Mapping spores on intact bark beetles using CytoViva Hyperspectral Imaging

Lori Eckhardt and Jim Beach

Spore detection was carried out by hyperspectral imaging on two species of Hylastes which had been rolled in spore material.

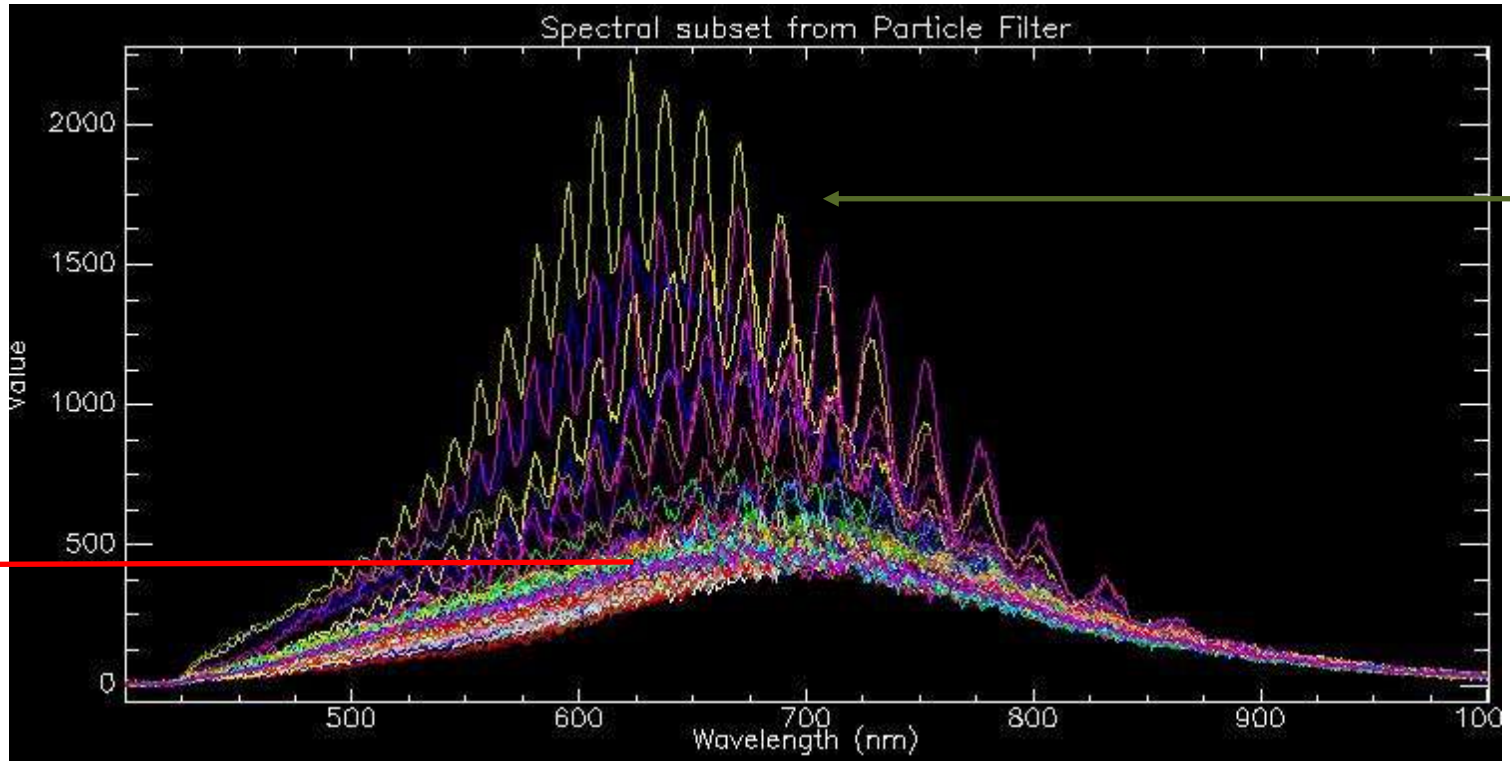
An oscillatory spectrum was found from regions of the reflected light image in all samples.

Only insects exposed to spores showed high frequency oscillations. The frequency pattern in the spectrum is presumed to be a spore signature.

Here we present the locations of oscillations in spectra on data cubes obtained from reflected light hyperspectral microscopy on whole beetles, obtained using different analysis methods.

a spectral matching method - spectral angle mapper
a frequency detection method - Fourier mapping

VISNIR reflected light spectra from *Hylastes tenuis* showing oscillatory patterns



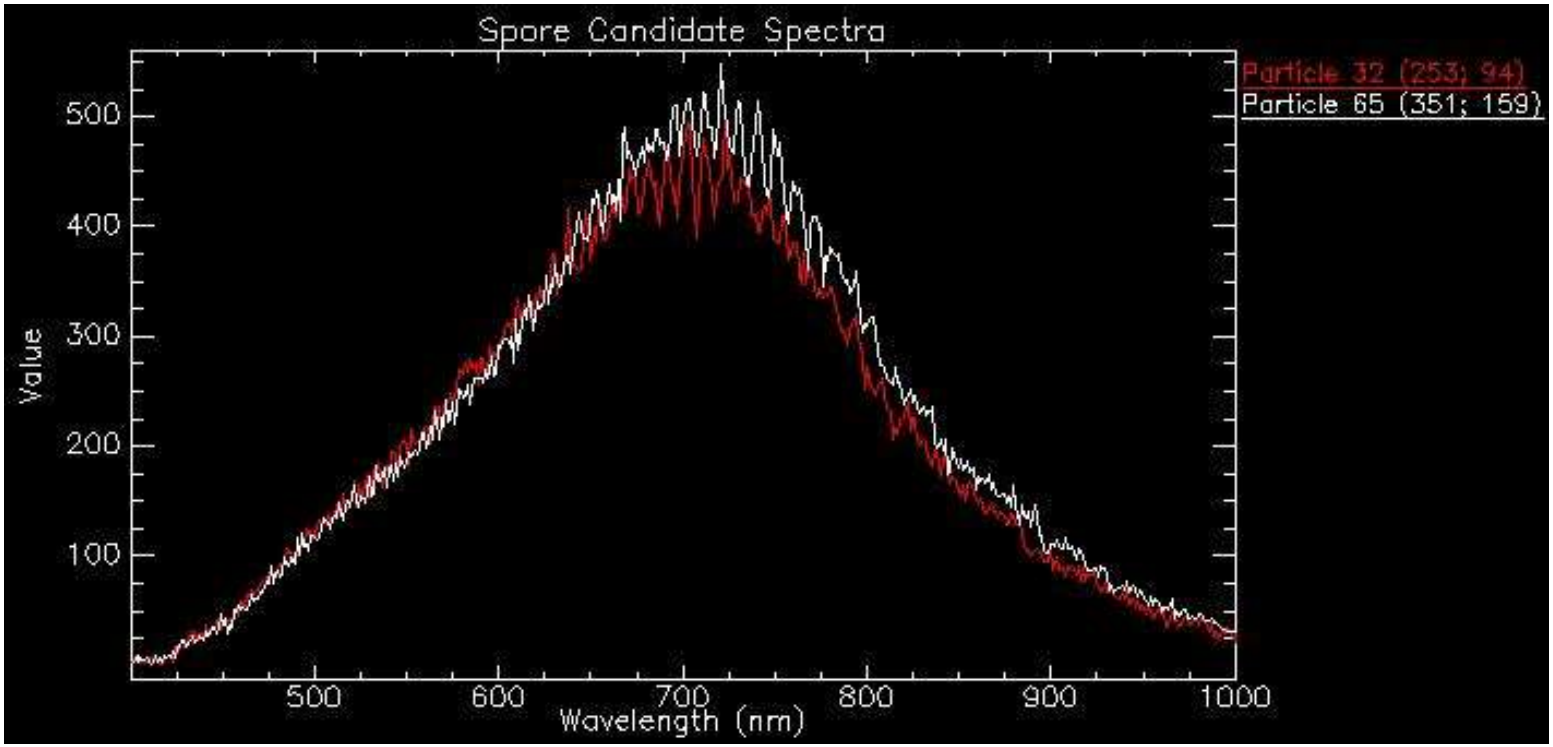
large amplitude oscillation, low frequency, strong reflection

w and w/o spore

small amplitude oscillation, high frequency, weak reflection

only w spore

Small amplitude high frequency oscillation from Ht these kinds of oscillations were seen only from beetle with spore



Spore Detection on *Hylastes tenuis* and *Hylastes salebrosus*

- using method of particle filter followed by spectral angle mapper -

Acquire reflected 10X data cube

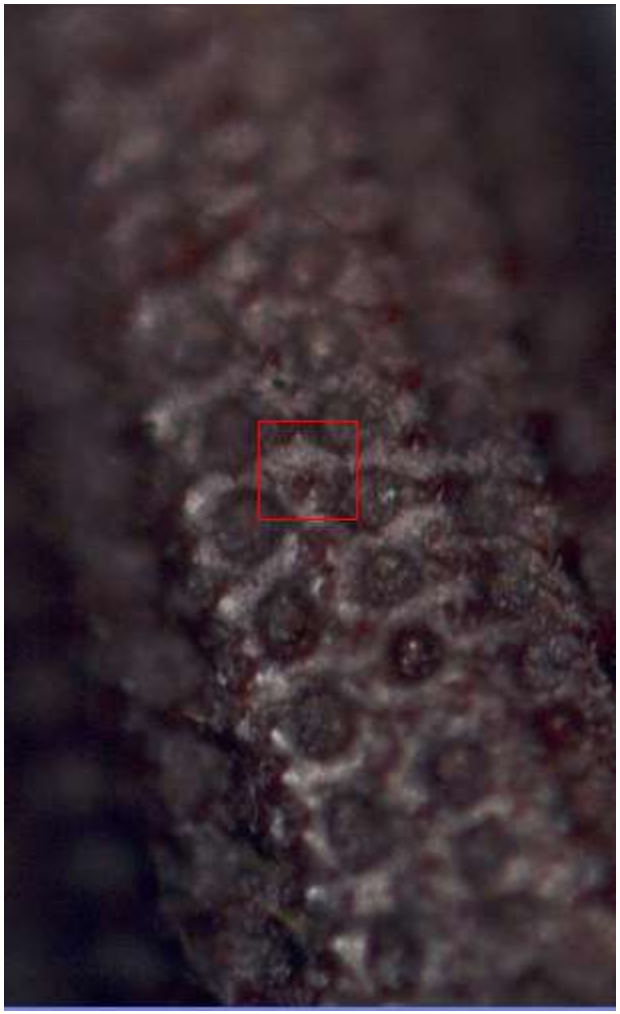
Find clusters of similar pixels using particle filter

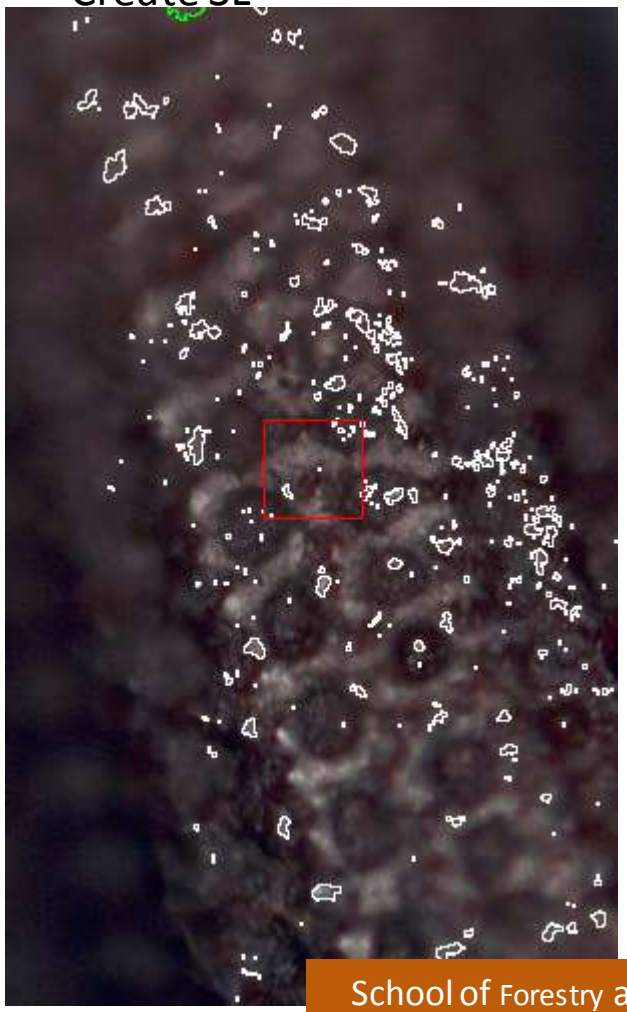
Can adjust brightness and size of particle

