

Review

Analysis Methods for the Detection of Plastic Particles in Biological–Environmental Samples

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Abstract

The detection of microplastics (MPs) and nanoplastics (NPLs) in biological samples is critical for understanding their environmental distribution and investigating human exposure, bioaccumulation and their potential health effects. This review provides an overview of current approaches used to detect plastic particles in *in vitro* and *in vivo* studies, focusing on microscopic, spectroscopic, spectrometric, chromatographic and flow-cytometry-based methods. Microscopy techniques, including optical, confocal, fluorescence, scanning electron (SEM), transmission electron (TEM), cryogenic electron (cryo-EM), and atomic force microscopy (AFM), enable the visualization and characterization of MPs and NPLs. Spectroscopic approaches, such as Fourier transform infrared (FT-IR) and Raman spectroscopy, are widely employed for polymer identification through characteristic molecular fingerprints. Spectrometric techniques, including single-cell inductively coupled plasma mass spectrometry (scICP-MS) and single-cell inductively coupled plasma time-of-flight mass spectrometry (scICP-TOFMS), provide sensitive elemental analyses, while flow cytometry offers high-throughput particle detection. Chromatographic approaches, particularly double-shot gas chromatography–mass spectrometry (Py-GC/MS), enable sensitive and specific polymer characterizations. Recent technological advances, including automated and high-resolution analytical approaches, are also discussed together with practical considerations for selecting appropriate methods according to the sample type and analytical objective. Collectively, these methods contribute to the assessment of plastic particle occurrence, bioaccumulation, and biological effects, supporting future environmental monitoring, human biomonitoring and risk assessment.

Keywords: microplastics; nanoplastics; analytical methods; toxicity



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1. Introduction

From the beginning of the middle of the 20th century, the use of plastic materials started to gradually displace other traditional materials in fabric materials [1], healthcare equipment [2], packaging [3], and other applications, resulting in their increasing popularity. During the 50s of 20th century, the global production of plastics was 1.5 million tons, while in 2022, it reached a level of about 400.3 million tons.

Plastics are synthetic polymers, with the majority consisting of carbon–carbon backbones, providing them with adaptability, water- and corrosion-resistant properties, high strength-to-weight ratios, and thermal and insulation properties [4] as well as low costs

and environmental persistence. Due to this last listed property, plastic debris is currently ubiquitous [5].

Plastic pollution is a global challenge without territorial boundaries. Plastics are very lightweight, and consequently, they can migrate between water bodies, disperse through the air, and can be spread to locations at long distances due to human activities [6]. In the environment, plastics can be fragmented into smaller particles that can be classified into the following: (i) macroplastics (MAPLs), with dimensions larger than 20 mm, resulting from plastic bottles, packages, fishing nets, etc.; (ii) mesoplastics (MEPLs), with a diameter between 5 and 10 mm, meaning plastic pellets; (iii) microplastics (MPs), with dimensions between 100 nm and 5 mm; and (iv) nanoplastics (NPLs), with diameters less than 100 nm. Recent evidence suggests that the environmental behaviour of plastics is even more complex than simple fragmentation into MPs and NPLs. Mechanical and environmental factors may induce phase separation within polymer matrices, promoting the release of amorphous polymer micropollutants into aquatic environments. These findings indicate that plastic degradation generates a broader spectrum of polymer-derived contaminants than previously recognized, further emphasizing the need for sensitive analytical methods capable of detecting diverse polymeric particles and degradation products [7].

Regardless of the analytical technique employed, reliable MP and NPL analyses strongly depend on an appropriate sample. Sample digestion and density separation are essential for reducing matrix interference while preserving particle integrity. In addition, rigorous quality assurance and quality control (QA/AC) measures, including contamination control, procedural blanks and recovery tests, are necessary to minimize false-positive results and improve the reproducibility and comparability of analytical data. Consequently, sample pretreatment should be regarded as an integral component of the analytical workflow rather than merely a preliminary preparation step [8,9].

Regardless of their type, all plastics present hazards to wildlife and humans. MAPLs can entangle or trap various animals, resulting in injury, malnutrition, increased vulnerability to predators, and even death, whereas MEPLs can be mistaken for food, resulting in death from asphyxiation, malnutrition, or toxic exposure over time. Also, MPs and NPLs are readily swallowed or inhaled, producing oxidative stress, inflammation, and microbiota dysregulation; however, the long-term consequences remain to be determined. Moreover, MPs and NPLs could adsorb harmful substances, pathogens, or viruses and act as vectors for their transport onto their surfaces, enhancing their toxicity to various organisms [10,11].

According to a recent study performed at the Alfred Wegener Institute for the World Wildlife Fund (WWF), MAPLs were found in the natural habitat of 2141 marine species. Aside from the ingestion of larger plastics or entanglement, which can lead to death, other repercussions of the interaction between MPs and/or NPLs and marine organisms were constricted locomotion, modified feeding behaviour, altered growth and physiology, inflammation, and changes in reproductive systems [12].

Experimental studies, predominantly conducted using fish and rodent models, have shown that exposure to MPs and NPLs may affect multiple organ systems due to their ability to accumulate in tissues, inducing inflammation and oxidative stress and/or releasing toxic additives [13]. Inhaled NPLs may be deposited in the lungs, leading to potential tissue damage [14]. Ingested MPs and NPLs have been reported to disrupt gut microbiota, induce inflammation and compromise intestinal barrier function [10]. Following systemic takeover, NPLs may promote cardiovascular diseases such as atherosclerosis [15]. Experimental evidence also indicates that these particles may cross the blood–brain barrier, raising concerns regarding neuroinflammation and neurotoxicity, which could potentially affect cognitive function and mental health [16]. Furthermore, MPs and NPLs have been associated with adverse effects on the reproductive system, including endocrine disruption

and oxidative stress in reproductive tissues [17]. Although MPs and NPLs have been detected in a wide range of human biological samples, current evidence remains insufficient to establish definitive conclusions regarding their effects on human health.

Considering the ubiquitous contamination of the environment by plastic debris and the risks generated with respect to ecosystems and human health [18], the quantification of their concentration and the compositions' identification represent a matter of utmost importance for assessing the correlation between concentrations, types of plastic and biological effects. Apart from this, considerable attention is given to the techniques used to detect plastics in various samples.

Recently, using an analytical technique based on double-shot pyrolysis–gas chromatography/mass spectrometry (Py-GC/MS), MPs and NPLs (>700 nm) were detected in human blood at a total polymer mass concentration of 1.6 µg/mL [19]. Additionally, these particles were found in sand, seawater, mussels, cockles [20], and wastewater [21]. Different enzymatic methods have been optimized or are currently used to detect MPs [22,23] in human blood samples. Also, Fourier transform infrared microspectroscopy (µFT-IR) was used to detect MPs in human lungs [24] and stool, and an average of 20 pieces of MPs was found in a 10 g sample [25]. This method was also used in detecting MPs in environmental samples, such as soil [26], sediment [27], sand [28], water [29], and wastewater [30], as well as fish [31] and turtles [32]. A few years ago, the first analytical study using laser direct infrared (LDIR) was published, which detailed the levels of some specific MPs (polyamide (PA) and polyurethane (PU)) in the placenta, infant stools, meconium, breast milk, and baby formula [33].

Also, plastics have been detected in human [34,35], cat and dog samples [36] using Raman spectroscopy. The results revealed that polypropylene/polyethylene terephthalate-MPs (PP-/PET-MPs) were not only present in the cat liver and ileum but also in the dog lungs [33]. The same method was used for MP analysis in water, sediments [37–39], aquatic organisms [31,40,41], food and drinks [42–45], and cosmetics [46].

These are just a glimpse of the recent breakthroughs in this field and some of the detection methods utilized. Addressing the complexities of this problem necessitates precise and economical approaches for identifying plastics in a variety of shapes and sizes.

Understanding the effects of plastic pollution on the environment and human health is critical for influencing legislative decisions, educating the scientific community, and increasing awareness among the public. Considering this significant impact, the detection of MPs and NPLs in any type of sample must be done by a standardized procedure that is accessible worldwide.

A variety of reviews about MP and NPL detection methods exist, but most are focused on environmental samples [47–49]. Thus, a systematic review centred on the analytical detection techniques of currently accessible MPs and NPLs present in *in vitro* and *in vivo* samples could constitute a benefit for the members of the scientific community focused on this topic. Therefore, this review engages in the presentation of a variety of such methods with the goal of providing valuable insights into the identification and quantification of MPs and NPLs in different samples.

2. Sources, Distribution and Impact of Nano- and Microplastics on Biota

2.1. Sources and Distribution

Plastic is a synthetic compound made primarily from petroleum, natural gas or coal, and it is generally not biodegradable. Thus, it ends up in the soil or waters of lakes, rivers, seas and oceans, where it is degraded by UV radiation, waves and wind exposure, finally resulting in MPs and NPLs. The fundamental issue is that the constituent elements resulting from the breakdown of plastic particles remain in nature for several hundred or thousands

of years. Meanwhile, they pollute ecosystems, contaminating soils, bodies of water, and air, causing toxicity in wildlife [50] and in humans.

Based on their environmental spread, MP and NPL sources can be categorized as primary (15–31%) or secondary, representing the majority of MPs found in ecosystems [51]. Primary sources include synthetic fabrics, care and beauty products (microbeads, glitter, etc.), plastic pellets, and other items intentionally designed to perform these functions, as well as urban dust. The secondary sources of MPs and NPLs are represented by the degradation of primary ones through the fragmentation of larger plastic pieces unintentionally released into the environment. These plastic wastes are known as “secondary microplastics” and are created by the wear and tear of car tires or the breakdown of larger items (fish nets, bottles, plastic bags, etc.). MPs are released into the sewage water system during factory cleaning procedures or laundry. On the ground or in various bodies of water, these particles are transported by wind and waves, respectively. Rainwater also carries these particles into the sewers. Filtering smaller particles, such as those found in sewage treatment facilities, is very challenging. Tons of MPs are distributed everywhere, like on the surface of global waterways, where it is estimated that 93–236 thousand metric tons of plastic float [52].

Being insignificant in size and present in any ecosystem, MPs and NPLs have been found in various habitats—mountainous, unpopulated areas [53], and deep oceans, including the Mariana Trench [54]—reaching a unique global level of distribution. Their transport is sustained by air or sea currents, as well as sediments, etc.

The first airborne particle measurements, with the objective of establishing the quantity of atmospheric MPs, was carried out in London and revealed that these particles represent the vast majority (92%) of fine dust, reaching a concerning level of accumulation in the city centre that ranged between 575 and 1008 MPs/m²/day [55]. In more isolated mountainous places, for instance, the Pyrenees Mountains in France, sedimentation rates were lower but still increased, with an overall mean daily of 365 MPs/m²/day [56]. Additionally, Arctic snow was compared against European snow, namely an urban location in Germany and a mountainous region in the Swiss Alps. The concentration of MPs in the Arctic area was significantly lower ($0\text{--}14.4 \times 10^3 \text{ N L}^{-1}$) compared to Europe ($0.19 \times 10^3\text{--}154 \times 10^3 \text{ N L}^{-1}$). The transport of plastic particles by the wind across vast distances must be highlighted and taken into consideration regarding their impact even in unpopulated regions. The most common polymers detected in Europe were acrylates and PU, followed by nitrile, polyethylene (PE), and PA, whereas a small number of plastic particles, such as polystyrene (PS), polyvinyl chloride (PVC), polycarbonate (PC), polylactic acid (PLA), and polyimide (PI), were exclusively identified in Arctic snow [53].

