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Potential for Detection of Plant Pathogens using Raman Spectroscopy

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April 18, 2013

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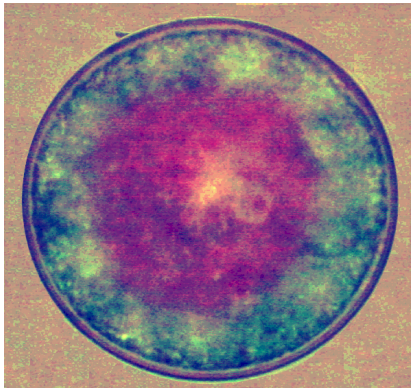
Motivation

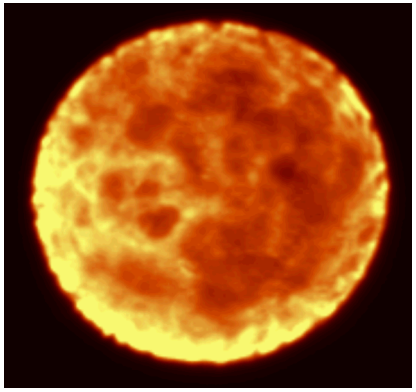
Spatially and Temporally Resolved Biochemical Information at the Cellular Level

Light Micrograph



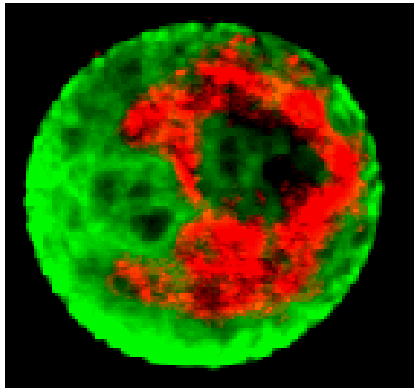
Confocal Raman Microscopy

Integrated Raman Image



Multivariate Curve Resolution

Chemical Image



Light Microscopy

Each pixel in the image is a combination of 3 (RGB) colors (morphology, refractive properties)

Spectral/Hyperspectral Imaging

Each pixel in the image is a spectrum relating to chemical and/or molecular structure within

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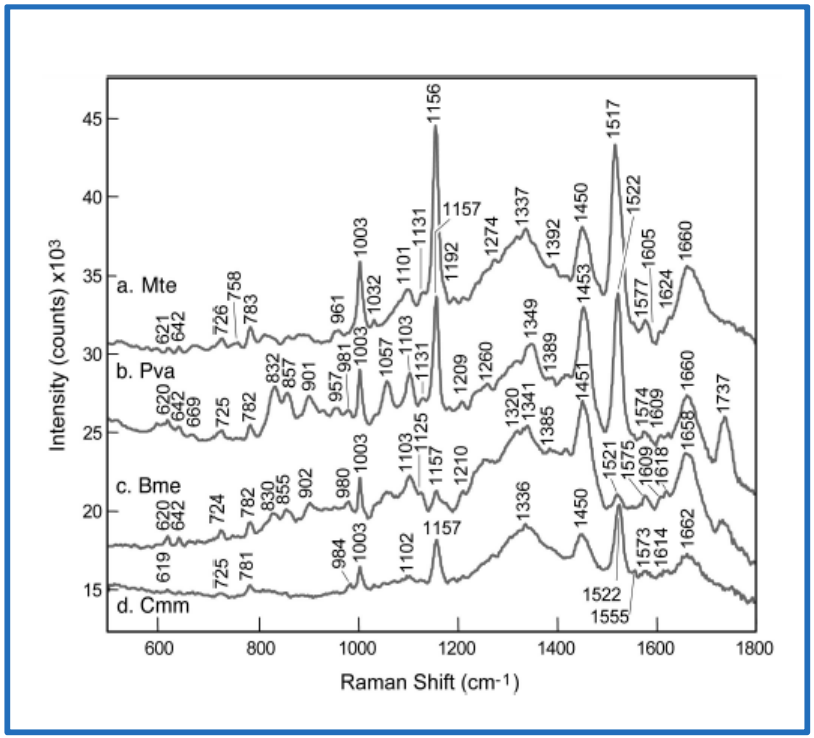
Raman Spectroscopy and Spectral Imaging in Plant Science

- Non-destructive, live-cell friendly
- Raman in plants
  - *Baranska M, Roman M, Dobroowolski JC, Schulz H, Baranski R, Recent Advances in Raman Analysis of Plants: Alkaloids, Carotenoids, and Polyacetylenes. Curr. Anal. Chem. 2013; 9: 108-127.*
- Bacterial detection has been demonstrated
- Fungal, viral pathogens less explored
- Very little imaging, if any



Paret ML, Sharma SK, Green LM, Alvarez AM, Biochemical Characterization of Gram-Positive and Gram-Negative Plant-Associated Bacteria with Micro-Raman Spectroscopy. *Appl. Spectrosc.* 2010; 64: 433-441.

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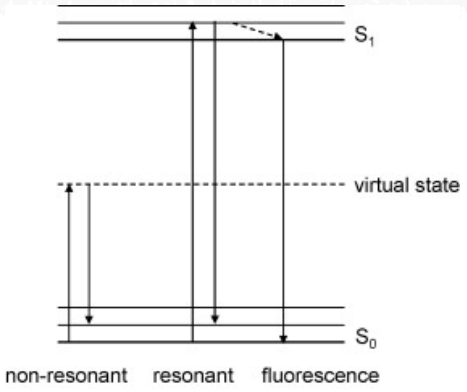
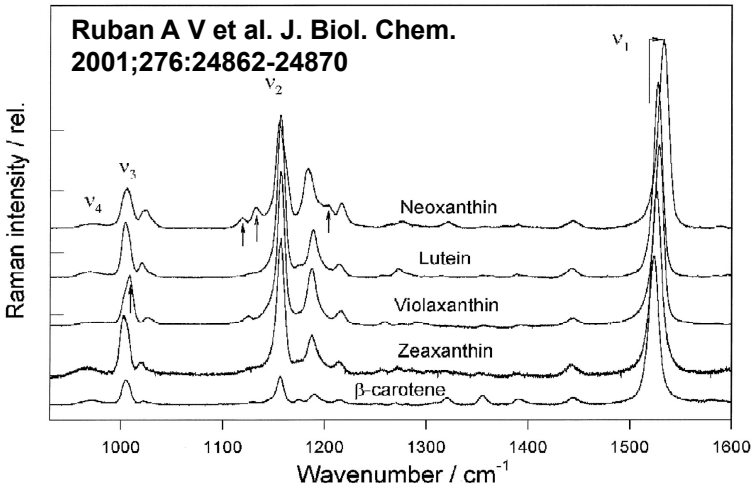
Raman Spectroscopy and Spectral Imaging in Plant Science

