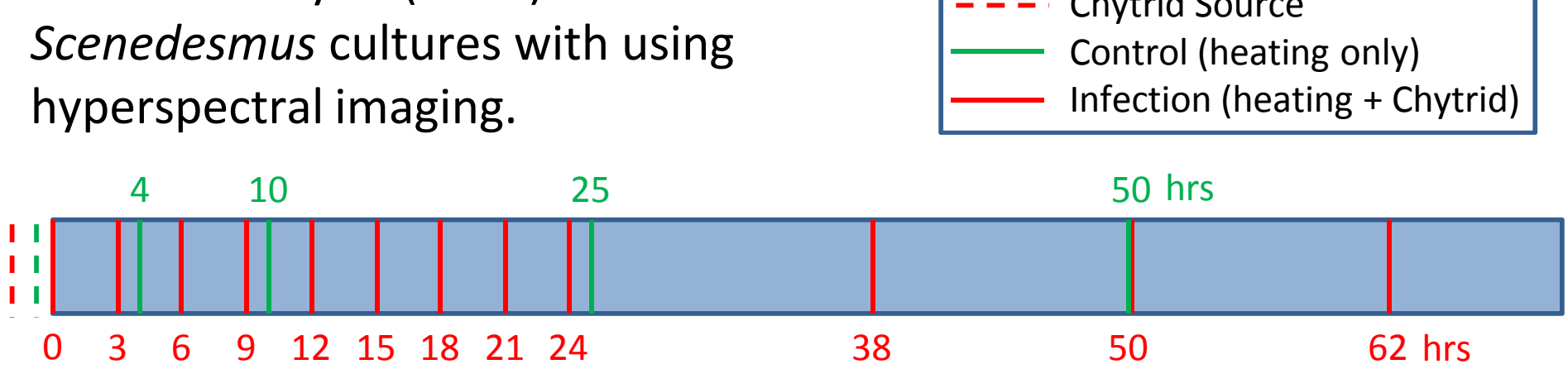
SAND2012-8080P

Temporal Investigation of the Chytrid Infection of Scenedesmus

Sapphire update meeting

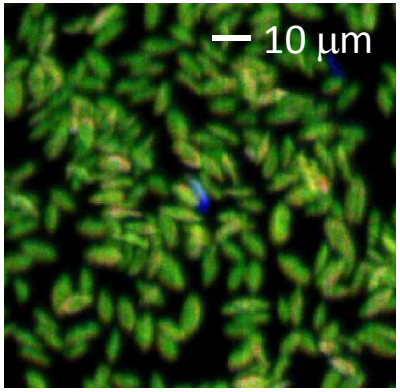
04/13/2012

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

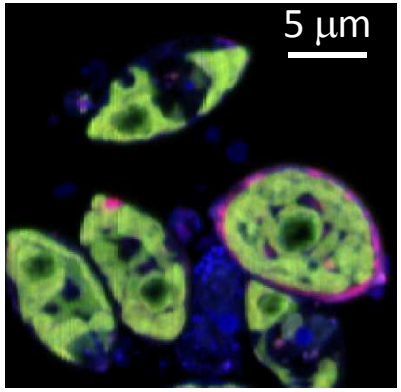
10X Objective Investigation



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

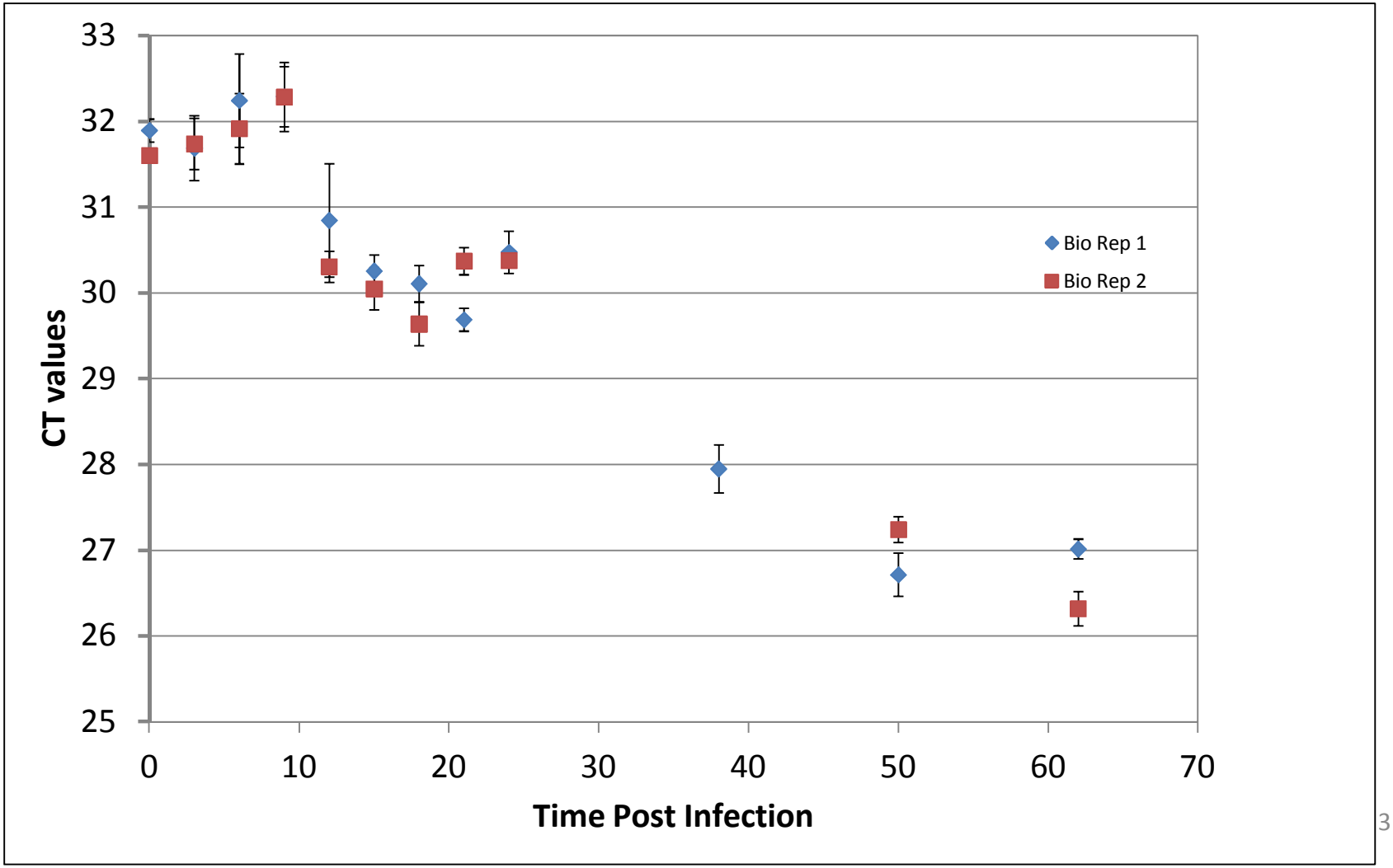


Goal: To develop better understanding of the mechanism of infection using chemical, spatial and temporally resolved imaging.

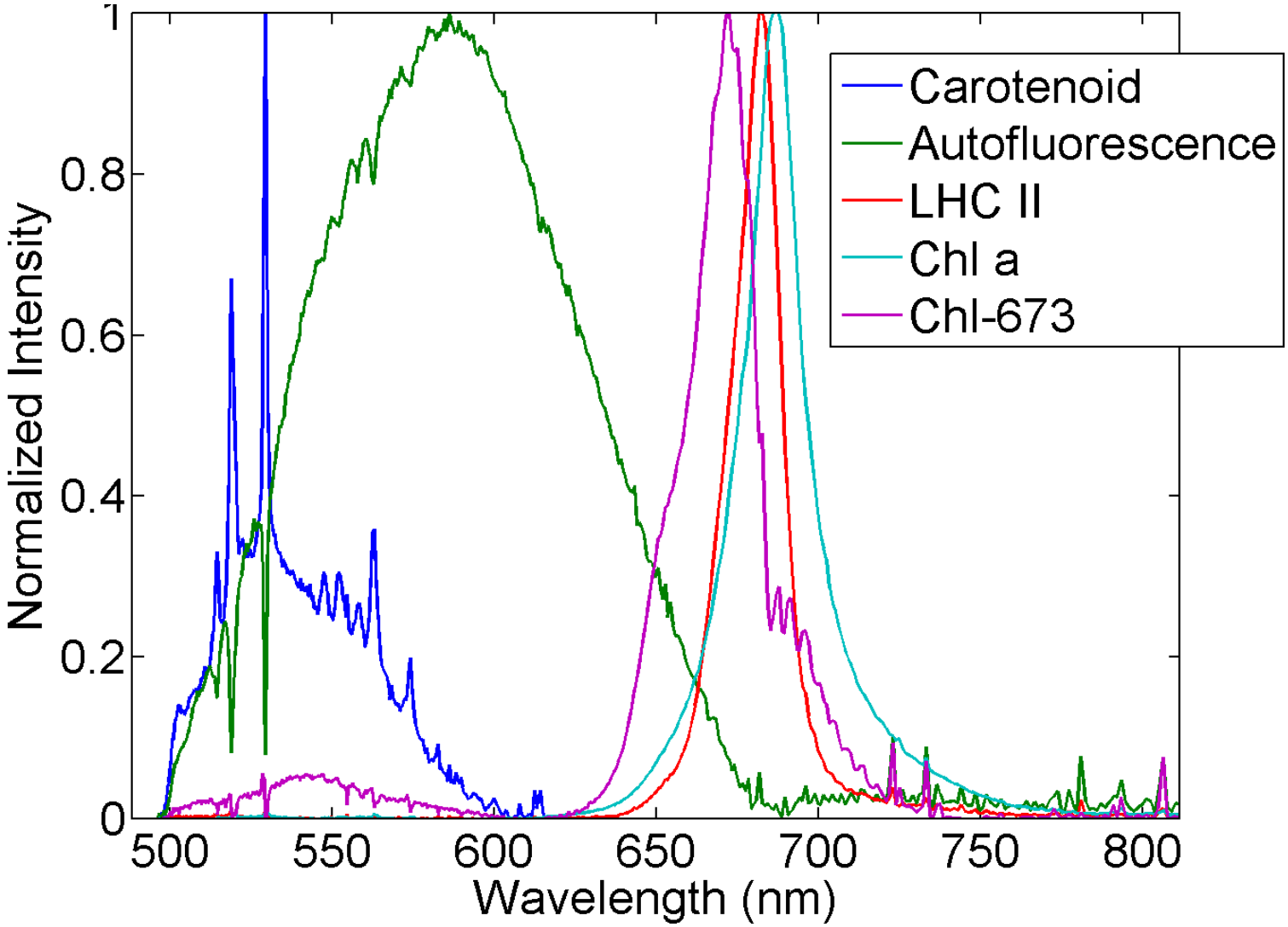
- 244 images collected
- Only 1 biological replicate imaged (chosen at random)
- All images were combined, compressed 16x (4x4), MCR model was generated and then applied to uncompressed data.
- Only cellular material was analyzed.