2.2. Environmental and Human Health Impacts

There are three main routes of exposure to plastics: inhalation, ingestion, and skin contact.

More than 25 years ago, 43% of marine mammals were already affected by plastic pollution [57]. Moreover, the analysis of the stomach content of three True’s beaked whales (*Mesoplodon mirus*) stranded in Ireland revealed the presence of PE, polypropylene (PP), rayon and polyester MPs. Even if the exposure route was not defined, inhalation, water column exposure, targeted feeding and food chain exposure were considered possible pathways [58].

Only very limited information regarding the effects of MPs after consumption is available, even if MPs have been identified in freshwater and marine fish all over the world [59,60]. This could be correlated with the different gastrointestinal tract (GIT) morphologies of various fish species. Ingestion is the most common NPL and MP exposure route of aquatic species [61].

MP pollution and their impact on freshwater or marine fish species have already been addressed in numerous studies [62–65], but the effects are still incompletely

understood [66]. PS particles of 100 to 400 μm , administered to rainbow trout species for 4 weeks, generated no modifications in ion transport, paracellular permeability, and physiology of tight junctions in the intestine. Also, from a transcriptomic point of view, no difference in expression in cytokine genes has been noticed [67]. Some MPs induced hyperplastic epithelial layers in fish muscles, high neutrophil infiltration, morphological alterations, and elevated inflammatory cell infiltration scores [68].

Despite its importance to human existence, freshwater has not received adequate attention from the research community on this specific topic, even though MPs and NPLs enter the food chain via freshwater systems to a large extent [63].

People's exposure to MPs and NPLs is daily and constant because they inhale house dust or polluted air, ingest plastic-wrapped food and water, and, more recently, use protective masks against the COVID-19 virus [69]. Statistically, a person inhales 26 to 130 MPs per day [70] and ingests between 39×10^3 and 52×10^3 MPs, among which 4×10^3 particles are added by drinking water or 9×10^3 particles in the case of bottled water [71]. Additional exposure may occur through chewing gum, as after one hour of chewing, human saliva was found to contain approximately 25×10^4 MPs, together with detectable NPLs [72]. Evidence of human exposure has also been confirmed through the direct detection of MPs and NPLs in a wide range of biological samples, such as human blood [19], placenta [35], breast milk [33], arterial tissues [73], lungs [24,34], brain, liver [74], follicular fluid, and testes and semen [75]. This demonstrates that plastic particles can reach multiple organs following chronic exposure. This widespread occurrence highlights the importance of developing reliable and standardized analytical methods for human biomonitoring.

Dermal exposure to plastic particles remains difficult to quantify, and no reliable estimates of daily exposure through skin contact are currently available. However, potential health effects associated with dermal contact are more frequently linked to plastic additives, such as bisphenol A and phthalates, rather than to the particles themselves. In intact skin, penetration of MP particles appears to be limited, with most studies suggesting that particles larger than nanoscale ones remain largely confined to the stratum corneum. Nevertheless, dermal exposure may occur through the widespread use of cosmetic and personal care products containing synthetic polymer particles, particularly leave-on formulations that remain in prolonged contact with the skin, thus increasing the potential for exposure and interaction [76]. Analyses of cosmetic formulations indicate that plastic ingredients are frequently present, including polymers such as PE, PP, and various acrylate copolymers used as film-forming agents, viscosity regulators, or exfoliating particles [77]. For instance, an analysis of more than 7000 cosmetic and personal care products (sunscreens, hand sanitizers, shampoos, face creams, etc.) reported that over 60% contained MPs [78]. Experimental studies have also investigated the potential biological effects of MPs. A recent study evaluating three commercially available sunscreens containing plastic microspheres on HaCaT keratinocyte cells showed that a dose of 100 mg/L led to a significant decrease in cell viability and lysosomal and mitochondrial numbers and a 0.1 μm^2 increase in lysosomal size [79].

Recently, the presence of MPs (PET, PA, PVC, and PE) in coronary and carotid arteries, which presented atherosclerotic plaques, and the aorta without these plaques was identified at a concentration of approximately 118.66 μg per gram of tissue. In the case of arteries affected by atherosclerosis, MP concentrations were higher (by 50–80%) compared to healthy ones. These data suggested that the accumulation of MPs in the cardiovascular system is higher for people with atherosclerosis, and more detailed studies are needed to evaluate the real hazards of MPs with respect to arterial diseases [73].

A concerning finding is that the accumulation of MPs in the brain is selective and increases with time compared to other organs [73]. Moreover, studies on human tumour

samples (gastric, colorectal, cervical, and pancreatic) revealed that pancreatic tumour samples exhibit a significantly high concentration of MPs compared with the others. Additionally, the presence of PS-, PE-, and PVC-MPs in these samples was generally linked to an accumulation of neutrophils, which can perturb immune system function [74].

The most prevalent consequence noticed in all cases is an inflammatory reaction in several cell types, particularly affecting the respiratory, digestive, immune, and cardiovascular systems and also the endocrine and reproductive systems [13].

3. Overview of Detection Methods

Over the past several years, novel techniques and approaches have been developed to reduce both the expense and time required while enhancing the efficiency of the sampling process and the separation and detection of MPs and NPLs [51,80,81].

In vitro MP detection techniques (Figure 1) provide information regarding the presence, characteristics, and environmental impacts of MPs and NPLs. They facilitate research without relying on living organisms. Plastic particles can be accurately identified and quantified in a wide range of samples. These techniques assist in gaining insights into plastic pollution and developing appropriate mitigation strategies.

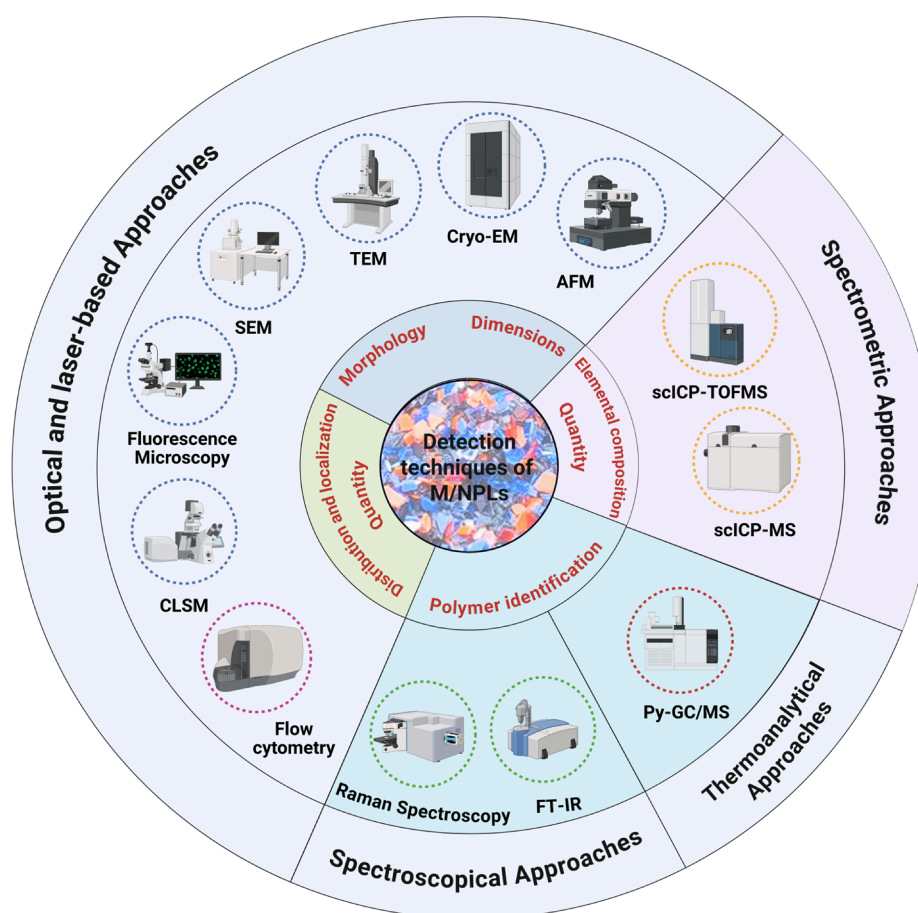


Figure 1. Schematic figure of detection techniques of microplastics and nanoplastics, grouped by type of approach. The figure illustrates the principal analytical techniques currently employed for the detection and characterization of MPs and NPLs, including transmission electron microscopy (TEM), scanning electron microscopy (SEM), cryogenic electron microscopy (cryo-EM), fluorescence microscopy, confocal laser scanning microscopy (CLSM), atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FT-IR), Raman spectroscopy, single-cell inductively coupled plasma–time-of-flight mass

spectrometry (scICP-TOFMS), single-cell inductively coupled plasma–mass spectrometry (scICP-MS), flow cytometry, and double-shot pyrolysis–gas chromatography/mass spectrometry (Py-GC/MS). The outer ring classifies the techniques into four major categories: optical and laser-based approaches, spectroscopic approaches, spectrometric approaches, and thermoanalytical approaches. The inner circle summarizes the main analytical information provided by these techniques, including morphology, dimensions, elemental composition, quantification, polymer identification, and distribution/localization. Created in BioRender. Balas, M. (2026) (<https://BioRender.com/rrodj65>, accessed on 20 July 2026) is licensed under CC BY 4.0 [82].

As shown in Figure 2, generally, three primary steps are needed for detecting MPs and NPLs: (i) discerning the type of sample (environmental, in vivo, or in vitro); (ii) processing it via multiple intermediate procedures to ensure that plastic particles can be separated by filtration, differentiation, and/or density gradient centrifugation; (iii) detection and assessment of the particles by performing different techniques, including but not limited to (a) transmission electron microscopy (TEM); (b) scanning electron microscopy (SEM); (c) cryogenic electron microscopy (cryo-EM); (d) fluorescence microscopy; (e) confocal microscopy; (f) atomic force microscopy (AFM); (g) Fourier transform infrared spectroscopy (FT-IR); (h) Raman spectroscopy; (i) single-cell inductively coupled plasma–time-of-flight mass spectrometry (scICP-TOFMS); (j) single-cell inductively coupled plasma–mass spectrometry (scICP-MS); (k) flow cytometry; and (l) thermoanalytical techniques (double-shot pyrolysis–gas chromatography/mass spectrometry (Py-GC/MS)).

