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Preliminary Investigations of EtOH Producing Cyanobacteria

Work conducted as part of a scoping experiment in collaboration with Algenol

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Algenol scientists: Mitchell Rosner, Peter Heifetz

Sandia National Laboratories

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Goal: Determine feasibility of hyperspectral Raman microscopy/imaging approach for detection of EtOH production in cyanobacteria toward the long term aim of early detection/high throughput screening (either single cell or small colony)

Approach:

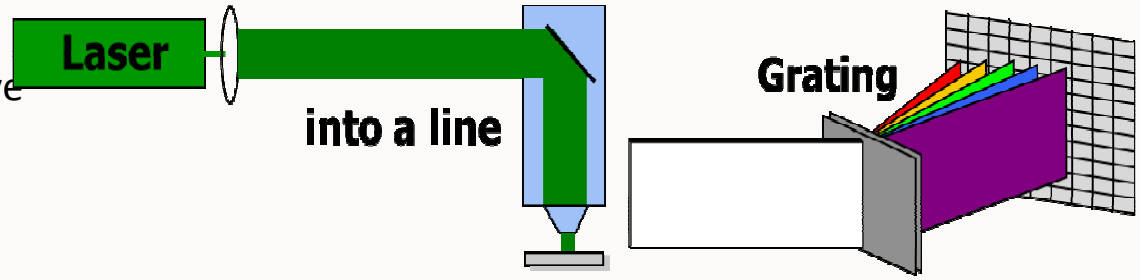
- Acquire Raman spectra of EtOH dilution series to determine lower limit of detection
- Acquire Raman spectra and line-scan Raman images of several cyanobacteria species including WT and EtOH producing strains under inducing and repressed conditions.
- Analyze these data to determine advantages and disadvantages and advise on next steps

Raman Line Scan Approach

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

- 532 nm excitation
- 10x, 20x, 40x or 60x dry objective
- Lateral resolution = 1 µm
- Axial resolution = ~2-6 µm
- Spectral range = 500-1800 cm^{-1}
- Spectral resolution = 1 cm^{-1}
- Acquisition rate = ≤2000 spectra/s

SNL's Hyperspectral Raman Line Scanner



Move line across sample to build up image

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

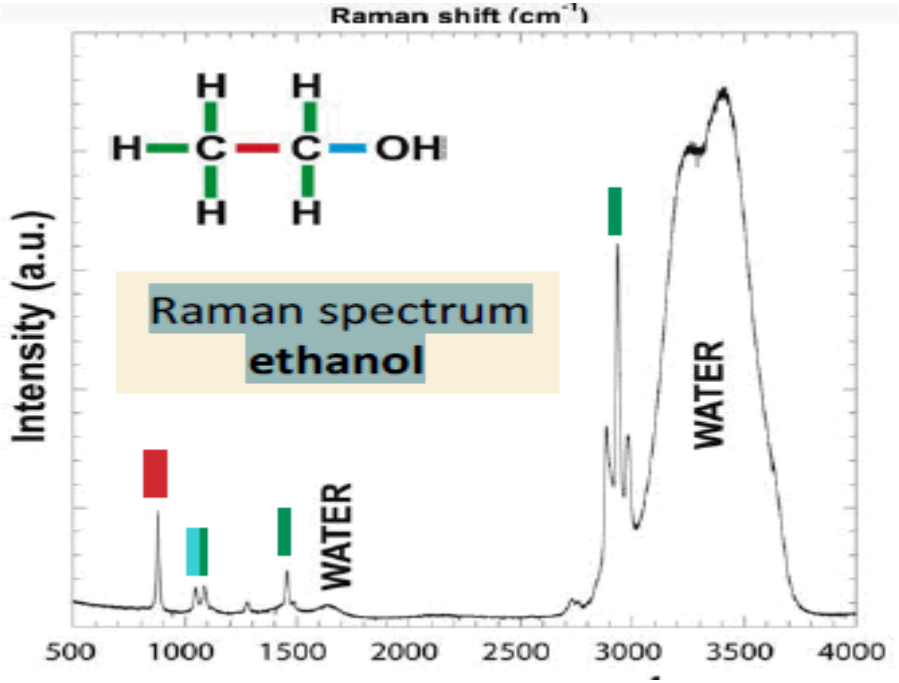
Algal Strains

- Synechococcus 7002 #1 (Algenol strain)
  - Induced at Algenol
  - Induced at SNL
  - Repressed
- Synechococcus 7002 #2 (Algenol strain)
  - Induced at Algenol
  - Induced at SNL
  - Repressed
- Synechococcus 7002 WT (SNL grown)

