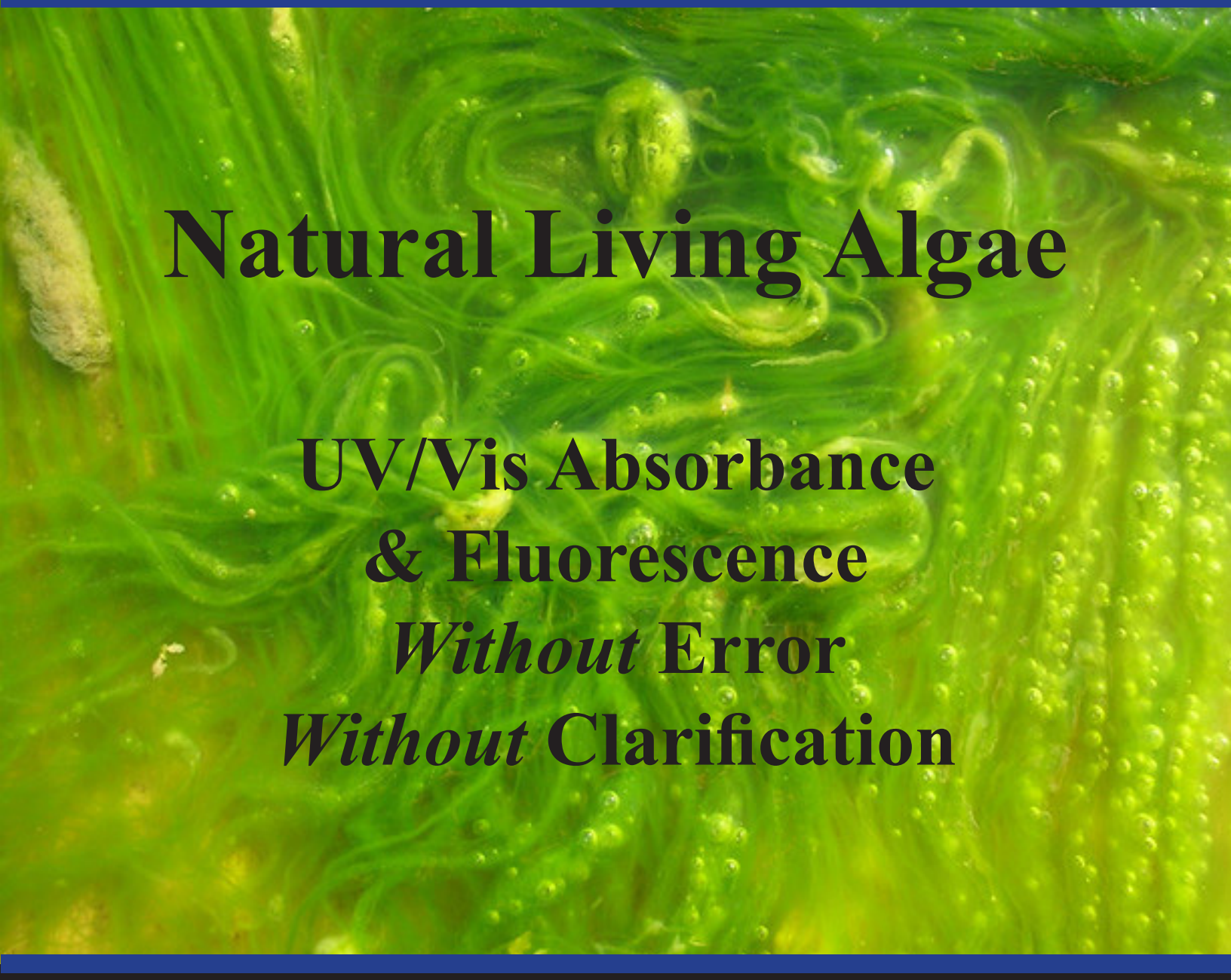

Natural Living Algae

UV/Vis Absorbance & Fluorescence *Without* Error *Without* Clarification

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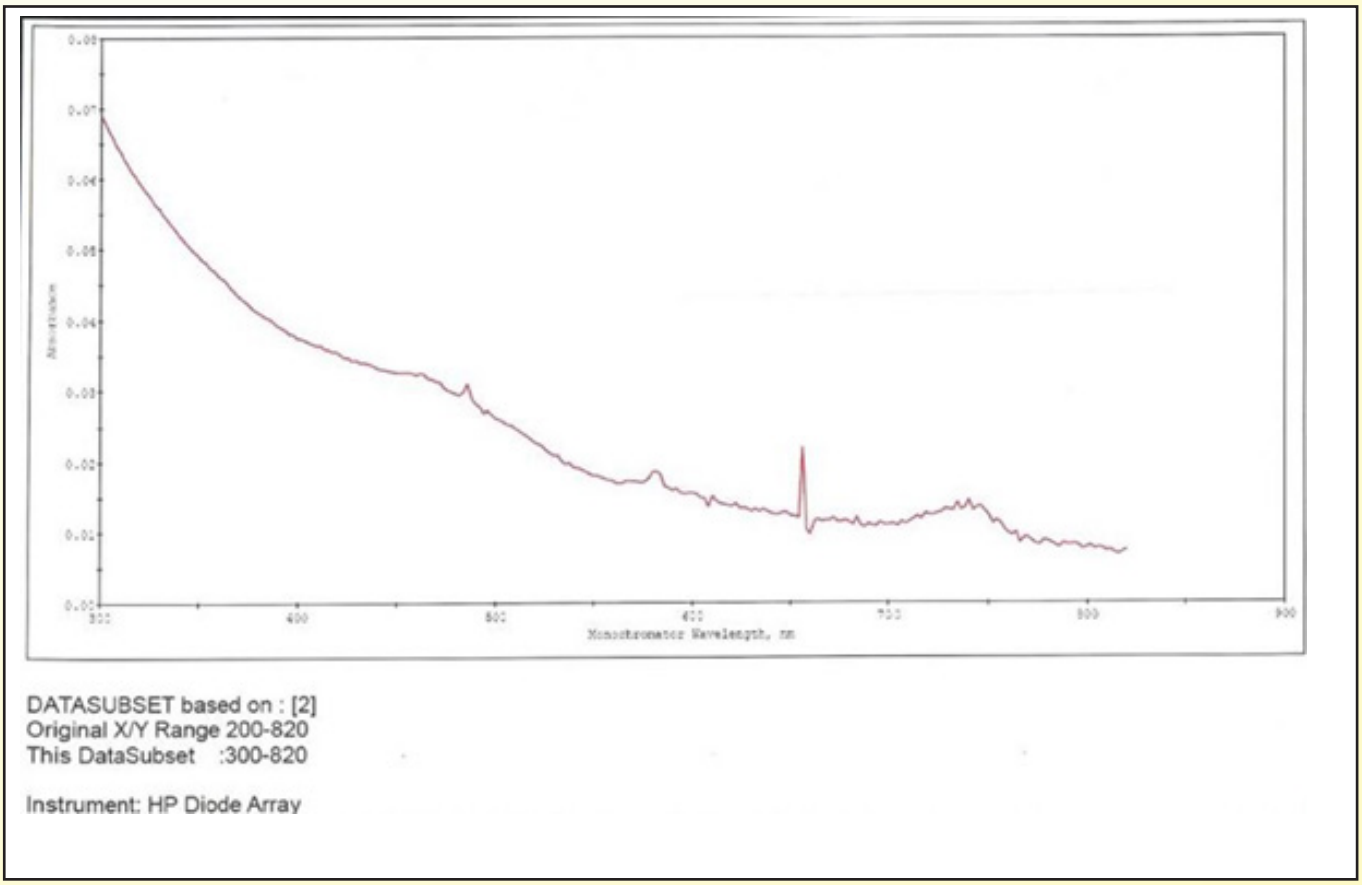
Clarification of a turbid sample is essential when using a cuvette spectrophotometer.

If there is turbidity in your sample, a diode array spectrometer collects no meaningful spectral information, as seen from this turbid algae sample. The dramatic rise in absorbance from the visible to the ultraviolet is the fingerprint of scatter, not of the sample.



