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Tracking Early Infection Events of *Paramecium bursaria Chlorella* Virus with Confocal Fluorescence Microscopy

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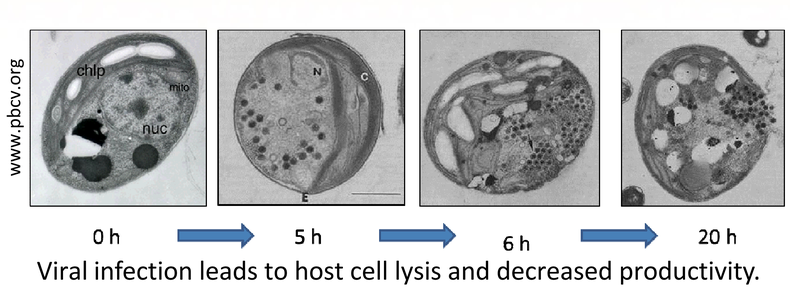
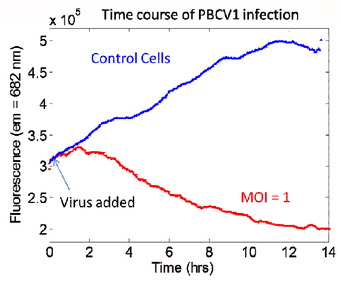
Introduction

A significant challenge for algal cultivation in open, large-scale systems is the detection of invading species, infection, and other algal stressors. Early detection of these undesirable contaminants is of particular importance if mitigation strategies are to be implemented. Viruses are undoubtedly the most abundant biological agent in ocean waters and algal viruses in particular can account for 10-100% of phytoplankton mortality, depending on algal species. Moreover, viral infections have been observed in biofuels production systems and therefore, a recognized need exists for the early detection of viral infection in algal cultivation due to the detrimental effect of infection.

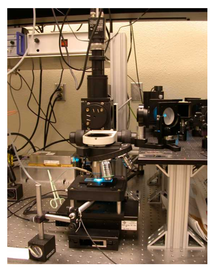
We have studied the early infection events of the model system, *Paramecium bursaria Chlorella* virus (PBCV) 1, with its algal host *Chlorella* using confocal fluorescence microscopy.



Viral infection leads to host cell lysis and decreased productivity.

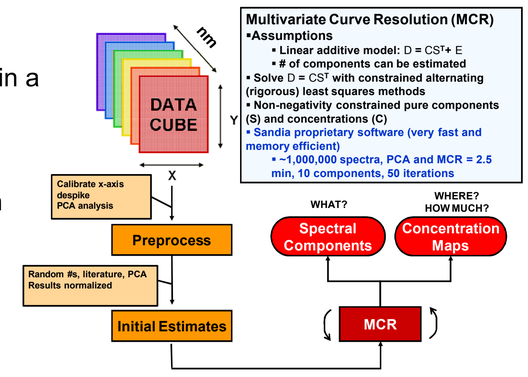


Hyperspectral fluorescence microscopy and Multivariate Curve Resolution



Multivariate Curve Resolution (MCR)

- Discover & quantify all emitting species in a sample simultaneously with no *a priori* knowledge
- Mathematical isolation of pure spectral components, independent concentration maps
- Jones et al., (2008) J Chemom. 22:482-490 and references therein

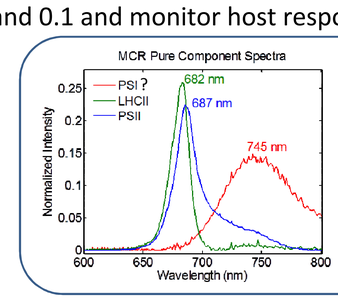


Can we develop spectral signatures for the early detection of viral infections?

