# 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

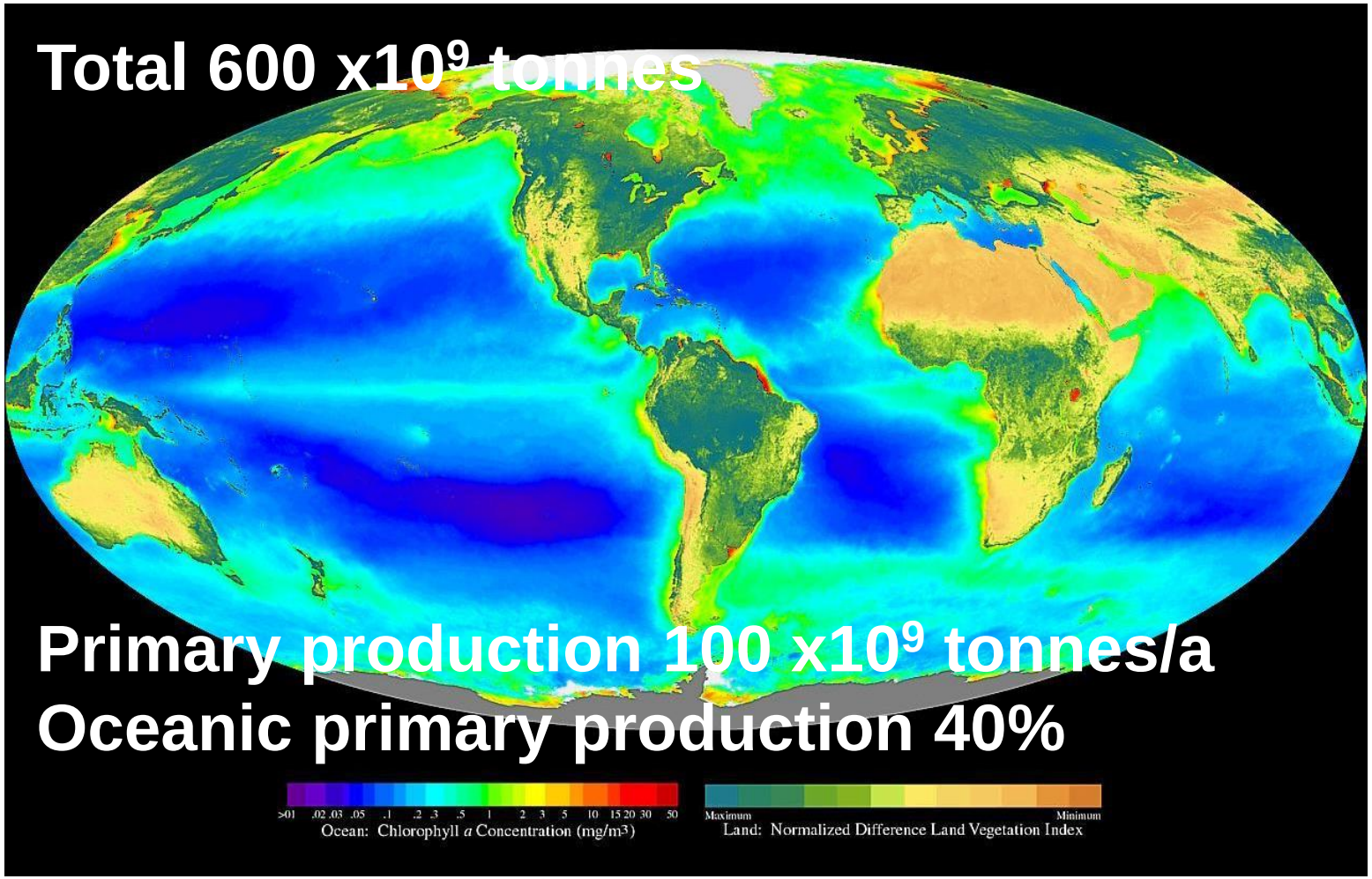
## Why do we  measure Chlorophyll a?

## Diverse methods used for determination

## Comparison, Benefits and Drawbacks

## Feedback & Follow up

# Biomass distribution

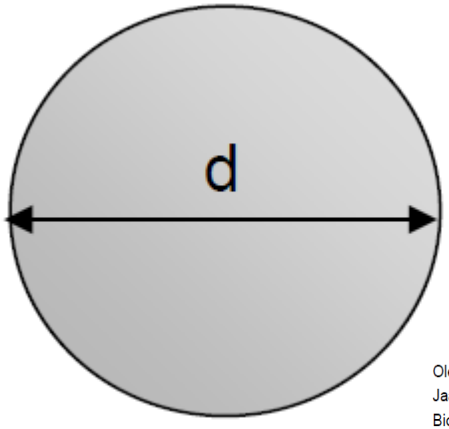


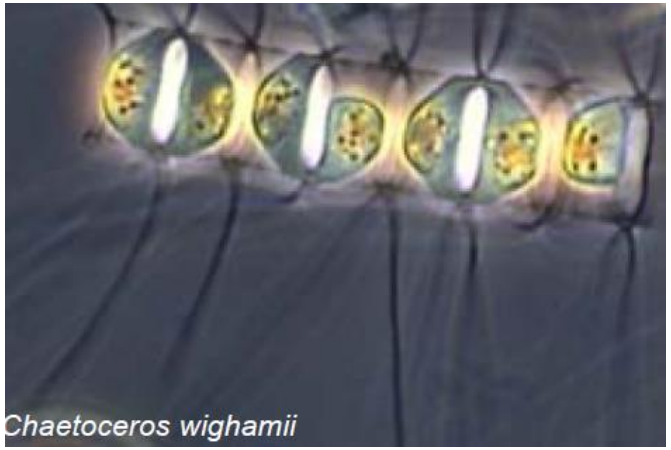
# Biomass Evaluation

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($mm^3/L$)

***Sphere***
Volume: $V = \pi/6 * d3$
V: volume
d: diameter





*Chaetoceros wighamii*

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.

