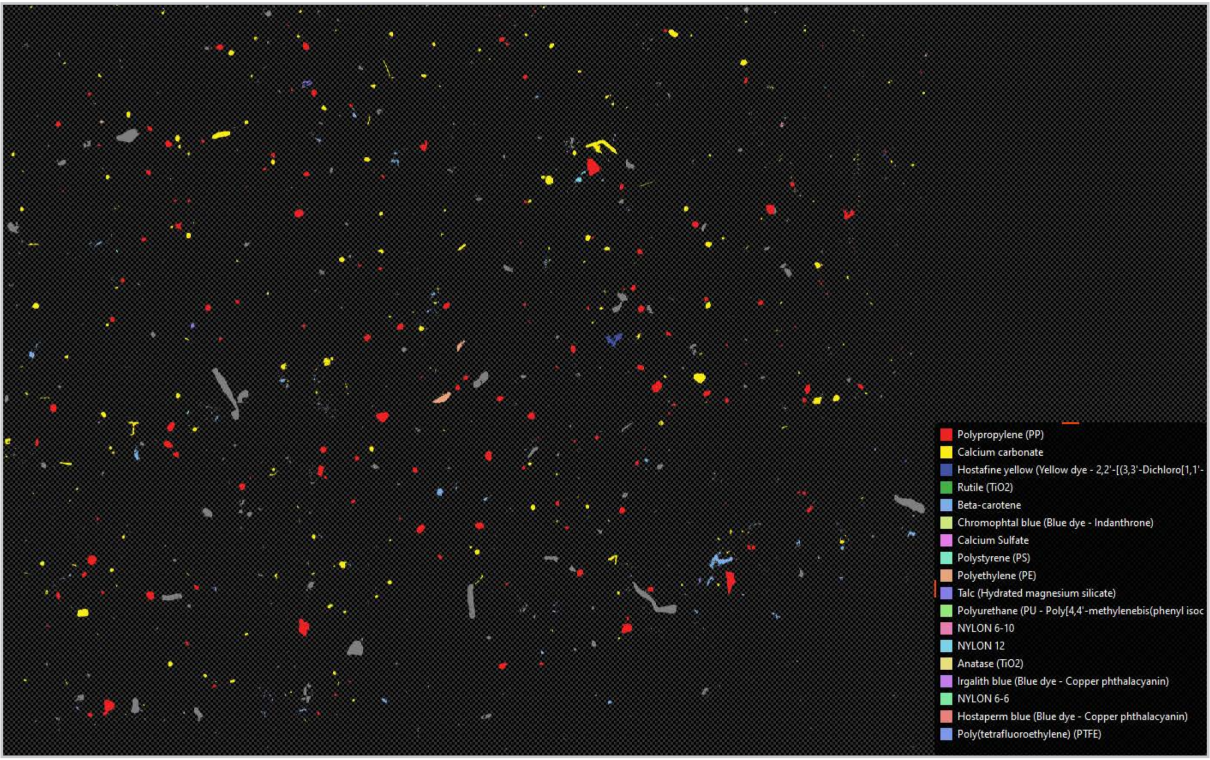

Figure 4 - Composite image illustrating the size and composition of particles, as identified by Raman spectroscopy. Some particles in this sample were too fluorescent to be identified, at this laser wavelength. These are indicated in grey in the image, and were excluded from analysis.

Detailed results

The analysis revealed the presence of 18 different materials, including inorganics, pigments and dyes, as well as a range of microplastics. Figure 3 shows the distribution of the equivalent circle diameters (ECD) for each of the materials.

Particles can be coloured on the image based on their size metrics or chemistry. This visually illustrates the data, revealing trends that are less apparent from the table. Figure 4 shows the 18 identified materials, including the 8 polymers.

The majority of the particles are either polypropylene or calcium carbonate. Analysing the relationship between the particle size and composition (Figure 5) shows the ratio of polypropylene particles to calcium carbonate particles changes based on the particle size.

Small particles (up to 8 µm in ECD) are dominated by calcium carbonate. Other polymers, present in low numbers, such as polystyrene and polyurethane also fall into this size range. As particle size increases, more polypropylene is observed relative to calcium carbonate and other polymers. Above 16 µm (ECD), polypropylene becomes dominant (the very small number of large particles doesn't make the ratio comparison statistically significant above 32 µm ECD).

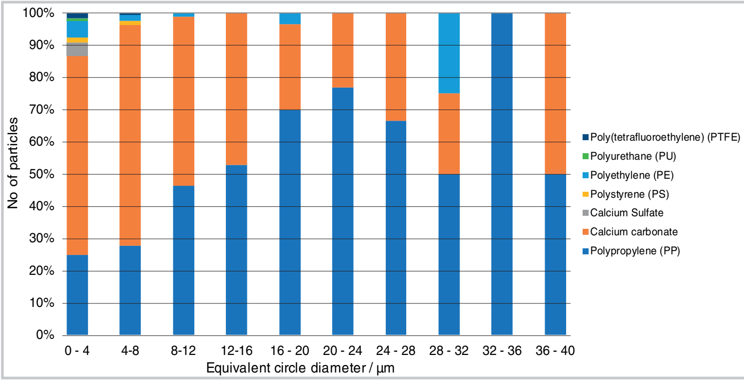

Figure 5 - A graph (produced using Microsoft® Excel from exported Particle Analysis values) showing the relationship between the particle size and composition.

