Exceptional service in the national interest

SAND2015-8667PE

Hyperspectral Confocal Fluorescence Microscopy of *Acaryochloris* sp. R61 and *Acaryochloris marina*

Imaging conducted on 8/4/15

SAND2015-XXXX

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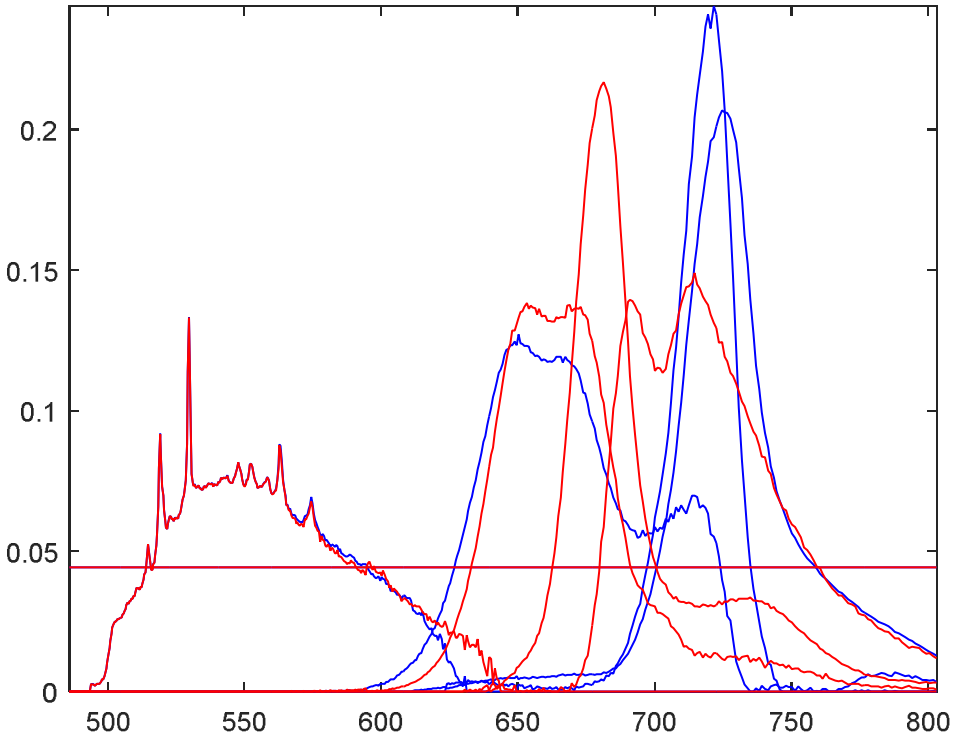
Experimental Details

- Samples were received from SNL receiving on 7-29-15 and were placed in a shaking incubator at 30 C and 150 rpm under 30 uEi of light in 125 mL glass beveled Erlenmeyer flasks. (note – R61 doesn't like 30 deg as per Jeremy – did we ever move it?)
- Samples were imaged on 8-4-15
- Prior to imaging samples were removed and 1 mL was placed in a centrifuge tube and spun down to concentrate. The majority of the supernatant was removed and the resulting pellet was gently shaken to resuspend in remaining media. 4uL of the solution was placed on an agar coated slide containing BG-11 media (we were piggy-backing from another experiment and this was what we had that day). The slide was coverslipped and gentle pressure was applied to seat cells to agar. Nail polish was used to seal the coverslip and the sample was allowed to sit for 5 min for cells to settle.
- Images were taken immediately after that, for about 15-30 minutes.
- 488 nm excitation, OD setting of 1 (laser power < 10 uW/um^2)
- Data was compiled several different ways for analysis.

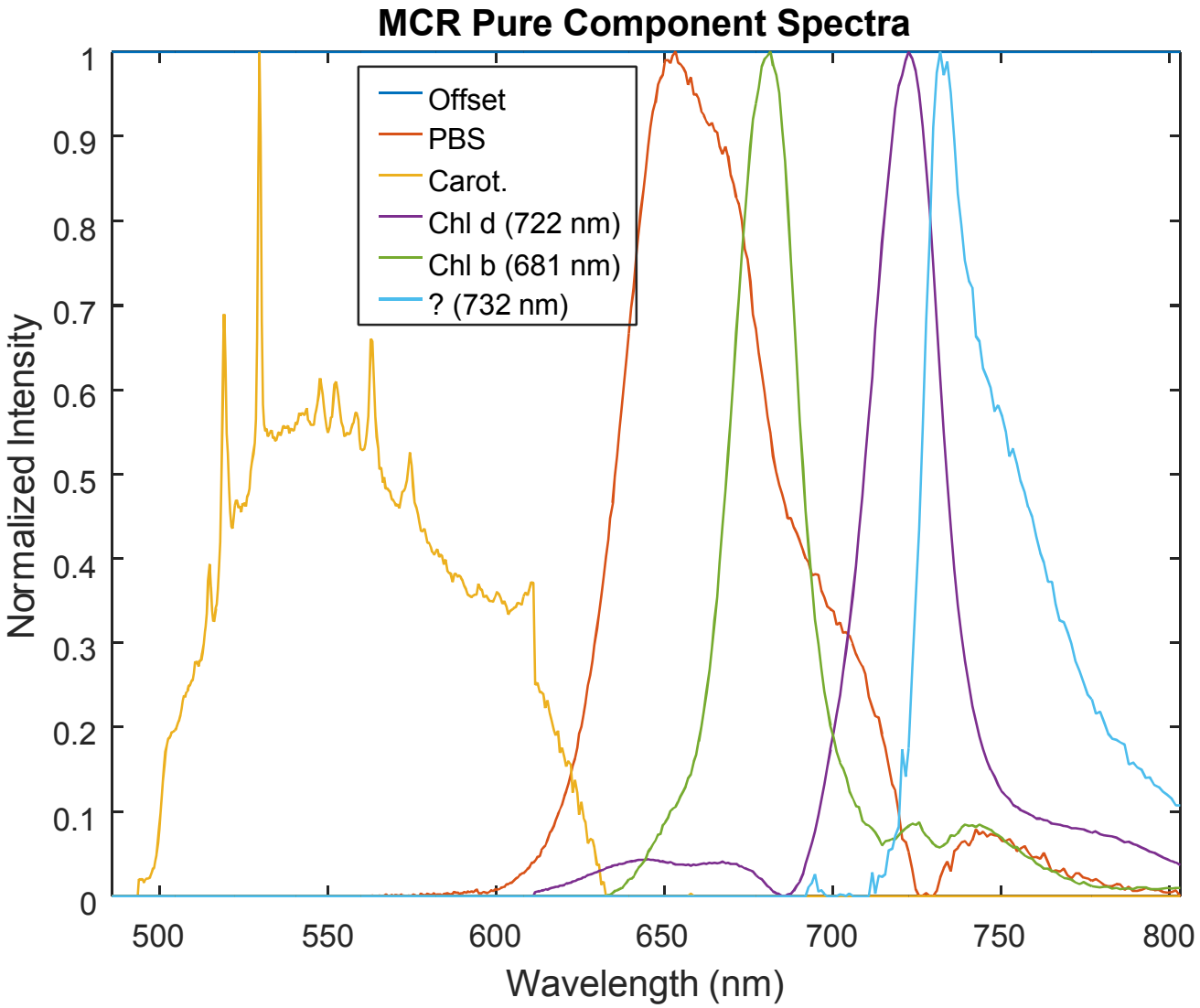
Independent Models for Each of the Species are not Identical



