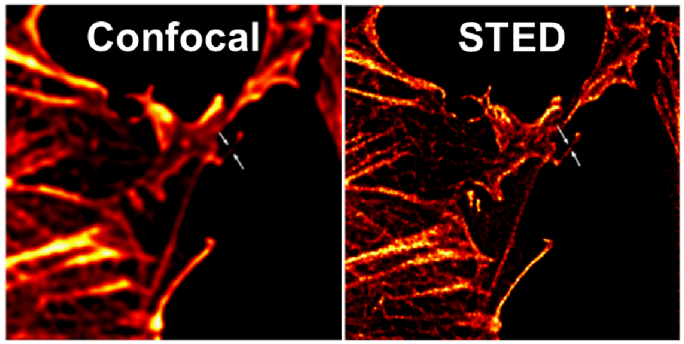
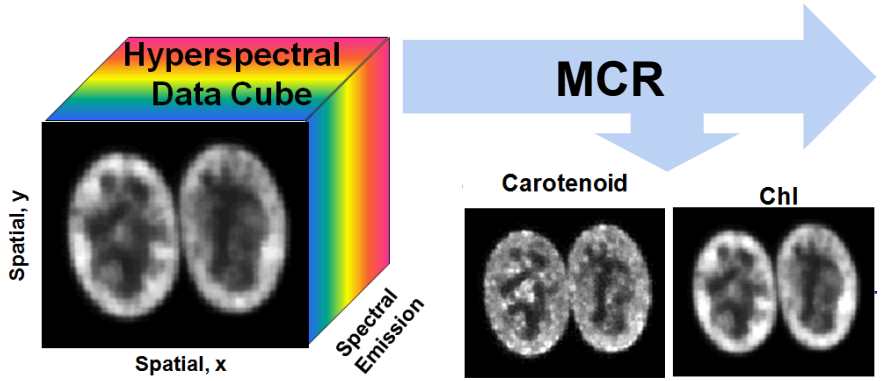
SAND2017-3611C



Adapted from Howard Vindin - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=40722030



Hyperspectral Super-Resolution Imaging and Data Analysis

Stephen M. Anthony

U.S. DEPARTMENT OF ENERGY
NNSA National Nuclear Security Administration

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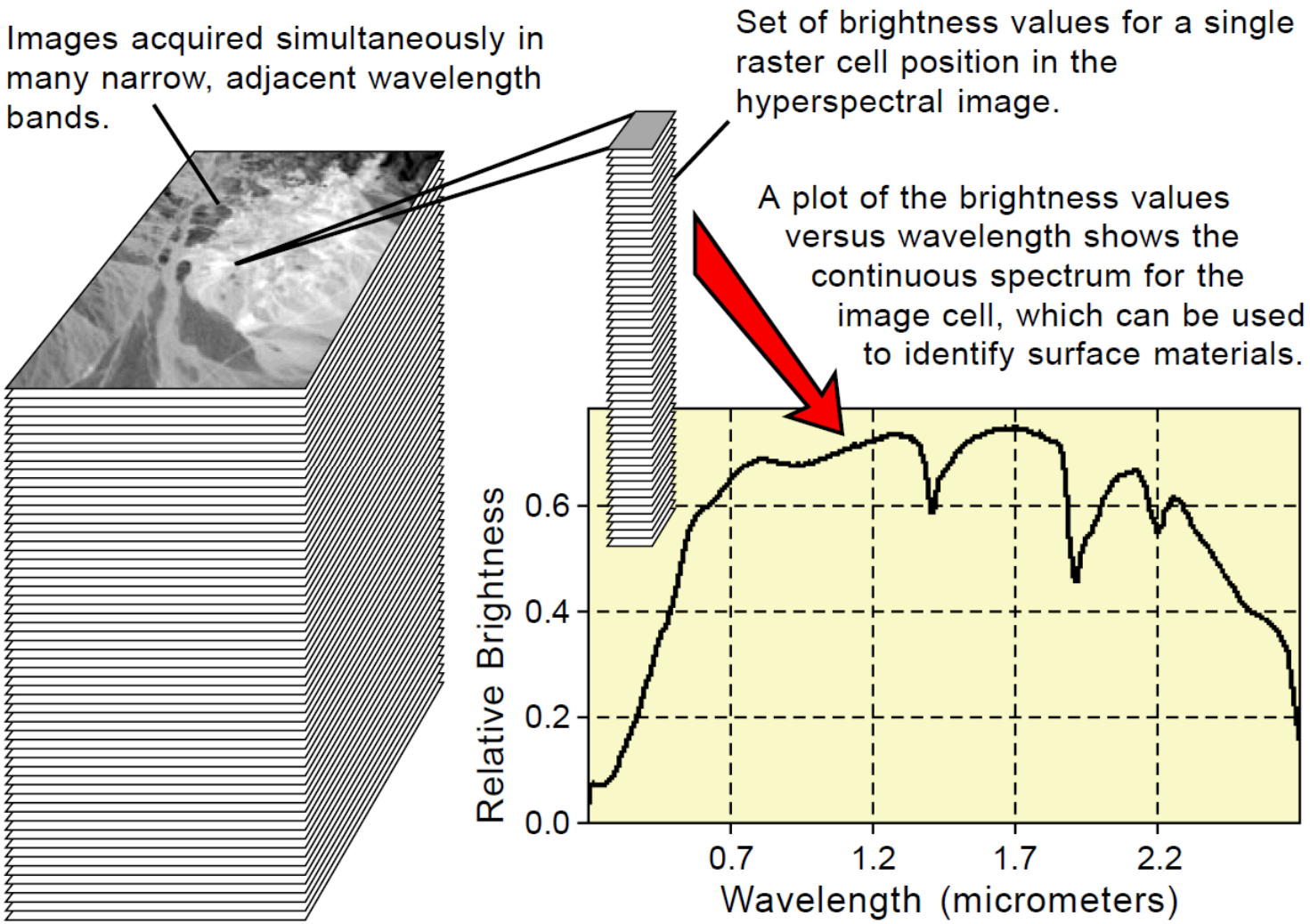
Outline

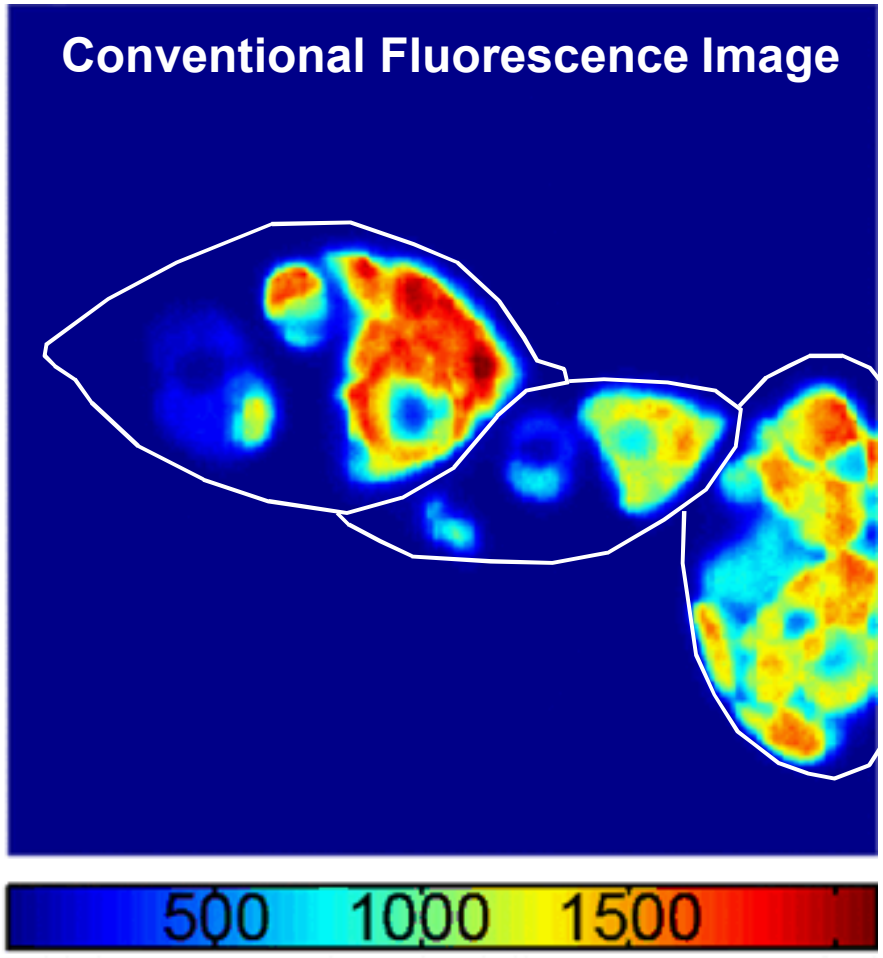
- **Why hyperspectral?** – What is hyperspectral imaging and what are its benefits?
- **Sandia's hyperspectral microscopes** – What are the systems I typically work with?
- **Analyzing hyperspectral data** – What can multivariate curve resolution (MCR) do for you?
- **Improving MCR** – Ongoing work to improve its capabilities.
- **Trilinear Data** – How to leverage additional information.

Introduction to Hyperspectral Imaging



Smith, R. B. (2012) Introduction to Hyperspectral Imaging. Microimages
http://www.microimages.com/documentation/Tutorials/hyprspec.pdf

Why Use Hyperspectral Imaging?

