SAND2013-0525P

Chytrid FD01 infection time course of *Scenedesmus dimophus*

Experiment data Collection (12/12/2012)

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Analysis – Aaron Collins

Sandia National Laboratories is a multi program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

Study Purose

With the original FD01-*Scenedesmus* infection study (03/27/2012), we were effectively "blind" to spectral signatures for the chytrid due to its low pigment levels.

The new investigation was proposed to perform a similar infection time couse, only this time an exogenous label would be added to the sample to label the chytrid cell wall.

After initial screening, 1.5 ug/ml Nile Red was chosen. The unique characteristics of this lipid label are that it labels control cells (uninfected?) very weakly and we can distinguish neutral and polar lipids due to a spectral shift that is associated with the different dielectric constants of the lipid environments.

In other words;

TABLE 2. Fluorescence properties of nile red in various solvents

Solvent	Dielectric Constant[a]	Excitation Maximum	Emission Maximum	Relative Fluorescence Intensity
		nm	*nm*	
Water	78.5	591	657	18
Ethanol	24.3	559	629	355
Acetone	20.7	536	608	687
Chloroform	4.8	543	595	748
iso-Amylacetate	—	517	584	690
Xylene	2.4	523	565	685
n-Dodecane	2.0	492	531	739
n-Heptane	—	484	529	585

Nile red was analyzed at a concentration of 1 μg/ml in all solvents. Excitation and emission maxima were determined and the relative fluorescence intensity in each solvent was measured at the corresponding excitation and emission maxima.

[a]Derived from (27).

Greenspan and Fowler. Spectrofluorometric studies of the lipid probe, nile red. J. Lipid Res. 1985 26:(7) 781-9.

Time course of Infection

Colors represent unique infections (in duplicate) as we are trying to squeeze 24 hours of imaging into a 1 day experiment.
Infection conditions; ~32C, 400 uE*m^2*s^{-1} shaking on an orbital shaker.

Infected? Yes	No	Date & Time of Infection	ID	Time of Imaging		PAM	Oxygraph
X		12/11, 4 pm	Infected t16	12/12	8 am	X	X
	X		Control t0	12/12	9 am	X	X
X		12/12, 6 am	Infected t4	12/12	10 am	X	X
X		12/10, 4 pm	Infected t48	12/12	12 pm	X	X
	X		Control t4	12/12	1 pm	X	X
X		12/12, 6 am	Infected t8	12/12	2 pm	X	X
X		12/11, 4 pm	Infected t24	12/12	4 pm	X	X
	X		Control t8	12/12	5 pm	X	X
X		12/12, 6 am	Infected t12	12/12	6 pm	X	X

*Control cells will be cultured under same conditions as infected cultures.
For each time point there will be biological duplicates that will assessed on the oxygraph and PAM however, only a single bio rep will be used for imaging.
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1500 µl total will be removed.
1) 50 µl samples will be retained for DNA extraction

Protocol from R. McBride; mixing 50 µl of sample with 50 µl of 0.25X lysis buffer in PCR tubes. The mixture is then placed in a PCR block and heated to 95°C for 10 min, cooled to 25°C for 5 min, heated to 95°C for 10 min and then cooled to 25°C for 5 min. The DNA lysis buffer (1X) is composed of: 50 mM Tris-HCl, pH 8.0; 200 mM NaCl; 20 mM EDTA, pH 8.0; 1.0% (v/v) SDS

2) 1000 µl used for Oxygraph measurement. Triplicate PAM measurements will be made *in situ*.

3) 400 µl will be retained to prepare sample for imaging
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Imaging and Analysis

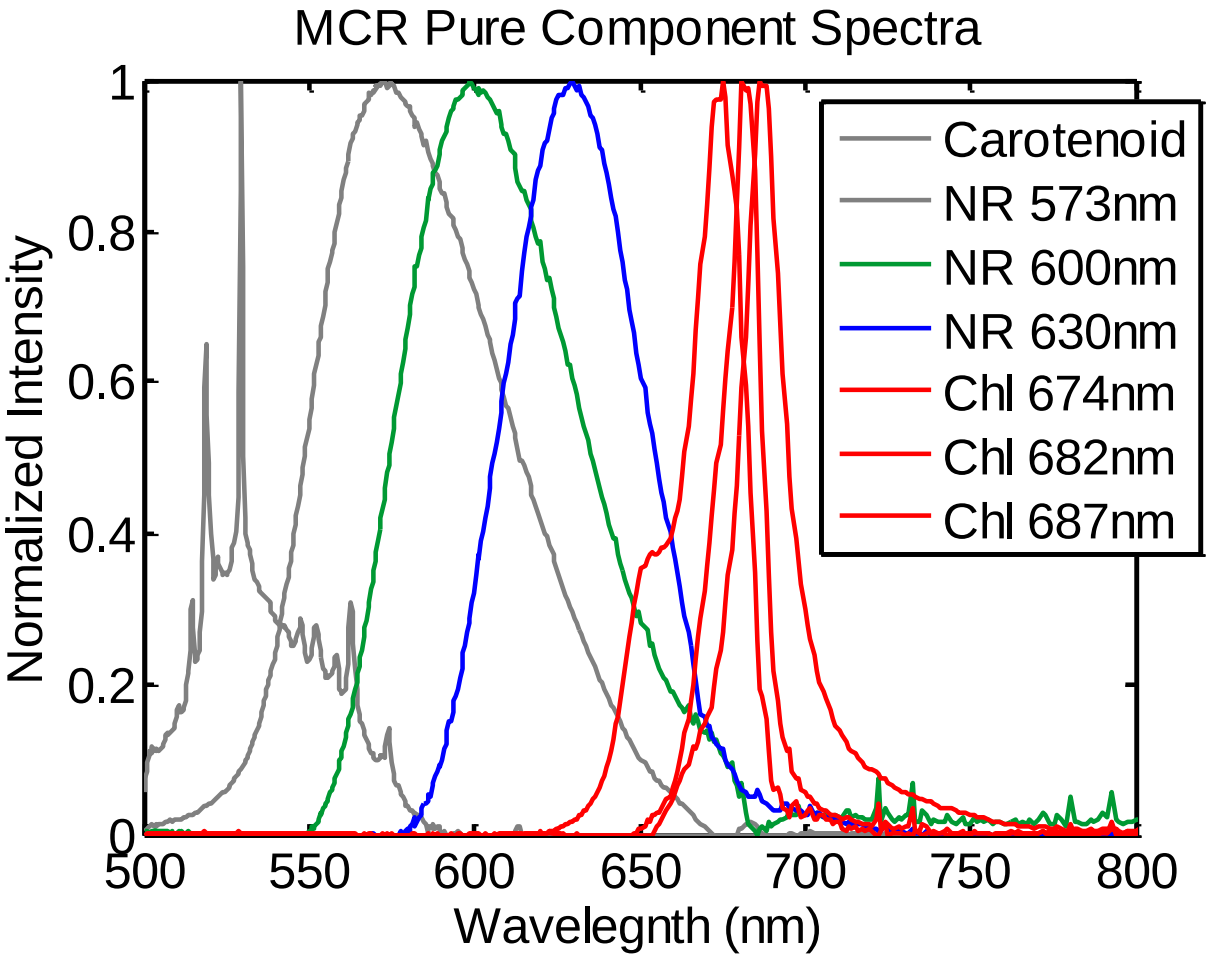
Imaging

- 400 µl of culture was incubated with 1.5 µg/ml Nile Red for 10 minutes in darkness
- Sample was centrifuged (700 x g) for 3 minutes and 375 µl of supernatant was removed.
- Remaining soft pellet was resuspended and 4 µl was loaded to microscope slide and covered with a #1.5 coverglass.
- Imaging commenced on SNL's HSI microscope
  - → 60 x magnification (Nikon, plan apochromat, NA 1.4)
  - → Laser OD = 0 (~200 µW laser power into microscope)
  - → BG-38 optical filter placed in front of detector.
  - → At least 25 cells were imaged per time point and a 2-3 optical sections were taken.

Analysis

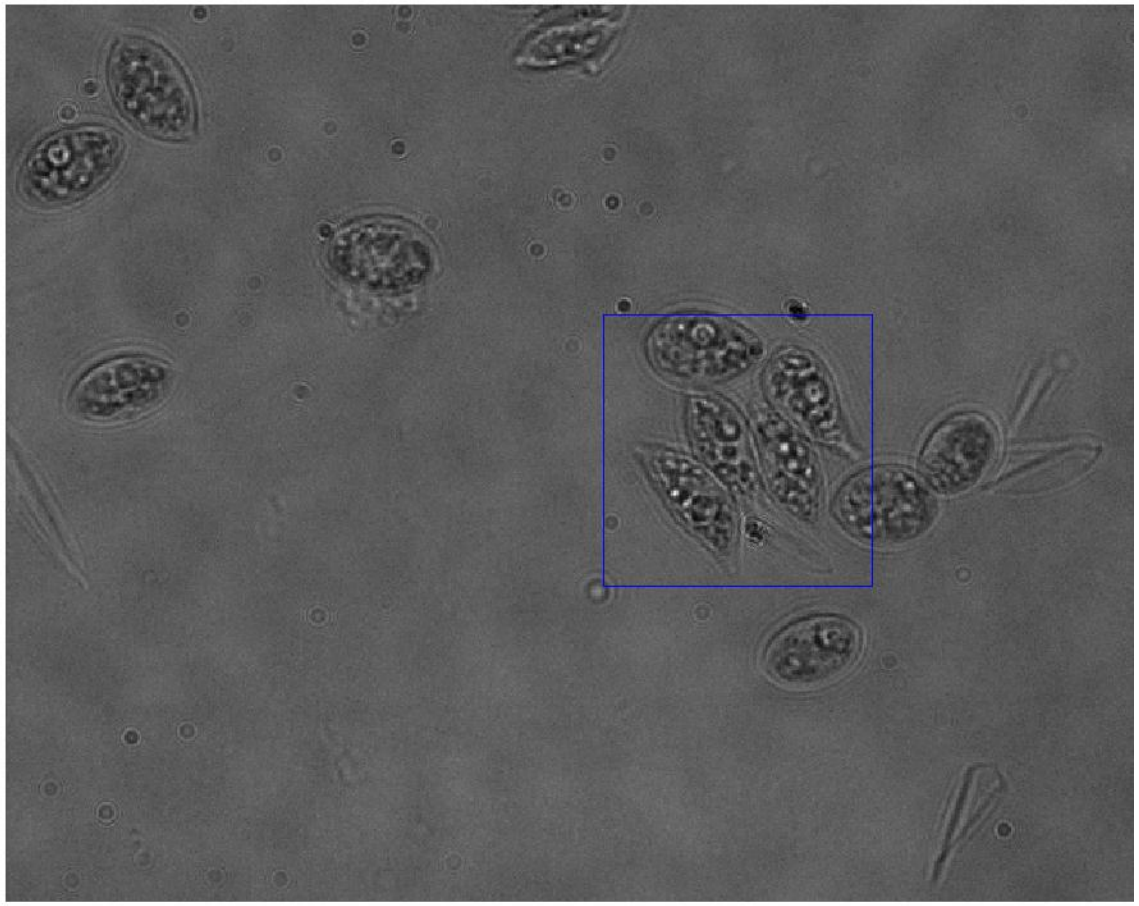
- Imaging generated 18.1 Gb of raw data!
- Images were pre-processed via in-house routines
- Due to the size of the dataset, control cells and cells infected for 48 hours were combined into a composite dataset and MCR algorithms were used to model the spectral data. After a sufficient model was generated, the spectral model was constrained and applied to the remaining data.

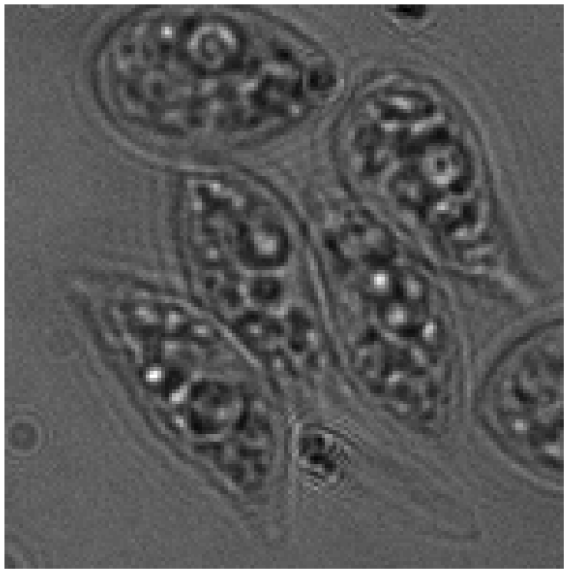
MCR generated model

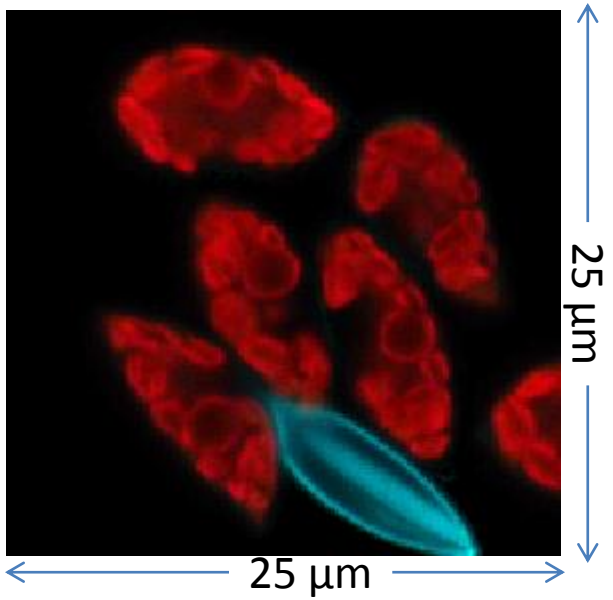


One the following slides, I will use RGB coloring, referenced above, to display the spectral images

Representative control cells t = 0 hours

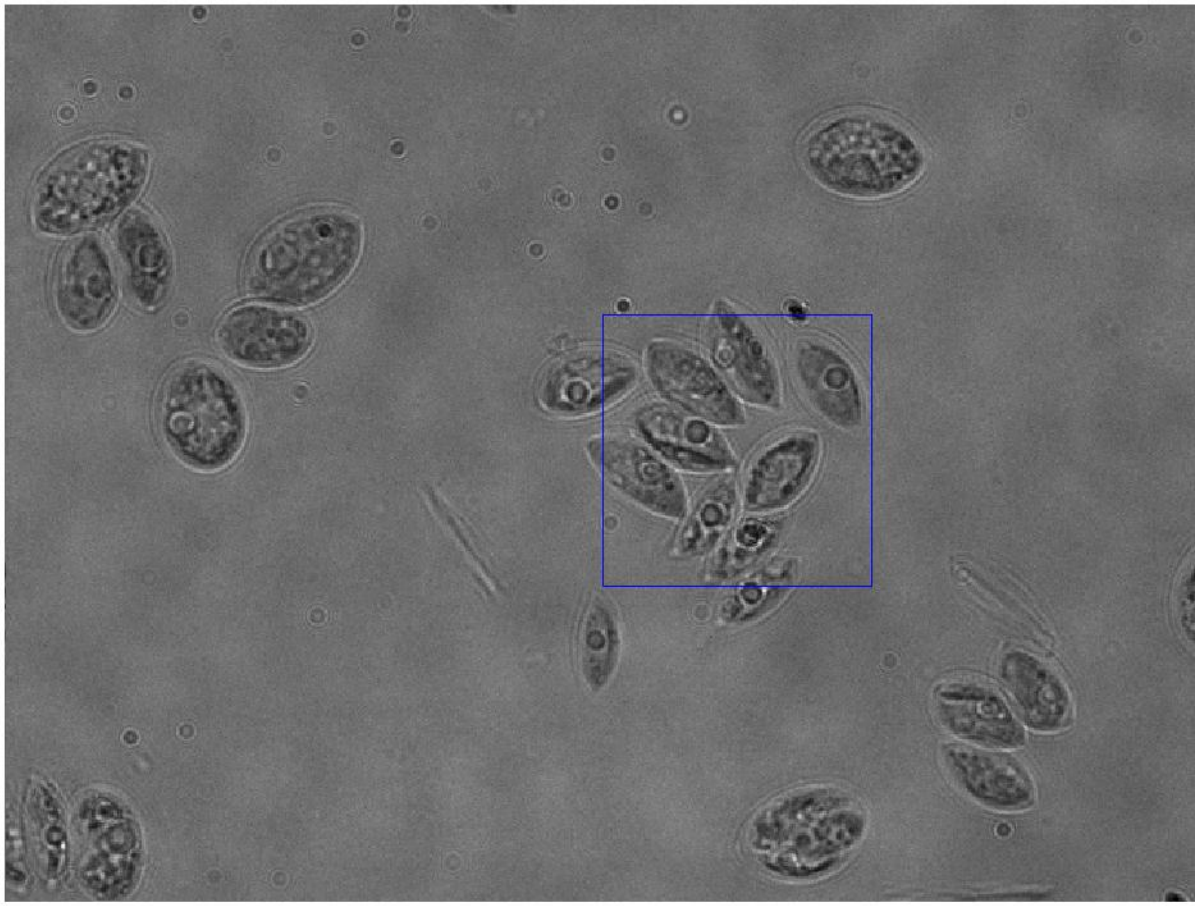


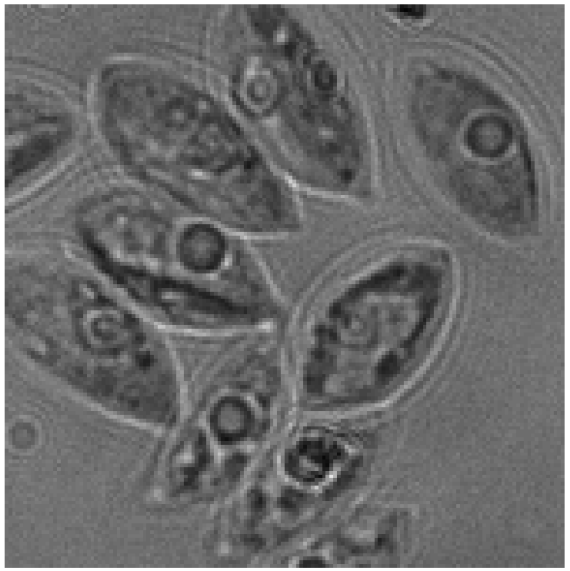


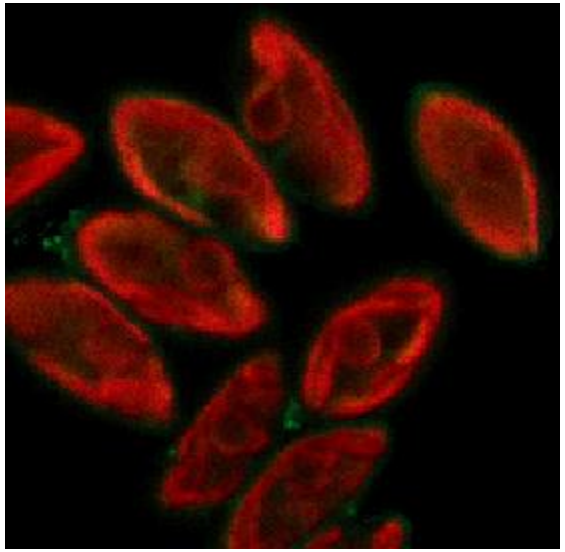


Note the empty cell wall in this image. While not abundant, it was present in control cells. Also, note obvious pyrenoid in each cell.

