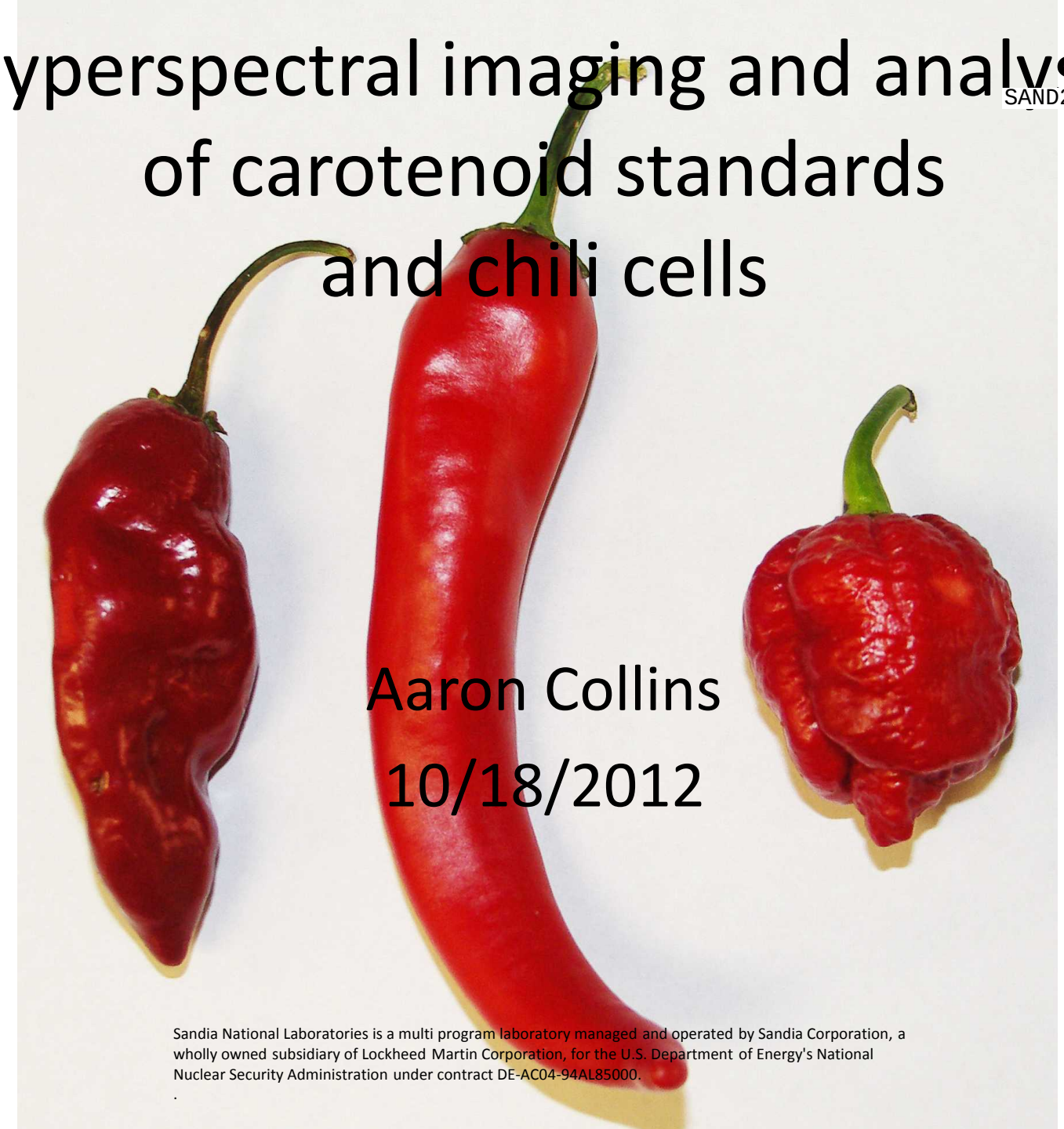
# Hyperspectral imaging and analysis of carotenoid standards and chili cells

SAND2012-9233P



Aaron Collins

10/18/2012

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# Methodology – for standards

Sample Prep

Carotenoid standards were sent to SNL from Mart O'Connell on 10/9
Standards were stored at -80C in the dark

For analysis, 5 ul of standard solution was spotted on a slide and allowed to dry.

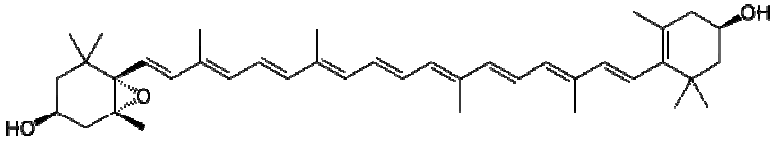
Imaging Acquisition

20x objective (Nikon, PlanApo NA = 0.75 )
50 um x 50 um area interrogated
>5 images acquired for each standard.
Laser power ~ 200 uW (OD = 0, before entering microscope)
488 nm excitation

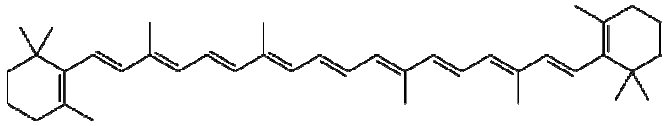
Image analysis

Mask applied to omit all non-pigmented portion of image
Multivariate Curve Resolution algorithms used to determine spectral components for each standard individually (no combining of datasets across different standard)

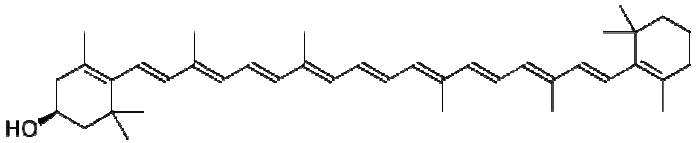
In the following slides only the Carotenoid components are displayed.



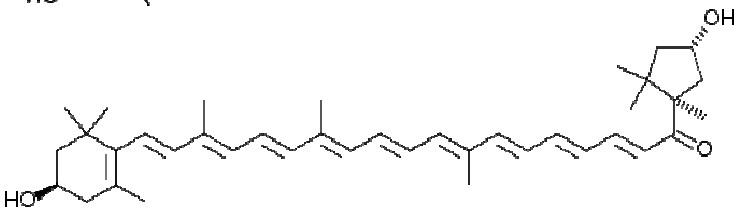

Antheraxanthin



Beta-carotene

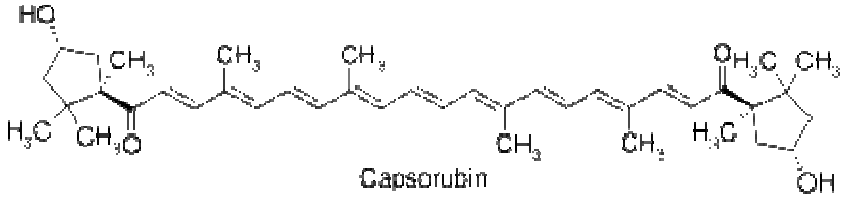



Beta-cryptoxanthin

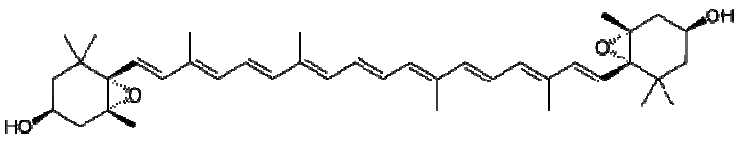



Capsanthin

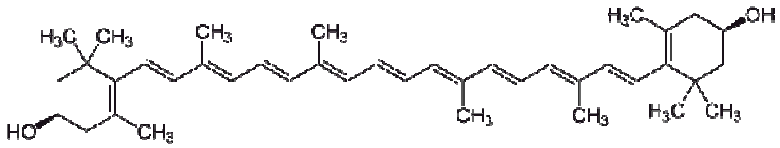
I update a slide that now includes this data



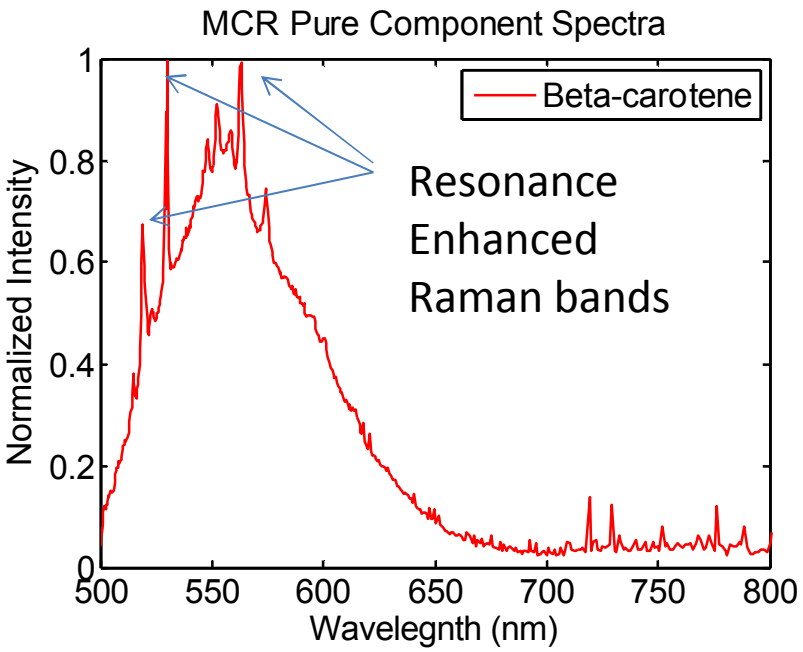

Capsorubin

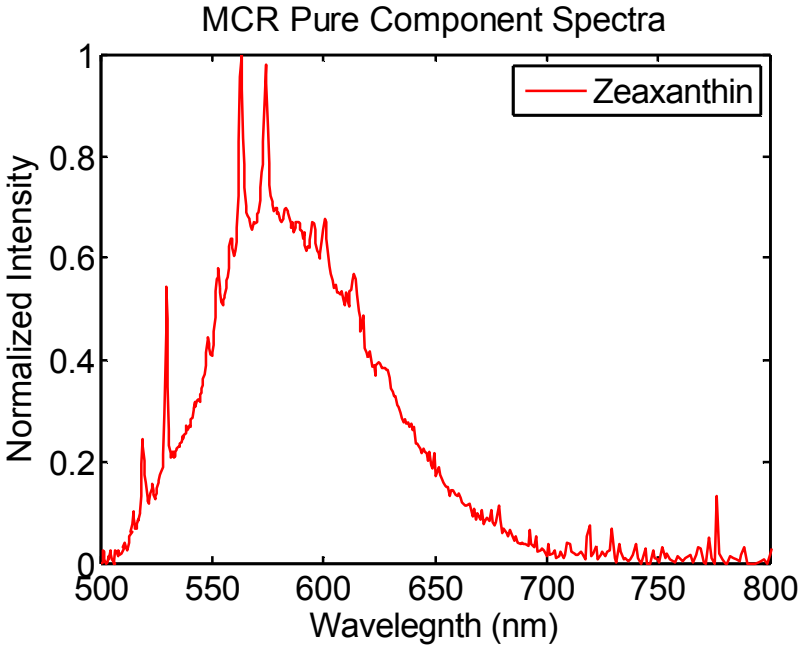


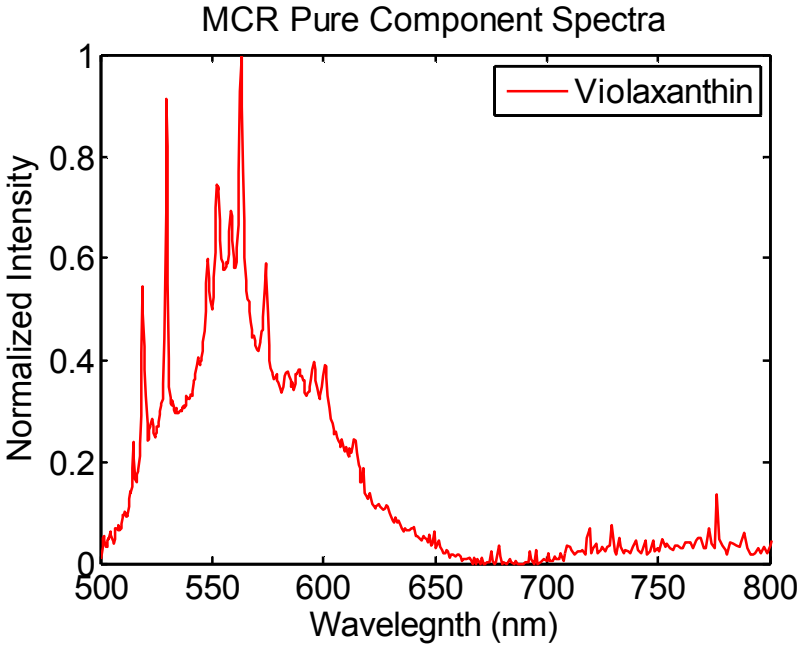

Violaxanthin

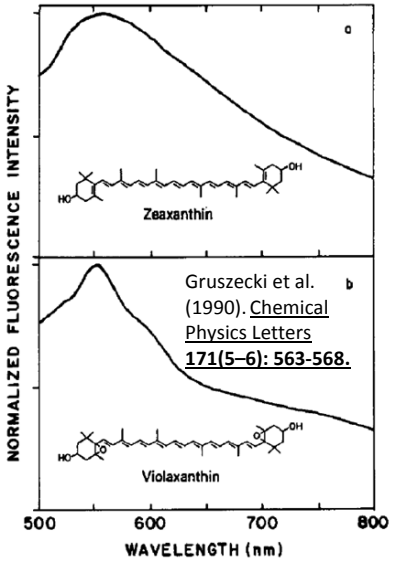



Zeaxanthin



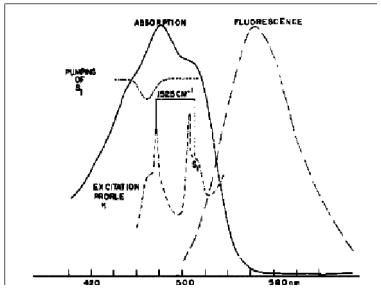




Gruszecki et al. (1990). Chemical Physics Letters 171(5–6): 563-568.

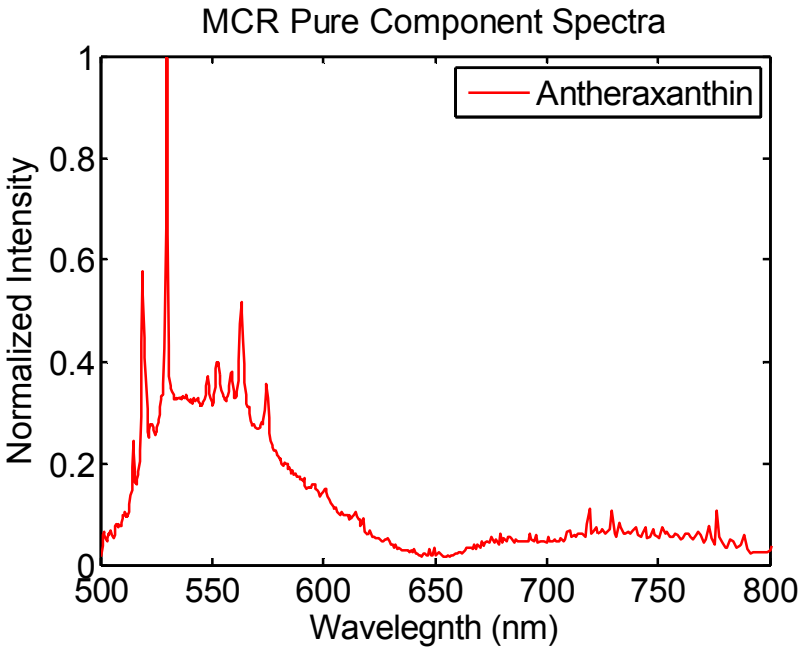
Fig. 3. Corrected fluorescence spectra of (a) zeaxanthin and (b) violaxanthin aggregates in 10% ethanol in water ($\lambda_{exc}$=400 nm).

