

## What determines accuracy of chemical identification when using microspectroscopy for the analysis of microplastics?

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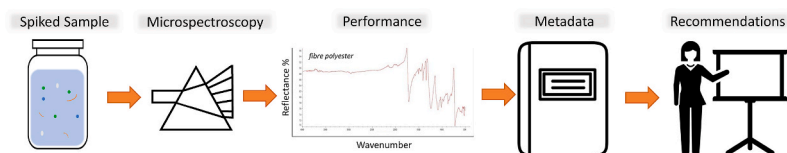
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## HIGHLIGHTS

- Harmonization of methods is a priority in microplastics research.
- FTIR and Raman microspectroscopy are common methods used to analyze microplastics.
- Variables for successful identification using microspectroscopy were identified.
- Particle characteristics and instrumental parameters influence identification success.
- Technical method recommendations for using FTIR and Raman microspectroscopy are provided.

## GRAPHICAL ABSTRACT



## ABSTRACT

Fourier transform infrared (FTIR) and Raman microspectroscopy are methods applied in microplastics research to determine the chemical identity of microplastics. These techniques enable quantification of microplastic particles across various matrices. Previous work has highlighted the benefits and limitations of each method and found these to be complimentary. Within this work, metadata collected within an interlaboratory method validation study was used to determine which variables most influenced successful chemical identification of un-weathered microplastics in simulated drinking water samples using FTIR and Raman microspectroscopy. No variables tested had a strong correlation with the accuracy of chemical identification ( $r = \leq 0.63$ ). The variables most correlated with accuracy differed between the two methods, and include both physical characteristics of particles (color, morphology, size, polymer type), and instrumental parameters (spectral collection mode, spectral range). Based on these results, we provide technical recommendations to improve capabilities of both methods for measuring microplastics in drinking water and highlight priorities for further research. For FTIR microspectroscopy, recommendations include considering the type of particle in question to inform sample presentation and spectral collection mode for sample analysis. Instrumental parameters should be adjusted for certain particle types when using Raman microspectroscopy. For both instruments, the study highlighted the need for harmonization of spectral reference libraries among research groups, including the use of libraries containing reference materials of both weathered plastic and natural materials that are commonly found in environmental samples.

## 1. Introduction

The field of microplastics research has seen an exponential growth over the last two decades (Granek et al., 2020; Wootton et al., 2021), since this pressing environmental problem was first studied in the 1970's and microplastics were defined in 2004 (Carpenter and Smith, 1972; Thompson et al., 2004). Our ability to measure and characterize microplastics has evolved with our continued understanding of this diverse and multifaceted environmental contaminant. Early investigations relied primarily on counting and characterizing larger particles ( $>333 \mu\text{m}$ ) based on their visual or tactile properties (e.g., Doyle et al., 2011; Eriksen et al., 2013; Hidalgo-Ruz et al., 2012; Lusher et al., 2020; Wang and Wang, 2018). These studies largely relied on optical microscopes (Shim et al., 2017; Wang and Wang, 2018). However, research has since demonstrated that using microscopy alone results in subjectivity and bias in microplastic estimates, often where small particles are missed, and natural particles are falsely identified as plastic (Isobe et al., 2019; Löder and Gerdt, 2015; Shim et al., 2017).

The introduction of vibrational spectroscopic techniques such as Raman and FTIR microscopes allow for the chemical identification of suspected plastic particles. Vibrational characteristics of chemical functional groups are identified from a spectrum (Ricci et al., 2015). FTIR microspectroscopy generates a fingerprint of the material being analyzed by measuring the change of the dipole moment of the molecule excited by infrared radiation to produce a spectrum which can be used to identify materials. Raman microspectroscopy is another vibrational technique that provides information about the change of polarizability of the molecules via a sample spectrum which can be used to identify materials (Araujo et al., 2018; Ivleva et al., 2017). The use of microspectroscopy in microplastics research allows researchers to confirm the material type of particles (i.e., plastic or natural), increasing the reliability of particle count estimates from environmental samples (Brander et al., 2020; Cowger et al., 2020a; Kooi et al., 2021; Primpke et al., 2017). Using polymer confirmation, researchers can also adjust their

visual microscopy counts to better reflect their true representative number (De Frond et al., 2022a). Spectral data also provides researchers with critical information on specific polymer types found in a sample, which can be used to understand their relative abundances, flows and comparisons in different environmental matrices (Kooi et al., 2021), and trends in space and time. As researchers gain a greater understanding of the types of plastics in environmental samples, they will also understand their potential origins for more successful and targeted source reduction strategies. Similarly, polymeric data can be used to inform the design of laboratory experiments and ultimately risk assessments based on the toxicity of specific polymers and their documented abundance in the environment (Kögel et al., 2020).

Despite their advantages, analyses using FTIR and Raman microspectroscopy still encounter challenges due to the unique and varied nature of microplastic particles (Rochman et al., 2019). Within current literature, the capabilities, and limitations of both FTIR and Raman microspectroscopy have been discussed in detail (Anger et al., 2018; Cabernard et al., 2018; Cowger et al., 2020b; Hidalgo-Ruz et al., 2012; Käßler et al., 2016; Löder and Gerdt, 2015; Prata et al., 2019; Shim et al., 2017; Song et al., 2015). However, a thorough comparison of performance for analyzing specific particle types has not yet been investigated quantitatively, particularly for particle sizes below  $20 \mu\text{m}$ , for which only one study to date has measured and compared performance among the two methods (Müller et al., 2020). There is also no consensus on best practice for selecting instrument parameters and particle preparation methods to acquire the best spectra.

Passed in 2018, State of California Senate Bill 1422 requires the development of standard methods to analyze microplastics in drinking water for monitoring purposes. Thus, it has become necessary to identify and optimize the capabilities and minimize limitations for the most promising spectroscopic methods so that their use can be further standardized for monitoring and regulation. An interlaboratory method validation study was carried out from 2019 to 2021 to measure the performance of commonly used methods for identifying and

characterizing microplastics from drinking water.

From the study's overall results (De Frond et al., 2022b), it was concluded that both FTIR and Raman microspectroscopy had high accuracy in identifying plastic particles above 50  $\mu\text{m}$  (95% and 91% accuracy respectively). Evaluation of the extensive metadata collected within this study presented additional opportunities to identify which experimental parameters were most influential for successful chemical identifications, to determine where methods could be improved to increase the likelihood of achieving accurate chemical identification results for future research, and to improve limits of detection.

