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Analysis of carotenoid lipid droplet and zebra finch retina

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Goals/Notes:

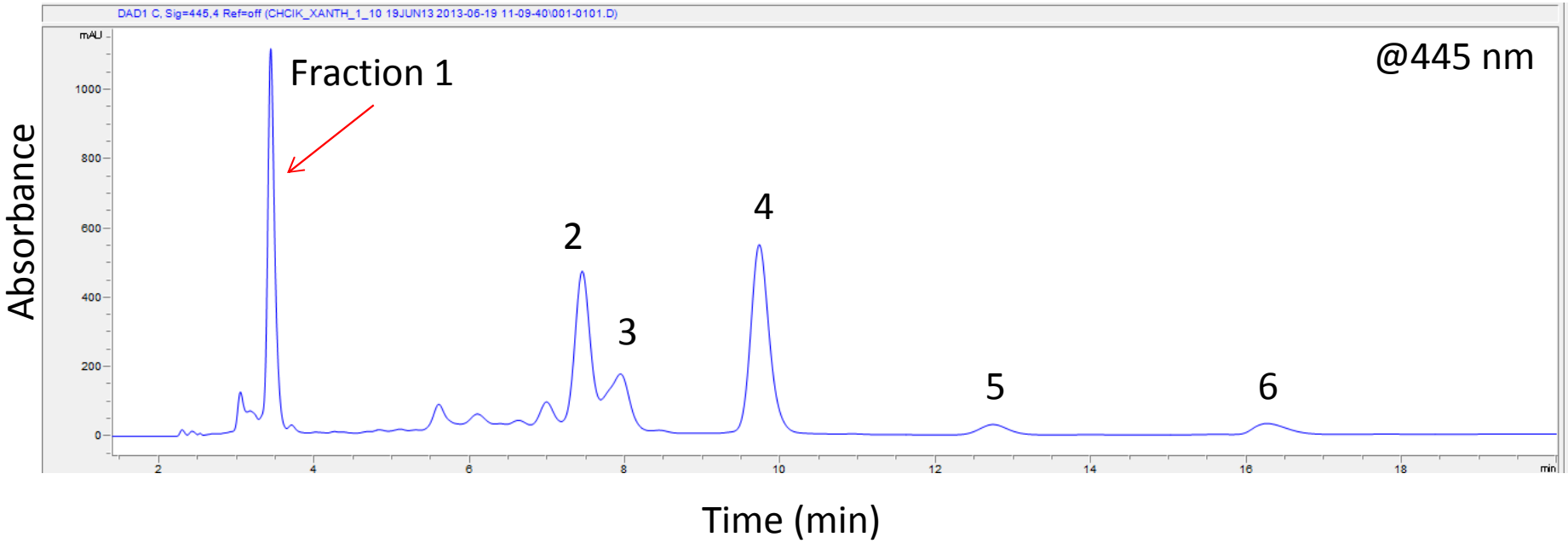
Initial Raman analysis of chicken retina gave promising results and spurred additional Interest in investigating the Zebra Finch retina.

The purpose of this study was two fold;

1) Build a Raman library with purified carotenoids suspended in artificial lipid droplets

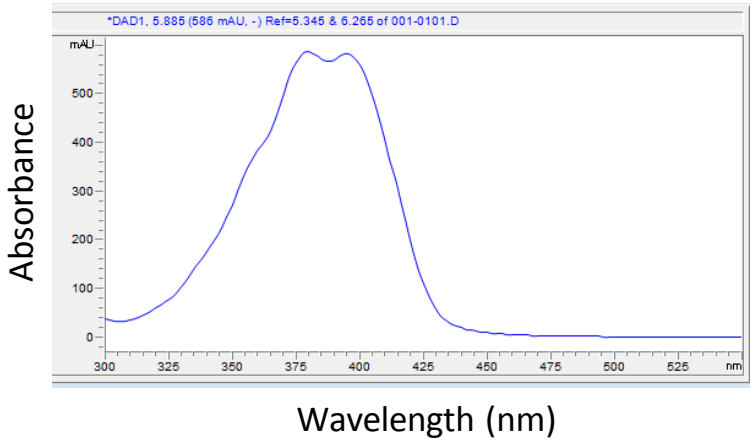
2) Obtain Raman spectral images on the Zebra Finch retina paying special attention to the pale oil droplets.

Reverse-phase HPLC of chicken retina saponified with 0.2M NaOH (recovers apocarotenoids and xanthophylls)

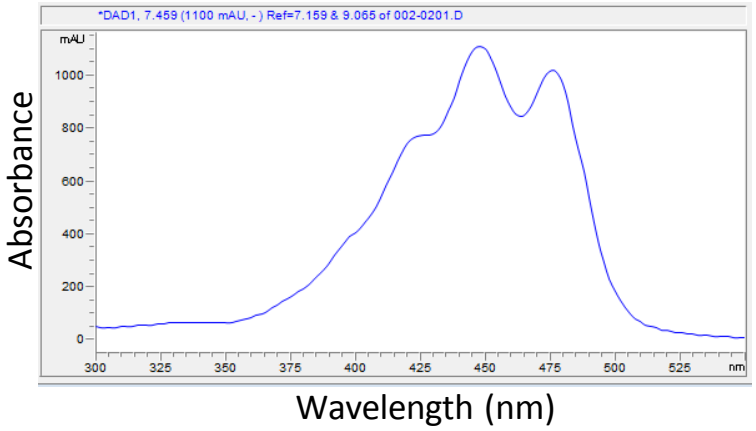


Data from M. Toomey

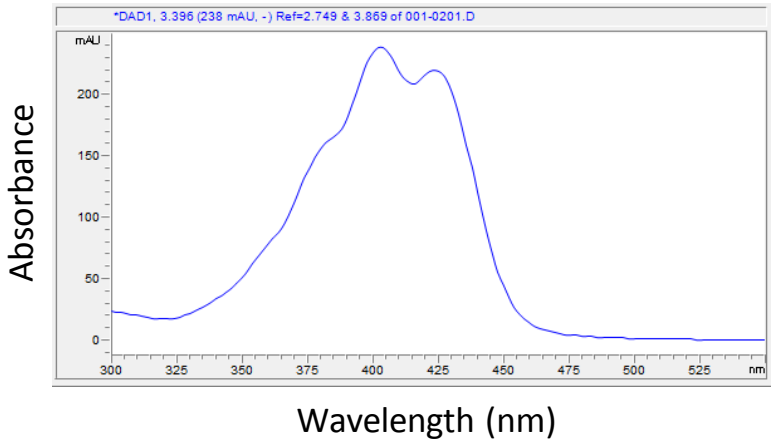
Fraction 1d – dihydrogalloxanthin from zebra finch retina



