Fast repetition reate Fluorometry

FRRF nebo také FRRf

Miloš Barták

1992

FAST REPETITION RATE (FRR) FLUOROMETER FOR MAKING *in situ* MEASUREMENTS OF PRIMARY PRODUCTIVITY

Zbigniew S. Kolber and Paul G. Falkowski
Oceanographic and Atmospheric Science Division, Brookhaven National Laboratory,
Upton, New York 11973

1. ABSTRACT

Understanding the ocean carbon cycle and predicting how climate-induced changes in ocean circulation will affect ocean productivity requires that (a) primary productivity be measured with high spatial and temporal resolution, and (b) natural variability in primary productivity be parameterized with regard to environmental factors such as nutrient availability, irradiance, and temperature. Instrumentation to measure primary productivity from the stimulated *in vivo* fluorescence of phytoplankton chlorophyll is currently being developed at Brookhaven National Laboratory. The instrumentation is based on fast repetition rate (FRR) fluorometry, and provides a robust technique for deriving the photosynthetic rates *in situ*. Moreover, the FRR methodology directly measures several photosynthetic parameters such as effective absorption cross section, photoconversion efficiency, and turnover time of photosynthesis, and relates them to primary productivity. Since photosynthetic parameters are affected by environmental factors

As an alternative approach, fluorescence methods of assessing primary productivity are based on a functional relationship between the *in vivo* fluorescence signal of marine phytoplankton and their photosynthetic efficiency. Two basic methods have evolved during the last few years: passive fluorescence sensors, utilizing a solar-stimulated fluorescence signal [1], and pump-and-probe fluorometry, using flash-stimulated fluorescence [2,3]. The passive fluorescence method is based on empirical relationship between the yield of fluorescence and photosynthesis. Active fluorescence, on the other hand, is based on measurements of photosynthetic parameters such as the effective absorption cross-section, photoconversion efficiency, and turnover time of photosynthesis [4]. These parameters can be incorporated into a mechanistic model of photochemistry, based on the kinetics of electron flow between Photosystems II and I.

Fast Repetition Rate (FRR) fluorometry is based on the

Limnol. Oceanogr., 49(4), 2004, 1006–1012

Fast repetition rate fluorometry is not applicable to studies of filamentous cyanobacteria from the Baltic Sea

Mika Raateoja,[1] *Jukka Seppälä, and Pasi Ylöstalo*
Finnish Institute of Marine Research, P.O. Box 33, FIN-00931 Helsinki, Finland

Abstract

Fast repetition rate (FRR) fluorometry has been used successfully to investigate the variable fluorescence characteristics of cyanobacteria in oceanic Case 1 waters. In these waters, the effective absorption bands of the light-harvesting pigments for photosystem II (PSII) of the dominant cyanobacterial taxa overlap with the peak of the FRR excitation at 470 nm. The effective PSII absorption of the ecologically-significant filamentous cyanophytes in the Baltic Sea—*Nodularia spumigena* (Mertens ex Bornet and Flahault 1886) and *Aphanizomenon* sp. ((L.) Ralfs ex Bornet and Flahault 1886)—is, however, restricted to beyond 550 nm. We tested the applicability of a currently commercially available FRR fluorometer to studies of these two cyanobacterial taxa. We propose that the FRR technique should not be used in studies on these taxa, or on any cyanophyte containing phycoerythrocyanin instead of phycoerythrin, the former having inefficient PSII light harvesting in the wave band of the FRR excitation. This issue should be taken into account whenever field studies utilizing the FRR system are planned in those Case 2 water bodies, in which cyanobacteria lacking phycoerythin are among the dominant phytoplankton groups.

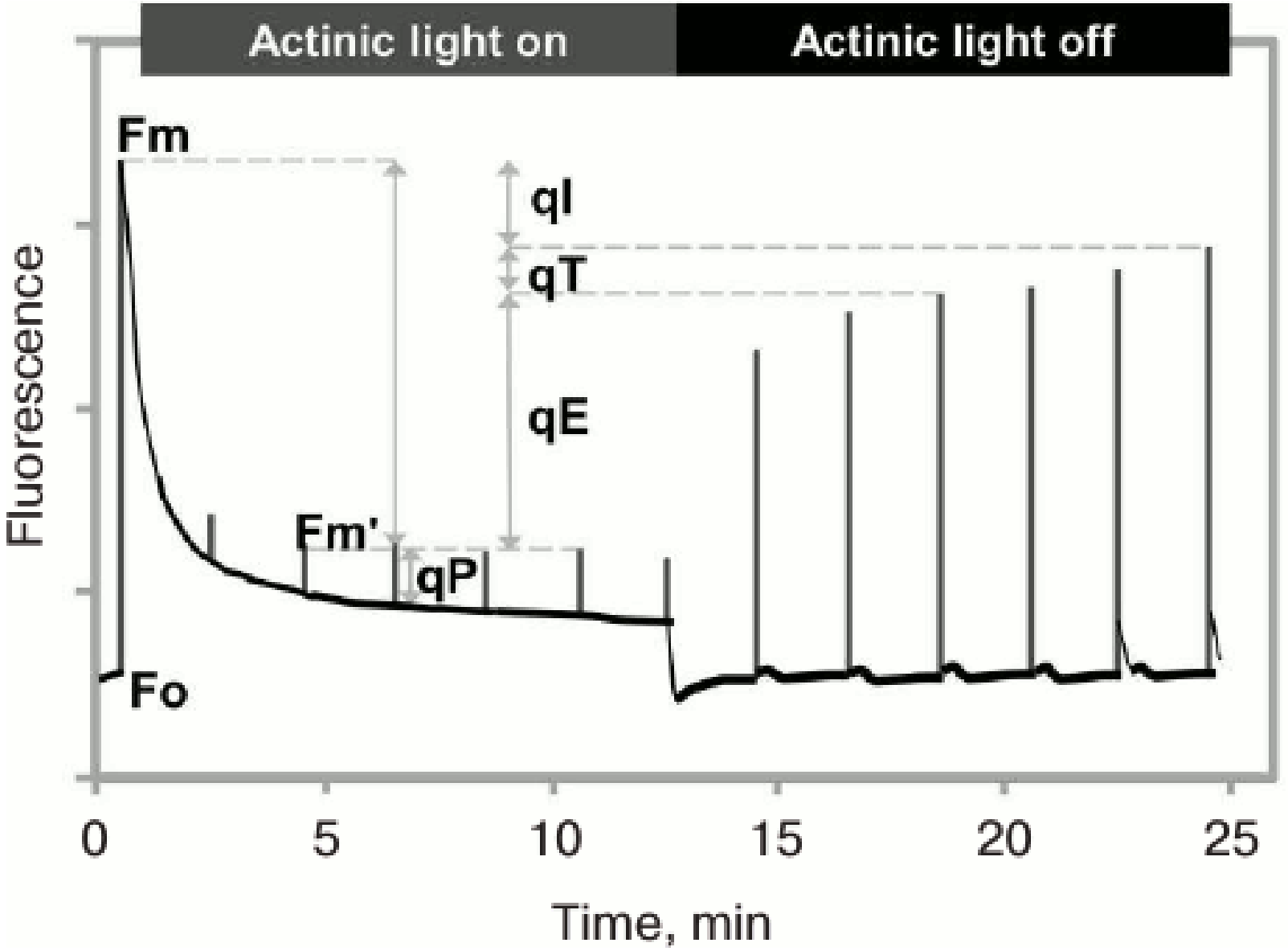
Since the introduction of its commercial version in the mid-1990s, the fast repetition rate (FRR) fluorometer (Kolber and Falkowski 1992) has been rapidly adopted in the field oceanographic research. Being capable of nearly instantaneous high-frequency in situ determinations of a suite of algal photophysiological parameters, this technique has helped us to comprehend, for example, the extent of iron limitations on phytoplankton growth in the world's oceans (Boyd et al. 2000).

