Fluorescence polarimetry for microplastics identification

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ABSTRACT

Microplastics (MPs) are emerging pollutants that pose potential threats to global ecosystems due to their widespread distribution and resistance to degradation. Conventional identification methods, such as spectroscopy and chromatography, often require complex and time-consuming procedures. In this study, we present a novel optical approach based on fluorescence anisotropy for the identification and characterization of MPs. By analyzing the polarization properties of fluorescence emission under polarized light excitation, the system is capable of distinguishing common MP types and providing information of particle size. The results indicate that exogenous fluorescence signals exhibit polarization information which can reflect the physical and chemical properties of MPs. This method provides a cost-effective, minimally invasive, and time-efficient perspective for MP detection, with potential applications in environmental monitoring.

Keywords: Microplastics, fluorescence anisotropy, environmental monitoring, non-invasive measurement, optical metrology

1. INTRODUCTION

Plastics have become indispensable in modern society. Despite their low price and versatility, their persistent existence in the environment can thus generate microplastics (MPs, <5 mm) and nanoplastics (NPs, <1 µm), which have raised urgent ecological concerns.[1] MPs and NPs have been broadly detected in the atmosphere, soils, oceans, rivers, and even in food and drinking water.[2] Their small particle size could potentially lead to toxicological effects, including inflammation, tissue accumulation, and oxidative stress.[3,4] However, in spite of their wide distribution, it remains challenging to reliably detect and characterize MPs and NPs in complex environmental matrices.[5]

Previous studies have demonstrated the effectiveness of a variety of analytical approaches to detect MPs and NPs, such as microscopy, vibrational spectroscopy (FTIR, Raman), and thermal analysis (Py-GC-MS).[6,7] These techniques can provide information on size distribution and chemical composition, but also are labor-intensive and time-consuming. Particularly, for sub-micron plastics, interference from organic matter and sample contamination could hinder robust analysis. Therefore, there is an increasing need for developing more rapid and minimally invasive techniques that can complement or even surpass existing approaches.

Fluorescence polarization (FP), which is also called fluorescence anisotropy, is a ratiometric technique that measures the degree of polarization (DoP) of fluorescently labeled or intrinsically emissive particles under the excitation of polarized light. FP techniques have been leveraged in the fields of drug discovery, clinical diagnostics, virus detection, and biomolecular interaction, exhibiting potential advantages such as fast speed, high sensitivity, and homogeneity.[8,9,10] FP signals are sensitive to viscosity, particle size, and binding states, which are directly related to physical and chemical properties.[11] These characteristics indicate that fluorescence polarimetry has the potential to serve as a promising, yet underexplored tool for MP identification.

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Currently, FP techniques have rarely been used for detection and characterization of plastic particles. Existing optical methods mainly focus on spectral fingerprints, while neglecting polarization states that possess particle size and molecular interaction with fluorochrome.[2,12] Motivated by this research gap, we present a fluorescence polarimetry approach to identify MP particles in this study. We conducted preliminary verification on the effectiveness of using FP techniques to distinguish MPs of different types and particle sizes. This method shows strengths of being rapid, minimally invasive, and cost-effective, offering a complementary pathway for environmental monitoring of MPs.

2. METHODS

The schematic diagram in Figure 1 presents the experimental setup and a sample image of fluorescence emission excited by laser. In the experiment, we used a laser source to emit linearly polarized light of 532 nm. An optical isolator was placed in the beam path to protect the laser source from back-reflected light. By allowing light to pass only in the forward direction and blocking laser light propagating backward, the isolator prevents optical feedback in the laser cavity which can potentially cause power instabilities, frequency drift, or even physical damage. A plane mirror was employed to reflect the excitation light onto the sample slide.

For the MP samples, we selected Rhodamine 6G (R6G) as the fluorescence reagent for MP staining. The included types of plastics are: polystyrene (PS), polylactic acid (PLA), and polymethyl methacrylate (PMMA). For all these types of plastics, we prepared microsphere suspension samples stained by R6G, with a particle diameter of 800 nm at a concentration of 25 mg/ml. For PS microsphere, we additionally prepared samples of 300, 500, 2000, and 10 000 nm, with the same concentration, respectively. In the sample slide, we diluted the suspension samples with distilled water in a 1:1 ratio.

