High Throughput FTIR Analysis of Macro and Microplastics with Plate Readers

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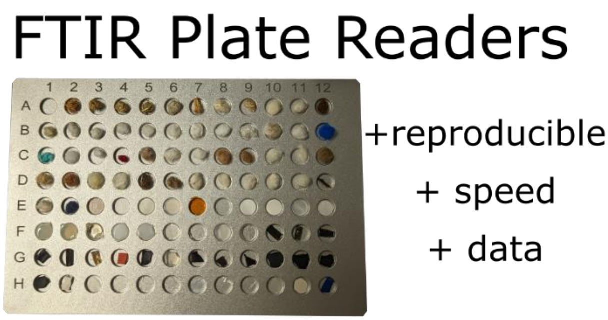
Graphical Abstract



Abstract

FTIR spectral identification is today's gold standard analytical procedure for plastic pollution material characterization. High-throughput FTIR techniques have been advanced for small microplastics (10 um - 500 um) but less so for large microplastics (500 um - 5 mm) and macroplastics (> 5 mm). These larger plastics are typically analyzed using ATR, which is highly manual and can sometimes destroy particles of interest. Furthermore, spectral libraries are often inadequate due to the limited variety of reference materials and spectral collection modes. We advance a new high-throughput technique to remedy these problems. FTIR plate readers are high throughput devices for measuring large particles (> 500 um). We created a new reference database of over 6000 spectra for transmission, ATR, and reflection spectral collection modes with over 600 plastic, organic, and mineral reference materials relevant to plastic pollution research. We also streamline analysis in plate readers by creating a new particle holder for transmission measurements using off-the-shelf parts and fabricating a non-plastic 96-well plate for storing particles. We validated the new database using Open Specy and demonstrated that transmission and reflection spectra reference data are needed in spectral libraries.

Keywords

Plastic pollution, microplastics, ftir, database, high throughput, spectroscopy

Introduction

Spectroscopy is currently a gold standard procedure for material characterization of microplastic (1 um – 5000 um) particles [1–3]. Fourier transform infrared (FTIR) spectroscopy is a non-destructive technique that provides rich information about chemical bonds in materials and can accurately differentiate plastics from non-plastics[4,5]. High-throughput spectroscopy techniques like mapping FTIR are now gaining widespread use[6,7]. These techniques have improved sample throughput by orders of magnitude and made plastic particles in the nanometer range possible to characterize[8]. However, the optimal particle size range for the most widely used high throughput FTIR techniques is 10-500 um and there does not currently exist a proposed technique for high-throughput FTIR analysis of large microplastic particles (500 um - 5 mm), sometimes referred to as mesoplastics[9], or macroplastic particles (> 5 mm). These larger particles often comprise most of the plastic mass in many samples[10,11]. They can also be highly abundant[12], leading to a significant amount of time in manual spectral characterization. Standard attenuated total reflection (ATR) measurement techniques for these larger particles require an average of 10 minutes per particle to collect a quality particle spectrum, which must be redone if anything goes wrong during spectral collection. This is because the spectroscopist must physically focus the ATR on every particle individually and stay with the device while it collects the spectra. FTIR plate readers have been used extensively in other fields to characterize samples in high-throughput (< 1 min per particle), including biology[13–16] and soil research[17,18]. Plate readers have been piloted for plastic pollution research[19], but have not been tested at scale. Our first study goal was to develop a technique for using FTIR plate readers for large microplastic and macroplastic characterization.

The lack of reference spectral libraries for reflection and transmission spectral collection modes is one of the largest barriers to utilizing FTIR plate readers in plastic pollution research. Reflection and transmission spectra can be quite different from the ATR spectra commonly included in commercial and open-source databases[20]. Spectral database development has been a huge challenge even for leading industry spectral database suppliers[21], due to the diversity of the microplastic materials[22] and spectra[23]. Our second goal was to use the high throughput technique to develop a harmonized database for ATR, reflection, and transmission spectra of relevant materials for studying plastic pollution (i.e., plastic, natural organics, and minerals).

