Leveraging Microscopy To Characterize Morphology And Autofluorescence Of Lignocellulose Degrading Microbes

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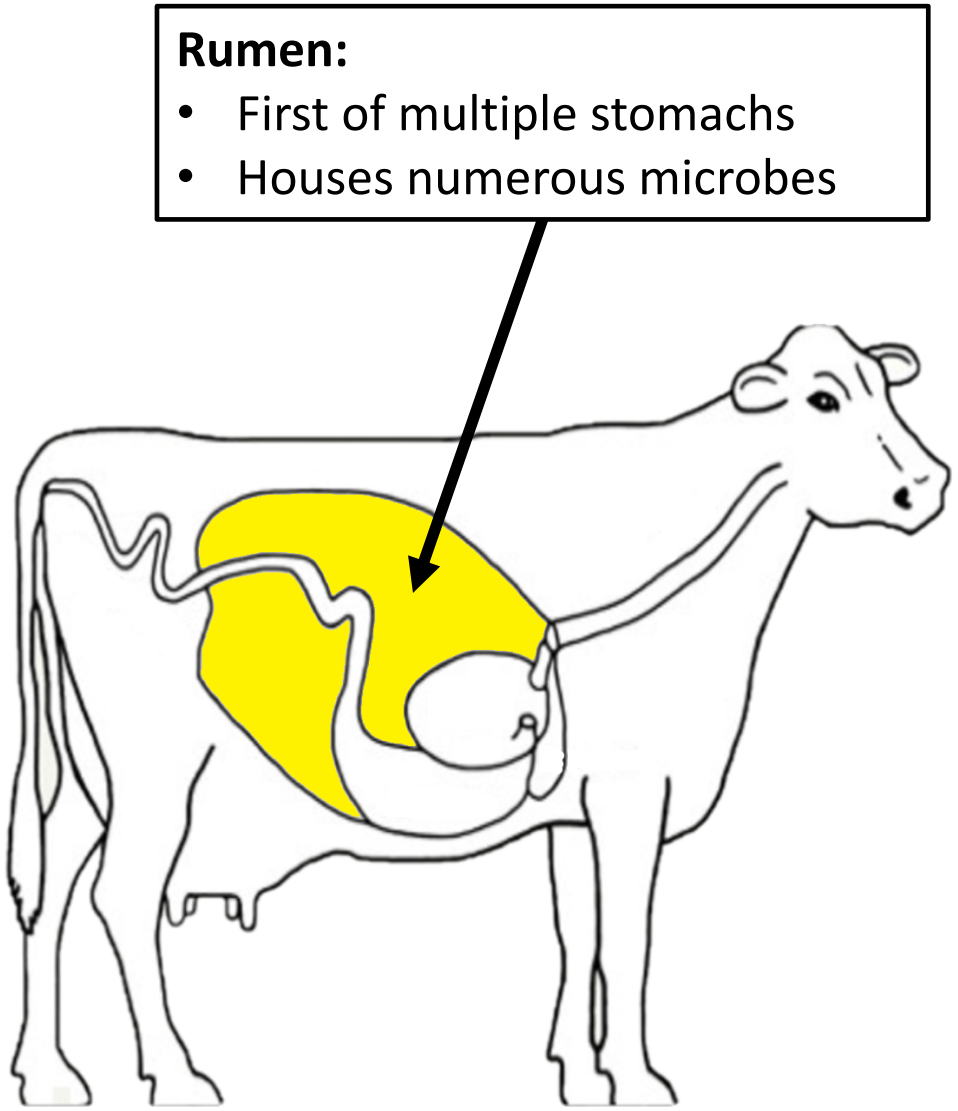
CHEMICAL ENGINEERING

