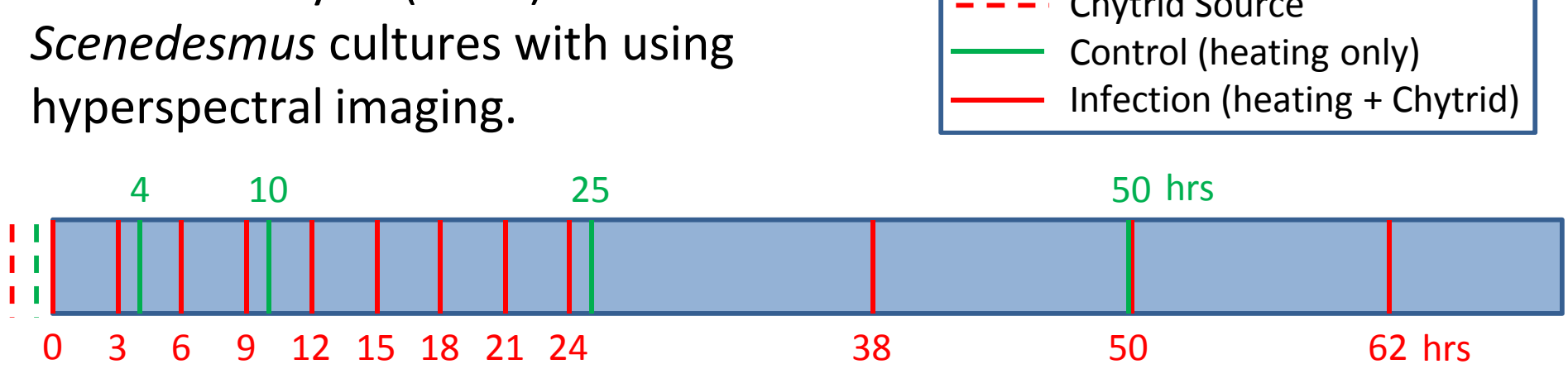
SAND2012-3085P

# Temporal Investigation of the Chytrid Infection of Scenedesmus

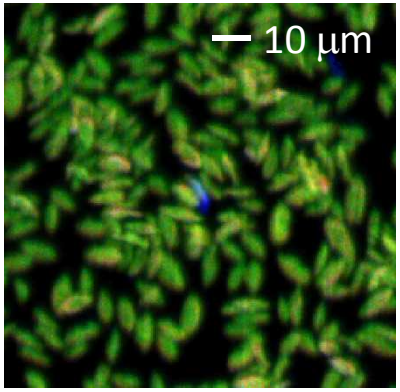
Sapphire update meeting

Purpose of Experiment: To temporally follow the chyrid (FD01) infection of *Scenedesmus* cultures with using hyperspectral imaging.




* Representative samples and controls from each time point were sent to Sapphire of QPCR analysis

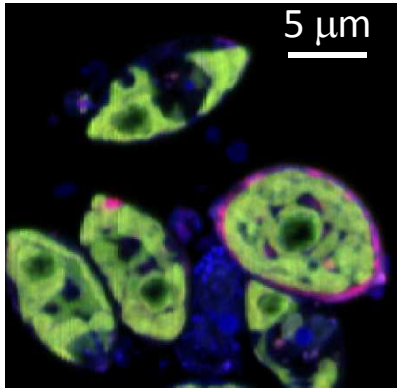
## 10X Objective Investigation




Goal: Can we detect the chytrid infection using a "bulk sample like" fluorescence measurement?

- 296 images collected
- Approximately 7-10 images per sample or control
  - 2 bioreps per sample, 1 biorep per control
- All images combined together and analyzed with MCR
- Only cellular material was analyzed

## 60X Objective Investigation

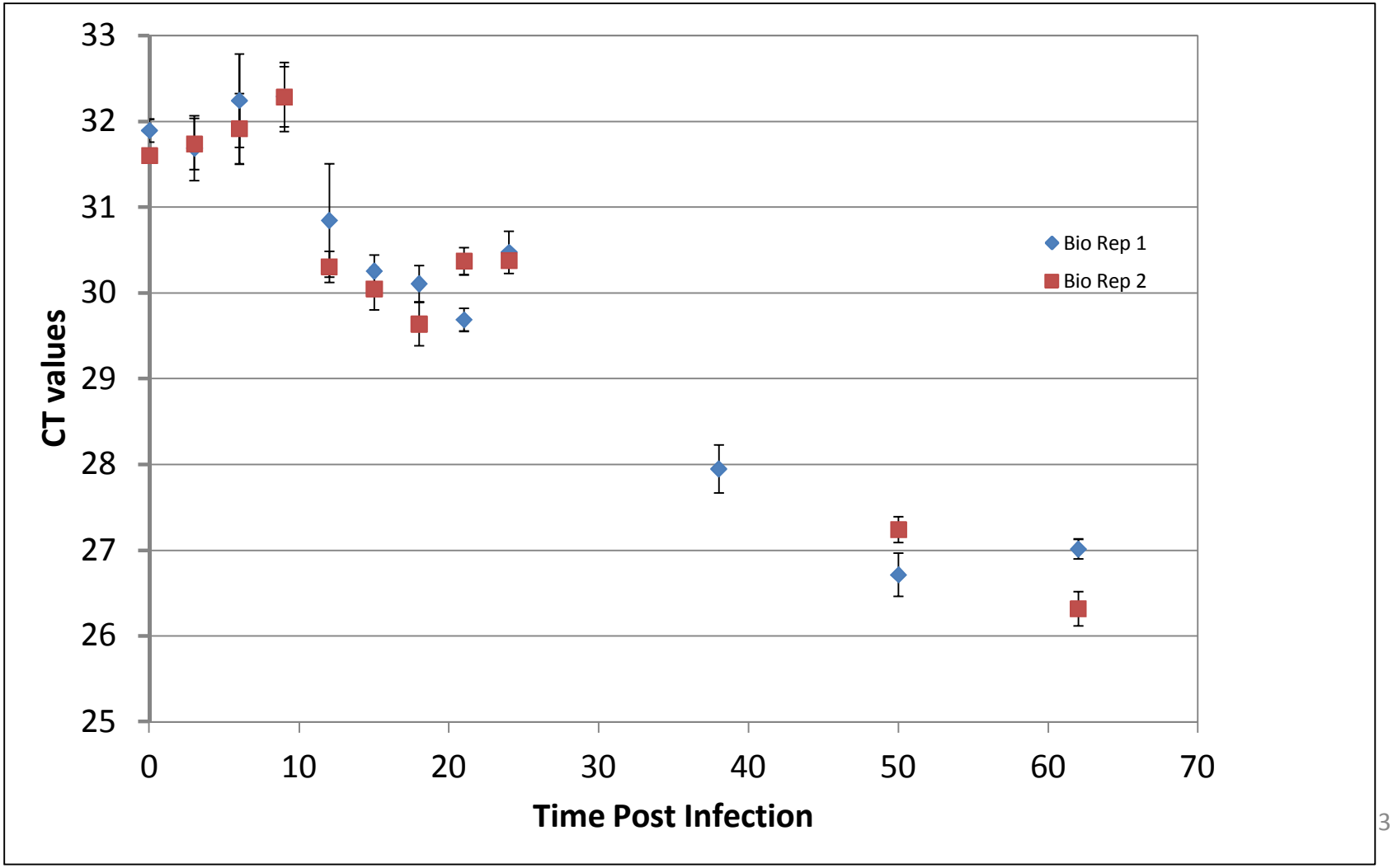



Goal: To develop better understanding of the mechanism of infection using chemical, spatial and temporally resolved imaging.