Metadata collected within the interlaboratory method validation study (publicly available to download via: <https://microplastics.sccwrp.org/>) was utilized to determine the variables most correlated with accuracy of chemical identification when using FTIR and Raman microspectroscopy for the analysis of spiked microplastics in simulated drinking water. From the results, recommendations are provided to improve the performance of these techniques for the analysis of microplastics, and priorities for future research are identified.

## 2. Methods

The Southern California Water Research Project (SCCWRP) led an interlaboratory method validation study intending to measure the performance (accuracy and precision) of three commonly used methods in microplastic research; optical microscopy, FTIR microspectroscopy and Raman microspectroscopy (De Frond et al., 2022b). In total, 22 laboratories from six countries participated and submitted the clean water matrix results. All participating laboratories used optical microscopy for their analyses, and results for optical microscopy are discussed within Kotar et al. (2022), with recommendations for improving methods and reporting for future research and monitoring. Microspectroscopy was used by 20 laboratories to chemically identify suspected microplastics that were first counted using microscopy; 11 used FTIR microspectroscopy and nine used Raman microspectroscopy. The results and metadata from groups using microspectroscopy will be discussed here.

### 2.1. Sample analysis

Methods for sample preparation and processing are outlined in detail within (De Frond et al., 2022b). In brief: three spiked samples of simulated clean water and a laboratory blank were sent to each laboratory with a prescribed Standard Operating Procedure (SOP) for extraction, quantification, and characterization. Simulated clean water samples were spiked with a known amount of microparticles within four size fractions (1–20  $\mu\text{m}$ , 20–212  $\mu\text{m}$ , 212–500  $\mu\text{m}$ , >500  $\mu\text{m}$ ), four polymer types (PE, PS, PVC, and PET) and several colors (clear, white, green, blue, red and orange), and morphologies (sphere, fragment and fiber). Plastics spiked into samples consisted of specifically designed soda capsules containing microplastics. These were produced by the Norwegian Institute of Water Research (NIVA) (further details regarding the creation of reference materials can be found within Martínez-Francés et al., in prep.). Plastics were sourced as raw plastic materials from Goodfellow and Cospheric LLC © in the form of PE, PS and PVC spheres, particles were sieved to obtain required size fractions. PET fibers (300–1000  $\mu\text{m}$ ) were generated at NIVA by collecting fibers released via washing PET fabric sourced from IKEA. In addition to the capsules, green PE spheres from Cospheric LLC © were manually added to samples. All plastic particles used in the study were un-weathered (virgin plastic). ‘False positive’ particles (natural hair, fibers and shells) that may be mistaken for microplastics, were also added to each sample in known quantities. Sample processing involved multiple steps, including sample extraction using sieving and/or vacuum filtration, and particle counting, imaging, measurement and visual characterization using stereomicroscopy. Within the SOP, a subsampling procedure for microspectroscopy was outlined (S1), in which labs were required to select up to 30 particles of each color and morphology combination (e.g.,

30 orange fibers, 30 white fragments), within each size fraction. In cases where less than 30 particles were identified/recovered of a certain particle type, participants were instructed to subsample as many as were found for chemical identification (e.g., if only 8 orange fibers were counted, participants selected all 8 for microspectroscopy). Laboratories were asked to chemically identify all subsampled particles using FTIR and/or Raman microspectroscopy. Specific guidance was not provided for using microspectroscopy other than the particles to be identified and suggestions for particle preparation (e.g., small particles that cannot be manually picked may be analyzed directly from the filter surface). For novice labs that were using the instruments situated at SCCWRP, specific instructions were provided on how to operate each instrument to run manual particle by particle analyses.

### 2.2. Data submission

Data submission variables for the interlaboratory method validation study were chosen based on the ‘Microplastic Reporting Guidelines’ within Cowger et al. 2020a, and further refined through discussion among the participating laboratories. Data were submitted for each suspected plastic particle counted during microscopy, and analyzed by FTIR and/or Raman, which included: sample ID, particle ID, size fraction, particle color, morphology, chemical identification result and the instrument used to carry out chemical identification. Each lab reported the materials they used (e.g., filter or slide type), instrument settings, analysis parameters, the time taken to process each size fraction and sample using spectroscopy and self-reported experience level (Tables S3 and S4).

Following submission of results, further detail on methods used for microspectroscopy was acquired by completing a survey sent to participating laboratories (See Supplementary Information; Kotar et al., 2022). Survey questions were composed by the working groups that were analyzing the metadata for each method (e.g., FTIR microspectroscopy or Raman microspectroscopy), and participating laboratories were instructed to answer all relevant questions with as much detail as possible. In this work, survey responses were utilized to provide context to the patterns observed within the metadata.

### 2.3. Data analysis

#### 2.3.1. Calculating bias in particle selection for chemical identification

Due to logistical limitations such as time and personnel availability, largely influenced by the ongoing COVID-19 pandemic, several laboratories were not able to follow the subsampling protocol for chemical identification, and thus the total number of particles chemically identified by each lab was highly varied (De Frond et al., 2022b). These differences among laboratories were further investigated to determine how the particles selected for chemical identification impacted overall results. Bias in particle selection for chemical identification was calculated using data from all reported particles. This includes any particles suspected to be plastic by participating laboratories, including false positive particles miscategorized as plastic, particles introduced to samples from laboratory contamination, and spiked particles that were miscategorized by color and/or morphology (discussed further in Kotar et al., 2022). These particles were included within the bias analyses as they still provide information on the potential selectivity that laboratories have in analyzing particles with particular characteristics using microspectroscopy. Also, one laboratory used different size fractions to the SOP provided (20–250  $\mu\text{m}$  and 250–500  $\mu\text{m}$ ). These results are included in the bias calculations, but these two size fractions are not included in other results due to low sample size. To calculate bias, first the total number of particles was calculated of a given shape, size, and color visually identified as plastic and counted using microscopy. The total number of particles in each category was then divided by the number that were identified using either FTIR or Raman microspectroscopy. These metrics can be directly inferred as the probability of

a particle to be identified using Raman or FTIR in this study given its particle properties.

