Funded by
the European Union

InPlasTwin

Increasing expertise in micro- and nanoplastics analysis through twinning action

Horizon Europe Grant agreement ID: 101160289

D5.2 Knowledge exchange on MNPs analysis strategies: Developed enhanced understanding of the applicability of various complementary analytical techniques

inplastwin.eu

Author(s)/Organisation(s)	André Marcel Bienfait /IMR
Contributor(s)	Majda Nikezić and Janja Vidmar/JSI
Reviewers	All authors and contributors
Work Package Title	WP5 – Knowledge exchange in analytical techniques for the MNPs detection
Linked task title	Task 5.1 Improve knowledge on the analysis of MNPs with spectroscopic techniques Task 5.2 Improve knowledge on the analysis of MNPs with mass spectrometric techniques Task 5.3 Virtual training: Enhancing MNPs analysis techniques knowledge exchange
Partner responsible	IMR
Actual Delivery Date	30.3.2026
Dissemination Level	Public
Abstract:	This deliverable summarizes the knowledge acquired through hands-on and online training activities carried out within Work Package 5 (WP5), focusing on analytical strategies for the identification and characterization of micro- and nanoplastics (MNPs). It emphasizes the enhanced understanding of the applicability, advantages, and limitations of complementary spectroscopic and mass spectrometric techniques, supporting the informed selection of appropriate methods for specific research objectives. Furthermore, it provides a foundation for selecting suitable analytical approaches to detect MNPs in soil and strawberry samples resulting from mulching film degradation, thereby informing upcoming research activities within the InPlasTwin project.

Document Revision History

Date	Version	Author/Contributor/ Reviewer	Summary of main changes
27-1-2026	1	Janja Vidmar	Drafted Deliverable outline and Chapter 3 summary
11-3-2026	2	Majda Nikezić	Prepared the first draft on the applicability of different techniques used at IMR
27-3-2026	3	Janja Vidmar	Finalizing the Introduction section, slightly reorganizing the structure of the deliverable, describing µRaman spectroscopy and electron microscopy
28-3-2026	4	Janja Vidmar	Finalizing the Conclusions section, describing the multi-detector FFF method
30-3-2026	5	André Marcel Bienfait, Janja Vidmar	Final revision

Participant Number	Participant organization name	Short name	Country
1.	INSTITUT JOZEF STEFAN	JSI	Slovenia
2.	GEOPONIKO PANEPISTIMION ATHINON	AUA	Greece
3.	UDRUZENJE ZA PREDUZETNISTVO I INOVACIJE FOODSCALE HUB	FSH	Serbia
4.	VLAAMSE INSTELLING VOOR TECHNOLOGISCH ONDERZOEK N.V.	VITO	Belgium
5.	HAVFORSKNINGSINSTITUTTET	IMR	Norway
6.	DANMARKS TEKNISKE UNIVERSITET	DTU	Denmark

Disclaimer

...

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them.

Title: Increasing expertise in micro- and nanoplastics analysis through twinning action

Type of action: HORIZON Coordination and Supporting Action

Topic:HORIZON-WIDERA-2023-ACCESS-02-01

Start date of project: 1 October 2024 • Duration: 36 months

Table of Contents

List of Figures

List of Tables

List of Abbreviations and Acronyms

FPA	Focal plane array
NPs	Nanoplastics
MPs	Microplastics
MNPs	Microplastics and nanoplastics
IR source	Infrared source
ATR-FTIR	Attenuated Total Reflection Fourier Transform Infrared spectroscopy
µFTIR	Fourier Transform Infrared microscopy
QCL-µIR	Quantum Cascade Laser Infrared microscopy
py-GC-MS	Pyrolysis–gas chromatography–mass spectrometry

JSI	Jožef Stefan Institute
AUA	Agricultural University of Athens
IMR	Institute of Marine Research
VITO	Flemish Institute for Technological Research
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
FFF	Field-flow fractionation
AF4	Asymmetrical flow FFF
EDS	Energy-dispersive X-ray spectroscopy
MALS	Multi-angle light scattering
ICP-MS	Inductively coupled plasma mass spectrometry
SERS	Surface-enhanced Raman spectroscopy
TERS	Tip-enhanced Raman spectroscopy
WP	Work package
DLS	Dynamic light scattering

1. Introduction

Mulching films are extensively used in crop cultivation to enhance soil temperature, conserve moisture, and suppress weed growth, with strawberry production being a key application (Salama & Geyer, 2023). However, in the environment, these polymers undergo fragmentation and degradation under environmental stresses (e.g., UV radiation, mechanical abrasion, microbial activity), generating micro- and nanoplastics (MNPs) that may accumulate in soil and potentially be taken up by plants (Bai et al., 2024; Carneiro et al., 2025). The presence of such low-concentration MNPs in soil and plant matrices presents significant analytical challenges due to their small size, heterogeneous properties, and complex sample composition (Cowger et al., 2020).