- Resonant Raman vs. non-resonant Raman





ν_1: C = C stretching vibrations
ν_2: C-C stretches coupled to C-H in-plane bending or C-CH_3 stretching
ν_3: CH_3 in-plane rocking vibrations
ν_4: C-H out-of-plane bending modes

- RR-based spectral imaging is particularly exciting
  - Discriminate, quantify, and localize carotenoids *in situ.*
  - Bioenergy, environment, human health applications

- SERS (Surface Enhanced Raman)
  Use adsorption to substrate to enhance certain vibrations
  SERS probes are increasing in popularity

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Future Opportunities

Raman spectral imaging – compliments 'omics with spatial resolution

- Spectral imaging plant –pathogen interactions
  - Understanding mechanism
  - Effectiveness of treatment
  - Effect of environment
  - Microbial communities

Raman spectroscopy - "warning system" based on spectral response

- Early detection, screening of crops
  - Altered host response or pathogen signature
  - Non-specific vs. specific

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Commercially Available Systems

Raman Imagers

ChemImage®

Raman Microscopes
Raman Microprobes

KAISER OPTICAL SYSTEMS, INC.
A ROCKWELL COLLINS COMPANY

WITec
focus innovations

Hand Held,
Portable Raman Spectrometers

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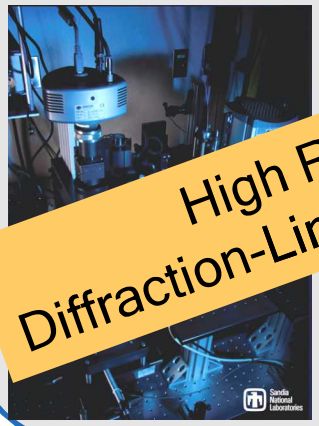
Technology Available at SNL

SNL's Hyperspectral Confocal Microsc



- 488 nm excitation
- 60x (1.4 N
- L
- 900 cm^{-1}
- tral resolution = 35 - 100 cm^{-1} (1-3 nm)
- Acquisition rate = ≤8300 spectra/s

Sinclair, et. al., Applied Optics, 45, 6283-6291 (2006).

High Read-out Rate, Diffraction-Limited Spatial Resolution

WiTec alpha300R

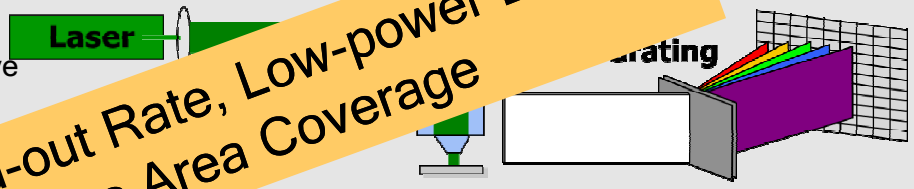
- 532 nm excitation
- 100x (0.9 NA) dry objective
- Lateral resolution = 4
- Axial resolu
- Spec 700 cm^{-1}
- tion = 1 cm^{-1}
- tion rate = 100 spectra/s

http://www.witec.de/products/raman/alpha300-r/

Exquisite Spectral Resolution

SNL's Hyperspectra e Scanner

- 532 nm excitation
- 4x, 10x, 40x or 100x dry objective
- Lateral resolution = 1 µm
- Axial resolution = ~2-6 µm
- Spectral range = 500-1
- Spectral resolu
- Acquisition



Christensen & Morris, Applied Spectroscopy, 52, 1145-1147 (1998) & Sinclair, et. al., Applied Optics, 43, 2079-2089 (2004)

High Read-out Rate, Low-power Density Large Area Coverage

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Advantages of SNL Technology

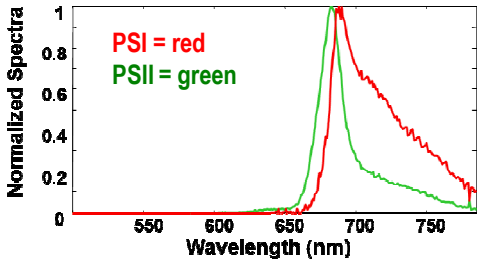
- Flexible platform for design optimization
- Line scan design
  - Lower power density
  - Least temporal sacrifice due to mutliplexing
- Coupled to multivariate curve resolution algorithms

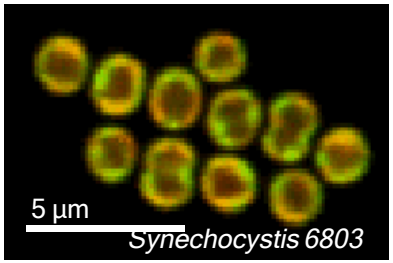
Challenges in Raman Spectral Imaging of Plants

- Additional wavelengths
- Chlorophyll/pigment emission
- Determination of detection limits
- Line scan is not confocal

Examples of Chemical Imaging in Plant and Algal Research

Hyperspectral Confocal Fluorescence Microscopy

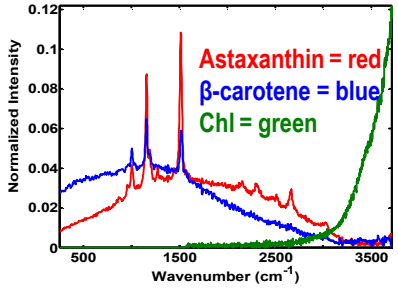


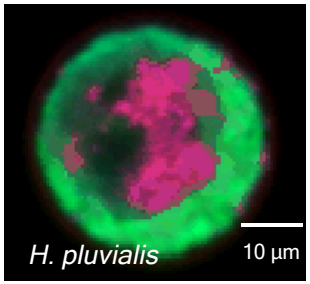


Subcellular localization, discrimination, and quantification of photosynthetic pigments

Collins AM, Liberton M, Garcia OF, Jones HDT, Pakrasi HB, Timlin JA, *Plant Physiology* 2012; 158(4): 1600-1609.

