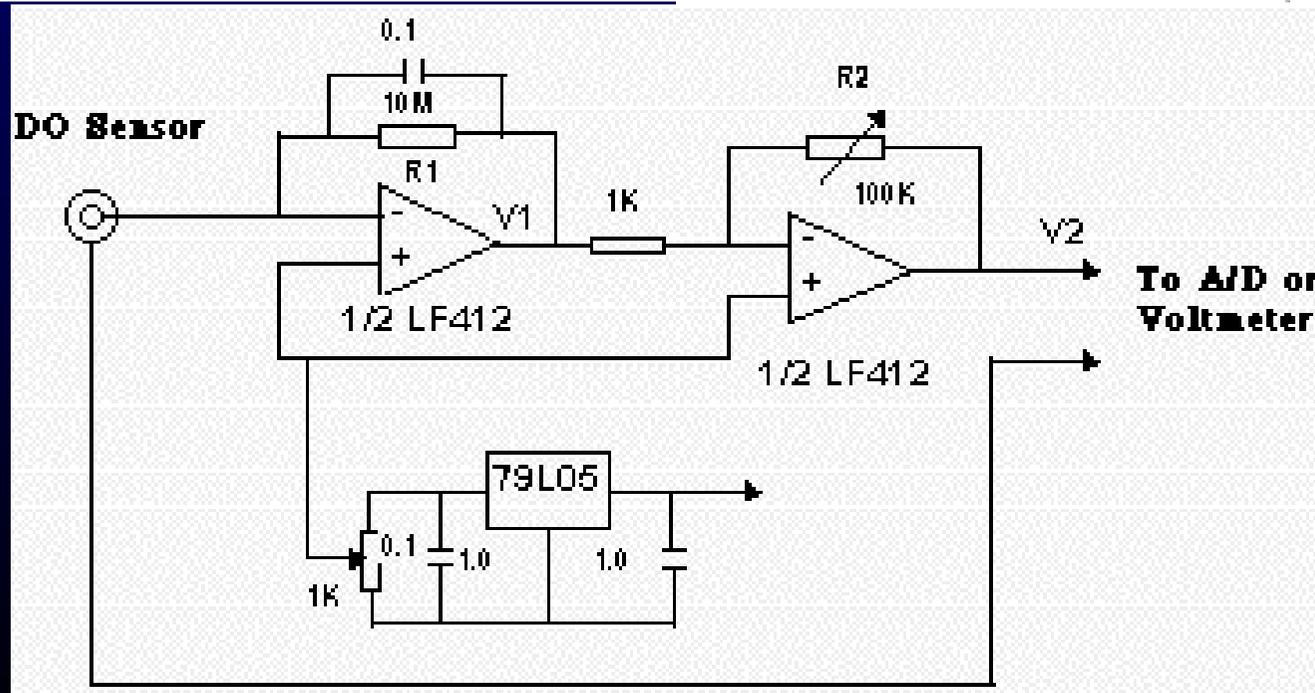
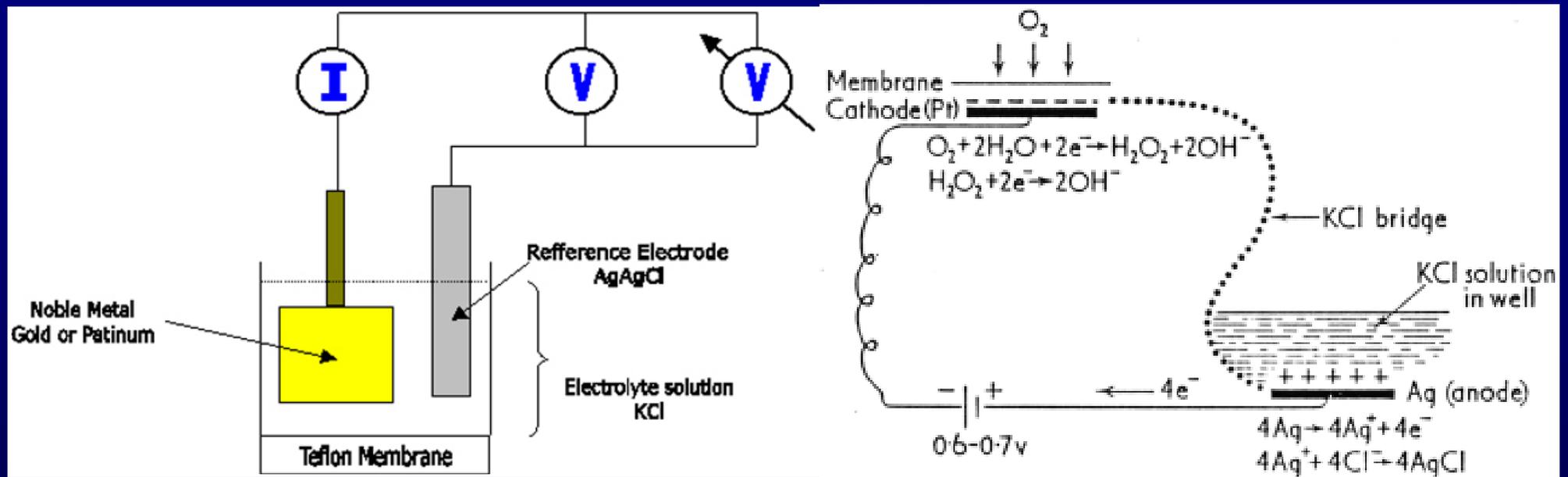


Biophysical & physicochemical methods
for analyzing plants *in vivo* and *in situ*:

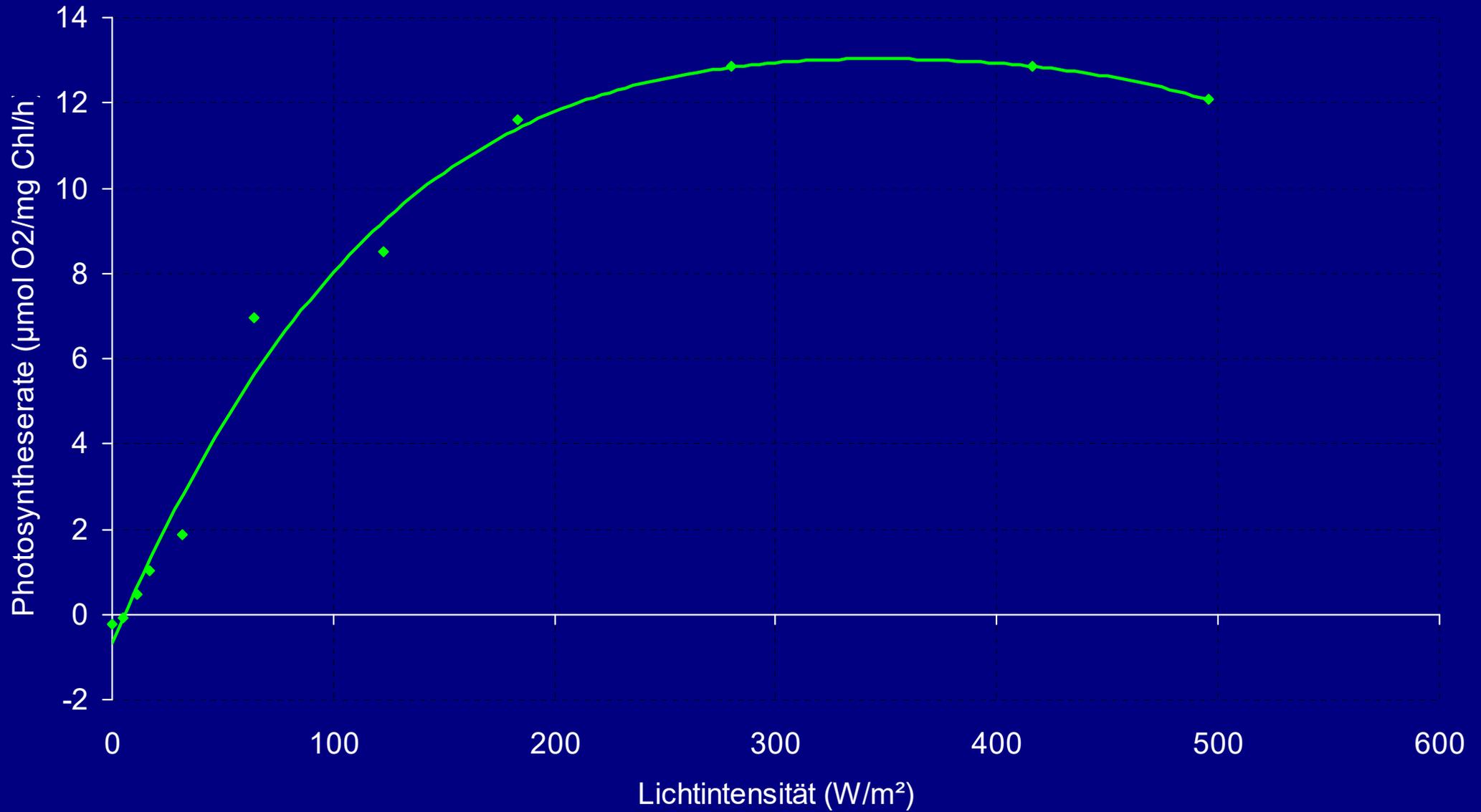
Methods for Analysis of Photosynthesis - a few important examples

Measurement of photosynthesis activity

Polarographic measurement of O₂ exchange

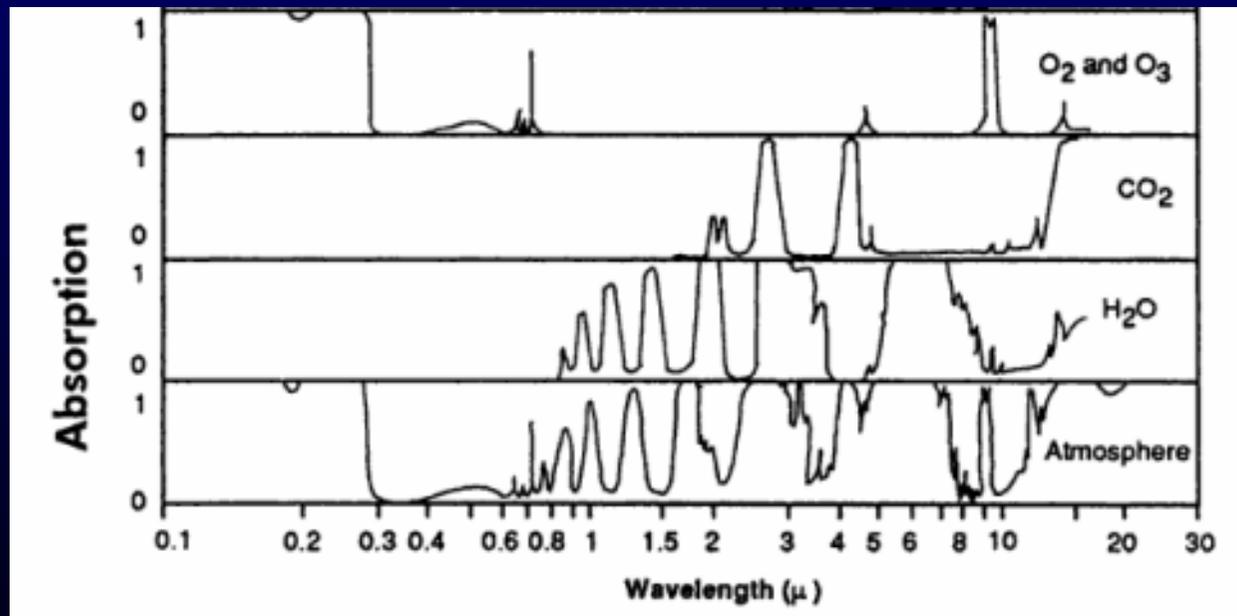
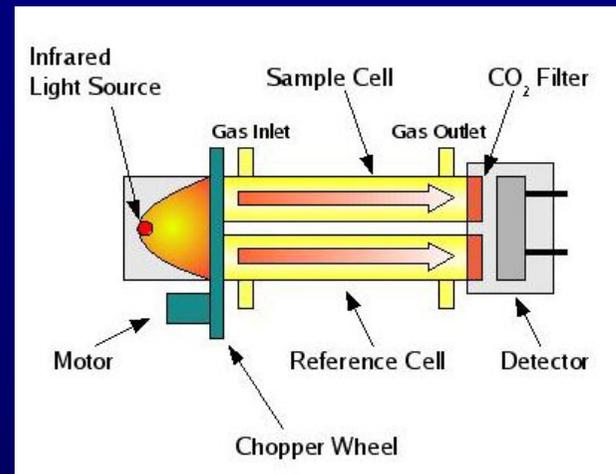
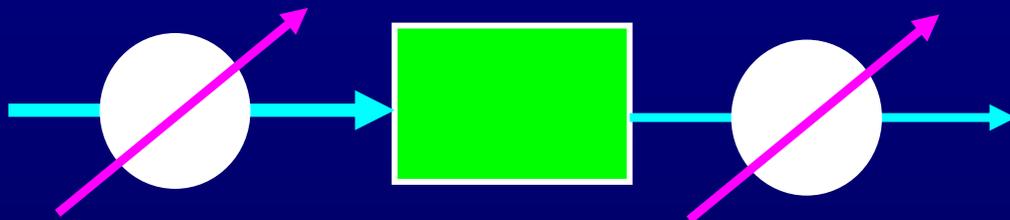


