CALIBRATION OF OPTICAL SENSORS: OUTCOMES FROM THE HELSINKI WORKSHOP OF FEBRUARY 9TH, 2012

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(SYKE)

Work package number [53]	WP4	Type of activity [54]	COORD
Work package title	HARMONIZING OPERATION AND MAINTENANCE METHODS		

TASK 4.1: CALIBRATION (M1 – M42), (HZG, OGS, SMHI, SYKE, NERC(POL), HCMR, CNR, IH)

SubTask 4.1.2: Optical sensors Chl-a, Turbity, PAR (SMHI, IH, OGS, SYKE).

1) Harmonization of calibration practices through documentation and assessment of existing calibration methodologies, equipment, and reference material currently in use within JERICO
2) Sharing of calibration facilities including: a) joint meetings for documentation of existing calibration infrastructures within JERICO b) identification/definition of potential trans-network “nodes” for these services.
3) Designation of best practices for the use of optical sensors. This includes recommendations on time of day and frequency for sampling, calibration procedures, anti fouling measures and procedures to combine different data to produce high quality products.

TASK 4.2: BIO FOULING PREVENTION (M1 – M42), (CNR, HCMR, SYKE, NERC(POL), HZG, NIVA, IFREMER, CNRS)

SubTask 4.2.2: Optical sensors Chl-a, Turbity, PAR (SYKE, NIVA, HCMR)

1) All different methods and approaches will be described and evaluated in terms of costs;
2) The impacts of biofouling on the data quality will be evaluated;
3) Recommendations for the best practice will be given.

Participant list

Participant	institute
Seppo Kaitala	PP2: SYKE
Jukka Seppälä	PP2: SYKE
Petri Maunula	PP2: SYKE
Pasi Ylöstalo	PP2: SYKE
Stefan Simis	PP2: SYKE
John Olsson	PP2: SYKE
Kai Sörensen	PP5: NIVA
Marit Norli	PP5: NIVA
Rajesh Nair	PP8: OGS
Mauro Bastianini	PP9: CNR
~~George Petihakis~~	~~PP11:HCMR~~
Manolis Ntoumas	PP11:HCMR
Dimitris Podaras	PP11:HCMR
Panos Drakopoulos	PP11:HCMR
Athanasios Gkritzalis-Papadopoulos	PP12: NERC
Wilhelm Petersen	PP14:HZG
Hendrik Rust	PP14:HZG
Bengt Karlson	PP17: SMHI
Kieran Adlum	PP20:MI, P&OMaritime
Carlos Hernandez	PP22:AZTI
Francisco Calisto de Almeida	PP24:IH
David Bowers	Univ. Of Bangor UK

Aims for the workshop:

How to perform the primary instrument calibration for fluorometers?

- Algae cultures / Solid secondary standards / Chemical standards
- Comparison of instruments

How to perform validation with field samples?

- How to deal with the variable fluorescence yield

How to prevent bio fouling ?

Can we identify best practices, harmonize protocols, and disseminate Jerico know-how?

Jerico WP4 workshop: Thursday 9th February, 2012 Helsinki

Timetable

9:00-12:00 Scientific session: ***Principles of calibration and bio fouling prevention of optical instruments, especially fluorometers***

9:00-9:10 Welcome (Kaitala)
9:10-9:40 Seppälä: *Challenges in matching up concentration and fluorescence data*
9:40 – 10:10 Karlson: *Diversity of phytoplankton and implications for the use of fluorescence of photosynthetic pigments as biomass proxies*
10:10-10:30 Coffee
10:30-10:50 Sörensen: *Calibration of Chla-Flu*
10:50-11:10 Petersen: *Bio-fouling prevention and experiences with the solid-standard in HZG*
11:10-11:30 Gkritzalis-Papadopoulos: *Experience on chlorophyll sensors - calibrations, applications and data - and on bio-fouling of various sensors*
11:30-11:50 Petihakis: *Fluorescence sensor metrology : Main issues and Ifremer's actions & Biofouling protection for in situ oceanographic sensors by local chlorination*
11:50-12:20 Kaitala: *Calibration, validation and bio fouling prevention of optical sensors in Alg@line project*

12:20-13:30 Lunch (at your own cost in cafeteria next to the meeting room)

13:30-15:00 ***Demonstration of Alg@line-project calibration activities at SYKE***

Chl-a & turbidity (Maunula, Kaitala)
Phycocyanin & CDOM (Seppälä)
Recent developments in optical measurements at SYKE (Simis, Ylöstalo, Olsson) *with coffee*

15:00-18:00 ***Discussions: harmonization of calibration activities, current practices and way forward***

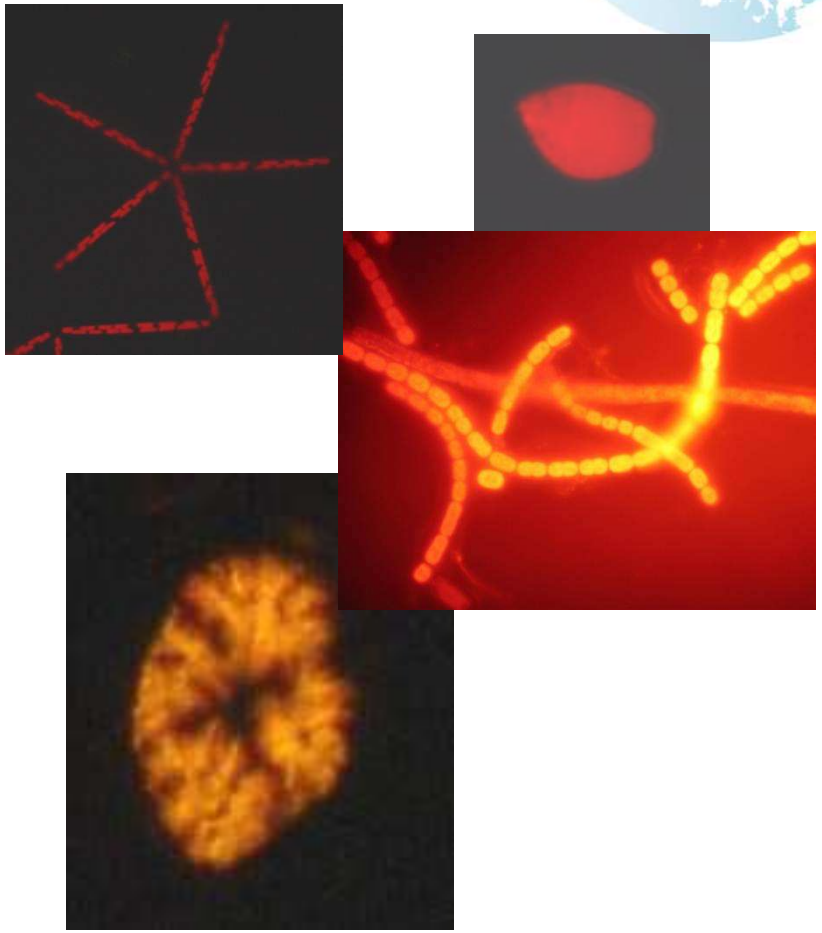
18:00 – Evening buffet and sauna

Diversity of phytoplankton and implications for the use of fluorescence of photosynthetic pigments as biomass proxies