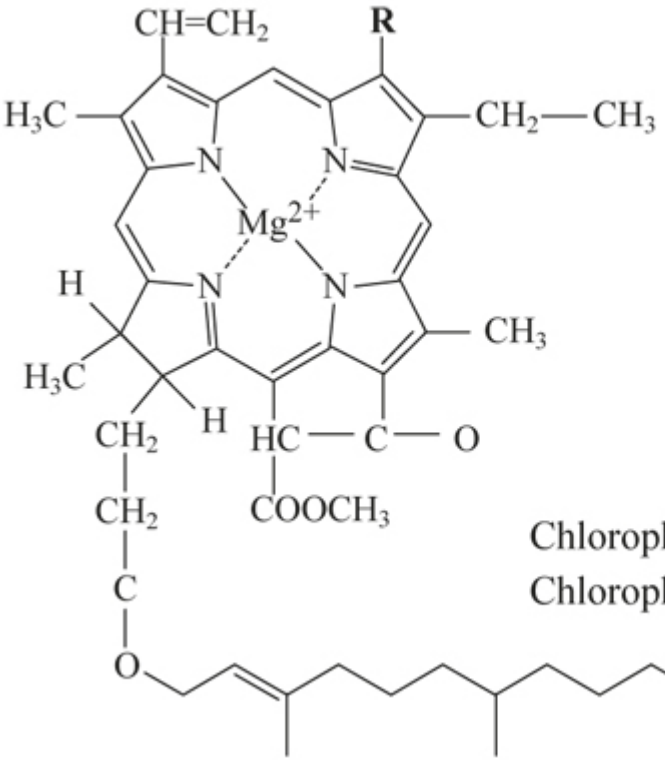
# Chlorophyll a as a proxy for biomass

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

  Biomass = Chlorophyll a x 67

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

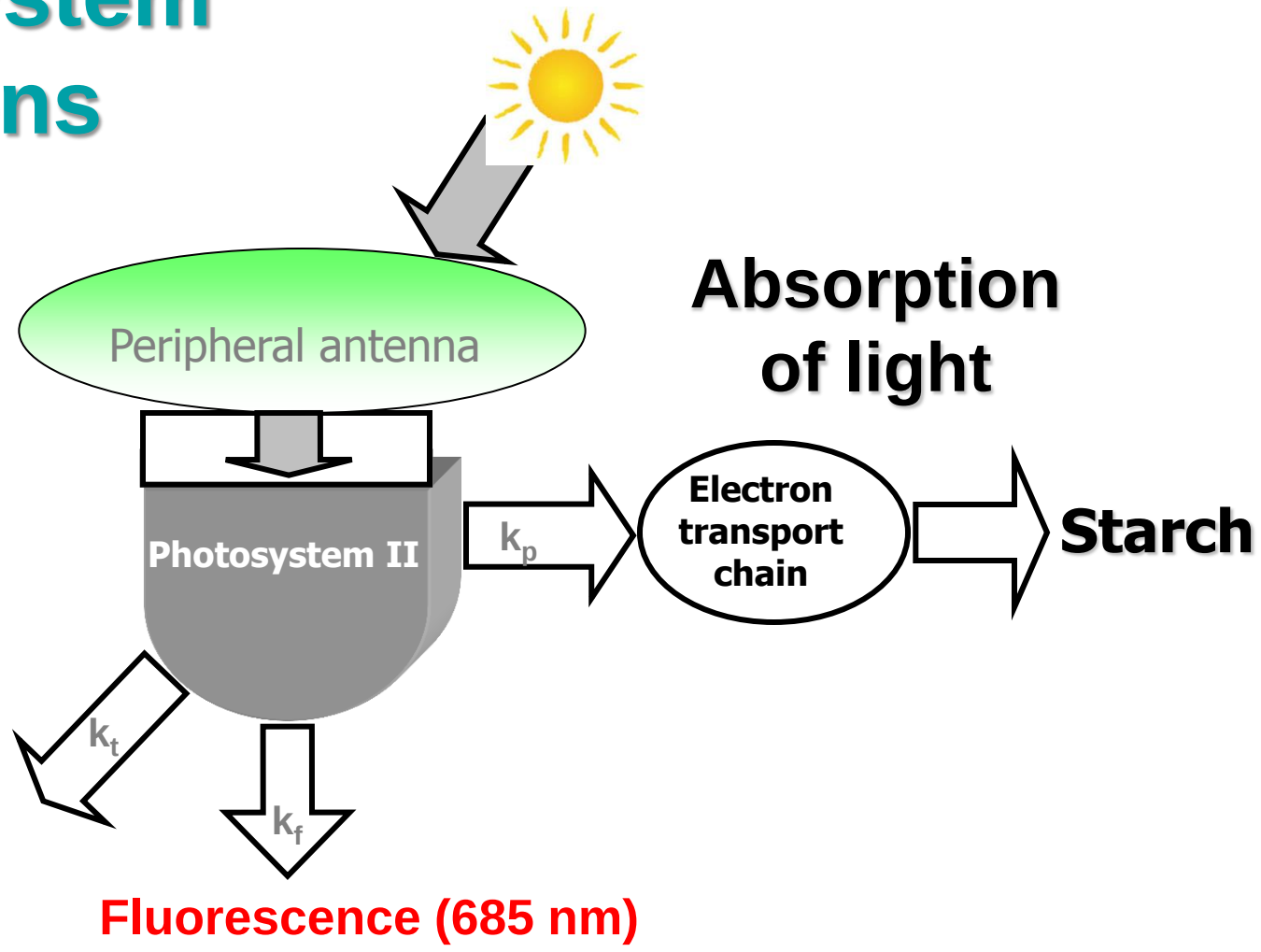
# Chlorophyll a in Photosynthesis




Chlorophyll a: R = $CH_3$
Chlorophyll b: R = CHO

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

# Photosystem Reactions





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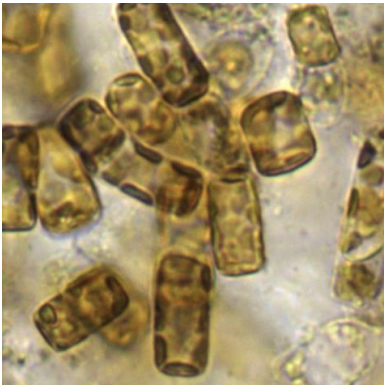


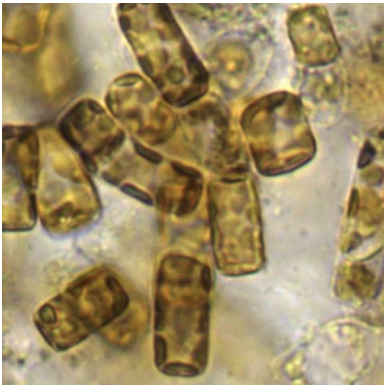
# Microalgae & Chlorophyll a

Samples

Extraction

Chorophyll a *in vitro*

Fluorometry or Photometry



# Microalgae & Chlorophyll a

Samples

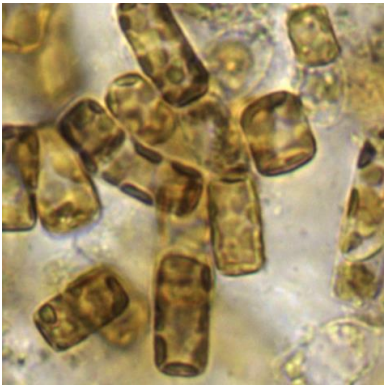
Extraction

Chorophyll a *in vitro*

Fluorometry or Photometry

Fixation

Microscopy Counting



# Microalgae & Chlorophyll a

Samples

Extraction

Chorophyll a *in vitro*

Fluorometry or Photometry

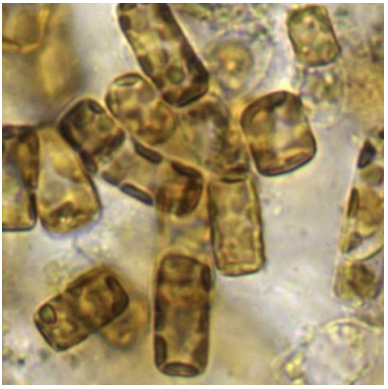
Fixation

Microscopy

*In vivo / in situ*

Chorophyll a *in vivo*

Fluorometry





# Chorophyll a *in vitro*

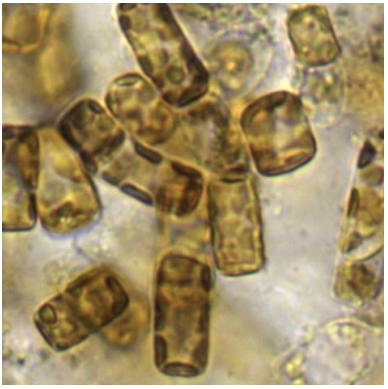
**Water quality — Measurement of biochemical parameters — Spectrometric determination of the chlorophyll-a concentration**