UC SANTA BARBARA

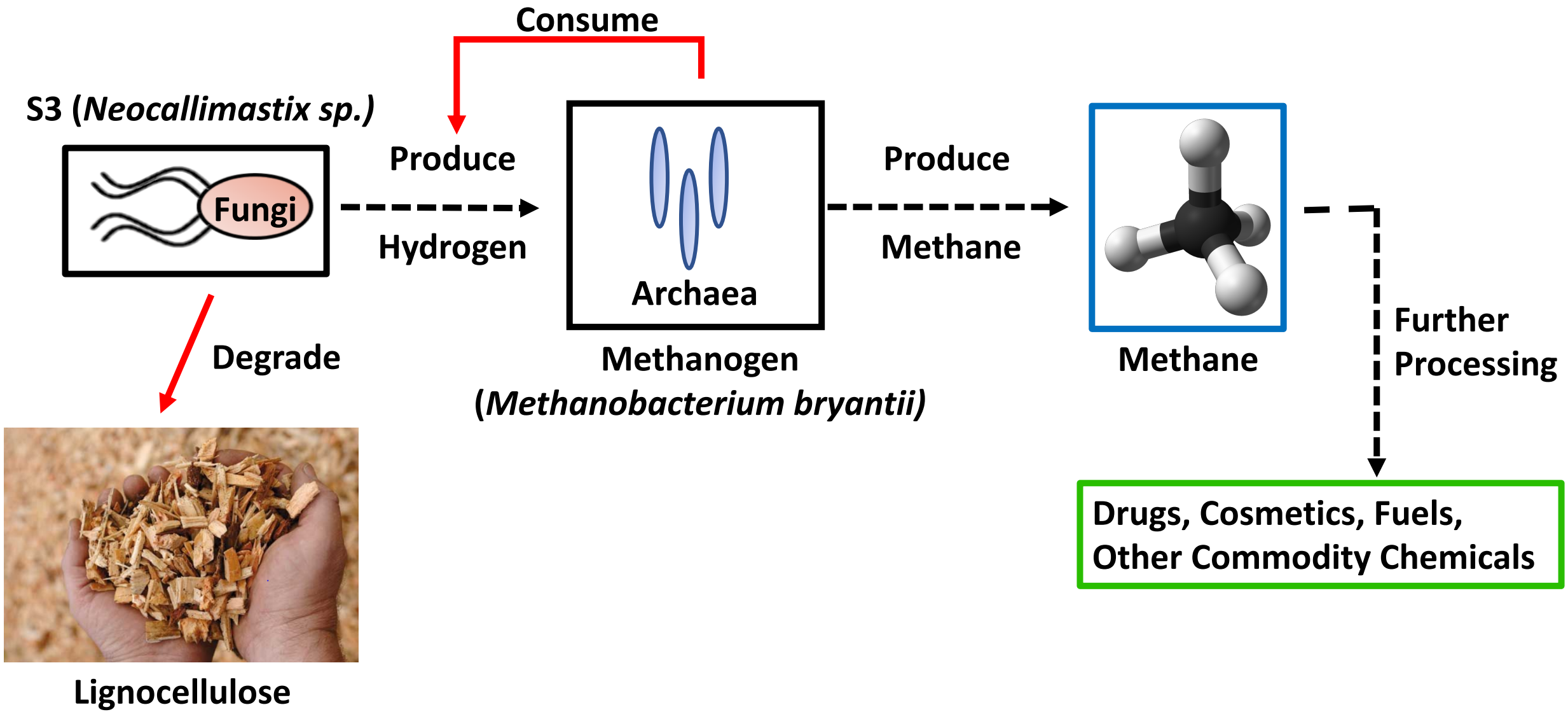
Lignocellulose is the Most Abundant Renewable Resource