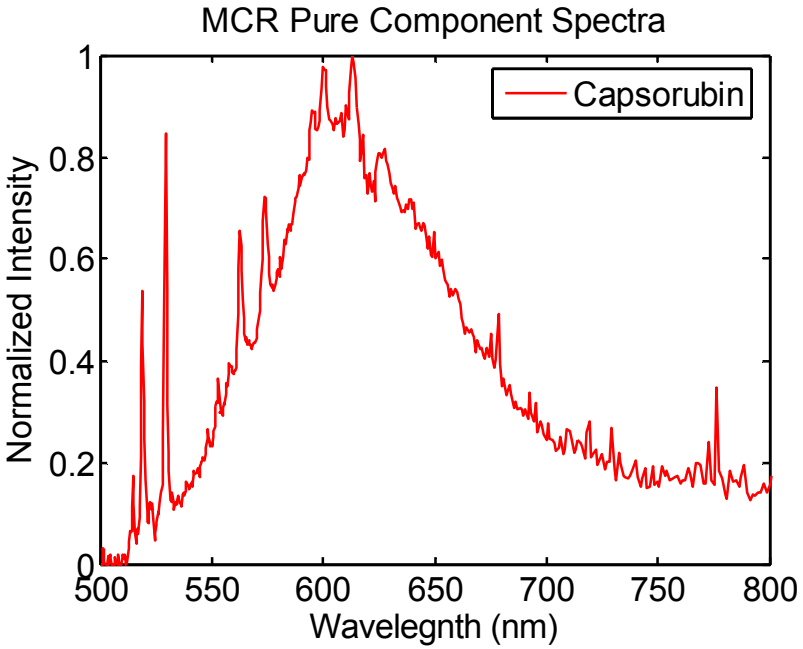


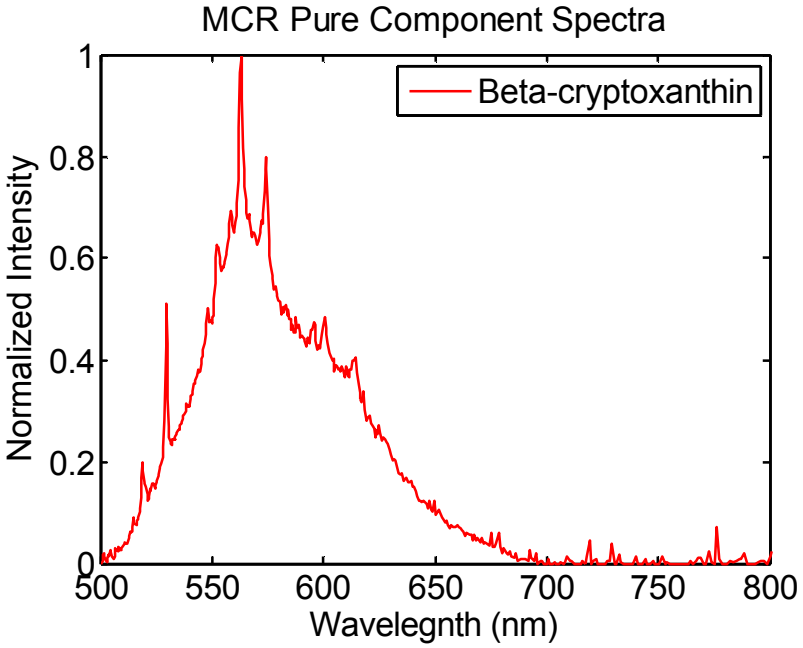
Beta-carotene

L. V. Haley , J. A. Koningstein
J. Phys. Chem., 1983, 87 (4), pp 621–62

Good agreement between Literature and measured spectral shapes

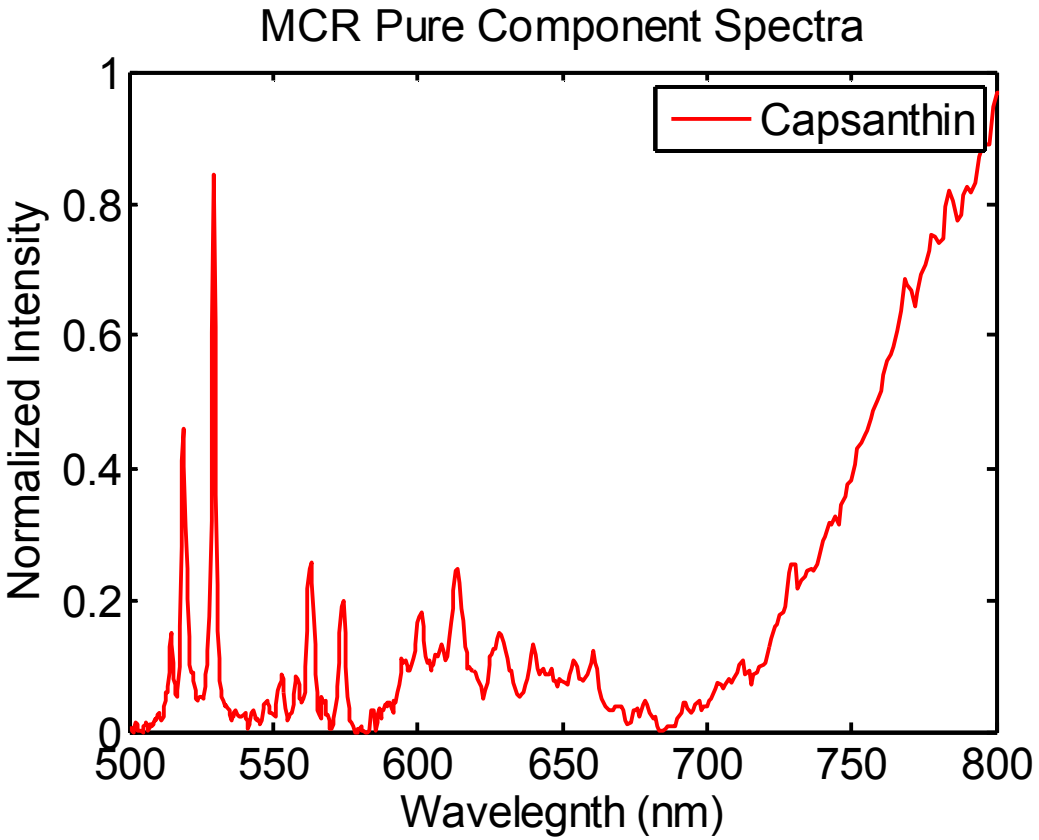

MCR Pure Component Spectra
Antheraxanthin
Normalized Intensity
Wavelegnth (nm)
0
0.2
0.4
0.6
0.8
1
500
550
600
650
700
750
800


MCR Pure Component Spectra
Capsorubin
Normalized Intensity
Wavelegnth (nm)
0
0.2
0.4
0.6
0.8
1
500
550
600
650
700
750
800

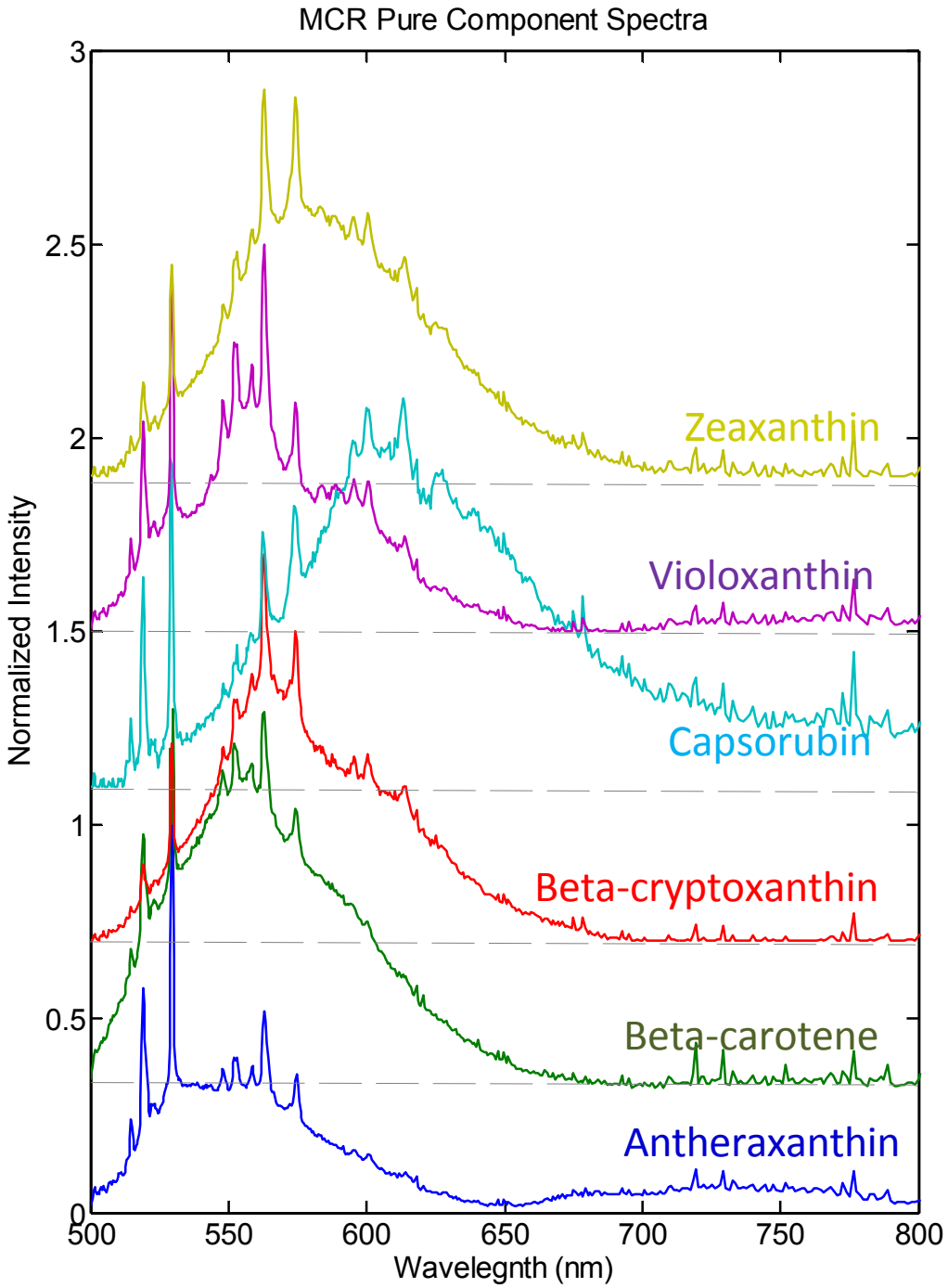

MCR Pure Component Spectra
Beta-cryptoxanthin
Normalized Intensity
Wavelegnth (nm)
0
0.2
0.4
0.6
0.8
1
500
550
600
650
700
750
800

# Update – Capsanthin standard

With 4x the integration time, a weak capsanthin component was obtained




This spectrum is slightly smoothed and may not be the best spectrum we can do. Use of the Raman scope will help resolve this pigment further.




Each dried carotenoid sample gave a unique fluorescence/Raman profile.

Notice the Raman bands for all samples are more-or-less in the same position (same wavelength) while the underlying fluorescence shapes are different.

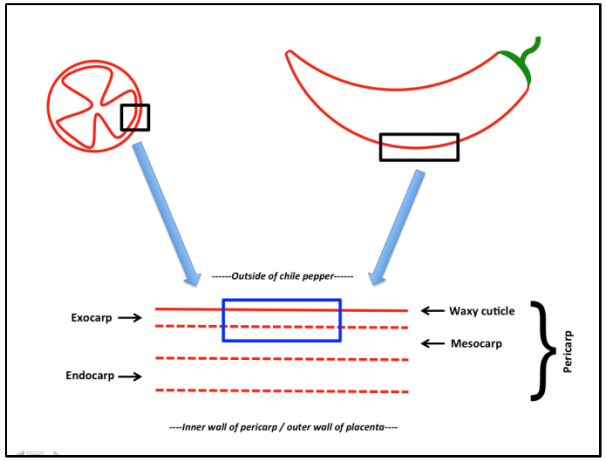
Zeaxanthin, violaxanthin and beta-carotene gave very good signatures. In other words, these carotenoids must have higher quantum yields on our microscope.

# Live chili imaging

Sample Prep

A very thin section of flesh was cut using a double-sided razor blade. The sample was placed cuticle side down in a well-frame, covered with a few drops of water and sealed with a #1.5 coverslip. Our imaging setup uses an upright microscope so that the mesocarp is directly under the coverslip.

Image courtesy of James P. Kilcrease




Imaging Acquisition

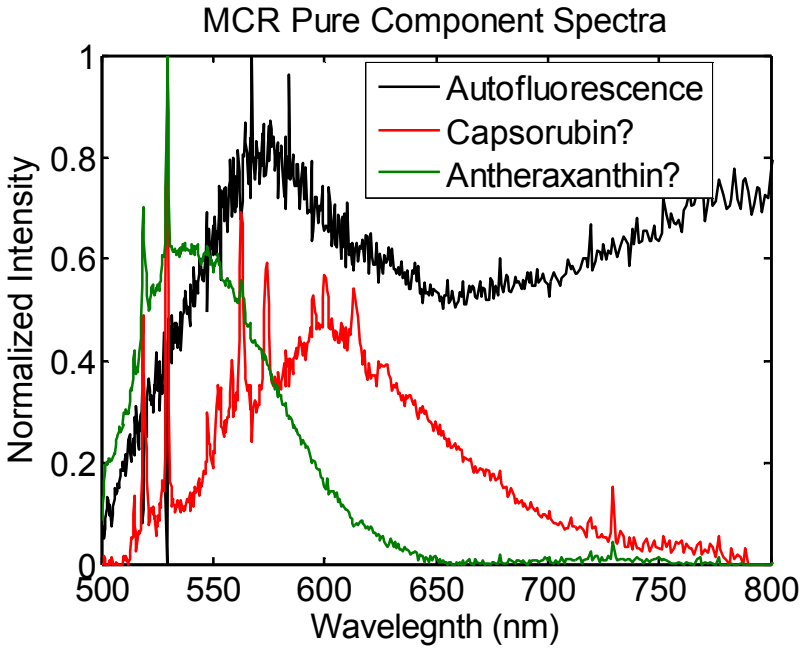
20x objective (Nikon, PlanApo NA = 0.75 )
60x oil objective (Nikon, PlanApo NA = 1.4 )
Laser power ~ 200 uW (OD = 0, before entering microscope)
488 nm excitation
Some images required double the typical integration time to increase signal/noise

Image analysis

Mask applied to omit all non-pigmented portion of image
Multivariate Curve Resolution algorithms used to determine spectral components for each chili type individually (no combining of datasets across different standard)

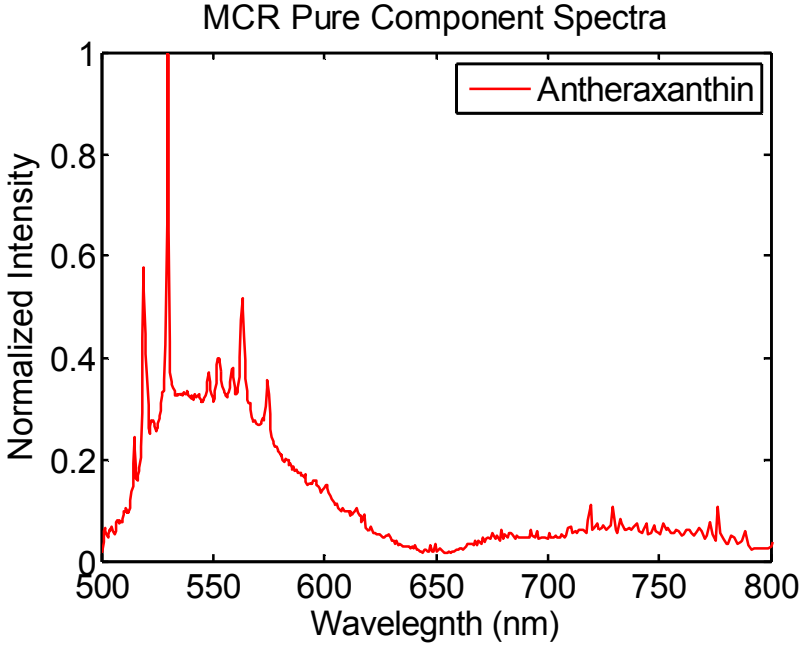
I am only showing the 60X data as it had the greatest resolution.

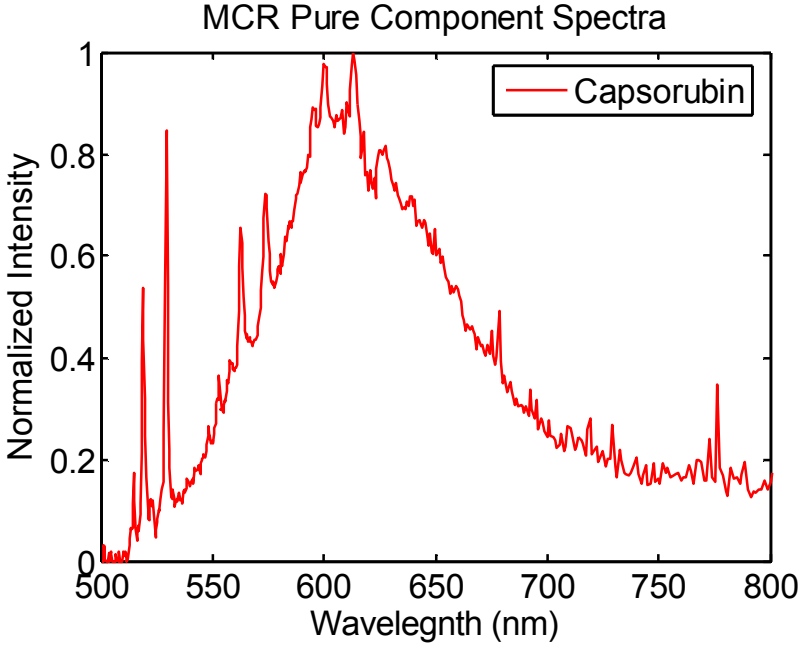
# Red sweet Pepper – 60X magnification



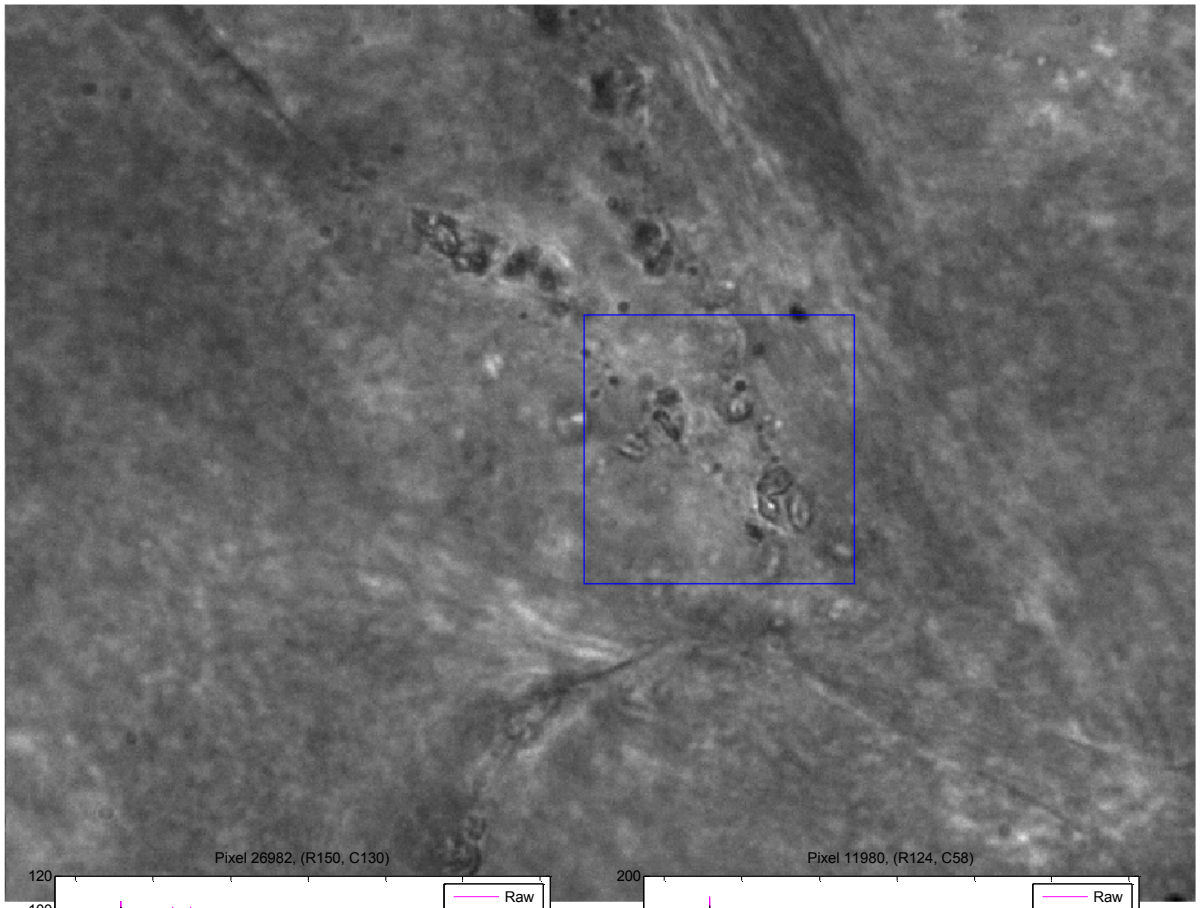

The red banana pepper spectral data could be described by 3 components (plus and offset). I tentatively assigned these components based on the signatures of the pure pigment standards.