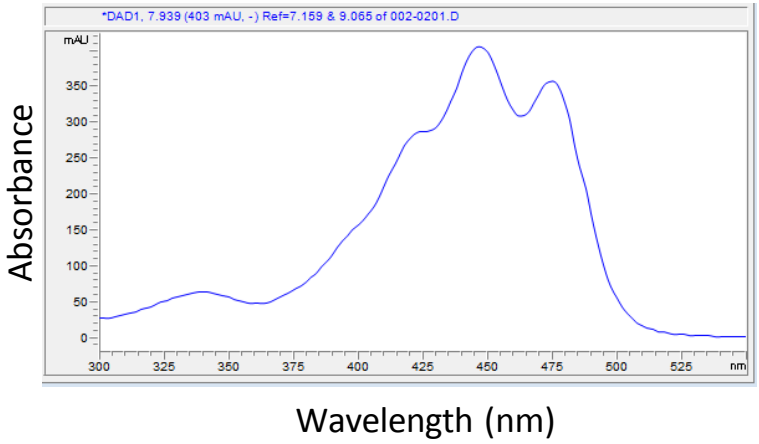
Fraction 2 - Lutein



Fraction 1 – Galloxanthin from chicken retina

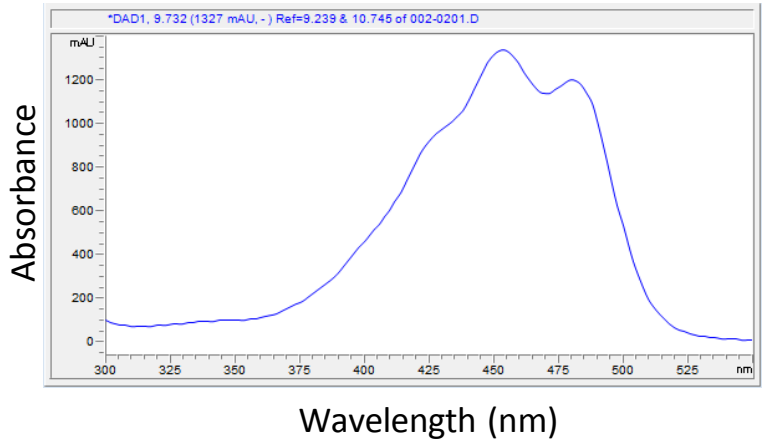


Fraction 2 – *cis*-Lutein

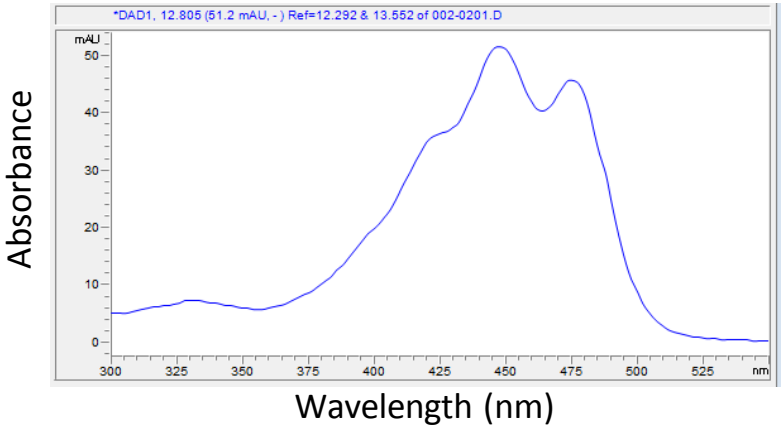


Data from M. Toomey

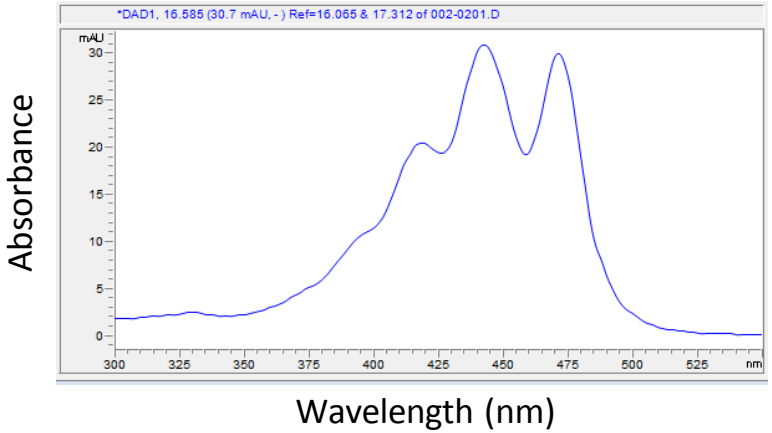
Fraction 4 - Zeaxanthin



Fraction 5 - unknown

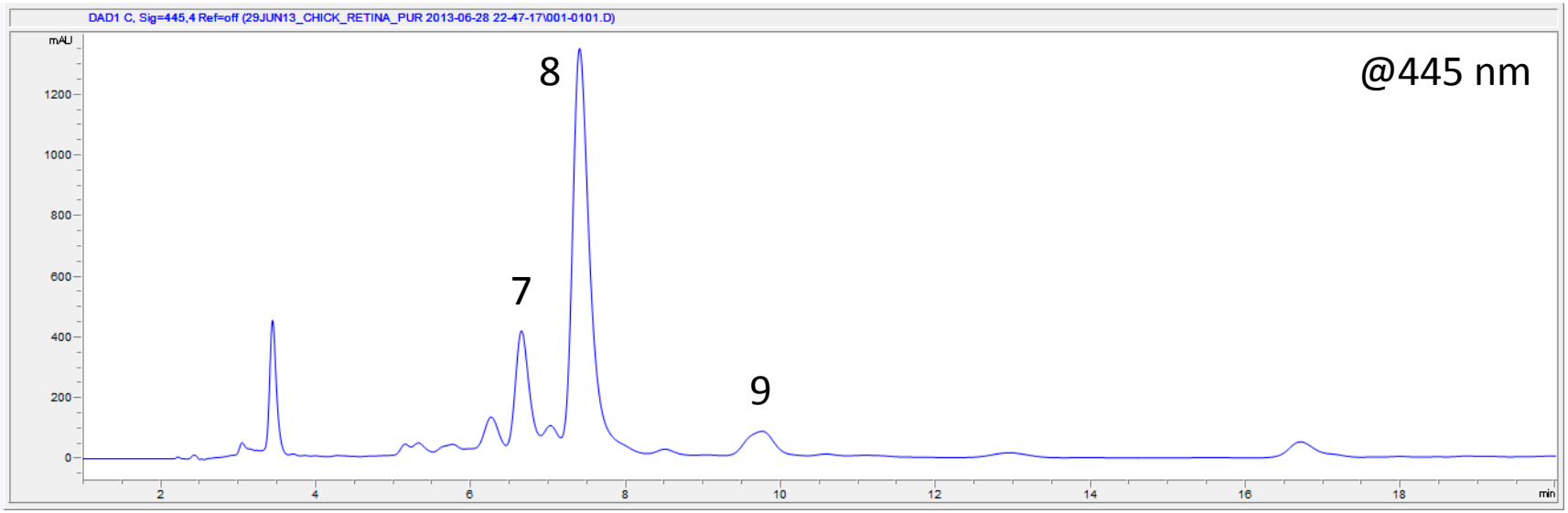


Fraction 6 – ε,ε - carotene



Data from M. Toomey

Reverse-phase HPLC of chicken retina saponified with 0.02M NaOH (recovers ketocarotenoids)