Bengt Karlson SMHI

PIGMENTS WE ARE TRYING TO DETECT USING FLUORESCENCE

- Chlorophyll a – found in all phytoplankton except for *Prochlorococcus*
- *Phycocyanin* – found in some cyanobacteria but also in some cryptophytes
- *Phycoerythrin* – found in some cyanobacteria and in some cryptophytes, dinoflagellates and a ciliate



Photos by Bengt Karlson and Kevin Vikström

Chlorophyll *a in vivo* vs. *in vitro*

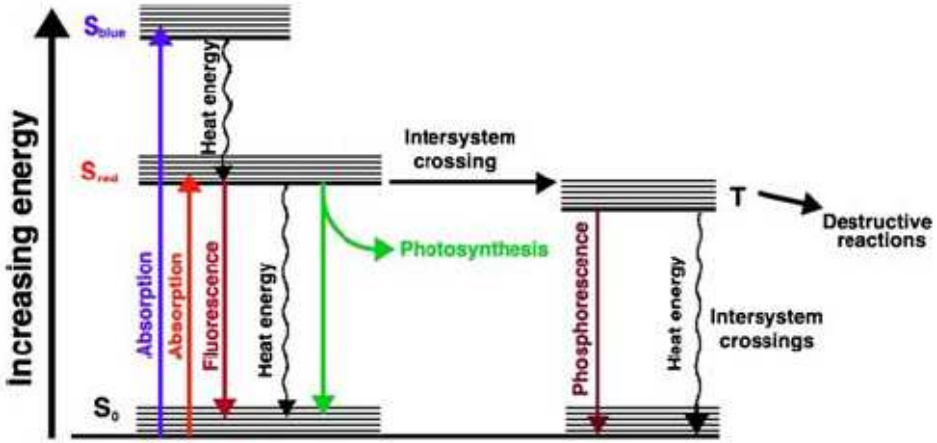
Fluorescence yield, ϕ_F = *fluorescence emission / light absorption*

*Chl*a in vitro: $\phi_F = k_f / (k_f + k_d + k_i) \approx 0.3$

k_f , k_d *and* k_i *are rate constants for excited state decay by fluorescence, thermal emission and triplet formation.*

*Chl*a in vivo: $\phi_F = k_f / (k_f + k_d + k_i + k_p + k_q) \approx 0.005\text{-}0.05$ ***i.e. not constant***

where k_p *and* k_q *are rate constants for photochemistry and for other non-photochemical processes*



Björn et al. 2009 Photosynth Res

From *in vivo* Chl*a* fluorescence [F] to Chl*a* concentration [Chl*a*]

$F = [Chla] \cdot R$

R varies 2-4 fold for single species,
and up to 50-fold between different species.

$$F(\lambda_{ex/em}) = [Chla] \cdot E_{ex} \cdot a^*_{psl} \cdot Q_a^*(\lambda_{em}) \cdot \phi_f$$

Biomass | Instrument | Species | Pigmentation | Physiology

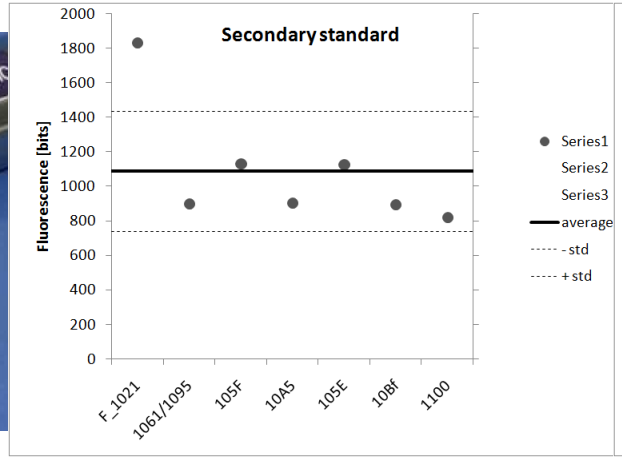
Challenges in matching up concentration & fluorescence data

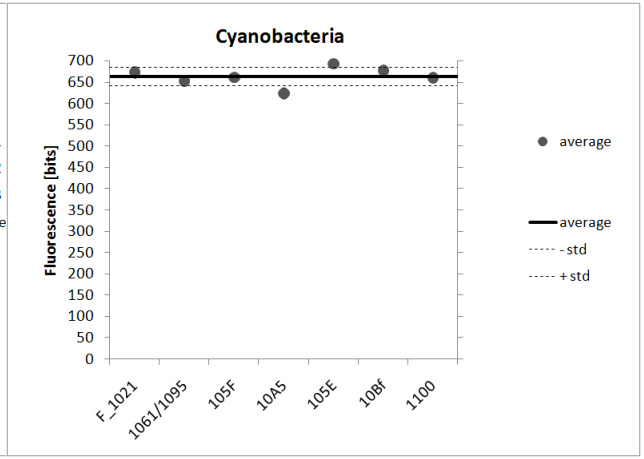
Jukka Seppälä. SYKE

From *in vivo* Chl*a* fluorescence [F] to Chl*a* concentration [Chl*a*]; *Calibration / validation*

Calibration with stable chemical standard or with secondary standard recommended over the use of cultures

- stable and traceable signal, thus instrument performance can be tracked
- instruments (with similar optics) can be compared
- secondary standard does not, however, always allow direct instrument-instrument comparisons

