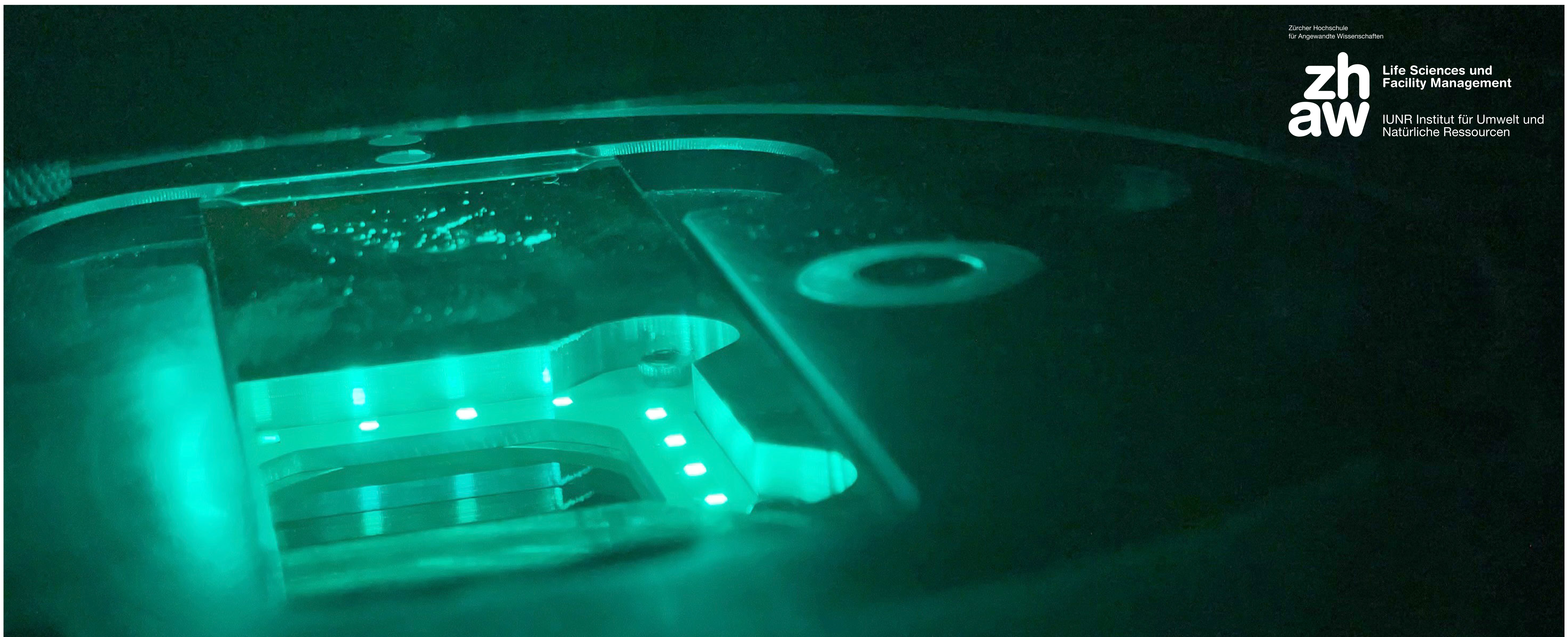


Improved Detection and Identification of Microplastics in Soils through the Combination of Nile Red Staining and FTIR-Analysis

Basil Thalmann[1]
[1]Institute of Natural Resource Sciences, Zurich University of Applied Sciences (ZHAW), Wädenswil, Switzerland

Purification and identification of microplastic particles (MP) in complex matrices such as soils is analytically challenging. Most purification protocols remove inorganic particles through density separation and mitigate organic particles via oxidation before analysis by Fourier-transform infrared (FTIR) spectroscopy. However, chemical oxidation of lignified biomass is not possible without degradation of common plastic polymers (e.g. PE, PS). Thus, high numbers of non-plastic particles persist in purified samples and all remaining particles have to be measured to identify the presence of MP resulting in increased analytical time and efforts.
Selective staining of MP with Nile red facilitates the identification of polymeric among other particles such as lignified biomass or mineral constituents (Shim et al., 2016). However, for the identification of the type of polymer, further analytical tools such as FTIR is needed. To combine both technics a slide-on ring-light was developed, fitting the existing slot for the attenuated total reflection (ATR) tip of the Nicolet iN10 Infrared Microscope (Thermo Scientific, USA). The here presented protocol and improvement of the FTIR-microscope reduces the particle number to be automatically analysed (in our example resulting in a 20-fold time reduction) and, hence, is a step toward a high-throughput method appropriate for private environmental laboratories.