qPCR data

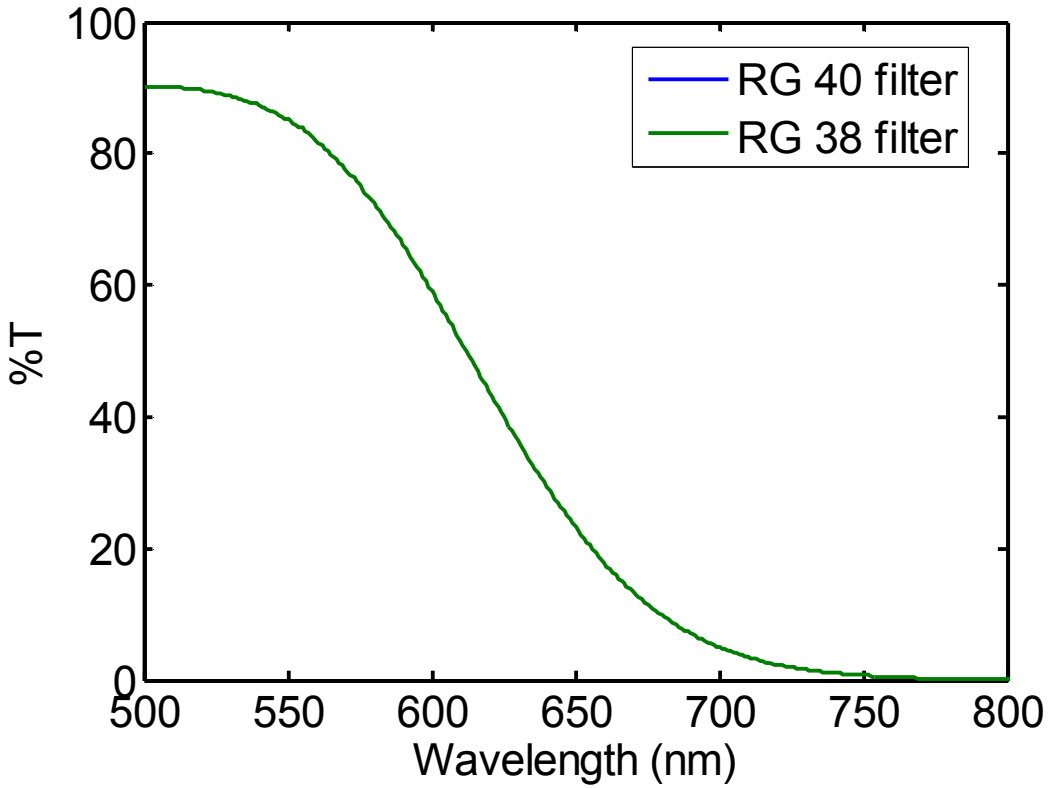
During time-course image experiments, 50 ul aliquots were removed for qPCR analysis.
When does chytrid infection begin to increase? 9-12 hours?
Does this infection follow Sapphire results?
Note: Chytrid DNA is evident at t=0 (CT = 40 for uninfected controls)



10x MCR Pure Spectral Components



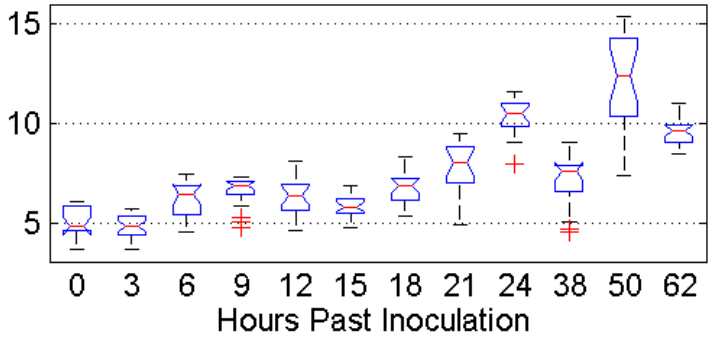
An optical filter is place in front of the detector to allow us to use higher laser power to down-weight the Chl-emission while revealing subtle changes in autofluorescence.



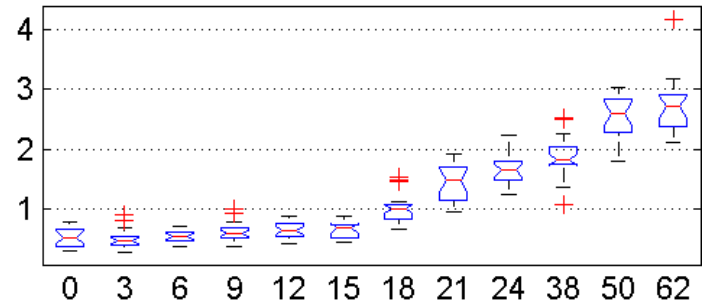
Statistical Box Plots

(Inoculation Data, Mean Image Data)