Combined Hyperspectral Confocal Raman & Fluorescence Microscopy

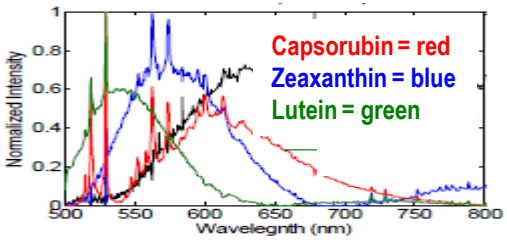


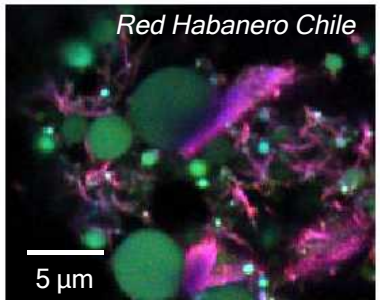


Subcellular localization, discrimination, and quantification of carotenoids and chlorophylls

Collins AM, Jones HDT, Han D, Hu Q, Beechem TE, Timlin JA, *PLoS One* 2011; 6(9): e24302.

Hyperspectral Confocal Raman Microscopy





Subcellular localization, discrimination, and quantification of carotenoid, lipids, and precursors

In preparation for publication

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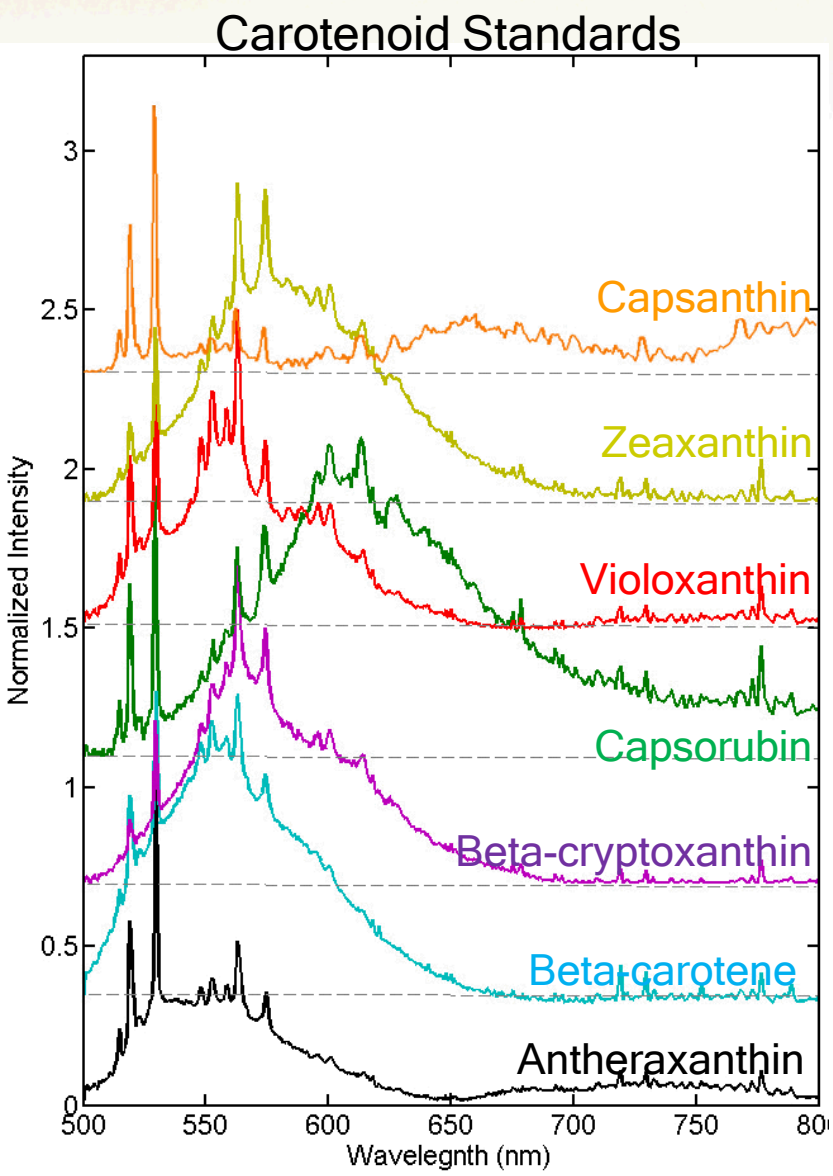
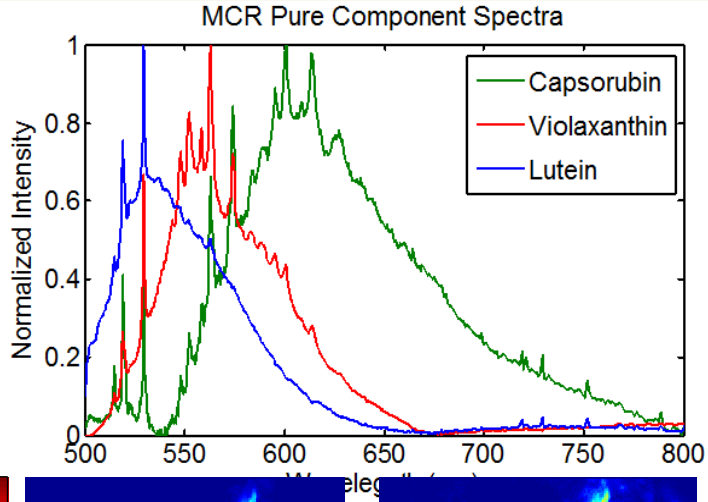
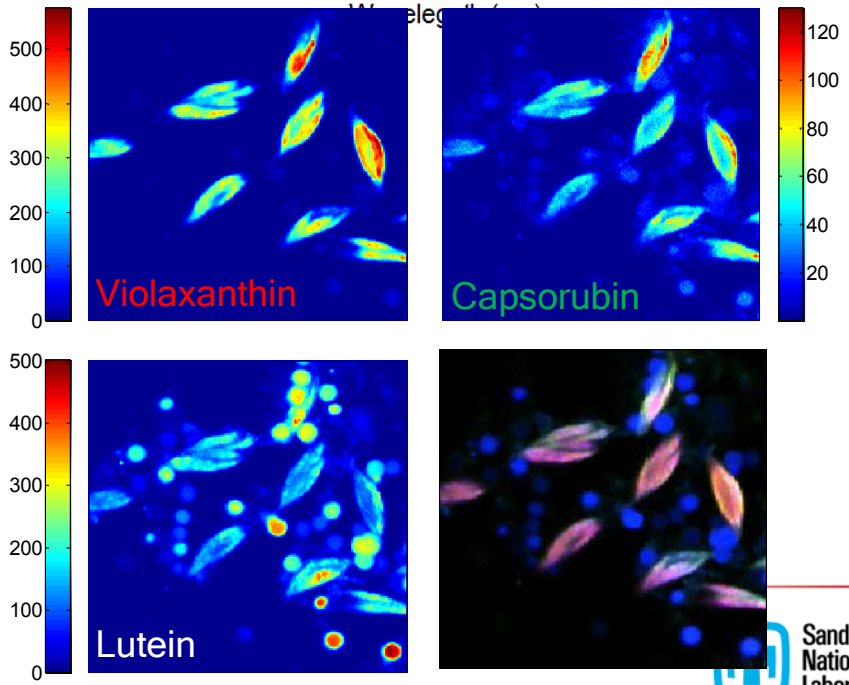
Carotenoid Localization and Abundance in Chile Peppers