Representative control cells t = 4 hours

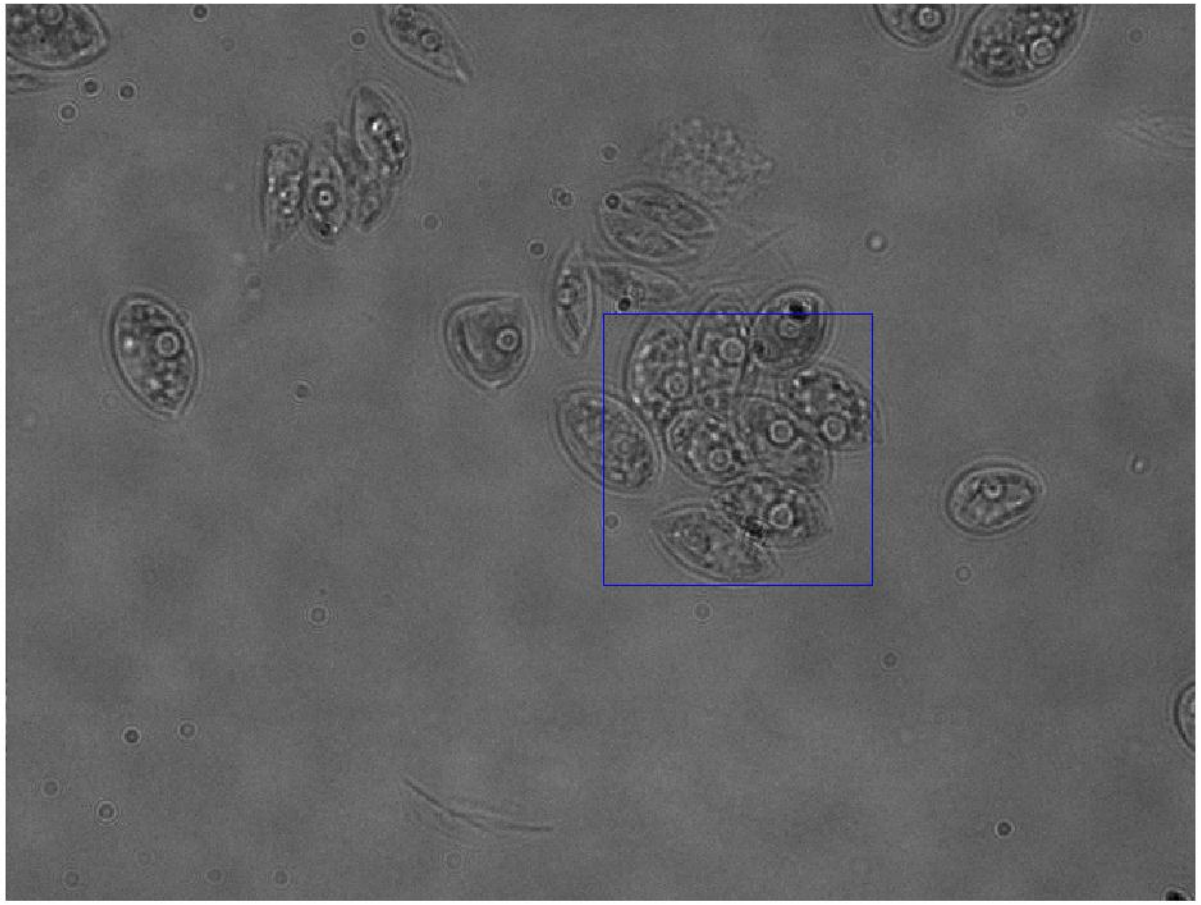


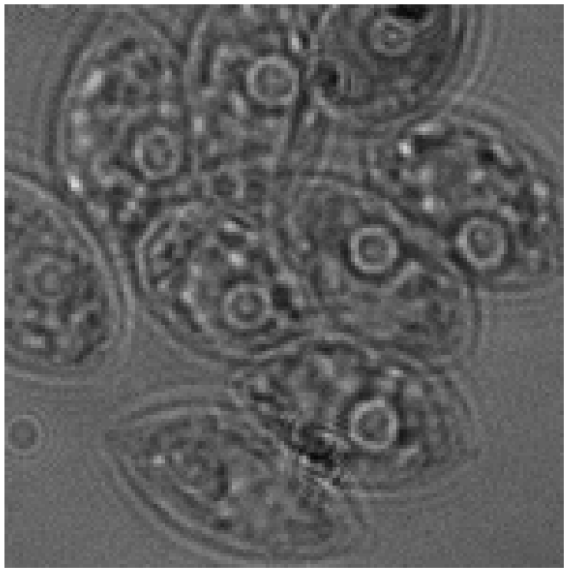


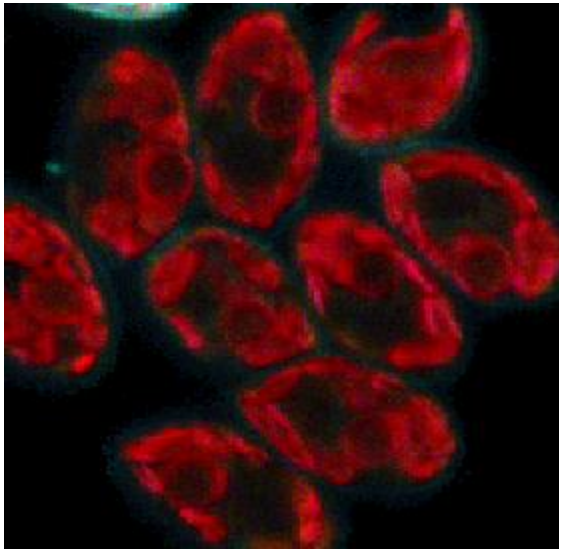


Similar to t = 4 hour sample. Very faint NR labeling
is more prevalent at this time compared to t = 0 hours

Representative control cells t = 8 hours

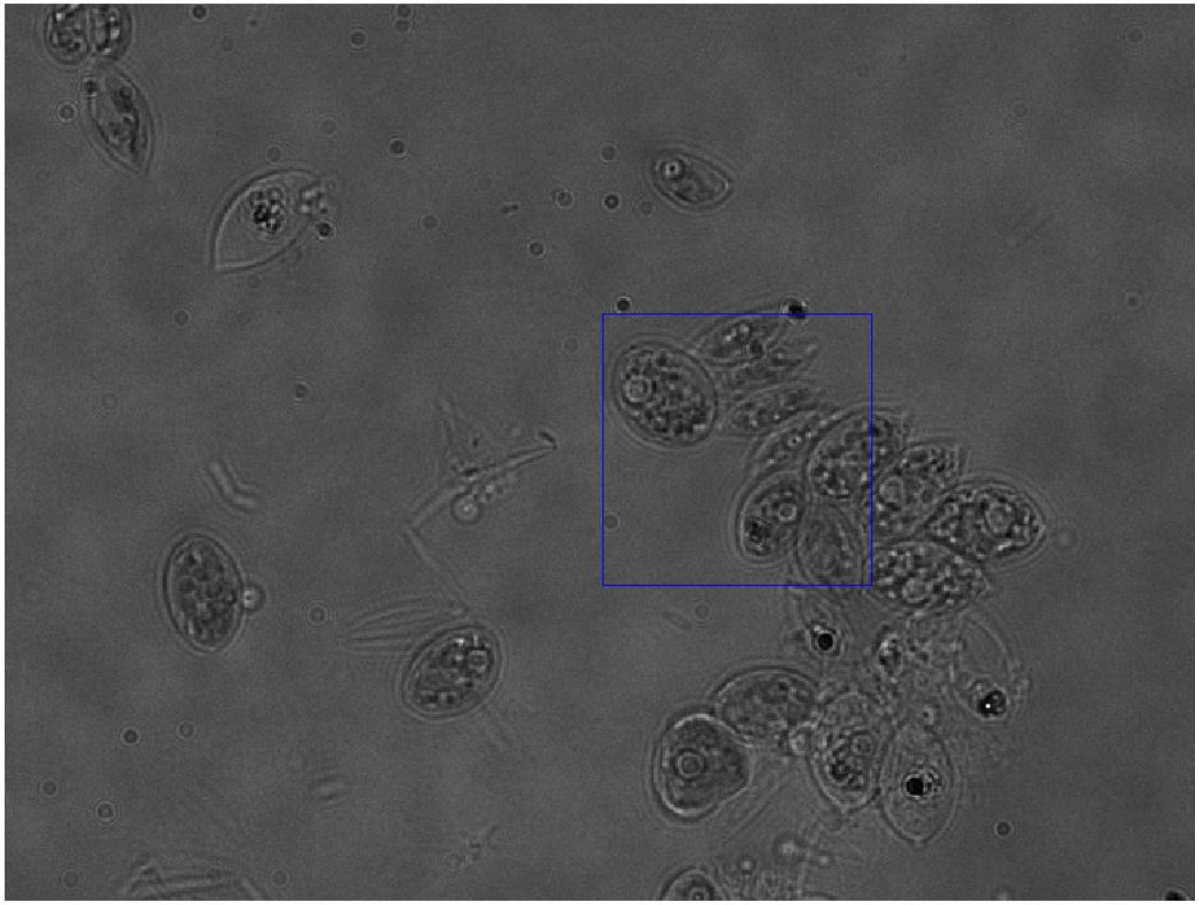


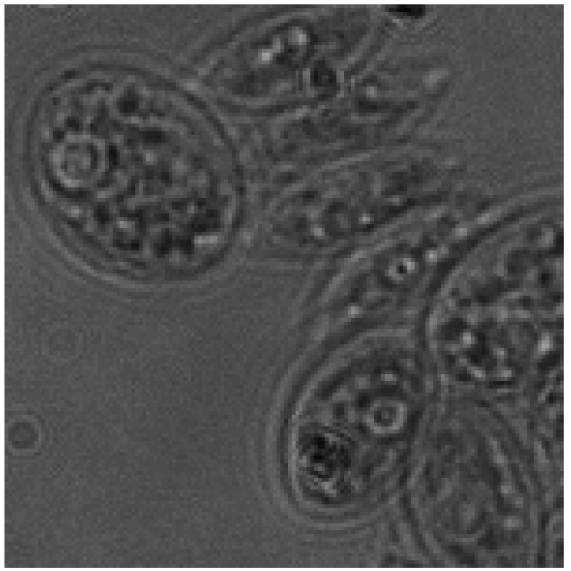


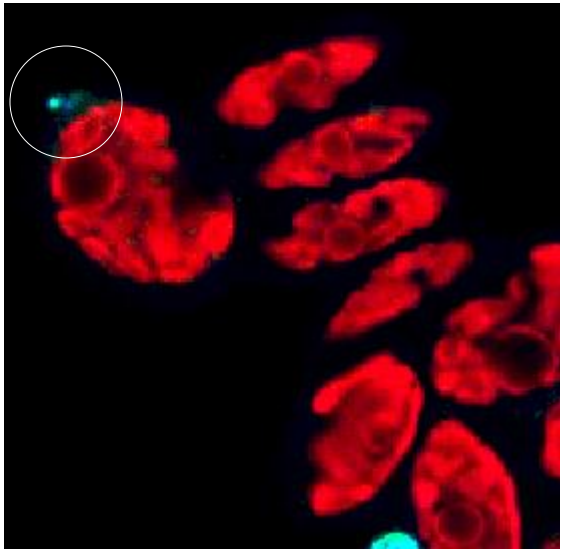


At t = 8 hours not substantial change from t = 4 or 0 however, more cells are being labeled, perhaps from the elevated temperature and irradiance?

Representative infected cells t = 4 hours

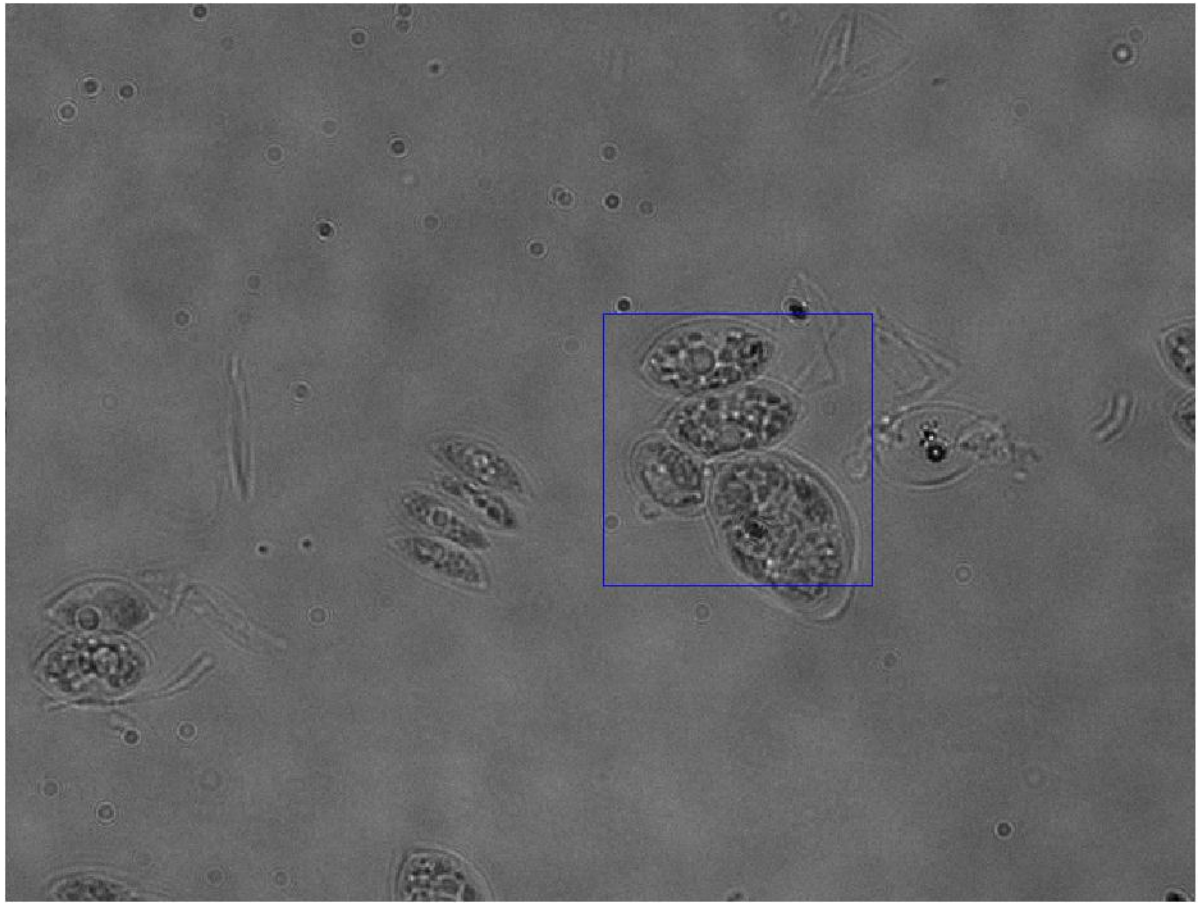


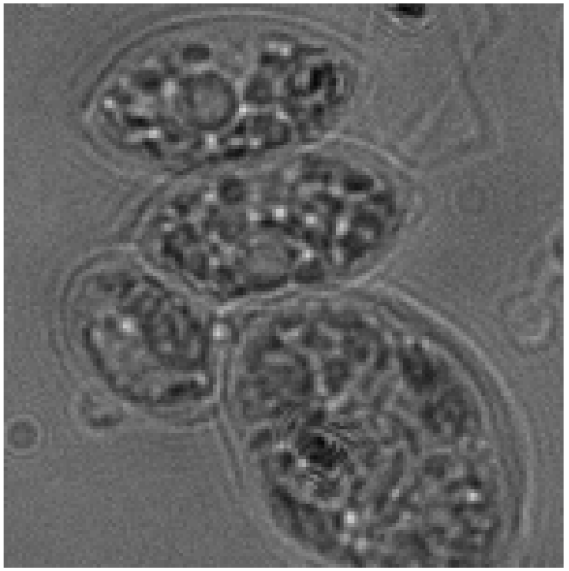


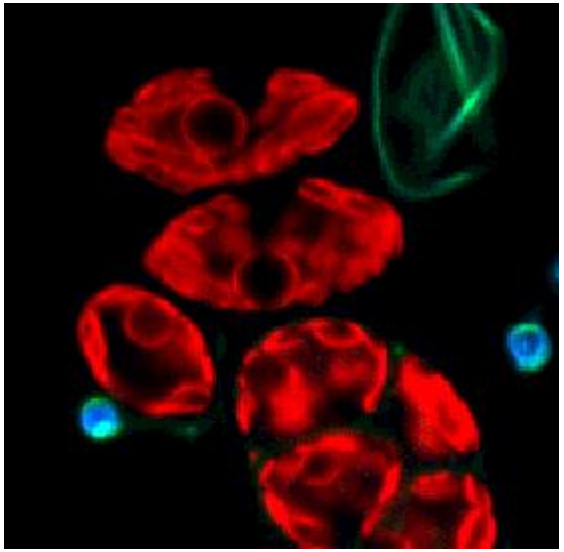


Encircled portion could be a early stage cyst?

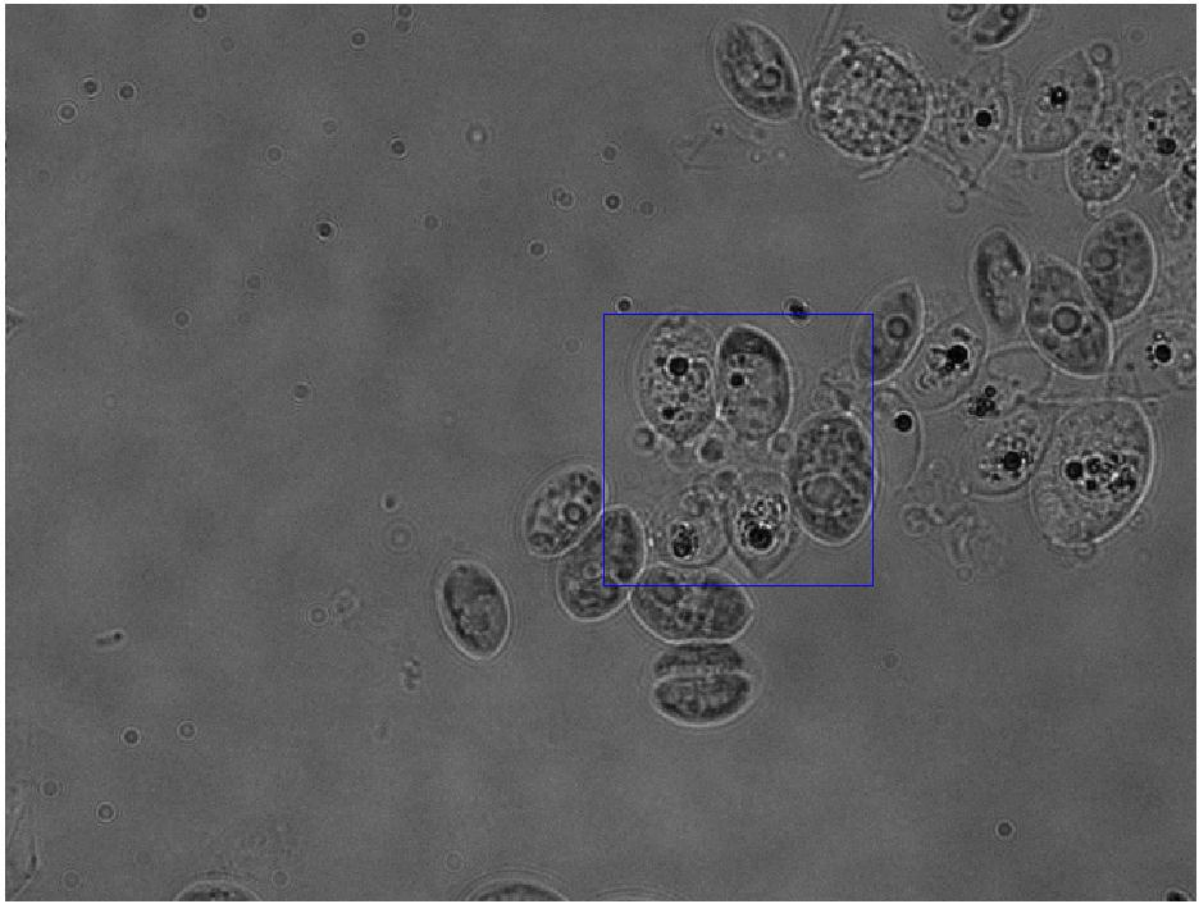
Representative infected cells t = 4 hours