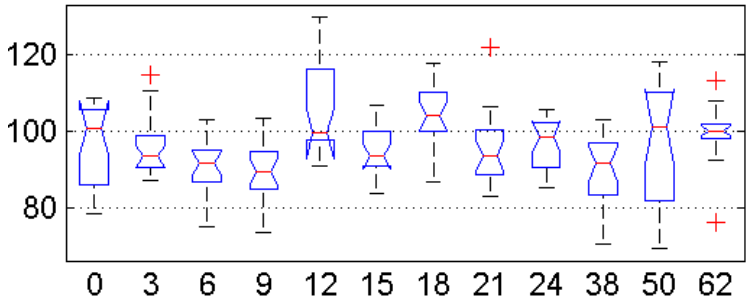
Carotenoid



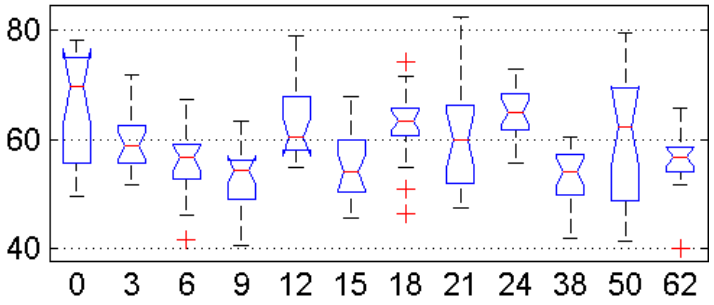
Autofluorescence



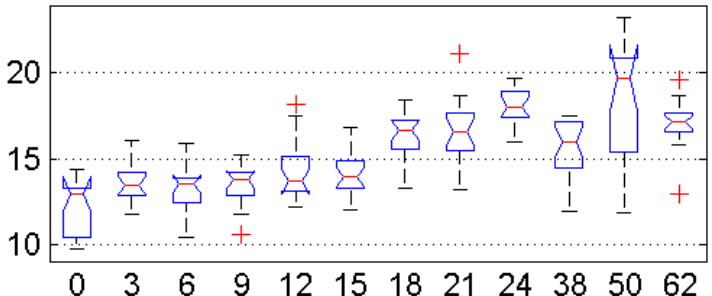
LHC II



Chl a

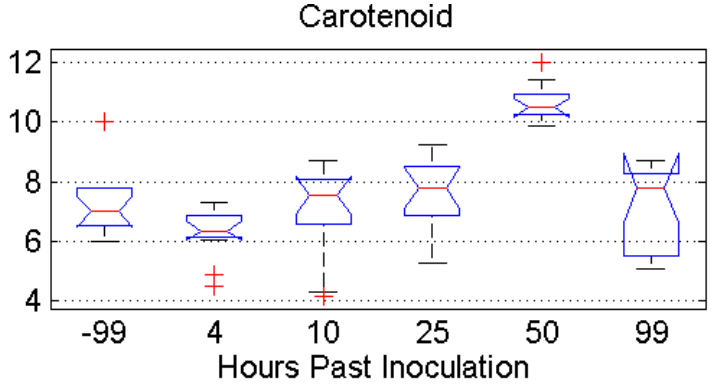


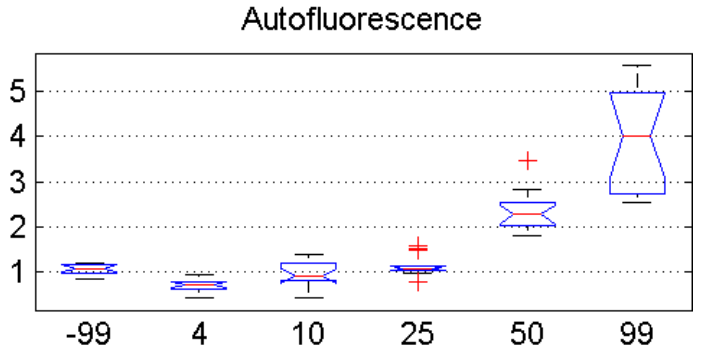
Chl-673

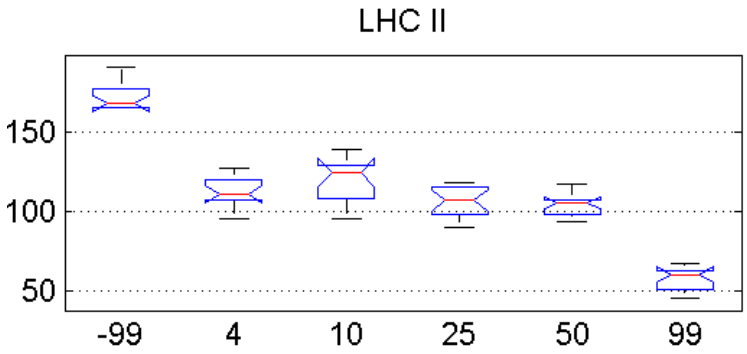


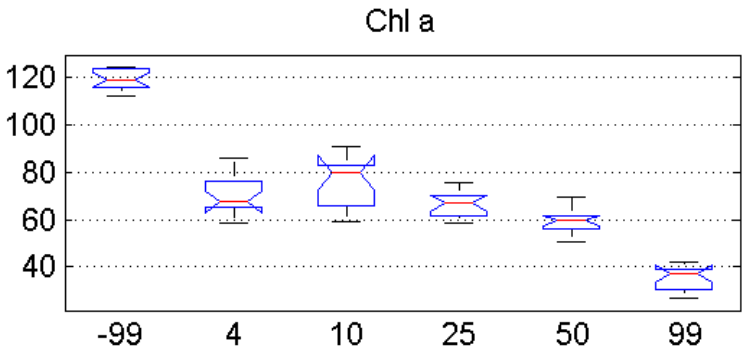
Statistical Box Plots

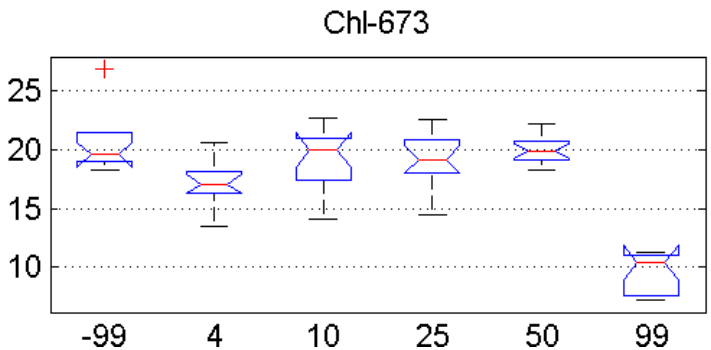
(Control Data, Mean Image Data)







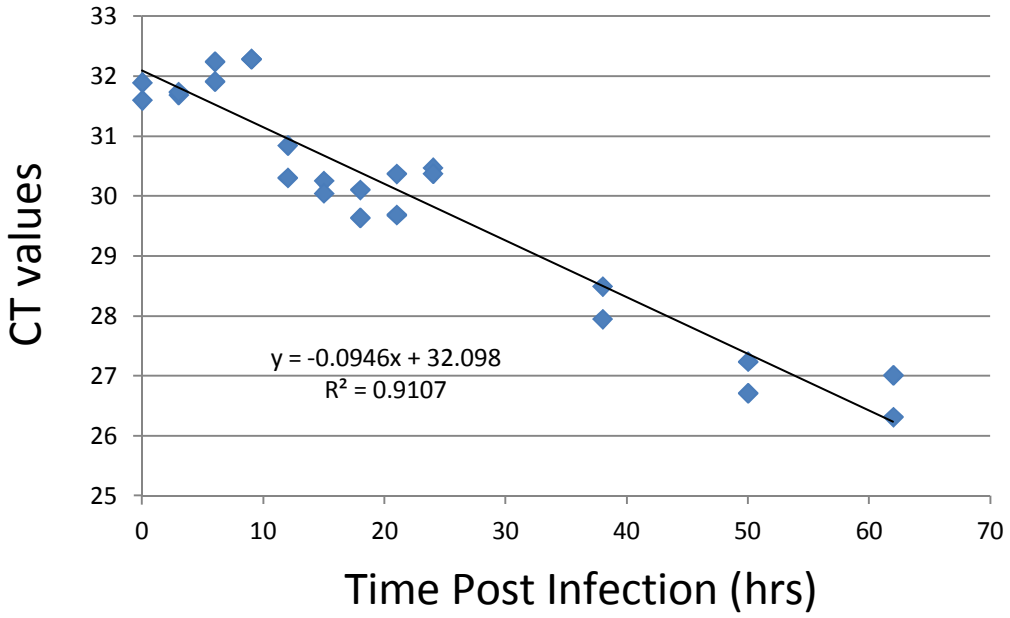




-99 = Scenedesmus Source

99 = Chytrid Source

Can we generate a qPCR spectroscopic calibration using PLS



Reference Values

- Try using the actual reference measurements.
- Try fitting a trend line through these measurements and use these for the reference measurements

Full Spectral Region
(no outliers removed)

Type: PLS1 Start Time: 08:14:21 Stop Time: 08:14:23 Version: 1.16 Date: 1/17/08

#Spectra: 231 #Components: 2 #Regions: 1 Sampling: All

#Points: 512 Variance Scaling: NO Mean Center: YES No Corr

Pathlength Corr: NO Custom: NO Baseline: NO

Max PRESS factors: 20 Frequencies: 488.4 811.7

Rotations: 6 8 10 10 10 10 10 10 9 8 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10

Rotation Component: 6 Tolerance: 0

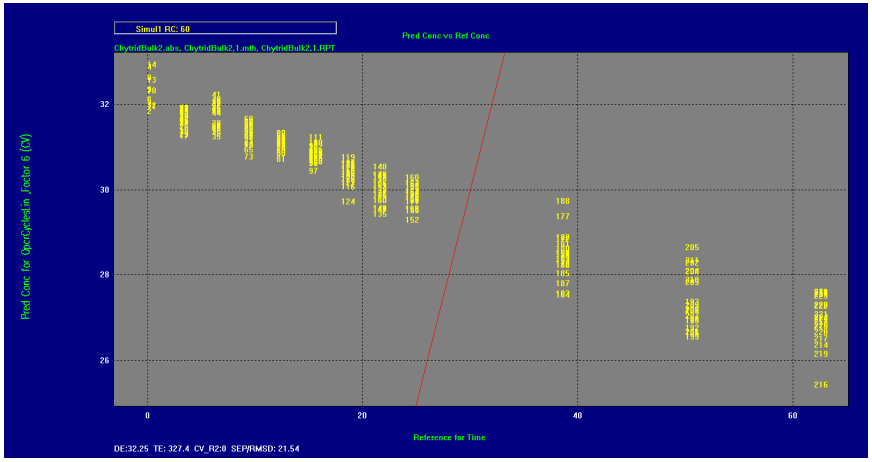
Method file: ChytridBulk2,1.mth Calibration file: ChytridBulk2.abs

PLS-1 FACTORS

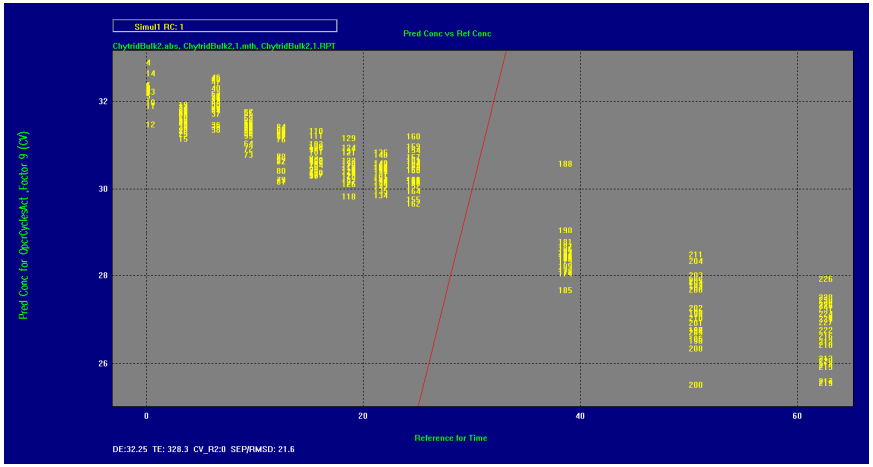
Components	Used	F-R Press	SE_ Press	NonP Press	R2	SEP
QpcrCyclesLin	6	6	6	5	.9434	.4162