Up to the present time, FRR-based estimates of the photosynthetic competence (F_v/F_m) of cyanobacteria (Cyanophyceae) at the species level are scarce, as the bulk of the published FRR datasets originate from field studies conducted umn measurements on a *Prochlorococcus*-dominated community (Babin et al. 1996). Berman-Frank et al. (2001) used FRR metrics on a filamentous genus *Trichodesmium* spp., and noted F_v/F_m to vary from 0.25 to 0.55, depending on the severity of Fe-limitation in the strain IMS101.

The Baltic Sea is categorized as a Case 2 water body (Morel and Prieur 1977; Babin et al. 2003) due to its high load of suspended matter, but especially due to its high concentration, for a marine system, of chromophoric dissolved organic matter (CDOM) (Kirk 1994). The pelagic Baltic Sea represents a coastal type 3 in the Jerlov classification system (Jerlov 1976), with its peak transmittance from 550 to 570 nm, depending on the time of the year (Seppälä and Raateoja

Fast Repetition Rate Fluorometry (FRRf)

- Development from conventional chlorophyll fluorimetry
- Specific tool to study phytoplankton physiology
- Used for open ocean research
  - Photosynthesis efficiency, biomass, primary photosynthesis/productivity
  - Carbon dioxide fixation, climate modelling
  - Bloom detection
- Applications:
  - Homeland security
  - Industrial contamination detection
  - Environmental monitoring
  - Bioreactor process monitoring


Actinic light on
Actinic light off
Fm
qI
qT
qE
Fm'
qP
Fo
Fluorescence
0
5
10
15
20
25
Time, min

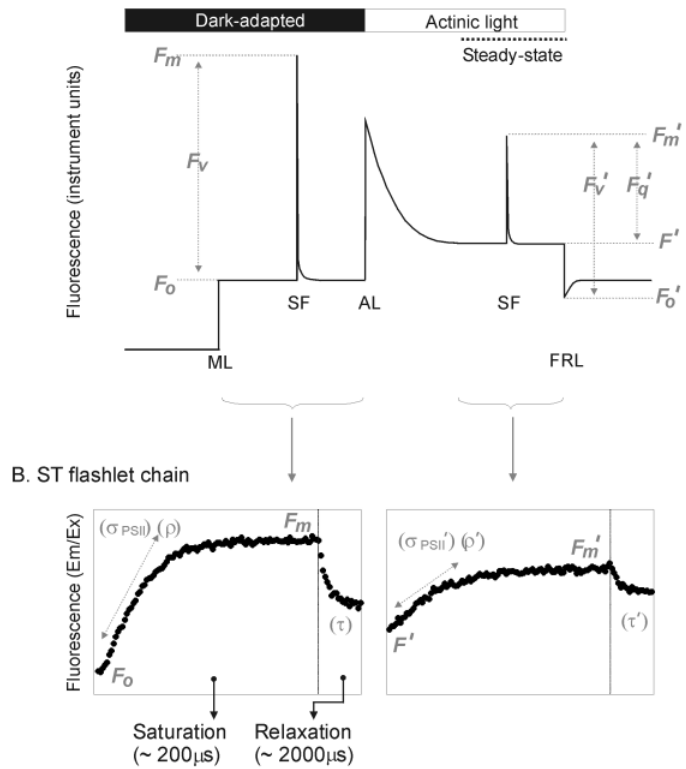


Figure 2. Multiple turnover (MT) and single turnover (ST) fluorescence induction techniques employed by the Pulse Amplitude Modulation and FRR fluorometers, respectively. Examples are shown for phytoplankton in the dark (dark-adapted) and under actinic light. (A) The MT technique delivers a single high intensity saturating flash. (B) The ST technique delivers a series of sub-saturating flashlets. The interval between flashlets is altered to examine the saturation and relation kinetics of photosystem II. All symbols are described in Table 1 except ML (modulating light), SF (saturating flash), AL (actinic light) and FRL (far-red light).

Úplně základní definice nezbytné pro pochopení
Metody FRRF

Právě jednou jsou všechna RC PSII zahlcena (zavřená)
a velmi rychle se ´překlopí´zpět do otevřeného stavu

Single turnover flash

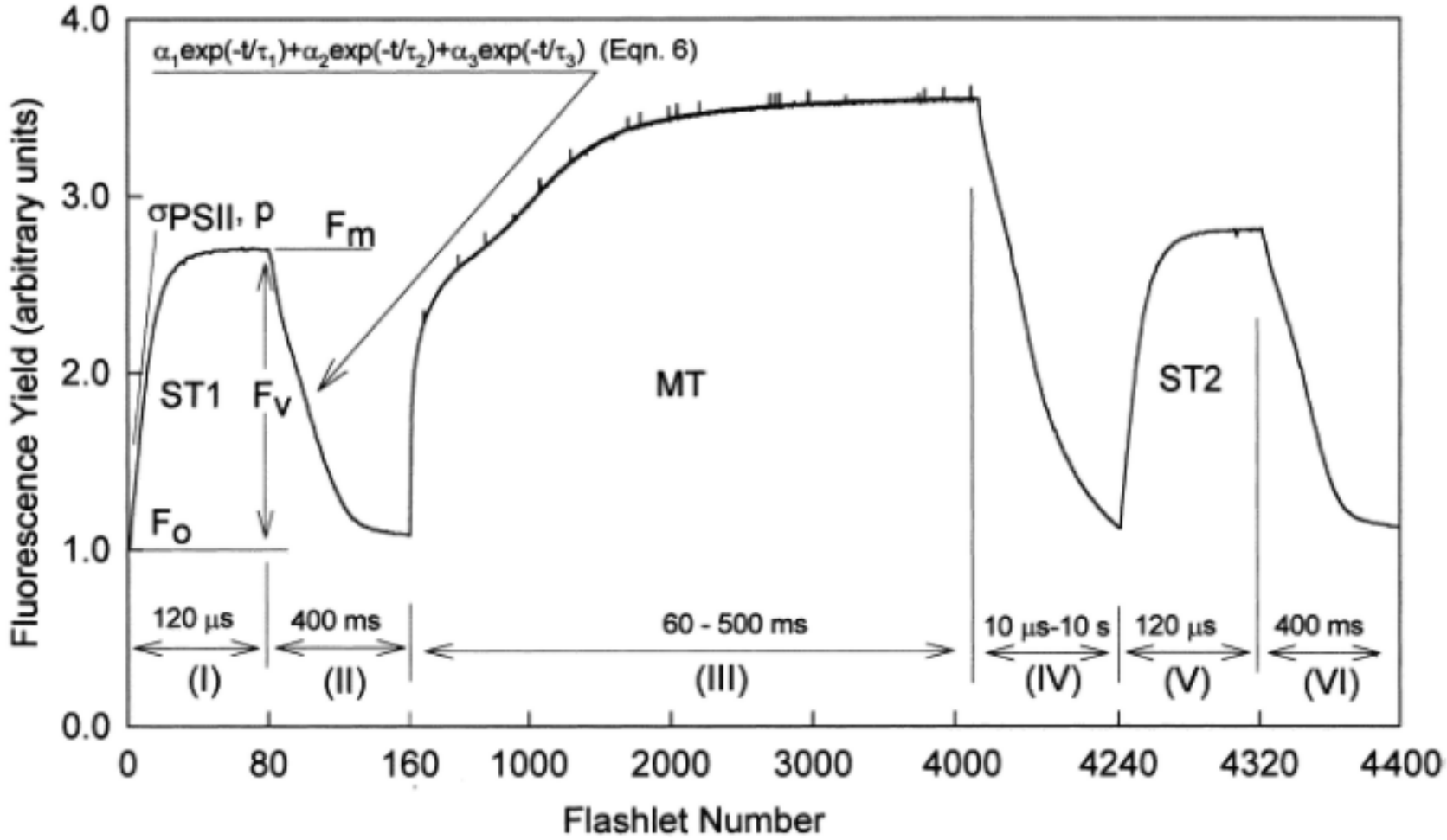
The QA primary acceptor can be reduced with a minimal perturbation of the photosynthetic electron transport chain by using a saturating flash of light that is short enough to ensure a single turnover of Photosystem II reaction centers. The duration of the flash should be shorter than **few tens of microseconds**. Such flashes have been experimentally realized by pulsed lasers, xenon flashlamps