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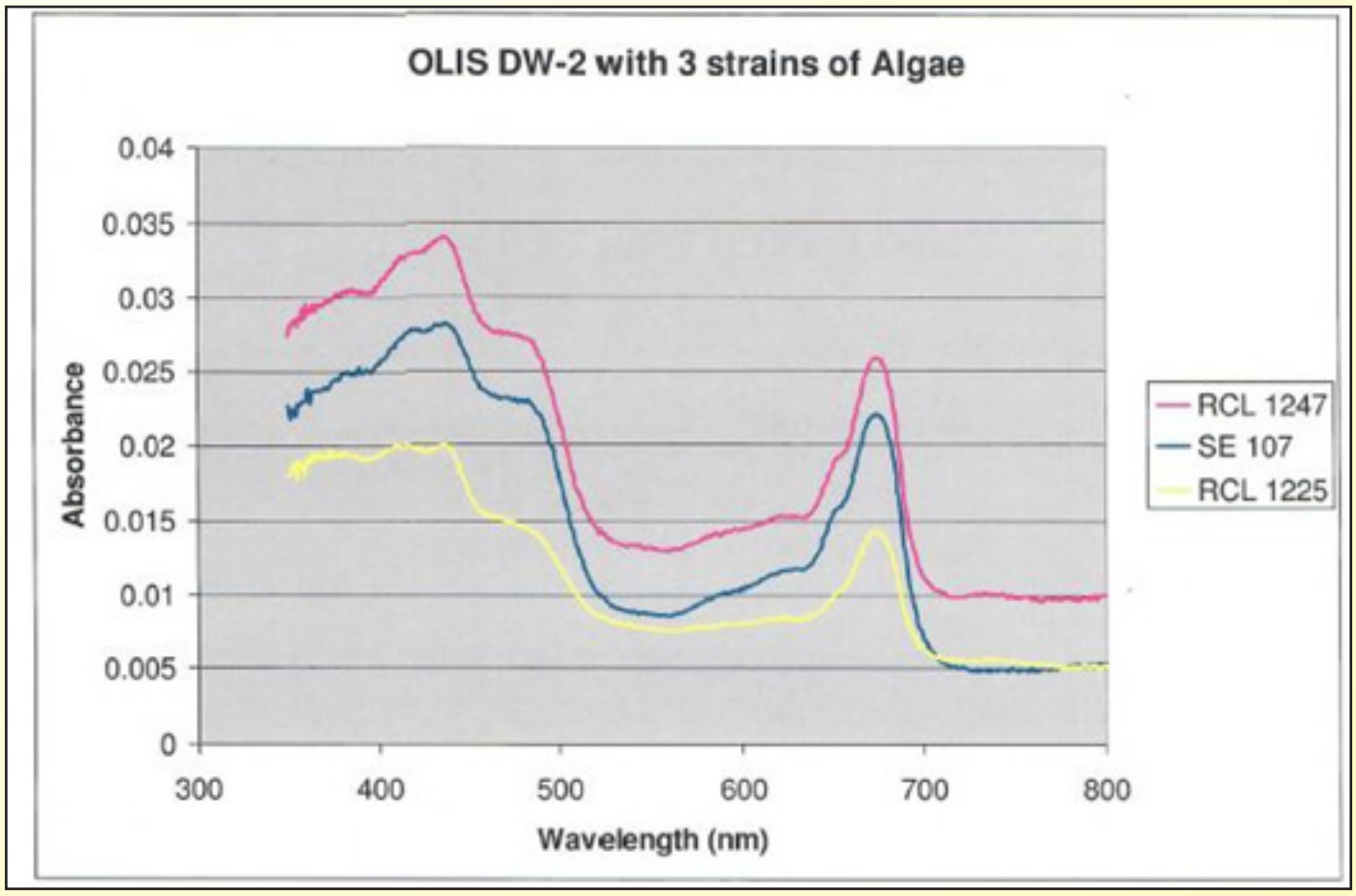
Dual-wavelength spectrophotometers were developed to be more successful with turbid samples.

In a dual wavelength spectrometer, difference spectra are calculated using “chemistry + scatter” and “scatter” spectra. And, to capture as much scatter as possible, a large faced detector is used. Results in the visible region are useful at modest levels of turbidity. Here, RCL 1247 has more scatter than the other algae and thus the most flawed answer of the three.



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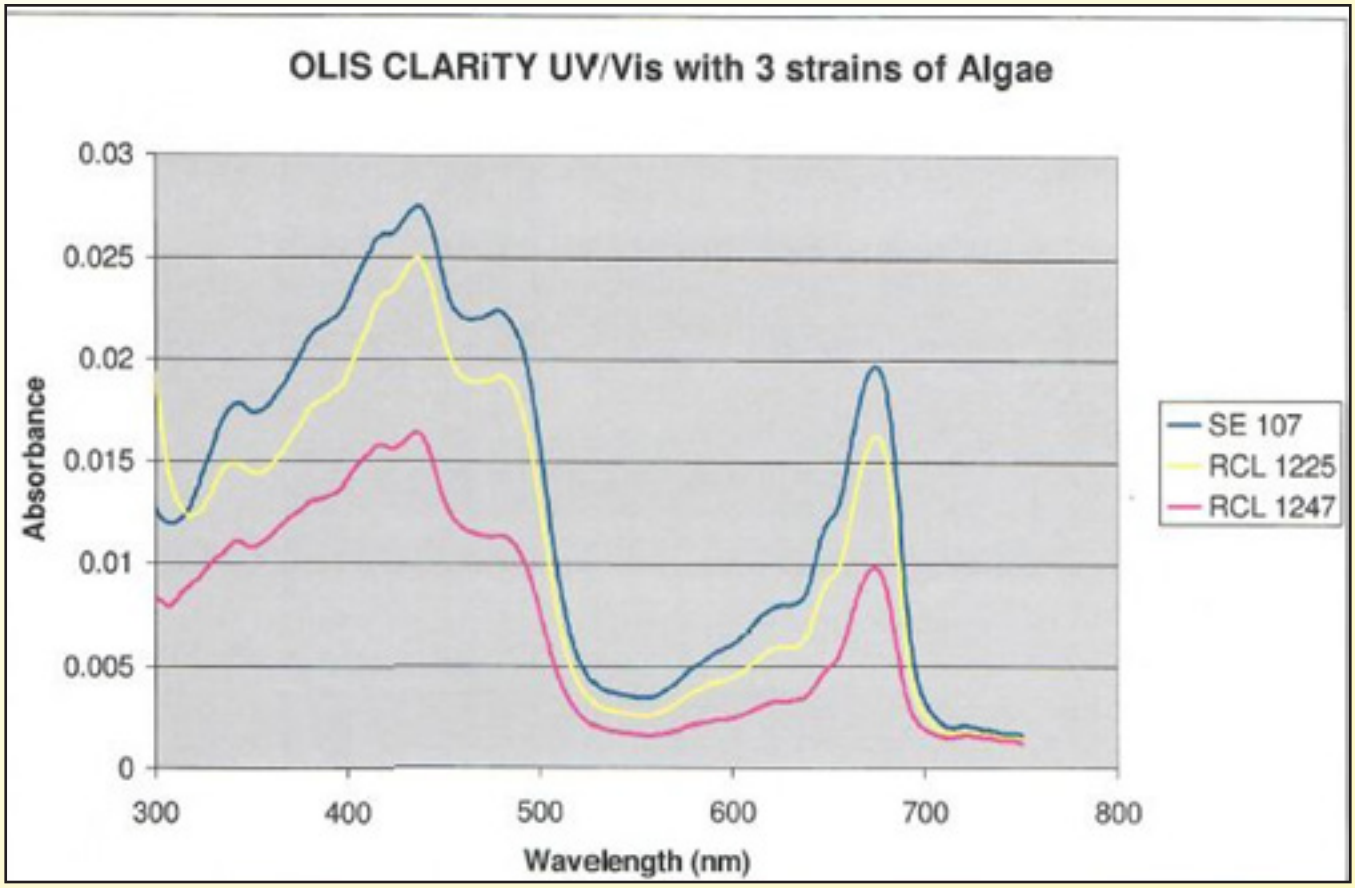
CLARiTY spectrophotometers *cannot see scatter*, so their answers are correct with or without turbidity in the sample.