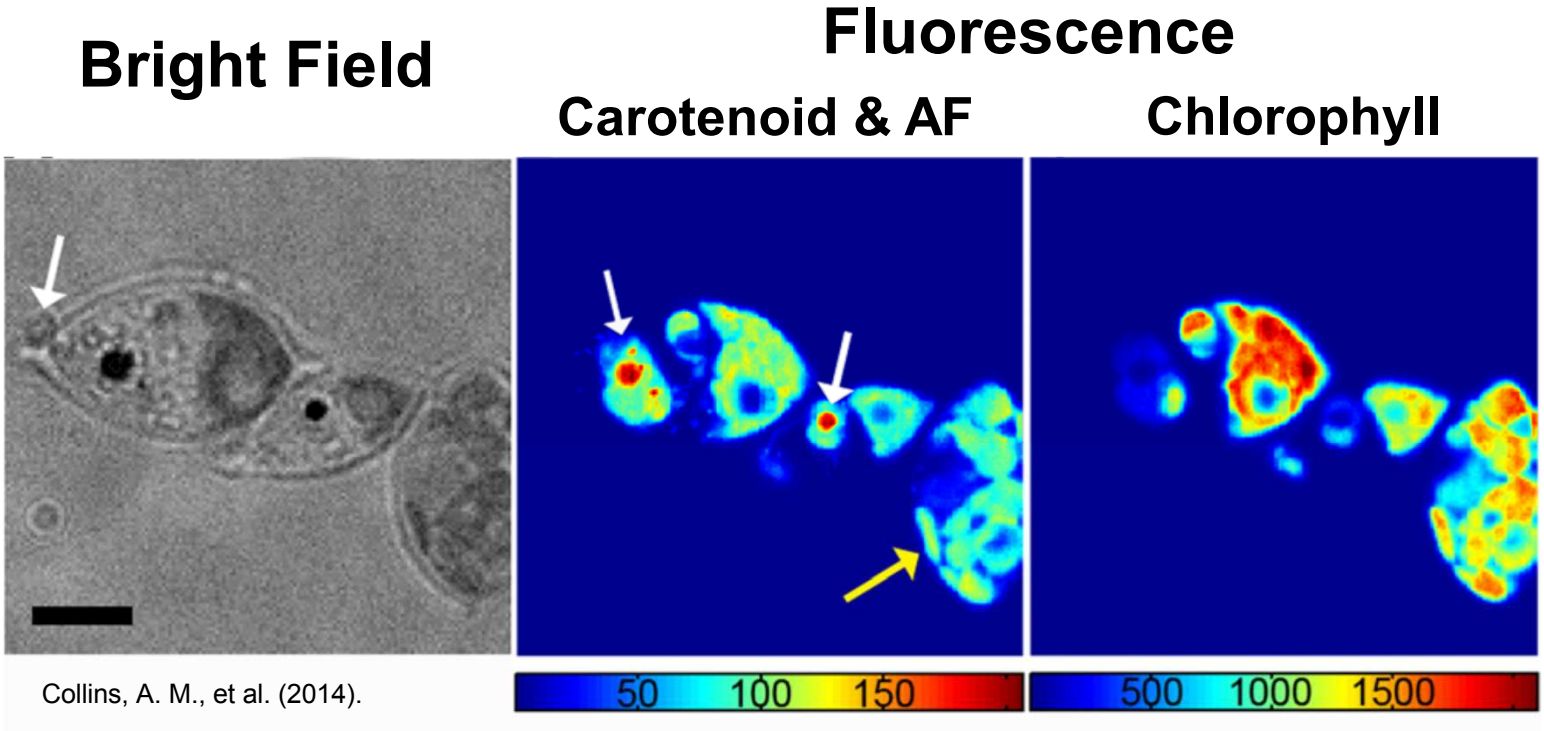


Adapted from Collins, A. M., et al. (2014). "Host Cell Pigmentation in Scenedesmus dimorphus as a Beacon for Nascent Parasite Infection." Biotechnology and Bioengineering **111(9): 1748-1757.**

Image of the endogenous fluorescence from *S. dimorphous* (algae) undergoing parasitic infection by *A. protococcarum*.

- Approximate cell borders are hand-drawn in white.
- Two of the cells contain parasitic vacuoles.
- **Can you spot the parasitic vacuoles?**

Why Use Hyperspectral Imaging?

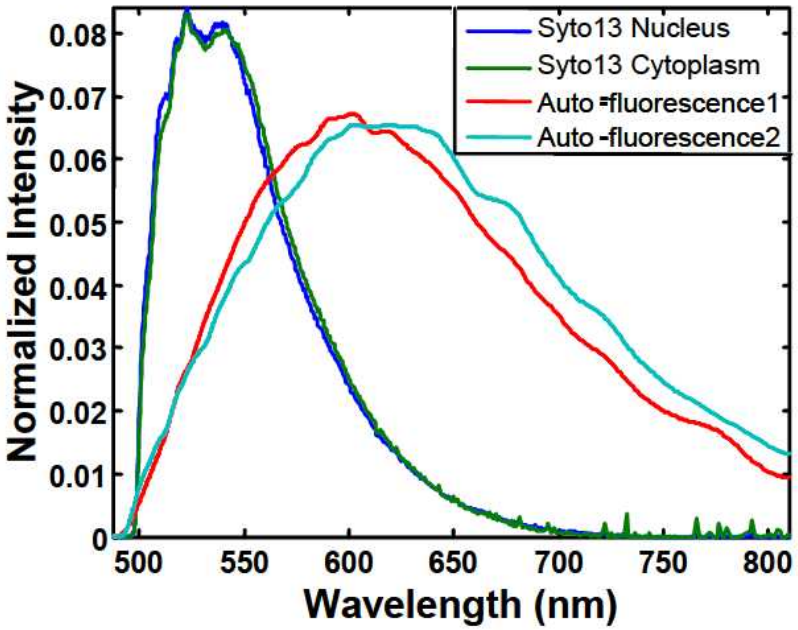


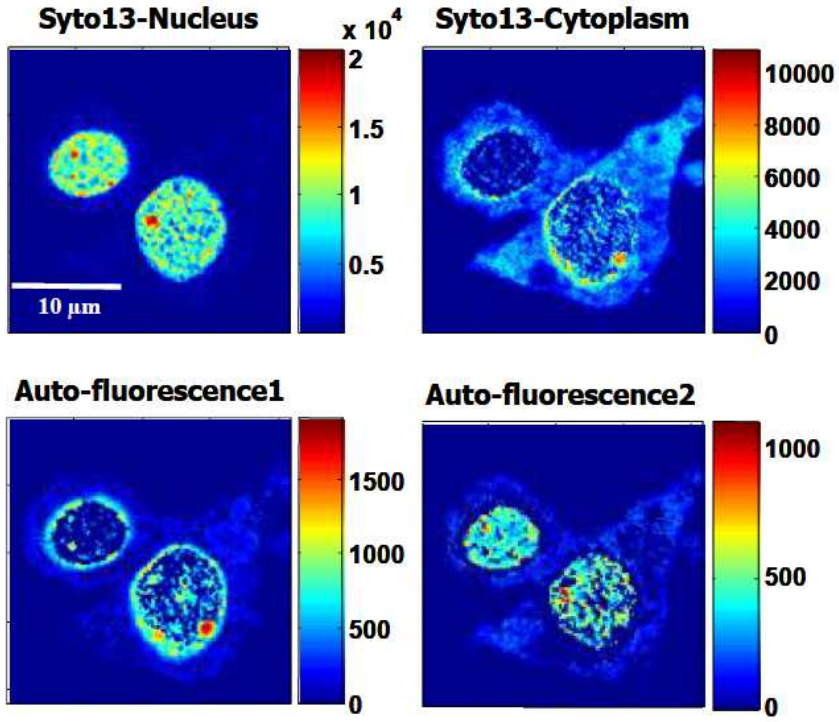
Collins, A. M., et al. (2014).

- Parasitic vacuoles (white arrows) are easily spotted using the combined carotenoid and autofluorescence signal.
- Spotting them is nearly impossible when examining all the fluorescence together as the chlorophyll signal dominates.
- **Hyperspectral imaging reveals otherwise hidden features.**

Why multispectral is not enough

Haaland, D. M., et al. (2007). "Hyperspectral confocal fluorescence imaging of cells" Next-Generation Spectroscopic Technologies **6765: 76509-76509.**





Left) Fluorescence spectra for two Syto 13 and two autofluorescence emission components. Right) Relative concentration of the components' spatial distributions in mouse macrophage cells (Raw 264.7).

- Multispectral imaging (e.g. filter-based microscopes) would only distinguish Syto 13 from autofluorescence – two components.
- **Hyperspectral imaging can distinguish nearly identical spectra.**

Hyperspectral Imaging Applications

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- Fluorescence or Raman microscopy – cell signaling
- Agriculture (satellite or drone-based) – monitoring crop locations (poppy fields), crop health, disease outbreak
- Chemical detection – Detect airborne chemical hazards at ppm levels up to 5 km away
- Mineralogy – disturbed ground indicative of improvised explosive devices

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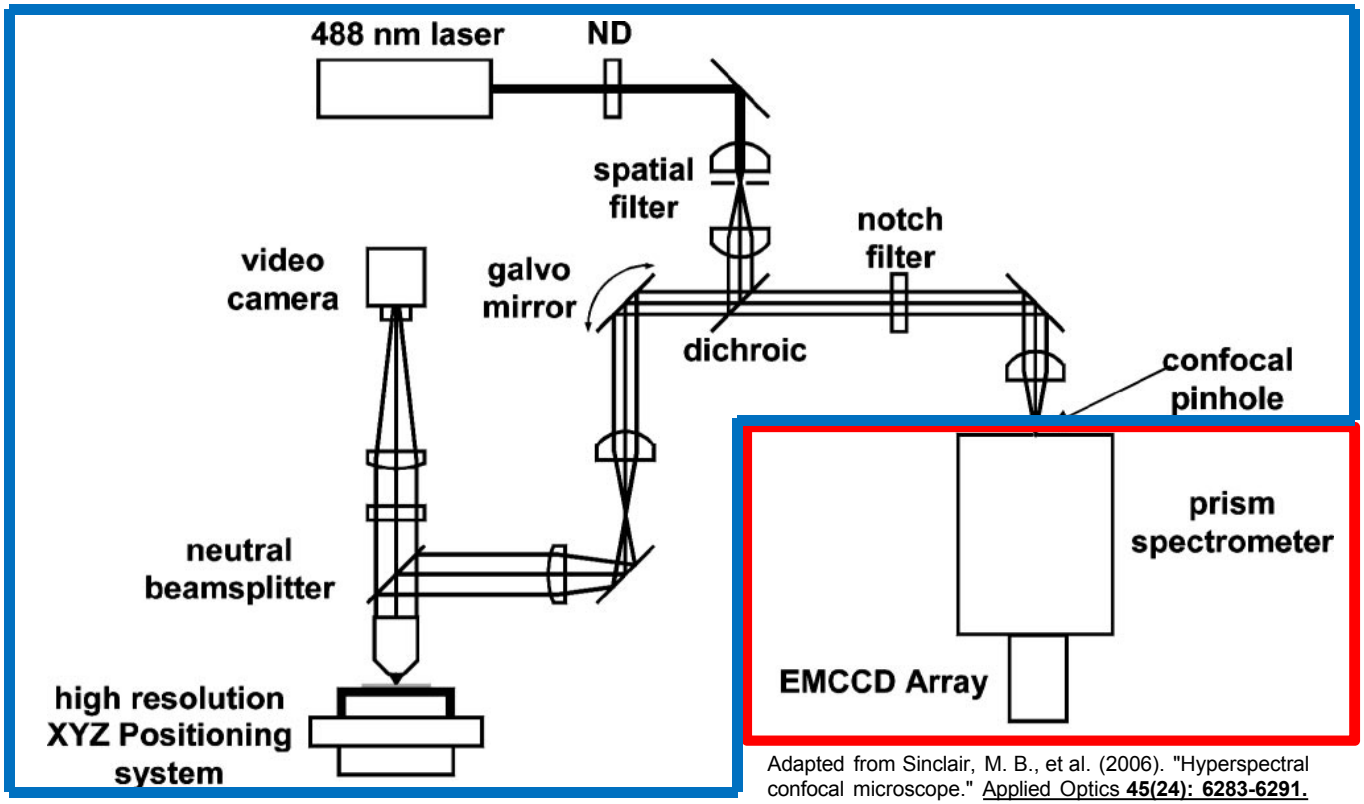
How to Build a Hyperspectral Microscope



