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Analysis of chicken retina and carotenoid standards with spectral imaging

Imaging and analysis -Aaron Collins

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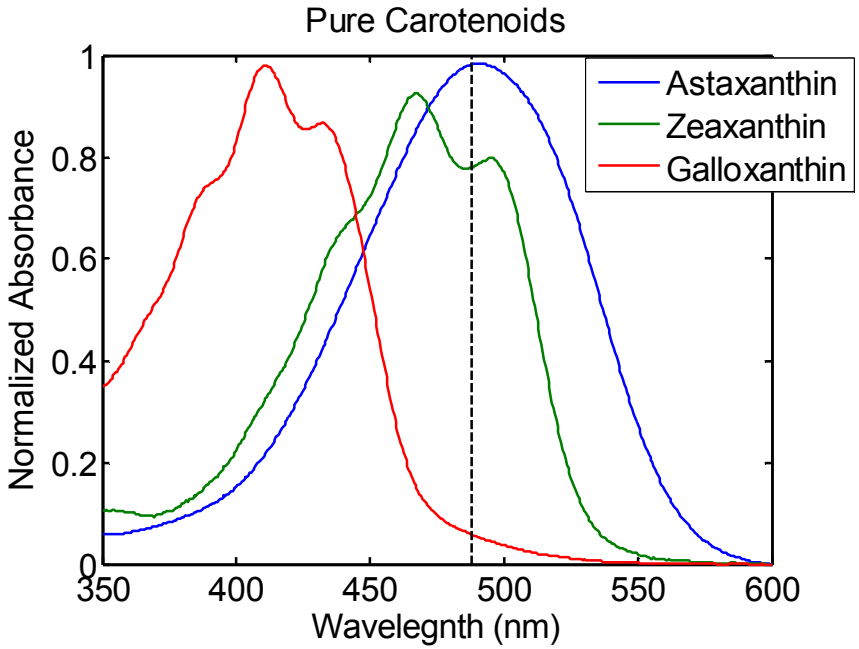
Absorbance of pure carotenoids dissolved in pyridine.



Laser wavelength for spectral imaging is at 488 nm and is indicated by the vertical dashed line.

Using extinction coeffs for
Astaxanthin = 1.12 E 5 M^{-1} cm^{-1}
Zeaxanthin = 1.3 E 5 M^{-1} cm^{-1}
Galloxanthin = 0.41 E 5 $M^{-1}cm^{-1}$

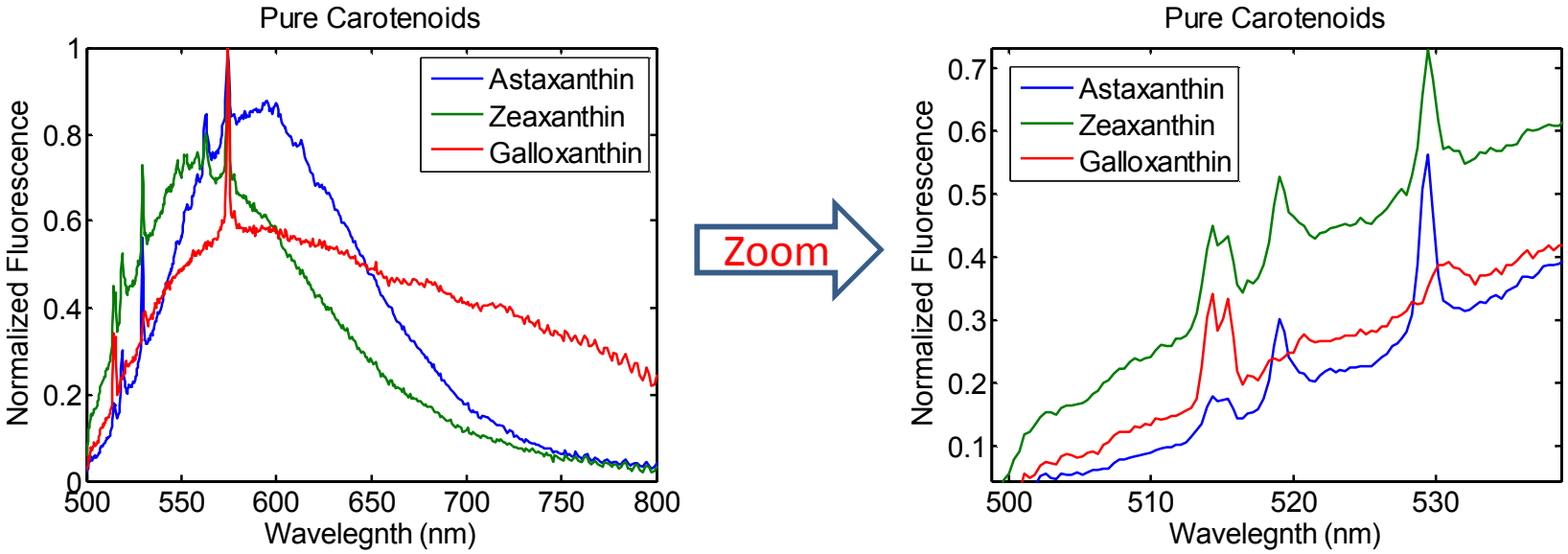
For galloxanthin this value assumes M. Toomey sent me 10 ug of pure galloxanthin which I dissolved in 400 uL of pyridine.