The successful sample holder is not a cuvette but **an integrating cavity**. The sample, the measurement light, and the scattered light are held within this white-encased cavity. Here, *there is no light which is not detected*. Here, *nothing escapes measurement as lost intensity* ("scatter").



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Absorbance & fluorescence (optical) spectroscopy is only as good as the measure of the sample's effect on the measurement light:

LIGHT-IN *MINUS* SAMPLE'S ABSORPTION [SHOULD!] *EQUAL* LIGHT-OUT

Incorrect answers result when light-out is misdirected or scattered before the detector. This scatter registers as diminished intensity, but is actually deflection of the light to an angle outside of the detector's ability to see it.

The dual-wavelength method captures more of the deflected light than does a diode array; the CLARiTY method captures all of the light.

With normal & dual wavelength cuvette spectrometers, **scatter renders the answer worthless to flawed.** ***Only perfect clarification makes a perfectly correct answer possible.***

With a CLARiTY integrating cavity spectrometer, scatter & thus error are eliminated. ***Clarification of turbid samples is not required to obtain a perfectly correct answer.***

Thus, you are free to work with intact systems which do not need to be disrupted because your spectrophotometer needs clear samples.

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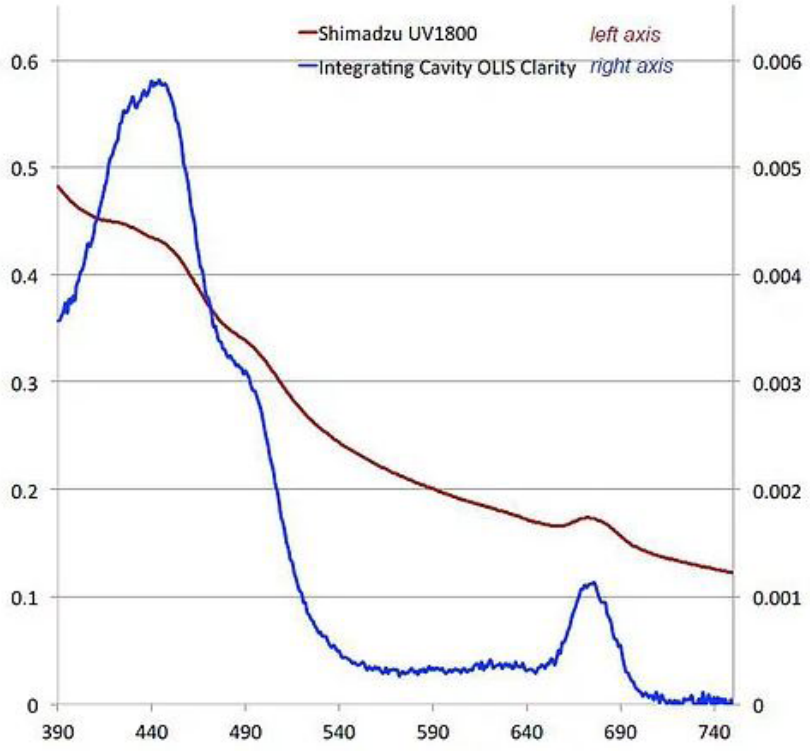
Comparison of spectra by a cuvette spectrometer (Shimadzu 1800 in red) & an integrating cavity spectrometer (CLARiTY in blue)

OLIS Clarity Integrating cavity spectrophotometer, for accurate absorbance spectra of dilute phytoplankton suspensions.