Lichtsättigungskurve der Photosynthese

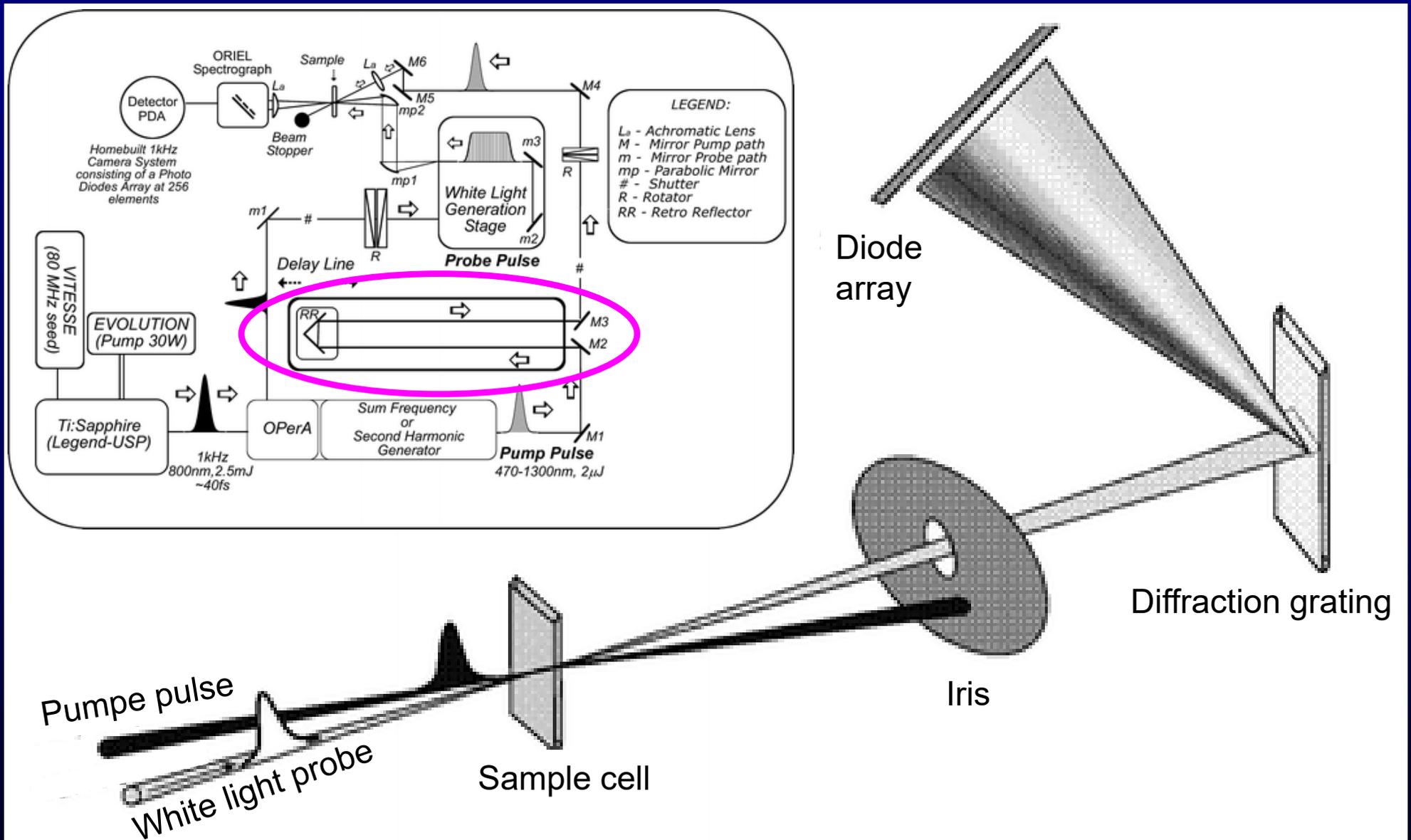


Measurement of photosynthesis activity

IR-Measurement of CO₂ assimilation

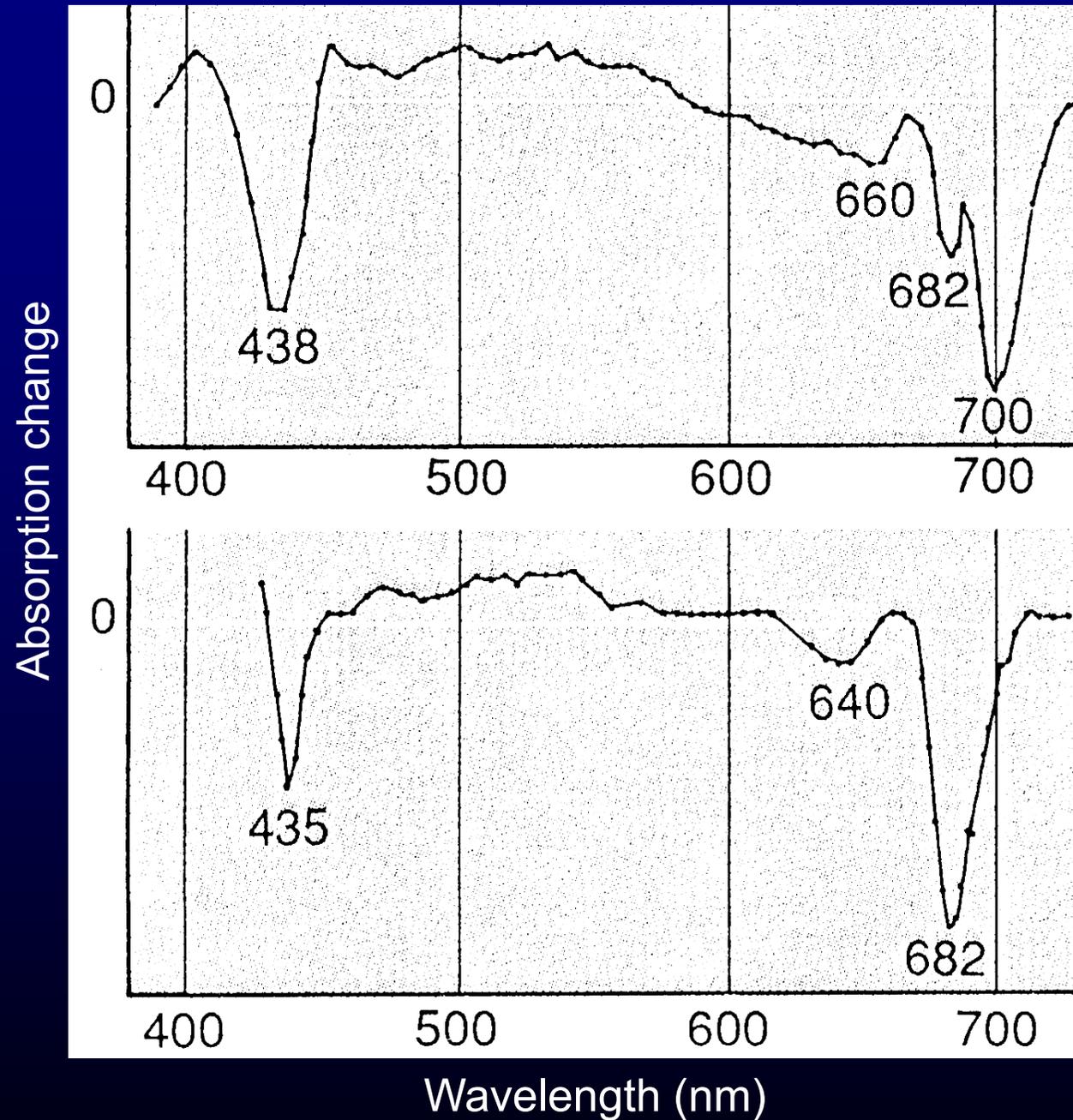


Measurement of fast events, e.g. primary charge separation



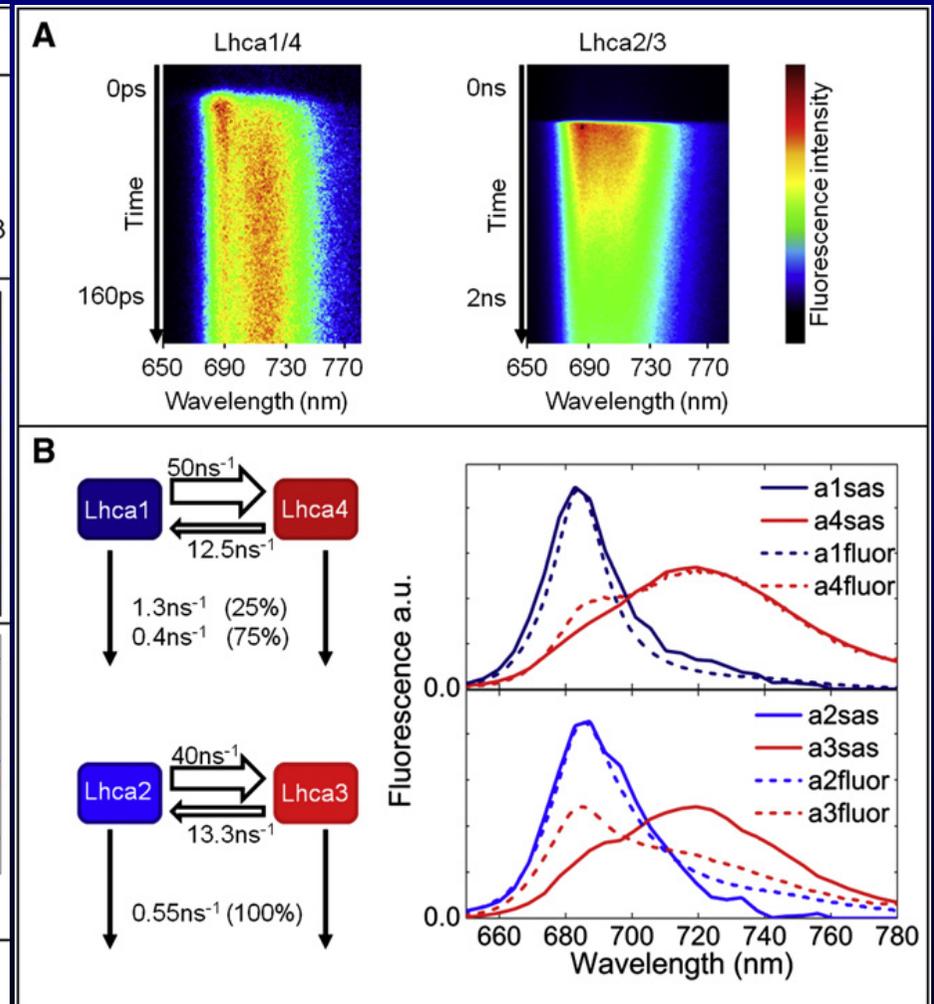
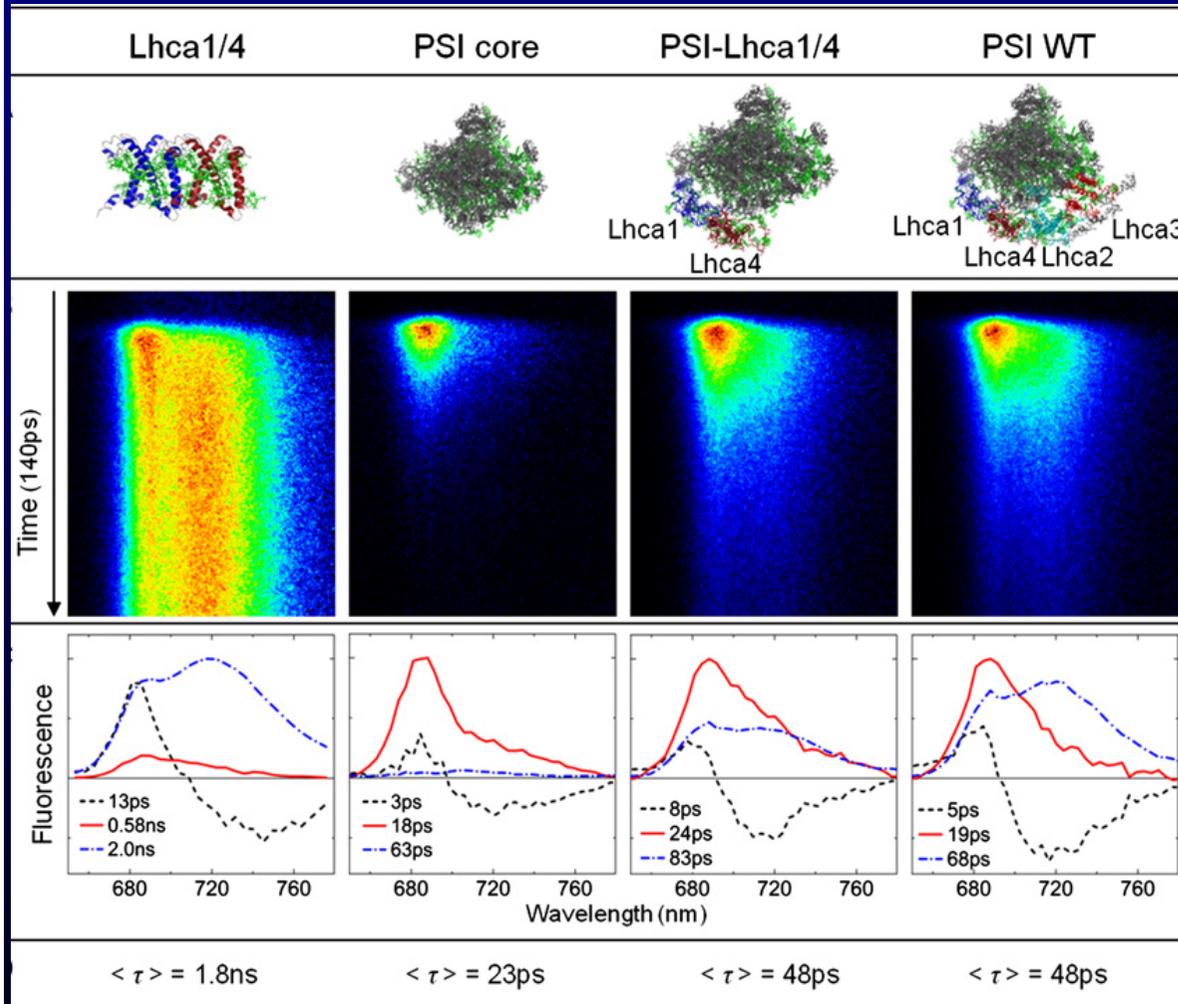
From: Berera R_vanGrondelle R_Kennis JTM_Ultrafast transient absorption spectroscopy_PhotosynthRes101_105-118

Measurement of primary charge separation



Ultrafast UV/VIS spectroscopy

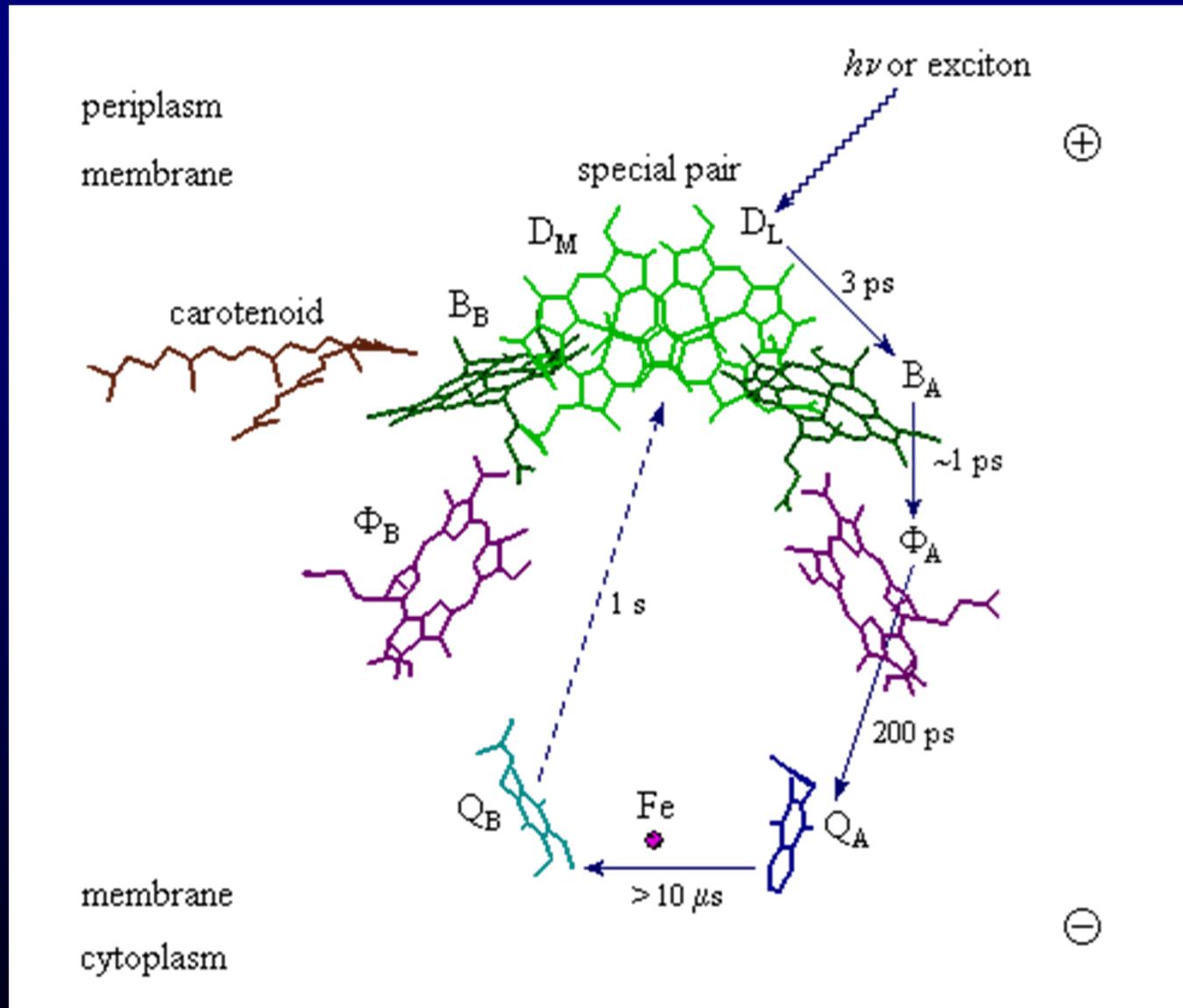
Excitation transfer times between light harvesting complexes



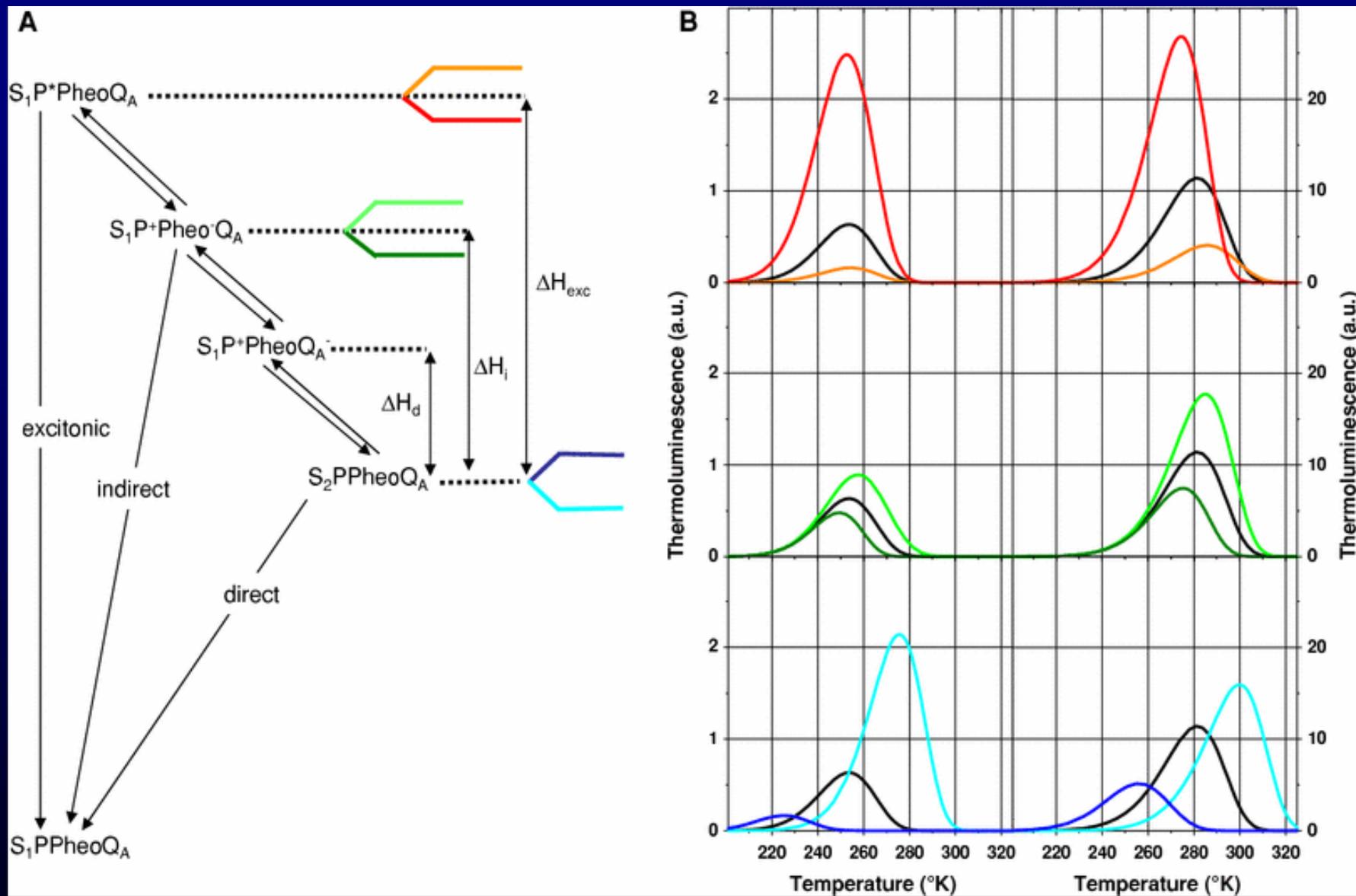
From: Wientjes E_et al (2011) BiophysJ101, 745-54

From: Wientjes E_et al (2011) BiophysJ 100, 1372-80

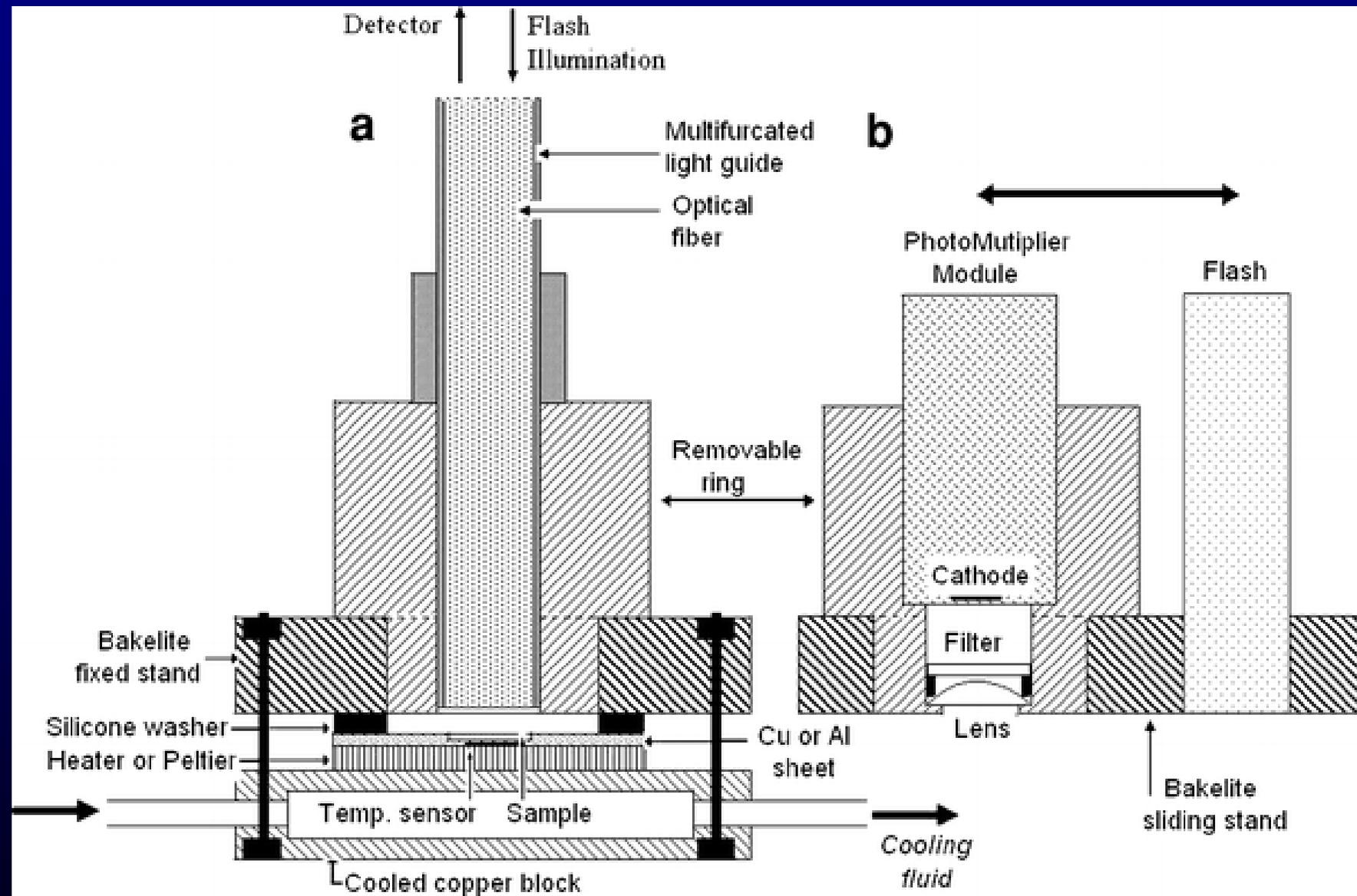
Photosystem II reaction centre: steps of electron transfer → to be analysed with thermoluminescence measurements