**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**

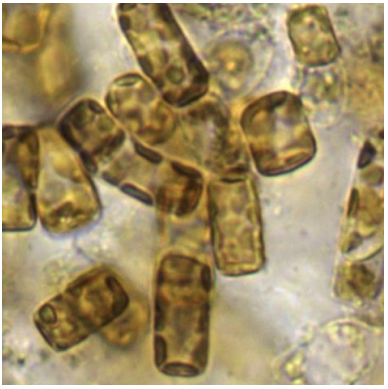


# Spectrophotometric determination

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 damags 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 emperical formula

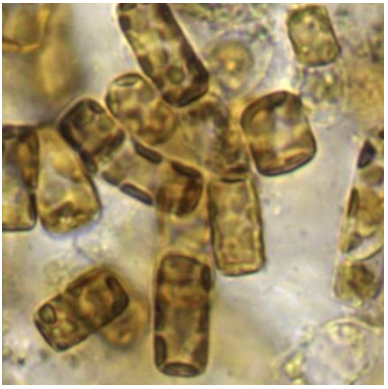


# Spectrophotometric determination in Robin tests

**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,





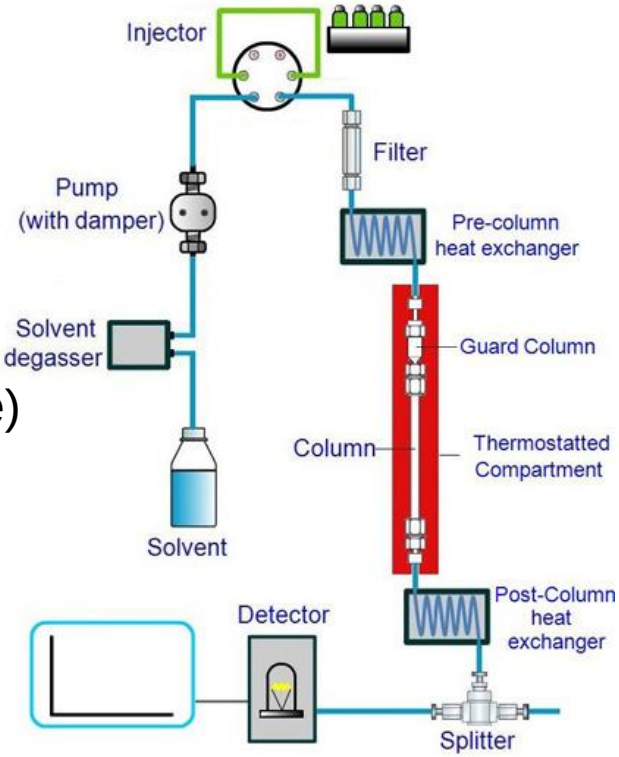
# Spectroscopic determination

**Method 447.0**

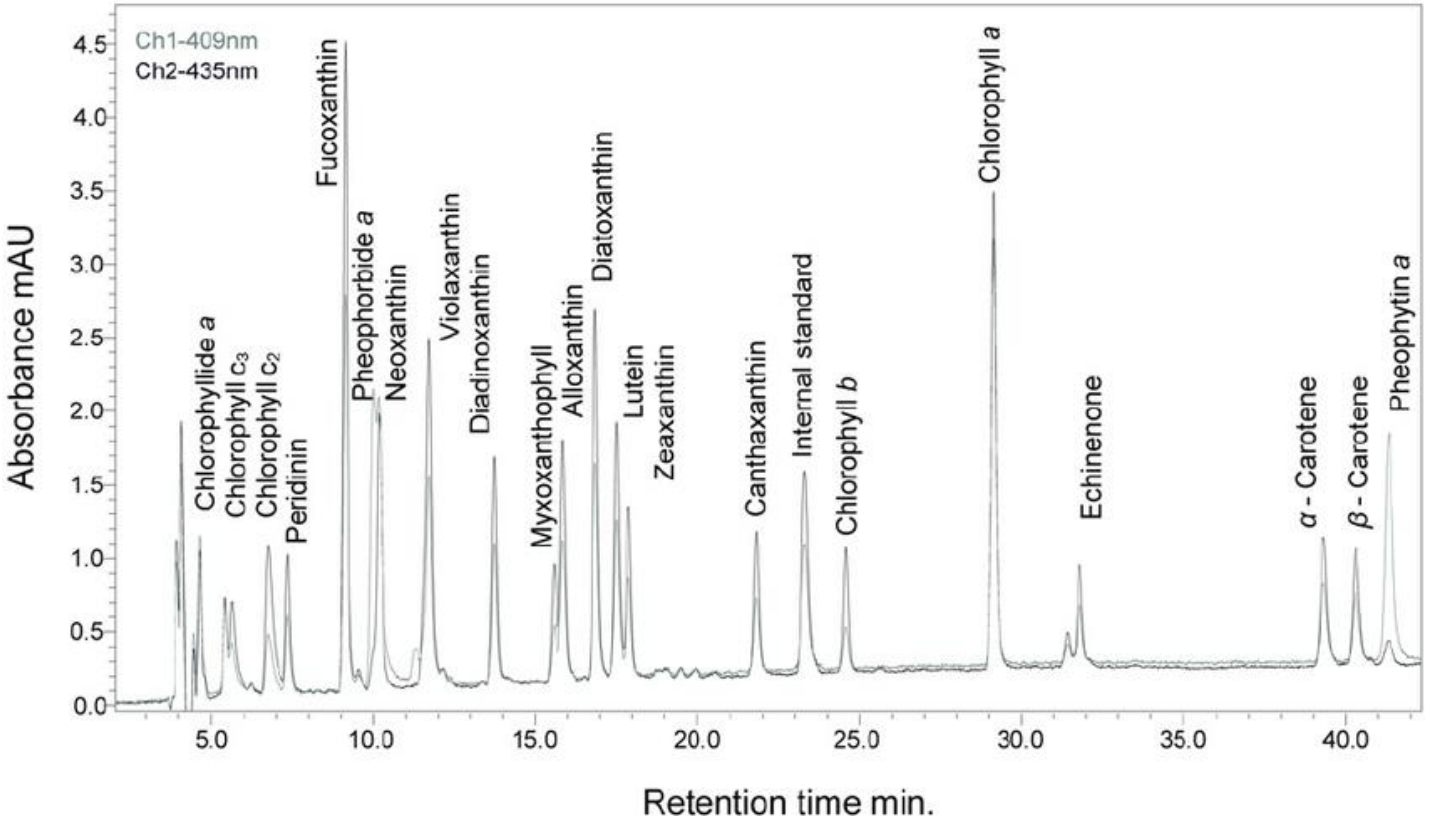
**Determination of Chlorophylls *a* and *b* and Identification of Other Pigments of Interest in Marine and Freshwater Algae Using High Performance Liquid Chromatography with Visible Wavelength Detection**

## Procedure

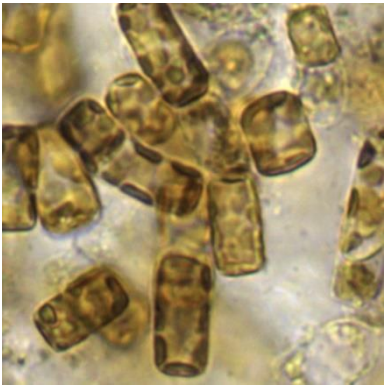
- Filtration of suspended phytoplankton
- Extraction from filter with organic solvent (acetone)
- Filtration of dissolved pigments
- Pigment separation by HP liquid chromatography
- Spectrophotometric determination of chlorophylls and phaeophytins
- Use of flow-through photometer or fluorometer