### 2.3.2. Assessment of performance

A fraction of the particles that were recovered, counted and chemically identified by study participants were not spiked particles, but the result of laboratory contamination. In these cases, it was not possible to determine the true material type of these particles. Thus, to evaluate the performance of spectroscopy we used only results from spiked particles (including false positives) that were recovered and analyzed using microspectroscopy. To confirm which particles reported were spiked particles for this purpose, the submitted data from each lab was carefully reviewed by those involved in preparing the clean water samples. The specifications of the spiked standard particles were documented for the quality assurance checks. The particle image, when available, was firstly compared with a representative photo of the spiked standard material. Following this, if the visible appearance, size fraction, dimension values, morphology and color also matched that of one of the spiked particle type, then the reported particle ID was confirmed. If the particle ID was unable to be matched with the information of the reported image and data, a “ns” (representing ‘not sure’) was assigned to the reported particle. Next, the reported material ID was checked to score the result as “y” for correct ID or “n” for incorrect ID. Within this refined dataset, any false positive particles that were counted and characterized (i.e., mistaken for plastic particles) in the study were included within the results and were used to determine accuracy for identifying natural particles using the methods investigated.

Following checks to verify the spiked particles, data were then filtered using the quality assured dataset to include only recovered spiked particles (both plastic and natural) that were analyzed using microspectroscopy (either FTIR or Raman). With this refined dataset, we compared the true material of each spiked particle to the reported chemical assignment and calculated the proportion of accurate results for each method and particle type (no. accurate results/no. particles identified) (Fig. S1). This result was calculated both among and within labs. Data analysis was carried out in R software version 4.0.3 (R Core Team, 2020) using several packages (base packages: stats, graphics, grDevices, datasets, utils, methods, base. Other attached packages are stated in Appendix A, S.4).

### 2.3.3. Determining influential variables for accurate chemical identification

Results for the proportion of accurate chemical ID's, data submission and survey response results were used to determine which variables (e.g., instrument settings, or particle characteristics) had the greatest correlation with the proportion of accurate chemical identification results among laboratories. Strong correlations would be indicative of the predictive power of a variable on determining accurate spectral identification. Here, we used the Cramers V statistic (bias corrected) which measures the correlation between two categorical variables. Each variable was measured for correlation to the accurate identification (TRUE, FALSE). All numeric variables were turned into categorical variables for this exercise because there was no clear way to derive a correlation metric that could be comparable between categorical-categorical and numeric-categorical relationships. Cramers V values were bootstrapped (resampling with replacement,  $n = 10,000$ ) for every relationship to create 95% confidence intervals. From these results, the top five variables that were most correlated with obtaining an accurate chemical ID were identified for each method and investigated further.

Next, an investigation was performed to evaluate how differences in mean accuracy drove the correlations for accurate chemical identification. The mean probability of an accurate chemical identification result (0 = never accurate, 1 always accurate) was calculated and bootstrapped (resampling with replacement,  $n = 10,000$ ) the 95% confidence intervals from the bootstrap distribution. Categories that did not have overlapping confidence intervals in their mean accuracy scores were said to be statistically different. For figures and results, this

probability was converted into % accuracy of chemical identification by multiplying by 100.

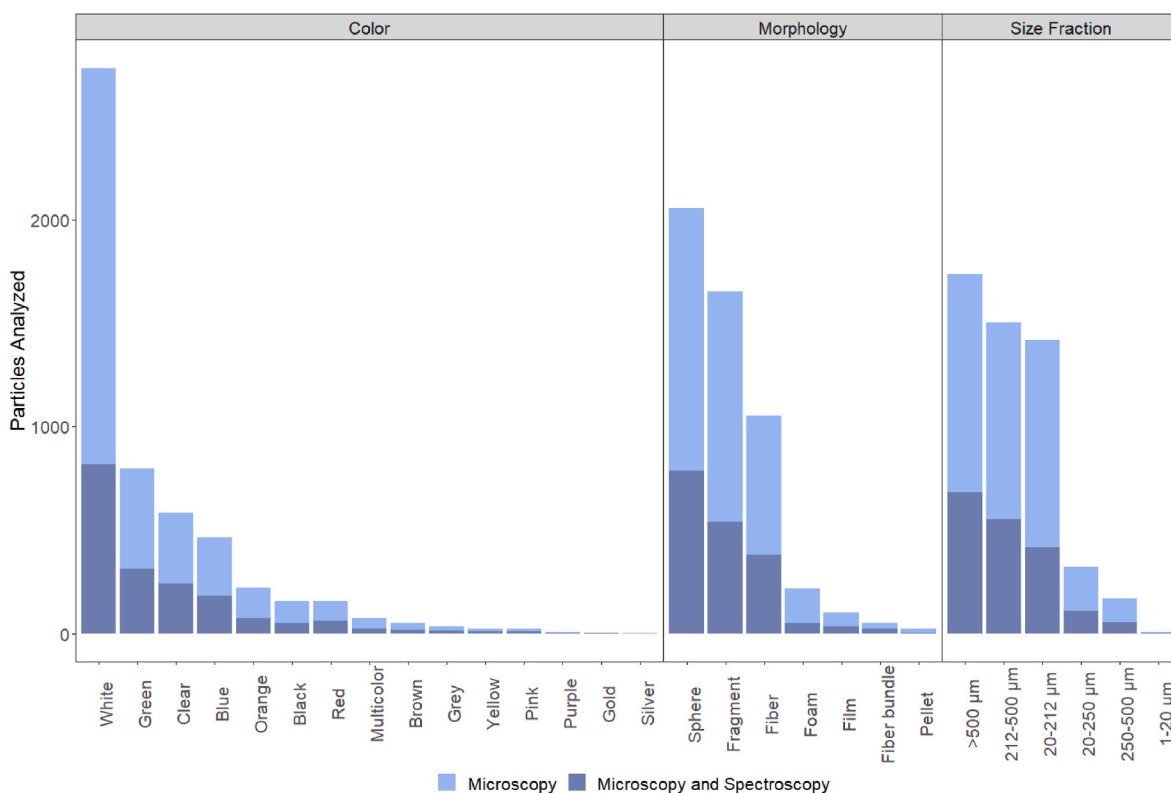
The top five most correlated variables with accuracy for each method were then compared among laboratories. Here, the proportion of accurate results for each laboratory was used to compare performance among labs using different method parameters. For example, where particle morphology was a highly correlated variable with accurate chemical identification using FTIR, we then compared accuracy for each lab with each particle morphology category. From these results, any trends within the data were identified and used to inform method recommendations.