The Absorbance data and extinction coeffs yield standards of

Astaxanthin = 50 uM
Zeaxanthin = 8 uM
Galloxanthin = 25 uM

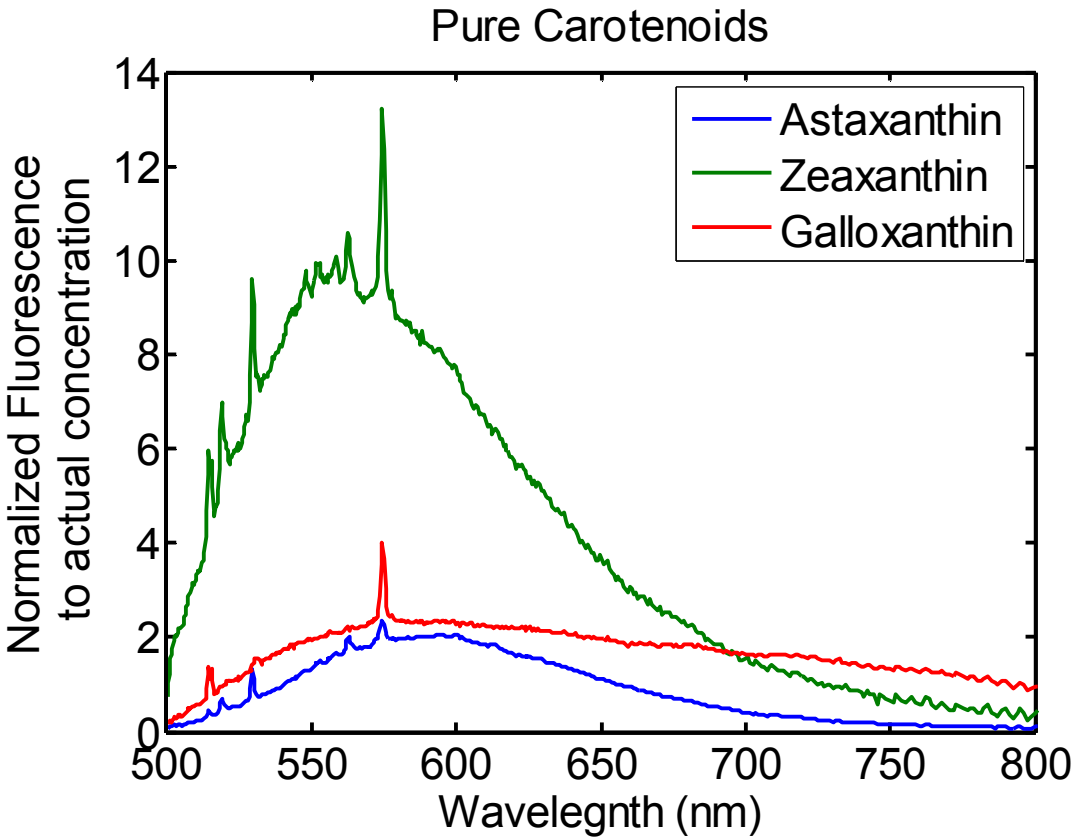
It would great to get the actual extinction coefficient for galloxanthin. Maybe it is in Carotenoids :handbook by G. Britton? We don't have access to this reference at SNL.