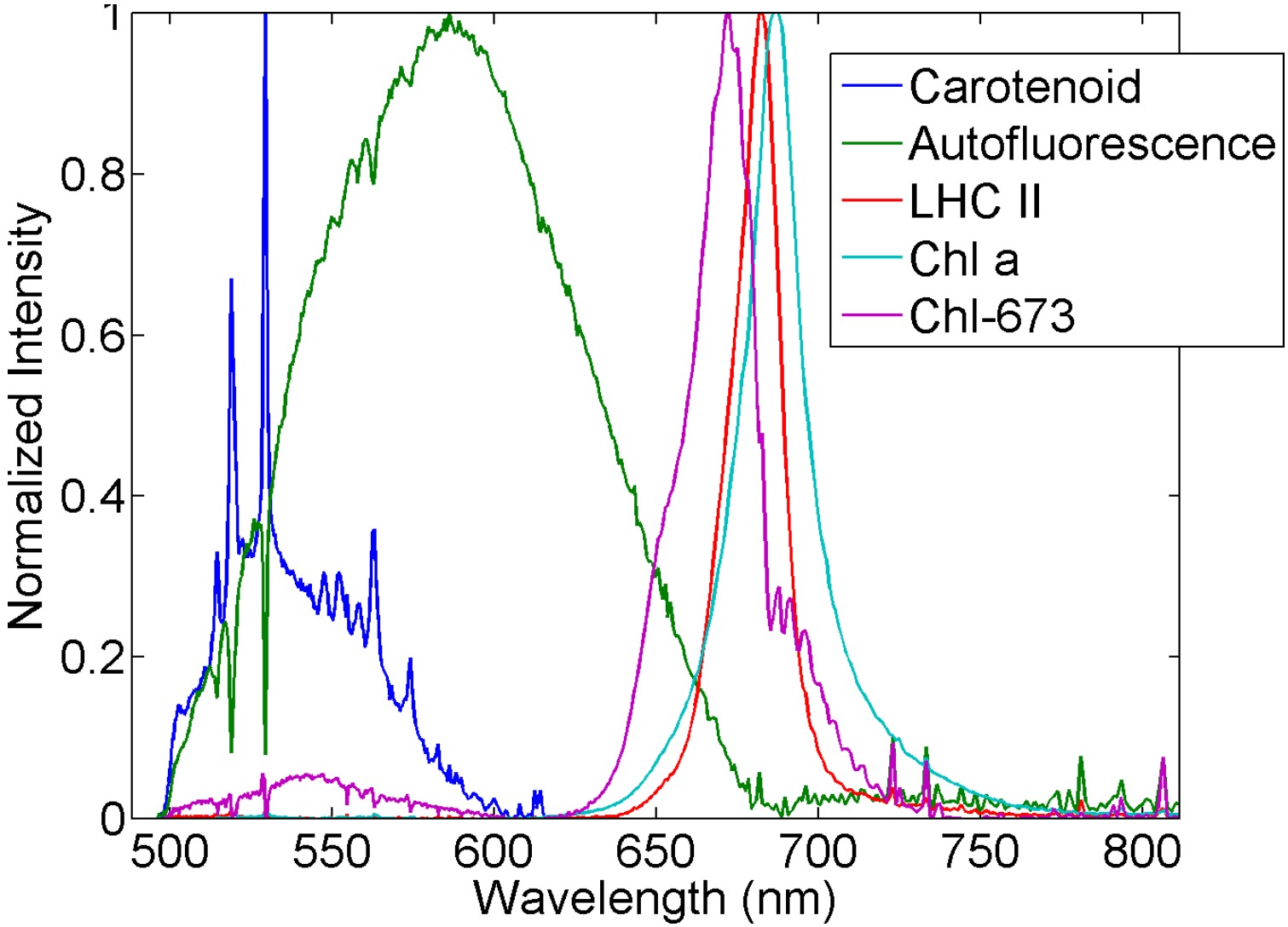
- 244 images collected
- Only 1 biological replicate imaged (chosen at random)
- All images were combined, compressed 16x (4x4), MCR model was generated and then applied to uncompressed data.
- Only cellular material was analyzed.

# qPCR data

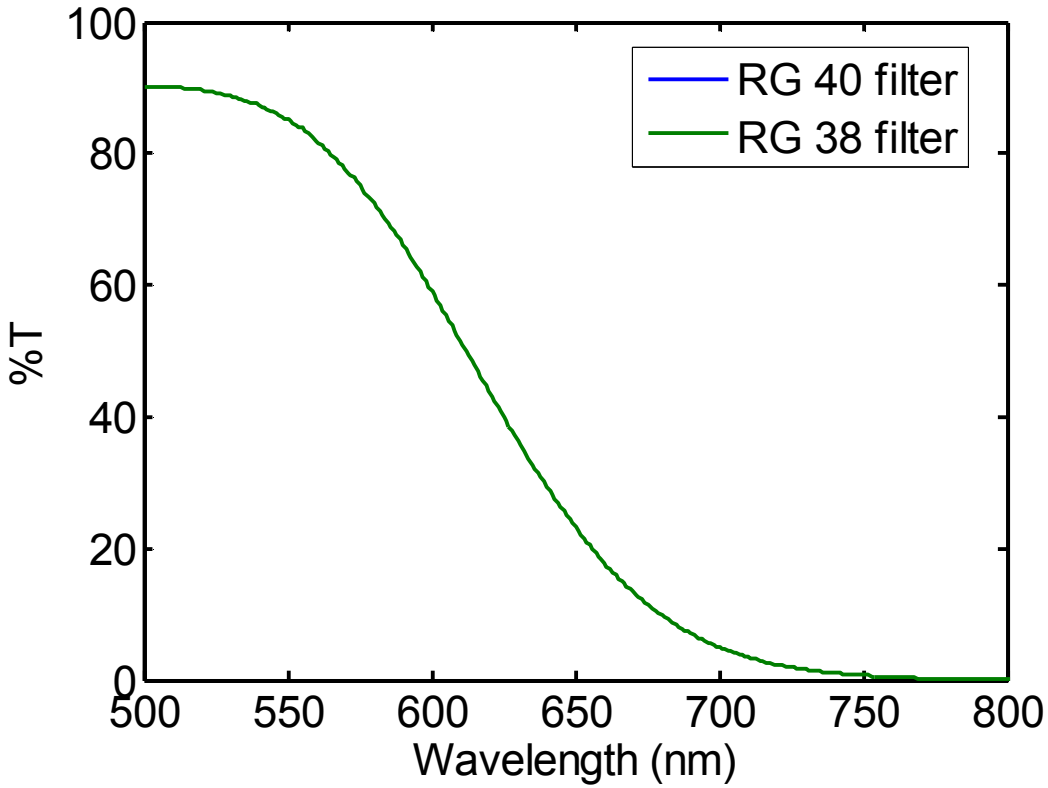
During time-course image experiments, 50 ul aliquots were removed for qPCR analysis.
When does chytrid infection begin to increase? 9-12 hours?
Does this infection follow Sapphire results?
Note: Chytrid DNA is evident at t=0 (CT = 40 for uninfected controls)



# 10x MCR Pure Spectral Components



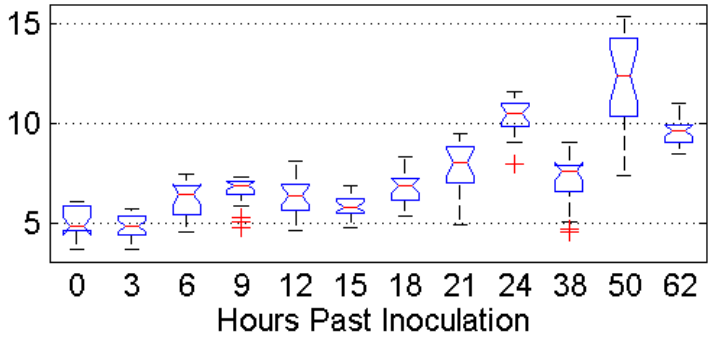
An optical filter is place in front of the detector to allow us to use higher laser power to down-weight the Chl-emission while revealing subtle changes in autofluorescence.