Thermoluminescence measurement



Thermoluminescence measurement

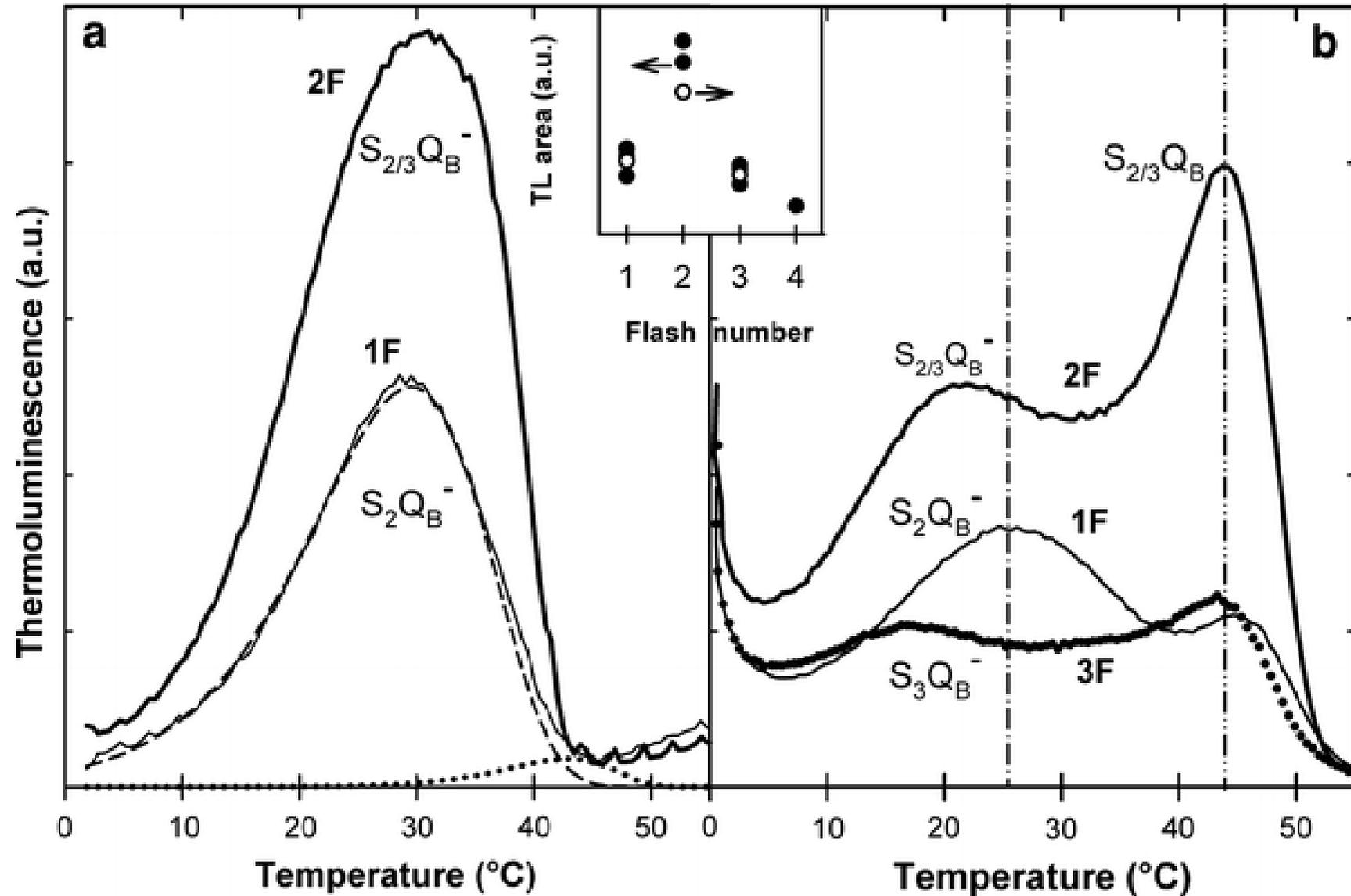


Thermoluminescence measurement: parameters

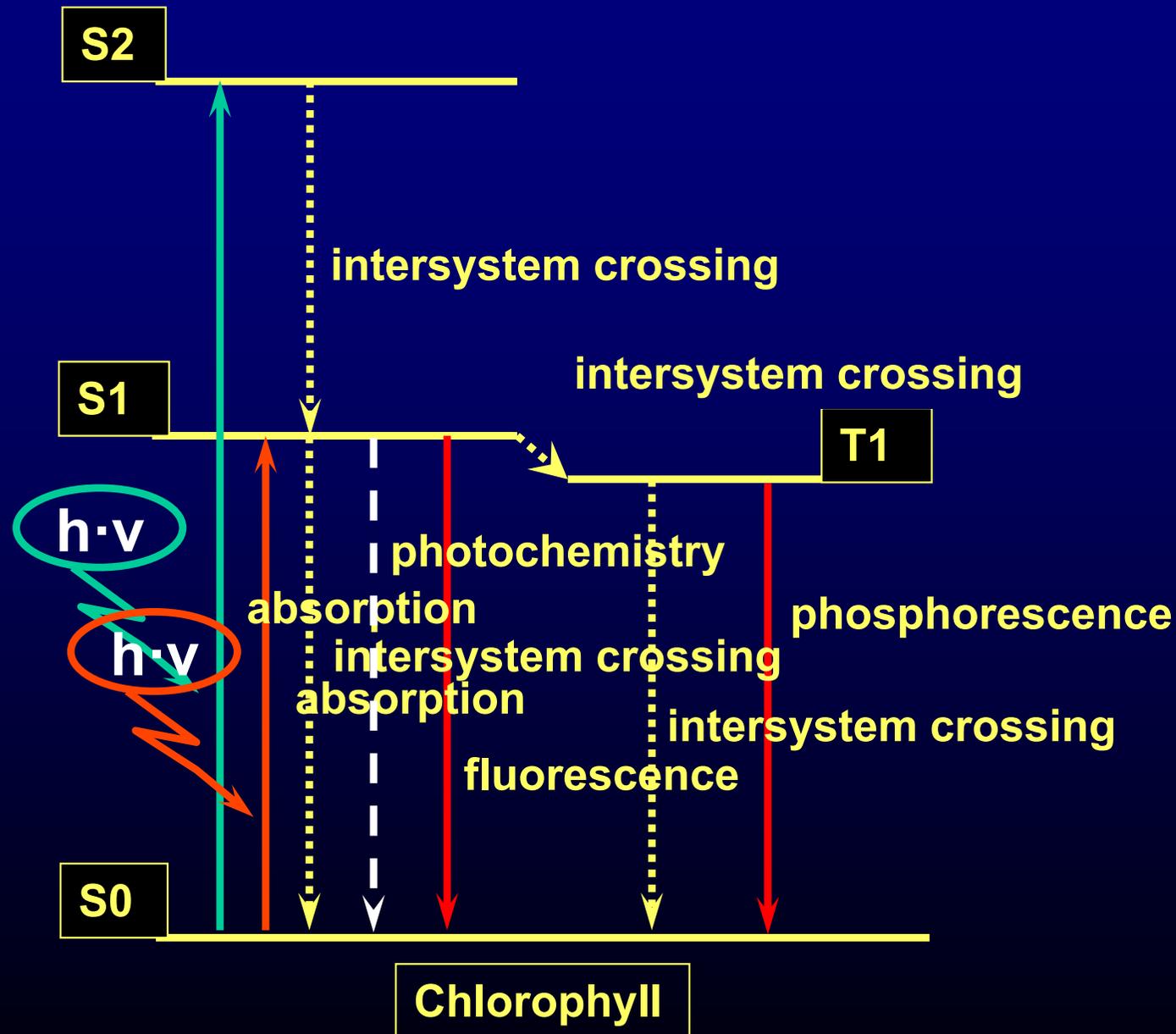
Name	T_M range	Origin	Origin PS II	Comments
Z	-160°C	Pigments	-	Low temperature pigment photochemistry
Z _V	-70 to -100°C	(P ₆₈₀ ⁺ Q _A ⁻ ?)	+	T _m depends on illumination temperature
A _T	-10 to -20°C	TyrZ ⁺ Q _A ⁻	+	Damage to Mn oxygen-evolving complex (TyrZ is the functional donor to PS II center)
A	~-15°C	S ₃ Q _A ⁻ ?	+	
Q	+2 to 10°C	S ₂ Q _A ⁻	+	Damage to secondary Q _B quinonic acceptor or inhibition by DCMU-like herbicides
B	30 to 38°C	S _{2/3} Q _B ⁻	+	Lumen pH > 7
B ₂	28 to 32°C	S ₂ Q _B ⁻	+	Lumen pH < 7
B ₁	20 to 30°C	S ₃ Q _B ⁻	+	Lumen pH < 7
AG	+45°C (→ +35°C)	S ₂ /S ₃ Q _B + e ⁻	(+)	Electron from stroma, in intact chloroplasts or cells
C	+52/55°C	TyrD ⁺ Q _A ⁻	+	Minor band, increased by DCMU or damage (TyrD is the non functional donor to PS II center)
HTL1	60 to 85°C	?	-	Different bands of unknown origin, without illumination
HTL2	120 to 140°C	Lipid peroxides	-	Thermolysis: -C-O-O- → *C=O + Chl → *Chl

T_M values are given for data obtained with a 0.5°C/s TL heating rate

Thermoluminescence measurement: example



The basis for measurement of photosynthesis via fluorescence kinetics: competition for the S1 excited state



Measurement of *in vivo* chlorophyll fluorescence kinetics

Why?

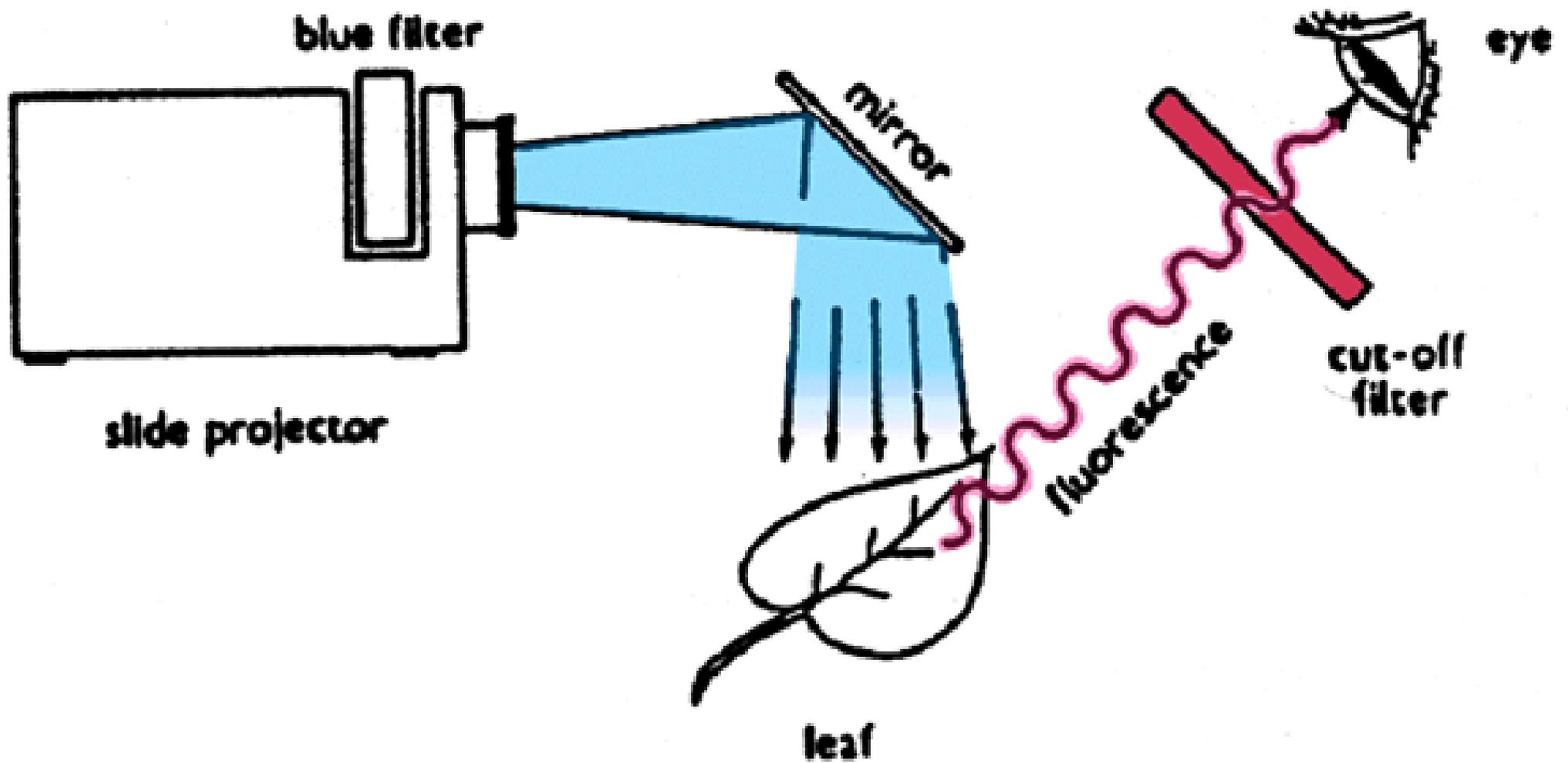
The quantum yield of *in vivo* chlorophyll fluorescence depends on a competition for excitons between photochemistry (including electron transport after PSII via feedback), thermal relaxation ("nonphotochemical quenching") and fluorescence.

--> The fluorescence quantum yield and especially its change in response to changes in actinic irradiance allows a detailed assessment of photosystem II function and thus the vitality of a cell, tissue, plant or even ecosystem.

Examples of Applications

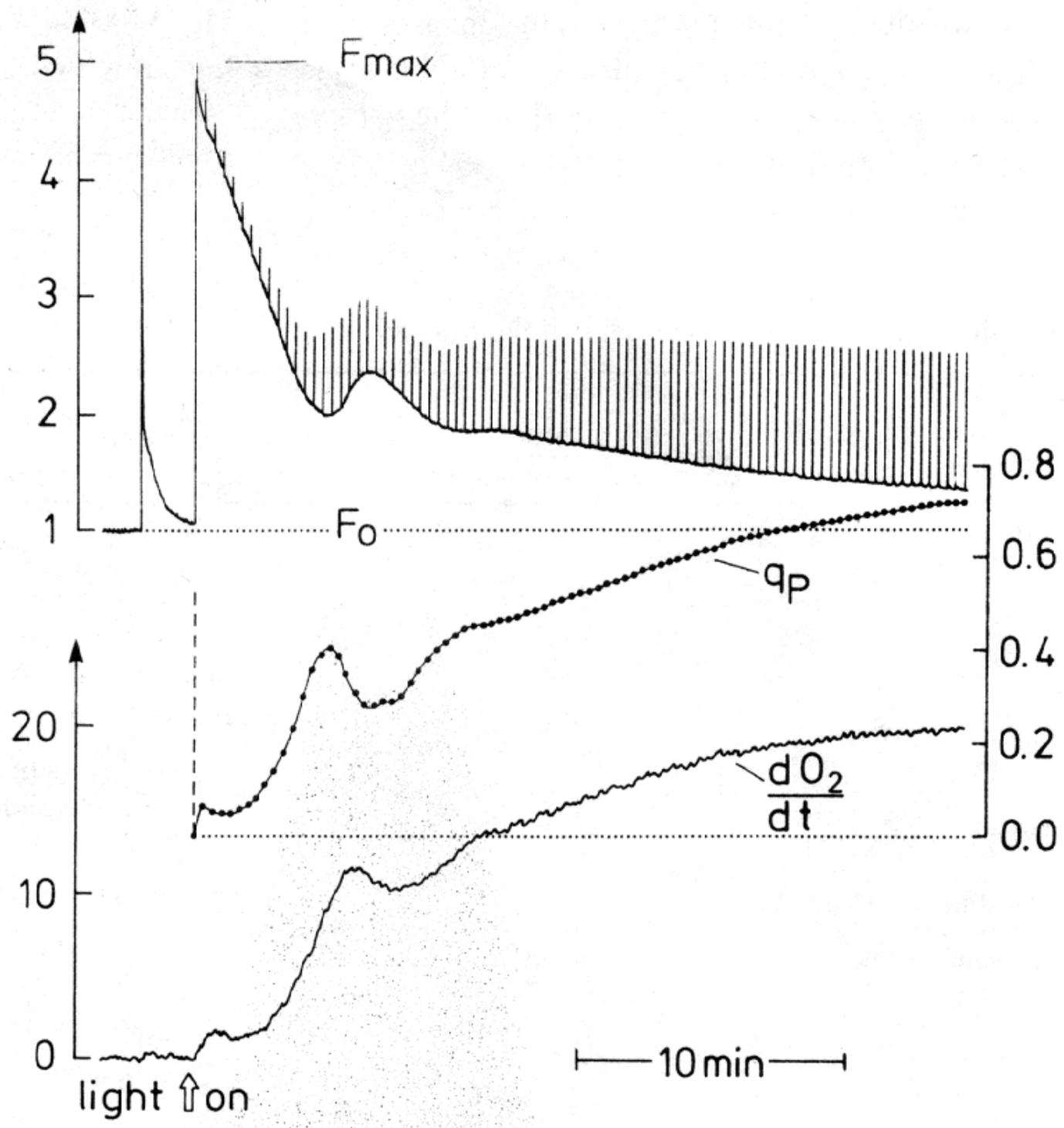
- biophysical investigations of mechanisms of photosynthesis
- studies of the effects of abiotic and biotic stress on plants
- ecophysiological studies
- fruit quality assessment

Chlorophyll-Fluoreszenz



Fluorescence yield, rel. units

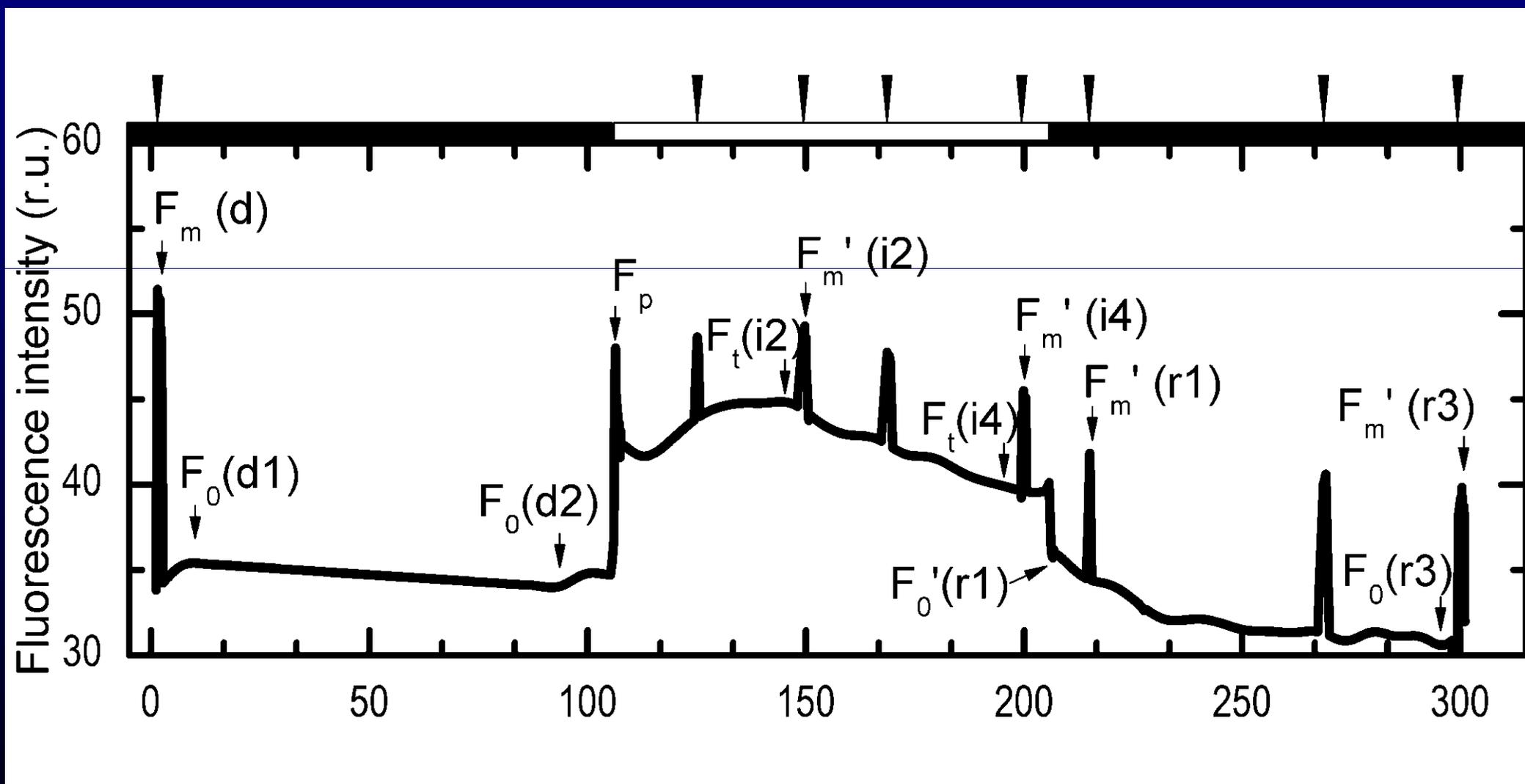
O_2 -evolution rate, $\mu\text{mol m}^{-2}\text{s}^{-1}$

