Received: 18 November 2015 ■ Revised: 17 April 2016 ■ Accepted: 30 April 2016 ■ Publication: 9 May 2016

JNIRS JOURNAL OF NEAR INFRARED SPECTROSCOPY

Hyperspectral imaging and data analysis for detecting and determining plastic contamination in seawater filtrates

Therese M. Karlsson,[a,b] Hans Grahn,[a,c] Bert van Bavel[a,d] and Paul Geladi[c,e]

[a]University of Örebro, MTM, School of Science and Technology, SE 701 82 Örebro, Sweden. E-mail: therese.karlsson@marine.gu.se

[b] University of Gothenburg, Department of Marine Sciences, Sven Loven Research Centre, Kristineberg 566, 45178 Fiskebäckskil, Sweden

[c]Corpus Data & Image Analysis AB, Rålambsvägen 66 B, suite 1408, SE 112 56 Stockholm, Sweden

[d] Norwegian Institute for Water Resaerch, Gaustadalléen 21, NO-0349 Oslo, Norway

[e]Forest Biomaterials and Technology, Swedish University of Agricultural Sciences, SE 90183 Umea, Sweden

One possible way of monitoring plastic particles in sea water is by imaging spectroscopic measurements on filtrates. The idea is that filters from seawater sampling can be imaged in many wavelengths and that a multivariate data analysis can give information on (1) spatial location of plastic material on the filter and (2) composition of the plastic materials. This paper reports on simulated samples, with spiked reference plastic particles and real seawater filtrates containing microplastic pollutants. These real samples were previously identified through visual examination in a microscope. The samples were imaged using three different imaging systems. The different wavelength ranges were 375–970 nm, 960–1662 nm and 1000–2500 nm. Data files from all three imaging systems were analysed by hyperspectral image analysis. The method using the wavelength span 1000–2500 nm was shown to be the most applicable to this specific type of samples and gave a 100% particle recognition on reference plastic, above 300 µm, and an 84% pixel recognition on household polyethylene plastic. When applied to environmental samples the technique showed an increase in identified particles compared with visual investigations. These initial tests indicate a potential underestimation of microplastics in environmental samples. This is the first study to demonstrate that hyperspectral imaging techniques can be used to study microplastics down to 300 µm, which is a common size limit used in microplastic surveys.

Keywords: visualisation of multivariate results, interactive visual data handling, plastic identification, visual spectroscopy, near infrared spectroscopy, microplastics

Introduction

Microplastics, commonly defined as synthetic polymers with a size below 5 mm, which are found in seawater samples from all over the globe,[1–3] are making headlines as emerging, widespread pollutants. For sampling microplastics in surface water, a trawl with a mesh size around 300 µm is often used. The methodological lower size limit is thereby 300 µm, although some studies measure smaller particles through using finer mesh sizes.[4] The plastic pollution of our oceans has proven to be of societal, environmental and economic concern[5,6] and is included as one of the descriptors for good environmental status (GES) in the marine strategy framework directive (MFDS).[7]

Plastic polymers commonly found in the environment are polypropylene (PP), polyethylene (PE), polyethylene

ISSN: 0967-0335
doi: 10.1255/jnirs.1212

terephthalate (PET), polystyrene (PS) and polyvinylchloride (PVC).[8] Together these comprise 72.9% of the plastic produced globally.[9]

Quantitative measurements are important for risk assessment and monitoring purposes. They are also important in allowing temporal and spatial comparison of pollutants. Currently microplastic surveys require visual analysis, often performed using a microscope. This technique is time consuming and investigations show that results differ between researchers.[7] A faster and more objective method of analysis, suitable for environmental samples, would therefore be beneficial in future microplastic studies. An increasing number of publications are combining microscopy with spectral analysis of identified particles to avoid misidentification.[2,10] Often Raman or Fourier transform infrared (FT-IR) spectroscopy is used in combination with visual identification in a microscope. These techniques require individual particle analysis, meaning that the suspected plastic particle has to be visually identified as plastic, or suspected plastic, and then tested spectroscopically. It has, however, been shown that for plastic particles and fragments the particles are likely underestimated.[11]

Methods for reliable and objective quantitative and qualitative analysis of plastic particles in environmental samples are needed. Hyperspectral imaging of large filter areas (1–100 cm^2) combines spectral and spatial information that can be used to detect and identify plastic particles and discriminate them from biological material found in sea water filtrates. The measurement is fast but produces a large data file that has to be submitted to an optimal chemometric analysis to extract relevant information. The software used is often based on interactive visualisation and brushing between images.