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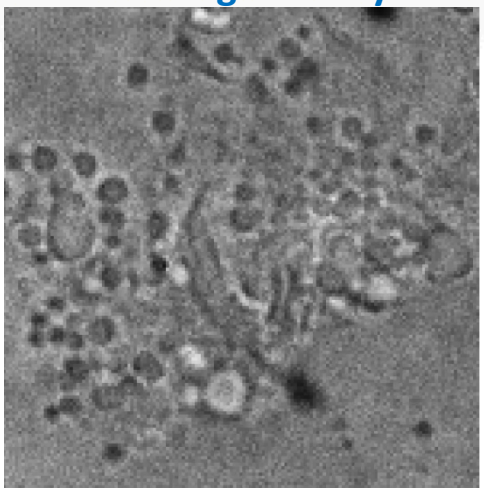
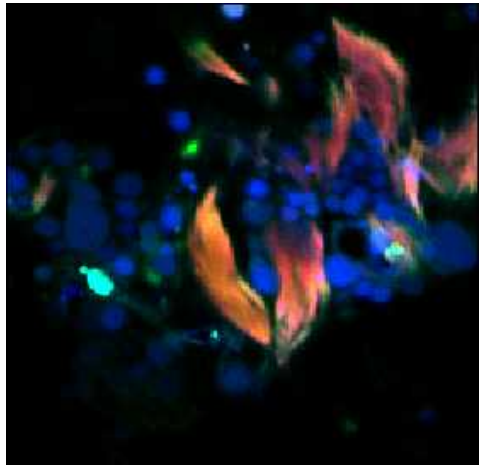
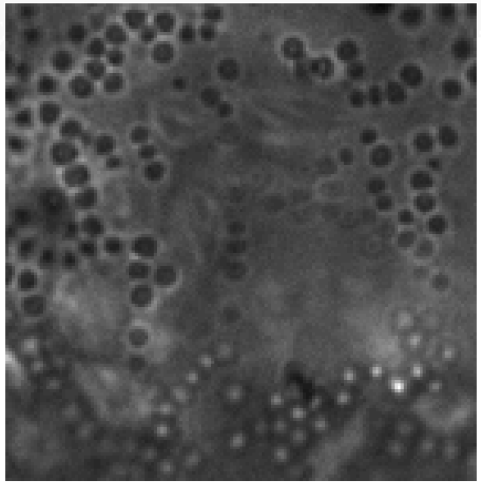
Comparison Across Chile Species

