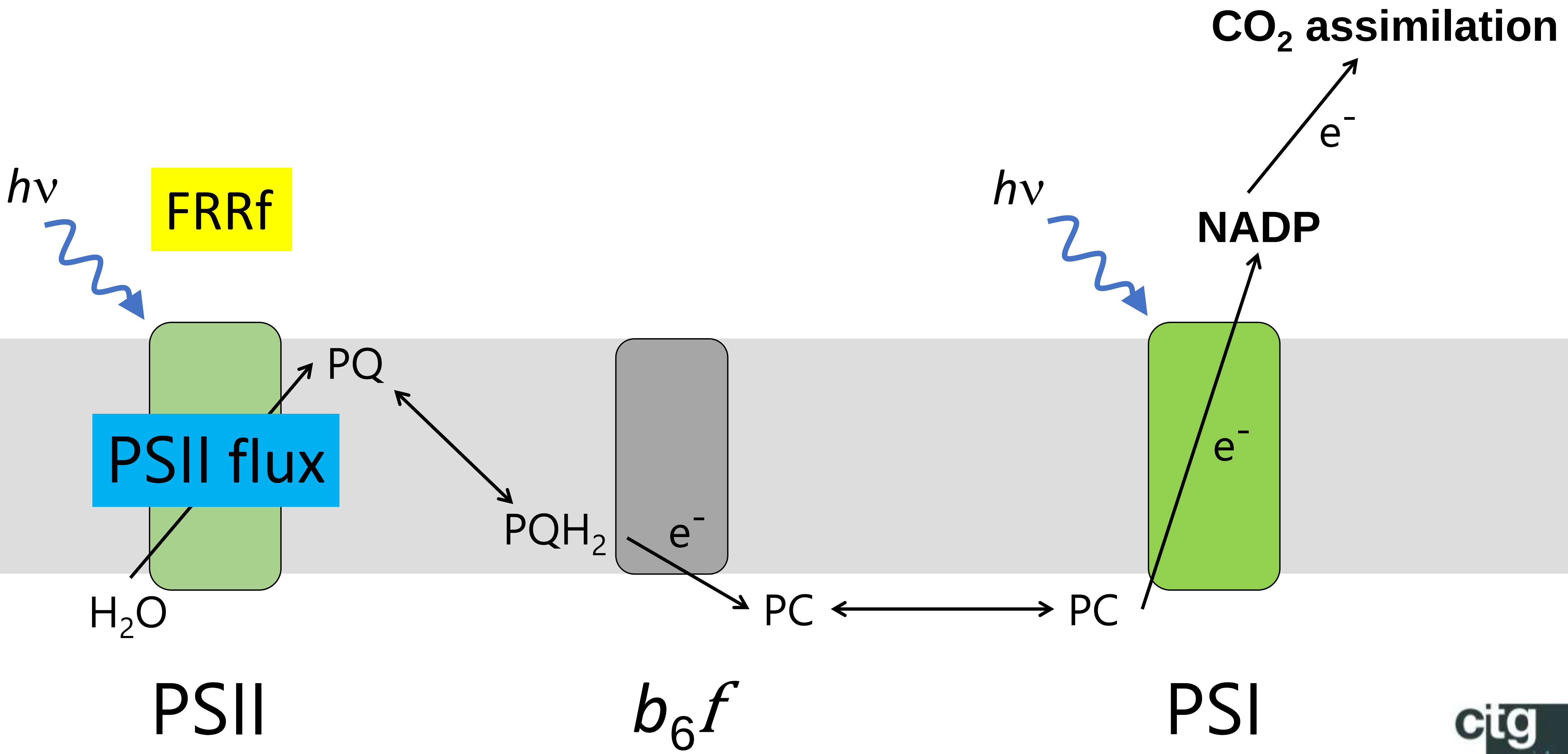
Using Fast Repetition Rate fluorometry to estimate PSII electron flux per unit volume: A purely optical method for estimating GPP?

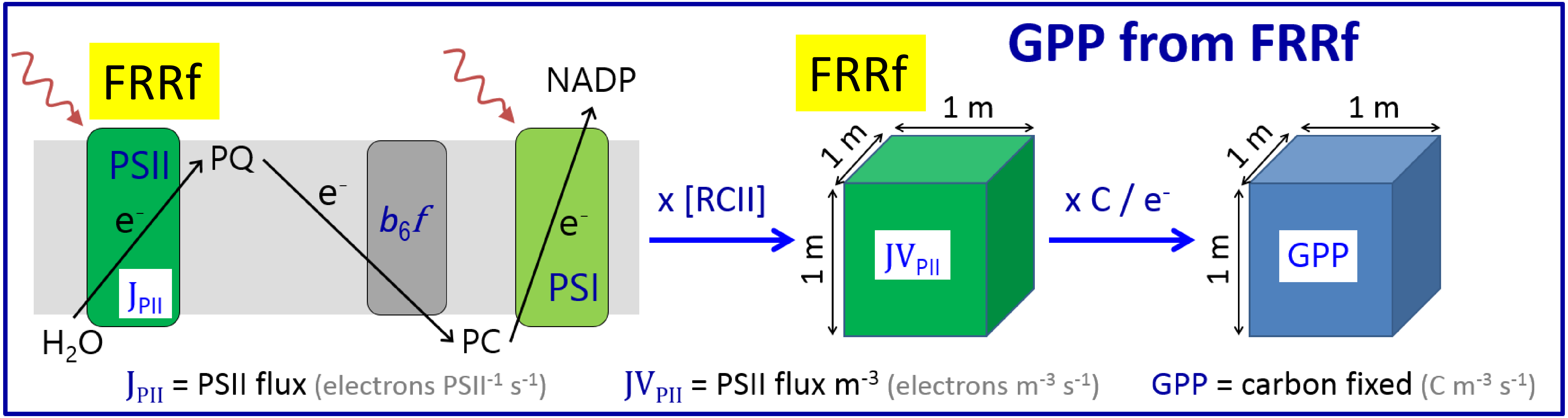
Kevin Oxborough
Principal Scientist

Kevin Oxborough (CTG)
Mark Moore (Southampton)
Dave Suggett, Richard Geider (Essex)

Photosynthetic electron transport



From PSII electron flux to GPP



$$J_{PII} = \sigma_{PII}' \cdot E$$

$$JV_{PII} = a_{PII}' \cdot E$$

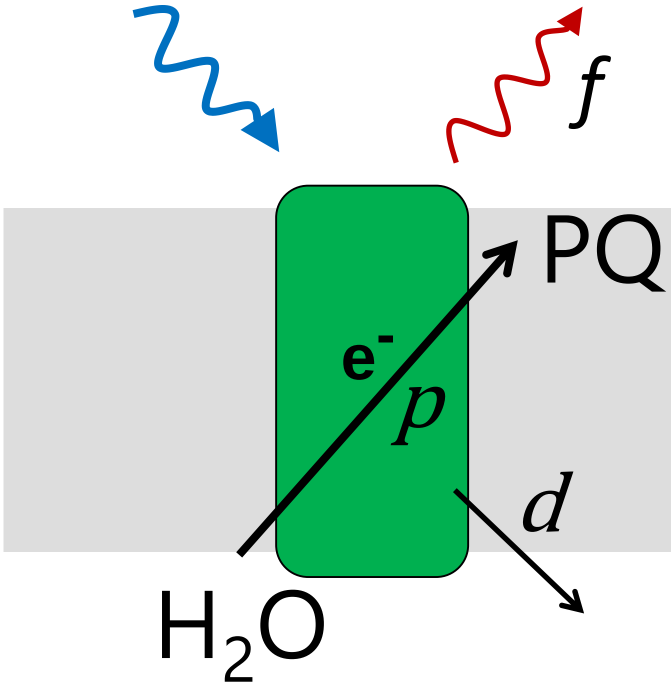
σ_{PII}' = absorption cross section of PSII photochemistry (m^2 $PSII^{-1}$)

a_{PII}' = absorption coefficient for PSII photochemistry (m^{-1})

E = Photon irradiance (photons m^{-2} s^{-1})

Photochemistry and fluorescence

Open RCII



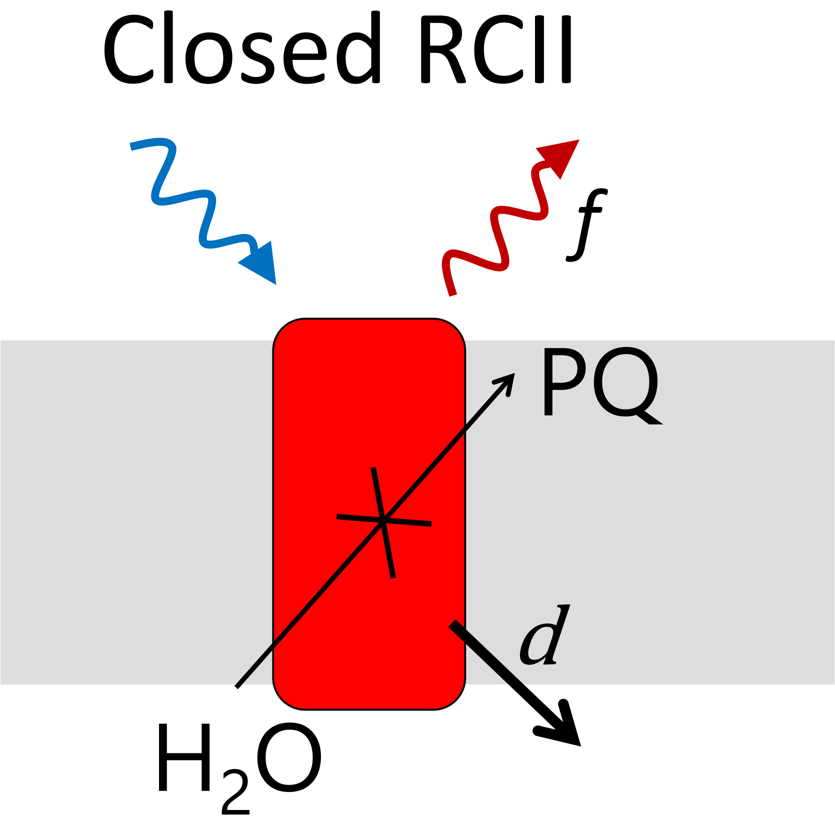
$$\Phi_f = \frac{k_f}{k_p + k_f + k_d}$$

$$\Phi_p = \frac{k_p}{k_p + k_f + k_d}$$

$$\frac{P}{F} \propto \frac{k_p}{k_f}$$

How consistent is this relationship?

Photochemistry and fluorescence



$$\phi_f = \frac{k_f}{k_f + k_d}$$

$$\phi_p = 0$$

The FRRf technique

ELSEVIER

Biochimica et Biophysica Acta 1367 (1998) 88–106

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols

Zbigniew S. Kolber, Ondřej Prášil [1], Paul G. Falkowski *

Environmental Biophysics and Molecular Biology Program, Rutgers University, 71 Dudley Rd, New Brunswick, NJ 08901-8521, USA

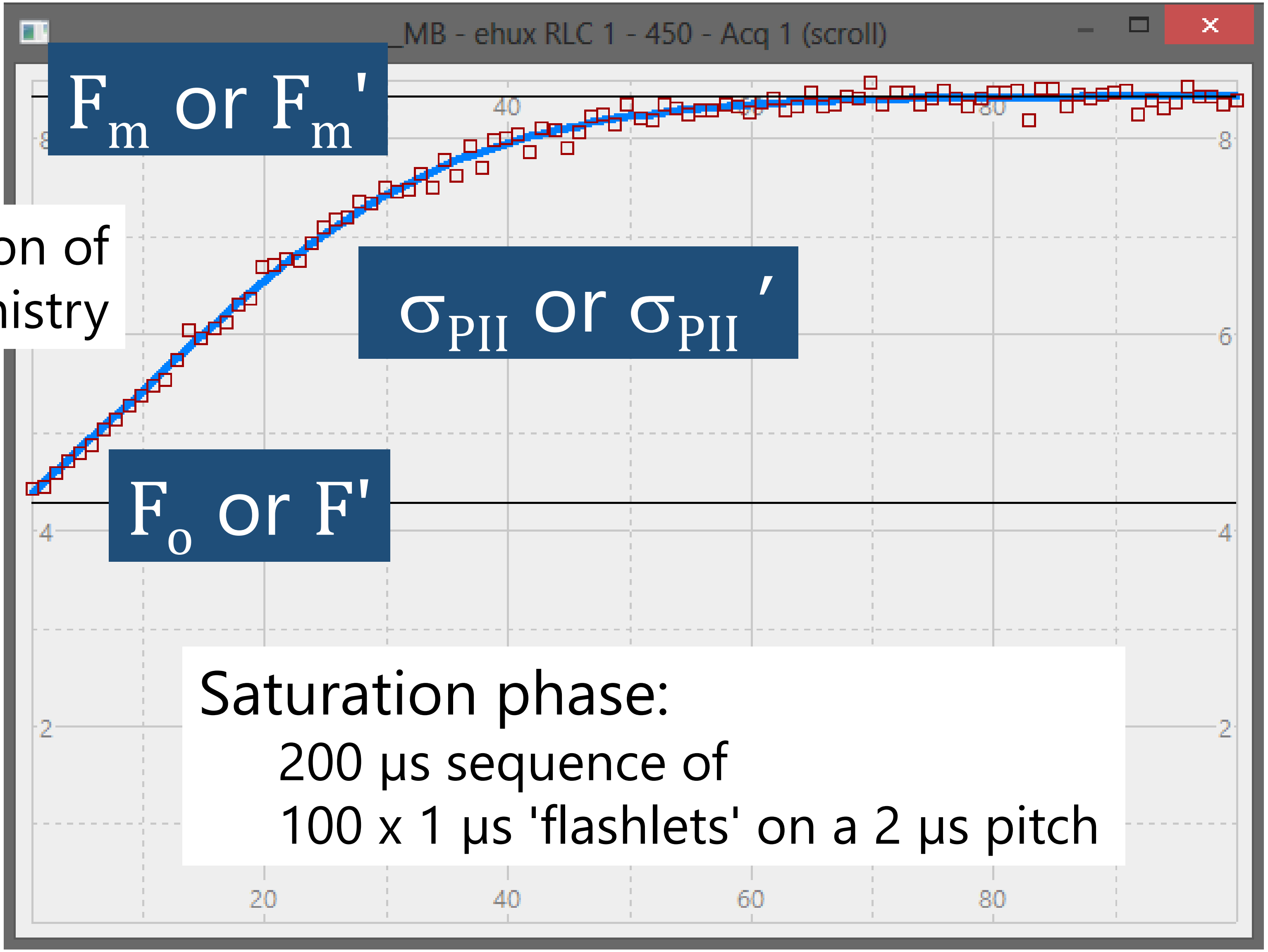
Received 12 December 1997; revised 15 June 1998; accepted 23 June 1998