Experimental Section

Sample Preparation

Particles were collected from the in-house reference standards available at Alfred Wegener Institute in Dr. Pimpke’s Lab, the National Renewable Energy Laboratory, the Moore Institute for Plastic Pollution Research, Hawaii Pacific University’s Center for Marine Debris Polymer Kit 1.0, and microplastic samples from environmental samples from Roscher et al.[24,25]. The standard materials contained 554 plastic materials, 56 natural organic, 3 minerals, 7 other materials, and 31 unknown, totaling 637 materials. Small particles (< 5 mm) were placed in the well without additional preparation (Figure 1). Large particles (> 5 mm) were prepared by reducing them to a size that would fit in the 5 mm plate reader wells. Fibrous particles were hand-rolled into small balls (2-5 mm). Ridgid large plastic was clipped using a standard hole punch (3-5 mm) for paper. Film particles were cut with scissors by hand. Pellets were chopped with scissors if they were too large to fit in the well. No granule or liquid particles were assessed with this technique because the transmission plate could not prevent cross-contamination since the wells did not have complete walls. A needle was used for extracting and inserting particles that fit snugly in the wells. Plates were cleaned with 99.9% ethanol (Merck, Germany) pre-filtered using 0.2 µm GTTP membranes (Merck, Germany) before measurements or between transfers of one batch of particles to the next. Position A1 was always kept free and used for background measurements.

