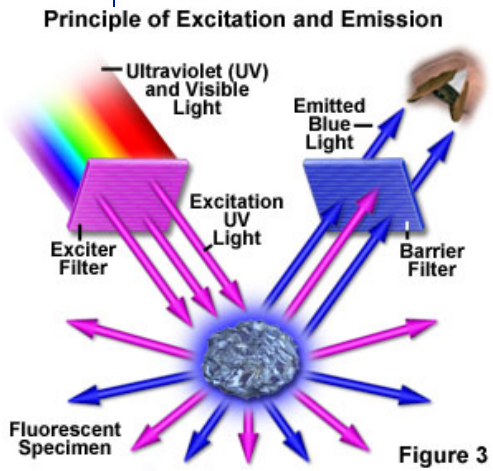
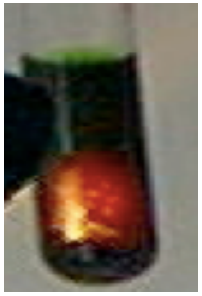
SMS 598: Calibration and Validation for Ocean Color Remote Sensing

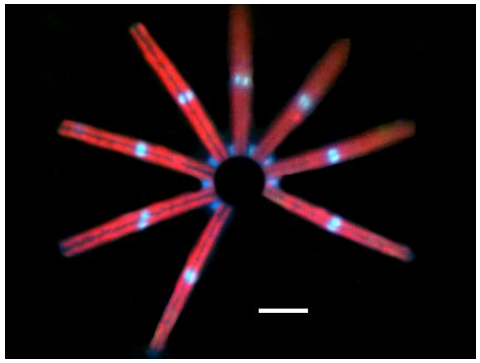
Lecture 10 Fluorescence

Mary Jane Perry

12 July 2011

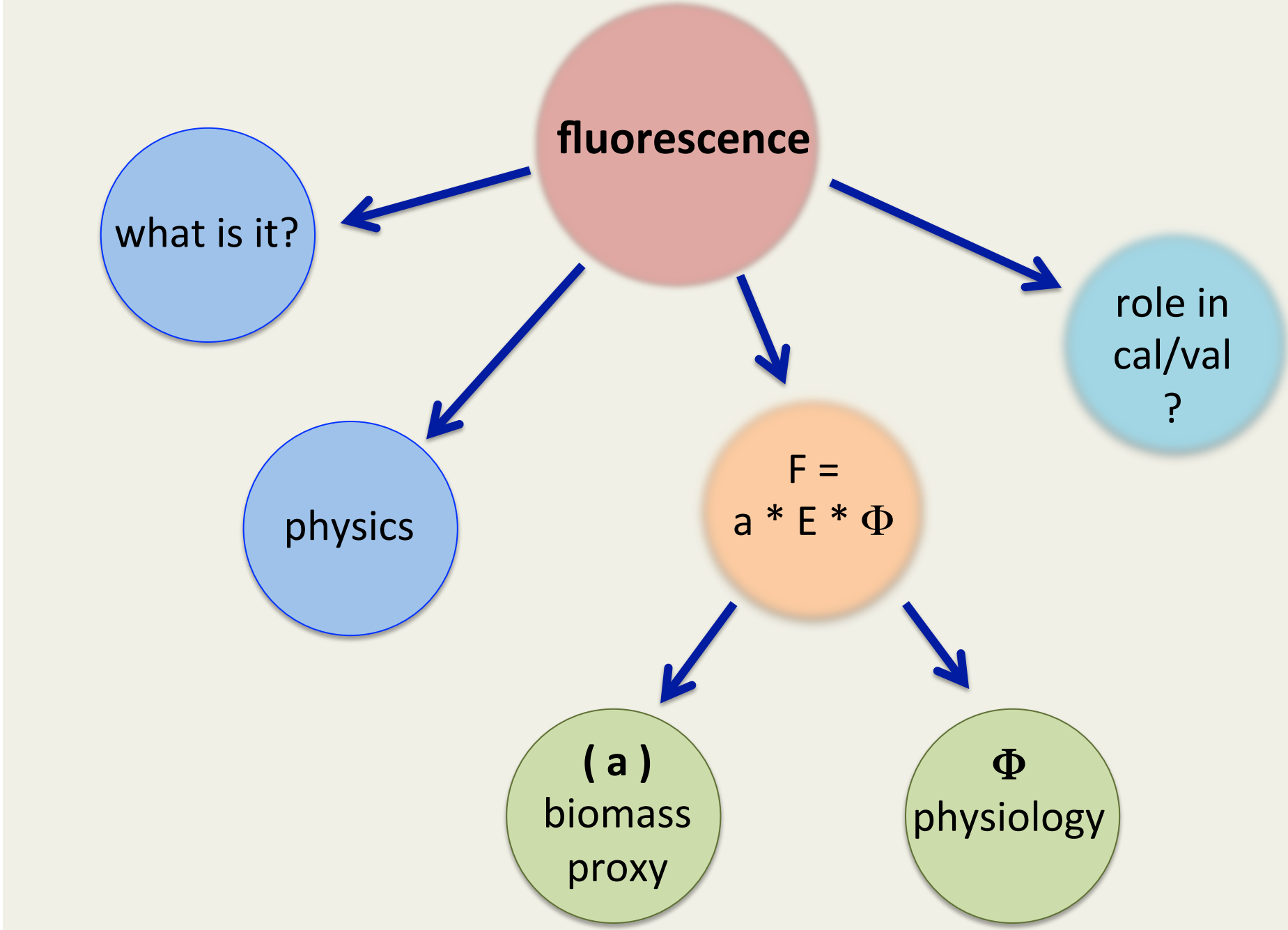






http://micro.magnet.fsu.edu/primer/lightandcolor/fluorointroduction.html

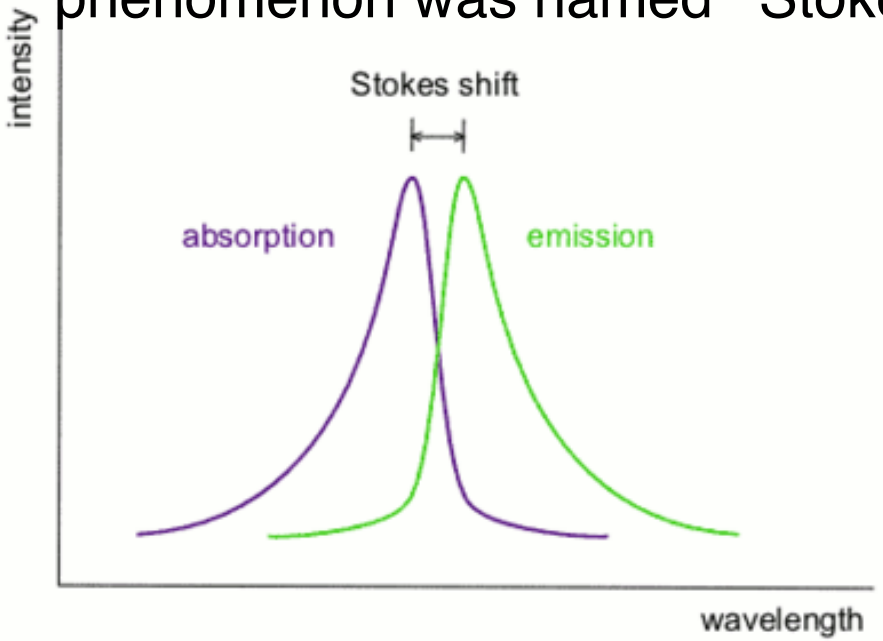
Epifluorescence microscope: chlorophyll fluorescence in *Thalassionema* (courtesy of M. Sierackiz0


fluorescence
what is it?
physics
F =
a * E * Φ
role in
cal/val
?
(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'.

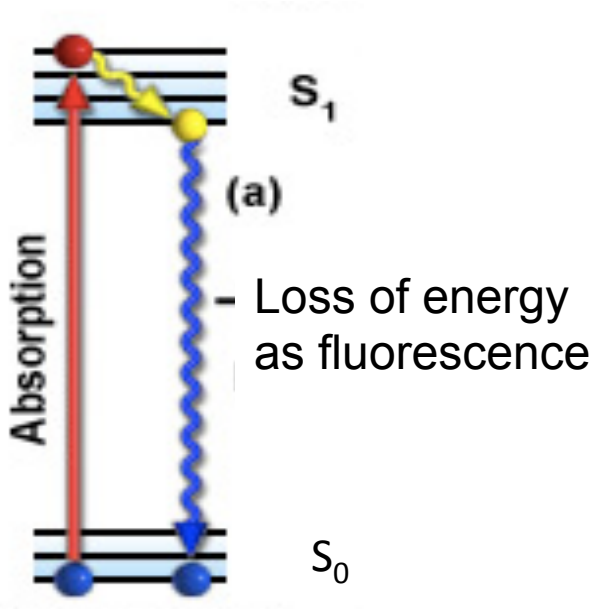


A fraction of energy absorbed at **shorter wavelength** (higher frequency, higher energy) is re-emitted as a photon at **longer wavelength** (lower frequency, lower energy).

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

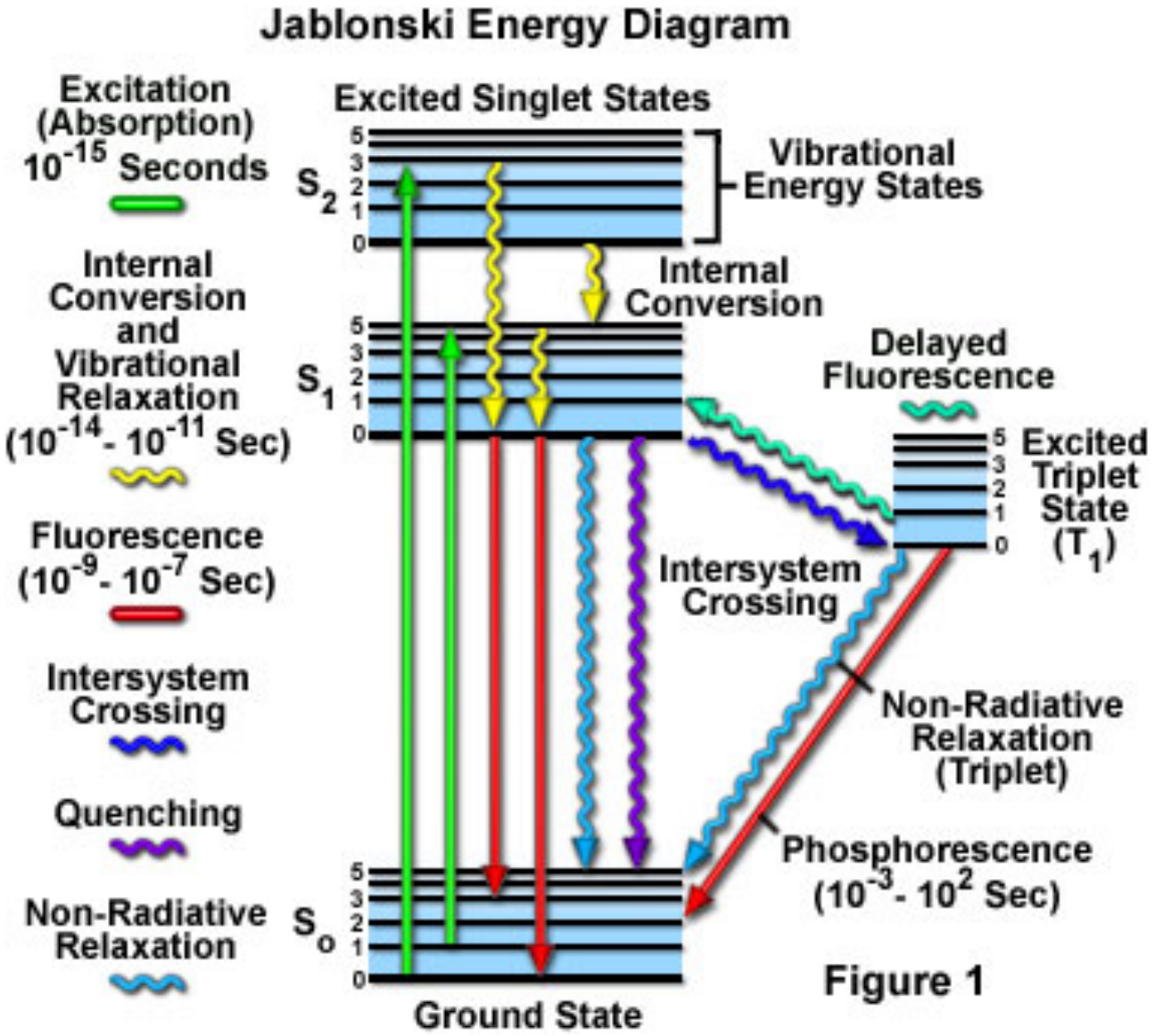
Fluorescence: A fraction of energy absorbed at a shorter wavelength (higher frequency, higher energy) is re-emitted as a photon at a longer wavelength (lower frequency, lower energy).

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.

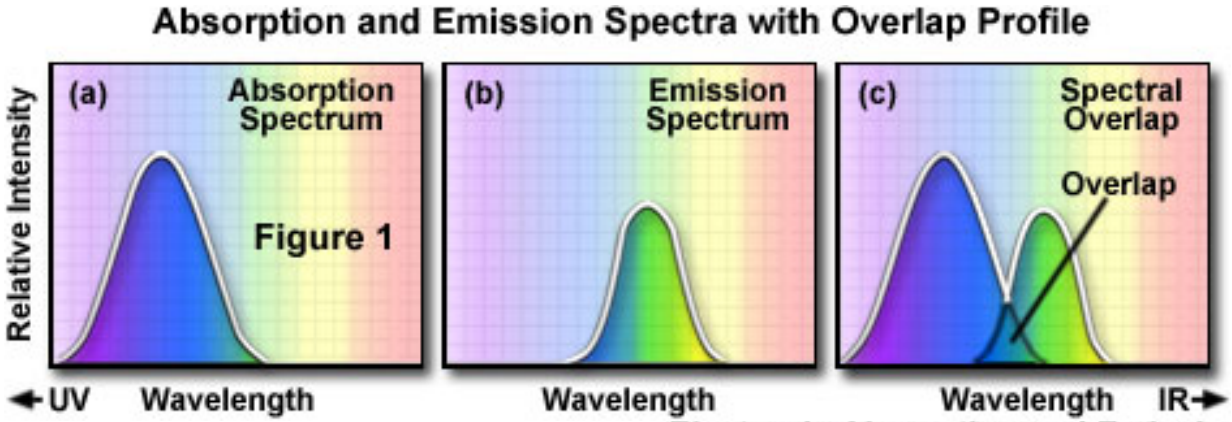
Neat applet: http://www.micro.magnet.fsu.edu/primer/java/jablonski/lightandcolor/