Lignocellulose

- Dry woody complex in plants
- Most abundant renewable resource



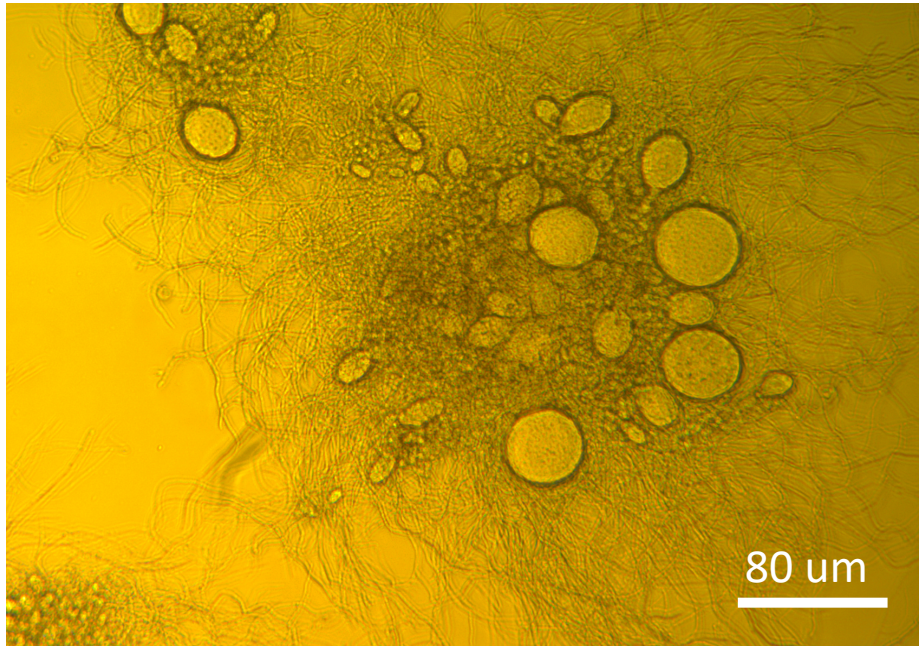
Using A Co-Culture To Maximize Degradation and Methane Production



Investigating S3: Non-Model Fungal Organisms

Why are these fungi "non-model" organisms?

- Little known about their biological functions
- Very few established methods for analysis



S3 Fungal Cells (Brightfield Microscope)

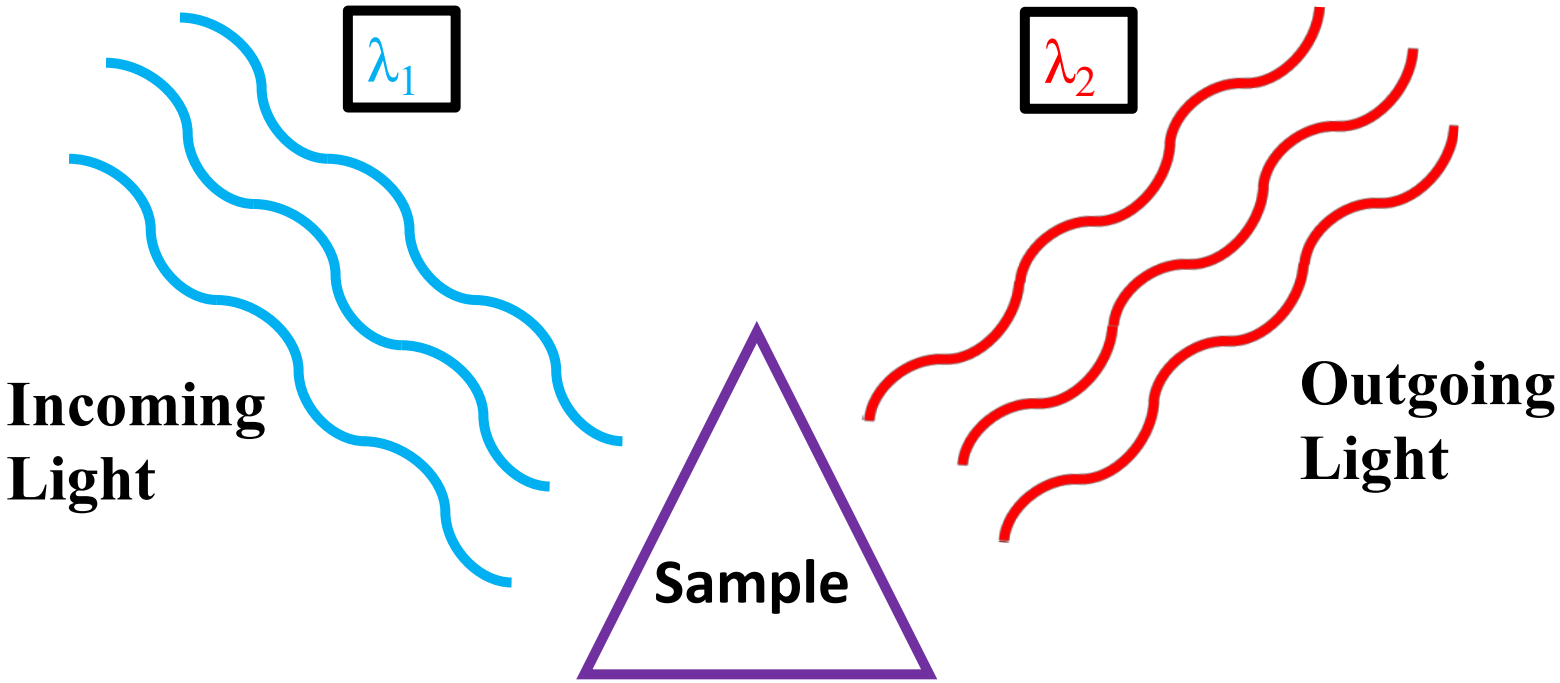
What we need to investigate:

