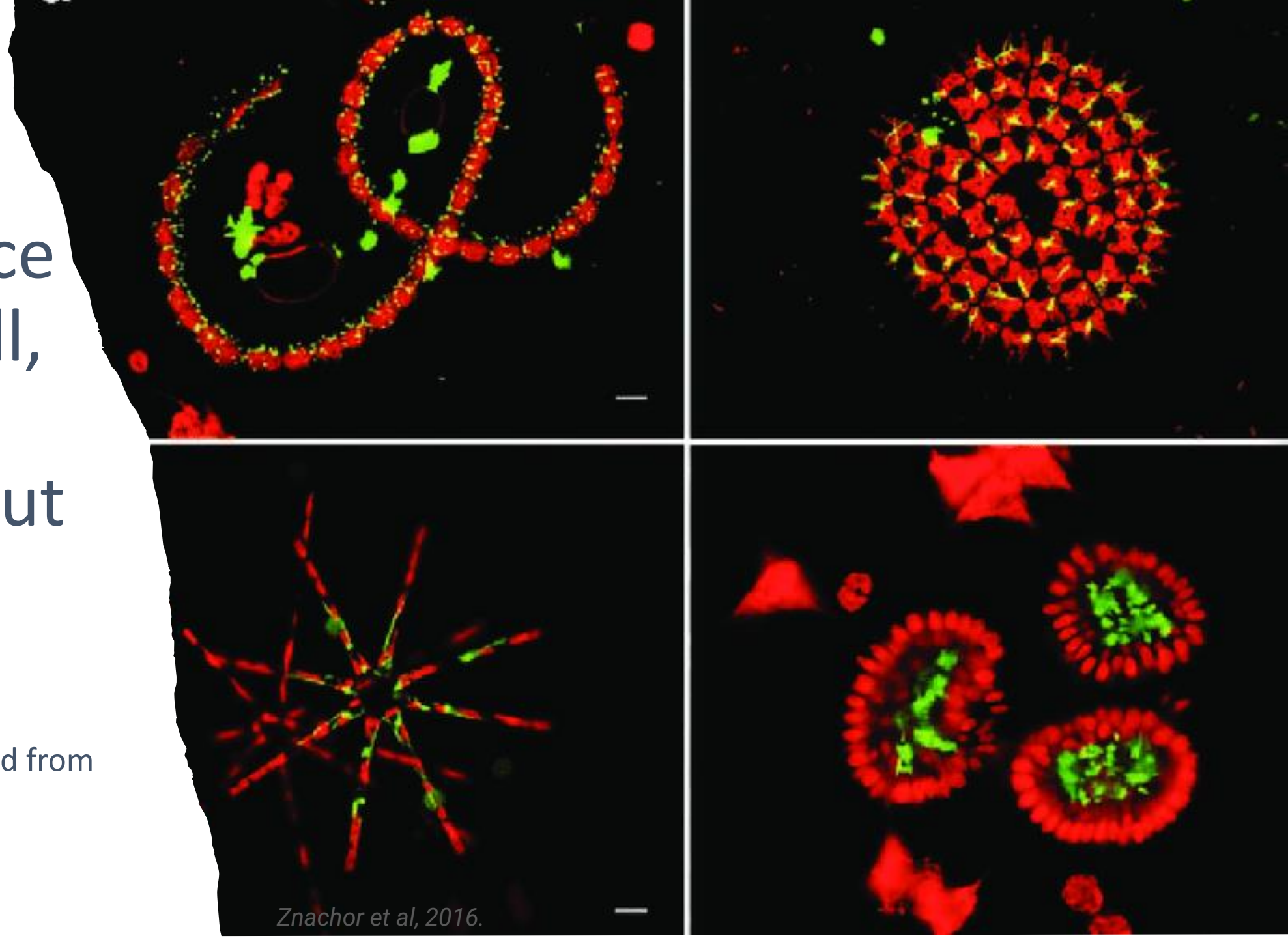
Fluorescence (Chlorophyll, mostly, and tiny bit about CDOM)

Ivona Cetinić

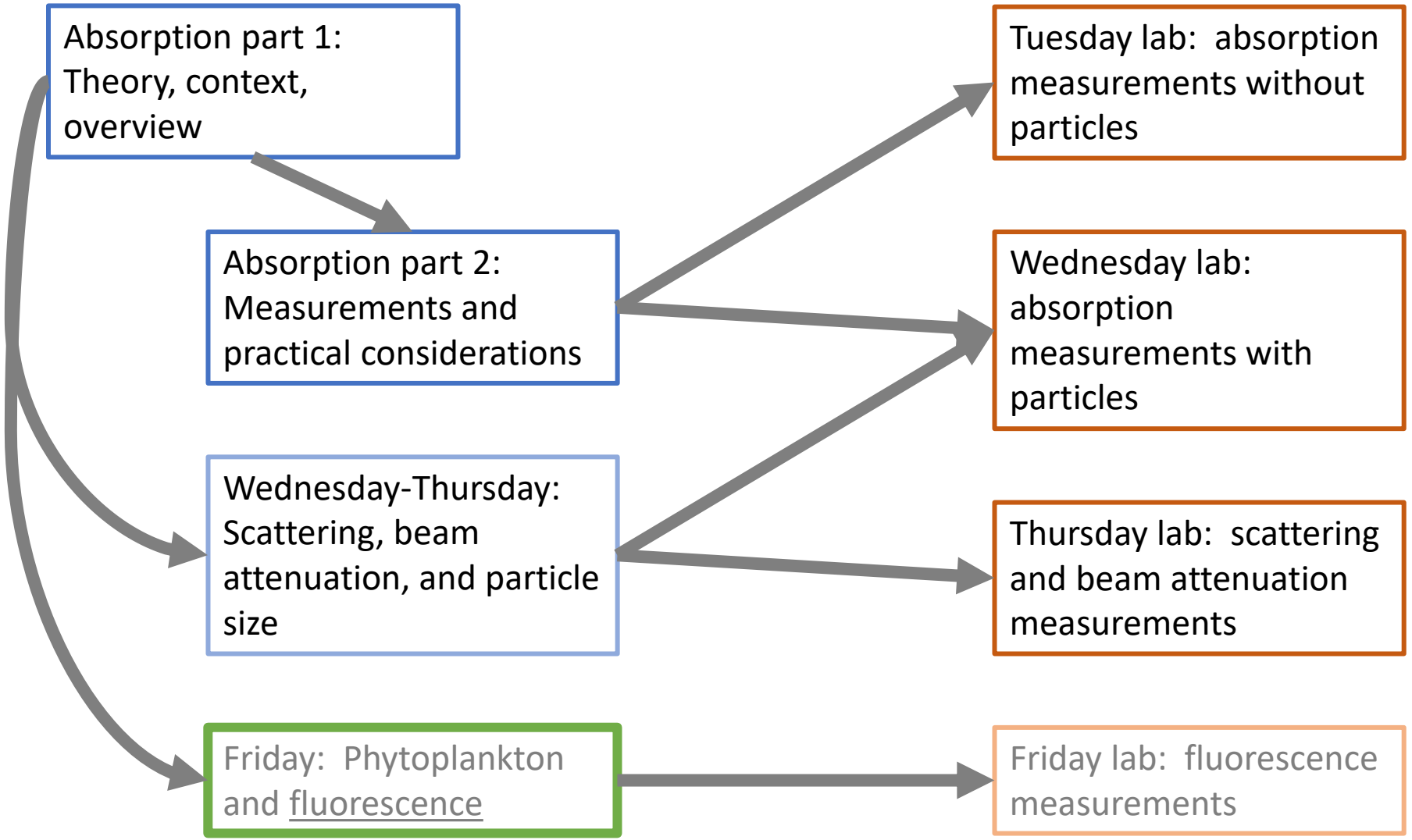
Slides and ideas borrowed from Collin & Mary Jane

GODDARD EARTH SCIENCES



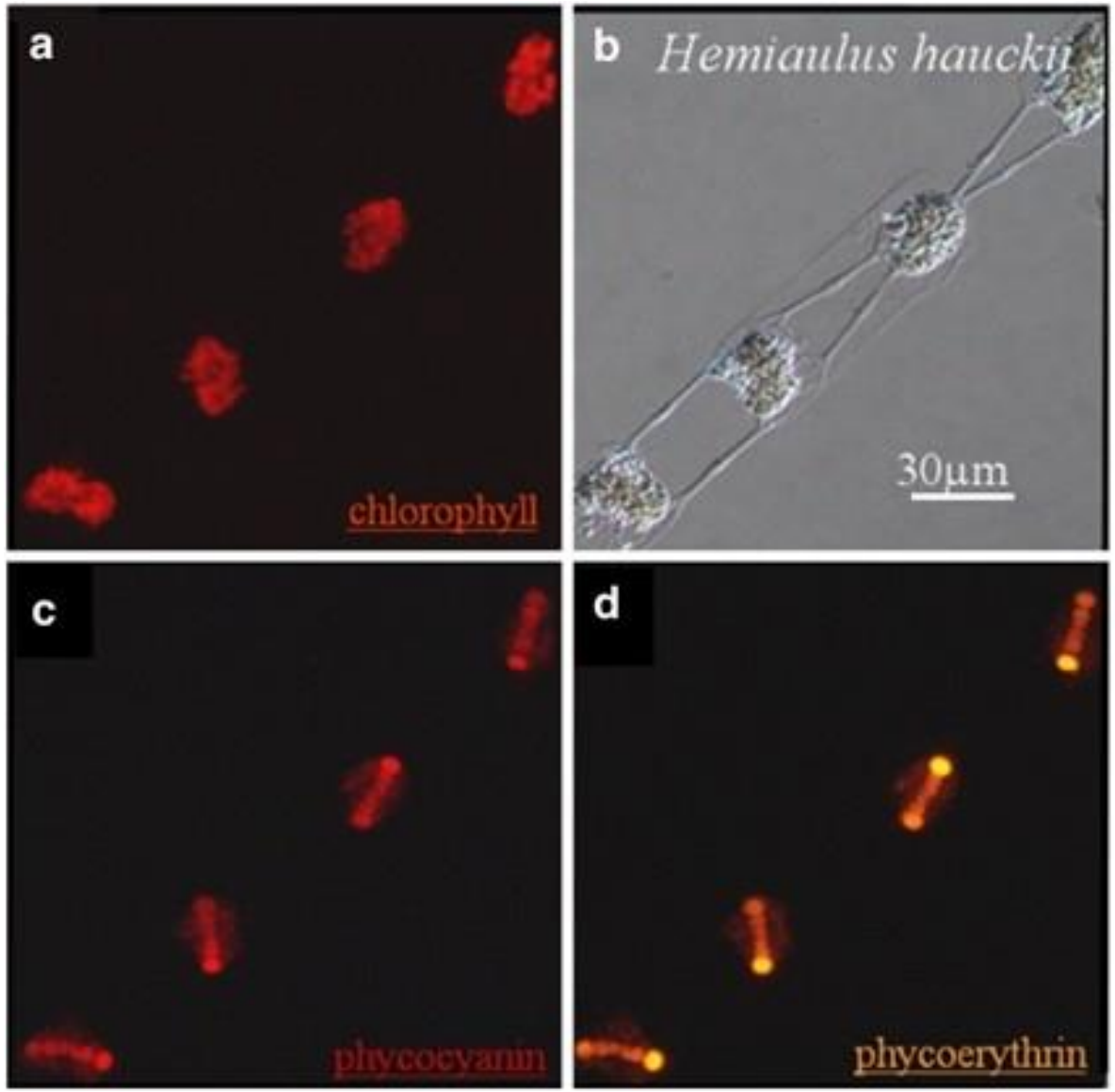
Znachor et al, 2016.

Class context: Week 1 roadmap



Fluorescence lecture

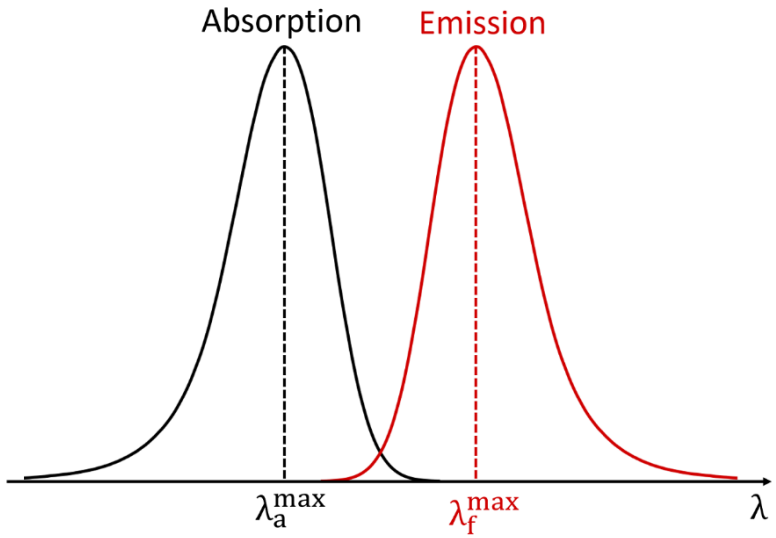
- Fluorescence is the property of a molecule to re-emit absorbed light energy at a longer wavelength (lower energy)
- Things that fluoresce in ocean
  - Pigments – Chlorophyll (s), phycoerythrin, phycocyanin
  - Organic components in dissolved world
  - Oil
  - Certain minerals (sediment)



Zev et al, ISME 2008

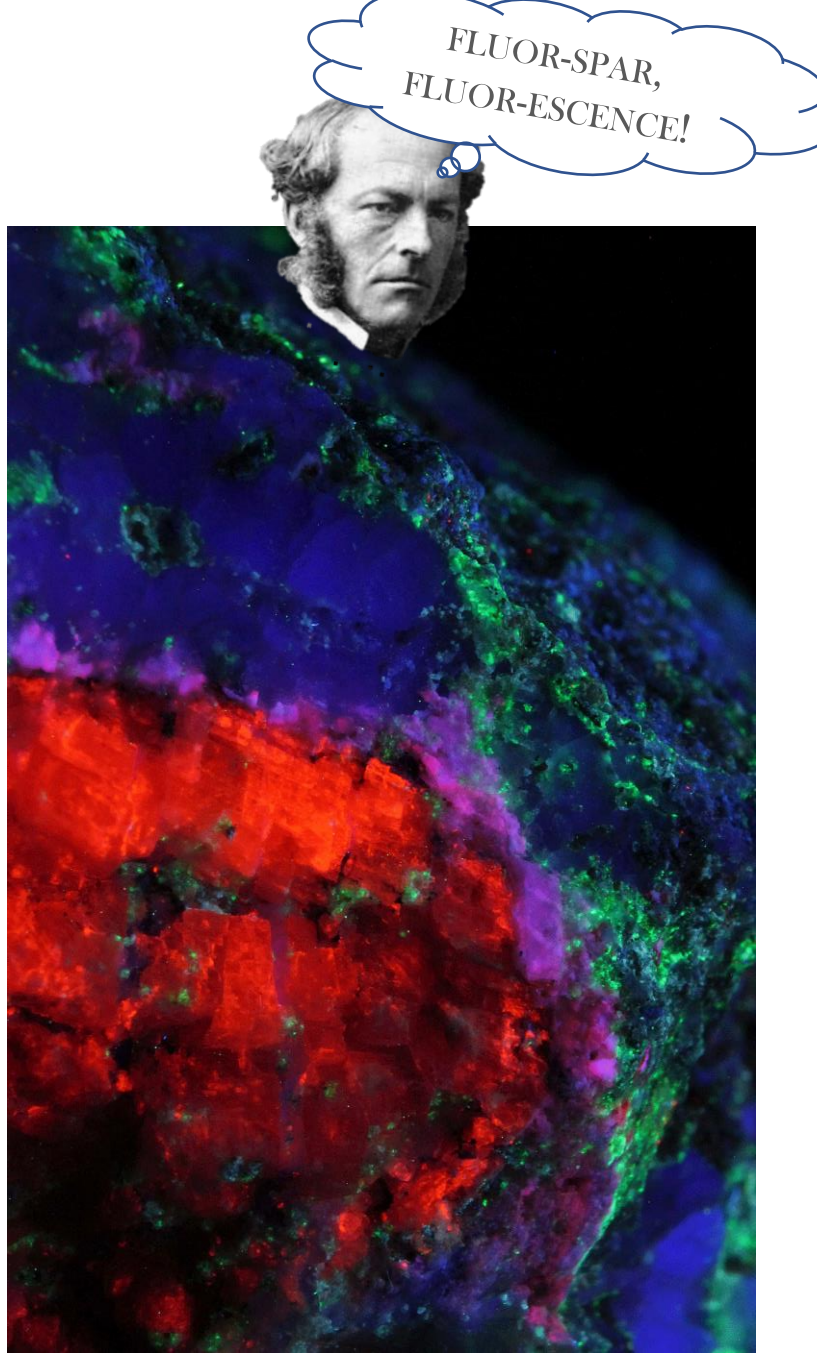
What is Fluorescence?