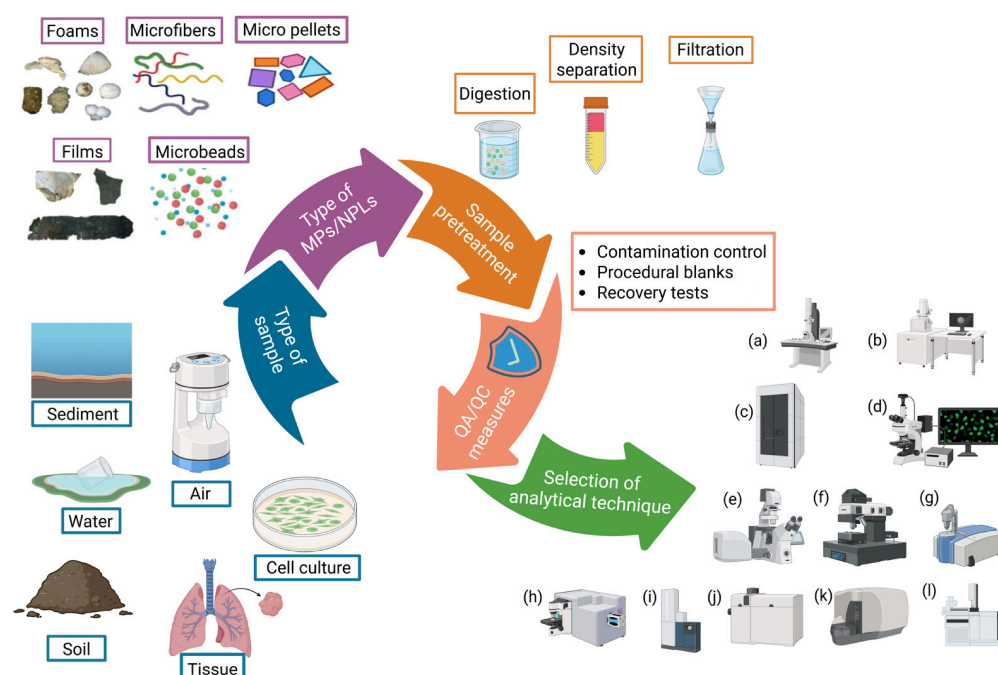


Figure 2. Schematic figure of the detection process of MPs and NPLs. Various types of samples (sediment, water, soil, air, tissue, and cell culture) may contain different forms of MPs and NPLs, including microbeads, micro pellets, microfibers, foams, and films. Depending on the sample matrix and particle characteristics, appropriate sample pretreatment procedures, such as digestion, density separation, and filtration, are applied prior to analysis. To ensure reliable and reproducible results, quality assurance and quality control (QA/QC) measures, including contamination control, procedural blanks, and recovery tests, should be implemented throughout the analytical workflow. The pretreated samples can subsequently be analyzed using the principal techniques currently employed for MP and NPL detection and characterization: (a) TEM, (b) SEM (c) cryo-EM, (d) fluorescence microscopy, (e) confocal microscopy, (f) AFM, (g) FT-IR, (h) Raman spectroscopy, (i) scICP-TOFMS, (j) scICP-MS, (k) flow cytometry, and (l) GC-MS. Created in BioRender. Balas, M. (2026) (<https://BioRender.com/mseer3t>, accessed on 20 July 2026) is licensed under CC BY 4.0 [83].

3.1. Microscopy and Laser-Based Methods

3.1.1. Transmission Electron Microscopy (TEM)

TEM is an imaging method that analyses the internal structure of biological and non-biological samples using a beam of electrons transmitted through an ultrathin specimen to visualize their internal ultrastructure. Owing to its excellent spatial resolution, TEM is widely used in cell biology, nanotoxicology, virology, and materials science [84,85]. The resulting image is primarily formed from electrons transmitted through the specimen [86].

Recently, TEM has been used to detect the effects of PS-NPLs (2 μm) on human kidney cells *in vitro* and mouse kidneys [87].

In addition, this technique was used to confirm the internalization of PS-NPLs doped with palladium in A549 (human alveolar type II epithelial cell line) and THP-1 cells after a novel and complex technique, scICP-TOFMS, was applied [88]. Similarly, the uptake of gold-doped PE and PVC nanoplastics by Caco-2 and RAW 264.7 cells was confirmed using TEM and SEM, while quantitative information on cellular internalization was obtained through single-cell inductively coupled plasma mass spectrometry (scICP-MS), a highly sensitive analytical technique [89]. Moreover, TEM was used to detect the accumulation of PE-NPLs/MPs in VK2 E6/E7 cells (normal vaginal mucosal cells) after exposure to particles at concentrations of 25 and 250 $\mu\text{g}/\text{mL}$ in chronic and acute conditions, proving the existence of vesicular structures formed near the nuclei of cells exposed to the higher dose. Furthermore, the TEM acquisitions demonstrated the accumulation of PE-NPLs/MPs between cell junctions when a dose of 25 $\mu\text{g}/\text{mL}$ was applied [90].

Zhao et al. [91] investigated the alveolar toxicity of amine-functionalized PS-NPLs (50 nm) and FITC-labelled PS-NPLs of various diameters (20 nm, 50 nm, 100 nm, and 200 nm) on A549 cultured cells using a biomimetic platform, particularly a stretchable electrochemical sensor, integrated into a microfluidic device that mimicked inhalation and expiration at the alveolar level. TEM was used to locate internalized NPLs under cyclic stretching conditions, revealing that these particles were present in both the cytoplasm (edge and centre) and the cell nucleus [91]. Using this method, Wan et al. [92] succeeded in visualizing 50 nm PS-NPLs incorporated in trophoblast Swan 71 cells, as well as apoptotic bodies, karyopyknosis, wrinkles in the nuclear membrane, and other processes, suggesting that the exposed cells underwent apoptosis [92].

Although TEM is a valuable technique for detecting the uptake of NPLs or MPs in many model systems, the characterization of these particles (size, surface, thickness, etc.) is challenging. The analysed particles are not electron-dense and have an amorphous structure. Consequently, heavy-metal stains are required to analyse NPLs or MPs using TEM [93]. As indicated, scanning electron microscopy (SEM) and/or fluorescence microscopy are suitable options for these types of experiments.

From a practical perspective, TEM is particularly valuable when confirming the intracellular localization of NPLs or investigating particle–organelle interactions at the ultrastructural level. However, because it provides limited chemical information and requires labour-intensive sample preparation, TEM is usually used as a complementary imaging technique rather than as a standalone analytical method for routine MP/NPL identification.

3.1.2. Scanning Electron Microscopy (SEM)

SEM is an electron imaging technique that generates high-resolution images of the surface morphology and topography of biological and non-biological specimens. The beam is directed onto and scanned over the specimen, generating different signals, each captured by a special detector. Each detector can offer various data about the specimen. The main advantage over a light microscope is the combination of high resolutions and a large depth of field [94]. In SEM, the electron beam is focused on a fine spot, while the

signals are collected by detectors. Even though SEM can produce a high-resolution image and does not damage the samples, the threshold for detection is at least 5 nm, and the sample's preparation—which is crucial to the technique's performance—is a challenging procedure [95].

SEM is usually used in combination with energy-dispersive X-ray spectroscopy (SEM-EDX) to characterize NPLs from a physical point of view (morphology, size, ageing, etc.) rather than to detect their general uptake [96]. Moreover, this method can be used to confirm the results obtained by scICP-TOFMS [88].

This technique can also be applied in conjunction with other analysis techniques such as surface-enhanced Raman spectroscopy (SERS). This approach was utilized for the first time to detect single-particle-level PS-MPs with diameters between 500 nm and 1 µm, as well as PMMA-MPs measuring 300 nm–1 µm (polymethyl methacrylate-MPs), in muscle tissue isolated from bighead carp (*Aristichthys nobilis*) [97]. Furthermore, NPLs have recently been identified in human gallbladder bile using the same combination of methodologies. It was discovered that 24 NPLs fragments (PS-, PP-, PET-, and PVC-NPLs) with sizes ranging from 100 nm to 10 µm were detected in 10 human gallbladders [68].

Also, in a previous study, SEM was used to detect the accumulation of 200 to 800 µm fibrous MPs in organoids formed from human lung-tissue-derived stem cells. The analysis revealed no influence on their development or morphology for any MP concentrations after 17 days, probably because the extended exposure period provided sufficient time for the organoids to adapt to the presence of plastic particles in the media compared to a 6–24 h interval [98].

Also, this method was used to measure the uptake of 50 µm amine-/carboxylate-modified PS-NPLs, tagged with rhodamine and unlabelled, in neonatal rat ventricular myocytes and to analyse the acute effects of differentially charged plastic particles on the contractile force of myocytes. To distinguish between the PS-NPLs taken up into the cytosol and their aggregates on the cellular membrane surface, SEM and confocal imaging have been used. It was proven that amine-modified PS-NPLs presented a higher internalization rate, which affected contractility due to multiple metabolic and electrophysiological alterations [99].

SEM is most appropriate for studies focused on particle morphology, aggregation states, surface weathering and interactions with biological tissues. Since SEM alone cannot reliably identify polymer compositions, it is frequently coupled with complementary techniques such as EDX, FT-IR or Raman spectroscopy to obtain both morphological and chemical information.

3.1.3. Cryogenic Electron Microscopy (cryo-EM)

Cryogenic electron microscopy (cryo-EM) comprises a group of electron microscopy techniques in which specimens are rapidly frozen and imaged at cryogenic temperatures. This approach preserves the native ultrastructure of biological samples by minimizing dehydration and reducing preparation-induced artefacts. In addition, cryogenic conditions help reduce radiation-induced damage and maintain the spatial organization of cellular and molecular components. Cryo-EM has become an important tool in structural biology and nanomaterial research due to its ability to visualize specimens in a near-native state. Some advantages of this procedure are maintaining sample hydrated, preventing radiation damage, and preserving the samples' natural activity and functional state, as well as post-translational modifications [100]. Up to this point, no study has been identified in the literature that uses simple cryo-EM to evaluate NPL accumulation in cells; however, a recent research employed a version of this technique, cryogenic scanning

electron microscopy (cryo-SEM), to investigate the accumulation of 500 nm PS-NPLs in two-dimensional cultured keratinocytes [101].

Cryo-SEM is particularly advantageous for hydrated biological samples because it minimizes structural alterations caused by dehydration and conventional sample preparation. Nevertheless, its high cost and specialized instrumentation currently limit its routine application in MP/NPL analysis.

3.1.4. Fluorescence Microscopy

Fluorescence microscopy is based on the excitation of fluorophores using light of a specific wavelength, followed by the emission of fluorescence at a longer wavelength. The difference between the excitation and emission wavelengths is known as the Stokes shift [102]. This technique is suitable for fluorescent particles obtained by labelling with Nile Red [103], Rhodamine B [104] or other more complex fluorophores, such as a tetraphenylethylene functional group fused with xanthene [105], as well as those produced by the combined swelling-diffusion effect, which refers to the entrapment of fluorescent molecules in a polymer matrix, depending on temperature and solubility [104]. MPs are difficult to identify without labelling in biological and environmental samples due to their small dimensions and heterogeneity.