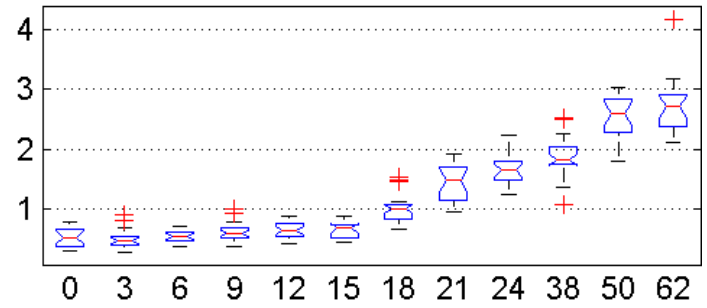
# Statistical Box Plots

## (Inoculation Data, Mean Image Data)

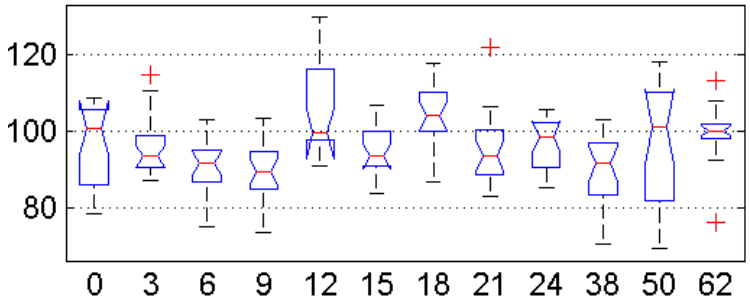
Carotenoid



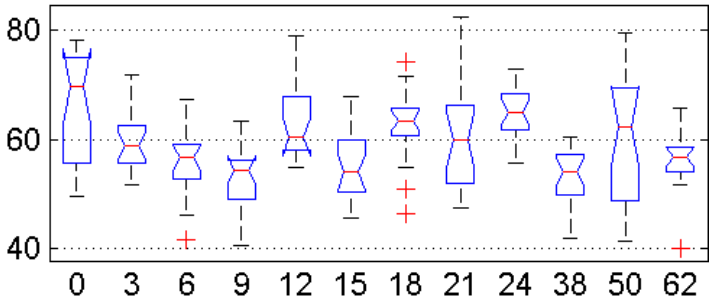

Autofluorescence



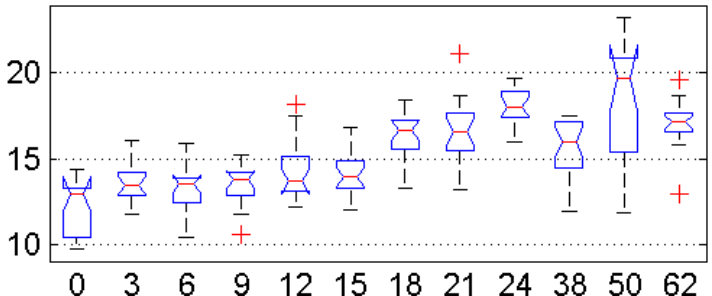

LHC II




Chl a

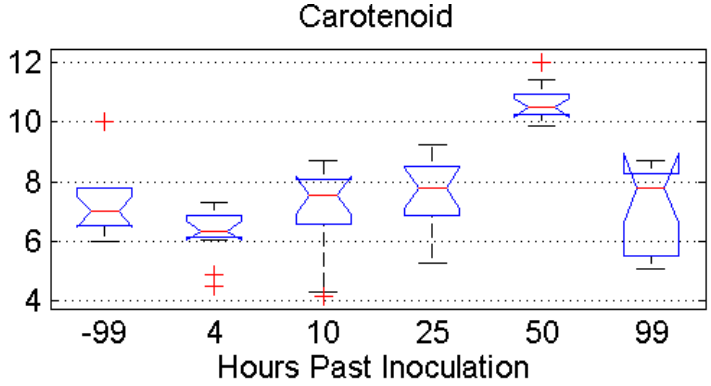


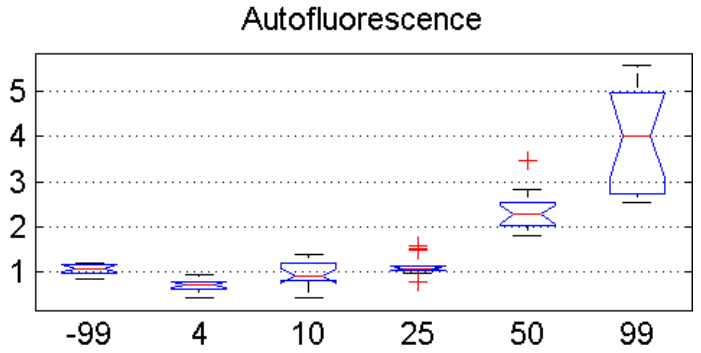

Chl-673

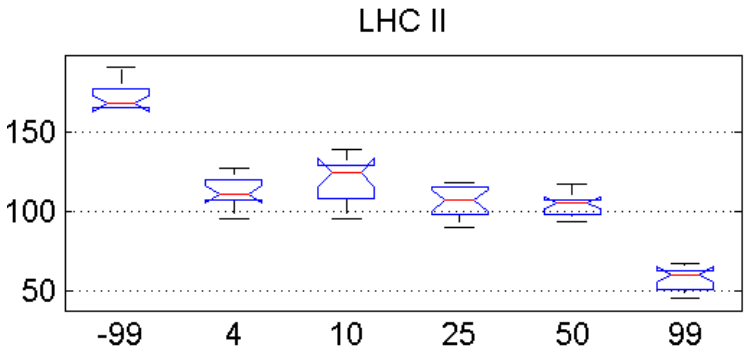


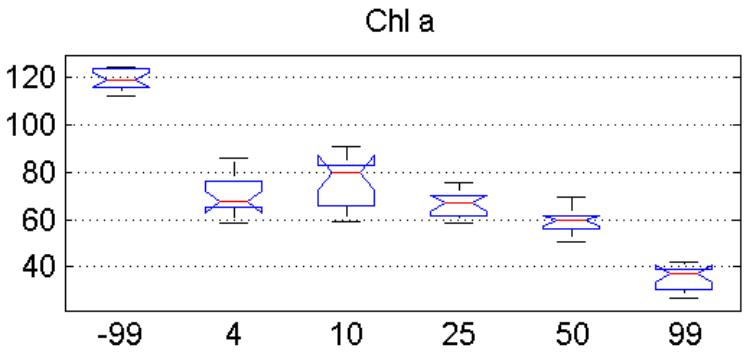
# Statistical Box Plots

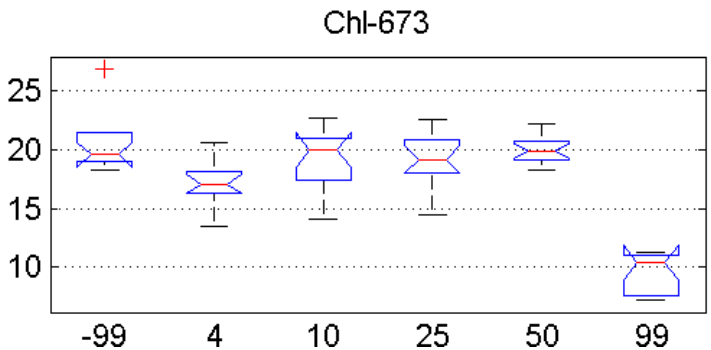
## (Control Data, Mean Image Data)