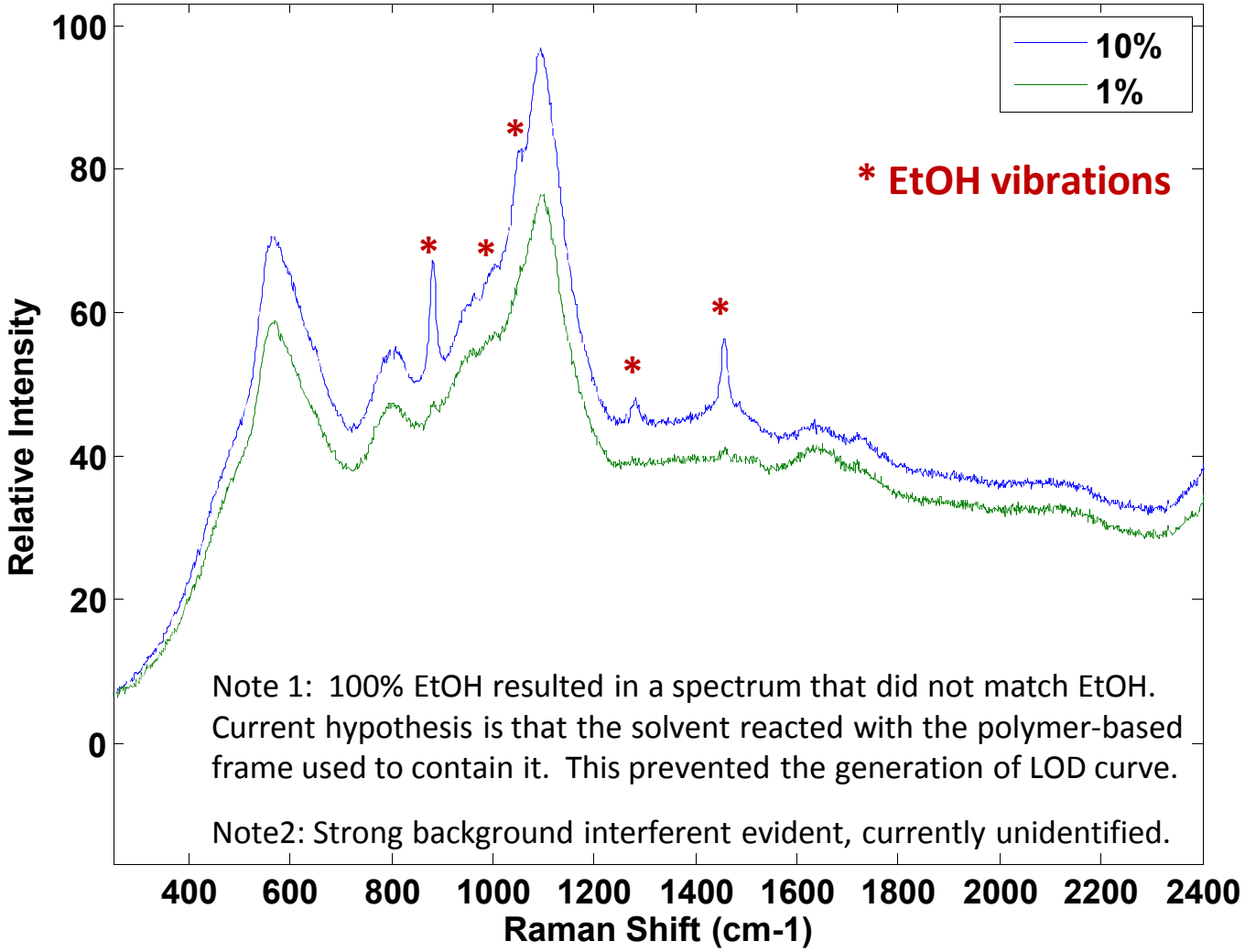
Note: Investigation of these samples was conducted on 6-27-2013 during a visit form Algenol. Prior to this visit preliminary investigations of Synechocystis and Synechococcus WT strains were conducted on two occasions (2-27-2013, and 2-28-2013)

