biological · biophysical · engineering

bbe
moldaenke

If you know what is right,
you also know what is wrong,
but if you do not know what is wrong,
you will never achieve what is right:
chlorophyll-a analysis under discussion

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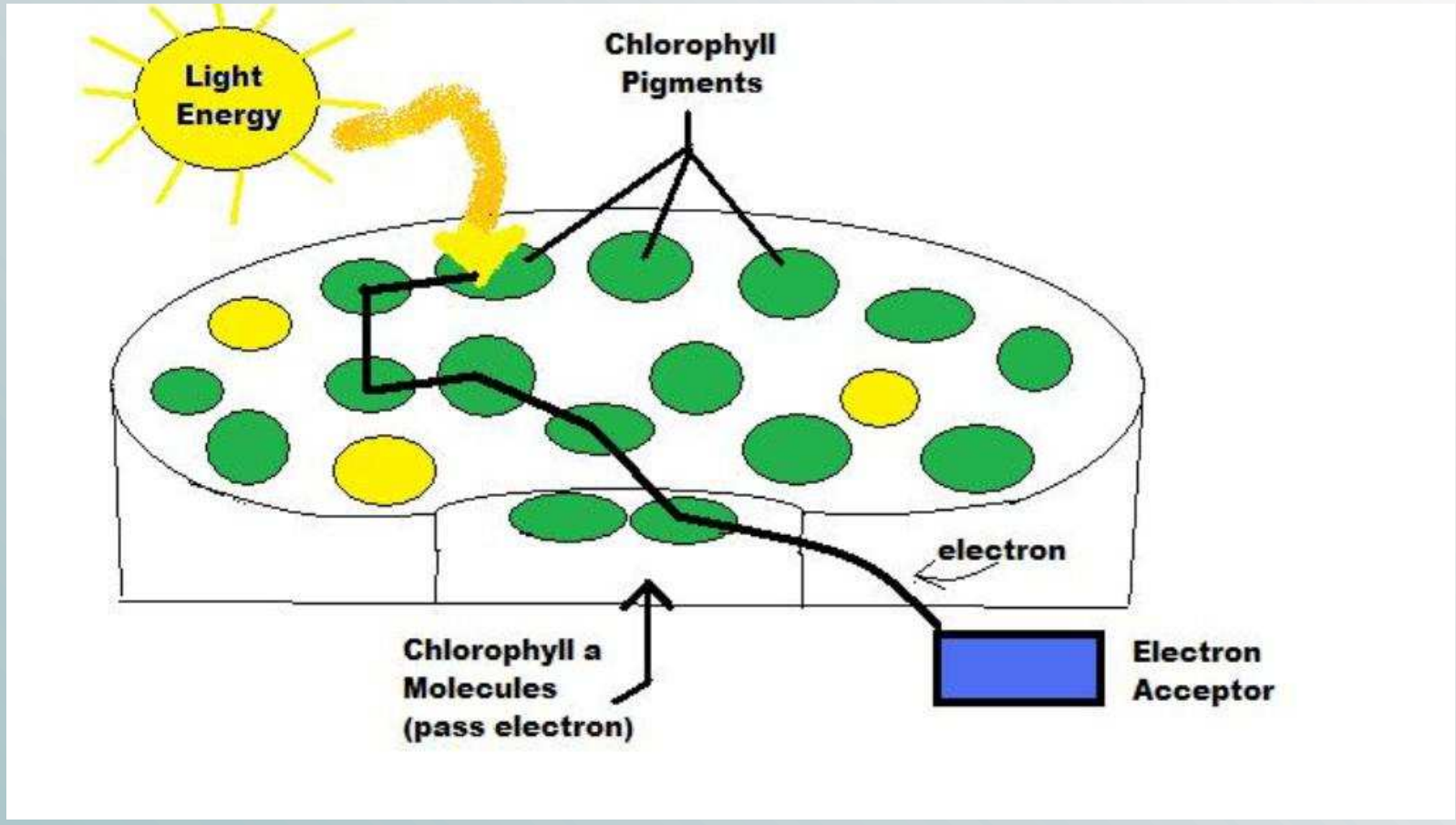
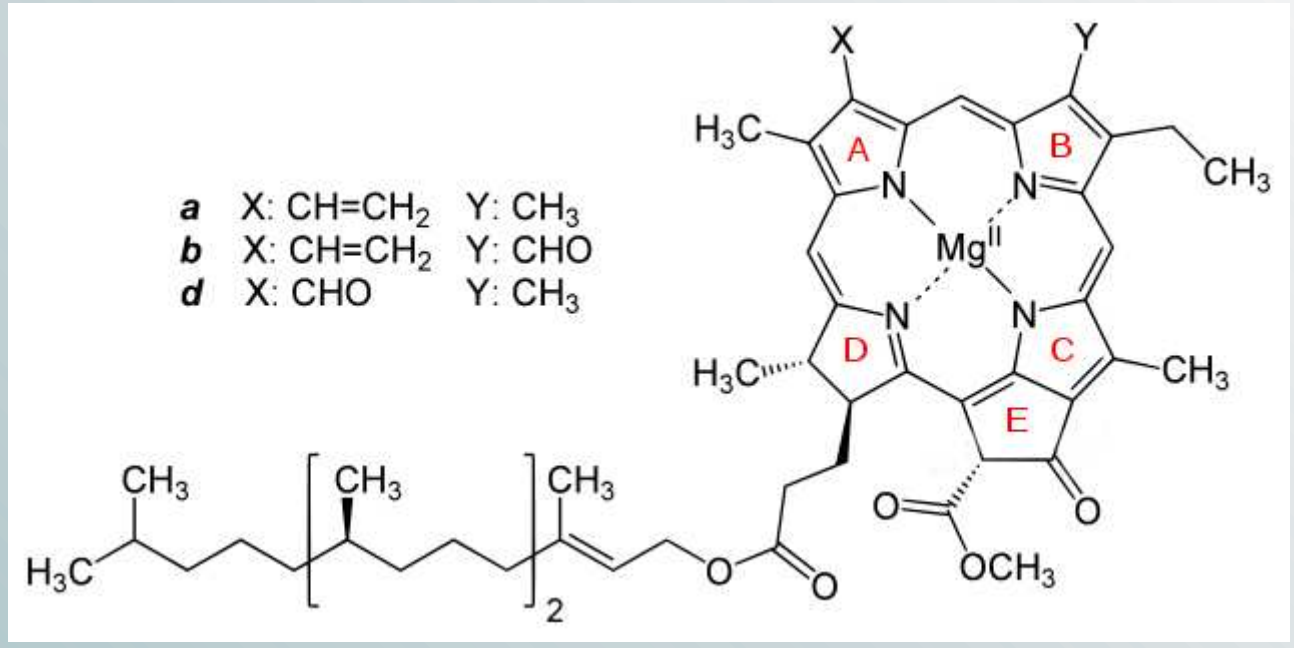
Microalgae – Why chlorophyll?