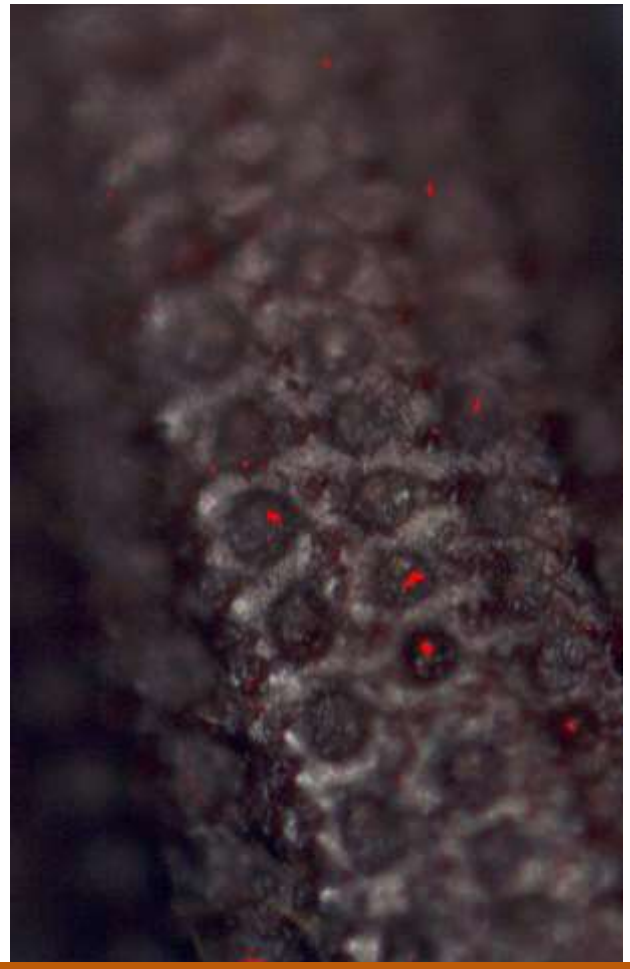
Create SL

A fraction of particles have spore signature

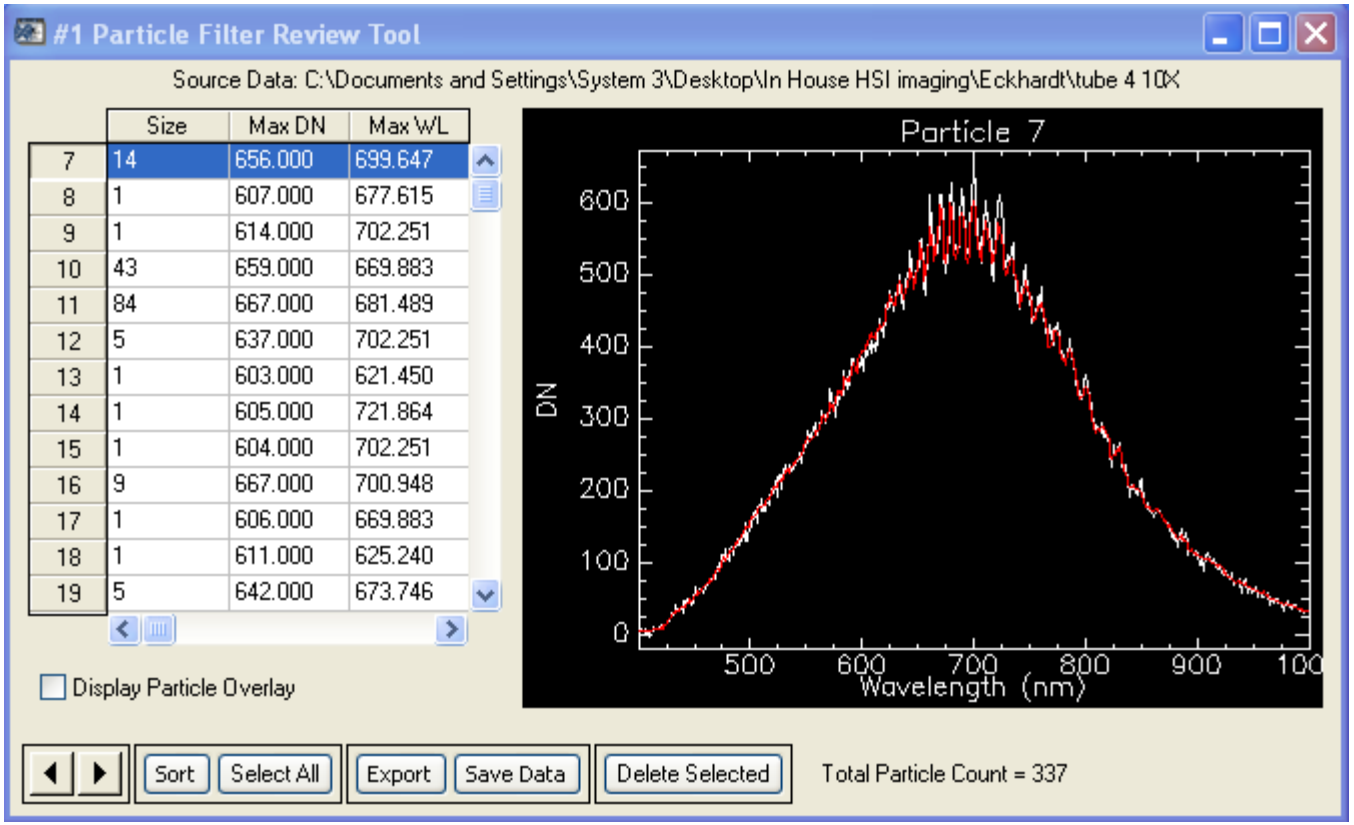
Use SAM to find pixels matching signature



