

14th Webinar

**Some considerations on the determination
of an important biological parameter**

Chlorophyll determination of microalgae and cyanobacteria

Welcome, today we are



Ylva Tischler Detlev Lohse
bbe Team

14th Webinar

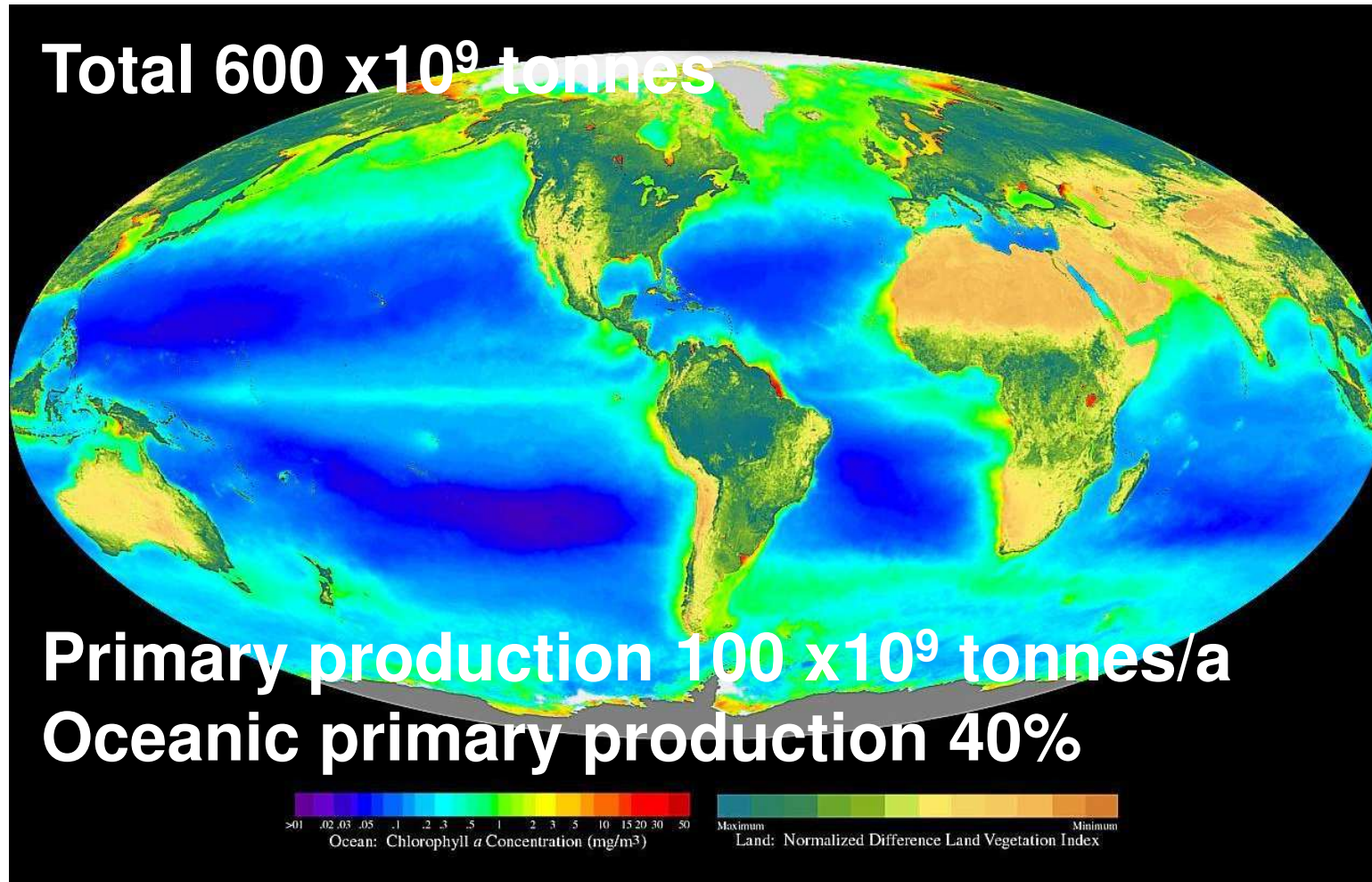
Why do we measure Chlorophyll a?

Diverse methods used for determination

Comparison, Benefits and Drawbacks

Feedback & Follow up

Biomass distribution



Biomass Evaluation

The most accurate method for biomass evaluation is the microscopic count, the mean volume of each phytoplankton species being calculated and multiplied by the number of cells for that species, on different size classes. C. SANDU et al. 2003

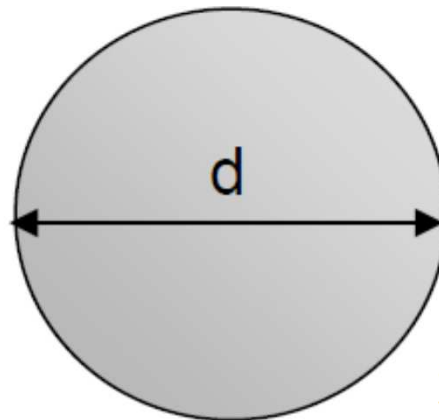
The volume of cells in a unit amount of water(mm³/L)

Sphere

Volume: $V = \pi/6 * d^3$

V: volume

d: diameter



Olenina, I., Hajdu, S., Edler, L., Andersson, A., Wasmund, N., Busch, S., Göbel, J., Gromisz, S., Huseby, S., Huttunen, Jaanus, A., Kokkonen, P., Ledaine, I. and Niemkiewicz, E. 2006
Biovolumes and size-classes of phytoplankton in the Baltic Sea
HELCOM Balt.Sea Environ. Proc. No. 106, 144pp.

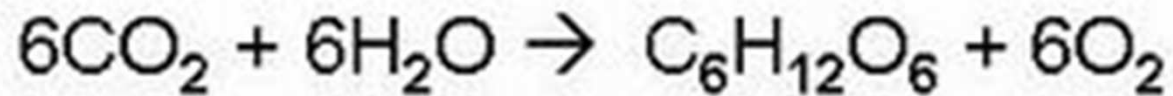
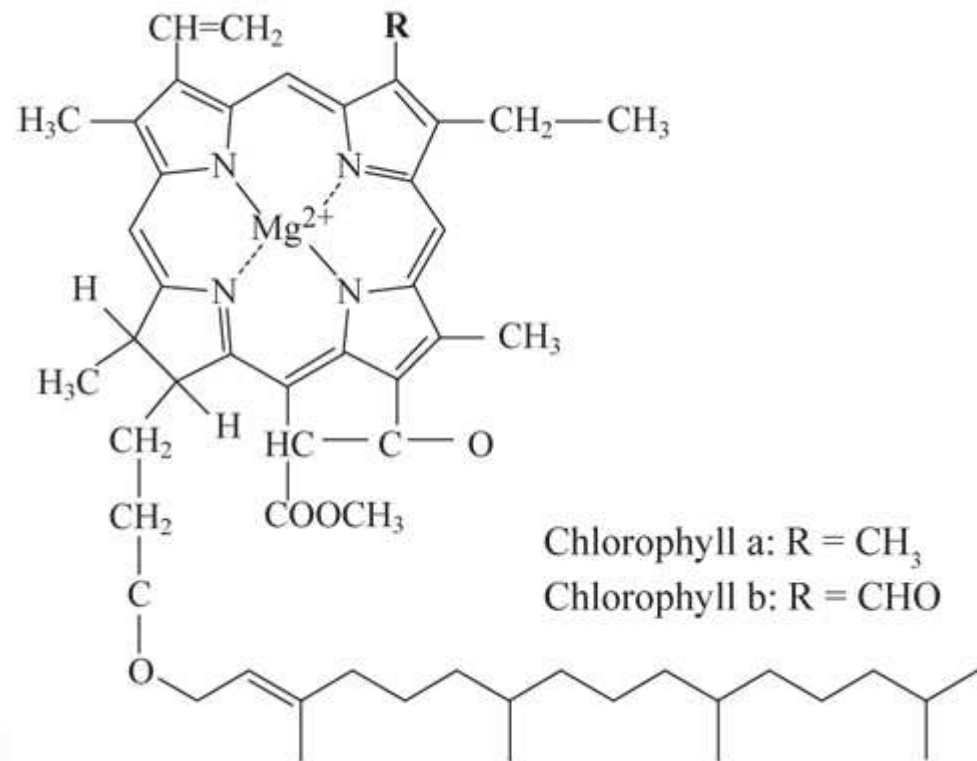
Chlorophyll a as a proxy for biomass

- Strong relation between biomass and chlorophyll a from phytoplankton (Raschke 1993)

