Use of Imaging Flow Cytometry (FlowCam®) in the Study of Microplastics

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Synopsis

Microplastics are ubiquitous in marine environments yet remain vastly uncharacterized. Methodologies for the detection, characterization, and quantification of microplastics continue to be developed. In response to the need to automate the characterization of microplastics, researchers have turned to the FlowCam® (Fig. 1), an imaging particle analyzer used globally for phytoplankton analysis, to image, characterize, and quantify microplastics (Fig. 2, 3). The FlowCam was used in two recent studies to quantify, image, and measure microplastics.

The prevalence of microplastics in marine environments has lead to question the effects of microplastics on aquatic organisms, particularly filter feeders. Woods et al. (2017) used the FlowCam to quantify the microplastic fiber uptake, ingestion, and egestion rates in the blue mussels *(Mytilus edulis)*. Mussels were placed in filtered seawater treatments with *Rhodomonas salina* (food source) and varying concentrations of polyethylene terephthalate microplastic fibers. The FlowCam was used to quantify the uptake of *Rhodomonas* and microplastic fibers in each treatment, as well as the number of microplastic fibers in mussel tissue, feces, and pseudofeces.

As naturally-occurring marine microplastics exhibit a variety of shapes and sizes (beads, fibers, fragments, etc.), they can be cumbersome to analyze using only image recognition software. Therefore, to improve and simplify microplastic analysis, Lorenz et al. (2017) evaluated the ability of an enzymatic-oxidative treatment (Löder et al., 2017) to digest organic materials in marine samples and, in doing so, isolate the microplastics present. The FlowCam was used to calculate the total area-based diameter of all particulate matter following each digestive step to evaluate its efficacy.

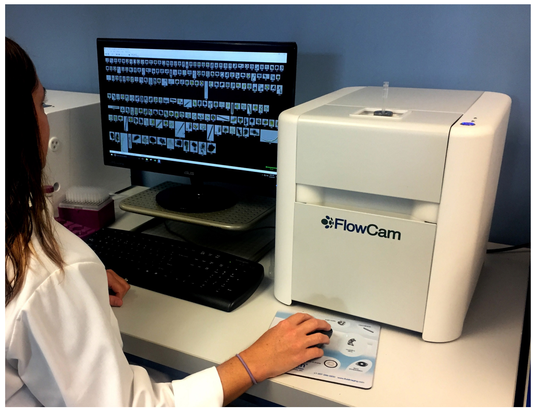

Figure 1. The FlowCam is a particle analyzer that is effective at analyzing microplastics using its imaging and analysis capabilities. Credit: Fluid Imaging Technologies, Inc.

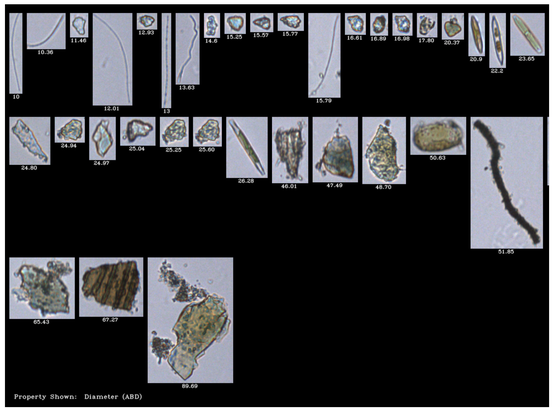

Figure 2. Naturally-occurring marine microplastics, and some diatoms, as imaged by the FlowCam at 10X. Credit: Fluid Imaging Technologies, Inc.

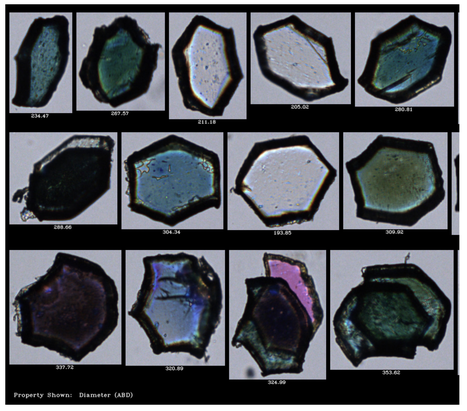

Figure 3. Multi-colored plastic glitter as imaged by the FlowCam at 4X. Credit: Fluid Imaging Technologies, Inc.

Identify and Enumerate Microplastic Fibers Using FlowCam In Feeding Experiment

Woods et al. (2017) quantified the uptake, digestion, and egestion of polyethylene terephthalate microplastic fibers (MPF) (Fig. 4) in blue mussels *(Mytilus edulis)* using the FlowCam. Mussels were placed in filtered seawater with MPF concentrations ranging from 3,000 MPF/L to 30,000 MPF/L and fed a diet of *Rhodomonas salina* (Fig. 5).