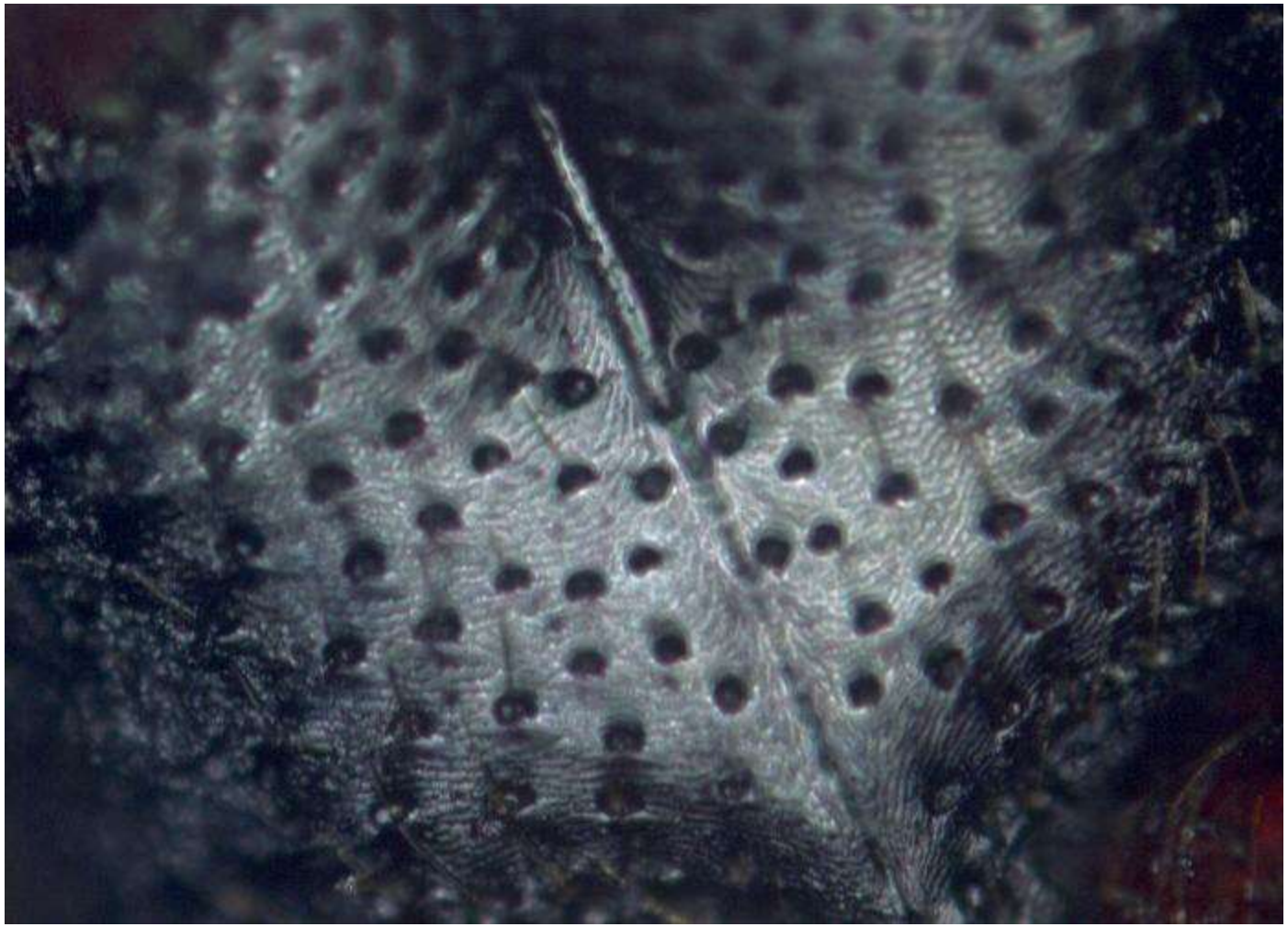




Particle 7 has a spore signature so add the curve to the spectral library

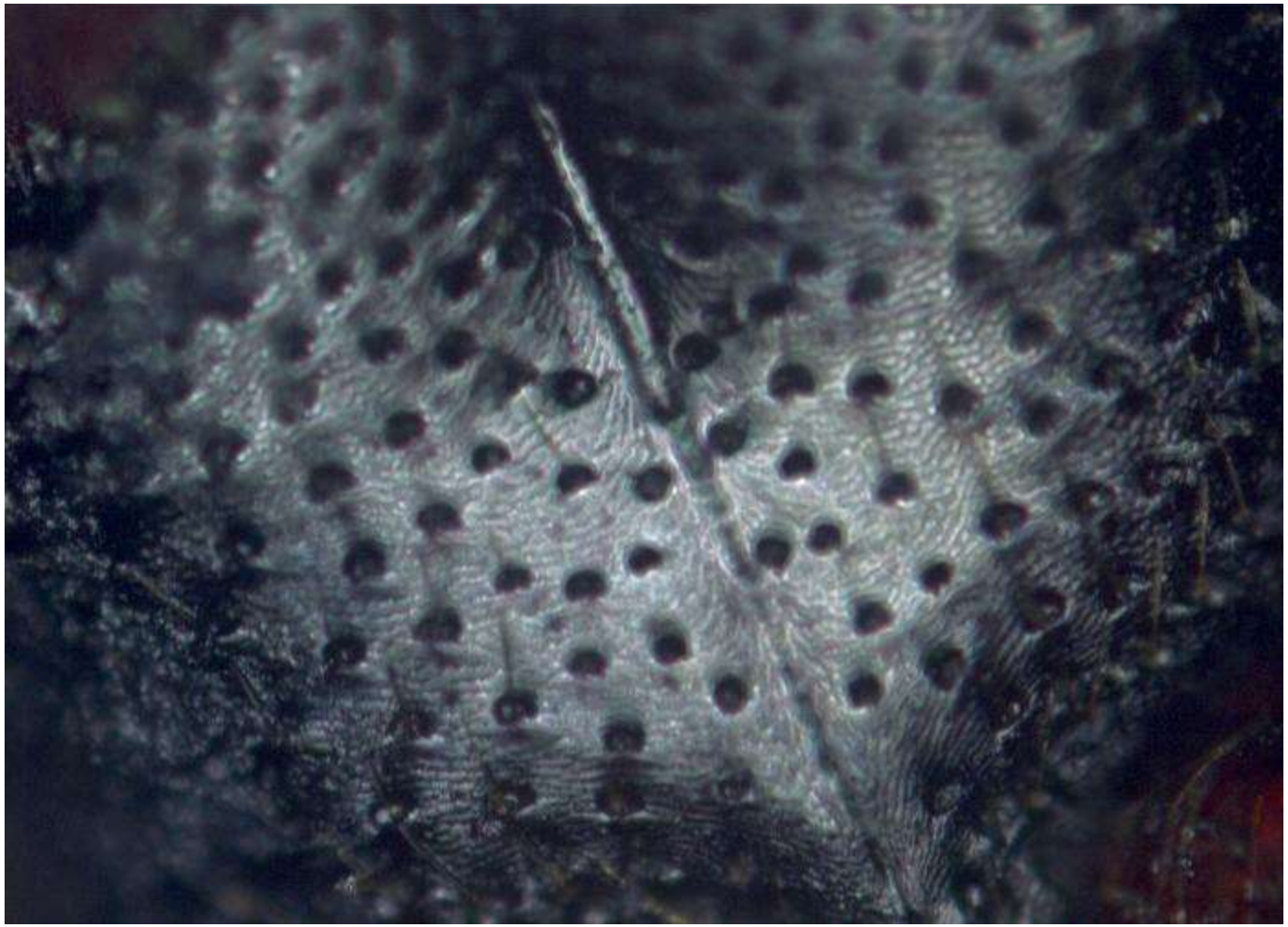


Hylastes salebrosus w/o Spores

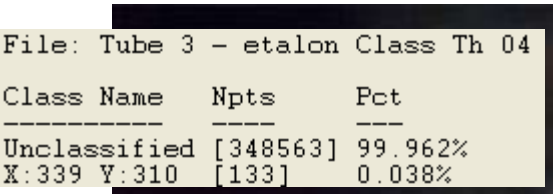


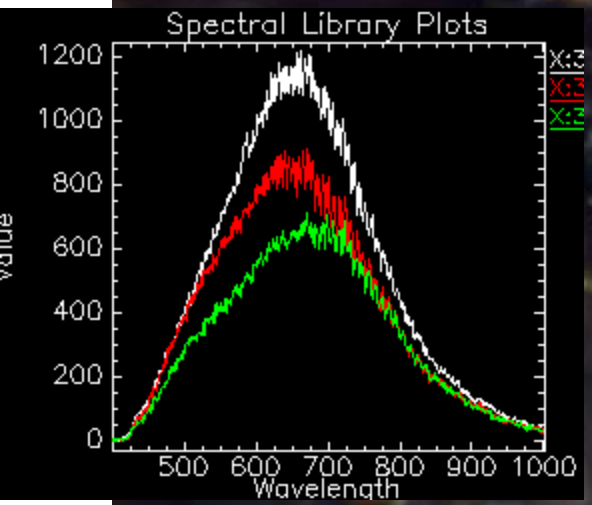
Hylastes salebrosus w/o Spores

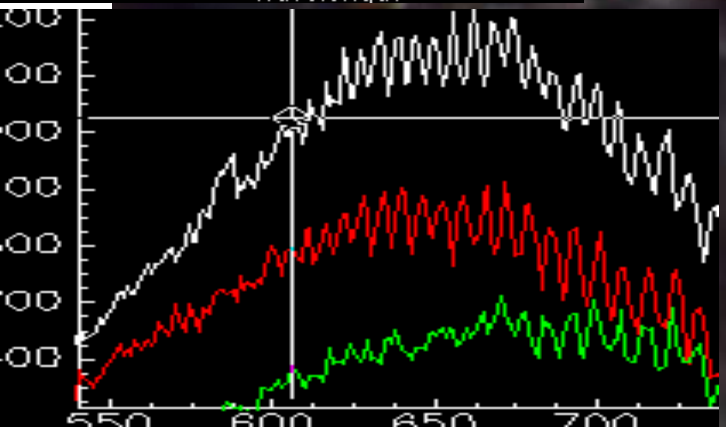
Using *G. alacris* spectral library



H. salebrosus rolled in *G. alacris*

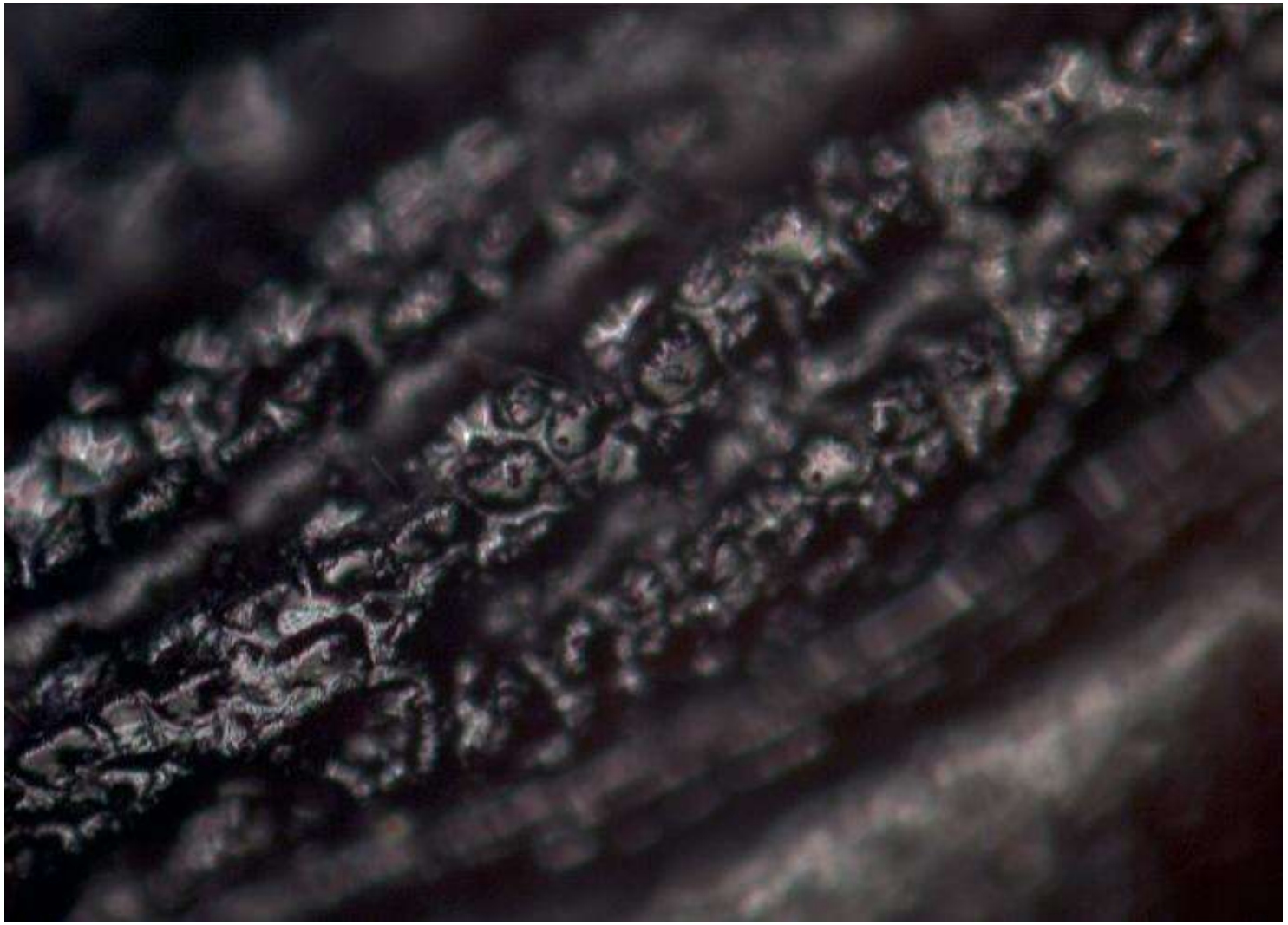


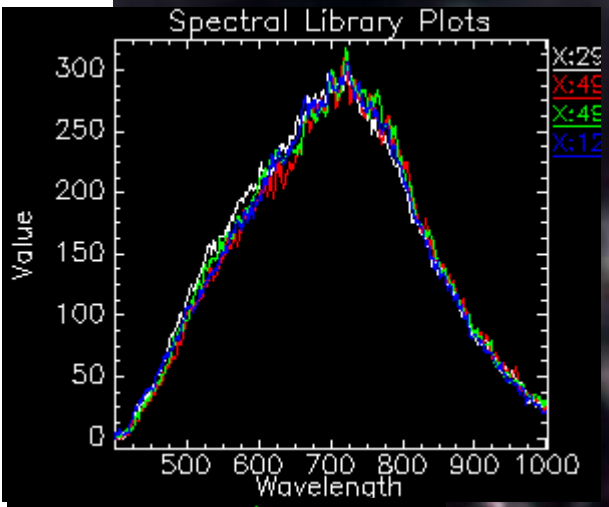


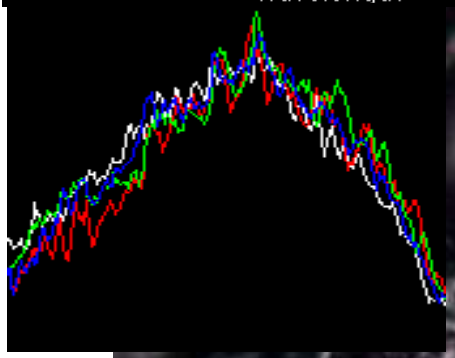


Hs: *G. alacris* map **Hs: *G. alacris* SL**

H. salebrosus rolled in *L. procerum*





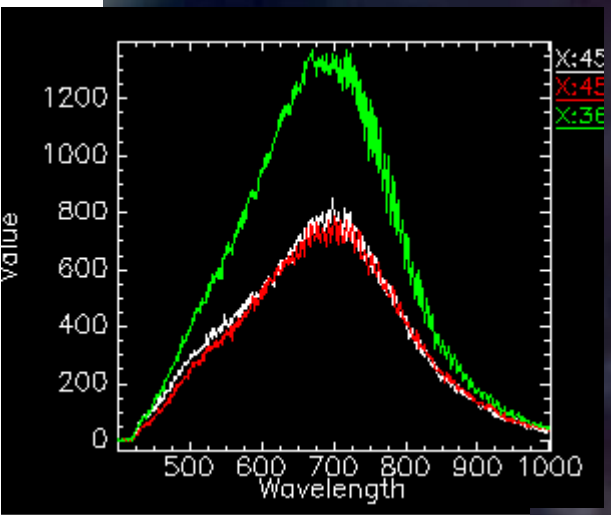


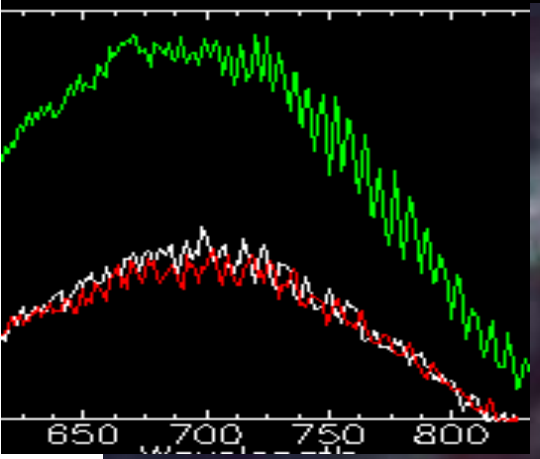
File: Hs procerum etalon CLASS T

Class Name	Npts	Pct
Unclassified	[348542]	99.956%
X:292 Y:90	[154]	0.044%

Hs: *L. procerum* map **Hs: *L. procerum* SL**

H. salebrosus rolled in *L. terebrantis*





File: Hs terebrantis etalon CLA

Class Name	Npts	Pct
Unclassified	[348221]	99.864%
X:496 Y:405	[475]	0.136%

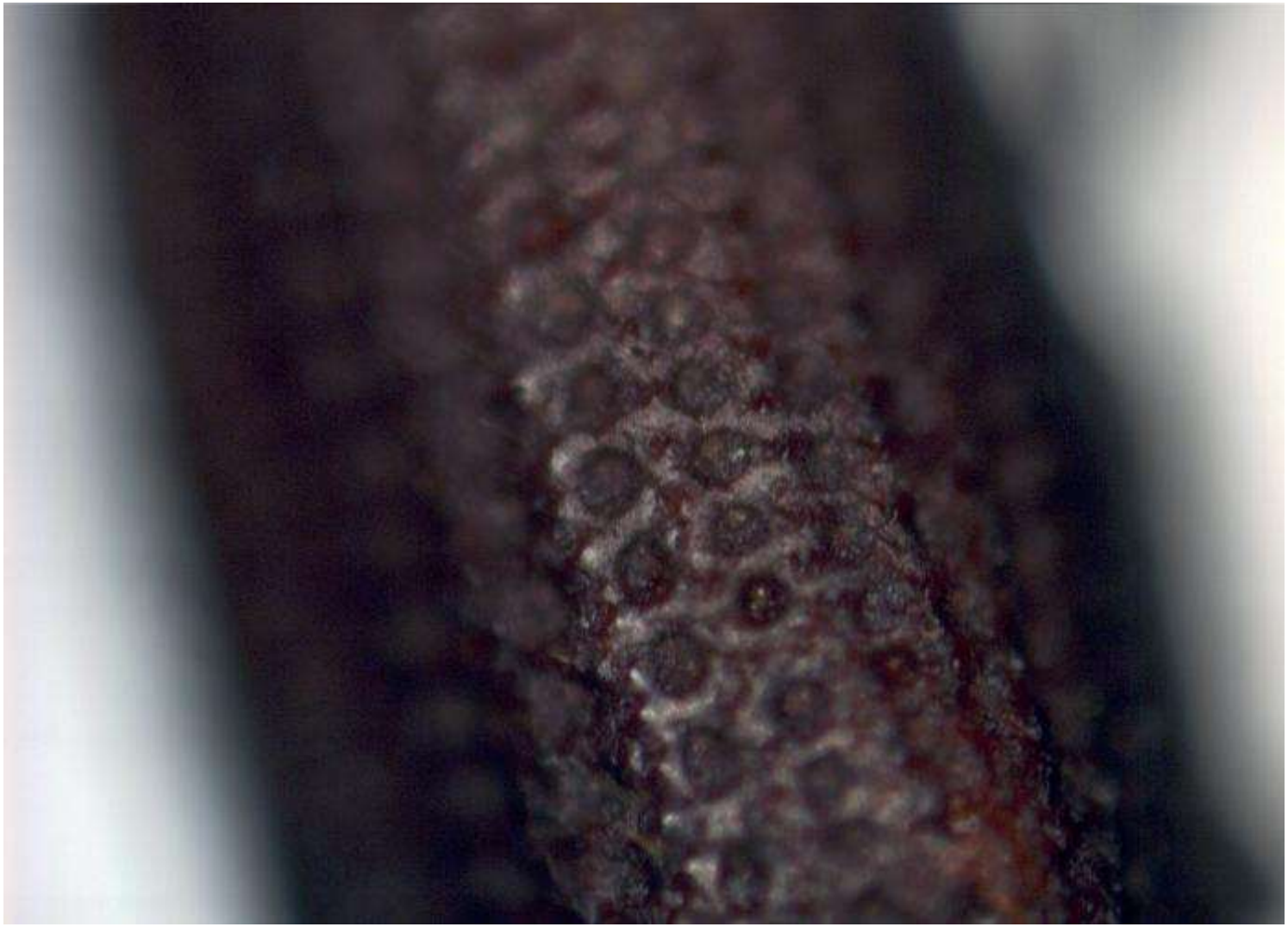
Hs: *L. terebrantis* map **Hs: *L terebrantis* SL**