-~100X more sensitive than traditional spectrophotometer -intrinsic scatter correction

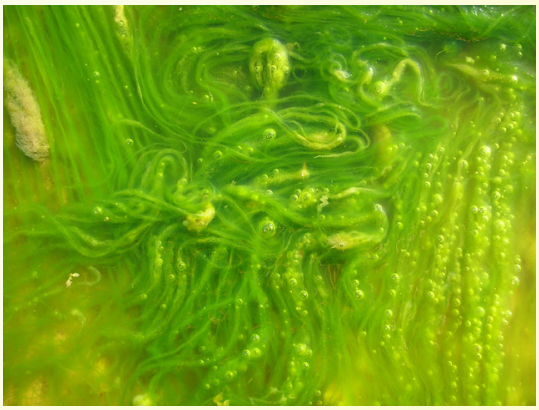
We can measure total light capture by a phytoplankton suspension.

Data collected in Professor Doug Campbell's laboratory comparing the OLIS CLARiTY 17 and a Shimadzu UV1800



The CLARiTY gives you freedom to obtain correct results using turbid NATURAL systems!

Nature evolved algae to be intact. Ideally, one studies it intact.



- Cuvette spectrophotometers obtain worthless to flawed results from a light scattering sample.
- Clarifying the natural sample to a clear form is changing the sample for the benefit of a cuvette spectrophotometer.
- Clarification required by a cuvette spectrophotometer strips away color and/or key environment that nature put there, which likely has **value & utility to the organism.**
- Steps between natural sample and a clear(er) version of it are time-consuming, messy, space intensive, expensive, and fallible.
- ***Perfect clarification is virtually impossible. The best one achieves is reduced scatter.***

- **Perfect immunity to scatter exists in the CLARiTY integrating cavity spectrophotometer.**
- **SCATTER DOES NOT MATTER!**
- **THE RIGHT ANSWER IS THE ONLY ANSWER**
- **YOU, NOT THE SPECTROPHOTOMETER, DETERMINE ANY CHANGE FROM NATURAL**

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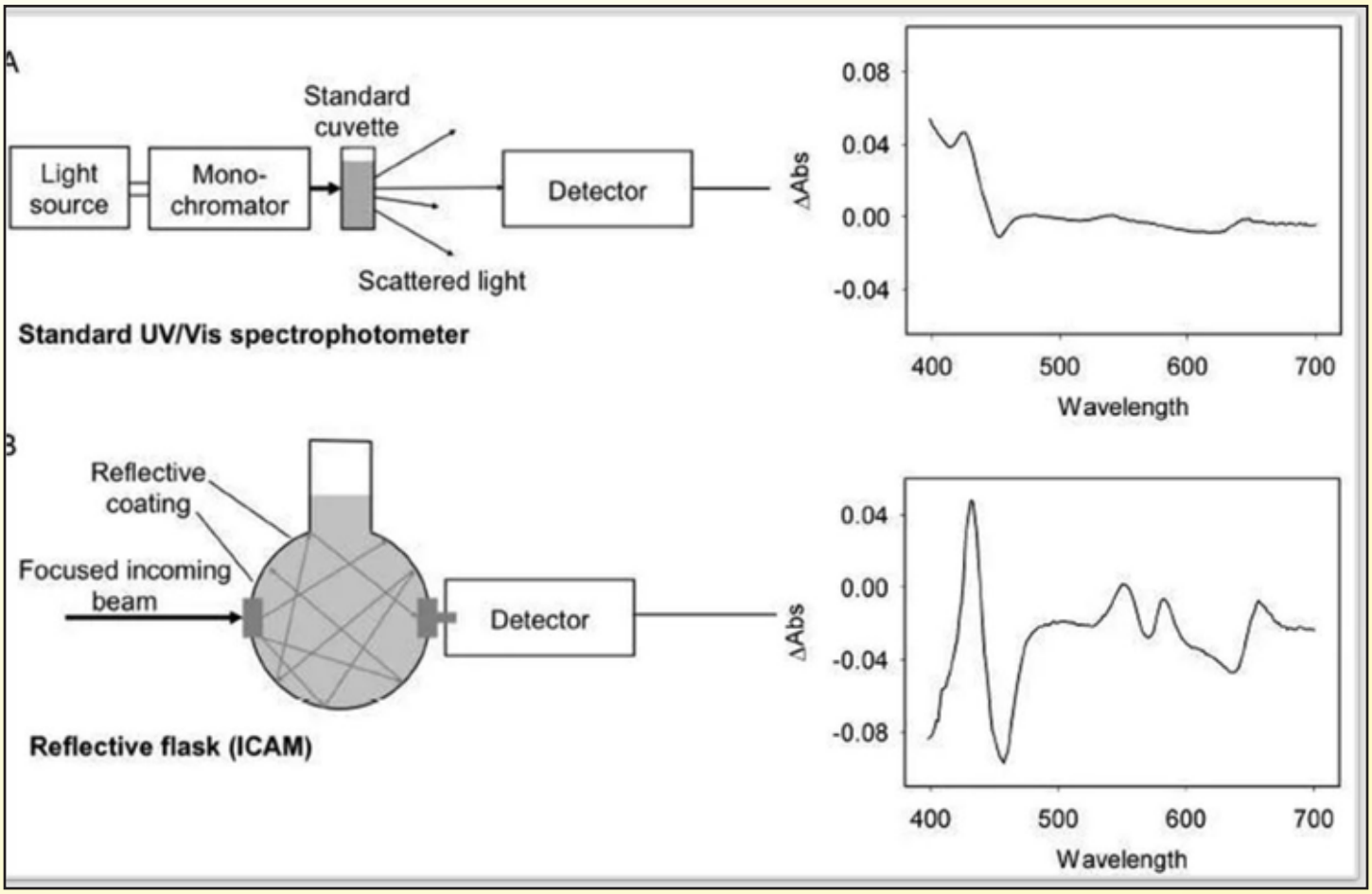
Comparing captured light from cuvette & integrating cavity

(ICAM = Integrating Cavity Absorption Meter)

Graphic from "… In situ measurements of CO binding by integrating cavity dual beam spectrophotometry."

