Host Pathogen Interaction: A study of the pigment response of *Chlorella* when exposed to *Paramecium bursaria* chlorella virus (PBCV-1)

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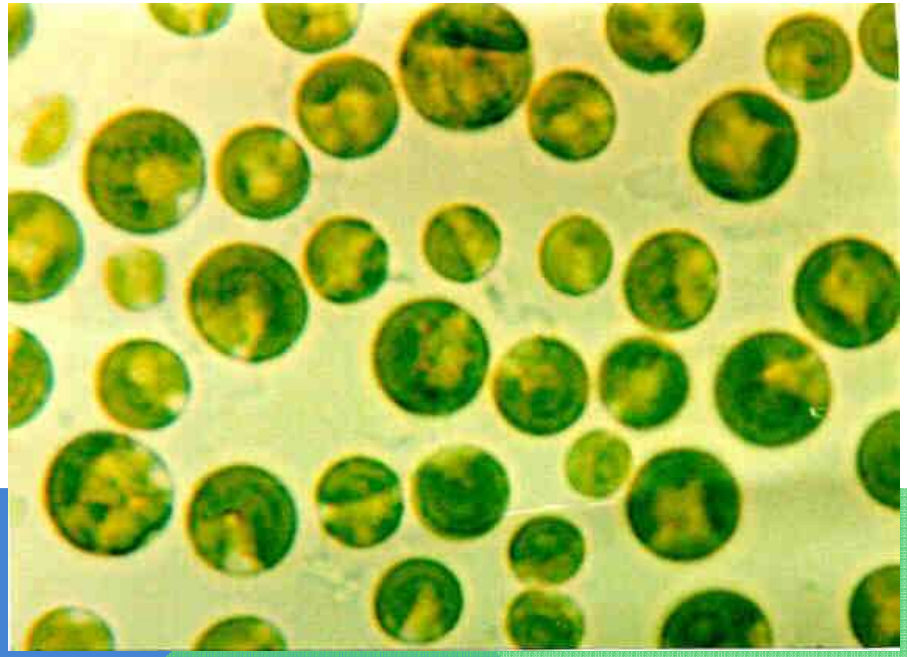
THE HOST

Chlorella NC64A

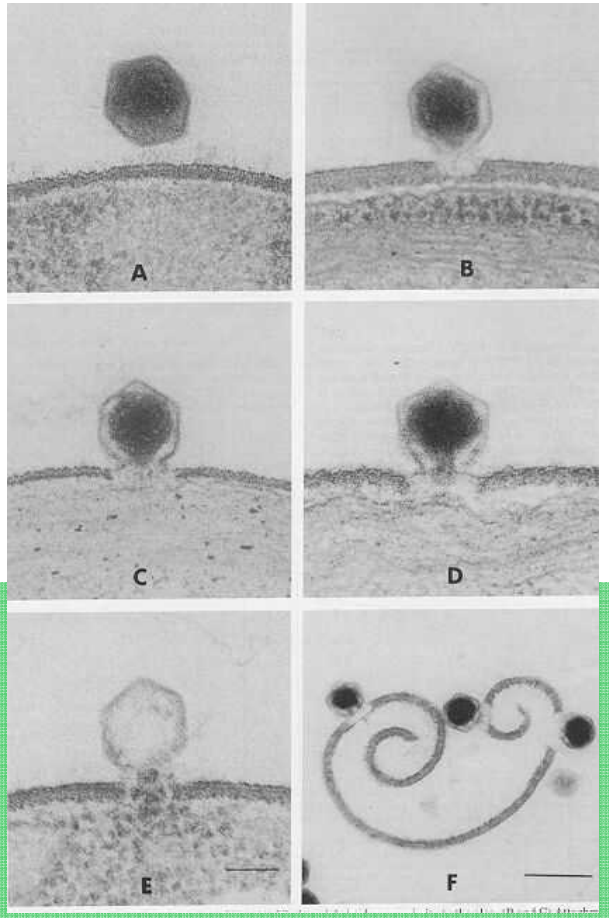
- Single Celled green algae
- 2-10um in diameter
- Potential biofuel candidate



THE PATHOGEN

Paramecium bursaria Chlorella virsus (PBCV-1)