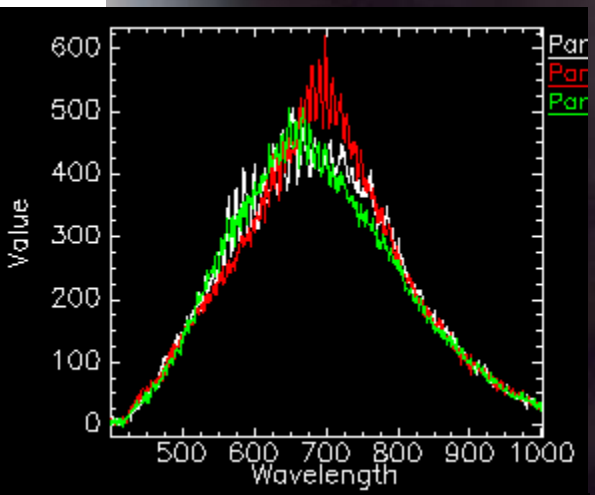
H. tenuis w/o Spores

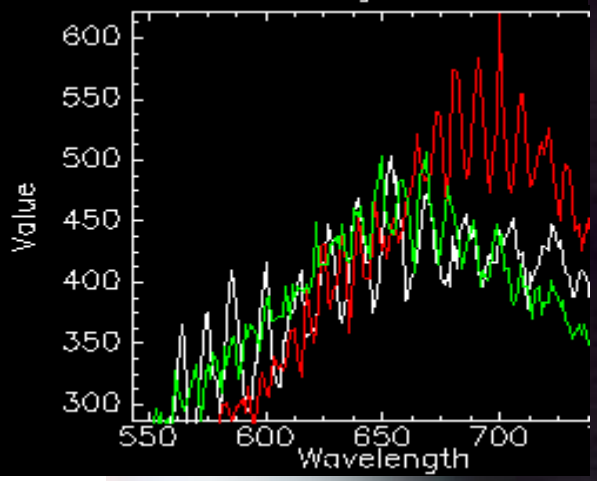
H. tenuis w/o Spores

Using *G. alacris* spectral library

H. tenuis rolled in *G. alacris*







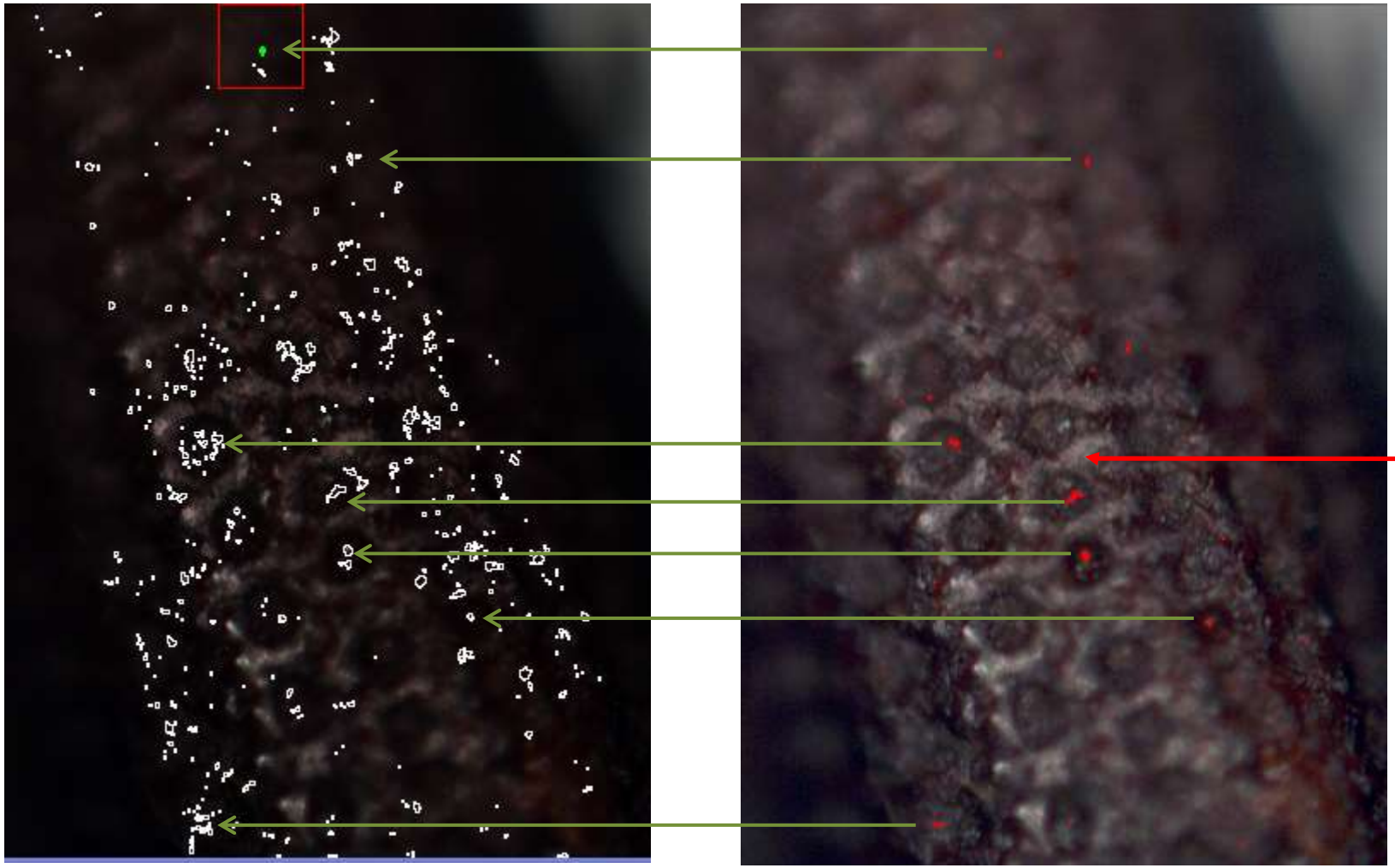

```
File: etalon CLASS size 50 max 500 T

Class Name       Npts      Pct
----------       ----      ---
Unclassified     [348516] 99.948%
Particle 10 (36 [180]     0.052%
```

Ht: *G. alacris* map **Ht: *G. alacris* SL**

Particle Filter map

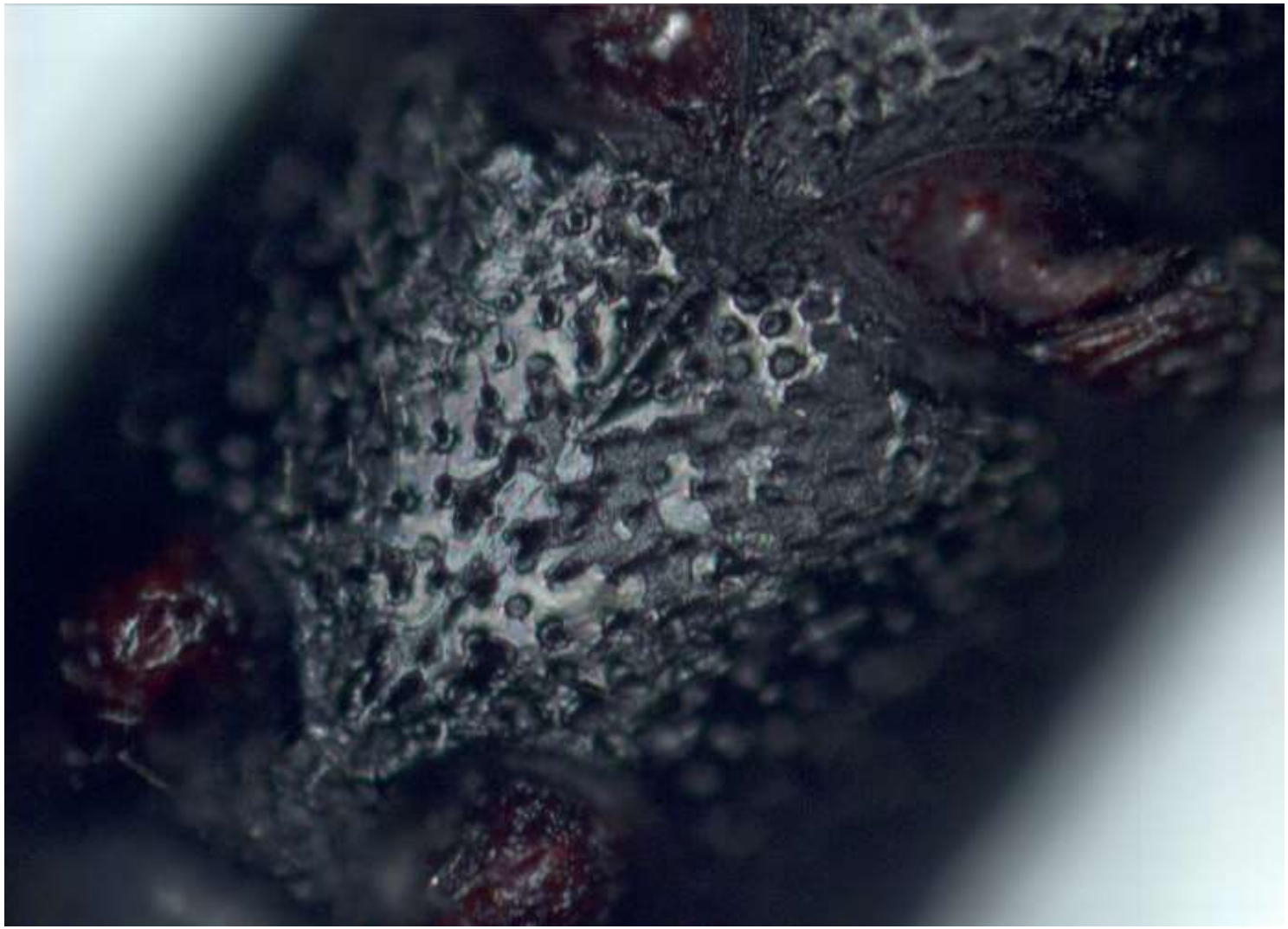
SAM map

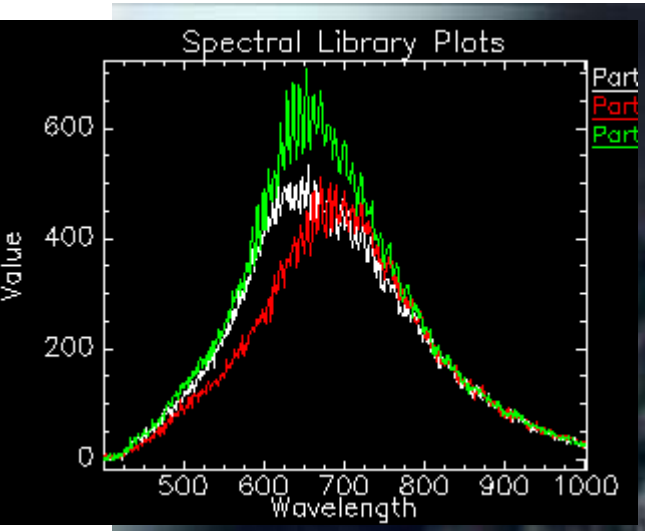


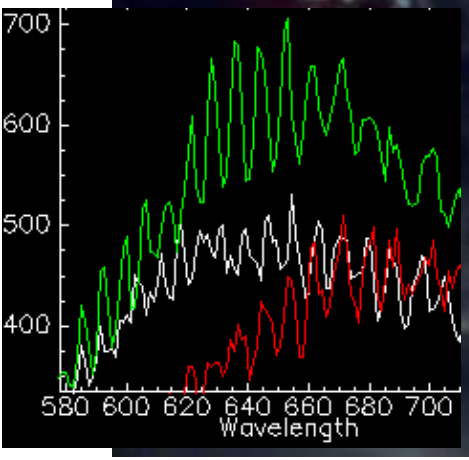
~ 7 %, or 25 out of 382 particles, contained oscillatory spectra

area missed by particle filter

H. tenuis rolled in *L. procerum*





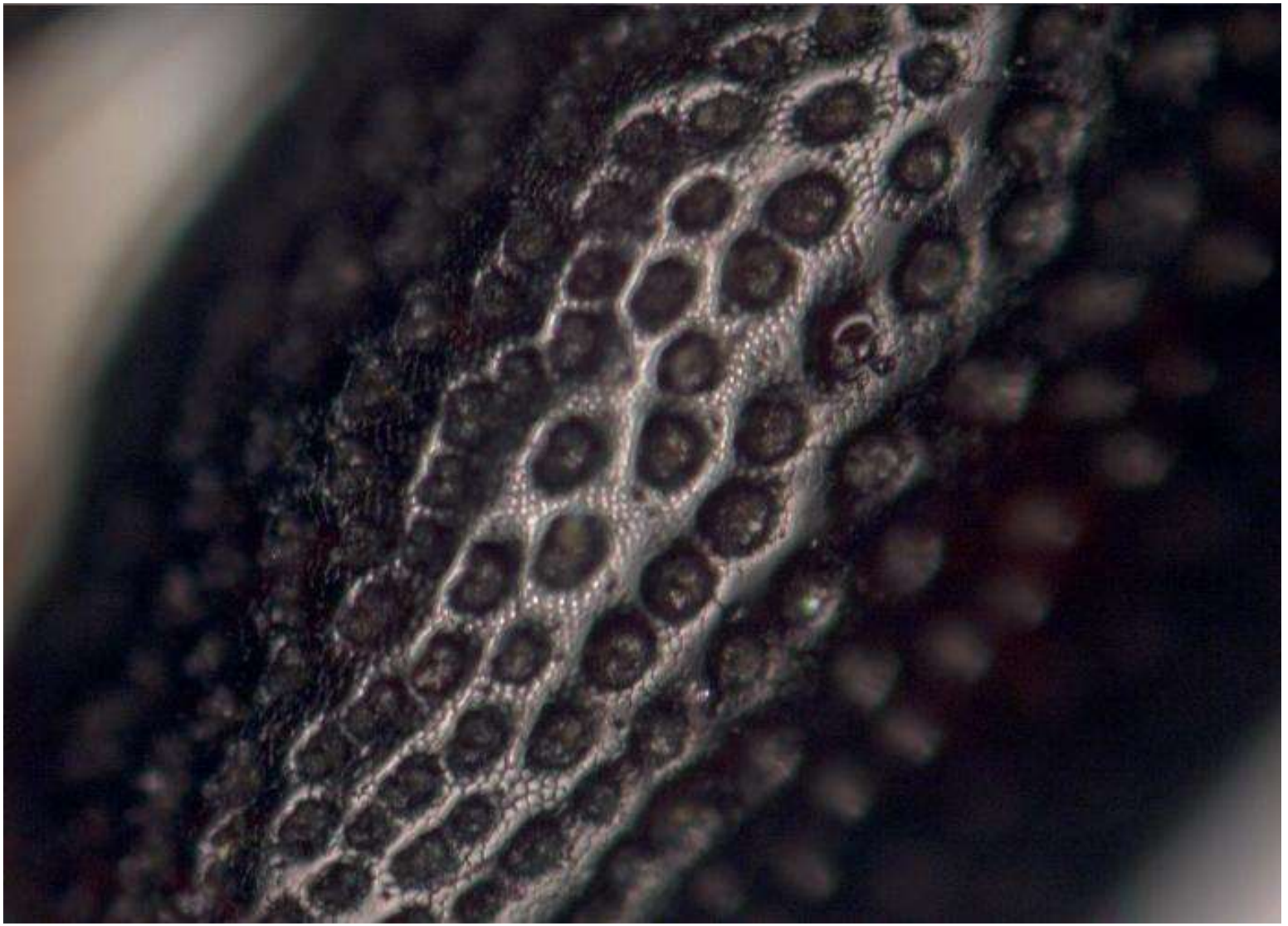


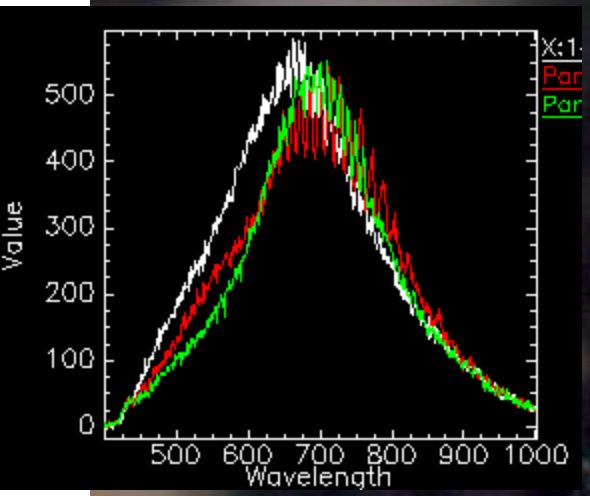
File: [Memory609] [348,696 points]

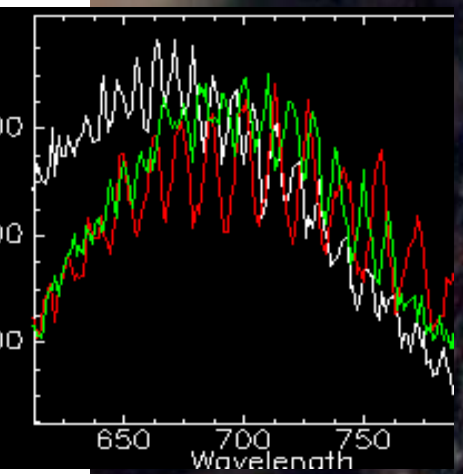
Class Name	Npts	Pct
Unclassified	[348657]	99.989%
Particle 165 (6	[39]	0.011%