As shown in the sample image of Figure 1, being excited by the green laser (532 nm), the MP samples emitted orange light. A band-pass (BP) filter was placed after the sample slide to only filter out orange light of 580 nm. A polarization camera (CREVIS MG-A500P-22) was utilized for capturing images of four polarization directions: 0° ,45°, 90°, and 135°. The exposure times for PS samples of 300 nm and 500 nm are set as 2000 ms and 1200 ms, respectively. For other particle sizes and types of plastics, the exposure time was uniformly set as 500 ms.

The acquired images of four polarization directions were used for DoP calculation. The Stokes parameters were computed as:

$$S_0 = I_0 + I_{90}, \quad S_1 = I_0 - I_{90}, \quad S_2 = I_{45} - I_{135}, \tag{1}$$

where I_0, I_{45}, I_{90}, and I_{135} denote the fluorescence intensities recorded at the corresponding polarization angles.

The DoP was then obtained as:

$$\mathrm{DoP} = \frac{\sqrt{S_1^2 + S_2^2}}{S_0}. \tag{2}$$

To ensure robustness, the intensity values were first averaged over the central 80% region of interest (ROI) and further divided into a 9×9 grid. The intensity values within each sub-ROI were averaged before calculating the Stokes parameters, and the DoP was computed separately for each sub-ROI. This approach characterizes the anisotropy of fluorescence emission, thereby reflecting the orientation distribution and rotational mobility of fluorophores within the suspension samples.