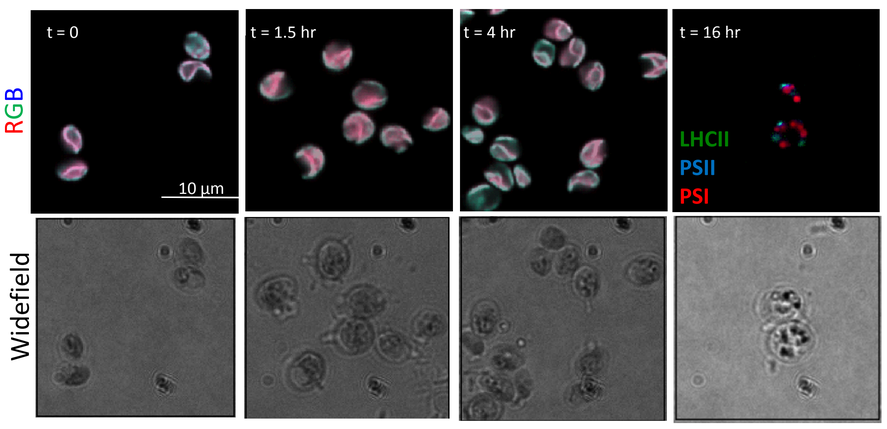
Infect cultures of Chlorella at MOI = 1 and 0.1 and monitor host response at t = 0, 1.5, 4 and 16 hr.

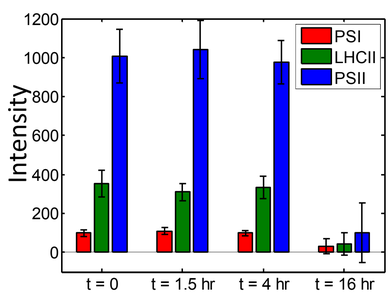
Culture conditions

Chlorella variabilis NC64A was grown on MBBM media in the presence of 10 μg/ml tetracycline in 80 μE of light with a 16:8 photoperiod.



54 total spectral images were combined into a composite dataset and MCR algorithms were used to develop a pure component model.





Per image (n=54) mean intensities for PSII, PSI, and LHCII

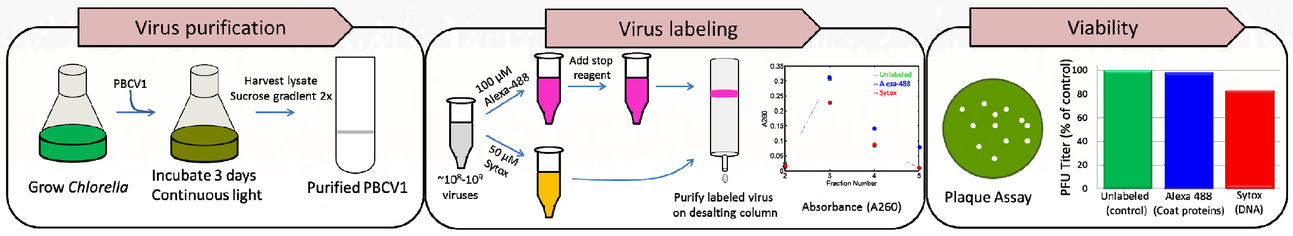
Viral infection leads to:

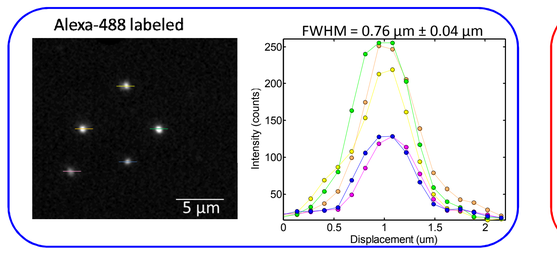
- Altered chloroplast morphology
- Decrease in PSII and LHCII *after* cell lysis
- Can't discriminate between infected cells and uninfected from host signal only
- Does cell lysis or viral infection cause pigment intensity decrease?

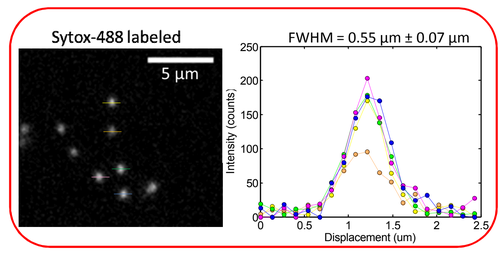
Chlorella virus labeling methodology

To study the initial infection of the PBCV with *Chlorella* a general virus labeling strategy was developed based on Zhang et. al. 2010. Journal of Virological Methods 167(2): 172-177.