Fluorescence Light

A slide-on ring-light module with two holes, one for recording the fluorescence of stained MP and the other one for the FTIR-measurements, was designed and machined out of aluminium (Figure 1). The module was further equipped with: (i) a custom-made printed circuit board (PCB) containing 16 high-power LEDs (Osram, GC VJLPE1.13) with a typical wavelength of 490 nm (optimum according to Shim et al. (2016)) and (ii) a longpass filter with a cut-on wavelength of 525 nm (Edmund Optics, OD >4.0), to exclude non-florescent light reflected from the probe (Figure 2).

Test of Light

To test the capabilities of the fluorescence light pure polymers with different hydrophobicity were stained with Nile red (5 mg/L Nile red in water, pH 2.5 for 12 h (Sturm et al., 2021)) and imaged under the FTIR-microscope (Figure 3).
To visualize the improvement in MP detection, spherical PE particles were spiked to soil extracts, obtained through a purification protocol consisting of an oxidation step (Fenton's reagent) and density separation (NaBr, ρ = 1.5), and filtered onto aluminium oxide FTIR-filters. After Nile red staining, the filters were analyzed with the FTIR-microscope. First, to analyze the total count of particles, a section of the baklit filters was imaged (Figure 4). Automatic image analyzis resulted in 641 particles.
Second, the same filter area was analyzed using the developed ring light with the slide-on module set to position one (fluorescence light turned on). The fluorescent particles on the filter were recorded with the microscopes integrated camera (Figure 5). This resulted in a reduction of the total particle count to 31.
Third, to identify the particles polymers, the module was set to position two, the open hole, and the fluorescent particles were automatically detected and measured by the FTIR-microscopes integrated Particle Wizard. The 31 particle spectra were then identified using Open Specy (Cowger et al., 2021) resulting in 29 being PE spheres and 2 unidentifiable (Figure 6).