Light Energy
Chlorophyll Pigments
electron
Chlorophyll a Molecules (pass electron)
Electron Acceptor





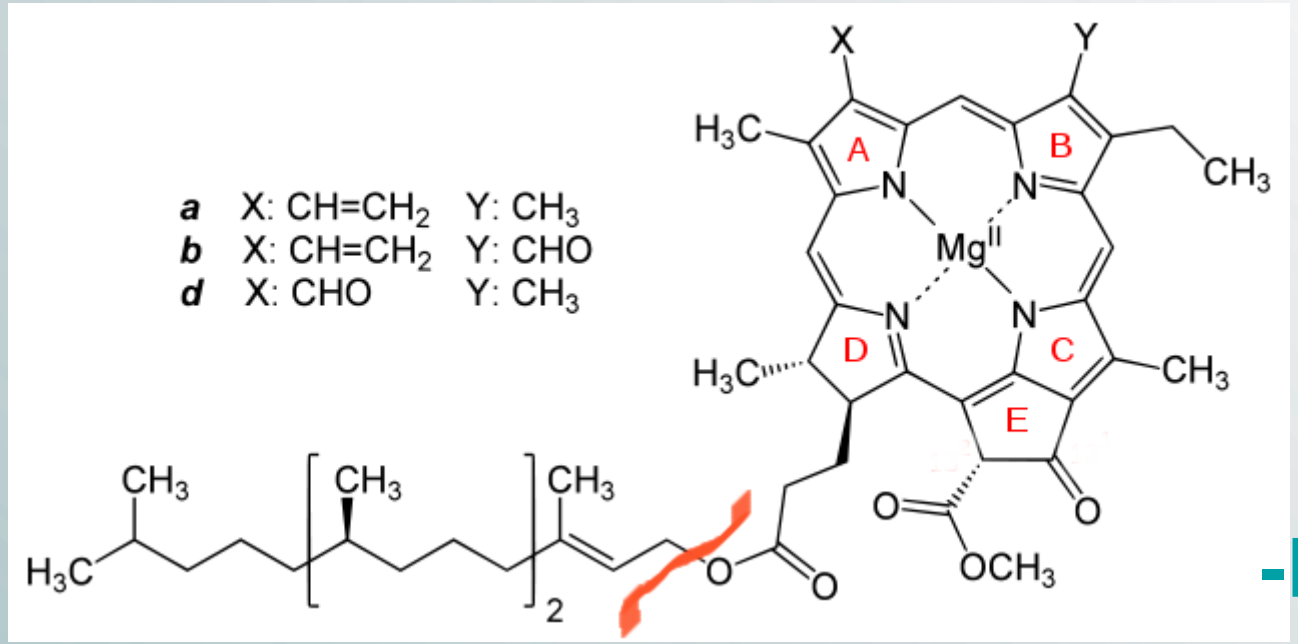
Chlorophyll



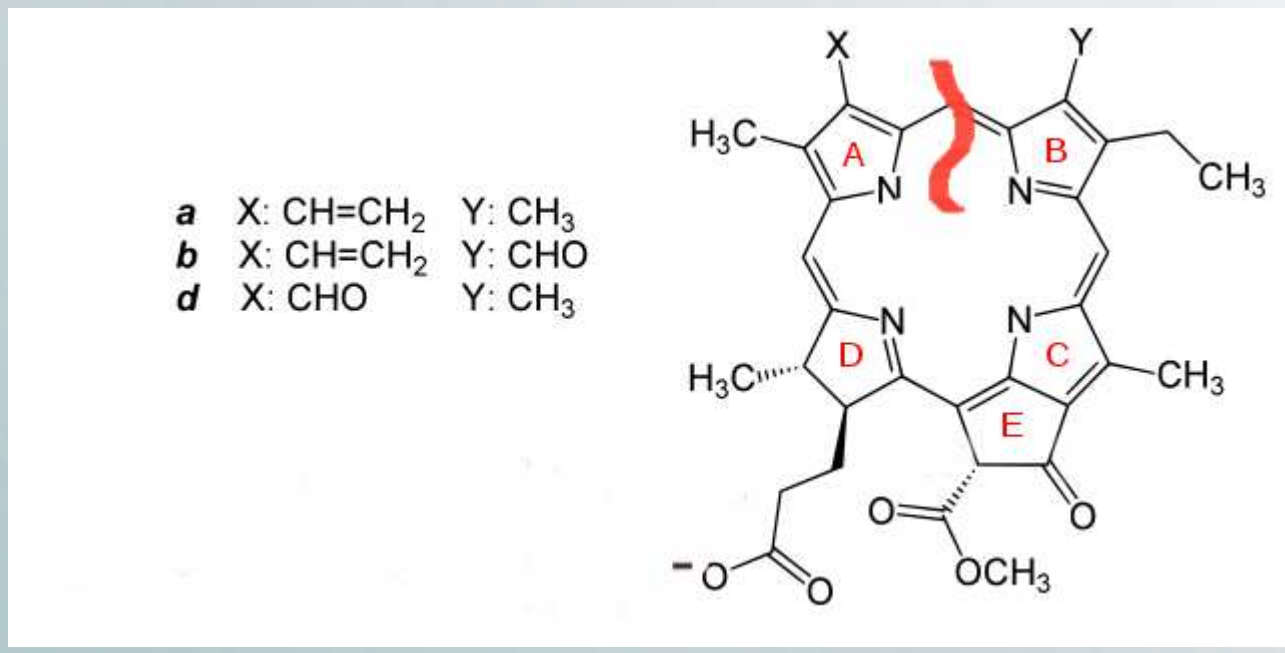




Chlorophyll and chlorophyll degradation



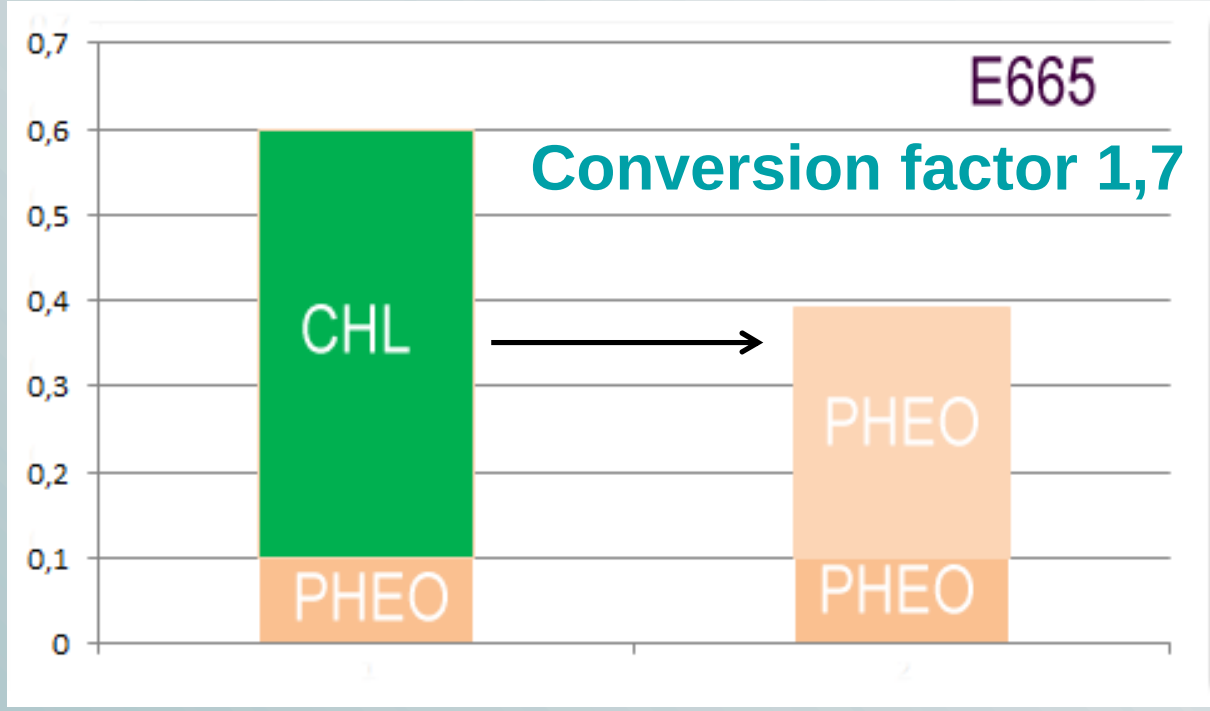
-Mg^{2+}


a X: CH=CH2 Y: CH3
b X: CH=CH2 Y: CHO
d X: CHO Y: CH3
X
Y
H3C
CH3
A
B
N
N
N
N
D
C
E
H3C
CH3
O
O
O
O
OCH3





ISO 10260 Spectrometric determination of the chlorophyll-a concentration







specific absorbtion coefficient at 665 nm		
	%	L/µg cm
Et-OH	90	82
Et-OH	96	83,4
Acetone	90	89
Me-OH	100	75-77,9