Adapted from Sinclair, M. B., et al. (2006). "Hyperspectral confocal microscope." Applied Optics **45(24): 6283-6291.**

Schematic diagram of Sandia's hyperspectral confocal microscope

Hyperspectral Confocal Microscope =

Confocal Microscope + Spectrometer

Hyperspectral STED Microscope

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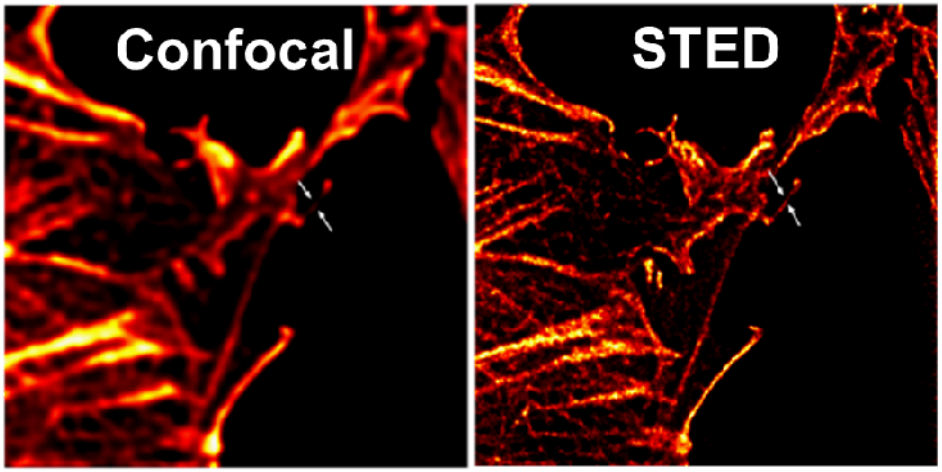
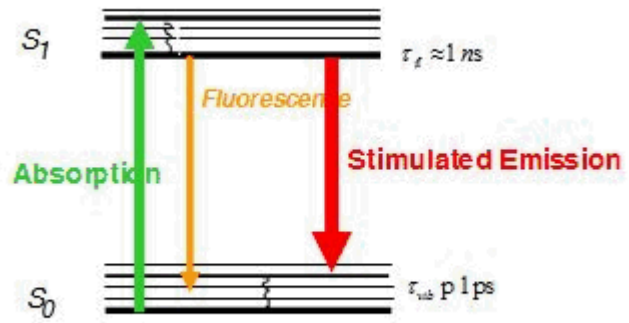
- Stimulated Emission Depletion (STED) is a super-resolution microscopy technique
  - Super-resolution microscopy won the 2014 Nobel prize in chemistry
- STED dramatically improves the spatial resolution (~30 nm typical, <3 nm reported)



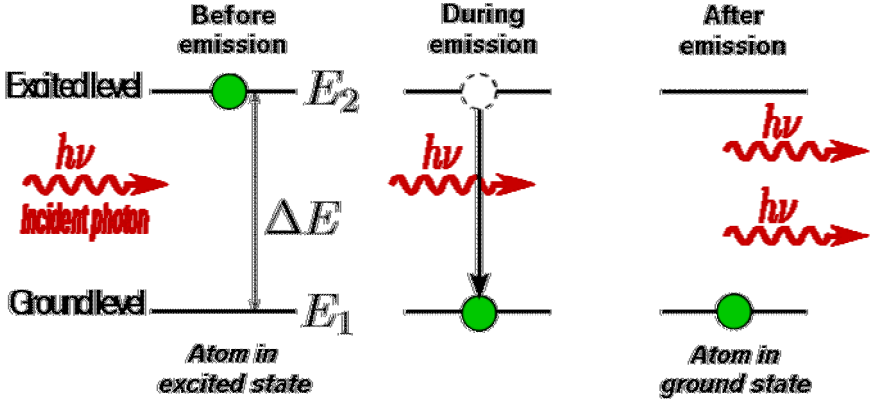
- Building world's first hyperspectral STED microscope
  - Patented by Jeri Timlin (8631) and Jesse Aaron

Stimulated Emission Details

Basic Principle of Stimulated Emission

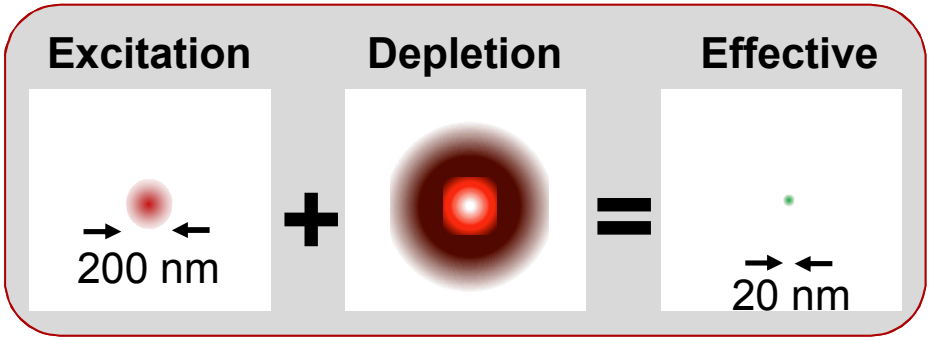


https://en.wikipedia.org/wiki/File:STED_Jablonski.jpg



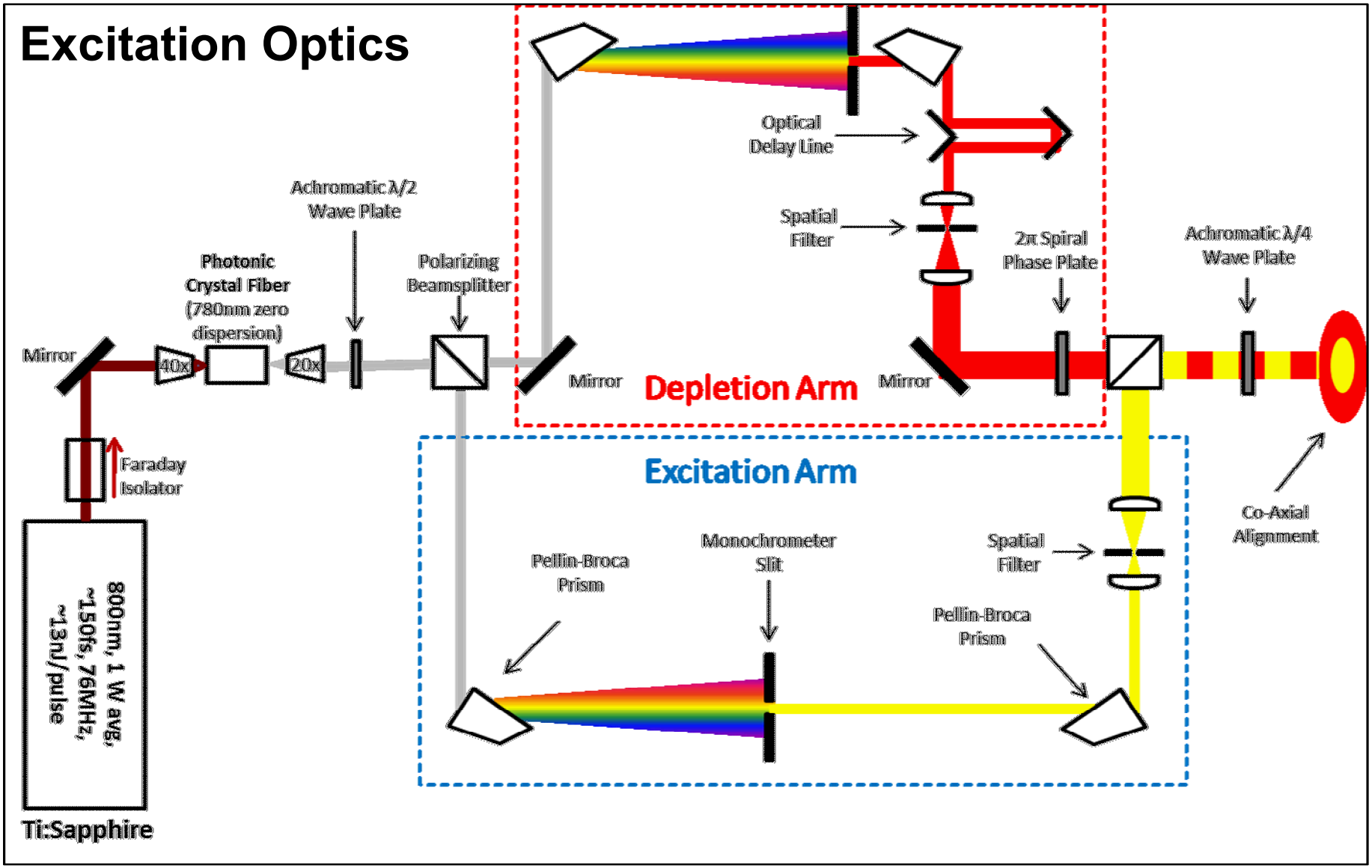
By V1adis1av - Own work, GFDL, https://commons.wikimedia.org/w/index.php?curid=3983414

Generating the STED Point Spread Function (PSF)