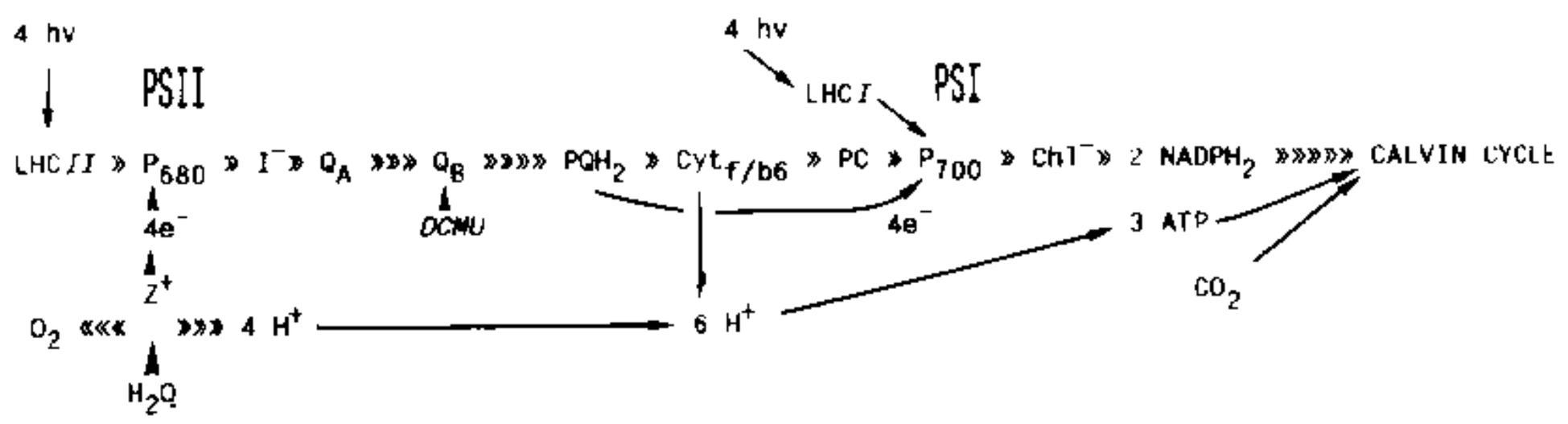
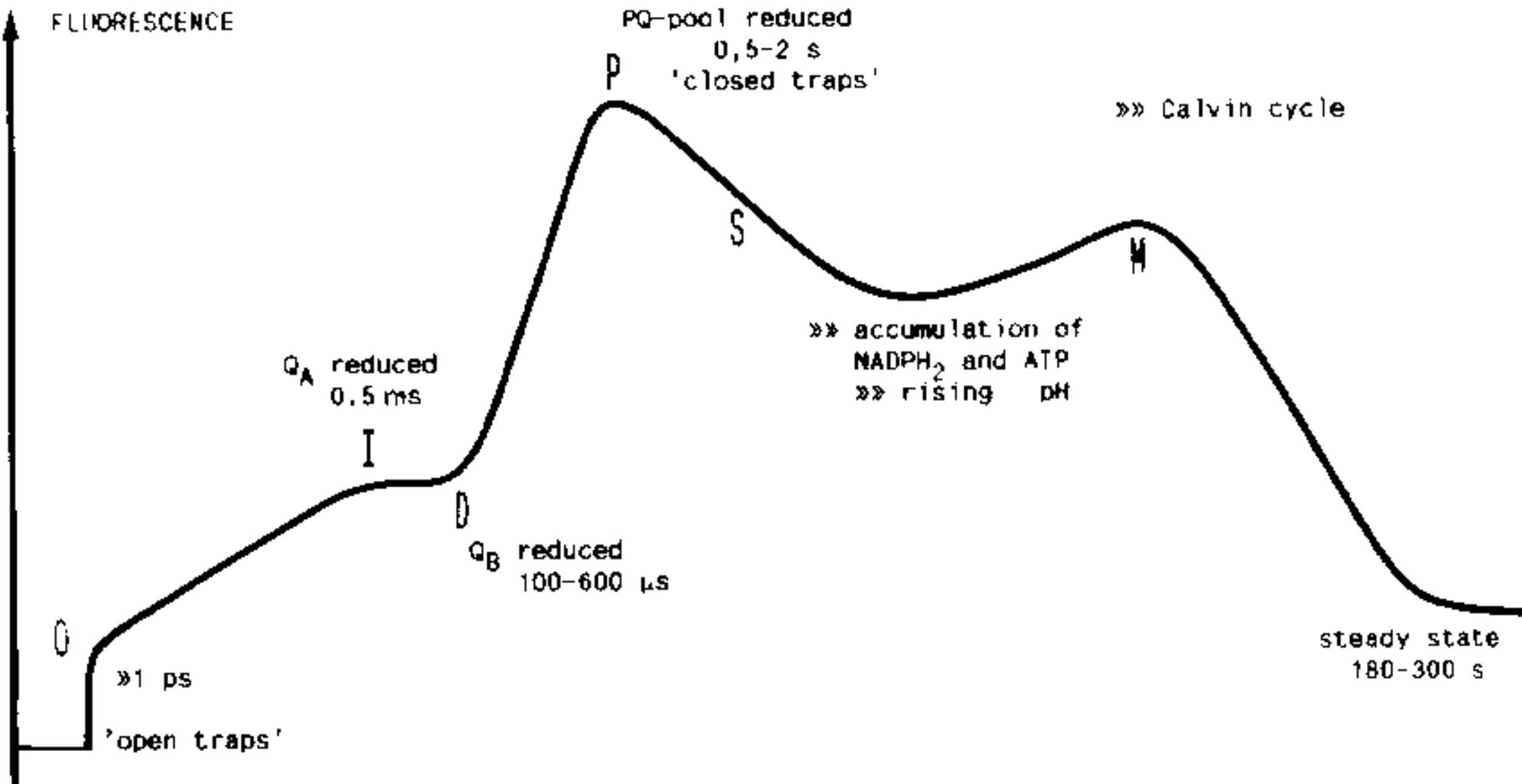


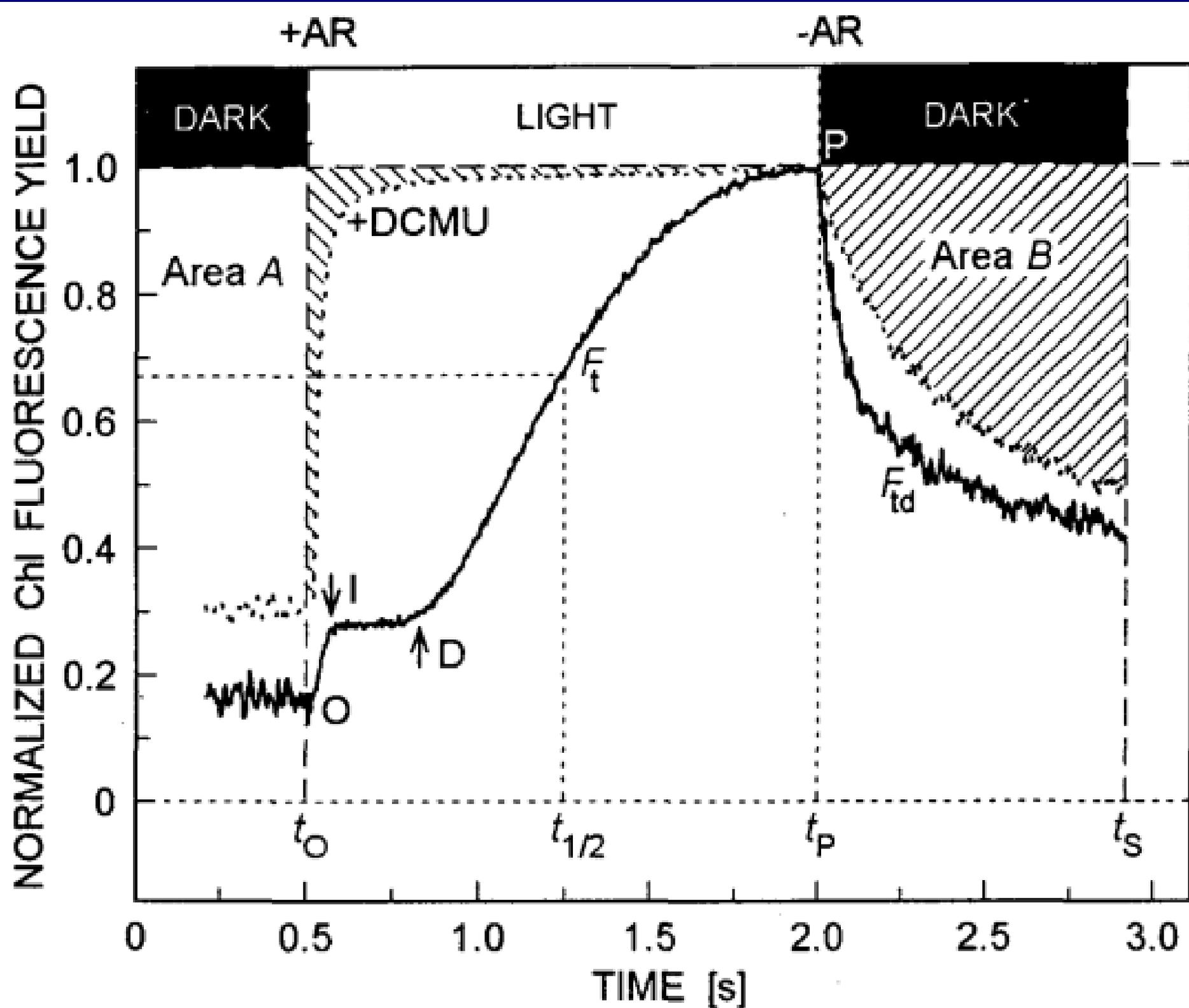
Quenching coefficient, q_p

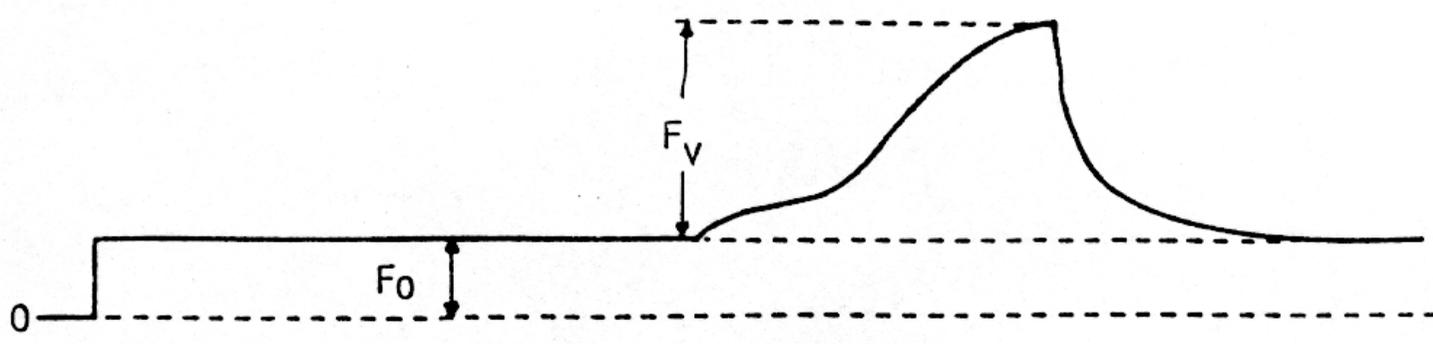
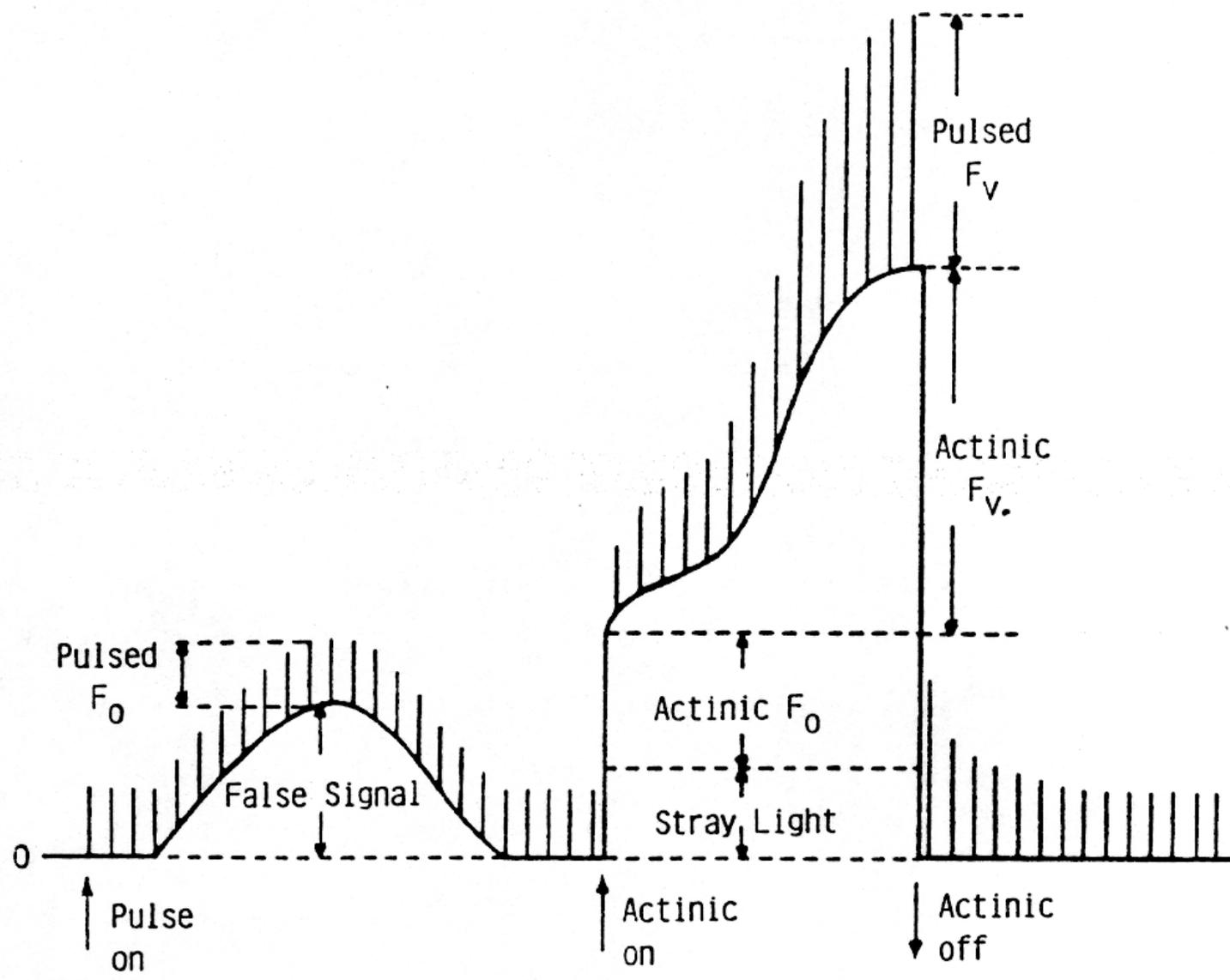
10 min

Wichtigste Symbole in der Chlorophyll-Fluoreszenzmessung



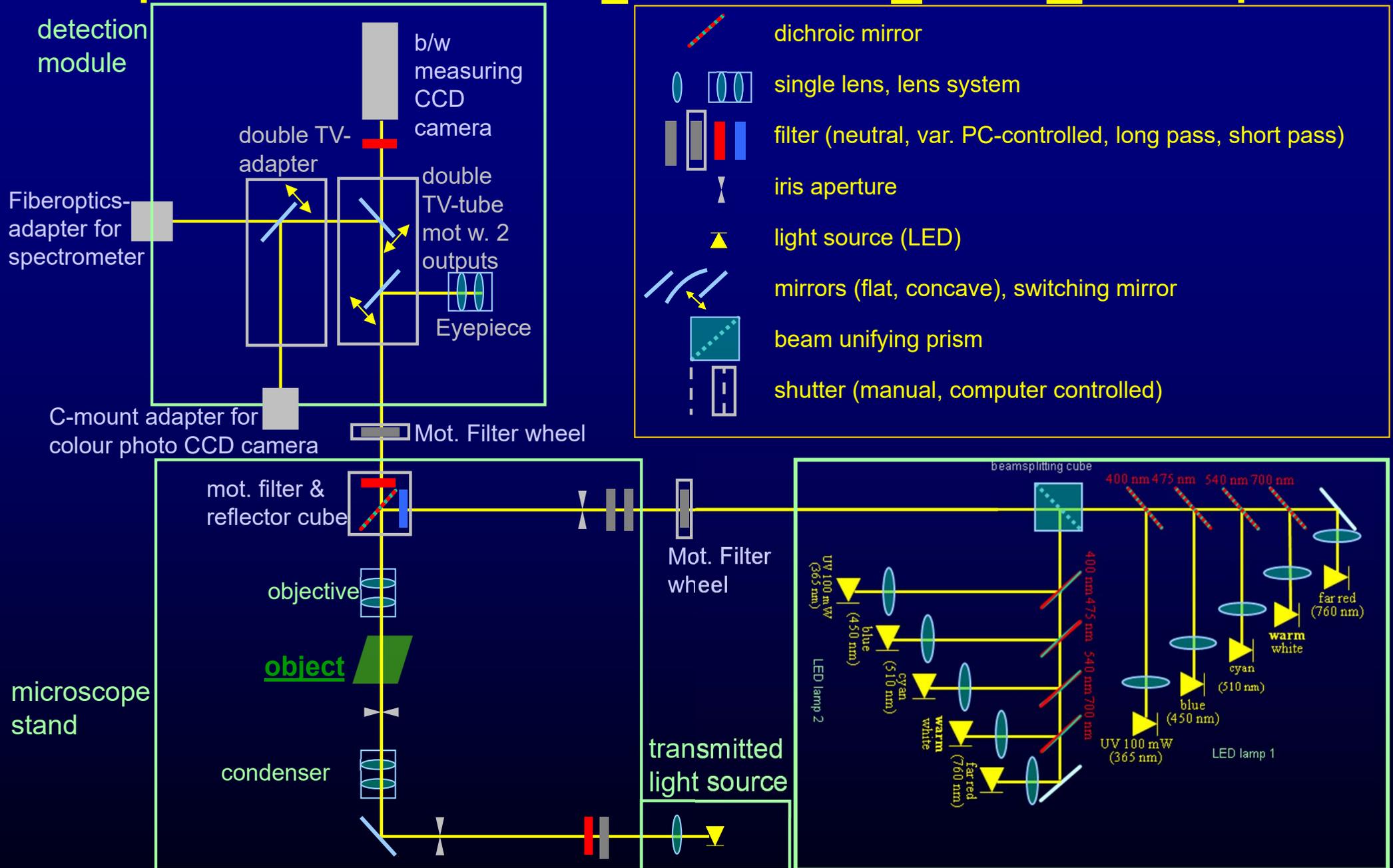








Biophysical measurements *in vivo* with temporal, spatial and spectral resolution: the Fluorescence Kinetic Microscope



Küpper H, Aravind P, Leitenmaier B, Trtilek M, Šetlík I (2007) *New Phytol* 175, 655-74