Ht: *L. procerum* map **Ht: *L. procerum* SL**

H. tenuis rolled in *L. terebrantis*





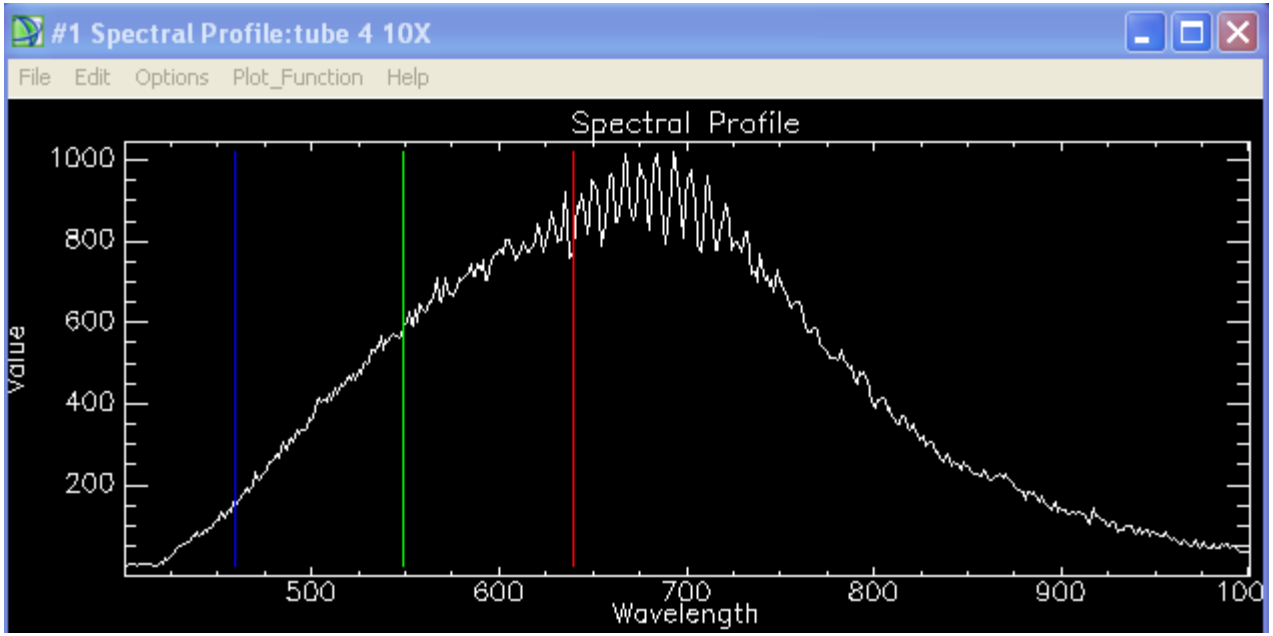


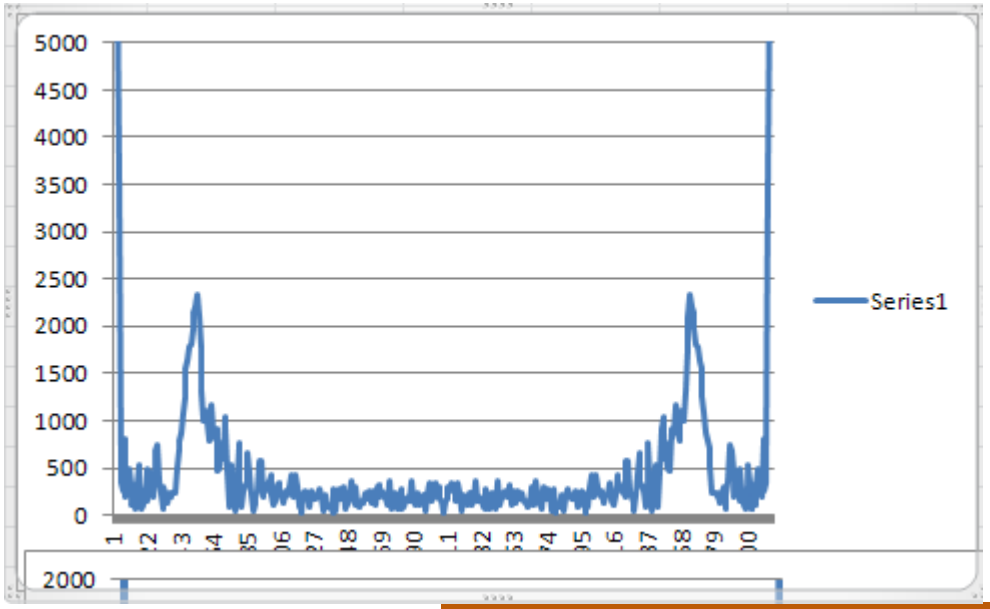
Ht: *L. terabrantis* map **Ht: *L. terebrantis* SL**

Spore Detection on *Hylastes tenuis* and *Hylastes salebrosus*

- using method of Fourier Mapping -

Fourier analysis gives a peak at the frequency of oscillation



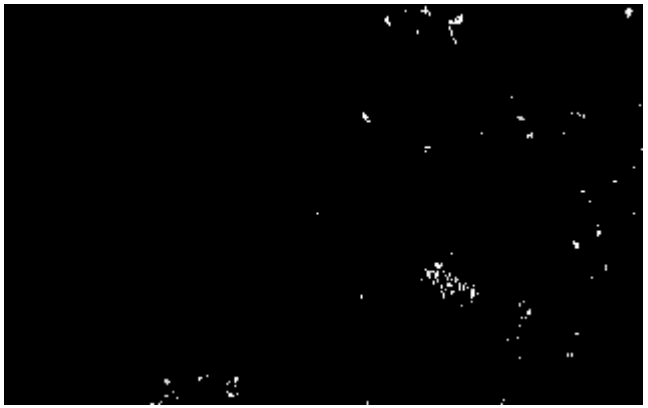


Fourier Mapping - Comparison with and without spore: *Hylastes salebrosus* showing locations of high frequency oscillations

Hs: w/o spore

Hs: *L. procerum*

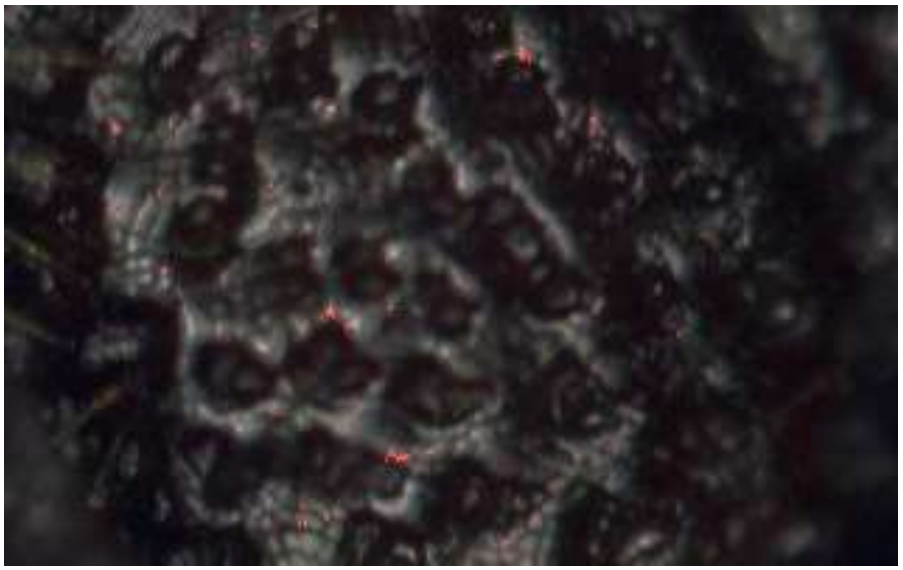
Hs: *G. alacris*



Hs: *L. terebrantis*

Fourier Mapping – Hs: *G. alacris*

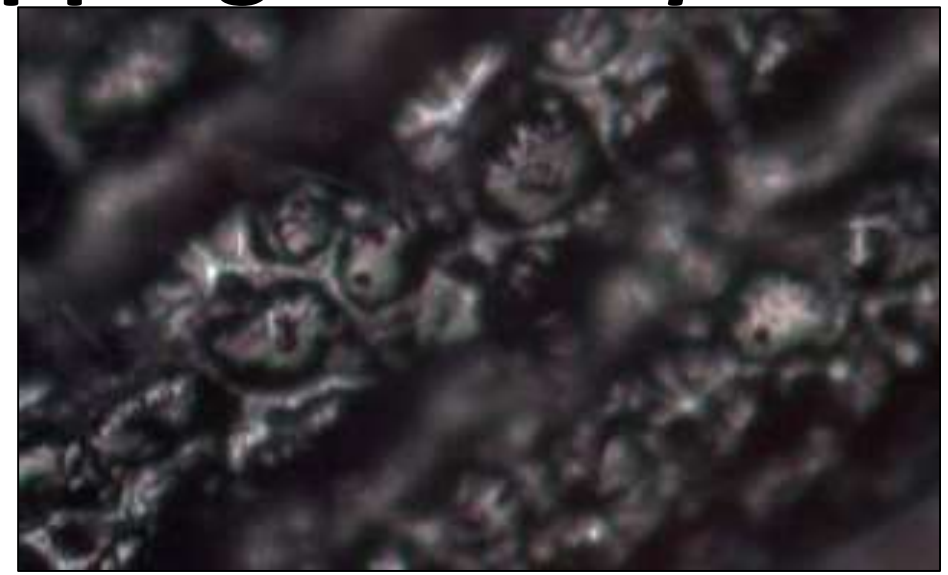
10X reflected image



spore map

Fourier Mapping – Hs: *L. procerum*

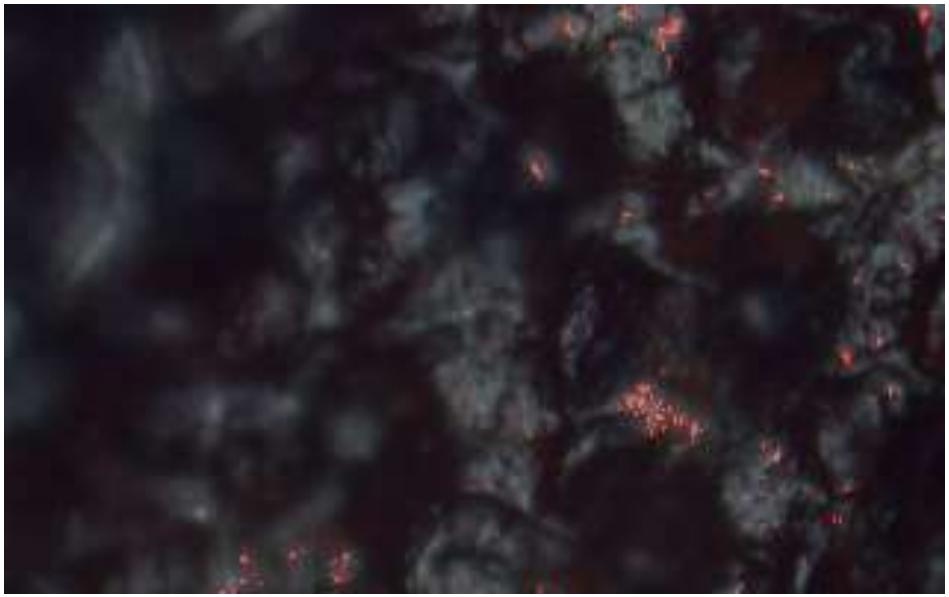
10X reflected image



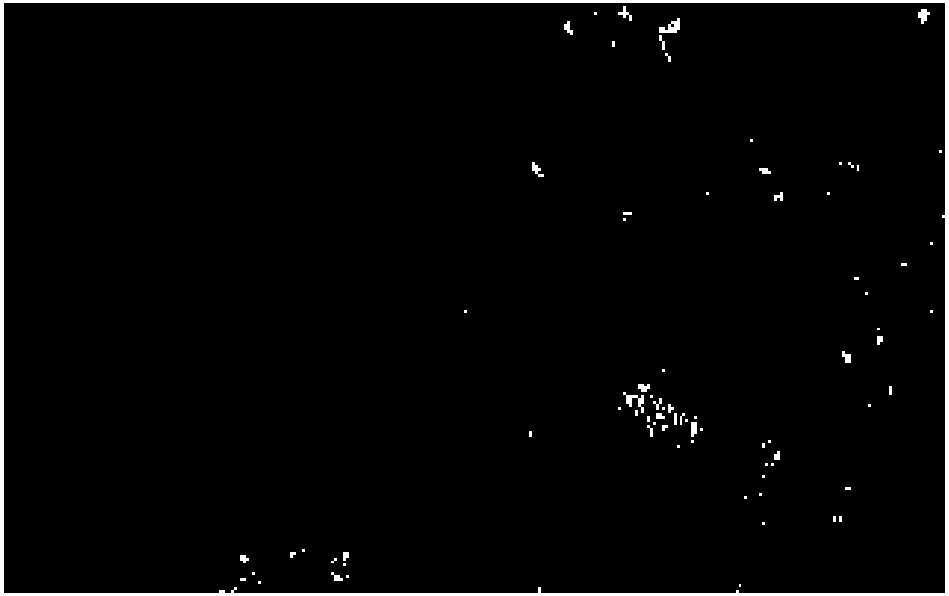
spore map

Fourier Mapping – Hs: *L. terebrantis*

10X reflected image



spore map



Fourier Mapping - Comparison with and without spore: Hylastes tenuis showing locations of high frequency oscillations

