Can you identify spores of fungal species on Coleoptera with hyperspectral interferometry?

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Vocabulary

- Hyperspectral interferometry
  - Hyperspectral = involving the electromagnetic spectrum
  - Interferometry = technique that uses superimposed waves (electromagnetic for example) to gain information

- Fabry-Perot etalon model
  - Consisting of 2 reflecting mirrors, it measures small differences in wavelength by the interference produced, including an adjustable distance between mirrors

- Power spectrum
  - Plots a signal's power (energy over time) within a frequency category

Vocabulary

- Near infrared (NIR) spectrograph
  - Near infrared = 780 – 2500 nm of the electromagnetic spectrum, just above visible light
  - Spectrograph = a tool for photographing/recording spectra with a camera by separating light

- Fast Fourier Transform (FFT)
  - Used to calculate power spectra
  - An algorithm that converts a signal from its original form to a representation in a frequency category

Introduction

Previous interferometry work used a Fabry-Perot etalon model to locate and size features on the surface of *Hylastes tenuis* (Beach et al. 2015)

- – Measured the length of setae from the tip
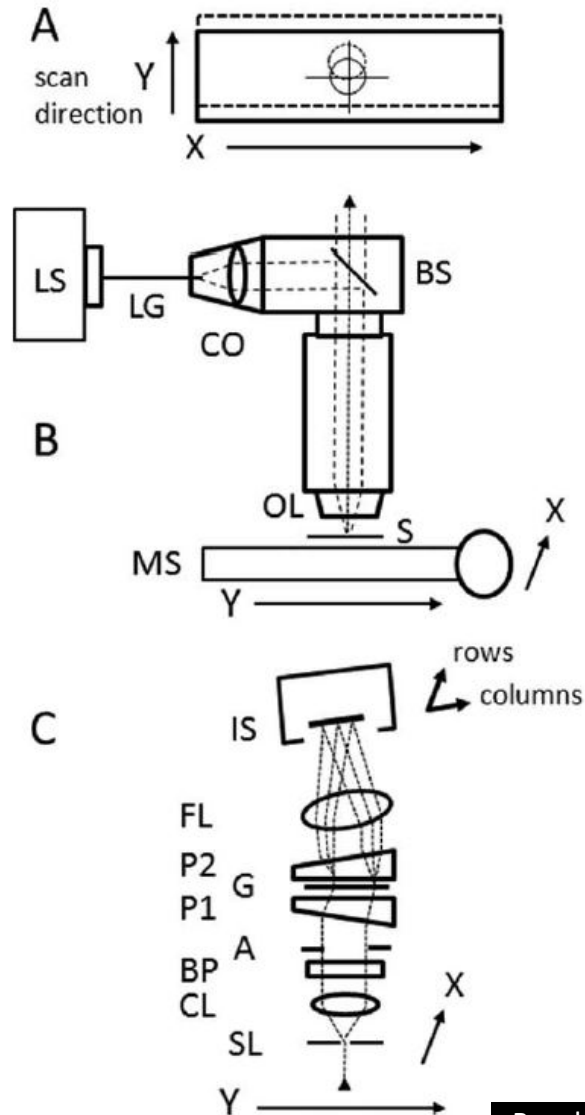- – Located regions containing spores
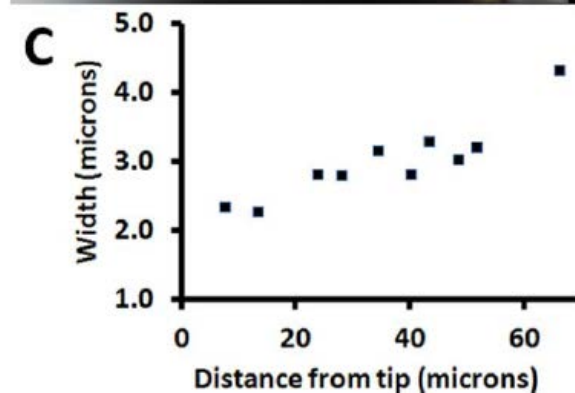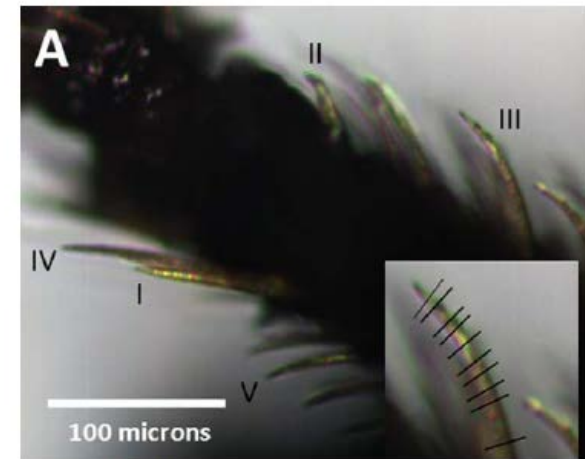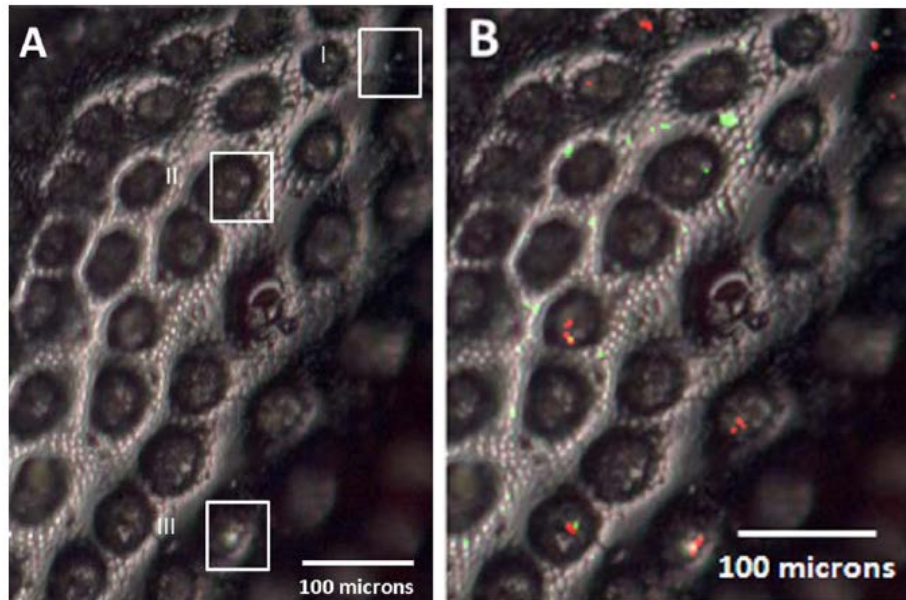
Objective