In this paper three hyperspectral imaging[12,13] solutions are presented and compared for the study of a number of commonly produced plastic polymers. The aim was to test if hyperspectral imaging can replace or complement the less objective visual counting in microscopes for microplastic particles down to 300 µm. To test this, we investigated if the techniques can accurately identify reference plastics, common household plastics and finally if they can differentiate between the often more degraded plastic particles and organic material found in real seawater filtrates.

Through comparing instruments with different spectral ranges and resolutions, it was possible to assess what spatial and spectral resolution would be required to achieve robust analysis results.

Material and methods

Industrial reference plastics

A number of plastic particles that are often used in industry and found in sea water samples were selected to use as references (Table 1). A few less common polymer types were also analysed, including a set of bioplastics as described in Table 1.

Household reference plastics

The models and the spectra were tested and compared with household plastic scanned on a white reflective Teflon background (Table 2). Different colours and properties of the plastics were tested, as additives and colouring agents have been shown to affect spectroscopic identification when using other spectroscopic methods such as Raman.[10]

Table 1. Names, composition and particle size of the reference plastics.

Reference plastic	Size	Abbreviation	Supplier
Polyamide	350 µm	PA	Goodfellow
Polyamide unfilled	3-5 mm	PA	Erteco rubber
Polyamide 4.6 fibre	1 mm wide	PA	Goodfellow
Polyhydroxyalkaonate-biopolymer	3 mm	PHA	Goodfellow
Polyhydroxybutyrate-biopolymer	5 mm	PHB	Goodfellow
Poly L lactic acid-biopolymer	5 mm	PLLA	Goodfellow
Bioon-biopolymer	3 mm	Bioon	Bioon
High impact polystyrene	5 mm	HIPS 485	Erteco rubber
Polystyrene	900 µm	PS	Goodfellow
Polystyrene	250 µm	PS	Goodfellow
Polypropylene	3 mm	PP	Sigma Aldrich
Polypropylene	3 mm	PP	Goodfellow
Polyvinylchloride unplasticised	250 µm	PVC	Goodfellow
Low density polyethylene	3-5 mm	PE-LD	Erteco rubber
High density polyethylene	3-5 mm	PE-HD	Erteco rubber
Polyethyleneterephtalate	3 mm	PET	Goodfellow
Polycarbonate	3 mm	PC	Goodfellow

Table 2. Household plastic, classified from resin number, used to test models and to compare properties with reference plastics.

Plastic	Colour	Description	Origin
PP	Black	Hard	Food packaging
	Transparent	Hard	Food packaging
	Tranparent	Soft	Product packaging
	White	Soft	Food packaging
	Yellow	Hard	Tobacco packaging
	White	Hard	Tobacco packaging
	Black	Hard	Tobacco packaging
	Orange/white/transparent/green	Soft	Food packaging
PE	Pink	Soft	Product packaging
	Transparent	Soft	Plastic bag
	White	Soft	Food packaging
	Blue/white	Soft	Food packaging
PET	Green	Hard	Drinking bottle
	Transparent	PETE Hard	Food packaging
	Transparent	R-PET Hard	Food packaging
	Transparent	Hard	Drinking bottle
PS	Black/brown	Hard	Food packaging
	Transparent	Hard	Drinking cup
	White	Expanded PS	Food packaging

Surface water samples

The imaging techniques were applied on samples that were collected from the SV *Sea Dragon* in the Baltic Sea during the month of August 2014. Sampling was performed using a 3 m long manta trawl with a 300 μm mesh and a collection sock at the end. A flow meter was attached to the aperture of the trawl. The trawl was attached at the spinnaker pole and towed for 60 min, along the side of the boat, at a speed of 0.5–1.5 m s^{-1}.