Küpper H, Šetlík I, Trtilek M, Nedbal L (2000) *Photosynthetica* 38(4), 553-570

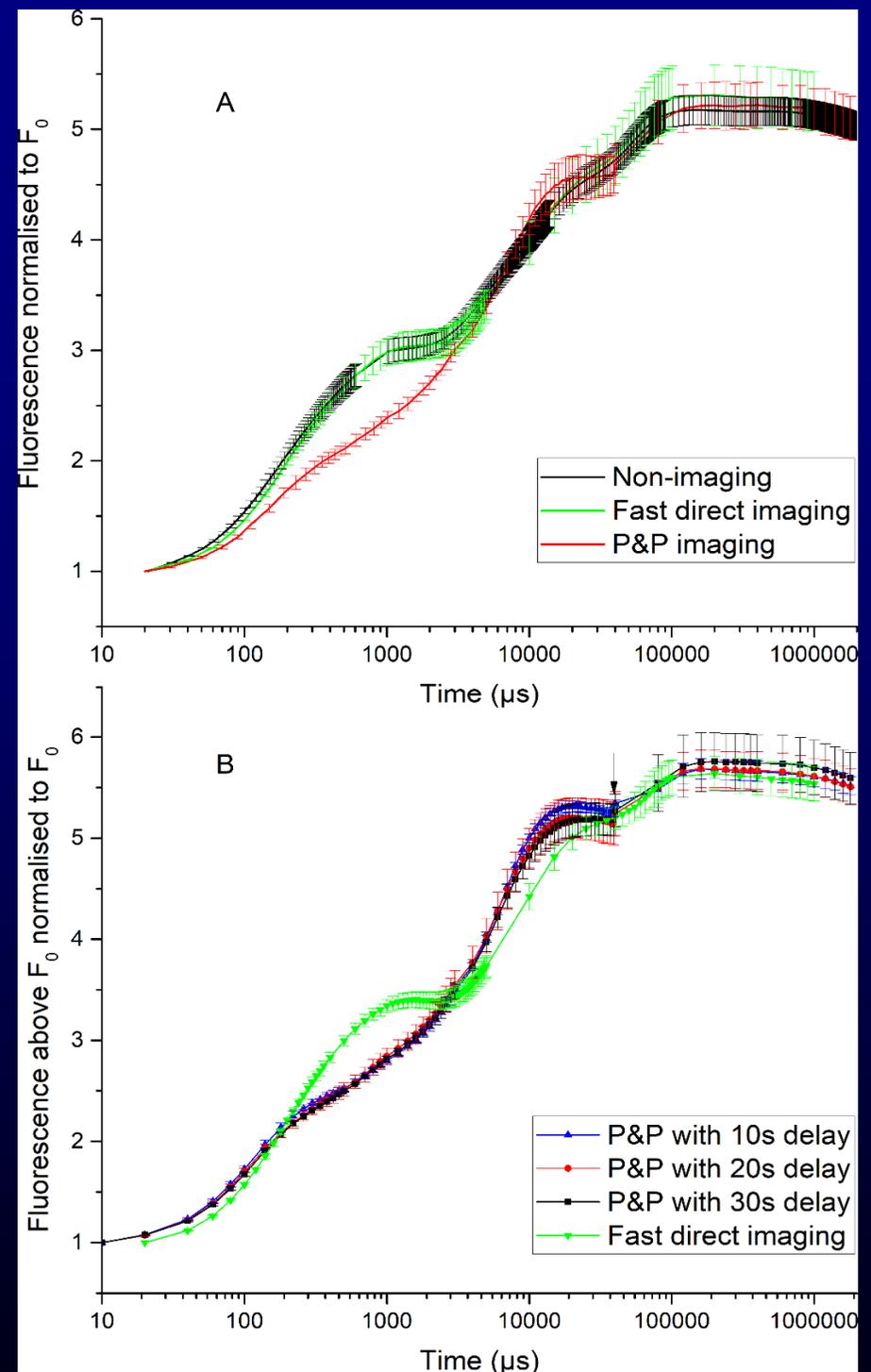
excitation module

Analysis of OJIP chlorophyll fluorescence kinetics & QA re-oxidation kinetics by direct fast imaging

Comparison of different OJIP measuring methods using *Arabidopsis thaliana* leaves

A) Comparison of non-imaging direct measurement, imaging measurement with pump-and-probe (P&P), and fast direct imaging. The values represent the average \pm SD of five independent measurements (leaves of similar age on different plants).

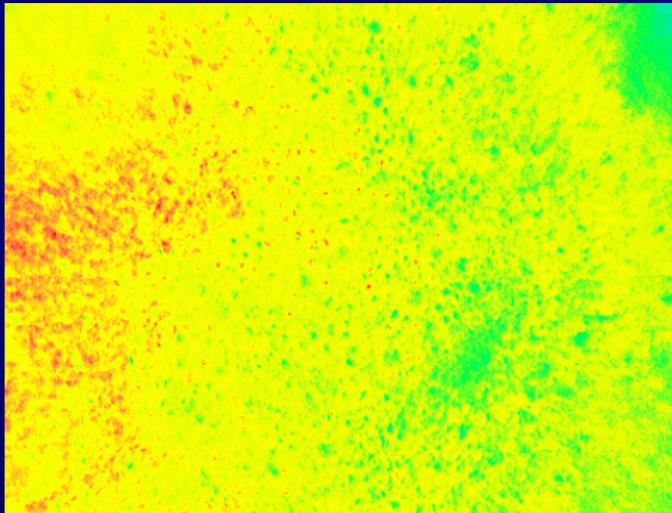
B) Comparison of different delay times for the P&P sequences with fast direct imaging. The values represent the average \pm SD of four independent measurements (leaves of similar age on different plants).



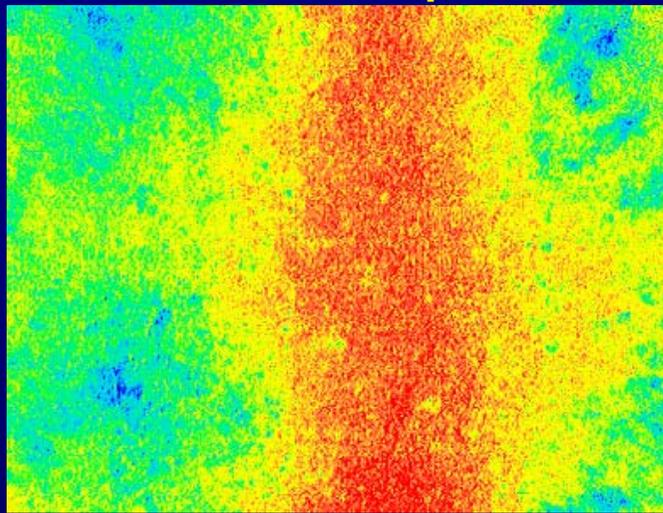
Fluorescence kinetic microscopy

Methods of data processing

Method 1: images of fluorescence parameters



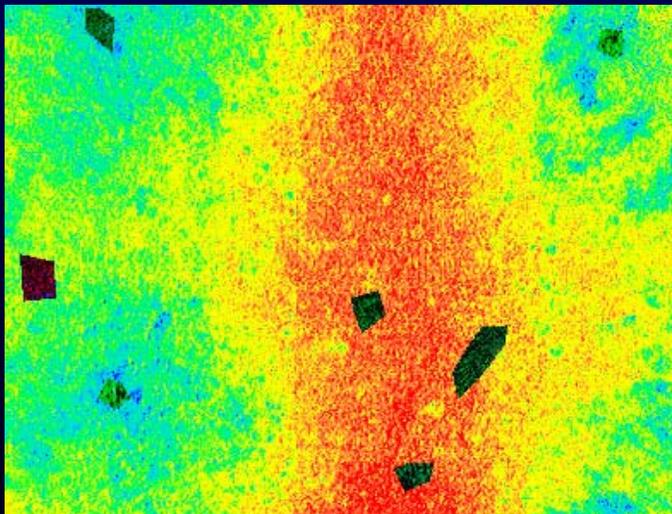
False colour image of F_m Chl fluorescence calculated from fluorescence kinetic film



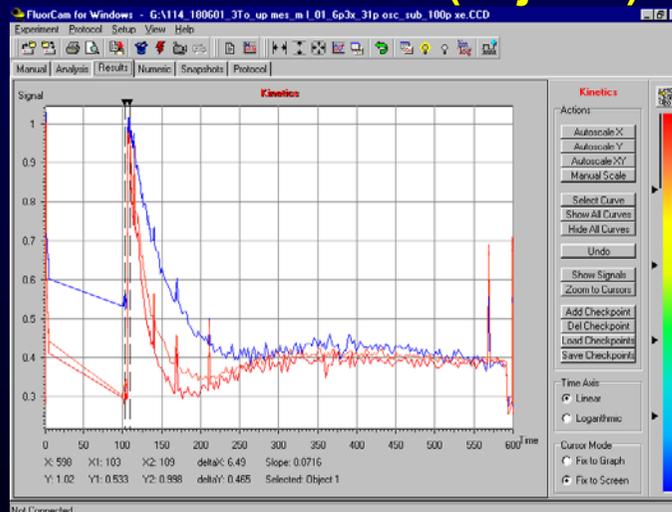
False colour map of F_v/F_m , showing the **differences in this parameter over the entire image.**

To obtain images of fluorescence parameters, frames within the relevant time periods are selected and the necessary mathematical operations are performed on every pixel.

Method 2: kinetics of selected areas (objects)



Manual selection of objects for kinetic analysis.

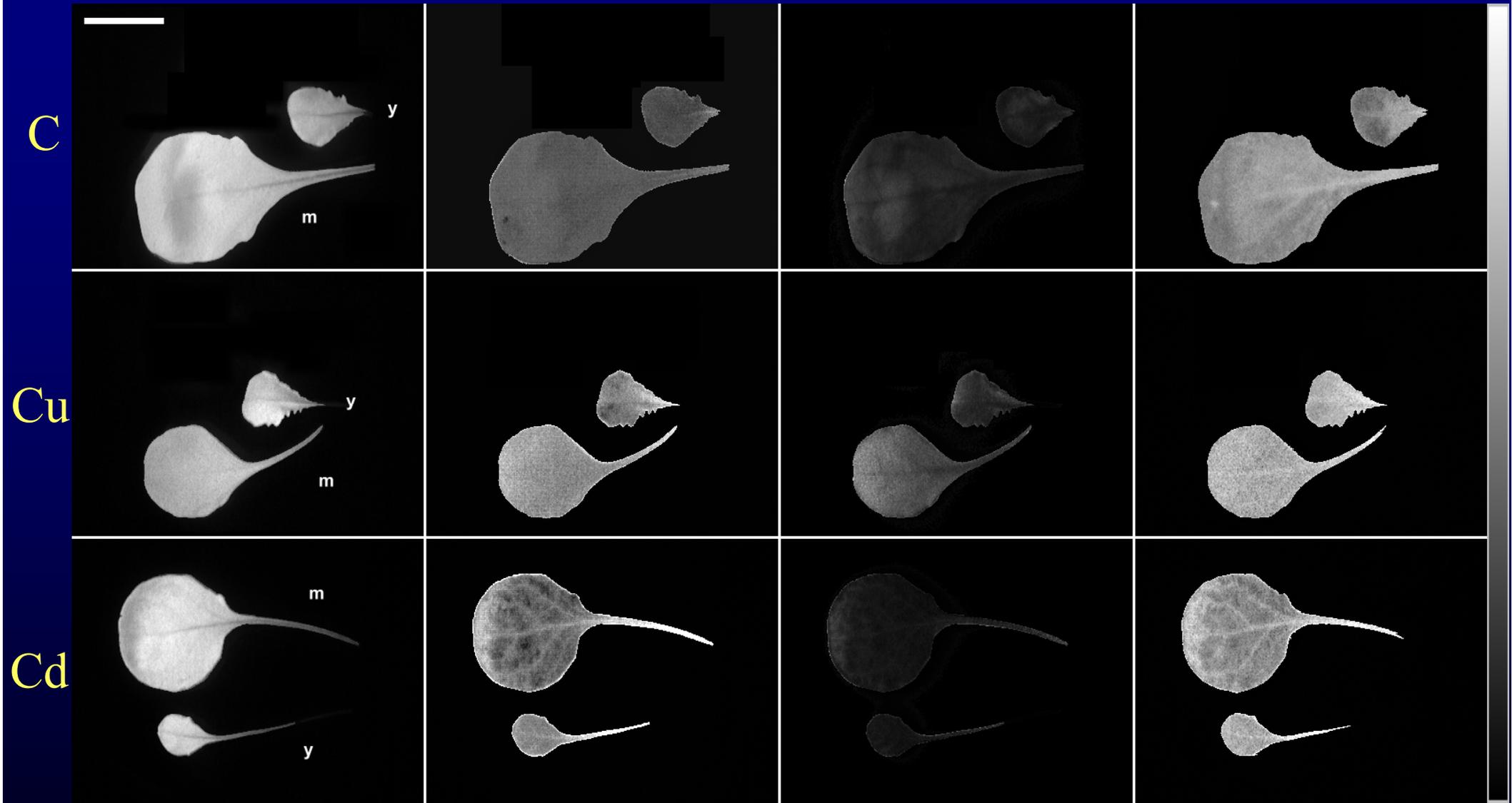


Fluorescence induction of selected objects, showing **all differences in kinetics for representative cells.**

To obtain kinetic traces, the relevant regions are selected on a captured frame or parameter image. The kinetics of all pixels within the selected areas are averaged.

Cd-stressed *Thlaspi caerulescens*

Images of PS II activity parameters



Fluorescence yield
during F_m

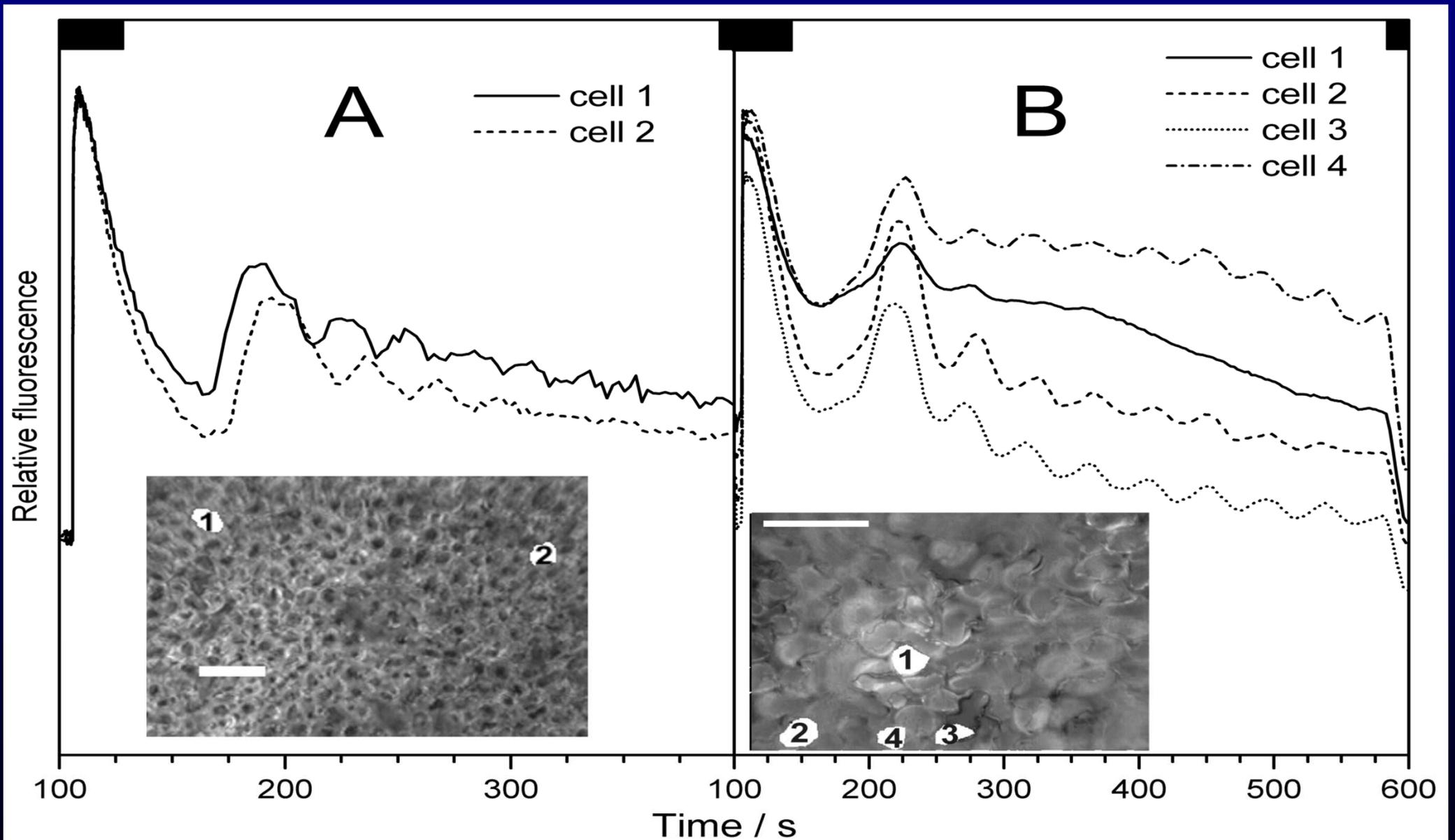
Efficiency of PS II
 F_v/F_m

Light saturation
 F_m/F_p

Electron flow through
PS II during actinic
irradiance $(F_m' - F_t)/F_m'$

Spatial heterogeneity of photosynthetic oscillations over the leaf surface

Insets: fluorescence emission images (F_p); the white bar represents 100 μm



Cd-stress in the Zn-/Cd-hyperaccumulator *T. caerulescens*: images of PSII activity parameters