A type of inelastic scattering where fraction of energy absorbed at **shorter wavelength** (higher frequency, higher energy) is re-emitted as a photon at **longer wavelength** (lower frequency, lower energy).



$$\downarrow E = h\nu = \frac{hc}{\lambda} \uparrow$$

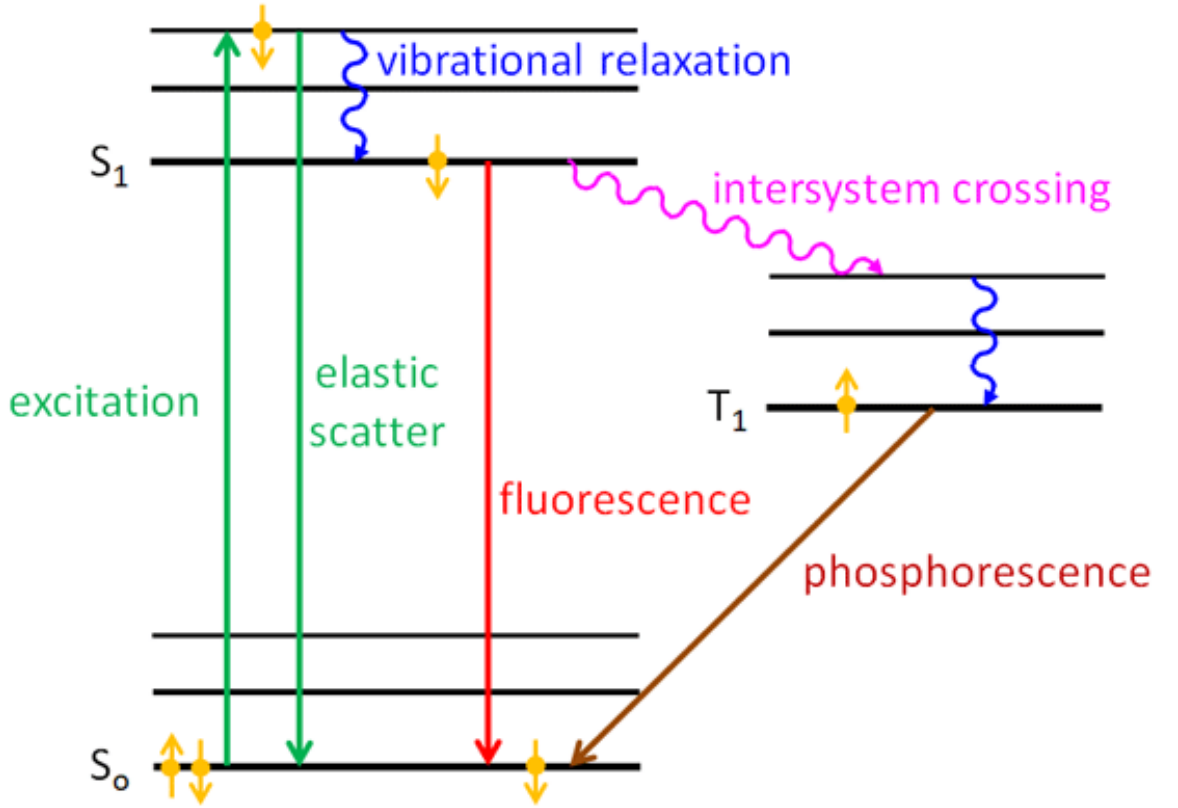
Only certain molecules can fluoresce (e.g., chlorophyll a, some organic molecules, minerals), unique fingerprint.



You have seen this on Tuesday …

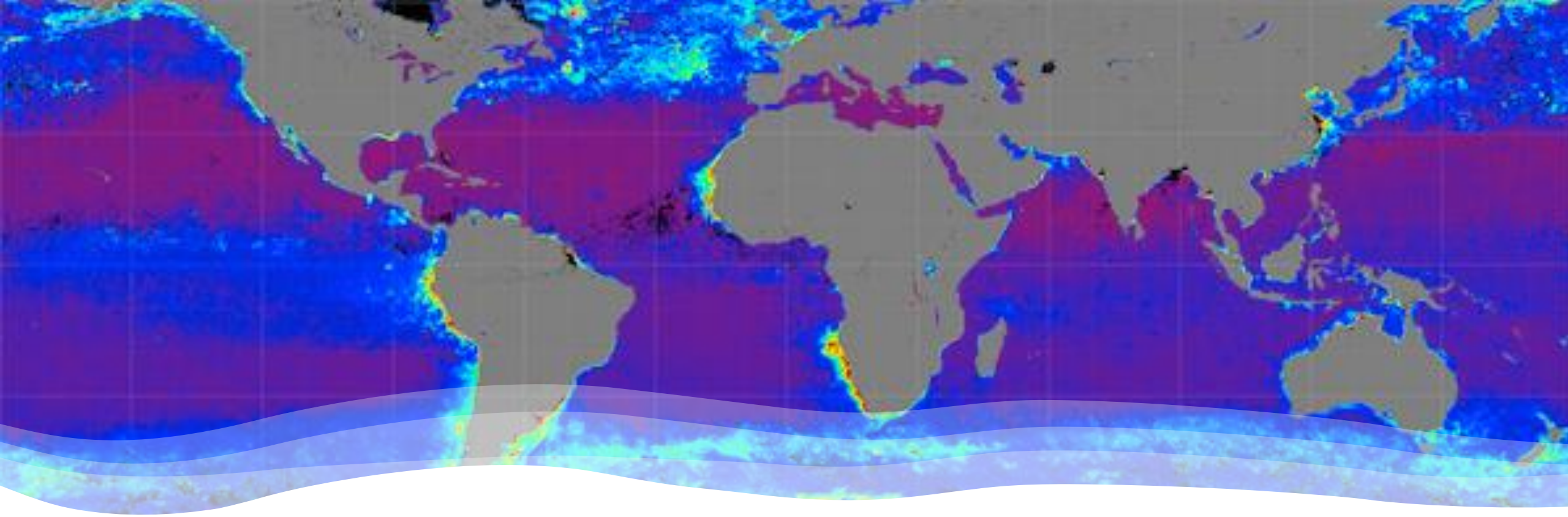
Absorption only happens if the energy of the photon is equal to Δ energy between an electron in the ground electronic state (S0) and in a higher electronic state (Sn).

($O(10^{-15}$ s))



Fluorescence only happens from lowest electronic state of S1 (not Sn).
$O(10^{-7} – 10^{-10}$ s)

Mobley' Optics book, 2022



What fluoresces in the ocean/aquatic environment?

- Chlorophyll
- Colored dissolved organic matter
  - Oil (think oil spills..)
- Phycoerythrin
- Some minerals?

Using fluorescence as a proxy for biogeochemical property

Chlorophyll fluorescence as proxy for

- Chlorophyll concentration
- *Phytoplankton* biomass
- photosynthesis
- Physiological state of the cell

Phycoerythrin fluorescence as proxy for

- •Phycoerythrin concentration
- •*Cyanobacterial* biomass

CDOM fluorescence as proxy for

- •“CDOM” concentration or absorption
- •Dissolved organic carbon concentration (DOC)

https://sarahefawcett.wordpress.com/

Using fluorescence as a proxy for biogeochemical property

Chlorophyll fluorescence as proxy for

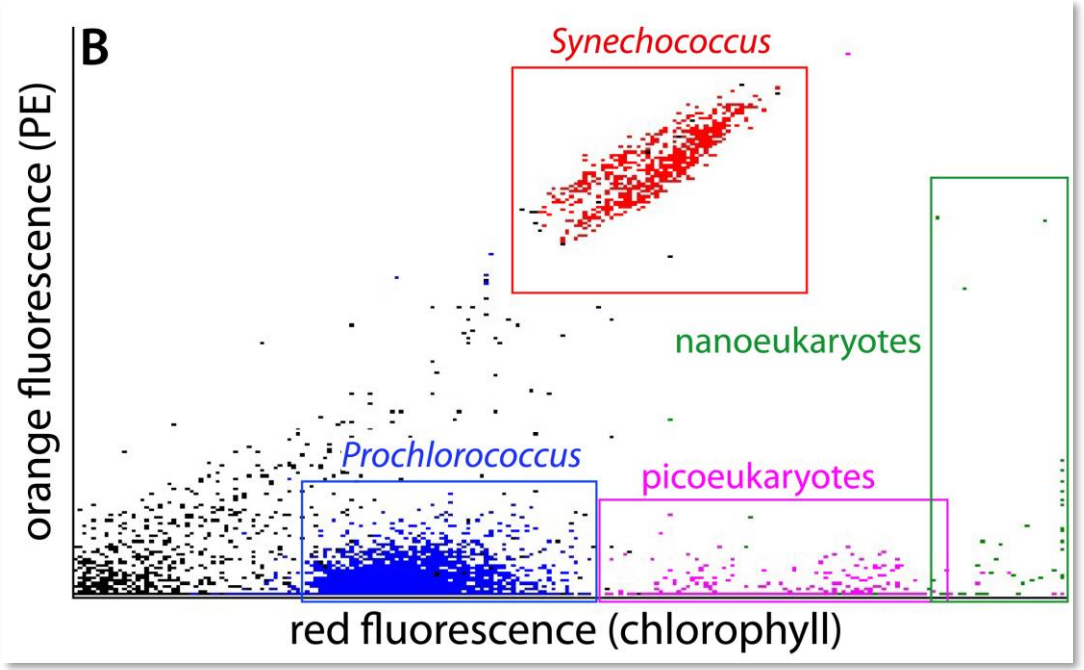
- Chlorophyll concentration
- *Phytoplankton* biomass
- photosynthesis
- Physiological state of the cell

Phycoerythrin fluorescence as proxy for

- •Phycoerythrin concentration
- •*Cyanobacterial* biomass

CDOM fluorescence as proxy for

- •“CDOM” concentration or absorption
- •Dissolved organic carbon concentration (DOC)



https://sarahefawcett.wordpress.com/

Measuring fluorescence

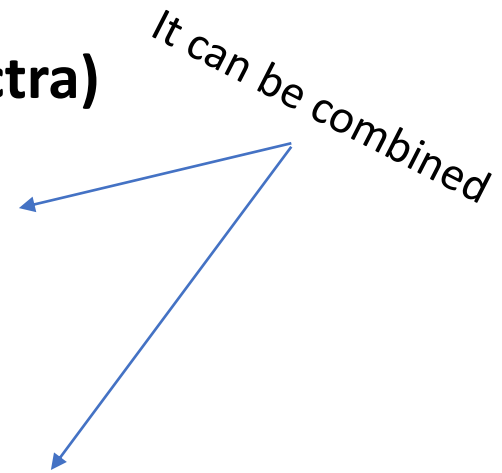
Fluorescence Excitation Spectrum (it looks a bit like absorption spectra)

- Monitor fluorescence signal at some emission wavelength (e.g., 695 nm)
- Excite the sample with light along the spectrum
- Plot the magnitude of fluorescence associated with each excitation wavelength
- Ensure or correct for constant excitation energy across spectrum

Measuring fluorescence

Fluorescence Excitation Spectrum (it looks a bit like absorption spectra)

- Monitor fluorescence signal at some emission wavelength (e.g., 695 nm)
- Excite the sample with light along the spectrum
- Plot the magnitude of fluorescence associated with each excitation wavelength
- Ensure or correct for constant excitation energy across spectrum



Fluorescence Emission Spectrum

- Excite fluorescence at some excitation wavelength (e.g., 420 nm)
- scan the emission signal in response to excitation along the emission waveband
- Ensure uniform detection response across emission

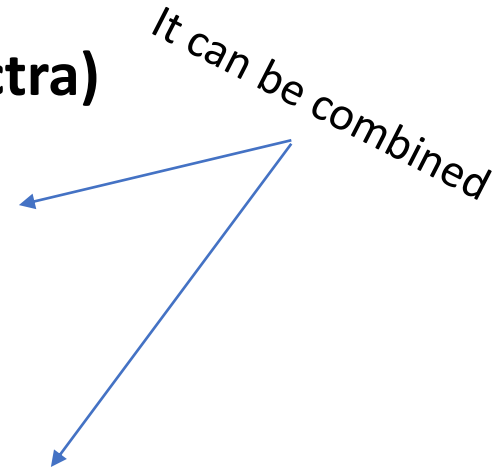
Measuring fluorescence

Fluorescence Excitation Spectrum (it looks a bit like absorption spectra)

- Monitor fluorescence signal at some emission wavelength (e.g., 695 nm)
- Excite the sample with light along the spectrum
- Plot the magnitude of fluorescence associated with each excitation wavelength
- Ensure or correct for constant excitation energy across spectrum

Fluorescence Emission Spectrum

- Excite fluorescence at some excitation wavelength (e.g., 420 nm)
- scan the emission signal in response to excitation along the emission waveband
- Ensure uniform detection response across emission



FROM SPACE (sun induced ChI F) – check out Behrenfeld et al 2009

Measuring fluorescence

Fluorescence Excitation Spectrum (it looks a bit like absorption spectra)

- Monitor fluorescence signal at some emission wavelength (e.g., 695 nm)
- Excite the sample with light along the spectrum
- Plot the magnitude of fluorescence associated with each excitation wavelength
- Ensure or correct for constant excitation energy across spectrum

Fluorescence Emission Spectrum

- Excite fluorescence at some excitation wavelength (e.g., 420 nm)
- scan the emission signal in response to excitation along the emission waveband
- Ensure uniform detection response across emission

Single Ex/Em(e.g., ECO sensor)

- Excite at one wavelength (e.g., 420 nm)
- Measure emission at one wavelength (e.g., 695 nm)
- Calibrate to known chlorophyll concentration

Measuring fluorescence

Fluorescence Excitation Spectrum (it looks a bit like absorption spectra)

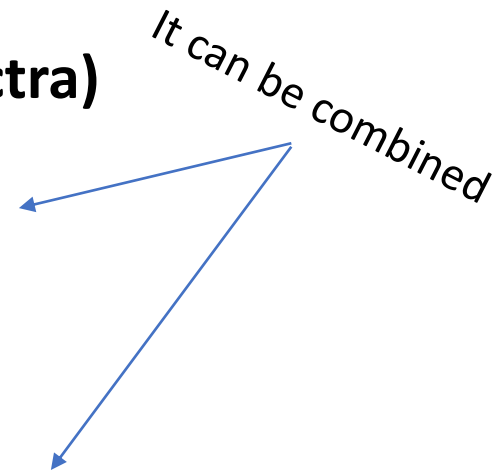
- Monitor fluorescence signal at some emission wavelength (e.g., 695 nm)
- Excite the sample with light along the spectrum
- Plot the magnitude of fluorescence associated with each excitation wavelength
- Ensure or correct for constant excitation energy across spectrum

Fluorescence Emission Spectrum

- Excite fluorescence at some excitation wavelength (e.g., 420 nm)
- scan the emission signal in response to excitation along the emission waveband
- Ensure uniform detection response across emission

Single Ex/Em(e.g., ECO sensor)

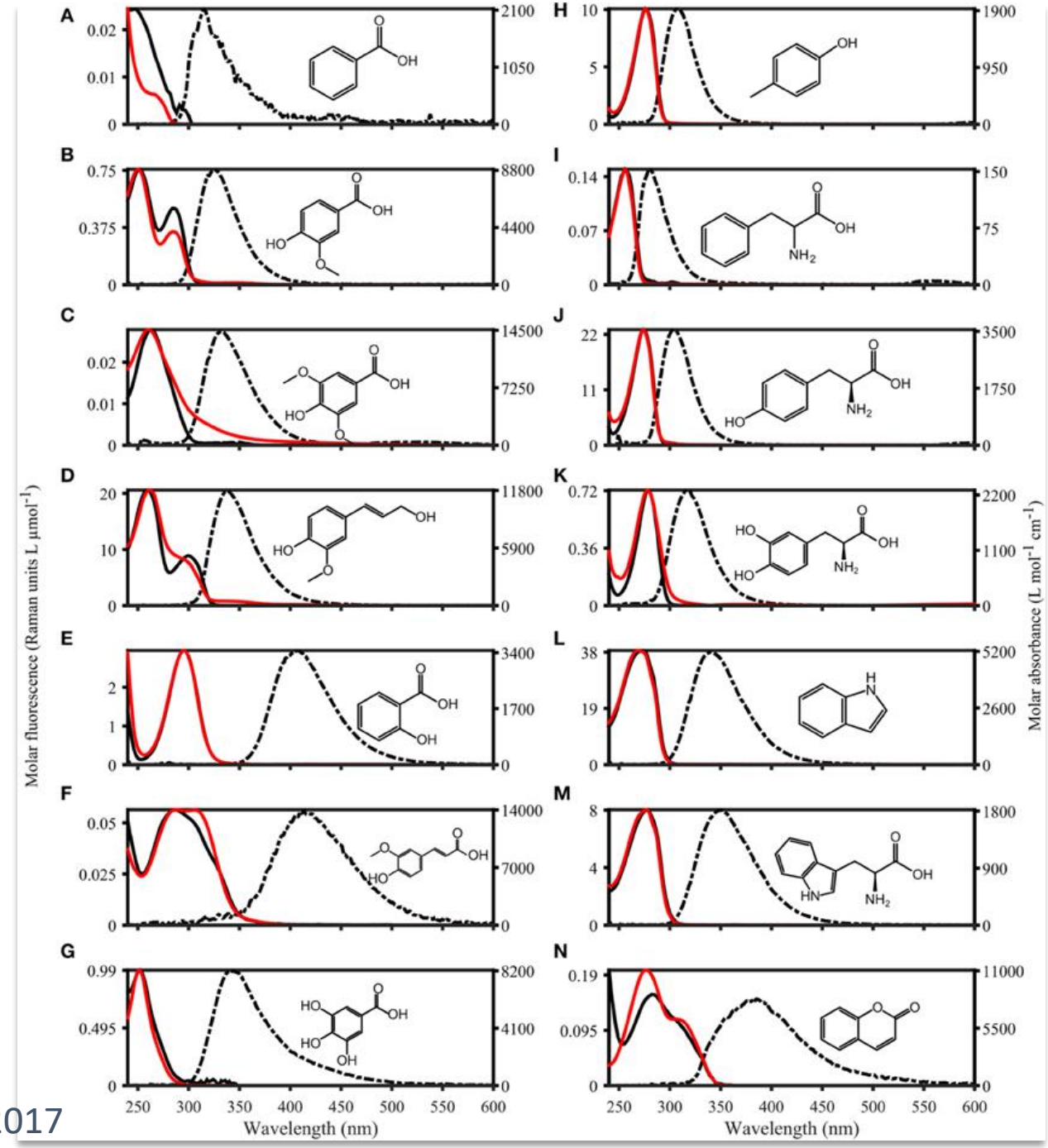
- Excite at one wavelength (e.g., 420 nm)
- Measure emission at one wavelength (e.g., 695 nm)
- Calibrate to known chlorophyll concentration