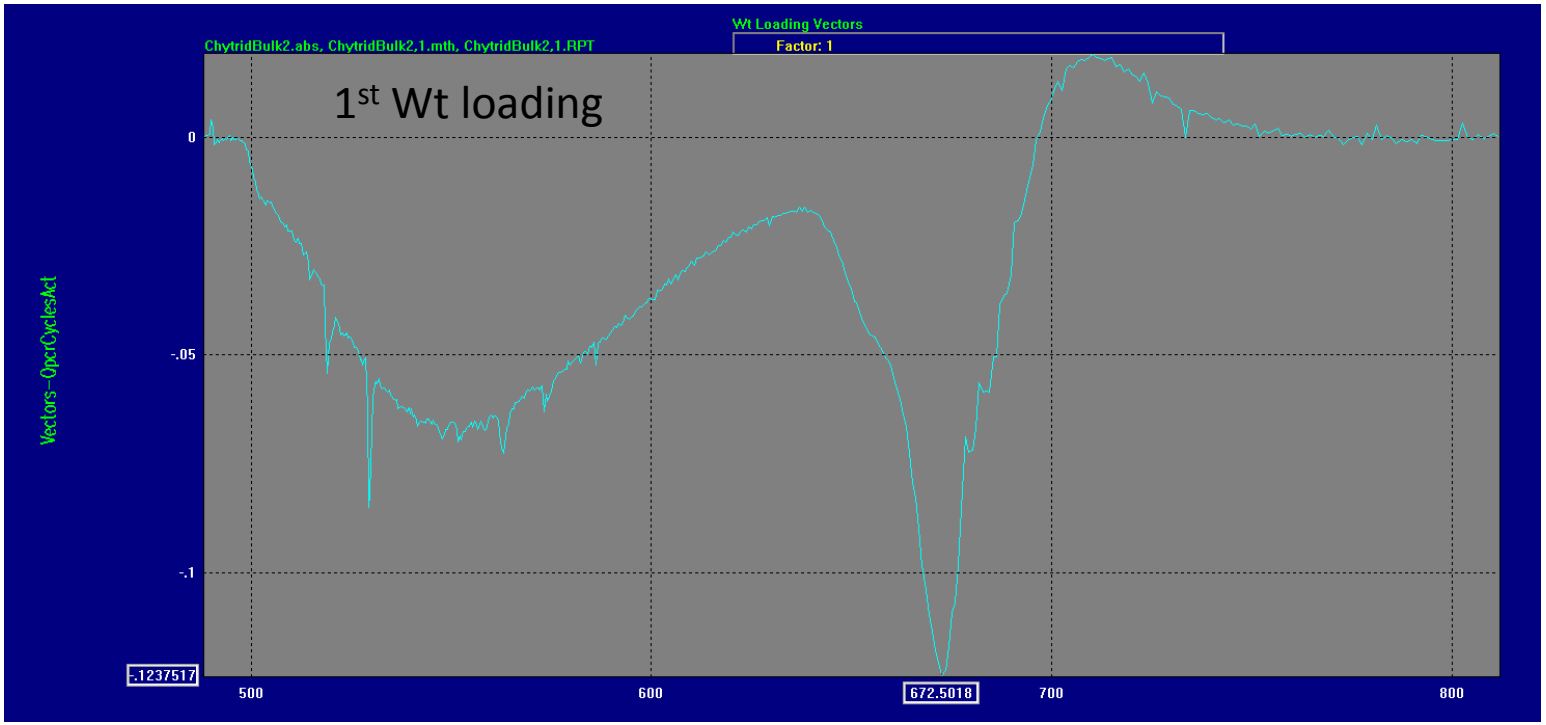
Components	Used	F-R Press	SE_ Press	NonP Press	R2	SEP
QpcrCyclesAct	9	9	8	9	.8783	.6443

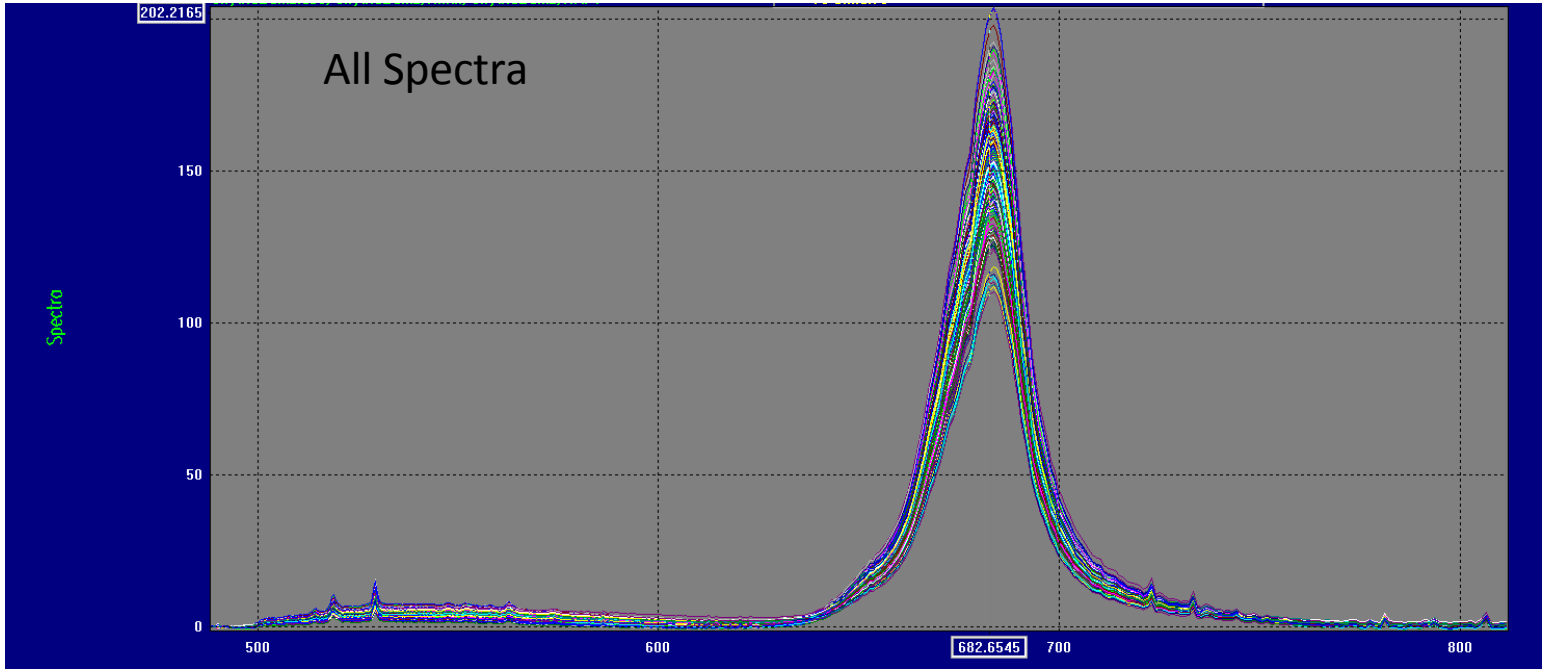
Linear Reference



Actual Values




Wt Loading Vectors
ChytridBulk2.abs, ChytridBulk2,1.mth, ChytridBulk2,1.RPT
Factor: 1
1st Wt loading
Vectors-OpcrCyclesAct
0
-.05
-.1
-.1237517
500
600
672.5018
700
800


All Spectra
Spectra
202.2165
150
100
50
0
500
600
682.6545
700
800

Chl Spectral Region (outliers removed)

