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# Identification of the locale of tolyporphins, unusual tetrapyrroles in a microbial photosynthetic community

Meghan C. Barnhart-Dailey[1*], Yunlong Zhang[2*], Ran Zhang[2], Eric S. Miller[3], Philip G. Williams[4], Jonathan S. Lindsey[2], and Jerilyn A. Timlin[1]

[1] Sandia National Laboratories, Albuquerque, NM; [2] Department of Chemistry, North Carolina State University, Raleigh, North Carolina ; [3] Department of Plant and Microbial Biology, North Carolina State University, Raleigh, North Carolina ; [4]Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii

*Authors contributed equally to this work

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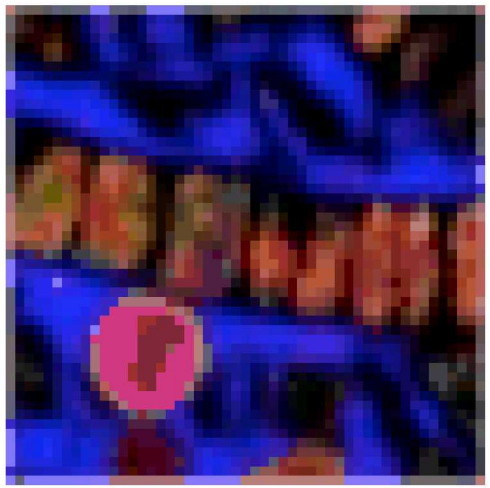
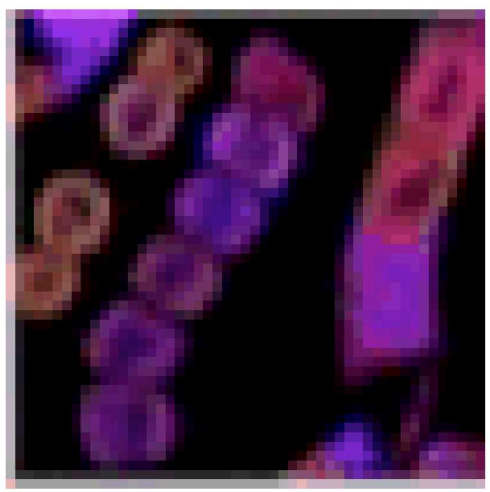
SAND No. 201X-XXXX.

# Outline

- Introduction:
  - Why study tolyporphins and the organism that produces them?
- Question:
  - *Where* is tolyporphin A localized within the cyanobacterial *Tolypothrix nodosa* strain HT-58-2?
- Methods to answer this question:
  - Hyperspectral confocal fluorescence microscopy
- Results:
  - 30 vs. 60 days in culture
  - Effect of nitrate in the media on tolyporphin A quantities
  - Dark adaptation





Scientific Achievement

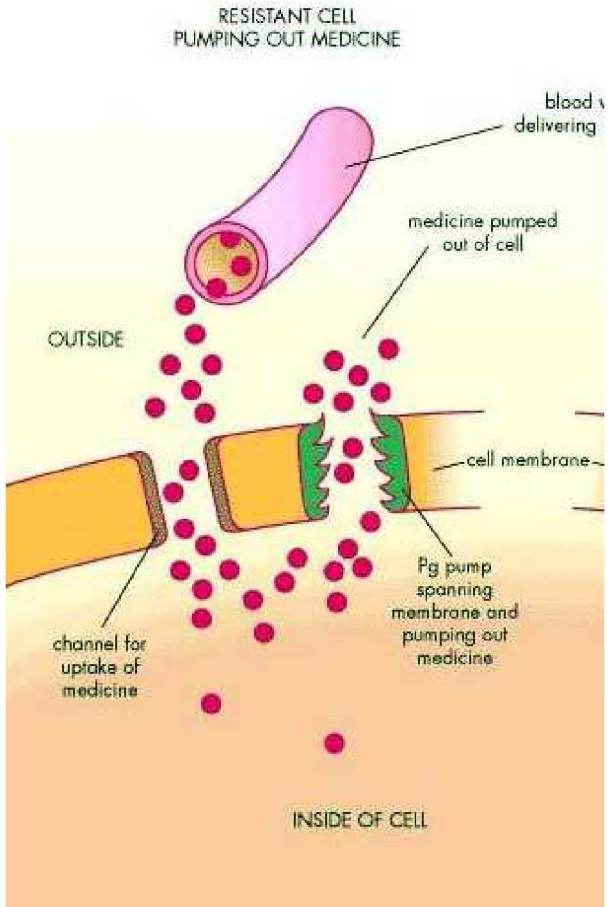
Determined tolyporphin locale and abundance in intact, living HT-58-2 cells.

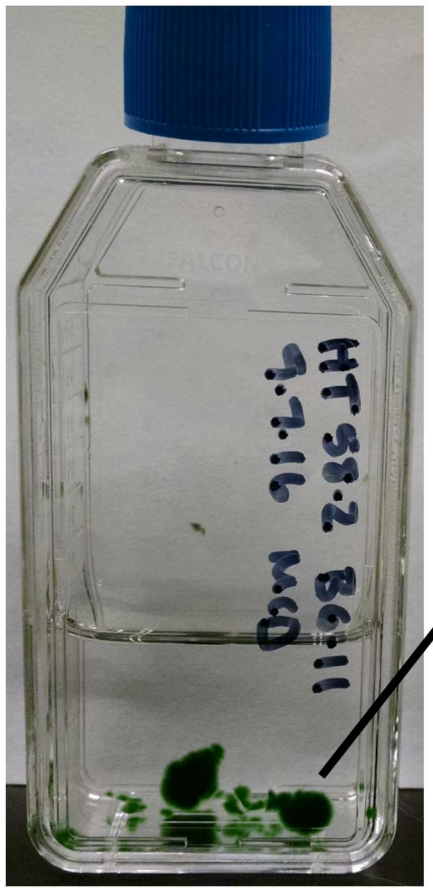
Significance and Impact

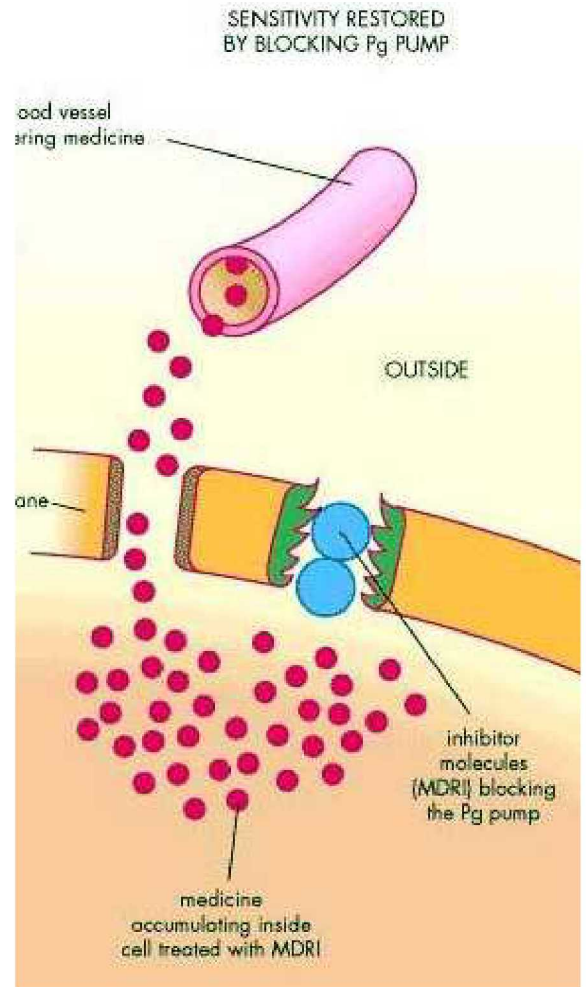
Tolyporphins are spectrally similar to chlorophylls and the location of tolyporphins in cells could not be independently assessed *in vivo* prior to this work.

# Cyanobacteria and Treating Multi-Drug Resistance in Cancer?

Originally collected as part of a screen done by the University of Hawaii in a search for cyanobacteria strains that produce bioactive compounds.