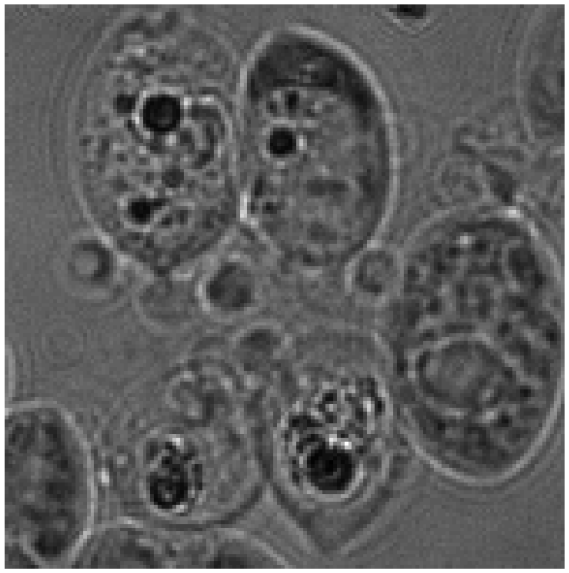


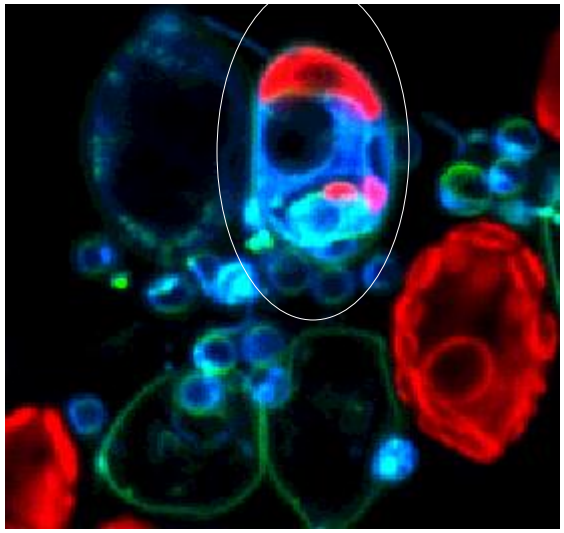




Representative infected cells t = 4 hours

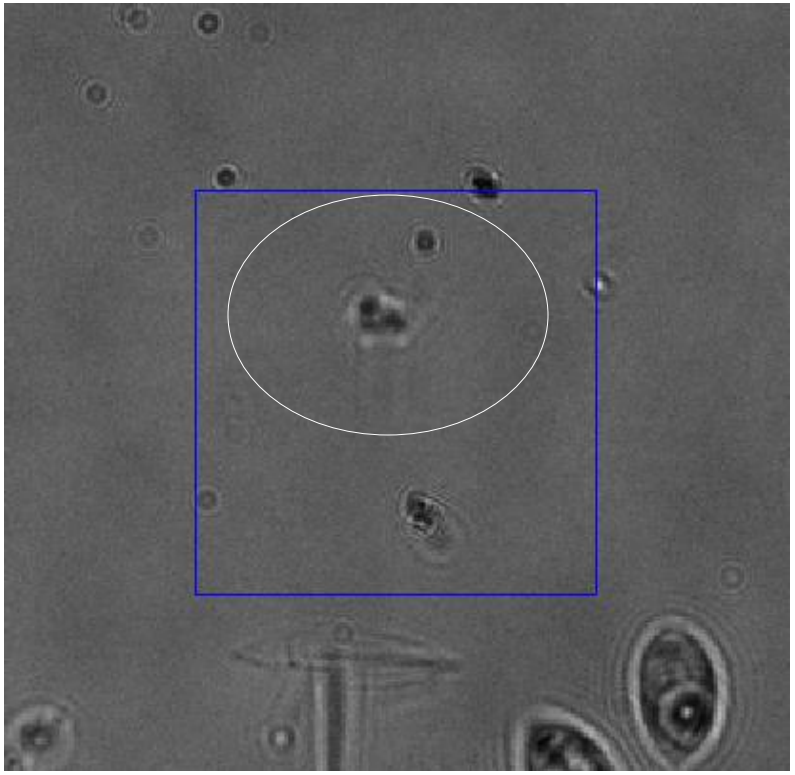


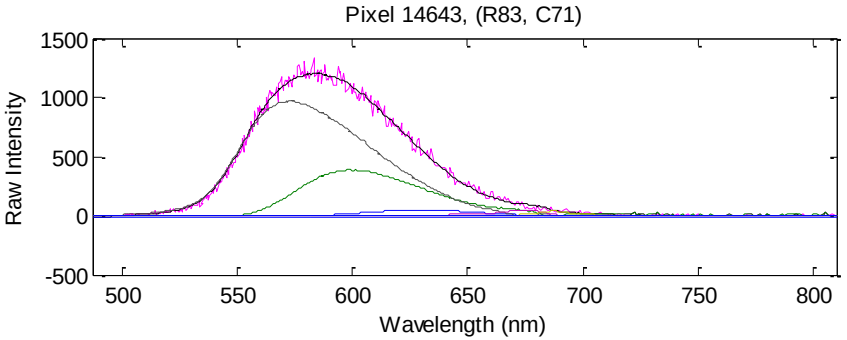


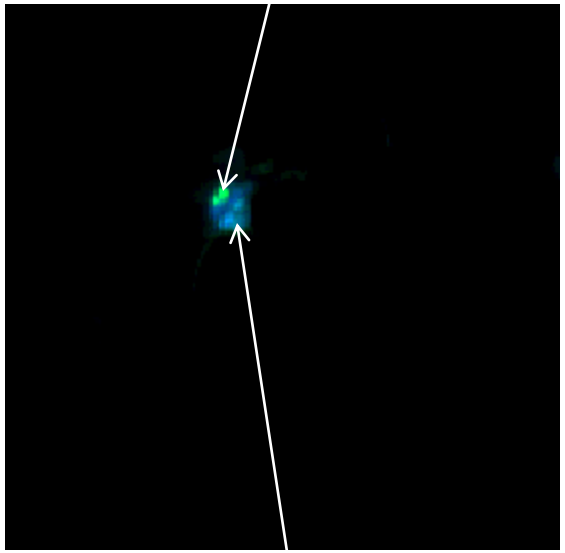


NR labeling allows for better delineation of cell membranes, both algal and chytrid. Note that likely lipid bodies are obvious in sporangia. Encircled host cell shows chloroplast reduction and chytrid propagation inside of host. It is possible that these cells are from the infection source itself and not new infection.

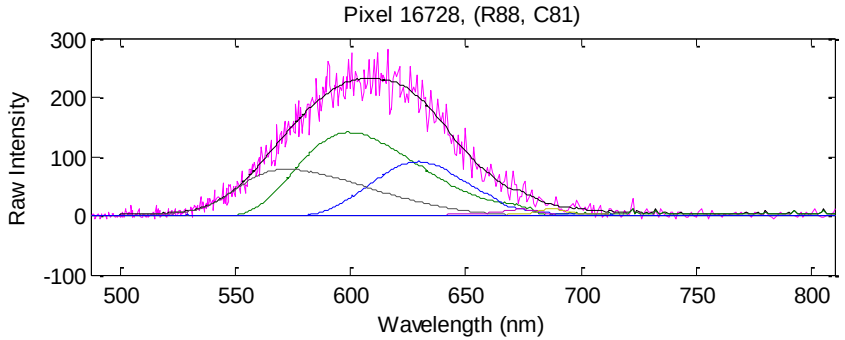
Chytrid in absence of host



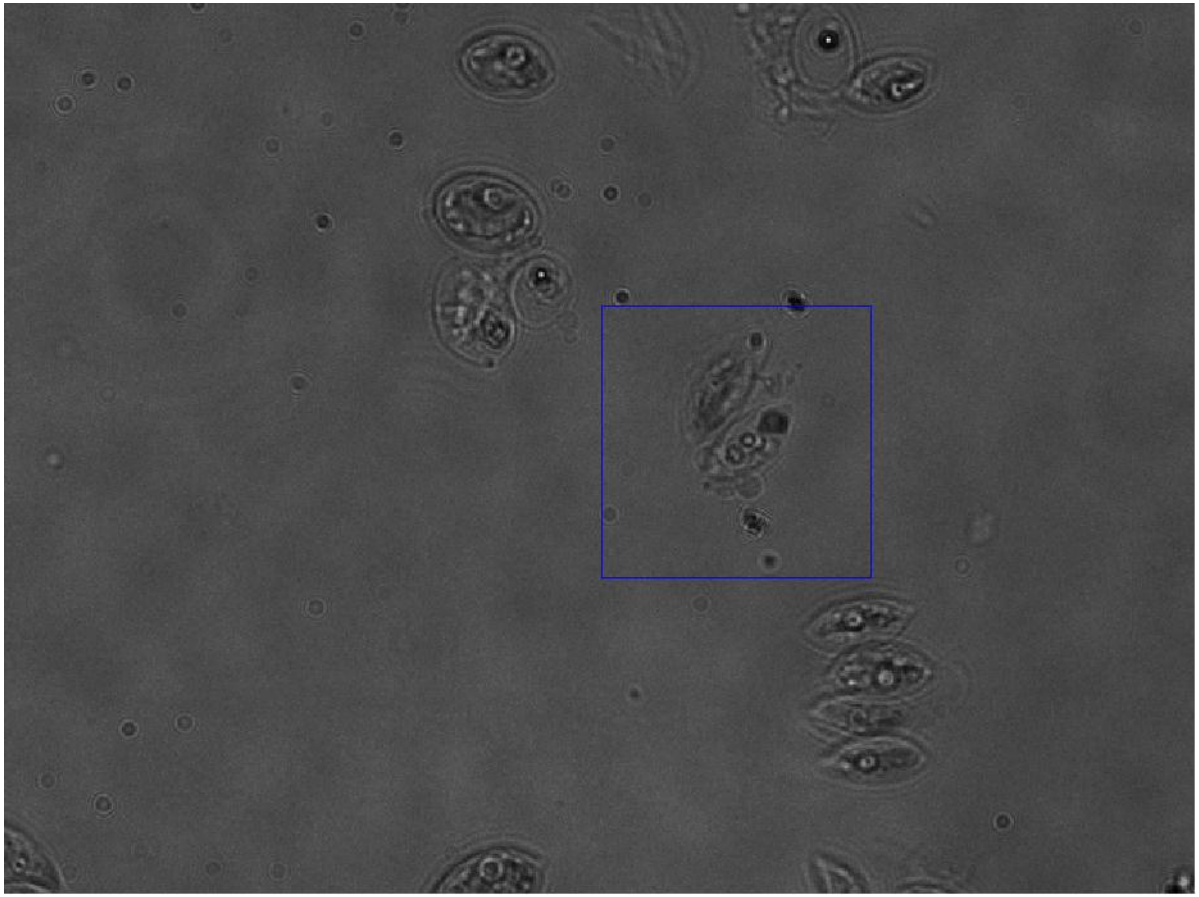


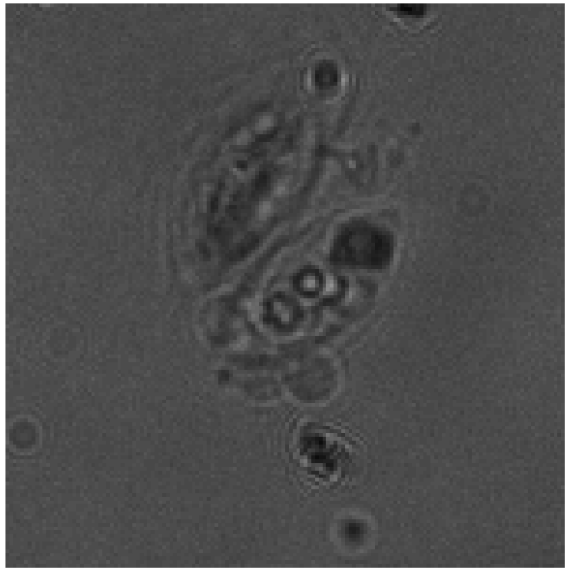


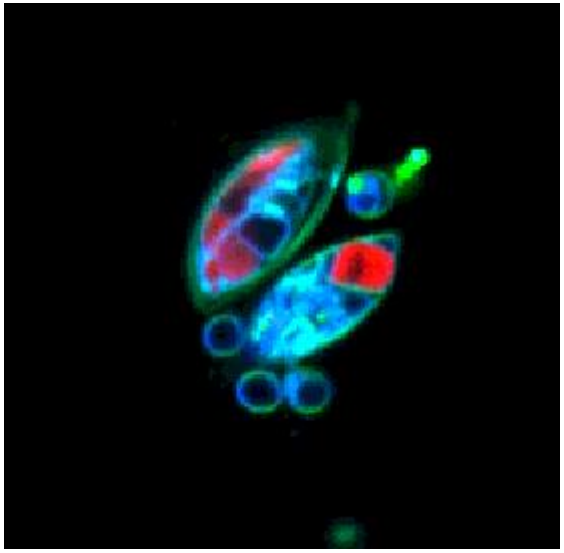
Filimentous chytrid does not show uniform labeling. A small punctate region is highly labeled by NR. We have seen something similar in unlabeled chytrid and it contains a carotenoid signature. I am not sure what organelle or subcellular location would be responsible for these observations.



Representative infected cells t = 8 hours

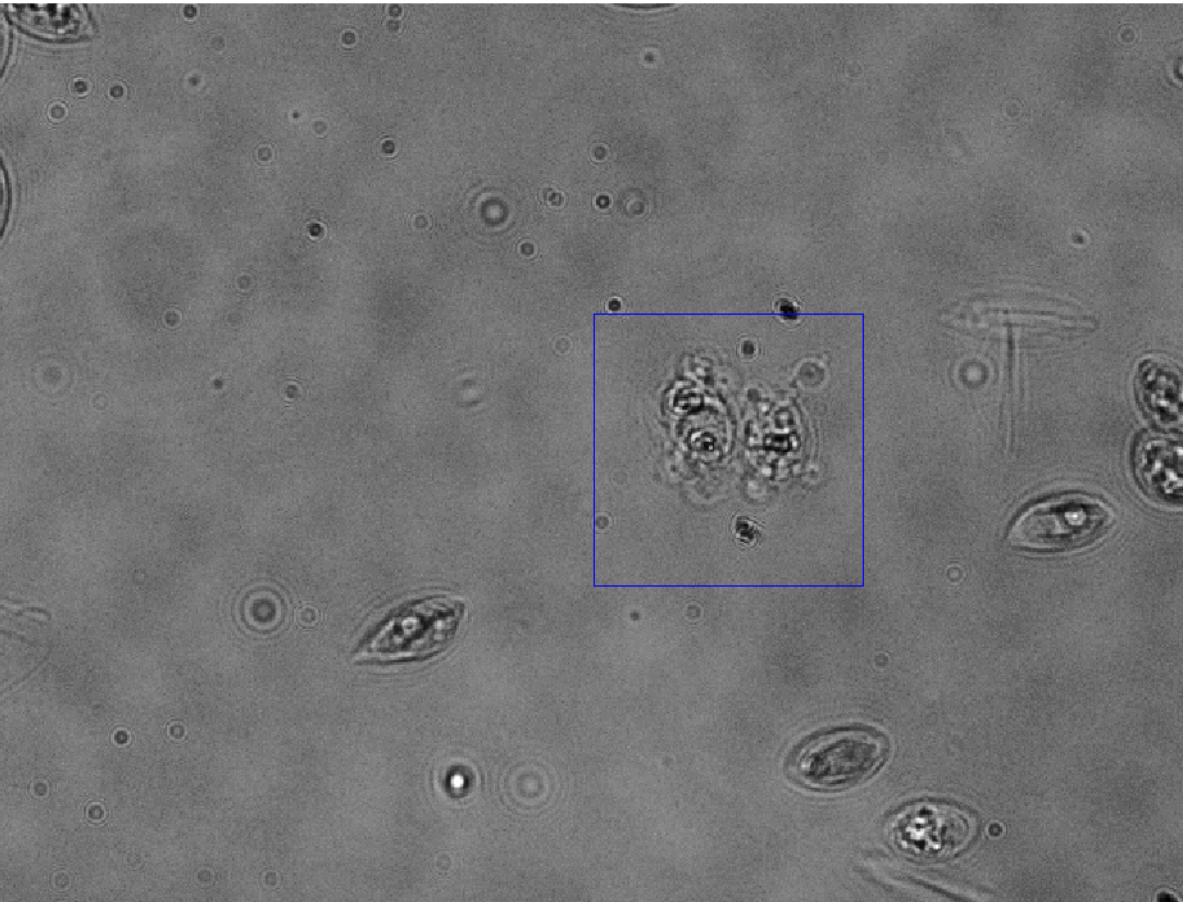


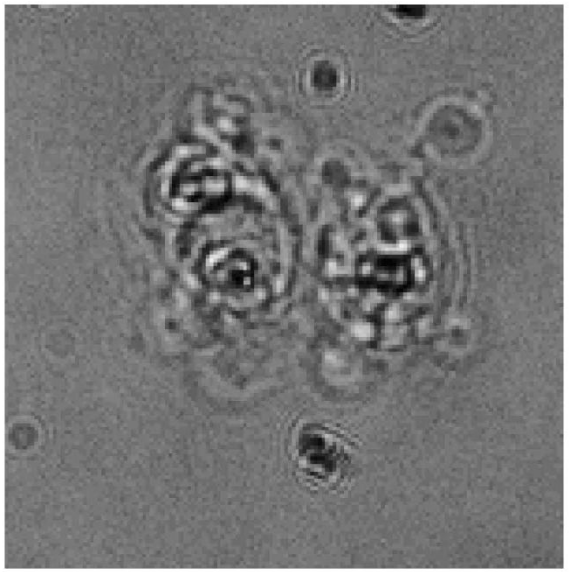


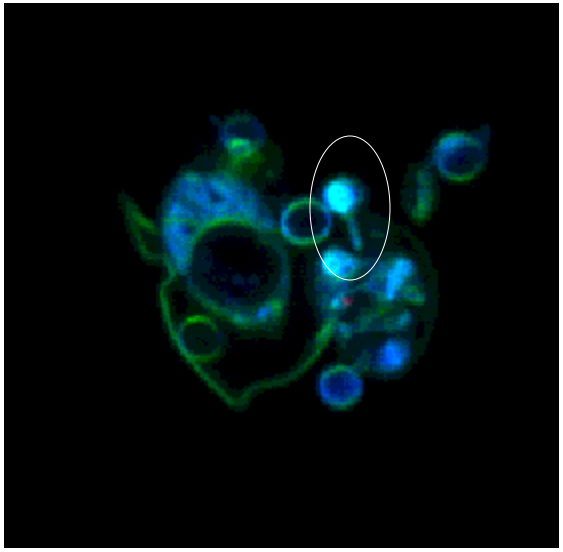


Portions of the chloroplast still remain in these fluorescence images.