Alexa Fluor® Succinimidyl Esters – amine reactive dye, allows for covalent coat protein labeling.
Sytox ® green stain – DNA intercalating dye, allows for viral DNA labeling.

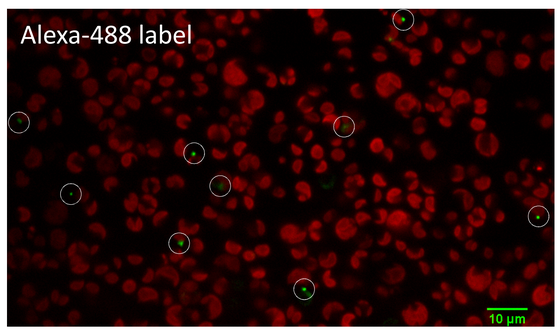


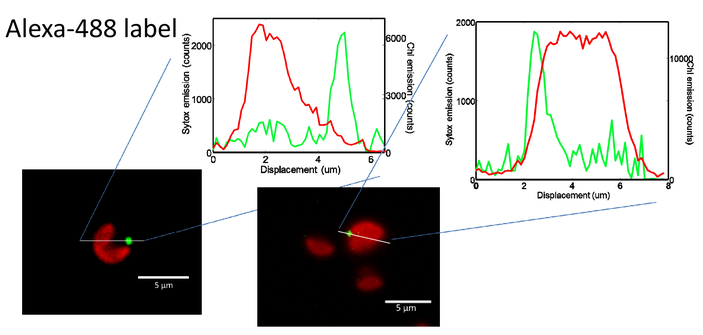




Labeling efficiency and virus purity were assayed by visualizing single virus particles with confocal fluorescence microscopy. Single particles (~125 nm diameter) are smaller than the diffraction limit.

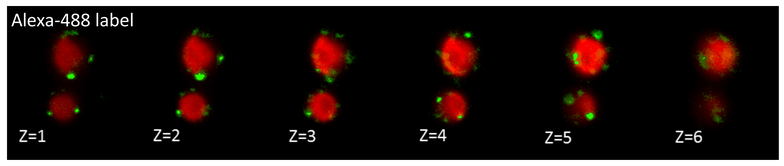
Chlorella/PBCV1 interactions – live cells imaging





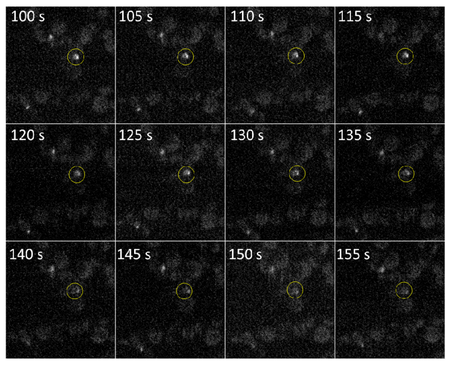
Employing the labeling strategies, individual virus (green) can not be visualized among a field of uninfected *Chlorella* cells (chl., red). Infection at the single cell level shows virus capsid remaining on host cell wall.

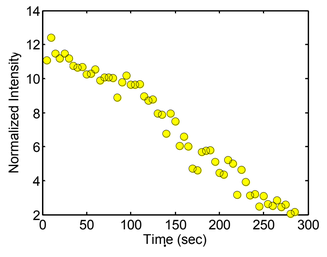
At high MOI (~10), multiple viruses can be observed on the host cell by optical sectioning.



PBCV1 Infections in real time

Real-time monitoring of viral infection was observed with Sytox-DNA labeling. When viral DNA is injected into the host cell, the label becomes diluted and disappears.





Intensity vs. time for particle Identified in left panel.

Images were acquired every 5 s. Single particle tracking was achieved with MOSAIC (Sbalzarini and Koumoutsakos. 2005, Journal of Structural Biology 151(2):182-195,

Conclusions

- **Live cell imaging of *Chlorella* during time course of infection**
- **Virus labeling allows for monitoring infection in real time as well as determine MOI on a per cell basis**
- **On going experiments are incorporating virus labeling with spectral imaging**

Acknowledgements

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