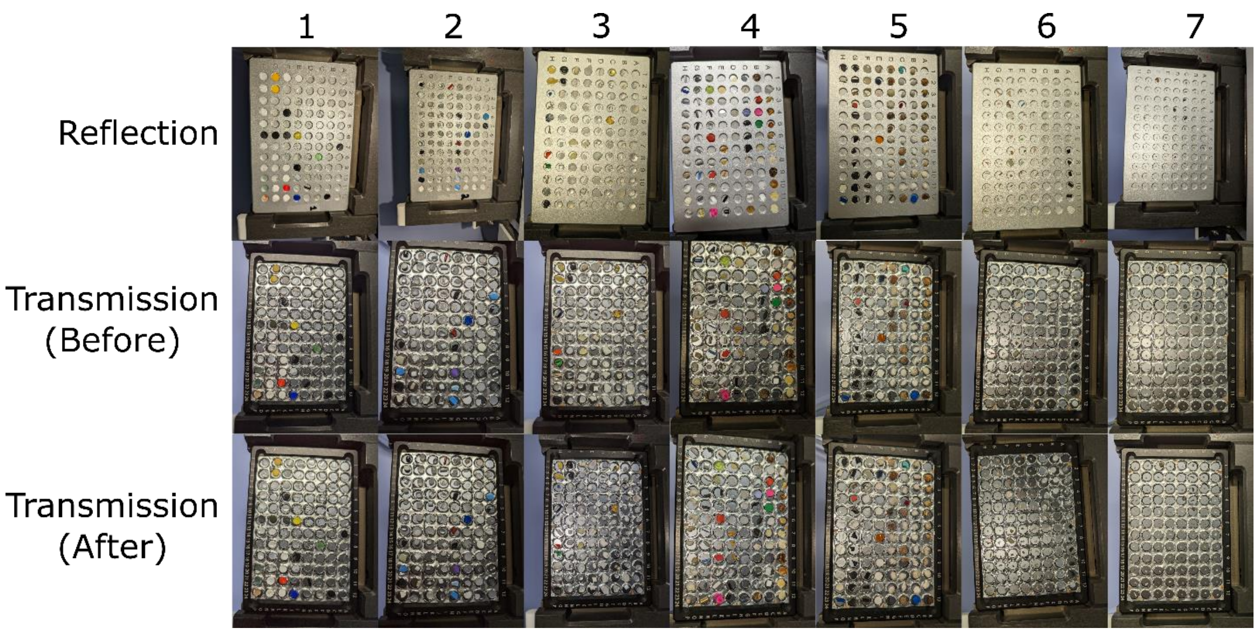


Figure 1: Images of particles in plates for transmission and reflection measurements. Each well holds a different particle. Spectral collection mode is labeled on the left axis, and the plate number is on the top axis. Transmission before is the transmission plate before going into the plate reader and transmission after is the same plate after it came out of the plate reader. The transmission images can be compared to assess whether particles moved during the measurement. Transmission plates have a custom-made well overlay from heavy aluminum foil. No particles are observed to be missing or crossing into another well.

Transmission Cover Creation

The standard transmission plate for the Bruker HTS-XT had a flat surface that could not prevent particle cross-contamination. The vibration of the plate would cause particles to roll into other wells and thus lose their reference in the data. There are other transmission plates with edges on the wells[26], but we were unaware of one that existed for the Bruker HTS-XT. We fabricated an overlay using heavy aluminum foil to prevent particle movement, which we hand-cut using a rubber mallet and a circular hole punch (Figure 2). We created a template for the hammering by putting a transparent piece of plastic on top of the transmission plate, tracing out where the wells were, and then taping the template to a piece of heavy aluminum foil for cutting (Figure 2: Step 1). Hammering was done on top of a hard plastic plate to prevent curling of the aluminum when hit and to prevent cutting through the floor (Figure 2: Step 2). The aluminum foil cutout was flattened by hand to fit tight against the silicon plate (Figure 2: Step 3). The heavy aluminum foil was then fixed as close to the silicon transmission plate surface as possible using a minimal amount of tape (Figure 2: Step 4). The tape was positioned to avoid overlapping with the wells by placing it between the wells. We photographed all plates before and after measurement to ensure that particles were not drifting between the wells (Figure 2: Step 5 & Figure 1). There were a few cases where the particle got extremely close to the edge of the well or became sandwiched between the cover and the silicon. Still, we found no evidence of particles leaving the wells in the measurements or spilling over into another well.