Although Nile Red and Rhodamine B are among the most widely used dyes for plastic particle detection because they adsorb onto plastic surfaces and emit fluorescent signals under UV light, they have certain limitations. Nile Red can be efficiently utilized to identify NPLs but may also be associated with organic materials. Furthermore, the detection of MPs that exceed 5 mm and are smaller than 3 µm remains challenging [106]. On the other hand, Rhodamine B is less suitable for detecting MPs smaller than 10 µm because the weak fluorescence signal generated by very small particles, together with the resolution limits of conventional fluorescence microscopy, reduces detection sensitivity [107].

Recently, pregnant female rats were exposed via inhalation to 20 nm fluorescent PS-NPLs on gestational day 19. Twenty-four hours after exposure, maternal tissues (uterus, lung, heart, liver, kidney, and spleen) and foetal samples, including the placenta, foetal abdomen, liver, heart, and whole-body preparations, were collected and analysed. In each of these samples, an elevated plastic deposition was noticed, especially in the uterus and whole pup, which indicated that NPLs translocated from the maternal lungs to the rest of the organism, the foetus, and its organs [108].

This technique was also used in different studies to examine the uptake of NPLs in various bidimensional [109–114] and three-dimensional cultures [101], as well as in microfluidic systems [115,116].

Nonetheless, this approach lacks the necessary resolution to properly identify NPLs in tissues and distinguish them from free dye. This led to the development of a derivative technique that can minimize these drawbacks, i.e., stimulated emission depletion microscopy (STEDM). Using passive sorption, the plastic particles are labelled with Atto 647N, a cationic dye particularly created for single-molecule detection within the red spectral range [117].

Fluorescence microscopy is best suited for rapid visualization of labelled MPs and NPLs and for preliminary assessments of cellular uptake. However, because fluorescence staining may generate false-positive signals and does not provide polymer-specific information, confirmatory analyses using spectroscopic techniques are generally recommended.

3.1.5. Confocal Microscopy

Confocal laser scanning microscopy (CLSM) is a fluorescence imaging technique that uses a focused laser beam to excite fluorophores within a specimen. The emitted fluorescence is collected through the objective lens and passes through a spatial pinhole

positioned in the detection pathway, which eliminates out-of-focus light and improves image contrast and resolution. The detected signal is converted into an electrical signal by photomultiplier tubes or other detectors and reconstructed into high-resolution optical images. Compared with conventional fluorescence microscopy, CLSM provides improved optical sectioning capabilities and enables three-dimensional reconstructions of biological specimens. However, its spatial resolution remains limited by optical diffraction, and image quality may be affected by photobleaching, phototoxicity, and reduced signal intensity in thick tissues [118].

In vitro studies used CLSM to detect various MPs and NPLs, especially PS, in cell cultures or complex cell systems. This technique was used to detect PS particles of 1–10 µm taken up after 24 h of exposure in a mucus co-culture model of Caco-2 and HT29-MTX E12 cells, M cell models of Caco-2 and Raji B-lymphocytes, and THP-1 (human leukemia monocytes) models for investigating a possible risk of intestinal immune cells exposed to NPLs. The results showed that the mucus model accumulated a higher amount of PS-NPLs [115] compared to the colon epithelial cell monoculture. CLSM was also used in a microfluidic-based model of BEAS-2B to study the uptake of NPLs that were internalized in cells in various ways depending on the culture conditions [116] or the lung's mechanical microenvironment [91]. Other studies utilized this technique to assess intracellular accumulation of NPLs in HeLa [112], Caco-2, HepG2, HepaRG, [111], NRVMs [99], A549 [119], and trophoblast Swan 71 cells [92].

Compared with conventional fluorescence microscopy, CLSM provides superior 3D imaging and enables accurate co-localization of particles with intracellular organelles or tissue structures. Nevertheless, its application remains dependent on fluorescent labelling and therefore does not directly identify polymer compositions.

3.1.6. Atomic Force Microscopy (AFM)

AFM aims to obtain the topography of a sample with nanometre resolutions by detecting the interaction between a sharp probe and the surface of the sample. It measures the attractive and repulsive forces between the atoms of a sharp tip and those of the sample's surface. The tip is attached to a very flexible, microscopic cantilever that bends toward or away from the sample in a manner depending on the presence of different types of forces, i.e., attractive or repulsive ones. In terms of the Lennard-Jones potential, the probe enters a repulsive domain and experiences substantial surface repulsion as the distance between it and the sample decreases [120,121]. The cantilevers are etched at the side of a macroscopic silicon chip. The latter can be attached to a piezoelectric positioner, allowing the cantilever to be positioned vertically. The bending of the cantilever is detected optically by a photodiode [122].

AFM is often employed to evaluate the surface of the samples; hence, it is not well suited for detecting the internalization of NPLs in cells or tissues. Together with SEM, Raman spectroscopy, and CLSM, it may be used to evaluate the surface potentials and porosity of cell walls to discriminate between functionalized PS-NPL adsorption and uptake [123]. However, recently, a new method employing AFM, namely peak force tapping nanomechanical AFM, was created to detect PS-NPLs in human fibroblasts. This approach operates by analysing several characteristics, such as deformation and adhesion, after the image has been acquired. Previously, the same research team utilized AFM to physically characterize the surface of PS-NPLs (with diameters of 100, 200, 500, and 1000 nm), which were consistent with the manufacturer's description. The internalization of particles was verified using dark-field hyperspectral microscopy. By comparing the data provided by both approaches, it was determined that this AFM mode had a resolution that could

only evaluate the internalization of larger particles above 500 nm in the cytoplasm of fibroblasts [124].

AFM is particularly useful when nanoscale surface morphology or nanomechanical properties are of interest. However, the limitations of AFM in identifying and characterizing NPLs, such as its relatively slow imaging speed and limited capacity for providing chemical composition data, reduce its suitability for high-throughput screening of environmental samples. While advanced techniques like AFM-IR or coupling with Raman spectroscopy [125,126] can help alleviate some of these constraints, they necessitate specialized equipment and expertise. Additionally, the possibility of tip-induced artefacts during measurement underlines the importance of appropriate sample preparation [127,128]. Despite these drawbacks, AFM remains an effective technology for studying the morphology and mechanical characteristics of NPLs at high resolutions.

3.2. Spectroscopical Methods

Spectroscopic methods have been widely used in MP and NPL research, demonstrating their versatility in a variety of studies. The efficacy of these techniques has been proven through the detection of plastic particles in environmental samples [129–131] and the analysis of food and beverage samples [45,132] and human stool samples [25].

FT-IR and Raman spectroscopy can both be coupled with microscopy techniques to analyse the chemical composition and the physical characteristics of MPs [133]. Nevertheless, numerous criteria must be evaluated to maximize sample analysis and ensure the credibility of the results. The key issues include adjusting for particle size restrictions and ensuring that solvent or support materials (such as filters or sample containers) do not produce interference signals that overlap with those of the target sample. Raman spectroscopy and infrared spectroscopy (IRS) are two forms of vibrational spectroscopy. The process that produces the spectroscopic signal is different for each of them, but the information obtained is similar. The Raman technique instead presents the advantage of better size resolution up to 1 μm .

3.2.1. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR represents the most used type of IRS. In this method, light from the emitting source is split by a beamsplitter. One part of the beam is reflected in a fixed mirror, and on its way back, it passes the beamsplitter and reaches the detector. The other part passes through the beamsplitter on its first encounter; then, it is reflected by a movable mirror and by the beamsplitter before it hits the detector. When the two beams recombine, they interact with each other, and constructive or destructive interference will occur depending on the length difference in the two paths. The instrument measures the dependence of light intensity on the position of the movable mirror.

The Fourier transform of the spectrum, which is a mathematical model used to translate signals between two different domains—for example, translating a function of time or space into a function of frequency—is represented by this light intensity. The measured data are transformed back into a spectrum using another Fourier transform. The concept of the Fourier transform involves decomposing the signal into its constituent frequencies, providing a frequency spectrum that represents the signal in the frequency domain. The main advantage of a Fourier transform spectrometer is the high light intensity of the detector and, as a consequence, the high signal-to-noise ratio. Therefore, a spectrum can be recorded within as little as 10 ms [134].

Recent studies have used this technique or adapted versions to detect the presence of MPs in human samples, as can be seen in Table 1.

Table 1. Recent studies that used μ FT-IR to detect MPs in various human samples.

Sample	Types of Polymers Found	Size	Amount Found	Reference
Human stool	PA, PC, PE, polyethylene terephthalate (PET), PP, PS, PU, PVC, polyoxymethylene (POM)	50–500 μ m	2 MPs/g of sample	[25]
Sputum from patients suffering from respiratory diseases	PU, polyester, chlorinated PE, alkyd varnish, acrylates, PP, rubber, PE, polyacetal, PMMA, polyvinyl alcohol, silicone, polysulfones, polytetrafluoroethylene, PVC, acrylonitrile butadiene, ethylene vinyl acetate, PI, chlorinated polyisoprene, polybutadiene, polycarbonate	44.67–210.64 μ m	39.5 particles/10 mL	[135]
Healthy human lung samples	PE, PET, PMMA, PP, PS—11 types	>3 μ m	1.42 MPs/g of tissue	[24]
Human saphenous vein tissue	Alkyd resin, polyvinyl propionate/acetate, nylon, ethylene vinyl alcohol, polyvinyl alcohol, tie layer, PU, polyurethane vitrimer, ethylene–vinyl acetate	16–1074 μ m length and 7–300 μ m width	14.99 $\pm$ 17.18 MPs/g tissue	[136]

Another group used a different experimental FT-IR configuration, namely ATR-FTIR (attenuated total reflectance -FT-IR), along with Raman spectroscopy to detect the presence of PS-NPLs in HepG2 cells and to identify their cellular effects. The alterations generated by three types of 70 nm plastic particles, pristine PS-NPLs, amine-modified PS-NPLs, and carboxylate-modified PS-NPLs, were investigated, revealing that the amine-modified particles exhibited a dose-dependent negative effect (modifying the genetic material, proteins, and lipids), whereas the other two generated a non-linear dose–response effect [137].

Although FT-IR is widely used for its speed, accuracy, and preservation of samples during analysis, sample preparation is a more laborious and complex step that requires a longer period than Raman imaging. In addition to conventional FT-IR, approaches such as attenuated total reflectance (ATR) and LDIR spectroscopy have been introduced to reduce sample processing and analysis time while maintaining reliable polymer identification of MPs and NPLs, and they have been successfully applied for the identification of MPs in environmental matrices, including water and sediments, while offering faster analysis than conventional FT-IR spectroscopy [138]. LDIR spectroscopy has also been used for the identification of common polymer types such as PE, PP, PET, PS, and PVC in environmental samples [139]. FT-IR is generally considered the preferred technique for routine polymer identification of larger MPs because it combines reliable chemical composition with relatively high automation potential. However, its spatial resolution limits the detection of the smallest particles, making Raman spectroscopy more suitable when sub-10 μ m MPs are analysed.