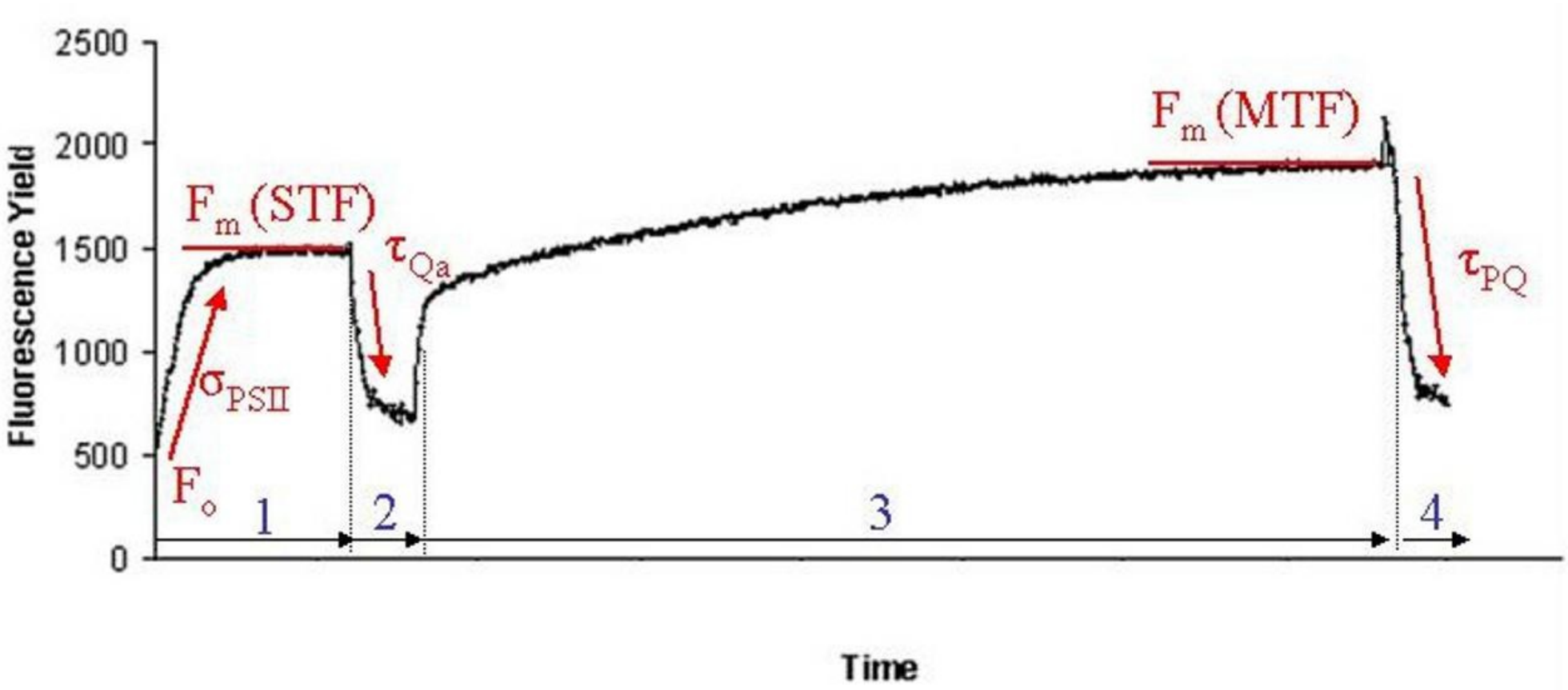
Úplně základní definice nezbytné pro pochopení
Metody FRRF

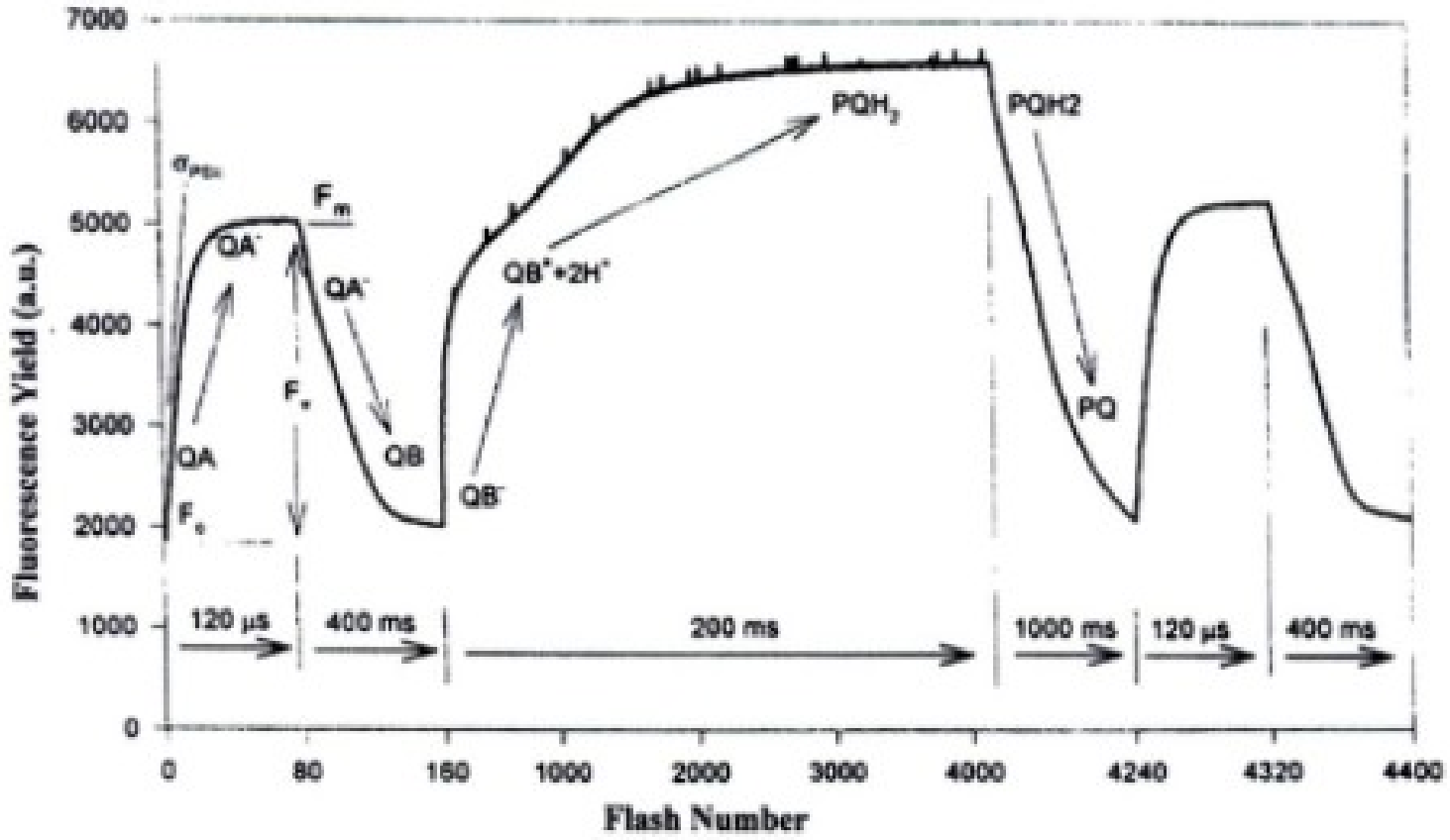
Multiple turnover flash

RC PSII jsou postupně zahlcena (zavřená) a primární akceptor přechází Z oxidovaného (ready to transfer) do plně redukovaného stavu (not ready)