# Spectrophotometric determination

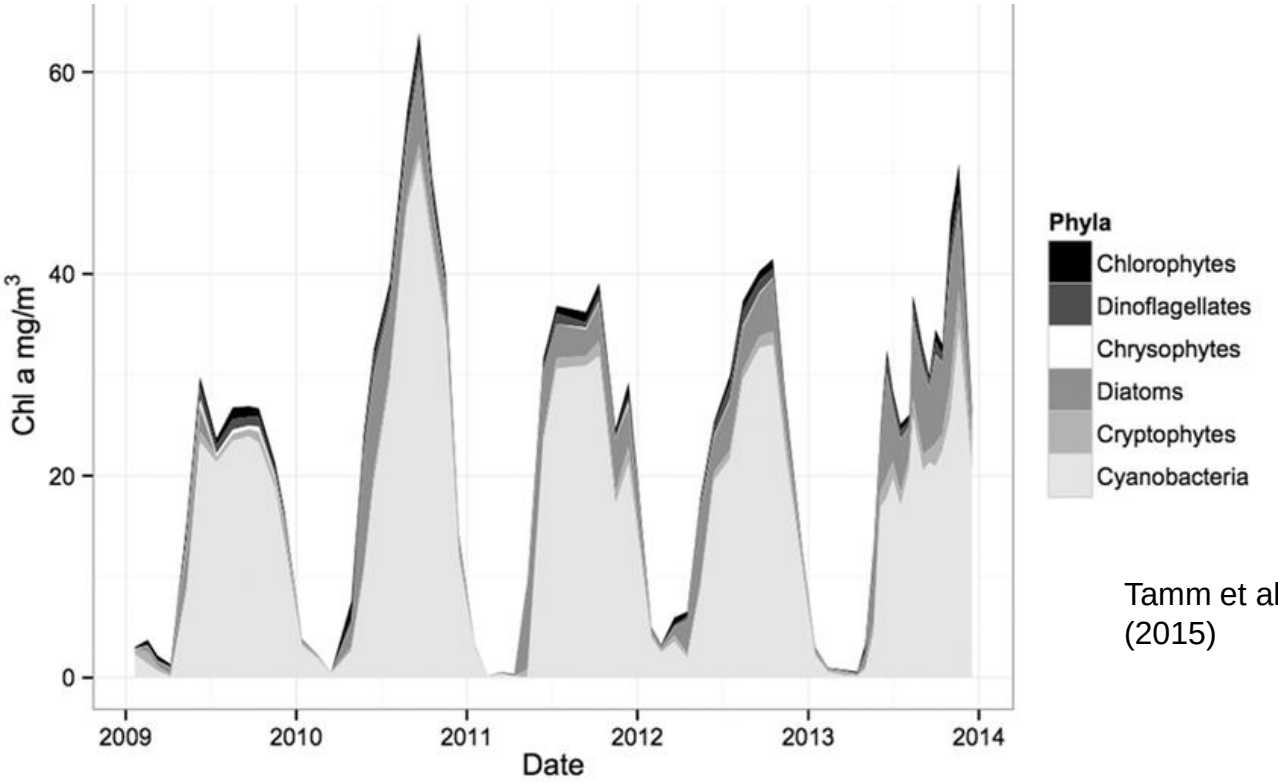



Tamm et al., (2015)



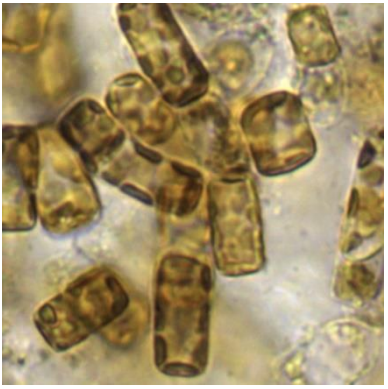


# Spectrophotometric determination and CHEMTAX




Tamm et al., (2015)

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





# Spectrophotometric determination with HPLC

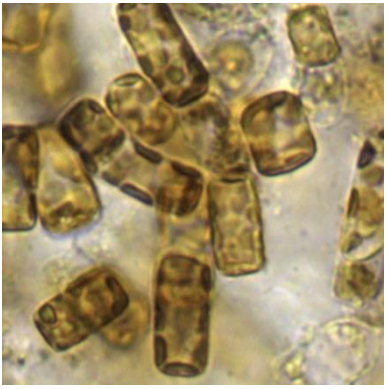
**Advantages**

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

**Disadvantages**

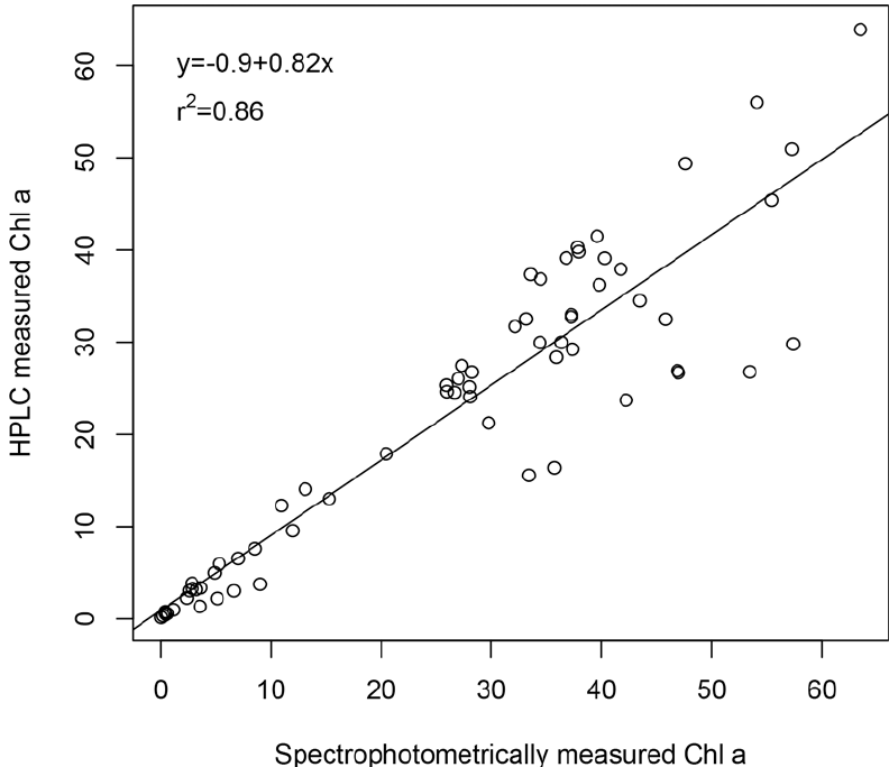
- Artefacts (Chlorophyllides)
- High costs procurement and maintenance
- Harmful organic solvents
- Needs expert level