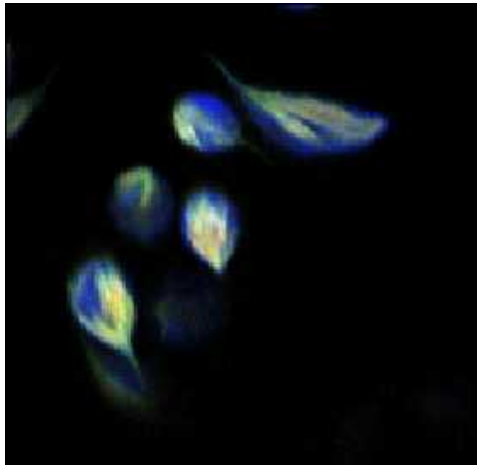
Costeño Amarillo
Orange variety



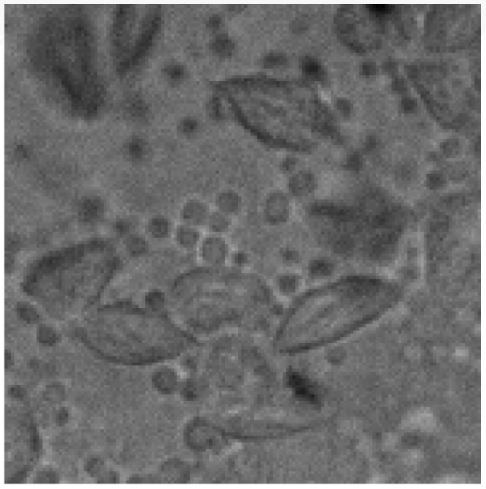


Costeño Amarillo
Red variety





NuMex Heritage 6-4



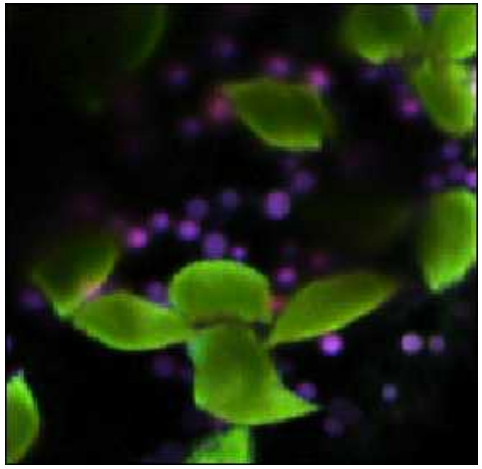


Image dimensions: 25 x 25 µm

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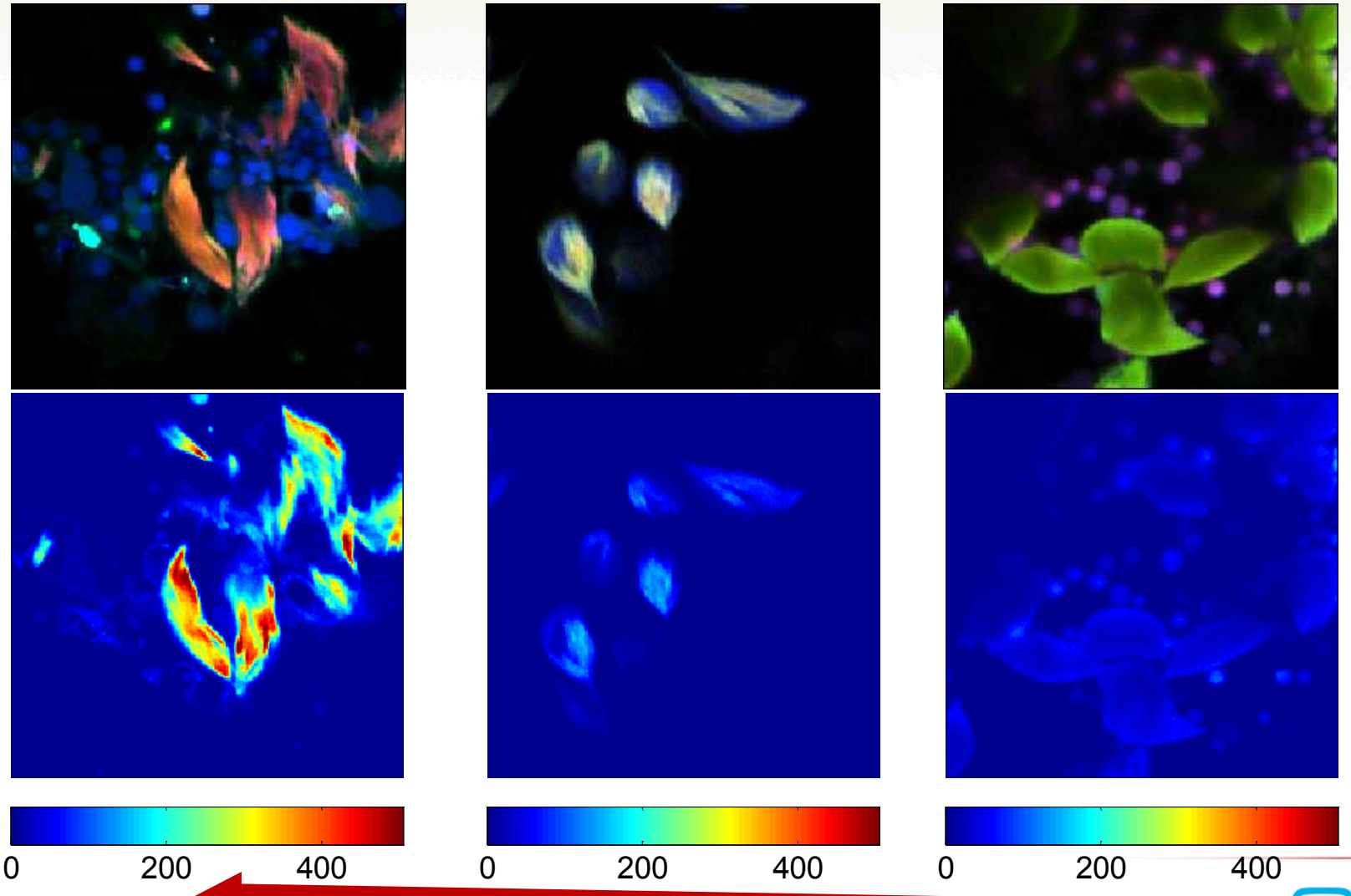
Relative Quantification of Violaxanthin

Costeño Amarillo
Orange variety

Costeño Amarillo
Red variety

NuMex Heritage 6-4



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Conclusions and Opportunities

- Hyperspectral imaging based upon Raman spectral signatures offers an excellent chemical and spatial resolution for important plant biomolecules.
  - Plant and algal biology
  - Environmental response
  - Bioenergy, algal biofuels
- Opportunities abound –
  - Spatially resolved information largely unexplored for plant pathogens
  - Basic research for fundamental understanding
  - Applied research for development of prototype
  - Scoping studies are critical
    - The field is not at a state where specific tools can be developed without basic science first.

SNL

Acknowledgements

- **Aaron M. Collins**
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- **Ryan W. Davis**
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- Omar F. Garcia

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- Michelle Liberton and Himadri Pakrasi, Biology Dept., WUStL

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- Laboratory Directed Research and Development program at Sandia National Laboratories

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- U.S. Department of Energy funded Sustainable Algal Biofuels Consortium (Award # DE-EE0003372).