## 3. Results and discussion

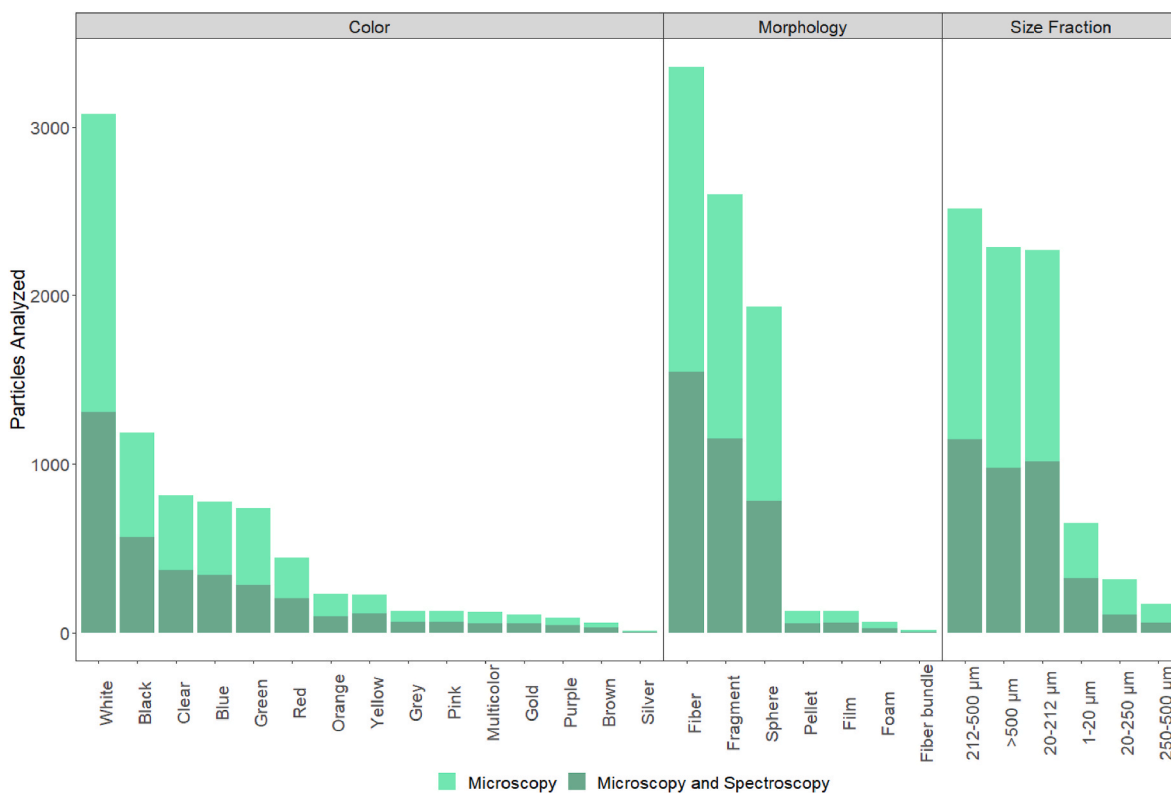
### 3.1. Selection of particles for chemical analysis

Among laboratories using FTIR microspectroscopy, certain particle colors were selected for analysis less often than others (Fig. 1, Appendix A; Fig. S2, Appendix B). Of 819 white particles counted among laboratories using microscopy, 43% of particles were identified. Further, 46% of 108 black particles and 49% of 53 multicolor particles that were counted were analyzed. Dark colors can reflect infrared radiation weakly (Andrade et al., 2020), which can explain potential bias against analysis of such colors. Among morphology categories, laboratories appeared to prioritize analysis of fiber bundles, with 88% of 26 fiber bundles counted. Laboratories showed a bias against analysis of particles categorized as pellets, where 20% of 20 particles counted were analyzed, and particles categorized as foam where 30% of 168 particles counted were analyzed. Particles with surface irregularities such as pellets and foam can be challenging to analyze using FTIR (Käppler et al., 2015), so this may be the cause of these particle selection biases. However, several spiked plastic particles were miscategorized by morphology (e.g., fragments miscategorized as foam, and spheres miscategorized as pellets, discussed further in (Kotar et al., 2022)). Foams and pellets were therefore less common in general within the dataset, and thus any biases against selection of these categories appeared more obvious. Particles in larger size fractions were also selected for FTIR analysis more often than smaller particles. For example, 1054 particles in the  $>500\ \mu\text{m}$  size fraction were counted and 65% were analyzed, however of 1003 particles were counted in the  $20\text{--}212\ \mu\text{m}$  size fraction, 41% were analyzed. FTIR microspectroscopy can characterize particles down to  $10\text{--}20\ \mu\text{m}$  in size, but when using certain analysis modes such as ATR, analysis is limited to particles over approximately  $100\ \mu\text{m}$  in size. (Primpe et al., 2020a). As 75% of laboratories used ATR mode for spectral collection in at least some of their analyses, it is expected that the limit of detection led to more larger particles being analyzed.

When using Raman microspectroscopy, the proportion of particles selected for analysis ranged from 62 to 100% among color categories, with green particles chosen least often for analysis (62%) (Fig. 2, Appendix A; Fig. S3, Appendix B). Although particle analysis using Raman microspectroscopy can have issues with fluorescence from bright colors or shiny particles (e.g., gold or silver in color), 100% of particles counted that were categorized as silver, gold and purple in color were analyzed using Raman. Among particle morphology categories, the proportion analyzed ranged from 63 to 85%. Particles categorized as foam and spheres were selected the least (63 and 67% analyzed, respectively). Similar to FTIR microspectroscopy, as no foam particles were spiked it is likely that particles categorized as foam were miscategorized by participating laboratories, and therefore these particles were less common overall. Among discussions with laboratories after the study, it was noted that the curved surface of the spheres caused difficulties in obtaining a spectrum from these particles, and thus it is possible that laboratories avoided selecting these particle types for analysis compared to other morphologies. Laboratories using Raman spectroscopy showed no bias against analysis of small particles, as 99% of particles counted in the  $1\text{--}20\ \mu\text{m}$  size fraction were analyzed. This is in line with previous



**Fig. 1.** Number of all recovered particles that were counted using microscopy, and the number that were chemically analyzed using FTIR microspectroscopy. Number of particles analyzed is summed among all participating laboratories for each category.



