JOINT EUROPEAN RESEARCH INFRASTRUCTURE NETWORK FOR COASTAL OBSERVATORIES

CHALLENGES IN MATCHING UP CONCENTRATION & FLUORESCENCE DATA

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Aims for the workshop:

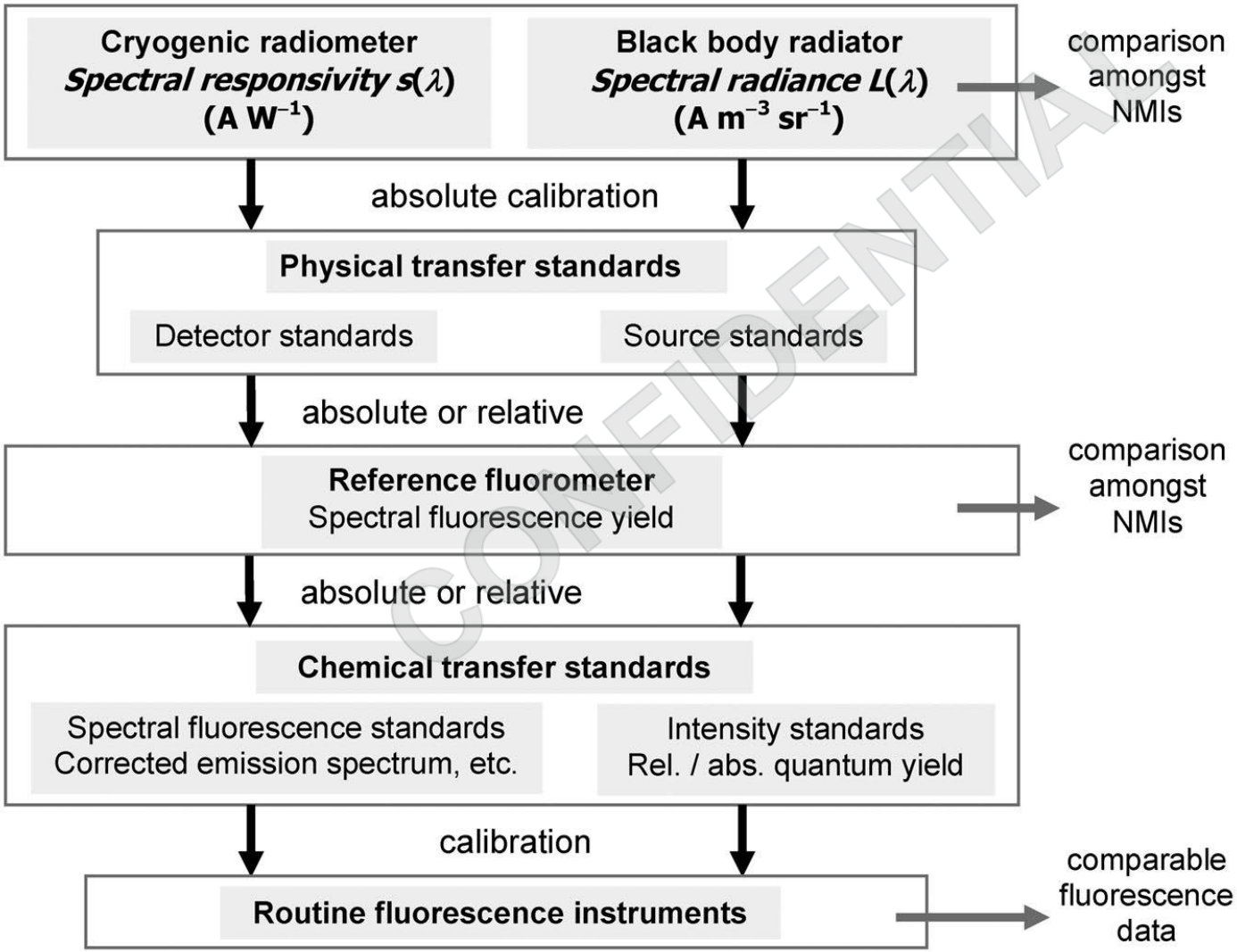
How to perform the primary instrument calibration?
- Algae cultures / Solid secondary standards / Chemical standards
- Comparison of instruments

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?

Fluorometer calibration, view from IUPAC



Resch-Genger & DeRose 2010 Pure Appl. Chem.

Fluorometer calibration, view from manufacturer

in vivo data is normally "calibrated" after the field measurements have been taken by correlating the in vivo data with extracted chlorophyll a data obtained from water samples collected during in vivo sampling

…

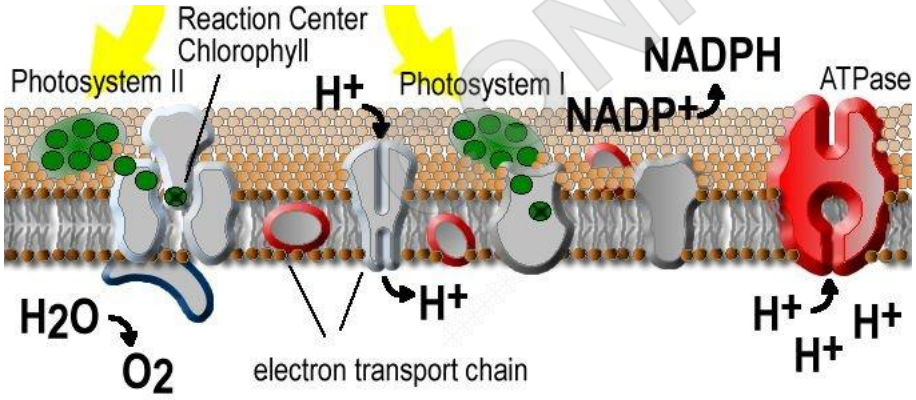
The fluorometer can then be set to read actual chlorophyll *a but it must be understood that changing environmental conditions can introduce significant changes in readings even within one day.*