To be considered:
Chlorophyll-b, Chlorophyllids
Correction at 750 nm

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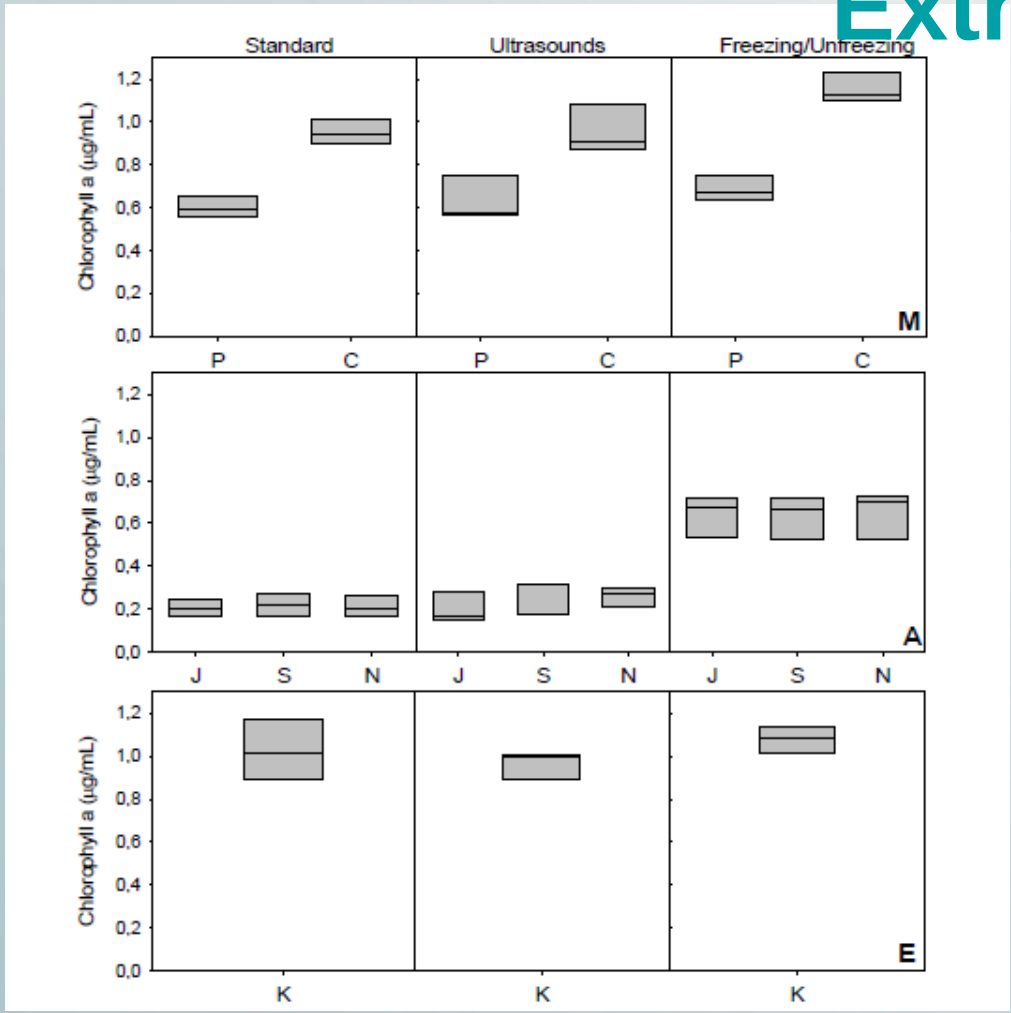
Methanol		**(M)**
Mackinney [12]	$\mu g_{chlorophyll} / mL_{medium} = 13.43\, A_{665}\, v/(lV)$	(C)
Porra et al. [13]	$\mu g_{chlorophyll} / mL_{medium} = (16.29 A_{665} - 8.54 A_{652}) v/(lV)$	(P)
	$\mu g_{chlorophyll} / mL_{medium} = 15.65\, A_{666}$	
Acetone		**(A)**
Jeffrey and Humphrey [9]	$\mu g_{chlorophyll} / mL_{medium} = (11.85 A_{664} - 1.54 A_{647} - 0.08 A_{630}) v/(lV)$	(J)
Strickland and Parsons [15]	$\mu g_{chlorophyll} / mL_{medium} = (11.66 A_{665} - 1.31 A_{645} - 0.14 A_{630}) v/(lV)$	(S)
UNESCO [8]	$\mu g_{chlorophyll} / mL_{medium} = (11.64 A_{663} - 2.16 A_{645} - 0.10 A_{630}) v/(lV)$	(N)
Ethanol		**(E)**
Kaczmar [16]	$\mu g_{chlorophyll} / mL_{medium} = (11.64 A_{663} - 2.16 A_{645} - 0.10 A_{630}) v/(lV)$	(K)