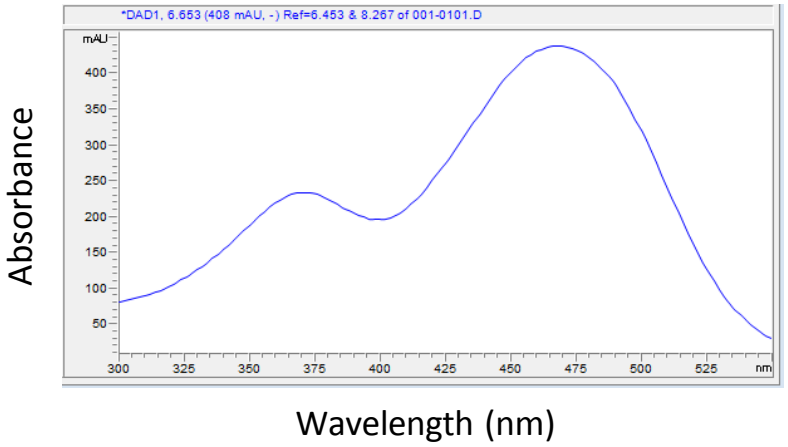


Absorbance

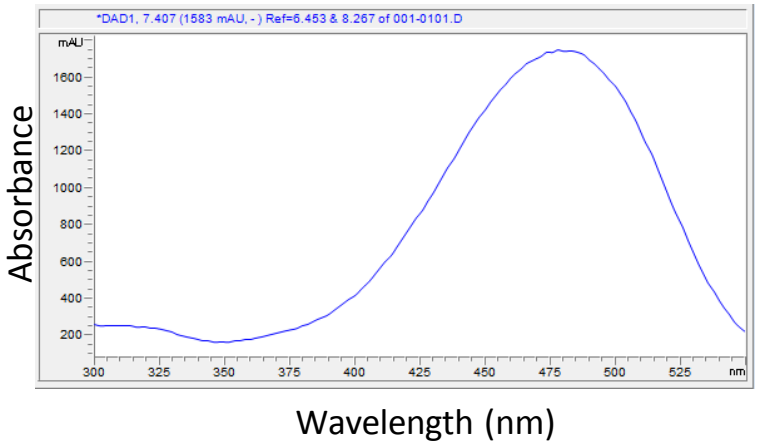
Time (min)

Data from M. Toomey

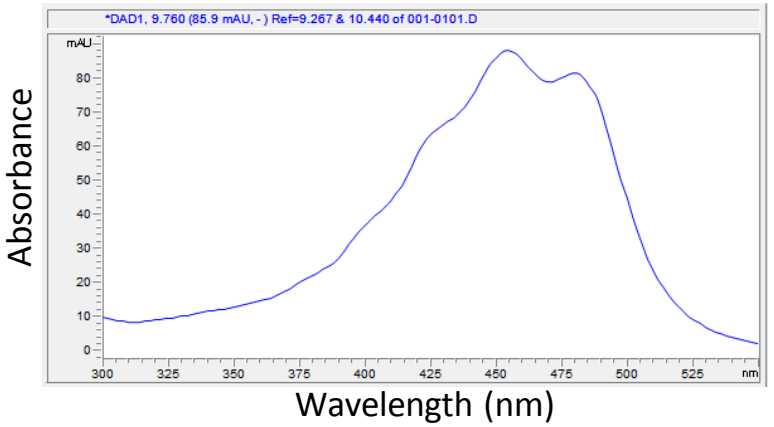
Fraction 7 – *cis*-Astaxanthin



Fraction 8 – Astaxanthin



Fraction 9 - Zeaxanthin



Data from M. Toomey

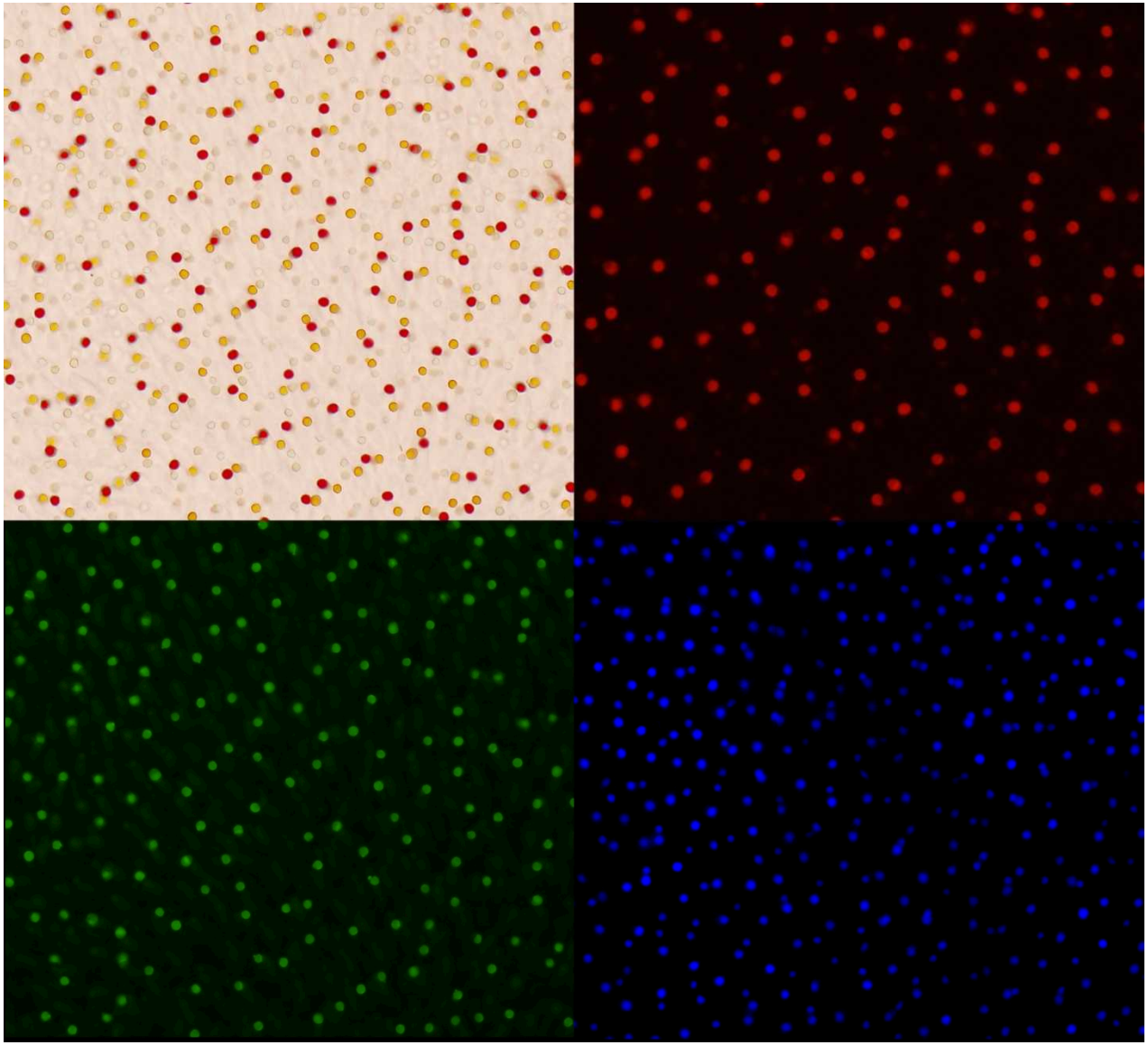
These were the samples sent to me.
I split the galloxanthin into 2x 8.2 ug fractions and zeaxanthin into 3 x 3.1 ug