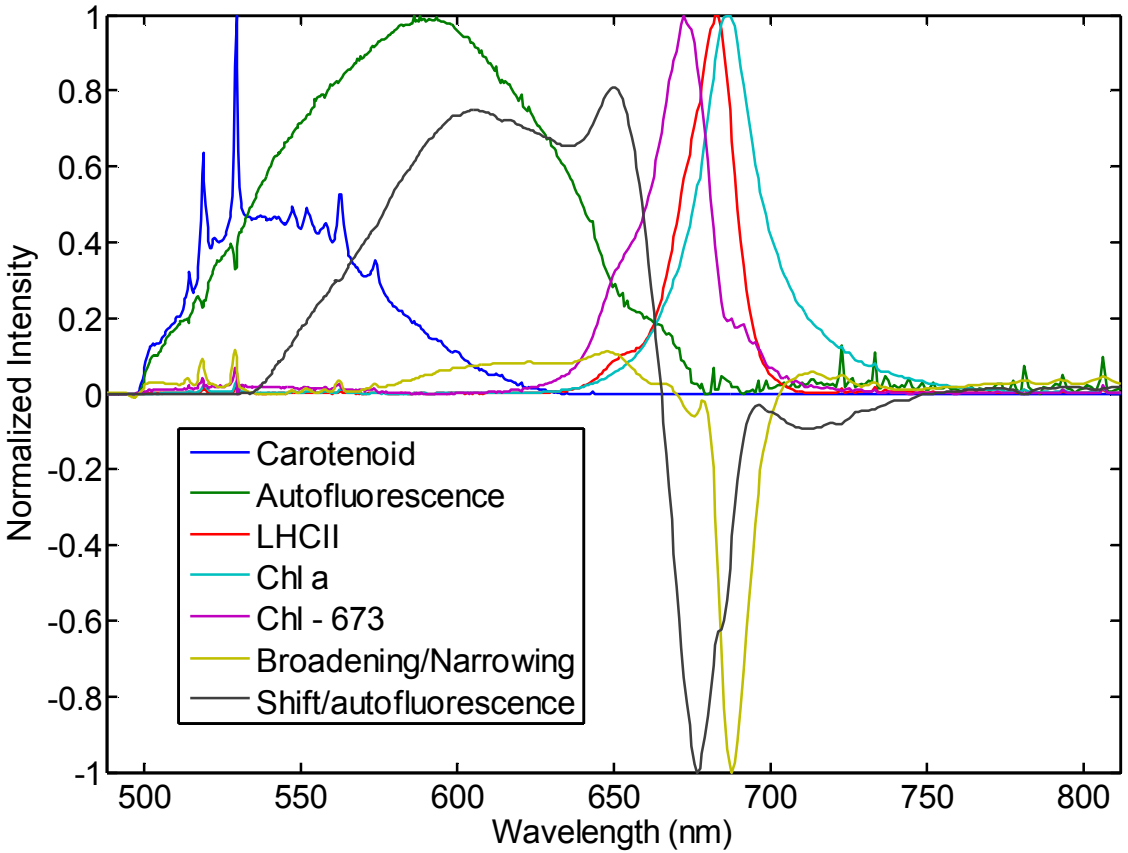

-99 = Scenedesmus Source

99 = Chytrid Source

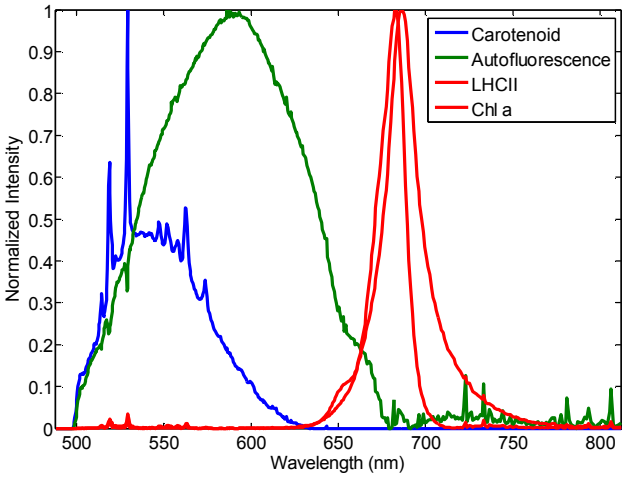
# 10x Investigation Summary

- Starting at 18 hours, we see a statistically significant increase in the amount of autofluorescence and blue-shifted chlorophyll (Chl-673) components
  - Carotenoid is increased starting at 21 hours
- Control data for the carotenoid and autofluorescence is relatively unchanged until somewhere between 25-50 hours
  - Could be due to heat stress
- The control data for the blue-shifted chlorophyll (Chl-673) component is relative unchanged throughout the experiment
  - This could be a good marker for chytrid infection
- The major chlorophyll features (LHCII and Chl a) are not good indicators for <u>early</u> detection of a chytrid infection

# 10x MCR Pure Spectral Components

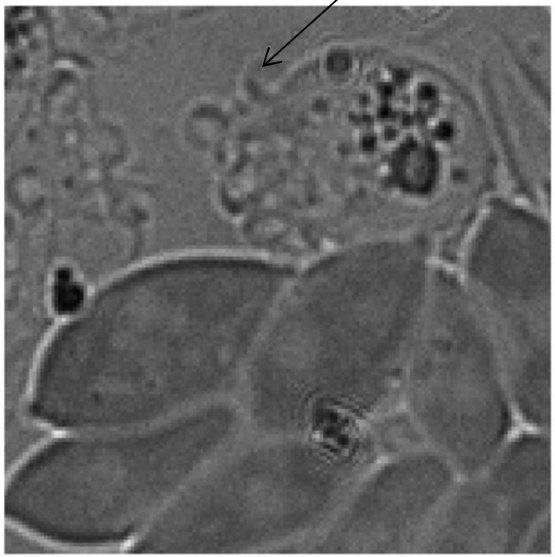


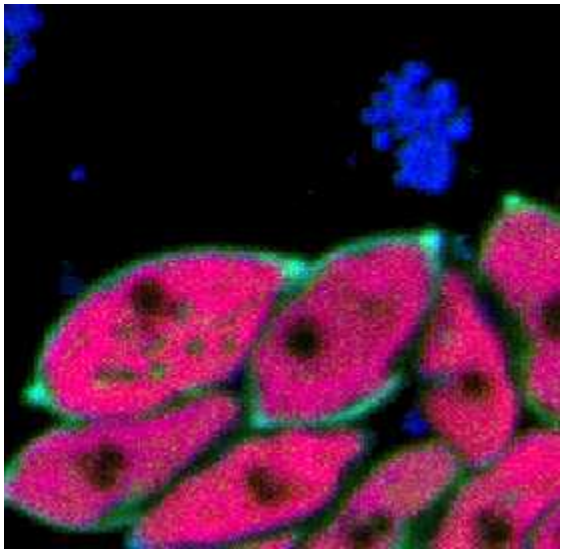

Very similar to 10 X model however two additional comps (**Broadening/narrowing** and **shift/autofluorescence**) which arise due to additional spatial resolution of 60x objective.

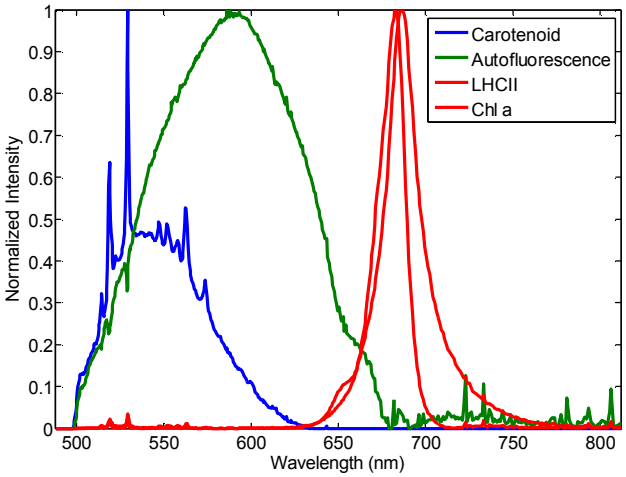



Select images - T0B2_60x_16.dat

Note sporangia in widefield image but not detected in fluorescence image.
These cells are very likely the infection source as these images were acquired at t = 0 hr (immediately following infection).

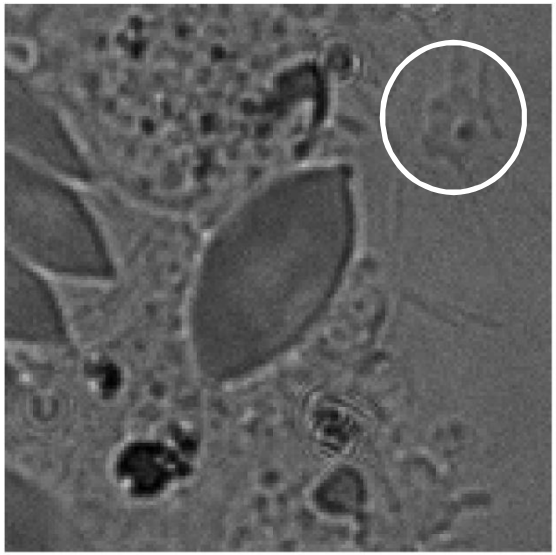


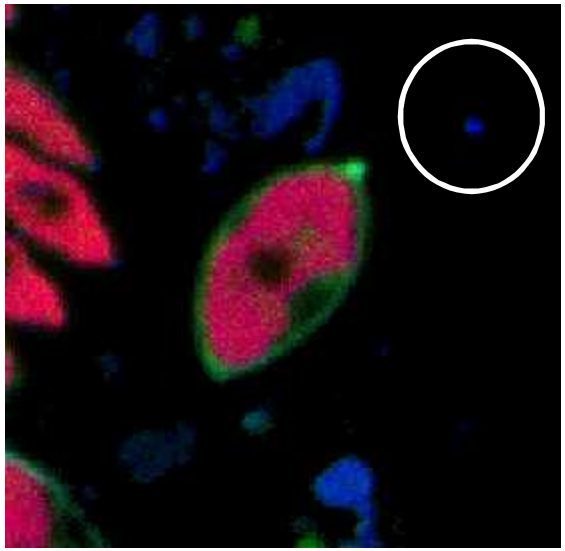


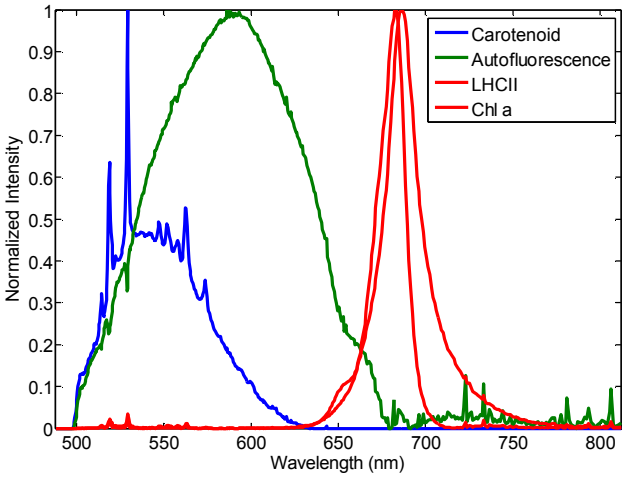



Select images - T0B2_60x_18.dat

Likely filamentous chytrid. We note that even though the we can see the chytrid in widefield only a small portion is visible in fluorescence and contains significant carotenoid. This is consistent with previous observations.