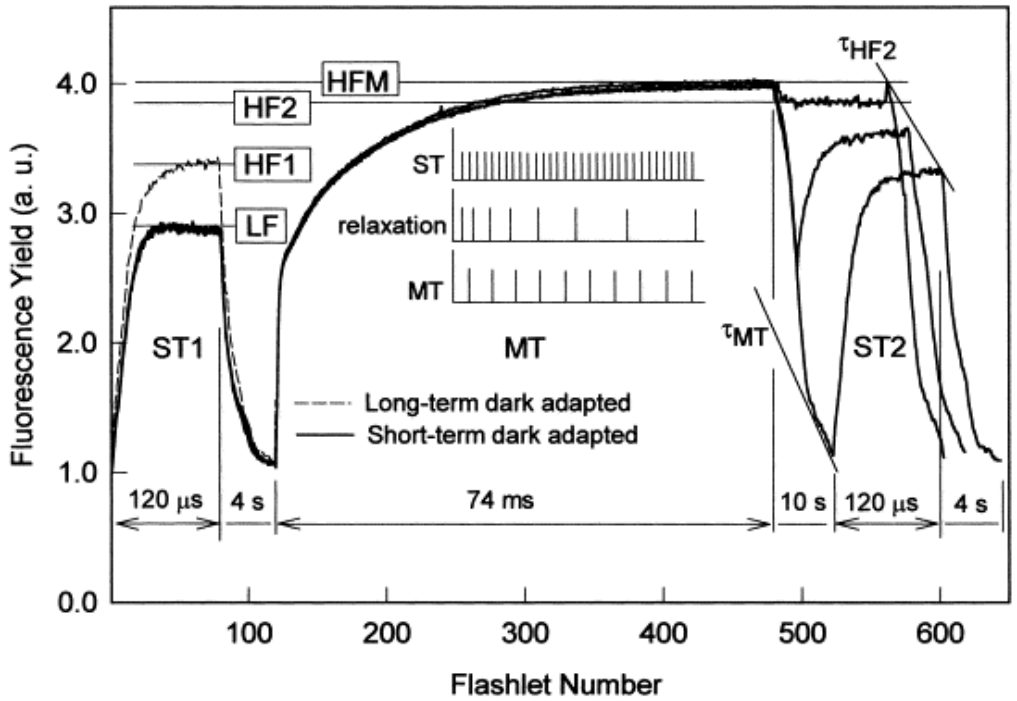
The QA primary acceptor is reduced with a further transfer of entery to the photosynthetic electron transport causing gradual reduction of plastoquinone pool. The duration multiple turnover flash should is about **hundreds of miliseconds**.

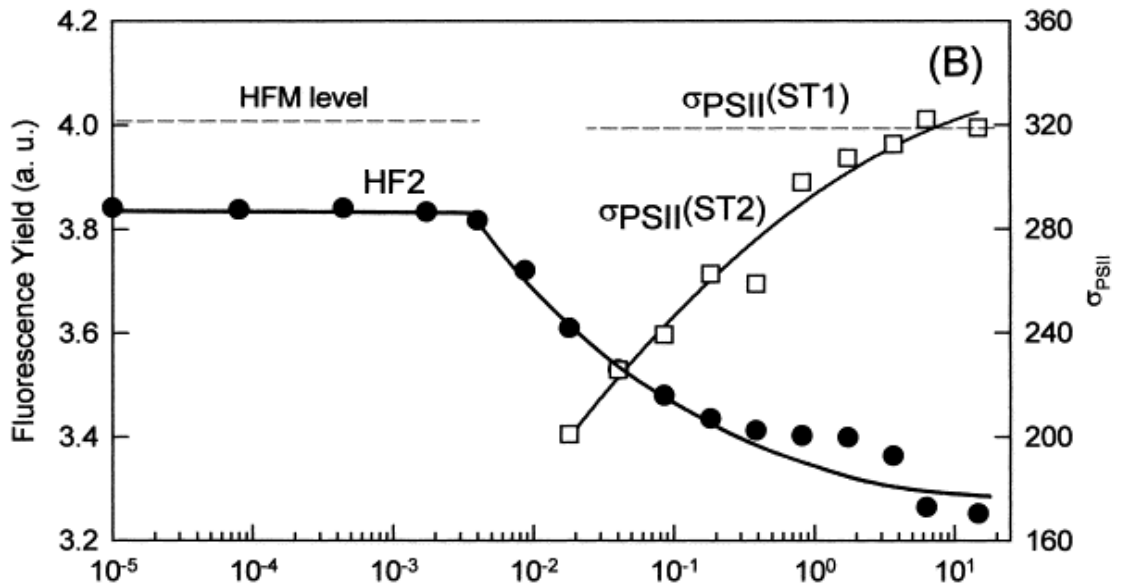

$\alpha_1 exp(-t/\tau_1)+\alpha_2 exp(-t/\tau_2)+\alpha_3 exp(-t/\tau_3)$ (Eqn. 6)
σPSII, p
F_m
F_v
F_o
ST1
MT
ST2
120 µs
400 ms
60 - 500 ms
10 µs-10 s
120 µs
400 ms
(I)
(II)
(III)
(IV)
(V)
(VI)
Fluorescence Yield (arbitrary units)
Flashlet Number
0.0
1.0
2.0
3.0
4.0
0
80
160
1000
2000
3000
4000
4240
4320
4400