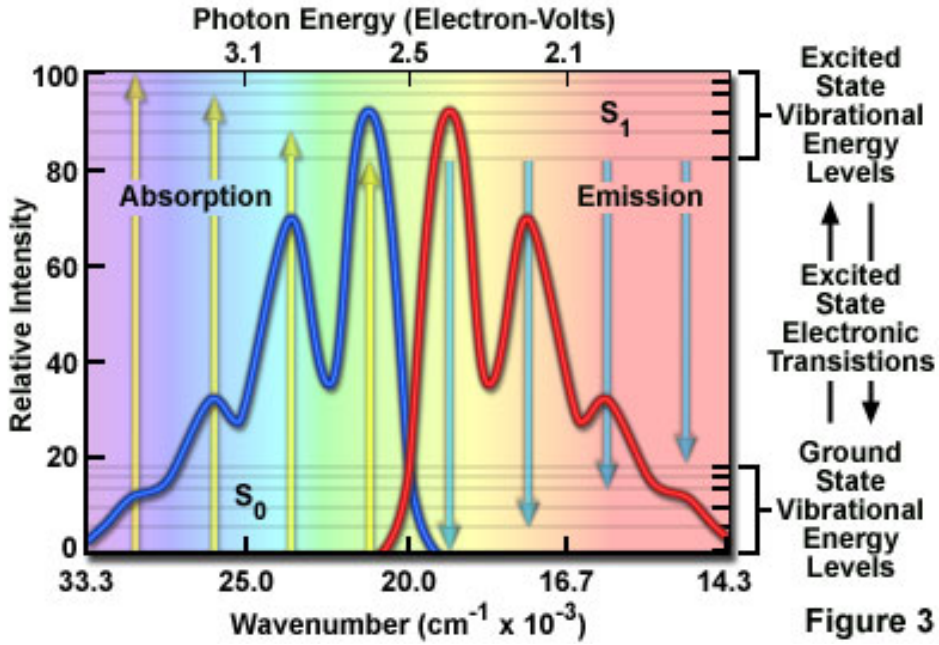


http://www.micro.magnet.fsu.edu/optics/timeline/people/jablonski.html

Summary: **fluorescence emission**

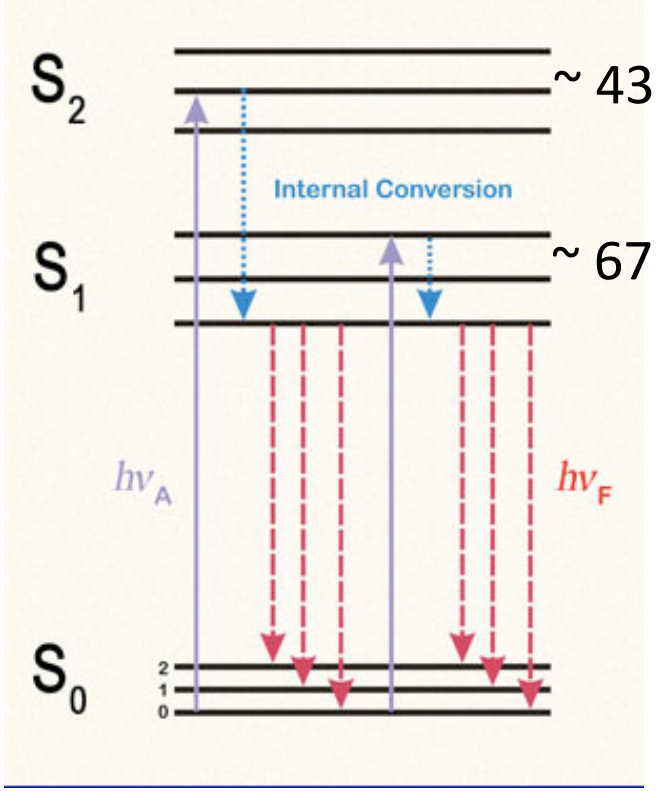
1. always from lowest vibrational state of S_1
2. red shifted – Stokes shift (higher λ, lower E)
3. mirror image of absorption

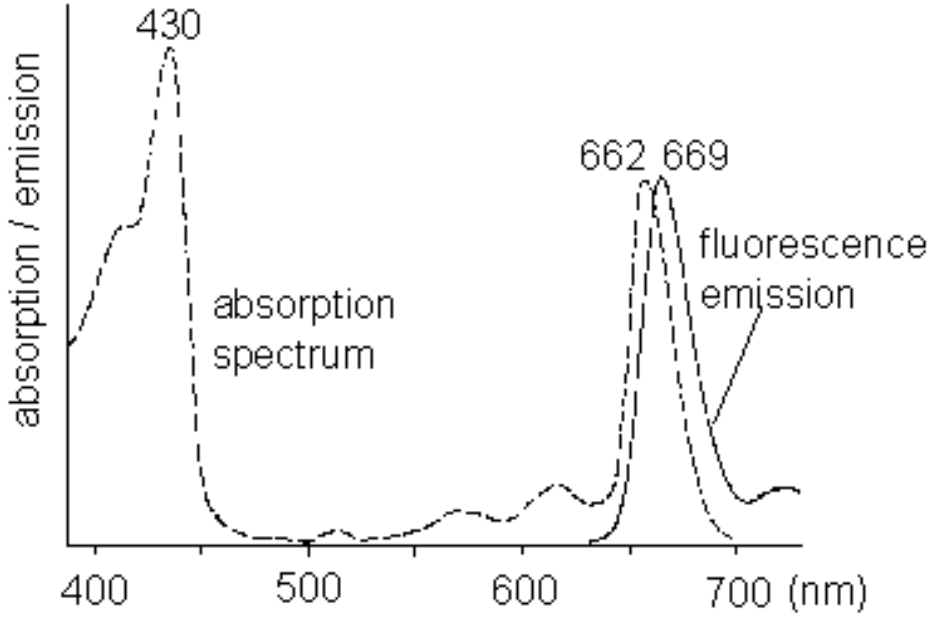




Chlorophyll *a* example

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



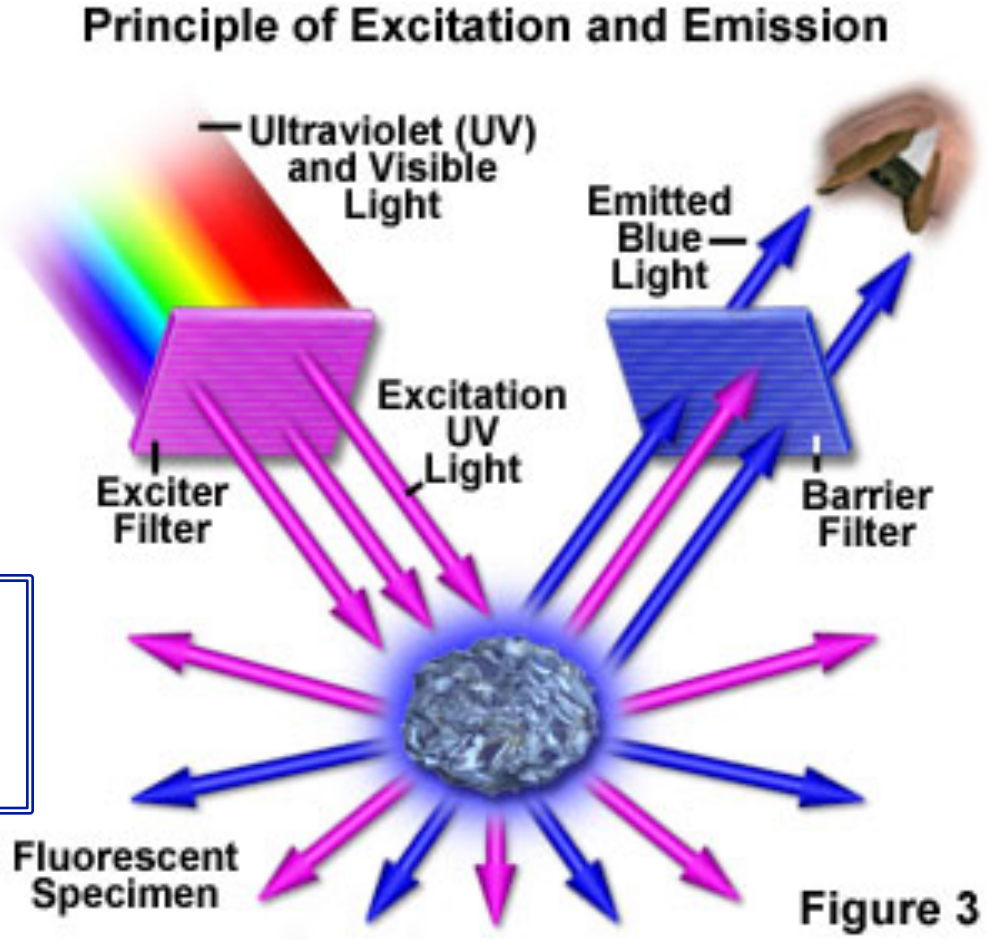


Note: on left, absorption λ maxima are *in vivo*; right, λ maxima are *in vitro*

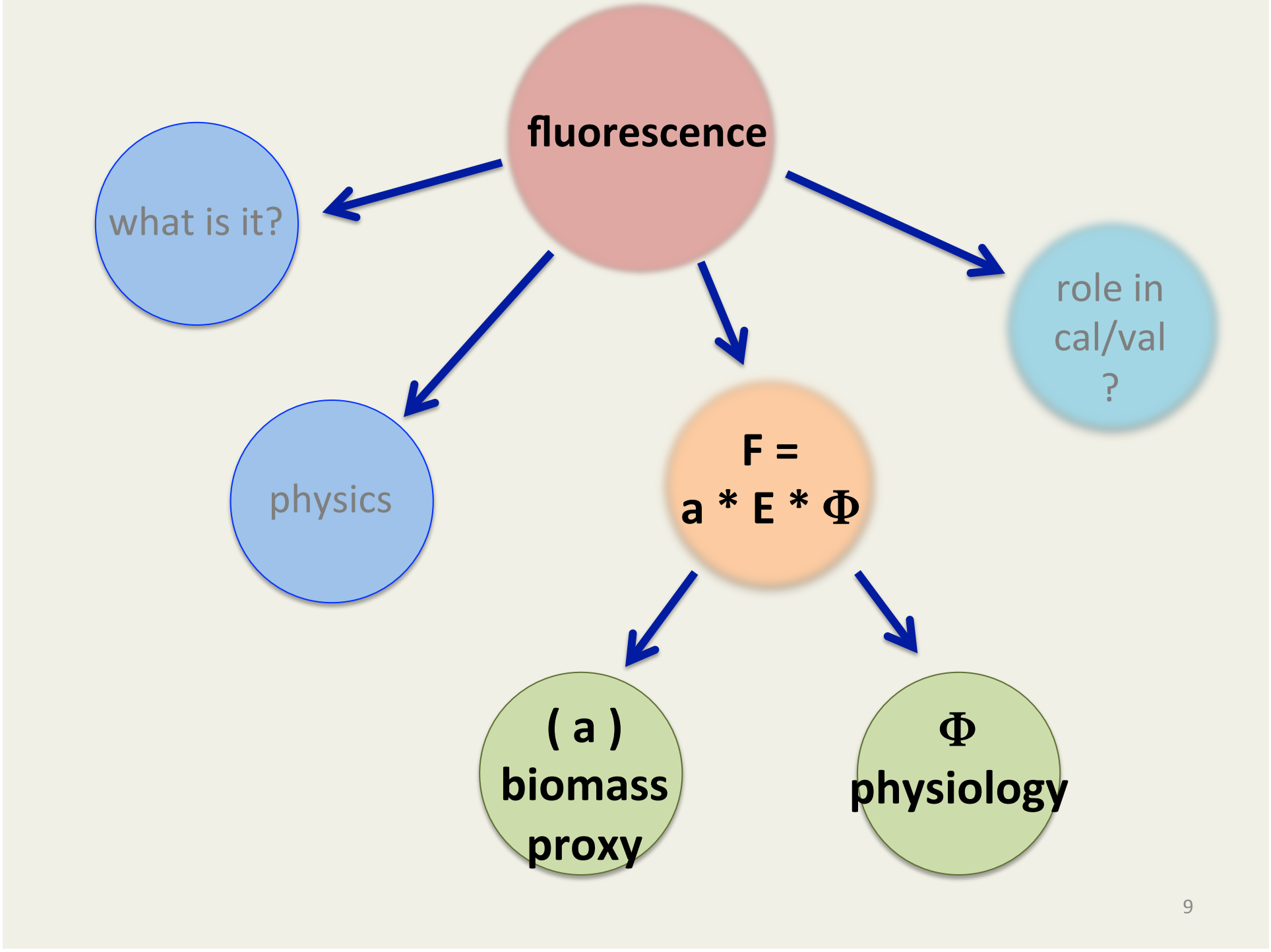
Chlorophyll *a* example *(other ocean compounds: CDOM & PE)*
– single pigment molecule with two primary absorption bands: blue Soret band (S2) and red Q band (S1), with fluorescence emission only from Q band (with Stokes' shift – see preceding slide).

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.

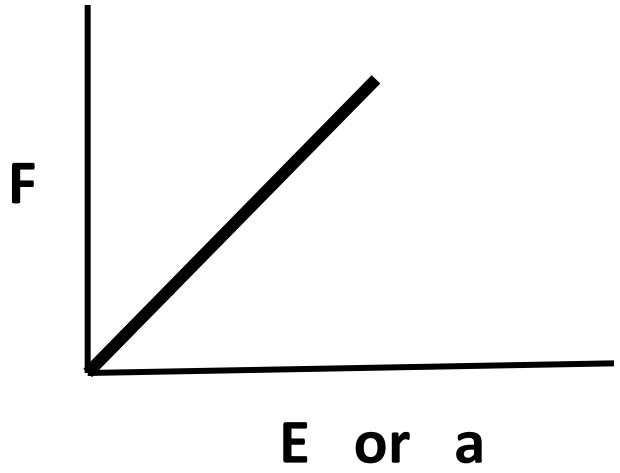


http://micro.magnet.fsu.edu/primer/lightandcolor/fluorointroduction.html


fluorescence
what is it?
physics
F =
a * E * Φ
role in
cal/val
?
(a)
biomass
proxy
Φ
physiology

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

Beer's Law, A = conc *ε* L
Hold ε* L constant, A ~ conc.
Rearrange equation to measure a (or conc.)



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*)

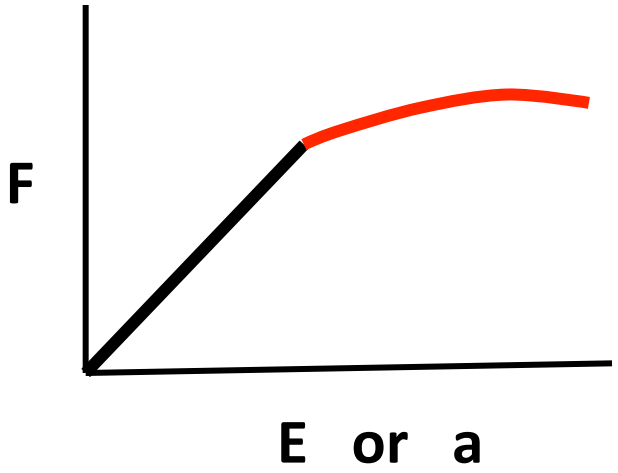
E = energy of excitation light

λ = wavelength

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

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

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



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

E = energy of excitation light

λ = wavelength

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

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

Examine **three terms** in the fluorescence equation:

1. a = absorption coefficient (not concentration); in acetone extract 'a' ~~ chl concentration, but not in live cells.

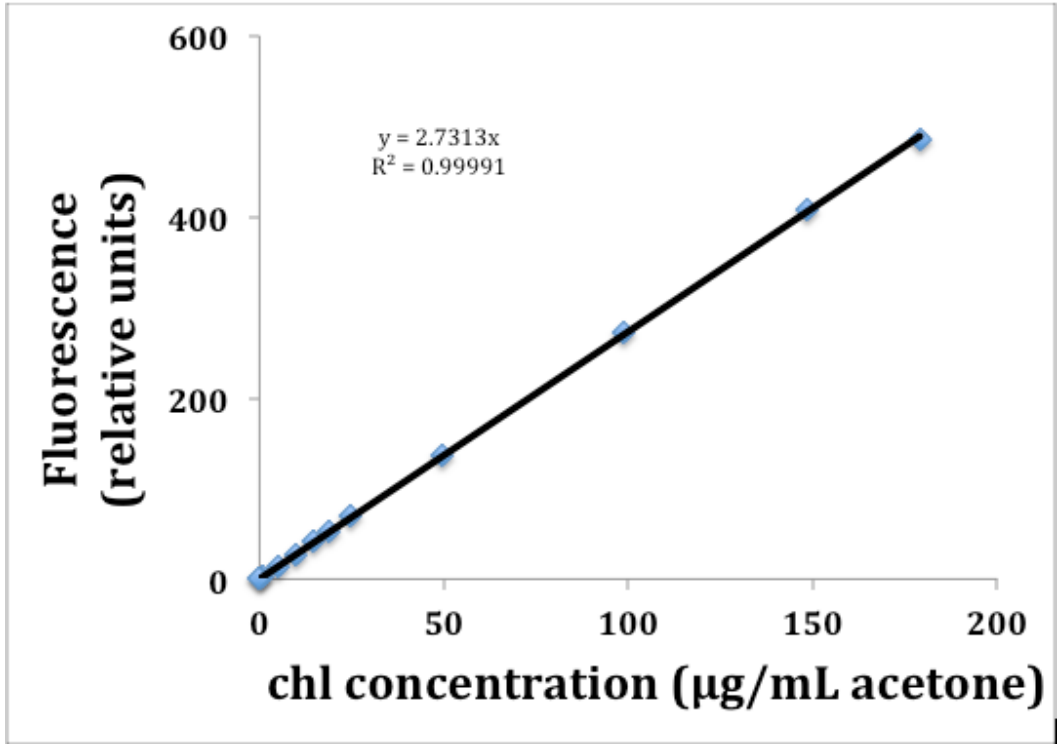
2. λ term for both absorption and E, excitation energy

3. quantum yield of fluorescence, Φ_f , varies:
   - in solution (*in vitro)*, F is a function of solvent and temperature
   - in living cell (*in vivo),* F is a function of physiology

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

1) 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 (MSUT be same temperature). Track daily changes with secondary standard.