Determine if hyperspectral interferometry can speciate fungal spores on the bodies of bark beetles

Methods – The Microscope


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The Microscope

A) Specimen (circle) mounted on a glass slide, Y and X axes illustrated as the slide moves on a motorized stage

B) Microscope components with reflected light illumination: light source (LS), liquid light guide (LG), collimator (CO), beam splitter (BS), objective lens (OL), sample (S), motorized X/Y stage (MS)

C) Inspector prism-grating-prism spectrograph setup; camera mounted above beam splitter: entrance slit (SL), collimating lens (CL), higher order blocking band pass filter (BP), aperture stop (A), prism 1 (P1), holographic transmission grating (G), prism 2 (P2), focusing lens (FL), and camera image sensor (IS)
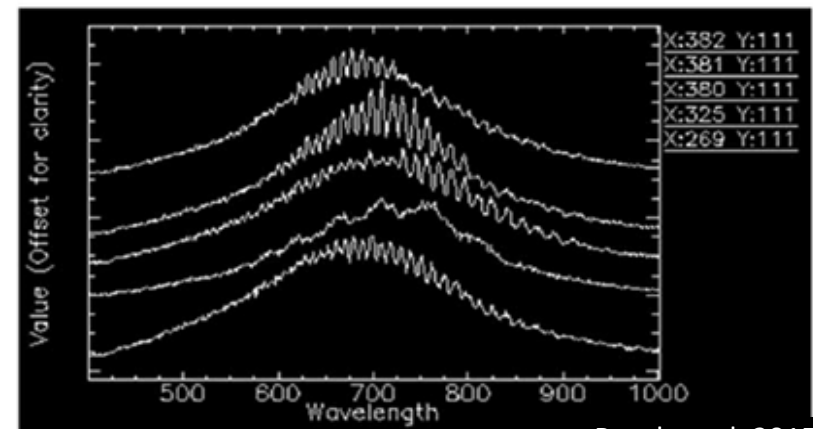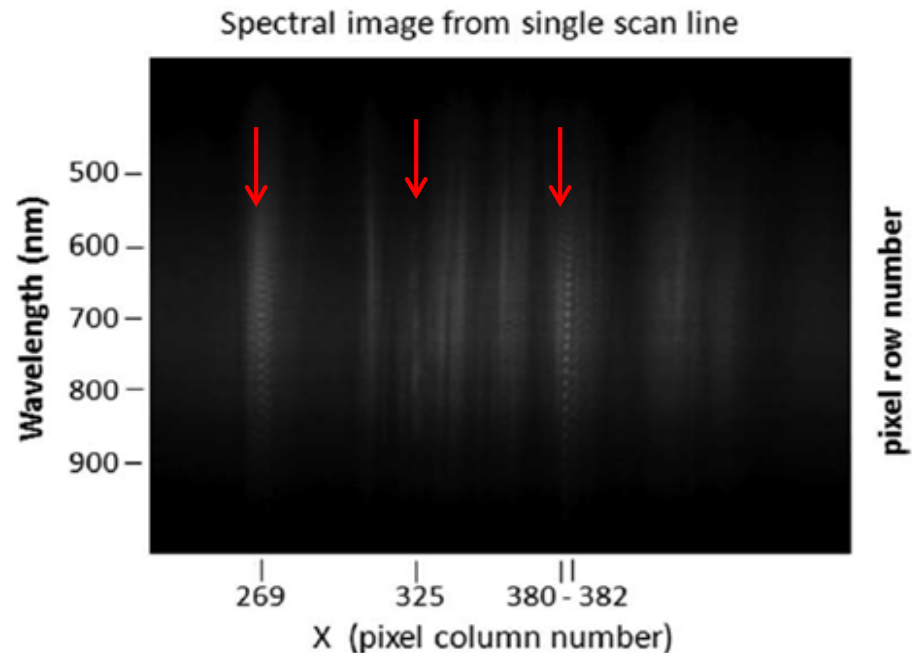
Beach et al. 2015

