Lightnovo

APPLICATION NOTE

Microplastics detection in seawater using 2D Raman mapping of a filter (membrane) with residue

KEYWORDS

Microplastics; Raman microscopy; 2D mapping; Raman hyperspectral imaging; Multivariate Curve Resolution – Alternating Least Squares (MCR-ALS); Essential Spatial Pixels (ESPs); Essential Spectral Variables (ESVs).

INTRODUCTION

Nowadays, microplastics (MPs) – synthetic particles less than 5 mm in size – are widely present in the environment, and seen as a potential global threat to biodiversity, food safety and public health [1]. The problem is especially acute for seawater, since it is one of the main sources of foodstuff, and, at the same time, it accumulates a huge amount of plastic waste. As a result, MPs have already been found in the tissues and organs of many marine species, as well as in sea salt. To control and correct the situation, it is necessary the systematic extensive monitoring of MPs in seawater is including their type/size distribution, spreading over the water area/depth, weathering, degradation, interaction with chemical pollutants etc.

Raman spectroscopy is an effective analytical technique for this task [1, 2]. It is a rapid, non-contact and non-destructive method that uses monochromatic light to excite molecules in a material, and the spectrum of inelastically scattered light (Raman spectrum) to recognize the types of these molecules, intra- and intermolecular bonds. The method has high selectivity and sensitivity, allowing to identify different types of MPs, even if their number are low, they are mixed, or the irrelevant (matrix) components are present. In addition, one can observe minor changes in the MPs chemical composition and structure. Combination with scanning optical microscopy (Raman microscopy) yields the distribution of locally measured Raman spectra over the sample (hyperspectral imaging) that in turn can be used for simultaneous visualization of the MPs chemical composition, geometry and spatial distribution (chemical imaging).

BACKGROUND

Usually, when analyzing MPs in waterbodies, the initial sample represents the residue collected by some filter (net, sieve, membrane etc.) during trawling, sieving or pumping [1]. Besides MPs, the residue may include soil, sediments, plankton and other additives. Therefore, most of the often-used relevant analytical methods – e.g., Fourier-transform infrared (FTIR) microcopy;

fluorescence microcopy; scanning electron microscopy (SEM); pyrolysis gas chromatography with mass spectrometry (Pyr-GC/MS) – require to take off the residue from the filter and additionally process it in order to obtain from MPs the analytical signals free from the matrix influence. Visual sorting, flotation/density separation, chemical digestion, sieving/filtration, oven-drying and other techniques may be needed to remove additives. Nevertheless, such procedures can lead to the loss of a certain number of MPs, their aggregation, changes in morphology, structure and chemical composition. Moreover, the residue processing is difficult to carry out "in the field", and samples should be transported to a laboratory. All this negatively affects the cost, complexity and duration of the analysis.

Compact backscattering Raman microscopes from Lightnovo ApS allow to bypass aforesaid obstacles and analyze the residue "on site", without taking off it from the filter and removing additives. The approach involves: (i) hyperspectral imaging of the filter with residue by 2D mapping; (ii) processing of this image to obtain the "pure" spectra and concentration profiles for different filter/residue chemical components; (iii) identification of the MPs relevant data by comparing "pure" spectra with the library ones; (iv) construction of chemical images for MPs distributed over the filter.

The microscope uses the same objective to deliver excitation monochromatic light from the in-built laser to the analyzed object and to collect the backscattered light, Figure 1a.