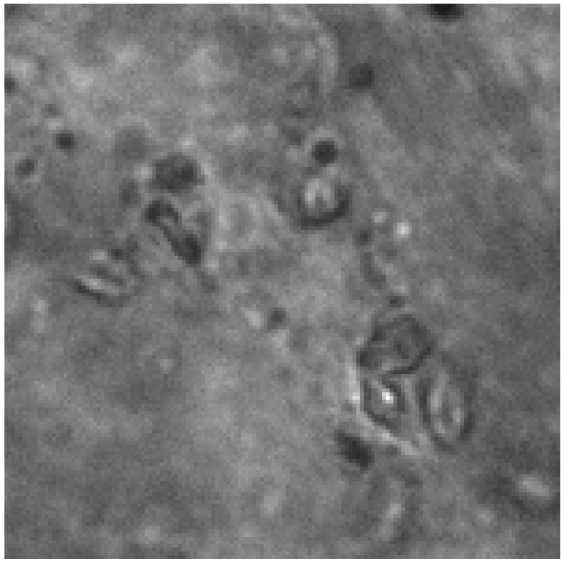
In following slides, the RGB coloring of the Composite images are colored according to the MCR model to the left ←

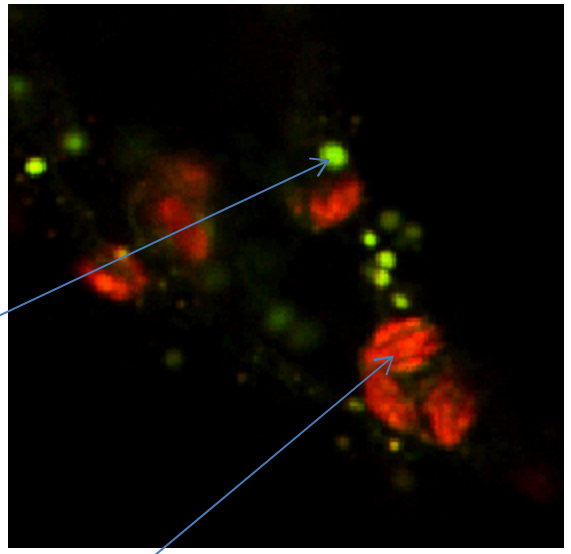


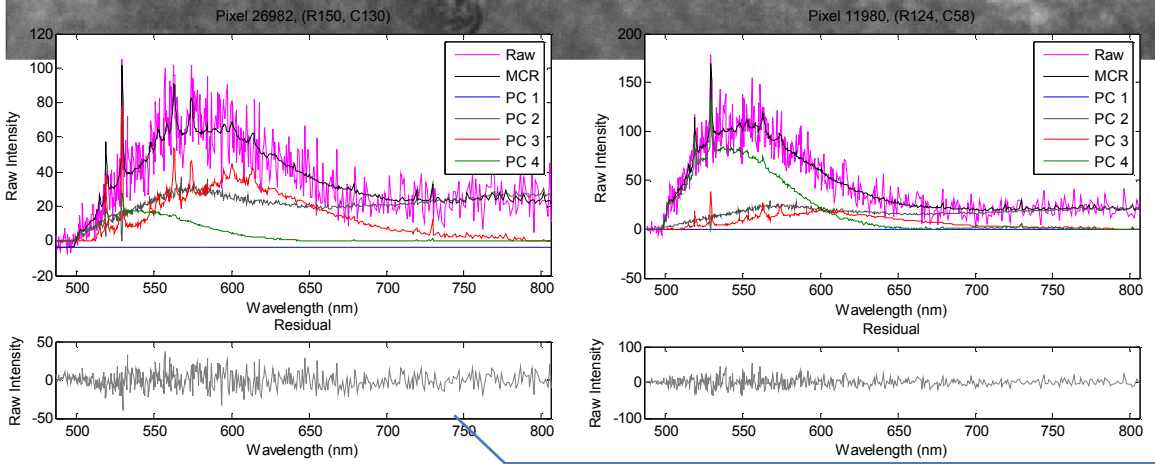



# Sweet Red Pepper – 60X magnification







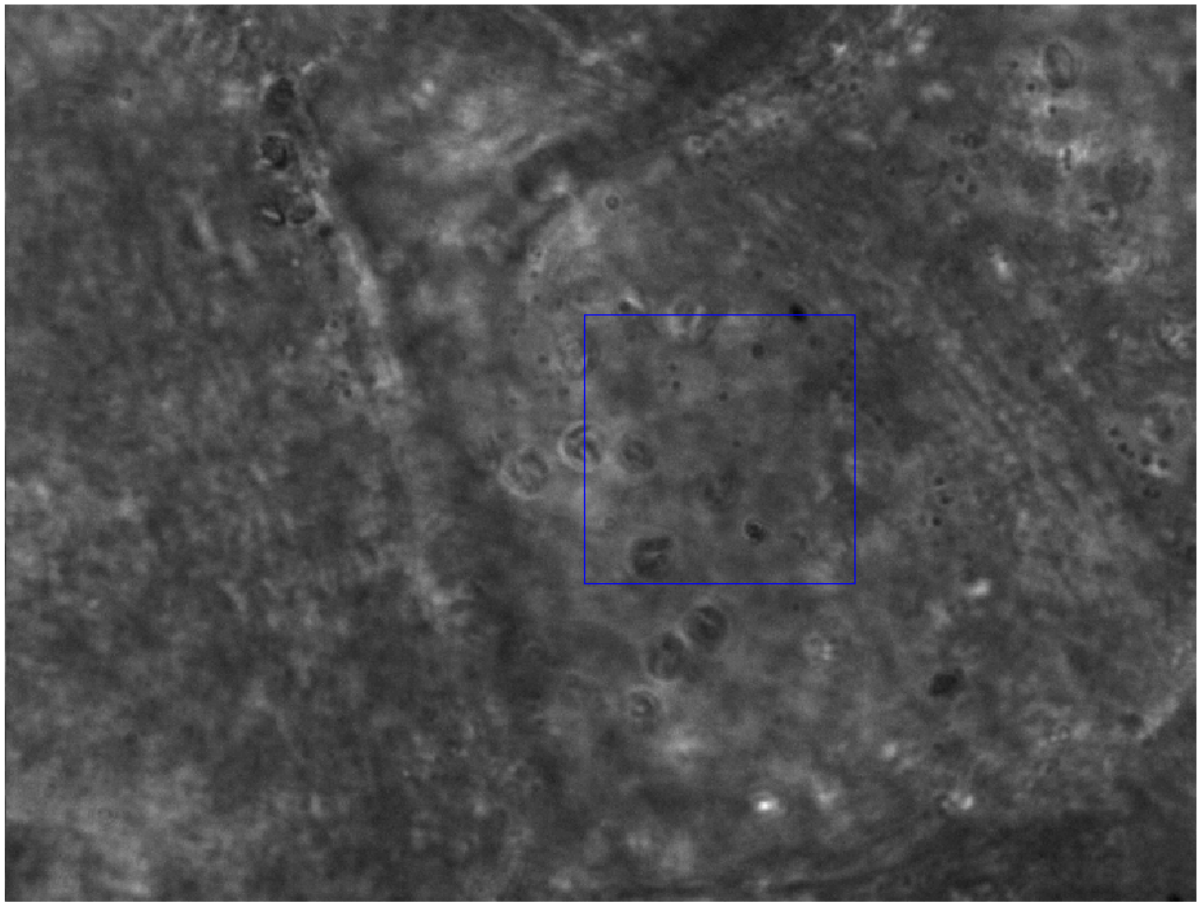


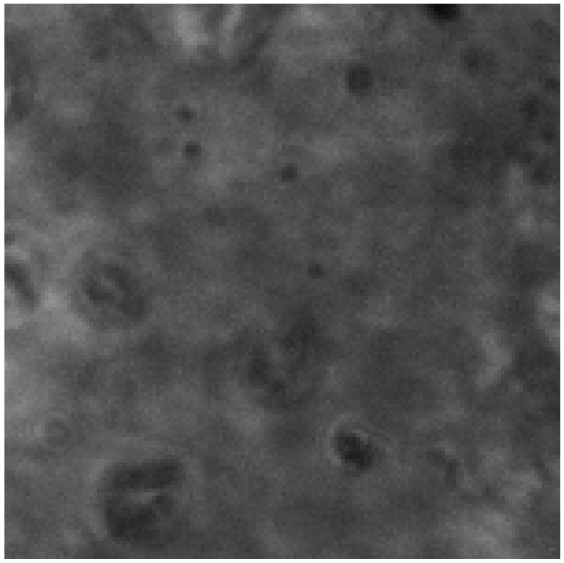


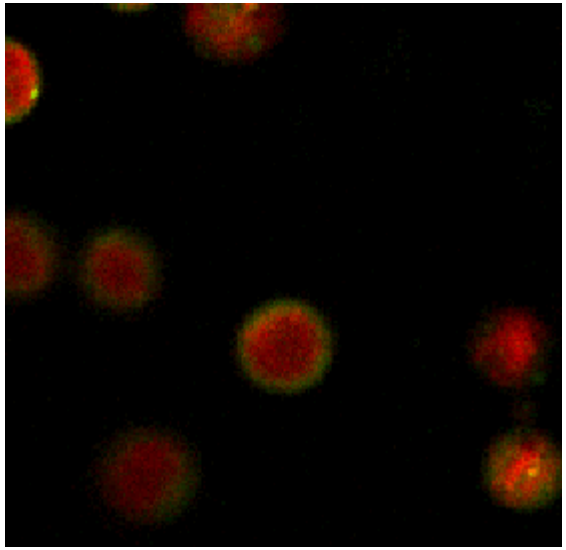


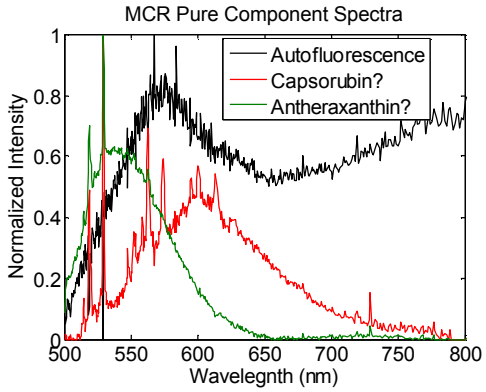
Single pixel spectra

# Sweet Red Pepper – 60X magnification

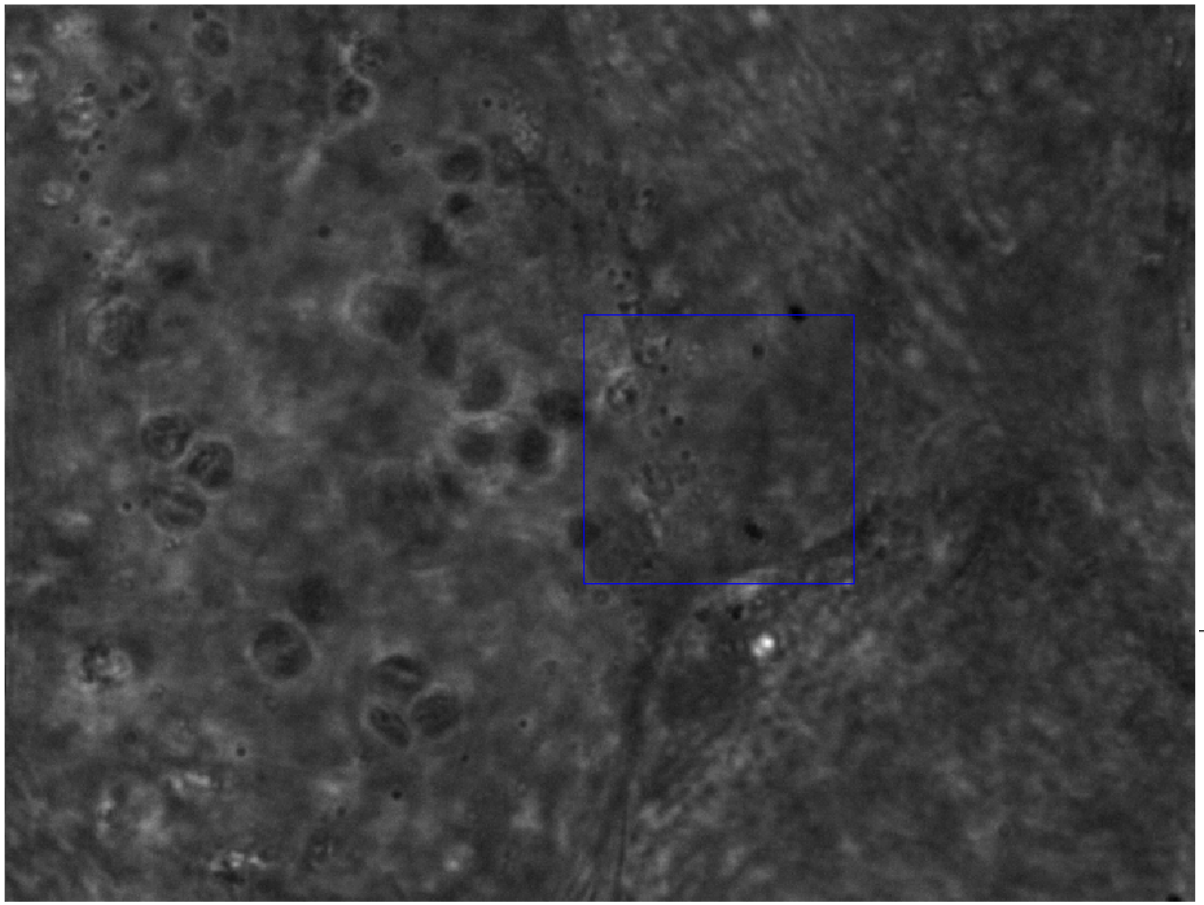


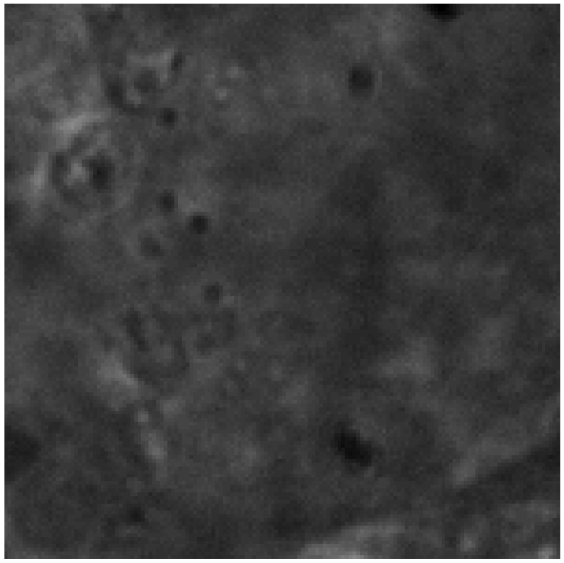


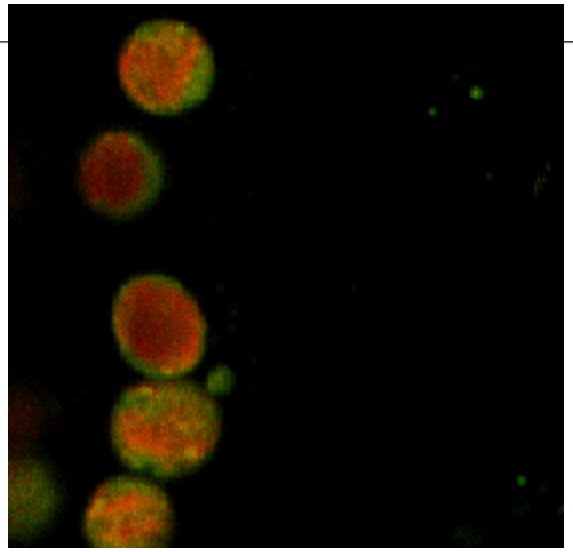


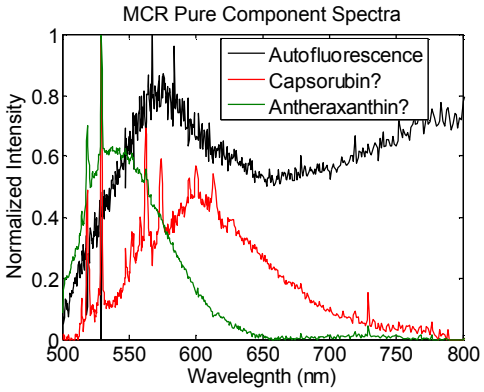


# Sweet Red Pepper – 60X magnification

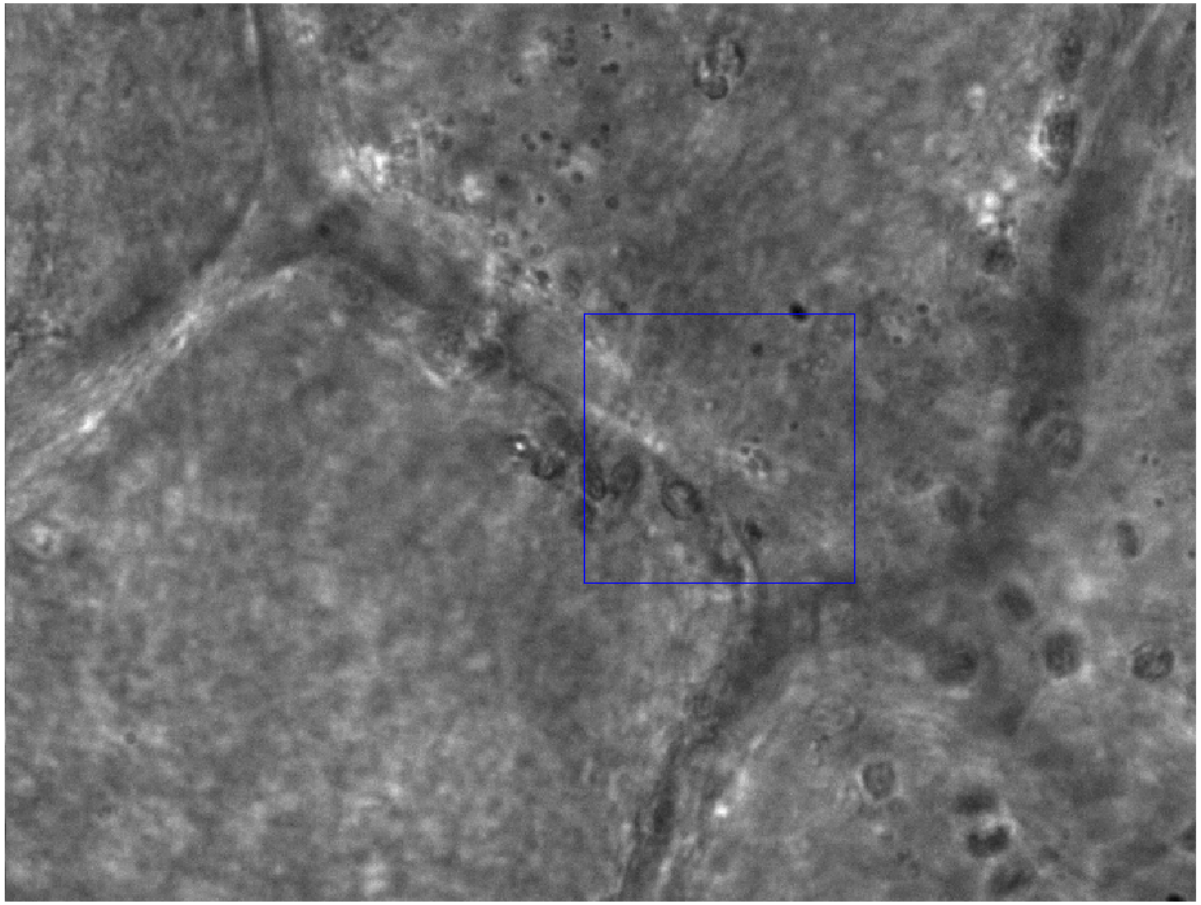


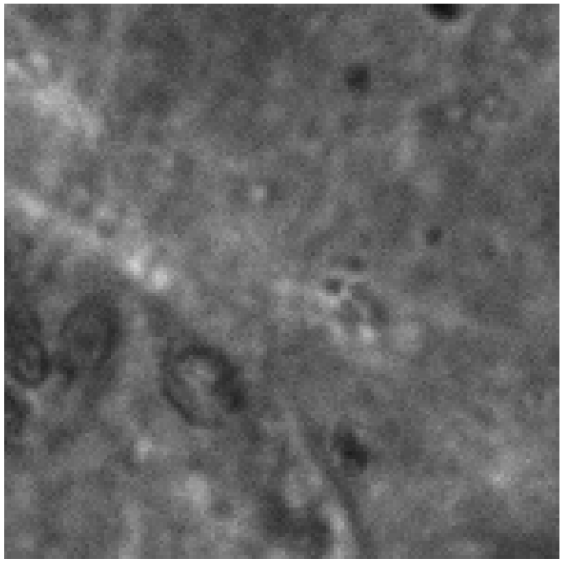