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

1) a = absorption coefficient

in vivo (living cells): F is ~ to 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**)

Perry & Porter 1989

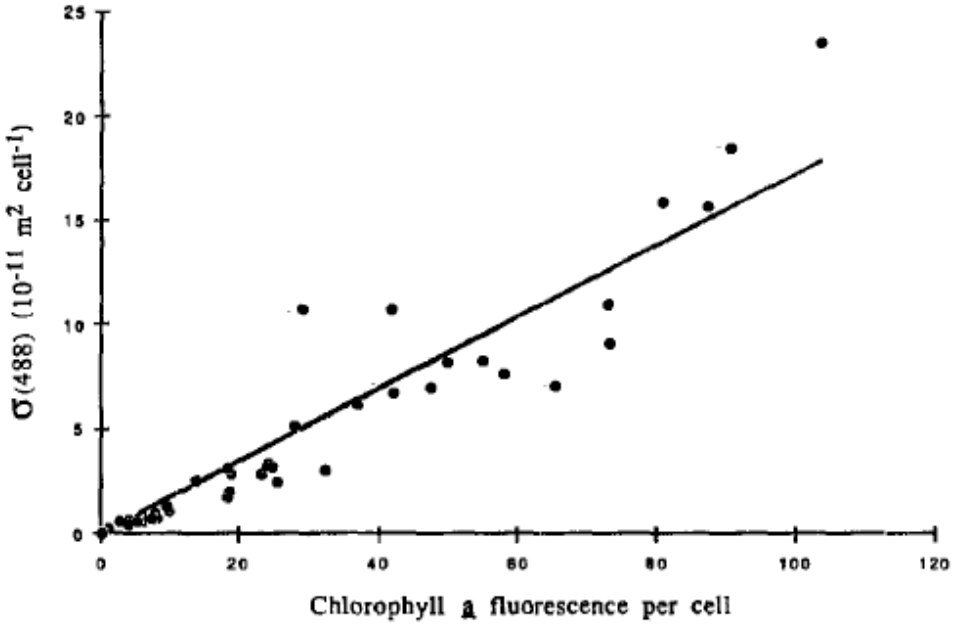


Fig. 5. Regression of geometric mean of Chl *a* fluorescence per cell (converted to linear units) measured in the flow cytometer vs. $\sigma(488)$ for all species and growth irradiances listed in Table 1; $\sigma(488) = 0.173 \times 10^{-11} \times$ Chl *a* fluorescence per cell; $r^2 = 0.93$.

At lower excitation energy (typical fluorometers), fluorescence /chlorophyll changes due to pigment packaging (cell size ~ pathlength) and photo-adaption (more chlorophyll/cell at low growth irradiances).

***a = absorption/chlorophyll.** What's the effect of ***a** on fluorescence? (lower *a = more F/chl or less F/chl?)

Pigment Packaging impact on absorption

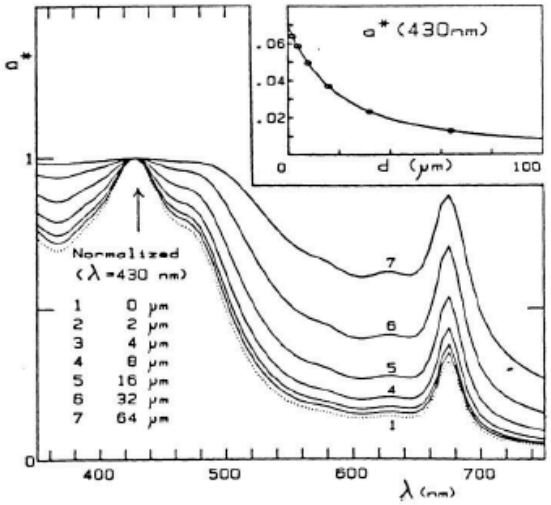


Fig. 2. Change in spectral absorption values with variable cell size (diameter, d, in μm) whereas the cell material forming the cells remains unchanged. The spectral absorption values of this material, somewhat arbitrarily adopted, are shown as the dotted curve. All curves are normalized, at $\lambda = 430$ nm, to evidence the progressive deformation. The variations with size of the specific absolute value at 430 nm (m^2 mg^{-1} Chl a) are shown in inset, under the same assumption of a constant absorption of the cell material ($a_{cm} = 2 \times 10^3$ m^{-1} at 430 nm) and with the additional assumption of a constant intracellular pigment concentration ($c_i = 2.86 \times 10^6$ mg Chl a m^{-3}).

Morel and Bricaud 1981 DSR

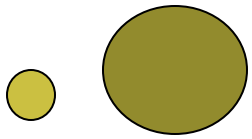
(1) vary size, maintain constant intracellular pigment concentration

or

(2) maintain size, vary intracellular pigment concentration

Collin's lecture Tuesday

F/Chl was a function of cell size in San Francisco Bay,
due to greater pigment packaging in larger cells.



Sizes were separated w/ screens

- netplankton (>22 µm)
- nanoplankton (5–22 µm)
- ultraplankton (<5 µm)

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

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

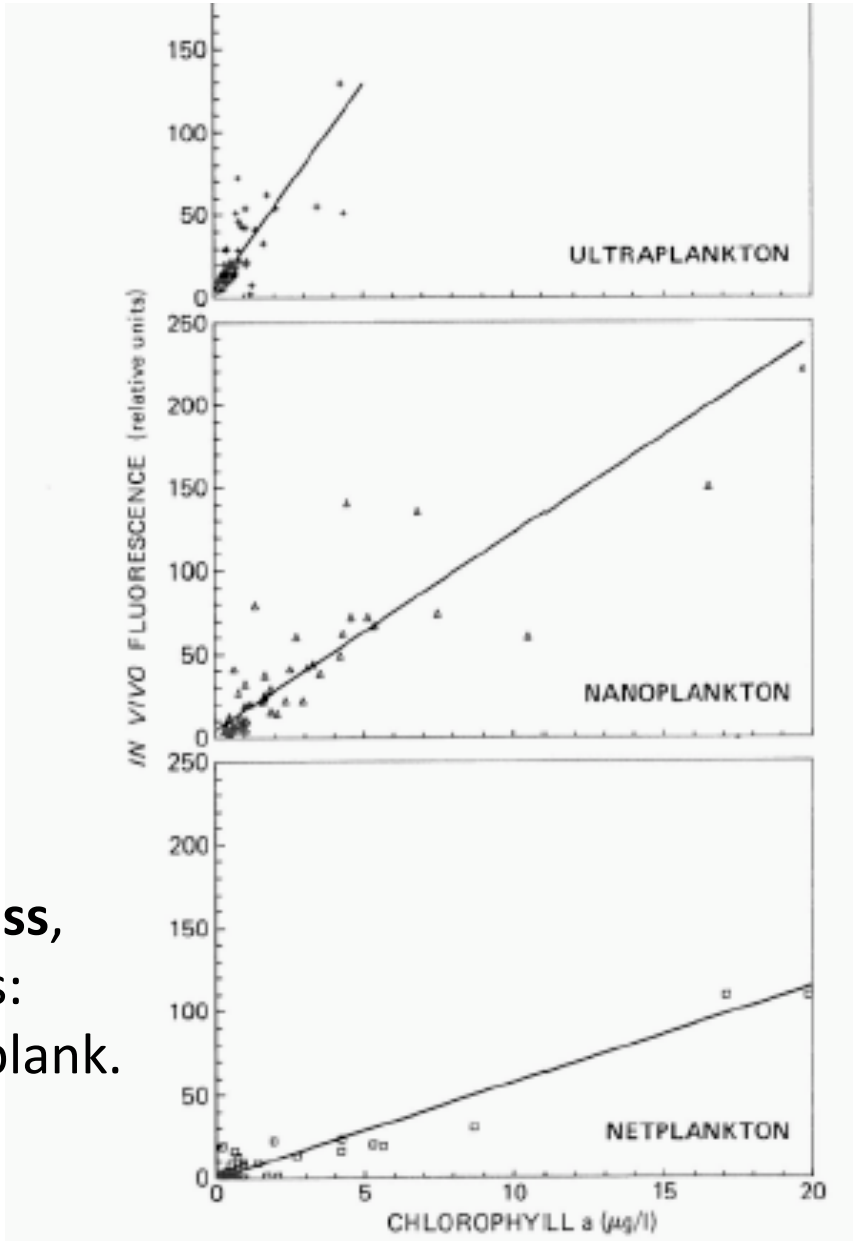
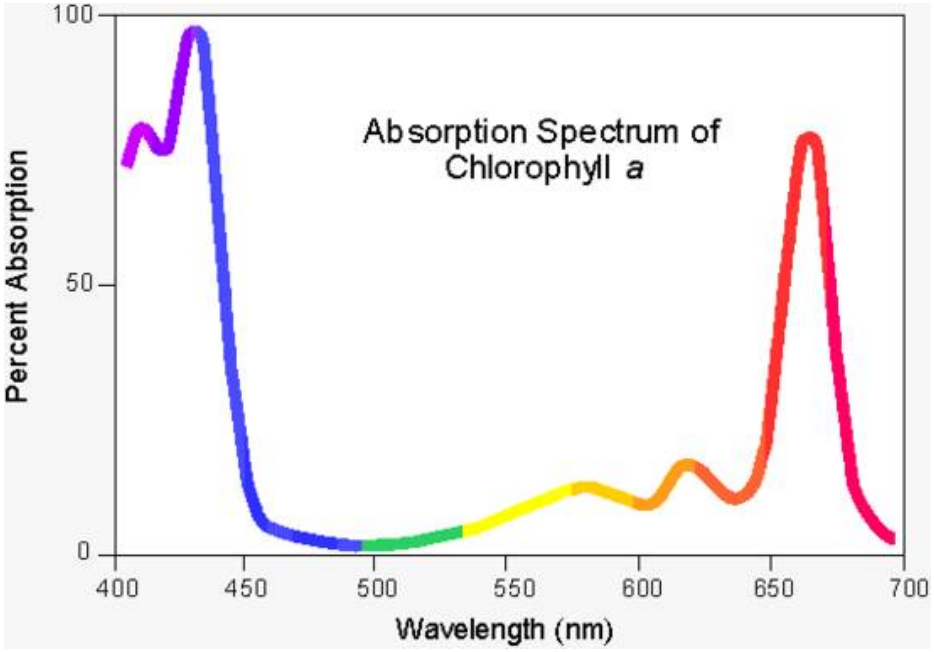


Fig. 2. *In vivo* fluorescence plotted against chlorophyll *a* for each size class. Data are pooled sites in San Francisco Bay over a year long period. Regression lines are shown for each size

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

2) λ− dependence for both absorption and E, excitation energy:

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



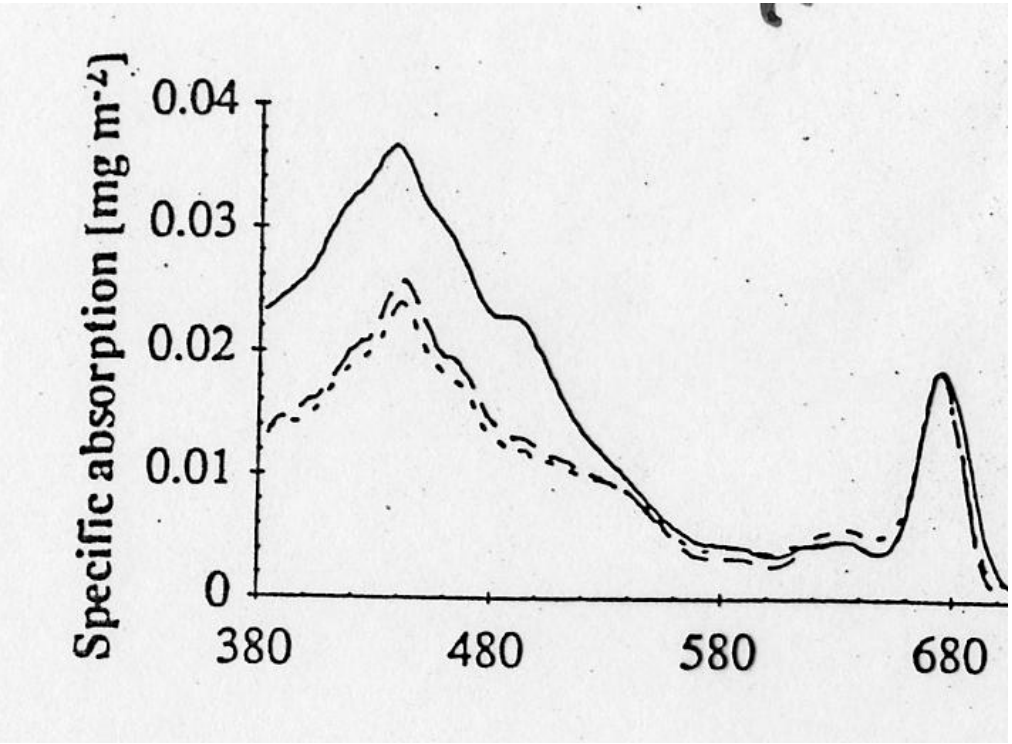
What is the excitation spectrum of a typical *in situ* fluorometer?

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

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

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. Here, fluorescence is proportional to a_ps.



solid line = a_phyt

dotted line = a_ps

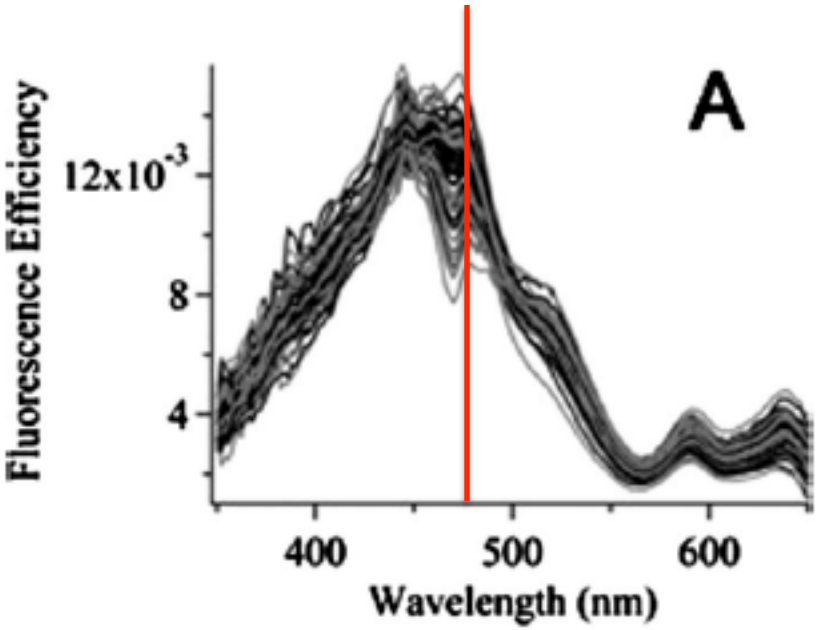
difference = a_pp

Data from Culver & Perry

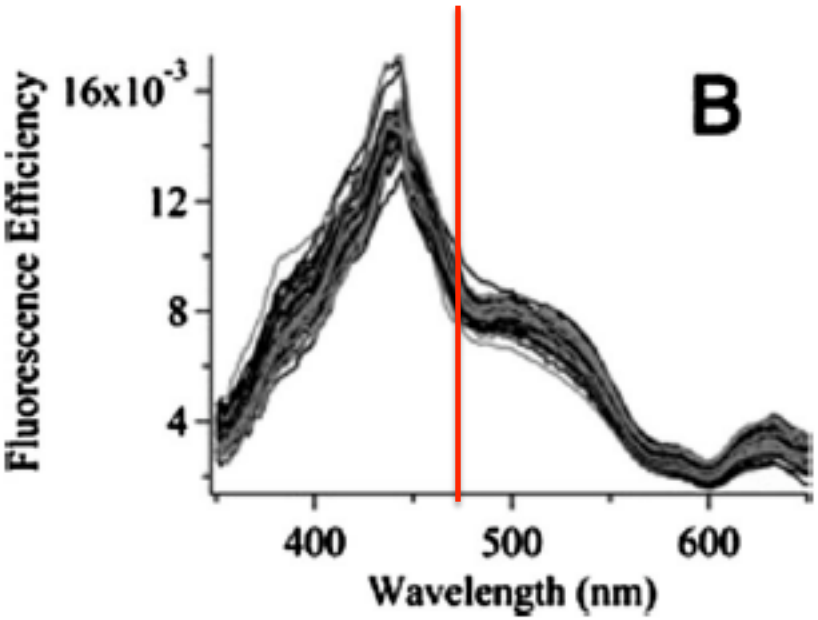
$$F = a(\lambda) * E(\lambda) * \Phi_f$$

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))

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

3) quantum yield of fluorescence varies (Φ_f = moles photon fluoresced/ moles photon absorbed):

– in solution (*in vitro)*, F is a function of environment (solvent, pH, **temperature**, ionic strength, etc.); $\Phi_f \sim 33\%$ for Chl *a* acetone extract.