- Icosahedral DS DNA virus
- ~0.19 um diameter



MOTIVATION

- Higher energy yield per acre
- Can be grown on low value land
- Can be grown with waste CO2 and/or wastewater
- Can be refined into a myriad of products including
  - Jet fuel
  - Bio-diesel

http://www.makebiofuel.co.uk/

POND CRASHING

- Major impediment to commercial viability of operations
- Rapid loss of biomass
- Commonly attributed to
  - Fungi
  - Viruses
  - Zooplankton.

https://share.sandia.gov/news/resources/news_releases/pond-collapse

SANDIA'S APPROACH

Multiple individual research thrusts to tackle problem including

- Rapid Threat Organism Recognition (RapTOR)
  - Uses ultra-high-throughput DNA sequencing
  - Characterizes organisms
  - Identifies threats
- Hyperspectral fluorescence microscopy
  - Characterize stress induced pigment fluctuations
- Pulse Amplitude Modulation (PAM)
  - Monitors photosynthetic activity
- Confocal fluorescence microscopy
  - Monitors morphological changes in chloroplasts
  - Can be used to view viral infection and propagation

CONFOCAL FLUORESCENCE MICROSCOPY

- Patented by Marvin Minsky in 1957
- Pinhole aperture eliminates of out of focus light
  - Excludes out of focus flare in thick fluorescently labeled specimens
  - Enables automated collection of three dimensional data

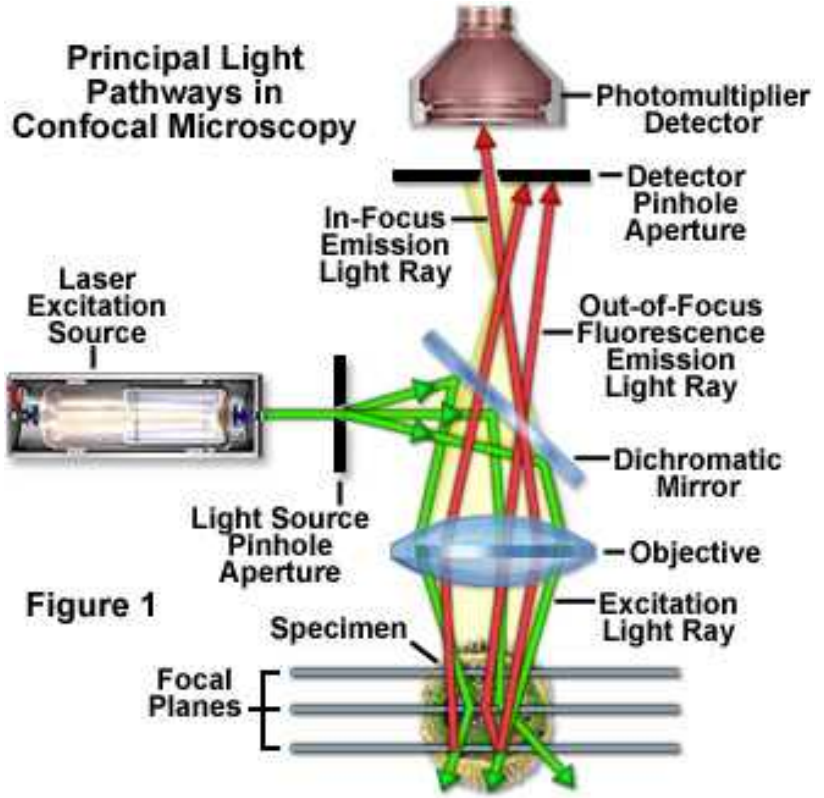


Figure 1

https://www.microscopyu.com/articles/confocal/confocalintrobasics.html

PULSE AMPLITUDE MODULATION OF CHLOROPHYLL FLUORESCENCE

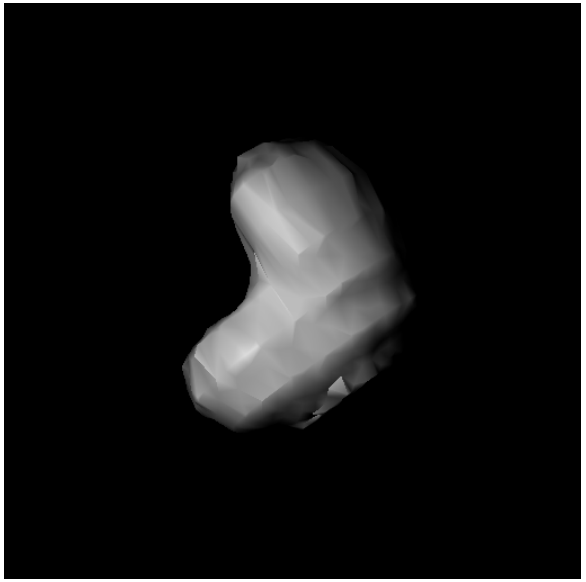
- In vivo method for analyzing linear electron flux, CO2 assimilation and Photosynthetic activity
- Utilizes modulated measuring beams in which phase and frequency decoding are used to detect fluorescence yield changes
- Measurements/Terms:

$$F_m' = maximum\ fluorescence\ (Q_A\ completely\ reduced)$$

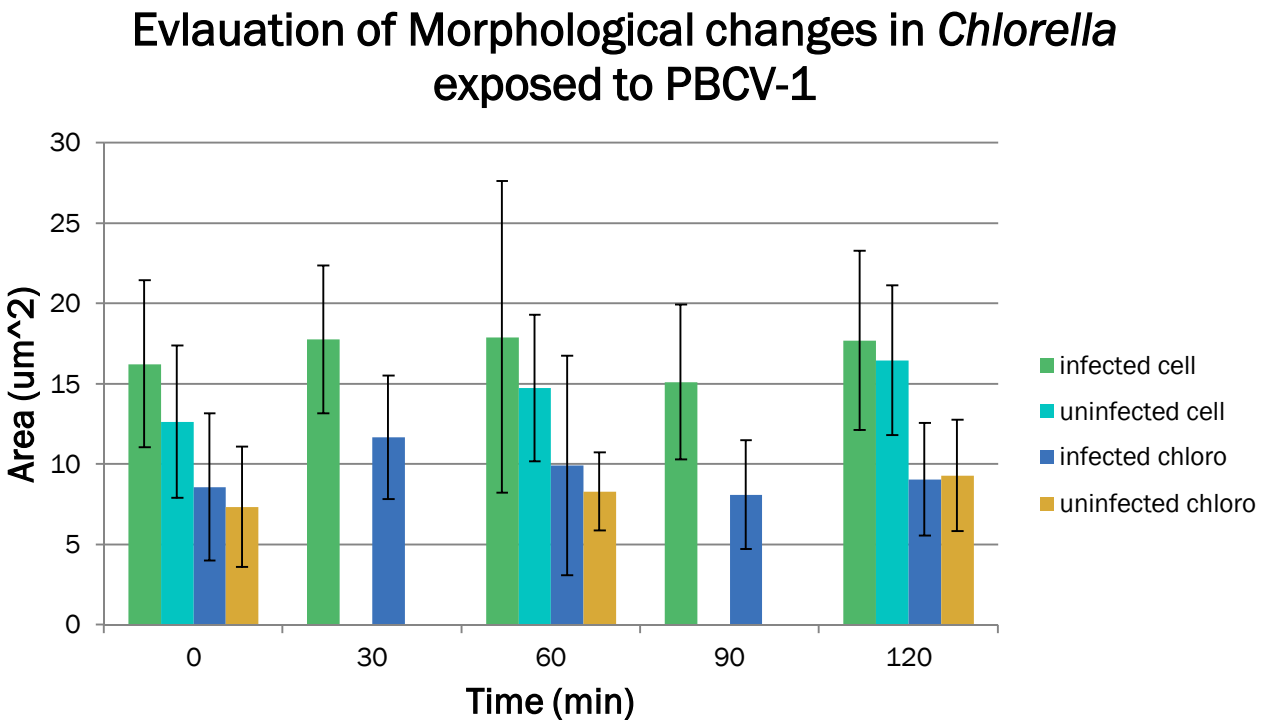
$$F_o' = minimum\ fluorescence\ (Q_A\ completely\ oxidized)$$

$$F_v' = F_m' - F_o' \qquad \frac{F_v'}{F_m'} = Maximum\ efficiency\ of\ PS\ II$$