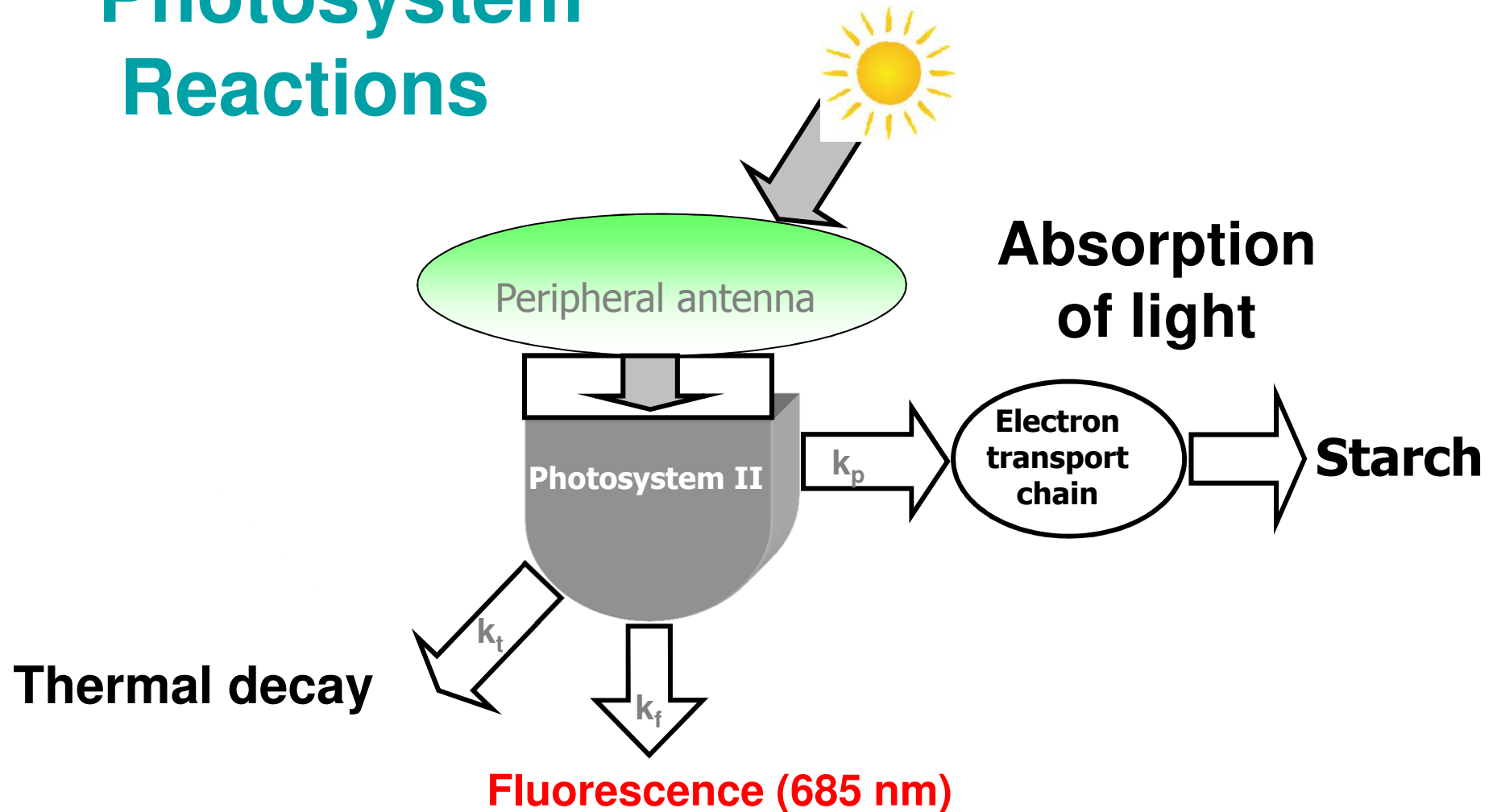
$$\text{Biomass} = \text{Chlorophyll a} \times 67$$

- Conversion ratio of phytoplankton biomass carbon to chlorophyll-a 26 – 250, mean 56 (Shuguo Lü et al. 2009)