Microbiology 2014

Robert K. Poole, *et. al.*, University of Sheffield



Graphic from 2019 Plant and Cell Physiology by Eva-Maria Aro, et.al., University of Turku using a CLARiTY 17

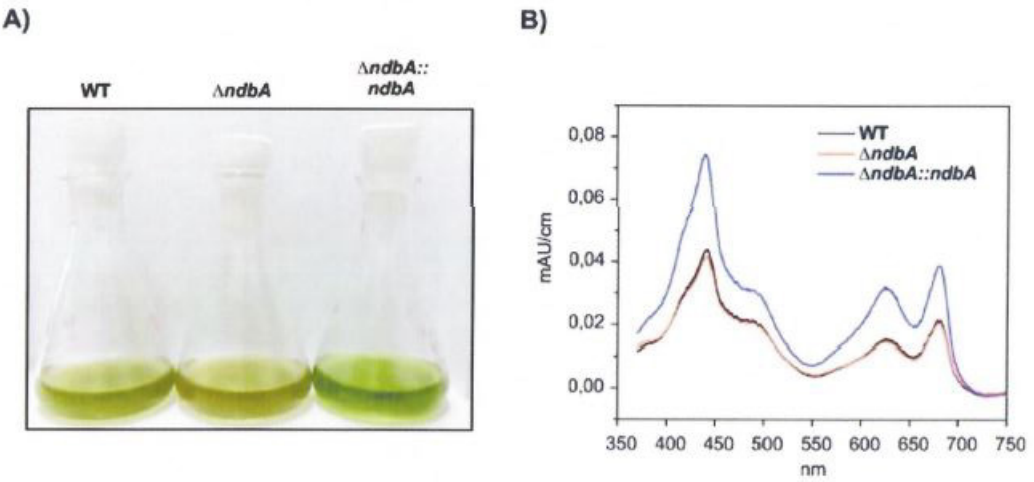


Figure 5. The color difference between WT, ΔndbA and ΔndbA::ndbA grown under light activated heterotrophic growth (LAHG) conditions. A) The color phenotype of WT, ΔndbA and ΔndbA::ndbA cultures grown under LAHG conditions for 4 days. B) The whole cell spectra at room temperature from WT (black line), ΔndbA (red line) and ΔndbA::ndbA (blue line) grown under LAHG conditions. OD750 was adjusted to 0.3 before recording the absorption spectra. Each curve is an average from 4 biological repetitions.

Graphic from 2013 PLoS ONE by Doug Campbell, et.al., University of Mt. Allison using a CLARiTY 17