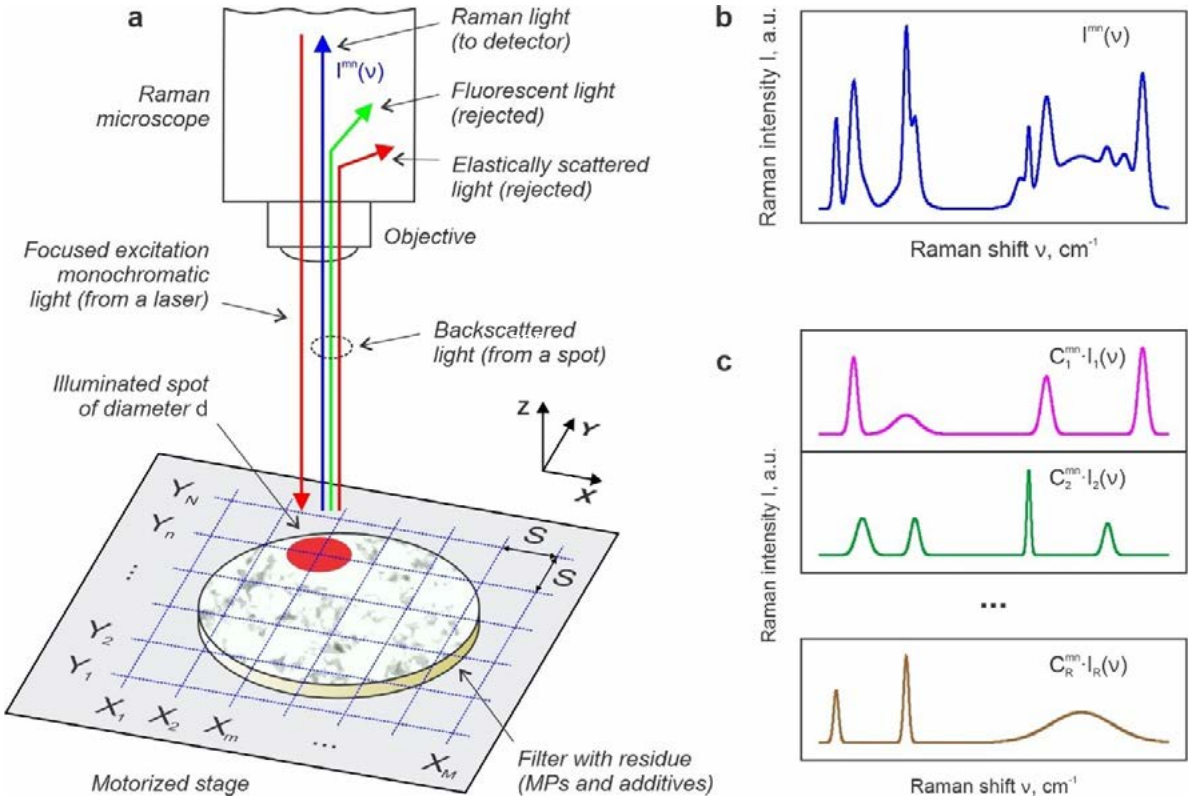

Figure 1. 2D Raman mapping:
a) general measurement scheme;
b) model Raman spectrum for the mixture "obtained" from one illuminated spot;
c) "pure" Raman spectra for individual components present within the spot.

A motorized stage with a base plane XY normal to the optical axis serves to mount the filter with residue so that the last one faces to the objective. Due to the high numerical aperture of the objective NA, the excitation light can be focused into a spot of small diameter d. It can scan the filter surface with a preset step size S via the stage moving in the XY plane. Accordingly, when the spot center has coordinates (X_m, Y_n), m = 1…M, n = 1…N (the cross points of the blue dotted lines on Figure 1a.), the microscope measures local Raman spectrum $I_{mn}(\nu)$, where I – light intensity,

ν – wavenumber, which represents the correspondent "spectral pixel" of the hyperspectral image.

The optical design with a number of original technical solutions efficiently rejects the main parasitic signals: elastically scattered light; fluorescent light; stray light came from the out-of-focus regions. This allows to obtain the spectra with rather clear peaks and suppressed background. And additional post-processing is usually not needed.

Nevertheless, the illuminated spot affects several Raman-active components that conditions the complex form of the $I_{mn}(\nu)$ spectrum, as it shown by the model spectrum on Figure 1b. And decomposition is required to isolate the MPs spectral contributions.

In chemometrics, it is accepted that the mixture's spectrum is the sum of the products of the "pure" spectrum and the concentration for each component of the mixture (a linear mixed model). Then, the measured Raman hyperspectral image can be presented as:

$$
\begin{aligned}
&I^{11}(\nu) = C_1^{11} \cdot I_1(\nu) + \ldots + C_r^{11} \cdot I_r(\nu) + \ldots + C_R^{11} \cdot I_R(\nu) \\
&\ldots \\
&I^{mn}(\nu) = C_1^{mn} \cdot I_1(\nu) + \ldots + C_r^{mn} \cdot I_r(\nu) + \ldots + C_R^{mn} \cdot I_R(\nu) \qquad \text{(Eq.1)} \\
&\ldots \\
&I^{MN}(\nu) = C_1^{MN} \cdot I_1(\nu) + \ldots + C_r^{MN} \cdot I_r(\nu) + \ldots + C_R^{MN} \cdot I_R(\nu) \\
&m = 1 \ldots M; \quad n = 1 \ldots N; \quad r = 1 \ldots R
\end{aligned}
$$

Here: m, n – indexes of the spot coordinates (X_m, Y_n);
M, N – their maximum values (they are actually the measure of the field of view);
r – component number;
R – total number of components;
$I_r(\nu)$ – "pure" spectrum for the r-th component;
C_r^{mn} – concentration of r-th component in the (X_m, Y_n) position.