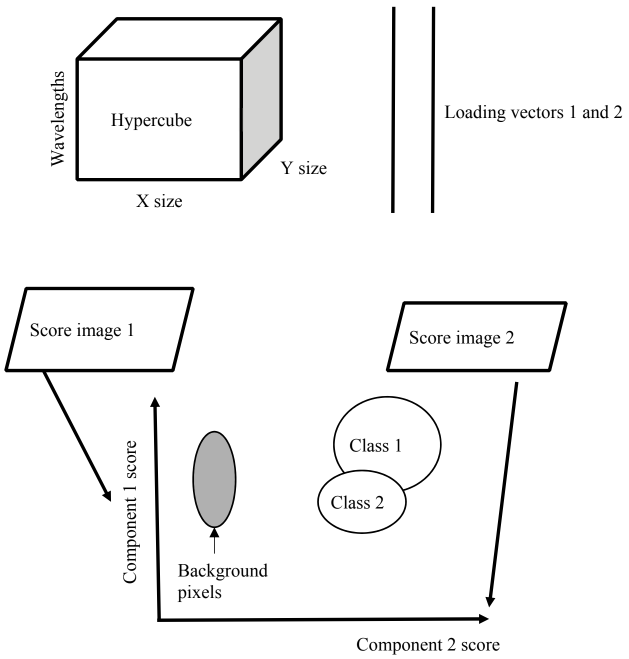

Figure 1. Hyperspectral images have at least three dimensions. Two are the physical dimensions width and length, usually called X and Y (IxJ). The third dimension is spectral, very often wavelength (K). Principal component analysis of mean-centred data produces score images and loading vectors. The loading vectors can be used for spectral interpretation. The score images can be studied as images, but they can also be used in score plots. Brushing is an interactive means of marking points in the score plots and finding their spatial position or the other way around. Usually points of similar chemical composition group together in the score plot. The example shows three clusters: background, class 1 and class 2. These could be Filter background, plastic 1 and plastic 2.

Upon retrieval the sides of the trawl were rinsed and the sock was removed. The contents of the sock were transferred to a glass jar with a polypropylene lid. Particles that were visually identified as plastic and suspected particles that could not be confirmed visually to be plastic, were transferred to glass petri dishes and scanned with a white Teflon reflecting background.

Hyperspectral imaging

Hyperspectral images are collected as hypercubes (Figure 1), which contain a large number of data points. This makes multivariate analysis and data reduction necessary. Figure 1 shows the decomposition of a hypercube to get two score images and two loading vectors. In many cases a few components represent 99% of the data and the remainder is noise. This is a huge data reduction. Just looking at score images is not enough. A more interesting technique is making scatter plots where each pixel is represented as a point. Because of the vast amount of data, the plots have to be visualised as density plots. In such plots clustering can be easily recognised. In most cases a cluster of pixels representing background can be detected and removed. Furthermore, different materials may form different clusters if their chemical composition and spectroscopic behaviour are different. Figure 1 gives a conceptual example of such a case. Two components have been plotted against each other resulting in three clusters. The pixels representing background are shown in green and two other clusters show samples with different characteristics and a small overlap.

Videometer

The Videometer instrument (Videometer A/S, Lyngsø Allé 3, DK-2970 Hørsholm, Denmark) makes 2050 × 2050 pixel images of a sample size of 120 × 120 mm in 19 wavelength bands. This is done by illuminating the sample with a sequence of radiation bursts generated from light emitting diodes. An integrating sphere is used for distributing the illumination evenly over the sample. The wavelength range is 375–970 nm. The spatial resolution is about 60 µm and the magnification is determined by the objective used. Only one objective was available. The measuring time is less than a minute.

Malvern

The Malvern (previously MatrixNIR, Enigma Business Park, Grovewood Road, Malvern, WR14 1XZ, United Kingdom) is an InGaAs camera. The sample is illuminated by four quartz halogen lamps. The image is collected through a lens and a filter (monochromator) for selecting wavelengths. The monochromator is a liquid crystal tuneable filter. The resulting images are of size 256 × 320 pixels for a sample of 49 × 55 mm. The wavelength range is 960–1662 nm with a band every 6 nm. The spatial resolution is 0.17 mm in the setup (objective) used, but other setups are possible, and the measuring time varied between 5 min and 10 min.

Umbio Inspector

The Umbio Inspector instrument (modified by Prediktera AB, Riddaregatan 8, SE 903 36 Umeå, Sweden from Sisuchema Specim, Oulu, Finland) instrument is a line scan camera based on an HgCdTe detector array. The monochromator element is a prism–grating–prism. Whole images are made by moving the sample on a synchronised belt and adding scanned lines. In this way, images of a width of 320 pixels are made in up to 256 wavelength bands over 1000–2500 nm. The length of the images is determined by how many lines are scanned. A 22.5 mm lens was used on the camera. The spatial resolution is dependent on line width, but typically 300 µm is easily achievable. The measuring time is around 1 min dependent on chosen settings such as measuring length and integration time.

Software and data analysis

The Evince software (Prediktera AB, Riddaregatan 8, SE 903 36 Umeå, Sweden) for hyperspectral image analysis was used for the calculations. This software is interactive by using screen and cursor brushing. The software combines the multivariate methods principal component analysis, classification and regression analysis. A special feature is the use of graphical interaction in plots called "brushing".[14] Tests on reference materials and household plastic were used to evaluate the methods through counting percentage of successfully identified particles and percentage correctly identified pixels.

Results and discussion

Software and data analysis

Analysis was performed through a multi-image import, with the reference plastic and the sample. Subsequently a principal component analysis (PCA) plot was created, from the imported images and their spectra, which was then used to analyse for microplastics.