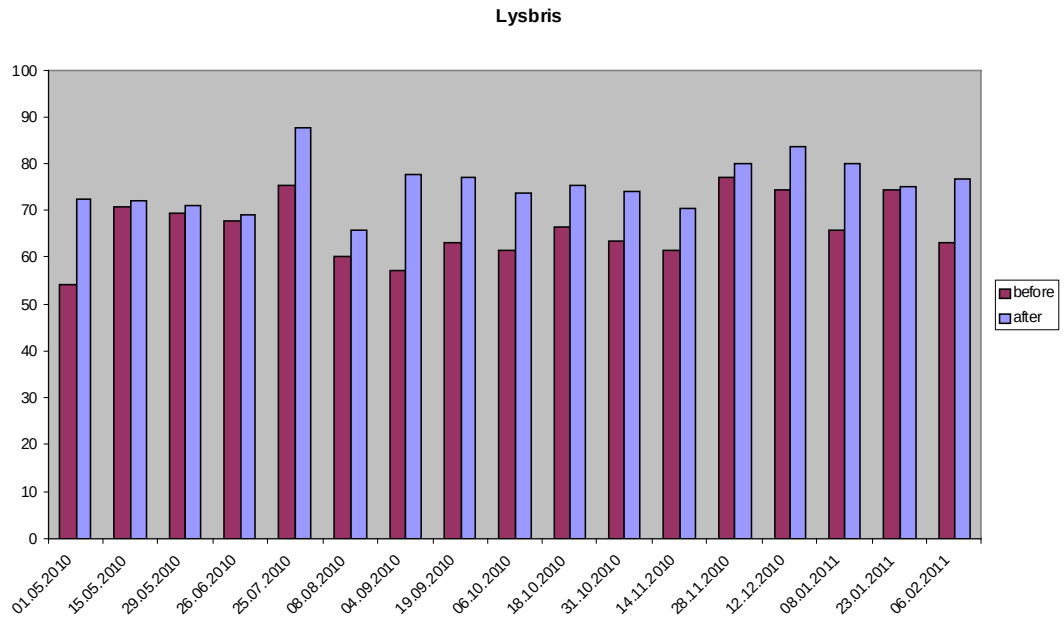




Bio-fouling prevention and experiences with the solid-standards in HZG

Wilhelm Petersen HZG

Experiences with SCUFA-II secondary solid standard

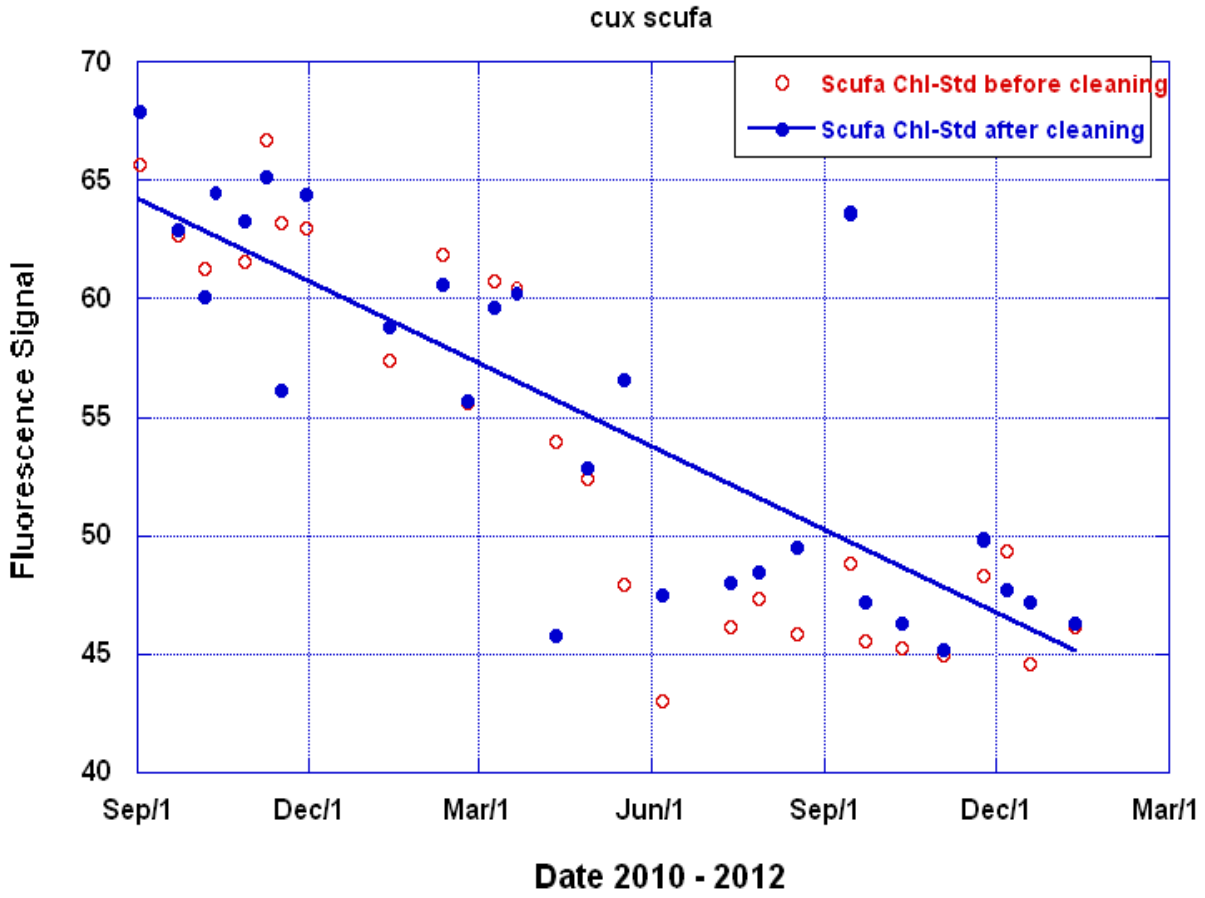


➔ High variability but no significant trend

Bio-fouling prevention and experiences with the solid-standards in HZG