Marina
R61

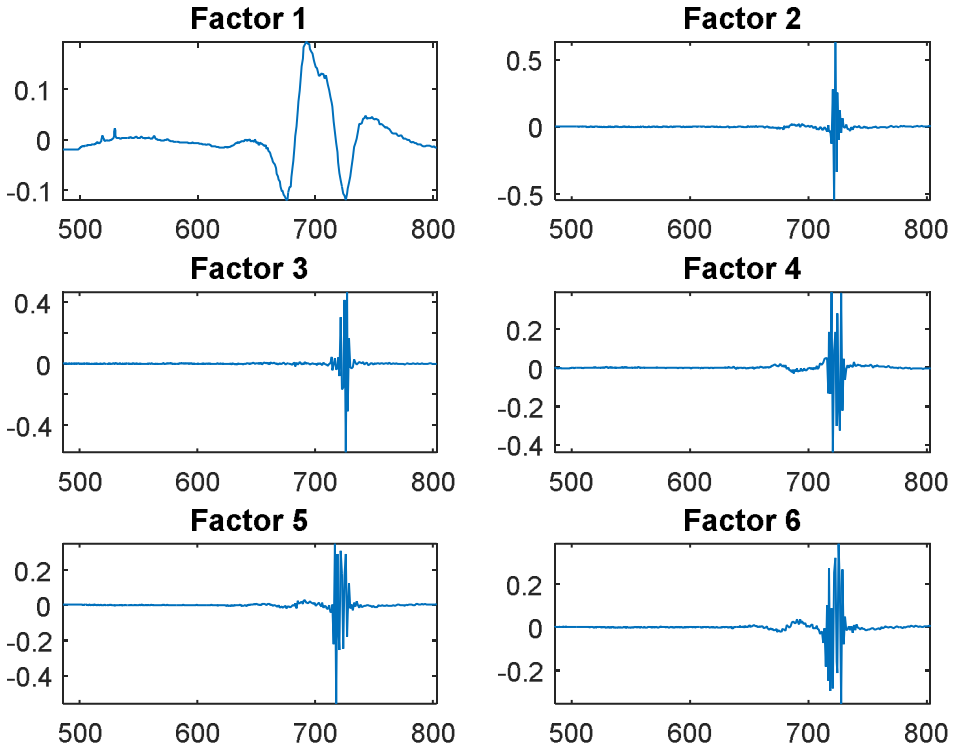
Both of these have a significant residual at the point of the spectrum of the highest intensity, most likely from the non-linear detector response. These were taken at OD 1.0 and the laser power may have been a bit too high. Spectral deconvolution is challenging because there's not enough variation in the data set. Would be improved if we had cells under two conditions that promoted phycobilsome /chl ratio changes.

Combined Model for Both Species



This Model is not Perfect Either

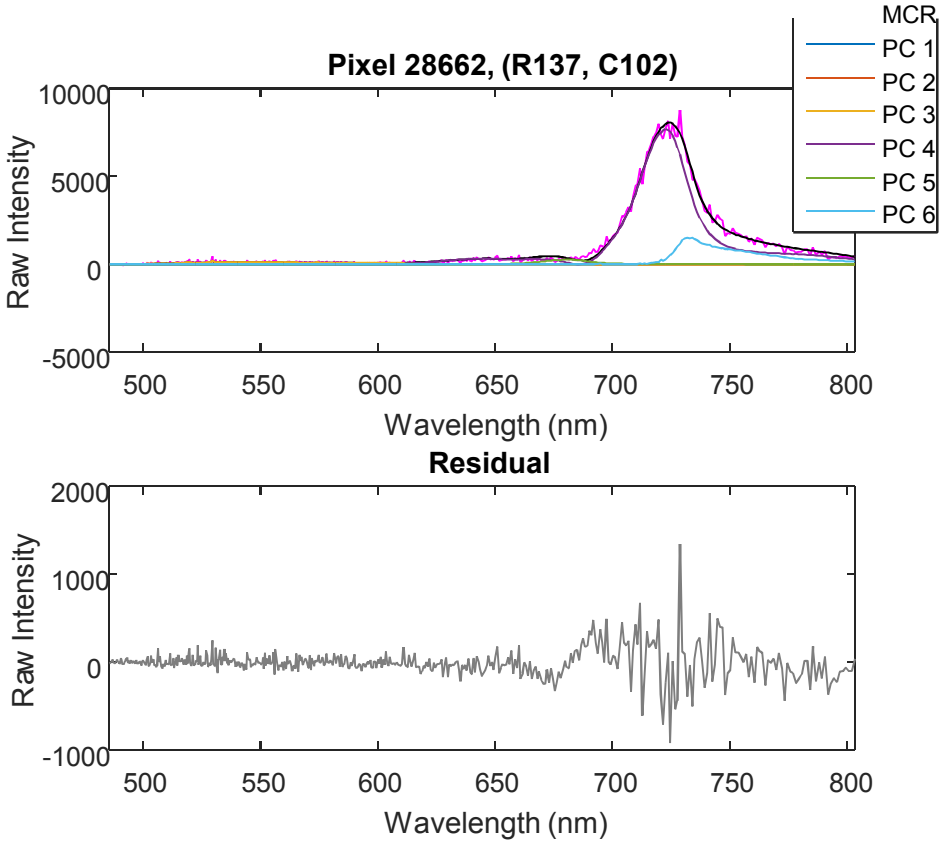
There's one residual. It could be from intensities being out of the linear range or it could be from slight shifts due to underlying Chl a – 685 or it could be due to the fact that the two species have inherently slightly different spectra emissions or a combination of any of the above.