200 ul of each carotenoid was loaded to 1mm pathlength cuvette and placed under the microscope with a 10X objective. Several images were taken to assess our sensitivity to each of the carotenoids.



Here I am showing the normalized spectral (to max fluorescence peak) data for each carotenoid. Note that the galloxanthin $\nu_1$band is red-shift compared to the others. This is the expected direction of such a shift. The most intense galloxanthin Raman band at 575 nm is likely also is the ν_1 vibronic mode but is unique that ν_2 or ν_3 do not accompany it.

If we take the unnormalized data and weight it by the concentration then the resulting data look as follows

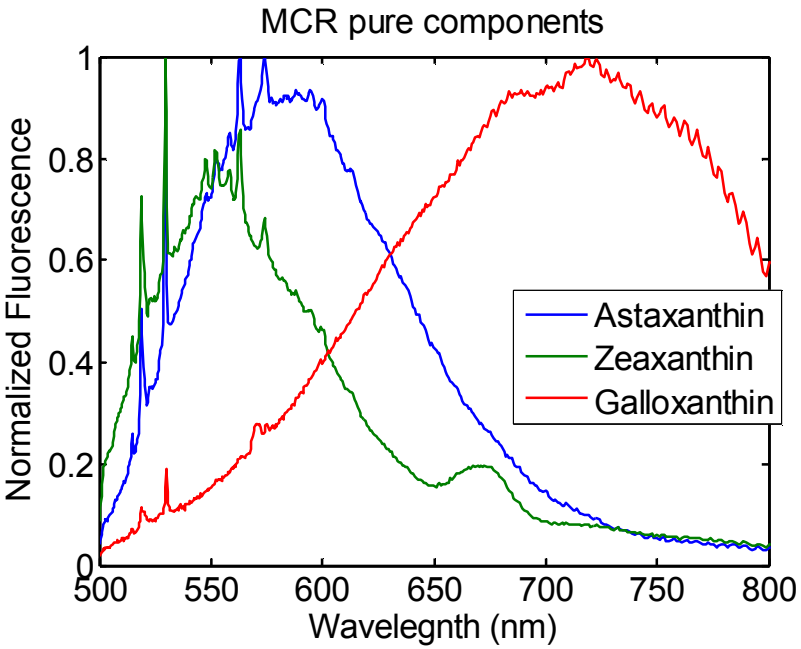


This plot shows that we should be sensitive to all three carotenoids but most sensitive to zeaxanthin by about 5X.

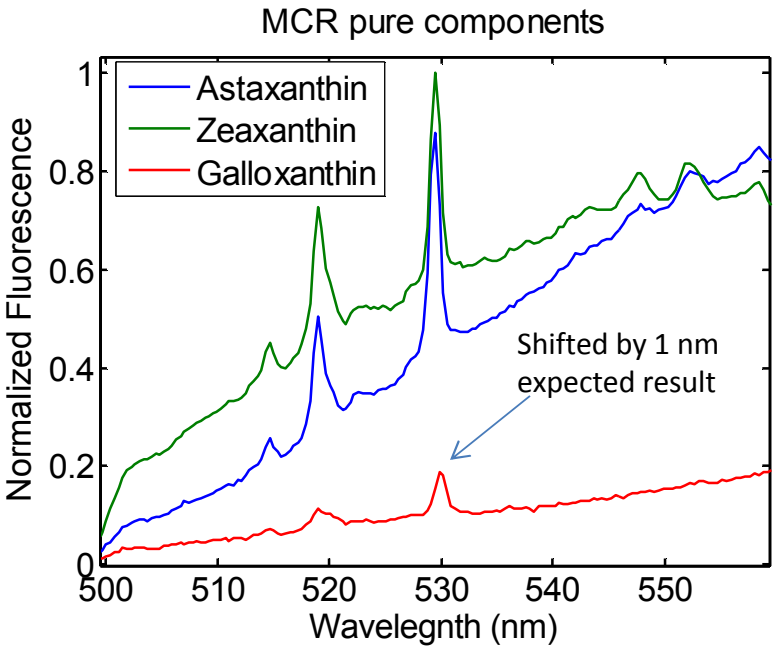
The units for the y-axis would be something like fluorescence per uM of carotenoid but this is not a metric that can be used quantitatively when analyzing retina images.