Single turnover (ST) FRRf measurement

Closed RCIIs →

Absorption cross section of PSII photochemistry

Open RCIIs →

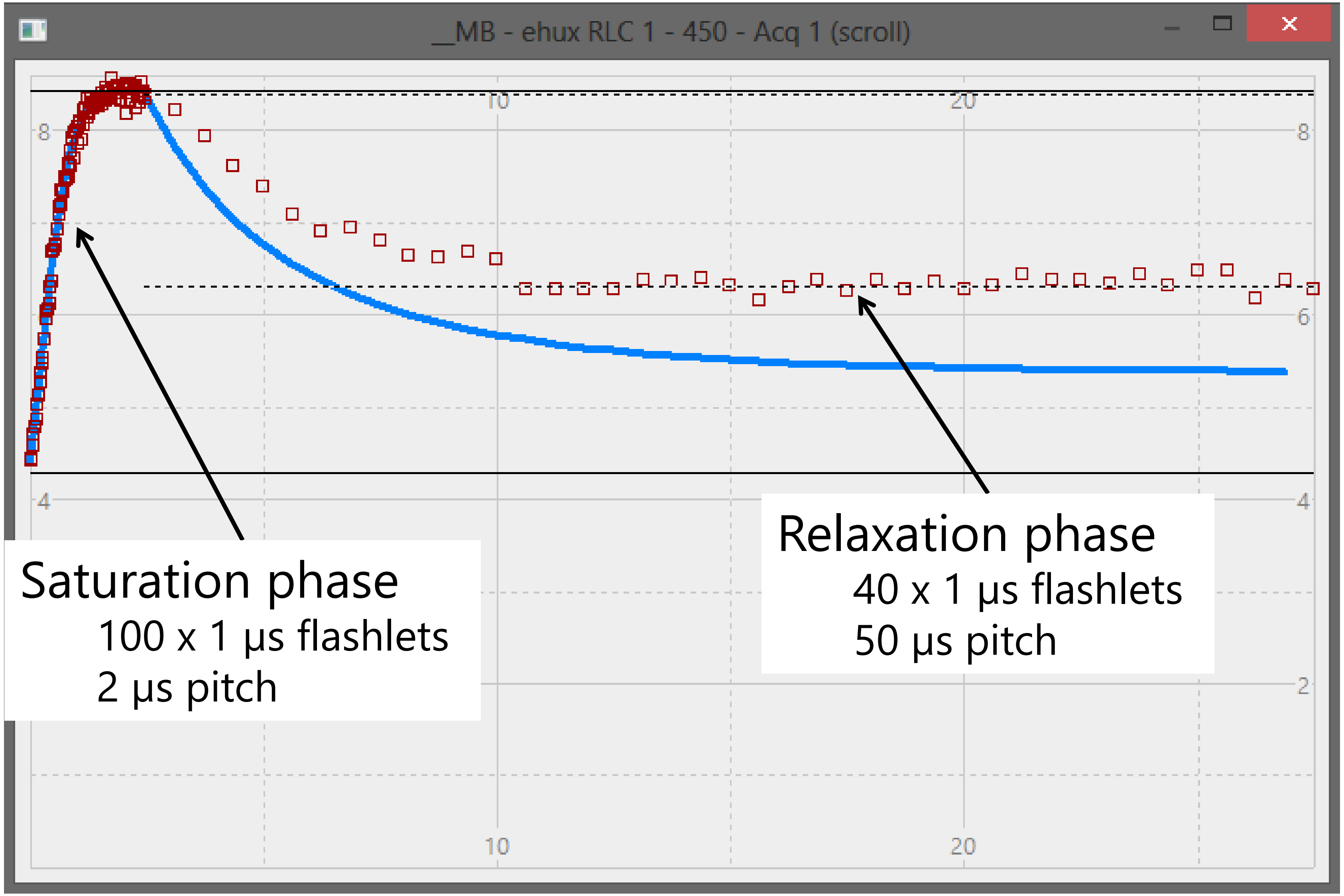


Saturation phase:

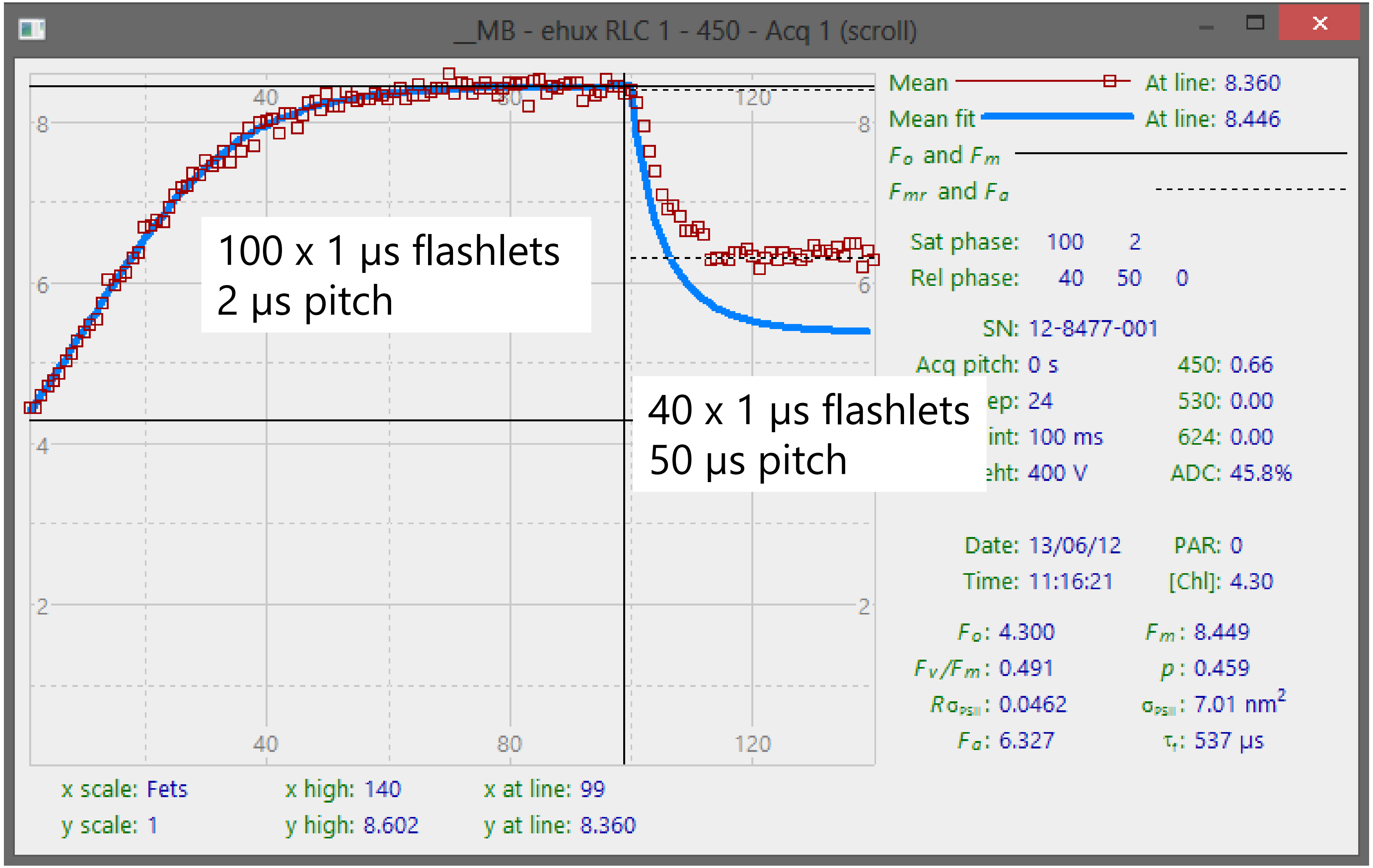
200 µs sequence of

100 x 1 µs 'flashlets' on a 2 µs pitch

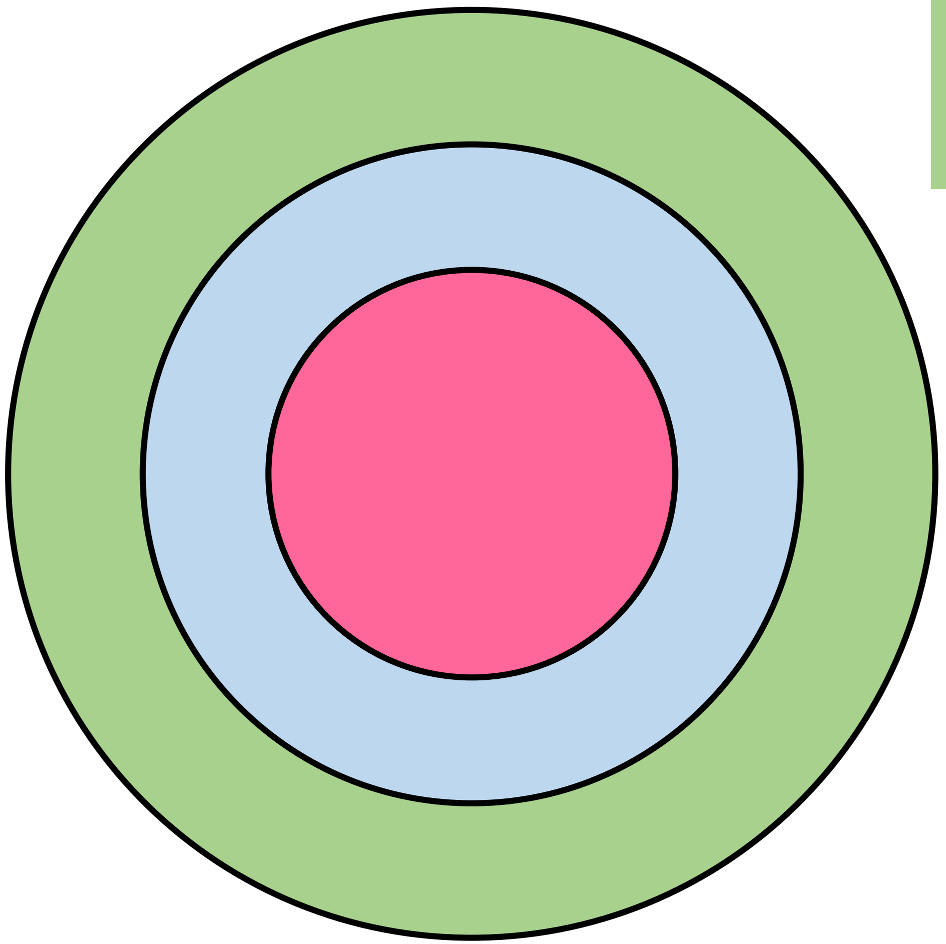
Saturation and relaxation phases



Saturation and relaxation phases



Absorption cross section (Sigma)



Physical cross section of the PSII light-harvesting system (LHII)

Absorption cross section of LHII (σ_{LHII})

Absorption cross section of PSII photochemistry (σ_{PII})

All unit area (m^2 $PSII^{-1}$)

The FRR-ST data fit

$$C_n = C_{n-1} + R\sigma_{\mathrm{PII}} \cdot \frac{1 - C_{n-1}}{1 - C_{n-1} \cdot p}$$

$$\mathrm{F_n} = \mathrm{F_o} + (\mathrm{F_m} - \mathrm{F_o}) \cdot C_n \cdot \frac{1 - p}{1 - C_n \cdot p}$$

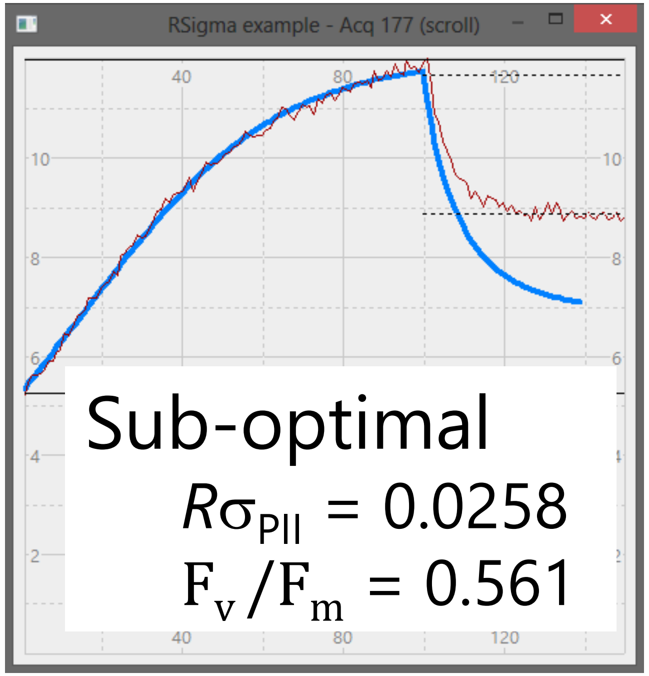
C_n = closed RCII at flashlet n
$R\sigma_{\mathrm{PII}}$ = RCII closed by first flashlet
p = RCII connectivity

