

First JERICO Fixed Platforms Workshop

Rome 29th February – 1st March 2012



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

***JUKKA SEPPÄLÄ & SEPPÖ KAITALA
(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, Turbidity, 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, Turbidity, 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

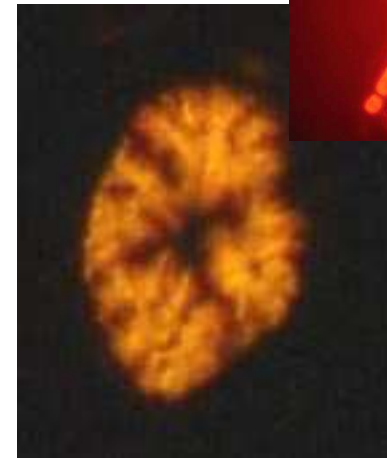
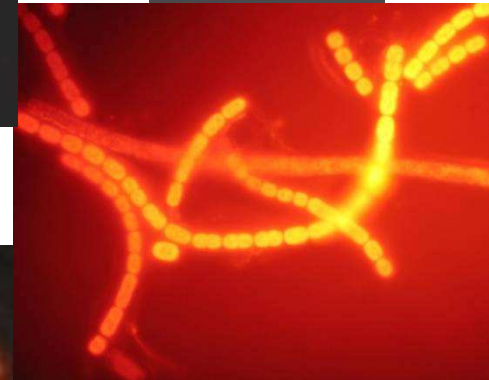
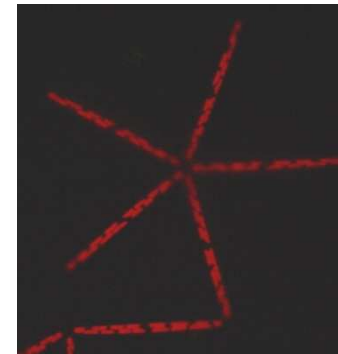
Diversity of phytoplankton and implications for the use of fluorescence of photosynthetic pigments as biomass proxies

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

Challenges in matching up concentration & fluorescence data

Jukka Seppälä. SYKE



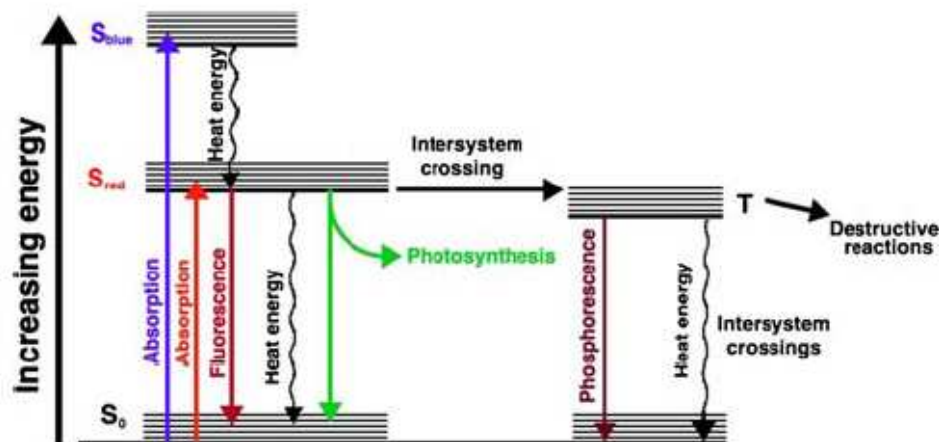
Chlorophyll *a* in vivo vs. in vitro

Fluorescence yield, $\phi_F = \text{fluorescence emission} / \text{light absorption}$

Chla 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.

Chla 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

Challenges in matching up concentration & fluorescence data

Jukka Seppälä. SYKE



From *in vivo* Chla fluorescence [F] to Chla concentration [Chla]

$$F = [\text{Chla}] \cdot R$$

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

$$F(\lambda_{ex/em}) = [\text{Chla}] \cdot E_{ex} \cdot \bar{a}_{PSII}^* \cdot Q_a^*(\lambda_{em}) \cdot \phi_F$$

Biomass Instrument Species Pigmentation Physiology

Challenges in matching up concentration & fluorescence data

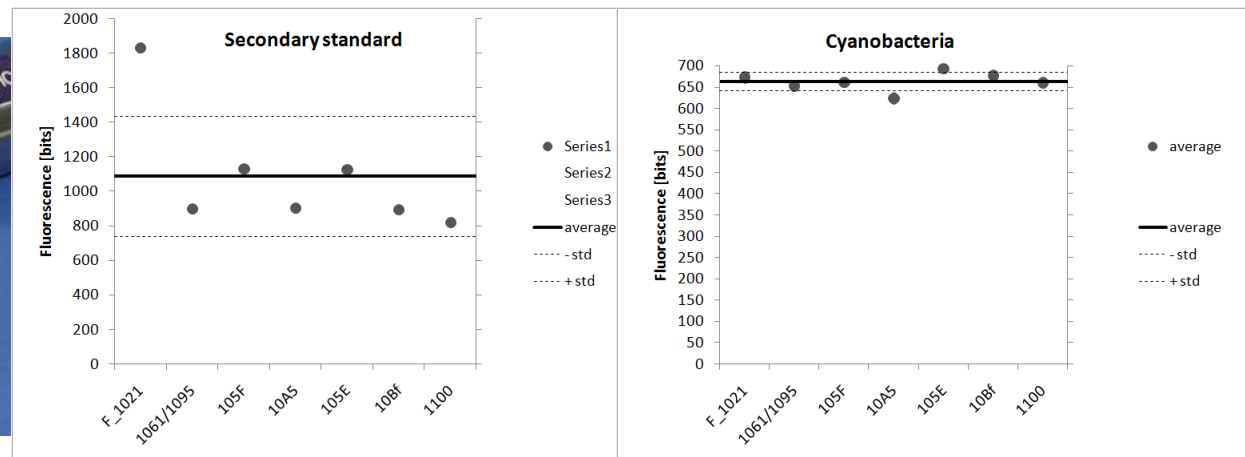
Jukka Seppälä. SYKE



From *in vivo* Chla fluorescence [F] to Chla concentration [Chla]; 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