For an initial background removal the spectra were limited to 1667.3–2086.4 nm, which were the areas that seemed less perturbed by degradation processes. In this area the first overtones of C–H stretching[15] can be seen which showed pronounced peaks for all tested plastics.

A multiplicative scatter correction (MSC)[12] was then applied to decrease the baseline shifts and slope variations between reference plastic and environmental samples. Additionally a first derivative transformation was added. Background and organic material could then be removed through a careful simultaneous analysis of the spectral information and cluster formation using five different components.

Once the background was removed, the wavelength scope could be widened to include wavelengths 1082.9–2248.4 nm, thereby including the second overtone of C–H and the first overtone of the C–H combination bands (Figure 2). This facilitated polymer identification, through a cluster formation with the respective reference plastic (Figure 3).

The spectra of the identified particles were then used to confirm the polymer type. The combination bands for C–H were, however, excluded due to the amount of noise perturbing their signal in most samples.

Data handling of spectroscopic imaging has been acknowledged as one of the main problems with the technique, by several authors.[16] The software used in this article provides

Table 3. Summary of the hyperspectral techniques used.

Instrument	X size	Y size	# wavelengths	Wavelengths (nm)	Measuring time (mins)	Spatial resolution
Videometer	2050	2050	19	375-970	1	60 µm
Malvern	320	256	118	960-1662	5–10	300 µm, 1000 µm
Umbio	320	free	256	1000-2500	1	300 µm

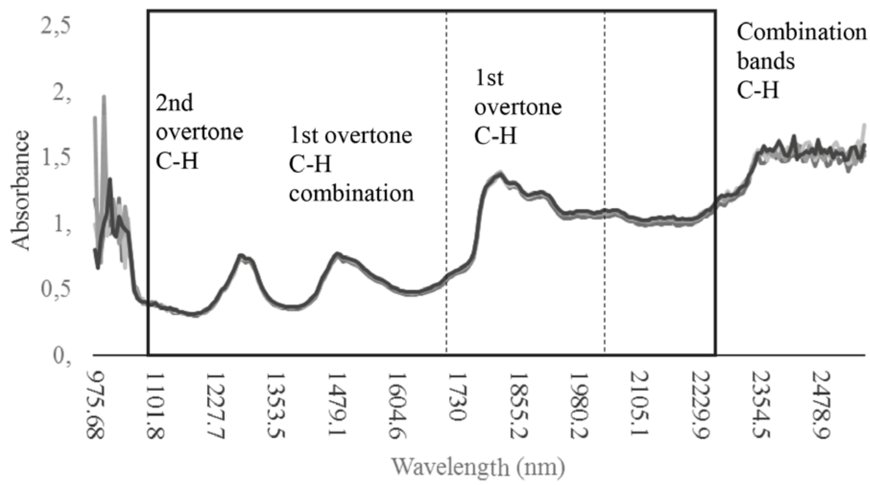

Figure 2. The first distinction between plastic and the background was best achieved through including the part of the spectra showing the first overtone of the CH-stretch of the polymers,[15] as illustrated by the dotted box in the figure. This area showed a pronounced peak and gave a spectral fingerprint for each of the tested polymers. Once the background was removed the particle identification was improved if a wider wavelength span was included as illustrated by the full-line box in the figure.

the possibility to work simultaneously with several aspects of the data while maintaining an overview (Figure 3).

A partial least squares discriminant analysis prediction model can be constructed for recognising reference plastics. However, when applied to household plastics or plastic pieces found in the marine environment, and hence subject to degradation processes, the model shows large classification errors. This was likely due to the large difference in peak intensity and the increased relative level of background and noise observed.

Using a PCA model approach, as described, where the scan of the sample was combined with a scan of reference plastics was found to be more reliable. After transforming the data, the plastic particles in the samples could be separated from other material such as shells, animals and algae, through clustering with the reference plastic.

Through the usage of a validation system with confirming polymer type, not only through the clusters formed with PCA, but through a continuous cross validation of the spectral match, the reliability of the method increased.

Reference materials

Spectra from reference plastic, household plastic and environmental samples were tested with the equipment with the highest spectral range (Umbio Inspector) to compare the spectral quality. Tests on household plastic and environmental samples show several spectral dissimilarities compared to the ones obtained from reference plastics. This is exemplified using different types of polyethylene in Figure 4, other polymers showed similar patterns.

Notably the absorbance intensity decreased for the household plastic and an even further decrease, relative to the reference, was seen for the plastic found in marine samples. The relative influence of background and noise also increased. Areas in the beginning and the end of the wavelength interval seemed more affected, which has also been noted in other applications of NIR spectroscopy.[16] Areas that were less affected were used for initial differentiation between the plastic and other material as described under data handling.