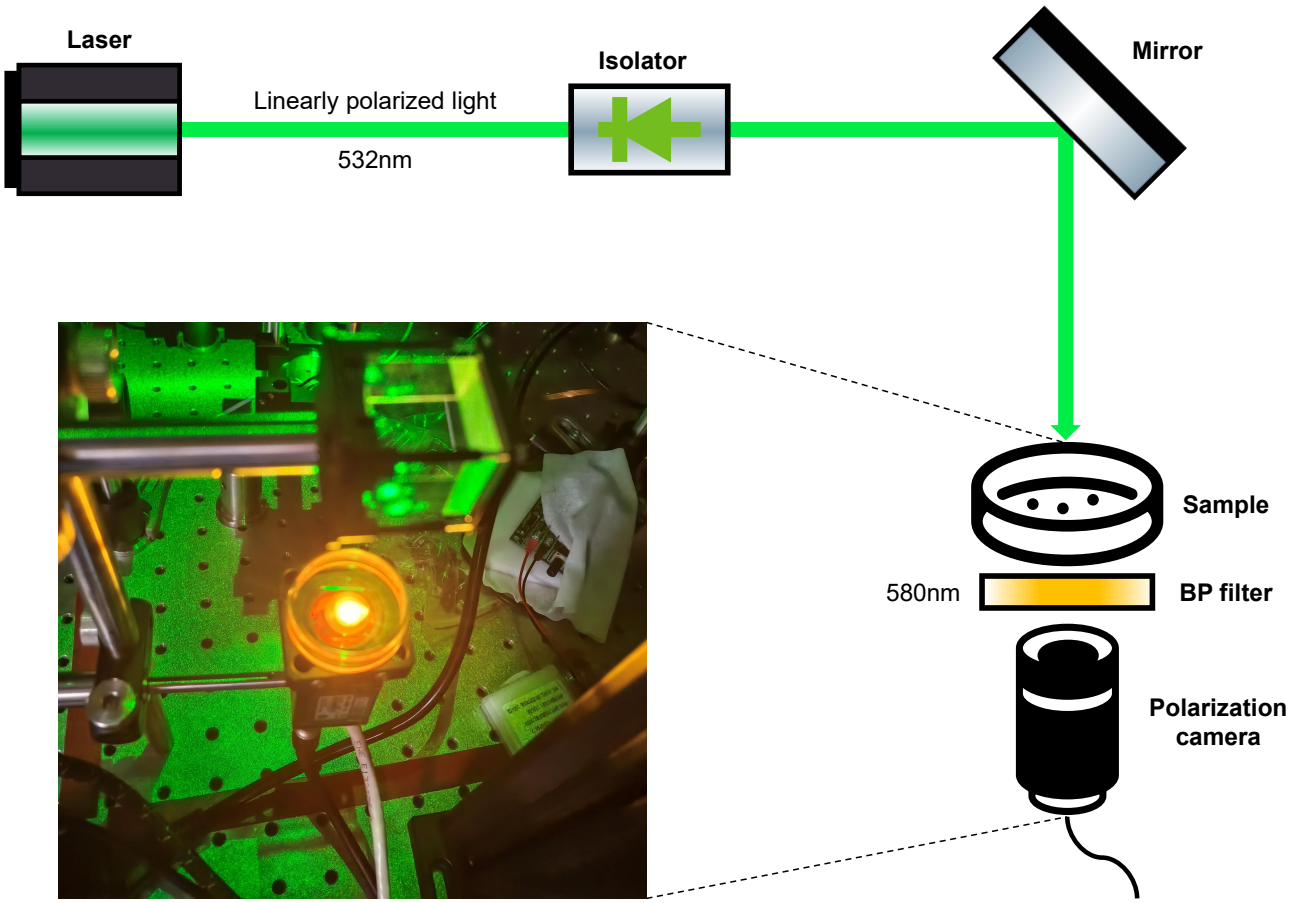

Figure 1. Schematic diagram and sample image of fluorescence polarization measurement.

3. RESULTS AND DISCUSSION

Figure 2 presents the raw images of different sizes (300 nm, 500 nm, 800 nm, 2000 nm, and 10 000 nm) of PS particles under four polarization directions. Similarly, Figure 3 presents the raw images of different types of plastics samples (PS, PLA, and PMMA) under the same particle size of 800 nm. The visualization resuts demonstrate that the intensity variations of different polarization directions are difficult to distinguish only by human eyes. It is much more difficult to differentiate them through visual identification. Thus, it is necessary to leverage post processing and statistical tools.

In Figure 4, we employed two boxplots to visualize the DoP distribution of different particle sizes of PS microspphere suspension samples and different types of plastics (PS, PLA, and PMMA) at the same particle size of 800 nm. The Wilcoxon signed-rank test results in Figure 4(a) demonstrates that, the proposed FP approach cannot effectively distinguish different particle sizes in nanoscale (300 nm, 500 nm, and 800 nm). However, when the PS particle size increased to 2000 nm, the DoP was statistically higher than that of nanoscale particles. As the particle size further increased into 10 000 nm, the DoP increased drastically, compared with other four groups of particle samples.

This behavior can be interpreted from the perspective of fluorescence polarization. For nanoscale particles, the fluorophores undergo rapid rotational diffusion during their excited-state lifetime, which tends to randomize the emission dipole orientation and leads to low polarization levels. As the particle size increases, the rotational mobility of fluorophores embedded within the matrix becomes progressively restricted, resulting in a slower depolarization rate and therefore a higher observed DoP. In microscale particles (e.g., 2000 nm and above), the restriction of fluorophore rotation is much stronger, leading to a pronounced increase in fluorescence anisotropy