- Turner Designs-

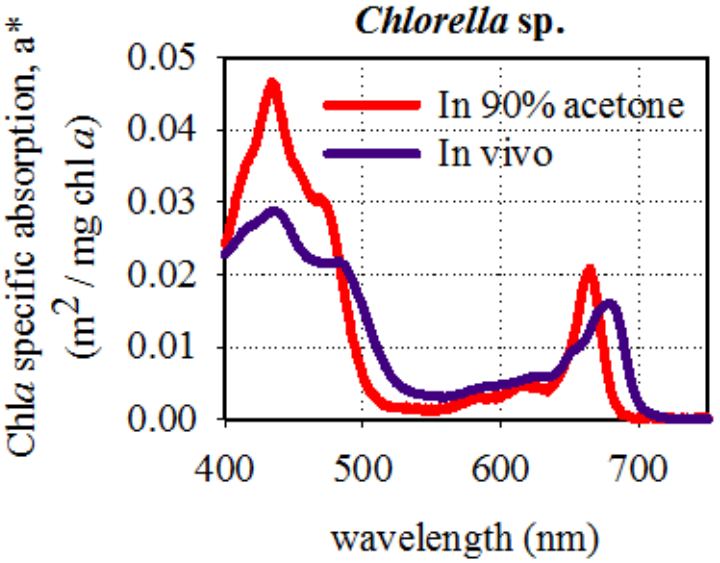
Q: If one has to make field measurements to get meaningful data out from in vivo fluorescence signal, then why to bother with calibration in lab?

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

Absorption: The proteins binding the pigments in cells modify their absorption properties and absorption peaks are shifted towards longer wavelengths.
In vivo, pigments partly shade each other and the actual absorption efficiency per pigment is less than if the pigments were uniformly distributed, like *in vitro*.