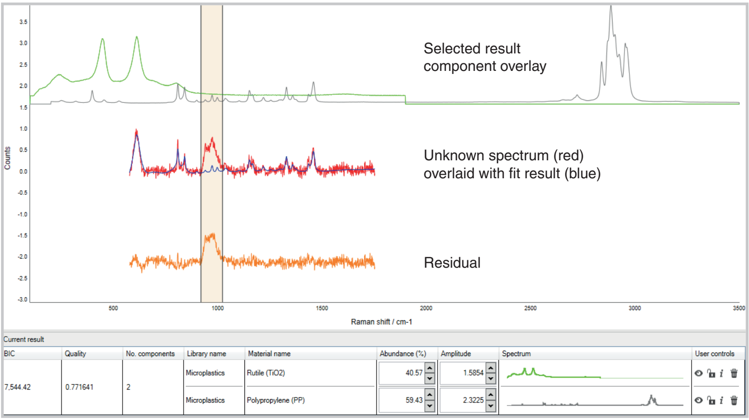

Figure 6 - The particle spectrum (red) has been analysed with mixture analysis. This reveals the presence of polypropylene (grey) and TiO_2 (rutile – green).

Specificity and precision

Dyes and pigments can also be identified and reported, in addition to particle material. This information can provide an ideal input into any filtration strategy for the bottled water supplier. Some particles exhibit Raman data only containing pigments/dyes, while others have mixed spectra. Renishaw's Spectrum Search module within the WiRE software enables mixed spectra to be split into multiple library results. In Figure 6, the particle spectrum from one particle (ID59)(red) has been analysed with mixture analysis. This reveals the presence of polypropylene (grey) and TiO_2 (rutile – green).

This particle (Figure 7) is only 2.6 µm in diameter, showing the level of high chemical specificity, sensitivity and positional accuracy, necessary to conduct such studies.

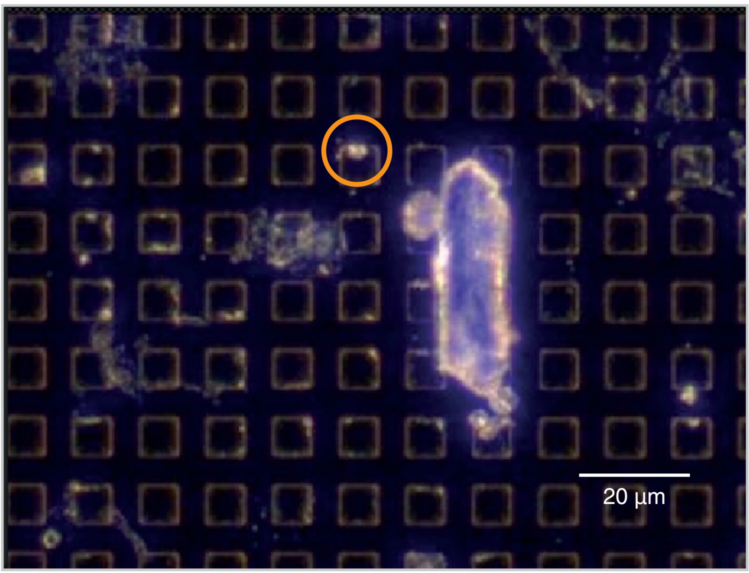

Figure 7 - The particle identified in Figure 6.

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Figure 8 - The inVia™ Qontor confocal Raman microscope.

Targeted microplastic analysis using Particle Analysis

The inVia Raman microscope is not just a tool to enable micro-plastic analysis. It brings the performance and precision expected from a world leading metrology company. The Particle Analysis software module is a dedicated simple-to-use system that exploits many advanced capabilities of the inVia microscope. Everything you need for detailed, fast and automated microparticle studies is present so that you not only get the best data possible but can report on your findings with confidence.

inVia with Particle Analysis has everything you need for targeted microplastic and trace material analysis.

References

1 SmartMembranes GmbH Heinrich-Damerow-Str. 4 06120 Halle (Saale), Deutschland (10 mm × 10 mm) with a 5 µm hole size.

A range of related Renishaw literature is available. Please ask your local Renishaw representative for more information.

Renishaw. The Raman innovators

Renishaw manufactures a wide range of high performance optical spectroscopy products, including confocal Raman microscopes with high speed chemical imaging technology, dedicated Raman analysers, interfaces for scanning electron and atomic force microscopes, solid state lasers for spectroscopy and state-of-the-art cooled CCD detectors.

Offering the highest levels of performance, sensitivity and reliability across a diverse range of fields and applications, the instruments are designed to meet your needs, so you can tackle even the most challenging analytical problems with confidence.

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Please visit www.renishaw.com/raman for more information.