Type: PLS1 Start Time: 13:12:39 Stop Time: 13:12:40 Version: 1.16 Date: 1/17/08

#Spectra: 215 #Components: 2 #Regions: 1 Sampling: All

#Points: 231 Variance Scaling: NO Mean Center: YES No Corr

Pathlength Corr: NO Custom: NO Baseline: NO

Max PRESS factors: 20 Frequencies: 599.819 801.212

Rotations: 6 8 10 10 10 10 10 10 9 8 10 10 10 10 10 10 10 10 10 7 5 9 9 4

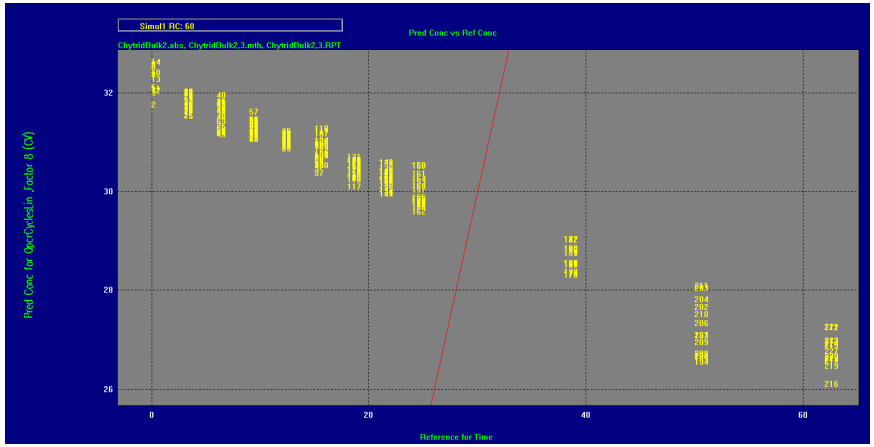
Rotation Component: 6 Tolerance: 0

Method file: ChytridBulk2,3.mth Calibration file: ChytridBulk2.abs

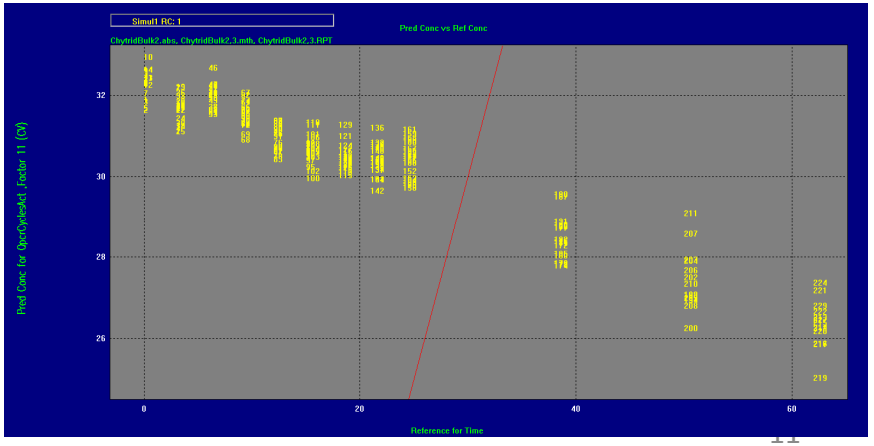
PLS-1 FACTORS

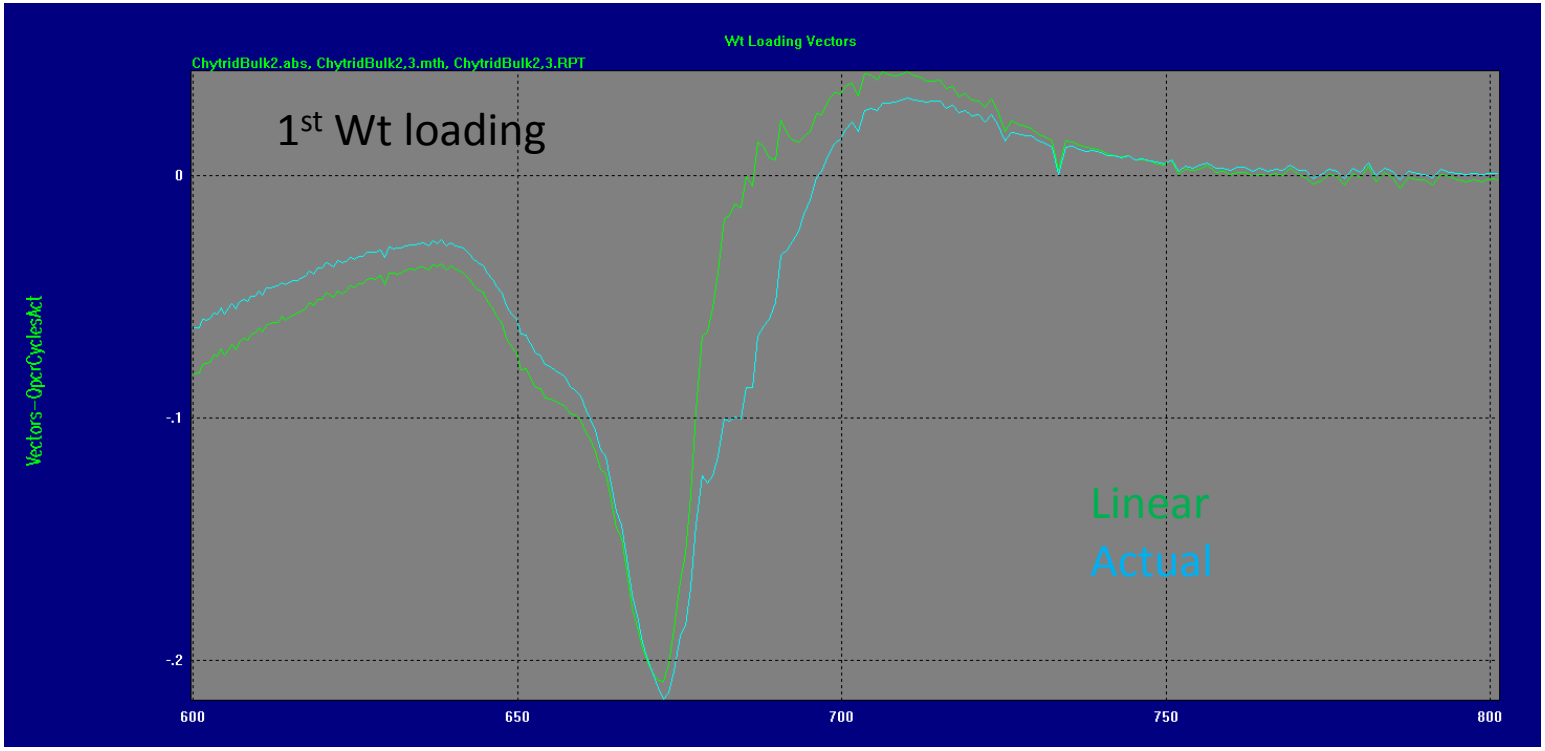
Components	Used	F-R Press	SE_ Press	NonP	R2	SEP	Components	Used	F-R Press	SE_ Press	NonP	R2	SEP
QpcrCyclesLin	8	8	7	7	.9686	.2826	QpcrCyclesAct	11	11	11	10	.8666	.6181

Linear Reference



Actual Values



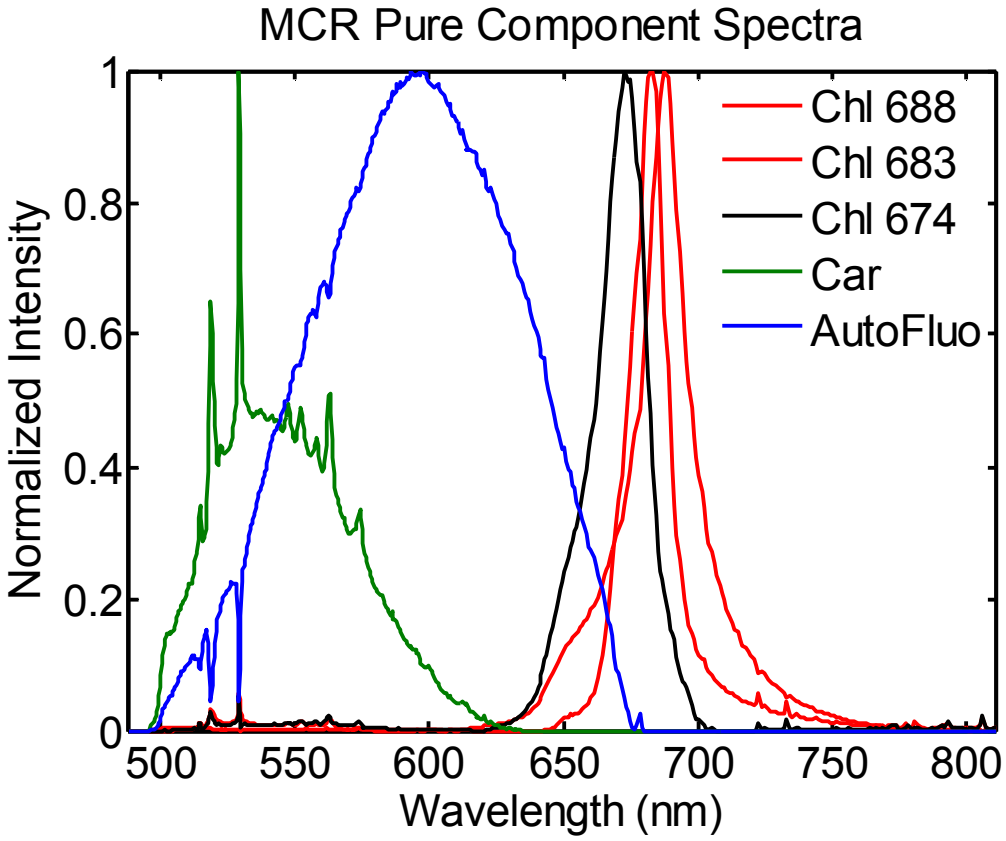

Wt Loading Vectors
ChytridBulk2.abs, ChytridBulk2.3.mth, ChytridBulk2.3.RPT
1st Wt loading
Vectors-OpctCyclesAct
0
-.1
-.2
600
650
700
750
800
Linear
Actual

10x Investigation Summary

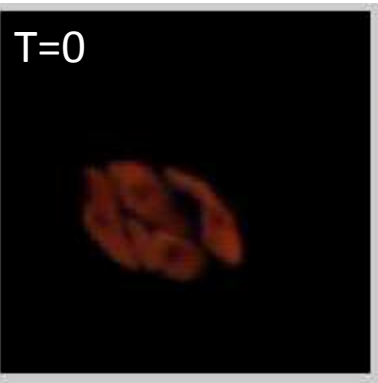
- Starting at 18 hours, we see a statistically significant increase in the amount of autofluorescence and blue-shifted chlorophyll (Chl-673) components
  - Carotenoid is increased starting at 21 hours
- Control data for the carotenoid and autofluorescence is relatively unchanged until somewhere between 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

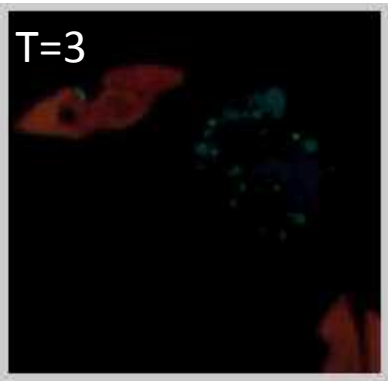
60x Investigation

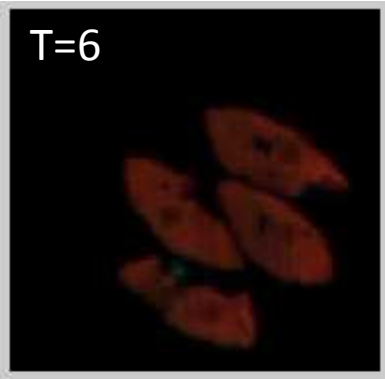
Unlabeled SD and Chytrid FD001 for HJ poster

