UMaine, SMS 598: Calibration and Validation
for Ocean Color Remote Sensing
11-29 July 2011

Lecture 4 – Phytoplankton

What are phytoplankton?
How are phytoplankton assessed?
Why are phytoplankton important?

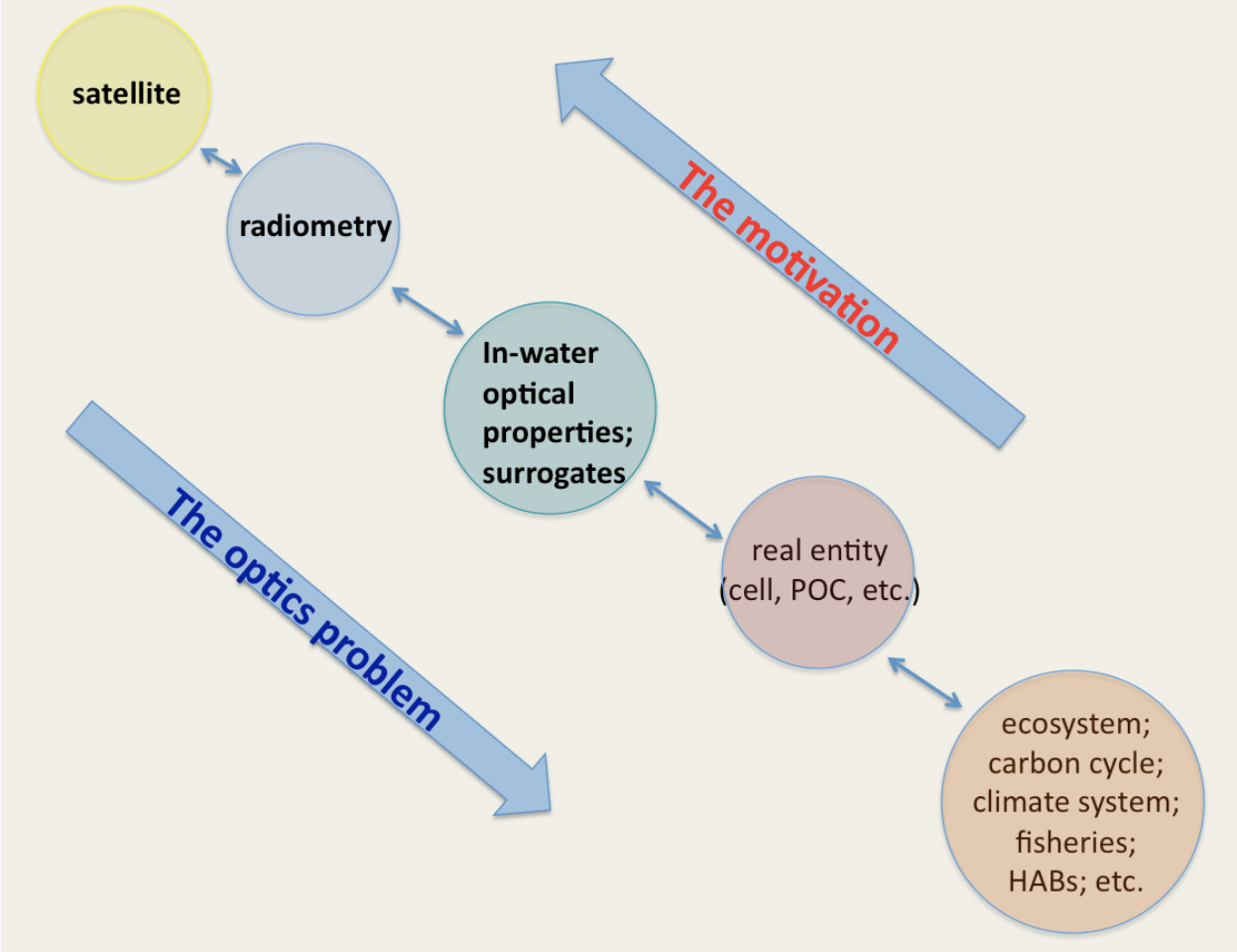
Mary Jane Perry
12 July 2011

On the piece of paper provided,
answer these three questions:

What are phytoplankton? (one sentence max)
How are phytoplankton assessed? (top approach)
Why are phytoplankton important? (top reason)

What's your question?

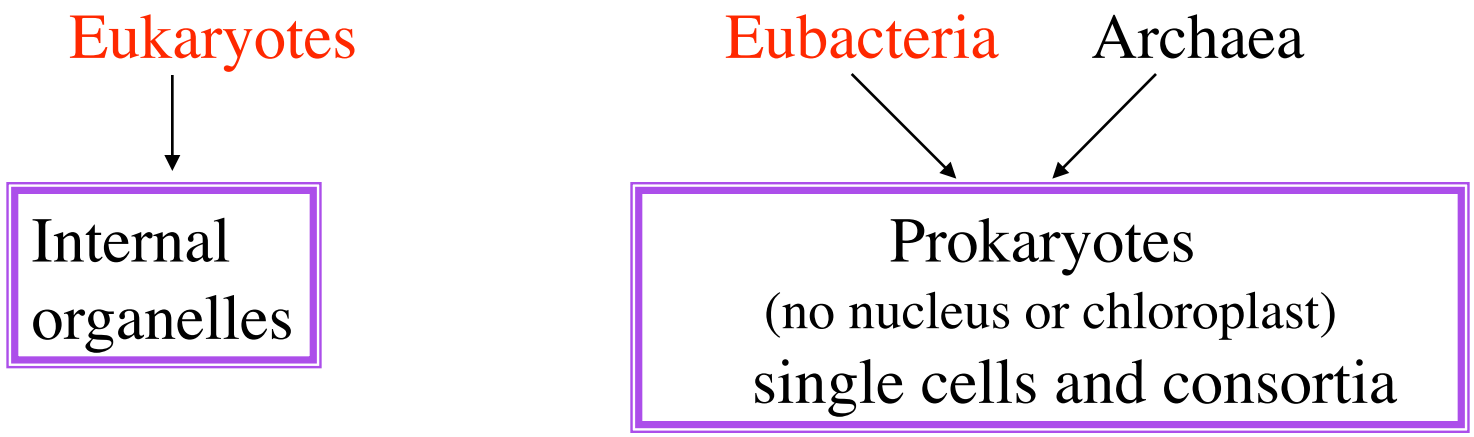
Your question places boundary conditions on your answer.



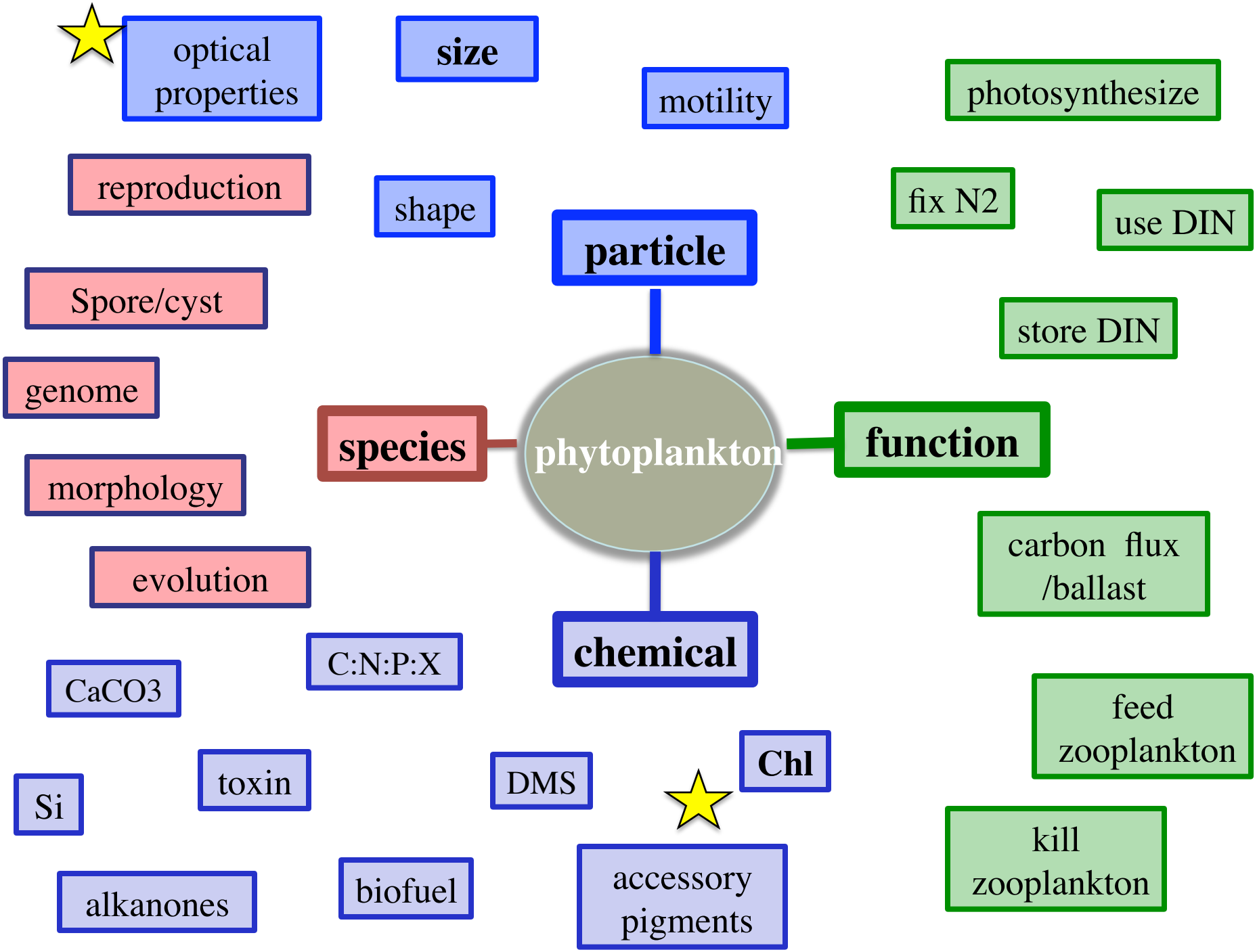
What are phytoplankton?

Aerobic (oxygenated environment)
Photosynthetic (pigmented)
Oxygenic (oxygen producing; use sunlight)
Small, single-celled particle (usually; some form chains)

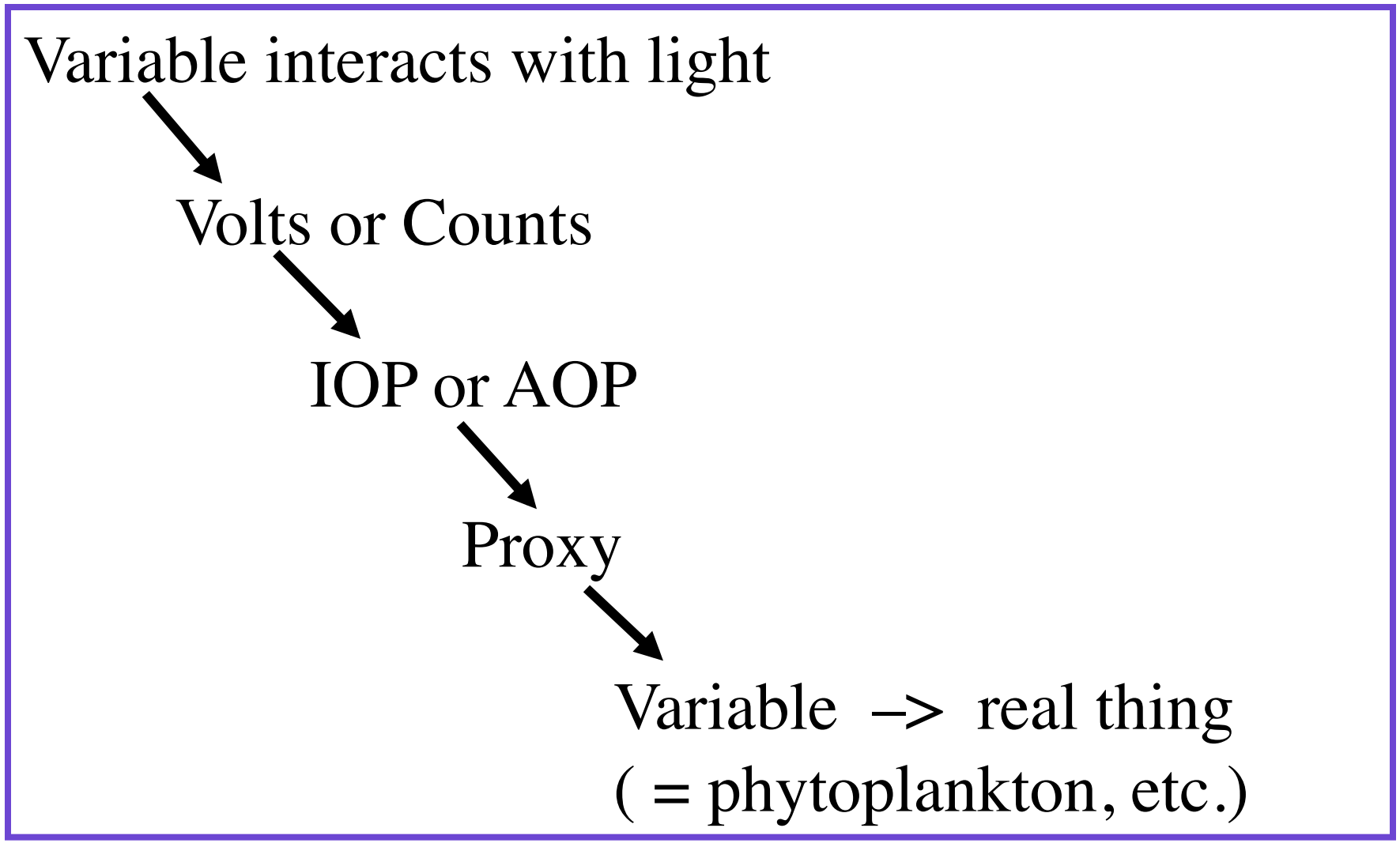
Three Super Kingdoms (phytoplankton in 2)



Bottom line:
great diversity of organisms that interact with light in the ocean
(See Keeling et al. 2004, Science 306: 2191, endosymbiotic evolution)


optical properties
size
motility
photosynthesize
reproduction
shape
fix N2
use DIN
particle
Spore/cyst
store DIN
genome
species
phytoplankton
function
morphology
carbon flux /ballast
evolution
chemical
C:N:P:X
CaCO3
feed zooplankton
Chl
toxin
DMS
Si
kill zooplankton
accessory pigments
biofuel
alkanones

Optical Properties as Proxies or Surrogates
– what optics? which aspect of phytoplankton?



Today:

1. Introduce you first to phytoplankton,
   and a little bit about their role in the ocean

2. What are the proxies/surrogates are based on interaction w/ light?
   - particles scatter light
   - pigments absorb light
   - chlorophyll *a* and phycoerythrin fluoresce light

3. How does physiology changes the relationship between
   phytoplankton and some of their optical proxies?
   (plasticity is intrinsic to their survival, potential annoyance to us)

Phytoplankton as particles

– in the ocean, **size matters** and is related to function

historical nomenclature:

net $> 20\mu$m nano $< 20\mu$m

pico $< 5\mu$m ultra $< 2\mu$m (smallest mostly prokaryotes)

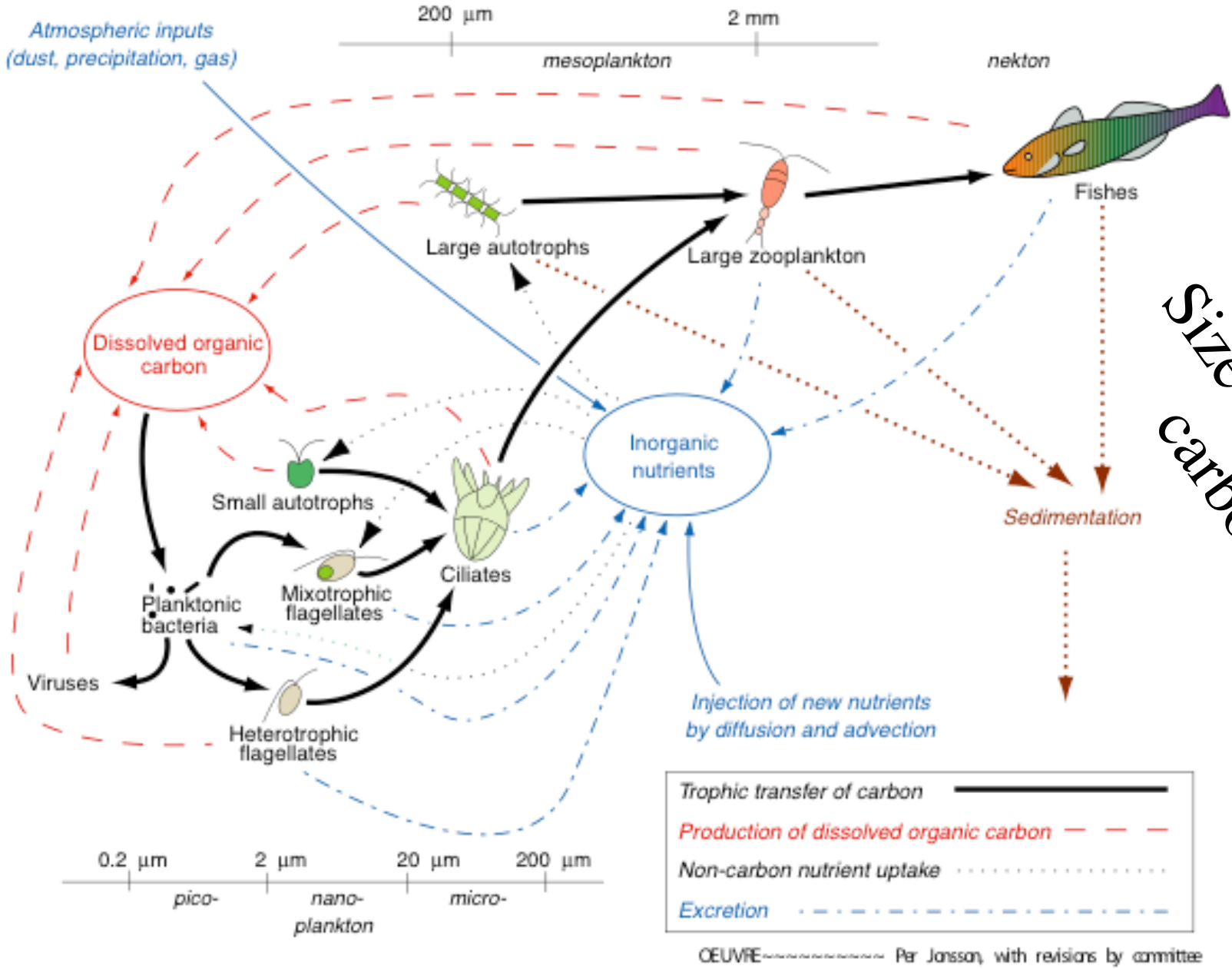
Size

* small cells are mostly spherical; larger cells often non-spherical
* efficiency of dissolved solute capture (diffusion – smaller cells better)
* efficiency of encounter – surface area for contact
* exposure to light (packaging; a*) and UV damage greater for small cells
* carbon content - # cells/volume higher for small cells
* metabolic rates scale to size (specific rate decreases w/size)
* settling – Stokes Law (carbon cycling – small cells don't sink)
* efficiency of aggregation
* larger cells motile on scales measured by optics

*** points regarding interaction with light**

– b: scattering (cross sectional area)

– a: absorption (cell volume affects absorption efficiency)



Size – food webs; carbon cycling

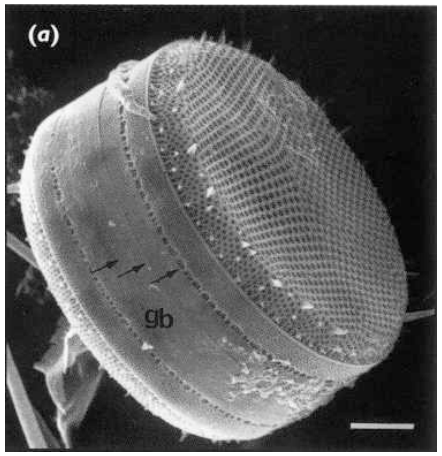
A community consensus view; details and color at <http://www.joss.ucar.edu/joss_psg/project/oce_workshop/oeuvre/report/>

Phytoplankton as species (or taxa) –when does knowing species matter?

