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SAND2012-1284P

Advanced Spectroscopic Imaging Techniques in Support of the Biomass Conversion Team

Howland Jones, Jerilyn Timlin and Michael Sinclair

SABC Review 02/22/2012

Sandia National Labs

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Microscopic characterization

- Study the real-time biochemical conversion of algal biomass residual and whole algal cells to fermentable sugars during chemical and enzymatic pre-treatment of cells at the microscopic level

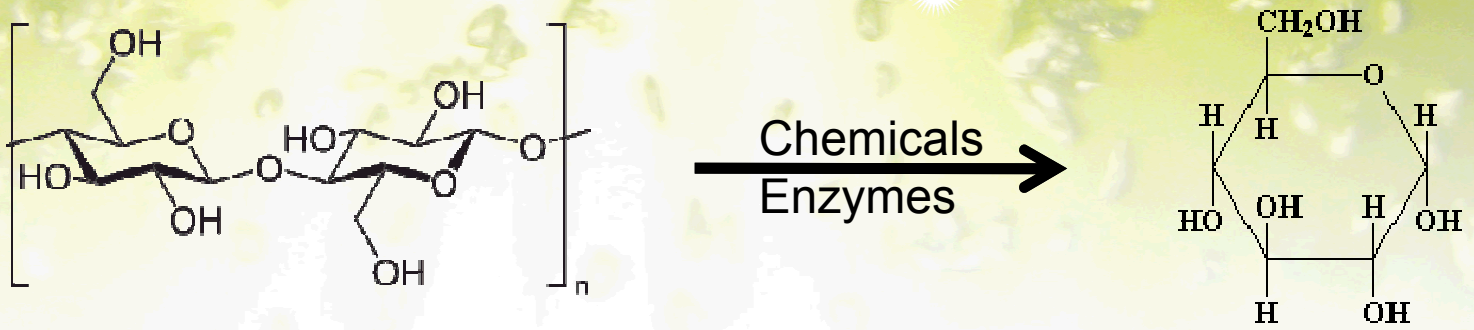
- Provides unique spatial, temporal and biochemical information that is required for the support of the biochemical conversion task (1.3)

Outcome from the Last Review

- Gary Dirks requested that we reach out and apply our advanced spectroscopic imaging techniques beyond the Ionic Liquid investigations
  - Acid pretreatment studies
    - NREL - Nick Nagel, Lieve Laurens, Stefanie Van Wychen
  - Enzyme conversion studies
    - SNL – Amy Powell, Kylea Parchert
      - To be presented by Amy Powell

Microscopic characterization

Study the real-time biochemical conversion of algal biomass residual and whole algal cells to fermentable sugars during pre-treatment of cells at the microscopic level.



Imaging provides unique spatial, temporal and biochemical information

- Fluorescence Imaging
  - Endogenous Photosynthetic pigments (Chlorophyll components, carotenoid)
  - Fluorescent labeling
- Raman Imaging
  - Improved molecular specificity to measure endogenous components
    - Cellular and conversion products

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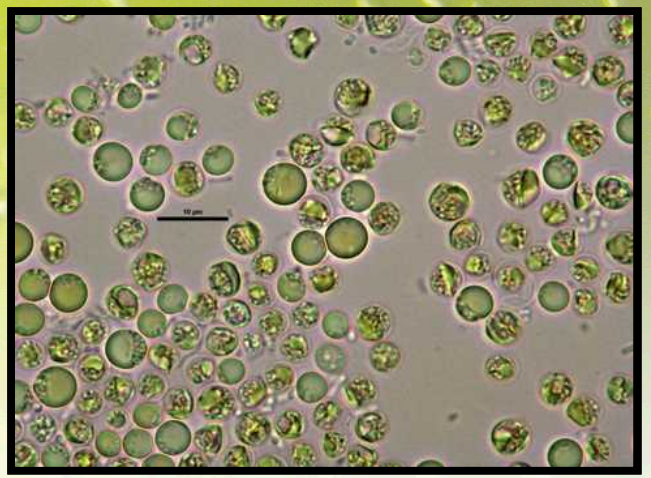
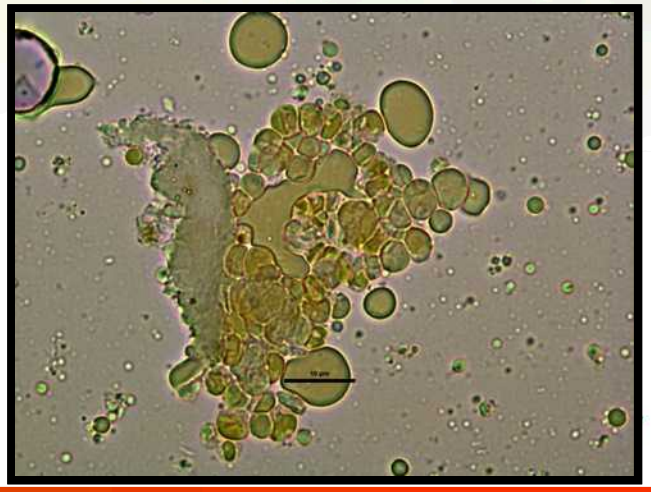
Acid Pretreatment Studies