- Morphology (shape and size) - to elucidate spatial organization
- Autofluorescence - to distinguish in mixed culture of methanogens and fungi**

What Is Autofluorescence?

Autofluorescence: The emission of light from molecules within a biological sample that have been excited by some light source.

- Can be used to easily distinguish constituents in a sample or culture



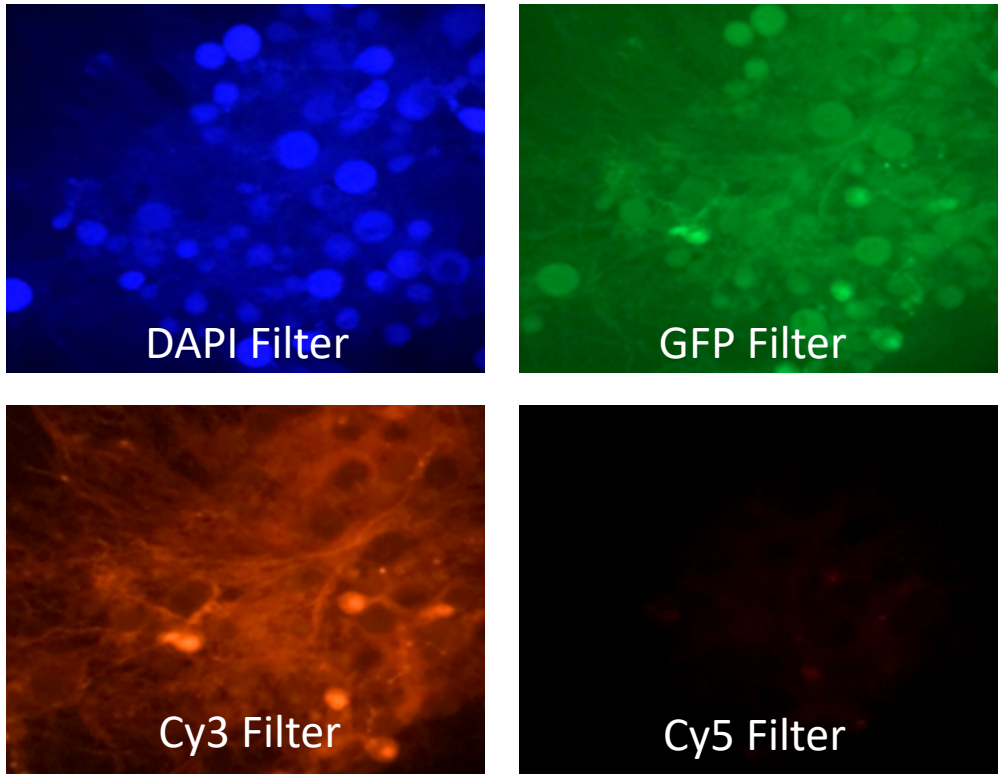
Characterizing S3 Shape, Size, and Autofluorescence

