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Hyperspectral Imaging of Endogenous Pigments in the Bioenergy Sciences

J.A. Timlin*, H.D.T. Jones*, M. Liberton**, R.W. Davis***, R.M. Noek*, J.B. Ricken*, M.B. Sinclair****, M. Hadi***, H.B. Pakrasi**, D.M. Haaland*

* Biofuels & Defense Technologies, Sandia National Laboratories, PO Box 5800, Albuquerque, NM 87185-0895
** Department of Biology, Box 1137, Washington University, St. Louis, MO 63130
*** Biomass Science & Conversion Technologies, Sandia National Laboratories, 7011 East Avenue, Livermore, CA
**** Electronic & Nanostructured Materials, Sandia National Laboratories, PO Box 5800, Albuquerque, NM 87185-1411

To meet the 21st century needs for clean, renewable, and efficient energy sources, fundamental research into biotechnology-based strategies to produce energy from a diverse range of biological feedstocks is critical. Though these feedstocks, commonly referred to as biomass, can vary widely in composition from woody plants to animal waste, all are dependent on harvesting light from the sun then transferring that light into energy to grow and sustain itself. Exploiting these natural processes for light harvesting and energy and biomass conversion to create affordable, renewable energy sources for the future is a major challenge.

Many of the fundamental processes driving energy production and biomass conversion rely on photosynthesis and thus contain endogenous photosynthetic pigments. These endogenous pigments provide unique fluorescence emission spectra which can be followed in time and/or space to elucidate fundamental relationships between light capture and energy storage and conversion within the organism. Unfortunately identification of the pigment emission spectra in living organisms is complicated due to the high degree of both spatial and spectral overlap, as well as the sensitivity of the emission to changes in local environment including protein association and energy transfer. Hyperspectral imaging coupled with multivariate spectral image analysis is well-suited to tackle this complex problem.

A hyperspectral confocal fluorescence microscope collects an entire fluorescence emission spectrum with high spectral resolution from each voxel within an image [1]. This methodology is extremely powerful when combined with multivariate analysis tools to identify and/or isolate overlapped spectral signatures and estimate their abundances within each image voxel. We have previously demonstrated the utility of the hyperspectral imaging technology and multivariate analysis for isolating multiple exogenous fluorescent labels in the presence of strong cellular autofluorescence [2]. More recently we demonstrated the power of hyperspectral confocal fluorescence microscopy for visualizing multiple, endogenous pigments in the living cyanobacterium, *Synechocystis* sp. PCC 6803 [3].

In this paper we will present several novel bioenergy related applications in which hyperspectral imaging and multivariate analysis are providing an unprecedented view at multiple spatial scales of the light harvesting, energy transfer, and energy storage processes in biomass feedstocks. This new insight permits improved understanding of fundamental biological processes such as lipid

biosynthesis in algae (Figure 1) and light harvesting in living photosynthetic organisms without the use of labels [4].

References

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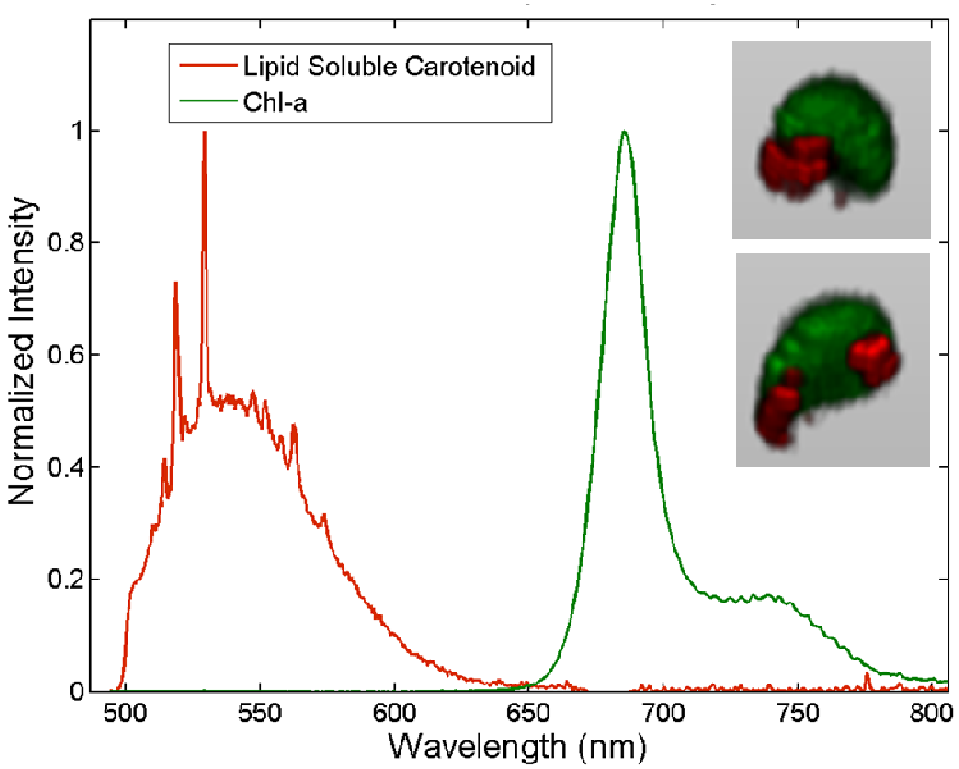

FIG. 1. Hyperspectral imaging and multivariate curve resolution [2, 3] provide volumetric comparison of chloroplast (as delineated by chlorophyll) and lipid with living *Nannochloropsis salina* cells without the use of traditional Nile red label for lipid determination. Main plot: Spectral signatures isolated from multivariate curve resolution of four-dimensional image data cube. Red trace: Spectral signature contains features from both fluorescence and resonance enhanced Raman scattering from carotenoid. Green trace: Chlorophyll A fluorescence Inset: Volumetric renderings of chloroplasts (green) and lipid soluble caroetnoid (red) created from relative concentration maps corresponding to spectral signatures.

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