EtOH Dilution Series

- 100% EtOH was diluted with distilled water and pipetted onto a glass slide equipped with an adhesive in-situ frame. A glass coverslip was immediately applied and the slide imaged.
- Raman spectroscopy was conducted (in imaging mode) using 532 nm laser excitation at various excitation powers and acquisition times and a 20x objective (NA 0.75).
- 100%, 10%, and 1% EtOH



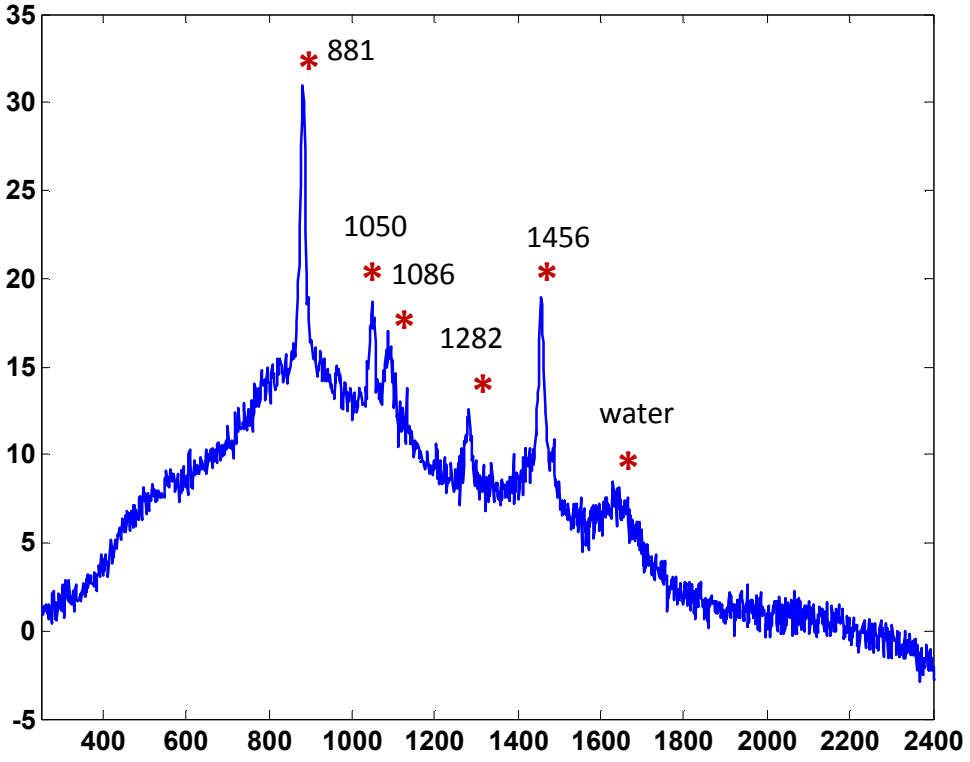
Ethanol Peaks Clearly Visible in 10% and Somewhat in 1%