Methods

- Specimen is moved incrementally with a motorized stage
  - Spectrographic camera takes images with every stop
    - Has a visible near-infrared (NIR) spectrograph
    - The travel of the stage is guided by a motion algorithm so it is equivalent to the spacing of the image pixels for correct image geometry
  - 696 lines → processed into a final image and data cube
- Program ENVI 4.8 performs spectral analysis while ImageJ is used for spore sizing

lookfordiagnosis.com
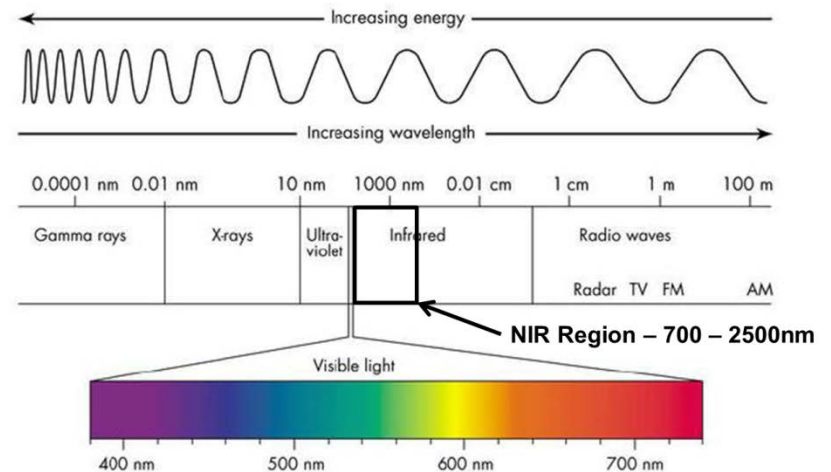
Methods

- Example:
  - Regions of interest appear as bars resembling venetian blinds
  - Wavelengths correspond to sizes

Spectral image from single scan line

Beach et al. 2015

Methods - Fungal Spores

- *Grosmannia alacris*
  - Oblong shaped, with truncate bases and rounded apices
  - Ranges from (3.3 – 7.8) x (1.4 – 2.8) microns
- Pine twig agar (PTA), oatmeal

Jacobs and Wingfield 2001

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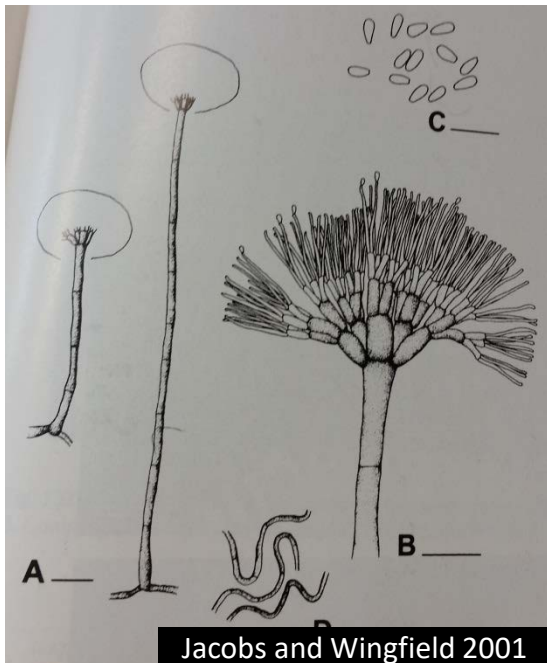
Fungal Spores

How can we get the size of a spore we see?

Transmitted Light Spectrum

- Peaks/valleys occur at 495.11 (n_1) and 449.75 (n_2) nm
- Step 1: 1 / ($n_1 - n_2$) = 0.02205
- Step 2: 0.02205 x n_1 x n_2 = 4909.08
- Step 3: 4909.08 / (2 x 1.53) = 1583.57 nm

And thus . . . 1.583 microns

Methods

- Spores are sticky!
- Glass seed beads to reduce glare
  - Able to roll on media plates
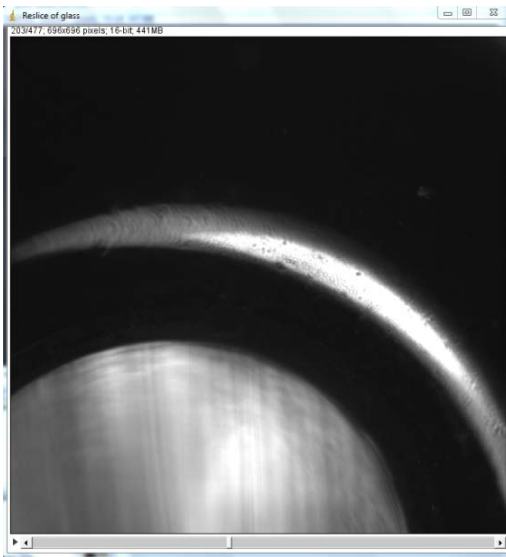
Control bead
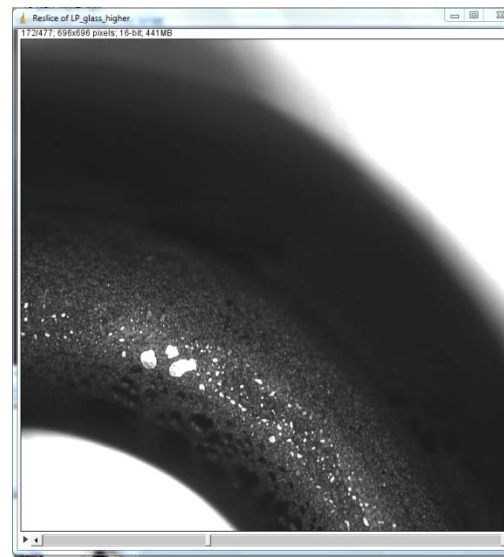
Beads with spores
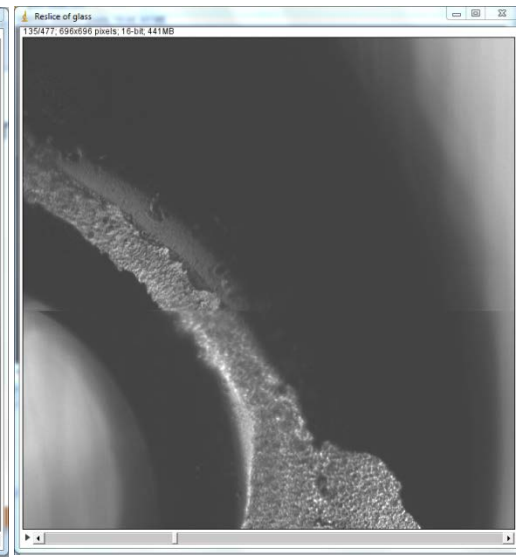
Methods