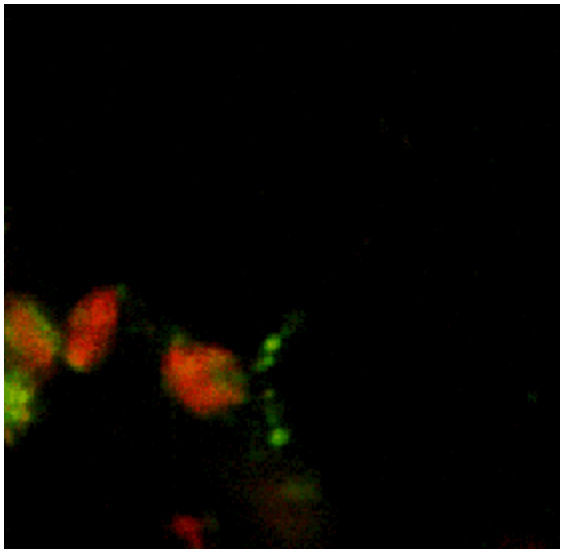


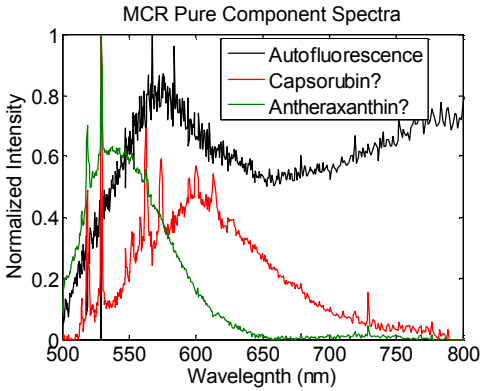


# Sweet Red Pepper – 60X magnification

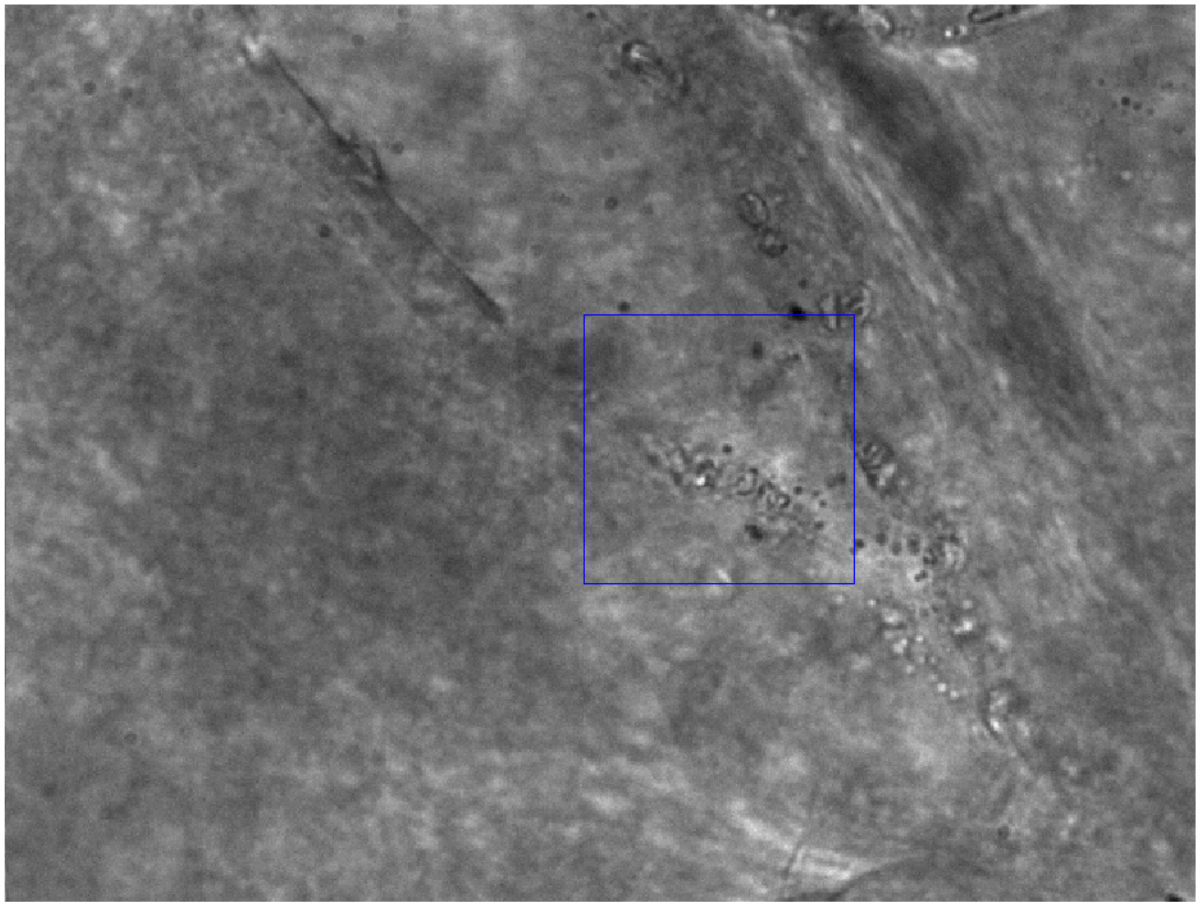


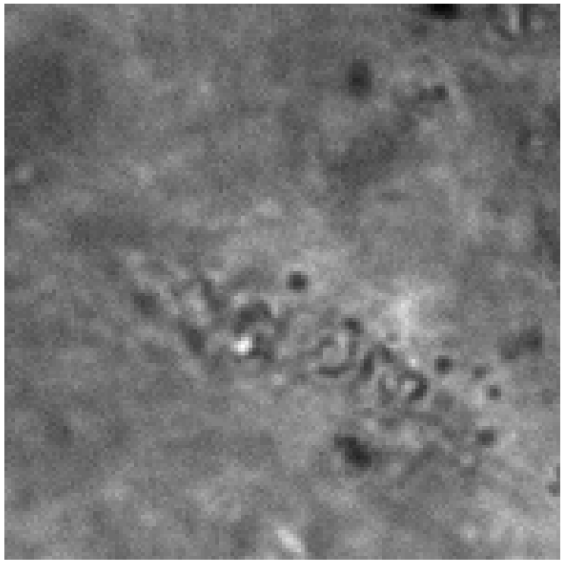


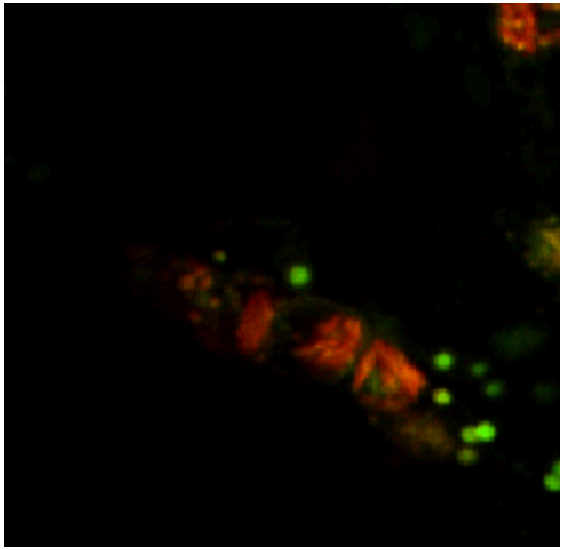


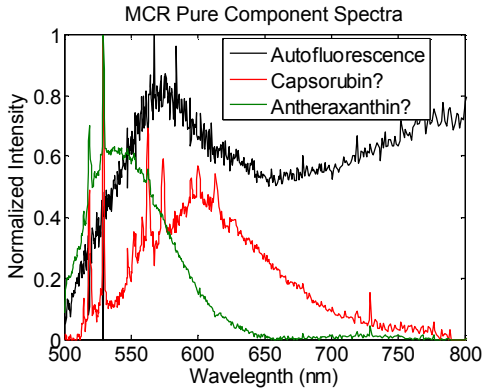


# Sweet Red Pepper – 60X magnification

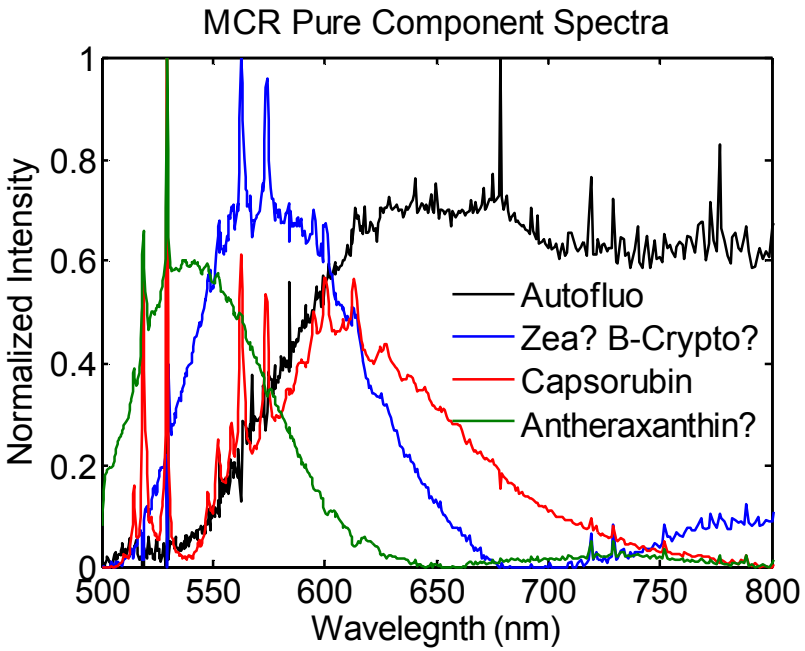




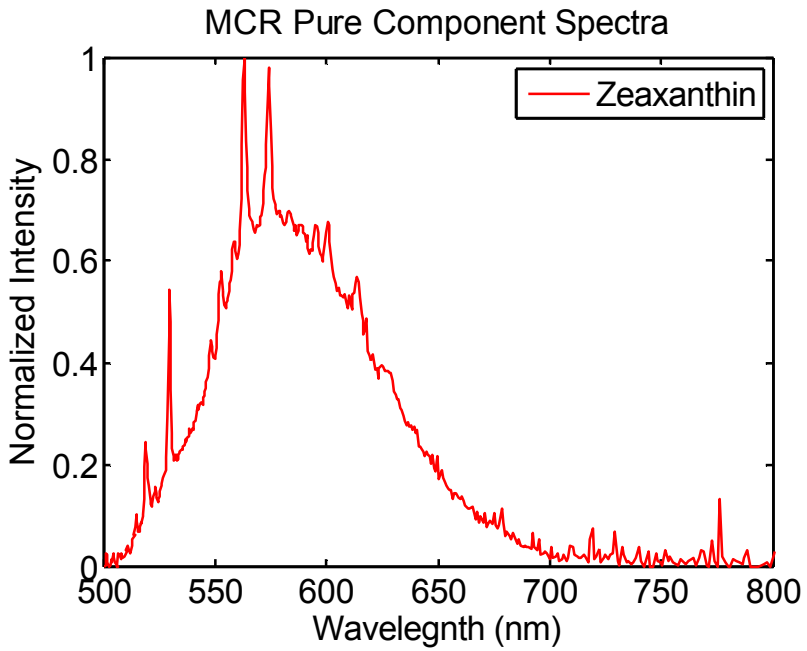


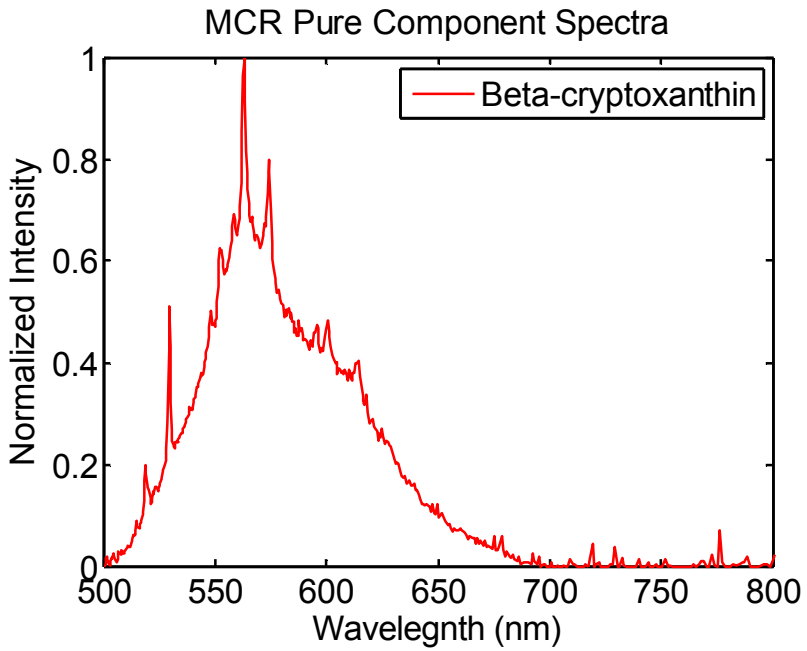


# Red Habanero – 60X magnification

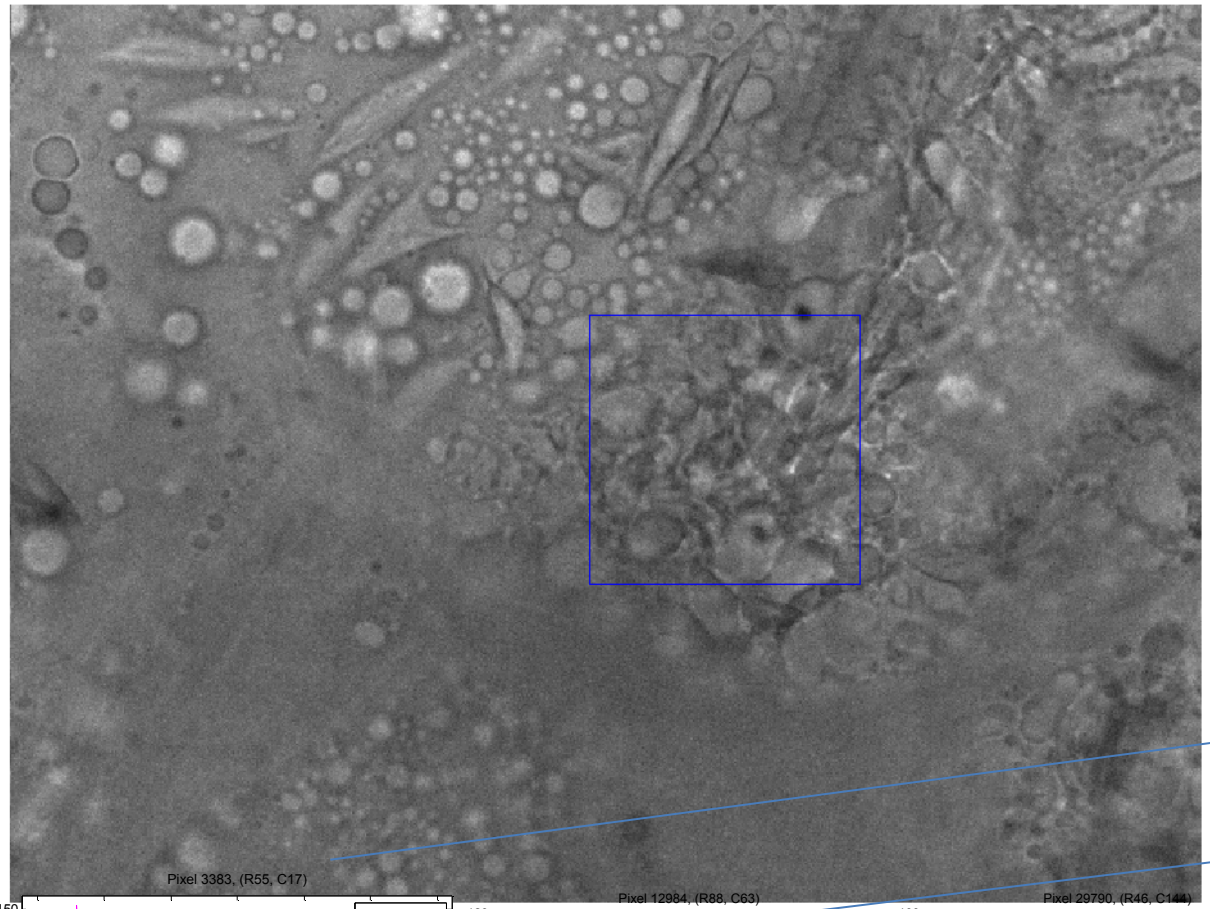


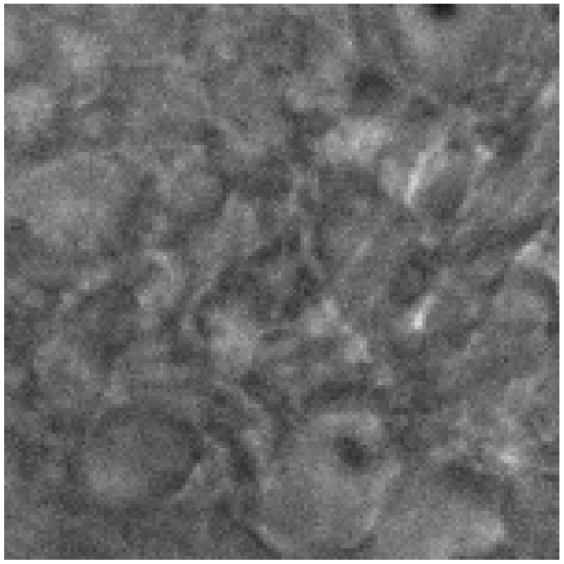

For the Habanero chili, two of the components are nearly identical to the antheraxanthin and capsorubin from the sweet red pepper analysis. An additional component as modeled and based on its shape it could be zeaxanthin or B-cryptoxanthin?