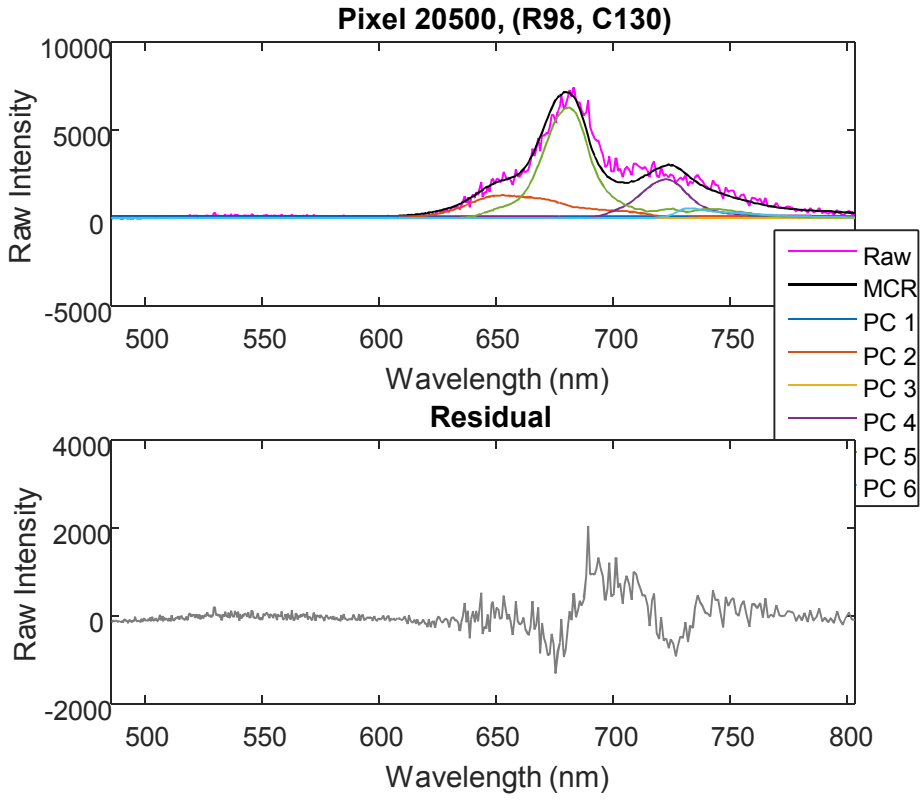
Residuals = 1.41 %

Comparison of Model Fit

Model fit is acceptable for A marina, but doesn't describe the R61 strain very well. Attempts to model the two strains independently were only partially successful.