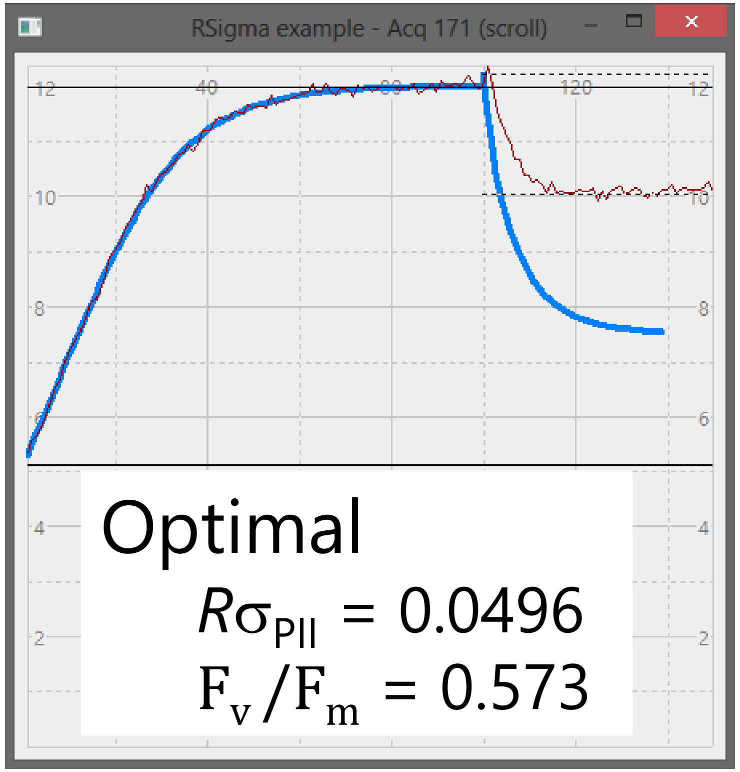
Kolber, Prášil and Falkowski (1998)

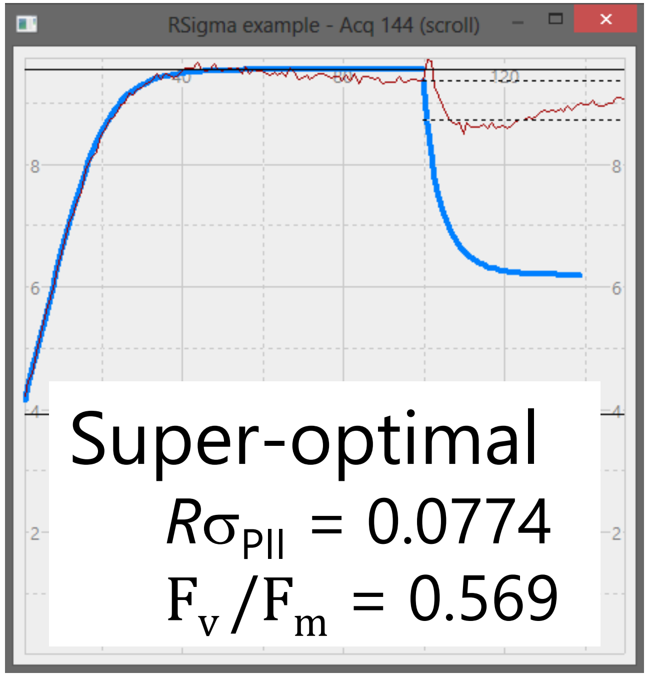
$R\sigma_{\mathrm{PII}}$

- Probability of an RCII being closed by the first flashlet in an FRR-ST sequence
- Dimensionless parameter
- Workable range of 0.03 to 0.06
- Can be 'set' by changing E_{LED}