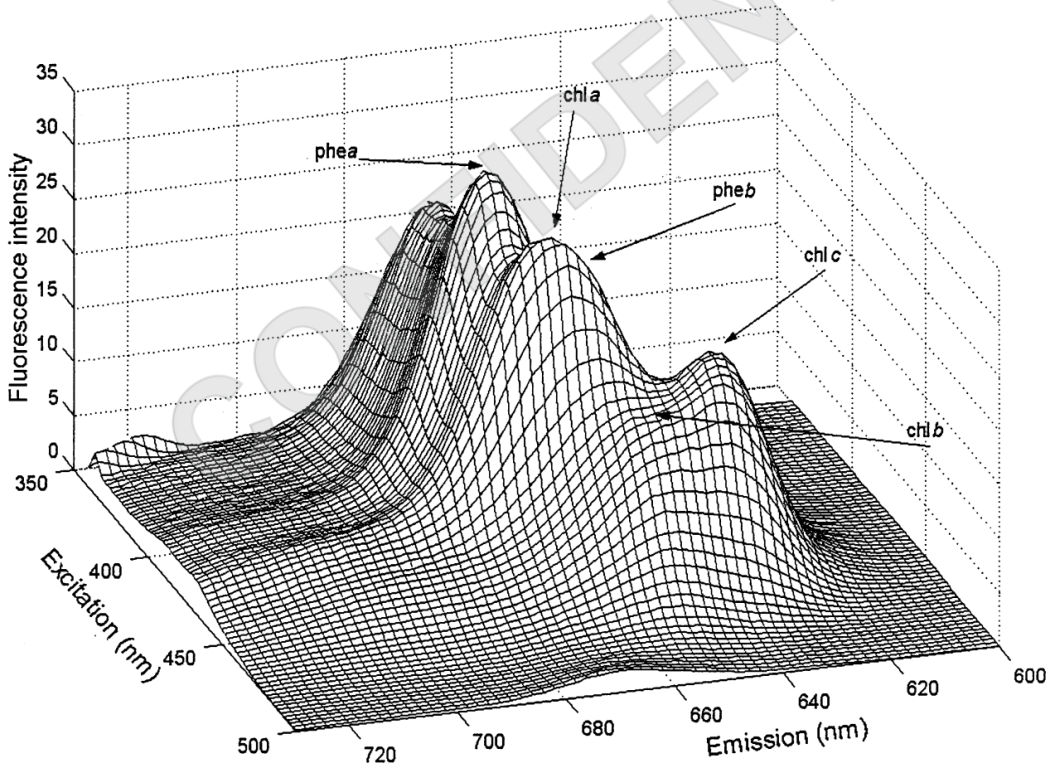


From bioweb.wku.edu/pix/pix.htm



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

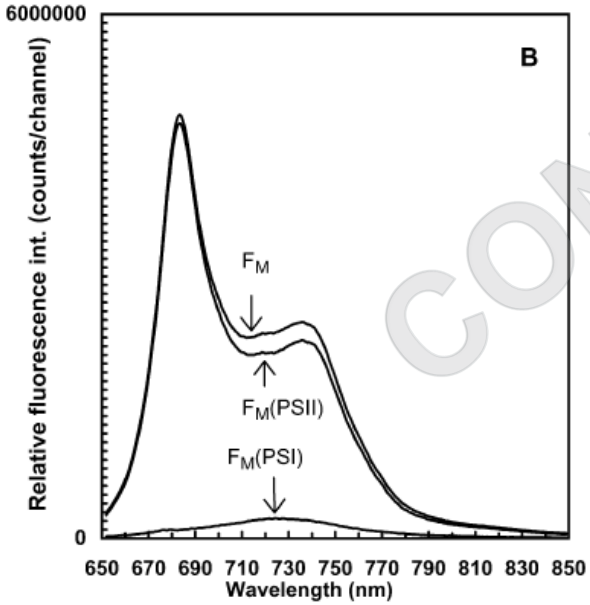
Fluorescence: *In vitro*, all chlorophylls show their own fluorescence, at specific excitation and emission wavelengths.



Moberg et al 2001, Talanta

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

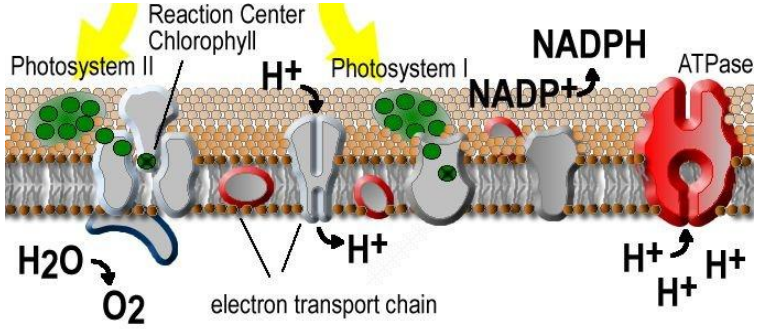
Fluorescence: *In vivo*, only Chl*a* in photosystem II and phycobilin pigments emit fluorescence. Accessory pigments in photosystem II transfer energy to Chl*a* but do not emit fluorescence.



Franck etal 2002 BBA

Emission wavelength [nm]

>95% of fluorescence comes from PSII , although PSI may contain ~50% of Chla



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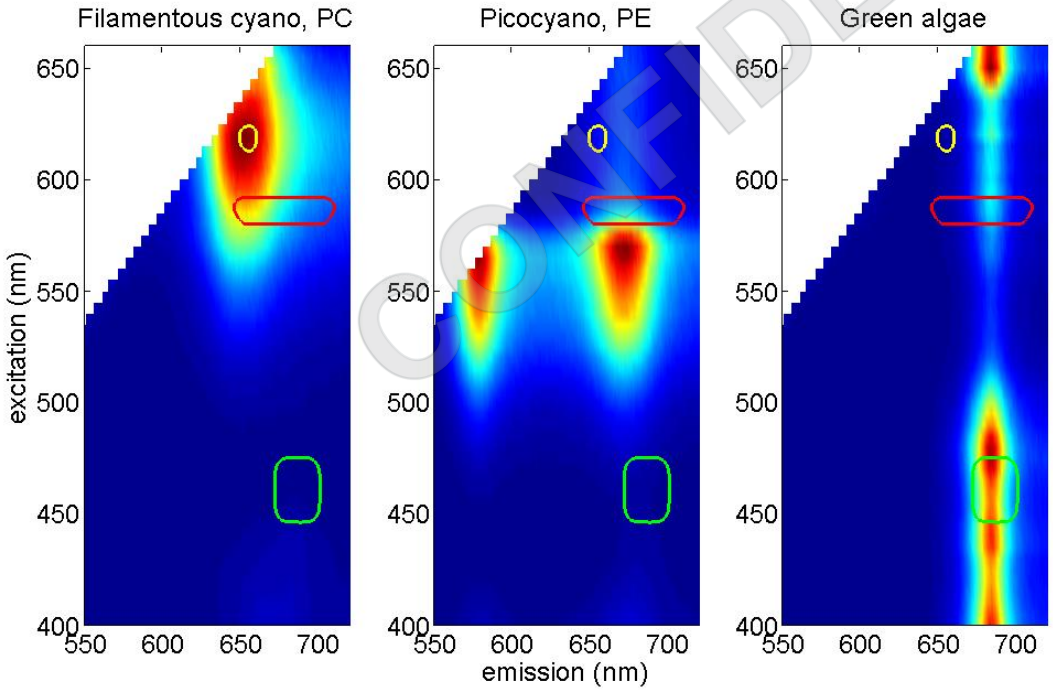


Cyanobacteria has most Chl*a* in PSI, thus low Chl*a* fluorescence.

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

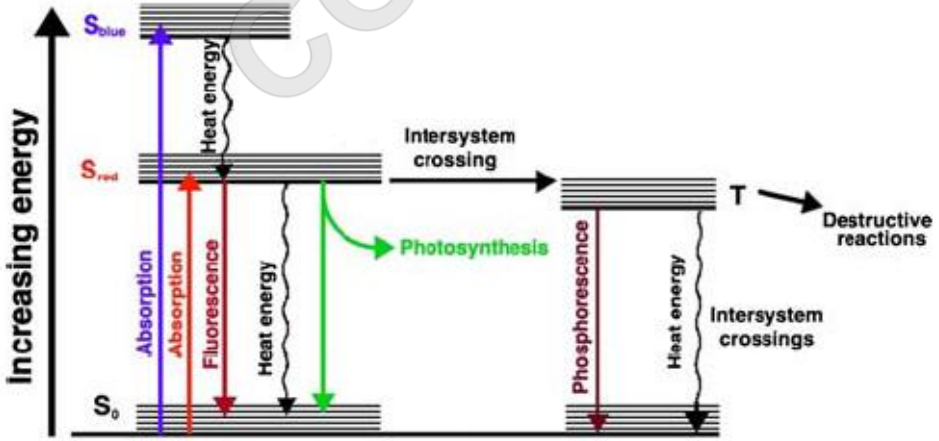
Fluorescence yield, ϕ_F = *fluorescence emission / light absorption*

*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.