Extraction method



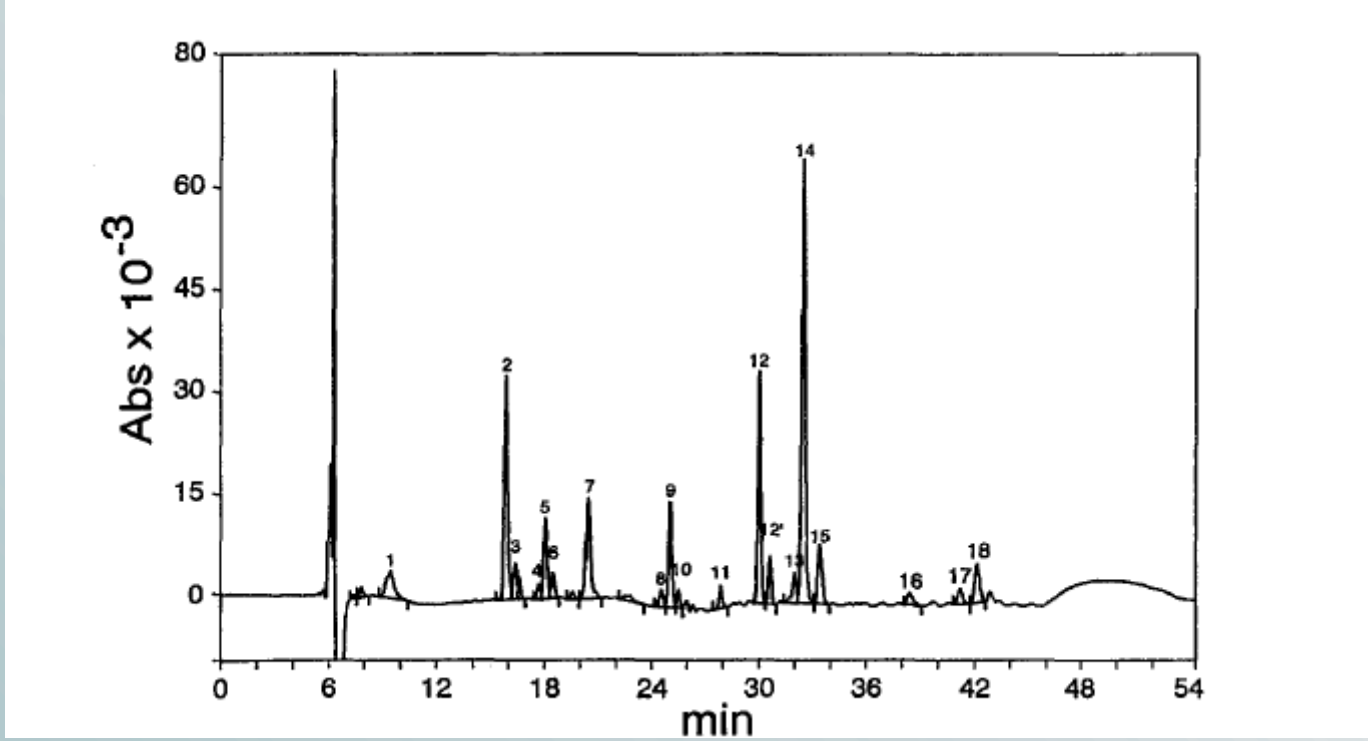
M=methanol
A=acetone)0% v /v
E=ethanol

P=Porra correlation
C=Mackinney correlation
J=Jeffrey-H. correlation
S=Strickland-P. correlation
N=Unesco
K=Kacmar correlation