Chlorophyll a in Photosynthesis

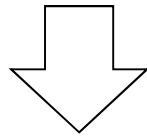


Photosystem Reactions

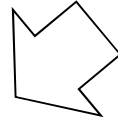




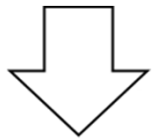
Microalgae & Chlorophyll a



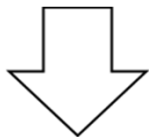
Samples



Extraction



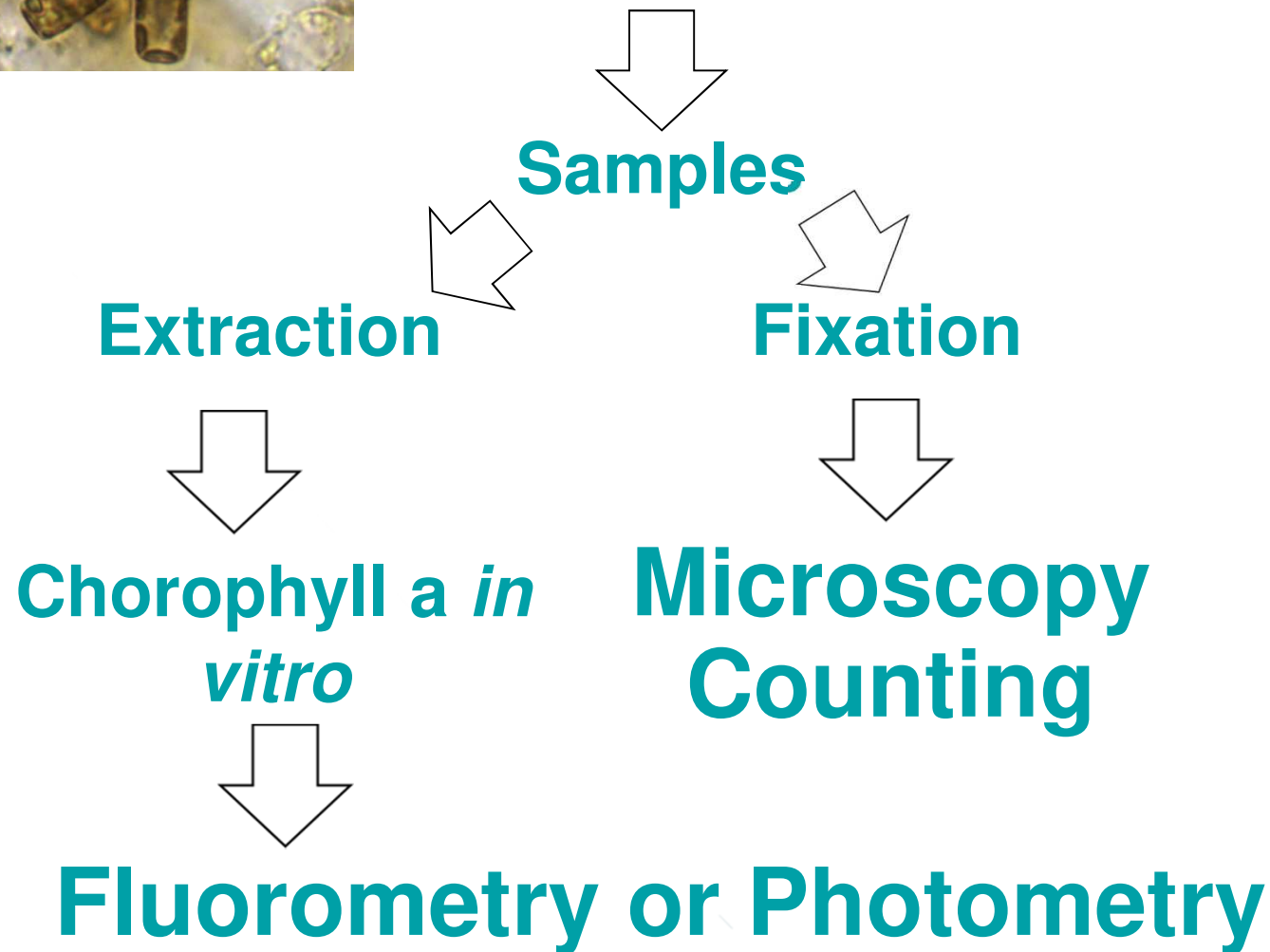
Chlorophyll a *in vitro*



Fluorometry or Photometry

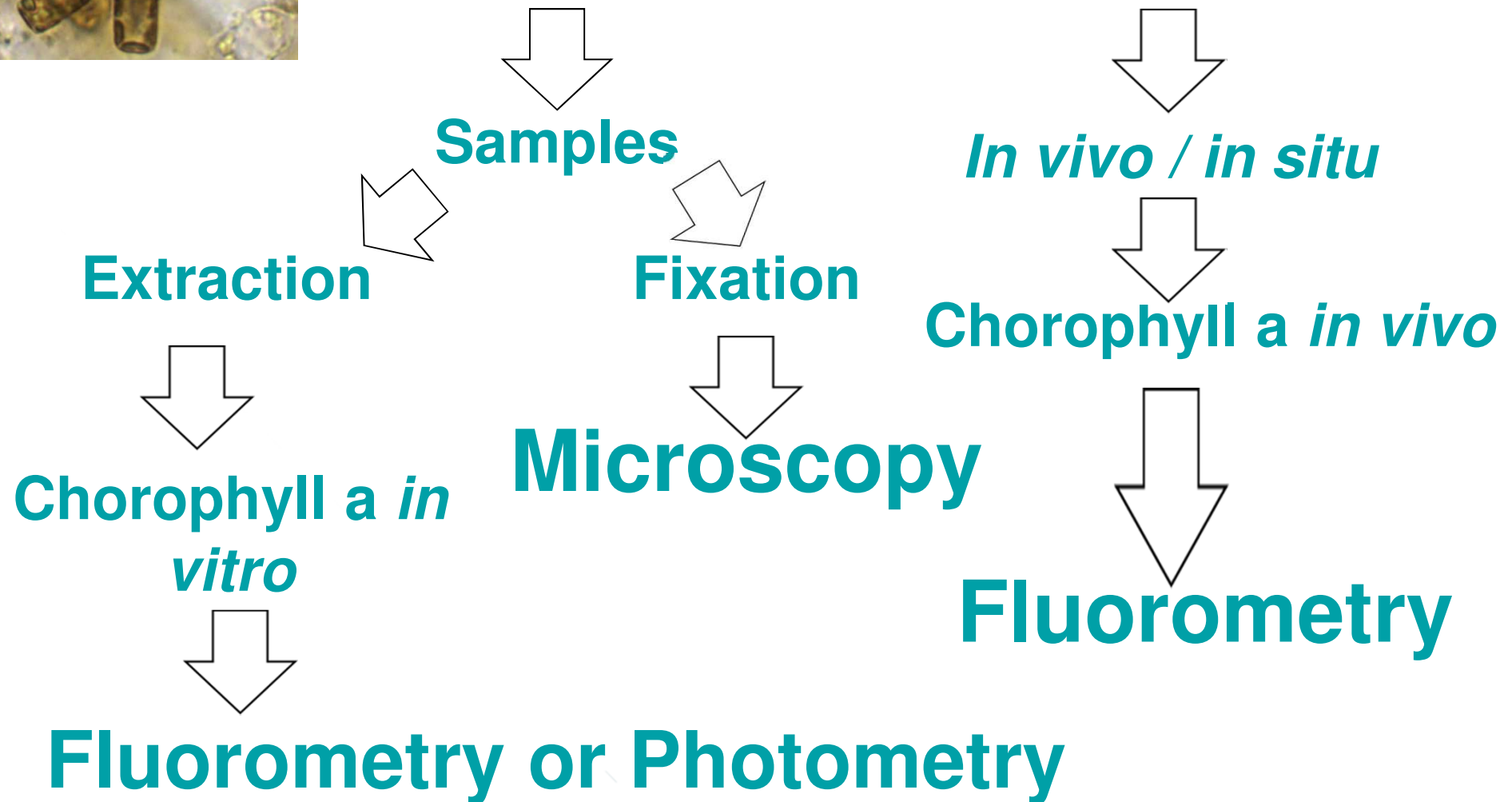


Microalgae & Chlorophyll a





Microalgae & Chlorophyll a





Chlorophyll *a in vitro*

Water quality — Measurement of biochemical parameters — Spectrometric determination of the chlorophyll-a concentration

**ISO
10260**

Method 447.0

Determination of Chlorophylls *a* and *b* and Identification of Other Pigments of Interest in Marine and Freshwater Algae Using High Performance Liquid Chromatography with Visible Wavelength Detection

U.S. Environmental Protection Agency



Spectrophotometric determination

Water quality — Measurement of biochemical parameters — Spectrometric determination of the chlorophyll-a concentration

Procedure

- Filtration of suspended phytoplankton
- Extraction from filter with organic solvent
- Filtration of dissolved pigments
- Spectrophotometric determination of chlorophyll a and phaeophytins
- Acidification converts chlorophyll a into phaeophytin
- Application of empirical formula to determine the chlorophyll a



Spectrophotometric determination

Water quality — Measurement of biochemical parameters — Spectrometric determination of the chlorophyll-a concentration

Pitfalls and problems

- Variety of provisions, national differences in the descriptions
- Complex procedure, many worksteps
- Filtering may damage algal cells
- Water content of filtered sample
- Extraction complete?
- Filtration times, temperature, contact with air & light
- Wavelengths accuracy of photometer
- Acidification may create phaeopigments
- Uncertainty of empirical formula



Spectrophotometric determination in Robin tests

Water quality — Measurement of biochemical parameters — Spectrometric determination of the chlorophyll-a concentration

Parameter		Mean value µg/l	relative Standard deviation %	Number valid labs	Number valid single measurements
Chloropyll a	Probe1	104,9	16,6	13	64
Chloropyll a	Probe2	32,2	40,5	14	70
Chloropyll a	Probe3	4,7	10,8	12	59
Phaeopigment	Probe1	48,6	25,1	12	60
Phaeopigment	Probe2	5,6	60,7	14	70
Phaeopigment	Probe3	1,9	54,7	14	68

- Non conformity,
- Filter material,
- Temperature.
- Filter homogenisation
- Extraction,

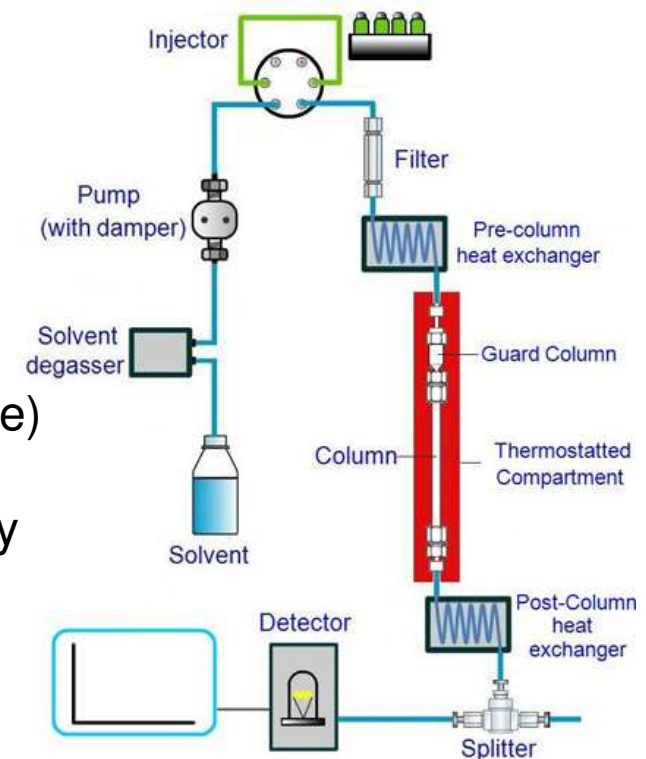


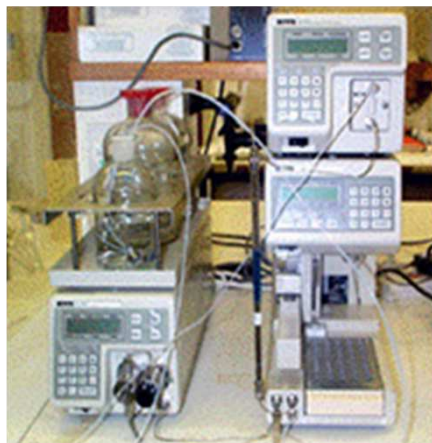
Spectroscopic determination

Method 447.0
Determination of Chlorophylls *a* and *b* and Identification of Other Pigments of Interest in Marine and Freshwater Algae Using High Performance Liquid Chromatography with Visible Wavelength Detection