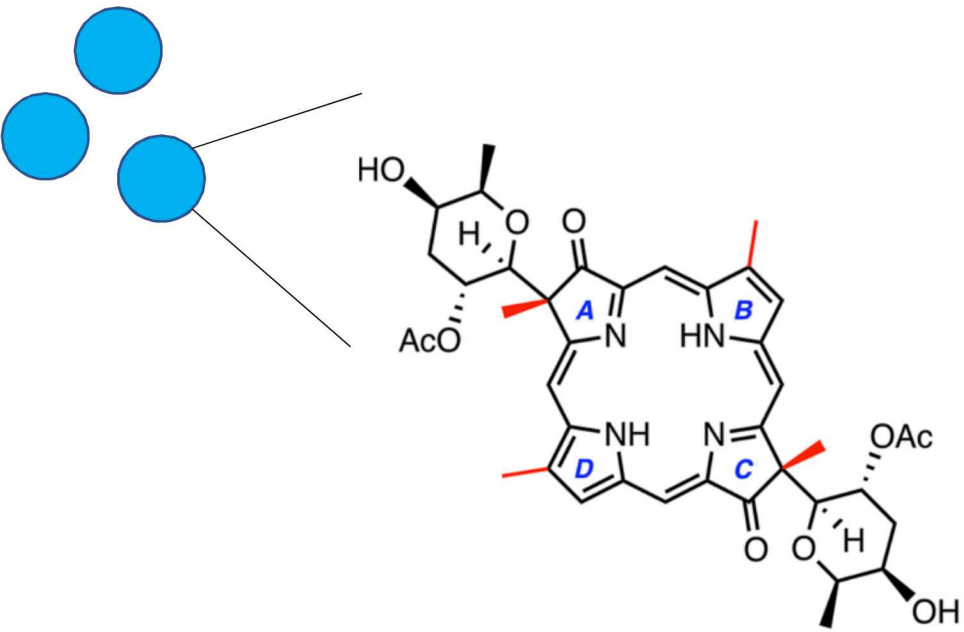


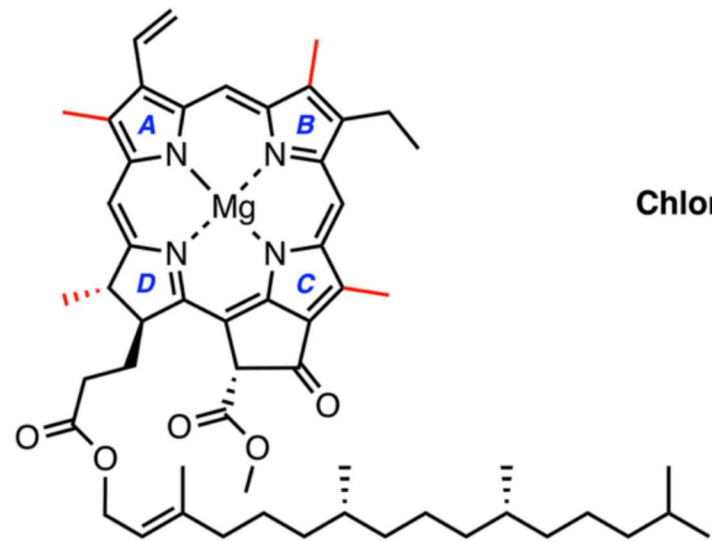



Chakraborty, 2013

# Tetrapyrrole macrocycles




**Tolyporphin A**

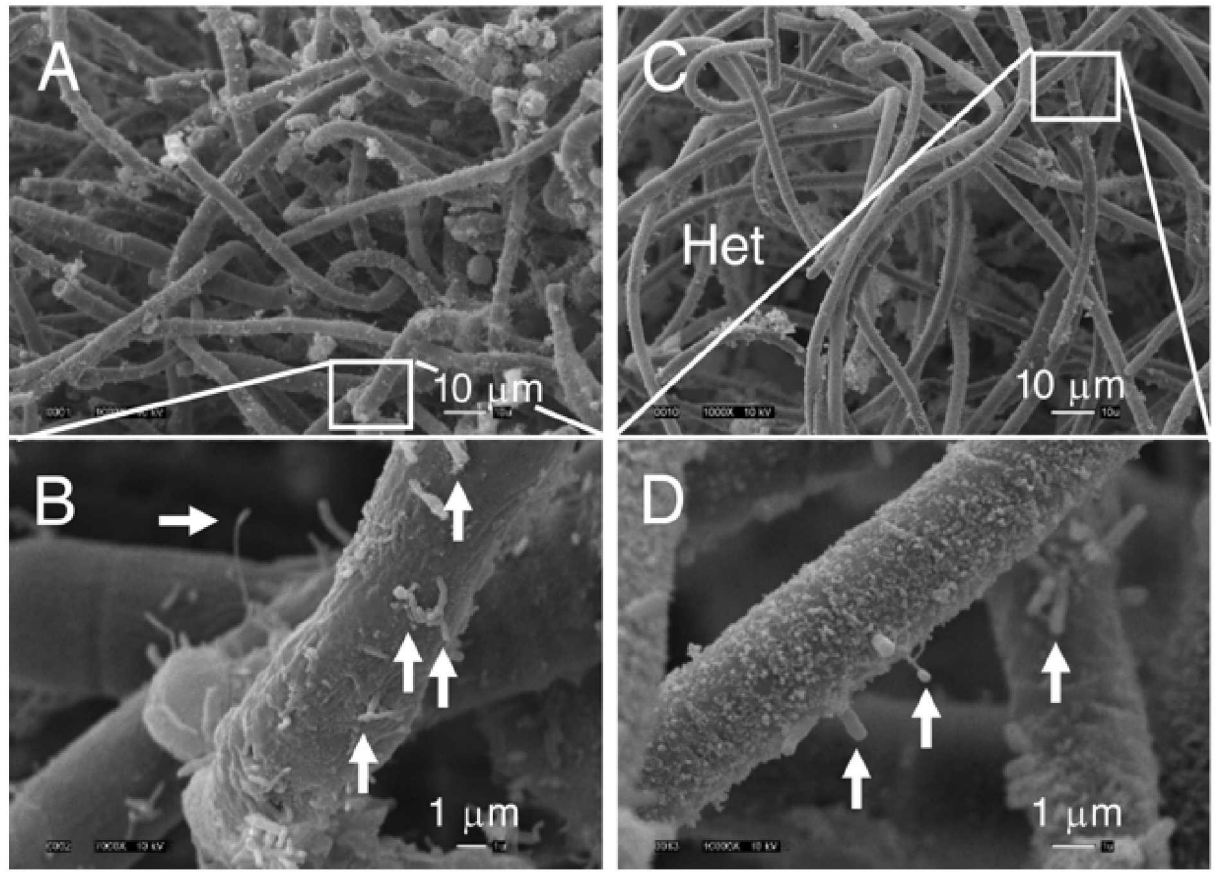



**Chlorophyll *a***

Lipophilic extract of *Tolypothrix nodosa* reverses MDR in a vinblastine-resistant human ovarian adenocarcinoma cell line

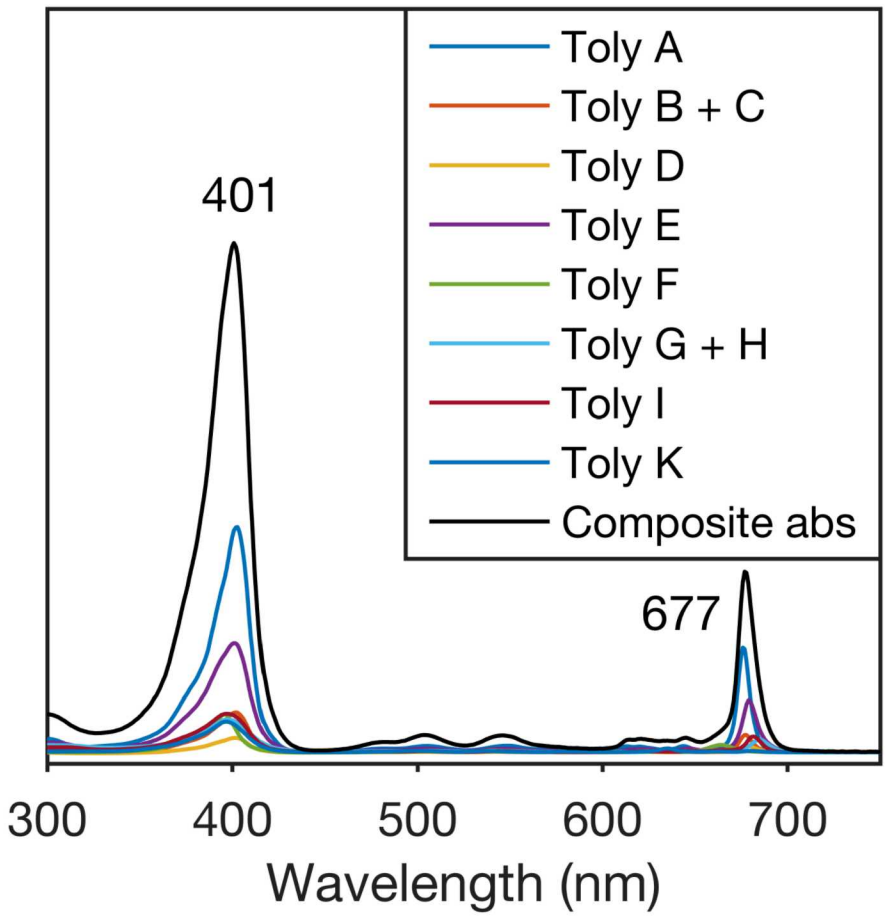
Via P-glycoprotein inhibition by Tolyporphin A

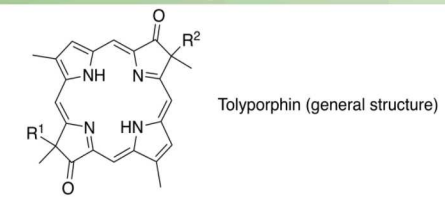
# HT-58-2 cyanobacteria and their community

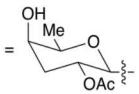
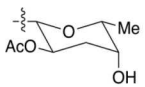
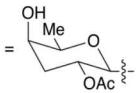
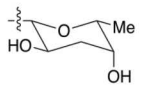
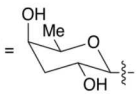
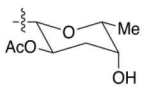
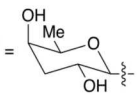
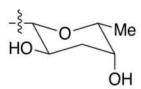
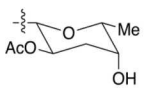
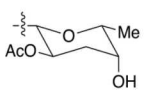
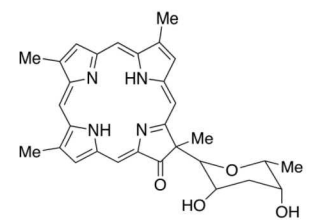



Hughes, et. al 2017

# Absorption spectra of tolyporphins A-K

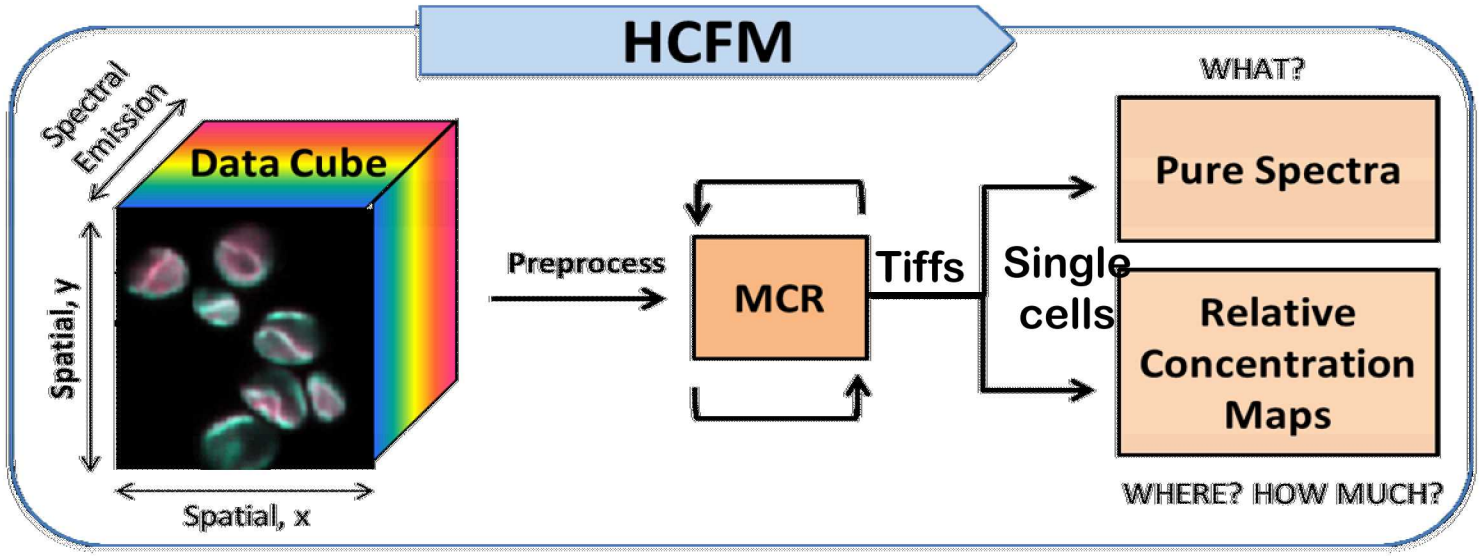


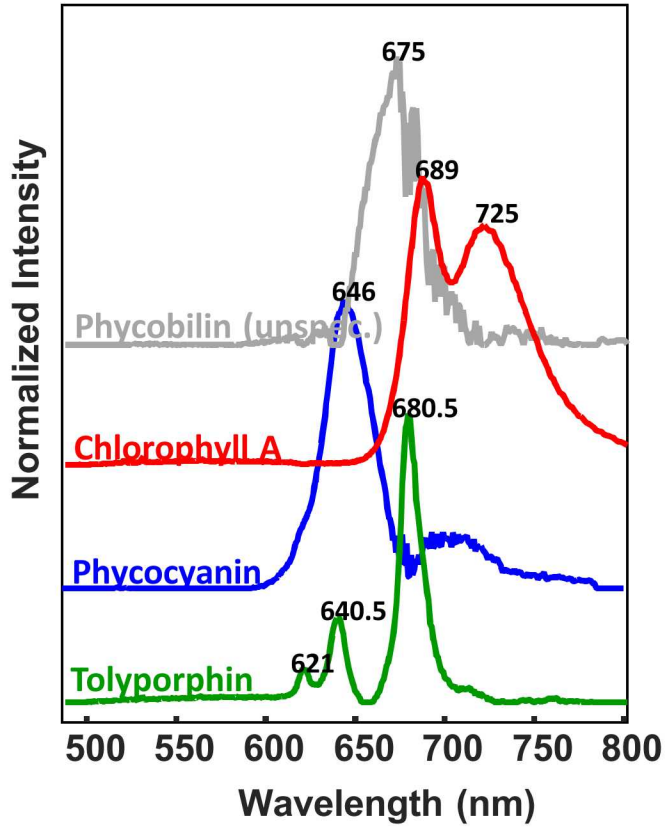

| | | |
|---|---|---|
| A | $R^1$ =  | $R^2$ =  |
| B | $R^1$ =  | $R^2$ =  |
| C | $R^1$ =  | $R^2$ =  |
| D | $R^1$ =  | $R^2$ =  |
| E | $R^1$ = AcO– | $R^2$ =  |
| F | $R^1$ = HO– | $R^2$ =  |
| G | $R^1$ = AcO– | $R^2$ = –OH |
| H | $R^1$ = HO– | $R^2$ = –OAc |
| I | $R^1$ = AcO– | $R^2$ = –OAc |
| J | $R^1$ = HO– | $R^2$ = –OH |
| K |  | |

# Sandia's Hyperspectral Confocal Fluorescence Microscopy Workflow

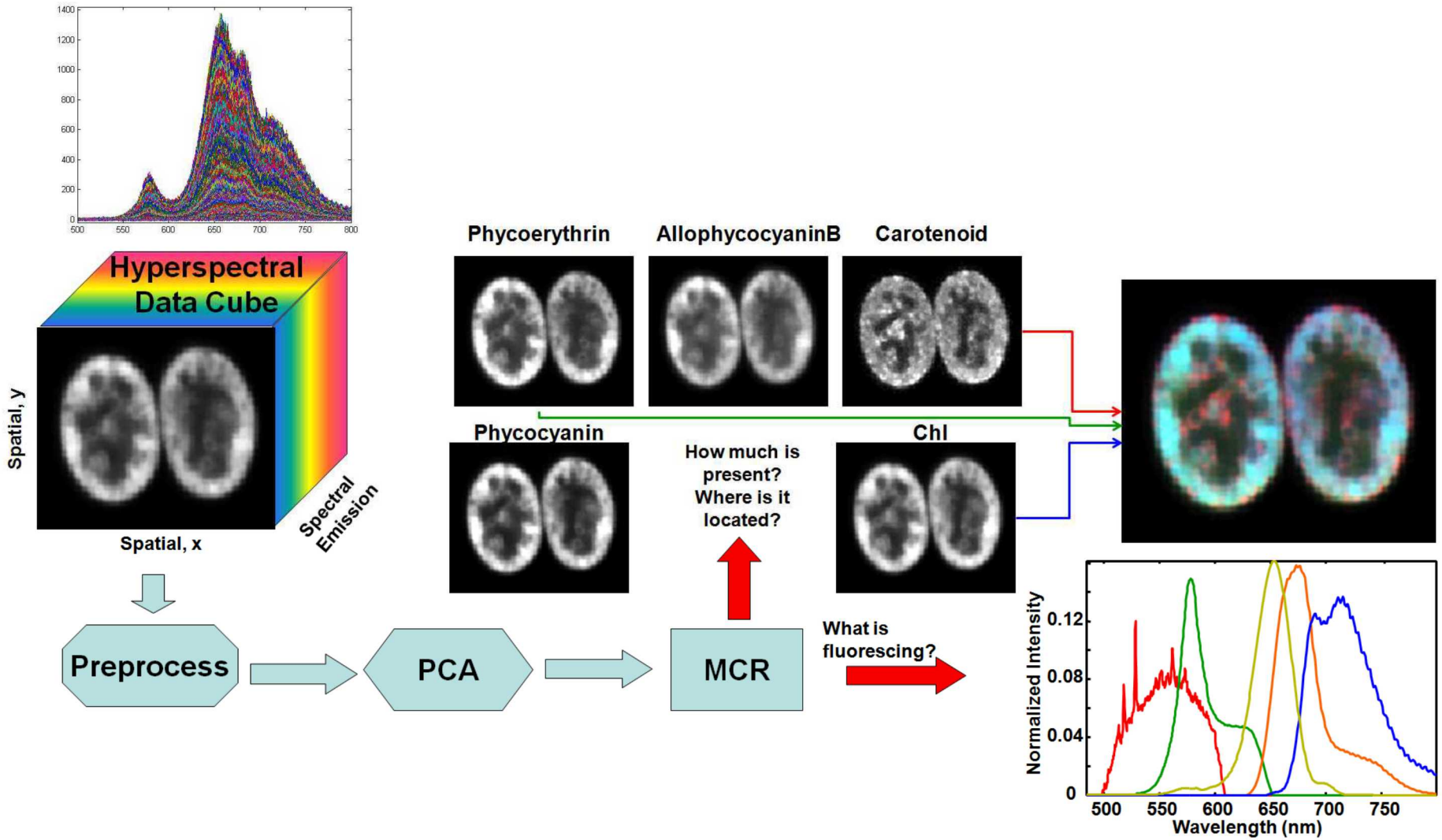
- *Identity of pigments*
- *Location, relative abundance*
- *Spatial relationship*
- *Single cell statistics*