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Extra Slides

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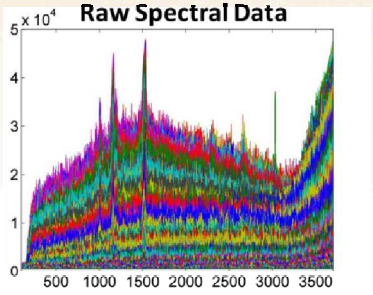
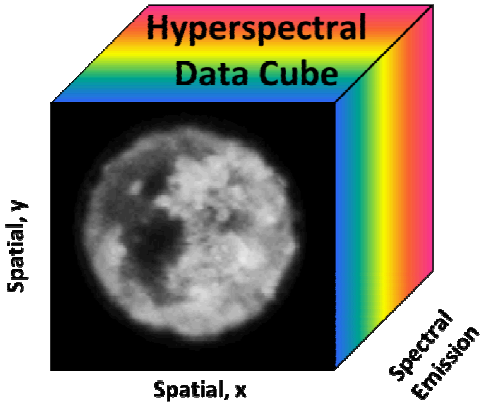
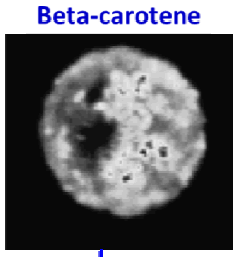
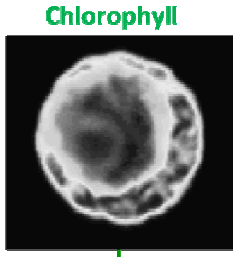
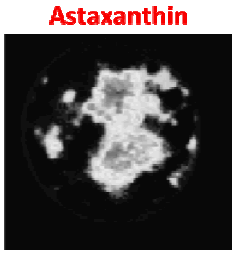
Multivariate Curve Resolution (MCR) Example

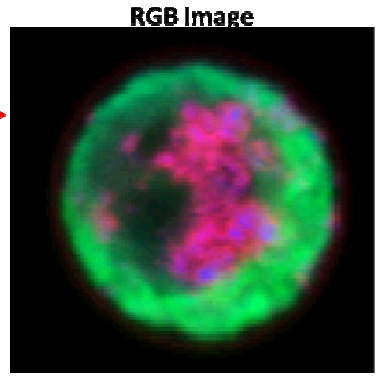












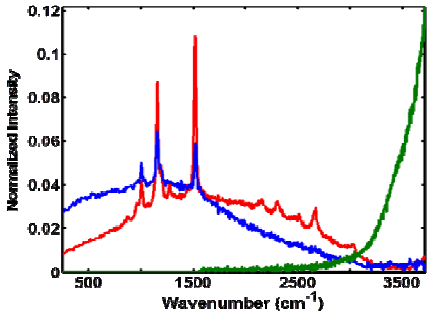
How much is present? Where is it located?

Preprocess → PCA → MCR → What is present?

- Calibrate wavelength axis
- Remove cosmic spikes

- Weight data for Poisson noise
- Estimate # of components
- Initial guess spectral signatures

- Constrained alternating least squares analysis
- Linear additive model, $D = CS^T$



Algorithim details: *Haaland, et al., Proc. SPIE, Vol. 4959, 55 (2003) ; Jones, et. al., J Chemom, 22:482-490 (2008); Jones, et. al., J Chemom, 117:149-158 (2012)*

Biological relevance: *Collins, et al., Plos ONE,* 6:e24302 (*2011*)

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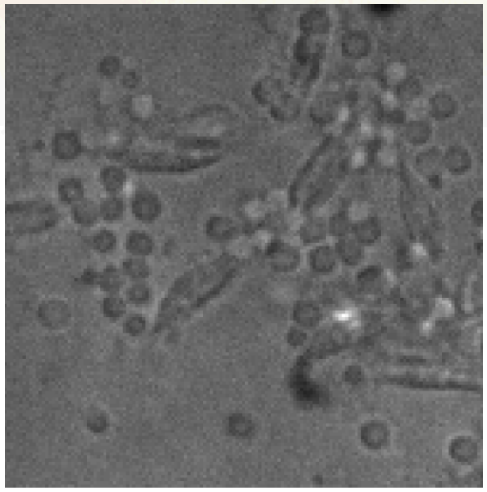
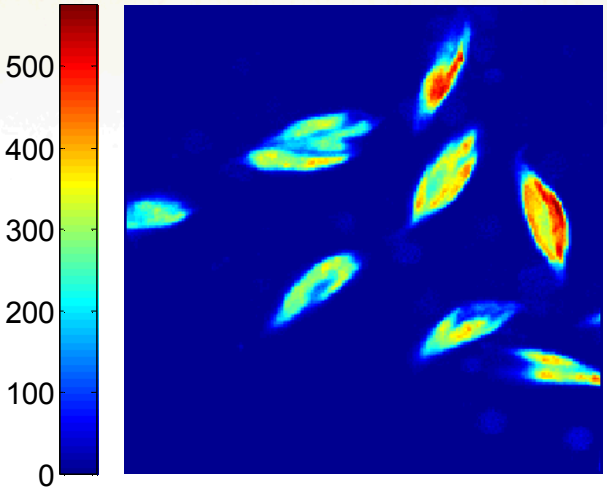
Costeño Amarillo - orange variety