- Purpose and Goals
  - Understand the effect of acid pretreatment procedures (cellular level)
  - Understand aggregate formations at the high acid level
  - Determine Laser requirements to guide development of Raman line-imaging microscope
- Biomass
  - NREL supplied
  - High lipid Chlorella (HLCZ)
  - Control – untreated biomass
  - High acid – 2% H_2SO_4, microwave digestion (145°C for 1 minute)
- Imaging
  - Fluorescence and Raman Hyperspectral

Control



High Acid



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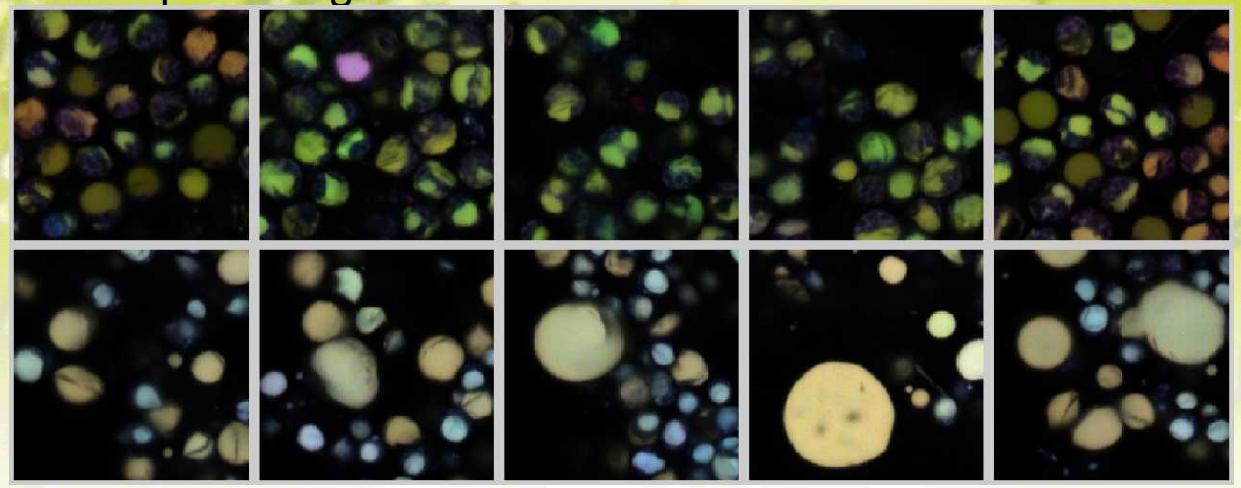
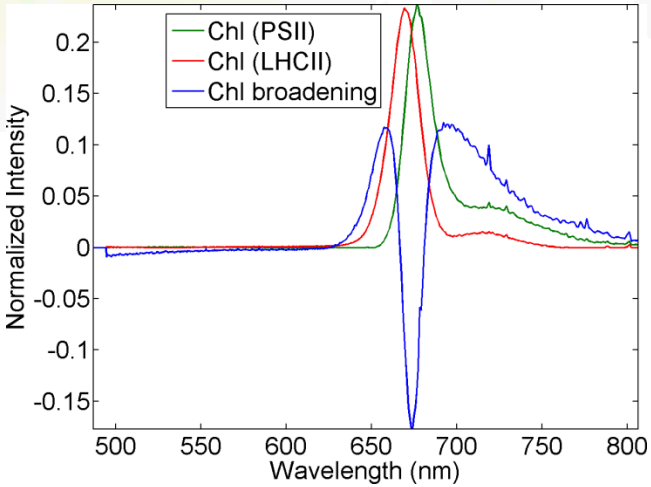
Fluorescence Imaging

Five repeat images of each biomass



Untreated HLCZ Biomass

HLCZ Biomass
2% H_2SO_4, 145°C, 1 min



- Large differences between the untreated and treated biomass
- Approximately equal parts of chlorophyll components present in large oil droplets
- Understanding how the photosynthetic pigments degrade and change upon treatment