Data collected with 2 sec acquisition times, 20x objective

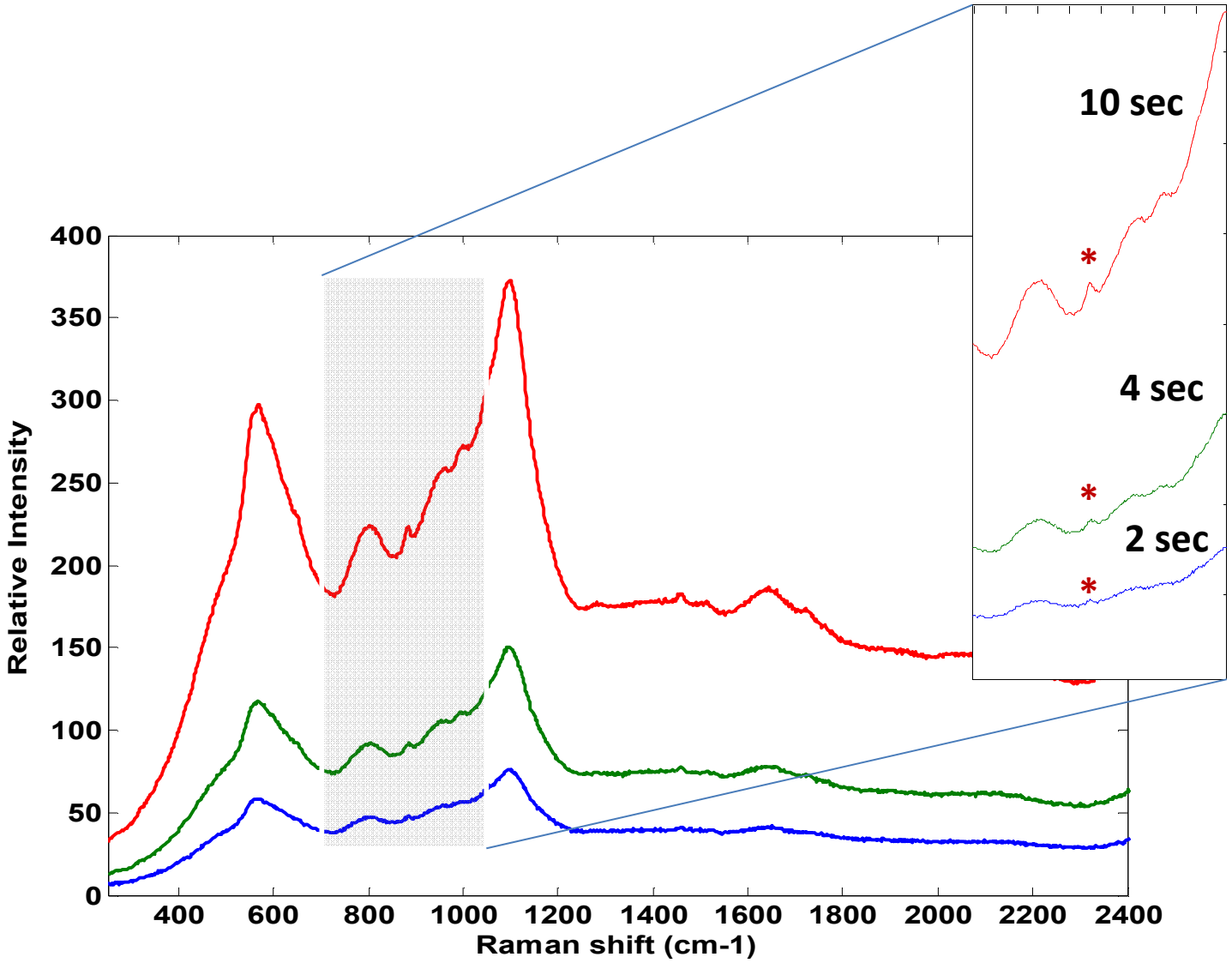
10% EtOH Spectrum Following Background Removal Matches Literature

S/N = ~15, but S/bkgd of original data much worse due to interferent



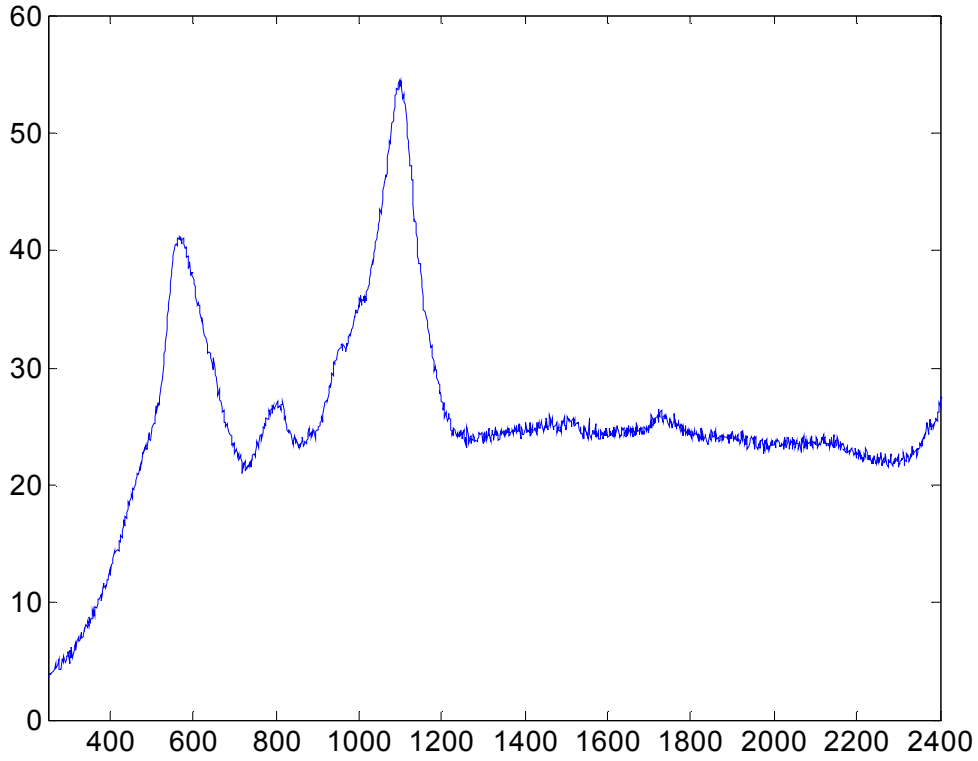
Raman Shift (cm-1)