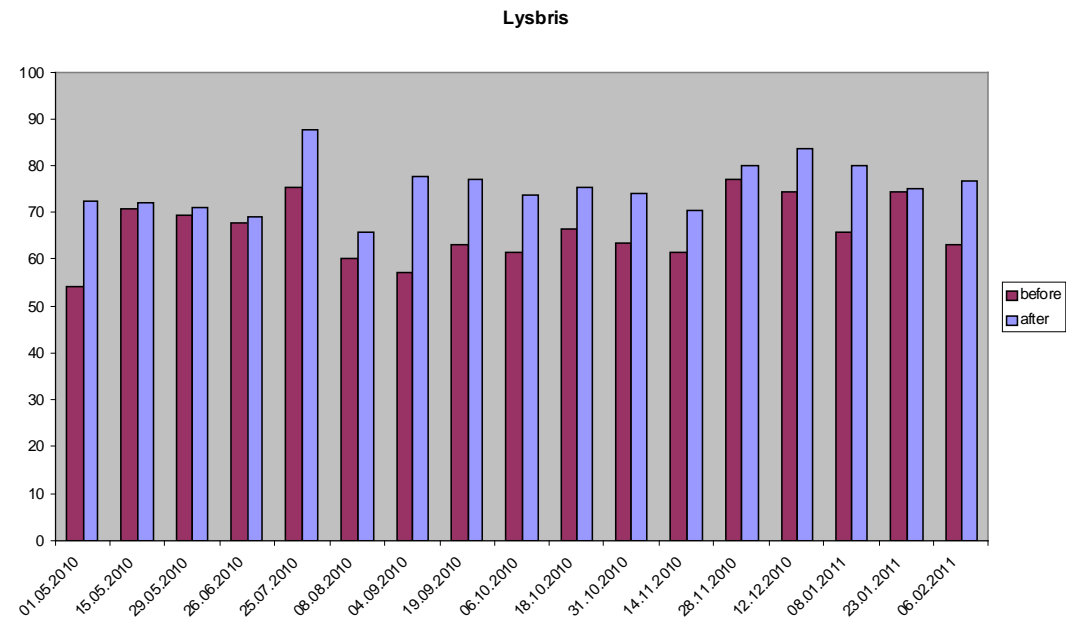


Bio-fouling prevention and experiences with the solid-standards in HZG

Wilhelm Petersen HZG



Experiences with SCUFA-II secondary solid standard



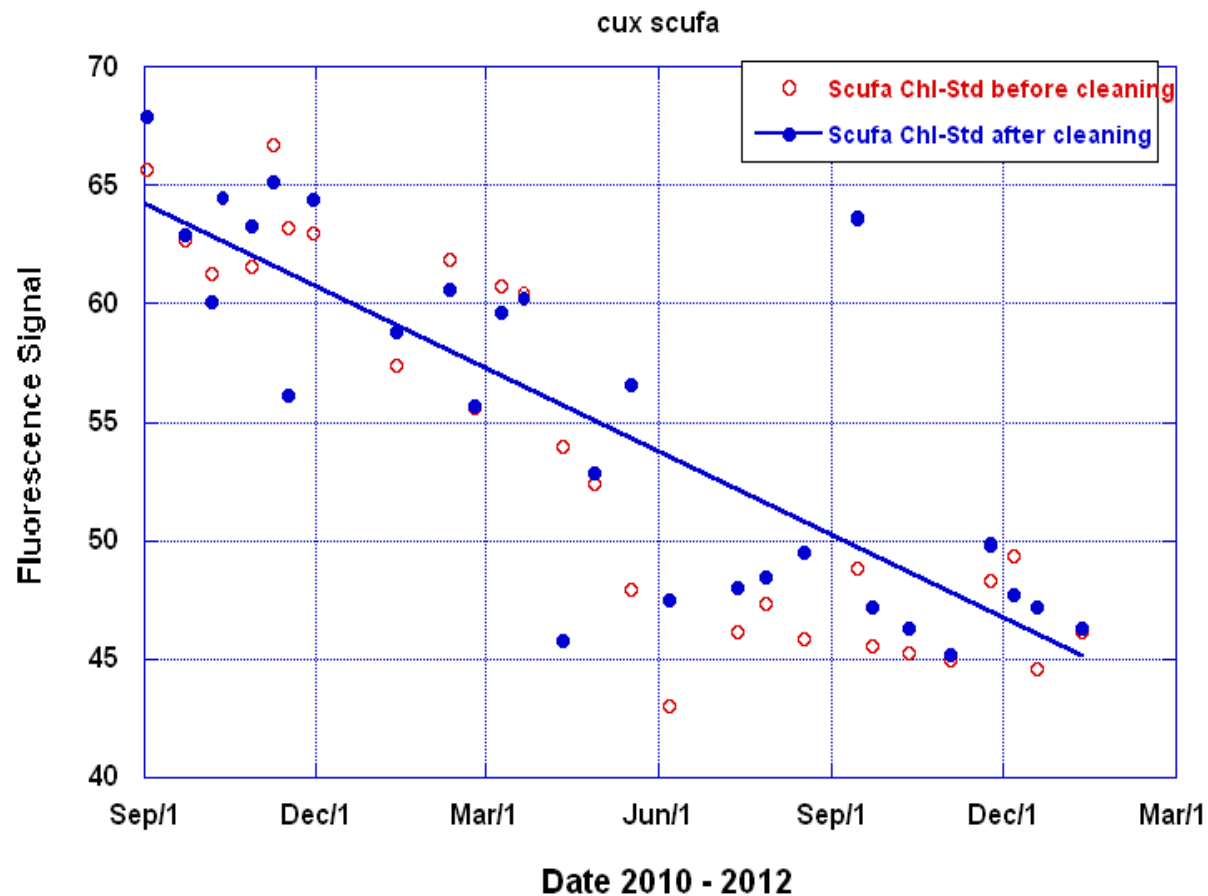
→ High variability but no significant trend