The set of C_r^{11}, … ,C_r^{MN} presents the concentration profile (distribution map) of r-th component over the sample.

For illustrative purpose, Figure 1c shows some of "pure" spectra $I_r(\nu)$, r = 1, 2, R, that enter into the composition of the model spectrum $I_{mn}(\nu)$ from Figure 1b.

To detect MPs, it is necessary to find the full sets of $I_r(\nu)$ and C_r^{mn}. The Multivariate Curve Resolution – Alternating Least Squares (MCR-ALS) method is the powerful tool for this. Unlike many other, it can provide physically meaningful concentration profiles and spectra, rather than just the results of some formal calculation. The method has convenient software realization for the MATLAB® environment known as the "MCR-ALS Graphic User Interface 2.0" (MCR-ALS GUI) [3].

The MCR-ALS relies on the matrix representation of (Eq.1) and is the iterative method. First, the total number of components R are chosen. This can be done in various ways, including manually. Some restrictions are also specify for C_r^{mn} and $I_r(\nu)$. At least, both can't be negative: $C_r^{mn} \geq 0$ and $I_r(\nu) \geq 0$. Then, initial values for one of the C_r^{mn} or $I_r(\nu)$ sets are setting and substituting into (Eq.1), whereas the second set is calculating from there under the least square criterion. Obtained set is correcting in accordance with specified restrictions, and again substituting into (Eq.1) to calculate new values of the first set. The iterations are repeated until a convergence criterion is met.

A lack of computing power may be a problem for the MCR-ALS implementation to the MPs detection task. This is because, the analysis of a spacious filter with residue requires a wide field of view of Raman microscope, i.e., large M and N values, and accordingly a large number of lines in (Eq.1). At the same time, for example, when working "in the field" only a standard laptop is usually available. In order to solve this issue, the Essential Spatial Pixels (ESPs) and Essential Spectral Variables (ESVs) selection methods can be used jointly [4]. This approach eliminates the "redundant" spatial points and intensity values of the measured Raman spectra from (Eq.1), but saves information that is critical to correct calculations to $I_r(\nu)$ and C_r^{mn} with MCR-ALS.

SAMPLE PREPARATION

The Mediterranean Sea water was taken nearby Naples (Italy), in 100 m from the coast from a depth of 1 m using a grab sampler.

To filter the water, it was used a porous silicon membrane having the square pores with sizes of (5×5) μm^2 and a pitch of 10 µm.

To prepare a sample, 10 liters of water were passed through the edge-fixed membrane under the influence of gravity. After this, the membrane was dried for 24 hours. Figure 2 demonstrates the photo of the membrane fragment with residue made before the measurements.

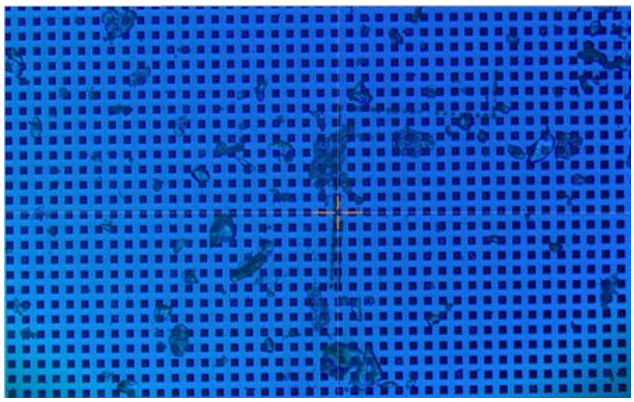

Figure 2. Membrane (a fragment) with residue.

MEASURING SYSTEM

Lightnovo RG Raman microscope, Figure 3, equipped with RG 532 spectrometer and a motorized platform was used for the analysis.

Measurements were carried out with the Olympus LMPLFLN50X objective having a magnification of 50x and a numerical aperture of NA = 0.5.