Fraction	putative identity	Amount (micrograms)
1	galloxanthin	16.4
2	Lutein	7.3
3	cis-Lutein	4.1
4	Zeaxanthin	9.3
5	Unknown	0.9
6	e-carotene	0.7
7	cis-Astaxanthin	1.1
8	Astaxanthin	5.5
9	Zeaxanthin	0.8
1d	dihydrogalloxanthin	2.8
at-ROL	all-trans-retinol	10

Data from M. Toomey

Flat mounted zebra finch retina

Brightfield



520-550 nm excitation

460-490 nm excitation

327 nm excitation

Data from M. Toomey

Lipid droplet protocol

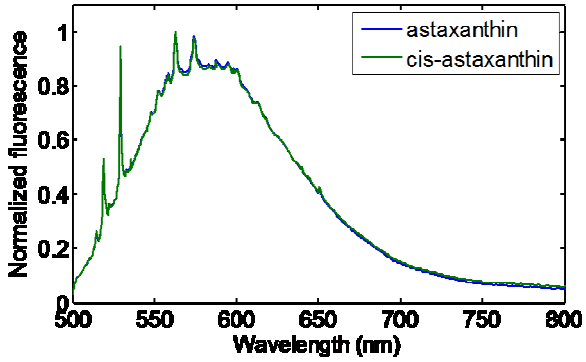
50 mg of glyceryl trilinoleate dissolved in 5 ml of 2:1 chloroform:methanol
Aliquoted into 500 ul fractions and capped under N_2. Stored at –20C.

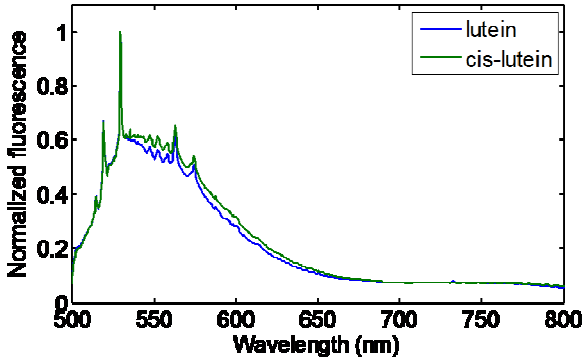
25 ul of stock TAG (above solution) and an additional 50 ul of 2:1 chloroform:methanol added to dried carotenoid. Sample was vortexed and then dried under a stream of N_2.

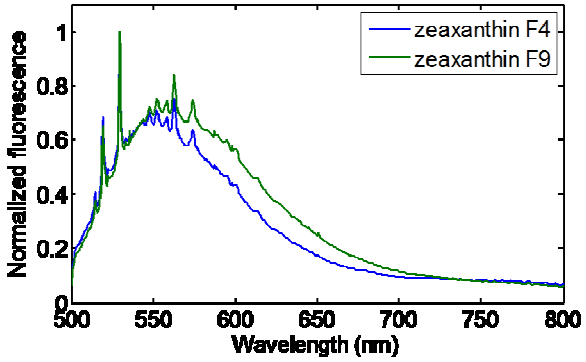
20-50 ul of PBS buffer was added to the above sample, vortex for 20 seconds and 10 ul was added to the microscope slide. Covered with #1.5 cover glass and sealed with nail polish.

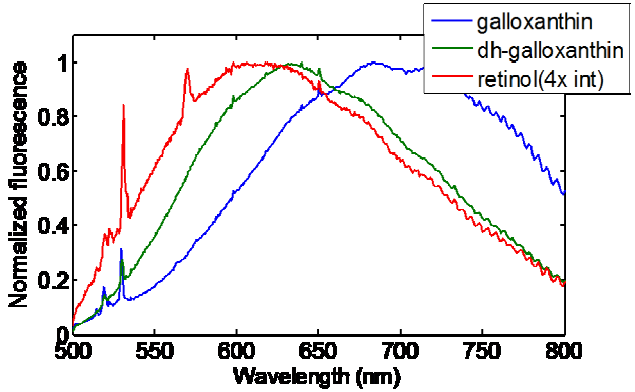
Imaging conditions
Laser OD = 0 (~200 uW laser power)
Integration time = 0.24 ms
FOV = 12.5 um
Objective = 60x (NA =1.4).

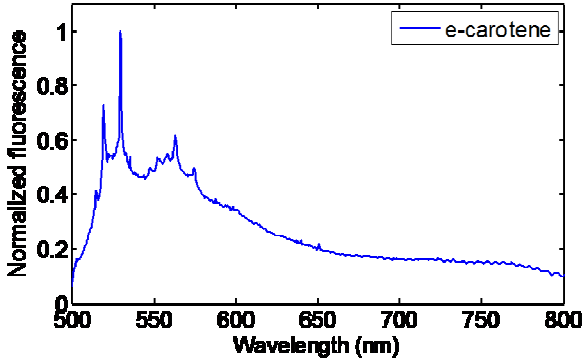
Spectral components for the pure carotenoids indicated suspended into lipid droplets.