3.2.2. Raman Spectroscopy

Raman spectroscopy offers the same information as infrared spectroscopy regarding molecules and/or macromolecules. The difference lies in the detected vibrations and experimental requirements. Raman spectroscopy detects light that scatters inelastically. Unlike Rayleigh scattering, which is an elastic process in which the scattered light has the same energy and wavelength as the incident light, Raman scattering involves an energy exchange between light and molecular vibrations or rotations. This interaction causes a shift in the wavelength of the scattered light, producing either Stokes lines (lower energy) or anti-Stokes lines (higher energy). While Rayleigh scattering is much stronger and involves no

change in energy, Raman scattering is weaker but provides detailed molecular information through these spectral shifts. Due to this fact, Raman scattering is a powerful tool for analysing molecular structure and composition. The difference between the Raman and Rayleigh photons indicates the energy needed for vibrational transitions of the molecule of interest. A limitation of this technique is the generation of the Raman signal by a small part of the incident photons because Raman scattering has a lower probability than Rayleigh scattering, which is already unlikely. This limits the signal-to-noise ratio [140].

Stokes Raman scattering occurs when the scattered photon has less energy than the incident photon, while in anti-Stokes Raman scattering, the scattered photon has more energy due to the molecule initially being in an excited vibrational state. Raman scattering involves UV, visible, or near-infrared light, enabling the use of standard UV/VIS spectrometer components [141]. A laser serves as the light source, and Rayleigh scattering is filtered out. The Raman spectrum is analysed using a monochromator or a Michelson interferometer [142].

Recent advances have further expanded the analytical capabilities of Raman spectroscopy through the development of SERS and tip-enhanced Raman spectroscopy (TERS). SERS enhances the inherently weak Raman signal through localized surface plasmon resonance generated by metallic nanostructures, substantially improving detection sensitivity for trace amounts of MPs and NPLs [143–145]. In contrast, TERS combines Raman spectroscopy with scanning probe microscopy to achieve chemical imaging with nanometre-scale spatial resolutions beyond the optical diffraction limit, making it particularly promising for the characterization of individual NPLs [143]. Despite these advantages, both techniques require specialized instrumentation and remain technically demanding, which currently limits their routine application in environmental and biological MP/NPL analysis.

Previous studies have used Raman spectroscopy and adaptations of it to detect different polymers in various samples, as seen in Table 2.

Table 2. Recent studies that used Raman microspectroscopy to detect MPs in various samples.

Sample	Types of Polymers Found	Size	Amount Found	Reference
Human placenta	PP	5–10 µm	12 MPs/4 placentas	[35]
Healthy human lung tissues	PP, PE, PA, cellulose acetate, polyamide, PE co-PP, PS, PS-co-PVC, PU	1.6–16.8 µm	0.56 MPs/g of tissue.	[34]
Human saliva, hair, face and hand skin	PE, PET, PP, PS, PVC	Mostly <100 µm	0.32 MPs/individual/day (saliva); 3.73 MPs/individual/day (hair); 2.13 MPs/individual/day (face skin); 2.03 MPs/individual/day (hands skin).	[146]
Mouse kidney tissues	PS	2 µm	3.07 ± 0.15 mg/g kidney.	[87]
A549, CaCo-2 and THP-1 cell cultures	PS	1 µm	-	[114]

Unlike FT-IR, Raman imaging enables analyses of non-transparent or dark-coloured particles and is less sensitive to interference from water signals. However, the fluorescence background may limit its application. This can result from pigments or additives contained

in plastic particles. Inorganic and organic compounds can also interfere with NPL and MP identification due to their intense fluorescence. These limitations can often be reduced by optimizing acquisition parameters, such as the excitation laser wavelength, laser power, acquisition (integration) time, and the number of spectral accumulations, according to the sample's characteristics [147]. Another downside of Raman spectroscopy is that some polymers, such as PET, can produce fluorescent peaks that can overwhelm a spectrum and are a result of exposure to ultraviolet radiation and hydrolysis. This extends the time required to acquire high-quality spectra [138]. Although longer acquisition times and fluorescence interference may reduce analytical throughput, Raman spectroscopy is often selected when high spatial resolutions are required. This technique can complement FT-IR by enabling polymer identification of considerably smaller particles.

3.3. Spectrometric Methods

The spectrometric methods used for the study of plastic particles are scICP-TOFMS and scICPMS. Both techniques require the analysis of individual cells exposed to these particles by introducing them into a plasma source where they are ionized. The detection method used after the cells are atomized and ionized determines the difference between the two types of spectrometric analyses. In the case of scICPMS, a quadrupole mass spectrometer is used to sequentially scan across different mass-to-charge ratios (m/z), which might take longer and limit the ability to detect ions in real time during the rapid analysis of single cells [89].

On the other hand, scICP-TOFMS employs a time-of-flight mass spectrometer (TOFMS), which measures the time needed for ions to pass through a flight tube to the detector. This allows a significantly faster mass spectra capture and real-time analysis of multiple elements, making it more suitable for high-throughput single-cell research.

Regarding sample preparation, after exposure, the cells are rinsed with phosphate-buffer saline to remove any unbound particles, fixed with 4% paraformaldehyde, and kept at 4 °C until analysis. Then, these are washed and diluted with MilliQ water to obtain the required particle concentrations for scICP-TOFMS analyses. Metal-doped NPLs can be analysed by standard trace metal measurement procedures, allowing researchers to investigate their activity in biological tissues and cells.

Traditional methods such as microwave-induced acid digestion followed by ICP-MS analysis are adequate for determining overall particle fate but lack cell-specific resolutions. ICP-TOFMS can be used to investigate the multi-elemental fingerprints of each particle event, providing high-sensitivity, full-spectrum mass analysis at high temporal resolutions. During data processing, the fingerprints generated might be assigned to different subpopulations, such as cells, NPLs, or linked events (cells and NPLs) [88].

ScICP-MS measurements depend heavily on sample preparation, although this aspect has not received enough attention. The highly complex media necessary for mammalian cell growth (which include proteins, lipids, amino acids, and inorganic salts) [148] make it difficult to distinguish single particle/cell ionization events from the background. Direct insertion into the instrument is thus not possible. Chemical fixation is necessary to resuspend mammalian cells in a more appropriate medium for ICP-MS (e.g., water) while retaining intracellular components and cellular structures, resulting in a higher signal-to-noise ratio [149,150]. Using ^{13}C isotope monitoring, scICPMS can identify MPs because it is possible to fully volatilize and atomize the particles—making it possible to analyze size distributions and to detect particles—using aqueous dissolved carbon standards. The outcome is a temporal scan that includes numerous particle events above the baseline depending on the frequency chosen [151].

Hendriks et al. [88] used scICP-TOFMS to assess the accumulation of 200 nm PS-NPLs doped with palladium in A549 and THP-1 cell cultures. Also, scICPMS was applied in the quantification of 50–350 nm PE-/PVC-NPLs doped with gold and taken up by Caco-2 and RAW 264.7 cell cultures, demonstrating scICPMS to be a fast and efficient method of detecting plastic debris in bio-distribution assessments. Furthermore, it has been proven that model NPLs can be doped with compounds to enhance traceability and to enable sensitive detection, such as ultra-small gold nanoparticles of ~3 nm [89].

Both these techniques are especially suitable for quantitative biodistribution studies using metal-labelled NPLs at the single-cell level. However, their dependence on labelled particles currently limits their application to experimental rather than environmental samples.

3.4. Flow Cytometry

Flow cytometry is a technique that provides multiple analyses of single cells in solutions in a rapid manner. The basic principle is that the light source of the instrument is a laser that can produce two types of signals, namely scattered and fluorescent light. These signals are recognized by a detector and converted to electronic signals, which are interpreted by computer software. There are a variety of cytometers with several lasers and fluorescence channels, such as cell sorters, mass cytometers, acoustic focusing cytometers, etc. [152].

Recently, a team of researchers used an adaptation of this technology, namely nano cytometry, to analyse and detect MPs in human and mouse peripheral blood. They detected the number of events/L (NPL/ μ L). The samples were provided by healthy donors and patients suffering from different types of cancers (myeloma, leukaemia, and lung cancer), idiopathic nephrotic syndrome, or type 1 diabetes. The highest value of NPL/ μ L was found in acute lymphoblastic leukaemia, while the lowest was identified in individuals with type 1 diabetes [153].

This technique has additional applications in *in vitro* investigations. Flow cytometry was applied to both two-dimensional cultures [111] and microfluidic systems [116] to evaluate the uptake efficiency of PLA-/PMMA-/PS-NPLs.

To differentiate the uptake of PS-NPLs of 25 nm and 70 nm in A549 cells, the smaller particles were labelled with FITC, and the others were labelled with Nile Red [119]. Also, it has been proven that the 20 and 100 nm Nile Red-labelled fluorescent PS-NPLs taken up in AML-12 (Alpha Mouse Liver 12) cells induced apoptosis and inflammation [154]. The internalization of FITC-labelled 50 nm PS-NPLs in trophoblast Swan 71 cells generated placental apoptosis [92], whereas the uptake of PS-NPLs had an important morphological impact on RAW264.7 cells compared to other cell types [113].

Flow cytometry is particularly advantageous for high-throughput quantitative studies of particle uptake and cell-particle interactions, allowing the rapid analysis of thousands of events within a short period of time. However, because it provides limited information regarding polymer composition, it is commonly combined with imaging or spectroscopic techniques for comprehensive characterization. Regarding the drawbacks of this approach in detecting NPLs and MPs, the detection limit of conventional flow cytometry is insufficiently low for the reliable analysis of smaller NPLs. To mitigate this limitation, samples could be pre-concentrated, which would significantly increase the time and labour required for the procedure. Furthermore, traditional flow cytometry can be used to examine materials with particle dimensions between 100–200 nm and 30–40 μ m, whereas nanoflow cytometry extends the detectable size range to 7–1000 nm. However, nanoflow cytometry still presents certain restrictions regarding filter membrane materials and elution procedures [155].

3.5. Thermoanalytical Methods—Double-Shot Pyrolysis–Gas Chromatography/Mass Spectrometry (Py-GC/MS)

As described above, microscopy-based and spectroscopic techniques such as FT-IR and Raman spectroscopy are widely used to detect and determine polymer structures and the shapes and sizes of particles that are important for toxicological investigations, but they have limitations in terms of polymer mass and advanced chemical composition. To address these constraints, thermoanalytical spectrometric methods, including Py-GC/MS, are frequently used.

This technique, as the name suggests, combines three separate methods: pyrolysis (the sample is degraded by heat), gas chromatography (the sample is separated into components based on size and chemical properties), and mass spectrometry (the portions of molecules are separated based on mass and charge). This approach quantifies mass fractions and enables a more comprehensive chemical analysis of the plastics present in samples [156]. However, it is essential to emphasize that the analysis of MPs presents a particular set of concerns due to their inhomogeneous distribution and the diversity of the polymeric elements contained [157]. Therefore, great attention is required when grinding and homogenizing the samples to avoid the formation of agglomerates. Even though this technique does not require solvents, the registration and analysis can be automatic, which makes it reliable and highly sensitive. However, there is a limited database on the interpretation of signals for polymers, and it can be used especially for MPs with larger dimensions. Although the identification and quantification of MPs/NPLs in the environmental samples is difficult, the use of multiple methodologies, such as spectroscopic and thermoanalytical techniques, can provide useful information regarding their composition and, possibly, their toxicological impact.

