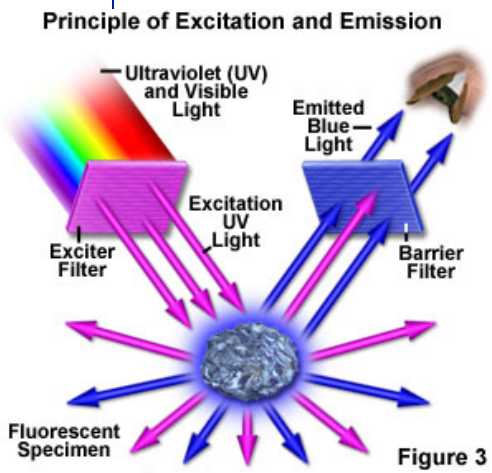
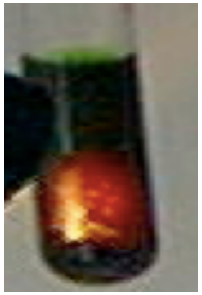
SMS 598: Calibration and Validation for Ocean Color Remote Sensing

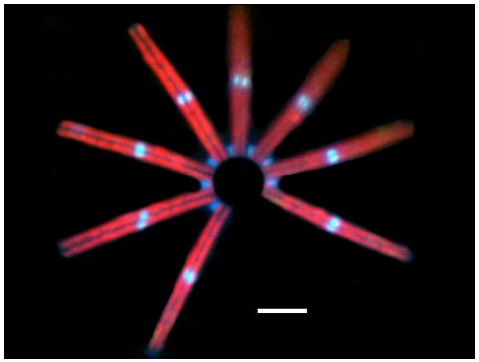
Lecture 10 Fluorescence

Mary Jane Perry

10 July 2015







http://micro.magnet.fsu.edu/primer/lightandcolor/fluorointroduction.html

Epifluorescence microscope: chlorophyll fluorescence in *Thalassionema* (courtesy of M. Sierackiz0

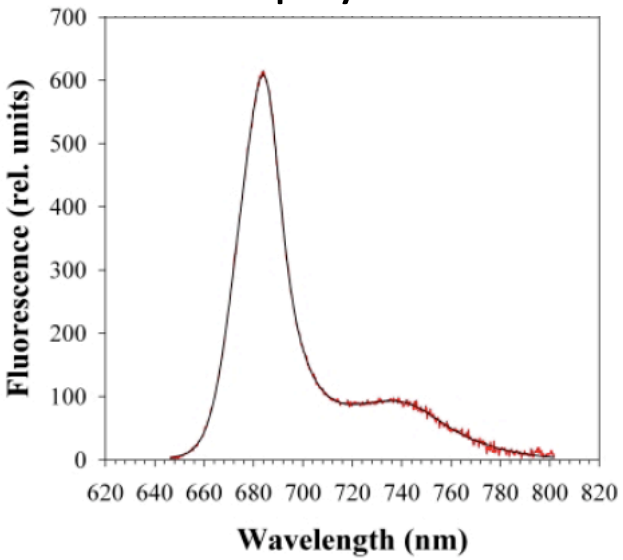
Take away points

1. Fluorescence is re-emission of absorbed light

2. Fluorescence in the ocean from
   - CDOM
   - Chlorophyll a
   - Phycoerthyrin

3. With some caveats, fluorescence can be used to infer mass

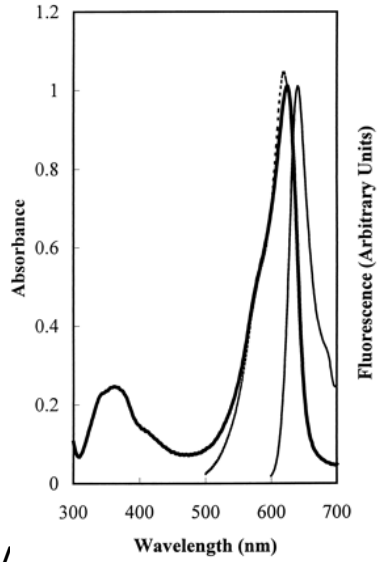
Chlorophyll *a* - red



What fluoresces in the ocean?

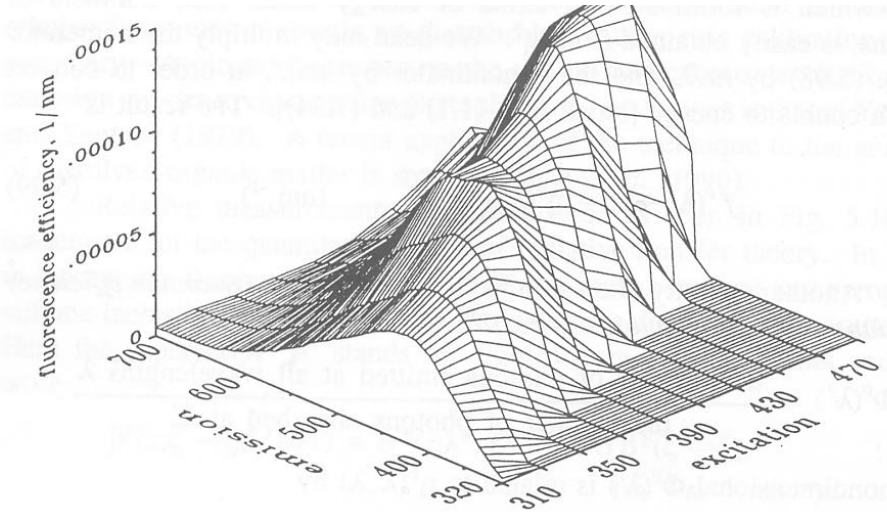
both Chl *a* and CDOM exhibit natural or solar stimulated fluorescence;
PE – lidar stimulation.

Phycoerythrin – orange

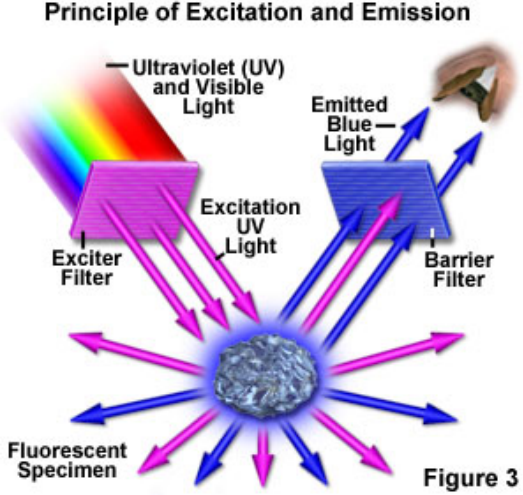


http://www.pnas.org/content/98/19/10560/F4.expansion.html

CDOM – broad excitation and emission spectra (with some peaks)



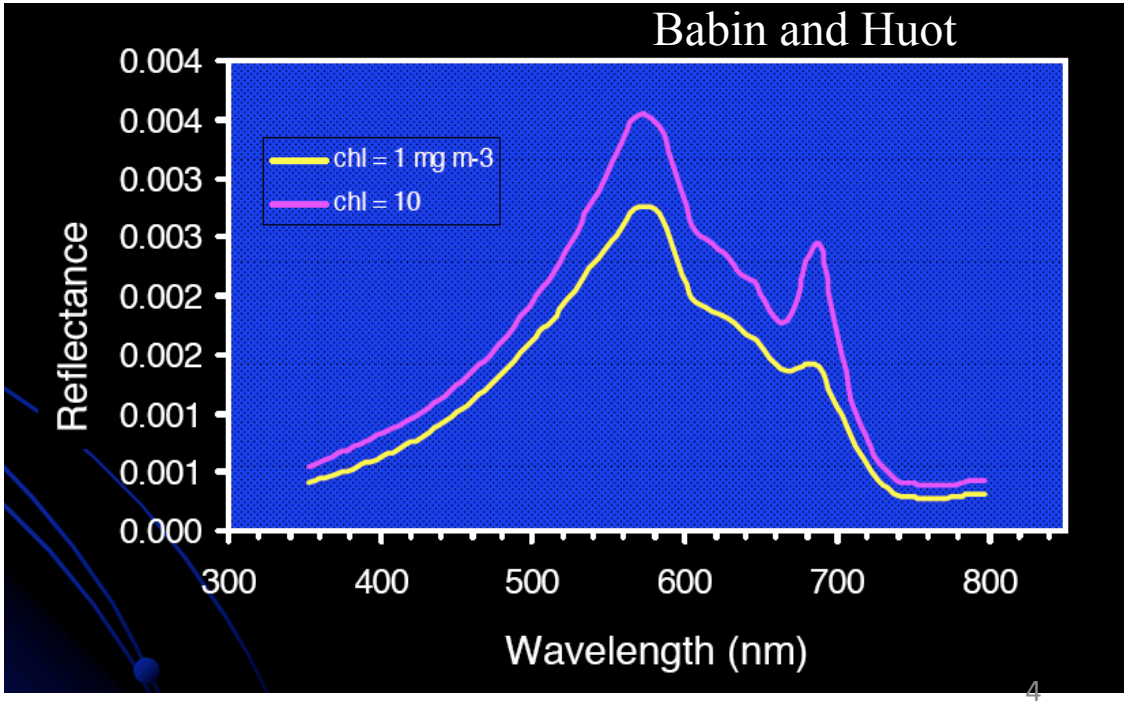
Two types of fluorescence measurements

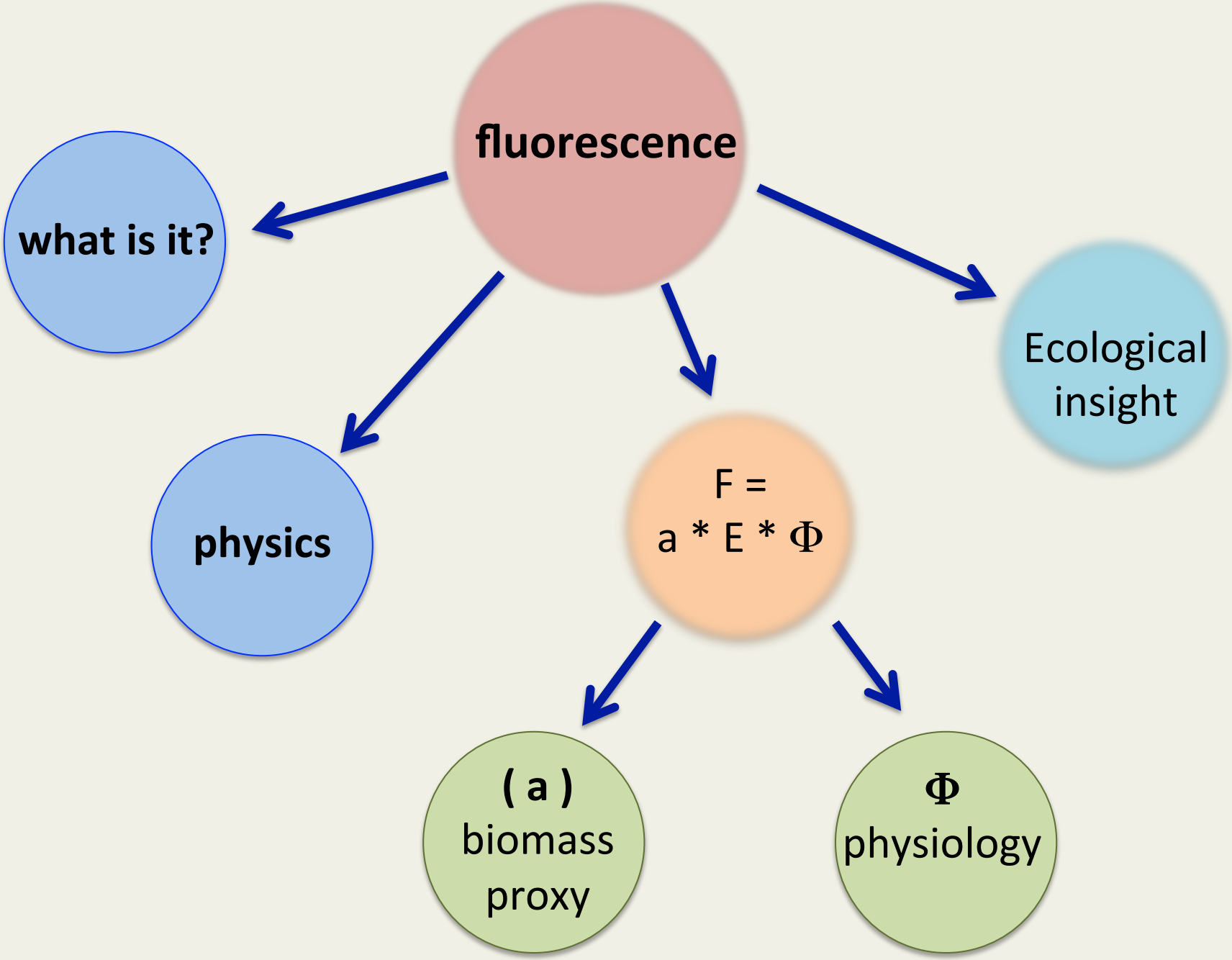


1) **active – artificial light source for E(λ)**
   - <u>static:</u> use for profiles of chlorophyll fluorescence; moorings; mobile platforms – **TODAY'S LAB**
   - <u>time resolved</u> (true τ_F is ~ femos and picos for chemistry, like hole burning in CDOM; pump & probe and variable F ~ μs – more later, in productivity lecture.)

2) **passive**
   - sun is light source for E(λ)

YOU WILL SEE SOLAR FLUORESCENCE IN FIELD RADIOMETRIC DATA AND HYDROLIGHT

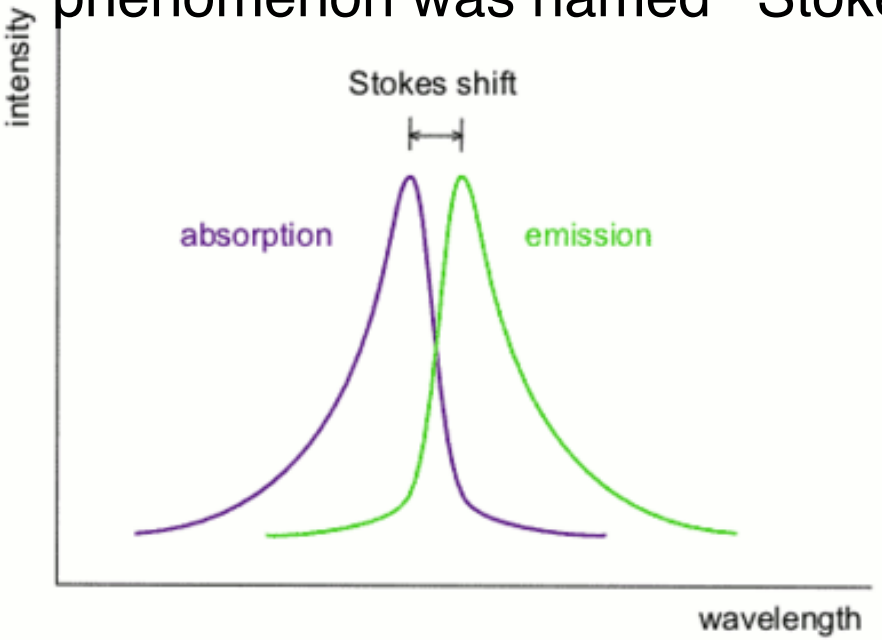



fluorescence
what is it?
physics
F =
a * E * Φ
Ecological
insight
(a)
biomass
proxy
Φ
physiology

Sir George Gabriel Stokes

"I am almost inclined to coin a word and call the appearance fluorescence, from fluor-spar, as the analogous term opalescence is derived from the name of a mineral." –Phil. Trans. 479 (1852)

In 1852 Stokes described fluorescence, as exhibited by fluorspar and uranium glass. **He noted emission of visible light when he exposed them to UV light.** This phenomenon was named 'Stokes shift'.