CONFOCAL RESULTS

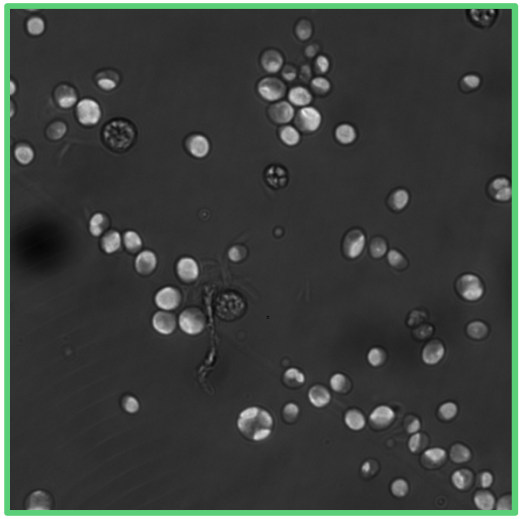


3-D model of chloroplast utilizing Fiji's 3d projection plugin - ImageJ extension



- Analyzed 25 cells at each time point using ImageJ
- Took area measurements of freehand selected z-projections.
- Found no statistically significant changes in cell size over the course of 2 hour infection.
- Large variation in cell size makes statistically significant volumetric changes difficult to detect.

CONFOCAL RESULTS CONT.



Bright field view of algae with excitation source on.