1% EtOh at Several Integration Times



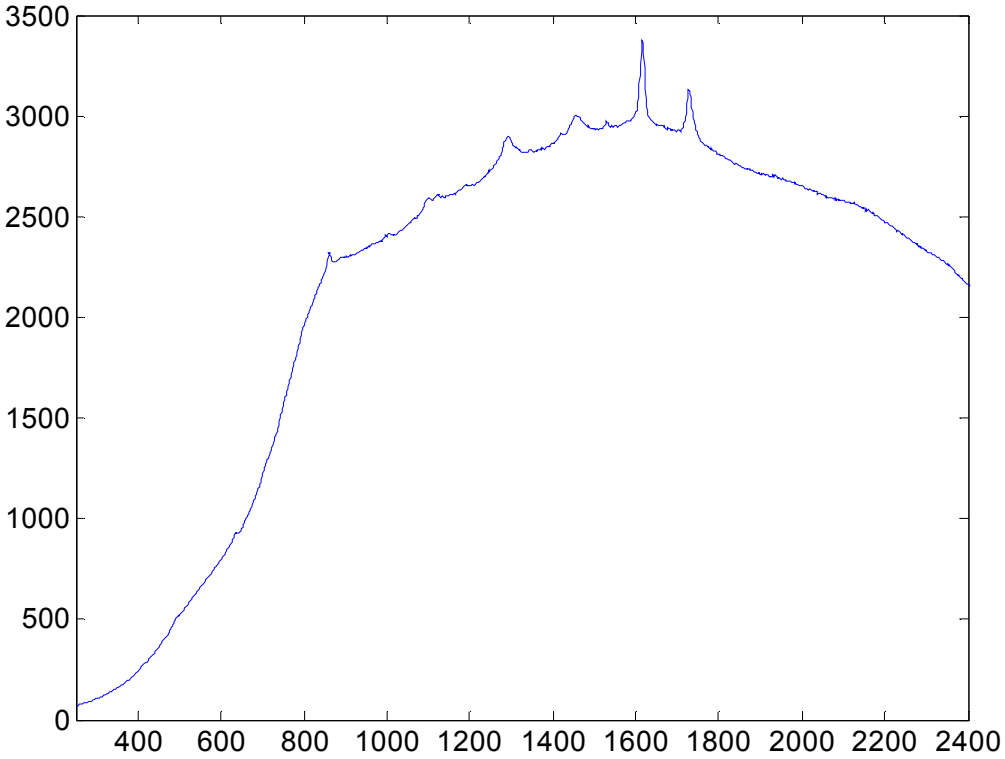
Background Interferent

Yet to be identified and present in differing amounts in all samples from this day. Possibilities include: contamination on the objective, dopants in the glass slides or coverslips. It is not from either of the samples or the EtOH itself since it occurs in both cyano samples and EtOH dilution set.