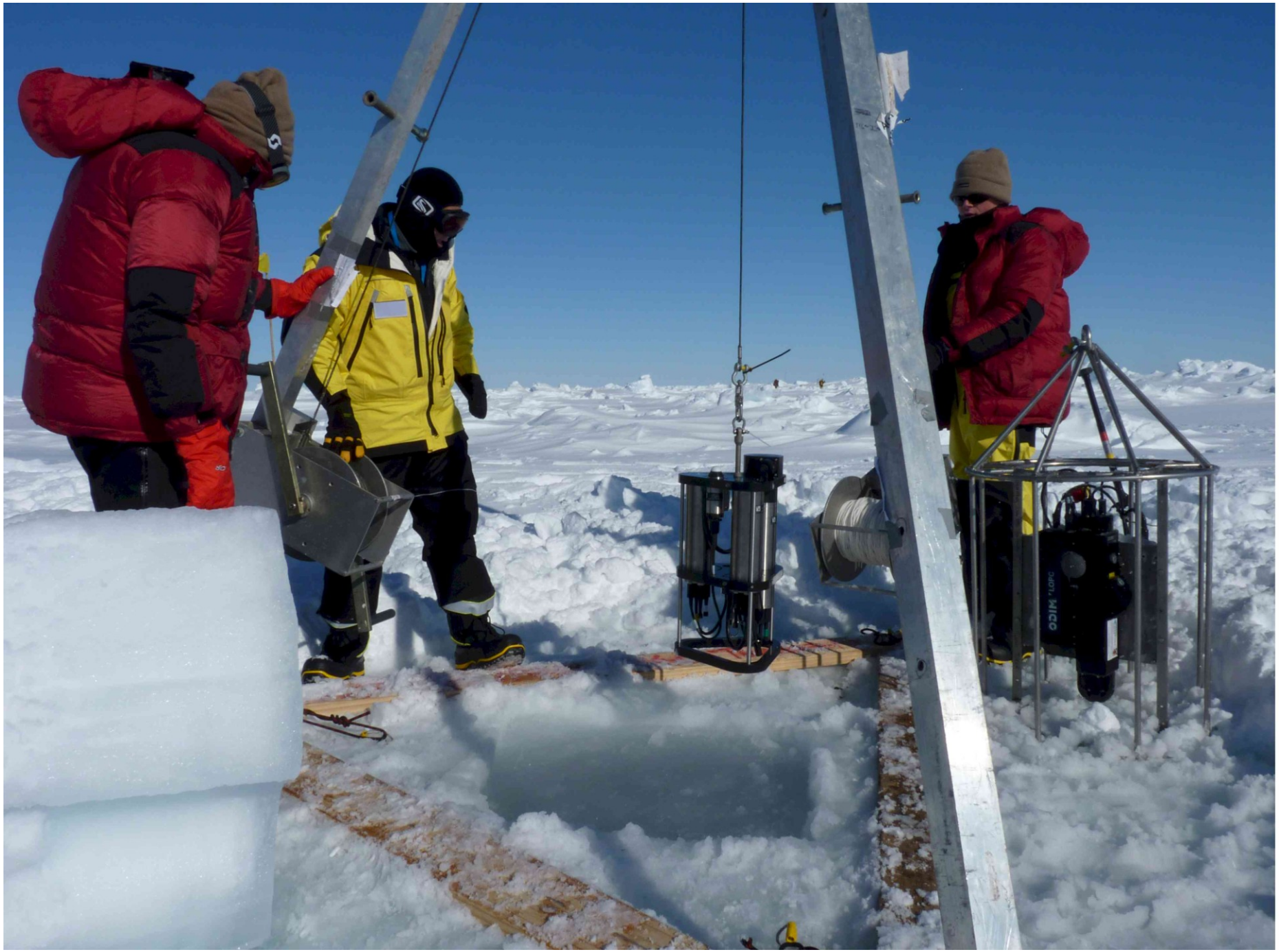

2500
2000
1500
1000
500
0
Fluorescence Yield
F_m (STF)
τ_{Qa}
F_m (MTF)
τ_{PQ}
σ_{PSII}
$F_\circ$
1
2
3
4
Time



that can be derived from Fast Repetition Rate Fluorometry (FRRF). In the initial 120 µs the effective extent of reduction of the primary electron acceptor in PS II (Q_A) can be inferred from the kinetics profile Following the saturation profile, a weak series of pulses are applied and the electron transfer from is then followed by long term pumping with subsaturating flashes to reduce the PQ pool. The over the next 1000 ms with very weak pulses, and the cycle is repeated.

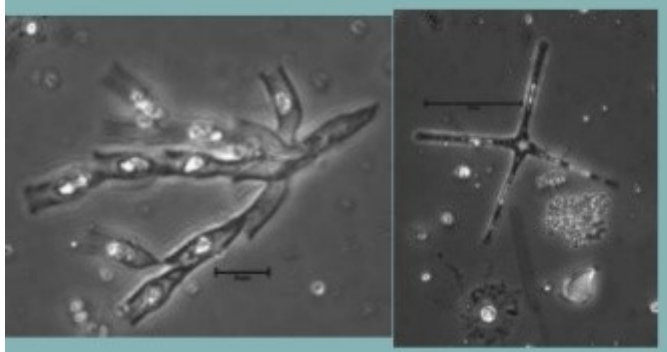

Fluorescence Yield (a. u.)
Flashlet Number
HFM
HF2
HF1
LF
ST
relaxation
MT
ST1
MT
ST2
τHF2
τMT
Long-term dark adapted
Short-term dark adapted
120 µs
4 s
74 ms
10 s
120 µs
4 s