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

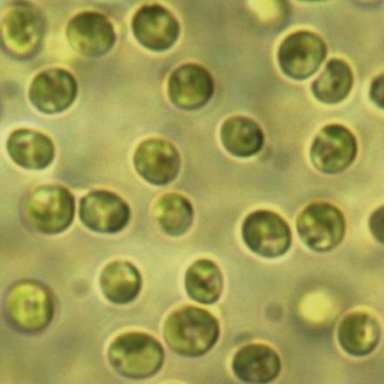




# Spectrophotometric determination ISO vs. HPLC

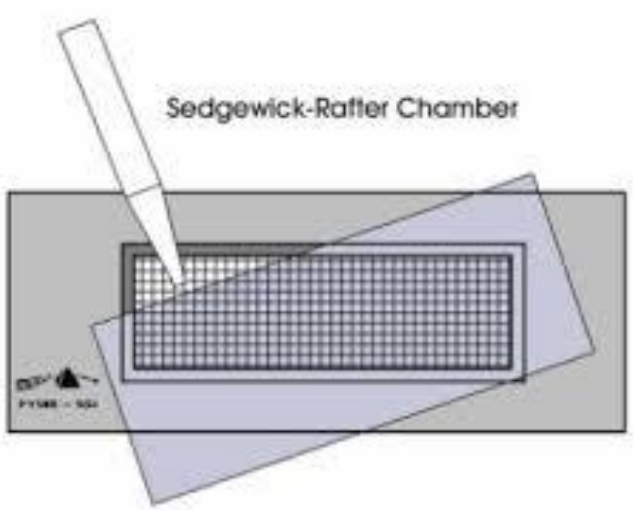



Tamm et al., (2015)





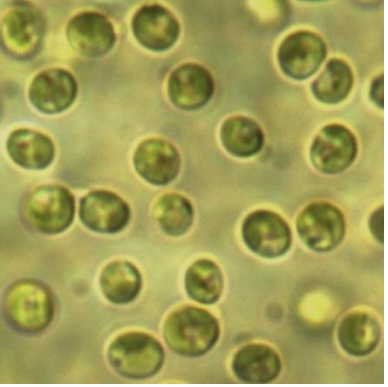
# Cell counting




What do you actually need?

- Counting chamber
- Skilled staff
- Microscope

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



# Cell counting

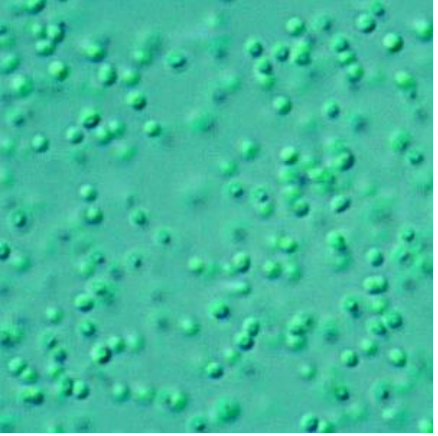
New York Microscope Co

## 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

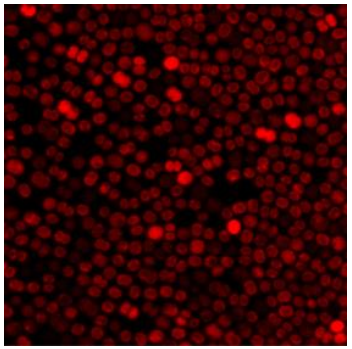


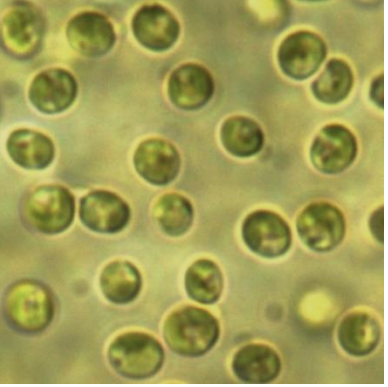


# Missed

*Prochlorococcus* are the smallest organisms in the world able to carry out photosynthesis (they measure only ~0.6µm), as well as being the most numerous (Partensky et al, 1999 ).

**Synechocystis measure < 5 µm**







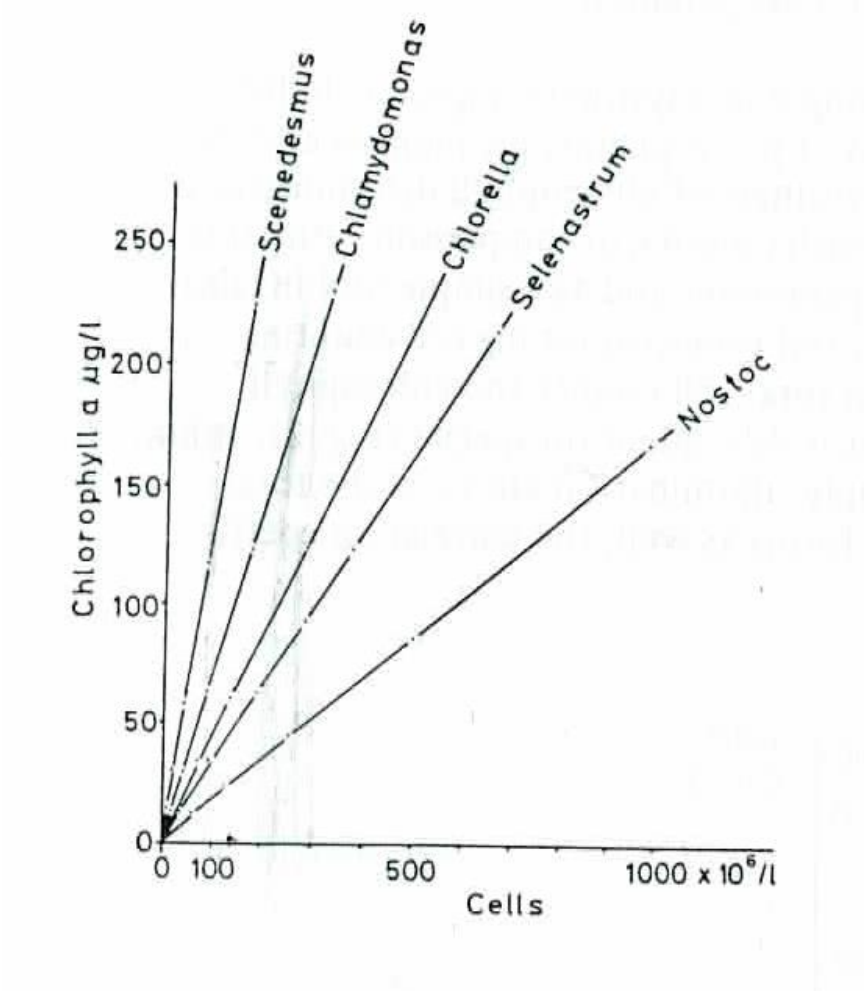
# Cell counting results

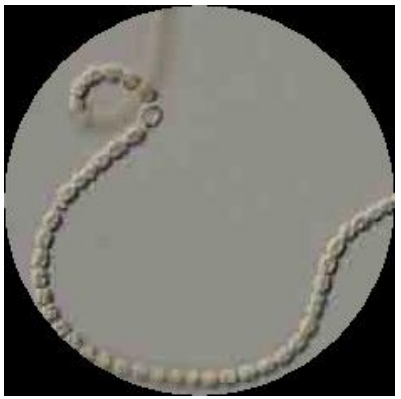
**cyanobacteria counting (cells/ml) by three different laboratories**