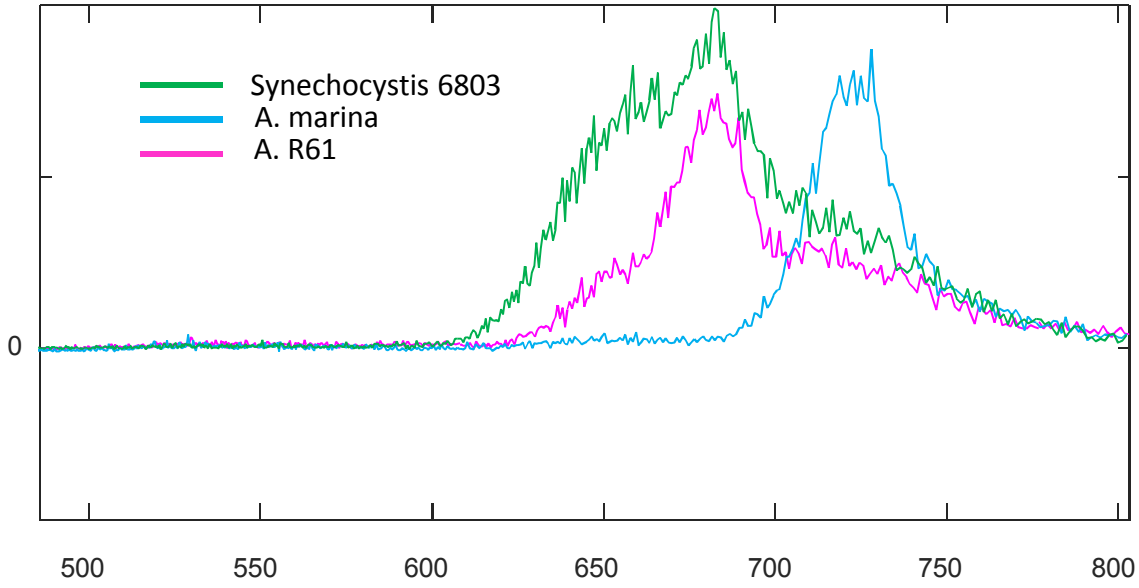


Marina – Trace 7 showing excellent model fit over entire emission spectrum

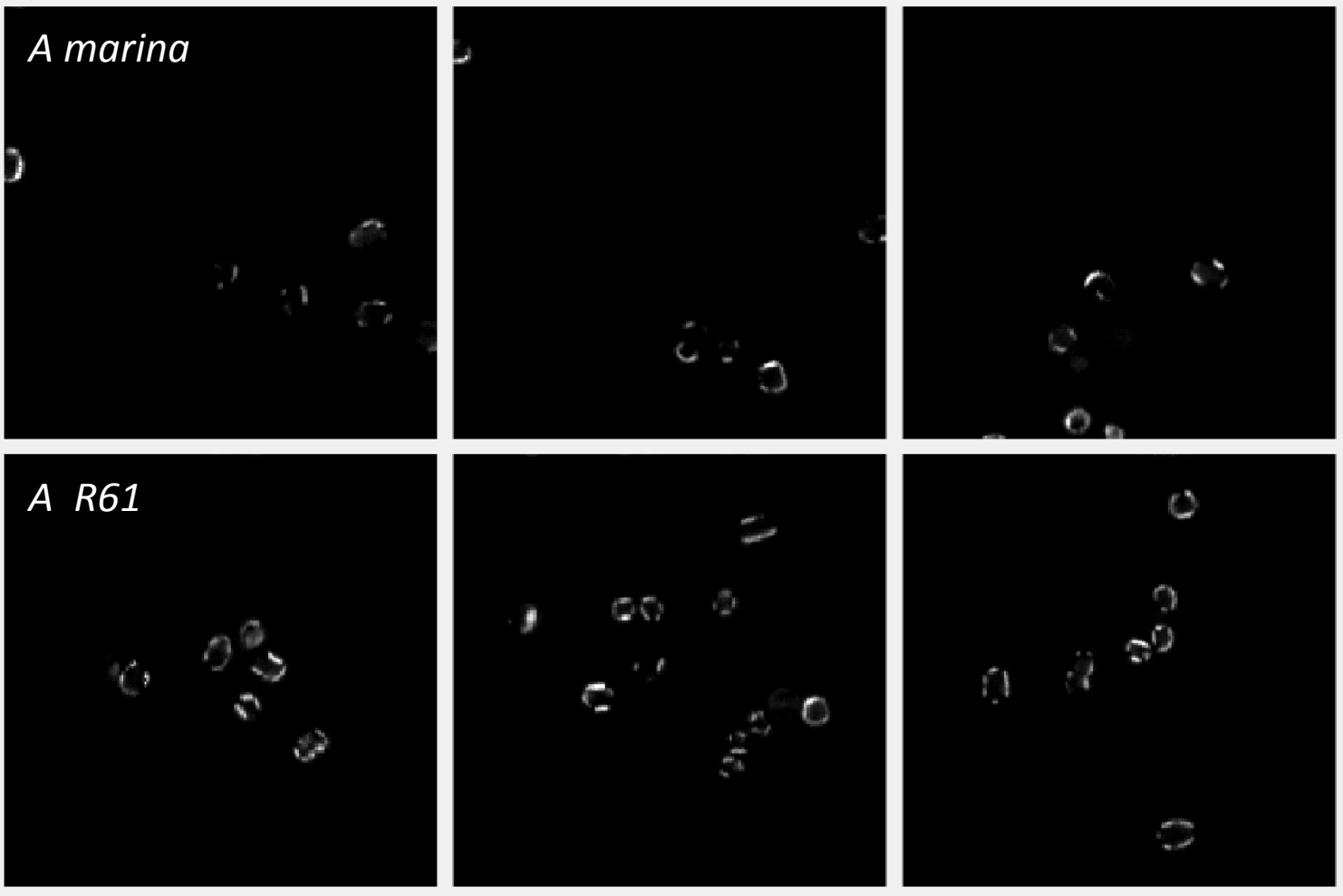


R61 – Trace 22 showing poor fit in Chld area of the emission spectrum

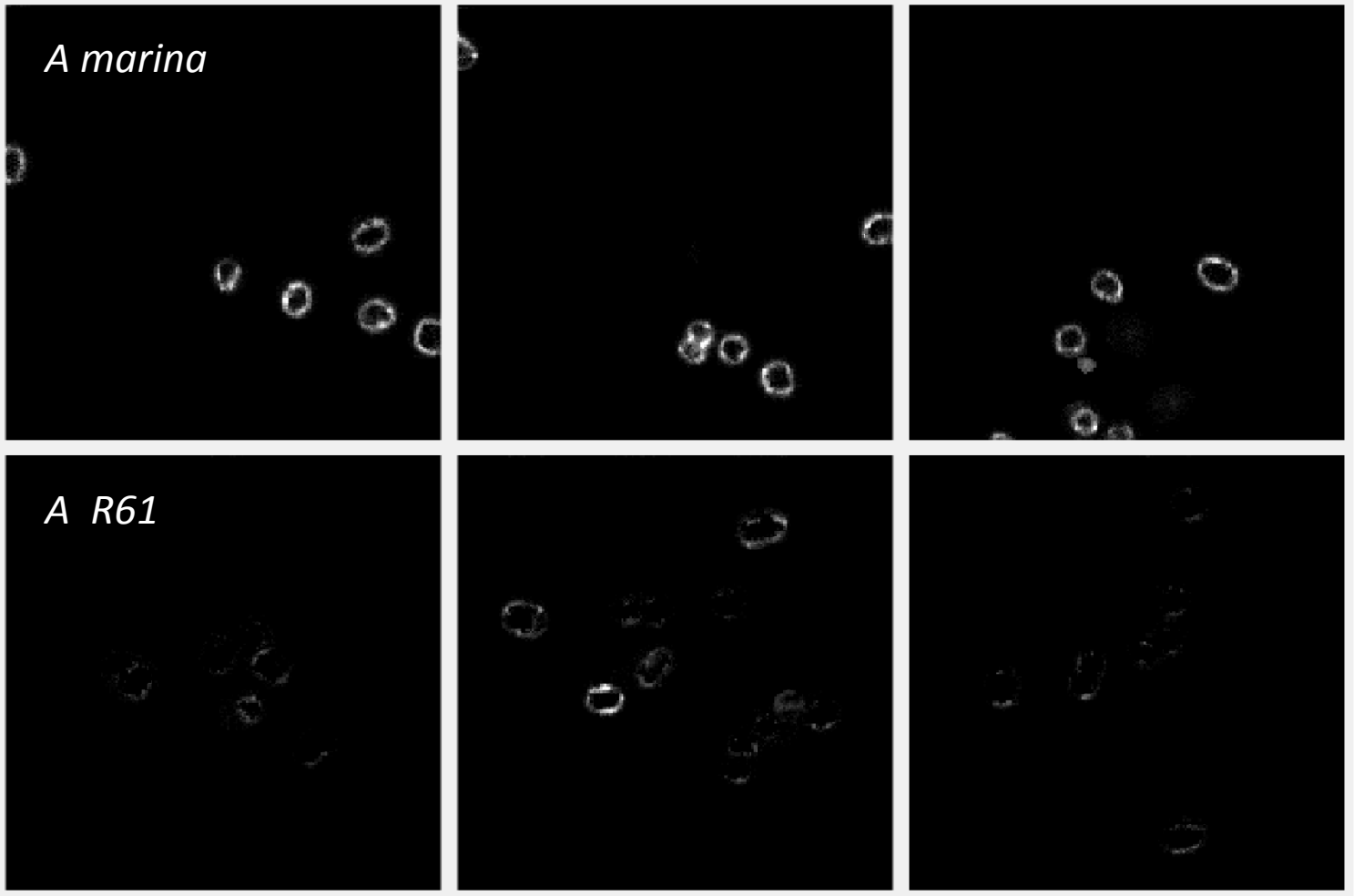
Comparison of Raw Spectra from Several Strains