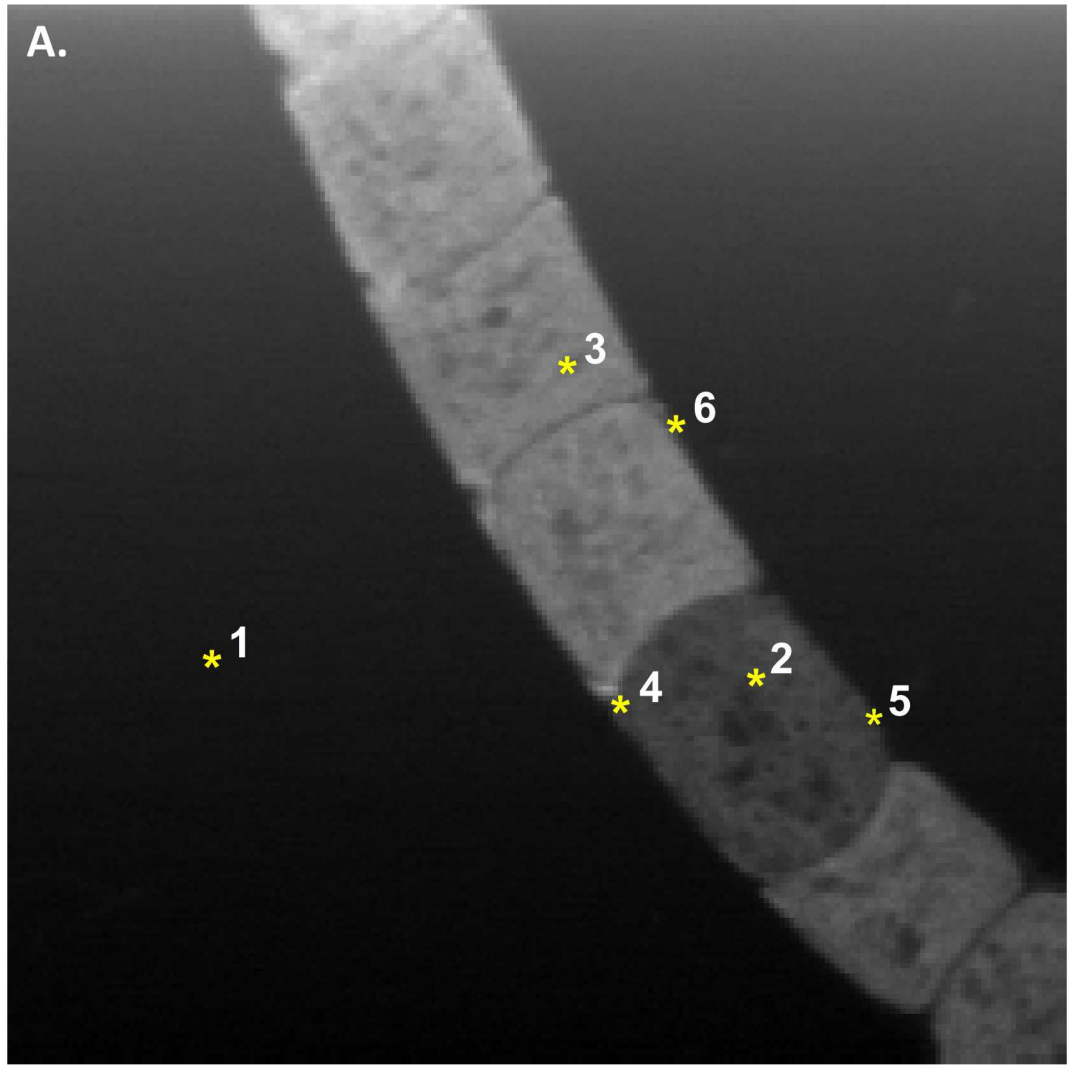



**Hyperspectral Confocal Fluorescence Microscopy (HCFM):** Also known as spectral imaging. Excites sample with a 488 nm laser and collectes an entire emission spectrum at each image voxel.



# Multivariate Image Analysis Example





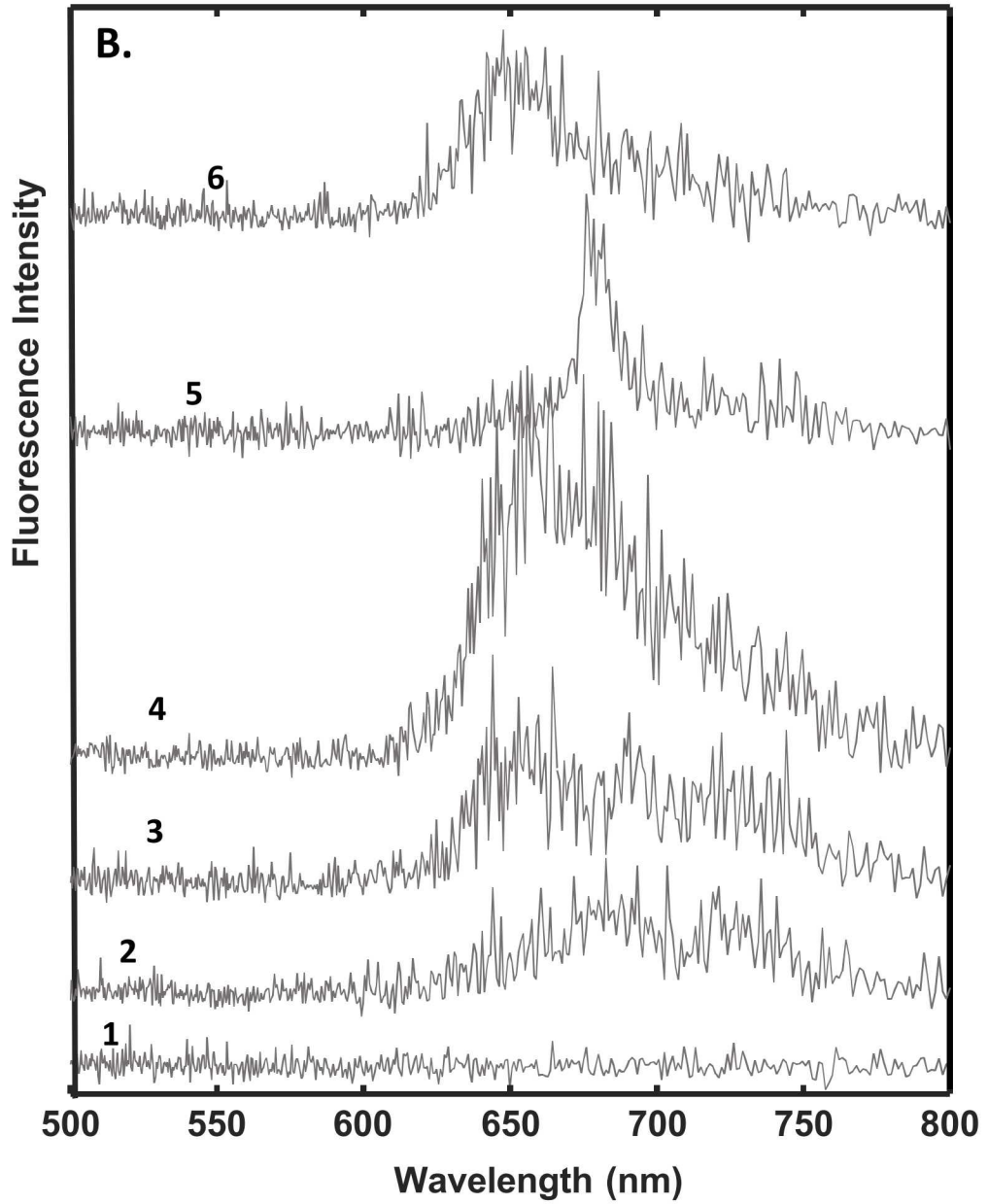
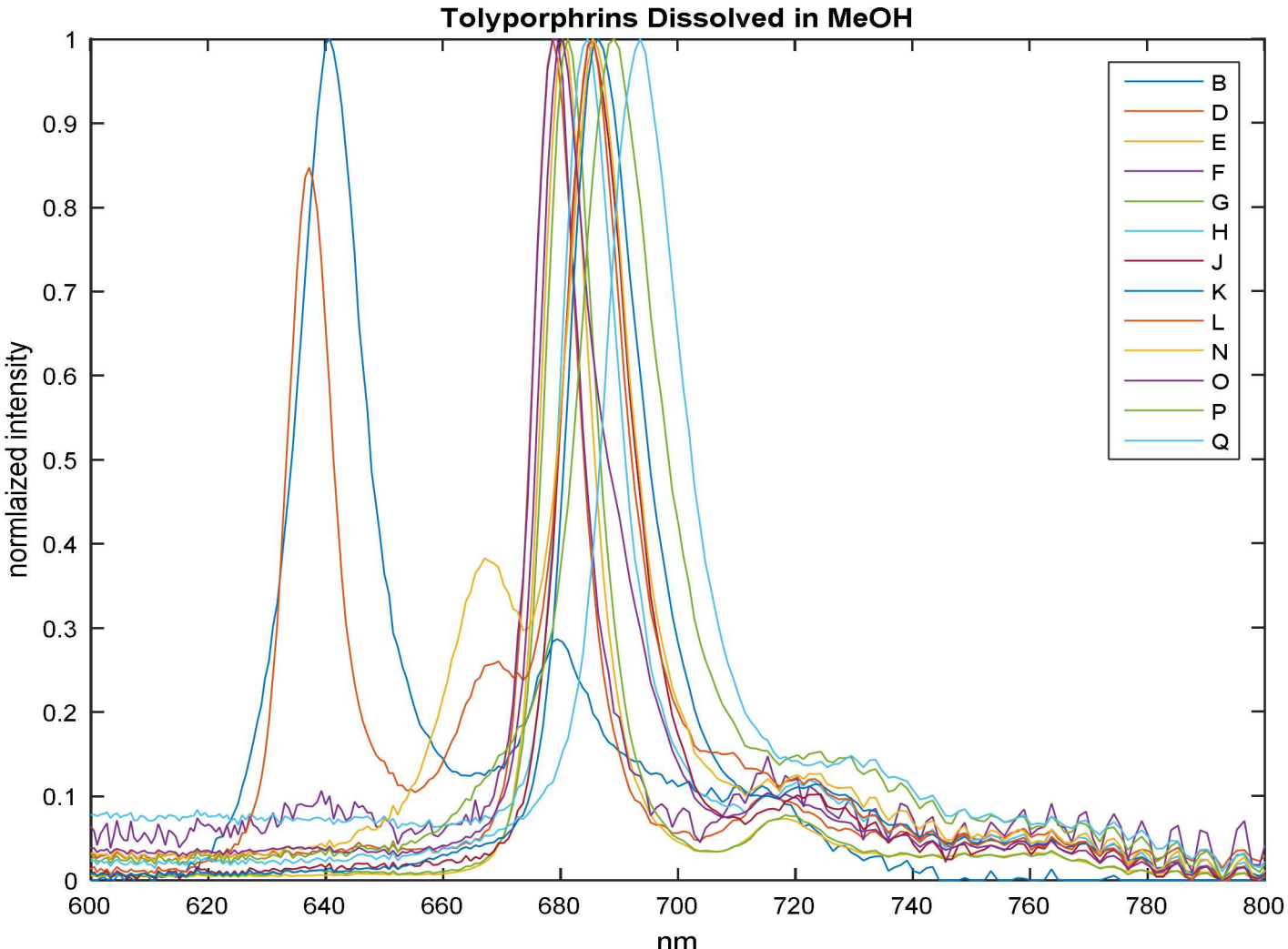




Figure 1: Raw HCFM data from HT-58-2 grown in BG-11o for 29 days, highlighting spectral differences between cyanobacteria filament and bacteria. A. Grayscale image created by summing intensities from 500-800 nm. B. Fluorescence emission spectra from corresponding pixels labeled with stars in A.