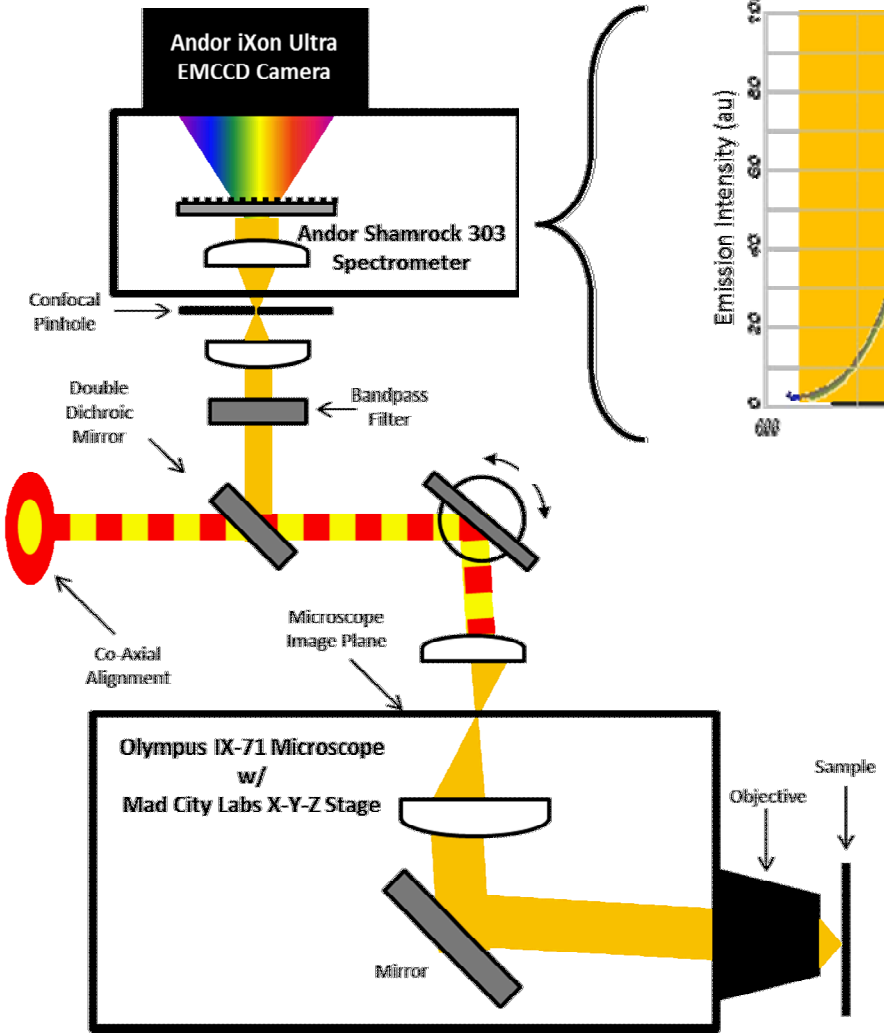
Neither beam PSF can exceed the diffraction limit, but the effective PSF can!

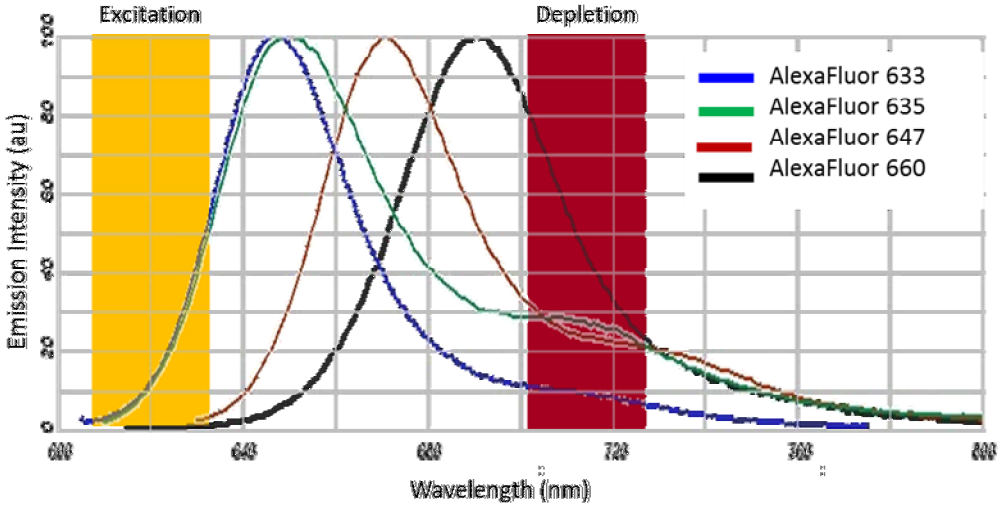
Building a Hyperspectral STED



Building a Hyperspectral STED

Detection Optics





Design Considerations

- Tunable wavelength for both excitation and depletion beams
- Can be optimized for any STED fluorophore with exchange of a single optic (the dichroic)

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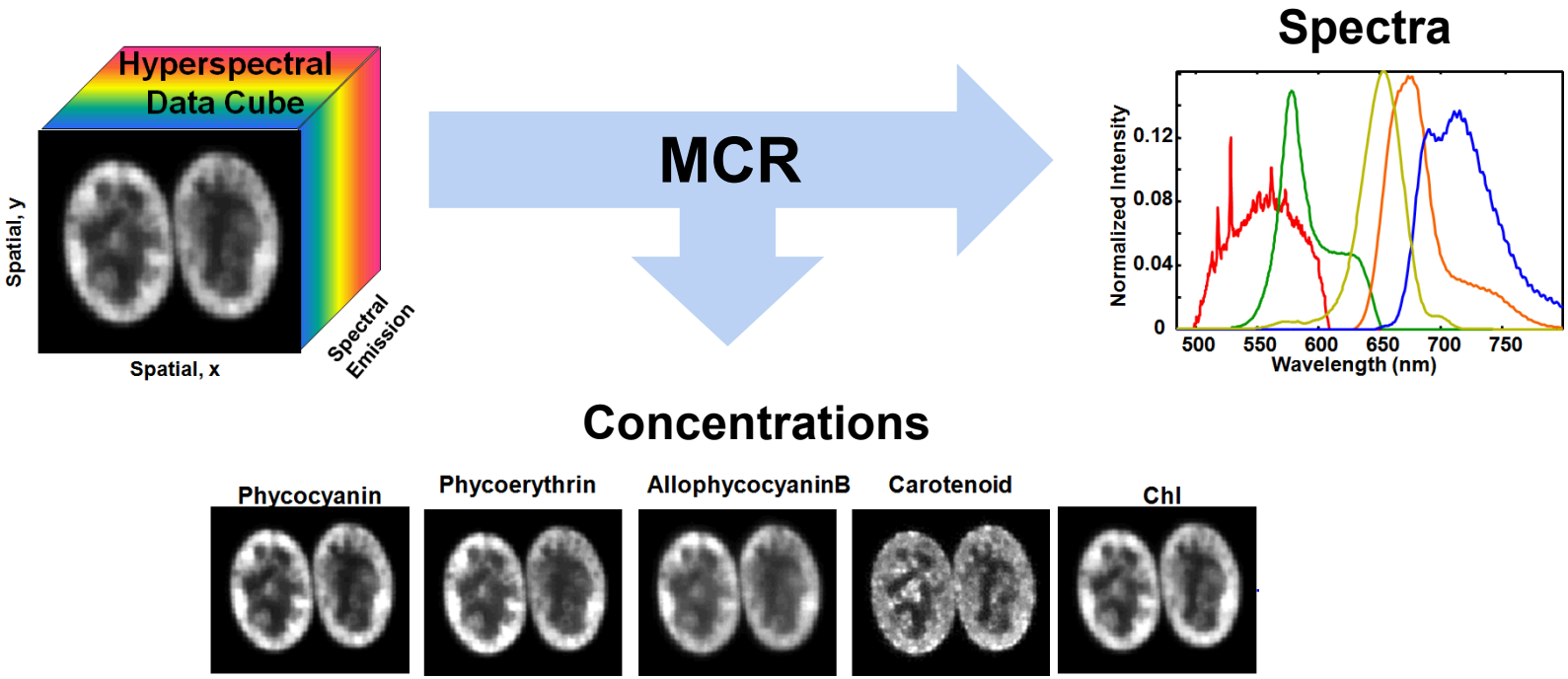
Multivariate Curve Resolution (MCR)

Chemometric factor analysis (such as MCR) extracts:

1) the spectra of the pure compounds &

2) their relative concentrations at each position.



Common Spectral Image Analysis Methods

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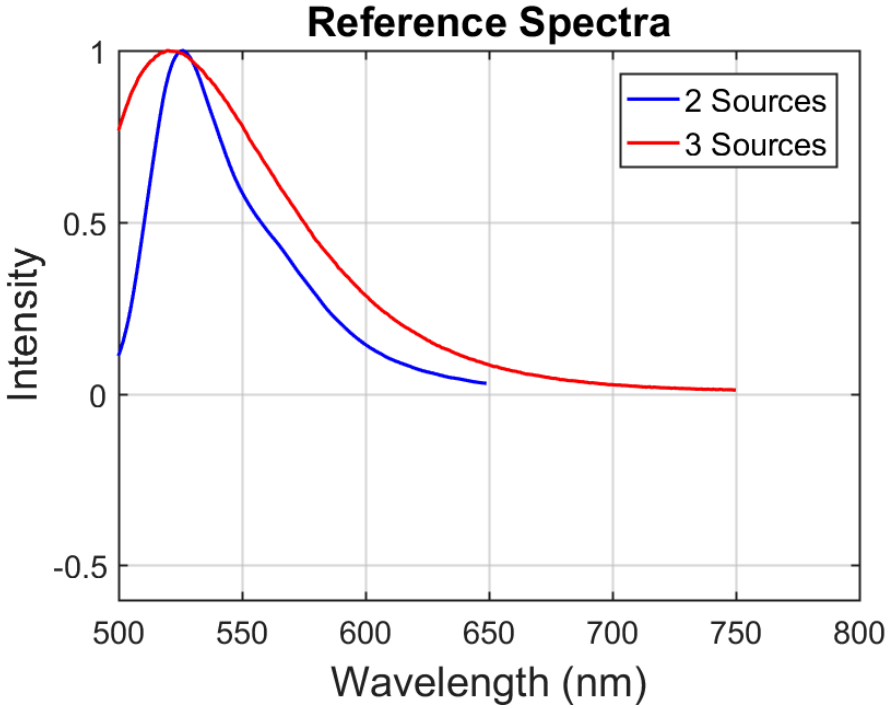
Required knowledge of system behavior