Seeley and Lynch [158] offered a comprehensive review of the applications, varieties, and potential of the technique in quantifying and identifying different plastic polymers in environmental samples. A team of researchers used an adaptation of Py-GC/MS, namely double-shot Py-GC/MS, to analyse MPs and NPLs from human whole-blood samples using two temperature programs (“shots”). The first shot is “thermal desorption”, i.e., the sample is loaded in the pyrolyzer from 100 °C to 300 °C, known as the “temperature ramp”. This step provides the possibility to analyse any volatile compounds present on the filter. Due to the low temperature, evaporation can happen only to additives or monomers. After this stage, the second shot follows, in which the pyrolyzer increases the temperature to 600 °C so that the plastic polymers can be volatilized and analysed [159]. The first study to quantify MPs in human whole blood using this approach employed, for blood purification, a sequence of pretreatment steps, including protein denaturation, enzymatic digestion, filtration, and extensive contamination control prior to Py-GC/MS analysis, allowing selective identification and quantification of polymer-specific pyrolysis products. Using this workflow, the authors reported a total polymer mass concentration of 1.6 µg of plastic particles per millilitre of blood. Among the identified polymers were styrene, PE, PET, and PMMA [19].

Recently, an average of 126.8 to 147.5 µg of MPs per gram of placental tissue was reported in 62 samples subjected to this technique. The most prevalent were PE, PVC, and nylon, but nine other polymer types were also found in varying amounts, as well as PS [159]. Another study used Py-GC/MS to identify and quantify MPs in human kidneys, livers, and brains, observing that the brains contained higher levels of MPs, particularly PE [73]. A recent human biomonitoring study demonstrated the utility of Py-GC/MS for polymer-specific quantification in biological matrices. Using whole-blood samples from 100 participants, the researchers detected MPs/NPLs in all samples, reporting a median total concentration of 427.5 ng/mL. PVC, PET, and PE were the most abundant polymers identified, highlighting the suitability of Py-GC/MS for polymer-specific quantification [160].

Unlike microscopy-based techniques, Py-GC/MS does not preserve particle morphology but instead provides accurate quantification of total polymer mass independent of particle size. Therefore, it is particularly valuable for exposure assessment studies in which determining the total amount of polymer present in the sample is more relevant than particle morphology or intracellular localization.

3.6. Multi-Method Approaches

After extraction and cleaning, the separated particles are analyzed from both physical and chemical perspectives [161]. For an advanced investigation of MPs and NPLs, researchers generally combine various analytical methods to properly characterize these particles in terms of their size, shape, quantity, chemical composition, and physical characteristics. Since each strategy has specific strengths and limitations, implementing multiple methods aids in developing a more thorough comprehension.

AFM and Raman spectroscopy are often used to confirm morphology and chemical composition [125], whereas SEM and/or TEM are typically used together with energy-dispersive X-ray spectroscopy (EDX) for elemental analysis [162]. To determine the topography, particle size, and chemical functional groups of NPLs, atomic force microscopy-based infrared spectroscopy (AFM-IR) can be employed, as this method provides a very high spatial resolution. Py-GC/MS can complement this method, confirming plastic varieties and abundances without regard for particle size. This complementary approach allows for a more comprehensive analysis of NPLs, ensuring accurate identification and quantification regardless of their size [126].

Another hybrid approach, between SEM and SERS, combines the ultrahigh spatial resolution of SEM alongside the advantages of Raman spectroscopy, such as structural fingerprints. This technological coupling could be a useful tool in future research because of its simplicity, short duration, and efficiency [97].

In addition, AFM, SEM, and TEM are complementary techniques for analysing NPLs, each providing unique advantages in resolution, cost, and applications. Used together, they offer comprehensive information about particle size and morphology, across different scales. The accuracy of AFM imaging depends on the shape and finite size of the probe tip, which can lead to artefacts [163,164]. Instead, SEM identifies cluster formation and particle shapes, generally for over 50 nm NPLs, while TEM provides the highest resolution and detailed morphological analyses, offering the possibility to study smaller NPLs. Considering the method's familiarity, instrument availability, and cost, the SEM setup is more widespread, while TEM and AFM are more expensive, often limiting access [165].

To analyze particles in the range of 10 μm , FTIR is frequently combined with microscopy (μFTIR). Pairing microscopy techniques like SEM or TEM with μFTIR [166] or confocal Raman allows for confirmation of particle shape and chemical identification [167]. Some studies used TEM or SEM to confirm the findings of newly developed techniques such as scICP-TOFMS [88] or scICPMS [89]. For complex samples, FTIR and/or Raman spectroscopy are employed in conjunction with Py-GC/MS to establish the polymer composition, whereas Py-GC/MS validates the nature of the plastic [20,159].

Other scholars employed AFM in addition to DLS (dynamic light scattering) studies to characterize NPLs. DLS is a well-established technique for determining the size distribution of monodisperse NPLs, but it is less effective for smaller particles in mixed-size samples. Still, it is excellent for highlighting aggregates of MPs. In contrast, AFM, usually used as a morphological analysis tool, is excellent for evaluating heterogeneous materials and providing accurate measurements of NPL dimensions by directly visualizing individual particles with high precision. This combination of approaches provides information on the size range and stability of NPLs [164].

For a complete physical, chemical, and functional characterization of M/NPLs across various samples, researchers can address the limitations of individual methods by combining multiple complementary techniques. This integrative approach provides a more comprehensive and accurate characterization.

3.7. Comparison and Selection Criteria of Analytical Techniques

Although each analytical technique has distinct advantages, none can simultaneously provide comprehensive information regarding particle morphology, polymer composition, intracellular localization, quantitative analysis, and high-throughput screening. Therefore, selecting the most appropriate analytical workflow should be guided primarily by the aim of the study, the expected particle size range, and the complexity of the investigated matrix. Table 3 provides a practical comparison of the principal analytical techniques according to their analytical performance, polymer identification accuracy, quantitative potential, analysis time, automation capability, and recommended applications.

The selection of an analytical technique should consider the sample matrix, expected particle size, the type of information required (morphological, chemical, or quantitative), and the overall objectives of the study.

Regardless of the selected analytical technique, appropriate sample pretreatment and standardized QA/QC procedures remain essential prerequisites for obtaining reliable, reproducible, and comparable analytical results across different environmental and biological matrices [8].

Table 3. Practical comparison of analytical techniques used for the detection and characterization of MPs and NPLs. The table summarizes the practical analytical performance of the principal techniques used for MPs and NPLs analysis, including typical detectable particle size, polymer identification capability, quantitative potential, analysis time, automation level, and recommended applications. The reported characteristics represent typical performances described in the literature and may vary depending on the analytical configuration, sample preparation protocol, and instrument specifications.

Technique	Typical Detectable Particle Size	Polymer Identification	Quantification	Analysis Time *	Automation **	Recommended Use
TEM	NPLs and very small MPs	None	None	Long	Low	Intracellular localization and ultrastructural analysis, organelle interaction
SEM	Typically >50 nm	Indirect (after coupling with complementary techniques)	None	Moderate	Moderate	Surface morphology, aggregation, weathering
Cryo-SEM	Nanometer to micrometer range	None	None	Long	Low	Native hydrated biological samples, biological interfaces
Fluorescence microscopy	Fluorescently labelled particles; typically >3 µm (environmental MPs)	None	Semi-quantitative	Short	High	Rapid screening, preliminary uptake studies, biodistribution
CLSM	Fluorescently labelled MPs/NPLs	None	Semi-quantitative	Moderate	Moderate	3D fluorescence localization, co-localization with organelles, tissue imaging
AFM	Nanometer to micrometer range	None	None	Low	Low	Surface topography & nanomechanics, roughness, mechanical properties, particle size measurements
FT-IR/µFT-IR	Typically >10–20 µm	Excellent	Good	Moderate	High	Chemical fingerprinting

Table 3. Cont.

Technique	Typical Detectable Particle Size	Polymer Identification	Quantification	Analysis Time *	Automation **	Recommended Use
Raman	$\geq 1 \mu\text{m}$	Excellent	Good	Long	Moderate	Chemical fingerprinting at high spatial resolution, identification of small MPs
scICP-MS	Metal-labelled NPLs	Limited	Excellent	Short	High	Quantification of metal-labelled NPL uptake at the single-cell level
scICP-TOFMS	Metal-labelled NPLs	Limited	Excellent	Very short	Very high	High-throughput biodistribution and uptake studies, multi-element analysis
Flow cytometry / Nanoflow cytometry	Conventional: 200 nm–40 μm Nanoflow: 7–1000 nm	None	Excellent	Very short	Very high	Uptake studies, biodistribution, particle–cell interaction studies, high-throughput particle detection
Py-GC/MS	Independent of particle size	Excellent	Excellent	Moderate	High	Total polymer uptake, exposure assessment, polymer mass composition

* Analysis time refers to the approximate duration of the analytical measurement itself and does not include sample collection, extraction, purification, digestion, or other sample preparation procedures. ** Automation refers to the potential for automated sample acquisition, particle recognition, and data processing using commercially available analytical workflows.

Strengths and Limitations of Techniques

A description of some of the most important strategies in identifying MPs and NPLs, including their benefits and drawbacks, as well as bibliographic references for various applications and their TRL (technology readiness level) parameters, is available in Table 4.

Table 4. Comparison of analytical techniques used for MP and NPL detection, visualization, and characterization.

Detection Technique	Strengths	Limitations	TRL	Application Bibliographic Source
TEM	• Ultrahigh magnification resolution • Provides ultrastructural and morphological information at nanometer resolution • Allows visualization of individual nanoparticles and nanoparticle aggregates	• Embedding in epoxy resin • Difficulty in identification without labelling with a compound with an electron density higher than the sample, such as heavy metals • Lack of selectivity • Time-constraining • Highly expensive setup • Operation and analysis require special training • A sample preparation step is needed	1–2	[79,168]
SEM	• High resolution and large depth of field • When coupled with other techniques, it acquires new advantages (detection of smaller particles, lack of sample preparation stage, etc.) • Easy data analysis and image processing	• Cannot identify the chemical structure of polymers • High expense • Time- and effort-consuming • Examination occurs only under a high vacuum	4–6	[96]

Table 4. Cont.