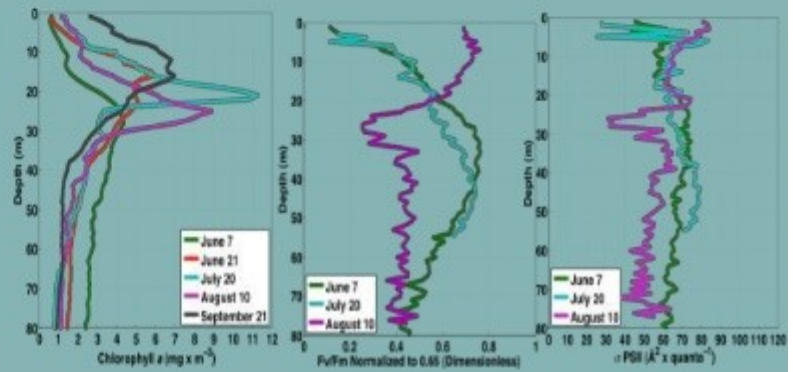

(B)
HFM level
σPSII(ST1)
HF2
σPSII(ST2)
Fluorescence Yield (a. u.)
σPSII

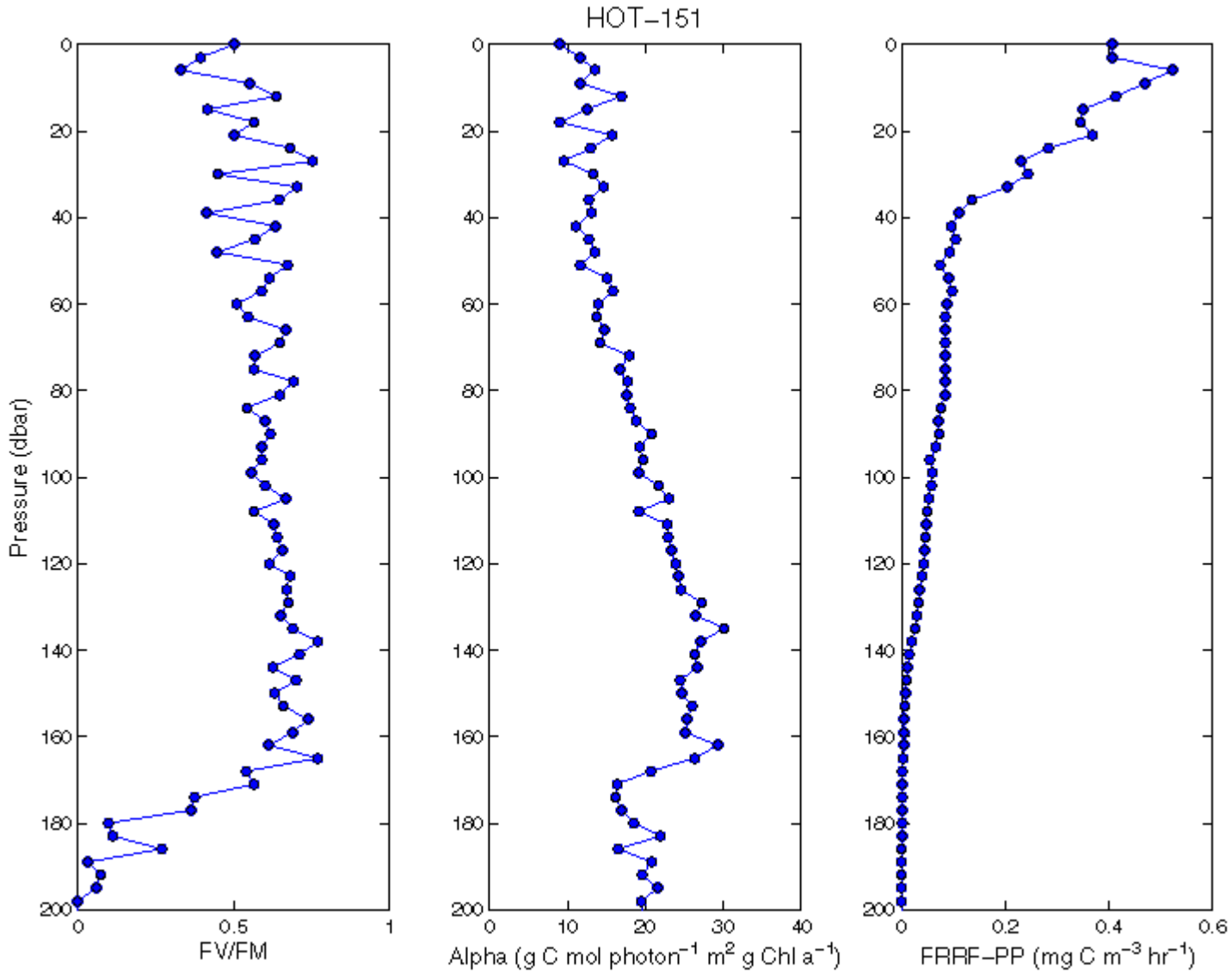

MICO

In-situ fluorometry used to estimate phytoplankton composition & productivity

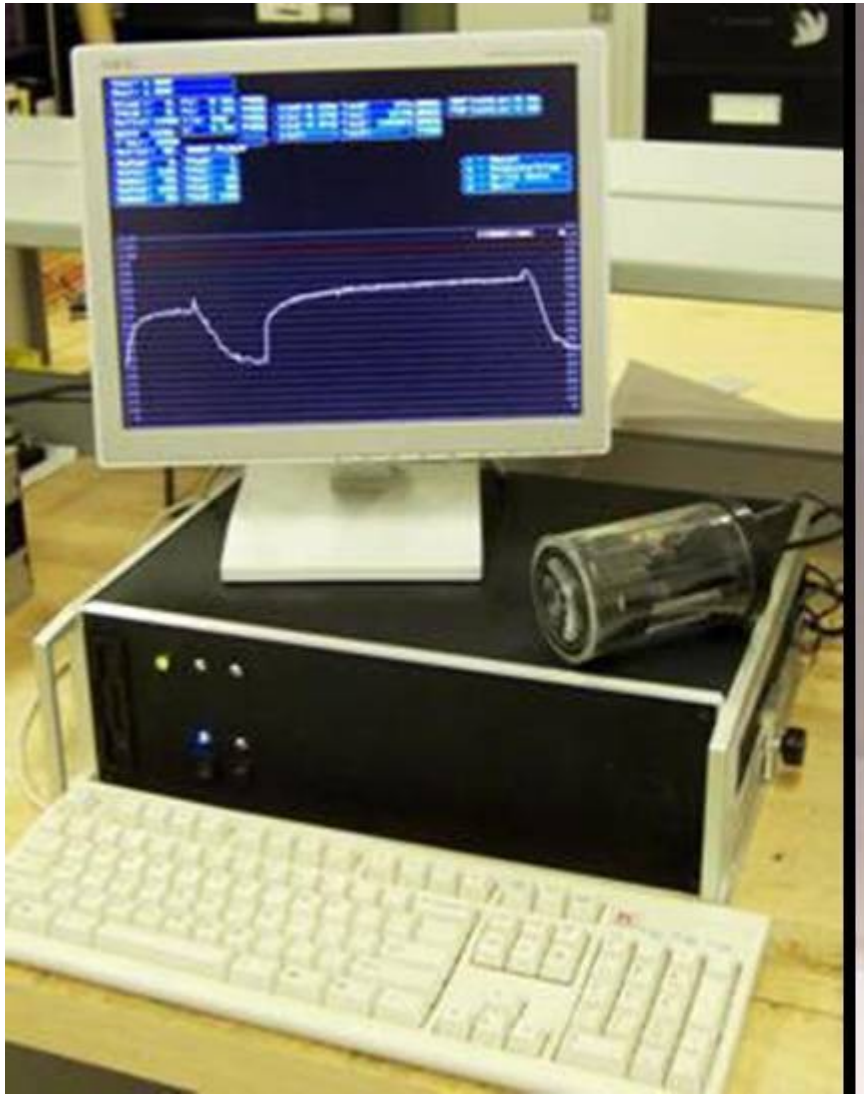
- Some of the instruments we use include FluoroProbe, Fast Repetition Rate Fluorometer, Flow Cytometer and Inverted Microscopy