Representative infected cells t = 8 hours

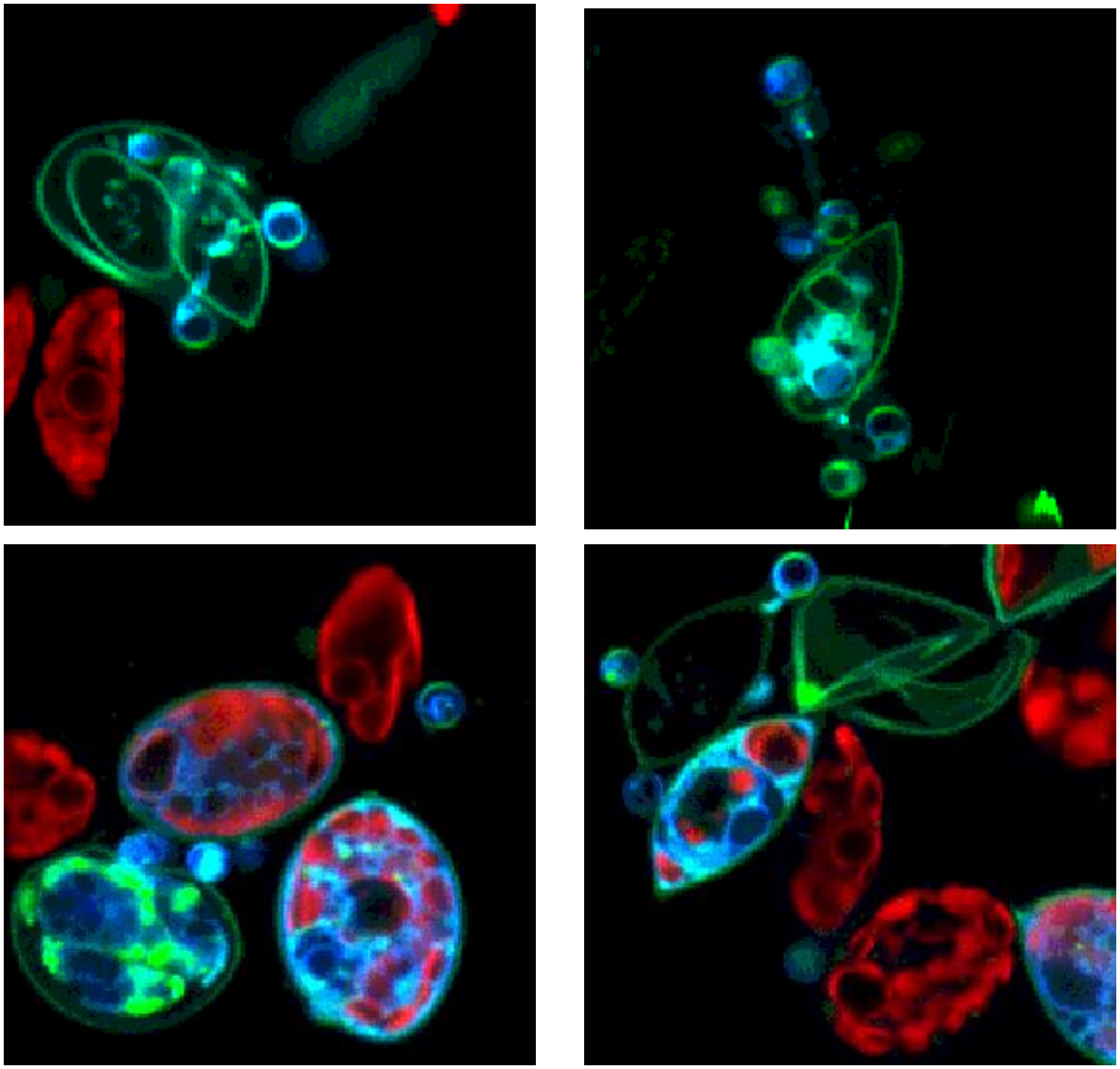




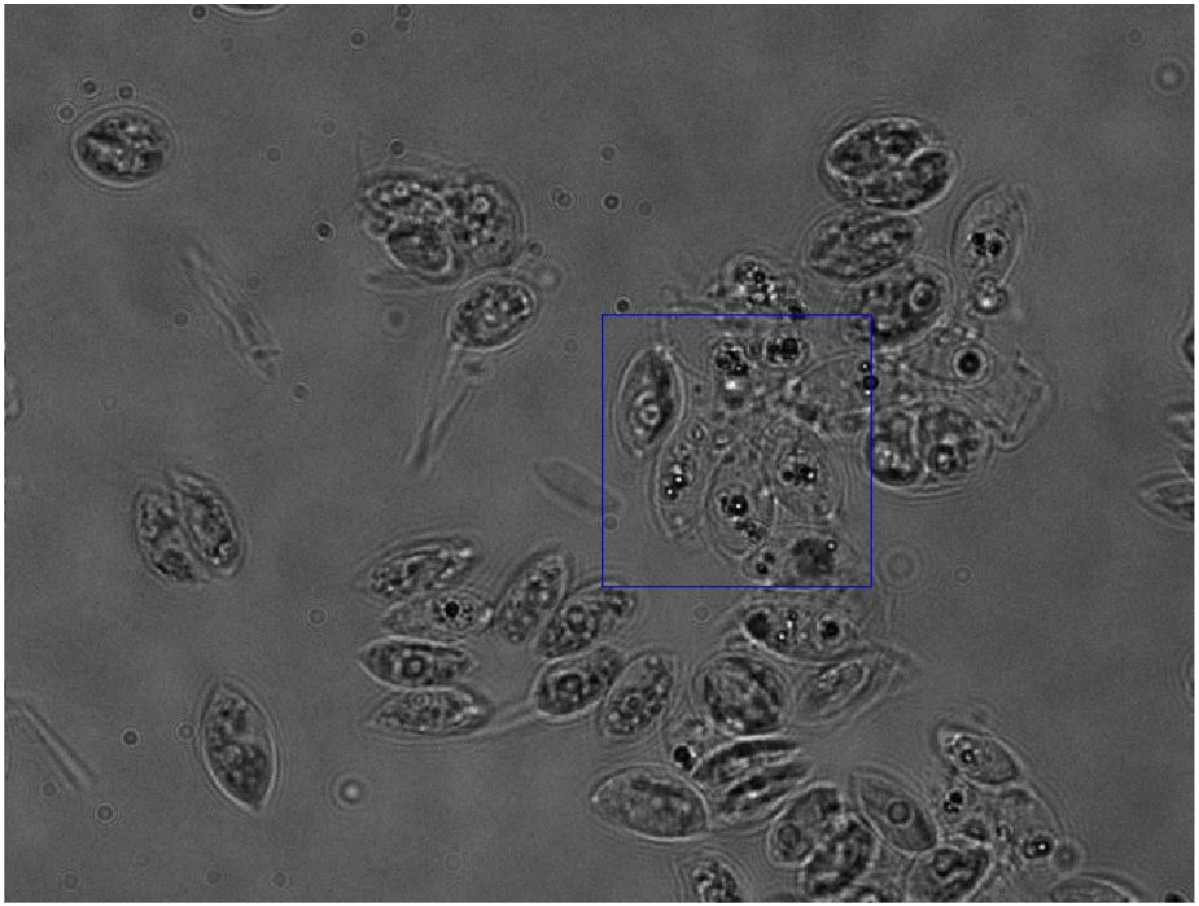


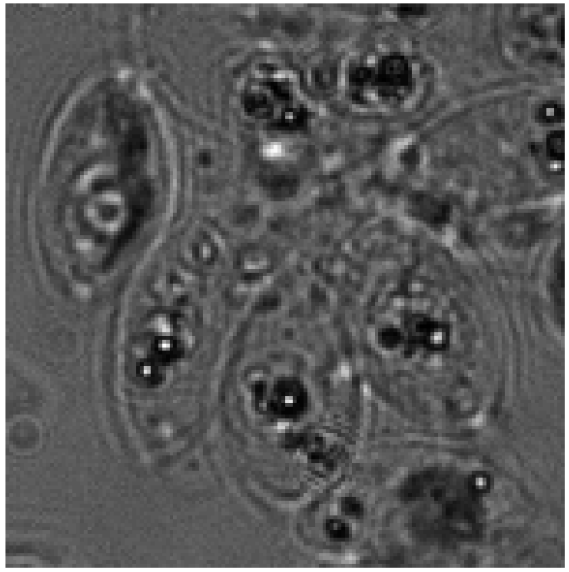
Encircled region could be showing penetration into host cell. Both cells show some evidence for chytrid progeny within host cell.

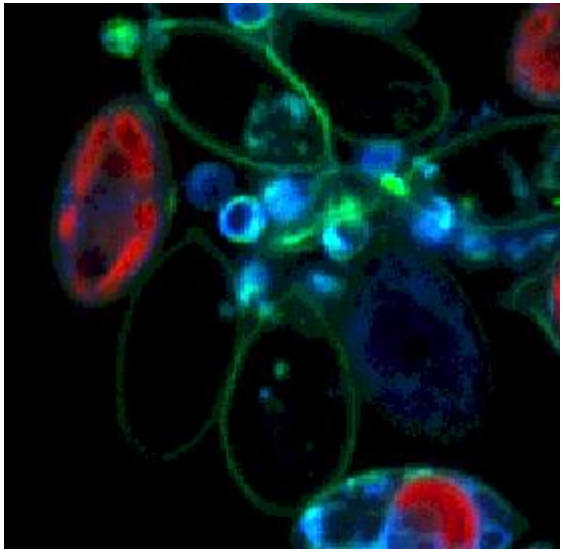
Representative infected cells t = 8 hours



Representative infected cells t = 12 hours

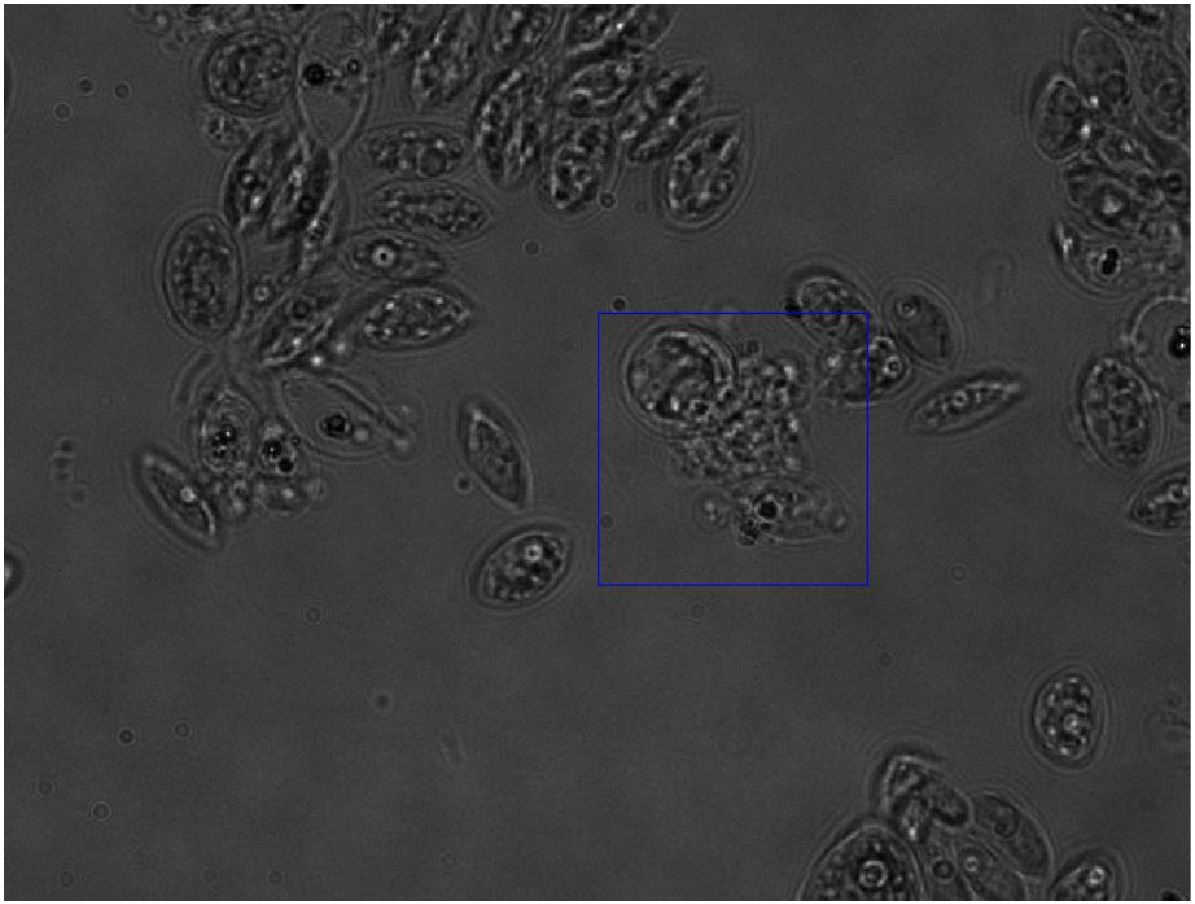


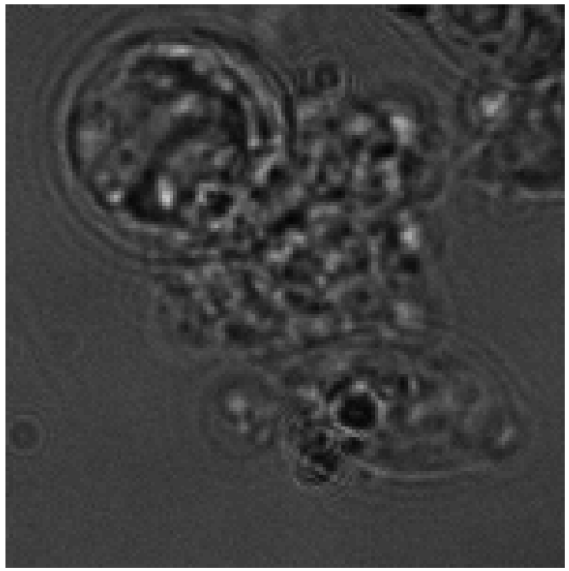


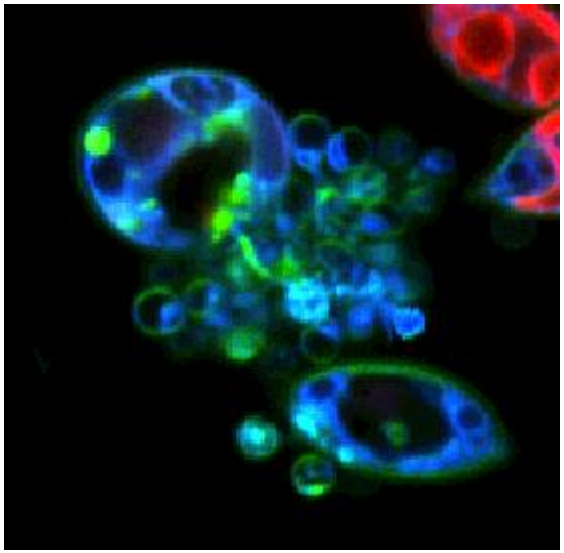


In the widefield image, more of the host cells show signs of infection.

Representative infected cells t = 12 hours

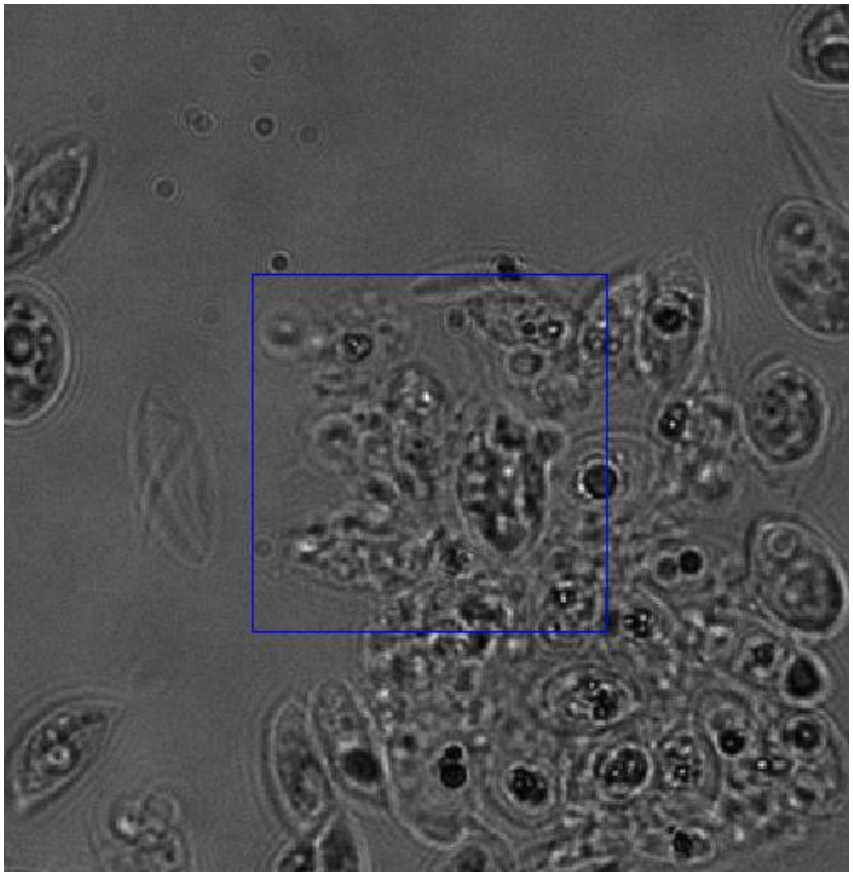


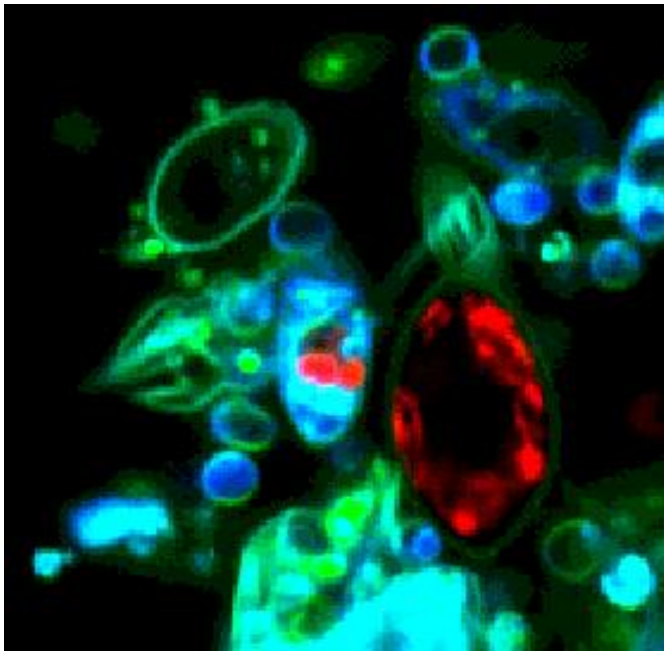




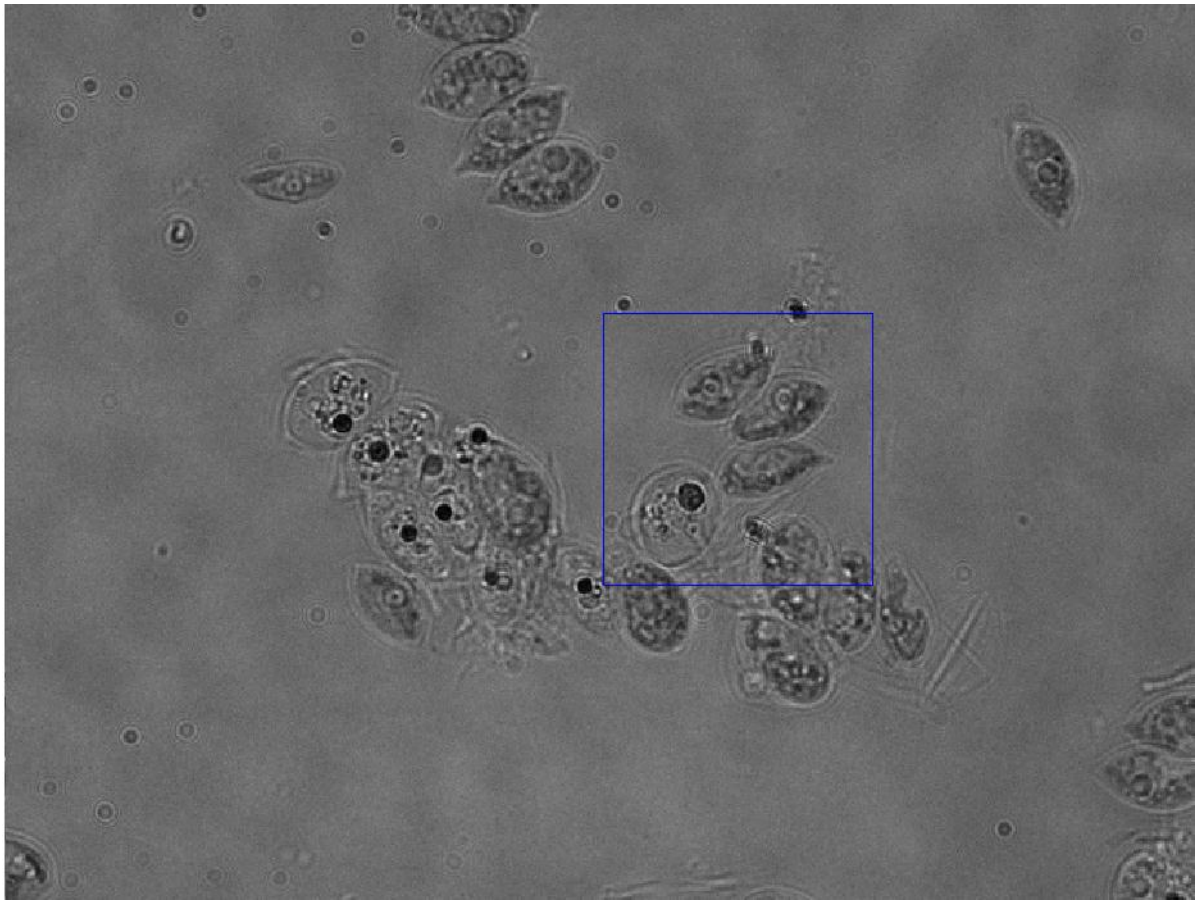
Quite a bit of sporangia! Complete absence of chlorophyl in Infected cells.