Variable Chlorophyll Fluorescence
Chapter 9, IOCCG 2022

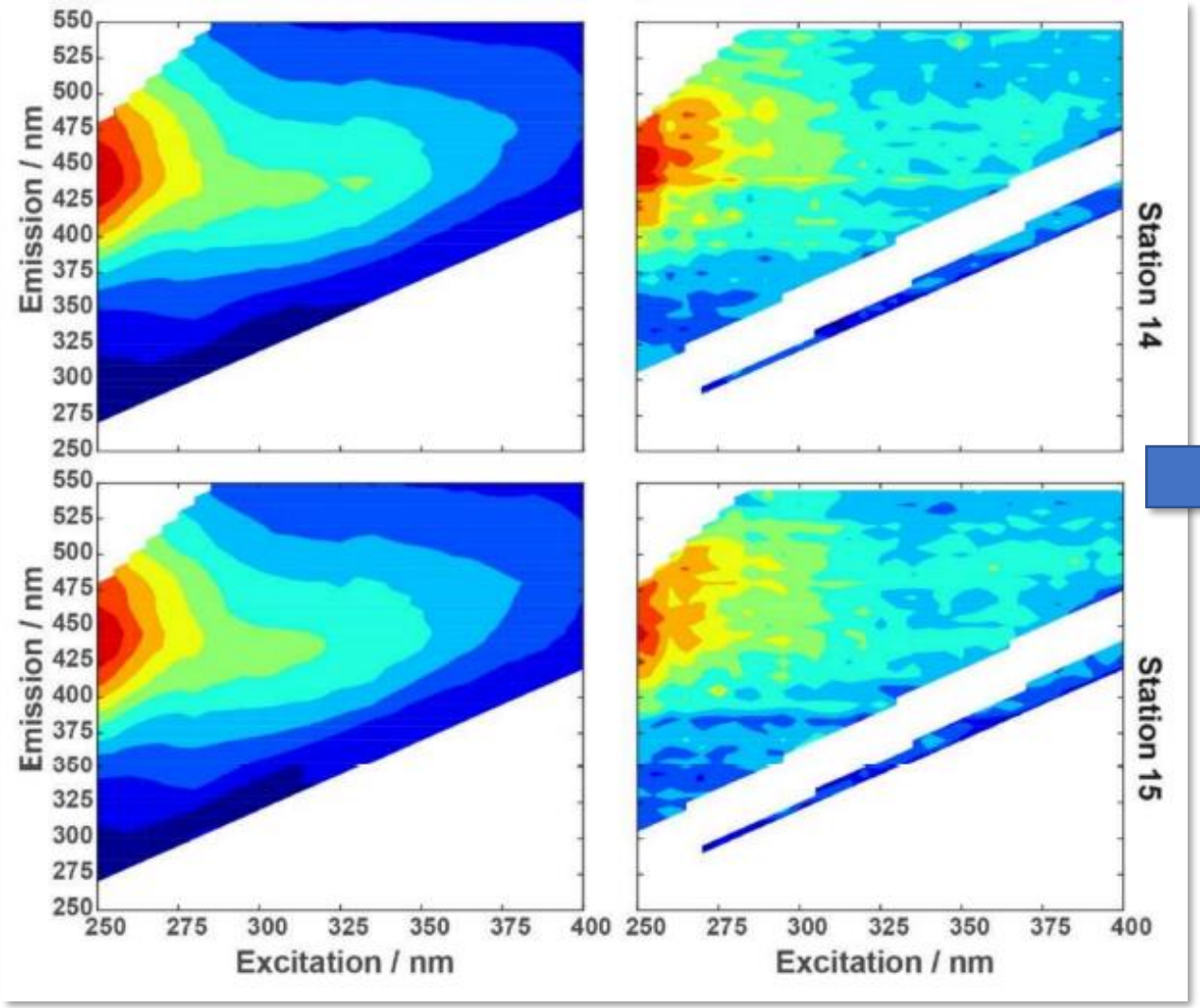
CDOM fluorescence

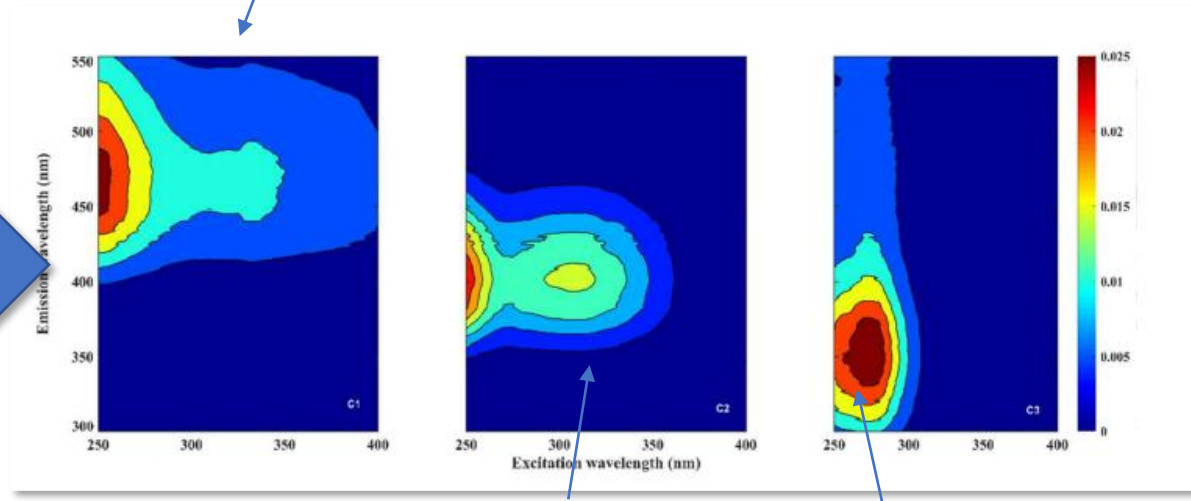
- Really complex
  - Think about how many things can be called “CDOM”
- Molar absorption (red); Fluorescence (black dash)
  - Range of absorption/fluorescence emission spectra



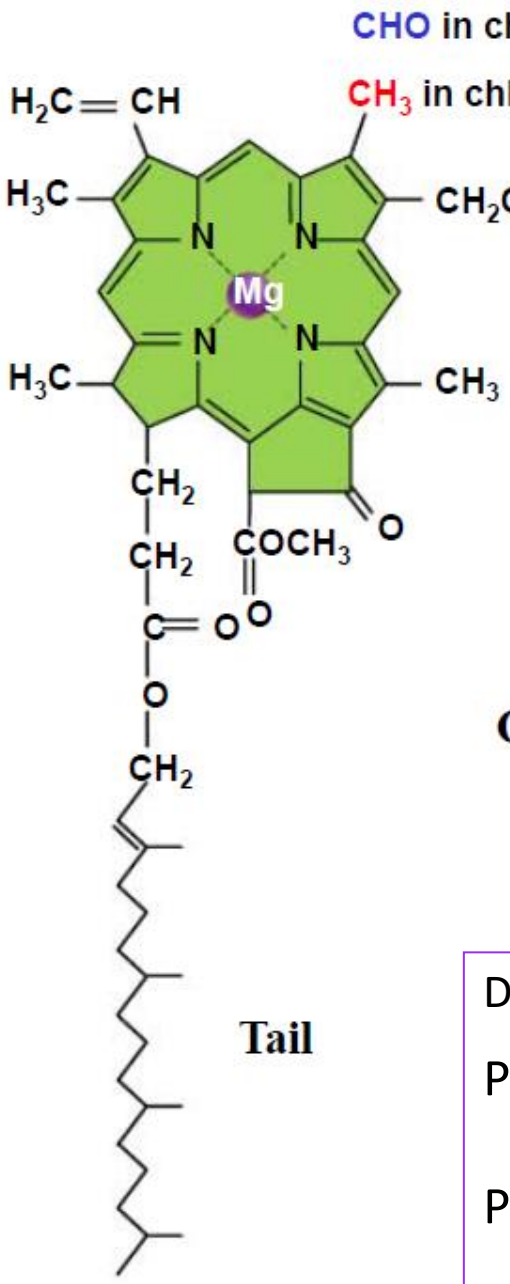
Wünsch et al, 2017

CDOM emission/excitation – CDOM composition





Zielinski et al 2018



HRH CHLOROPHYLL

CHLOROPHYLL a & b

Degraded pigments:

Pheophytin

lost Mg^{++}; peak shifts to ~415

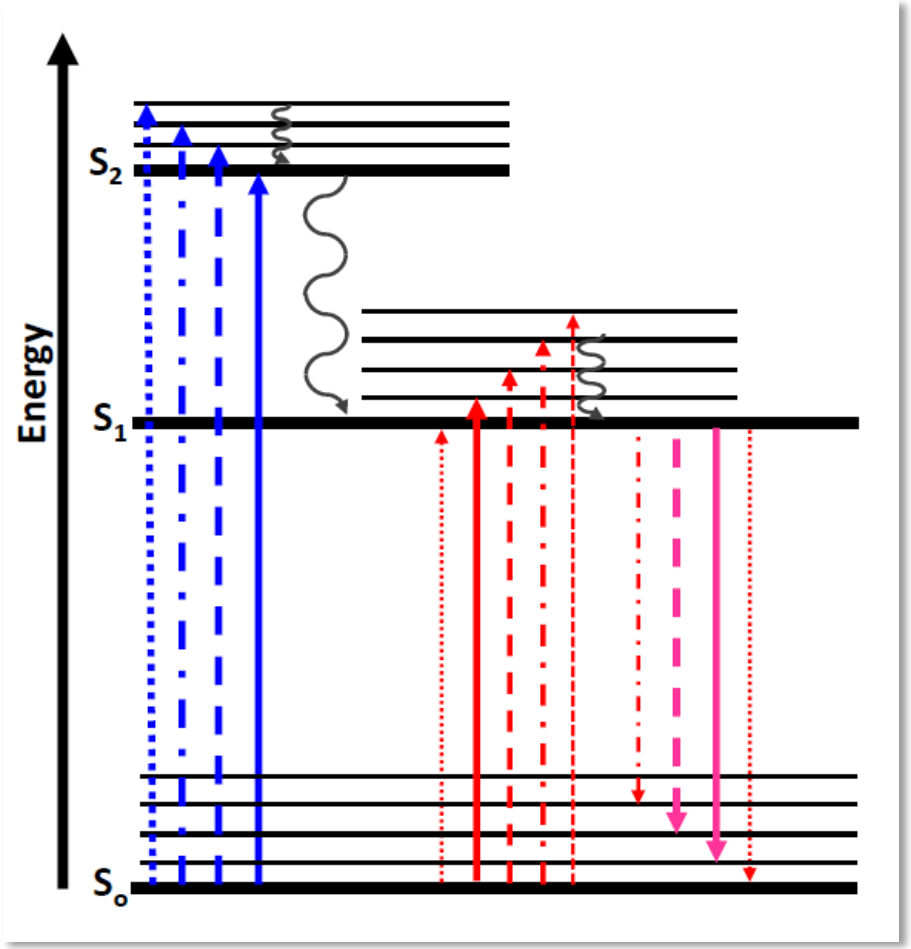
Pheophorbide

lost Mg^{++} and phytol tail

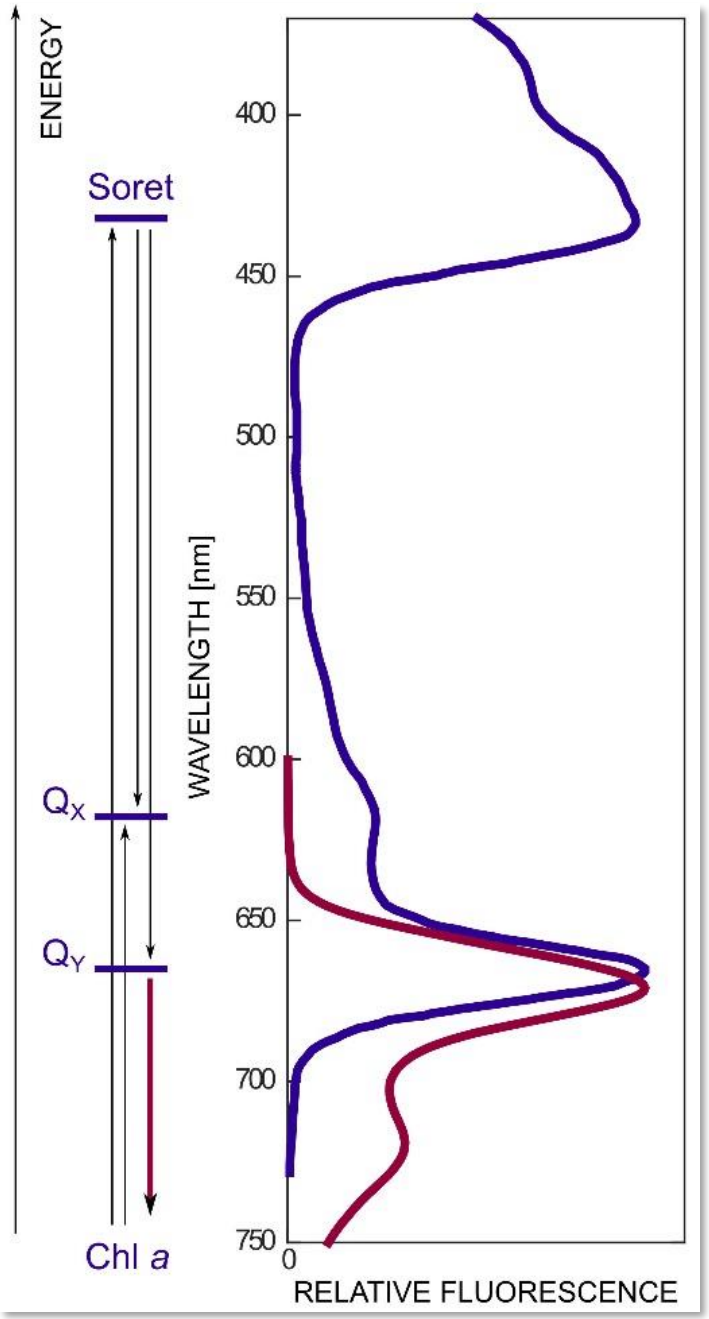
Chlorophyll *a (only molecule, in solvent, aka in-vitro)*

has <u>two</u> primary absorption bands:

- blue Soret band (S2) and red Q band (S1)
- fluorescence emission Stokes' shift is only from Q band (that is why chl. fluorescence is red, not blue).



Do [check Collin's lecture] from 2021



In vitro Fluorescence, chlorophyll proxy (aka only molecule, and solvent)

$$F_{chl}(\lambda_2) = E(\lambda_1) \times a_{chl}(\lambda_1) \times \Phi f(\lambda_1, \lambda_2)$$

$$F_{chl} = E \times a_{chl}{}^{*} \times \mathbf{[Chl]} \times \Phi_f$$

Chl molecules in solution, $\boldsymbol{Chl}$

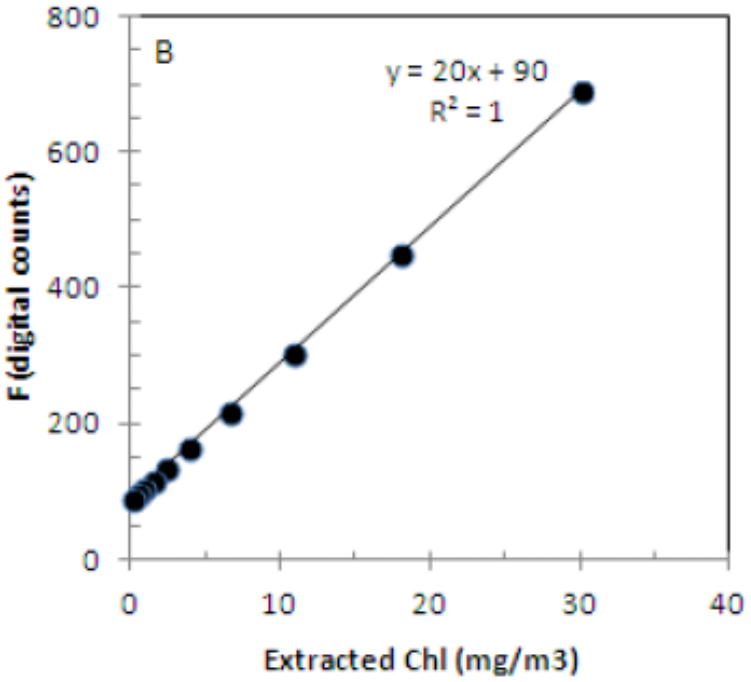
Constant mass-specific absorption, $a_{chl}(\lambda_1)^{*}$ $(m^2 mg^{-1})$

Constant quantum yield, Φf (environment specific, no physiology, ratio of energy fluoresced and energy absorbed)

Maintain constant E

$F_{chl} \sim \mathbf{[Chl]}$

In vitro Fluorescence, chlorophyll proxy (aka only molecule, and solvent)



$$F_{chl}(\lambda_2) = E(\lambda_1) \times a_{chl}(\lambda_1) \times \Phi f(\lambda_1, \lambda_2)$$

$$F_{chl} = E \times a_{chl}{}^{*} \times \mathbf{[Chl]} \times \Phi_f$$

Chl molecules in solution, $\boldsymbol{Chl}$

Constant mass-specific absorption, $a_{chl}(\lambda_1)^{*}$ $(m^2 mg^{-1})$

Constant quantum yield, Φf (environment specific, no physiology, ratio of energy fluoresced and energy absorbed)