Eubacteria – **cyanobacteria** (oxygenic eubacteria)

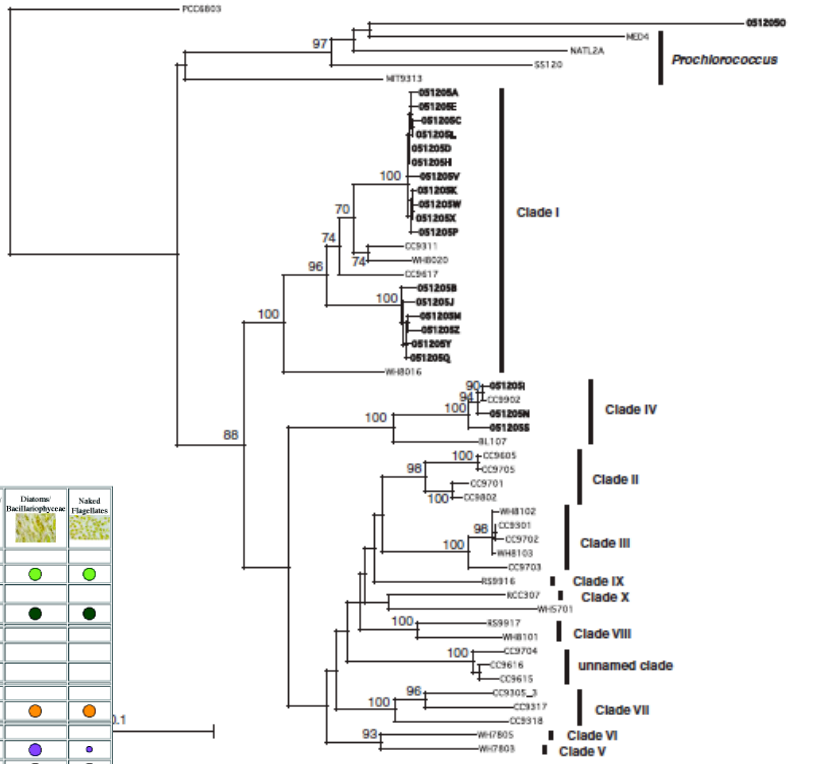
also aerobic, anoxygenic species and anaerobic, anoxygenic (sulfur bacteria)

Eukaryotes – **protists** (very diverse) and **chlorophytes** (closer to land plants)

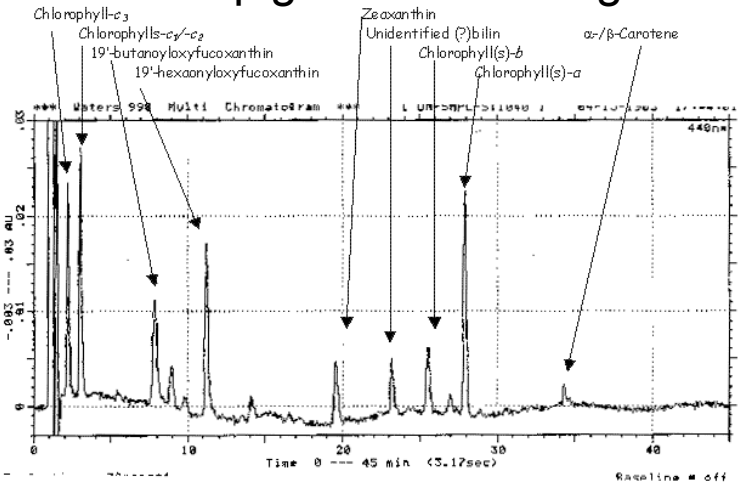


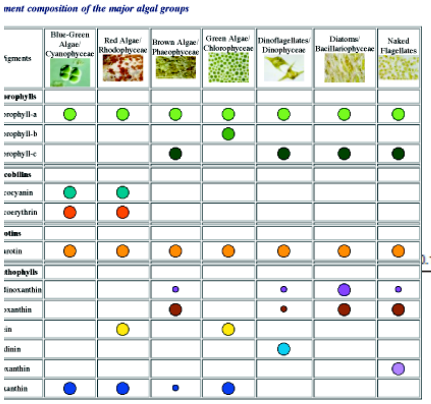
Morphological characterization of species (diatom frustule structure)

Molecular characterization (clades of *Synechococcus)*



HPLC pigment clustering & Chemtax





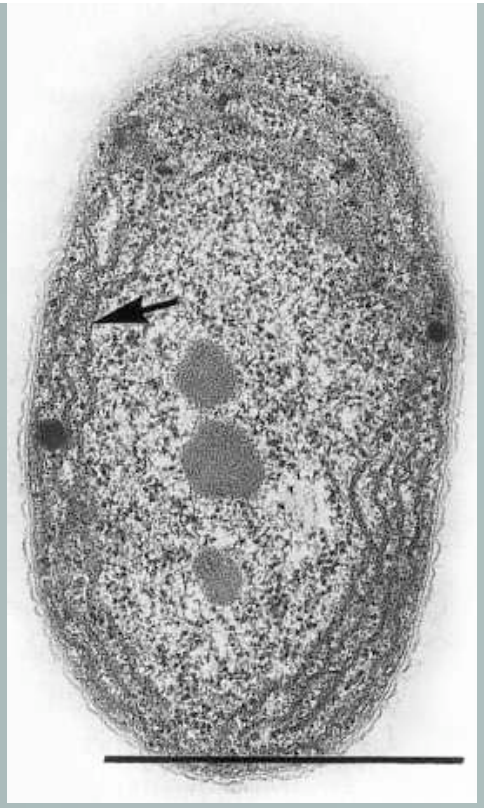
Tai & Palenik, 2009, ISME 3: 908

Phytoplankton as functional types:

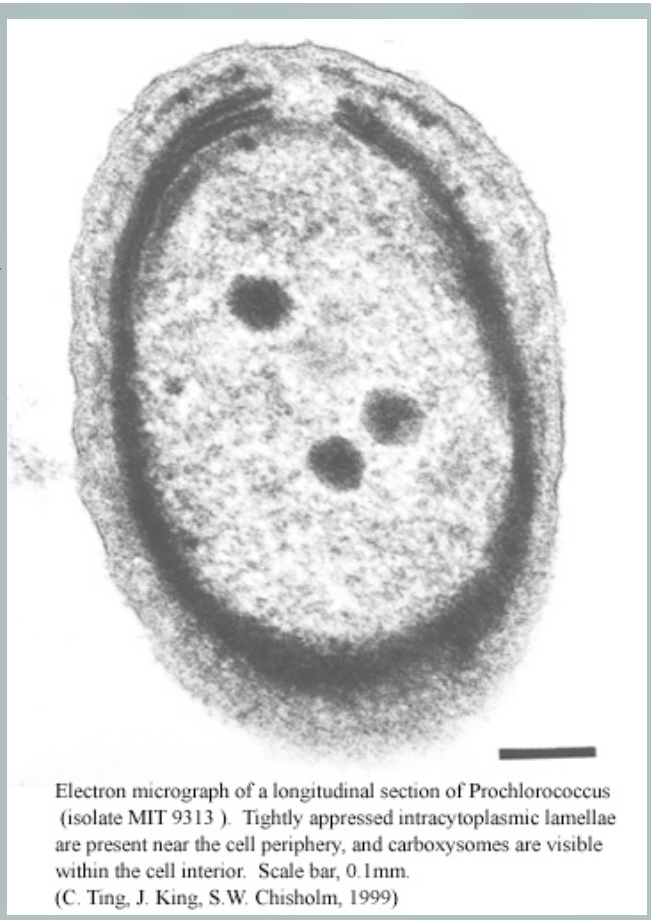
Functional type

- autotrophic, oxygenic, oxygen evolving
- size and shape, each with specific carbon or nutrient concentration
- transformer of specific nutrient (N_2 fixer, $CaCO_3$ precipitator, silica polymerizer, etc.); ballasting to enhance C flux; specialized nutrient-up take pathways, sequestering mechanisms; unique C:N:P:trace metal ratio
- nutritional value to higher trophic organisms, such as essential fatty acids, toxins or development disrupters, paleo markers
- ability to live in turbulent vs. stratified environment
- motility for enhancing nutrient acquisition, encounter gametes, avoiding predation
- what else ??

Chemical composition – relates to function, species, etc. For optics, pigments are key(& sometimes unique) chemicals. But do we really want C?



electron micrographs of prokaryotic phytoplankton



Electron micrograph of a longitudinal section of Prochlorococcus (isolate MIT 9313). Tightly appressed intracytoplasmic lamellae are present near the cell periphery, and carboxysomes are visible within the cell interior. Scale bar, 0.1mm.
(C. Ting, J. King, S.W. Chisholm, 1999)

Synechococcus (~ 1 μm)

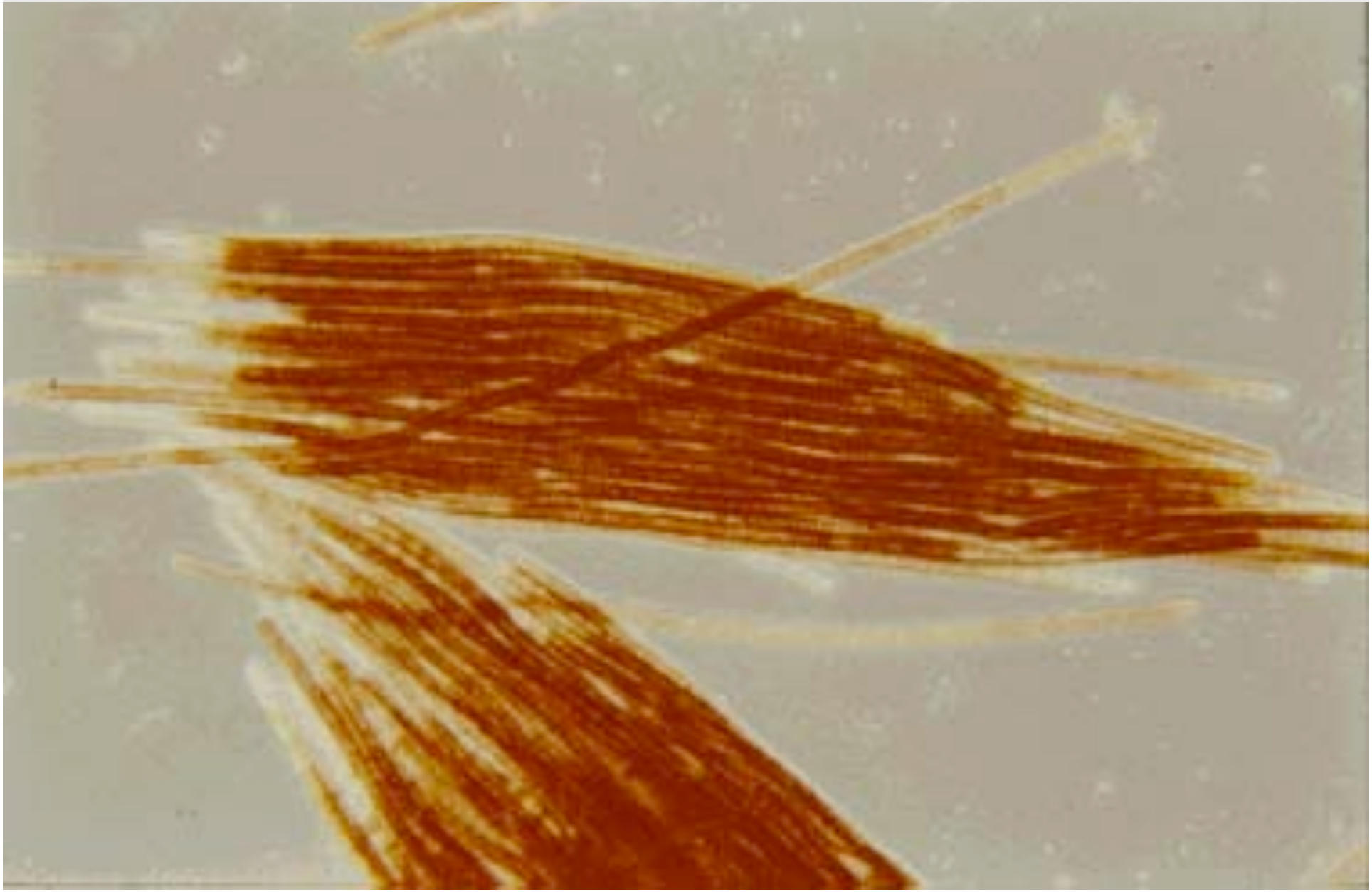
Arrow denotes thylakoid membrane which has both photosynthetic and respiratory functions.

Diagnostic: phycoerthyrin pigment fluoresces orange (in contrast to chlorophyll, which fluoresces red.

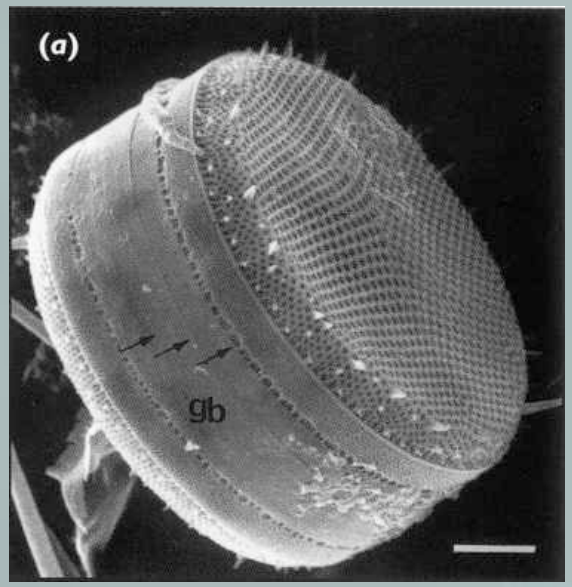
Prochlococcus (~ 0.7 μm)

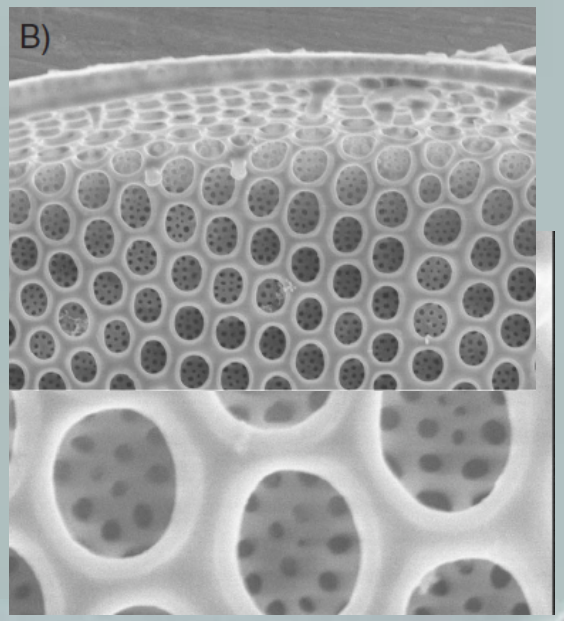
Diagnostic: very small size, lack of orange fluorescence, divinyl chlorophyll a & b. Found only in tropics/subtropics.

Trichodesmium (cyanobacterial nitrogen fixer;
warm waters; patchy; Fe may regulate abundance)

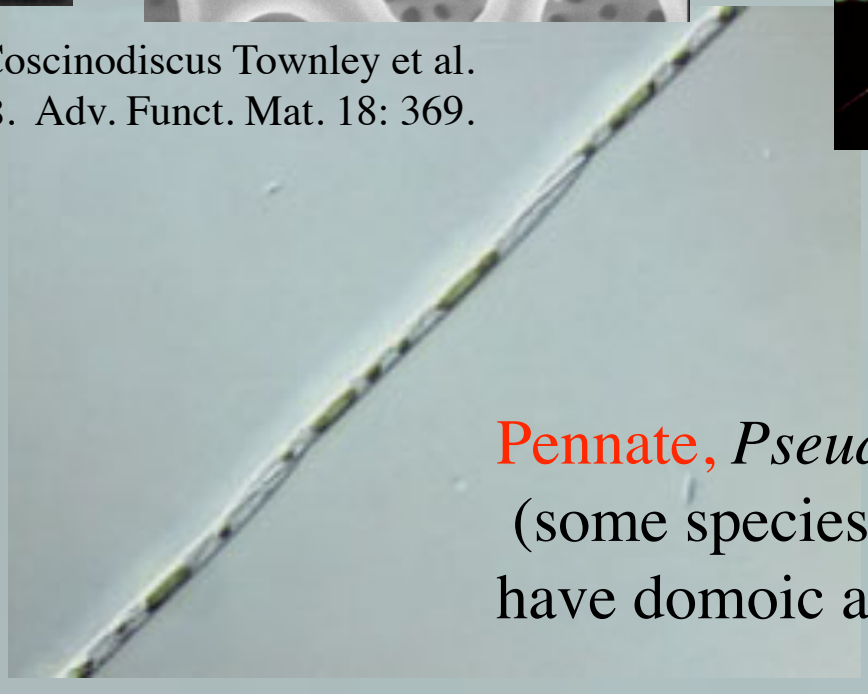


Centric Diatoms, single cell *Thalassiosira* and chained *Chaetoceros*





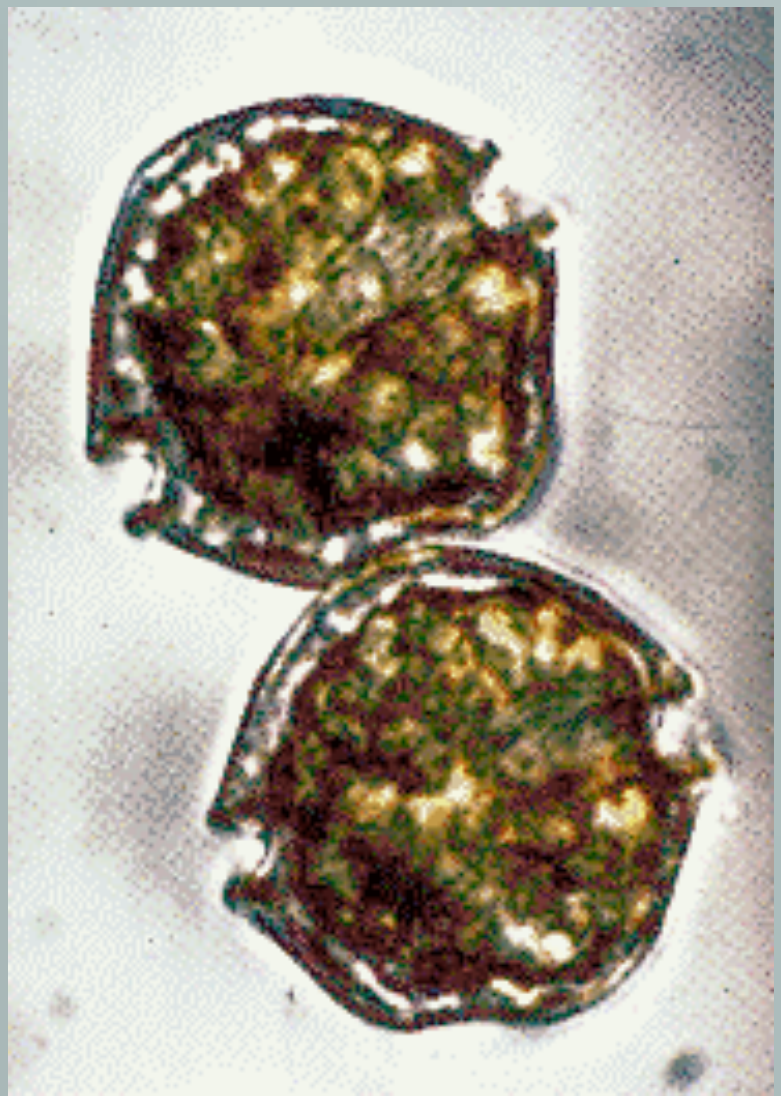
SEM –Coscinodiscus Townley et al. 2008. Adv. Funct. Mat. 18: 369.