The MPF and microalgal uptake rates of each mussel were determined by calculating the change in *R. salina* and MPF concentrations in the treatment water at regular intervals for the duration of the study period. MPF in treatment water, pseudofeces, and feces were quantified using the FlowCam.

The image recognition function in VisualSpreadsheet discriminates particles based on morphology. Woods et al. selected a sampling of fiber images from all particulate matter imaged and executed the image recognition function in VisualSpreadsheet. Images of similar, fiber-like particles were filtered from images of other particulate matter. Both MPF and naturally-occurring fibers present in the treatment (marine fibers, laboratory fibers) were collected. The MPF used in this study were obtained by shearing fibers from polyethylene terephthalate fabric with a blade and therefore showed sharp, non-weathered edges which were used to distinguish MPF from naturally occurring fibers which showed frayed, weathered edges. After MPF were identified using VisualSpreadsheet's image recognition capability, the total number of images within that set were enumerated by VisualSpreadsheet (Fig.4).

Woods et al. found that microalgal uptake rates were greatly reduced in mussels exposed to concentrations of 15,000 MPF/L or greater. Pseudofeces production showed positive correlation with MPF uptake rates at 30,000 MPF/L. A single fecal pellet showed up to 70 MPF.

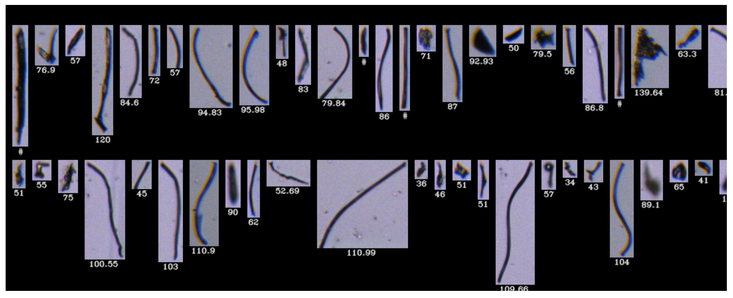

Figure 4. (Above) Polyethylene terephthalate microplastic fibers (MPF) as imaged by the FlowCam. The crisp, non-weathered edges of the above MPF distinguished these from other naturally-occurring, weathered fibers. Credit: Woods et al. (2017).

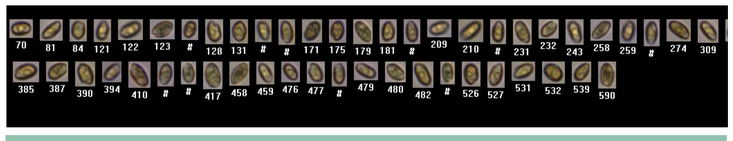

Figure 5. (Above) *Rhodomonas salina* imaged by the FlowCam at 10X. Credit: Fluid Imaging Technologies, Inc.

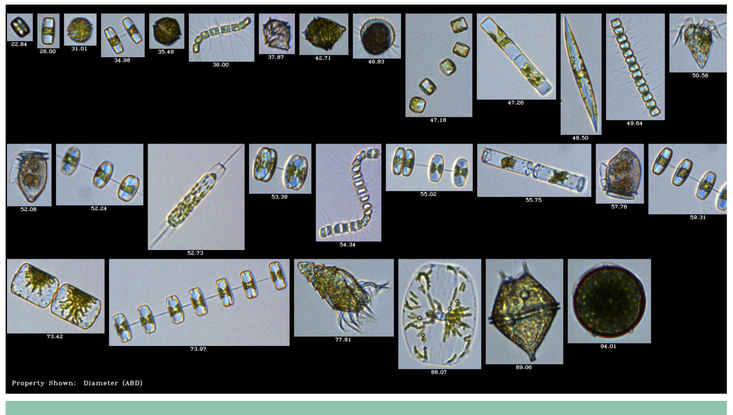

Figure 8. (Above) A phytoplankton community from the Gulf of Maine imaged by the FlowCam at 10X. Credit: Fluid Imaging Technologies, Inc.