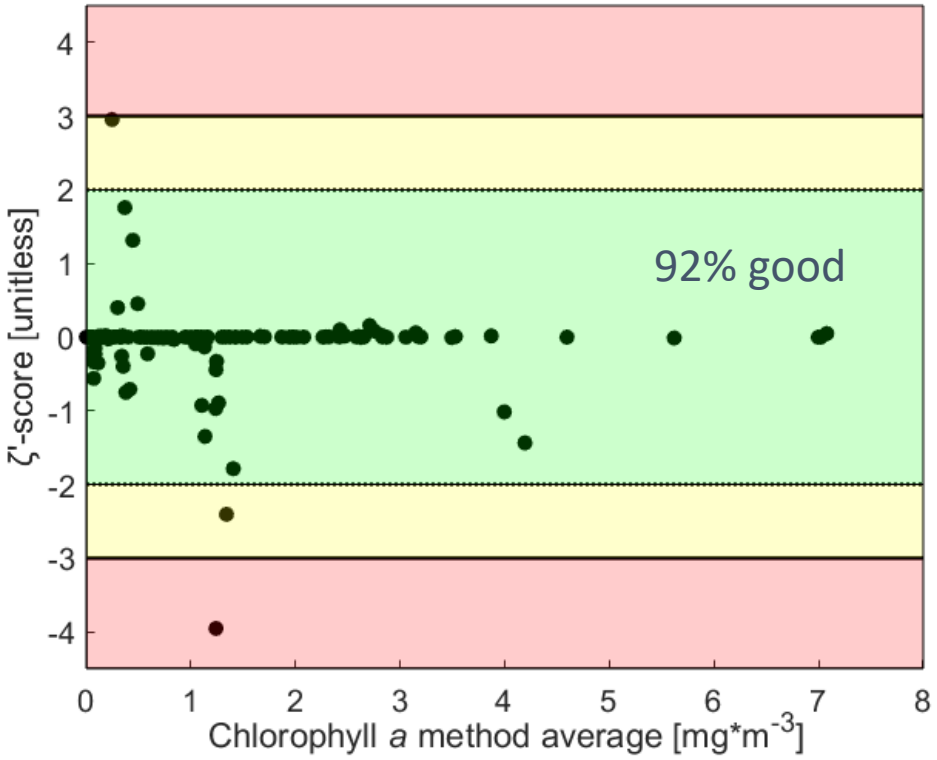
Maintain constant E

$F_{chl} \sim \mathbf{[Chl]}$

Fluorometers relative units, (dc or volts)

Calibrate with Chl standard solution

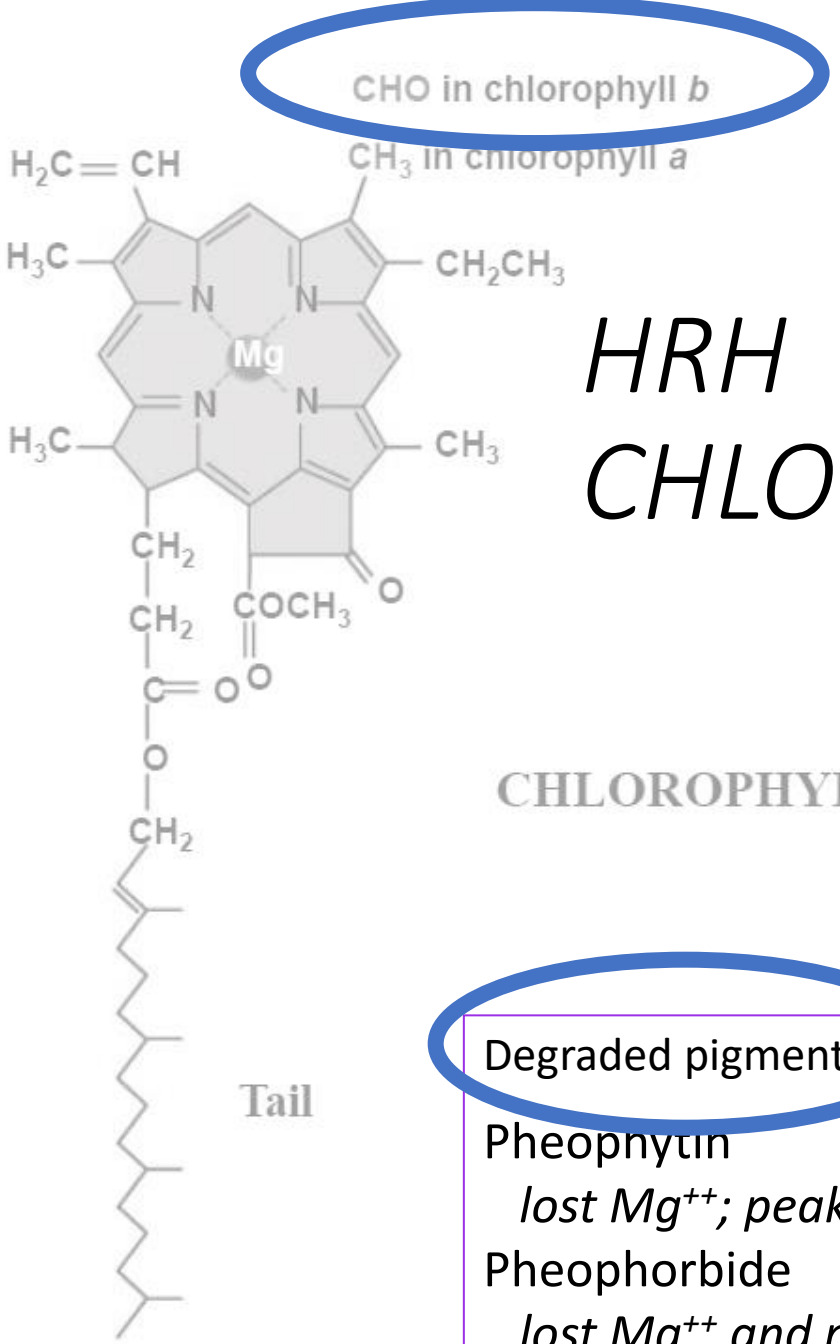
How good is this measurement (in comparison to gold standard, HPLC Chl a)?



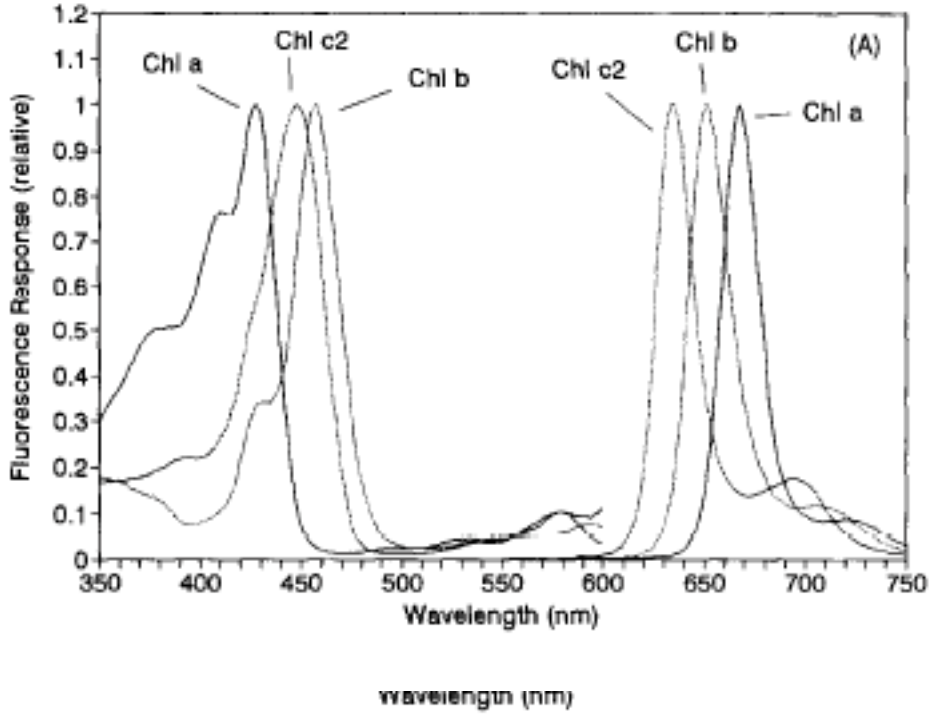
Source of data	n	Slope (lower, upper)	r^2	RMSE	SSE	Bias (mg/m^3)	Bias' (mg/m^3)	MAE (mg/m^3)	MAE' (mg/m^3)
All	1731	0.983 (0.981, 0.986)	0.996	0.191	62.84	0.002	0.002	0.329	0.227

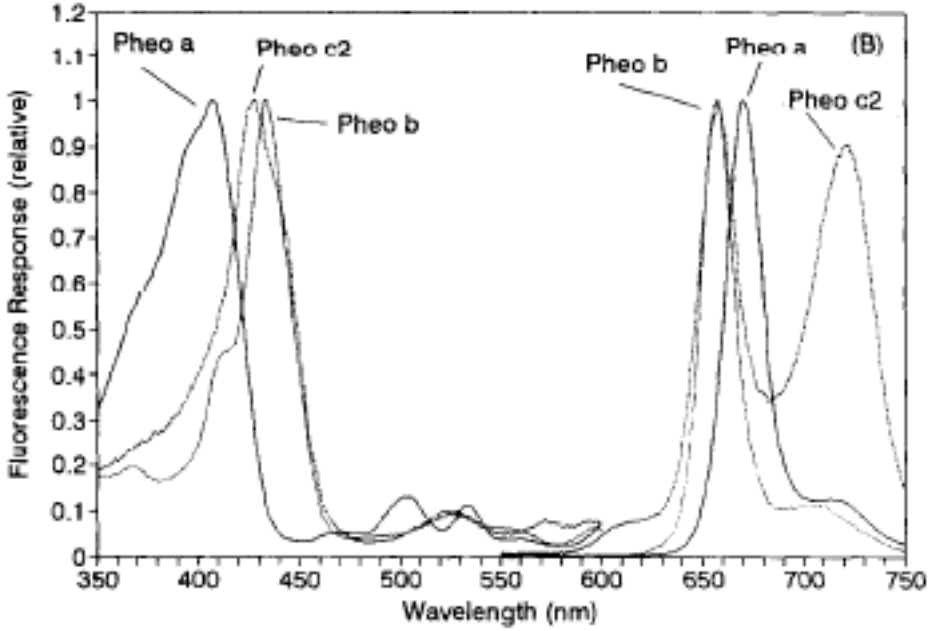
LOOKS GOOD, BUT WHY ARE THE DIFFERENCES THERE?

Neeley, in prep



HRH CHLOROPHYLL





Degraded pigments:

Pheophytin

lost Mg^{++}; peak shifts to ~415

Pheophorbide

lost Mg^{++} and phytol tail

In vitro Fluorescence, chlorophyll proxy (aka only molecule, and solvent)

In Lab today!

Exploring the uncertainties associated with this measurement!!!

$$F_{chl}(\lambda_2) = E(\lambda_1) \times a_{chl}(\lambda_1) \times \Phi f(\lambda_1, \lambda_2)$$

$$F_{chl} = E \times a_{chl}{}^{*} \times \mathbf{[Chl]} \times \Phi_f$$

Chl molecules in solution, $\boldsymbol{Chl}$

Constant mass-specific absorption, $a_{chl}(\lambda_1)^{*}$ $(m^2 mg^{-1})$

Constant quantum yield, Φf (environment specific, no physiology, ratio of energy fluoresced and energy absorbed)

Maintain constant E

$F_{chl} \sim \mathbf{[Chl]}$

Fluorometers relative units, (dc or volts)

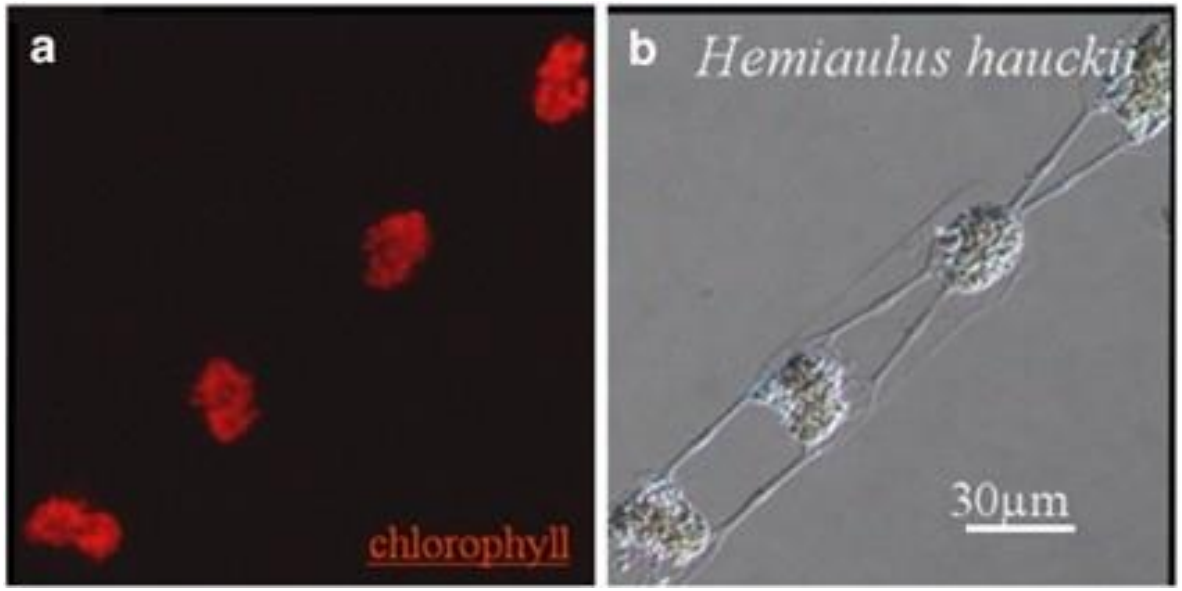
Calibrate with Chl standard solution

Yet, Biology…

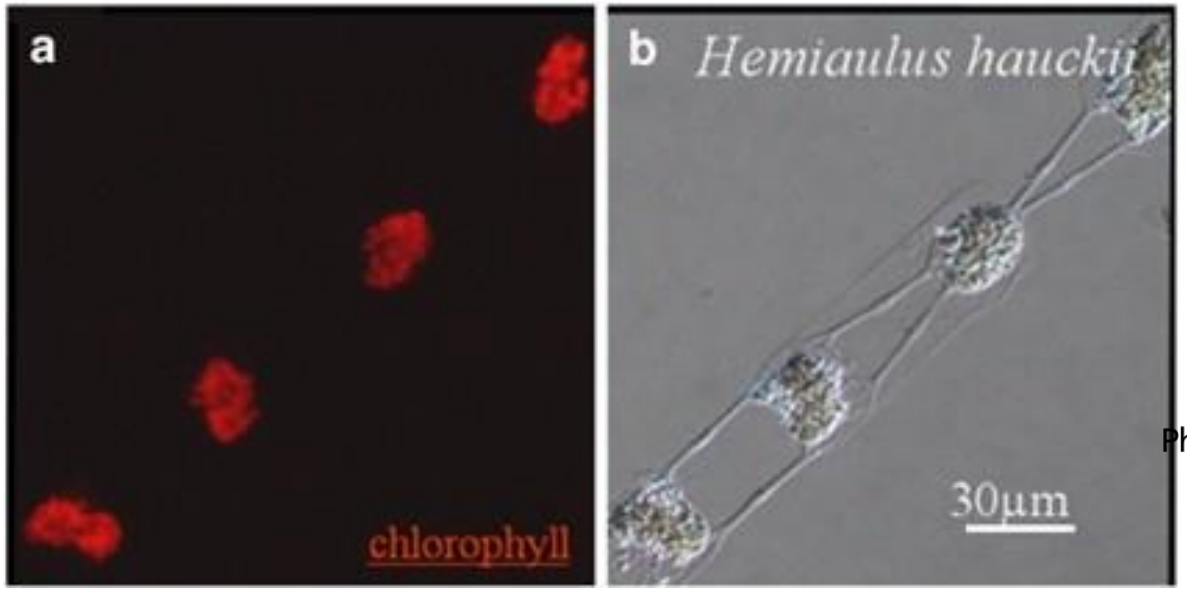
"My ally is the Force, and a powerful ally it is. Life creates it, makes it grow. Its energy surrounds us, binds us. Luminous beings are we, not this crude matter. "

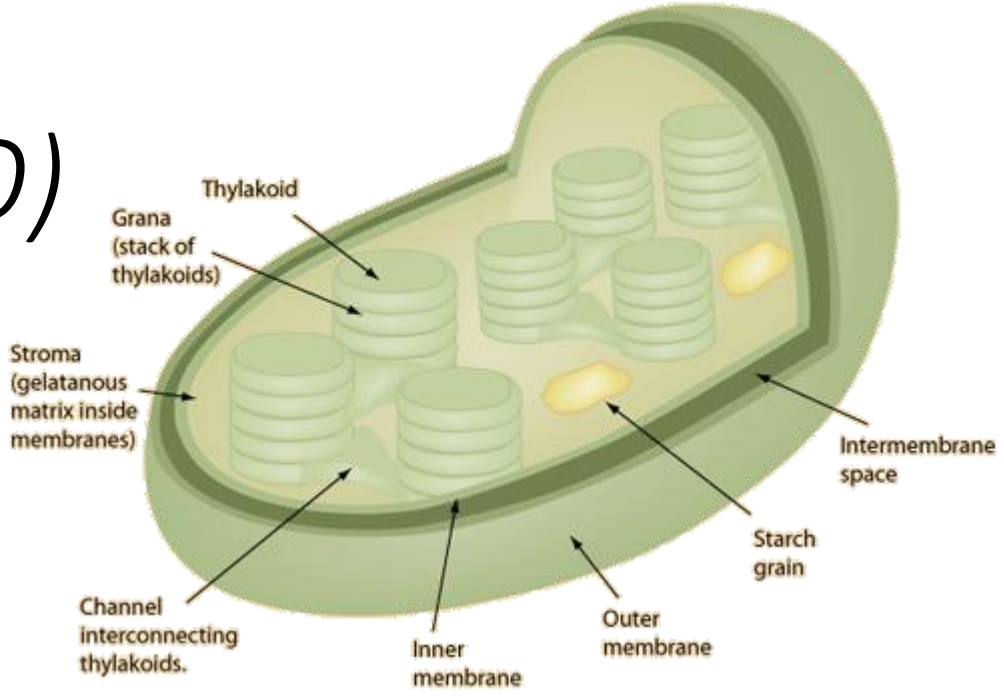
Yoda, *Episode V: The Empire Strikes Back*