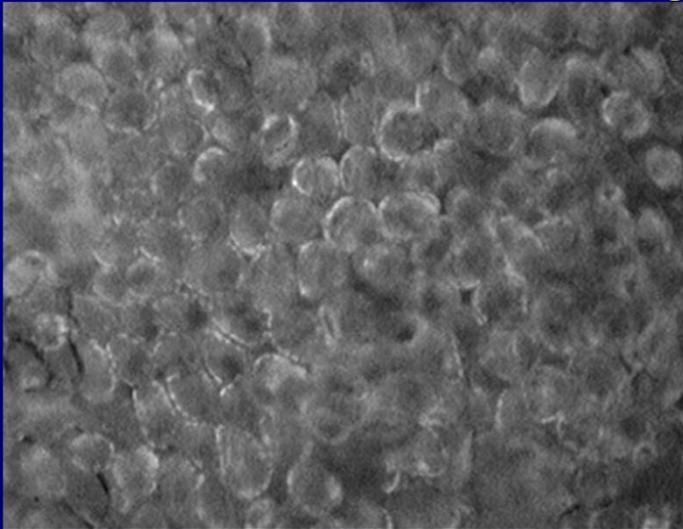


Image of F_m of an unstressed mature leaf

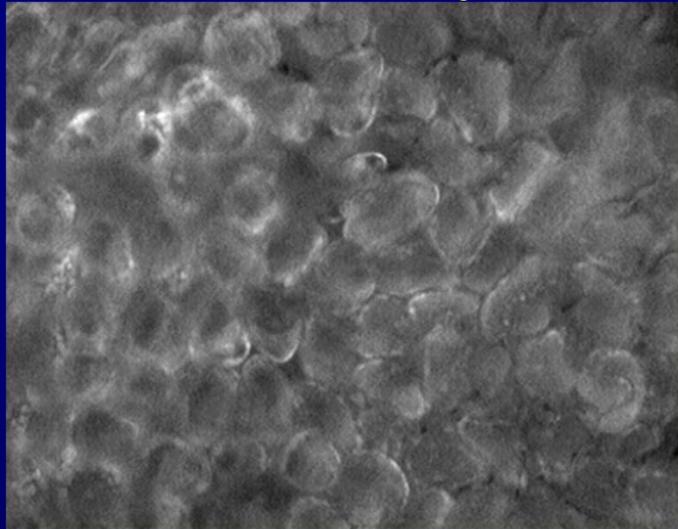


Image of F_m of a leaf stressed with $50\mu\text{M Cd}^2$, showing bright cells

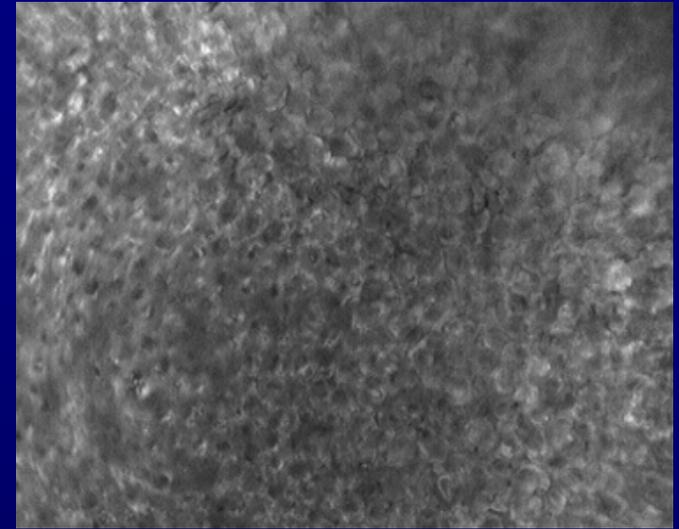


Image of F_m of the same sample as on the left, lower magnification

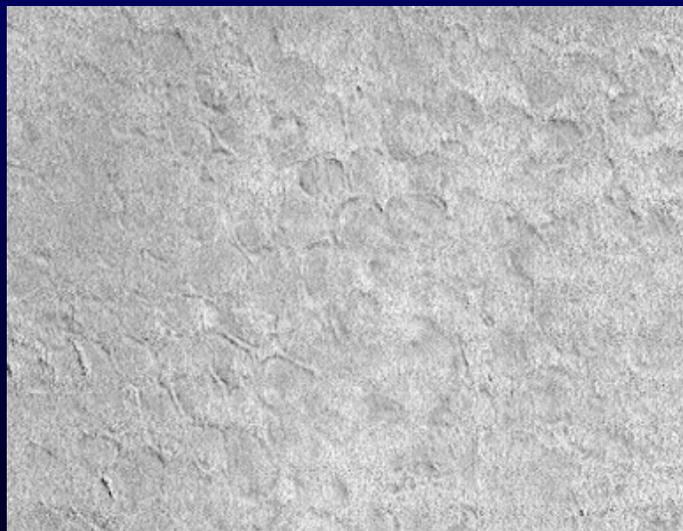


Image of F_v/F_m of the same sample as above, showing the homogeneously high photosynthetic activity of a healthy leaf of this plant

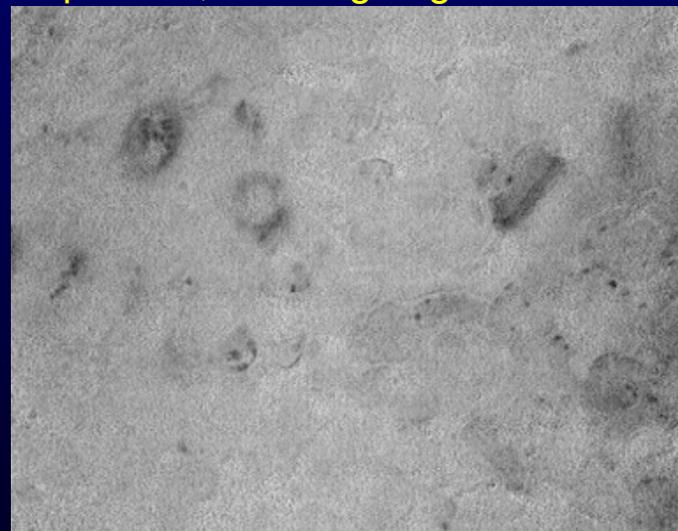


Image of F_v/F_m of the same sample as above, showing the low photosynthetic activity of the bright cells

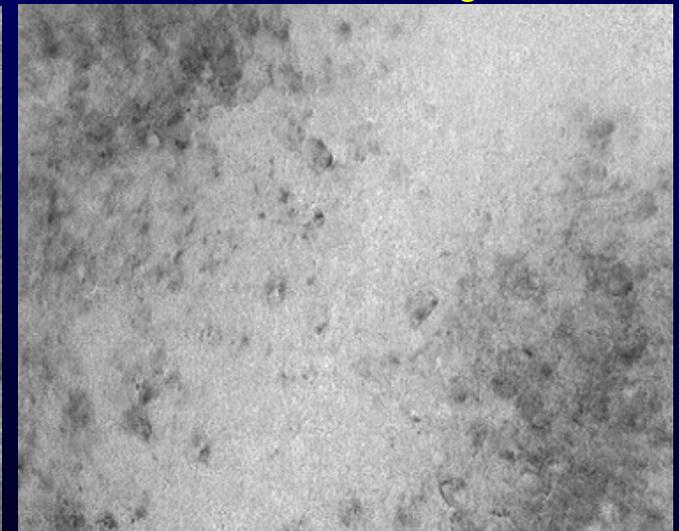
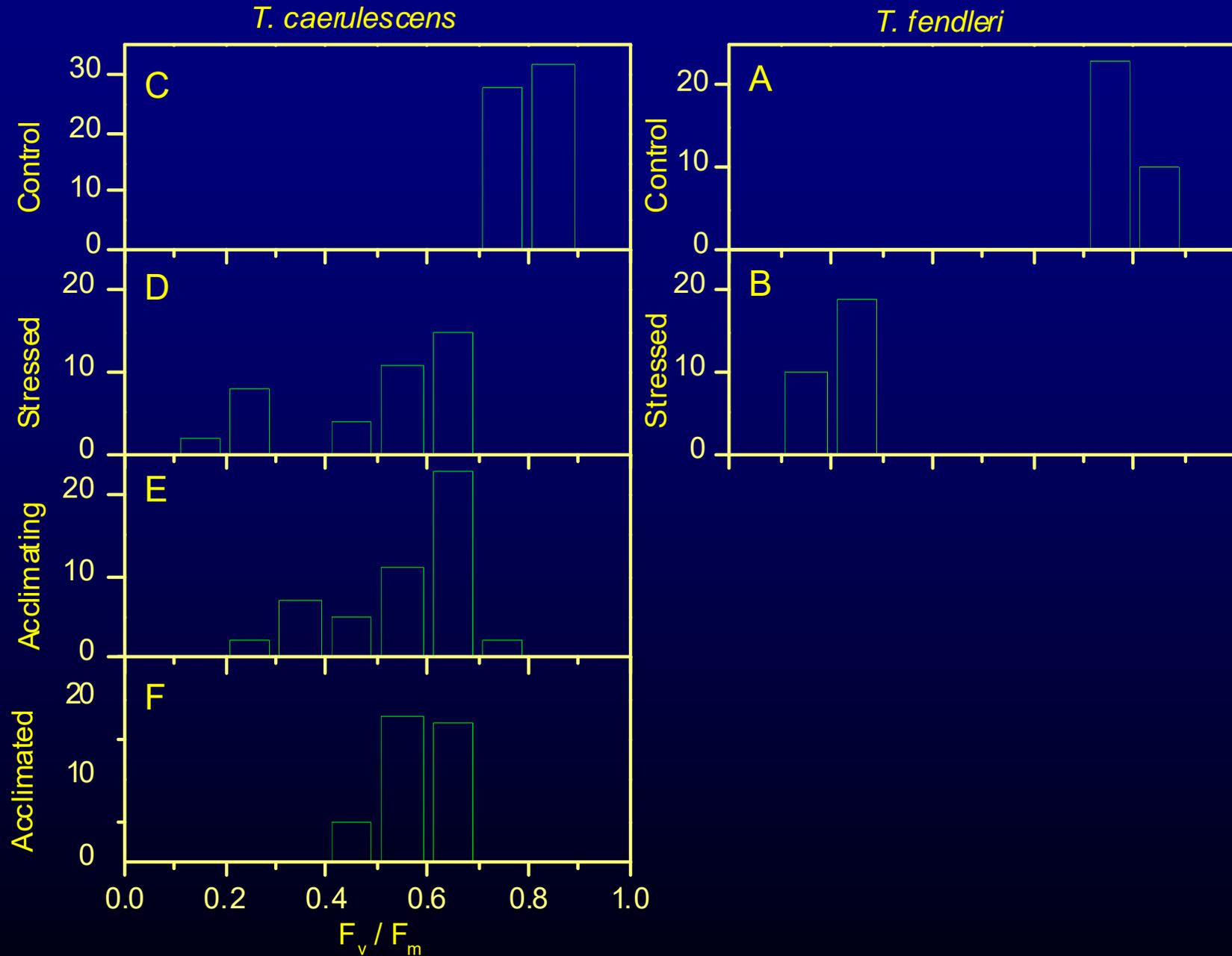
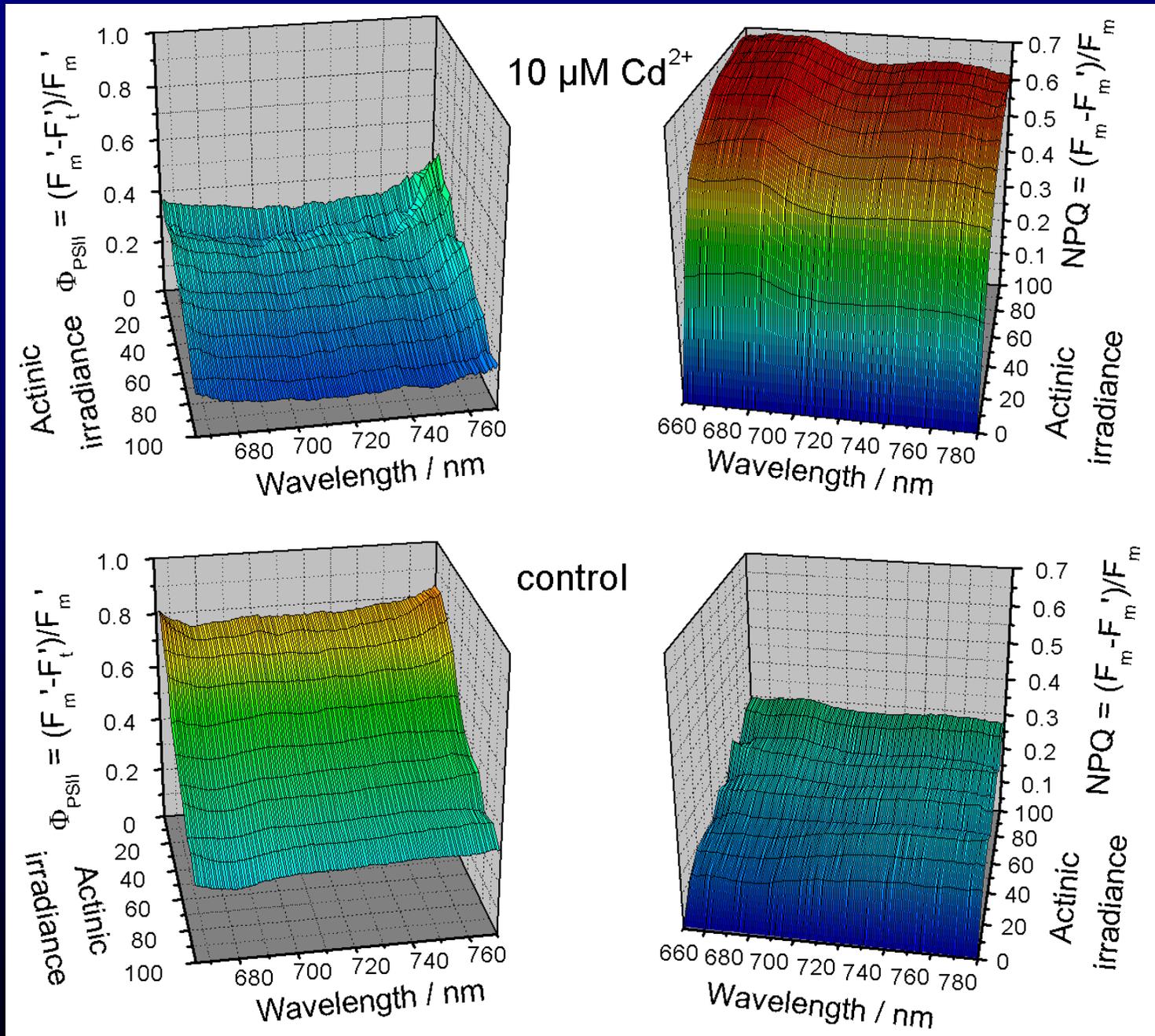


Image of F_v/F_m of the same sample as on the left, but with lower magnification

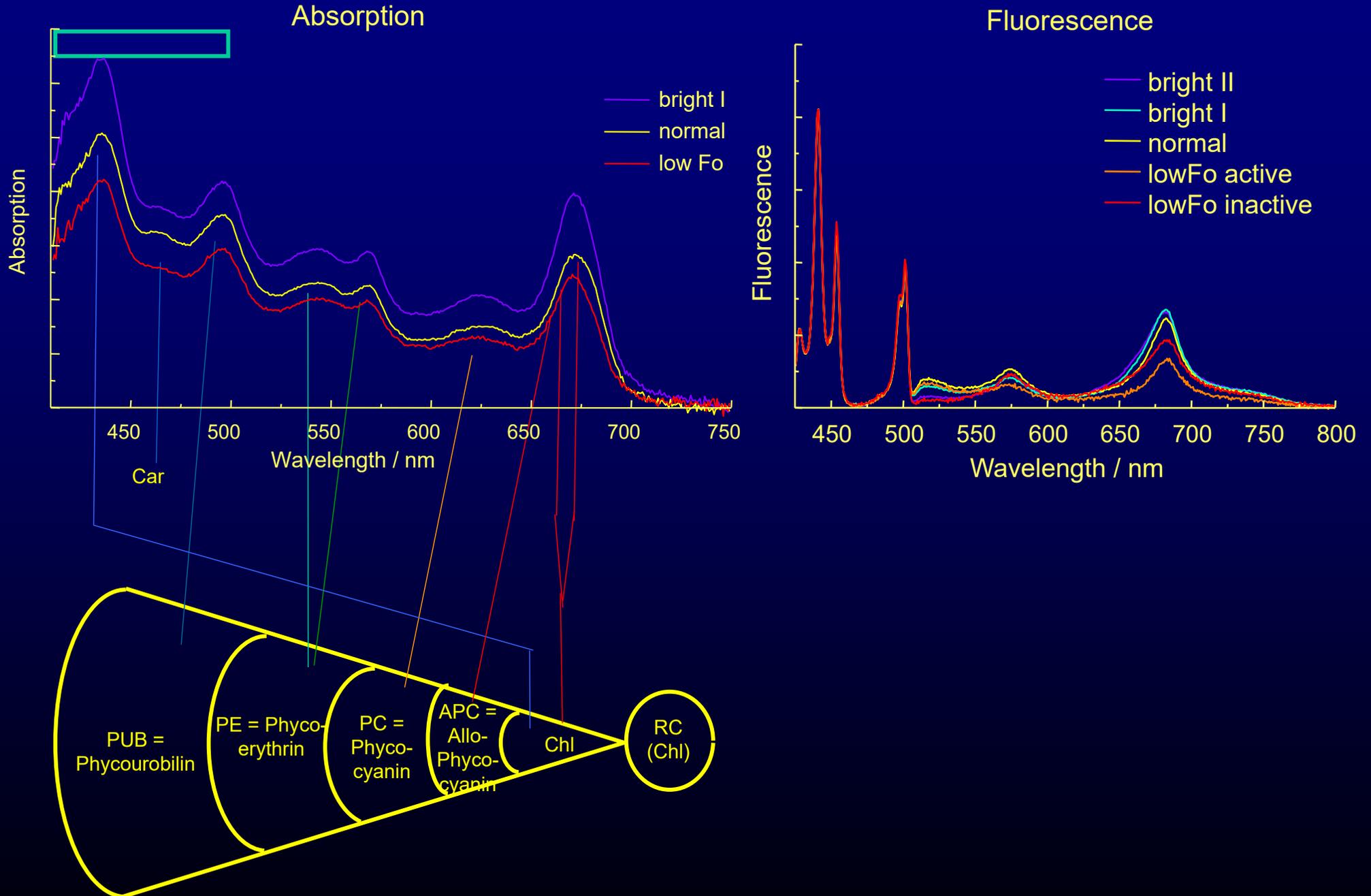
Cd-stress and acclimation in *T. caerulescens* & *T. fendleri*: histograms of F_v/F_m



Spectrally resolved fluorescence kinetic parameters (II)



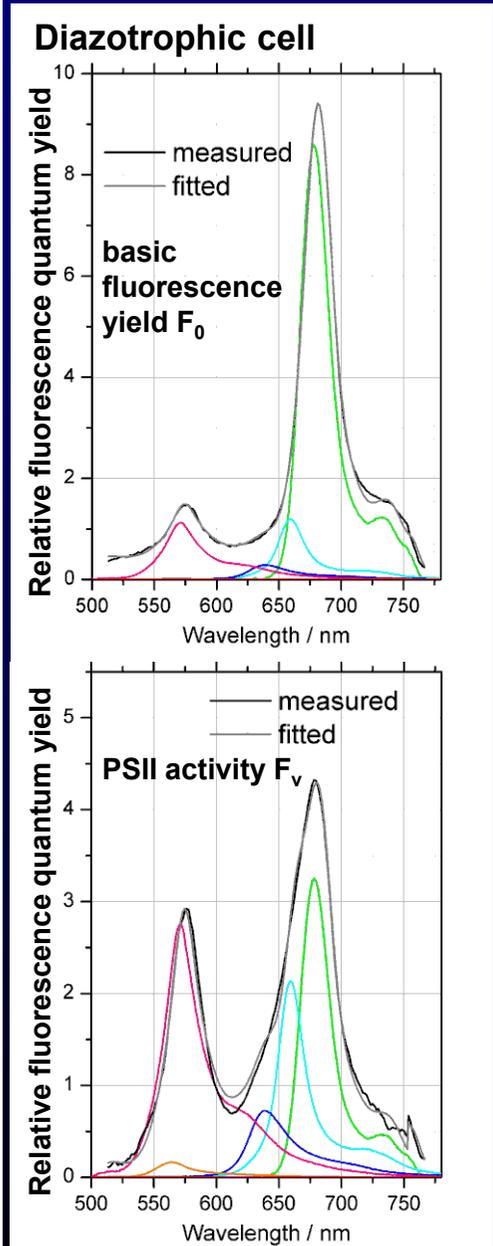
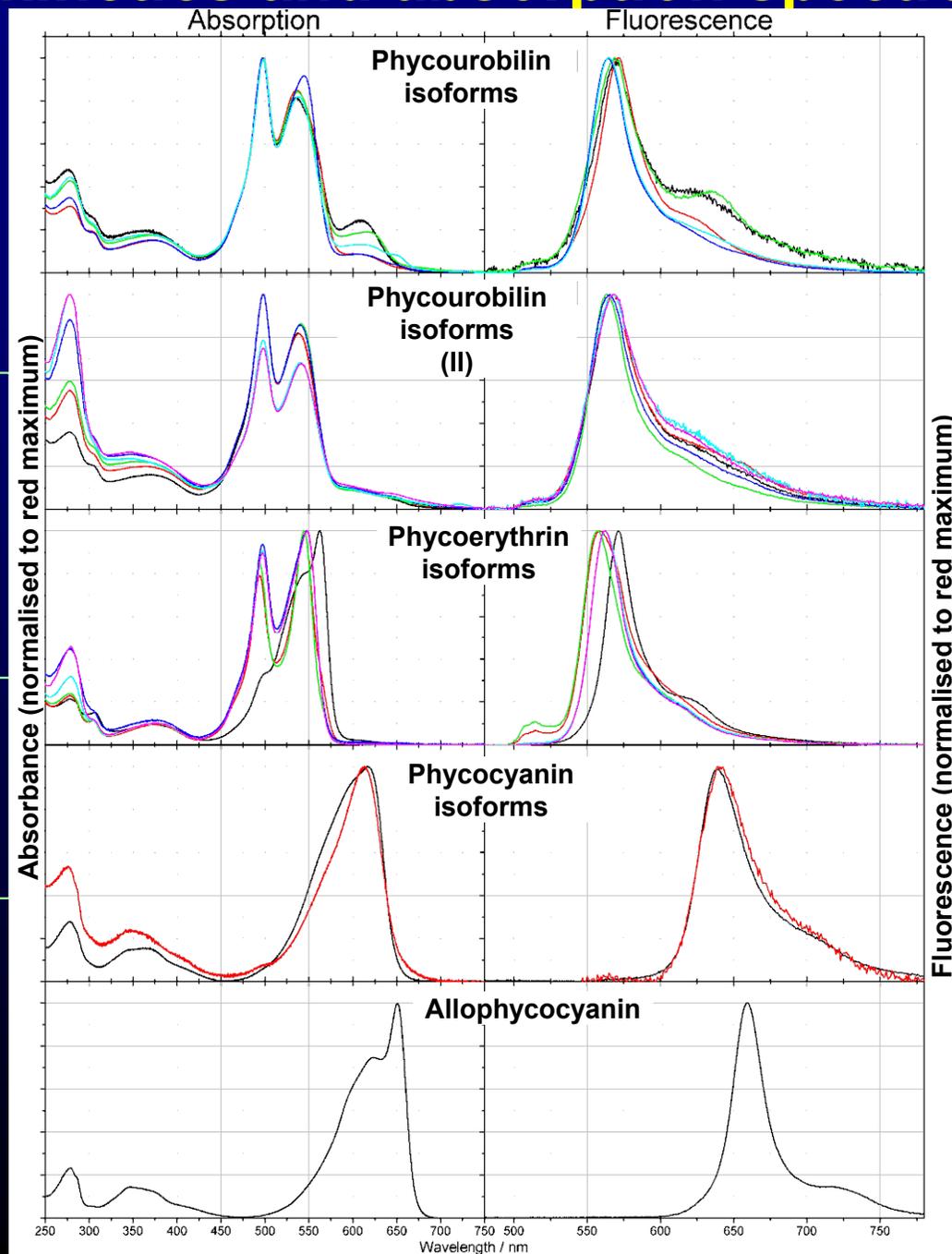
Spectrally resolved fluorescence kinetic parameters (I)