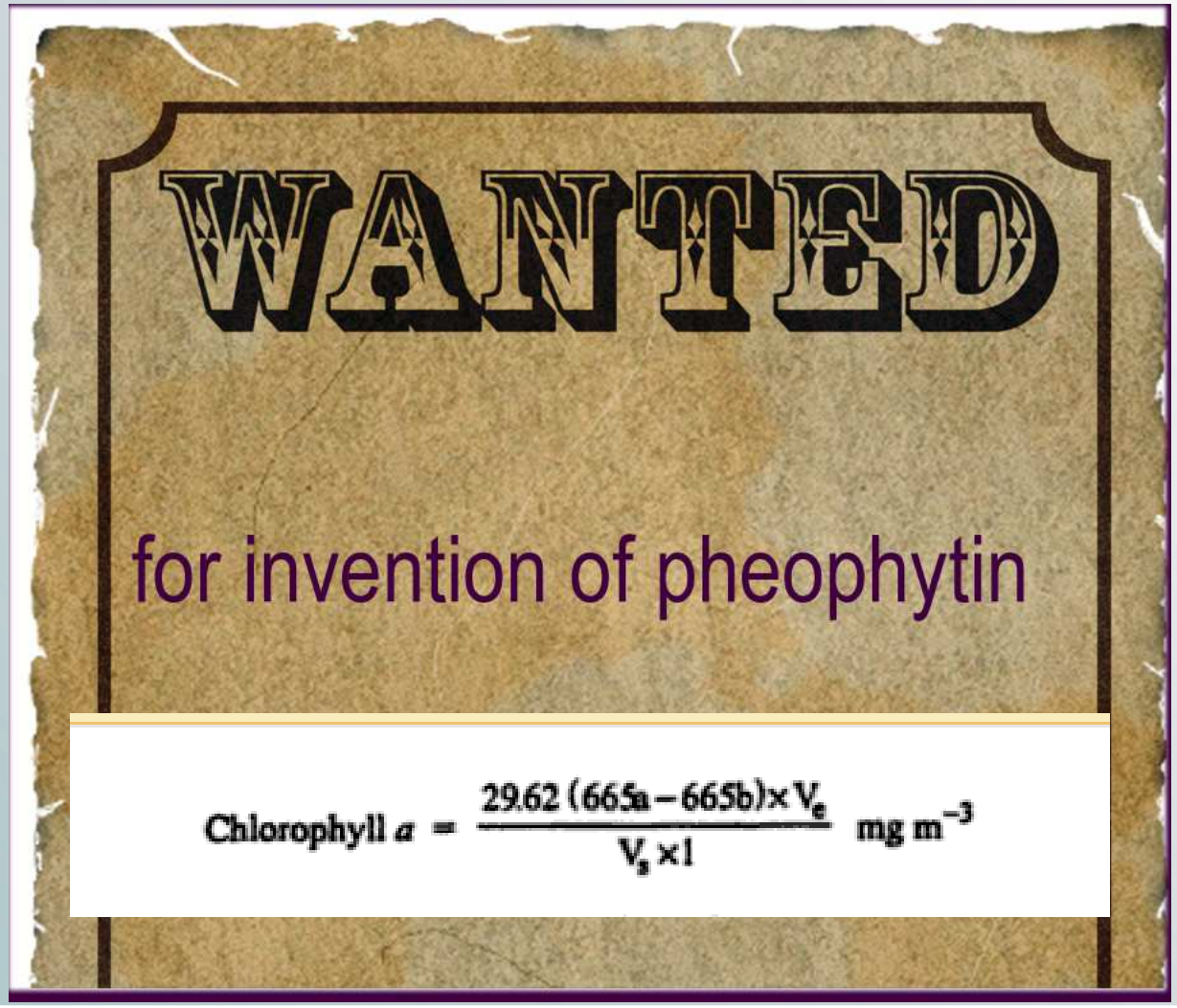
HPLC Chromatogram



People working with HPLC, almost never see a peak of pheophytin.



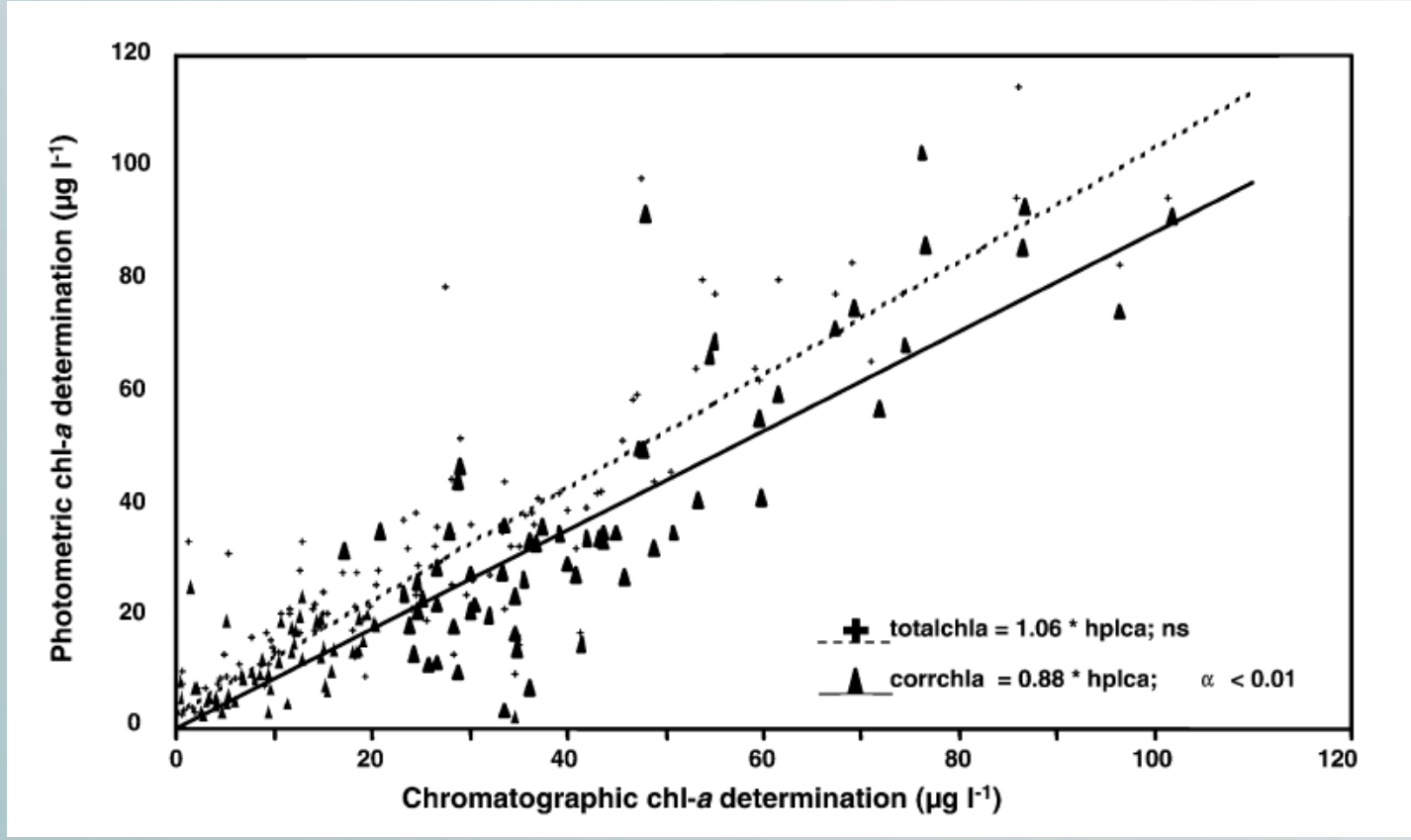



WANTED
for invention of pheophytin
Chlorophyll a = 29.62 (665a − 665b) × V_e / V_s × l mg m^-3