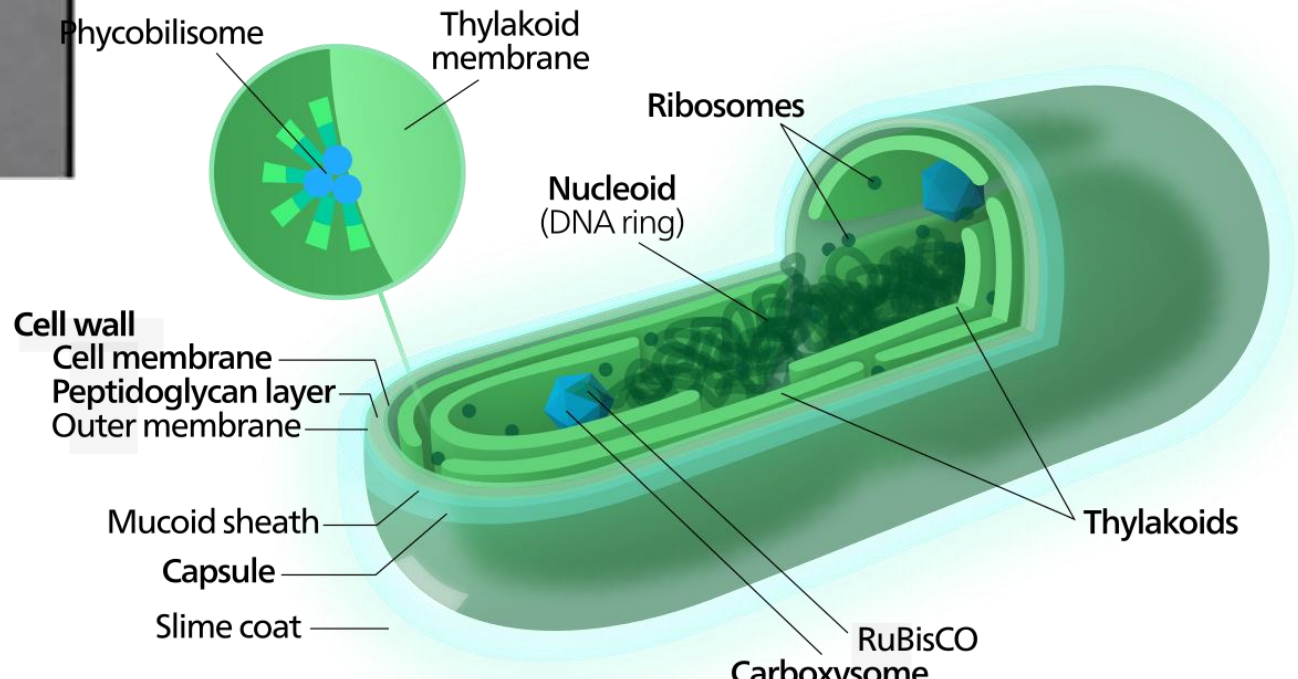
CHLOROPHYLL (IN VIVO)



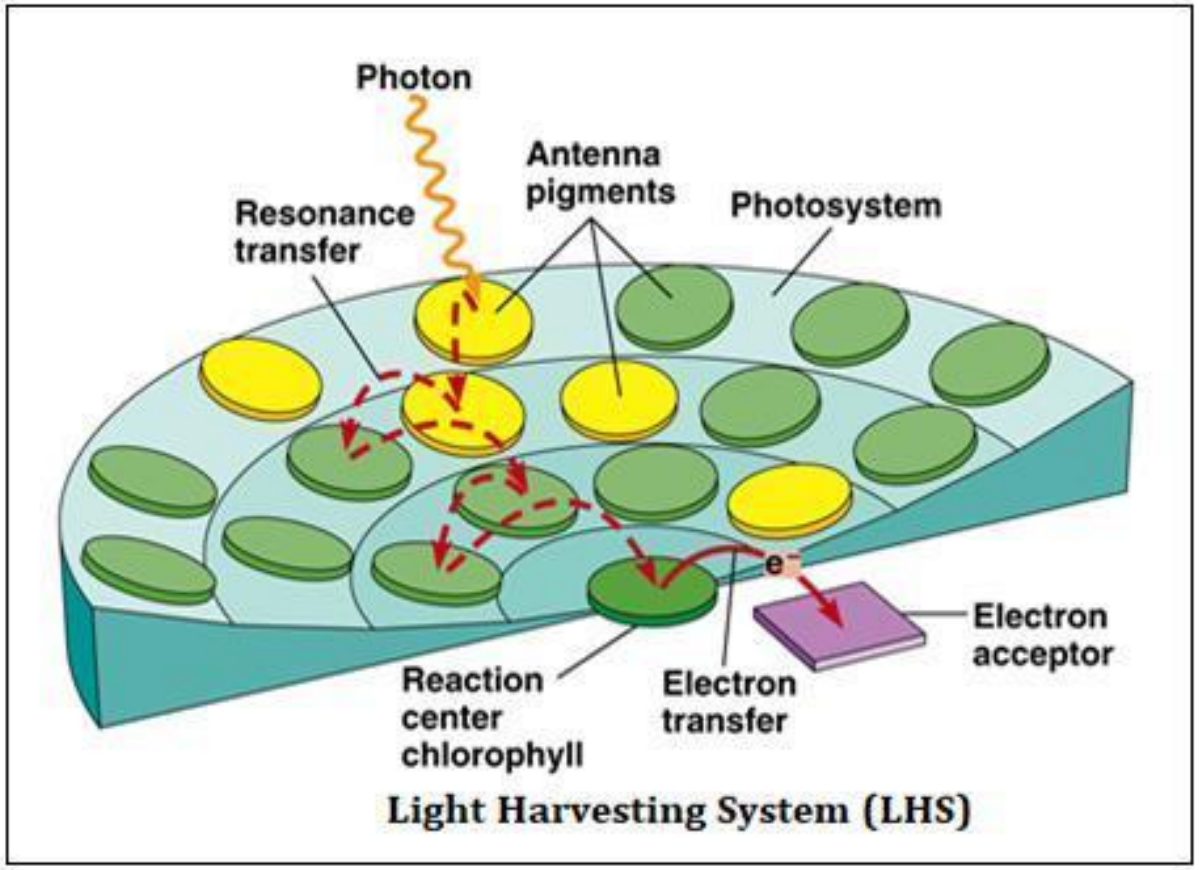
CHLOROPHYLL (IN VIVO)





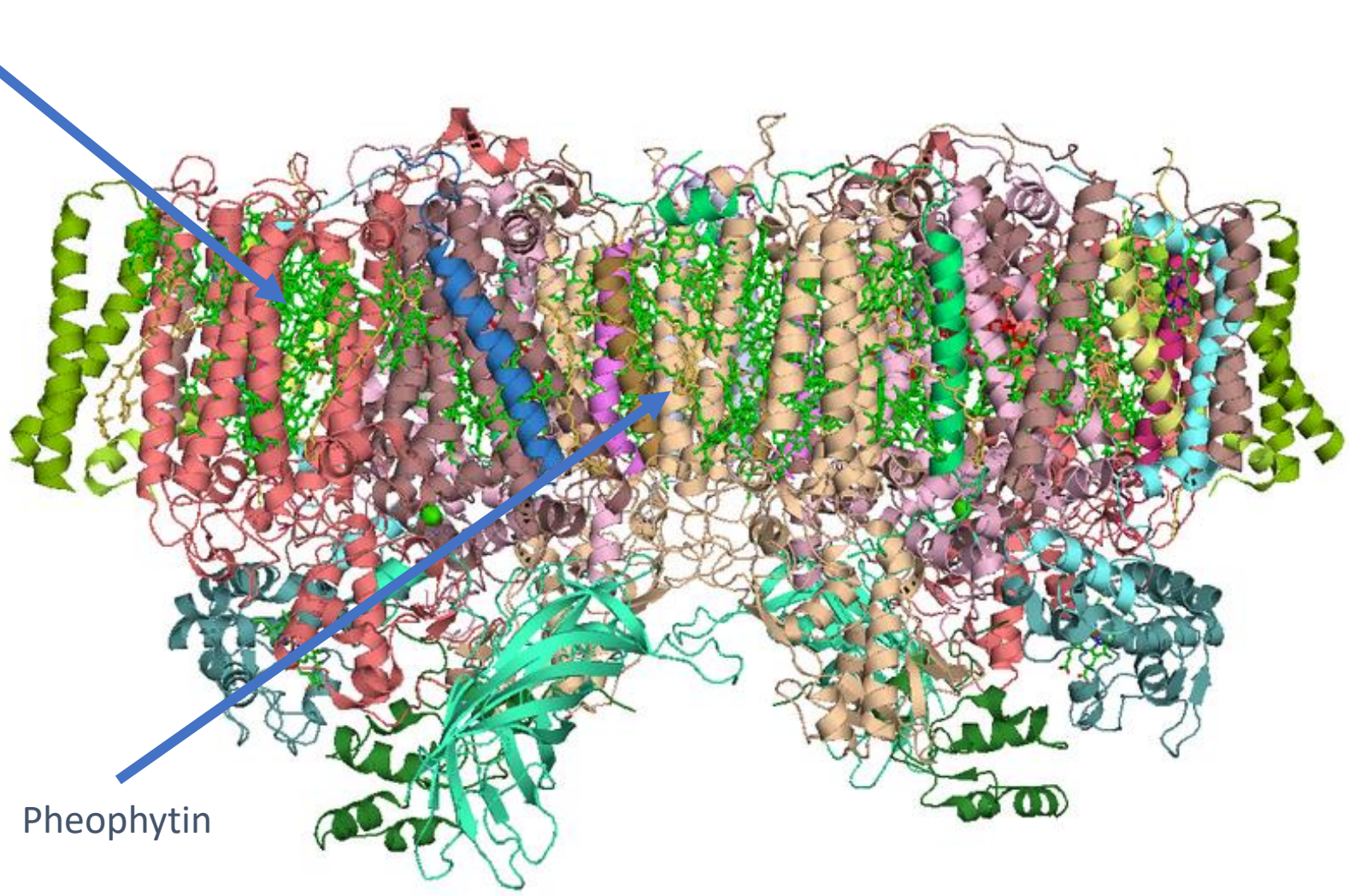


CHLOROPHYLL (IN VIVO) – light harvesting complex

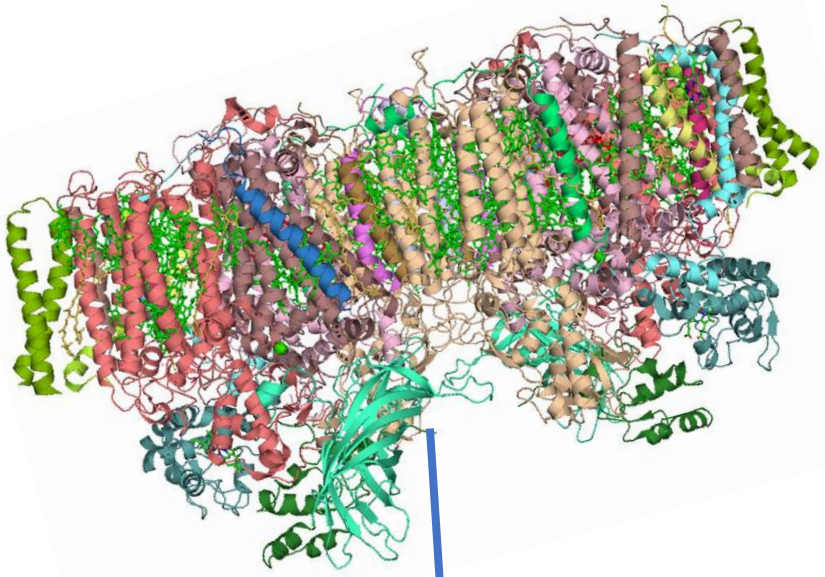


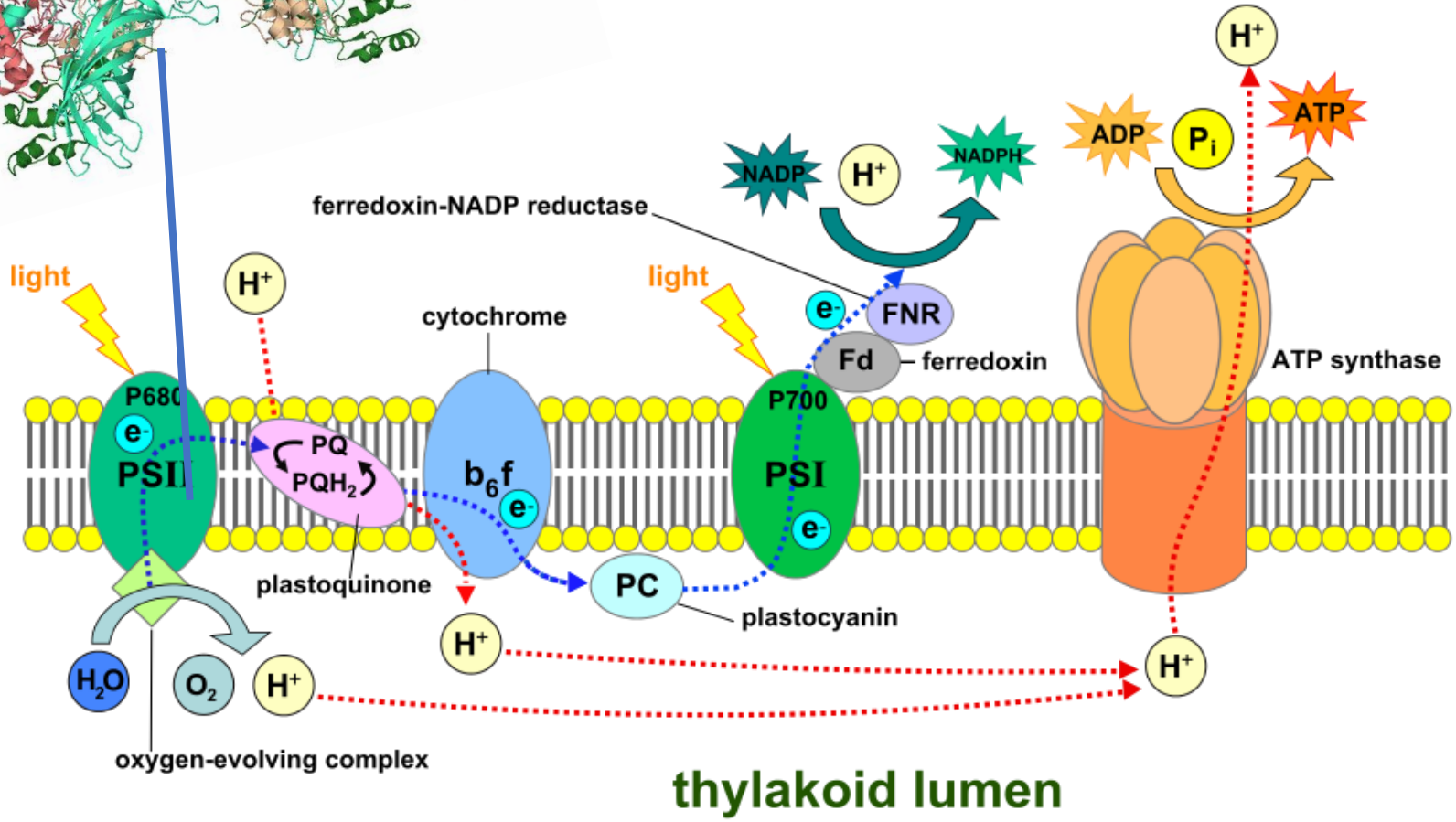
- The chlorophyll *a* molecules responsible for absorbing blue light are called the antenna chlorophylls
- Carotenoids and other chlorophylls (b and c) absorb longer wavelengths towards the green range (and red)
- In cyanobacteria – this is happening in phycobilosomes – different pigments
- Photosynthetic pigments transfer energy to the reaction center chlorophyll molecules
- the transfer between adjacent molecules is an efficient radiationless and lossless thanks to overlapping absorption spectra

PHOTOSYSTEM II



Photosystems





Three fates of absorbed photon

- 1. the energy is used for photochemisty, e.g., for photosynthesis
- 2. the energy goes into vibrational modes of the molecule, i.e., into heat;
- 3. the energy is re-emitted as light via fluorescence

- Chlorophyll concentration

Three fates of absorbed photon

- 1. the energy is used for photochemisty, e.g., for photosynthesis
- 2. the energy goes into vibrational modes of the molecule, i.e., into heat;
- 3. the energy is re-emitted as light via fluorescence