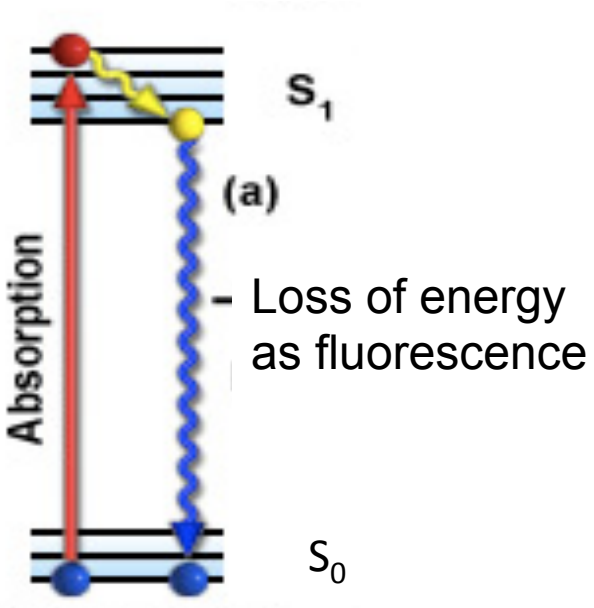


A fraction of energy absorbed at **shorter wavelength** (higher frequency, higher energy) is re-emitted as a photon at **longer wavelength** (lower frequency, lower energy).

$E = h\nu = hc/\lambda$

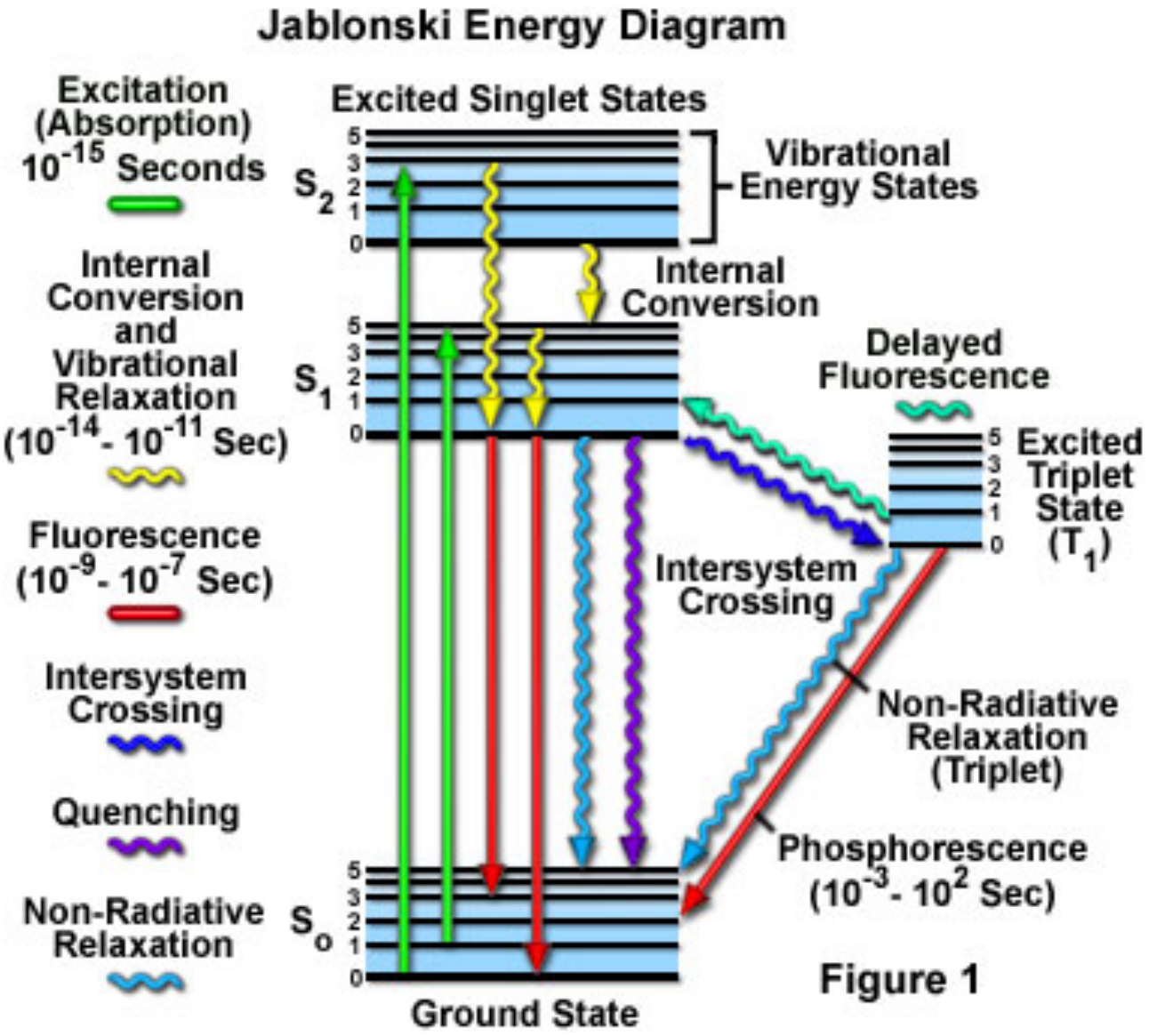
Fluorescence: A fraction of energy absorbed at a shorter wavelength (higher frequency, higher energy) is re-emitted as a photon at a longer wavelength (lower frequency, lower energy).

Energy (as a photon) can be absorbed **IF and ONLY IF** the energy of the photon ($E = h\nu = hc/\lambda$) is equal to <u>Δ energy</u> between an electron in the ground electronic state (S_0) and in a higher electronic state (S_n).



<u>Absorption</u> is an "electronic transition" ($O(10^{-15}$ s)), leading to an excited state. The excited electron returns to ground state by vibrational loss of energy (radiation-less decay). Certain molecules can lose some energy through photon loss, e.g., fluorescence. Note: from lowest electronic state of S_1 (not S_n). Other processes on next slide, but we'll stick to F.

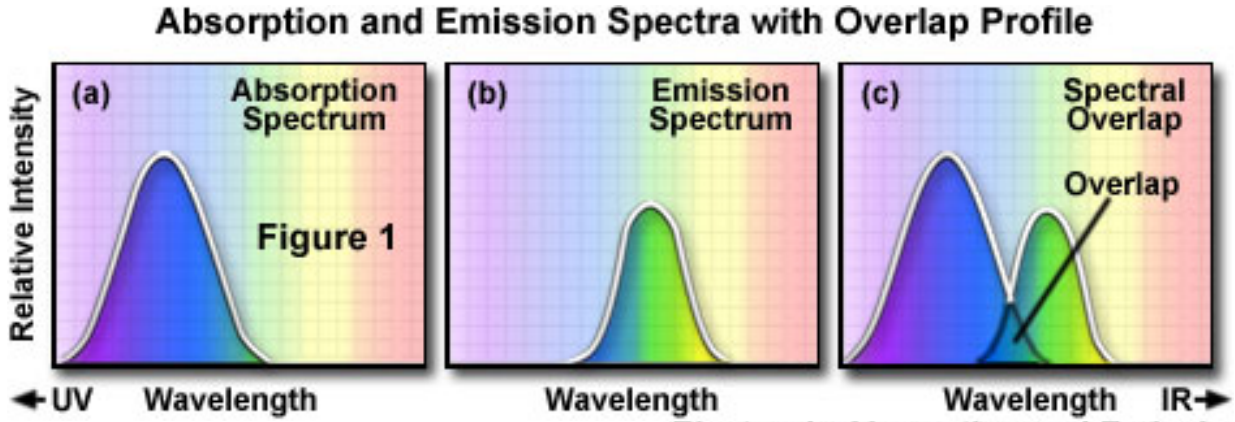
Neat applet: http://www.micro.magnet.fsu.edu/primer/java/jablonski/lightandcolor/

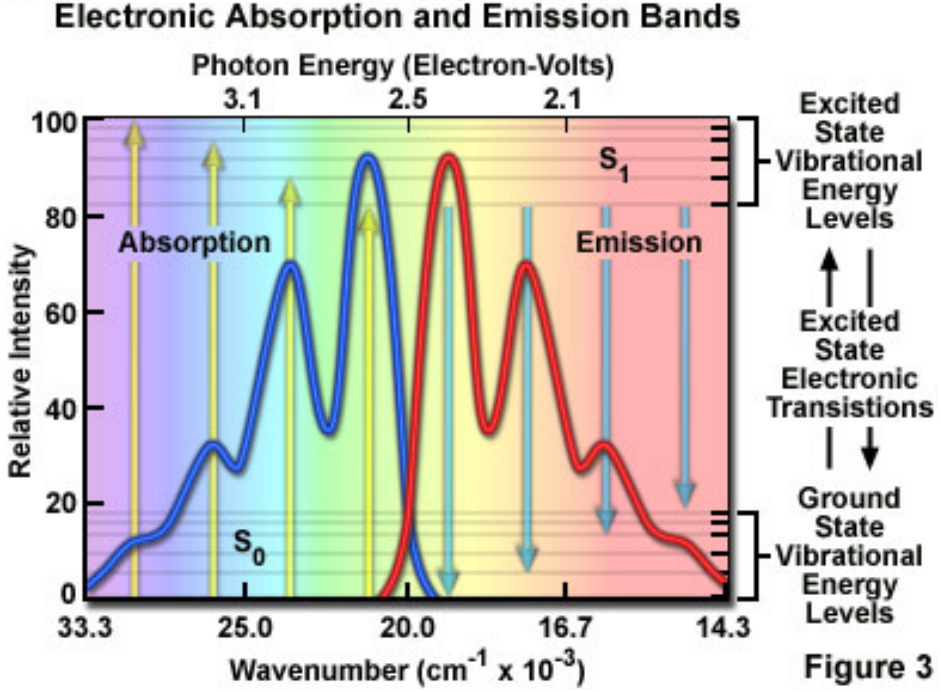


http://www.micro.magnet.fsu.edu/optics/timeline/people/jablonski.html

Summary: **fluorescence emission**

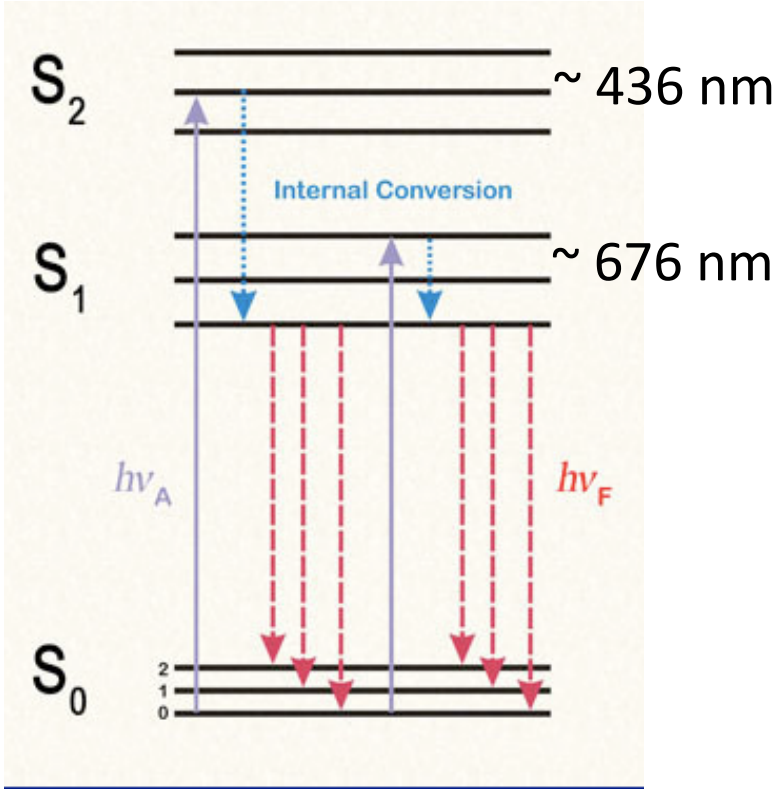
1. always from lowest vibrational state of S_1
2. red shifted – Stokes shift (higher λ, lower E)
3. mirror image of absorption

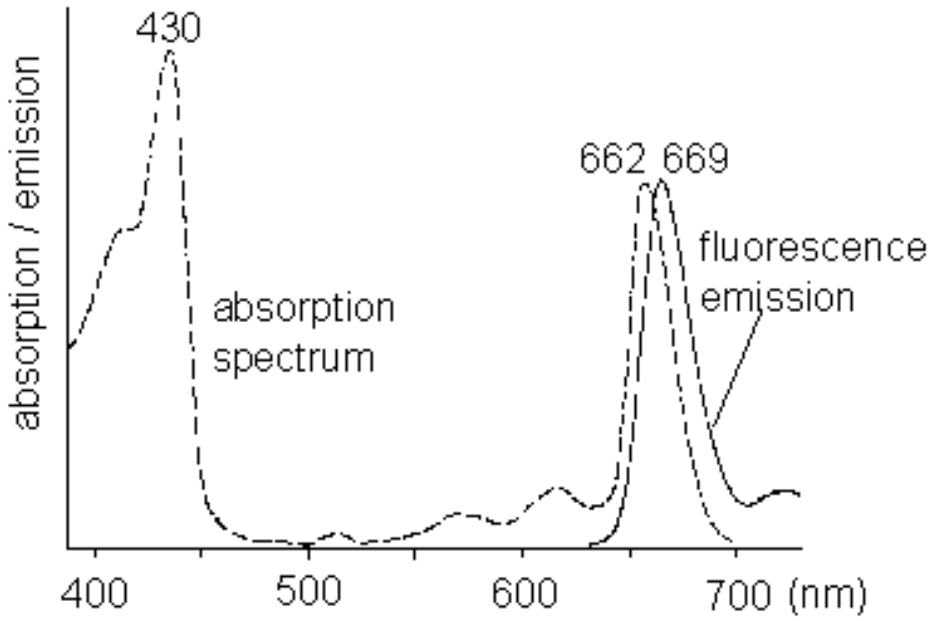




Chlorophyll *a* example

– single pigment molecule with two primary absorption bands: blue Soret band (S2) and red Q band (S1), with Stokes' shift to Q band and fluorescence emission only from Q band.