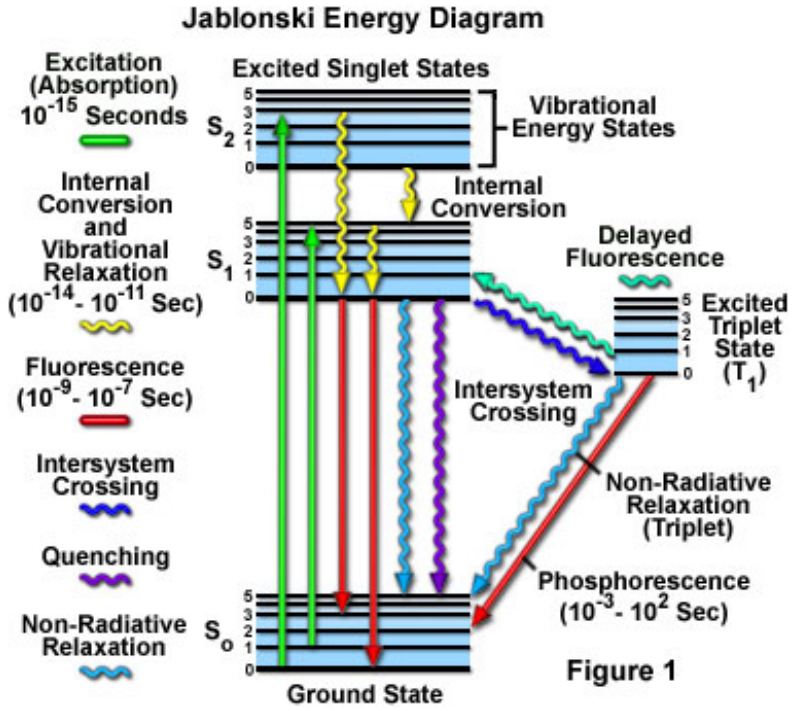
$$\Phi_f = \frac{K_F}{K_F + K_{IC} + K_{IS}}$$

Where

K_F = rate of fluorescence,

K_{IC} = rate of internal conversion (radiationless decay),

K_{IS} = rate of intersystem crossing

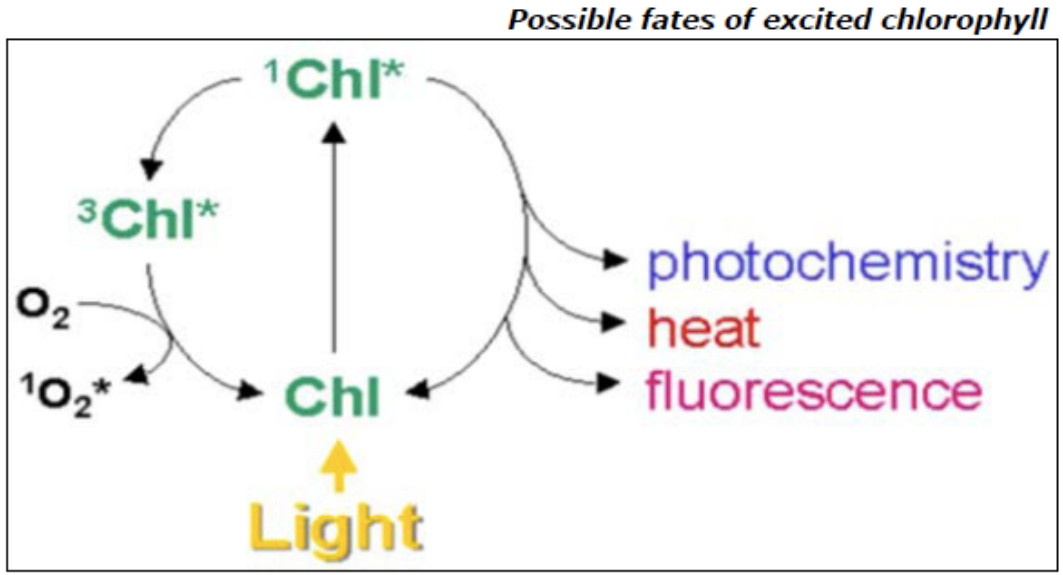


http://biologie.univ-mrs.fr/upload/p222/1_fluorescence.pdf

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

3) quantum yield of fluorescence varies (Φ_f = moles photon fluoresced/ moles photon absorbed):

– 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) and nutrient limitation (so it will vary spatially).; Typically in living cell Φ_f is ~ 0.5% – 2%



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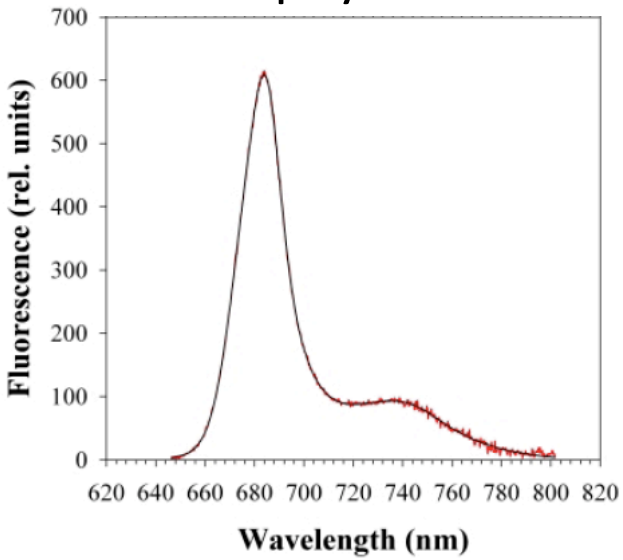
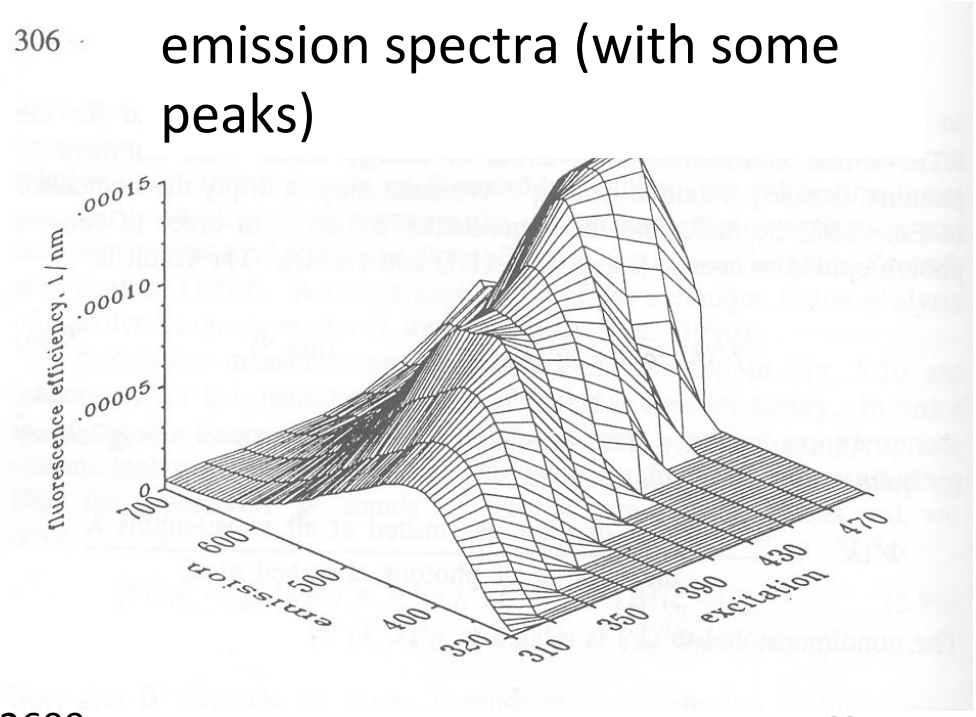
Chlorophyll *a* - red



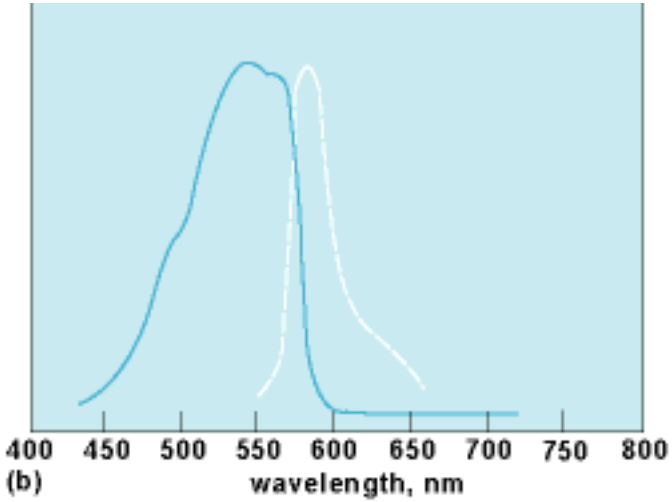
What fluoresces in the ocean?

both Chl *a* and CDOM exhibit natural or solar stimulated fluorescence;
PE – lidar stimulation (solar – not sure).

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

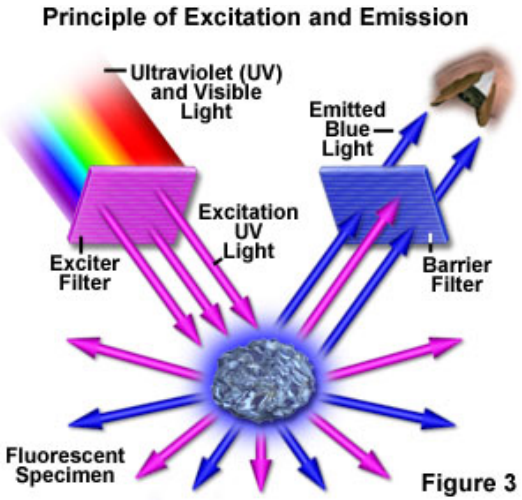


Phycoerythrin – orange



http://accessscience.com/content/Phycobilin/512600

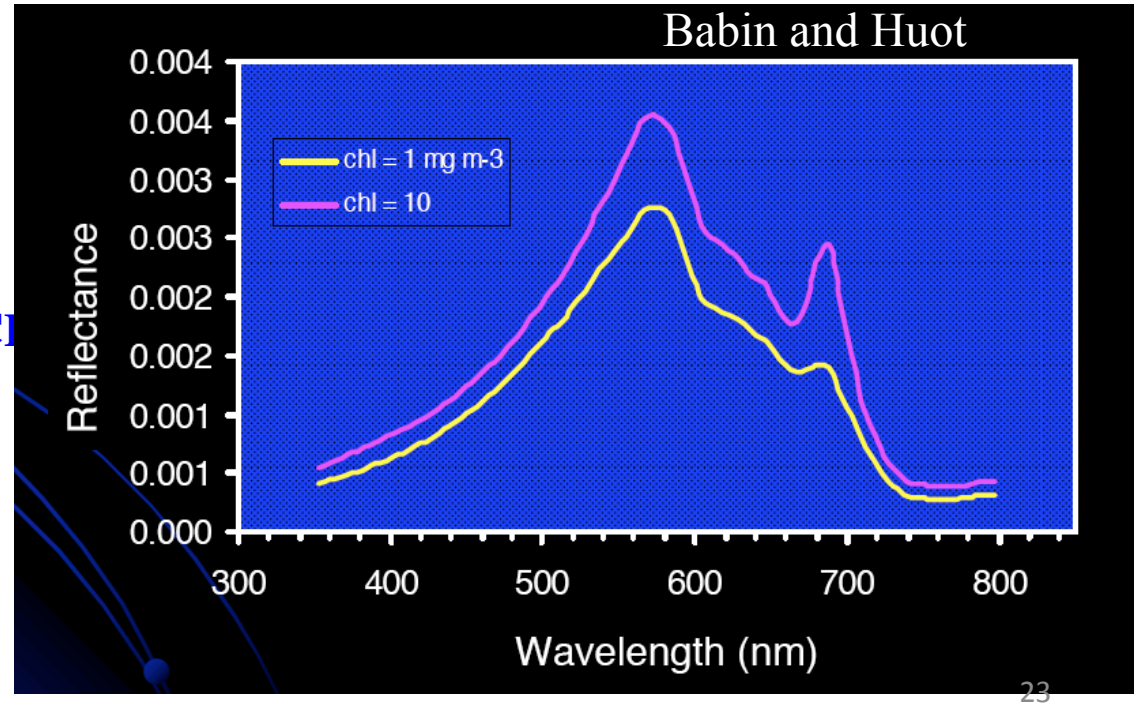
Two types of fluorescence measurements

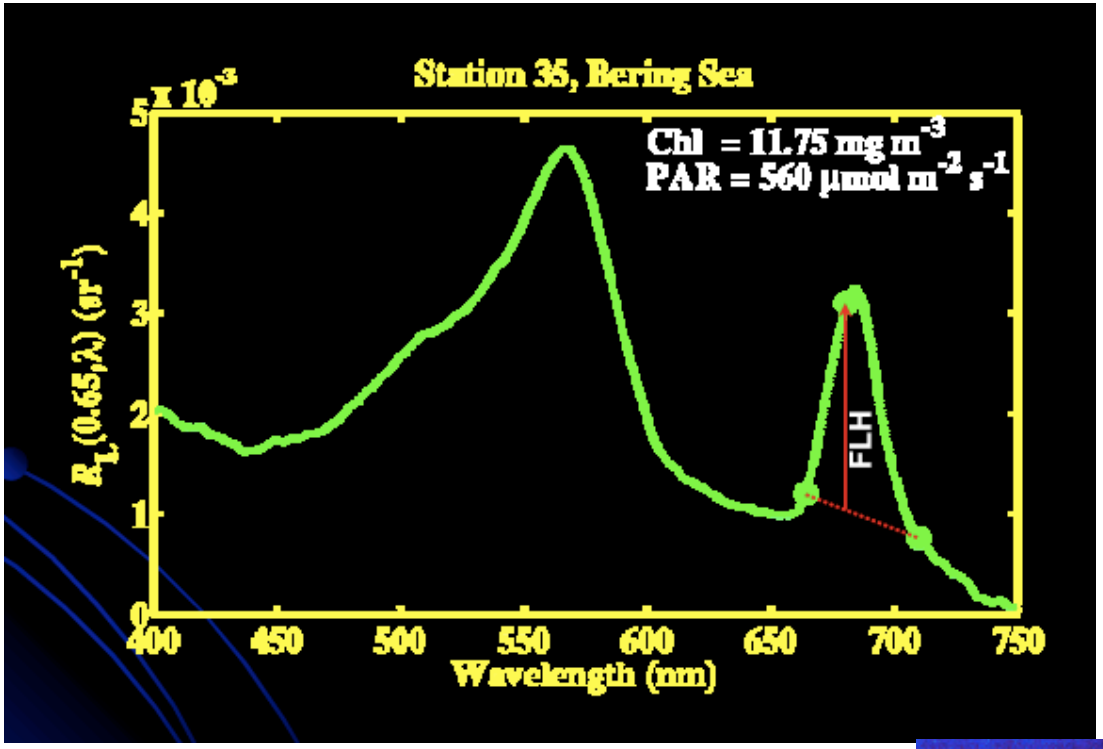


1) **active – artificial light source for E(λ)**
   - static: use for profiles of chlorophyll fluorescence; moorings; mobile platforms – **TODAY'S LAB**
   - time resolved (true τ_F is ~ femos and picos for chemistry, like hole burning in CDOM; pump & probe and variable F ~ μs – more later, in productivity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