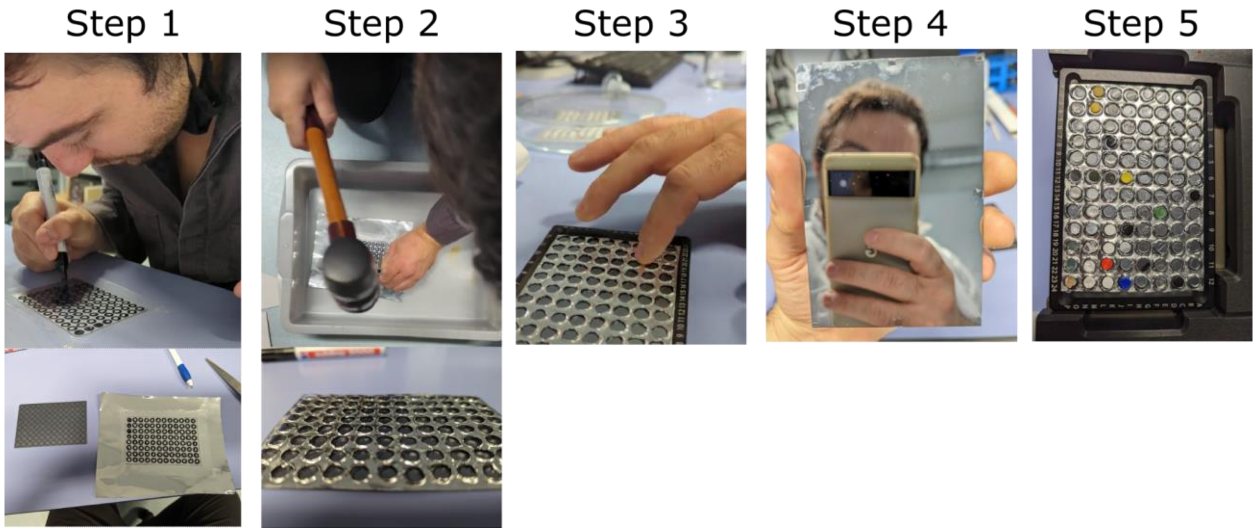


Figure 2: Visual instructions for creating the aluminum overlay for the transmission plates. Step 1: Trace wells and outline of transmission plate on thick plastic and transfer the plastic overlay to a piece of heavy aluminum foil with tape. Step 2: Pound a gaged stamp of the well size with a rubber mallet on top of a hard plastic platform and cut the aluminum to size with scissors. Step 3: Tamp the aluminum flat by hand on top of the silicon plate. Step 4: Tape the aluminum cover to the silicon plate with small slivers of tape. Step 5: Load the plate into the HTS-XT with particles to analyze.

Spectral Acquisition Parameters

We follow recommendations by Andrade et al. 2020 [27] for minimum information for the publication of infrared spectra in microplastic research. Spectra were collected with a Bruker Tensor 27 with the HTS-XT plate reader attachment. The device was flushed with air scrubbed of water and carbon dioxide to prevent atmospheric artifacts. The device used the OPUS software to collect the data. We used the device's three spectral collection modes: ATR, transmission, and reflection. The database contained 1-8 spectra per particle from ATR, transmission, and reflection. All particles were assessed with transmission and reflection, but some in plates 3, 4, and 5 were not assessed with ATR due to how time intensive the ATR data collection was. 637 materials were measured in total, with some materials replicated in wells up to 5 times.

ATR spectra were collected for each particle on two sides of the particles with the ATR attachment of the Tensor 27 with a room temperature detector RT-DLaTGS and a mirror speed of 10 KHz, 32 scans, a 4 wavenumber spectral resolution, from 4000 to 400 wavenumbers, and 6 mm aperture. The background measurement was done before every particle measurement on an open and clean ATR surface and automatically subtracted from the spectra. Fourier transformation was conducted with Mertz phase correction and an apodization function of Blackman-Harris 3 term and 2 zero filling factor. We observed every spectrum collected, and if a particle had drastically different spectra on each side, we noted that. The ATR crystal and tip were cleaned with ethanol between particles.

Transmission spectra were collected with the HTS-XT plate reader using the HTS XT transmission room temperature detector using a 5 mm aperture, a mirror speed of 10 KHz, 32 scans, and a 4 wavenumber spectral resolution from 4000 to 400 wavenumbers. The background was done before every measurement on an empty transmission well (position A1). Fourier transformation was conducted with Mertz phase correction and an apodization function of Blackman-Harris 3 term and 4 zero filling factor. We tested the impact of changing spectral wavenumber resolution to 8 and collecting only one spectrum per material. We found a high Pearson correlation between the data sets (0.92), suggesting that changing the parameters slightly to others commonly used does not drastically change the quality of the database produced and that replicates of wells are not strictly mandatory.

Reflection spectra were collected with the HTS-XT plate reader with an LN MCT detector cooled with liquid nitrogen with a 6 mm aperture and a 20 KHz mirror speed, 32 scans, and a 4 wavenumber spectral resolution from 4000 to 620 wavenumbers. Before every measurement, a background measurement was done on the empty reflection plate well (position A1). Fourier transformation was conducted with Mertz phase correction and an apodization function of Blackman-Harris 3 term and 4 zero filling factor.