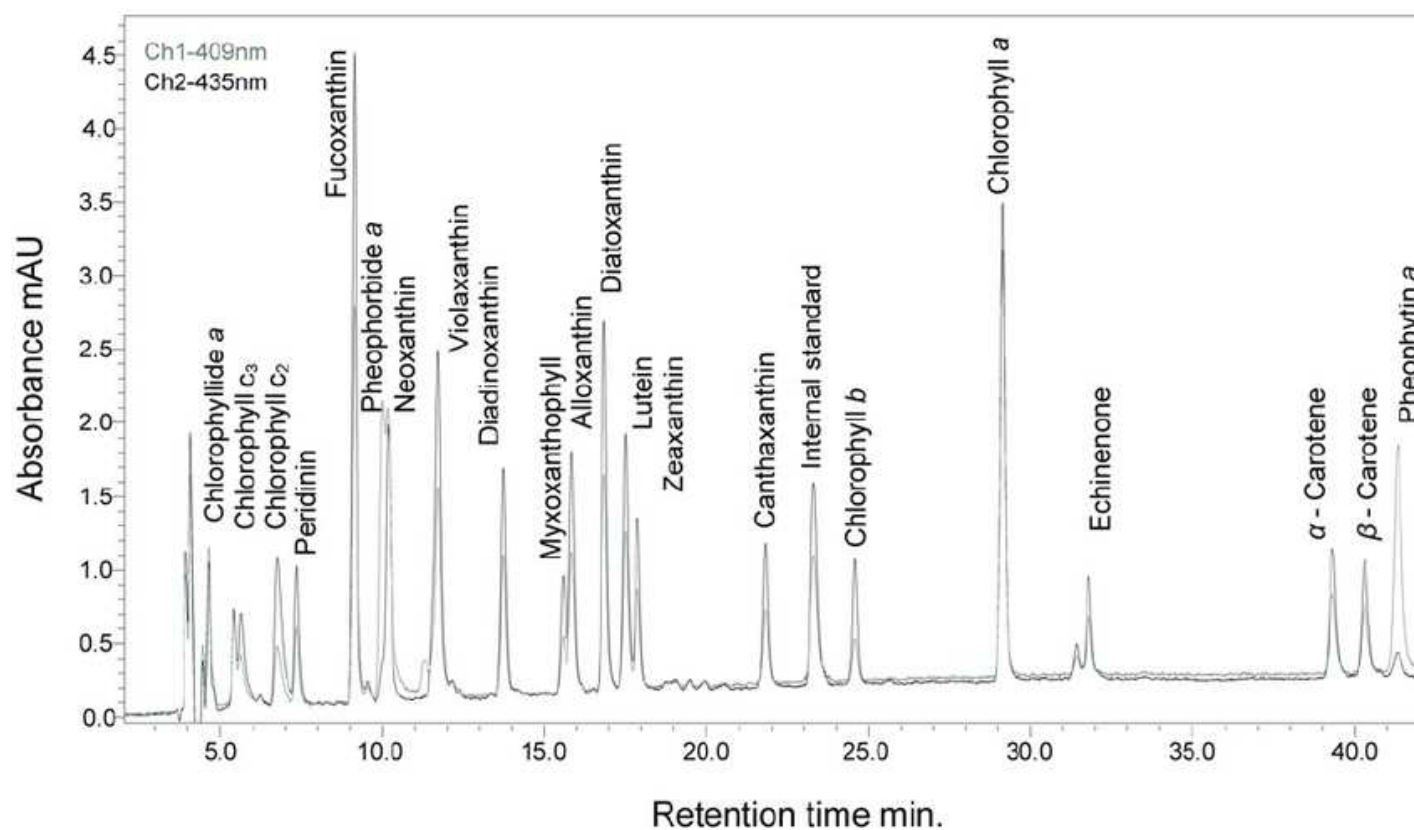
Procedure

- Filtration of suspended phytoplankton
- Extraction from filter with organic solvent (acetone)
- Filtration of dissolved pigments
- Pigment separation by HP liquid chromatography
- Spectrophotometric determination of chlorophylls and phaeophytins
- Use of flow-through photometer or fluorometer





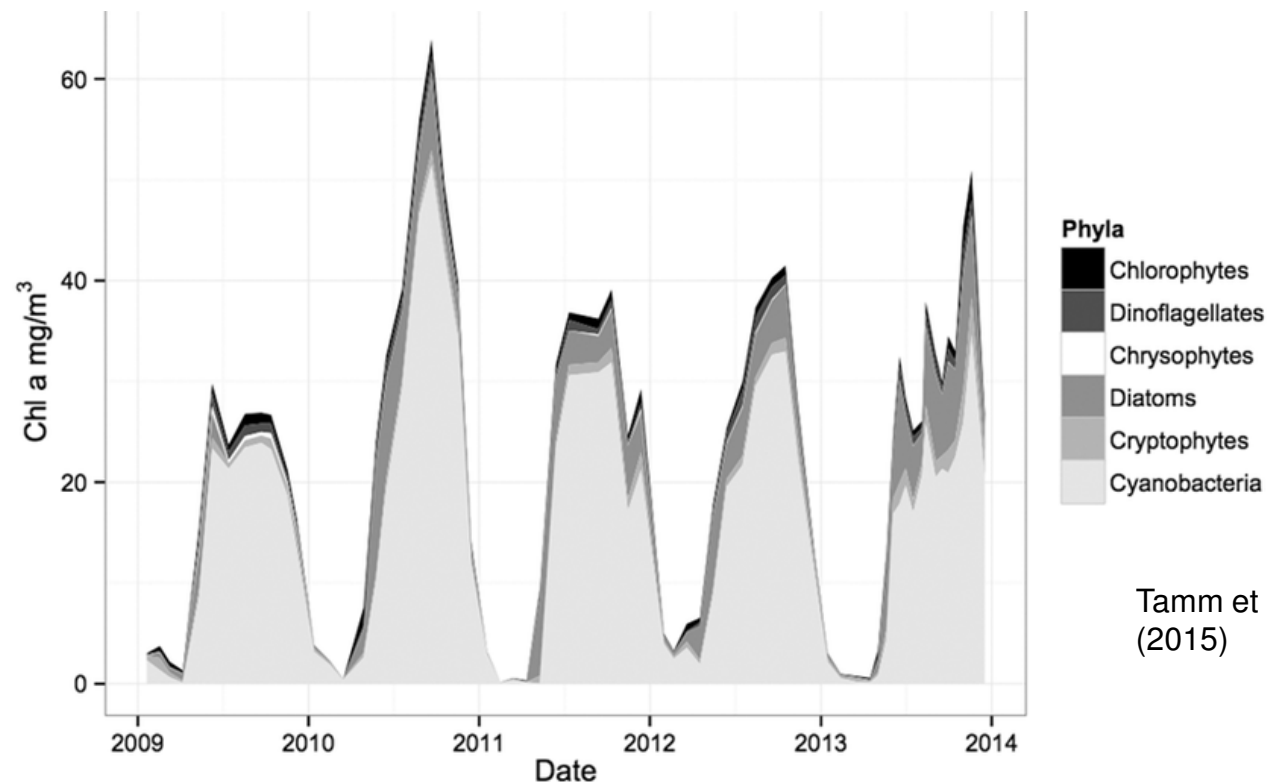
Spectrophotometric determination



Tamm et al.,
(2015)



Spectrophotometric determination and CHEMTAX



Tamm et al.,
(2015)

Estimates class abundances from phytoplankton pigments (Mackey et al. 1996)



Spectrophotometric determination with HPLC

Advantages

- High resolution & quantification
- Differentiation of all chlorophylls and decay products
- Internal standard applicable
- Rapid separation, UHPLC

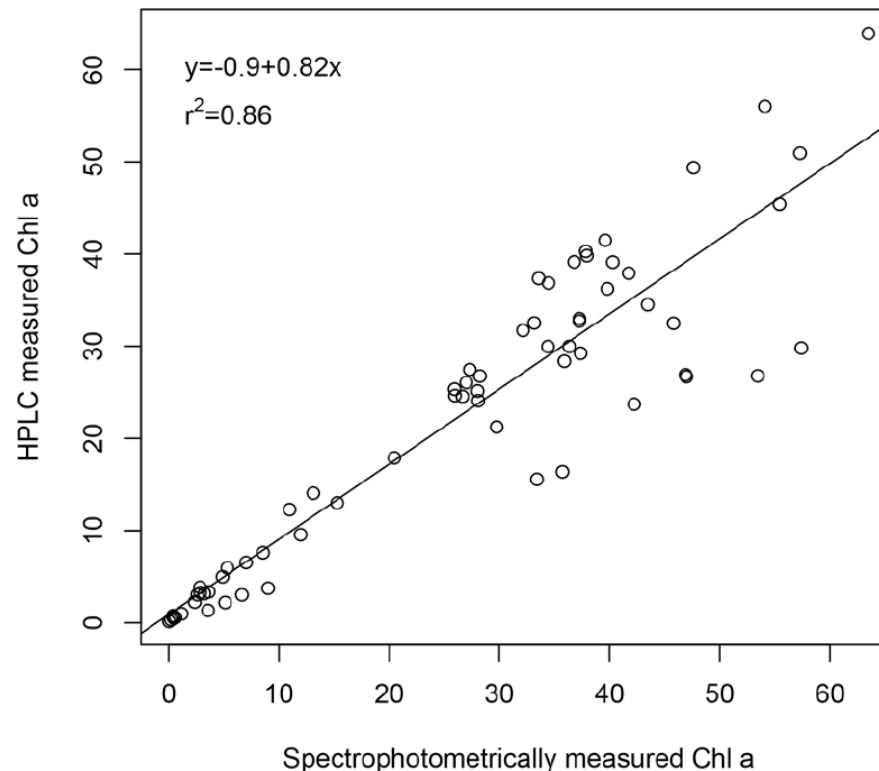
Disadvantages

- Artefacts (Chlorophyllides)
- High costs procurement and maintenance
- Harmful organic solvents
- Needs expert level