**Fig. 2.** Number of all recovered particles that were counted using microscopy, and the number that were chemically analyzed using Raman microspectroscopy. Number of particles analyzed is summed among all participating laboratories for each category.



works demonstrating that Raman microspectroscopy accurately analyzes smaller particles down to 1–10  $\mu\text{m}$  in size, due to its good spatial resolution (Araujo et al., 2018; Cabernard et al., 2018; Oßmann et al., 2018; Schymanski et al., 2018; Sobhani et al., 2020).

Along with particle characteristics such as color, surface texture and size, it was noted by some participants that pattern recognition may have also contributed to lower rates of analysis for certain particle types. Although this is not general practise in the analysis of ‘real world’ samples, it was reported by some laboratories that considering the large numbers of particles required for analysis in this study, to increase efficiency of sample analysis certain patterns were noted and particles with the same characteristics were grouped together within results. For example, after analyzing 10 green spheres within a certain size range, one could be reasonably confident that other green spheres of the same size within test samples were likely to be of the same polymer type. In these cases, the remaining green spheres may not have been analyzed by a small number of participants.

### 3.2. Influential variables for accurate chemical identification using microspectroscopy

A strong positive correlation amongst certain variables was expected, as has previously been presented in the scientific literature. However, our analysis found no variables to have a correlation greater than 0.63 (Cramers V) with accuracy of chemical identification using FTIR or Raman microspectroscopy (Figs. 3 and 4). For FTIR spectroscopy, the top five correlated variables with accuracy among laboratories were: Polymer type (e.g., Polypropylene or Polyethylene Terephthalate) ( $r = 0.52 + 0.08/-0.07$ ), Material type (e.g., plastic or false positive) ( $r = 0.29 + 0.09/-0.09$ ), particle morphology ( $r = 0.27 + 0.06/-0.07$ ), spectral collection mode ( $r = 0.21 + 0.05/-0.04$ ) and particle color ( $r = 0.21 + 0.07/-0.05$ ) (Fig. 3). For Raman spectroscopy the top five correlated variables with accuracy were material type ( $r = 0.63 + 0.04/-0.05$ ), polymer type ( $r = 0.51 + 0.03/-0.02$ ), particle color ( $r = 0.47 + 0.03/-0.03$ ), particle morphology ( $r = 0.42 + 0.04/-0.03$ ), and spectral range ( $r = 0.30 + 0.02/-0.02$ ) (Fig. 4). From these results, several notable findings can be derived to provide method recommendations and findings from the study related to using microspectroscopy for the analysis of microplastic particles (Appendix C). Trends among laboratories for these top five correlated variables were investigated for each method, and notable findings are discussed below.

## 4. Method recommendations for FTIR microspectroscopy

### 4.1. Particle storage and presentation

FTIR microspectroscopy is generally reported to have a size limitation for analysis of around 10  $\mu\text{m}$  (Primpke et al., 2020a), therefore particle size was expected to be correlated with accuracy of chemical identification when using this method. Particle size was identified as one of the variables most correlated with accuracy (Fig. 3), likely due to decreased accuracy for particles  $<20 \mu\text{m}$  (De Frond et al., 2022b), however particle morphology and color were more strongly correlated with accuracy within this study. When compared across color and morphology categories, the lowest accuracy was achieved for red and orange fiber particles, with 88 and 66% accuracy respectively (Fig. S4, Fig. S5, Table S5). From the metadata obtained in the study, laboratories reported issues in analyzing small particles and fibers using FTIR not due to their color, but due to particle extraction and storage methods. Several laboratories stored all particles on a layer of double-sided tape between particle counting using microscopy, and analysis using FTIR microspectroscopy. Survey responses reported that fibers and particles smaller than 212  $\mu\text{m}$  became coated with adhesive when stored on double-sided tape, making it more difficult to obtain a clean spectrum and risking damage to the diamond or germanium (softer and easier to damage) ATR tip. Laboratories reported successful analysis when particles were presented to the instrument directly on a glass or gold slide or where particles were measured directly on the filter surface. The use of one substrate for storage and analysis is the least complex method and has the added benefits of minimized equipment costs, reduced time for sample processing, and minimal opportunities for particle loss. Alternatively, particles that are more likely to be lost (such as fibers) can be either adhered to a glass slide using 2% dextrose solution (Ross et al., 2021; Vassilenko et al., 2021) or stored between glass slides that are taped together. As fibers tend to bend in all three dimensions, they can be placed onto a filter or mirror and covered by an IR transparent window such as BaF<sub>2</sub> for measurement by transmission (Primpke et al., 2019) or reflection. For the application of chemical imaging approaches, particles can be assessed using polypropylene supported Anodisc filters (Löder and Gerdt, 2015) or silicone membranes (Käppler et al., 2015). These types of filters have been already used in various types of analysis and especially supported Anodisc filter provide a broad range of applications ranging from sample storage for reanalysis towards analyses with other techniques like pyrolysis-GC/MS (Primpke et al., 2020c). Such an approach can be applied by all types of instruments and is already used in an increasing number of studies generating data which is well suitable for meta-analyses (Kooi et al., 2021).

