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Short Communication

Rapid and direct detection of small microplastics in aquatic samples by a new near infrared hyperspectral imaging (NIR-HSI) method

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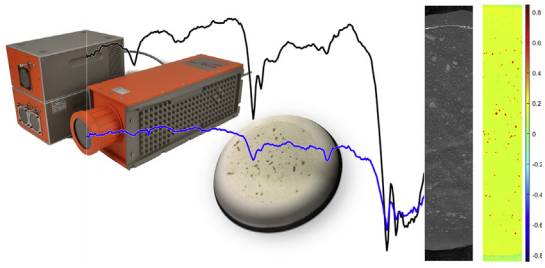
HIGHLIGHTS

- A new hyperspectral NIR imaging method for analysis on microplastics is presented.
- Microplastics in aquatic samples are identified directly on the filters.
- Large portions of filters or whole filters can be analysed automatically.
- Fast chemical mapping can be applied on large number of samples.
- Procedural steps, time and costs are drastically reduced for extensive monitoring.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastic (MP) contamination is a critical environmental challenge with a strong impact on the ecosystems, economy and potentially for human health. The smaller the MP size, the greater is the environmental risks as well as the analytical difficulties in detecting and characterising the particles. .We propose a rapid near infrared hyperspectral imaging (NIR-HSI) method that enables the chemical identification and characterisation of small MP (down to 80 μm) in aquatic samples, directly on filters, with no pre-sorting step needed. By considerably reducing the procedural steps, the time of analysis and costs our method addresses the urgent need of cost-effective and robust tools for extensive monitoring of MP in natural systems.

1. Introduction

Microplastics (MP) are plastic particles characterised by very small dimensions (≤5 mm) (Arthur et al., 2009). They have two main origins: i) those that are manufactured in very small sizes

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(primary MP) such as the precursors of plastic products (e.g. virgin industrial pellets); or synthetic microspheres found in cosmetics (e.g. exfoliates) or detergents, and ii) particles that originate from the physical, biological or chemical degradation (e.g. ultraviolet radiation, wind or water erosion, microorganisms) of larger pieces of plastic waste released into the environment (secondary MP) (Carbery et al., 2018). MP are ubiquitous contaminants and have been found in terrestrial, freshwater and marine ecosystems (Carbery et al., 2018; de Souza Machado et al., 2018; Ivleva et al., 2017).

MP contamination in aquatic environments is particularly worrying, due to both the high concentrations in these systems as well as the ingestion by a variety of organisms. The smaller the synthetic particles, the greater the risk, as they become available to a much wider range of species, entering the food chain and being transported at upper trophic level potentially up to humans (de Souza Machado et al., 2018). Once ingested by organisms, MP particles and their potential associated toxic contaminants (additives and persistent organic pollutants) can cause physical (e.g., obstructions, abrasions and inflammation) and adverse physiological effects (e.g. decreased food consumption, weight loss, decreased growth rate and fecundity, energy depletion) (Zhang et al., 2019).

To address the requirement of the Marine Strategy Framework Directive there is an urgent need to develop costs-effective monitoring protocols to provide information on i) quantities and typologies, ii) sources and iii) biological harm caused my marine litter, including MP (Galgani et al., 2013).

The effective monitoring of MP in aquatic systems, however, still faces numerous methodological limitations associated to the long-time of analysis and costs that do not allow an adequate sampling replication, thereby preventing an accurate evaluation of spatial and temporal patterns of MP abundance and composition at global scale (Underwood et al., 2017).

The smaller the particles, the greater the difficulty in detecting and quantifying them in the environment (Ivleva et al., 2017). This is an issue, as there is increasing evidence that the bulk (number of particles) of MP in aquatic ecosystems consists of small MP particles. A recent study on MP distribution in the North Sea showed that on average 99% of MP in sediments and 94% in surface waters are smaller than 500 μm (Lorenz et al., 2019). Further, the high physicochemical heterogeneity (in terms of the size, shape and polymer composition) makes the identification of MP very challenging (Peiponen et al., 2019).

Conventional methods involve the selection of potential MP particles by visually examining the pre-filtered and digested sample. The selected particles are then subjected to single-point spectroscopic analyses (such as Raman or FTIR microscopy and/or spectroscopy). The visual identification of particles required by these techniques is subjective and may lead to erroneous results, particularly for particles <500 μm. Although in the last years also micro-FTIR imaging methods are becoming increasingly used, they are very time-consuming and require extensive manipulation of the samples (Paul et al., 2019), which may lead to the risk of particle loss and/or contamination of samples by airborne fibres.