$R\sigma_{PII}$ – optimum E_{LED} intensity



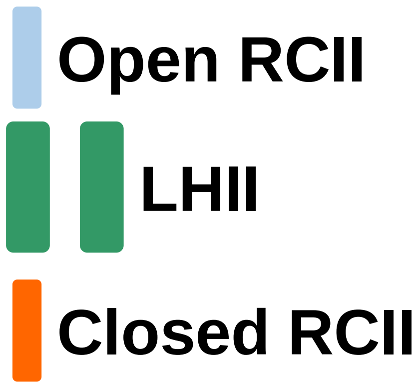


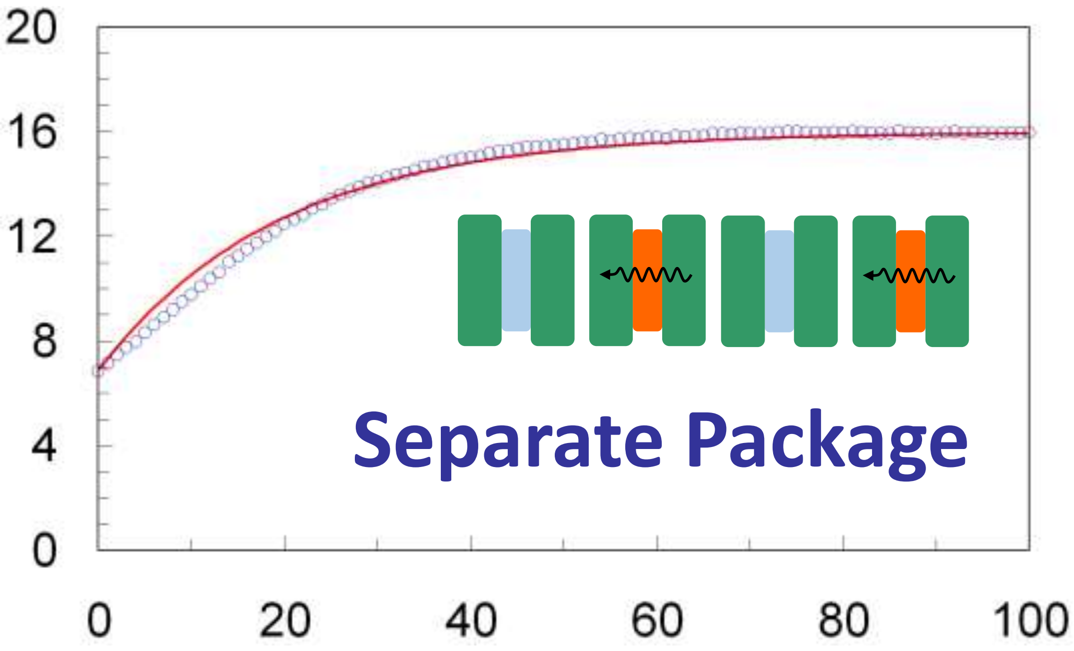


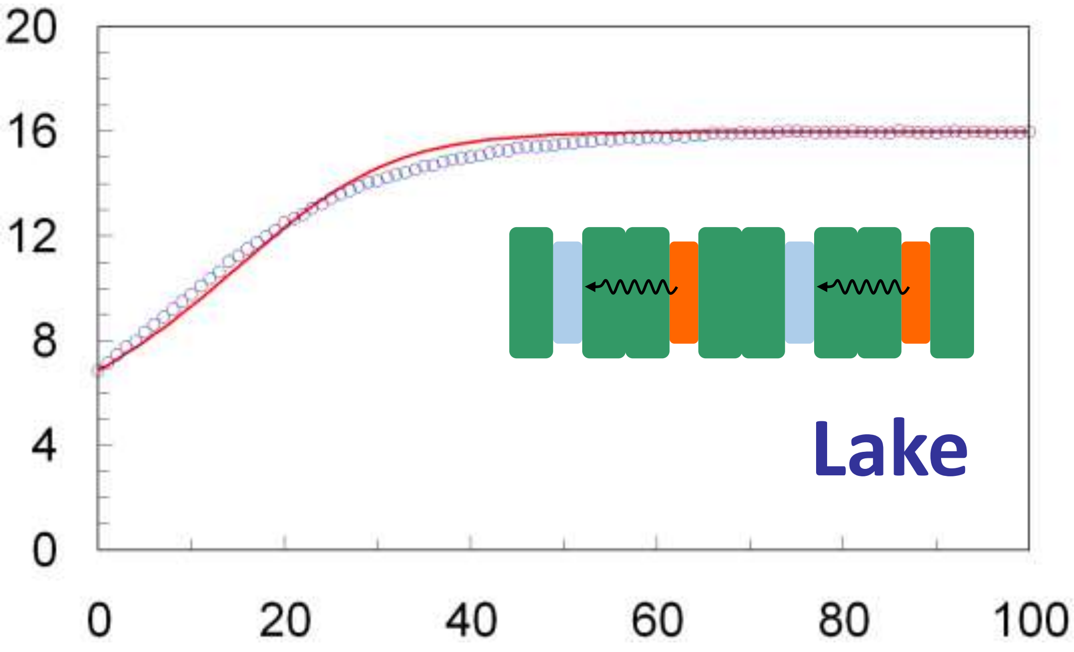
Increasing E_{LED}

ctg

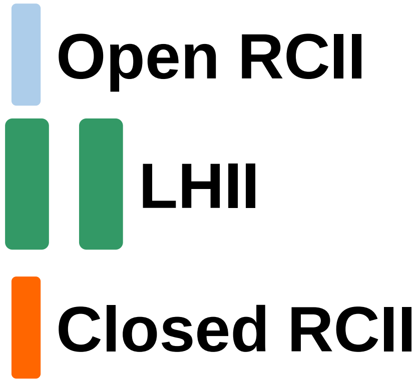
Connectivity among RCIIs

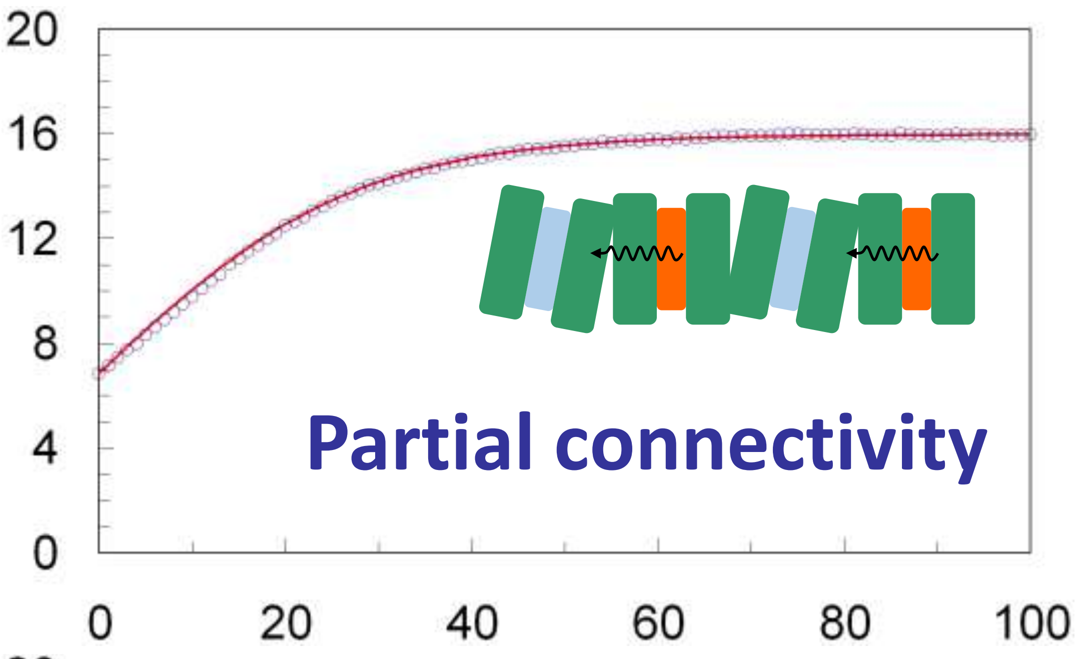


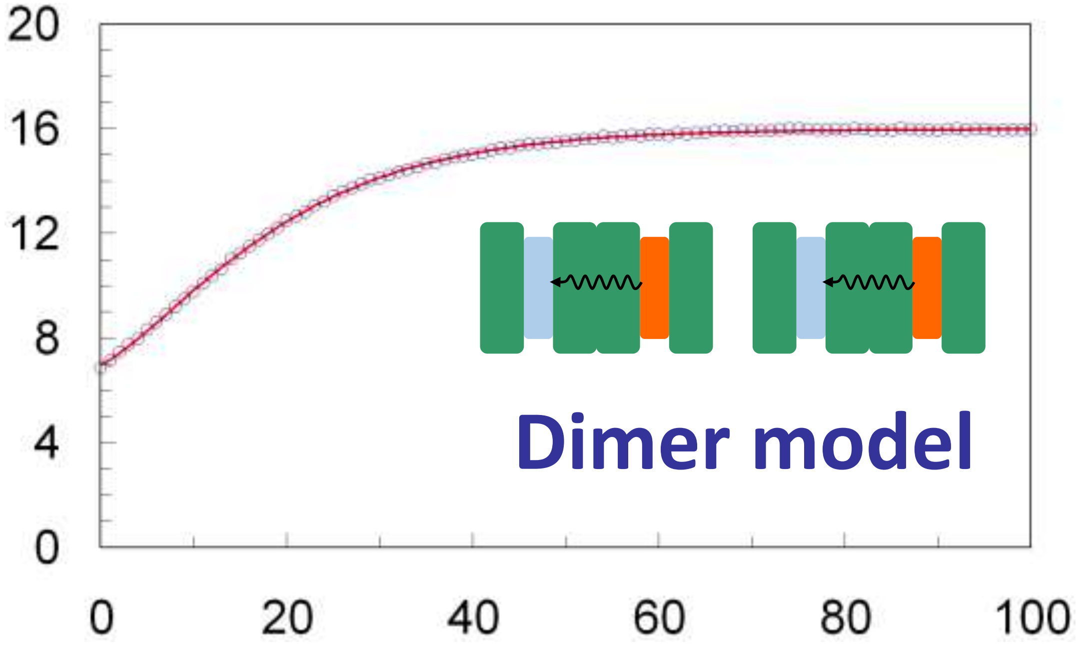




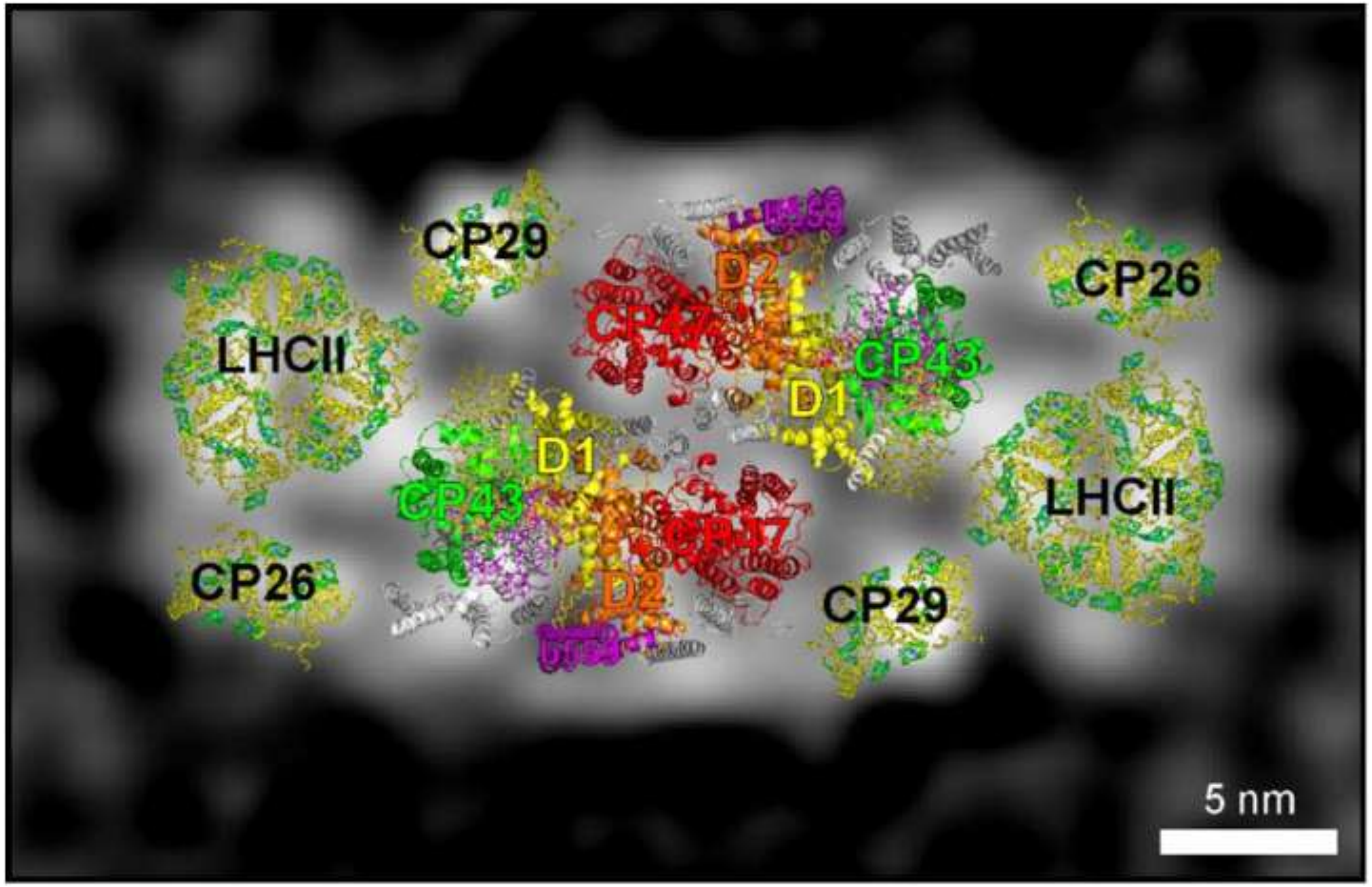
Connectivity among RCIIs







Photosystem II dimer



- X-ray crystallography
  - Core from cyanobacterium
  - LHCII from spinach
- Cryo-EM from spinach

taken from Nield & Barber (2006)

Basic terminology

Parameter	PSII photochemistry	PSII light harvesting
Absorption cross section (m^{-2} $PSII^{-1}$)	$\sigma_{PII}^{(\prime)}$	σ_{LHII}
Absorption coefficient (m^{-1})	$a_{PII}^{(\prime)}$	a_{LHII}
Photon efficiency (dimensionless)	$\phi_{PII}^{(\prime)}$	

$$\sigma_{PII}{}' = \sigma_{LHII} \cdot \phi_{PII}{}'$$

$$a_{PII}{}' = a_{LHII} \cdot \phi_{PII}{}'$$

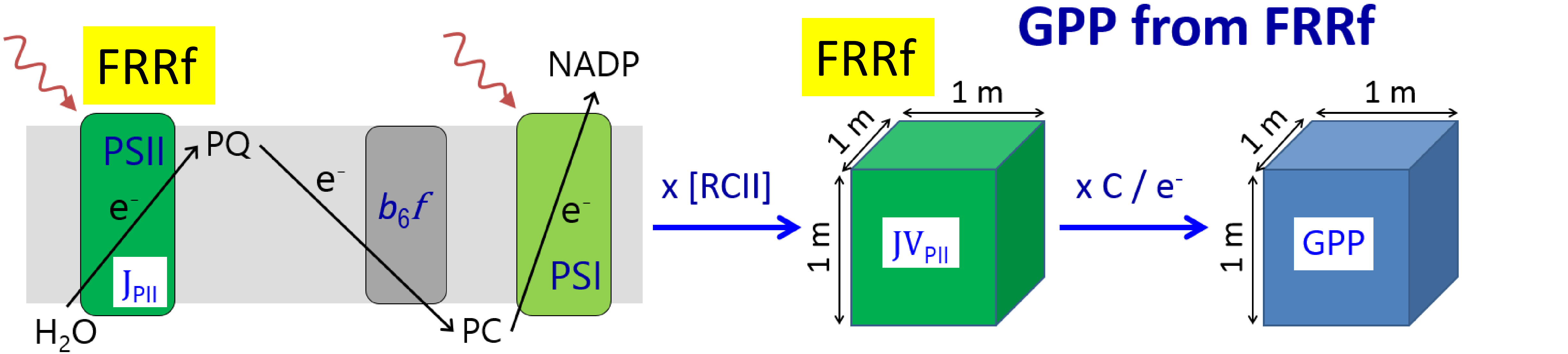
Flux terminology

Flux	PSII electron flux	Photon flux
Defined area	J_{PII} (e^{-} $PSII^{-1}$ s^{-1})	
Unit area		E (photons m^{-2} s^{-1})
Unit volume	JV_{PII} (e^{-} m^{-3} s^{-1})	

e^{-} $PSII^{-1}$ s^{-1} $$J_{PII} = \sigma_{PII}{}' \cdot E$$ m^{2} $PSII^{-1}$ · photons m^{-2} s^{-1}

e^{-} m^{-3} s^{-1} $$JV_{PII} = a_{PII}{}' \cdot E$$ m^{-1} · photons m^{-2} s^{-1}

From PSII electron flux to GPP



J_{PII} = PSII flux (electrons $PSII^{-1}$ s^{-1}) JV_{PII} = PSII flux m^{-3} (electrons m^{-3} s^{-1}) GPP = carbon fixed (C m^{-3} s^{-1})

$$J_{PII} = \sigma_{PII}' \cdot E$$

Sigma method

$$JV_{PII} = a_{PII}' \cdot E$$
$$= a_{LHII} \cdot \phi_{PII}' \cdot E$$

Absorption method

Calculation of PSII electron flux per unit volume (JV_{PII}) using the sigma method

$$JV_{PII} = \sigma_{PII}' \cdot [RCII] \cdot (1 - C) \cdot E$$

σ_{PII}' → from iterative curve fit to FRR-ST data

[RCII] → from chlorophyll determination (n_{PSII})

$(1 - C)$ → from light + dark FRR data

E → from PAR sensor

Kolber, Prášil and Falkowski (1998)

The sigma method (Kolber et al. 1998)

✓Can be applied on wide spatial and temporal scales

✓Can be used to probe PSII photochemistry

✓ Provides a good estimate of PSII electron flux (J_{PII})

✓Estimate of rETR (relative electron transport rate to NADP)

- JV_{PII} requires an independent estimate of [RCII]
- Requires an assumed level of connectivity to quantify C

The absorption method (Oxborough et al. 2012)

LIMNOLOGY
and
OCEANOGRAPHY: METHODS

Limnol. Oceanogr.: Methods 10, 2012, 142–154

Direct estimation of functional PSII reaction center concentration and PSII electron flux on a volume basis: a new approach to the analysis of Fast Repetition Rate fluorometry (FRRf) data

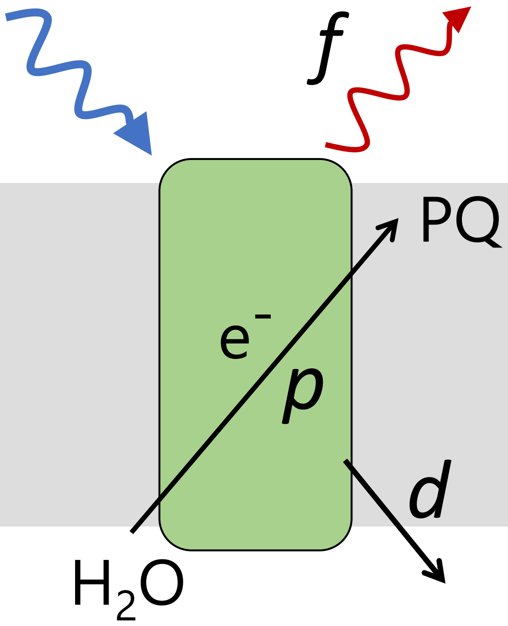
Kevin Oxborough[1], C. Mark Moore[2], David J. Suggett[3], Tracy Lawson[3], Hoi Ga Chan[1], and Richard J. Geider[3]*
[1]CTG Ltd., 55 Central Avenue, West Molesey, KT8 2QZ, UK
[2]Ocean and Earth Science, University of Southampton, National Oceanography Centre, Southampton, SO14 3ZH, UK
[3]School of Biological Sciences, University of Essex, CO4 3SQ, UK

Basic principle of the absorption method

$$\phi_f = \frac{k_f}{k_p + k_f + k_d}$$

$$\phi_p = \frac{k_p}{k_p + k_f + k_d}$$

$$\frac{P}{F} \propto \frac{k_p}{k_f}$$



Open RCII

Absorption method requires that this is a consistent relationship

Calculation of $[\mathrm{RCII}]$ using the absorption method

Hypothesis:

$$\frac{k_p}{k_f}$$ falls within a very narrow range

Consequence:

$$[\mathrm{RCII}] = \frac{F_o}{\sigma_{\mathrm{PII}}} \cdot \frac{\mathrm{K_R}}{E_{\mathrm{LED}}}$$

NIOZ PROTOOL workshop, Yerseke (2012)

Organised by: Greg Silsbe & Jacco Kromkamp

12 phytoplankton species

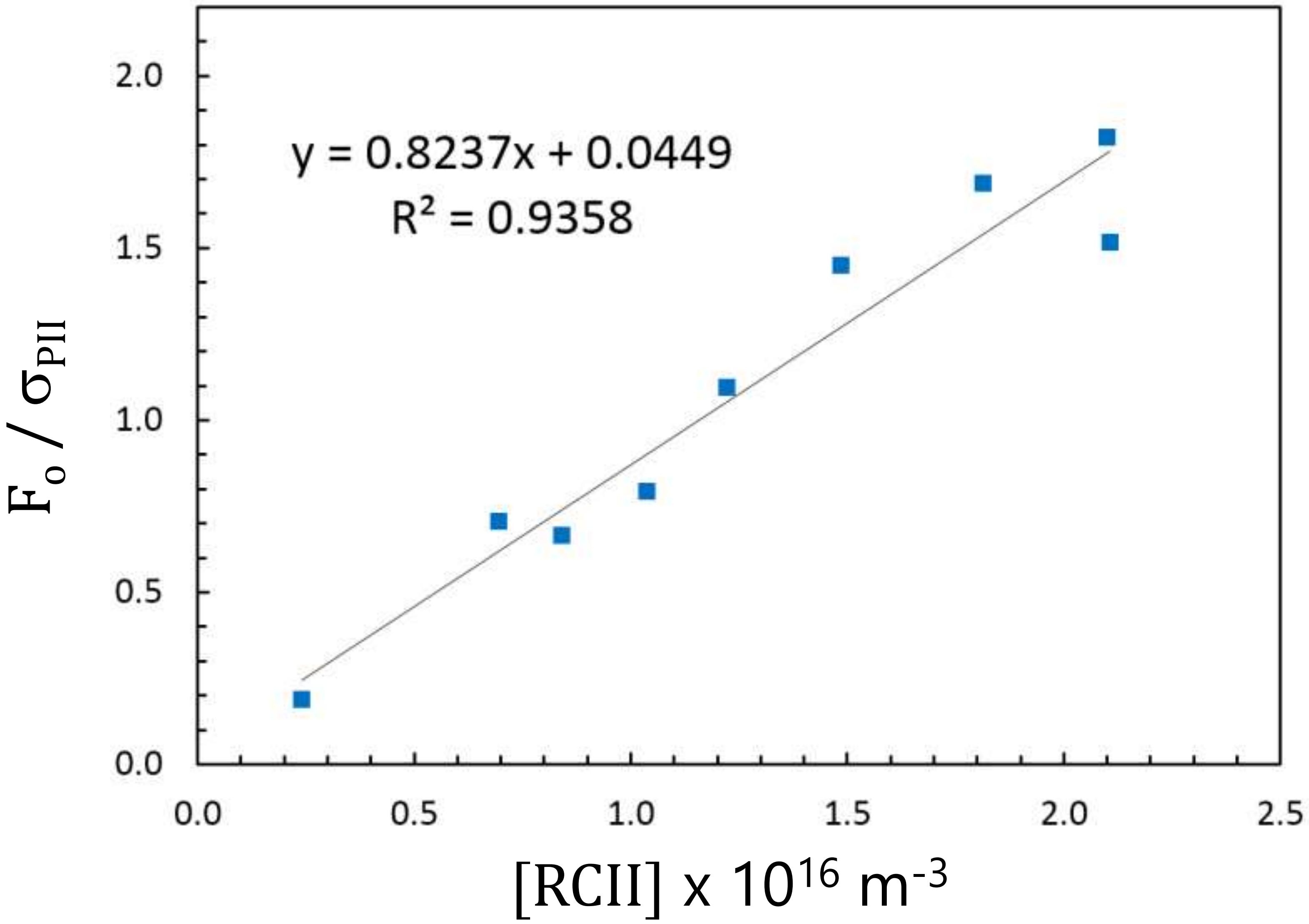
Chaetoceros sp.
Ditylum brightwellii
Emiliania huxleyi
Nannochloropsis gaditana
Phaeocystis glabosa
Prorocentrum sp.
Skeletonema sp.
Synechococcus (red 9903 + green 0417)
Tetraselmis sp.
Thalassiosira pseudonana (-Fe / +Fe)
Thalassiosira westfloggii (-Fe / +Fe)

