

## A novel, automated method to identify microplastic polymers based on fluorescent staining with Nile Red

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There has been a growing concern about the microplastic levels in the marine environment and their potential adverse effects for ecosystems and human health. Due to the increased demand for microplastic pollution monitoring at national and global levels, there is an urgent need for a standardized, reliable method that reduce the time, efforts and costs spent on plastic identification in the marine environment and allow for long-term monitoring. An optical microplastic detection method that has gained considerable attention lately because of its time- and cost- effectiveness makes use of the hydrophobic fluorescent dye Nile Red (NR). This method has proven useful for discriminating plastic particles from non-plastic materials in several matrices, but the possibility to use a fluorescent dye to obtain polymer-specific identification has not been explored intensely. In this ongoing study, an automated approach is being developed that makes use of the solvatochromic nature of Nile Red and allows for the identification of individual plastic polymers using fluorescence microscopy. The theoretical principle behind the new method is that the fluorescence emission spectrum of NR shifts depending on the polarity of its environment. The latter means that based on a large and qualitative training set of emission spectra of different polymers (collected under standardized conditions) automated algorithms can be developed for identification of polymers. Hence, the new method combines imaging of fluorescent particles with machine learning algorithms for the accurate and automated detection of different plastic polymers in various matrices at microscale. Based on the developed preliminary algorithms, this study show that polymers can be categorized based on their coloration following staining with Nile Red. We demonstrated that the identity of individual plastic particles can be predicted using this new technique. Based on the results so far this method could be a promising approach for the rapid, cost-effective and reliable identification of microplastics, and it could support the long-term monitoring of microplastics in various matrices.

Keywords: Microplastics; Polymer identification; Nile red; Fluorescence; Machine learning; Automation; Monitoring