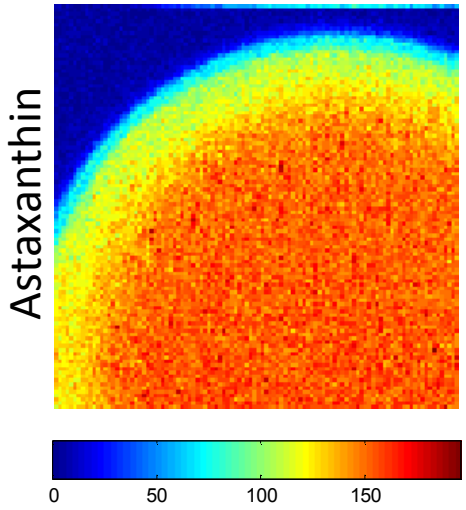
Also note that each carotenoid has a unique fluorescence that is superimposed on the Raman bands. This will likely aid in identifying these components of the retina images.

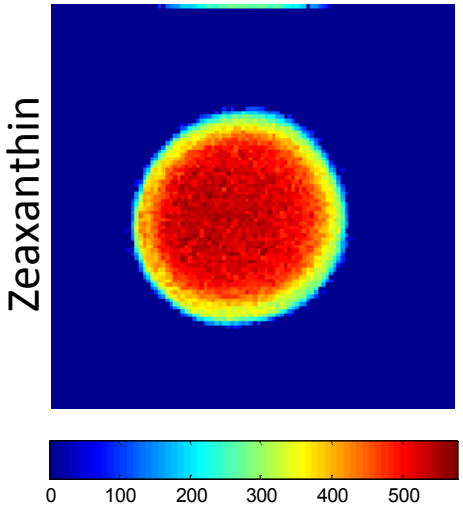
Carotenoids were dissolved in microscope immersion oil and then emulsified in 50 mM tris (pH=8). 4 ul aliquots were loaded to a microscope slide, covered with a #1.5 cover glass and imaged on the HSI using the 60x (NA=1.4) objective. Each carotenoid was analyzed individually using MCR.