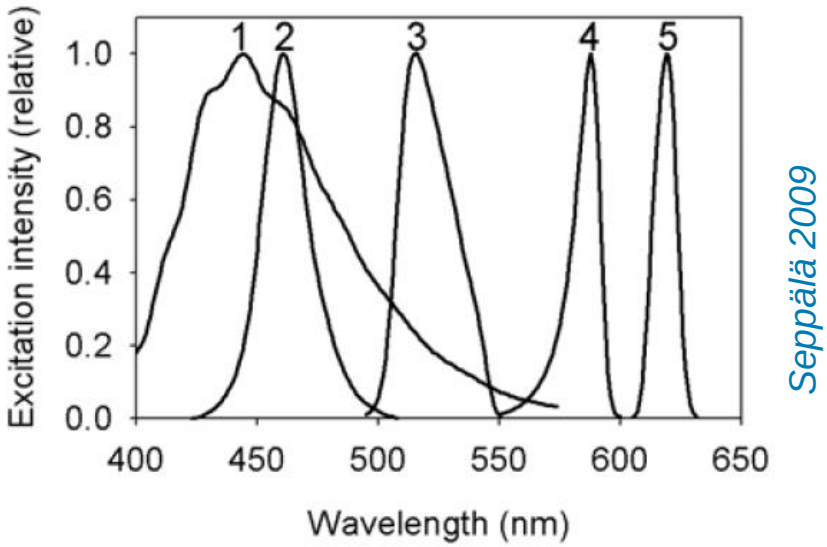
$$F(\lambda_{ex/em}) = [Chla] \cdot E_{ex} \cdot \bar{a}_{PSII}^{*} \cdot Q_a^{*}(\lambda_{em}) \cdot \phi_F$$

Biomass | Instrument | Species | Pigmentation | Physiology

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

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

Fluorometer light



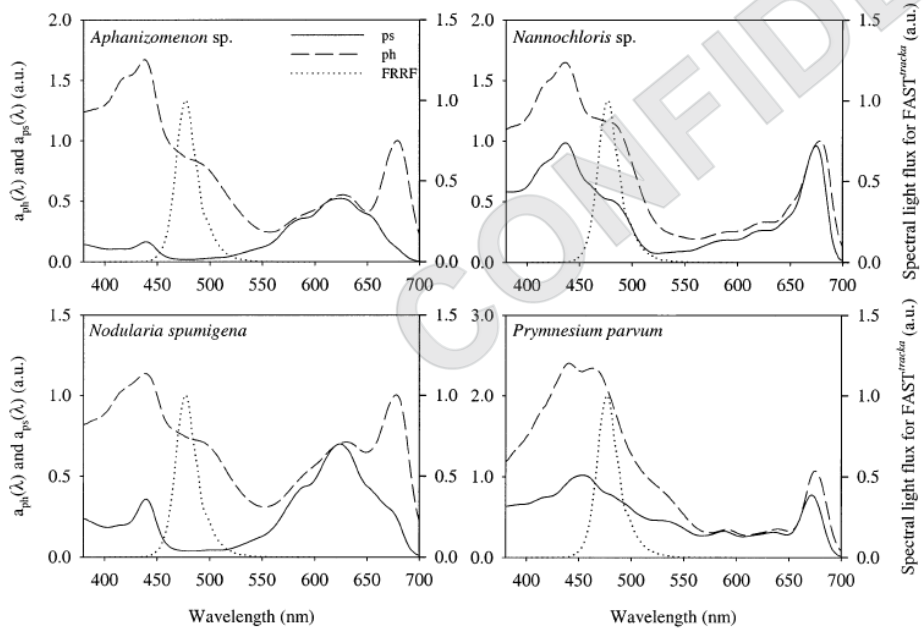
Seppälä 2009

Figure 12. Excitation spectra of commercial field fluorometers used in study UP-II. 1. Chl*a* channel of miniBackScat SII (Dr.Haardt Optik Mikroelectronic), 2. Cyclops-7 for Chl*a* 3. Cyclops-7 for PE 4. Cyclops-7 for PC (Turner Designs Inc.) and 5. PC channel of TwinFlu (TriOS GmbH). Each spectrum is scaled to maximum intensity.