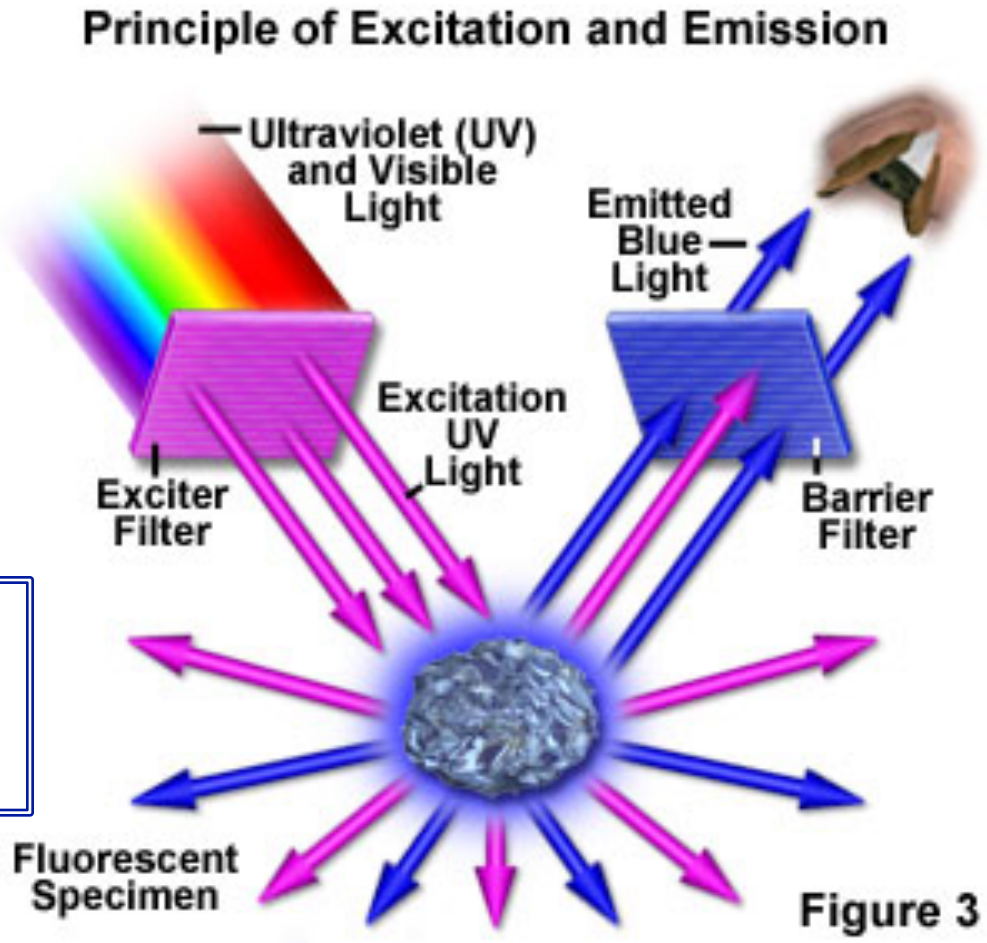




Note: on left, absorption λ maxima are *in vivo*; right, λ maxima are *in vitro*

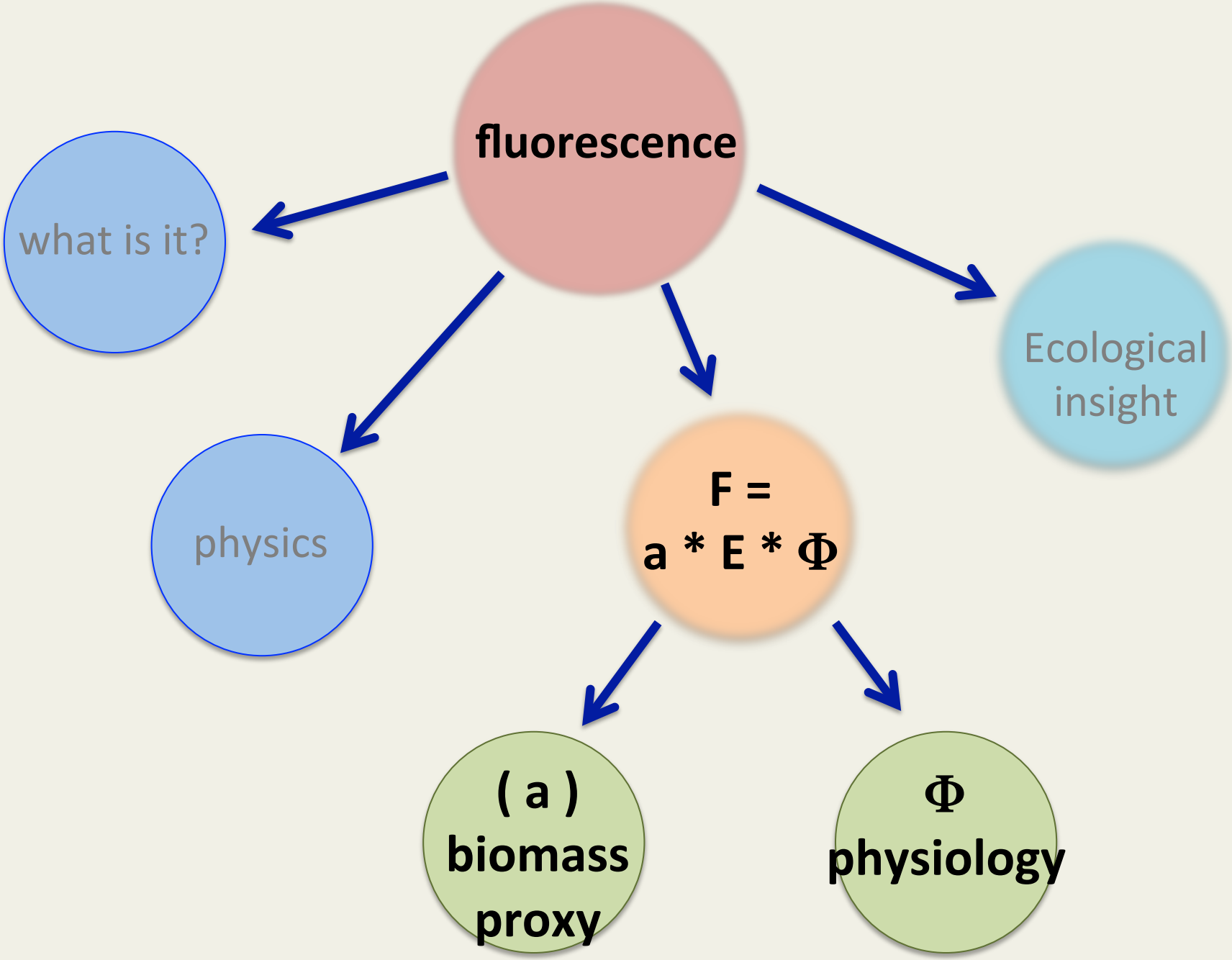
Chlorophyll *a* example *(other ocean compounds: CDOM & PE)*
– single pigment molecule with two primary absorption bands: blue Soret band (S2) and red Q band (S1), with fluorescence emission only from Q band (with Stokes' shift – see preceding slide).

Two absorption bands of chlorophyll provides a great technical advantage
– allow better separation of excitation (blue) and emission (red) light.

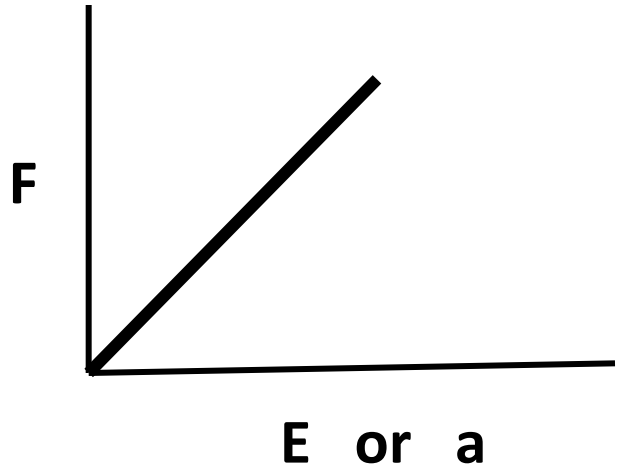


Technical note: excite and detect fluorescence orthogonally; fluorescence is isotropic.

http://micro.magnet.fsu.edu/primer/lightandcolor/fluorointroduction.html


fluorescence
what is it?
physics
F =
a * E * Φ
Ecological insight
(a)
biomass
proxy
Φ
physiology

$$F(\lambda) = a(\lambda) * \Phi_f * E(\lambda)$$



Analogy: Beer's Law, A = conc *ε* L
Hold ε* L constant, A ~ conc.
For fluorescence, hold E and Φ_f constant

F = fluorescence emission
measure as photons or energy – difficult to get absolute measurement, so typically measured as relative fluorescence, in digital counts or analogue detector in volts)

a = absorption coefficient; 'related' to **C**, concentration (and a*)

E = energy of excitation light

λ = wavelength

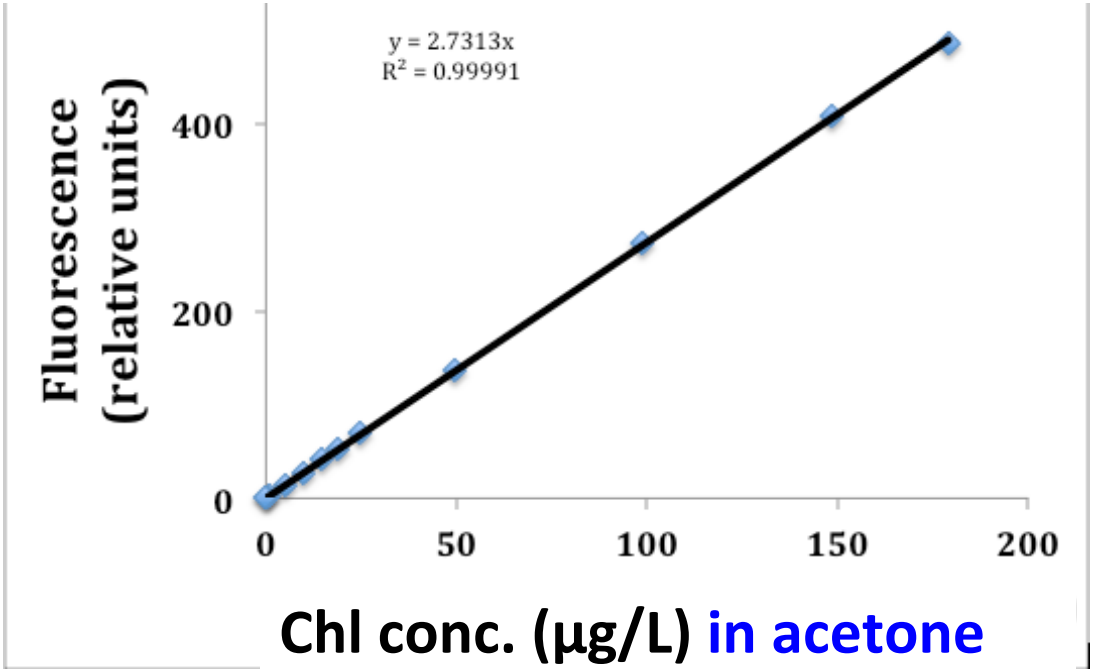
$\mathbf{\Phi_f}$ = quantum yield of fluorescence = $\frac{\text{moles photons emitted}}{\text{moles absorbed}}$

a (λ)

$$F = a(\lambda) * \Phi_f * E(\lambda)$$

a = absorption coefficient

in vitro (e.g., in acetone extract), a ~ chl conc., hence F ~ conc; Turner Designs 10-AU calibration protocol requires E and Φ_f to be constant (measure at same temperature as calibration temperature). Track daily changes with secondary standard.



a (λ)

$$F = a(\lambda) * \Phi_f * E(\lambda)$$

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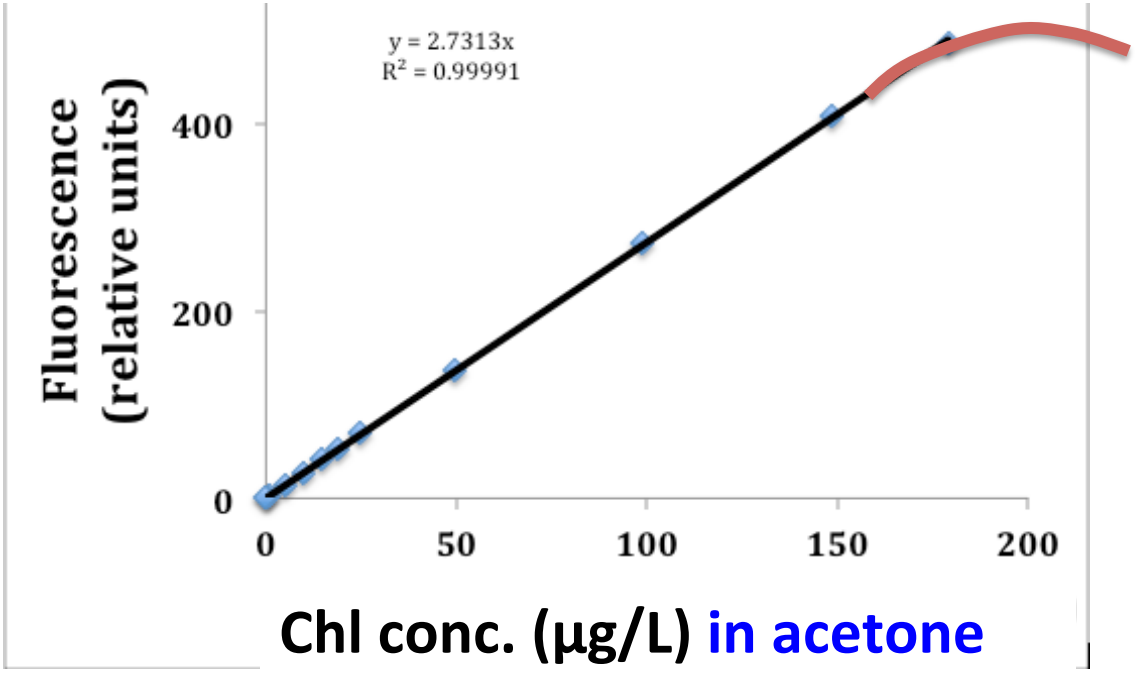
in vitro (e.g., in acetone extract), a ~ chl conc., hence F ~ conc; Turner Designs 10-AU calibration protocol requires E and Φ_f to be constant (measure at same temperature as calibration temperature). Track daily changes with secondary standard.