The 2D Raman scanning parameters: laser wavelength 532 nm; light power 1 mW; spot diameter d = 2 µm; lateral step size S = 16.6 µm; field of view (10×10) mm^2; number of the scanning points along X and Y axes M = N = 600. Raman spectra were recorded in the wavenumber range ν = (100...3600) cm^{-1}. Each spectrum consists of 4056 readouts. Registration time of one spectrum 200 ms. Total scanning time for the entire field of view 20 hours.

Figure 3. Lightnovo RG Raman microscope.

RESULTS

A preliminary overlook of the measured Raman hyperspectral image allowed to reveal some intense spectral peaks, which are most often found in the image spatial plane XY. Figure 4 demonstrates the spatial distributions of their intensities. The peaks at ν = 521.1 cm^{-1} and ν = 960.3 cm^{-1} are strong and, based on a comparison, are conditioned by the silicon membrane. The rest ones may be assigned to MPs.

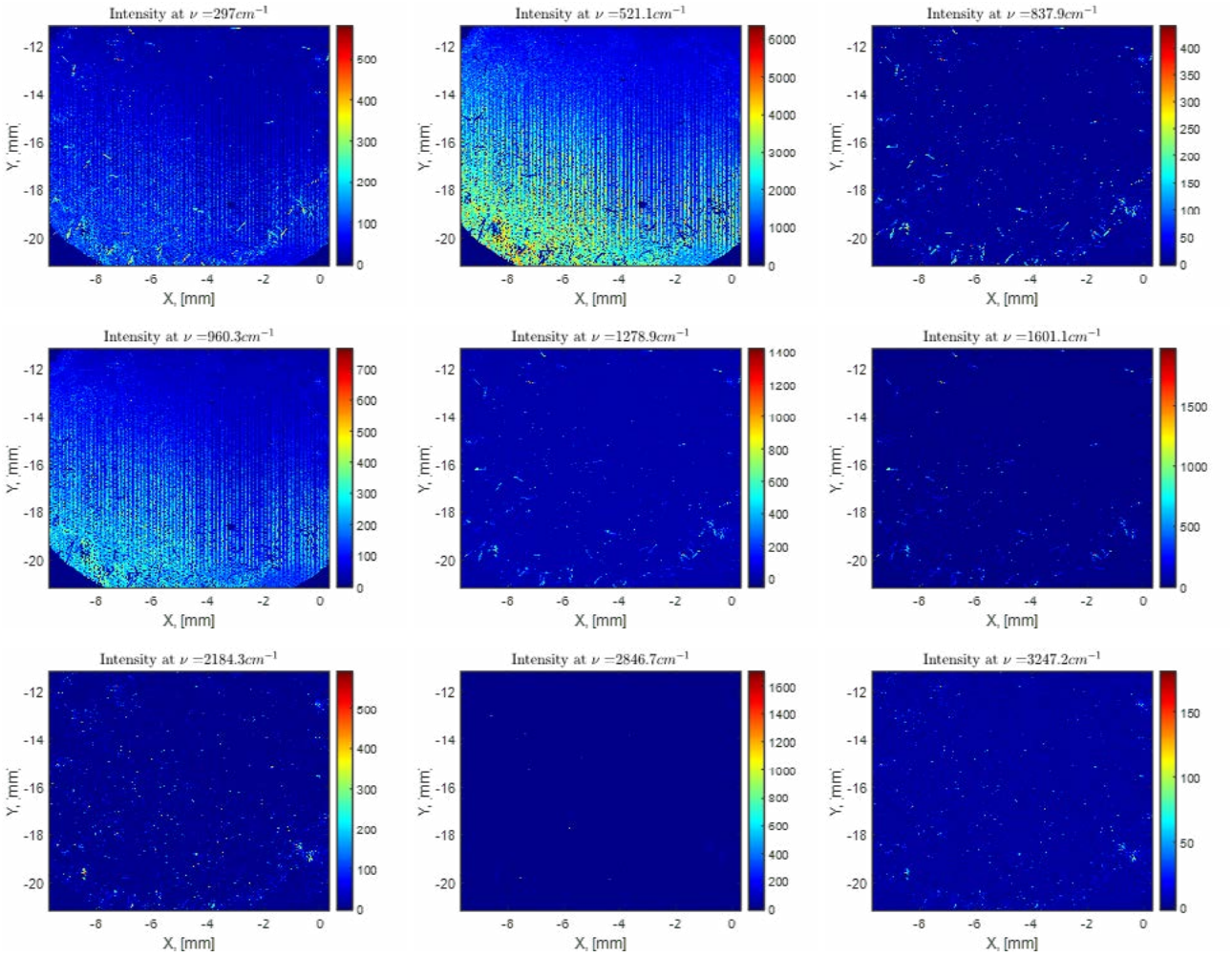