Shape

- Approximately spherical
- Branching legs/roots (rhizoids)
- **Average Size:** 39.41 +/- 1.59 um (diameter)

~2/5 of a human hair!

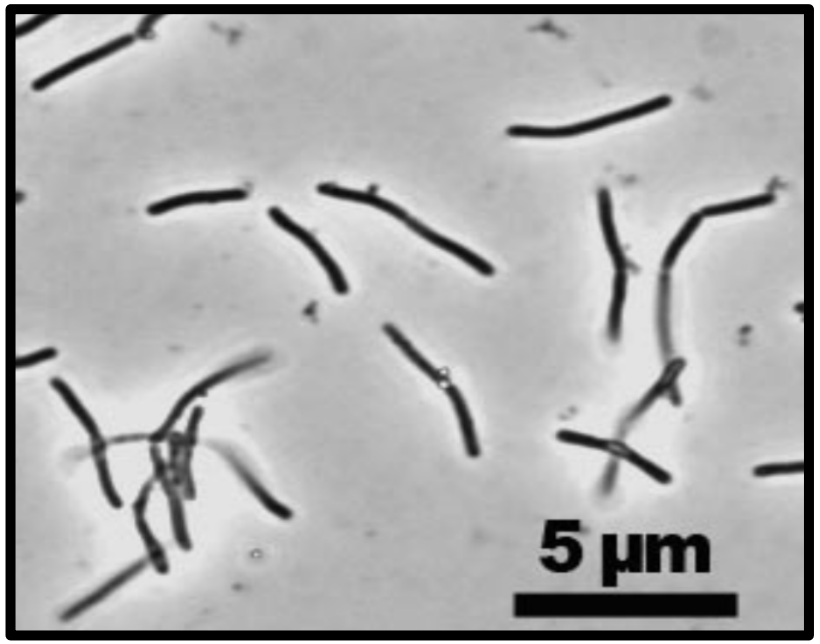
Autofluorescence:



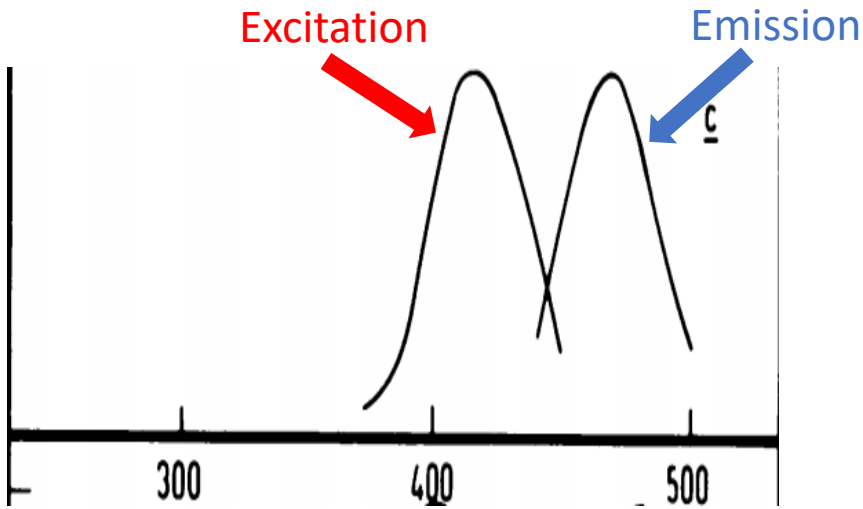
Leggieri et al. - In preparation

Characterizing Methanogen Shape, Size, and Autofluorescence

Shape[1]