Nonlinearity at higher concentrations due to absorption of excitation light and/ or absorption of fluorescence light (inner filter effect) or chemical reaction in excited state



a (λ)

$$F = a(\lambda) * \Phi_f * E(\lambda)$$

a = absorption coefficient

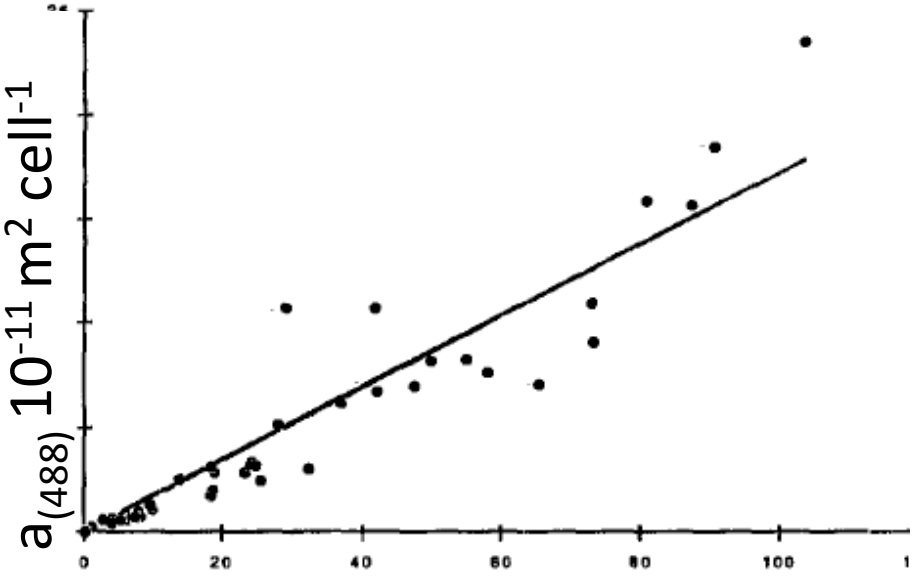
in vivo (living cells):

F ~ absorption,

with other caveats,

such as constant Φ_f

Linear relationship between phytoplankton absorption(488 nm) and fluorescence (measured in a flow cytometer with **high energy laser excitation**)



Chl F / cell – living cells

Fig. 5. Regression of geometric mean of Chl *a* fluorescence per cell (converted to linear units) measured in the flow cytometer vs. $\sigma(488)$ for all species and growth irradiances listed in Table 1; $\sigma(488) = 0.173 \times 10^{-11} \times$ Chl *a* fluorescence per cell; $r^2 = 0.93$.

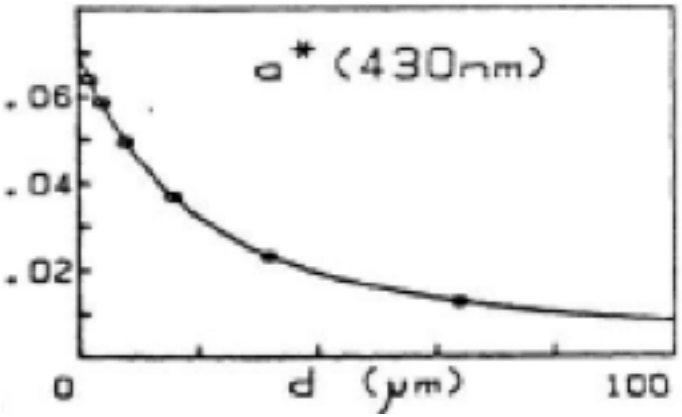
Perry & Porter 1989

a (λ)

In typical fluorometers (with relatively low excitation energy), **fluorescence /Chl a** varies due to

1) pigment packaging (cell size ~ pathlength)
   - cell size and
   - photo-adaption (more chl/cell at low growth irradiances).
2) photo-quenching and photo-damage (lab today).

$$\frac{a_{(488)}}{\text{chl conc.}}$$



(1) vary size, maintain constant intracellular pigment concentration

or

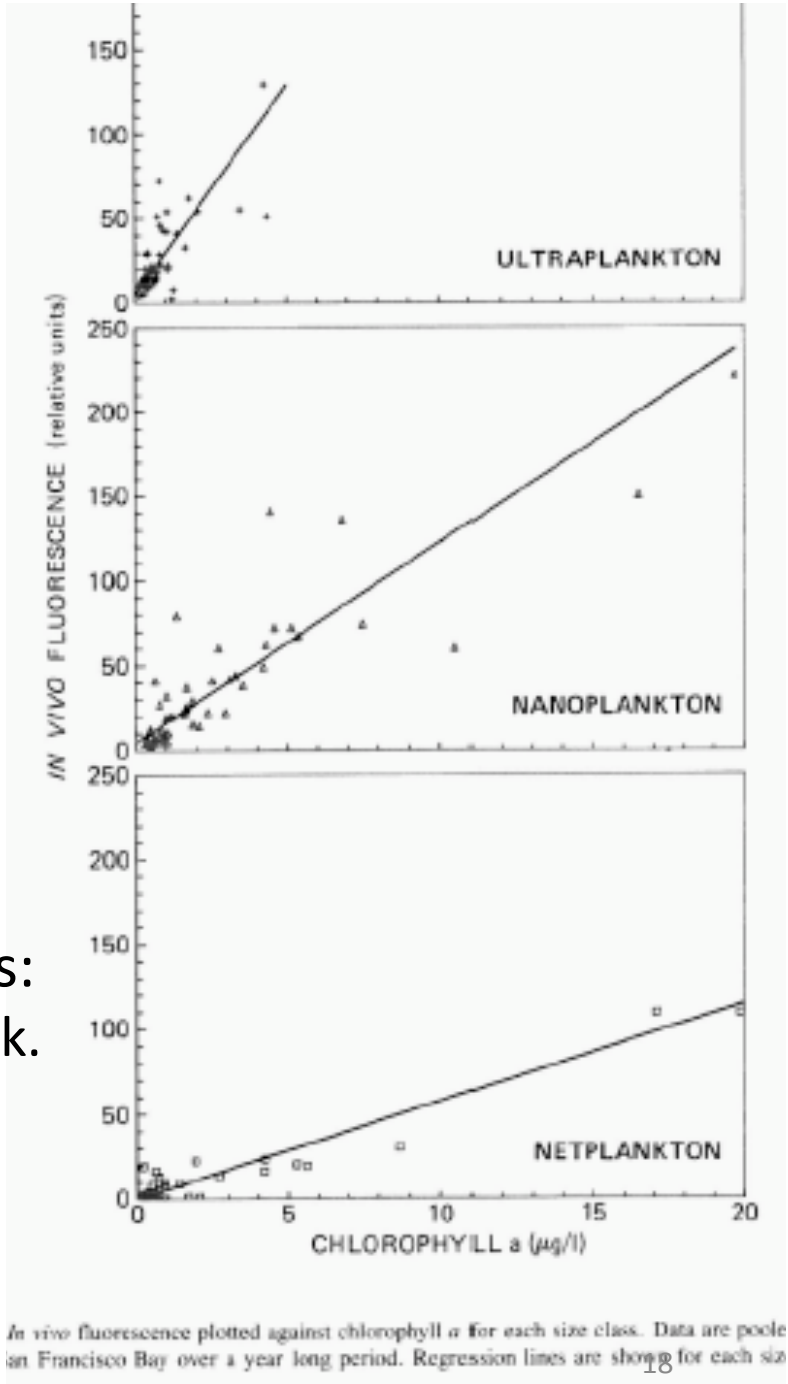
(2) maintain size, vary intracellular pigment concentration

a (λ)

Higher pigment packaging decreases F/Chl

San Francisco Bay:
sizes were separated w/ screens
- netplankton (>22 µm)
- nanoplankton (5–22 µm)
- ultraplankton (<5 µm)

F / Chl was linearly related to extracted Chl concentration within size class,
but was significantly different among sizes:
ultraplankton = 2 * nanoplank. = 2 * netplank.



In vivo fluorescence plotted against chlorophyll *a* for each size class. Data are pooled
an Francisco Bay over a year long period. Regression lines are shown for each size

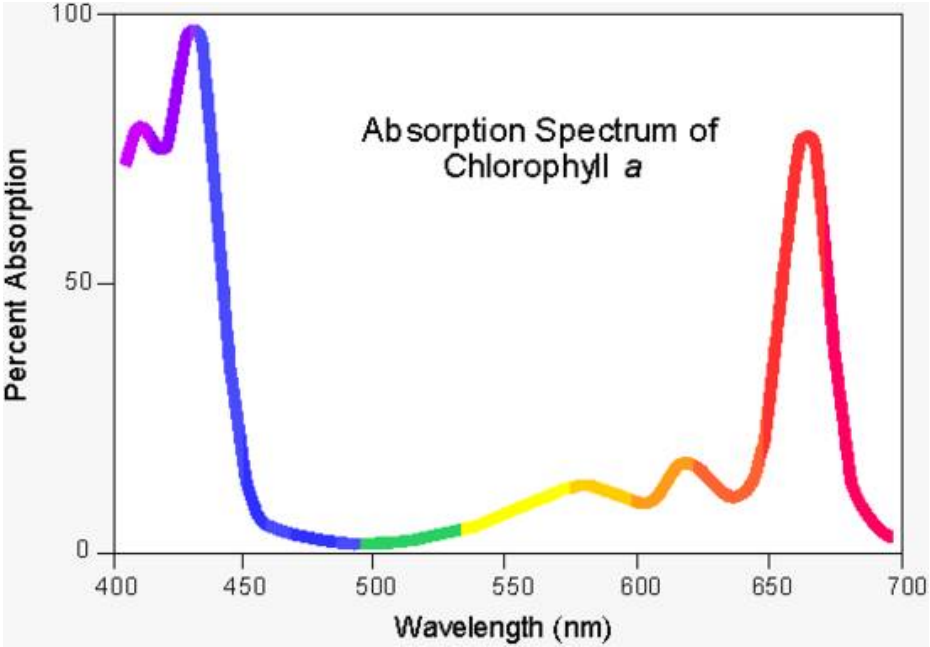
Data from San Francisco Bay;
Alpine and Cloern (1985). J Plankton Research 7: 318.

(λ)

$$F = a(\lambda) * \Phi_f * E(\lambda)$$

λ– dependence for both absorption and E, excitation energy:

there must be a match between wavelengths of phytoplankton absorption spectrum and lamp excitation spectrum.



What is the excitation spectrum of a typical *in situ* fluorometer?

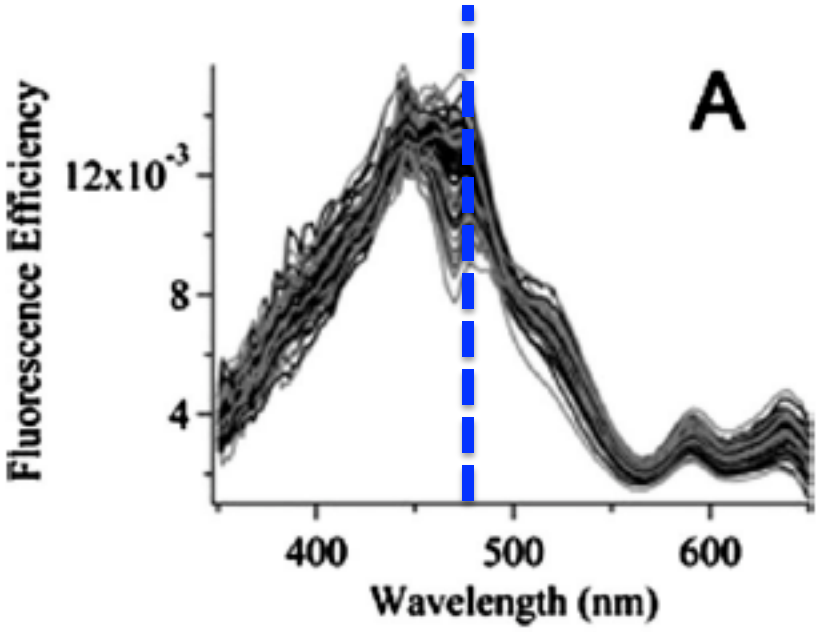
(Models do differ in λ; also, calibration issues associated with changes in E and/or λ over time)

(λ)

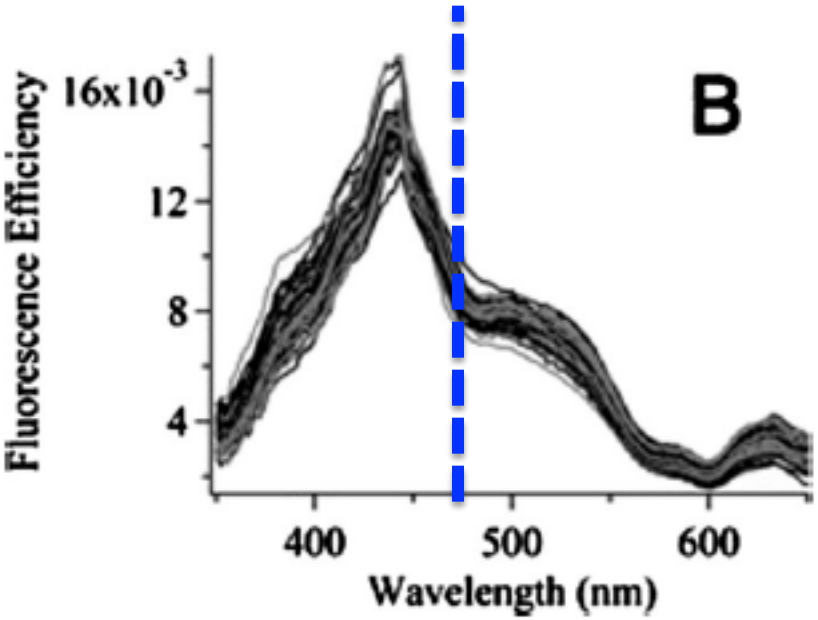
$$F = a(\lambda) * \Phi_f * E(\lambda)$$

λ– dependence for both absorption and E, excitation energy:

Is the ratio between chlorophyll *a* and accessory pigments constant?