Figure 4. Spatial distributions of the spectral peak intensity at ν = 297 cm^{-1}; 521.1 cm^{-1}; 837.9 cm^{-1}; 960.3 cm^{-1}; 1278.9 cm^{-1}; 1601.1 cm^{-1}; 2184.3 cm^{-1}; 2846.7 cm^{-1}; 3247.2 cm^{-1}.

The hyperspectral data array consists of 600 × 600 × 4056 ≈ $1.46 \cdot 10^9$ points that obviously required some data reduction. The results of the joint implementation of ESPs and ESVs methods [4] are visualized on Figure 5. The red dots correspond to data essential in terms of keeping spectral information: the spatial pixels (left map) and spectral variables (right graph). The resulting optimized data array contains ≈ $8.55 \cdot 10^5$ points, which is 3 orders of magnitude less than the original one.

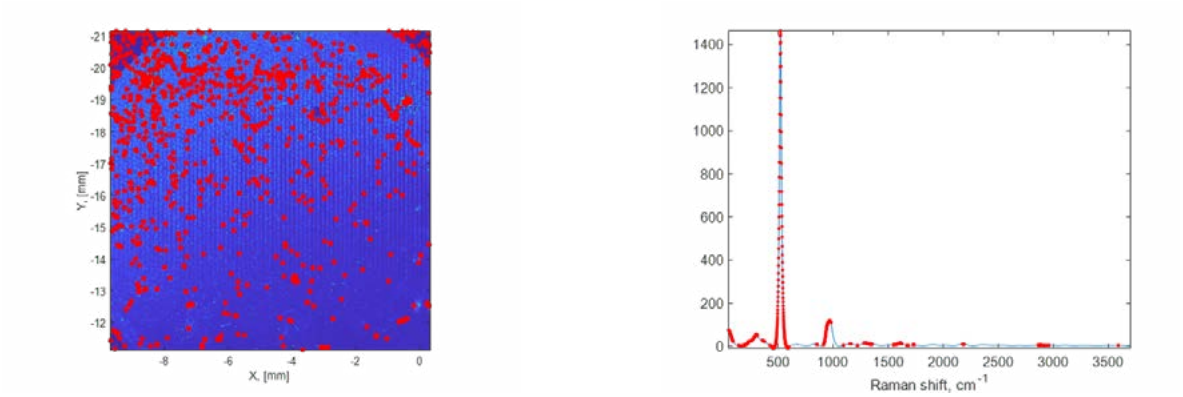

Figure 5. Results of joint using of ESPs (left) and ESVs (right) methods.

Optimized data set was decomposed with MCR-ALS method onto R = 7 components. The correspondent concentration profiles and "pure" Raman spectra are presented in Figure 6. The spatial distribution and spectra for the components with r = 2, 3, 6 indicate that they belong to the silicon membrane.