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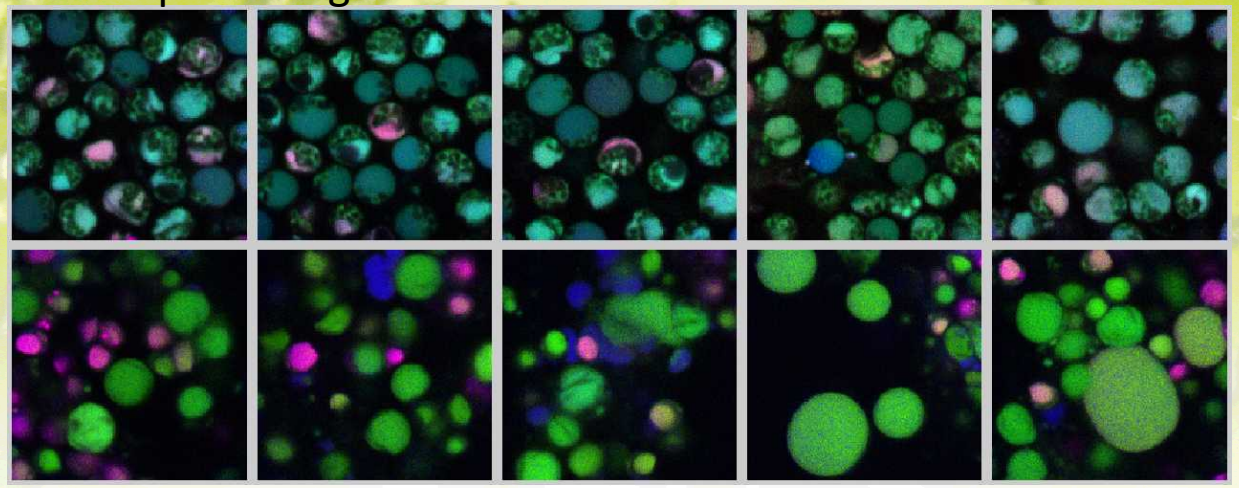

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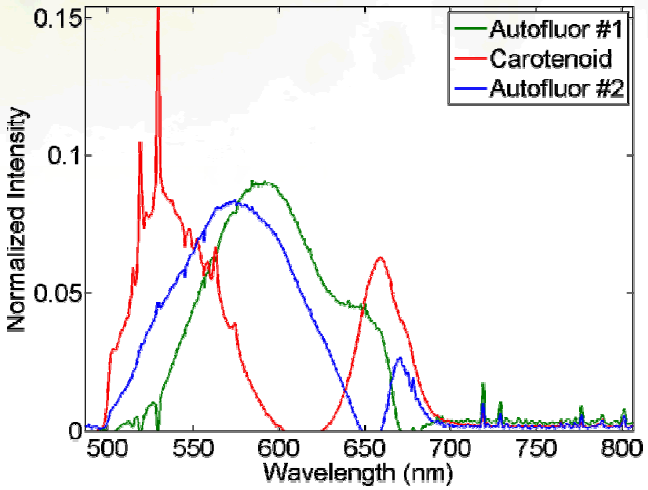

- Large differences between the untreated and treated biomass
- Decomposition components are more intense in the treated biomass
- Difficult to interpret the meaning of these results

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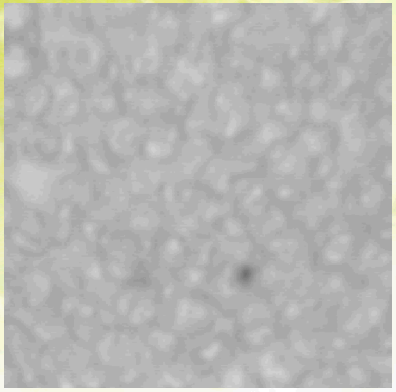
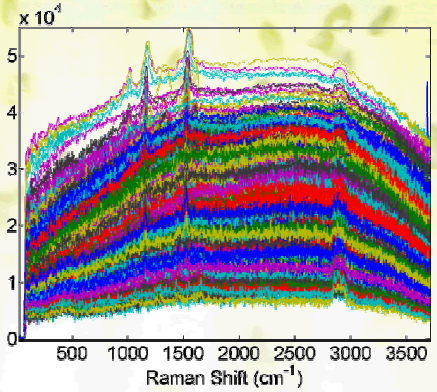
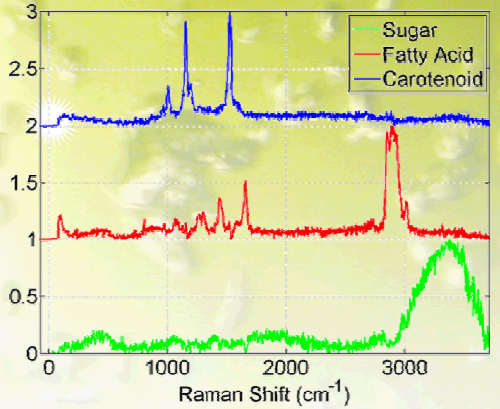
Raman Imaging

