Micro- and Nanoplastics Analysis by Raman Microscopy and Imaging

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Introduction

Microplastics (< 5 mm) and nanoplastics (< 1 µm) are of increasing concern in environmental, food, and health-related research. Their reliable characterization requires particle-resolved information on chemical composition, size, and morphology. Raman microscopy provides a non-destructive analytical approach with submicron spatial resolution, enabling detailed chemical identification and imaging of micro- and nanoplastics. Based on inelastic light scattering, Raman spectroscopy delivers molecular fingerprints that allow unambiguous material classification. This application note compares two complementary Raman-based approaches for nanoplastics analysis: particle-by-particle micro-spectroscopy and Raman imaging.

Comparing Measurement Strategies

In **particle-by-particle micro-spectroscopy**, particles are selected optically and measured individually. This approach is flexible and suitable for targeted analysis but suffers from selection bias, limited statistical relevance, and increasing measurement time with higher particle counts.

When using **Raman imaging**, spatially resolved chemical datasets are created. This allows identification based on chemical contrast, supports analysis of heterogeneous particles, and improves classification reliability through spectral averaging.

Experimental Setup

All measurements were performed using the confocal RAMANtouch Raman microscope equipped with a high numerical aperture objective. As careful optimization of the measurement is essential for reliable nanoplastics characterization, parameters were optimized to ensure a balance between acquisition speed, spectral quality, and spatial resolution.

- Laser wavelength: 532 nm
- Laser power: 8.7 mW (point mode) / 115 mW (line mode)
- Integration time: 2 x 0.5 s (point mode) / 3 s (line mode)
- Objective: 100×, NA 0.9
- Step size (imaging): 200 nm
- Identification method: library matching (particle mode) / correlation (imaging)

Results and Discussion

The reference sample of nanoplastic particles was analyzed on a flat glass surface to minimize the influence of a filter substrate for the comparison. Both particle-by-particle and imaging approaches were applied to compare performance in detecting, identifying and characterizing particles.

Particle-by-Particle Micro-Spectroscopy

In the first step, nanoplastic particles were localized based on their visual contrast in the optical image. Particle detection was optimized by adjusting key parameters such as intensity thresholds, size filters, and autofocus conditions. These settings directly influence the resulting particle metrics and thus require careful tuning. Laser power and integration time were defined prior to the measurement.

Once the measurement was initiated, the extracted particle coordinates were analyzed sequentially. For each detected particle (Fig. 1, top right), a Raman spectrum was acquired and linked to its corresponding morphological parameters from the visual image.

Finally, material identification was performed through database matching of the acquired spectra, completing the automated analysis workflow from particle detection to chemical classification. The identified particles were correctly classified as polystyrene.