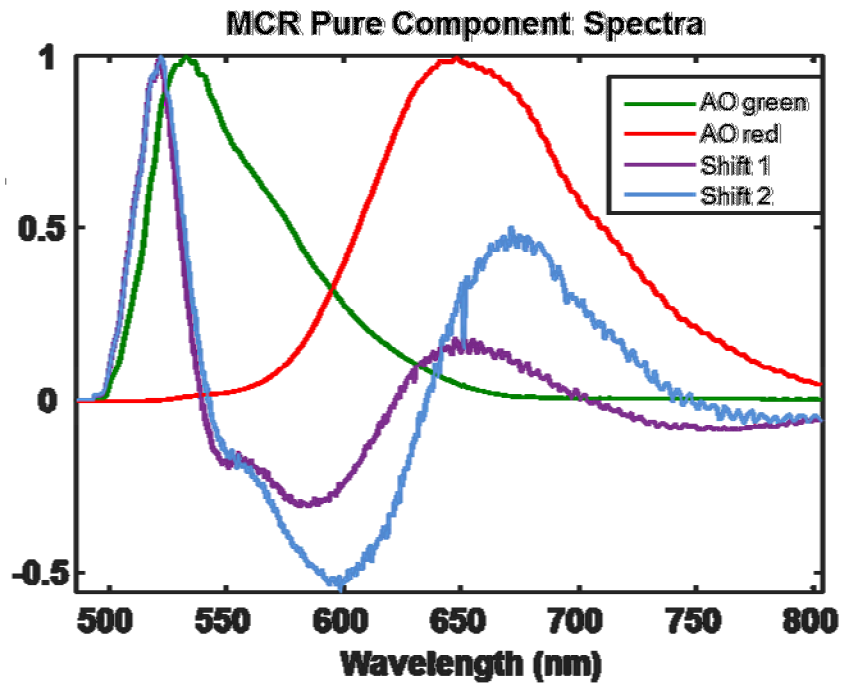
Mathematical Complexity, overall accuracy

- Univariate methods
  - → Band integration, peak height, peak positions
  - → **Isolated bands, no spectral interference**
- Multivariate methods
  - Unmixing methods
    - → Least squares prediction based (e.g. classical least squares)
    - → ***A priori* knowledge**
      - → Spectral shapes or pure image pixels
  - Factor Analysis methods
    - → Principle components analysis (PCA) , Factor analysis, SIMPLISMA, self modeling curve resolution, multivariate curve resolution **(MCR)**
    - → Data defines
    - → **No *a priori* knowledge** of spectral shapes/pure pixels
      - → Need number of components
      - → Constraints to narrow solution space

Why Aren't the Spectra Known?

"Acridine Orange is a cell-permeant nucleic acid binding dye that emits **green fluorescence** when bound to dsDNA and **red fluorescence** when bound to ssDNA or RNA." - ThermoFisher Scientific





Reference spectra are not always available, and when available do not always capture the complete spectral properties.

Broadly Applicable

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Works for any data satisfying the linear additive model:

$$D = CS^T + E$$

- **Fluorescence spectroscopy**
- **Raman spectroscopy**
- **Mass spectroscopy**
- Infrared satellite imagery

MCR Assumptions

1. **Assumes a linear additive model:**

$$\mathbf{D = CS^T + E}$$

D = Data matrix — nPoints X nWavelengths

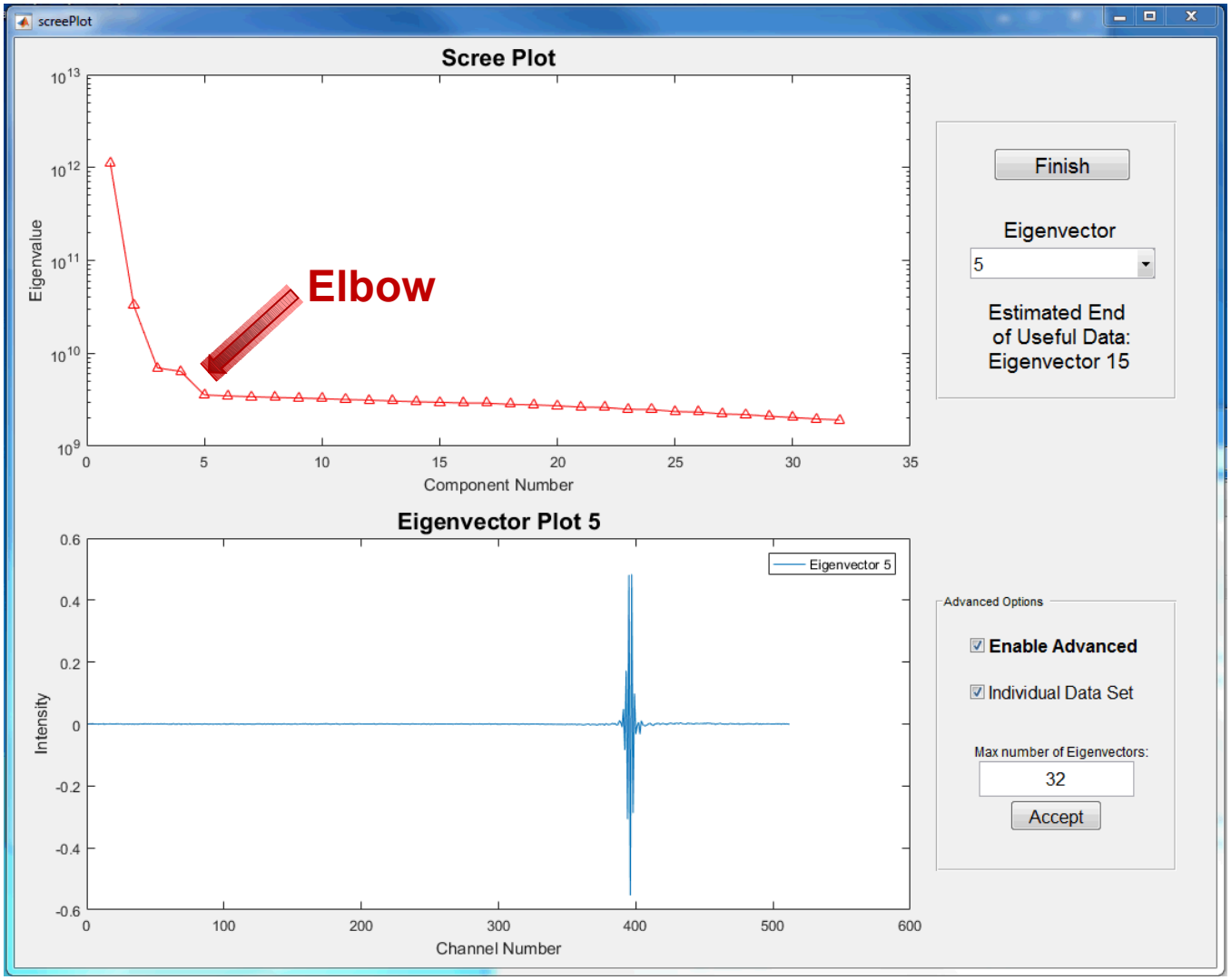
C = Concentrations matrix — nPoints X nComponents

S^T = (Spectra matrix)^{Transpose} — nComponents X nWavelenths

E = Noise (error) matrix — nPoints X nWavelengths

2. The # of spectral components is known or can be estimated

Determining the # of Spectra



Scree plots allow rough estimation of the number of spectra.

Elbow (transition to flat) in scree and eigenvectors that look like noise indicate no more components.