Video Images | Spectral Data | MCR Pure Components

Untreated HLCZ Biomass







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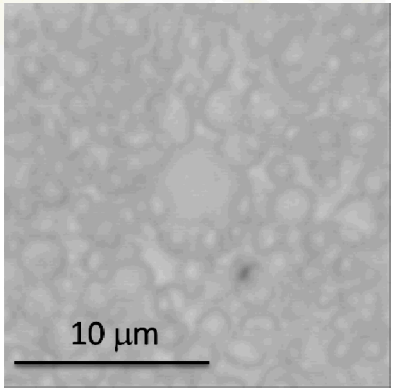
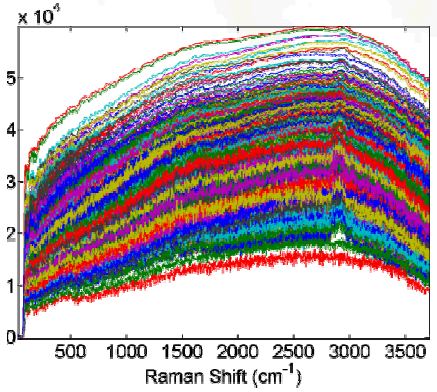
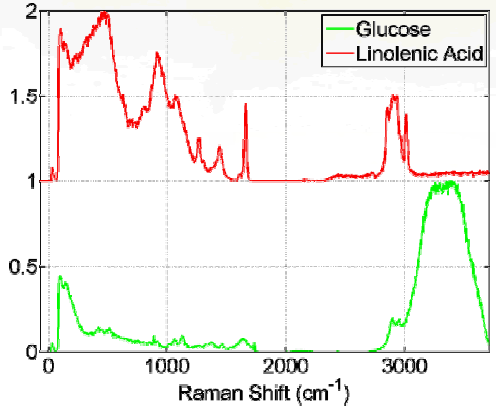




Standards



Witec Raman confocal microscope used for preliminary measurements.

14 spectral components

Measured standards confirms the presence of fatty acid deposits and free sugars in the biomass.