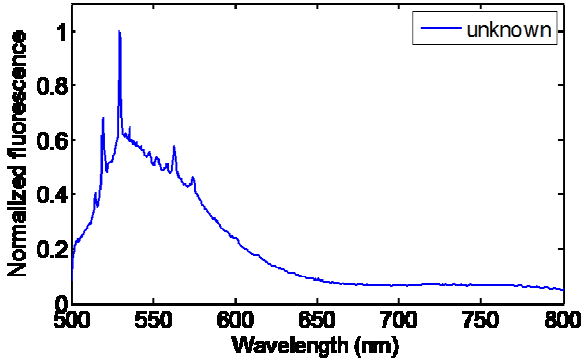


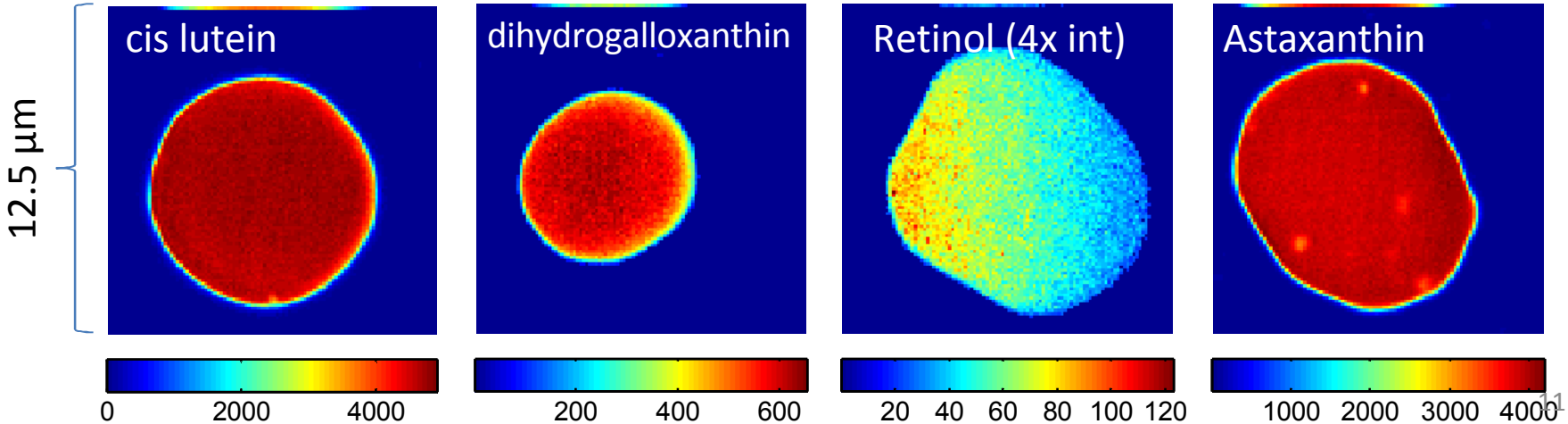




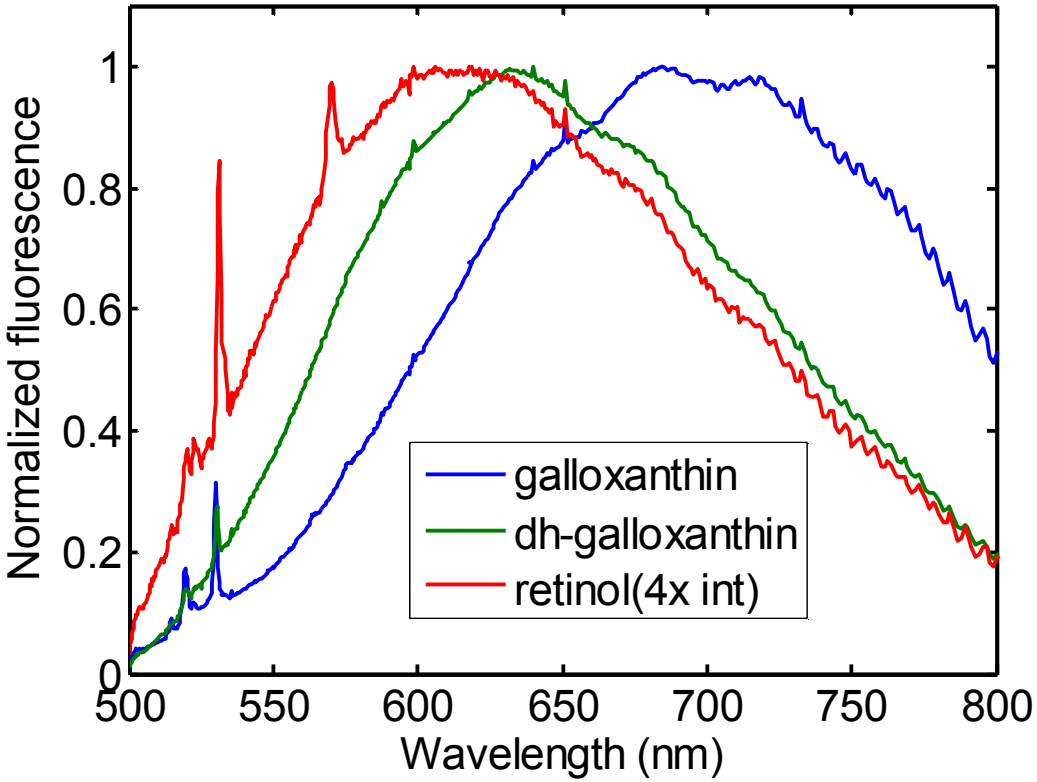




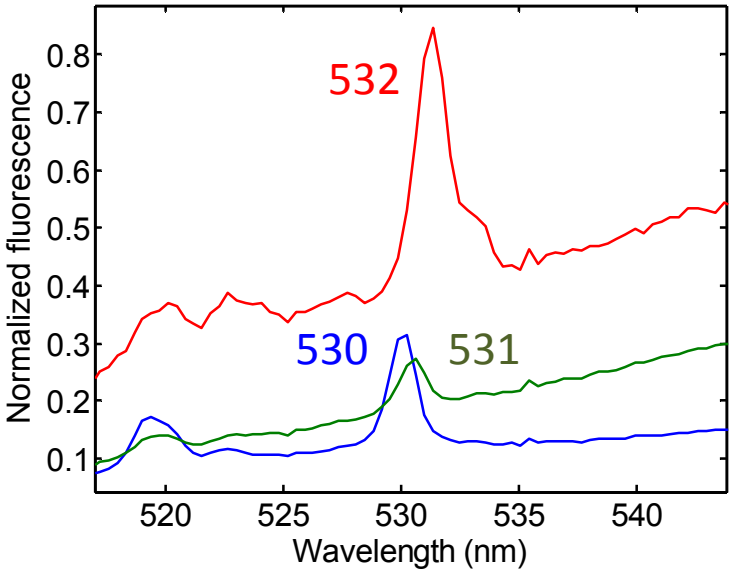




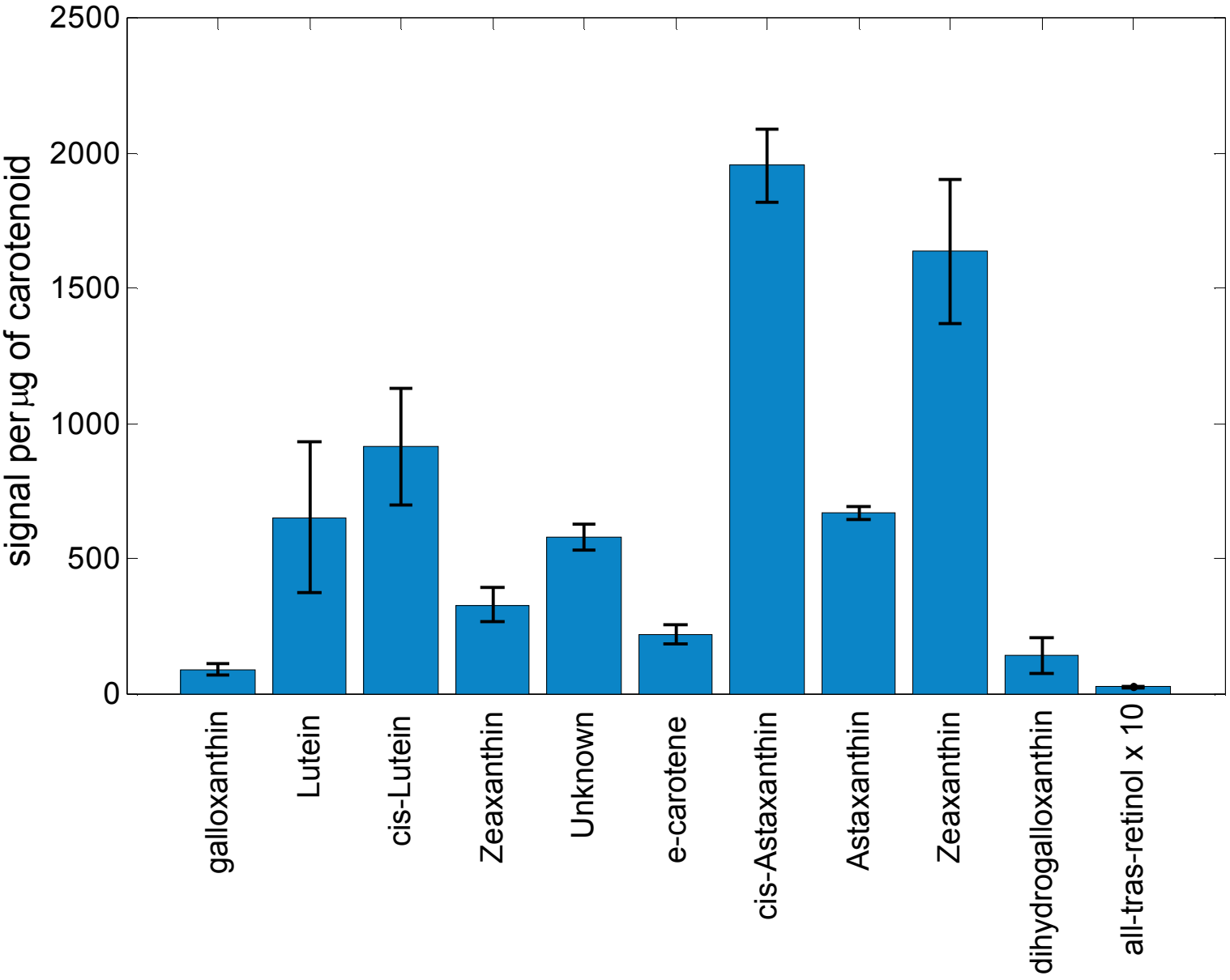
Expansion of the weakest emitting carotenoids.