Long-term storage

Reflection and transmission plates were expensive, so we fabricated non-plastic 96-well plates to hold the particles long-term. Metal 96-well plates were fabricated in-house in the scientific workshop of the Alfred Wegener Institute in corresponding positions to where they would be in the reflection or transmission plates for the plate readers (Figure 3). The plates were stored face up in glass Petri dishes (Ø 18 cm), which prevent the loss of the particles from blowing wind. Storage in this way allowed all particles to be rapidly transferred to a reflection or transmission plate and reanalyzed if needed. The total time for transferring 95 particles from one plate to another was less than 15 min. Alternatively, additional reflection or glass plates could be purchased and used for long-term storage.

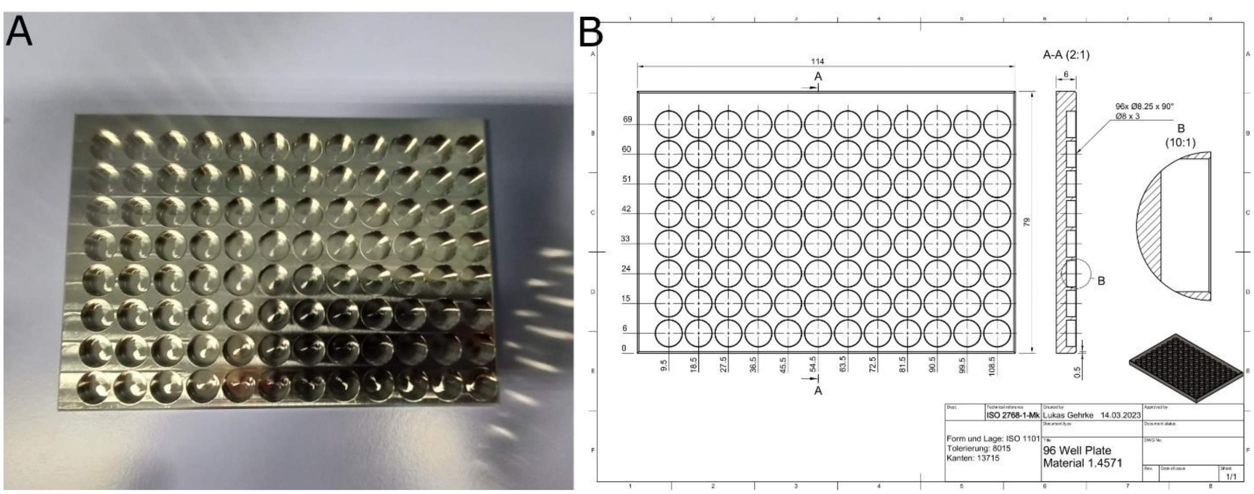


Figure 3: A long-term storage setup for particles from the plate reader using a metal 96-well plate. The numbers can be labeled along the left and top axis in the blank space. (A) An image of the 96-well plate made from stainless steel. (B) Blueprints for the creation of the 96-well plates.

Validation Statistics

The technique was validated for its spectral quality by comparing the spectra collected with the Open Specy library[20], a collection of several open-access spectral databases for FTIR[4,23]. Out-of-the-box accuracy was tested using the Open Specy package[28] and several other data cleaning and visualization packages[29–35] in R[36] with the default settings for smoothing (Savitzky–Golay filter with 11 points and a 3rd-order polynomial)[37], baseline correction (imodpolyfit 8th order polynomial)[38], and correlation (Pearson). Unknown materials were not used in assessing the validity of the library. The identification was said to be accurate if the top match returned by Open Specy was identical to the known identity of the material. The correlation values were used to infer the rationale behind lower hit qualities for some spectral collection modes (Supplemental Information). A hit quality threshold was not used to calculate out-of-the-box accuracy.