Wilhelm Petersen HZG

SCUFA-II Cuxhaven Change of sensitivity due to high sediment load

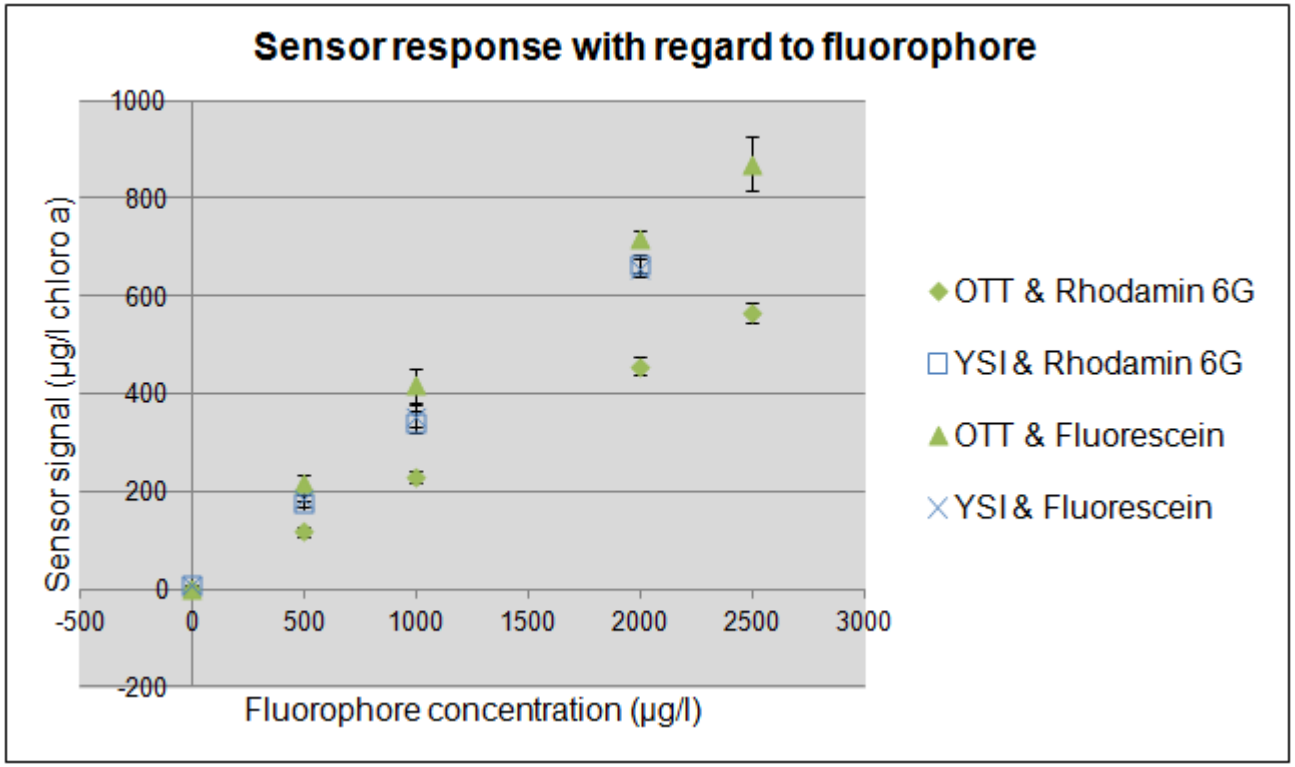


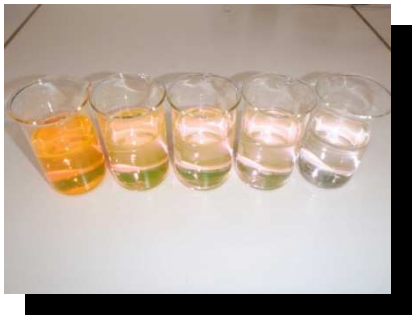
Fluorescence sensor metrology : Main issues and Ifremer's actions

Florence Salvetat IFREMER

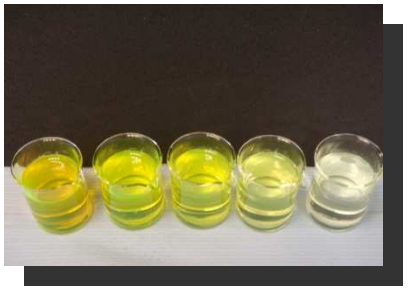
Fluorescence calibration with chemical standards:

- Sensor comparison impossible: sensor response dependent on technology





Rhodamin 6G



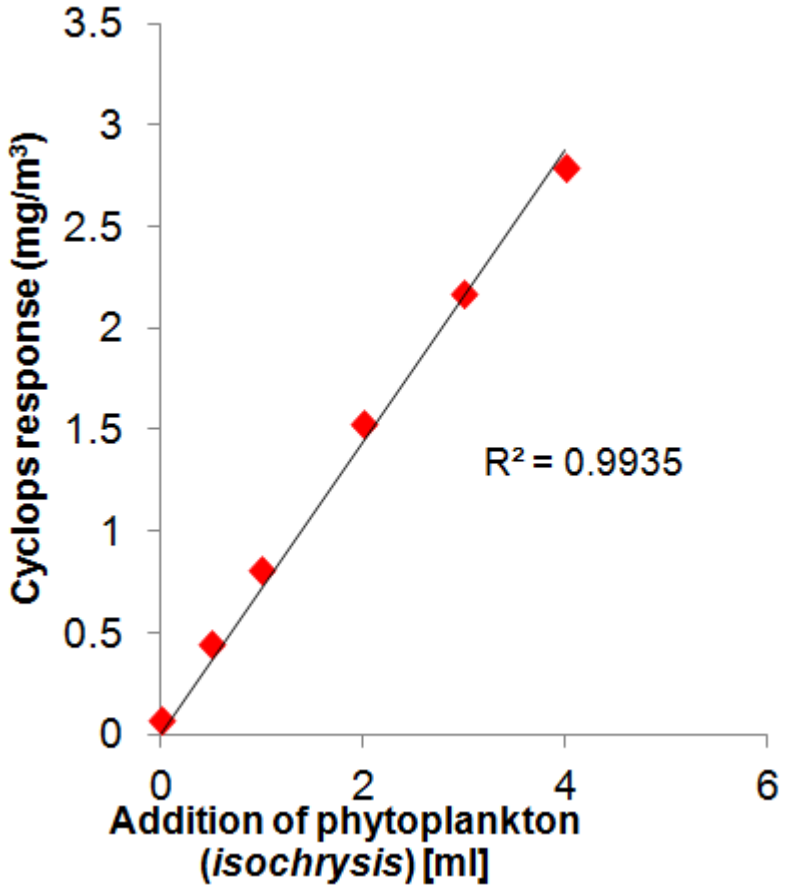
Fluorescein

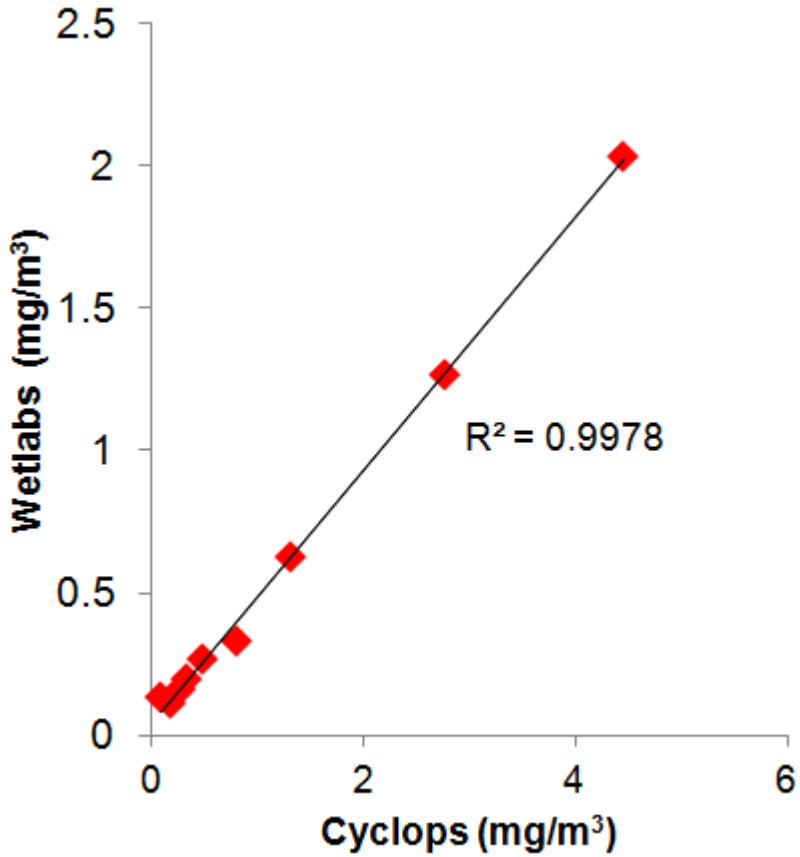
The perfect chromophore-based fluorescence standard should

- be simple to use,
- be sufficiently stable in solution or as a solid
- absorb and emit in the same general regions as the compounds under study,
- display a spectral shape for the emission or excitation spectrum suitable for its scope
- have a constant fluorescence quantum yield
- reveal a negligible small temperature dependence of its fluorometric properties,
- be easy to purify
- dissolve in solvent compatible with field fluorometers

Modified from
Resch-Genger & DeRose 2010 Pure Appl. Chem.

NOCS *Experience on chlorophyll sensors - calibrations, applications and data - and on bio-fouling of various sensors* Thanos Gkritzalis et al NOC

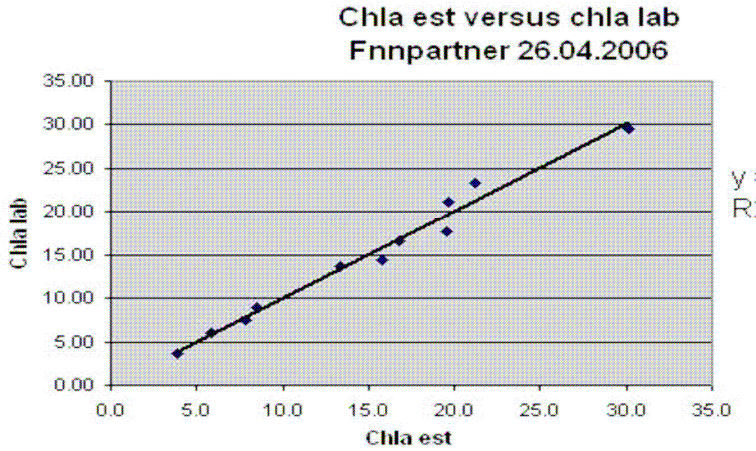




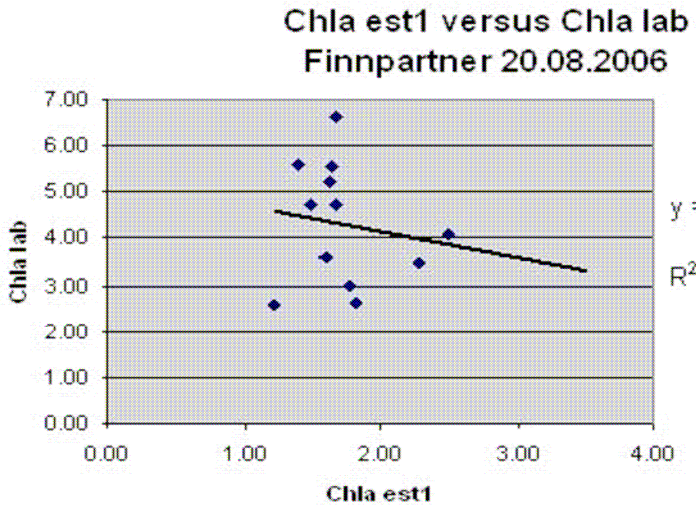
Calibration, validation and bio- fouling prevention of optical sensors in Alg@line project

Seppo Kaitala, Jukka Seppälä, Petri Maunula SYKE

Chlorophyll a validation of chlorophyll-a fluorescence against chlorophyll-a analysis with extraction.