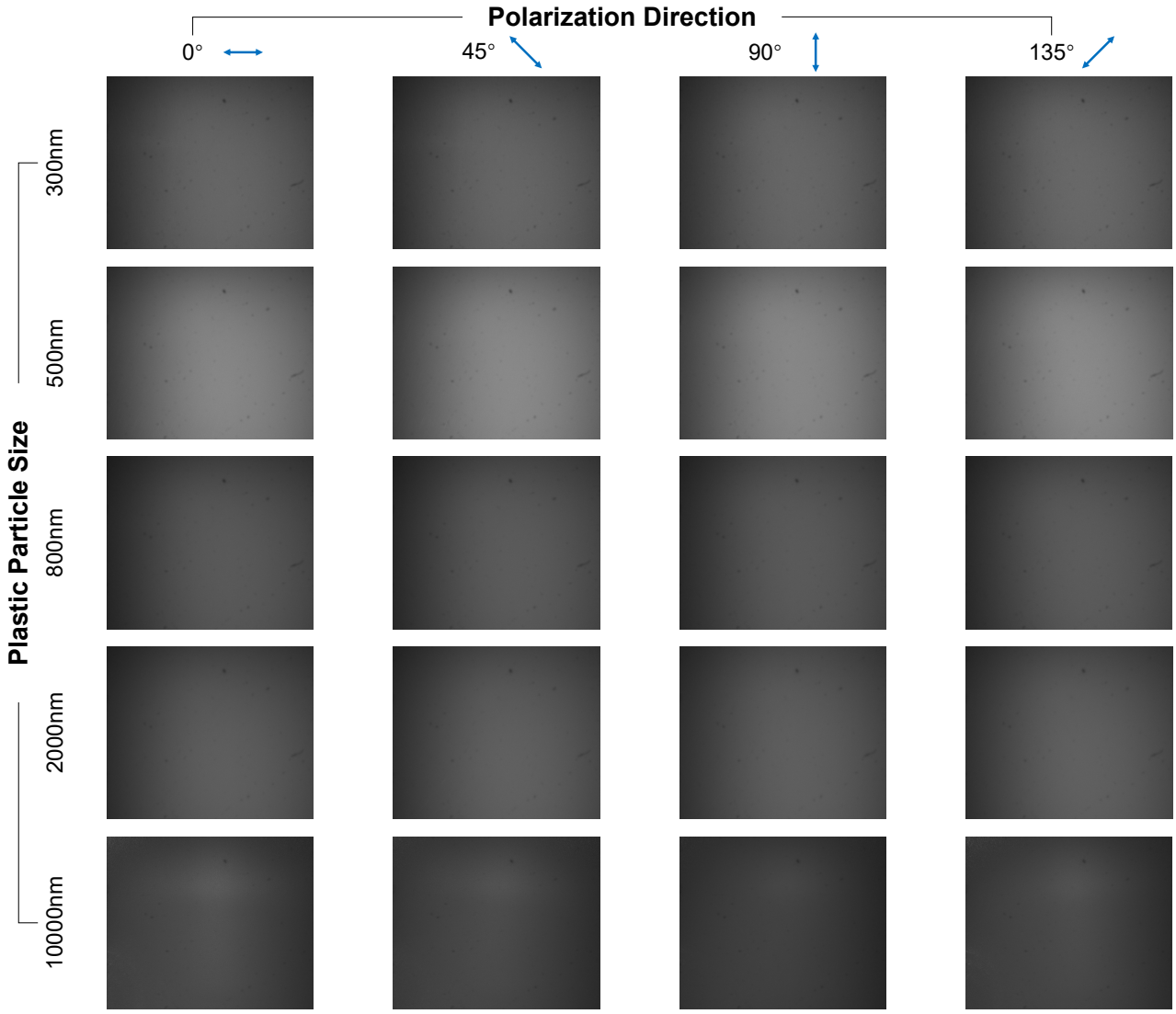

Figure 2. Raw images of polystyrene (PS) microsphere suspension samples of different particle sizes (300 nm, 500 nm, 800 nm, 2000 nm, and 10 000 nm) acquired under four polarization directions (0°, 45°, 90°, and 135°).