Results and Discussion

Validation of technique

Out-of-the box accuracy for Open Specy in identifying the spectra we collected was best for ATR Spectra (62%) followed by Transmission (25%) and Reflection (21%) (Figure 4). This was unsurprising to us since Open Specy's library primarily consisted primarily of ATR spectra (as do most commercial products[21]) and ATR spectra are quite different from Transmission and Reflection (Figure 5). Correlation values for reflection and transmission spectra were, for the most part, below the recommended threshold of 0.7 (Figure S1), and the largest particles assessed appeared to have worse correlation values for reflection and transmission (likely due to near total absorbance)(Figure S2). As a note, a careful user would likely achieve higher accuracy using Open Specy than out-of-the-box accuracy by counting correct “unknown” ids as accurate ids and manipulating the parameters in Open Specy to improve baseline subtraction and smoothing. We recommend declaring hits below 0.7 as “unknown materials” and making particles as thin as possible when conducting plate reader measurements.

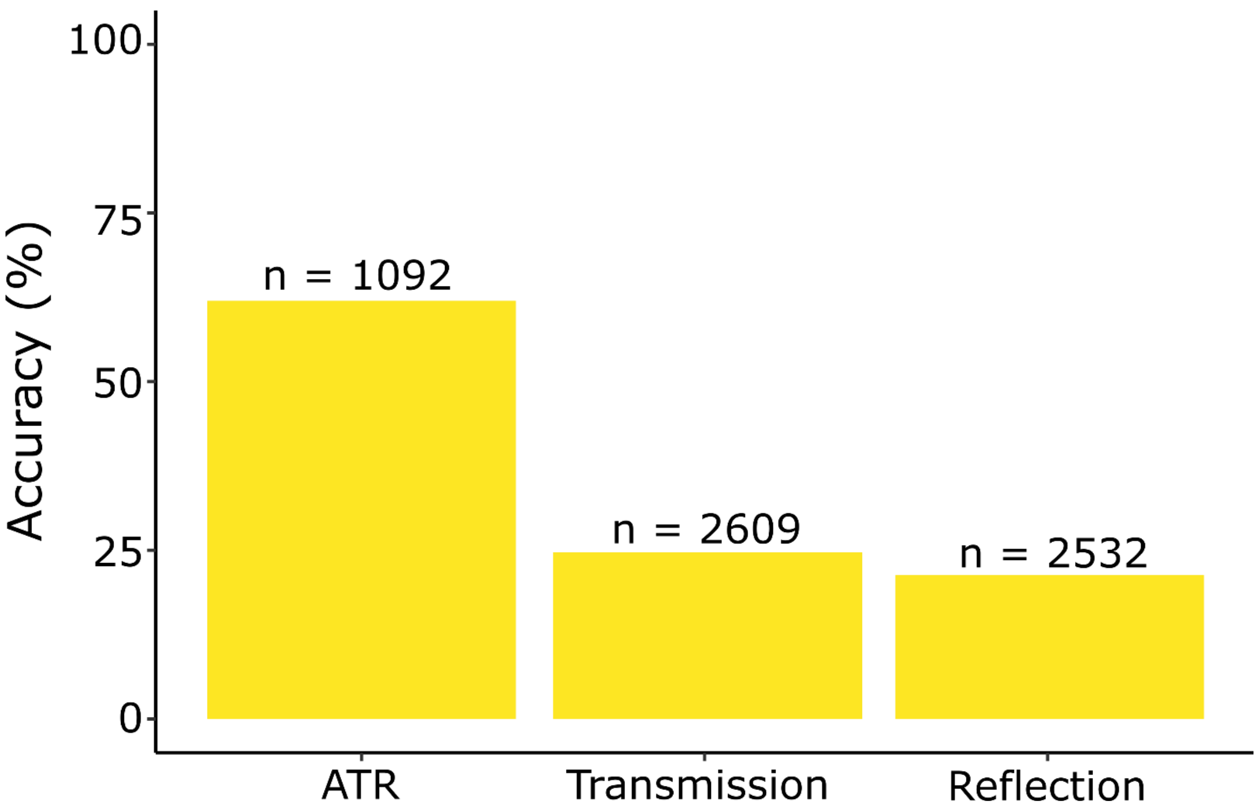


Figure 4: Validation of the database produced using Open Specy’s out-of-the-box settings to identify the material type. X axis is the spectral collection mode employed in collecting the database. Y axis is the accuracy in percent of correct identifications of Open Specy in identifying

spectra from the spectral collection mode group. The total number of spectra tested for each spectral collection mode is listed above the bars. The height of the bars is the accuracy. Spectra counts are not identical across the techniques because not all particles were measured in all modes, and some particles were measured more times than others.