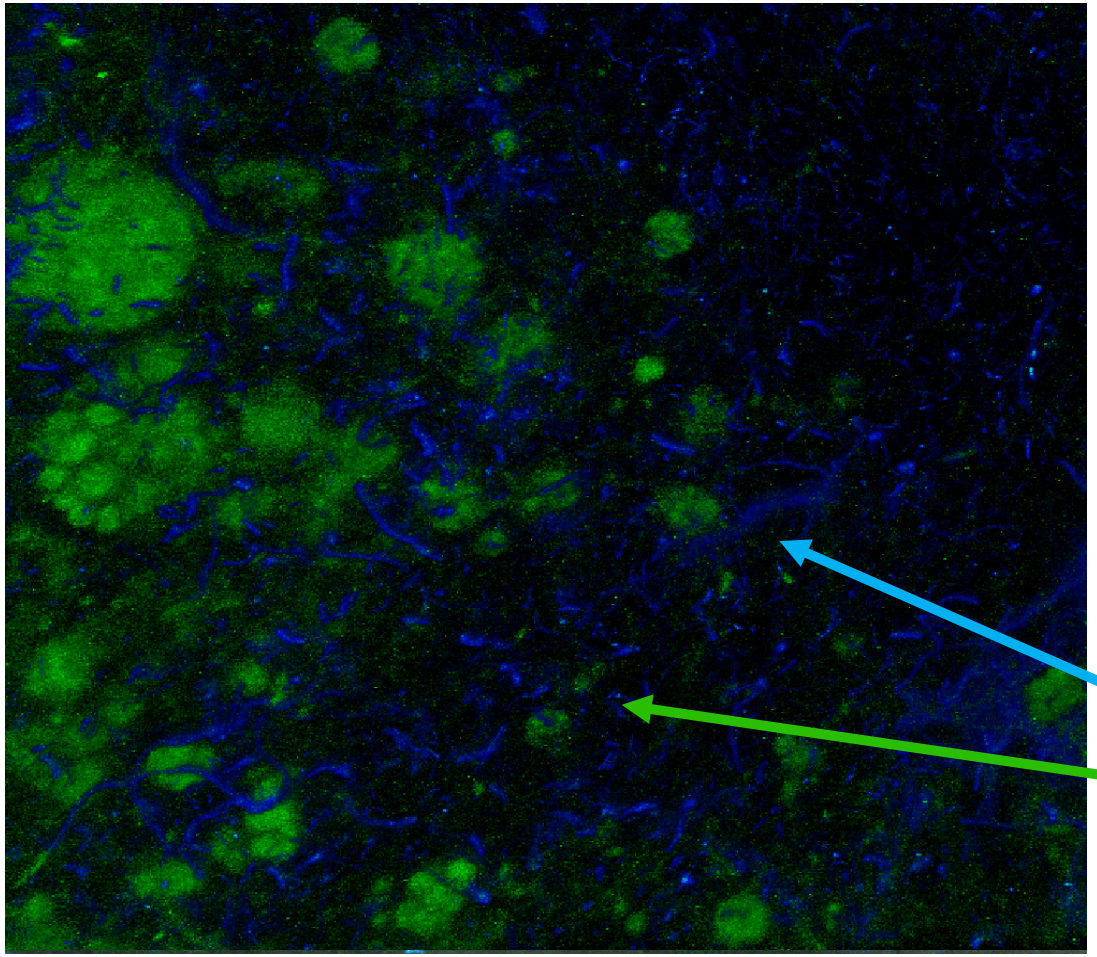
- Approximately cylindrical (rod-like)
- **Average Size:** To Be Determined