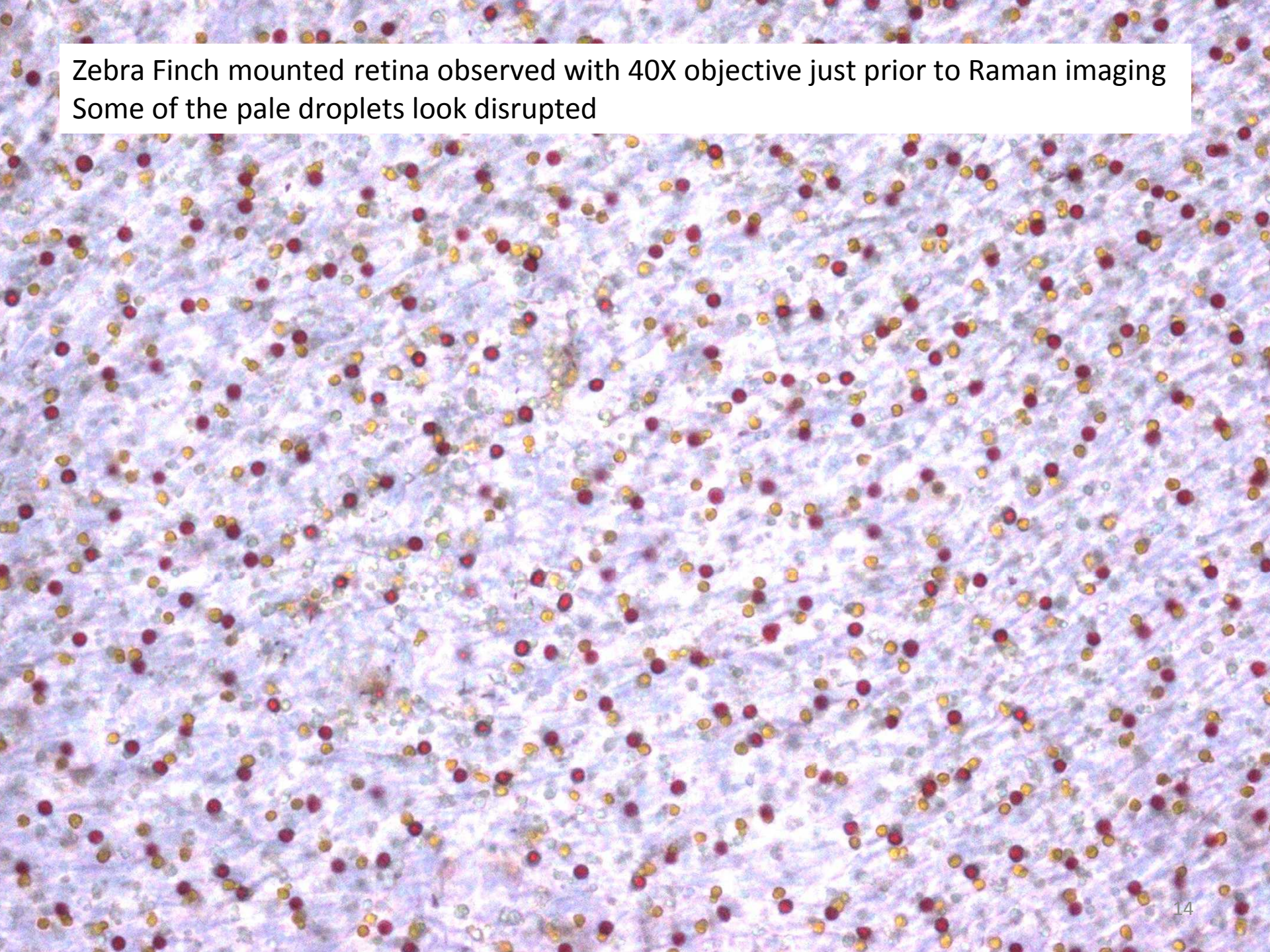
Zoom to v_1 region



This plot represents the average per-lipid droplet signal strength normalized on a per microgram of carotenoid basis. This plot assumes the lipid droplet preparation were identical between samples.



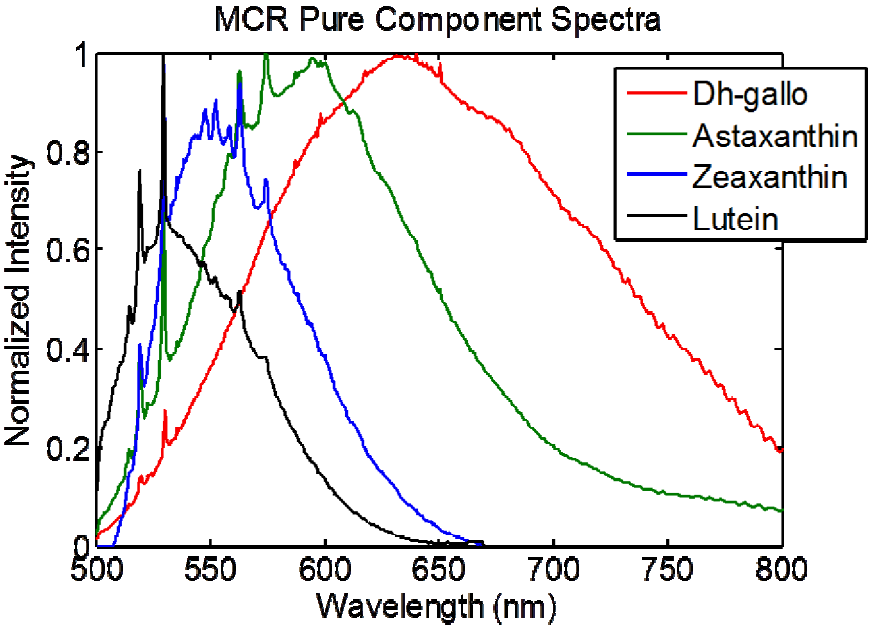
Zebra Finch mounted retina observed with 40X objective just prior to Raman imaging
Some of the pale droplets look disrupted