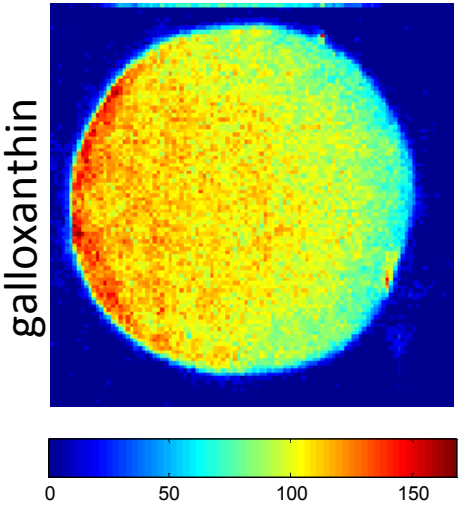


Zoom



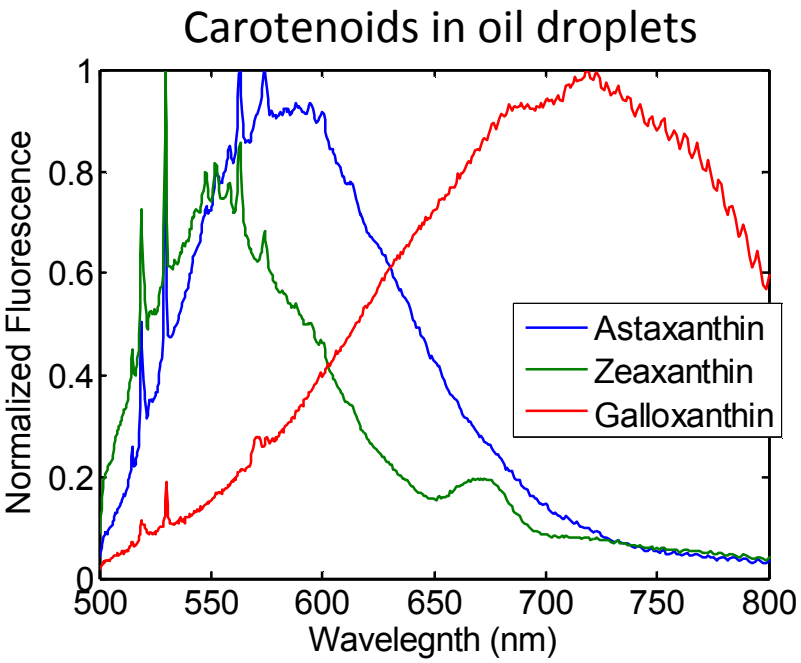


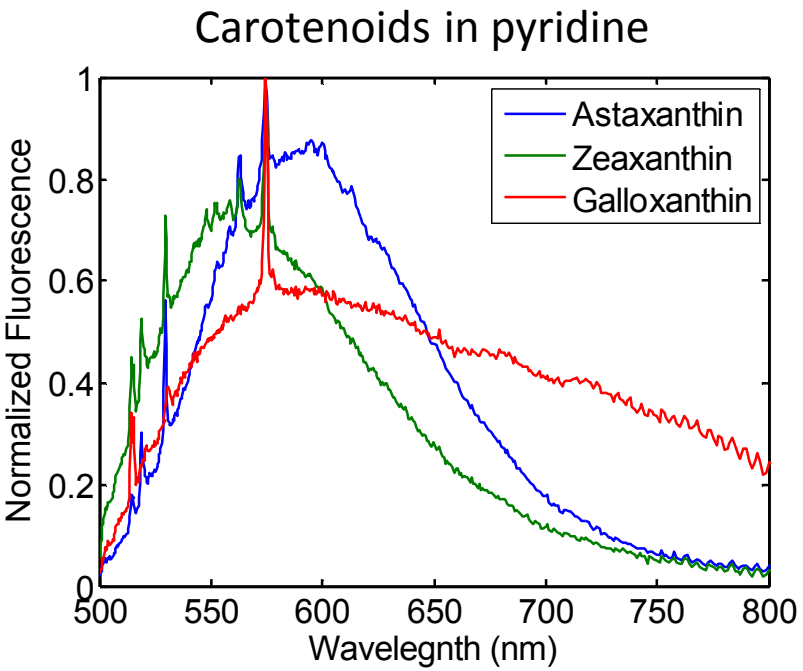




Each image is 12.5 um on edge

Comparison of carotenoid spectral signatures in oil droplets (left) and pyridine (right)