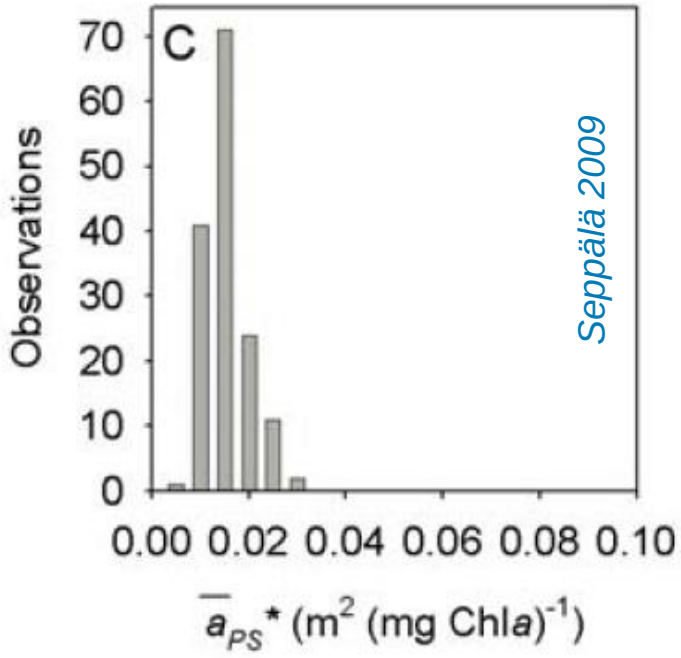
From *in vivo* Chl*a* fluorescence [F] to Chl*a* concentration [Chl*a*]; Species specific pigmentation

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

Chlorophyll a specific absorption coefficient for PSII



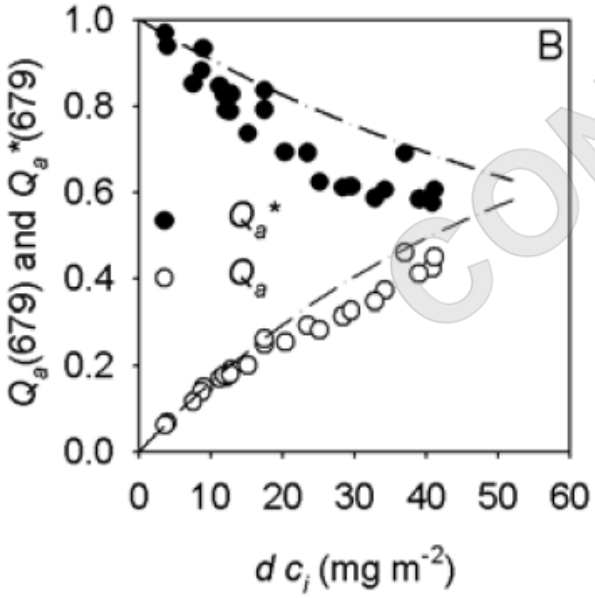
From: Raateoja, Seppälä & Ylöstalo (2004) Limnol. Oceanogr

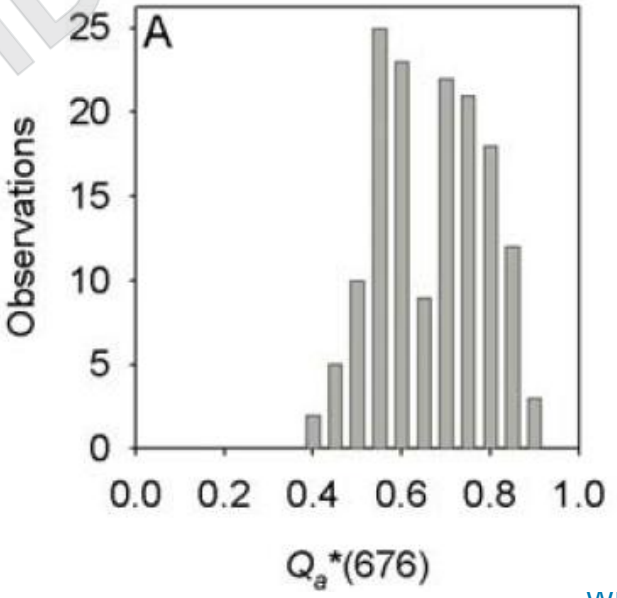


From *in vivo* Chl*a* fluorescence [F] to Chl*a* concentration [Chl*a*]; reabsorption / package effect

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

Reabsorption of fluoresced light





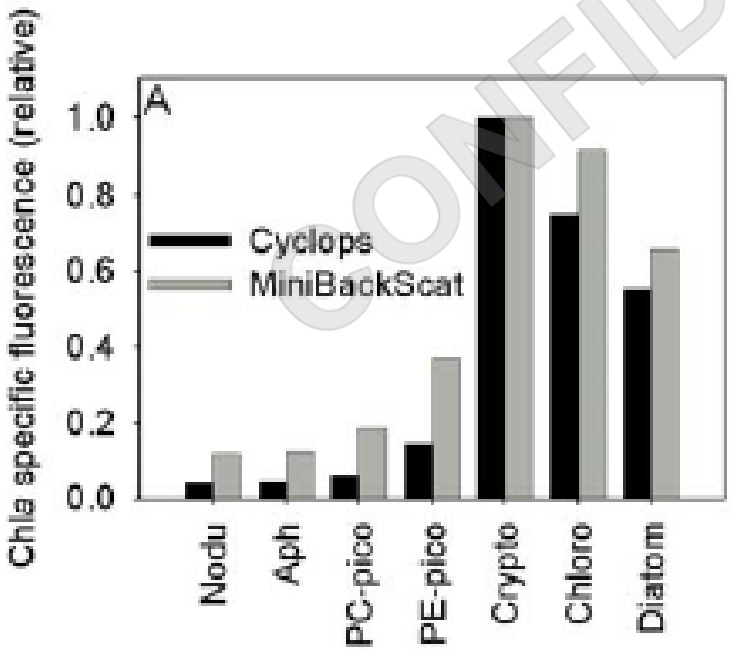
Seppälä 2009

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

Comparison of instruments with different optics may be a mess...