Methods

Fast Repetition Rate fluorometers
- Mk I and Mk II FAST*tracka*
- FastOcean

Flash O_2 release
Thermoluminescence
MIMS
^{13}C
Absorption spectrometry
Fluorescence excitation spectrometry

NIOZ PROTOOL workshop, Yerseke (2012)



Implications for the sigma algorithm

$$\mathrm{JV_{PII}} = \sigma_{\mathrm{PII}}' \cdot [\mathrm{RCII}] \cdot (1 - C) \cdot E$$

σ_{PII}' → from iterative curve fit to FRR-ST data

[RCII] → from FRR-ST data

$(1 - C)$ → from light + dark FRR data

E → from PAR sensor

No longer a requirement for independent measurement of [RCII]

The absorption algorithm

Hypothesis:

$$\frac{k_p}{k_f}$$ falls within a very narrow range

Consequence:

$$\mathrm{JV_{PII}} = \mathrm{oF'} \cdot \frac{\mathrm{K_R}}{\mathrm{E_{LED}}} \cdot E$$

Where oF' is the emission from open RCII under ambient light

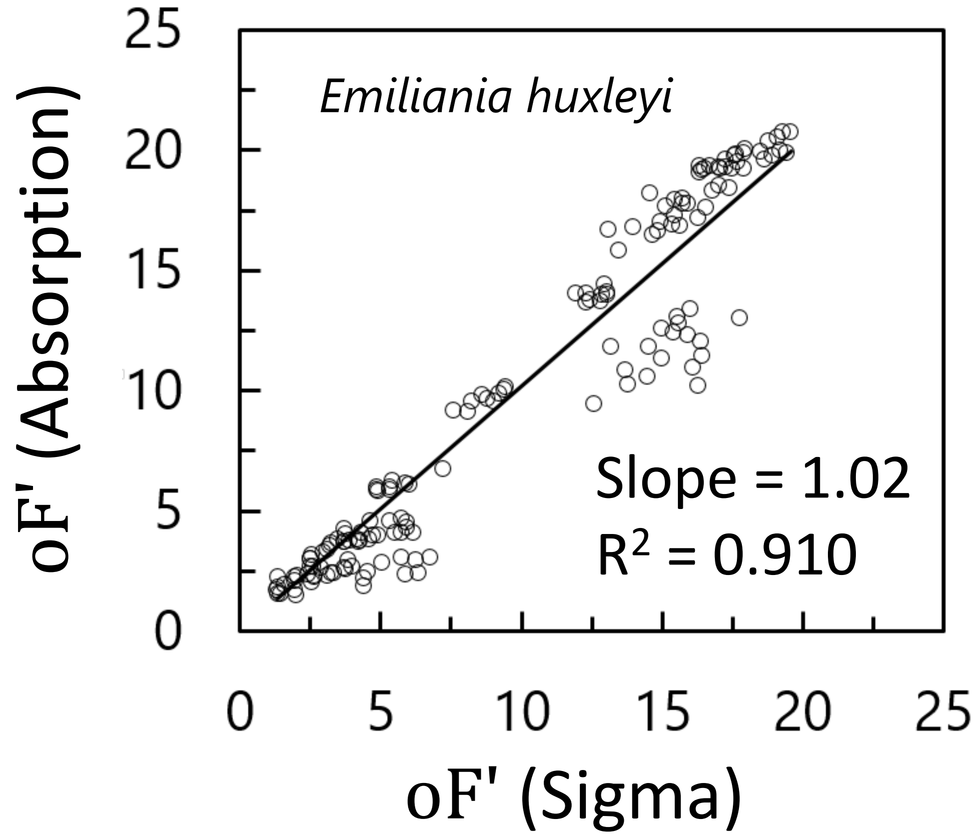
Comparing the sigma and absorption algorithms

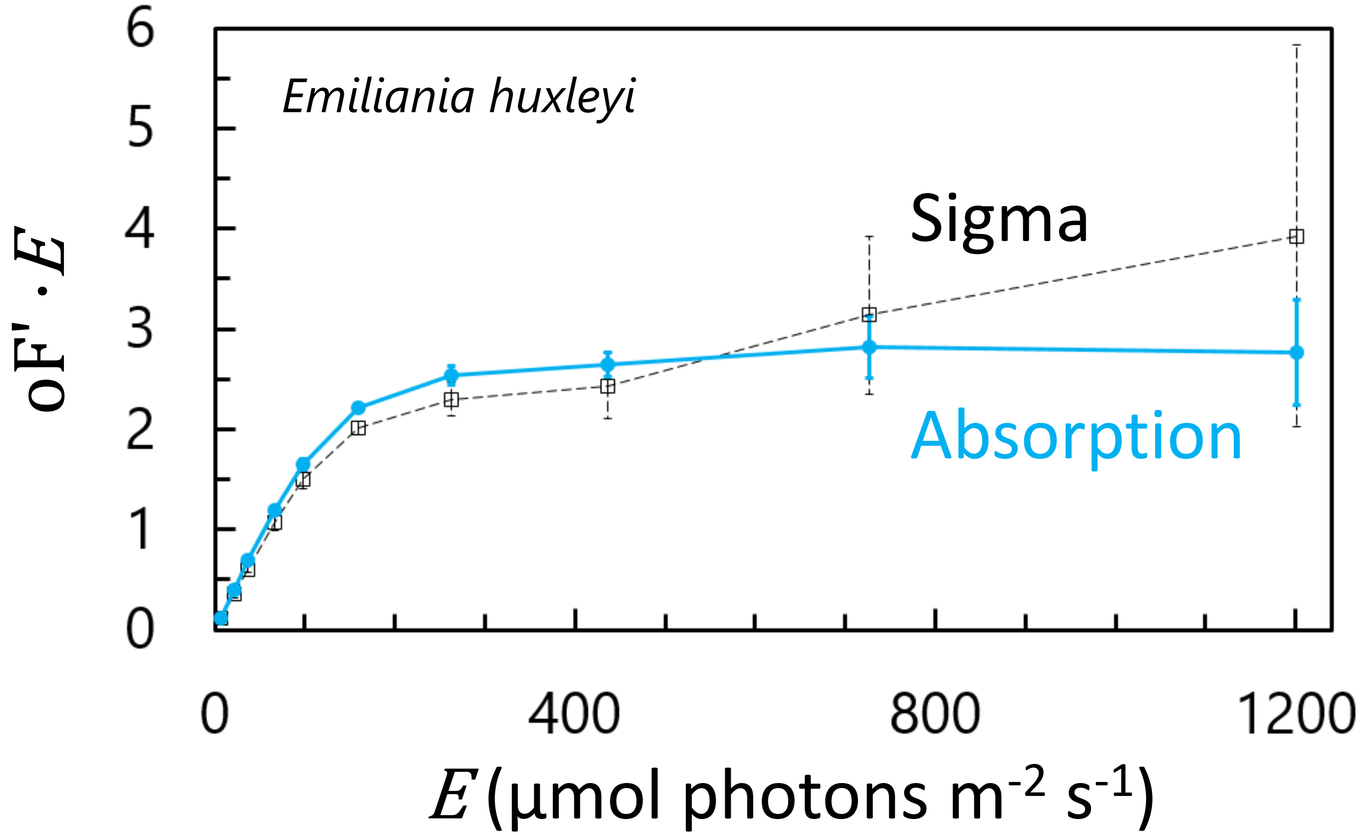
$$\mathrm{oF}' = \mathrm{F_o} \cdot \frac{\sigma_{\mathrm{PII}}{}'}{\sigma_{\mathrm{PII}}} \cdot (1 - C)$$ (Sigma algorithm)

$$\mathrm{oF}' = \frac{\mathrm{F_m} \cdot \mathrm{F_o}}{\mathrm{F_m} - \mathrm{F_o}} \cdot \frac{\mathrm{F_m'} - \mathrm{F'}}{\mathrm{F_m'}}$$ (Absorption algorithm)

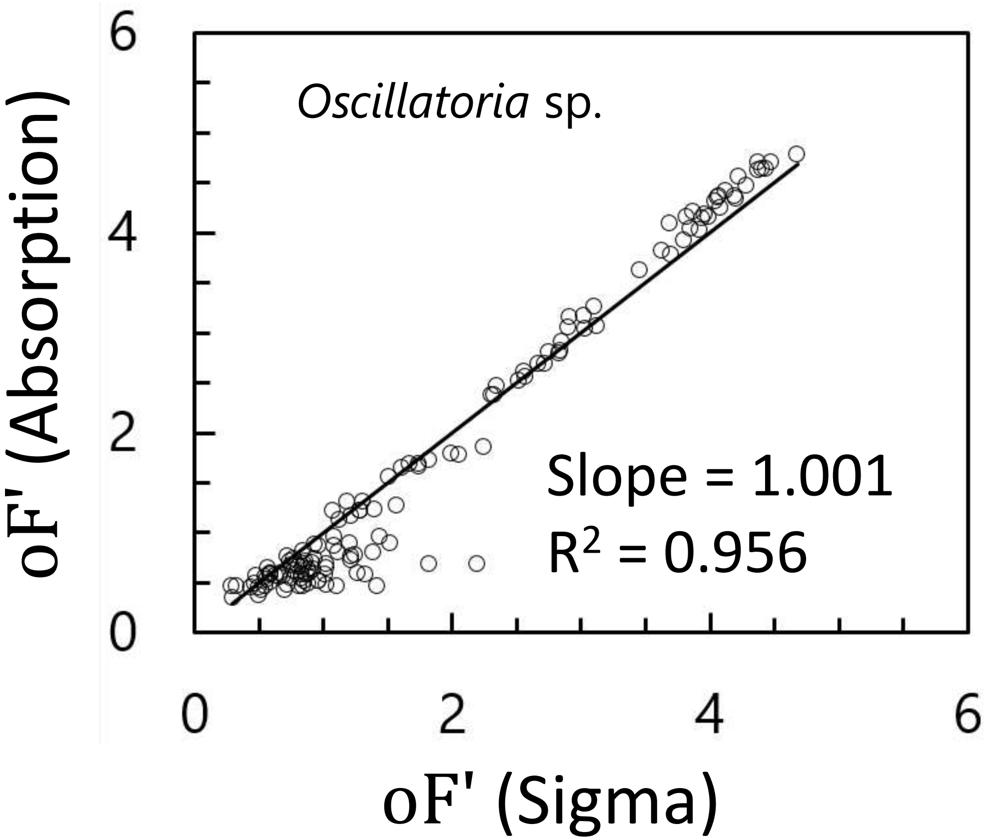
Absorption algorithm doesn't require σ_{PII}, σ_{PII}' or $1 - C$