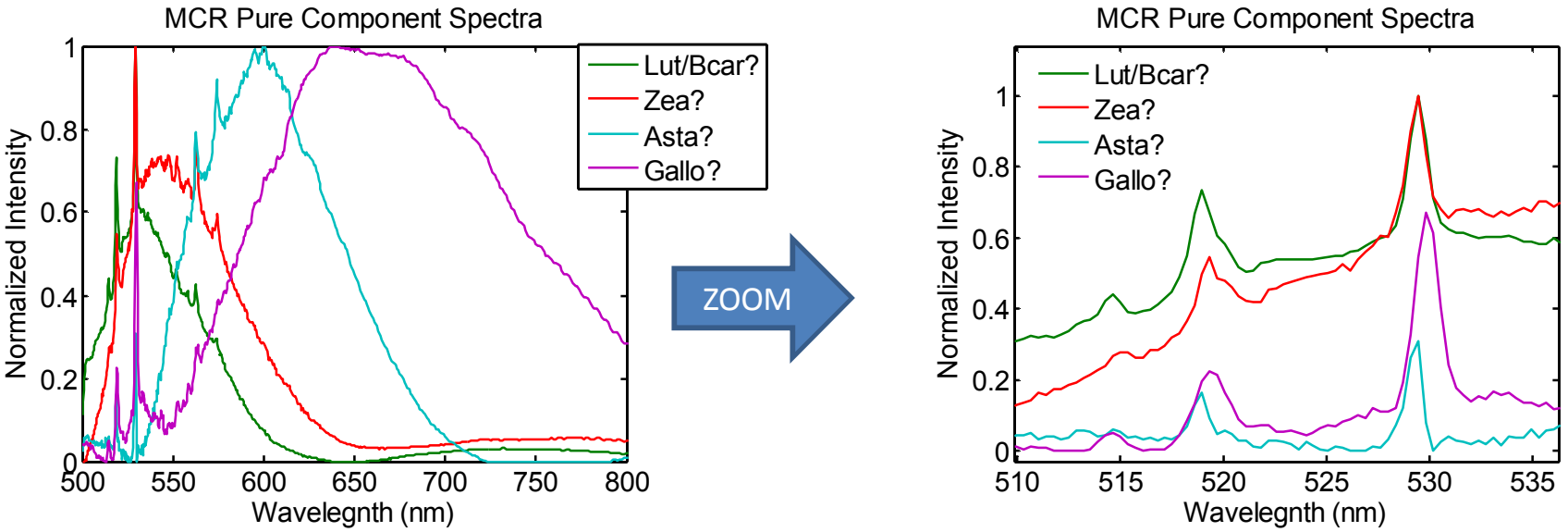




Astaxanthin and zeaxanthin show relatively little change when dissolved in the two solvents however, galloxanthin shifts its fluorescence significantly. I am not sure why.

Multivariate analysis of retina spectral images

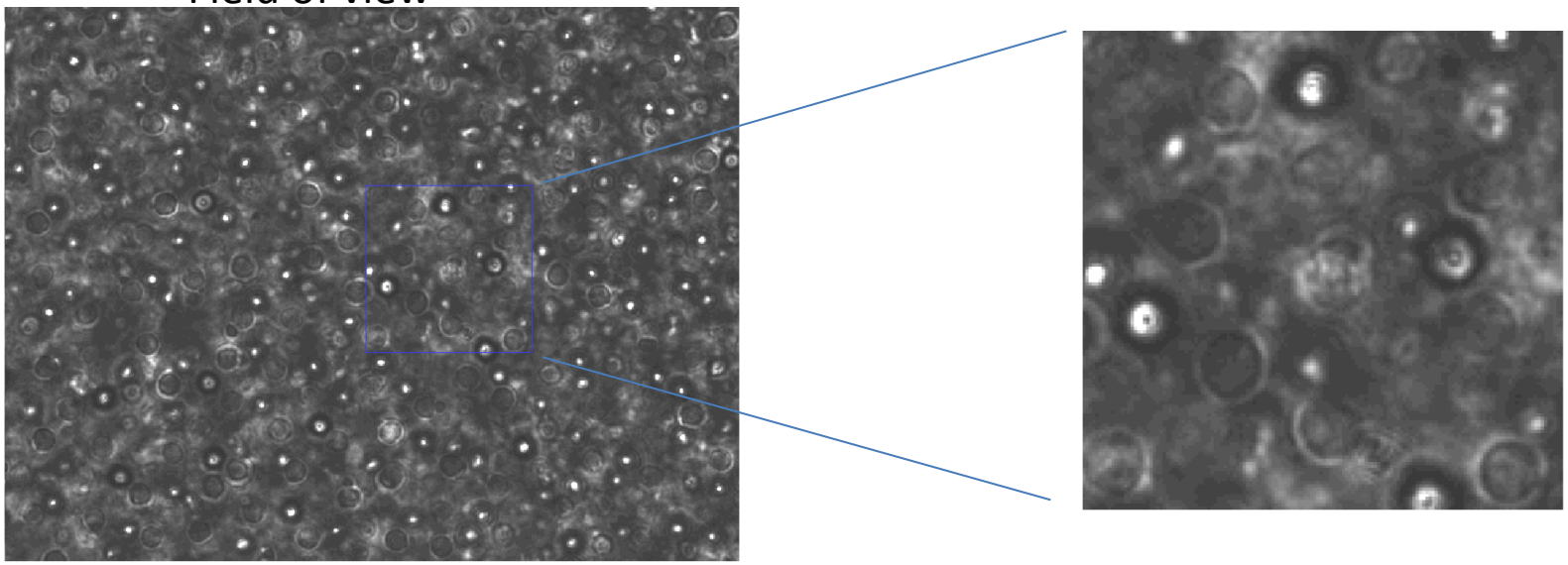
~20 different optical sections with axial spacing of 1 um were taken. Spectral images were combined into a composite dataset. The data were preprocessed to mask off regions not of interest (devoid of signal), despiked of cosmic rays and corrected for the microscope noise structure. Multivariate curve resolution was used to unmix the spectral data. Below are the spectral components that can describe >99% of the spectral variance in the data. This model was developed de novo, in other words, I did not use any guesses to fit this data.