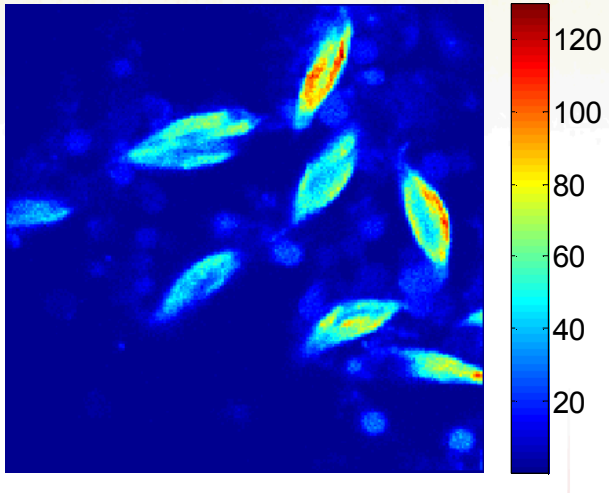
Widefield



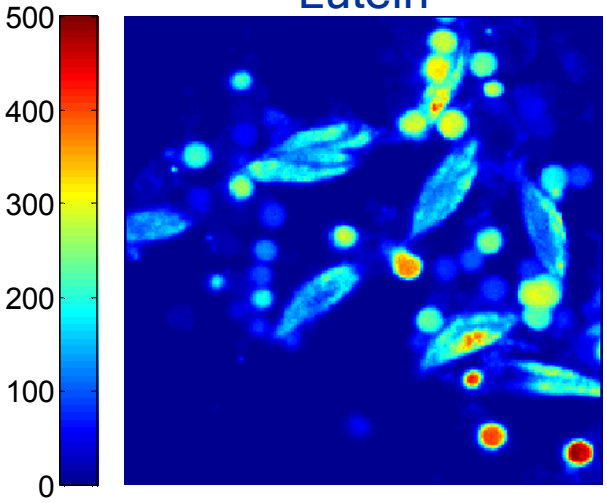
Violaxanthin



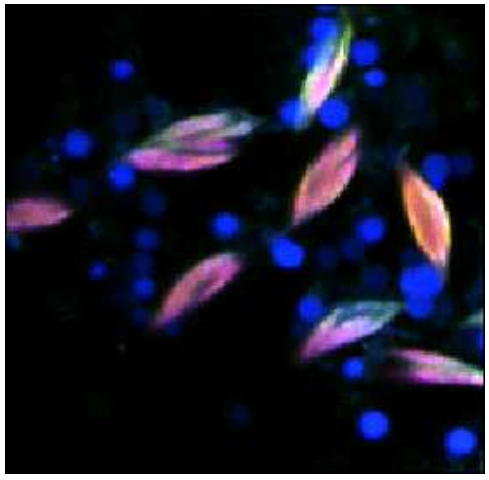
Capsorubin

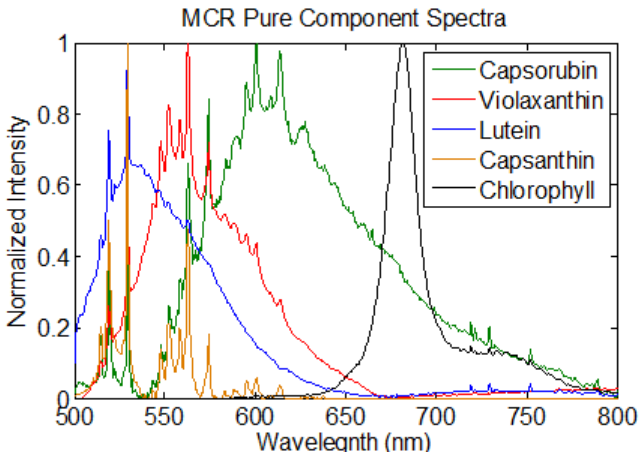


Lutein



RGB





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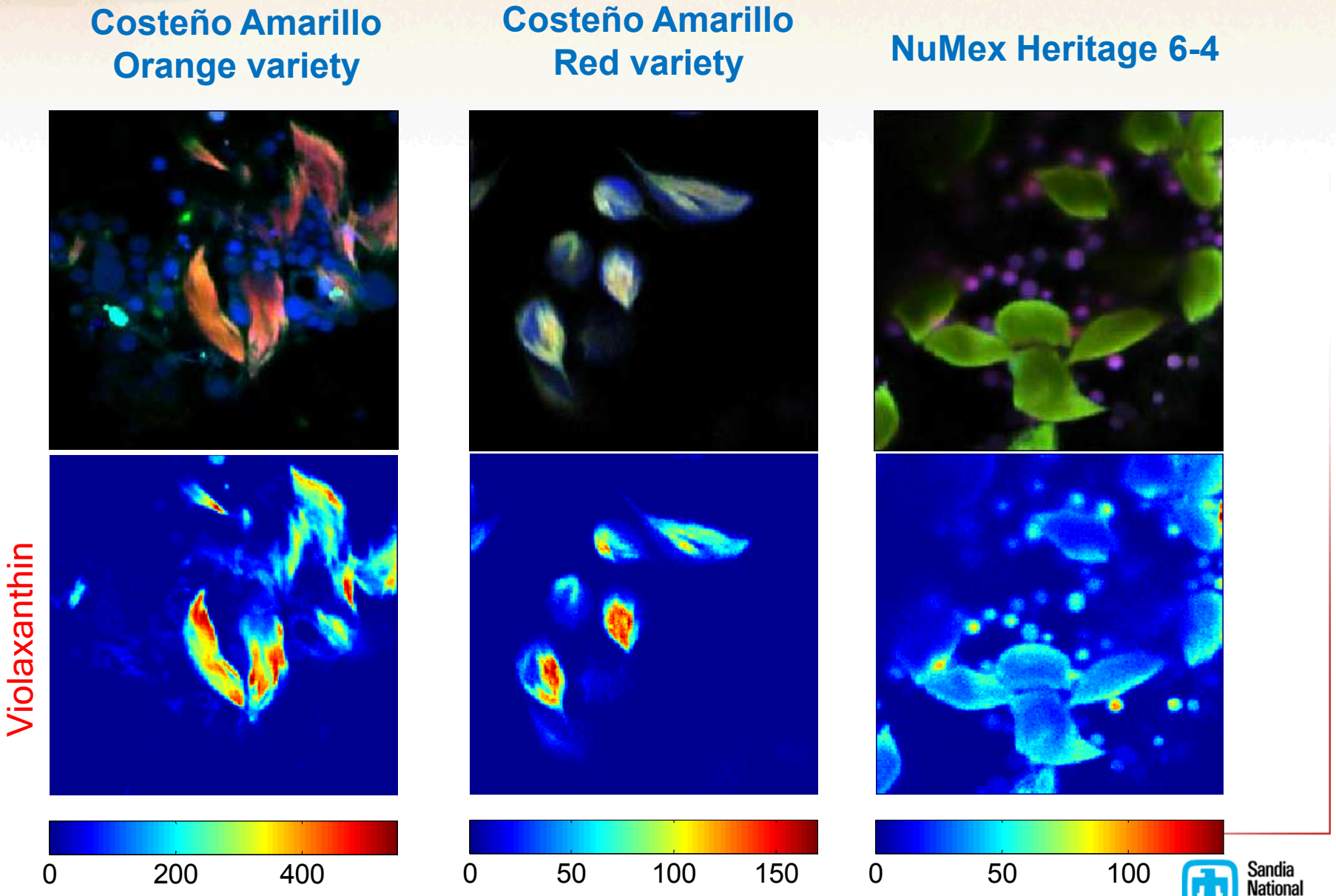
Relative Quantification of Violaxanthin