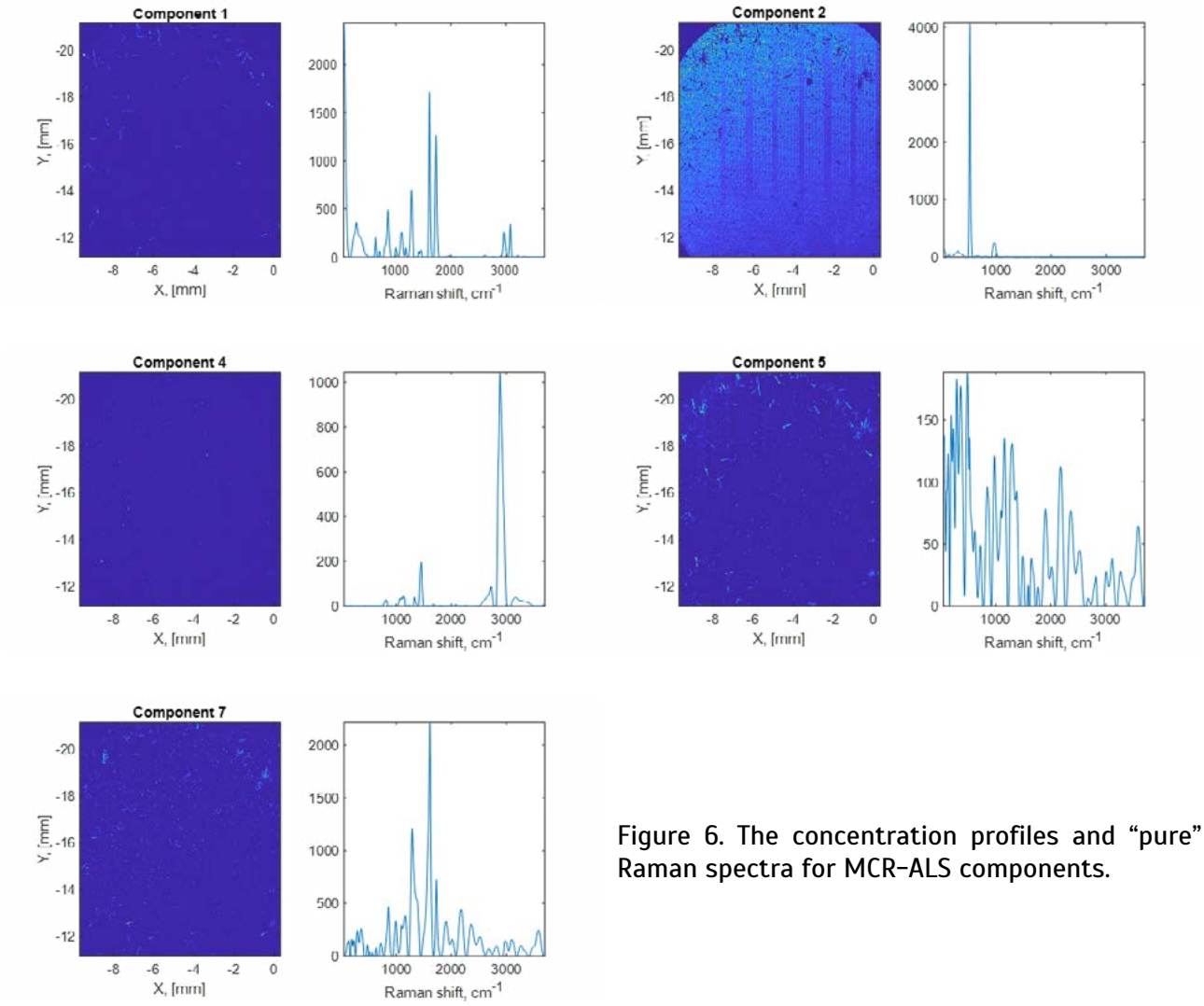

Figure 6. The concentration profiles and "pure" Raman spectra for MCR-ALS components.

In order to identify other components, «A Raman database of microplastics weathered under natural environments» [5] was used. The database spectra were background corrected with the same parameters as for the measured data. Also, the obtained "pure" spectra were interpolated

to match spectral region of the library ones. The Pearson correlation coefficient was chosen to characterize the hit quality index (HQI), a typical parameter describing the correlation between the unknown and reference spectra.

Figure 7 shows a comparison of normalized spectra: the "pure" ones for MCR-ALS components r = 1, 4, 5, 7 (blue), and their best matches (originally named as "wea-100", "sta-16", "wea-44", "sta-15" correspondingly) from the database library [5] (in red). The abbreviation "wea" stands for "the weathered microplastic debris"; the abbreviation "sta" means "the standard microplastic debris and particles".