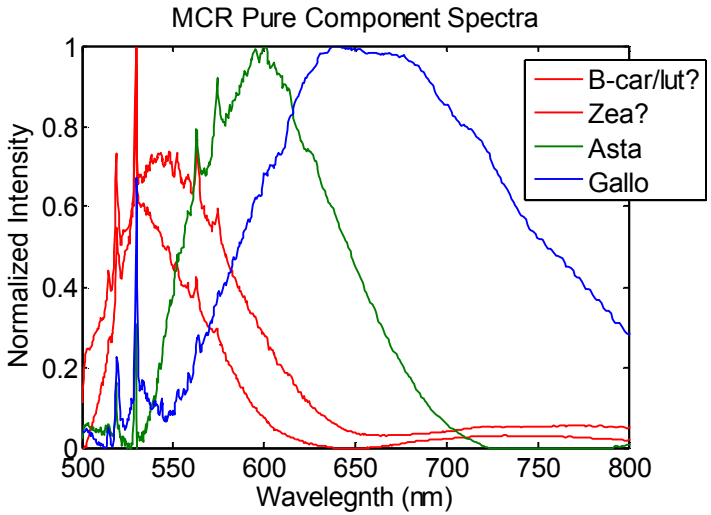


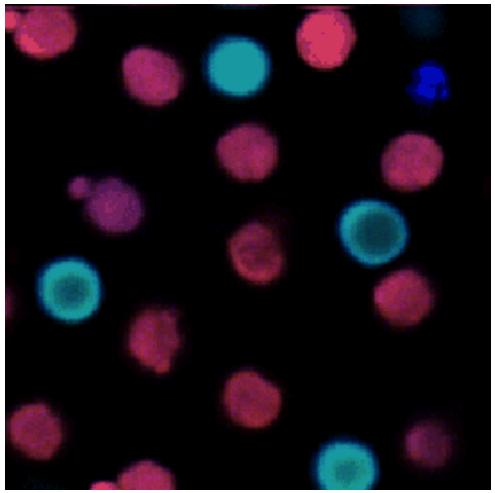
Very encouraging to see the 1 nm shift in v_1 for galloxanthin!

Example visualization of the data… more will be separately.