Accurate detection and characterization of MNPs in complex matrices require a combination of complementary analytical approaches. Vibrational spectroscopic methods such as Fourier Transform Infrared spectroscopy (FTIR) and Raman spectroscopy are powerful for polymer identification and, when coupled with microscopy, can provide particle-level chemical imaging. FTIR is non-destructive and suitable for many polymer types, but its diffraction-limited spatial resolution (≈5–20 µm) restricts its applicability for smaller particles and very low concentrations in environmental and food samples (Rosso et al., 2023). Raman spectroscopy offers higher spatial resolution (down to ≈1 µm) and improved fingerprint specificity, and advanced modalities such as surface-enhanced Raman spectroscopy (SERS) and tip-enhanced Raman spectroscopy (TERS) can extend detection into the nanoscale; however, Raman is sensitive to fluorescence interference, requires careful sample preparation, and remains challenging for quantification at trace levels (Dong et al., 2024). Emerging innovations such as quantum cascade laser (QCL)-based IR microscopy and machine learning–assisted spectral classification further enhance detection limits, throughput, and polymer discrimination, though such approaches involve complex instrumentation and significant data processing demands.

Mass spectrometric approaches, including pyrolysis–gas chromatography–mass spectrometry (py–GC–MS), provide highly sensitive and specific quantitative information on polymer composition and associated additives by thermally decomposing particles into diagnostic fragments. The method is effective across a wide size range and independent of particle morphology, enabling detection of low-mass contaminants; however, it is inherently destructive, requires extensive sample preparation and calibration, and does not yield particle-level size or shape information (Okoffo et al., 2023 and 2024). Electron microscopy techniques (scanning electron microscopy - SEM and transmission electron microscopy - TEM) and field-flow fractionation (FFF) coupled with multi-detector systems allow, on the other hand, detailed size-resolved and morphological characterization, offering valuable insights into particle shape, aggregation state, and size distributions. These physical characterization methods are sensitive to nanoscale features, but they are time-intensive, require high expertise, and typically provide limited chemical identification without corroborating spectroscopic or mass spectrometric data (Huber et al., 2023).

WP5 aims to strengthen the analytical capacity of researchers at JSI and AUA for the characterization of MNPs following their extraction from complex matrices, as established in Work Package (WP) 4. Targeted hands-on training activities implemented within Tasks 5.1 and 5.2, as well as virtual training and consultations organized under Task 5.3, included both spectroscopic and mass spectrometric approaches, enabling researchers to select the most appropriate analytical strategy for specific research and monitoring needs. Together, these activities facilitated the transfer of expertise,

discussion of methodological challenges, and a broader understanding of the practical application of complementary analytical techniques used for MNP identification and characterization.

Deliverable D5.2 supports this objective by providing an enhanced and comprehensive understanding of the applicability of complementary analytical techniques. The analytical techniques presented in this deliverable encompass the primary instruments and approaches employed in WP5 for the analysis of MNPs. It compares instrument configurations, performance, strengths and limitations, and their suitability for specific applications, particularly in relation to the needs and long-term strategies of the participating research organizations.

The deliverable reflects the progress made in strengthening methodological knowledge and analytical capacity within the consortium, thereby contributing to the broader goal of improving research capabilities in the field of MNP analysis. Rather than identifying a single “best” technique, the focus is on evaluating how different instrumental setups and configurations perform in practice and how they address specific analytical requirements. Such comparisons are not intended to rank different analytical techniques, but to assess how each approach aligns with defined analytical objectives and institutional contexts.

This work also represents an important step towards deciding which analytical technique(s) to apply to the analysis of MNPs expected in soil and strawberry samples arising from mulching film degradation during strawberry cultivation. These applications will be addressed in subsequent phases of the InPlasTwin project through hydroponic (WP7) and field-scale (WP8) experiments.

2. Analytical Techniques

2.1. µRaman spectroscopy

Micro-Raman (µRaman) spectroscopy is based on the inelastic scattering of monochromatic laser light, where the interaction between the incident light and molecular vibrations produces a characteristic Raman spectrum. When coupled with a microscope, this technique enables spatially resolved chemical analysis of individual particles, allowing precise targeting and identification within heterogeneous samples.

The main analytical output consists of Raman spectra that provide distinct molecular fingerprints of polymers, as well as chemical maps when imaging modes are applied. The technique is typically applicable to particles in the size range of approximately 1 µm up to several hundred micrometers. Under optimized conditions, detection below 1 µm is possible, although reliable characterization of true nanoplastics (NPs) remains challenging.

µRaman spectroscopy provides detailed information on polymer type and chemical composition, and can also reveal the presence of pigments and certain other additives. When combined with mapping approaches, it enables visualization of particle distribution within complex matrices. Its key strengths include high spatial resolution, mainly non-destructive analysis, and suitability for analyzing low-micrometer plastic particles. It is often used as a complementary technique to FTIR spectroscopy for particle-level identification.