| 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

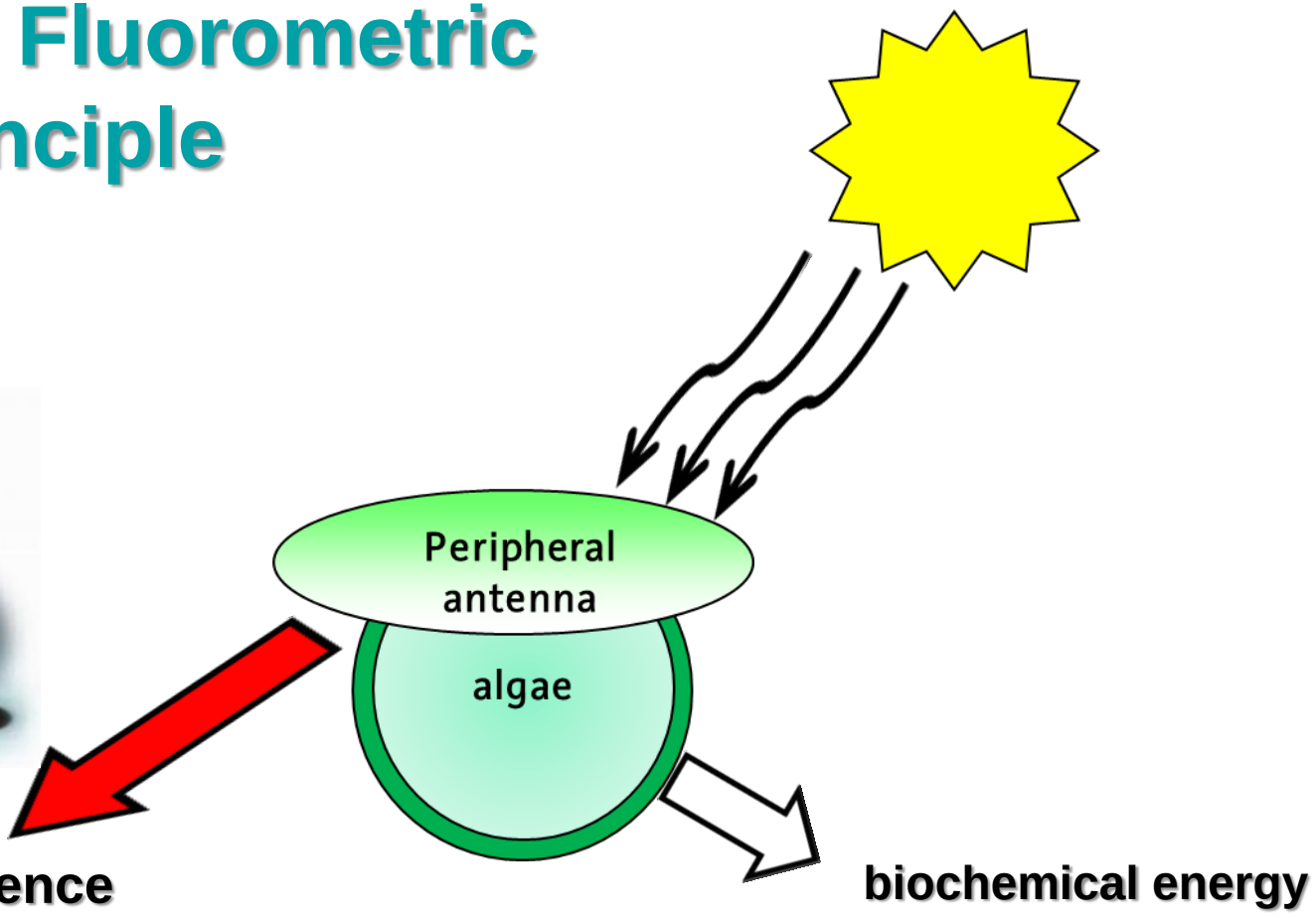


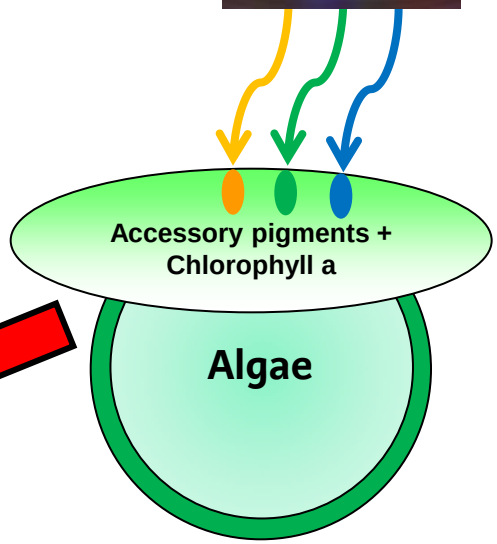
*Nostoc verrucosum*

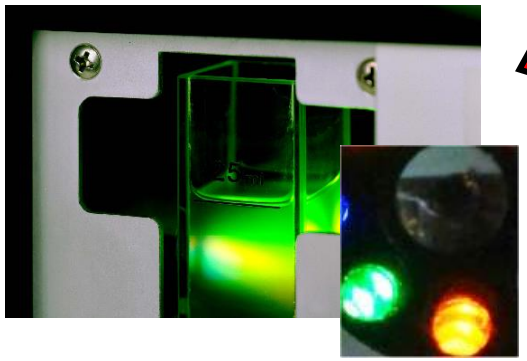
E.A. Nusch (1980)

# The Fluorometric Principle



# Multicolor Excitation

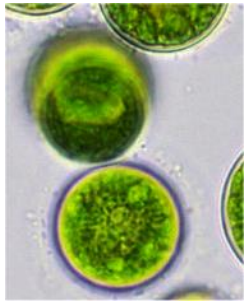


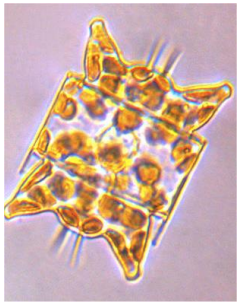
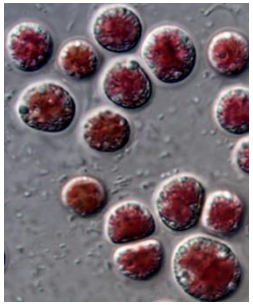
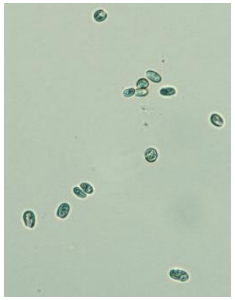



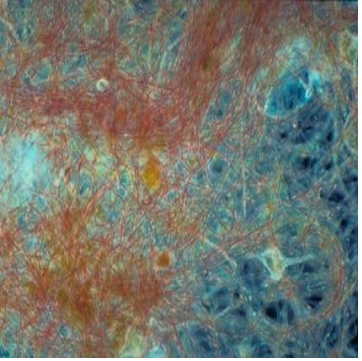
Determination of chlorophyll fluorescence

# Classification of Phytoplankton

| Spectral Group | Green | Blue | Brown | Red | Mixed |
|---|---|---|---|---|---|
| Peripheral Antenna | Chlorophyll a/b-Xanthophyll | Phycobilisomes (Phycocyanin) | Chlorophyll a/c-Xanthophyll | Phycobilisomes (Phycoerythrin) | Chlorophyll a/c-Phycobiliprotein |
| Division | Chlorophyta | Cyanophyta Glaucophyta | Heterokonto-phyta Haptophyta Dinophyta | Rhodophyta | Cryptophyta |