Field of view



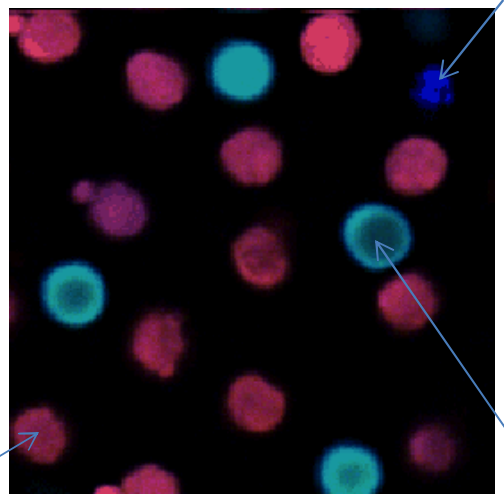


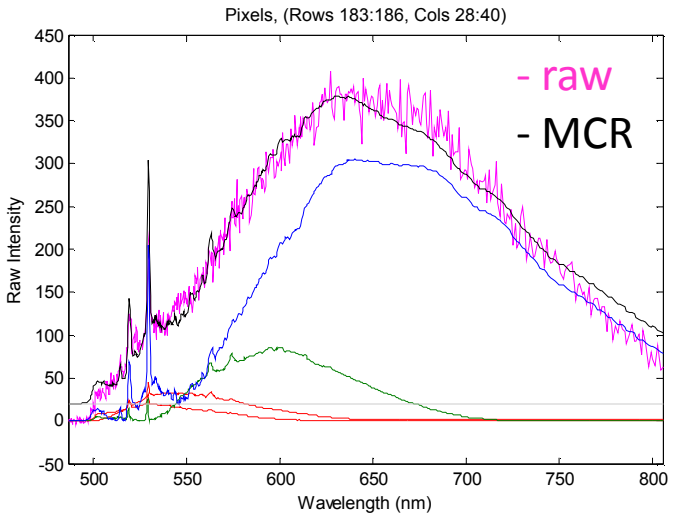


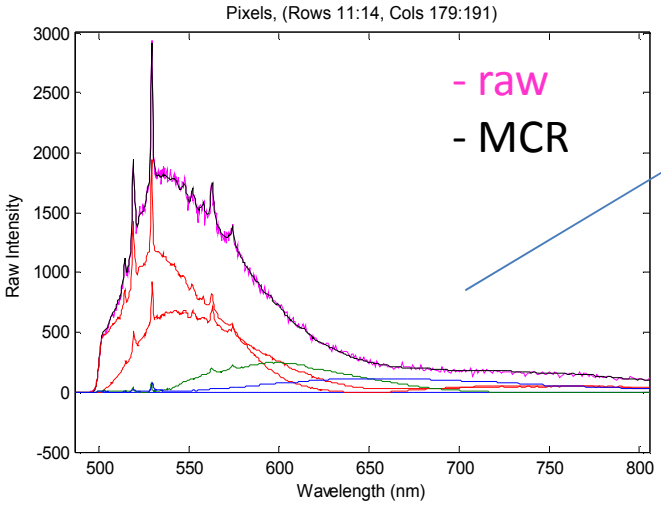
RGB color scheme for RGB image →

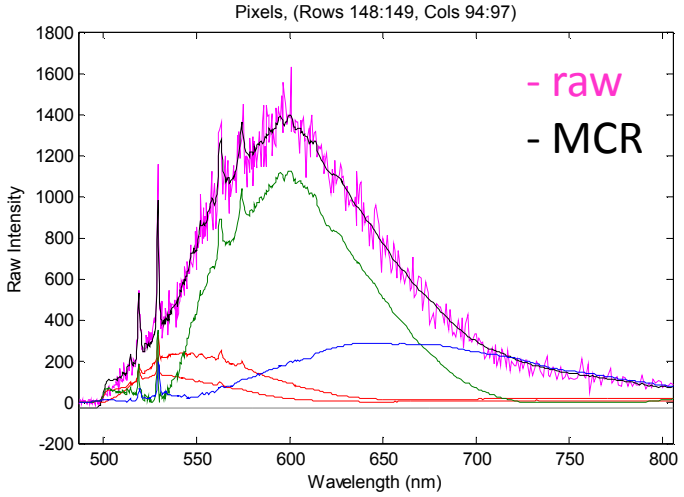
How well are we fitting the data?

From this single image, we can see there are at least 3 oil droplet types and each has a unique spectral profile. Note: A little bit of each spectral shape is in each oil droplet. Is this real i.e. are the oil droplets heterogeneous or is this spectral mixing due to the MCR model being insufficient?









Here, I am averaging several pixels within an individual oil droplet and showing how the MCR model is fitting the data.

Comments:

So here is where we stand. I have more data that I could analyze but I wanted to get something to you in the interim. Almost all of the data I have are optical sections into the retina. As I mentioned to you on the phone, there appears to be a depth dependence for the oil droplet type.
We are very sensitive to zeaxanthin due to the resonance effect. Also, the carotenoid that I labeled lutein/b-carotene is a guess based on prior experience with the spectral shape for this carotenoid. I need you to tell me if that is reasonable or not. We would be expected to be rather sensitive to either on this instrument.

Other analysis we could do would be per-oil droplet statistics for the MCR components or construct 3-d rendered images…but of course all of this will take time.

I am going to send you stacked *.tif files for each image stack that I have analyzed to far. These will represent the concentrations for each MCR component and not be RGB images. I figured you can visualize the data however you see fit. Please let me know if you can access them, they will be sent from an FTP server.

Let’s chat sometime next week about a path forward; do these results make sense? Do they represent something new and how can we finalize the analysis.