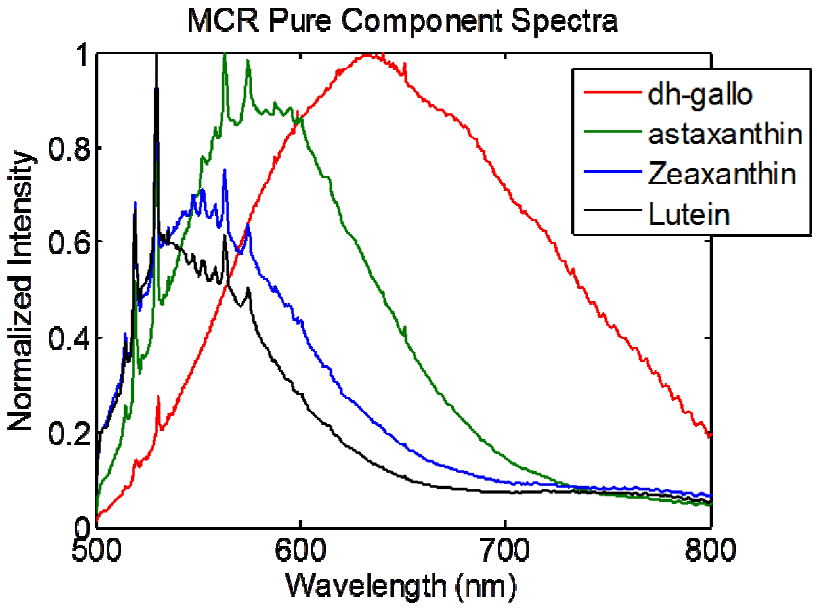
I used the first retina fragment to develop an initial MCR model. 12 z-stack image series were spatial compressed by 4 (2 x 2) to allow all of the data to be analyzed at once and to decrease noise.

In order to develop a model that was consistent with the composition of the retina, I spectrally constrained the dihydrogalloxanthin component to be identical to the artificial TAG droplet and then a 4-component model was developed that seemed to model most (but not all) of the spectral variance.

Zebra finch retina analysis



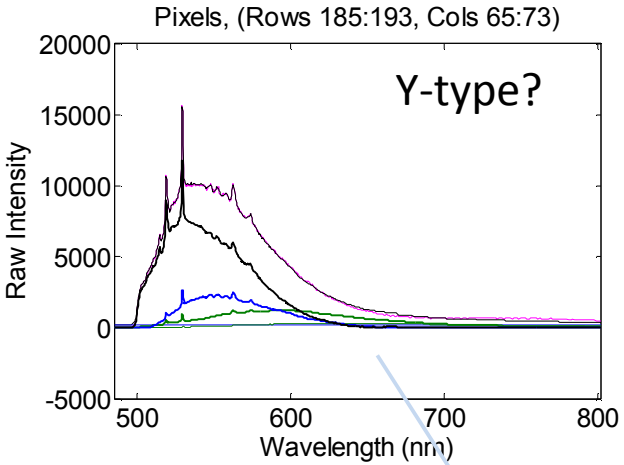
Pure carotenoids in TAG



Note: These assignments are based on similarity to the pure carotenoids in TAG droplets.

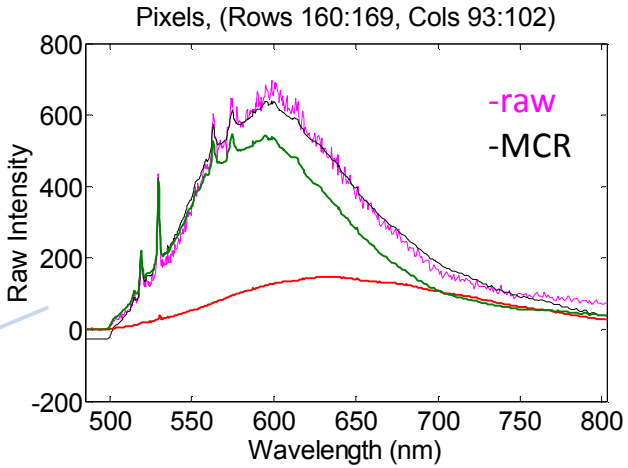
Example images and spectra

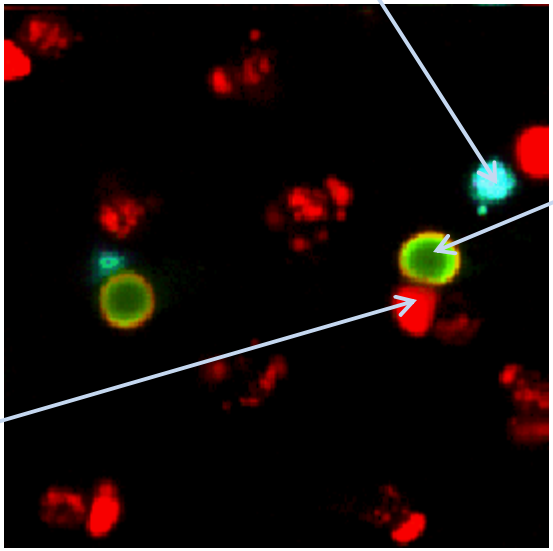
Section taken from a plane nearer to the top surface



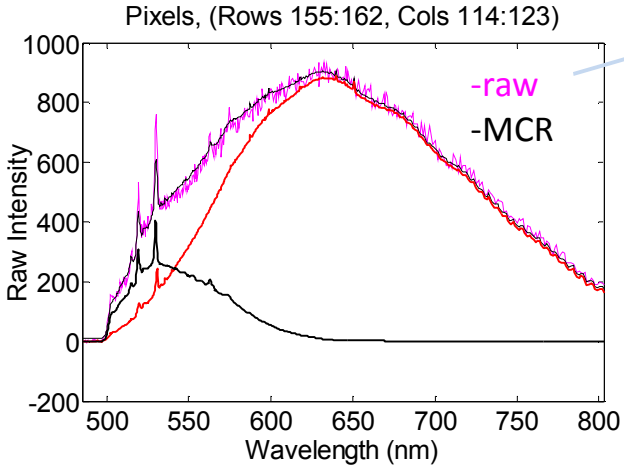
Color scheme
Dh-galloxanthin
Astaxanthin
Zeaxanthin

R-type?





P-type?