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

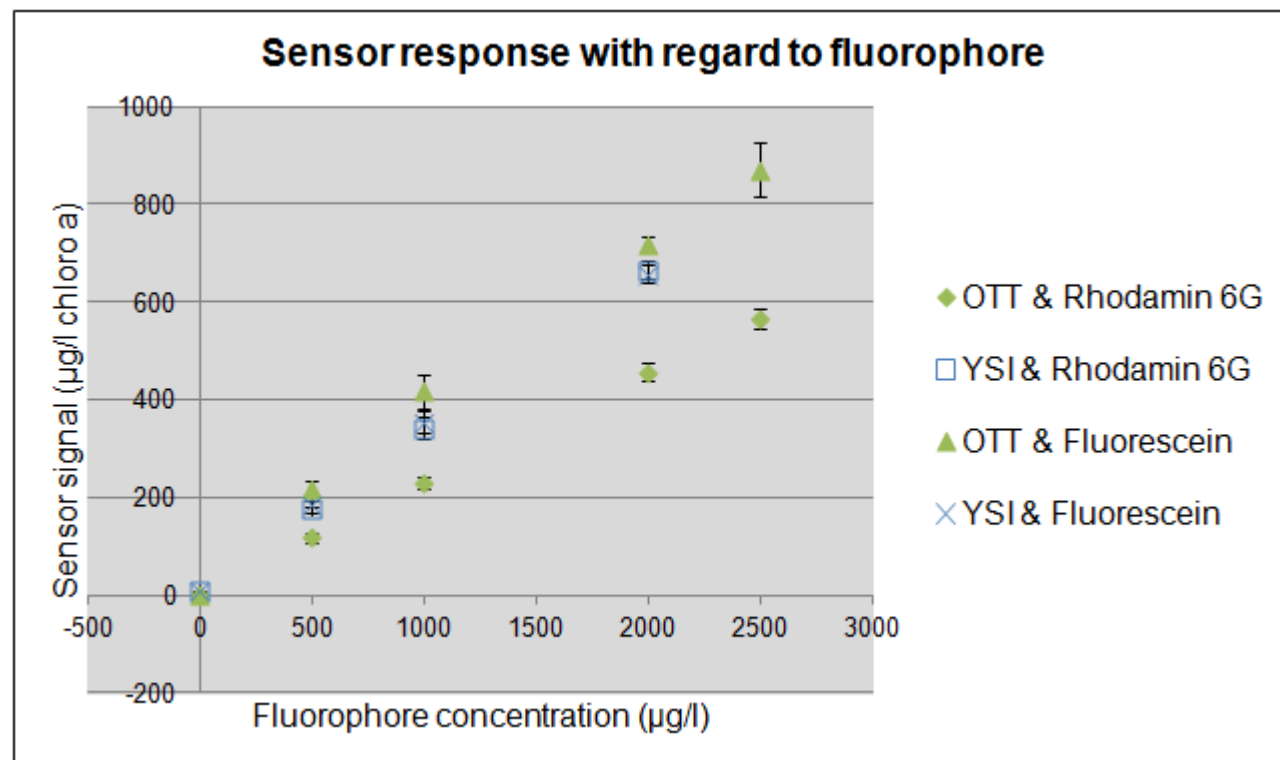


Fluorescence sensor metrology : Main issues and Ifremer's actions *Florence Salvetat* IFREMER

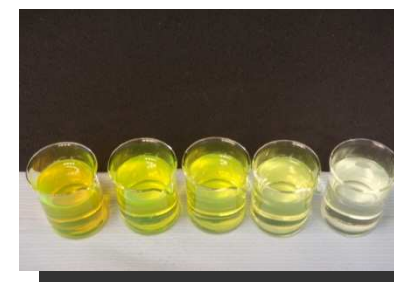


Fluorescence calibration with chemical standards:

- Sensor comparison impossible: sensor response dependent on technology



Rhodamin 6G



Fluorescein

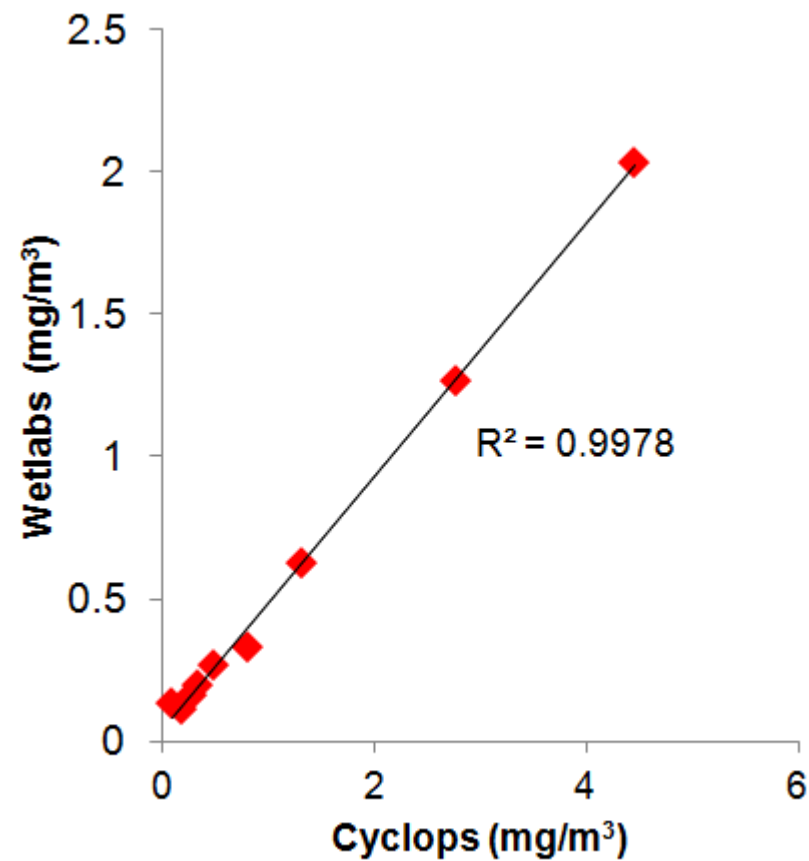
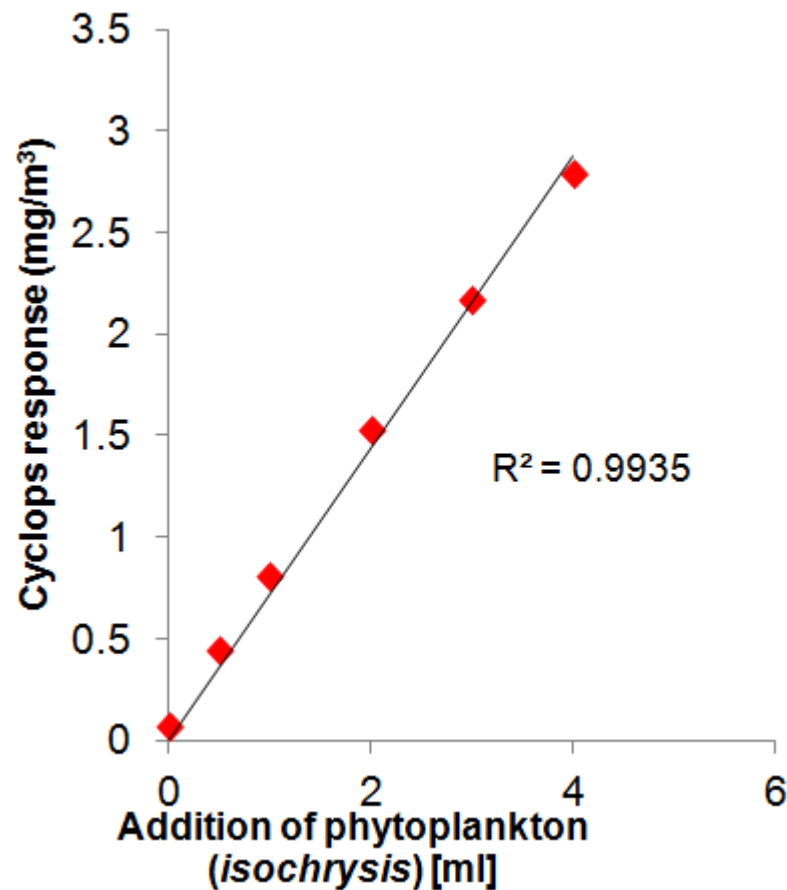
The perfect chromophore-based fluorescence standard should



- 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

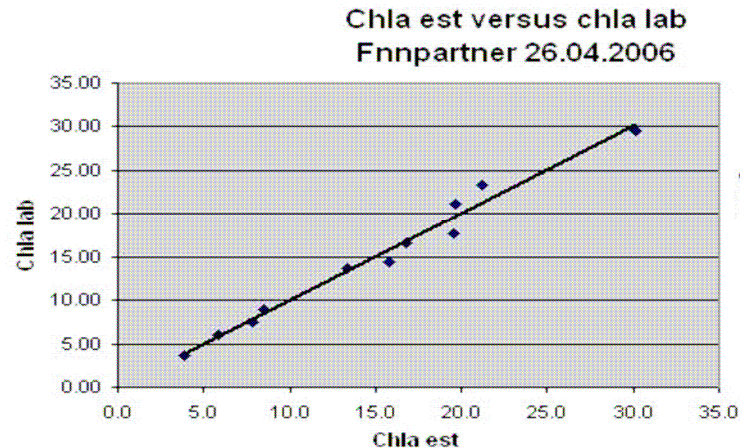


Calibration, validation and bio-fouling prevention of optical sensors in Alg@line project