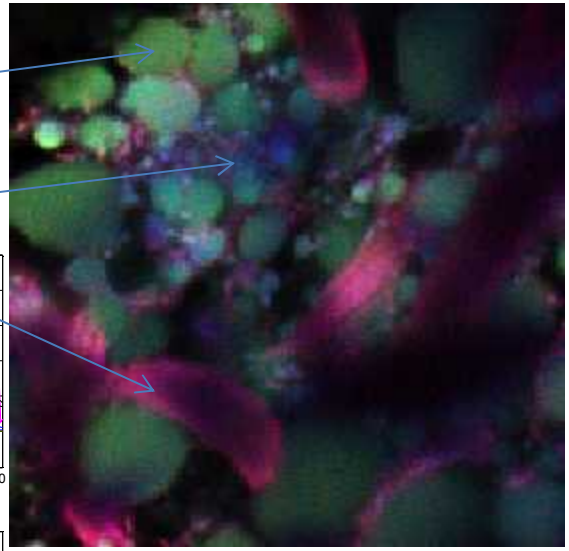


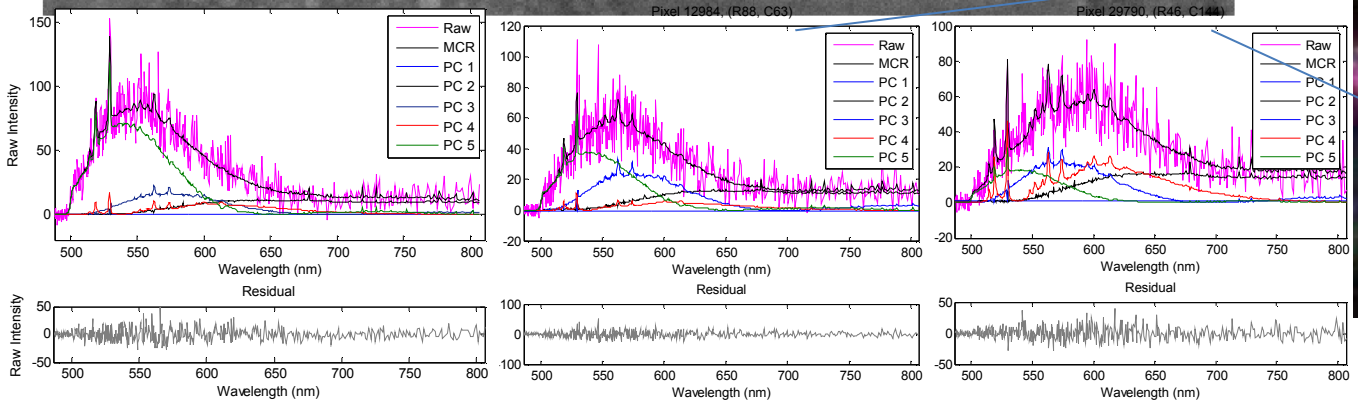



# Red Habanero – 60X magnification

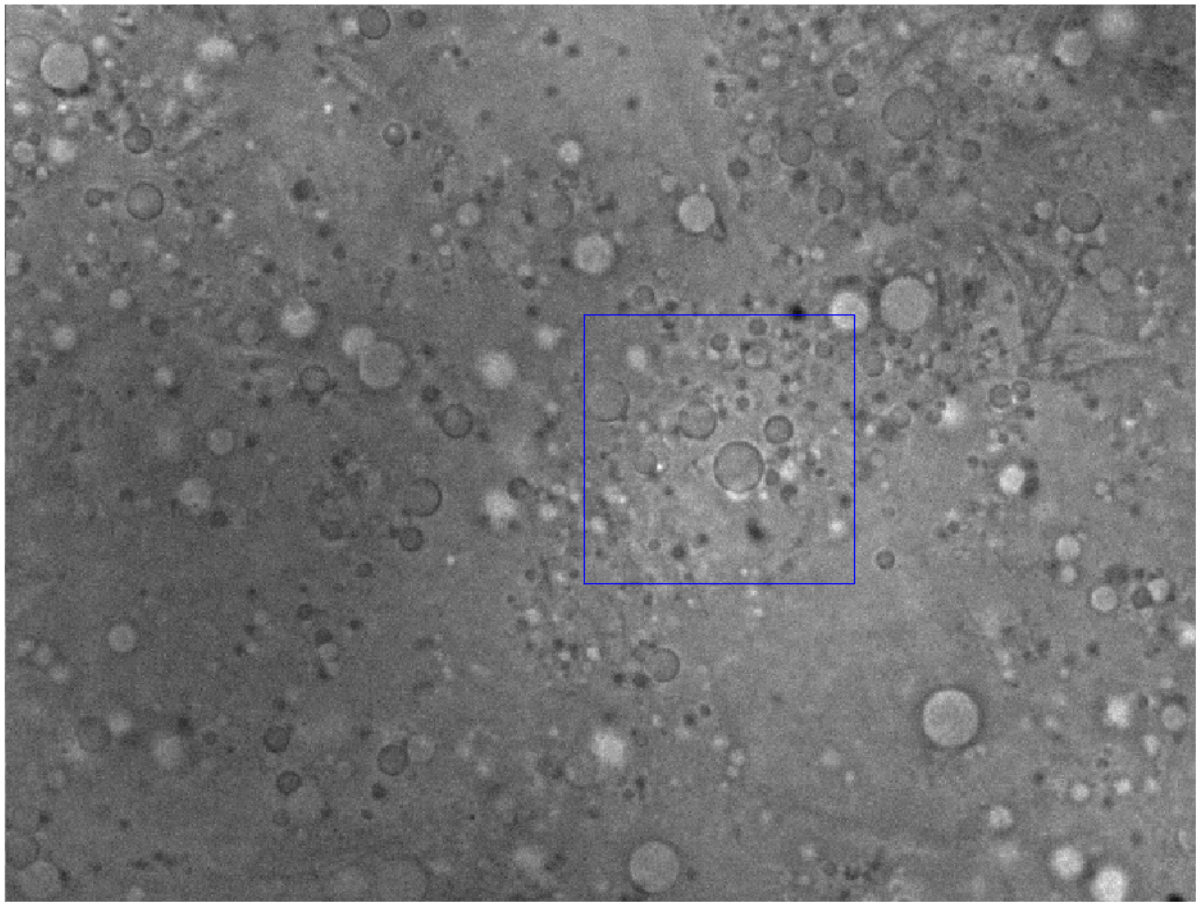


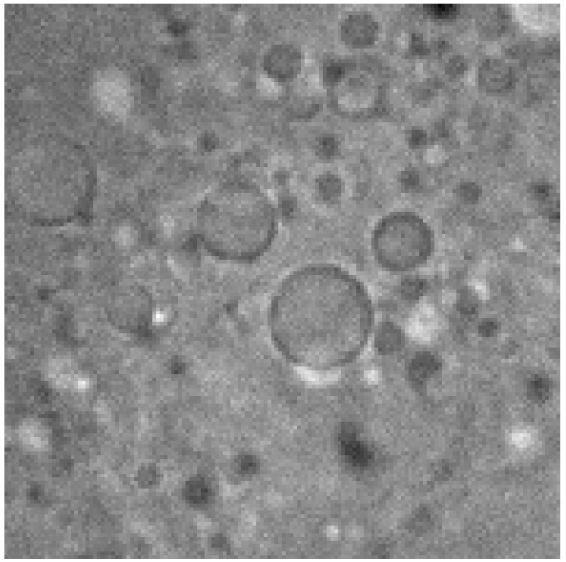


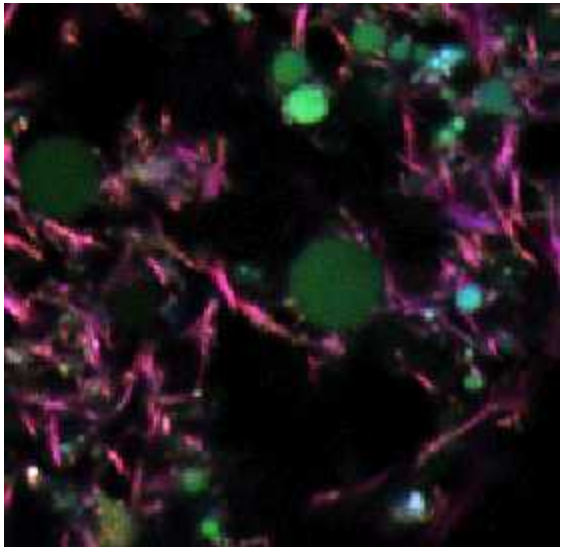


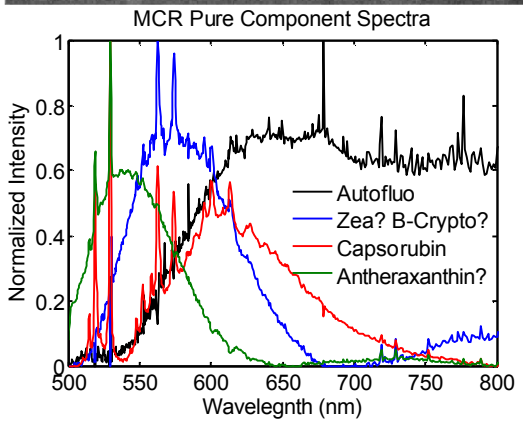


# Red Habanero – 60X magnification

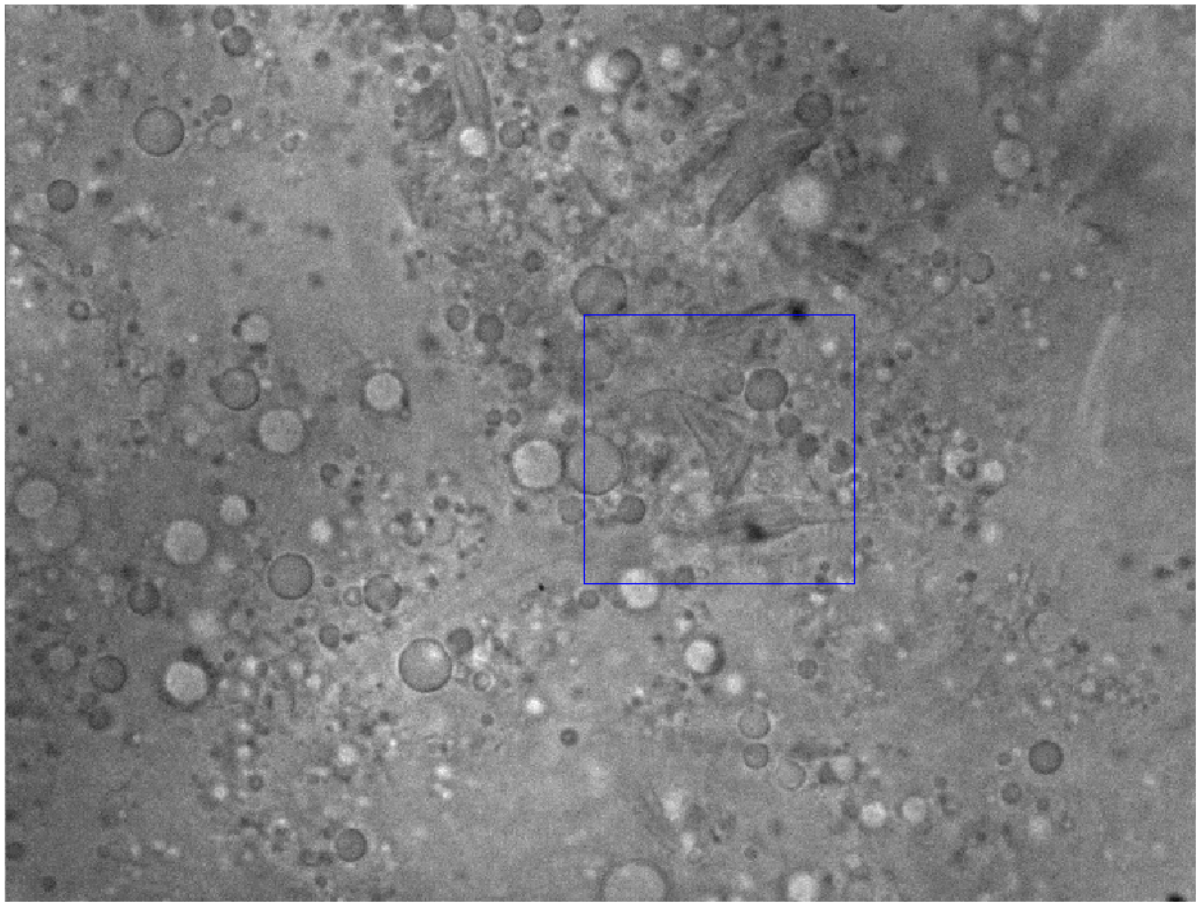


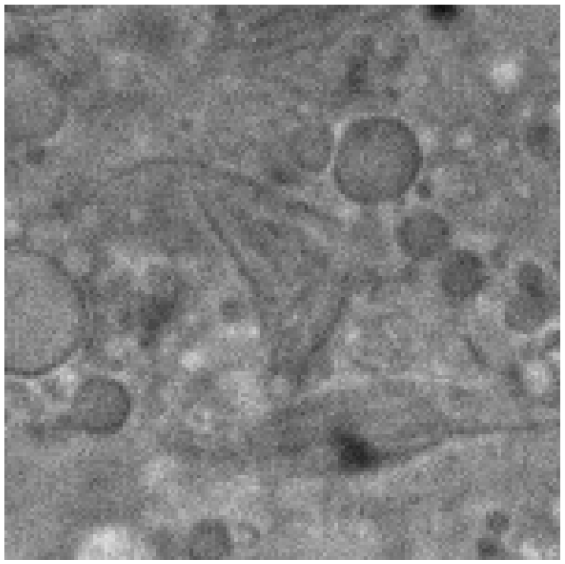


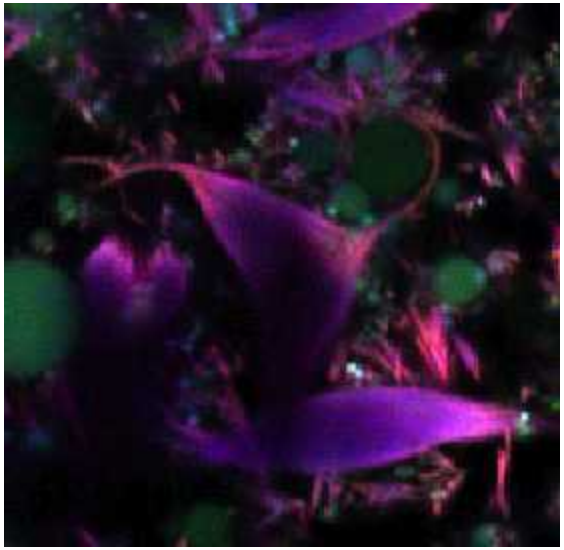


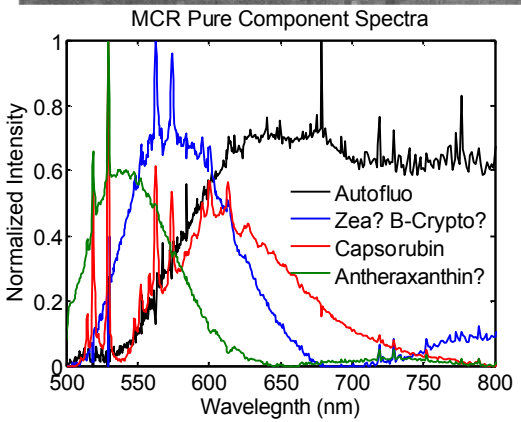


# Red Habanero – 60X magnification

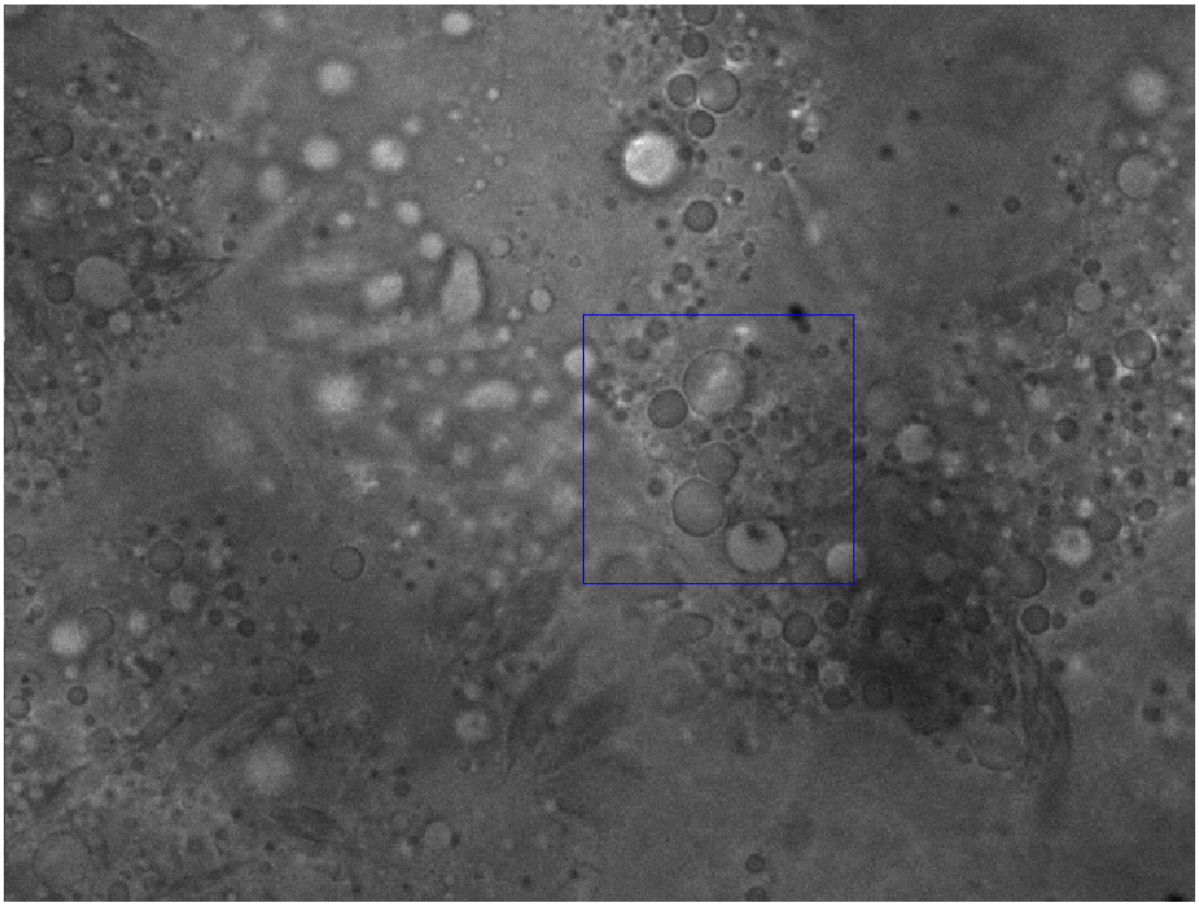


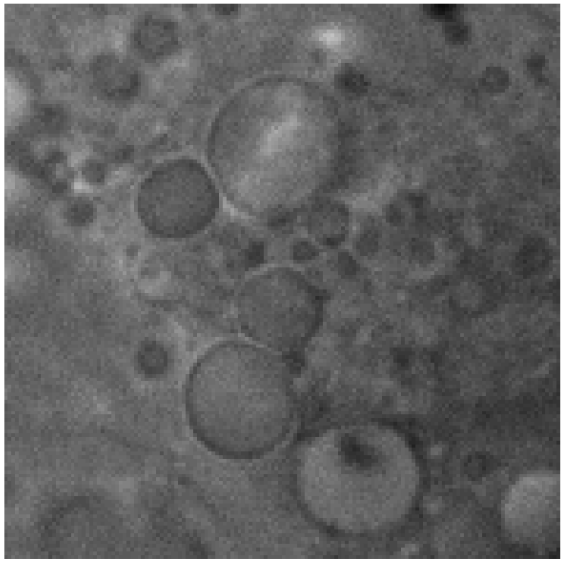


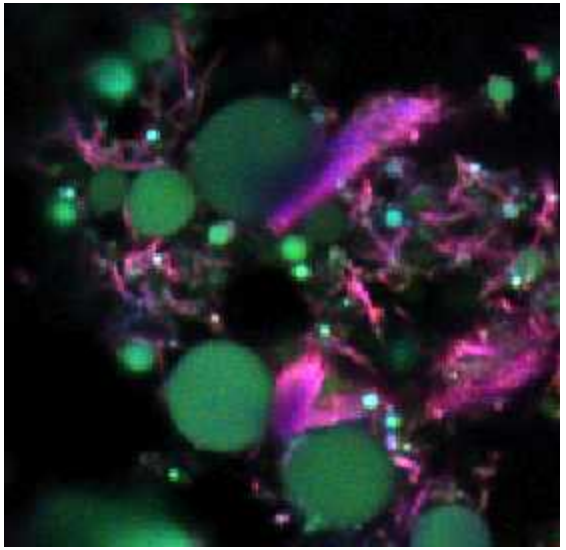


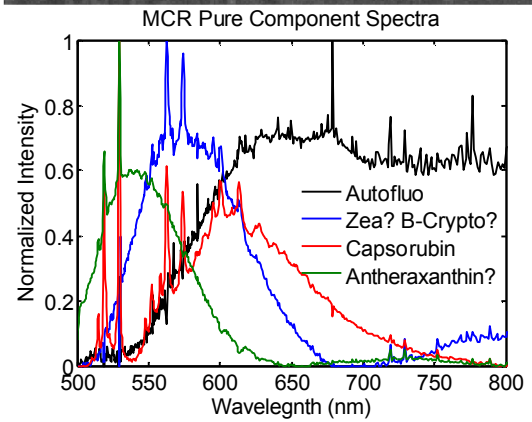


# Red Habanero – 60X magnification

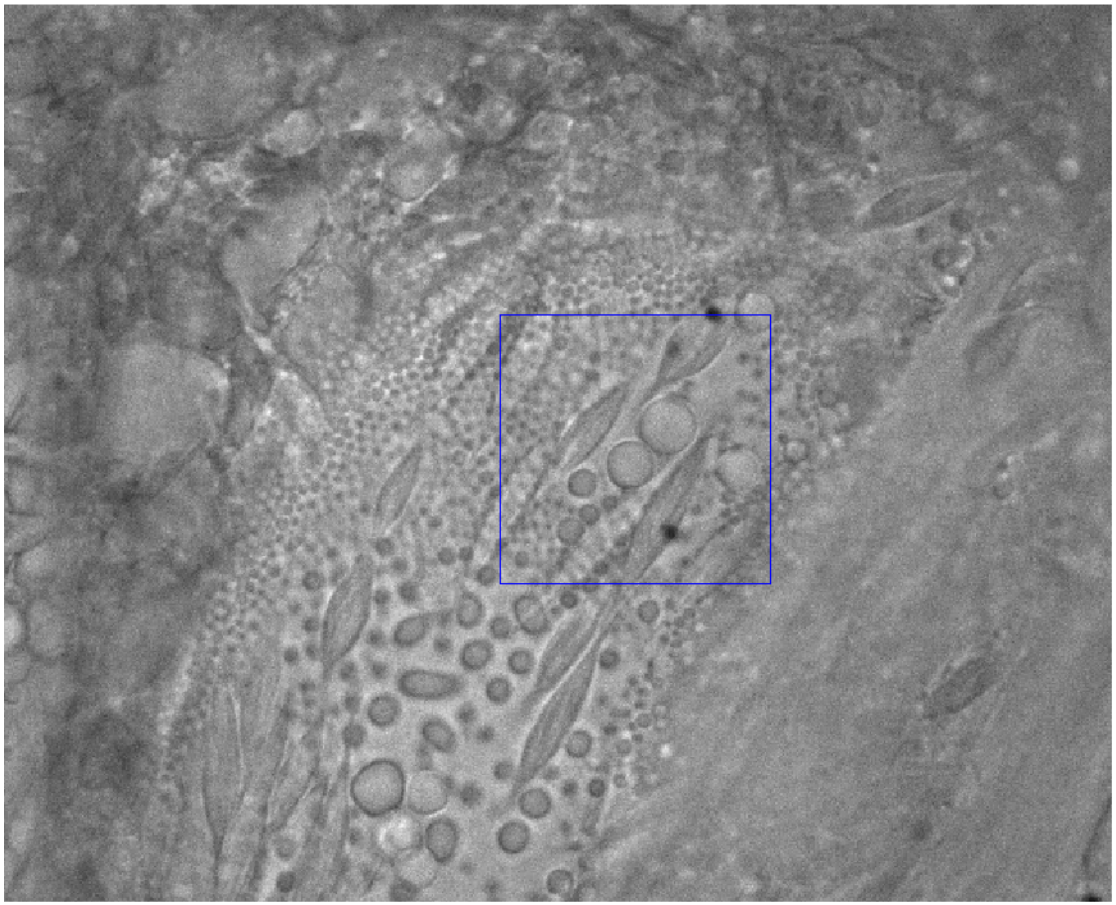