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

YOU WILL SEE SOLAR FLUORESCENCE IN FIELD RADIOMETRIC DATA AND HYDROLIGHT



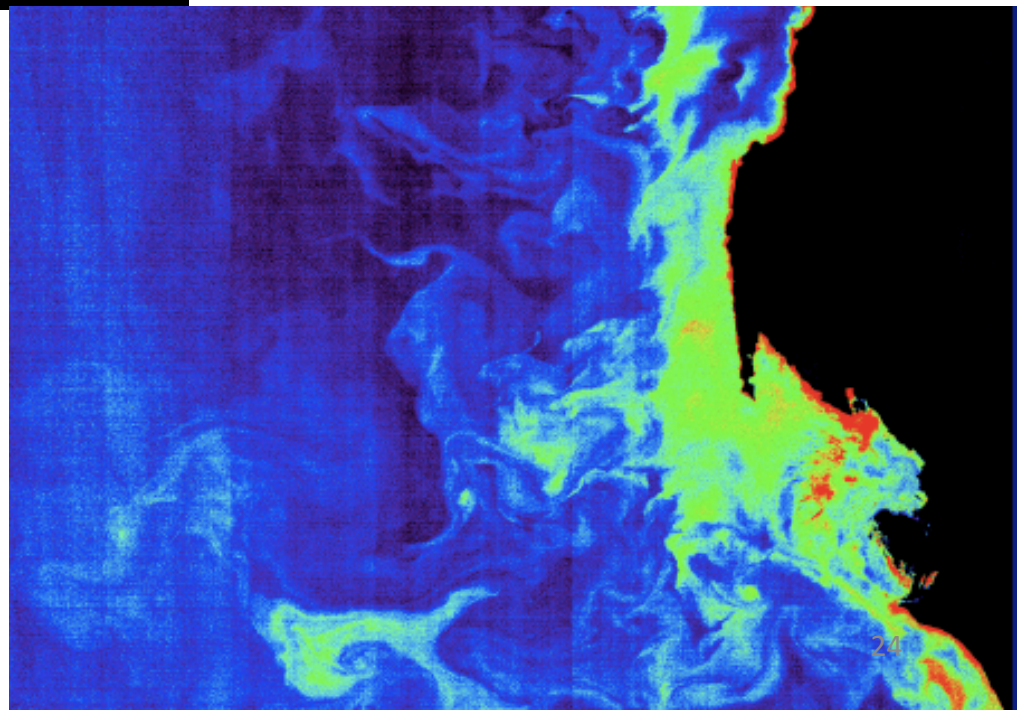


Not going to say much now about solar-stimulated fluorescence. Slides from Babin and Huot; they caution its use in turbid waters (bbp, not all F)

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



Won't say much about fluorescence induction curve: rapid rise and slow decline. Input for productivity models.

Fast rise (< second); #1 – low light; #2 – high light adapted; #3 DCMU

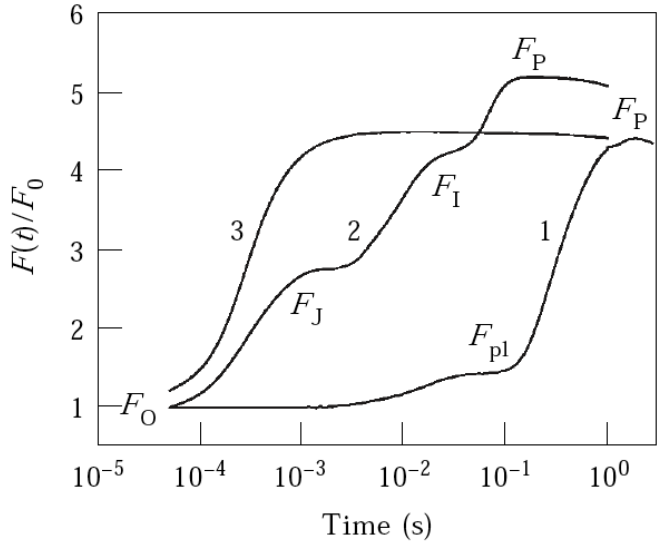
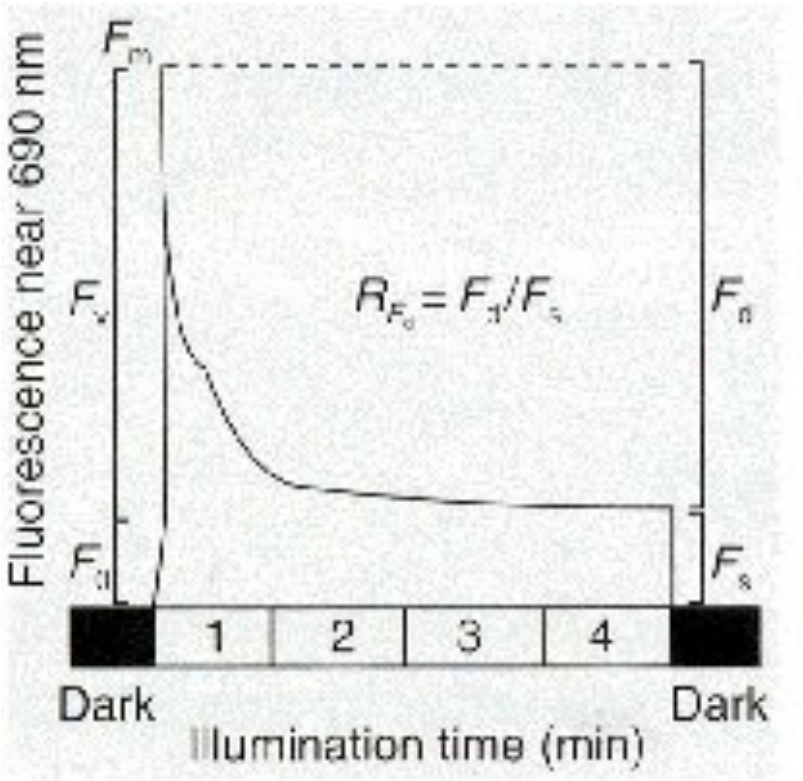


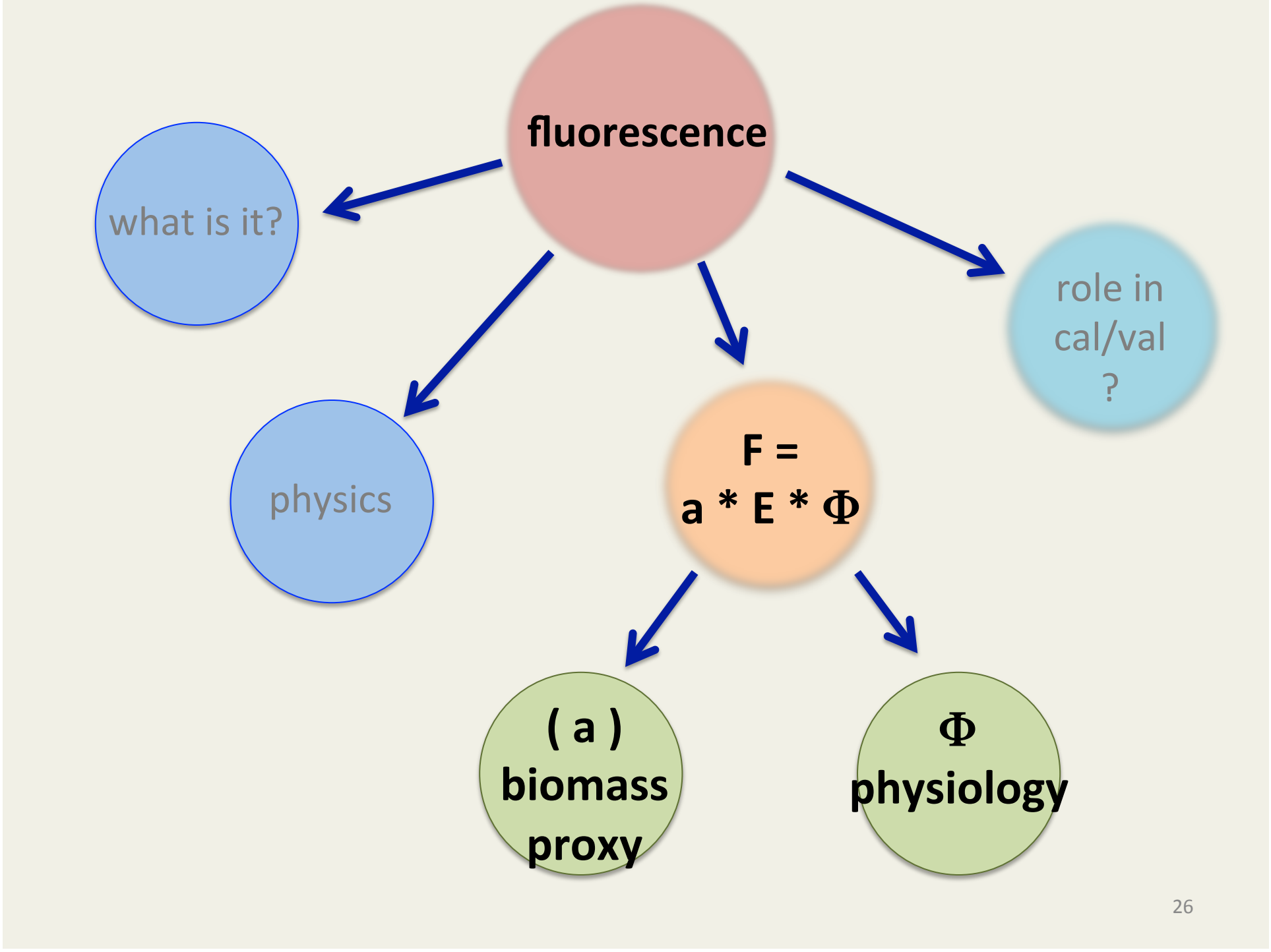
FIG. 1. Fast Chl *a* fluorescence induction curves (fluorescence as a function of time—from 50 μs to 1 s) measured on dark adapted *Pisum sativum* leaves illuminated with 12 Wm^{-2} (curve 1), 600 Wm^{-2} (curve 2), and 600 Wm^{-2} in the presence of DCMU (curve 3). Wavelength of illumination, 650 nm. For definition of symbols, see Glossary.

photoreduction of QA to QA^- and connectivity among Reaction Centers

Slow rise (< minute)

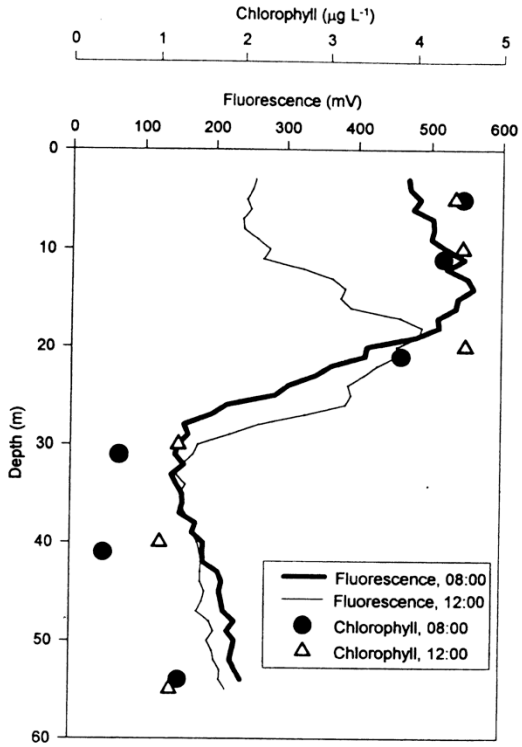


photochemical, thermal and other quenching


fluorescence
what is it?
physics
F =
a * E * Φ
role in
cal/val
?
(a)
biomass
proxy
Φ
physiology


KNORR

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



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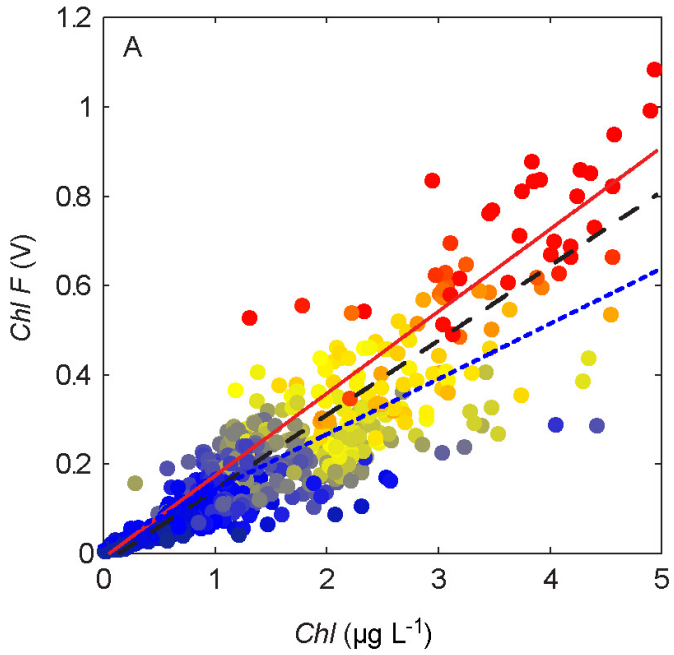
From Falkowski and Raven 1997
Chlorophyll fluorescence and extracted concentration of chlorophyll early AM vs. noon.

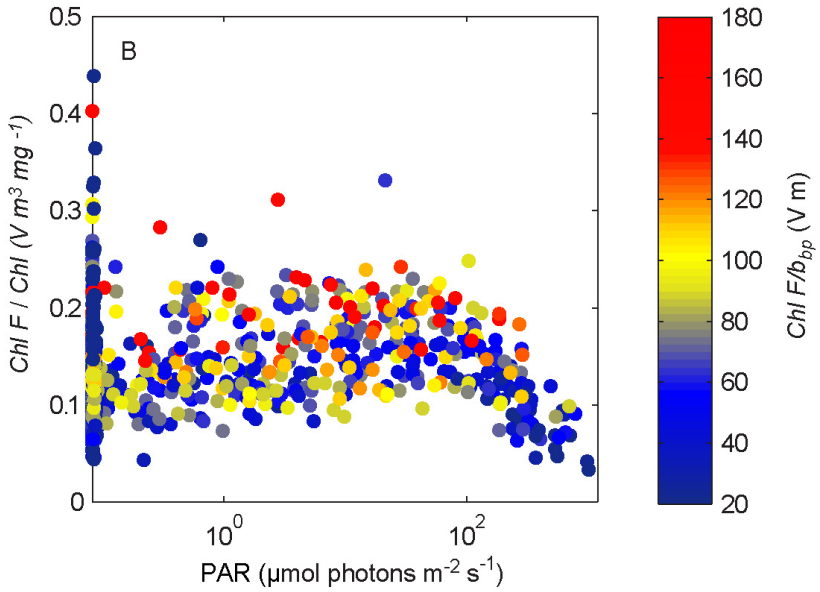
This profile shows the effect of day-time fluorescence quenching on mid-day fluorescence profile. Symbols are extracted chlorophyll from bottle samples.