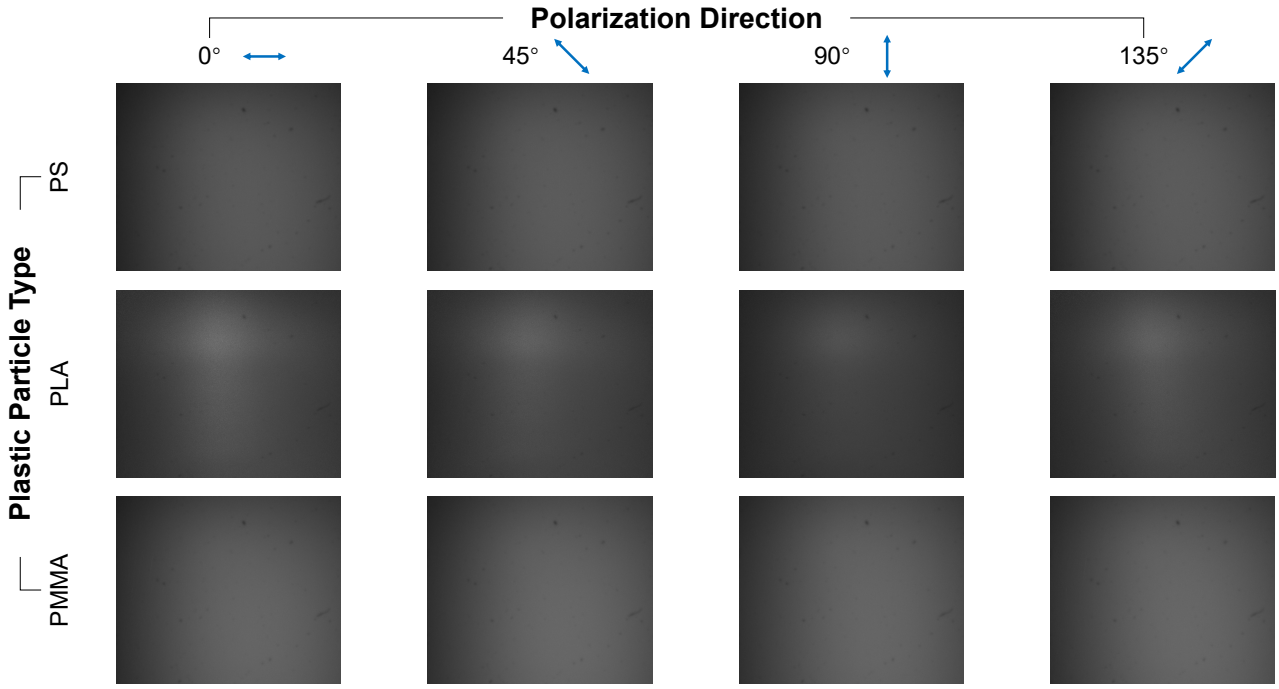

Figure 3. Raw images of microsphere suspension samples of different types of plastic acquired under four polarization directions (0°, 45°, 90°, and 135°). All particles had a uniform diameter of 800 nm. Plastic types were polystyrene (PS), polylactic acid (PLA), and polymethyl methacrylate (PMMA).