# Spectra From Purified/Isolated Tolyporphins




From these fractions A, C, I, M and R were too weakly fluorescent for reliable analysis

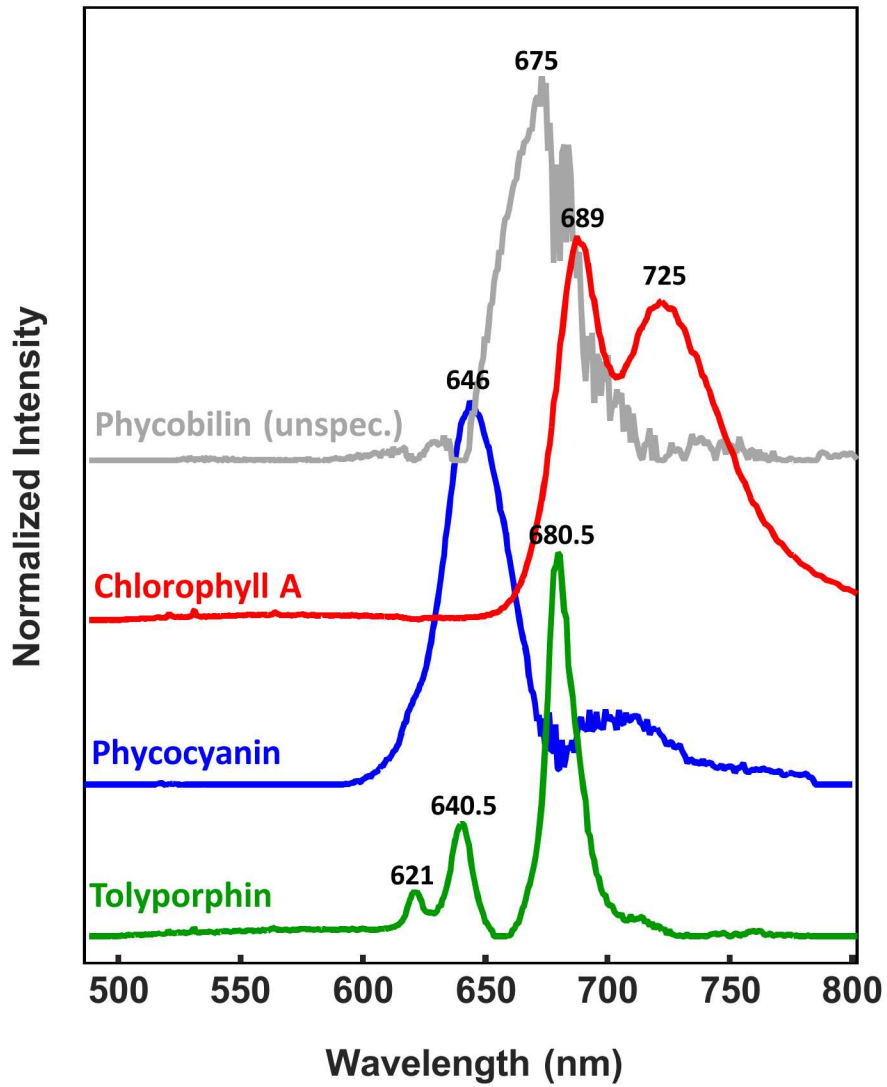



Figure 2: MCR extracted spectral components from HCFM data of HT-58-2 cultures grown in BG-11 and BG-11o. An additional linear offset was used in the model (not shown).

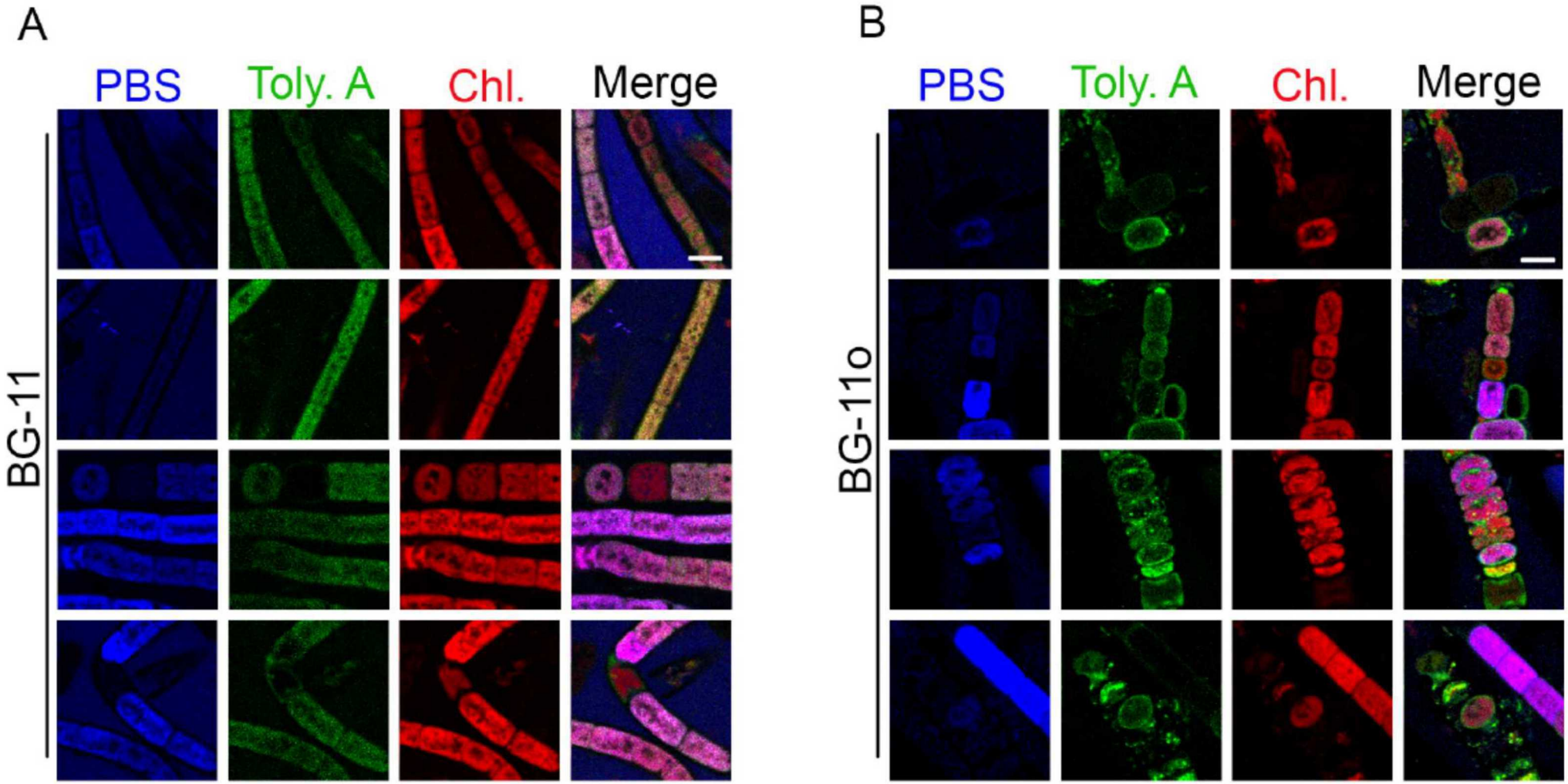
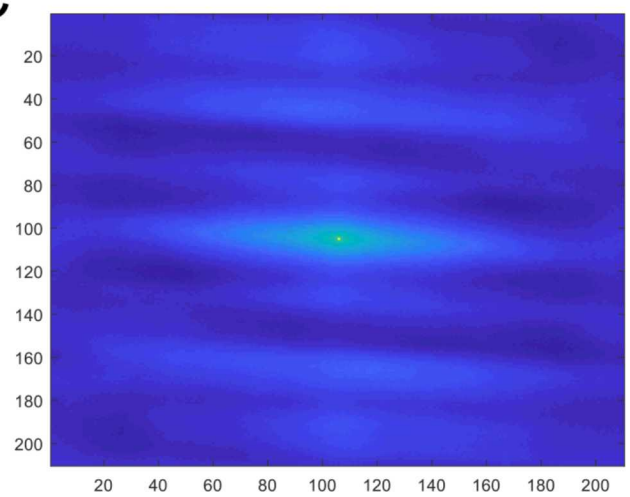
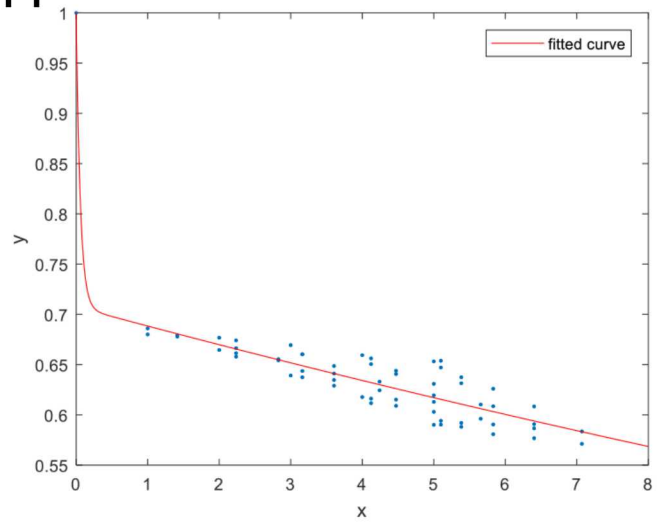



Figure 3. Representative MCR concentration maps of 30 day HT-58-2 cultures. The three main components (PBS - phycobilisomes, Toly A - tolyporphin A, and Chl - chlorophyll) from the spectral model are displayed. The scale bar represents 5µm. A. HT-58-2 grown in BG-11 media. B. HT-58-2 cultures grown in BG-11o media. Image intensities in A&B are scaled equally. The scale bar represents 5µm

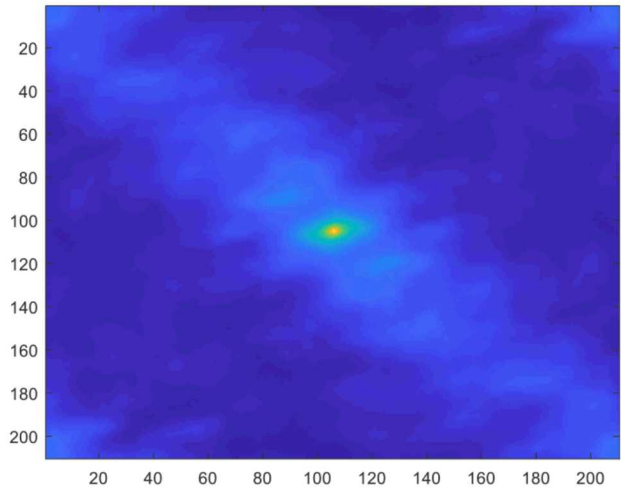
C



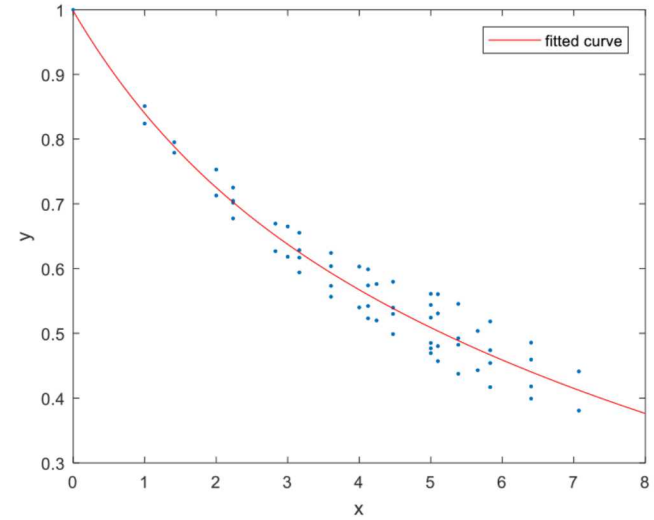
BG-11




D



BG-11o




E

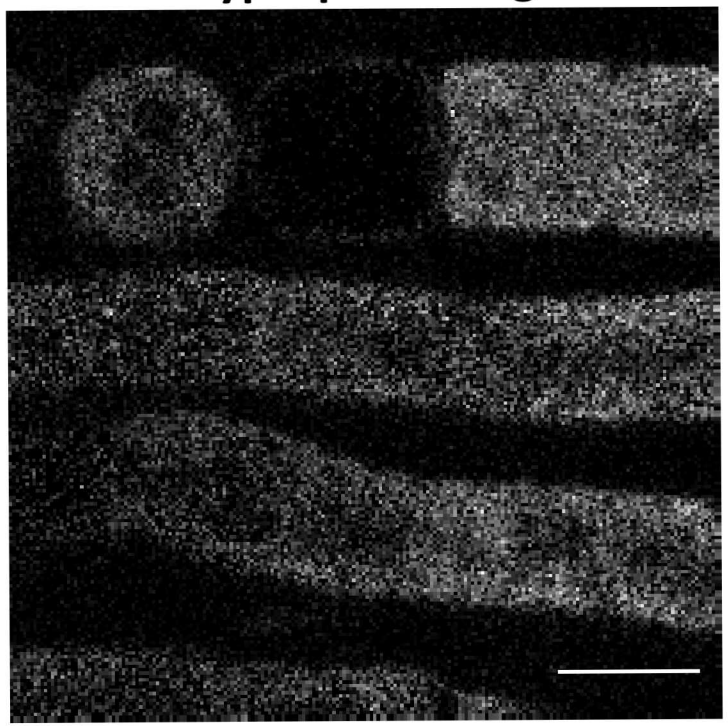
Graph of tolyporphin A intensities surrounding Chlorophyll signal in BG-11 vs BG-11o 30 days images.