Pennate, *Pseudonitzschia* (some species have domoic acid)

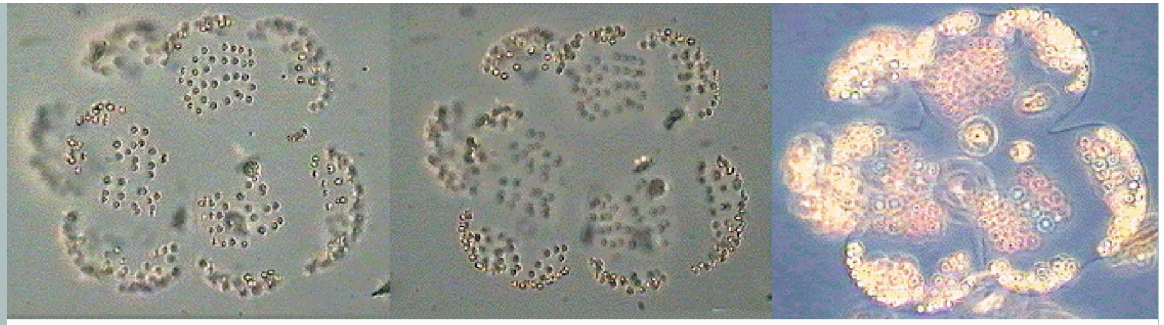
Dinoflagellates

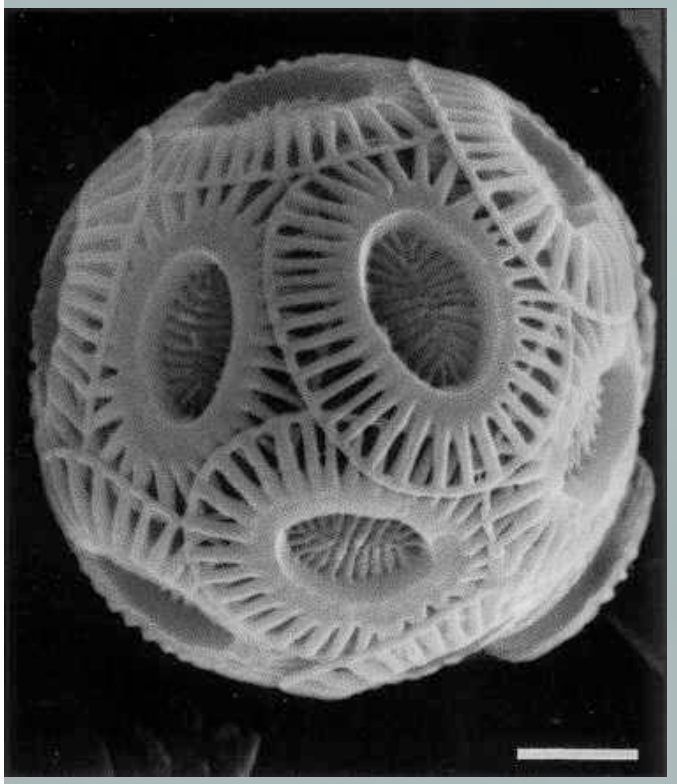
Alexandrium tamarense

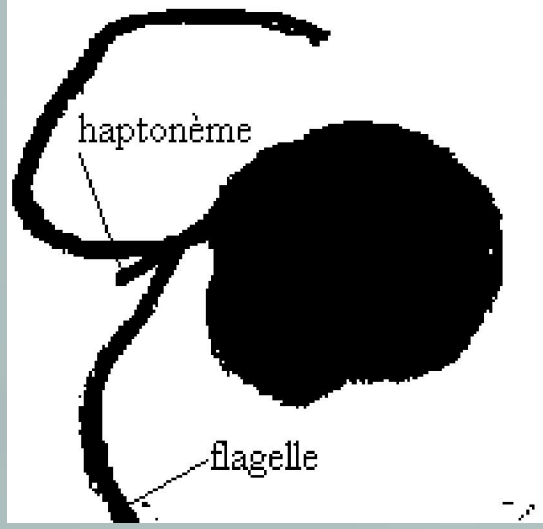


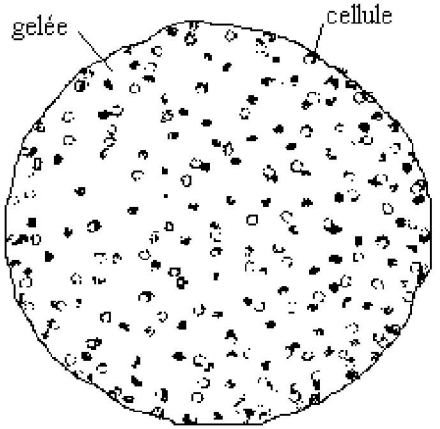
Ceratium

Coccolithophorid, with calcite plates or coccoliths (blooms visible from space)





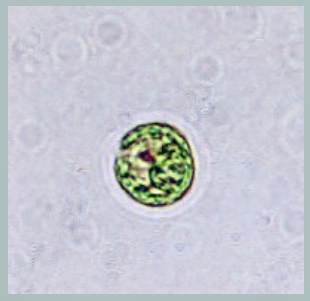


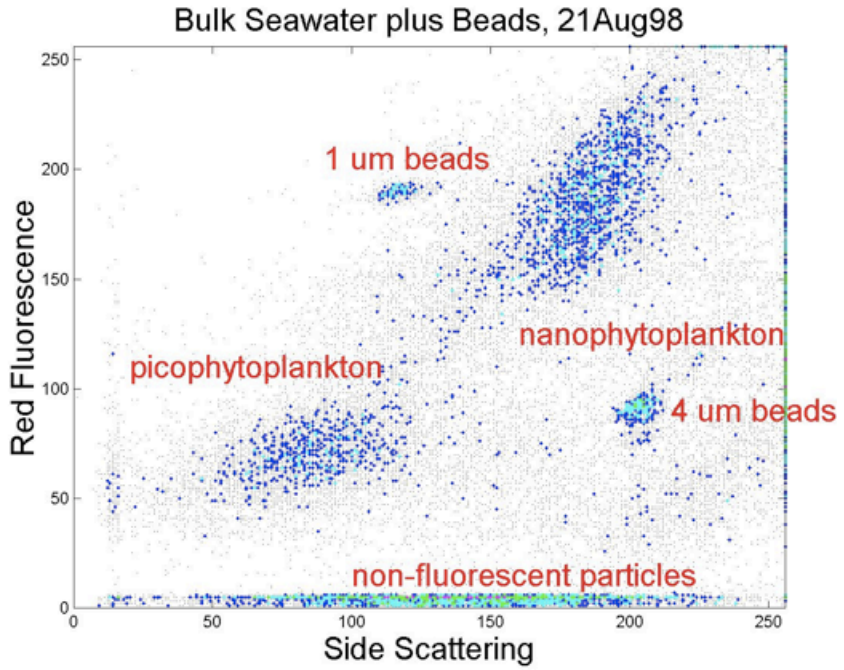


Phaeocystis

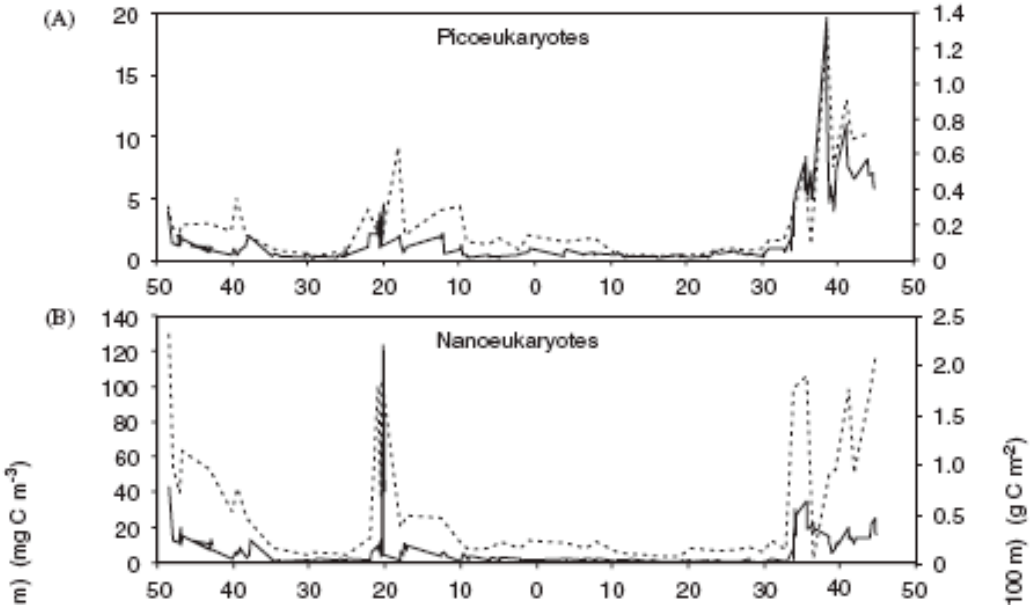
(colonical and single cell) famous for producing foam on northern European beaches

Lots of cells that look like this
(small and non-descript under microscope)

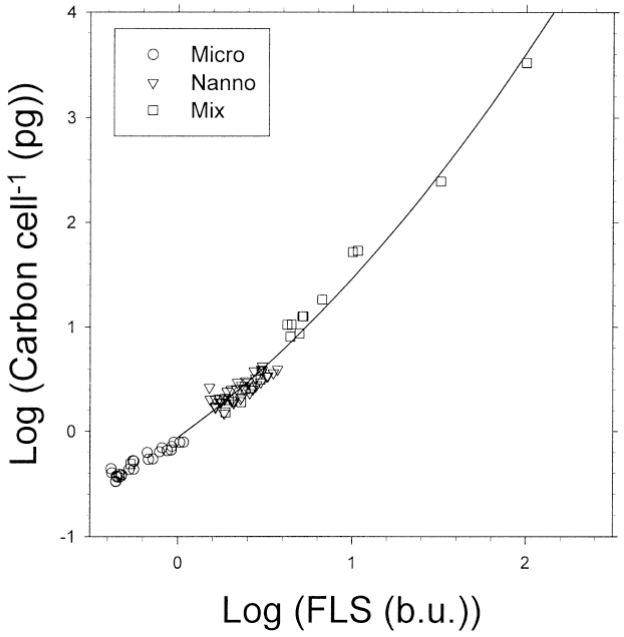




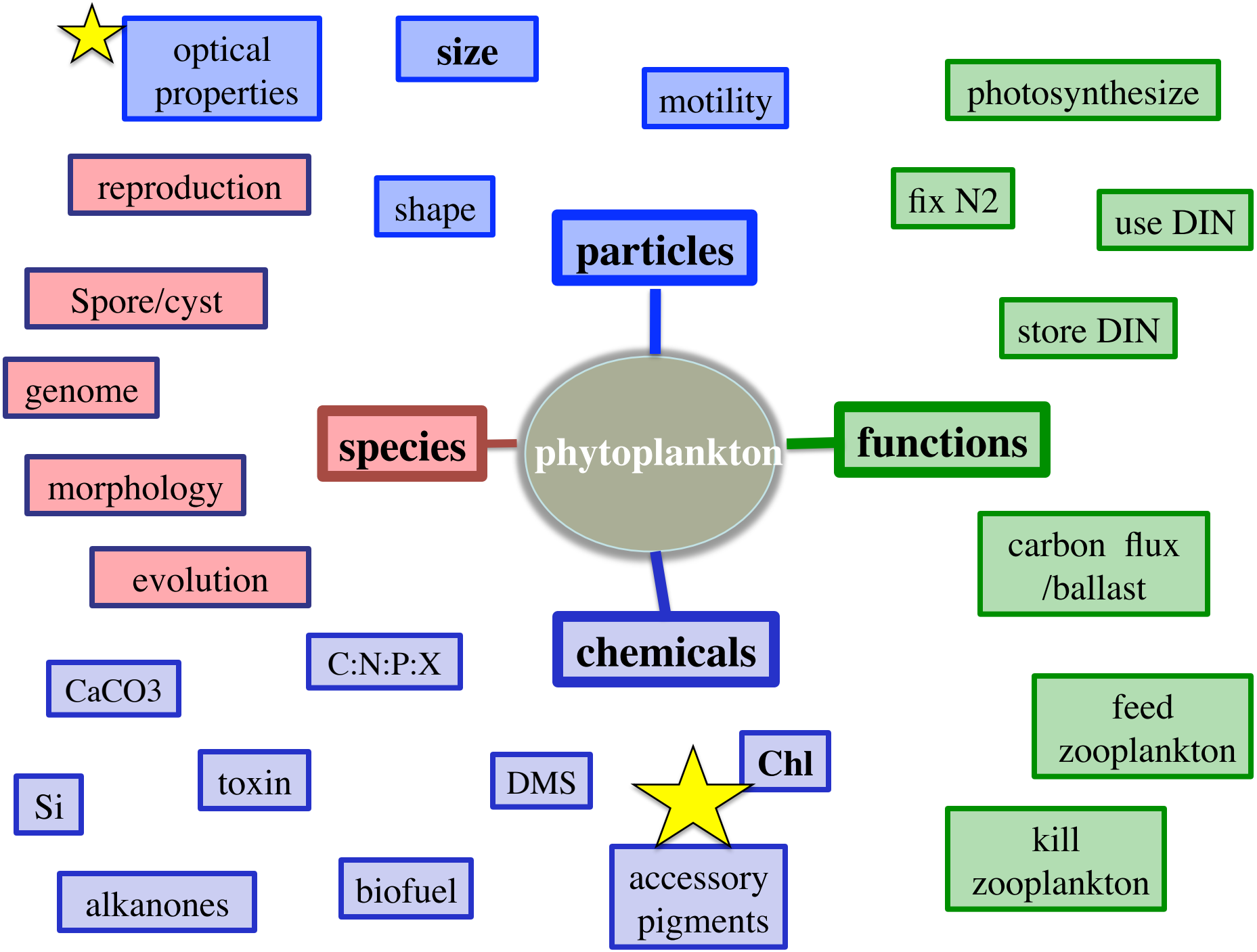
Common way to measure picoeukaryotes, as well as determine their carbon, is by flow cytometry – measures single particle (chl and PE fluorescence and FS optical size)



Tarran et al. 2006 DSR II 53: 1516

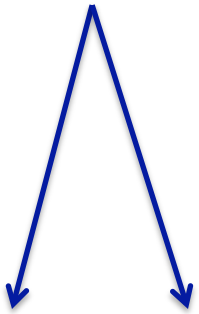


DuRand 2002 J. Phycol. 39: 1132

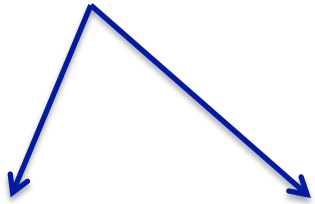

optical properties
size
motility
photosynthesize
reproduction
shape
fix N2
use DIN
particles
Spore/cyst
store DIN
genome
species
phytoplankton
functions
morphology
carbon flux /ballast
evolution
chemicals
C:N:P:X
CaCO3
feed zooplankton
Chl
toxin
DMS
Si
kill zooplankton
accessory pigments
biofuel
alkanones

Conceptualize (and measure) phytoplankton as:

State variable vs. Rate variable



Individual or Mass (or proxy)



Productivity or Physiology
(function)

Cell counts

Mass of phytoplankton ('bulk' measurement – not individuals)

gene sequence – presence/absent or now quantitative?

molecule – carbon, (but not unique, but that might be what we want;
chlorophyll is unique

optics – related to chlorophyll & other pigments, proxy for mass

Chlorophyll *a* – most common entity used to denote presence of phytoplankton and attempt to quantify concentration (mass).

Term 'chlorophyll' biomass often used – anathema to some.

Is chlorophyll a perfect proxy for phytoplankton? Yes / No

Chlorophyll a (or divinyl Chl a) is found in all phytoplankton and not in heterotrophs (exception: mixotrophs, digesting predators).

Relationship between carbon and Chl allows estimation of phytoplankton carbon. But beware Mobley's Law of Conservation of Misery; C/Chl ratio influenced by physiology.

Some measure of assessing chlorophyll can be used at all scales – from mooring, ship, autonomous platform, satellite.

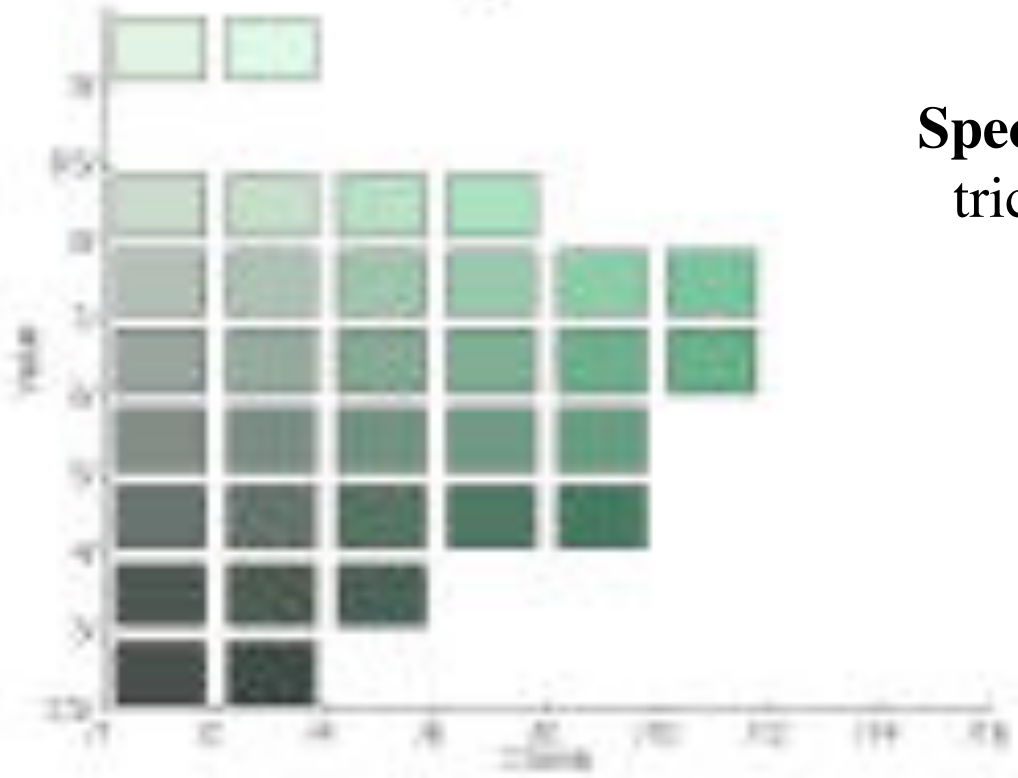
Different measures of assessing 'chlorophyll' need to be aligned; not measuring exactly same thing.

Brief history of measurement of ‘bulk’ chlorophyll & related entities

Harvey Plant Pigment Unit (HPPU) - up to ~ 1950

– standardized color on filters (Munsell chart);

still used for soils and tobacco.



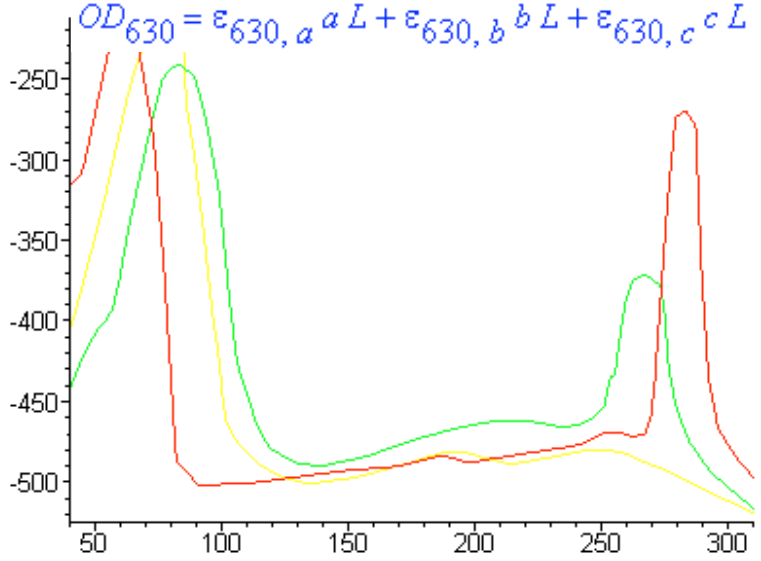
Spectrophotometry, extracts in solvent; trichromatic eq. to separate pigments.