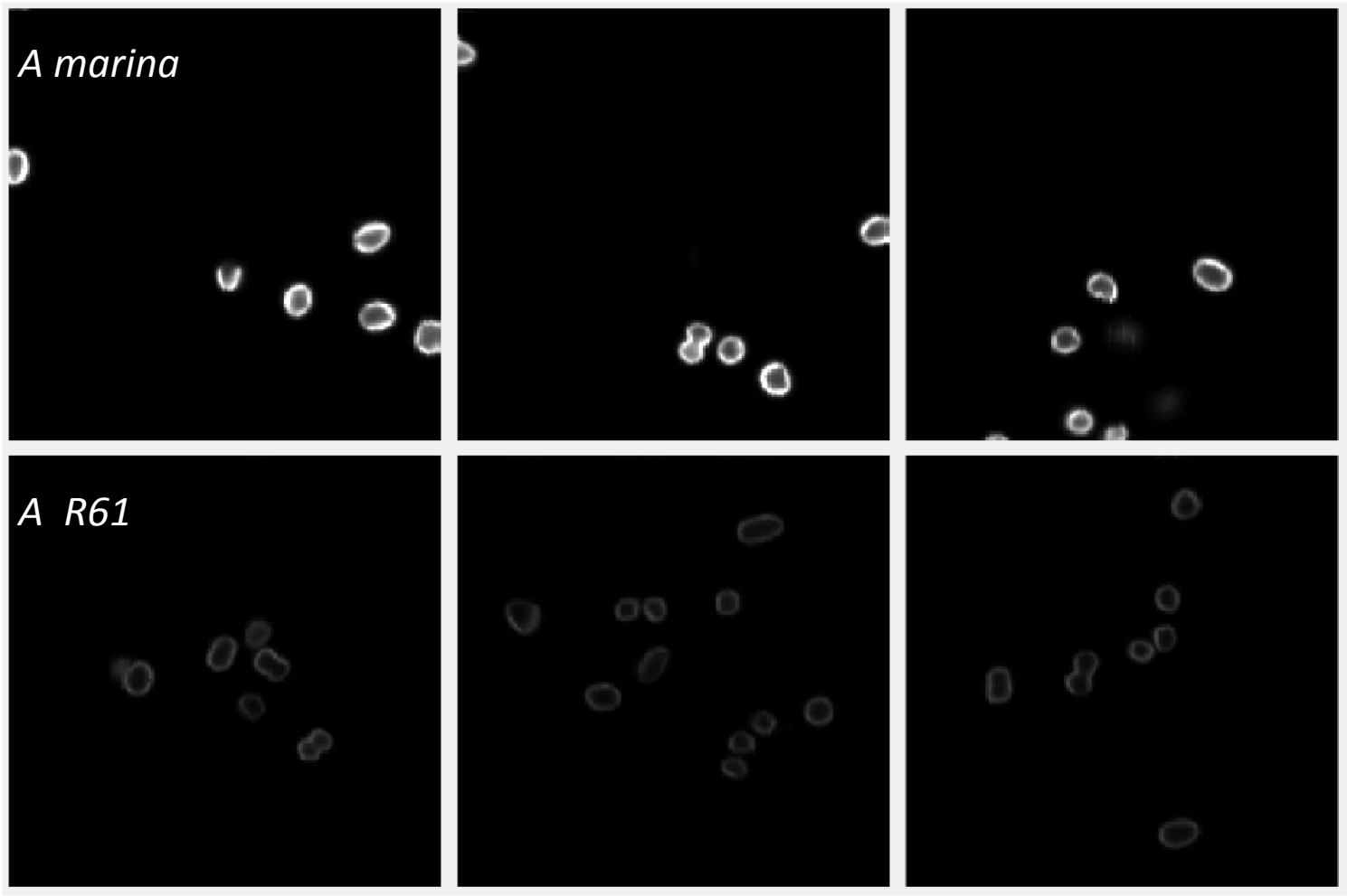
PBS



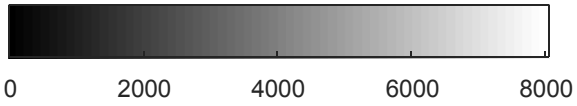
Carotenoids



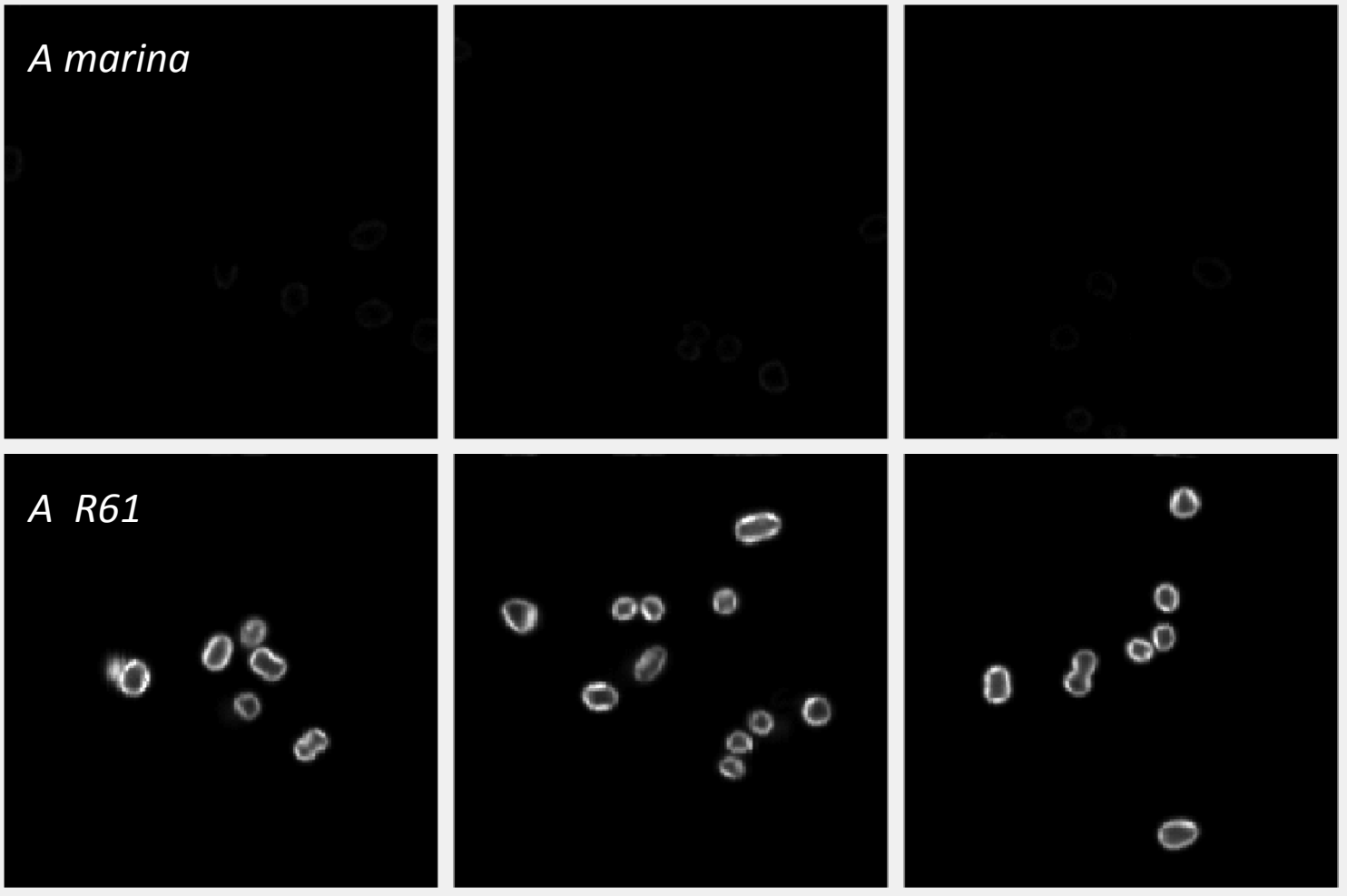
Chl d (722 nm)