Detection Technique	Strengths	Limitations	TRL	Application Bibliographic Source
Cryo-SEM	• Analysis of single particles, without size limitations or need for crystallization • Ability to visualize samples in their native state • Compatible with computational analysis	• Highly expensive setup • Operation and analysis require special training • Difficulty in characterizing larger samples • Resolution limitations (for their increase, a certain homogeneity of the particle populations is required) • Large datasets generated (computationally demanding)	4–6	[89]
Fluorescence microscopy	• Unchallenging • Can detect colourless MPs and NPLs • Real-time rapid viewing of samples • Cost-effective • No additional pre-steps	• Additives from NPLs and MPs can interfere with the analysis • Can be destructive under UV laser • Polymers must be fluorescently labelled	4–6	[169]
CLSM	• Highly quantitative • High spatial resolution • High contrast • High-quality multispectral images • Control over depth of field • Low background • Clear images from a thin section of a thick sample	• Fluorescent samples required • Resolution limited by optical diffraction • Photobleaching and phototoxicity • Relatively slow image acquisition for high-resolution imaging • Phototoxicity and photobleaching due to high-intensity laser • Image quality is susceptible to interference • Higher quality acquisitions require more time	5–7	[72,170]
AFM	• High spatial resolution • Label-free detection • Surface morphology analysis • Can measure physical properties, being a quantitative analysis • It offers versatility regarding the conditions of operating. • Minimal sample preparation • Non-destructive analysis • It is able to detect and analyse individual NPLs, ensuring high specificity and sensitivity • It can investigate the surface of non-conducting polymers • It can offer 3D images of the surface structure of NPLs	• Time-consuming, costly, and requires expensive maintenance • It cannot provide data regarding chemical composition; therefore, it must be combined with other techniques (e.g., tip-enhanced Raman spectroscopy (TERS)) • Limited area that can be analysed • Operations and data interpretation require specialized training • Even though minimal sample preparation is needed, surface cleanliness and flatness can impact results, making it difficult to work with biological and environmental sample • Sample size constraints • Artifacts may arise from interactions between the sample and the AFM tip, affecting data accuracy • Tip-induced artefacts may affect measurements • Sample deformation may occur in soft biological specimens	4–6	[127,128]

Table 4. Cont.

Detection Technique	Strengths	Limitations	TRL	Application Bibliographic Source
FT-IR spectroscopy	- Can determine the composition of particles - Harmless for samples - Can be adapted to analyse particles < 20 µm - No false positive or negative values - High signal-to-noise ratio - Multiplex advantage - Wavelength precision - Simple procedure of sample preparation in standard measurements - Short time needed for recording an infrared spectrum (less than a minute) - High time resolution	- The technique is both time-consuming and costly, thereby reducing its accessibility - Limited wavelength radiation - The small dimensions of the sample chamber constrain the quantity or volume of the sample that can be analysed - The infrared (IR) beam may be obstructed by components of the mounting assembly - Absolute intensity measurements may deviate by a small percentage	5–7	[25,117]
Raman spectroscopy	- Can detect NPLs - Harmless for samples - Is not limited by the dispersion medium of the sample - High spatial resolution - Wide spectral coverage - Low interference with water - Can be used to identify the location of MPs in living organisms - Simple procedure of sample preparation in standard measurements	- Time- and cost-intensive - Pigments and fluorescence can interfere - Ratio between signal and noise is low - Sample can heat up - Scattered intensity is often very low - Requires high concentrations of the molecule of interest, as the signal is weak	5–7	[35]
Flow cytometry	- Simultaneous analysis of multiple parameters - Eliminates debris or dead cells from the data provided. - Offers the possibility to characterize cells on a single-cell level	- Time- and cost-intensive - Requires qualified technicians - May offer false results if the debris is aggregated	5–7	[171]
Py-GC/MS	- Useful in analytical characterization - Fast separation - Little requirements for samples - High sensitivity and selectivity	- Time- and cost-intensive - Not suitable for thermally vulnerable samples or those that require extensive preparation. - The sample will be lost after analysis	4–6	[19]

3.8. Emerging Analytical Trends in MP/NPL Detection

Although conventional analytical techniques remain the cornerstone of MP and NPL characterization, their application is often limited by long acquisition times, operator-dependent workflows, insufficient spatial resolution, and increasingly complex data processing. These limitations become evident when analyzing heterogeneous environmental samples or biological matrices containing NPLs. Consequently, recent technological developments have focused on improving analytical throughput, automation, and standardized analytical workflows [139,143,172]. Rather than replacing established analytical techniques, these emerging approaches extend the analytical capabilities of conventional microscopy and vibrational spectroscopy by overcoming several limitations associated with particle

detection, polymer identification, and data interpretation. A summary of the principal emerging analytical technologies, together with their main advances over conventional analytical methods and their current limitations, is presented in Table 5.

Table 5. Emerging analytical technologies for MP and NPL detection: applications, advantages, and current limitations.

Emerging Technology	Best Suited For	Advantages Over Conventional Techniques	Limitations	Sources
FPA-FTIR Imaging	Automated chemical mapping of MPs in environmental and biological samples	Simultaneous acquisition of thousands of IR spectra using focal plane array detectors, enabling rapid chemical imaging, improved reproducibility and automated particle identification compared with conventional FT-IR.	Diffraction-limited spatial resolution, limited detection of particles below ~10–20 µm and generation of large hyperspectral datasets requiring extensive computational processing.	[173,174]
AFM-IR	Nanoscale chemical characterization of individual MPs/NPLs, heterogeneous biological matrices	Combines nanometer-scale AFM imaging with IR spectroscopy, enabling simultaneous morphological and chemical characterization beyond the diffraction limit of conventional FT-IR. Particularly suitable for particles <500 nm.	Low analytical throughput, expensive instrumentation, complex operation, and contact-based measurements currently limit routine, large-scale applications.	[143,144,175,176]
O-PTIR	Label-free characterization of small MPs/NPLs in biological and environmental samples	Provides submicrometre IR imaging beyond the diffraction limit while generating spectra directly comparable with FT-IR libraries. Enables high-resolution analysis with minimal fluorescence-related artefacts.	High instrumentation cost, limited accessibility, restricted optical penetration depth, and insufficient validation across diverse biological matrices.	[143,144,175–177]
LDIR	High-throughput routine monitoring of MPs in environmental and biological samples	Enables automated particle detection and polymer identification using QCL-based IR spectroscopy. Significantly reduces analysis time and minimizes operator intervention compared with conventional µFT-IR mapping.	Narrower spectral range than FT-IR, reduced sensitivity for the smallest NPLs, and relatively high instrumentation costs.	[139,143]
Automated Raman Imaging	Large-scale particle screening and automated polymer identification	Integrates particle localization, spectral acquisition, and polymer identification into a single workflow, substantially increasing analytical throughput while reducing operator-dependent variability.	Long acquisition times for highly heterogeneous samples and potential laser-induced heating of sensitive materials.	[172,178]
Hyperspectral Raman Imaging	Detailed chemical mapping of heterogeneous samples and small MPs/NPLs	Acquires a complete Raman spectrum for every pixel, enabling simultaneous chemical mapping and morphological characterization with high spatial resolution.	Generates very large datasets requiring advanced computational resources, sophisticated data processing and prolonged acquisition times.	[144,178]
Artificial Intelligence-Assisted Particle Classification	Automated particle recognition and polymer identification from large imaging and spectroscopic datasets	Automates particle detection, spectral interpretation, and polymer classification while reducing operator bias, improving reproducibility and facilitating standardized analytical workflows across multiple analytical platforms.	Requires large, well-curated reference datasets, extensive algorithm validation, and standardized training procedures before widespread implementation.	[143–145,172,178]

3.8.1. Advances in Infrared (IR) Spectroscopy

Recent developments in IR spectroscopy have primarily focused on improving spatial resolution, analytical throughput, and automation while preserving the non-destructive chemical identification of polymer particles. Among the most recent developments, focal plane array Fourier transform IR imaging (FPA-FTIR), atomic force microscopy–infrared spectroscopy (AFM-IR), laser direct infrared imaging (LDIR), and optical photothermal infrared spectroscopy (O-PTIR) have emerged as complementary IR-based approaches that address different limitations of conventional FT-IR microscopy.

FPA-FTIR represents a major advancement over conventional point-by-point FT-IR microscopy by simultaneously collecting thousands of IR spectra using a focal plane array detector. This parallel spectral acquisition substantially accelerates chemical imaging, improves reproducibility, and enables automated analysis of large sample areas without requiring prior visual particle selection. Consequently, FPA-FTIR has become one of the most widely adopted approaches for automated MP identification in environmental samples and is increasingly being explored for biological matrices. Recent studies have further demonstrated its superior capability for detecting small MPs (10–100 μm), revealing significantly higher particle abundances than conventional ATR-FTIR in complex environmental samples. However, FPA-FTIR remains limited by the IR diffraction limit, which restricts the detection of smaller NPLs, and generates large hyperspectral datasets that require considerable computational resources for data processing and spectral interpretation [173,174].

AFM-IR combines the nanometre-scale spatial resolution of AFM and chemical characterization of individual plastic particles beyond the diffraction limit of conventional FT-IR microscopy [176,177]. Compared with traditional FT-IR imaging, AFM-IR provides substantially improved spatial resolution, making it suitable for the characterization of NPLs < 500 nm and heterogeneous biological samples [175,177]. Furthermore, AFM-IR enables localized chemical analysis without fluorescent labelling, providing valuable information on particle composition and surface chemistry. Despite these advantages, its routine implementation remains limited by relatively low analytical throughput, expensive instrumentation, complex operation, and the contact-based nature of AFM measurements, which restrict large-scale monitoring applications [176,177].

LDIR represents one of the most important recent developments in automated IR analysis for MP detection. By combining quantum cascade laser (QCL)-based IR spectroscopy with automated particle recognition and spectral library matching, LDIR substantially reduces acquisition times compared with conventional $\mu\text{FT-IR}$ imaging while maintaining reliable polymer identification [139]. Unlike conventional FT-IR mapping, which acquires complete spectra over the entire scanned area, LDIR selectively analyses detected particles, considerably increasing analytical throughput while minimizing operator intervention. Consequently, this technique is particularly attractive for monitoring MPs in water, sediments, soils, and, increasingly, biological samples. Nevertheless, its application remains constrained by a narrower spectral range ($\sim 1800\text{--}900\text{ cm}^{-1}$), reduced sensitivity for the smallest NPLs, and relatively high instrumentation costs [139].

O-PTIR represents another important advancement by overcoming the diffraction-limited spatial resolution of conventional IR microscopy. Compared with FT-IR, O-PTIR enables submicrometre chemical imaging while producing spectra directly comparable with commercial FT-IR spectral libraries [176,177]. In addition, the technique minimizes fluorescence-related artefacts and provides high signal-to-noise ratios, facilitating the label-free characterization of MPs and NPLs in complex environmental and biological matrices [175,177]. O-PTIR has successfully been applied to the detection of plastic particles in mammalian tissues, snow, and other challenging matrices, demonstrating detection capabilities down to $\sim 200\text{--}250\text{ nm}$ [177]. However, its routine implementation is currently

limited by high instrumentation costs, restricted optical penetration depth, and the need for further validation across diverse biological sample types [175,176].