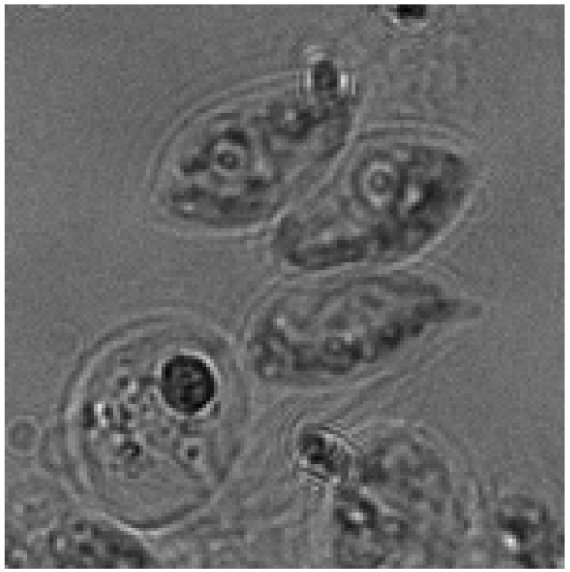
Representative infected cells t = 12 hours

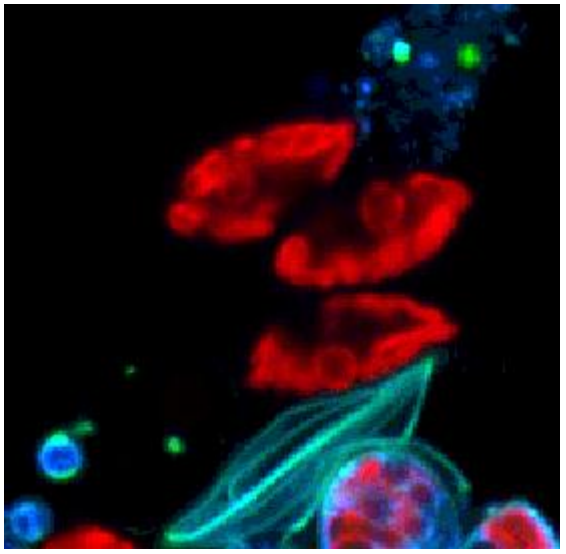




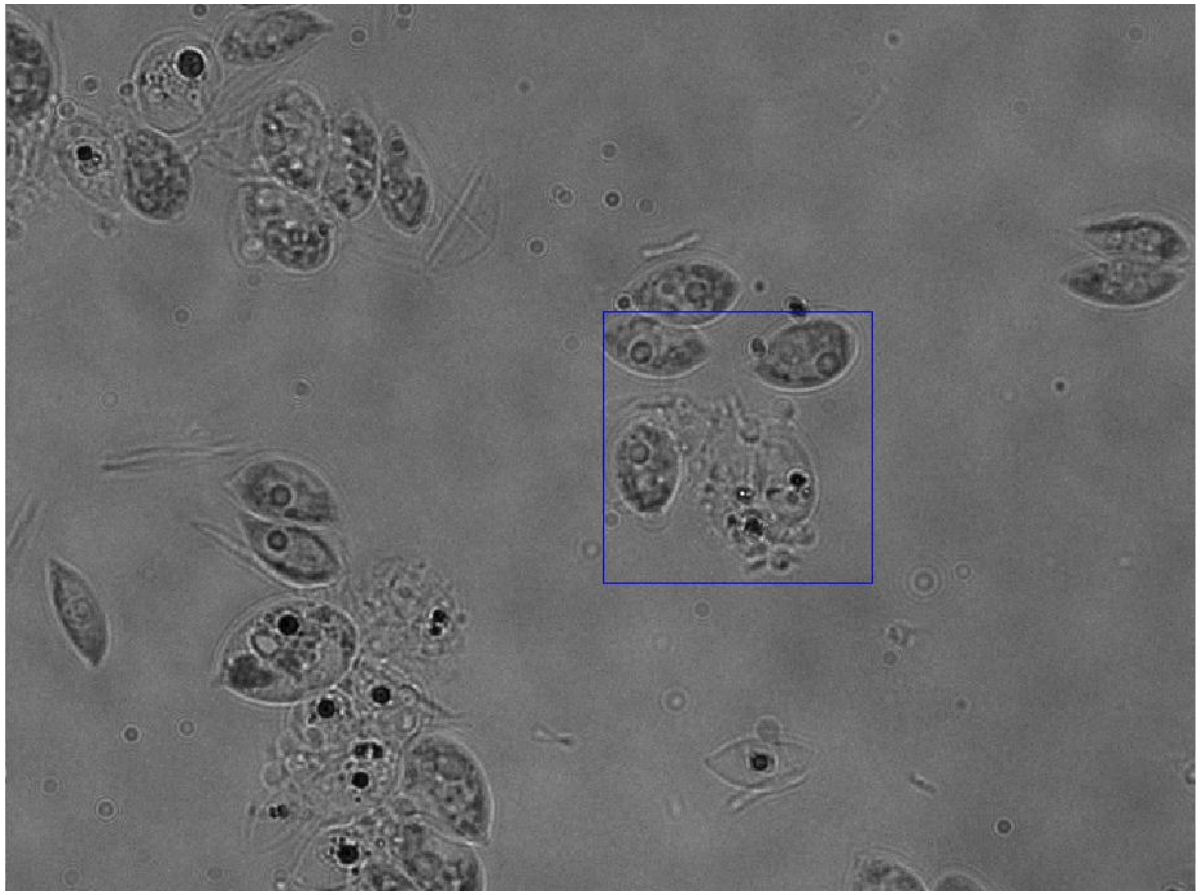
Representative infected cells t = 16 hours

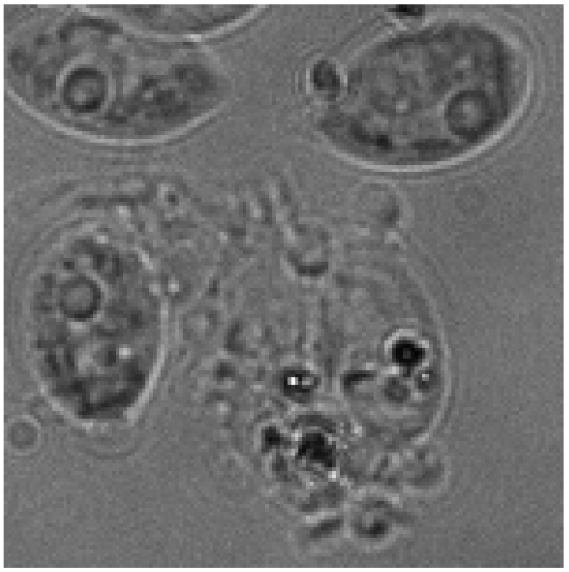


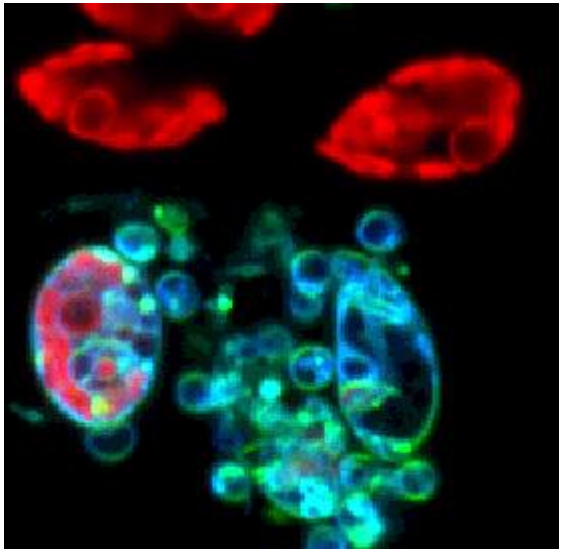




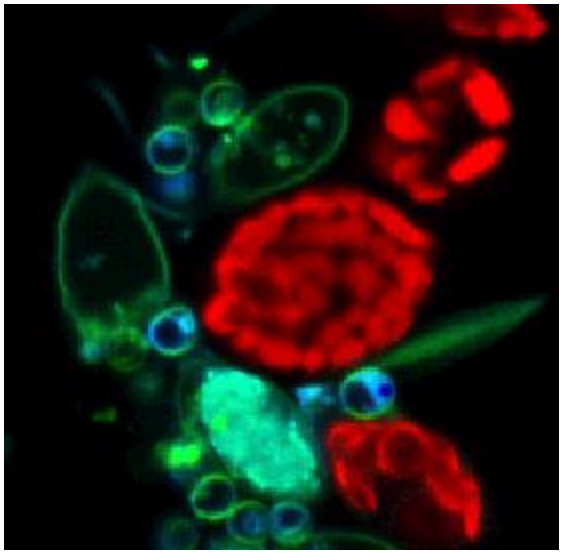
Representative infected cells t = 16 hours

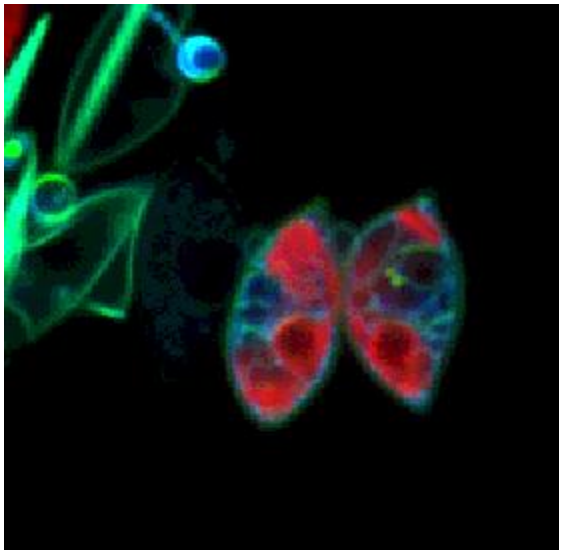


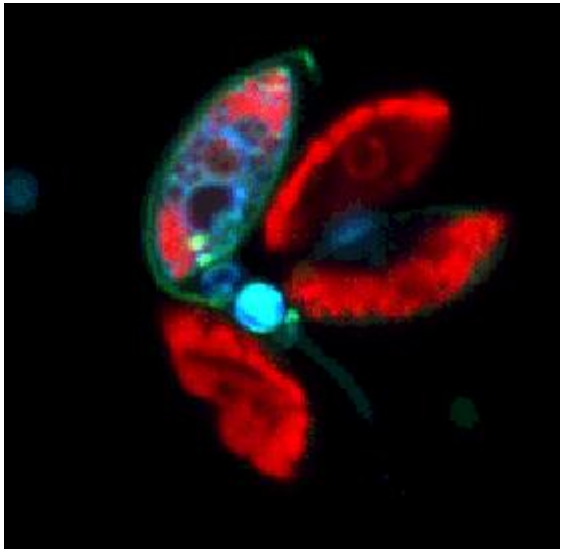




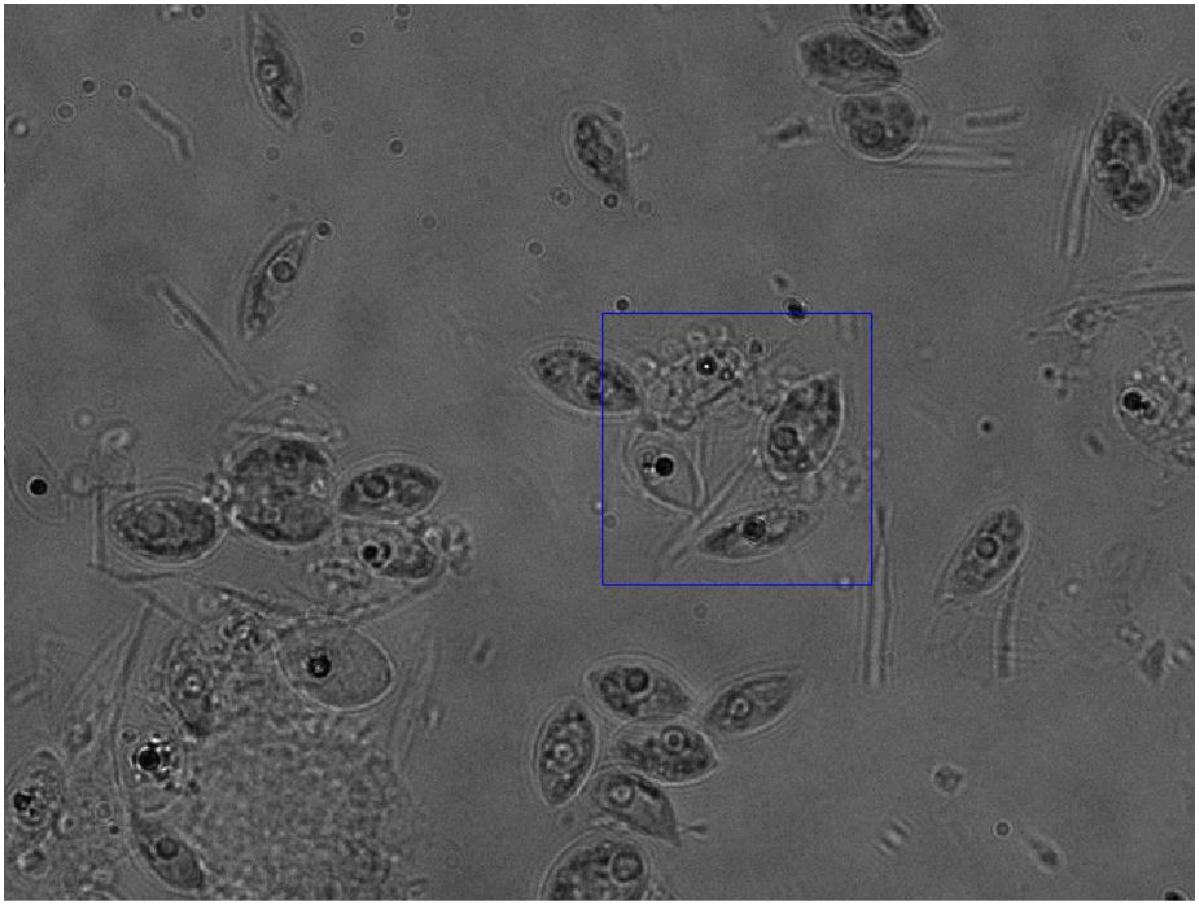
Representative infected cells t = 16 hours

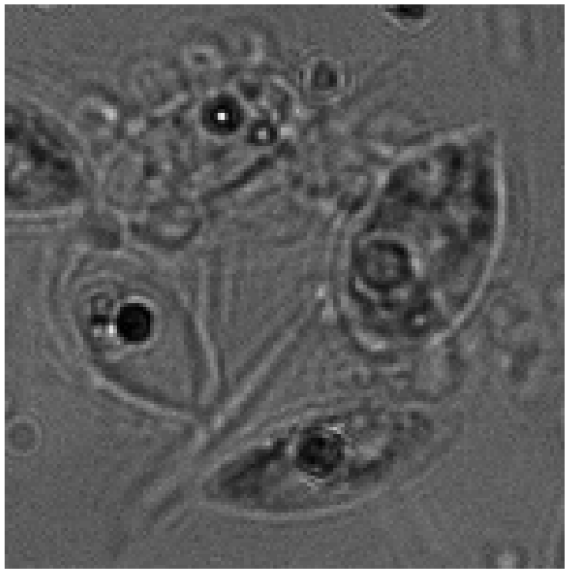


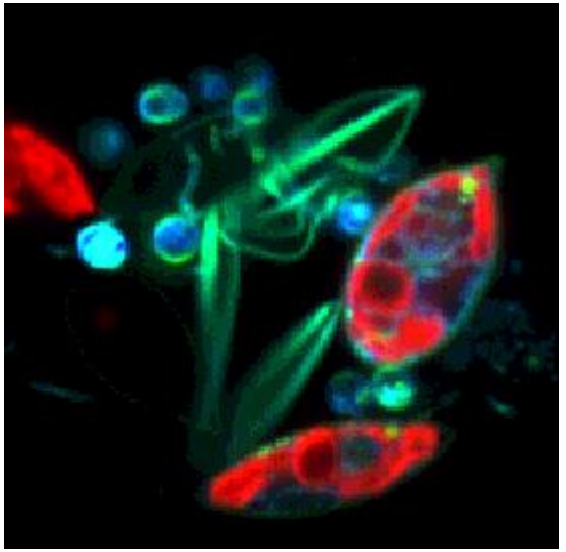




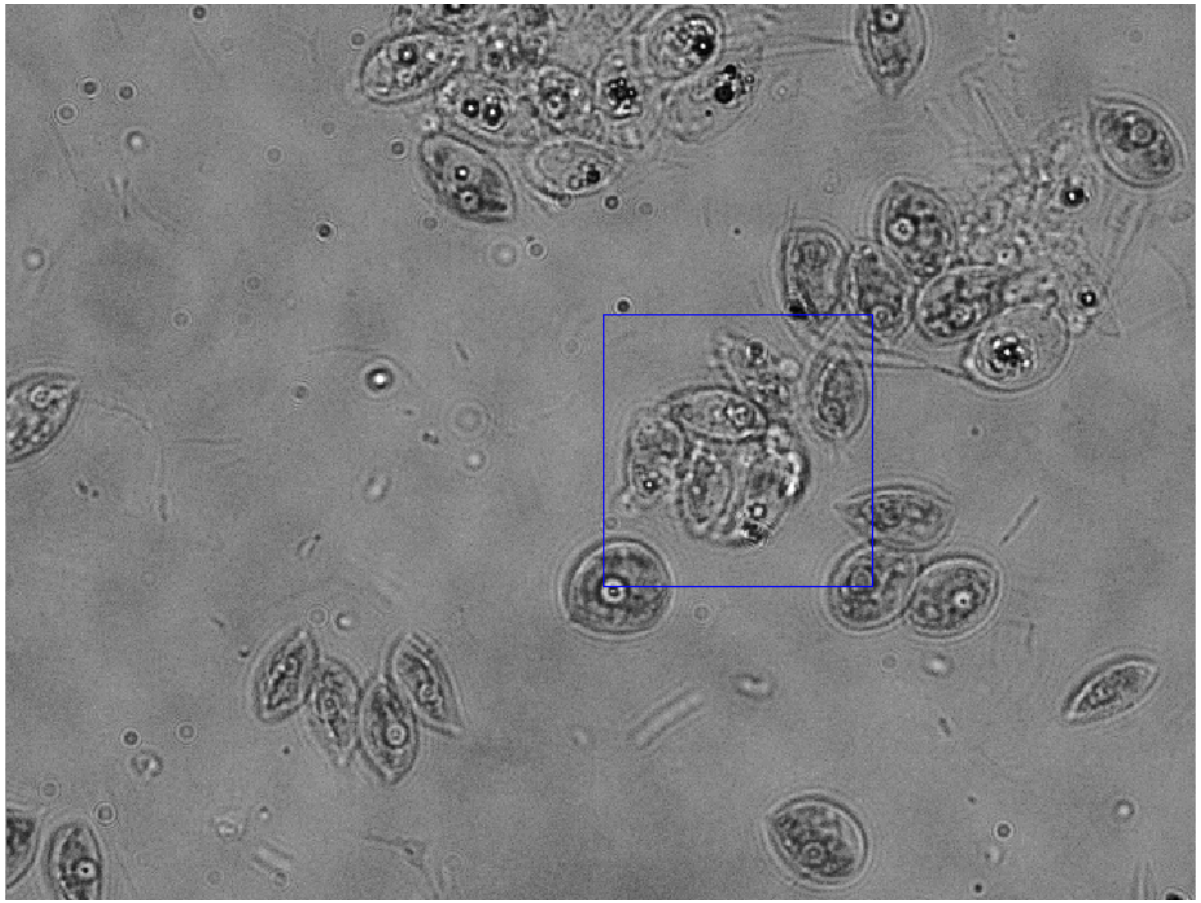
Representative infected cells t = 24 hours