Excitation - Emission Spectra for Methanogenic Autofluorescence

Dodema and Vogels, 1978

We Can Distinguish Fungi From Methanogens Using Autofluorescence



Confocal Microscope Image of Co-Culture

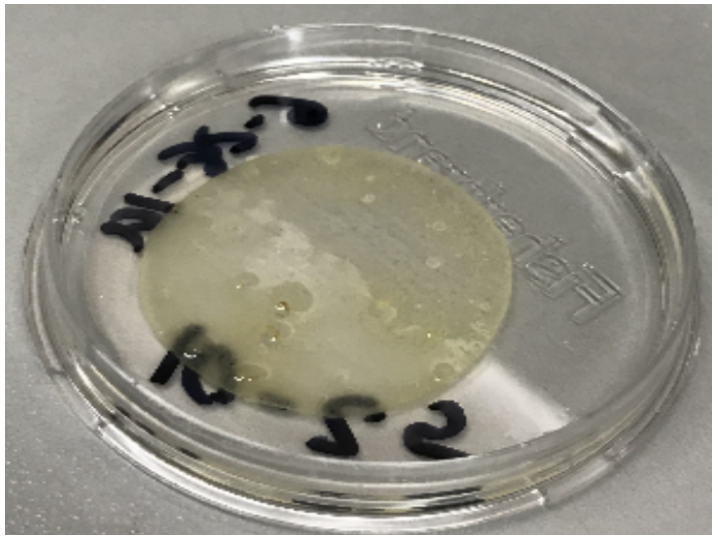
Saves us an immense amount of time:

- No need to engineer a genetic transformation
- No need to use fluorescent tagging
- No need to use stains

Blue = Methanogens (Wavelength: ~460 nm)

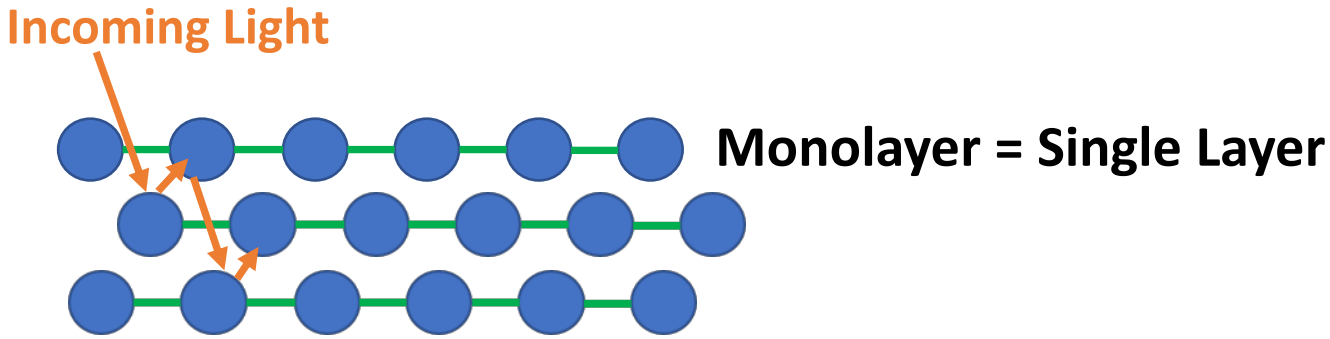
Green = Fungi (Wavelength: ~510 nm)

We Need Thin Films Before We Can Begin Investigating Co-Cultures



S3 Fungal Biofilm

Major Complication!



Need to develop:
- Repeatable method for forming biofilms
- Monolayer formation <100 um

- **Want:** Single-layer film
  - Thicker films = noisy images
- **Parameters:** Substrate volume and concentration, film adhesion to surface

Thin Fungal Biofilms Will Enable Further Analysis

- Begin co-culture monolayer film analysis
- Determine what causes the cells to autofluorescing (Proteins? Carbohydrates? Etc.)
- Quantitative characterization of autofluorescence parameters:
  - Fluorescence lifetime
  - Fluorescence emission intensities
  - Fluorescence bleaching

References

1. Concept Map - Mind Map. Retrieved from https://www.mindomo.com/mindmap/concept-map-e370e2afb381427485de9c7469b5f268

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