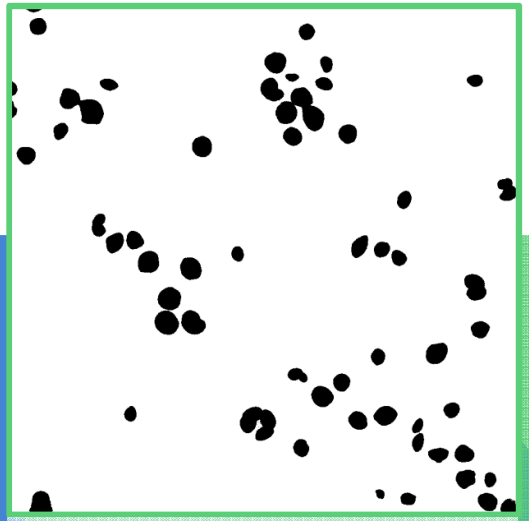
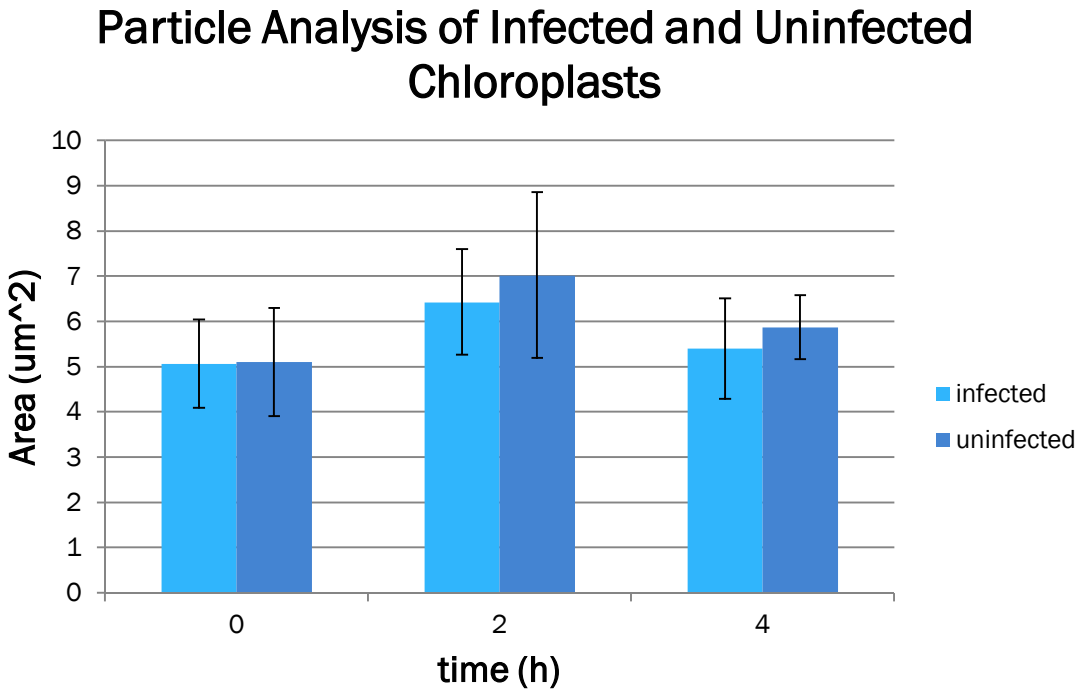
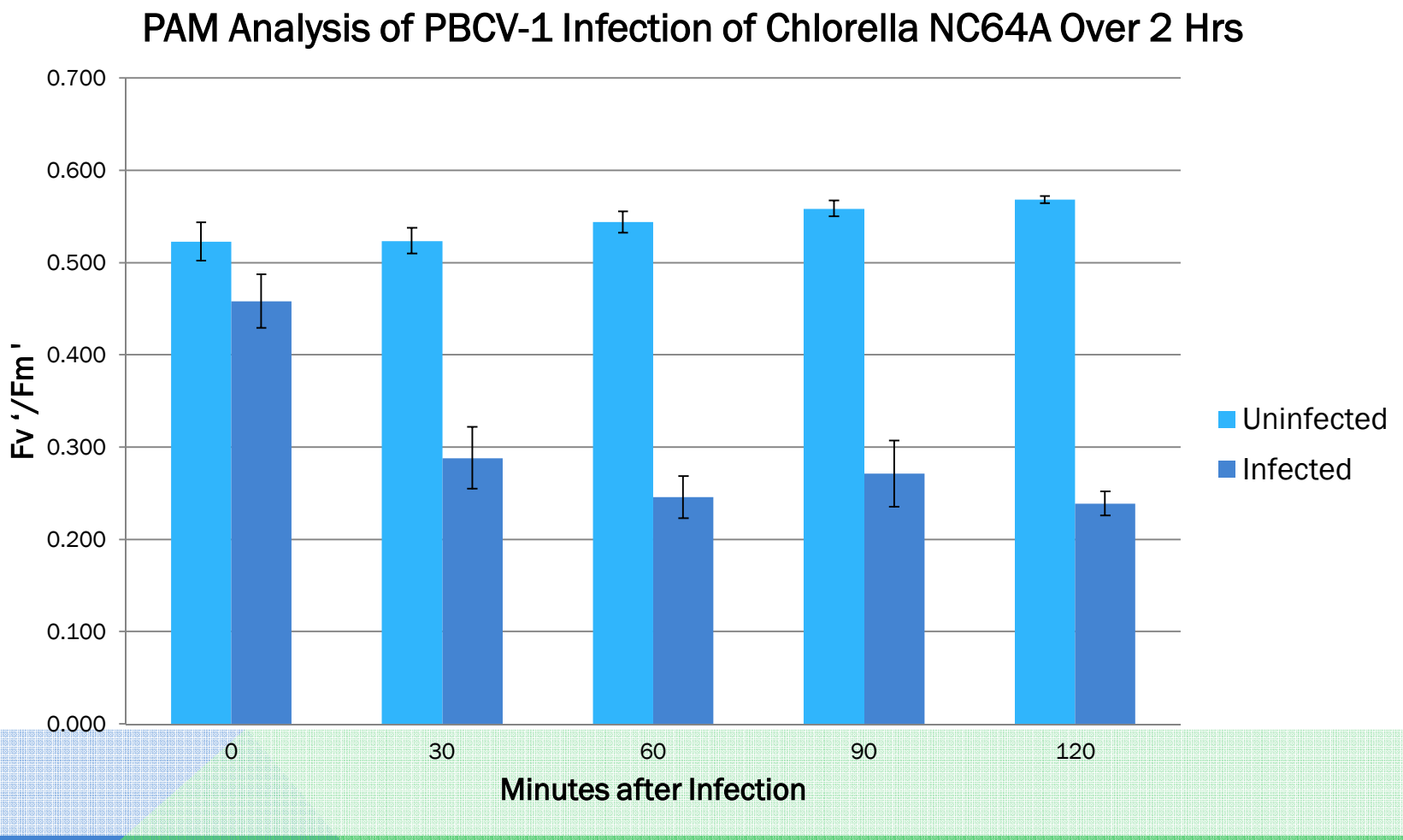