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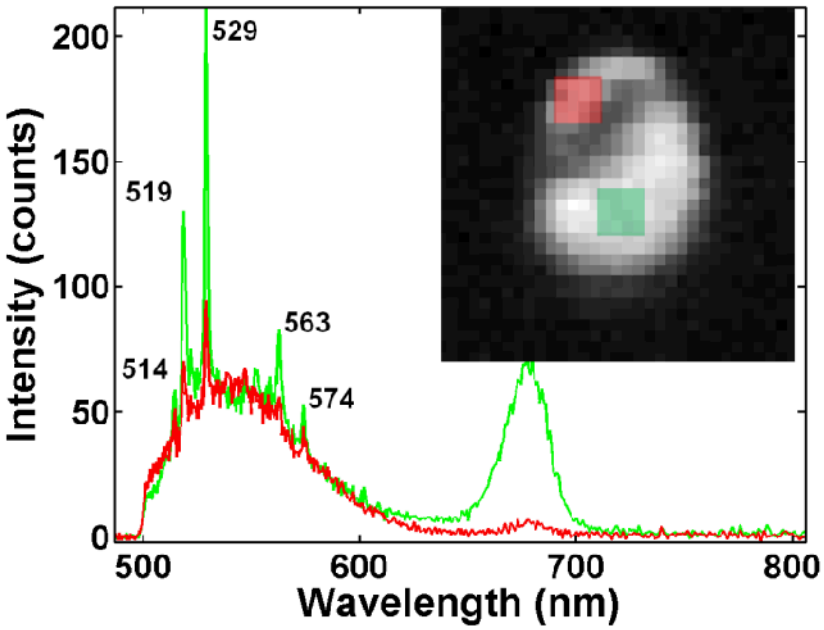
Carotenoid Biogenesis: Importance in Algal Biofuels

- Carotenoid biogenesis is intimately tied to the lipid production pathways in algae.
- Carotenoids partition into lipid droplets as well as lipid bilayers in membranes.
- RR offers ability to discriminate various carotenoids and their precursors within algal lifecycle
  - Basic understanding of primary lipid production, membrane recycling
  - Applied utility as a method for label-free lipid determination within single cell levels



Small changes in the carotenoid signal can allow the separation of lipid bodies from the chloroplast

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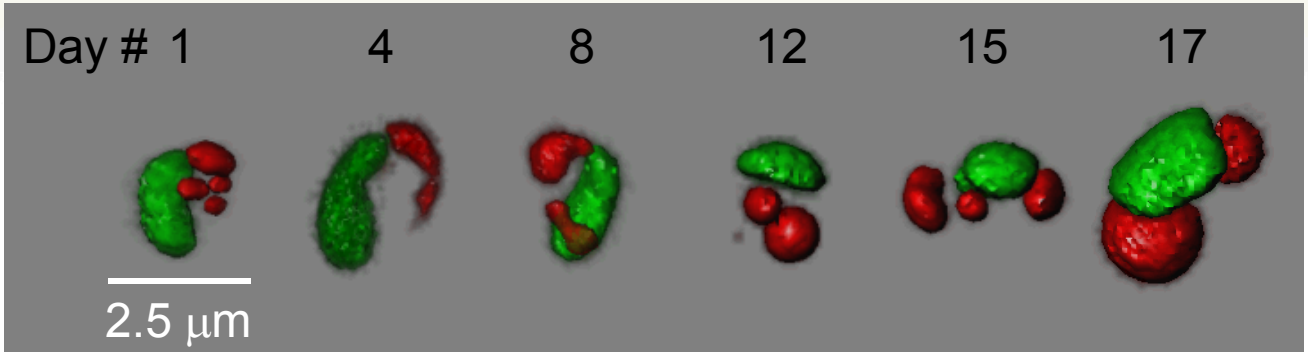
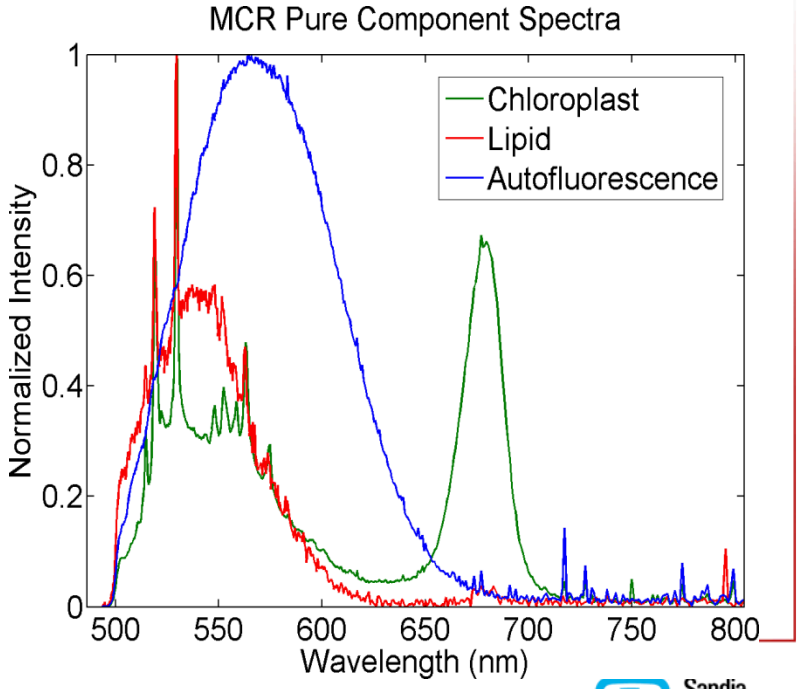
Lipid Production in *Nannochloropsis salina*



14 day multifactorial study of N. Salina under nitrogen limitation and CO_2 stress

- Using hyperspectral fluorescence and RR imaging for the *in-situ* monitoring of algal cells
- Insights into lipid production and accumulation at the sub-cellular level
- Develop spatial/temporal relationship of lipid and photosynthetic pigments



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