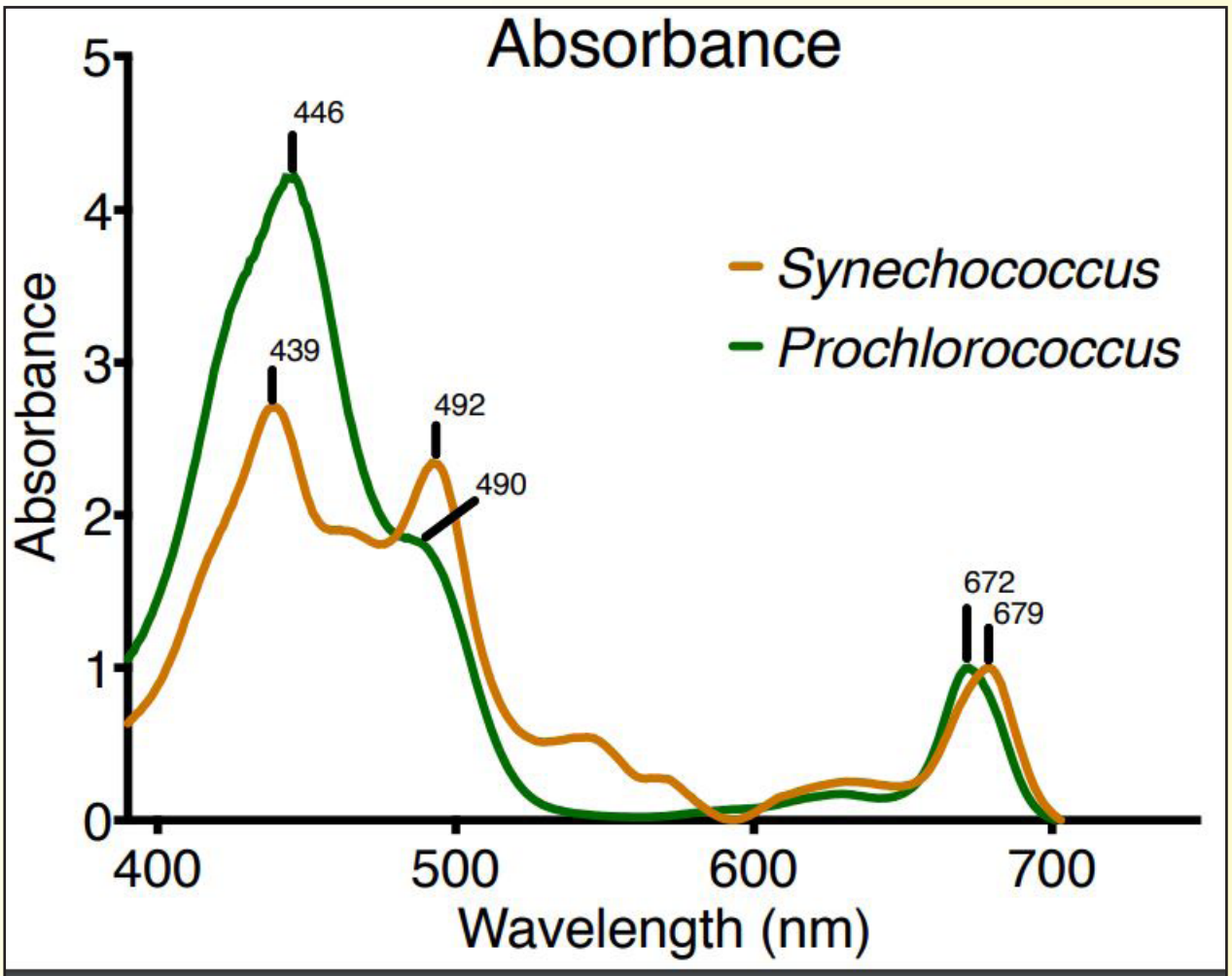
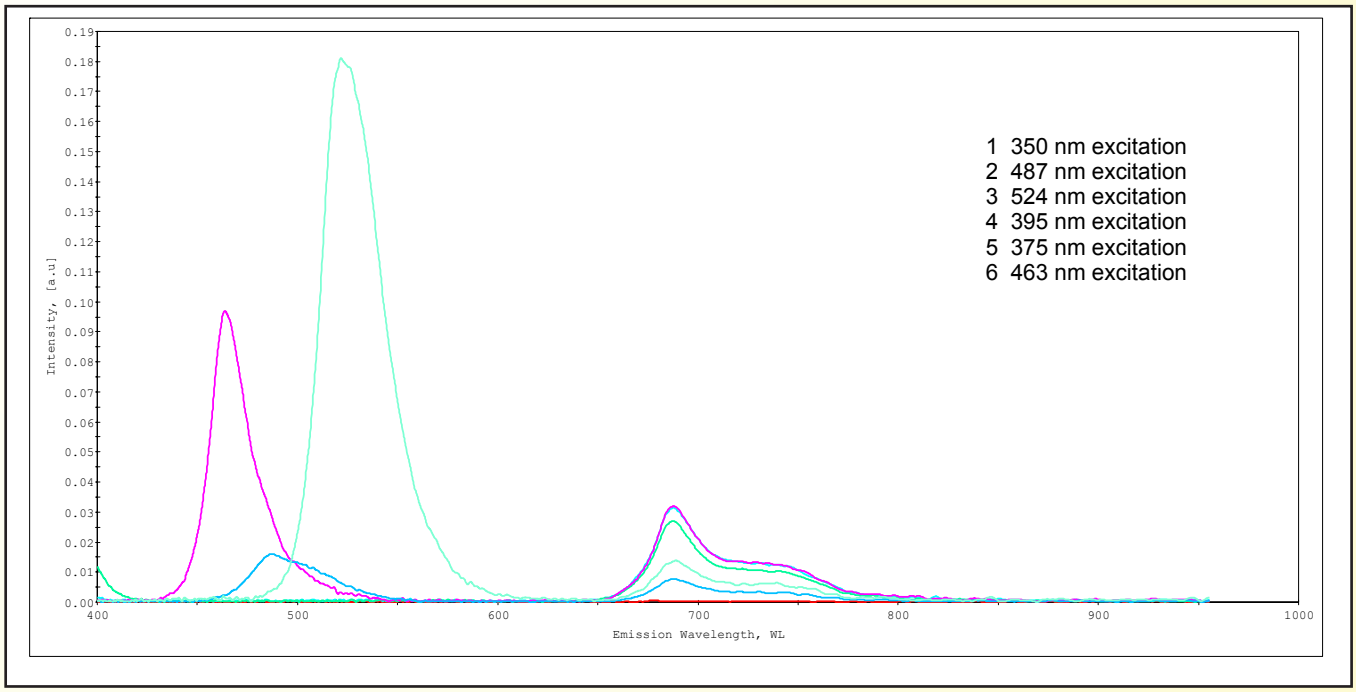