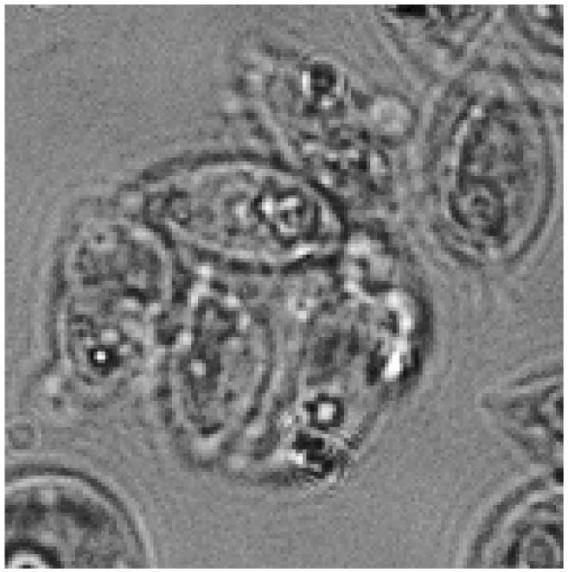


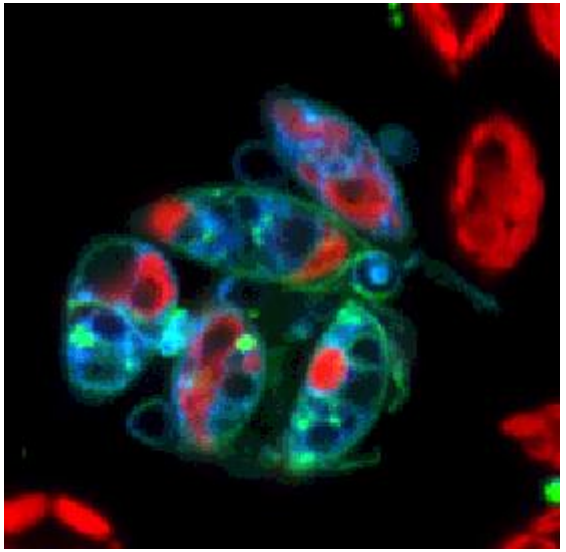




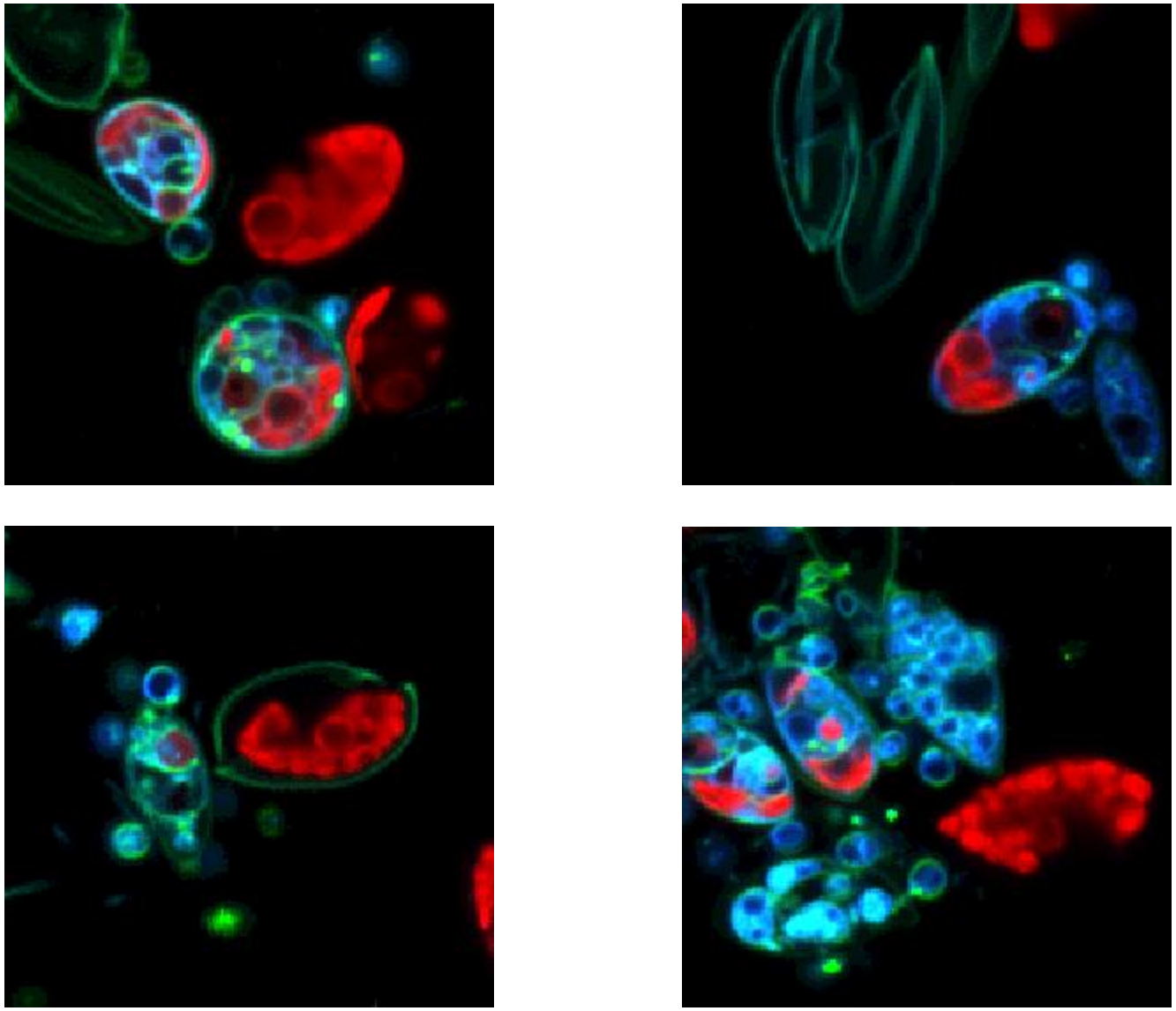
Representative infected cells t = 24 hours



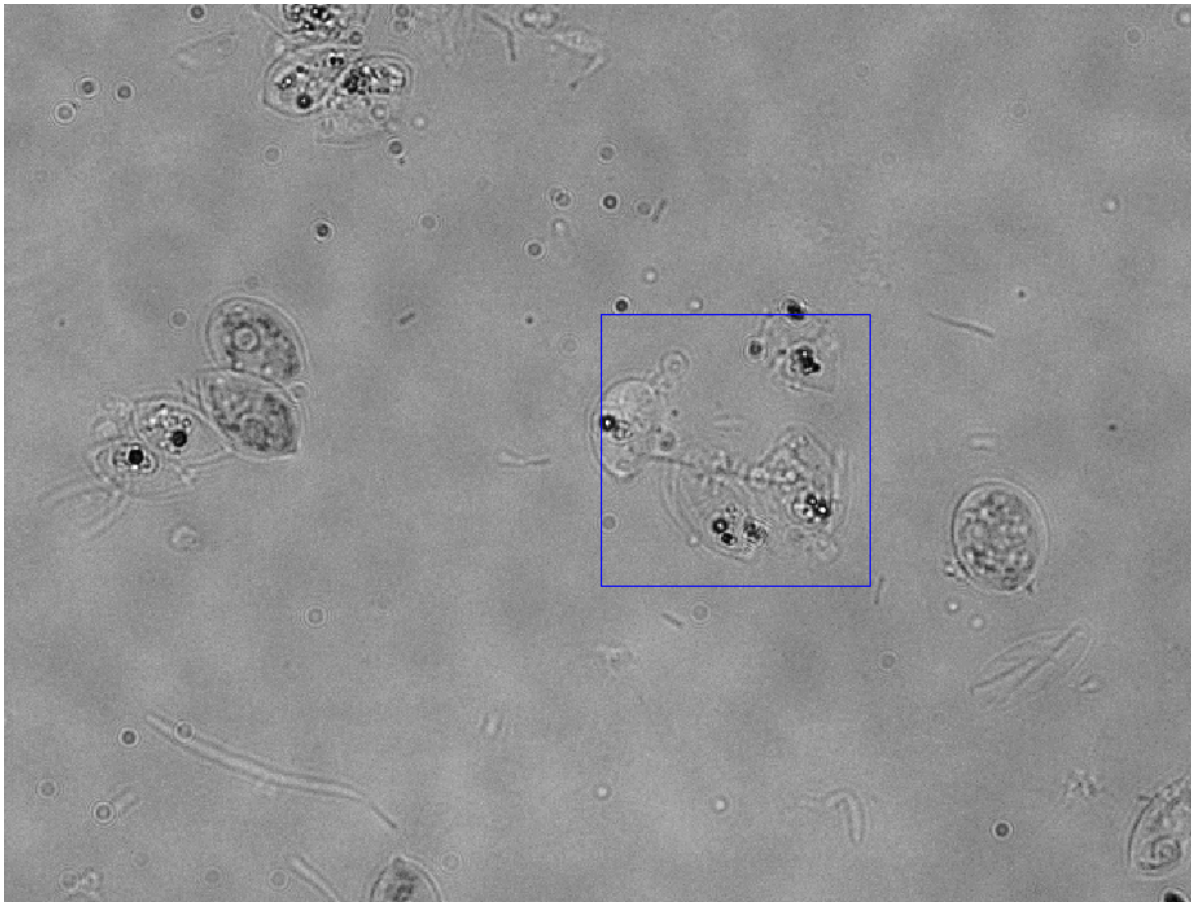


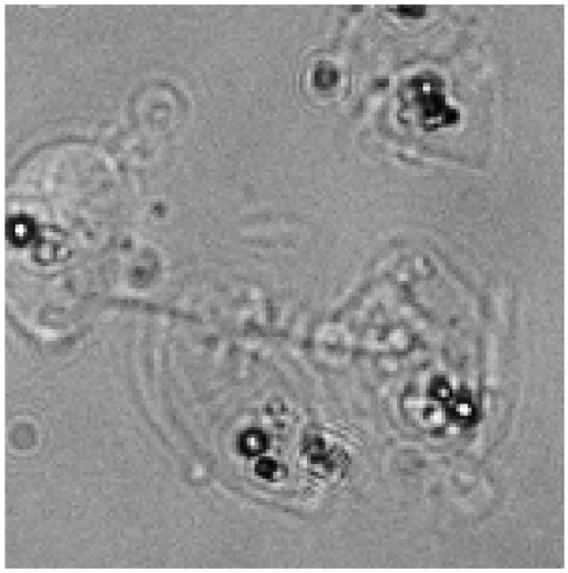


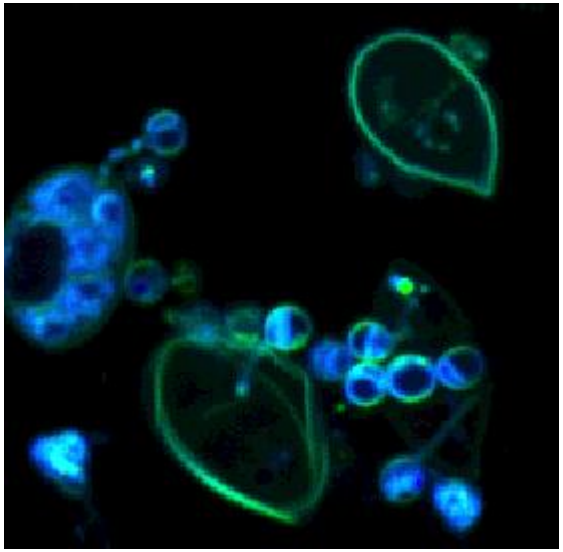
Representative infected cells t = 24 hours



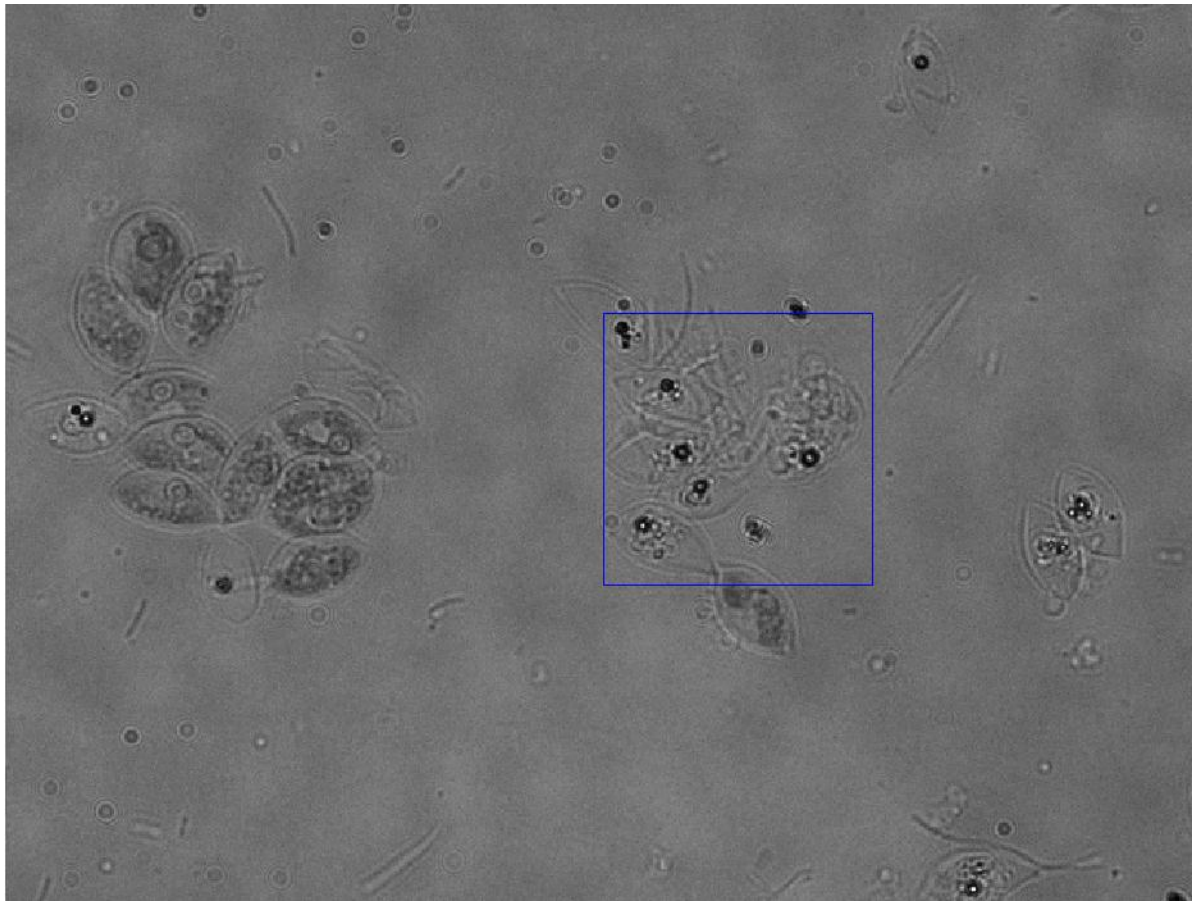
Representative infected cells t = 48 hours

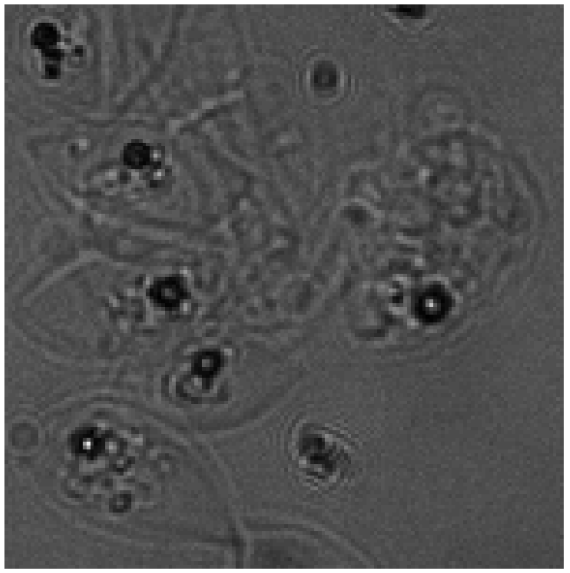


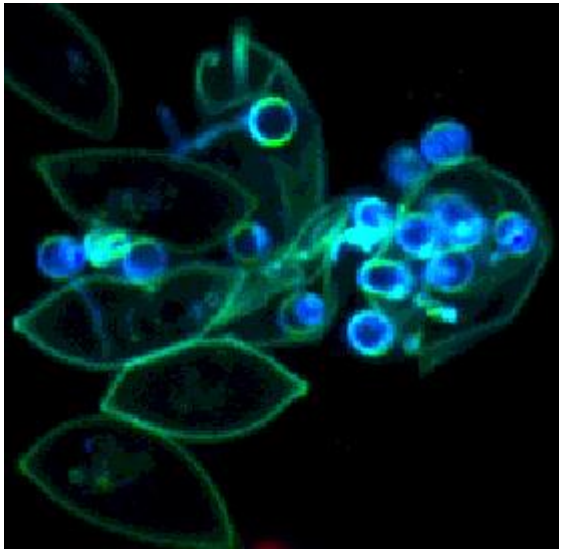




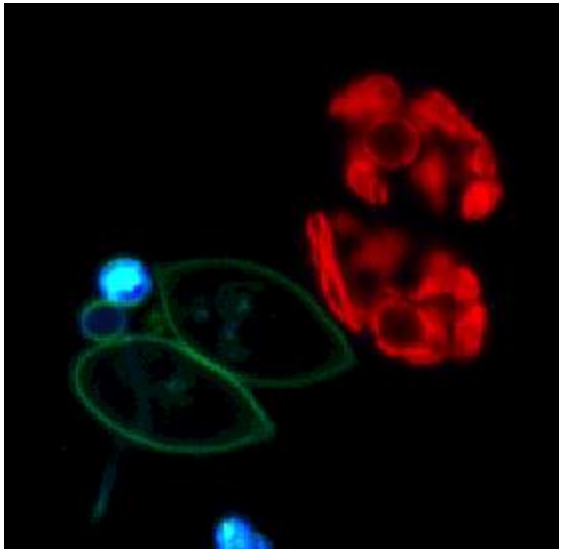
Representative infected cells t = 48 hours