~ 1950’s

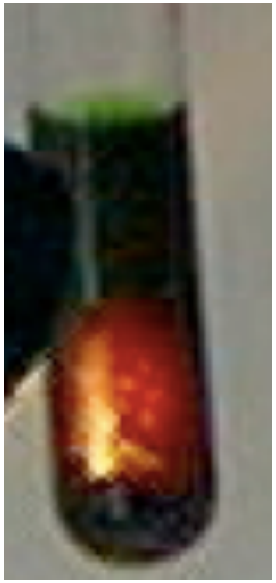
$$OD_{664} = \varepsilon_{664,a}\, a\, L + \varepsilon_{664,b}\, b\, L + \varepsilon_{664,c}\, c\, L$$

$$OD_{647} = \varepsilon_{647,a}\, a\, L + \varepsilon_{647,b}\, b\, L + \varepsilon_{647,c}\, c\, L$$

$$OD_{630} = \varepsilon_{630,a}\, a\, L + \varepsilon_{630,b}\, b\, L + \varepsilon_{630,c}\, c\, L$$

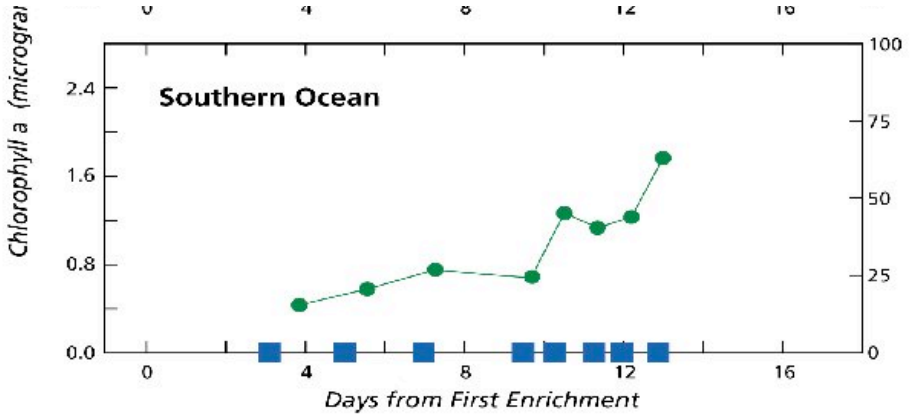


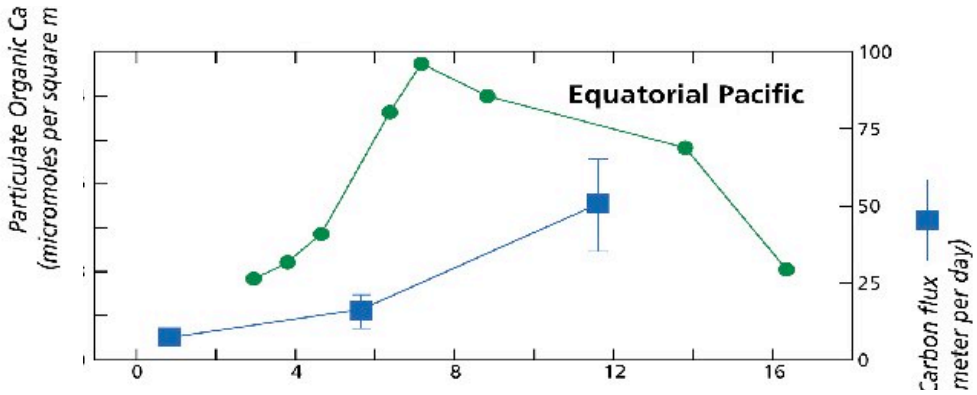
Brief history of measurement of 'bulk' chlorophyll & related entities



Early 1960's, **fluorescence extracts** from water samples. Reasonably fast.

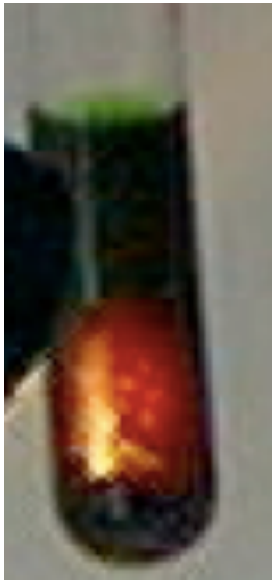
This method was used in iron-fertilization experiments; Chl *a* (μg L^{-1}) provided an index of bulk phytoplankton response: Southern Ocean vs. Equatorial Pacific.





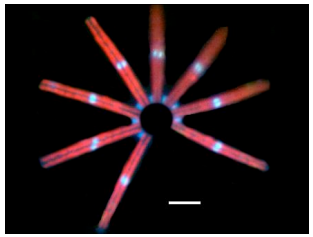
http://cafethorium.whoi.edu/Fe/1999-Annualreport.html

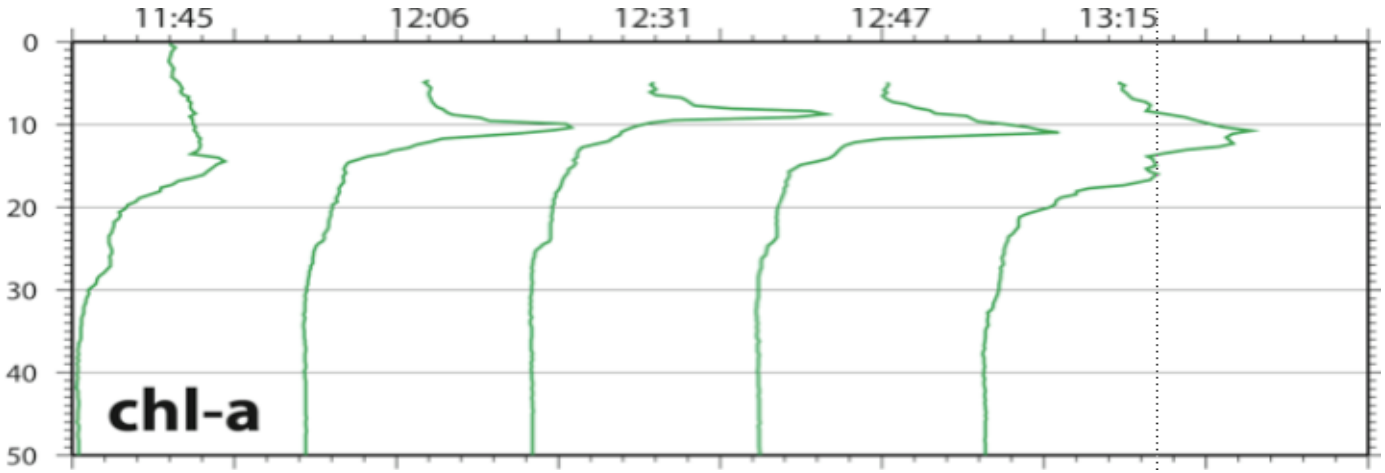
Brief history of measurement of ‘bulk’ chlorophyll & related entities



Early 1960’s, **fluorescence extracts** from water samples. Reasonably fast.

Late 1960’s, **fluorescence profiles of fluorescence in living cells** – in the ocean.
Fast! and high vertical resolution.
Used on CTD, mooring, floats, gliders, etc.
Example below of thin layers in Monterey Bay.

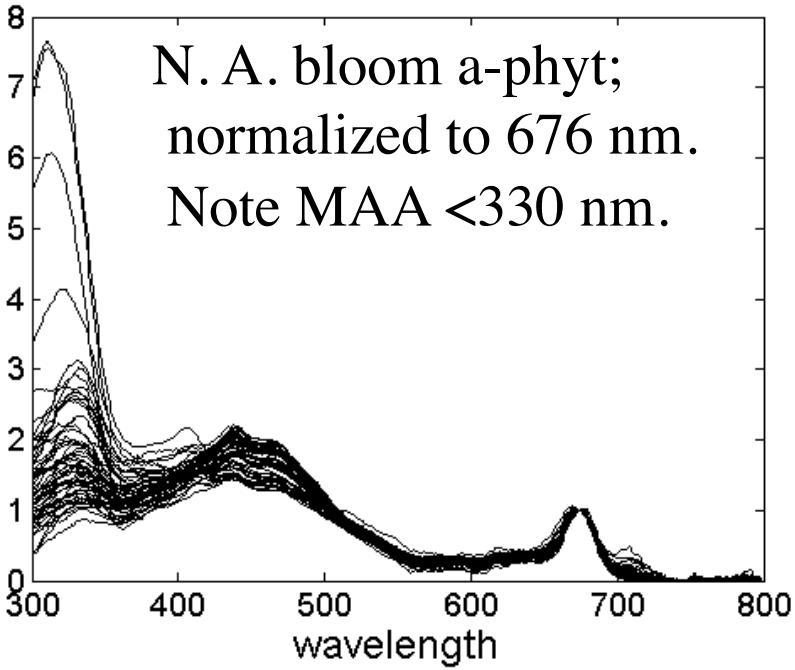


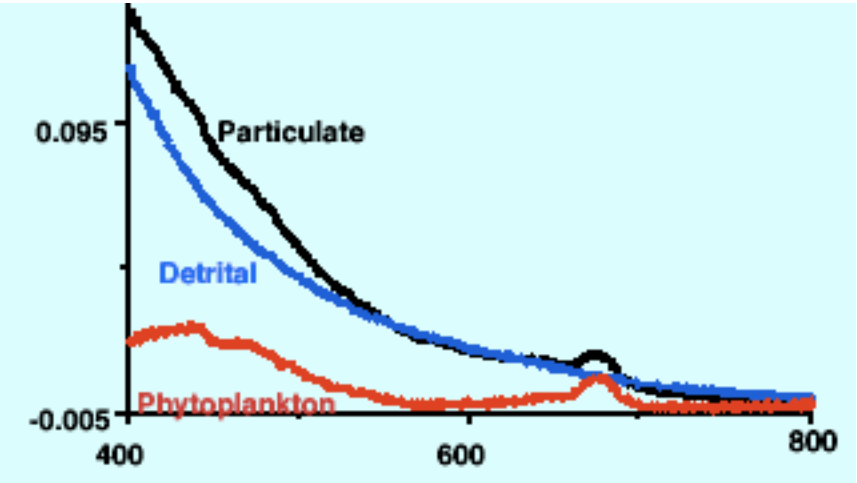


Brief history of measurement of 'bulk' chlorophyll & related entities

QFT – Quantitative Filter Technique (filter pad absorption) ~ 1980's

(Quantitative version of HPPU)





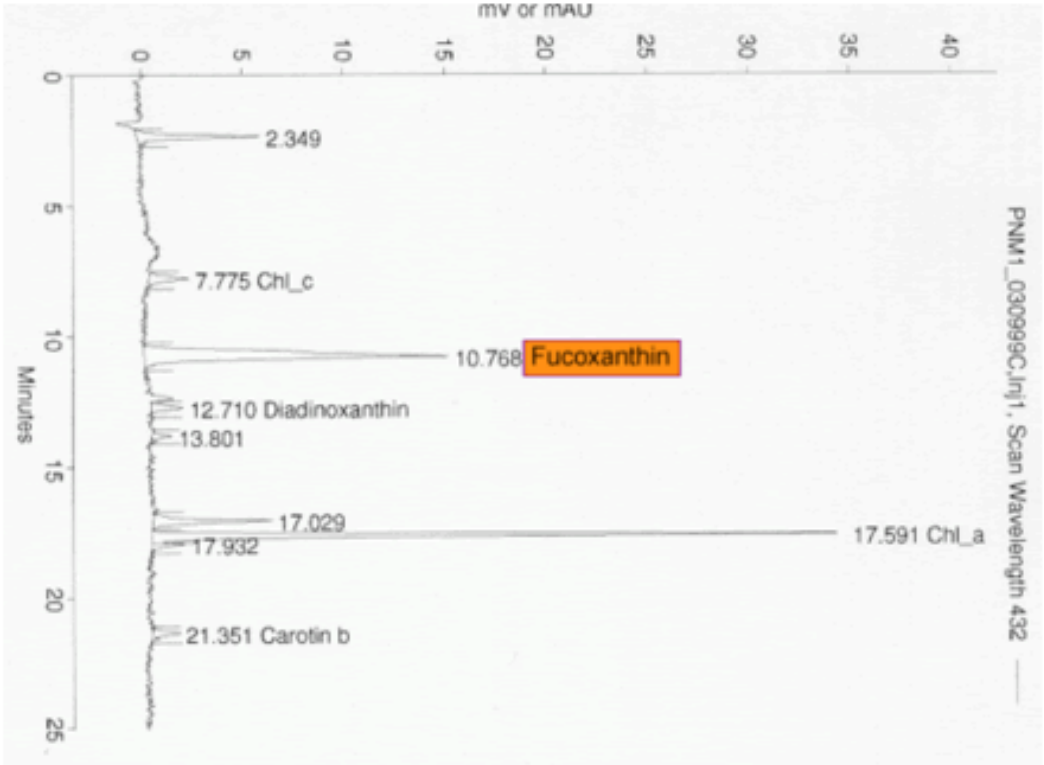
a-particulate

a-phytoplankton: a-PS and a-PP

a-NAP: a-mineral and a-dead stuff

Brief history of measurement of ‘bulk’ chlorophyll & related entities

HPLC pigments – resolve most of phytoplankton pigments. ~1990’s.
Chemtax – for taxonomic assessment (requires training).



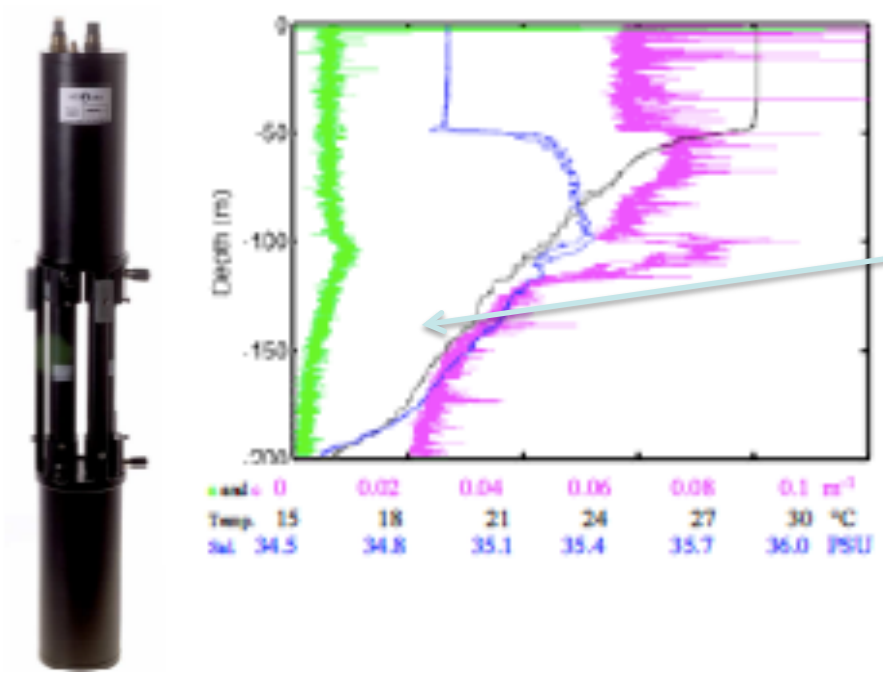
Pigment composition of the major algal groups

Pigments	Blue-Green Algae/ Cyanophyceae	Red Algae/ Rhodophyceae	Brown Algae/ Phaeophyceae	Green Algae/ Chlorophyceae	Dinoflagellates/ Dinophyceae	Diatoms/ Bacillariophyceae	Naked Flagellates
Chlorophylls							
Chlorophyll-a	●	●	●	●	●	●	●
Chlorophyll-b				●			
Chlorophyll-c			●		●	●	●
Phycobilins							
Phycocyanin	●	●					
Phycoerythrin	●	●					
Carotins							
ß-Carotin	●	●	●	●	●	●	●
Xanthophylls							
Diadinoxanthin			●		●	●	●
Fucoxanthin			●		●	●	●
Lutein		●		●			
Peridinin					●		
Alloxanthin							●
Zeaxanthin	●	●	●	●			

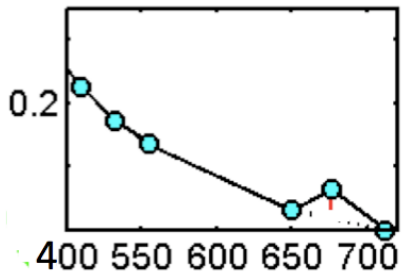
(Quantitative version of trichromatic equations)

Brief history of measurement of ‘bulk’ chlorophyll & related entities

ac-9 and acs - absorption and attenuation meters for profiles ~ 1990’s



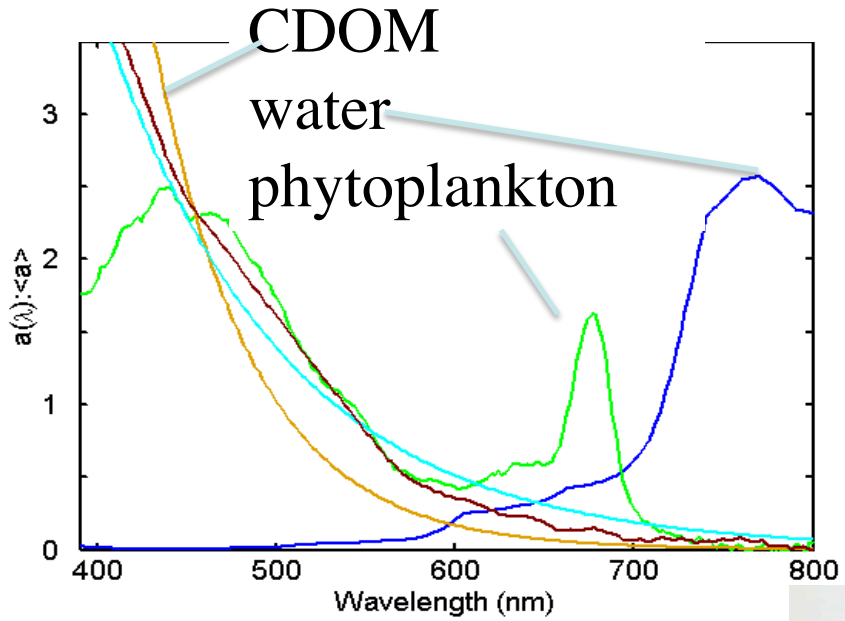
a_phyt (676) is a good estimator of chlorophyll concentration in cell (Roesler)



In-situ measurements demonstrate instrument stability and precision. Absorption (673nm, green line), Beam attenuation (650nm, magenta line), Temperature (black line) and Salinity (blue line) profiles taken at the Hawaii Ocean Time Series (HOTS) Aloha site near 22.75°N, 158°W (approximately 100 km north of Oahu, Hawaii) on August 11, 2004. The data were obtained during one down and up profile.

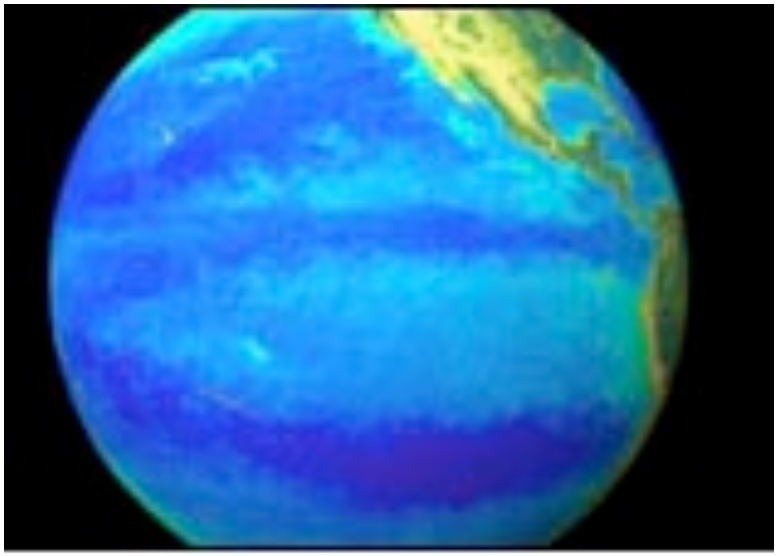
http://www.wetlabs.com/Research/presentations/ONR%20ac-s.pdf

Brief history of measurement of ‘bulk’ chlorophyll & related entities

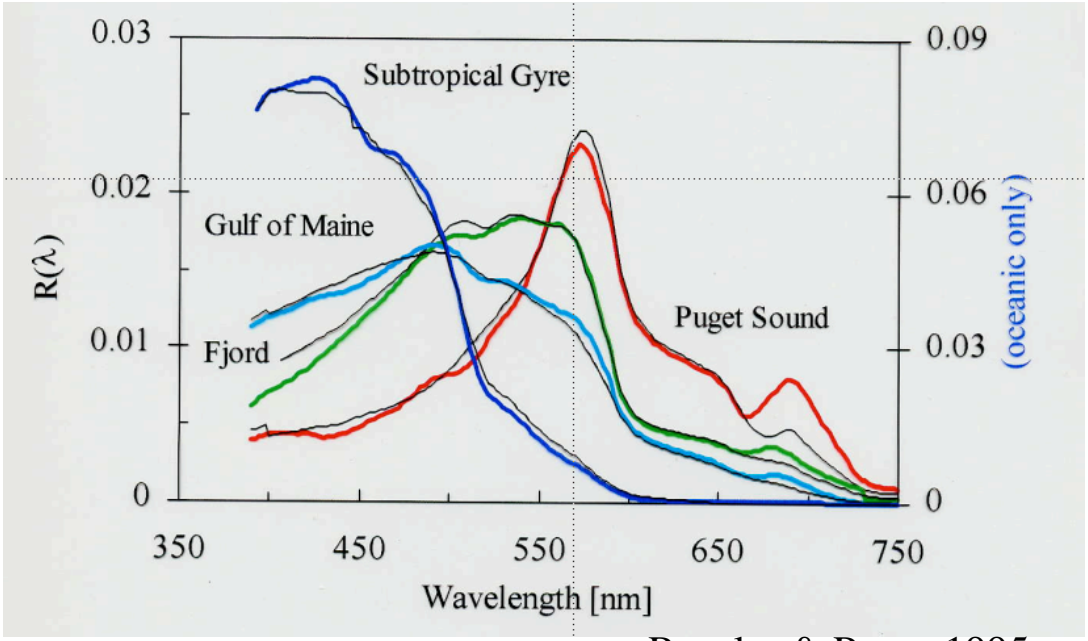


Remote sensing reflectance is based on selective absorption by phytoplankton pigments; empirical algorithms; local tuning.