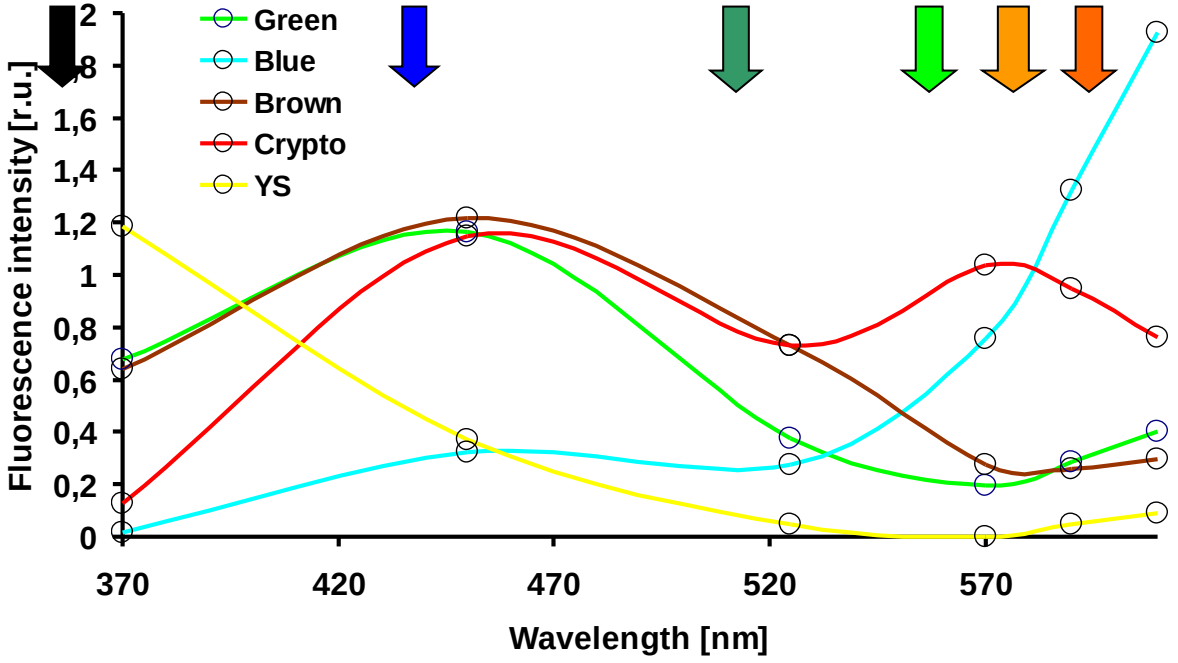


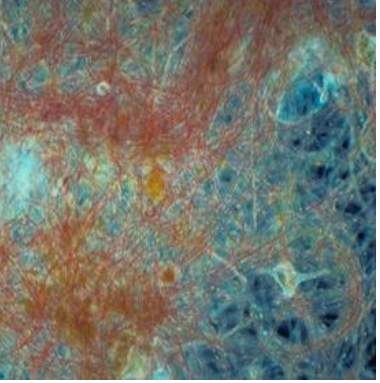




# Algae excitation spectra (fingerprints)




**……enables to calculate the quantity**





# *In Vivo* Fluorometry

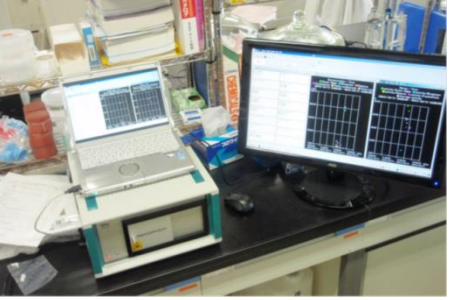

AlgaeTorch by bbe

**Advantages**

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

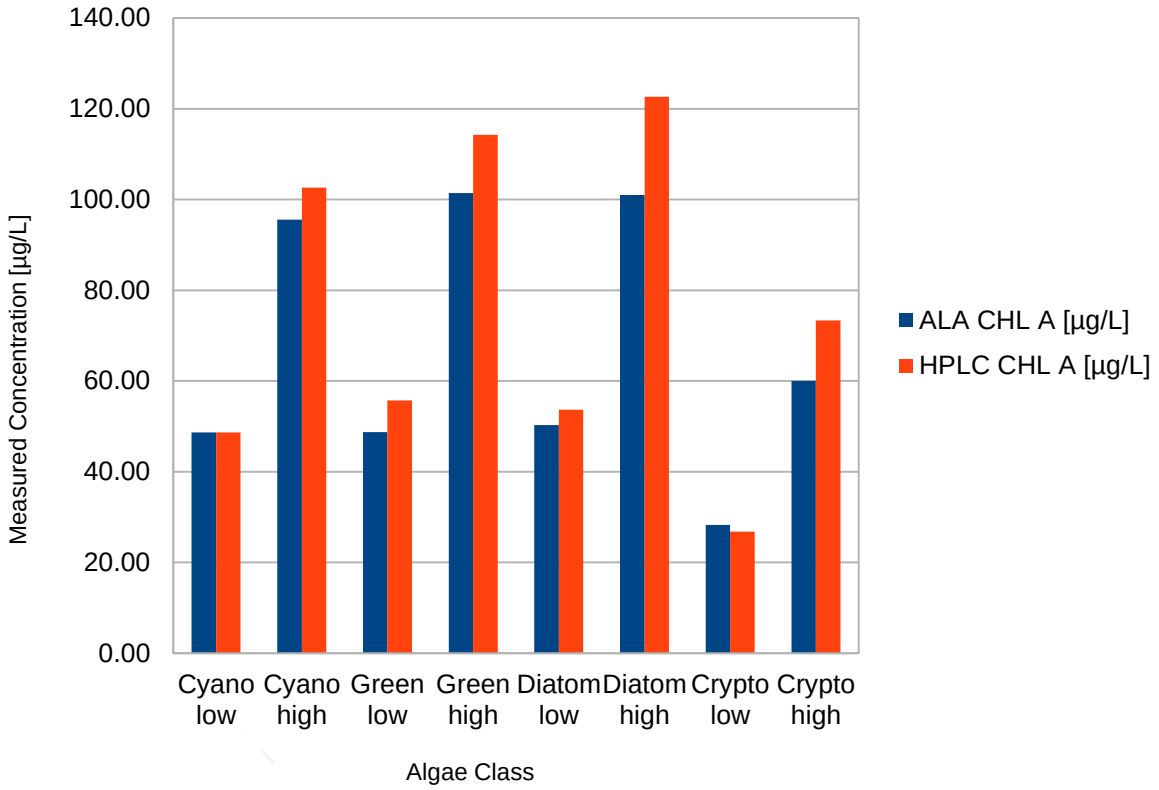
**Disadvantages**

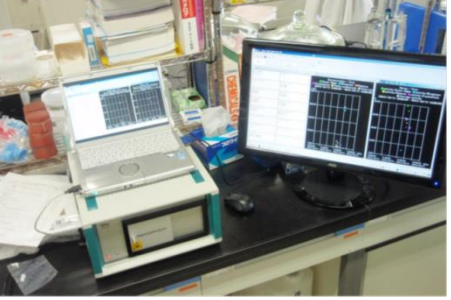
- Interferences with FDOM
- Problems with filamentous  algae and colonies
- Does not work at high turbidity
- Nutrient supply affects chlorophyll complexes
- Ambient light affects chlorphyll fluorescence