Emiliania huxleyi cells



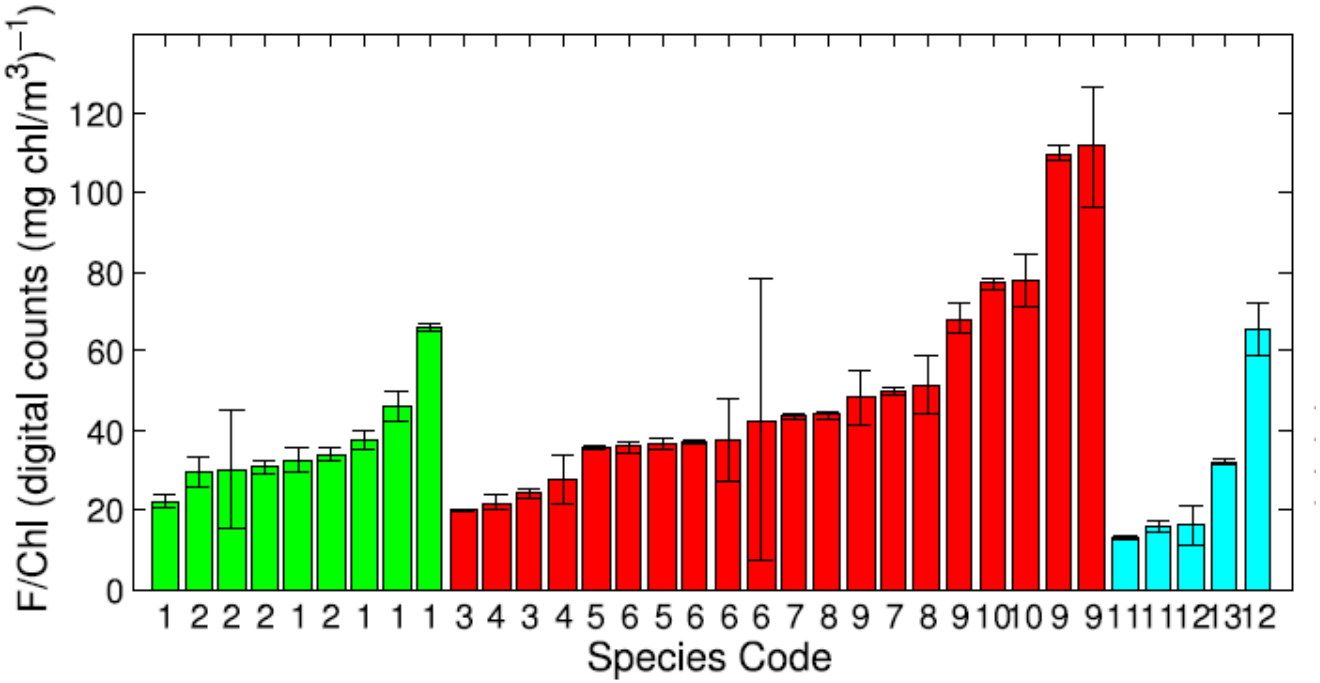
Thalassiosira pseudonana

Richardson et al. 2010. Rev. Sci. Instrum. 81, 013103

Single-cell excitation spectra (O(50-100 individual cells))

(λ)

Fluorescence/Chl varies with species



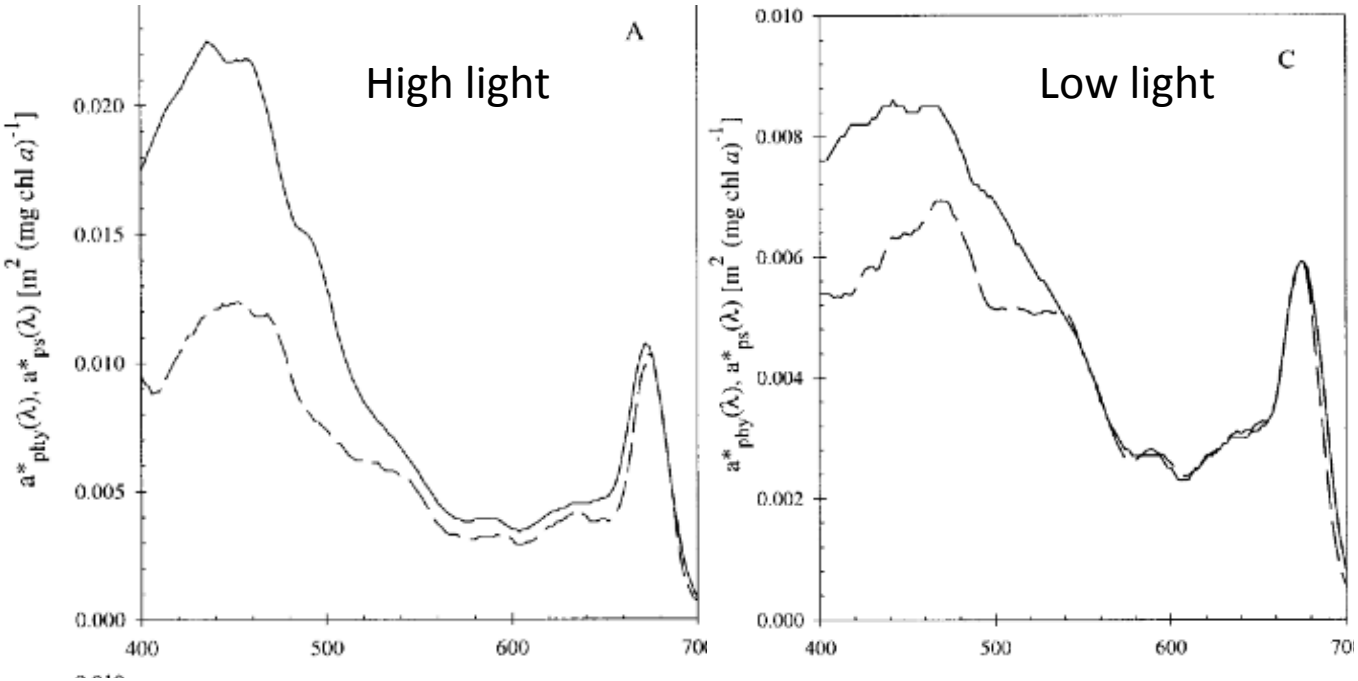
Roesler and Bernard, 2013;
Proctor and Roesler, 2010

(λ)

$$F = a(\lambda) * \Phi_f * E(\lambda)$$

λ– dependence for both absorption and E, excitation energy:

phytoplankton absorption at 470 nm can be separated into absorption by **photosynthetic pigments(a_ps)** and photoprotective pigments (a_pp). Only photosynthetic pigments are capable of transferring energy to chlorophyll Q-band, resulting in fluorescence. fluorescence ~ a_ps.



solid line = a_phyt

dotted line = a_ps

difference will be a_pp

Data from Culver & Perry

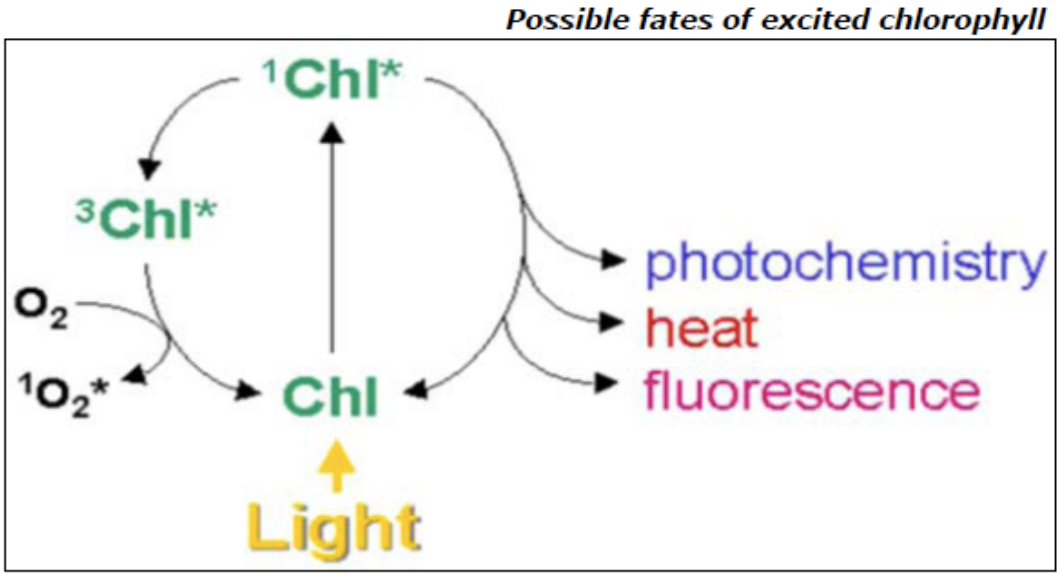
Φ_f

$$F = a_{ps}(\lambda) * \Phi_f * E(\lambda)$$

quantum yield of fluorescence varies (Φ_f = moles photon fluoresced/ moles photon absorbed by photosynthetic pigments):

– in living cell (*in vivo),* F is a function of photosynthetic physiology, and is influenced by

light (photo-quenching and photo-damage – so it will vary temporally) & **nutrient** limitation (so it will vary spatially).;

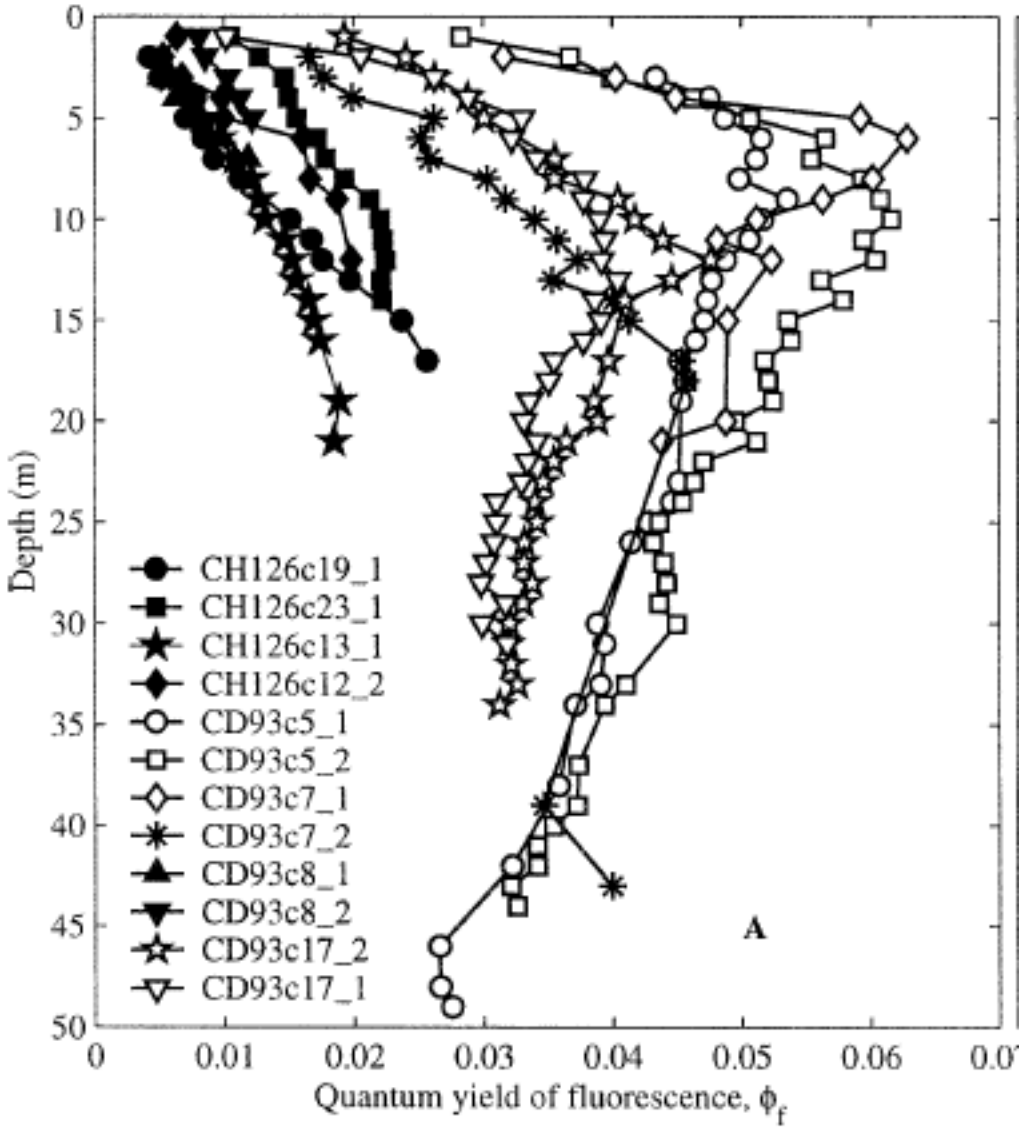


Typically in living cell

Φ_f is ~ 0.5% – 2%

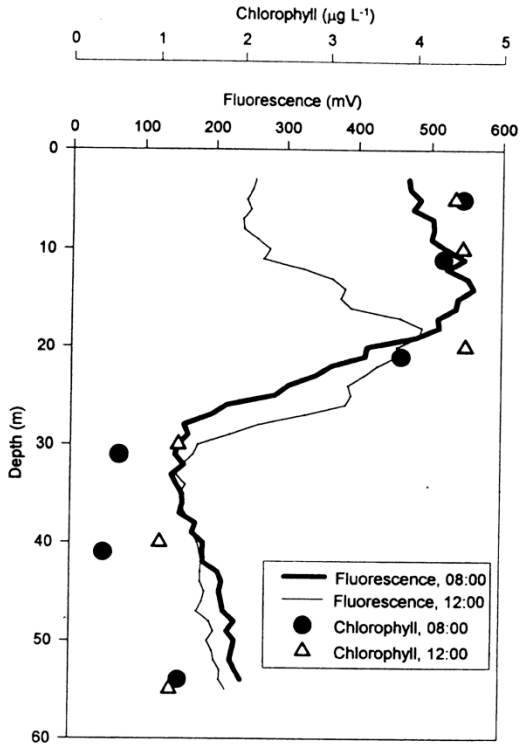
http://biologie.univ-mrs.fr/upload/p222/1_fluorescence.pdf

Φ_f Chl fluorescence vs. depth shows photoquenching near surface



Morrison 2003

Fluorescence measurements on a ship can be well calibrated, because you can collect frequent water samples.



From Falkowski and Raven 1997
Chlorophyll fluorescence and extracted concentration of chlorophyll early AM vs. noon.

This profile shows the effect of day-time fluorescence quenching on mid-day fluorescence profile. Symbols are extracted chlorophyll from bottle samples.