$R_{rs} \sim [b_b / (a + b_b)]$



El Nino



Roesler & Perry, 1995

Let's explore more pigments

Definition: absorbing compound

Role: light harvesting for photosynthesis (PS)
light protection when too much light (PP)

Types:

chlorophylls

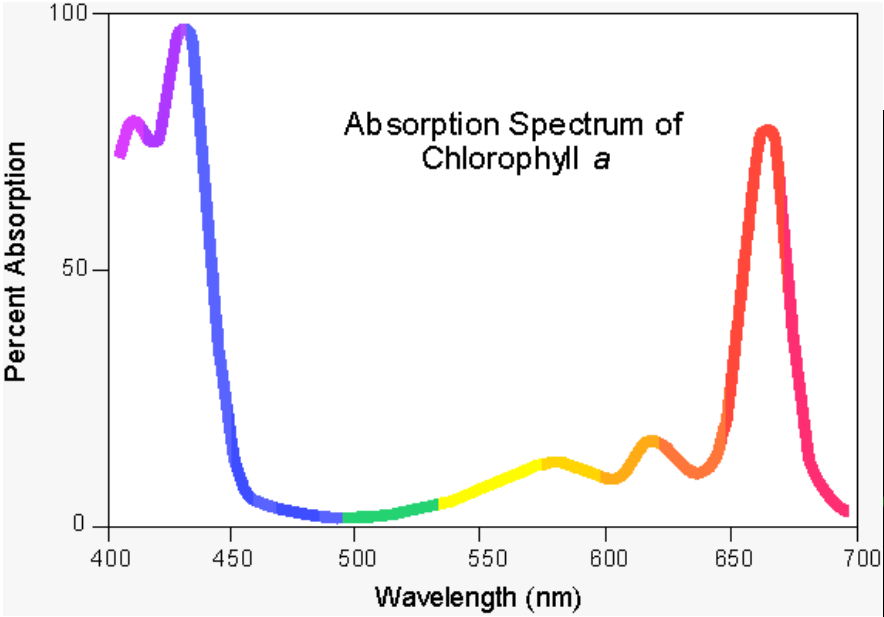
chlorophyll *a* - primary PS pigment in all oxygen producers
chlorophyll *b* or *c* - accessory PS pigments; expand λ range;
transfer energy to chlorophyll *a*
(divinyl chl *a* and *b*)

carotenoids

light harvesting for photosynthesis (PS)
light protection when too much light (PP)

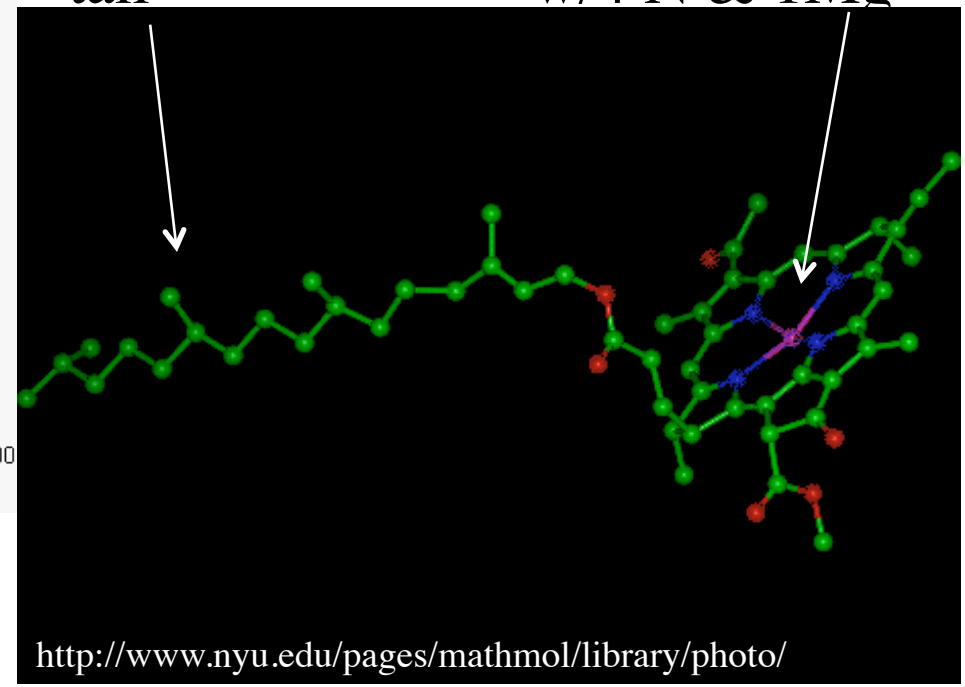
phycobilins

water soluble pigments; phycoerythrin can fluorescence



phytol tail

Porphyrin ring w/4 N & 1Mg^{++}



Chlorophyll *a*

(absorption peaks will vary, depending on environment – protein complex in membrane, polarity of solvent);
in vivo fluorescence

Degraded pigments:

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

Pheophorbide
lost Mg^{++} and phytol tail

www.ch.ic.ac.uk/local/projects/ steer/cloroads.gif

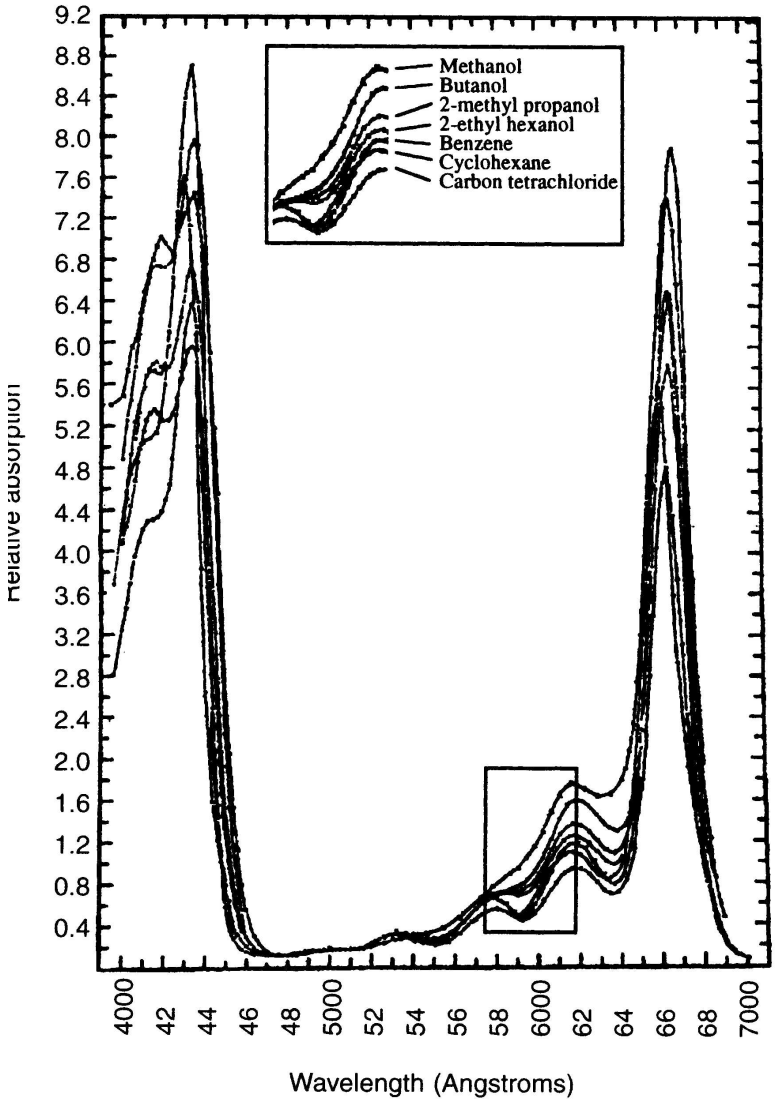
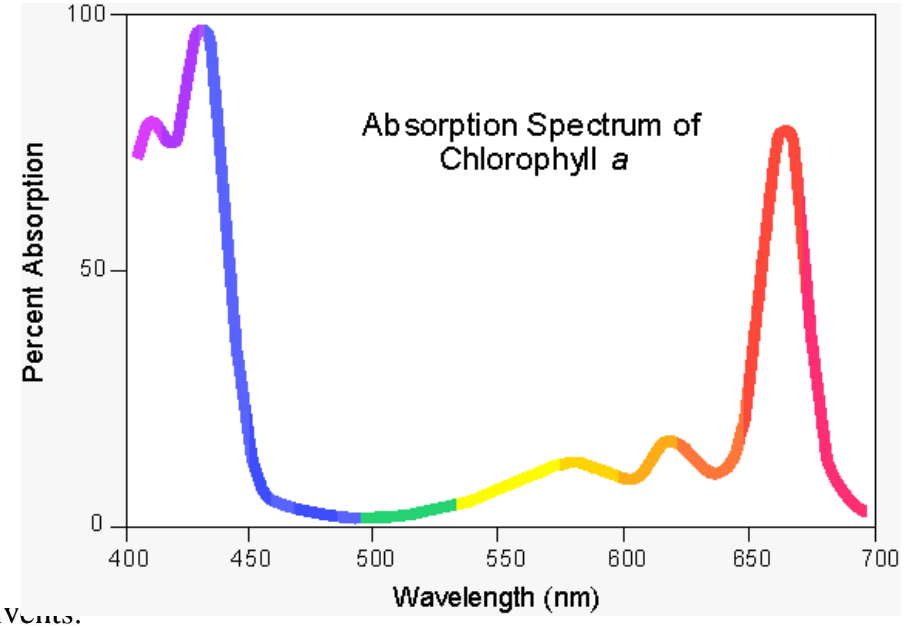


Figure 7.2
Absorption spectra of highly purified chlorophyll *a* in different solvents.
Original, after Harris and Zscheile (1943).

Note of caution:
solvent effects
position of absorption
peaks



Accessory pigments:

Chl *b* and *c*

inside chl a max peaks

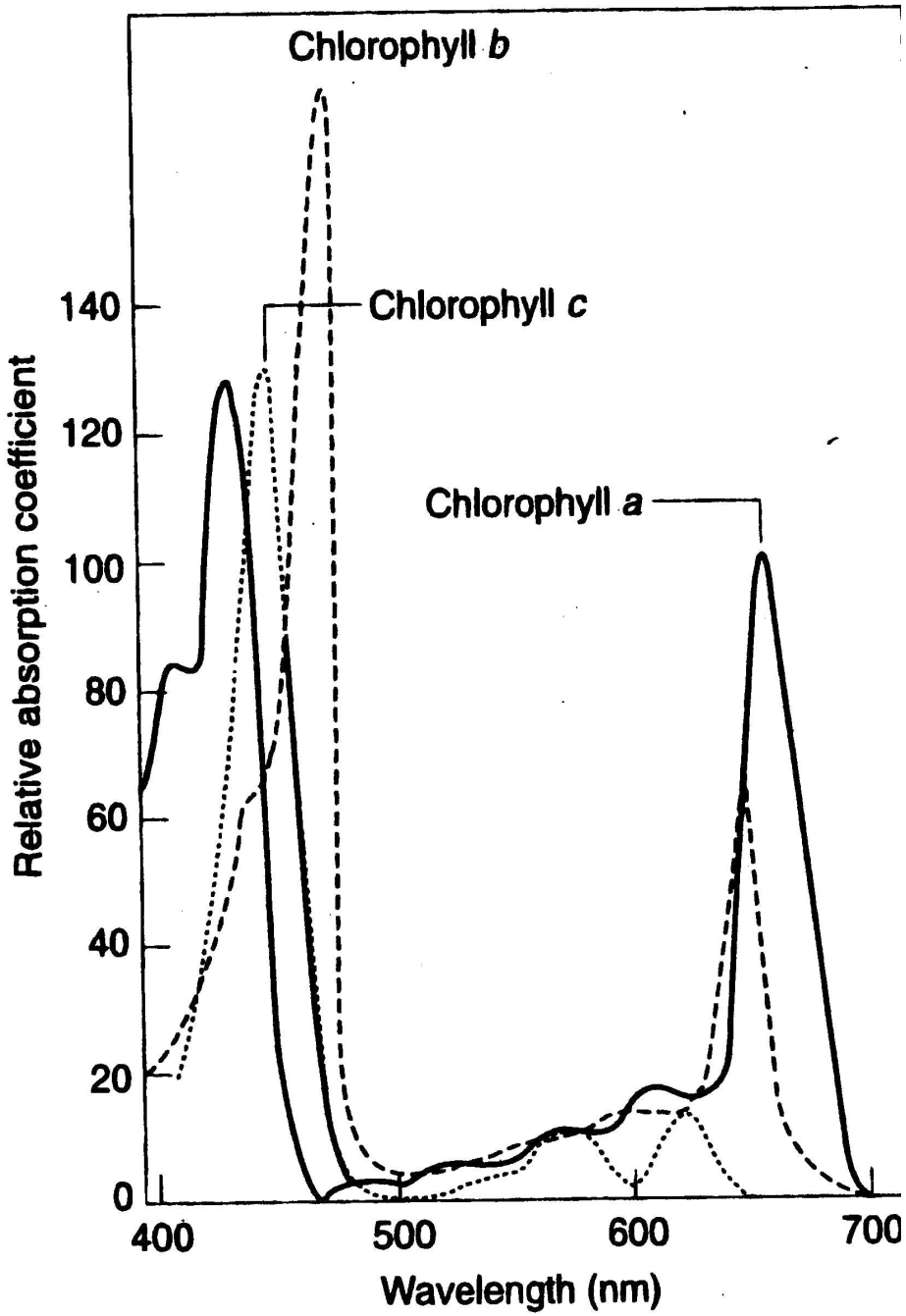
minor modification of ring

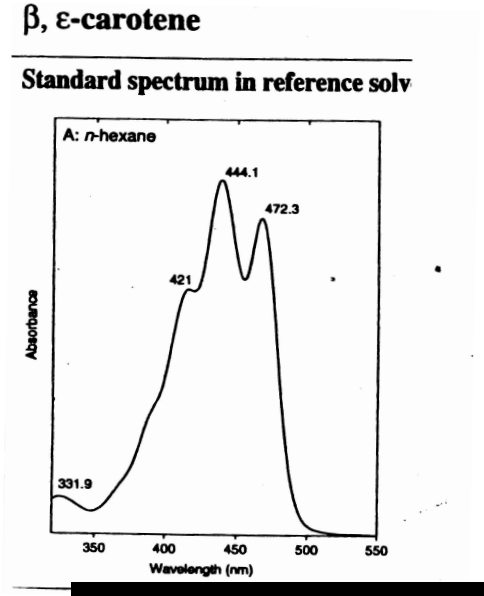
Chl *b*

in vitro fluorescence

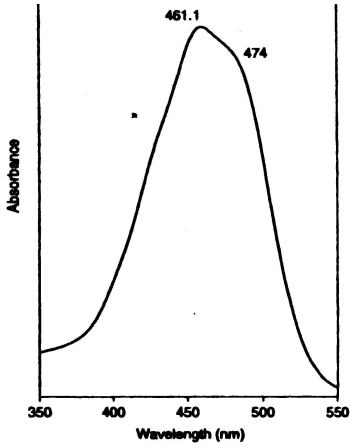
Chl *c*

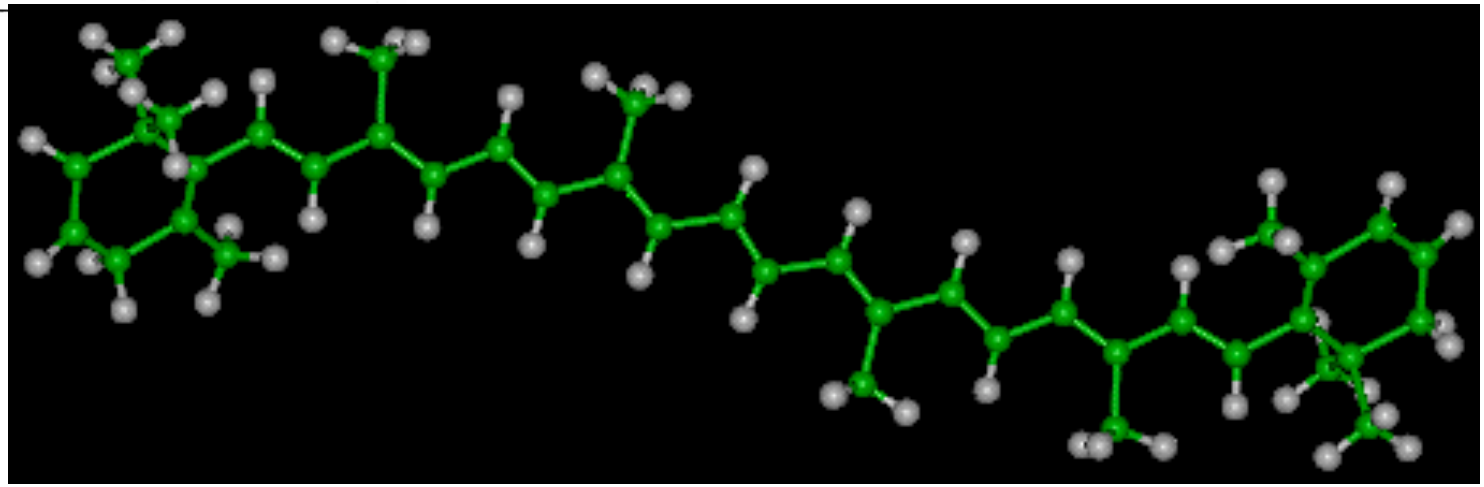
lacks phytol tail





Carotenoids





conjugated double bonds; some taxon specificity; role in photosynthesis (PS - absorb blue-green-yellow λs) and photoprotection (PP - absorb excess photons, quench free radicals & triplet oxygen)

Phycobilins (phycobiliproteins) – water soluble

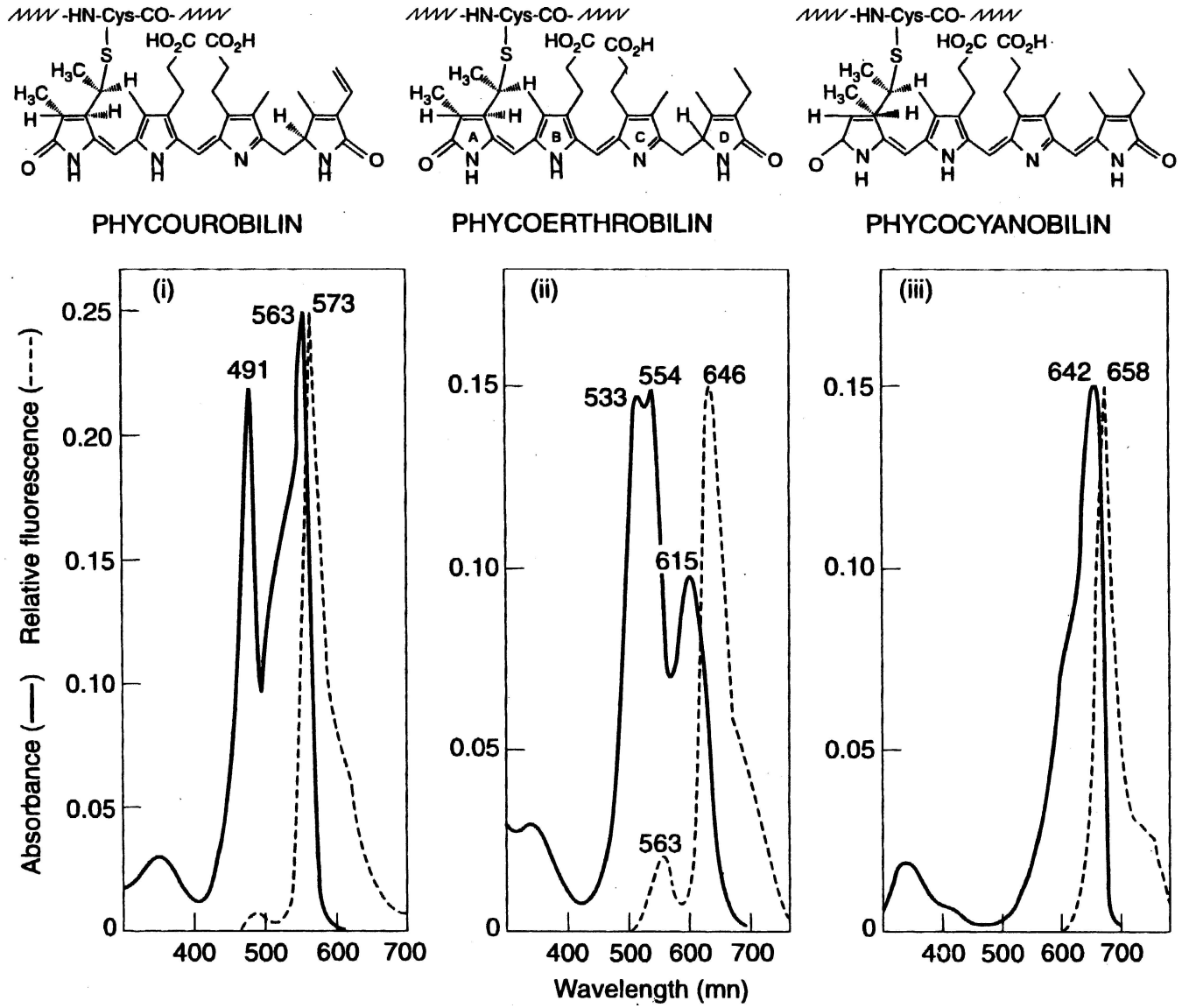
cyanobacteria and chryptomonads

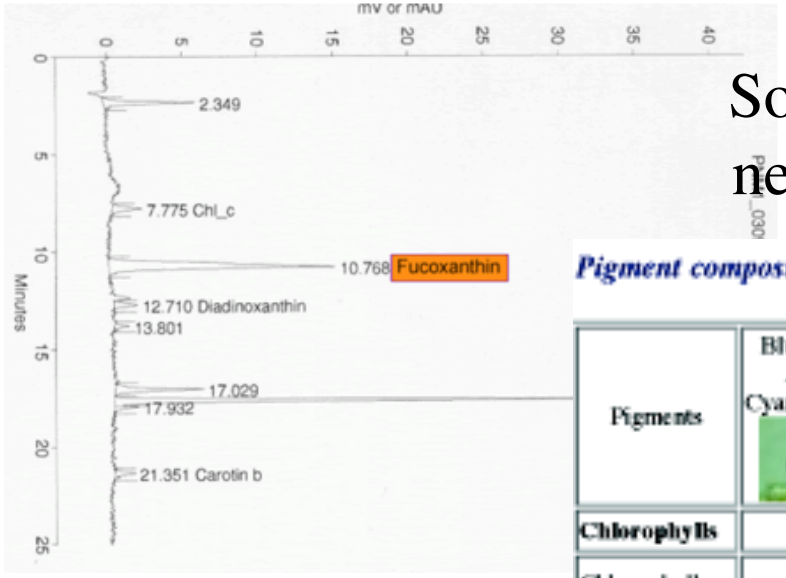
PUB
phycourobilin

PE
phycoerthyrin
(fluoresces
orange)