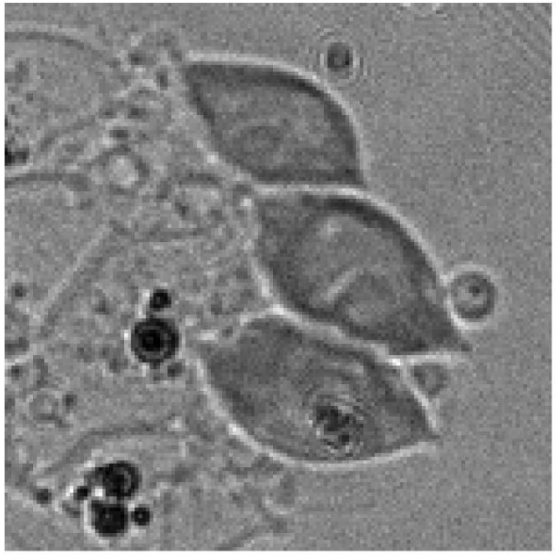


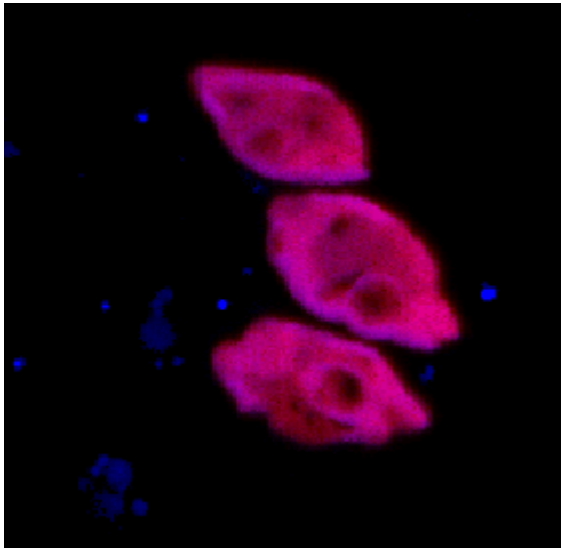


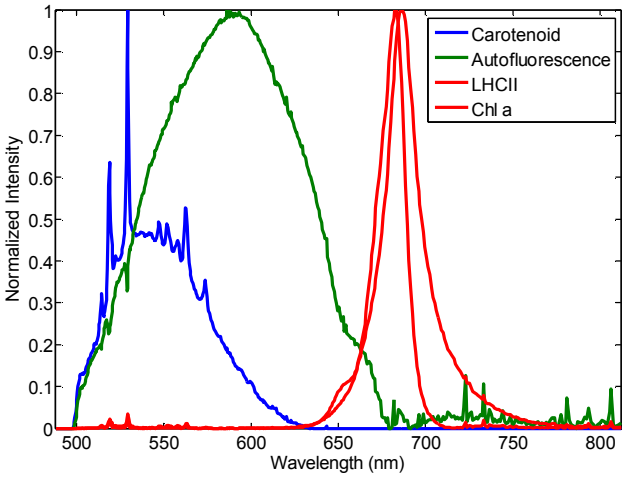

Select images - T3B2_60x_7.dat

Stressed cells have large pyrenoid

Chytrid visualized as small and round blue objects

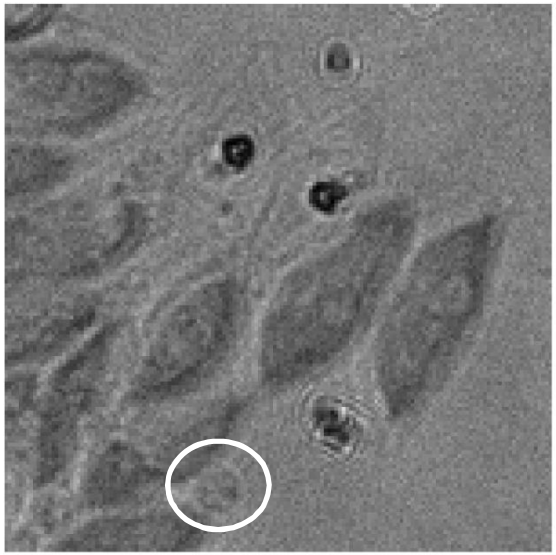


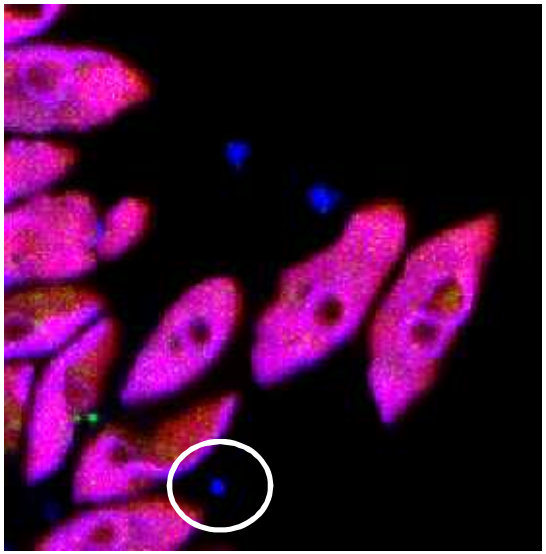


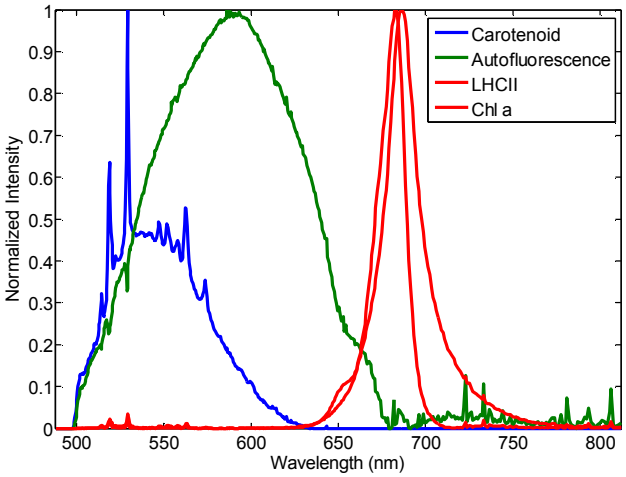



Select images - T18B2_60x_7.dat

Chytrid is present in image

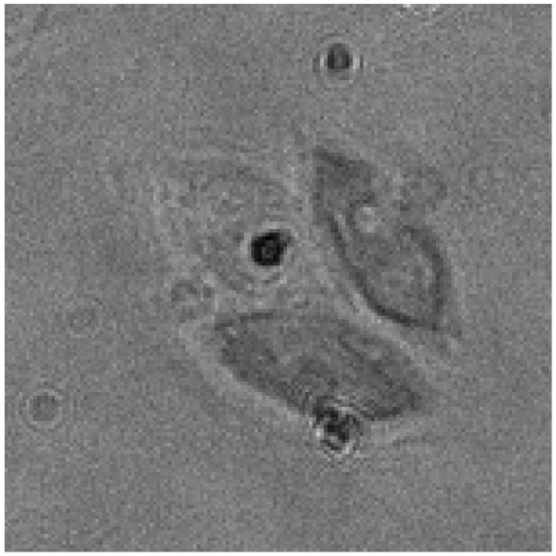


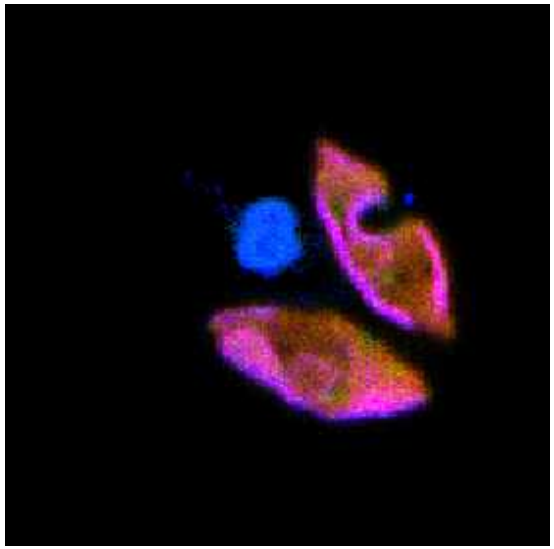


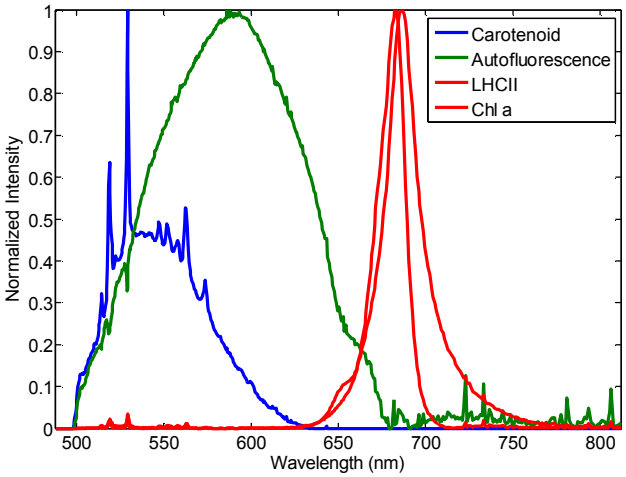



Select images - T18B2_60x_11.dat

Chytrid is present in image.
Note: Chloroplast retreats from site of chytrid.
Because we don't see the cytoplasm "fill" will Chl, I think the chloroplast is intact still.