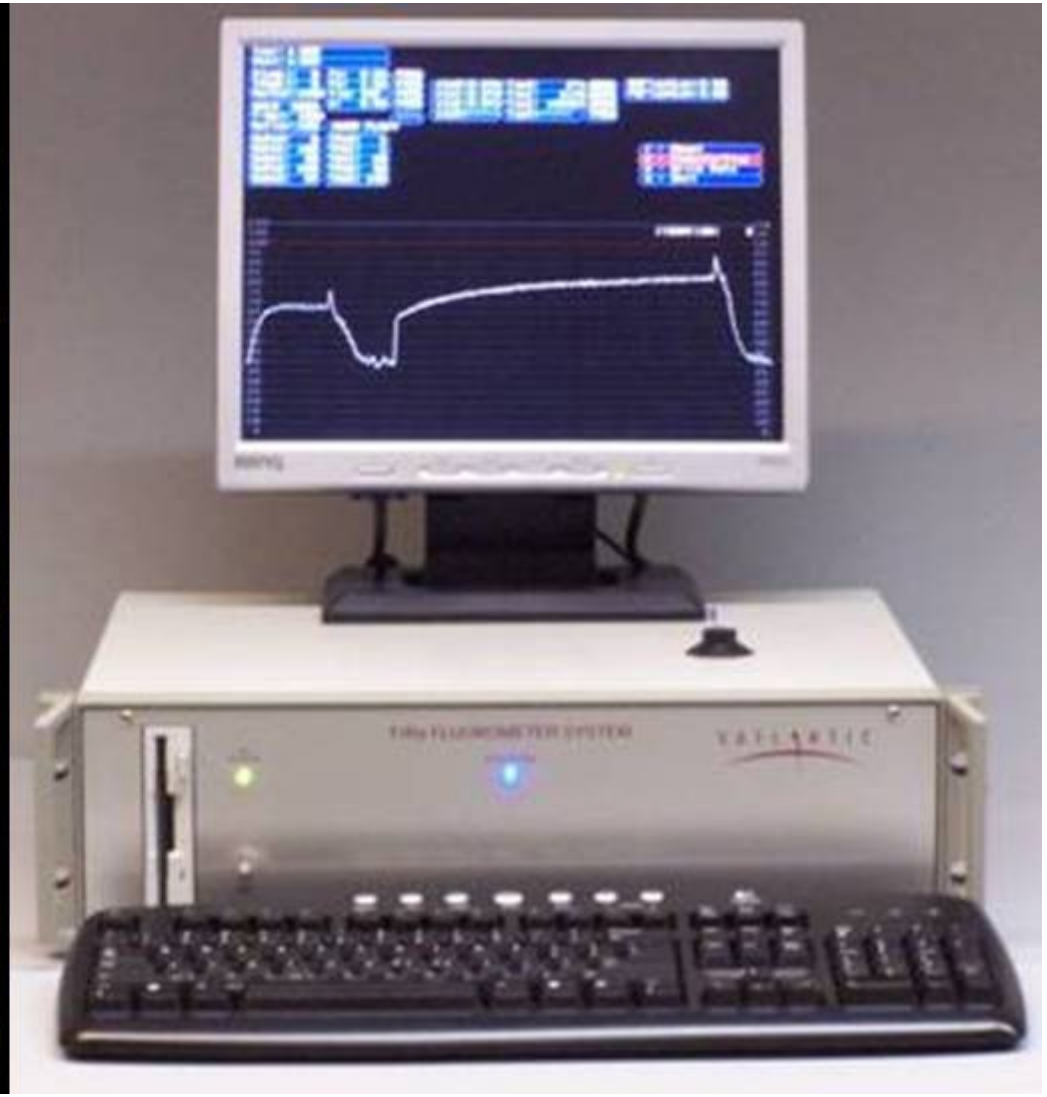


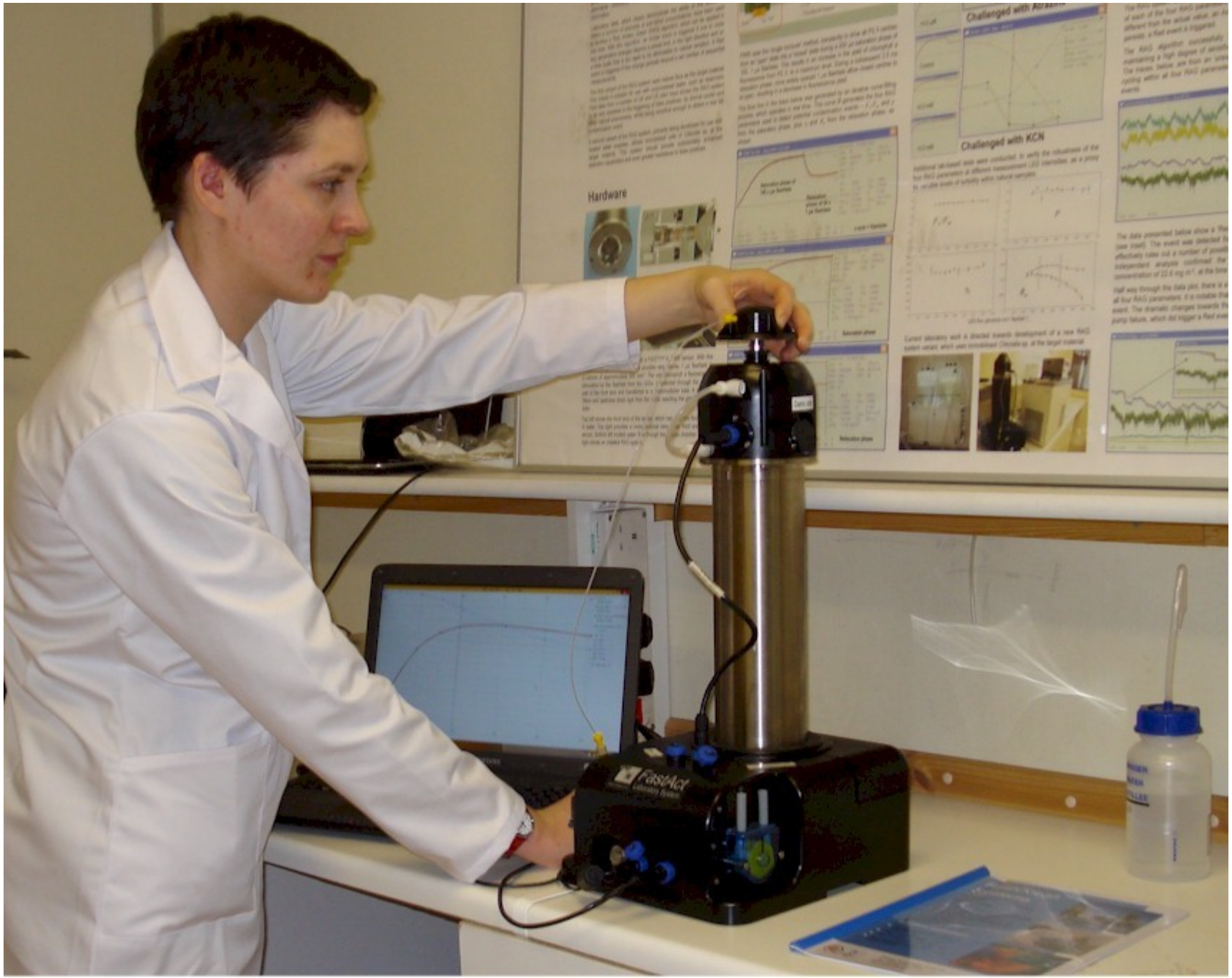


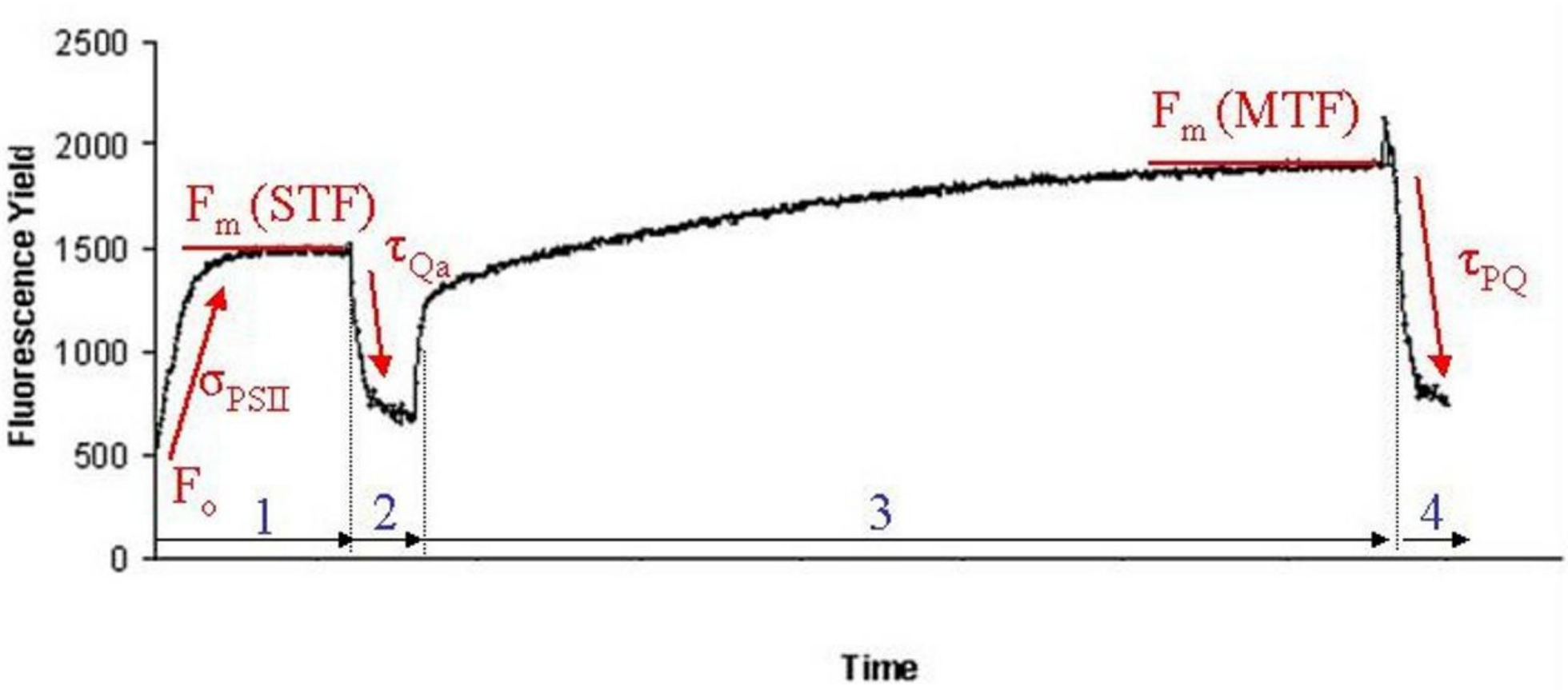

HOT−151
Pressure (dbar)
0
20
40
60
80
100
120
140
160
180
200
0
0.5
1
FV/FM
0
10
20
30
40
Alpha (g C mol photon^{-1} m^2 g Chl a^{-1})
0
0.2
0.4
0.6
FRRF−PP (mg C m^{-3} hr^{-1})