- photosynthesis

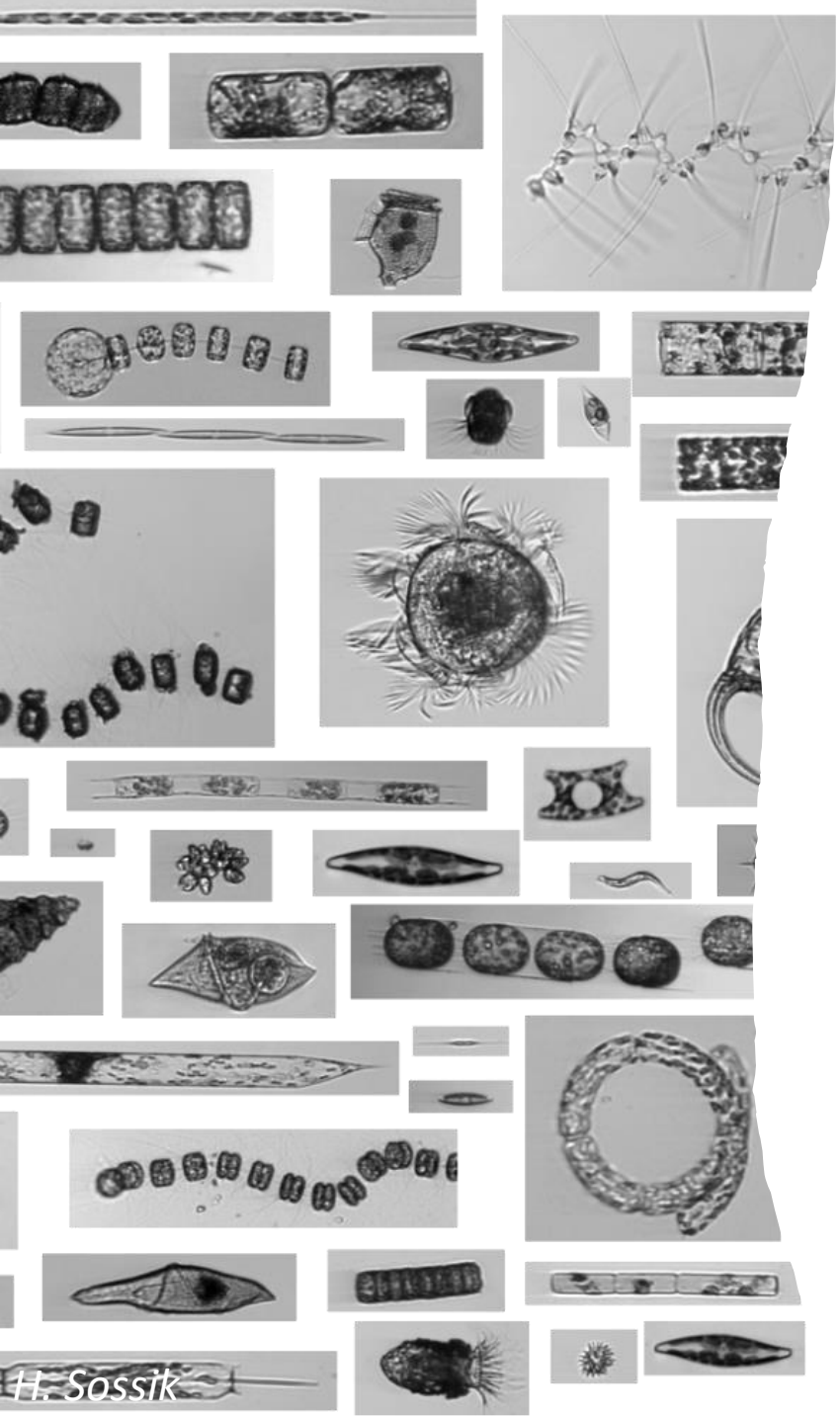
- Chlorophyll concentration

Three fates of absorbed photon

- 1. the energy is used for photochemisty, e.g., for photosynthesis
- 2. the energy goes into vibrational modes of the molecule, i.e., into heat;
- 3. the energy is re-emitted as light via fluorescence

- (photochemical quenching)
- (non-photochemical quenching)

Phytoplankton actively regulate photochemistry and NPQ, such that measured changes in the quantum yield of ChlF are directly linked to changes in the quantum yields of photochemistry and NPQ

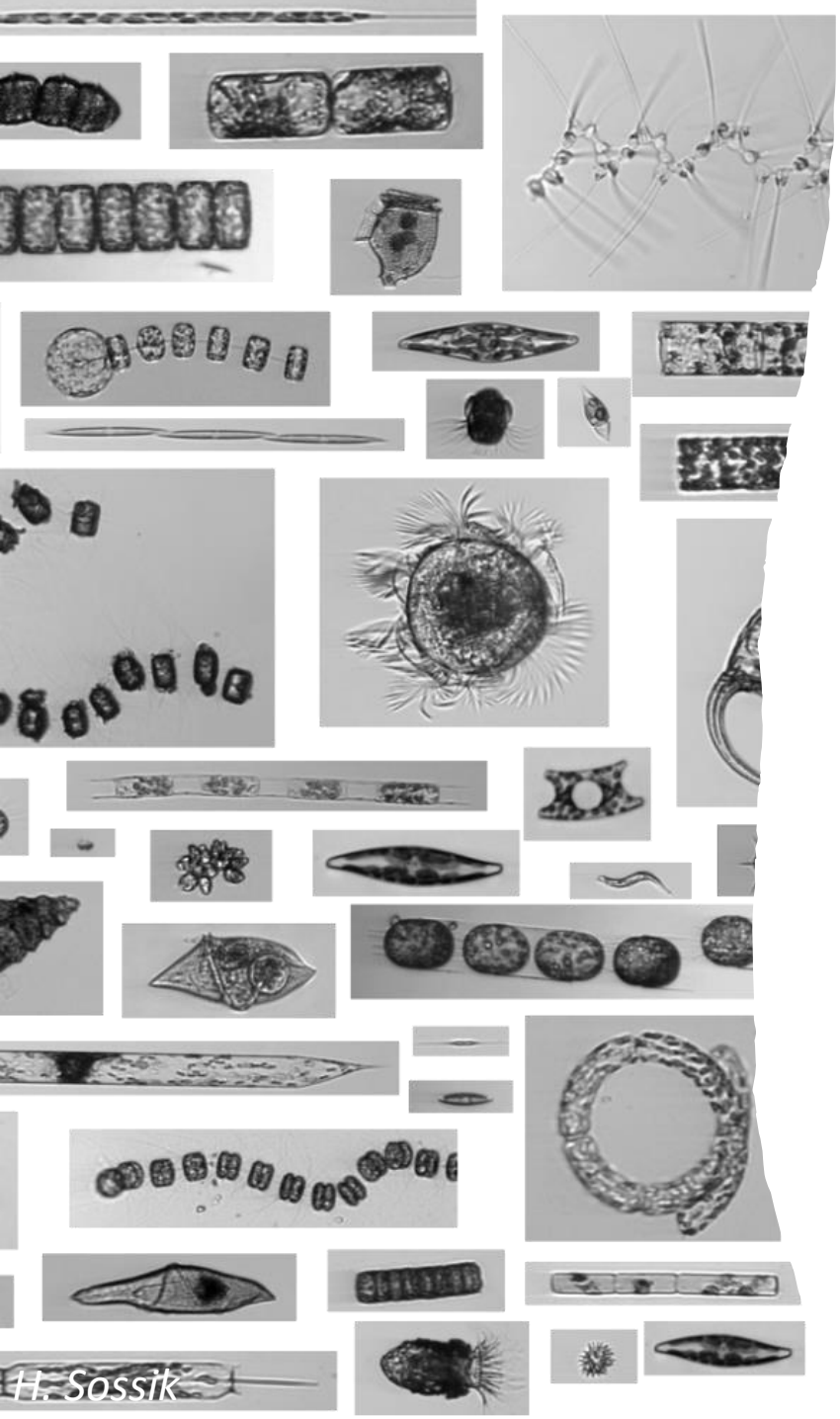


In vivo Fluorescence, chlorophyll proxy? (molecule + pigment friends + physiology)

$$F_{chl}(\lambda_2) = E(\lambda_1) \times a_{chl}(\lambda_1) \times \Phi f(\lambda_1, \lambda_2)$$

$$F_{chl} = E \times a_{chl}{}^{*} \times \mathbf{[Chl]} \times \Phi_f$$

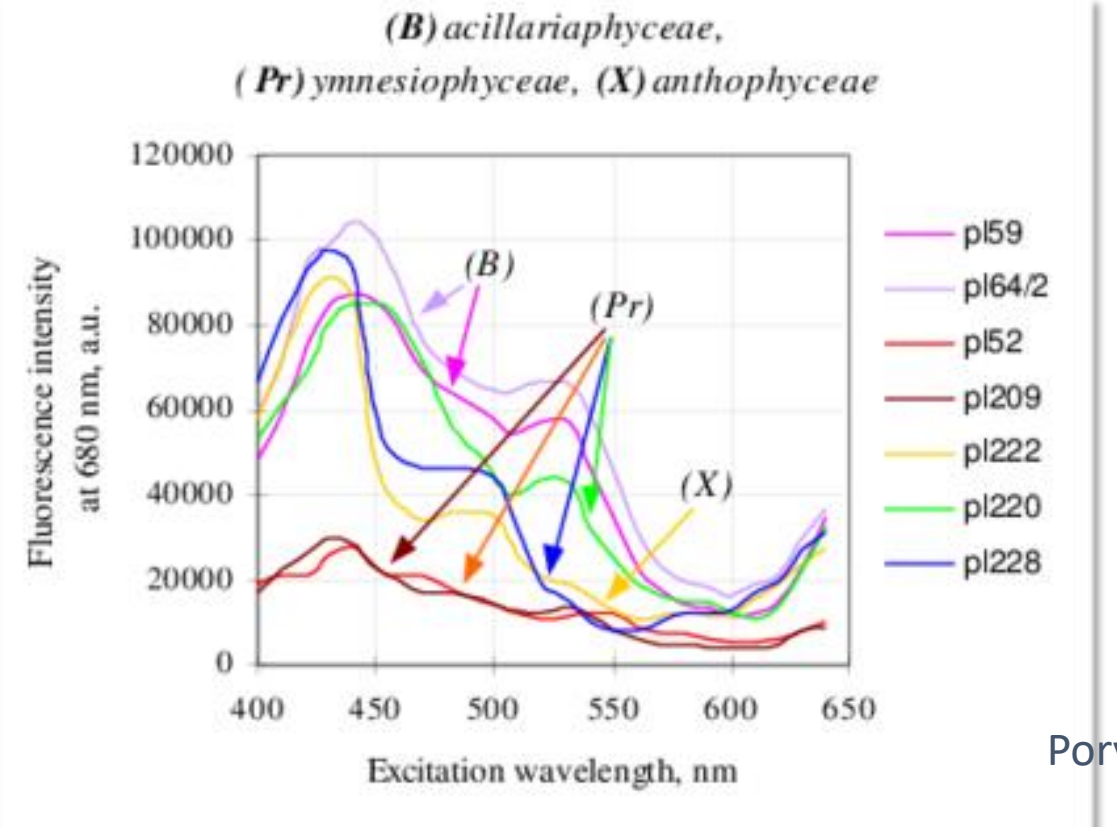
Poryvkina et al 2001



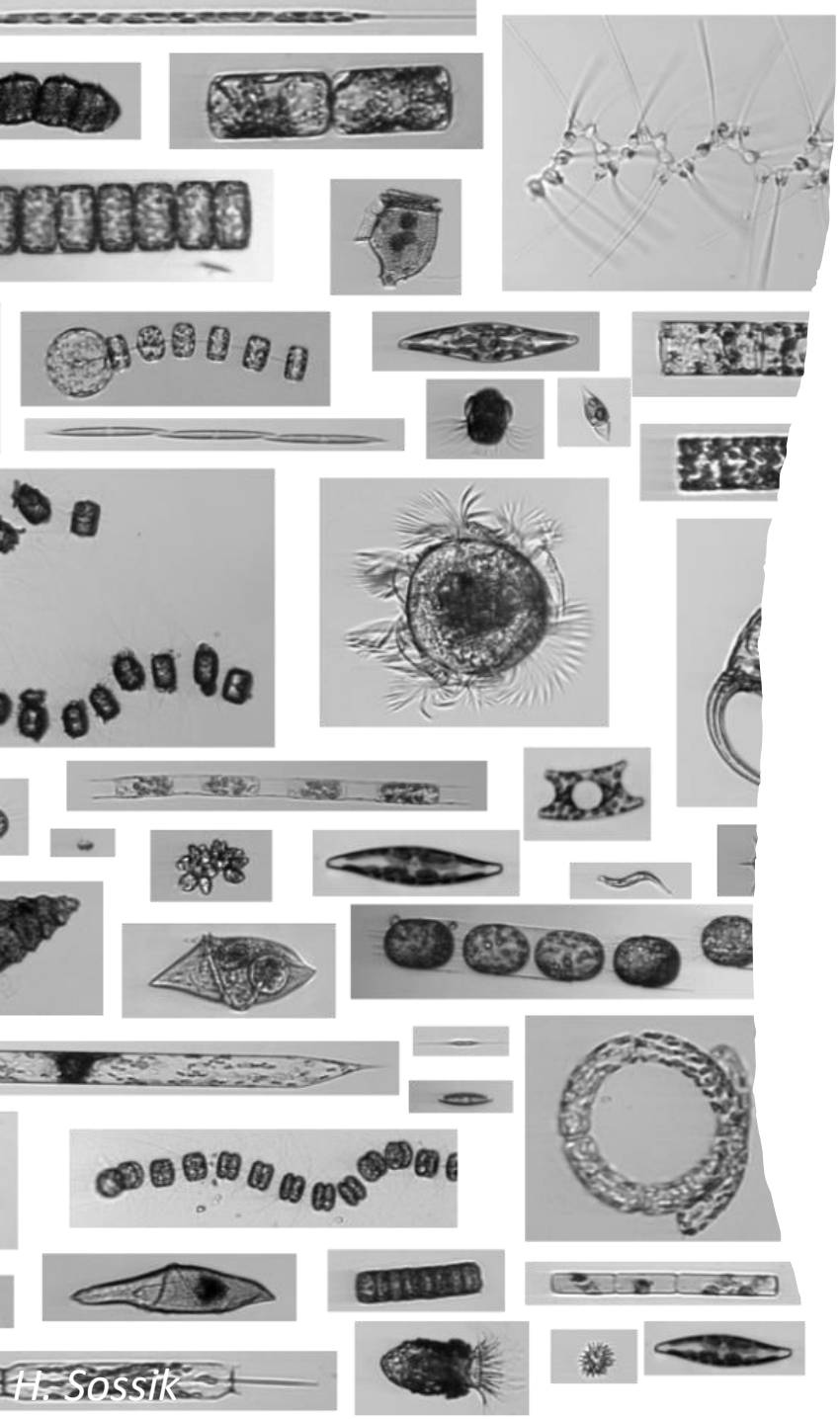
In vivo Fluorescence, chlorophyll proxy? (molecule + pigment friends + physiology)

$$F_{chl}(\lambda_2) = E(\lambda_1) \times a_{chl}(\lambda_1) \times \Phi f(\lambda_1, \lambda_2)$$