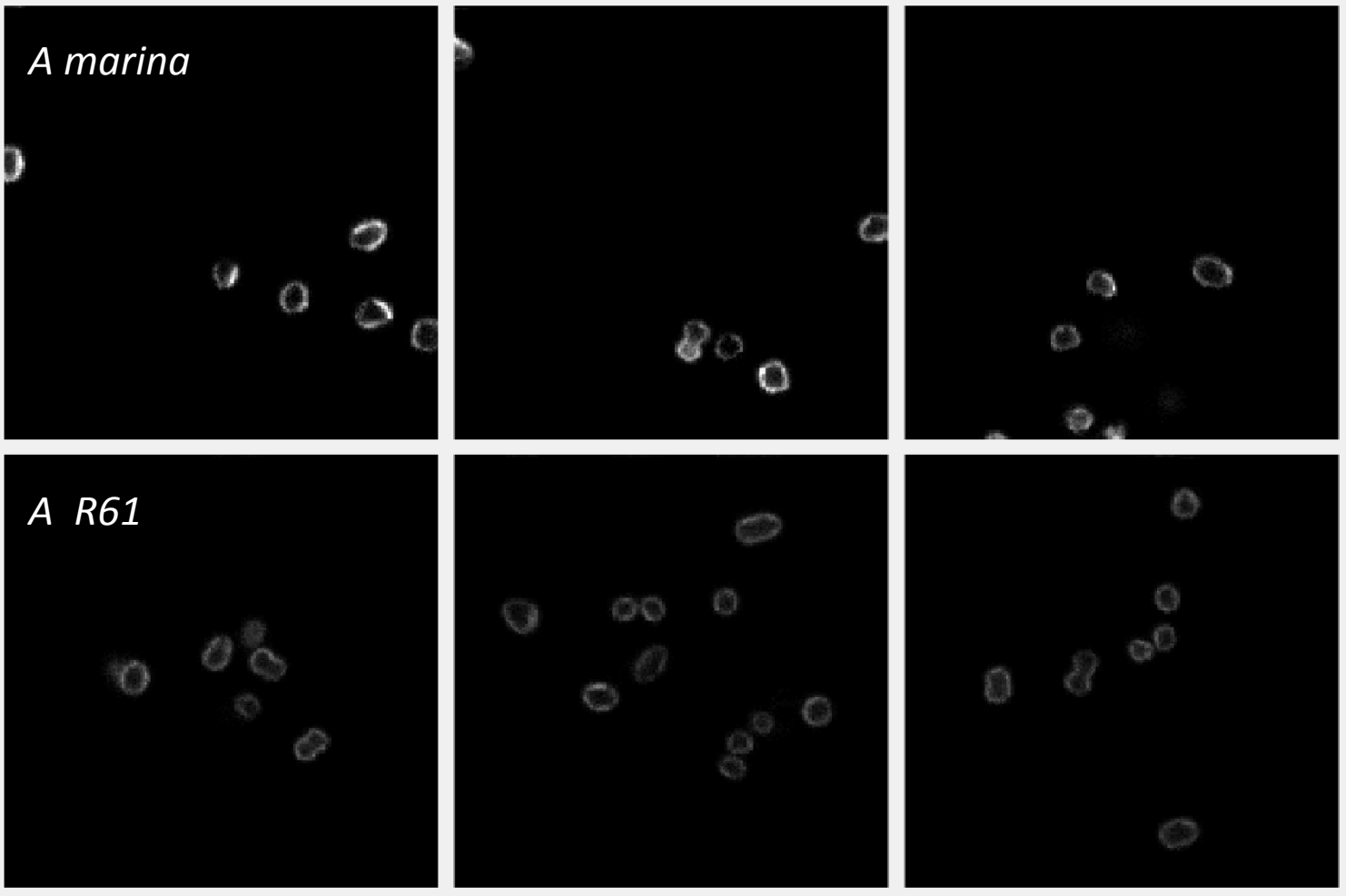
The minor signal for A R61 is a result of poor spectral model fit.



Chl b (681 nm)