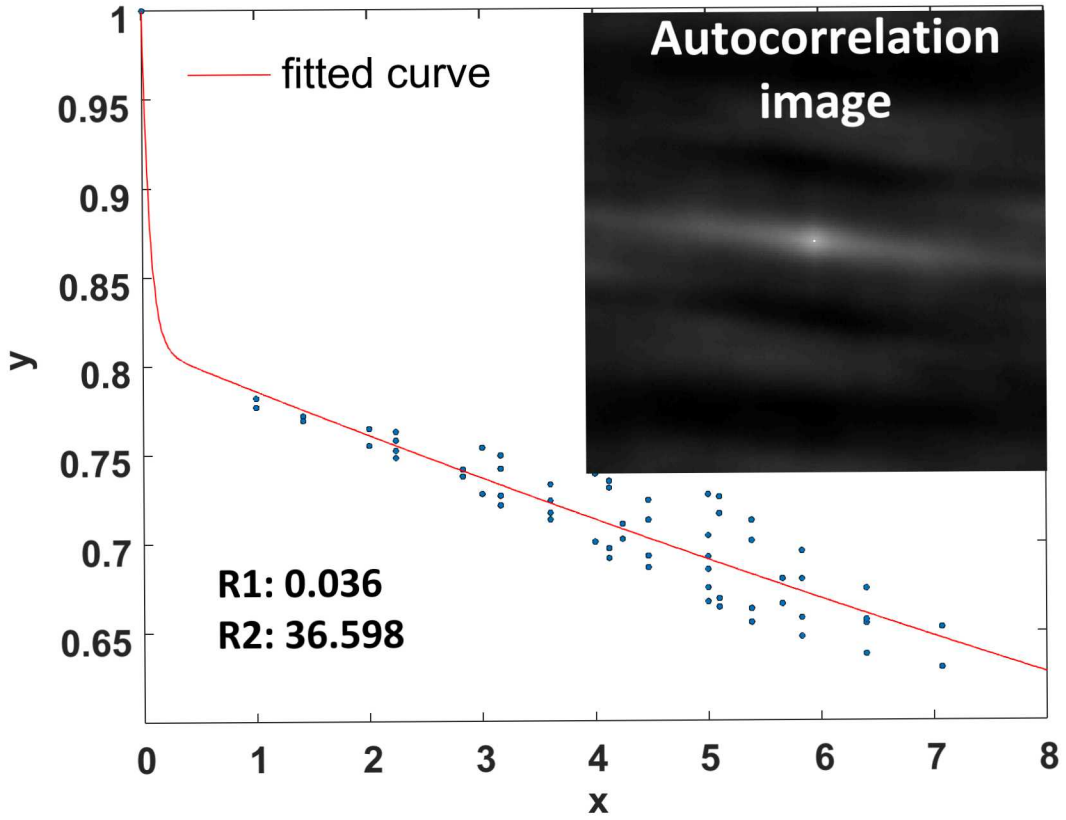
Progress: Chlorophyll masks are made, now need to measure tolyporphin in surrounding 5 pixels of that mask and subtract out the intensities that overlap with chlorophyll

Figure 3 continued. C. FFT analysis of tolyporphin A signal in BG-11 cells at 30 days. D. FFT analysis of tolyporphin A signal in BG-11o cells at 30 days. E. Tolyporphin A intensity surrounding chlorophyll mask in BG-11 vs. BG-11o cells at 30 days.