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Raman Imaging

Video Images

MCR Pure Components

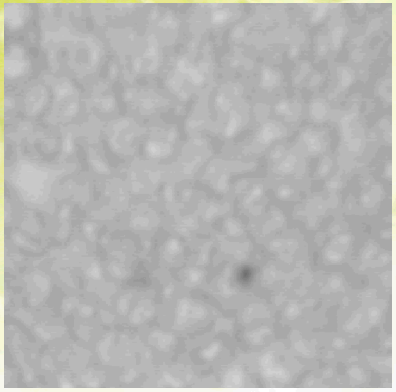
MCR Concentrations

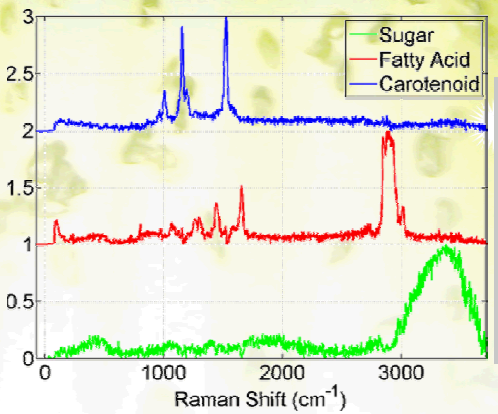
Free Sugars

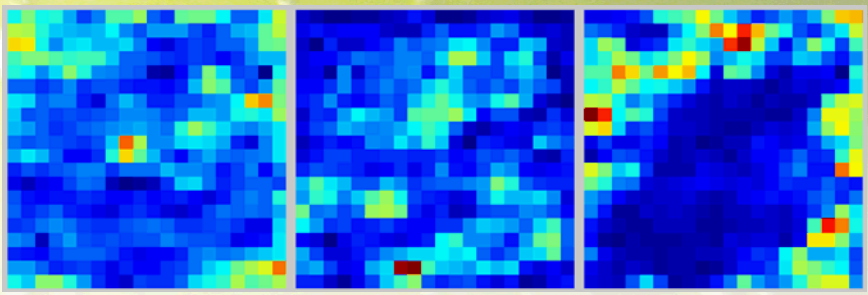
Fatty Acids

Carotenoid

Untreated HLCZ Biomass

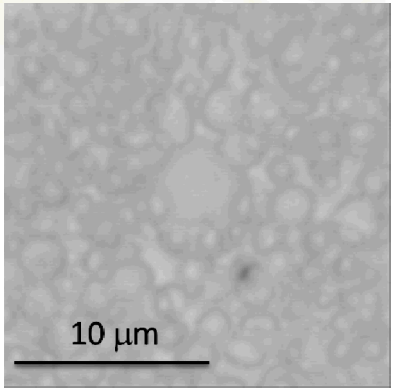






HLCZ Biomass

2% H_2SO_4, 145°C, 1 min



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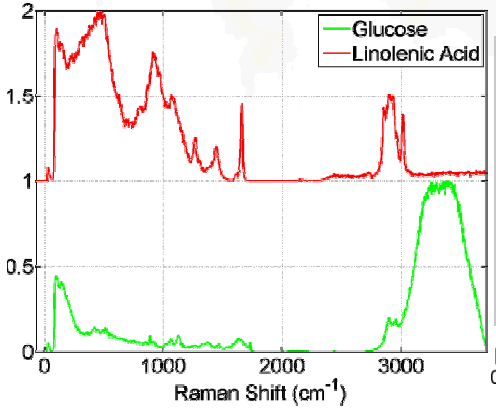

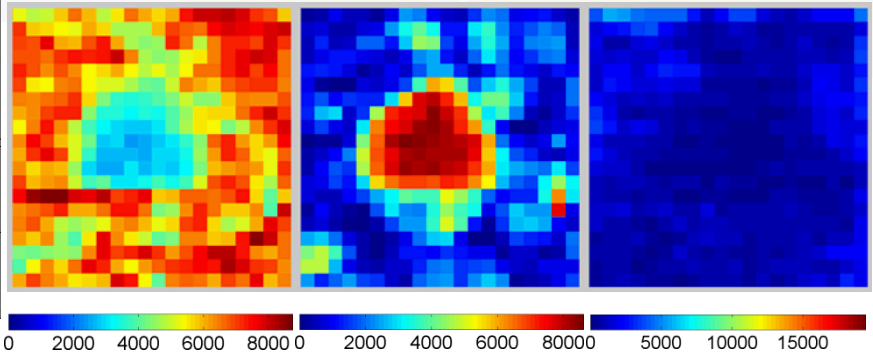

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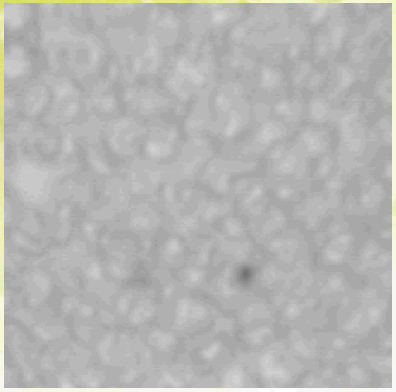
Raman Imaging

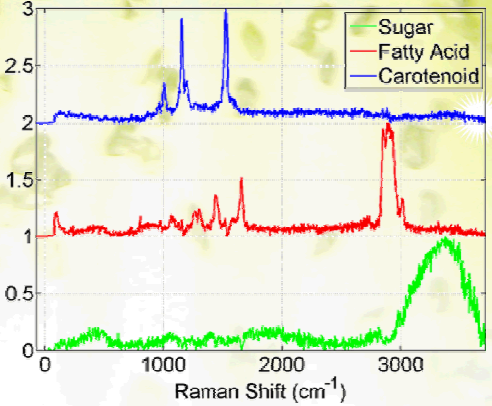
Video Images

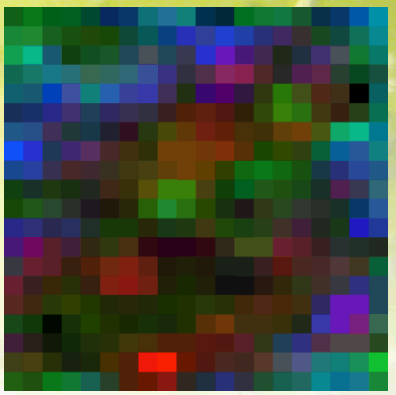
MCR Pure Components

RGB Images

Untreated HLCZ Biomass





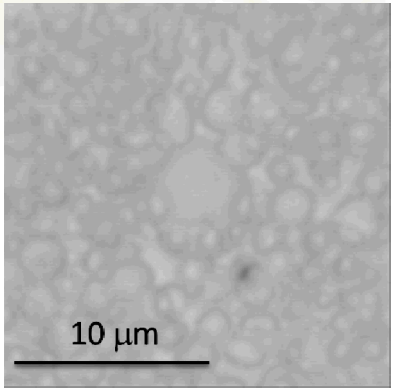


High chemical specificity!

