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Hyperspectral Raman and Fluorescence Microscopy of Individual Algal Cells for Biochemical Analysis

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Highlights

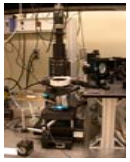
- **Hyperspectral confocal Raman and fluorescence microscopy of pigments in living algal cells**
- **Subcellular localization of multiple, overlapping carotenoids in individual cells**

Introduction

We show that the combination of Hyperspectral confocal Raman and fluorescence microscopy paired with multivariate analysis, can provide critical spatially resolved biochemical relationships in algal cells. Raman spectroscopy aided by resonance enhancement distinguishes between chemically similar carotenoids, while fluorescence spectral information provides complimentary and confirmatory information about the photosynthetic pigments. Vegetative cells of *Haematococcus pluvialis* synthesize predominantly β-carotene and lutein however, under abiotic or biotic stressors, astaxanthin is accumulated outside of the chloroplast and the cells form large cysts. We track the accumulation of carotenoids and the distribution of chlorophyll in living *H. pluvialis* cells. Cells of *Dunaliella salina* are included to show the robustness of the analysis.

Experimental Parameters

Fluorescence Microscope



- Custom built
- 488 nm laser excitation
- 20x dry objective
- Lateral res. = 325 nm
- Axial res. = ~3.4 μm
- Spectral range 490-800 nm
- Spectral res. = 1-3 nm
- Acquisition rate = 4100 spectra/s
- Sinclair, MB., et al. (2006). Appl. Opt. 45, 3283-3291.

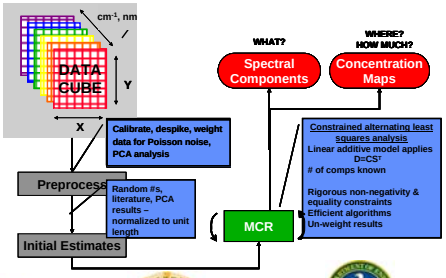
Raman Microscope

- WiTec alpha300R
- 532 nm laser excitation
- 100x dry objective
- Lateral res. = ~300 nm
- Axial res. = ~2.5 μm
- Spec. range = 100-3700 cm^{-1}
- Spec. res. = 1 cm^{-1}
- Acquisition rate = 100 spectra/s

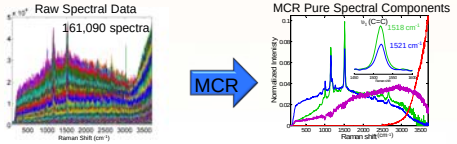
Image Analysis Flow Chart

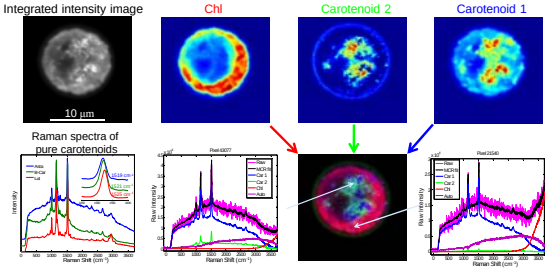
Multivariate Curve Resolution (MCR)

- Discover & quantify all emitting species in a sample simultaneously with no a priori knowledge
- Mathematical isolation of pure spectral components, independent concentration maps
- Jones et al., (2008) J Chemom. 22:482-490 and references therein

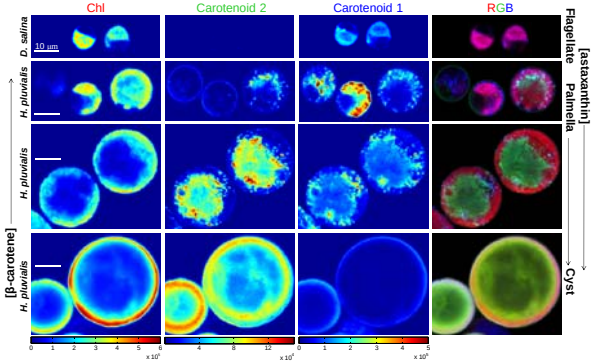


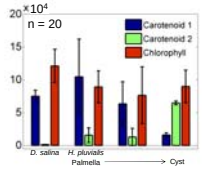
Confocal Raman Microscopy on Single Algal Cells





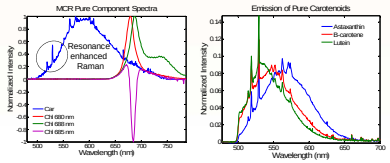
Confocal Raman microscopy of individual cells of *H. pluvialis* together with multivariate analysis allows for mathematical isolation of independently varying chemical species. Two unique carotenoids, autofluorescence and chlorophyll fluorescence are identified. Comparison of the MCR isolated species to the Raman spectra of pure carotenoids suggest these components are astaxanthin (carotenoid 2) and β-carotene (carotenoid 1). Chlorophyll emission occurs in the chloroplast. Carotenoid 1 and 2 are found outside of the chloroplast in distinct globular regions.



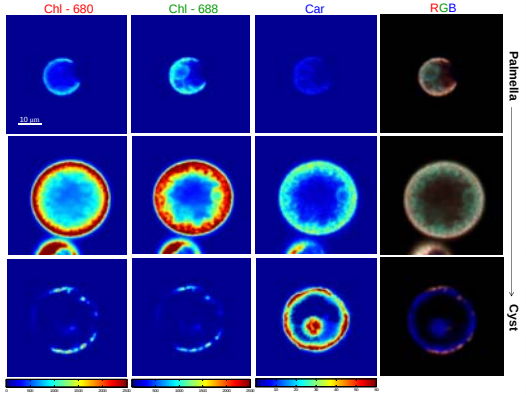


Localization and distribution of carotenoids and chlorophyll during the life cycle of *H. pluvialis*. Under environmental stress (ROS, high-light, nutrient deficiency), cells of *H. pluvialis* convert β-carotene to astaxanthin, accompanied by the transformation of cells into the resting cyst phase. For comparison, cells of *D. salina* are presented and are known to not synthesis astaxanthin.

Hyperspectral Confocal Fluorescence Microscopy



HCFM of individual *H. pluvialis* cells and multivariate analysis reveals two chlorophyll emission features (plus a band narrowing) and fluorescence attributed to astaxanthin.



The two chlorophyll emission features are found in distinct cellular regions with Chl-680 nm being located towards the periphery of the cell. Astaxanthin fluorescence is most prominent in the cyst-forming cell. Note the resonance enhancement mixed with astaxanthin fluorescence however, limiting resolution does not allow for carotenoid discrimination from the data.

Summary

- **Multivariate analysis of confocal Raman and fluorescence images of individual algal cells demonstrate the ability to distinguish the subcellular location and identity of carotenoids and chlorophylls.**
- **First demonstration of the separation of multiple carotenoids in single indiviadual algal cells.**
- **Quantification and tracking of pigment distribution during the cellular phases of *H. pluvialis***

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