Sample preparation for µRaman analysis is generally relatively simple compared to many other techniques, but it remains a critical step, especially for environmental and food samples. Typically,

samples require pre-treatment to remove organic matter and isolate particles, often involving digestion (e.g., enzymatic, oxidative, or chemical treatments) followed by filtration onto suitable substrates such as aluminum-coated filters, glass fiber filters, or silicon wafers. Clean, flat, and non-fluorescent substrates are preferred to minimize background interference. Care must be taken to avoid contamination and to ensure that particles are well-dispersed and not overlapping, as this can affect spectral quality and identification accuracy.

However, the technique also has limitations. Fluorescence interference from organic residues or weathered particles can obscure Raman signals, and data acquisition, especially for mapping, can be time-consuming. Sensitivity decreases for nanoscale particles, and there is a risk of laser-induced sample degradation. In addition, quantitative analysis remains challenging.

In the context of MNPs analysis, µRaman spectroscopy is widely used for the identification and characterization of microplastics (MPs) in environmental and food samples. It is particularly valuable for confirming polymer composition and assessing particle distribution in complex matrices, especially when appropriate sample preparation ensures clean and representative particle isolation.

In terms of maintenance and overall cost, µRaman spectroscopy generally involves a higher initial investment and slightly higher maintenance expenses compared to techniques such as Fourier Transform Infrared microscopy (µFTIR). However, these costs can be partly compensated by reduced sample preparation requirements, lower consumption of reagents and consumables, and greater analytical versatility. µFTIR remains more cost-effective and easier to maintain, making it well-suited for routine analyses, whereas µRaman spectroscopy is typically preferred when higher spatial resolution or more specialized analytical capabilities are needed.

2.2. Infrared (IR) spectroscopic techniques

2.2.1. Macroscopic IR spectroscopy (ATR-FTIR)

Attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) is one of the most widely used and accessible techniques for polymer identification in microplastics laboratories. In this approach, infrared radiation undergoes total internal reflection within an ATR crystal, typically made of diamond or another high-refractive-index material, generating an evanescent wave that penetrates a few micrometers into the sample in contact with the crystal surface. The resulting spectrum provides characteristic infrared absorption bands that allow reliable identification of polymer types based on their molecular structure.

In the context of MPs analysis, ATR-FTIR is primarily used for the characterization of bulk plastic materials and microplastic particles typically larger than ~300 µm. The technique requires minimal sample preparation, as fragments, films, or particles can be analyzed directly by pressing them against the ATR crystal. This enables rapid measurements, high sample throughput, and reduced contamination risk, which are important considerations in microplastics laboratories. However, ATR-FTIR requires particles to be brought into direct contact with the ATR crystal and relies on sufficient sample volume within the infrared interaction depth to generate a measurable signal. For this reason, the technique is unsuitable for the analysis of smaller MPs and NPs. Consequently, it is typically complemented by techniques such as µFTIR imaging or mass-spectrometric approaches that allow analysis of smaller particle size ranges.

For laboratories establishing microplastics analysis capabilities, a macroscopic ATR-equipped FTIR system represents a valuable foundational instrument. Although it does not replace microscopic techniques required for small-particle analysis, it provides an efficient, cost-effective, and robust solution for screening, training, and routine polymer identification. For these reasons, ATR-FTIR is widely considered a core analytical tool in microplastics laboratories.

2.2.2. Microscopic IR spectroscopy and imaging

2.2.2.1. µFTIR

Micro-Fourier transform infrared spectroscopy (µFTIR) represents one of the most widely applied analytical techniques for the identification and characterization of MPs in environmental and food-related samples. During the training, the Agilent Cary 620/670 µFTIR imaging system was used, which combines a Cary 670 FTIR spectrometer with a Cary 620 infrared microscope equipped with a focal plane array (FPA) detector. This configuration enables automated chemical imaging of sample surfaces, allowing the simultaneous acquisition of thousands of infrared spectra across a defined measurement area. In a typical microplastics workflow, samples are first subjected to appropriate preparation steps, such as organic matter digestion, density separation, and filtration onto infrared-transparent membranes (e.g., aluminum oxide or PTFE filters). The prepared filters are then placed directly under the microscope, where hyperspectral infrared images are collected in reflection or transmission mode. Each pixel in the resulting image corresponds to a full infrared spectrum, enabling the chemical identification of individual particles across the analyzed area. Data processing involves spectral pre-treatment, comparison with polymer spectral libraries, and automated particle detection using specialized software or machine-learning-based classification tools. This approach allows the simultaneous determination of particle number, size distribution, morphology, and polymer composition within a single measurement.

Compared with macroscopic ATR-FTIR, µFTIR significantly extends the analytical capability toward smaller particle sizes, typically enabling reliable identification of MPs down to approximately 10-20 µm depending on the detector configuration and measurement parameters. The technique, therefore, represents a core analytical tool for microplastics research and monitoring laboratories. The Agilent Cary 620/670 system is particularly advantageous due to its FPA-based imaging capability, which enables relatively rapid acquisition of large hyperspectral datasets compared with point-by-point measurements. However, µFTIR analysis remains time- and data-intensive, as large datasets require substantial processing and interpretation. In addition, the instrumentation and maintenance costs are considerably higher than for macroscopic FTIR systems, and careful sample preparation is essential to avoid filter contamination and spectral interferences. Despite these challenges, µFTIR provides comprehensive chemical and morphological information for hundreds or even thousands of particles within a single sample, making it one of the most powerful and widely accepted techniques for routine MPs analysis.