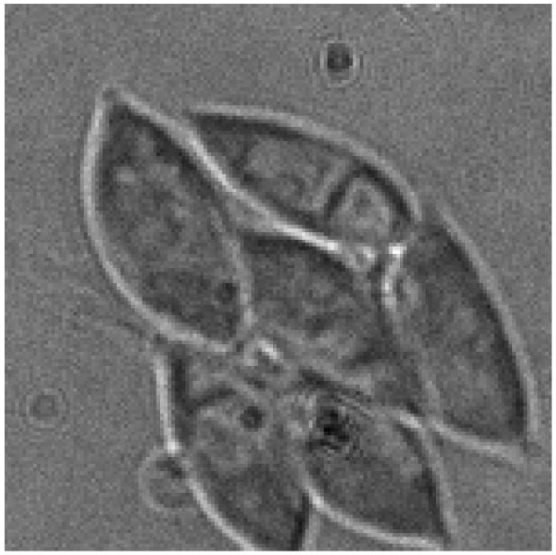


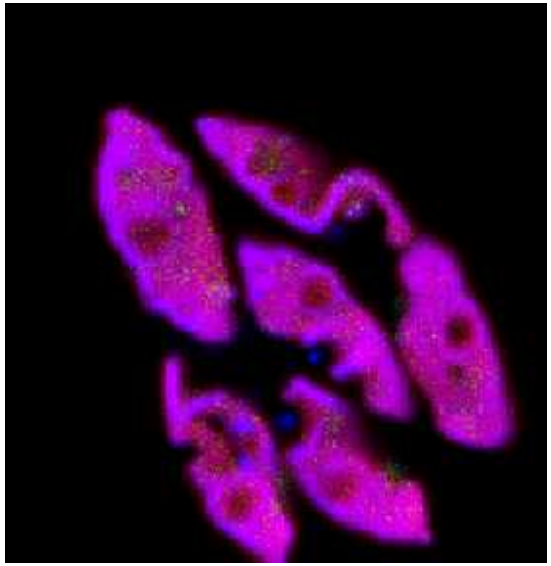


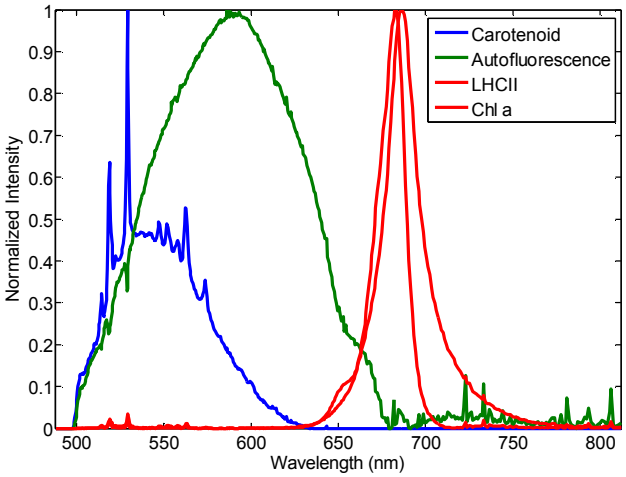



Select images - T18B2_60x_14.dat

Chytrid is present in image.
Note: Chloroplast retreats from site of chytrid.
Because we don't see the cytoplasm "fill" will Chl, I think the chloroplast is intact still.

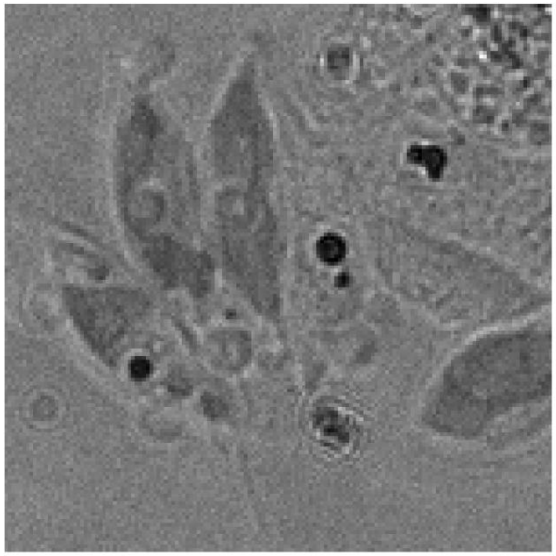


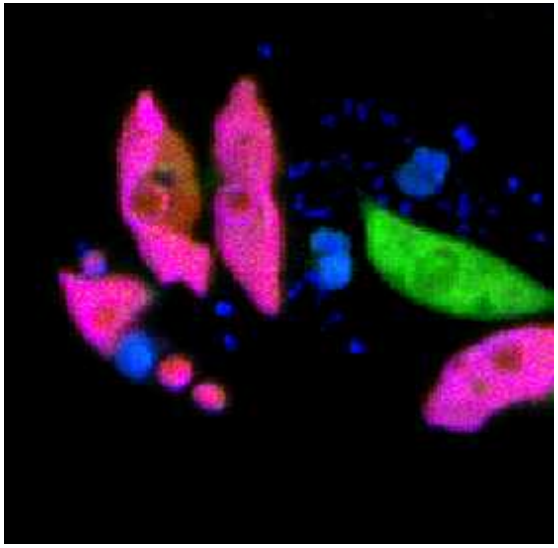


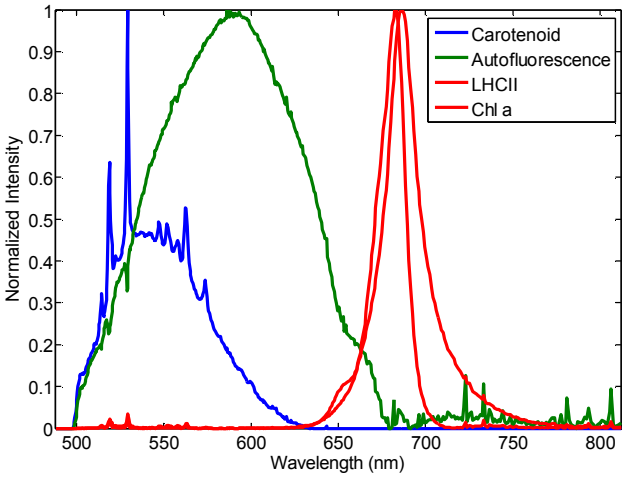



Select images - T21b1_60x_8.dat

Note the green cell. No Chl no Carotenoid but lots of autofluorescecne.