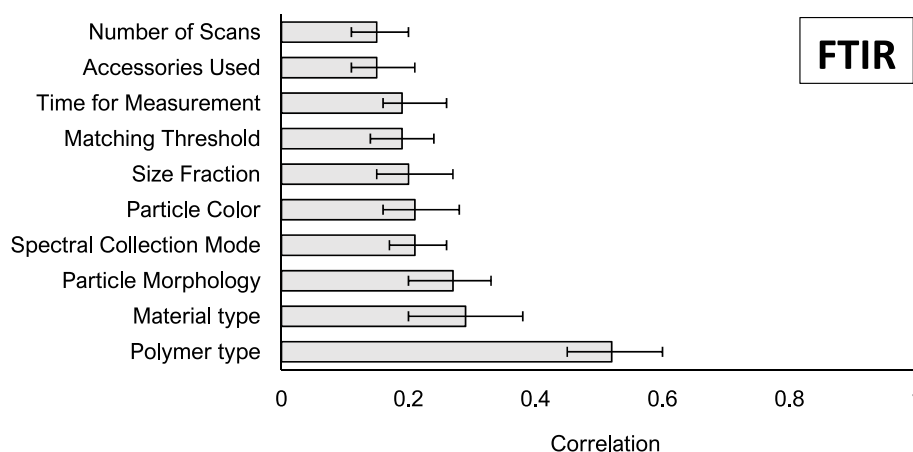
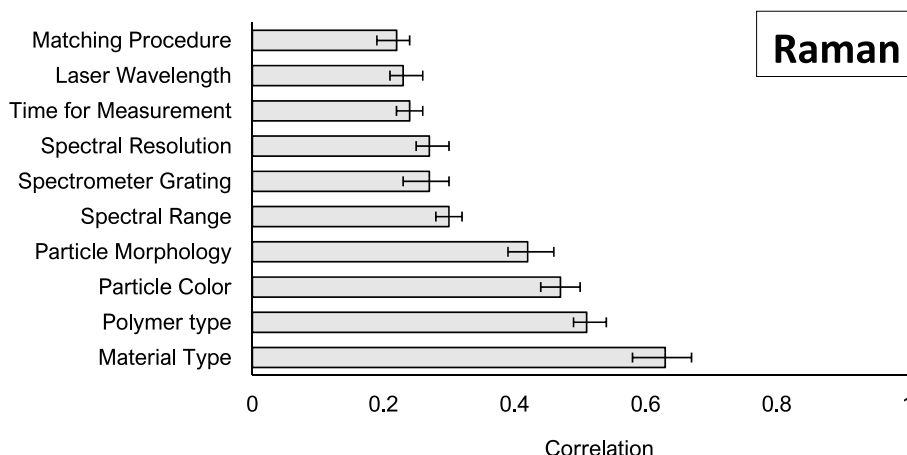


Fig. 3. Correlation between instrumental and particle characteristic variables with accuracy of chemical identification using FTIR microspectroscopy. The ten most correlated variables are shown here, and correlation values can be found within Table S1.



**Fig. 4.** Correlation between instrumental and particle characteristic variables with accuracy of chemical identification using Raman microspectroscopy. The ten most correlated variables are shown here, and correlation values can be found within Table S1.

#### 4.2. Spectral collection

Spectral collection mode was also identified as a variable most correlated with accuracy when using FTIR spectroscopy for the analysis of microplastics (Fig. 3). Thus, combination of particle preparation approaches and spectral collection mode tailored to the particle size and shape in question are important factors to consider for optimal results. Attenuated total reflection (ATR) allows greater precision in differentiating among similar materials compared to other modes of spectral collection (Comnea-Stancu et al., 2017), and should be the preferred method of spectral analysis where particle size or shape is not limiting. Laboratories that used a combination of spectral collection modes, (ATR in combination with reflectance or transmission modes) had comparably accurate results (Fig. S6) and reported the least amount of issues with particle analysis (Detailed within survey responses, (Kotar et al., 2022)). To avoid sticking or breaking of smaller particles during analysis, it is recommended to use ATR and/or reflectance for particles >200  $\mu\text{m}$ , and reflectance or transmission for particles <200  $\mu\text{m}$ . If using Focal Plane Array (FPA) based systems, particles in the range 500 to 200  $\mu\text{m}$  are recommended to be measured in transmission (Löder et al., 2017). Alternatively, if losing the particle on the tip is a possibility because it is small, one can conduct reflectance/transmission first as a back-up, and then proceed to ATR. Thus, if the particle cannot be retrieved following ATR, the spectroscopic data have not been completely lost.

FTIR microspectroscopy has been described as an accessible easy to use technique for the analysis of microplastics (Tagg et al., 2015; Zhang et al., 2020), yet it was still expected that more experienced users would obtain greater accuracy in results compared to novice laboratories. In this work, experience level was not identified as a variable strongly correlated with accuracy using FTIR, which indicates that even novice researchers can obtain accurate results using this instrument. Other instrumental parameters such as spectral resolution and number of scans were also not strongly correlated with accuracy using FTIR. Both settings can contribute to the length of time spent on analysis per particle, however the evidence in this work indicates that high resolution or multiple scans are not necessarily required to obtain accurate results. This is a promising outcome for using lower resolution mapping techniques which are becoming more widespread in the field (Primpke et al., 2020c; Cowger et al., 2020a). In general, laboratories that obtained high accuracy using FTIR microspectroscopy did not spend longer than 10 min per particle (Fig. S7). This length of time should be a rough guide to compare spending too much time analyzing each particle, without benefits in identification accuracy.

#### 4.3. Spectral matching

Accuracy in using FTIR microscopy for chemical identification of spiked particles was high among labs (92%), however accuracy varied by polymer type (Fig. S8). It should also be noted that all plastic particles used in the study were un-weathered (virgin plastic). Analysis of weathered plastics introduces a higher likelihood of identification error due to physical and chemical changes to the particles (De Frond et al., 2021; Fernández-González et al., 2021). Errors in identification were mostly observed for false positive particles (i.e., natural particles added intentionally at 65% accuracy), rather than plastic particles (95% accuracy). In particular, accuracy for identifying animal fur particles was 14%. Most animal fur fibers that were incorrectly identified were still identified as natural particles, meaning FTIR microspectroscopy can reliably differentiate between fibers of plastic or natural origin. However, laboratories reported difficulty in spectral matching due to the absence of fur and other keratin-based materials within reference libraries. For example, different types of fur or hair (e.g., wool, pet fur, human hair) all match the spectra for keratin, limiting the specificity of the result. To improve results for such particles, reference libraries used for spectral matching should be specific to the types of materials, polymeric, semi-synthetic, and natural that are often found within environmental samples including commonly detected (Primpke et al., 2018), and even weathered microplastics (De Frond et al., 2021; Munno et al., 2020). To encourage harmonization of methods among groups, where libraries of this kind are developed in-house that may be applicable to other laboratories and their samples, the libraries should be made open-access to minimize costs where possible. Examples of such libraries and their accessibility can be found via open-source programs such as Open Specy (Cowger et al., 2021) and siMple (Primpke et al., 2020b).