It was noted that the Cary 620/670 system will no longer be in active production, raising considerations regarding long-term instrument sustainability, including the availability of spare parts, service support, and software updates. Although the instrument remains scientifically robust and fully functional, these factors are important when planning new laboratory investments. Taken together with the limited throughput and lack of full automation, the system may be less suitable for laboratories currently establishing or expanding MP analysis infrastructure, where long-term reliability, scalability, and operational efficiency are key strategic requirements.

2.2.2.2. QCL-µIR

Quantum cascade laser–based micro-infrared spectroscopy (QCL-µIR) represents a newer alternative to conventional µFTIR imaging for the analysis of MPs. While both techniques operate in the mid-infrared region and rely on the detection of characteristic polymer absorption bands, their operating principles differ substantially. In contrast to µFTIR systems that use a broadband thermal IR source and collect full spectra simultaneously, QCL-µIR instruments employ tunable quantum cascade lasers that emit high-intensity radiation at selected wavelengths. Spectral information is therefore acquired sequentially across diagnostically relevant parts of the infrared spectrum, typically within the fingerprint region. This targeted acquisition approach - in combination with the high light intensity of the QCL - significantly reduces measurement times and enables faster chemical imaging compared with traditional µFTIR systems. While this approach is generally sufficient for the identification of common polymer types, it may present limitations in more complex cases involving degraded materials, additives, or uncommon polymers. This is particularly relevant for emerging polymer types such as biodegradable plastics, where commercially available spectral libraries may be incomplete, requiring laboratories to generate dedicated reference datasets for reliable classification.

Sample preparation for QCL-µIR analysis follows a workflow similar to that used for µFTIR imaging, including matrix digestion, density separation, and filtration of the extracted particles onto infrared-compatible filters. The filters are then placed in the instrument for automated imaging and polymer identification based on spectral libraries and classification algorithms. During the training, it was demonstrated that batch analysis of multiple filters could be achieved through a custom Python script developed by the hosting laboratory, enabling sequential automated analysis of up to six samples. This approach substantially increased analytical throughput compared with conventional µFTIR workflows, where measurements are performed on a sample-by-sample basis.

From an operational perspective, QCL-µIR offers several practical advantages for routine laboratory use. The system operates with a room-temperature detector and does not require liquid nitrogen cooling, which simplifies daily operation and reduces consumable costs. Based on the experience shared by the hosting staff, the instrument also demonstrated high operational stability, with very limited downtime and minimal troubleshooting requirements during routine use. However, QCL-µIR remains a relatively recent technology in the field of microplastics research, and its adoption is still limited compared with established µFTIR systems. Consequently, fewer laboratories currently employ this technique, which may present challenges when comparing results across studies or establishing broadly standardized analytical workflows. Despite these limitations, the high acquisition speed, automation potential, and operational robustness make QCL-µIR a promising complementary technique for high-throughput MPs analysis.

2.3. Py-GC-MS

Pyrolysis gas chromatography-mass spectrometry (py-GC-MS) is a widely used analytical technique for the identification and quantification of MNPs based on their chemical composition. In this approach, polymer particles are thermally decomposed in a pyrolyzer, generating characteristic volatile degradation products that are subsequently separated by gas chromatography and detected by mass spectrometry. The resulting chromatograms and mass spectra provide information on polymer type, as well as the presence of additives and degradation products. Unlike spectroscopic techniques, py-GC-MS does not rely on particle size and is therefore applicable to a broad range of MNPs materials.

Due to its high sensitivity and quantitative capabilities, py-GC-MS is particularly suitable for the analysis of complex environmental and food matrices. In addition, the instrumentation is often relatively accessible compared with specialized microplastic imaging systems. At the same time, several limitations must be considered. Because the method relies on thermal degradation of the sample, the analysis is inherently destructive and does not provide information on particle size, number, or morphology. In addition, py-GC-MS workflows require careful control of contamination and blank measurements, as polymer-related signals may appear in blanks due to background contamination or carry-over effects, potentially leading to false-positive results if not properly addressed. The interpretation of pyrolysis chromatograms also requires substantial expertise, as multiple polymers, additives, and degradation products may produce overlapping signals that complicate data evaluation. Despite these challenges, py-GC-MS represents a promising complementary technique for MNPs analysis, particularly for mass-based identification and quantification in complex samples.

Within the project, participants had the opportunity to receive hands-on training on py-GC-MS at two partner institutions, IMR and VITO. Both laboratories operated the same pyrolysis unit (Frontier EGA/PY-3030D Multi-Shot Pyrolyzer), ensuring comparable thermal degradation and sample introduction conditions. However, the pyrolyzer was coupled to different GC-MS platforms at the two institutions, allowing trainees to gain experience with two complementary analytical configurations. At VITO, the system was coupled to a conventional single-quadrupole GC-MS instrument, representing a widely used and robust setup for routine polymer identification and quantification. In contrast, the system at IMR was coupled to a high-resolution Orbitrap GC-MS platform, providing enhanced mass accuracy and resolution that enable more detailed characterization of pyrolysis products and improved selectivity in complex matrices.