Spore size
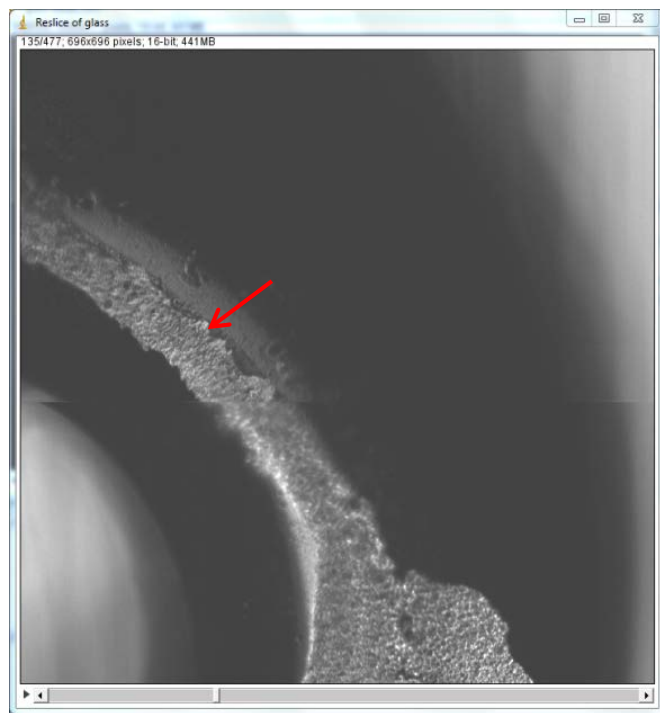
Bead with spores
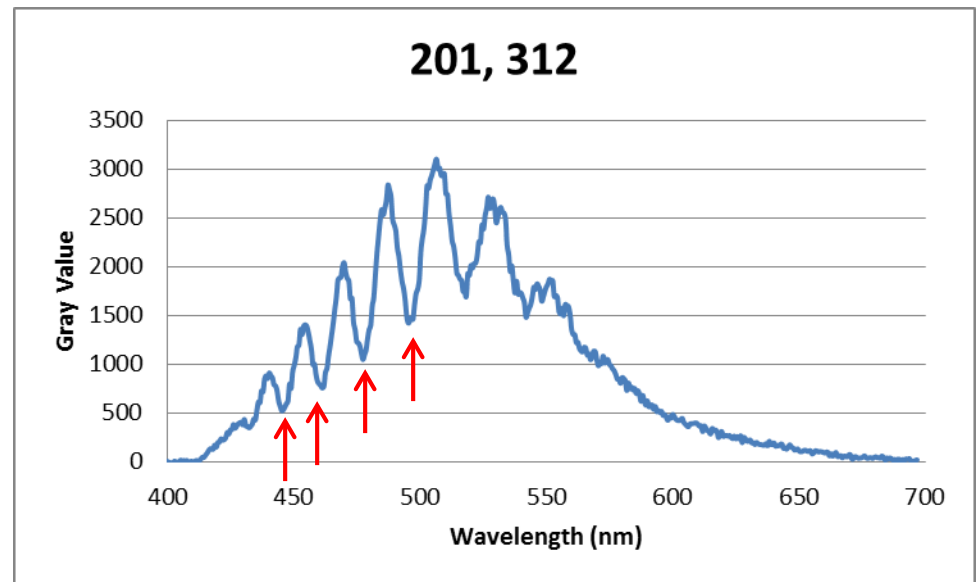
- Valleys occur at 444.1, 460.77, 478.08, and 496.04 nm
- Following the formula, this gives us an average spore size of 4.16 microns for this coordinate

Methods - Beetle Capture

- *Hylastes salebrosus*
- Two locations:
  - Louise Kreher Forest Ecology Preserve
  - Mary Olive Thomas Demonstration Forest
- Baited with 95% ethanol + 90% 3-carene
  - Replenished every 3 days