Ritgers School of Biological Sciences



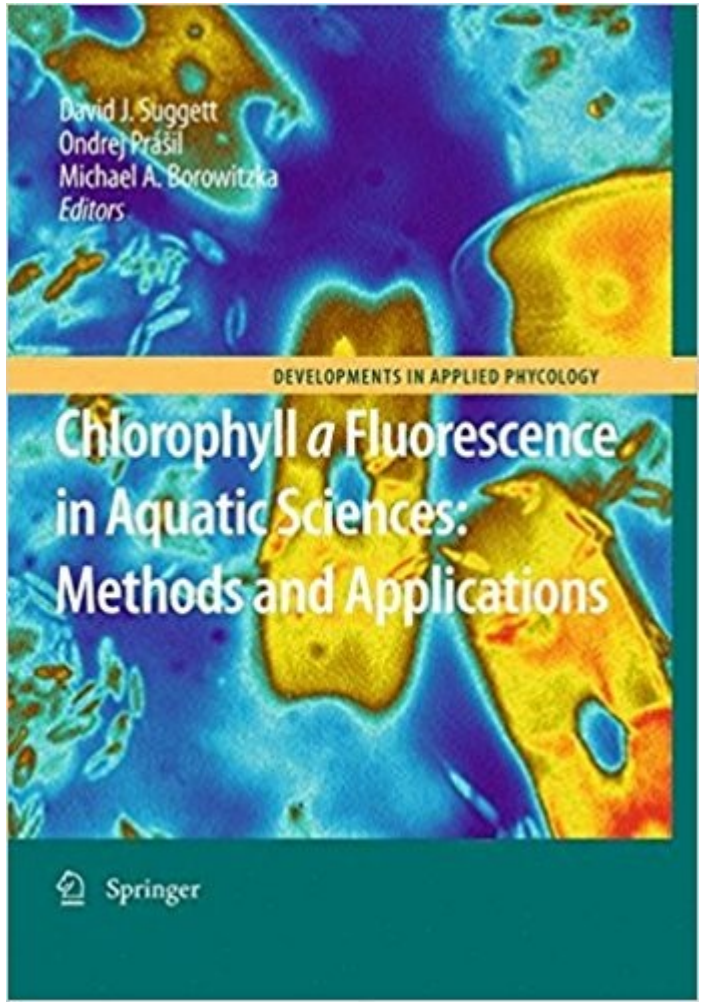


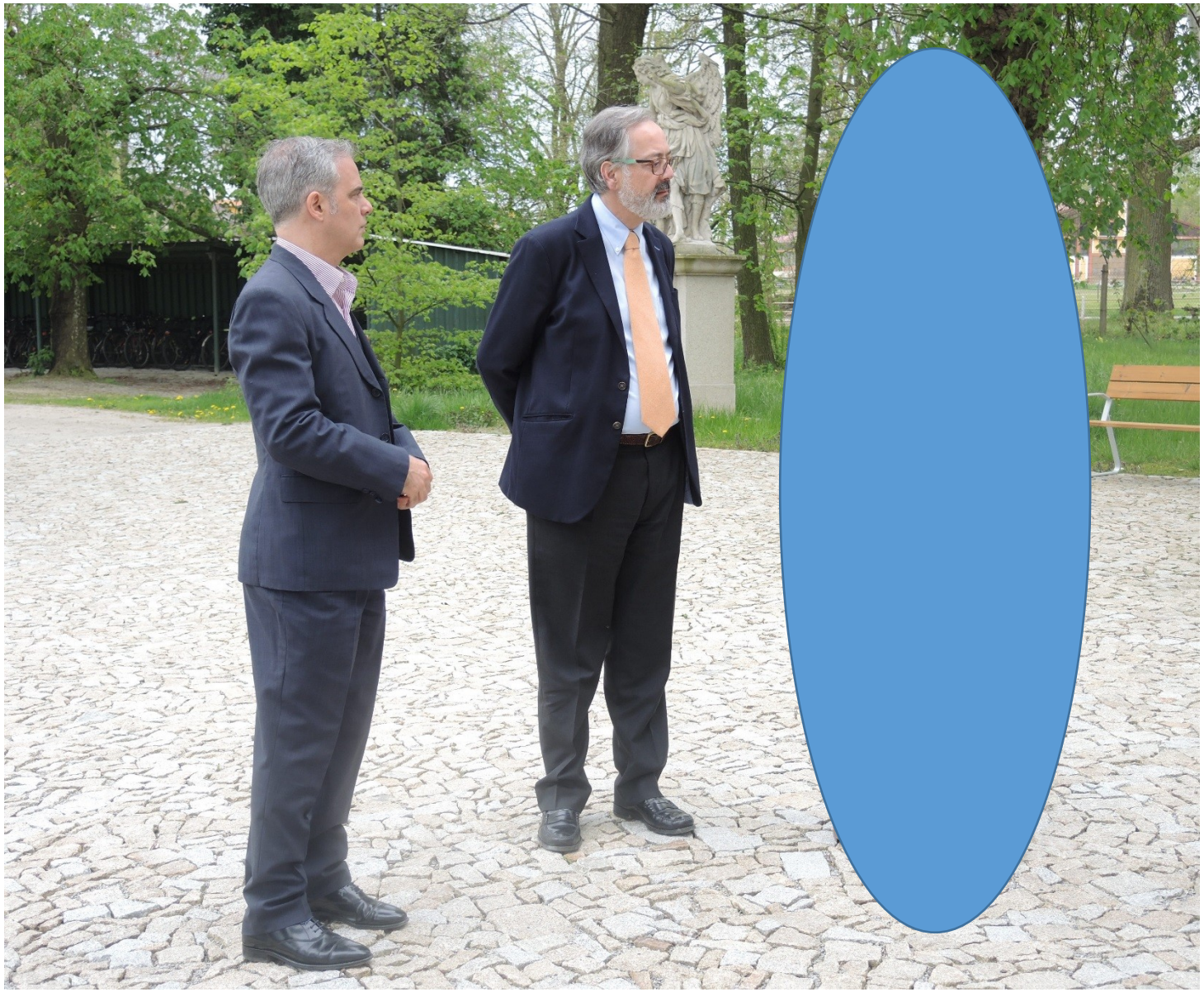

Hardware
Challenged with KCN
FastAct


2500
2000
1500
1000
500
0
Fluorescence Yield
F_m (STF)
τ_{Qa}
F_m (MTF)
τ_{PQ}
σ_{PSII}
$F_\circ$
1
2
3
4
Time

Variable	Definition
PC_{FRR}	Rate of gross photosynthesis (mg C (mg Chl-*a*)$^{-1}$ h^{-1})
E	Photosynthetically active radiation (400–700 nm) (µmol photons m^{-2} s^{-1}) of light (wavelength dependent)
σ_{PSII}	Functional absorption cross section nm^2 quanta^{-1}
σPSII’	Functional absorption cross section nm^2 quanta^{-1} in actinic light
$1 - C$	Fraction of open reaction centres ($C = 1 - qJ$)
PSU	Photosynthetic unit size is the concentration of functional PSII reaction centres (mol Chl-*a* m^{-3}/mol RCII m^{-3})
[RCII]	Number of functional reaction centres (mol RCII m^{-3}) = KRELED×ChlFRRfσPSII
$[Chl]^{FRRf}$	Gain normalised minimum fluorescence signal parameter calculated by *Fast*^{PRO}
K_R	Instrument specific constant (mol photons m^{-3} s^{-1})
$\Phi_{E:C}$	Electron requirement for carbon uptake (molecule CO_2 (mol electrons)$^{-1}$)

Further reading





Ladies and gentleman,

Thank you for your attention