About the FlowCam

Developed at Bigelow Laboratory for Ocean Sciences, the FlowCam is a continuous imaging flow cytometer and particle analyzer designed for conducting research and monitoring of microorganisms and particles in both marine and freshwater systems. By providing high resolution digital images of discreet particles ranging in size from 2µm to 5mm (Fig. 8), the FlowCam can provide cell counts, size data, including length, width, area, various diameter readings, as well as biovolume measurements, along with some 40 additional image parameters of imaged particles. The FlowCam has proprietary software that includes a pattern recognition algorithm allowing the user to 'train' the instrument to identify organisms or particles of interest. This also provides for the capability to automatically classify organisms and/or particles in samples based on image analysis. Today there are over 400 FlowCams being used in 50+ countries to study and monitor microorganisms in aquatic systems.

Digestive Isolation of Microplastics Evaluated Using FlowCam

Lorenz et al. (2017) evaluated the efficacy of microplastic isolation in a natural marine water sample (Fig. 6) using a six-step enzymatic-oxidative digestion developed by Löder et al. (2017).

The efficiency of each digestive step was evaluated by calculating the change in total surface area of particulate matter from before to after the digestive step (Fig. 7). The FlowCam captured an image of each particle (microplastic or plankton) from which the area-based diameter (ABD) was calculated by VisualSpreadsheet. The efficacy of each digestive step is indicated by the reduction in total particulate matter ABD. Lorenz et al. observed that the complete digestion process (all six steps) resulted in a 98.6% reduction in total particle area.

Following the digestion, the remaining particulate matter was filtered from the water matrix. The total area of particulate matter per mL of sample was calculated using the FlowCam to determine an appropriate volume to be filtered so as not to overburden the filter.

References

Woods, M.N., Stack, M., Fields, D.M., Matrai, P., (2017) Microplastic fiber uptake, ingestion, and egestion rates in the blue mussel *(Mytilus edulis)*. Unpublished raw data, Marine and Environmental Research Institute, Blue Hill, ME, and Bigelow Laboratory for Ocean Sciences, Boothbay, ME.

Lorenz, C., Speidel, L., Primpke, S., Gerdts, G., (2017) Using the FlowCam to Validate an Enzymatic Digestion Protocol Applied to Assess the Occurrence of Microplastics in the Southern North Sea. MICRO 2016: Fate and Impact of Microplastics in Marine Ecosystems doi: 10.1016/B978-0-12-812271-6.00094-6.

Löder, M.G.J., Imhof, H.K., Ladehoff, M., Löschel, L.A., Lorenz, C., Mintenig, S., et al. (2017) Enzymatic Purification of Microplastics in Environmental Samples. Environ Sci Technol 51:14283-14292 doi:10.1021/acs.est.7b03055.

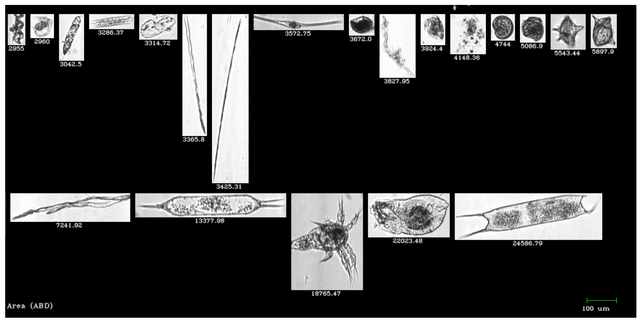

Figure 6. (Above) Images of particulate matter in sample prior to enzymatic digestion. Note images of diatoms, dinoflagellates, zooplankton, and detritus, much of which is removed by the digestive process. Credit: Lorenz et al. (2017).

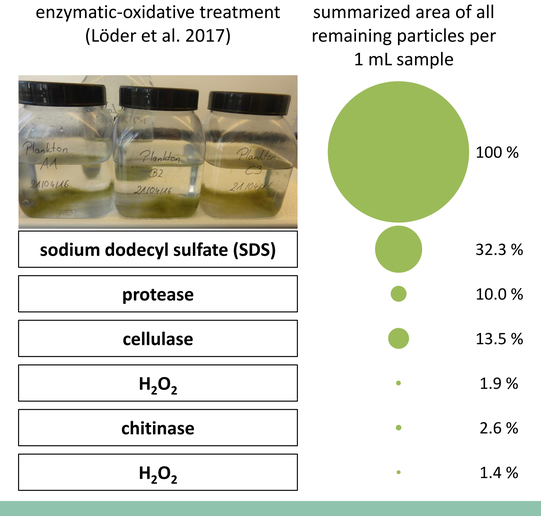

Figure 7. (Above) The six-step digestion used by Lorenz et al. to isolate microplastics from organic matter in marine water samples. Each step shows a reduction in area of remaining particulate matter, as calcuated by VisualSpreadsheet using FlowCam images. Credit: Lorenz et al. (2017).