The differences complicate using an automatic model approach to the analysis. Using transformation techniques such as multiplicative scatter correction (MSC) can help to correct for these differences and plastic particles can then be distinguished from the background (Figure 3) using the more manual approach described in the data analysis section.

A further understanding of the degradation effects on the spectra of different polymers, as well as an inclusion of the spectral effects of additives, is important to include for future studies of plastic particles in environmental samples. Particularly for adopting a semi-automatic recognition system or model as a model based on reference plastic would not recognise changes introduced by degradation processes.

Surface water samples

In the analysed samples 50 of 51 of the particles, which had visually been identified as plastic, were confirmed as plastic polymers with the hyperspectral imaging analysis. Additionally, 13 other particles, which were not identified as plastic pieces with visual analysis, were identified as plastic resulting in an average increase of 50% in the number of particles in the samples (Table 4).

This further confirms the suspicion that particles are often at risk of being underestimated and fits the results as shown by Song and colleagues.[11] It should, however, be noted that these particles in question were separated from the sample as suspicious anthropogenic particles, the actual underestimation might be higher.

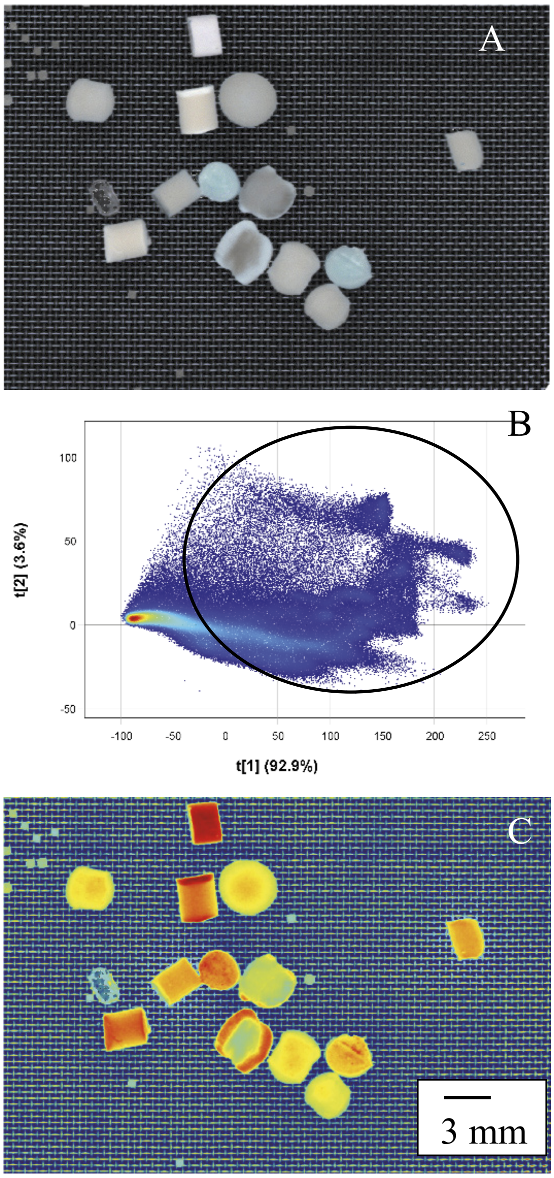

Figure 3. Reference plastic on a metal grid seen as a RGB image in 3A obtained from a Malvern scan. Although the metal grid disturbs the signal through causing reflections, a rough distinction between background and plastic can easily be made in the PCA plot in 3B as marked by the black circle. When the PCA contour plot (3C) is plotted for the first component the plastic pieces stand out against the background.