Fluorescence quenching a challenge

(sometimes have to look at night time data only, or try correction)

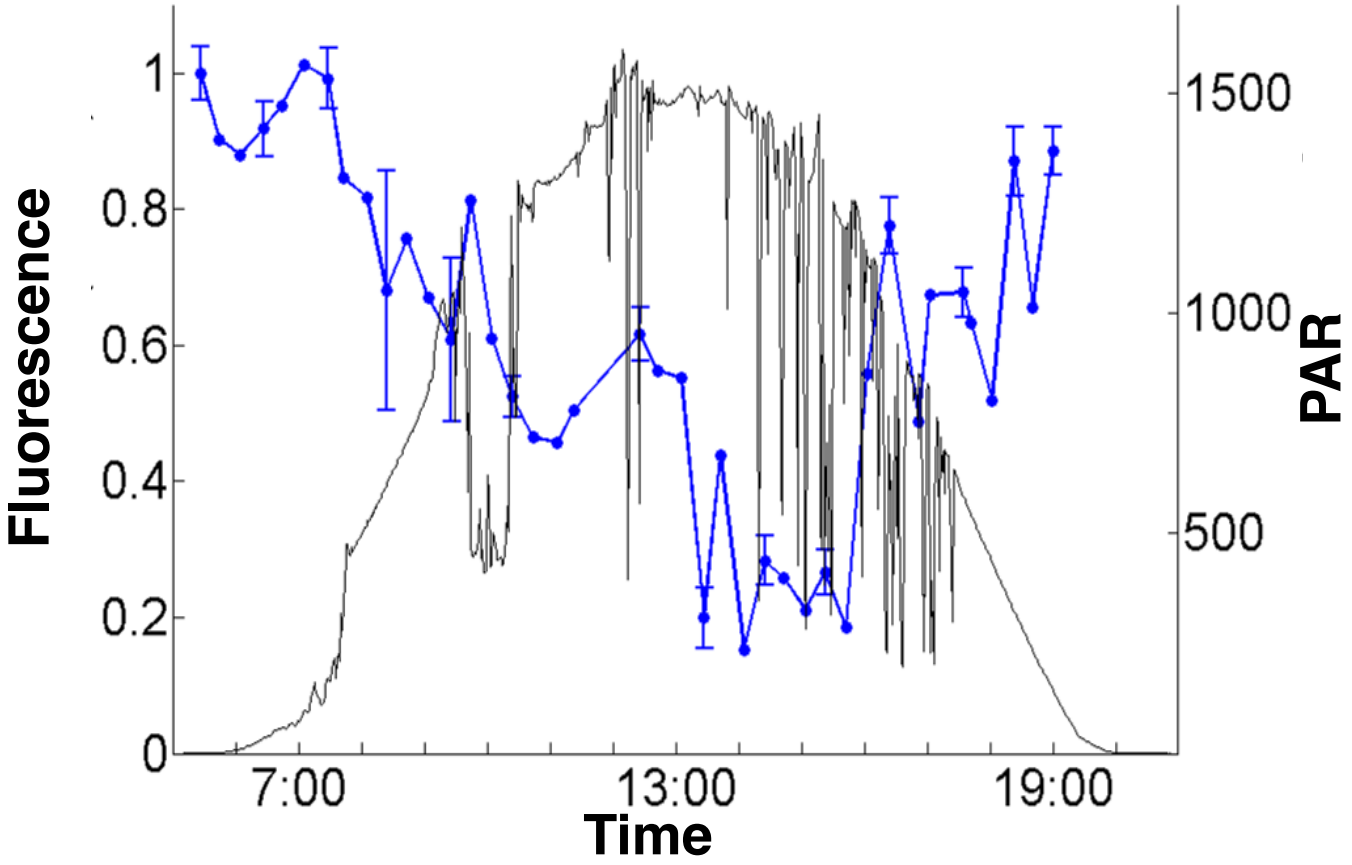
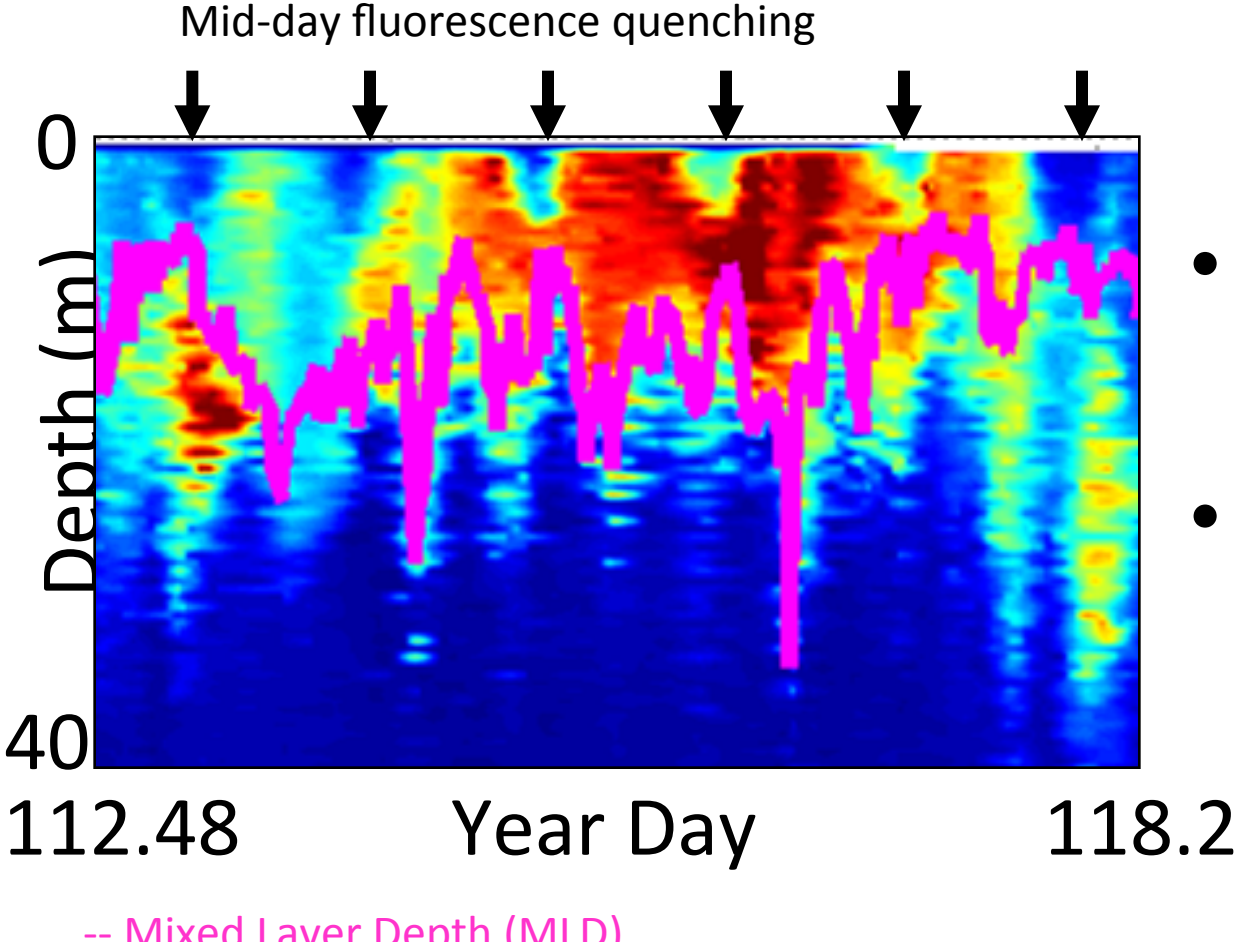


Figure 2: Damariscotta River *in situ* chlorophyll a fluorescence and PAR (µmol photons/s/m^2) vs. time.

Drzewianowski 2008 – MS thesis

Another example of mid-day fluorescence quenching, from autonomous glider data (Washington coast)

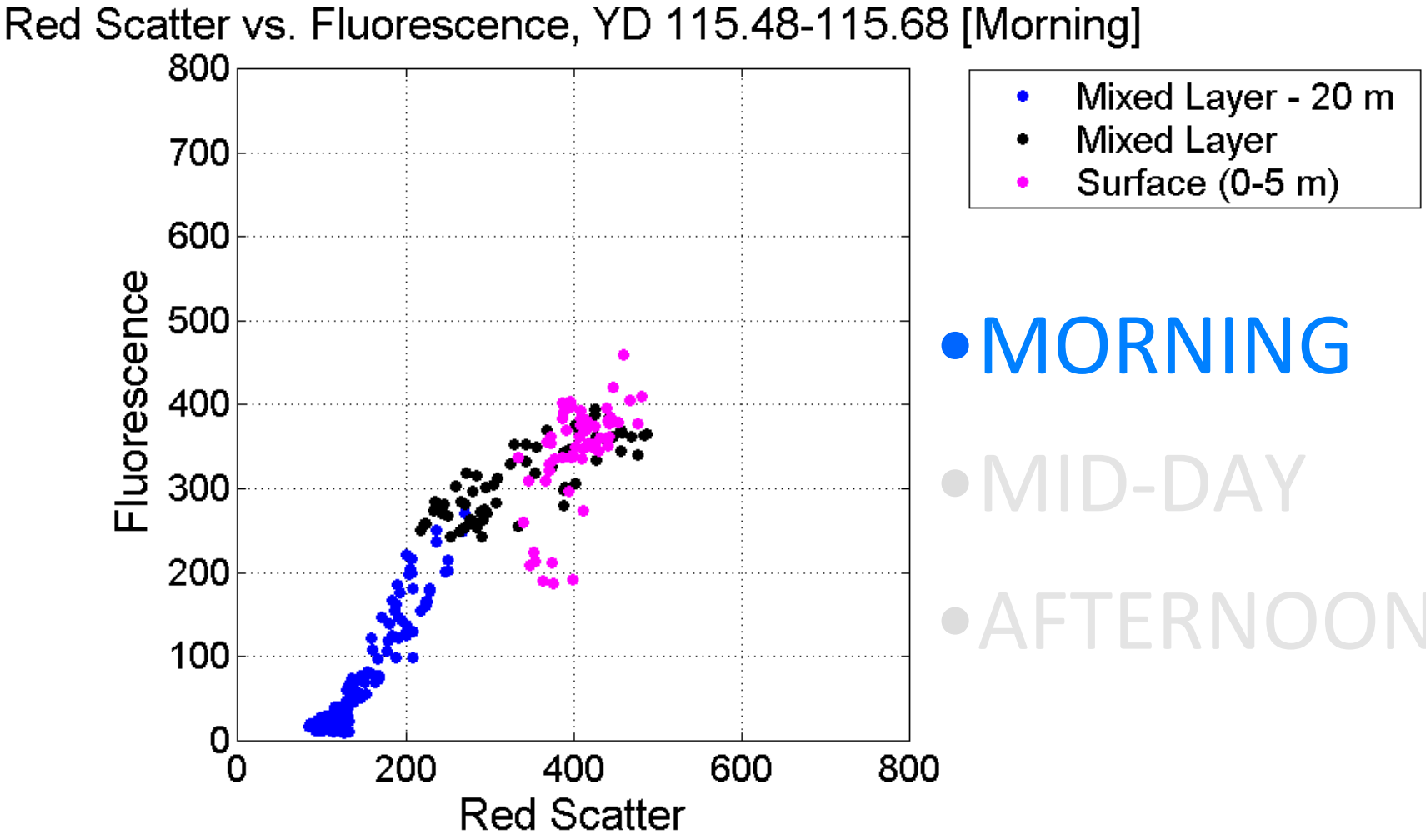


- Quenching observed to 11m
- Fluoresence quenched up to 80% at surface

So maybe for biomass, should we concentrate on night-time measurements *in vivo* fluorescence measurements?

Sackmann 2007 PhD.

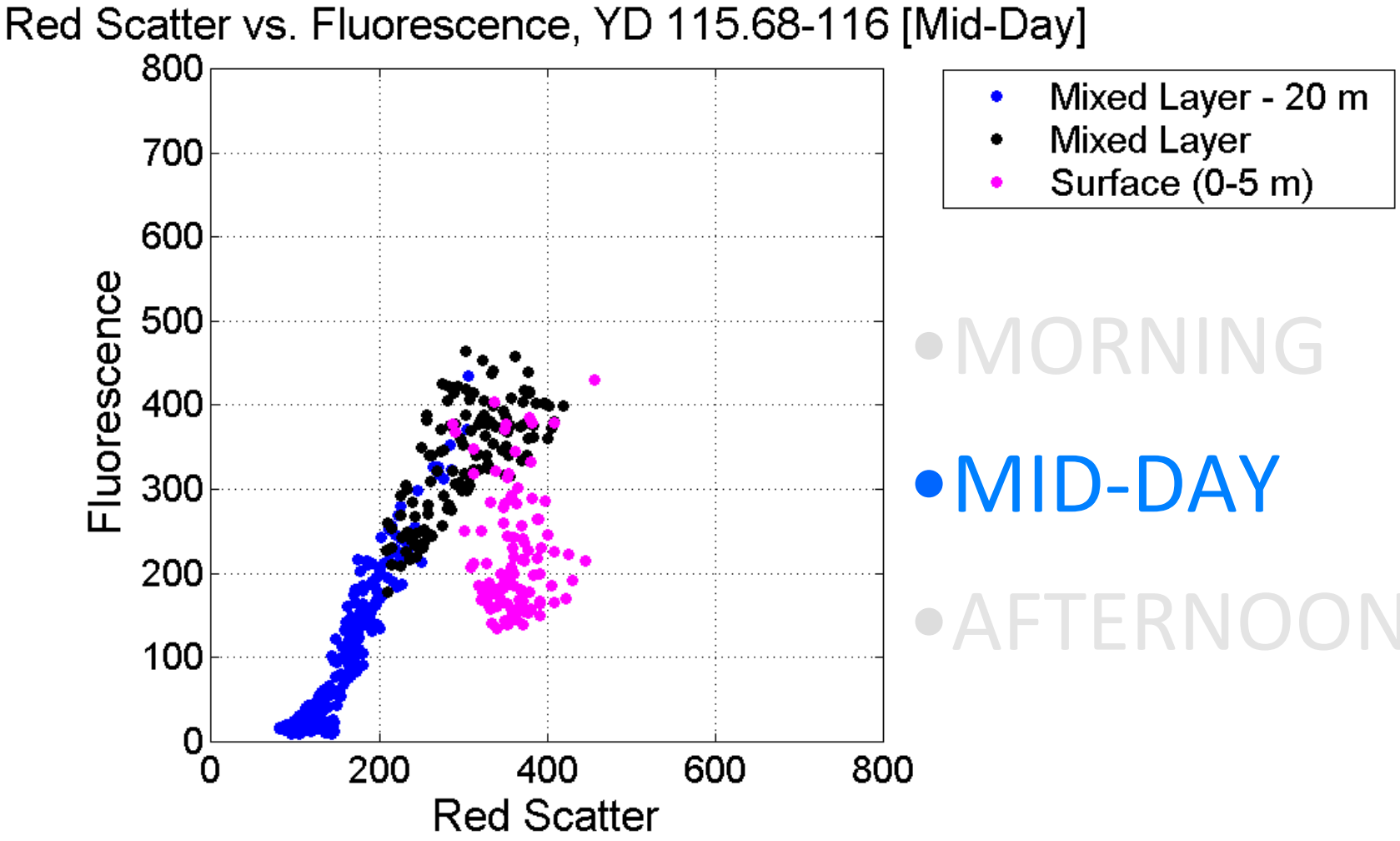
Mid-day fluorescence quenching



- MORNING
- MID-DAY
- AFTERNOON

Sackmann et al., unpub.