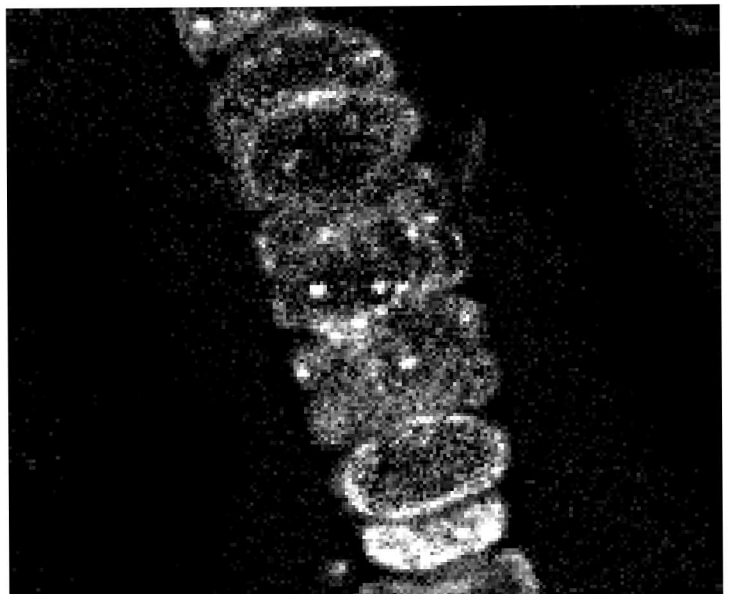
Tetraporphin image

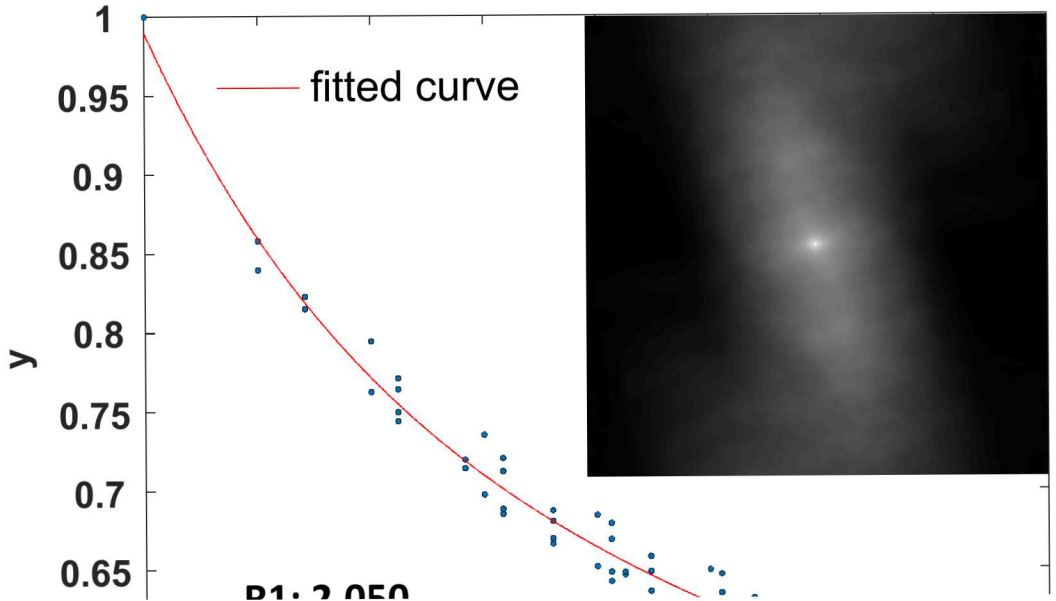
BG11



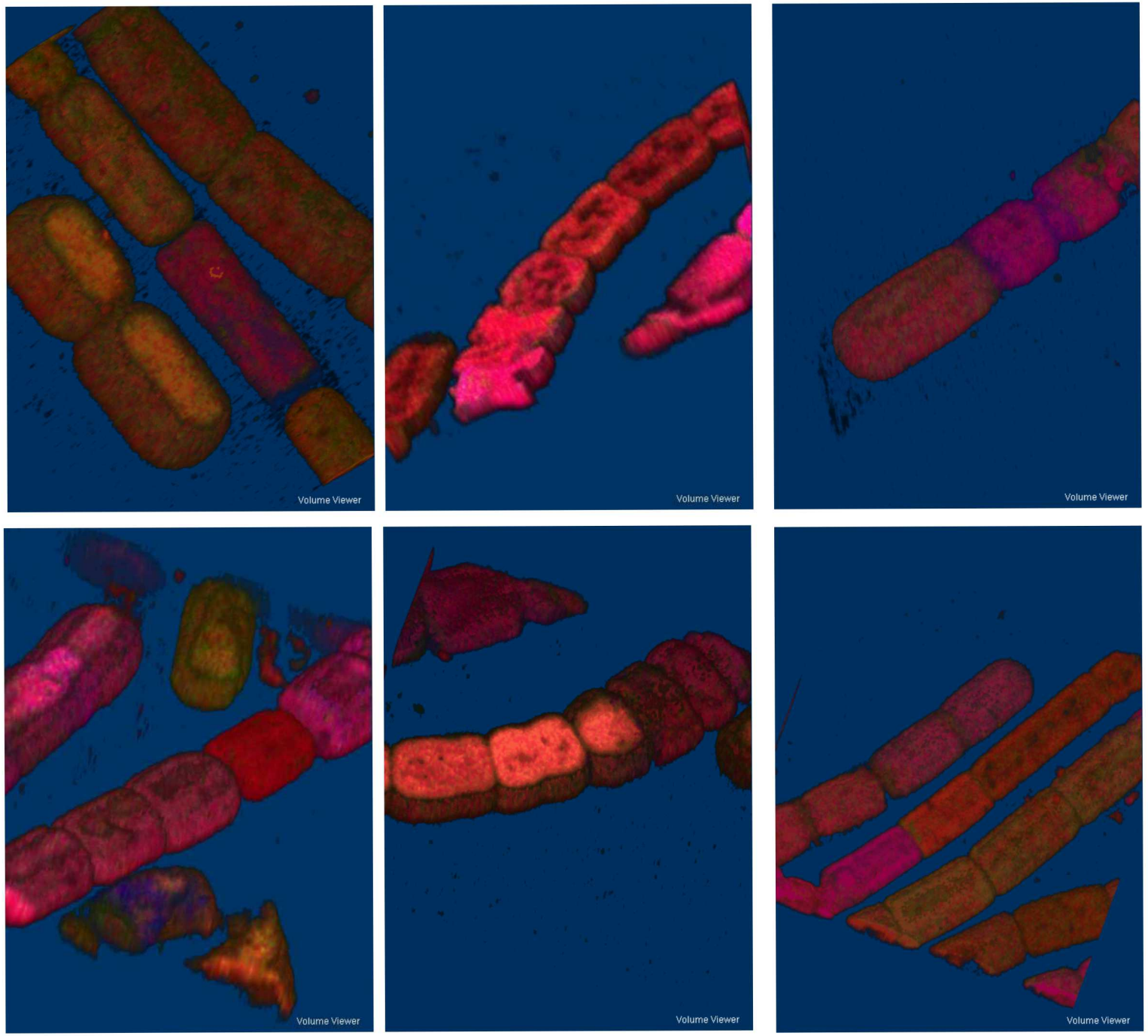



BG11o

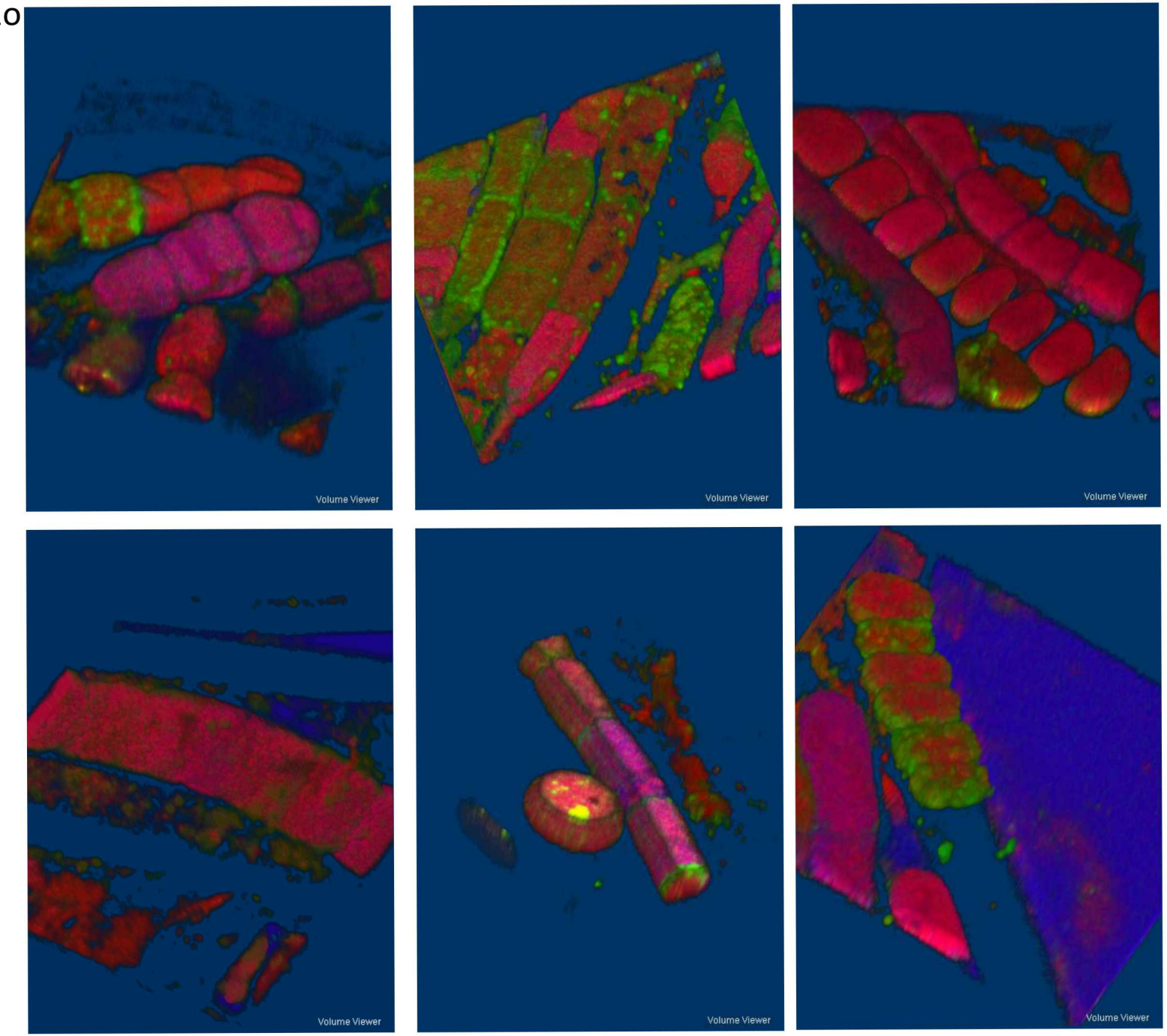




31 Day BG11


Volume Viewer
Volume Viewer
Volume Viewer
Volume Viewer
Volume Viewer
Volume Viewer

31 Day BG11o


Volume Viewer
Volume Viewer
Volume Viewer
Volume Viewer
Volume Viewer
Volume Viewer

Quantitative Analysis

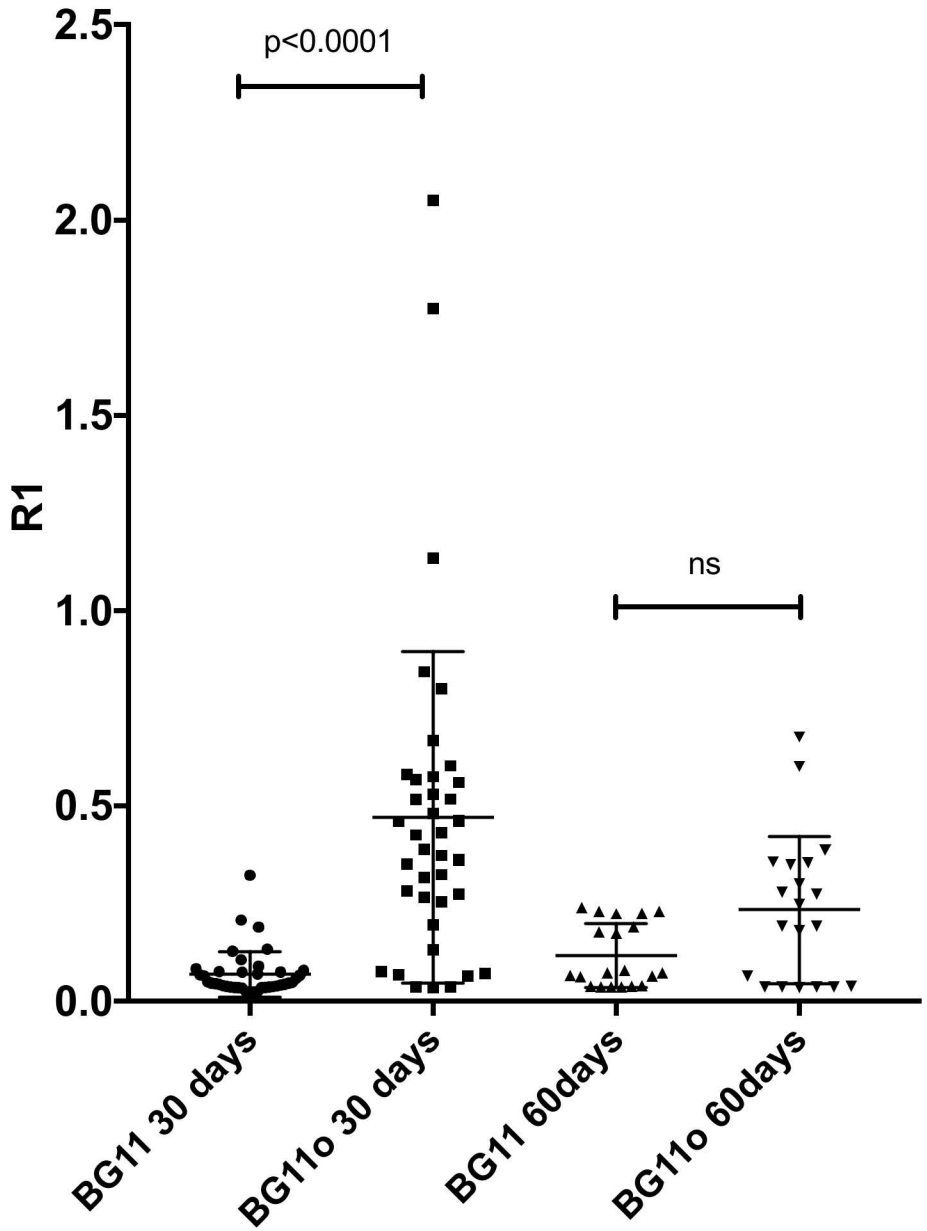
Attempted to use a autocorrelation method to perform texture analysis

- Perform a FFT analysis of the tolyp image and then fit the 2D autocorrelation function
- If the texture is coarse, then the autocorrelation function will drop off slowly
- Otherwise, it will drop off very rapidly.
- For regular textures or regularly spaced features, the autocorrelation function will exhibit periodic peaks and valleys
- We get essentially two terms a “fast” and a “slow”
- Believe the “slow” corresponds to the cell size/spacing.
- The fast one corresponds to the texture within the cells.
- Distinct differences between images in BG11 and BG11o

Averages over ALL Images

| | | |
|---|---|---|
| BG11 | 0.069+/- 0.06 | 35.57 |
| BG11o | 0.469 +/-0.42 | 19.26 |

| | | R1 | R2 |
|---|---|---|---|
| 30 days | BG11 | 0.062 ± 0.042 | 35.844 ± 11.991 |
| | BG11o | 0.471 ± 0.424 | 19.260 ± 7.496 |
| 60 days | BG11 | 0.117 ± 0.082 | 25.721 ± 7.968 |
| | BG11o | 0.234 ± 0.188 | 18.995 ± 6.025 |

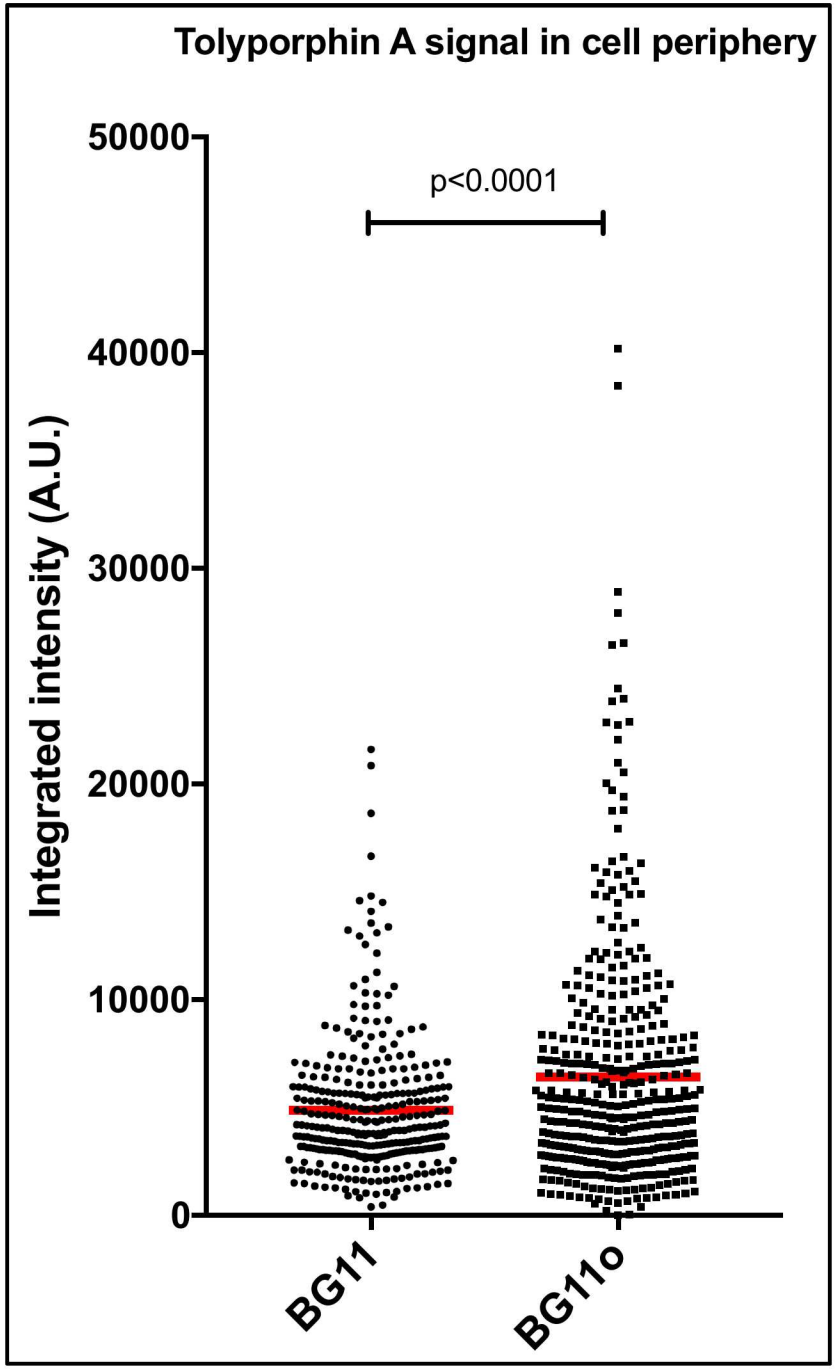


## Analysis steps:

1. CellFinder to segregate single cells using Chlorophyll signal.
2. Enlarge that chlorophyll mask by 5 pixels around the periphery of the cells (>>expandCells)
3. Use that expanded mask to measure tolyporphin A signal around the periphery of the chlorophyll signal (>>switchChannel)
4. Subtract tolyporphin A signal that overlaps with chlorophyll to yield signal around the periphery of the chlorophyll signal.

5. T test used to generate p<0.0001. I used this despite data being non-normally distributed because I had >300 values per condition (needed at least 15 to make t test valid due to normality assumptions used in this test, which are mostly influential with small data sets).

6. If run a nonparametric test, p=0.01 so still significant but less so. Nonparametric test always have less statistical power.