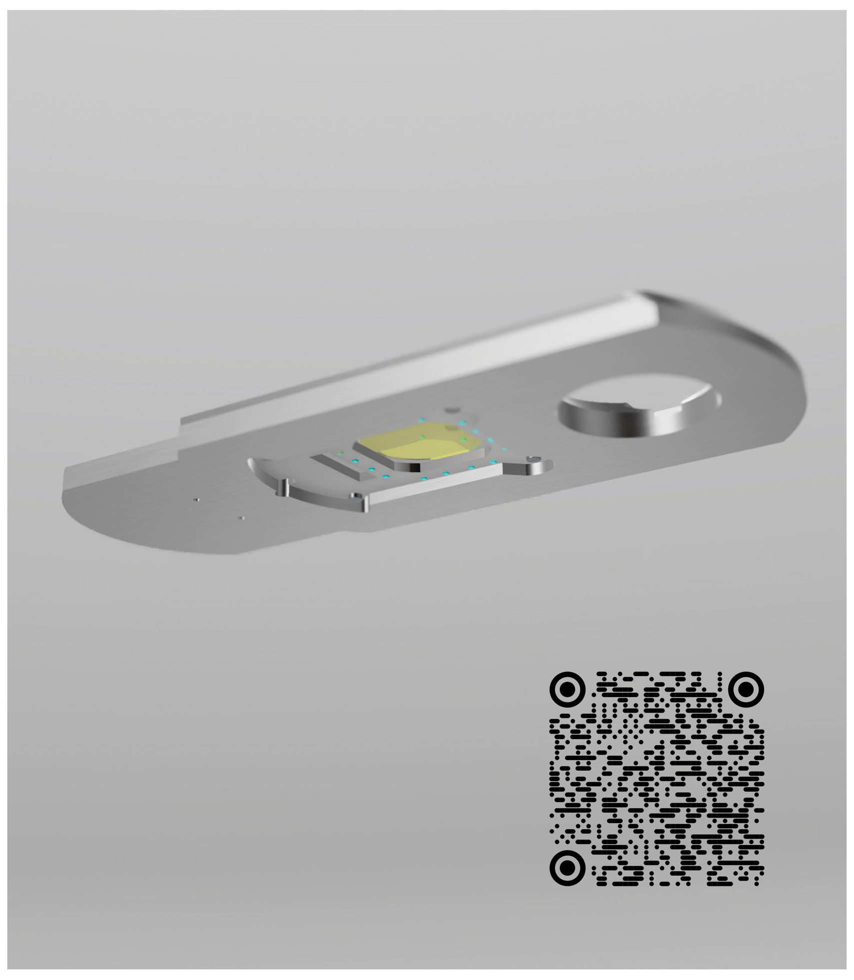


Figure 1. 3D model of ring-light module (scan QR-code for AR model)

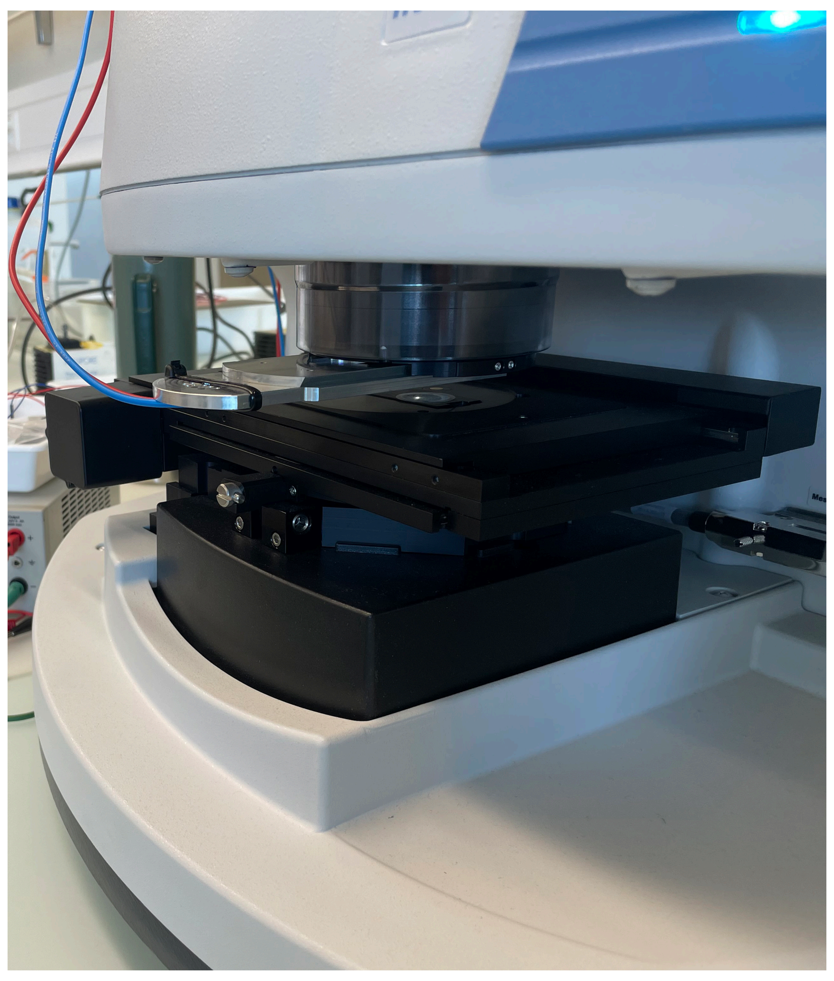
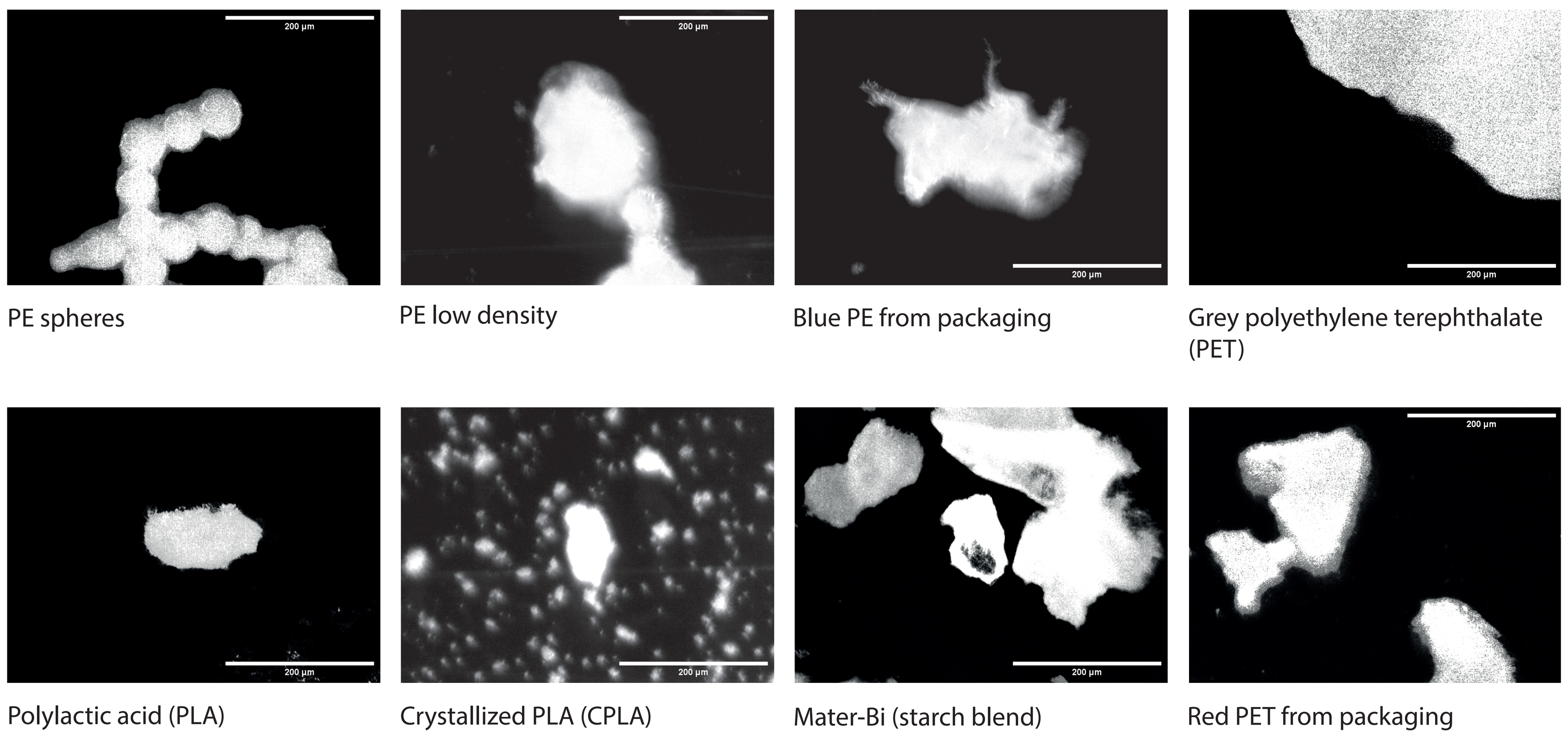