Basic Operation

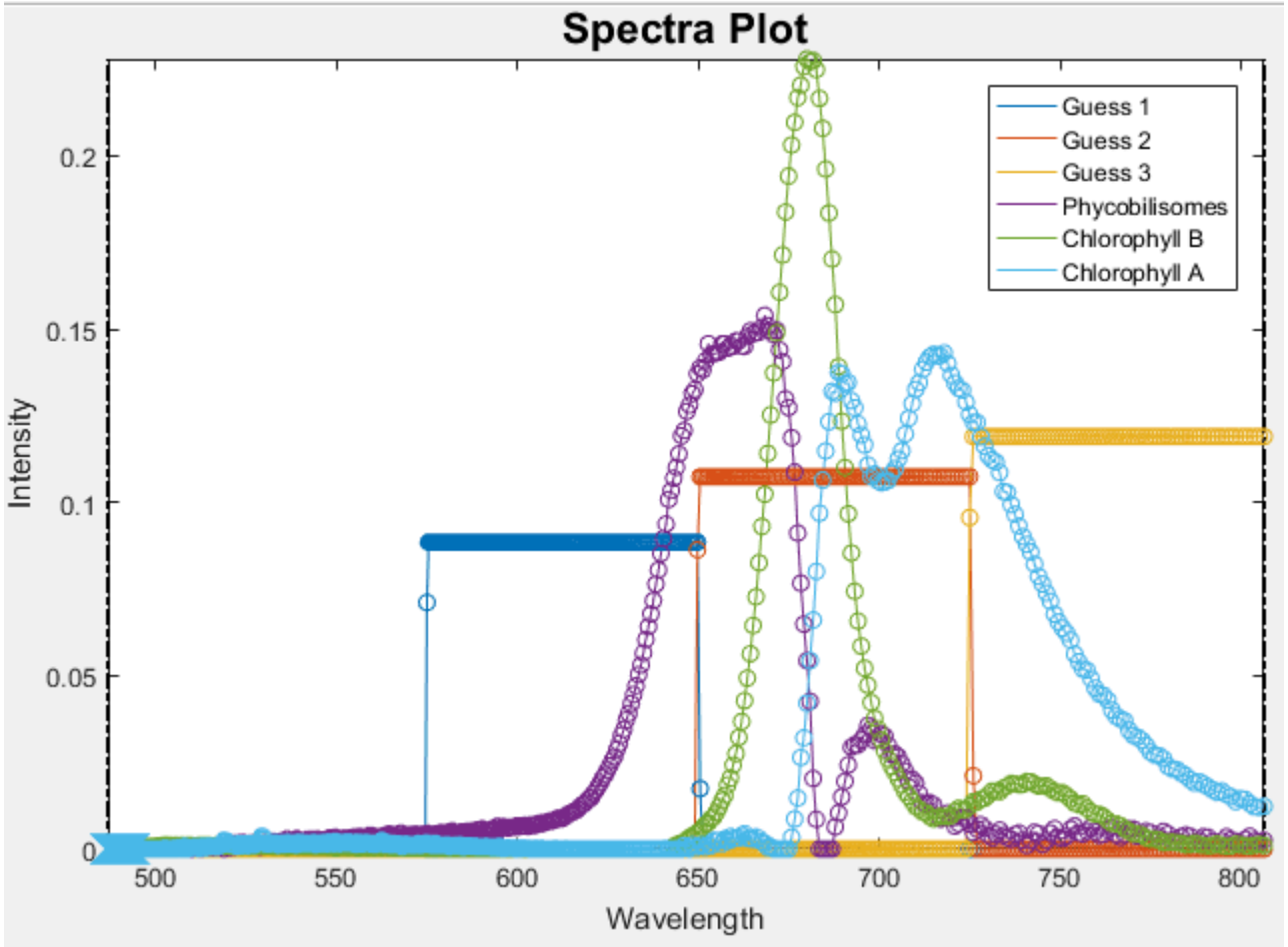
$$D = CS^T + E$$

- D is known
  - If C were known, could solve for S
  - If S were known, could solve for C
- Constrained Alternating Least Squares
  1. Provide an initial guess for S (or C)
  2. Solve for C based upon current S guess, enforcing constraints
  3. Solve for S based upon current C guess, enforcing constraints
  4. Repeat steps 2 & 3
  5. **Iterations converge on solution**

Advantages of MCR

- Extracts underlying relationships from complex data sets
- No *a priori* knowledge needed
- Signals below the noise level can be detected!
- Physically meaningful constraints
  - Negative concentrations not allowed
  - Negative intensities not allowed
- Efficient algorithms developed at Sandia
  - Keenan, M. R. and P. G. Kotula (2003). Apparatus and system for multivariate spectral analysis, Google Patents.

No *A Priori* Spectra Required



Hyperspectral Confocal Fluorescence Microscopy of R61 *Acaryochloris*

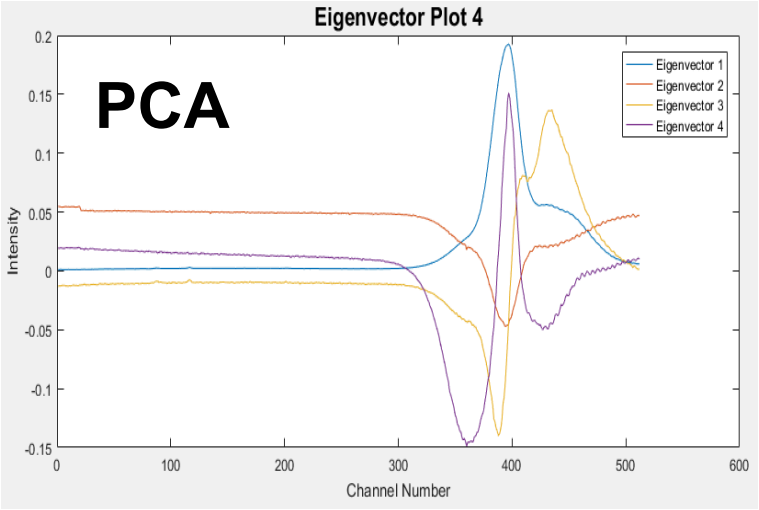
Even when initialized with naïve guesses,

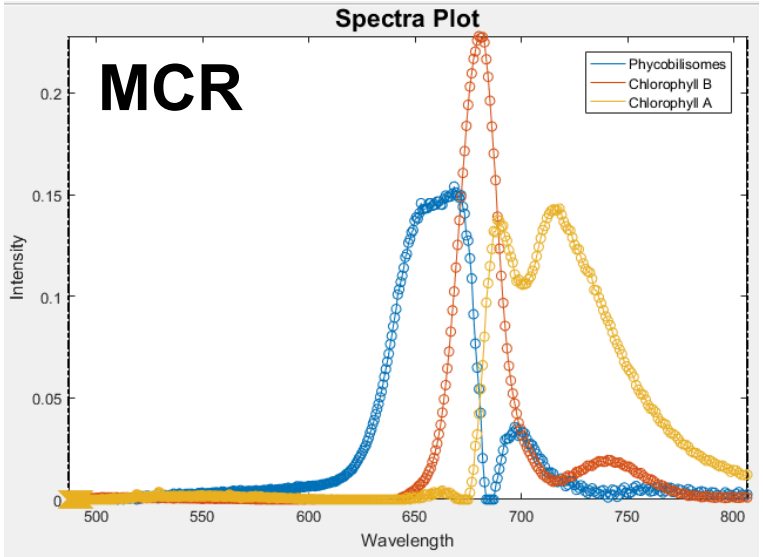
MCR determines the spectrum of chlorophyll B and localizes where it is present.

Why MCR vs. PCA?

- Three related factor analysis methods
  - Multivariate Curve Resolution (MCR)
  - Principal Component Analysis (PCA)
  - Independent Component Analysis (ICA)
- All resolve the data into pure spectral components and concentrations without a priori information
- **Different Constraints**
  - MCR – Physical and Chemical Constraints (e.g. no negative concentrations, no negative intensities)
  - PCA – Linearly uncorrelated
  - ICA – Statistically Independent

Why MCR vs. PCA?





- PCA
  - Recovers 4 spectral components when only 3 are present
  - Eigenvectors do not look like normal spectra – major negative portions
- MCR
  - Recovers the correct number of components
  - Components generally correspond to actual spectra

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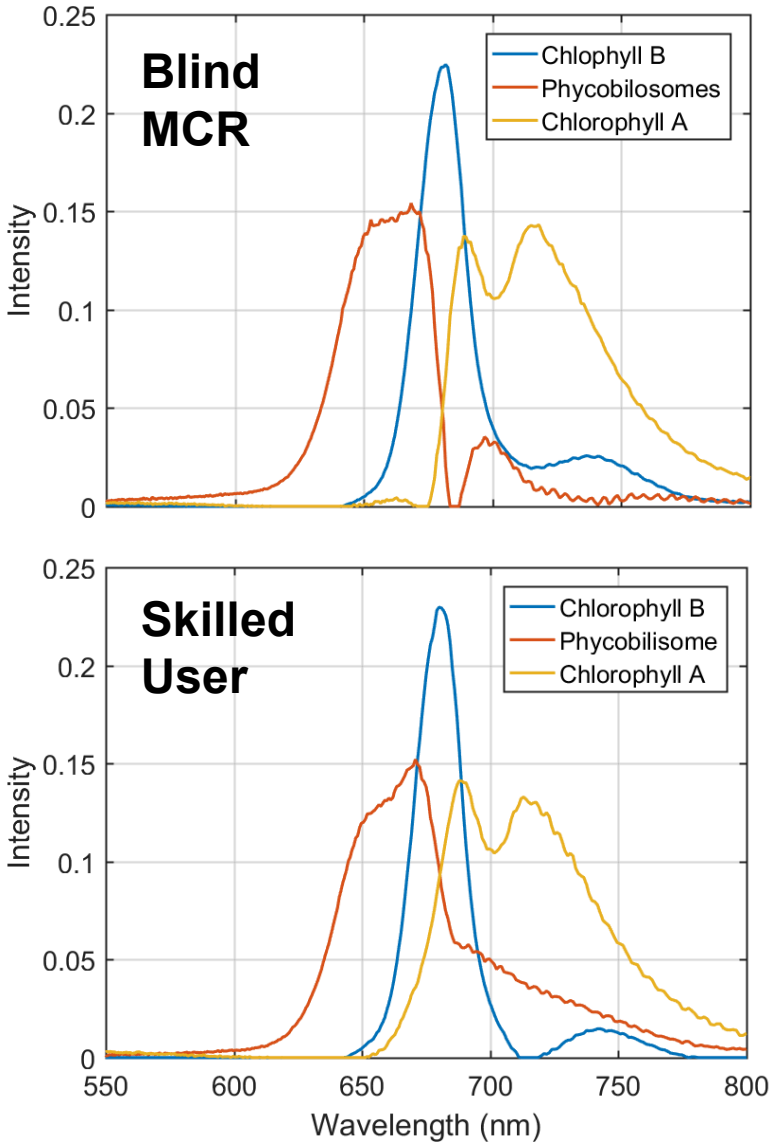
The Art of MCR



While MCR can provide excellent results, it is currently more of an art than a science.

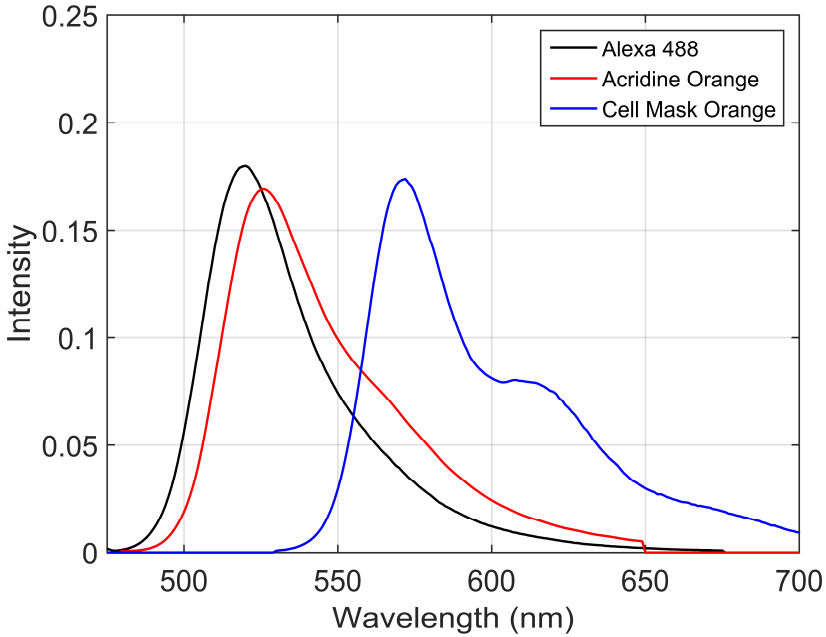
A skilled user will obtain better spectra from MCR, particularly for weaker spectral components.