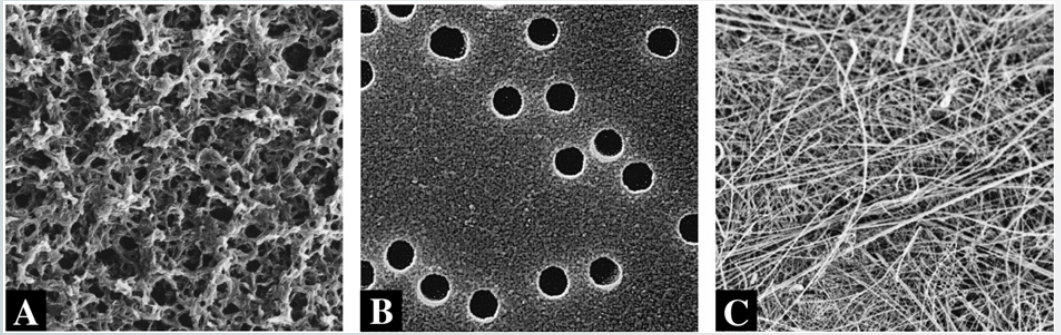
Less is better: Uncorrected versus pheopigmentcorrected photometric chlorophyll-a estimation







Filter & vacuum pump



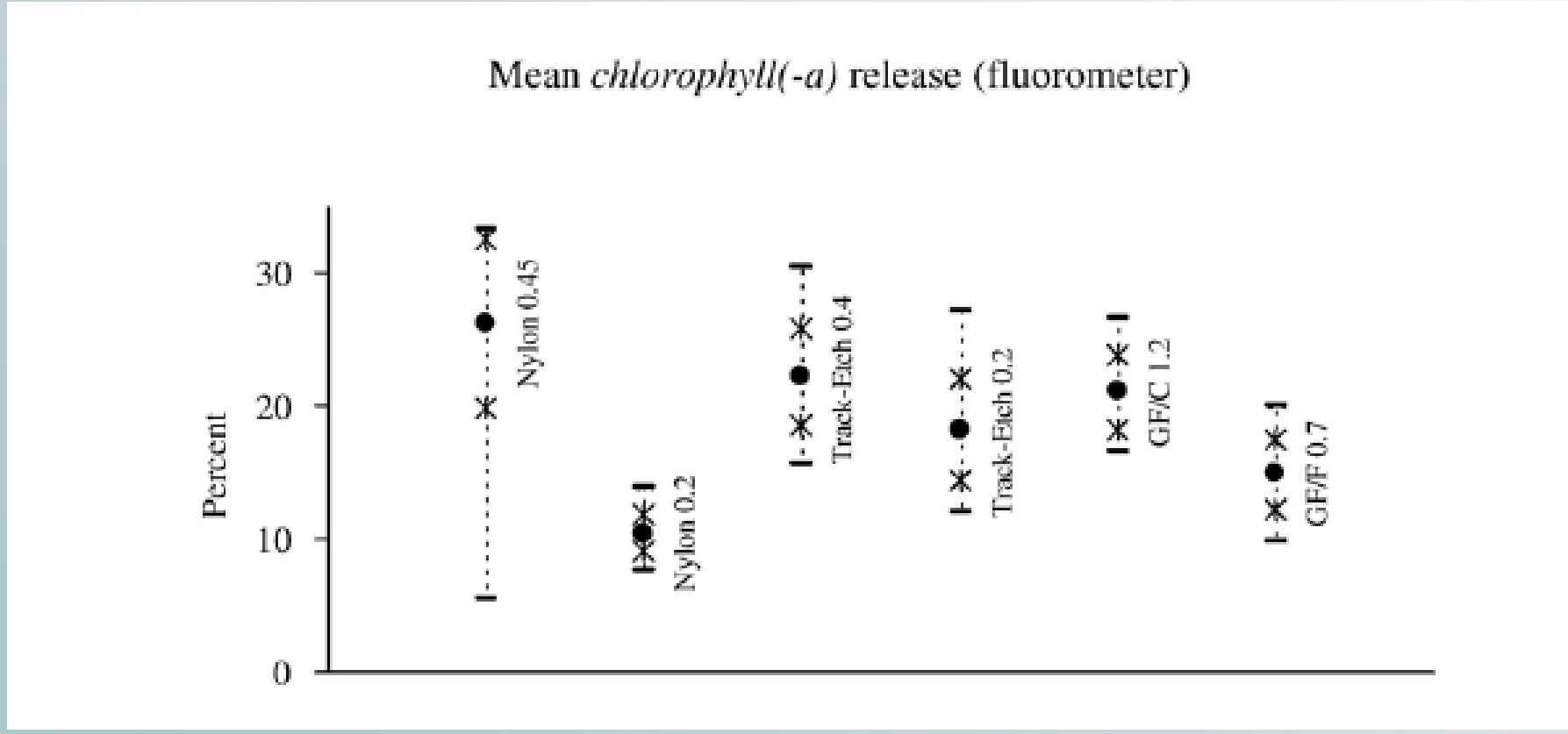
Phaeophytins

indicate degradation of chlorophyll *a* either in scenescent field populations or during analysis. When samples are concentrated by filtration for the purposes of analysis, the cells die. Consequently, the chlorophyll immediately starts to degrade to phaeopigments. If filters are not rapidly extracted or frozen, chlorophyll *a* concentrations are thus reduced.