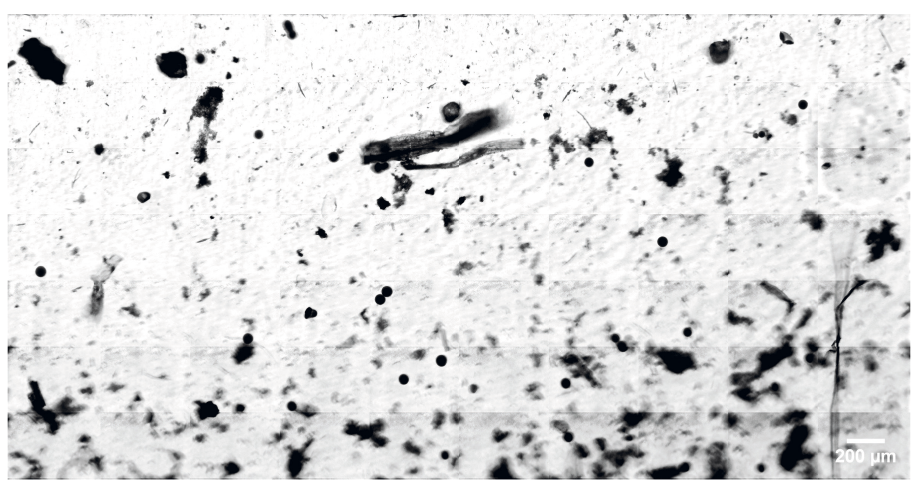
Figure 2. Manufactured module inserted in the FTIR-microscope.