$$F_{chl} = E \times a_{chl}{}^{*} \times \mathbf{[Chl]} \times \Phi_f$$



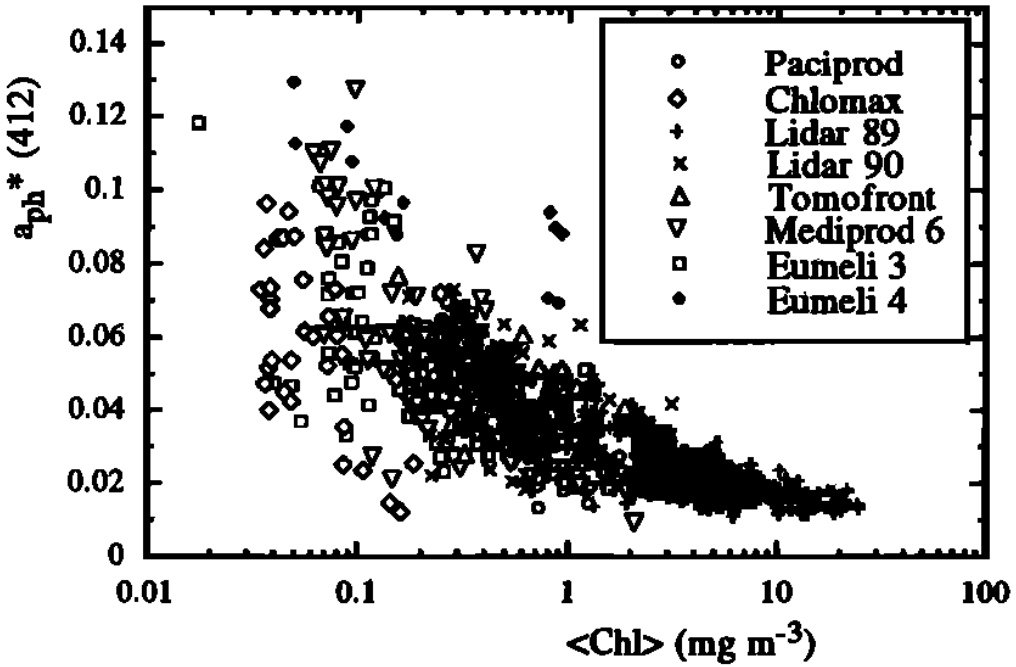
Poryvkina et al 2001



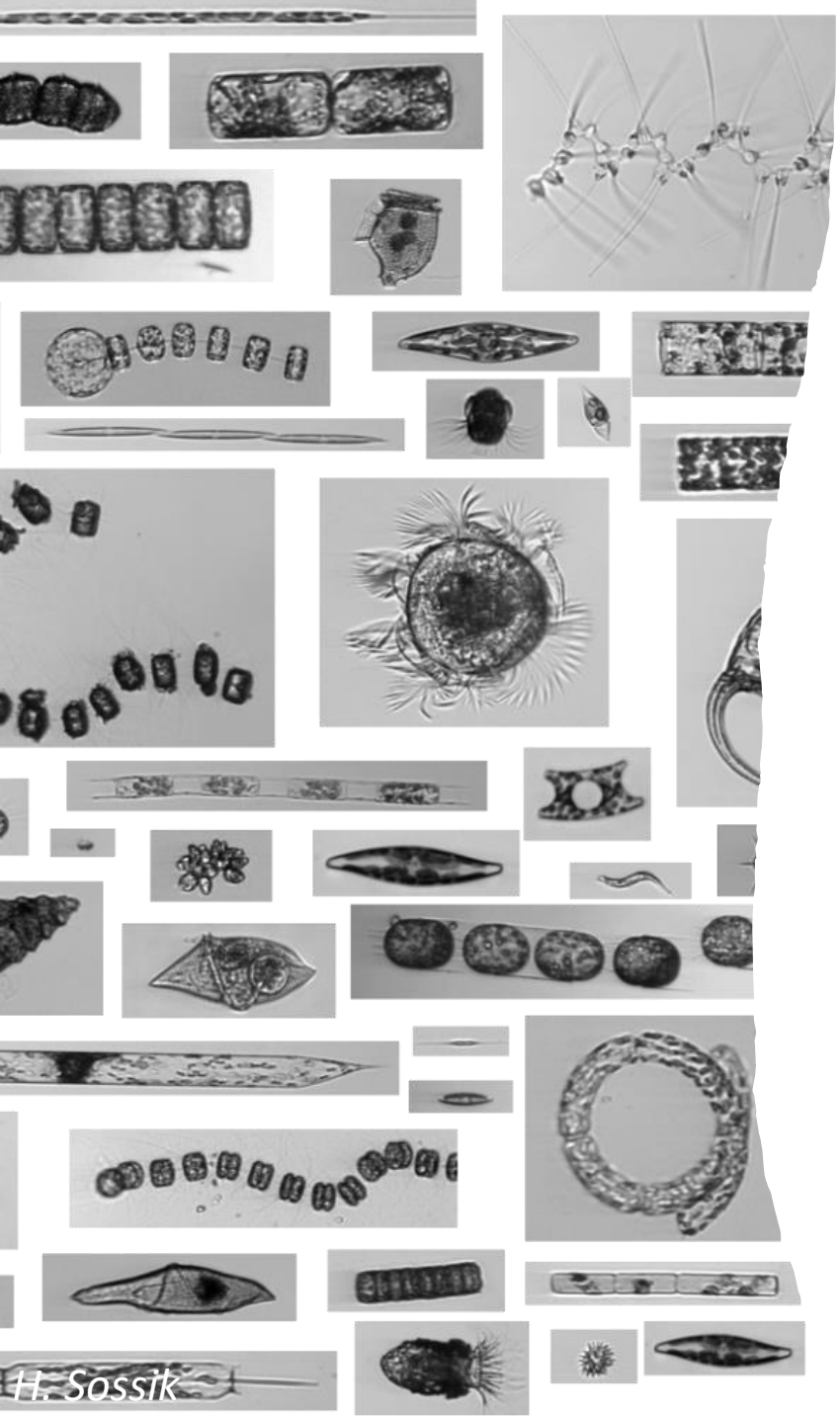
In vivo Fluorescence, chlorophyll proxy? (molecule + pigment friends + physiology)

$$F_{chl}(\lambda_2) = E(\lambda_1) \times a_{chl}(\lambda_1) \times \Phi f(\lambda_1, \lambda_2)$$

$$F_{chl} = E \times a_{chl}{}^{*} \times \mathbf{[Chl]} \times \Phi_f$$



Bricaud et al 1995



In vivo Fluorescence, chlorophyll proxy? (molecule + pigment friends + physiology)

$$F_{chl}(\lambda_2) = E(\lambda_1) \times a_{chl}(\lambda_1) \times \Phi f(\lambda_1, \lambda_2)$$

$$F_{chl} = E \times a_{chl}{}^{*} \times \mathbf{[Chl]} \times \Phi_f$$

Super variable → Constant mass-specific absorption, $a_{chl}(\lambda_1)^*$ $(m^2 mg^{-1})$

Super variable → quantum yield, Φf (environment specific, no physiology, ratio of energy fluoresced and energy absorbed)

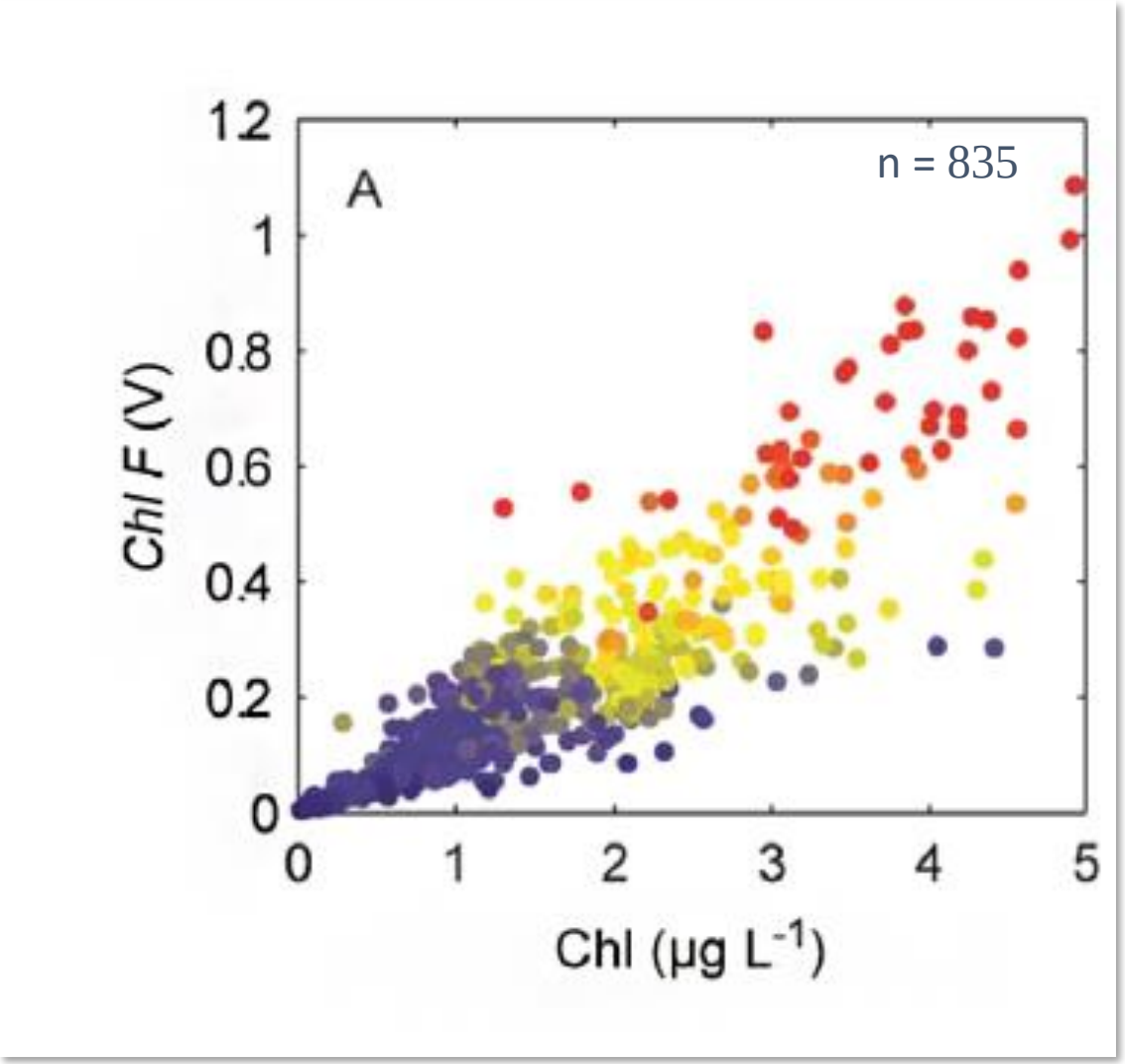
constant E ?

F_{chl} ~ **[*Chl*] ?**

Fluorometers relative units, (dc or volts)

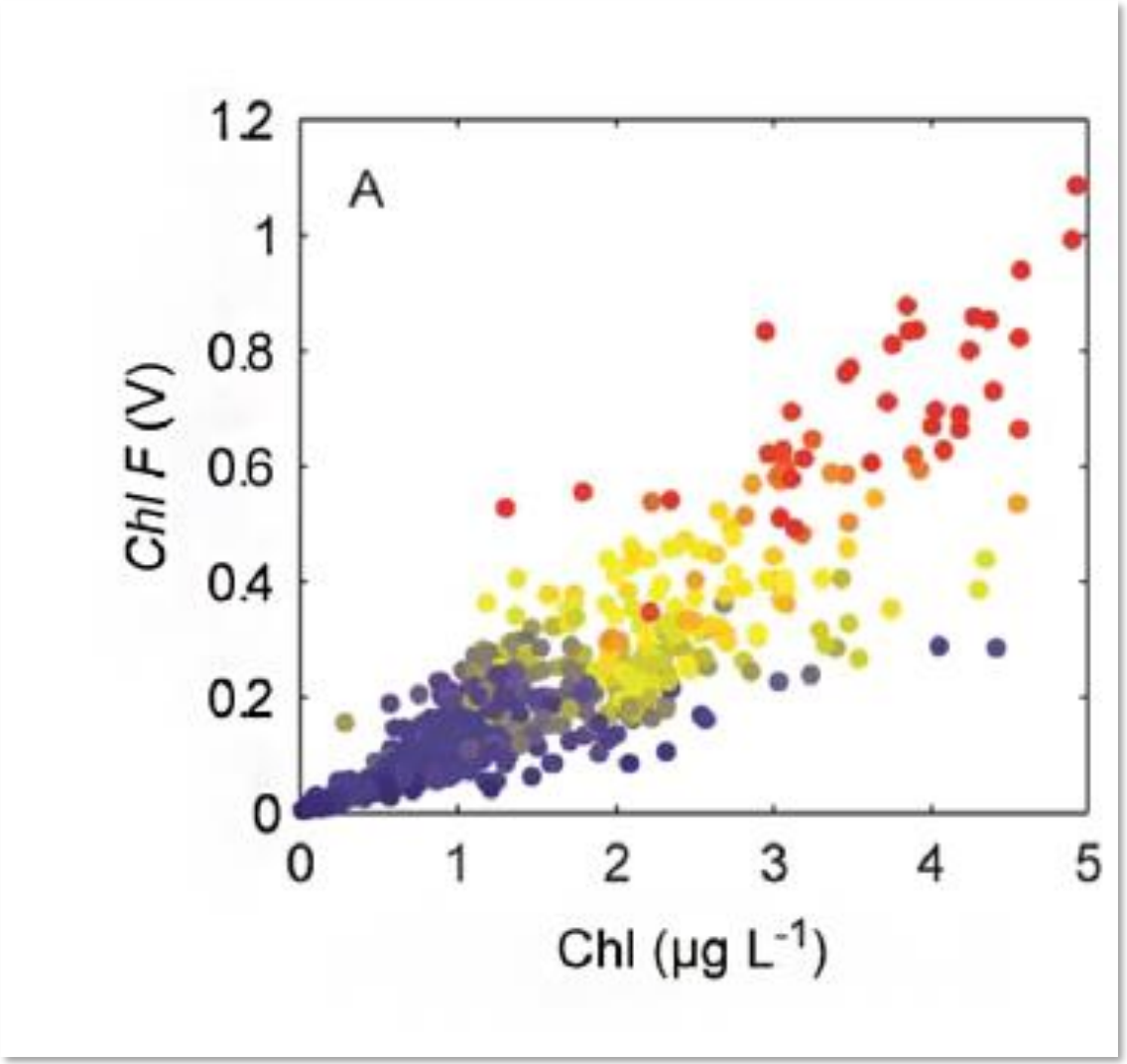
Calibrate with Chl standard solution

$F_{chl} \sim [Chl]$?

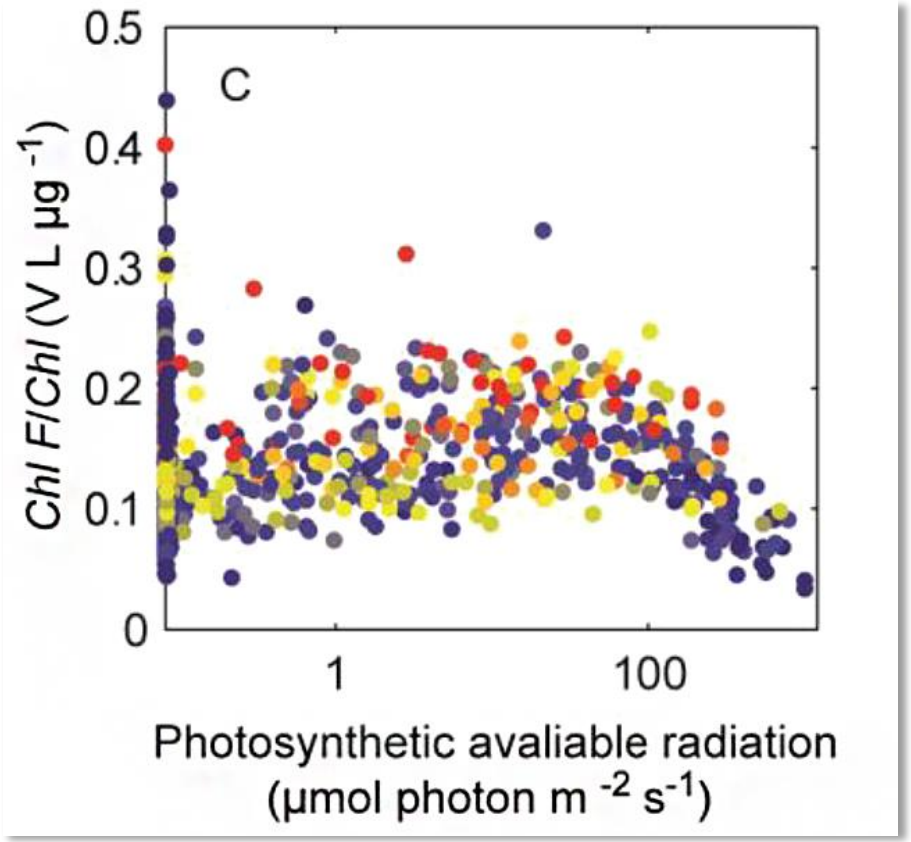


D'asaro 2011, Cetinic et al 2015

$F_{chl} \sim [Chl]$?

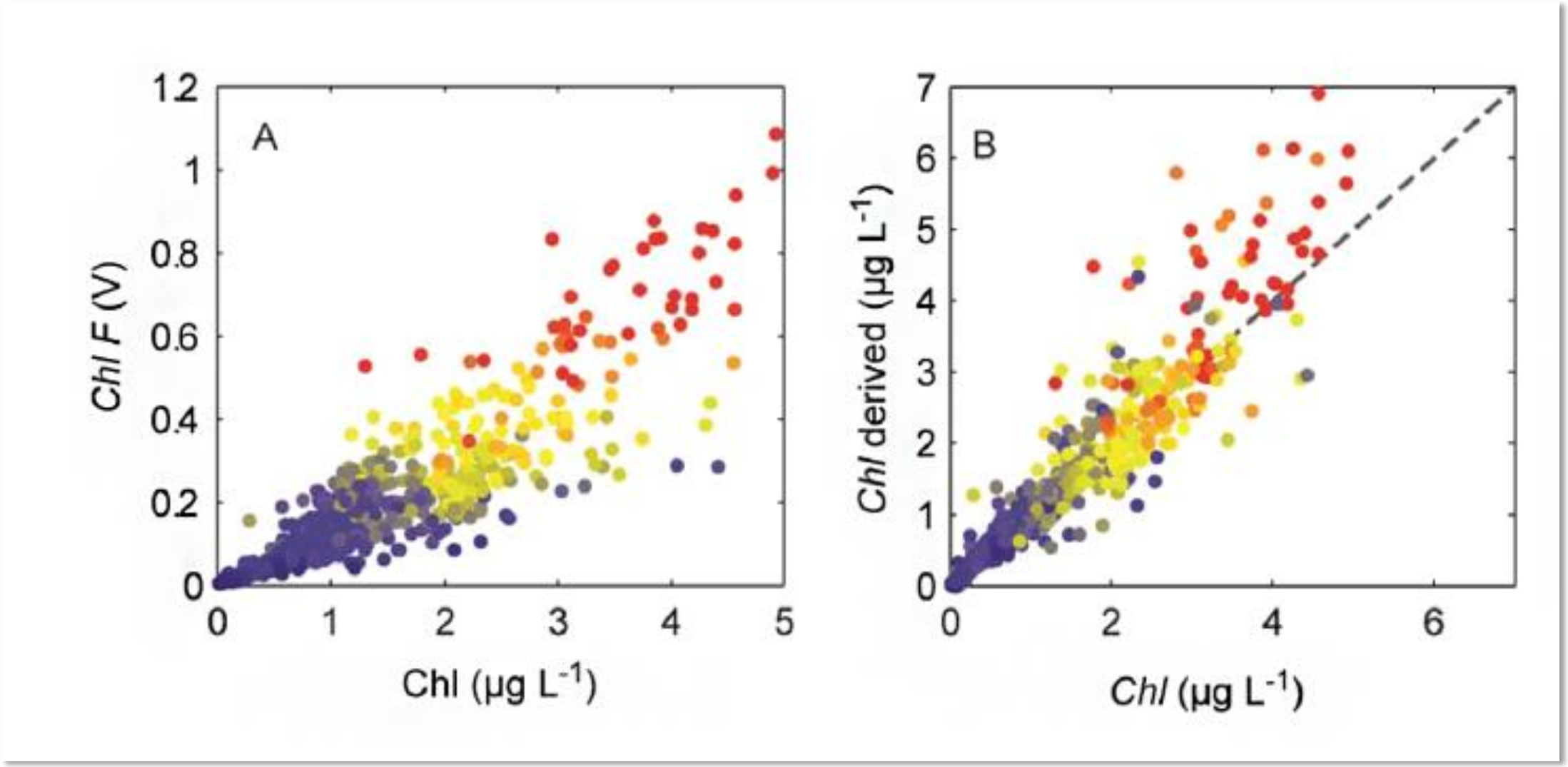


Used a nonlinear best-fit function of temperature, PAR, depth and YD to make the fit better.