Image used for particle analysis
Side length = 80um

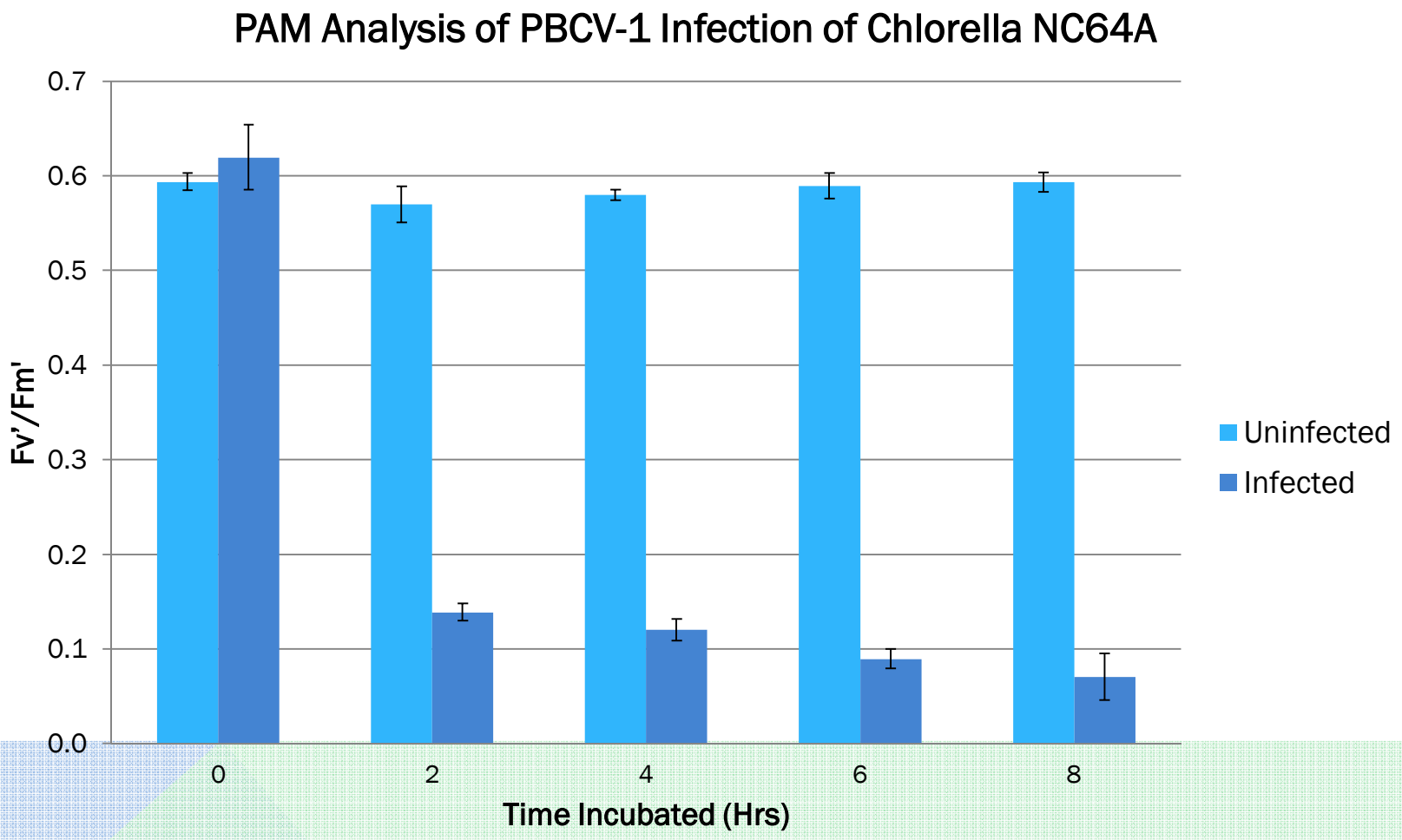


- Utilized imageJ's particle analysis capabilities to gather large amounts of data on chloroplast size.
- Did not find evidence of chloroplast shrinkage over course of infection.

PAM RESULTS


PAM Analysis of PBCV-1 Infection of Chlorella NC64A Over 2 Hrs
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Fv '/Fm'
0
30
60
90
120
Minutes after Infection
Uninfected
Infected

PAM RESULTS CONT.



CONCLUSION

- Morphological changes in cell and chloroplast proved difficult to detect due to large sample variation
- PAM can detect reduced photosynthetic activity due to viral infection in <30 minutes after inoculation with MOI=1

REFERENCES

- MacAllister ED, Myers J. 1940. The time course of photosynthesis and fluorescence observed simultaneously. *Smithson. Inst. Misc. Collect.* 99:1–37
- Baker. 2008. Chlorophyll Fluorescece: A Probe of Photosynthesis In Vivo