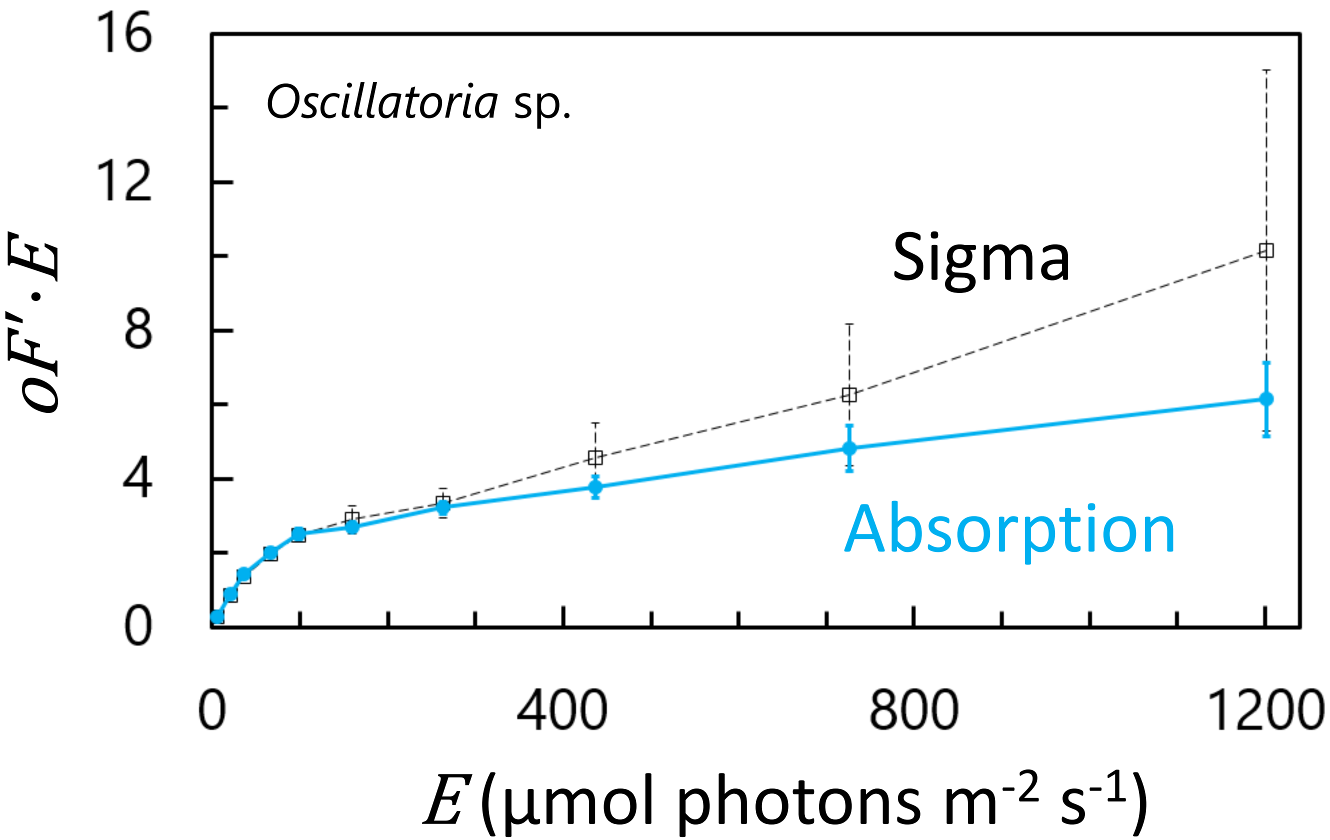
Comparing the sigma and absorption algorithms



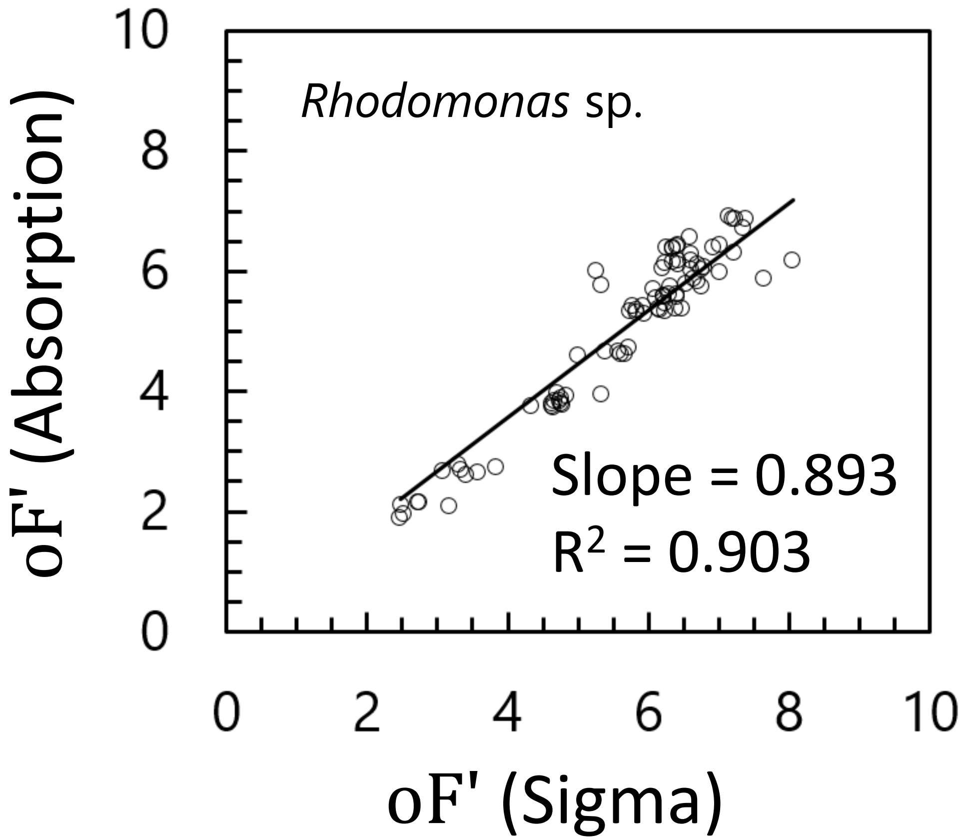


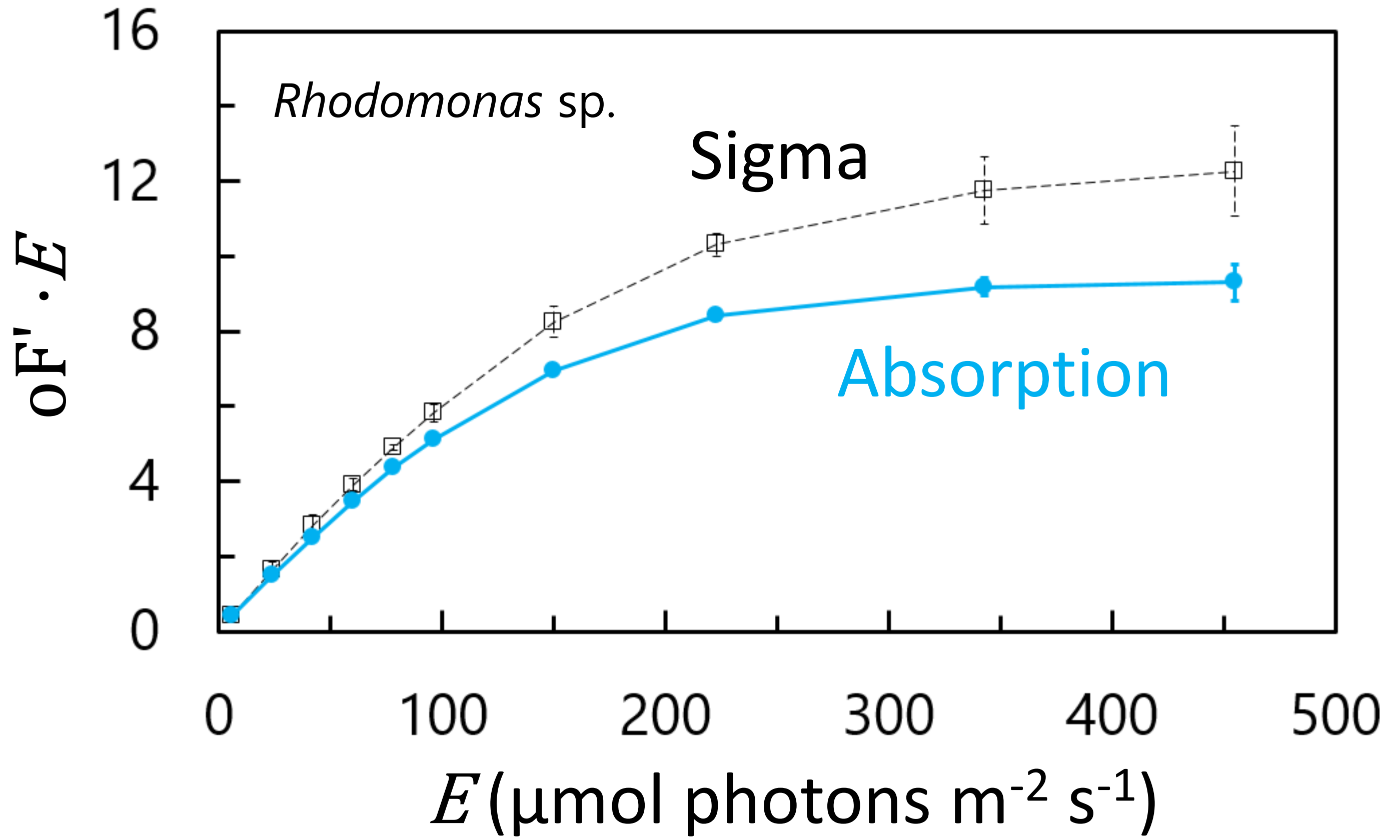
Comparing the sigma and absorption algorithms





Comparing the sigma and absorption algorithms





Instrument calibration

Oxborough et al. (2012):

$$[\mathrm{RCII}] = \frac{F_o}{\sigma_{\mathrm{PII}}} \cdot \frac{\mathrm{K_R}}{E_{\mathrm{LED}}}$$

$\mathrm{K_R}$ is an instrument specific constant, which cannot be used with other instruments of the same design

FastOcean calibration:

$$[\mathrm{RCII}] = \frac{F_o}{\sigma_{\mathrm{PII}}} \cdot \mathrm{K_a}$$

$\mathrm{K_a}$ is an instrument type-specific constant, which can be used with all FastOcean sensors

Practical overview of the sigma method

Instrument calibration –

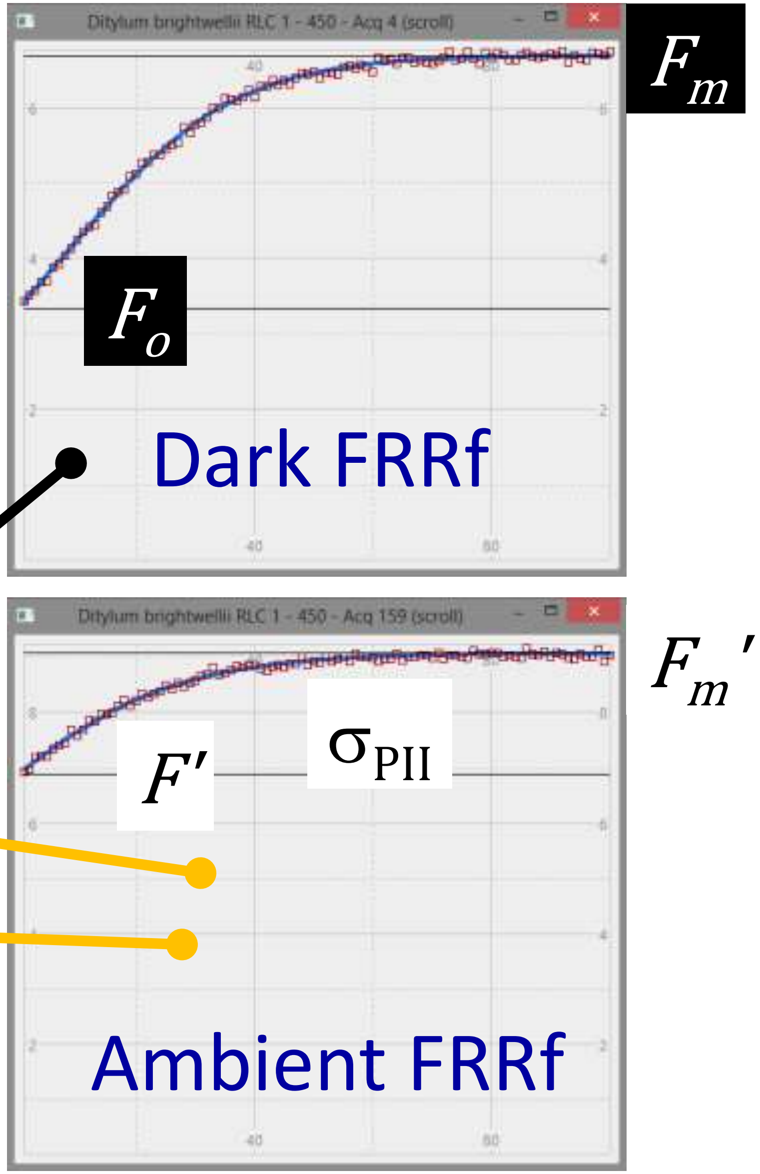
- Gain against chlorophyll *a*
- E_{LED} (photons m^{-2} s^{-1})

GPP estimated through –

$$JV_{PII} = \sigma_{PII}' \cdot [RCII] \cdot (1 - C) \cdot E$$

Each measurement requires –

- σ_{PII}' (m^2) from ambient FRR-ST data
- [RCII] (m^{-3}) from chlorophyll (n_{PSII})
- $1 - C$ (proportion) = $(F_m' - F')/(F_m' - F_o')$
- E (photons m^{-2} s^{-1}) from a PAR sensor



Practical overview of the absorption method

Instrument calibration –

Same as Sigma method plus:

K_a (m^{-1}) = $[RCII] \cdot (\sigma_{PII} / F_o)$

Derivation of K_a requires –

$[RCII]$ (m^{-3}) from flash O_2 release

F_o (dimensionless) and...