Collectively, AFM-IR, LDIR, and O-PTIR demonstrate the current evolution of IR-based MP/NPL analysis toward higher spatial resolution, greater analytical throughput, and increased automation, thereby overcoming several limitations of conventional FT-IR microscopy while preserving reliable polymer identification.

3.8.2. Advances in Raman Spectroscopy

Recent developments in Raman spectroscopy have focused on improving analytical throughput, spatial resolution, and automation while reducing operator-dependent variability during particle detection and polymer identification.

Automated Raman imaging systems integrate particle localization, spectral acquisition, and polymer identification into a single analytical workflow using dedicated software platforms capable of automatically detecting hundreds to thousands of particles within a scanned area [143,178]. Compared with conventional point-by-point Raman analysis, automated Raman imaging substantially reduces operator-dependent variability, increases analytical efficiency, and improves reproducibility, making the technique particularly suitable for large-scale environmental monitoring and routine analytical laboratories [143,172]. Nevertheless, long acquisition times for highly heterogeneous samples and the potential for laser-induced heating or degradation of sensitive materials remain important limitations [144,178].

Hyperspectral Raman imaging acquires a complete Raman spectrum for every pixel within the scanned area, generating a hyperspectral data cube containing both spatial and chemical information [144]. This approach enables simultaneous morphological visualization and polymer identification with high spatial resolutions, facilitating the detection and characterization of small MPs and NPLs, including particles approaching 100 nm under optimized analytical conditions [144,175,178]. However, hyperspectral Raman imaging generates extremely large datasets requiring advanced computational resources and sophisticated data processing algorithms, while acquisition times may extend from several minutes to several hours depending on sample complexity [144].

Together, automated and hyperspectral Raman imaging techniques considerably expand the analytical capabilities of conventional Raman spectroscopy by enabling faster, more reproducible, and chemically comprehensive characterization of MPs and NPLs.

3.8.3. Artificial Intelligence-Assisted Particle Analysis

Artificial intelligence (AI) has rapidly emerged as one of the most promising developments in MP and NPL analysis by enabling automated particle recognition, spectral classification, and polymer identification while substantially reducing operator-dependent variability [143,145]. Machine learning and deep learning algorithms are capable of processing large imaging and spectroscopic datasets while identifying subtle spectral differences that may be difficult to distinguish through conventional manual interpretation [172,178,179]. Consequently, AI-assisted analysis substantially reduces manual data processing time, improves classification accuracy, and enhances the reproducibility of polymer identification, particularly when integrated with Raman spectroscopy and IR imaging techniques [144,172,178].

Beyond improving high-capacity analysis, AI also represents a promising strategy for the standardization of MP and NPL analysis by facilitating automated spectral interpretation and the development of comprehensive reference libraries for polymer identification [143]. Nevertheless, current AI-based approaches still require extensive vali-

dation using high-quality reference datasets and may encounter difficulties when analyzing highly heterogeneous mixtures of previously uncharacterized polymer types [145,175,178].

Rather than replacing conventional analytical techniques, AI is expected to become an essential component of future automated analytical workflows by complementing existing analytical platforms and improving both the speed and reliability of MP and NPL characterization.

Collectively, these technological developments demonstrate a clear transition from conventional, operator-dependent analytical workflows to increasingly automated, standardized, and faster analysis approaches for MP and NPL detection. Recent advances in IR spectroscopy have primarily improved spatial resolution and analytical speed, whereas developments in Raman spectroscopy have focused on automation and comprehensive chemical mapping. The integration of AI further complements these techniques by facilitating automated particle recognition, spectral interpretation, and standardized polymer classification. Although these emerging technologies do not replace established analytical methods, they significantly extend their analytical capabilities and are expected to play an increasingly important role in future environmental monitoring and human biomonitoring studies.

3.9. Role in Risk Assessment

MPs are complex materials that require comprehensive risk evaluations to prevent potential environmental impacts. Current risk assessment methods compare projected exposures with known hazards or toxicity thresholds. However, particle risk assessment methodologies are still in their early stages of development, and assumptions are therefore commonly applied. There are certain knowledge gaps regarding the environmental concentrations of various polymer types, their size distributions and morphologies, and their impact on various organisms. Notably, current findings suggest that environmental exposure levels are usually lower than the concentrations associated with particle uptake into cells and organs and the onset of measurable biological effects. Preliminary estimations indicate that MP levels are within acceptable limits in most situations. Nevertheless, the impacts of chronic exposures, as well as the consequences of the growth in plastic manufacturing and use, remain insufficiently understood. Human exposure to MPs is closely associated with their presence in aquatic environments, particularly through the consumption of marine products. Future research is therefore required to address these knowledge gaps.

4. Future Directions and Challenges

Emerging trends in MP/NPL detection involve research topics and technological advancements aimed at increasing detection sensitivity, optimizing and/or simplifying sample processing, and broadening analysis areas. Key priorities include developing innovative detection technologies, such as nanoparticle-based sensors and spectroscopic imaging techniques that offer greater sensitivity and specificity than previous methods. The number of studies regarding the use of magnetic resonance imaging, photoacoustic imaging [168], computed tomography, or positron emission tomography [169] to detect labelled NPLs/MPs is low. Also, the emergence of microfluidic systems is enhancing the accuracy of particle detection and separation by incorporating specialized flow sensors within microchannels to aid real-time monitoring [170].

One crucial knowledge gap is the development of standardized protocols and reference materials available that can be used to ensure consistency and comparability across studies. Additionally, the improvement of sampling techniques to capture MPs/NPLs in different environmental matrices in an accurate manner requires greater attention. Furthermore,

supplementary research is needed to elucidate the mechanisms of MP/NPL uptake and accumulation in organisms, as well as their potential health effects.

Understanding the fate and transport of MPs/NPLs in ecosystems, including their interactions with other pollutants, is another important research priority and is crucial for implementing effective mitigation strategies to reduce their impact on ecosystems and human health. This will help the development of policies and practices aimed at reducing pollution of MPs and protecting environmental and human health. Growing environmental and health concerns regarding the release of synthetic polymer particles from cosmetic products have led to regulatory measures in several regions, including restrictions and bans on intentionally added microbeads in rinse-off cosmetic products. In the United States, the Microbead-Free Waters Act of 2015 prohibits the manufacture, from 2017, and sale, from 2018, of rinse-off cosmetics containing intentionally added plastic microbeads [180]. In the European Union, recent restrictions under Regulation (EU) 2023/2055 target intentionally added MPs in consumer products, with immediate bans on microbeads used as abrasive exfoliants and phased restrictions for other cosmetic applications [181]. Despite these regulatory measures, synthetic polymers remain widely used in cosmetic formulations, highlighting the need for further research on exposure pathways and potential health risks.

Investigating the potential long-term health impacts of MPs/NPLs on organisms and on their uptake and accumulation mechanisms could provide valuable insights for policy-makers and regulators. Studying the interactions between MPs/NPLs and other pollutants in ecosystems could help identify synergistic effects that may exacerbate environmental degradation. Developing standardized protocols for measuring and analysing MPs/NPLs across studies could enhance comparability and facilitate more robust conclusions in scientific research.

Future challenges and opportunities in MP/NPL research might involve overcoming existing obstacles while embracing new prospects for innovation and collaboration. One significant challenge lies in standardizing detection methods and data analysis protocols to ensure consistency and comparability across studies. Furthermore, additional research regarding the long-term effects of MPs/NPLs on various ecosystems and organisms is needed to better understand the potential consequences for the environment and human health. On the other hand, the rise of interdisciplinary research and technological advancements offers exciting opportunities for developing novel solutions to tackle the issue of MPs/NPLs pollution in a more effective manner. By working together and leveraging diverse expertise, scientists can make significant steps in addressing this environmental concern. Interdisciplinary collaboration among scientists from various fields, such as environmental science, chemistry, biology, and engineering, is crucial in effectively addressing complex questions related to MP/NPL pollution.

Despite these challenges, there are promising opportunities for innovation and collaboration in MPs/NPLs research. For example, collaboration between chemists and engineers could lead to the development of new materials able to break down MPs/NPLs more efficiently in the environment. By combining their knowledge and skills, novel solutions could be developed to reduce the impact of MP/NPL pollution on ecosystems. Technological advancements, including the development of high-throughput detection techniques and advanced imaging technologies, offer new possibilities for more accurate and efficient analysis of MPs/NPLs. Additionally, integrating big data analytics and machine learning algorithms can enhance data processing and interpretation, leading to deeper insights into MPs/NPLs behaviour and impacts.

5. Conclusions

On a final note, research on in vitro and in vivo methods for detecting MPs and NPLs represents a valuable advancement in our understanding of the prevalent effects of plastic pollution in the medium and long term. Additionally, monitoring and addressing global plastic pollution issues will require the development of more sensitive and accurate detection technologies. Although these methods could provide valuable information, it is essential to consider any potential limits and select the most suitable one for each research according to several specific factors. Ensuring that the data obtained are accurate and reliable is crucial in understanding the advantages and disadvantages of each technique. Through constant improvement of detection techniques and adaptation to diverse study requirements, progress toward setting up better strategies may be achieved.

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Abbreviations

The following abbreviations are used in this manuscript:

MPs	Microplastics;
NPLs	Nanoplastics;
SEM	Scanning electron microscopy;
TEM	Transmission electron microscopy;
Cryo-EM	Cryogenic electron microscopy;
Cryo-SEM	Cryogenic scanning electron microscopy;
CLSM	Confocal laser scanning microscopy
AFM	Atomic force microscopy;
FT-IR	Fourier transform infrared spectroscopy;
scICP-MS	Single-cell inductively coupled plasma mass spectrometry;
scICP-TOFMS	Single-cell inductively coupled plasma–time-of-flight mass spectrometry;
Py-GC/MS	Double-shot gas chromatography–mass spectrometry;
MAPLs	Macroplastics;
MEPLs	Mesoplastics;
μFT-IR	Fourier-transform infrared microspectroscopy;
LDIR	Laser direct infrared;
PA	Polyamide;
PU	Polyurethane;
PP	Polypropylene;
PET	Polyethylene terephthalate;
PE	Polyethylene;
PS	Polystyrene;

PVC	Polyvinyl chloride;
PC	Polycarbonate;
PLA	Polylactic acid;
PI	Polyimide;
GIT	Gastrointestinal tract;
SEM-EDX	Scanning electron microscopy–energy-dispersive X-ray spectroscopy;
SERS	Surface-enhanced Raman spectroscopy;
TERS	Tip-enhanced Raman spectroscopy;
PMMA	Polymethyl methacrylate;
STEDM	Stimulated emission depletion microscopy;
POM	Polyoxymethylene;
ATR-FTIR	Attenuated total reflectance-FT-IR;
AFM-IR	Atomic force microscopy-based infrared spectroscopy;
DLS	Dynamic light scattering;
TRL	Technology readiness level;
FPA-FTIR	Focal plane array Fourier-transform IR imaging;
O-PTIR	Optical photothermal infrared spectroscopy;
LDIR	Laser direct infrared imaging;
AI	Artificial intelligence.

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