‘Stat on pixels’: An automated counting method for selective fluorescent-stained microplastics using Nile Red dye

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Abstract: The NSW marine debris threat and risk assessment identified microplastics as a priority threat to the environmental assets and social values of the marine estate. There are increasing interests in microplastics and calls for a greater understanding of their presence and impacts. However, traditional environmental microplastic assessment is expensive, labour intensive, and lacks standardisation in sampling and analysis protocols. Most commonly, microplastic assessment involves (1) an expert manually counting each piece through a microscope, or (2) the more expensive approach, Fourier transform infrared (FTIR) and Raman micro spectroscopy.

To increase the efficiency of large-scale microplastic monitoring, we propose an automated counting method for microplastics which are fluorescent stained using Nile Red (NR) dye. NR dye treatment is found to be the most effective in terms of absorption and fluorescence intensity for microplastics (Maes et al. 2017) making it easier to identify in an image taken over an orange filter.

The method we propose has 5 main steps:

1. The raw image is split into the respective RGB colour bands.
2. A Gaussian blur is applied with a user-defined kernel size and an in-situ variance depending on the kernel size.
3. The blurred image undergoes an adaptive thresholding step where the image is segmented to a binary image based on the object pixel intensity within a user-defined neighbourhood.
4. A contour extraction algorithm based on border following (Suzuki et al. 1985) is used on the binary image to extract the outer contours of the objects.
5. A filter based on the contour size specified by the user is applied to count the appropriated objects within a certain size range.

The results from the proposed method were validated against (1) visual counting of the images by the naked eye, (2) counting under a microscope, (3) manual counting on an enhanced image derived from the software ImageJ, and (4) comparing total counts from an FTIR count to the automated count. The preliminary results show similar counts, with a negligible difference when compared with the above validation methods. This method creates a standardised, rapid, and low-cost assessment for microplastic counts, that can be used for large-scale monitoring. Current extensions of the methodology involve categorizing microplastics by size, shape, and fluorescent intensity.

REFERENCES

Maes, T., Jessop, R., Wellner, N. 2017. et al. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Sci Rep 7, 44501.

Suzuki, S., Abe, K. 1985. Topological structural analysis of digitized binary images by border following, Computer Vision, Graphics, and Image Processing, 30(1), 32–46, https://doi.org/10.1016/0734-189X(85)90016-7

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