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 quenching a challenge

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

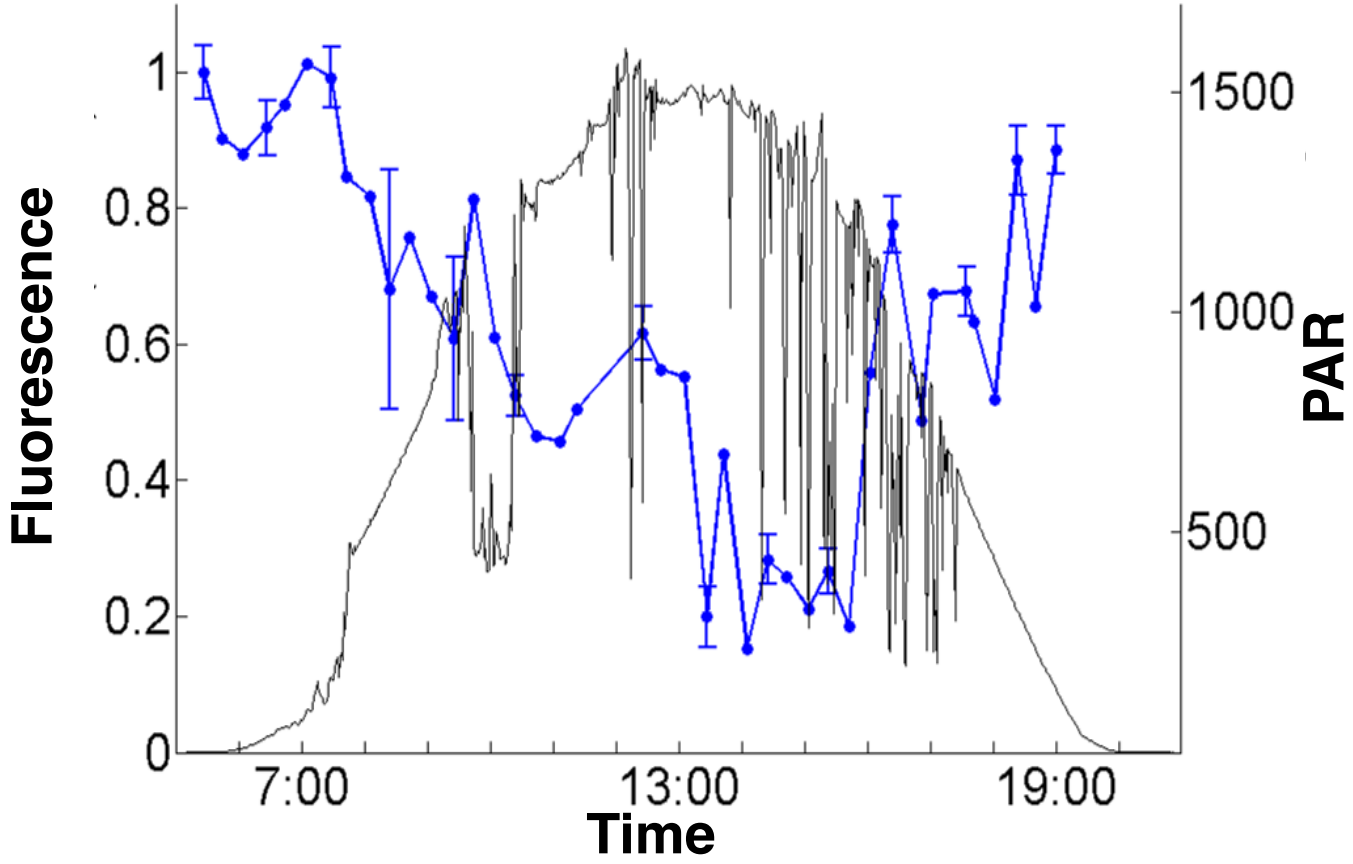
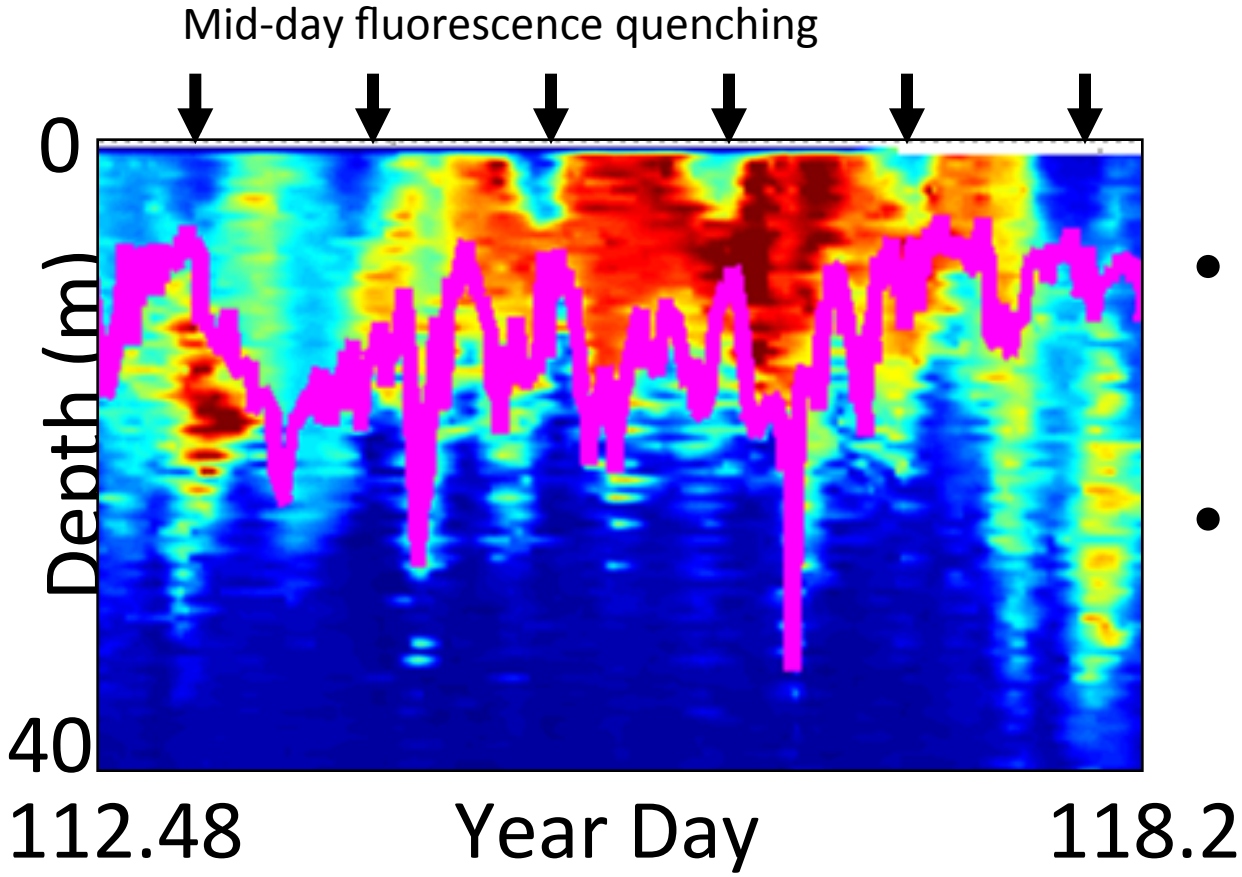


Figure 2: Damariscotta River *in situ* chlorophyll a fluorescence and PAR (µmol photons/s/m^2) vs. time.

Drzewianowski 2008 – MS thesis

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



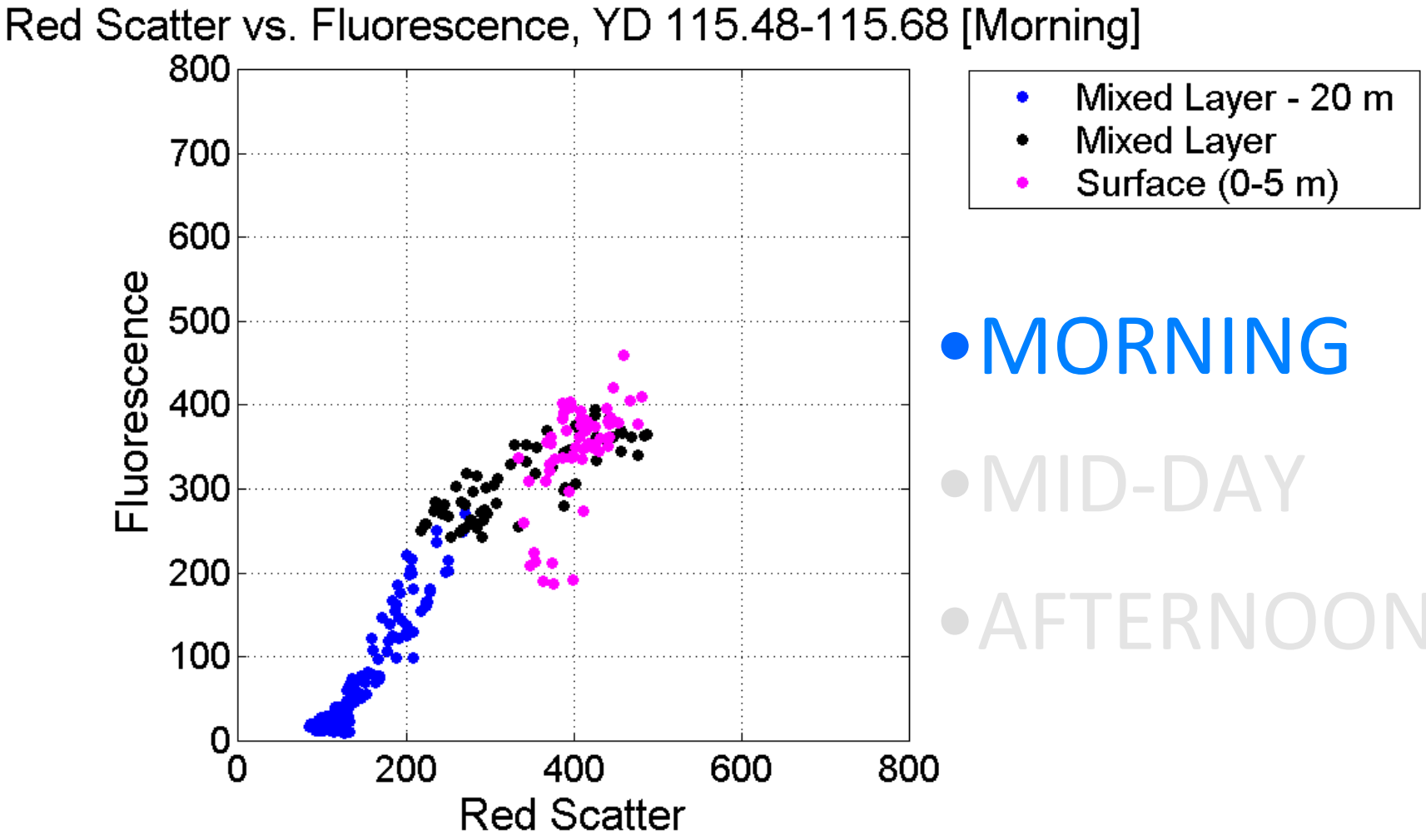
- Quenching observed to 11m
- Fluoresence quenched up to 80% at surface

-- Mixed Layer Depth (MLD)

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

Sackmann 2007 PhD.

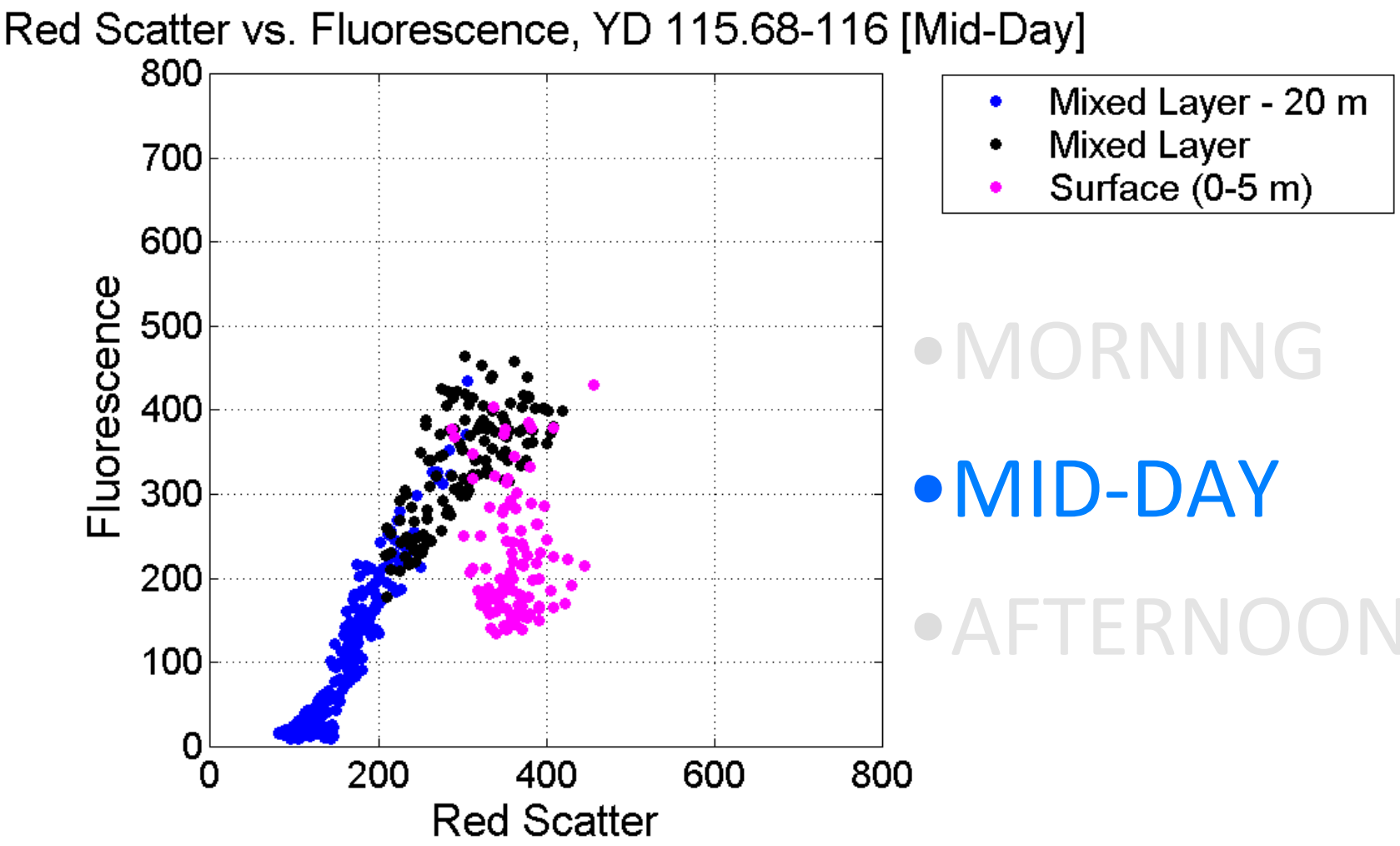
Mid-day fluorescence quenching



- MORNING
- MID-DAY
- AFTERNOON

Sackmann et al., unpub.