PC
phycocyanin

APC
allophycocyanin





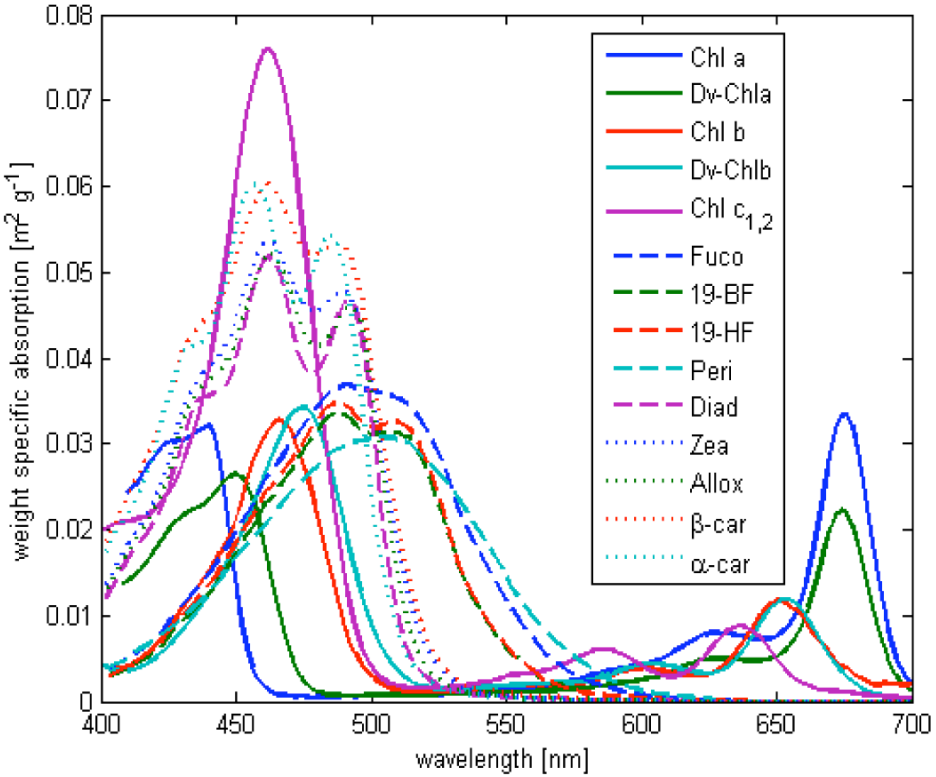
Some taxonomic information in pigments, need to assess against species information

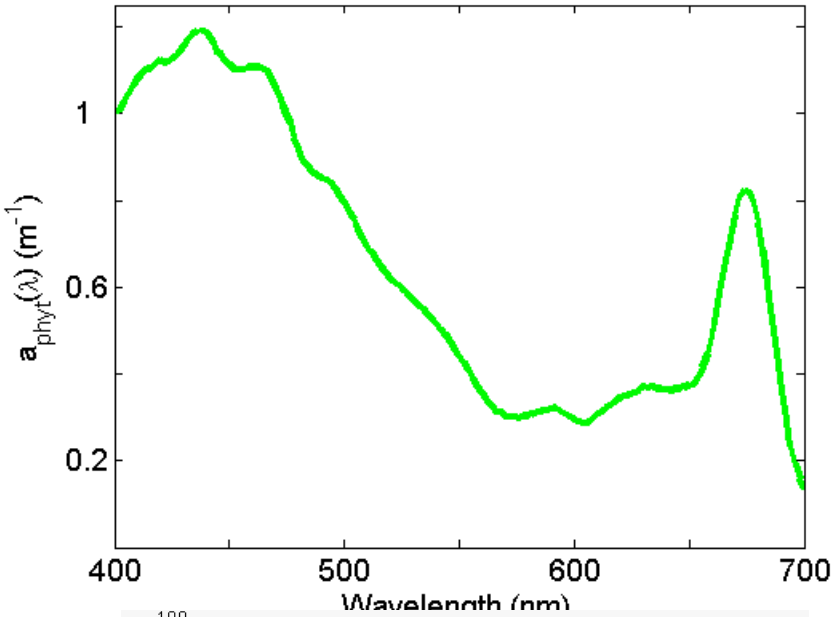
Pigment composition of the major algal groups

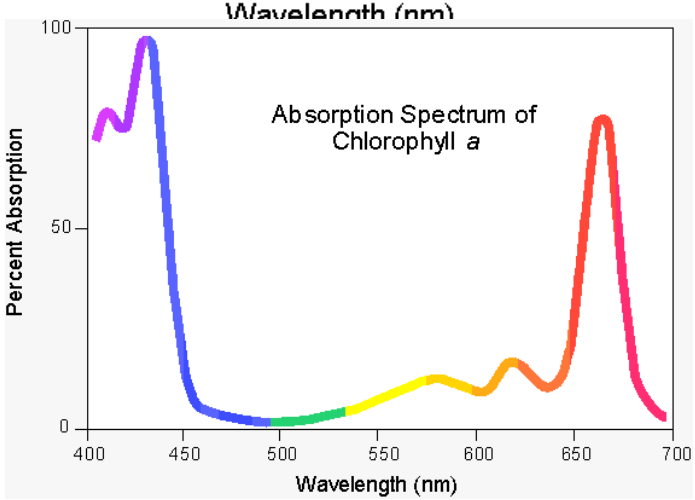
Pigments	Blue-Green Algae/ Cyanophyceae	Red Algae/ Rhodophyceae	Brown Algae/ Phaeophyceae	Green Algae/ Chlorophyceae	Dinoflagellates/ Dinophyceae	Diatoms/ Bacillariophyceae	Naked Flagellates
Chlorophylls							
Chlorophyll-a	●	●	●	●	●	●	●
Chlorophyll-b				●			
Chlorophyll-c			●		●	●	●
Phycobilins							
Phycocyanin	●	●					
Phycoerythrin	●	●					
Carotins							
ß-Carotin	●	●	●	●	●	●	●
Xanthophylls							
Diadinoxanthin			●		●	●	●
Fucoxanthin			●		●	●	●
Lutein		●		●			
Peridinin					●		
Alloxanthin							●
Zeaxanthin	●	●	●	●			

Composite absorption – why have multiple pigments?

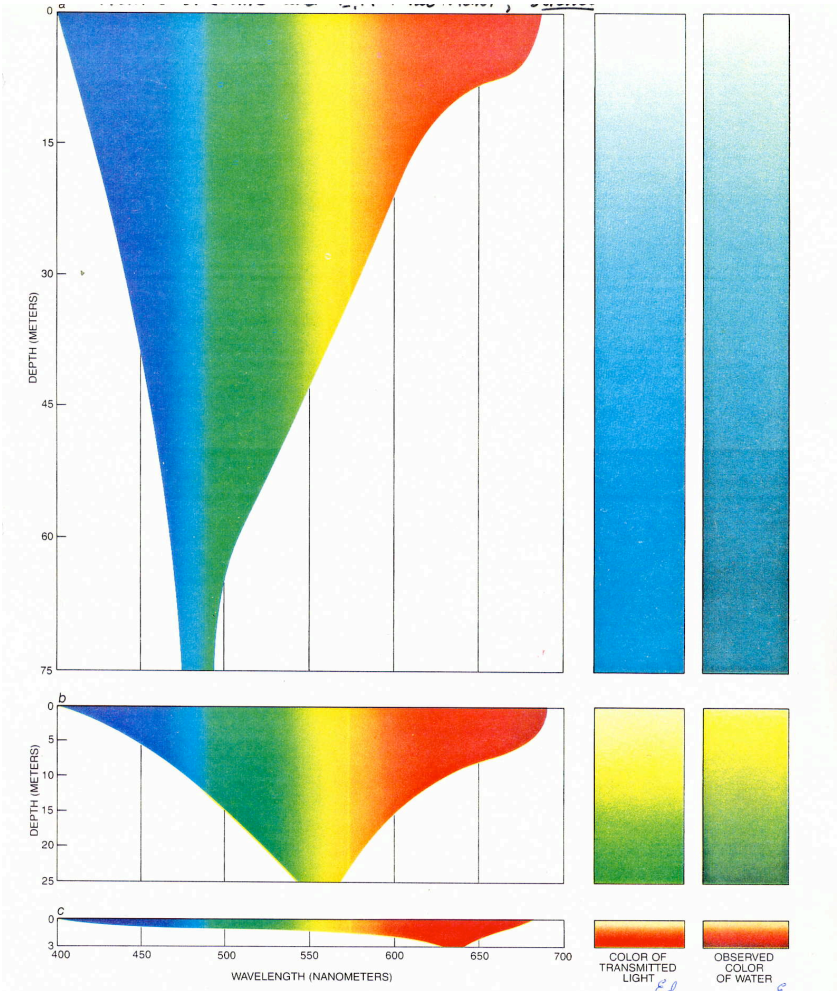
Chlorophyll *a* and *b* is good enough for spinach.



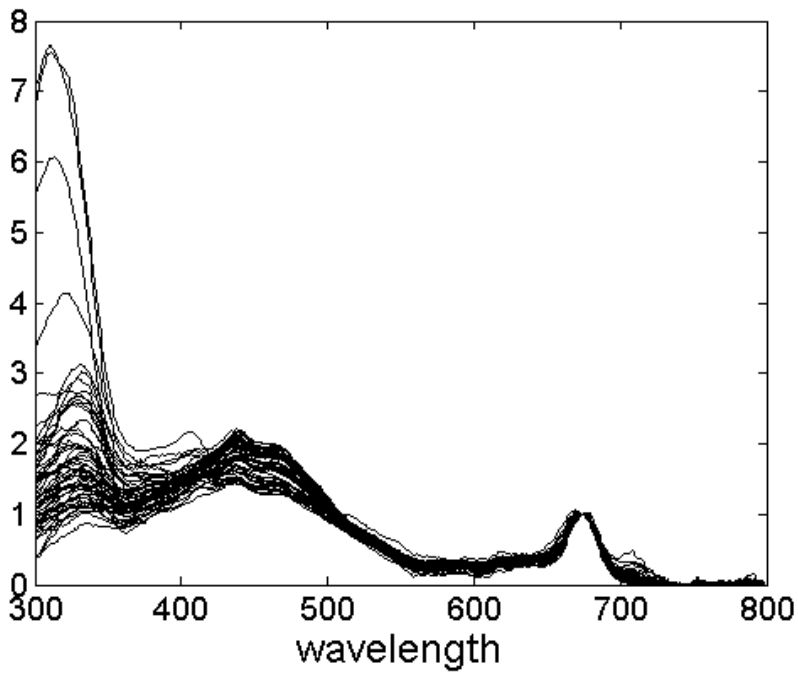




Composite absorption – multiple pigments expand environment



TRANSMISSION OF LIGHT by water is dependent on the color or wavelength of the light. In clear oceans and lakes (*a*) the light becomes increasingly monochromatic and blue as its path length increases. In fresh water that carries green organic matter (*b*) light at all wavelengths is absorbed more quickly than it is in clear water, but the light becomes greener with path length. In rivers, swamps and marshes that carry large amounts of the products of plant and animal decay (*c*) absorption is rapid and the spectral distribution of the light shifts to the red. Such waters are called black because the human eye is relatively insensitive to light at long wavelengths; a less anthropomorphic name would be infrared water. The depths given for the maximum penetration of light are typical, but they vary widely.



Chlorophyll *a* – most common entity used to denote presence of phytoplankton and attempt to quantify concentration (mass).

.

Is chlorophyll a good proxy for phytoplankton?

Chlorophyll a (or divinyl Chl a) is found in all phytoplankton and not in heterotrophs (exception: mixotrophs, digesting predators).

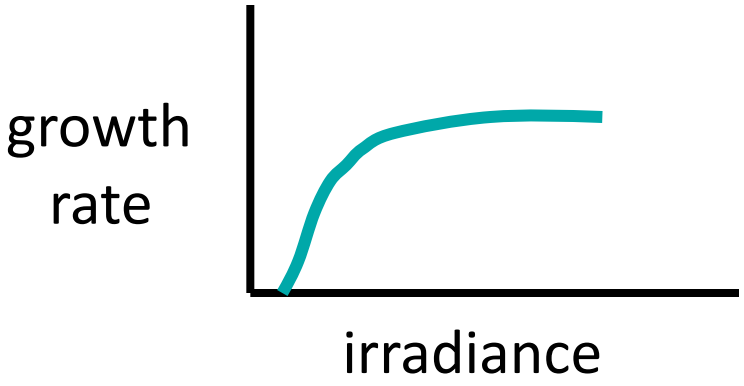
Relationship between carbon and Chl allows estimation of phytoplankton carbon. But beware Mobley's Law of Conservation of Misery: **C/Chl ratio influenced by physiology**.

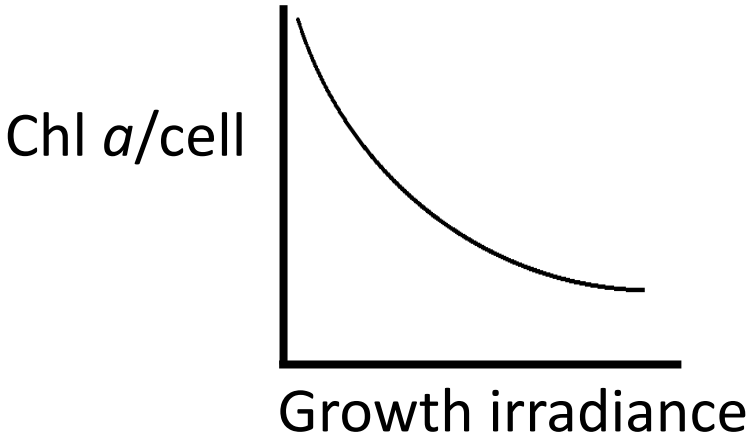
Some measure of assessing chlorophyll can be used at all scales – from mooring, ship, autonomous platform, satellite.

Different measures of assessing 'chlorophyll' need to be aligned; not measuring exactly same thing.

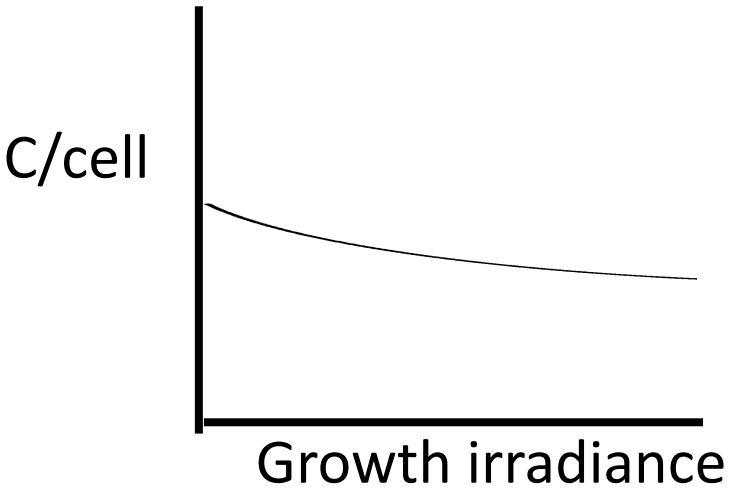
Variability in Chl / cell - cartoon

This is physiological adaptation to low light
– increase amount of light collectors (chlorophyll molecules).

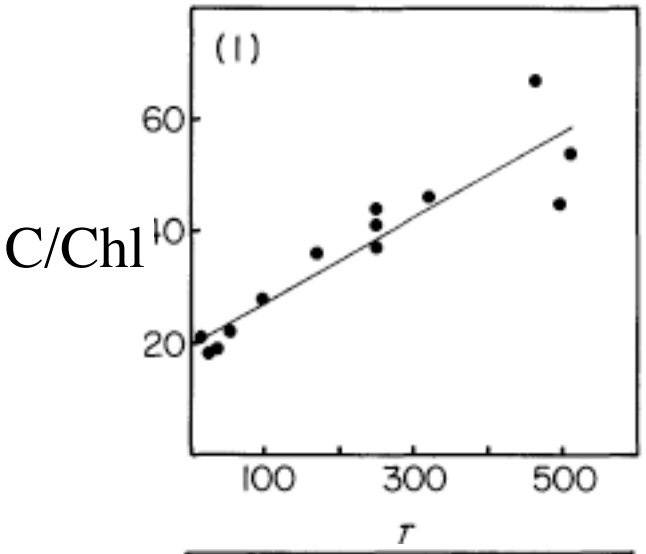


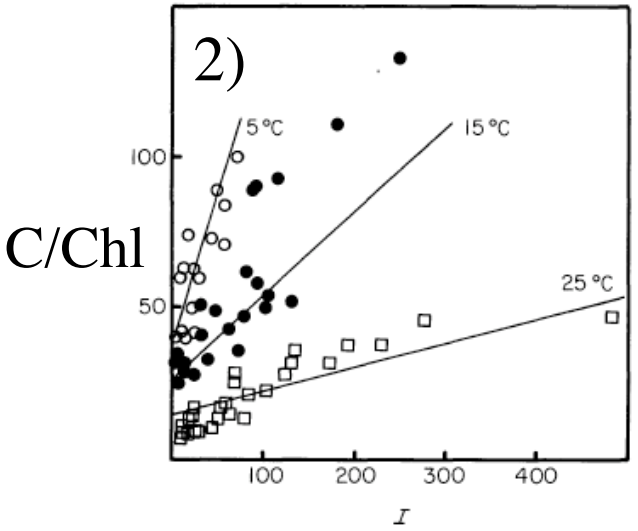


Greater variability in Chl/cell (vs. C/cell) translates into a plastic C/Chl ratio (next slide).