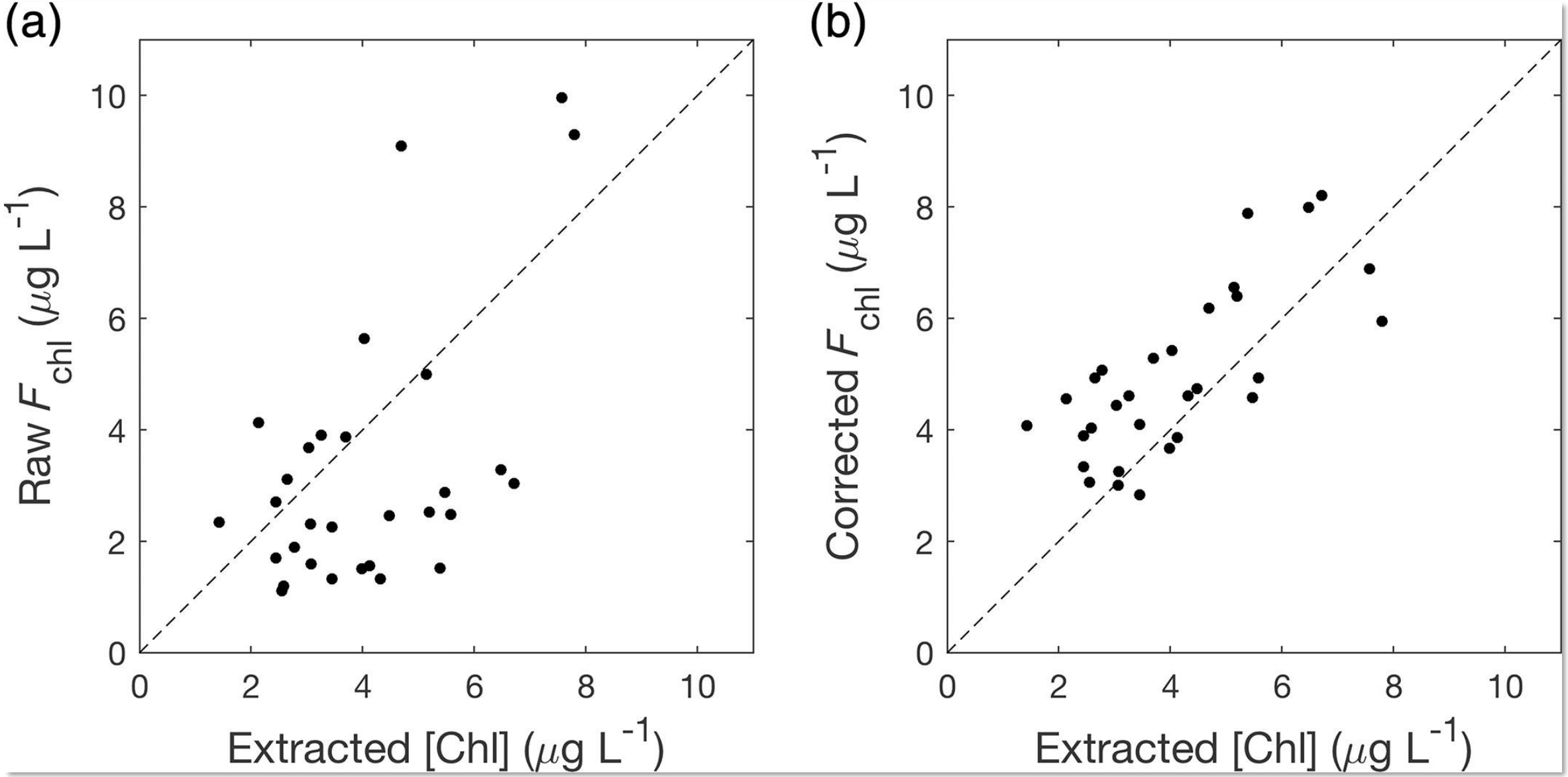


D'asaro 2011, Cetinic et al 2015

$F_{chl} \sim [Chl]$?

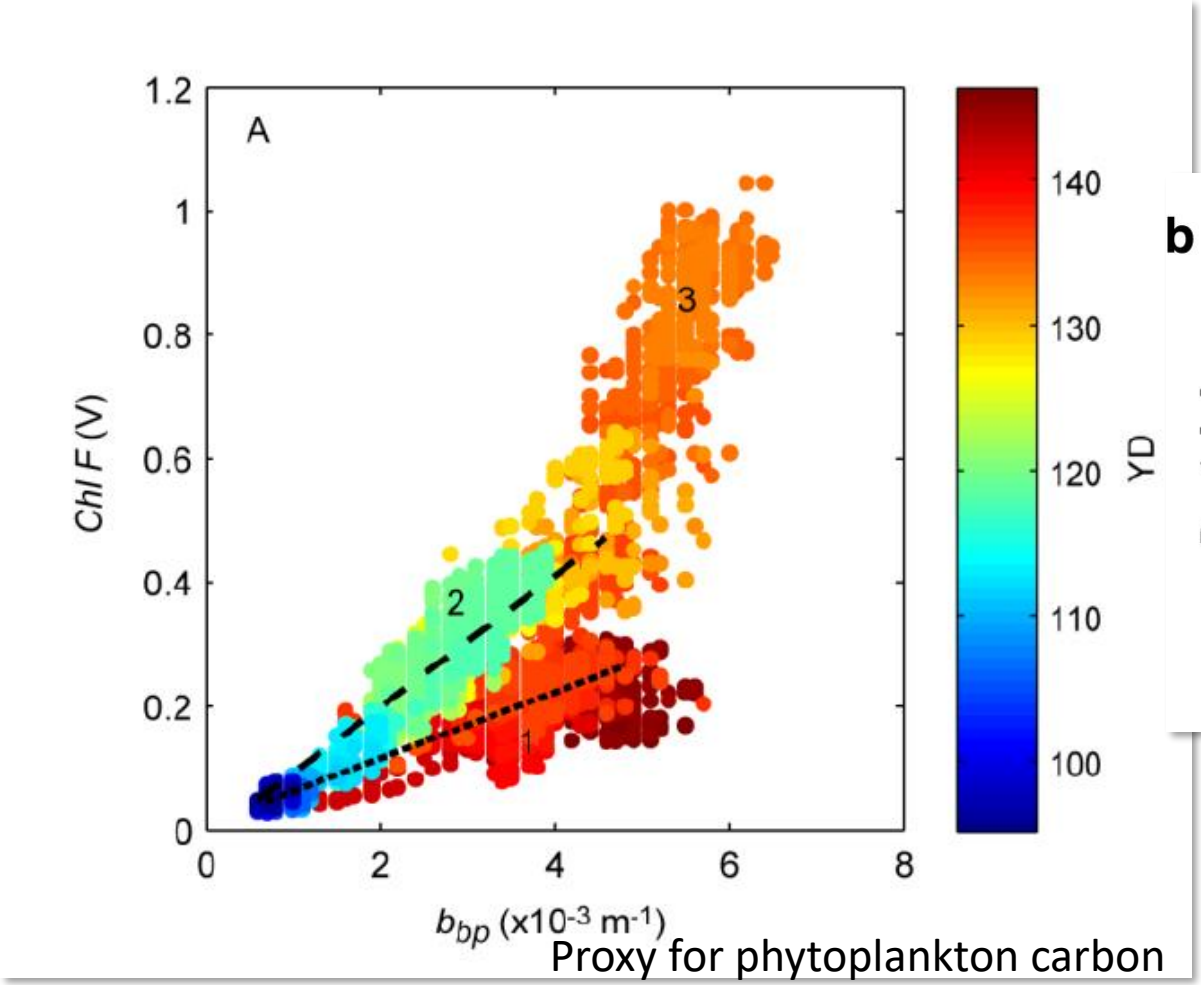


D'asaro 2011, Cetinic et al 2015



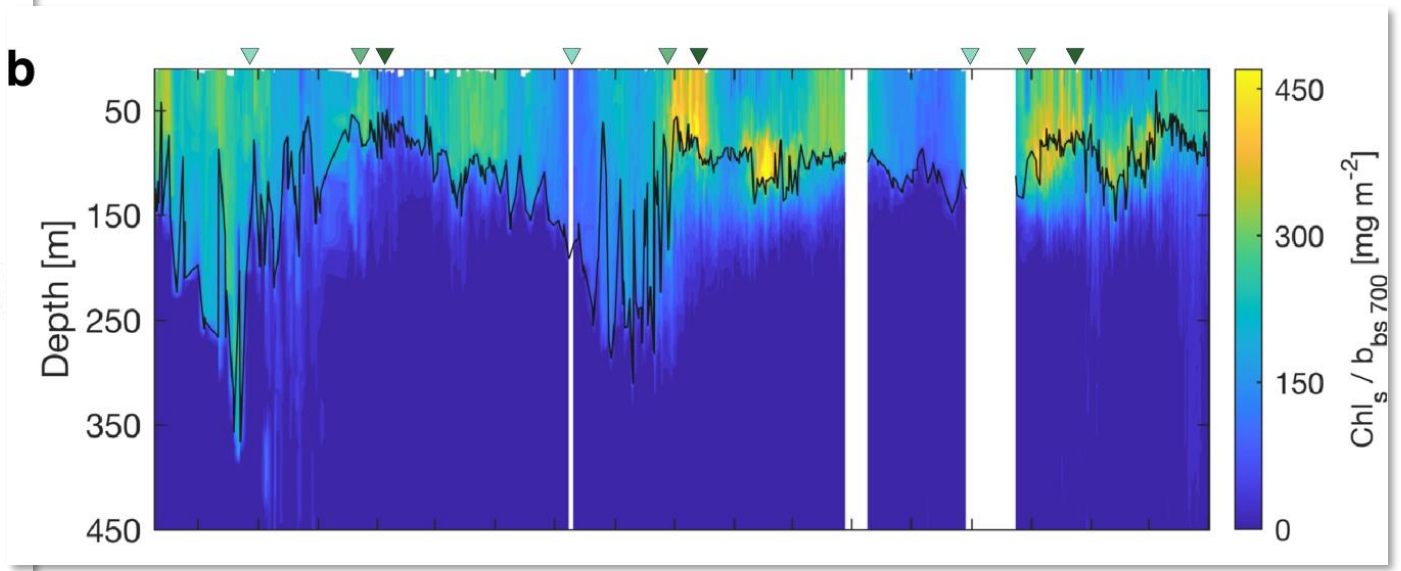
Carberry et al, 2019

Chl F as phytoplankton biomass?



Proxy for phytoplankton carbon

Cetinic et al 2015



Lacour et al, 2023

In vivo Fluorescence, chlorophyll proxy? (molecule + pigment friends + physiology)

In Lab today!

Trying to calibrate chlorophyll fluorometer in vivo!!!

$$F_{chl}(\lambda_2) = E(\lambda_1) \times a_{chl}(\lambda_1) \times \Phi f(\lambda_1, \lambda_2)$$

$$F_{chl} = E \times a_{chl}{}^{*} \times \mathbf{[Chl]} \times \Phi_f$$

Super variable → Constant mass-specific absorption, $a_{chl}(\lambda_1)^*$ $(m^2 mg^{-1})$

Super variable → quantum yield, Φf (environment specific, no physiology, ratio of energy fluoresced and energy absorbed)

constant E ?

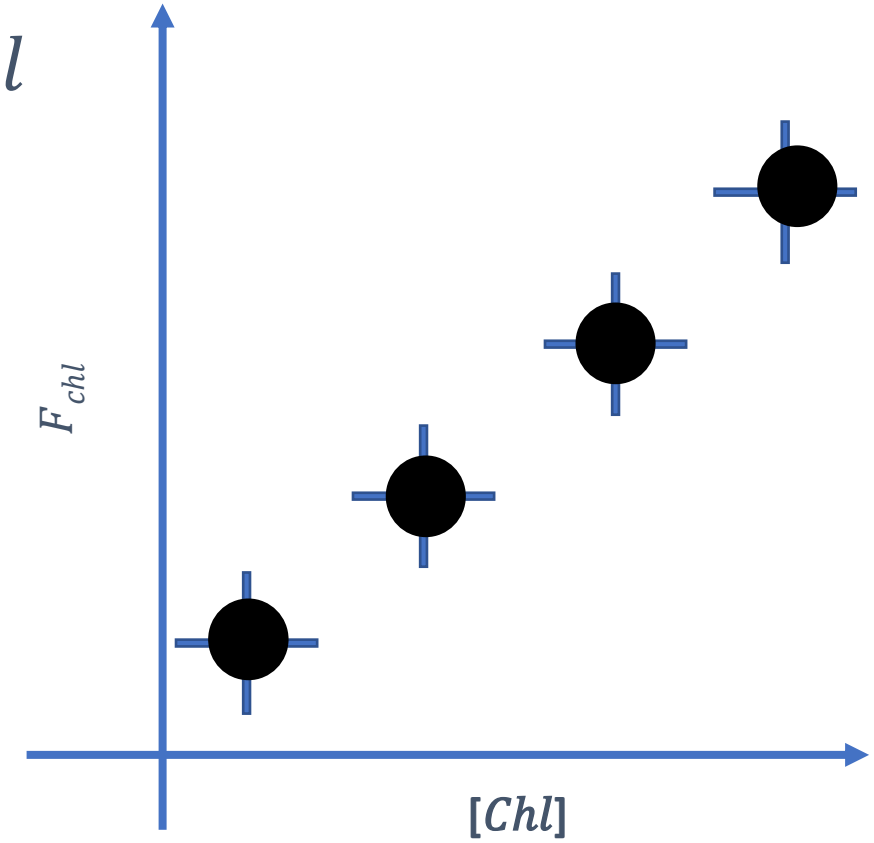
$F_{chl} \sim$ **[*Chl*] ?**

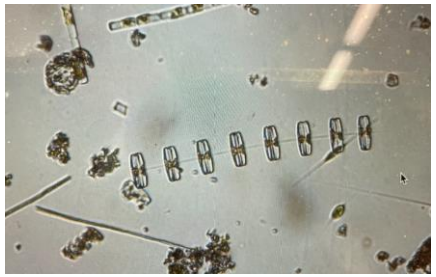
Fluorometers relative units, (dc or volts)

Calibrate with Chl standard solution

Calibration curve for the F_{chl}

1) Phytoplankton dilution series
2) Measure dark counts (F_{dark})
3) Measure of F_{chl} of each dilution
4) Sample and measure extracted $[Chl]$



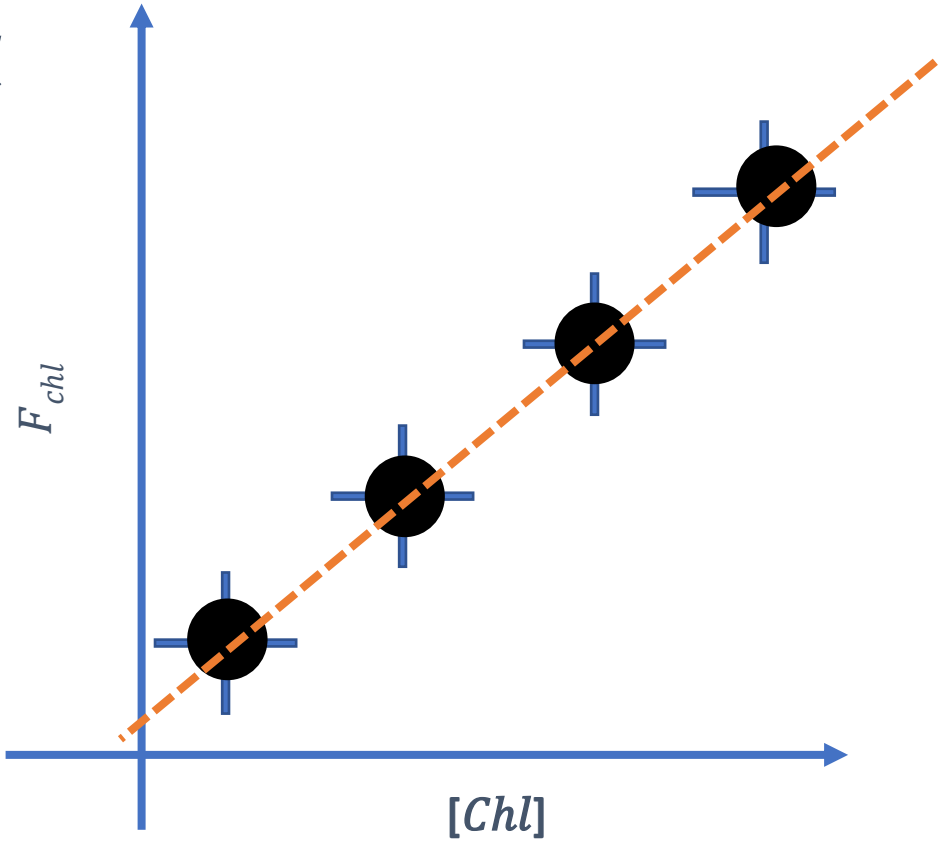


Calibration curve for the F_{chl}

1. Calculate linear regression statistics

   Choose your fitting wisely, you have error in both terms

2. Calibration slope (m) is
   - m= F_{chl} /**[*Chl*]**
3. Calibration offset (b),
4. Calculate Chl in vivo following:



$$Chl\ \left(\frac{mg}{m^3}\right) = \frac{(F_{measured} - F_{dark})}{scale\ factor}$$

CONGRATS,
YOUR FIRST BGC PROXY!