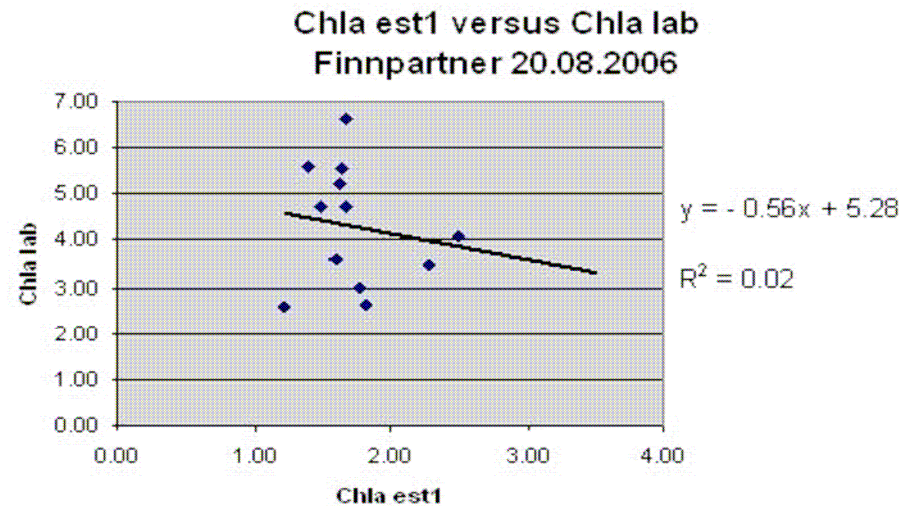
Seppo Kaitala, Jukka Seppälä, Petri Maunula SYKE



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



$$y = 1.4391x - 0.7031$$
$$R^2 = 0.98$$



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

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)

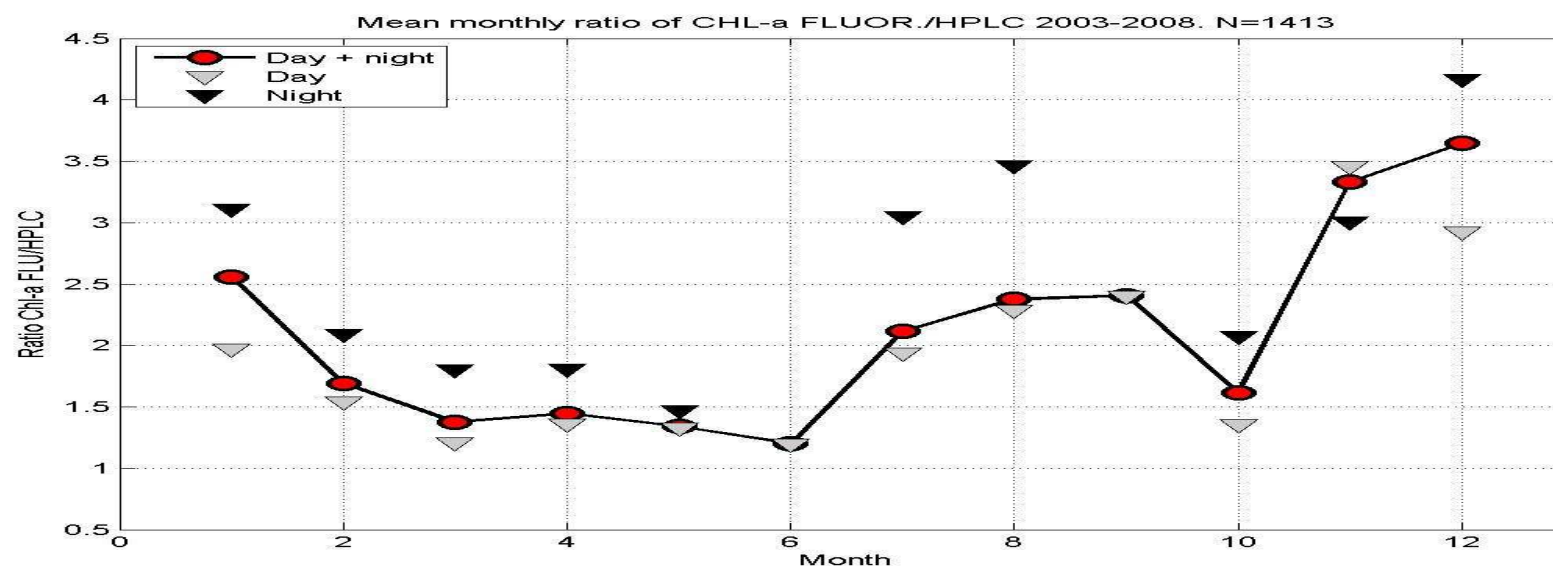
Prim. production (not in this study)

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 fluorescence using all the Chl-a_hplc water samples

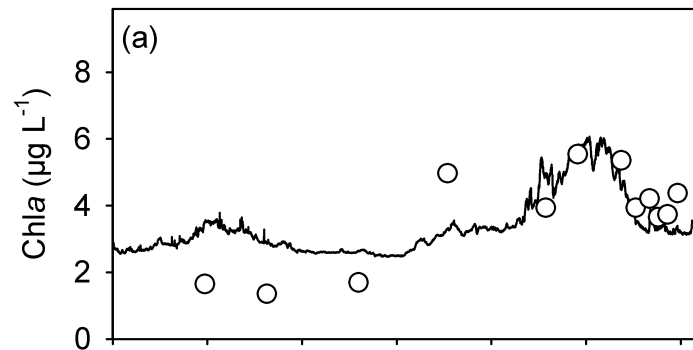
- *Most months show the same trend:*
 - *High ratio at night*
 - *Lower ratio at daytime*