The use of a minimum spectral matching threshold is often a consideration in microplastic analysis methods (Cowger et al., 2020b; Weisser et al., 2022). In this study, match threshold was not strongly correlated with accuracy ( $r = 0.19$ , Fig. 3), and when results are compared among the thresholds used, accuracy was lower for laboratories using a minimum threshold of 70% Hit Quality Index, compared to laboratories that did not use a threshold at all (Fig. S9). Thus, we do not recommend a specific matching threshold to be used as standard because different research groups use different hit quality indices and different spectral matching libraries (commercial, open-access, or in-house) that affect spectral matching success. If in the future the use of spectral libraries is more harmonized among research groups, a standardized spectral matching threshold may be appropriate.

## 5. Method recommendations for Raman microspectroscopy

### 5.1. Spectral collection

In this study, particle color was identified as an influential variable for chemical identification using Raman microspectroscopy. Current literature has noted that spectral interferences (Ivleva et al., 2017; Silva et al., 2018) resulting from fluorescence, additives, and pigments within plastic particles impede the likelihood of achieving an accurate identification result (Ivleva et al., 2017; Prata et al., 2019; Sobhani et al., 2019). Therefore it was expected that particle characteristics such as color would influence accurate chemical identification using Raman (Anger et al., 2018; Munno et al., 2020; Xu et al., 2019). When comparing accuracy across color categories, accuracy for red particles decreased to 5% (Fig. S10, Table S6). Among morphology categories, accuracy of chemical identification for fibers decreased to 30% (Fig. S11, Table S6). One laboratory misidentified numerous red cotton fibers as PET in this study, which likely influenced results. The cause of this specific misidentification could not be determined from the meta-data. However, in post-study discussions other laboratories that achieved accurate results for dyed fibers reported adjusting settings based upon the particle in question according to its characteristics (color and morphology) to minimize spectral interference. For example, it is recommended to use a lower laser power (e.g., 5 or 10 mW) for brightly colored particles to minimize particle fluorescence, or where darkly colored particles were identified as susceptible to burning (Xu et al., 2019).

Of other instrument settings, spectral range was identified as an influential variable for accurate identification of particles using Raman (Fig. 4). A typical molecular species will have vibrational transitions between 0  $\text{cm}^{-1}$  and 4000  $\text{cm}^{-1}$ . Thus, it is desirable to capture the fingerprint (400  $\text{cm}^{-1}$  to 2000  $\text{cm}^{-1}$ ) and high frequency regions (>2700  $\text{cm}^{-1}$ ) within spectra for accurate identification. In this study, all laboratories acquired spectra that included the fingerprint and high frequency regions, accessing the full spectral range available and relevant for polymer identification. The minimum spectral range employed that achieved the highest accuracy in this study was 800–3300  $\text{cm}^{-1}$  (Table S2) and this should be considered an acceptable spectral range for accurate polymer identification. Although an important consideration, high spectral resolution was not critical to accurate polymer identification with accurate results achieved with a minimum resolution of 1  $\text{cm}^{-1}$  (Table S2), indicating an opportunity possibly to save time during spectral acquisition where lower resolutions are used.

### 5.2. Spectral processing

Although often used prior to spectral matching, spectral processing variables were not vital for achieving accurate results using Raman microspectroscopy in this study. For example, a mixture of baseline correction methods was used (polynomial, automated, manual) however there was no significant difference in accuracy among laboratories that did or did not use baseline correction (Fig. S12). Therefore, correction should be carried out to minimize spectral noise and interference prior to spectral matching where appropriate, but it is not recommended as an imperative step for all spectra. In addition to baseline correction, other methods to minimize spectral noise are also useful to aid accurate spectral matching e.g., spectral normalization and relative intensity correction. The results of this work show that these steps may not always be necessary, and it is recommended to minimize time spent on spectral processing, and rather focus on ensuring the appropriate settings are used for spectral acquisition. Accurate results can be achieved when spending less than 10 min per particle (De Frond et al., 2022b).

### 5.3. Spectral matching

Accuracy using Raman microspectroscopy was high among labs (83%) and varied across material types (Fig. S13). Although unweathered particles were used in this study, it is unclear how accuracy would have differed for weathered particles, as results of how degradation can alter Raman spectra are mixed (Dong et al., 2020; Phan et al., 2022). The greatest error in identification observed for false positive (natural) particles, specifically natural fibers animal fur, and dyed cellulose (cotton). Laboratories noted within post-study discussions that reference libraries containing spectra from dyed polymers and dyed anthropogenic particles (e.g. Munno et al., 2020) were useful where dye overlay was unavoidable in spectral acquisition, and inclusion of natural materials was useful for confident differentiation among polymers and natural particles. As with FTIR microspectroscopy, the inclusion of reference spectra that represent the types of particles that are often found in environmental samples (i.e., polymers, natural materials, and weathered particles) will benefit accurate identification.

## 6. Recommendations for further research

From the results of this work and the result of the interlaboratory method validation study (De Frond et al., 2022b), it can be concluded that both FTIR and Raman microspectroscopy are accurate techniques for the identification of microplastics in drinking water. Owing to the differences amongst the techniques, both methods can be considered complementary in agreement with how they are often described within published literature (Cabernard et al., 2018; K  ppler et al., 2016). FTIR has the potential to be a relatively fast technique, with less adjustment of instrument settings per particle required to obtain a high-quality spectrum. It is also a flexible technique, with different modes available for different particle types. Raman microspectroscopy requires less consideration for particle preparation of larger particles compared to FTIR and can more accurately identify small particles <20  $\mu\text{m}$  in size (Table S5, Table S6). Some challenges and data gaps remain for both techniques which have been highlighted within this study. These will require further research and method development.

The development of spectral libraries with a variety of materials commonly found in microplastics research is beneficial for accurate and confident spectral matching. To further minimize operational costs, open-access spectral libraries and open-source tools such as Open Specy (Cowger et al., 2021) provide the community with spectra, and will become more useful as more open-access libraries are included. In addition to the content of the reference libraries, options for researchers to distinguish between polymer groups or used harmonized nomenclature for identifying polymer groups (e.g. HDPE and LDPE being classified together as Polyolefins) dependant on their research objectives will help researchers quickly interpret the outcome of spectral matches in a meaningful way and should be advanced for policy related groupings starting with work that has begun on the topic (e.g., Cowger et al., 2022; Wiesinger et al., 2021).