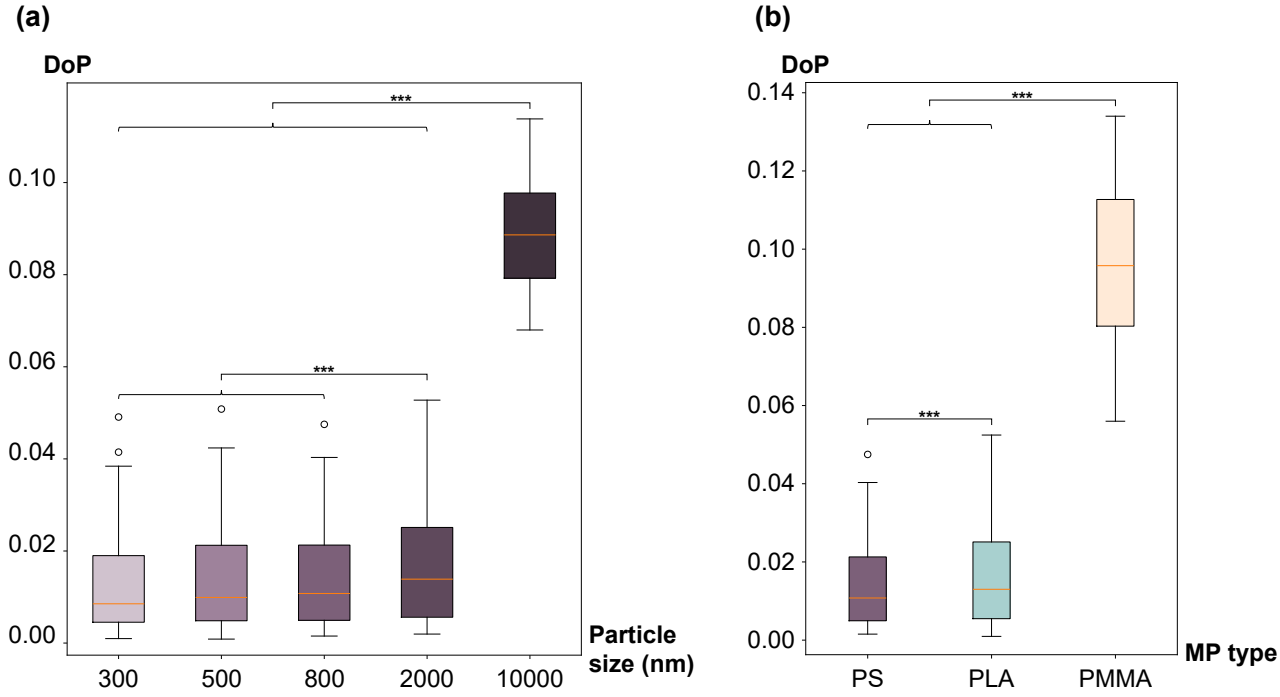

Figure 4. Boxplots of the degree of polarization (DoP): (a) Different particle sizes of polystyrene (PS) microsphere suspension samples; (b) Different types of plastics at a fixed particle size of 800 nm. *** indicates that $P \leq 0.001$ under the Wilcoxon signed-rank test.

and polarization preservation.

The results in Figure 3(b) demonstrate the capability of the proposed method to differentiate PS, PLA, and PMMA samples. At a fixed particle size of 800 nm, PLA and PMMA samples exhibited significantly higher DoP against PS. And the DoP of PMMA was also significantly higher than PLA.

These differences arise from the intrinsic photophysical properties of the polymer matrices. PLA and PMMA have more rigid molecular backbones, which impose stronger restrictions on the rotational freedom of the embedded fluorophores. In contrast, the aromatic rings in PS provide relatively flexible microenvironments that facilitate partial depolarization through π–π interactions and local chain motions. Consequently, the fluorescence polarization signal reflects both particle size–dependent rotational constraints and polymer-specific microenvironments, enabling discrimination between different plastic types.

4. CONCLUSION

In this work, we presented a fluorescence polarimetry approach for the identification and characterization of microplastics. By analyzing fluorescence emission under four polarization orientations, we demonstrated that the degree of polarization (DoP) can serve as a sensitive optical parameter to reflect both particle size and polymer type. Experimental results revealed that nanoscale PS particles (300 nm–800 nm) exhibited weak polarization due to rapid rotational diffusion of fluorophores, while microscale particles (2000 nm and 10 000 nm) showed significantly higher DoP owing to restricted molecular mobility. Furthermore, material-specific differences were observed at a fixed particle size of 800 nm, where PLA and PMMA samples exhibited higher DoP values than PS, reflecting their more rigid molecular backbones and stronger restriction on fluorophore rotation.

These findings suggest that fluorescence polarization provides an additional contrast mechanism that complements existing spectroscopic and imaging methods for microplastics analysis. The proposed technique is rapid, minimally invasive, and cost-effective, making it a promising tool for environmental monitoring and potential large-scale screening of microplastics in complex samples. Future work will focus on combining fluorescence polarization with spectral fingerprinting and advanced machine learning algorithms to enhance classification accuracy, as well as extending the methodology to real-world environmental samples beyond controlled laboratory conditions.

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