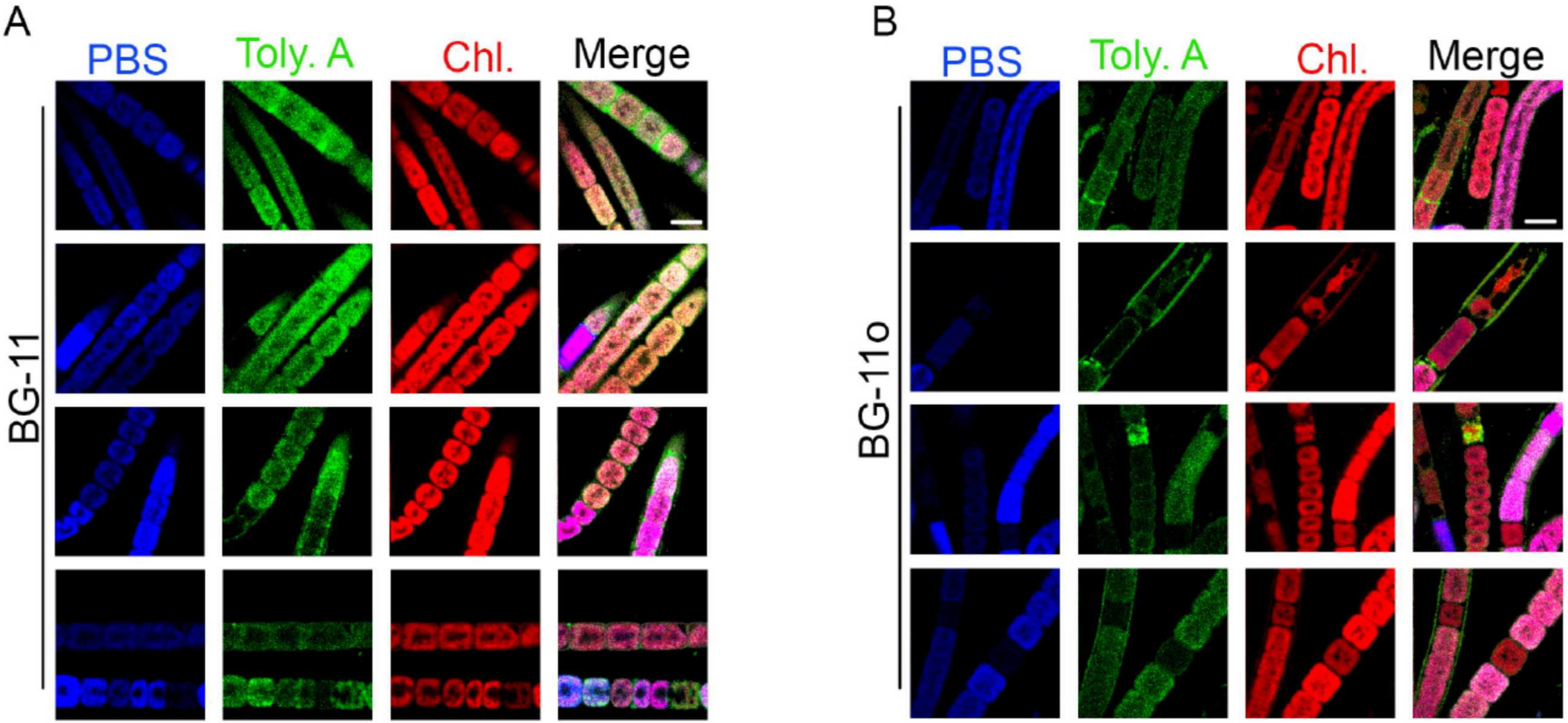



Figure 4. Representative MCR concentration maps of 60 day HT-58-2 cultures. The three main components (PBS - phycobilisomes, Toly. A - tolyporphin A, and Chi. - chlorophyll) from the spectral model are displayed. The scale bar represents 5µm. A. HT-58-2 grown in BG-11 media. B. HT-58-2 cultures grown in BG-11o media. Image intensities in A&B are scaled equally. The scale bar represents 5µm

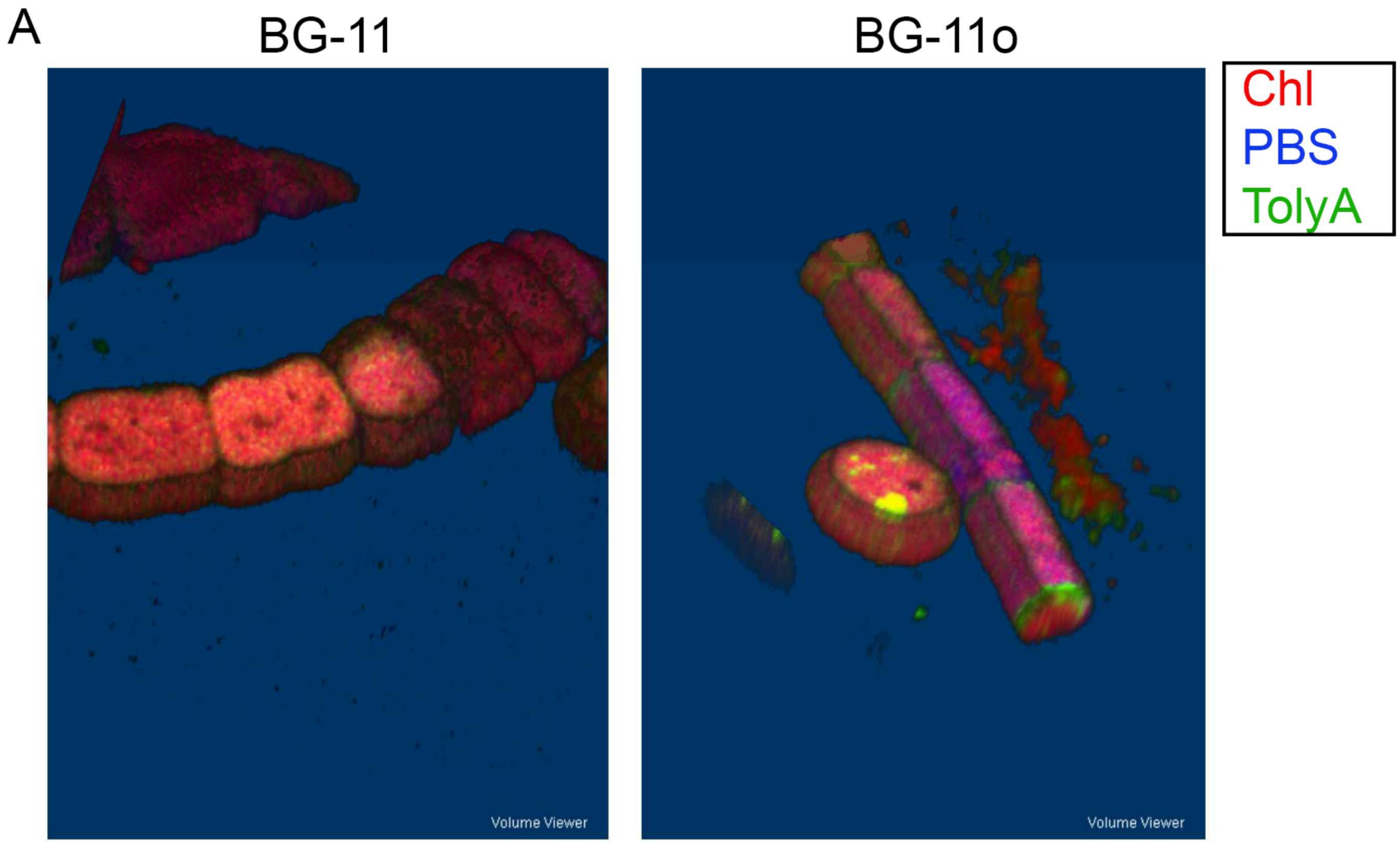



Figure 5: 3D volume renderings of HT-58-2 filaments grown in BG-11 or BG-11o media at 31 days.

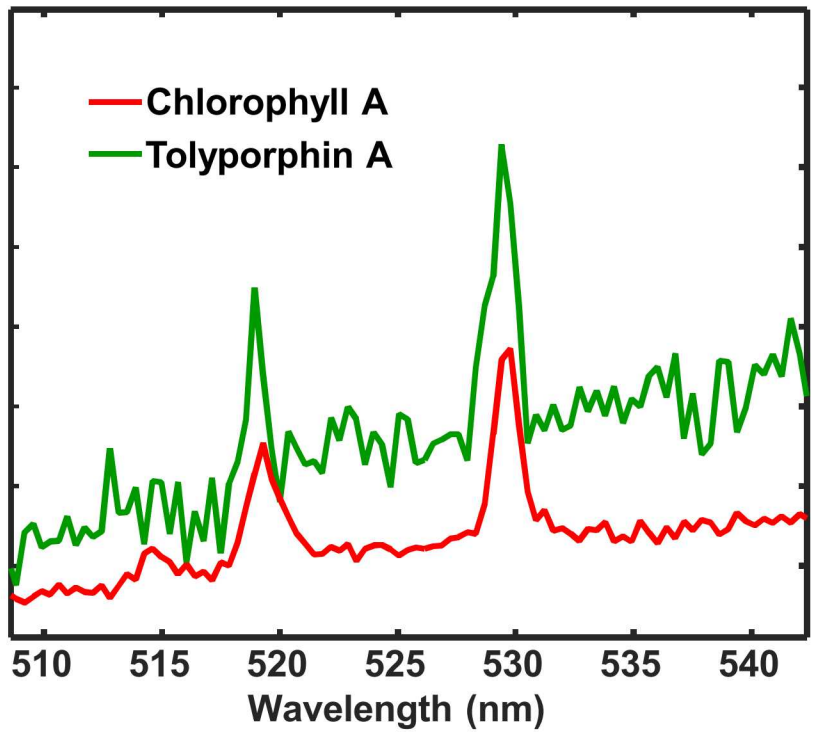



Figure S1: Zoom in of the spectral region 500- 542 nm shows evidence of carotenoid association with both chlorophyll A and tolyporphin pigments. Peaks shown are the resonance enhanced Raman vibrations (v1 and v2) of carotenoids.

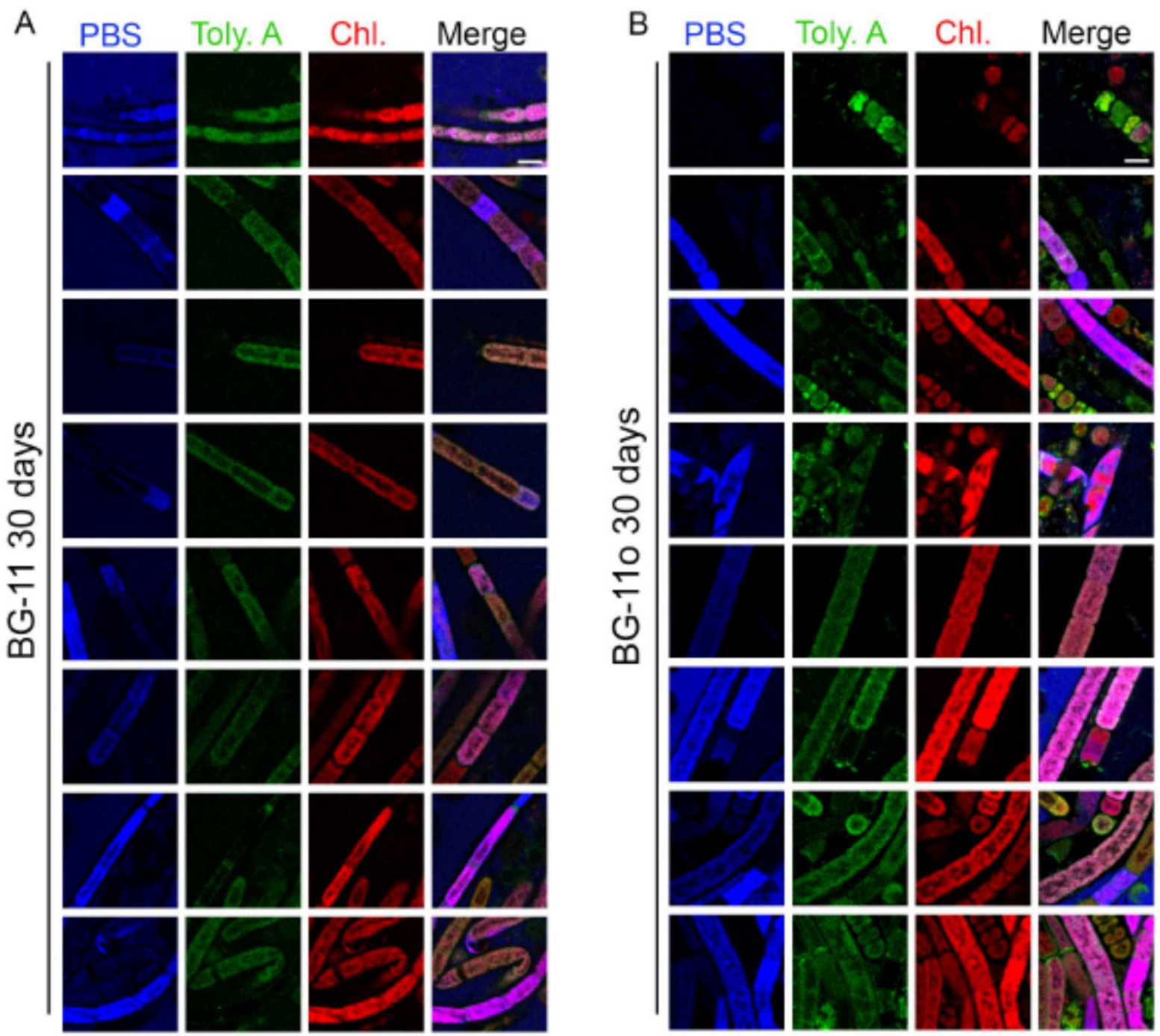



Figure S2.. Additional representative MCR concentrations maps of 30 day HT-58-2 cultures A. Cultures grown in BG-11 media. B. Cultures grown in BG-11o media. The three main components (PBS - phycobilisomes, Toly. A - tolyporphin A, and Chi. - chlorophyll) from the spectral model are displayed. Image intensities in A & B are scaled equally. The scale bar represents 5µm.