Ht: w/o spores

Ht: w/ *L. procerum*

Ht: w/ *G. alacris*

Ht: w/ *L. terebrantis*

Fourier Mapping – Ht w/o Spores

10X reflected image

spore map

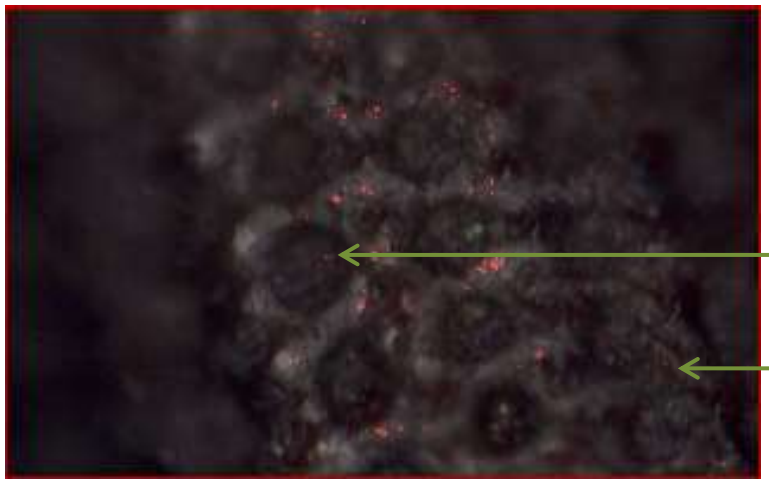
Fourier Mapping – Ht: *G. alacris*

10X reflected image

spore map

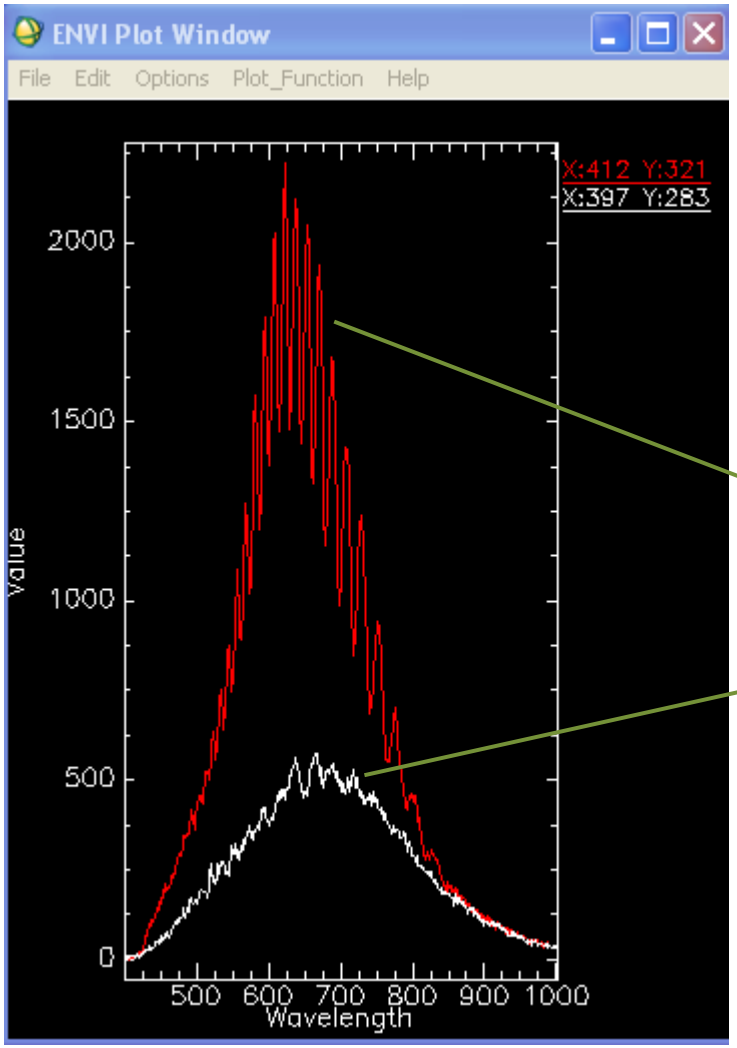
Comparison of detected regions using Fourier and Spectral Angle Mapper

Fourier map
matches stay in focused areas better



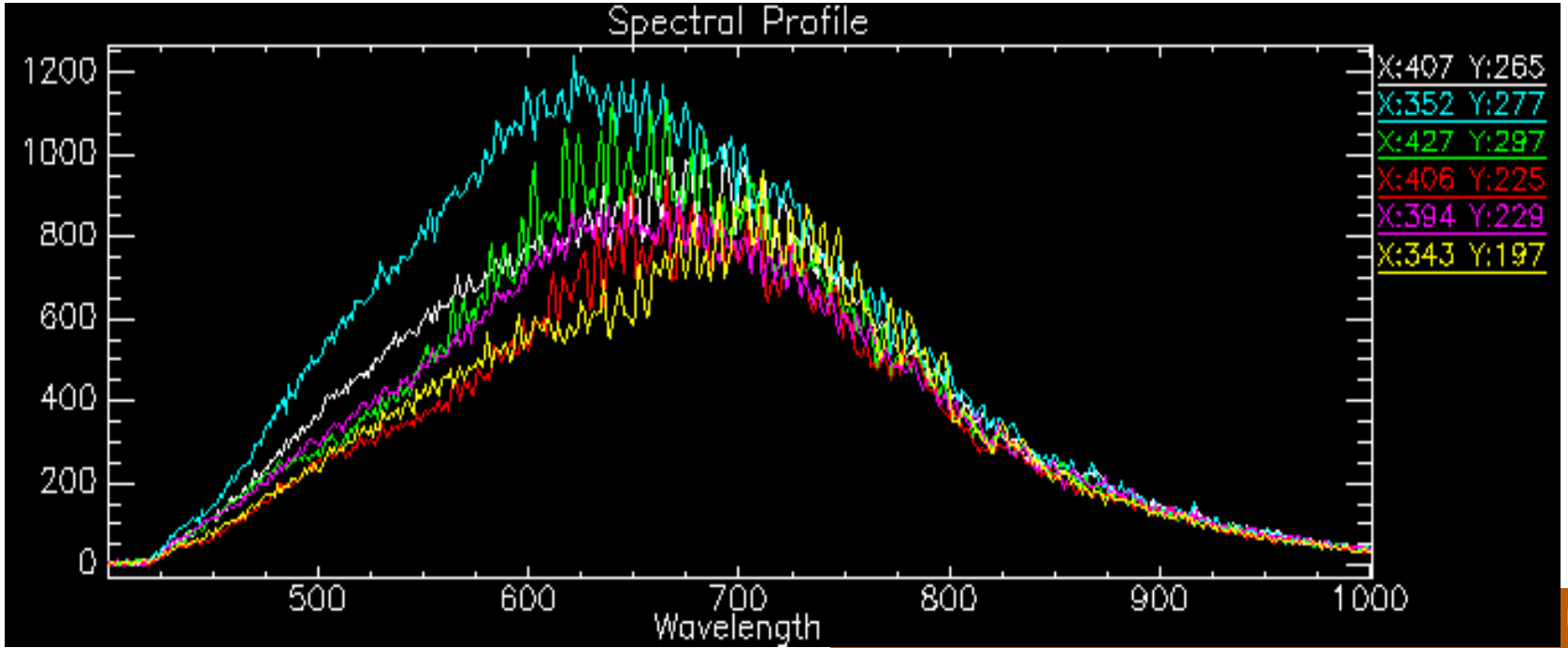
Reasons for discrepency

1. match by SAM not necessarily an oscillatory signature
2. Fourier method used filtering to select frequencies above a lower limit



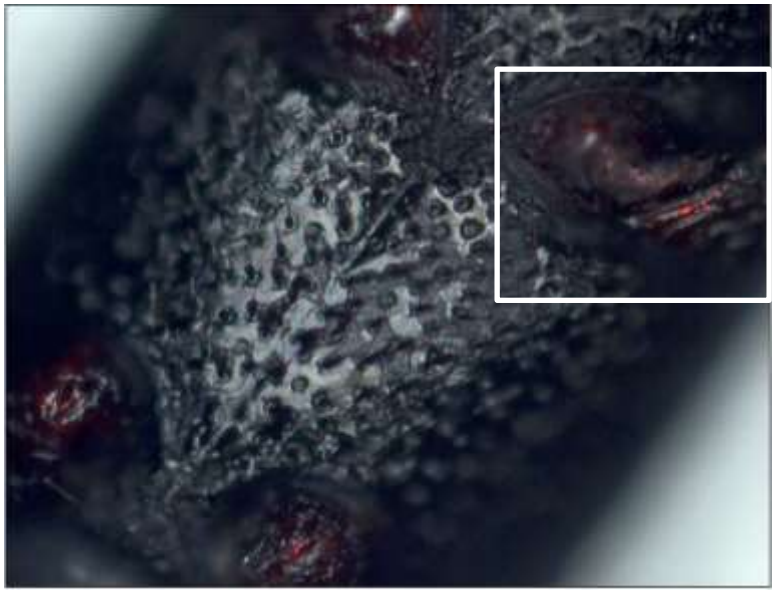
Two areas found from the particle filter-SAM came from low frequency spectra, and were not detected by the Fourier implementation which selected only higher frequencies. Frequencies were tightly controlled only in Fourier method.

Spectra from Fourier-mapped pixels



Fourier Mapping – Ht: *L. procerum*

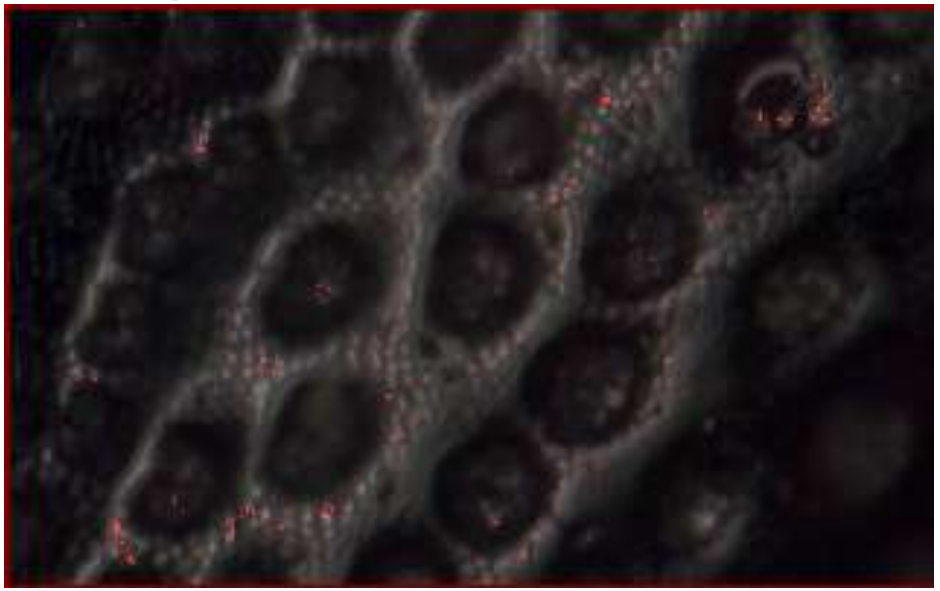
10X reflected image



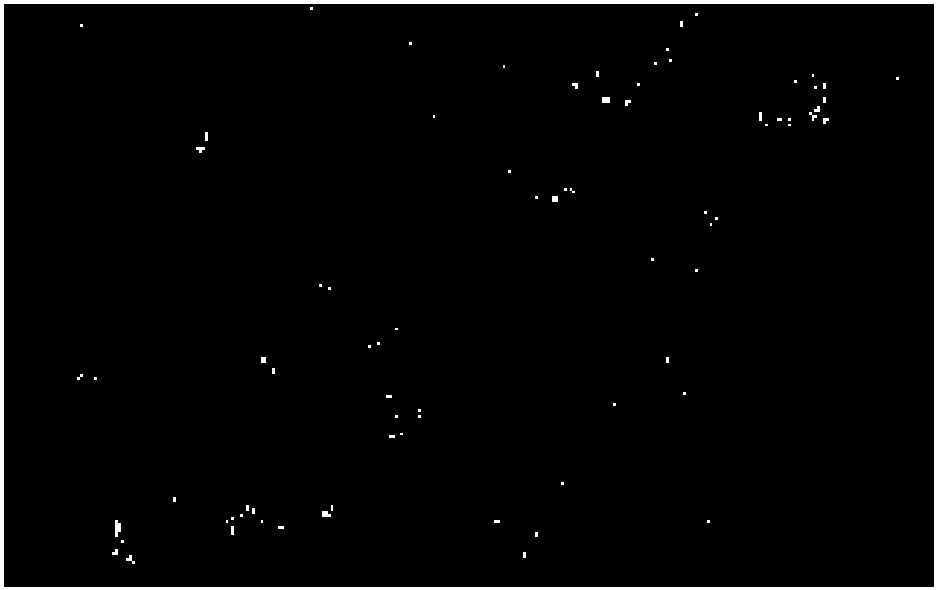
spore map

Fourier Mapping – Ht: *L. terebrantis*

10X reflected image



spore map

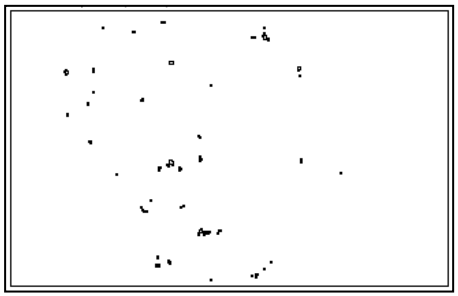


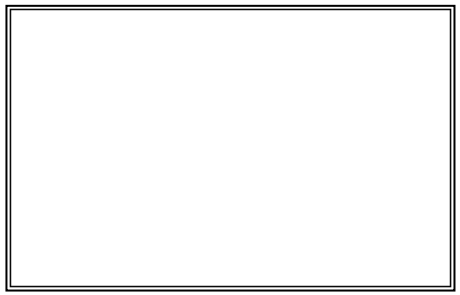
Particle Analysis

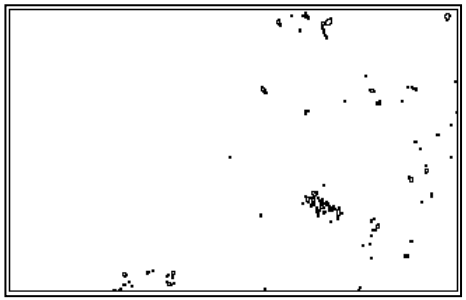
tube	sample	count	average Size (micron ^{2})	% area	mean freq (rel. scale)
1	Hs: w/o spore	0	0	0	0
2	Ht: w/o spore	0	0	0	0
3	Hs: G. alacris	49	4.278	0.197	14.856
5	Hs: L. procerum	0	0	0	0
7	Hs: L. terebrantis	83	5.172	0.404	17.764
4	Ht: G. alacris	57	3.619	0.194	16.046
6	Ht: L. procerum	115	4.240	0.091	16.39
8	Ht: L. terebrantis	79	3.075	0.229	14.981