In the present study, a near infrared hyperspectral imaging (NIR-HSI) technique (Malaspina et al., 2018; Malegori et al., 2016; Oliveri et al., 2019) is proposed for identifying MP down to 80 μm in aquatic samples. Analysis is performed directly on the filters that are commonly used to concentrate the digested organismal soft tissue and it does not require any particle pre-sorting. The presented technique, therefore, limits the risk of losing or incorrectly sorting the particles and reduces the time of analysis, costs, and procedural bias.

2. Material and methods

2.1. Samples preparation

Six specimens of Mediterranean mussels (*Mytilus galloprovincialis*, Lamarck), collected in Marina di Ravenna (Northern Adriatic Sea, Italy, (44°29′32 0.6″N, 12°17′15.2″E), were dissected to separate the soft tissue from the shell. The soft tissues were then digested using 10% potassium hydroxide (KOH), similarly to the method previously reported by Piarulli et al. (2019, 2020). After an incubation of 24 h at 50 °C, 1.5 mg of 10–500 μm low density polyethylene (LDPE) powder, produced by fragmenting LDPE pellets (Goodfellow Cambridge Ltd. UK), was added to each of the six digestate solutions. Three digestates were vacuum-filtered on Nylon© filters (mesh size: 20 μm, Ø: 5 cm, PLASTOK®) and three on cellulose filters (mesh size: 11 μm, Ø: 5 cm, Whatman®). The two different types of filter were tested to identify the most suitable support membrane for the characterisation of MP through NIR analyses. To validate the NIR-HSI procedure, we also prepared six analytical blanks (three for each type of Nylon© or cellulose filter) by filtering a solution of 10% KOH without the digested mussel tissue, and six procedural controls (three for each type of Nylon© or cellulose filter) by individually filtering the digested tissues of six mussels without adding MP.

2.2. NIR-HSI analysis and data processing

All the filters were subjected to NIR-HSI analysis. We used a SWIR-3 HSI camera equipped with a 40 × 20 cm Lab Scanner (Specim Ltd, Finland) to record a near-infrared (NIR) spectrum in the region between 1000 up to 2500 nm for each pixel. To obtain a high spatial resolution and ensure an efficient chemical characterization of the MP, we used a macro lens (OLES MACRO, 73.3 mm focal length, 10 cm working distance) with a nominal pixel size of 21 μm. Hyperspectral images were acquired at a 0.7 mm/s scan rate. To identify the diagnostic NIR bands of LDPE, a reference sample of the powder was analysed.

Chemical maps were obtained by calculating the normalised difference image (NDI) (Malegori et al., 2020) and by applying the following formula:

$$NDI = \frac{R_{\lambda f} - R_{\lambda s}}{R_{\lambda f}}$$

where $R_{\lambda s}$ is the reflectance value at a wavelength characteristic of the filters (2121 nm for cellulose and 2071 nm for Nylon©) and $R_{\lambda f}$ is the reflectance value at 2332 nm characteristic for LDPE MP. The counting of the MP, as well as the evaluation of the particle size distribution, was performed manually by selecting each particle and evaluating the dimensional range at the largest cross-section.

3. Results and discussion

The reference powder showed characteristic and well-defined bands at 2332 nm and 2371 nm, ascribable to the C–H stretching and bending combination bands. The absorption at 1747 nm was associated with the 1st overtone of C–H stretching, while the band at 1412 nm related to the 1st overtone of O–H stretching vibration (Fig. 1).

The spectra of Nylon© and cellulose filters are also shown in Fig. 1 for comparison. Due to the peculiar physical texture of the filter determining highly scattered diffuse reflection, both filters were characterised by low-intensity NIR signals (Burns and Ciurczak, 2008) and their characteristic bands did not significantly overlap with those of LDPE.

