SAND2016-1747C

Multivariate Curve Resolution for Hyperspectral Images

Stephen M. Anthony

Bioenergy and Defense Technologies, Sandia National Laboratories, Albuquerque , NM 87185, USA

Introduction

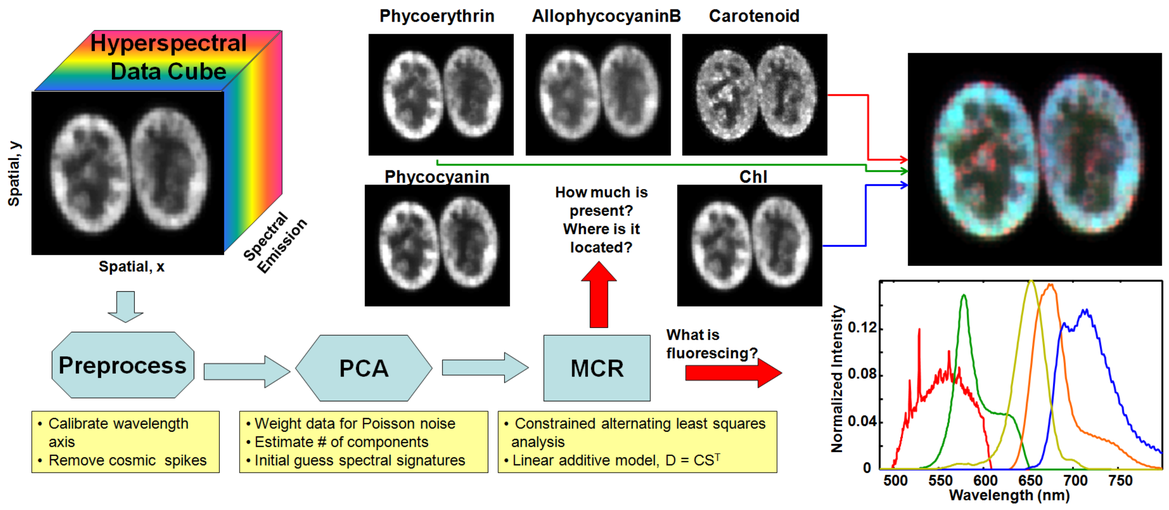
The application of Multivariate curve resolution (MCR) to hyperspectral images allows the determination of the number of different spectral components, their pure spectra, and their spatial distribution even for spectrally similar compounds. MCR is related to both principal component analysis (PCA) and independent component analysis (ICA), where all techniques resolve hyperspectral data into pure spectral components and concentrations while requiring little or no prior information. However. the three algorithms all find different solutions, each of which maximizes the explained variance of the data under the influence of a particular set of constraints. Whereas PCA and ICA which impose mathematically and statistically meaningful constraints, MCR applies physically and chemically meaningful constraints. Examples include requiring that concentrations cannot be negative and that fluorescence spectra cannot be negative.

Leveraging the physical and chemical constraints reduces the rotational ambiguity (non-uniqueness of the solution), facilitating the determination of the real spectra and concentrations. However, the alternating least squares optimization performed by MCR may sometimes become trapped in local rather than global minima, and a certain amount of rotational ambiguity generally remains. Additionally, MCR generally assumes that the noise is independent and ideally distributed (perfect Gaussian white noise),[1] whereas Poisson noise becomes highly significant for single-molecule biophysics experiments. I am currently extending Sandia's existing MCR algorithm to properly account for the noise, minimize the uncertainty, and quantify the remaining uncertainty.

Biophysical Applications of Hyperspectral Microscopy

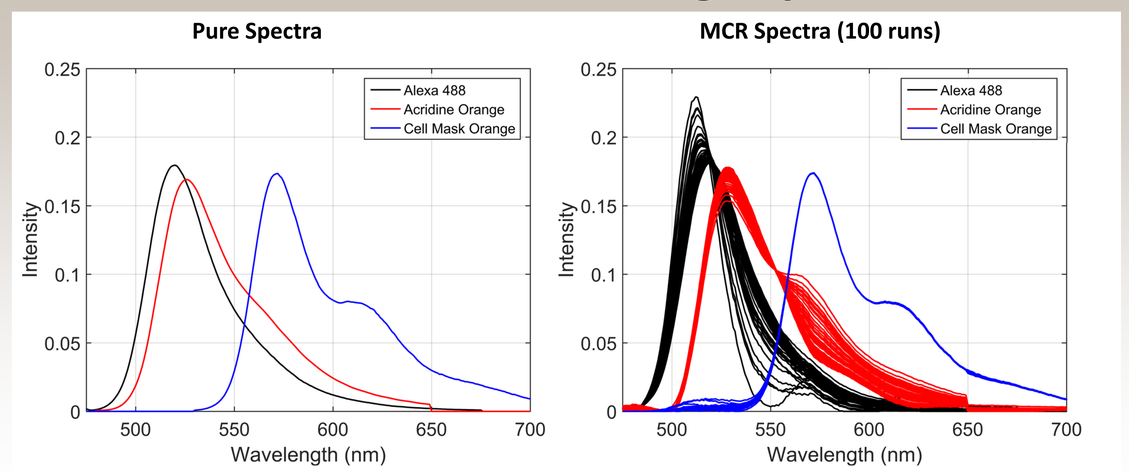
Hyperspectral microscopy greatly improves upon the spectral resolving power of traditional microscopy by measuring the entire emission spectrum for each voxel instead of at most a few distinct spectral bands for traditional, filter-based microscopes. The limited spectral resolution of traditional microscopes places severe constraints on the number of labels which can be used and the spectral similarity between labels, constraints which hyperspectral microscopy relaxes. Further, when only a few spectral bands are observed, auto-fluorescence can be nearly impossible to distinguish from the contributions of fluorescent probes measured at the single-molecule level. The combination of hyperspectral microscopy with MCR allows the contributions from the auto-fluorescence to be isolated. Currently, along with Jeri Timlin I am building a hyperspectral Stimulated Emission Depletion (hyperspectral STED) microscope.[2] Hyperspectral STED microscopy builds off the advances in spatial resolution made in recent years by various super-resolution microscopy techniques, providing analogous improvements in spectral resolution.