y = 1.4391x - 0.7031
R2 = 0.98



y = - 0.56x + 5.28

R^2 = 0.02

Kai Sørensen, Marit Norli and Are Folkestad NIVA

FLUCTUATIONS IN $CHLA_{FL}$ / $CHLA_{CONC}$

Problem for using Chla_fl as phytoplankton biomass estimation

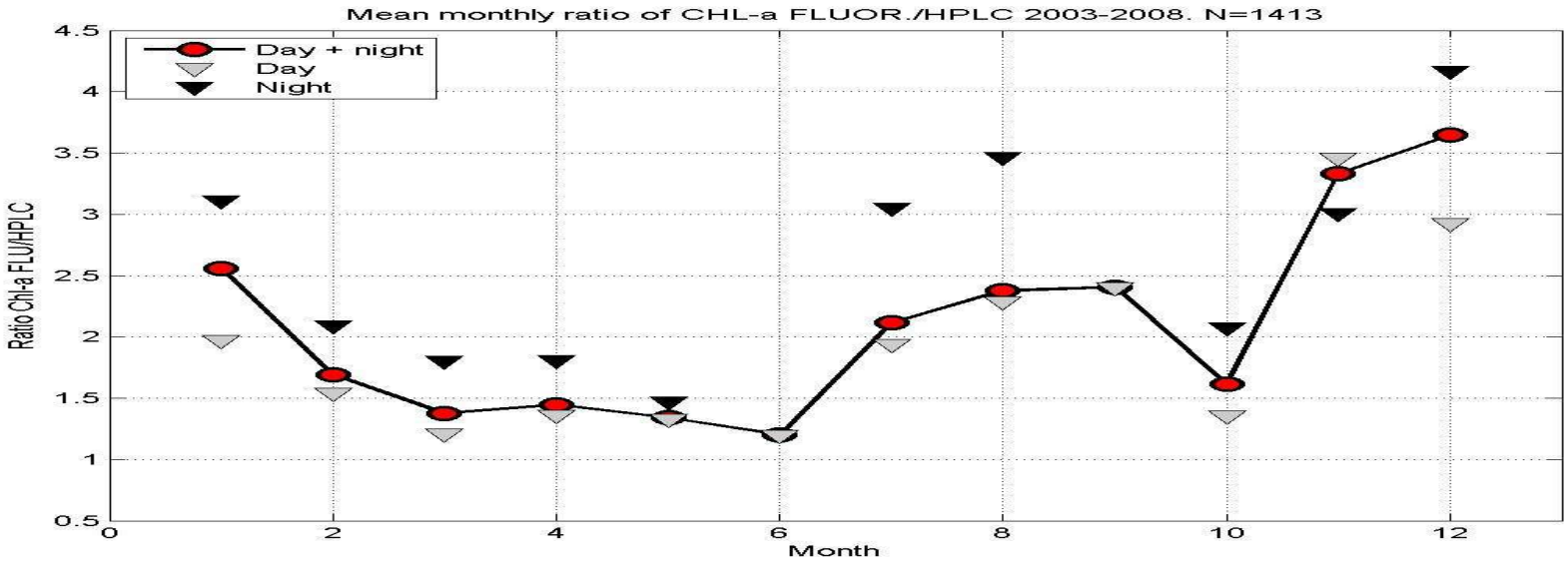
Search for "solutions" in Ferrybox data series and other dataset/time-series from other investigations using data on:

- *PAR*
- *Day length*
- *Temperature*
- *Species composition*
- *Nutrients (not in this study)*
- *Prim. production (not in this study)*

EXPERIENCE FROM CONVERSION («CALIBRATION») OF CHL-A FLUORESCENCE DATA TO CHL-A CONCENTRATION IN FERRYBOX SYSTEMS

Kai Sørensen, Marit Norli and Are Folkestad NIVA

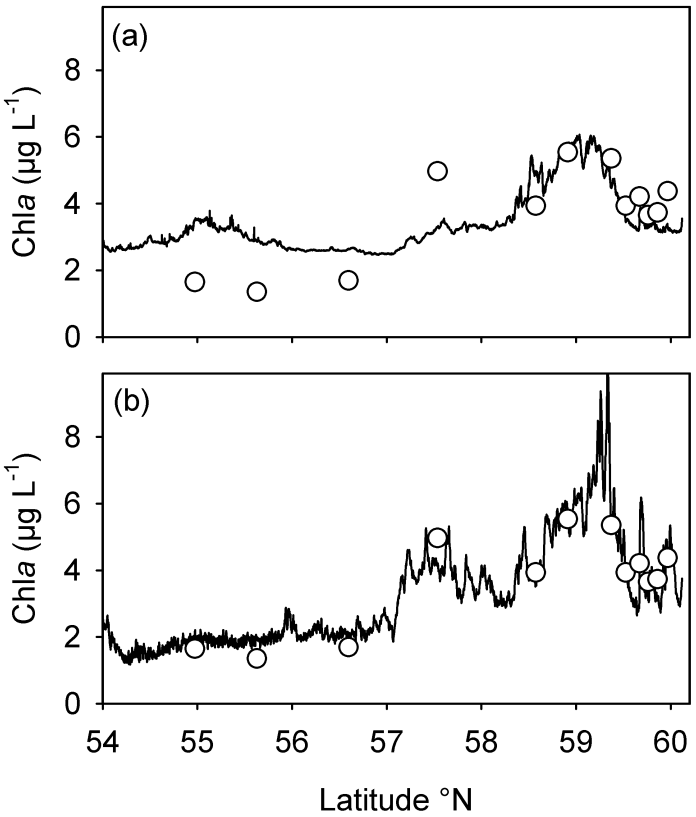
FerryBox seasonal and night and day variation



Yearly calibration of the Chl-a fluorescense using all the Chl-a_hplc water samples

- *Most months show the same trend:*
  - *High ratio at night*
  - *Lower ratio at daytime*

Measuring both Phycocyanin and Chl*a* fluorescence will improve Chl*a* concentration estimates.