Comparing techniques

Comparing the spectra acquired between ATR, reflection, and transmission, we see that all three techniques can provide similar quality spectra under ideal scenarios like film plastic spectra (Figure 5B). In some cases, transmission and reflection spectra have additional peaks that ATR does not (Figure 5A, 5C, 5D, 5E). This can be partly explained due to the technique's penetration depth. ATR collects spectra of a thin surface of the material while transmission and reflection techniques have deeper penetration which can change the relative intensities of peaks[39] and collect signals through polymer composite materials[40]. Other differences between the signals include derivative like distortions[41] of reflection spectra (Figure 5C) and relative positive shift in absorbance intensity towards lower wavelengths for ATR of thicker samples[39] (Figure 5A and 5C). Sometimes one technique produced less variable spectra than the other two for a given particle (Figure 5A). The shape and form of transmission and reflection spectra appear more similar to each other than to ATR spectra, suggesting that the two could be used complementarily in reference libraries (Figure 5D).

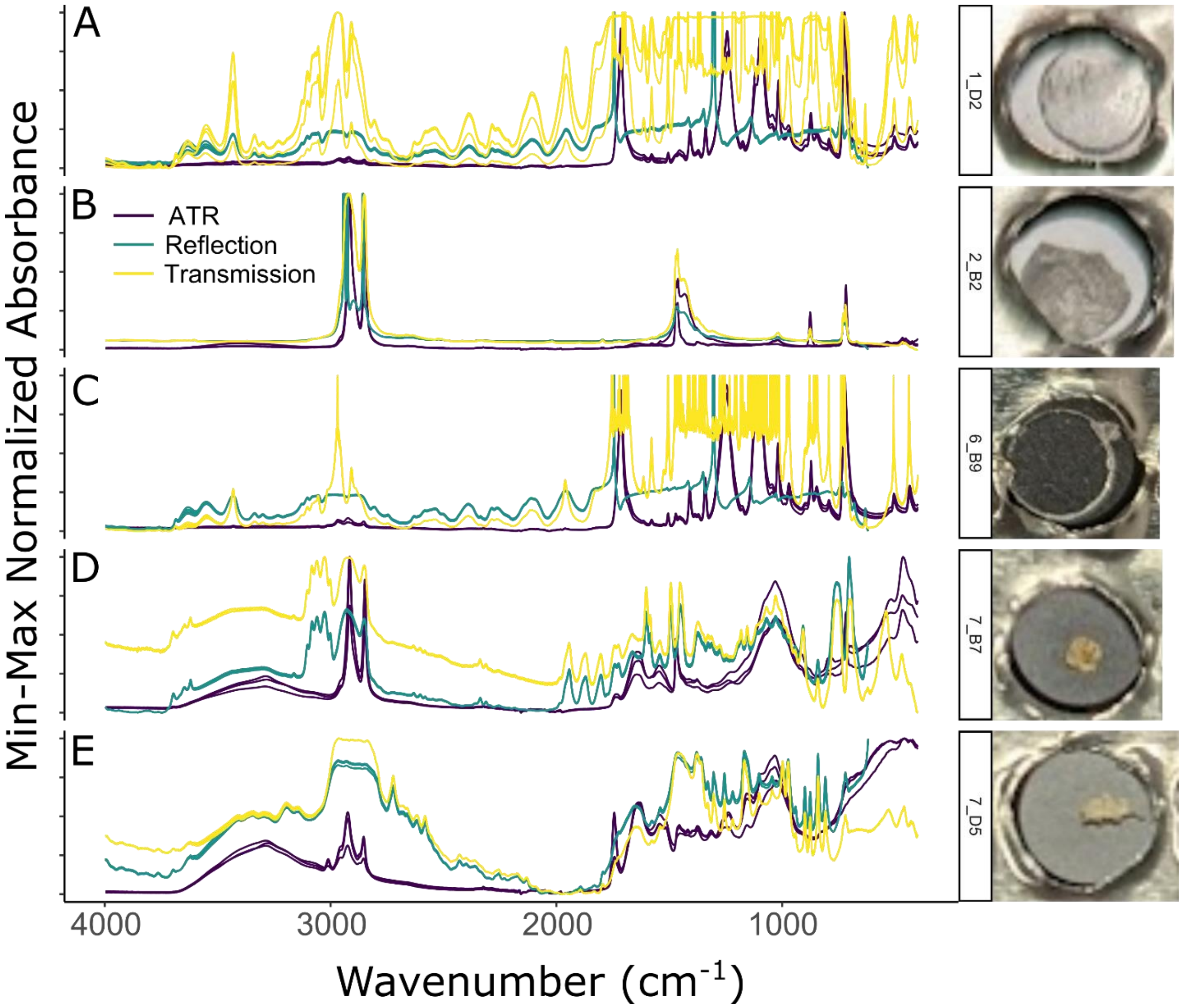


Figure 5: Comparison of spectra from the same particle for transmission (yellow), reflection (green), and ATR (purple). The y-axis is min-max normalized intensity values for each spectrum. The x-axis is wavenumbers in units cm^{-1}. When multiple spectra were collected in a mode they are overlaid. On the right axis, the plate number is followed by the well id and an image of the particle extracted from Figure 1 is shown. These particles were randomly selected from particles that had all three spectral collection modes.

The primary advantage of the plate reader method is increased speed for analyzing large microplastic and macroplastic particles compared to ATR. Based on our work with these techniques, we estimate the plate reader technique takes 1 minute per particle, on average, to prepare the sample; this could then rapidly be reassessed with any number of spectral collection parameters. This method is much faster than ATR, typically 10 minutes per particle, and must be