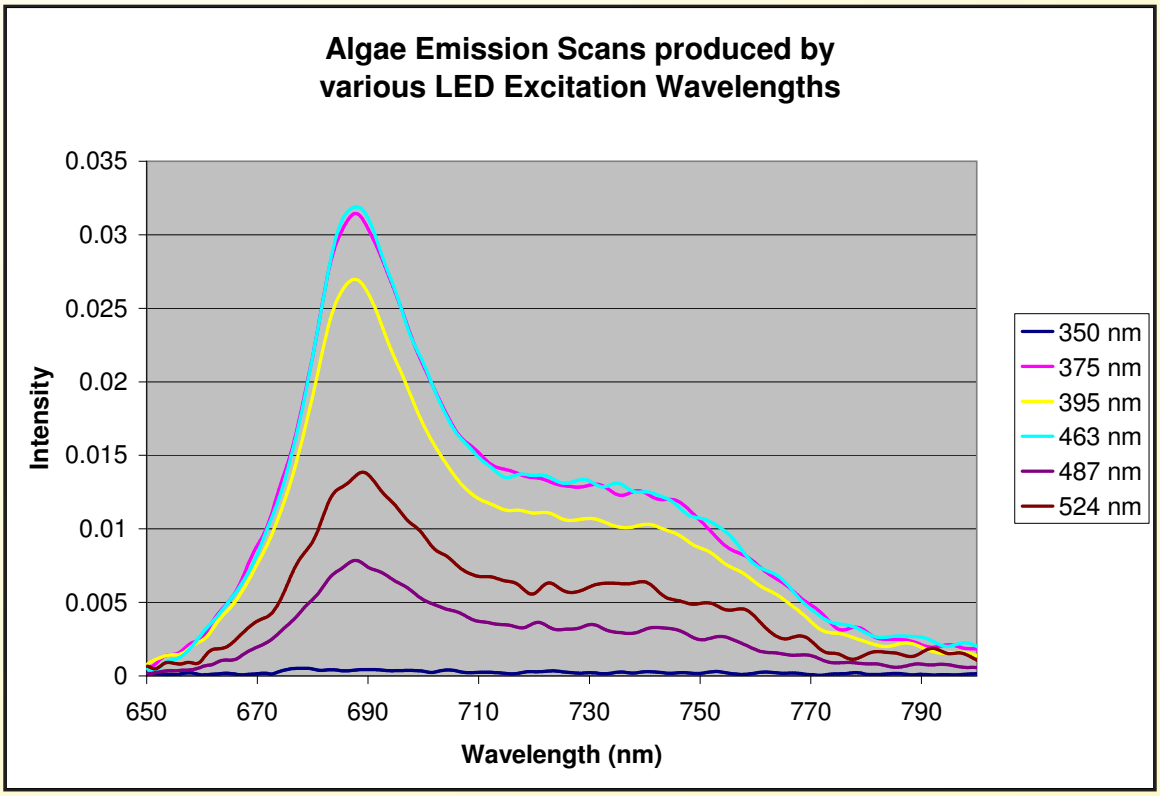