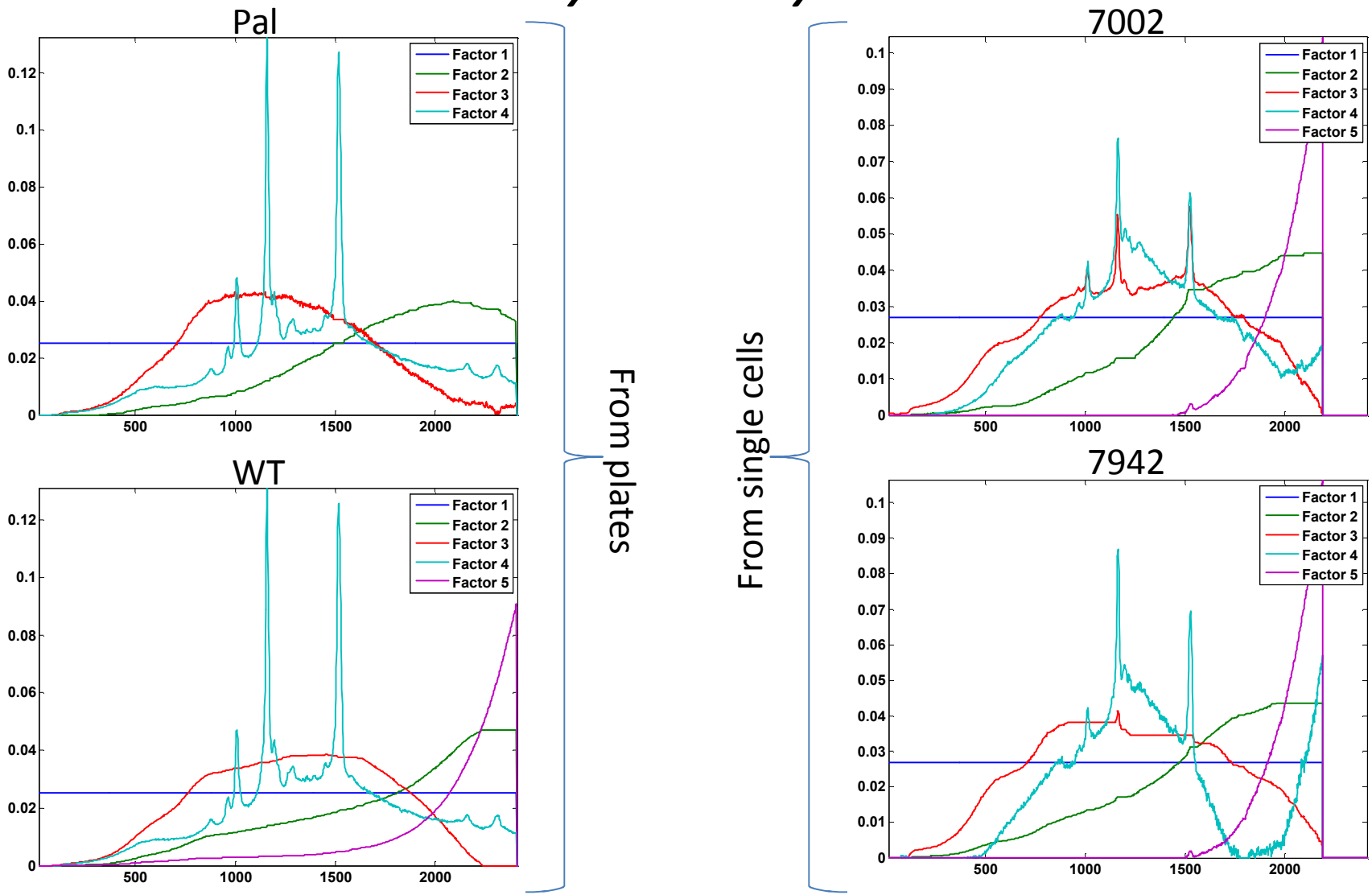


100% EtOH Spectrum

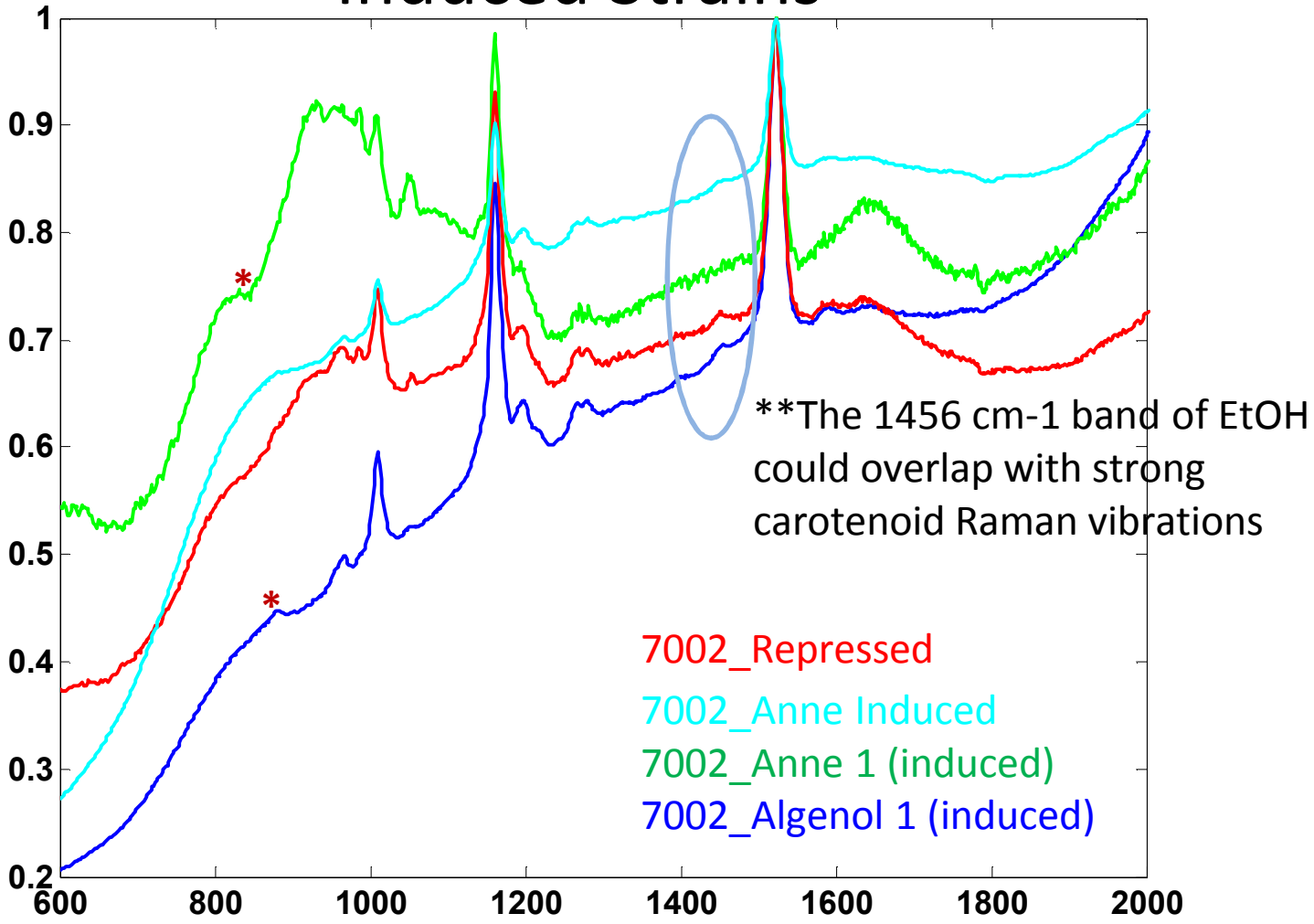
Yet to be confirmed. Current hypothesis is that this is the result of the EtOH reacting with the polymer frame used to create the well on the slide.



Previously Identified Raman Spectra from Cyanobacteria: Complexities with *Synecococcus* vs. *Synechocystis*



Preliminary Evidence of EtOH Peak (883 cm-1) in Presence of Strong Carotenoid Vibrations in Induced Strains



Mean spectra of image data set with crude normalization to carotenoid peak at 1583 cm-1.

Conclusions & Discussion Points

- EtOH LOD: Detection of 1% EtOH using this instrument and configuration is possible, but possibly not quantitative (S/N~1-2). Detection of 2-10% EtOh should be robust. Strategies to boost detection of EtOH are possible
  - More laser power
  - Higher NA objectives
  - Natural concentration from the cells
  - SERS sensor (?)
- Identifying EtOH production in living cells: Evidence for weak EtOH peaks was noted for several of the induced samples. (comparing mean spectra from the 40x objective) The detection could be enhanced by comparing cell area to area outside the cell as well as multivariate curve resolution. Unfortunately, these approaches were challenging give the limited data collected in the few hours of imaging.