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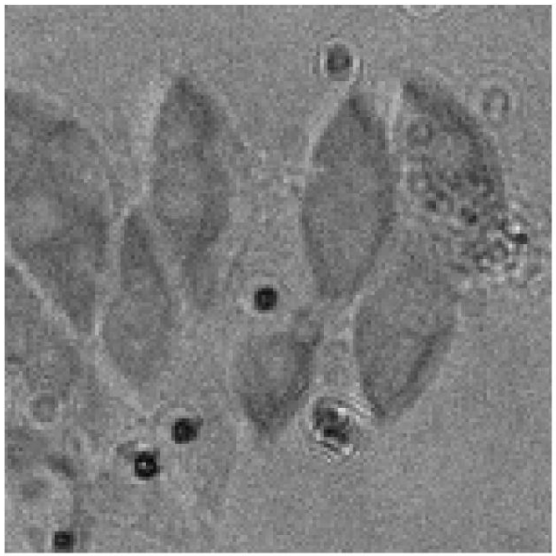

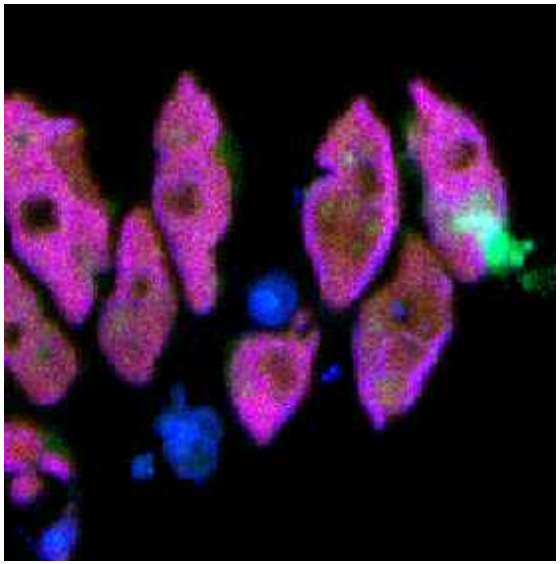

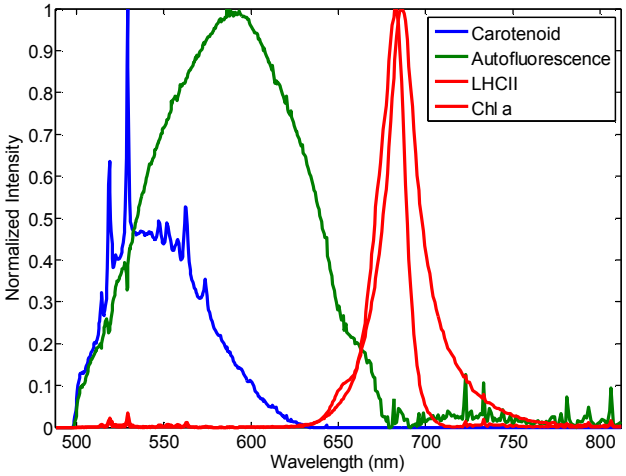


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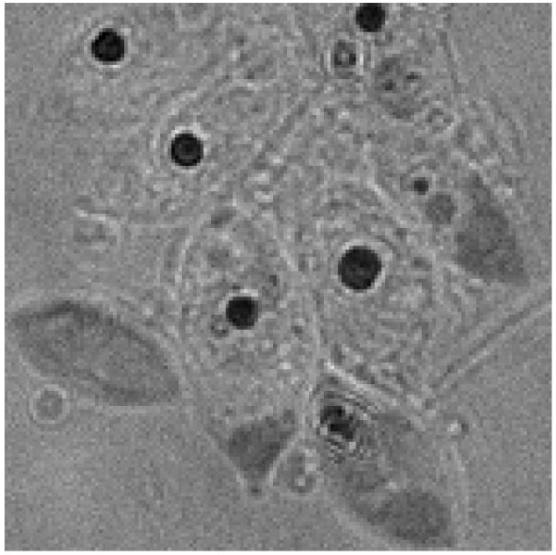
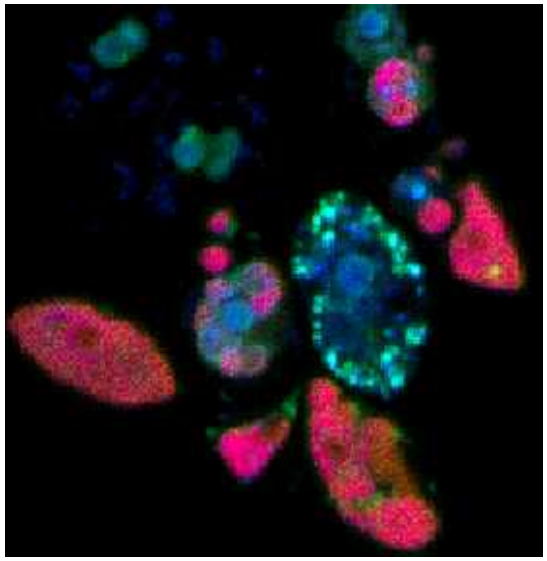
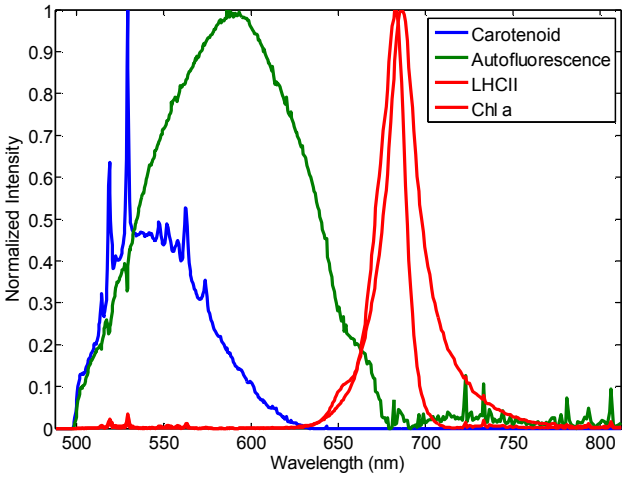
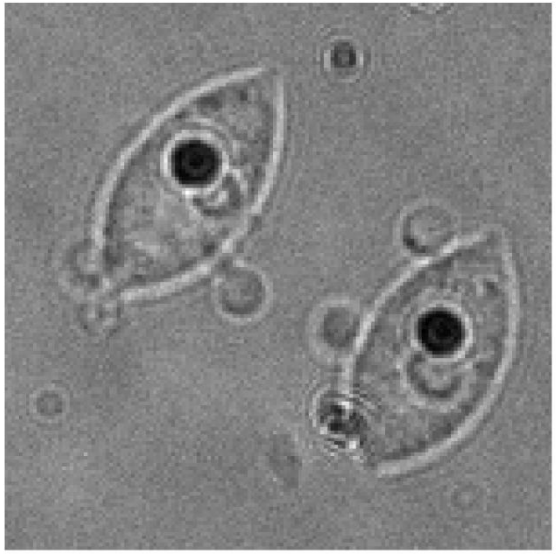


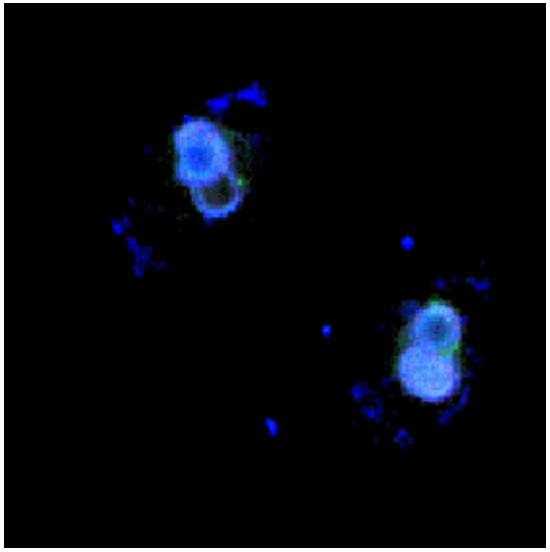


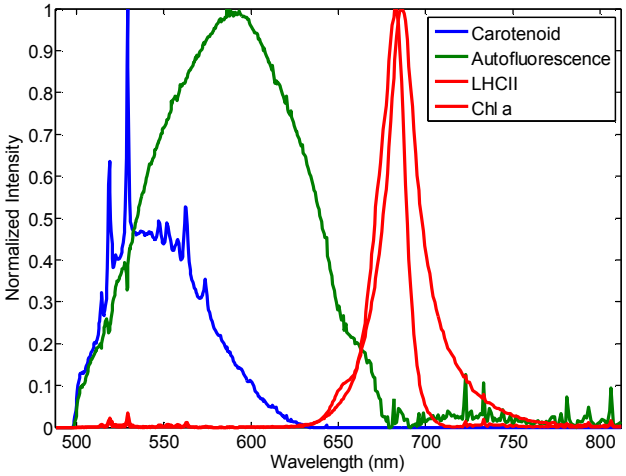



Select images

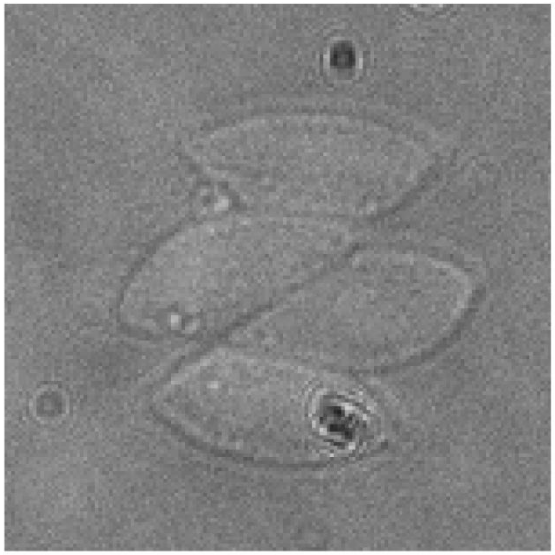
Again, sporangia hard to see in fluorescence image