In this study, particles were first visually sorted and identified using microscopy, an appropriate pre-screening method for particles >50  $\mu\text{m}$  to minimize chances of false positive results (De Frond et al., 2022b; Kotar et al., 2022). Below this size, extracted particles are often identified using microspectroscopy only, increasing the likelihood of both false positive and false negative chemical identification results. Raman microspectroscopy had high accuracy in identifying particles of all sizes including particles <20  $\mu\text{m}$  such as those found in treated drinking water O  mann et al. (2018); Pivokonsky et al., 2018; Schymanski et al. (2018); (Wyer et al., 2020). However, data was limited for the analysis of particles below 20  $\mu\text{m}$  in size for both FTIR and Raman techniques (Table S5, Table S6). Reliable methods that allow efficient but accurate identification of a variety of small particles (different materials, colors, morphologies) are imperative to gain a thorough understanding of how well we can detect, count, and identify such particles from a variety of



matrices. Thus, further data on the applicability of each method for analysis and accurate identification of particles <20 µm is needed and should be a priority for further research. This includes the applicability of methods to ensure the occurrence of both false positives and false negatives is kept to a minimum (e.g., Nile Red staining (Prata et al., 2021), automated selection for particles on a filter (von der Esch et al., 2020)). Analysis of these small particles that are often most numerous in the environment (Koelmans et al., 2022) can be time-consuming even with automated techniques, and several laboratories reported a limited capacity to complete this work during the COVID-19 pandemic. Small microplastics are not only more numerous in the environment (Collard et al., 2018; K  ppler et al., 2016), but they are the particles most likely to enter biological blood systems and organs (Ma et al., 2022; Yuan et al., 2022) and thus have been highlighted as a priority characteristic to consider in toxicological testing both for aquatic and human health effects (Thornton Hampton et al., 2022).

In this study, un-weathered (virgin plastic) particles were added to test samples. Weathering of microplastics can lead to both physical and chemical changes to the particles. Physical changes to particles may impede successful identification e.g., using ATR-FTIR on fragile or brittle particles that may be subject to fragmentation during analysis. Chemical changes to particles can alter the IR spectra of microplastics (De Frond et al., 2021; Fern  ndez-Gonz  lez et al., 2021; Simon et al., 2021), however this effect is less clear for spectra obtained using Raman microspectroscopy (Dong et al., 2020; Phan et al., 2022). In drinking water, particles are exposed to minimal weathering. However, when considering the use of these methods for monitoring microplastics in other environmental compartments (e.g., surface water, sediment and seafood) consideration of how results may change due to different particle extraction procedures is vital. Additionally, investigation into how accuracy and thus appropriate methods may differ for the analysis of weathered particles is recommended, particularly for Raman microspectroscopy, where studies on this are currently limited.

In many cases, including monitoring, efficiency of sample processing is vital, and subsampling of particles for chemical identification can minimize time expenditure. For results among studies to be comparable, subsampling methods do not necessarily need to be identical among laboratories, but they must be representative. To date, subsampling approaches for the automated analysis of small particles have been investigated (Brandt et al., 2021; Thaysen et al., 2020), although due to the heterogeneity of particle spread on the filter surface, further research is still required to recommend best practices. Further, subsampling approaches for larger particles that can be manipulated using forceps have recently been recommended to reduce time for analysis where particle counts are high (De Frond et al., 2022a, Cowger et al., 2022 (in prep.)). The harmonization of such methods would not only improve efficiency of sample processing, but also the comparability of data and results among research groups. A further way to decrease time spent on analysis and subjectivity in the selection of particles for analysis is the development of rapid screening techniques, either through automated image analysis and mapping (e.g., Appendix C, methods used in this study) or through the development of new technologies (e.g. Su et al., 2022). Moreover, further research is required to determine the most appropriate sample preparation methods for size fractionation. Questions regarding what size ranges of particles should be analyzed together, appropriate filter loadings, how to subsample from the sample matrix, or use size fractionation to minimize particle overlay on the filter for efficient and accurate automated analyses using microspectroscopy should be addressed.

Recommendations for appropriate filter types for analysis are also required for both techniques. The choice of filter substrate differs depending on sample type, method of analysis and costs, but further testing will support the choice made by individual research groups. Studies can inform this for silicone, aluminum, gold, and black filters (K  ppler et al., 2015; O  mann et al., 2017), and the first direct comparison on the effectiveness of each for the analysis of a variety of small

microplastics has recently been published using FTIR microspectroscopy (Sukumaran et al., 2022; in prep.), with recommendations detailed within.

## 7. Conclusion

In this work the influence of both particle and instrumental variables were correlated with accuracy of chemical identification using microspectroscopy with the aim to identify method recommendations and priorities for future research. FTIR microspectroscopy is a versatile technique, and thus methods should be adjusted depending on the particle type (size and morphology) to provide the greatest likelihood of obtaining spectra of high quality. Raman spectroscopy is reliable for the analysis of a variety of particle types, although care should be taken to adjust instrument parameters to minimize chance of fluorescence and particle burning for dyed particles, and spectral processing methods may be utilized to improve spectral quality prior to matching with reference libraries. Further research should focus on testing and developing harmonized methods for the efficient but accurate analysis of particles below 20 µm, that are most challenging to analyze using these techniques, but are the most prominent and concerning particle types found in both drinking water and environmental samples.

## Author contributions

Hannah De Frond, Win Cowger: Data Curation, Formal Analysis, Visualization, Investigation, Writing – Original Draft, Writing – Review & Editing.

Sebastian Primpke, Susanne Brander, Violet Renick, Suja Sukumaran, Amy Lusher, Bridget O'Donnell, Dounia Elkhatib, Steve Barnett, Florian Vollnhals, Wenjian Lao, Maria Navas-Moreno, Keith Rickabaugh, Eunah Lee, Gaurav Amarpuri, George Sarau, Silke Christiansen: Investigation, Writing – Original Draft, Writing – Review & Editing.

## 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.

## Data availability

All data used in this work is publicly available via: <https://microplastics.sccwrp.org/>. This link is also included within the manuscript.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2022.137300>.

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