Many of the p-type droplets appear fragmented

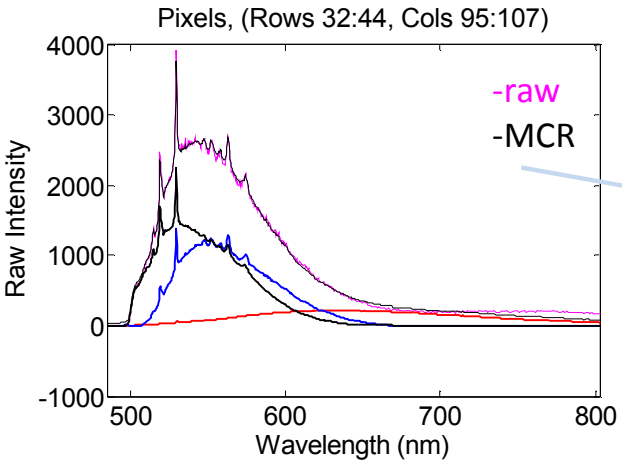
Image field is 25 um x 25 um

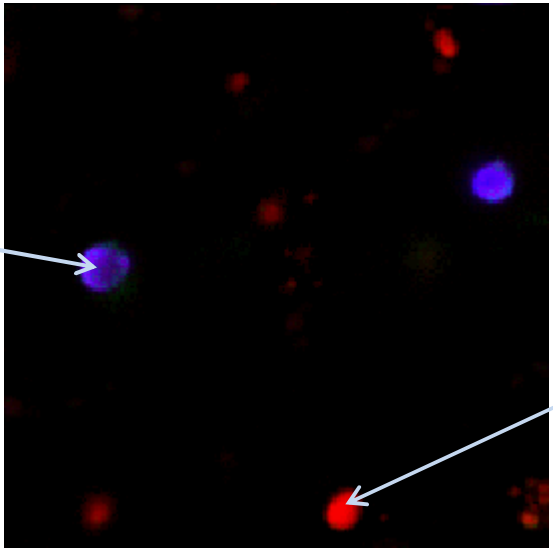
Example images and spectra

Section taken from a plane nearer to the bottom surface of same image stack

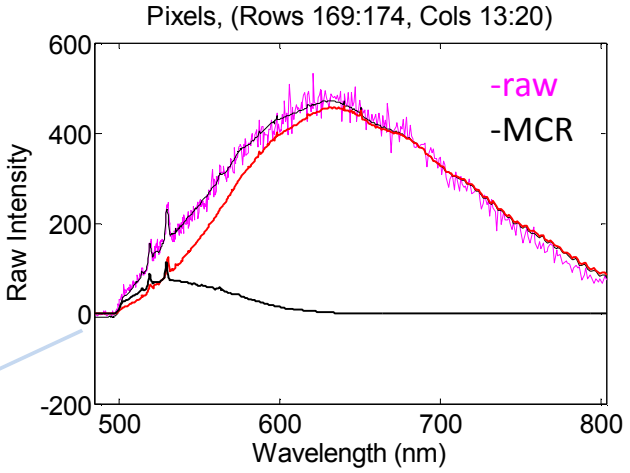
Color scheme
Dh-galloxanthin
Astaxanthin
Zeaxanthin

Y-type?





C-type?

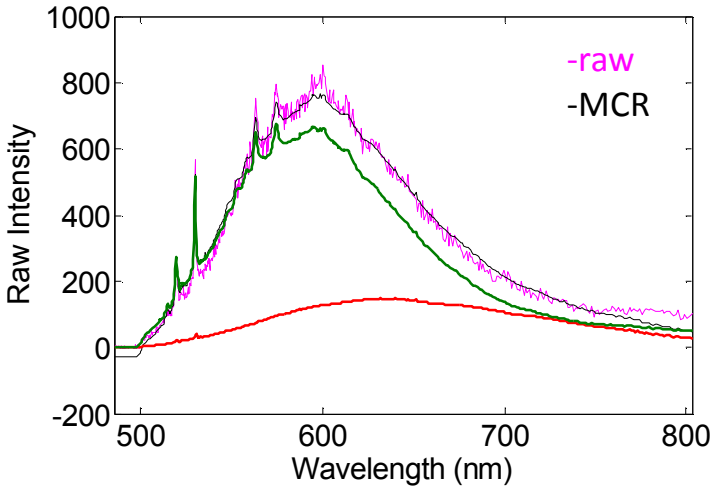


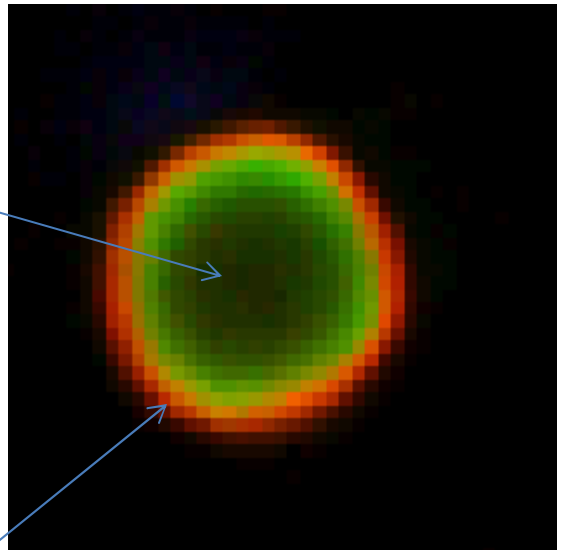
Recall that almost none of the pure carotenoids in TAG have 0 intensity at 800 nm (see slide 15). I suspect that the model needs some refinement as dh-galloxanthin is being fit at the longer wavelengths and this could be incorrect.

Interesting effect in R-type droplets.

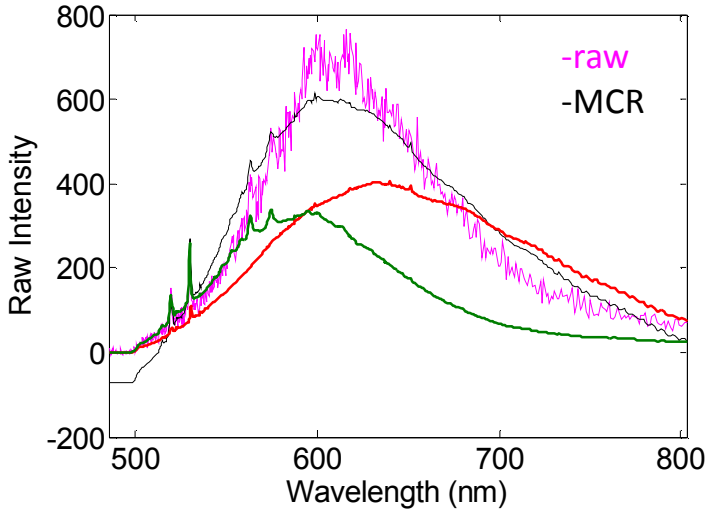
Color scheme
Dh-galloxanthin
Astaxanthin
Zeaxanthin

center of droplet





Edge of droplet



Note a good fit to the raw data for the edge of the droplet. Either the droplet is heterogeneous in spatial composition or this is an aberration.

Conclusions: I think this is a good starting point but the model needs refinement.

1) The zea, asta, and lutein comps should be broadened and should have intensity around 800 nm. This might clean up the spectral mixing
2) The R-type droplets have an interesting "edge-effect." Is this real or an artifact of the spectral imaging? Going back, this was observed in the chicken retina images but it seemed to be less pronounced.
3) The analysis of pure carotenoids in TAG was very profitable.
   → e-carotene has a very unique spectral shape. It should be used to better model the chicken retina data as it is suspected that those samples contained e-carotene in the P-type droplets.
4) We can resolve galloxanthin, dh-galloxanthin and all-trans retinol.

I have many gbs of data that I can process into stacked tif files and send to Matt but these are not overly useful of the MCR model is not correct.

One thing we will have to work out is how to proceed once I leave SNL. I will likely not be able to do any more experiments and Jeri and I will have to work out how to finish the analysis.