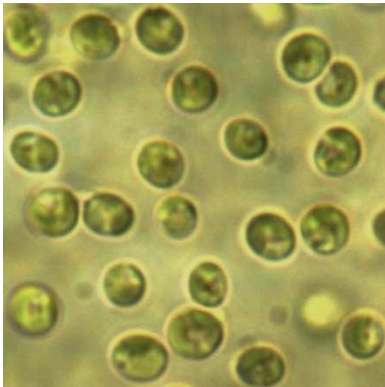
Pheophytin a was virtually lacking in all the samples, Yosef Z.Yacobi et al. 1996



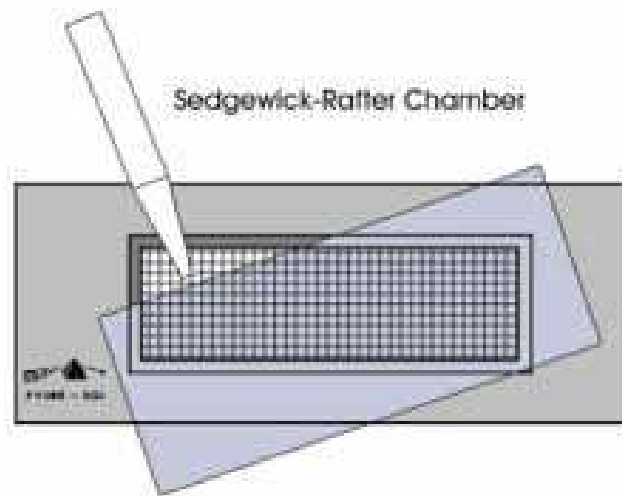
Spectrophotometric determination ISO vs. HPLC



Tamm et al.,
(2015)



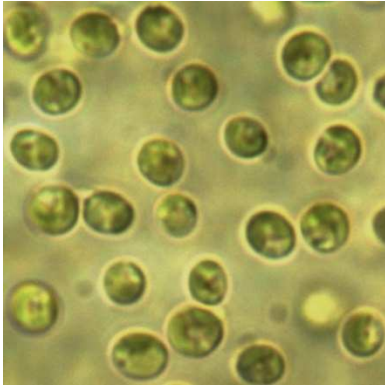
Cell counting



What do you actually need?

- Counting chamber
- Skilled staff
- Microscope

alternatively **use of inverse
microscopical counting
(UTERMÖHL method)**



Cell counting

Advantages

- Analysis down to species level
- Low amount of consumables
- Affordable

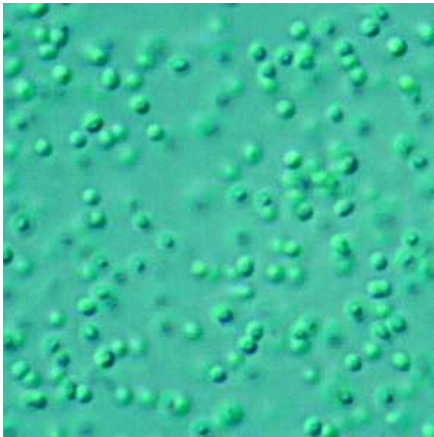
Disadvantages

- Artefacts
- Individual errors
- Laborious, time consuming
- Overlooks some nano- and picoplankton
- Problems with filamentous algae and colonies



[New York Microscope Co](http://www.newyorkmicroscope.co)

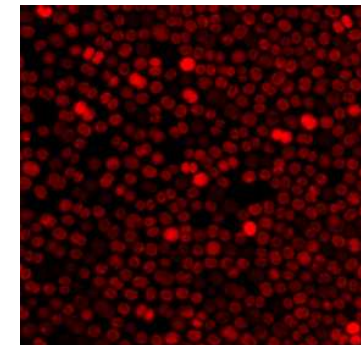


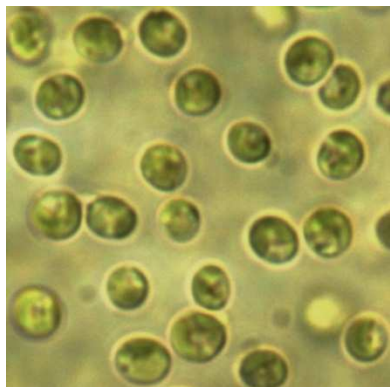


Missed

Prochlorococcus are the smallest organisms in the world able to carry out photosynthesis (they measure only $\sim 0.6 \mu\text{m}$), as well as being the most numerous (Partensky et al, 1999).

Synechocystis measure $< 5 \mu\text{m}$





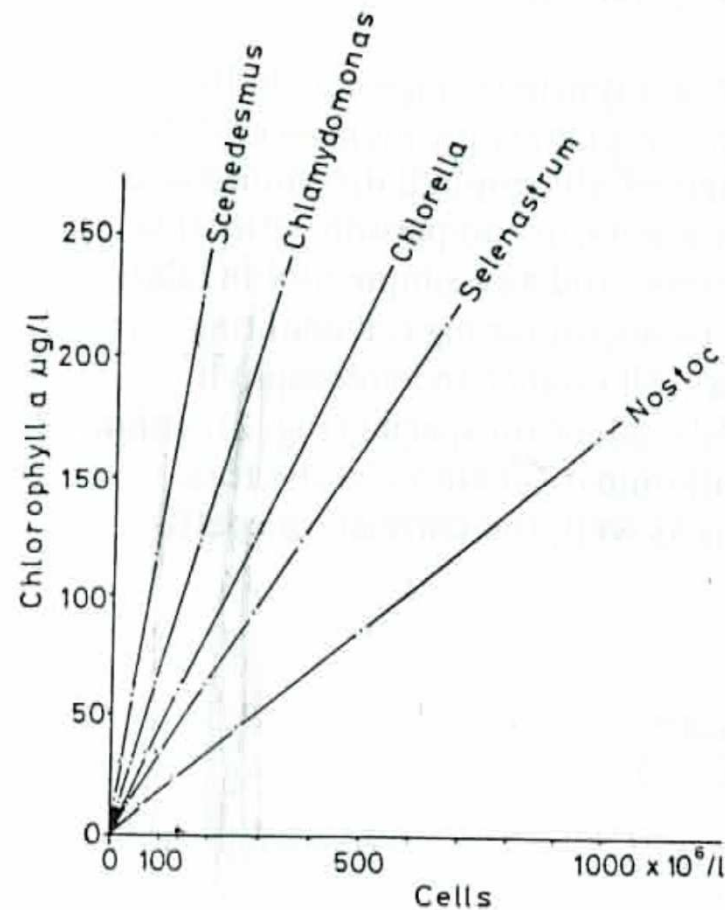
Cell counting results

cyanobacteria counting (cells/ml) by three different laboratories

code	MYC UvA	PLA UvA	ANA UvA	APH UvA
Lab 1	614000	481000	788000	188000
Lab 2	1325000	565000	674000	303000
Lab 3	2267000	438000	1293000	193000
Mean value	1402000	494000	918000	228000
standard deviation in %	59%	13%	36%	28%