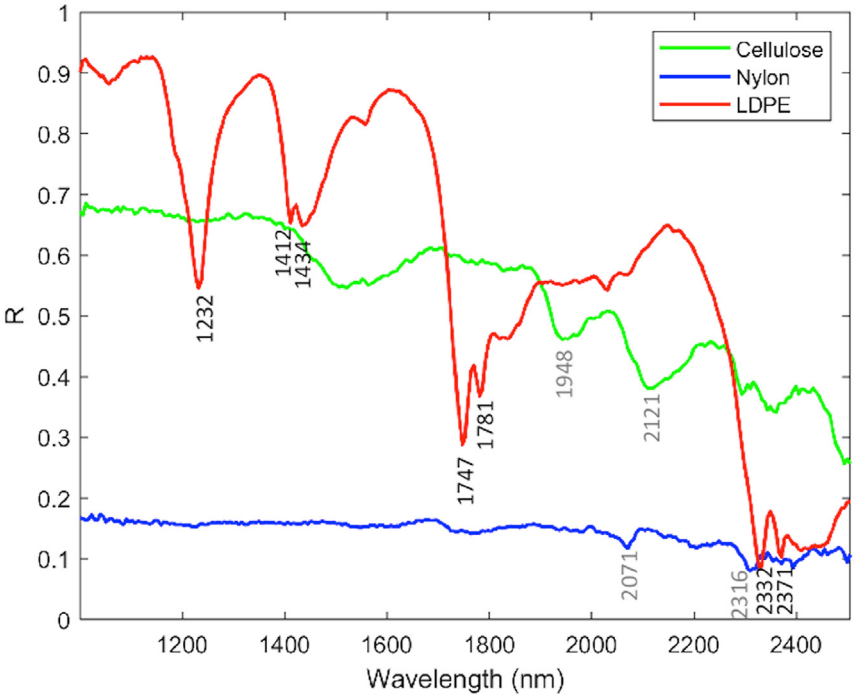

Fig. 1. Average NIR spectral profile of standard cellulose, Nylon© and LDPE.

In the cellulose filter, the band at 2121 nm was ascribable to the cellulose O—H stretching and bending combination band, while the signal at 1948 nm was related to the O—H combination band of water. The broad absorption at 2360 nm was associated to the C—H stretching and deformation band (Schwanninger et al., 2011).

Nylon© filters showed very low intense signals within the investigated spectral region: the absorption at 2071 nm was linked to the N—H combination band, while the band at 2316 nm was ascribable to the C—H stretching and bending combination band (Xu and Wu, 2007).

The difference between the MP and the support filter is represented by a colour-scale image whose range is between the maximum (red) and the minimum (blue) reflectance values.

Figs. 2 and 3 report the MP distribution maps obtained on cellulose and Nylon© filters after the filtration of the mussels' tissues with added MP.

According to this representation, MP particles are identified by red pixels that stand out from the green background. Each chemical map was acquired in few seconds guaranteeing an affordable application of the method for the characterization of a high number of samples. Thus, filters can be automatically analysed in total or large areas. The lowest detectable MP size was 80 μm, thus significantly lower than the ranges previously reported and obtained by near infrared spectroscopy (Karlsson et al., 2016). The NIR spectra can be interactively extracted by selecting the pixels of interest from the chemical maps. A straightforward identification of the diagnostic bands of LDPE was achieved on both the cellulose and Nylon© filters, thanks to the easy visualization and comparability of the associated NIR spectra. On cellulose filters, the aggregation of particles was less evident compared to the Nylon© support. This effect may be due to the different physical texture and to the different hydrophilic degree of the two materials. Cellulose filters are more porous and more hydrophilic, thus enhancing the efficiency of filtration of water-based suspensions resulting in the maintenance of the original size and shape of the MP particles, that is fundamental for the subsequent identification of the MP contamination sources.

To the best of our knowledge, only one previous study has evaluated the possibility to use a NIR-HSI system for detecting MP (Karlsson et al., 2016). Karlsson's method, however, involves a preliminary visual recognition and sorting of the largest particles, potentially ranging from 300 μm up to 5 mm, and excluding from the investigation smaller particles that represent the greatest environmental concern. In addition, the visual sorting may lead to a non-negligible risk of particle loss and sample exposure to airborne contamination.

The chemical maps of the analytical blanks revealed the total absence of LDPE spectral signals on both cellulose and nylon filters (data not shown), confirming the effectiveness of the method (absence of false positives) in identifying MP directly on the filters. Indeed, thanks to the low intensity of the NIR spectral signals associated with cellulose and Nylon© filters, absorption bands of MP were clearly detectable. In addition, the procedural control filters showed no evident spectral signals derived from the digestate solution (data not shown), thus highlighting that the method does not require strong or multiple sample purifications that are usually time consuming and leading to degradation or loss of particles.

4. Conclusion

In conclusion, the results reported in the present paper constitute a proof of concept for the application of the NIR-HSI system to identify MP particles down to a size of 80 μm, directly on filters. The method does not require any manual sorting of the particles, thereby considerably reducing the analysis time, sample manipulation, and the potential disruption or loss of particles. The method is particularly efficient when applied on cellulose filters, which limited the formation of MP aggregates compared to nylon filters, thus maintaining the original size and shape of the MP particles. This is fundamental for the subsequent identification of MP sources in natural systems.

