Performance Comparison of Spectral Unmixing Methods for Stimulated Raman Scattering Microscopy

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Stimulated Raman scattering (SRS) microscopy is regarded as a powerful technique of label-free, real-time biomedical imaging. In particular, hyperspectral SRS microscopy allows us to detect molecular vibrational spectra as a source of image contrast. The resultant hyperspectral datacube should be processed with a spectral unmixing method, which extracts meaningful information including vibrational spectra and concentration profiles of different components. So far, various methods of spectral unmixing, such as multivariate curve resolution (MCR) [1], independent component analysis (ICA) [2], and vertex component analysis (VCA) [3] have been adopted to SRS imaging but their pros and cons are still not clarified. In this work, we compare these three methods in terms of the extracted data and investigate their tolerance to low signal-to-noise ratio (SNR).

We conducted SRS imaging of HeLa cells in the CH stretching region between 2800 – 3100 cm^{-1} followed by spectral unmixing with MCR, ICA, and VCA. The number of components is set to 3. Figures 1a and 1b show the concentration profiles of lipids and protein, respectively, extracted with VCA. Figures 1c and 1d show the extracted vibrational spectra of lipids and protein, respectively, extracted with MCR, ICA, and VCA. The spectra of lipids are almost identical for different algorithms, while those of protein exhibit certain differences. The differences of extracted spectra may result from the existence of water, which slightly distorted spectra unmixed with VCA and MCR, while influenced ICA less since it tries to retrieve independent components. By adding Gaussian noise into SRS dataset, extracted spectra were compared to the pure spectra without adding noise, and then the mean relative absolute error (MRAE) between the both was calculated (Figs. 1e and 1f). We found that VCA presented higher noise tolerance in the case of lipids while ICA and MCR performed better in the case of protein. This kind of difference may give us important insights into the mechanisms behind the algorithms.

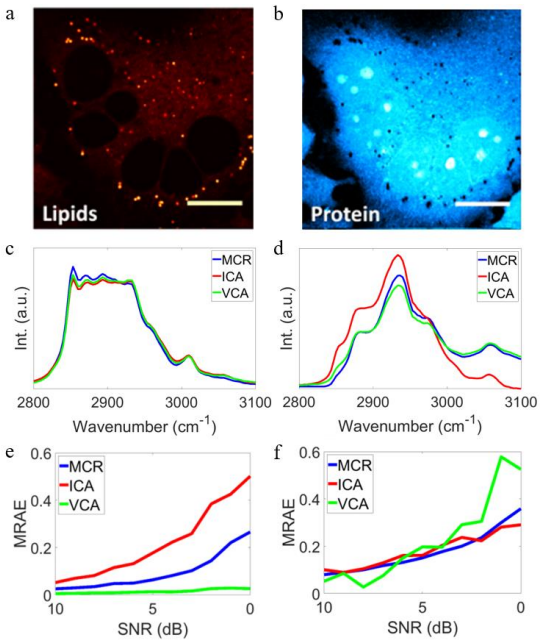

Fig. 1. Evaluation of the spectral unmixing methods. (a) Concentration profile of lipids extracted with VCA. (b) Concentration profile of protein extracted with VCA. (c) Spectra of lipids extracted by MCR, ICA and VCA. (d) Spectra of protein extracted by MCR, ICA and VCA. (e) MRAE of the three methods for lipids. (f) MRAE of the three methods for protein.

References [1] D. Zhang *et al.*, Anal. Chem. **85**, 98 (2013). [2] Y. Ozeki *et al.*, Nat. Photon. **6**, 845 (2012). [3] Nascimento *et al.*, IEEE Trans. Geosci. Remote Sens. **43**, 898 (2005)