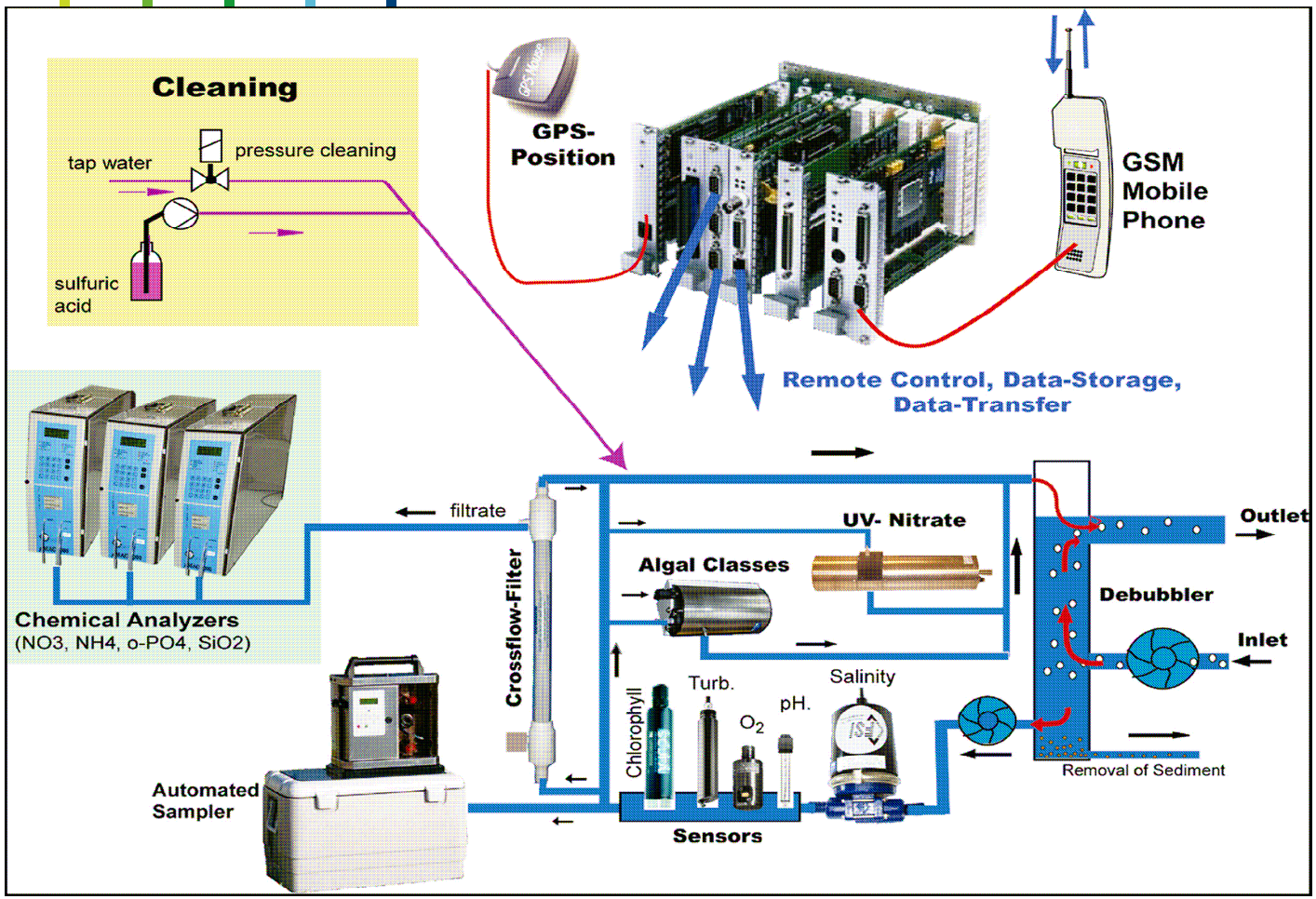


$[Chla] = b_0 + b_1 * Chla\ Fl$

$[Chla] = b_0 + b_1 * Chla\ Fl + b_2 * PC\ Fl$

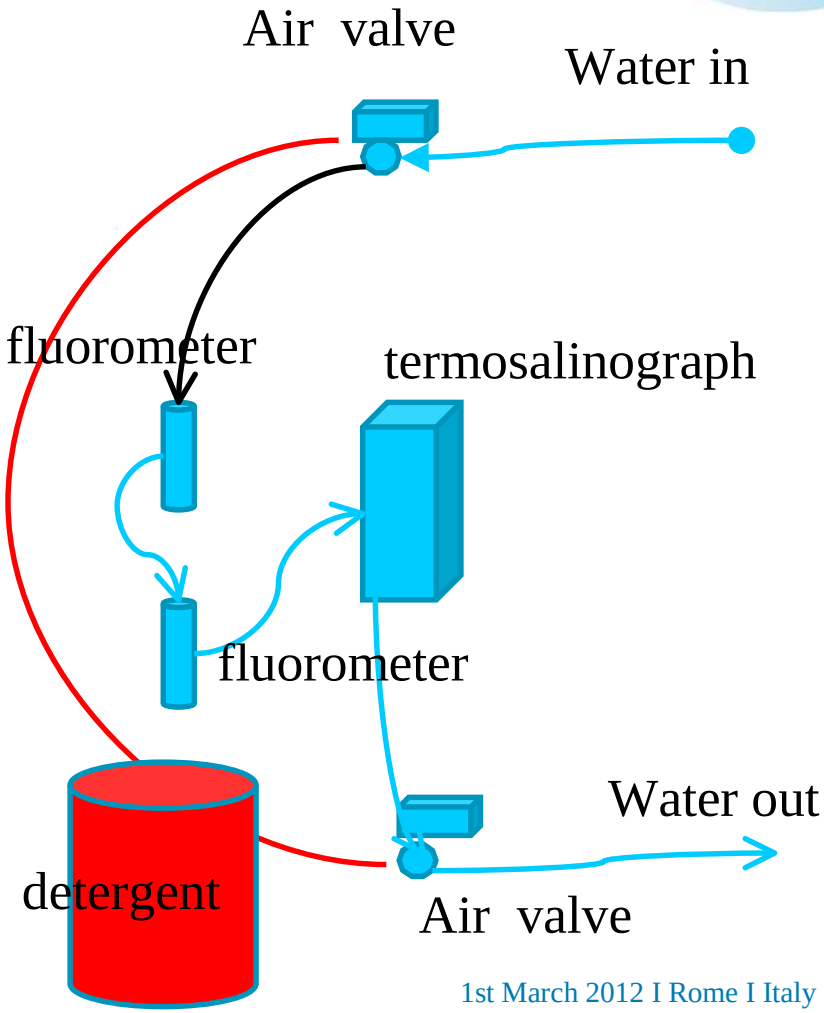
Bio-fouling prevention and experiences with the solid-standards in HZG

Wilhelm Petersen HZG



Calibration, validation and bio- fouling prevention of optical sensors in Alg@line project