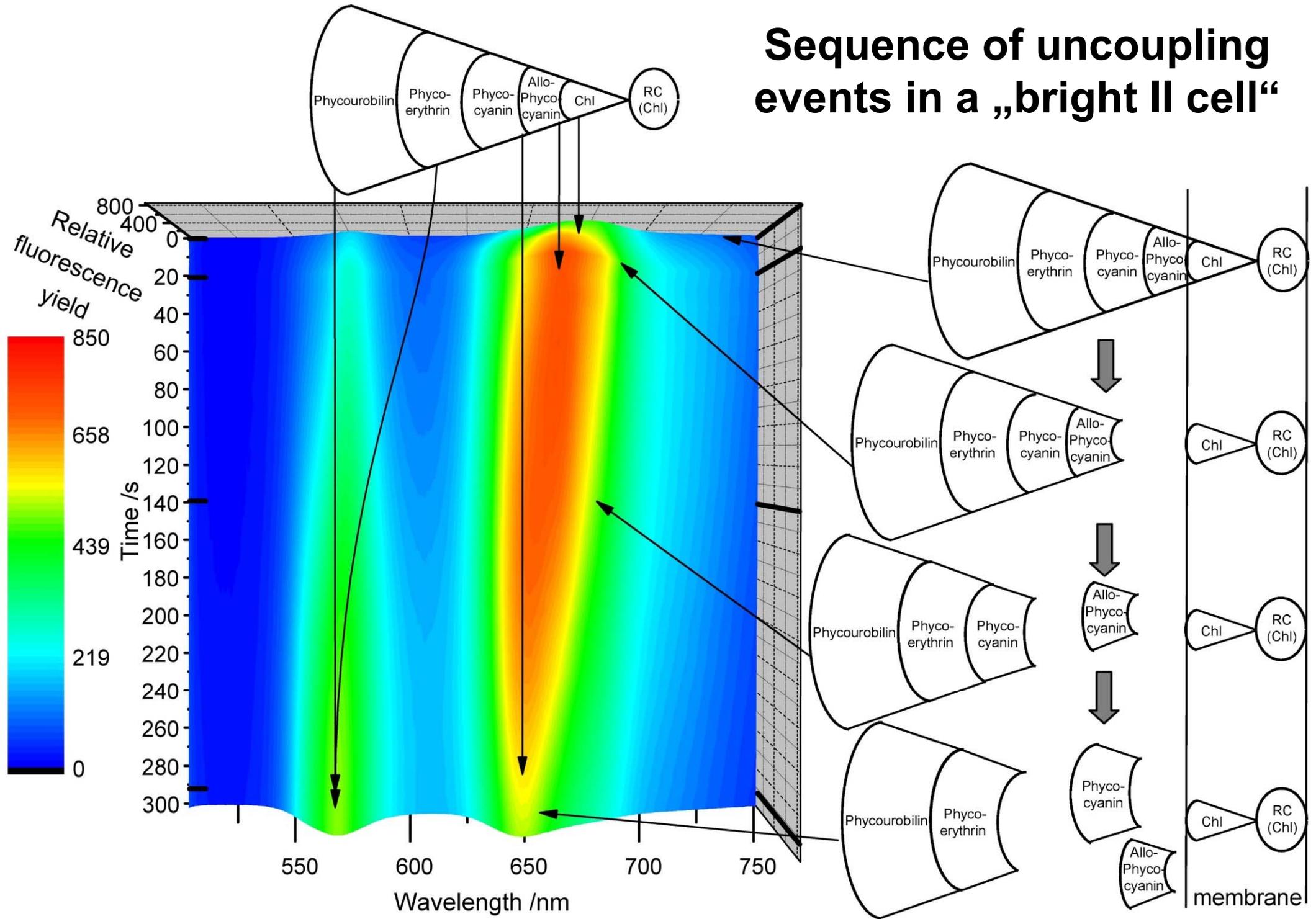
Purification of *Trichodesmium* phycobiliproteins for deconvoluting spectrally resolved *in vivo* fluorescence kinetics and absorption spectra

Phycobiliprotein purification + characterisation: Küpper H, Andresen E, Wiegert S, Šimek M, Leitenmaier B, Šetlík I (2009) *Biochim. Biophys. Acta (Bioenergetics)* 1787, 155-167

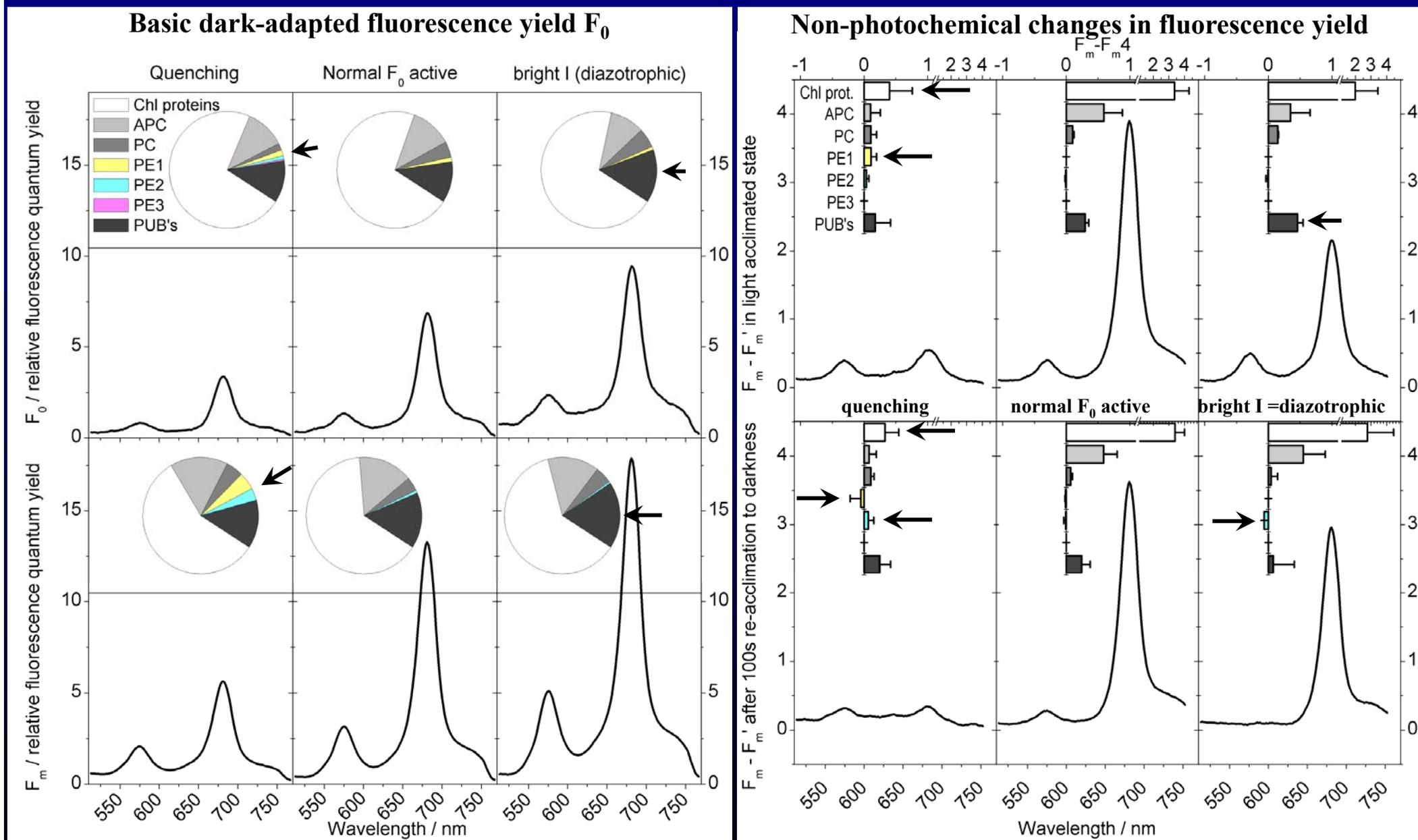
Method of deconvolution: Küpper H, Seibert S, Aravind P (2007) *Analytical Chemistry* 79, 7611-7627

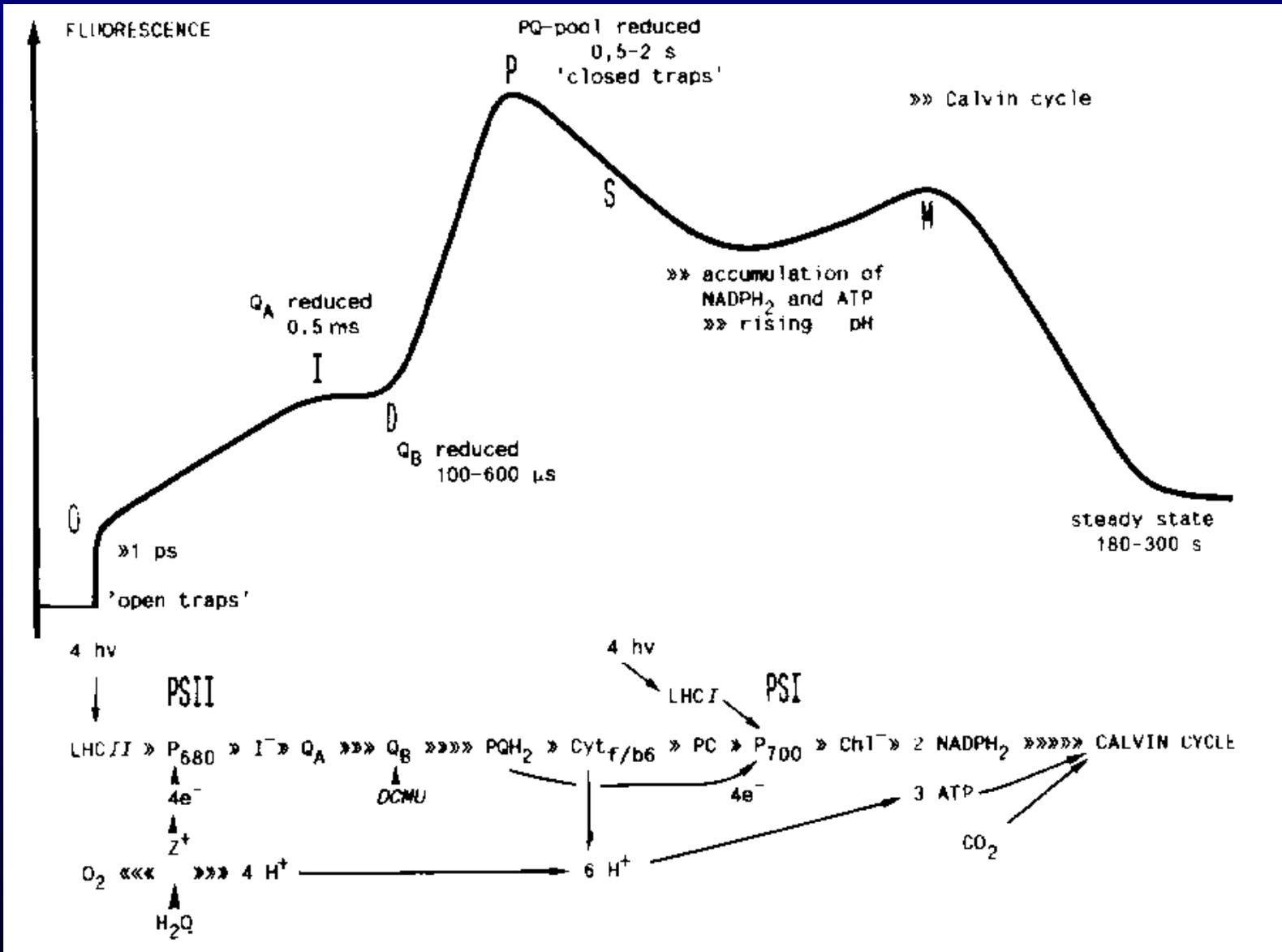


Sequence of uncoupling events in a „bright II cell“

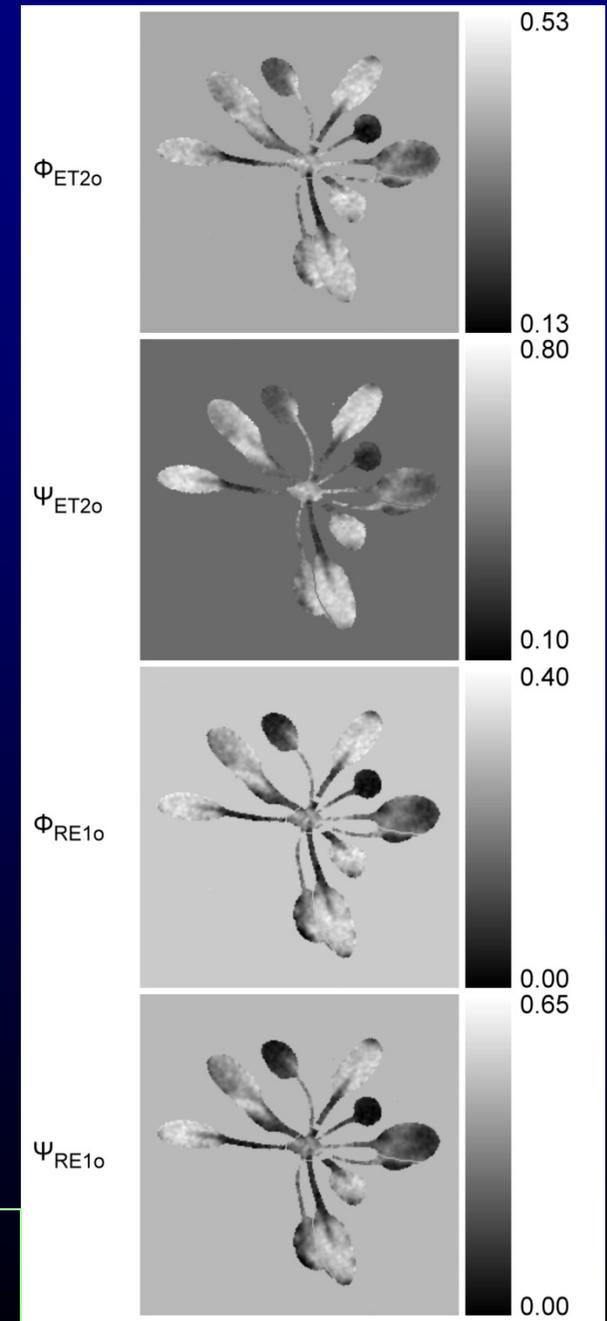
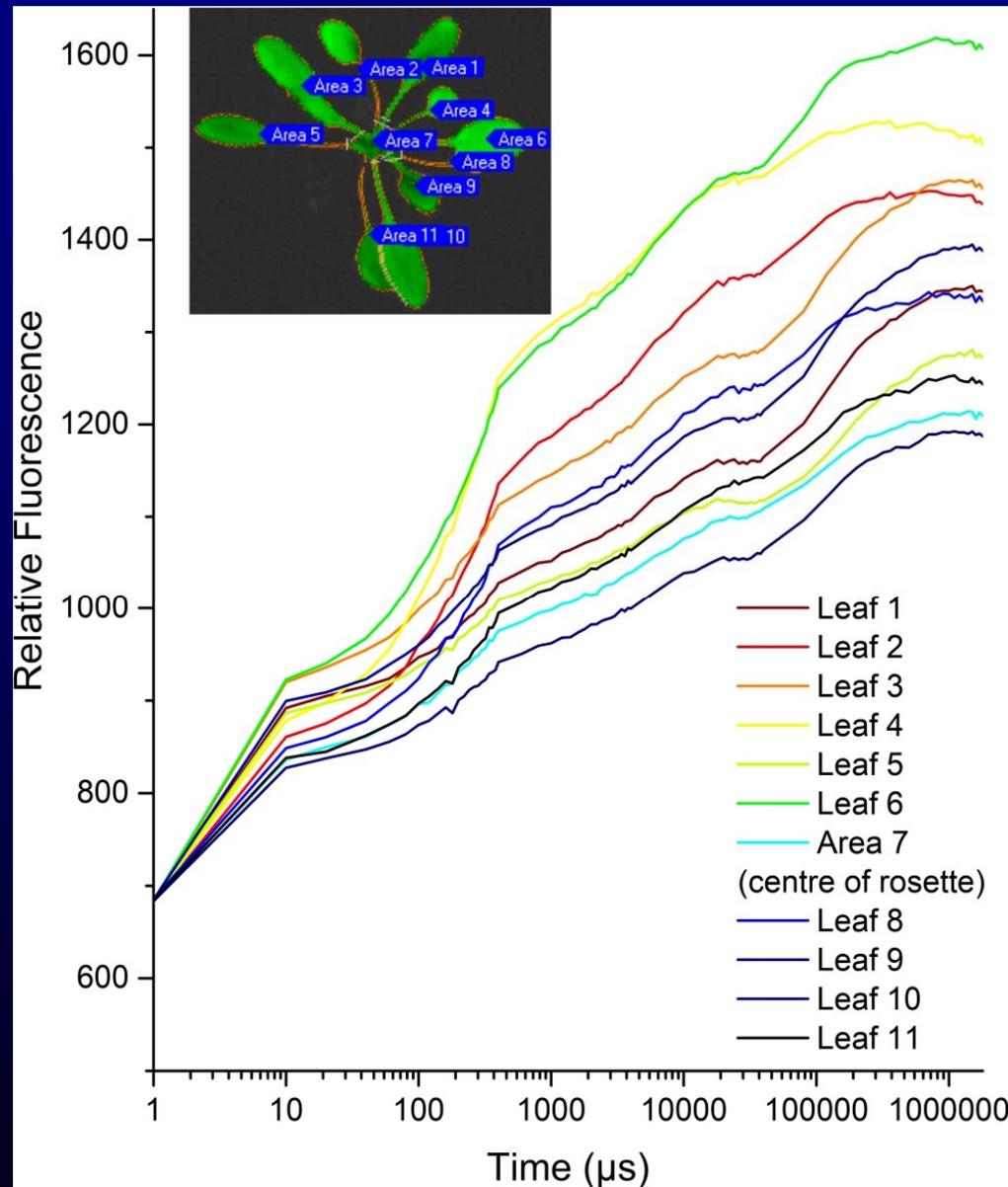