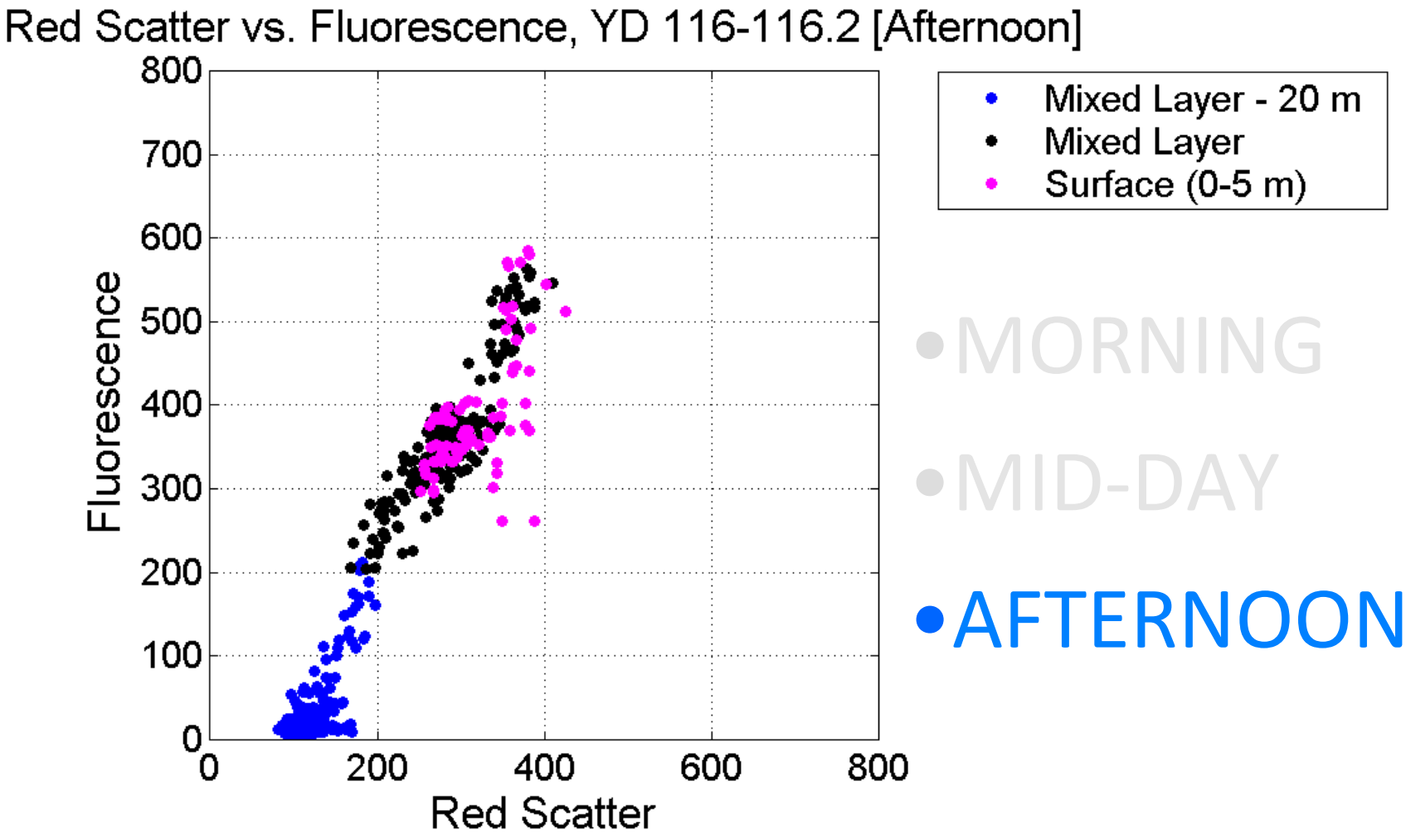
Mid-day fluorescence quenching



- MORNING
- **MID-DAY**
- AFTERNOON

Sackmann et al., unpub.

Mid-day fluorescence quenching

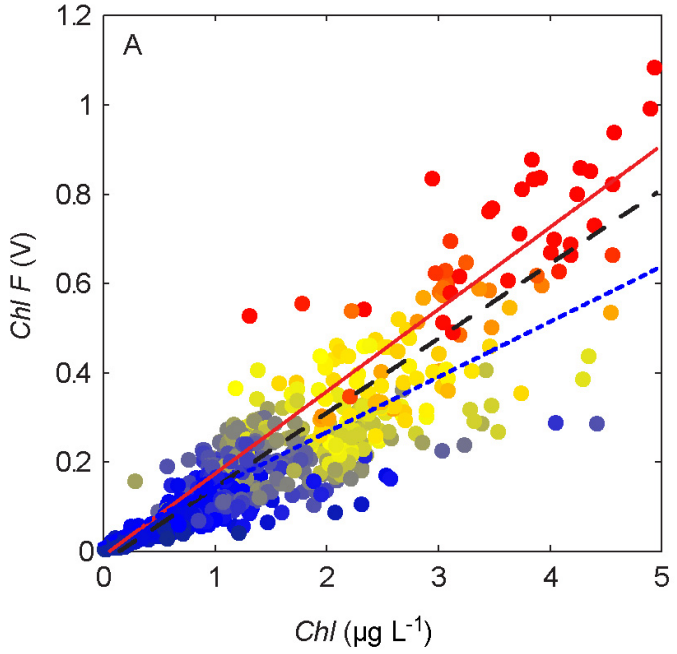


- MORNING
- MID-DAY
- AFTERNOON

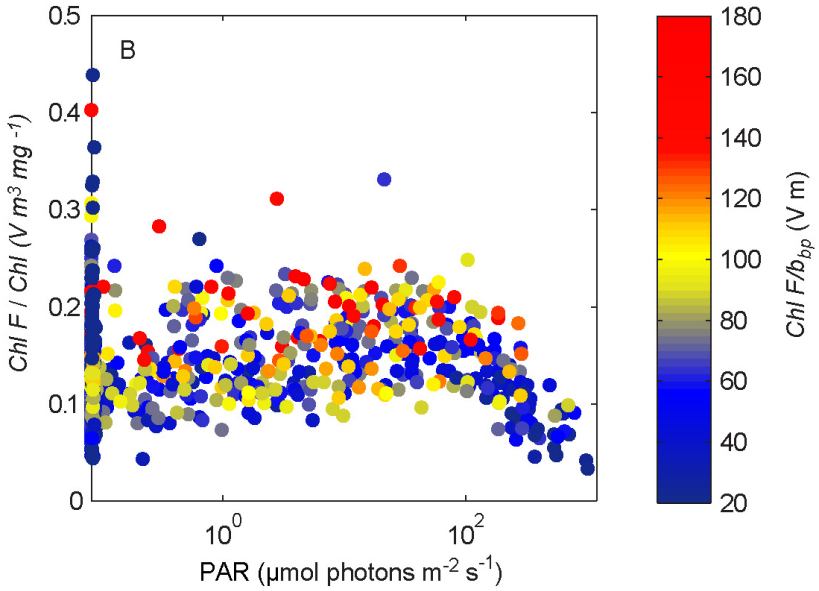
Sackmann et al., unpub.

Yesterday's lecture – data from North Atlantic, calibrated with ship samples.

Chlorophyll fluorescence to chlorophyll concentration



Raw data: fluorescence vs. extract

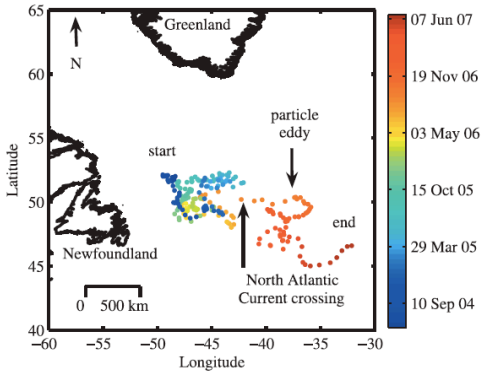


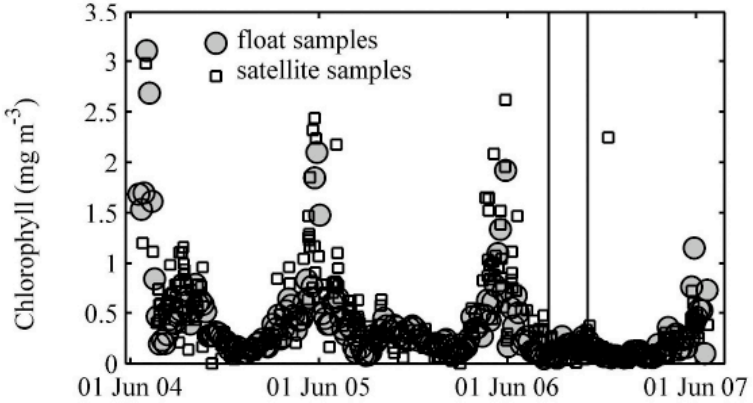
Part of variability in Chl fluorescence/ extracted chlorophyll is due to solar quenching

Fluorescence to chlorophyll cal. difficult on remote, autonomous platforms

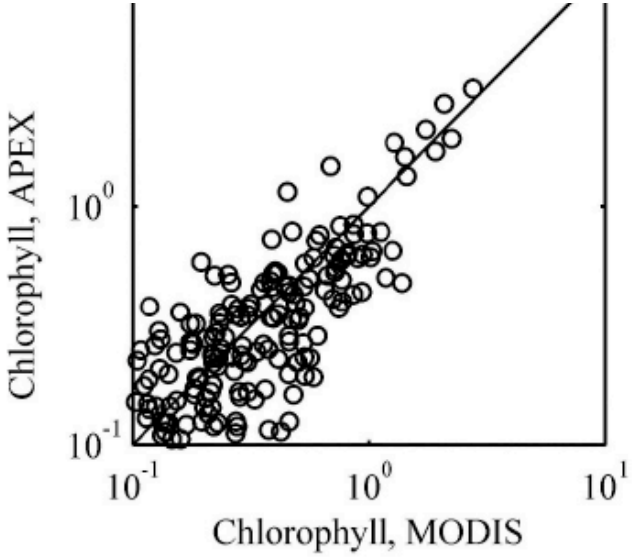
Boss et al. (2008) Limnol. Oceanogr.

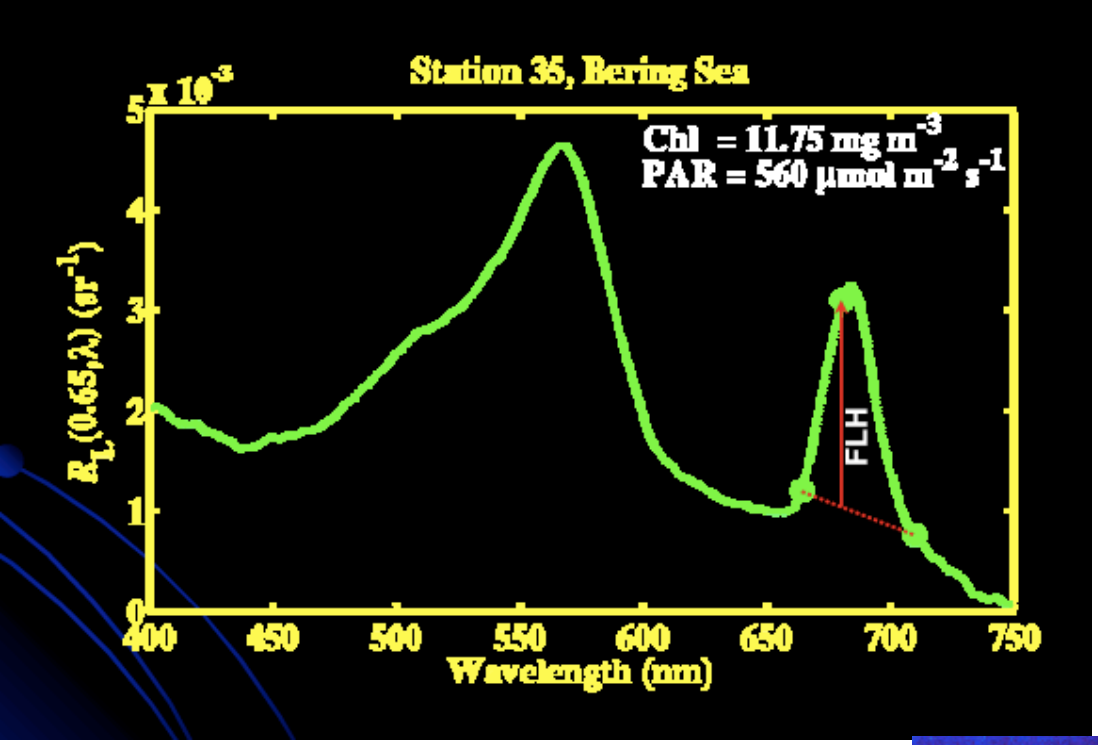
Observations of pigment and particle distributions in the western North Atlantic from an autonomous float and ocean color satellite





Time series and comparison of chlorophyll concentration as measured by the float and satellite ocean color sensors.