Seppo Kaitala, Jukka Seppälä, Petri Maunula SYKE

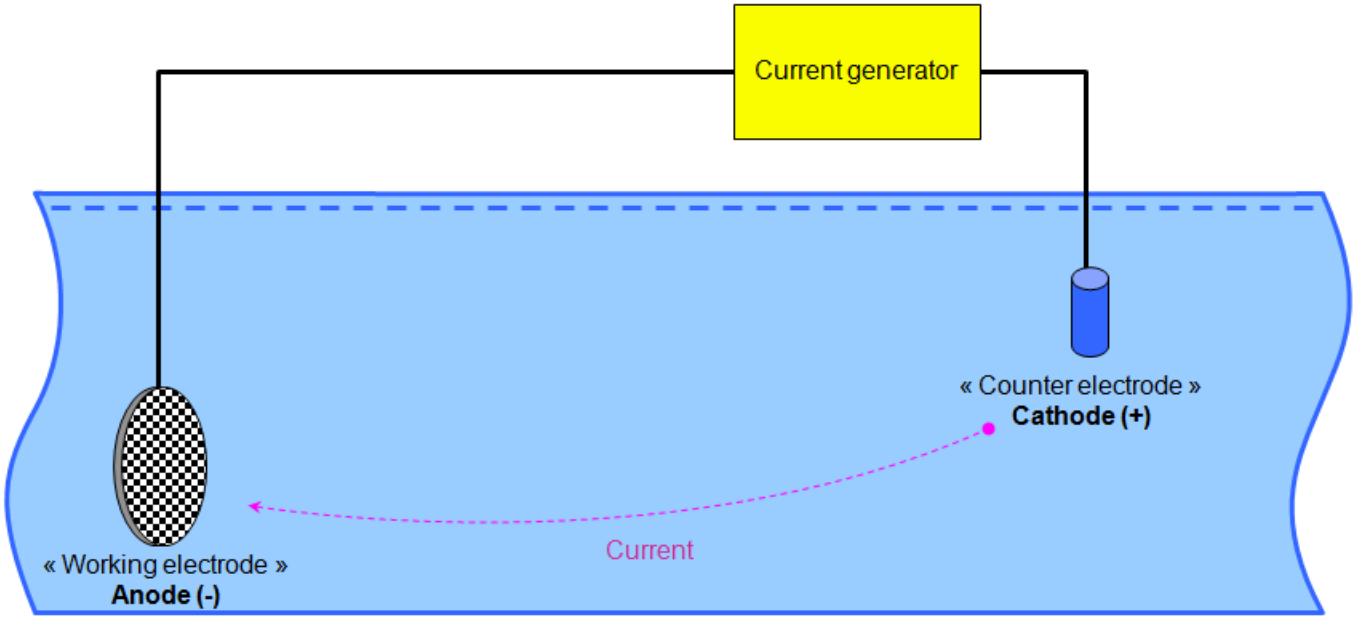


➢ Bio-fouling

- Yes it is present, but...
- Conventional (copper shields, wipers) techniques work
- Equipment and performance of moving parts can b compromised, but … we cannot evaluate whether it affects data quality

Chlorine Generation System In Sea Water

➢ **Sea water electrolysis : Hypochlorous Acid generation.**



$2\ Cl^- \longrightarrow Cl_2 + 2\ e^-$

Then in function of pH and Temperature : Hypochlorous Acid

Note : Anode and Cathode naming is electrochemistry convention, electricity convention is the opposite.

Calibration of optical sensors: outcomes from the Helsinki workshop of February 9th, 2012

1st level problem
Reference materials for Chla calibrations.

Secondary standards:

• Best practice to use solid standard to follow instrument performance
•Traceability of secondary standard (contact manufacturers)

Chemical standards:

• Chla in acetone (or other solvent) may be solution for some instruments but may not be compatible with other
• Should find better chemical standards for primary calibration (artificial Chla proposed by Rajesh)
•Are there special problems with instruments working in low range (stability of standards, offset)

Calibration of optical sensors: outcomes from the Helsinki workshop of February 9th, 2012

2nd level problem
Conversion from fluorescence to Chla concentration

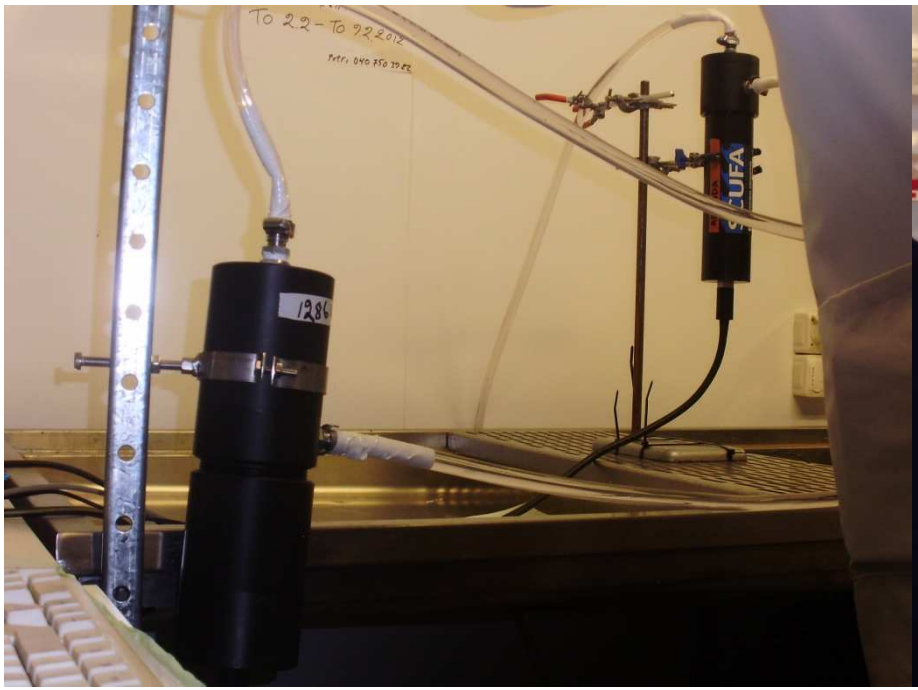
Many alternatives to estimate Chla concentration from fluorescence:

•Importance of keeping raw data
•Importance of archiving
•Optimal data treatment solutions may be site-specific, time-specific , event specific, user specific …

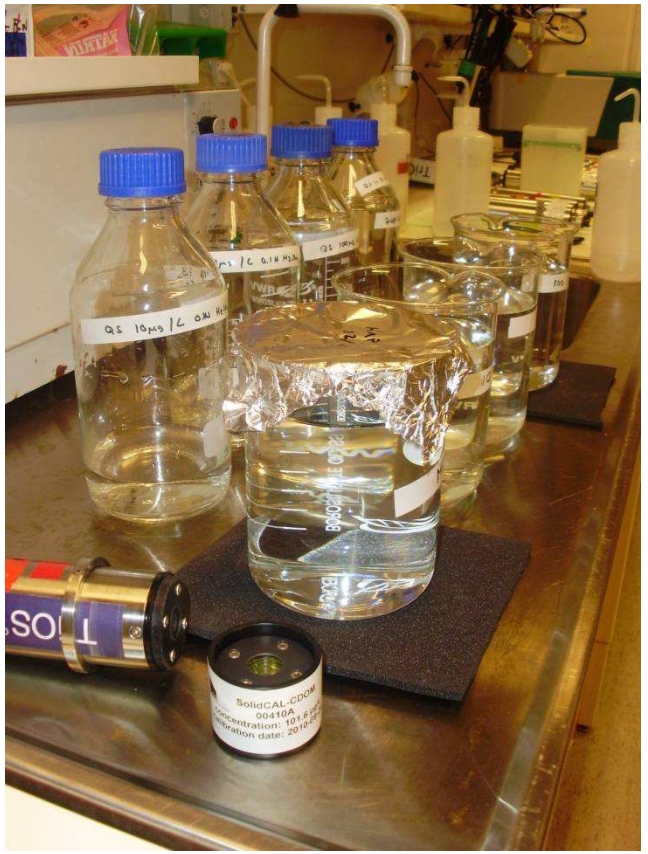
New methods may provide new solutions

• measuring light, variable fluorescence, community structure may improve validation
• WP4 – WP10 communication

Demonstration of Alg@line-project calibration activities at SYKE



Demonstration of Alg@line-project calibration activities at SYKE



Demonstration of Alg@line-project calibration activities at SYKE

Thanks / Kiitos / Grazie!