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

Spectral variability between calibration and field samples



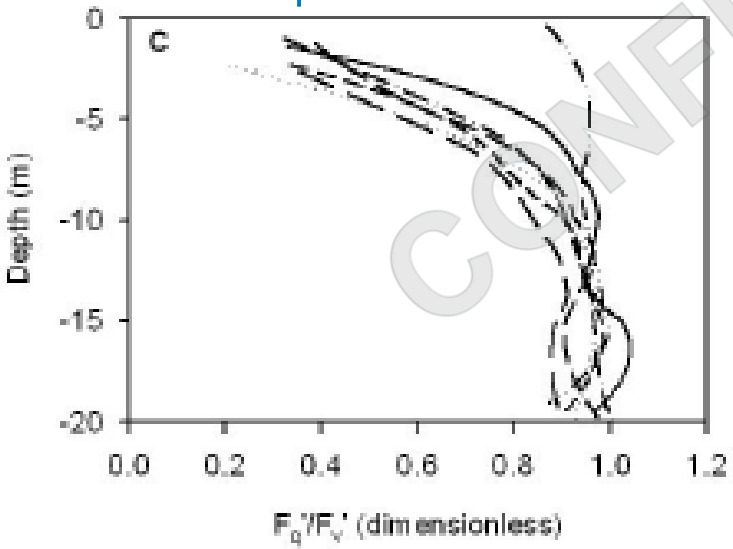
Seppälä 2009

From *in vivo* Chl*a* fluorescence [F] to Chl*a* concentration [Chl*a*]; physiological state of cells

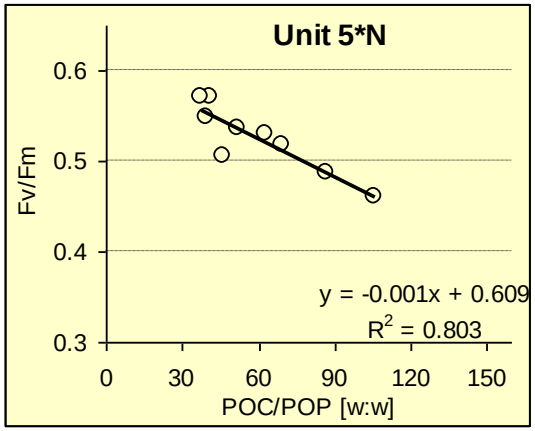
$$F(\lambda_{ex/em}) = [Chla] \cdot E_{ex} \cdot \bar{a}_{PSII}^{*} \cdot Q_a^{*}(\lambda_{em}) \cdot \phi_F$$

Quantum yield of fluorescence

fraction of open reaction centers



Raateoja, Ylöstalo, Seppälä, in prep.