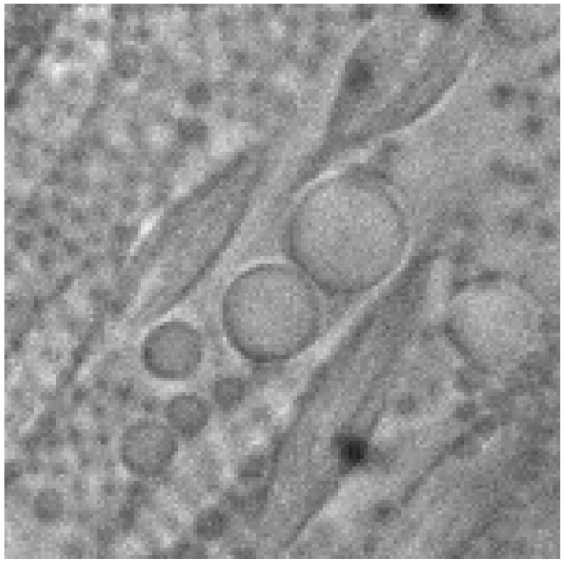


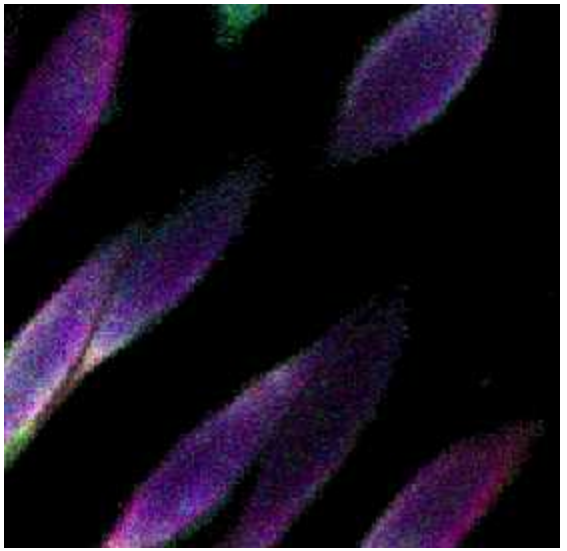


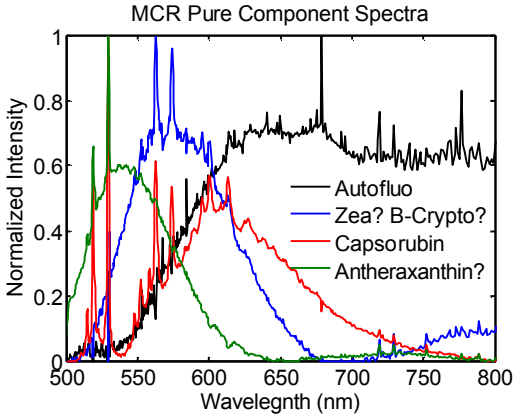


# Red Habanero – 60X magnification

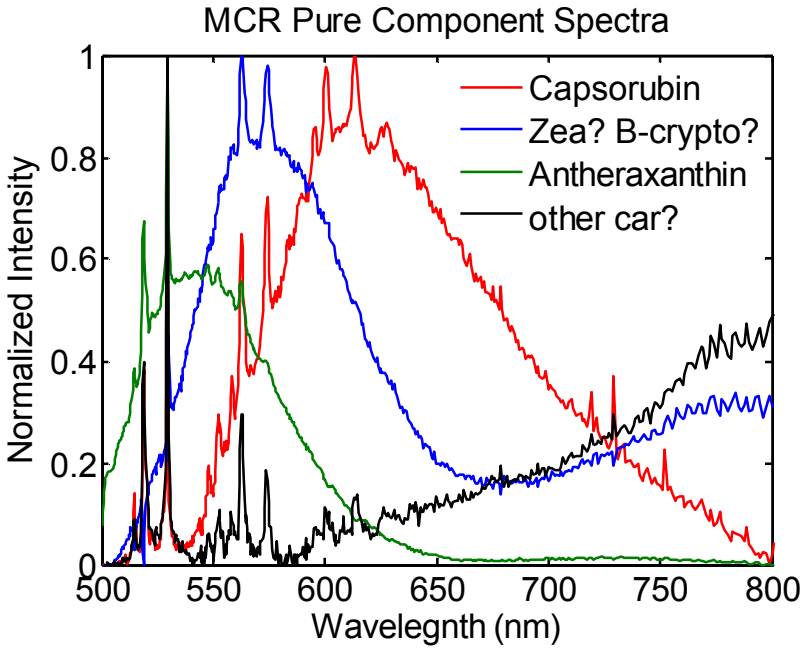


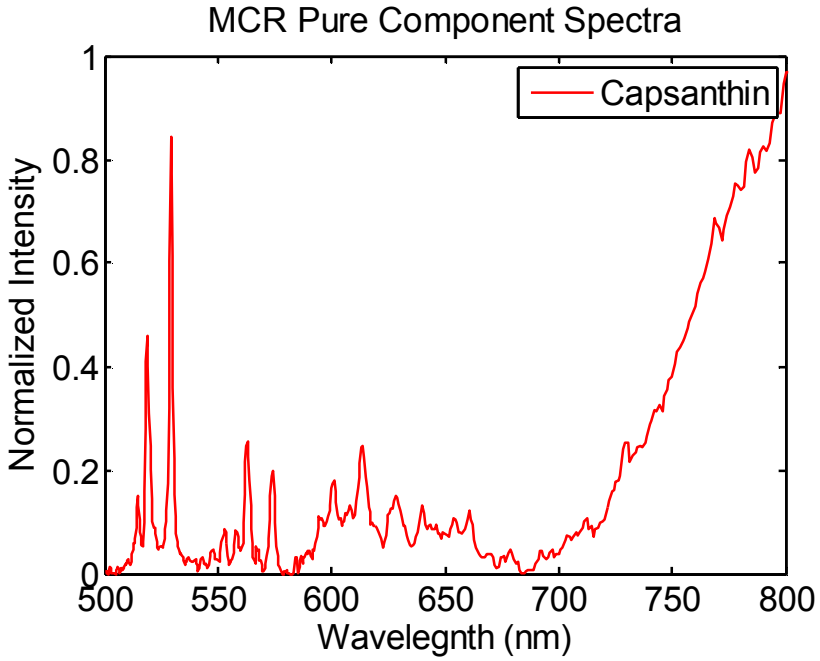






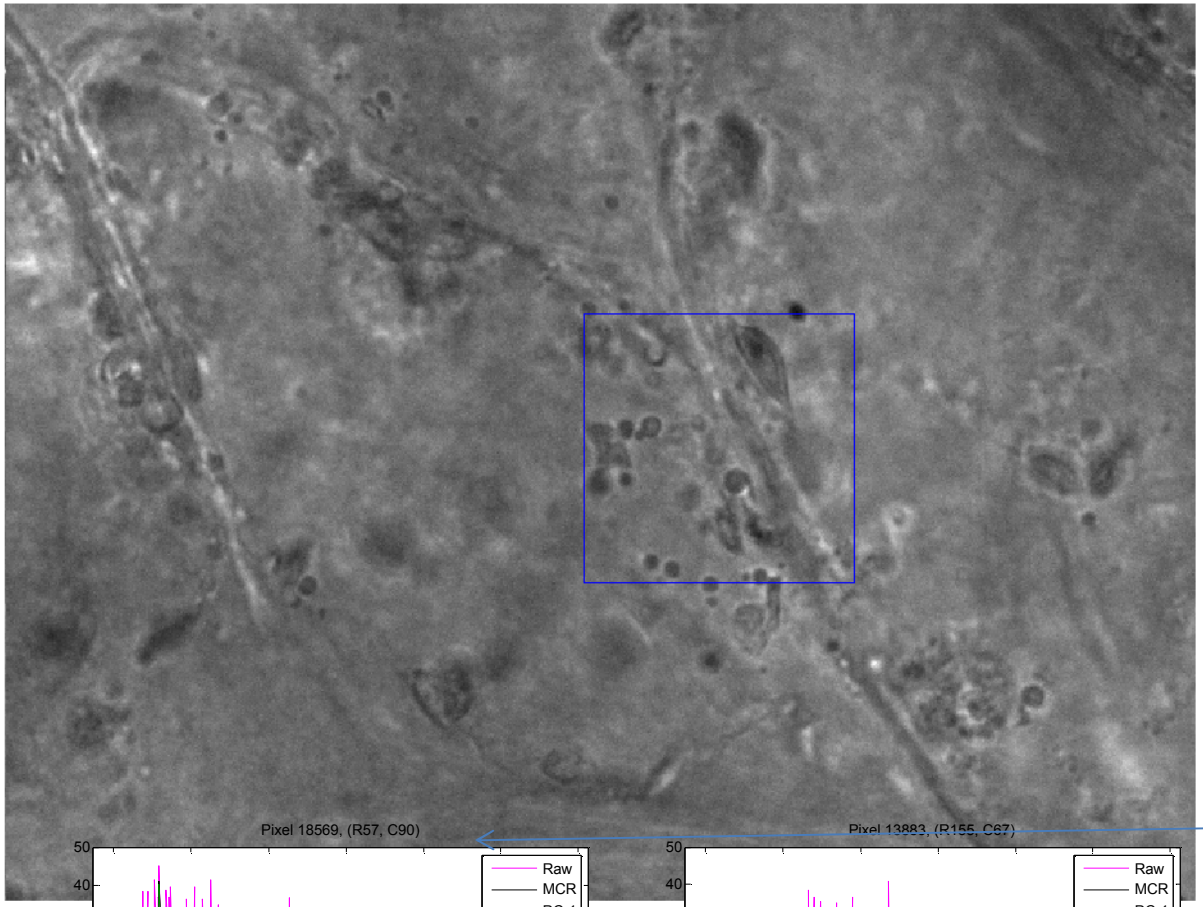
# Scorpion – 60X magnification

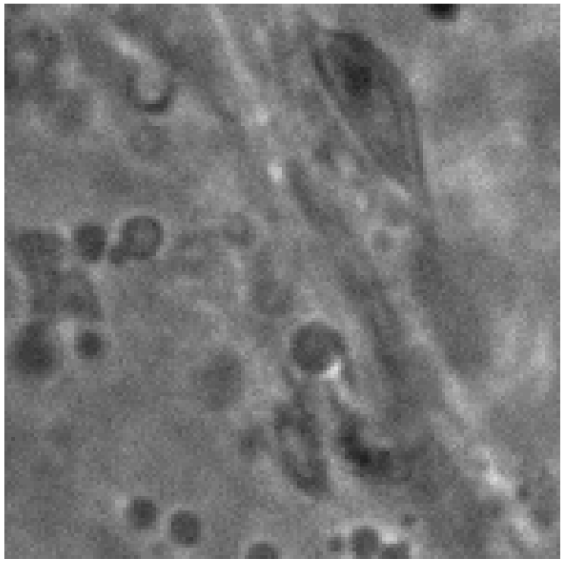


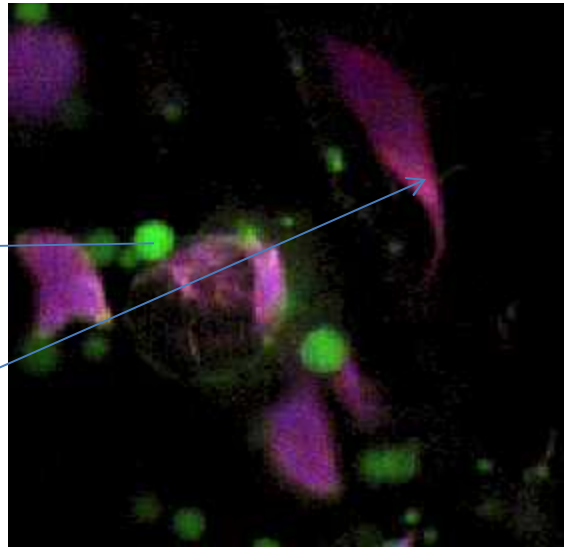



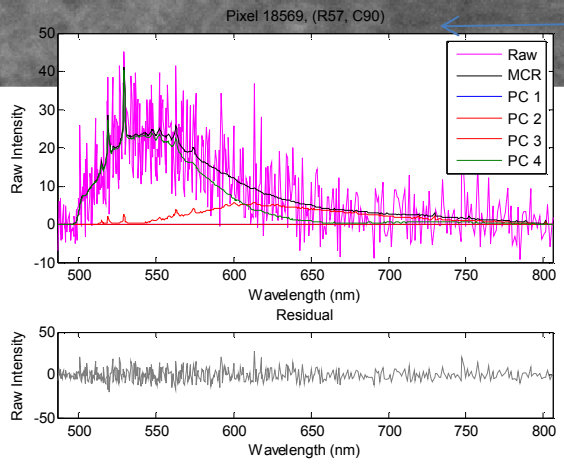

The analysis of the scorpion chili gave three components nearly identical to the red habanero; antheraxanthin and capsorubin and zea or B-cryptoxanthin. The additional component could be an additional carotenoid such as capsanthin but that is only a guess at this point.