Real data - higher concentrations of chlorophyll and other pigments allow cells to grow better at lower irradiances

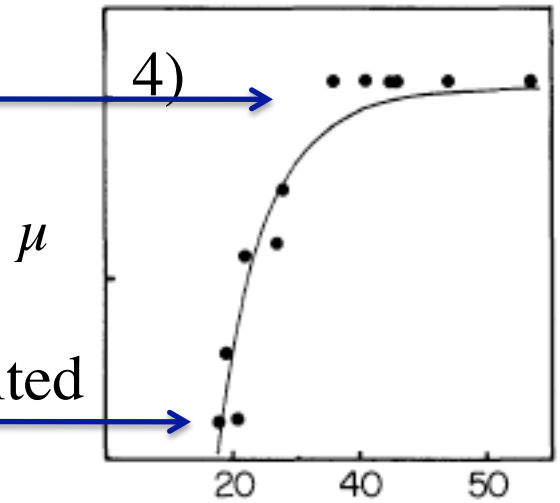


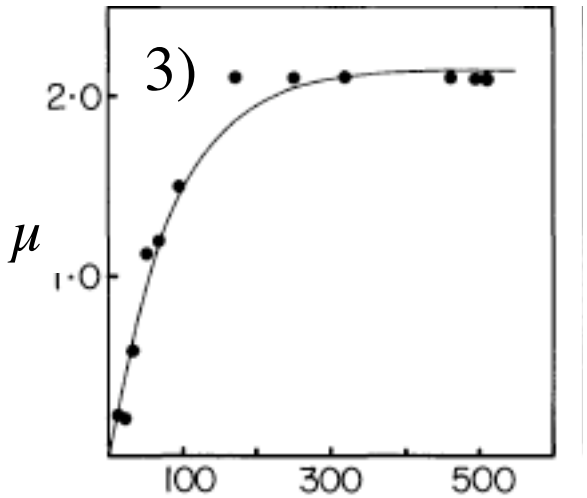


Temp

Light not limited

Light limited

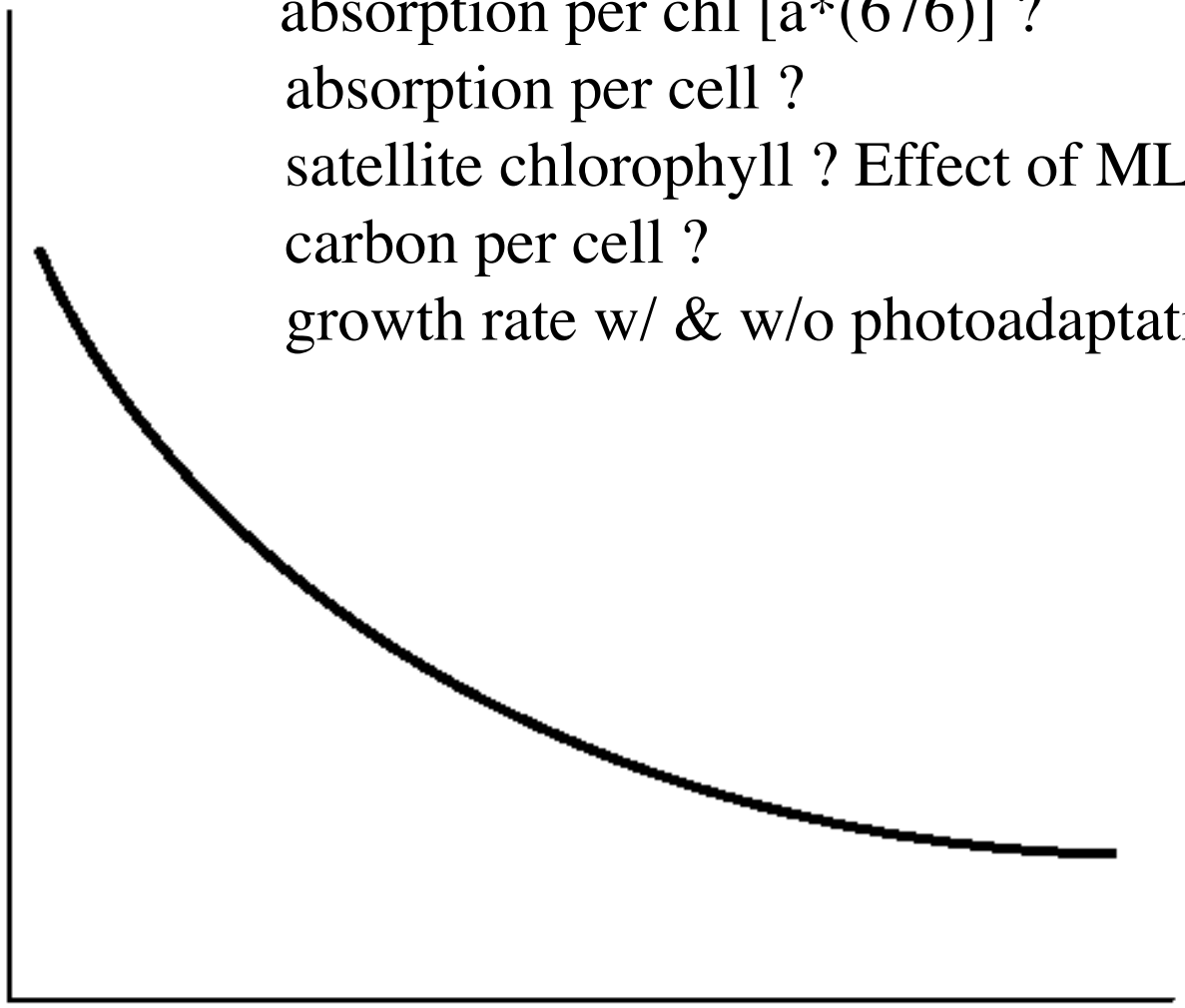


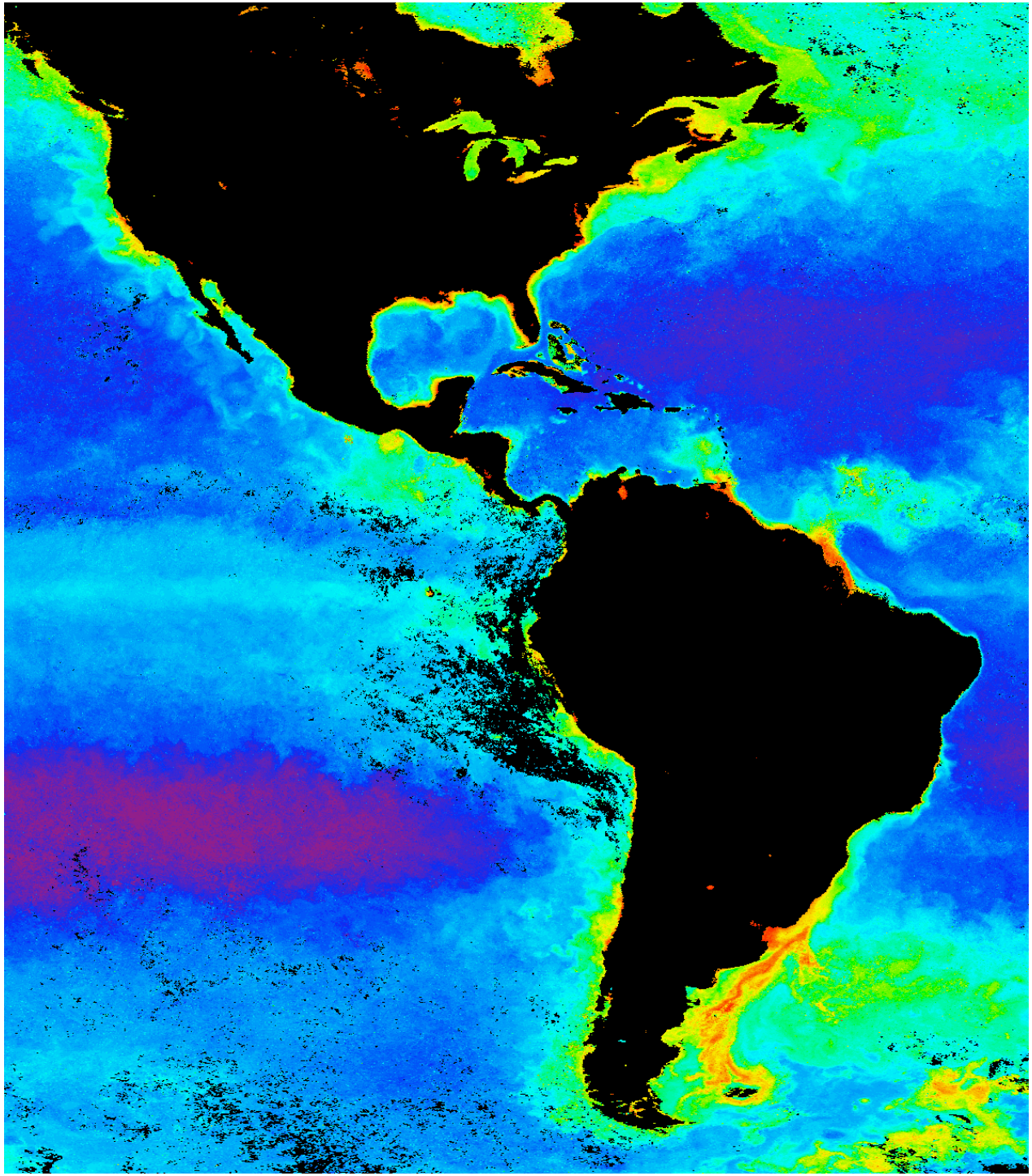


Geider. 1987. New Phytologist 106 :1

What is the consequence of photo adaptation on:

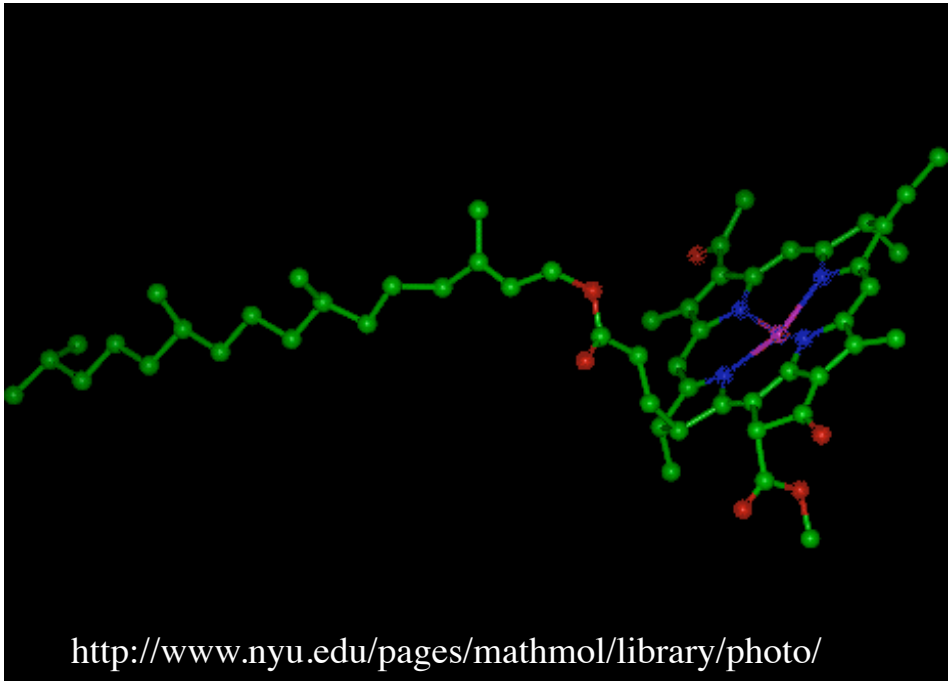
absorption per chl [a*(676)] ?
absorption per cell ?
satellite chlorophyll ? Effect of MLD?
carbon per cell ?
growth rate w/ & w/o photoadaptation ?

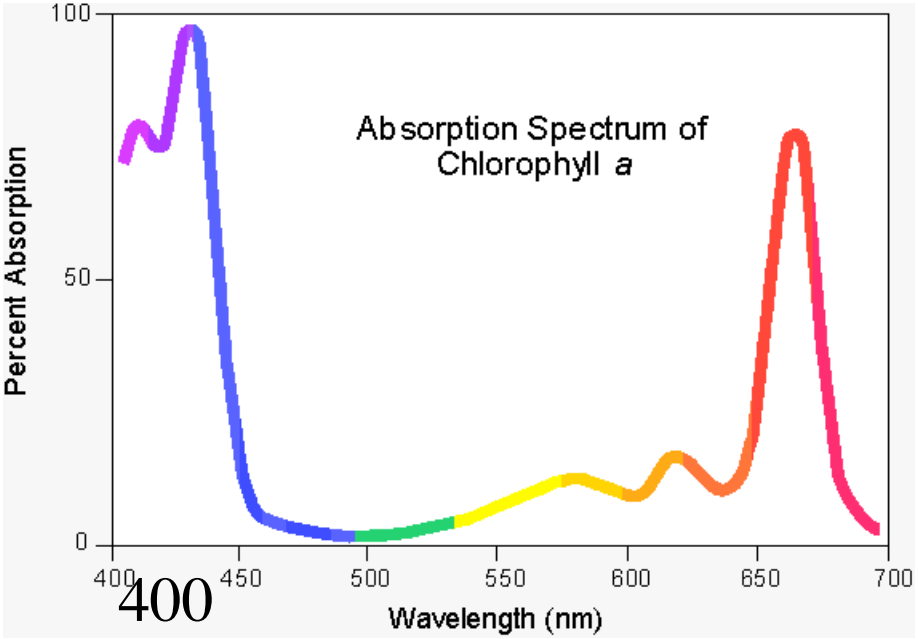




Organization of pigments
– what's the relationship between what we 'see' from space and
– chlorophyll?
– phytoplankton?

Chlorophyll *a* – chemical structure & absorption spectrum

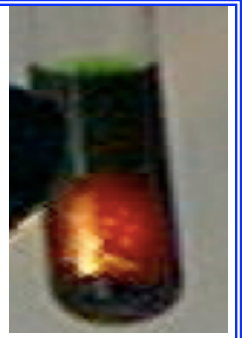




400 700

Extract chlorophyll *a*:

* filter cells GF/F filter
* extract w/ 90% acetone
* measure in fluorometer blue source, red emission
* concentration of molecule ~ red light emitted



What's the relationship of extracted Chl to its organization in cell?

Chlorophyll molecule is attached to binding protein.

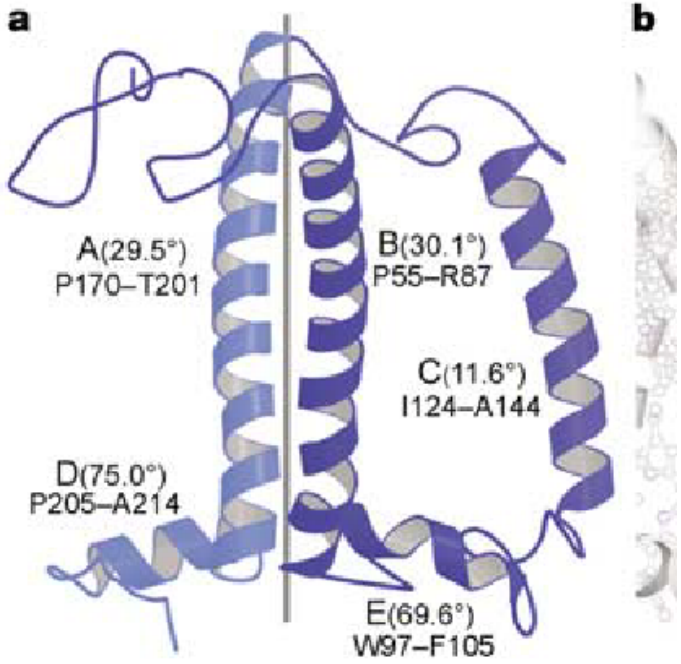
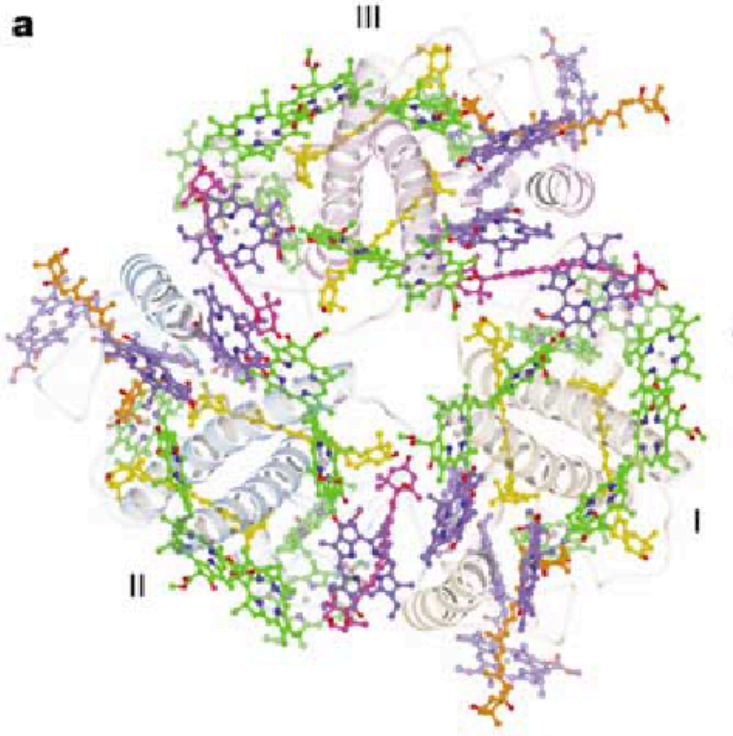


Figure 3 Secondary structure of monomeric LHC-II

protein backbone of monomeric LHC-II protein complex, from electron density mapping

Trimeric complexes of Chl and binding protein.



3 monomers = 1 trimer

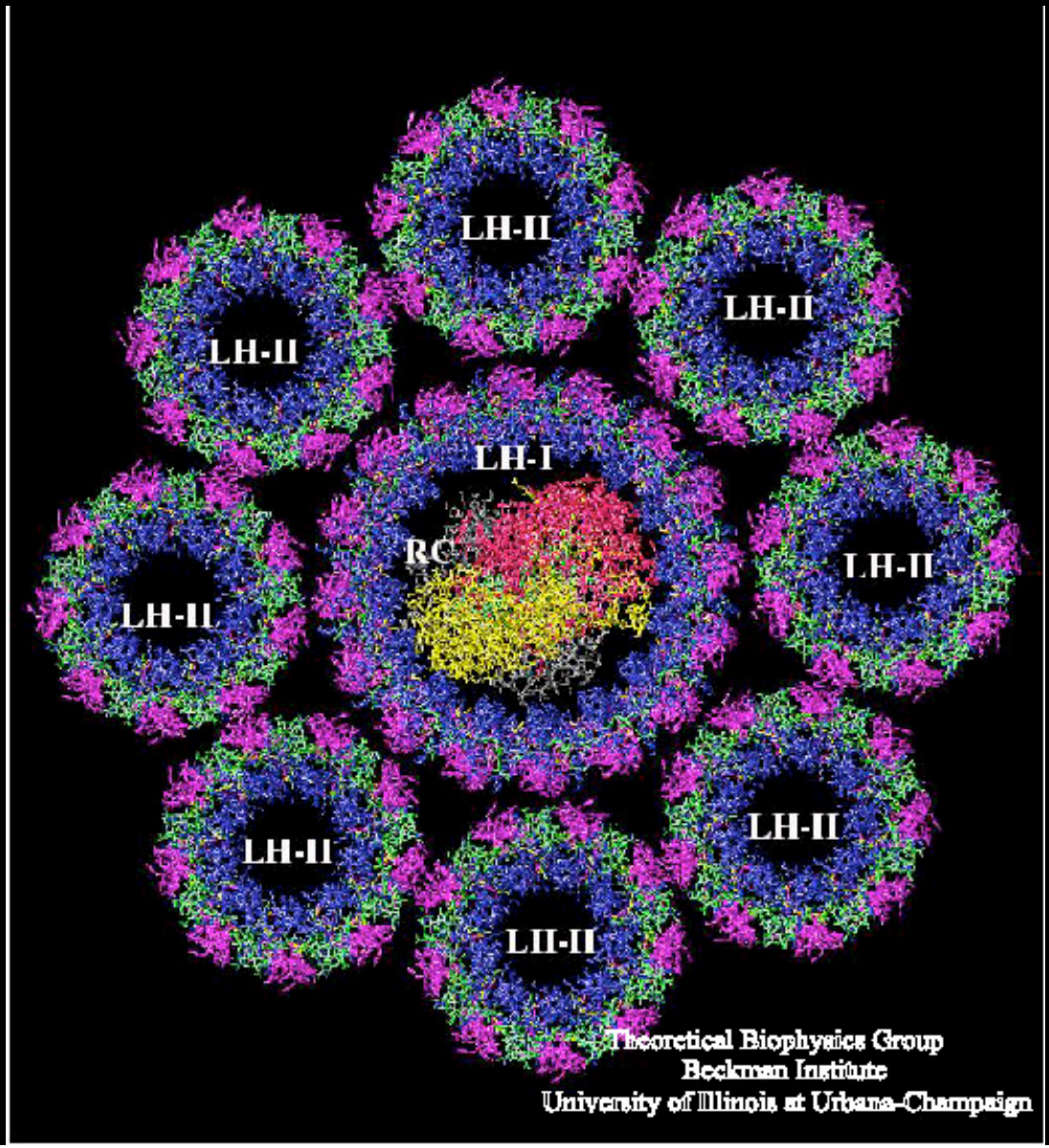
green: chl *a*; blue: chl *b*

yellow/orange: P carotenoids

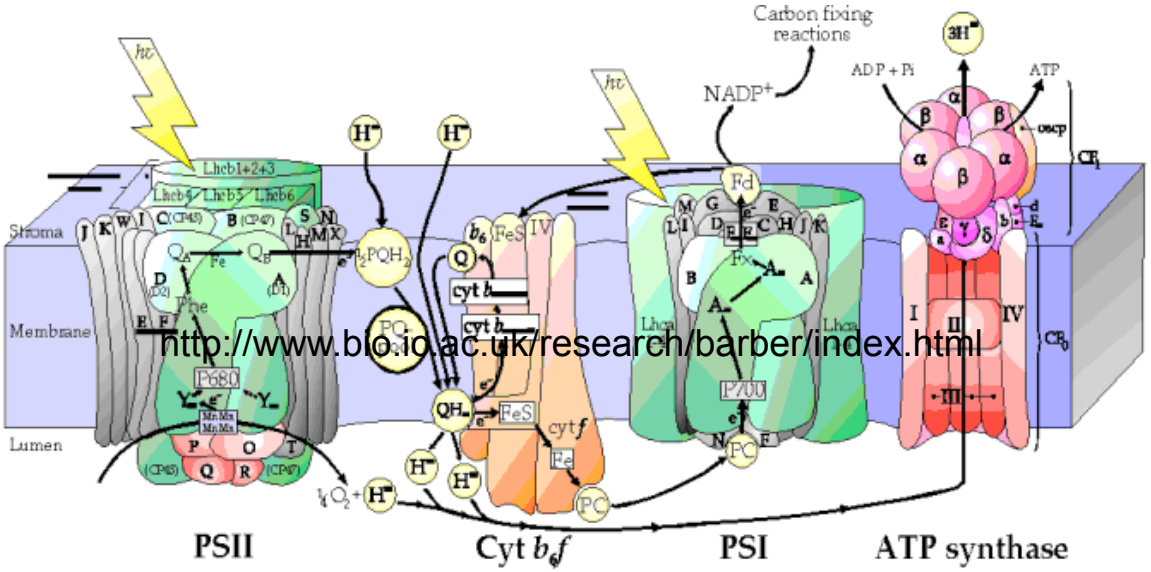
magenta: PP carotenoids

Lui et al., 2004, Nature 428: 287ff for spinach LHC-II)

Many light harvesting trimers around reaction center (PS II)
to form a light harvesting complex.