This provided a valuable opportunity to compare the analytical capabilities of both configurations. While the quadrupole-based system represents a cost-effective and well-established solution suitable for routine targeted analysis of common polymers, the Orbitrap-based system offers advanced analytical performance for research-oriented applications, including additive identification and non-targeted screening of degradation products. At the same time, high-resolution systems are associated with substantially higher acquisition and operational costs and require greater expertise in data interpretation. Exposure to both configurations, therefore, allowed participants to better understand the trade-offs between analytical performance, operational complexity, and cost when selecting instrumentation for MNPs analysis. This comparison was particularly valuable for the JSI team, as it provided practical insights supporting future decisions related to the development of analytical infrastructure for MNPs research.

2.4. Stereomicroscopy

Stereomicroscopy supports the preliminary assessment of particle characteristics such as size, shape, and color. The technique is particularly useful for particles in the several hundred µm size range and above, where visual characteristics can provide initial indications of polymeric origin. In addition, stereomicroscopy contributes to quality control by helping to identify potential contamination, non-plastic materials, or artifacts introduced during sample preparation.

From an operational perspective, stereomicroscopes are simple to operate, require minimal sample preparation, and are widely available in analytical laboratories. As such, they represent a cost-effective and practical component of MNP analytical workflows, improving overall efficiency by

guiding more targeted and informed use of more advanced and resource-intensive analytical techniques.

Although visual inspection under a stereomicroscope can be time-consuming for particle-rich samples, it generally improves overall workflow efficiency by allowing analysts to pre-select suspicious particles and exclude obvious non-plastic materials before more time-intensive spectroscopic analyses such as µFTIR imaging.

2.5. Electron microscopy

Electron microscopy (EM) techniques are commonly used for the characterization of MNPs, providing detailed morphological and structural information. Scanning Electron Microscopy (SEM) operates by scanning the sample surface with an electron beam and detecting secondary or back-scattered electrons, producing high-resolution images of particle surface topography. SEM is applicable to particles from approximately 10 nm to several micrometers and provides information on particle shape, size, surface texture, and aggregation state. When coupled with energy-dispersive X-ray spectroscopy (EDS), elemental composition can also be obtained. Its main strengths are very high spatial resolution and detailed surface characterization, allowing visualization of features such as cracks, pits, roughness, and biofilm formation that are particularly typical of weathered MPs. Limitations of SEM include minimal chemical information without EDS, the need for conductive samples or metal coating, and potential sample alterations in the vacuum. Typical applications in MNPs analysis include morphology assessment, aggregation studies, and size verification, with sample preparation involving drying, mounting, and coating of non-conductive polymers.

Transmission Electron Microscopy (TEM) uses an electron beam transmitted through ultra-thin sample sections to generate high-resolution images of internal particle structures. TEM can resolve particles from ~1 nm up to several hundred nanometers, providing information on internal morphology, aggregation, and in some cases crystallinity or elemental composition when combined with EDS or electron energy loss spectroscopy (EELS). Strengths include ultra-high resolution and the ability to visualize NPs and internal structures, whereas limitations involve complex and destructive sample preparation, analysis restricted to thin or small particles, and potential alterations due to the vacuum environment. TEM is typically applied in MNPs analysis for characterization of NPs, assessment of internal morphology, and identification of sub-100 nm particles. Sample preparation requires deposition of ultra-thin sections or dispersed NPs on TEM grids, often with contrast agents to improve visualization of low-density polymers.

Together, SEM and TEM provide complementary information on MNPs' morphology and size. Both techniques, however, have limitations, providing minimal chemical identification, so they are generally used in combination with spectroscopic methods such as Raman or FTIR for polymer type confirmation. When applied to the MNPs in complex environmental or food samples, embedded in organic matter or present at very low concentrations, careful isolation of MNPs is required to avoid their alteration. TEM in particular is time-intensive and low-throughput, limiting its suitability for large-scale screening.

2.6. Field-flow fractionation

A field-flow fractionation (FFF) is a non-destructive technique that provides high-resolution size fractionation of complex, polydisperse MNP samples. Through size-based separation under mild

conditions, FFF enables the analysis of heterogeneous particle populations while preserving their physicochemical integrity. FFF separates particles based on their size-dependent hydrodynamic behavior in a laminar flow channel without the use of a stationary phase. In asymmetrical flow FFF (AF4), the most commonly used variant for MNP analysis, particles are directed by a cross-flow toward a semipermeable membrane, where they establish equilibrium positions according to their size and diffusivity. Smaller particles diffuse further from the membrane and elute earlier, while larger particles remain closer to the wall and elute later, achieving size-based separation.