Mid-day fluorescence quenching



- MORNING
- **MID-DAY**
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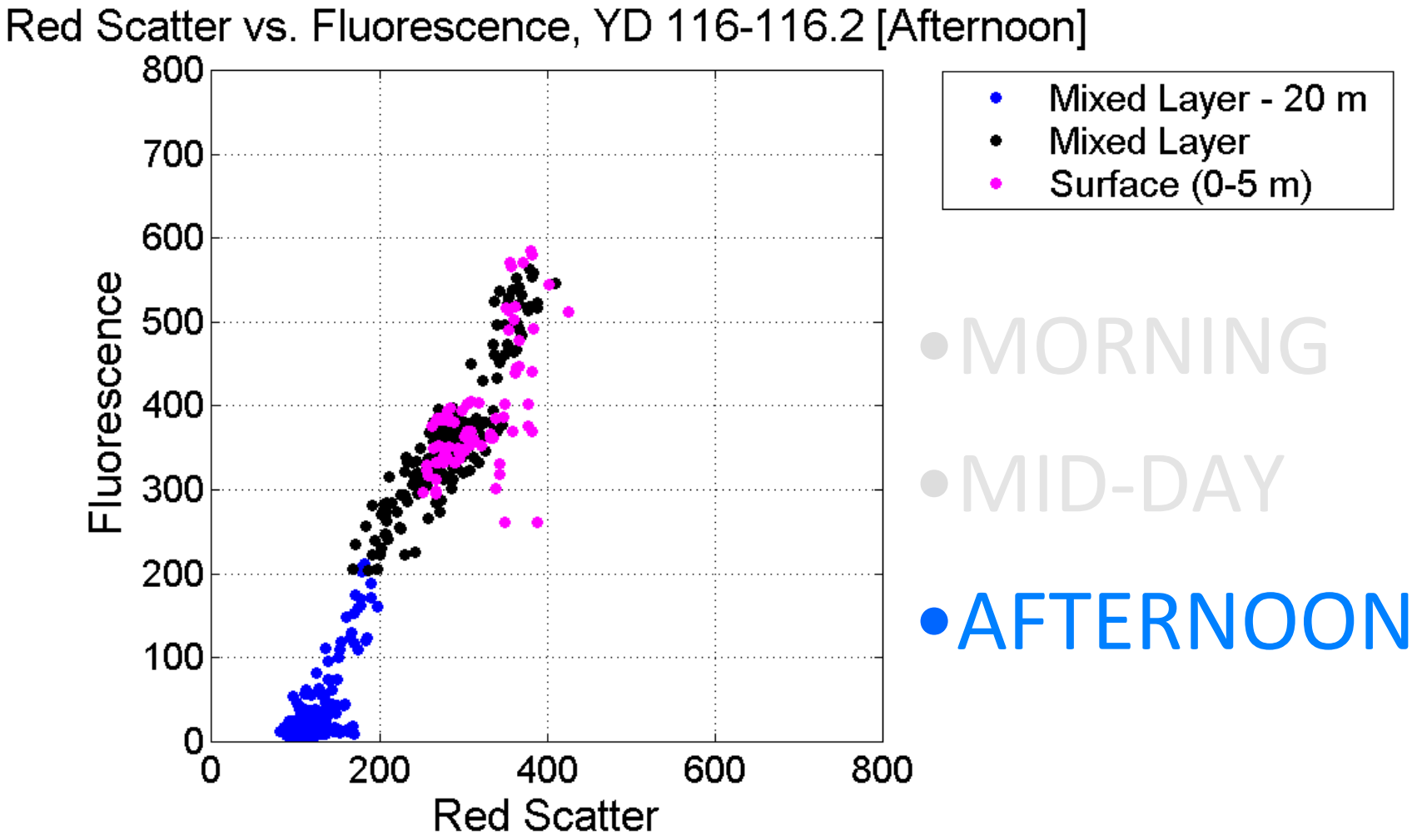
Sackmann et al., unpub.

Mid-day fluorescence quenching



- MORNING
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Boss et al. (2008) Limnol. Oceanogr.

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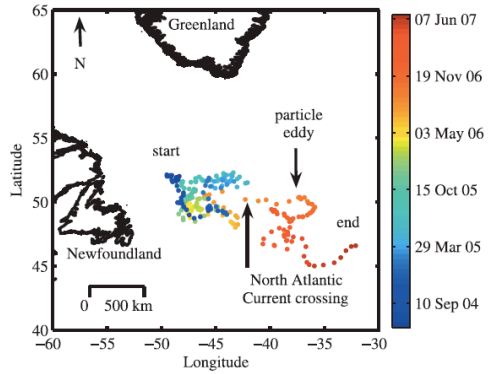

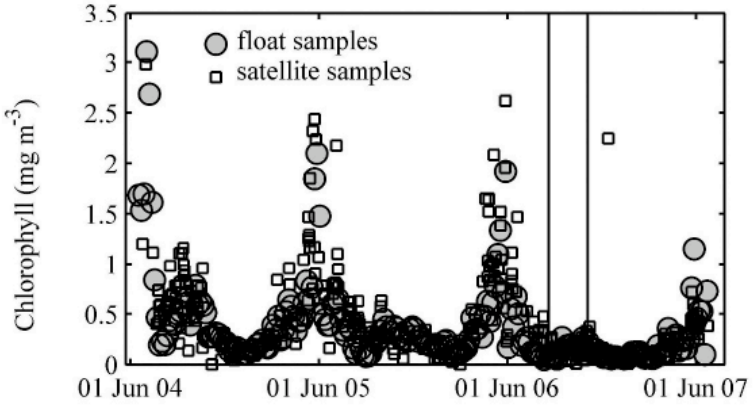

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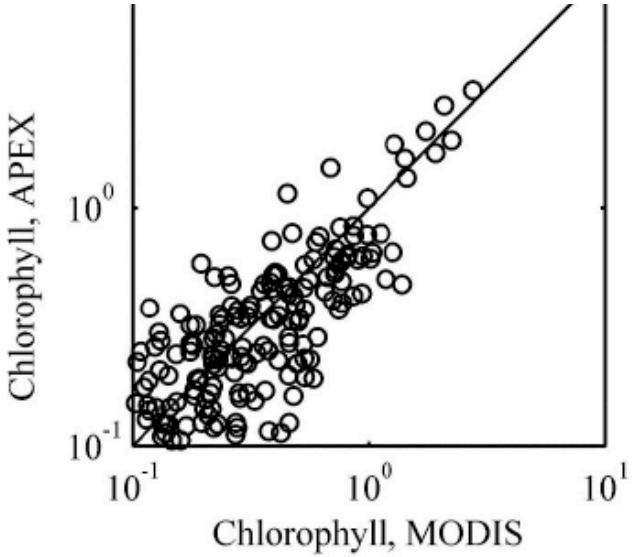
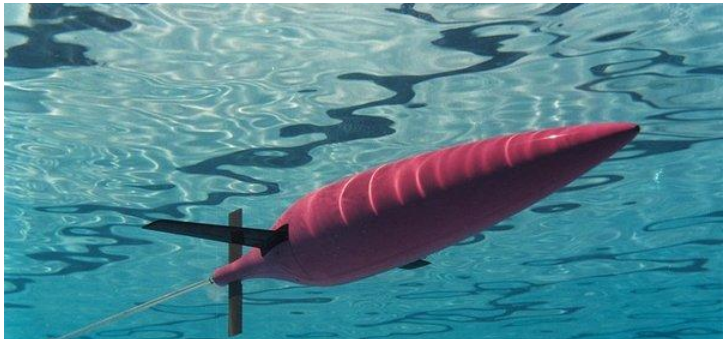
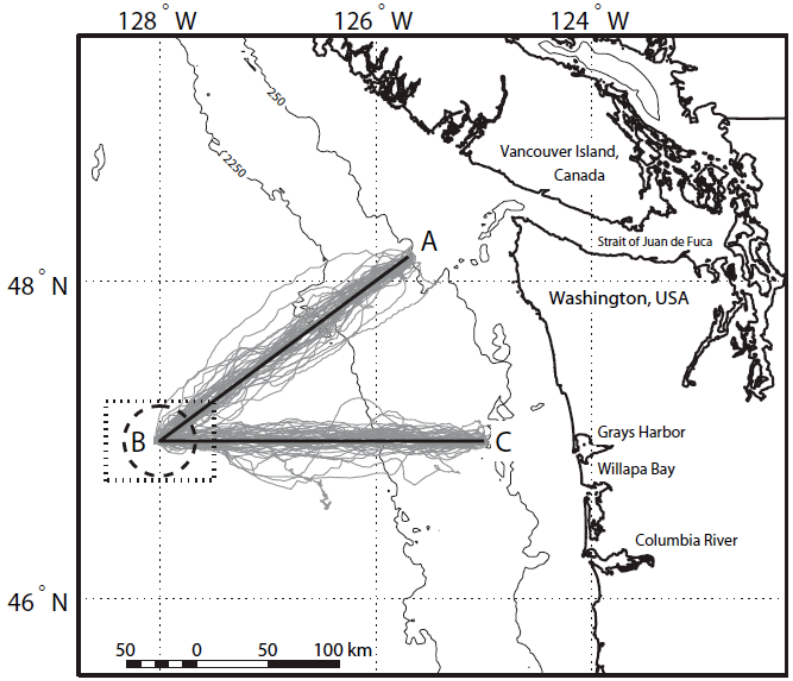
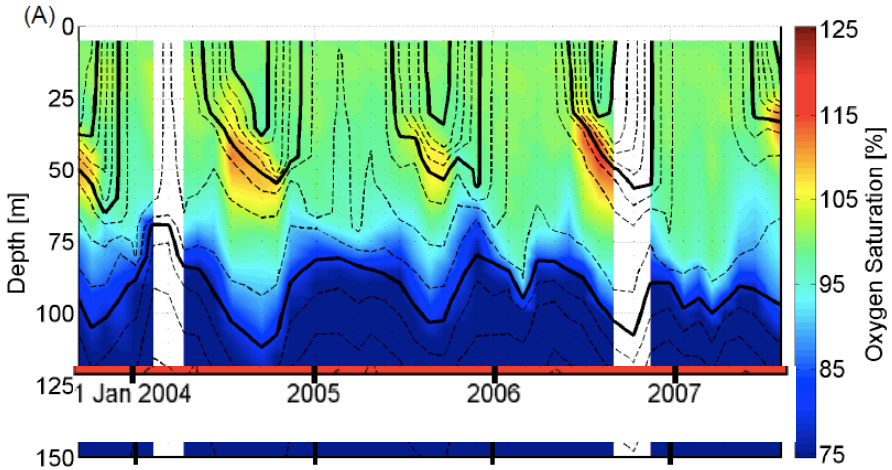
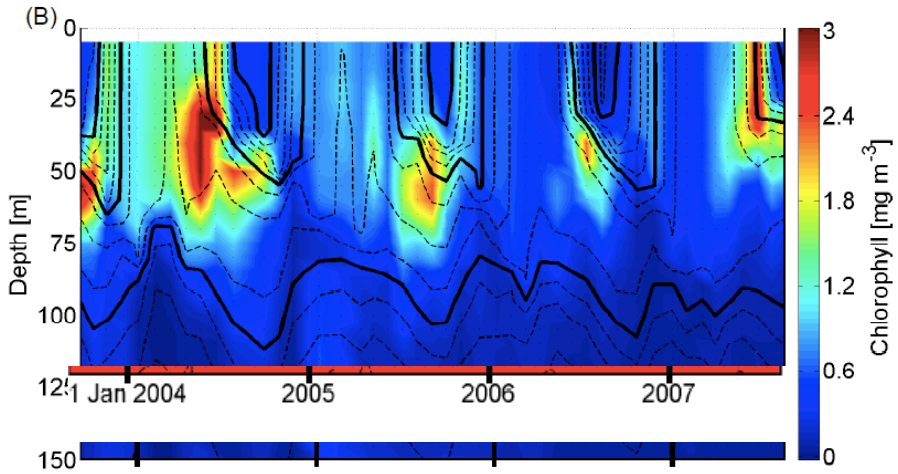






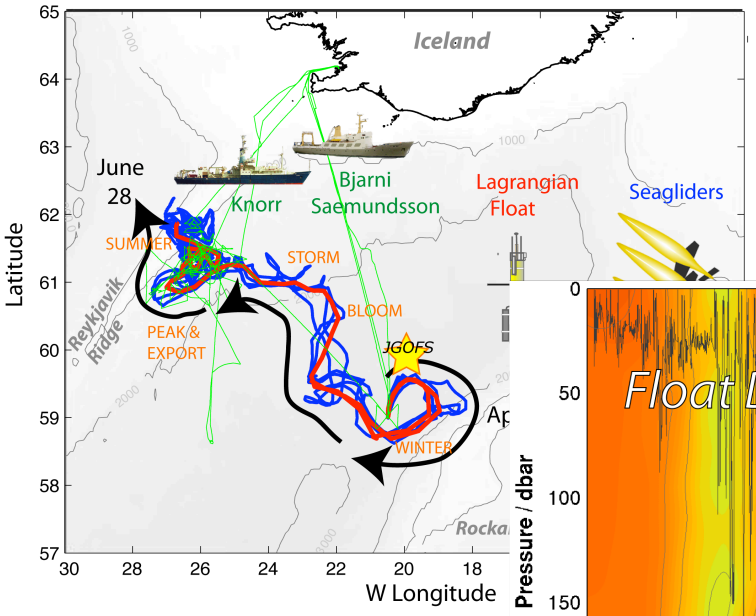
But … F reveals important patterns: interannual variability in evolution of subsurface chlorophyll maximum layer. Seaglider oxygen and chlorophyll fluorescence measurements to 150 m for four years off Washington coast .



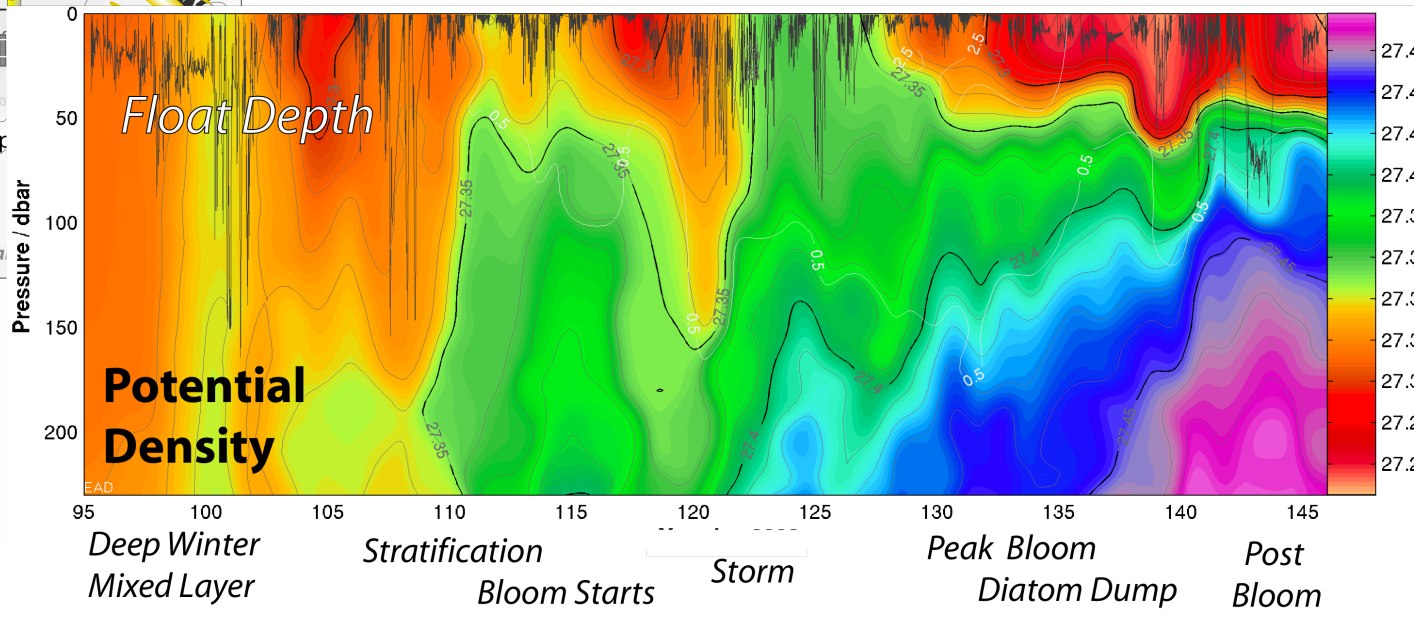


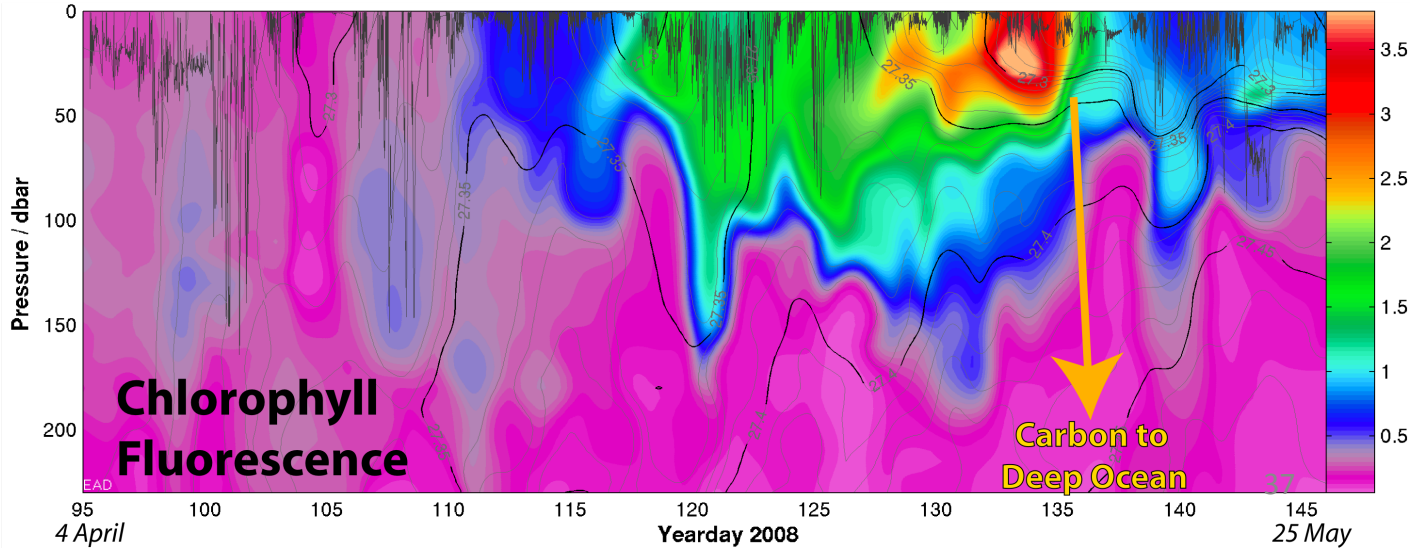
Perry et al. (2008) Limnol. Oceanogr.

