Inter-study comparison of Nile Red-based staining protocols for the detection of microplastics in environmental samples

Srumika Konde[1], J. Ornik[1], J. A. Prume[1], J. Taiber[1] and M. Koch[1]

[1]Fachbereich Physik/Halbleiterphotonik, Philipps-Universität Marburg, Germany

Introduction

- Microplastics have recently become a major environmental issue due to their omnipresence in aquatic systems.
- Several studies proposed the solvatochromic dye Nile Red as a simple approach to detect plastics in an environmental matrix [1-4].
- In this work, we present a comparison of four already published staining protocols and a novel one.

Staining Procedure

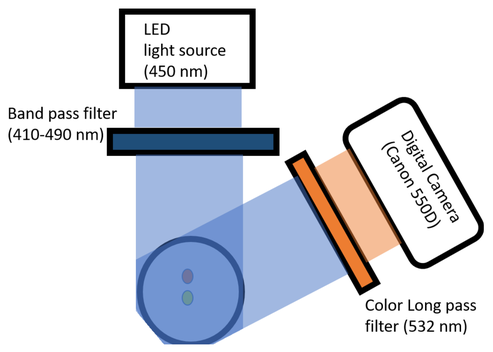
- Concentration of Nile red, polarity of solvent and staining procedure influence the fluorescence intensity and chemical shift of stained plastic.
- Foils, pellets and fragments from polar (PET, PVC) and non-polar (PP, PE) plastics were stained with below mentioned protocols to compare the fluorescence behavior of stained plastics

Protocols	Authors	Solution concentration NR/Solvent (µg/mL)	Solvent	Staining procedure
1	Cole (2016)	500	Acetone	Shortly vortexed and incubated for 10 min
2	Our method	20	Ethanol and acetone	Heated at 60 °C for 10 min
3	Maes et al. (2016)	10	Acetone	Incubated in a shaker for 30 min at 100 rpm
4	Shim et al. (2016)	5	Acetone	Incubated for 10 min and washed with n-hexane
5	Erni-Cassola et al. (2017)	1	Methanol	Heated at 60 °C for 10 min

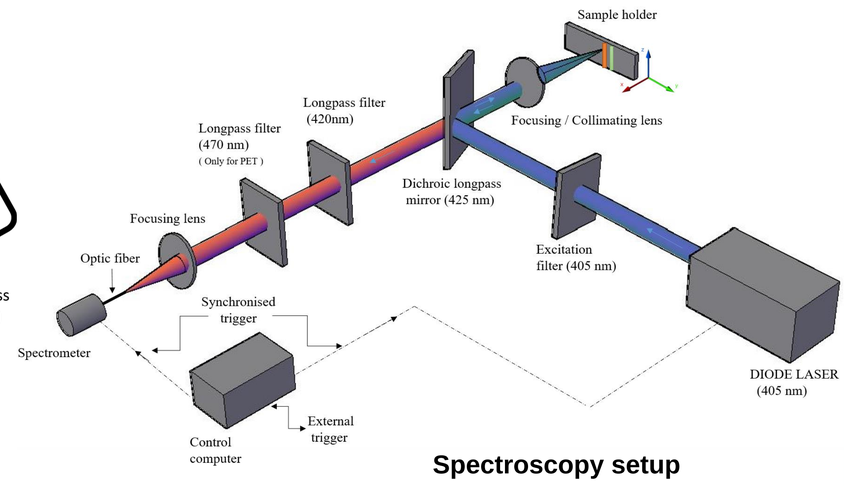
Fluorescence Analysis

- Fluorescence intensity and chemical shift (color) of the stained plastics were determined visually (photos) and spectroscopically (spectra).

Parameters	Visual inspection (Photograph)	Spectroscopic measurement
Excitation	450 nm-LED diode	405 nm-LASER diode
Emission	532 nm Color long pass filter	No emission filter