Deconvolution of spectrally resolved *in vivo* fluorescence kinetics shows reversible coupling of individual phycobiliproteins

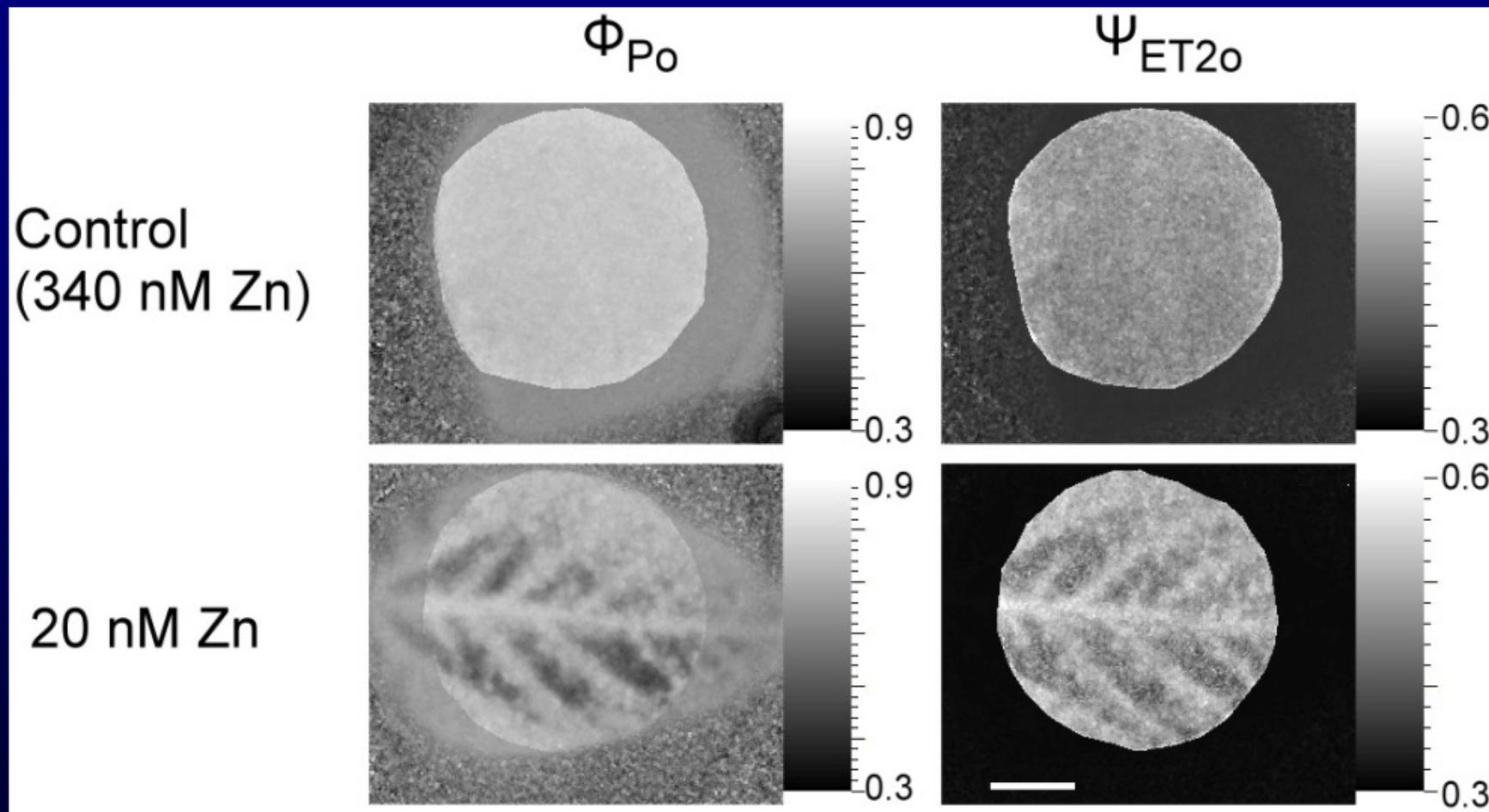




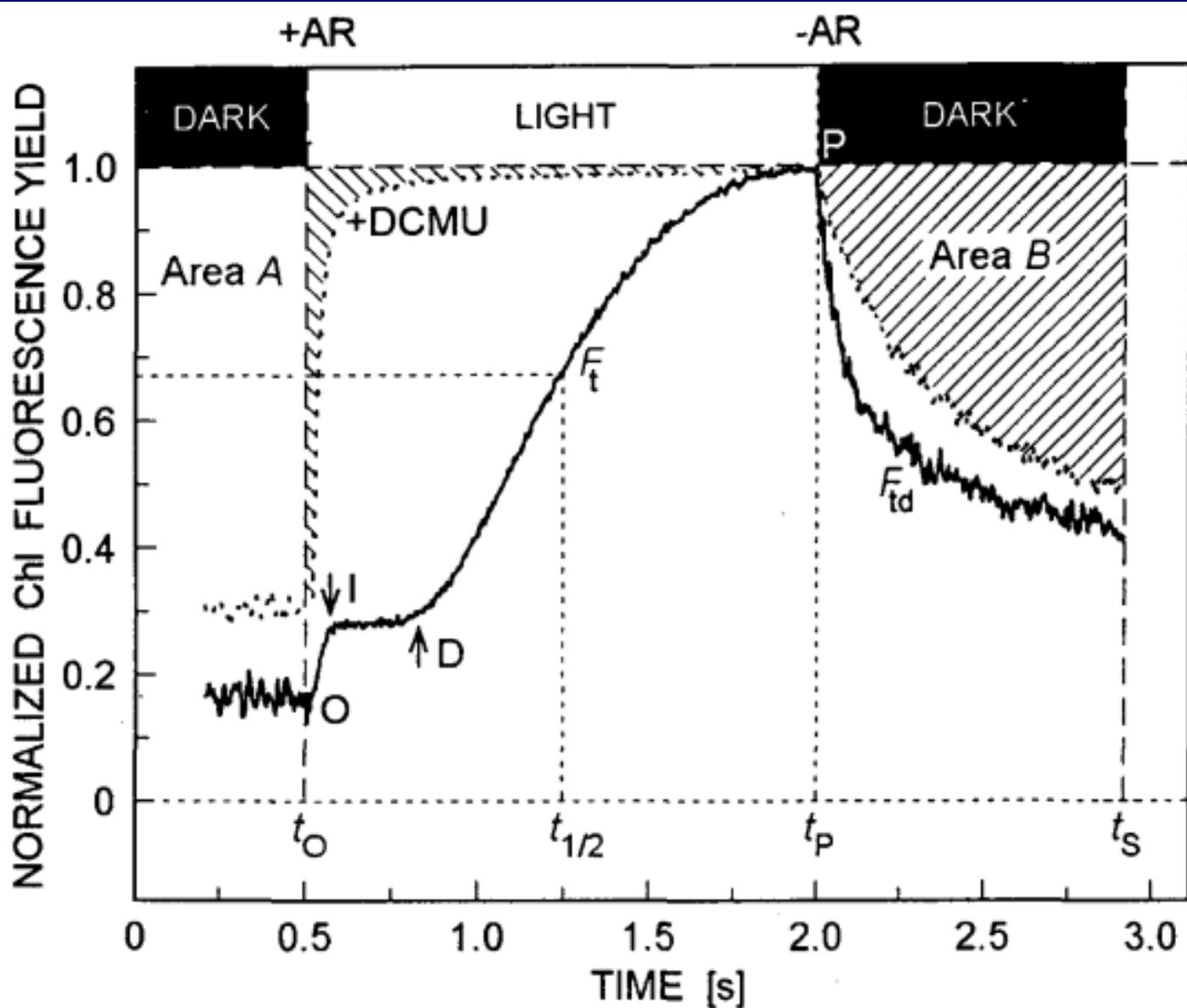
Differences in OJIP kinetics between leaves of an *A. thaliana* plant measured by direct fast imaging



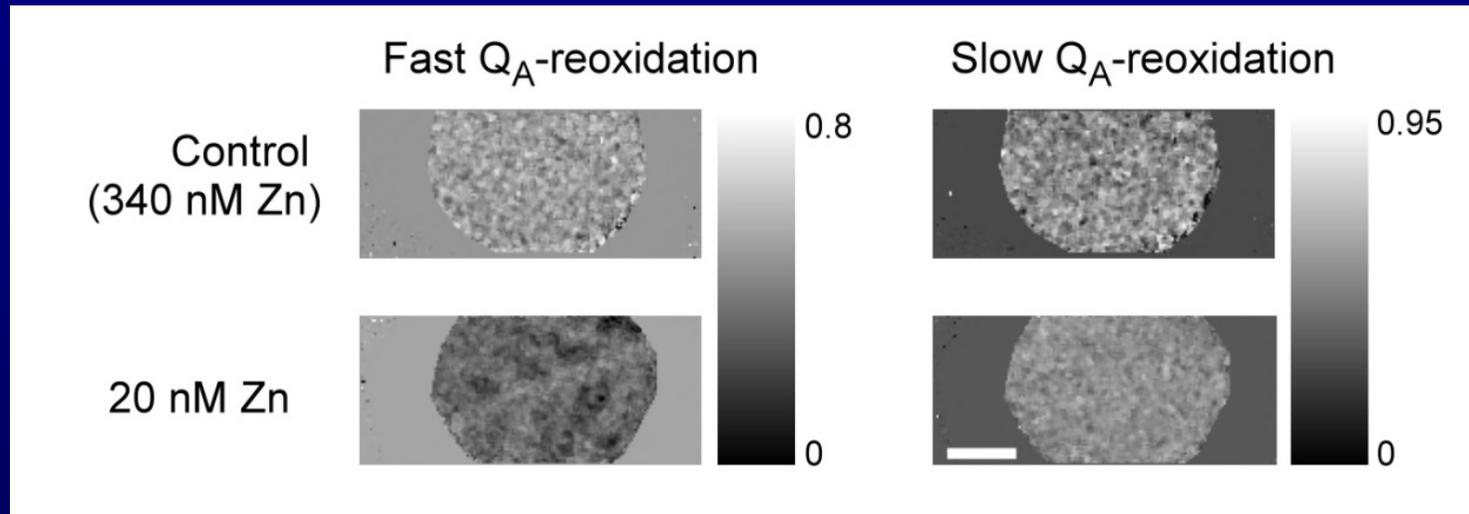
Changes of Ψ_{ET20} and Φ_{P0} in response to zinc deficiency stress



Soybeans treated with "0" Zn addition (20 nM residue from chemicals and water) compared to "control" (340 nM Zn) for 6 weeks. Scale bar is 1 cm.

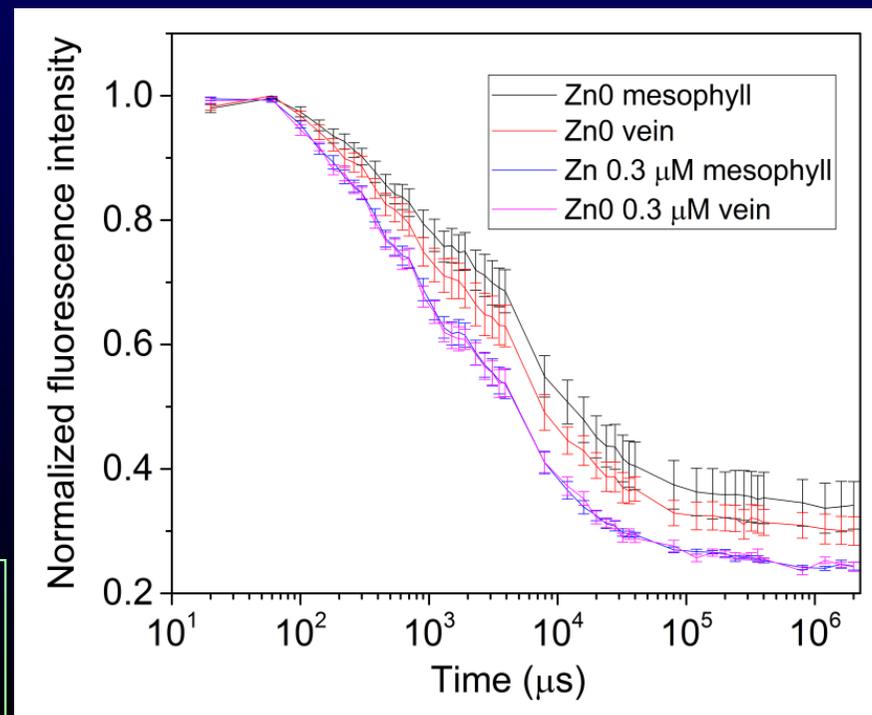


Differences in Q_A re-oxidation kinetics of veins (bundle sheath cells) and regular mesophyll cells in response to zinc deficiency stress



Soybeans treated with "0" Zn addition (20 nM residue from chemicals and water) compared to "control" (340 nM Zn) for 6 weeks.

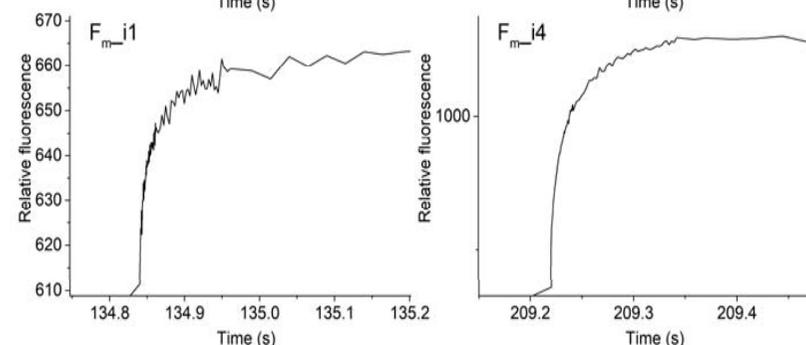
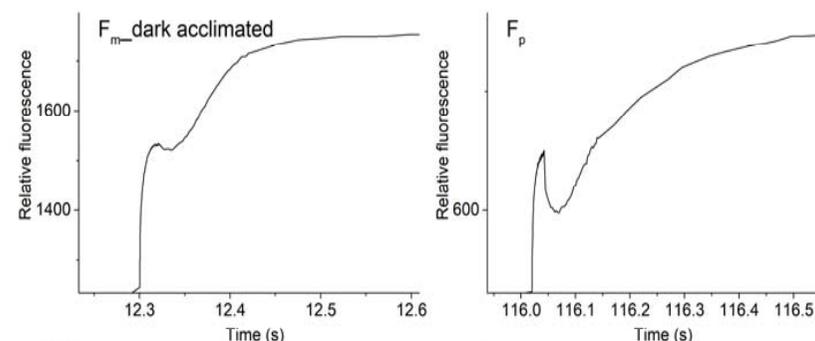
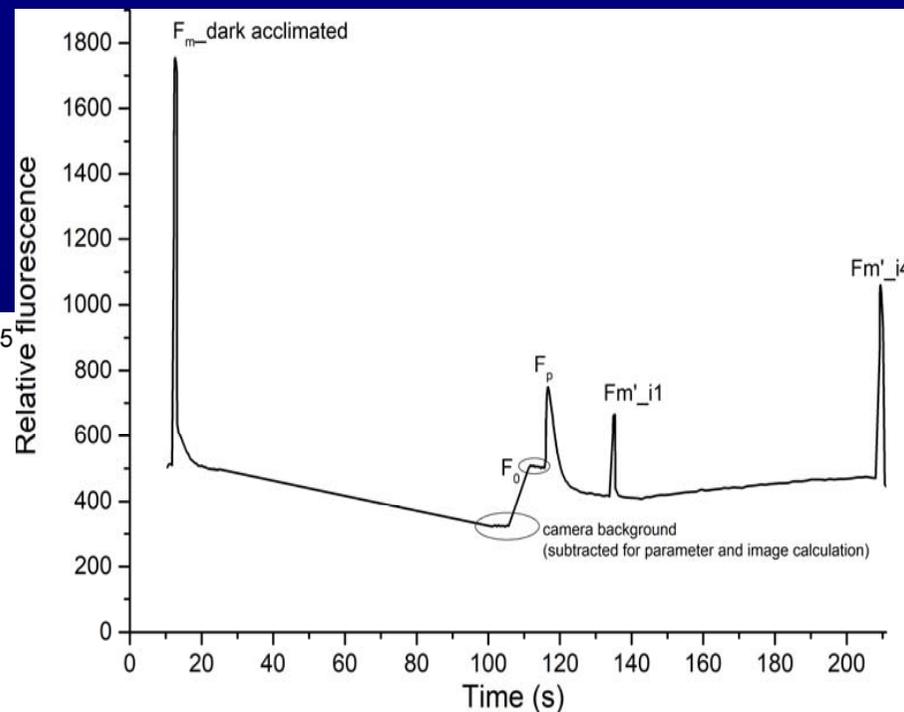
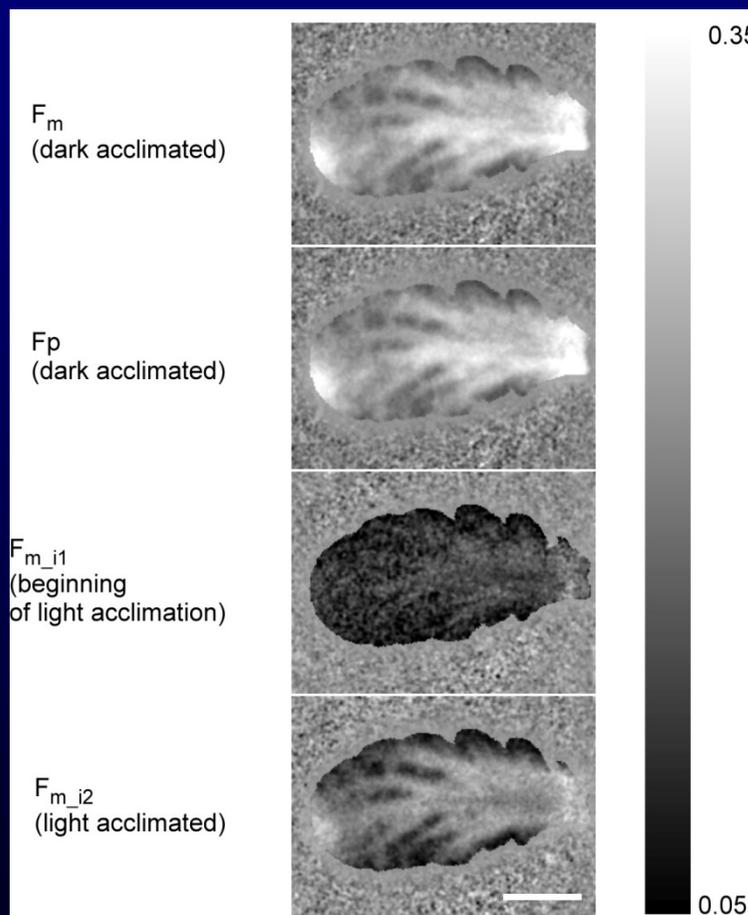
Scale bar is 1 cm.



Multi-OJIP protocol for analysis of adaptation to actinic light

Right: The shape of the fluorescence rises of each peak are shown below the main graph. The measured sample was a young-mature leaf of the Cd/Zn hyperaccumulator *Noccaea caerulescens* grown with replete (non-toxic) 100 μM Zn^{2+} for three months.

Left: $\Phi_{\text{ET}20}$ parameters derived from the multi-OJIP protocol of *Noccaea caerulescens* leaves grown with replete (non-toxic) 100 μM Zn^{2+} for three months. Scale bar is 1 cm.



**All slides of my lectures can be downloaded
from my workgroup homepage**

Biology Centre CAS → Institute of Plant Molecular Biology → Departments
→ Department of Plant Biophysics and Biochemistry,
or directly

http://webserver.umbr.cas.cz/~kupper/AG_Kuepper_Homepage.html