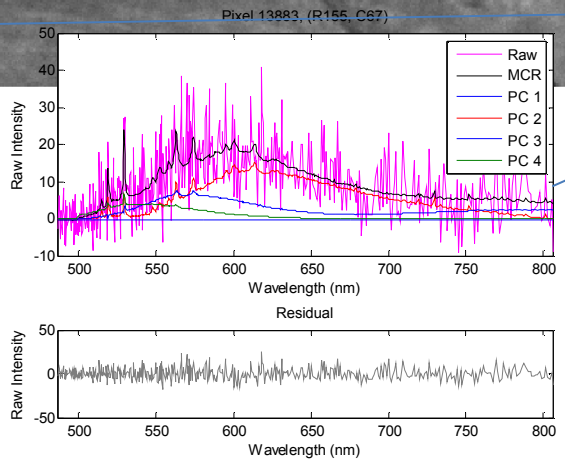
# Scorpion – 60X magnification



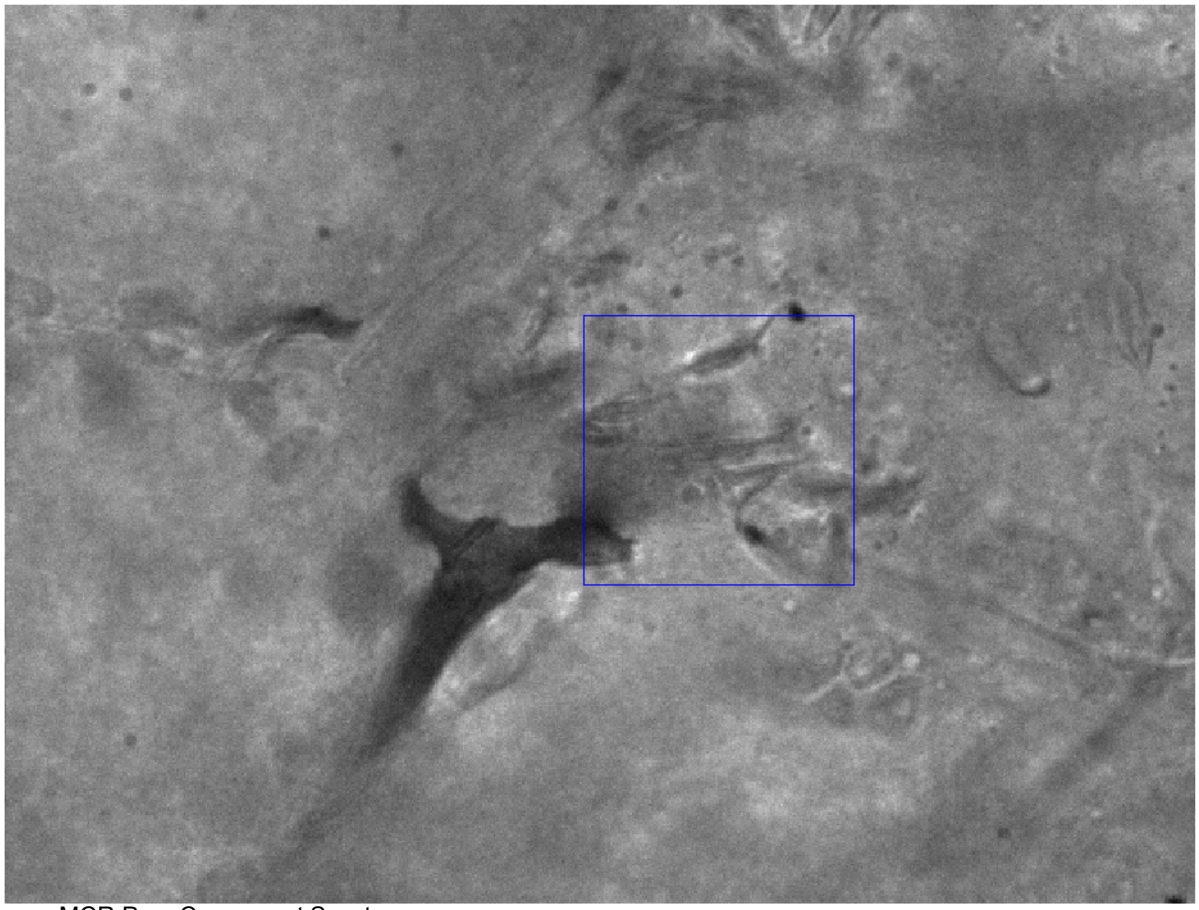


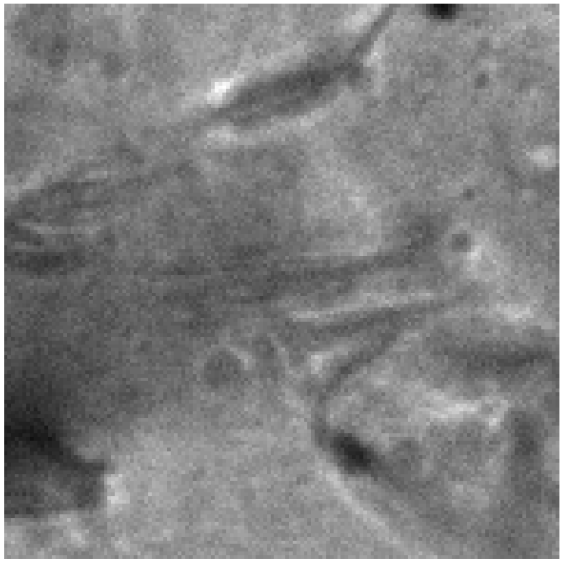


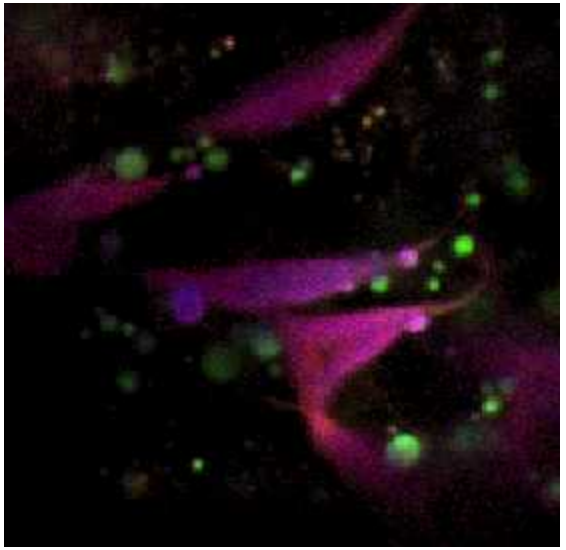


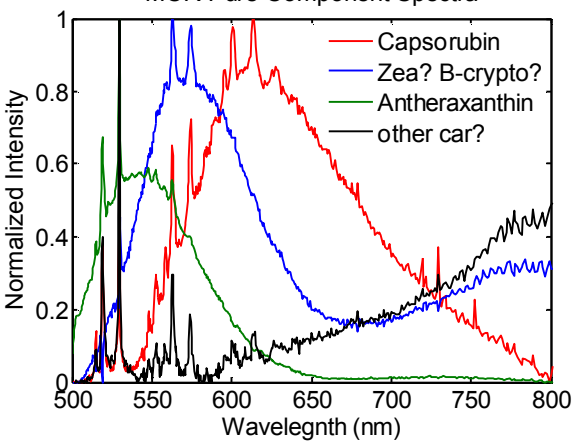
# Scorpion – 60X magnification



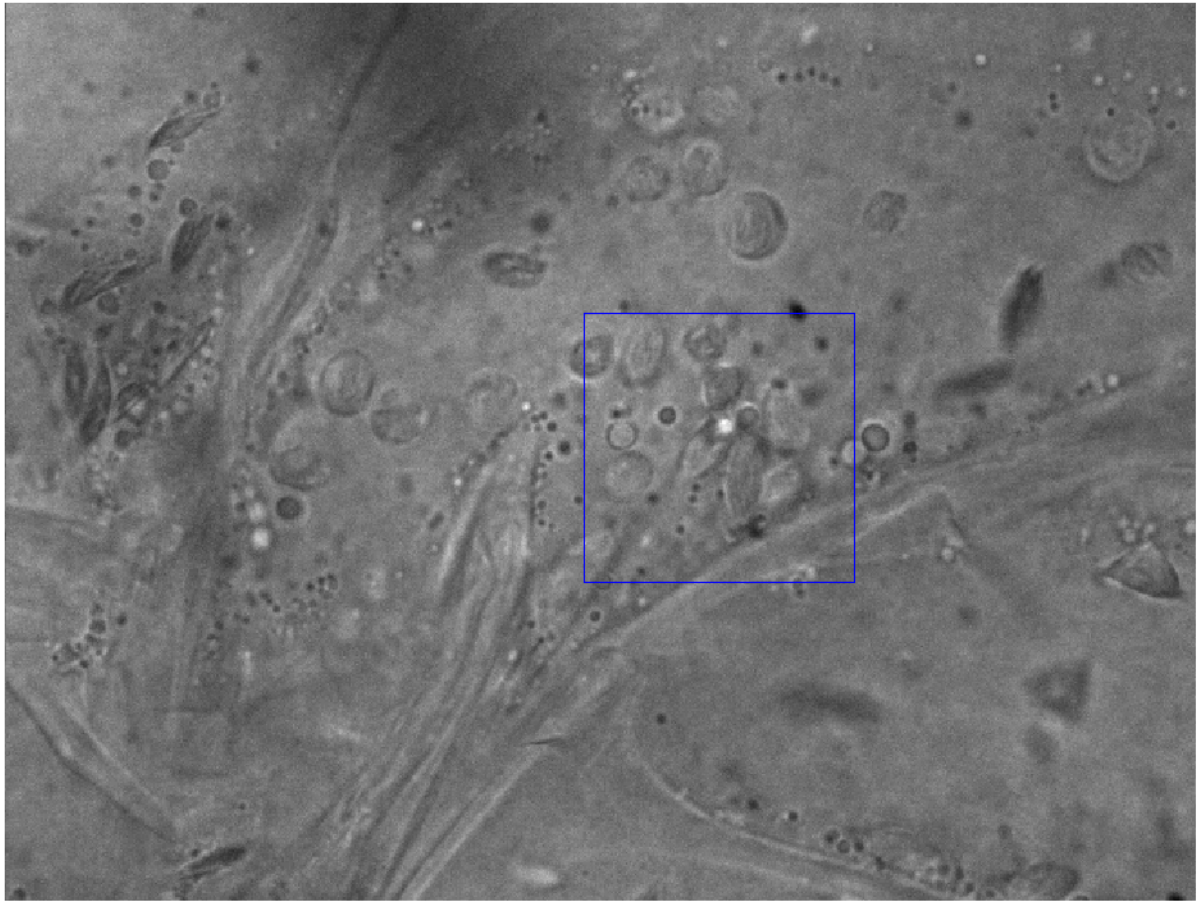


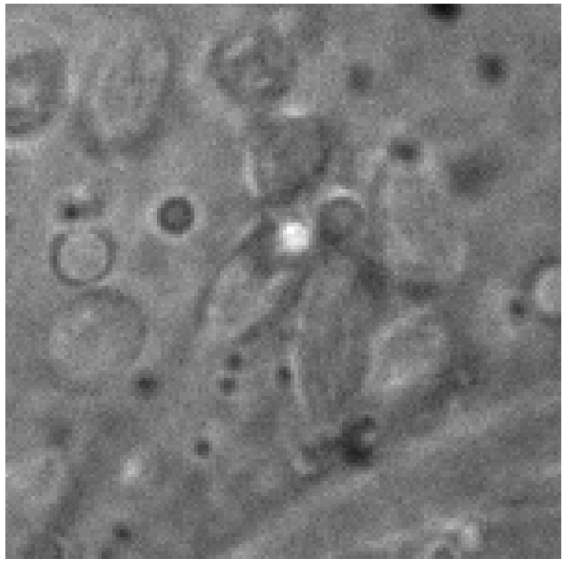


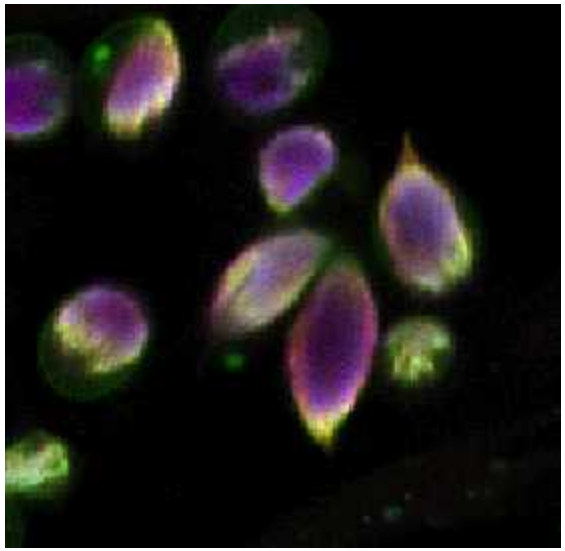
MCR Pure Component Spectra

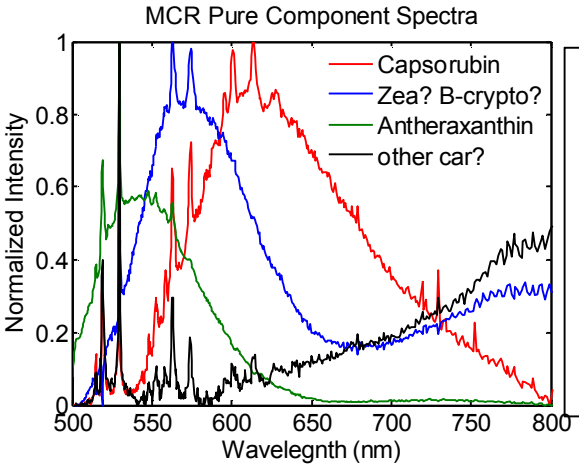


# Scorpion – 60X magnification

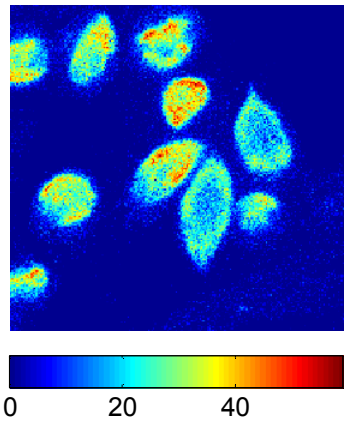




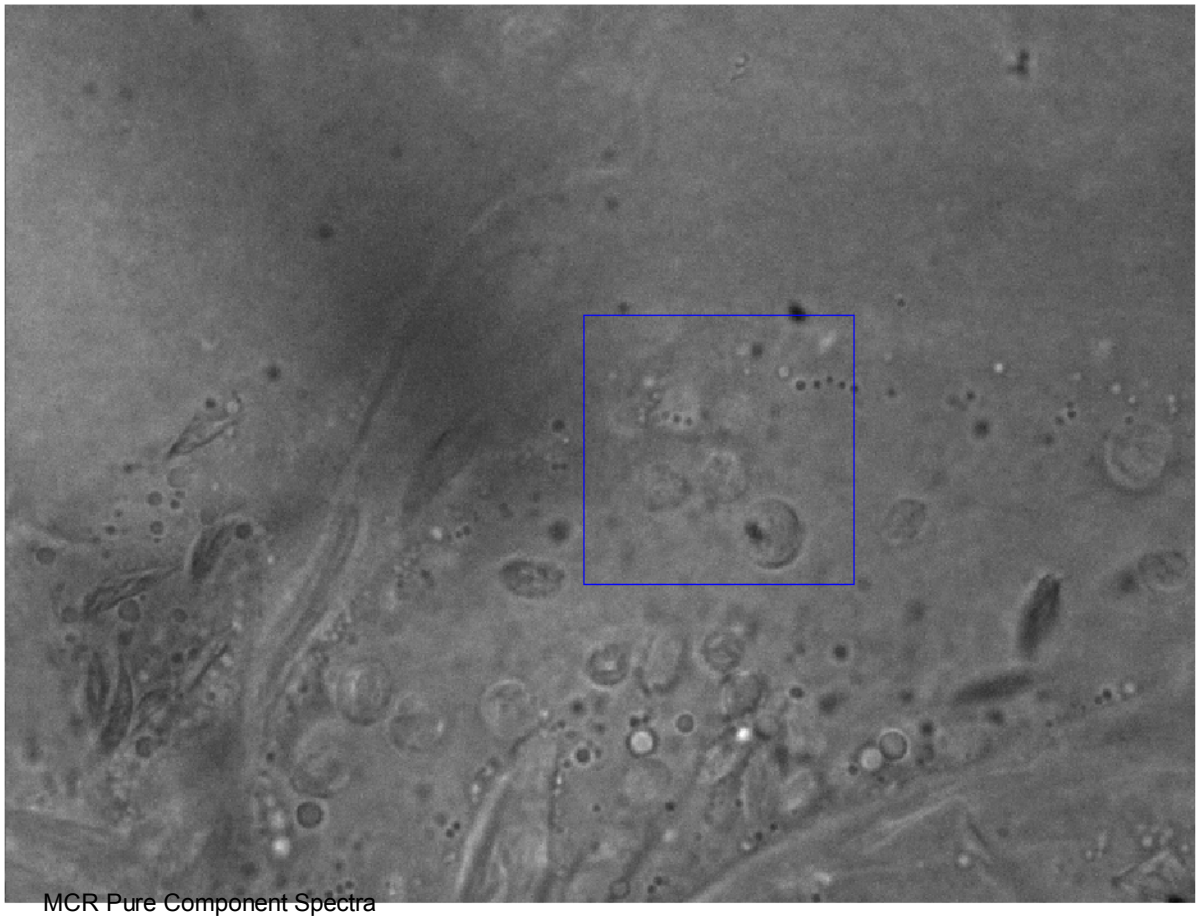


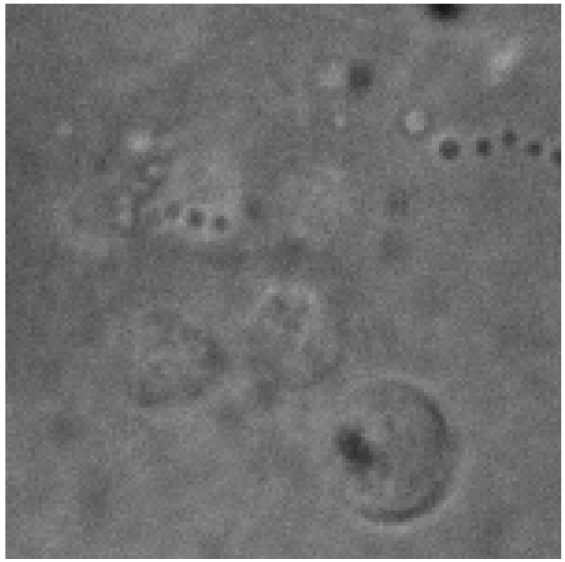


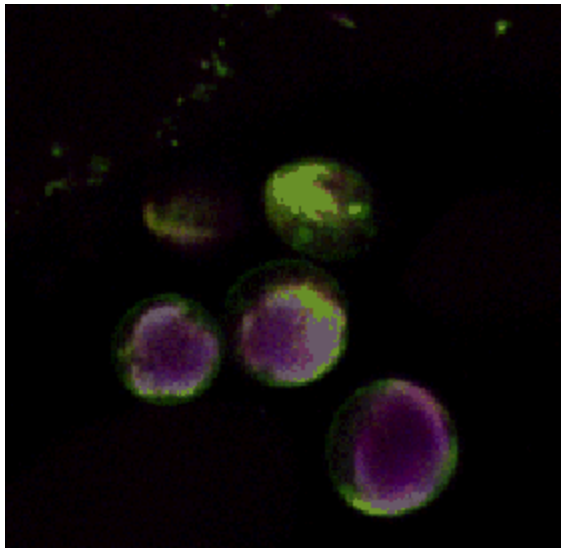