When coupled with multiple detectors, FFF provides a versatile platform for the comprehensive characterization of MNPs. When hyphenated with detectors such as dynamic light scattering (DLS) or multi-angle light scattering (MALS), it allows the determination of particle size distributions and, in combination, supports the assessment of particle conformation and shape. For instance, when coupled with DLS, which provides intensity-weighted hydrodynamic size distributions for particles in the 1 nm to 1 µm range, FFF can be effectively applied to estimate the size of NPs in suspensions, with matrix constituents being removed by FFF prior to DLS analysis. The integration of spectroscopic techniques, including UV–Vis, FTIR, or Raman spectroscopy, further enables polymer identification, while offline coupling with py-GC-MS provides detailed chemical fingerprinting (Loeschner et al., 2022). In addition, coupling with inductively coupled plasma mass spectrometry (ICP-MS) enables the detection of elemental tracers, additives, or metal-containing components associated with MNPs, thereby supporting particle quantification and offering complementary compositional information. Overall, concentration-related data can be obtained through optical detectors or complementary quantitative approaches, depending on the analytical configuration.

However, the FFF method is complex and requires careful sample preparation and measurement optimization. Separation efficiency can be affected by particle–matrix interactions, necessitating careful tuning of cross-flow and carrier conditions depending on the sample type. Detection limits depend on the sensitivity of the coupled detectors, making the quantification of low concentrations of NPs challenging without sample pre-concentration. When combined with offline polymer identification analyses, this approach lowers throughput and makes data integration across techniques more complex.

3. Summary of Key Characteristics of Analytical Techniques for MNP Analysis

To facilitate comparison of analytical techniques presented in the deliverable, the key characteristics of these techniques are summarized in Table 1. The table provides an overview of the analytical technique, main analytical output, applicable particle size range, type of information obtained, as well as their principal strengths and limitations. In addition, typical applications of each technique in MNP analysis are highlighted. This summary provides a concise reference for the complementary roles of the analytical approaches presented in this Deliverable and can be used as a guide for selecting the most appropriate technique based on the research objective and the characteristics of the targeted MNPs, including polymer type and particle size.

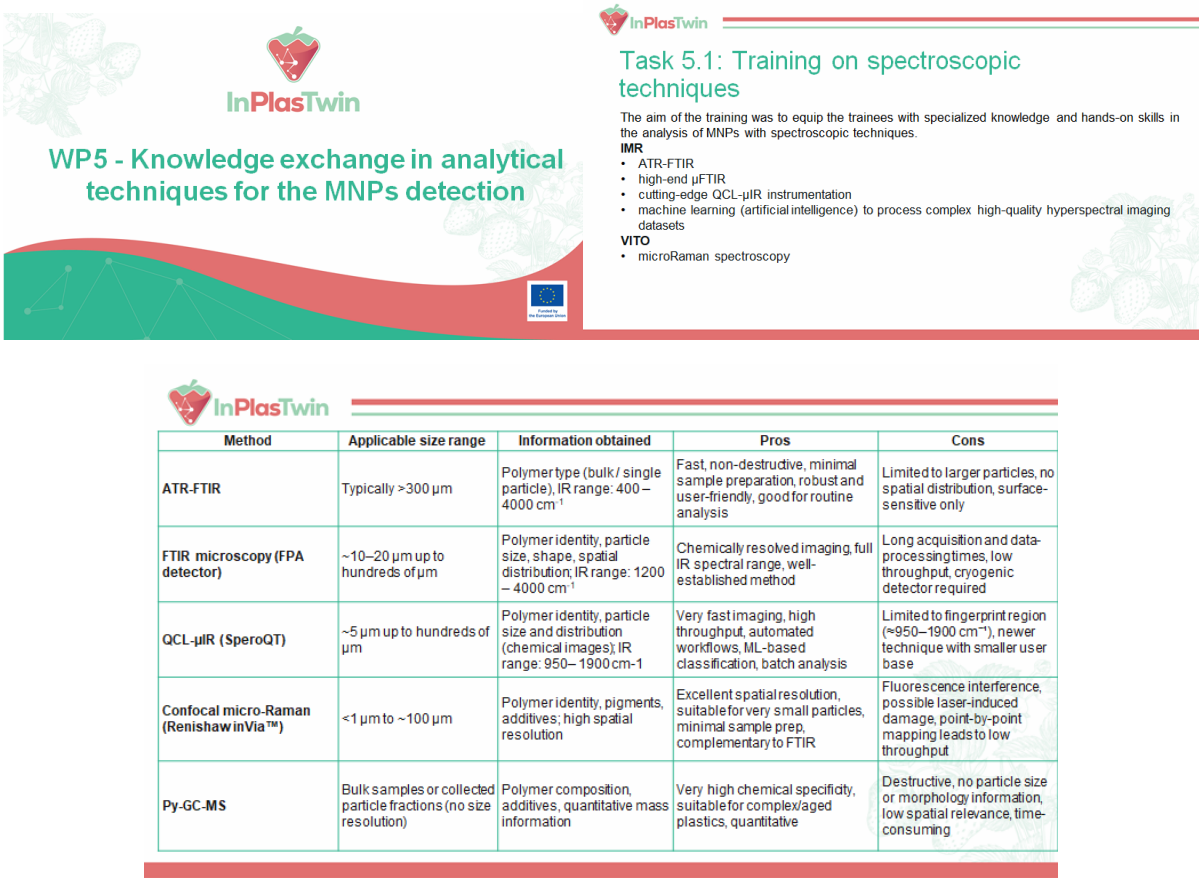
Table 1. Comparison of analytical techniques for micro- and nanoplastics (MNPs) analysis