manually redone if a mistake is made. Although all these techniques are generally considered non-destructive, there were cases where particles were altered using ATR from the force of the press, or particles had to be cut to use in the plate reader. In a few cases, particles were geometrically complex and rigid, preventing us from collecting a high-quality ATR spectrum, but transmission and reflection were not impacted. When it is critical not to alter the particle and to collect a good-quality spectrum, great care must be taken to assess which technique is most appropriate.

Conclusions

We presented a new technique for analyzing large microplastic and macroplastic FTIR signatures in reflection and transmission modes and compared it to traditional ATR measurement. FTIR plate readers can provide higher throughput analysis of large microplastics and macroplastic samples than ATR. The spectra acquired in transmission and reflection modes from plate readers are of sufficient quality for spectral analysis but are substantially different from ATR spectra commonly available in spectral reference libraries. We provide one of the largest and most extended open-access spectral libraries to date to accelerate the adoption of this technique. We created an off-the-shelf plate cover for transmission plate readers to keep particles in position, which could be improved in future studies if a walled well plate design were developed or a rigid metal cover was fabricated to fit the silicon plates. Last, we demonstrated that out-of-the-box identification is not appropriate for accurate spectral characterization at this time and propose that better automated routines for spectral analysis continue to be advanced.

Data Availability

Data and source code come with a CC BY NC license allowing copying and reuse for non-commercial purposes. Commercial licenses may be sought by contacting the corresponding authors. Raw data, source code, and spectral database developed in this manuscript are available DOI: 10.5281/zenodo.7772572.

Acknowledgments

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Statements and Declarations

The authors declare no conflicts of interest.

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