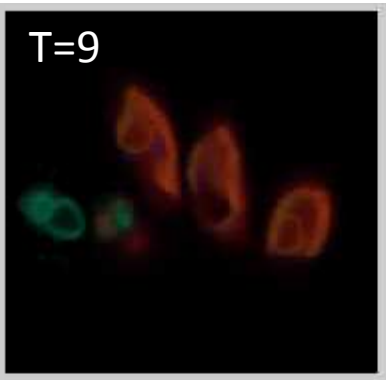


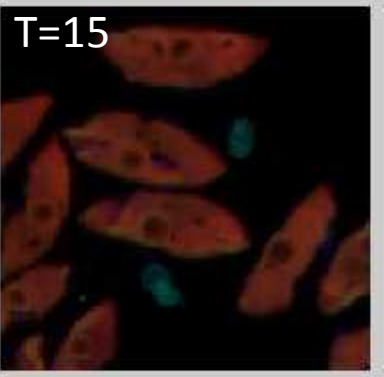
Autoscale off

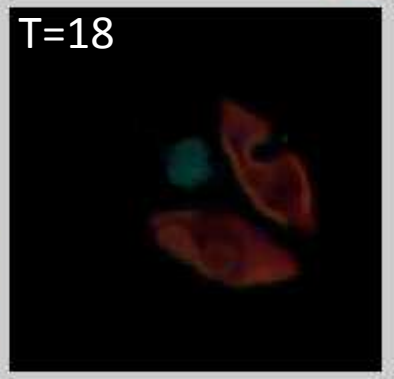


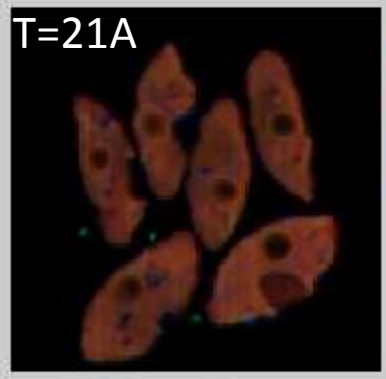


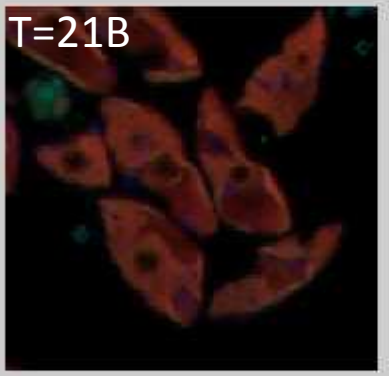


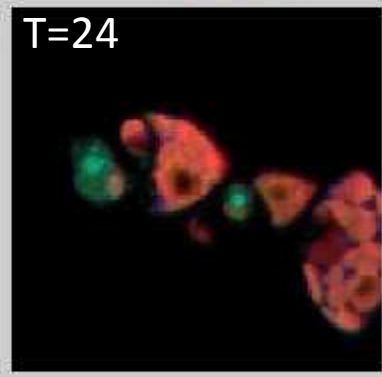


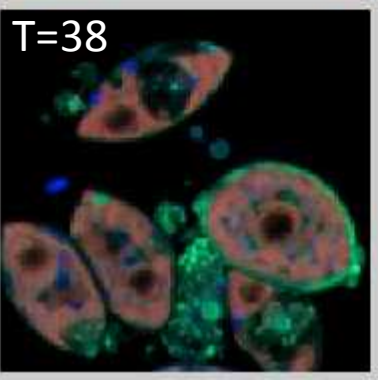


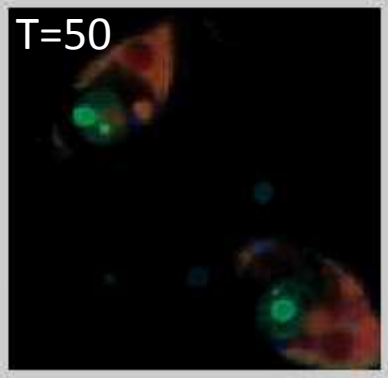