Light harvesting complexes and other functional complexes are located in thylakoid membrane.



http://www.bio.ic.ac.uk/research/barber/index.html

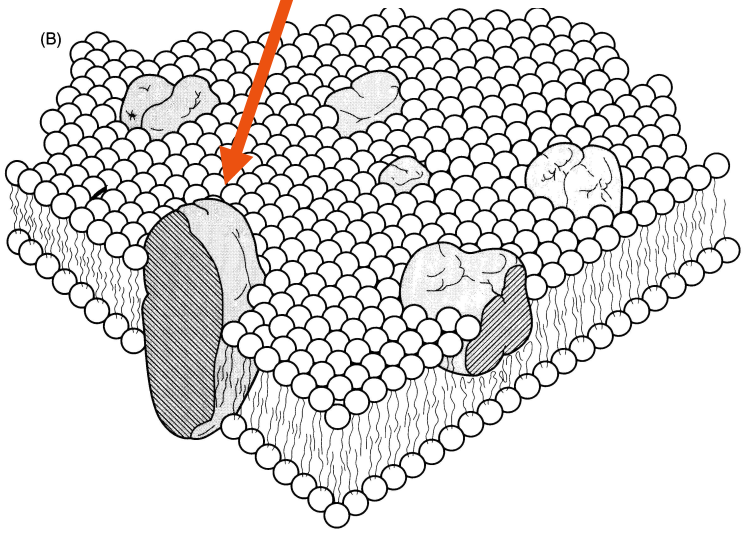
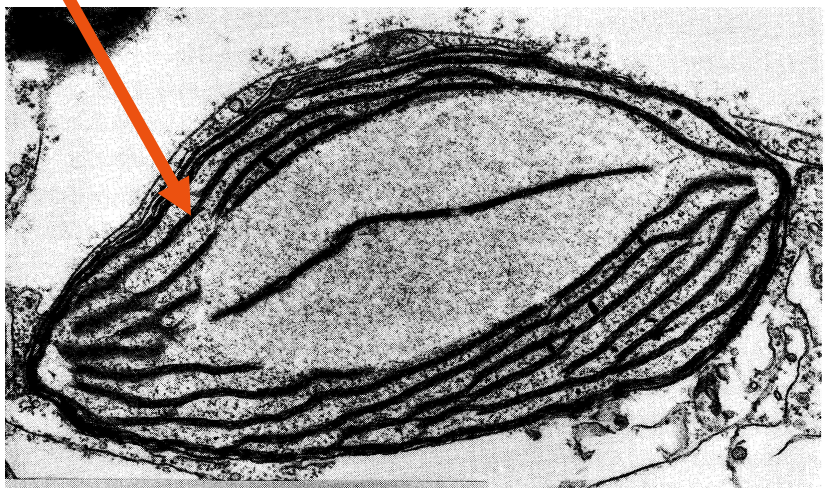
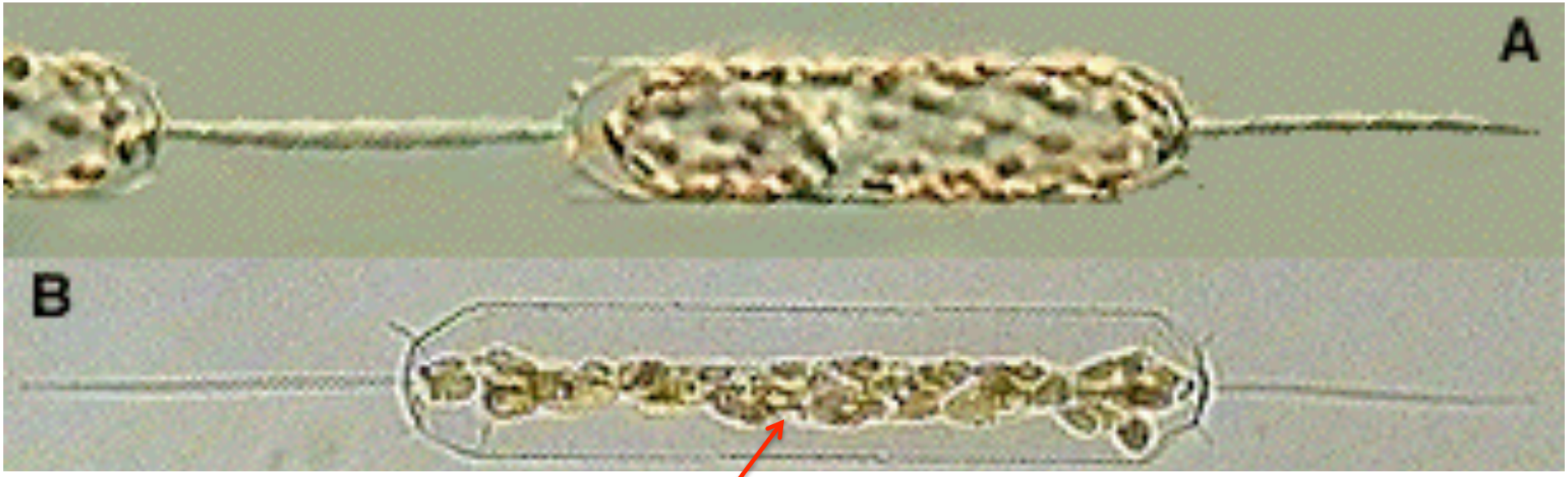


Figure 1.2 (A) Structure of two of the most important lipids that make up thylakoid membranes: monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG). In the formation of membranes, the polar sugar groups face the aqueous phases, while opposing nonpolar alkyl groups are oriented toward each other to form a lipid bilayer. The width of the bilayer is approximately 4 nm. (B) A schematic diagram of a thylakoid membrane (modified from Singer, Nicolson 1972). Thylakoid membranes are largely composed of MGDG and DGDG with other polyunsaturated fatty acids. Proteins are oriented within the membrane in a nonrandom fashion. Some proteins span the membrane, whereas others may only partially protrude. The proteins will have specific "sidedness," with some functional groups facing the lumen and others facing the stroma.

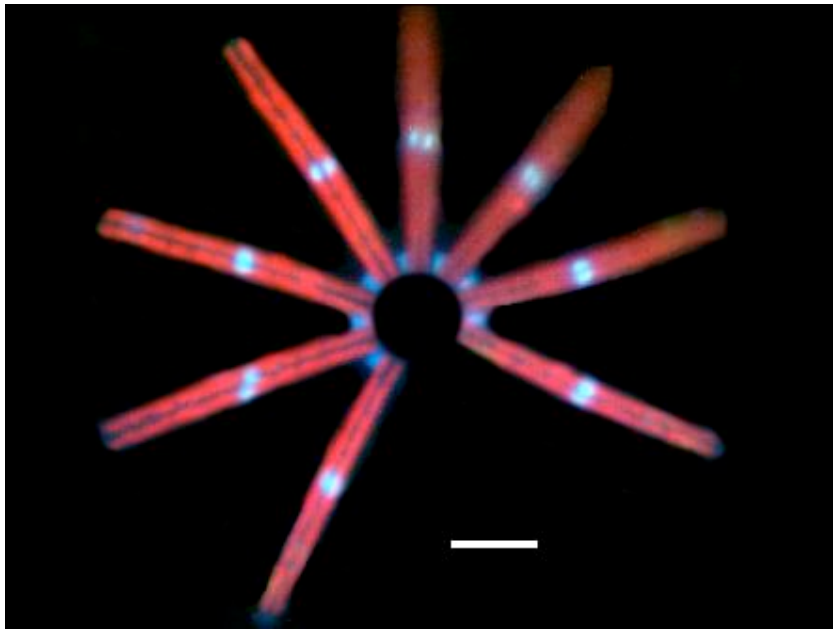
Thylakoid membranes in chloroplast

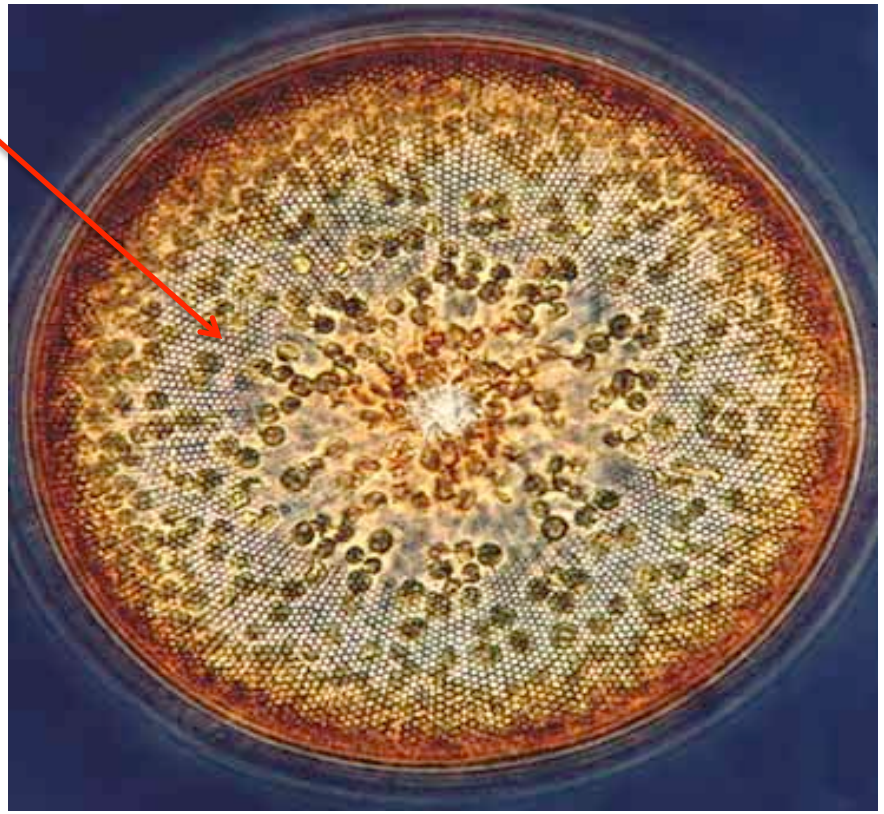




Diatom chloroplasts

In vivo chlorophyll fluorescence





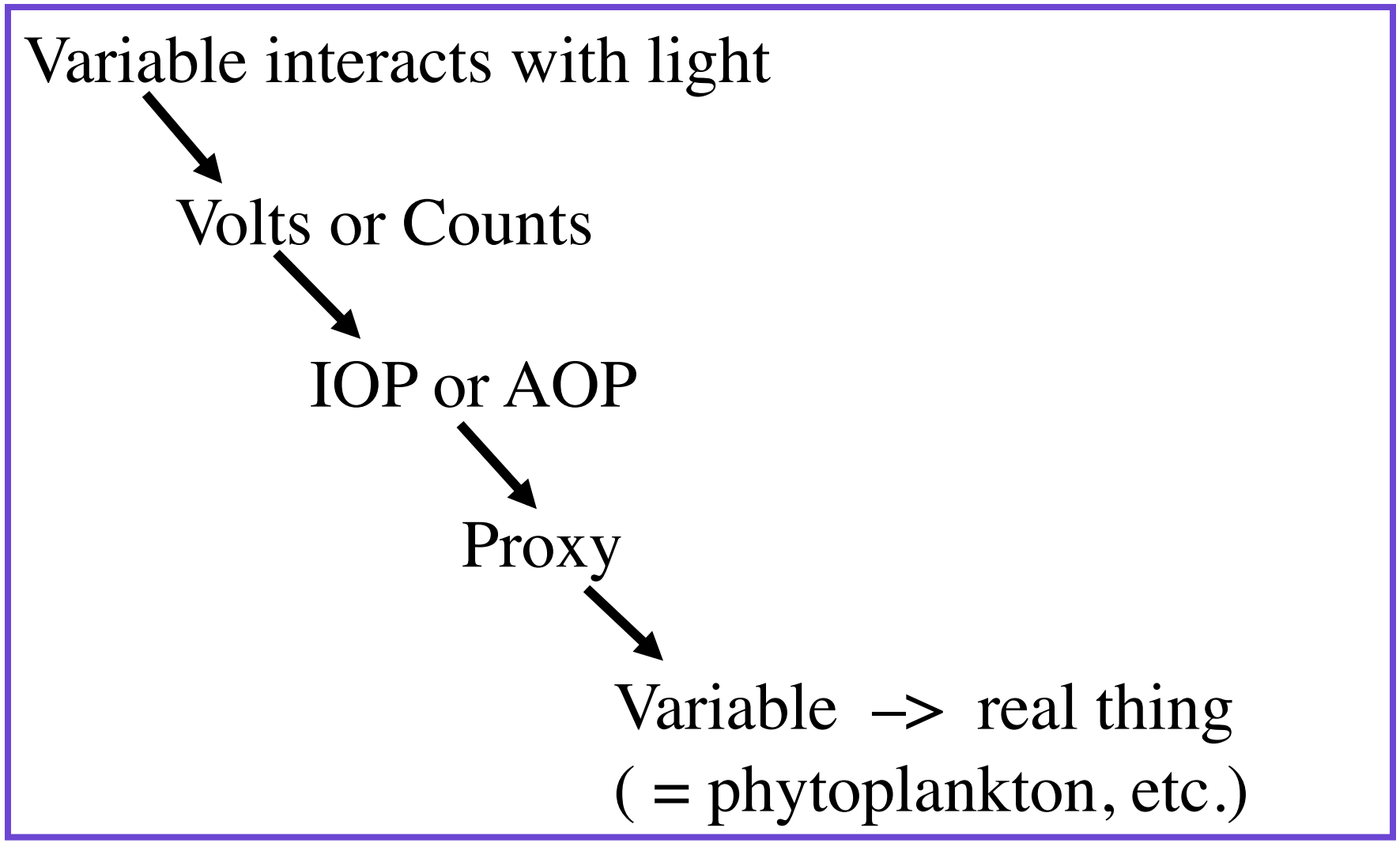
Proxies or surrogates

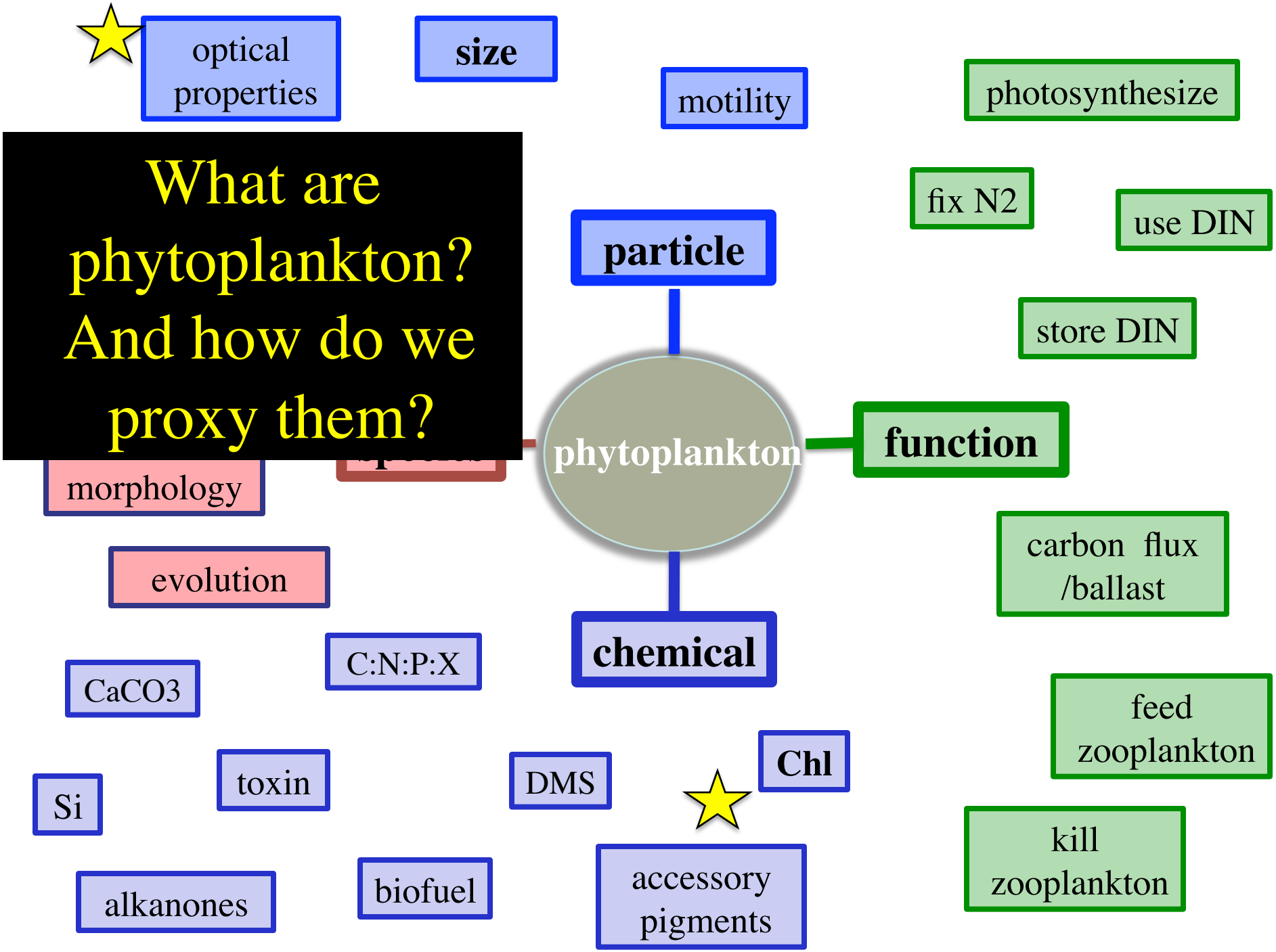
What is a phytoplankton? Cell or chlorophyll or C or ???

What are potential surrogates for phytoplankton:

* extracted chlorophyll or other pigments (HPLC)
* chlorophyll fluorescence
* absorption coefficients
  - a_{phyt}, all pigments
  - a_{ϕ} photosynthetically competent pigments
* beam c or backscatter
* particle size distribution
* particle size distribution
* what else ?

Optical Properties as Proxies or Surrogates
– what optics? which aspect of phytoplankton?




What are phytoplankton? And how do we proxy them?
phytoplankton
particle
function
chemical
optical properties
size
motility
photosynthesize
fix N2
use DIN
store DIN
morphology
evolution
carbon flux /ballast
C:N:P:X
CaCO3
feed zooplankton
Chl
toxin
DMS
Si
kill zooplankton
alkanones
biofuel
accessory pigments