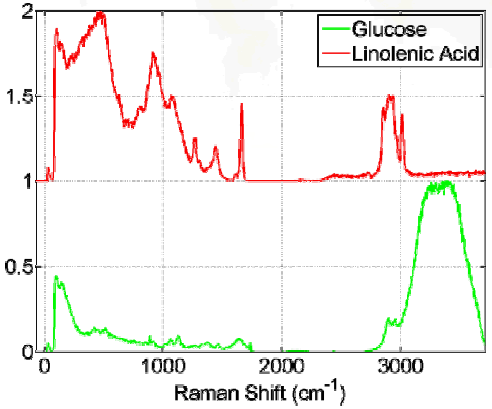
Poor spatial resolution is due to slow microscope mapping.

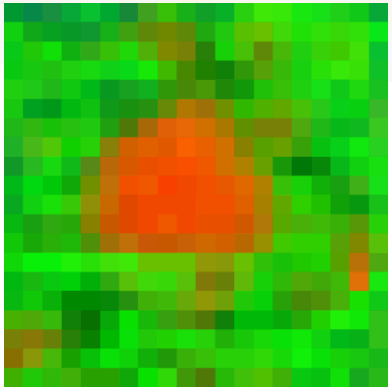
HLCZ Biomass

2% H_2SO_4, 145°C, 1 min



Standards





Spatial resolution can be improved either on the commercial scope or using our research scope.

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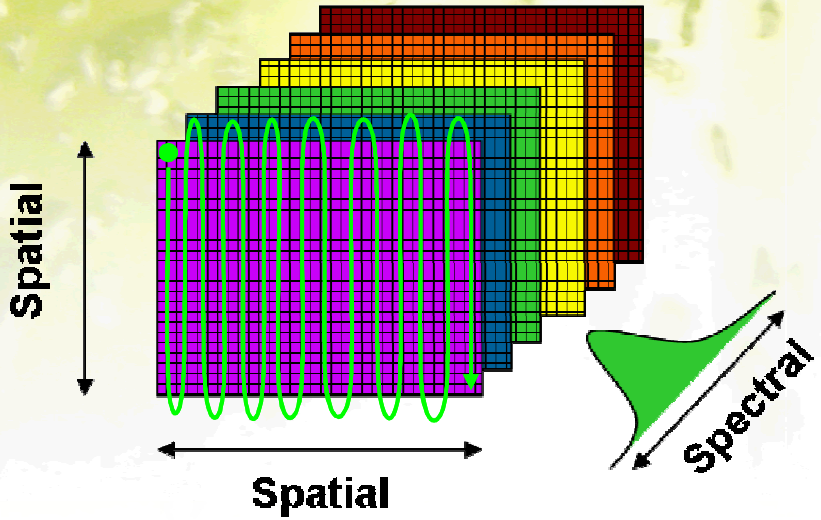
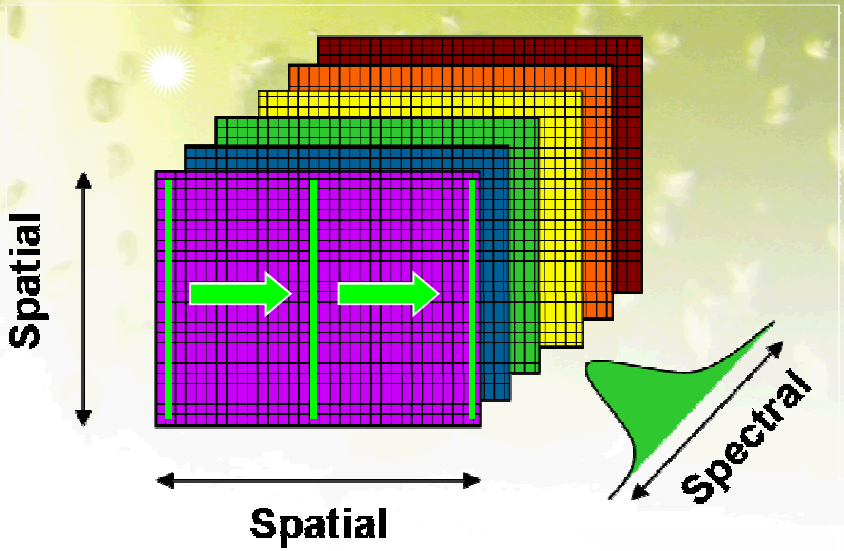
Line imaging is the key to increase our data acquisition speed!

S/N is proportional to Laser Power x Integration Time



Most commercial instruments employ spot mapping to image a sample by illuminating and collecting a spectrum at each pixel.



We augmented our current fluorescence line imager to do Raman line imaging which illuminates and collects 200 pixels simultaneously.