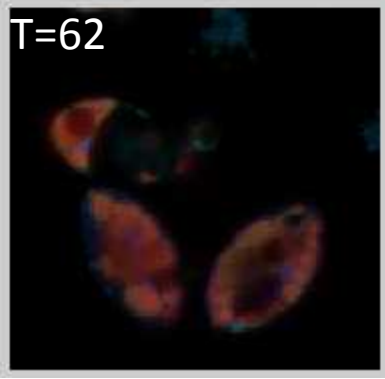


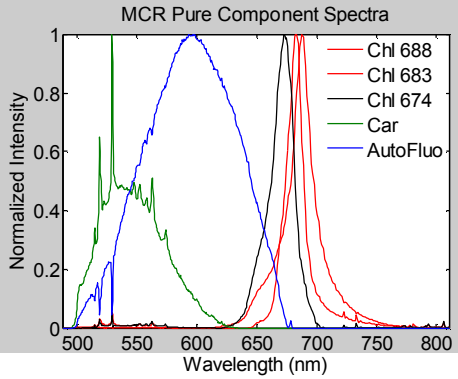




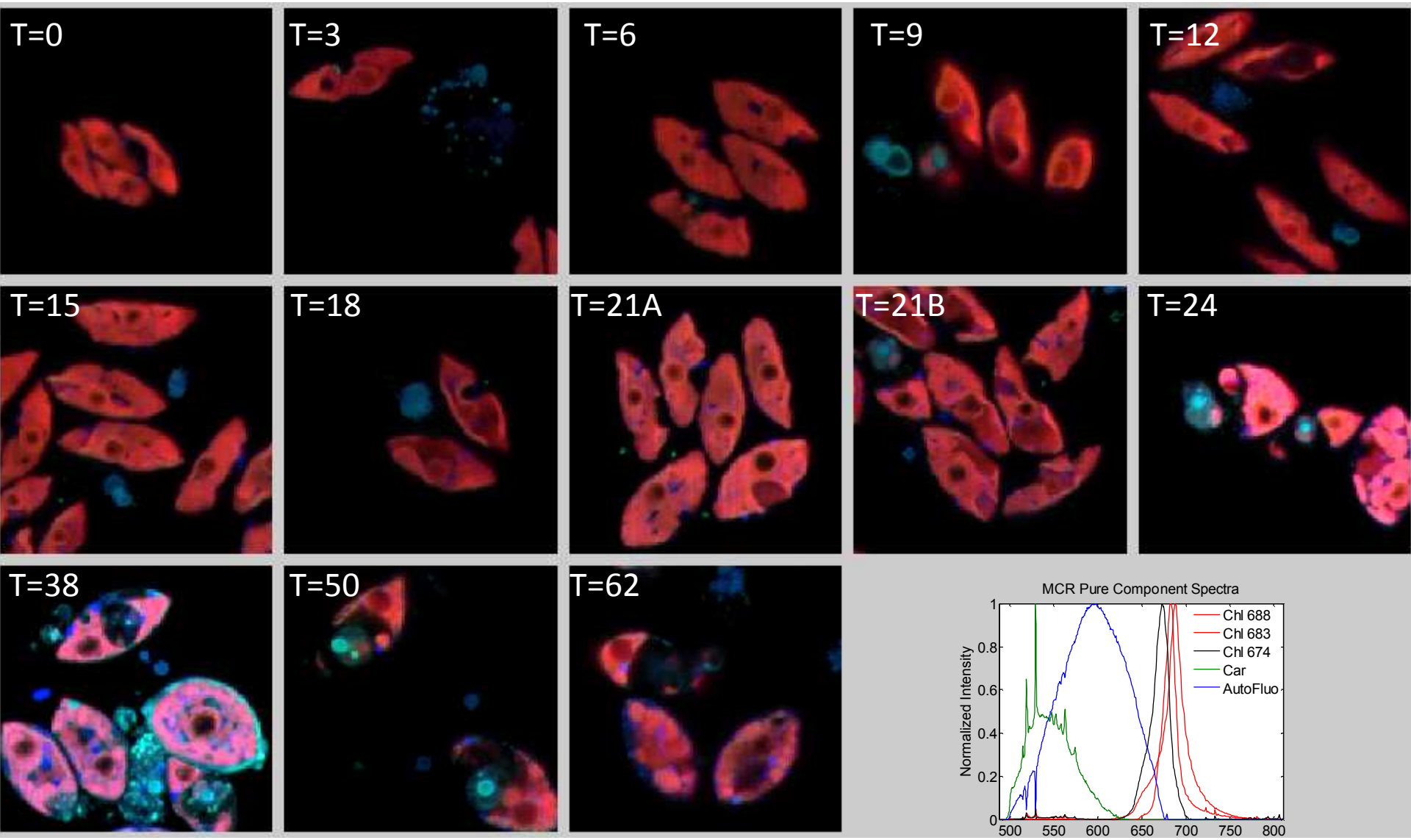




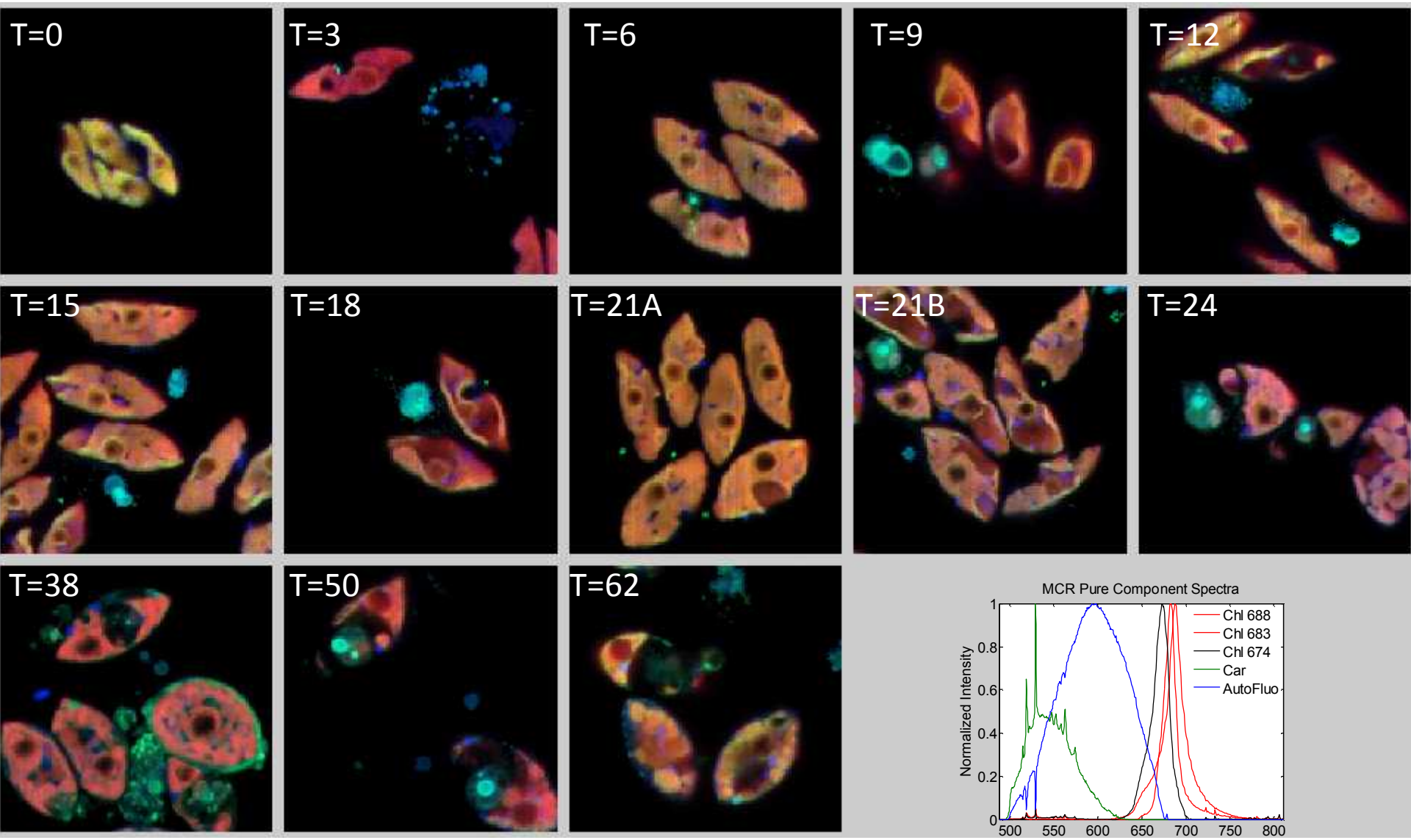




Contrast adjusted by AC

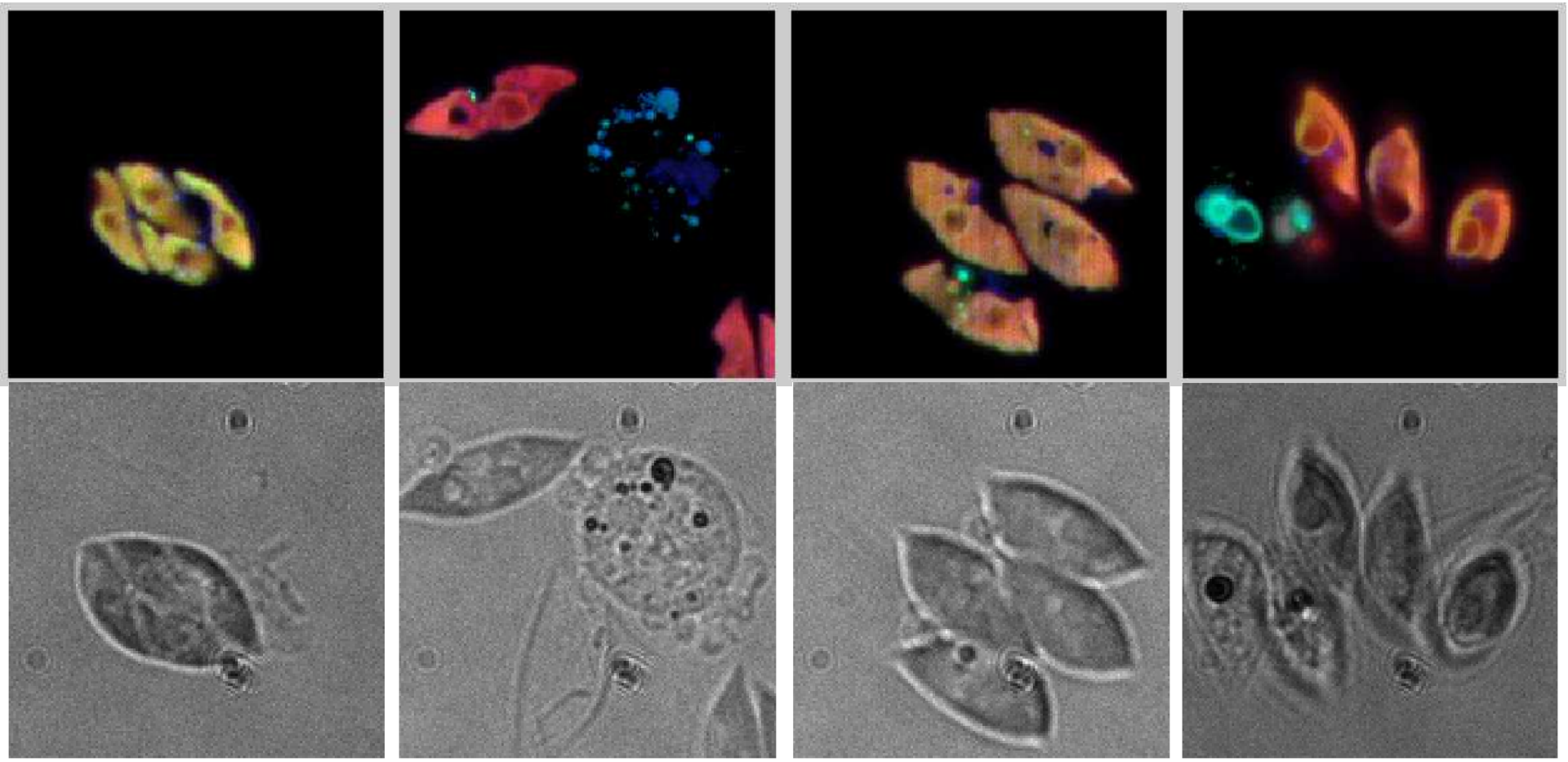


Full Autoscale

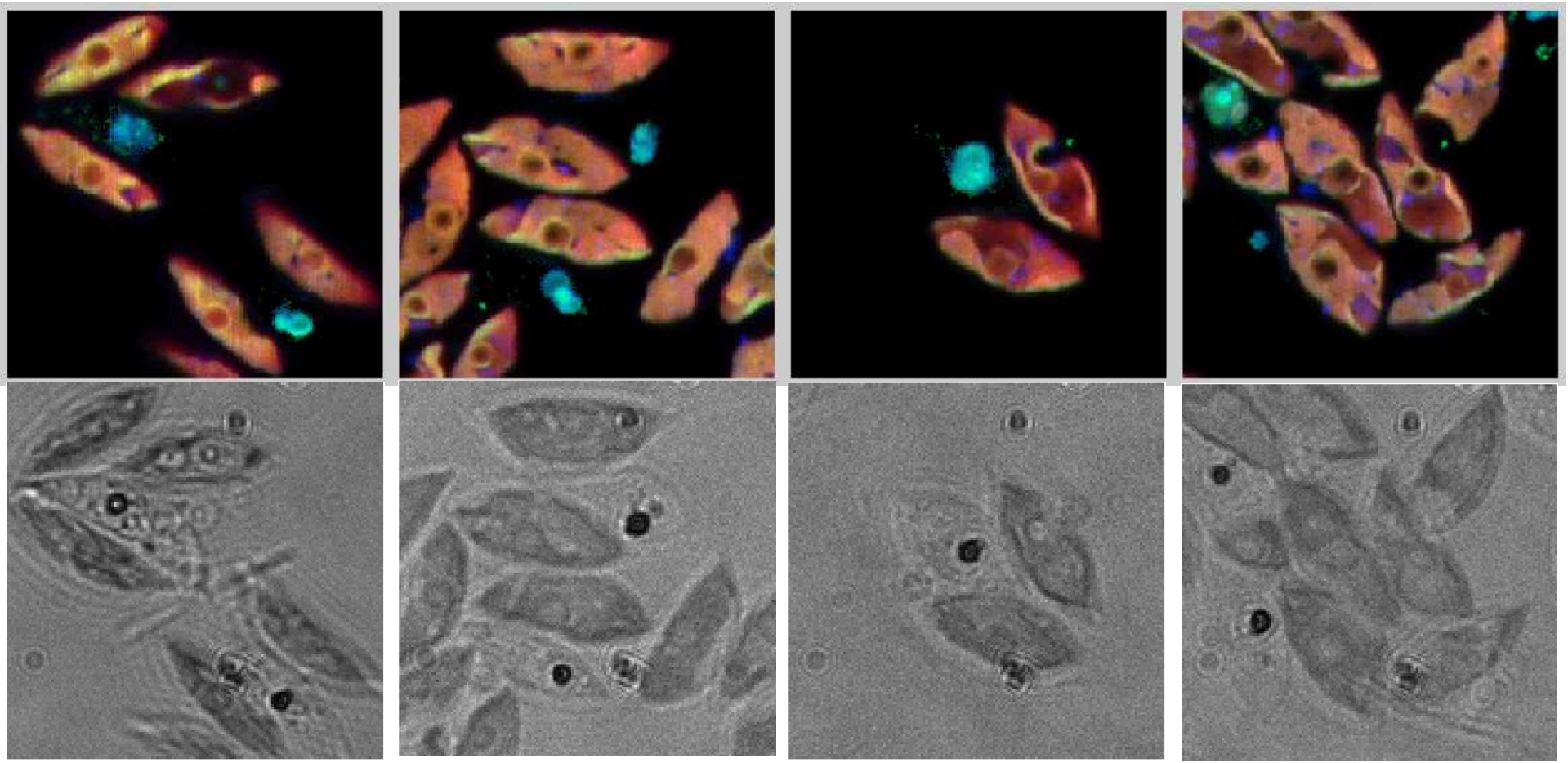


Look at T=21B and 24, autofluorescence shape “looks” like internal sporangia from TEM?