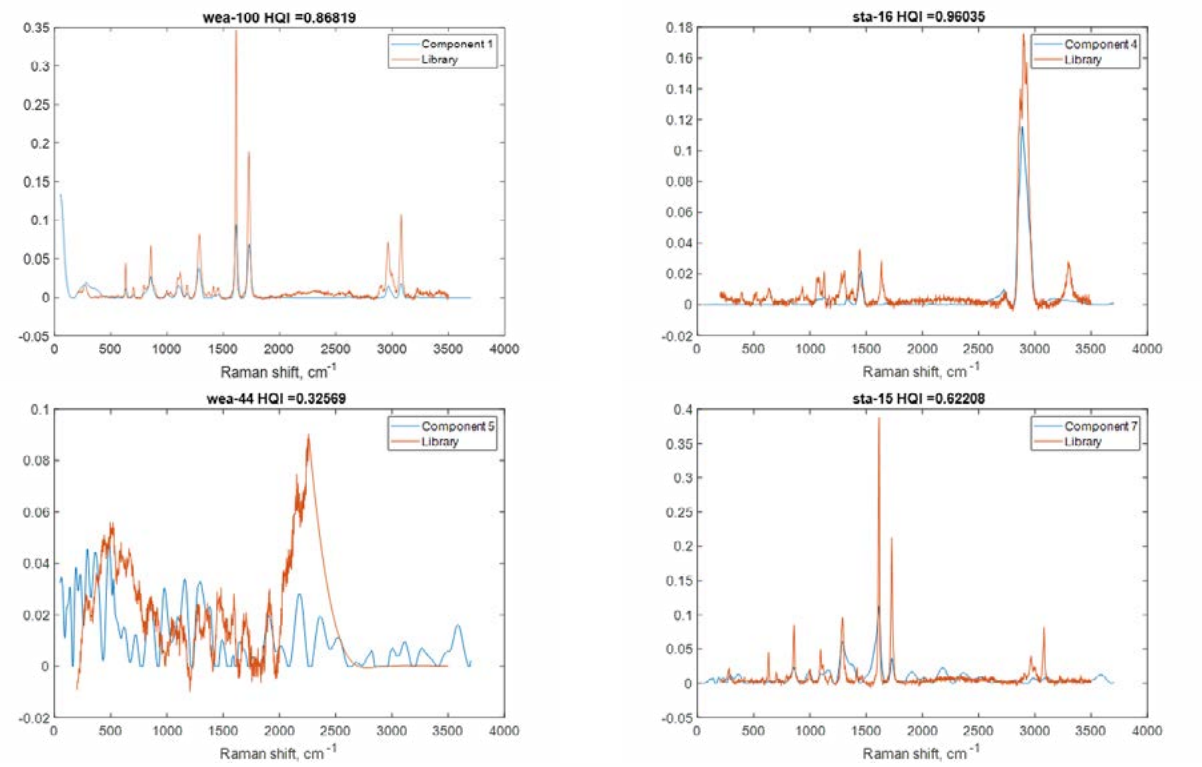

Figure 7. Comparison of the Raman spectra for MCR-ALS components r = 1, 4, 5, 7 with their best matches from database library [5].

As a result of comparison, one can conclude that the components:

- r = 1 is polyester due to high correlation with the library spectrum HQI = 0.87;
- r = 4 is polyamine (nylon), high correlation HQI = 0.97;
- r = 7 probably is a plastic, medium correlation HQI = 0.62;
- r = 5 is not a plastic, at least for the used library, low correlation HQI=0.32.

Different polyester MPs are clearly separated on the concentration profile for r = 1, Figure 6. For most of them, the morphology is close to the pieces of fibers (or filaments) with a diameter of several tens of microns. This corresponds to the results of systematic studies, where, by the way, microfibers, including the polyester ones, are considered the major pollutant of the aquatic environment. For example, in the Mediterranean Sea nearby Spain, the fraction of filaments is > 60% of MPs [1].

Concentration profiles for r = 5 and r = 7 also contain the objects that have morphology, size, positions and orientations close to the correspondent polyester MPs from the r = 1 profile. This suggests that all three profiles are associated with the same polyester MPs having some changes in surface composition/structure due to weathering or degradation. And the lack of correct library spectra does not allow obtaining high HQI values for r = 5 and r = 7 components.

In turn, the component with r = 4 demonstrates rather granule-like nylon MFs of small diameter, uniformly distributed across the field of view. Nevertheless, the higher spatial resolution required to estimate their geometry and size.

CONCLUSION

The scanning Raman microscopes from Lightnovo ApS demonstrate a great potential for analyzing microplastics in the environment, particularly in seawater. The results described above confirm capabilities to determine the type of material, as well as the shape and size of different microplastics. The microscopes are compact, inexpensive, do not require additional sample preparation, and provide fast data collection. Ability to synergize with the state-of-the-art data processing techniques allows the instruments to remain relevant, and at the same time easily adapt to a wide range of tasks and using scenarios: from routine "in the field" monitoring to the operation as a part of complex analytical systems.

LITERATURE

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Harness the power of Raman spectroscopy and make it widely accessible for the benefit of mankind.

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