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

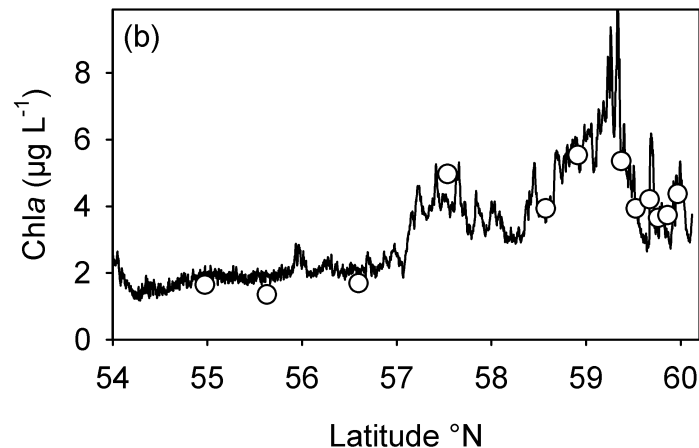
Seppo Kaitala, Jukka Seppälä, Petri Maunula SYKE



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



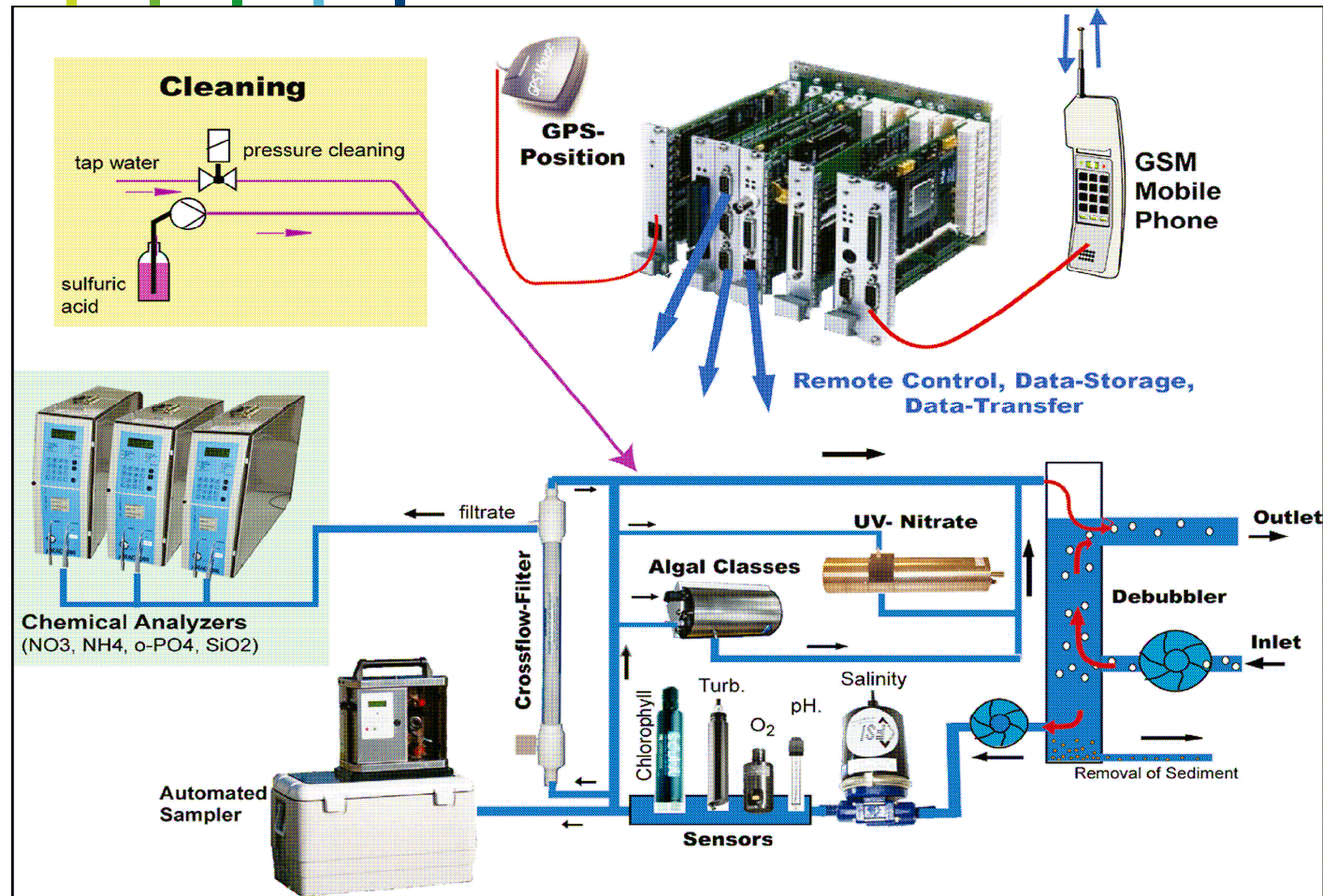
$$[\text{Chl}a] = b_0 + b_1 * \text{Chl}a \text{ Fl}$$