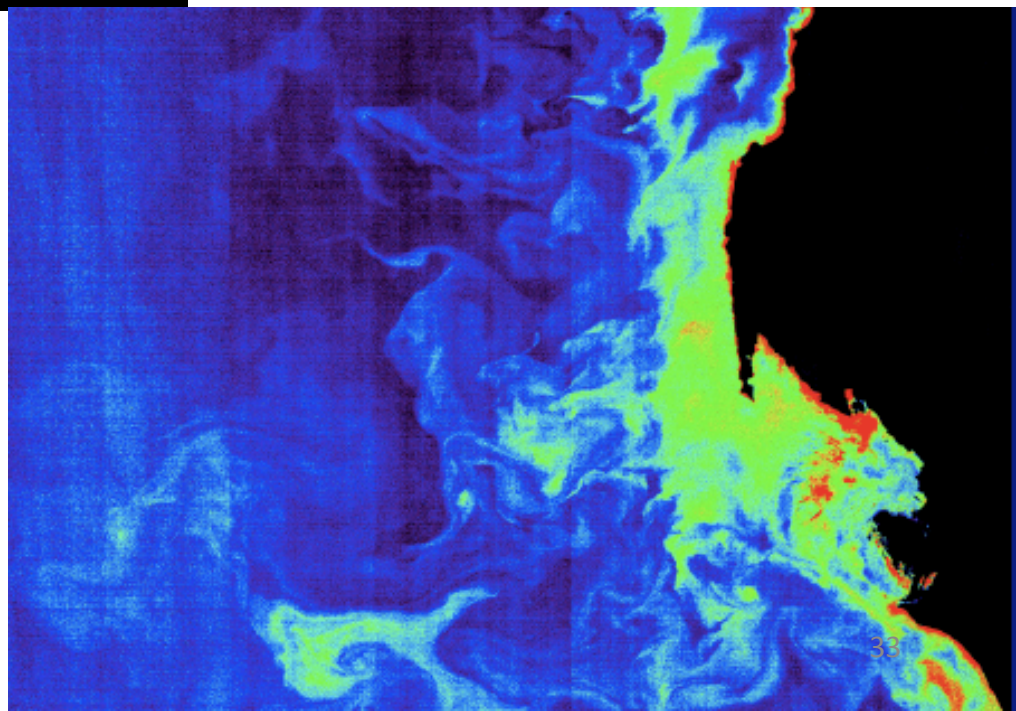


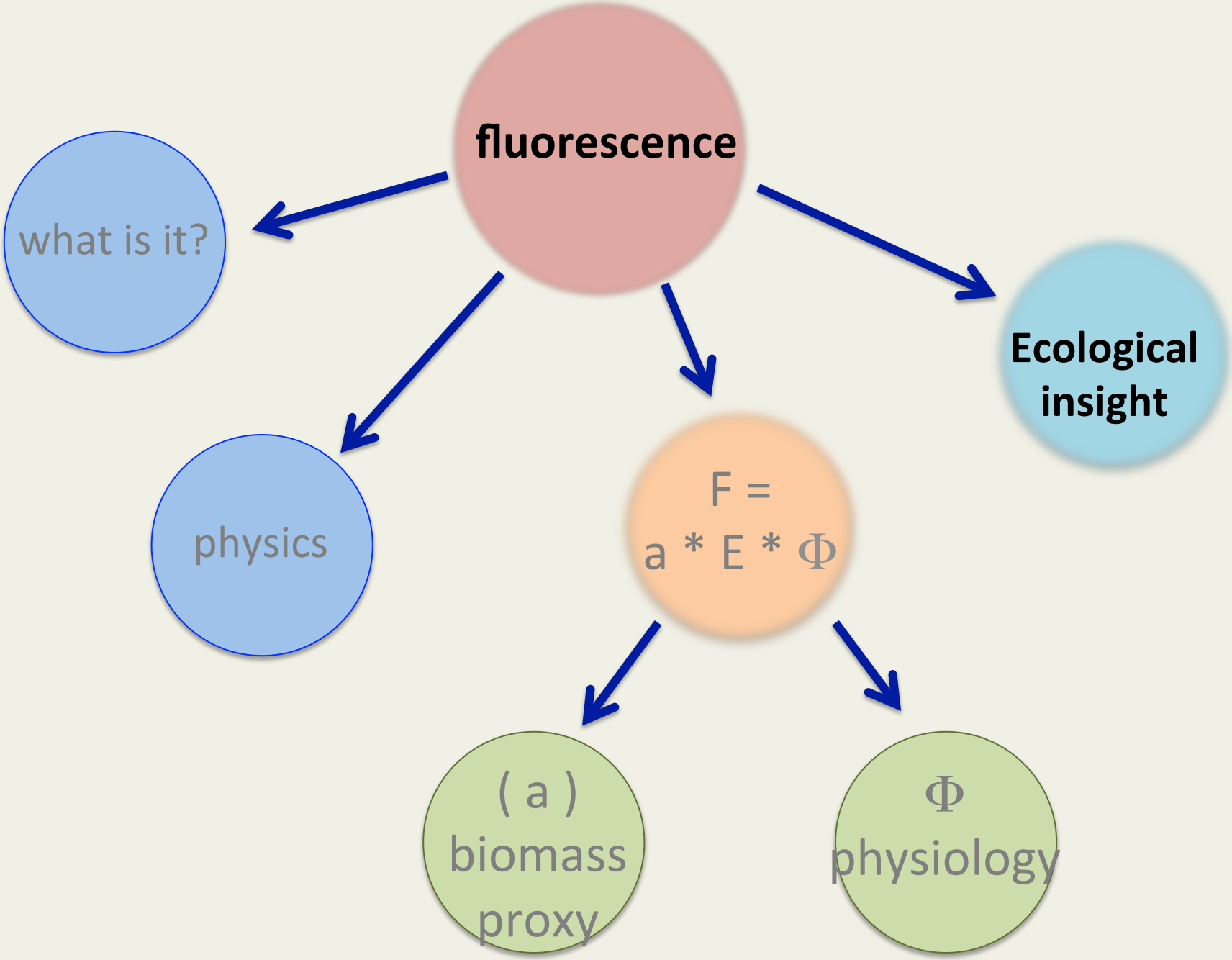
Not going to say much now about solar-stimulated fluorescence. Slides from Babin and Huot; they caution its use in turbid waters (b_{bp}, not all F)

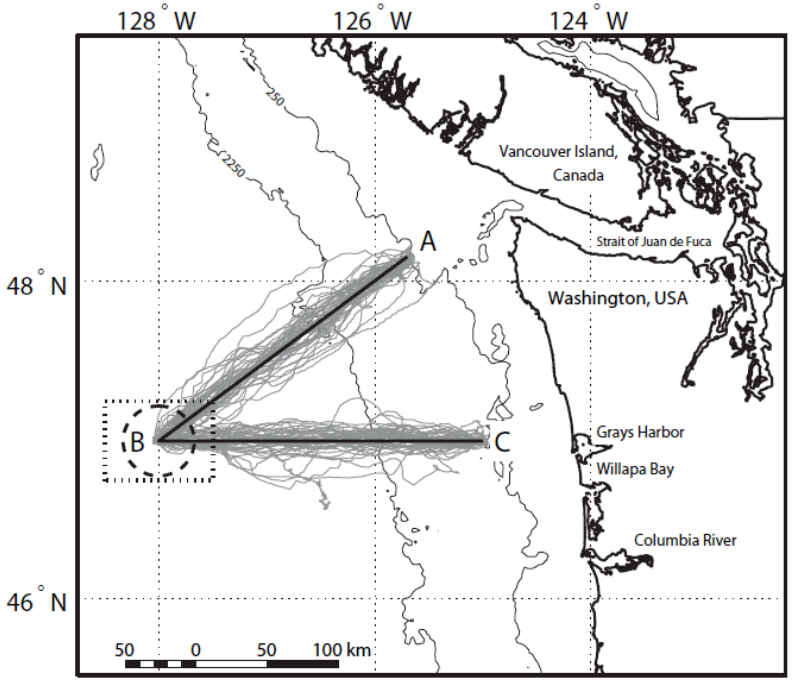
Other issues:

1) satellite images only available on clear days; bias of high light/ quenching; what is Φ_f?

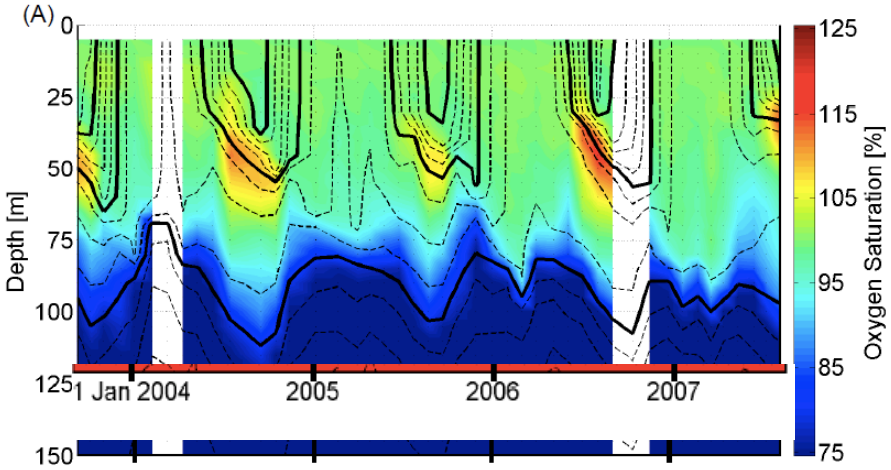
2) how to interpret, E(λ), a (λ), depth resolution

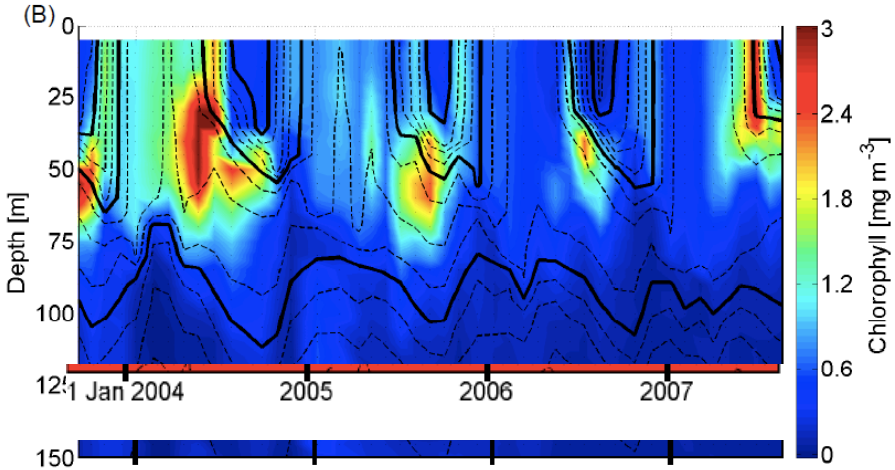



fluorescence
what is it?
physics
F =
a * E * Φ
Ecological insight
(a)
biomass
proxy
Φ
physiology



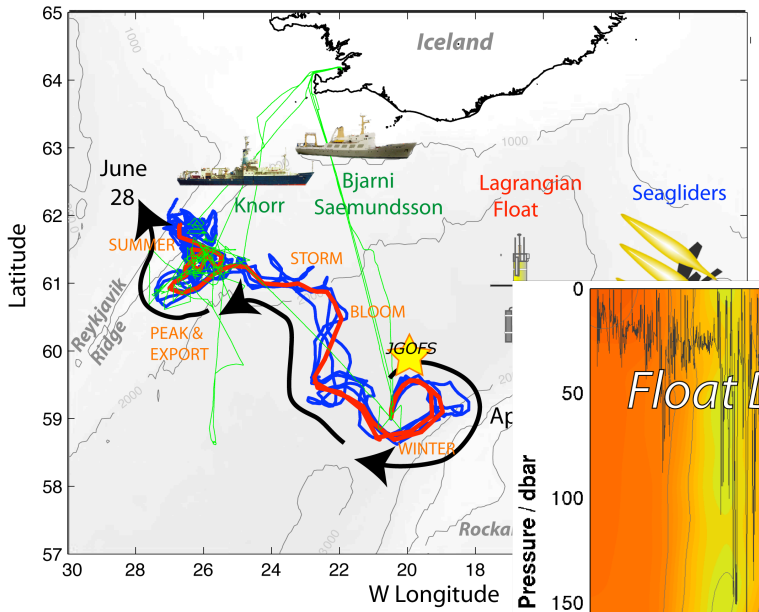
But … F reveals important patterns: interannual variability in evolution of subsurface chlorophyll maximum layer. Seaglider oxygen and chlorophyll fluorescence measurements to 150 m for four years off Washington coast .

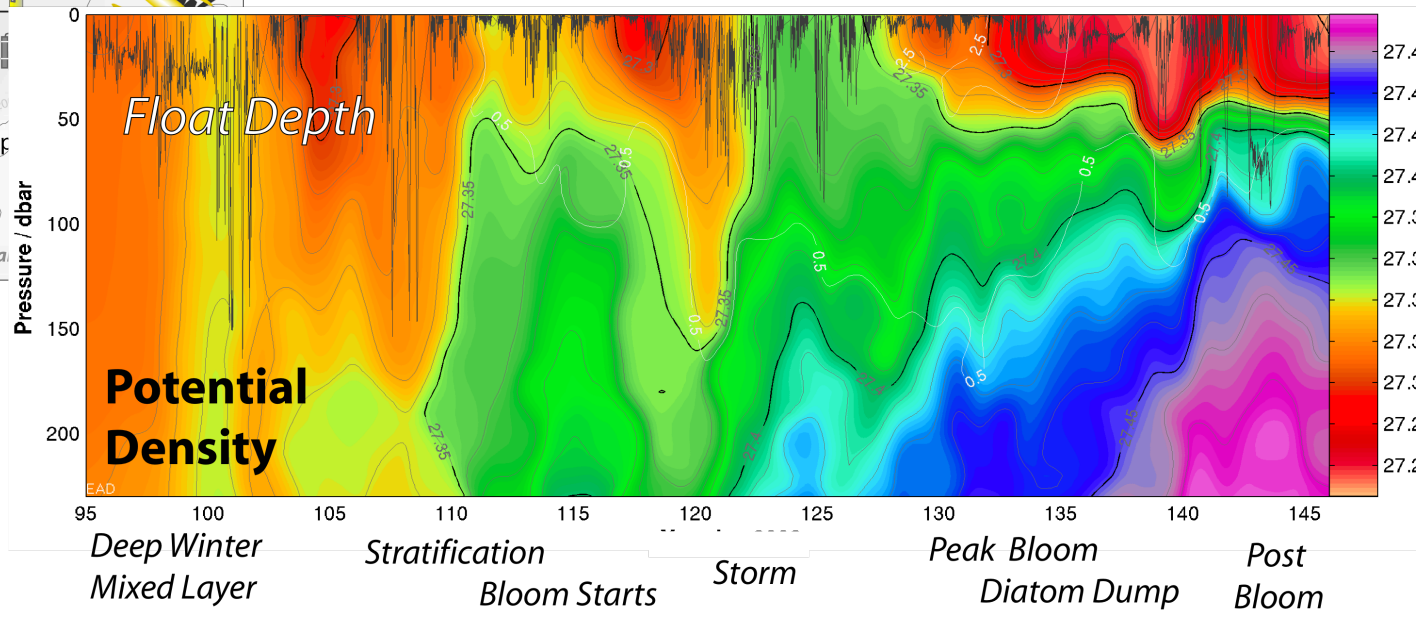


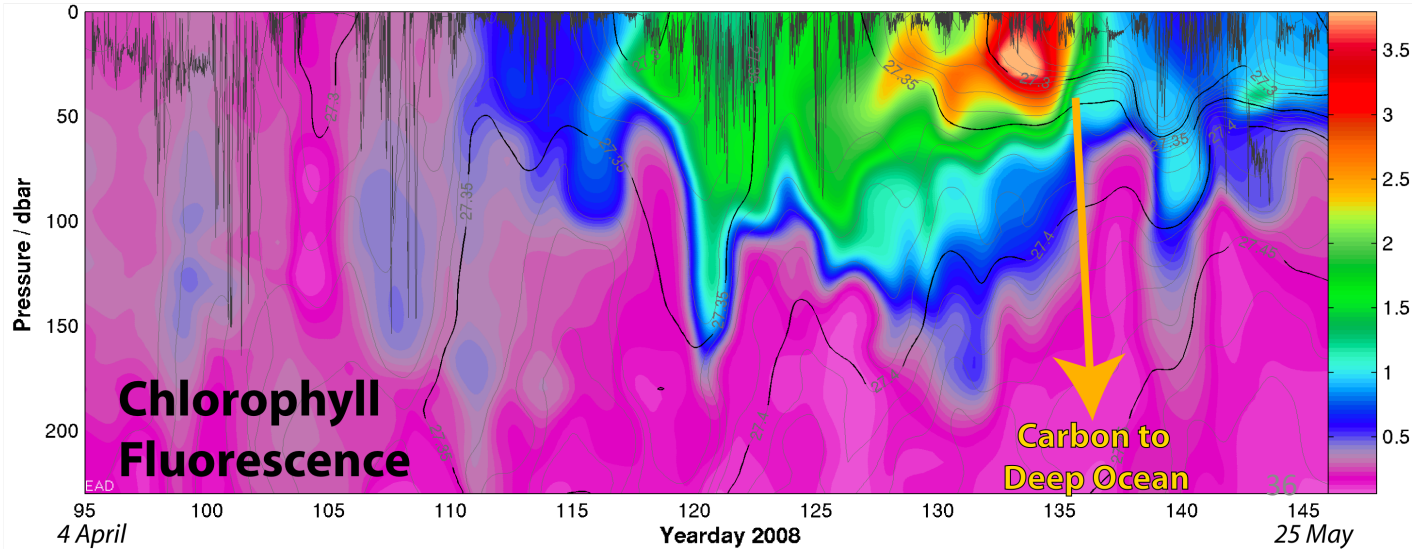


Perry et al. (2008) Limnol. Oceanogr.

Timing of a bloom from a float – evolution of float patch from early April to late May.







Alkire et al., sub.

$$F(\lambda) = a(\lambda) * E(\lambda) * \Phi_f$$

Today's lab

1) Station 1 – Mitchell Lab

Chlorophyll and CDOM fluorometers:

dark values and blanks; contamination by solar irradiance?

linearity

effect of other fluorescing material and scatterers

wavelength of excitation

2) Station 2 – MJP lab

Solar quenching of fluorescence (living cells)

HPLC vs. fluorometric analysis of acetone extracts – 2 filter sets