Figure 2.5 - Absorbance Spectra of Synechococcus and Prochlorococcus
The spectra for Synechococcus sp. WH8102 (orange) was collected on March 13, 2014 and the spectra for Prochlorococcus marinus MED4 (green) was collected on March 18, 2014. Both cultures were grown at 260 µmol photons m-2 s-1 and tested in late exponential growth phase using the Olis CLARiTY DSPC photometer (Bogart, Georgia). Spectra were corrected as described in Materials and Methods, section 3.5, and normalized to a value of 1.00 at their red peaks.

In-house emission results using a CLARiTY CCD

Done @ request of Tuo Shi, Xiamen University





More Technical Details

Inside the integrating cavity, the measurement light is an isotropic "gas of photons" which cannot be further dispersed or scattered by a turbid sample. The consequence is a huge improvement over normal focused light systems in the study of turbid samples *(see Bateman & Monk, Science, 121, 441, (1955)).*

The integrating cavity chamber also enjoys a **huge increase in effective pathlength** over a cuvette and thus a **huge increase in sensitivity.** The increase in sensitivity is related to the volume of the sample and the character of the cavity and is as long as 30 cm using 9 mL of sample.

The pathlength is not linear and thus does not follow Beer's Law. Instead, the pathlength is volume and absorbance dependent. The acquired "raw apparent absorbance" is converted to the final AU/cm using the method of Javorfi et al, *(Jrl Photochem Photobiol B, Biology 2006; 82(2): 127-31).*

Many components of cells and subcellular organelles – e.g., proteins – can exhibit fluorescence. On suitably configured CLARiTY, **emission spectra can be collected with or instead of absorbance.**

The host spectrophotometers equipped with the integrating cavity chamber (aka, **DSPC – DeSa Suspension Presentation Cavity)** include our rapid-scanning OLIS RSM 1000, the upcycled Cary 14/17 UV/Vis/NIR, OLIS DM 45, OLIS DM 245, and a CCD model.

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