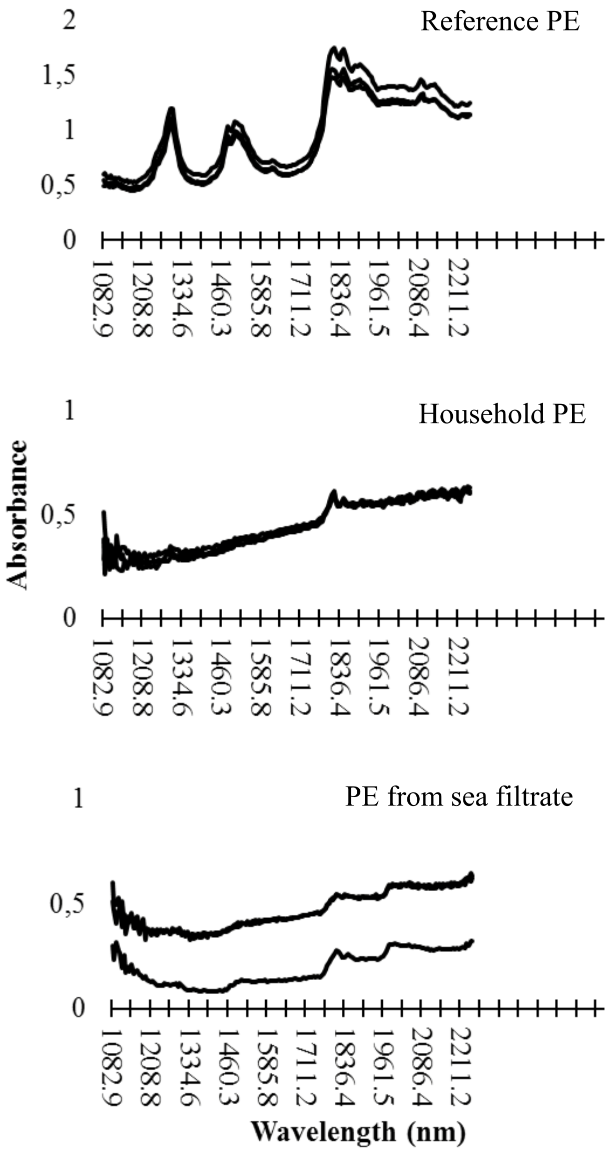

Figure 4. Obtained polyethylene spectra from reference plastic, household plastic and plastic samples from sea filtrate.

Hyperspectral imaging techniques

The Videometer showed promise for distinguishing between different polymer types when applied on reference plastics, as it separated the different polymer types in different clusters. It also had the benefit of adding a high resolution to the images. The spectral information with 19 wavelengths 375–970 nm (Figure 5) was, however, too limited for creating a model able to distinguish between the reference plastics when mixed, and also for household plastics. Much of the interesting information is above 1000 nm.

Measurements of reference plastic material showed good result for the Malvern and the Umbio Inspector (Figure 5). The Malvern measured 119 wavelengths and therefore gave a significantly higher spectral resolution than the Videometer, which facilitated polymer identification. It showed clear distinction in the PCA plot and good classification based on the second overtone of the C–H and the first combination overtone.

The Umbio Inspector showed both the second and the first overtone of the C–H as well as the combination band and the first overtone of the combination band. The first overtone and the combination band have a stronger signal and are therefore appropriate for analysing samples that are expected to show a higher degree of degradation than the

Table 4. Summary of the 10 samples that were investigated with visual and hyperspectral image analysis. An average increase of 65% in particle recognition was achieved with hyperspectral image analysis. When only particles that could be identified as a specific polymer was included a 22% increase compared to visual analysis was still achieved. Of those, 74% were polyethylene, 21% were polypropylene and 4% were polystyrene.

		Particle counts using hyperspectral imaging analysis			
Sample number	Visual counts	Particles identified as plastic	% Increase compared to visual analysis	Particles identified to polymer type	% Increase compared to visual analysis
1	1	3	200	2	100
2	4	7	75	5	25
3	7	8	14	8	14
4	18	19	6	15	–17
5	5	6	20	1	–80
6	5	7	40	4	–20
7	3	3	0	3	0
8	6	6	0	6	0
9	0	2	200	2	200
10	1	2	100	1	0