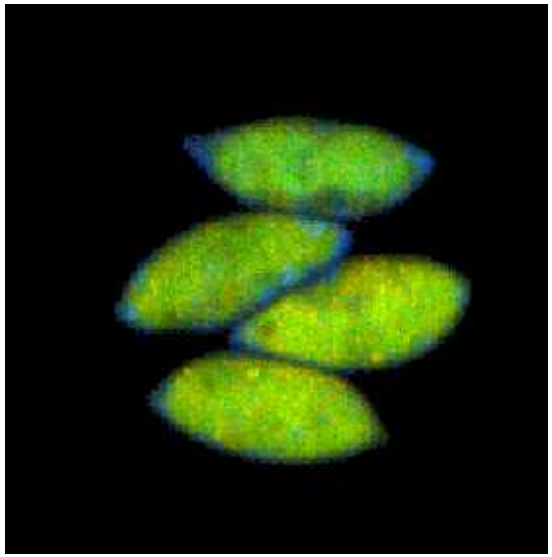


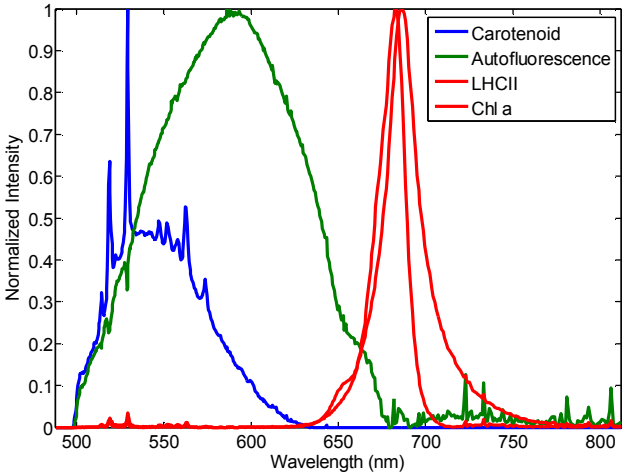




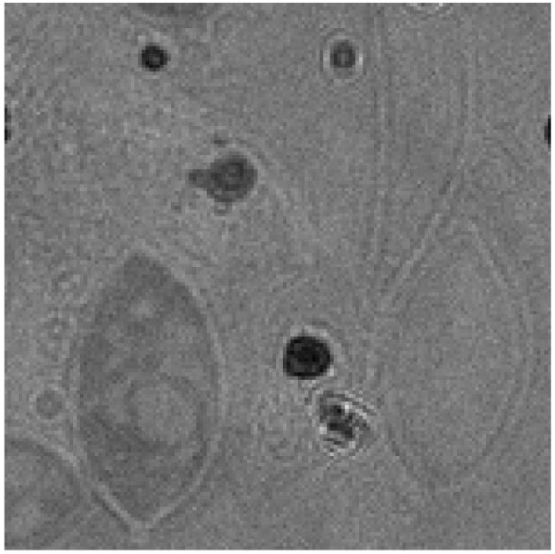

# Select images - T38b2_60x_13.dat

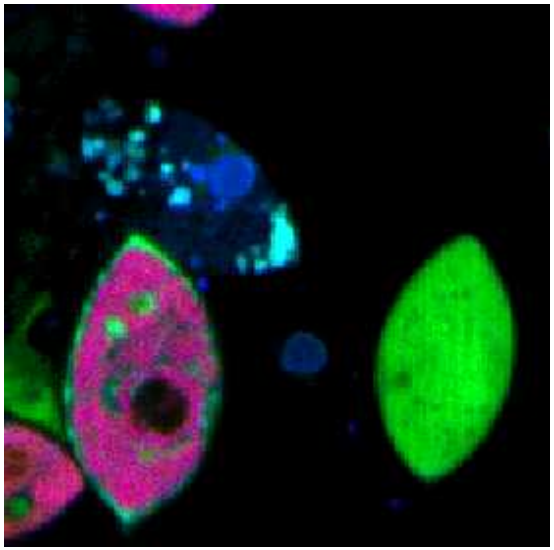


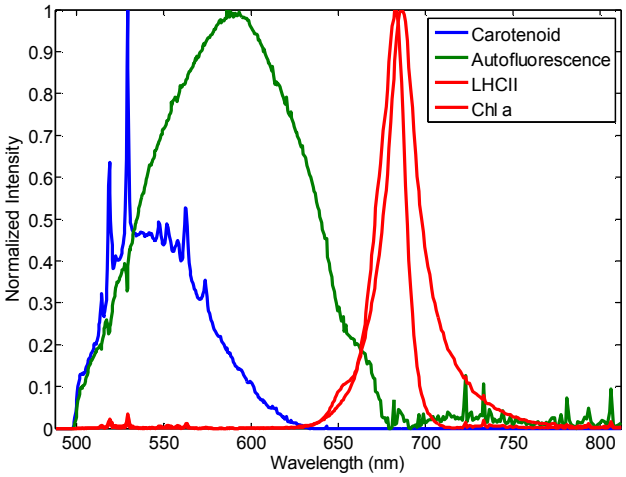





# Select images - T38b2_60x_23.dat

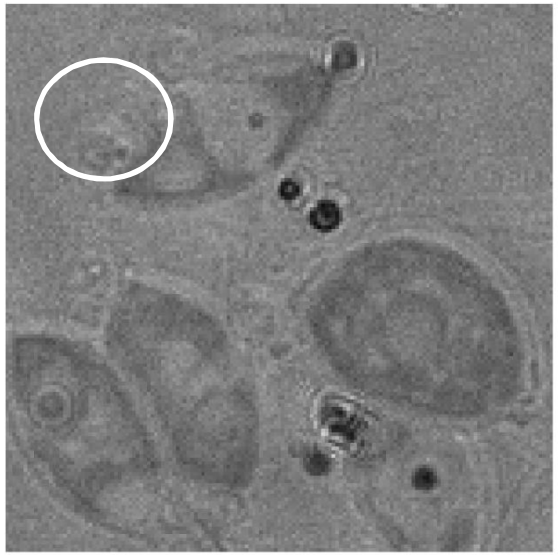


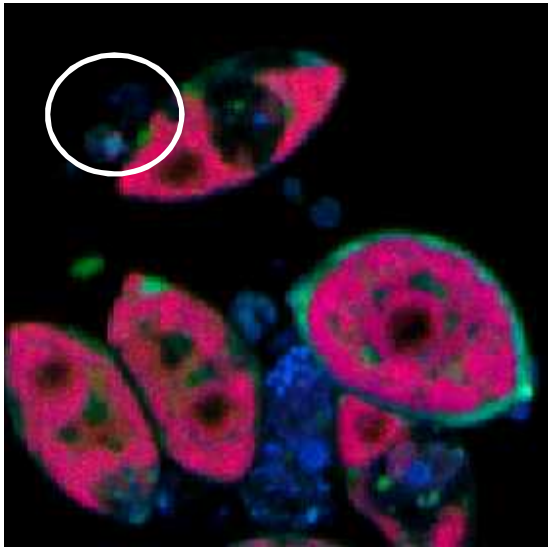





Select images

Sporangia?





# Summary of 60 X data

For the 60X data, we focused on stressed-looking cells.

- Morphological changes associated with infection although stress cannot be ruled out
  → I think condensed, pigmented bodies in center of hollowed out cells is the remnant of the pyrenoid (PSI enriched regions). Our filter cuts off PSI emission in this study but we have seen it before. Also, PSI has more Car then PSII and we definitely see more Car in these regions.
  → Cells have large pyrenoid that is visible in control cells

- Filamentous chytrid is visible in fluorescence microscopy however, there appears to be one (sometimes multiple) carotenoid-containing granules that is much smaller than chytrid.

- Sporangia are not very visible in fluorescence.
- No obvious chytrid on cell interior.
- Occasional observation of chytrid on cell exterior with chloroplast condensed away from chytrid.

- General observation of infection;

→ Heterogeneous23

→ Autofluorescence and Car occupying space between chloroplast and cell wall (mid infection?)

→ Later time points show almost no fluorescence – no pigments.