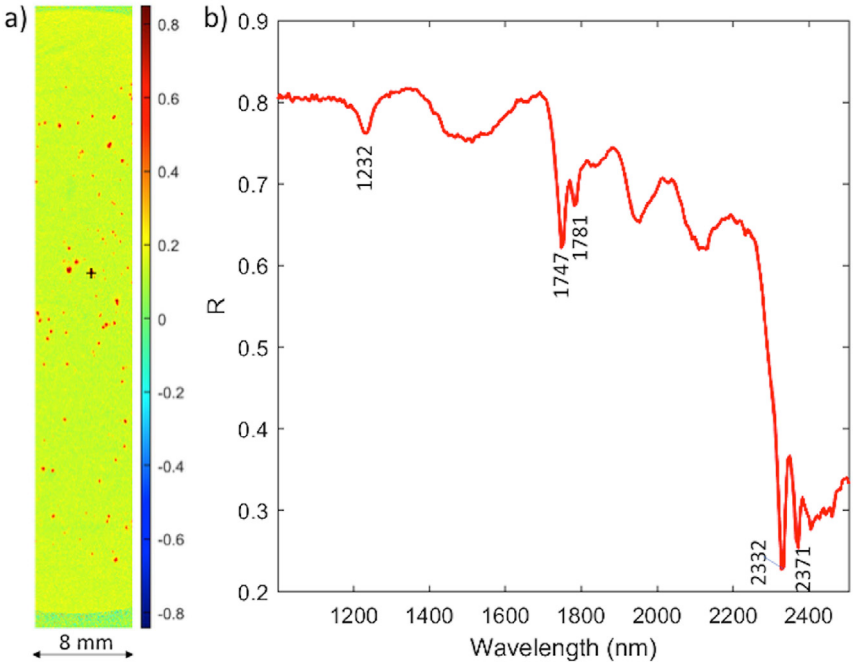

Fig. 2. a) NDI maximizing the contrast between LDPE microparticles (in red) and the cellulose support. b) NIR spectral profile of a selected particle aggregate (indicated by the black cross in the NDI). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

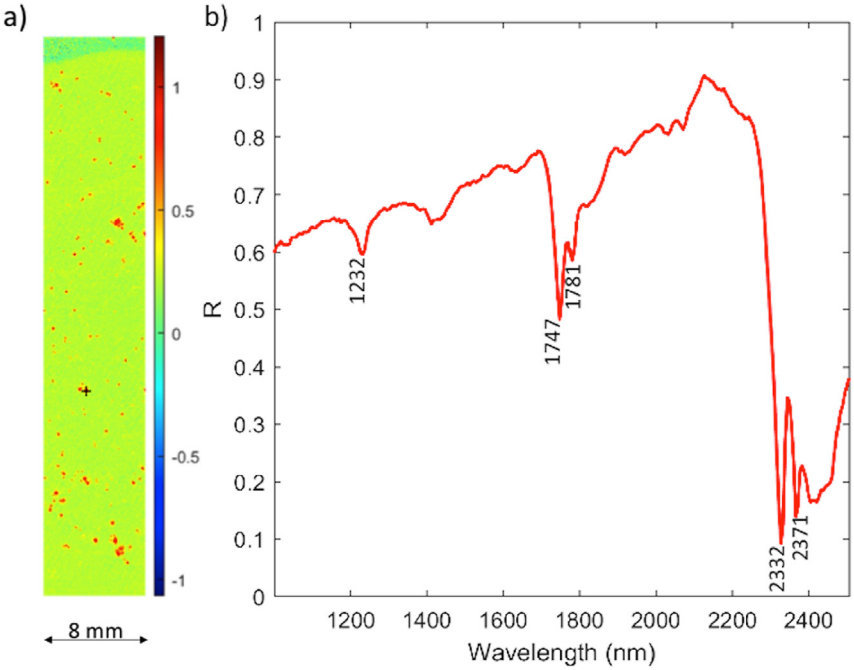

Fig. 3. a) NDI maximizing the contrast between LDPE microparticles (in red) and the Nylon© support. b) NIR spectral profile of a selected particle aggregate (indicated by the black cross in the NDI). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

By considerably simplifying the analyses, our method addresses the urgent need for cost/effective and rapid methods for analysing a large number of biological and/or environmental samples.

Further research efforts are currently dedicated to demonstrate the applicability of the method on a wider range of polymers and shapes of particles (e.g. fibres), as well as to evaluate its performance in detecting MP from different environmental matrices. An automated image analysis procedure for particle counting and size distribution assessment will also be implemented to improve the applicability of the method on a large-scale.

The benefits of a large-scale implementation of the NIR-HSI systems include being able to design sampling monitoring programmes to determine the spatial distribution and/or temporal dynamics of MP in different aquatic species and environments.

Credit author statement

Stefania Piarulli: Conceptualization, Resources, Writing - original draft, Giorgia Sciutto: Conceptualization, Methodology, Writing - review & editing, Supervision, Visualization, Paolo Oliveri: Conceptualization, Methodology, Writing - review & editing, Supervision, Software, Cristina Malegori: Investigation, Data curation, Silvia Prati: Writing - review & editing, Validation, Rocco Mazzeo: Writing - review & editing, Validation, Laura Airoldi: Writing - review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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