PE spheres | PE low density | Blue PE from packaging | Grey polyethylene terephthalate (PET)

Polylactic acid (PLA) | Crystallized PLA (CPLA) | Mater-Bi (starch blend) | Red PET from packaging

Figure 3. Nile red stained MP under fluorescence light.



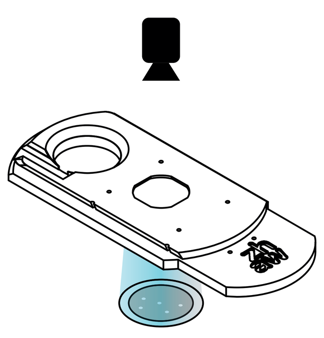


Figure 4. A total of 641 particles on backlit filter.

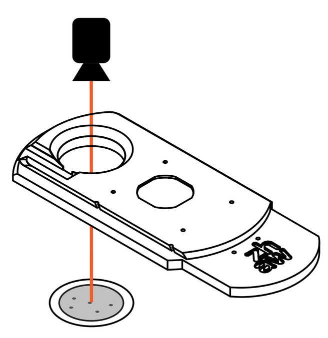


Figure 5. A total of 31 fluorescent particles.

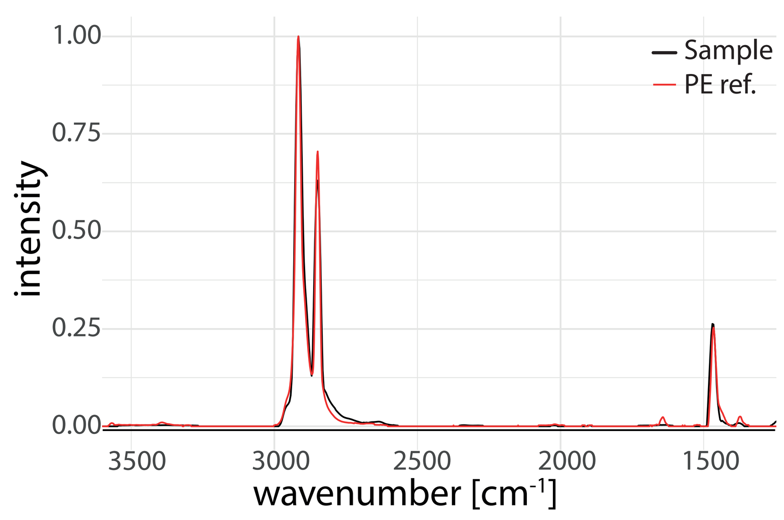


Figure 6. FTIR spectra of PE sphere particle and PE refernece.

Literature:
Cowger, W., Steinmetz, Z., Gray, A., Munno, K., Lynch, J., Hapich, H., Primpke, S., De Frond, H., Rochman, C., Herodotou, O., 2021. Microplastic Spectral Classification Needs an Open Source Community: Open Specy to the Rescue! Anal. Chem. 93, 7543–7548. https://doi.org/10.1021/acs.analchem.1c00123
Shim, W.J., Song, Y.K., Hong, S.H., Jang, M., 2016. Identification and quantification of microplastics using Nile Red staining. Mar. Pollut. Bull. 113, 469–476. https://doi.org/10.1016/j.marpolbul.2016.10.049
Sturm, M.T., Horn, H., Schuhen, K., 2021. The potential of fluorescent dyes—comparative study of Nile red and three derivatives for the detection of microplastics. Anal. Bioanal. Chem. 413, 1059–1071. https://doi.org/10.1007/s00216-020-03066-w

Acknowledgments:
Thanks to Christoph Koller for the support in the design and manufacturing of the LED-PCB.
This work is part of the larger project „Einfluss und Nachweis von biol.ogisch abbaubaren Werkstoffen in der Umwelt (BAWIU)" financed by the Federal Office for the Environment (FOEN).

Contact: thai@zhaw.ch