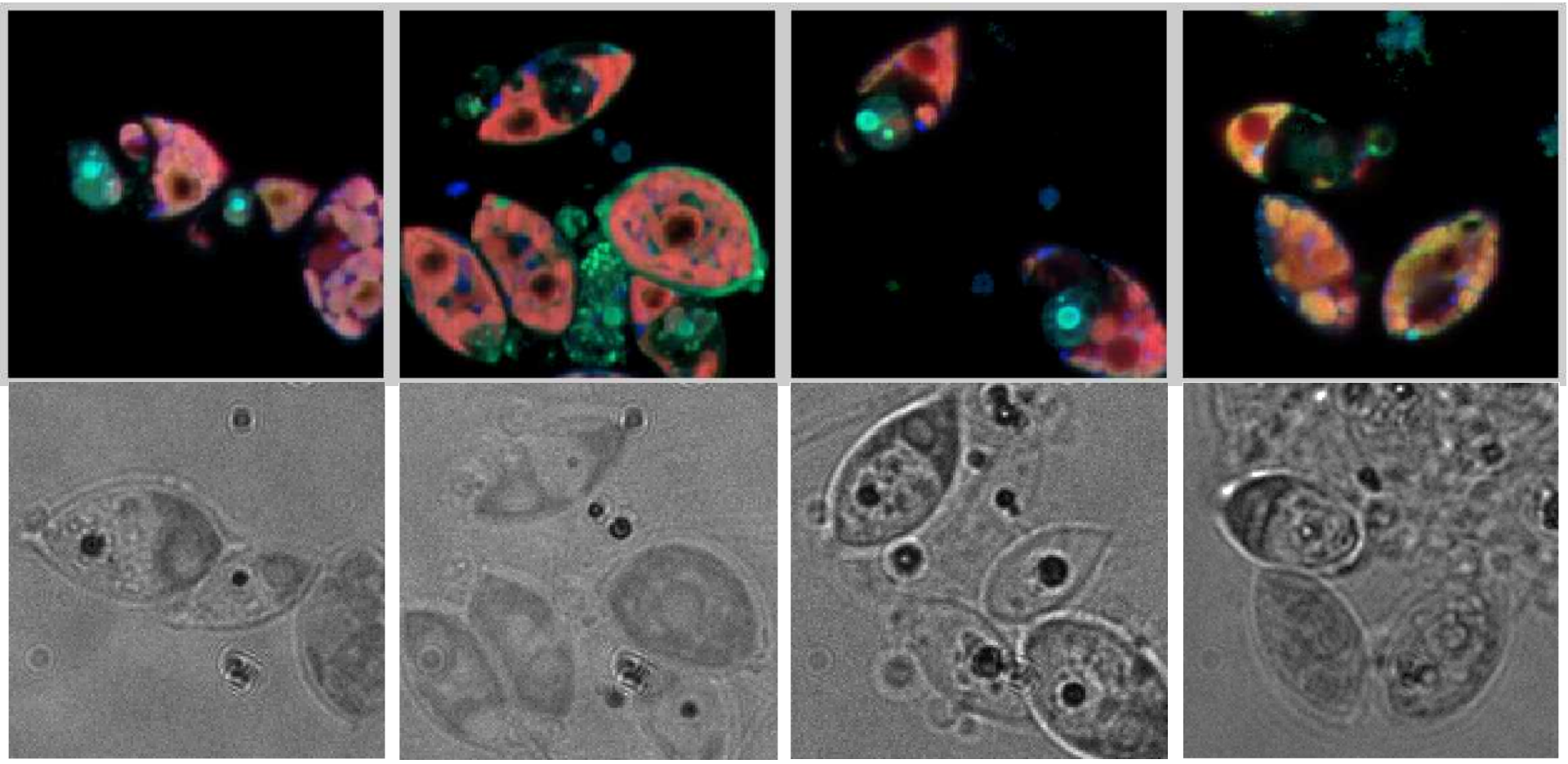
T0, T3, T6, T9



T12, T15, T18, T21



T24, T38, T50, T62



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;

→ Heterogeneous22

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

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

Nile Red label
Chytrid */ Scenedesmus*

Aaron Collins

Data Acquired 09-12-2012

Details

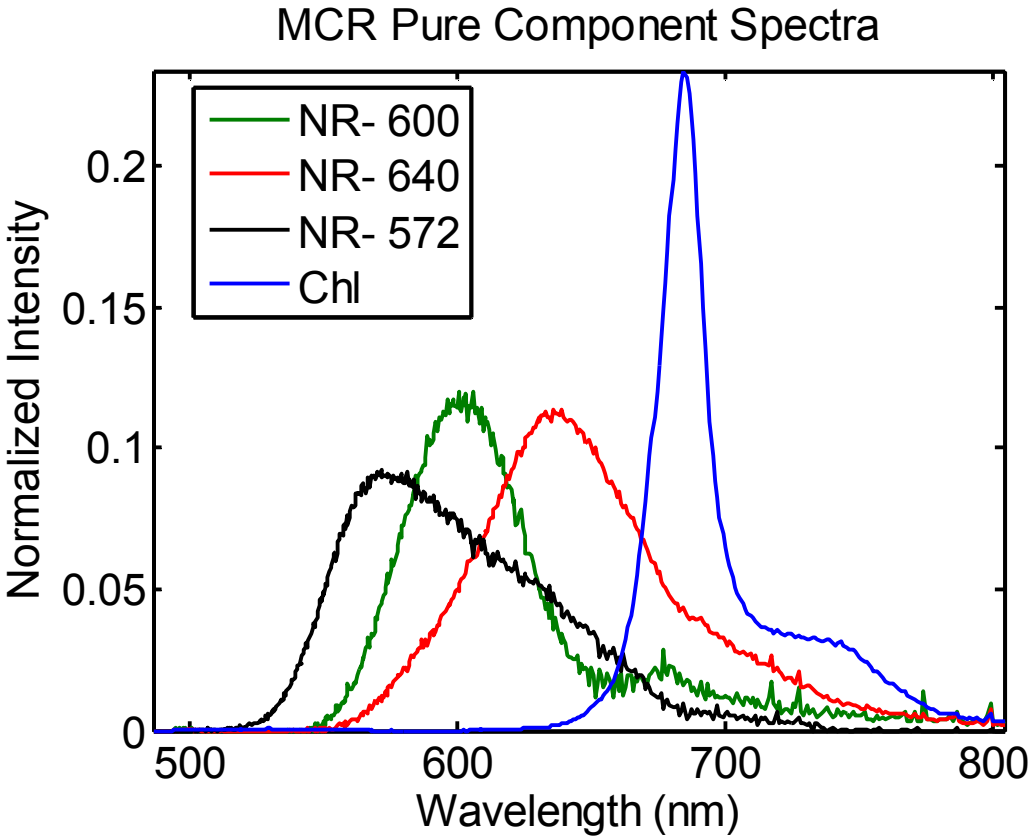
- Scenedesmus dimorphus culture crashed with chytrid FD001 at 32C and 400 uE m^{-2} s-1
- Nile red added to the culture from a 1 mg/ml stock (in DMSO) to achieve a 1ug/ml final concentration. Incubated in the dark for 10 minutes
- Imaged…hyperspectrally

  60X objective
- Analysis

  MCR

Due to Chl emission > 2000 counts, an inverse mask approach was applied to mask on non-Chl comp. Pure comps were then generated for Nile Red. These were then constrained and Chl-containing pixels were fit to a single component.

MCR model



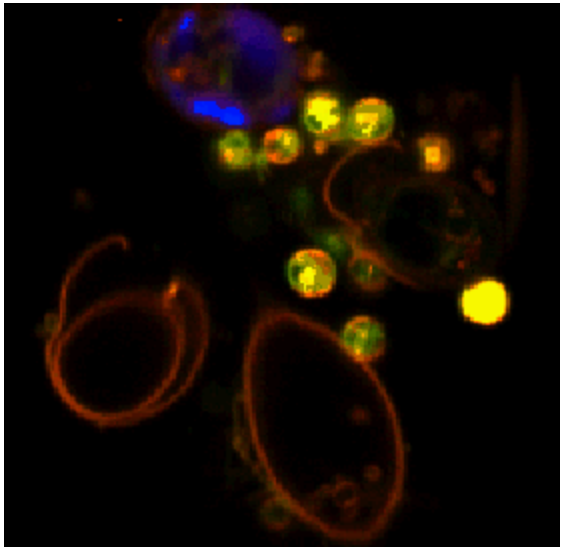
NR – 600 shows up in sporangia Interior – TAG-like

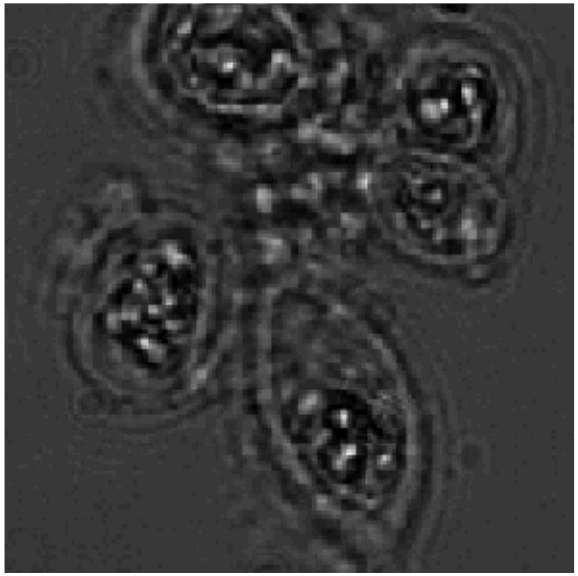
NR – 640 – shows up in membranes – phospholipid-like

NR – 572 – Punctate granules on sporangia similar to Car from non-labeled studies

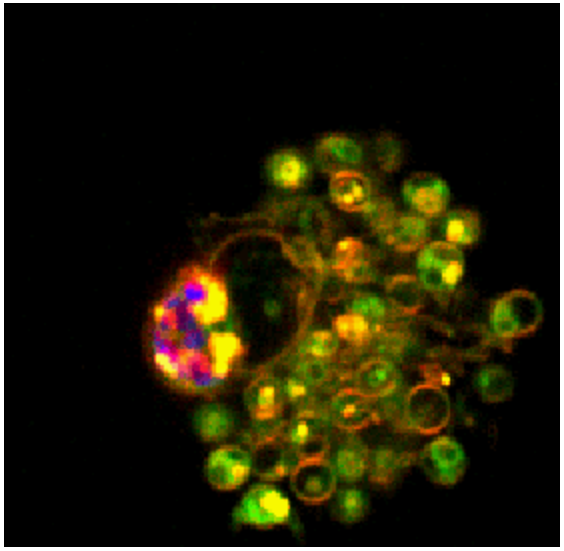
Chl - Chlorophyll

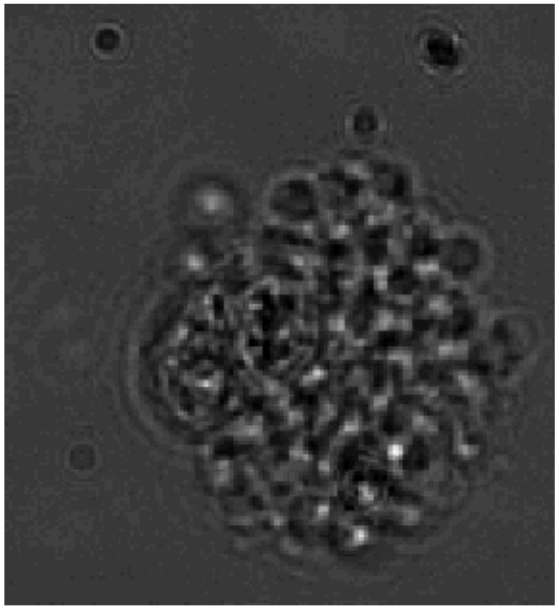
Best images…ever.





Best images…ever.





NR – 572 nm component location. Found in punctate regions like Carotenoids…