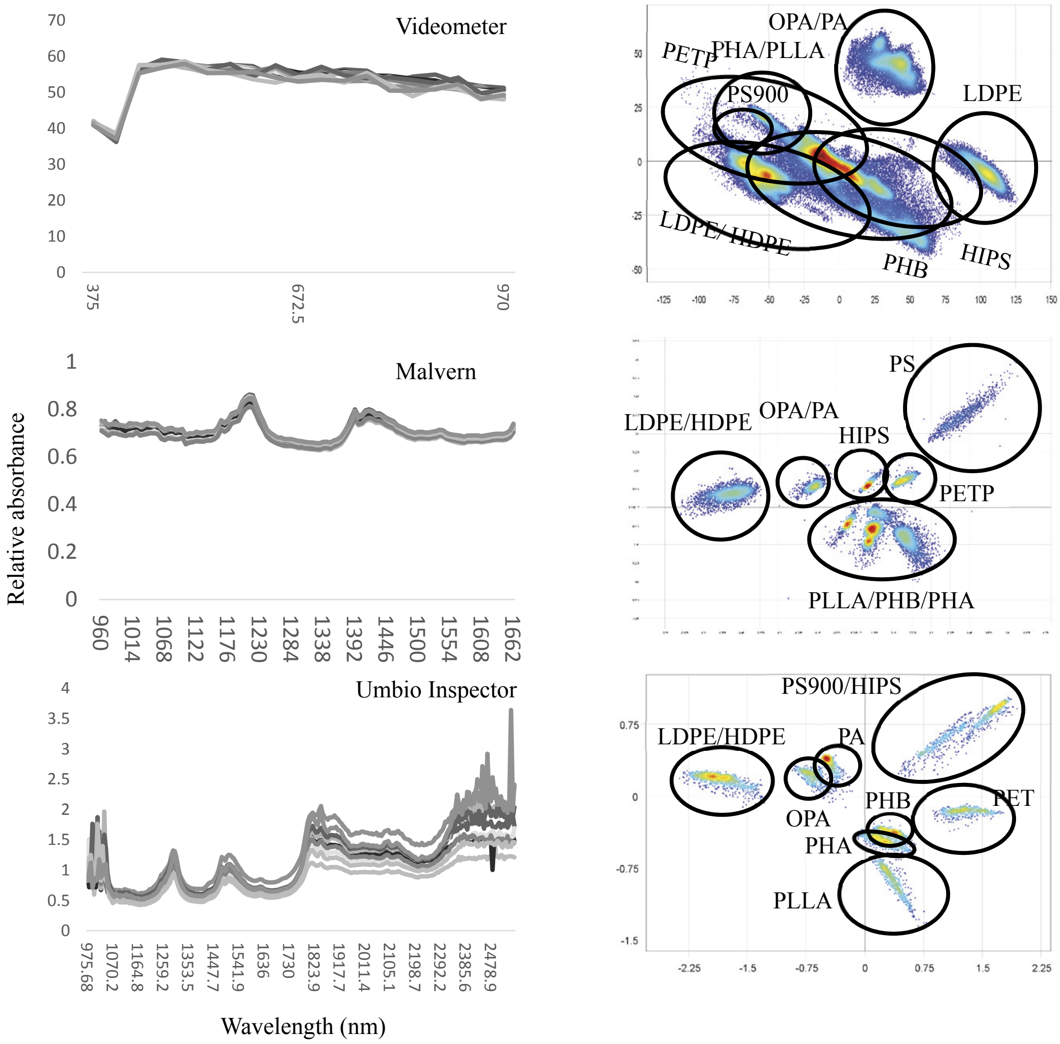

Figure 5. Spectra of polyethylene from the three different techniques presented next to the PCA plot of 10 of the different reference plastics. The plots show an increasing level of separation as the spectral resolution and span increases.

reference material. Additionally the Umbio Inspector showed the fastest image acquisition. The image resolution on the other hand was not as high as with the Videometer, but due to the high spectral resolution and interchangeable lenses an adequate resolution to analyse particles of 300 µm was obtained.

Data handling and pre-processing

Analysis was performed through a multi-image import with the reference plastic. As a first step, for an easier removal of the background, only the first overtones of the C–H stretches were included in the analysis. This area (Figure 2) showed pronounced peaks for all reference plastics and was less perturbed by degradation (Figure 4).

Different correctional tools can also be used, such as MSC, to decrease the baseline shifts and slope variations between reference plastic and environmental samples.[12]

Once the background has been removed, the wavelength scope should be widened to include the second overtone of C–H and the first overtone of C–H combination band[15] (Figure 2). This facilitates polymer identification through cluster formation with the respective reference plastic.

Data handling of spectroscopic imaging has been acknowledged as one of the main problems with the technique.[16] The software and techniques used in this article provides the possibility to work simultaneously with several aspects of the data while maintaining an overview.

Conclusions

Hyperspectral imaging techniques can be a useful complement for monitoring purposes. These techniques can provide an objective and comparable analysis of microplastics in environmental samples. Additionally they provide compositional information through polymer identification. Comparisons with visual identifications also showed that the technique can identify particles that were not visually identified as plastic; hence the method reduces the risk of underestimating certain types of microplastics. The increased objectivity achieved with the method could improve spatial and temporal comparisons between results from different research groups; this has previously been complicated due to the discrepancy observed between visual identifications. It was, however, shown that certain aspects such as the spectral effects of polymer degradation have to be taken into account when calculating prediction models. It is suggested that these techniques are initially used complementary to the traditional visual methods, to further assess possibilities and limitations in different types of samples. Of the three hyperspectral instruments tested, the Videometer showed very high spatial resolution, but less clear discrimination between plastic types. Both the other instruments, Malvern and Umbio Inspector, had a lower spatial resolution but a better potential for plastic discrimination and identification due to their higher spectral resolution.

Acknowledgements

We acknowledge funding from the European Union Seventh Framework Program (FP7/2007-2013) under Grant Agreement 308370. HG would like to acknowledge grants from the Swedish Knowledge Foundation (KK-Stiftelsen) DNR 20140024.