location of “other car” component. Seems to be associated with blue and red pixels.

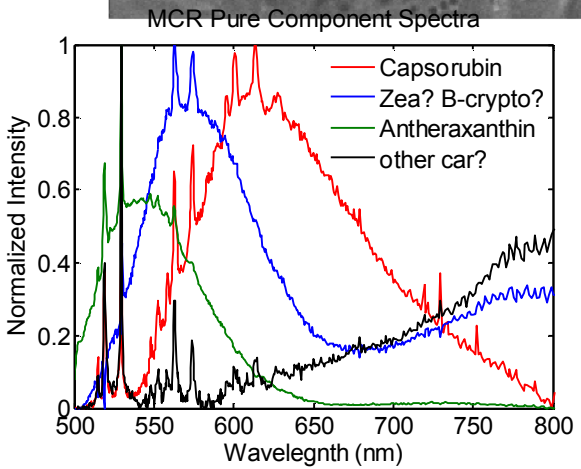


# Scorpion – 60X magnification

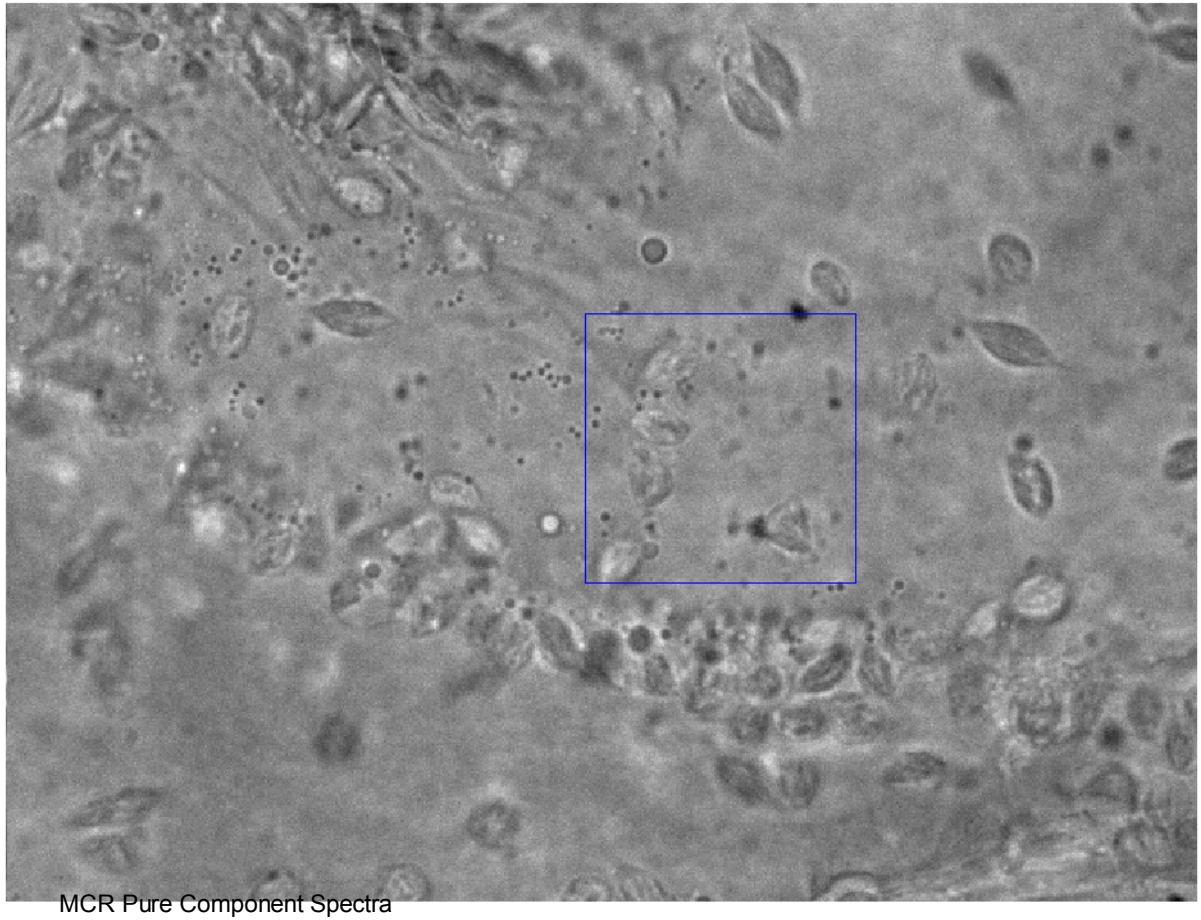


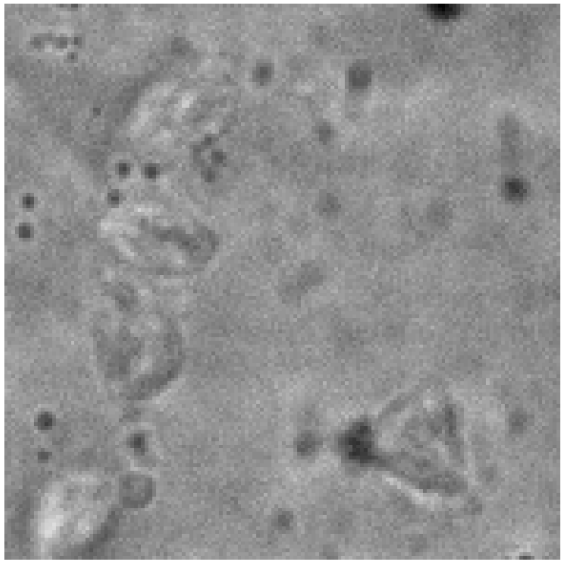


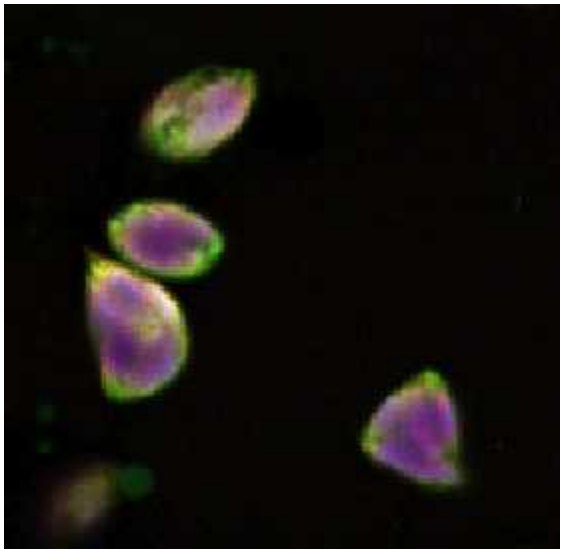


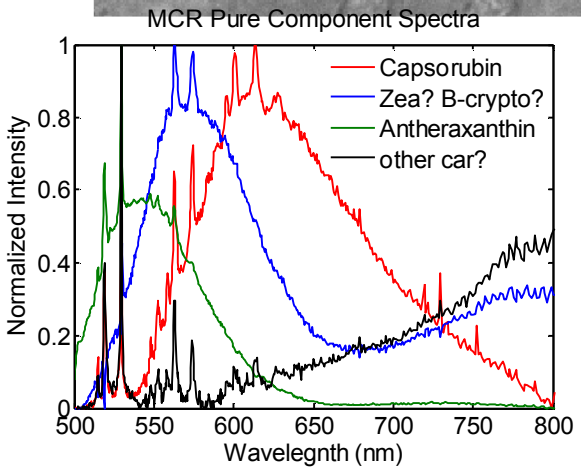


# Scorpion – 60X magnification









- We can resolve several carotenoids from a each chili type although assignment of the component spectra needs verification
- These peppers contained no Chlorophyll. Chl is very spectrally distinct from the carotenoids that resolving it should be straightforward.

- Next steps: Raman spectroscopy in carotenoid standards and Raman imaging on chili flesh.