$$[\text{Chl}a] = b_0 + b_1 * \text{Chl}a \text{ Fl} + b_2 * \text{PC Fl}$$

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

Wilhelm Petersen HZG

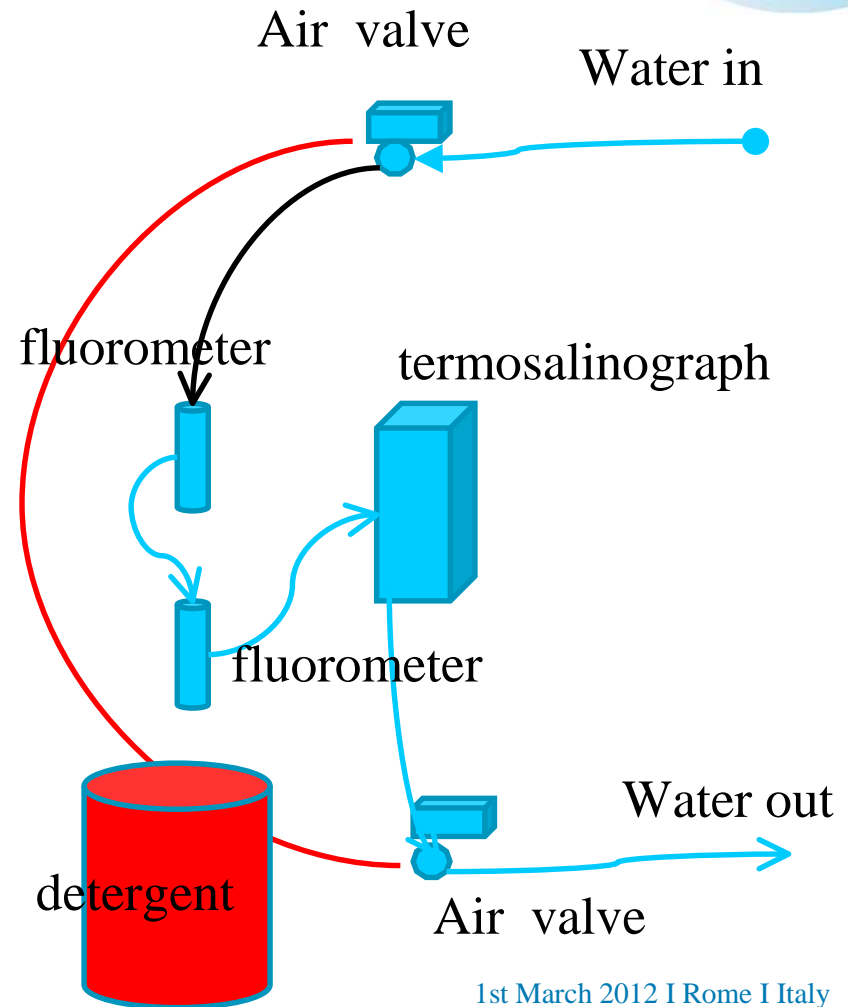


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Calibration, validation and bio-fouling prevention of optical sensors in Alg@line project

Seppo Kaitala, Jukka Seppälä, Petri Maunula SYKE



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



➤ **Bio-fouling**

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



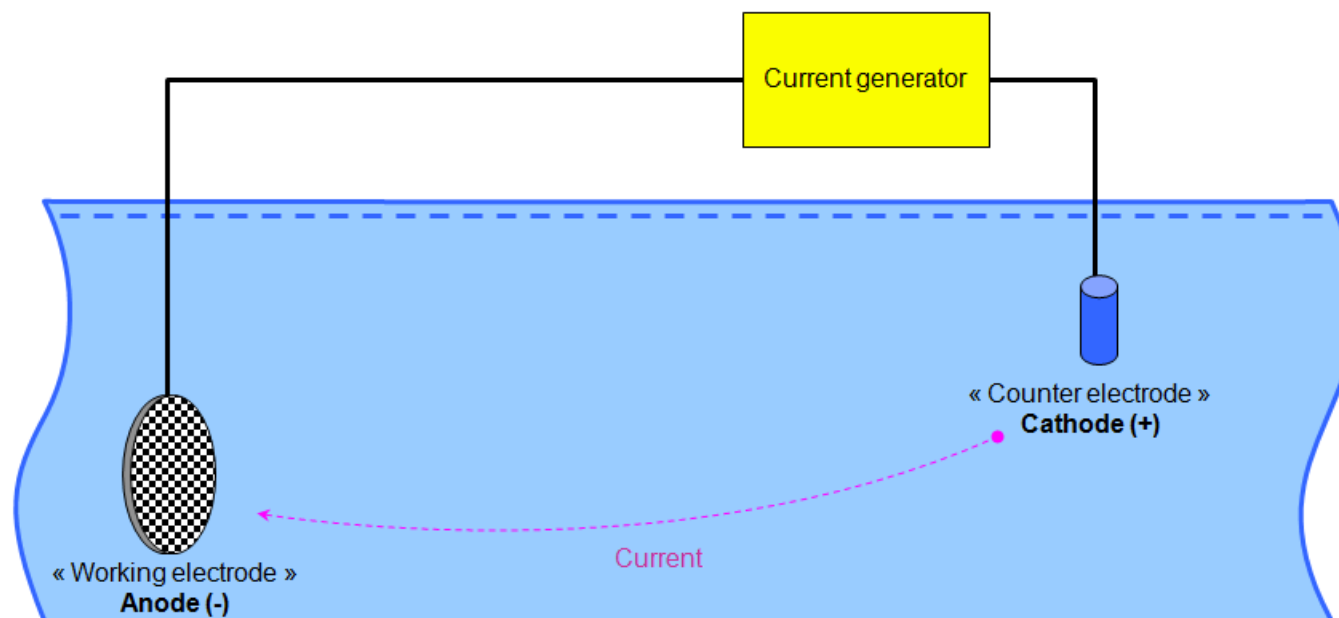
Biofouling protection for *in situ* oceanographic sensors by local chlorination

L.Delauney IFREMER



Chlorine Generation System In Sea Water

➤ Sea water electrolysis : Hypochlorous Acid generation.