Relative amounts:

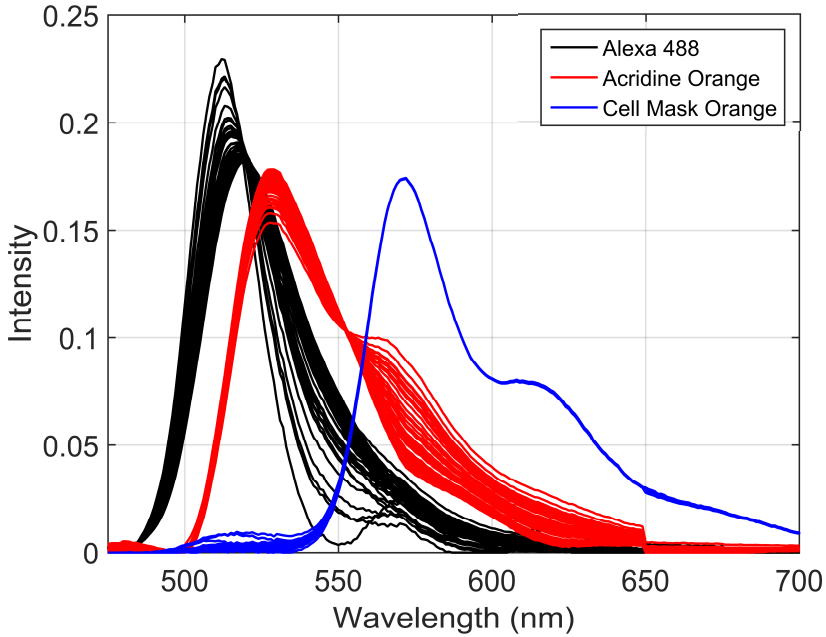
Chlorophyll B:	3.7
Chlorophyll A:	2.2
Phycobilosomes:	1.0

Multitude of Results

Pure Spectra



MCR Spectra (100 different runs)



MCR results for 100 runs on a simulated data set for a 100 x 100 pixel hyperspectral image averaging ~55000 counts for each spectrum initialized with random spectra.

Why multiple results? Two possibilities:

- Failure to converge – trapped in a local minimum
- Rotational ambiguity – results mathematically equally good

Rotational Ambiguity

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Also known as the rotation problem:

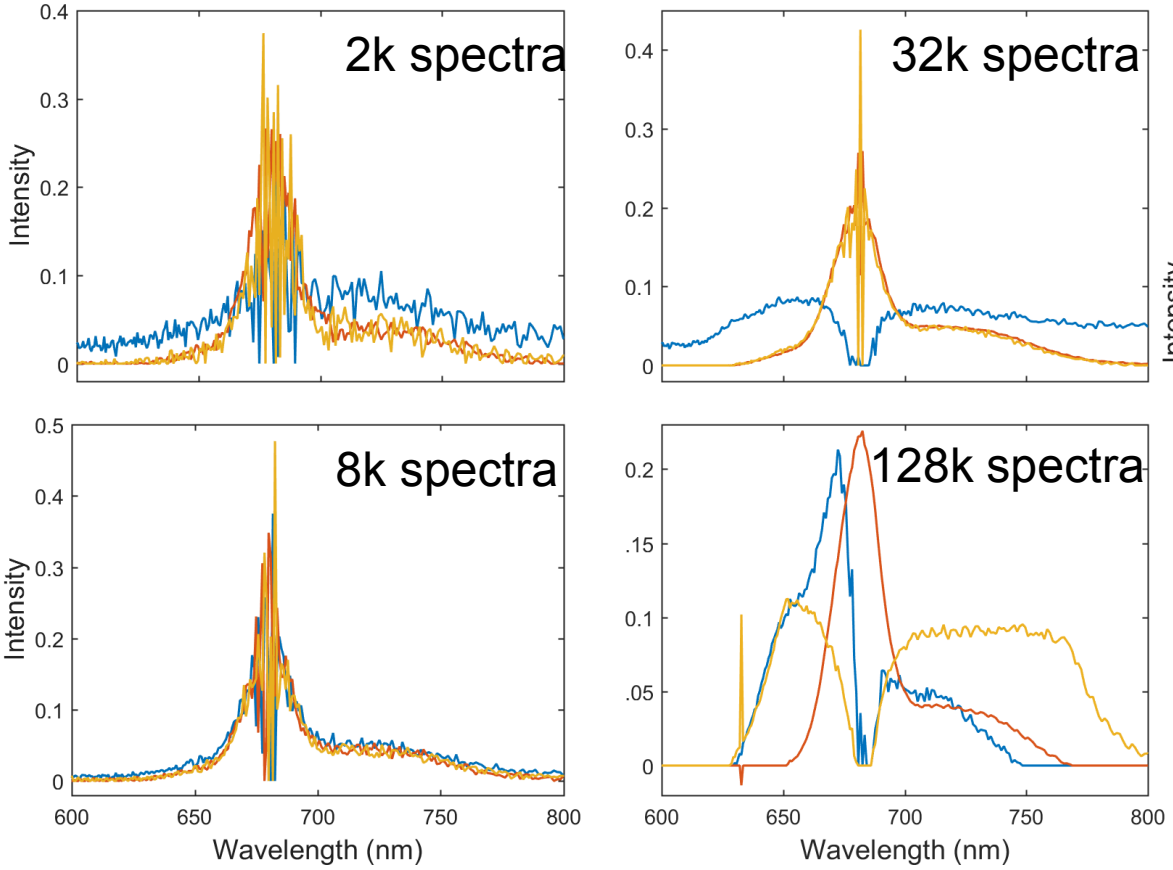
- Construct an invertible transformation matrix M_i to operate on the matrices C and S.

$$D = CS^T = (CM_i^{-1})\,(M_iS^T)$$

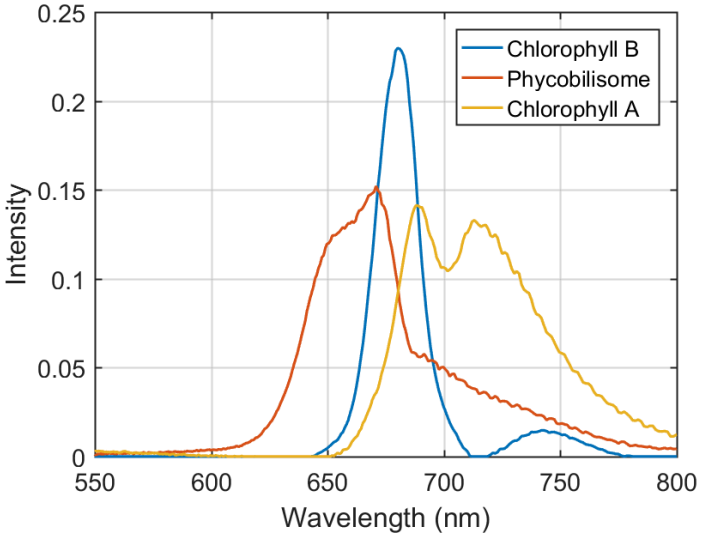
- The resulting matrices (CM_i^{-1} and M_iS^T) are an alternate solution with identical residuals

Constraints may reduce or eliminate rotational ambiguity.

Data Size



Desired Results
653k spectra

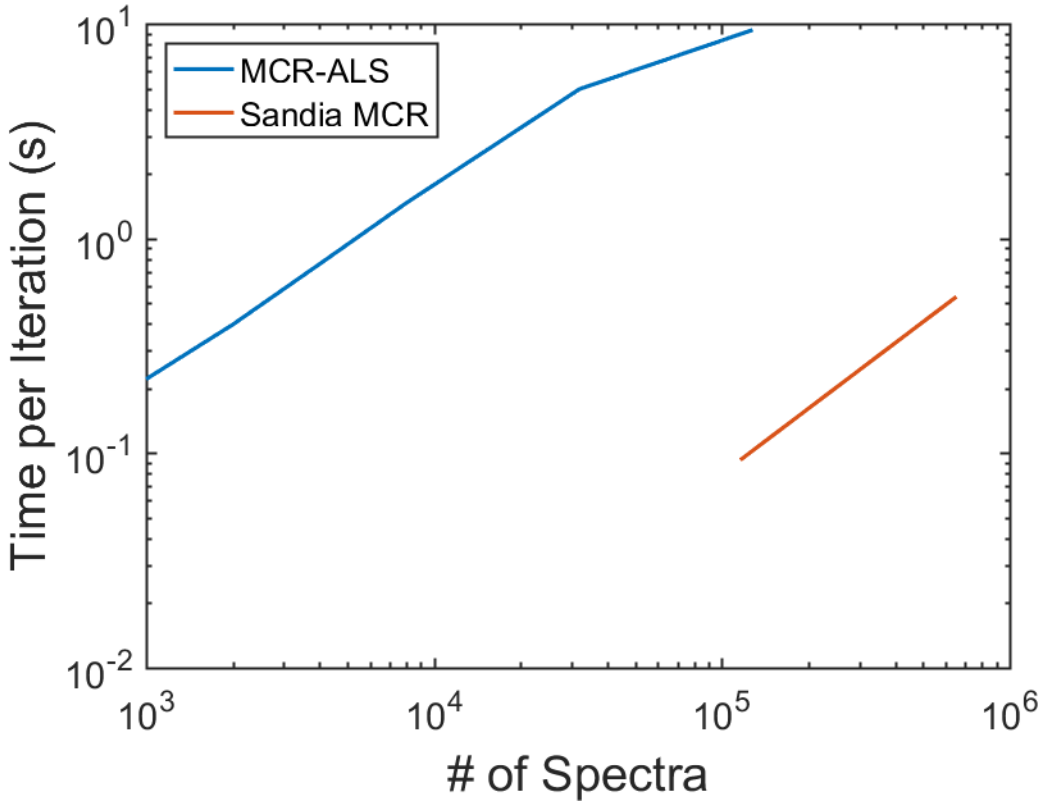


Relative amounts:

Chlorophyll B:	3.7
Chlorophyll A:	2.2
Phycobilosomes:	1.0

Working with more data provides better results

Computational Requirements



Sandia's MCR algorithm runs ~50 times faster than competing code[1] released in 2015.

Competing code[1] is widely used – cited 46 times in 2 years.

1) Jaumot, J. et al., "MCR-ALS GUI 2.0: new features and applications." *Chemometrics and Intelligent Laboratory Systems* 140 (2015): 1-12.