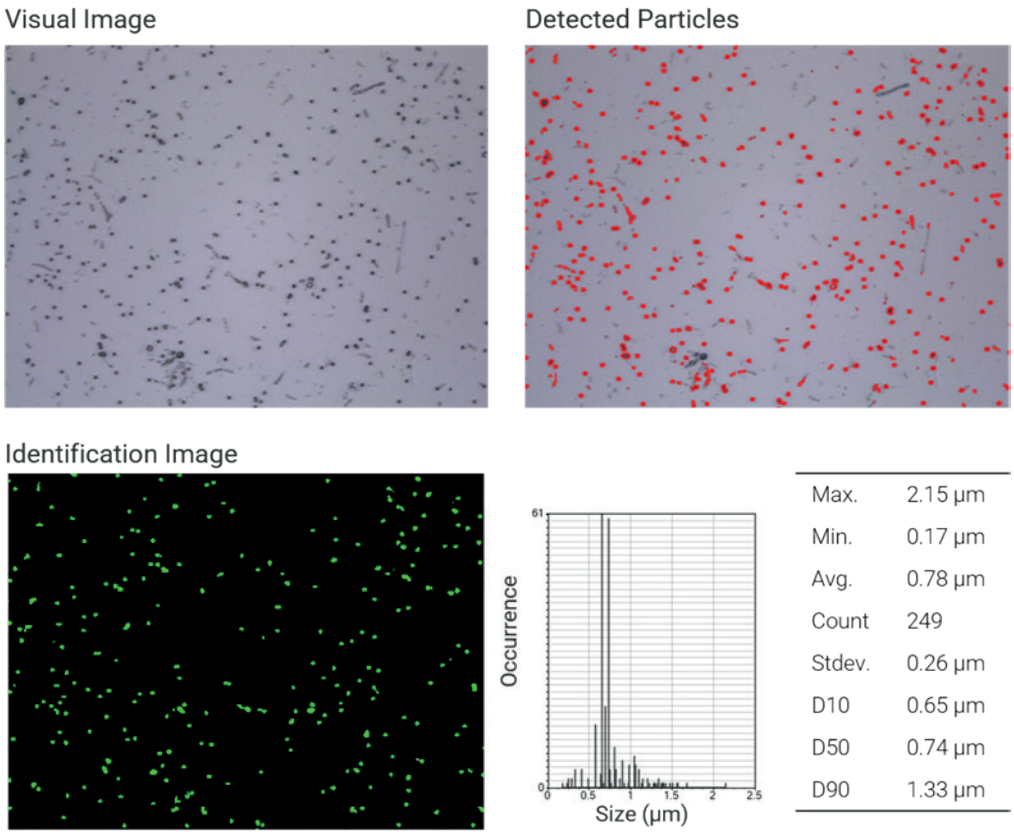

Fig. 1
NP analysis particle-by-particle.
Top left: Visual image of nanoplastics (100x objective lens).
Top right: Map of detected particles based on visual contrast.
Bottom left: Identified polystyrene particles based on the Raman spectrum.
Bottom right: Particle and size statistics.

Raman Imaging

For Raman imaging, the same field of view was analyzed using laser line scanning. In this approach, the laser power and integration time needed to be optimized to achieve a balance between fast acquisition and sufficient signal-to-noise ratio, as well as the scanning step size.

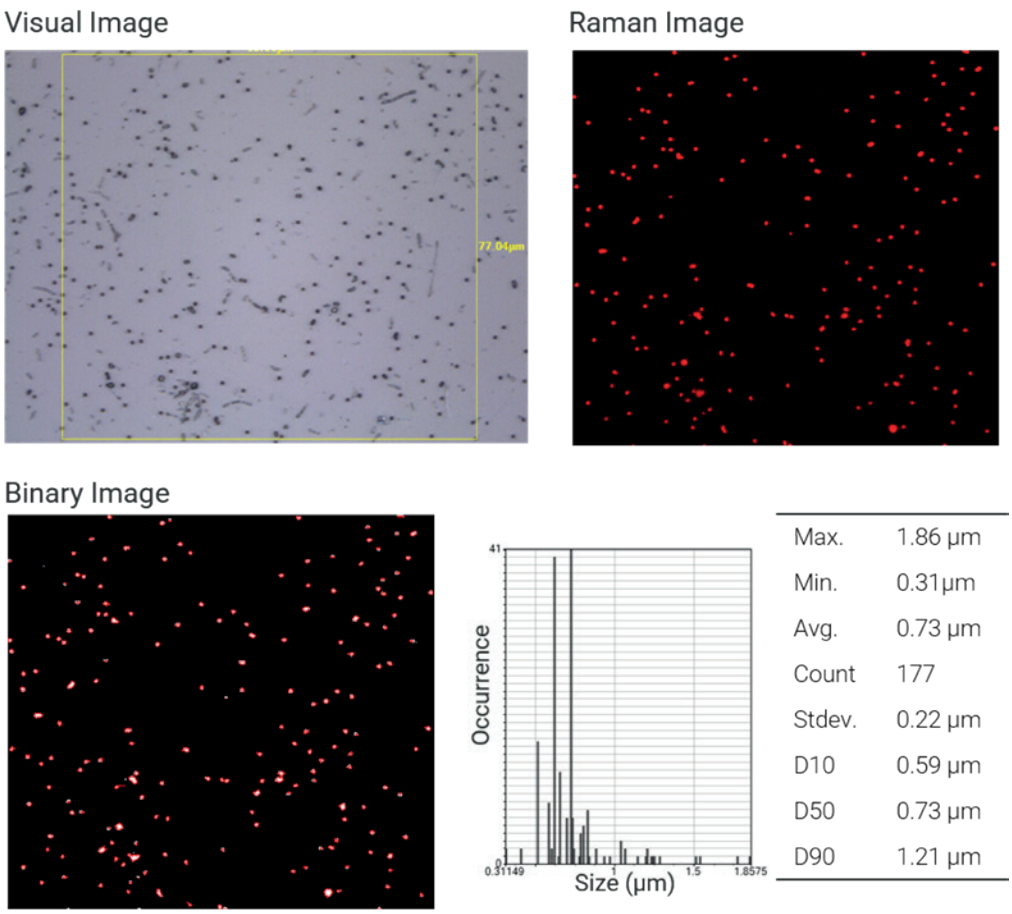

Fig. 2
NP analysis by imaging. Top left: Visual image of nanoplastics with region of interest (100x objective lens). Top right: Raman image of polystyrene particles based on correlation analysis. Bottom left: Binarization of the Raman image to extract particle statistics. Bottom right: Particle and size statistics.

The resulting Raman dataset (Fig. 2) was evaluated using correlation analysis, where reference spectra of different polymer types were applied. A strong correlation with the polystyrene reference spectrum is visualized in red, confirming the expected material composition.