G. alacris ***L. procerum*** ***L. terebrantis***

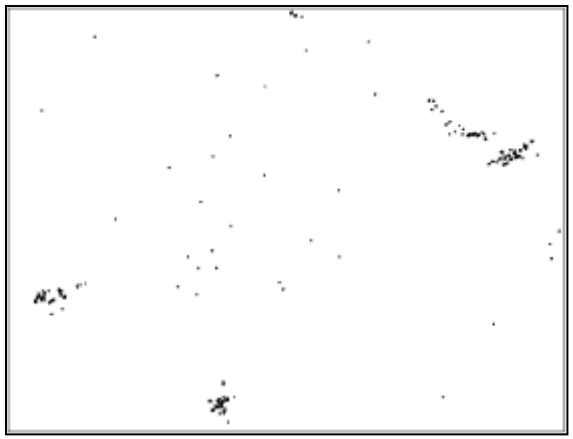
Hs

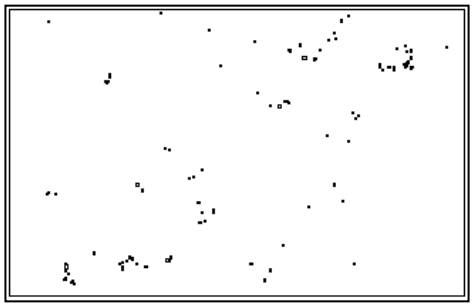






Ht

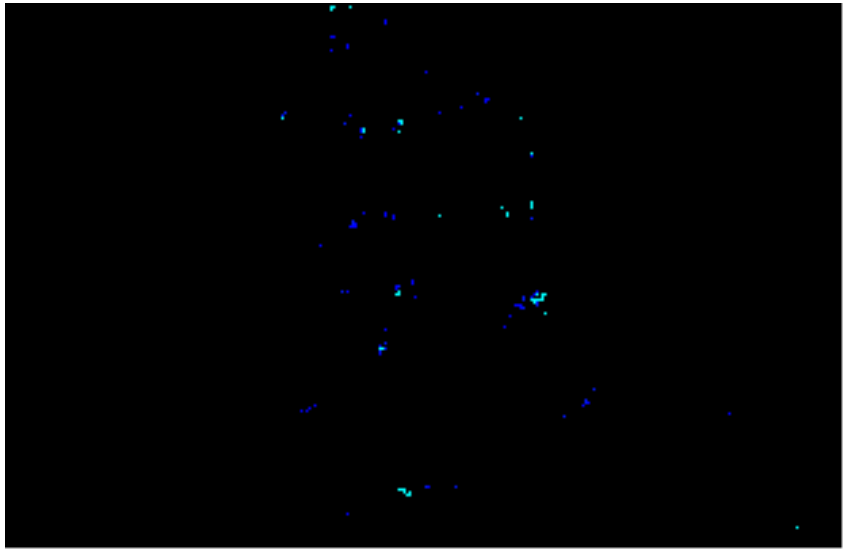




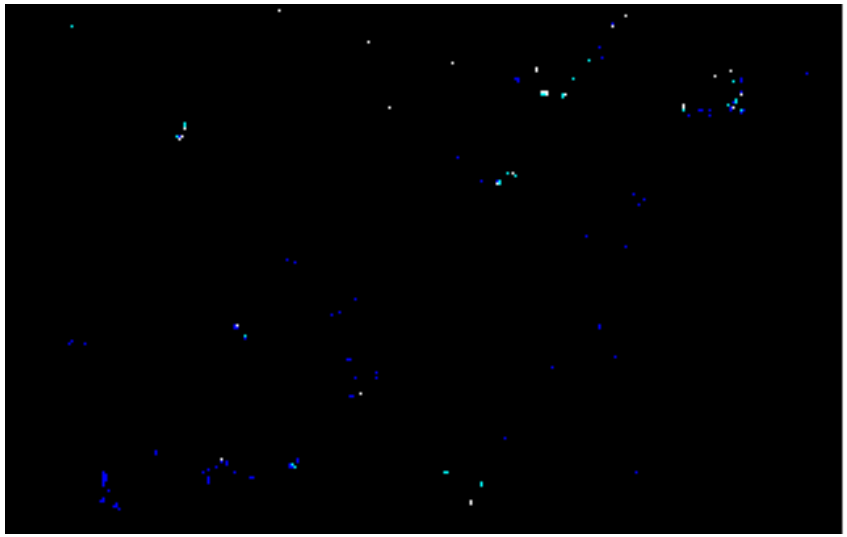
Frequency Analysis

not calibrated to Hz

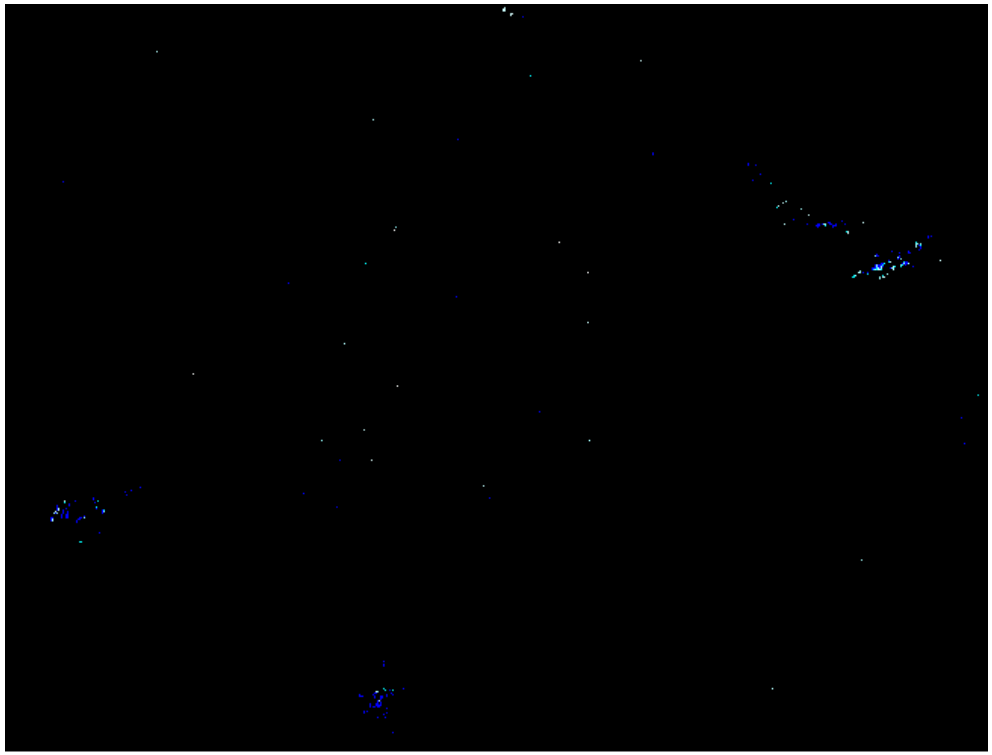
blue 12 - 20
green 21 - 50



Ht: *G. alacris*

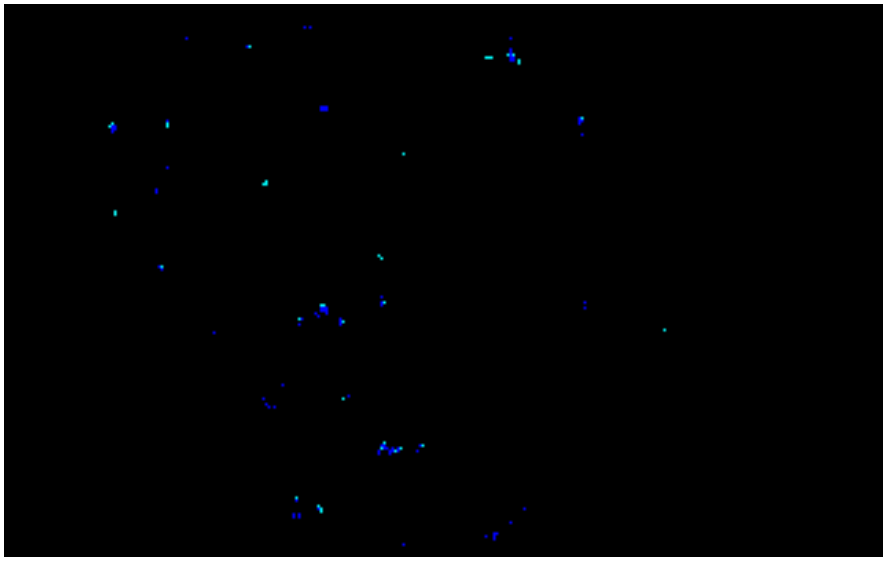


Ht: *L. terebrantis*



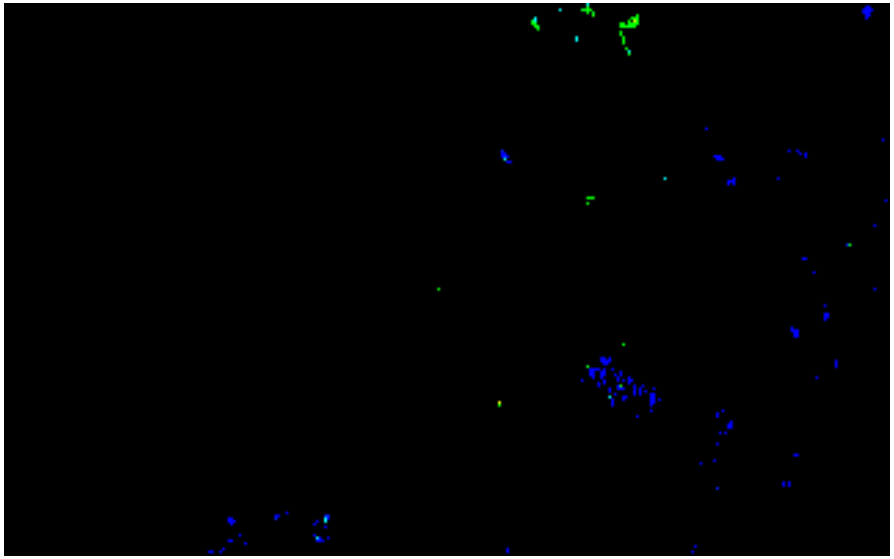
Ht: *L. procerum*

Frequency Analysis



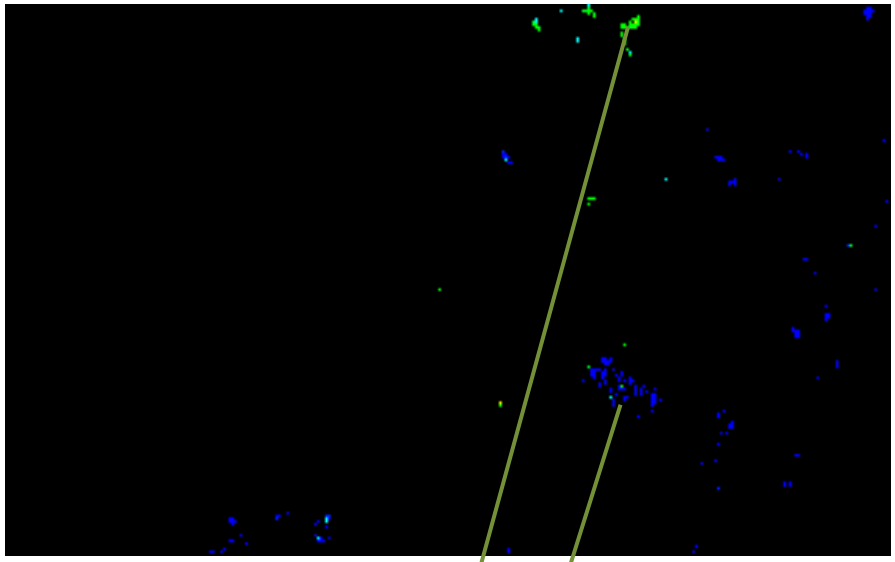
Hs: *G. alacris*

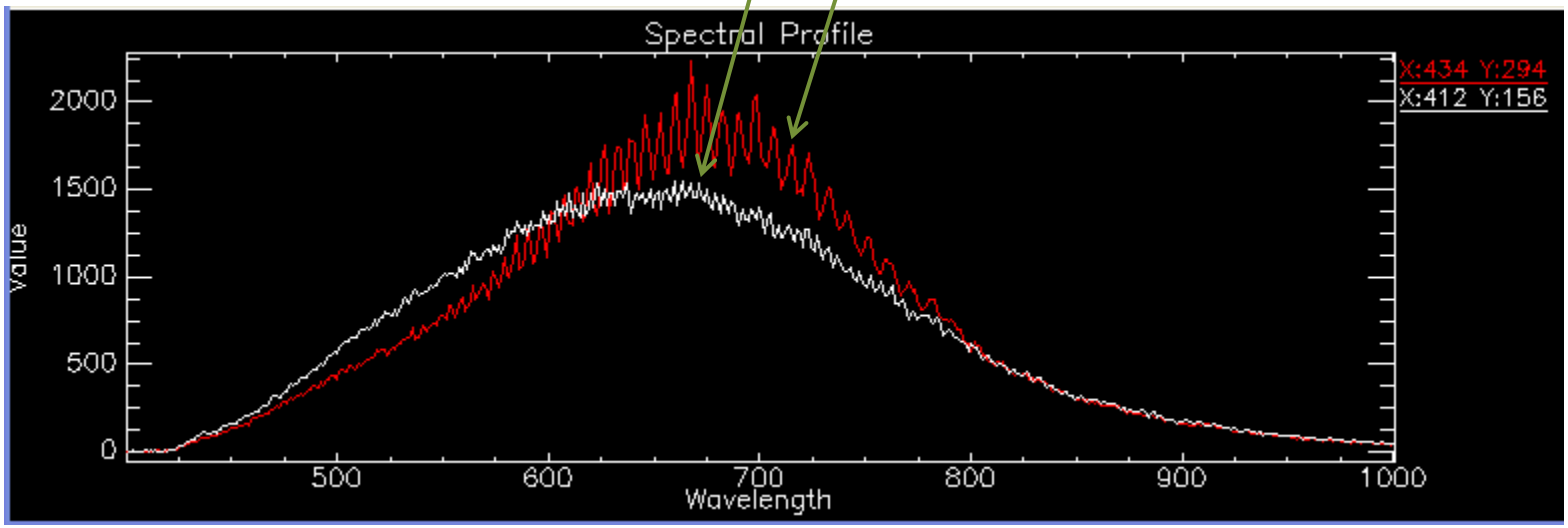
Hs: *L. procerum*



Hs: *L. terebrantis*

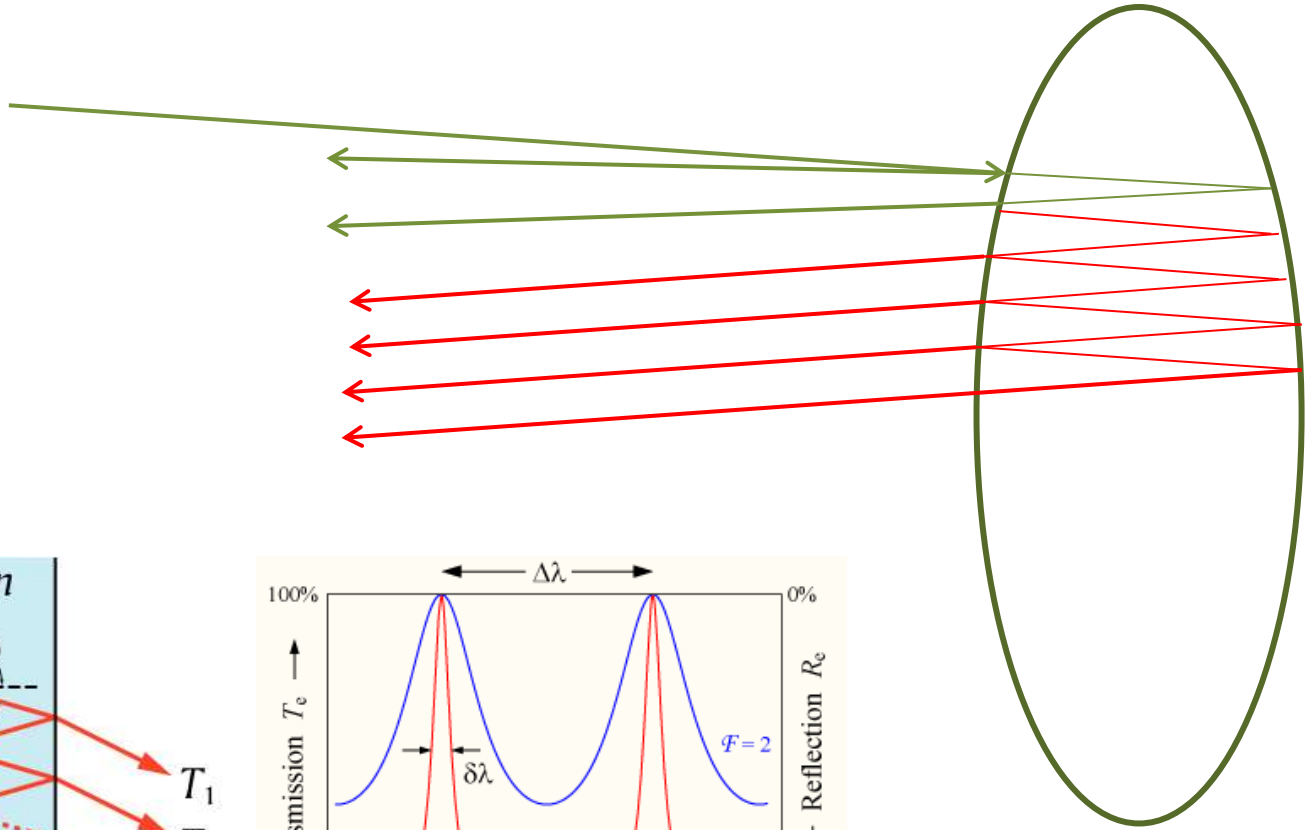
Hs: *L. terebrantis*



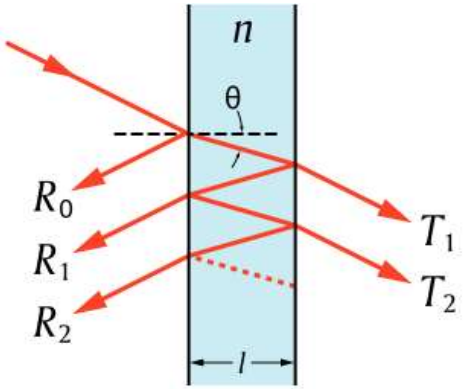


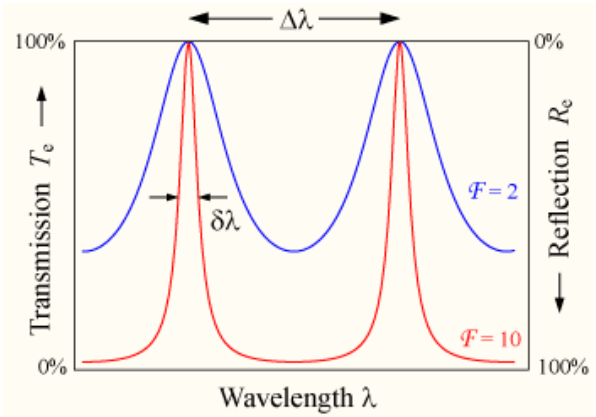
Why would a spore create an oscillation with respect to the wavelength of the reflected light?

Answer: interferences from periodic changes in light path length.



spore

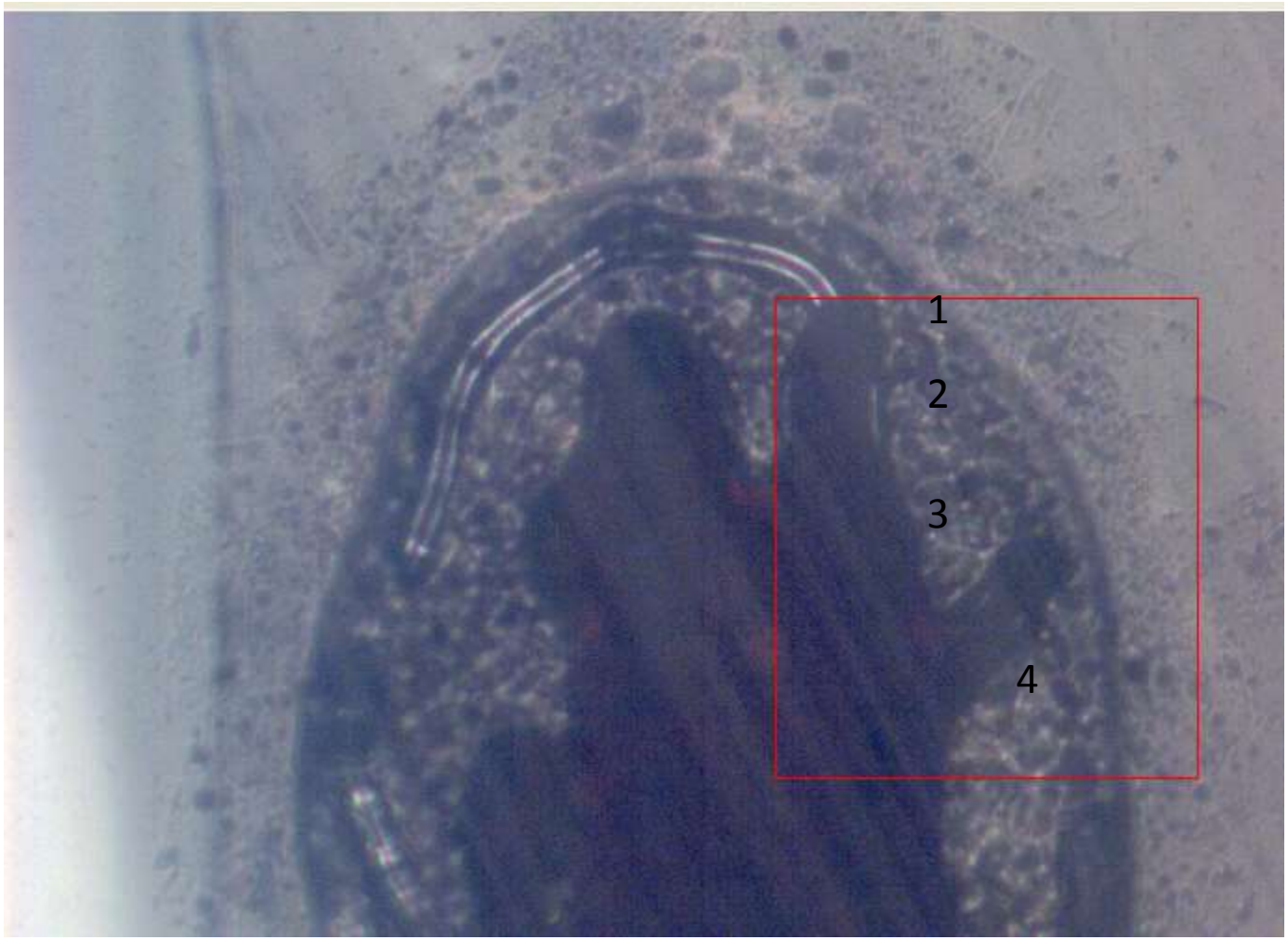