Ron van der Oost,
Waternet, STOWA report 2010

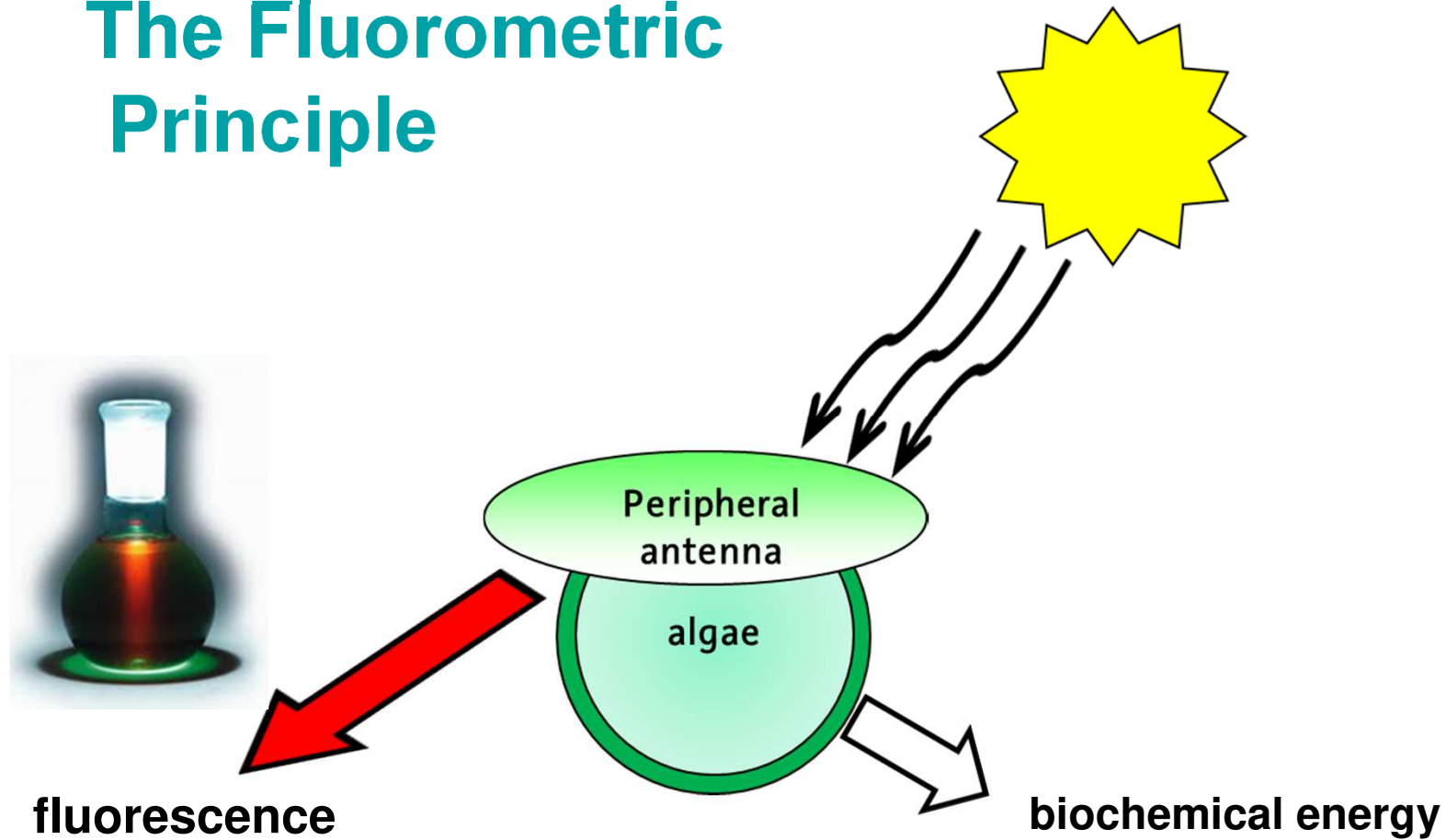
Cell counting and Chlorophyll a



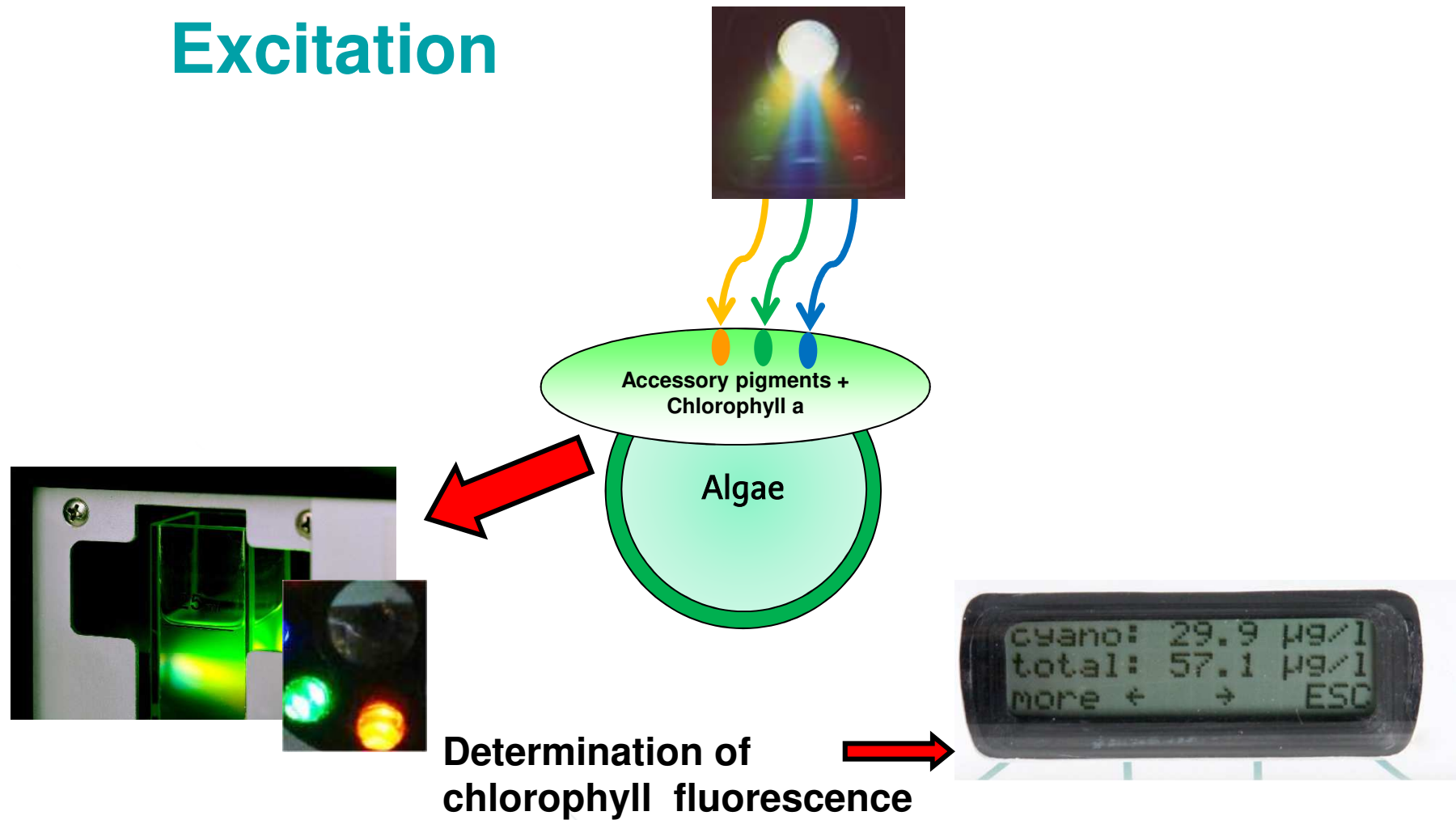
Nostoc verrucosum

E.A. Nusch (1980)

