Deconstruction of Obscure Features in SVD Spectral Components for Biological Raman Imaging Applications

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Raman spectroscopy is an optical spectroscopy technique with applications in biology and medicine.[1] Different functional biomolecules bear characteristic functional groups and hence their optical responses come at different frequencies in the Raman spectrum. This opens a possibility of simultaneous detection of multiple biomolecules in single cell microorganisms. However, highly overlapped signatures in the collected Raman data defy an easy analysis. Singular-value decomposition (SVD) and principal component analysis (PCA) are routinely used for analyzing Raman images. Often, as a general strategy, classification of specimens based on overall spectral variance explained by specific spectral vectors have been adopted. Consequently, such analysis methods unfortunately destroy the molecular information in the Raman data. This often leads to speculative interpretations based on the relative intensities of the bands in the SVD/PCA vectors. To circumvent this problem, we have developed a combined multivariate approach, *viz.*, deconstruction of SVD vectors applying MCR (DSAM).[3] By applying this methodology, we have extracted the contribution of five biomolecular constituents of the *Penicillium chrysogenum* filamentous cell[4] to the SVD vectors. Our study showed complex mixing of Raman spectra and the background, which makes relative intensities in the SVD spectral vectors difficult to interpret. We also show that the relative intensity ratio of biomolecule specific bands in SVD components, if observed clearly, are not reflective of their relative contributions. Similarly, image contrast in the SVD-decomposed Raman images from *Penicillium chrysogenum* was also misleading. However, by applying DSAM, we succeeded in accurately interpreting SVD spectra and images in molecular terms.

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