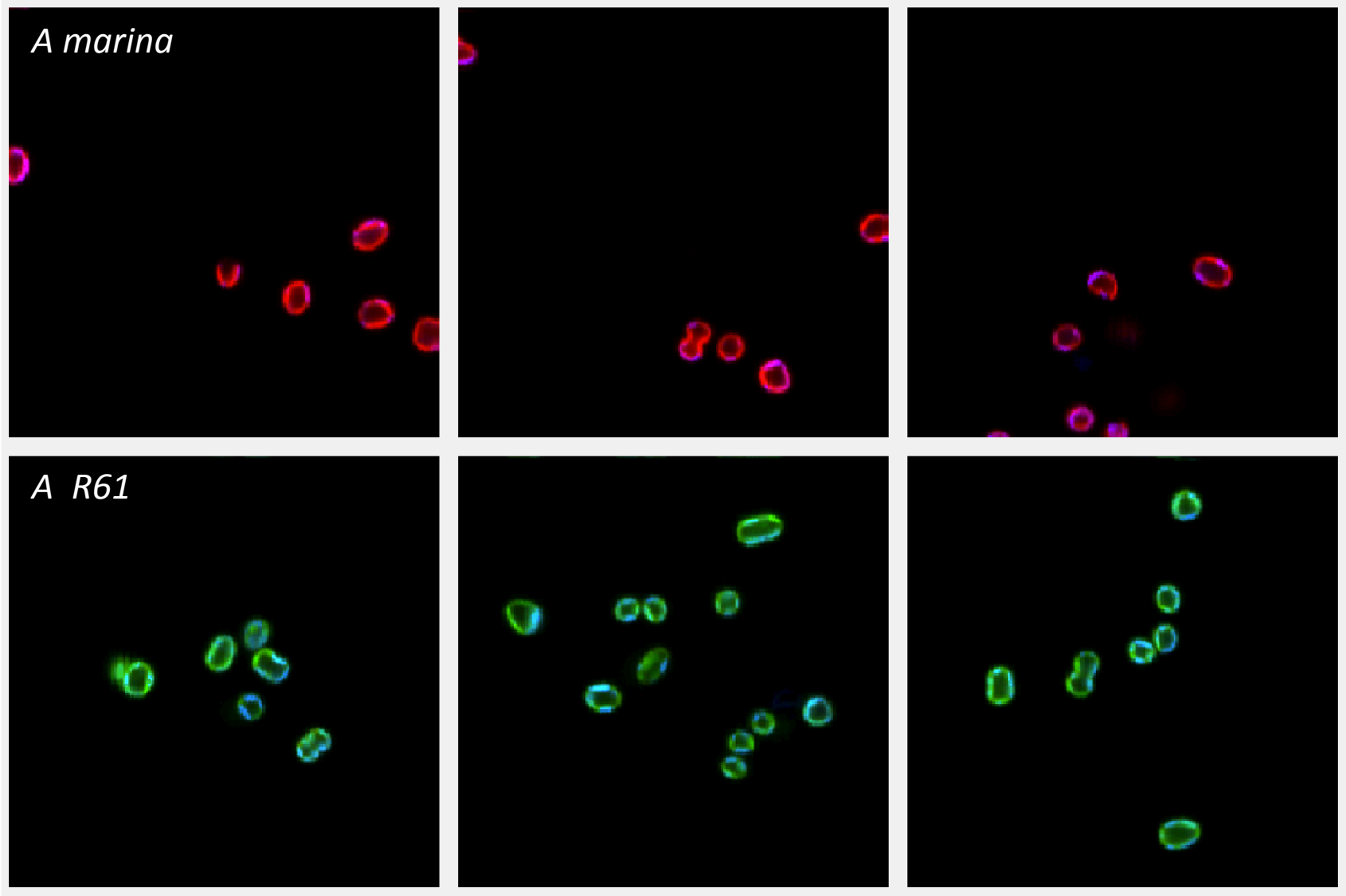


Chl d (733 nm)

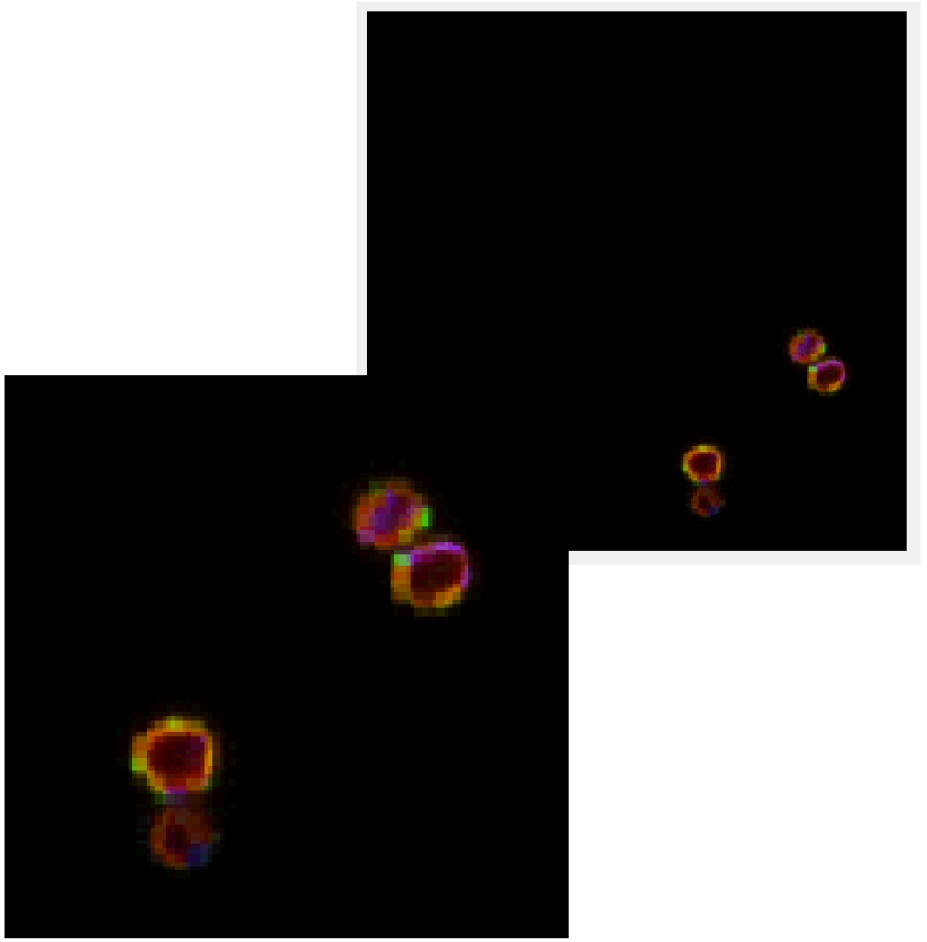


The minor signal for A R61 is a result of poor spectral model fit.