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FHDL
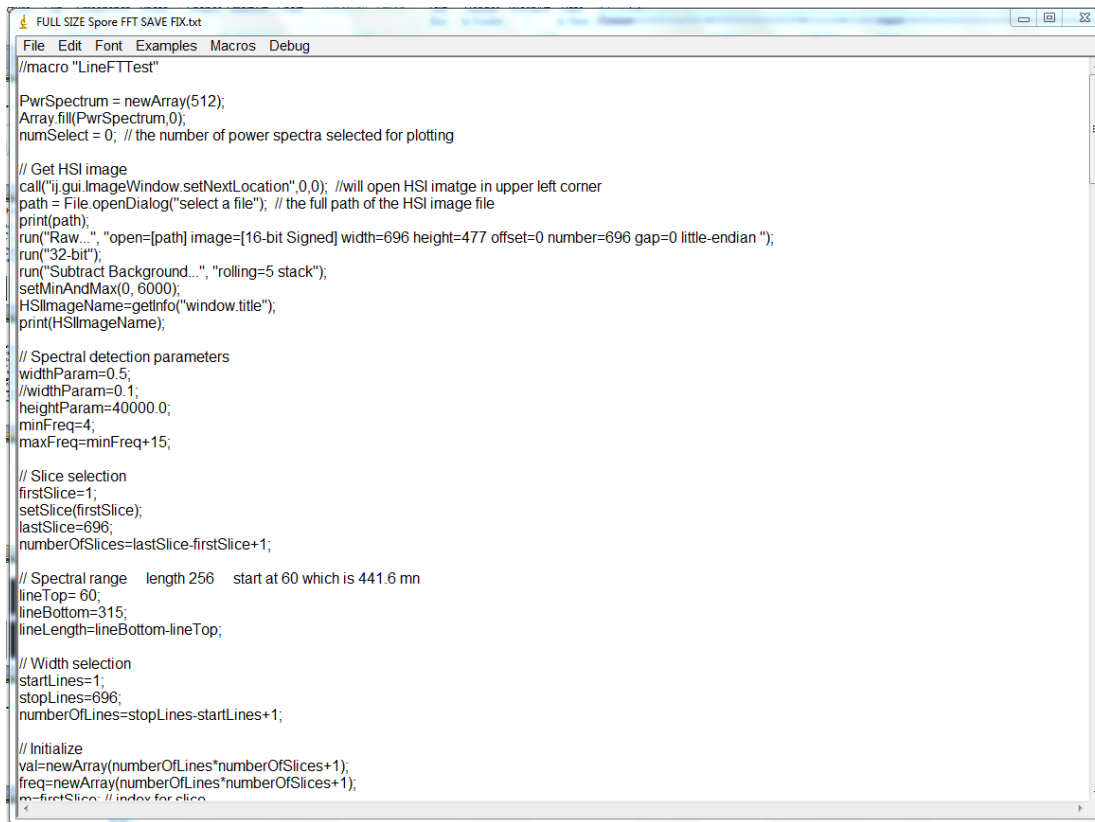
Methods

- Captured beetles kept in the cooler (4°C) for up to 30 days
- Sterilized in 95% ethanol for 30 seconds prior to prep
- Rolled on plates of sporulating fungal cultures
    - Simulate how beetles naturally pick up spores
- Spore loaded beetles taken to CytoViva for imaging
- Resulting images were captured with ENVI and spore maps obtained with the use of ImageJ
    - Developed program (FFT map) for use in ImageJ to pick up objects (spores) that had wavelengths above certain amplitudes after observing control images

Methods

FFT map → into ImageJ

– "Macros" → "Run Macro" → open a captured ENVI image file
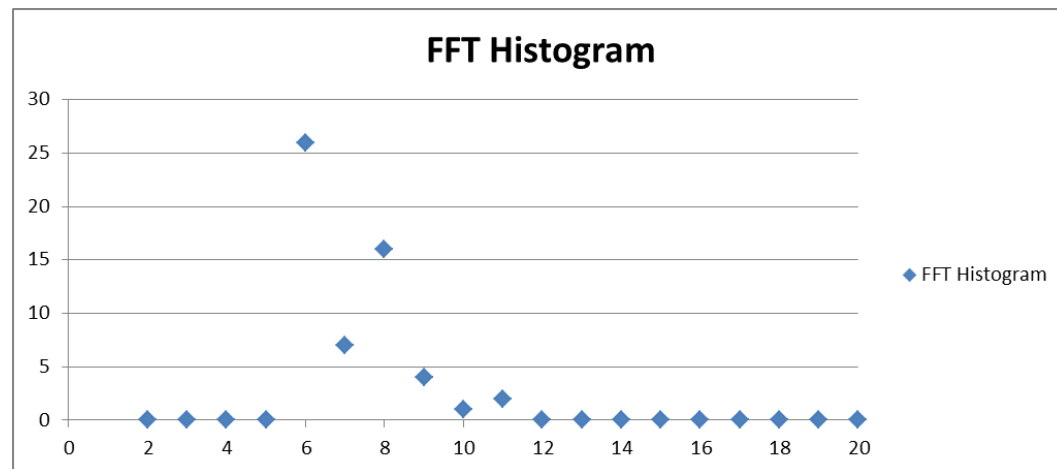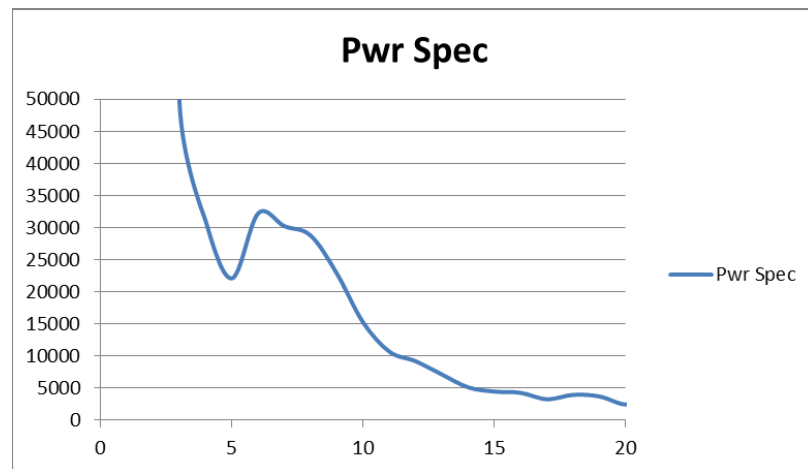
Methods