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

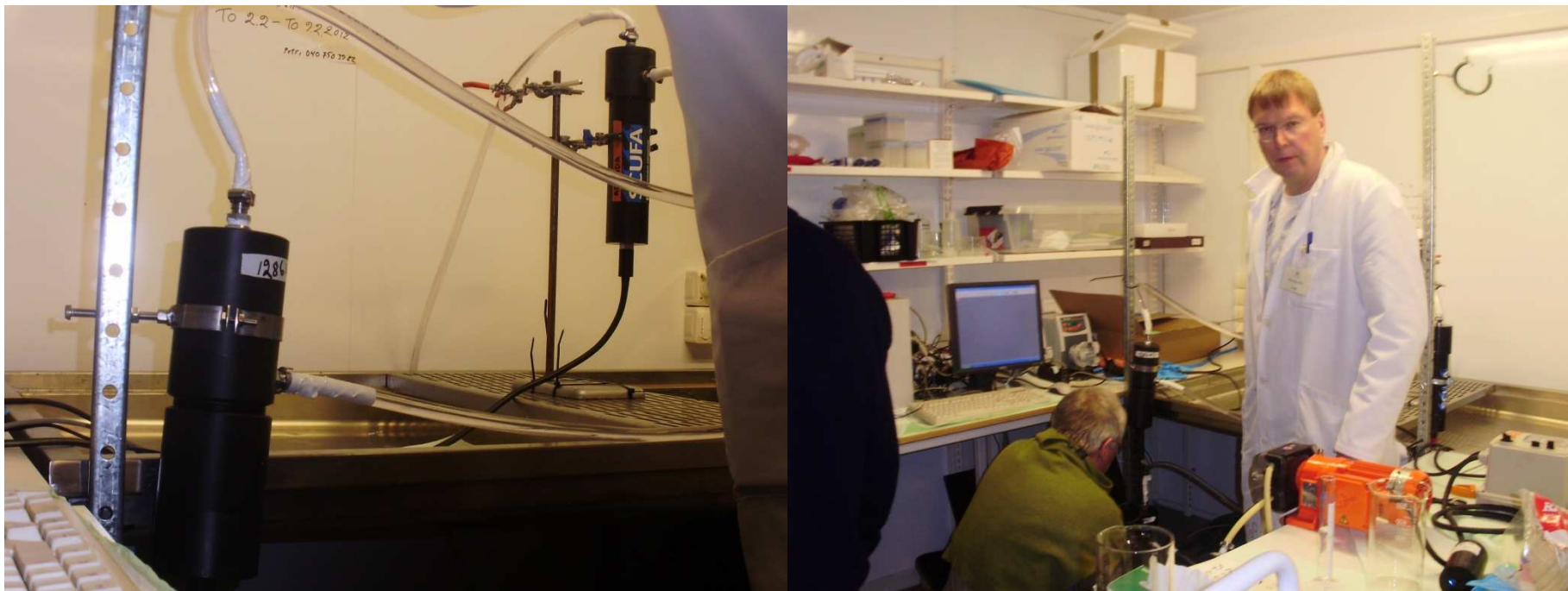
Many alternatives to estimate Chla concentration from fluorescence:

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

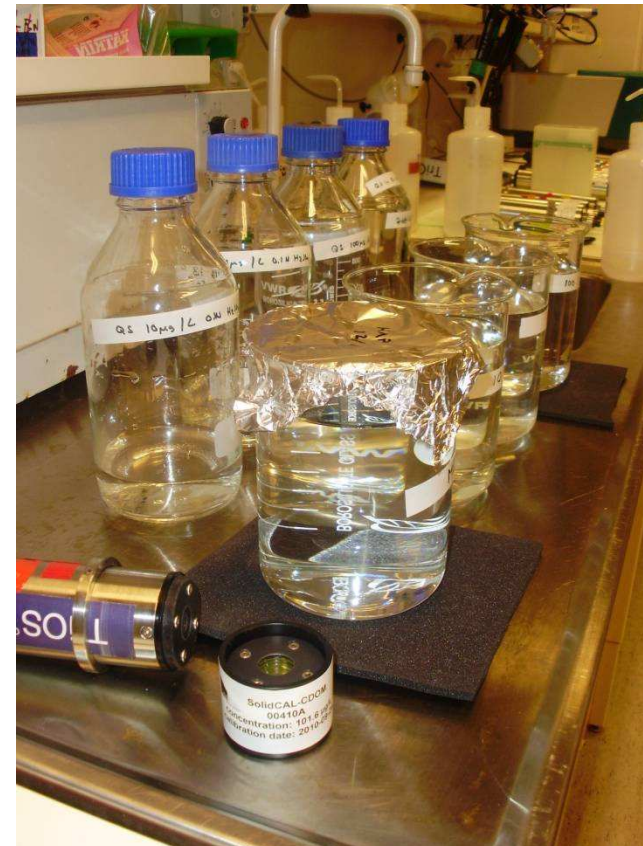
New methods may provide new solutions

- 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!