σ_{PII} (m^2) from dark FRRf data

GPP estimated through –

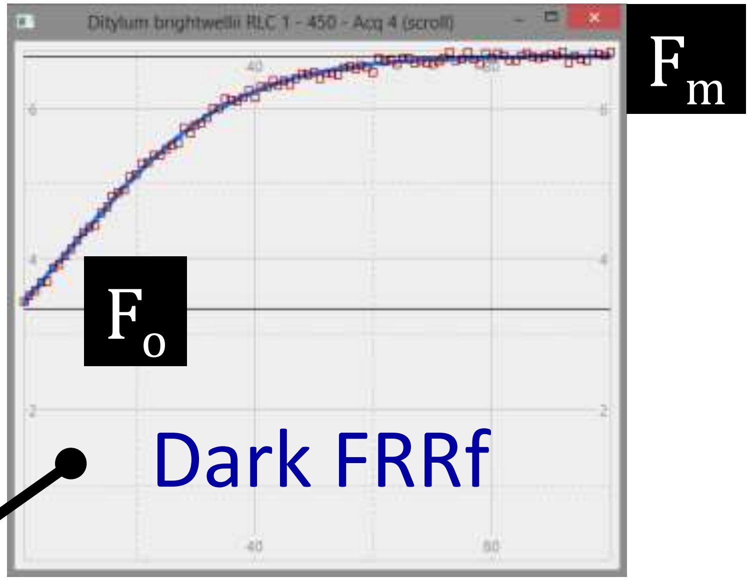
$$JV_{PII} = a_{LHII} \cdot \phi_{PII}' \cdot E$$

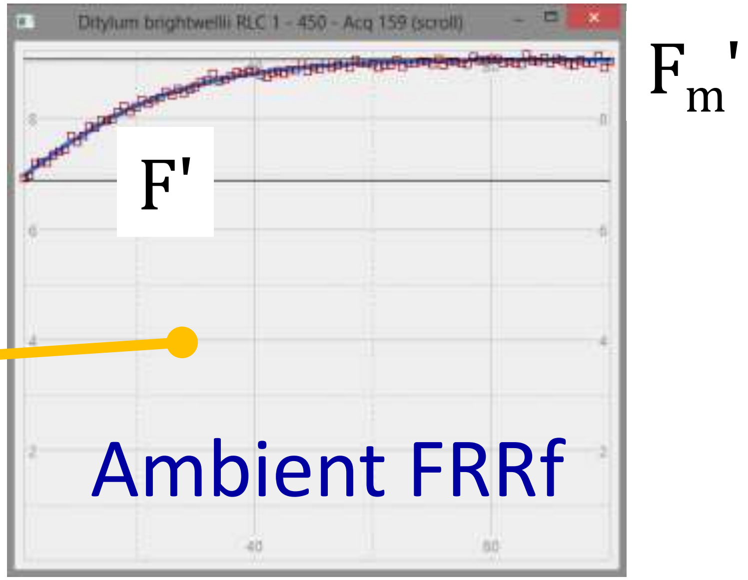
Each measurement requires –

a_{LHII} (m^{-1}) = $([F_m \cdot F_o] / [F_m - F_o]) \cdot K_a$

$\phi_{PII}' = 1 - (F' / F_m')$

E (photons m^{-2} s^{-1}) from a PAR sensor





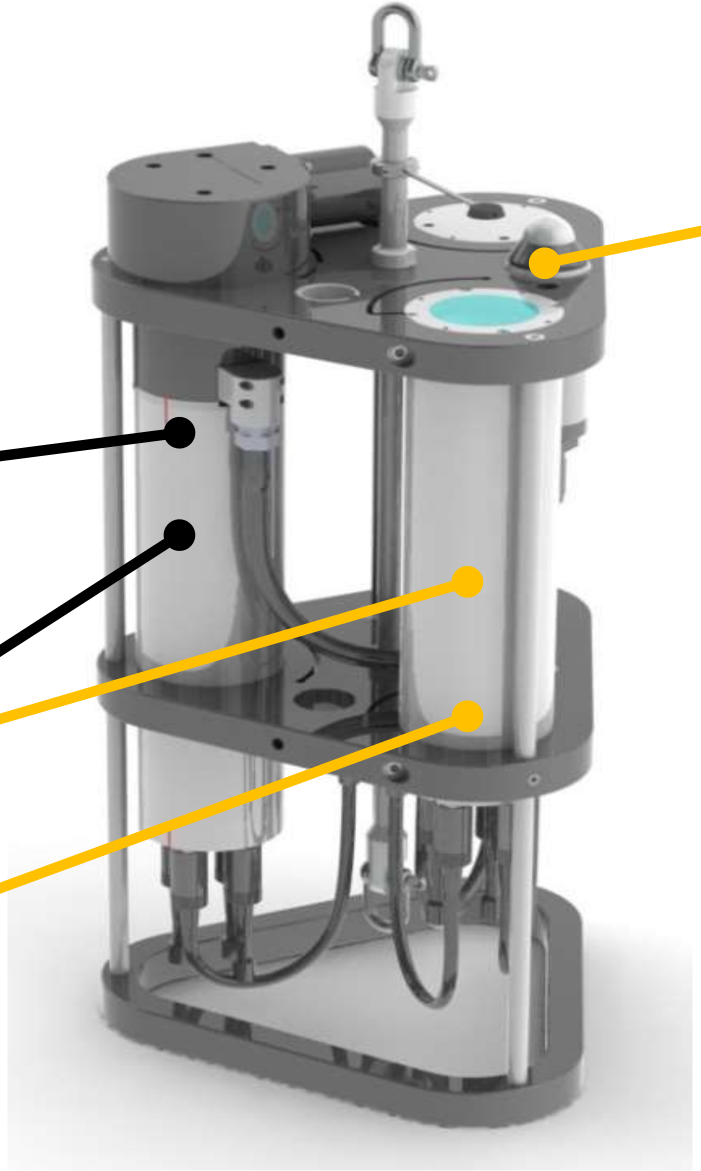
Field measurements - sigma

$$JV_{PII} = \sigma_{PII}' \cdot [RCII] \cdot (1 - C) \cdot E$$

$$[RCII] = K_a \cdot \frac{F_o}{\sigma_{PII}}$$

$$1 - C = \frac{F_m' - F'}{F_m' - F_o'}$$

σ_{PII}'



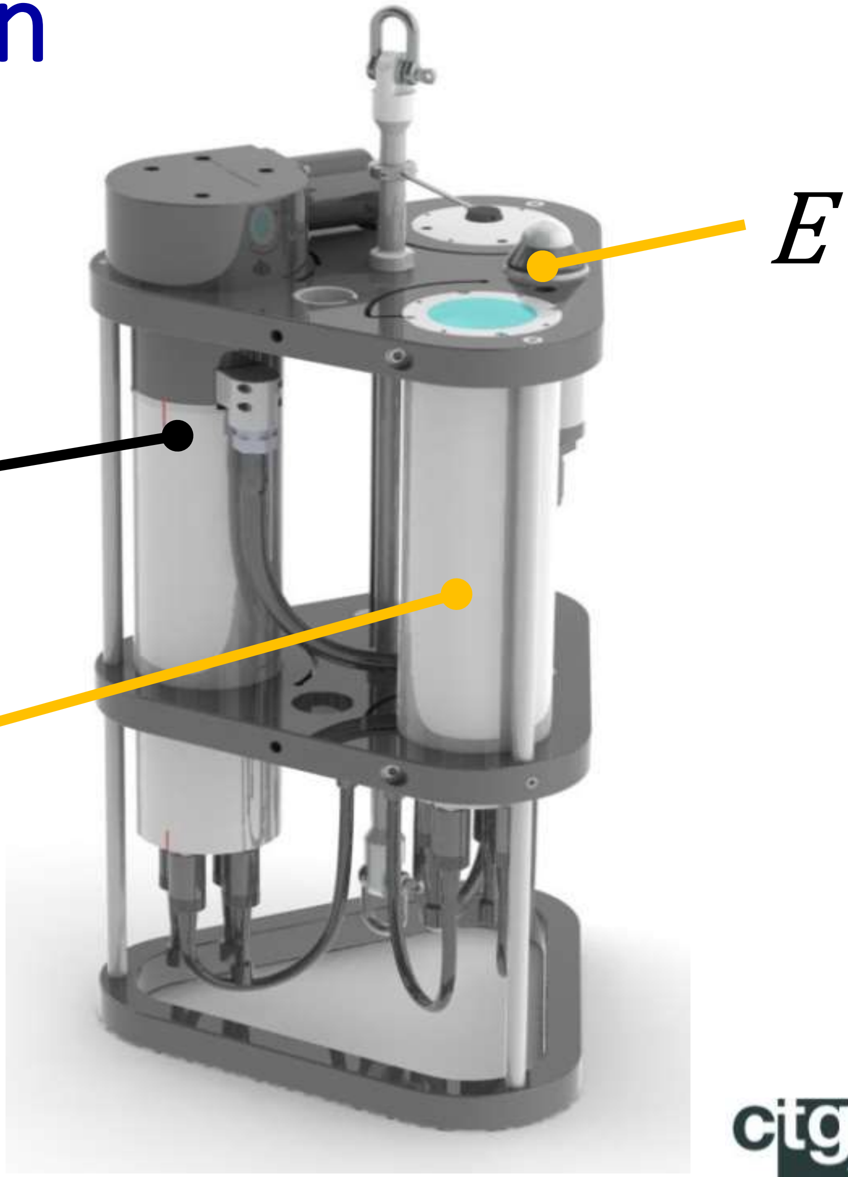
E

Field measurements - absorption

$$JV_{PII} = a_{LHII} \cdot \phi_{PII}' \cdot E$$

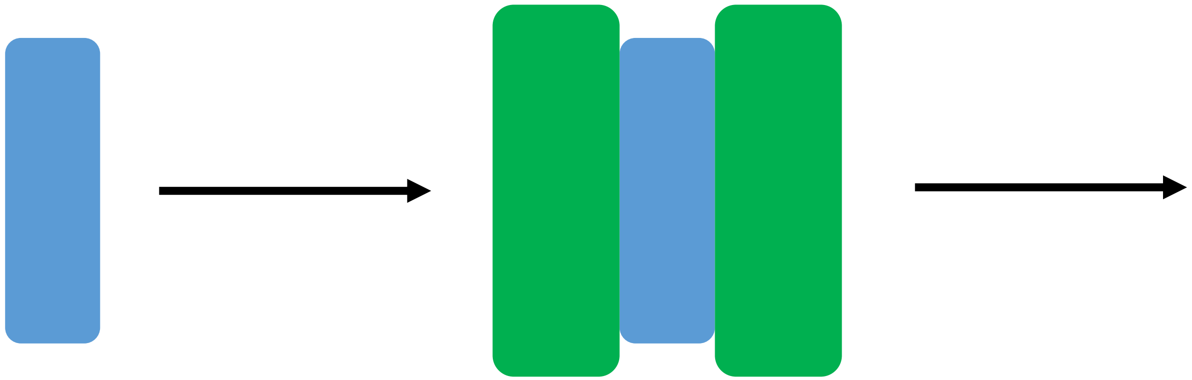
$$a_{LHII} = ([F_m \cdot Fo] / [F_m - Fo]) \cdot K_a$$

$$\phi_{PII}' = 1 - (F' / Fm')$$



Baseline fluorescence

RCII | RCII + LHII | FRRf



$\phi_{PII} \approx 0.9$ | $\phi_{PII} \approx 0.6$ | $\frac{F_v}{F_m} \approx 0.5$

$\frac{F_v}{F_m} < 0.5$ due to:

- Photoinactivation of RCII (photoinhibition)
- Downregulation (non-photochemical quenching)