Example: Using MCR to Determine Chemical Species



Mathematical isolation of independently varying chemical species is accomplished using a fast multivariate curve resolution algorithm with robust constraints.[3] Example shown: hyperspectral confocal[4] (not STED) imaging of endogenous pigments in cyanobacterium *Cyanothece*

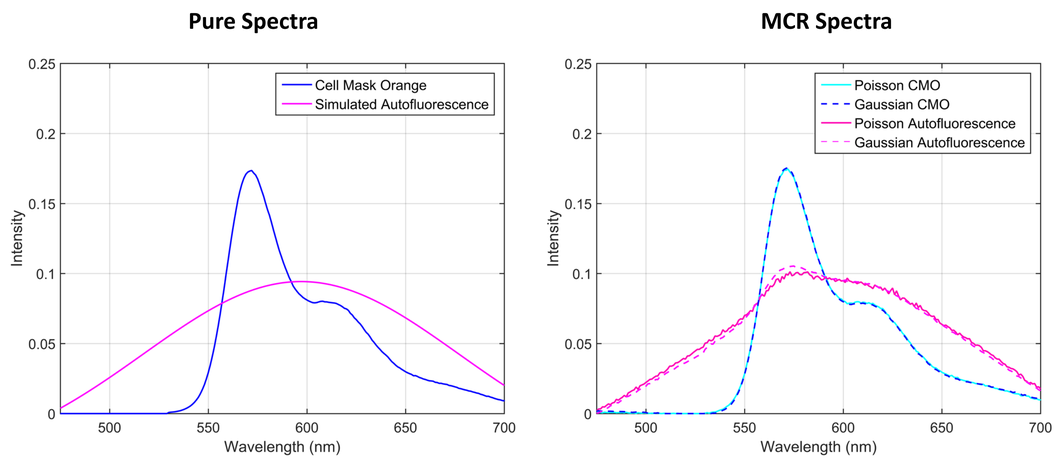
Rotational Ambiguity



MCR results for a simulated data set for a 100 x 100 pixel hyperspectral image averaging ~55000 counts for each spectrum. Random initializations were used for each of the 100 MCR runs, where the random initialization indicated the general location of the spectral peak but not the spectral shape.

The MCR algorithm is deterministic, producing a unique output for a given set of inputs. However, if the pure spectra are not known *a priori* and the spectra must be randomly initialized, the algorithm will produce a range of outputs which depend upon the random initialization. The observed range of outputs indicates remaining rotational ambiguity as well as possible trapping of the optimization in local rather than global minima. Alternate optimization schemes are being explored to avoid becoming trapped by local minima. Separately, the remaining rotational ambiguity is being evaluated and the uncertainty quantified.

Wavelength Dependent Noise



MCR results for a simulated data set for a 30 x 30 pixel hyperspectral image averaging ~55000 counts for each spectrum with either Poisson or Gaussian (5 counts) noise. The simulated autofluorescence signal was 2.5 times weaker than the CMO signal. The same random initialization was used for both, where the random initialization indicated the general location of the spectral peak but not the spectral shape.

Typically, MCR algorithms assume that the noise is independent and ideally distributed (i.e. Gaussian white noise). In the presence of noise violating this assumption, such as the simulated Poisson noise above, leads to over-fitting where part of the noise becomes embedded in the spectral model. In a separate issue, when MCR determines the spectra of weaker components, often some of any stronger spectra will be mixed with it.

Conclusions

Multivariate Curve Resolution is a valuable tool for the analysis of hyperspectral images, allowing the recovery of the number of different spectral components, their pure spectra, and their spatial distribution with minimal *a priori* knowledge. However, interpretation of the results can be complicated, where some areas of potential improvement are highlighted.

Acknowledgements

I would like to acknowledge Mark Van Bentham and Jeri Timlin for their helpful discussions .

References

1. Ruckebusch, C. and L. Blanchet (2013). "Multivariate curve resolution: A review of advanced and tailored applications and challenges." Analytica Chimica Acta **765: 28-36.**
2. Timlin, J. A. and J. S. Aaron (2014). Hyperspectral stimulated emission depletion microscopy and methods of use thereof, Patent US8686363 B1
3. Jones, H. D. T., et al. (2008). "Weighting hyperspectral image data for improved multivariate curve resolution results." Journal of Chemometrics **22(9-10): 482-490.**
4. Sinclair, M. B., et al. (2006). "Hyperspectral confocal microscope." Applied Optics **45(24): 6283-6291.**

U.S. DEPARTMENT OF ENERGY

National Nuclear Security Administration

Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.