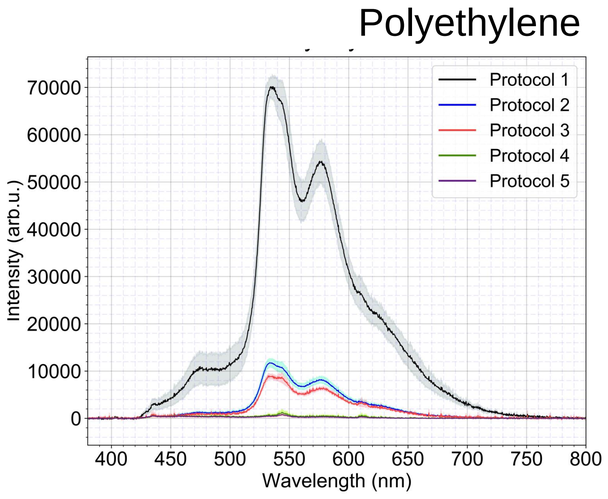
Visual inspection setup

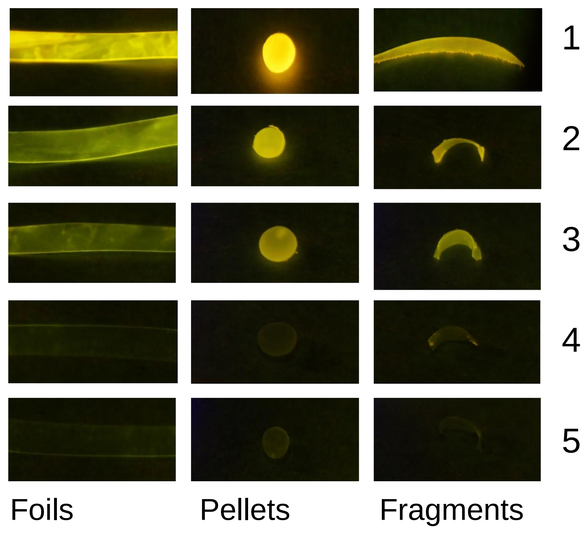


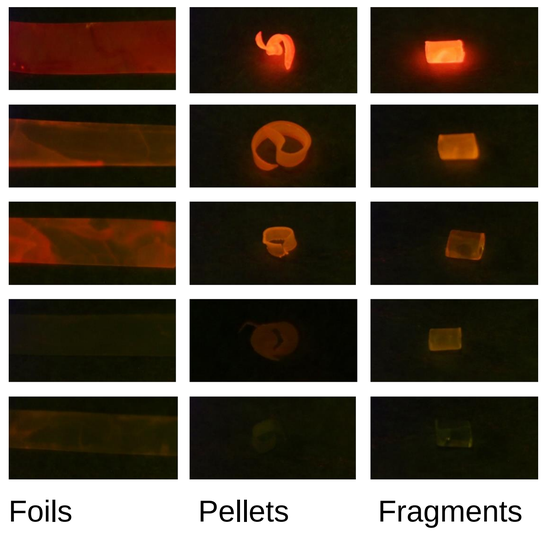
Spectroscopy setup

Results

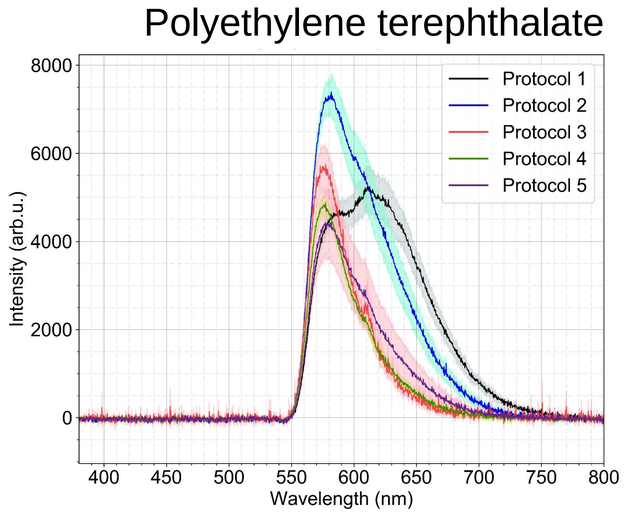
Polyethylene



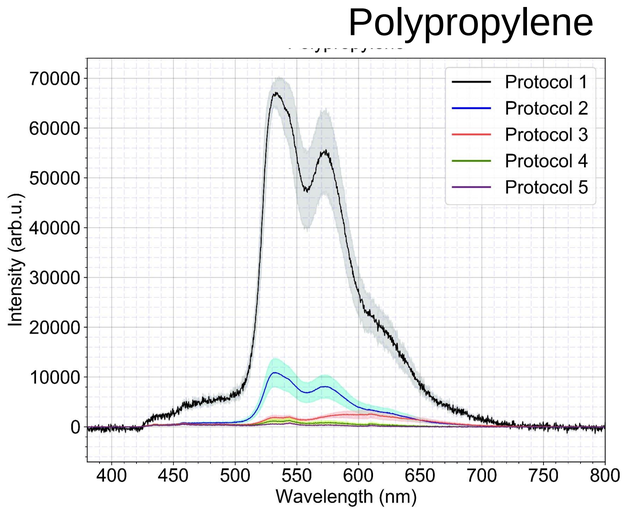


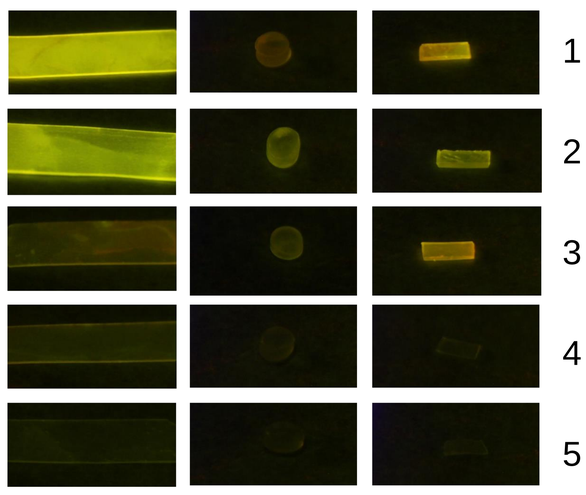


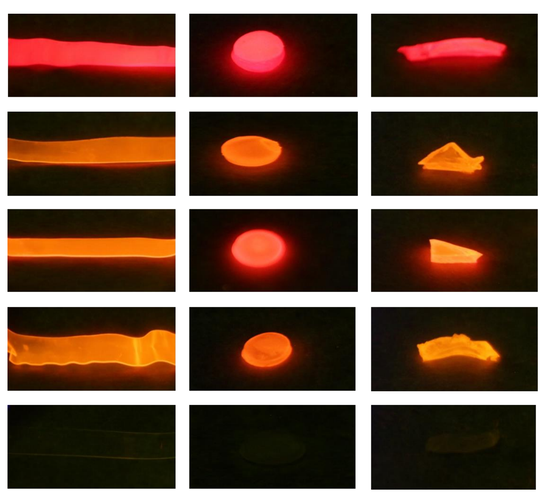
Polyethylene terephthalate



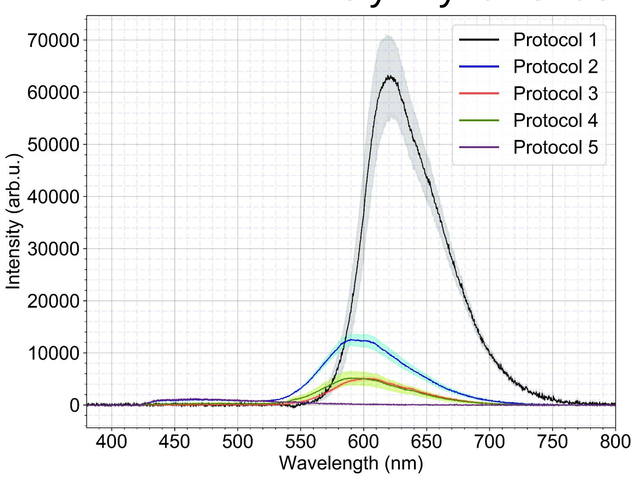
Polypropylene







Polyvinyl chloride



- Higher concentrations of Nile red do not only influence the fluorescence intensity but also the chemical shift
- In our method, acetone and ethanol were combined to avoid polymer degradation and to better differentiate polar and non polar plastics.

References

1. Cole, Matthew. (2016). A novel method for preparing microplastic fibers. Scientific Reports. 6. 10.1038/srep34519.
2. Maes, Thomas & Jessop, Rebecca & Wellner, Nikolaus & Haupt, Karsten & G Mayes, Andrew. (2017). A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Scientific Reports. 7. 10.1038/srep44501.
3. Joon Shim, Won & Song, Young-Kyoung & Hong, S & Jang, Mi. (2016). Identification and quantification of microplastics using Nile Red staining. Marine Pollution Bulletin. 113. 10.1016/j.marpolbul.2016.10.049.
4. Erni-Cassola, Gabriel & Gibson, Matthew & C. Thompson, Richard & Christie-Oleza, Joseph. (2017). Lost, but found with Nile red; a novel method to detect and quantify small microplastics (20 µm–1 mm) in environmental samples. Environmental Science & Technology. 51. 10.1021/acs.est.7b04512.