Comparison of different filter types on *chlorophyll-a* retention and nutrient measurements



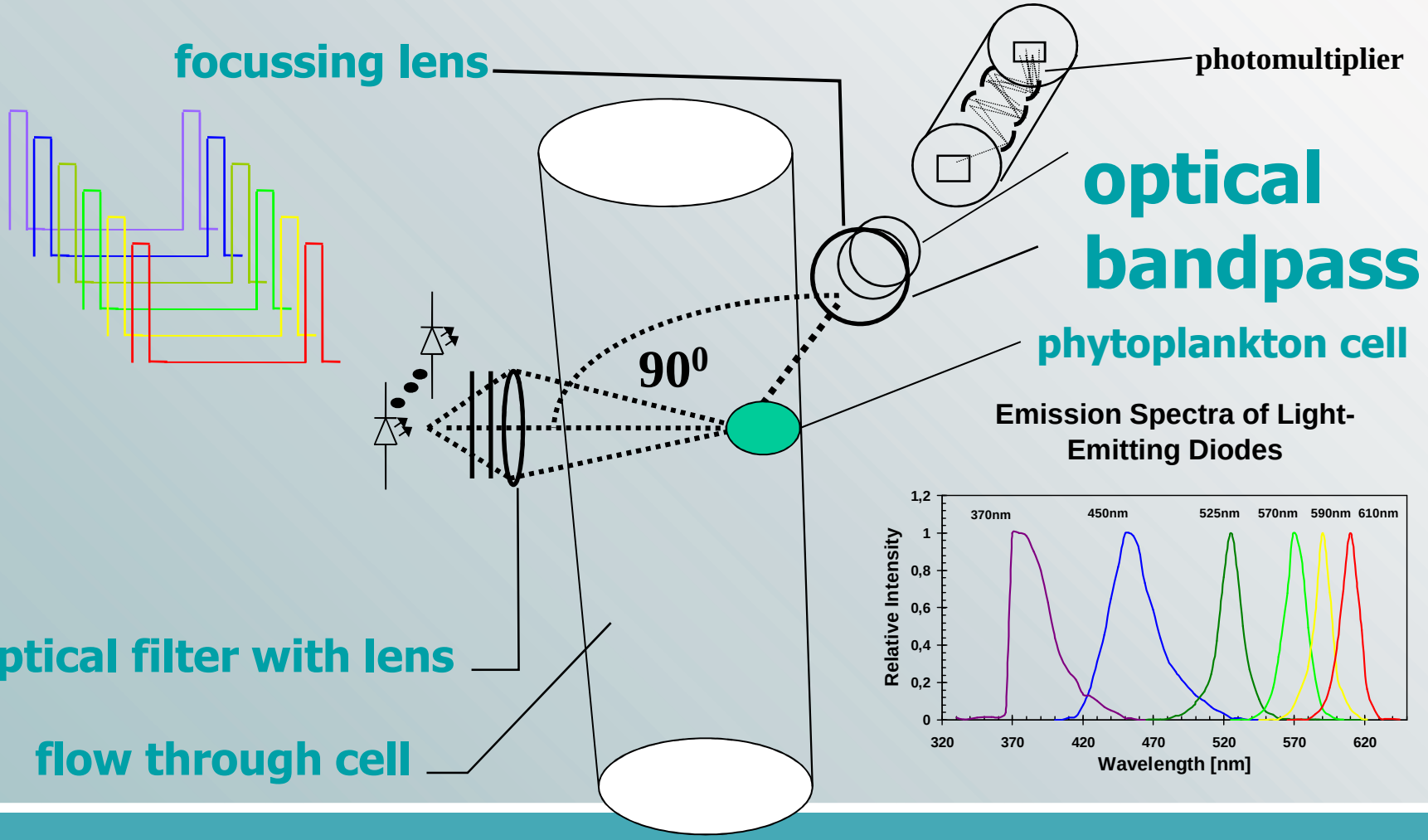
In situ fluorescence

- Sample preparation
- Temperature dependency
- Other chlorophylls
- Breakdown products
- CDOM, FDOM
- Humic acids
- Sensitivity





In situ fluorescence



Emission Spectra of Light-Emitting Diodes





Algae specific features	Cellmembranes, colonies, mucilage
Pretreatment	Cooling, freeze-thaw, ultrasound, temperature
Solvent effects	Extraction: EtOH, MeOH, Acetone, DMF, DMSO Water content Extraction times
Diverse extractions	ISO 10260, EPA Method 445, 447
Filter materials	Nylon, GF/F Knefelkamp et al. 2007
Empiric formulas	Jeffrey, Humphry; UNESCO: Lorenzen; Kacmar
Spectral Absorptionscoeffizient	Nusch
Conversionfactor phaopigment	pH dependent
Acidification step and chl b , chl c	Effect on chlorophyll a measurement
Acidification	Dimerisaqtion
HPLC	Integration of chl a peak area, internal standard
Cell counting	Different6 chloropyll content per cell, result dependent on observer
Biovolumen	Dependent on form factors, colonies
Wavelength accuracy	Photometer
Turbidity	Turbidity correction

So if you also know what is wrong you´re closer to “right”





Thanks for listening