

Segmentation integration in multivariate curve resolution applied to coherent anti-Stokes Raman scattering

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Coherent anti-Stokes Raman scattering (CARS) is a vibrational spectroscopy method based on a coherent $\chi^{(3)}$ nonlinear interaction between light and matter through four-wave mixing. CARS process has been extensively used as a microscopy technique in biology. However the shape of CARS spectra is generally complex because of the combination of both resonant and non-resonant contributions, which limits the development of CARS microspectroscopy applications. In this context, phase retrieval methods (PRM) like the maximum entropy method (MEM) [1] are commonly used to extract the vibrationally resonant signal, corresponding to $\text{Im}[\chi^{(3)}]$, and thus to provide Raman-like spectra, which are more suitable for interpretation.

Another method widely used in spectroscopy is multivariate curve resolution (MCR) [2], a matrix decomposition method aiming at finding compounds spectra and concentrations of analyzed samples. MCR can operate with constraints applied to computed matrices to ensure convergence and improve results. Several solvers exist to resolve the matrix decomposition problem. Here we show that non-negative least squares (NNLS) [3] is a new and convenient approach for processing congested CARS data. We implement NNLS and supplement it with a normalization constraint on the concentration so as to obtain the ratio between concentrations for each location. We then add a segmentation step and a regularization treatment based on partial differential equation to reduce the impact of the sample environment, take into account the spatial aspect of the acquisition method and reduce the noise that creates undesired variance between close pixels. This hyperspectral segmentation and regularization constraint (HSRC) is directly integrated into the MCR-NNLS algorithm and thus impacts the projection coefficients.

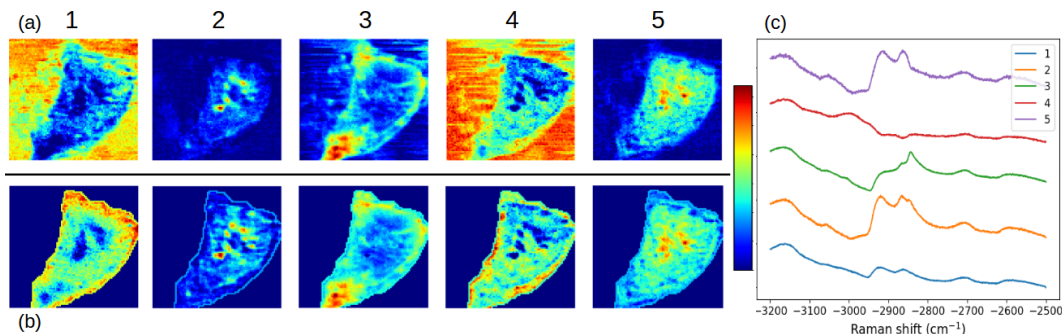


Fig. 1 HEK cell images for the first five dimensions obtained by MCR-NNLS (a) without HSRC and (b) with HSRC, showing the extraction of five characteristic compounds. (c) Corresponding CARS spectra extracted without HSRC.

Fig. 1 illustrates the analysis of a fixed human embryonic kidney (HEK) cell. Dimension 1 (without HSRC) highlights the presence of water since a higher concentration is visualized in the aqueous environment surrounding the cell (Fig. 1a), in agreement with the predominance of OH stretching in spectrum 1 (Fig. 1c). With the same approach, dimensions 2 and 3 are assigned to proteins (CH_3 stretching) and lipids (CH_2 stretching), respectively. In Fig. 1b, we can see that the HSRC allows to efficiently extract the cell from the environment and to better identify cellular compartments.

Recently, long short-term memory networks (LSTMs) have been used as PRM [4]. Convolutional neural networks (CNNs) have also proven their ability to solve classification problems, including Raman spectroscopy [5]. For example, CNNs allow to identify, for each pixel, the class to which the associated spectrum belongs. At the conference, we will introduce the use of CNN classification in the segmentation step to drive the MCR process.

References

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