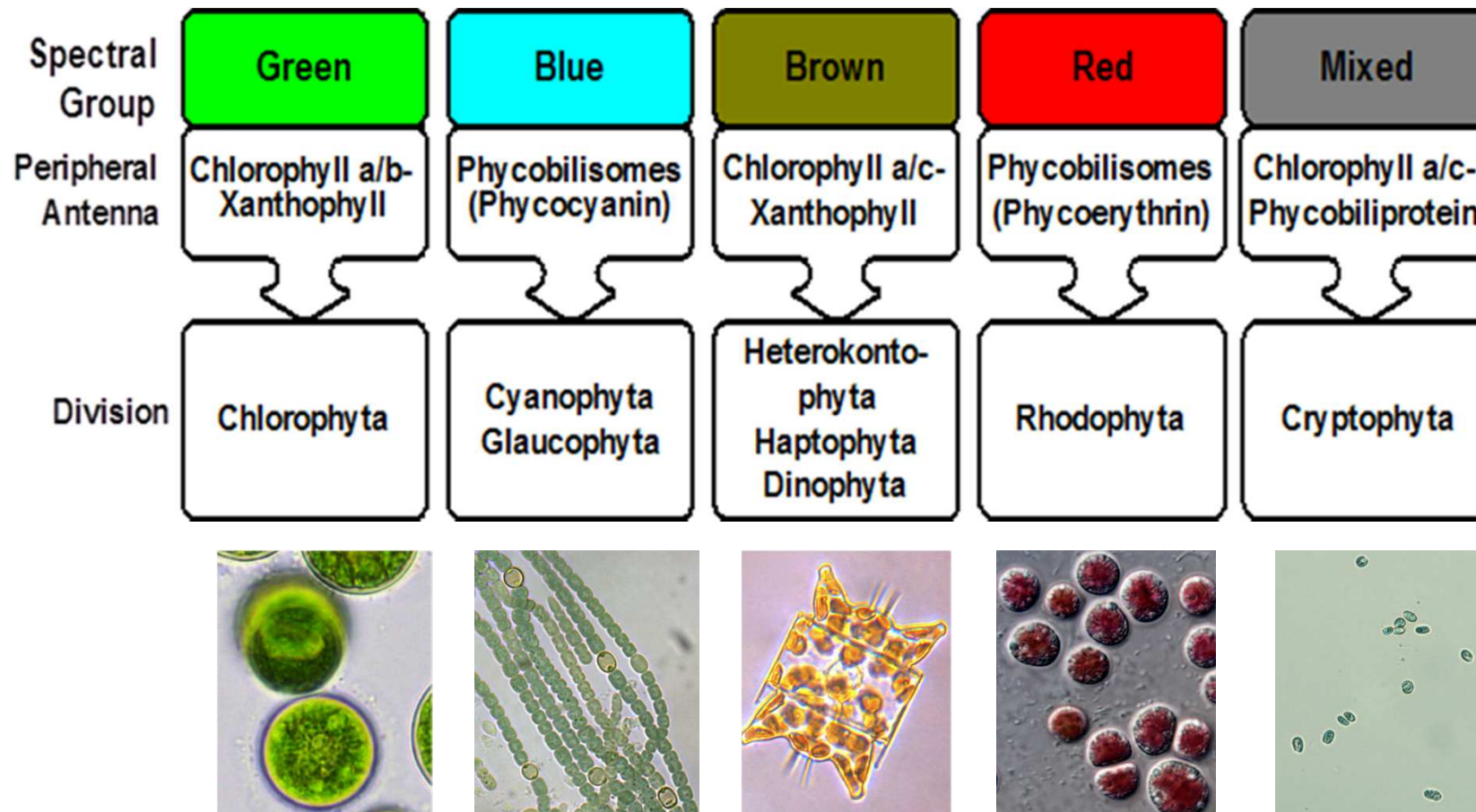
The Fluorometric Principle

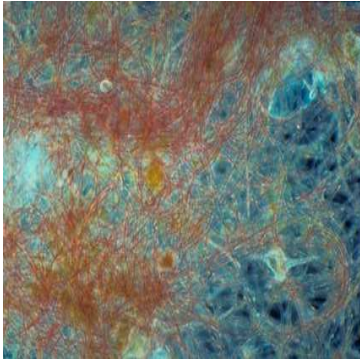


Multicolor Excitation

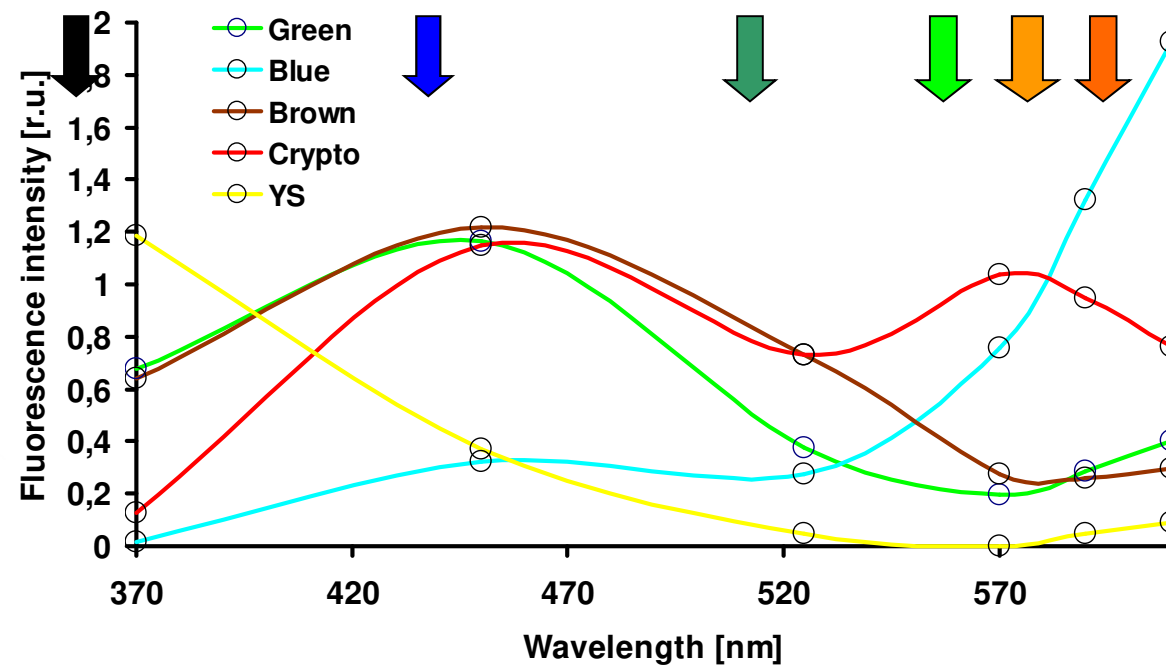


Classification of Phytoplankton

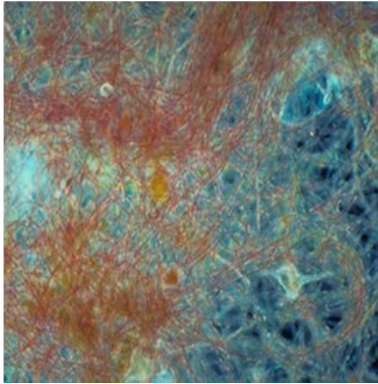




Algae excitation spectra (fingerprints)



.....enables to calculate the quantity



In Vivo Fluorometry

Advantages

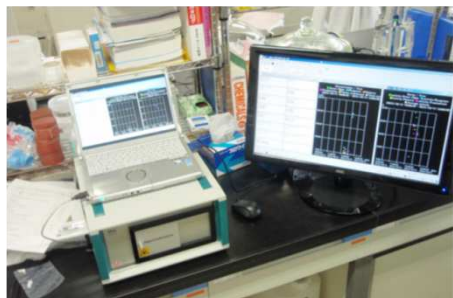
- Rapid analysis
- High sensitivity
- Algae classification
- No cell disruption
- Detects nano- and picoplankton

Disadvantages

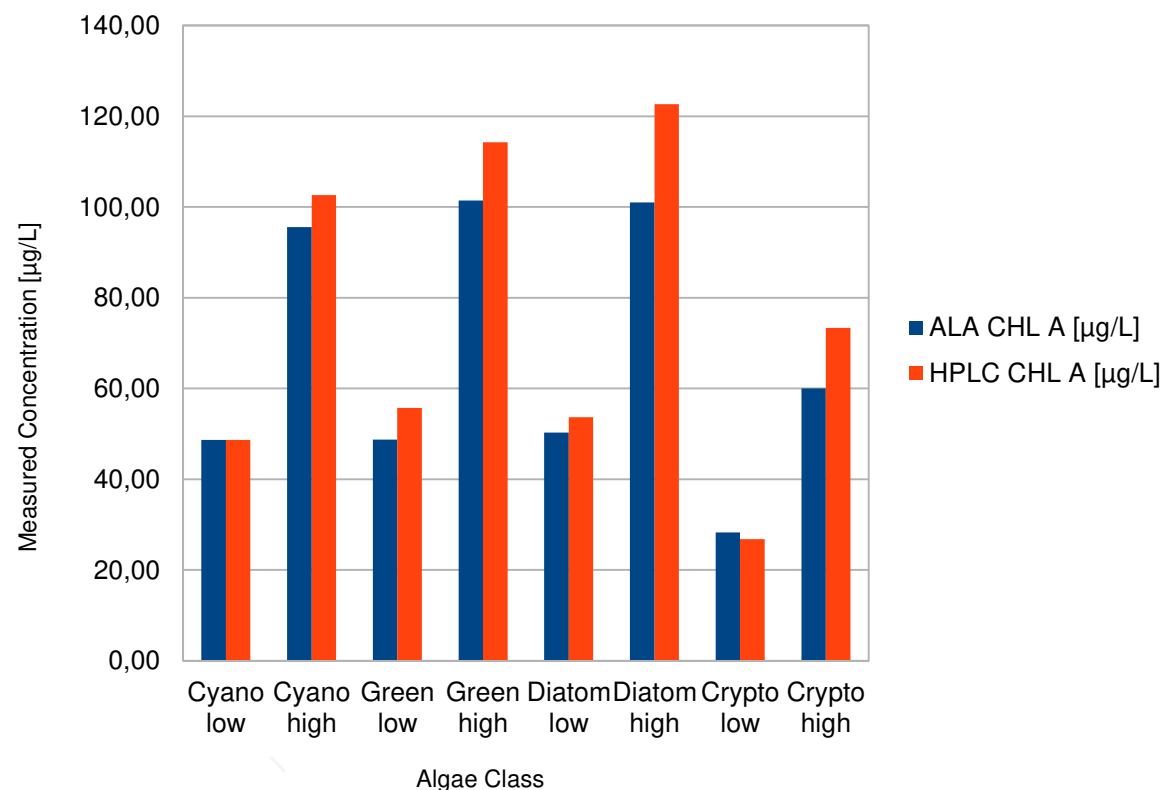
- Interferences with FDOM
- Problems with filamentous algae and colonies
- Does not work at high turbidity
- Nutrient supply affects chlorophyll complexes
- Ambient light affects chlorophyll fluorescence