The authors thank the Baltic Sea expedition 2014 for collaboration with samples. We also thank Christine Schönlau and Helena Nilsson (MTM Örebro University) for their assistance.

References

1. C.K. Pham, E. Ramirez-Llodra, C.H. Alt, T. Amaro, M. Bergmann, M. Canals, J.B. Company, J. Davies, G. Duineveld, F. Galgani, K.L. Howell, V.A.I. Huvenne, E. Isidro, D.O.B. Jones, G. Lastras, T. Morato, J.N. Gomes-Pereira, A. Purser, H. Stewart, I. Tojeira, X. Tubau, D.v. Rooij and P.A. Tyler, "Marine litter distribution and density in European Seas, from the shelves to deep basins", *PloS one* **9(4),** e95839 (2014). doi: http://dx.doi.org/10.1371/journal.pone.0095839
2. M. Cole, P. Lindeque, C. Halsband and T.S Galloway, "Microplastics as contaminants in the marine environment: a review", *Mar. Pollut. Bull.* **62,** 2588–2597 (2011). doi: http://dx.doi.org/10.1016/j.marpolbul.2011.09.025
3. A.L. Andrady, "Microplastics in the marine environment", *Mar. Pollut. Bull.* **62,** 1596–1605 (2011). doi: http://dx.doi.org/10.1016/j.marpolbul.2011.05.030
4. V. Hidalgo-Ruz, L. Gutow, R.C. Thompson and M. Thiel, "Microplastics in the marine environment: a review of the methods used for identification and quantification", *Environ. Sci. Technol.* **46,** 3060–3075 (2012). doi: http://dx.doi.org/10.1021/es2031505
5. UNEP, *Valuing Plastics: The Business Case for Measuring, Managing and Disclosing Plastic Use in the Consumer Goods Industry* (2014).
6. C. Leggett, N. Scherer, M. Curry, R. Bailey and T. Haab, "Assessing the economic benefits of reductions in marine debris: a pilot study of beach recreation in Orange County, California", *NOAA Mar. Debris Progr. Ind. Econ. Inc.* 45 (2014).
7. S. Olenin, F. Alemany, A.C Cardoso, S. Gollasch, P. Goulletquer, M. Lehtiniemi and B. Aleksandrov, *Marine Strategy Framework Directive. Task Group, 2* (2010). doi: http://dx.doi.org/10.2788/87092
8. T. Rocha-Santos and A.C. Duarte, "A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment", *TrAC Trends Anal. Chem.* **65,** 47–53 (2015). doi: http://dx.doi.org/10.1016/j.trac.2014.10.011
9. *Plastics–The Facts 2013: An Analysis of European Latest Plastics Production, Demand and Waste Data*. Plastics Europe, Brussels, pp. 1–40 (2013). http://bit.ly/1WEZXXY
10. L.V. Cauwenberghe, A. Vanreuse, J. Mees and C.R. Janssen, "Microplastic pollution in deep-sea sediments",

Environ. Pollut. **182,** 495–499 (2013). doi: http://dx.doi.org/10.1016/j.envpol.2013.08.013

11. Y.K. Song, S.H. Hong, M. Jang, G.M. Han, M. Rani, J. Lee and W.J. Shim, "A comparison of microscopic and spectroscopic identification methods for analysis of microplastics in environmental samples", *Mar. Pollut. Bull.* **93,** 202–209 (2015). doi: http://dx.doi.org/10.1016/j.marpolbul.2015.01.015

12. H. Grahn and P. Geladi, *Techniques and Applications of Hyperspectral Image Analysis*. Wiley, Chichester (2007). doi: http://dx.doi.org/10.1002/9780470010884

13. P. Geladi, H. Grahn and M. Manley, "Data analysis and chemometrics for hyperspectral imaging", in *Raman, Infrared and Near-Infrared Chemical Imaging*, Ed by S. Sasic and Y. Ozaki. Wiley, Chichester, pp. 93–107 (2010). doi: http://dx.doi.org/10.1002/9780470768150.ch5

14. P. Geladi and H. Grahn, *Multivariate Image Analysis*. Wiley, Chichester (1996). ISBN: 978-0-471-93001-3

15. J.R. Workman and L. Weyer, *Practical Guide and Spectral Atlas for Interpretive Near-Infrared Spectroscopy*. CRC Press (2012). doi: http://dx.doi.org/10.1201/b11894

16. G. Elmasry, N. Wang and C. Vigneault, "Detecting chilling injury in Red Delicious apple using hyperspectral imaging and neural networks", *Postharvest Biol. Technol.* **52,** 1–8 (2009). doi: http://dx.doi.org/10.1016/j.postharvbio.2008.11.008