MICROBIAL SPECTRAL SIGNATURES

IS A MICROBE MORE THAN THE SUM OF ITS PARTS?

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My Project…

* Use deep UV Raman spectroscopy to establish the similarities and differences between a signature of microbial cells and a mixture of organics.
Motivation…

To understand what causes the microbial signature and if we can take the spectra from the microbial components, add them together, and receive the microbial signature that we began with.
Different Paths…

- Going forward: Take microbes and break them apart into their various components and find the spectrum corresponding to each microbial component.
Different Paths…

- Going backward: Create mixtures of microbial components and find the spectrum corresponding to each.
Outcome...

- Understand how reliable the DUVRS method is for detecting microbes from organics in their environments.

- Assist in advancing the spectral library to be discussed.
The microbe consists of the following components:

- Amino Acids-Proteins
- Carbohydrates
- Nucleic Acids-DNA
- Lipids

We Know...
We Know…

Structure of the Cell Membrane

Outside of cell
- Lipid Bilayer
- Transport Protein
- Proteins
- Carbohydrate chains
- Phospholipids

Inside of cell (cytoplasm)
- Capsule
- Cell wall
- Plasma membrane
- Cytoplasm
- Ribosomes
- Plasmid
- Pili
- Bacterial Flagellum
- Nucleoid (circular DNA)
MOBIUS is a spectroscopy instrument very similar to SHERLOC.

For each spot/pixel/area analyzed:

1. Deep UV Laser illuminates surface with 40µs pulse

2. During Laser Pulse: interaction with sample results in Fluorescence Emissions and Raman Scattered photons return back to & collected by the instrument

3. Collected Light is diffracted by a grating and imaged on to a detector
Deep UV Raman Spectroscopy is...

- The process of measuring the amount of light scattered, or the vibrational motions of a molecule, using monochromatic light as an illuminator device.

- The monochromatic light, when shone on an object or substance, yields a spectrum of scattered light due to the photonic interactions with various particles of matter.
Methods...

- Defined Media
- *Shewanella oneidensis* microbes
- Amino acids, carbohydrates, and proteins—Crystallized and stock solutions
Methods...

- MOBIUS-Deep UV Raman Spectroscopy
- Cell fractionation-Ultrasonic homogenizer
Observations…
Results...

- 1200-1700: Lipid
- 2300: Nitrogen Line
- 3300: C-H stretch; Cell Membrane
Results...

- Pellet: Membrane bound proteins, lipids
- Supernatant: Free amino acids, nucleic acids
- Pellet: Not heavily contributing to whole cell spectra.
Observations...

![Graph showing Raman shift intensity for Whole Cells and Amino Acid Mixture]
A Closer Look…

Amino Acid Mixture

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Microliters</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Alanine</td>
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<tr>
<td>L-Arginine</td>
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<tr>
<td>L-Asparagine</td>
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<td>L-Aspartic Acid</td>
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<td>L-Cysteine</td>
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<td>L-Glutamic Acid</td>
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<td>L-Histidine</td>
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<td>L-Tyrosine</td>
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<tr>
<td>L-Valine</td>
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</tbody>
</table>
Results…

- 1400 & 1500: Nucleic Acids
- 2900-3300: Microbial Cell Wall
- 3300: C-H Stretch
The Future…

- Create stock solutions of nucleic acids, lipids, DNA
- Collect spectra corresponding to dried and crystallized forms of nucleic acids, lipids, DNA
- Add nucleic acids, lipids, DNA to amino acid mixture
- Understand the peaks
- Amino acid sensitivity testing
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