Computational time is on a high-end PC

Ongoing Work – Improved Weighting

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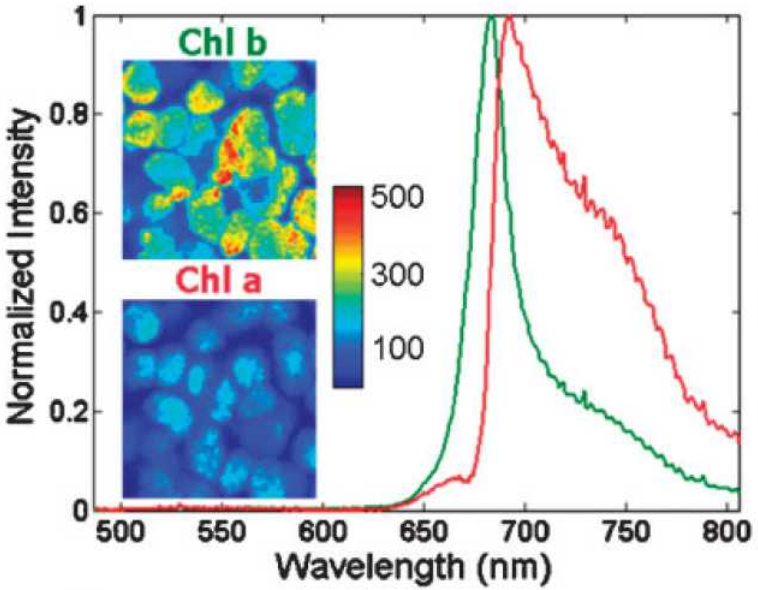
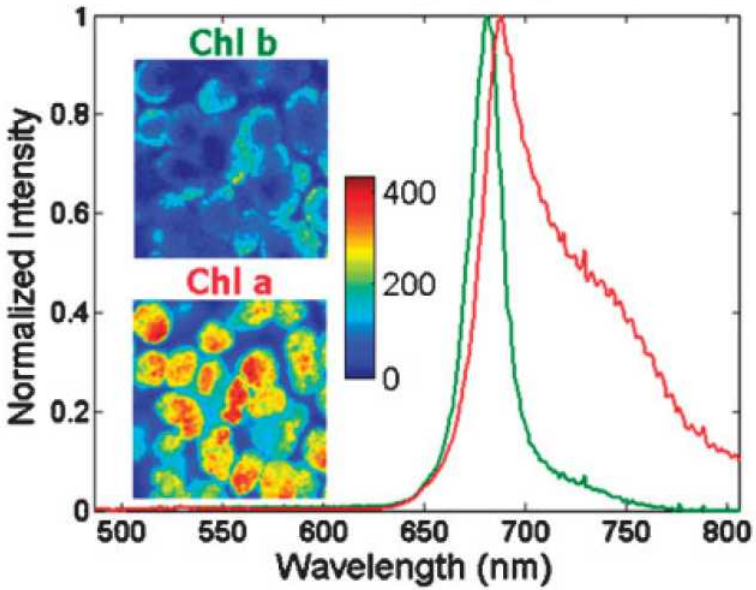
Without Weighting



Weighted for Poisson & Gaussian Noise



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.**

Proper weighting makes a major difference!

Working on improving the weighting to correctly account for all sources of noise, including the pre-processing steps.

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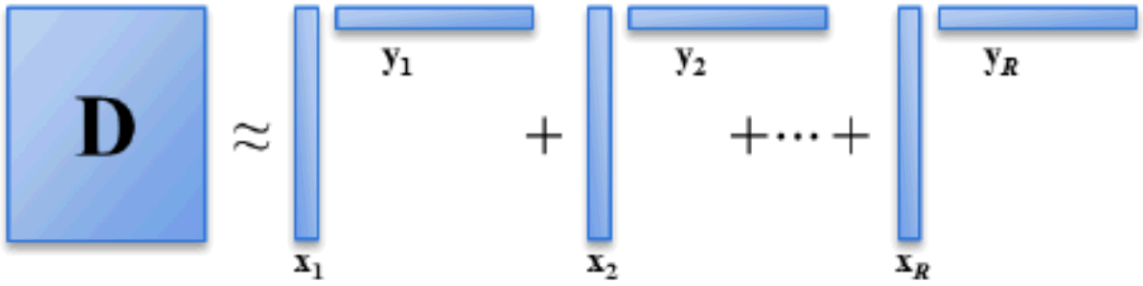
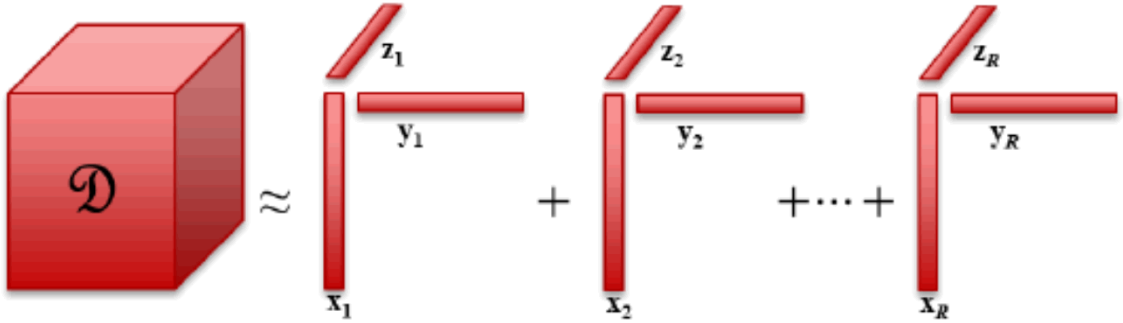
Bilinear vs. Trilinear Data

$$\mathbf{D} \approx \sum_{i=1}^{R} \mathbf{x}_i \mathbf{y}_i^{\mathrm{T}}$$



$$\mathcal{D} \approx \sum_{i=1}^{R} \mathbf{x}_i \circ \mathbf{y}_i \circ \mathbf{z}_i$$

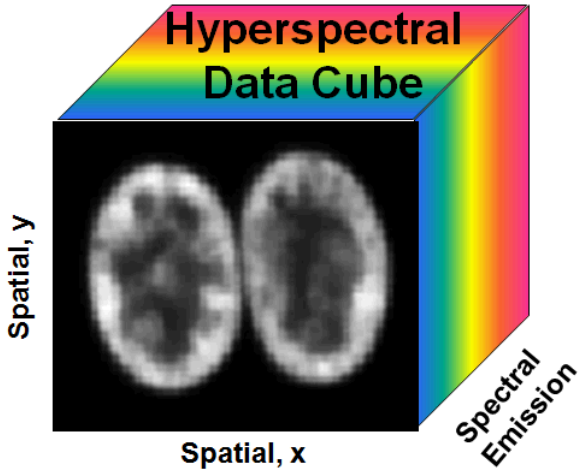


Bilinear Data – Each component in the data matrix can be expressed as the product of two vectors.

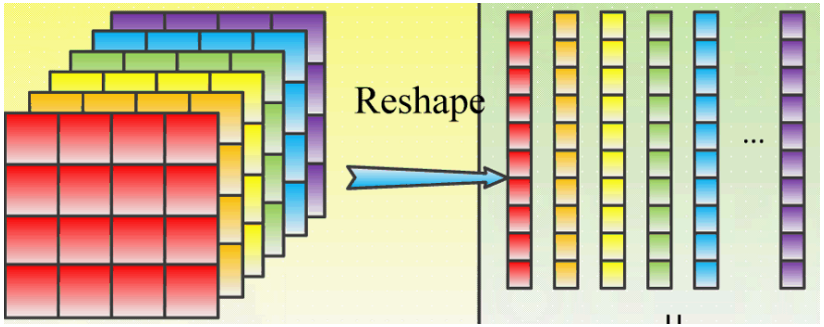
Trilinear Data – Each component in the data tensor can be expressed as the product of three vectors.

Adapted from SAND2014-1825

Hyperspectral Data Cube = Bilinear

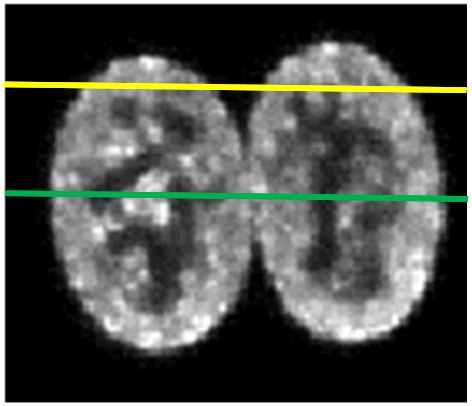


While hyperspectral data cubes are 3-dimensional, typically the data is only bilinear, not trilinear. Prior to processing with MCR, the data must be reshaped.



Adapted from Wu, Zhaojun, et al. *Journal of Electronic Imaging* 25.1 (2016): 013037-013037.

Carotenoid



In order to be trilinear, for an individual component, the cross sections at different y positions (yellow and green lines) would need to be identical.

Examples of Trilinear Data

- Hyperspectral fluorescence lifetime
  - Spatial position
  - Wavelength
  - Lifetime
- Gas Chromatography
  - Elution Time
  - Mass spectrum
  - Multiple possibilities (sample number, run temperature)

Analyzing Trilinear Data

- MCR can be applied to trilinear data, but better methods exist
  - Trilinear data can always be reshaped to generate a bilinear data set.
  - Doing so forfeits one of the great advantages of trilinear data. Trilinear analysis eliminates the rotational ambiguity problem common to bilinear data.
- Trilinear methods exist
  - Parafac, Tucker3 algorithms are examples
  - For trilinear data analysis, talk to Mark van Benthem (1814)

Summary

- Hyperspectral microscopy and MCR are valuable tools
- Hyperspectral STED will provide super-resolution capability
- Improvements to MCR will:
  - Make MCR less of an art
  - Improve estimation of the weaker components

Acknowledgements

Collaborators or prior research:

Microscopy: Jeri A. Timlin, Michael B. Sinclair, Jesse S. Aaron

MCR: Mark Van Benthem, Jeri A. Timlin, Michael R. Keenan, Paul G. Kotula

Funding

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. Support is acknowledged from Sandia National Laboratories' Laboratory Directed Research and Development project "Unmasking Hidden Compounds within Hyperspectral Images"