Particle metrics (Fig. 2, bottom right) were subsequently derived by binarization of the correlation image. It should be noted that these metrics are highly dependent on the spatial resolution of the measurement, particularly the selected step size, which directly influences the accuracy of particle size and shape determination, as well as the binarization thresholds.

Direct Comparison

As a final verdict, both approaches enabled reliable detection and identification of nanoplastics down to submicron sizes. After excluding particles outside the Raman imaging area, comparable particle counts were obtained for both methods.

Minor differences were observed:

- Two particles detected in Raman imaging are not identified in the particle-by-particle approach (Fig. 3, white circles), and vice versa (Fig. 3, blue circles)
- Particle sizes derived from optical detection tend to be larger due to inclusion of neighboring features (Fig. 3, orange circles)
- Raman imaging-based sizing depends strongly on step size and pixel resolution

These differences arise from fundamentally different detection and evaluation strategies. Particle-by-particle analysis relies on optical pre-selection and may miss low-contrast particles, while Raman imaging provides an unbiased chemical map of the entire measurement area.

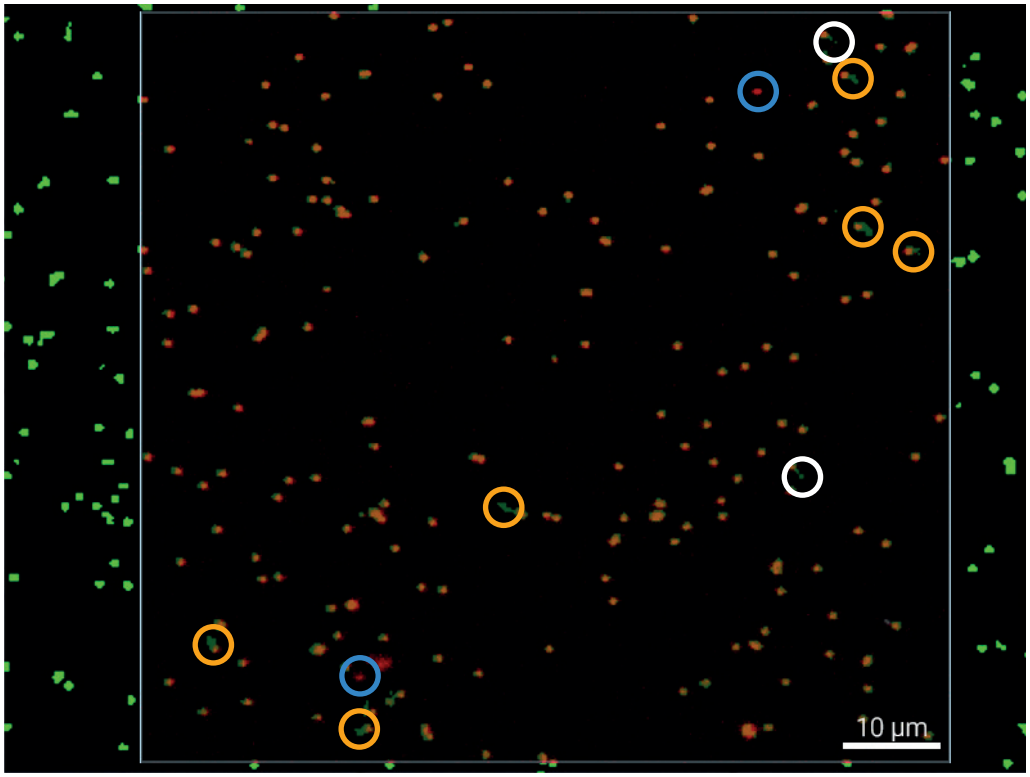

Fig. 3
Particle Analysis Overlay. Merge of particle-by-particle and Raman imaging analysis results. White circles indicate particles detected by micro-spectroscopy but missing in the Raman imaging result, while light blue circles highlight particles identified by Raman imaging but not captured in the particle-by-particle approach. Yellow circles mark discrepancies in particle size, arising from differences in the respective size determination methods.

Conclusion

Raman microscopy is a powerful and versatile tool for nanoplastics characterization, enabling chemical identification and particle-resolved analysis at the submicron scale.

Both particle-by-particle micro-spectroscopy and Raman imaging demonstrate reliable performance and yield highly comparable results in a controlled model system.

While particle-by-particle analysis offers fast and targeted measurements, Raman imaging provides unbiased, spatially resolved chemical information. The strong agreement between both approaches highlights their complementary nature.

Together, these techniques form a robust and flexible analytical workflow for nanoplastics analysis and provide a strong foundation for future investigations of complex mixtures and real-world samples.

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