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

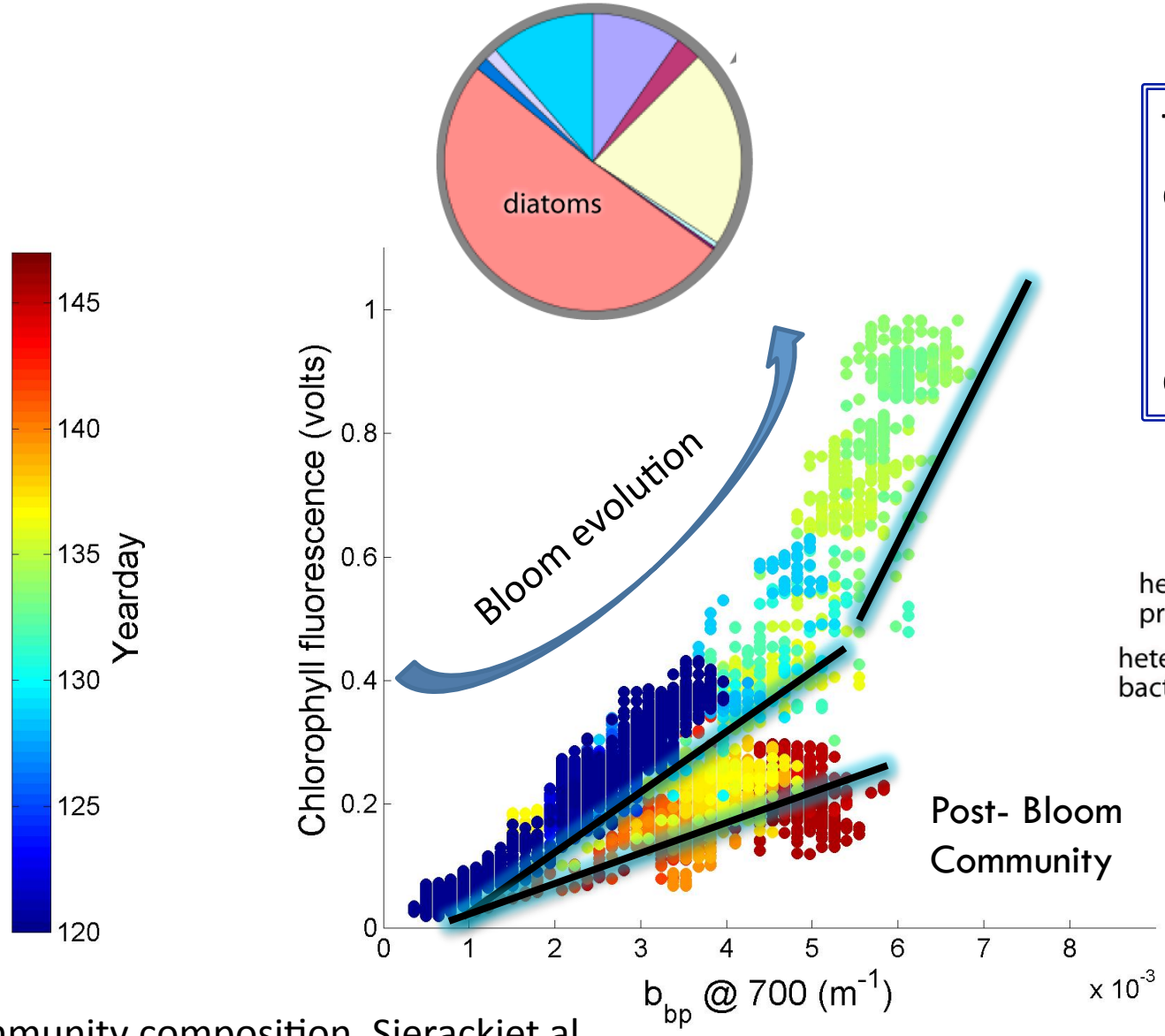


Alkire et al., sub.

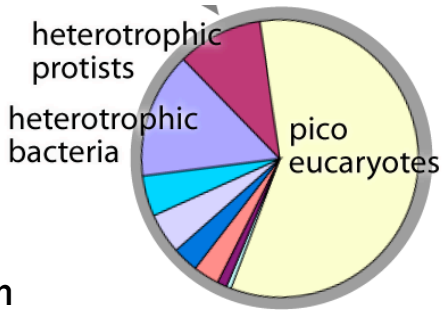




Change in Chl F/ b_{bp} – diagnostic of phytoplankton community composition? (shift from diatoms after Si depletion to pico-eukaryotes)



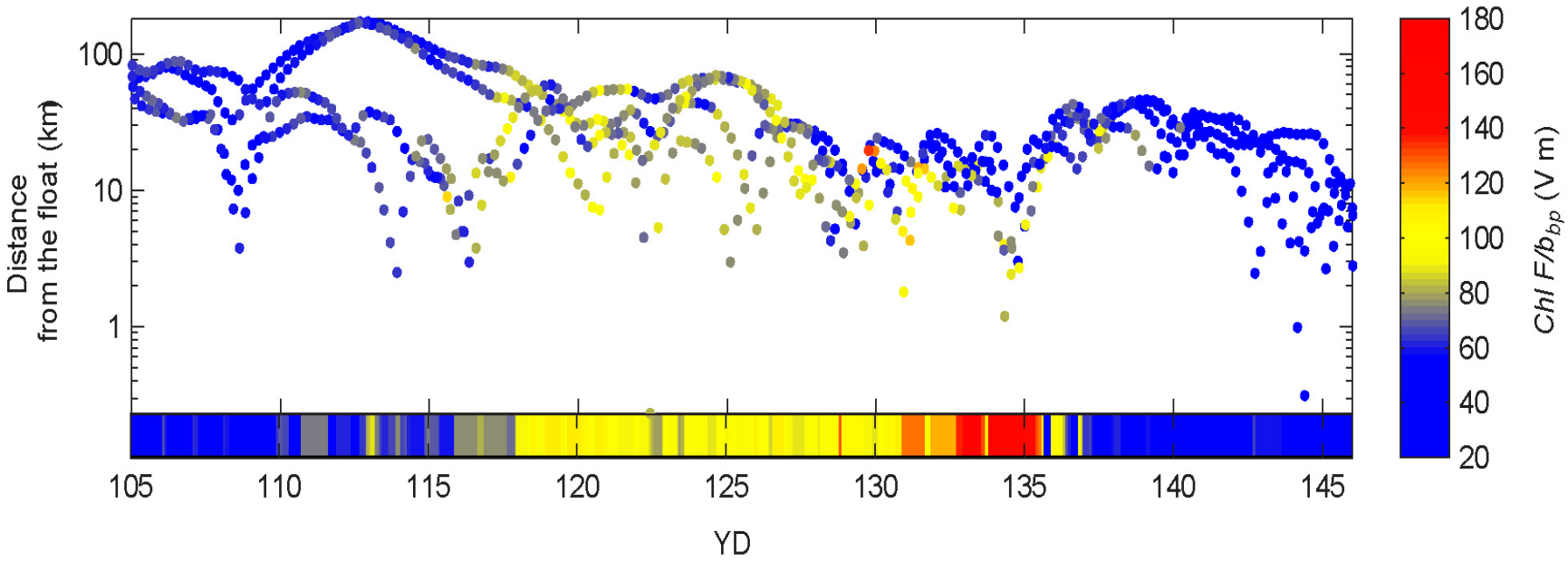
Three distinct optical regimes; phytoplankton 'pie' scaled to cell carbon



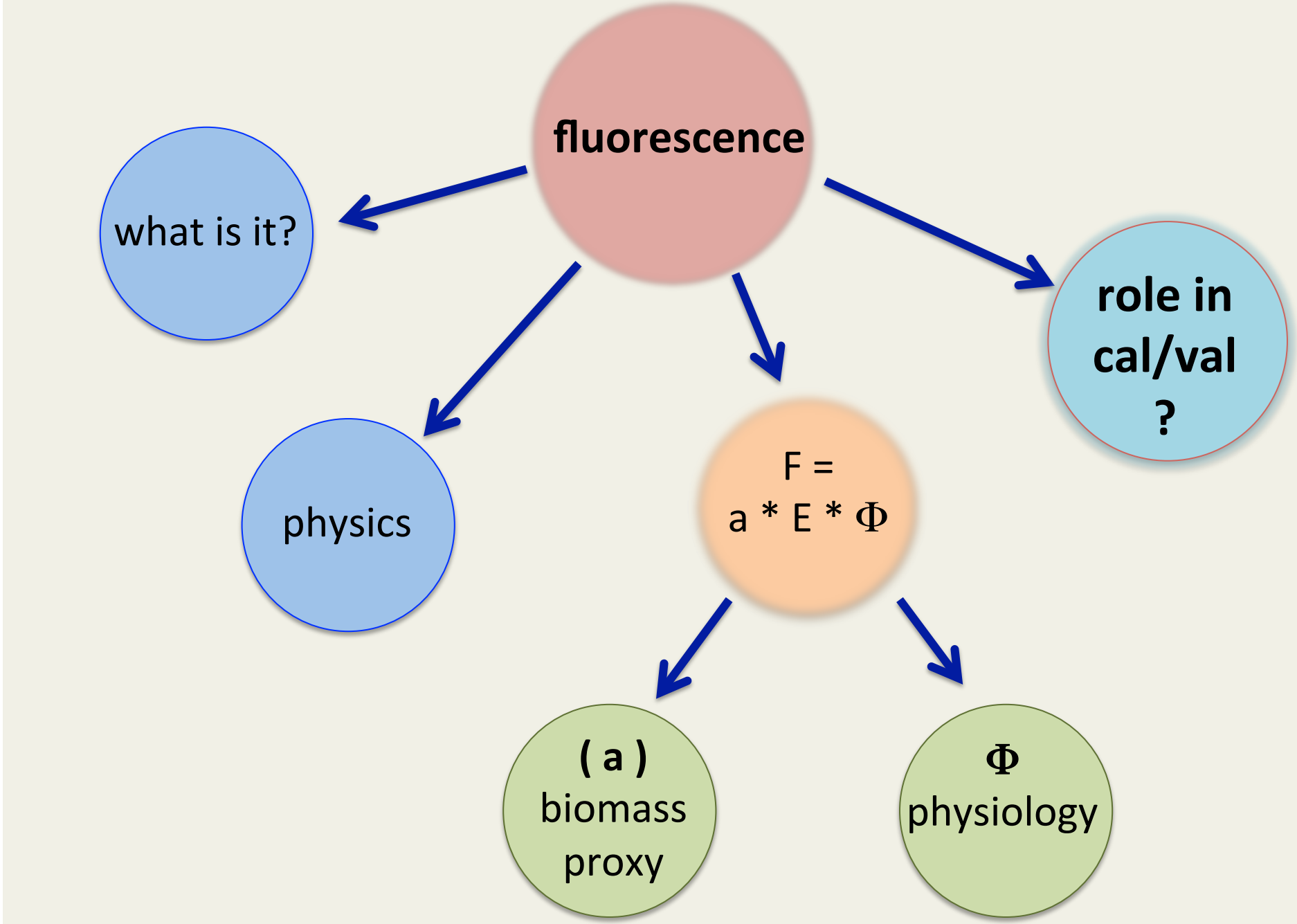
Ship data

Community composition, Sierackiet al.

Seaglider measurement of Chl F/ b_{bp} shows diatom patches



Cetinic et al.


fluorescence
what is it?
physics
F =
a * E * Φ
role in
cal/val
?
(a)
biomass
proxy
Φ
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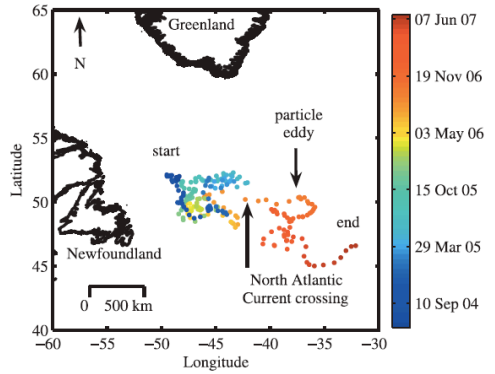

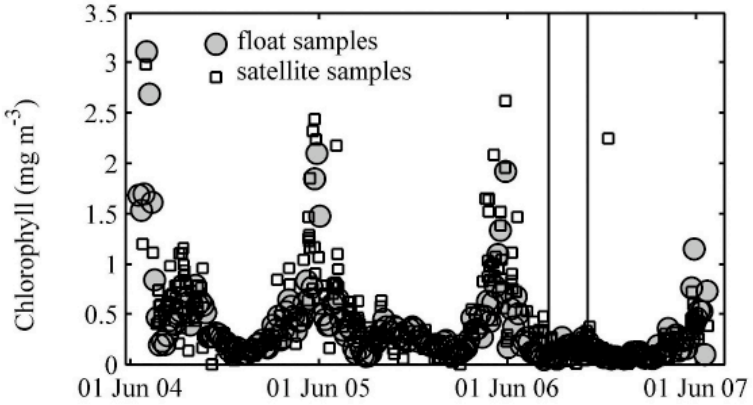

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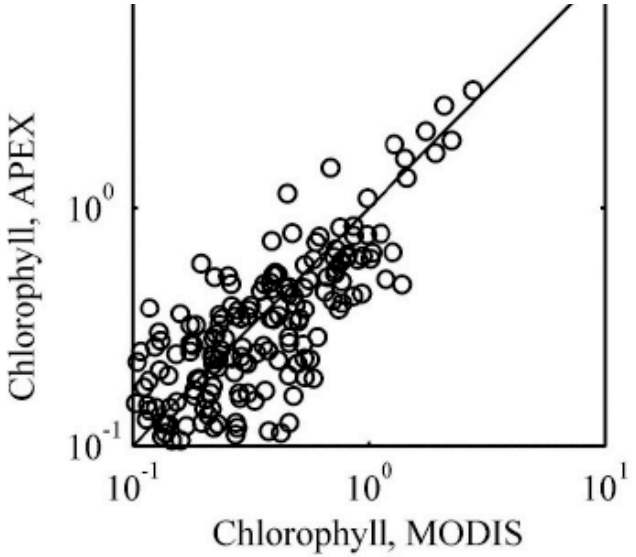

Today's lab

1) Station 1 – Mitchell Lab

Chlorophyll and CDOM fluorometers:

linearity

effect of other fluorescing material

effect of scatterers

wavelength of excitation

contamination by solar irradiance

2) Station 2 – MJP lab

Solar quenching of fluorescence (living cells)

Sampling variability for measurement of chlorophyll by standard filtration/acetone extraction method