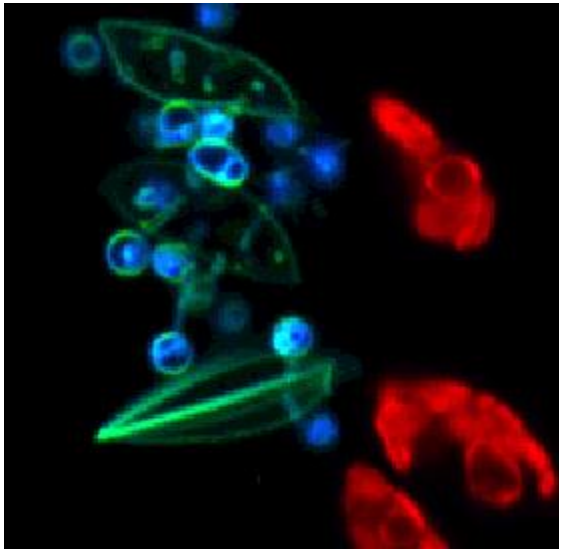


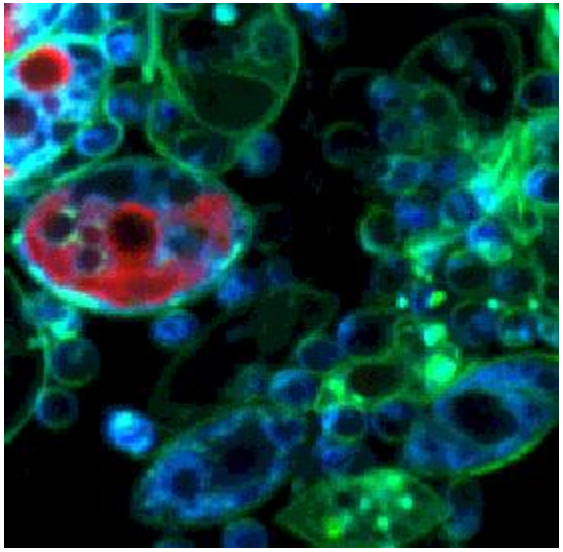


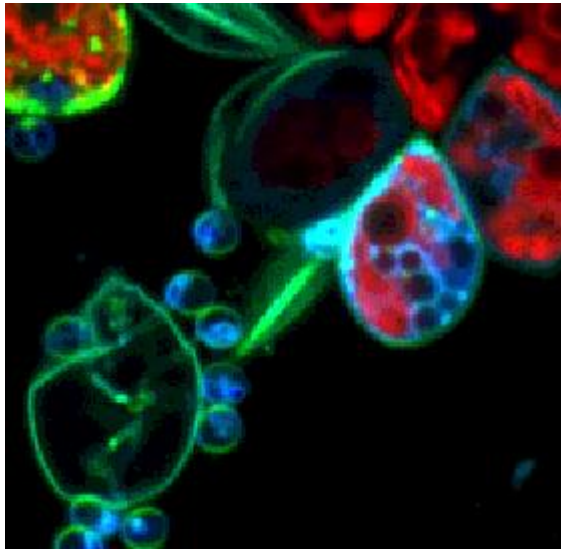


Representative infected cells t = 48 hours



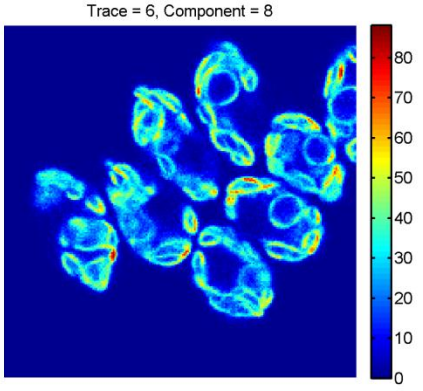




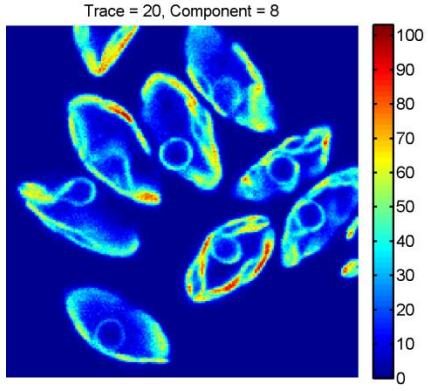


Component comparison – Carotenoid

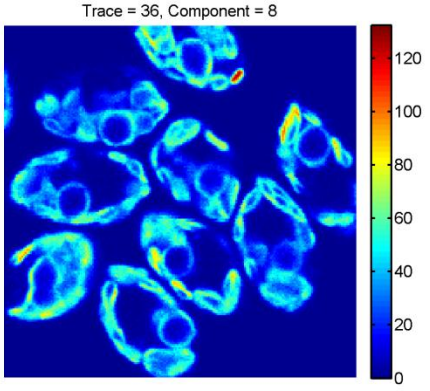
Control t0



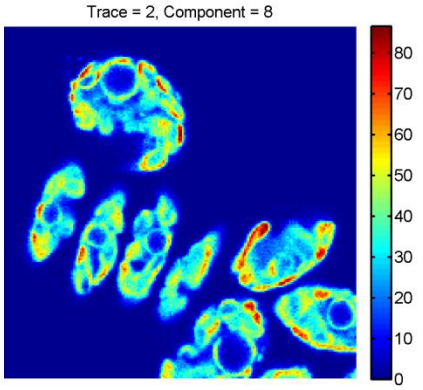
Control t4



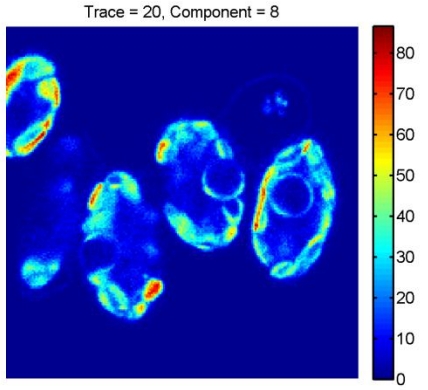
Control t8



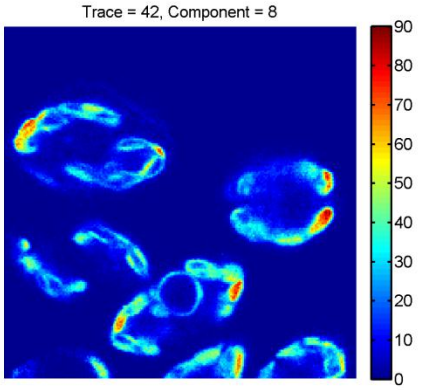
Infected t4



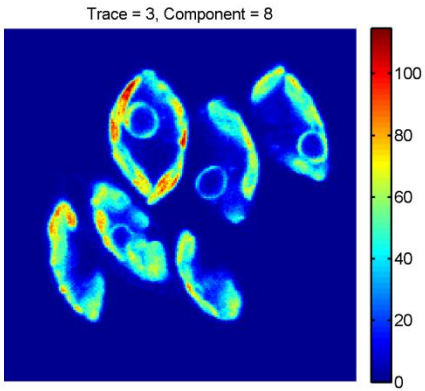
Infected t8



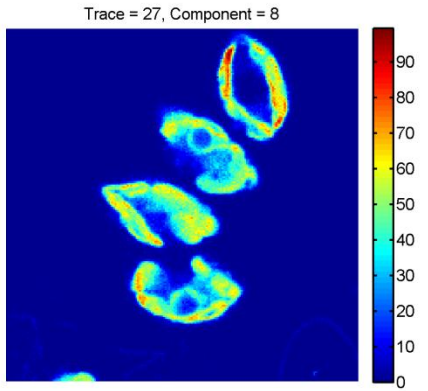
Infected t12



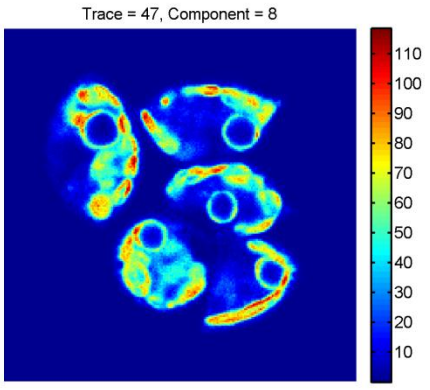
Infected t16



Infected t24

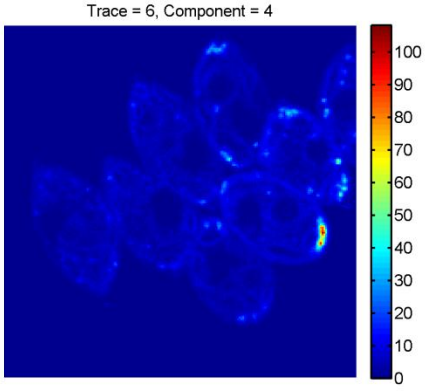


Infected t48

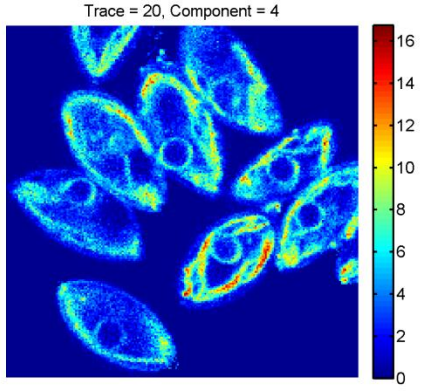


Component comparison – NR 573nm

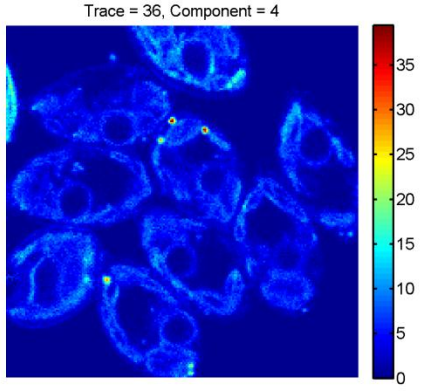
Control t0



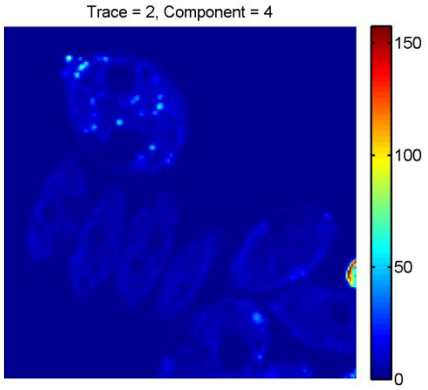
Control t4



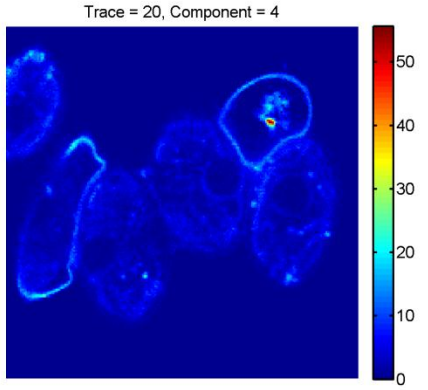
Control t8



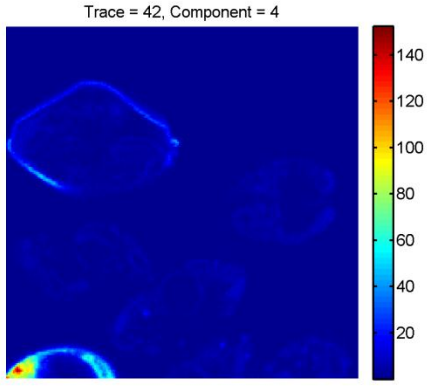
Infected t4



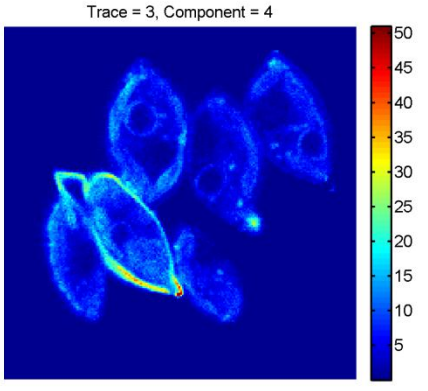
Infected t8



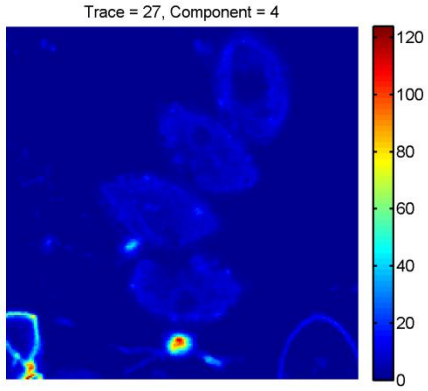
Infected t12



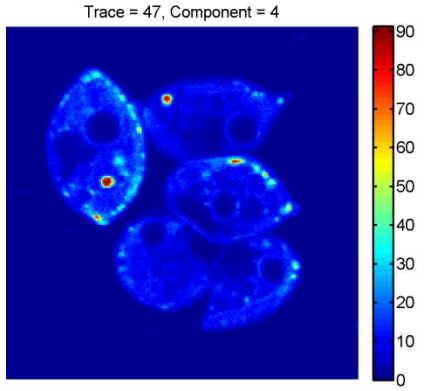
Infected t16



Infected t24

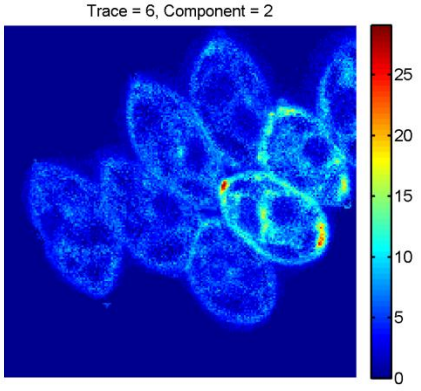


Infected t48

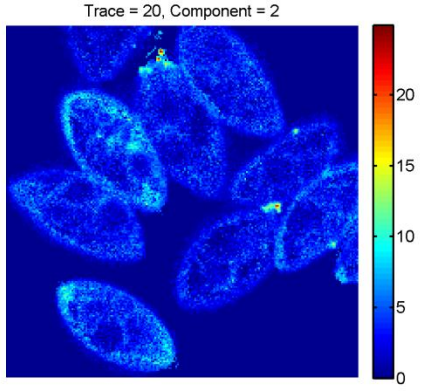


Component comparison – NR 600nm

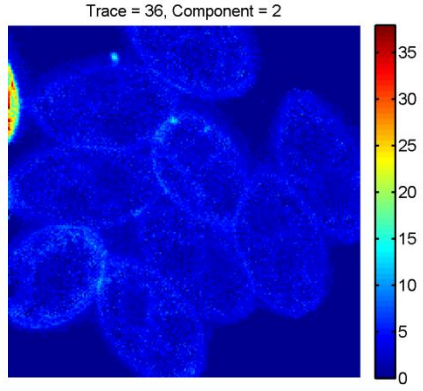
Control t0



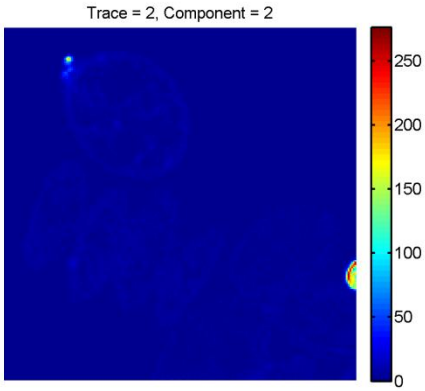
Control t4



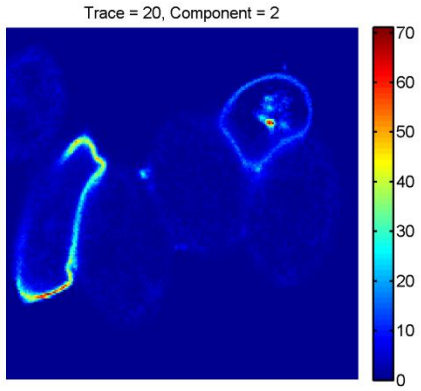
Control t8



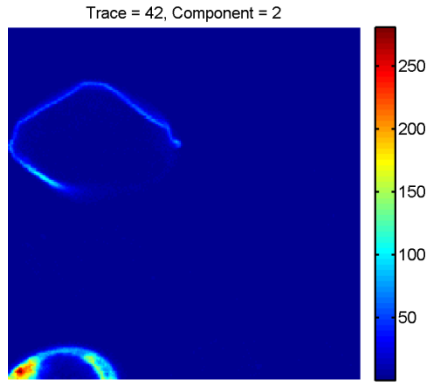
Infected t4



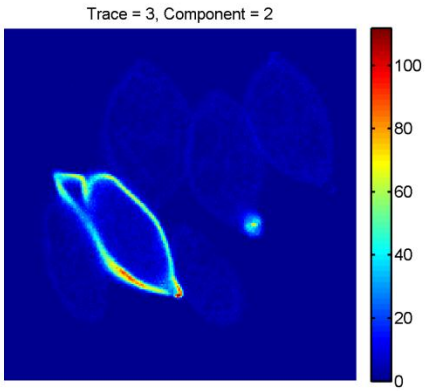
Infected t8



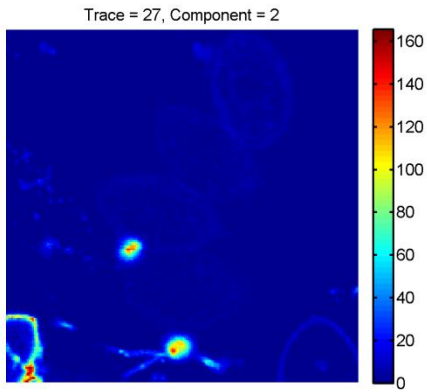
Infected t12



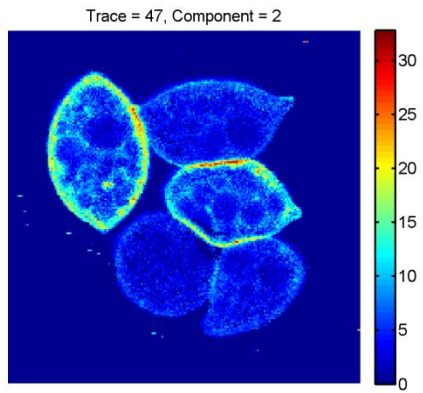
Infected t16



Infected t24

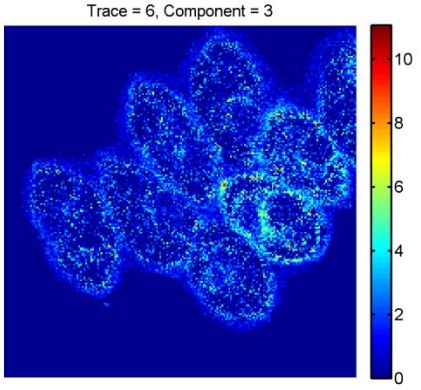


Infected t48

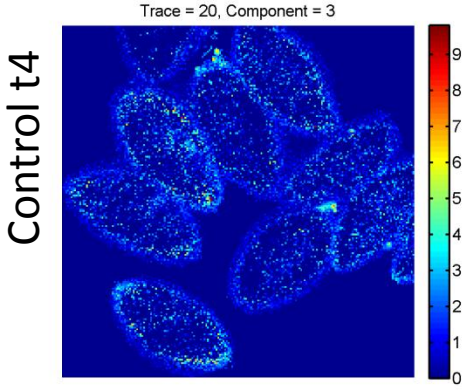


Component comparison – NR 630nm

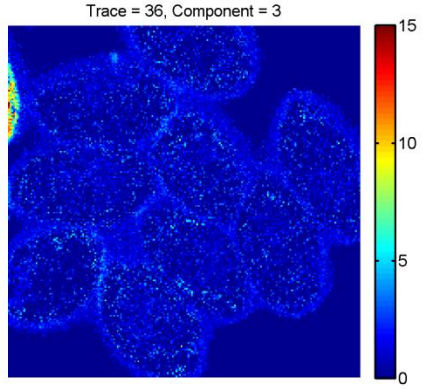
Control t0



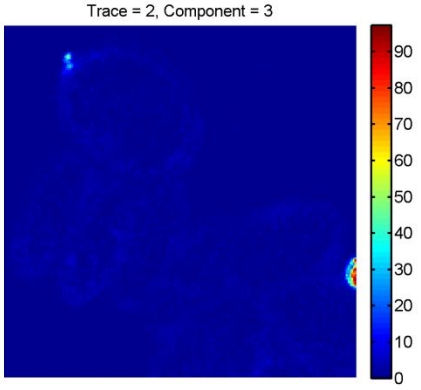