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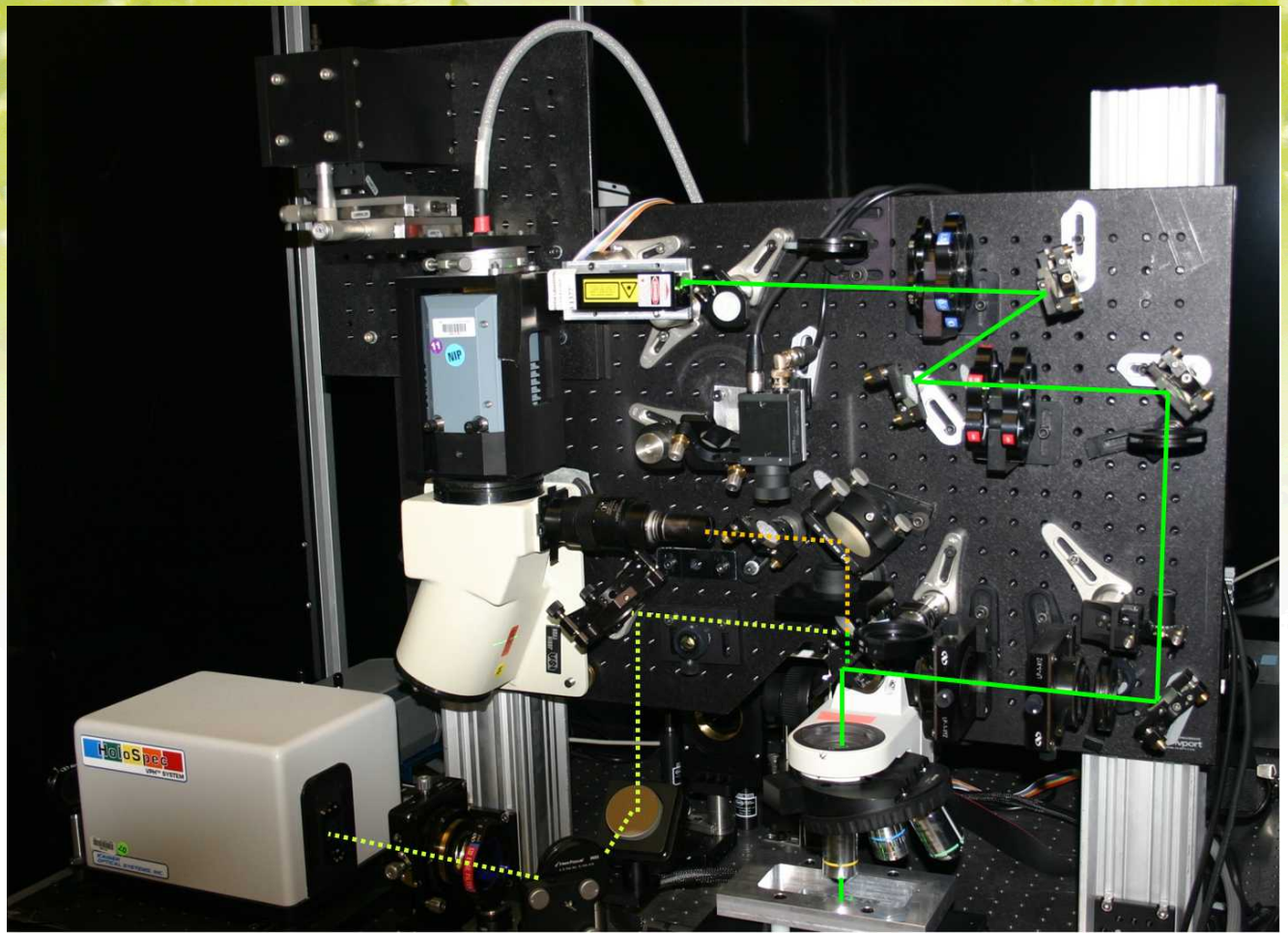
Fluorescence and Raman line-imaging microscope



Optical assembly completed and adjustments are being made to improve the SNR.

3 watt, 532 nm laser is being purchased to improve the excitation of our biomass samples.

Software being modified to operate in stop and stare modality.

Initial Raman spectra have been collected.