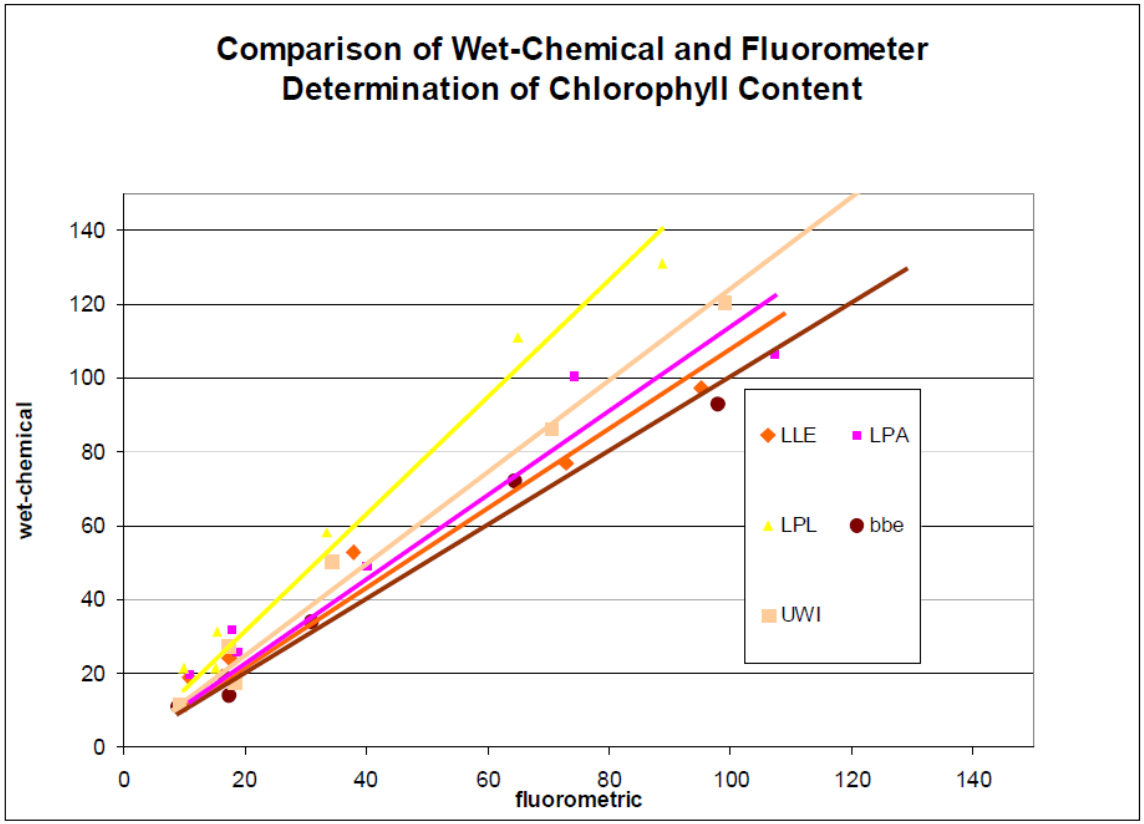
# Comparison: Fluorometry and HPLC/photometry







# 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 adaptions are 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

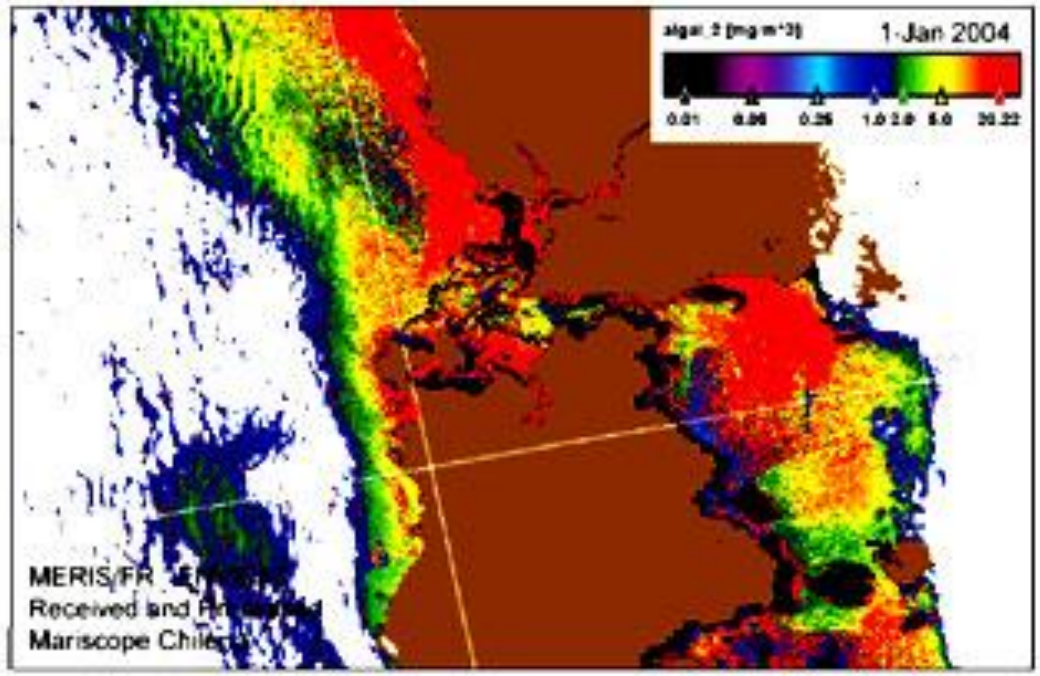
1:1 cellcount algae class distribution

# Chlorophyll a vs. Biovolume

1:1 chl-a algae class distribution

1:1 biovolume algae class distribution

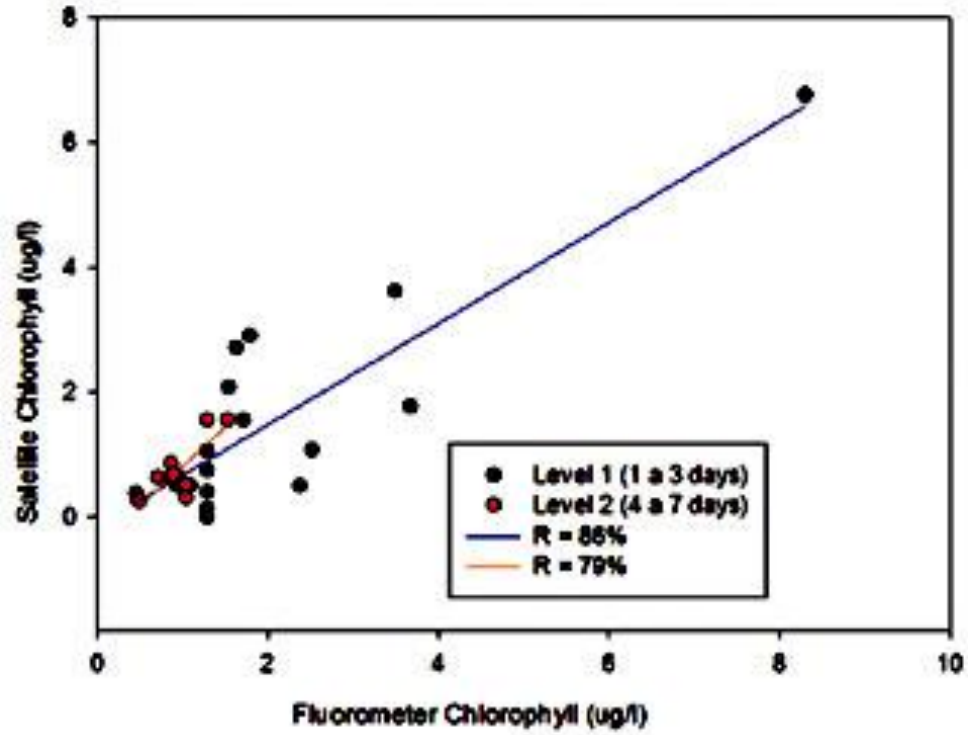
# Algal bloom in the Chacao Channel




MERIS Satellite remote observation

Submersible FluoroProbe

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




Thank you