FFT test results

- Power spectrum
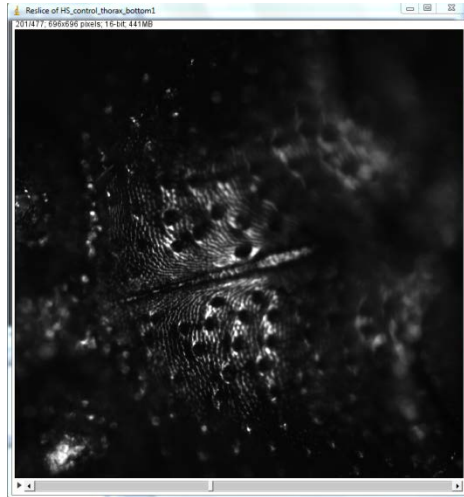- Histogram
- Data file
- Log box
- Map
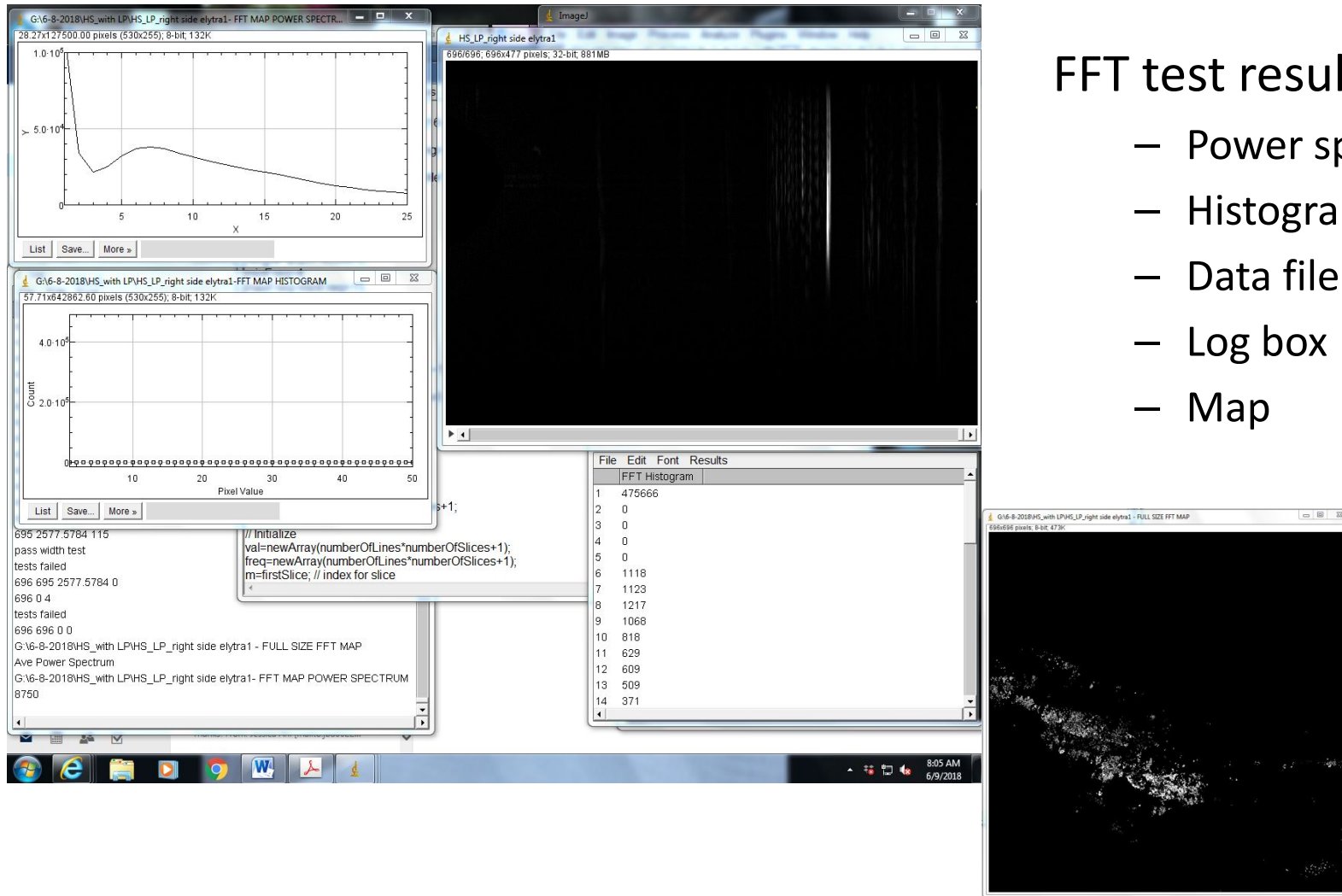
Methods