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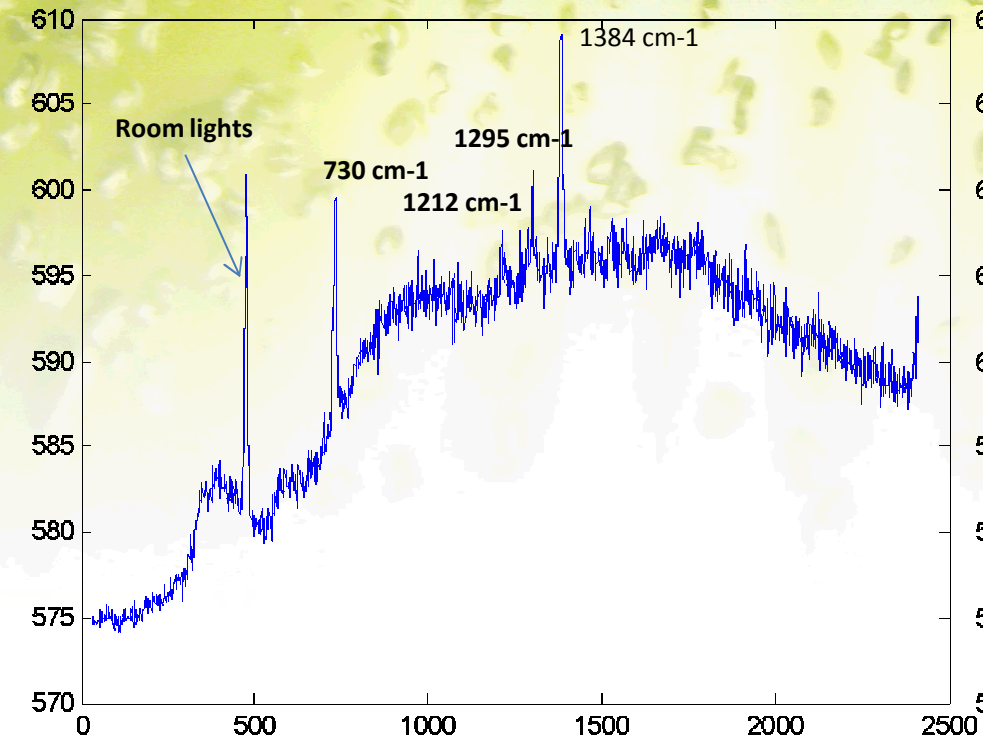
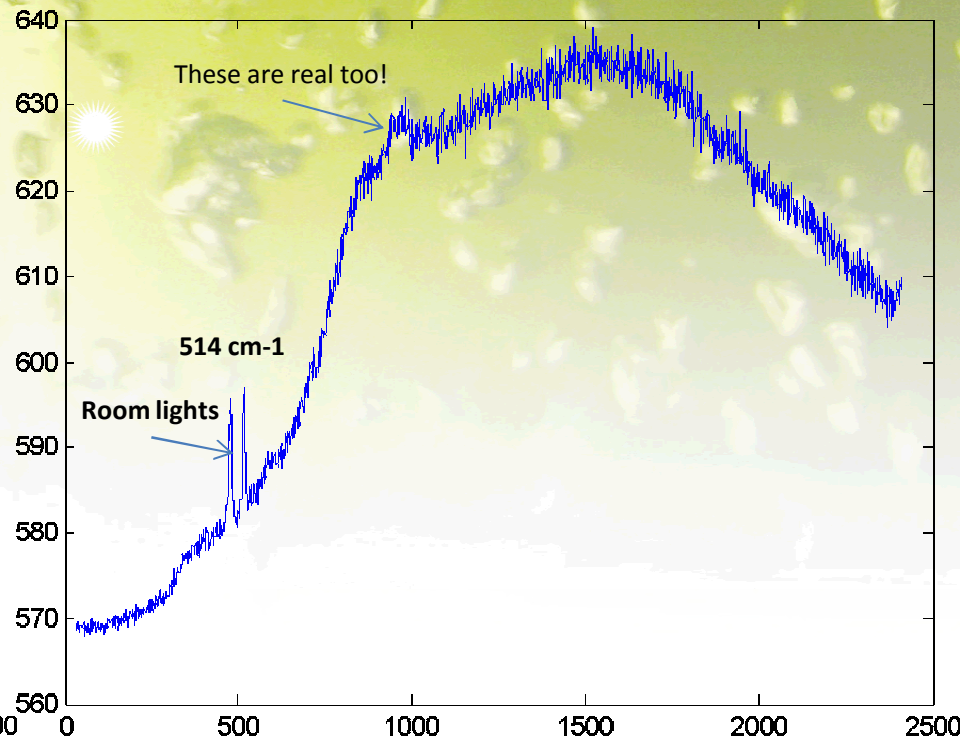
First Raman Spectra from Line-Imaging System

Teflon Tape



Silicon



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Conclusions / Next Steps

- Conclusions
  - Working with conversion team to improve the understanding of the conversion processes at the cellular level.
  - Large differences seen between the control and the acid and enzyme pretreated biomass.
  - Raman imaging provides molecular specificity
    - Increased FFA after pretreatment
    - Increased free sugars
  - High-speed Raman microscopy becoming a reality!

- Next Steps
  - Continue to work with Amy Powell and NREL to provide critical conversion insights at the cellular level.
  - Acid pretreatment studies
    - Test different treatment schemes
    - Conduct temporal studies
    - Provide higher spatial resolution for improved relational understanding
    - Identify additional Raman components (e.g., Protein)
  - Provide Amy with Raman imaging

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