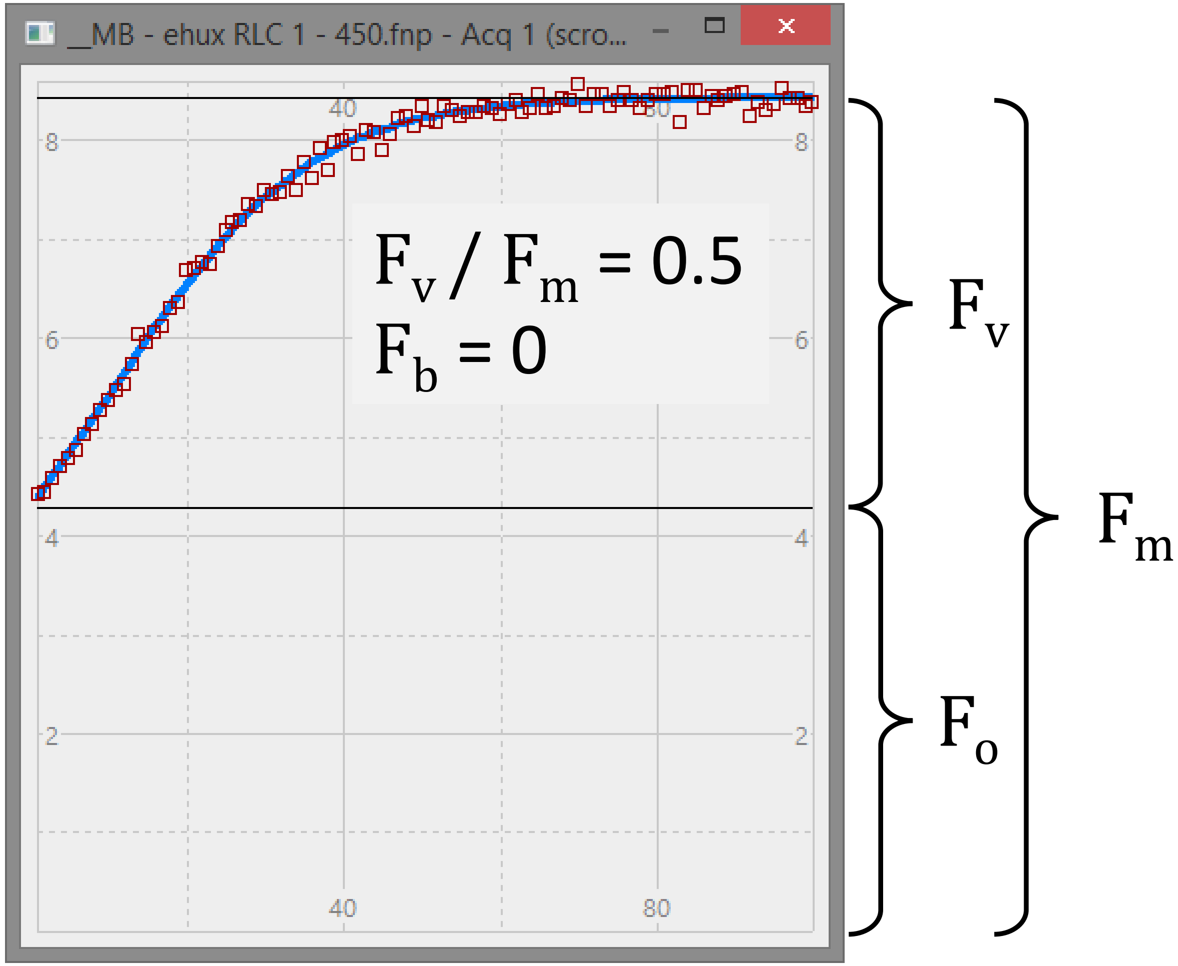
Baseline fluorescence

$$F_b = F_m - F_v / (F_v/F_m)^*$$

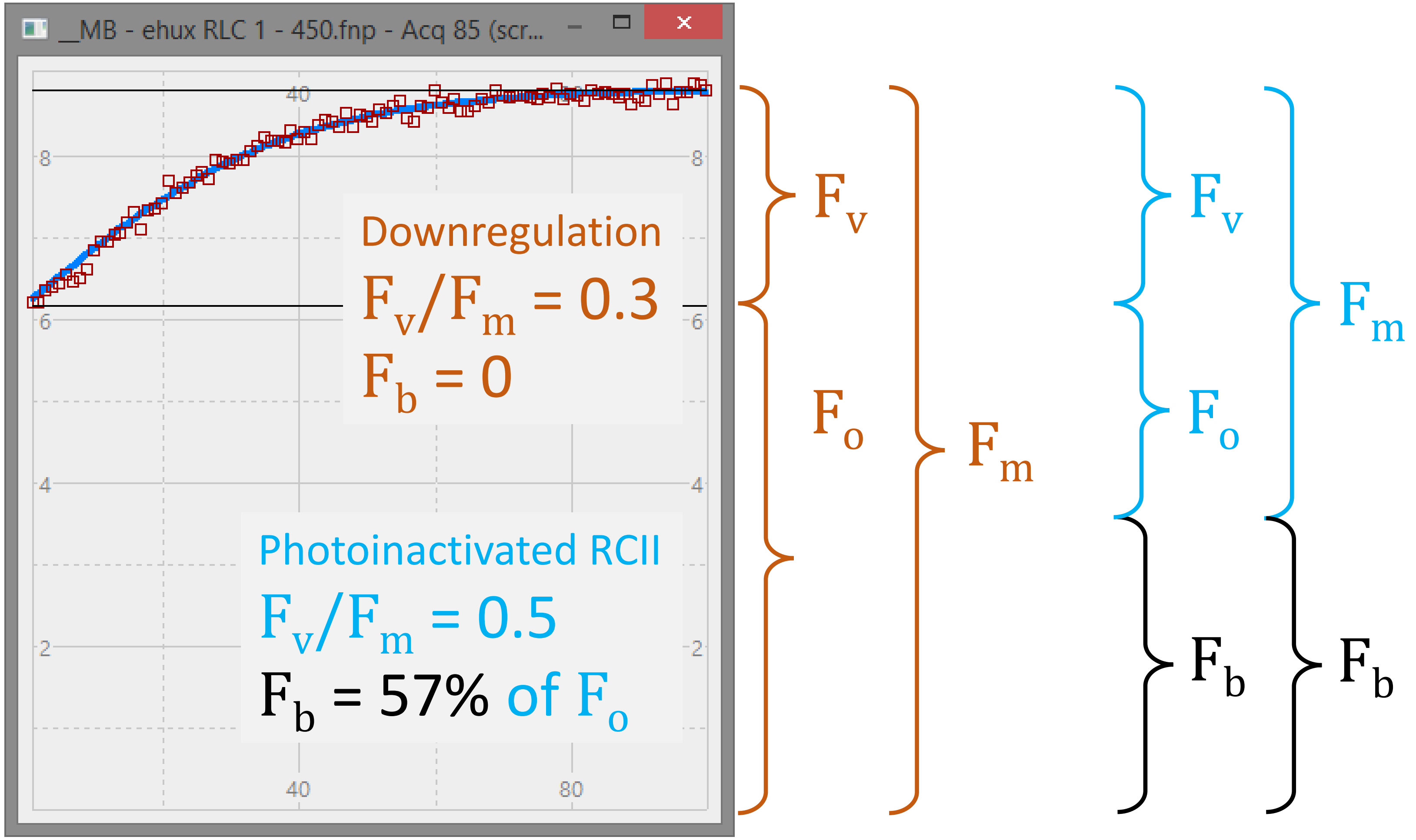
Where:

- $(F_v/F_m)^*$ is the assumed 'true' F_v/F_m from active RCII
- F_b is baseline fluorescence

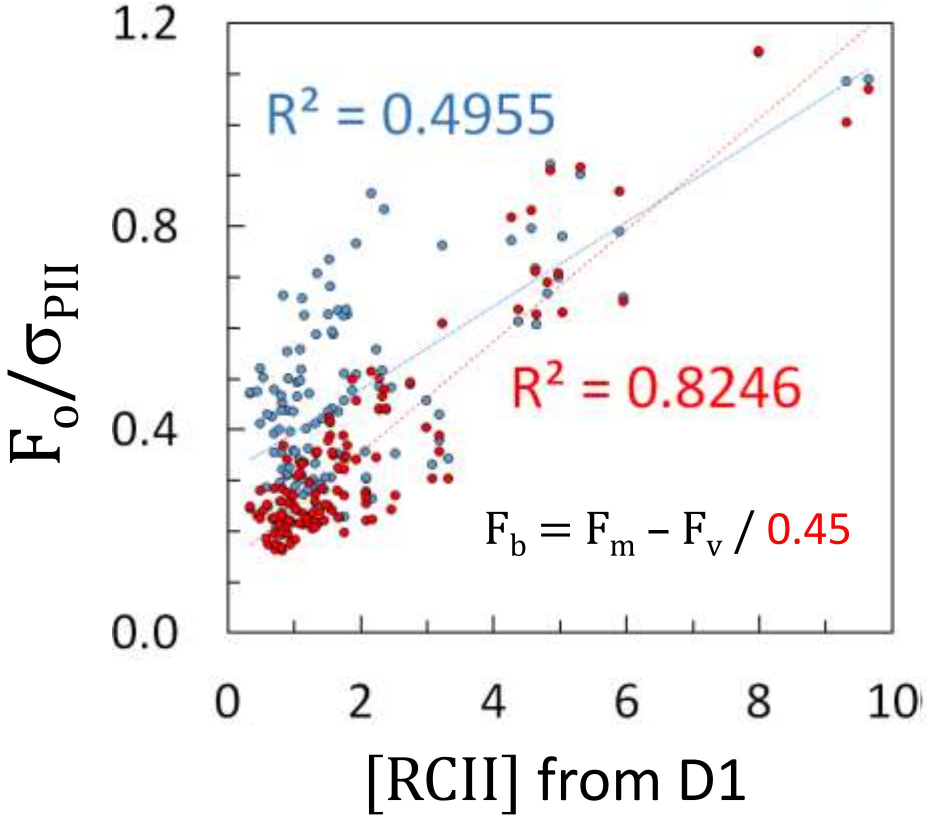
Baseline fluorescence = 0

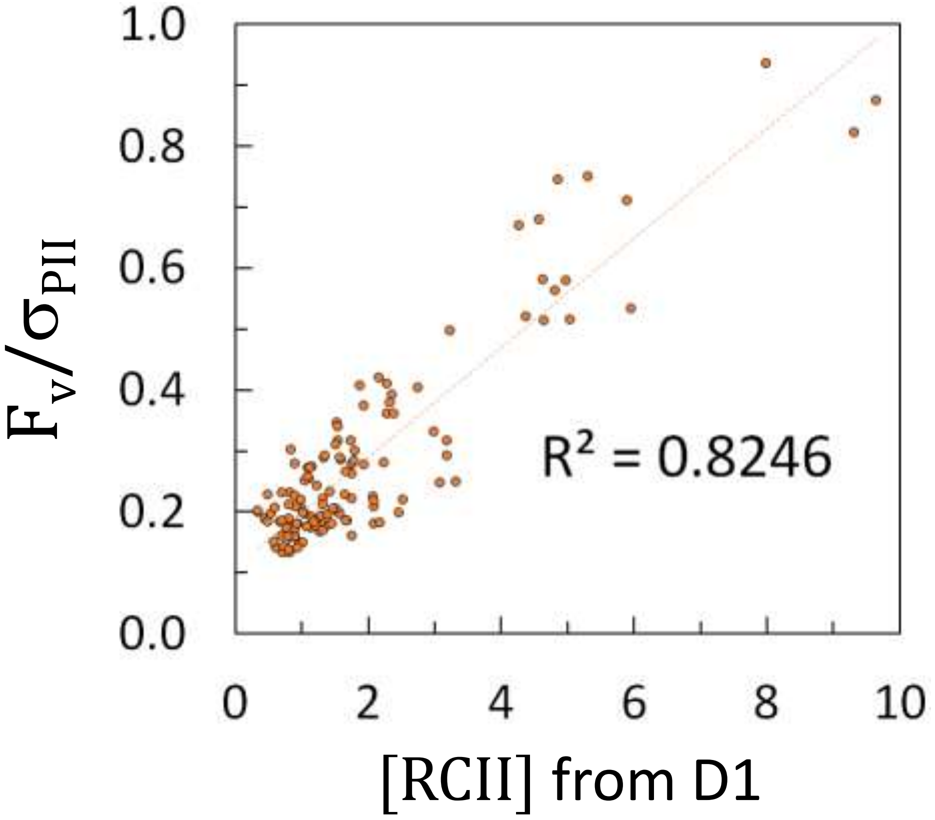


Baseline fluorescence = 0 or F_b



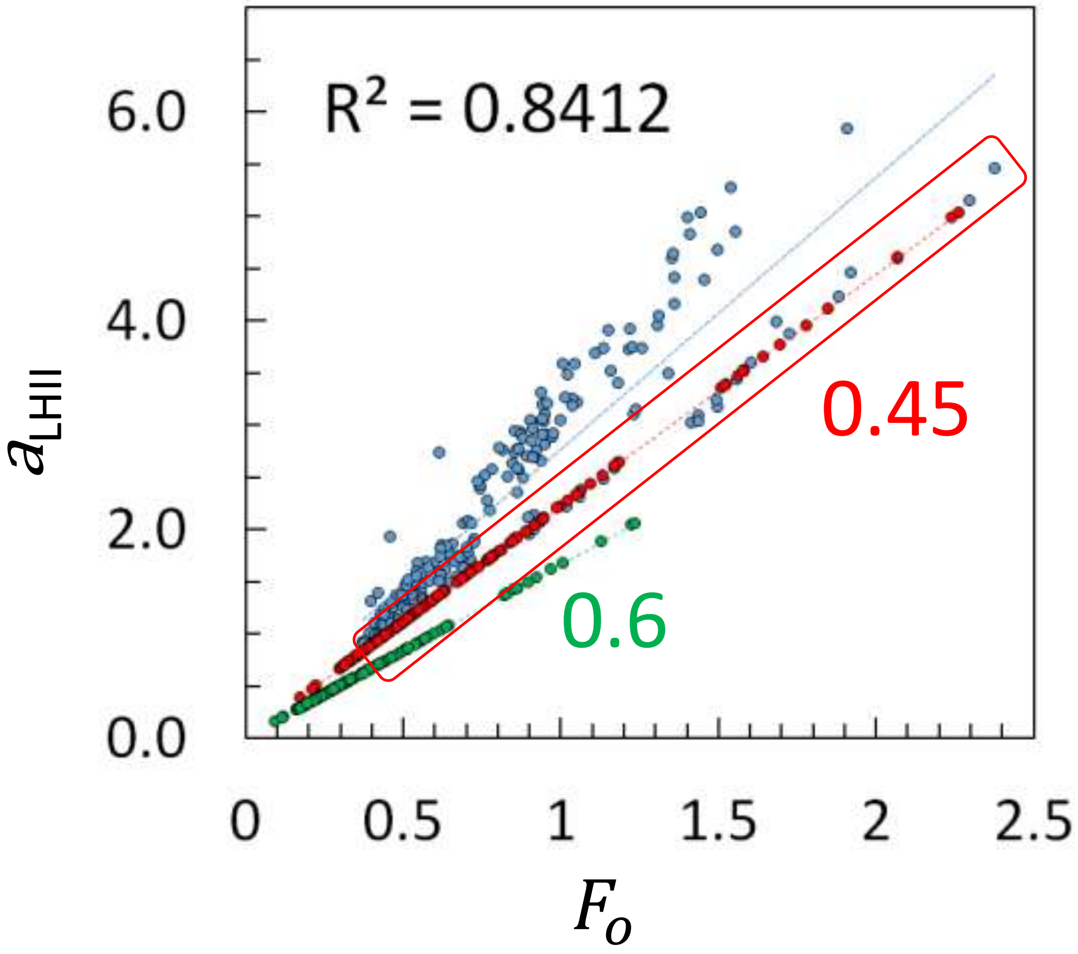
Fe-limited, oligotrophic conditions – natural phytoplankton population





Anna Macey, Tom Bibby and Mark Moore (University of Southampton, NOC)

Fe-limited, oligotrophic conditions – natural phytoplankton population



F_v/F_m = 0.45 gives the best fit to the +Fe points

F_v/F_m = 0.6 would give lower JV_{PII} values than 0.45

Summary

The absorption method:

- Can be used to estimate $\mathrm{JV_{PII}}$ on wide spatial and temporal scales
- Provides a much better S:N than the sigma method – particularly at high ambient photon irradiance (E)
- Baseline 'correction' of data looks to be a viable method for dealing with low $\mathrm{F_v/F_m}$ from dark-adapted material
    - More data are required to test this idea