Technique	Main analytical output	Applicable size range	Information obtained	Strengths	Limitations	Typical use in MNPs analysis
µRaman spectroscopy	Raman spectra of distinct molecular fingerprints of polymers	<1 µm to ~100 µm	Polymer type, particle size and shape, particle counting	High spatial resolution, suitable for very small particles	Fluorescence interference, possible laser-induced damage, point-by-point mapping lead to low throughput	Identification and size characterization of small microplastics
ATR-FTIR	IR spectra (surface-sensitive)	>~300 µm	Polymer type (surface chemistry)	Minimal sample preparation, rapid, non-destructive analysis, user-friendly, suitable for routine analysis	Limited to larger particles, no spatial distribution, surface-sensitive only	Bulk or single-particle polymer identification
FTIR Microscopy (µFTIR)	IR spectra with spatial resolution	~10 µm–up to hundreds of µm	Polymer type, particle size and shape, particle counting	Established method, automated particle analysis, robust libraries, full IR spectral range (1200 – 4000 cm^{-1})	Limited for nanoplastics, lower spatial resolution, long acquisition and data-processing times, low throughput	Identification and counting of microplastics on filters
QCL-µIR	IR spectra with high spatial resolution	~>5 µm–up to hundreds of µm	Polymer type, particle size and shape, particle counting	High spatial resolution compared to conventional µFTIR, fast imaging, high throughput, automated workflows	Limited availability with a smaller user base, emerging method, limited to fingerprint region (≈950–1900 cm^{-1})	Advanced Identification and counting of microplastics on filters
Py-GC-MS	Chromatograms and mass spectra	No size resolution (bulk analysis)	Polymer composition, additives, degradation products, mass quantification	High sensitivity and selectivity, quantitative, suitable for complex matrices	Destructive, no particle size or morphology information	Mass-based identification and quantification of MNPs
FFF coupled to multiple detectors	Size-separated fractions of particles and associated signals (depending on the detector used)	~1 nm–several µm (depending on the FFF variant)	Hydrodynamic size distributions, particle shape, molecular weight, chemical composition, and particle concentration (depending on the detector used)	Gentle, non-destructive size separation of particles in polydisperse and heterogeneous samples, suitable for both micro- and nanoscale particles	Method complexity, requires careful sample preparation and measurement optimization .	Size fractionation and characterization of heterogeneous MNP mixtures
Microscopy-based techniques	Images	nm–mm (technique-dependent)	Particle size, shape, surface morphology, particle counting	Direct visualization, wide size coverage	Limited or no chemical identification (without any coupling to e.g. EDS)	Visual identification, morphology assessment, size classification

4. Overview of Virtual Trainings

Within this framework, Task 5.3 (Virtual training: Enhancing MNPs analysis techniques knowledge exchange) complemented the hands-on training activities carried out in Tasks 5.1 and 5.2. The task provided a platform for online interactions among consortium partners to discuss methodological challenges, analytical strategies, and practical aspects related to MNP analysis. These virtual exchanges support the interpretation of experimental results, facilitate the sharing of expertise across institutions, and ensure continuous communication throughout the implementation of the training activities. Overall, these virtual trainings and consultations effectively supported knowledge exchange and collaboration among the project partners

Following the training, the main learning outcomes were presented in an online session held on the 27th of January 2026, where Janja Vidmar (JSI) and Majda Nikezić (JSI) shared their experiences from the training at IMR. They emphasized the insights gained into emerging spectroscopic and mass spectrometric techniques and provided a critical evaluation and comparison of various analytical methods, considering their applicable size range, types of information obtained, strengths, and limitations (Figure 1).