- ## What does the power spectrum tell us?
  - Gives index of frequencies – a relative measure
- ## What does the histogram tell us?
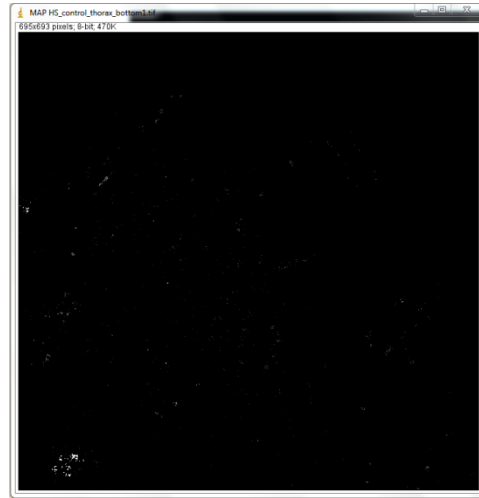 - Tells us where most objects rank in the image
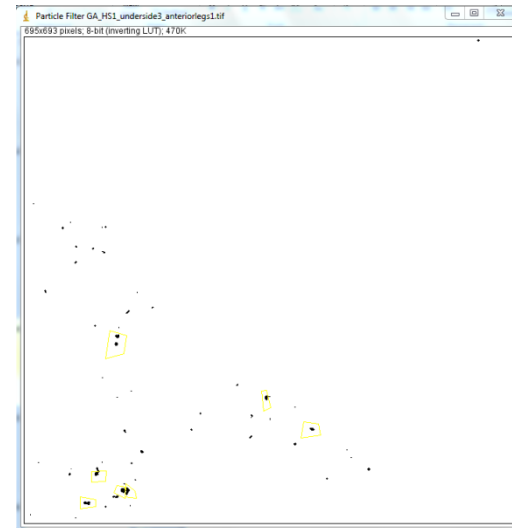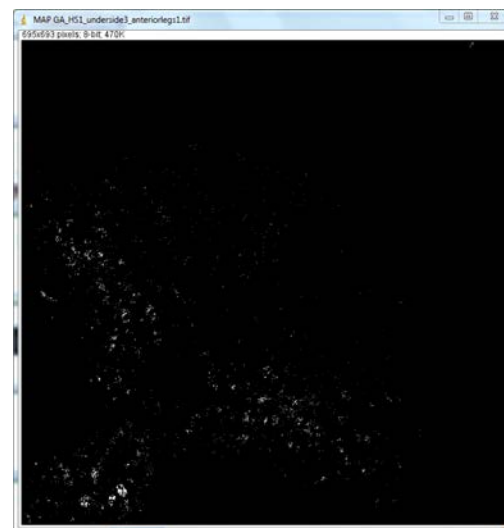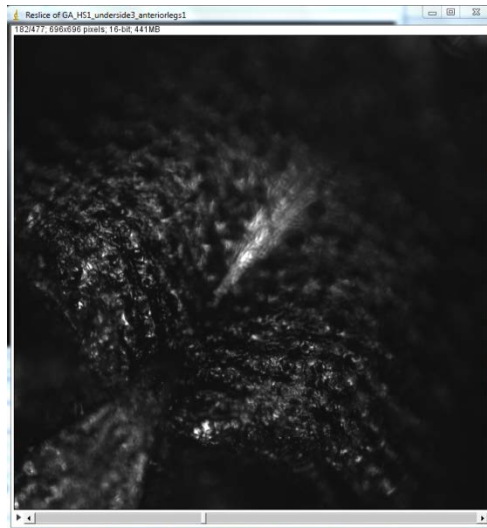
Results

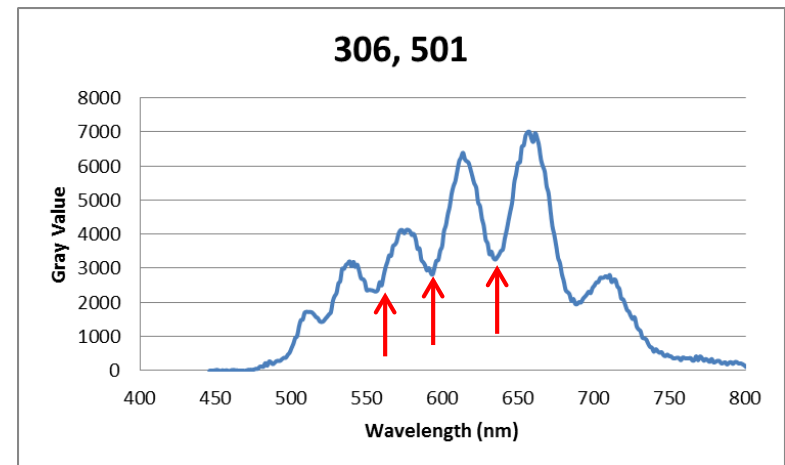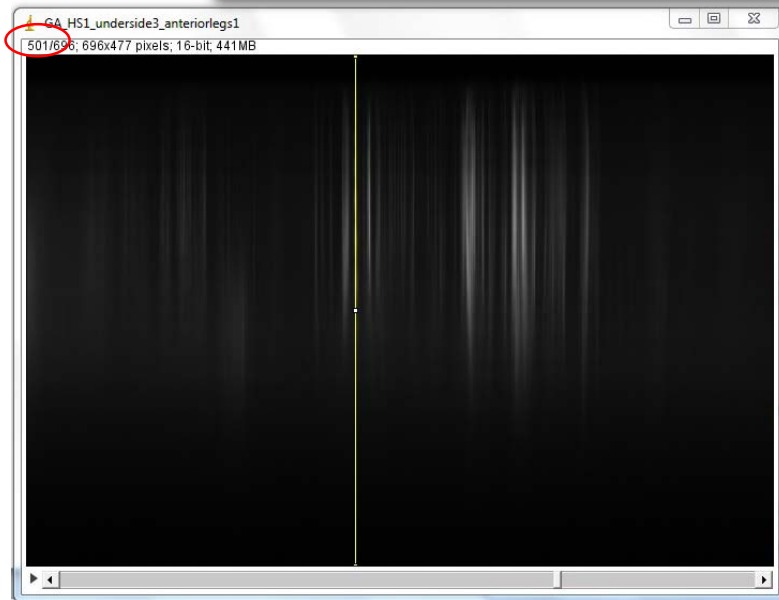ENVI images   FFT maps
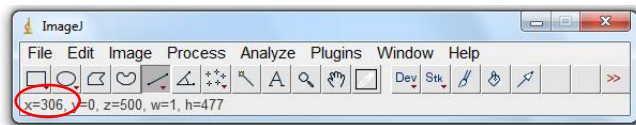