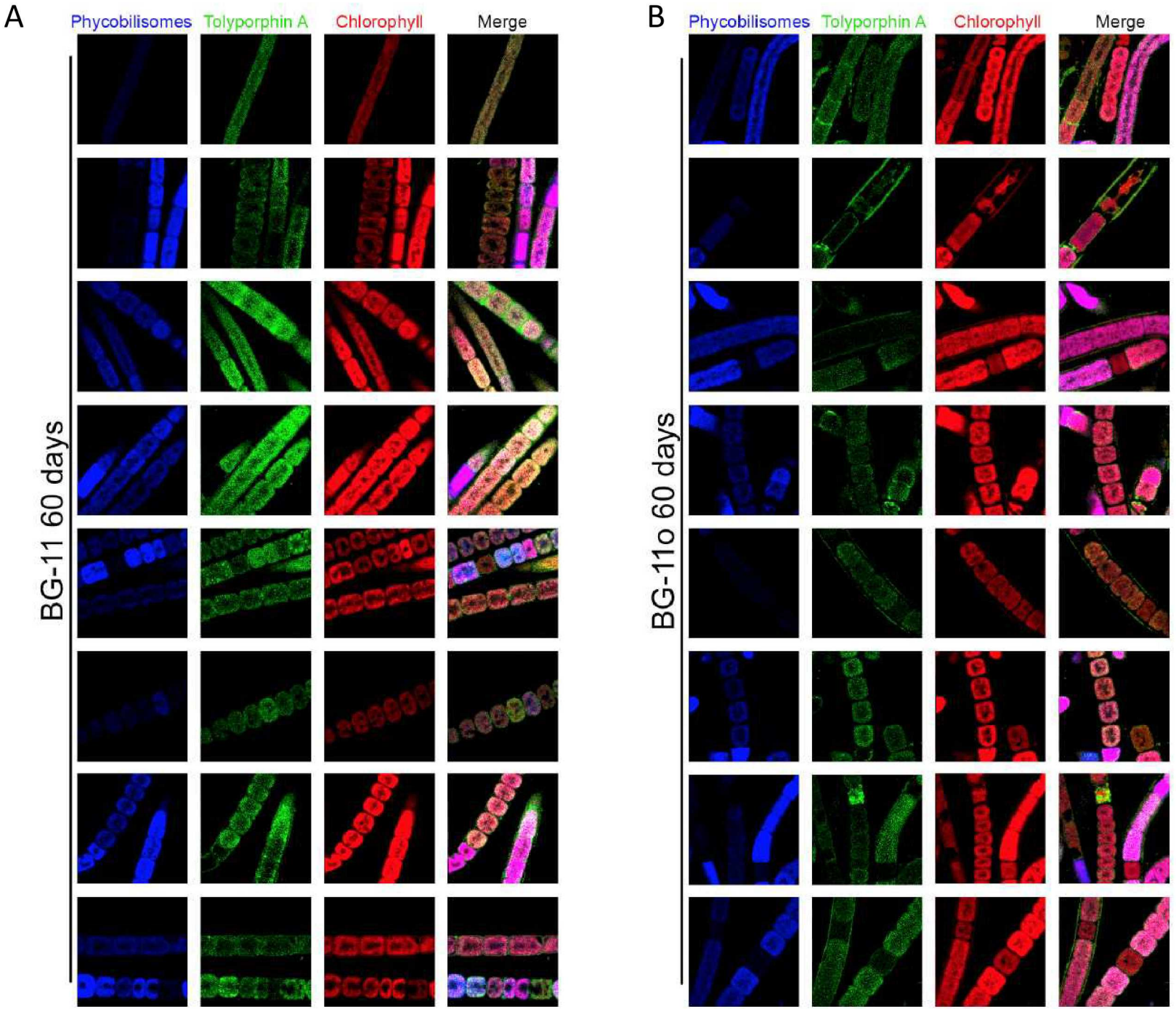



Figure S3.. Additional representative MCR concentrations maps of 60 day HT-58-2 cultures A. Cultures grown in BG-11 media. B. Cultures grown in BG-11o media. The three main components (PBS - phycobilisomes, Toly. A - tolyporphin A, and Chi. - chlorophyll) from the spectral model are displayed. Image intensities in A & B are scaled equally. The scale bar represents 5µm.

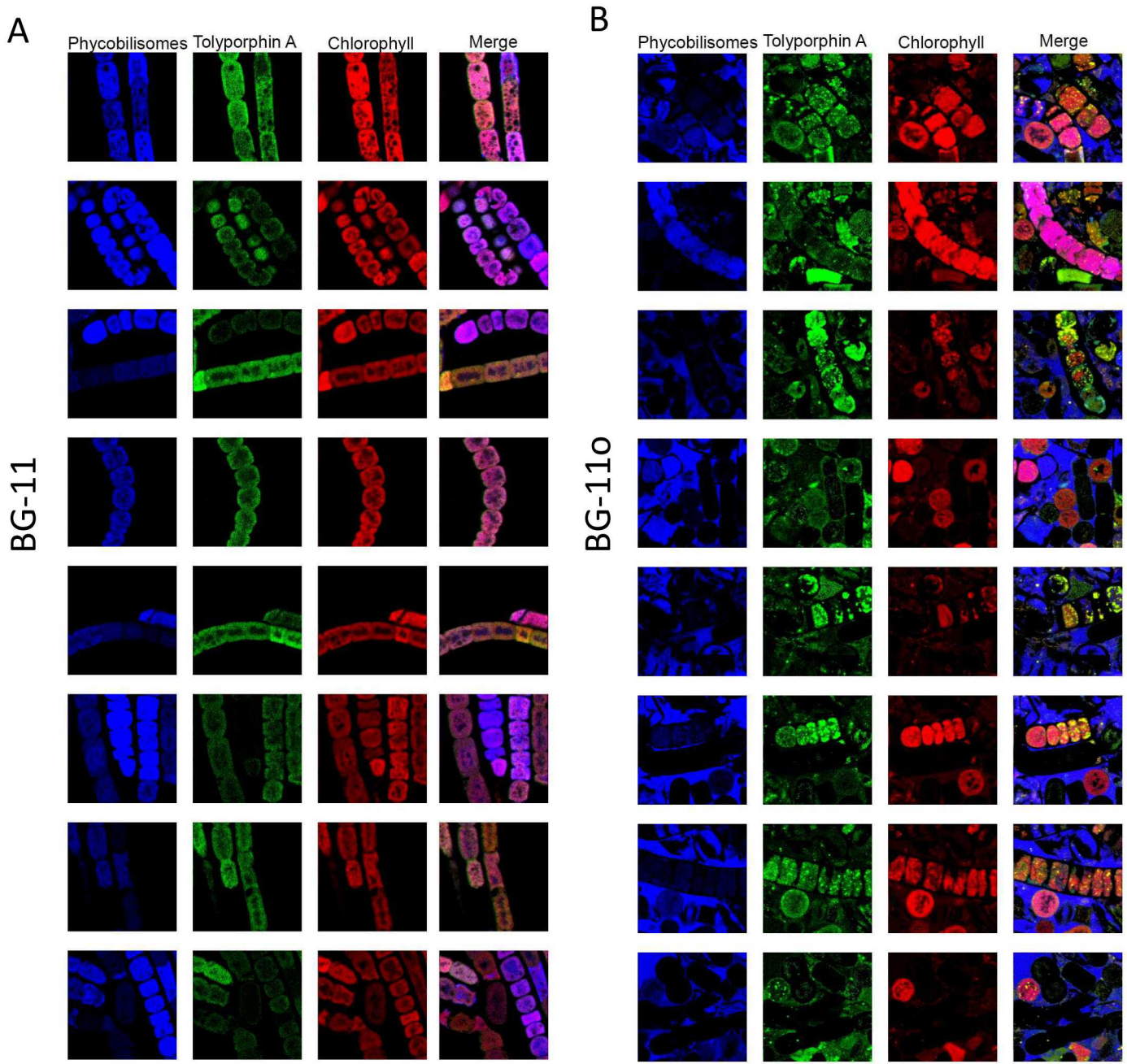



Figure S4. Representative MCR concentrations maps of 30 day HT-58-2 cultures that were dark-adapted for 24 hours. A. Cultures grown in BG-11 media. B. Cultures grown in BG-11o media. The three main components (PBS - phycobilisomes, Toly. A - tolyporphin A, and Chi. - chlorophyll) from the spectral model are displayed. Image intensities in A & B are scaled equally. The scale bar represents 5µm.

# Conclusions

- Hyperspectral confocal fluorescence imaging
- *HT-58-2* cultures in BG11 and BG11o (nitrogen limited) media at 30 and 60 days
- Dark-adapted HT-58-2 cultures were also imaged (30 day sample in dark for 18 hrs)
- 488 nm excitation, >2,000,000 spectra

- Tolyporphin A is punctate and enriched in 30 day cultures in the absence of nitrate

# Acknowledgements


- Lindsey and Miller labs at NC State
  - Yunlong Zhang
  - Xiaohe Jin

- Michael Sinclair, SNL
- Stephen Anthony, SNL
- Jeri Timlin, SNL

# Location and Abundance of Tolyporphin in HT-58-2

## Scientific Achievement

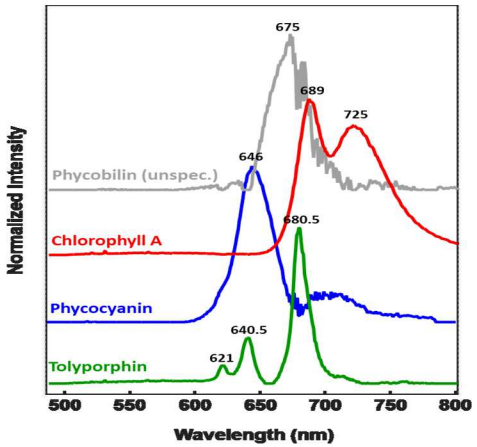
**Determined tolyporphin locale and abundance in intact, living HT-58-2 cells.**

## Significance and Impact

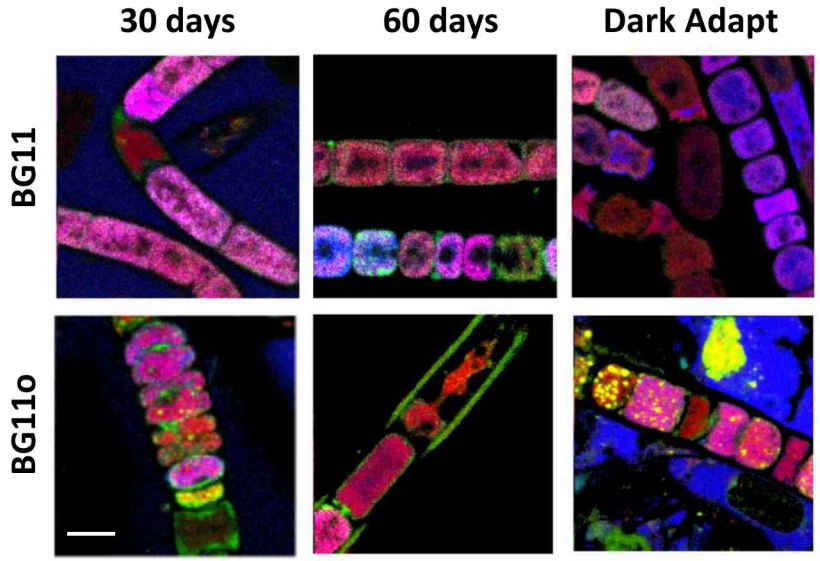
**Tolyporphins are spectrally similar to chlorophylls and the location of tolyporphins in cells could not be independently assessed *in vivo* prior to this work.**

## Research Details

- Hyperspectral confocal fluorescence imaging
- *HT-58-2* cultures in BG11 and BG11o (nitrogen limited) media at 30 and 60 days
- Dark-adapted HT-58-2 cultures were also imaged (30 day sample in dark for 18 hrs)
- 488 nm excitation, >2,000,000 spectra




**Top left: Spectral components Lower panels: Abundance and locale of tolyporphin in HT-58-2.** Tolyporphin localized to exterior of cell and cell septa, bright puncta within some cells are visible. Increased tolyporphin production in BG11o vs. BG11, with time, and upon dark adaptation in BG11o. Image quantification underway.




Scale bar = 5 µm. RGB pseudocoloring corresponds to Spectral plot.

Collaboration between: Yunlong Zhang, Meghan Dailey, Jonathan Lindsey, Eric Miller, Jeri Timlin

Imaging was performed at Sandia National Laboratories by Yunlong Zhang and Meghan Dailey (PARC scientific exchange).

PARC

NC STATE UNIVERSITY

Sandia National Laboratories