Method	Applicable size range	Information obtained	Pros	Cons
ATR-FTIR	Typically >300 μm	Polymer type (bulk / single particle), IR range: 400 – 4000 cm⁻¹	Fast, non-destructive, minimal sample preparation, robust and user-friendly, good for routine analysis	Limited to larger particles, no spatial distribution, surface-sensitive only
FTIR microscopy (FPA detector)	~10–20 μm up to hundreds of μm	Polymer identity, particle size, shape, spatial distribution; IR range: 1200 – 4000 cm⁻¹	Chemically resolved imaging, full IR spectral range, well-established method	Long acquisition and data-processing times, low throughput, cryogenic detector required
QCL-μIR (SperoQT)	~5 μm up to hundreds of μm	Polymer identity, particle size and distribution (chemical images); IR range: 950– 1900 cm-1	Very fast imaging, high throughput, automated workflows, ML-based classification, batch analysis	Limited to fingerprint region (≈950–1900 cm⁻¹), newer technique with smaller user base
Confocal micro-Raman (Renishaw inVia™)	<1 μm to ~100 μm	Polymer identity, pigments, additives; high spatial resolution	Excellent spatial resolution, suitable for very small particles, minimal sample prep, complementary to FTIR	Fluorescence interference, possible laser-induced damage, point-by-point mapping leads to low throughput
Py-GC-MS	Bulk samples or collected particle fractions (no size resolution)	Polymer composition, additives, quantitative mass information	Very high chemical specificity, suitable for complex/aged plastics, quantitative	Destructive, no particle size or morphology information, low spatial relevance, time-consuming

Figure 1. Example of the PowerPoint slides presented and discussed during the online presentation on the 27th of January 2026.

5. Conclusions and Outlook

The activities carried out within WP5 significantly strengthened the analytical capacity of the participating institutions in the field of MNPs analysis. Through hands-on training and knowledge exchange activities, participants gained practical experience with several complementary analytical techniques. This provided a comprehensive understanding of the strengths and limitations of different analytical approaches and their role within the overall MNP analytical workflow.

A key outcome of the training was the recognition that no single analytical technique can address all aspects of MNP characterization. Spectroscopic imaging techniques such as µFTIR and QCL-µIR provide detailed information on particle number, size distribution, morphology, and polymer identity, whereas py-GC-MS enables mass-based polymer identification and quantification independent of particle size. The combination of these techniques therefore represents a robust strategy for comprehensive MNP analysis, particularly in complex matrices such as food and soil samples. Understanding these complementary capabilities allows researchers to select the most appropriate method or combination of methods depending on whether the focus is on particle characterization or total polymer mass. The choice of analytical technique for MNP analysis, therefore, strongly depends on the specific analytical question being addressed.

Despite many advances, several challenges still remain. Further efforts are required to optimize and harmonize sample preparation procedures for complex food and soil matrices, where high organic content complicates particle isolation. In addition, continued development of spectral libraries is needed, particularly for emerging polymer types such as biodegradable plastics. The activities also underlined the importance of strict contamination control and careful laboratory practices throughout all stages of analysis, as background contamination and procedural artifacts can influence both spectroscopic and mass spectrometric results. Strengthening these methodological aspects will be essential to improve data reliability and comparability across laboratories and analytical platforms.

6. References

Bai, Y., Zhang, H., Chen, Q., 2024. The reciprocity principle in mulch film deterioration and microplastic generation, Environ. Sci.: Processes Impacts, 2024, 26, 8-15

Carneiro, F., Martins, R., Lopes, I., 2025. Biodegradable Microplastics from Agricultural Mulch Films: Implications for Plant Growth-Promoting Bacteria and Plant's Oxidative Stress, Antioxidants (Basel), 14(2):230

Cowger, W., Steinmetz, Z., Gray, A., Munno, K., Lynch, J., Hapich, H., Primpke, S., De Frond, H., 2020. Microplastic spectral classification needs an open-source community: Open Specy to the rescue! *Anal. Chem.*, 92, 16730–16736.

Dong, X., et al., 2024. Enhancing Raman spectroscopy for micro- and nanoplastic detection using SERS and TERS. *Anal. Chim. Acta*, 1234, 341256

Huber, M.J., Ivleva, N.P., Booth, A.M., Beer, I., Bianchi, I., Drexel, R., Geiss, O., Mehn, D., Meier, F., Molska, A., Parot, J., Sørensen, L., Vella, G., Prina-Mello, A., Vogel, R. & Caputo, F., 2023. Physicochemical characterization and quantification of nanoplastics: applicability, limitations and complementarity of batch and fractionation methods. *Anal. Bioanal. Chem.*, 415, 3007–3031.

Loeschner, K., Vidmar, J., Hartmann, N. B., Bienfait, A. M., Velimirovic, M. (2023). Multi-detector asymmetrical flow field-flow fractionation for the characterization of micro- and nanoplastics. Analytical and Bioanalytical Chemistry, 415, 7–16.

Okoffo, E.D., Rauert, C., Thomas, K.V., 2023. Mass quantification of microplastic at wastewater treatment plants by pyrolysis–gas chromatography–mass spectrometry. *Sci. Total Environ.*, 856(Pt 2), 159251.

Okoffo, E.D., Thomas, K.V., 2024. Quantitative analysis of nanoplastics in environmental and potable waters by pyrolysis–GC–MS. *J. Hazard. Mater.*, 464, 133013

Rosso, P., et al., 2023. Fourier Transform Infrared Spectroscopy: An analytical technique for microplastic identification and quantification. *Infrared Phys. Technol.*, 136, 105070.

Salama, R., Geyer, R., 2023. Plastic Mulch Films in Agriculture: Their Use, Environmental Problems, Recycling and Alternatives, Environments,2023, *10*(10), 179