← Control beetle

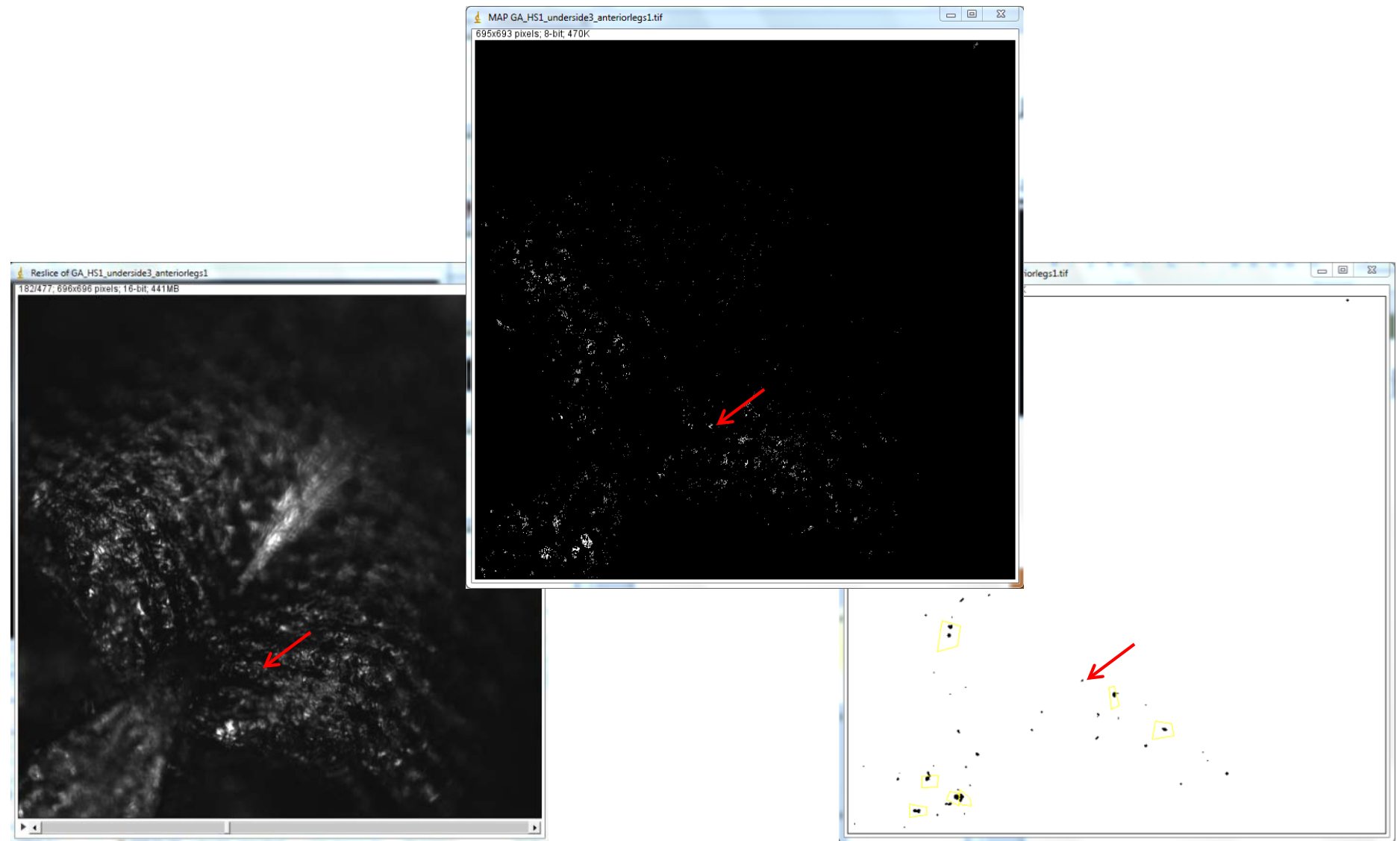Beetle with *G. alacris* spores
↓

Results

Using maps and data file, go through and locate coordinates of interest

- From the 1st peak to the 2nd, the size is 2.59 microns
- From the 2nd peak to the 3rd, the size is 2.805 microns
- The average –and final – is 2.7 microns

Results

Results

From two images taken off a *Grosmannia alacris* spore loaded beetle, we obtained 5 points of interest

- Each image had an average spore size of 2.485 and 4.052 microns, respectively
- Overall average was 3.268 microns

Discussion

- We know *G. alacris* (GA) spores are irregularly shaped, like pills/eggs
  - Sizes range from (3.3 – 7.8) x (1.4 – 2.8) microns
- Our average spore size for two hyperspectral images on a beetle was 3.3 microns, and falls in this range
- From an image of the GA spore loaded glass bead, we got an average size of 4.4 microns
- Next step is to compare with *Leptographium procerum* on beetles and compare with *G. alacris*
  - Look at sizes as well as power spectra patterns/curves
  - Then can run a statistical analysis with a t test to look for a difference

Acknowledgements

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