Control t4



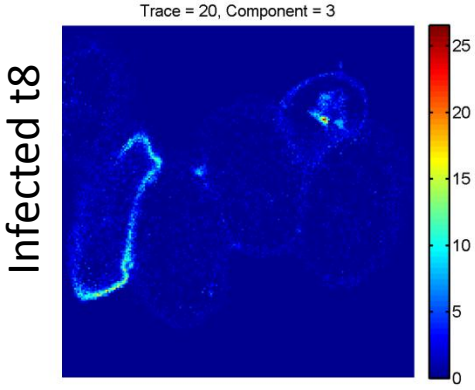
Control t8



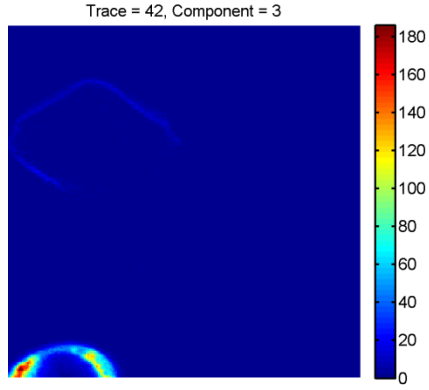
Infected t4



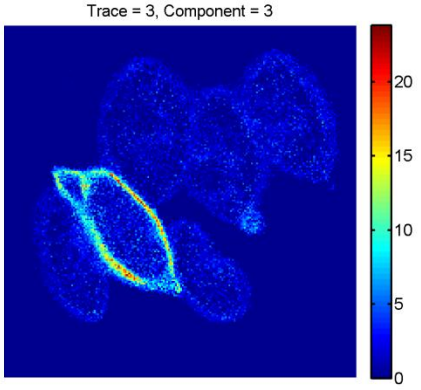
Infected t8



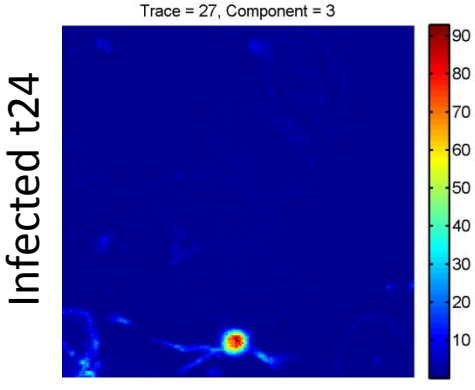
Infected t12



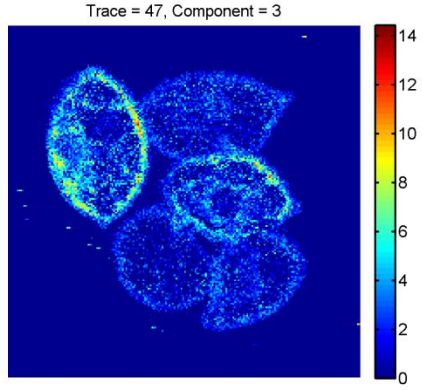
Infected t16



Infected t24

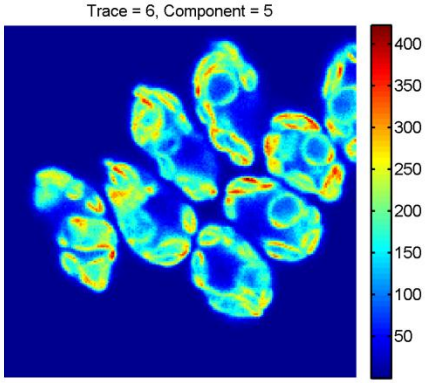


Infected t48

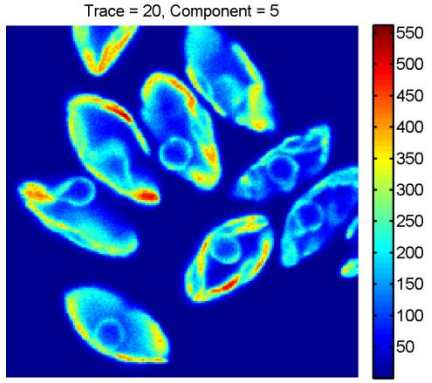


Component comparison – Chl 673nm

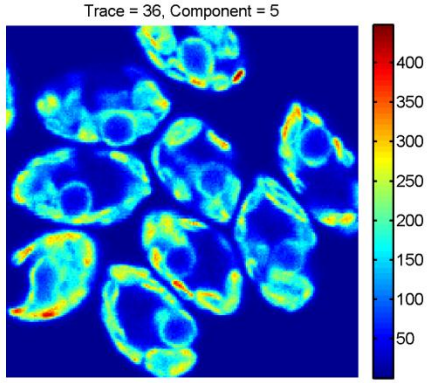
Control t0



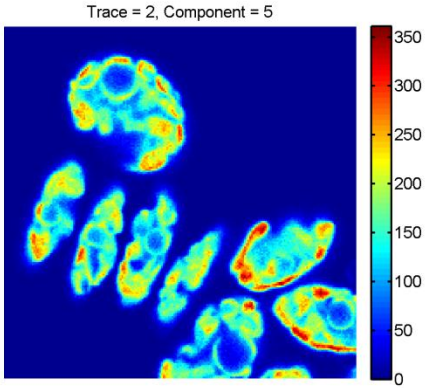
Control t4



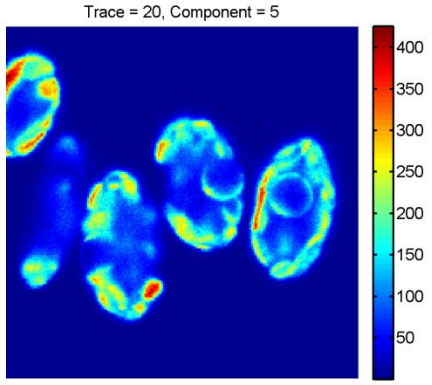
Control t8



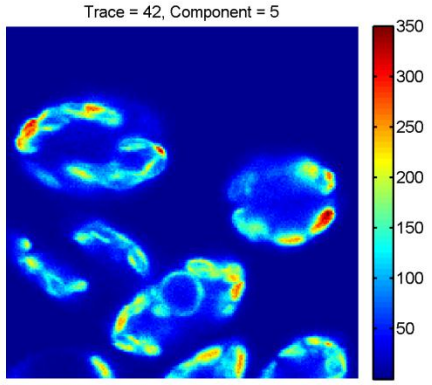
Infected t4



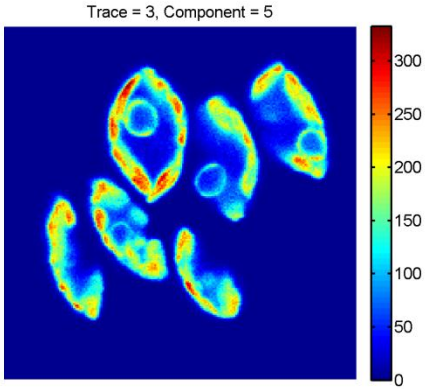
Infected t8



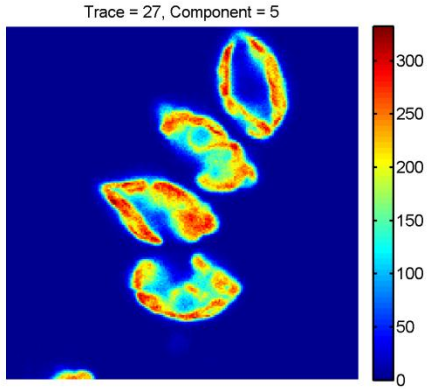
Infected t12



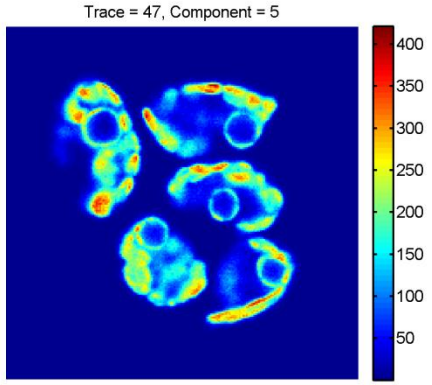
Infected t16



Infected t24

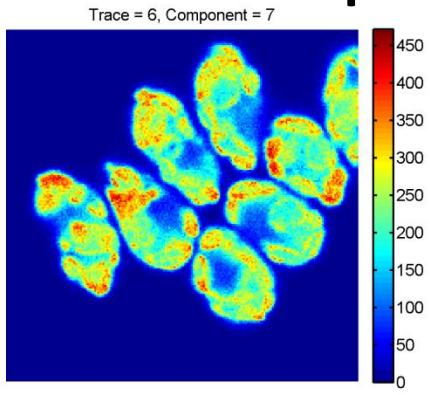


Infected t48

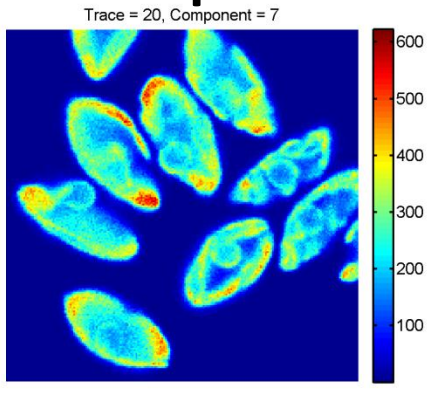


Component comparison – Chl 682

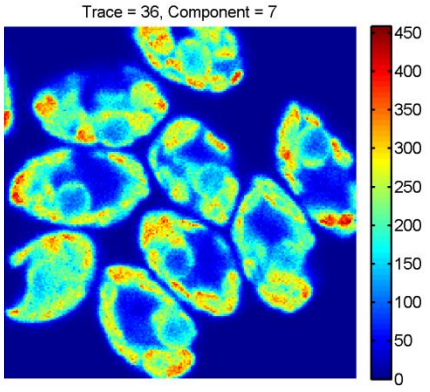
Control t0



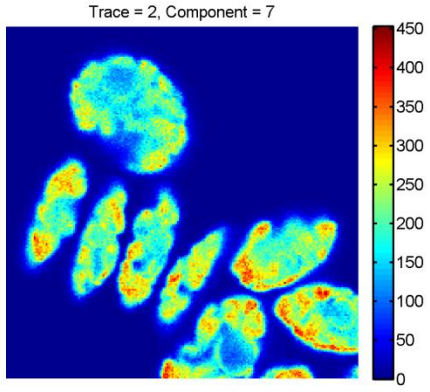
Control t4



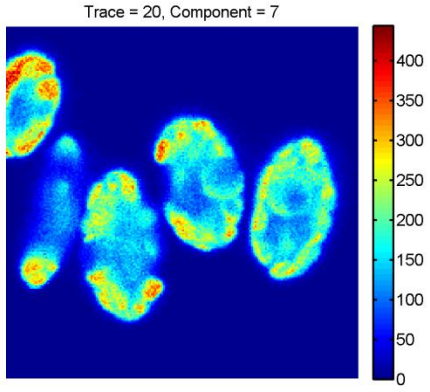
Control t8



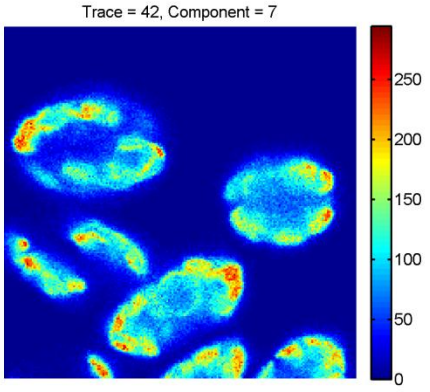
Infected t4



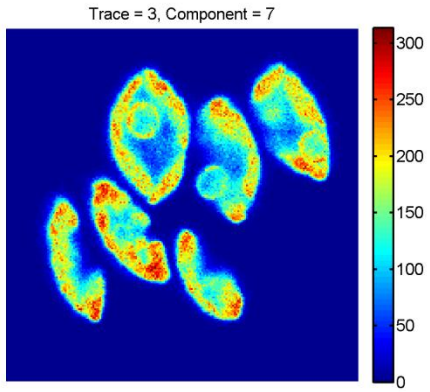
Infected t8



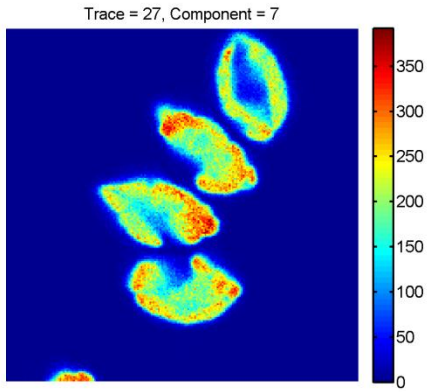
Infected t12



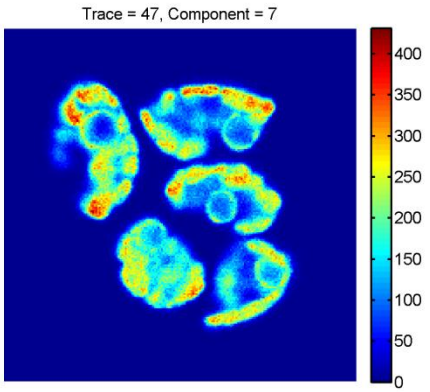
Infected t16



Infected t24

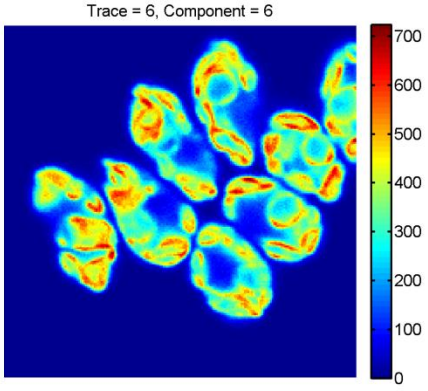


Infected t48

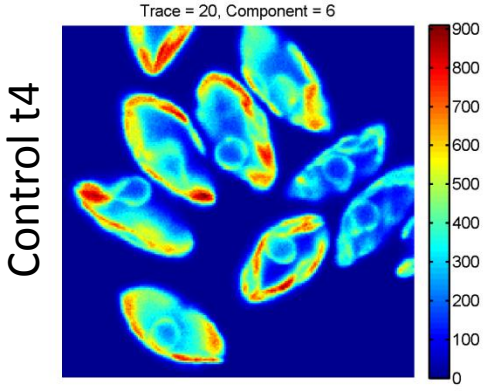


Component comparison – Chl 687nm

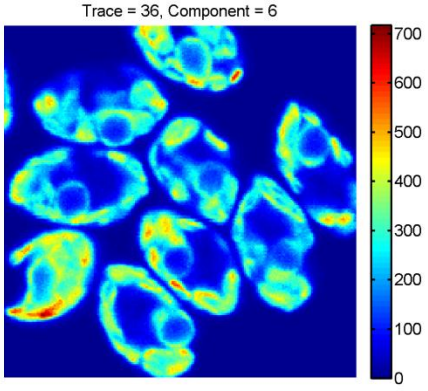
Control t0



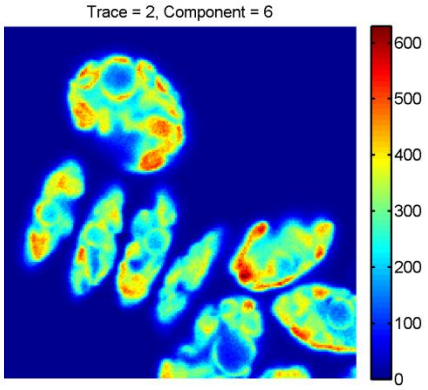
Control t4



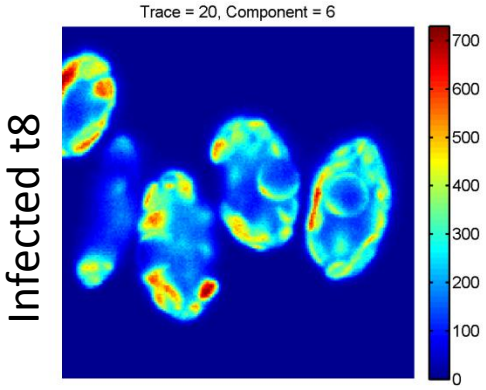
Control t8



Infected t4



Infected t8



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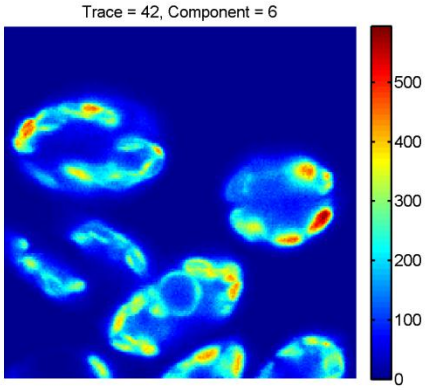

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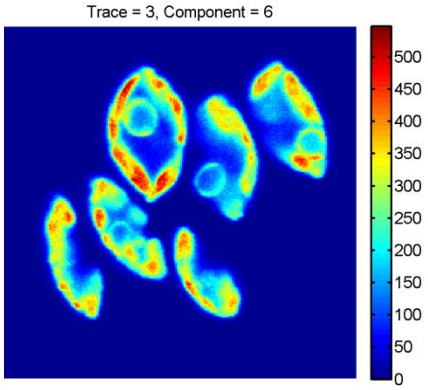

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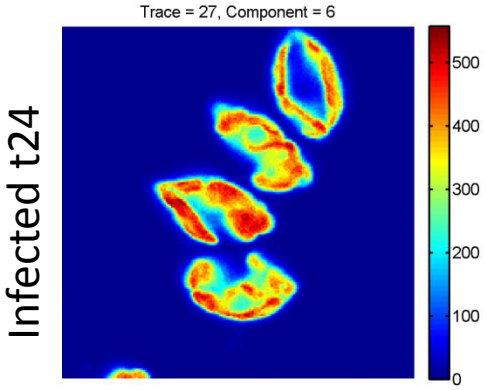

Infected t48