Fabry-Perot etalon

Verification that spores on slide match spectral profiles.

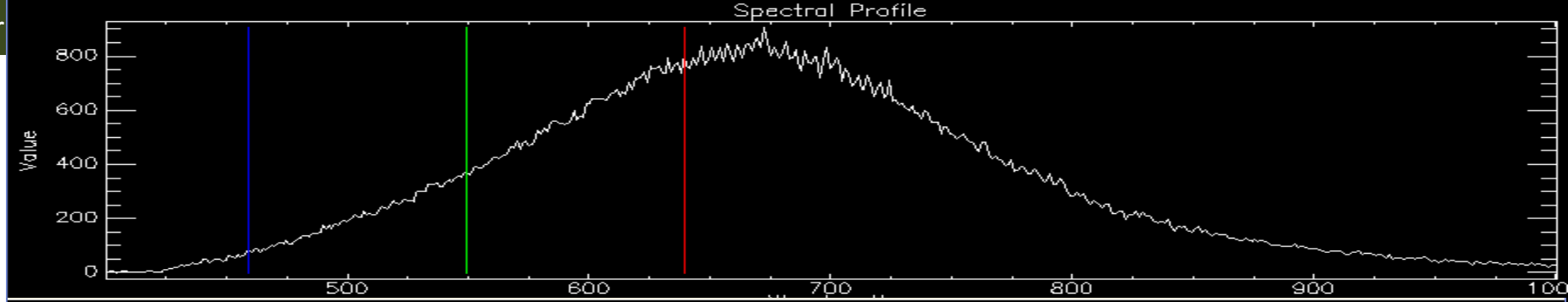
HSI scan of *G. alacris* spores on slide



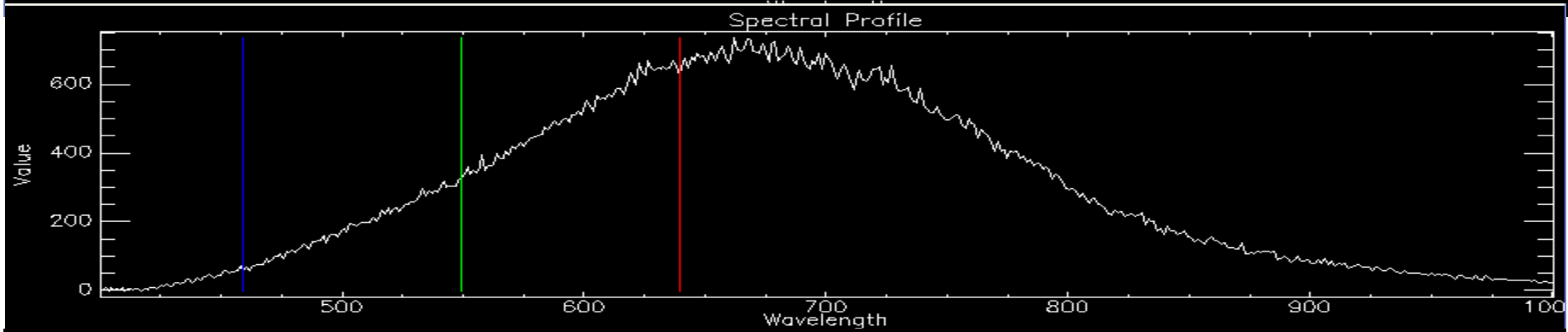
Osc spectra were found at pixels below and to left of each marker, usually on or near a bright spot in highly textured areas of the sample.

For

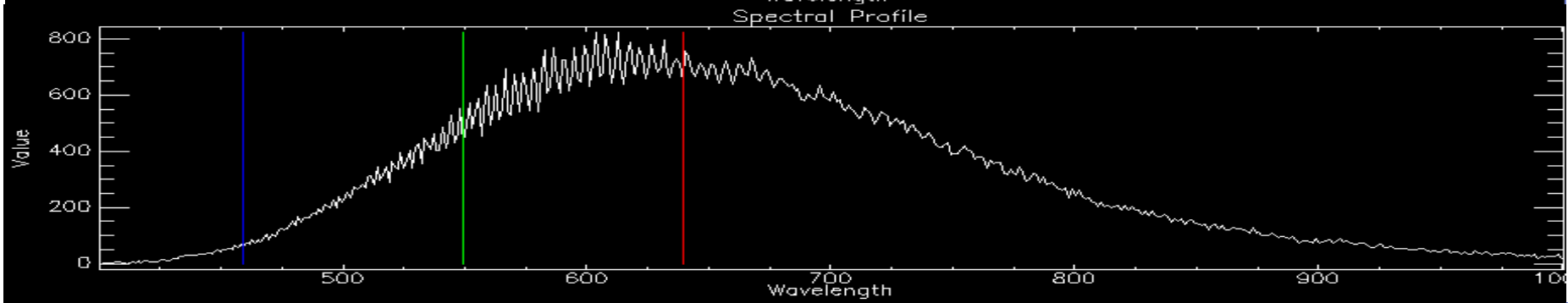
1


Spectral Profile
Value
0
200
400
600
800
500
600
700
800
900

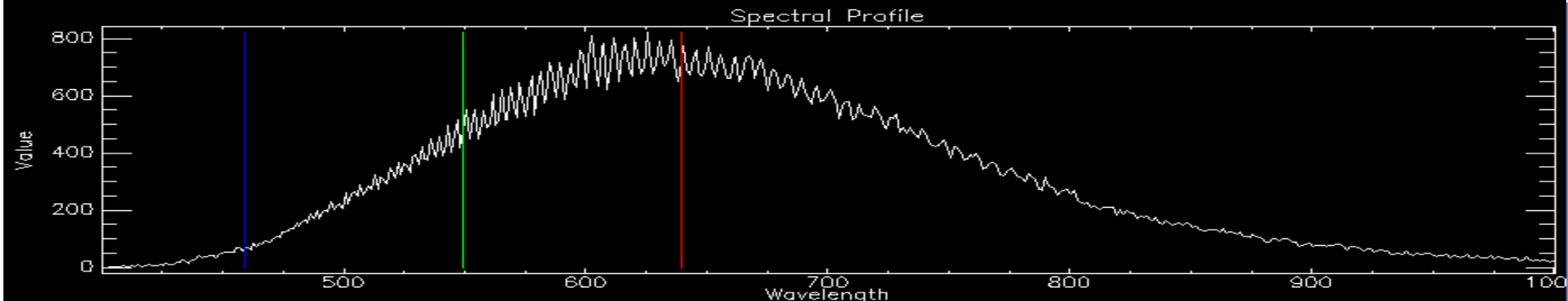
2


Spectral Profile
Value
0
200
400
600
500
600
700
800
900
Wavelength

3


Spectral Profile
Value
0
200
400
600
800
500
600
700
800
900
Wavelength

4


Spectral Profile
Value
0
200
400
600
800
500
600
700
800
900
Wavelength

Summary

Frequency analysis of reflected light from bark beetles, using CytoViva hyperspectral imaging, is able to detect spores in situ without histology.

Next step is to determine if we can speciate spores directly using CytoViva hyperspectral imaging.

CytoViva®