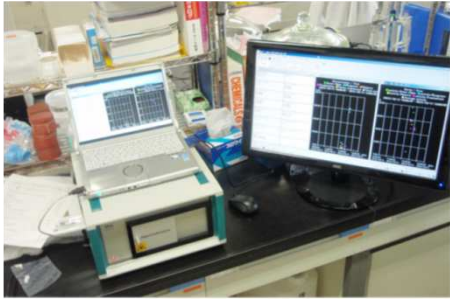


AlgaeTorch by bbe

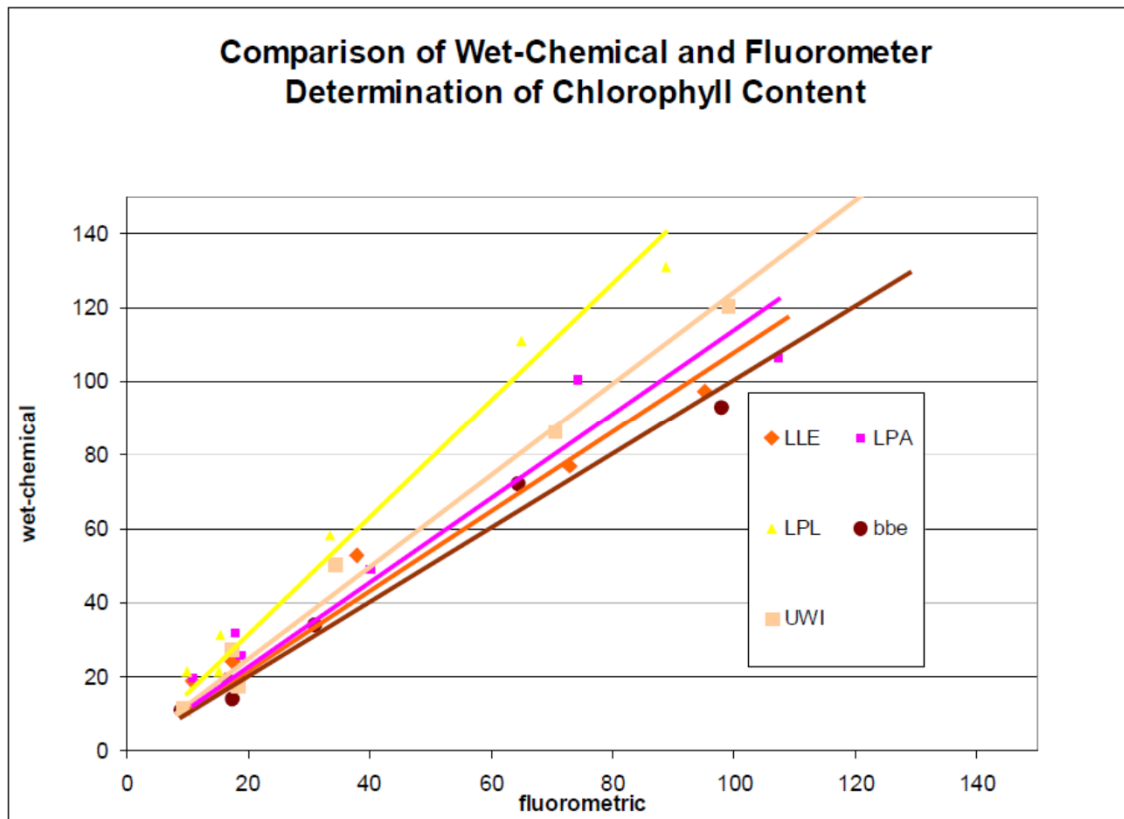


Comparison: Fluorometry and HPLC/photometry





Comparison: Fluorometry vs. Wet Chemical Analysis



Laboratory	Slope	R ²
LLE	1,08	0,95
LPA	1,14	0,9
LPL	1,58	0,98
BBE	1,01	0,98
UWI	1,24	0,99

- **The different chlorophyll determination methods show deviations while correlation might be quite good**
- **For intercalibration adaption is necessary (factor)**
- **Complete concordance between methods cannot be expected**
- **Chlorophyll a value cannot substitute biomass determination directly**

Chlorophyll a vs. Cellcounts

**1:1 chl-a algae
class
distribution**



**1:1 cellcount
algae class
distribution**

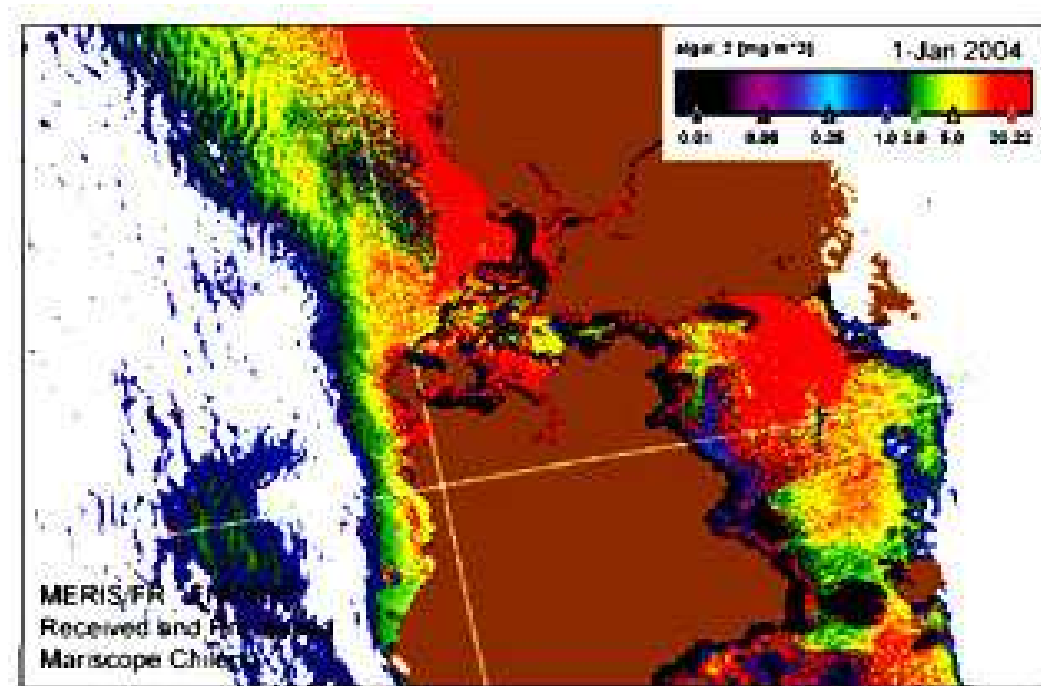
Chlorophyll a vs. Biovolume

**1:1 chl-a algae
class
distribution**



**1:1 biovolume
algae class
distribution**

Algal bloom in the Chacao Channel



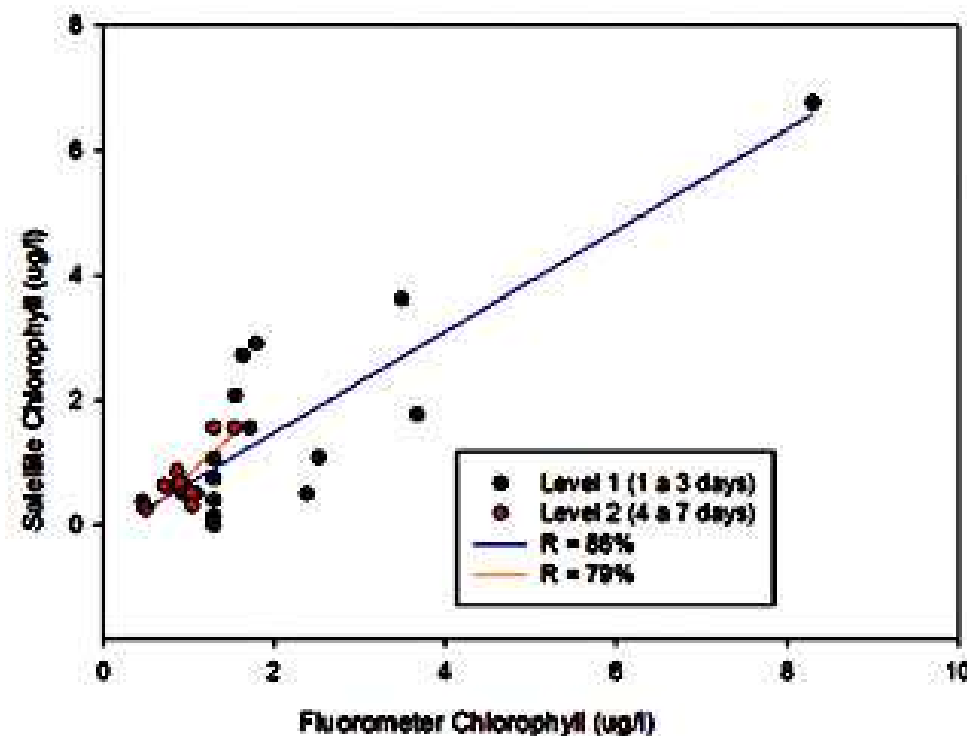
MERIS Satellite remote observation



Submersible FluoroProbe

Rodríguez.-Benito, C. and Haag, C. APLICATIONS OF ENVISAT DATA IN THE SOUTH OF CHILE: MONITORING ALGAL BLOOMS AND OTHER COASTAL OCEAN FEATURES USING MERIS AND AATSR IMAGERY. Gayana (Concepc.), 2004, vol.68, no.2, suppl.TIIProc, p.508-513. ISSN 0717-6538

Correlation between remote sensing (satellite) and in situ measurement with submersible FluoroProbe



Ocean color data in relation to the in situ data

C. Rodriguez-Benito and C. Haag (2004)

Thank you