Red = Chl d, Green Chl b, Blue = PBS

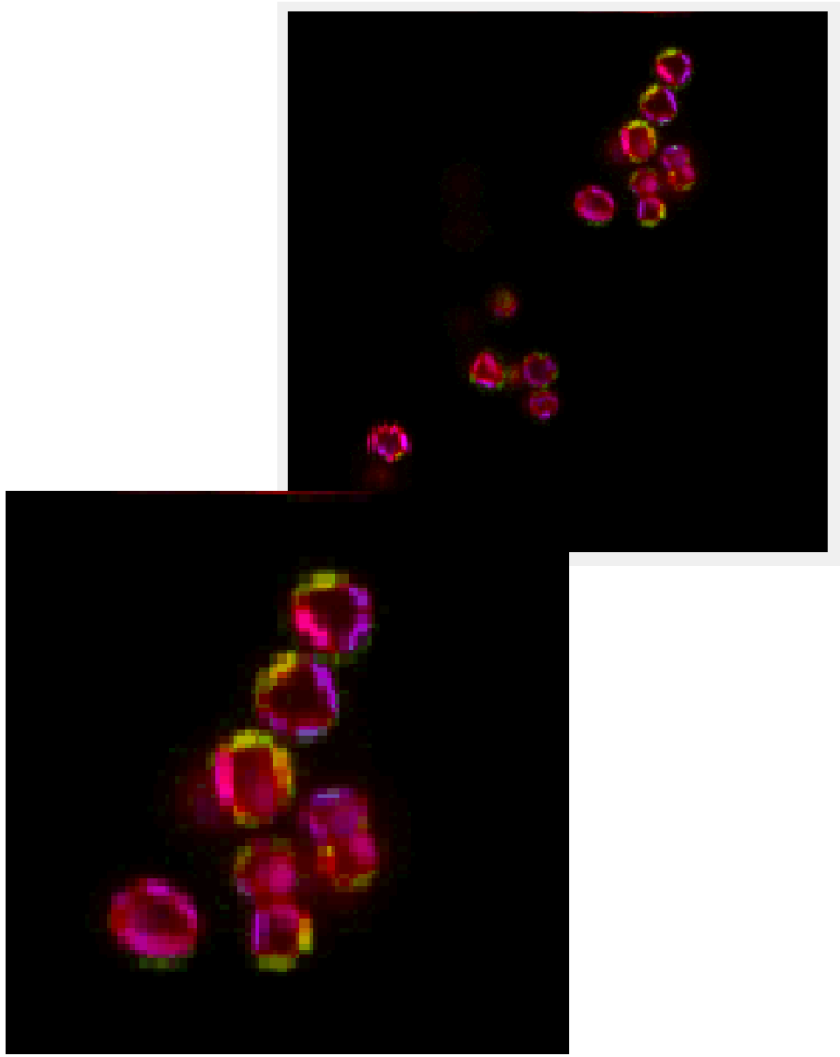


Marina



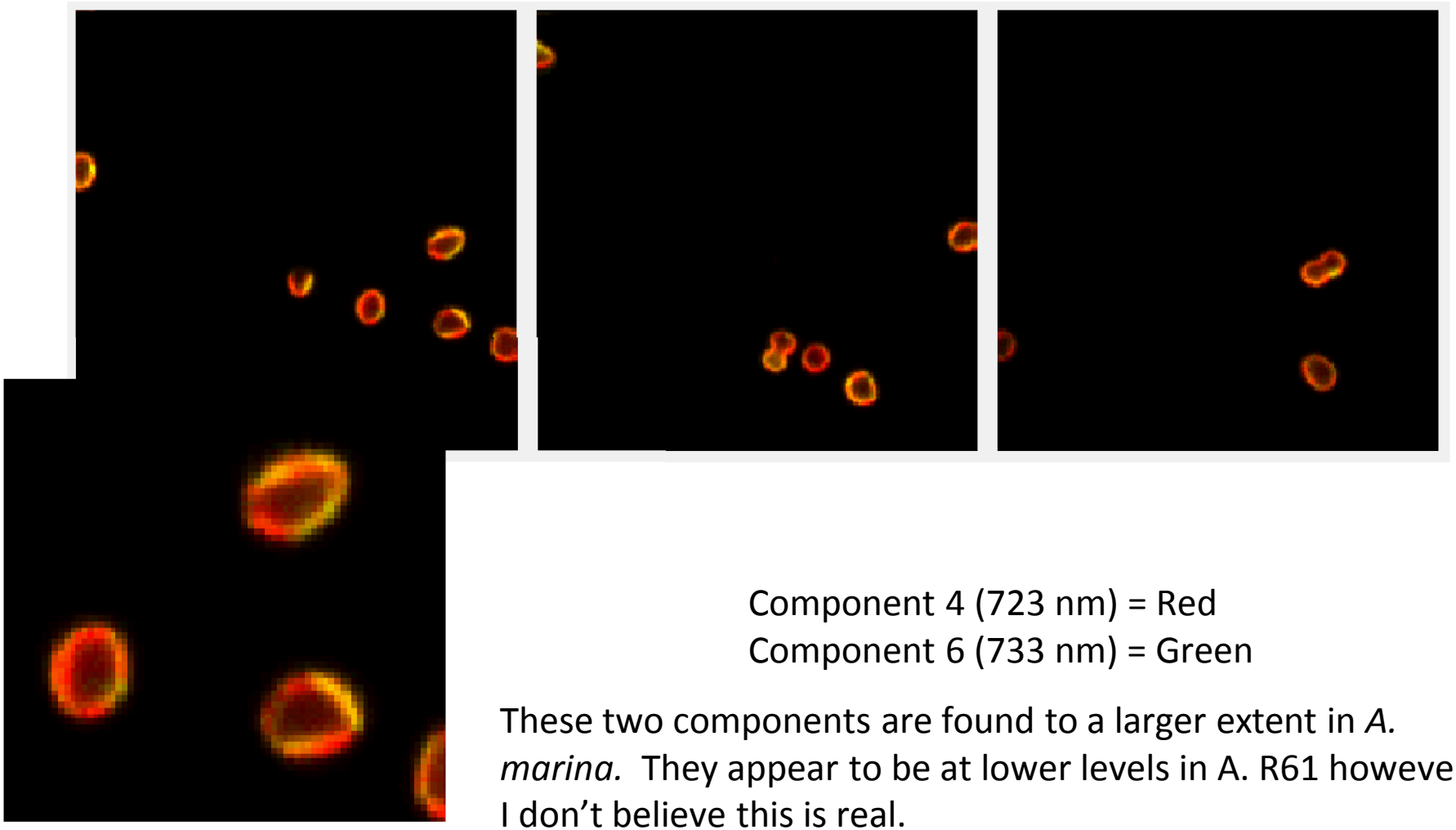
Red [Chl d] Green [Carotenoid] Blue [PBS]

R61



Red [Chl b] Green [Carotenoid] Blue [PBS]

A marina: Component 4 & 6 are not Uniformly Distributed, nor Colocalized



Component 4 (723 nm) = Red
Component 6 (733 nm) = Green

These two components are found to a larger extent in *A. marina.* They appear to be at lower levels in A. R61 however, I don't believe this is real.

Observations

- No clear Chl-685 identified? This could be hidden in that residual that looks like a shift.
  - For reference we didn't see a Chl 685 component in our previous *A marina* imaging either.
- PBS spectrum is a composite of multiple PBS components as evidenced by the multiple peaks
- Spatial organization of PBS appears the same in both organisms.
- Carotenoid and PBS are in separate membrane domains
- None of the components appear uniformly distributed in the cell membrane. Significant puncta and patches are visible – sometimes even single component patches – discreet localiations
- Two Chl d peaks at 722 and 732 nm are not colocalized. What is the second peak?

Next Steps

- Get new cultures, be sure to put R61 at lower temp (24 C) and keep marina at 30 C
- Repeat with lower laser power to avoid the possibility of non-linear detector response
- Is there a way to induce changes in the PBS/Chl ratio within a strain to improve the ease of spectral separation? Would different light intensities work or dark acclimation?
- Why are there two Chl-d components?