Flow cytometry for microplastic observation in the marine environment

Timperman Jade[1], Meyers Nelle[2], Asselman Jana[3], Janssen Colin[4], De Witte Bavo[3], Catarino Ana Isabel[2], De Rijcke Maarten[2] and Everaert Gert[2]

[1] Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Gent, Belgium
E-mail: jade.timperman@ugent.be
[2] Flanders Marine Institute (VLIZ), Wandelaarkaai 7, 8400 Oostende, Belgium
[3] Aquatic Environment and Quality Research Area, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Ankerstraat 1, 8400 Oostende, Belgium
[4] Department of Animal Sciences and Aquatic Ecology, Faculty of Bioscience Engineering, Ghent University, Bluebridge, Wetenschapspark 1, 8400 Oostende, Belgium

In recent years, there is a growing concern about the accumulation of microplastics in the environment and the associated risks for ecological and human health. To date, multiple physical and chemical identification methods are widely used to assess plastics concentrations in different environmental matrices. However, current methods are usually time consuming, subjective to human bias and often focussed on the analysis of relatively large particles (> 100 µm). However, in ecotoxicological assessments the focus is often on smaller sized particles (< 100 µm). There is a growing need to develop time- and cost-effective methodologies to identify, characterise and quantify microplastics in the field. The combination of flow cytometry with fluorescent dyes, specifically Nile red, is a promising methodology that can enable high-throughput detection of micro- and nanoplastics. Flow cytometry is an extremely fast method with which thousands of cells can be identified in a few seconds, and that is widely used in different ecological applications such as the screening of phytoplankton in seawater. The fluorescent dye Nile red, frequently used to stain lipophilic cellular components in histology, is strongly fluorescent when adsorbed to plastics. Although the combination of flow cytometry and Nile red is expected to be suitable for the detection, quantification and identification of smaller size ranged microplastics, only few studies have investigated this technology in microplastic research.

In this work, we aim to optimize the observation and identification of microplastics (20 µm – 250 µm) using a combination of Nile red staining methods and flow cytometry. As a first step, we developed a microplastic quantification method for small particles to enable verification of the flow cytometer results. In a next step, we assessed how to stain microplastics in a way suitable for flow cytometric analysis, but without affecting the plastics themselves. We developed a method to concentrate the sample volumes and to ensure that the Nile red-stained plastics are homogeneously distributed in the sample volume taken up by the flow cytometer. Autofluorescent polyethylene (PE) and polystyrene (PS) beads will be used to trial the methodology, and this analysis will be followed by Nile red-staining of PE and PS pristine beads, heterogeneously shaped PE and PS particles, and eventually heterogeneously shaped particles of other, abundantly produced polymer types. In the coming months, spiked artificial seawater samples with mixtures of known particles will be tested. We expect our approach to detect and identify polydisperse plastic particles of unknown size and concentration, and we think it can pave the way for a fast and robust in-situ monitoring method of microplastics in the marine environment.

Keywords: Microplastics; Surveys; Marine environment; Flow cytometry; Nile red, In-situ quantification; method development