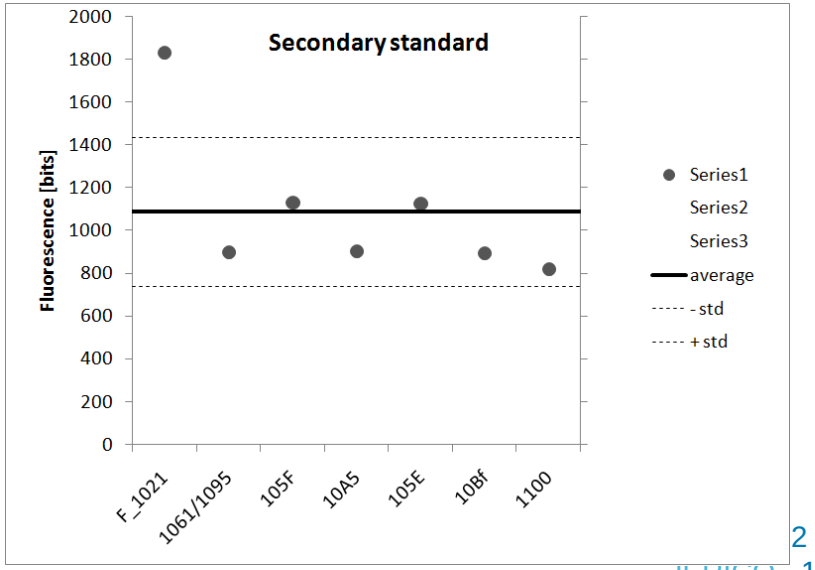


Seppälä, Le'Floch,Geider & Lignell, unpublished

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



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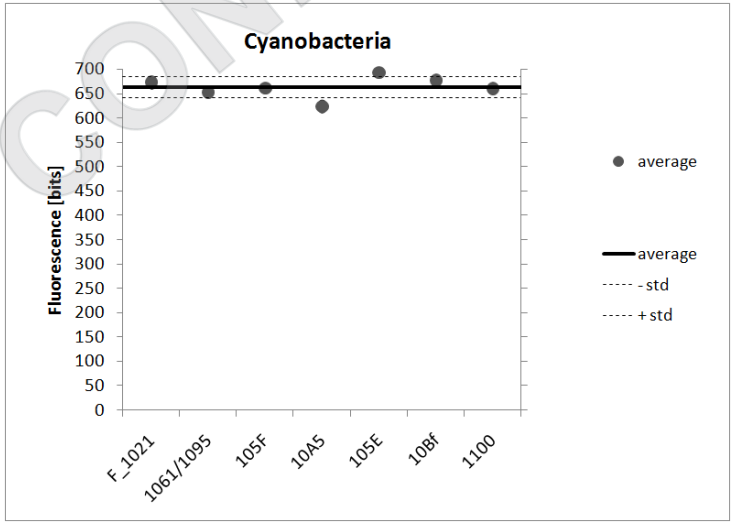
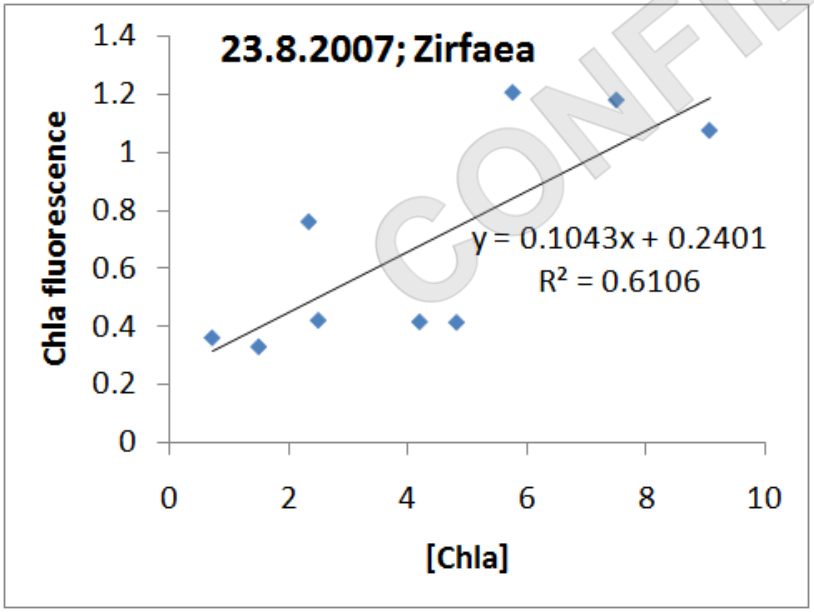
- stable and traceable signal, thus instrument performance can be tracked
- instruments (with similar optics) can be compared
- secondary standard does not, however, always allow direct instrument-instrument comparisons
- for that purpose, intercomparison with cultures/dyes should be carried out

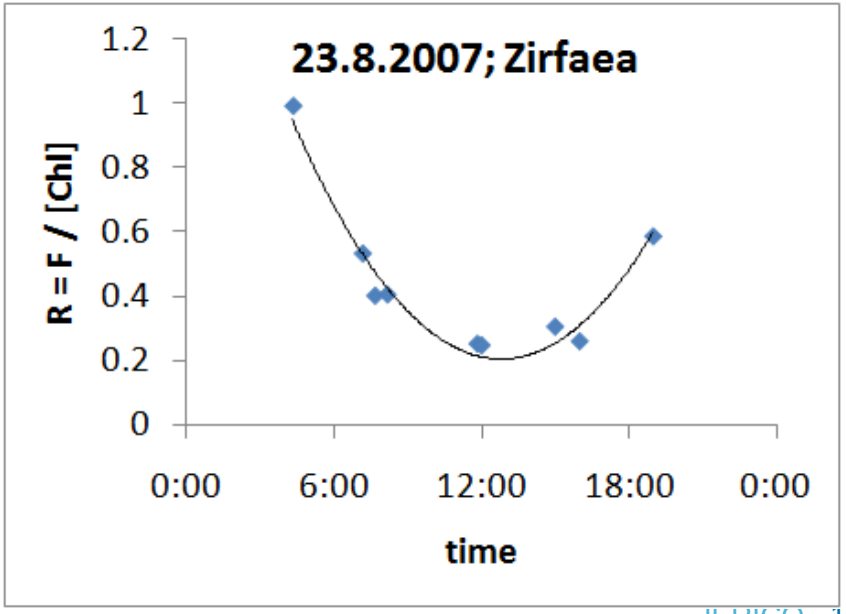


From *in vivo* Chl*a* fluorescence [F] to Chl*a* concentration [Chl*a*]; *Calibration / validation*

As F / [Chla] ratio varies due to phytoplankton physiology & community structure, “enough” validation samples must be provided and possibly other data should be provided

Irradiance / time





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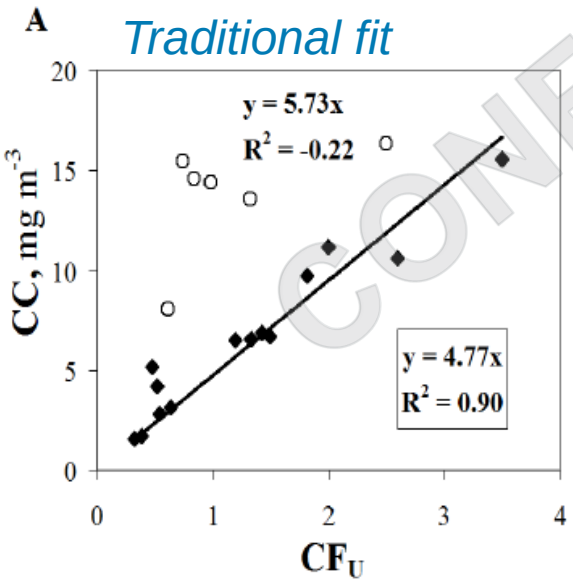
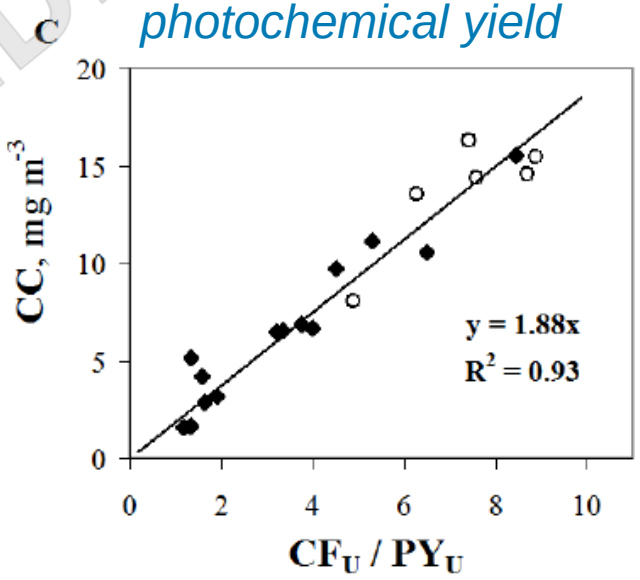
Traditional fit



Fit including PSII photochemical yield



Chekalyuk & Hafez, Optics Express 2011

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

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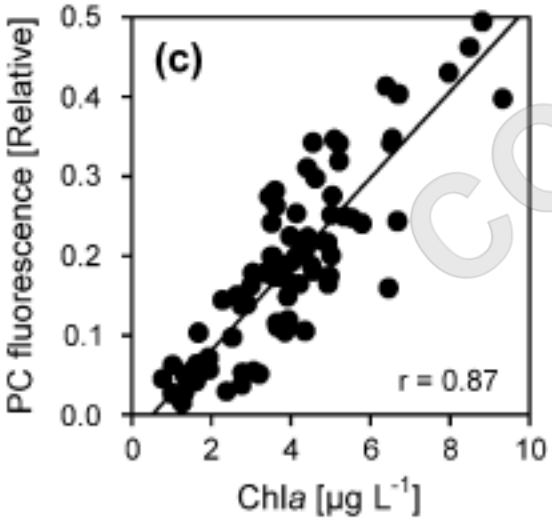
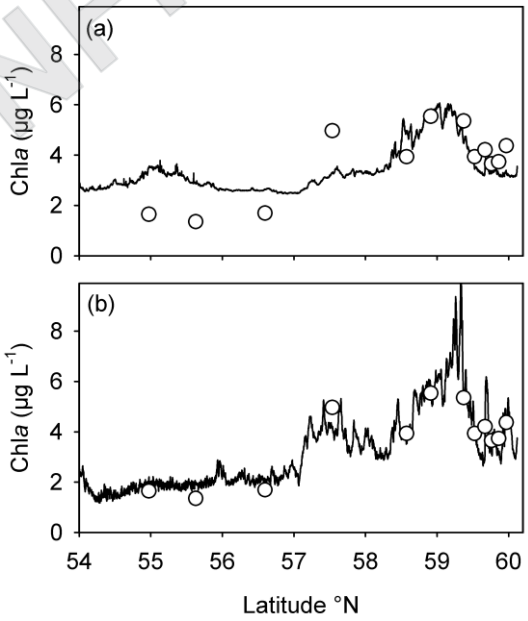
In Baltic Sea, during cyanobacteria dominance, [Chla] may be better explained by phycobilin fluorescence



Seppälä et al. (2007) ECSS 73



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

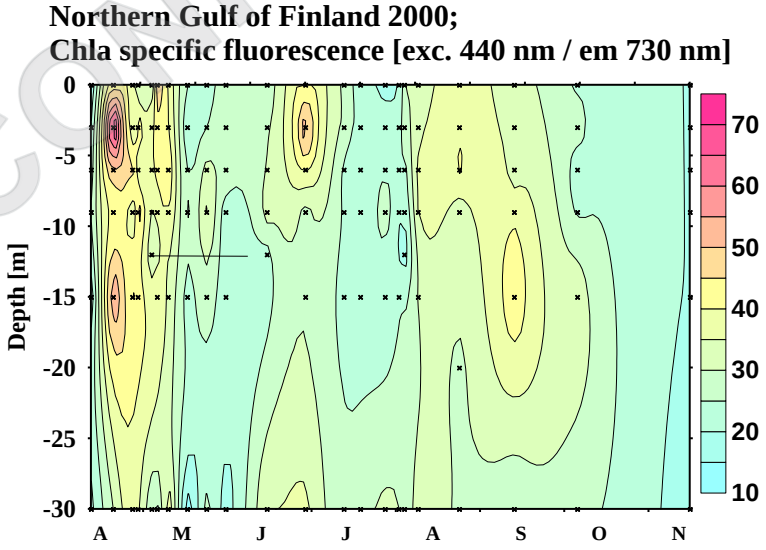
$[Chla] = b_0 + b_1 * Chla\ Fl + b_2 * PC\ Fl$

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

Transfer of the validation model from

- ***one instrument to another (among same type)***
- ***from one day to another***
- ***from one year to another***

is possible if primary calibration is traceable.



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- ***one instrument to another (among same type)***
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And more accurate estimation of Chl*a* can be obtained if some additional measurements are carried out

- ***other spectral fluorescence data (taxonomy)***
- ***light (photophysiology)***
- ***PSII photochemical yield (photophysiology)***

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.

Challenges in matching up concentration & fluorescence data

Thanks !

Co-authors:
Seppo Kaitala, Pasi Ylöstalo, Petri Maunula